Tremella umbilicariae (Tremellomycetes, Basidiomycota), a new lichenicolous species on *Umbilicaria* from Peru

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Abstract. The new *Tremella umbilicariae*, lichenicolous on *Umbilicaria*, is described from Peru. It is characterized by large, dark brown, convex basidiomatal galls, long and narrow basidia, and relatively large basidiospores.

1. Introduction

The genus Tremella Pers. includes mainly mycoparasitic species, most of them being highly host-specific and confined to a single fungal genus or species. Over fifty lichenicolous species have been recognized so far growing exclusively on lichenized fungi (Diederich 1986, 1996, 2003, Sérusiaux et al. 2003, Diederich 2007, Zamora et al. 2011, Millanes et al. 2012, Millanes et al. 2014). The lichenicolous Tremella species often induce the formation of conspicuous galls on their host thalli or ascomata, whilst some intrahymenial taxa do not produce any external symptoms (Diederich 1996, Zamora et al. 2011). The phylogenetic position of the lichen-inhabiting representatives has been tested by molecular methods by Millanes et al. (2011), who confirmed that they are nested within the genus Tremella. In this paper we will describe a new licheninhabiting species of Tremella inducing large galls on the thallus of Umbilicaria.

2. Material and Methods

2.1. Molecular studies

In addition to the specimen studied, 18 specimens representing 12 *Tremella* species were included in the molecular study (Table 1).

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The sampling included the type of the genus *Tremella* (*T. mesenterica*), terminals of the Fuciformis and Foliacea groups distinguished by Chen (1998) and terminals representing three groups of lichenicolous species distinguished by Millanes et al. (2011), in particular, lichenicolous species with a micro-morphology similar to that of the specimen growing on *Umbilicaria. Filobasidium floriforme* and *F. uniguttulatum* were used as outgroup. Species names, voucher information, and GenBank accession numbers are given in Table 1.

DNA extraction and PCR amplifications were achieved following protocols in Millanes et al. (2012), except that in this case we used the primers ITS1F (Gardes & Bruns 1993) and BasidLSU3-3 (Millanes et al. 2011), to amplify only the internal transcribed spacer I, the 5.8 rDNA gene, the internal transcribed spacer II and a small fragment of approximately 120 bp in the nLSU rDNA gene.

Bayesian analyses were conducted following Millanes et al. (2012). Maximum likelihood analyses were achieved in RAxMLGUI 1.3, a graphical front-end for RAxML (Stamatakis, 2006), using the GTRCAT model of nucleotide substitution. We performed a total of 100 runs and assessed node support via 1000 bootstrap replicates.

2.2. Morphological examination

Dry herbarium specimens were examined and measured under a binocular microscope Leica MZ 7.5. Macroscopic photographs were done using a Canon 40D camera with a Canon MP-E 65 mm lens, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Hand-made sections and squash preparations of basidiomata were studied in a mixture of Phloxin B, Congo Red and 10% KOH. Microscopic photographs were prepared using a Leica DMLB microscope with DIC optics at a magnification of 1000× and a Leica EC3 camera. Measurements of basidia and basidiospores are indicated as (minimum-) $\overline{X} - \sigma_x - \overline{X} + \sigma_x$ (-maximum), followed by the number of measurements (N); the ratio length/width of ascospores is indicated as l/w and given in the same way.

3. Results

3.1. Phylogenetic results

One ITS sequence of *Tremella umbilicariae*, including also ca. 120 nucleotides of the nuLSU, was aligned together with sequences already available in GenBank (Table 1). We produced a combined data matrix of the ITS and nLSU rDNA sequences, which comprised 1212 characters. A majority rule consensus tree was constructed from the 2500 trees of the stationary tree sample from the Bayesian analysis. Convergence among runs was assessed following Millanes et al. (2012). The best tree obtained from the ML analysis had an ln-likelihood value of -4569.7999.

The new species *Tremella umbilicariae* is included within the so-called "Clade II" in Millanes et al. (2011), together with *T. cladoniae*, *T. lobariacearum* and *T. phaeophysciae* (Fig. 1), although the relationships with these species is not resolved with support.

Table 1. Sequence newly produced corresponding to the type of the	e new species (bold), and
sequences downloaded from GenBank, with specimen data or cultu	ire references.

Species names	Specimen data / culture references	ITS	nLSU
Tremella caloplacae	France, Sérusiaux s.n. (S-F102489)	JN053469	JN043574
Tremella candelariellae	Luxembourg, Diederich 12808 (S-F102492)	JN053470	JN043575
Tremella cetrariicola-a	Finland, Suija s.n. (S-F102413)	JN053490	JN043596
Tremella cetrariicola-b	Latvia, 2005, Suija s.n. (TU)	JN053491	JN043597
Tremella cladoniae-a	France, Diederich 16031 (S-F102550)	JN053478	JN043584
Tremella cladoniae-b	Estonia, Suija 872 (TU-45019)	JN053477	JN043583
Tremella coppinsii-a	UK, Diederich 15628 (S-F102414)	JN053495	JN043601
Tremella coppinsii-b	Estonia, <i>Suija</i> 38a (TU-38637)	JN053496	JN043602
Tremella dendrographae	USA, Tehler 7068 (S-F102415)	JN053471	JN043576
Tremella foliacea	Sweden, Wiklund 018 (S-F102409)	JN053502	JN043609
Tremella hypogymniae-a	Sweden, Wedin 6892 (UPS)	JN053484	JN043590
Tremella hypogymniae-b	Estonia, Suija s.n. (TU-39402)	JN053485	JN043591
Tremella lobariacearum-a (Type)	Madeira, Diederich 4935 (S-F102418)	JN053473	JN043579
Tremella lobariacearum-b	Canary Islands, <i>Diederich</i> 16468 (S-F102419)	JN053474	JN043580
Tremella mesenterica-a	Sweden, Ryman 9146 (S-F102411)	JN053463	JN043568
Tremella phaeophysciae-a	Luxembourg, Diederich 12429 (S-F102505)	JN053479	JN043585
Tremella phaeophysciae-b	Estonia, Suija s.n. (TU-55041)	JN053480	JN043586
Tremella umbilicariae (Type)	Peru, Santesson & Moberg P62:55 (UPS)	KM507564	-
Outgroup			
Filobāsidium floriforme	CBS 6241	AF190007	AF075498
Filobasidium uniguttulatum	CBS 1730	AF444302	AF075468



Fig. 1. Fifty per cent majority rule Bayesian consensus tree with average branch lengths from the combined analyses of ITS and nnLSU datasets. PP values \geq 0.95, obtained in the Bayesian analysis, are indicated over the branches, and maximum likelihood bootstrap values \geq 70%, below the branches. Branch lengths are scaled to the expected number of nucleotide substitutions per site. The new species *Tremella umbilicariae* is highlighted in bold and bigger font. The clade corresponding to "Clade II" in Millanes et al. (2011) is indicated to the right.

3.2. Taxonomy

Tremella umbilicariae Diederich & Millanes sp. nov. (Figs. 2–3) Mycobank MB 810174 Characterized by large, dark brown, convex basidiomatal galls, long and narrow, 1-transseptate basidia, $19.7-25.2 \times 7.2-7.8 \mu m$, and relatively large basidiospores, $7.6-9.4 \times 6.1-7.6 \mu m$.



Fig. 2. Tremella umbilicariae on Umbilicaria (holotypus). Scale bar = 1 mm (the same for both photographs).

Type: Peru, dept. Ancash, prov. Huaraz, pass W of Conococha (road Huaraz-Pativilca), 10°7' S, 77°23' W, alt. 3900 m, on *Umbilicaria*, 1 iii 1981, *R. Santesson* & *R. Moberg* P62:55 (UPS–holotype; herb. Diederich–isotype).

Basidiomata inducing the formation of distinct convex galls on the host thallus, base not or slightly constricted, dark brown to blackish, relatively smooth, matt, frequently pruinose near the base, 0.6–2.5 mm diam., when very old sometimes almost tuberculate and with a cracked surface. *Context hyphae* thin-walled, often with clamp connections, $2.5-3 \mu m$ diam.; haustorial branches present, mother cells subspherical, $3-5 \times 3-4 \mu m$, haustorial filament $1-1.5 \mu m$ diam. *Hymenium* hyaline, containing numerous probasidia; hyphidia present, but not abundant, thick-walled, with numerous septa and ramifications, $2.5-5.0 \mu m$ diam.; probasidial initials clavate, proliferations occurring through the basal clamp. *Basidia*, when mature, 2-celled, with one transverse septum, slightly constricted



Fig. 3. *Tremella umbilicariae* (holotypus, in a mixture of Phloxin B, Congo Red and 10% KOH, using DIC optics). A, Basidiospores. B, Young, mature and old basidia, and epibasidia (right). C, Hymenium, showing basidia and numerous context hyphae. D, Context hyphae and clamp (arrow). E, Hyphae with haustorial branches (arrows). Scale bar = $10 \mu m$ (the same for all photos).

at the septum, $(19.0-)19.7-25.2(-28.0) \times (6.7-)7.2-7.8(-8.0) \ \mu\text{m}$ (excl. epibasidia), ratio l/w (2.5-)2.6-3.4(-3.7) (N=15), rarely with an attenuated stalk-like base; epibasidia subcylindrical, up to 45 μ m long, 2.5-4 μ m diam. *Basidiospores* ellipsoid to subspherical, with a distinct apiculus, (6.5-)7.6-9.4(-10.0) \times (5.5-)6.1-7.6(-8.3) μ m, ratio l/w (1.1-)1.2-1.3(-1.4) (N=15). *Anamorph* not observed.

Distribution and host. Known only from the type locality in Peru at 3900 m, gall-inducing on the thallus of *Umbilicaria* sp.

Discussion. Tremella umbilicariae has to be compared with the other species with 1-transseptate, narrow basidia, relatively long epibasidia and relatively large basidiospores described by Diederich (1996). T. cladoniae Diederich & M. S. Christ. is distinguished by pinkish brown to reddish brown basidiomatal galls, the absence of hyphidia, often longer and narrower basidia, $20-30 \times 4.5-8 \mu m$, and the presence of asteroconidia in some specimens. T. lobariacearum Diederich & M. S. Christ. is distinguished by the absence of hyphidia, much broader basidia, $14-23 \times 7-11 \mu m$, with transverse, oblique or longitudinal septa, and the presence of asteroconidia in some specimens. T. phaeophysciae Diederich & M. S. Christ. is distinguished by more flat to slightly convex, olivaceous galls, the absence of hyphidia, often narrower basidia, 4.5-8 µm diam., shorter epibasidia, 25-30 µm long, more roundish basidiospores, ratio l/w c. 1.1, and the frequency of asteroconidia, which are absent in the type specimen of the new species. T. psoromicola Diederich is distinguished by smaller basidiomata, 0.2-0.8 mm diam., not inducing galls, and much broader basidia, $17-24 \times 8.5-11.5$ um. T. santessonii Diederich has much smaller, reddish brown basidiomata, 0.2-0.3 mm diam. T. hypogymniae Diederich & M. S. Christ. has shorter and broader basidia, $11-16 \times 7-12 \,\mu\text{m}$.

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