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- 1 Short title: New lichenicolous *Tremella*
- 2 Understanding lichenicolous heterobasidiomycetes: new taxa and reproductive
- 3 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 diderichiana* is a name proposed for a species micromorphologically close to *T. christiansenii*, but inducing the formation of very small, pale galls on the thallus and apothecia of *Lecidea* aff. *erythrophaea*. *Tremella varia* grows on *Lecanora varia* thallus, instead of on the apothecia, as 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 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

INTRODUCTION

Tremella Pers. is a genus of heterobasidiomycetous fungi, mainly characterized by a parasitic lifestyle on fungal hosts through specialized haustoria, dolipore septa with cupulate-vesiculose parenthesomes, and complex basidia, often longitudinally, obliquely or transversely septate (exceptionally single-celled) in which single epibasidia develops from each hypobasidial cell (Bandoni 1984; Diederich 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* s.l., a number of described species are restricted to parasitize lichen-forming fungi, showing a high host-parasite specialization (Diederich 1996). Approximately 50 lichenicolous species are currently recognized (Diederich 1986, 1996, 2003, 2007; Diederich and Marson 1988; Diederich and Christiansen 1994; Sérusiaux et al. 2003; Zamora et al. 2011; Millanes et al. 2012, 2014a, 2015; Diederich et al. 2014), and four more are awaiting formal description (Diederich 1996, 2007). Lichenicolous *Tremella* species are usually inconspicuous and commonly overlooked by both mycologists and lichenologists (Millanes et al. 2012). Since their study is considerably recent, many new species are expected to be discovered.

Tremella s.l. is remarkable by its wide variety of reproductive strategies. Besides the sexual reproduction via basidiospores, *Tremella* species are often able to change from its filamentous nature to a yeast state (dimorphic fungi, which is rather 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, e.g., the asteroconidia

and lunate conidia of various lichenicolous species (Diederich 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 (“endospores”) was found in one of the species and is also described 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 previous works (Zamora et al. 2011, 2014), being summarized here.

Micromorphological study was done using 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 during 3–5 min, then washed with distilled water until the leftover staining was removed; alternatively, a solution of 1% sodium dodecyl sulphate was used to remove the excess of stain. Spore amyloidity was tested using Melzer’s reagent. Measurements were made in KOH solution. Basidiospore length:width ratio was expressed as Q, and basidiospore shape terminology follows Bas (1969). Drawings were prepared by direct observation under a Jeulin light microscope. Microscopic photographs were taken in a Zeiss AX10 microscope fitted with differential interference contrast (DIC) and coupled to an AxioCam digital camera.

Specimens used for comparison or DNA extractions.—*Celatogloea simplicibasidium* (Lindsey & Gilb.) P. Roberts. GREAT BRITAIN, ENGLAND, NORFOLK: Swanton

Novers, near Fakenham, Great Wood, in sterile basidiome of *Corticium roseum* on dead attached *Salix* twigs, 29-X-2003, A. M. Ainsworth, K (M) 122005.

Tremella macrobasidiata J.C. Zamora, Pérez-Ortega & V.J. Rico. PORTUGAL, MINHO: Castro Laboreiro, near hotel Miracastro, 42°01'39"N, 08°09'37"W, 950 m, in *Lecanora chlarotera* apothecia, on *Populus* sp. bark, 11-IX-2014, J.C. Zamora, DNA: AM 453, MAF-Lich 19755. SPAIN, CASTILLA Y LEÓN, ÁVILA: El Tiemblo, El Castañar, 40°21'26"N, 04°30'34"W, 1120 m, in *L. chlarotera* apothecia, on fallen *Castanea sativa* bark, 10-XI-2010, B. Zamora & J. C. Zamora, DNA: AM 452, ISOTYPE—MAF-Lich. 16877.

Tremella tuckerae Diederich. SPAIN, ANDALUCÍA, JAÉN: Montizón, Venta de Los Santos, near Dañador dam, 38°24'06"N, 03°02'42"W, 695 m, on *Ramalina farinacea* thallus, on *Quercus faginea* trunk and branches, 25-VII-2011, B. Zamora & J. C. Zamora, DNA: AM 372, MAF-Lich 19754. COMUNIDAD DE MADRID, MADRID: San Lorenzo de El Escorial, La Herrería forest, near Ermita de la Virgen de Gracia, 40°34'24"N, 04°09'18"W, 973 m, on *Ramalina farinacea* thallus, on *Quercus pyrenaica* bark, 18-XII-2011, M. Vivas, B. Zamora & J.C. Zamora, DNA: AM 373, MAF-Lich 19753.

Molecular study.—Taxon sampling consisted of two specimens per new species, in addition to two specimens of *Tremella macrobasidiata*, two specimens of *T. tuckerae*, and a group of 19 specimens from the genus *Tremella* (Table I). These included the genus type species (*Tremella mesenterica* (Schaeff.) Retz.), as well as representatives of the Aurantia, Fuciformis and Indecorata groups distinguished by Chen (1998), representatives of three groups of lichenicolous species distinguished by Millanes et al. (2011), and lichenicolous taxa with a similar micro or macro-morphology to that of the new species. *Tremella foliacea* Pers. was used as outgroup based on Millanes et al. (2011,

2012). Species names, voucher information, and GenBank accession numbers are given in Table I.

DNA extractions and PCR amplifications were achieved following Millanes et al. (2012). We amplified the nuc rDNA internal transcribed spacer 1 (ITS1), 5.8S, and internal transcribed spacer 2 (ITS2) using the primer pair ITS1F (Gardes and Bruns 1993)/BasidLSU3-3 (Millanes et al. 2011), and a fragment, starting in 5', of approximately 1000 bp of the rDNA 28S region (28S) using 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 as significant if a significantly supported clade (bootstrap support $\geq 70\%$; 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 some modifications. We considered ITS1, 5.8S, and ITS2 as independent partitions, as has been suggested by Gaya et al. (2011) and Petkovits et al. (2011). Likelihood models were selected for each of the three gene regions using the Bayesian Information Criterion (BIC) as implemented in jModeltest (Posada 2008). A GTR+ Γ model was selected for the ITS1, a K80+I model was selected for the 5.8S, a SYM+ Γ model was selected for ITS2, and a GTR+I+ Γ model was selected for 28S. Three parallel runs were executed, each with five chains four of 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 RAxMLGUI 1.3 (Stamatakis 2006), using the GTRGAMMAI model of nucleotide substitution applied to all partitions because of constraints of the software RAxML. We performed a thorough ML search with 100 runs and assessed node support by thorough bootstrap using 1000 bootstrap pseudo-replicates.

RESULTS

Molecular phylogenetic analyses.—We generated 24 new sequences (12 ITS and 12 nLSU rDNA), which were aligned together with sequences available from GenBank (Table I). Two data matrices were produced, one including ITS and one including nLSU rDNA.

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 15000 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 shown in 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 to their hosts.

TAXONOMY

Tremella celata J.C. Zamora, Millanes, V.J. Rico & Pérez-Ortega sp. nov. FIGS. 2, 4A

MycoBank MB 814014

Similar to *Tremella tuckerae*, but with distinct ITS–nLSU ribosomal sequences, growing in the thallus of the fruticose lichen *Ramalina fraxinea*, and developing smaller $9\text{--}18 \times 7.5\text{--}11.5 \mu\text{m}$ sessile to stalked basidia and spur-like pseudoclamps instead of clamp-connections.

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

Basidiomata resupinate, inducing the formation of diffuse and often inconspicuous, waxy, $0.5\text{--}4 \text{ mm}$ long, cream-coloured 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 $2.5\text{--}4 \mu\text{m}$ diam., slightly thick-walled, clampless but conspicuous spur-like pseudoclamps often observed; subbasidial hyphae rather similar or with slightly shorter cells and thicker walls. Haustoria frequent, mother cell $2.5\text{--}3.5 \times 2.5\text{--}3(-3.5) \mu\text{m}$, broadly ellipsoid, haustorial filaments unbranched or sometimes with two short branches, $2\text{--}5 \times 1 \mu\text{m}$. 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, $9\text{--}18 \times 7.5\text{--}11.5 \mu\text{m}$ ($n = 25$, including the attenuated base but not the stalk, when present), stalk $1\text{--}8 \times 1.5\text{--}3 \mu\text{m}$. Epibasidia thin-walled, $9\text{--}22 \times 2\text{--}4 \mu\text{m}$, subcylindrical to narrowly fusiform, developing an apical sterigma of $2\text{--}4 \mu\text{m}$ long. Basidiospores $5.5\text{--}7 \times 5.5\text{--}7(-8) \mu\text{m}$ ($n = 20$), $Q = 0.86\text{--}1$, globose to subglobose, usually broader than long, hyaline, inamyloid, with a conspicuous and non-refractive hilar appendix, $1\text{--}1.5 \mu\text{m}$ long. Basidiospore germination not seen. Anamorph unknown.

- 186 *TYPE*. SPAIN, COMUNIDAD DE MADRID, MADRID: San Lorenzo de El
 187 Escorial, La Herrería forest, near Ermita de la Virgen de Gracia, 40°34'24"N,
 188 04°09'18"W, 973 m, in the thallus of *Ramalina fraxinea*, on bark of *Quercus pyrenaica*,
 189 18-XII-2011, *M. Vivas, B. Zamora & J.C. Zamora*, DNA: AM 370, GenBank accession
 190 numbers: KT334577 and KT334589, HOLOTYPE—MAF-Lich. 19726, ISOTYPES—
 191 AH, UPS.
- 192 *Additional specimens studied* (all in *Ramalina fraxinea* var. *fraxinea* thalli unless
 193 otherwise indicated). SPAIN, CASTILLA Y LEÓN, ÁVILA: El Tiemblo, El Castañar,
 194 40°21'26"N, 04°30'34"W, 1120 m, in the thallus of *R. fraxinea* var. *calicariformis*, on
 195 *Castanea sativa* trunk, 18-IX-2010, *B. Zamora & J.C. Zamora*, MAF-Lich. 19727. Ibid.,
 196 27-III-2011, *B. Zamora & J.C. Zamora*, MAF-Lich. 19728. SEGOVIA: Real Sitio de San
 197 Ildefonso, La Pradera de Navahorno, near the sawmill, 40°52'50"N, 04°00'54"W, 1210
 198 m, on *Aesculus hippocastanum* trunk, 30-IV-2010, *J.C. Zamora, J. Señoret & B. Zamora*,
 199 DNA: AM 371, MAF-Lich. 19729. Ibid., CENEAM, 40°52'16"N, 04°01'03"W, 1230 m,
 200 on *Quercus pyrenaica* trunk, 30-IV-2011, *J.C. Zamora, B. Zamora & J. Señoret*, MAF-
 201 Lich. 19730. COMUNIDAD DE MADRID, MADRID: Aoslos, 41°03'30"N,
 202 03°35'18"W, 1038 m, on *Fraxinus angustifolia* trunk, 30-IV-2011, *J.C. Zamora, B.*
 203 *Zamora & J. Señoret*, MAF-Lich. 19731. Montejo de la Sierra, 41°04'33"N,
 204 03°31'18"W, 1254 m, on *Q. pyrenaica* trunk, 16-IV-2011, *J.C. Zamora & B. Zamora*,
 205 MAF-Lich. 19732. San Lorenzo de El Escorial, vicinity of Silla de Felipe II, 40°34'04"N,
 206 04°09'06"W, 1053 m, on *Crataegus* sp. branches, 12-II-2011, *J.C. Zamora & B. Zamora*,
 207 MAF-Lich. 19733. PRINCIPADO DE ASTURIAS, ASTURIAS: Bezanes, Redes forest,
 208 43°06'16"N, 05°18'23"W, 1227 m, on *Fagus sylvatica* branch, 6-IX-2012, *J.C. Zamora*,
 209 MAF-Lich 19756. SWEDEN, VÄSTERGÖTLAND: Varnhem parish, Varnhem church,

58°23'05"N, 13°39'25"E, 153 m, on decayed unidentified bark, 01-XI-2011, *S.I. Sunhede*
& *J.C. Zamora*, MAF-Lich. 19734.

Observations. *Tremella celata* is an inconspicuous but probably common species that grows in the thallus of the rather frequent 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, since stalked basidia are more common in young galls, while sessile basidia are often seen in older ones. *Tremella ramalinae* Diederich shares the same host, which is extremely uncommon among lichenicolous heterobasidiomycetes. This species can be distinguished by forming well-developed, pinkish basidiomata or galls with a constricted base, and by its 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*, as both species do not cluster together in one lineage in our phylogenetic tree (FIG. 1). *Tremella tuckerae* (Diederich 2007) is morphologically very 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 very 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 isidia 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 non-lichenicolous *T. giraffa* Chee J. Chen shares the stalked basidia and hyphae with pseudoclamps, but grows intrahymenially in species of the non-lichenized 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 MB 814015

Characterized by distinct ITS–nLSU ribosomal sequences, growing within the crustose lichen apothecia *Lecanora carpineae* 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.

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.

Fungus growing within the host apothecia, first inconspicuous, soon inducing the formation of convex, waxy to gelatinous, cream-coloured to yellowish or greenish galls, 0.2–1.3 mm diam., shrunked when dried. Hyphal system monomitic; context hyphae 2–5 μm diam., slightly to clearly thick-walled, clampless; subbasidial hyphae with shorter cells and thicker walls. Haustoria frequent, mother cells 2–4.5(–8) $\times$ 2–4(–6) μm , subglobose to broadly ellipsoid, haustorial filaments 0.5–1(–1.5) $\times$ 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) $\times$ 9.5–17(–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, $\pm$ cylindrical, thin-walled epibasidium, 70–130 $\times$ 5–8 μm , often poorly delimited from the hypobasidial part, with an apical sterigma, 2–4.5 μm long. Basidiospores 9.5–15.5 $\times$ 10–16 μm ($n=25$), $Q = (0.83\text{--})0.87\text{--}1.08$, globose to subglobose, rarely broadly ellipsoid, often broader 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) $\times$ 7–12(–17) μm , producing thick-walled conidia by apical budding, 4–7.5 $\times$ (2.5–)3–5 μm . Additionally, old basidia sometimes producing conidial “endospores”, (4.5–)5–9(10) $\times$

(2.5–)3–6 μm , that are morphologically very similar to the thick-walled conidia of the conidiogenous cells, and seem to be generated by internal budding from the basidium base.

TYPE. SPAIN, COMUNIDAD DE MADRID, MADRID: Collado Villalba, 40°39'12"N, 04°00'01"W, 890 m, in *Lecanora carpinea* apothecia, on *Prunus* sp. branches, 31-XII-2010, J.C. Zamora & B. Zamora, DNA: AM 376, GenBank accession numbers: KT334579 and KT334591, HOLOTYPE—MAF-Lich. 19742.

Additional specimens studied (all in *Lecanora carpinea* apothecia). SPAIN, COMUNIDAD DE MADRID, MADRID: Horcajo de la Sierra, 41°03'34"N, 03°35'17"W, 1620 m, on *Quercus pyrenaica* branch, 30-IV-2011, J.C. Zamora, B. Zamora & J. Señoret, MAF-Lich. 19743. Montejo de la Sierra, 41°04'33"N, 03°31'18"W, 1254 m, on *Q. pyrenaica* trunk, 16-IV-2011, J.C. Zamora & B. Zamora, MAF-Lich. 19744. San Lorenzo de El Escorial, vicinity of la Silla de Felipe II, 40°34'04"N, 04°09'06"W, 1053 m, on *Crataegus monogyna* branch (*Heteroacanthella ellipsospora* J. C. Zamora, Pérez-Ortega & V. J. Rico also present in some apothecia of *L. carpinea*), 3-VI-2010, B. Zamora & J.C. Zamora, MAF-Lich. 19745. Ibid., 11-I-2015, J.C. Zamora, M. Prieto & I. Olariaga, MAF-Lich. 19746. Ibid., on *Crataegus* sp. branches, 12-II-2011, B. Zamora & J.C. Zamora, DNA: AM 375, MAF-Lich. 19747.

Observations. *Tremella endosporogena* is a remarkable species in being one of the few heterobasidiomycetes with monosporic basidia. The recently described *Heteroacanthella ellipsospora* often grows close by 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 ($5\text{--}7 \times 4.5\text{--}6$ 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. endosporogena* nests close to other *Tremella* s.l. species in our phylogenetic analyses (FIG. 1).

Tremella diderichiana Pérez-Ortega, Millanes, Wedin, V.J. Rico & J.C. Zamora sp. nov. FIGS. 5, 7A

MycoBank MB 814016

Similar to *Tremella christiansenii*, but with distinct ITS–nLSU ribosomal 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, $(2.5\text{--})3\text{--}4$ μm diam. haustoria, and two- to four-celled basidia.

Etymology. This species is dedicated to 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.

Fungus growing in the host apothecia and thallus, basidiomata inducing the formation of convex to bullate, rounded, waxy, pale cream to pale brown, rarely brown or greenish galls, $0.05\text{--}0.5$ ($\text{--}0.7$) mm diam. Hyphal system monomitic; context hyphae 3.5--

334 8 μm diam., more or less moniliform, strongly thick-walled, clamps absent or
 335 inconspicuous, rarely few clear clamps visible in young probasidial initials of specimens
 336 with numerous four-celled basidia; subbasidial hyphae very similar or with slightly
 337 shorter cells and thicker walls. Haustoria frequent, mother cell (2.5–)3–4 μm diam.,
 338 subglobose, haustorial filaments normally unbranched, up to $7 \times 1 \mu\text{m}$. Hymenium
 339 amphigenous, hyaline, well-developed, with numerous subglobose to ellipsoid
 340 probasidia; basal clamps almost indistinct and rarely seen. Hyphidia probably absent or
 341 not clearly differentiated, but young fertile hyphae may resemble hyphidia prior to
 342 basidia development. Basidia two- to four-celled at maturity (each type may dominate in
 343 different galls), with longitudinal septa (exceptionally oblique); individual hypobasidial
 344 cells elongating and growing separately, thin- to slightly thick-walled, each 14.5–34(–38)
 345 $\times 6$ –13 μm ($n = 30$). Epibasidia thin-walled, 20 – 50×3.5 – $7.5 \mu\text{m}$, subcylindrical,
 346 sometimes not clearly differentiated from the hypobasidial part, developing an apical
 347 sterigma, 2.5–5 μm long. Basidiospores (7–)10–12.5(–13.5) $\times$ (8.5–)10–14(–16) μm ($n =$
 348 50), $Q = (0.82$ –)0.87–1.05, globose to subglobose, rarely broadly ellipsoid, often broader
 349 than long, hyaline, inamyloid, with a conspicuous and non-refractive hilar appendix, 1–
 350 1.5(–2) μm long. Basidiospores capable of germinating by producing a sterigma and a
 351 secondary ballistospore (ballistoconidium), similar to a basidiospore but somewhat
 352 smaller, 9–10 μm diam.; blastic conidia (yeast-like cells) also observed, 5 – 7.5×4 –6 μm ,
 353 ovoid to broadly ellipsoidal; germination by the formation of an hypha rarely seen. Other
 354 anamorphs unknown.

355 *Note on the host.* The host lichen of *Tremella diderichiana* is similar to *Lecidea*
 356 *erythrophaea* (Smith et al. 2009; Hertel and Printzen 2004; Boqueras 2000), but differs in
 357 the wider, never fusiform spores of 10–13(–14) $\times$ 6–7.5 μm and branched, brown,
 358 thickened to 6 μm diam. capitate paraphysal tips. Moreover, studied specimens mainly

grow on the branches of shrub plants 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, as it occurs with North American material (cf. Hertel and Printzen 2004). According to observed morphological characters, such as its corticolous habit, $\pm$ *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 (Rodríguez Flakus and Printzen 2014; Pérez-Ortega et al. 2010; Hertel and Printzen 2004).

TYPE. SPAIN, COMUNIDAD DE MADRID, MADRID: Villanueva de la Cañada, Guadamonte residential, 40°24'49"N, 03°57'47"W, 630 m, on *Lecidea* aff. *erythrophaea* apothecia and thalli, on *Cistus ladanifer* branches (*Tremella macrobasidiata* also present in *Lecanora chlarotera* apothecia, and incipient *T. dactylobasidia* basidiomata associated with *Vuilleminia macrospora*), 7-II-2015, J.C. Zamora & B. Zamora, HOLOTYPE—MAF-Lich. 19735, ISOTYPES—AH, UPS.

Additional specimens studied (all in *Lecidea* aff. *erythrophaea* thalli and/or apothecia). SPAIN, CASTILLA-LA MANCHA, TOLEDO: Los Navalucillos, Parque Nacional de Cabañeros, near the parking at the park entrance, 39°34'32"N, 04°39'36"W, 750 m, jaral, on *Cistus ladanifer*, 23-X-2010, S. Pérez-Ortega 1749 & M. Arróniz-Crespo, DNA: AM 378, MAF-Lich. 19737. Ibid., on *C. ladanifer* branch, V-2013, J.C. Zamora, I. Villa, V. Ferrero, J.E. Rodríguez & P. Jiménez, MAF-Lich. 19736. COMUNIDAD DE MADRID, MADRID: Cadalso de los Vidrios, surroundings of the forest track near El Boquerón stream, 40°19'25"N, 04°24'10"W, 724 m, on *C. ladanifer* branches (*Tremella macrobasidiata* also present in the apothecia of *Lecanora*

384 *chlarotera*), XI-2013, A. Otero & E. Cano, MAF-Lich. 19738. Collado Villalba, Canchal
 385 de los Moros, 40°38'36"N, 03°58'24"W, 1020 m, on *C. ladanifer* branches (*T.*
 386 *macrobasidiata* also present in the apothecia of *L. chlarotera*), 29-XII-2010, J.C.
 387 Zamora, Á. González & F. Prieto, DNA: AM 374, MAF-Lich. 19739. San Martín de
 388 Valdeiglesias, road from Pelayos de la Presa to Cadalso de los Vidrios, 40°20'17"N,
 389 04°22'06"W, 813 m, on *C. ladanifer* branch, 28-XII-2010, J.C. Zamora & B. Zamora,
 390 MAF-Lich. 19740. Valdemorillo, Mojadillas residential, 40°31'51"N, 04°05'56"W, 890
 391 m, on *C. ladanifer* branch (*T. varia*e also present in *L. varia*, see below), 12-II-2011, B.
 392 Zamora & J.C. Zamora, MAF-Lich. 19741.

393 *Observations.* *Tremella diderichiana* is a well-defined species,
 394 micromorphologically very similar to *T. christiansenii* Diederich. Both taxa have
 395 clampless or inconspicuously clamped hyphae, basidia in which cells elongate and grow
 396 independently before forming the epibasidia, and basidiospores of similar size and shape
 397 (Diederich 1996). We have studied some specimens of *T. christiansenii* and, according to
 398 Diederich (1996) and our own observations, it has mostly two-celled basidia (four-celled
 399 basidia are very rare), smaller haustoria (2.5–3 µm diam.), and context hyphae that tend
 400 to be narrower and with thinner walls. In addition to these somewhat subtle differences,
 401 they are strikingly different based on their macromorphological traits. *Tremella*
 402 *christiansenii* typically develops big (sometimes more than 5 mm when fresh) and
 403 cerebriform, brown to dark brown galls, in contrast with the minute, convex and paler
 404 galls of the new *T. diderichiana* species. Moreover, *T. christiansenii* is only known to
 405 parasitize species of the foliose genus *Physcia*. Our phylogenetic analysis placed both
 406 species in two distant clades (FIG. 1). There are few other *Tremella* species in which
 407 hypobasidial cells elongate and grow separately before forming the epibasidia: *Tremella*
 408 *hypocenomyces* Diederich is a rare species that forms gelatinose, blackish basidiomata 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 $\times$ 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 $\times$ 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 non-lichenicolous species that grows associated with the hymenomycetous fungus *Vuilleminia 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 MB 814017

Similar to *Tremella macrobasidiata*, but with distinct ITS–nLSU ribosomal sequences and growing in the thalli and apothecia exciple of the crustose lichen *Lecanora varia*, developing smaller basidia, 15–26 $\times$ 12–22 μm , subglobose to broadly ellipsoid or clavate, sessile and mainly longitudinally septate.

Etymology. Growing on *Lecanora varia*.

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 creamish 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) $\times$ 1 μm .

434 Hymenium amphigenous, not always well-developed, hyaline, with numerous globose to
 435 broadly clavate probasidia. Hyphidia, if present, almost indistinct from fertile hyphae, 2–
 436 4 μm diam., with many septa with clamps. Basidia two- to four-celled at maturity, with
 437 longitudinal septa, rarely slightly oblique, sessile, thin- to slightly thick-walled, 15–26 $\times$
 438 12–22 μm ($n = 30$), subglobose to broadly ellipsoid or clavate. Epibasidia thin-walled,
 439 subcylindrical, pointed, sometimes forked, 12–28 $\times$ (2.5–)3–5 μm , developing an apical
 440 sterigma, 3–4.5(–7) μm long, rarely more than one sterigma present. Basidiospores (6.5–
 441)7–10.5 $\times$ (6.5–)7–11(–12) μm diam. ($n = 15$), $Q = 0.88$ –1.13, globose to subglobose,
 442 hyaline, inamyloid, with a conspicuous, not to slightly refractive hilar appendix, 1–1.5
 443 μm long. Basidiospore germination not seen. Anamorph unknown.

444 *TYPE*. SPAIN, CASTILLA-LA MANCHA, TOLEDO: Los Navalucillos, Parque
 445 Nacional de Cabañeros, near the parking at the park entrance, 39°34'32"N, 04°39'36"W,
 446 750 m, jaral, on *Lecanora varia* on *Cistus ladanifer*, 23-X-2010, S. Pérez-Ortega 1748 &
 447 M. Arróniz-Crespo, DNA: AM 377, GenBank accession numbers: KT334587 and
 448 KT334599, HOLOTYPE—MAF-Lich. 19748.

449 *Additional specimens studied* (all in *Lecanora varia*). SPAIN, ANDALUCÍA,
 450 JAÉN: Montizón, Aldeahermosa, El Avellanar mine, 38°21'50"N, 03°11'10"W, 555 m,
 451 on *Cistus ladanifer* branches, 7-XII-2011, J.C. Zamora, MAF-Lich. 19750. CASTILLA-
 452 LA MANCHA, TOLEDO: Los Navalucillos, Parque Nacional de Cabañeros,
 453 39°34'32"N, 04°39'36"W, 750 m, on *C. ladanifer* branches, V-2013, J.C. Zamora, I.
 454 Villa, V. Ferrero, J.E. Rodríguez & P. Jiménez, MAF-Lich. 19749. COMUNIDAD DE
 455 MADRID, MADRID: San Martín de Valdeiglesias, road from Pelayos de la Presa to
 456 Cadalso de los Vidrios, 40°20'17"N, 04°22'06"W, 813 m, on *C. ladanifer* branches, 28-
 457 XII-2010, J.C. Zamora & B. Zamora, MAF-Lich. 19751. Valdemorillo, Mojadillas

residential, 40°31'51"N, 04°05'56"W, 890 m, on *C. ladanifer* bark, 12-II-2011, B.

Zamora & J.C. Zamora, DNA: AM 379, MAF-Lich. 19752.

Observations. *Tremella variaie* 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 rimicola* 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 chlarotera* 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 extremely 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). The present study aims to contribute to fill this gap by describing four species that parasitize common epiphytic lichens.

Lichenicolous heterobasidiomycetes tend to be highly specific in 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. diderichiana* (hesitantly, but see notes on the host), *T. endosporogena*, *T. variaie*, 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 hypobasidial cells that can or not elongate and grow independently before forming epibasidia, and with transverse, longitudinal or oblique basidial septa. The topology of the phylogenetic tree suggests that a cophylogenetic study with lichen-forming crustose species in the *Lecanoraceae* would 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* (*Tremella celata*, *T. tuckerae*, *T. ramalinae*) are not closely related in our phylogeny (Fig. 1). Millanes et al. (2014b) showed 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 bring further evidences 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 parasymbionts, 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. 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 behaviour?, as is the case of *Tremella varia* 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 levels of plasticity in basidium habit, basidium septation, and conidium types, which are characters traditionally considered as important in the Tremellales classification. On the contrary, some smaller groups recovered using molecular data have been also characterized by morphological synapomorphies (Chen, 1998). At the species level, morphology tends to be worth of value, and often allows the distinction of different taxa without the necessity of molecular data. As an example, the four species described in the present study are all well-characterized using 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 if morphology or host specificity could be useful to characterize any monophyletic group above the species level.

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 Clmenon (2004)) is an extremely unusual form of reproduction among the Basidiomycota. So far, this endospore formation is only known 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 *Sporidiobolus* (Bandoni 1984) endospore formation is known in the teliospores that function as basidial precursors. Only in *Naohidea*, endospore ontogeny has been carefully investigated, 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 if these spores are viable or may have a significant role in the reproductive success of the species, since they have been observed only once (Bandoni and Ginns 1993). On the contrary, 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 more deeply, our observations indicate that they are most likely occurring from internal budding at the attachment point of the basidium with 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 at the attachment point of the basidium may represent an effective way to spare resources for the fungal hyphae, since 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 has not been previously reported for this taxon. As commented above, *Celatogloea* is morphologically very close to *Tremella endosporogena*, and the repetitive basidial budding of the former is rather similar to the

endospore production of the latter; this particularity emphasises the potential relatedness of these two species. However, this hypothesis still awaits verification using molecular data.

From a morphological point of view, endospores are not significantly different to 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 very 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

The study contribute 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 purposes, but it is more unreliable for characterizing major clades obtained through molecular data analyses. Host specificity should also be taken into account for systematic uses, despite it has shortcomings as well, due to the existence of distantly related taxa sharing a 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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LEGENDS

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 $\geq 70\%$ in the ML analysis. Newly described species are in bold font. Other groups in the Tremellales distinguished by Chen (1998), and Millanes et al. (2011), are indicated with grey bars. Branch lengths are scaled to the expected number of nucleotide substitutions per site.

FIG. 2. *Tremella celata* holotype. A. Dry galls induced in the host thallus by the basidioma. B. Detail of the hymenium. C. Two-celled sessile basidium. D. Spur-like pseudoclamp. E. Sessile basidium with epibasidia initials. F. Mature stalked basidium with epibasidium in one of the cells. G. Stalked basidium with epibasidia in both cells. H. Short-stalked mature basidium with epibasidia. I, J. Basidiospores. B–J: Phloxin B in 5% KOH after KOH pretreatment. Microscopic images are by DIC. Bars: A = 1 mm; B, F, G, H = 10 μm ; C, D, E, I, J = 5 μm .

FIG. 3. *Tremella endosporogena*. A. Dry galls induced in the host apothecia. B. Wet galls. C. Young basidium. D, E, F. Mature basidia with epibasidia. G. Vessel-like conidiogenous cell with conidium. H, I. Old basidia with ‘endospores’. J, L, M. Basidiospores. K. Thick-walled conidia. C–H: Congo-red and phloxin B in 5% KOH. I: 1% crystal violet. J–M: Phloxin B in 5% KOH after KOH pretreatment. C–M: Microscopic images are by DIC. All images from MAF-Lich. 19742 (holotype), except J, L, M from MAF-Lich. 19745. Bars: A, B = 500 μm ; C, J, L, M = 5 μm ; D, E, F, H, I, K = 10 μm .

752 FIG. 4. Micromorphology of *T. celata* and *T. endosporogena*. A. *Tremella celata*
 753 holotype. A1–5. Basidiospores. A6–20. Basidia at different stages of development. A21.
 754 Context hyphae with haustoria and pseudoclamps. B. *Tremella endosporogena* holotype.
 755 B1–5. Basidiospores, in B2 with a secondary ballistospore. B6, B9, B11–19. Basidia at
 756 different stages of development. B7, B8, B10. Old basidia filled with thick-walled
 757 conidial “endospores”. B20, B23–27. Conidiogenous cells forming thick-walled conidia.
 758 B21–22. Clampless hyphae with haustoria. Bar = 20 μ m.

759 FIG. 5. *Tremella diderichiana* holotype. A, B. Dry basidiomata and galls in host thalli.
 760 C. Clampless hyphae, haustoria, probasidia and one almost mature basidium. D. Two-
 761 celled basidium and clampless hypha. E–F. Two-celled basidia. H. Three-celled young
 762 basidium. I. Four-celled basidium (one hypobasidial cell with a late development). G, J,
 763 K. Two-celled mature basidia with epibasidia initials. L. Three-celled mature basidia with
 764 epibasidia. M. Two-celled basidia at different stages of development (one cell collapsed
 765 and not stained). N–O. Basidiospores. P, Q, S. Basidiospores forming yeast-like cells. R.
 766 Basidiospore germinating to form a hypha. C–S: Phloxin B in 5% KOH after KOH
 767 pretreatment; microscopic images are by DIC. Bars: A, B = 500 μ m; C–S = 10 μ m.

768 FIG. 6. *Tremella variaie* holotype. A, B. Dry galls in host thalli. C. Three-celled young
 769 basidium. D–F, I. Two-celled young basidia. G. Four-celled young basidium. H. Two-
 770 celled mature basidium with epibasidium in one cell. J–L. Basidiospores. C–L: Phloxin B
 771 in 5% KOH after KOH pretreatment; microscopic images are by DIC. Bars: A, B = 500
 772 μ m; C–I = 10 μ m; J–L = 5 μ m.

773 FIG. 7. Micromorphology of *T. diderichiana* and *T. variaie*. A. *Tremella diderichiana*
 774 MAF-Lich. 18737, except A1, A14, A16, A20 of holotype. A1–A7. Basidiospores, in A1
 775 with a blastic conidia (yeast-like cell) and in A4–5 and A7 forming secondary
 776 ballistospores. A8–19. Basidia at different stages of development. A20. Clamped hyphae.

777 A21. Clampless hyphae, haustoria, probasidia and one almost mature basidium. B.
778 *Tremella varia* holotype. B1–7. Basidiospores. B8–9. Clamped hyphae with haustoria
779 and young basidia. B10–28. Basidia at different stages of development. Bar = 20 μ m.
780 FIG. 8. *Celatogloea simplicibasidium* K (M) 122005. A. Young basidia. B. Young basidia
781 and mature basidium with epibasidium. C, D. Repetobasidia. E. Basidiospores. F. Clamp-
782 connection. A–F: Congo-red in 5% KOH; microscopic images are by DIC. Bars: A–C, E
783 = 20 μ m; D = 10 μ m; F = 5 μ m.

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785 FOOTNOTES

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