Cortinarius ochrolamellatus **(Agaricales, Basidiomycota): a new species in *C*. sect. *Laeti*, with comments on the origin of its European-Hyrcanian distribution**

ISAAC GARRIDO-BENAVENT[1], JOSEP BALLARÀ[2], KARE LIIMATAINEN[3], BÁLINT DIMA[4], TOR ERIK BRANDRUD[5] & RAFAEL MAHIQUES[6]

[1] *Department of Biogeochemistry and Microbial Ecology, National Museum of Natural Sciences, CSIC, E-28006, Madrid, Spain. E-mail: igbenavent@mncn.csic.es https://orcid.org/0000-0002-5230-225X*

[2] *C/ Tossalet de les Forques, 44, E-08600, Berga, Catalonia, Spain. E-mail: jbcortinarius@telefonica.net https://orcid.org/0000-0003-0276-0815*

[3] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3AB, United Kingdom. E-mail: k.liimatainen@kew.org https://orcid.org/0000-0002-5422-2384*

[4] *Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/c, Budapest 1117, Hungary. E-mail: cortinarius1@gmail.com http://orcid.org/0000-0003-2099-3903*

[5] *Norwegian Institute for Nature Research (NINA), Gaustadalléen 21, 0349 Oslo, Norway. E-mail: tor.brandrud@nina.no https://orcid.org/0000-0001-9005-9679*

[6] *C/ Dr. Climent, 26, E-46837, Quatretonda, València, Spain. E-mail: rmahiquessan@gmail.com https://orcid.org/0000-0003-0107-0468*

Correspondence to: igbenavent@mncn.csic.es

Abstract

Geographic distributions of mushroom-forming fungi usually remain elusive because of the scarcity of occurrence data. However, the increasing number of environmental and ectomycorrhizal (EcM) root tip DNA sequences deposited in public repositories provides a unique opportunity to expand our knowledge about fungal geographic distributions, even at the intercontinental scale. Here, we present *Cortinarius ochrolamellatus*, a new species of an EcM fungus likely associated to a number of oak

species (*Quercus spp.*) in the Iberian Peninsula, Sardinia, Central Italy, France, and Norway. The morphological and phylogenetic study of the collections allowed us to include it within *C.* section *Laeti.* Furthermore, the combination of specimen-based and EcM root tip ITS data in the phylogenetic analysis revealed the presence of this species in the Hyrcanian *Quercus* forests of northern Iran. Hence, the present study adds further evidence for a biogeographic link between Europe and these Asian mountainous forests concerning fungi. The inferred Pleistocene temporal framework for the origin and diversification events in *C. ochrolamellatus* suggests that the distribution of this EcM fungus was likely driven by the distribution of its *Quercus* tree hosts during the complex Eurasian Quaternary history.

Introduction

Cortinarius (Pers.) Gray (1821: 627) is a genus of basidiomycetous fungi with nearly 3000 species distributed in boreal (subarctic) and temperate and subtropical forests and certain shrublands of both hemispheres (Niskanen *et al*. 2018) that form ectomycorrhizae with a range of woody and herbaceous plants (e.g. Bjorbækmo *et al*. 2010, Geml *et al*. 2011, Nouhra *et al*. 2013). Since the early 2000s, combined morphological and phylogenetic research in *Cortinarius* has been bolstered by the use of the ITS genomic fragment as a DNA barcode (Garnica *et al*. 2016). Detailed studies have been carried out mainly in North America and Europe (Frøslev *et al*. 2007, Harrower *et al*. 2011, Niskanen *et al*. 2011, 2013, Liimatainen *et al*. 2014, Dima *et al*. 2016, Brandrud *et al*. 2018, Mahiques *et al*. 2018), whereas various regions in the Southern Hemisphere have been surveyed in depth more recently (e.g. Salomón *et al*. 2018, Soop *et al*. 2019). These works and other works have uncovered nearly fifty new species, and dealt with many long-lasting nomenclatural issues (Liimatainen *et al*. 2014, Dima *et al*. 2016). Yet, a large gap of knowledge about *Cortinarius* diversity still exists for many areas around the globe, especially Africa and Asia.

The past decade has witnessed tremendous advances in the study of fungal diversity and distribution thanks to the increasing amounts of environmental and,

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

especially, ectomycorrhizal (EcM) root tip DNA sequence data (e.g. Bahram *et al.* 2012, Reis *et al.* 2018). Similarly, the widespread use of high-throughput sequencing (HTS) technologies has made it possible to survey the mycobiome of little explored or even unexplored areas and vegetation types (e.g. Buée *et al.* 2009, Hui *et al.* 2017, Rojas-Jiménez *et al.* 2017, Xu *et al.* 2017), contributing to the global assessment of diversity and the distribution of fungi as well as drawing inferences about their potential ecological roles (Tedersoo *et al.* 2014, Peay *et al.* 2016). Regarding *Cortinarius*, studies using HTS have demonstrated that it is a widespread and abundant component of below-ground fungal communities (Bahram *et al.* 2012, Teasdale *et al.* 2013, Reis *et al.* 2018, Truong *et al.* 2019). Although the use of environmental and EcM root tip sequences, or other sequence data obtained from voucher-less fungi, is, for taxonomic purposes, still hotly debated (Hawksworth *et al.* 2016, Zamora *et al.* 2018), using these data in combination with traditional specimen-based taxonomic analyses (Truong *et al.* 2017) may open potential avenues for exploring the geographic distribution of new fungal lineages. In fact, several recent works in *Cortinarius* have combined these data sources and revealed a biogeographic pattern of shared species between Europe and the Hyrcanian forests in northern Iran (Dima *et al.* 2014, Brandrud *et al.* 2017, Liimatainen *et al.* 2017).

Here, we report an integrative taxonomic study of several *Cortinarius* samples collected in the Mediterranean Region (Iberian Peninsula, Sardinia and Central Italy), Scandinavia (Norway) as well as publicly available EcM root tip sequences to describe a new species, and to add empirical evidence for a biogeographic connection between the Mediterranean, Northern Europe and Hyrcania. Motivated by our expectation of a strong link between the biogeographic histories of EcM fungi and their plant hosts (e.g. Murat *et al.* 2004, Põlme *et al.* 2012, Looney *et al.* 2020), we also estimated divergence times to gain further insight into the evolution in space and time of the target species.

Material and methods

Sample collection, morphological studies and isolation

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.
Cortinarius ochrolamellatus (Agaricales, Basidiomycota): a new species in C. sect. Laeti, with comments on the origin of its European-Hyrcanian distribution. *Phytotaxa* 2020 ;Volum 460.(3) s. 185-200

A total of 10 collections were examined in this study. Macroscopic descriptions were made from fresh basidiomata and microscopic observations from dried material rehydrated in 1% NH$_4$OH. Handmade sections of pilei and cross sections of lamellae were prepared in water, and 3% KOH or Congo Red mixed with 1% NH$_4$OH and were observed under a Nachet microscope fitted with a Nikon D80 digital camera. At least fifty spores per collection and from the top of the stipe were mounted in water and measured. The Q value represents the length/width ratio (Q = L/w). The minimum and maximum values are given in parentheses. Macrochemical reactions were performed following standard protocols using 20% KOH, Phenolaniline (Ph.A) and Guaiac Tincture. Cailleux (1981) has been followed for the colour names of basidiomata. A preliminary, morphology-based taxonomic hypothesis for our collections was constructed with information from the following works: Brandrud *et al*. (1990–2018) and Knudsen & Vesterholt (2012). The studied material is deposited in the Royal Botanical Garden of Madrid (MA), the Royal Botanic Gardens, Kew (K), University of Helsinki (H), Botanical Museum, University of Oslo (O), and the personal herbaria of J. Ballarà (JB) and B. Dima (DB) (Table 1).

Laboratory procedures, sequence alignment and phylogenetic analysis
Genomic DNA isolation and PCR amplification followed the protocols described in Mahiques *et al*. (2018). Briefly, DNA extraction was based on a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle 1987). The entire Internal Transcribed Spacer (ITS1 and ITS2, including 5.8S) region of the nuclear rDNA was amplified using the universal fungal primer pair ITS1F–ITS4 (White *et al*. 1990, Gardes & Bruns 1993). Sequencing was performed at Macrogen Europe (The Netherlands) using the same primers. Chromatograms were checked and assembled using SeqManII v.5.07$^{©}$ (Dnastar Inc.). DNA extraction and sequencing of the type collection of *C. ochrophyllus* Fr. (1861: 26) and two Sardinian collections followed Liimatainen *et al*. (2014). The Norwegian collections were either sequenced in collaboration with the Norwegian Barcode of Life Network (NorBOL) as part of BOLD

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

(Ratnasingham & Hebert 2007) or following the methods described in Papp & Dima (2018).

We used the BLAST online tool (Altschul *et al.* 1997) to check for possible PCR-product contamination and to identify and retrieve available, highly similar *Cortinarius* ITS sequences to our 10 newly produced sequences (see accession numbers in Table 1). GenBank (http://www.ncbi.nlm.nih.gov/), UNITE (Nilsson *et al.* 2018) and BOLD (Ratnasingham & Hebert 2007) nucleotide databases were used as references. Thirty-five sequences were downloaded, of which 14 were the most genetically similar to our queries and belonged in *C.* sect. *Laeti* (= sect. *Fulvescentes* Melot) (see Soop *et al.* 2019). The remaining sequences corresponded to representatives of *C.* sect. *Infracti* (Kühner & Romagn.) Moënne-Locc. & Reumaux (3 *spp.*), *C.* sect. *Anomali* Konrad & Maubl. (3 *spp.*), *C.* sect. *Obtusi* Melot (6 *spp.*), *C.* sect. *Dermocybe* (Fr.) Gillot & Lucand (2 *spp.*), *C.* sect. *Illumini* (Liimat., Niskanen & Kytöv.) Soop, B. Oertel & Dima (2 *spp.*), and *C.* sect. *Calochroi* M.M. Moser & E. Horak (2 *spp.*). To root the inferred phylogenetic trees, we selected three taxa within *C.* sect. *Purpurascentes* M.M. Moser: *C. collocandoides* Reumaux (2009: 1377), *C. purpurascens* Fr. (1838: 265), and *C. subporphyropus* Pilát. (1954: 6) Twenty-four sequences used in this study were obtained from type specimens.

A multiple sequence alignment (MSA) was built with MAFFT 7.308 (Katoh *et al.* 2002, Katoh & Standley 2013) using the FFT-NS-I x1000 algorithm, the 200PAM/k = 2 scoring matrix, a gap open penalty of 1.5 and an offset value of 0.123. Manual editing of the resulting alignment was carried out in Geneious 11.0.5 and consisted in trimming alignment ends of longer sequences that included part of the 18S–28S rDNA subunits, and replacing gaps at the ends of shorter sequences with N (i.e. IUPAC code representing any base). The software GBlocks 0.91b (Castresana 2000) was subsequently used to automatically deal with ambiguously aligned regions, implementing the least stringent parameters but allowing gaps in 50% of the sequences. The trimmed alignment was deposited in TreeBASE under TB2:S25323 (http://purl.org/phylo/treebase/phylows/study/TB2:S25323). Then, a Maximum Likelihood (ML) phylogeny was inferred with the online version of RAMXL-HPC2

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

hosted at the CIPRES Science Gateway (Stamatakis 2006, Stamatakis *et al.* 2008, Miller *et al.* 2010). The analysis used the GTR + Γ substitution model for two delimited partitions within the ITS (ITS1 + ITS2 and 5.8S), and nodal support was evaluated with 1000 bootstrap pseudoreplicates. Tree nodes showing Bootstrap Support (BS) values equal or higher than 70% were regarded as significantly supported. The tree was drawn using the ITOL web tool (Letunic & Bork 2019).

Dating analysis

Inference of divergence ages for our study group was done within a Bayesian framework with BEAST 1.8.1 (Drummond *et al.* 2012). The analysis used a secondary calibration imposed on the ITS substitution rate because *Cortinarius* lacks a suitable fossil record. Specifically, the BEAST analysis used the average rate of 5.84×10^{-3} substitutions per site per million years (s/s/Ma) inferred for *Cortinarius s.l.* in Ryberg & Matheny (2012). This analysis will be referred to as Dating A. To take into account the uncertainty associated to that rate, we re-ran the analyses using the estimates representing the minimum (3.96×10^{-3} s/s/Ma, Dating B) and maximum (7.92×10^{-3} s/s/Ma, Dating C) values of the 95% credibility interval provided by Ryberg & Matheny (2012). Furthermore, we conducted preliminary Bayes Factor comparisons (Kass & Raftery 1995) of Maximum Likelihood Estimates (MLE) calculated with Path Sampling and Stepping-Stone (Lartillot & Philippe 2006, Xie *et al.* 2011) to choose among different BEAST molecular clocks and tree priors. The use of an uncorrelated lognormal relaxed molecular clock over the strict clock was strongly supported. As for the tree priors, models incorporating the Coalescent-Constant Size and Birth-Death (BD) Process produced comparable MLE values, which were substantially higher than the ones obtained using the Yule Process prior (not shown). Therefore, we run BEAST analyses in duplicate in order to use the former two tree priors (Coalescent and BD) independently and compare the resulting age estimates. Before analyses, redundant sequences were removed from the GBlocks-trimmed dataset. Optimal substitution models for the two ITS partitions were inferred with PartitionFinder 1.1.1 (Lanfear *et al.* 2012) considering a model with linked branch lengths and the Bayesian Information

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

Criterion (BIC). This analysis favoured the HKY + Γ model for the ITS1 + ITS2 partition, and the K80 for the 5.8S. Runs using chain lengths of 4×10^7 steps were implemented and parameters were logged every 4000 steps. Resulting log files were checked in Tracer 1.7 to ensure that all parameters had Estimated Sample Sizes (ESSs) above 200 after removing the first 20% of saved trees as burn-in. Then, the median heights of the 8000 post-burn-in tree samples were annotated with TreeAnnotator 1.8.1, and the chronograms were drawn with FigTree 1.4 to prepare them for further stylistic refining. The latter three programs are available at http://tree.bio.ed.ac.uk/. We set the value of Bayesian posterior probabilities (PP) at a minimum of 0.97 to consider tree nodes as well-supported.

Results

The original MSA (multiple sequence alignment) produced with MAFFT was 696 base pairs in length (247 variable and 64 singleton sites), whereas the GBlocks-trimmed MSA comprised 594 positions (85% of the original length with 216 variable and 55 singleton sites) distributed in 46 blocks. The ML analysis in RAXML based on the GBlocks-trimmed dataset generated a phylogeny with Ln = –3327.582549 (Figure 1a), which showed no supported conflicts with regard to a phylogeny estimated with the original, non-trimmed MSA (data not shown). The statistics estimated in the Bayesian dating analysis had average EESs well above 200, and statistically-supported topological conflicts were not observed when compared to the ML phylogeny (Figure 1b).

In general, higher statistical support values were estimated for terminal clades than for those at the phylogeny backbone (inner nodes). Analyses, however, retrieved BS > 70% and PP > 0.97 for clades that in our study represent several sections, such as *C.* sections *Infracti*, *Anomali*, *Laeti*, *Dermocybe*, *Illumini* and *Obtusi* (Figure 1). The nine sequenced specimens from the Iberian Peninsula, Sardinia and Norway belonged within *C.* sect. *Laeti*, and formed a highly supported clade together with an environmental sample sequence from Iran obtained from EcM root tips (FR852264, 826 bp; Bahram *et*

al. 2012). Nucleotide variation across the 10 ITS sequences was found to be low. Thus, the GBlocks-trimmed alignment revealed two variable sites, one shared between the Iranian, the Iberian MES-4519 and the three Norwegian samples, and the other being unique to the Iranian. In the original alignment, the Sardinian sample TN04-1113 showed a likely 5-nuc deletion in region ITS1. Sister relationships of this clade to the rest of laetioid/fulvescentoid species remained elusive due to the low support. Furthermore, the morphologically similar *C. ochrophyllus* Fr. was phylogenetically well separated based on 25 nucleotide differences in the GBlocks-trimmed dataset. The number of differences in the original MSA was even greater, including the existence of indels.

The two alternative tree priors implemented in the BEAST models (Coalescent-Constant size and BD) did not cause topological incongruences and had a very subtle impact on the estimated median age values and associated 95% Highest Posterior Density (HPD) intervals. Table 2 shows such effect: for Dating A, B and C, implementing a Coalescent prior generated slightly lower age estimates compared with those obtained using the BD. Table 2 also shows the effect of considering the three ITS substitution rates on the age estimates for the crown of *Cortinarius* sect. *Laeti* and the crown of the target species. As expected, higher substitution rates produced younger age estimates. For simplicity, we will base our discussion on results of Dating A (Figure 1b) that used the mean ITS substitution rate of 5.84×10^{-3} s/s/Ma (Ryberg & Matheny 2012), because it produced overlapping 95 % HPD intervals to those inferred in Dating B and C. According to our results, the diversification of *C. sect. Laeti* started probably in the Pliocene, *ca*. 3.52 million years ago (Ma) (4.76–2.51 Ma, 95% HPD; Table 2) and extended into the Pleistocene (Figure 1b). Divergence of the two subclades within the target species dated back to the Pleistocene, *ca*. 0.42 Ma (0.83–0.14 Ma, 95% HPD; Table 2 and Figure 1b). Although divergence among *C.* sections *Infracti*, *Anomali*, *Dermocybe*, *Illumini* and *Obtusi* cannot be dated with confidence due to the lack of support in the tree backbone, the BEAST analysis suggested a Middle to Late Miocene scenario ranging from 9.61 to 6.52 Ma (Figure 1b). The few species composing each section likely originated during the Late Pliocene and Pleistocene.
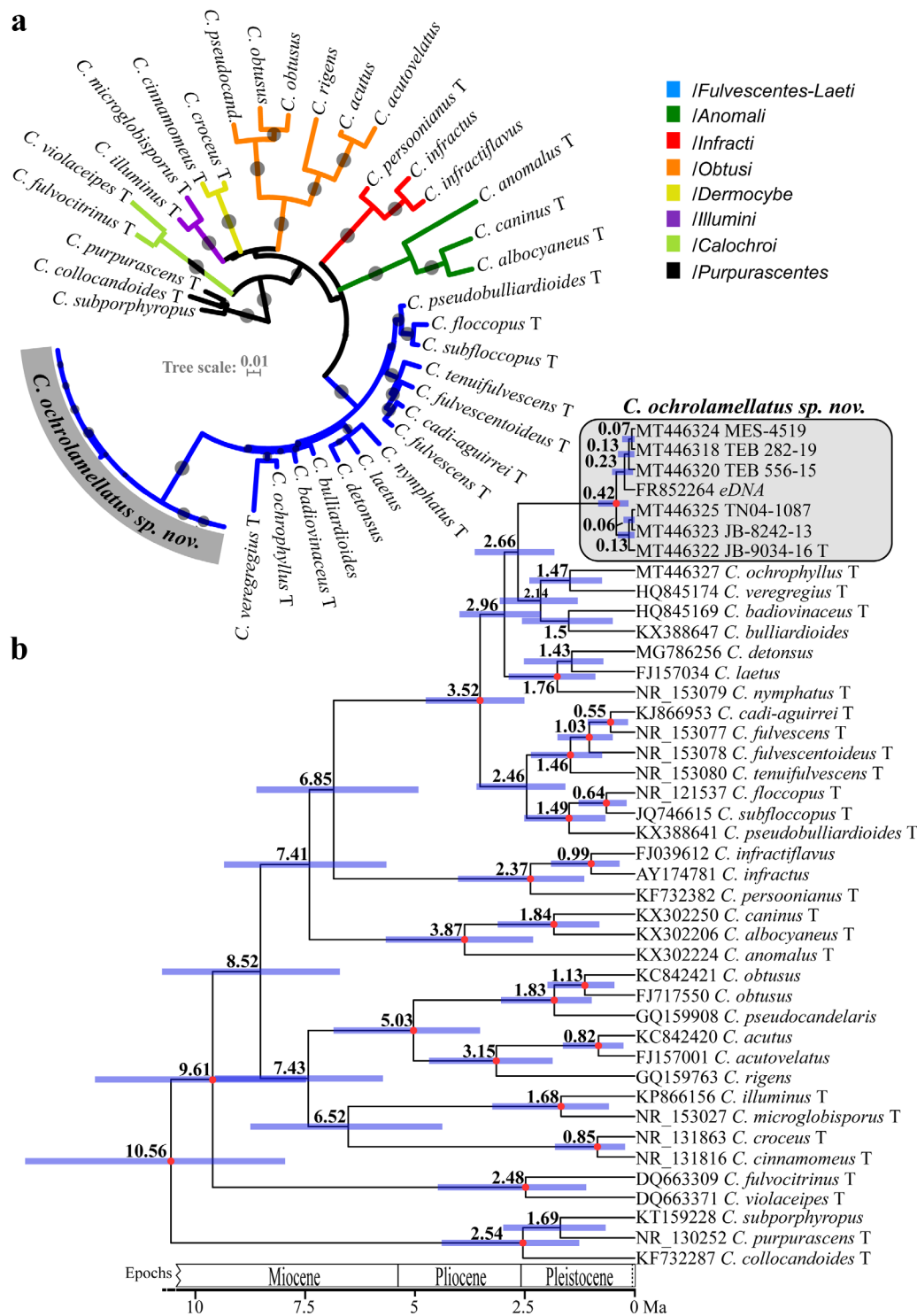
FIGURE 1. a RAXML phylogram obtained with the GBlocks-trimmed ITS dataset that depicts relationships among the new species *Cortinarius ochrolamellatus* and members of additional sections. Branches corresponding with different sections are coloured

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

variously (see colour legend on the right), and supported branches (BS ≥ 70%) are labelled with a grey dot, whose size is positively correlated with the absolute BS value. **b** BEAST time-calibrated MCC tree based on the same molecular dataset but without duplicated sequences, and estimated with a secondary calibration on the mean ITS substitution rate of *Cortinarius* inferred in Ryberg & Matheny (2012). Median age estimates (in million years, Ma) and the corresponding 95% HPD intervals are provided for all nodes. Each terminal comes with its corresponding GenBank ITS accession number and a specification of whether the sequence was obtained from the type material (T). Collection numbers are provided only for the new species' collections. Branches showing strong support (PP ≥ 0.97) display a red dot on the node. Colours in right column correspond with those used in Figure **1.a**. Analyses and illustration: I. Garrido-Benavent

Taxonomy

Cortinarius ochrolamellatus Ballarà, Liimat., Brandrud & Mahiques, *spec. nov.* (Figure 2a–d)

MycoBank number: MB 833221.

Etymology:—The epithet refers to the ochraceous colour of the lamellae.

Diagnosis:—Basidiomata growing in *Quercus spp*. forests with olivaceous ochre brown, discolouring pale ivory cream pilei with a finely fibrillose surface, olivaceous ochre yellow to olivaceous grey-brown lamellae, and whitish to very pale beige stipe surface covered with whitish to ochraceous universal veil remnants; spores 8–10.5 × 5–6.5 μm on average.

Holotype (here designated):—SPAIN. Catalunya, Barcelona, Berguedà region, Montmajor, Mare de Déu del Bosc, 42º 02' 33" N, 1º 44' 10" W, 880 m asl, in a *Quercus ilex* and *Q. humilis* forest with an understorey composed of *Buxus*

sempervirens and *Juniperus communis*, on calcareous soil, 22 October 2015, leg. J. Ballarà *JB-9034-16*. Holotype deposited in the Royal Botanical Garden of Madrid herbarium under the accession MA-90455. GenBank ITS accession number: MT446322.

Description:—*Basidiomata* small to medium-sized, but stout. *Pileus* (15)20–40 mm diam., at first campanulate (to hemispherical), later convex with a persistent obtuse and low umbo; margin first incurved and later extended; surface hygrophanous, matt, finely fibrillose, dark olivaceous (ochre) brown when moist discoloring to pale ivory cream when dry (Caill. K71, L71, M70), usually with persistent whitish to ochraceous, fine partial veil remnants, which are, with age, more evident at the margin. *Lamellae* moderately crowded, broad, uncinated, olivaceous ochre yellow to olivaceous grey-brown with a whitish attaching area; lamellar edges slightly serrate and paler; *lamellulae* present. *Stipe* (20)25–50(60) mm long, 6–11 mm wide at the apex, cylindrical and more or less claviform; surface whitish to very pale beige; when young, pale ochraceous yellow universal veil often copious, forming a discontinuous girdle, but later often indistinct. *Context* of pileus and stipe tinged olivaceous ochre brown or with hygrophanous streaks when fresh and moist, drying whitish to pale beige; taste mild and smell slightly acidic. *Macrochemical reactions*: slow, yellowish brown reaction to KOH; negative with Guaiac Tincture, phenol-aniline (Ph.A), $AgNO_3$ and methol.

Basidiospores of a moderate size, 8–10.5(11) × 5–6.5(7) μm, Q = (1.35)1.40–*1.55*–1.6, ellipsoid in side view, sometimes more broadly ovoid in front view; surface densely ornamented with relatively low, not prominent warts. *Basidia* 34–45 × 9–11 μm, 4-spored; lamellar edge fertile accompanied with claviform cells. *Pileipellis* duplex, thin, composed of a layer of 3–7 μm wide, clamped hyphae, displaying a relatively regular arrangement, with scattered pale ochraceous granular wall pigmentation; hyphae of veil remnants 2–3 μm wide; subcutis composed of short, inflated ("subcellular") septate hyphae, 35–65 × 24–35 μm in size, and irregularly arranged, with more or less brownish yellow walls.

Habitat and distribution:—This species is found mainly in calcareous, thermophilic oak forests. It apparently associates with *Quercus ilex*, *Q. suber* and *Q. pubescens* (= *Q.*

humilis) in the north-eastern Iberian Peninsula and Sardinia (Figure 3a–b), in calcareous, mixed *Quercus* and *Carpinus* forests in France (Cheptainville, Paris region; F. Valade, pers. comm.) and in calcareous *Tilia cordata* and *Q. robur*-dominated forests in SE Norway. The SE Norwegian calcareous *Tilia* forests are usually devoid of *Quercus*, but in all four, south-facing, thermophilic localities of *C. ochrolamellatus*, a substantial amount of *Q. robur* was also present. Therefore, the species here is more likely associated with *Quercus* than *Tilia* (although many telamonioid cortinarii here are preferentially associated with *Tilia*, Brandrud *et al.* 2017). Finally, in the Hyrcanian forests of north Iran the species seems associated with *Q. castaneifolia* and/or *Q. macranthera* (Figure 3a,c).

From a bioclimatic perspective, the species shows a broad range, including the mediterranean-submediterranean colline-montane zone of north-eastern Spain and Sardinia, the nemoral-temperate zone of the Paris region, the montane-temperate zone in northern Iran, and the boreo-nemoral zone of south-eastern Norway. It should be added that the Norwegian sites are relatively and particularly warm and coincide with Europe's northernmost occurrences of thermophilic fungal species, such as *Rubroboletus rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang (2014: 70), *Cortinarius chevassutii* Rob. Henry (1982: 7) and *C. sodagnitus* Rob. Henry (1935: 22). The forests in which the species was found in north-eastern Iberian Peninsula occurred in the mesomediterranean belt from an altitude of 250 m asl (Canyamars, sample *JB-8242-13*) up to submediterranean sites at 1105 m asl (Bernués, *MES-4519*). According to Bahram *et al*. (2012), the two species of *Quercus* occurring in the Iranian localities Asalem, Nowshahr and Savadkuh grow at an altitude ranging between 50–1000 m asl (*Q. castaneifolia*) and 2000–2400 m asl (*Q. macranthera*). In Savadkuh and Nowshahr, the latter oak species dominates the transition zone between forest and grassland. With regard to edaphic requirements, *C. ochrolamellatus* seems to be strictly calciphilous in boreo-nemoral and nemoral zones, whereas the taxon in the Mediterranean area can be found in siliceous soils as well. This pattern is observed in a number of *Cortinarius* species, such as *C. splendens* Rob. Henry. In our opinion, the xerothermous conditions in the Mediterranean allow these fungal species to take enough nutrients and

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

electrolytes, even on siliceous soils, whereas the same species are confined to calcareous soils further north.

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

FIGURE 2. a–b *Cortinarius ochrolamellatus* when dry (holotype, MA-90455 collected in a *Quercus ilex* and *Q. humilis* mixed forest, Spain). **c** *Cortinarius ochrolamellatus* when moist (TEB 282-19, in a mixed *Tilia cordata-Q. robur* forest, Norway). **d** *Cortinarius ochrolamellatus* when moist (DB1493, in a *Q. ilex* forest, Sardinia). **e** *Cortinarius ochrolamellatus* (H7017993, in a *Q. ilex* forest, Sardinia). **f** *Cortinarius ochrolamellatus* (MA-90447, in a *Q. ilex* and *Q. suber* mixed forest, Spain). **g–h** Spores on lamellae mounted in water (holotype, MA-90455). *Scale bars* 10 μm. Photo credits: J. Ballarà (**a–b**, **f–h**), T. E. Brandrud (**c**), B. Dima (**d**), K. Liimatainen (**f**). Illustrator: I. Garrido-Benavent.

Additional specimens examined:—SPAIN. Catalunya, Barcelona province, Maresme, Dosrius, Canyamars, 41º 35' 33.25" N, 2º 27' 54.96" E, 250 m asl, in a *Quercus ilex* and *Q. suber* forest with *Pistacia lentiscus*, *Erica arborea* and *Cistus salviifolius*, on siliceous soil, 21 December 2013, leg. J. Ballarà *JB-8242-13* (MA-90447); Sagàs, La Quar, 42º 06' 05.31" N, 1º 57' 21.89" E, 1010 m asl, in a *Q. ilex* forest, on calcareous soil, 13 November 2016, leg. J. Ballarà *JB-9160-16* (MA-90456); Aragón, Huesca, Jaca, Bernués, 41º 29' 40.63" N, 0º 34' 52.56" W, 1105 m asl, in a *Q. pubescens* and *Corylus avellana* forest, 11 October 2014, leg. P. García, det. R. Mahiques *MES-4519* (MA-90457). ITALY. Sardinia, Nuoro, Gavoi, Lago di Gusana, by the hotel Taloro, 40º 07' 09.16" N, 9º 12' 32.36" E, 668 m asl, in a *Quercus ilex* and *Cistus* forest, 03 November 2004, leg. K. Liimatainen, B. Dima & T. Niskanen *04-1087* (H7017993, dupl. K and in B. Dima pers. herb. as *DB1493*); *loc. cit.*, 04 November 2004, leg. K. Liimatainen & T. Niskanen *04-1113* (H7017817, dupl. K); Marche, Urbino, near Urbino, *Quercus* spp. forest, on calcareous soil, leg. G. Hensel, det. B. Dima *DB5975*. NORWAY. Telemark, Porsgrunn, Brattås, 59.0913º N, 9.6769 º E, 65 m asl, in warm, steep, calcareous *Tilia cordata* forest with *Q. robur* and *C. avellana*, 21 September 2011, leg. T.E. Brandrud *TEB 939-11* (O); Hitterød NW, 59.0757 º N, 9.6480 º E, 37 m asl, in warm, steep calcareous *T. cordata* forest with *Q. robur*, 15 September 2011, leg. T.E. Brandrud *TEB 847-11* (O); Blekebakken NR, 59.0552º N, 9.6768º E, 37 m asl, in

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

warm, calcareous *T. cordata-Q. robur* forest with some *C. avellana*, 25 September 2015, leg. T.E. Brandrud *TEB 556-15* & B. Dima (O); Kongkleivåsen S, Frierflogene nature reserve, 59.0673º N, 9.6531º E, 22 m asl, in warm, steep, calcareous *T. cordata-Q. robur* dominated forest with some *C. avellana*, 30 August 2019, leg. T.E. Brandrud *TEB 282-19* & B. Dima (O).

Additional species examined:—*Cortinarius ochrophyllus* Fr. NORWAY. Oppland, Valdres, Vang, Øye, at west end of lake Vangsmjøsa, 61º 09' 40" N, 08º 24' 38" E, 523 m asl, in a *Picea abies* forest, on siliceous soil, 10 August 2019, leg. J. Ballarà *JB-N19-10-03*.
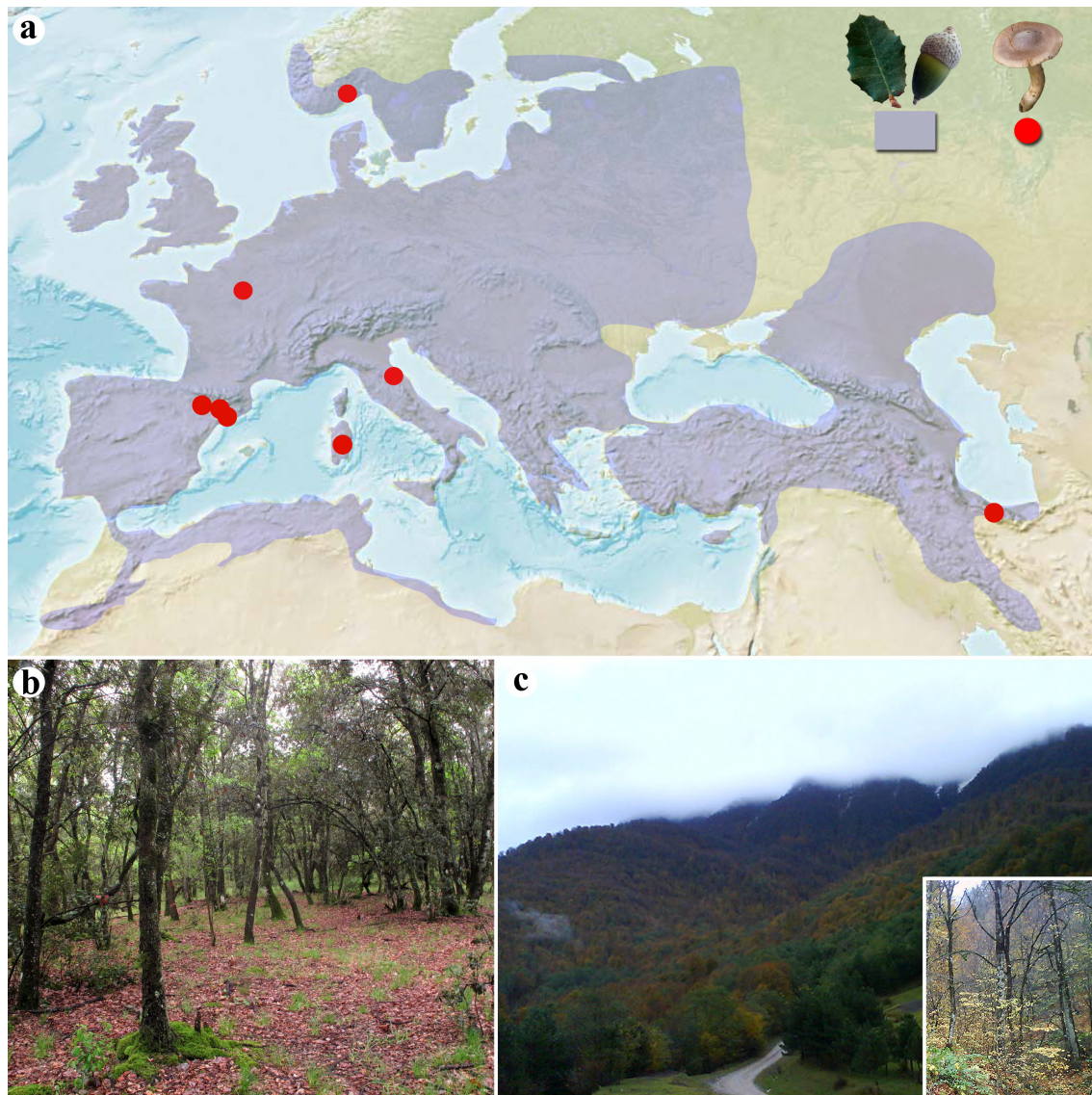
FIGURE 3. a Geographic distribution of the new species *Cortinarius ochrolamellatus* across Eurasia (red dots); in purple, rough distribution of potential tree symbionts in *Quercus* subgenera *Quercus* and *Cerris* in the same geographical area (adapted from Figure 2.3 in Denk *et al*. 2017). **b** *Quercus ilex* forest close to the type locality (Mare de Déu del Bosc, Catalunya, north-eastern Iberian Peninsula). **c** Forest dominated by *Quercus* and *Fagus* in Kūh-e Narū (eastern part of the Hyrcanian forests, Iran); inset shows a detail of a forest in Golestan National Park. Photo credits: J. Ballarà (**b**), M. Bahram (**c**). Illustrator: I. Garrido-Benavent

Discussion

The integrative taxonomic approach implemented in the present study has allowed us to delineate and describe *Cortinarius ochrolamellatus*, which is characterized by its stout but rather small basidiomata having dark olivaceous brown pilei which soon become paler on drying. The lamellae are typically pale yellowish brown to ochraceous yellow, usually with an olivaceous tinge, and the veil remnants are pale ochraceous yellow. Furthermore, the species is characterized by its habitat in thermophilic *Quercus* forests. According to the inferred ITS phylogeny, *C. ochrolamellatus* is a member of *C.* sect. *Laeti s. lat.* (= sect. *Laeti* + sect. *Fulvescentes sensu* Brandrud *et al*. 1990–2018). Within this section, the new species might be especially confused with *C. ochrophyllus* due to the morphological resemblance. The latter species has very similar olive ochre colours to the new species, but whereas *C. ochrolamellatus* is a thermophilic, more or less calciphilous *Quercus* forest species, *C. ochrophyllus* is a species occurring in boreal coniferous forests that associates preferentially with *Picea* on siliceous soils. Morphologically, *C. ochrophyllus* has more pronounced olivaceous veil remnants on the stipe (Fig. 4) and, microscopically produces ovoid to subglobose spores, which are smaller than those of *C. ochrolamellatus* (Brandrud *et al*. 1990–2018). Other species in *C.* sect. *Laeti* may be compared with *C. ochrolamellatus*. *Cortinarius detonsus* (Fr.) Fr. (1838: 313) has a different pigmentation, with somewhat more vivid yellowish brown tinges, including the lamellae and veil, and it produces slightly narrower spores, and grows in humid, subalpine-boreal *Picea* forests (Brandrud *et al*. 1990–2018). *Cortinarius nymphatus* Kytöv., Niskanen, Liimat. & Bojantchev (2016: 232) displays a brown to deep red-brown pigmentation on the pileus, forms incomplete girdles on the stipe, has smaller spores (6.8–8.2 × 4.3–4.8 μm), and it inhabits humid coniferous forests (FACESOFFUNGI 2017: FoF02467).

The inclusion of EcM root tip DNA sequence data in our phylogenetic analysis has shown that *Cortinarius ochrolamellatus* occurs in the Hyrcanian forests of Iran. This

area covers *ca.* 18500 km^2 of mountainous slopes along the southern shores of the Caspian Sea from the Talish region in Azerbaijan (at longitude 48º E) to the Golestan National Park in Iran (at longitude 56º E) and spans latitudes between 38º 55' and 35º 05' N. This region was an important refugium during the Quaternary glaciations for temperate broad-leaved trees and, today, it includes alluvial and lowland to submontane and subalpine forests (reviewed by Akhani *et al.* 2010). As indicated in Figure 3a, this montane region represents the most southeastern portion of the European-Anatolian-Caucasian distribution of *Quercus* (Denk *et al.* 2017). Although the information about the diversity of *Cortinarius* in North Iran is scarce (Asef 2007, 2009, Asef & Etemad 2016), a number of recent works have explored EcM communities by sequencing root tips below different vegetation types and found *Cortinarius* to be one of the most abundant genera (Bahram *et al.* 2012, Zamani *et al.* 2018, Aghajani *et al.* 2019). Furthermore, the EcM root tip ITS data provided by Bahram *et al.* (2012) has been used to feed phylogenies across several sections of *Cortinarius*, uncovering genetic affinities with European taxa. For example, Dima *et al.* (2014) found Iranian sequences to be identical to those obtained from specimens of *C. uraceonemoralis* Niskanen, Liimat., Kytöv., Bojantchev. & H. Lindstr. (2014: 876, in Dima et al. 2014) (*C.* sect. *Uracei* Kühner & Romagn. ex Melot) and *C. uraceomajalis* Dima, Liimat., Niskanen & Bojantchev (2014: 874, in Dima et al. 2014) (*C.* sect. *Colymbadini* Melot) collected across several European countries spanning a wide latitudinal range (Italy, France, Hungary, Bulgaria, Czech Republic and Sweden). Similarly, a monophyletic clade under the name of *C.* cf. *sertipes* Kühner (1955: 40) was inferred by Brandrud *et al.* (2017), showing nearly identical ITS sequences from Austria, Italy, Denmark and Iran. And, finally, Liimatainen *et al.* (2017) revealed the presence of two Iranian sequences in a monophyletic clade including *C. confirmatus* Rob. Henry (1983: 67), within the telamonioid *C.* sect. *Saturnini* Rob. Henry ex Moënne-Locc. & Reumaux. In the present study, the existence of this European-Hyrcanian biogeographic link in *Cortinarius* is extended to include *C. ochrolamellatus*, a species growing in *Quercus* forests in the Iberian Peninsula, Sardinia, Central Italy, France and Norway. The occurrence of the new species in the mountainous regions north of Turkey and in the Caucasus must be

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.

further explored, as these regions have been shown to host a significant number of basidiomycete species mainly known from northern, western and southern Europe (Sesli & Moreau 2015, Corfixen & Parmasto 2018, Papp & Dima 2018, Malysheva *et al.* 2019).
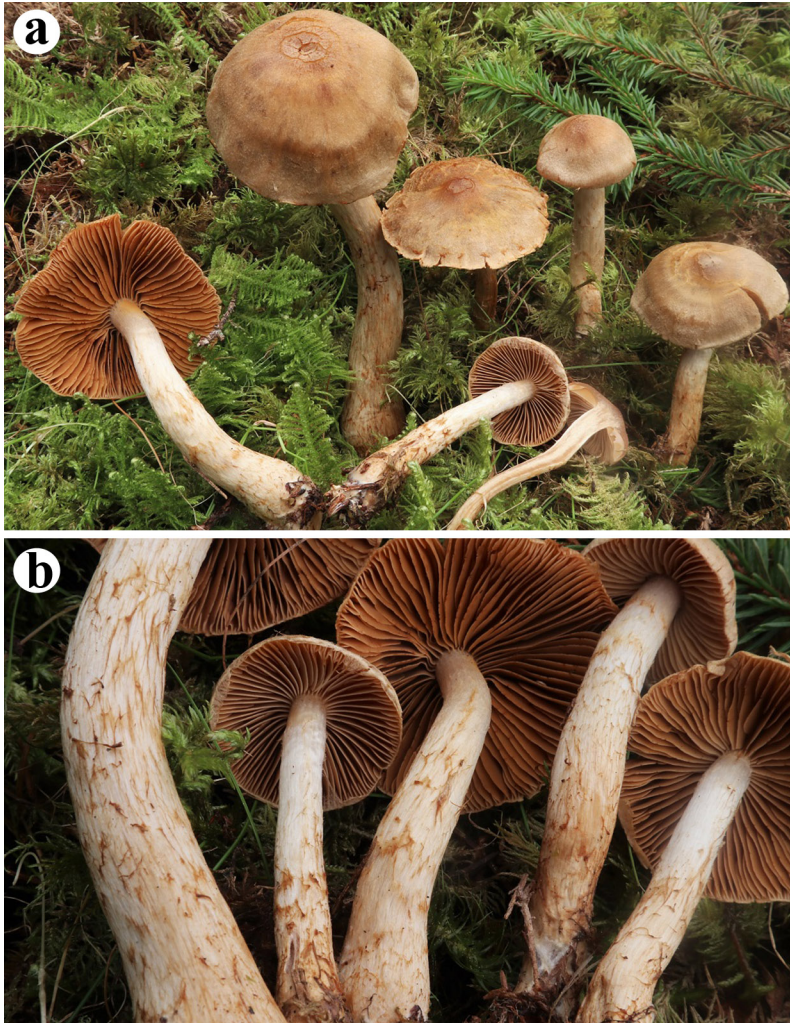

FIGURE 4. *Cortinarius ochrophyllus* (JB-N19-10-03) collected in a coniferous forest in Oppland, Norway. Photo credits: J. Ballarà. Illustrator: I. Garrido-Benavent

Our dating study placed the crown node of *Cortinarius ochrolamellatus* in the Pleistocene, *ca*. 0.42 Ma (0.83–0.14 Ma, 95% HPD). This temporal window is much younger compared to the Miocene-Pliocene interval inferred for the diversification of the various *Quercus* sections that accommodate species associated with *C. ochrolamellatus*, such as *Q. ilex*, *Q. pubescens*, *Q. suber* or *Q. castaneifolia* (Hubert *et al*. 2014, Simeone *et al*. 2016). However, this Miocene-Pliocene scenario is in part concomitant with the timing of diversification of *C.* sect. *Laeti*, which our dating analysis placed in the Pliocene, *ca*. 3.52 Ma (4.76–2.51 Ma, 95% HPD). Moreover, the strict association of *C. ochrolamellatus* to *Quercus* species suggests that its known Eurasian distribution was shaped by following the plant hosts' postglacial migration routes (Wang & Qiu 2006). Past codispersal of EcM fungi and their hosts in Europe has been suggested for the Perigord truffle (*Tuber melanosporum* Vittad. (1831: 36), Murat *et al*. 2004), *Alnus spp.*-associated EcM fungal communities (Põlme *et al*. 2012), members in *Cortinarius* sect. *Calochroi* (Mahiques *et al*. 2018) and, at a wider geographic scale, in *Russula* subsect. *Roseinae* (Looney *et al*. 2020). The occurrence of *C. ochrolamellatus* in southern Europe (Iberian and Italic peninsulas) and Hyrcania reinforces this view because these regions have been proposed as refugia during Quaternary glaciations for many plant species (Thompson 2005, Akhani *et al*. 2010, Feliner 2011), and specially for European *Quercus spp*. (Taberlet *et al*. 1998, Petit *et al*. 2003). However, migration of EcM fungi via occasional long-distance spore dispersal should be also considered (Hallenberg & Küffer 2001), as this mechanism has been shown to be important in shaping the biogeographic history of EcM species in, for example, the family Inocybaceae (Matheny *et al*. 2009) and the genus *Strobilomyces* Berk. (1851: 78) (Han et al. 2018). All in all, the phylogeographic history proposed for our target species might also explain the European-Hyrcanian distributions of the other *Cortinarius* taxa mentioned above (Dima *et al*. 2014, Brandrud *et al*. 2017, Liimatainen *et al*. 2017). To further delve into this hypothesis, future work must consider alternative calibration strategies and the use of datasets assembled with a population genetics scope and encompassing more variable molecular markers.

In conclusion, the results of the present work demonstrate that the combination of specimen-based and EcM root tip DNA sequence data in phylogenetic surveys may help to elucidate wider and more complex distribution ranges for EcM fungi, like *Cortinarius*, which in principle are influenced by the biogeographic history of the associated plant hosts. Moreover, our study provided crucial insights into the Eurasian biogeographic connections, which appear to be widespread for EcM fungi.

Acknowledgements

References

Aghajani, H., Hojjati, S.M., Tajick-Ghanbari, M.A., Puormajidian, M.R. & Borhani, A. (2019) Molecular identification of ectomycorrhizal fungal communities associated with oriental beech trees (*Fagus orientalis* Lipsky) in Hyrcanian Forest of Iran. *Iran Journal of Science and Technology, Transactions A* 43: 25–32. doi: 10.1007/s40995-017-0435-2

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.
Cortinarius ochrolamellatus (Agaricales, Basidiomycota): a new species in C. sect. Laeti, with comments on the origin of its European-Hyrcanian distribution. *Phytotaxa* 2020 ;Volum 460.(3) s. 185-200

Akhani, H., Djamali, M., Ghorbanalizadeh, A. & Ramezani, E. (2010) Plant biodiversity of Hyrcanian relict forests, N Iran: an overview of the flora, vegetation, palaeoecology and conservation. *Pakistan Journal of Botany* 42: 231–258.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402. doi: 10.1093/nar/25.17.3389

Asef, M.R. (2007) Macrofungi flora of Arasbaran 1. *Cortinarius* subgenus *Myxacium*. *Rostaniha* 8: 178–185.

Asef, M.R. (2009) Macrofungi of Arasbaran (N. W. Iran) 3. *Cortinarius* subgenus *Phlegmacium*. *Rostaniha* 10: 80–90.

Asef, M.R. & Etemad, V. (2016) Identification of agaric fungi of Kheyroud Research Forest, Noshahr (Mazandaran province, N. Iran). *Rostaniha* 17: 19–27.

Bahram, M., Põlme, S., Kõljalg, U., Zarre, S. & Tedersoo, L. (2012) Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist* 193: 465–473. doi: 10.1111/j.1469-8137.2011.03927.x

Berkeley, M.J. (1851) Decades of fungi. Decades XXXIV. Sikkim Himalaya fungi, collected by Dr. J.D. Hooker. *Hooker's Journal of Botany and Kew Garden Miscellany* 3: 77–84.

Bjorbækmo, M., Carlsen, T., Brysting, A., Vrålstad, T., Høiland, K., Ugland, K.I., Geml, J., Schumacher, T. & Kauserud, H. (2010) High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. *BMC Plant Biology* 10: 244. doi: 10.1186/1471-2229-10-244

Brandrud, T.E., Lindström, H., Marklund, H., Melot, J. & Muskos, S. (1990–2018) *Cortinarius*, Flora Photographica. Sweden: Editions Matfors. 5 fasc. English version

Brandrud, T.E., Dima, B., Liimatainen, K. & Niskanen, T. (2017) Telamonioid *Cortinarius* species of the *C. puellaris* group from calcareous *Tilia* forests. *Sydowia* 69: 37–45. doi: 10.12905/0380.sydowia69-2017-0037

Brandrud, T.E., Schmidt-Stohn, G., Liimatainen, K., Niskanen, T., Frøslev, T.G., Soop, K., Bojantchev, D., Kytövuori, I., Jeppesen, T.S., Bellù, F., Saar, G., Oertel, B., Ali, T., Thines, M. & Dima, B. (2018) *Cortinarius* sect. *Riederi*: taxonomy and phylogeny of the new section with European and North American distribution. *Mycological Progress* 17: 1323–1354. doi: 10.1007/s11557-018-1443-0

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S. & Martin, F. (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* 184: 449–456. doi: 10.1111/j.1469-8137.2009.03003.x

Cailleux, A. (1981) *Code des couleurs des sols*. Boubée, France, 15 pp.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552. doi:10.1093/oxfordjournals.molbev.a026334

Corfixen, P. & Parmasto, E. (2018) *Hymenochaete* and *Hymenochaetopsis* (Basidiomycota) in Europe. *Karstenia* 57: 49–80. doi: 10.29203/ka.2017.483

Denk, T., Grimm, G.W., Manos, P.S., Deng, M. & Hipp, A.L. (2017) An updated infrageneric classification of the oaks: review of previous taxonomic schemes and synthesis of evolutionary patterns. *In*: Gil-Pelegrín, E., Peguero-Pina, J. & Sancho-Knapik, D. (Eds.) *Oaks physiological ecology: exploring the functional diversity of genus* Quercus *L*. Tree Physiology, vol 7. Springer, Cham, pp. 13–38.

Dima, B., Liimatainen, K., Niskanen, T., Kytövuori, I. & Bojantchev, D. (2014) Two new species of *Cortinarius*, subgenus *Telamonia*, sections *Colymbadini* and *Uracei*, from Europe. *Mycological Progress* 13: 867–879. doi: 10.1007/s11557-014-0970-6

Dima, B., Lindström, H., Liimatainen, K., Olson, Å., Soop, K., Kytövuori, I., Dahlberg, A. & Niskanen, T. (2016) Typification of Friesian names in *Cortinarius* sections *Anomali*, *Spilomei*, and *Bolares*, and description of two new species from northern Europe. *Mycological Progress* 15: 903–919. doi: 10.1007/s11557-016-1217-5

Doyle, J.J. & Doyle, J.L. (1987) A rapid procedure for DNA purification from small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973. doi: 10.1093/molbev/mss075

FACESOFFUNGI (2017) *Cortinarius nymphatus* Kytöv., Niskanen, Liimat. & Bojantchev. Available from: http://www.facesoffungi.org/cortinarius-nymphatus-facesoffungi-number-fof-02467/ (accessed: 24 April 2020).

Feliner, G.N. (2011) Southern European glacial refugia: a tale of tales. *Taxon* 60: 365–372. doi: 10.1002/tax.602007

Fries, E.M. (1838) Epicrisis systematis mycologici. Uppsala, Sweden, 610 pp.

Fries, E.M. (1861) Hymenomycetes novi vel minus cogniti, in Suecia 1852-1860 observati. *Öfversigt af Kongl. Vetenskaps-Akademiens Forhandlingar* 18: 19–34.

Frøslev, T.G., Jeppesen, T.S., Læssøe, T. & Kjøller, R. (2007) Molecular phylogenetics and delimitation of species in *Cortinarius* section *Calochroi* (Basidiomycota, Agaricales) in Europe. *Molecular Phylogenetics and Evolution* 44: 217–227. doi: 10.1016/j.ympev.2006.11.013

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

Garnica, S., Schön, M.E., Abarenkov, K., Riess, K., Liimatainen, K., Niskanen, T., Dima, B., Soop, K., Frøslev, T.G., Jeppesen, T.S., Peintner, U., Kunhert-Finkernagel, R., Brandrud, T.E., Saar, G., Oertel, B. & Ammirati, J.F. (2016) Determining threshold values for barcoding fungi: lessons from *Cortinarius* (Basidiomycota), a highly diverse and widespread ectomycorrhizal genus. *FEMS Microbiology Ecology* 92: fiw045. doi: 10.1093/femsec/fiw045

Geml, J., Timling, I., Robinson, C.H., Lennon, N., Nusbaum, H.C., Brochmann, C., Noordeloos, M.E. & Taylor, D.L. (2011) An Arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal

basidiomycetes in Svalbard based on soil and sporocarp DNA. *Journal of Biogeography* 39: 74–88. doi: 10.1111/j.1365-2699.2011.02588.x

Gray, S.F. (1821) *A natural arrangement of British plants*. United Kingdom, London: Baldwin, Cradock and Joy, 824 pp.

Hallenberg, N. & Küffer, N. (2001) Long-distance spore dispersal in wood-inhabiting basidiomycetes. *Nordic Journal of Botany* 21: 431–436. doi: 10.1111/j.1756-1051.2001.tb00793.x

Han, L.H., Feng, B., Wu, G., Halling, R.E., Buyck, B., Yorou, N.S., Ebika, S.T.N. & Yang, Z.L. (2018) African origin and global distribution patterns: Evidence inferred from phylogenetic and biogeographical analyses of ectomycorrhizal fungal genus Strobilomyces. *Journal of Biogeography* 45: 201–212. doi: doi.org/10.1111/jbi.13094

Harrower, E., Ammirati, J.F., Cappuccino, A.A., Ceska, O., Kranabetter, K.M., Kroeger, P., SeaRa, L., Taylor, T. & Berbee, M.L. (2011) *Cortinarius* species diversity in British Columbia and molecular phylogenetic comparison with European specimen sequences. *Botany* 89: 799–810. doi: 10.1139/b11-065

Hawksworth, D.L., Hibbett, D.S., Kirk, P.M. & Lücking, R. (2016) 308–310. Proposals to permit DNA sequence data to serve as types of names of fungi. *Taxon* 65: 899–900. doi: 10.12705/654.31

Henry, R. (1935) Étude de quelques Cortinaires du groupe des *Scauri* . Deux espéces nouvelles. *Bulletin de la Société Mycologique de France* 51: 34–101 + pI. 1–2.

Henry, R. (1982) *Cortinarius chevassutii*. *In*: Chevassut, G. & Henry, R. (Eds.) Cortinaires nouveaux ou rares de la région Languedoc - Cévennes - 2. *Documents Mycologiques* 12: 1–86.

Henry, R. (1983) Cortinaires rares ou nouveaux. *Bulletin de la Société Mycologique de France* 99: 5–92.

Hubert, F., Grimm, G.W., Jousselin, E., Berry, V., Franc, A. & Kremer, A. (2014) Multiple nuclear genes stabilize the phylogenetic backbone of the genus *Quercus*. *Systematics and Biodiversity* 12: 405–423. doi: 10.1080/14772000.2014.941037

Hui, N., Jumpponen, A., Francini, G., Kotze, D.J., Liu, X., Romantschuk, M., Strömmer, R. & Setälä, H. (2017) Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. *Environmental Microbiology* 19: 1281–1295. doi: 10.1111/1462-2920.13660

Kühner, R. (1955) Complements à la Flore Analytique IV. Espèces nouvelles ou critiques des Cortinaires. *Bulletin Mensuel de la Société Linnéenne de Lyon* 24: 39–54.

Kytövuori, I. (2016). *Cortinarius nymphatus*. *In*: Hyde, K.D., Hongsanan, S., Jeewon, R. & 109 additional authors (Eds.) Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 80: 1–270. doi: 10.1007/s13225-016-0373-x

Kass, R.E. & Raftery, A.E. (1995) Bayes Factors. *Journal of the American Statistical Association* 90: 773–795.

Katoh, K. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066. doi: 10.1093/nar/gkf436

Katoh, K. & Standley, D.M. (2013) MAFFT: Iterative Refinement and additional methods. *Methods in Molecular Biology* 1079: 131–146. doi: 10.1007/978-1-62703-646-7_8

Knudsen, H. & Vesterholt, J. (2012) *Funga Nordica*, 2on ed. Copenhague: Nordsvamp. 2 vols.

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. doi: 10.1093/molbev/mss020

Letunic, I. & Bork, P. (2019) Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research* 47:W256–W259. doi: 10.1093/nar/gkz239

Liimatainen, K., Niskanen, T., Dima, B., Kytövuori, I., Ammirati, J.F. & Frøslev, T.G. (2014) The largest type study of *Agaricales* species to date: bringing identification

and nomenclature of *Phlegmacium* (*Cortinarius*) into the DNA era. *Persoonia* 33: 98–140. doi: 10.3767/003158514x684681

Liimatainen, K., Carteret, X., Dima, B., Kytövuori, I., Bidaud, A., Reumaux, P., Niskanen, T., Ammirati, J.F. & Bellanger, J-M. (2017) *Cortinarius* section *Bicolores* and section *Saturnini* (Basidiomycota, *Agaricales*), a morphogenetic overview of European and North American species. *Persoonia* 39:175–200. doi: 10.3767/persoonia.2017.39.08

Looney, B.P., Adamčík, S. & Matheny, P.B. (2020) Coalescent-based delimitation and species-tree estimations reveal Appalachian origin and Neogene diversification in *Russula* subsection *Roseinae*. *Molecular Phylogenetics and Evolution* doi: 10.1016/j.ympev.2020.106787

Mahiques, R., Ballarà, J., Salom, J.C., Bellanger, J-M. & Garrido-Benavent, I. (2018) Morphogenetic diversity of the ectomycorrhizal genus *Cortinarius* section *Calochroi* in the Iberian Peninsula. *Mycological Progress* 17: 815–831. doi: 10.1007/s11557-018-1394-5

Malysheva, E., Moreno, G., Villarreal, M., Malysheva, V. & Svetasheva, T. (2019) The secotioid genus *Galeropsis* (Agaricomycetes, Basidiomycota): a real taxonomic unit or ecological phenomenon? *Mycological Progress* 18: 805–831. doi: 10.1007/s11557-019-01490-6

Matheny, P.B., Aime, M.C., Bougher, N.L., Buyck, B., Desjardin, D.E., Horak, E., Kropp, B.R., Lodge, D.J., Soytong, K., Trappe, J.M. & Hibbett, D.S. (2009) Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. *Journal of Biogeography*, 36: 577–592. doi: 10.1111/j.1365-2699.2008.02055.x

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In*: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, pp. 1–8.

Murat, C., Díez, J., Luis, P., Delaruelle, C., Dupré, C., Chevalier, G., Bonfante, P. & Martin, F. (2004) Polymorphism at the ribosomal DNA ITS and its relation to

postglacial re-colonization routes of the Perigord truffle *Tuber melanosporum*. *New Phytologist* 164: 401–411. doi: 10.1111/j.1469-8137.2004.01189.x

Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U. & Abarenkov, K. (2018) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* 47: D259–D264. doi: 10.1093/nar/gky1022

Niskanen, T., Kytövuori, I. & Liimatainen, K. (2011) *Cortinarius* sect. *Armillati* in northern Europe. *Mycologia* 103: 1080–1101. doi: 10.3852/10-350

Niskanen, T., Liimatainen, K., Ammirati, J.F. & Hughes, K. (2013) *Cortinarius* section *Sanguinei* in North America. *Mycologia* 105: 344-356. doi: 10.3852/12-086

Niskanen, T., Douglas, B., Kirk, P., Crous, P., Lücking, R., Matheny, P.B., Cai, L., Hyde, K. & Cheek, M. (2018) New discoveries: species of fungi described in 2017. *In*: Willis, K.J. (Ed.) *State of the World's Fungi 2018, Report*. Royal Botanic Gardens, Kew, pp. 18–23.

Nouhra, E., Urcelay, C., Longo, S. & Tedersoo, L. (2013) Ectomycorrhizal fungal communities associated to *Nothofagus* species in Northern Patagonia. *Mycorrhiza* 23: 487–496. doi: 10.1007/s00572-013-0490-2

Papp, V. & Dima, B. (2018) New systematic position of *Aurantiporus alborubescens* (*Meruliaceae*, Basidiomycota), a threatened old-growth forest polypore. *Mycological Progress* 17: 319–332. doi: 10.1007/s11557-017-1356-3

Peay, K.G., Kennedy, P.G. & Talbot, J.M. (2016) Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology* 14: 434. doi: 10.1038/nrmicro.2016.59

Petit, R.J., Csaikl, U.M., Bordács, S., Burg, K., Coart, E., Cottrell, J., van Dam, B., Deans, J.D., Dumolin-Lapègue, S., Fineschi, S., Finkeldey, R., Gillies, A., Glaz, I., Goicoechea, P.G., Jensen, J.S., König, A.O., Lowe, A.J., Madsen, S.F., Mátyás, G., Munro, R.C., Olalde, M., Pemonge, M.-H., Popescu, F., Slade, D., Tabbener, H., Taurchini, D., de Vries, S.G.M., Ziegenhagen, B. & Kremer, A. (2003) Chloroplast DNA variation in European white oaks: Phylogeography and patterns

of diversity based on data from over 2600 populations. *Forest Ecology and Management* 156: 5–26. doi: 10.1016/S0378-1127(01)00645-4

Pilát, A. (1954) Pavučinec fialovoporfyrovy—*Cortinarius subporphyropus* s. nov. *Ceská Mykologie* 8: 4–6.

Põlme, S., Bahram, M., Yamanaka, T., Nara, K., Dai, Y. C., Grebenc, T., Kraigher, H., Toivonen, M., Wang, P.-H., Matsuda, Y., Naadel, T., Kennedy, P.G., Kõljalg, U. & Tedersoo, L. (2013) Biogeography of ectomycorrhizal fungi associated with alders (*Alnus* spp.) in relation to biotic and abiotic variables at the global scale. *New Phytologist* 198: 1239–1249. doi: 10.1111/nph.12170

Ratnasingham, S. & Hebert, P.D. (2007) BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular Ecology Notes* 7: 355–364. doi: 10.1111/j.1471-8286.2007.01678.x

Reis, F., Valdiviesso, T., Varela, C., Tavares, R.M., Baptista, P. & Lino-Neto, T. (2018) Ectomycorrhizal fungal diversity and community structure associated with cork oak in different landscapes. *Mycorrhiza* 28: 357–368. doi: 0.1007/s00572-018-0832-1

Reumaux, P. (2009) *Cortinarius collocandoides* Reumaux. *In*: Bidaud, A., Moënne-Loccoz, P., Reumaux, P. & Carteret, X. (Eds.) *Atlas des Cortinaires*, Pars XVIII. FMDS Eds, Marlioz, p. 1377.

Rojas-Jiménez, K., Wurzbacher, C., Bourne, E.C., Chiuchiolo, A., Priscu, J.C. & Grossart, H.P. (2017) Early diverging lineages within Cryptomycota and Chytridiomycota dominate the fungal communities in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. *Scientific Reports* 7: 15348. doi: 10.1038/s41598-017-15598-w.

Ryberg, M. & Matheny, P.B. (2012) Asynchronous origins of ectomycorrhizal clades of *Agaricales*. *Proceedings of the Royal Society of London, Series B-Biological Sciences* 279: 2003–2011. doi: 10.1098/rspb.2011.2428

Salomón, M.E.S., Dresch, P., Horak, E., Galleguillos, F., Barroetaveña, C. & Peintner, U. (2018) The enigmatic *Cortinarius magellanicus* complex occurring in

Nothofagaceae forests of the Southern Hemisphere. *Fungal Biology* 122: 1077–1097. doi: 10.1016/j.funbio.2018.08.009

Sesli, E. & Moreau, P.A. (2015) Taxonomic studies on some new fungal records from Trabzon, Turkey. *Turkish Journal of Botany* 39: 857–866. doi:10.3906/bot-1409-45

Simeone, M.C., Grimm, G.W., Papini, A., Vessella, F., Cardoni, S., Tordoni, E., Piredda, R., Franc, A. & Denk, T. (2016) Plastome data reveal multiple geographic origins of *Quercus* Group *Ilex*. *PeerJ* 4: e1897. doi: 10.7717/peerj.1897

Soop, K., Dima, B., Cooper, J.A., Park, D. & Oertel, B. (2019) A phylogenetic approach to a global supraspecific taxonomy of *Cortinarius* (Agaricales) with an emphasis on the southern mycota. *Persoonia* 42: 261. doi: 10.3767/persoonia.2019.42.10

Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. doi:10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A fast bootstraping algorithm for the RAxML web-servers. *Systematic Biology* 57: 758–771. doi:10.1080/10635150802429642

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G. & Cosson, J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453–464. doi: 10.1046/j.1365-294x.1998.00289.x

Teasdale, S.E., Beulke, A.K., Guy, P.L. & Orlovich, D.A. (2013) Environmental barcoding of the ectomycorrhizal fungal genus *Cortinarius*. *Fungal Diversity* 58: 299–310. doi: 10.1007/s13225-012-0218-1

Tedersoo, L., Bahram, M., Põlme, S. and 55 additional authors (2014) Global diversity and geography of soil fungi. *Science* 346: 1256688. doi: 10.1126/science.1256688

Thompson, J.D. (2005) *Plant evolution in the Mediterranean*. UK, Oxford: Oxford University Press, 304 pp.

Truong, C., Mujic, A.B., Healy, R., Kuhar, F., Furci, G., Torres, D., Niskanen, T., Sandoval-Leiva, P.A., Fernández, N., Escobar, J.M., Moretto, A., Palfner, G., Pfister, D., Nouhra, E., Swenie, R., Sánchez-García, M., Matheny, P.B. & Smith, M.E. (2017) How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. *New Phytologist* 214: 913–919. doi: 10.1111/nph.14509

Truong, C., Gabbarini, L.A., Corrales, A., Mujic, A.B., Escobar, J.M., Moretto, A. & Smith, M.E. (2019) Ectomycorrhizal fungi and soil enzymes exhibit contrasting patterns along elevation gradients in southern Patagonia. *New Phytologist* 222: 1936–1950. doi: 10.1111/nph.15714

Vittadini, C. (1831) Monographia Tuberacearum. Mediolani, 1–88.

Wang, B. & Qiu, Y.L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299–363. doi: 10.1007/s00572-005-0033-6

White, T.J., Bruns, T.D., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols*. San Diego: Academic Press, pp. 315–322.

Xu, W., Gong, L.F., Pang, K.L. & Luo, Z.H. (2017) Fungal diversity in deep-sea sediments of a hydrothermal vent system in the Southwest Indian Ridge. *Deep-Sea Research* 131: 16–26. doi: 10.1016/j.dsr.2017.11.001

Zamani, S.M., Goltapeh, E.M., Safaie, N. & Pedram, M. (2018) Diversity of ectomycorrhizal fungi recovered from the roots of oak trees in Hyrcanian forests of Iran. *Iranian Journal of Forest and Poplar Research* 26: 291–305.

Zamora JC, Svensson M, Kirschner R, and more than 100 hundred additional authors (2018) Considerations and consequences of allowing DNA sequence data as types of fungal taxa. IMA Fungus 9:167. doi: 10.5598/imafungus.2018.09.01.10

Zhao, K., Wu, G. & Yang, Z.L. (2014) A new genus, *Rubroboletus*, to accommodate *Boletus sinicus* and its allies. *Phytotaxa* 188: 61–77. doi: 10.11646/PHYTOTAXA.188.2.1

TABLE 1. List of taxa used in phylogenetic analyses. Newly studied collections are in bold. Collection data (country, vouchers) and ITS GenBank accession numbers are provided.

Cortinarius section and species		Collection information	ITS
Anomali	*C. albocyaneus* (T)	Sweden, Jmt, CFP1177 (S)	KX3022 06
	C. anomalus (T)	Sweden, Ang, CFP1154 (S)	KX3022 24
	C. caninus (T)	Sweden, Jmt, CFP627 (S)	KX3022 50
Calochroi	*C. fulvocitrinus* (T)	France, Doubs, CFP893 (O)	DQ6633 09
	C. violaceipes (T)	France, Vaucluse, *P. Möenne-Loccoz*, PML4731 (PC)	DQ6633 71
Dermocybe	*C. cinnamomeus* (T)	Sweden, F44851 (S)	NR_131 816
	C. croceus (T)	Finland, *K. Liimatainen & T. Niskanen*, H6031266 (H)	NR_131 863
Fulvecentes/La	*C. badiovinaceus* (T)	Austria, *M. Moser*,	HQ8451

eti		MM1950/0061 (IB)	69
	C. bulliardioides	Finland, *T. Niskanen*, TN04-582 (H)	KX3886 47
	C. cadi-aguirrei (T)	Spain, Lleida province, Isil and Alòs, Bordes d'Alòs, *J. Ballarà*, JB-3089/98	KJ86695 3
	C. detonsus	Canada, British Columbia, Motase Peak, field collection 78	MG7862 56
	C. floccopus (T)	France, G00052633 (G)	NR_121 537
	C. fulvescens (T)	Finland, *T. Niskanen*, TN04-935 (H)	NR_153 077
	C. fulvescentoideus (T)	Slovakia, *K. Liimatainen & T. Niskanen*, TN03-1634 (H)	NR_153 078
	C. laetus	Canada, UBC-F15817 (UBC)	FJ15703 4
	C. nymphatus (T)	Finland, *I. Kytövuori*, IK95-1549 (H)	NR_153 079
	C. ochrolamellatus	**Norway, Telemark, Porsgrunn, Kongkleivåsen S, *T.E. Brandrud, B. Dima*, TEB 282-19 (O)**	**MT4463 18**
	C. ochrolamellatus	**Norway, Telemark, Porsgrunn, Brattås, *T.E. Brandrud*, TEB 939-11 (O): O-F-248861**	**MT4463 19**
	C. ochrolamellatus	**Norway, Telemark, Porsgrunn, Blekebakken NR, *T.E. Brandrud, B. Dima*, TEB 556-15 (O); (BOLD database**	**MT4463 20**

		code CAFUN071-17)	
	C. ochrolamellatus	**Spain, Catalunya, Barcelona province, Sagàs, La Quar, *J. Ballarà*, JB-9160-16, MA-90456 (MA)**	**MT4463 21**
	C. ochrolamellatus **(T)**	**Spain, Catalunya, Barcelona province, Montmajor, Mare de Déu del Bosc, *J. Ballarà*, JB-9034-16, MA-90455 (MA)**	**MT4463 22**
	C. ochrolamellatus	**Spain, Catalunya, Barcelona province, Dosrius, Canyamars, *J. Ballarà*, JB-8242-13, MA-90447 (MA)**	**MT4463 23**
	C. ochrolamellatus	**Spain, Aragón, Huesca province, Jaca, Bernués, *P. García*, MES-4519, MA-90457 (MA)**	**MT4463 24**
	C. ochrolamellatus	**Italy, Sardinia, Nuoro, Gavoi, Lago di Gusana, *K. Liimatainen & T. Niskanen*, TN04-1087, 7017993 (H)**	**MT4463 25**
	C. ochrolamellatus	**Italy, Sardinia, Nuoro, Gavoi, Lago di Gusana, *K. Liimatainen & T. Niskanen*, TN04-1113, 7017817 (H)**	**MT4463 26**
	C. ochrolamellatus	Iran, Hyrcanian Forests, Gilan or Mandazaran province (precise locality not indicated)	FR85226 4 (EcM root tip ITS data)
	C. ochrophyllus **(T,**	**Sweden, Smoland, Femsjö,**	**MT4463**

	isoneotype)	Kyrkemosse, *M. Moser, 19790265 (IB)*	27
	C. pseudobulliardioides (T)	USA, Washington, *J. Ammirati & K. Liimatainen*, TN11-452 (H)	KX388641
	C. subfloccopus (T)	France, *P. Moënne-Loccoz*, GK432473 (G)	JQ746615
	C. tenuifulvescens (T)	Canada, Newfoundland and Labrador, *K. Liimatainen & T. Niskanen*, 7000956 (H)	NR_153080
	C. veregregius (T)	France, 0090322 (PC)	HQ845174
Illumini	*C. illuminus* (T)	Sweden, F44877 (S)	KP866156
	C. microglobisporus (T)	Italy, Borgo val di Taro, *E. Borghi,* IB20110123 (IB)	NR_153027
Infracti	*C. infractiflavus*	Canada, SMI286, UBC-F16391 (UBC)	FJ039612
	C. infractus	Germany, Eschweiler, TUB011441 (TUB)	AY174781
	C. persoonianus (T)	France, *A. Bidaud*, AB97-11-496 (PC)	KF732382
Obtusi	*C. acutovelatus*	Canada, *J. Ammirati*, UBC-F15818 (UBC)	FJ157001
	C. acutus	Norway, OS576	KC842420
	C. obtusus	Norway, OS577	KC842421
	C. obtusus	USA, SAT00-298-30	FJ71755

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.
Cortinarius ochrolamellatus (Agaricales, Basidiomycota): a new species in C. sect. Laeti, with comments on the origin of its European-Hyrcanian distribution. *Phytotaxa* 2020 ;Volum 460.(3) s. 185-200

			0
	C. pseudocandelaris	Canada, British Columbia, OC93, UBC-F17165 (UBC)	GQ1599 08
	C. rigens	Canada, British Columbia, OC101, UBC-F17173 (UBC)	GQ1597 63
Purpurascente s	*C. collocandoides* (T)	France, *G. Redeuilh & P. Reumaux*, PML5087 (PC)	KF73228 7
	C. purpurascens (T)	Sweden, *I. Kytövuori*, IK98-2121 (H)	NR_130 252
	C. subporphyropus	Hungary, *B. Dima*, M0275858	KT1592 28

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.
Cortinarius ochrolamellatus (Agaricales, Basidiomycota): a new species in C. sect. Laeti, with comments on the origin of its European-Hyrcanian distribution. *Phytotaxa* 2020 ;Volum 460.(3) s. 185-200

TABLE 2. Estimated divergence ages for the crown nodes representing *Cortinarius* sect. *Laeti* and the new species *C. ochrolamellatus* based on different analyses implementing alternative BEAST model settings. Dating A, B and C used the mean, minimum and maximum values, respectively, for the inferred *Cortinarius*-specific ITS substitution rate calculated in Ryberg & Matheny (2012). "Coal." indicates the use of a Coalescent: Constant size tree prior; "BD", a Speciation: Birth-Death Process; "relaxed", an uncorrelated lognormal relaxed clock. Age units are million years ago (Ma). Values within parentheses represent estimated 95% HPD age intervals).

	Dating A (coal./relaxed)	Dating A (BD/relaxed)	Dating B (coal./relaxed)	Dating B (BD/relaxed)	Dating C (coal./relaxed)	Dating C (BD/relaxed)
Crown *Cortinarius* sect. *Laeti*	3.45 (4.86–2.4)	3.52 (4.76–2.51)	5.08 (7.1–3.41)	5.2 (7.08–3.74)	2.52 (3.54–1.71)	2.6 (3.52–1.86)
Crown *C. ochrolamellatus*	0.38 (0.74–0.11)	0.42 (0.83–0.14)	0.56 (1.13-0.18)	0.62 (1.22–0.19)	0.28 (0.55–0.09)	0.31 (0.62–0.11)

Garrido-Benavent, Isaac; Ballara, Josep; Liimatainen, Kare; Dima, Bálint; Brandrud, Tor Erik; Mahiques, Rafael.