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Tricholosporum goniospermum, genetic diversity and phylogenetic relationship with the Tricholomatineae [formerly tricholomatoid clade]

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The species *Tricholosporum goniospermum* is mainly known from north Europe and north Italy. However, recently, the presence of this species was reported also in central Italy. In this study we examined samples collected in central Italy that showed a spore size smaller than typically reported for this species. In agreement with morphological observations we found genetic polymorphisms in the ITS region suggesting that populations from central Italy are genetically different from those of northern Italy. Moreover, we detected intra-individual polymorphism in the ITS region. These finding may be explained with the extremely fragmented habitat of this species that may represent a limit for gene flow. Based on morphology the genus *Tricholosporum* is currently classified in the *Tricholomataceae*, however the presence of cruciate basidiospores represents a distinctive character with respect to other *Tricholomataceae*. Thus, we used nuclear ITS, LSU, SSU and *rpb2* DNA sequences to evaluate the phylogenetic position of this genus within the clade of "tricholomatoid" fungi. This analysis showed a low affinity of *Tricholosporum* with the clade of the *Tricholomataceae* and an isolated position of this genus within the *Tricholomatineae*. Thus the analyses performed in this study provided further insight for the revision of the species and family in the *Tricholomatineae*.

Keywords: ITS, LSU, SSU *rpb2*, *Tricholomataceae*.

Tricholosporum is a rare fungal genus with a mainly pantropical distribution (Contu & Mua 2000, Heaton 2014). This genus comprises 14 species according to Index Fungorum (http://www.indexfungorum.org/names/Names.asp) although only nine species were included in the key provided by Angelini et al. (2014). It is represented in Europe by the following species: *T. goniospermum*, *T. subgoniospermum*, *T. tetragonosporum,* and *T. cassonianum* (Baroni 1982, Bohus et al. 1999, Contu & Mua 2000, Moreau & Contu 2007, Boisselet & Moreau 2008). *Tricholosporum goniospermum* is the type species of the genus. It is mainly distributed in northern and central Europe, and is considered a saprotroph that colonizes small soil patches near deciduous trees. The first Italian record of this species is reported from northern Italy, in the region of Trentino Alto Adige (Bresadola 1892); only a few and scattered localities in northern Italy were known until 1995, then it was reported also on high altitude grasslands in central Italy (Tafini 1995, Contu & Mua 2000, Onofri et al. 2005). These findings suggest a disjunct geographic distribution and prompted for a more in-depth investigation of the habitat types and distribution of this species in Italy (Di Massimo & Donnini 2008). In the Umbria-Marche Apennines in central Italy *T. goniospermum* is commonly referred to as "Cervino" and appreciated for its culinary qualities (Tafini 1995).

Although *Tricholosporum* differs from *Tricholoma* by the unusual cruciate or staurosporous shaped basidiospores and in some species for presence of purplish, violaceous or lilac-vinaceous pigments in some portion of the basidioma (Baroni 1982), these fungi were initially included in the genus *Tricholoma* (Bresadola 1892, Singer 1945). The genus *Tricholosporum* was established by Guzmán (1975) for those *Tricholoma*-like agarics which Singer placed in *Tricholoma* sect. *Iorigida,* such as *T. goniospermum* (*typus generis*), *T. porphyrophyllum* from the temperate zone of Japan (Imai 1938, Bohus et al. 1999), and *T. subporphyrophyllum* from tropical areas of Mexico (Guzmán 1975). Subsequently, several other species have been described from Central and

North America (Baroni 1982; Guzmán et al. 1990, 1994; Halling & Franco-Molano 1996), Malaysia (Watling 1997), South Africa (Reid et al. 1988), Hungary (Bohus et al. 1999), France (Moreau & Contu, 2007), La Palma (Canary Islands, Spain) (Fernandez Vincente et al. 2010) and the Dominican Republic (Angelini et al. 2014).

The genus *Tricholosporum* has been included in the family of *Tricholomataceae* based on morphological characters, however, recently molecular studies have provided increasing evidence that this family, defined based on the morphology of basidiomata (e.g. the white or rarely pale cream colour of spore deposit, the adnexed, sinuate-emarginate to decurrent type of lamellae), is polyphyletic (Sánchez-García et al. 2014). A number of molecular studies have been performed to re-evaluate taxonomy, phylogeny and relationship of tricholomatoid fungi now classified as *Tricholomatineae* (Dentinger et al. 2016). In particular, Sánchez-García et al. (2014) proposed to include in the *Tricholomataceae* the following seven genera: *Dennisiomyces*, *Porpoloma*, *Corneriella*, *Albomagister*, *Tricholoma*, *Pseudotricholoma* and *Leucopaxillus*. The other well resolved clades were the *Entolomataceae,* including the genera *Entoloma, Rhodocybe*, *Clitopilus,* and the *Lyophyllaceae* including the genera *Lyophyllum*, *Tephrocybe*, *Calocybe, Tricholomella, Ossicaulis*, *Termitomyces*. Many other species resulted phylogenetically distinct from these three main clades, but were not strongly supported in phylogenetic reconstructions and referred as the residual tricholomatoid clades (Sánchez-García et al. 2014). With this background, the aims of the present study were to: i) assess whether samples harvested in central Italy belong to *T. goniospermum* by studying the fast evolving internal transcribed spacer (ITS) region; ii) clarify the phylogenetic position of the genus *Tricholosporum* within the *Tricholomatineae*. For this purpose both the ITS, the genes for the ribosomal small (SSU) and large (LSU) subunit of rDNA and the gene for the RNA polymerase II second largest subunit (rpb2) were used.

Materials and methods

Sampling and morphological identification

Basidiomata were collected between 2010 and 2013 in central Italy on both the western and eastern sides of the Apennine (Sibillini Mountains) in the Umbria and Marche regions, respectively, between 1600 and 1750 m a.s.l. More specifically the basidiomata were collected in two sites (Assisi and Spello) within the Regional Park of Mount Subasio, in one site (Norcia) on Mount Vettore and in one site (Ascoli) on Mount Monaco (Tab. 1). The collection sites are marked by semi-circular belts of greener, lusher grass, the so-called "witches' circles". All collected specimens were dried and deposited in the herbarium of Perugia University (PERU). A voucher specimen of *T. goniospermum* collected in Trieste (North Italy) was also examined and compared with specimens collected in central Italy.

For species determination, both fresh samples and exsiccata were examined for their macroscopic and microscopic morphological characters. Dried specimens were rehydrated by soaking in 3 % KOH for 10 min. Dried and fresh samples were stained with 1 % Congo Red and 1 % Floxina solutions in water. Spores, cystidia and other morphological structures were observed and photographed with a Zeiss light microscope. Biometric analyses were carried out with a graduated reticule and using the software Mycomètre v. 2.02 (http://mycolim.free.fr/DOC_SML/mycm202/Charg_Mycm202.htm) by averaging 30 independent measurements. To test for differences in spore size pairwise t-test were performed using R software (https://www.r-project.org/).

DNA isolation and molecular analyses

Genomic DNA was isolated from fresh and dried specimens according to Angelini et al. (2012). DNA quality and quantity were evaluated by agarose gel electrophoresis (0.8 %) in the presence of ethidium bromide and a DNA mass ladder (GeneRuler DNA Ladder Mix, Fermentas, Hannover, MD). The internal transcribed spacer (ITS) region was amplified with the ITS1f and ITS4 primers (White et al. 1990, Gardes & Bruns 1993). The 5' end of the large subunit (LSU) rDNA region was amplified using the LR0R/LR7 primers pair (Moncalvo et al. 2000, Tedersoo et al. 2006). The small subunit (SSU) of rDNA was amplified using the primers NS1-NS4 and NS5-NS8 (White et al. 1990). Polymerase chain reactions (PCR) were performed according to Rubini et al. (2011). The *rpb*2 gene was amplified using the primers pair bRPB25f-bRPB27r and the PCR conditions described by Matheny (2005). The PCR products were analysed by electrophoresis on 2 % agarose gels stained with ethidium bromide in presence of size standards (GeneRuler DNA Ladder Mix, Fermentas, Hannover, MD).

Sequencing and phylogenetic analyses

The PCR amplified products were purified using the JetQuick PCR purification Kit (Genomed, Löh-

ne, Germany) and directly sequenced. ITS fragments from samples MA41, MS44, MS45, AR122 and AR123 were also cloned in *E. coli* using the pGEM-T Easy Vector System (Promega Corp., Madison, Wisconsin, USA) following standard procedures (Sambrook et al. 1989). From each of the two ITS clone libraries obtained, ten colonies were picked randomly, heated for 5 min in 50 µl of sterile water, PCR amplified with ITS1F/ITS4 primer pair and sequenced.

Sequences were carried out with BigDye terminator sequencing kit (Life Technologies, USA) according to the supplier's instructions and by using an ABI 3130 genetic analyzer. All the ITS, SSU, LSU and *rpb2* sequences were checked for similarity with sequences deposited in GenBank using the BLASTn service at NCBI. The sequences showing the highest similarity were retrieved and aligned using Muscle v. 3.7 (Edgar 2004). For combined dataset sequences of rpb2, LSU and SSU genes of *T. goniospermum* sample MS41 were concatenated and realigned with sequences of the tricholomatoid species used by Sánchez-García et al. (2014) and retrieved from TreeBase web site. Sequences obtained in this study were deposited in GenBank under the following accession numbers: KU559844-KU559865.

Phylogenetic trees were inferred with maximum-likelihood (ML) method using RaxML software (Stamatakis 2014) at the CIPRES Science Gateway V. 3.3 web service (https://www.phylo.org/portal2/login). The rapid bootstrapping option of RaxML and the CAT model of rate heterogeneity were used. The combined dataset was analysed by considering three partition of 685 (*rpb2*), 1736 (SSU) and 1508 (LSU) bp, respectively, and using the same substitution model.

The alignments and phylogenetic trees were deposited in TreeBase (https://treebase.org/treebase-web/home.html).

Results

Taxonomy

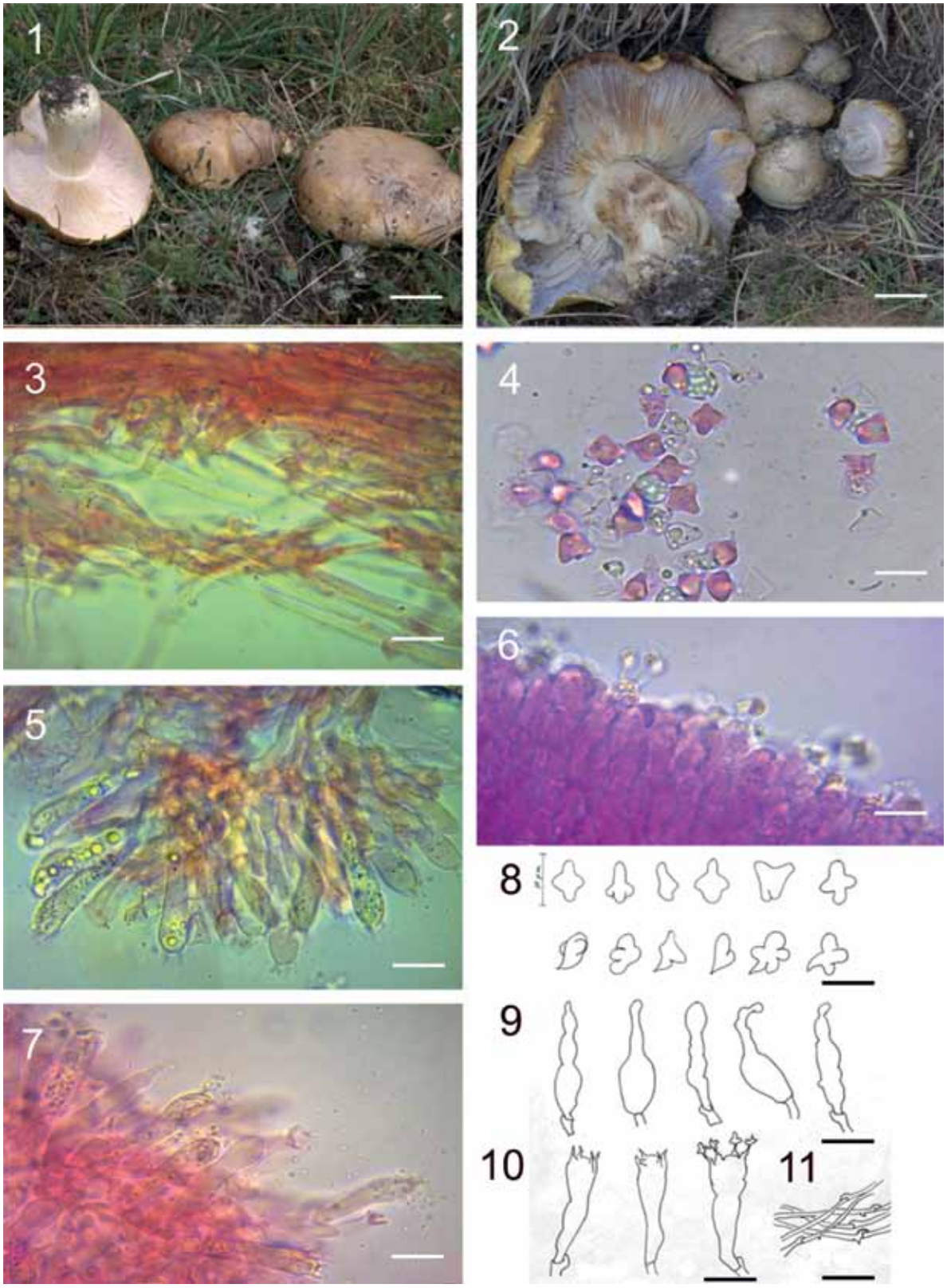
All the samples collected in central Italy (Tab. 1) showed the following macro-morphological characters: habit tricholomatoid. Pileus 4–15 cm wide, hard and compact, white, darkening with cutting and handling, rather fleshy, evolving from almost globate to semi-globate, then flattened, margin involute, colour pale brown, then fading to yellowish brown, bruises reddish brown (Fig. 1). Cuticle not viscid. Lamellae ranging from very crowded to completely tighten in older individuals, emarginate, whitish, greyish or pinkish, finally slightly lilac (Fig. 2). Stipe 5–10 × 1–2 cm, cylindrical, sometimes slightly ventricose, attenuated towards the base, white, sometimes yellow with shades of lilac, becoming darkish with manipulation (Fig. 2). Stipe base tending to retain residue of soil mixed with lilac mycelium. Basidioma smell pleasant of flour, somewhat spermatic. Pileipellis composed of hyphae of 2-6 µm diam. and showing clamp connections (Figs. 3, 11). Spore deposit white. Spores hyaline, elongated cruciform, generally with four, but sometimes with three or five prominences (Figs. 4, 8). Basidia 30–38 × 6.5–9 µm, clavate (Figs. 5, 10), four sterigmata, rarely two (Figs. 6, 10). Cystidia present as cheilocystidia 30–40 × 6.5–10 µm, fusiform, lageniform, clavate or moniliform (Figs. 7, 9), pleurocystidia not observed.

The *T. goniospermum* samples collected in central Italy showed features similar to those described by Contu & Mua (2000) and those of the sample

Tab. 1. List of *Tricholosporum goniospermum* samples used in this study.

Code	Collection sites (Italy)	Date	Voucher (PERU)	ITS haplotype[1]						
				h1	h2	h3	h4	h5	h6	h7
Ms41	Ascoli	2011	A005711API			x	x			
Ms42	M. Subasio, Assisi	2011	A005811PGI			x	x			
Ms43	M. Vettore, Norcia	2011	A005911PGI	x						
Ms44	M. Subasio, Spello	2012	A006012PGI	x	x					
Ms45	M. Vettore, Norcia	2010	A005510PGI				x	x		
AR122	Trieste	2010	A005610PGI						x	x
AR123	Ascoli	2011	A006211PGI	x	x					

[1] the x indicates the presence of the haplotype



Figs. 1–11. Morphological features of *Tricholosporum goniospermum* samples collected in central Italy. **1–2.** Pileus and stipe. Bars 12 mm. **3, 11.** Cuticle hyphae. Bars 16 µm. **4, 8.** Spores. Bars 7 µm. **5, 10**. Basidia. Bars 16 µm. **6, 9.** Cheilocystidia. Bars 16 µm.

AR122 collected in north Italy. However, a careful examination showed that spores of samples from central Italy were slightly smaller than those described in the literature and those of the sample AR122. In particular, the spores of AR122 were (6.29)8.60±0.21(10.6) × (5.16)6.37±0.18(8.59) µm whereas, in samples from central Italy the spore size was in the range of 5.07–8.56 × 4.04–6.55 µm (Tab. 2). The T-test reveals no significant differences among the spore size of samples from central Italy, whereas these resulted significantly different from that of sample AR122.

Tab. 2. Spore size of T. gionospermum. av = average.

Code	Spore size (µm)
MS41	5.29–7.60 × 4.04–6.55 (av 6.53 ± 0.24 × 5.28 ± 0.23)
MS42	5.07–7.66 × 4.08–6.24 (av 6.35 ± 0.22 × 5.26 ± 0.24)
MS43	5.12–7.26 × 4.96–6.23 (av 6.12 ± 0.21 × 5.42 ± 0.16)
MS44	6.21–7.69 × 4.85–6.46 (av 6.82 ± 0.44 × 5.83 ± 0.49)
MS45	5.26–7.34 × 4.38–6.55 (av 6.43 ± 0.24 × 5.28±0.21)
AR122	6.29–10.6 × 4.1–8.59 (av 8.60±0.21 × 6.37±0.18)
AR123	5.29–8.56 × 4.04–6.39 (av 6.64±0.16 × 5.16±0.13)

ITS analysis

In order to test if samples from central Italy and the *T. goniospermum* sample AR122 belong to the same species, analysis of the ITS region was performed. PCR resulted in an amplicon of approximately 600 bp in all samples considered. With the only exception of MS43, direct sequencing with the forward primer ITS1f resulted in additive sequence, starting approximately from the nucleotide residue 220. This suggested the presence of more than one ITS copy, differing for the presence of insertion/deletions. Sequence additivity was confirmed when the ITS4 was used as reverse sequencing primer.

In order to obtain ITS sequences without ambiguities the PCR products from samples MS41, MS42, MS44, MS45, AR122 and AR123 were cloned and ten clones for each sample sequenced. Sequencing revealed in each of these samples the presence of two divergent haplotypes, differing from each other for short insertions and/or A/T nucleotide substitutions, all located in the same region. In sum, seven haplotypes arranged in various combinations were detected (Tab. 1). Some of the haplotypes were shared between samples from central Italy. More specifically, the samples AR123 and MS44 showed the haplotypes h1 and h2 and both shared the haplotype h1 with MS43. The samples MS41 and MS42 showed the haplotypes h3 and h4 and both shared the haplotype h4 with MS45. The haplotype h5 was detected in the sample MS45, whereas the haplotypes h6 and h7 were detected in the sample AR122 from Trieste only (Tab. 1). The percentage of similarity between these haplotypes was about 98 %. Blast analysis resulted in the similarity with sequence KC969668 belonging to the species *T. porphyrophyllum*.

Phylogenetic analysis

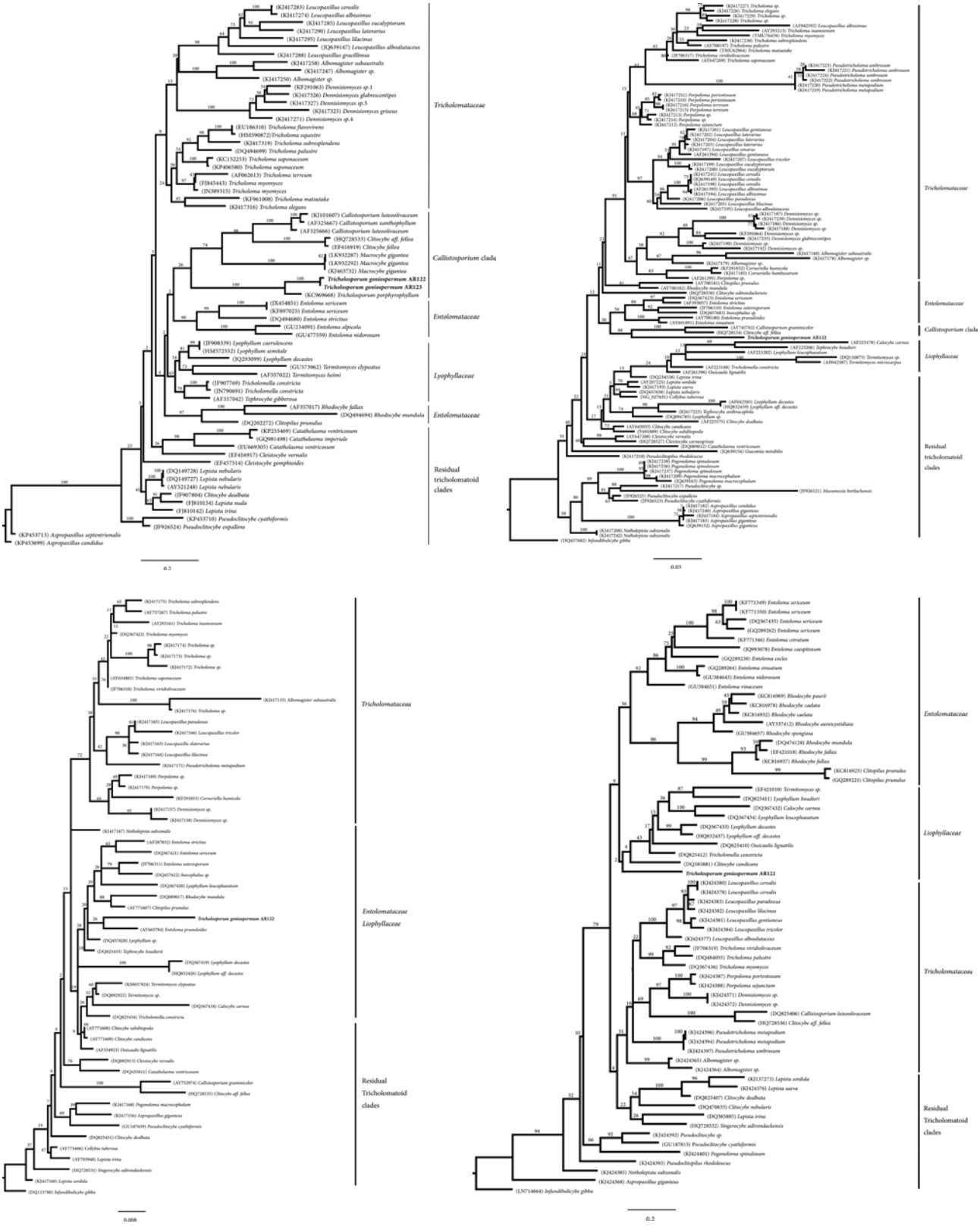
In order to evaluate the position of genus *Tricholosporum* within the tricholomatoid clade, we performed a maximum likelihood phylogenetic analysis using the ITS, LSU, SSU and *rpb*2 sequences. We used these genes to perform both single gene and multiple gene phylogenies. For each marker a set of sequences representative of the species belonging to the *Tricholomatineae* were retrieved from GenBank and aligned. The species in the different clades were selected according to the phylogenetic studies of Moncalvo et al. (2002) and Sánchez-García et al. (2014) and by considering the results of BlastN analysis.

In the ITS tree the *T. goniospermum* samples formed a clade with *T. porpyrophyllum* (KC969668) and this clade appears closely related to that including the species *Callistosporium* spp., *Clitocybe fellea* and *Macrocybe gigantea*. These two clades were related to the clade formed by *Entoloma* species although with a low bootstrap support (Fig. 12). Similarly, in the LSU tree *T. goniospermum* appears related to *Callistosporium* and the *Entolomataceae* (Fig. 13). Conversely in SSU (Fig. 14) and *rpb*2 (Fig. 15) trees *T. goniospermum* showed affinity with *Entolomataceae* and *Lyophyllaceae*. The combined *rpb*2-SSU-LSU tree (Fig. 16) placed, although with low bootstrap support, *Tricholosporum* within a clade including the *Callistosporium* group, the *Entolomataceae* and *Lyophyllaceae*.

Discussion

Genetic diversity of *T. goniospermum*

In the present study careful morphological examination of basidiomata collected from different localities in central Italy revealed some distinctive



Figs. 12–15. Phylogenetic trees based on ITS (**12**) LSU (**13**) SSU (**14**) *rpb2* (**15**) sequences. GenBank accession numbers are given in brackets. Numbers near the branches indicate the bootstrap values.

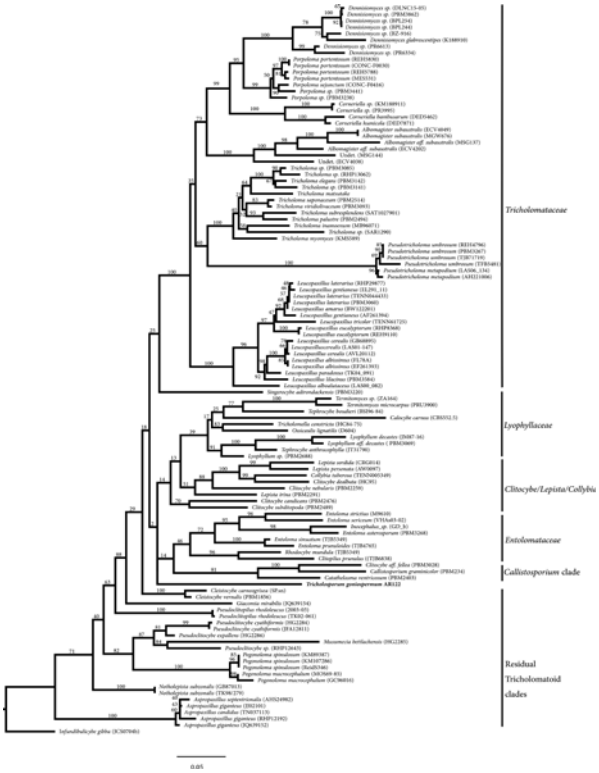


Fig. 16. Phylogenetic trees based on combined *rpb*2-LSU-SSU sequences. Numbers near the branches indicate the bootstrap values. Strains names according to Sánchez-García et al. (2014) in brackets.

characteristics compared to those reported in the literature. In particular, microscopic examination has revealed the presence of cheilocystidia, not always reported for *T. goniospermum* (Bresadola 1927, Contu & Mua 2000). Moreover, the basidiospore size of these specimens appears smaller than that reported for *T. goniospermum* according to various authors without significant differences among these samples. In the examined samples, the spores reached 8.56 µm in length and 6.5 µm in diam., whereas for *T. goniospermum* a spore size of 8–10 × 5–7 µm has been previously reported (Bresadola 1927, Contu & Mua 2000, Angelini et al. 2014). The comparison carried out with the *T. goniospermum* sample (AR123) collected in Trentino has confirmed this difference, highlighting in this sample spores up to 10.6 µm in length and up to 8.59 µm in diam., therefore significantly bigger than those of samples collected in central Italy. Considering the spore size, the samples from central Italy may resemble the species *T. subgoniospermum*, *T. pseudosordidum* and *T. subporphyrophyllum*. However, the samples examined differed from these species in other morphological characters: *T. subgoniospermum* tends to get stained vividly yellow when handled (Bohus et al. 1999, Angelini et al. 2014), *T. pseudosordidum* (Baroni 1982) has a different pileus colour, while *T. subporphyrophyllum* differed for the size of the pileus and the stipe (Baroni 1982).

In order to assess whether the morphological differences observed between samples from north and central Italy were reflected at a genetic level, an analysis of the ITS region of rDNA has been carried out. Sequencing of ITS revealed the presence of polymorphism among the samples of central Italy as well as differences from the sample of north Italy. However, as the ITS sequences share about 98 % of similarity, these differences are likely to be considered at an intraspecific level. Further to this, the analysis of ITS region evidenced polymorphisms at an intra-individual level. In fact, direct sequencing and subsequent analysis using a clone library approach showed in each individual, with the only exception of MS43, the presence of at least two different ITS haplotypes. Overall, seven haplotypes differing for a short region of 10–12 bp were identified. Interestingly, the two ITS haplotypes of the sample collected in northern Italy are different from all those detected in the individuals of central Italy. Conversely, individuals collected from different localities of central Italy share some of these haplotypes, such as the haplotype h1, h3 and h4. The presence of intra-individual heterogeneity of the ITS region was reported in different organisms: plants, insects and even fungi. For example, Hughes & Petersen (2001) reported the presence of intra-individual ITS polymorphism in *Flammulina* as a result of hybridization of two different species (*F. rosea* and *F. velutipes*). Hughes et al. (2013) reported the occurrence of both intra- and inter-specific hybridization in several fungal species such as *Armillaria mellea*, *Amanita citrina*, *Gymnopus dichrous* and *Hygrocybe flavescens*. According to Hughes et al. (2013) these divergences and successive hybridization may have originated from the re-contact of populations that have been isolated in different refugia during the last glacial period. Furthermore, Hughes et al. (2013) speculated that, later, other factors such as the adaptation to different ecological niches may have maintained this population's genetic diversity giving rise to phenomena of hybridization occurring in the contact zones (i.e. the periphery of their distribution areas). To this regard, it has been observed that the concerted evolution that is known to be responsible for the homogenization of the ribosomal genes arrays not always works so efficiently as generally assumed for these genes (Si-

mon & Weiß 2008). In other words, the homogenization process depends on the rate of speciation and the hybrid organism initially may retain copies of both parental ITS sequences (Sang et al. 1995, Cronn et a1. 1996). In the case of *T. goniospermum* samples analysed in this study, it can be hypothesized that, because of the fragmented distribution, the populations of these fungi are characterized by hybridization processes. More specifically, the divergence may have been generated by a barrier such as the areal fragmentation, but the absence of reproductive incompatibility still allows rare episodes of crossing between individuals of neighbouring populations. This is in agreement with the fact that haplotypes are shared between individuals of close areas of central Italy, but not with samples from more distant areas such as northern Italy.

As an alternative hypothesis, paralog ribosomal genes fixed in the same haploid genome may be present. However, because no more than two haplotypes in each individual have been identified, it is likely that the observed ITS heterogeneity resulted from crossing between divergent genotypes. In order to better clarify the origin of the intra-individual ITS polymorphism observed in this species, studies of segregation using single spore mycelial cultures are in progress.

The genetic divergence of populations due to fragmented geographical distribution was also in agreement with the observed morphological differences (i.e. the spore size) between individuals of central and northern Italy.

Inferring the phylogenetic relationship of *Tricholosporum* with tricholomatoid fungi

The genus *Tricholosporum* was established to accommodate those *Tricholoma*-like species with unusual bumped/cruciate basidiospores as well as distinctive purplish, violaceous or lilac-vinaceous colour of some part of the basidioma (Guzmán 1975). These fungi were in fact previously classified in the genus *Tricholoma* (Bresadola 1892) but later, because of the presence of these features, unusual for the *Tricholomataceae*, subgenus, sections and subsections were created. For example, Singer (1945) included *Tricholosporum* in the genus *Tricholoma* but created the subgenus *Contextocutis* within the *Iorigida* section. This view was followed by e.g. Bohus (1985) and Bon (1991) while more recently many authors considered *Tricholosporum* a valid genus (Angelini et al. 2014 and references therein). However, this genus was never included in a molecular phylogenetic study, thus the relationship of *Tricholosporum* with the other tricholomatoid fungi remained to be evaluated.

In the present study the ITS, SSU, LSU and *rpb*2 genes of *T. goniospermum* were sequenced and analysed within the phylogenetic framework recently proposed for tricholomatoid fungi by Sánchez-García et al. (2014). The phylogenetic trees obtained (Figs. 12–16) seem to indicate that the genus *Tricholosporum* does not have great affinity with the *Tricholomataceae* within the *Tricholomatineae*. The trees based on SSU and *rpb*2 genes in fact placed *Tricholosporum* within the *Entolomataceae/Lyophyllaceae*. The LSU and ITS trees placed *Tricholosporum* within a group of morphologically heterogeneous species such as *Macrocybe gigantea*, *Clitocybe fellea, Pleurocollybia brunnescens* and *Callistosporium* spp. According to Moncalvo et al. (2002) these species belong to the "callistosporoid" group and appeared closely related to the *Entolomataceae*. Successive studies, however, produced contrasting results: Matheny et al. (2006) excluded the affinity of *Callistosporium* with the *Entolomataceae,* whereas according to Ammirati et al. (2007) and Sánchez García et al. (2014) it belong to the *Catathelasma* clade detected as sister clade to *Entolomataceae* but with poor bootstrap support.

Finally, the phylogenetic tree based on combined rpb2-SSU-LSU genes showed a relationship of *Tricholosporum* with the clade *Entolomataceae, Lyophyllaceae,* the "*Clytocybe/Lepista/Collybia"* and the "callistosporoid" groups, although this relationship was poorly resolved and had a low bootstrap support.

Overall, the phylogenetic analyses performed placed *Tricholosporum* outside the family *Tricholomataceae*, however the position of this genus within a given group of Tricholomatineae is scarcely supported statistically. In the combined tree, *Tricholosporum* is placed within the clade that include *Entolomataceae* and *Lyophyllaceae* thus it can be speculated that this genus may belong to a new distinctive family phylogenetically related to this clade. It is interesting to note the presence in *Tricholosporum* of some morphological characters such as spores with bumped ornamentations to suggest an affinity with the *Entolomataceae*. However, these similarities must be considered with caution. Co-David et al. (2009) in fact demonstrate that the spore walls forming the ornamentation of *Entolomataceae* are not homologous to those of other tricholomatoid species with bumped spores (e.g. *Tephrocybe tylicolor*, *Lepista nuda*, *L. irina*, *Omphaliaster asterosporus*), and that these bumped ornamentations may have arisen independently in the

Entolomataceae. More specifically, differently from these species, in the *Entolomataceae* the spore ornamentation originates from the modification of the epicorium layer of the spore wall (Co-David et al. 2009). Thus, future study of spore ultrastructure will be important to evaluate the origin of basidiospore ornamentation of *Tricholosporum* and to make comparisons with those of other species in the *Tricholomatinae*.

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References

Ammirati J.F., Parker A.D., Matheny P.B. (2007) *Cleistocybe*, a new genus of Agaricales. *Mycoscience* **48**: 282–289.

Angelini C., Contu M., Vizzini A. (2014) *Tricholosporum caraibicum* (Basidiomycota, *Tricholomataceae*), a new species from the Dominican Republic. *Mycosphere* **5**: 430–439.

Angelini P., Rubini A., Gigante D., Reale L., Pagiotti R., Venanzoni R. (2012) The endophytic fungal communities associated with the leaves and roots of the common reed (*Phragmites australis*) in Lake Trasimeno (Perugia, Italy) in declining and healthy stands. *Fungal Ecology* **5**: 683–693.

Baroni T.J. (1982) T*richolosporum* and notes on *Omphaliaster* and *Clitocybe*. *Mycologia* **74**: 865–871.

Bohus G. (1985) *Tricholoma* sectio Iorigida Sing in Europe and North Africa. *Agarica* **6**: 381–386.

Bohus G., Vasas G., Locsmàndi C. (1999) Two new fungus species from Hungary (*Basidiomycetes, Agaricales*). *Annales Historico Naturales Musei Nationalis Hungarici* **91**: 37–44.

Boisselet P., Moreau P.A. (2008) *Tricholosporum tetragonosporum* (Maire) Contu & Mua récolté en Ardèche. *Bulletin mycologique et botanique Dauphiné-Savoie* **189**: 17–21.

Bon M. (1991) Flore Mycolologique d'Europe 2. Tricholomataceae (Fayod Heim 1 (ère Partie) *Documents Mycologique Memorie Hors Serie* 2.

Bresadola G. (1892) *Fungi Tridentini novi, vel nondum delineati*. Vol. II: 6–7 Trento.

Bresadola G. (1927) *Iconographia Mycologica*. Vol. I–III. Pizzi & Pizio, Milano

Co-David D., Langeveld D., Noordeloos M.E. (2009) Molecular phylogeny and spore evolution of Entolomataceae. *Persoonia* **23**: 147–176.

Contu M., Mua A. (2000) Il Genere *Tricholosporum* Guzmán (Basidiomycotina,Agaricomycetes) in Europa. *Bollettino dell'Associazione Micologica Bresadola* **43**: 249–257.

Cronn R.C., Zhao X., Paterson A.H., Wendell J.F. (1996) Polymorphism and concerted evolution in a tandemly repeated gene family: 5S ribosomal DNA in diploid and allopolyploid cottons. *Journal of Molecular Evolution* **42**: 685–705.

Dentinger B., Gaya E., O'Brien H., Suz L.M., Lachlan R., Díaz-Valderrama J.R., Koch R.A., Aime M.C. (2016) Tales from the crypt: genome mining from fungarium specimens improves resolution of the mushroom tree of life. *Biological Journal of the Linnean Society* **117**: 11–32.

Di Massimo G., Donnini D. (2008) *Tricholosporum goniospermum* (Bres.) Guzmán nell'Appennino Marchigiano. *Micologia Italiana* **37**: 12–17.

Edgar R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Fernandez Vicente J., Iglesias P., Hidalgo F., Oyarzabal M. (2010) Aportaciones al conocimiento micologico de la Isla de La Palma II y una nueva especie de *Tricholosporum*. *Errotari* **7**: 84–131.

Gardes M., Bruns T.D. (1993) ITS primers with specificity for Basidiomycetes: application to the identification of mycorrhizae and rust. *Molecular Ecology* **2**: 113–118.

Guzmán G. (1975) Un nuevo genero y dos nuevas especies de Agaricaceos Mexicanos. *Boletín de la Sociedad Mexicana de Micología* **9**: 61–66.

Guzmán G., Bandala V., Montoya L. (1994) *Tricholosporum* in Mexico and description of a new species. *Mycotaxon* **50**: 355–363.

Guzmán G., Montoya L., Bandala V. (1990) Observations on the genera *Asproinocybe* and *Tricholosporum* and description of a new species of *Tricholosporum* (*Agaricales, Tricholomataceae*). *Mycotaxon* **38**: 485–495.

Halling R., Franco-Molano A.E. (1996) *Agaricales* from Costa Rica: new taxa with ornamented spores. *Mycologia* **88**: 666–670.

Heaton K. (2014) *Phylogenetic placement of the genus Tricholosporum by DNA sequencing. USU student showcase*. Student Showcase (http://digitalcommons.usu.edu/student_showcase/45)

Hughes K.W., Petersen R.H. (2001) Apparent recombination or gene conversion in the ribosomal ITS region of a *Flammulina* (Fungi, Agaricales) hybrid. *Molecular Biology and Evolution* **18**: 94–96.

Hughes K.W., Petersen R.H., Lodge D.J., Bergemann S.E., Baumgartner K., Tulloss R.E., Lickey C.K., Cifuentes J. (2013) Evolutionary consequences of putative intra-and interspecific hybridization in agaric fungi. *Mycologia* **105**: 1577–1594.

Imai S. (1938) Studies on the *Agaricaceae* of Hokkaido. 1. *Journal of the Faculty of Agriculture of the Hokkaido Imperial University* **43**: 69.

Moncalvo J.M., Lutzoni F.M., Rehner S.A., Johnson J., Vilgalys R. (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* **49**: 278–305

Moncalvo J.M., Vilgalys R., Redhead S.A., Johnson J.E., James T.Y., Aime M.C., Hofstetter V., Verduin S.J.W., Larsson E., Baroni T.J., Thorn R.G., Jacobsson S., Clémençon H., Miller O.K. Jr. (2002) One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution* **23**: 357–400.

Moreau P.A., Contu M. (2007) Une espèce remarquable de létage thermoméditerranéen de Corse et de Sardaigne: *Tricholosporum cossonianum* (Maire) comb. nov. *Bulletin Semestriel de la Fédération des Associations Mycologiques Méditerranéennes* **32**: 41–52.

Matheny P.B. (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; *Agaricales*). *Molecular Phylogenetics and Evolution* **35**: 1–20.

Matheny P.B., Curtis J.M., Hofstetter V., Aime M.C., Moncalvo J.M., Ge Z.W., Yang Z.L., Slot J.C., Ammirati J.F., Baroni T.J., Bougher N.L., Hughes K.W., Lodge D.J., Kerrigan

R.W., Sedl M.T., Aanen D.K., DeNitis M., Daniele G.M., Desjardin D.E., Kropp B.R., Norvell L.L., Parker A., Vellinga E.C., Vilgalys R., Hibbett D.S. (2006) Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia* **98**: 982–995.

Onofri S., Bernicchia A., Filipello V., Padovan F., Perini C., Ripa C., Salerni E., Savino E., Venturella G., Vizzini A., Zotti M., Zucconi L. (2005) *Checklist of Italian fungi*. Carlo Delfino, Sassari.

Reid D.A., Eicker A., Clémençon H., Roux C. (1998) South Africa Fungi 7: *Tricholosporum laeteviolaceum* sp. nov., a representative of a genus new to southern Africa. *Mycotaxon* **69**: 409–418.

Rubini A., Belfiori B., Passeri V., Baciarelli Falini L., Arcioni S., Riccioni C., Paolocci F. (2011) The AD-type ectomycorrhizas, one of the most common morphotypes present in truffle fields, result from fungi belonging to the *Trichophaea woolhopeia* species complex. *Mycorrhiza* **21**: 17–25

Sambrook J., Fritsch E.F., Maniatis T. (1989) *Molecular Cloning. A Laboratory Manual*. 2 edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sánchez-García M., Matheny P.B., Palfner G., Lodge D.J. (2014) Deconstructing the *Tricholomataceae* (*Agaricales*) and introduction of the new genera *Albomagister, Corneriella, Pogonoloma* and *Pseudotricholoma*. *Taxon* **63**: 993–1007.

Sang T., Crawford D.J., Stuessy T.F. (1995) Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proceedings of the National Academy of Science USA* **92**: 6813–6817.

Simon U.K., Weiß M. (2008) Intragenomic variation of fungal ribosomal genes is higher than previously thought. *Molecular Biology and Evolution* **25**: 2251–2254.

Singer R. (1945) New and interesting species of Basidiomycetes. *Mycologia* 425–439.

Stamatakis A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* **10**: 1093

Tafini M. (1995) *Il nome dei funghi in Umbria*. Circolo micologico perugino, Perugia.

Tedersoo L., Hansen K., Perry B.A., Kjøller R. (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. *New Phytologist* **170**: 581–596

Watling R. (1997) *Tricholosporum superbum*: a new Malesian agaric. *Bollettino del Gruppo Micologico Bresadola* **40**: 485–490.

White T.J., Bruns T., Lee S., Taylor J. (1990) *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: PCR protocols. A Guide to Methods and Applications. (eds. Gelfand M.A., Sninski D.H., White T.J.), Academic Press, San Diego: 315–322.

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