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Phylogeny of the *Pluteaceae* (*Agaricales*, *Basidiomycota*): taxonomy and character evolution

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Abstract

The phylogeny of the genera traditionally classified in the family *Pluteaceae* (*Agaricales*, *Basidiomycota*) was investigated using molecular data from nuclear ribosomal genes (nSSU, ITS, nLSU) and consequences for taxonomy and character evolution were evaluated. The genus *Volvariella* is polyphyletic, as most of its representatives fall outside the Pluteoid clade and shows affinities to some hygrophoroid genera (*Camarophyllus*, *Cantharocybe*). *Volvariella gloiocephala* and allies are placed in a different clade, which represents the sister group of *Pluteus*, and a new generic name, *Volvopluteus*, is proposed to accommodate these taxa. Characters such as basidiospore size and pileipellis structure can be used to separate *Pluteus*, *Volvariella* and *Volvopluteus*. The genus *Pluteus* is monophyletic and includes species with partial veil traditionally classified in the genus *Chamaeota*. The evolution of morphological features used in the infrageneric taxonomy of the genus, such as metuloid cystidia and pileipellis structure, was analyzed. Agreement between the molecular

phylogeny and morphological subdivision of *Pluteus* is, generally speaking, good, though some rearrangements are necessary: (i) species with non-metuloid pleurocystidia and pileipellis as a cutis are placed either in sect. *Celluloderma*, together with the species characterized by a hymenidermal pileipellis, or in sect. *Pluteus*, with the metuloid bearing species; (ii) subdivision of sect. *Celluloderma* according to the presence/absence of cystidioid elements in the pileipellis is not supported by molecular data.

Keywords

- Character evolution;
- Phylogeny;
- *Pluteus*;
- *Volvariella*;
- *Volvopluteus*

Introduction

The family *Pluteaceae* Kotl. & Pouzar (*Basidiomycota*, *Agaricales*) comprises three genera of non-mycorrhizal agaric fungi (*Chamaeota* (W.G. Sm.) Earle, *Pluteus* Fr. and *Volvariella* Speg.) that share the following combination of morphological characters (Fig 1): basidiocarps with lamellae that are free from the stipe; pink or pinkish brown spore print; basidiospores smooth, inamyloid, non-dextrinoid, cyanophilic; and inverse hymenophoral trama. *Pluteus* and *Chamaeota* species grow mostly on wood or other decaying plant material (sawdust, wood chips). The majority of species of *Volvariella* grow terrestrially in the litter layer of the soil, in grasslands or in woods, but one mycoparasitic and some lignicolous taxa are also known. The recently described *Volvariella terrea* Musumeci & A. Riva and *Volvariella koreana* Seok *et al.* grow among basidiocarps of *Agaricus xanthodermus* Genev. and *Clitocybe albofundibuliforme* Seok *et al.* respectively, but the exact nature of the relationship between both pairs of fungi is not yet known (Musumeci and Riva, 2007 and Seok *et al.*, 2009). The family is readily morphologically distinguishable among the *Agaricales*, especially based on the inverse hymenophoral trama and basidiospore characteristics, and a close relation to the *Amanitaceae* R. Heim ex Pouzar has been repeatedly postulated (Singer 1986). Separation of the three genera in the *Pluteaceae* has relied on the presence or absence of partial and universal veil on the basidiocarps, with *Volvariella* having a well-developed volva, *Chamaeota* showing a distinct annulus on the stipe and *Pluteus* lacking both volva and annulus (Singer 1986).



Fig 1. Morphological characters of the *Pluteaceae*. a. *Pluteus petasatus* (sect. *Pluteus*); b. *Pluteus plautus* (sect. *Hispidoderma*); c. *Pluteus aurantiorugosus* (sect. *Celluloderma*); d. *Pluteus mammillatus* (= *Chamaeota*); e. *Volvopluteus gloiocephalus*; f. *Volvariella volvacea*; g–h. Metuloid cystidia (*Pluteus* sect. *Pluteus*); i–j. Non-metuloid cystidia (*Pluteus* sect. *Hispidoderma* and *Celluloderma*); k–n. Pileipellis types in *Pluteus*: k. Cutis (sect. *Pluteus*), l. Trichoderm (sect. *Hispidoderma*), m. Hymeniderm (sect. *Celluloderma* subsect. *Eucellulodermini*), n. Hymeniderm with cystidioid elements (sect. *Celluloderma* subsect. *Mixtini*); o. Basidiospores of *Volvopluteus*; p. Basidiospores of *Volvariella*; q. Pileipellis (ixocutis) of *Volvopluteus*; r. Pileipellis (cutis-trichoderm) of *Volvariella*. Photos a, b, c, e and f courtesy of Agustín Caballero. Photo d courtesy of Walter J. Sundberg.

Pluteus, typified by *Pluteus cervinus*, includes approximately 300 species and is distributed worldwide (Kirk *et al.* 2008). Infrageneric taxonomy is primarily based on the characteristics of the hymenial cystidia and the pileipellis (Fig 1). Singer (1986) recognized three sections: Sect. *Pluteus* (with metuloid pleurocystidia and pileipellis as a cutis), Sect. *Hispidoderma* Fayod (with non-metuloid pleurocystidia and pileipellis composed of elongated elements organized as a cutis, an hymeniderm or a trichoderm) and Sect. *Celluloderma* Fayod (with non-metuloid pleurocystidia and a pileipellis composed of short, clavate or spheropedunculate elements organized as an hymeniderm, with transitions to an epithelium). Sect. *Celluloderma* is further subdivided into two subsections according to the presence (subject. *Mixtini* Singer) or absence (subject. *Eucellulodermini* Singer) of elongated cystidioid elements in the pileipellis. A variation on this taxonomic scheme was proposed by Vellinga & Schreurs (1985) that subdivided sect. *Hispidoderma* into two taxonomic units: the new sect. *Villosi* Vellinga & Schreurs, which accommodates the species with non-metuloid cystidia and a pileipellis as a cutis, and the subsection *Hispidodermini* Vellinga & Schreurs in sect. *Celluloderma*, for the remaining species, characterized by the pileipellis as a hymeniderm or trichoderm made up of elongated elements.

A total of 12 taxa have been classified in *Chamaeota*, though some have been shown to belong in other genera, like *Chamaeota dextrinoidespora* Z.S. Bi and *Chamaeota sinica* J.Z. Ying, that are in fact species of *Leucoagaricus* Locq. ex Singer (Yang 2007). The type species, *Chamaeota xanthogramma* (Ces.) Earle, is only known from the plate accompanying the original description and its true identity has been subjected to debate through the decades (Corriol and Moreau, 2007 and Singer, 1986). Only *Chamaeota mammillata*, from North America, and *Chamaeota fenzlii*, from Europe, are relatively well known.

Volvariella comprises about 50 species worldwide (Kirk *et al.* 2008), including the cultivated *Volvariella volvacea* ('paddy straw mushroom'). Several morphological/ecological groups can be differentiated within the genus, more or less corresponding to the 'stirps' recognized by Singer (1986): *Volvariella gloiocephala*-group (basidiospores >11 µm long, pileipellis as an ixocutis), *Volvariella bombycina*-group (pileus covered with conspicuous fibrills, lignicolous), *V. volvacea*-group (medium to large species, i.e. pileus >50 mm in diameter, with darkly colored, usually grey-brown, basidiocarps), *Volvariella taylorii*-group (small species, i.e. pileus <50 mm in diameter, with darkly colored, usually grey-brown, basidiocarps) and *Volvariella pusilla*-group (small species, with white basidiocarps, including the type species of the genus, *Volvariella argentina* Speng. and the mycoparasitic *Volvariella surrecta*).

Moncalvo *et al.* (2002) performed a phylogenetic analysis of the *Agaricales* based on nLSU data that included 17 sequences of *Pluteus* and two of *Volvariella*. *Pluteus* appeared well supported as monophyletic and subdivided into two major clades: one with only representatives of sect. *Pluteus* and the other a mixture of species of sections *Celluloderma* and *Hispidoderma* sensu Singer (1986). No representatives of subject. *Mixtini* were included. The genus *Melanoleuca* Pat., represented by two sequences, was placed as the sister group of *Pluteus*, which was rather unexpected based on morphological data. *Melanoleuca* has been traditionally classified in the *Tricholomataceae* R. Heim ex Pouzar (Singer 1986) and is characterized by a white spore print, basidiospores with amyloid ornamentation and regular hymenophoral trama. More surprising was the placement of *Volvariella* (*V. volvacea*, *Volvariella hypophytis*) in a very distant position, clustering (with no statistical support) with *Fistulina* Bull. and *Schizophyllum* Fr.

Matheny *et al.* (2006) presented the results of a six gene phylogeny of the *Agaricales*, including three taxa of *Pluteus*, one of *Volvariella* (*V. gloiocephala*) and one of *Melanoleuca*. In this analysis *Pluteus* appears as monophyletic with *V. gloiocephala* as its sister group, and *Melanoleuca* closely related. The three genera were placed together with members of the *Amanitaceae*, *Pleurotaceae* Kühner, the

aquatic basidiomycete *Limnoperdon* G.A. Escobar and some ‘orphan’ agaric genera (*Tricholomopsis* Singer, *Cantharocybe* H.E. Bigelow & A.H. Sm., *Macrocystidia* Joss.), in one major group named the ‘Pluteoid’ clade. However, the authors pointed out that this general grouping was poorly supported and some of its constituents fell outside the Pluteoid clade in some of the analysis. Binder *et al.* (2010) recovered a similar topology for *Pluteus*, *V. gloiocephala* and *Melanoleuca*, with *Amanita* Pers. as the sister group of the ‘core’ pluteoid genera, but in this study *Tricholomopsis* and *Cantharocybe* were placed outside the Pluteoid clade.

Minnis *et al.* (2006) generated new nLSU data for the American *C. mammillata* and performed a phylogenetic analysis, showing that this annulate species clusters inside *Pluteus*, and questioned the taxonomic status of the genus *Chamaeota*. *Chamaeota mammillata* and the other North American species of the genus, *Chamaeota sphaerospora*, were placed in synonymy and the new combination *Pluteus mammillatus* was proposed. Based on morphological data alone, the European *C. fenzi* was also transferred to *Pluteus*, and the subsection *Annularia* (Schulzer) Corriol & P.-A. Moreau was introduced in sect. *Hispidoderma* to accommodate this species (Corriol & Moreau 2007). Morphology-based phylogenies of *Pluteus* (Banerjee and Sundberg, 1995 and Minnis and Sundberg, 2009) and description of new taxa based on morphological and molecular data (Menolli *et al.*, 2010, Rodríguez *et al.*, 2009 and Rodríguez *et al.*, 2010) have also been published in recent years.

The present study has two major objectives. First, it aims to resolve the conflicting results about the evolutionary relationships of the genera *Pluteus* and *Volvariella*; second it focuses on character evolution in *Pluteus*, especially in relation to the proposed infrageneric classifications (Singer, 1986 and Vellinga and Schreurs, 1985). For these purposes, new molecular data of three nuclear ribosomal loci, 18S (nSSU), ITS1 + 5.8S + ITS2 (ITS), and 25S (nLSU), were generated for all the major morphological/ecological groups of *Pluteus* and *Volvariella*. Phylogenetic analyses were performed on single gene and multi-gene datasets and consequences for morphological evolution and taxonomy in the *Pluteaceae* were examined. During the course of this study we gained some insights about several species-complexes in the *Pluteaceae*, including newly discovered taxa, but they will be discussed in detail in a separate paper (Justo *et al.* 2010).

Materials and methods

Fungal collections

A total of 182 fresh or dried specimens of *Pluteus* and *Volvariella* were selected for molecular sampling (Table 1). The geographic origin of the collections includes Africa, Asia, Europe, North and South America. In many cases, morphological study and identification of the collections were done previously to this work (Justo and Castro, 2007a, Justo and Castro, 2010, Menolli *et al.*, 2010 and Minnis and Sundberg, 2010), but most specimens were re-examined during the course of this study. Newly collected materials, and all the Asian and African herbarium collections, were studied using standard procedures for morphological examination of the *Pluteaceae* (e.g. Justo and Castro, 2007b and Minnis and Sundberg, 2010). Descriptive terms for morphological features follow Vellinga (1988). ‘Q’ is used for the quotient of length and width, and ‘avQ’ is the average quotient. Herbarium acronyms follow Thiers (2010).

Table 1. New sequences generated for this study with GenBank accession numbers. *P* = *Pluteus*, *V* = *Volvariella*; *Vp* = *Volvopluteus*. An asterisk (*) indicates names being published concurrently with the present article. The symbol '!' indicates a type collection (holotypus, epitypus or paratypus).

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. aff. cervinus</i>		HM562098		AJ181 (MICH)	USA (MA)
<i>P. aff. cervinus</i>		HM562085	HM562242	AJ148 (MICH)	USA (FL)
<i>P. aff. cervinus</i>		HM562034	HM562220	AJ78 (LOU)	Spain
<i>P. aff. cervinus</i>		HM562101		TNSF12351	Japan
<i>P. aff. cervinus</i>		HM562100		TNSF12361	Japan
<i>P. aff. cervinus</i>		HM562126		TNSF16091	Japan
<i>P. aff. cervinus</i>		HM562102		TNSF12352	Japan
<i>P. aff. cervinus</i>		HM562103		TNSF12370	Japan
<i>P. aff. cervinus</i>		HM562128		TNSF897	Japan
<i>P. aff. cervinus</i>		HM562168		REG13664	Germany
<i>P. aff. cervinus</i>		HM562151		REG13658	Germany
<i>P. aff. cervinus</i> (white basidiocarp)		HM562178		SF7 (BPI)	USA (IL)
<i>P. aff. cinereofuscus</i>		HM562115		TNSF12400	Japan
<i>P. aff. ephebeus</i>		HM562080		Shaffer4673 (MICH)	France
<i>P. aff. ephebeus</i>		HM562198		Pearson s.n. (MICH, as <i>P. plautus</i>)	UK (England)
<i>P. aff. leoninus</i> I		HM562190		SF21(BPI)	USA (MO)
<i>P. aff. leoninus</i> I		HM562188		SF19 (BPI)	USA (IL)
<i>P. aff. leoninus</i> II		HM562139		TNSF11908	Japan
<i>P. aff. nothopellitus</i>		HM562177		SF5 (BPI)	USA (MI)
<i>P. aff. nothopellitus</i>		HM562060		AHS42452 (MICH)	USA (MI)
<i>P. aff. phlebophorus</i>		HM562186		SF16 (SIU)	USA (IL)
<i>P. aff. podospileus</i>		HM562196		Lundell2541 (MICH)	Sweden
<i>P. aff. pouzarianus</i>		HM562156		REG13683	Germany

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. aff. pouzarianus</i>		HM562167		REG13620	Germany
<i>P. aff. semibulbosus</i>		HM562090		TNSF12393	Japan
<i>P. allostipitatus</i> (Dennis) Singer	HM562262	HM562057	HM562090	AJ154 (MICH)	USA (FL)
<i>P. allostipitatus</i> (Dennis) Singer		HM562106	HM562243	AJ187 (MICH)	USA (FL)
<i>P. allostipitatus</i> (Dennis) Singer		HM562130		GF5374 (BR)	Democratic Rep. of Congo
<i>P. atromarginatus</i> (Singer) Kühner		HM562083		Sundberg3657 (SIU)	USA (OR)
<i>P. atromarginatus</i> (Singer) Kühner		HM562040	HM562219	AJ76 (LOU)	Spain
<i>P. atromarginatus</i> (Singer) Kühner	HM562274	HM562061	HM562218	AJ75 (LOU)	Spain
<i>P. atropungens</i> A.H. Sm. & Bartelli		HM562059		A.H.Smith62033!(MICH)	USA (MI)
<i>P. aurantiorugosus</i> (Trog) Sacc.		HM562121		TNSF12391	Japan
<i>P. aurantiorugosus</i> (Trog) Sacc.		HM562074		Hoseny1740 (MICH)	USA (MI)
<i>P. aurantiorugosus</i> (Trog) Sacc.		HM562072		Bigelow19232 (NY)	USA (MA)
<i>P. aurantiorugosus</i> (Trog) Sacc.		HM562081		ILLS42433	USA (IL)
<i>P. aurantiorugosus</i> (Trog) Sacc.		HM562041		AJ219 (LOU)	Spain
<i>P. aureovenatus</i> Menolli & Capelari		HM562160		SP394388	Brazil
<i>P. brunneidiscus</i> Murrill		HM562042		AJ61 (MA)	Spain
<i>P. castri</i> Justo & E.F. Malysheva*		HM562099		TNSF17081	Japan

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. castris</i> Justo & E.F. Malysheva*		HM562092		TNSF17602!	Japan
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562176		SF4 (BPI)	USA (MI)
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562043		AJ106 (MICH)	USA (MA)
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562136		AJ77 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562261	HM562035	HM562221	AJ82 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562133		AJ191 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562135		AJ79 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562134		AJ192 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562104		TNSF12347	Japan
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562153		REG13618	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562169		REG13622	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562171		REG13661	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562152		REG13641!	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562150		REG16651	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562155		REG13616	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562166		REG13662	Germany

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. cervinus</i> (Schaeff.) P. Kumm.		HM562165		REG13652	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm. (white basidiocarp)		HM562200		SF1 (BPI)	USA (IL)
<i>P. cervinus</i> var. <i>scaber</i> J.E. Lange		HM562075		A.H.Smith 39846 (MICH)	USA (WA)
<i>P. cf. eugraptus</i>		HM562116		TNSF12042	Japan
<i>P. cf. nanus</i>		HM562046	HM562247	AJ216 (LOU)	Spain
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.		HM562181		SF11 (SIU)	USA (IL)
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.		HM562182		SF12 (BPI)	USA (IL)
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.		HM562180		SF10 (BPI)	USA (IL)
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562269	HM562064	HM562241	AJ45 (MA)	Spain
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.		HM562088		TNSF12388	Japan
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.		HM562125		TNSF12383	Japan
<i>P. cinereofuscus</i> J.E. Lange	HM562271	HM562124	HM562248	AJ34 (MA)	Spain
<i>P. cinereofuscus</i> J.E. Lange		HM562108		AJ229 (LOU)	Portugal
<i>P. conizatus</i> var. <i>africanus</i> E. Horak		HM562142		Thoen5250	Democratic Rep. of Congo

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. densifibrillosus</i> Menolli & Capelari		HM562159		SP393696!	Brazil
<i>P. dietrichii</i> Bres.		HM562143	HM562239	JLS1624 (J.L. Siquier)	Spain
<i>P. eliae</i> Singer		HM562076		Isaacs2460 (MICH)	USA (FL)
<i>P. eludens</i> E.F. Malysheva, Minnis & Justo*		HM562185		SF15 (BPI)	USA (IL)
<i>P. eludens</i> E.F. Malysheva, Minnis & Justo*		HM562118	HM562240	MA50497!	Portugal (Madeira)
<i>P. ephebeus</i> (Fr.) Gillet	HM562265	HM562044	HM562237	AJ234 (LOU)	Spain
<i>P. fenzlii</i> (Schulzer) Corriol & P.-A. Moreau		HM562111	HM562236	Kotlaba F1020647 (F)	Slovakia
<i>P. fenzlii</i> (Schulzer) Corriol & P.-A. Moreau		HM562091		TNSF12376	Japan
<i>P. glaucotinctus</i> E. Horak		HM562132	HM562251	Thoen5546 (BR)	Democratic Rep. of Congo
<i>P. glaucotinctus</i> E. Horak		HM562157		SP394384	Brazil
<i>P. glaucotinctus</i> E. Horak		HM562147		SP394380	Brazil
<i>P. glaucotinctus</i> E. Horak		HM562131		GF5274! (BR)	Democratic Rep. of Congo
<i>P. granularis</i> Peck		HM562069		Strack7 (SIU)	USA (IL)
<i>P. granularis</i> Peck		HM562189		SF20 (BPI)	USA (IL)
<i>P. granulatus</i> Bres.	HM562276	HM562048	HM562226	AJ203 (LOU)	Spain
<i>P. heteromarginatus</i> Justo*	HM562275	HM562058	HM562249	AJ172! (MICH)	USA (FL)

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. leoninus</i> (Schaeff.) P. Kumm.		HM562187		SF17 (BPI)	USA (MI)
<i>P. leoninus</i> (Schaeff.) P. Kumm.		HM562045	HM562234	AJ212 (LOU)	Spain
<i>P. leoninus</i> (Schaeff.) P. Kumm.		HM562071		Halling6546 (NY)	USA (NY)
<i>P. leoninus</i> (Schaeff.) P. Kumm.		HM562077		Josserand s.n (MICH, as <i>P. luteomarginatus</i>)	France
<i>P. leoninus</i> (Schaeff.) P. Kumm.		HM562215		DrewH (BPI)	USA (ID)
<i>P. longistriatus</i> (Peck) Peck		HM562082		Minnis309203 (SIU)	USA (MO)
<i>P. longistriatus</i> (Peck) Peck		HM562158		SP393700	Brazil
<i>P. longistriatus</i> (Peck) Peck		HM562172		SP394386	Brazil
<i>P. longistriatus</i> (Peck) Peck		HM562149		SP394004	Brazil
<i>P. losulus</i> Justo*(= <i>P. cervinus</i> var. <i>ealaensis</i> Beeli)		HM562129	HM562250	GF5273 (BR)	Democratic Rep. of Congo
<i>P. magnus</i> McClatchie		HM562087	HM562245	CBM36790	Japan
<i>P. mammillatus</i> (Longyear) Sundb. & Methven	Minnis, HM562266	HM562086		Minnis309202 (SIU)	USA (MO)
<i>P. mammillatus</i> (Longyear) Sundb. & Methven	Minnis,	HM562120		Singer244A (F)	USA (FL)
<i>P. mammillatus</i> (Longyear) Sundb. & Methven	Minnis,	HM562119		ASM7916! (EIU)	USA (MO)
<i>P. multiformis</i> Justo, A. Caball. & G. Muñoz*		HM562201		AC4249! (AH)	Spain

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. nothopellitus</i> Justo & M.L. Castro		HM562063	HM562235	AJ58!(MA)	Spain
<i>P. pallescens</i> P.D. Orton	HM562272	HM562056	HM562231	AJ214 (LOU)	Spain
<i>P. pallidus</i> Homola		HM562193		A.H.Smith62487! (MICH)	USA (MI)
<i>P. pantherinus</i> Courtec. & M. Uchida		HM562089		TNSF12882	Japan
<i>P. pellitus</i> (Pers.) P. Kumm.		HM562037	HM562225	AJ202 (LOU)	Spain
<i>P. pellitus</i> (Pers.) P. Kumm.		HM562047	HM562223	AJ74 (LOU)	Spain
<i>P. pellitus</i> (Pers.) P. Kumm.		HM562036	HM562222	AJ72 (LOU)	Italy
<i>P. petasatus</i> (Fr.) Gillet		HM562179		SF9 (BPI)	USA (MO)
<i>P. petasatus</i> (Fr.) Gillet		HM562073		Harrison10325 (MICH)	USA (MI)
<i>P. petasatus</i> (Fr.) Gillet		HM562070		Mueller-Strack1 (SIU)	USA (IL)
<i>P. petasatus</i> (Fr.) Gillet		HM562175		SF3 (BPI)	USA (IL)
<i>P. petasatus</i> (Fr.) Gillet		HM562109		AJ145 (MICH)	USA (FL)
<i>P. petasatus</i> (Fr.) Gillet	HM562273	HM562038	HM562224	AJ201 (LOU)	Spain
<i>P. petasatus</i> (Fr.) Gillet		HM562065		AJ143 (MICH)	USA (MA)
<i>P. petasatus</i> (Fr.) Gillet		HM562084		Ammiranti3103 (MICH)	USA (MI)
<i>P. phaeoleucus</i> E. Horak		HM562141		GF5102! (BR)	Democratic Rep. of Congo

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. phlebophorus</i> (Ditmar) P. Kumm.		HM562184		SF14 (SIU)	USA (MI)
<i>P. phlebophorus</i> (Ditmar) P. Kumm.		HM562144		AJ193 (LOU)	Spain
<i>P. phlebophorus</i> (Ditmar) P. Kumm.		HM562137		AJ194 (LOU)	Spain
<i>P. phlebophorus</i> (Ditmar) P. Kumm.		HM562138		AJ228 (LOU)	Spain
<i>P. phlebophorus</i> (Ditmar) P. Kumm.		HM562117		TNSF12394	Japan
<i>P. phlebophorus</i> (Ditmar) P. Kumm.	HM562268	HM562039	HM562227	AJ81 (MA)	Spain
<i>P. phlebophorus</i> (Ditmar) P. Kumm.		HM562112		Homola1849 (MICH)	USA (MI)
<i>P. plautus</i> (Weinm.) Gillet		HM562055		AJ209 (LOU)	Spain
<i>P. podospileus</i> Sacc. & Cub.	HM562267	HM562049	HM562228	AJ204 (LOU)	Spain
<i>P. podospileus</i> Sacc. & Cub.		HM562122		TNSF12398	Japan
<i>P. pouzarianus</i> Singer		HM562050		AJ208 (LOU)	Spain
<i>P. pouzarianus</i> Singer		HM562096		TNSF12371	Japan
<i>P. pouzarianus</i> Singer		HM562154		REG13626	Germany
<i>P. pouzarianus</i> Singer		HM562170		REG13619	Germany
<i>P. puttemansii</i> Menolli & Capelari		HM562164		SP393698!	Brazil
<i>P. riberaltensis</i> var. <i>conquistensis</i> Singer		HM562162		SP393704	Brazil
<i>P. romellii</i> (Britzelm.) Sacc.	HM562264	HM562062	HM562238	AJ232 (LOU)	Spain
<i>P. romellii</i> (Britzelm.) Sacc.		HM562105		Sundberg24198112 (SIU)	USA (MO)

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P. romellii</i> (Britzelm.) Sacc.		HM562078		Shaffer3715 (MICH)	USA (MI)
<i>P. romellii</i> (Britzelm.) Sacc.		HM562183		SF13 (SIU)	USA (IL)
<i>P. romellii</i> (Britzelm.) Sacc.		HM562054		AJ215 (LOU)	Spain
<i>P. romellii</i> (Britzelm.) Sacc.		HM562123		TNSF12387	Japan
<i>P. rugosidiscus</i> Murrill		HM562079		Homola109 (MICH)	USA (MI)
<i>P. salicinus</i> (Pers.) P. Kumm		HM562174		SF2 (BPI)	USA (MI)
<i>P. salicinus</i> (Pers.) P. Kumm	HM562263	HM562051	HM562233	MA67874	Spain
<i>P. sandalioticus</i> Contu & Arras		HM562107	HM562246	AJ60 (MA)	Spain
<i>P. sandalioticus</i> Contu & Arras		HM562052	HM562229	AJ200 (LOU)	Spain
<i>P. saupeii</i> Justo & Minnis*		HM562113		ILLS42441!	USA (IL)
<i>P. seticeps</i> (G.F. Atk.) Singer		HM562191		SF23 (SIU)	USA (MO)
<i>P. seticeps</i> (G.F. Atk.) Singer		HM562192		SF24 (SIU)	USA (WI)
<i>P. seticeps</i> (G.F. Atk.) Singer		HM562199		Shaffer798 (MICH)	USA (IL)
<i>P. sp. I</i> (sect. Hispidoderma)		HM562114		TNSF12365	Japan
<i>P. sp. I</i> (sect. Hispidoderma)		HM562127		TNSF12372	Japan
<i>P. sp. II</i> (sect. Celluloderma)		HM562146		SP394387	Brazil
<i>P. sp. II</i> (sect. Celluloderma)		HM562148		SP394382	Brazil

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>P.</i> sp. III (sect. Celluloderma)		HM562145		SP394379	Brazil
<i>P.</i> sp. IV (sect. Celluloderma)		HM562173		SP394383	Brazil
<i>P.</i> sp. V (sect. Celluloderma)		HM562161		SP394389	Brazil
<i>P.</i> sp. VI (sect. Celluloderma)		HM562216		DrewU (BPI)	USA (IL)
<i>P. spetzianus</i> Singer		HM562194		SingerM3377 (MICH)	Argentina
<i>P. stirps subcervinus</i>		HM562217		DrewP (BPI)	USA (ID)
<i>P. stirps subcervinus</i>		HM562095		TNSF12360	Japan
<i>P. stirps subcervinus</i>		HM562097		TNSF12356	Japan
<i>P. stirps subcervinus</i>		HM562094		TNSF12348	Japan
<i>P. stirps subcervinus</i>		HM562093		TNSF12349	Japan
<i>P. stirps subcervinus</i> (white basidiocarp)		HM562068		Thiers39341 (SIU)	USA (CA)
<i>P. thomsonii</i> (Berk. & Broome) Dennis		HM562197		Homola930 (MICH)	USA (MI)
<i>P. thomsonii</i> (Berk. & Broome) Dennis		HM562067		AMD120 (SIU)	USA (IL)
<i>P. thomsonii</i> (Berk. & Broome) Dennis		HM562066		MA54629	Spain
<i>P. thomsonii</i> (Berk. & Broome) Dennis		HM562053	HM562230	AJ206 (LOU)	Spain
<i>P. umbrosus</i> (Pers.) P. Kumm.	HM562277	HM562140	HM562232	AJ213 (MA)	Spain
<i>P. viscidulus</i> Singer		HM562110		SingerT797! (MICH)	Argentina
<i>P. xylophilus</i> (Speg.) Singer		HM562163		SP393707	Brazil
<i>V. aff. pusilla</i>			HM562258	AEF735 (MICH)	USA (AZ)

Taxon	nSSU	ITS	nLSU	Collection (Herbarium)	Geographic origin
<i>V. bombycina</i> (Schaeff.) Singer	HM562279	HM562212	HM562256	AJ244 (LOU)	Spain
<i>V. caesiotincta</i> P.D. Orton	HM562278	HM562211	HM562255	MA54717	Spain
<i>V. lepiotospora</i> Singer	HM562281	HM562214	HM562259	AJ155 (MICH)	USA (FL)
<i>V. pusilla</i> (Pers.) Singer			HM562257	AJ245 (LOU)	Spain
<i>V. surrecta</i> (Knapp) Singer	HM562282	HM562213	HM562254	AJ55 (MA)	Spain
<i>V. taylorii</i> (Berk. & Broome) Singer	HM562280	HM562210	HM562260	AJ54 (MA)	Portugal
<i>Vp. asiaticus</i> Justo & Minnis*		HM562206		TNSF15191!	Japan
<i>Vp. earlei</i> (Murrill) Vizzini, Contu & Justo	HM562270	HM562204	HM562253	MA22816	Spain
<i>Vp. gloiocephalus</i> (DC.) Vizzini, Contu & Justo		HM562203		PBM2272	USA (CA)
<i>Vp. gloiocephalus</i> (DC.) Vizzini, Contu & Justo		HM562202	HM562252	AJ239 (LOU)	Spain
<i>Vp. michiganensis</i> (A.H. Sm.) Justo & Minnis		HM562195		A.H.Smith32-590! (MICH)	USA (MI)

Table options

Taxon sampling for the molecular analyses

Selection of taxa for the analyses was aimed to maximize the representation of the different morphological and ecological groups ('stirps') of *Pluteus* and *Volvariella* as recognized by Singer (1986) and, in the case of *Pluteus*, a wide geographic representation. After examining the results from a preliminary analysis of the nLSU dataset, the gene was found to be too highly conserved to address the infrageneric taxonomy of *Pluteus* and to resolve the relationships between *Pluteus* and the *Volvariella gloiocephala* group, and molecular sampling then focused on the ITS. Representatives of the major lineages recognized in the analysis of the ITS dataset of *Pluteus* and in the nLSU dataset of *Volvariella*, were selected for obtaining nSSU data. A total of 22 nSSU, 180 ITS and 42 nLSU sequences were generated for this study (Table 1).

Additional sequences (2 nSSU, 32 ITS and 36 nLSU) were retrieved from GenBank (Table 2). The nLSU sequence labeled as *Pluteus pallidus* (Moncalvo *et al.* 2002), a member of sect. *Celluloderma*, corresponds in fact to *Pluteus albineus*, a member of sect. *Pluteus* (Menolli *et al.* 2010). Comparison with the sequences generated in this study indicates that at least an additional four nLSU and four ITS sequences deposited in Genbank are also misidentified, and they were renamed according to their placement in the phylogenetic analyses (Table 2). In order to find unidentified environmental samples potentially belonging to *Pluteus* or *Volvariella*, all newly generated ITS sequences were used for BLAST searches (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), yielding seven additional sequences of *Pluteus* that were included in the ITS dataset.

Table 2. Sequences retrieved from GenBank. Original names for misidentified sequences are indicated in parentheses. *P* = *Pluteus*, *V* = *Volvariella*; *Vp* = *Volvopluteus*. An asterisk (*) indicates names being published concurrently with the present article. The symbol '!' indicates a type collection (holotypus, epitypus or paratypus).

Taxon	nSSU	ITS	nLSU	Collection	Geographic origin
<i>P. abmirabilis</i> (Peck) Peck			AF261578	DAOM 197226	–
<i>P. abmirabilis</i> (Peck) Peck			AF264577	DAOM 193532	–
<i>P.</i> aff. <i>nothopellitus</i> (as ' <i>P. pellitus</i> ')		FJ774078		LE 217548	Russia
<i>P.</i> aff. <i>romellii</i> (as ' <i>P. romellii</i> ')			EU522788	TM02_69	Canada
<i>P.</i> aff. <i>romellii</i> (as ' <i>P. romellii</i> ')		AY854065	AY634279	ECV3201	USA (CA)
<i>P.</i> aff. <i>umbrosus</i> (as ' <i>P. umbrosus</i> ')			AF261580	DAOM 197235	–
<i>P. albineus</i> Bonnard (as ' <i>P. pallidus</i> ')			AF261569	JB 90/27	Switzerland
<i>P. allostipitatus</i>		FJ816656		SP393713	Brazil

Taxon	nSSU	ITS	nLSU	Collection	Geographic origin
<i>P. albstipitatus</i>		FJ816661	FJ816647	SP 393714	Brazil
<i>P. atromarginatus</i>			AF261572	JB97/14	–
<i>P. atromarginatus</i>		EF530926		UBC F16254	Canada
<i>P. atromarginatus</i>		FJ774075		LE 246081	Russia
<i>P. atromarginatus</i>		DQ494687	DQ094788	HKAS 31573	China
<i>P. atromarginatus</i> (as ' <i>P. pellitus</i> ')			AF261571	JB 93/3	–
<i>P. aurantiorugosus</i>			AF261579	DAOM 197369	–
<i>P. aureovenatus</i>		FJ816663	FJ816649	SP 393697	Brazil
<i>P. brunneoradiatus</i> Bonnard			AF261567	JB 97/3	–
<i>P. castri*</i> (as ' <i>P. aurantiorugosus</i> ')		FJ774077		LE 216873	Russia
<i>P. cervinus</i>			AF261570	JB 97/19	–
<i>P. cervinus</i>		EU486448		UBC F16293	Canada
<i>P. cf. nanus</i>		FJ774081		LE 213093	Russia
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.			AF261581	DAOM 190194	–
<i>P. dominicanus</i> var. <i>hyalinus</i> Menolli & Capelari		FJ816665	FJ816651	SP 393695!	Brazil
<i>P. eludens*</i> (as ' <i>P. podospileus</i> ')		FJ774085		LE 213015	Russia
<i>P. ephebeus</i>			AF261574	JB 97/23	–
<i>P. fenzlii</i>		FJ774082		LE 246083	Russia
<i>P. fluminensis</i> Singer		FJ816664	FJ816650	SP 393711	Brazil
<i>P. fluminensis</i> Singer		FJ816655		SP 393710	Brazil
<i>P. fuligineovenosus</i> E. Horak		FJ816662		SP 393705	Brazil

Taxon	nSSU	ITS	nLSU	Collection	Geographic origin
<i>P. granulatus</i>		FJ774086		LE 212990	Russia
<i>P. harrisii</i> Murrill		FJ816666	FJ816652	SP 393709	Brazil
<i>P. harrisii</i> Murrill		FJ816654	FJ816644	SP 393708	Brazil
<i>P. jamaicensis</i> Murrill		FJ816657		SP 393706	Brazil
<i>P. mammillatus</i>			DQ451549	ASM 7916!	USA (MO)
<i>P. petasatus</i>			AF042611	JB 91/21	–
<i>P. petasatus</i>		AF085495		CBS441.85	Czech. Rep.
<i>P. plautus</i>		FJ774076		LE 213024	Russia
<i>P. pouzarianus</i>			AF261568	JB 94/26	–
<i>P. primus</i> Bonnard			AF042610	JB 94/24	–
<i>P. riberaltensis</i> var. <i>conquistensis</i>			FJ816648	SP 393704	Brazil
<i>P. romellii</i>		FJ774073		LE 217944	Russia
<i>P. romellii</i>			AF261575	JB 97/26	–
<i>P. salicinus</i>		FJ774087		LE 215427	Russia
<i>P. salicinus</i>			AF261573	JB 97/6	–
<i>P. semibulbosus</i> Lasch Gillet		FJ774080		LE 227534	Russia
<i>P. sp. VII</i> (as ' <i>P. exiguus</i> ')		FJ774083		LE 226543	Russia
<i>P. sublaevigatus</i> (Singer) Menolli & Capelari		FJ816667	FJ816653	SP 393694	Brazil
<i>P. thomsonii</i>		FJ774084		LE 234787	Russia
<i>P. xylophilus</i>		FJ816659	FJ816645	SP 393701	Brazil
<i>P. xylophilus</i>		FJ816660	FJ816646	NMJ147 (SP)	Brazil
Uncultured Basidiomycete		DQ672275		Environmental sample (soil)	Australia
Uncultured Basidiomycete		AM901879		Environmental sample (house dust)	Finland

Taxon	nSSU	ITS	nLSU	Collection	Geographic origin
Uncultured Basidiomycete		AY969369		Environmental sample (hardwood litter)	USA (NC)
Uncultured fungus		FM999644		Environmental sample (sporocarp)	USA (OH)
Uncultured fungus		FM999650		Environmental sample (sporocarp)	USA (OH)
Uncultured fungus		FM999562		Environmental sample (sporocarp)	USA (OH)
Uncultured fungus		FM999557		Environmental sample (sporocarp)	USA (OH)
<i>V. bombycina</i>			EU920669	SP 393637	Brazil
<i>V. caesiotincta</i>			DQ071726	FO 46681	–
<i>V. hypopithys</i> (Fr.) Shaffer			AF261532	JMleg.AIM	–
<i>V. nullicystidiata</i> Menolli & Capelari			EU920670	SP 393639!	Brazil
<i>V. volvacea</i>		AY632077		OE-55	–
<i>V. volvacea</i>	DQ851588		AF261531	JMleg.SRL	–
<i>Vp. gloiocephalus</i>	AY45710		AY45710	PBM 2272	USA (CA)

Table options

Genbank sequences, for taxa other than *Pluteus* and *Volvariella*, employed in the combined nSSU + ITS + nLSU datasets, are the same ones used in the study of Matheny *et al.* (2006). Accession numbers for the sequences used in the nLSU analysis are given on the tree (Fig 2).

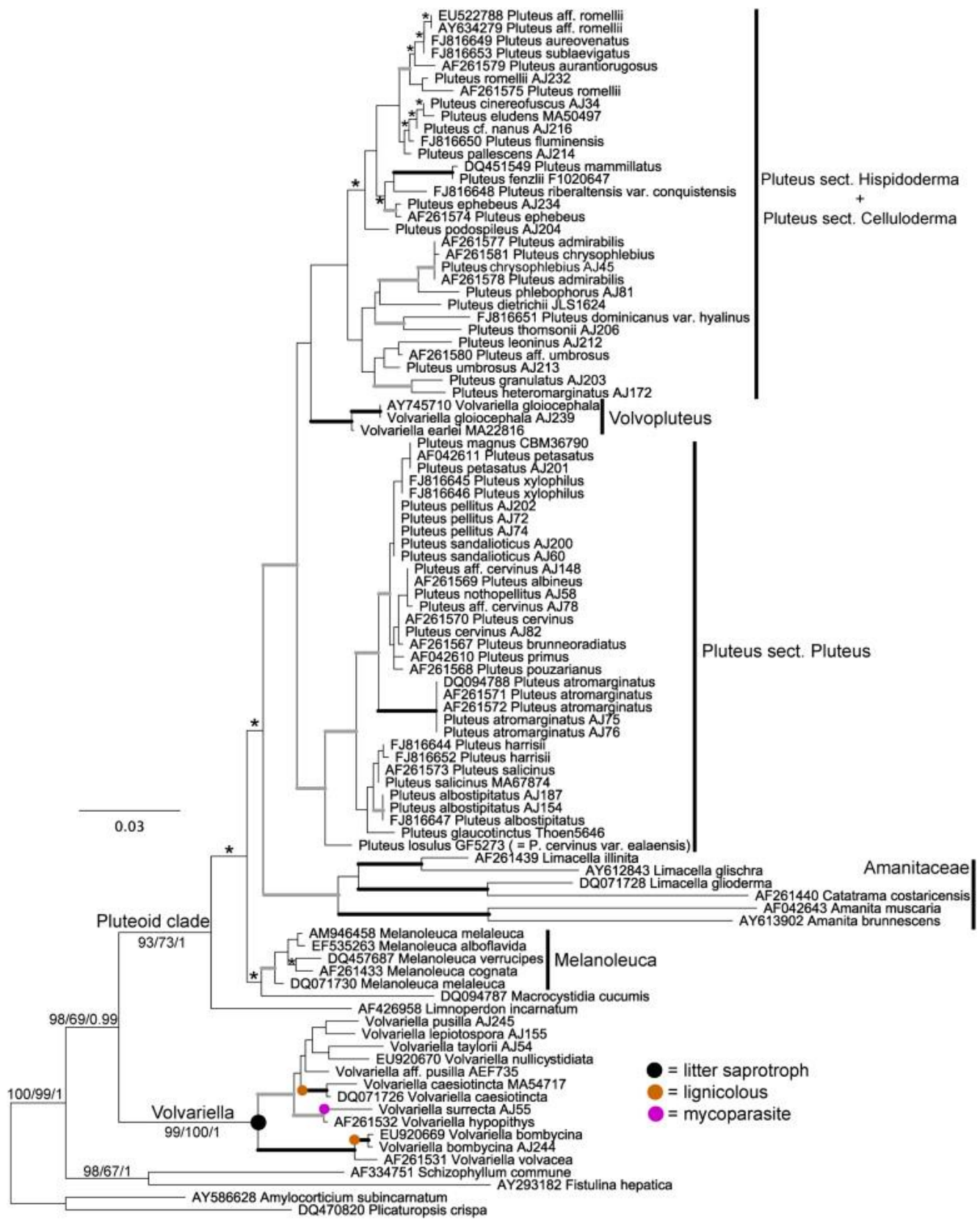


Fig 2. Best tree from the ML analysis of the nLSU dataset. BS and PP values for internal nodes are given on the branches (ML/MP/PP). Thick black branches are supported by BS values $\geq 90\%$ in both ML and MP analyses and PP ≥ 0.95 . Thick grey branches are supported in at least two of the analyses with BS $\geq 70\%$ and/or PP ≥ 0.90 . An asterisk (*) indicates that the branch collapses in the strict consensus tree of the MP analysis.

DNA extraction, PCR, sequencing and alignments

Approximately 0.05–0.10 g of tissue (preferably gills) from each collection were ground directly in a 1.5 ml eppendorf tube, using plastic pestles, or in a mortar with liquid nitrogen. DNA was extracted using 3 % SDS extraction buffer and then isolated by the sequential addition of phenol–chloroform and chloroform–isoamyl alcohol; finally, isopropyl alcohol and 3 M sodium acetate were added to precipitate the DNA, which was washed with 70 % ethanol and resuspended in sterile water.

Primer pairs LR0R–LR5 or LR0R–LR7 were used to amplify a portion of the nLSU gene (Vilgalys Lab, <http://www.biology.duke.edu/fungi/mycolab/primers>); ITS1F–ITS4 for the ITS (Gardes and Bruns, 1993 and White et al., 1990) and PNS1–NS8 for the nSSU (Hibbett, 1996 and White et al., 1990). For some samples, the nSSU was amplified in two separate fragments using primer pairs PNS1–NS41 and NS19b–NS8 (Bruns Lab, <http://plantbio.berkeley.edu/~bruns/tour/primers.html>). The amplification products were sequenced using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction reagents with the following primer combinations: nLSU forward primers (LR0R, LR3R), nLSU reverse primers (LR3, LR5 and LR7 if that primer was used for PCR) (Vilgalys Lab); ITS1F (forward) and ITS4 (reverse) were used for the ITS; nSSU forward primers (PNS1, NS19b, NS51), nSSU reverse primers (NS19bc, NS41, NS8 or alternatively NS6, if NS8 did not yield a good result) (White *et al.* 1990; Bruns Lab). Sequencing was carried out on an ABI model 3130 Genetic Analyzer. Raw data were processed using Sequencher 4.7 (GeneCodes, Ann Arbor, Michigan).

Sequences were aligned using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/online/server/>). The strategy G–INS–i was selected for the nLSU and nSSU datasets, and the Q–INS–i option for the ITS dataset. The alignments were then examined and manually corrected using MacClade 4.05 (Maddison & Maddison 2002). For all the combined datasets each gene was aligned separately and then concatenated in MacClade. In these datasets only the 5.8S portion of the ITS region was used in the analyses. Alignments have been deposited in TREEbase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S10635>).

Three different datasets were assembled for the phylogenetic analyses:

- (i) nLSU dataset. The dataset includes 66 *Pluteus* and 15 *Volvariella* sequences. Members of the *Amanitaceae* and other representatives of the Pluteoid clade sensu Matheny *et al.* (2006), as well as *Fistulina* and *Schizophyllum* were also included. Two members of the *Amylocorticiales*, *Amylocorticium subincarnatum* (Peck) Pouzar and *Plicaturopsis crispa* (Pers.) D.A. Reid, were used as an outgroup.
- (ii) Combined nSSU + ITS + nLSU dataset of the *Agaricales*. The dataset includes 16 species of *Pluteus*, two of *Volvopluteus* (see Results), six of *Volvariella* and a wide representation of the major lineages of the *Agaricales* recognized in the works of Matheny *et al.* (2006) and Binder *et al.* (2010). *Plicaturopsis crispa* and *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. were used as an outgroup.
- (iii) ITS dataset of *Pluteus*. The dataset includes 207 sequences of *Pluteus* and four of *Volvopluteus* that were used as an outgroup.

Phylogenetic analyses

Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Analysis (BA) were performed with the following parameters: (i) MP: Equally weighted parsimony analysis was performed using PAUP *4.0.b10 (Swofford 2002). One thousand heuristic search replicates were performed with starting trees generated by stepwise addition with random addition sequences followed by Tree Bisection Reconnection branch swapping. Up to two trees were kept in each replicate. Parsimony bootstrap analysis was performed with 1000 replicates, each with 10 random taxon addition sequences and branch swapping set to subtree pruning and regrafting; (ii) ML: The analysis was run in the RAxML servers (<http://phylobench.vital-it.ch/raxml-bb/index.php>; which implements the search protocol of Stamatakis *et al.* 2008), under a GTR model with one hundred rapid bootstrap replicates; (iii) BA: The analysis was run using MrBayes 3.1 (Ronquist & Huelsenbeck 2003) for 10 million generations, under a GTR model, with four chains, and trees sampled every 100 generations; after examining the graphic representation of the likelihood scores, using Tracer (<http://tree.bio.ed.ac.uk/software/tracer/>), the burn-in period was set to 1.5 million generations for all datasets, except for the combined dataset of the major clades of *Agaricales*, in which the burn-in period was set to 2 million generations. The three analyses were performed in all the datasets presented here.

Ancestral state reconstruction (ASR)

ASR was performed using parsimony in Mesquite 2.72 (Maddison & Maddison 2009).

To investigate the evolution of the different trophic strategies in *Volvariella*, characters were considered as ‘unordered’ and coded as: saprotrophic (not directly associated with wood), lignicolous and mycoparasitic. The fifty percent majority rule consensus tree resulting from the MP analysis of the nLSU dataset was used for the ASR. For the study of the evolution of the morphological features that define the infrageneric classifications of *Pluteus*, characters were considered as ‘unordered’ and coded as follows (see also Fig 1). Pleurocystidia: (i) metuloid, (ii) poorly developed metuloid, (iii) non-metuloid, (iv) absent. Pileipellis: (i) cutis, (ii) ixocutis, (iii) trichoderm or hymeniderm made up of elongated elements ($avQ \geq 3$), (iv) hymeniderm or epithelium composed of short, clavate or spheropedunculate elements ($avQ \leq 3$), without elongated cystidioid elements, (v) the same as the precedent but with elongated cystidioid elements. ASR was performed on the best tree from the ML analysis and on the fifty percent majority rule consensus tree resulting from the MP and BA analyses of the ITS dataset of *Pluteus* and the combined nSSU + ITS + nLSU of the *Agaricales*.

Results

Analyses of the nLSU dataset

The final dataset consists of 96 sequences of 1364 characters (gaps included), of which 225 are parsimony informative. In the MP analysis 212 equally most parsimonious trees (MPT) were recovered (Length = 1023; CI = 0.43; RI = 0.78). The best tree from the ML analysis is shown in Fig 2.

Members of the Pluteoid clade sampled in this study (*Pluteus*, *Volvariella gloiocephala*-group, *Amanitaceae*, *Melanoleuca*, *Macrocystidia* and *Limnoperdon*) are placed together with moderate to high statistical support in all the analyses (Bootstrap (BS) = 93%(ML), 73%(MP); Posterior Probability (PP) = 1) though the internal topology of this clade is not well resolved. To avoid confusion between the ‘*V. gloiocephala*-group’ and the remaining *Volvariella* species, the former group is referred to as *Volvopluteus* from this point onwards (see Taxonomy section). The genus

Volvariella, excluding the species placed in *Volvopluteus*, forms a highly supported group that is placed outside the Pluteoid clade, and they did not cluster with *Schizophyllum* or *Fistulina*.

An inclusive clade of *Pluteus* sect. *Pluteus*, *Volvopluteus* and an assemblage of the remaining species of *Pluteus* (sections *Hispidoderma* and *Celluloderma*) was recovered, and relatively well supported in all the analyses. The relation between these three major groups was the same in the different analyses, but in none of them the position of *Volvopluteus* as the sister taxon of Sect. *Hispidoderma* + *Celluloderma* was supported. The internal topology of this latter clade was poorly resolved.

The *Amanitaceae* is placed as the sister group of the *Pluteus* + *Volvopluteus* clade in the ML and BA analyses, though this relation only receives significant support in the latter. In the strict consensus tree from the MP analysis, the relations among all major groups in the Pluteoid clade are totally unresolved, and in the MP fifty percent majority rule consensus tree, *Melanoleuca* is placed as the sister group of *Pluteus* + *Volvopluteus*, though this relation receives no BS support.

Analyses of the combined nSSU + ITS + nLSU dataset of the *Agaricales*

The final dataset consists of 117 combined sequences (nSSU + ITS + nLSU) of 3117 characters (gaps included), of which 592 are parsimony informative. In the MP analysis, two MPT were recovered (Length = 4467; CI = 0.26; RI = 0.48). The fifty percent majority rule Bayesian phylogram is shown in Fig 3.

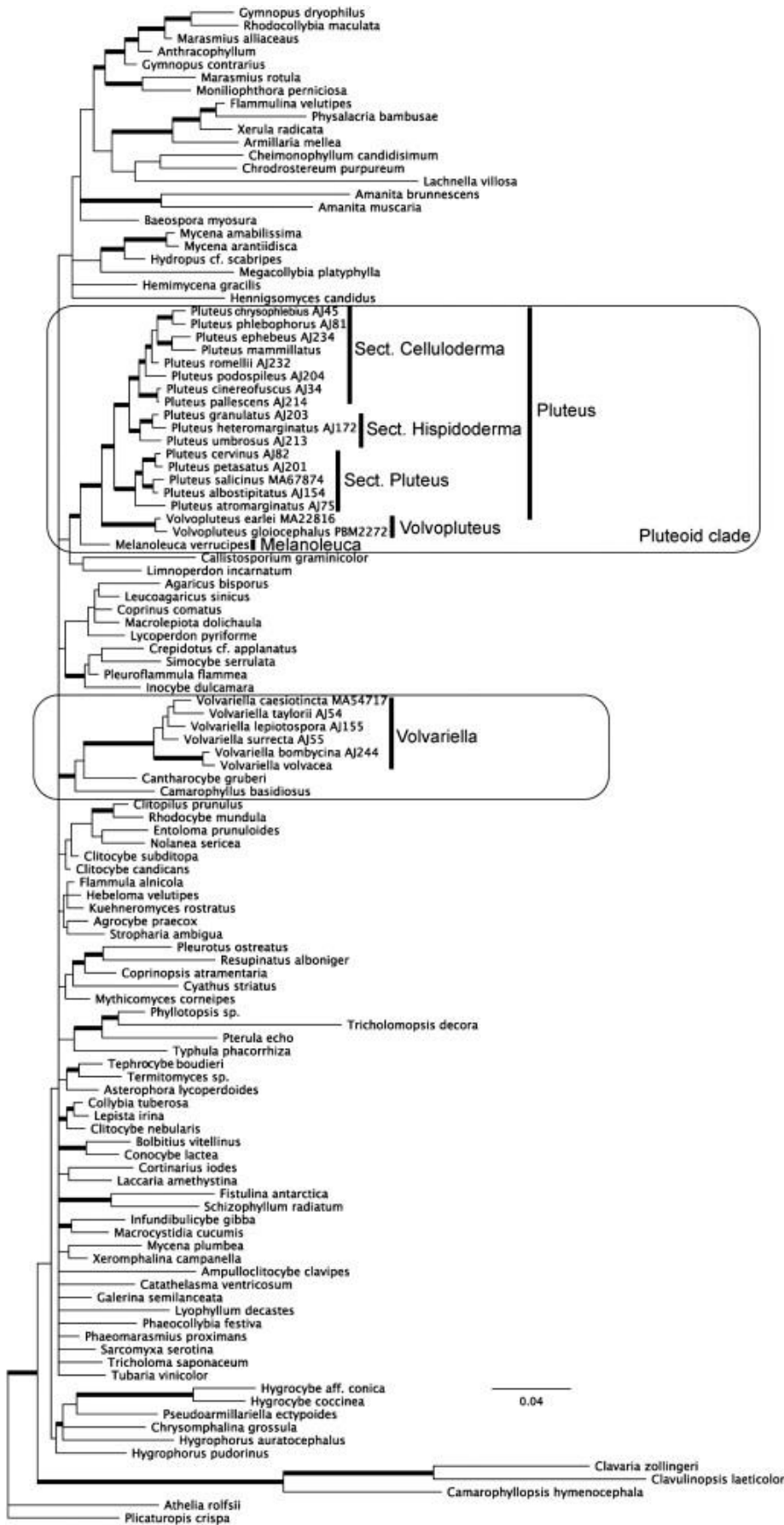


Fig 3. Fifty percent majority rule Bayesian phylogram for the nSSU + ITS + nLSU dataset including all major lineages of *Agaricales*. Thick branches are supported by PP \geq 0.90.

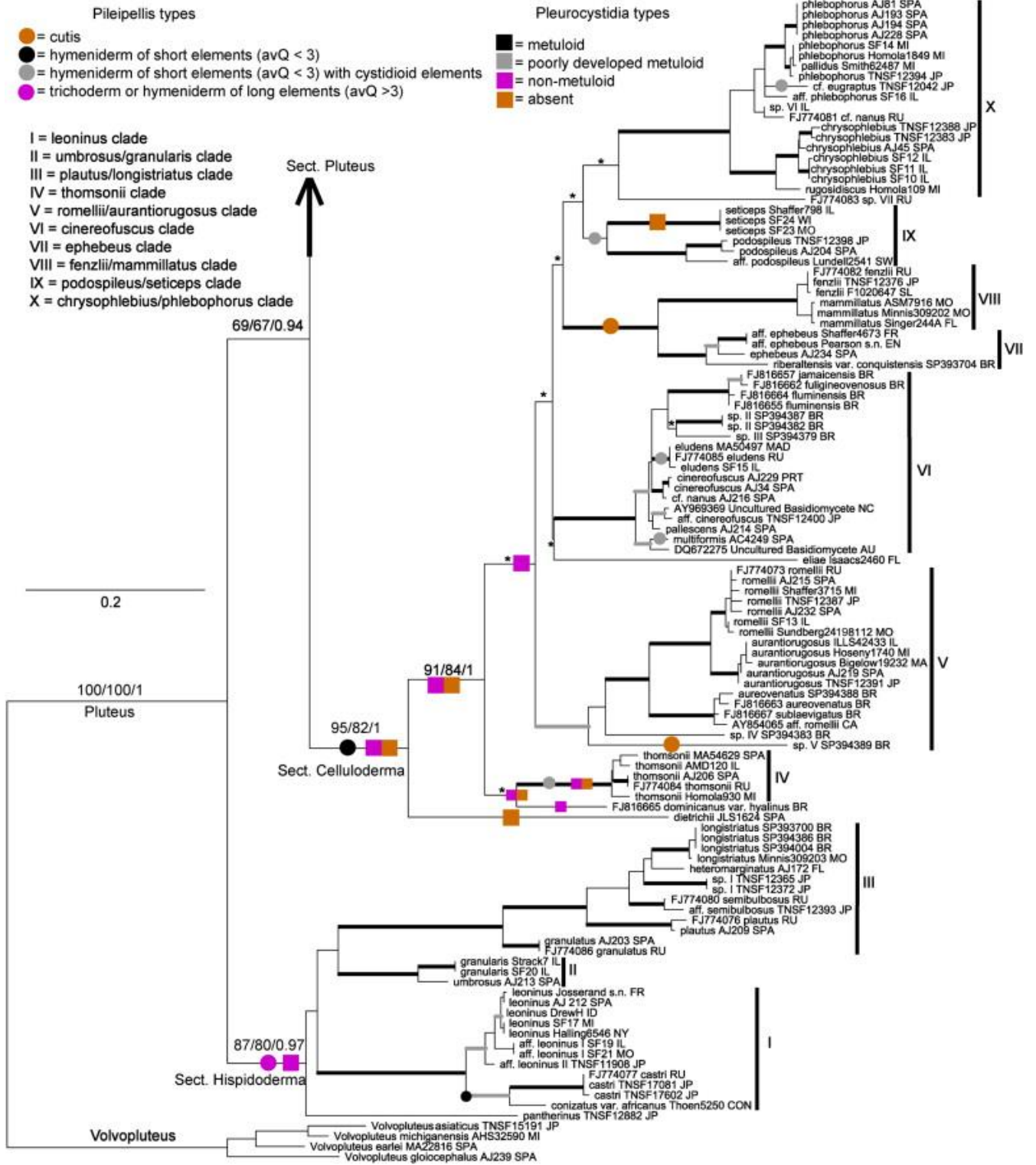
The general topological relations among the different groups of agarics are very poorly resolved. In all analyses *Pluteus* and *Volvopluteus* are supported as monophyletic and sister taxa. *Pluteus* itself is highly supported as monophyletic, and it is subdivided into three major lineages, more or less corresponding to the sections *Pluteus*, *Hispidoderma* and *Celluloderma* in the sense of Singer (1986), though the placement of some taxa (*Pluteus albobostipitatus*, *Pluteus ephebeus*, *Pluteus mammillatus*) needs further comment (see Discussion). *Melanoleuca* is placed as the sister group of *Pluteus* and *Volvopluteus* (Fig 3).

Volvariella clusters with *Cantharocybe gruberi* (A.H. Sm.) H.E. Bigelow & A.H. Sm. and *Camarophyllus basidiosus* (Peck) Murrill in all the analyses, but this only receives statistical support in the BA analysis. *Volvariella* is subdivided into two distinct clades: one with *Volvariella volvacea* and *Volvariella bombycina* and the other with the remaining species sampled in this study (Fig 3).

To further test the phylogenetic position and relationships of *Pluteus*, *Volvopluteus* and *Volvariella*, three additional combined nSSU + ITS + nLSU datasets were also analyzed: (i) Dataset only with *Volvariella* and representatives of the Agaricoid, Hygrophoroid, Marasmioid, Pluteoid and Tricholomatoid clades; (ii) Dataset only with *Volvariella* and representatives of the Hygrophoroid clade; (iii) Dataset only with representatives of the Pluteoid clade. Since the results from the phylogenetic analyses of these datasets are essentially the same as in the *Agaricales* dataset they are only shown in the Supplementary Information.

Analyses of the ITS dataset of *Pluteus*

The final dataset consists of 211 sequences of 693 characters (gaps included), of which 286 are parsimony informative. In the MP analysis 936 MPT were recovered (Length = 2070; CI = 0.30; RI = 0.89). The best tree from the ML analysis is shown in Fig 4.



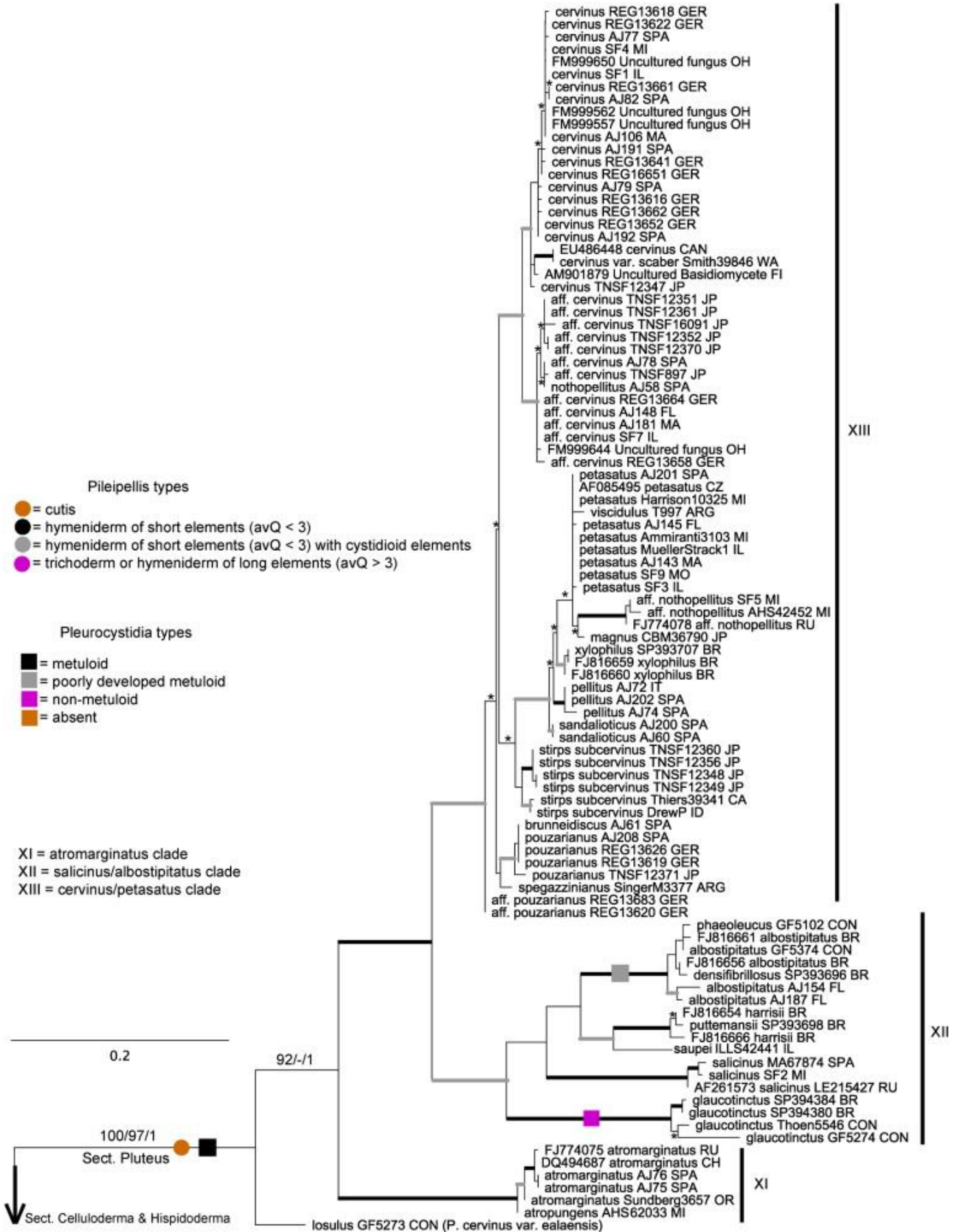


Fig 4. Best tree from the ML analysis for the ITS dataset of *Pluteus*. BS and PP values for internal nodes are given on the branches (ML/MP/PP). Thick black branches are supported by BS values $\geq 90\%$ in both ML and MP analyses and PP ≥ 0.95 . Thick grey branches are supported in at least two of the analyses with BS $\geq 70\%$ and/or PP ≥ 0.90 . An asterisk (*) indicates that the branch collapses in the strict consensus tree of the MP analysis. ARG (Argentina), AU (Australia), BR (Brazil), CA (California), CAN (Canada), CH (China), CON (Democratic Republic of Congo), CZ (Czech Republic), EN (England), FI (Finland), FL (Florida), FR (France), GER (Germany), ID (Idaho), IL (Illinois), IT (Italy), JP (Japan), MA (Massachusetts), MAD (Madeira Island, Portugal), MI (Michigan), MO (Missouri), NC (North Carolina), NY (New York), OH (Ohio), OR (Oregon), PRT (Portugal), RU (Russia), SL (Slovakia), SPA (Spain), SW (Sweden), WA (Washington), WI (Wisconsin).

Three major clades of *Pluteus* were recovered with high statistical support in all the analyses: sect. *Hispidoderma*, sect. *Pluteus*, and sect. *Celluloderma*. The internal topology of *Pluteus* was the same in the different analyses, with sect. *Hispidoderma* as basal to the sects. *Pluteus* and *Celluloderma*, though this relationship did not receive high support in any of the analyses.

Section *Pluteus* includes taxa with metuloid pleurocystidia and the pileipellis as a cutis, but some taxa with poorly differentiated metuloids (*Pluteus albstipitatus*) or non-metuloid cystidia (*Pluteus glaucotinctus*) are also placed here. Four major lineages can be recognized in sect. *Pluteus*: the *cervinus/petasatus* clade, the *salicinus/albstipitatus* clade, the *atromarginatus* clade and *Pluteus losulus* (= *Pluteus cervinus* var. *ealaensis*). The latter is placed as the earliest diverging member of this group in the ML and BA analyses, while in the fifty percent majority rule consensus tree from the MP analysis the *atromarginatus* clade is basal in the section.

Section *Celluloderma* includes taxa with the pileipellis as a hymeniderm, with transitions towards an epithelium, composed mostly by short clavate to spheropedunculate elements, intermixed or not with long cystidioid elements. Species with non-metuloid pleurocystidia and the pileipellis as a cutis, and either without (*ephebeus* clade, *Pluteus* sp. V SP394389) or with (*fenzlii/mammillatus* clade) a partial veil in the stipe, are also placed here. Several species-complexes received significant support in the analyses: *thomsonii* clade, *romellii/aurantiorugosus* clade, *cinereofuscus* clade, *ephebeus* clade, *fenzlii/mammillatus* clade, *podospileus/seticeps* clade and *chrysophlebius/phlebophorus* clade. Four taxa are placed outside any of these major lineages: (i) *Pluteus dominicanus* var. *hyalinus* is placed as sister to the *thomsonii* clade (ML/BA) or together with *Pluteus eliae* and *Pluteus* sp. VII (MP); (ii) *P. eliae* is placed as sister to the *cinereofuscus* clade (ML), sister to the *ephebeus*, *fenzlii/mammillatus* clades (BA) or together with *P. dominicanus* var. *hyalinus* and *Pluteus* sp. VII (MP); (iii) *Pluteus* sp. VII is placed as sister to the *chrysophlebius/phlebophorus* clade (ML/BA) or together with *P. eliae* and *P. dominicanus* var. *hyalinus* (MP), (iv) *Pluteus dietrichii* is placed as sister to all other members of sect. *Celluloderma*, with high statistical support in all the analyses. Except for the placement of *P. dietrichii* and the sister taxa relationship of the *ephebeus* clade with the *fenzlii/mammillatus* clade, all other topological relationships between the major lineages of sect. *Celluloderma* received no statistical support in the different analyses.

Section *Hispidoderma* includes taxa with pileipellis as a hymeniderm or trichoderm, though there is a considerable variation in the shape and size of the terminal elements of the pileipellis (see Discussion). Three major clades are recognized within sect. *Hispidoderma*: one includes *Pluteus leoninus* and allied taxa, a second with *Pluteus plautus*, *Pluteus longistriatus*, and related species, and a third clade with *Pluteus umbrosus* and *Pluteus granularis*. *Pluteus pantherinus* is placed outside these three clades in all the analyses. Relationships between the different lineages of sect. *Hispidoderma* are not resolved in any of the analyses.

Ancestral State reconstruction

The evolution of trophic strategies in *Volvariella* is unambiguously reconstructed in all the analyses (Fig 2). The pleurocystidia and pileipellis type of the ancestral *Pluteus* cannot be determined, in part because of topological differences between the different trees (ML, MP, BA) from the ITS analyses, and between the ITS and the combined nSSU + ITS + nLSU analyses (Fig 4, Suppl. Fig 3). Despite these differences, both the pleurocystidia and pileipellis types can be unambiguously reconstructed for each of the ancestral nodes of sections *Pluteus*, *Celluloderma* and *Hispidoderma*, with the exception of the pleurocystidia type for the ancestral *Celluloderma*, that has ‘non-metuloid’ and ‘absent’ as equally parsimonious states. The ancestral character states, and changes from the plesiomorphic state, for each section of *Pluteus* are depicted in Fig 4 and Suppl. Fig. 3.

Taxonomy

The genus *Volvariella*, in the traditional sense, is polyphyletic. *Volvariella gloiocephala* and allied species represent the sister group of *Pluteus* (Fig 3), and the new genus, *Volvopluteus*, is proposed here to accommodate these taxa. *Volvariella gloiocephala* was chosen as the type species of *Pseudofarinaceus* Earle. However this name is illegitimate under Art. 53 of the I. C. B. N. (McNeill *et al.* 2006) as it is a later homonym of *Pseudofarinaceus* Battarra ex Kuntze, which is a synonym of *Amanita* (Shaffer 1957).

Volvopluteus Vizzini, Contu & Justo gen. nov.

MycoBank 518591

Volvariella sect. *Macrosporae* Contu, Boletim da Sociedade Broteriana, Sér. 2, 65: 82 (1992)

Latin diagnosis. *A genero Pluteo differt basidiosporis saepe elongatis et conspicue crassotunicatis, epicute pilei gelatinosa, volva conspicua et structura molecularis.*

Type species (designated here): *Volvopluteus gloiocephalus* (DC.: Fr.) Vizzini, Contu & Justo

Basidiocarps pluteoid with universal veil remnants present as a volva at stipe base. Pileus conical to plano-convex, viscid. Lamellae free, whitish when young, later pink or pinkish brown. Stipe central, cylindrical, usually wider towards the base. Volva membranous, saccate, white. Spore print pink or pinkish brown.

Basidiospores ellipsoid to oblong, thick walled (up to 1–1.5 μm), smooth, without germ pore, inamyloid, non-dextrinoid, cyanophilus, with guttular content, average length over 11 μm . Basidia commonly 4-spored, but 2-spored and 1-spored basidia also occur. Pleurocystidia present or absent, thin-walled. Cheilocystidia present or absent, thin-walled. Hymenophoral trama inverse. Pileipellis an ixocutis composed of relatively thin, cylindrical hyphae, 2–10 (15) μm wide, embedded in a thick (100–450 μm) gelatinous matrix. Stipitipellis a cutis, composed of cylindrical hyphae 5–15 μm wide. Caulocystidia present, as cylindrical septate hairs, or absent. Universal veil composed of densely interwoven cylindrical hyphae. Clamp-connections absent.

Habitat – saprotrophic, terrestrial, in gardens, grassy fields, in or outside forests, on accumulations of vegetable matter (compost, wood chips).

Distribution – widespread. *Volvopluteus gloiocephalus* has been reported from all continents except Antarctica, and molecular data confirms that this species occurs, at least, in Europe and California (Justo *et al.* 2010).

Volvopluteus gloiocephalus (DC.: Fr.) Vizzini, Contu & Justo, comb. nov.

MycoBank 518592

Agaricus gloiocephalus, DC. in De Candolle & Lamarck, Fl. Franc., ed.3, 6: 52. 1815 (basionym).

Agaricus gloiocephalus, DC.: Fr., Syst. Mycol. 1: 278. 1821.

Volvariella gloiocephala (DC.: Fr.) Boekhout & Enderle, Beitr. Kennt. Pilze Mitteleur. 2: 78. 1986.

Volvariella speciosa (Fr.: Fr.) Singer, Lilloa 22: 401. 1951.

For a complete list of synonyms, see Boekhout (1990) and Shaffer (1957).

Volvopluteus earlei (Murrill) Vizzini, Contu & Justo, comb. nov.

MycoBank 518593

Volvariopsis earlei Murrill, Mycologia 3: 282. 1911 (basionym).

Volvaria earlei (Murrill) Murrill, Mycologia 4: 332. 1912.

Volvariella earlei (Murrill) Shaffer, Mycologia 49: 550. 1957.

Volvopluteus michiganensis (A.H. Sm.) Justo & Minnis, comb. nov.

MycoBank 518594

Pluteus michiganensis A.H. Sm., Annales Mycologici 32: 482. 1934 (basionym).

The main morphological characters that separate *Volvopluteus* from *Pluteus* and *Volvariella* are: (i) average basidiospore length over 11 μm ; (ii) pileipellis as an ixocutis, composed of relatively thin hyphae (on average less than 15 μm wide), embedded in a very conspicuous gelatinous matrix. The degree of gelatinousness of the pileipellis is variable among collections of *Volvopluteus* (even of the same species), but a gelatinous matrix is present in all the specimens examined in this study and in all collections of *Vp. gloiocephalus* and *Vp. earlei* from Europe examined by us (Contu, 2007 and Justo and Castro, 2010). Some species of *Pluteus* sect. *Pluteus*, e.g. *Pluteus petasatus*, sometimes present a slightly gelatinous cutis, but in this case, the hyphae are wider than in *Volvopluteus*, the basidiospores shorter than 10 μm , the cystidia are metuloid and a thick saccate volva is lacking. Thus, morphological separation of these taxa is easy. The presence of a volva can be used as an additional character to separate *Volvopluteus* and *Pluteus*, but it should be mentioned that ‘volva-like’ remnants have been described for *Pluteus stephanobasis* Singer, a species of sect. *Pluteus*. Singer (1958) described these remnants as ‘volva fragmentary, consisting of an inconspicuous, but constant and persistent, deep brown to deep fuscous fibrillose line which continuously or intermittently runs around the base of the stipe’. *Pluteus stephanobasis* is, otherwise, a ‘typical’ member of sect. *Pluteus*. The presence of this type of structure, and whether it is really the same tissue that constitutes the universal veil of *Volvopluteus*, should be checked on new collections

of *P. stephanobasis* when they become available. So far, *P. stephanobasis* is only known from the three Argentinean collections mentioned by Singer (1958) in the original description, and no similar structures have been reported elsewhere in the genus.

Revision of the holotype of *P. michiganensis* revealed that this taxon is in fact a member of *Volvopluteus*. The basidiospore size ('11–13 × 7–8 μm') and the viscid pileus mentioned in the original description (Smith 1934) are anomalous for a *Pluteus* species. There was no mention of universal veil at the stipe base, though a conspicuous volva is present in the holotype. A detailed description of this species, and an additional *Volvopluteus* from Japan, is given in Justo *et al.* (2010).

Several taxa traditionally placed in *Volvariella*, with basidiospores >11 μm long, are briefly summarized here:

- (i) *Volvariella canalipes* (Murrill) Shaffer is characterized by a furrowed stipe and the very long (15–22 μm) basidiospores. According to the revision of the type (Shaffer 1957) there is no gelatinous matrix in the pileipellis. This species is only known from the type collection (U.S.A., FL, Clay Co., Green Cove Springs, March 3, 1926, Murrill FLAS9975).
- (ii) *Volvariella alabamensis* (Murrill) Shaffer is characterized by the dark brown pileus, and relatively long basidiospores (14.7–20.3 μm). According to Shaffer (1957) there is no gelatinous matrix in the pileipellis. The type specimen at NY (U.S.A., AL, Lee Co., Auburn, March 10, 1898) has been destroyed by insects, and only a portion of the pileus is conserved at CUP–A (Shaffer 1957).
- (iii) *Volvariella cnemidophora* (Mont.) Singer and *Volvaria microchlamida* (Speg.) Sacc., described from Brazil and Argentina respectively, were considered as synonyms of *V. speciosa* (= *Vp. gloiocephalus*) by Shaffer (1962).
- (iv) *Volvariella acystidiata* N.C. Pathak, described from Africa, is characterized by the absence of hymenial cystidia (Heinemann 1975). The type collection at BR (Democratic Rep. of Congo, Binga, November 1941, Goossens–Fontana 2068) was revised by us, but unfortunately it is in very bad condition. A modern collection of this species made in Italy (Vizzini & Contu 2010) was sampled for molecular data, showing that this taxon is a synonym of *Vp. earlei* (Justo *et al.* 2010).
- (v) *Volvariella cookei* Contu from Italy, looks like a small form of *Vp. gloiocephalus* and is characterized by clavate cystidia with a very long and thin appendage (Contu, 1998 and Contu and Signorello, 2004). Molecular data shows that this taxon is also a synonym of *Vp. earlei* (Justo *et al.* 2010).
- (vi) *Volvariella insignis* Heinem. is separated from *Vp. gloiocephalus* by the dark brown pileus (Horak & Heinemann 1978). This species is only known from the two African collections mentioned in the original description.
- (vii) *Volvariella macrospora* Singer is characterized by the very small basidiocarps (pileus about 10 mm in diameter) and the presence of clamp-connections (Singer 1989). It is only known from the type collection deposited at F (Brazil, Amazonas, 30 km north of Manus, May 11, 1977, Singer B9863).
- (viii) *Volvariella arenaria* (Pat.) Singer was described from the Arabian desert, and it is characterized by the relatively small basidiocarps (pileus up to 30 mm in diameter) and arenicolous habitat (Singer 1962).
- (ix) Additional species listed by Singer (1986) as belonging to this group are: *Volvariella californica* (Earle) Singer and *Volvariella stercoraria* (Peck) Singer.

None of the taxa listed above are formally recombined in *Volvopluteus*, since they are known only from their respective original collections and/or descriptions and have never been reported again in the mycological literature of the past century (with the exception of *V. acystidiata* and *V. cookei*). Based on morphological characters, some may represent autonomous species, but for others it is

doubtful that they are really different from *Vp. gloiocephalus*. Only with new collections, preferably from their type localities, will it be possible to clarify the taxonomic status of these taxa, and some of their morphological characters like the apparent absence of a gelatinous matrix in the pileipellis of *V. canalipes* and *V. alabamensis* or the presence of clamp-connections in *V. macrospora*.

Recently, Menolli & Capelari (2008) described *Volvariella heterospora*, with dimorphic basidiospores: one type of less than 8 µm long and a second type of elongated, usually deformed, basidiospores, up to 12 µm long. However, this species is better placed in *Volvariella* and not in *Volvopluteus* because of the pileipellis type.

The generic name *Volvariella* should be kept for the species characterized by basidiospores shorter than 11 µm (in most species less than 10 µm) and the pileipellis as a cutis, or an intermediate cutis-trichoderm, with hyphae wider than 15 µm and no gelatinous layer. *Volvariella* is typified by *Volvariella argentina* Speg. (Spegazzini 1899) but unfortunately there are no molecular data, and as far as we know, no modern collections, for this species. The author of the species later placed *V. argentina* in synonymy with *Volvariella parvula* (Spegazzini 1926), which is accepted by most modern authors (Boekhout, 1990, Shaffer, 1957, Shaffer, 1962 and Singer, 1986) as a synonym of *Volvariella pusilla*. After studying the type collection of *V. argentina*, Shaffer (1962) found no morphological differences between this species and modern collections of *V. pusilla*. Regardless of how many species can be recognized around *V. pusilla*, all of them share the basidiospore and pileipellis characteristics of the genus *Volvariella* as recognized here, and therefore the generic name is maintained for this clade.

Due to the phylogenetic positions of *Pluteus*, *Volvariella* and *Volvopluteus* recovered in our analyses, it appears that the rather unique morphological feature shared by these three genera, the inverse hymenophoral trama, has originated at least twice during the evolution of the *Agaricales*.

Discussion

Phylogenetic position of *Volvariella*

The placement of *Volvariella* outside the Pluteoid clade is supported by the analyses of the nLSU (Fig 2) and the combined nSSU + ITS + nLSU datasets (Fig 3, Suppl. Information). The previous result of Moncalvo *et al.* (2002) that placed *Volvariella* together with *Schizophyllum* and *Fistulina* was not recovered here (Fig 2 and Fig 3). In all analyses of the different combined datasets, *Volvariella* consistently clusters with *Cantharocybe* and *Camarophyllus*, though this relation does not always receive statistical support (Fig 3, Suppl. Information).

The genus *Cantharocybe* was created to accommodate *Clitocybe gruberi* A.H. Sm., which differs from other representatives of that genus in the yellow spore print, elongated basidiospores (11–16 µm long) and the presence of cheilocystidia (Bigelow & Smith 1973). This species has been reported from North America and China, on soil under conifers or broad-leaved trees (Bi *et al.* 1993; Bigelow & Smith 1973). *Cantharocybe* was described as ‘apparently saprotrophic’, though to this day uncertainty remains about its trophic strategy (Matheny *et al.* 2006). There is no obvious morphological connection between *Cantharocybe* and *Volvariella*. Many macroscopic (yellow, decurrent and anastomosing lamellae; absence of universal veil) and microscopic characters (regular lamellar trama, elongated basidiospores, presence of clamp-connections) of *C. gruberi* are not found in *Volvariella*.

The genus *Camarophyllus*, represented in the combined datasets by *Camarophyllus basidiosus*, has been treated as separate genus (Singer 1986) or as an infrageneric group of *Hygrocybe* (Arnolds

1990). Current molecular data support its separation from *Hygrocybe* (Matheny *et al.* 2006). Basidiocarps of *Camarophyllus* have decurrent lamellae, white or cream spore print, and predominantly white or brownish-orange colors. Microscopically, the irregular hymenophoral trama is the most defining character of the genus. *Camarophyllus* is generally considered as saprotrophic (Singer, 1986 and Arnolds, 1990) though it has been also mentioned as mycorrhizal (Lawrey *et al.* 2009). As with *Cantharocybe*, there is no obvious morphological connection between *Camarophyllus* and *Volvariella*.

With the current molecular sampling, *Cantharocybe* and *Camarophyllus* are the only taxa that show affinities with *Volvariella*, but more research on additional genes and taxa is still needed to further clarify the position of *Volvariella* among the *Agaricales*.

Character evolution in *Volvariella*

Species of *Volvariella* can grow directly associated with wood, as mycoparasites or terrestrially, associated with the litter layer of the soil or with accumulations of vegetable matter. Results from the ASR (Fig 2) indicate that two independent transitions towards a lignicolous habitat (*Volvariella bombycina*, *Volvariella caesiointincta*) have occurred in the genus. The mycoparasitism of *Volvariella surrecta*, on basidiocarps of *Clitocybe nebularis*, is also a derived character within the genus. The remaining species of *Volvariella* sampled in this study have maintained what appears to be the plesiomorphic trophic strategy of the genus, as litter saprotrophs.

The subdivision of the genus into two major clades one with *V. bombycina* and *Volvariella volvacea* and the other with the remaining species sampled in each dataset, was highly supported in all the analyses (Fig 2 and Fig 3, Suppl. Information). There is no obvious combination of morphological or ecological features that can be used to characterize both clades. Generally speaking, *V. bombycina* and *V. volvacea* tend to form relatively large basidiocarps (pileus up to 20 cm in diameter), with strongly fibrillose pilei and volvas that cover up to the half of the stipe. However, these characters have a wide range of variation in the genus.

Infrageneric taxonomy of *Pluteus*

In the analyses of the ITS dataset, *Pluteus* is subdivided into three major lineages:

Sect. *Pluteus*

It contains all sampled taxa with metuloid pleurocystidia and a pileipellis as a cutis, and therefore it corresponds to the morphological concept of sect. *Pluteus* of both Singer (1986) and Vellinga & Schreurs (1985). However, one species with indistinct metuloids (*Pluteus allostipitatus*) and one with non-metuloid cystidia (*Pluteus glaucotinctus*) are also placed here. Four major lineages are recognized in all analyses of the ITS dataset (Fig 4):

- (i) *cervinus/petasatus* clade. It includes *Pluteus cervinus* (the type species of the genus), *Pluteus petasatus* and allied taxa. Members of this clade are diverse in ecological characteristics, growing in both hardwood and conifer wood, or more rarely terrestrially, possibly from buried wood or other organic debris. Geographically, members of the clade are widely distributed, with almost identical ITS sequences occurring on both sides of the Atlantic Ocean (e.g. *P. cervinus* AJ82 from Spain and AJ106 from MA), and also on both northern and southern hemispheres (e.g. *P. petasatus* AJ201 from Spain and '*Pluteus viscidulus* T797' from Argentina). The correspondence

between morphological and phylogenetic species recognition in this group will be discussed separately in Justo *et al.* (2010).

- (ii) *salicinus/albostipitatus* clade. It includes *P. albostipitatus*, *P. glaucotinctus*, *Pluteus harrisii*, *Pluteus salicinus* and *Pluteus saupei*. Except for *P. salicinus*, all taxa in this clade have somewhat atypical pleurocystidia. In *P. harrisii* and *P. saupei* the metuloids generally lack well-developed hooks at apices, though they can still be easily recognized as metuloids because of the thick wall and general morphology. In *P. albostipitatus* the pleurocystidia have relatively thin walls (up to 1 µm) and the apices are usually truncate with no hooks on them. Because of this character, *P. albostipitatus* has been traditionally placed in sect. *Hispiderma*, though the unusual shape of the cystidia, and its possible relation to the metuloids in sect. *Pluteus*, was already noticed by Singer (1958) and Pegler (1983) and also, with molecular evidence, by Menolli *et al.* (2010). The cystidia in *P. glaucotinctus* are thin-walled and morphologically similar to the cystidia found in sect. *Hispiderma* and sect. *Celluloderma*.

The presence of psilocibin in *P. salicinus* has been demonstrated using North American (Saupe 1981) and European collections (Stijve & Bonnard 1986), and it is correlated with blue or blue-green tinges in the basidiocarp. One of the American collections used by Saupe (ILLS 42441) was included in the ITS dataset and does not represent *P. salicinus*, but it is in fact a new species, formally described as *P. saupei* in Justo *et al.* (2010). *Pluteus salicinus* itself is actually present in both Europe and North America (Fig 4). Blue-green tinges have been also described for *P. albostipitatus* var. *poliobasis* and *P. glaucotinctus*.

Pluteus albostipitatus and *P. glaucotinctus* occur in Brazil and the Democratic Rep. of Congo, which indicates that either vicariance or long distance dispersal events must be invoked to explain the distribution of these taxa. The fact that some ITS sequences from both sides of the Atlantic are almost 100 % identical suggests long distance dispersal, human mediated or not, as a more likely scenario. The biochemical and biogeographical connections in this clade should be addressed in detail in future studies.

- (iii) *atromarginatus* clade. Includes collections of *Pluteus atromarginatus*, from Asia, Europe and North America, and the holotype of *Pluteus atropungens*. Both taxa are characterized by the dark brown lamellar edges, caused by the presence of pigmented cheilocystidia, however this character is also found in some representatives of the *cervinus/petasatus* clade.
- (iv) *Pluteus losulus* (*P. cervinus* var. *ealaensis*) appears as the more basal taxon in sect. *Pluteus*, in the ML and BA analyses. This African taxon was originally described as a variety of *P. cervinus* but it clearly represents a separate species. A detailed taxonomic discussion is given in Justo *et al.* (2010).

Sect. *Celluloderma*

Taxa included here are characterized by the non-metuloid (or absent) pleurocystidia and, most of them, by a pileipellis composed of short clavate or spheropedunculate cells, mixed or not with elongated cystidioid elements. The species with this type of pileipellis have been placed in subsect. *Eucellulodermini* (without cystidioid elements) and *Mixtini* (with cystidioid elements) in the classifications of Singer (1986) and Vellinga & Schreurs (1985). The *thomsonii* clade and the *podospileus/seticeps* clade contain only taxa with a mixed pileipellis, but this character is also found in some representatives of the *chrysophlebius/phlebophorus* clade (*Pluteus* cf. *eugraptus*) and the *cinereofuscus* clade (*Pluteus eludens*, *Pluteus multiformis*). Though the internal topology of sect. *Celluloderma* is not well resolved in any of the analyses, it seems unlikely that the subsections traditionally recognized are in fact natural units.

Species with non-metuloid cystidia and the pileipellis as a cutis have been placed in sect. *Hispidoderma* by Singer (1986), together with taxa with trichodermal pileipellis. Vellinga & Schreurs (1985) emphasized the differences in pileipellis structure and created sect. *Villosi* to accommodate these taxa. The results from the molecular analyses show that the species with this type of pileipellis (*ephebeus* clade, *Pluteus* sp. V) are not members of sect. *Hispidoderma*, but they should be classified in sect. *Celluloderma* instead of a different section. The *ephebeus* clade includes the European *Pluteus ephebeus*, which is likely a species complex rather than a single species, and the American *Pluteus riberalensis* var. *conquistensis*. This clade is placed as the sister group of *Pluteus fenzlii*/*Pluteus mammillatus* that were classified in the genus *Chamaeota* because of the presence of partial veil. Both clades share the pileipellis as a cutis. This character is also present in the Brazilian collection SP394389, that probably represents an undescribed species, here provisionally named as *Pluteus* sp. V.

Sect. *Hispidoderma*

This clade corresponds to sect. *Hispidoderma* sensu Singer (1986) excluding the species with the pileipellis as a cutis discussed before. Species included here are characterized by a pileipellis composed of long, elongated elements (with $avQ \geq 3$), very variable in shape and size, organized as a hymeniderm or trichoderm. Short clavate elements with $avQ \leq 3$ occur in *Pluteus longistriatus*, *Pluteus pantherinus* and *Pluteus heteromarginatus*, but they are scattered at the base of the elongated elements and do not constitute the main component of the pileipellis layer. Only in *Pluteus castri* and *Pluteus conizatus* var. *africanus* a different pattern is found: the elements of the pileipellis are predominantly clavate or narrowly clavate with an $avQ \leq 3$. Morphological distinction of this type of pileipellis from the one found in sect. *Celluloderma* can be challenging, though in the latter case a high proportion of the elements are sphaeropedunculate (almost isodiametrical) and transitional states between a hymeniderm and an epithelium are frequently found.

Some taxa in this group are apparently widely distributed, with almost identical ITS sequences occurring in geographically distant areas: *P. longistriatus* (Brazil and Missouri), *Pluteus plautus* (Russia and Spain), *Pluteus granulatus* (Russia and Spain), *Pluteus leoninus* (Europe and North America).

Evolution of the pleurocystidia, pileipellis and partial veil in *Pluteus*

The discussion about ASR in *Pluteus* is based on the results from the analyses of the ITS dataset since it has more sampled taxa. However, there is one significant topological difference between the ITS and the combined nSSU + ITS + nLSU analyses. In the ITS trees, sect. *Hispidoderma* is the earliest diverging clade with sections *Pluteus* and *Celluloderma* as sister taxa (Fig 4). In the combined datasets trees, sect. *Pluteus* is the earliest diverging clade, with sections *Hispidoderma* and *Celluloderma* as sister taxa (Fig 3, Supplementary Information).

Even with this difference, and keeping in mind the different level of taxon sampling between the two analyses, some aspects of ASR are the same in both cases:

- (i) There is one single origin for the metuloid pleurocystidia found in sect. *Pluteus*. This character was present in the common ancestor of the section and the poorly developed metuloids or non-metuloid pleurocystidia found in the *salicinus/albostipitatus* clade are derived states (Fig 4). The common ancestor of sect. *Pluteus* had a pileipellis as a cutis and no changes in this character have occurred later in the evolution of this group.
- (ii) The common ancestor of sect. *Celluloderma* had the type of pileipellis that is more commonly found in that group, a hymeniderm or epithelium composed of short, commonly

sphaeropedunculate elements with $avQ \leq 3$. Cystidioid elements in the pileipellis have appeared at least five independent times (Fig 4). Two independent transitions towards a cutis have occurred during the evolution of this group (Fig 4). The ancestral *Celluloderma* had non-metuloid or absent pleurocystidia.

- (iii) The common ancestor of sect. *Hispidoderma* had the type of pileipellis that is more commonly found in that group, a trichoderm or hymeniderm composed of elongated elements (with $avQ \geq 3$). The predominantly narrowly clavate pileipellis elements with $avQ \leq 3$, found in *Pluteus castri* and *Pluteus conizatus* var. *africanus*, are a derived state within the section. The ancestral *Hispidoderma* had non-metuloid pleurocystidia.
- (iv) The partial veil has appeared at least once in the genus, during the evolution of sect. *Celluloderma* (*fenzlii/mammillatus* clade). This character has been described for two other taxa, not sampled in this study, *Chamaeota tropica* Pegler and *Chamaeota subolivascens* Courtec. (Pegler, 1983 and Courtecuisse, 1991). Both taxa have a hymenidermal pileipellis like most representatives of sect. *Celluloderma*, instead of a cutis like *Pluteus fenzlii* and *Pluteus mammillatus*, and they could represent independent origins of the partial veil within *Pluteus*.

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