
A contribution to the ITS-LSU phylogeny of the genus *Leucopaxillus* (/tricholomatoid clade, *Agaricales*), with three new genera and notes on *Porpoloma*

Vizzini A^{1*}, Ercole E¹ and Contu M²

¹Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Viale P.A. Mattioli 25, 10125-Torino, Italy

²Via Marmilla 12, 0702- Olbia (OT), Italy

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Phylogenetic analyses based on ITS-LSU rDNA sequences dataset indicate that *Leucopaxillus*, as currently defined, is a highly polyphyletic genus. The new genera *Giacomia*, *Notholepista* and *Pseudoclitopilus* are introduced to accommodate *Leucopaxillus mirabilis*, *L. subzonalis* and *L. rhodoleucus*, respectively. *Leucopaxillus* subg. *Aspropaxillus* also seems to represent an independent evolutionary line in the /tricholomatoid clade, for which we suggest resurrecting the genus *Aspropaxillus*. Furthermore, the morphologically allied genus *Porpoloma* is also polyphyletic.

Key words – *Agaricomycetes* – new combinations – ITS-LSU sequences – taxonomy

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*Corresponding author: Alfredo Vizzini – e-mail – alfredo.vizzini@unito.it

Introduction

The basidiomycete genus *Leucopaxillus* Boursier, typified by *L. paradoxus* (Costantin & L.M. Dufour) Boursier and traditionally placed, together with *Melanoleuca* Pat., in the subtribus *Leucopaxillineae* Singer (tribus *Leucopaxillae* Singer, family *Tricholomataceae* R. Heim ex Pouzar of the *Agaricales* Underw., Singer 1986), consists of cosmopolitan species with usually terrestrial basidiomata. It is characterized by the following: clitocyboid to tricholomatoid habit; convex to slightly depressed pilei; adnate to decurrent lamellae easily separable from the pileus context; veils usually absent; white to pale yellowish spore print; cutis to trichoderm pileipellis; hyaline, smooth to verruculose spores, smooth spores weakly amyloid (subg. *Aspropaxillus* (Kühner & Maire) Bon = sect. *Aspropaxillus*), verruculose spores with strongly amyloid ornamentations and without a

well differentiated plage (subg. *Leucopaxillus* = sect. *Leucopaxillus*); cheilocystidia absent or hyphoid (not well developed); presence of clamp connections (Singer & Smith 1943, Pegler & Young 1973, Singer 1986, Bon 1991, Gulden 1992, Noordeloos 1984, 1995, Consiglio & Contu 2000, Horak 2005, Christensen 2008, Watling & Turnbull 2008, Vizzini 2009). Regarding its trophic status, Bryan & Zak (1961) reported on a ectomy-corrhizal synthesis between *Leucopaxillus albissimus* var. *piceinus* and *Pinus* sp. but with a poorly developed myoclena and Hartig net. This taxon probably represents a *Tricholoma* sp. (Matheny et al. 2006). Stable isotopes (Kohzu et al. 1999, Hart et al. 2006) and synthesis experiments (Yamada et al. 2001) suggest that *Leucopaxillus* species are non-ectomy-corrhizal but saprotrophic in forest and grassland (Tedersoo et al. 2010). Species of

Leucopaxillus subg. *Aspropaxillus* (*L. candidus* (Bres.) Singer, *L. giganteus* (Sowerby) Singer, *L. lepistoides* (Maire) Singer) may produce very large fairy rings; Kaiser (1998) studied the relationships between *L. giganteus*, microfungi and herbaceous plants. *Leucopaxillus* species turned out to be easy to cultivate in vitro: mycelia of some species are characterized by forming chlamydoconidia (rhexolytically seceding conidia) in pure culture (Pantidou et al. 1983, Buchalo 1988, Ingaramo 2002).

Melanoleuca, a morphologically allied genus, differs from *Leucopaxillus* mainly in lacking clamp connections, by spores with a well differentiated plage area, and usually having well-developed hymenial thick-walled cystidia (Singer 1986, Bon 1978, 1991, Boekhout 1999). But, according to recent molecular analyses (Moncalvo et al. 2000, 2002, Matheny et al. 2006, Vizzini et al. 2011a), *Melanoleuca* and *Leucopaxillus* are not phylogenetically closely related: *Melanoleuca* species cluster within the Pluteoid clade (*Pluteaceae* Kotl. & Pouzar partim + *Amanitaceae* R. Heim ex Pouzar + *Lynnoperdaceae* G.A. Escobar + *Macrocystidiaceae* Kühner + *Pleurotaceae* Kühner) (Moncalvo et al. 2002, Bodensteiner et al. 2004, Binder et al. 2006, Matheny et al. 2006, Vizzini et al. 2011a) sister to a monophyletic group formed by *Pluteus* Fr. species and *Volvopluteus* Vizzini, Contu & Justo (Justo et al. 2011), whereas *Leucopaxillus* belongs to the /tricholomatoid clade, close to *Porpoloma* sp. + *Tricholoma* (Fr.) Staude (Moncalvo et al. 2002) or sister to *Tricholoma* (Matheny et al. 2006). *Leucopaxillus*, together with *Clitocybe* (Fr.) Staude, *Collybia* (Fr.) Staude, *Lepista* (Fr.) W.G. Sm., and *Tricholoma* (Fr.) Staude, forms the family *Tricholomataceae* s.s. (Moncalvo et al. 2002, Matheny et al. 2006). Therefore, morphological similarities between *Leucopaxillus* and *Melanoleuca* are due to evolutionary convergence.

Porpoloma Singer, typified by *P. sejunctum* Singer, differs in having a clear tricholomatoid habit, non-decurrent lamellae that are not separable from the pileus context, and always smooth amyloid spores (Raithelhuber 1980, Singer 1986, Bon 1991). The unique *Porpoloma* sequence (*Porpoloma* sp. AF261395) used in a phylogenetic analysis

(Moncalvo et al. 2002), clustered sister to *Leucopaxillus*, forming with *Tricholoma* and *Leucopaxillus* the /tricholomatoid clade.

The genus *Leucopaxillus* is not yet well covered by DNA studies and only a few species have been sequenced. The present study, based on a wider ITS-LSU sequence dataset, sequences retrieved both from public databases (GenBank, www.ncbi.nlm.nih.gov/genbank/ and UNITE, unite.ut.ee/) and from newly sequenced collections, is the first to examine this genus extensively. The aim was to check whether *Leucopaxillus* is monophyletic as traditionally circumscribed.

Methods

Morphology

All *Leucopaxillus* collections were identified or redetermined using specific monographs (Singer & Smith 1943, Bon 1991, Consiglio & Contu 2000). Watling & Turnbull (1983), Horak (2005), and Christensen (2008) were also consulted. When not identifiable, collections are cited in Table 1 and Figs. 1–2 as *Leucopaxillus* sp. Author citations follow the Index Fungorum-Authors of Fungal Names (www.indexfungorum.org/authorsoffungalname.s.htm) and the names of new taxa are deposited in MycoBank (www.mycobank.org/Default-Page.aspx). Herbarium acronyms follow Thiers (2011) except for “GC” that refers to the personal herbarium of Giovanni Consiglio.

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of herbarium specimens (Table 1) by using the DNeasy Plant Mini Kit (Qiagen, Milan, Italy) following the manufacturer’s instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990, Gardes & Bruns 1993) and primers LR0R/LR7 (Vilgalys & Hester 1990, Vilgalys lab, unpublished, www.botany.duke.edu/fungi/mycolab) for the LSU rDNA amplification. Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20 mM

Table 1 *Leucopaxillus* and *Porpoloma* new sequenced collection used in this study for the molecular analyses.

Species	GenBank acc. Numbers		Source, country
	ITS	LSU	
<i>Leucopaxillus alboalutaceus</i> (F.H. Møller) F.H. Møller	JQ639147	—	GC 97076, Italy
<i>Leucopaxillus cerealis</i> (Lasch) Singer	JQ639148	JQ639149	TO AVL20112, Italy
<i>Leucopaxillus giganteus</i> (Sowerby) Singer	JQ639150	—	GC 94133, Italy
<i>Leucopaxillus giganteus</i>	JQ639151	JQ639152	GC 98046, Italy
<i>Leucopaxillus mirabilis</i> (Bres.) Konrad & Maubl.	JQ639153	JQ639154	GC 94141, Italy
<i>Leucopaxillus mirabilis</i> var. <i>nigrescens</i> Fontenla & Para	JQ639155	—	GC 07186, Italy
<i>Leucopaxillus monticola</i> (Singer & A.H. Sm.) Bon	JQ639156	—	TO AVL20111, France
<i>Leucopaxillus paradoxus</i> (Costantin & L.M. Dufour) Boursier	JQ639157	JQ639158	TO AVL20113, Italy
<i>Leucopaxillus</i> sp. 1	JQ639159	—	TO AVL20114, Italy
<i>Leucopaxillus</i> sp. 2	JQ639160	JQ639161	TO AVL20115, Italy
<i>Porpoloma macrocephalum</i> (Schulzer) Bon	JQ639162	JQ639163	GC 96016, Italy
<i>Porpoloma metapodium</i> (Fr.) Singer	JQ639164	—	TO AVL20116, France

Tris/HCl pH 8.4, 50 mM KCl), 1 μ M of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 unit of *Taq* polymerase (Promega). The PCR program was as follows: 3 min at 95°C for 1 cycle; 30 s at 94°C, 45 s at 50°C, 2 min at 72°C for 35 cycles, 10 min at 72°C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. PCR products were purified and sequenced by DiNAMYCODE srl (Turin, Italy). Sequence assembly and editing were performed using Geneious v5.3 (Drummond et al. 2010). The sequences are deposited in GenBank under the accession numbers given in Table 1 and Figs. 1–2.

Sequence alignment and phylogenetic analysis

Sequences included in the phylogenetic analyses were either generated in this study (Table 1) or retrieved from GenBank and UNITE databases, according to recent studies on *Agaricales* (Moncalvo et al. 2002, Matheny et al. 2006, Vizzini 2011b).

Two separate analyses of ITS and LSU sequences were carried out. Alignments were generated using MAFFT v6.814b (Kato et al. 2002) with default conditions for gap openings and gap extension penalties. Alignments were slightly edited using MEGA 5.0 (Tamura et al. 2011). Molecular-phylogenetic analyses were performed using the Maximum likelihood (ML) and Bayesian inference (BI) approaches. ML

estimation was performed through RAxML (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the -f a option of RAxML and -x 12345 as a random seed to invoke the novel rapid bootstrapping algorithm. BI of phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes (Huelsenbeck & Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 10 000 000 generations, under GTR+G model assumption. Trees were sampled every 1000 generations resulting in an overall sampling of 10 001 trees. The “burn-in” value was evaluated using Tracer (Rambaut & Drummond 2007). The first 20% of trees was discarded as “burn-in”. For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees.

Xeromphalina campanella (Hygrophoroid clade, GenBank accessions GU320006 and GU320009) was used in both datasets analysis as outgroup taxon. Only MLB and BPP values over 50% and 0.75, respectively, are reported in the resulting trees (Figs. 1–2). Pairwise % identity values of ITS sequences

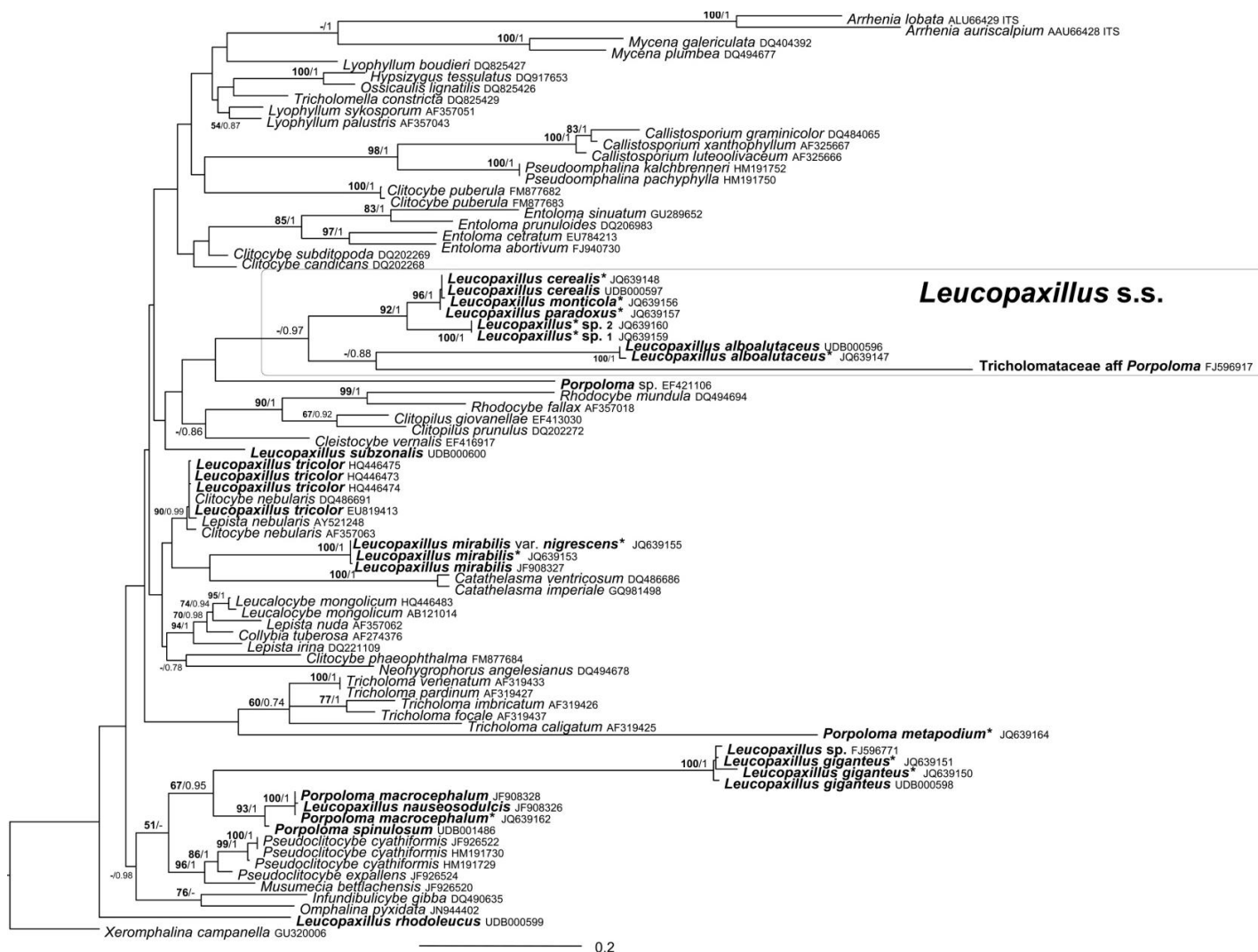


Fig. 1 – Maximum likelihood phylogram obtained from the ITS sequence dataset of the tricholomatoid clade. *Xeromphalina campanella* (Hygrophoroid clade) was used as outgroup. Support values (MLB, in bold and BPP) are given above branches. *Leucopaxillus* and *Porpoloma* sequences are in bold. * refers to samples sequenced in this work and reported in Table 1. The bar indicates number of substitutions per site.

were calculated using MEGA 5.0 (Tamura et al. 2011).

Results

Maximum likelihood and Bayesian inferences were performed on a total of 81 sequences of the ITS dataset, including 69 sequences available from GenBank and UNITE public databases. Final alignment length was 916 bp. The LSU dataset consists of 60 sequences, including 54 available from GenBank and UNITE databases. Final alignment length was 1614 bp. Topology of Maximum likelihood and Bayesian phylogenies is congruent.

In the ITS phylogram (Fig. 1) *Leucopaxillus paradoxus* (the type species of the genus) clusters together with *L. monticola*, *L. cerealis*, *L. alboalutaceus*, *Leucopaxillus* sp. 1 and sp. 2 and “Tricholomataceae aff. *Porpoloma*” forming a monophyletic genus *Leucopaxillus* s.s. The genus is sister to *Porpoloma* sp. EF421106 (DUKE-PR3995). *L. giganteus*, *L. rhodoleucus*, *L. subzonalis*, *L. mirabilis* and *L. mirabilis* var. *nigrescens* fall outside *Leucopaxillus* s.s. *Porpoloma spinulosum*, *P. metapodium*, *Porpoloma* sp. EF421106, “Tricholomataceae aff. *Porpoloma*” and *P. macrocephalum* do not form a coherent group. The four *Leucopaxillus tricolor*

sequences from GenBank clearly do not represent a *Leucopaxillus* but a taxon very close to *Lepista nebularis* (pairwise % identity value 99.4). The two *Porpoloma macrocephalum* sequences and the *Leucopaxillus nauseodulcis* sequence showed a pairwise % identity value of 99.0, and are conspecific.

In the LSU phylogram (Fig. 2) *L. paradoxus*, *L. cerealis*, *L. albissimus* (Peck) Singer (= *L. cerealis* (Lasch) Singer, fide Singer 1986), *L. gentianeus* and *Leucopaxillus* sp. 2 form a monophyletic group (*Leucopaxillus* s.s.). *Leucopaxillus* s.s., together with *Porpoloma* AF261395 (JLPR3395), is sister to *Tricholoma*. *L. mirabilis* and *L. giganteus* are independent from *Leucopaxillus*. *Porpoloma* AF261395 (JLPR3395) is not related to *Porpoloma macrocephalum*.

Discussion

Our molecular analyses clearly show (Figs. 1–2) that *Leucopaxillus*, as currently defined, is polyphyletic. Species traditionally ascribed to this genus (Singer 1986, Bon 1991, Consiglio & Contu 2000, Christensen 2008) do not form a monophyletic assemblage and are distributed over the /tricholomatoid clade. According to the LSU analysis (Fig. 2) *Leucopaxillus* s.s. and *Porpoloma* AF261395 (JLPR3395) are sister to *Tricholoma*, in agreement with Moncalvo et al. (2002) and with the multilocus phylogenetic overview by Matheny et al. (2006). The *Leucopaxillus/Tricholoma* connections were already highlighted, even if only on morphological bases, by Kühner (1980). So it is evident that the “leucopaxilloid” facies (basidiomata with a clitocyboid to tricholomatoid habit, clamp-connections and spores with amyloid ornamentations) have arisen many times in more or less independent lines of evolution. Within *Leucopaxillus* s.s., *Leucopaxillus* sp. 1 and *Leucopaxillus* sp. 2 are collections representative of a yet undescribed taxon characterized by clampless basidiomata and mainly bisporic basidia. *Leucopaxillus giganteus*, *L. mirabilis*, *L. subzonalis* and *L. rhodoleucus* are not phylogenetically connected with *Leucopaxillus* s.s. and are unrelated to each other (Figs. 1–2). They represent new genera in the /tricholomatoid clade.

Resurrecting the genus *Aspropaxillus*

Leucopaxillus giganteus is the type species of *Aspropaxillus* Kühner & Maire. Kühner & Maire (1934) established the genus to accommodate the smooth-spored species, with a clitocyboid habitus and a perisporium characterized by a weak amyloid reaction. Singer & Smith (1943), in their monographic treatment of *Leucopaxillus*, maintained this separation but only at a sectional level. Bigelow (1982) considered it as a subsection of section *Clitocybe* within his heterogeneous definition of *Clitocybe* (Fr.) Staude. Finally, Bon (1990, 1991) accepted it as a distinct subgenus of *Leucopaxillus*. According to both ITS and LSU analyses (Figs. 1–2), we suggest using the genus *Aspropaxillus* for *L. giganteus* and allied species. Therefore, we propose the following new combinations:

Aspropaxillus Kühner & Maire, Bull. trimest. Soc. mycol. Fr. 50: 13, 1934.

≡ *Leucopaxillus* subgen. *Aspropaxillus* (Kühner & Maire) Bon, Docum. Mycol. 20(79): 57, 1990.

Type: *Aspropaxillus giganteus* (Sowerby) Kühner & Maire

Aspropaxillus septentrionalis (Singer & A.H. Sm.) Vizzini, **comb. nov.**

Mycobank MB 564422

≡ *Leucopaxillus septentrionalis* Singer & A.H. Sm. Mycologia 39: 726, 1948 [1947] (basionym).

≡ *Clitocybe septentrionalis* (Singer & A.H. Sm.) H.E. Bigelow, Canad. J. Bot. 37: 772, 1959.

Aspropaxillus sainii (Singer) Vizzini, **comb. nov.**

Mycobank MB 564424

≡ *Leucopaxillus sainii* Singer, Fieldiana, Bot, new ser. 21: 19, 1989 (basionym).

Aspropaxillus jageshwariensis (Dhanch., J.C. Bhatt & S.K. Pant) Vizzini, **comb. nov.**

Mycobank MB 564426

≡ *Leucopaxillus jageshwariensis* Dhanch., J.C. Bhatt & S.K. Pant, Acta Bot. Indica 19: 107, 1991 (basionym).

Both *A. sainii* and *A. jageshwariensis* were described from India and are apparently known only from that region. We did not have

the chance to study authentic material of them but, judging by the protologues (op. cit.) they are well characterized and deserve specific rank on their own right.

On the taxonomic placement of *Tricholoma mirabile* Bres.: a new genus.

Leucopaxillus mirabilis is a striking European taxon easy to recognize in the field due to a dark brown pileus and stipe, a hairy pileus margin, a wrinkled stipe apex, an araneous partial veil forming a thin ring on stipe apex, abundant cheilocystidia (Moser 1963, Kühner 1977, Bon 1978, 1987a, 1991, Consiglio & Contu 2000), and heterogeneous spores variable in size, form and degree of ornamentation, which ranged from coarsely verrucose to smooth (Moser 1963). The type of ornament is composed of isolated, hemispherical verrucae, similar to that of *Melanoleuca cognata* (Fr.) Konrad & Maubl. (Pegler & Young 1973). It is not easily culturable in vitro (Moser 1963). Due to its peculiar features, Bon (1991) classified the species in the monospecific subsection *Mirabilini* (Bon) Bon (stipe with an arachnoid ring-like velum) of sect. *Mirabiles* Bon (presence of cheilocystidia) of *Leucopaxillus*. *L. mirabilis* var. *nigrescens* differs only in having a darker pileus (Bresadola 1927, Bon 1991). The two *L. mirabilis* sequences and the one of *L. mirabilis* var. *nigrescens* are clearly identical (pairwise % identity value = 99.9). This variety is here reduced to a form. The species is not closely related either to *Leucopaxillus* s.s. or to other taxa of the /tricholomatoid clade; consequently we accept this lineage to represent a distinct genus and establish the new genus *Giacomia* for accommodating *L. mirabilis*.

***Giacomia* Vizzini & Contu, gen. nov.**

Mycobank MB 564428

Etymology – named in honour of Abbé Giacomo Bresadola, eminent Italian mycologist, and father of the species name.

A Leucopaxillo differt basidioma velo araneoso ornato et in stuctura molecularis (ITS-spatiiis internis transcriptis et LSU DNA).

Basidiomata agaricoid (with distinct pileus, lamellae and stipe), partial veil present as an arachnoid cortina, basidiospores with amyloid warts, cheilocystidia often abundant, filamentous, pileal surface a cutis of repent to interwoven, cylindrical hyphae, clamp-connections present, no sarcodimitic texture in any part of the basidioma. On the ground, never

on wood.

Type: *Tricholoma mirabile* Bres.

***Giacomia mirabilis* (Bres.) Vizzini & Contu, comb. nov.**

Mycobank MB 564429

≡ *Tricholoma mirabile* Bres., Fungi Tridentini I: 16, 1881 (basionym).

≡ *Leucopaxillus mirabilis* (Bres.) Konrad & Maubl., Encyclop. Mycol. 20: 191, 1952.

≡ *Melanoleuca mirabilis* (Bres.) Singer, Lloydia 5: 121, 1942.

***Giacomia mirabilis* f. *nigrescens* (Bres.) Vizzini & Contu, comb. nov. et stat. nov.**

Mycobank MB 564430

≡ *Tricholoma mirabile* Bres. var. *nigrescens* Bres., Iconographia Mycologica II: 92, 1927 (basionym).

≡ *Melanoleuca nigrescens* (Bres.) Bon, Docum. Mycol. 9(33): 47, 1978.

≡ *Leucopaxillus mirabilis* var. *nigrescens* (Bres.) Fontenla & Para, Rivista di Micologia 50(3): 233, 2007.

On the natural taxonomic placement of *Agaricus (Clitocybe) subzonalis* Peck: a new genus.

Leucopaxillus subzonalis is a rare species first described from North America (Peck 1873, Singer & Smith 1943, 1947, Bigelow 1965) and then reported also from Europe (Josserand 1953, Henze 1970, Bon 1991, Bidaud & Cavet 2006, Christensen 2008) and China (Horak 1987). It is characterized by entirely yellow basidiomes with a *Lepista gilva*/*Hygrophorus lucorum*-like habit and a hygrophanous pileus (Singer & Smith 1943, 1947, Josserand 1953, Bigelow 1965, Henze 1970, Horak 1987, Bon 1987b, 1991, Bidaud & Cavet 2006, Christensen 2008, Soop, karl.soop.org/English/galled8.html). Lavorato & Contu (2001) reported from Italy as *L. subzonalis* a collection clearly referable to a different taxon, probably a yellowish form of *L. cerealis*.

***Notholepista* Vizzini & Contu, gen. nov.**

Mycobank MB 564431

Etymology – refers to the habit being reminiscent of *Lepista gilva*.

A Lepista differt sporis verrucis amyloides obtectis. A Leucopaxillo differt habitu clitocyboideo vel lepistoido, Lepista

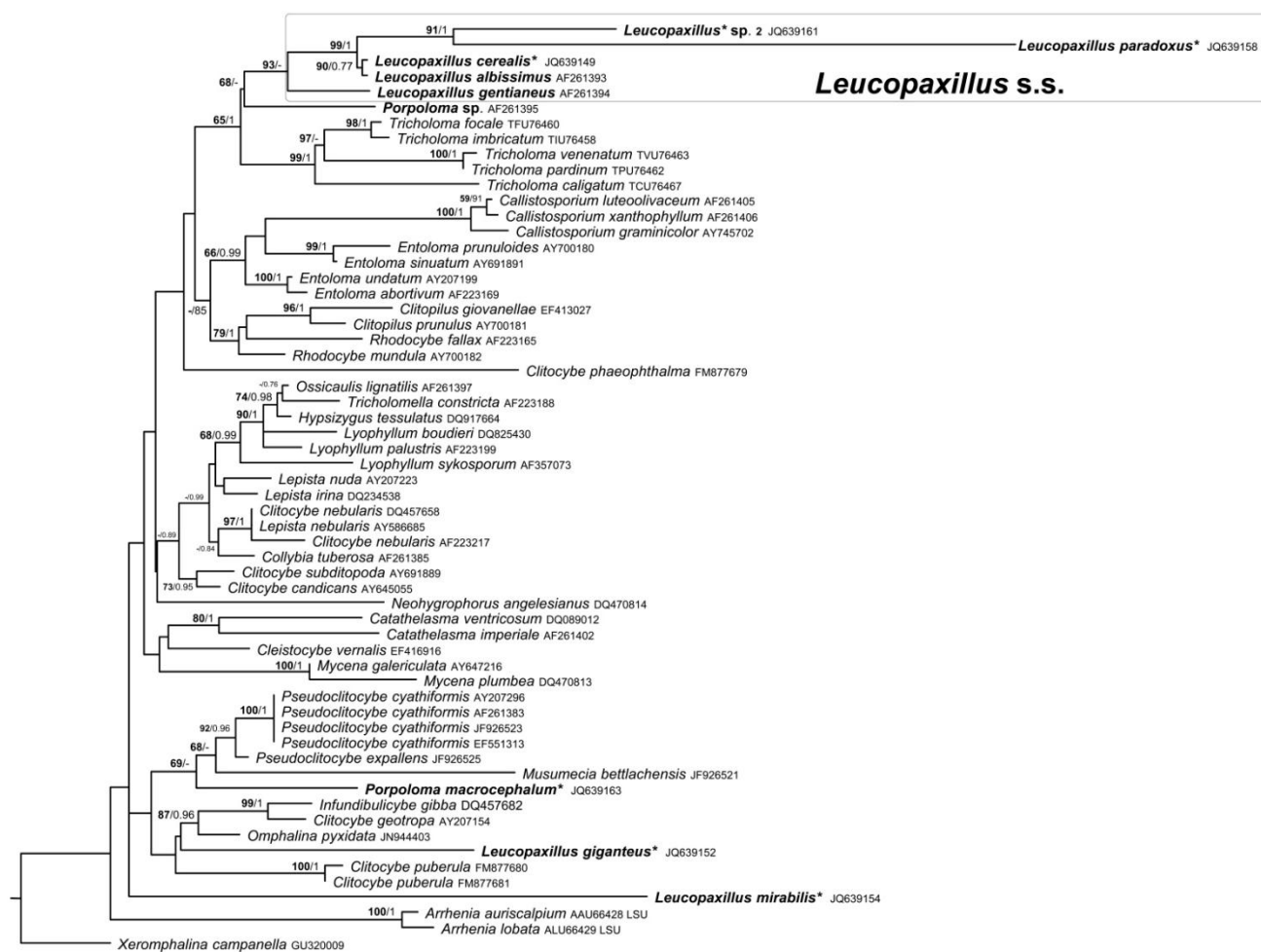


Fig. 2 – Maximum likelihood phylogram obtained from the LSU sequence dataset of the /tricholomatoid clade. *Xeromphalina campanella* was used as outgroup. Support values (MLB, in bold and BPP) are given above branches. *Leucopaxillus* and *Porpoloma* sequences are in bold. * refers to samples sequenced in this work and reported in Table 1. The bar indicates number of substitutions per site.

gilva in mente revocante et in stuctura molecularis (ITS-spatiiis internis transcriptis DNA).

Basidiomata agaricoid (with distinct pileus, lamellae and stipe), resembling those of *Lepista gilva* (Pers.) Pat., veils absent, basidiospores with amyloid warts, cystidia and pseudocystidia absent, pileal surface a cutis of repent to interwoven, cylindrical hyphae, clamp-connections present, no sarcodimitic texture in any part of the basidioma. On the ground, never on wood.

Type: *Agaricus (Clitocybe) subzonalis* Peck.

Notholepista subzonalis (Peck) Vizzini & Contu, **comb. nov.**

Mycobank MB 564432

≡ *Agaricus (Clitocybe) subzonalis* Peck, Bull. Buffalo Soc. Nat. Sci. 1: 46, 1873 (basonym).

≡ *Clitocybe subzonalis* (Peck) Saccardo, Syll. Fung. 5: 184, 1887.

≡ *Leucopaxillus subzonalis* (Peck) H.E. Bigelow, Lloydia 28: 179, 1965.

= *Clitocybe pulcherrima* Peck, Journ. Mycol. 14: 1, 1908.

≡ *Leucopaxillus pulcherrimus* (Peck) Singer & A.H. Smith, Pap. Mich. Acad. Sci. Arts, Letters 28: 116, 1943 [1942].

On the taxonomic placement of *Agaricus rhodoleucus* Romell: a new genus for a species mimicking an unusually fleshy *Clitopilus* species.

Leucopaxillus rhodoleucus, a species recorded so far only from Europe, looks like *Clitopilus prunulus* (Scop.) P. Kumm. or *Hygrophorus karstenii* Sacc. & Cub. because of its strongly decurrent lamellae with a pinkish tint, especially in young basidiomata, and a white, somewhat hygrophanous pileus (Szemere 1966, Pegler & Young 1973, Trimbach 1978, Fanelli 1984, Bon 1991, Watling R, Turnbull 1998, Consiglio & Contu 2000, Anon. 2001, Markones 2003, Christensen 2008). *L. salmonifolius* M.M. Moser & Lamoure (Moser 1979, Bidaud 1993) differs mainly in having shorter spores (6.0–8.0 µm vs 4.5–6 µm).

***Pseudoclitopilus* Vizzini & Contu, gen. nov.**

MycoBank MB 564433

Etymology – refers to the habit being reminiscent of *Clitopilus prunulus*.

A Clitopilo differt sporis verrucis amyloideis obtectis, hilo sporalis haud typi Entolomatacaearum. A Leucopaxillo differt in stuctura molecularis (ITS-spatiis internis transcriptis DNA).

Basidiomata agaricoid (with distinct pileus, lamellae and stipe), resembling a stout and fleshy white *Clitopilus*, veils absent, basidiospores with amyloid warts, cystidia and pseudocystidia absent, pileal surface a cutis of repent to interwoven, cylindrical hyphae, clamps present, no sarcodimitic texture in any part of the basidioma. On the ground, never on wood.

Type: *Clitocybe rhodoleuca* Sacc.

***Pseudoclitopilus rhodoleucus* (Sacc.) Vizzini & Contu, comb. nov.**

MycoBank MB 564434

≡ *Agaricus rhodoleucus* Romell, Bot. Notiser: 66, 1895, nom. illeg., non Lèv. 1855.

≡ *Clitocybe rhodoleuca* Sacc., Beibl. Hedwigia 35(7): II (1896) (basionym).

≡ *Lepista rhodoleuca* (Romell) Maire, Bull. trimest. Soc. mycol. Fr. 40: 305, 1926.

≡ *Leucopaxillus rhodoleucus* (Romell) Kühner, Bull. mens. Soc. linn. Lyon 5: 126, 1926.

***Pseudoclitopilus salmonifolius* (M.M. Moser & Lamoure) Vizzini & Contu, comb. nov.**

MycoBank MB 564435

≡ *Leucopaxillus salmonifolius* M.M.

Moser & Lamoure, Beihefte zur Sydowia 8: 268, 1979 (basionym).

The status of *Porpoloma* Singer

The genus *Porpoloma* seems also polyphyletic (Fig. 1). Singer (1952) erected the genus for three Argentinian species of *Nothofagus* forests, then combined the European *Tricholoma spinulosum* Kühner & Romagn, *Hygrophorus metapodius* (Fr.) Fr. and *Agaricus elytroides* Scop. in *Porpoloma* in 1962 and 1973, respectively. According to our analysis, the closely related *Porpoloma macrocephalum* and *P. spinulosum* are very distant from the other sequenced taxa of *Porpoloma*. According to the ITS phylogeny (Fig. 1) *P. metapodium* is sister to *Tricholoma*; this species was treated as *Hygrophorus* by Hesler & Smith (1963, as *Hygrophorus* sect. *Amylohygrocybe*), as *Hygrocybe* by Moser (1967), and as *Tricholoma* by Papetti (1999). Nevertheless, we refrain from erecting new genera until re-examination and sequencing of *P. sejunctum* Singer, the type species of *Porpoloma*, can better determine its characters and phylogenetic affinities.

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References

- Anon. 2001 – Fungal portraits: No. 6. *Leucopaxillus rhodoleucus*. Field Mycology 2(2), 39.
- Bidaud A. 1993 – *Leucopaxillus salmonifolius* Moser & Lamoure. Bulletin trimestriel de la Fédération mycologique Dauphiné-Savoie 130, 33–34.
- Bidaud A, Cavet J. 2006 – Journée des espèces rares ou intéressantes 2005, La Tour-du-Pin - 11 janvier 2006 - 1re partie. Bulletin Mycologique et Botanique Dauphiné-Savoie 181, 39–58.
- Bigelow HE. 1965 – The genus *Clitocybe* in North America. Section *Clitocybe*. Lloydia 28, 139–180.
- Bigelow HE. 1982 – North American species of *Clitocybe*. Part I. Beihefte zur Nova Hedwigia 72, 1–280.

- Binder M, Hibbett DS, Wang Z, Farnham W. 2006 – Evolutionary relationships of *Mycaureola dilseae* (Agaricales), a basidiomycete pathogen of a subtidal rhodophyte. *American Journal of Botany* 93, 547–556.
- Bodensteiner P, Binder M, Moncalvo JM, Agerer R, Hibbett DS. 2004 – Phylogenetic relationships of cyphelloid homobasidiomycetes. *Molecular Phylogenetics and Evolution* 33, 501–515.
- Boekhout T. 1999 – *Melanoleuca* Pat. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC (eds). *Flora Agaricina Neerlandica* 4. A.A. Balkema, Rotterdam, pp. 153–165.
- Bon M. 1978 – *Tricholomataceae* de France et d'Europe occidentale (*Leucopaxilloideae*). *Documents Mycologiques* 9(33), 1–79.
- Bon M. 1987a – Agaricomycètes étudiés au symposium de Val di Rabbi (Italie, Septembre 1983). *Documents Mycologiques* 18(69), 27–34.
- Bon M. 1987b – Macromycetes importées ou nouveaux pour le territoire français. *Beiträge zur Kenntnis de Pilze Mitteleuropas* 3, 307–311.
- Bon M. 1990 – Taxons nouveaux et validations. *Documents Mycologiques* 20(79), 57–62.
- Bon M. 1991 – *Tricholomataceae* (Fayod) Heim (1ère partie) (*Tricholomoideae* et *Leucopaxilloideae*). *Flore Mycologique d'Europe* Vol. 2. Les Tricholomes et Ressemblants. *Documents Mycologiques Mémoire Hors Série*. Amiens, 163 p.
- Bresadola G. 1927 – *Iconographia Mycologica*, vol. 2. Società Botanica Italiana – sezione Lombardia, Milano, Italy.
- Bryan WC, Zak B. 1961 – Synthetic culture of mycorrhizae of southern pines. *Forest Science* 7, 123–129.
- Buchalo AS. 1988 – [Higher Edible Basidiomycetes in Pure Culture.] *Naukova Dumka*, Kiev. [In Russian]
- Christensen M. 2008 – *Leucopaxillus* Boursier. In: Knudsen H, Vesterholt J (eds). *Funga Nordica*. Nordsvamp, Copenhagen, pp. 409–411.
- Consiglio G, Contu M. 2000 – Il genere *Leucopaxillus* Boursier in Italia, con brevi note sulle rimanenti specie europee. *Bollettino della Associazione Micologica Ecologica Romana* 51, 3–36.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. 2010 – Geneious v5.3. Available from www.geneious.com/.
- Fanelli AL. 1984 – Una Tricholomatacea non comune: *Leucopaxillus rhodoleucus* (Romell) Kühner. *Micologia Italiana* 13(3), 16–20.
- Felsenstein J. 1985 – Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Gardes M, Bruns TD. 1993 – ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113–118.
- Gulden G. 1992 – *Leucopaxillus* Bours. In: Hansen L, Knudsen H (eds). *Nordic Macromycetes* Vol. 2. Nordsvamp, Copenhagen, pp. 135–136.
- Hart SC, Gehring CA, Selmants PC, Deckert RJ. 2006 – Carbon and nitrogen elemental and isotopic patterns in macrofungal sporocarps and trees in semiarid forests of the south-western USA. *Functional Ecology* 20, 42–51.
- Henze G. 1970 – *Leucopaxillus pulcherrimus*. *Bulletin de la Fédération mycologique Dauphiné-Savoie* 38, 12–13.
- Hesler LR, Smith AH. 1963 – North American species of *Hygrophorus*. The University of Tennessee Press, Knoxville, Tennessee.
- Horak E. 1987 – *Agaricales* from Yunnan, China. I. *Transactions of the Mycological Society of Japan* 28, 171–188.
- Horak E. 2005 – Röhrlinge und Blätterpilze in Europa. Spektrum, Elsevier, Heidelberg.
- Huelsenbeck JP, Ronquist F. 2001 – MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Ingaramo A. 2002 – Caratterizzazione e

- conservazione ex-situ della biodiversità di basidiomiceti del bosco dell'Alevè a diverso trofismo. Tesi di laurea, Facoltà di Scienze Matematiche, Fisiche e Naturali, Università degli Studi di Torino.
- Josserand M. 1953 – Étude d'une Agaricacée nord-américaine récoltée en France, *Leucopaxillus pulcherrimus* (Peck) nob. Bulletin de la Société des naturalistes d'Oyonnax 7, 50-56.
- Justo A, Vizzini A, Minnis AM, Menolli Jr N, Capelari M, Rodriguez O, Malysheva E, Contu M, Ghingnone S, Hibbett DS. 2011 – Phylogeny of the *Pluteaceae* (*Agaricales*, *Basidiomycota*): taxonomy and character evolution. Fungal Biology 115, 1–20.
- Kaiser P. 1998 – Relations of *Leucopaxillus giganteus*, basidiomycete of fairy rings, with soil microflora and grassland plants. Cryptogamie Mycologie 19(1–2), 45–61.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002 – MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30, 3059–3066.
- Kohzu A, Yoshioka T, Ando T, Takahashi M, Koba K, Wada E. 1999 – Natural 13C and 15N abundance of field-collected fungi and their ecological implications. New Phytologist 144, 323–330.
- Kühner R. 1977 – Étude morphologique, anatomique et cytologique des carpophores de l'agaricale *Leucopaxillus mirabilis* (Bres.) Kuhn.-Romagn. Bulletin Mensuel de la Société Linnéenne de Lyon 46(1), 6–10.
- Kühner R, Maire R. 1934 – Étude de la réaction de la membrane sporique à l' iode dans les divers genres d'Agarics leucosporés. Bulletin Trimestriel de la Société Mycologique de France 50: 9–24.
- Lavorato C, Contu M. 2001 – *Leucopaxillus subzonalis* - specie nuova per l'Italia. Micologia e Vegetazione Mediterranea 15(2), 110–114.
- Markones R. 2003 – The first Bavarian record of *Leucopaxillus rhodoleucus*. Mycologia Bavarica 6, 37–40.
- Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo J-M, Ge Z-W, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006 – Major clades of *Agaricales*: a multilocus phylogenetic overview. Mycologia 98, 982–995.
- Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000 – Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systematic Biology 49, 278–305.
- Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Cléménçon H, Miller OK. 2002 – One hundred and seventeen clades of euagarics. Molecular Phylogenetics and Evolution 23(3), 357–400.
- Moser M. 1963 – Zur Variabilität von *Leucopaxillus mirabilis* (Bres.) Mos. Schweizerische Zeitschrift für Pilzkunde 41, 181–194.
- Moser M. 1967. Kleine Kryptogamenflora von Mitteleuropa - Die Blätter- und Baupilze (*Agaricales* und *Gastromycetes*) Volume: Iib/2. G. Fischer, Stuttgart.
- Moser M. 1979 – Über einige neue oder seltene *Agaricales*-Arten aus dem Pieniny und aus Bieszczade, Polen. Beihefte zur Sydowia 8(3), 268–275.
- Noordeloos ME. 1984 – Notulae ad Floram Agaricinam Neerlandicam. IV–V. *Clitopilus* and *Leucopaxillus*. Persoonia 12, 155–167.
- Noordeloos EM. 1995. *Leucopaxillus* Boursier. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC (eds). Flora Agaricina Neerlandica, vol. 3. Balkema, Rotterdam, pp. 76–77.
- Pantidou ME, Watling R, Gonou Z. 1983 – Mycelial characters, anamorphs and teleomorphs in genera and species of various families of *Agaricales* in culture. Mycotaxon 17, 409–432.

- Papetti C. 1999 – *Tricholoma (Porpoloma) metapodium* (Fr.) Papetti comb. nov. Validazione. Bollettino del Circolo micologico G. Carini 37, 48.
- Peck CH. 1873 – Descriptions of new species of fungi. Bulletin of the Buffalo Society of natural Sciences 1, 41–72.
- Pegler DN, Young TWK. 1973 – Basidiospore form in the British *Leucopaxilleae*. Kew Bulletin 28, 365–379.
- Raithelhuber J. 1980 – Die Gattung *Porpoloma* Sing. Metrodiana 9(1), 10–16.
- Rambaut A, Drummond AJ. 2007 – Tracer v1.4. Available from <http://beast.bio.ed.ac.uk/Tracer>
- Singer R. 1952 – The agarics of the Argentine sector of Tierra del Fuego and limitrophous regions of the Magallanes area. Sydowia 6, 165–226.
- Singer R. 1962 (“1961”) – Diagnoses fungorum novorum Agaricalium II. Sydowia 15(1-6), 45–83.
- Singer R. 1973 – Diagnoses fungorum novorum Agaricalium III. Beihefte zur Sydowia 7, 1–106.
- Singer R. 1986 – The *Agaricales* in Modern Taxonomy, 4th ed. Koeltz Scientific Books, Koenigstein, Germany.
- Singer R, Smith AH. 1943 – A monograph on the genus *Leucopaxillus* Boursier. Papers of the Michigan Academy of Science, Arts & Letters 28, 85–132.
- Singer R, Smith AH. 1948 (“1947”) – Additional notes on the genus *Leucopaxillus*. Mycologia 39, 725–736.
- Szemere L. 1966 – *Leucopaxillus rhodoleucus* (Romell) Kühner. Česká Mykologie 20, 30–31.
- Stamatakis A. 2006 – RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S. 2011 – MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28, 2731–2739.
- Tedersoo L, May TW, Smith Me. 2010 – Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20, 217–263.
- Thiers B. 2011 (continuously updated) – Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. sweetgum.nybg.org/ih/.
- Trimbach J. 1978 – Matériel pour une "checklist" des alpes maritimes (suite). Documents Mycologiques 8(29), 39–53.
- Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4238–4246.
- Vizzini A. 2009 – Revisione dei generi *Armillaria*, *Crinipellis*, *Chaetocalathus*, *Leucopaxillus* e *Omphalotus*. In: Maire J-C, Moreau P-A, Robich G (eds.). Compléments à la Flore des champignons supérieurs du Maroc de G. Malençon et R. Bertault. Confédération Européenne de Mycologie Méditerranéenne, Nice, pp. 419–446.
- Vizzini A, Para R, Fontenla R, Ghignone S, Ercole E. 2011a – A preliminary ITS phylogeny of *Melanoleuca* (*Agaricales*), with special reference to European taxa. Mycotaxon 118, 361–381.
- Vizzini A, Contu M, Ercole E. 2011b – *Musumecia* gen. nov. in the Tricholomatooid clade (*Basidiomycota*, *Agaricales*) related to *Pseudoclitocybe*. Nordic Journal of Botany 29, 734–740.
- Watling R, Turnbull E. 1998 – *Cantharellaceae*, *Gomphaceae* and amyloid-spored and xeruloid members of *Tricholomataceae* (excl. *Mycena*). British Fungus Flora, Agarics and Boleti 8. Royal Botanic Gardens, Edinburgh.
- White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, MA, Gelfand, DH, Snisky JJ, White TJ (eds). PCR Protocols. Academic Press, London, pp. 315–322.

Yamada A, Ogura T, Ohmasa M. 2001 –
Cultivation of mushrooms of edible
ectomycorrhizal fungi associated with

Pinus densiflora by in vitro mycorrhizal
synthesis. II. Morphology of mycorrhizas
in open-pot soil. *Mycorrhiza* 11, 67–81.