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Research article

FIRST RECORDS OF *LEPIOTA CYSTOPHORA* (AGARICALES, BASIDIOMYCOTA) IN HUNGARY

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Abstract: *Lepiota cystophora* is reported new to Hungary based on six collections identified by morphological and molecular genetic methods. The species is known from thermophilous oak forests on calcareous soil from the Buda and Vértes Hills. The former generic placement of the species in *Cystolepiota* is not supported by ITS phylogeny.

Keywords: *Cystolepiota*, Central Europe, phylogeny, Agaricaceae

INTRODUCTION

Lepiota (Pers.) Gray is a large genus in the family Agaricaceae with global distribution, characterized by white spore print, a hymenidermal, cutis-like, epithelial or trichodermal pileipellis and with the presence of clamp connections (Candusso and Lanzoni 1990; Vellinga 2003a). The shape of basidiospores and the structure of pileal elements are the most important taxonomic characteristics among *Lepiota* species (Singer 1986; Candusso and Lanzoni 1990; Bon 1993; Vellinga 2003a, b; Qasim *et al.* 2015) and sections were delimited based on these two characters (Singer 1986; Candusso and Lanzoni 1990; Bon 1993). In Hungary, Margit Babos had an outstanding work in the thorough assessing of the lepiotoid genera (Babos 1958, 1961, 1974, 1980).

The genus *Cystolepiota* (Agaricaceae) was described by Rolf Singer based on the type species *Cystolepiota constricta* Singer from Argentina (Singer and Digilio 1952). The genus was originally established for lepiotoid species with neither dextrinoid, nor amyloid basidiospores, and with epithelioid pileipellis. *Cystolepiota*

differs from *Lepiota* in the structure of the universal veil which is made up of loosely arranged globose or inflated elements in the former, while in most *Lepiota* species the veil consists of long, cylindrical or clavate elements (Vellinga 2001a; Noordeloos *et al.* 2001).

Lepiota cystophora Malençon was described from Morocco under *Quercus ilex* (Malençon and Bertault 1970). It is a tiny species with solitary growing basidiomes, appressed, whitish squamules, and pileipellis consisting of large, globose or subglobose cells (Bon 1993; Antonín 1994). Bon (1976) later transferred it to the genus *Cystolepiota*, based on morphological characteristics, such as the spherocysts of the pileipellis. However, recent molecular phylogenetic analyses indicate that the genus *Cystolepiota* as originally circumscribed is polyphyletic, and the taxonomic placement of *C. cystophora* and related species is also unclear (Qu *et al.* 2023; Vellinga 2003b).

The species was originally described from the Mediterranean region, however, later collections confirmed that it occurs in other habitats, too. The first extra-Mediterranean specimens were found in a thermophilous oak forest in the Czech Republic (Antonín 1994). In 2015, 2020 and 2023, fresh basidiomes were discovered in similar habitats in Buda Hills, and in 2022 in Vértes Hills in Hungary. The present work reports the first records of *L. cystophora* from Hungary based on detailed morphological characteristics and phylogenetic analysis.

MATERIALS AND METHODS

Morphology

The dried basidiocarps were deposited in the collection at the Hungarian University of Agriculture and Life Sciences and at the Department of Plant Anatomy of the Eötvös Loránd University. Macromorphological descriptions were based on field notes. Micromorphological data were obtained from dried, we used a Zeiss Axio Imager A2 microscope, and measurements were made with the AxioVision Release 4.8.2 program. Preparations were stained with 1% ammoniacal Congo Red and Melzer's reagents. In describing basidiospores the following abbreviations were used: W = mean spore width, L = mean spore length, Q = variation in the L/W ratios, n = number of spores measured.

Molecular study

We extracted total DNA from dried specimens by using Omega Bio-tek E.Z.N.A.® SP Plant DNA Kit, according to the manufacturer's instructions. The nrDNA ITS region was amplified by PCR using ITS1/ITS4 primers (White *et al.* 1990; Gardes and Bruns 1993). The successful amplification was confirmed by agarose gel electrophoresis using 1% gel. The amplicons were sequenced at the Biological Research Centre (Szeged, Hungary), with the same primers as used in PCR reactions. The chromatograms were inspected in Chromas 2.6.6 (Technelysium Pty Ltd).

The newly generated sequences were compared to GenBank (https://www.ncbi.nlm.nih.gov/). Sequences of *Lepiota cystophora* and closely related taxa based on BLASTn (Altschul *et al.* 1999) as well as additional sequences from various phylogenetic studies in *Lepiota* and *Cystolepiota* (e.g. Qu *et al.* 2023; Vellinga 2003b) were retrieved from GenBank in order to generate a phylogenetic tree. Sequences of *Macrolepiota mastoidea* (Fr.) Singer (AY243604) and *Macrolepiota procera* (Scop.) Singer (HM125514) were chosen as outgroups.

Altogether 37 ITS sequences were included in our phylogenetic analysis (*Table 1*). The alignment was performed with the online version of MAFFT version 7 (https://mafft.cbrc.jp/alignment/server), and then edited in SeaView version 5.0.5 (Gouy *et al.* 2020). The dataset was subjected to maximum likelihood (ML) analysis in raxmlGUI 2.0 (Edler *et al.* 2021) using 1000 rapid bootstrap searches. The phylogenetic tree was visualis ed in MEGA 11 (Tamura *et al.* 2021).

RESULTS

Phylogenetic analyses

We generated four new ITS sequences from the Hungarian samples of *Lepiota cystophora* (*Table 1*). The final alignment consisted of 782 nucelotide positions including gaps. The gaps were treated as missing data. The phylogenetic tree in *Figure 1* shows the relationship between the lepiotaceous species included in this study. All sequences of the specimens from Hungary clustered together with a *L. cystophora* sequence (GQ141550) originated from a specimen collected in Italy and published as *Cystolepiota cystophora*

(Vellinga 2010; Osmundson *et al.* 2013) in a strongly supported clade (MLBS = 99%). *Lepiota cystophora* formed a strongly supported sister clade (MLBS = 98%) in our analysis represented by a sequence of *Lepiota cystophoroides* Joss. & Riousset from France published by Vellinga (2001a). *Lepiota scaberula* Vellinga (2001a) described from the USA, California also belongs to this species complex forming a strongly supported clade (MLBS = 100%) together with *L. cystophora* and *L. cystophoroides*. Based on our ITS phylogeny, *L. cystophora* clearly belongs to the genus *Lepiota*, and shows distant relationship with other *Cystolepiota* sequences (*Figure 1*).

Table 1. Taxa used in the nrDNA ITS phylogenetic analysis. Species, GenBank accession number, country and specimen voucher are presented, along with references. Sequences produced in this study are indicated in **bold**.

Species	GenBank Accession no.	Locality	Specimen voucher	References
Chamaemyces fracidus	AY176343	Belgium	X-1977	Vellinga (2004)
Cystolepiota pseudofumosifolia	MN810152	China	KUN-HKAS 105918	Hou and Ge (2020)
Cystolepiota pseudofumosifolia	MW447313	Pakistan	FA101	GenBank, unpublished
Cystolepiota pulverulenta	AF391037	Netherlands	E.C. Vellinga 1763 (L)	Vellinga (2001a)
Cystolepiota pulverulenta	AF391036	Great Britain	E.C. Vellinga 1872 (L)	Vellinga (2001a)
Cystolepiota seminuda	AY176350	Netherlands	4-X-1989, H.A. Huijser s.n	Vellinga (2004)
Cystolepiota sp.	U85332	Costa Rica	DUKE-JJ87	Johnson (1999)
Lepiota albogranulosa	LK932285	Pakistan	T 19	Qasim *et al.* (2015)
Lepiota albogranulosa	LK932284	Pakistan	T 14	Qasim *et al.* (2015)
Lepiota apatelia	AY176462	Netherlands	26-IX-1990, H.A. Huijser	Vellinga (2003b)
Lepiota castaneidisca	AF391065	USA	E.C. Vellinga 2516 (UC)	Vellinga (2001b)
Lepiota castaneidisca	GQ203818	USA	E.C. Vellinga 3005 (UC)	Vellinga (2010)
Lepiota castaneidisca	AF391055	USA	E.C. Vellinga 2594 (UC)	Vellinga (2001b)
Lepiota clypeolaria	MZ005559	Italy	IZS182	Giusti *et al.* (2021)
Lepiota clypeolaria	AY176361	Germany	E.C. Vellinga 1683 (L)	Vellinga (2004)
Lepiota coloratipes	KC900376	Spain	SAV F-3212	Vizzini *et al.* (2014)
Lepiota coloratipes	KC900377	Spain	SAV F-3213	Vizzini *et al.* (2014)

Species	GenBank Accession no.	Locality	Specimen voucher	References
Lepiota cristata	OL527695	Germany	SeSa55	Sarawi *et al.* (2022)
Lepiota cystophora	GQ141550	Italy	14107	Osmundson *et al.* (2013)
Lepiota cystophora	**PP776109**	**Hungary**	**RA_221006_01**	**this study**
Lepiota cystophora	**PP776110**	**Hungary**	**DB-2020-11-12-6**	**this study**
Lepiota cystophora	**PP776111**	**Hungary**	**AL 20/205**	**this study**
Lepiota cystophora	**PP776108**	**Hungary**	**DB5896**	**this study**
Lepiota cystophoroides	AF391031	France	E.C. Vellinga 2142 (L)	Vellinga (2001a)
Lepiota erminea	AY176470	Netherlands	E.C. Vellinga 2290 (L)	Vellinga (2003b)
Lepiota lilacea	AY176379	USA	E.C. Vellinga 2451 (UCB)	Vellinga (2004)
Lepiota magnispora	AF391017	USA	AEF1015	Vellinga (2001a)
Lepiota psalion	MG581687	Austria	WU 5152	Vizzini *et al.* (2019)
Lepiota psalion	MG581688	Italy	CAG P.11_9/7.68a	Vizzini *et al.* (2019)
Lepiota pseudolilacea	AY176392	Netherlands	E.C. Vellinga 2278 (L)	Vellinga (2004)
Lepiota rufipes	AF391066	Netherlands	9-X-1991, H.A. Huijser	Vellinga (2001b)
Lepiota scaberula	AF391030	USA	E.C. Vellinga 2595 (UC, holotype)	Vellinga (2001a)
Lepiota sp.	OM522666	USA	PUL:00033450	GenBank, unpublished
Lepiota sp.	OM522737	USA	S.D. Russell iNaturalist #91685441	GenBank, unpublished
Lepiota subincarnata	AY176491	Netherlands	E.C. Vellinga 2234 (L)	Vellinga (2003b)
Macrolepiota mastoidea	AY243604	Netherlands	7-XI-1998, N.J. Dam / E.C. Vellinga 22949 (L)	Vellinga (2004)
Macrolepiota procera	HM125514	China	HKAS8108	Ge *et al.* (2010)

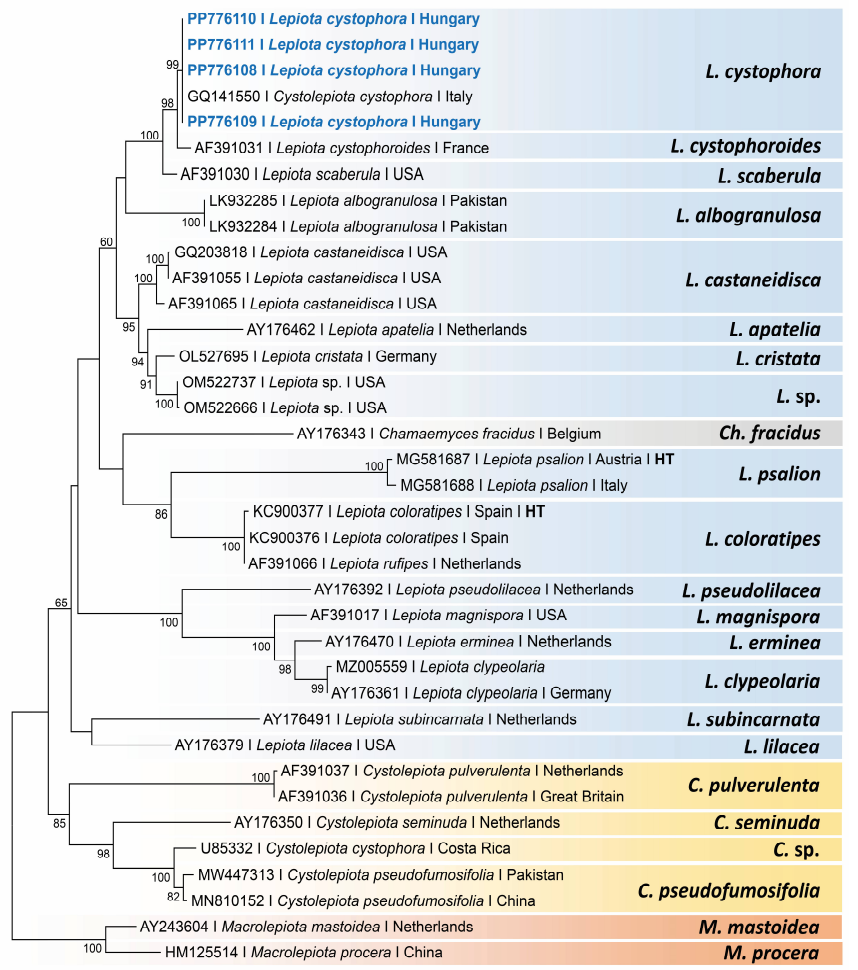

Figure 1. Phylogeny of *Lepiota* with emphasis on *L. cystophora* inferred from Maximum Likelihood and RAxML analyses of the nrDNA ITS sequences. Topology is from the best scoring Maximum Likelihood (ML) tree. *Macrolepiota mastoidea* and *M. procera* served as outgroup. ML bootstrap values >50% are shown above or below the branches. The bar indicates 0.05 expected change per site per branch.

Taxonomy

Lepiota cystophora Malençon, Flore des champignons supérieurs du Maroc 1: 124 (1970)
≡ *Cystolepiota cystophora* (Malençon) Bon, Documents Mycologiques 6 (24): 43 (1976) (*Figure 2*)

Figure 2. Macromorphology of *Lepiota cystophora*. A–D. Basidiomes **A**. DB-2020-11-12-6 **B**. AL 23/333 **C**. RA_221006_01 **D**. AL 20/205 (Photos: A: B. Dima, B, D: L. Albert, C: Á. Radnóti).

Pileus 2–4 cm, conical-hemispherical, then convex; squamulose, whitish when young, later ochre, yellow ochre, sometimes with rose tinges with white, floccose veil remnants at the margin. **Lamellae** crowded, free, whitish, cream-coloured when mature, slightly brownish when dry. **Stipe** 2–5 cm long, 0.2–0.4 cm in diameter, whitish with ochre or rosaceous colour towards the base. Ring zone floccose, woolly, fugacious. **Context** thin, whitish, with pinkish tinges (*Figure 2*). **Basidiospores** (6.2–)7.07–7.72(–8.5) × (3.5–)4.12–4.73(–5.7) µm, average: 7.4 × 4.5 µm, Q = (1.3–)1.52–1.79(–2.1), Qav = 1.7 µm, ellipsoid, thick-walled, hyalin, non-dextrinoid, not metachromatic in Cresyl Blue (*Figure 3E*). **Cheilocystidia**: 30–50 × 6–10 µm, clavate (*Figure 3B–C*). **Pileipellis**: composed of large, globose sphaerocysts, 20–30 µm in diameter (*Figure 3A*).

Habitat: Thermophilous oak forests on calcareous soil. The pH is presumed to be alkaline as a result of the carbonate bedrock. At the Széchenyi-hegy and Csillebérc localities, the vegetation is dominated by *Quercus pubescens* and *Fraxinus ornus* with scattered *Q. cerris* trees, at Szárliget *Q. cerris*, *Q. pubescens* and *Carpinus betulus*, at János-hegy *Q. cerris* with some *C. betulus*, while at Normafa the main species were *Fagus sylvatica*, *Quercus* sp. and *Tilia cordata*.

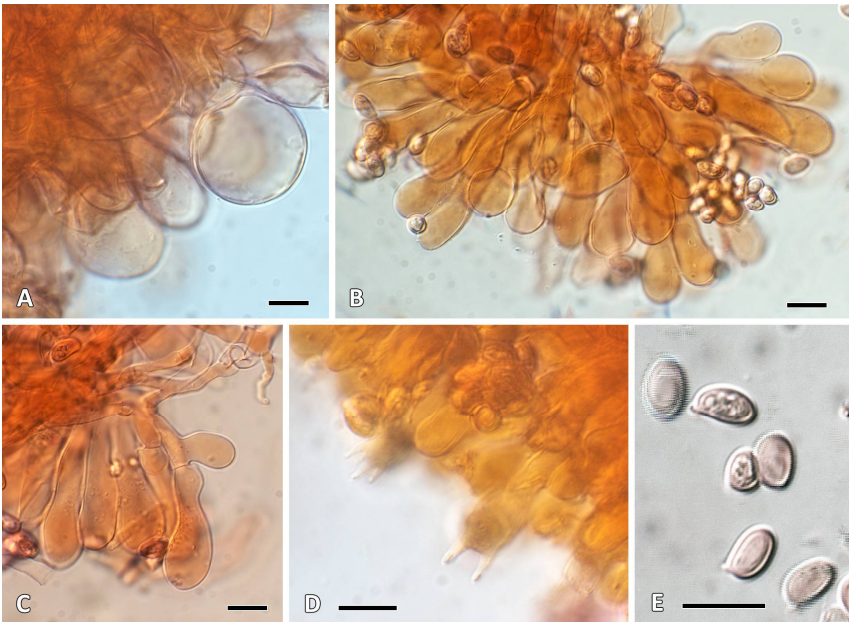

Figure 3. Micromorphology of *Lepiota cystophora*. **A**. Pileipellis. **B–C**. Cheilocystidia. **D**. Basidia. **E**. Basidiospores. Scale bars: A–D = 10 µm (Photos: Á. Radnóti).

Specimens examined: Hungary, Buda Hills, Budapest, Csillebérc, alt. 450 m, 18 Oct 2015, L. Albert, B. Dima (DB5896); Buda Hills, Budapest, Széchenyi-hegy, alt. 460 m, 15 Oct 2020, L. Albert (AL 20/205); Buda Hills, Budapest, Széchenyi-hegy, alt. 460 m, 12 Nov 2020, L. Albert, B. Dima (DB-2020-11-12-6); Buda Hills, Budapest, János-hegy, alt. 300 m, 9 Nov 2023, B. Dima (RA_231109_09); Buda Hills, Budapest, Normafa, alt. 477 m, 19 Nov 2023, L. Albert (AL 23/333); Vértes Hills, Szárliget, alt. 300 m, 6 Oct 2022, Á. Radnóti (RA_221006_01).

DISCUSSION

Six collection data from five new localities are reported, four from Buda Hills and one from Vértes Hills, representing the first records of *Lepiota cystophora* in Hungary, confirmed by morphological and molecular studies. All samples were collected in thermophilous oak forests on calcareous soil. The species might be overlooked in thermophilous deciduous habitats in Central as well as Western and South Europe.

Our analysis included a sequence from Costa Rica labelled incorrectly as *Cystolepiota cystophora* (U85332), which clustered distantly from the European *L. cystophora* sequences in the phylogenetic tree (*Figure 1*), representing another species. Qasim *et al.* (2015) suggested that *C. cystophora* might be identical to *L. cystophoroides*, however, the currently available sequence data suggest that these two are sister species (*Figure 1*). There are slight morphological differences between the two species*: L. cystophoroides* has no purplish or rosaceous tinges in the stipe base and has a rather hymeniform pileipellis with few sphaerocysts, while *L. cystophora* has more sphaerocysts and the stipe base possesses a purplish, rosaceous colour (Josserand and Riousset 1972). The known sequences of the morphologically identified *Cystolepiota* species did not form a monophyletic group across any phylogeny published to date (e.g. Vellinga 2003b, 2010; Qu *et al.* 2023). One of the key problems to understand the delimitation of *Cystolepiota*, is the lack of any sequence data of *C. constricta*, the type species of the genus. These facts indicate that a global phylogenetic and taxonomic revision is urgently needed to reveal the generic limits of lepiotaceous fungi.

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