



# Morphology and molecular phylogeny of four new species of *Entoloma* (*Entolomataceae*, *Agaricales*) from Europe and Western Eurasia

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## Abstract

*Entoloma* is one of the largest genera of *Agaricales* in terms of species diversity and is widespread throughout the world. In the present study, four new species, namely *Entoloma brunneofibrillosum*, *E. humidiphilum*, *E. ochraceodiscum*, and *E. colchicum*, are introduced as new to science. These species are described based on specimens collected in Cyprus, Georgia, Hungary, Italy, Russia, Spain, and Türkiye, including morphological characteristics and phylogenetic analyses of the nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. *Entoloma brunneofibrillosum* is recognized by its brown to dark brown pileus with conspicuous dark, radial fibrils, a pale brown stipe with glistening fibrils, and usually fusiform to broadly clavate cheilocystidia. It belongs to the *Undulatosporum* clade. *Entoloma humidiphilum* (subg. *Alboleptonia*) is close to *E. niveum* from New Zealand but differs by a completely pruinose or minutely squamulose pileus surface, narrowly cylindrical to cylindrical pileipellis elements with a deep median constriction, and by occurring in riparian habitats. *Entoloma ochraceodiscum* is characterized by funnel-shaped basidiomata with a deeply depressed yellowish-brown pileus and belongs to the section *Griseorubida*. *Entoloma colchicum* (subg. *Nolanea*) is similar to *E. ortonii* but differs by its distinctive radially fibrillose or velutinous pileus and the absence of odour. The new species are presented with photographs, line drawings, and comparisons with similar taxa.

**Keywords** *Basidiomycota* · Molecular systematics · Molecular marker · New species · Taxonomy

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## Introduction

The genus *Entoloma* (Fr.) P. Kumm. is one of the most species-rich genera in *Agaricales* (Noordeloos et al. 2018; Kalichman et al. 2020) with approximately 2000 species known so far. The genus is traditionally divided into up to 13 subgenera, based on morphological characteristics such as form of the basidiomata, structure of the pileipellis, shape of the basidiospores, presence/absence and shape of cystidia, and presence/absence of clamp connections (Romagnesi 1978; Noordeloos 1981, 2004; Noordeloos and Gates 2012). These traditionally defined subgenera are treated at the rank of genera by some authors (Largent 1994; Baroni and Lodge 1998; Aime et al. 2010; Baroni and Halling 2000; Karstedt et al. 2020; Niveiro et al. 2022). Phylogenetic studies (Co-David et al. 2009) show that these subgenera in many cases are not monophyletic. Several subgenera were revised, e.g. *Pouzarella* (Mazz.) Noordel. (He et al. 2013), *Leptonia* (Fr.) Noordel. (Morozova et al. 2014a), *Trichopilus* (Romagn.) Noordel. (Haelewaters et al. 2020), *Nolanea* (Fr.) Noordel.

(Reschke et al. 2022a), and *Cyanula* (Romagn.) Noordel. (Noordeloos et al. 2022a). The studies regarding other subgenera are still continuing. Some clades are currently not ascribed to a certain subgenus, such as the /*Rhombisporum* clade (Noordeloos et al. 2022b) and the clades containing *E. undulatosporum* Arnolds & Noordel. and *E. indutoides* (P.D. Orton) Noordel. (Noordeloos et al., unpublished data).

*Entoloma* species are assumed to be mostly saprotrophic, but some are ectomycorrhizal, and a few are parasitic on other mushrooms (Noordeloos 1992, 2004, 2012; Co-David et al. 2009; Noordeloos and Gates 2012). Their habitats span a broad range, from tropical to arctic regions, and from grasslands, wetlands to various types of forests and woodlands (Horak 1980; Largent 1994; Noordeloos 1992, 2004; Noordeloos and Gulden 2004; Horak 2008; Noordeloos 2012; Noordeloos and Gates 2012; Noordeloos et al. 2022c).

The knowledge about the genus *Entoloma* in Türkiye is rather limited and fragmented. To date, only 61 species of *Entoloma* have been reported from this country (Kaygusuz and Battistin 2020; Sesli et al. 2020; Solak and Türkoğlu 2022), accounting for nearly 3% of the worldwide diversity of the genus. On account of the species richness reported from Europe, i.e. approximately 400 species (Noordeloos 1992, 2004; Weholt et al. 2014; Noordeloos et al. 2020; Kokkonen 2021; Vila et al. 2021; Noordeloos et al. 2022a, b, c), it is to be expected that the actual number of species occurring in Türkiye is substantially larger. During the ongoing investigation of agarics in Türkiye, several specimens of *Entoloma* were collected. Separately, similar *Entoloma* collections had previously been gathered in some European (Hungary, Italy, and Spain) and Western Eurasian countries (Cyprus, Georgia, and Russia). Detailed investigations of these species revealed that they could not be assigned to any known species in the available databases. Among those, four new species are described here as new to science based on morphological characteristics and molecular sequence data from the ITS region.

## Materials and methods

### Morphological study

Fresh basidiomata for this study were collected in Türkiye, also in Cyprus, Georgia, Hungary, Italy, Russia, and Spain. Macro-morphological characteristics were recorded based on photographs taken in situ and field notes, including the morphological features of each basidioma, substrate type, ecology, and habitat. The specimens were dried in a dehydration machine at 30 °C for 24 h, packed in plastic bags, and then stored. The colour codes used follow the Munsell Soil Color Charts (Munsell 1975). Micro-morphological examinations of the dried specimens were done with free-hand

sections mounted in 5% potassium hydroxide (KOH) (w/v), 1% Congo red (w/v), and/or distilled water, followed by observation with a light microscope. For each specimen studied, at least 20 basidia and cystidia and 30 basidiospores were measured. The mean dimensions of the basidiospores are indicated as  $L^m$  for length and  $W^m$  for width, with the ratio of length-to-width represented by  $Q$ , and the mean ratio of the  $Q$  values for individual basidiospores signified as  $Q^m$ . The number of full-length lamellae ( $L$ ) and the number of series of lamellulae ( $l$ ) were used to calculate the spacing of the lamellae. The holotype specimens are deposited in the fungarium of Isparta University of Applied Sciences (ISUF). The Russian and Georgian collections are deposited in the Mycological Herbarium of the Komarov Botanical Institute RAS (LE), the Hungarian collection is deposited in the fungarium of Eötvös Loránd University, Budapest (ELTE), the Italian collection is deposited in the herbarium Naturalis, Leiden (L), and the Spanish specimen is deposited in the private fungarium of K. Reschke.

### DNA study and phylogenetic analyses

The genomic DNA of *Entoloma* specimens was isolated using the innuPREP Plant DNA Kit (Analytik Jena, Jena, Germany) and the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp, Ontario, Canada). Polymerase chain reaction (PCR) amplification and sequencing were done following Kaygusuz et al. (2020) and Reschke et al. (2022b). The nuclear rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) was amplified using the primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). PCR products were purified and sequenced using the same primers at Microsynth Seqlab (Göttingen, Germany), Sanger DNA Sequencing Service of Source Bioscience (Berlin, Germany), and the LGC Genomics (Berlin, Germany). Raw DNA data were edited and assembled using MEGA X v.10.0.5 (Kumar et al. 2018) and BioEdit (Hall 1999).

Thirty-two new nrDNA ITS sequences from *Entoloma* collections were generated in this study (GenBank no PP391296-PP391327). BLASTn searches were performed on the NCBI GenBank, and additional sequences with high similarity to the newly identified species were downloaded from the National Centre for Biotechnology Information (NCBI, [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) databases, as well as from recent publications (Co-David et al. 2009; Battistin et al. 2014; Morozova et al. 2014b; Raj et al. 2014; Vila et al. 2014, 2021; Kim et al. 2015; Kokkonen 2015; Crous et al. 2016; Raj and Manimohan 2016; He et al. 2019; Karstedt et al. 2020; Sammut 2021; Sleiman et al. 2021; Xing et al. 2021; Jayawardena et al. 2022; Noordeloos et al. 2022a, b; Reschke et al. 2022a, b; Voto 2023). The multiple sequence alignment was conducted using MAFFT 7.11 (Katoh et al. 2019) with the E-INS-i algorithm. The alignment was then

manually edited using AliView V.1.28 (Larsson 2014) where necessary. After alignment, ambiguous sites at the beginning and end of the dataset were trimmed. The best evolutionary model was estimated using MrModeltest 2.3 (Nylander 2004). Phylogenetic reconstructions were made using the methods of Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was performed with RAxML-HPC2 version 8.2.12 on XSEDE, using the CIPRES web portal (Miller et al. 2010) under the GTRGAMMA model with 1000 bootstrap iterations. The BI analysis was conducted using MrBayes 3.2.5 (Ronquist et al. 2012) with the Markov Chain Monte Carlo (MCMC) method under a GTR substitution model for 5,000,000 generations, with trees sampled every 1000th generation. FigTree v1.4.4 (Rambaut 2018) was used to show phylogenetic trees.

## Results

### Phylogeny

In this study, a total of thirty-two sequences of *Entoloma* spp. from Georgia, Hungary, Italy, Russia, Spain, and Türkiye were generated and deposited in GenBank. Together with 93 sequences downloaded from GenBank, the final ITS dataset comprises 126 sequences, including *E. coerulogracilis* G.M. Gates & Noordel., *E. haastii* G. Stev., and *E. perbloomii* Noordel. as outgroup. The aligned matrix comprises 929 characters (including gaps), of which 423 are parsimony-informative, 407 constant sites, and 99 singleton sites. The phylogenetic tree topologies obtained through both, ML and BI analyses, were largely consistent. The ML tree was chosen for presentation, with statistical support indicated through Maximum Likelihood Bootstrap (MLB) and Bayesian Posterior Probabilities (BPP) values (Fig. 1).

The phylogenetic analyses showed that the sequences of the new *Entoloma* species cluster in four distinct phylogenetic lineages that do not correspond to other species represented by data in our analysis. The first lineage, with high statistical support (MLB = 100%, BPP = 1.0), consists of five specimens from Türkiye and one from Hungary of the new species *E. brunneofibrillosum*, which clustered together with an undescribed species of *Entoloma* (LE315862) from Sweden. The second lineage is formed by sequences of the new species *E. humidiphilum* from Türkiye (MLB = 100%, BPP = 1.0), which is phylogenetically sister to *E. niveum* G. Stev. (PDD104142) from New Zealand. The third lineage, with high statistical support (MLB = 100%, BPP = 1.0), comprises the new species *E. ochraceodiscum* from Italy, Spain, and Türkiye, *E. contortisporum* Noordel. & Hauskn. (WU27137), *E. indutoides* (WU19603 and K(M)108968), *E. indutoides* var. *pleurocystidium* Noordel., Wölfel & Hauskn. (FA 3294), and an undescribed species of *Entoloma*

(FCG18). The fourth lineage consists of nine collections of *E. colchicum* from Cyprus, Georgia, Russia, and Türkiye and is distinct from other known *Entoloma* species with high statistical support (MLB = 100%, BPP = 1.0).

The newly presented *Entoloma* sequences are distinct from all sequences of the ITS region available for other *Entoloma* species. Since the specimens from which the sequences were obtained are also morphologically distinct from all other known *Entoloma* species, we propose these species here as new to science.

### Taxonomy

*Entoloma brunneofibrillosum* Kaya, Kaygusuz, Reschke, Noordel., Dima, Vrba & M. Piepenbr., sp. nov. (Figs. 2 and 3)

*Mycobank*: MB852521

*Holotype*: TÜRKİYE, Adıyaman Province, Central district, in Ziyaret village, at 37°45'N, 38°20'E, among grass on sandy soil, stream side, 560 m asl., 05 March 2009, leg. A. Kaya (K. 5928). GenBank ITS: PP391296.

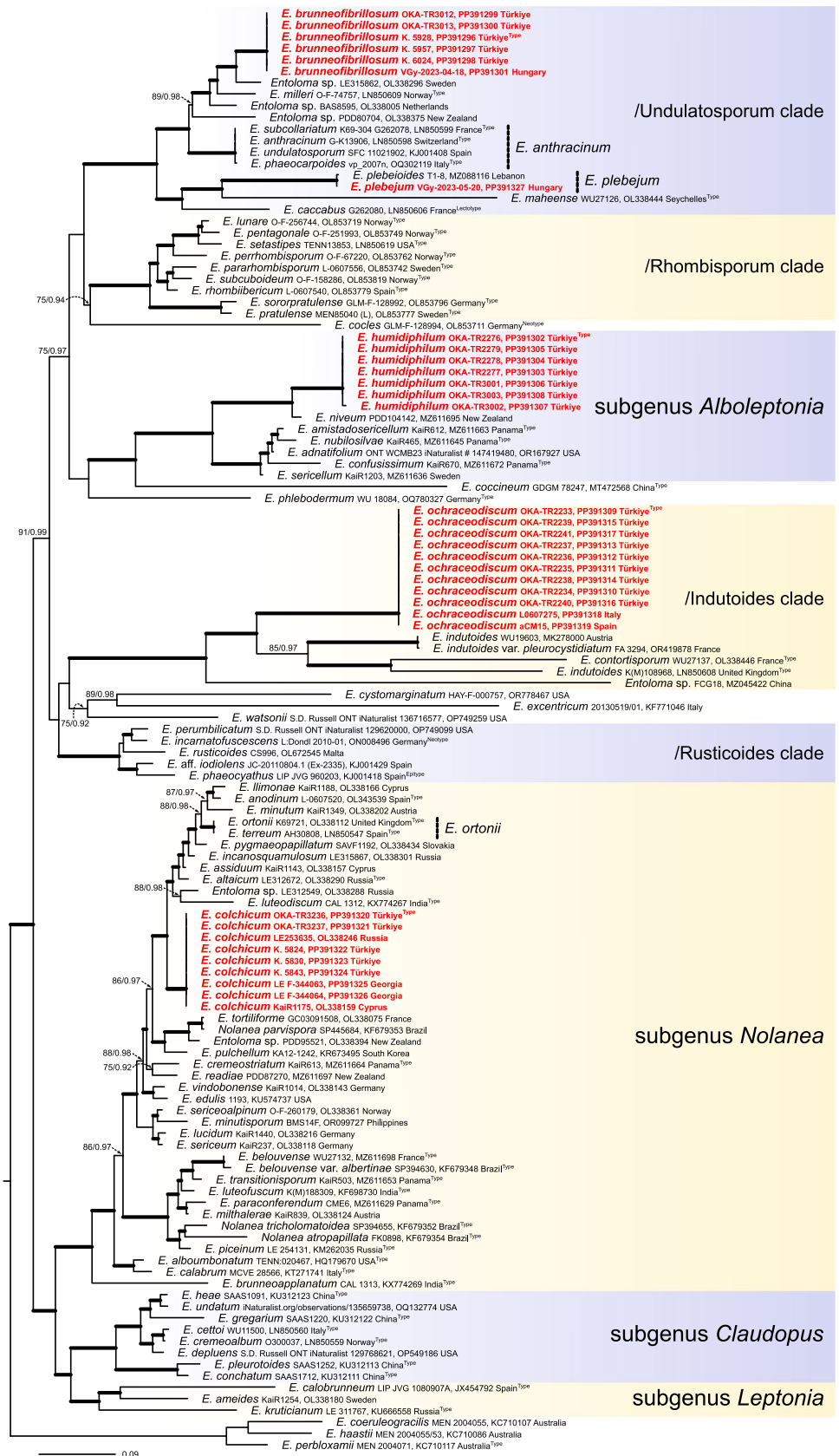
*Etymology*: Referring to the distinctly dark brown fibrillose pileus surface.

*Diagnosis*: Similar to the likewise vernal *Entoloma plebejum* with regard to the more or less tricholomatoid habit and prominent cheilocystidia, differing particularly by the smaller basidiospores and by distinct ITS sequences.

*Description*: Basidiomata small, tricholomatoid. Pileus 15–25 mm diam., conico-convex when young, expanding to convex or plano-convex, with or without low broad umbo, sometimes slightly depressed at the centre when old, with slightly deflexed then straight margin when young, the surface often completely opaque, not hygrophanous, not translucently striate, brown to dark brown (7.5YR 6–4/2) or tinged darker brown especially around the centre (5YR 5–3/2), with distinct radial dark fibrils on pale background, sometimes cracked in old basidiomata. Lamellae moderately crowded, L = 35–50, l = 3–5, predominantly adnexed, sometimes broadly adnate to slightly decurrent in basidiomata with depressed pileus, thin, ventricose, beige-brown, with an entire and concolorous edge. Stipe 25–35 × 2.0–3.0 mm, central, cylindrical, slightly curved at the base, surface pale brown, covered with longitudinal glistening fibrils. Basal mycelium white, cottony, sometimes with a few rather thick and cottony rhizomorphs. Context in stipe and pileus pale brown. Smell and taste indistinct.

Basidiospores (7.8)8.5–11.0(11.7) × 6.0–7.5 μm ( $n = 170$  of 4 coll.),  $L^m \times W^m = 9.5 \times 6.8$  μm,  $Q = (1.1)1.3–1.6(1.7)$ ,  $Q^m = 1.4$ , heterodiametrical, with 8–11, mostly nodulose angles in side view, guttulate, thick-walled. Basidia 38–55 × 8.0–13.0 μm, narrowly clavate to clavate, 4-spored, hyaline, thin-walled. Lamellar edge heterogeneous. Cheilocystidia (45)50–80 × 12–20 μm ( $n = 60$  of 3 coll.),  $L^m \times W^m = 63 \times 17$  μm, scarce, mostly fusiform

**Fig. 1** Phylogenetic tree derived from Maximum Likelihood (ML) analyses of nrDNA ITS sequences. The tree is rooted with a clade formed by *E. coeruleogracilis* G.M. Gates & Noordel. (MEN 2004055), *E. haastii* G. Stev. (MEN 2004055/53), and *E. perbloomii* Noordel. (MEN 2004071). Maximum Likelihood Bootstrap (MLB)  $\geq 75\%$  and Bayesian Posterior Probabilities (BPP)  $\geq 0.90$  values are indicated above branches. Branches highlighted in bold represent particularly strong support, representing  $MLB \geq 90\%$  and  $BPP \geq 0.95$ . Species names, collection numbers, GenBank accession numbers, and geographical origins of the sequences used are provided. The newly generated sequences are highlighted in bold red. Bar = number of estimated changes/nucleotide



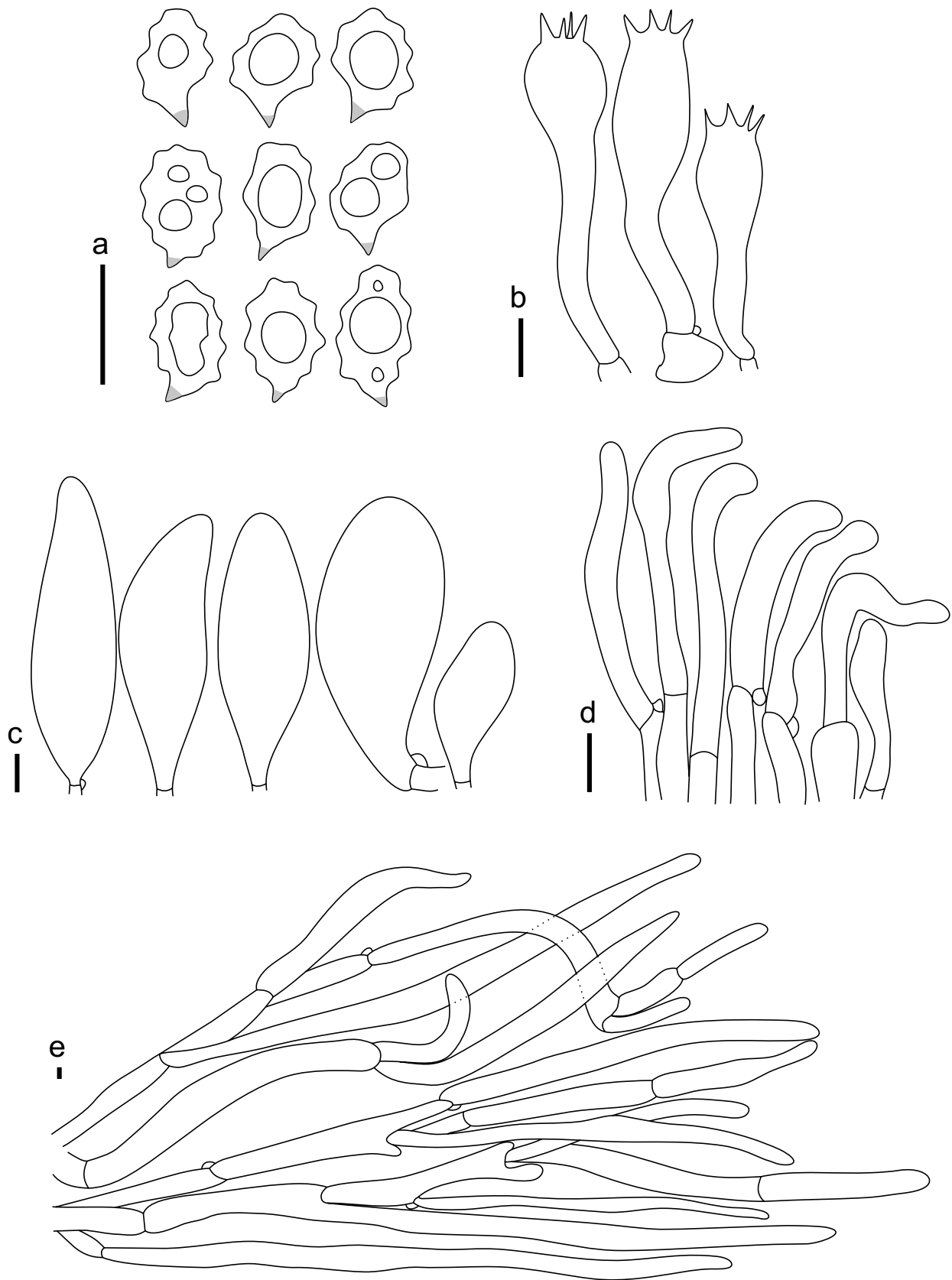


**Fig. 2** Basidiomata of *Entoloma brunneofibrillosum*. **a, b** Collection K. 5928 (holotype). **c, d** Collection K. 5957. **e, f** Collection K. 6024. Bars = 10 mm

to broadly clavate, hyaline, thin-walled. Pleurocystidia absent. Hymenophoral trama regular, made up of cylindrical hyphae, 8–15  $\mu\text{m}$  wide. Pileipellis a cutis with numerous fascicles of ascending hyphae forming a trichoderm composed of branched hyphae constricted at the septa; 65–320(–350)  $\times$  8–25  $\mu\text{m}$  terminal elements elongate cylindrical, with light brown intracellular pigment, thin-walled. Stipitipellis a cutis of elongate, cylindrical hyphae, 4–10  $\mu\text{m}$  wide. Caulocystidia 30–50  $\times$  5–7  $\mu\text{m}$ , narrowly cylindrical,

sometimes narrowly clavate, with obtuse or subcapitate apex, hyaline, thin-walled. Brilliant granules absent. Clamp connections present in all parts examined.

*Ecology, phenology, and distribution:* Basidiomata solitary or in small groups on the ground, in temperate grasslands with annual herbaceous plants, on calcareous loamy or sandy soil, at altitudes of 500–800 m asl. Mostly collected in the spring (March–May) but also in autumn (November). So far known from Hungary and Türkiye.



**Fig. 3** Microscopic features of *Entoloma brunneofibrillosum* (K. 5928, holotype). **a** Basidiospores. **b** Basidia. **c** Cheilocystidia. **d** Caulocystidia. **e** Pileipellis. Bars = 10  $\mu$ m

**Additional specimens examined:** HUNGARY, Komárom-Esztergom County, Vértes Hills, Tatabánya-Síkvölgy, 18 April 2023, leg. Gy. Vrba (VGy-2023–04-18, GenBank ITS: PP391301). TÜRKİYE, Adıyaman Province, Gerger district, in Budaklı village, at 38° 03' N, 38° 59' E, on grasslands, 590 m asl., 07 March 2009, leg. A. Kaya (K. 5957, GenBank ITS: PP391297); *ibid.*, Kahta district, Boğazkaya village, at 37° 53' N, 38° 32' E, on grasslands, 820 m asl., 12 March 2009, leg. A. Kaya (K. 6024, GenBank ITS: PP391298). Denizli Province, Pamukkale district, in Zeytin Upland, at 37° 43' 14.2" N, 29° 07' 21.9" E, on calcareous soil, under *Quercus coccifera* L., 800 m asl., 03 November 2016, leg. O. Kaygusuz (OKA-TR3012, GenBank ITS: PP391299); *ibid.*, at 37° 43' 20.8" N, 29° 07' 13.7" E, on calcareous soil, under *Q. coccifera*, 780 m asl., 20 November 2018, leg. O. Kaygusuz (OKA-TR3013, GenBank ITS: PP391300).

**Discussion:** *Entoloma brunneofibrillosum* is a member of the /Undulatosporum clade. Phylogenetically, *Entoloma brunneofibrillosum* is close to an undescribed species (LE315862) from Sweden and clusters with the holotype sequence of *E. milleri* Noordel. (O-F-74757) and two other undescribed taxa from the Netherlands (BAS8595) and New Zealand (PDD80704) (Fig. 1). The percentage of similarity of ITS sequences between *E. brunneofibrillosum* and *E. milleri* and three undescribed *Entoloma* species (BAS8595, LE315862, and PDD80704) is 92%, 92.4%, 94.5%, and 90%, respectively. Morphologically, *E. milleri* differs from *E. brunneofibrillosum* by its slightly shorter basidiospores (8.1–10.9 × 6.6–8.1 μm) and the absence of cheilo- and caulocystidia (Noordeloos and Gulden 2004; Kokkonen 2015).

*Entoloma plebejum* is most similar to *E. brunneofibrillosum*, as both species have a tricholomatoid habit and prominent cheilocystidia. It differs from *E. brunneofibrillosum* by larger basidiospores (12.0–12.8 × 8.0–8.5 μm) (Noordeloos et al. unpublished data).

Several other European species in the /Undulatosporum clade, which mostly have a very dark brown to black pileus, are superficially similar to *E. brunneofibrillosum*, e.g. *E. rancidodorum* Noordel. & Hauskn., *E. triste* (Velen.) Noordel., *E. undulatosporum*, and *E. winterhoffii* Wölfel & Noordel. However, *E. rancidodorum* differs from *E. brunneofibrillosum* by a slightly larger pileus (up to 40 mm diam.) with emarginate lamellae that are pinkish-reddish when mature, and a distinctive rancid-farinaceous odour (Noordeloos and Hausknecht 2002; Noordeloos 2004). *Entoloma triste* has a darker brown pileus than *E. brunneofibrillosum*, and it can have a glabrous or slightly plush-tomentose pileus, especially at the centre, longer basidiospores (8.5–13.5 × 6–9 μm), absence of the cheilocystidia, and a farinaceous odour (Wölfel and Noordeloos 1997; Noordeloos 2004). *Entoloma undulatosporum*, which is widely distributed in Europe, differs by its darker pileus that is distinctly hygrophanous, the absence of cheilocystidia, a remarkable farinaceous odour,

a preferential habitat in dunes, and most commonly occurs during the winter season (Noordeloos 2004). *Entoloma winterhoffii* is recognized by its mycenoid habit, a deeply striate pileus which is strongly hygrophanous, longer basidiospores (8.5–13.5 × 6.0–8.5 μm), and a lack of cheilocystidia (Wölfel and Noordeloos 1997; Noordeloos 2004).

Concerning other European species, *E. anthracinum* (J. Favre) Noordel. is similar morphologically to *E. brunneofibrillosum* but differs by its translucently striate pileus, a yellowish stipe, and 6-angled and broader basidiospores (8.7–11.7 × 7.7–9.5 μm) (Favre 1955; Noordeloos 1984).

***Entoloma humidiphilum*** Kaygusuz, Reschke, Kaya, Noordel. & M. Piepenbr., sp. nov. (Figs. 4 and 5)

**Mycobank:** MB852522

**Holotype:** Türkiye, Burdur Province, Bucak district, close to Karacaören, in Sweetgum Forest Nature Protection Area, at 37° 21' 39.4" N, 30° 49' 58.7" E, at the border of a river, on the soil in the Mediterranean forest dominated by *Liquidambar orientalis*, 275 m asl., 09 November 2019, leg. O. Kaygusuz (OKA-TR2276). GenBank ITS: PP391302.

**Etymology:** Referring to its habitat preference for the riparian environment.

**Diagnosis:** *Entoloma humidiphilum* differs from other species of the subgenus *Alboleptonia* by a unique combination of characteristics, i.e. small basidiomata, a completely pruinose or minutely squamulose pileal surface, mostly narrowly utriform cheilocystidia, narrowly cylindrical to cylindrical pileipellis elements with deep median constriction, and typically growing along riverbanks.

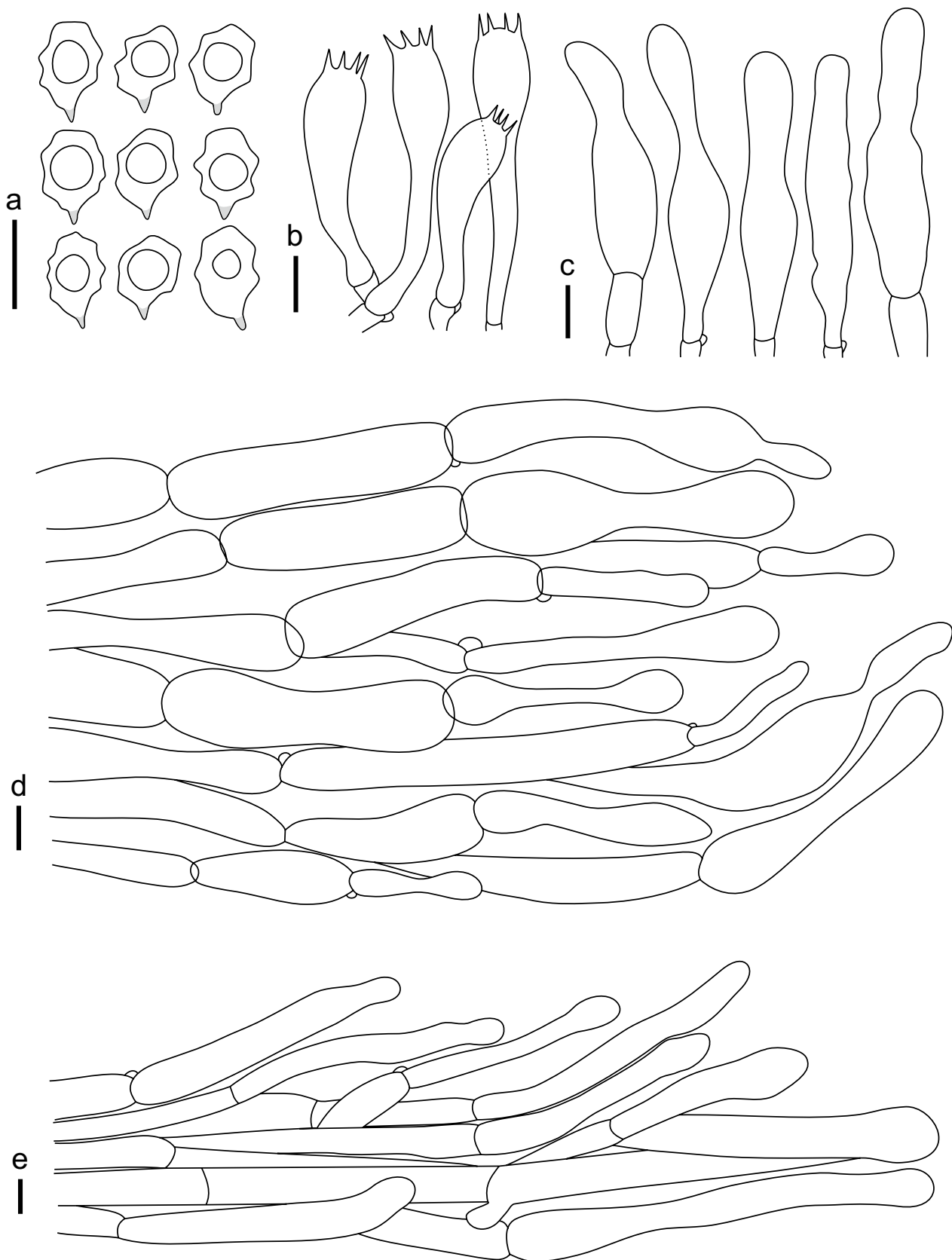
**Description:** Basidiomata collybioid. Pileus 3–15 mm diam., at first hemispherical, becoming convex, finally expanded to convex, with a slightly depressed to depressed centre, with inflexed margin when young, later straight, pileal surface entirely pruinose or minutely squamulose, opaque, not hygrophanous, initially not translucently striate but becoming slightly striate at the margin with age, white to snow-white in young basidiomata, pale creamish white with age. Lamellae distant to moderately crowded (L = 18–30, l = 1–3), adnate to decurrent, thin, ventricose, white, becoming creamish or pale pink at maturity, with an entire and concolorous edge. Stipe 7–20 × 0.6–1.7 mm, central, cylindrical, surface white to translucently whitish, slightly pruinose. Context in stipe and pileus white to whitish. Smell and taste indistinct.

Basidiospores (8.0)8.5–10.0(10.5) × (6.0)6.5–7.5 μm ( $n=155$  of 4 coll.),  $L^m \times W^m = 9.1 \times 7.0$  μm,  $Q = (1.1)1.2–1.4(1.5)$ ,  $Q^m = 1.3$ , heterodiametrical, 5–6(–7)-angled, sometimes nodulose-angled in side view, guttulate, thick-walled. Basidia 35–55 × 10–13 μm, narrowly clavate to clavate, 4-spored, hyaline, thin-walled. Lamellar edge heterogeneous. Cheilocystidia 45–60 × 7–16 μm ( $n=65$  of 3 coll.),  $L^m \times W^m = 51 \times 10$  μm, scarce, mostly narrowly utriform, sometimes narrowly



**Fig. 4** Basidiomata of *Entoloma humidiphilum*. **a** Collection OKA-TR2276 (holotype). **b** Collection OKA-TR2277. **c** Collection OKA-TR2278. **d** Collection OKA-TR2279. **e** Collection OKA-TR3001. **f** Collection OKA-TR3002. **h** Collection OKA-TR3003. Bars = 5 mm





**Fig. 5** Microscopic features of *Entoloma humidiphilum* (OKA-TR2276, holotype). **a** Basidiospores. **b** Basidia. **c** Cheilocystidia. **d** Pileipellis. **e** Caulocystidia. Bars = 10  $\mu$ m

cylindrical, hyaline, thin-walled. Pleurocystidia absent. Hymenophoral trama regular, consisting of cylindrical to inflated hyphae, 7–16 µm wide. Pileipellis a cutis to a trichoderm, composed of mainly narrowly cylindrical to cylindrical cells with deep median constriction or lageniform, narrowly clavate terminal cells, sometimes with an apical papilla, 50–80×3–19 µm, hyaline, thin-walled. Stipitipellis a cutis of elongate, cylindrical cell 5–15 µm wide. Caulocystidia 65–190×7–16 µm, subcylindrical to cylindrical with subcapitate apex or narrowly clavate, hyaline, thin-walled. Brilliant granules absent. Clamp connections present in all parts examined.

**Ecology, phenology, and distribution:** Basidiomata solitary or in small scattered groups, on submerged soil and then up to 4 mm deep within water or on very wet soil, on limestone sedimentary rocks formed by calcium carbonate in low-temperature fresh water, at an altitude of about 250–300 m asl., in a forest dominated by old *Liquidambar orientalis* with *Adiantum capillus-veneris* in the understory. Collected in autumn (November–October). Hitherto only known from Türkiye.

**Additional specimens examined:** Türkiye, Burdur Province, Bucak district, close to Karacaören, in Sweetgum Forest Nature Protection Area, at 37° 21' 38.0" N, 30° 49' 58.2" E, at the border of a river, on the soil, under *Liquidambar orientalis*, 285 m asl., 10 November 2019, leg. O. Kaygusuz (OKA-TR2277, GenBank ITS: PP391303); *ibid.*, at 37° 21' 37.6" N, 30° 50' 01.3" E, at the border of a river, on the soil, under *L. orientalis*, 300 m asl., 05 November 2020, leg. O. Kaygusuz (OKA-TR2278, GenBank ITS: PP391304); *ibid.*, at 37° 21' 29.5" N, 30° 50' 01.4" E, at the border of a river, on the soil, under *L. orientalis*, 277 m asl., 15 November 2020, leg. O. Kaygusuz (OKA-TR2279, GenBank ITS: PP391305); *ibid.*, at 37° 21' 31.7" N, 30° 50' 02.4" E, at the border of a river, on the soil, under *L. orientalis*, 293 m asl., 12 October 2021, leg. O. Kaygusuz (OKA-TR3001, GenBank ITS: PP391306); *ibid.*, at 37° 21' 35.2" N, 30° 49' 58.3" E, at the border of a river, on the soil, under *L. orientalis*, 277 m asl., 03 October 2022, leg. O. Kaygusuz (OKA-TR3002, GenBank ITS: PP391307); *ibid.*, at 37° 21' 38.7" N, 30° 49' 58.7" E, at the border of a river, on the soil, under *L. orientalis*, 268 m asl., 09 October 2022, leg. O. Kaygusuz (OKA-TR3003, GenBank ITS: PP391308).

**Discussion:** *Entoloma humidiphilum* belongs to the subg. *Alboleptonia* in the strict sense as treated by Reschke et al. (2022b). Phylogenetically, the species with available ITS closest to *E. humidiphilum* is *E. niveum*, described from New Zealand, from which it differs by 23 nucleotide positions, i.e. a similarity of 95%. Morphologically, *E. niveum* differs from *E. humidiphilum* by a larger pileus (up to 40 mm in diam.) and a stipe which is ochraceous at the base (Stevenson 1962).

*Entoloma humidiphilum* is phylogenetically closely related and morphologically similar to further members of the subg. *Alboleptonia*, such as *E. adnatifolium* (Murrill) Blanco-Dios,

*E. amistadosericellum* Reschke & Noordel., *E. confusissimum* Reschke & Noordel., *E. nubilosilvae* Reschke & Noordel., and *E. sericellum* (Fr.) P. Kumm. *Entoloma adnatifolium*, a North American species, differs from the new species by rhomboid basidiospores, vesiform cheilocystidia, a pileipellis made up of cylindrical-clavate cells, and rare and scattered clamp connections except for basidia (Largent and Benedict 1970). *Entoloma amistadosericellum*, *E. confusissimum*, and *E. nubilosilvae*, recently described from Panama (Reschke et al. 2022b), differ from *E. humidiphilum* by the absence of utriform cheilocystidia, and different habitats. *Entoloma sericellum* has a larger pileus (up to 40 mm in diam.), an ochraceous pileus with age, and slightly longer basidiospores (8–11.5×6–9 µm) (Noordeloos 1992).

Within the same subgenus, the European species *E. cuboidalbum* Noordel. & Hauskn. differs from *E. humidiphilum* by cuboid basidiospores (Noordeloos and Hausknecht 2009).

*Entoloma anamikum* Manim., A.V. Joseph & Leelav., *E. pruinocutis* Manim. & Noordel., *E. shwethum* Manim., A.V. Joseph & Leelav., *E. theekshnagandhum* Manim., A.V. Joseph & Leelav., and *E. vanajum* Manim., A.V. Joseph & Leelav., five other species belonging to the subg. *Alboleptonia* have been described from India. *Entoloma anamikum* differs from *E. humidiphilum* by a depressed to deeply infundibuliform pileus with a brownish tinge, considerably longer basidiospores (on av. 12.8×8.4 µm) and the absence of cheilo- and caulocystidia (Manimohan et al. 1995). *Entoloma pruinocutis* differs by a pruinose surface of its pileus and stipe, longer basidiospores (10–12×7–9 µm) and the absence of cheilocystidia (Manimohan et al. 2006). *Entoloma shwethum* differs by more robust basidiomata, larger pileus (up to 50 mm in diam.), longer basidiospores (on av. 10.4×7.8 µm) and ventricose, lageniform, and clavate cheilocystidia (Manimohan et al. 1995). *Entoloma theekshnagandhum* has a yellowish-white pileus, a strong and unpleasant odour, slightly broader basidiospores (7.5–10×7–9.5 µm), and versiform cheilo- and caulocystidia (Manimohan and Leelavathy 1988). *E. vanajum* is characterized by a conico-convex, pellucid-striate pileus, longer and cuboid basidiospores measuring on average 12.1×11.8 µm, and the absence of cheilo- and caulocystidia (Manimohan et al. 1995).

Most of the species of the subg. *Alboleptonia* that are macromorphologically similar to *Entoloma humidiphilum* have been described from tropical or subtropical parts of the world. The subtropical species *Entoloma aripoanum* Dennis from Trinidad and Tobago differs from *E. humidiphilum* by its longer (70–160×10–16 µm) and cylindrical cheilocystidia, and growth on decayed wood (Dennis 1953). *Entoloma crocotillum* Xiao L. He, described from China, differs by a longer stipe (up to 70 mm), slightly longer basidiospores (on av. 11.3×8.5 µm), cylindrical, subfusoid, or broadly clavate cheilocystidia, and the occurrence at high altitudes (up to

2700 m asl.) (He et al. 2015). The Papua New Guinean species *Entoloma maleolens* E. Horak has a pleasant odour and lacks of cheilocystidia (Horak 1980).

The other taxa of the subg. *Alboleptonia*, namely *E. davidii* Noordel. & Co-David and *E. subroseum* (T.J. Baroni & Lodge) Noordel. & Co-David from Puerto Rico (Baroni and Lodge 1998; Co-David et al. 2009), *E. minutoalbum* from New Zealand (Horak 1976), and *E.*

*overeemii* E. Horak from Indonesia (Horak 1976), differ by cuboid to subcuboid basidiospores.

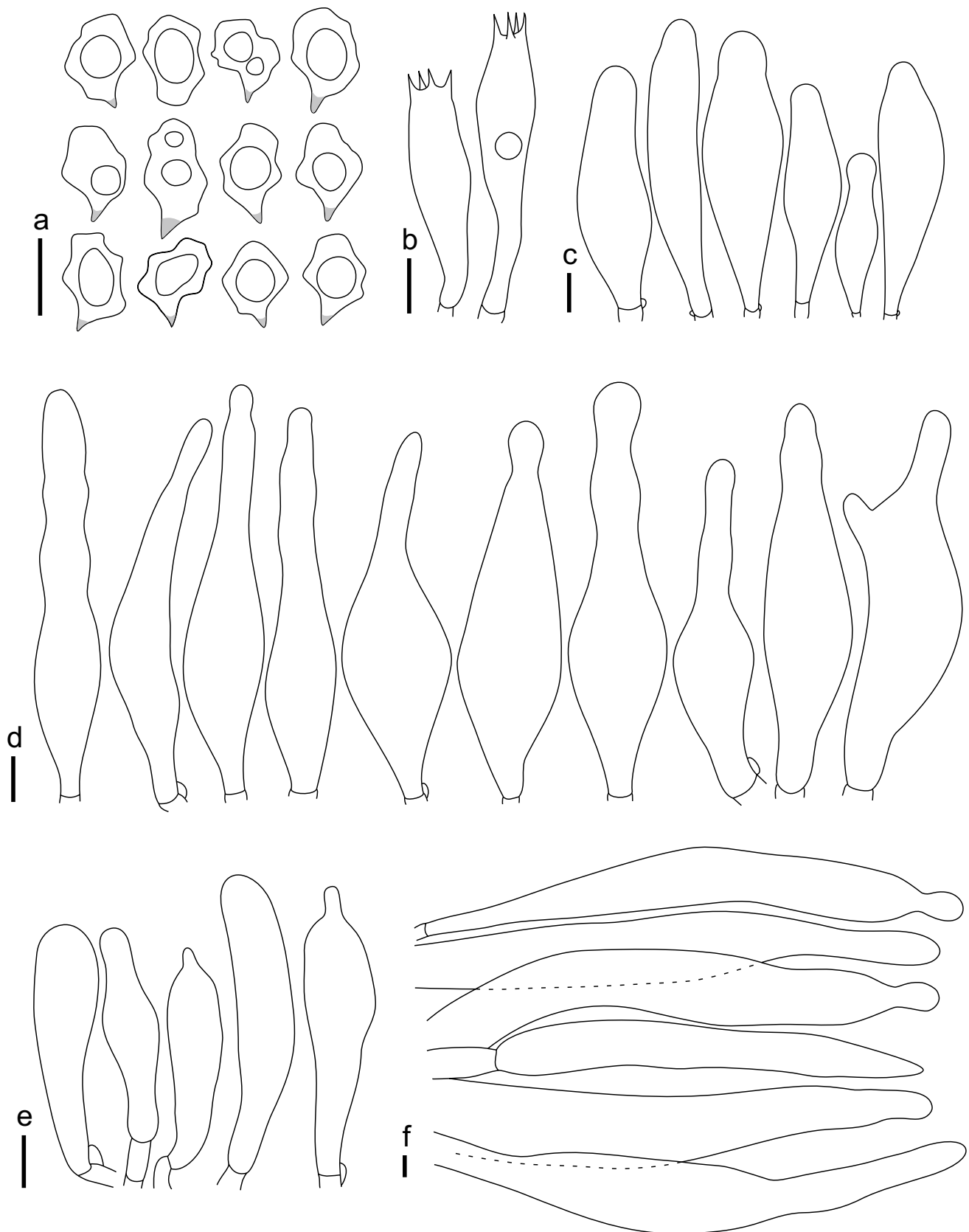
*Entoloma ochraceodiscum* Kaygusuz, Reschke, Kleine, M. Carbone, A. Battaglini, Kaya & M. Piepenbr., sp. nov. (Figs. 6 and 7)

*Mycobank*: MB852523

*Holotype*: Türkiye, Burdur Province, Bucak district, close to Karacaören, in Sweetgum Forest Nature Protection Area,



**Fig. 6** Basidiomata of *Entoloma ochraceodiscum*. **a** Collection OKA-TR2233 (holotype). **b** Collection OKA-TR2234. **c, e** Collection OKA-TR2235. **d** Collection OKA-TR2236. **f** Collection OKA-TR2237. Bars = 10 mm



**Fig. 7** Microscopic features of *Entoloma ochraceodiscum* (OKA-TR2233, holotype). **a** Basidiospores. **b** Basidia. **c** Caulocystidia. **d** Cheilocystidia. **e** Pleurocystidia. **f** Terminal cells of the pileipellis. Bars = 10  $\mu$ m

at 37° 21' 29.2" N, 30° 50' 03.5" E, on calcareous soil, in Mediterranean forest dominated by *Liquidambar orientalis*, 290 m asl., 20 October 2017, leg. O. Kaygusuz (OKA-TR2233). GenBank ITS: PP391309.

**Etymology:** The specific epithet refers to the yellowish-brown (ochraceous) coloration of the centre (discus) of the pileus.

**Diagnosis:** Similar to *E. indutoides*, but differs by the combination of funnel-shaped basidiomata with deeply depressed, yellowish-brown pileus, a pale greyish to yellowish stipe with a dark yellow tinge, 5–8-angled basidiospores, the presence of pleurocystidia and caulocystidia, and by distinct ITS sequences.

**Description:** Basidiomata collybioid to omphalinoid. Pileus 12–27 mm diam., deeply depressed or almost funnel-shaped, margin reflexed, surface opaque, not hygrophanous, not translucently striate, yellowish-brown with a darker yellow–brown centre (2.5Y 8/6, 7–6/8, centre 2.5Y 5/6), becoming dull yellowish upon drying, entirely pruinose to loose fibrillose. Lamellae crowded ( $L = 32\text{--}48$ ,  $l = 1\text{--}3$ ), adnate to somewhat decurrent, thin, ventricose, sometimes rather segmentiform, white, becoming light pink or pale greyish brown with pinkish tinge at maturity, with an entire and concolorous edge. Stipe 15–40 × 0.7–2.0 mm, central, cylindrical or somewhat broadened towards the base, straight or slightly curved, surface pale greyish to yellowish with a noticeable dark yellow tinge especially near the apex, pruinose at the apex. Basal mycelium white, cottony, often with some rather thick and cottony rhizomorphs. Context flexible and tough, brownish in the pileus and the stipe. Smell and taste indistinct.

Basidiospores (10.0)11.0–13.0(14.5) × (7.0)8.5–10.0 μm ( $n = 170$  of 4 coll.),  $L^m \times W^m = 12.0 \times 9.2$  μm,  $Q = (1.1\text{--})1.2\text{--}1.4(-1.5)$ ,  $Q^m = 1.3$ , heterodiametrical, 5–6(–8)-angled, sometimes nodulose-angled in side view, guttulate, thick-walled. Basidia 40–55 × 12–15 μm, clavate, 4-spored, hyaline, thin-walled. Lamellar edge heterogeneous. Cheilocystidia (50)55–80(90) × (11)13–17(18) μm ( $n = 60$  of 3 coll.),  $L^m \times W^m = 71.0 \times 14.5$  μm, abundant, frequently fusiform, ventricose-rostrate to lageniform, usually with a long neck and often a subcapitate apex, hyaline, thin-walled. Pleurocystidia 45–75 × 10–16 μm ( $n = 45$  of 2 coll.), narrowly utriform to narrowly clavate, sometimes with mucronate apex, hyaline, thin-walled. Hymenophoral trama regular, consisting of long, narrowly cylindrical to rather fusiform cells, 6–18 μm wide. Pileipellis a cutis with transitions to a trichoderm, composed of elongated fusiform or cylindrical terminal cells, with subcapitate apex, 120–260 × 13–35 μm, with pale brown intracellular pigment. Stipitipellis a cutis of cylindrical cells, 8.5–24 μm wide. Caulocystidia 40–75 × 10–17 μm, often grouped in clusters, narrowly utriform, fusiform, or clavate, sometimes with a subcapitate apex, hyaline, thin-walled. Brilliant granules absent. Clamp connections present in all parts examined.

**Ecology, phenology, and distribution:** Basidiomata solitary or in small, scattered groups, on moist, sandy soil with a pH range of 8.2 to 8.6, and roughly 6% calcium carbonate, at an altitude of about 300 m asl., in a forest of old *Liquidambar orientalis* trees. Mostly collected in autumn (October–December). So far known from Italy, Spain, and Türkiye.

**Additional specimens examined:** Italy, Tuscany, Bagno a Ripoli (FI), at 43° 43' 43.2" N, 11° 21' 17.8" E, growing on the mossy edge of a dirt road under young *Quercus ilex* L. and *Q. pubescens* Willd. with *Calluna vulgaris* (L.) Hull and few typical Mediterranean shrubs (e.g. *Rhamnus alaternus* L.), 205 m asl., 09 October 2022, leg. A. Battaglini & M. Carbone (L0607275, GenBank ITS: PP391318). Spain, Mallorca, near Son Gual, approx. at 39° 34' 6.2" N, 2° 49' 30.0" E, Mediterranean *Cistus* maquis on calcareous soil with *Cistus albidus* L., *C. monspeliensis* L., *Olea europaea* L., *Pistacia lentiscus* L., and *Rosmarinus officinalis* L., 20 December 2011, F. Hampe & J. Kleine (aCM15, GenBank ITS: PP391319). Türkiye, Burdur Province, Bucak district, close to Karacaören, in Sweetgum Forest Nature Protection Area, at 37° 21' 27.5" N, 30° 50' 03.7" E, on calcareous soil, in Mediterranean forest dominated by *Liquidambar orientalis*, 285 m asl., 28 October 2017, leg. O. Kaygusuz (OKA-TR2234, GenBank ITS: PP391310); *ibid.*, at 37° 21' 26.4" N, 30° 50' 03.9" E, on calcareous soil, under *L. orientalis*, 310 m asl., 19 October 2018, leg. O. Kaygusuz (OKA-TR2235, GenBank ITS: PP391311); *ibid.*, at 37° 21' 26.3" N, 30° 50' 06.5" E, on calcareous soil, under *L. orientalis*, 280 m asl., 15 October 2019, leg. O. Kaygusuz (OKA-TR2236, GenBank ITS: PP391312); *ibid.*, at 37° 21' 21.9" N, 30° 49' 60.0" E, on calcareous soil, under *L. orientalis*, 295 m asl., 12 October 2020, leg. O. Kaygusuz (OKA-TR2237, GenBank ITS: PP391313); *ibid.*, at 37° 21' 26.8" N, 30° 50' 02.6" E, on calcareous soil, under *L. orientalis*, 305 m asl., 04 October 2021, leg. O. Kaygusuz (OKA-TR2238, GenBank ITS: PP391314); *ibid.*, at 37° 21' 33.2" N, 30° 50' 02.8" E, on calcareous soil, under *L. orientalis*, 230 m asl., 17 October 2021, leg. O. Kaygusuz (OKA-TR2239, GenBank ITS: PP391315); at 37° 21' 23.4" N, 30° 49' 61.2" E, on calcareous soil, under *L. orientalis*, 271 m asl., 02 October 2022, leg. O. Kaygusuz (OKA-TR2240, GenBank ITS: PP391316); at 37° 21' 29.1" N, 30° 49' 50.5" E, on calcareous soil, under *L. orientalis*, 264 m asl., 11 October 2022, leg. O. Kaygusuz (OKA-TR2241, GenBank ITS: PP391317).

**Discussion:** *Entoloma ochraceodiscum* belongs to the /Indutoides clade within sect. *Griseorubida* (Noordeloos 1981) on account of its collybioid to omphalioid, funnel-shaped basidiomata with a deeply depressed, fibrillose, yellowish-brown pileus, heterodiametric basidiospores, fusiform to lageniform cheilocystidia, and the presence of clamp connections.

Phylogenetically, *E. ochraceodiscum* forms a distinct clade within the sect. *Griseorubida* which contains *E. contortisporum* Noordel. & Hauskn., *E. indutoides* var. *pleurocystidium*, and *E. indutoides*. This clade is strongly supported (MLB = 100%, BPP = 1.0, Fig. 1). ITS sequences generated from *E. ochraceodiscum* differ in 51 nucleotides (88.2% similarity) from those of *E. indutoides* (WU19603) and in 53 nucleotides (86.9% similarity) from those of *E. contortisporum* (WU27137). Morphologically, *E. indutoides* differs from *E. ochraceodiscum* by olivaceous tinges of pileus and stipe, a white, fimbriate lamella margin, and 5–9-angled basidiospores (Noordeloos 1992). *Entoloma contortisporum* from Réunion differs by small pilei (up to 12 mm diam.) which are grey-brown to dark brown, brown lamella edge, pale ochraceous brown stipe, remarkably contorted basidiospores, and the occurrence in tropical rain-forests (Noordeloos and Hausknecht 2007).

*Entoloma calaminare* Noordel., originally described from the Netherlands, is a further similar species in sect. *Griseorubida*. It is distinguished by its slender habit, pale ochraceous pileus, and smaller basidiospores (on average  $10.9 \times 7.0 \mu\text{m}$ ) (Noordeloos 1984).

*Entoloma insolitum* Noordel. is somewhat similar, but differs by a very dark brown pileus, slightly shorter basidiospores ( $11.0\text{--}12.5 \times 7.0\text{--}9.5 \mu\text{m}$ ), and by growing in sub-alpine areas (Noordeloos 1992). *Entoloma flavostipitatum* C.K. Pradeep & K.B. Vrinda, originally described from India, has a chocolate to teak-brown pileus, a butter-yellow stipe, much shorter basidiospores (on av.  $7.2 \times 6.5 \mu\text{m}$ ) and cylindroclavate cheilocystidia with yellowish-brown contents (Pradeep et al. 2016). *Entoloma cystidiosum* G.M. Gates & Noordel., described from Australia, is characterized by a brown, umbo or umbilicate pileus, slightly shorter basidiospores ( $9\text{--}12 \times 7\text{--}10 \mu\text{m}$ ), and the absence of clamp connections (Noordeloos and Gates 2009).

***Entoloma colchicum*** Kaygusuz, Reschke, O.V. Morozova, Kaya, M. Piepenbr. & Rebriev, sp. nov. (Figs. 8 and 9)

*Mycobank*: MB852524

*Holotype*: Türkiye, Denizli Province, Pamukkale district, in Zeytin Upland, at  $37^\circ 43' 28.6'' \text{N}$ ,  $29^\circ 07' 02.3'' \text{E}$ , on calcareous soil under *Quercus coccifera*, 725 m asl., 09 November 2012, leg. O. Kaygusuz (OKA-TR3236). GenBank ITS: PP391320.

*Etymology*: The epithet refers to the name of the ancient kingdom Colchis (Colchida) that was located partly in the territories of modern Georgia, Türkiye, and Russia, where the species was recorded.

*Diagnosis*: Similar to *E. ortonii* Arnolds & Noordel. but differs in its more robust basidiomata with a radially fibrillose or velutinous pileus surface, longer basidiospores (on av.  $10.1 \times 8.7 \mu\text{m}$ ), lack of odour, and unique ITS sequences.

*Description*: Basidiomata mycenoid, collybioid to tricholomatoid. Pileus 10–30 mm in diam., at first convex, expanding with age to plano-convex, with slightly depressed centre, with or without a weak low umbo, with an inflexed margin when young, then straight or deflexed, surface opaque, slightly hygrophanous, not translucently striate, uniformly light brown to pale brown (7.5YR 8–4/4) or moderately dark brown (5YR 4–3/2), sometimes with a slight brownish tinge at the centre, entirely radially fibrillose or velutinous. Lamellae crowded (L = 30–45, l = 1–3), emarginate, thin, ventricose, light brown, with entire, concolorous edge. Stipe 20–35  $\times$  1.5–3.0 mm, central, cylindrical, often distinctly broadened at the base, entirely longitudinally fibrillose-striate, with brown fibrils, becoming darker brown when damaged. Basal mycelium white, cottony, sometimes with some whitish rhizomorphs. Context in stipe and pileus light brown. Smell and taste indistinct.

Basidiospores  $(8.0)8.5\text{--}11.0(12.5) \times (7.2)7.7\text{--}10.0(10.9) \mu\text{m}$  ( $n = 110$  of 3 coll.),  $L^m \times W^m = 10.1 \times 8.7 \mu\text{m}$ ,  $Q = 1.0\text{--}1.2(-1.4)$ ,  $Q^m = 1.1$ , isodiametrical to subisodiametrical, 6–8 angled in side view, guttulate, slightly thick-walled. Basidia 40–55  $\times$  11–14  $\mu\text{m}$ , clavate to broadly clavate, rarely somewhat utriform, 4-spored, occasionally 2-spored, hyaline, thin-walled. Lamellar edge fertile. Cheilo- and pleurocystidia absent. Hymenophoral trama regular, made up of long cylindrical to inflated, 5–12  $\mu\text{m}$  wide cells, with some incrusting pigment especially on the narrower hyphae. Pileipellis a mostly loose cutis, consisting of narrow cells, 50–200  $\times$  4–14  $\mu\text{m}$ , with cylindrical to fusiform terminal elements, thin-walled, with brown, coarsely incrusting and sometimes in addition intracellular pigment, light brownish yellow in KOH. Pileitrasmus similar to hymenophoral trama. Stipitipellis a cutis of long cylindrical hyphae, 3–9  $\mu\text{m}$  wide. Caulocystidia absent. Clamp connections abundant in the hymenium, absent or rare elsewhere.

*Ecology, phenology, and distribution*: Basidiomata usually gregarious on the ground on grasslands or on extensively grazed meadows, often in open forest of *Quercus coccifera* or *Pinus brutia*, always on calcareous soil, at altitudes of 500–800 m asl. Mostly collected in the autumn (November–December). So far known from Cyprus, Georgia, Southwest Russia, and Türkiye, probably widespread in South-eastern Europe.

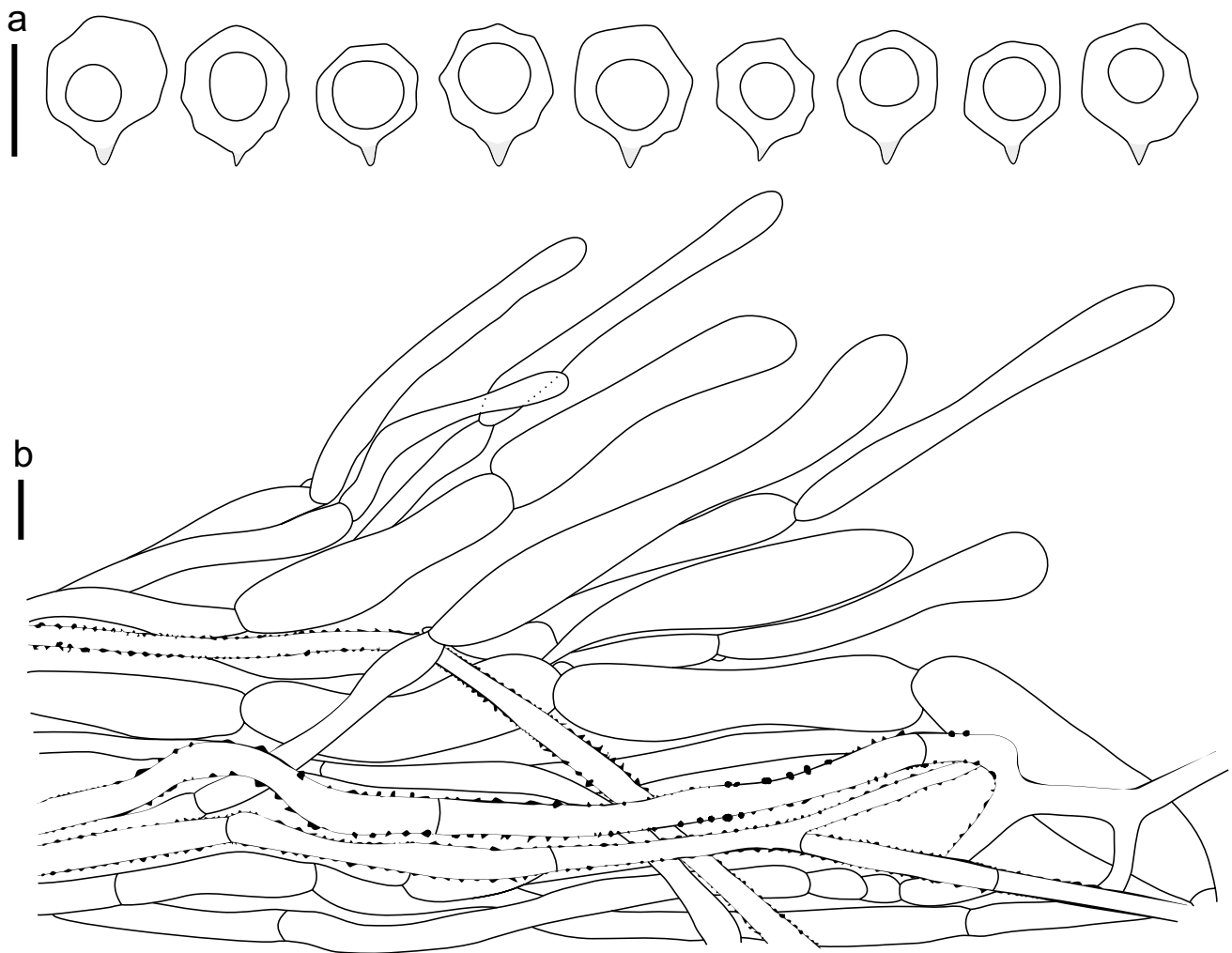
*Additional specimens examined*: Cyprus, Akamas forest, Adonis path, on naked soil in rather open vegetation with *Juniperus phoenicea* L. and *Cistus* spp., 20 December 2017, leg. K. Reschke (KaiR1175, GenBank ITS: OL338159). Georgia, vicinities of Tbilisi, Kojori, Azeula fortress, at  $41^\circ 38' 29.8'' \text{N}$ ,  $44^\circ 41' 33.0'' \text{E}$ , on soil in grassland, 1340 m asl., 06 October 2023, leg. O. Morozova (LE F-344063, GenBank ITS: PP391325); *ibid.*, at  $41^\circ 38' 29.8'' \text{N}$ ,  $44^\circ 41' 33.0'' \text{E}$ , on soil in grassland, 1340 m asl., 06 October 2023, leg. O. Morozova (LE F-344064, GenBank ITS: PP391326).



**Fig. 8** Basidiomata of *Entoloma colchicum*. **a** Collection OKA-TR3236 (holotype). **b** Collection K. 5830. **c** Collection K. 5843. **d** Collection LE F-344064. **e** Collection LE F-344063. **f** Collection OKA-TR3237. Bars = 10 mm

RUSSIA, Rostov Region, Orlovsky District, vicinities of the San Manych Village, at 46° 20' 40.0" N, 43° 12' 48.0" E, on soil in the tussock-grass steppe, 04 December 2004, leg. Yu. A. Rebriev (LE253635 as *E. sericeum* var. *cinereoopacum*,

GenBank ITS: OL338246). Türkiye, Şanlıurfa Province, Bozova district, in Dutluca village, at 37° 28' N, 38° 20' E, among moss or grass on calcareous soil, 630 m asl., 06 December 2008, leg. A. Kaya (K. 5824, GenBank ITS:



**Fig. 9** Microscopic features of *Entoloma colchicum* (OKA-TR3236, holotype). **a** Basidiospores. **b** Pileipellis. Bars = 10  $\mu$ m

PP391322); *ibid.*, Yaşlıca village, at 37° 27' N, 38° 23' E, in grass, 550 m asl., 06 December 2008, leg. A. Kaya (K. 5830, GenBank ITS: PP391323). Adıyaman Province, central district, in Akyazı village, at 37° 29' N, 38° 17' E, on calcareous soil, 740 m asl., 08 December 2008, leg. A. Kaya, (K. 5843, GenBank ITS: PP391324). Denizli Province, Pamukkale district, in Zeytin Upland, at 37° 43' 17.4" N, 29° 07' 00.6" E, on calcareous soil under *Q. coccifera*, 745 m asl., 17 November 2017, leg. O. Kaygusuz, (OKA-TR3237, GenBank ITS: PP391321).

**Discussion:** The molecular analyses inferred from ITS sequences revealed that *E. colchicum* forms a distinct phylogenetic branch within subsect. *Minuta* of subg. *Nolanea* (Reschke et al. 2022a). *Entoloma colchicum* shares some morphological and phylogenetic affinities with *E. altaicum* O.V. Morozova, Reschke, Noordel. & Ageev, *E. anodinum* Vila, Carbó, Valero, Reschke & Noordel., *E. assiduum* Vila, Reschke, Corriol, Polemis & Loizides, *E.*

*incanosquamulosum* (Largent) Noordel. & Co-David, and *E. ortonii*. *Entoloma altaicum*, originally described from Russia, differs from *E. colchicum* by a reddish brown to greyish brown pileus with a deeply translucently striate, silvery fibrillose stipe, much shorter basidiospores (on av. 8.1  $\times$  7.1  $\mu$ m), and the presence of caulocystidia (Reschke et al. 2022a). Two European species, *E. anodinum* and *E. assiduum*, are similar to *E. colchicum* by being predominantly associated with Mediterranean vegetation, but both differ by smaller basidiospores (on av. 9.5  $\times$  8.6 and 8.9  $\times$  8.0  $\mu$ m, respectively) and distinct ITS sequences (Vila et al. 2021). *Entoloma incanosquamulosum* has much shorter basidiospores and a holarctic distribution (Reschke et al. 2022a). *Entoloma ortonii* is morphologically similar to *E. colchicum* by the fibrillose aspect of its pileus surface. However, the basidiomata of *E. ortonii* are generally more fragile than those of *E. colchicum*, and the basidiospores are somewhat shorter (7.5–9.5  $\times$  5.5–8.0  $\mu$ m) and tend to



have fewer angles. In addition, *E. ortonii* has a strongly farinaceous-rancid odour (Noordeloos et al. 2022b), while *E. colchicum* has no distinct odour.

Further European species in the subsect. *Minuta* also differ from the new species. *Entoloma llimonae* Vila, F. Caball., Català & J. Carbó is readily characterized by its subspermatic odour and deeply translucently striate pileus (Vila et al. 2013), whereas *E. colchicum* has no remarkable odour and a pileus that is not translucently striate.

## Conclusions

In this study, four new *Entoloma* species were described from Europe and Western Eurasia using phylogenetic analyses and traditional morphological examinations. *Entoloma humidiphilum* and *E. ochraceodiscum* were collected in regions dominated by Mediterranean vegetation, while *E. brunneofibrillosum* and *E. colchicum* were found in both Mediterranean and non-Mediterranean habitats. The geographical distribution of *Entoloma* species is suggested to be influenced by climatic factors, such as temperature and precipitation. These species were primarily located in grasslands or open forests with calcareous soils, indicating their adaptation to and proliferation in such environments. To enhance our knowledge on the ecology and biogeographical distribution of *Entoloma* species, further studies should be conducted.

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**Author contribution** All authors contributed to the conception and design of the study. Oğuzhan Kaygusuz, Kai Reschke, Abdullah Kaya, Bálint Dima, Olga Morozova, and Machiel Evert Noordeloos contributed to material preparation or data collection. Morphological characteristics were examined by Oğuzhan Kaygusuz, Kai Reschke, Bálint Dima, Abdullah Kaya, and Olga Morozova. Molecular lab work and phylogenetic analyses were conducted by Oğuzhan Kaygusuz, Kai Reschke, Bálint Dima, and Olga Morozova. The first draft of the manuscript was written by Oğuzhan Kaygusuz, Kai Reschke, and Meike Piepenbring, which was then improved by changes, edits, suggestions, and comments from Abdullah Kaya, Bálint Dima, Olga Morozova, and Machiel Evert Noordeloos. All authors read and approved the final version of the manuscript.

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**Data availability** The DNA sequences produced in this study are available on NCBI GenBank (<https://www.ncbi.nlm.nih.gov>).

**Code availability** Not applicable.

## Declarations

**Ethics approval** Not applicable.

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

**Competing interests** The authors declare no competing interests.

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