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

Paul Diederich¹, Ana M. Millanes² & Mats Wedin³

¹ Musée national d’histoire naturelle, 25 rue Munster, L–2160 Luxembourg (paul.diederich@education.lu)
² Departamento de Biología y Geología, Universidad Rey Juan Carlos, C/ Tulipán s.n., E–28933 Móstoles, Spain (ana.millanes@urjc.es)
³ Department of Botany, Swedish Museum of Natural History, P. O. Box 50007, SE–104 05 Stockholm, Sweden (mats.wedin@nrm.se)


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 lichen-inhabiting 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). 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. Filobaсидium 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–) X-σx – X+σx (–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 nLSU, 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 new species (bold), and sequences downloaded from GenBank, with specimen data or culture references.

<table>
<thead>
<tr>
<th>Species names</th>
<th>Specimen data / culture references</th>
<th>ITS</th>
<th>nLSU</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tremella caloplacae</em></td>
<td>France, Sérusiaux s.n. (S-F102489)</td>
<td>JN053469</td>
<td>JN043574</td>
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<td><em>Tremella candeleriellae</em></td>
<td>Luxembourg, Diederich 12808 (S-F102492)</td>
<td>JN053470</td>
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<td><em>Tremella cetrariicola-a</em></td>
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<td>JN053490</td>
<td>JN043596</td>
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<tr>
<td><em>Tremella cetrariicola-b</em></td>
<td>Latvia, 2005, Suija s.n. (TU)</td>
<td>JN053491</td>
<td>JN043597</td>
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<tr>
<td><em>Tremella cladoniae-a</em></td>
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<td>JN053478</td>
<td>JN043584</td>
</tr>
<tr>
<td><em>Tremella cladoniae-b</em></td>
<td>Estonia, Suija 872 (TU-45019)</td>
<td>JN053477</td>
<td>JN043583</td>
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<tr>
<td><em>Tremella coppinsii-a</em></td>
<td>UK, Diederich 15628 (S-F102414)</td>
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<td>JN043601</td>
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<td><em>Tremella coppinsii-b</em></td>
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<td><em>Tremella dendrographae</em></td>
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<td><em>Tremella foliacea</em></td>
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<td><em>Tremella hypogymniae-a</em></td>
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<td><em>Tremella hypogymniae-b</em></td>
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<td><em>Tremella lobariacearum-a</em></td>
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<td><em>Tremella lobariacearum-b</em></td>
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<td><em>Tremella mesenterica-a</em></td>
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<td><strong>Tremella umbilicariae</strong></td>
<td>Peru, Santesson &amp; Moberg P62:55 (UPS)</td>
<td>KM507564</td>
<td>-</td>
</tr>
</tbody>
</table>

**Outgroup**

*Filobasidium 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 nLSU datasets. PP values ≥ 0.95, obtained in the Bayesian analysis, are indicated over the branches, and maximum likelihood bootstrap values ≥ 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 × 7.2–7.8 µm, and relatively large basidiospores, 7.6–9.4 × 6.1–7.6 µm.

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

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 µm diam.; haustorial branches present, mother cells subspherical, 3–5 × 3–4 µm, haustorial filament 1–1.5 µm diam. Hymenium hyaline, containing numerous probasidia; hyphidia present, but not abundant, thick-walled, with numerous septa and ramifications, 2.5–5.0 µm 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 µm (the same for all photos).
at the septum, (19.0–)19.7–25.2(–28.0) × (6.7-)7.2–7.8(–8.0) µm (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 µm long, 2.5–4 µm diam. Basidiospores ellipsoid to subpherical, with a distinct apiculus, (6.5–)7.6–9.4(–10.0) × (5.5–)6.1–7.6(–8.3) µm, 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-trans-septate, 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 × 4.5–8 µm, and the presence of astroconidia in some specimens. T. lobariacearum Diederich & M. S. Christ. is distinguished by the absence of hyphidia, much broader basidia, 14–23 × 7–11 µm, with transverse, oblique or longitudinal septa, and the presence of astroconidia 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 astroconidia, 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 × 8.5–11.5 µm. 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 × 7–12 µm.

Acknowledgments
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Literature