

## *Lyophyllum turcicum* (Agaricomycetes: Lyophyllaceae), a new species from Turkey

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**Abstract:** A new species, *Lyophyllum turcicum*, from the Kümbet plateau of the Dereli district in Giresun Province, Turkey, is described, taxonomically delimited, and illustrated based on morphological and molecular data. The new species belongs to a small group of species in the *Lyophyllum* sect. *Difformia* as traditionally circumscribed. *Lyophyllum turcicum* is easily distinguished mainly by the light tinges of the basidiomes, filiform-fusiform to cylindro-flexuose marginal cells, and elongate, ellipsoid spores.

**Key words:** Lyophyllaceae, *Lyophyllum turcicum*, new species, Turkey

### 1. Introduction

In recent years some new taxa have been described in the complex of the not blackening, caespitose-growing *Lyophyllum* P. Karst. species belonging to section *Difformia* (Bon, 1999). *Lyophyllum* sect. *Difformia* is presently known to include 14 caespitose and/or not blackening *Lyophyllum* species worldwide. Most of them are from Europe, with a few exceptions: *L. multifforme* from North America, *L. tucumanense* from Argentina, and *L. shimeji* from Japan. Two subsections have been distinguished: the first subsection, *Difformia*, encompasses the caespitose-growing species, which are the majority; the second subsection, *Lanzoniana* Consiglio & Contu, encompasses the not caespitose-growing species. No species belonging to the second subsection seem to be known outside Europe (Consiglio and Contu, 2002). Although new species are in print and the number of taxa will increase, the following species are currently known: *L. brunneum* Dähncke, Contu & Vizzini (Canary Islands); *L. calabrum* Lavorato & Contu (Italy); *L. cistophilum* Vila & Llimona (Spain, Italy); *L. conglobatum* (Vitt.) Bon (widespread in Europe); *L. decastes* (Fr.) Sing. (widespread in Europe); *L. fumosum* (Pers.) P.D. Orton (widespread in Europe); *L. lanzonii* Candusso (Italy); *L. multifforme* (Peck.) Bigelow (North America, Turkey); *L. pergamenum* (Cooke) Hornicek (= *connatum* ss Auct pl., widespread in Europe); *L. pseudoloricatum* Dähncke, Contu & Vizzini (Canary Islands); *L. shimeji* (Kawamura) Hongo (East Asia, Northern Europe); *L. subglobisporum* Consiglio & Contu (Italy, France, Spain);

*L. soniae* Picillo & Contu (Italy); and *L. tucumanense* Sing. (Argentina, South America). The genus *Lyophyllum* presently consists of about 230 taxa worldwide (Robert et al., 1999; Consiglio and Contu, 2002).

Seven *Lyophyllum* species [*L. decastes* (Fr.) Singer, *L. fumosum* (Pers.) P.D. Orton, *L. infumatum* (Bres.) Kühner, *L. loricatum* (Fr.) Kühner ex Kalamees, *L. multifforme* (Peck) H.E. Bigelow, *L. semitale* (Fr.) Kühner ex Kalamees, and *L. transforme* (Britzelm.) Singer] have been reported from Turkey prior to the present study (Sesli and Denchev, 2008). Four of them (*L. decastes*, *L. fumosum*, *L. loricatum*, and *L. multifforme*) belong to the section *Difformia*. According to recent studies, the other species recorded in Turkey are not classified in *Lyophyllum* [*L. ambustum* (Fr.) Singer = *Tephroclybe*, *L. connatum* (Schumach.) Singer = *Clitocybe*, *L. favrei* (R. Haller Aar. & R. Haller Suhr) R. Haller Aar. & R. Haller Suhr, and *L. leucophaeatum* (P. Karst.) P. Karst. = *Calocybe*].

*L. favrei* (R. Haller Aar. & R. Haller Suhr) R. Haller Aar. & R. Haller Suhr was collected from İzmir (Solak et al., 1999) and *L. decastes* (Fr.: Fr.) Singer from Samsun (Peşen and Karaca, 2000). "*L. connatum* (Schumach.: Fr.) Singer" was collected for the first time from Mersin-Mut (Doğan et al., 2003) and later from Gümüşhane (Uzun et al., 2006). In an earlier study, the first author collected *L. multifforme* (Peck) H.E. Bigelow from the roadside in a *Picea orientalis* forest near Giresun-Görelle-Deregözü (Sesli, 2007).

During field work in the Kümbet plateau of the Dereli district in Giresun Province in Turkey (8 October 2010),

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unusual *Lyophyllum* specimens were collected by the first author. Giresun is located in Turkey's Eastern Black Sea Region, having a steep, rocky area with elevations of 1500 to 1800 m in the west and 3000 to 4000 m in the east. The higher slopes facing the northwest are densely forested depending on natural conditions. The collection site is in the Euro-Siberian phytogeographical region of Turkey and the most common trees and shrubs are *Alnus glutinosa* (L.) Gaertn., *Carpinus betulus* L., *C. orientalis* Mill., *Corylus avellana* Thunb., *Fagus orientalis* Lipsky, *Picea orientalis* (L.) Peterm., *Rhododendron luteum* Sweet, and *R. ponticum* L.. The mild, damp oceanic climate with high and evenly distributed rainfall makes fungal biodiversity very rich. At the collection site, summers are warm and humid, and winters are cool and damp. Following a detailed study and molecular analysis of the described specimens, they were determined to belong to a new species, given the name *Lyophyllum turcicum*. The new species belongs to sect. *Difformia* as traditionally circumscribed (Figure 1).

## 2. Materials and methods

The basidiomes of *Lyophyllum turcicum* were collected in the Kümbet high plateau (Dereli, Giresun, Turkey) on 8 October 2010. Field notes and photos of the basidiomes

were taken at the collection site. Other photos were taken in the laboratory after evaporation of excess water of the basidiomes. One basidiome was used for a spore print and the rest were dried for future microscopic studies. A Zeiss Axio Imager A2 trinocular research microscope was used to observe microscopic structures. Microscopic sections of the basidiomes were reinflated in a concentrated ammonia solution and subsequently in Congo red; they were finally investigated under a light microscope. Iron chloride solution, acetocarmine, and chloral hydrate were used to observe carminophilous granules (Clémenton, 2009). Spore dimensions were measured with the Axio Imager software from 350 spores obtained from the spore print. Color terms in capital letters (e.g., Pale Ochraceous-Salmon) refer to Ridgway (1912). For identification of the species, Bon (1999), Consiglio and Contu (2002), Kalamees (2004), Knudsen and Vesterholt (2012), and Dähncke et al. (2010, 2011) were used. Author names are given according to Kirk et al. (2008), fungal names follow MycoBank (Robert et al., 1999), and nomenclature and taxonomy were made according to Bon (1999). The type materials are kept at Karadeniz Technical University (KATO) and the Department of Life Sciences and Systems Biology of the University of Turin (TO).

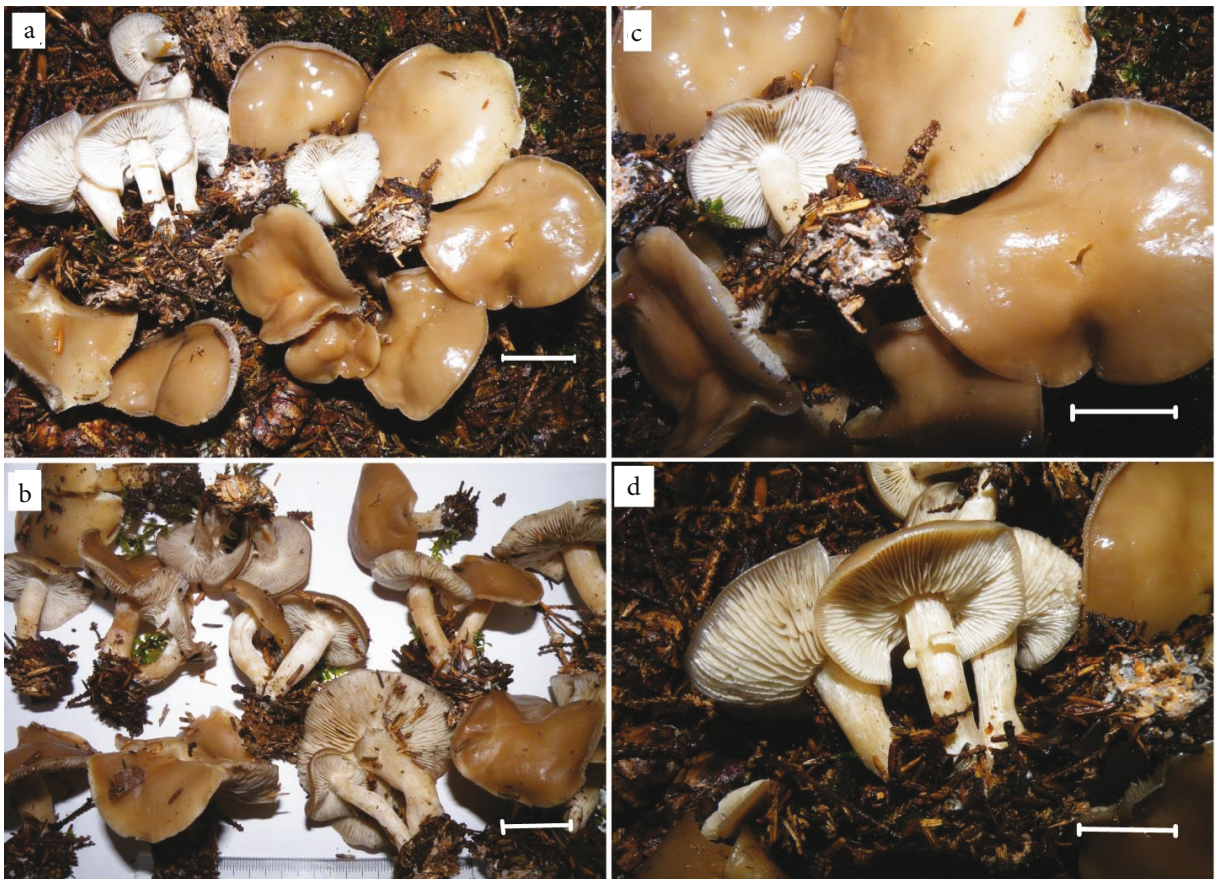
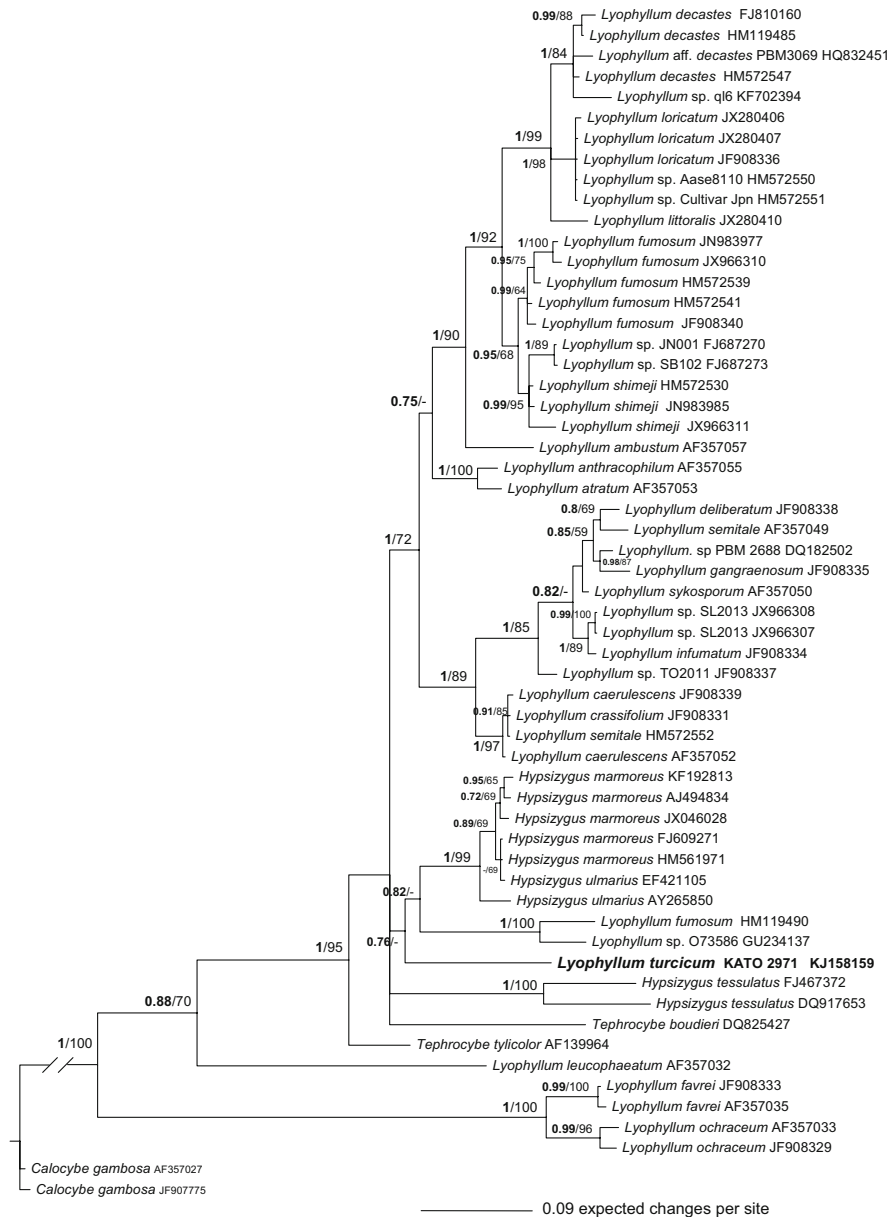


Figure 1. *Lyophyllum turcicum*: a, b, c, and d- basidiomes (scale bars: 2 cm).

**2.1. DNA extraction, PCR amplification, and DNA sequencing**

Genomic DNA was isolated from 1 mg of one herbarium specimen using the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy). Universal primers ITS1F/ITS4 were used for ITS region amplification (White et al., 1990; Gardes and Bruns, 1993). Amplification reactions were performed in a PE9700 thermal cycler (PerkinElmer, Applied Biosystems, USA) in a 25- $\mu$ L reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1X PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1  $\mu$ M of

each primer, 2.5 mM MgCl<sub>2</sub>, 0.25 mM of each dNTP, and 0.5 unit of Taq polymerase (Promega, USA). The PCR program was as follows: 3 min at 95 °C for 1 cycle; 30 s at 94 °C, 45 s at 50 °C, and 2 min at 72 °C for 35 cycles; and 10 min at 72 °C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. PCR products were purified and sequenced by MACROGEN (Seoul, Republic of Korea). Sequence assembly and editing were performed using Geneious v.5.4 (<http://www.geneious.com>). The sequence was deposited in GenBank under the accession number given in Figure 2.



**Figure 2.** Bayesian phylogram obtained from the general nrITS sequence alignment of Lyophyllaceae. *Calocybe gambosa* was used as the outgroup taxon. Support values in either the Bayesian [posterior probabilities values (BPPs)] or maximum likelihood [ML bootstrap percentage (MLB)] analyses are indicated. Only BPP values over 0.70 (in bold) and MLB values over 50 are given above clade branches. The new sequence is in bold.



## 2.2. Sequence alignment and phylogenetic analysis

The sequence obtained in this study was compared with those available in the GenBank database (<http://www.ncbi.nlm.nih.gov/>) by using the BLASTN algorithm. Based on these results, sequences with the greatest similarity were selected. Alignments were generated using MAFFT (Kato et al., 2002) with default conditions for gap openings and gap extension penalties. The sequence alignments were then imported into MEGA 5.10 (Tamura et al., 2011) for manual adjustment. *Calocybe gambosa* was used as the outgroup taxon. Best-fit models were estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada, 2008) to provide a substitution model for the alignment. Phylogenetic analyses were performed using the Bayesian inference (BI) and maximum likelihood (ML) approaches. The BI approach was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001), with four incrementally heated simultaneous Monte Carlo Markov chains run over 10 million generations under the GTR+ $\Gamma$  evolutionary model. Trees were sampled every 1000 generations, resulting in an overall sampling of 10,001 trees; the first 2500 trees were discarded as “burn in” (25%). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPPs). ML estimation was performed with RAxML v.7.3.2 (Stamatakis, 2006) with 1000 bootstrap replicates (Felsenstein, 1985), using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-fa” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al., 2010). Only BPP values over 0.70 and MLB values over 50 were reported in the resulting tree (Figure 2). Branch lengths were estimated as mean values over the sampled trees.

## 3. Results

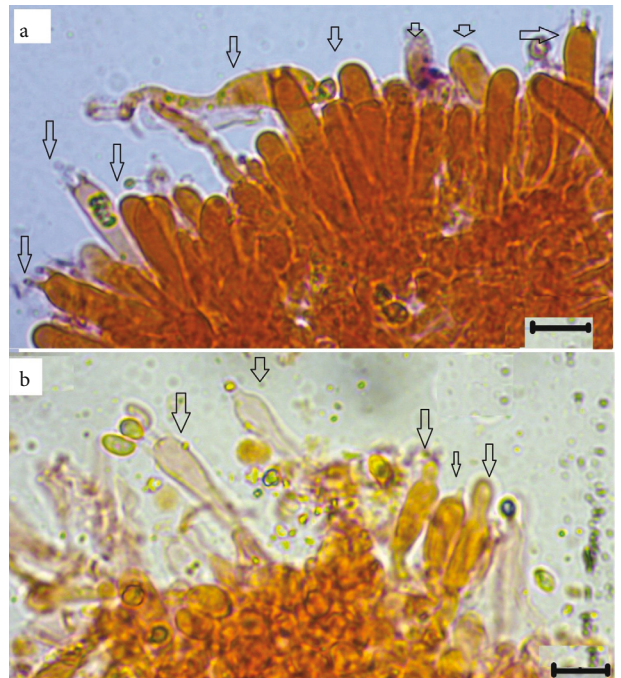
### 3.1. Molecular analysis

Both BI and ML analyses produced the same topology; therefore, only the Bayesian tree with both BPP and MLB values is shown (Figure 2). The ITS data matrix comprised a total of 58 sequences (including 57 from GenBank). In the Bayesian phylogram the sequence of *L. turcicum* occupies an isolated position and is sister to a clade formed by a *Lyophyllum* sp., a sequence labelled “*L. fumosum*”, and sequences of *Hypsizygus marmoreus* and *H. ulmarius*.

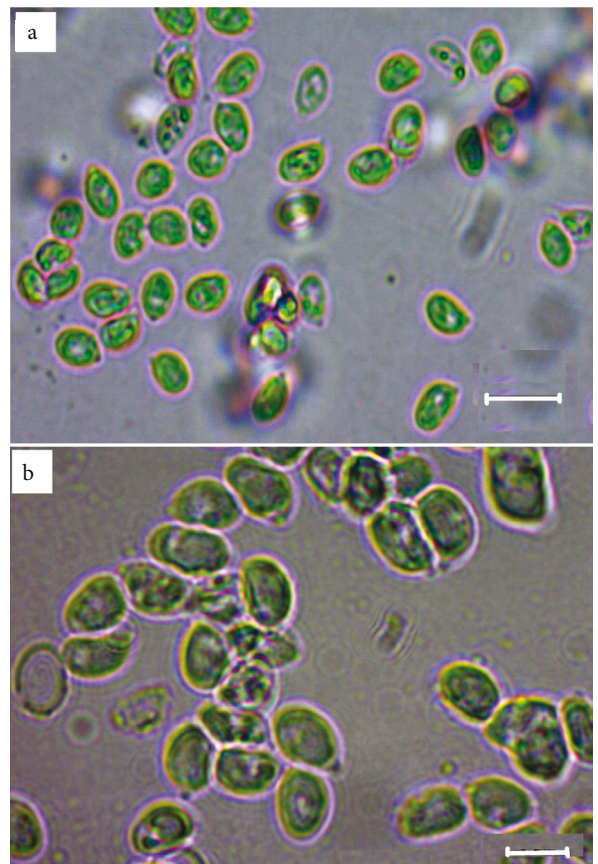
### 3.2. Taxonomy

#### 3.2.1. *Lyophyllum turcicum* Sesli, Vizzini & Contu sp. nov. (Figures 1, 3, 4, 5, and 6)

MycoBank MB 807330

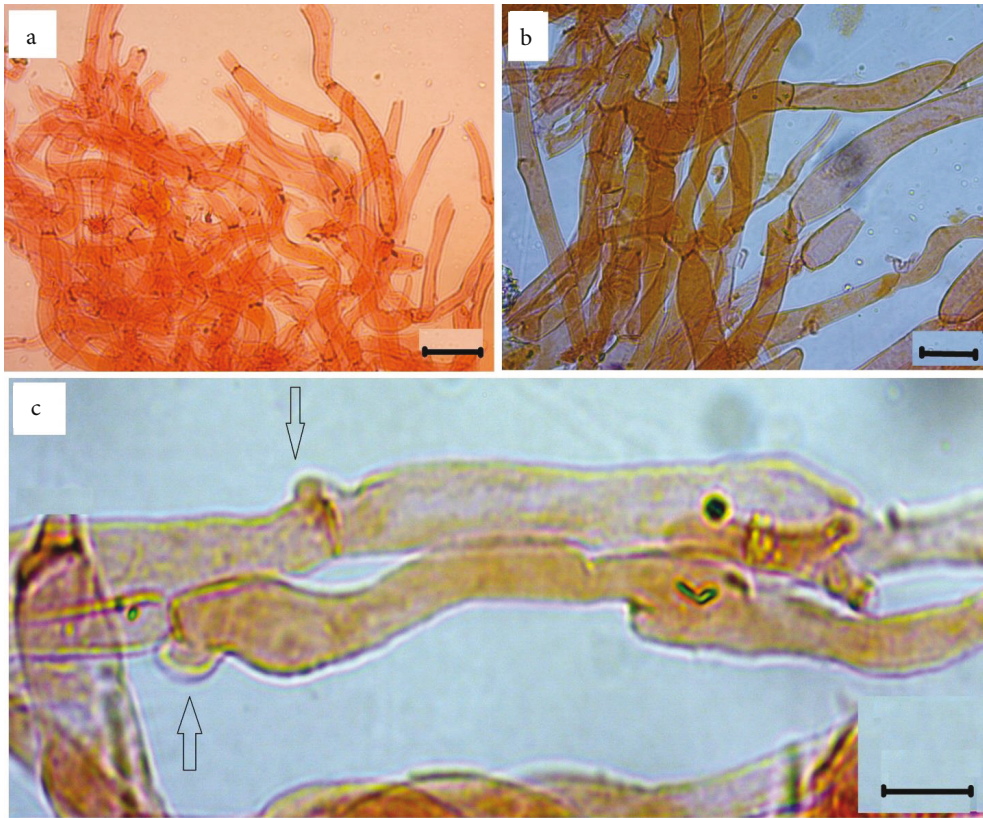


**Figure 3.** *Lyophyllum turcicum*: a and b- basidia (each arrow shows a separate basidium; scale bars: 10  $\mu$ m).



**Figure 4.** *Lyophyllum turcicum*: a and b- basidiospores (scale bars: a = 10  $\mu$ m, b = 5  $\mu$ m).





**Figure 5.** *Lyophyllum turcicum*: a- hyphae of subpileipellis; b- hyphae of subhymenium; c- clamp connections (each arrow shows a separate clamp connection; scale bars: a and b = 20  $\mu$ m, c = 10  $\mu$ m).

A tricholomatoid species with fleshy and fragile basidiomes that differs from *Hypsizyguus marmoreus*, *H. ulmarius*, and *Lyophyllum fumosum* in having ellipsoid, narrow ( $6 \times 4 \mu$ m) spores. Pileus 2–6 cm, broadly convex or flat with depressed or umbilicate center, beige-brown to wood color, lamellae slightly decurrent, basidia 2–4-spored and marginal cells filiform-fusiform to cylindro-flexuose.

Type: Turkey, Giresun, Dereli, Kümbet high plateau,  $40^{\circ}32.922'N$ ,  $38^{\circ}26.425'E$ . 08.10.2010, leg. E. Sesli (Holotype, KATO - Fungi 2971; isotype, TO AV410a).

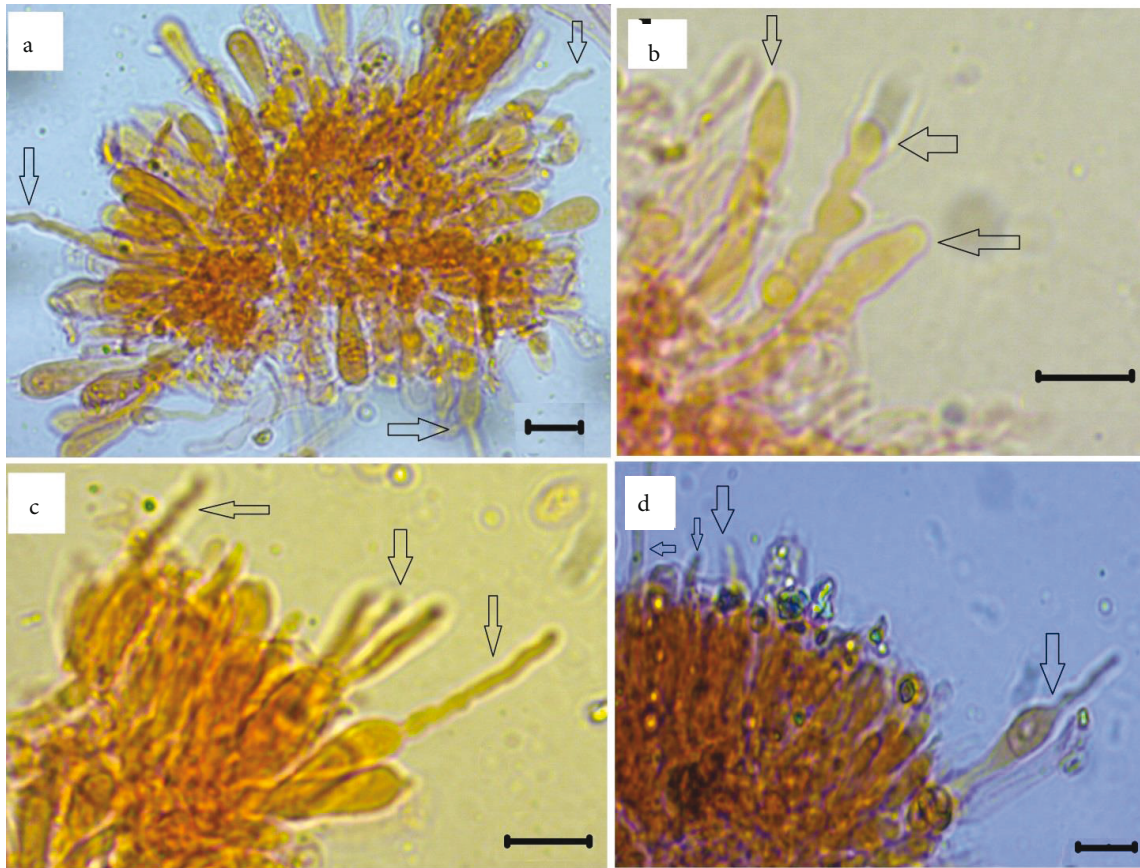
Etymology: From Latin *turcicum* = Turkish.

**Pileus** 2–6 cm wide, fleshy, fragile, variable in shape according to growth conditions, broadly convex when young, later more flat with depressed or umbilicate center, without umbo, sometimes seems saddle-shaped, pileus margin enrolled when young and curved outward in the course of time. Sometimes margin wavy, pileus context watery soaked in wet weather conditions,  $\pm$ pale brown, beige-brown to wood color (Pale Ochraceous-Salmon, Light Ochraceous Salmon, Plate XV; Buff-Pink, Plate XXVIII; Light Pinkish Cinnamon-Cinnamon, Plate XXIX; Avellaneous, Plate XL), typically smooth, glabrous and shiny, cuticle easily separable. **Lamellae** medium spaced, adnate to slightly decurrent, white or creamy to grayish,

moderately thin, easily removable from the context when fresh, edges smooth. **Stipe** 2–5.5 cm long and 0.5–1.5 cm wide, cylindrical to clavate, typically pruinose, especially at the base, fibrillose, sometimes tapered towards the base, often twisted, sometimes thickened towards the base or generally enlarged with the base because of large debris, sometimes slightly longitudinally grooved, whitish to pale gray or dingy white, turning slightly beige to pale gray to brownish on handling, remnants of mycelium typically present at the base. **Context** white, up to 5 mm thick at the pileus center, considerably thinner towards its margin. **Odor and taste** not distinctive.

**Spores**  $(5-5.5-7(-7.8) \times (3.1-3.5-4.5(-4.8) \mu$ m ( $n = 350$  and  $Q = 1.3-1.8$ ), on average  $6 \times 4 \mu$ m, ellipsoid, hyaline, smooth, always with oil drops, cyanophilous, nonamyloid in Melzer's reagent. Spore deposit white to cream. **Basidia**  $25-35 \times 5.5-6.5(-8) \mu$ m ( $n = 20$ ), (1-)2-4 spored, some with basal clamp connections, clavate, some with siderophilous granules. **Subhymenium** made up of moderately thin-walled hyphae, 4–20  $\mu$ m across. Occasional hyphal ends exerted and somewhat flexuose or forked. **Hymenophoral trama** regular, consisting of thin and hyaline hyphae, some with clamp connections.

**Marginal cells** not very conspicuous, thin-walled,



**Figure 6.** *Lyophyllum turcicum*: a, b, c, and d- marginal cells (each arrow shows a separate marginal cell; scale bars: 10  $\mu$ m).

hyaline, uni- to pluriseptate,  $15\text{--}40 \times 2.7\text{--}5 \mu\text{m}$ , filiform-fusiform to cylindro-flexuose ( $n = 20$ ). **Pileipellis** a cutis made up of parallel to slightly interwoven cylindrical hyphae,  $4\text{--}10\text{--}(16) \mu\text{m}$  across, mostly with intraparietal pigment, some with clamp connections, the outer hyphae is gelatinized. **Stipitipellis** a cutis of elongate hyphae. **Clamp connections** present at some septa. **Thromboplerous hyphae** not seen.

**Ecology and distribution:** Gregarious and often caespitose on debris under *Picea orientalis* (L.) Peterm. in *Picea-Fagus*-dominated forest. Autumn. Known only from Turkey.

#### 4. Discussion

*Lyophyllum turcicum* is an interesting tricholomatoid species with fleshy but fragile basidiomes. According to the ITS sequence analysis this species is close to *Hypsizygus marmoreus*, *H. ulmarius*, and a collection named *Lyophyllum fumosum* (Table). *Lyophyllum turcicum* differs from these relatives in being  $5.5\text{--}7 \times 3.5\text{--}4.5 \mu\text{m}$ , ellipsoid, and with narrow spores. In contrast to *L.*

*turcicum*, the basidiomes of *Hypsizygus ulmarius* and *H. marmoreus* grow directly on the wood of hardwood trees and their spores are globose to subglobose (Kalamees, 2004). *L. fumosum* is readily distinguished by the dark brown tinges of the pileus and the globose to subglobose spores (Consiglio and Contu, 2002).

In *Lyophyllum* sect. *Difformia*, characterized by caespitose-growing and not blackening taxa, two species with ellipsoid spores have been described, *L. brunneum* and *L. pseudoloricatum* (Dähne et al., 2010). Both are known only from the Canary Islands. *Lyophyllum brunneum* differs from *L. turcicum* by its stouter size, darker tinges in the pileus, nodulose marginal cells, and less elongate spores [ $6\text{--}7\text{--}(7.5) \times 4.5\text{--}5\text{--}(5.5) \mu\text{m}$ ] in the type-collection]. *L. pseudoloricatum* differs by its atypical elastic-cartilaginous context, darker tinges in the pileus, lack of marginal cells, larger basidia, and wider basidiospores [ $6\text{--}8\text{--}(8.1) \times 4.5\text{--}6 \mu\text{m}$ ] in the type-collection]. Sect. *Difformia*, as traditionally delimited, is polyphyletic (Figure 2). The species of the *L. decastes* complex (*L. decastes*, *L. loricatum*, *L. fumosum*, etc.) are not closely related to *L. turcicum*.



**Table.** Comparison of the diagnostic characteristics of *Lyophyllum turcicum* with similar species.

Character	<i>Lyophyllum turcicum</i>	<i>Lyophyllum brunneum</i>	<i>Lyophyllum fumosum</i>	<i>Lyophyllum pseudoloricatum</i>	<i>Hypsizygus marmoreus</i>	<i>Hypsizygus ulmarius</i>
Pileus	Pale brown to beige-brown, 2–6 cm	Fuscos-brown then brown, 3–7.5 cm	Dark to light gray, brown, 2–10 cm	Brown with a darker center	White to gray-brown, 4–15 cm	Cream colored to ocher, 8–13 cm
Stipe	Whitish to pale gray, 2–5.5 cm	White, 6–9 cm	Cream colored to brown, 2.5–10 cm	White, 6–7 cm	Whitish, 3–10 cm	Cream yellow to ocherish, 8–14 cm
Spores	Ellipsoid and narrow, 5.5–7 × 3.5–4.5 µm	Broadly ellipsoid to ellipsoid, 6–7 × 4.5–5 µm	Globose to subglobose, 5.5–7 × 5–7 µm	Broadly ellipsoid to ellipsoid, 6–8 × 4.5–6 µm	Ovoid to round, 5.5–6 × 5–5.5 µm	Subglobose, 5.5–7 × 4.5–6.5 µm
Basidia	25–35 × 5.5–6.5 µm	30–38 × 7–9 µm	40–45 × 8–10 µm	30–40 × 8–10 µm	25–32 × 5–6 µm	25–30 × 5–7 µm
Habitat	On debris	In mixed woods	Among grass	In mixed woods	On wood of hardwoods	On wood of hardwoods

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