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; Sessi and Denchev, 2014). Although approximately 2500 species of macrofungi have been recorded from Turkey (Şessi et al., 2016; Akata, 2017; Altuntaş et al., 2017; Işık and Türkakul, 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 characteristics of the specimens were recorded and photographed. The laboratory study included microscopic features 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
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 basidium .............................................................. 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 ...........

Ascomycota C. F. Müllern.

Peniophoraceae Dumort.

Sarcoppezia Loizides, Agnello & P. Alvarado

3.1.2. Sarcocpezia 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 Özür (FBozok00137, GenBank: nrITS MN097899).

Pyronemataceae Corda

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


*Basidiomycota* R.T. Moore

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


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

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 Noord., Hyporrhodius lampropus (Fr.: Fr.) Henn., Leptonia lampropus (Fr.: Fr.) Quél., Rhodophyllus lampropus (Fr.: Fr.) Quél.


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 lateritioroseus P. Karst., L. lilacinus subsp. eulilacinus Singer, L. lilacinus subsp. mitificus (Britzelm.) Singer, L. lilacinus var. minor Killerm., L. lilacinus var. mitificus (Britzelm.) Killerm.,
L. mitificus Britzelm. Lactifluus lateritioroseus (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 decurred 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. **Pleuromacrocytis** 60–100 × 8–10 µm, cylindrical, obtuse or mucronate. **Cheilocyctis** 50–80 × 8–9 µm, cylindrical, obtuse or mucronate. **Pilepellis** 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 3. *Conocybe dentatomarginata: a–c. Basidiomata, d, e. Basidium, f–g. Cheilocystidia, h. Basidiospores (LM), i. Basidiospores (SEM).*
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 dentatomarginata 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
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

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-1α gene regions. Before obtaining these 89 collections for four-gene dataset, 112 ITS and LSU rDNA sequences

**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).
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).
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 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 dentatomarginata* 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 dentatomarginata* develops on bare ground or plant remains, under broadleaved trees or shrubs in

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**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).
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. dentatomarginata* 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 fibrilllose 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).

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 acuminate* 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 terricoleous 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 cheilocystidia (≤ 45 μm) (Heilman-Clausen et al., 1998).

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