

New additions to Turkish mycota from Ankara, Balıkesir, and Kütahya provinces

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Abstract: The current research was conducted on the fungal materials collected from Ankara, Balıkesir, and Kütahya provinces between 2015 and 2019. As a result of the field and laboratory studies, *Conocybe dentatomarginata*, *Entoloma lampropus*, *Lactarius lilacinus*, *Otidea nannfeldtii* and *Sarcopeziza sicula* are reported for the first time from Turkey based on macro- and micromorphological studies and internal transcribed spacer (ITS) rDNA-based molecular phylogeny. Descriptions of new records are provided together with photos showing their macro and micro-morphology.

Key words: Biodiversity, internal transcribed spacer, macrofungi, new records, Turkey

1. Introduction

Fungi constitute significant members of the ecosystem by playing important roles not only in the ecosystem per se but also in various fields including pharmacology, food industry, and biodegradation. So far, more than 120.000 fungal species have been described and reported throughout the world, even though the global fungal biodiversity has been estimated to comprise between 2.2 and 3.8 million species (Hawksworth and Lücking, 2017).

Turkey has a very rich macrofungal diversity due to its different habitats, vegetation, and climate. The insight into the macrofungal diversity of Turkey is based on a research period exceeding 150 years. The number of such research studies has greatly increased over the last twenty years and the literature was reviewed and published as checklists at different times (Solak et al., 2015; Sesli and Denchev, 2014). Although approximately 2500 species of macrofungi have been recorded from Turkey (Sesli et al., 2016; Akata, 2017; Altuntaş et al., 2017; Işık and Türkekul, 2018; Akata et al., 2019; Acar et al., 2020) up until now, there is not any report about *Conocybe dentatomarginata* Watling, *Entoloma lampropus* (Fr.) Hesler, *Lactarius lilacinus* Fr., *Otidea nannfeldtii* Harmaja and *Sarcopeziza sicula* (Inzenga) Agnello, Loizides & P. Alvarado in Turkey. The current study aims to contribute to the mycobiota of Turkey by providing newly reported species with phylogenetic and morphological data.

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2. Materials and methods

In the current study, both conventional and molecular identification including macro- and micromorphological features of the specimens and internal transcribed spacer (ITS) sequences were exploited to characterize the five macrofungal samples.

2.1. Morphological study

Fungal specimens were taken from Ankara, Balıkesir, and Kütahya provinces between 2015 and 2019. During the field study, macroscopic and ecological characteristics of the specimens were recorded on site. In the laboratory, microscopic structures were provided with both light and scanning electron microscopy (SEM). For the light microscopy (LM), roughly 30 measurements were done with Euromex (Arnhem, The Netherlands) Oxion Trinocular microscope. Each structure was visualized under 100× objective and the combined data were assessed statistically. Some solutions including Melzer's reagent, 5% KOH, and Congo red were also employed. For SEM, pieces of mass from the lamellae were attached on stubs using double-sided sticky tape, coated with gold particles, and visualized using an EVO 40XVP (LEO Ltd., Cambridge, UK) scanning electron microscope with 20 kV accelerating voltage. Identification was performed with the help of the relevant literature (Heilmann-Clausen et al., 1998, Hausknecht, 2007; Agnello et al., 2013; 2018; Morozova et

al., 2014; Olariaga et al., 2015). Dried specimens are stored at the herbarium of Ankara University (ANK).

2.2. Molecular study

2.2.1. DNA extraction, PCR amplification, Sequencing, and Phylogenetic analysis

GeneMatrix Plant & Fungi DNA Purification Kit was utilized to extract the genomic DNA from dried specimens with slight modifications (Bozok et al., 2018, 2019, 2020; Doğan et al., 2018). ITS rDNA gene regions were amplified by using the universal ITS1F-ITS4 oligonucleotide primers with PCR method (White et al., 1990). The thermal cycling protocol was implemented as follows: 94 °C for 6 min, followed by 30 cycles of 30 s at 94 °C, 60 s at 51 °C and 90 s at 72 °C and the last elongation step of 6 min at 72 °C. The quality of the PCR amplicons was confirmed by electrophoresis on a 1.2% agarose gel and then the DNA sequencing of the amplicons was executed by using the BigDye Terminator v3.1 sequencing kit, again with using the same ITS1F-ITS4 primers for the ITS rDNA gene region. ABI 3730XL Sanger Sequencer (Applied Biosystems, Foster City, CA, USA) was employed for running of the sequencing reaction products. Raw sequences were edited and aligned by using Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA) and BioEdit 7.0.5.3 software. Phylogenetic trees were constructed by using maximum likelihood method based on the K-2 nucleotide substitution model (Gamma distributed with Invariant sites (G+I)) in MEGA 7.0 software (Kumar et al. 2016). Bootstrap support values were shown beside the branches. A discrete Gamma distribution was utilized to infer evolutionary rate differences among sites. All sequences that belong to this study were deposited in GenBank under the accession numbers MN097899 (*Sarcopeziza sicula*), MN097901 (*Otidea nannfeldtii*), MN097920 (*Lactarius lilacinus*), MN097903 (*Entoloma lampropus*), and MN097900 (*Conocybe dentatomarginata*).

3. Results

3.1. Systematic overview

3.1.1. Main key

- 1. Spores produced internally, inside the sporangium known as ascus 2
 - 1'. Spores produced externally, on the sporangium known as basidia 3
 - 2. Apothecia hypogeous or semihypogeous, cup-shaped which splitting into irregular rays and stipitate, hymenium purple sometimes with a lilac tinge, asci amyloid, up to 400 µm, spores elliptical with two drops (≥13 µm) *S. sicula*
 - 2'. Apothecia epigeous, ear-shaped, sessile, hymenium yellowish ochre to brown ochre, asci inamyloid up to 170 µm, spores elliptical with two drops (11 µm ≤) *O. nannfeldtii*

- 3. Gills exuding latex, spores amyloid, with reticulate ornamentation *L. lilacinus*
- 3'. Gills not exuding latex, spores inamyloid, without reticulate ornamentation 4
- 4. Spores angular, basidiocarps growing on the wood of black pine *E. lampropus*
- 4'. Spores elliptical, basidiocarps growing on soil *C. dentatomarginata*

Ascomycota Caval.-Sm.

Pezizaceae Dumort.

Sarcopeziza Loizides, Agnello & P. Alvarado *Ascomycete.org* 10(4): 179 (2018).

3.1.2. *Sarcopeziza sicula* (Inzenga) Agnello, Loizides & P. Alvarado (2018) (Figure 1).

Syn.: *Peziza sicula* Inzenga (1869), *Sarcosphaera sicula* (Inzenga) Pat. (1904).

Ascomata: 60–80 mm in diam. and 40–60 mm in height, stipitate, first hypogeous or semihypogeous, then subglobose or pear-shaped, partially or completely emerging from the soil, opening apically and form a cup shape which splits into irregular rays. **Hymenial surface:** Smooth, purple, sometimes with a lilac tinge. **External surface:** Smooth, vinaceous red, purple, purple-pink, or purple-brown, covered with a fine white bloom and tiny warts. **Context:** Pink, pinkish lilac or purplish lilac, thick and fragile. **Smell and taste:** Not distinctive. **Ascospores:** 13–15.5 × 8.5–9.5 µm, elliptical, with one, two, or several drops, smooth under the LM, but finely warted under the scanning electron microscope (SEM), hyaline, and uniseriate. **Asci:** 350–400 × 13–14 µm, cylindrical, operculate, eight-spored, and amyloid at the apex. **Paraphyses:** 3–5 µm broad, cylindrical, slightly thickened at apex, septate, often containing yellow to yellowish-brown intracellular pigments.

Material examined: TURKEY— Kütahya province, Tavşanlı district, on bare ground, 39°33' N, 29°29' E, 900 m alt., 15 April 2017, leg. Mustafa Özyurt (FBozok00137, GenBank: nrITS MN097899).

Pyronemataceae Corda

3.1.3. *Otidea nannfeldtii* Harmaja *Karstenia* 15: 31 (1976), (Figure 2).

Ascomata: 20–30 mm tall, split ear-shaped, and sessile. **Hymenial surface:** Smooth, yellowish ochre to brownish ochre, base covered with whitish cottony basal mycelium. **External surface:** Smooth to furfuraceous, almost concolorous, or slightly darker than hymenium. **Context:** Concolorous with apothecia, thick and fragile. **Smell and taste:** not distinctive. **Ascospores:** 9–10.5 × 6–7 µm, elliptical, with two drops, smooth, hyaline, and uniseriate. **Asci:** 150–170 × 8–9 µm, cylindrical, operculate, eight-spored and inamyloid. **Paraphyses:** 2.5–4 µm broad, slender, tips bent over and slightly thickened at the apex.

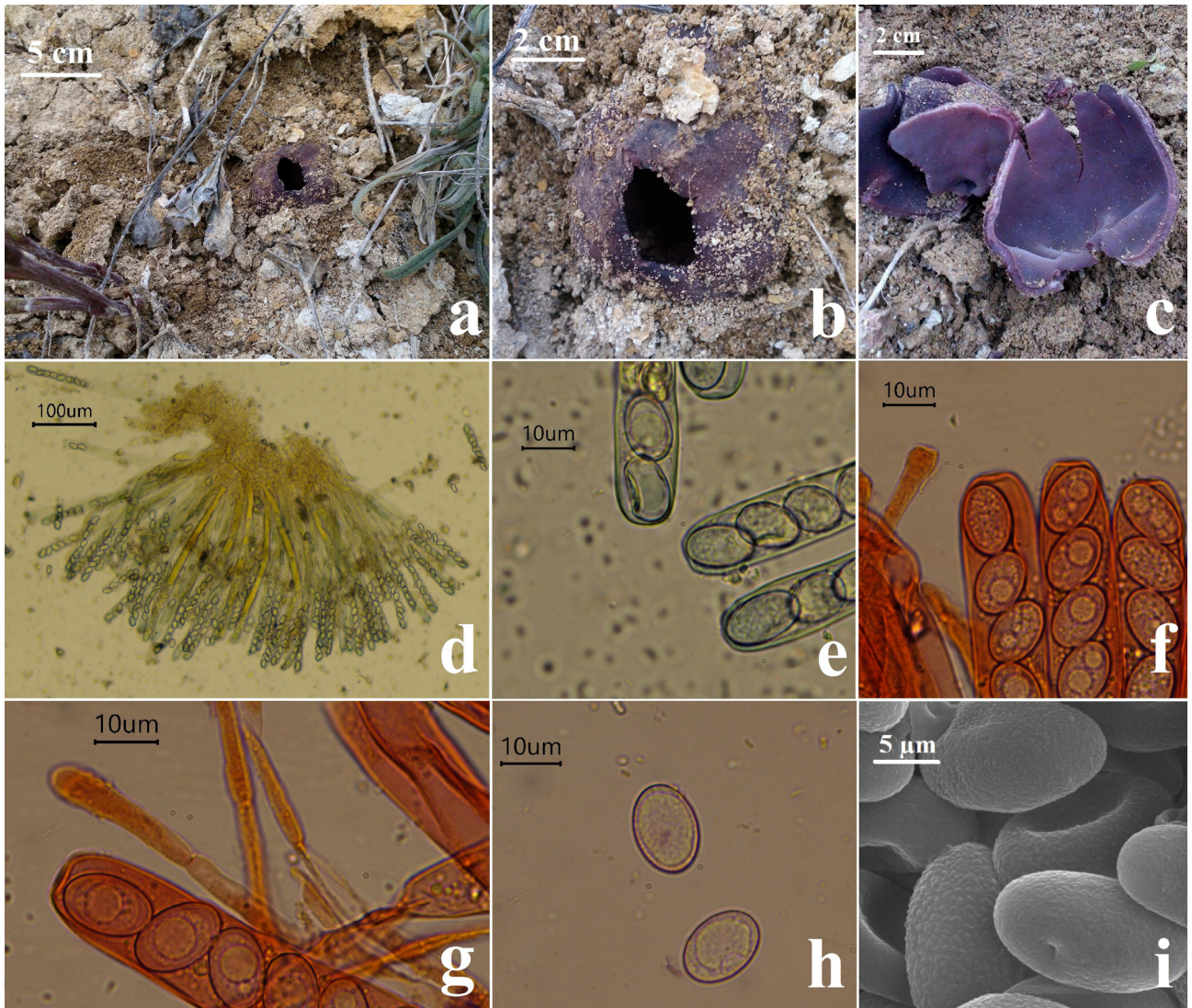


Figure 1. *Sarcopeziza sicula*: **a-c.** Ascomata, **d-g.** Asci and tips of paraphyses, **h.** Ascospores (LM), **i.** ascospores (SEM).

Material examined: TURKEY— Balıkesir province, Kaz Dağı National Park, in oriental plane (*Platanus orientalis* L.) and Turkish pine (*Pinus brutia* Ten.) mixed forest, 39°39' N, 26°56' E, 800 m alt., 14. December 2014, (ANK Altuntaş 439, GenBank: nrITS MN097901).

Basidiomycota R.T. Moore

3.1.4. *Conocybe dentatmarginata* Watling Notes R. bot. Gdn Edinb. 38(2): 333 (1980), (Figure 3).

Syn.: *Conocybe appendiculata* f. *macrospora* Kühner, *Pholiotina dentatmarginata* (Watling) Enderle, *P. nemoralis* var. *dentatmarginata* (Watling) Hauskn.

Pileus: 6–20 mm in diam., convex to campanulate at first, later plano-convex, with a small and broad umbo, hygrophanous, when moist, striate up to 3/4 the distance to the center, smooth, ochre brown to yellowish-brown, often darker in the centre, whitish veil remnants hanging

from the margin when young, often without veil when mature. **Lamellae:** Rather crowded, adnate to adnexed, ochre brown to yellowish-brown. **Stipe:** 30–60 × 2–3 mm, cylindrical, whitish yellow to pale brown when young whitish pruinose at the apex, in age ochre yellow, reddish to dark brown towards the base. Context thin, ochre to yellowish ochre. **Smell:** Mild. **Odor:** Not distinctive. **Basidiospores:** 9–10 × 5–6 µm, ellipsoid, smooth, and thick-walled. **Basidia:** 22–30 × 7–8 µm, clavate, with four sterigmata. **Pleurocystidia:** Absent. **Cheilocystidia:** 30–45 × 7–10 µm, lageniform with an elongated neck, often slightly enlarged towards the tips. **Caulocystidia:** 35–40 × 8–10 µm, similar to cheilocystidia. **Pileipellis:** A hymeniderm, with terminal elements 30–40 × 15–25 µm, composed of clavate to pyriform cells. **Clamp-connections:** Absent.

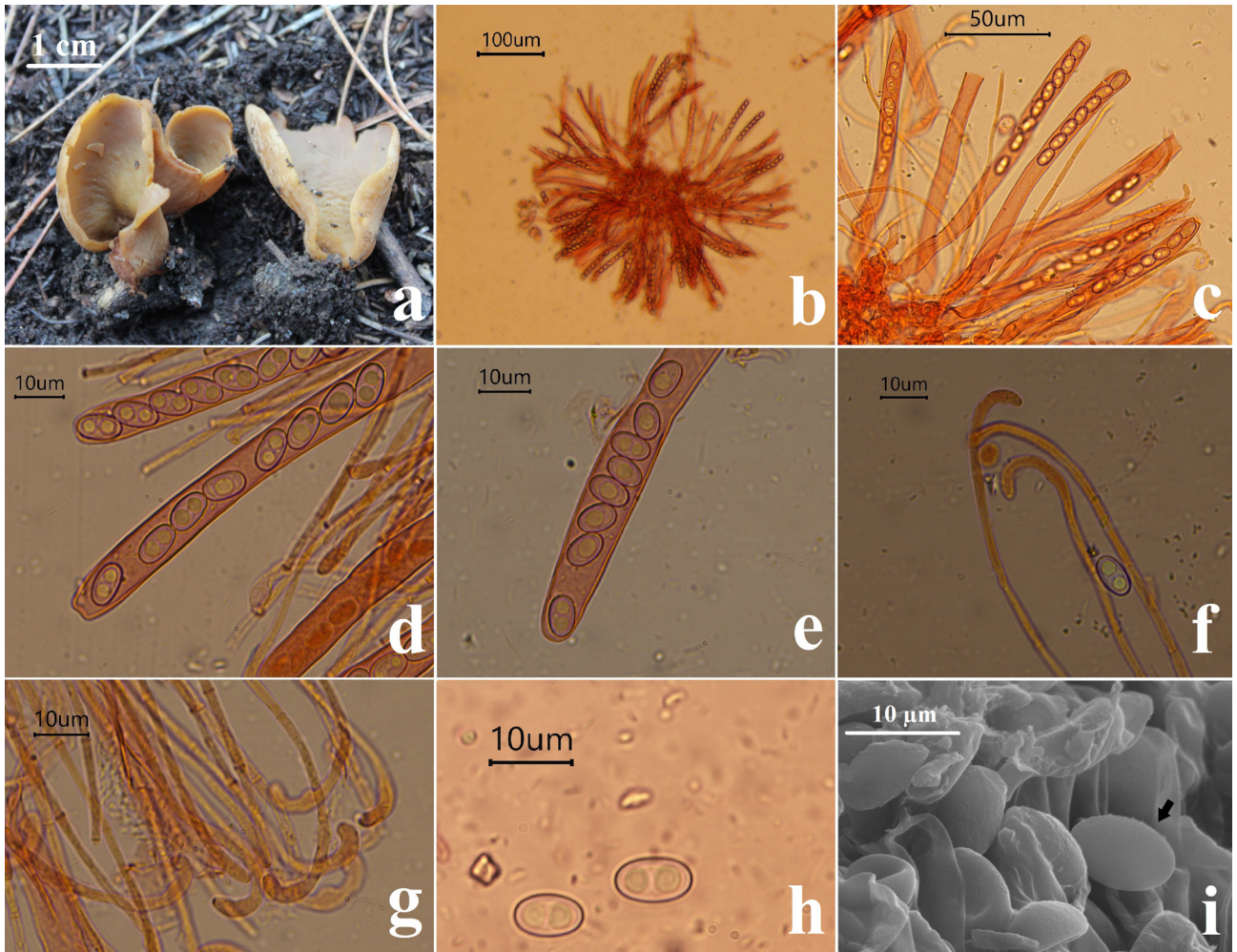


Figure 2. *Otidea nannfeldtii*: **a.** ascomata, **b-e** asci, **f-g.** tips of paraphyses **h.** ascospores (LM), **i.** ascospores (SEM).

Material examined: TURKEY— Ankara province, Ankara University Beşevler 10. yıl campus, under ash-leaved maple (*Acer negundo* L.), 860 m alt., 39°56' N, 32°50' E, 20.04.2019, (ANK Akata & Altuntaş 555, GenBank: nrITS MN097900).

Entolomataceae Kotl. & Pouzar

3.1.5. *Entoloma lampropus* (Fr. : Fr.) Hesler Beih. Nova Hedwigia 23: 154 (1967), (Figure 4).

Syn.: *Agaricus lampropus* Fr., *Entoloma lampropus* var. *monticola* Noordel., *Hyporrhodius lampropus* (Fr. : Fr.) Henn., *Leptonia lampropus* (Fr.: Fr.) Quél., *Rhodophyllus lampropus* (Fr. : Fr.) Quél.

Pileus: 20–40 mm in diam., conical or hemispherical at first, later convex, slightly depressed in the center, margin incurved, finally radially fibrillose, with small dark greyish brown squamules on beige gray to grayish background, sometimes with a lilac tinge, often darker in the center. **Context:** Thin and whitish. **Smell:** Mild. **Odor:** Not distinctive. **Lamellae:** Cream to grayish brown, adnate to

emarginate. **Stipe:** 30–40 × 2–3 mm, cylindrical, sometimes enlarged towards the base, longitudinally fibrillose to striate, light blue to grayish-blue. **Basidiospores:** 10–11 × 6.5–7.5 µm, 6–9 angled and thin-walled. **Basidia:** 30–35 × 8–10 µm, clavate, with four sterigmata and basal clamp. **Pleuro and Cheilocystidia:** Not seen. Pileipellis composed of cylindrical hyphae, some hyphae with clamps.

Material examined: TURKEY— Balıkesir province, Kaz Dağı National Park, in black pine (*Pinus nigra* J.F.Arnold) forest, on dead wood of black pine, 39°42' N, 26°53' E, 1350 m alt., 17 October 2015, (ANK Altuntaş 710, GenBank: nrITS MN097903).

Russulaceae Lotsy

3.1.6. *Lactarius lilacinus* Fr. Epicr. syst. mycol. (Upsaliae): 348 (1838), (Figure 5).

Syn.: *Agaricus lilacinus* Lasch, *Lactarius lateritoroseus* P. Karst., *L. lilacinus* subsp. *eulilacinus* Singer, *L. lilacinus* subsp. *mitificus* (Britzelm.) Singer, *L. lilacinus* var. *minor* Killerm., *L. lilacinus* var. *mitificus* (Britzelm.) Killerm.,

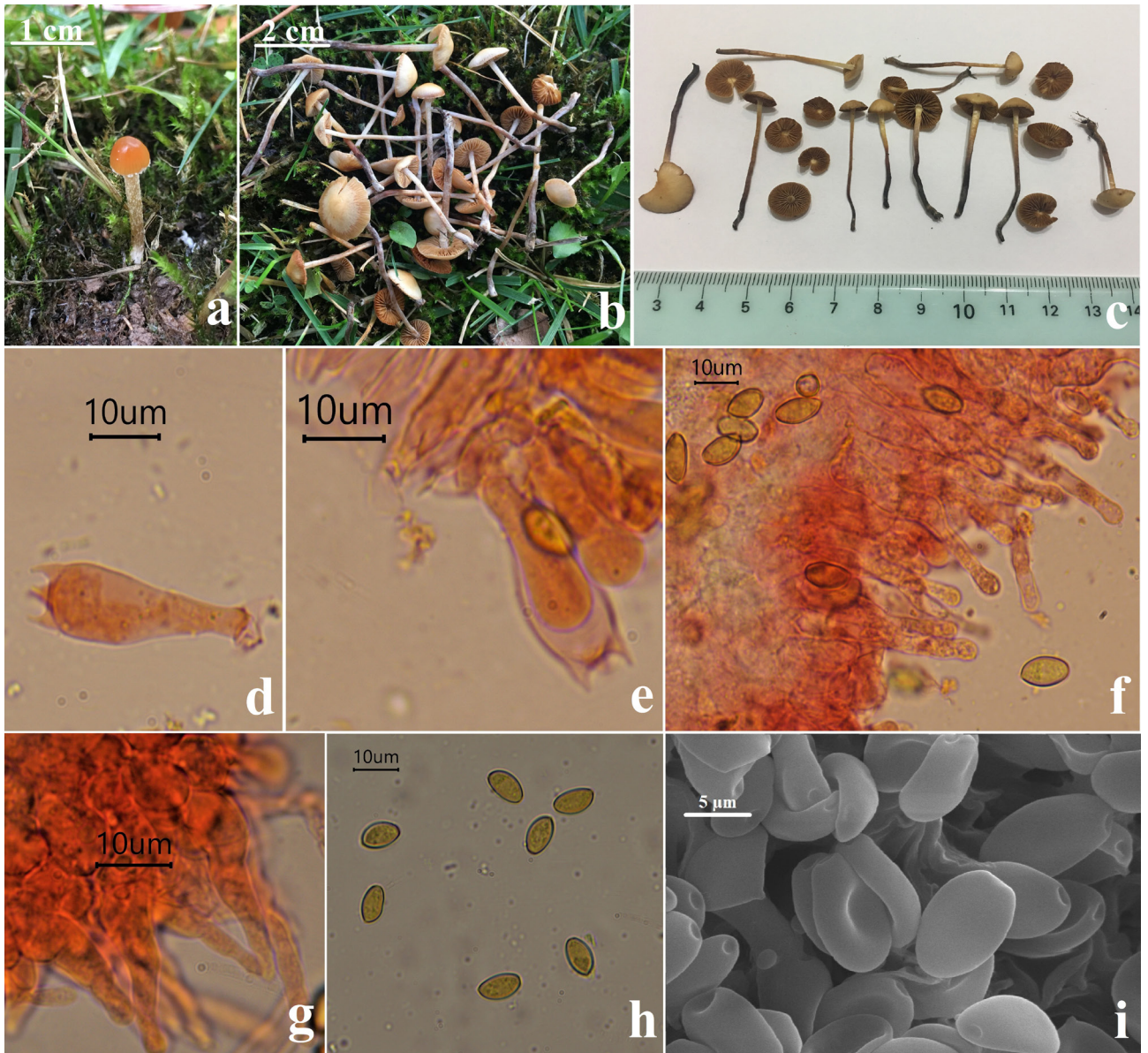


Figure 3. *Conocybe dentatmarginata*: a–c. Basidiomata, d, e. Basidium, f–g. Cheilocystidia, h. Basidiospores (LM), i. Basidiospores (SEM).

L. mitificus Britzelm. *Lactifluus lateritoroseus* (P. Karst.) Kuntze, *Lactifluus lilacinus* (Fr.) Kuntze).

Pileus 20–50 mm in diam., convex, often with umbo when young, soon plane with a depressed or indented center, finally funnel-shaped, with a decurved margin, surface smooth, dull, finely velutinous to squamulose, pink, salmon, sometimes with a lilac tint, often slightly zonate. **Lamellae** close, adnate to decurrent, pinkish buff at first, later ochraceous. **Stipe** 30–50 × 5–10 mm, cylindrical, sometimes furrowed or rugged, surface pale pinkish buff to salmon, solid at first, hollow at maturity. **Context** cream-colored to pale pink. **Smell**

mild to acrid. **Odor** fruity. **Latex** white, unchanging, acrid. **Basidiospores** 7.5–9 × 6–7 µm, subglobose to ellipsoid, amyloid, ornamentation up to 1 µm, consisting of warts and short ridges which form nearly a complete reticulum. **Basidia** 35–45 × 9–10 µm, clavate, four-spored. **Pleuromacrocyttidia** 60–100 × 8–10 µm, cylindrical, obtuse or mucronate. **Cheilomacrocyttidia** 50–80 × 8–9 µm, cylindrical, obtuse or mucronate. **Pileipellis** a cutis, sometimes a trichoderm, up to 13 µm broad, consisting of intertwined hyphae. **Clamp connection** absent.

Material examined: TURKEY—Balıkesir, Kaz Dağı National Park, near the stream, under common alder

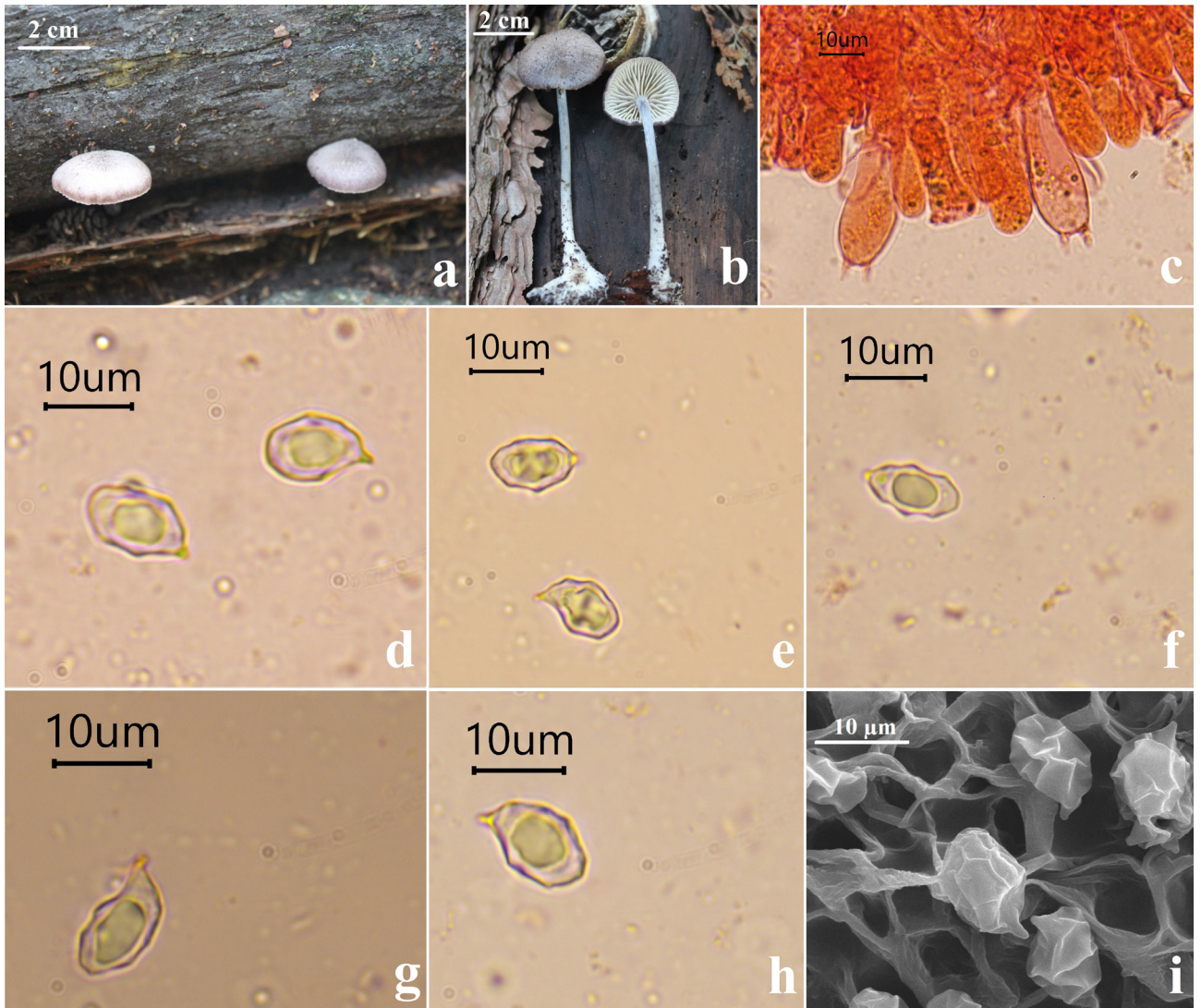


Figure 4. *Entoloma lampropus*: a–b. Basidiomata, c. Basidia, d–h. Basidiospores (LM), i. Basidiopores (SEM).

(*Alnus glutinosa* (L.) Gaertn.), 39° 42' N, 26° 53' E, 1340 m, 17 October 2015, (ANK Altuntaş 704, GenBank: nrITS MN097920).

3.2. Molecular phylogenetic characterization

ITS rDNA sequences obtained from different taxa selected from GenBank database were used for comparison with our samples and five new sequences were added to GenBank database during the present study (Figures 6–10). ITS rDNA gene region (639 bp) of *Sarcopeziza sicula* obtained from this study showed similarity at a rate of 99.83% with the sequence MH842196 (*S. sicula* collected from Cyprus, 595 bp) in GenBank database (Agnello et al., 2018). As seen in the phylogenetic tree drawn by using *Pachyphloeus oleiferus* as outgroup (Figure 6), *S. sicula* nesting as a sister of *Peziza polaripapulata*, had maximum supported clade value (100%). *Otidea nannfeldtii* sequence (810

bp) obtained from this study was clustered together with three sequences (KM010096, KM010097, and KM010098) belonging to *O. nannfeldtii* from Italy (Olariaga et al., 2015) in GenBank database with strong support value (100%) and showed similarity at a rate of 100%, 100% and 99.74% with these sequences, respectively (Figure 7). Phylogenetic analysis revealed that *Conocybe dentatmarginata* sequence (720 bp) obtained from this study (by taking maximum bootstrap value as 100%) was 99.85% similar to the sequence JX968257 (668 bp) and 96.14% similar with the sequence JF908599 (619 bp) in GenBank database (Toth et al., 2013; Osmundson et al., 2013), and it seems sister of the clade *C. aporos* in the phylogenetic tree drawn by using *Bolbitius titubans* as an outgroup taxon (Figure 8). In the ITS phylogenetic tree created by using *Lyophyllum decastes* as an outgroup taxon (Figure 9), our

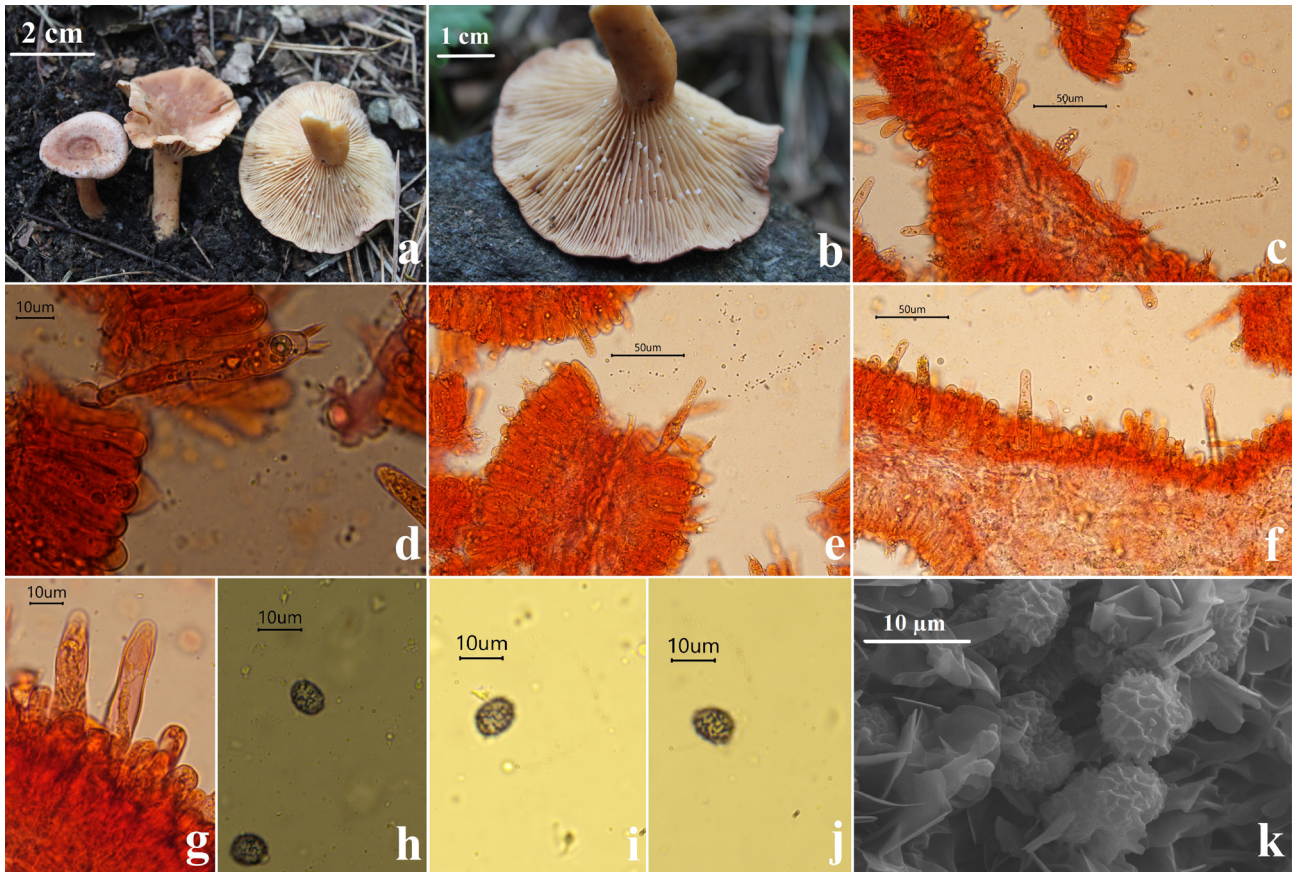


Figure 5. *Lactarius lilacinus*: a–b. Basidiomata, c–d. Basidia, e–f. Pleuromacrocystidia, g. Cheilomacrocystidia, h–j. Basidiospores (LM), k. Basidiopores (SEM).

sequence (673 bp) of the *Entoloma lampropus* clustered together with one sequence (KC898379) from Austria (Morozova et al., 2014) and showed similarity 99.83% with this sequence having the maximum support value (100%). *Lactarius lilacinus* sequence in this study showed similarity 100% with one sequence (KF133275) belong to *L. lilacinus* in GenBank database with high support value (97%) and clustered together with this sequence sampled from Belgium.

In the phylogenetic tree created by using *Russula crassotunicata* as an outgroup, it is obvious that the clade *L. lilacinus* was sister to the clade *L. lepidotus* (Figure 10).

4. Discussion

The genus *Peziza* has been reported as polyphyletic in recent phylogenetic studies (Agnello et al., 2018; Phookamsak et al., 2019). *Sarcopeziza* Loizides, Agnello & P. Alvarado was proposed as a new monotypic genus within *Pezizaceae* based on the multigene phylogenetic analyses including 28S rDNA, RPB2, and beta-tubulin loci by Agnello et al. (2018). In this regard, due to being phylogenetically distant from *Peziza*, *Peziza sicula* was

moved to the new genus *Sarcopeziza* (Agnello et al., 2018). The phylogenetic distance between *Peziza* and *Sarcopeziza* was indicated clearly by Agnello et al. (2018). This species was reported from Italy (Agnello et al., 2013, 2018), Cyprus, Greece, Tunisia (Agnello et al., 2018), Israel (as *Sarcosphaera coronaria* - Lewinsohn, 2008), and Spain (Phookamsak et al., 2019) so far. It was reported in the Mediterranean basin between January and April (Agnello et al., 2018). In Turkey, it was collected from Kütahya province (in the Central Western Anatolia) in April. The sequence submitted by Agnello et al. (2018) as MH842196 was used for comparison and verification. Our sequence was clustered in the same clade phylogenetically with the above-mentioned sequence (Figure 6).

S. sicula grows on bare ground from January to May. The species is solitary or gregarious, among grasses and herbs, sometimes under Aleppo pine (*Pinus halepensis* Mill.), olive tree (*Olea europaea* L.) and other sclerophyllous vegetation of the Mediterranean basin (Agnello et al., 2013, 2018). *Peziza ammophila* Durieu & Lév. and *Sarcosphaera coronaria* (Jacq.) J. Schröt. resemble *S. sicula* in terms of their similar appearance such as their subglobose, pear to

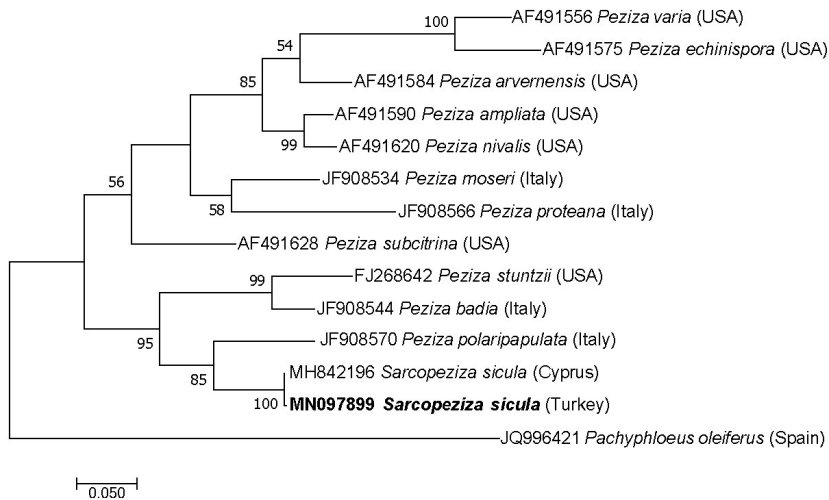


Figure 6. Phylogenetic tree created by using Maximum Likelihood analysis for ITS rDNA sequences of *Sarcopeziza sicula* in the present study and related species selected from GenBank database. Bootstrap values greater than 50% are given next to the branches. The highest log likelihood value of the tree is -7795.40. A discrete Gamma distribution was used to model evolutionary rate differences among sites [10 categories (+G, parameter = 0.4478)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.00% sites).

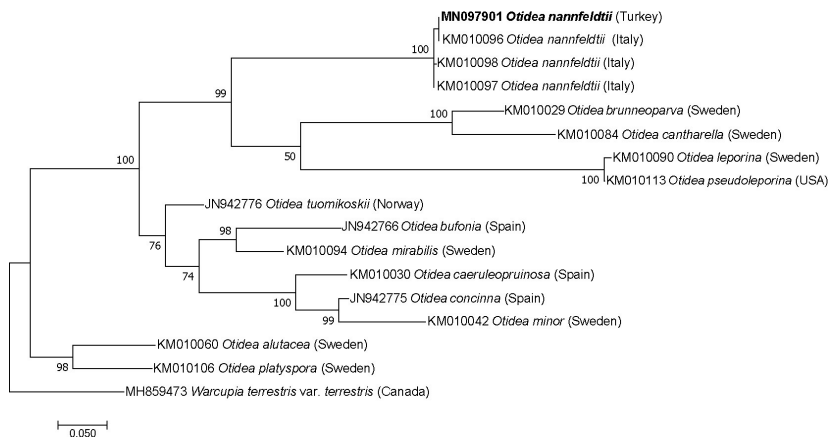


Figure 7. Phylogenetic tree created by using maximum likelihood analysis for ITS rDNA sequences of *Otidea nannfeldtii* in the present study and related species selected from GenBank database. Bootstrap values greater than 50% are given next to the branches. The highest log likelihood value of the tree is -7006.75. A discrete Gamma distribution was used to model evolutionary rate differences among sites [10 categories (+G, parameter = 1.4617)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 17.45% sites).

cup-shaped and sarcosphaeroid apothecia splitting into irregular rays. However, they are easily separated from *S. sicula* primarily by the color of their apothecia. While *P. ammophila* has brown to yellowish-brown, *S. coronaria* has whitish, gray-white, or pale violet apothecia (Agnello et al., 2013, 2018).

The genus *Otidea* was suggested as monophyletic as a result of many multilocus phylogenetic analyses (Hansen

et al., 2013; Olariaga et al., 2015). Recent extensive multilocus phylogenetic studies on this genus were conducted by Hansen and Olariaga (2015) and Olariaga et al. (2015). In the study carried out by Hansen and Olariaga (2015), molecular phylogeny of 89 specimens was revealed based on the LSU rDNA, RPB1, RPB2, and EF-1 α gene regions. Before obtaining these 89 collections for four-gene dataset, 112 ITS and LSU rDNA sequences

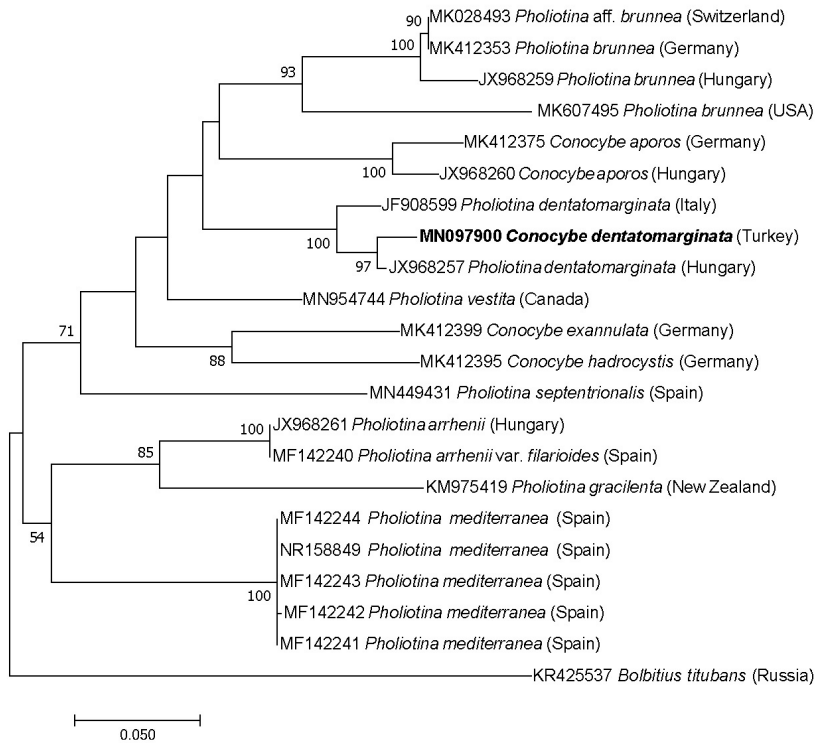


Figure 8. Phylogenetic tree created by using maximum likelihood analysis for ITS rDNA sequences of *Conocybe dentatomarginata* in the present study and related species selected from GenBank database. Bootstrap values greater than 50% are given next to the branches. The highest log likelihood value of the tree is -5194.63. A discrete Gamma distribution was used to model evolutionary rate differences among sites [10 categories (+G, parameter = 0.6602)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 19.45% sites).

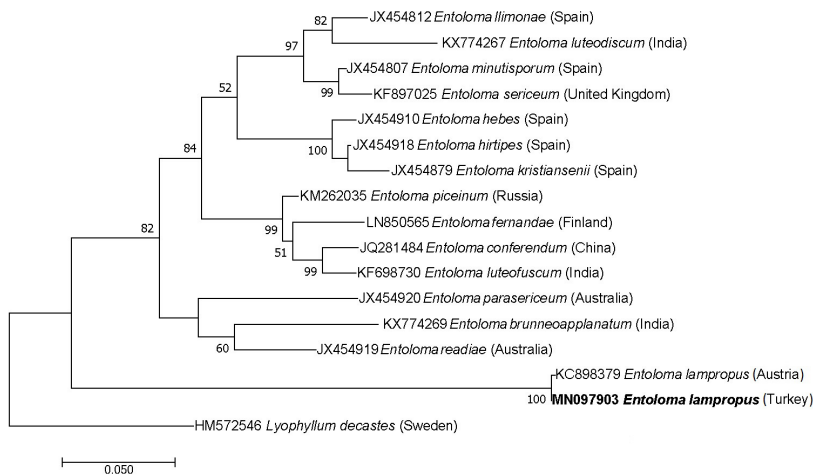


Figure 9. Phylogenetic tree created by using maximum likelihood analysis for ITS rDNA sequences of *Entoloma lampropus* in the present study and related species selected from GenBank database. Bootstrap values greater than 50% are given next to the branches. The highest log likelihood value of the tree is -5417.34. A discrete Gamma distribution was used to model evolutionary rate differences among sites [10 categories (+G, parameter = 0.6061)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 16.14% sites).

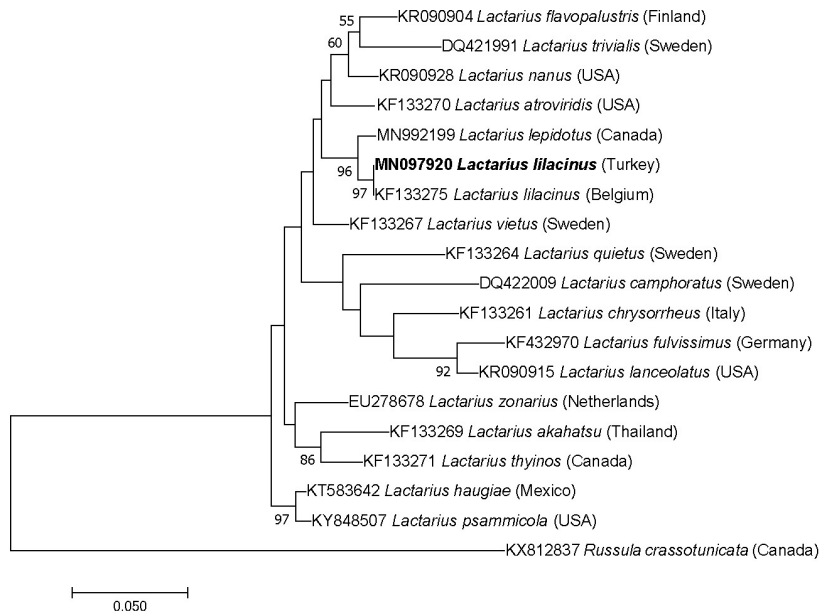


Figure 10. Phylogenetic tree created by using Maximum Likelihood analysis for ITS rDNA sequences of *Lactarius lilacinus* in the present study and related species selected from GenBank database. Bootstrap values greater than 50% are given next to the branches. The highest log likelihood value of the tree is 5376.11. A discrete Gamma distribution was used to model evolutionary rate differences among sites [10 categories (+G, parameter = 0.6387)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.00% sites).

were assessed together with the 34 ITS rDNA and 57 LSU rDNA sequences from GenBank. As a result, some species were assessed as synonyms such as *O. angusta* that nested within *O. nannfeldtii*. Also, in the study of Olariaga et al. (2015), five new species; *O. borealis*, *O. brunneoparva*, *O. oregonensis*, *O. pseudoleporina* were introduced and the species status of *O. subformicarum* was granted. Further on, *O. cantharella* var. *minor* and *O. onotica* var. *brevispora* were proposed. ITS rDNA and LSU rDNA sequences could be obtained for 12 of 30 type collections studied. Forty nine new ITS sequences were also reported in their studies. Phylogenetic placement of *O. nannfeldtii* was also addressed in these studies. In the study of Olariaga et al. (2015), nine *O. nannfeldtii* collections collected and obtained from Finland, France, Italy, Sweden, and the USA were analyzed phylogenetically. In our study, three of them were selected (accessed as KM010096, KM010097, and KM010097) for phylogenetic analyses and the phylogenetic position of our sample was proved as shown in Figure 7. In the study carried out by Hansen and Olariaga (2015), *O. nannfeldtii* has been nested in *O. formicarum* clade together with *O. formicarum* and *O. subformicarum* species. It has been observed that this clade is between *O. leporina* (*O. leporina*, *O. myosotis*, and *O. pseudoleporina* species) and *O. cantharella* (*O. cantharella*,

O. propinquata, and *O. brunneoparva* species) clades (Hansen and Olariaga, 2015).

O. nannfeldtii is a terricolous species that grows under broadleaved and coniferous trees as solitary or gregarious from September to December (Olariaga et al., 2015). The species is characterised by narrowly ear-shaped, yellowish ochre to brownish ochre and warty apothecia and small ascospores (less than 13 µm). Some *Otidea* members such as *O. formicarum*, *O. pseudoleporina*, and *O. tuomikoskii* resemble *O. nannfeldtii* due to their ear-shaped apothecia and small spores. Among them, *O. formicarum* is the most similar species but it usually has ear-shaped and darker colored apothecia. *O. pseudoleporina* is distinguished from *O. nannfeldtii* by a brighter ochre-orange hymenium while *O. tuomikoskii* by higher and more densely placed warts on the external surface of apothecia (Olariaga et al., 2015).

Very few sequences of *Conocybe dentatmarginata* were available in GenBank for the comparison of Turkish sequence data with the foreign samples. For this reason, the data obtained by Toth et al. (2013) was used. Confirmation of Turkish material was carried out using the ITS rDNA gene region and then they were compared with the sequences of Osmundson et al. (2013).

Conocybe dentatmarginata develops on bare ground or plant remains, under broadleaved trees or shrubs in

April (Hausknecht, 2007). It is an ex-annulate species, which has whitish veil remnants hanging from the margin, principally in young fruiting bodies. This species is mainly characterized by the large, ellipsoid spores, and lageniform cheilocystidia with a long, cylindrical neck and sometimes extended tip (Hausknecht, 2007). *C. dentatmarginata* may morphologically be confused with *C. nemoralis* Harmaja; however, the latter differs primarily by its variable shaped (cylindrical, subcylindrical, or fusoid-ventricose, sometimes slightly enlarged towards the tips) cheilocystidia (Harmaja, 1979; Hausknecht, 2007).

Ninety-eight new specimens were evaluated to figure out the taxonomic and phylogenetic positions of the 16 taxa of *Entoloma* subg. *Leptonia* together with the previous related collections analysed using ITS rDNA and LSU rDNA gene regions by Morozova et al. (2014). Neotypes were published for *E. dichroum*, *E. euchroum*, and *E. lampropus*. In this study, the collections of *E. lampropus* are originated from Austria (3), Germany (1), Sweden (1), and Russia (12). Genetic similarity among *Entoloma lampropus*, *E. placidum*, and *E. euchroum* has been reported by Morozova et al. (2014). Those three species belong to *Entoloma* subgenus *Leptonia* (Fr.: Fr.) Noordel. emend. O.V. Morozova, Noordel. & Vila, and Section *Leptonia* (Fr.: Fr.) Noordel. emend. O.V. Morozova, Noordel. & Vila. In our study, the GenBank accession submitted by Morozova et al. (2014) as KC898379 was used as a reference to compare the Turkish material.

Entoloma lampropus grows on soil or dead wood of coniferous trees from September to December. The species is close to *E. placidum* (Fr.: Fr.) Noordel. because of their similar macro and micro-morphology. Both species are characterized by greyish brown pileus, longitudinally fibrillose and blue stipe, four spored and clavate basidia with basal clamps, heterodiametrical spores with blunt angles, and the absence of cheilocystidia. Those two species, however, prefer different habitats. While the former grows both as terrestrial and on the deadwood of conifers, the latter only occurs on deadwood of deciduous trees such as beech, common hazel, and white birch (Morozova et al., 2014).

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- Rochet et al. (2011) phylogenetically evaluated 61 specimens of *Lactarius* collected under four European alder species in different locations of France and other European countries using ITS rDNA, RPB2, GPD, and SSU rDNA sequences. In their phylogenetic study, *Lactarius* collections nested within four lineages. *L. lilacinus* associated with *Alnus glutinosa* and *A. incana* clustered in the lepidotus lineage together with *L. lepidotus* associated with *Alnus alnobetula*. Montoya et al. (2014) described two new ectomycorrhizal *Lactarius* species located in pure *Alnus acuminata* subsp. *arguta* forest from central Mexico using ITS rDNA and RPB2 loci analyses and the phylogenetic position of *L. lilacinus* was considered together with these new species. For the phylogenetic analyses, two sequences of *L. lilacinus* submitted to GenBank and originated from Belgium and France were used. In the current study, the collected specimen, assessed as *L. lilacinus* by morphological characters, has been confirmed phylogenetically by comparing its ITS rDNA sequence with those of *L. lilacinus* available in NCBI GenBank database.
- Lactarius lilacinus* is a terricolous species that grows as gregarious or clustered from late August to November and it forms an ectomycorrhizal association with alder (*Alnus* Mill.) species. The species possess pink or salmon pileus with a velutinous to squamulose surface, pale pinkish buff to salmon stipe, and white and unchanging latex. *Lactarius lilacinus* could be confused with *L. spinosulus* Quél. & Le Bret. due to the similar color of their fruiting bodies, white and unchanging latex; however, the latter species is distinguished from *L. lilacinus* by its stronger zonate, more scaly pileus and shorter cheilomacrocytidia ($\leq 45 \mu\text{m}$) (Heilmann-Clausen et al., 1998).

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