Understanding lichenicolous heterobasidiomycetes: new taxa and reproductive innovations in *Tremella* s.l.

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Abstract: Four new lichenicolous *Tremella* species are described and characterized morphologically and molecularly. *Tremella celata* grows on *Ramalina fraxinea*, inducing the formation of inconspicuous galls, and having hyphae with incomplete clamps. *Tremella endosporogena* develops intrahymenially in the apothecia of *Lecanora carpinea*, having single-celled basidia and clampless hyphae. *Tremella diederichiana* is the name proposed for a species micromorphologically close to *T. christiansenii* but inducing the formation of small, pale galls on the thallus and apothecia of *Lecidea aff. erythrophaea*. *Tremella variae* grows on *Lecanora varia* thallus, instead of on the apothecia, as do the other known *Tremella* species parasitizing *Lecanora* s.l. Phylogenetic relationships and host specificity of these species are investigated and compared with other taxa that show morphological resemblances, phylogenetic affinities or similar hosts. The formation of mitotic conidia inside old basidia (endospores), which is a poorly known reproductive strategy in the Basidiomycota, is also a distinctive character of *Tremella endosporogena*. A discussion on the reproductive role and systematic implications of endospores is included.

Key words: endospores, host specificity, ITS, rDNA, Tremellomycetes

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Tremella Pers. is a genus of heterobasidiomycetous fungi, mainly characterized as being parasitic on fungal hosts through specialized haustoria, dolipore septa with cupulate-vesiculose parentheses, and complex basidia, often longitudinally, obliquely or transversely septate (exceptionally single-celled) in which single epibasidia develops from each hypobasidial cell (Bandoni 1984, Diedrich 1996). Millanes et al. (2011) showed that this genus, as conceived in all previous studies, is highly non-monophyletic, but the splitting into new genera is discouraged awaiting further knowledge. Within *Tremella* sensu lato a number of described species are restricted to parasitize lichen-forming fungi, showing a high host-parasite specialization (Diedrich 1996). Approximately 50 lichenicolous species are currently recognized (Diedrich 1986, 1996, 2003, 2007; Diedrich and Marson 1988; Diedrich and Christiansen 1994; Sérusiaux et al. 2003; Zamora et al. 2011; Millanes et al. 2012, 2014a 2015; Diedrich et al. 2014), and four more are awaiting formal description (Diedrich 1996, 2007). Lichenicolous *Tremella* species are usually inconspicuous and commonly overlooked by both mycologists and lichenologists (Millanes et al. 2012). Because their study is recent, many new species are expected to be discovered.

*Tremella* s.l. is remarkable by its wide variety of reproductive strategies. In addition to the sexual reproduction via basidiospores, *Tremella* species are often able to change from its filamentous nature to a yeast state (dimorphic fungi, which is common in Tremellomycetes), and many of them also produce conidia in natural conditions (Millanes et al. 2011). Conidia form from specialized conidiogenous cells or from rather undifferentiated hyphae by apical budding or subtended by clamp-connections and even by direct septation and differentiation of hyphal cells (Chen 1998, Chen et al. 1999, Roberts 2001). Outstanding types of conidial morphologies are, for example, the asteroconidia and lunate conidia of various lichenicolous species (Diedrich 1996) and the conidial clusters that build up the fruiting-bodies of *Tremella versicolor* Berk. (Bandoni and Ginns 1993).

The present study aims to describe four new parasitic *Tremella* species on epiphytic lichens, providing information about their morphological and phylogenetic relationships within the group. An unusual reproductive mode (as endospores) was found in one of the species and is also described in detail here.
MATERIALS AND METHODS

Morphology.—Morphological study was based on 32 specimens collected by the authors and deposited in AH, MAF-Lich. and UPS. General methodology and terminology follows Zamora et al. (2011, 2014) summarized here. Micromorphological study was done with hand-cut sections mounted and stained with Congo red in 10% ammonia, phloxin B in 5% KOH or a mixture of Congo red and phloxin B in 5% KOH. Selected samples of Tremella endosporogena were also stained with crystal violet to enhance the contrast of basidia filled with endospores. A solution of 1% crystal violet in distilled water was applied 3–5 min, then washed with distilled water until the leftover staining was removed; however, a solution of 1% sodium dodecyl sulfate was used to remove the excess. Spore amyloidity was tested with Melzer’s reagent. Spore amyloidity was also tested with crystal violet to enhance the contrast of basidiospores. A solution of 1% crystal violet in distilled water was applied in 5% KOH. Selected samples of Tremella endosporogena were mounted and stained with Congo red in 10% ammonia, phloxin B in 5% KOH or a mixture of Congo red and phloxin B in 5% KOH. Spore amyloidity was tested with Melzer’s reagent.

DNA extraction, PCR amplification, sequencing, and phylogenetic analyses.—Taxon sampling consisted of two specimens per new species, in addition to two specimens of Tremella macrobasid stigma, two specimens of T. tuckerae and a group of 19 specimens of the Tremellales (Table 1). These included: (i) the type species of the genus Tremella (Tremella mesenterica (Schaeff.) Retz.); (ii) Tremella fusiformis as representative of the Fuciformis group distinguished by Chen (1998); (iii) representatives of the Aurantia and Indecorata groups distinguished also by Chen (1998), which have recently been assigned the generic names Naematelia and Pseudotremaella, respectively (Liu et al. 2016); (iv) representatives of three groups of lichenicolous species distinguished by Millanes et al. (2011), and (v) lichenicolous taxa with a similar micro or macromorphology to that of the new species. Phaeotremella pseudofoliacea Rea was used as outgroup based on Millanes et al. (2011, 2012) and Liu et al. (2016). Species names, voucher information, and GenBank accession numbers are provided (Table 1).

DNA extractions and PCR amplifications were achieved following Millanes et al. (2012). We amplified the rDNA internal transcribed spacer 1 (ITS1), 5.8S and internal transcribed spacer 2 (ITS2) with the primer pair ITS1F (Gardes and Bruns 1993)/BasidLSU3-3 (Millanes et al. 2011), and a fragment, starting at 5’, of approximately 1000 bp of the rDNA 28S region (28S) with the primer pair BasidLSU1-5 (Millanes et al. 2011)/LR5 (Vilgalys and Hester 1990).

Sequence alignments (TreeBase accession 17326) were performed following Millanes et al. (2012). Dataset congruence was assessed manually by analyzing the datasets separately by parsimony bootstrapping. Conflict among clades was considered significant if a significantly supported clade (bootstrap support ≥ 70%; Hillis and Bull 1993) for one marker was contradicted with significant support by another. Datasets were combined if no incongruences were found.

Bayesian analyses were conducted following Millanes et al. (2012) with minor modifications. We considered ITS1, 5.8S and ITS2 independent partitions, as was suggested by Gaya et al. (2011) and Petkovits et al. (2011). Likelihood models were selected for each of the three gene regions with the Bayesian information criterion (BIC) as implemented in jModeltest (Posada 2008). A GTR+Γ model was selected for the ITS1, a k80+Γ model was selected for the 5.8S, a SYM+Γ model was selected for ITS2 and a GTR+Γ model was selected for 285S. Three parallel runs were executed, each with five chains from which were incrementally heated with a temperature of 0.15. The analysis was diagnosed for convergence every 100 000 generations, measured as the average standard deviation of splits across runs in the last half of the analysis. Every 100th tree was saved. The first half of the run was discarded as burn-in.

Maximum likelihood analyses were conducted in RAxML-GUI 1.3 ( Stamatakis 2006), with the GTR GAMMA model of nucleotide substitution applied to all partitions. We performed a thorough ML search with 100 runs and assessed node support thorough bootstrap using 1000 bootstrap pseudo-replicates.

RESULTS

Phylogenetic analyses.—We obtained rDNA ITS and partial 28S sequences from 12 new isolates. These sequences were aligned with sequences available from GenBank (Table 1). No incongruence was found, and the data were concatenated into a single dataset. The combined matrix contained 1278 characters (ITS1: 1–74; 5.8S: 75–229; ITS2: 230–360, 28S: 361–1278) and 29 terminals.

The Bayesian analysis halted after 1 000 000 generations, when the average standard deviation of split frequencies across runs reached 0.0086, indicating that the three runs had converged (< 0.01). A majority
rule consensus tree was constructed from the 15 000 trees of the stationary tree sample. The best tree obtained from the ML analysis had an ln likelihood value of $-7712.6399$. There was no incongruence between the Bayesian and ML trees. Therefore only the 50% majority rule consensus tree from the Bayesian analysis is illustrated (FIG. 1).

The phylogenetic analyses (FIG. 1) strongly support the delimitation of the four new species, which are described and discussed below, and demonstrate once more that lichenicolous *Tremella* species are highly specific regarding their hosts.

**TAXONOMY**

*Tremella celata* J.C. Zamora, Millanes, V.J. Rico & Pérez-Ortega, sp. nov.

MycoBank MB814014


**Etymology:** From the Latin word *celatus*, meaning hidden, concealed, because of the inconspicuous appearance of the species that is easily mistaken for *Tremella tuckerae*.

**Diagnosis:** Similar to *Tremella tuckerae* but with distinct ITS–28S rDNA sequences growing in the thallus of the fruticose lichen *Ramalina fraxinea* and developing smaller 9–18 × 7.5–11.5 μm sessile to stalked basidia and spur-like pseudoclamps instead of clamp-connections.

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**TABLE I.** Sequences newly produced (boldface), or downloaded from GenBank, with specimen data or culture references

<table>
<thead>
<tr>
<th>Species names</th>
<th>Cultures</th>
<th>Specimen data</th>
<th>Genbank accession No.</th>
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Basidiomata resupinate, inducing the formation of diffuse and often inconspicuous, waxy, 0.5–4 mm long, cream-colored galls visible on the thallus surface of host, becoming dark when old, sometimes coalescent. In some cases gall formation is almost absent. Hyphal system monomitic; context hyphae 2.5–4 μm diam, slightly thick-walled, clampless but conspicuous spur-like pseudoclamps often observed; subbasidial hyphae similar or with slightly shorter cells and thicker walls. Haustoria frequent, mother cell 2.5–3.5 × 2.5–3 (–3.5) μm, broadly ellipsoidal, haustorial filaments unbranched or sometimes with two short branches, 2–5 × 1 μm. Hymenium amphigenous, hyaline, well-developed, with numerous subglobose to cylindrical probasidia in young galls. Hyphidia not seen. Mature basidia two-celled, thin- to slightly thick-walled, often with an attenuated or stalked base but sometimes sessile, with a longitudinal or oblique septum, 9–18 × 7.5–11.5 μm (n = 25, including the attenuated base but not the stalk, when present), stalk 1–8 × 1.5–3 μm. Epibasidia thin-walled, 9–22 × 2–4 μm, subcylindrical to narrowly fusiform, developing an apical sterigma of 2–4 μm long. Basidiospores 5.5–7 × 5.5–7(–8) μm (n = 20), Q = 0.86–1, globose to subglobose, usually wider than long, hyaline, inamyloid, with a conspicuous and nonrefractive hilar appendix, 1–1.5 μm long. Basidiospore germination not seen. Anamorph unknown.


COMUNIDAD DE MADRID, MADRID: Aoslos, 41°03′30″N, 03°35′18″W, 1038 m, on *Fraxinus angustifolia* trunk, 30 Apr 2011, J.C. Zamora, B. Zamora & J. Señoret, MAF-Lich. 19731. Montejo de la Sierra, 41°04′33″N, 03°31′18″W, 1254 m, on *Pyrenula pseudofoliacea* trunk, 16 Apr 2011, J.C. Zamora & B. Zamora, MAF-Lich. 19732. San Lorenzo de El Escorial, vicinity of Silla de Felipe II, 0.06

**Fig. 1.** Fifty percent majority rule Bayesian consensus phylogram, with average branch lengths, from the combined analyses of ITS1, 5.8S, ITS2 and 28S datasets. Black dots represent branches supported in both Bayesian and ML analyses and white dots branches supported only either in the Bayesian analysis or in the ML analysis. Support is considered if BPP values ≥ 0.95 in the Bayesian analysis and bootstrap values ≥ 70% in the ML analysis. Newly described species are in boldface. Other groups in the Tremellales distinguished by Chen (1998), and Millanes et al. (2011) are indicated with gray bars. Branch lengths are scaled to the expected number of nucleotide substitutions per site.
Observations: *Tremella celata* is an inconspicuous but probably common species that grows in the thallus of the abundant epiphytic lichen *Ramalina fraxinea*. We detected an important morphological heterogeneity among different collections. In most specimens basidia tend to be stalked, with a conspicuous spur-like basal pseudoclamp, with both longitudinal and oblique septa. However, in some individuals most basidia are sessile, with obscure or inconspicuous pseudoclamps, and with longitudinal septa. Both kinds of basidia are nevertheless present in all specimens studied, and sometimes one type may dominate in a particular gall while the other type is dominant in another gall on the same thallus. For these reasons we considered that these morphotypes were part of the intraspecific variation and may be a product of age, because stalked basidia are more common in young galls while sessile basidia often are seen in older ones. *Tremella ramalinae* Diederich shares the same host, which is uncommon among lichenicolous heterobasidiomycetes. This species can be distinguished by forming well-developed, pinkish basidiomata or galls with a constricted base and by four-celled basidia that normally have two transverse septa and one apical longitudinal septum (Diederich 1996). Despite sharing the same host, *T. ramalinae* is phylogenetically divergent from *T. celata*, in that both species do not cluster in one lineage in our phylogenetic tree (Fig. 1). *Tremella tuckerae* (Diederich 2007) is morphologically similar, inducing the formation of diffuse although rather conspicuous galls on the fruticose thallus of different *Ramalina* species. It is particularly common in the Iberian Peninsula on *R. farinacea*, but we never found it growing on *R. fraxinea*. Its micromorphology also resembles *T. celata*, in particular when sessile basidia are profuse, but *T. celata* differs in the smaller basidia with cells that do not elongate at maturity and, according to
our own observations, *T. tuckerae* develops conspicuous clamp-connections and hyphae with thicker walls, especially in the subbasidial hyphae. Based on our molecular phylogenetic data, specimens of *T. tuckerae* (including the holotype) and *T. celata* form well-separated, independent phylogenetic lineages (Fig. 1). The widespread *T. lobariacearum* Diederich & M.S. Christ. shares several micromorphological characteristics with *T. celata*, but it forms conspicuous galls on isidiomata and other parts of the thallus of the foliose genera *Lobaria* and *Pseudocyphellaria*, often has asteroconidia and basidia with an attenuate base (Diederich 1996). *Tremella lobariacearum* and *T. celata* are well-separated in our phylogenetic tree (Fig. 1).

*Tremella nashii* Diederich is distinguished by inducing the formation of more or less conspicuous galls on several species of the fruticose genus *Usnea* and having clamped hyphae (Diederich 2007), but unfortunately we do not have molecular data for this species. Finally the nonlichenicolous *T. giraffa* Chee J. Chen shares the stalked basidia and hyphae with pseudoclamps but grows intrahymenially in species of the nonlichenized genus *Dacrymyces*, has thin-walled hyphae, basidia formed in chains and subglobose to broadly ellipsoid basidiospores (Chen 1996, Zamora 2009a). It is not closely related to *T. celata* in our phylogenetic analysis (Fig. 1).

**Tremella endosporogena** J.C. Zamora, Millanes, V.J. Rico & Pérez-Ortega, sp. nov. Figs. 3, 4B

*Mycobank* MB814015


*Etymology:* Compound epithet resulting of the combination of the terms *endo*—“inner”—, -spora, “spore”, and -genus, “to form” or “to generate”, meaning that the species produces internal spores in old basidia.

*Diagnosis:* Characterized by distinct ITS–28S rDNA sequences, growing within the crustose lichen
apothecia *Lecanora carpinea* and replacing the hymenium of its host by its own, lacking clamp-connections, and producing monosporic non-acanthoid basidia and internal conidia, or “endospores”, from old basidia.

Fungus growing within the host apothecia, first inconspicuous, soon inducing the formation of convex, waxy to gelatinous, cream-colored to yellowish or greenish galls, 0.2–1.3 mm diam, shrunk when dried. Hypothal system monomitic; context hyphae 2–5 μm diam, slightly to clearly thick-walled, clamped; subbasidial hyphae with shorter cells and thicker walls. Haustoria frequent, mother cells 2–4.5(–8) × 2–4(–6) μm, subglobose to broadly ellipsoid, haustorial filaments 0.5–1(–1.5) × 2–7 μm, often unbranched. Hymenium amphigenous, hyaline, made by a mixture of probasidia, mature basidia and conidiogenous cells, replacing the host hymenium; hyphidia absent or scarce and poorly differentiated. Probasidia often thick-walled, claviform to pyriform. Basidia (15–)17–40(–45) × 5.5–17(–19) μm (n = 50, including the stalk-like base), cylindrical to claviform or broadly fusiform, sessile or with a short stalk-like base up to 8 μm long, but normally much shorter, slightly to clearly thick-walled, with a somewhat rugose to cracked surface. When mature, each basidium develops a single, ± cylindrical, thin-walled epibasidium, 70–130 × 5–8 μm, often poorly delimited from the hypobasidial part, with an apical sterigma, 2–4.5 μm long. Basidiospores 9.5–15.5 × 10–16 μm (n = 25), Q = (0.83–)0.87–1.08, globose to subglobose, rarely broadly ellipsoid, often wider than long, hyaline, inamyloid, with a conspicuous and not to slightly refractive hilar appendix, 1–1.5(–2) μm long. Basidiospores forming secondary ballistoconidia (similar to small basidiospores) by the formation of a sterigma. Anamorph present in all specimens studied in the form of conidiogenous, thick-walled, vessel-like cells, 12–27(–41) × 7–12(–17) μm, producing thick-walled conidia by apical budding, 4–7.5 × (2.5–)3–5 μm. In addition, old basidia sometimes producing conidial “endospores”, (4.5–)5–9(10) × (2.5–)3–6 μm, that are morphologically similar to the thick-walled conidia of the conidiogenous cells and seem to be generated by internal budding from the basidium base.


*Observations:* *Tremella endosporogen*a is a remarkable species because it is one of the few heterobasidiomycetes with monosporic basidia. The recently described *Heteroacanthella ellipsospora* often grows nearby on the same host (both type specimens were found on the same tree) but is easily distinguished by the acanthoid, stalked basidia, ellipsoid basidiospores with a refractive hilar appendix, clamped hyphae and absence of conidiogenous cells (Zamora et al. 2014). *Tremella monospora* Diederich is the only other lichenicolous *Tremella* species with single-celled basidia. It is distinguished by the smaller, long-stalked basidia, smaller basidiospores (5–7 × 4.5–6 μm), thin-walled clamped hyphae, and by growing on the foliose thallus of *Leptogium* sp. (Diederich 1996). Furthermore none of these species are known to produce endospores. *Celatogloea simplicibasidium* (Roberts 2005) is probably the morphologically closest taxon. This species grows within the hymenium of the non-lichenized *Corticium roseum*, and it is distinguished by the often stalked basidia, thin-walled clamped hyphae and subglobose to broadly ellipsoid basidiospores (FIG. 8). In fact the new species fits quite well the diagnosis of *Celatogloea*, but we preferred to follow a conservative approach by describing it within *Tremella* s.l. since molecular data of *Celatogloea* are lacking, the phylogenetic and morphological boundaries among most of *Tremella* s.l. clades are not established with confidence (Millanes et al. 2011) and *T. endosporogen*a nests close to other *Tremella* s.l. species in our phylogenetic analyses (FIG. 1).

*Tremella diederichiana* Pérez-Ortega, Millanes, Wedin, V.J. Rico & J.C. Zamora sp. nov.  Figs. 5, 7A MycoBank MB814016

**Typification:** SPAIN, COMUNIDAD DE MADRID, MADRID: Villanueva de la Cañada, Guadamonte residential, 40°24′49″N, 03°57′47″W, 630 m, on *Lecanora chlorotera* apothecia and thalli, on *Cistus ladanifer* branches (*Tremella macrobasidiata* also present in *Lecanora chlorotera* apothecia, and incipient *T. dactylobasidium* basidiomata associated with *Vuilleminia macrospora*), 7 Feb 2015, J.C. Zamora & B. Zamora (*holotype* MAF-Lich. 19735). **Isotypes** AH, UPS.

**Etymology:** This species is dedicated to Dr Paul Diederich for his contribution to the field of lichenology, particularly to the knowledge of the lichenicolous Tremellomycetes, and in gratitude for his kindness and support.

**Diagnosis:** Similar to *Tremella christiansenii* but with distinct ITS–28S rDNA sequences and further
differentiated by growing in the thallus and apothecia of the crustose lichen *Lecidea* aff. *erythrophaea*, where it develops minute convex pale galls, (2.5–)3–4 µm diam haustoria, and two- to four-celled basidia.

Fungus growing in the host apothecia and thallus, basidiomata inducing the formation of convex to bulbate, rounded, waxy, pale cream to pale brown, rarely brown or greenish galls, 0.05–0.5 (–0.7) mm diam. Hyphal system monomitic; context hyphae 3.5–8 µm diam, more or less moniliform, strongly thick-walled, clamps absent or inconspicuous, rarely few clear clamps visible in young probasidial initials of specimens with numerous four-celled basidia; subbasidial hyphae similar or with slightly shorter cells and thicker walls. Haustoria frequent, mother cell (2.5–)3–4 µm diam, subglobose, haustorial filaments normally unbranched, up to 7 × 1 µm. Hymenium amphigenous, hyaline, well-developed, with numerous subglobose to ellipsoid probasidia; basal clamps almost indistinct and rarely seen. Hyphidia probably absent or not clearly

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differentiated, but young fertile hyphae may resemble hyphidia before basidia development. Basidia two- to four-celled at maturity (each type may dominate in different galls), with longitudinal septa (exceptionally oblique); individual hypobasidial cells elongating and growing separately, thin- to slightly thick-walled, each 14.5–34(–38) × 6–13 μm (n = 30). Epibasidia thin-walled, 20–50 × 3.5–7.5 μm, subcylindrical, sometimes not clearly differentiated from the hypobasidial part, developing an apical sterigma, 2.5–5 μm long. Basidiospores (7–)10–12.5(–13.5) × (8.5–)10–14(–16) μm (n = 50), Q = (0.82)–0.87–1.05, globose to subglobose, rarely broadly ellipsoid, often wider than long, hyaline, inamylloid, with a conspicuous and nonrefractive hilar appendix, 1–1.5(–2) μm long. Basidiospores capable of germinating by producing a sterigma and a secondary ballistospore (ballistoconidium), similar to a basidiospore but somewhat smaller, 9–10 μm diam; blastic conidia (yeast-like cells) also observed, 5–7.5 × 4–6 μm, ovoid to broadly ellipsoid; germination of the formation by an hypha rarely seen. Other anamorphs unknown.


Notes on the host: The host lichen of Tremella diederichiana is similar to Lecidea erythrophaea (Boqueras 2000, Hertel and Prinzen 2004, Smith et al. 2009) but differs in the wider, never fusiform spores of 10–13(–14) × 6–7.5 μm and branched, brown, thickened to 6 μm diam capitate paraphysal tips.

Moreover, studied specimens mainly grow on the branches of shrubs in a continental Mediterranean climate area, while L. erythrophaea s.str. is known from the humid climate of Eurosiberian western Europe, on the smooth bark of deciduous trees (Smith et al. 2009). It seems that the L. erythrophaea group needs a full revision to elucidate whether the Iberian specimens represent a distinct lichen-forming species, in that it occurs with North American material (cf. Hertel and Printzen 2004). According to observed morphological characters, such as its corticolous habit, ± Bacidia-type asci, paraphyses type and reduced exciple, the position of the L. erythrophaea group in Lecideaceae remains unclear and needs to be tested in a phylogenetic framework as described in other lecideoid lichens (Hertel and Prinzen 2004, Pérez-Ortega et al. 2010, Rodríguez Flakus and Printzen 2014).

Observations: Tremella diederichiana is a well-circumscribed species, micromorphologically similar to T. christiansenii Diederich. Both taxa have clampless or inconspicuously clamped hyphae, basidia in which cells elongate and grow independently before forming the epibasidia and basidiospores of similar size and shape (Diederich 1996). We have studied some specimens of T. christiansenii and, according to Diederich (1996) and our own observations, it has mostly two-celled basidia (four-celled basidia are rare), smaller haustoria (2.5–3 μm diam), and context hyphae that tend to be narrower with thinner walls. In addition, to these somewhat subtle differences, they are strikingly different based on their macromorphological traits. Tremella christiansenii typically develops big (sometimes more than 5 mm when fresh) and cerebriform, brown to dark brown galls, in contrast with the minute, convex and paler galls of the new T. diederichiana species. Moreover, T. christiansenii is known only to parasitize species of the foliose genus Physcia. Our phylogenetic analysis placed both species in two distant clades (Fig. 1). There are few other Tremella species in which hypobasidial cells elongate and grow separately before forming the epibasidia: Tremella hypocenomycae Diederich is a rare species that forms gelatinose, blackish basidimata on the thallus of Hypocenomyce scalaris, and has two-celled basidia, smaller basidiospores (5.5–6.5 μm diam.), and slightly larger haustoria (3.5–5 × 3–4 μm) (Diederich 1996). Tremella tuckerae grows on the thallus of Ramalina spp., inducing the formation of poorly delimited galls, and, according to our observations (see discussion above under T. celata) and the original description (Diederich 2007), it is readily distinguished by the presence of conspicuous clamp connections, often elongated probasidia and smaller basidiospores (6–9 × 5.5–8 μm); basidia are generally two-celled but a few four-celled basidia have been
observed in certain specimens. Finally *T. dactylobasidia* J.C. Zamora is a nonlichenicolous species that grows associated with the hymenomycetous fungus *Vuilleminiia macrospora*; it forms conspicuous yellow gelatinous basidiomata and possess clearly clamped thin-walled hyphae, normally four-celled basidia, and numerous thin- and thick-walled hymenial hyphidia (Zamora 2009b).

*Tremella variae* Pérez-Ortega, Millanes, V.J. Rico & J.C. Zamora, sp. nov.  Figs. 6, 7B  
*MycoBank* MB814017  
*GenBank accessions:* ITS KT334587, and 28S KT334599.  

Etymology: In reference to the regular occurrence of the species on the lichenized fungus *Lecanora varia.*

Diagnosis: Similar to *Tremella macrobasidiata* but with distinct ITS–28S rDNA sequences and growing in the thalli and apothecia exciple of the crustose lichen *Lecanora varia,* developing smaller basidia, 15–26 × 12–22 μm, subglobose to broadly ellipsoid or clavate, sessile and mainly longitudinally septate.

Fungus growing in the host thallus and apothecia exciple, inducing the formation of convex to bullate, rounded, waxy, greenish yellow at first (concolorous with host thallus) but soon cream to pale brown galls, 0.1–0.7 mm diam. Hyphal system monomitic; context hyphae 2–4(–5) μm diam, thick-walled, clamped; subbasidial hyphae with slightly shorter cells and thicker walls. Haustoria frequent, mother cells 3–4(–4.5) μm diam, subglobose, haustorial filaments mostly unbranched, 2–4(–8) × 1 μm. Hymenium amphigenous, not always well-developed, hyaline, with numerous globose to broadly clavate probasidia. Hyphidia, if

present, almost indistinct from fertile hyphae, 2–4 μm diam, with many septa with clamps. Basidia two- to four-celled at maturity, with longitudinal septa, rarely slightly oblique, sessile, thin- to slightly thick-walled, 15–26 × 12–22 μm (n = 30), subglobose to broadly ellipsoid or clavate. Epibasidia thin-walled, subcylintrical, pointed, sometimes forked, 12–28 × (2.5–)3–5 μm, developing an apical sterigma, 3–4.5(–7) μm long, rarely more than one sterigma present. Basidiospores (6.5–)7–10.5 × (6.5–)7–11(–12) μm diam (n = 15), Q = 0.88–1.13, globose to subglobose, hyaline, inamyloid, with a conspicuous refractive hilar appendix, 1–1.5 μm long. Basidiospore germination not seen. Anamorph unknown.


Observations: Tremella variae is the fourth Tremella species known to be associated with the crustose, lichen-forming fungal genus Lecanora s.l. Although some specimens have a considerable number of galls and basidia are easily seen, most basidiospores were scarce and collapsed. Tremella sp.2 in Diederich (1996) differs in having thinner two-celled basidia with a transverse septum, more ellipsoid basidiospores, and growing intrahymenially in Lecanora rimmcola H. Magn. apothecia; since only one old specimen is known, the species is still awaiting formal description. The most closely related species seems to be T. macrobasidiata, which is placed as a sister taxon in our phylogenetic reconstructions, despite the lack of support (Fig. 1). This species is distinguished by growing intrahymenially in Lecanora chlorotera apothecia, inducing the formation of conspicuous swellings and replacing the hymenium of the host, and by the much larger two- to four-celled basidia, sometimes with a stalk-like base, and with longitudinal, oblique or transverse septa (Zamora et al. 2011). As mentioned above, Tremella endosporogena, on Lecanora carpinea, is morphologically divergent by the one-celled, much larger basidia and several other morphological features (see above).

Discussion

Lichenicolous Tremella species: hyperdiversity linked with hosts.—The large species richness of lichenicolous heterobasidiomycetes, and particularly of Tremella s.l., was first illustrated in Diederich’s (1996) monograph. Recently the increasing interest in this group of fungi led to the discovery of numerous new species, several still awaiting formal description (Millanes et al. 2012).

Lichenicolous heterobasidiomycetes tend to be highly specific with respect to their hosts (Diederich 1996, Millanes et al. 2014b). In addition, the parasite seems to be exclusively fungicolous, not affecting the photobiont of the symbiosis (Grube and de los Ríos 2001). Our phylogenetic analyses showed that Tremella species parasitizing epiphytic crustose species of Lecanoraceae (T. diederichiana, hesitantly but see notes on the host, T. endosporogena, T. variae and T. macrobasidiata) form a highly supported monophyletic group (Fig. 1). Indeed host and host habitat similarity are the only synapomorphies characterizing this otherwise morphologically heterogeneous clade: species growing in the host thallus or hymenium, with or without clamps, with single-, two- or four-celled, sessile or stalked basidia, with hypobasidia cells that can or not elongate and grow independently before forming epibasidia, and with transverse, longitudinal or oblique basidia septa. The topology of the phylogenetic tree suggests that a cophylogenetic study with lichen-forming crustose species in the Lecanoraceae may reveal interesting patterns of speciation within this clade (Fig. 1). Additional molecular data, however, are needed to address this question. By contrast species parasitizing lichen-forming fruticose Ramalinaceae (Tre- mella celata, T. tuckerae, T. ramalinae) are not closely related in our phylogeny (Fig. 1). Millanes et al. (2014b) revealed that host switching, rather than cospeciation, was driving speciation in some highly specialized taxa in the Tremellales, some of these being characterized only by host specificity. Our results provide further evidence indicating that host species families and host habitat specificity are important characters that should be taken into consideration for systematic purposes.

The complex interactions that these parasites establish with their particular hosts are far from being well known. Some lichenicolous heterobasidiomycetes seem to function as parasybionts, not significantly altering the function or the appearance of the host. Many others produce galls or basidiomata in their host thallus or hymenium but without causing serious damages to the whole thallus but certainly reducing its fitness. The four new species described here produce infections that are often restricted to certain parts of the thallus or few apothecia. However, in
some cases these lichenicolous fungi can be very aggressive, showing a genuine pathogenic behavior, as is the case of *Tremella variae* that comes to infect the entire thallus of *Lecanora varia*.

**Morphological and phylogenetic boundaries in the Tremellales.**—Identifying morphological synapomorphies in the Tremellales is a challenging issue. In a large-scale study including both morphological and molecular data, Millanes et al. (2011) revealed high plasticity in basidium habit, basidium septation and conidium types, which are characters traditionally considered important in the Tremellales classification. Conversely, some smaller groups recovered with molecular data also have been characterized by morphological synapomorphies (Chen 1998). At species rank morphology tends to be of value and often allows the distinction of different taxa without the necessity of molecular data. For example, the four species described in this study are all well characterized with morphological data. However, some exceptions are known (Millanes et al. 2014b). All in all, with the current knowledge and before obtaining molecular data, it is hardly possible to know whether morphology or host specificity could be useful to characterize any monophyletic group above species rank.

**Endospore production in Tremella endosporogena basidia.**—Non-meiotic endospore formation in basidia acting as “sporangia” (do not confuse with the intrabasidial meiotic products that function as basidiospore precursors, as used by Bandoni [1984] and Clémençon [2004]), is an unusual form of reproduction among the Basidiomycota. So far this endospore formation is known only in *Naohidea sebacea* (Berk. & Broome) Oberw. (Oberwinkler 1990) and anecdotally in *Tremella versicolor* (Bandoni and Ginns 1993), while in *Cystofilobasidium* (Oberwinkler et al. 1983) and *Sporiidioholus* (Bandoni 1984) endospore formation is known in the teliospores that function as basidia precursors. Endospore ontogeny has been carefully investigated only in *Naohidea*, and endospores seem to be originated from an internal budding locus (Oberwinkler 1990). In *Tremella versicolor* endospore ontogeny is unknown and it is difficult to assess whether these spores are viable or may have a significant role in the reproductive success of the species, because they have been observed only once (Bandoni and Ginns 1993). Conversely, the constant presence of endospores in *T. endosporogena* (confirmed in all specimens despite their low abundance) may indicate that it is a well-established reproduction strategy. Those endospores are formed inside old basidia after the release of basidiospores. Although their ontogeny should be studied in more detail, our observations indicate that they most likely occur from internal budding at the attachment point of the basidium and the hypha. This ability of internal budding from old basidia is well known in the genera *Repetobasidium* J. Erikss. (Eriksson 1958), *Repetobasidiellum* J. Erikss. & Hjortstam (Eriksson et al. 1981), *Renatobasidium* Hauerslev (Hauerslev 1993) and *Heterorepetobasidium* Chee J. Chen & Oberw. (Chen et al. 2002), resulting in these cases in a new internal basidium instead of endospores. This budding ability may represent an effective way to spare resources for the fungal hyphae, because they do not need to differentiate in other structures. In addition, we observed a repetobasidial development in one specimen of *Celatogloea simplicibasidium* (FIG. 8C, D) described by Roberts (2005). As far as we know such ability had not been reported for this taxon. As noted above *Celatogloea* is morphologically close to *Tremella*.
endosporogena and the repetitive basidial budding of the former is similar to the endospore production of the latter; this particularity emphasizes the potential relatedness of these two species. However, this hypothesis still awaits verification with molecular data.

From a morphological point of view endospores are not significantly different from the thick-walled conidia formed in the conidiogenous vessel-like cells. Thick-walled conidia are formed by apical (rarely also lateral) budding of conidiogenous cells, in a similar way to endospore formation by budding inside old basidia. In addition, both thick-walled conidia from conidiogenous cells and endospores tend to germinate by forming a short filament, adopting then the form of a detached haustorium, which may indicate not only morphological but also behavior similarities.

CONCLUSIONS

This study contributes to the knowledge of Tremella diversity by describing four new species parasitizing common epiphytic lichens. Morphological and molecular characters clearly delineate the new species. Within lichenicolous Tremella species, morphology tends to be a good source of information for species delimitation, but it is more unreliable for characterizing major clades obtained through molecular data analyses. Host specificity also should be taken into account for systematic uses, despite its shortcomings due to the existence of distantly related taxa sharing the same host. Because of these uncertainties, molecular data are essential both to establish phylogenetic relationships and to test hypotheses in an evolutionary context.

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LITERATURE CITED


