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***Suillus lakei*, An Interesting Record For Turkish Mycobiota**

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Abstract: In this study, *Suillus lakei* (Murrill) A.H. Sm. & Thiers) was reported for the first time from Turkey. This species is characterized by its ectomycorrhizal features and the occurrence under *Pseudotsuga menziesii* (Douglas fir). Besides conventional identification methods, molecular methods (ITS rDNA) were also used and results were uploaded to GenBank. According to the Genbank results, our species shows 99% similarity to other data related to *Suillus lakei*. A short description with molecular analysis were given in the text and the results discussed briefly.

Key words: *Suillus lakei*, Douglas fir, ITs, new record, Turkey

***Suillus lakei*, Türkiye Mikobiyotası İçin İlginç Bir Kayıt**

Öz: Bu çalışmada, *Suillus lakei* (Murrill) A.H. Sm. & Thiers Türkiye'den ilk defa rapor edilmiştir. Bu tür, ektomikorhizal özellikleri ve *Pseudotsuga menziesii* (Douglas göknarı) altında yayılış göstermesi ile karakterize edilir. Geleneksel tanımlama yöntemlerinin yanı sıra moleküler yöntemler de (ITS rDNA) kullanılmış ve sonuçlar GenBank'a yüklenmiştir. Genbank sonuçlarına göre, örneklerimiz *Suillus lakei* ile ilgili diğer verilere %99 benzerlik göstermektedir. Metinde moleküler analizlerle birlikte kısa bir tanımlama verilmiş ve sonuçlar kısaca tartışılmıştır.

Anahtar kelimeler: *Suillus lakei*, Douglas göknarı, ITs, yeni kayıt, Türkiye

Introduction

Suillus includes approximately 50 species that form ectomycorrhizal relations mainly with Pine trees (Kirk et al., 2008). Even though *Suillus* members are mostly distributed in northern temperate regions, some of them have also been reported in southern hemisphere (Sarwar and Khalid, 2014).

Suillus lakei, commonly known as The Western Painted Suillus, forms ectomycorrhizal associations with Douglas fir and its fructifications could easily be observed on the ground in summer and autumn (Szczepekowski and Olenderek, 2017). The species is characterized by its dry, dark red to reddish-brown, fibrillose or scaly pileus, dingy yellow to ochre pores discolouring reddish brown when bruised, equal to slightly clavate, firm and solid stipe usually with veil (Arora, 1986).

According to checklist on Turkish macrofungi (Sesli and Denchev, 2008), 11 *Suillus* species (*S. bellinii* (Inzenga

Kuntze, *S. boudieri* (Quél.) Kuntze, *S. bovinus* (L.) Roussel, *S. collinitus* (Fr.) Kuntze, *S. flavidus* (Fr.) J. Presl, *S. granulatus* (L.) Roussel, *S. grevillei* (Klotzsch) Singer, *S. luteus* (L.) Roussel, *S. placidus* (Bonord.) Singer, *S. spraguei* (Berk. & M.A.Curtis) Kuntze and *S. variegatus* (Sw.) Richon & Roze) have thus far been reported from Turkey. But there is no record of *S. lakei* for Turkish mycobiota.

The aim of this study is to make a contribution to the Turkish mycobiota.

Materials and methods

Morphological study

Suillus samples were collected from İstanbul and Kocaeli provinces between 2014 and 2015. During field studies,



macroscopic and ecological characteristics were noted and they were photographed in their natural habitats. In the herbarium, macroscopic and microscopic investigations and micro-chemical reactions were carried out. Reagents such as melzer's reagent, 5% KOH, 10% NH₄OH, H₂SO₄, congo red etc. were used. Identification was performed according to the current literature (Assyov et al., 2006; Barroetaveña, 2007; Lavorato, 1999; Myra and Grace, 1986; Zahradka, 2005). Identified samples were kept in Mushroom Application and Research Centre Fungarium, Selçuk University.

Molecular study

Total genomic DNA isolation was carried out from dried samples by following the procedures of Eurx Genematrix Plant & Fungi DNA Purification Kit with small modifications. ITS1F-ITS4R primers were used for the amplification of ITS rDNA region (White et al., 1990). PCR conditions were set as follows: 94°C for 5 min, followed by 30 cycles of 30s at 94°C, 45s at 53°C, 60s at 72°C and a final extension of 10 min at 72°C. PCR amplifications were verified by electrophoresis on a 1.5% agarose gel. DNA sequence analyses of successful amplifications were performed by BMLabosis. Maximum Likelihood (ML) and Maximum Parsimony (MP) phylogenetic trees were drawn in MEGA7.0 by aligning the sequences in Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI).

Results

Suillaceae Besl & Bresinsky

Suillus lakei (Murrill) A.H. Sm. & Thiers (1964), (Figure 1,2).

Syn.: *Boletinus lakei* (Murrill) Singer (1945), *Boletinus lakei* subsp. *landkammeri* (Pilát & Svrček) Pilát & Dermek (1974), *Boletinus landkammeri* (Pilát & Svrček) Bon (1986), *Boletus lakei* Murrill (1912), *Boletus tridentinus* subsp. *landkammeri* Pilát & Svrček (1949), *Ixocomus lakei* (Murrill) Singer (1942), *Suillus lakei* var. *calabrus* Lavorato (2000), *Suillus lakei* var. *landkammeri* (Pilát & Svrček) H. Engel & Kľofac (1996), *Suillus lakei* var. *pseudopictus* A.H. Sm. & Thiers (1964).

Macroscopic features

Pileus 70-110 mm across, broadly convex at first, later plane, margin incurved and often with veil remnants, surface fibrillose to scaly, reddish-brown to pinkish-brown on a yellowish to dingy orange background. **Tubes** adnate to decurrent, yellow to dingy yellow. **Pores** angular, yellow when young, later dingy yellow to ochre, discolouring reddish brown to brownish when bruised (Figure 1). **Stipe** 40-70 × 10-20 mm, cylindrical to clavate, solid, firm, yellow at apex, with reddish-brown fibrils on lower part. **Flesh** thick, yellow, discolouring pinkish-red when bruised, **Odour** and **taste** not distinctive. **Veil** thin, membranous white to pale yellow.

Microscopic features

Basidia 25-30 × 9-10 µm, clavate, 2 to 4 spored (Figure 2a). **Spores** 8-9 × 3-4 µm, elliptic to subfusiform, thick-walled and smooth (Figure 2b). **Pleurocystidia** 40-60 × 9-10 µm, abundant to numerous and subclavate (Figure 3c,d). **Cheilocystidia** similar to pleurocystidia. **Caulocystidia** 70-80 × 10-14 µm, cylindrical to subclavate (Figure 2e). **Pileipellis** interwoven, floccose, homogenous, terminal cells 6-12 µm broad (Figure 2f).

Ecology: Summer to autumn, on poor and exposed soil associated with Douglas fir (Arora, 1986).

Distribution: Reported in Europe (Bosna and Herzegovina, Bulgaria, Denmark, England, Germany, Hungary, Italy, Poland, Slovakia and The Czech Republic.), America (Argentina, Chile and USA) and New Zealand (Assyov et al., 2006; Barroetaveña, 2007; Lavorato, 1999; Myra and Grace, 1986; Valenzuela and Esteve-Raventos, 1999; Vasas and Albert, 1990).

Material examined: TURKEY—İstanbul: Belgrad Forest, Atatürk Arboretum, under *Pseudotsuga menziesii* (Mirb.) Franco (planted Douglas fir), 41°10'N - 28°59'E, 130m, 27.09.2015, AKATA 6278; Kocaeli: Maşukiye, Sisliyadi, under (planted Douglas fir), 40°39'N-30°07'E, 1200m, 25.10.2014, HHDOĞAN 15048.

Phylogenetic affiliation

Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses

The evolutionary history was inferred by using the Maximum Likelihood and Maximum Parsimony methods (Figure 3). 35 nucleotide sequences were used for the analyses. The highest log likelihood of the tree was -1702.74 and length of 6 most parsimonious trees was 207. The consistency, the retention and the composite indexes were 0.670968, 0.894191 and 0.673883 for all sites and parsimony-informative sites, respectively. The percentage of trees was shown next to the branches. All positions containing gaps and missing data were eliminated. In the final dataset, there were a total of 440 positions. Shared branch length (SBL) and Transitions/Transversions (Ts/Tv) values were 0.51578639 and 1.6630, respectively. The average values for bases T, C, A and G (%) of sequences which used in the phylogenetic tree were 27.7, 23.7, 22.3 and 26.3, respectively (Table 1).

Discussion

Suillus lakei could be confused with *S. caerulescens* A.H. Sm. & Thiers and *S. ponderosus* A.H. Sm. & Thiers in terms of morphology and ecology. *S. lakei*, *S. caerulescens* and *S. ponderosus* are ectomycorrhizal with Douglas fir, while *S. imitatus* grows in mixed conifer forest, commonly associate with spruce. *S. caerulescens* differs from *S. lakei* by its more orangish pileus and slightly shorter spores.

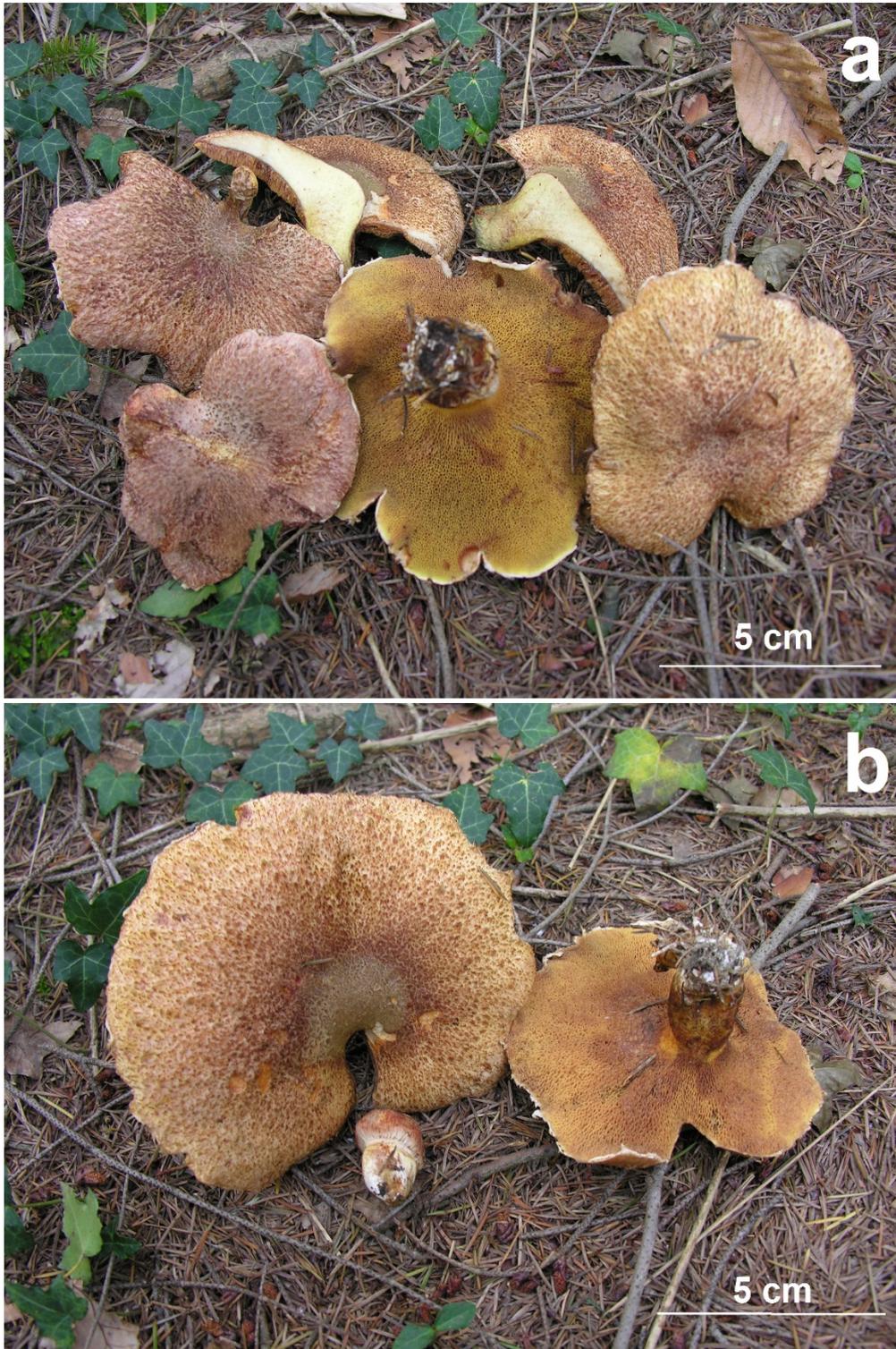


Figure 1. *Suillus lakei*: a, b-basidiomata.

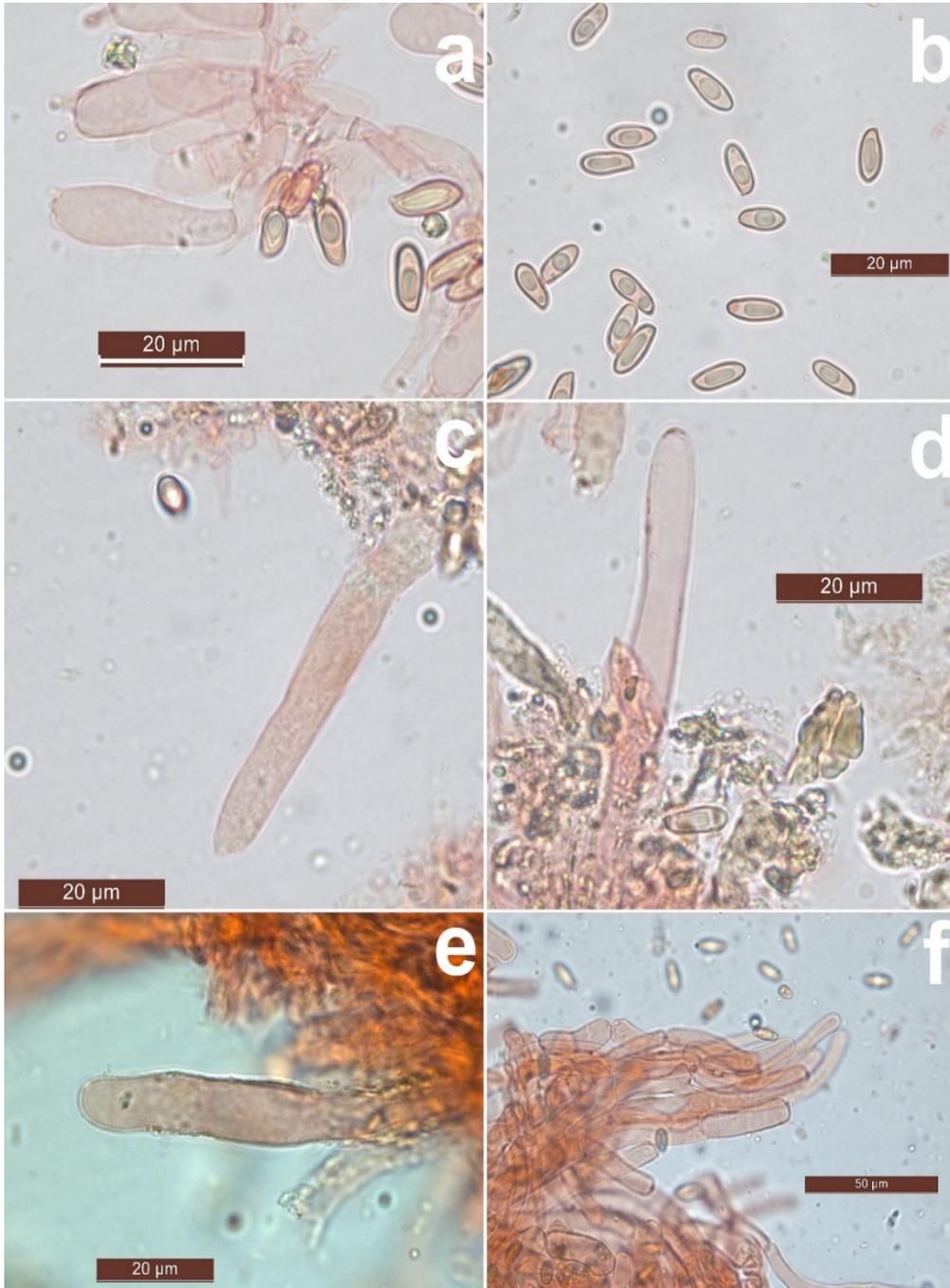


Figure 2. *Suillus lakei*: a-basidia. b-spores. c, d- pleurocystidium. e- caulocystidium. f- pileipellis

**Table 1.** Taxa, their accession number and T, C, A, G bases percentages (%) obtained from Genbank

Taxon	Location	T	C	A	G	Sequence Length (bp)	ITS Genbank Accession
<i>Rhizopogon luteolus</i>	UK	32.1	18.6	25.9	23.4	791	JQ888192
<i>Suillus tridentinus</i>	Germany	28.0	23.9	21.6	26.6	644	L54082
<i>Suillus tridentinus</i>	Italy	27.9	24.6	20.9	26.6	632	GU181836
<i>Suillus grisellus</i>	USA	27.7	23.7	22.7	25.9	679	KX230585
<i>Suillus grisellus</i>	USA	27.7	23.8	22.6	25.9	668	KX213732
<i>Suillus laricinus</i>	Not available	26.5	23.7	23.9	25.9	811	LC029032
<i>Suillus viscidus</i>	Korean	27.6	24.0	22.3	26.1	637	KJ415106
<i>Suillus elbenis</i>	USA	27.6	23.2	22.4	26.8	608	KX230646
<i>Suillus elbenis</i>	USA	27.4	23.2	22.5	26.9	609	KX230588
<i>Suillus viscidus</i>	Italy	27.6	24.8	21.3	26.3	456	JF908727
<i>Suillus viscidus</i>	Not available	28.2	24.3	21.4	26.2	544	JF908723
<i>Suillus cf. laricinus</i>	Nepal	28.1	24.3	21.6	26.0	643	L54120
<i>Suillus lariciphilus</i>	India	28.4	25.2	20.7	25.7	580	KJ778009
<i>Suillus clintonianus</i>	USA	28.0	22.8	22.8	26.4	711	KX230616
<i>Suillus clintonianus</i>	USA	27.7	23.0	22.8	26.5	710	KX230615
<i>Suillus grevillei</i>	Potugal	27.8	23.4	22.1	26.7	693	HM347659
<i>Suillus grevillei</i>	Sweden	27.4	23.1	21.8	27.7	628	EF493260
<i>Suillus cavipes</i>	USA	26.9	24.6	21.7	26.8	647	L54119
<i>Suillus cavipes</i>	Not available	26.1	23.9	23.2	26.9	830	JF899572
<i>Suillus cavipes</i>	China	27.2	24.1	22.9	25.8	717	AF166506
<i>Suillus cavipes</i>	China	26.6	24.0	22.9	26.5	721	AF166505
<i>Suillus asiaticus</i>	Finland	27.3	25.3	21.3	26.1	630	L54090
<i>Suillus asiaticus</i>	China	26.8	24.2	22.7	26.4	724	AF166504
<i>Suillus ponderorus</i>	USA	26.7	24.0	22.7	26.7	724	JQ958326
<i>Suillus ponderorus</i>	USA	26.5	23.9	22.9	26.7	716	JQ958325
<i>Suillus caerulescens</i>	USA	27.8	24.8	21.2	26.2	641	KX213728
<i>Suillus caerulescens</i>	USA	27.9	23.9	21.6	26.7	524	JQ958310
<i>Suillus lakei</i>	USA	27.4	23.7	22.3	26.5	679	KX213727
<i>Suillus lakei</i>	USA	27.6	24.1	21.8	26.5	675	KX230607
<i>Suillus lakei</i>	USA	27.1	23.9	21.9	27.0	691	DQ365643
<i>Suillus lakei</i>	USA	27.7	23.9	21.8	26.6	675	KX230593
<i>Suillus lakei</i>	USA	27.4	24.1	21.9	26.5	677	KX230610
<i>Suillus lakei</i>	USA	27.5	24.1	21.9	26.5	677	KX213757

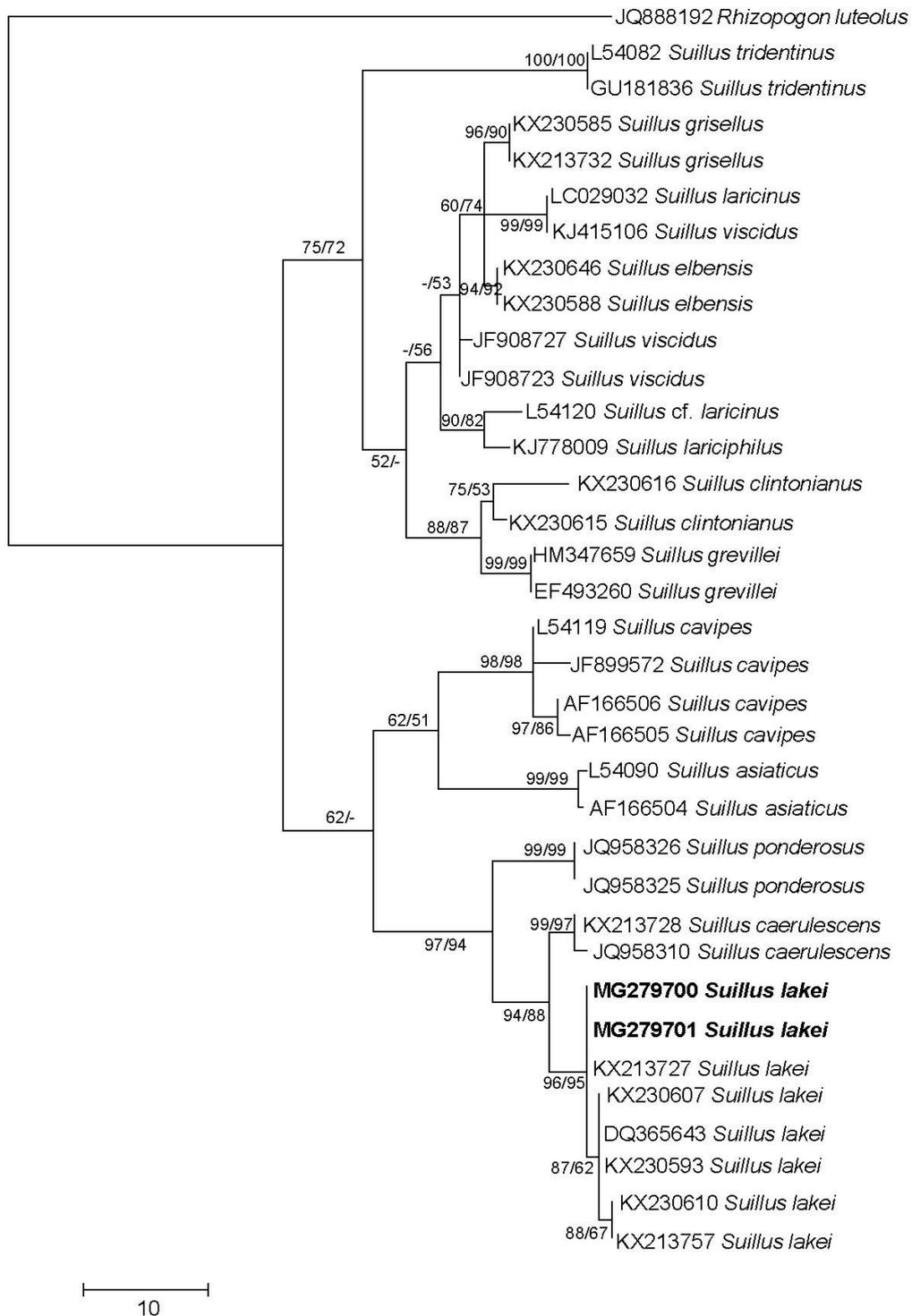


Figure 3. ITS Maximum Parsimony (MP) Tree of *Suillus lakei* in this study and other *Suillus* species in *Suillaceae* family selected from GenBank. *Rhizopogon luteolus* was used as outgroup. Support values (MP/ML bootstrap percentages) were shown next to the branches. The percentages >50% were shown. *Suillus lakei* sequences in the present study were shown as bold in the phylogenetic tree.



When the Genbank database was searched, there were 17 sequences (14 ITS and one atp6, SSU and LSU for each) in total for *S. lakei*.

In this study, 2 new ITS rDNA sequences belong to *S. lakei* were added to the Genbak database. When the ITS rDNA gene sequences of *S. lakei* samples collected from different regions of Turkey (Istanbul and Kocaeli) were compared with the sequences of samples in the Genbank database, it showed 99% similarity with ITS rDNA gene sequences of samples from California and

Colorado (USA) (Accession no KX213727, KX213727, KX230607, DQ365643, KX230593, KX230610, KX213757).

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