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Original paper

Rare findings from the chestnut forest of Monte Rocca Romana (Latium, Italy)

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Abstract

We report the identification of both *Hydnum ibericum* and *H. ovoideisporum* growing in late autumn, in the woods of Monte Rocca Romana, the highest peak (612 m asl) of Monti Sabatini, inside the Bracciano-Martignano Regional Natural Park in Latium, Italy. The Park includes large composite zones: the relatively humid climate and the fertile volcanic soils allow the growth of a manifold flora related to the Mediterranean scrub, to the deciduous (*Quercus* spp.) or chestnut (*Castanea sativa*) and depressed beech woods. The fungal findings were observed in an area of the Park characterised by a forest composed in prevalence of chestnut trees and underwood of *Ilex aquifolium* and *Ruscus aculeatus*. In the same habitat and period, two small fungal species were present: blue coloured *Entoloma* sp. and *Ramariopsis pulchella*. The *Entoloma* sp. belongs to the *Cyanula subgenus* but combined morphological and molecular criteria did not correspond to any described *Entoloma* species yet. *Ramariopis pulchella* is a rare and ecologically important species.

Keywords
Hydnum ibericum; Hydnum ovoideisporum; Entoloma; Cyanula; Ramariopsis pulchella; chestnut forest; Natural Parks

Introduction

The genus *Hydnum* L. (Basidiomycetes, Agaricomycetes, Cantharellales, Hydnaceae) was originally composed of six species present in both Europe and North America, on the basis of morphological characteristics: *H. repandum* L., *H. rufescens* Pers., *H. albidum* Peck, *H. umbilicatum* Peck *H. ellipsosporum* Ostrow & Beenken and *H. albomagnum* Banker (Ostrow and Beenken, 2004; Grebenc et al., 2009; Swenie et al., 2018). Various subsequent phylogenetic studies have raised this number currently to 34 global species (Feng et al., 2016; Buyck et al., 2017; Niskanen et al., 2018; Swenie et al., 2018). For example, the two species *H. ovoideisporum* and *H. vesterholtii* Olariaga, Grebenc, Salcedo & M.P. Martín were described as new taxa (Olariaga et al., 2012). Recently, another new species was defined: *H. ibericum* Olariaga, Liimat. & Niskanen, sister species of *H. vesterholtii* from which it deviates by eight substitution and indel positions in the genomic ITS sequences (Niskanen et al., 2018). Nonetheless, *Hydnum* remains one of the most understudied fungal genera (Feng et al., 2016). In fact, only 45 specimens of the genus have been officially recorded for Italy (https://www.gbif.org/species/2513763, consulted on 2021/05/15).

Hydnum ibericum is described as characterised by a small pileus, with cream or light ochre colours and orange tones, the hymenium with concoloured spines and ellipsoidal basidiospores. *Hydnum*

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ibericum is terricolous and ectomycorrhizal, like the other species of the genus (Agerer et al., 1996). It appears during late autumn and winter in coniferous forests and with *Castanea sativa* Mill. in Spain (Niskanen et al., 2018). *Hydnum vesterholtii* is characterised by an ochre to light ochre pileus with white stipe, spines slightly or not decurrent, sharply delimitated and conical not flattened, with pale ochre colour. It harbours ovoid to broadly ellipsoid basidiospores (Olariaga et al., 2012). Initially observed only in southern Europe, the Pyrenees and Andorra (Olariaga et al., 2012), *H. vesterholtii* was reported to be present also in Estonia (Vizzini et al., 2013). *Hydnum ovoideisporum* is described to accommodate brownish and deep orange pileus with conical and non-flattened spines, from orange-white to orange-ochre colours. The spines are non-decurrent, but in some cases small decurrent spines form a circular zone around the stipe. Its basidiospores vary from subglobose to broadly ellipsoid (Olariaga et al., 2012; Vizzini et al., 2013). This species appears in forests under *Pinus sylvestris* L. but also in *Quercus* woods, in Mediterranean areas of Europe and Italy (Olariaga et al., 2012; Vizzini et al., 2013).

We report the finding of *H. ibericum* and *H. ovoideisporum* species present in late autumn and winter, in a forest dominated by *Castania sativa* on the mountain slope of Monte Rocca Romana, the highest peak (612 m asl) of the Monti Sabatini, part of the Bracciano-Martignano Regional Natural Park, in the province of Viterbo, Latium, Italy (Supplementary Fig. 1).

The establishment of the Park in 1999 was aimed at promoting the conservation and enhancement of the territory and the natural and cultural resources of the vast area of the Sabatini Mountains. In fact, despite the past urban expansion, the Park still preserves stretches of riparian vegetation and extensive forest areas. The keeping of these habitats allows the presence of numerous species of flora and fauna, which should be a reason for continuous surveillance.

In the same habitat and season other two small and beautiful fungal species were found and described: blue coloured *Entoloma* sp., which determination through phylogenetic analyses will be discussed and *Ramariopsis pulchella* (Boud.) Corner, in the family of Clavariaceae, considered a rare and endangered species, enough to suggest inclusion in a fungal Red List (Onofri et al., 2005). Just two specimens of *R. pulchella* have been officially recorded for Italy (https://www.gbif.org/species/2540204, consulted on 2021/05/15) on the island of Sardinia, although it has also been reported in Tuscany (Salerni and Perini, 2007). Hence the importance of a continuous survey for rare and endangered macrofungi in the area.

Materials and Methods

Area of study and samples

The Monti Sabatini are a mountainous group originating from a currently quiescent volcanic district, which landscape is characterised by crater lakes and wooded hills, near the city of Rome, but partly extended into the province of Viterbo. The area is included in the Bracciano-Martignano Regional Natural Park and covered with vegetation characteristic of the Mediterranean forest and the Apennine beech woods, displaying the particular morphology of fertile volcanic soil in a relatively humid and rainy climate. The chemical compositions of the Sabatini volcanic products generally fall within the potassium series KS. The volcanic soils examined in the territory have a low content of calcium carbonate and a subacid to neutral reaction (pH = 5.3-7.8) and in general are fertile but fragile pedoenvironments. From the analysis of temperatures and rainfall, it emerges that the Bracciano-Martignano area figures as a transition zone from a more distinctly Mediterranean climate to a temperate climate, typical of the inland areas of the Lazio Apennines (Various authors, 2009).

The highest peak of 612 m asl is Monte Rocca Romana, a geological emergence formed by volcanic scoria cones, prevalently covered by *Quercus cerris* L. and other *Quercus* spp. on the south slope facing the lake. Instead, *C. sativa* grows on the more humid slope facing north confining with cultivations of hazelnut trees (*Corylus avellana* L.). Besides the prevalent chestnut trees, these woods (Supplementary Fig. 2) are composed of *Acer campestre* L., *Ostrya carpinifolia* Scop. and sporadic *Populus* sp., *Ulmus* sp. and *Fagus sylvatica* L. trees; the underwood is formed prevalently by holly (*Ilex aquifolium* L.) and *Ruscus aculeatus* L. Even if part of the pure chestnut woods is the result of human intervention, *C. sativa* is considered a representative species of the autochthonous flora of the district. In this study, the surveyed area stretched about 125×535 m along the escarpment at the side of the road cutting the *C. sativa* woods (Supplementary Fig. 1).

The first *Hydnum* specimen with orange colourings was collected on December 29th 2019, while in October and November of 2020 two morphotypes of *Hydnum* were found, one group with cream-light ochre colours and the other with orange colours (Fig. 1 and Fig. 3). The other two findings, *Entoloma* sp. (subgenus *Cyanula*) and *Ramariopsis pulchella*, were present in late autumn in 2020 in the same habitat on the berm of the road as the *Hydnum* specimens. *Ramariopsis pulchella* grew amidst moss, terricolous but with saprotrophic behaviour.

All specimens are conserved as part of the Mycological Collections of the Natural History Museum and Botanical Garden of the University of Tartu, Estonia (Table 1).

Table 1 - The collected specimen data, their herbarium accession number and nuclear ribosomal DNA sequence accession number. All specimens were collected in the same habitat in the woods of Monte Rocca Romana.

Species	Fungarium codes	UNITE Accession	Collection date
Hydnum ibericum	TUF135543	UDB0780327	2019/12/29
Hydnum ibericum	TUF105918	UDB0799298	2020/11/02
Hydnum ibericum	TUF105948	UDB0799303	2020/11/30
Hydnum ovoideisporum	TUF105919	UDB0799299	2020/11/02
Hydnum ovoideisporum	TUF105947	UDB0799302	2020/11/30
Entoloma sp.	TUF105920	UDB0799300	2020/11/02
Ramariopsis pulchella	TUF105921	UDB0799301	2020/11/02

Molecular analyses

Ribosomal DNA-based analysis was performed on all specimens listed in Table 1 in the frame of the UNITE project (Kõljalg et al., 2013; https://unite.ut.ee/). DNA extraction, PCR amplification of SSU partial, ITS1, 5.8S, ITS2, LSU partial regions and sequencing were performed as in Voitk et al., (2018). BLAST 2.8.1+ (Camacho et al., 2009) was used to assess sequence identity, when possible, and to select sequences for follow-up phylogenetic analyses. The selected sequences were aligned with the MUSCLE v3.8.425 algorithm (Edgar, 2004) in AliView 1.26 software (Larsson, 2014) and inspected manually. For the *Hydnum* sequences, two analyses were performed: one more extensive with 232 sequences (5 of this study, 2 as outgroup, 225 others, similar to Feng et al., 2016; sequences in Supplementary Table 1) and another more restrictive with 50 sequences (5 of this study, 1 as outgroup, 44 others; sequences in Supplementary Table 1). Only the second tree is shown here (the first tree is present in the Supplementary Fig. 3). For the analysis of the *Entoloma* sequence a total of 72 sequences were included (1 of this study, 2 as outgroup, 69 others; sequences in Supplementary Table 2). Maximum Likelihood and Bayesian inference were engaged, applying GTR as the model for nucleotide evolution after MrModeltest 2.3 (Nylander, 2004). In PhyML 3.1 (Guindon et al., 2010), Nearest Neighbour Interchange (NNI) was set up as topology research, initial trees obtained applying BIONJ and Neighbour-Joining to a matrix of pairwise distances. In MrBayes 3.2.6 (Ronquist et al., 2012), the Markov Chain Monte Carlo algorithm was run using four chains (three hot and one cold), setting a temperature of 0.1 and iterations for 2×10^6 generations on two trees with a diagnose frequency every 5,000 generations and a 25% burn-in. The final mean standard deviation of the separated frequencies was below 0.008 for both analyses of *Hydnum* spp. and *Entoloma* sp. multiple sequence alignments. The phylogenetic trees were produced using FigTree v1.4.3. The seven newly obtained sequences were submitted to the UNITE database (accession numbers in Table 1).

Morphological analyses
The photographs of basidiomata were taken in the field, apart from the *Hydnum* specimen collected in December 2019 (Fig. 1). Microscopic images were either obtained with a digital camera AmScope (Irvine CA, USA) MU 500 mounted on an AmScope B490 microscope, operating at ×800 and ×2000 magnifications or on a Nikon Eclipse Ni microscope, at ×2000 magnifications, with software NIS-Elements BR (Nikon Corporation, Tokyo, Japan). The mean length and width of free basidiospores were measured in lateral view.

Results

Upon the observation of *Hydnum* fungi with an adnate hymenium in 2019, which genomic and phylogenetic analyses proved to be *H. ibericum*, a further investigation of the area was performed in 2020. Various fungal species were present in late autumn and winter, in the same habitat together with reappeared *Hydnum* specimens under *C. sativa* woods, among which *Cortinarius*, *Macrolepiota* and *Helvella* species, *Geoglossum umbratile* Sacc., *Astraeus hygrometricus* (Pers.) Morgan, and *Strobilomyces strobilaceus* (Scop.) Berk., various Clavariaceae and others. *Astreus hygrometricus*, and *S. strobilaceus* were proposed to be inserted in the Global Fungal Red List. Two findings in particular were noteworthy and are described here: *Entoloma* sp. (subgenus *Cyanula*) and *Ramariopsis pulchella*.

Hydnum spp.
The first finding of this genus in 2019 grew isolated and terricolous under *C. sativa* trees. Macromorphology of basidiomes, in particular because of the orange pileus and the pink-orange adnate spines (Fig. 1a), indicated the collection of *H. rufescens*. Microscopical analyses showed basidiospores with ellipsoid and subglobose shapes (Fig. 1d), while it is reported that *H. rufescens* possess globose to subglobose basidiospores (Olariaga et al., 2012 and therein). In fact, phylogenetic analysis collocated the sequence in the *H. ibericum* clade (Fig. 2).

A year later (2020), in the same zone, we collected other *Hydnum* specimens, again morphologically very similar to *H. rufescens*. These had caps up to 6 cm wide, at first convex then plane or depressed, with deep orange tones of the pileus (Fig. 3a,b), often umbilicate, rather hygrophanous, with hymenophore composed by long conical hydni (spines) not flattened and well delimited, clearly adnate and not decurrent to the stipe, from whitish to pink-pale orange colours. Stipe was cylindrical, both central and eccentric, with peach orange colours of a lighter tone with respect to that of the cap. Contemporarily and colocalising (in some cases just one meter distanced from each other) with the orange cap specimens, we found other small *Hydnum* specimens with whitish-cream, light-ochre coloured pileus (diameter up to 3 cm) and well-separated conical spines not decurrent to the stipe, with whitish colours (Fig. 1b,c). Cylindrical stipe, sometimes thinned at the base or short, had the same tones of the cap. The genomic barcode of the orange specimens resulted to be *H. ovoideisporum*, instead those of the whitish-cream light ochre sporocarps fitted well with *H. ibericum*, as the basidioma collected in 2019 (Fig. 2).

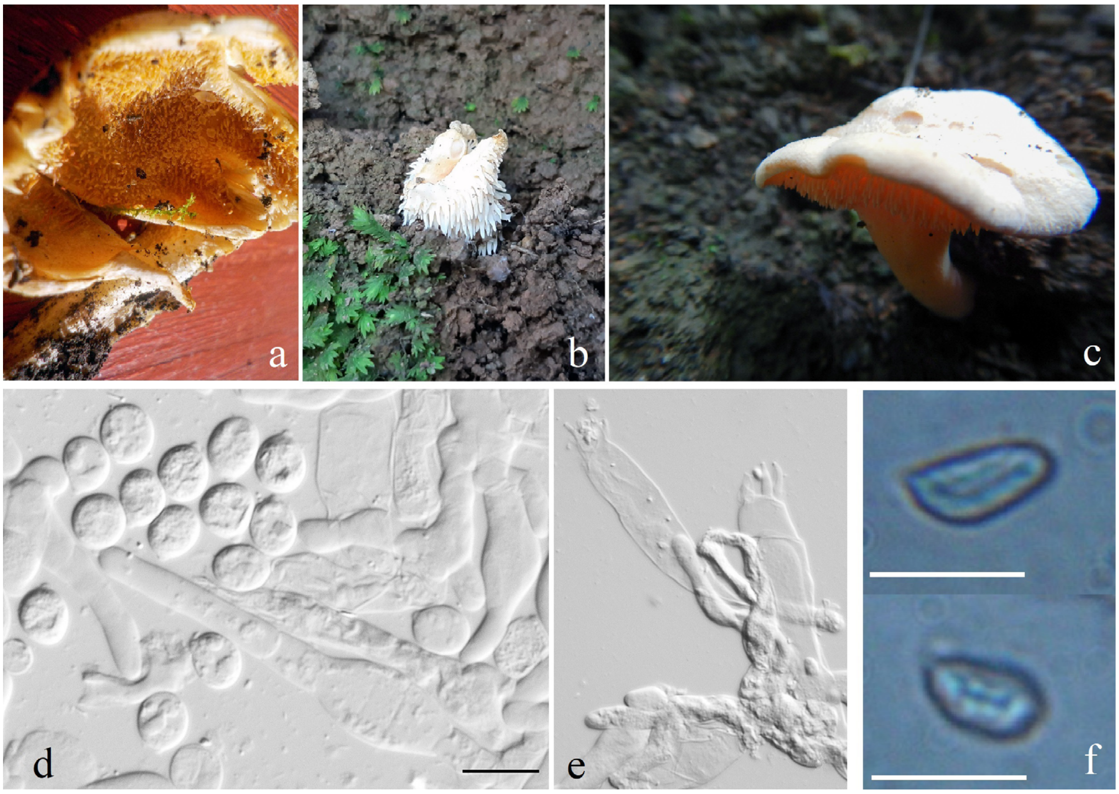

Fig. 1 – *Hydnum ibericum* found in a chestnut forest of Monte Rocca Romana: a) basidioma coloured with orange tones (December 2019, TUF135543); b-c) light-ochre coloured specimens (late autumn 2020, TUF105918 and TUF105948); d) TUF135543 basidiospores; e) TUF135543 basidia.; f) TUF105918 basidiospores. Bar = 10 µm.

Optical microscopy of the basidiospores of all *Hydnum* specimens (orange and light-ochre coloured specimens) showed smooth ellipsoid basidiospores with granular content. Microscopic measurements determined on basidiospores of *H. ibericum* fungi (Fig. 1f) gave means of 7.5 x 4.5 µm (n = 9) and Qm = 1.7; [(9.8)-7.5-(6.2) x (4.9)-4.5-(3.7) µm, Q = (2.0)-1.7-(1.4)]. Clamp connections were visible at the basis of basidia. Microscopic measurements determined on basidiospores of *H. ovoideisporum* (Fig. 3c) revealed basidiospores with means of 7.6 x 5.6 µm (n = 9), Qm = 1.3; [(8.7)-7.6-(7.1) x (6.3)-5.6-(5.0) µm, Q = (1.5)-1.3-(1.2)]. Basidia with two-four sterigmata (Fig. 3d). Clamp connections were evident.

Entoloma sp. subgenus *Cyanula*

The small blue fungi found had a mycenoid appearance (Fig. 4a), small conical, campanulate caps, afterward rather plane (up to about 1 cm). The pilei were translucently striate, showing bright blue lines alternating with light blue-whitish stripes, turning darker when dried. The pilei were squamulose in particular at the centre, with undulating margins. Adnate lamellae and lamellule, whitish with pale blue tones, moderately crowded. The long lithe stipe (about 6 cm) was smooth or slightly tomentose, azulene blue coloured and with white tomentum at the base.

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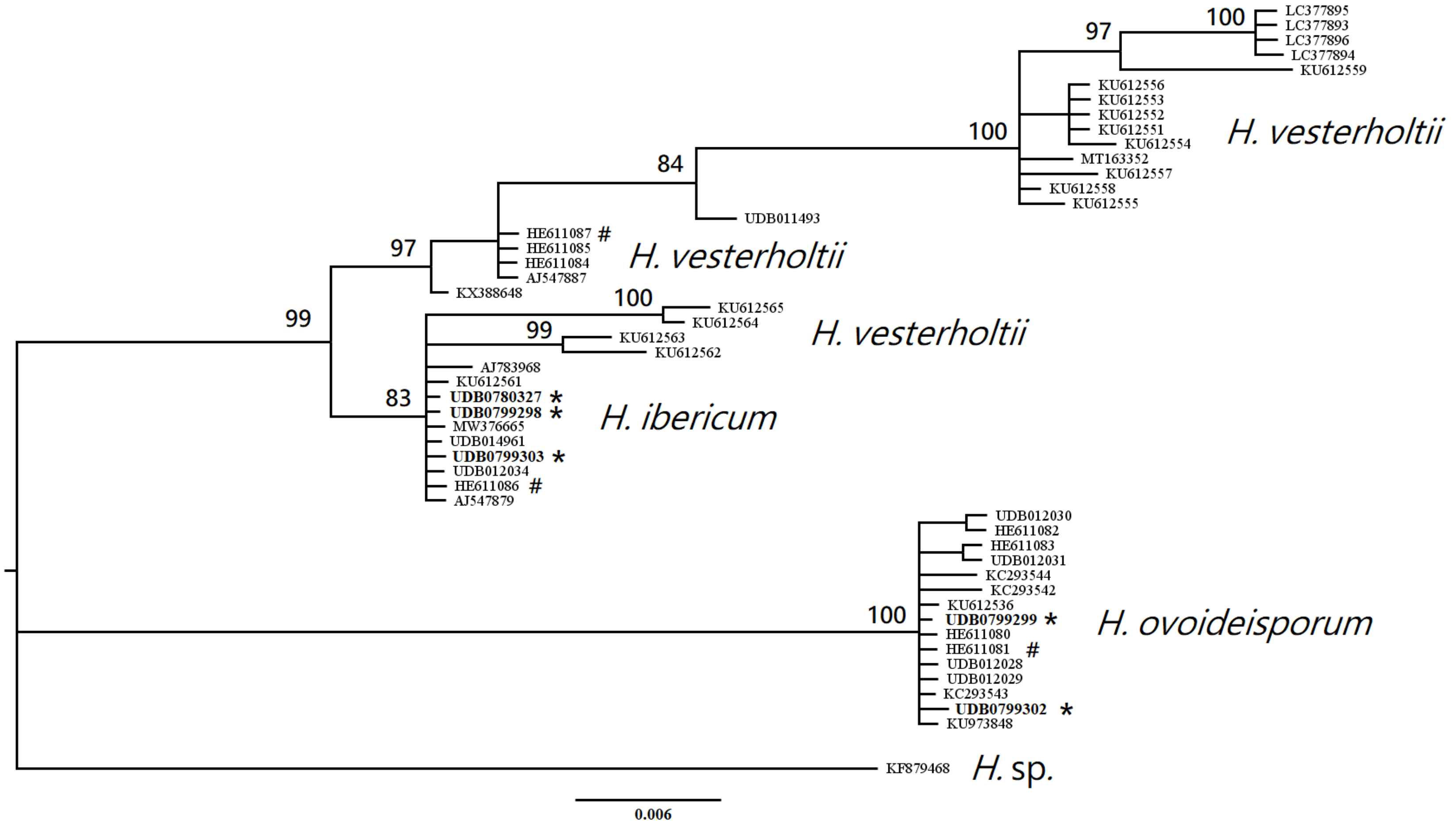

Fig. 2 – Phylogenetic tree upon alignment of the nuclear ribosomal ITS sequences of *Hydnum* species, identified by their UNITE/GenBank accession number and associated species. The sequence KF879468 from *Hydnum* sp. was used as outgroup. The new samples described in the article are indicated by asterisks and sequences from holotypes by hashes. Bayesian posterior probabilities >70% are reported on the main branches. The final mean standard deviation of the separated frequencies was < 0.008.

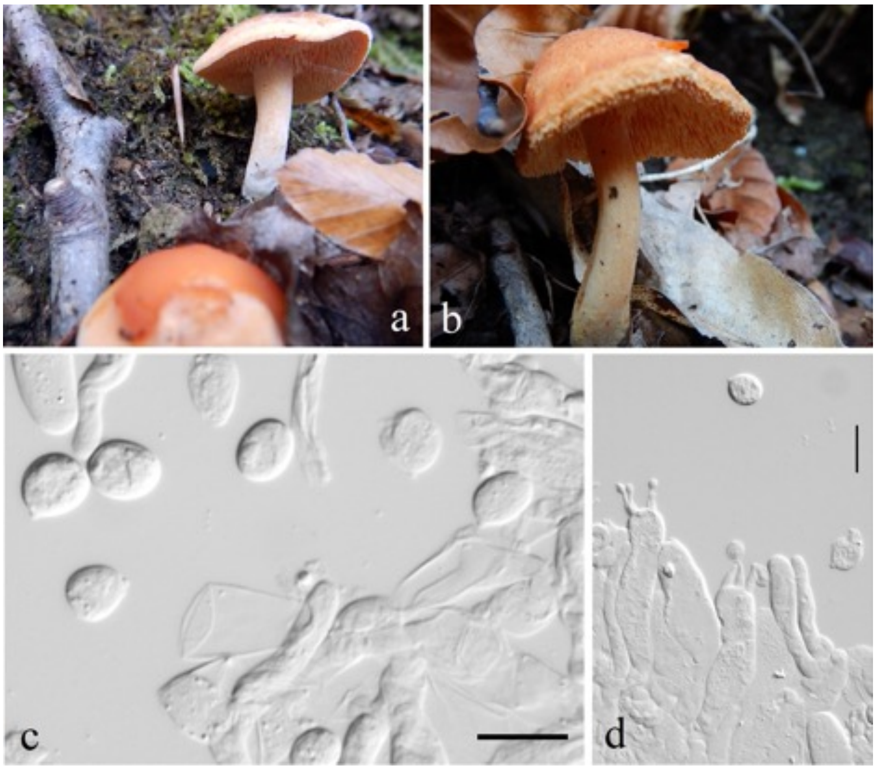

Fig. 3 – a-b) *Hydnum ovoideisporum* basidiomata found in late autumn 2020 on the edge of a road crossing a chestnut forest; c-d) specimen TUF105919: basidia and basidiospores. Bar = 10 µm.

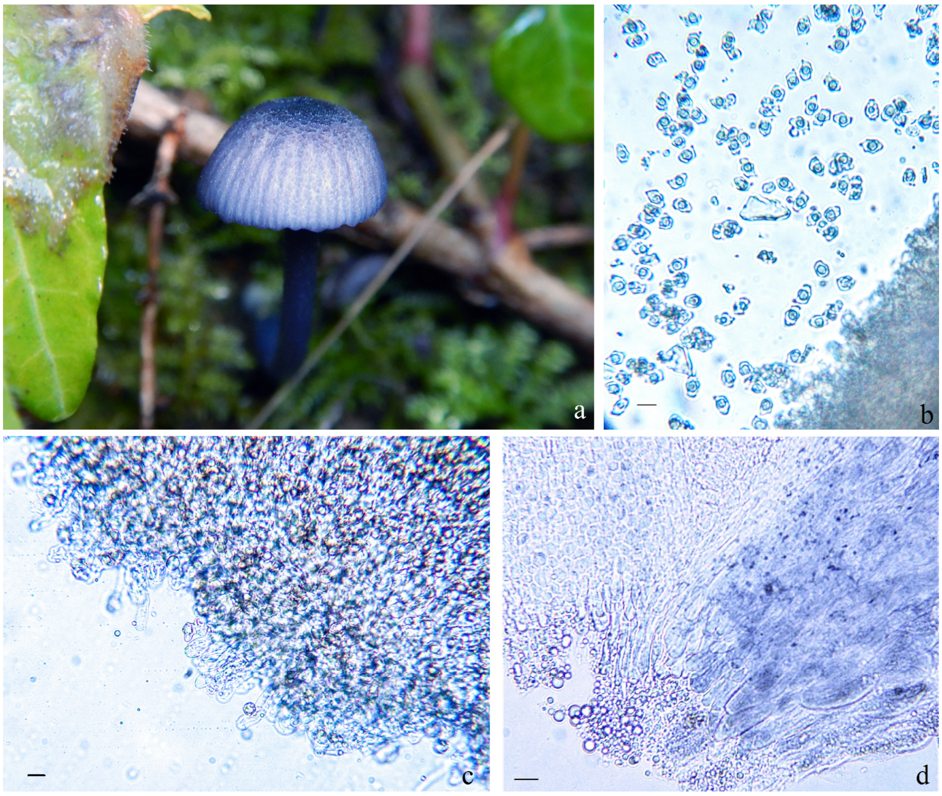

Fig. 4 – A specimen of the blue *Entoloma* sp. showing mycenoid appearance (a). Microscopic observations: (b) polygonal (5-7 angles) basidiospores with coloured lipid droplets; (c) lamella edge; (d) coloured cellular structures of the lamella and pileus edges. Bar = 10 µm.

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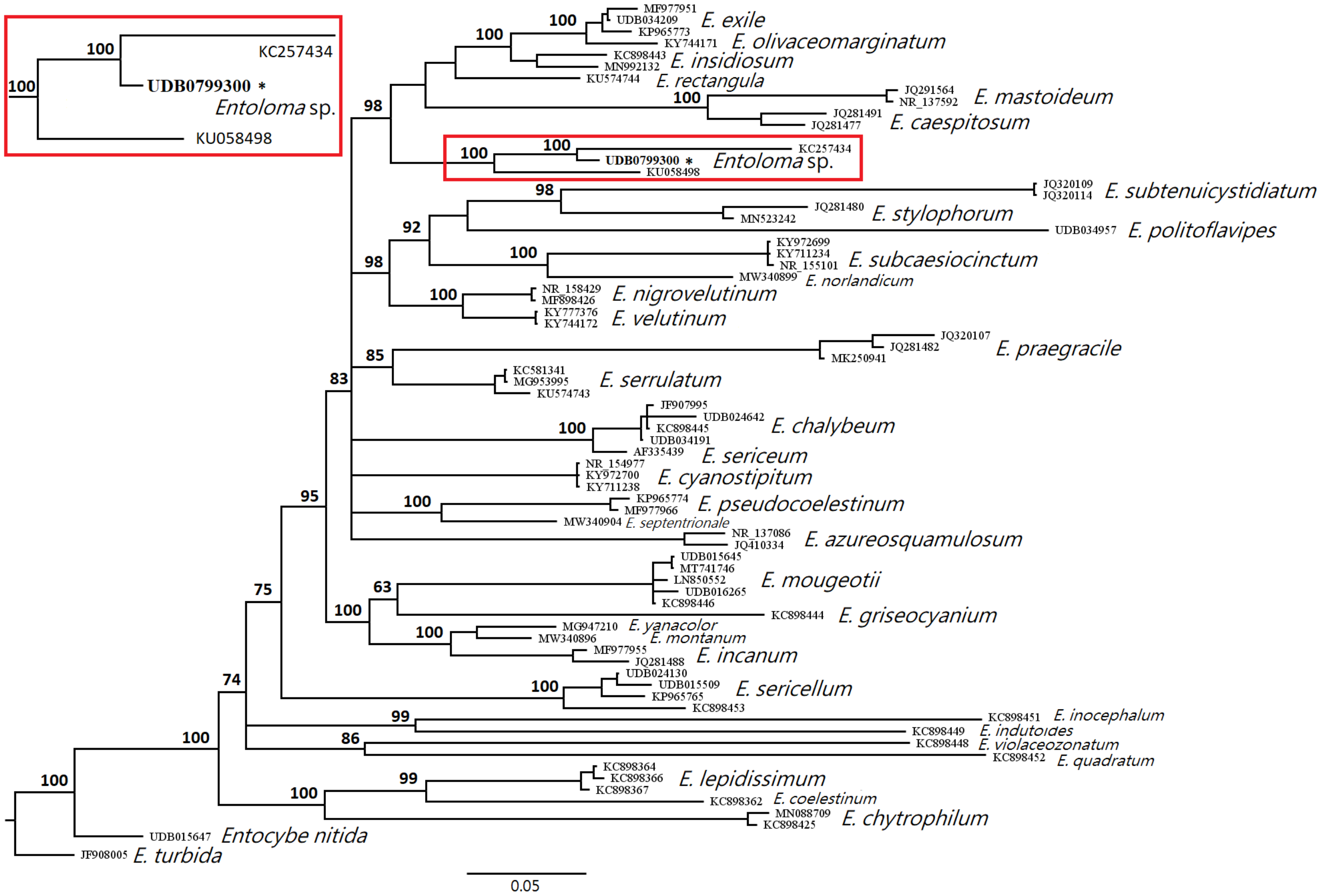

Fig. 5 – Phylogenetic tree with posterior probability values >70% obtained through Bayesian inference based upon alignment of the nuclear ribosomal ITS sequences of *Entoloma* species, identified by their UNITE/GenBank accession number and associated species. The sequences JF908005 from *Entocybe turbida* and UDB015647 from *Entocybe nitida* were used to root the tree. The final mean standard deviation of the separated frequencies was < 0.008. The new sample is indicated by an asterisk. The insert in the red box shows a vertical enlargement of the clade containing the new sample with the two nearest sequences.

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Upon microscopic analyses of the specimens, evident polygonal shaped basidiospores (Fig. 4b) directed their determination towards the genus *Entoloma*, tentatively as the species *E. lepidissimum*. However, phylogenetic analysis (Fig. 5) arranged the ITS sequence within the *Cyanula* subgenus. Microscopic observations of these *Entoloma* sp. specimens showed polygonal (5-7 angles) nodulose basidiospores (Fig. 4b) with apiculum and pigmented lipid droplets inside, average size 10.7 x 7.2 µm [(12.2)-10.7-(8.6) x (8.9)-7.2-(5.8) µm, Qm = 1.5 (n = 10), Q = (1.1)-1.5-(1.9)]. Some intermixed tufts of clavate cheilocystidia were present on lamella edges, pigments were visible inside the cellular structures (Fig. 4c,d). Clamp connections were not detected.

Ramariopsis pulchella

Small specimens of *Ramariopsis pulchella* were identified from their coralloid morphology (Fig. 6), basidiomata about 1 cm in height, smooth, with white feet and violet stems and filiform branches 2-3 times dichotomously separated and pale violet endings. The ends were simple or widened like short teeth. A BLAST search on the ITS sequence yielded a most similar match (96%) to the sequence KX812470 identified as *Ramariopsis pulchella*, collected in 2014 in the Guizhou Province in China.

Fig. 6 – Lilac *Ramariopsis pulchella* basidioma.

Discussion

This is the first record of *H. ibericum* fungi in the area of chestnut woods of Monte Rocca Romana inside the Regional Natural Park of Bracciano-Martignano, in Latium and the first record for Italy. The peculiar habitat formed by the particular volcanic soil and vegetation, together with the fact that the areas inside the Park are rather preserved, allow the presence of uncommon or in some case rare fungi (Ferretti et al., 2020). *Hydnum ibericum* grew scattered in the same environment in proximity, in some cases about one meter apart, of other *Hydnum* specimens identified as *H. ovoideisporum*. The sharing of the same habitat, which is relatively constant for several square meters of the area, suggests that the two species possess similar ecology, such as common ectomycorrhizal partner trees and soil requirement. Until a precise determination was obtained from DNA analysis and phylogeny of the different specimens, it could be thought that they belonged to the same species with differences in morphology due to variability.

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The *H. ibericum* samples show variability in colours of the pileus, varying from an orange tone to light ochre or whitish colours perhaps because the latter were young specimens. Also, the spines showed variable colouration from whitish to pink-orange, such as in the first specimen we identified. On the other hand, *H. ovoideisporum* was clearly characterised by an orange pileus as reported in the original description by Olariaga et al. (2012).

It is reported that the shape of the basidiospores is the most objective and constant character to distinguish the species (Ostrow and Beenken, 2004; Olariaga et al., 2012). However, both *H. ibericum* and *H. ovoideisporum* are described to possess ovoid to broadly ellipsoid basidiospores (Olariaga et al., 2012; Vizzini et al., 2013; Niskanen et al., 2018). In fact, the morphological characteristics that are constant in all the *H. ibericum* and *H. ovoideisporum* found in Monte Rocca Romana, were the hymenophore adnate to the stipe and the ellipsoid shape of the basidiospores, particularly evident in *H. ibericum* species. Probably the presence of these two species is underestimated in part because of the macromorphological similarity with other *Hydnums* (such as *H. rufescens*, *H. vesterholtii*) and also for the general lack of application of molecular barcoding to this genus.

In the context of the presence of uncommon or rare fungi in the Park areas, the discovery of an undetermined species of blue coloured *Entoloma* determined afterward in the clade *Cyanula* and of the *Ramariopsis pulchella* draws interest.

Genus *Entoloma* (Fr.) Kumm. contains more than 1,500 species, distributed in the most heterogeneous habitats with respect to longitude, latitude and altitude. The taxonomy of *Entoloma* is extremely difficult and still much in-progress with new species defined every year (He et al., 2020; Noordeloos et al., 2021). Molecular phylogeny is essential for the taxonomy of this genus because genetically different species do not always have significantly distinct morphological traits, on the contrary differences in their morphology as for instance in colour can occur in the same species. The high genetic variability of *Entoloma* (Vila et al., 2013) could lead to think that this genus has a favourable adaptive ability to produce modifications to its DNA, for example through the crossing of genetic sets coming from different but phylogenetically close *Entoloma* species.

The blue colourations, the striate cap and the size of the spores could lead to the hypothesis that the *Entoloma* specimens belong to *E. pseudocoelestinum* Arnolds (Arnolds, 1982) or to *E. lepidissimum* (Svrček) Noordel. in incertae sedis (Morozova and Vila, 2014). Both species are rare (Noordeloos, 1982; Clericuzio and Vizzini 2011). Phylogenetic analyses led to exclude the last identification and only collocates the *Entoloma* in the *Cyanula* subgenus. Noordeloos describes the Clade/group *Cyanula* as including bluish species, all clampless, in the subgenus *Leptonia* but shown to be phylogenetically rather distant from the *Leptonia* with clamp connections (Noordeloos et al., 2021). The two closest species *E. coelestinum* (Fr.) Hesler voucher HMLD1659 giving rise to the KC257434 sequence and the *Entocybe nitida* (Quél.) T.J. Baroni, Largent & V. Hofst. voucher TENN:067505 of the KU058498 sequence belong to groups that are genetically far from the position of the new sequence of *Entoloma* sp. found on Monte Rocca Romana, in accordance with the fact that *E. coelestinum* is described to have smaller spores and absent cheilocystidia (Morozova and Vila 2014) and the macroscopic and microscopic characteristics described for *Entocybe* species (Baroni et al., 2011) differ from that of our specimens. Anyhow, even these closest two relatives had a genetic distance of 69 (Accession KC257434) and 61 (Accession KU058498) nucleotide and indel variations. Unfortunately, neither specimen has been described morphologically. The *E. gomerense* Wölfel & Noordel. (Lorås et al., 2014) has a quite similar morphological appearance and is part of the *Cyanula* clade, therefore it would have been interesting to include its sequence in the DNA analysis however no sequence was available in the public databases. Therefore, we were not able to attribute the specific name to the *Entoloma* specimens from the Rocca Romana Forest.

Ramariopsis pulchella (Clavariaceae) is a globally distributed small coralloid species that can easily go unnoticed but are considered rare. Originally named *Clavaria pulchella* by Boudier in 1887, the species was transferred to *Ramariopsis* by Corner in 1950. Our observation of this fungus might have an important significance from an ecological point of view. In Sweden *R. pulchella* is included among the 130 fungal species, which have been designated as indicators or "signal species" of forests stand worthy of conservation (Senn-Irlet, 2005). The fungus is reported to be rare by some European studies (Arnolds et al., 1995; Shiryaev, 2009; Jindrich, 2012). In Italy a few specimens were described in some Reserves or Protected Areas of Tuscany (Ricci and Perini, 2001; Salerni and Perini, 2007). Because of its rarity in some European countries, it was also included in the list of species with threat categories, as in the Red List of Macromycetes of the Czech Republic in the CR category - critically endangered species. It is suggested that the reason for its rarity is mainly the loss of suitable habitats, i.e. native areas with bare clay among herbs, as well as underwood between moss or leaves (Volobuev and Bolshakov, 2017). Also in Switzerland the species is inserted in the 'Lista Rossa delle specie minacciate in Svizzera: macromiceti' (Senn-Irlet et al., 2007) and 'Lista delle specie prioritarie a livello nazionale' (UFAM, 2011).

The territory included in the Bracciano-Martignano Regional Natural Park is recognised to be characterised by a wealth of biodiversity with regards to flora and fauna, but also subjected to significant threats. For example, one species of aquatic quillwort was discovered and found only in the Bracciano Lake in 2013, *Isoetes Sabatina* Troìa & Azzella and is now critically endangered (Magrini et al., 2020). Furthermore, in 2018 the presence of the rare *Rosalia alpina* L. beetle was noted in the beech forest of Monte Raschio (Cerulli, 2019).

The findings reported are indicative of the multiplicity of fungal species and the relevant biodiversity of the habitats in the various volcanic areas included in the Bracciano-Martignano Regional Natural Park, and confirm the importance of environmental preservation, also to allow the continuous blooming of interesting fungi.

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References

Agerer R, Kraigher H, Javornik B (1996) Identification of ectomycorrhizae of *Hydnum rufescens* on Norway spruce and the variability if the ITS region of *H. rufescens* and *H. repandum* (*Basidiomycetes*). Nova Hedwigia 63:183-194.

Arnolds E (1982) Ecology and coenology of macrofungi in grasslands and moist heathlands in Drenthe, the Netherlands. Part I. Introduction and Synecology. Bibliotheca Mycologica 90:341-343.

Arnolds E, Kuyper ThW, Noordeloos EM (eds) (1995) Overzicht van de paddestoelen in Nederland. Nederlandse Mycologische Vereniging, Wijster.

Baroni TJ, Hofstetter V, Largent DL, Vilgalys R (2011) *Entocybe* is proposed as a new genus in the Entolomataceae (Agaricomycetes, Basidiomycota) based on morphological and molecular evidence. North America Fungi 6:1-19. https://doi.org/10.2509/naf2011.006.012

Boudier É (1887) *Clavaria pulchella* Boud. Bulletin de la Société Mycologique de France 3(2):146.

Buyck B, Duhen B, Das K, Jayawardena RS, Niveiro N, Pereira OL, Prasher IB, Adhikari S, Alberto' EO, Bulgakow TS, Castaneda-Ruiz RF, Hembrom ME, Hyde KD, Lewis DP, Michlig A, Nuithinck L, Parihar A, Popoff OF, Ramirez NA, Silva MD, Verma RK, Hofstetter V (2017) Fungi biodiversity profiles 21-30. Cryptogamie Mycologie 38: 101-146. https://doi.org/10.7872/crym/v38.iss1.2017.101

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421-429. https://doi.org/10.1186/1471-2105-10-421

Cerulli A (2019) Aspetti storico amministrativi, ambientali e gestionali forestali. La Faggeta di Monte Raschio, 1-18. Parco naturale regionale di Bracciano-Martignano.

Clericuzio M, Vizzini A (2011) Towards a better knowledge of the Tuscan mycological flora. Rare or interesting Basidiomycota from the Province of Grosseto. I. Micologia e Vegetazione Mediterranea 26:37-60.

Corner EJH (1950) A Monograph of *Clavaria* and Allied Genera. Annals of Botany Memoirs 1:645.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792-1797. https://doi.org/10.1093/nar/gkh340

Feng B, Wang XH, Ratkowsky D, Gates G, See Lee S, Grebenc T, Yang ZL (2016) Multilocus phylogenetic analyses reveal unexpected abundant diversity and significant disjunct distribution pattern of the Hedgehog Mushrooms (*Hydnum* L.). Scientific Reports 6:25586. https://doi.org/10.1038/srep25586

Ferretti A, Saar I, Knijn A (2020) On two endangered species (*Myriostoma coliforme* and *Battarrea* sp.) sharing the same microhabitat. Italian Journal of Mycology 49:1-7. https://doi.org/10.6092/issn.2531-7342/10039

Grebenc T, Martín MP, Kraigher H (2009) Ribosomal ITS diversity among the European species of the genus *Hydnum* (Hydnaceae). Anales del Jardín Botánico de Madrid 66(S1):121-132. https://doi.org/10.3989/ajbm.2221

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59:307-321. https://doi.org/10.1093/sysbio/syq010

He XL, Horak E, Wang D, Li TH, Peng WH, Gan BC (2019) Descriptions of five new species in *Entoloma* subgenus *Claudopus* from China, with molecular phylogeny of *Entoloma* s.l. MycoKeys 61:1-26. https://doi.org/10.3897/mycokeys.61.46446

Jindrich O (2012) *Ramariopsis pulchella* – a rare species of Czech nature. Mycologicky Sbornik 89:8-10.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiß M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of Fungi. Molecular Ecology 22:5271-5277. https://doi.org/10.1111/mec.12481

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30:3276-3278. https://doi.org/10.1093/bioinformatics/btu531

Lorås J, Weholt Ø, Eidissen SE (2014) *Entoloma gomerense* Wölfel & Noordel. – a new species to Northern Europe. Agarica 35:19-23.

Magrini S, Azzella MM, Bolpagni R, Zucconi L (2020) In vitro propagation of *Isoëtes sabatina* (Isoetaceae): A key conservation challenge for a critically endangered Quillwort. Plants 9: 887-902. https://doi.org/10.3390/plants9070887

Morozova O, Vila J (2014) *Entoloma* subgenus *Leptonia* in boreal-temperate Eurasia: Towards a phylogenetic species concept. Persoonia 32:141-169. https://doi.org/10.3767/003158514x681774

Niskanen T, Liimatainen K, Nuytinck J, Kirk P, Olariaga Ibarguruen I, Garibay-Orijel R, Novell L, Huhtinen S, Kytovuori I, Ruotsalainen J, Niemela T, Ammirati JF, Tedersoo L (2018) Identifying and naming the currently known diversity of the genus *Hydnum* with an emphasis on European and North American taxa. Mycologia 110:890-918. https://doi.org/10.1080/00275514.2018.1477004

Noordeloos ME (1982) *Entoloma* subgenus *Leptonia* in Northwestern Europa—I. Introduction and a revision of its section *Leptonia*. Persoonia 11:451-471.

Noordeloos ME, Lorås J, Eidissen SE, Brandrud Tor E, Bendiksen E, Morozova O, Jordal J B, Weholt Ø, Jansen GM, Larsson E, Dima B (2021) Three new *Entoloma* species of the *Cyanula* clade from (sub)alpine habitats in Northern Norway and Sweden. Sidowia 73:185-196. https://doi.org/10.12905/0380.sydowia73-2020-0185

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. https://github.com/nylander/MrModeltest2

Olariaga I, Grebenc T, Salcedo I, Martín MP (2012) Two new species of *Hydnum* with ovoid basidiospores: *H. ovodeisporum* and *H. vesterholtii*. Mycologia 104:1443-1455. https://doi.org/10.3852/11-378

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

Ostrow H, Beenken L (2004) *Hydnum ellipsosporum* spec. nov. (Basidiomycetes, Cantharellales) – ein Doppelganger von *Hydnum rufescens* Fr. Zeitschrift für Mykologie 70:137-156.

Ricci G, Perini C (2001) Ritrovamenti interessanti: *Ramariopsis pulchella* (Boud.) Corner. Rivista di Micologia 2:135–142.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539-542. https://doi.org/10.1093/sysbio/sys029

Salerni E, Perini C (2007) Mycodiversity of nature reserves in Central Italy. Acta Mycologica 42:5-19.

Senn-Irlet B (2005) The role of the ECCF in studies and conservation of fungi in Europe. Mycologia Balcanica 2:185-192. https://doi.org/10.5281/zenodo.2547027

Senn-Irlet B, Bieri G, Egli S (2007) Lista Rossa delle specie minacciate in Svizzera: macromiceti. Serie Pratica ambientale n. 0718. Editore Ufficio federale dell’ambiente, Berna e WSL, Birmensdorf.

Shiryaev A (2009) Diversity and distribution of clavarioid fungi in Estonia. Folia Cryptogamica Estonica 45:65–80. https://ojs.utlib.ee/index.php/FCE/article/view/13674

Swenie RA, Baroni TJ, Matheny PB (2018) Six new species and reports of *Hydnum* (Cantharellales) from eastern North America. MycoKeys 42:35-72. https://doi.org/10.3897/mycokeys.42.27369

Various authors (2009) Proposta di Piano di Gestione e delle Misure di Conservazione per la Gestione della ZPS IT6030085 “Bracciano-Martignano” e SIC annessi (Parco Nazionale Regionale ‘Bracciano-Martignano’ and Associazione HydranGea, eds).

UFAM (2011) Lista delle specie prioritarie a livello nazionale. Specie prioritarie per la conservazione e la promozione a livello nazionale, stato 2010. Ufficio federale dell’ambiente, Berna. Pratica ambientale n. 1103.

Vila J, Carbó J, Caballero F, Català S, Llimona X, Noordeloos M (2013) A first approach to the study of the genus Entoloma subgenus Nolanea s.l. using molecular and morphological data. Fungi non Delineati 66 (Studies on Entoloma): 3-62.

https://doi.org/10.6092/issn.2531-7342/12562

Vizzini A, Picillo B, Ercole E, Voyron S, Contu M (2013) Detecting the variability of *Hydnum ovoideisporum* (Agaricomycetes, Cantharellales) on the basis of Italian collections, and *H. magnorufescens* sp. nov. Mycosphere 4:32-44. https://doi.org/10.5943/mycosphere/4/1/2

Voitk A, Saar I, Trudell S, Spirin V, Beug M, Kõljalg U (2018) *Polyozellus multiplex* (Thelephorales) is a species complex containing four new species. Mycologia 109:975-992. https://doi.org/10.1080/00275514.2017.1416246

Volobuev S, Bolshakov S (2017) Host preferences and distributional patterns of rare and protected species of aphyllophoroid fungi in the Middle Russian Upland. In: Proceedings of the conference Fungal conservation in a changing Europe (International Society for Fungal Conservation Ss. Cyril and Methodius University, Skopje, Macedonian Mycological Society, ed), 1-6 October 2017, Ohrid, Republic of Macedonia, p 61.