

***Lyophyllum turcicum*, a first report for Austria, confirmed by molecular identification**

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Abstract: *Lyophyllum turcicum* is a species of *Agaricales* that had been described from Turkey only recently. In this study a yet unidentified specimen, which was collected in Carinthia (Austria) in 2012, has been re-examined. By comparison of ITS and LSU sequences as well as macro- and microscopic features it could be identified as *Lyophyllum turcicum*. This is the first report of this species outside of Turkey.

Zusammenfassung: *Lyophyllum turcicum* ist eine erst kürzlich beschriebene *Agaricales*-Art aus der Türkei. In dieser Studie wurde ein Beleg untersucht, der bereits 2012 in Kärnten (Österreich) gefunden, aber noch nicht identifiziert werden konnte. Mittels Vergleich von ITS und LSU Sequenzdaten sowie makro- und mikroskopischen Merkmalen konnte dieser Beleg als *Lyophyllum turcicum* identifiziert werden. Dies ist der erste bekannte Nachweis dieser Art außerhalb der Türkei.

Lyophyllaceae JÜLICH 1982 encompass about 160 species and are mainly distributed in the northern-temperate eco-region. They show parasitic and saprotrophic lifestyles and also include edible mushrooms like the cultivated *Hypsizygus ulmarius*, and the wild species *Lyophyllum decastes* and *Calocybe gambosa*. They are subject to systematic studies because a number of phylogenetic relationships within this group still remain enigmatic (MONCALVO & al. 1994, BELLANGER & al. 2015). In addition, there are still new species found and only recently SESLI & al. (2015) described a new species for Turkey, *Lyophyllum turcicum* SESLI, VIZZINI & CONTU.

In the present study a yet unidentified specimen that had already been collected in 2012 in Carinthia, Austria, had been re-examined using microscopy and molecular methods.

Material and methods

Specimen investigated: Austria, Carinthia, Karawanken/Ferlach, Windisch-Bleiberg, mapping grid square 9551/1, in a garden near a *Picea abies* tree at 1060 m a.s.l., leg. ANTON SGAGA, 24. September 2012, deposited in the herbarium KL accession number: KL-P0445 (see index herbariorum: <http://sweetgum.nybg.org/science/ih/>).

The region of the locality is characterized by Mesozoic limestones and dolomite, the soil type is a brown rendzina.

Microscopy

The dried material was rehydrated with Glamalc for microscopic characterization, subsequently transferred to 3% KOH and coloured with Phloxin B. The microscopic pictures were taken with a Zeiss Jenamed2 microscope using a Nikon Coolpix 4500 and a Moticam 2500. The measurement was taken by using an ocular-micrometre and the Motic Images 2.0 software. In total 20 spores were measured.

Molecular investigation

The DNA of the specimen was extracted using a modified protocol based on MICHELS & al. (2003). DNA amount was measured and adjusted to 16 ng μl^{-1} . PCR of the ribosomal large subunit (LSU) and the internal transcribed spacer (ITS) gene region was carried out using DF-Pfu DNA Polymerase (Bioron GmbH, Ludwigshafen, Germany) with a final concentration of 1× COMPLETE reaction buffer, 200 μM dNTPs, 0.8 $\mu\text{g ml}^{-1}$ (Bioline, Luckenwalder, Germany), bovine serum albumine (Sigma Aldrich, St. Louis, USA), 400 μM of the respective primers, and 0.1 units DF-Pfu DNA polymerase. The commonly used primer combinations for LSU (LR0R and LR5; VILGALYS & HESTER 1990) and the internal transcribed spacer region (ITS1F and ITS4B; GARDES & BRUNS 1993) were used. The reaction was conducted in an Eppendorf ProS Thermocycler with 240 sec initial denaturation at 95 °C, followed by 36 cycles of 40 sec at 95 °C, 40 sec at 54 °C, and 180 sec at 72 °C. The reaction was concluded by 8 min of final elongation at 72 °C and stored at 4 °C until sequencing. The sequencing was carried out by the Lab-centre of the Biodiversity and Climate Research Center (BiK-F, Frankfurt, Germany) in both directions using the PCR primers ITS1F and ITS4B, and LR0R and LR5 for ITS and LSU, respectively. The LSU sequence of the type specimen was provided by ALFREDO VIZZINI.

Phylogenetic analysis

The resulting forward and reverse sequences were merged using Geneious 4.0 (Biomatters Inc., USA) and deposited in GenBank (accession numbers KU183491-KU183493). Reference sequences were obtained from the NCBI database (<http://www.ncbi.nlm.nih.gov/>) and were aligned using Mafft with the G-INS-i algorithm (KATO & STANDLEY 2013). Phylogenetic inference was done with MEGA6 (TAMURA & al. 2013) using the Minimum Evolution algorithm (pairwise deletion, 1000 bootstraps) and with Trease (MISHRA & al., unpubl.) for Bayesian inference (GTR model, 10 Mio generations, 30% burnin). Phylogenetic inference was done for ITS and LSU separately as well as for a concatenated ITS-LSU dataset, after it was checked that no supported topological conflicts were present.



Fig. 1. *Lyophyllum turcicum*. a, b Habit, c detail of stipe apex and lamellae. – Phot. ANTON SGAGA.

Results

Lyophyllum turcicum SESLI, VIZZINI & CONTU 2015 found in Carinthia, Austria, 2012. (Figs. 1–2)

Field notes and macroscopic features (Fig. 1)

E c o l o g y : in coniferous forest, gregarious near *Picea abies* between needles and mosses.

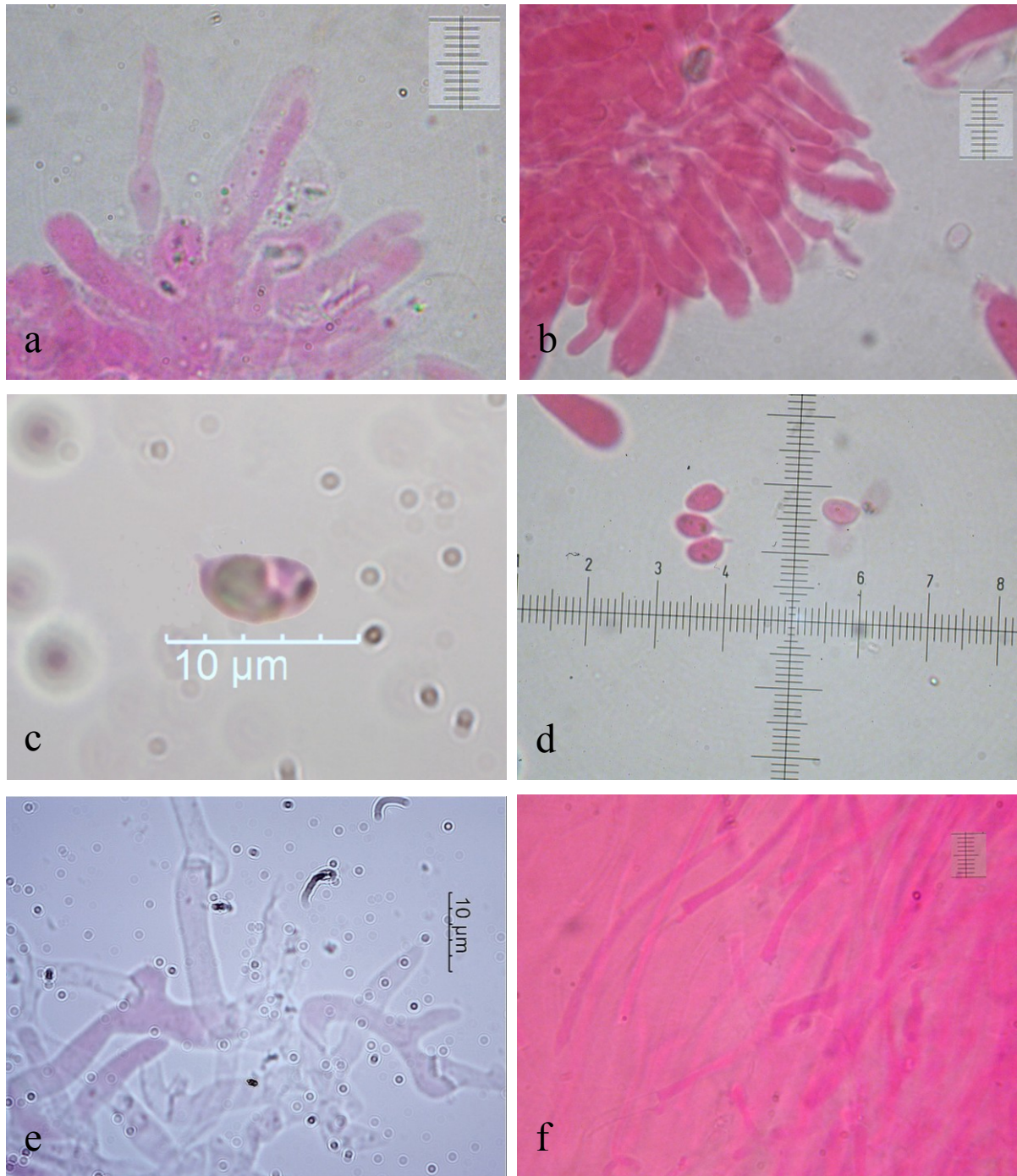


Fig. 2. *Lyophyllum turcicum*, microscopic features. *a, b* Marginal cells, *c, d* spores, *e* hyphae of subhymenium, *f* hyphae of pileipellis. – Bars: *a, b, d* 10 µm.

Pileus: 30–40 mm in diam., convex to expanded, slightly umbonate, surface smooth, bright beige-coloured, greasy when moist; margin up-rolled.

Lamellae: whitish to pale grey, undulating, shortly decurrent, generally with three lamellulae.

Stipe: 60–70 × 5–10 mm cylindrical, whitish, somewhat clavate at base, pruinose at apex (Fig. 1 c).

Context: texture, smell and taste not recorded.

Exsiccate: beige-brown.

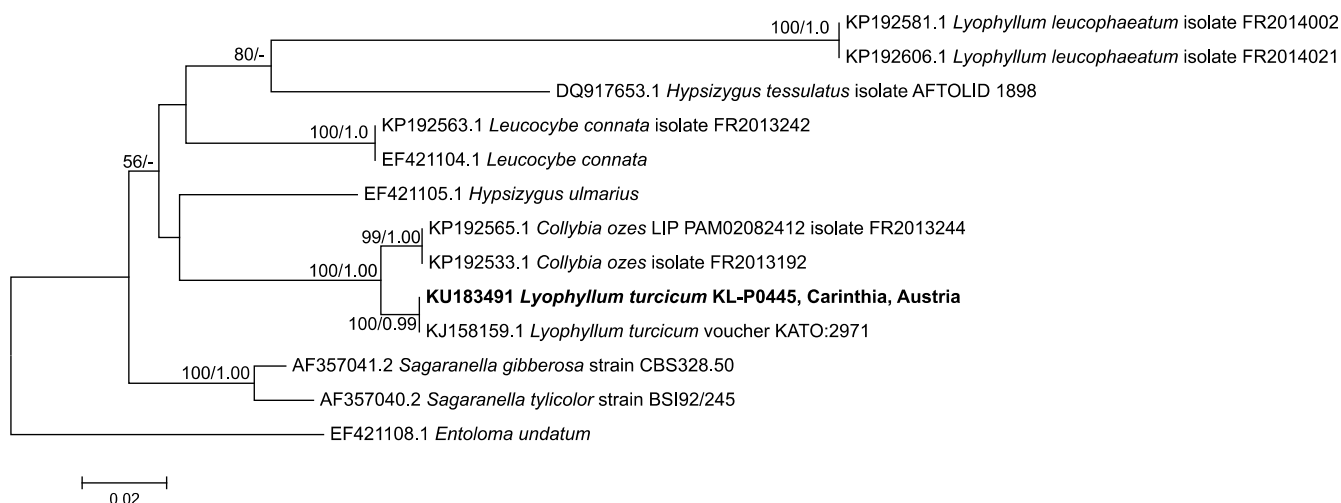


Fig. 3. Phylogenetic tree based on ITS sequence data; the values on the branches represent Minimum evolution bootstrap support / Bayesian posterior probabilities.

Microscopic features (Fig. 2)

Hymenophoral trama: regular, septa almost always with clamp connections; some scattered thromboplerous hyphae observed.

Spores: $5-7(-7.5) \times 3.5-4.5 \mu\text{m}$, on average $5.7 \times 3.8 \mu\text{m}$, spore quotient (length/width) 1.28–1.88; wide ellipsoid, hyaline, smooth, cyanophilous, with dark-reflecting oil droplets coalescing into one drop after some time.

Basidia: $25-30 \times 5-8 \mu\text{m}$, mostly 4-spored, siderophilous, with clamp connections.

Marginal cells: $20-30 \times 2-4 \mu\text{m}$, scattered, thin-walled, cylindrical to fusiform, flexuous or lageniform.

Pileipellis: a cutis of cylindrical parallel hyphae of $2.5-4 \mu\text{m}$ in diam., mostly with clamp connections; upper part gelatinized; some thromboplerous hyphae with refractive contents in the subcutis observed.

Molecular analysis

The sequences of the *Lyophyllaceae* specimen investigated here forms a maximum supported cluster with the recently described *Lyophyllum turcicum* from Turkey (SESLI & al. 2015) for ITS (Fig. 3), LSU and the concatenated data for ITS and LSU genes (data not shown). The closest relative to *L. turcicum* in the ITS dataset with maximum bootstrap and Bayesian posterior probability support is *Collybia ozes*. The type species of the genus *Lyophyllum*, *L. leucophaeatum* forms a distinct cluster in the ITS dataset (Fig. 3).

Discussion

Lyophyllum turcicum is characterized by fleshy and fragile basidiomata, which are not blackening and grow caespitose. This species is phylogenetically related with *Hypsizygus marmoreus* and *H. ulmarius* (SESLI & al. 2015), which differ from *Lyophyllum turcicum* in growing directly on wood and in having globose spores. In contrast to

these species *Lyophyllum turcicum* has ellipsoid spores and conspicuous cylindrical to fusiform and sometimes lageniform marginal cells. In the present collection clamp connections were frequent in all parts of the basidioma. Thromboplerous hyphae have been observed in the subpileipellis and in the hymenophoral trama. Basidia and marginal cells are as described by SESLI & al. (2015).

The phylogenetic analysis confirmed the morphological similarities and clustered the specimen from Carinthia to the type specimen of *Lyophyllum turcicum* with maximum support in Minimum Evolution and Bayesian Inference. The closest relative is *Collybia ozes* which belongs to clade I in the phylogenetic work of BELLANGER & al. (2015) and is distinct from the species that are traditionally grouped in *Lyophyllum* sect. *Difformia*. Already MONCALVO & al. (1993) proposed that the cluster that includes *Leucocybe connata* should not be included in *L.* sect. *Difformia*. Furthermore, the present phylogenetic data revealed that *L. turcicum* is not related to the major *Lyophyllum* clade including the type species of the genus *Lyophyllum*, *L. leucophaeatum*. These are striking hints that *L. turcicum* should not be included into the genus *Lyophyllum* but rather in the genus *Collybia* or an own group. But to completely understand these relationships within this group of *Lyophyllaceae* a comprehensive revision needs to be conducted, which was not the aim of the present study.

The identification of *Lyophyllum turcicum* in Carinthia, Austria is the first evidence of this just recently described species outside of the type locality in Turkey.

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Contributions

MK and MT designed the study. AS collected the specimen. HP and AS conducted macroscopic characterisation. MK and HP conducted the microscopic work. SP conducted the molecular work and phylogenetic analysis. AV provided the LSU sequence of the type specimen. SP and MK wrote the manuscript with contributions from MT.

References

- BELLANGER, J.-M., MOREAU, P.-A., CORRIOL, G., BIDAUD, A., CHALANGE, R., DUDOVA, Z., RICHARD, F., 2015: Plunging hands into the mushroom jar: a phylogenetic framework for *Lyophyllaceae* (*Agaricales*, *Basidiomycota*). – *Genetica* **143**: 169–194.
- GARDES, M., BRUNS, T. D., 1993: ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. – *Molec. Ecol.* **2**: 113–118.
- KATO, K., STANDLEY, D. M., 2013: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. – *Molec. Biol. Evol.* **30**: 772–780.
- MICHELIS, A., VAN DEN ENDE, W., TUCKER, M., VAN RIET, L., VAN LAERE, A., 2003: Extraction of high-quality genomic DNA from latex-containing plants. – *Anal. Biochem.* **315**: 85–89.
- MONCALVO, J. M., REHNER, S. A., VILGALYS, R., 1993: Systematics of *Lyophyllum* section *Difformia* based on evidence from culture studies and ribosomal DNA sequences. – *Mycologia* **5**: 788–794.
- SESLI, E., VIZZINI, A., CONTU, M., 2015: *Lyophyllum turcicum* (*Agaricomycetes*: *Lyophyllaceae*), a new species from Turkey. – *Turkish J. Bot.* **39**: 512–519.
- TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., KUMAR, S., 2013: MEGA6: Molecular Evolutionary Genetics Analysis, version 6.0. – *Molec. Biol. Evol.* **30**: 2725–2729.
- VILGALYS, R., HESTER, M., 1990: Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. – *J. Bacteriol.* **172**: 4239–4246.

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