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Tremella diploschistina (*Tremellales*, Basidiomycota, Fungi), a new lichenicolous species growing on *Diploschistes*

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Abstract: Several specimens of a lichen-inhabiting *Tremella*, inducing the formation of pale yellow, dark brown or black galls on species of *Diploschistes*, have been collected in three localities in Sweden and in one locality in the USA. Morphological and molecular studies confirm that this material represents a single species, which differs from other described *Tremella* species in the combination of gall morphology, basidium morphology, basidium and basidiospore sizes, presence of thick-walled hyphidia, and a different host-selection. We consequently describe this fungus as *Tremella diploschistina* sp. nov., on the basis of morphology and phylogenetic analyses of ITS and nLSU sequences. The phylogenetic analyses reveal that the fungus clearly belongs in *Tremella*, although the relationships with other species in the genus remain unclear.

Key words: lichens, molecular phylogeny, taxonomy, Tremellomycetes

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Introduction

The genus *Tremella* Pers. (Tremellomycetes, Basidiomycota, Fungi) includes predominantly mycoparasitic species, growing on a wide range of basidiomycete and ascomycete fungi (Chen 1998; Kirk *et al.* 2008). Each particular *Tremella* species is, however, highly host-specific, frequently being confined to a single fungal genus or species. Many taxa form conspicuous gelatinous basidiocarps and are well known representatives of 'jelly fungi', such as *Tremella mesenterica* or *Tremella foliacea*. Lichenicolous species are among the most poorly known representatives of the genus. Fifty-one *Tremella* species have been described so far, growing exclusively on lichenized fungi (Diederich 1986, 1996, 2003, 2007; Sérusiaux *et al.* 2003; Zamora *et al.* 2011), although five of these have not yet been formally named (Diederich 1996, 2007). The lichenicolous *Tremella* species often induce the formation of relatively conspicuous galls on their hosts, either on the thallus or on the hymenium of the ascocarps. Some intrahymenial taxa do not produce any external symptoms, at least not in early stages of growth (Diederich 1996; Zamora *et al.* 2011). The phylogenetic position of the lichen-inhabiting representatives had never been tested by molecular methods until the work by Millanes *et al.* (2011), who confirmed that they were nested within the genus *Tremella*. However, *Tremella* as currently circumscribed is strongly polyphyletic (Chen 1998; Fell *et al.* 2000; Scorzetti *et al.* 2002; Boekhout *et al.* 2011; Millanes *et al.* 2011), and both the genus and the family *Tremellaceae* are in need of a deep taxonomic revision (Boekhout *et al.* 2011; Millanes *et al.* 2011). In their study of Tremellomycetes, Millanes *et al.* (2011) found that the lichenicolous lifestyle was not restricted to a single clade, and they distinguished three monophyletic groups including mostly lichenicolous species.

It has been suggested that the diversity of lichen-inhabiting fungi is, in general,

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probably underestimated (Lawrey & Diederich 2003; Ihlen & Wedin 2008) and this is also the case in *Tremella* in particular, where the number of new descriptions of lichen-inhabiting taxa is certainly expected to increase in the future (P. Diederich, pers. obs.; Zamora *et al.* 2011). Moreover, the frequency of new lichenicolous *Tremella* records reported in recent years suggests that many of the species already described might have been largely overlooked in previous field surveys, and that the distribution of many species is possibly wider than currently considered (Diederich 2003; Sérusiaux *et al.* 2003; Ertz & Diederich 2008; Kukwa & Jabłonska 2008; Pippola & Kotiranta 2008; Puolasmaa *et al.* 2008; Westberg *et al.* 2008; Svensson & Westberg 2010).

During fieldwork on the island of Runmarö in the Stockholm archipelago (Sweden), the second author collected a specimen of the lichen *Diploschistes scruposus* showing unusual dark galls on the thallus. Further microscopic observations revealed the presence of basidia and basidiospores of the *Tremella* type inside these galls. The same author later found two other specimens growing on *Diploschistes scruposus* in Sweden, and a fourth sample, growing on *Diploschistes muscorum*, had been collected previously by Roger Rosentreter in the USA and sent to Paul Diederich. No other *Tremella* species had been previously recorded on these hosts, and we conclude that the four specimens correspond to a new species of lichen-inhabiting *Tremella*, which is described here as *Tremella diploschistina* sp. nov., on the basis of morphological and molecular studies. Its phylogenetic relationship to other species in the genus is also investigated by molecular methods.

Material and Methods

Morphological studies

Macromorphological traits were observed using an Olympus SZX16 dissecting microscope. Microscopic structures were studied using hand-cut sections stained with Phloxin (1% in water) after pre-treatment with KOH (5%), following the methods of Diederich (1996), and observed with an Olympus CX40 microscope. Drawings were performed using a drawing tube and by direct observation. Micrographs were taken using an Olympus BX53 microscope fitted with differential interference contrast (DIC) and an Olympus DP11 camera. Mycological terminology follows Diederich (1996) and Kirk *et al.* (2008). The apiculus was not included in basidiospore measurements. Sizes in parentheses represent minimum and maximum observed values. When the number of observations is less than 30, it is indicated in brackets.

Molecular studies

Choice of additional taxa and outgroup

In addition to the three specimens studied, 18 specimens representing 10 *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). We included two species of *Filobasidiales*, viz., *Filobasidium floriforme* and *F. uniguttulatum*, as outgroup.

Species names, voucher information, and GenBank accession numbers are given in Table 1.

DNA extraction and amplification

DNA was extracted directly from the three specimens examined (Table 1). The outer surface of the selected galls, in which most of the tremellalean hyphae and hymenial components are located, was sectioned and separated with a scalpel, in order to minimize the lichen tissue in the DNA extraction. Total DNA was extracted using the Qiagen DNeasy Plant MiniKit, according to the manufacturer's instructions.

For PCR amplification we used general fungal primers in combination with primers designed to selectively amplify the DNA from tremellalean fungi (Millanes *et al.* 2011). The primers ITS1F (Gardes & Bruns 1993), BasidLSU3-3 and BasidLSU1-5 (Millanes *et al.* 2011), and LR5 (Vilgalys & Hester 1990) were used to amplify the internal transcribed spacer I, the 5.8 rDNA gene, the internal transcribed spacer II and a fragment of approximately 1000 bp in the nLSU rDNA gene.

PCR amplifications were performed using Illustra™ Hot Start PCR beads, according to the manufacturer's instructions, with the following settings: for the primer pair ITS1F/BasidLSU3-3, we used initial denaturing at 95°C for 3 min, four cycles (95°C for 40 s, 53°C for 40 s and 72°C for 90 s), four cycles (95°C for 30 s, 50°C for 30 s and 72°C for 90 s), and finally 32 cycles (95°C for 30 s, 47°C for 30 s and 72°C for 90 s) with a final extension at 72°C for 480 s. For the primer pair BasidLSU1-5/LR5 we used initial denaturing at 95°C for 3 min, four cycles (95°C for 40 s, 56°C for 40 s and 72°C for 90 s), four cycles (95°C for 30 s, 53°C for 30 s and 72°C for 90 s) and finally 32 cycles (95°C for 30 s, 50°C for 30 s and 72°C for 90 s) with a final extension at 72°C for 420 s. Before sequencing, the PCR products were purified using the PCR-M® Clean-up System of

TABLE 1. *Sequences newly produced (bold) or downloaded from GenBank, with specimen data or culture references.*

Species names	Culture references	Specimen data	ITS	nLSU
Tremella caloplacae		France, *Sérusiaux* s. n. (S-F102489)	JN053469	JN043574
T. candelariellae		Luxembourg, *Diederich* 12808 (S-F102492)	JN053470	JN043575
T. cetrariicola-a		Finland, *Suija* s. n. (S-F102413)	JN053490	JN043596
T. cetrariicola-b		Latvia, 2005, *Suija* s. n. (TU)	JN053491	JN043597
T. cladoniae-a		France, *Diederich* 16031 (S-F102550)	JN053478	JN043584
T. cladoniae-b		Estonia, *Suija* 872 (TU-45019)	JN053477	JN043583
T. coppinsii-a		UK, *Diederich* 15628 (S-F102414)	JN053495	JN043601
T. coppinsii-b		Estonia, *Suija* 38a (TU-38637)	JN053496	JN043602
T. diploschistina-a *		Sweden, *Westberg & Berglund* 09-400 (S)	**JN790586**	**JN790588**
T. diploschistina-b		Sweden, *Westberg* 09-452 (S)	**JN790587**	**JN790590**
T. diploschistina-c		USA, *Rosentreter* 6836 (IMI-365462)	**JN790585**	**JN790589**
T. foliacea		Sweden, *Wiklund* 018 (S-F102409)	JN053502	JN043609
T. hypogymniae-a		Sweden, *Wedin* 6892 (UPS)	JN053484	JN043590
T. hypogymniae-b		Estonia, *Suija* s. n. (TU-39402)	JN053485	JN043591
T. lobariacearum-a		Madeira, *Diederich* 4935 (S-F102418)	JN053473	JN043579
T. lobariacearum-b		Canary Islands, *Diederich* 16468 (S-F102419)	JN053474	JN043580
T. mesenterica-a		Sweden, *Ryman* 9146 (S-F102411)	JN053463	JN043568
T. mesenterica-b		Sweden, *Wedin* 7612 (S-F102412)	JN053464	JN043569
T. phaeophysciae-a		Luxembourg, *Diederich* 12429 (S-F102505)	JN053479	JN043585
T. phaeophysciae-b		Estonia, *Suija* s. n. (TU-55041)	JN053480	JN043586
Outgroup				
Filobasidium floriforme	CBS 6241		AF190007	AF075498
F. uniguttulatum	CBS 1730		AF444302	AF075468

* Type specimen of the new species.

Viogene or the enzymatic method Exo-sap-IT© provided by USB Corporation.

Sequence alignment and phylogenetic analyses

Sequences were aligned using the Q-INS-i algorithm (Katoh & Toh 2008*a*) of the multiple sequence alignment software MAFFT version 6.611 (Katoh *et al.* 2002; Katoh & Toh 2008*b*), following Wedin *et al.* (2009), but aligning sequences in a single step. Major insertions and ambiguous regions were identified and eliminated with Gblocks version 0.91b (Castresana 2000).

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 & Bull 1993) for one marker was contradicted with significant support by another. No incongruence was found and the data were concatenated into a single dataset.

Maximum parsimony and parsimony bootstrap analyses were performed for the combined dataset using PAUP* 4.0b10 (Swofford 2002) with the following settings: gaps were treated as 'missing data', 1000 random addition sequence replicates, TBR branch swapping, steepest descent off, collapse branches if minimum length is 0, MulTrees on. Bootstrap (Felsenstein 1985): heuristic search settings identical to the above analysis but with 10 random addition replicates; bootstrap settings: 1000 bootstrap replicates, full heuristic search, retain groups with frequency >50%. Parsimony-uninformative characters were excluded from these analyses.

Bayesian inference of phylogeny (Huelsenbeck *et al.* 2001) was carried out by Markov Chain Monte Carlo (MCMC) sampling as implemented in the software MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Likelihood models were selected for each of the three gene regions using the Bayesian Information Criterion (BIC) as implemented in jModeltest (Posada 2008). We used full likelihood optimization and selected only among the 24 models implemented in MrBayes. Following this scheme, a SYM+Γ model was selected for the ITS, and a SYM+I+Γ for the nuclear LSU rDNA. The combined analysis treated the two gene regions as separate partitions with topology linked across partitions but separate model parameter values and proportional rates across partitions. The number of discrete gamma categories was kept at default four. Bayesian prior distributions included treating all tree topologies as equally likely, a uniform (0, 50) distribution for the gamma shape parameter, a uniform (0, 1) distribution for the proportion of invariable sites, and a flat (1, 1, 1, 1, 1, 1) Dirichlet for the rate matrix. For the combined dataset, two parallel

runs were performed, each with four chains, three 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.

The Species

Tremella diploschistina Millanes, M. Westb., Wedin & Diederich sp. nov.

MycoBank No: MB563327

Basidiomata lichenicola in thallo *Diploschistis*, gallas superficiales, luteas, atrobrunneas, vel atras, convexas, basim non constrictas 0·3–0·9 mm in diam. efficientia. Hymenium hyphidiis tumidis, elongatis, septatis, ramosis, 3·5–5 µm in diam. Basidia 2-cellularia, septo longitudinali, obliquo vel transversali (13–)14–30 (–34) × 8–14 µm. Basidiosporae 7–9 × (5–)6–9 µm. Conidia ignota.

Typus: Sweden, Uppland, Djurö par., Runmarö, Norestranden NE of Nore, 59°16′43″N, 18°47′47″E, 30 June 2009, *M. Westberg* & *T. Berglund* 09-400 (S—holotypus).

(Figs 1 & 2)

Basidiomata waxy, inducing the formation of galls on the thallus surface (Fig. 2A). Galls pale yellow, dark brown, or black, at first regularly convex to subglobose, 0·3–0·9 mm diam., often forming groups measuring up to 3 mm diam. *Context hyphae* thin-walled, often with clamp connections, 1·5–2·5 µm diam. (Fig. 1s'–v'); haustorial branches frequent, mother cells spherical to subspherical, 3–4 × 3–4 µm, haustorial filament 1 µm diam., up to 8 µm long (Fig. 1o'–r'). *Hymenium* hyaline, containing numerous probasidia; hyphidia present, thick-walled, with numerous septa and ramifications, 3·5–5·0 µm diam. (Fig. 1g'–n'), sometimes swollen with thin walls, then up to 6 µm diam. (n = 11). Probasidial initials clavate, proliferations occurring through the basal clamp (Fig. 1a & b). *Basidia*, when mature, 2-celled, with one transverse, oblique or longitudinal septum. The three types of basidium septation are often found within the same gall. When transverse, constricted at the septum, the lower cell with an attenuated stalk-like base, often longer than the upper cell, (13–)14–30(–34) × 8–14 µm (incl. stalk-like base; excl. epibasidia); lower part of the stalk-like base 2–4 µm diam.; epibasidia subcylindrical, at least up to 30 µm long, 2–4 µm diam. (Figs 1c–x and 2B–D). *Basidiospores* ellipsoid to subspherical, *c.* 7–9 × (5–)6–9 µm (n = 21) with a distinct apiculus, *c.* 1 µm diam. (Figs 1a'–f' & 2F).

Anamorph not observed.

Hosts. On the thallus of *Diploschistes*. Swedish samples grew on *Diploschistes scruposus* and the USA sample grew on *D. muscorum*.

Distribution and ecology. Known from northern Europe (Sweden) where it grows on exposed siliceous rocks, predominantly lake- or seashore rocks, but also in forested areas, and from North America (USA, Idaho) where it occurs in an *Artemisia tridentata* and *Agropyron spicatum* habitat on sandy loam soil.

Additional specimens examined. **Sweden:** *Dalsland*: Skållerud par., Lake Östebosjön, W side and near S tip of island Hinnön, 58°49′4″N, 12°28′51″E, 2009, *M. Westberg* 09-452 (S). *Uppland*: Djurö par., Runmarö, Norestranden NE of Nore, 59°16′43″N, 18°47′48″E, 14 v 2010, *M. Westberg* (hb. Diederich). *Södermanland*: Nacka par., SE part of Nyckelviken Nature Reserve, 59°19′6″N, 18°11′38″E, 14 v 2011, *M. Westberg* (S).— **USA:** *Idaho*: S of Emett, Old Freeze Out Hill, north facing hillside, T6N R1W, 1991, *R. Rosentreter* 6836 (IMI 35641, hb. Rosentreter).

Phylogenetic Results

We generated 6 new sequences (3 ITS and 3 nLSU rDNA), which were aligned together with sequences already available in GenBank (Table 1). Two data matrices were produced, one including ITS and one including nLSU rDNA. The three ITS sequences of *Tremella diploschistina* differed in two nucleotides among them, and 10 ITS positions differed from the rest of ITS *Tremella* sequences studied. Only 1 position differed among the three *T. diploschistina* nLSU sequences, and 16 positions in the nLSU were different from the other *Tremella* species studied.

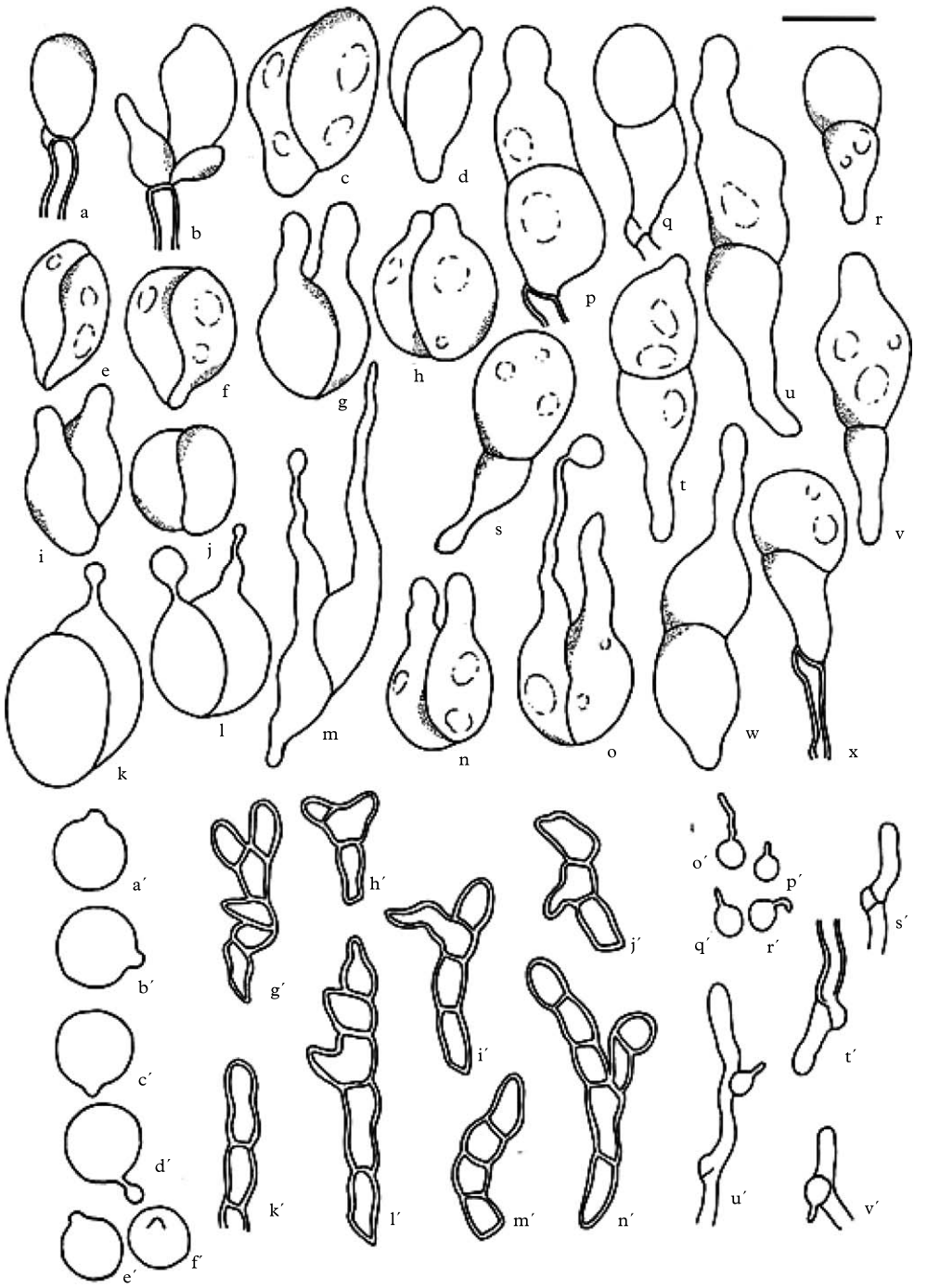

FIG. 1. *Tremella diploschistina*. a–x, basidia; a'–f', basidiospores; g'–n', hyphidia; o'–r', haustorial branches; s' & t', hyphae with clamps; u' & v', hyphae with haustorial cells; a, d, l–m, s, v–w, e'–f', i'–j', m'–n', and o'–r' (IMI 35641); j–k, n–q, t–u, e'–f', i'–j', m'–n' (*M. Westberg* 09-452 (S)); b–c, e–i, r, x, a'–b', k'–l' and s' (holotype). Scale = 10 μm.

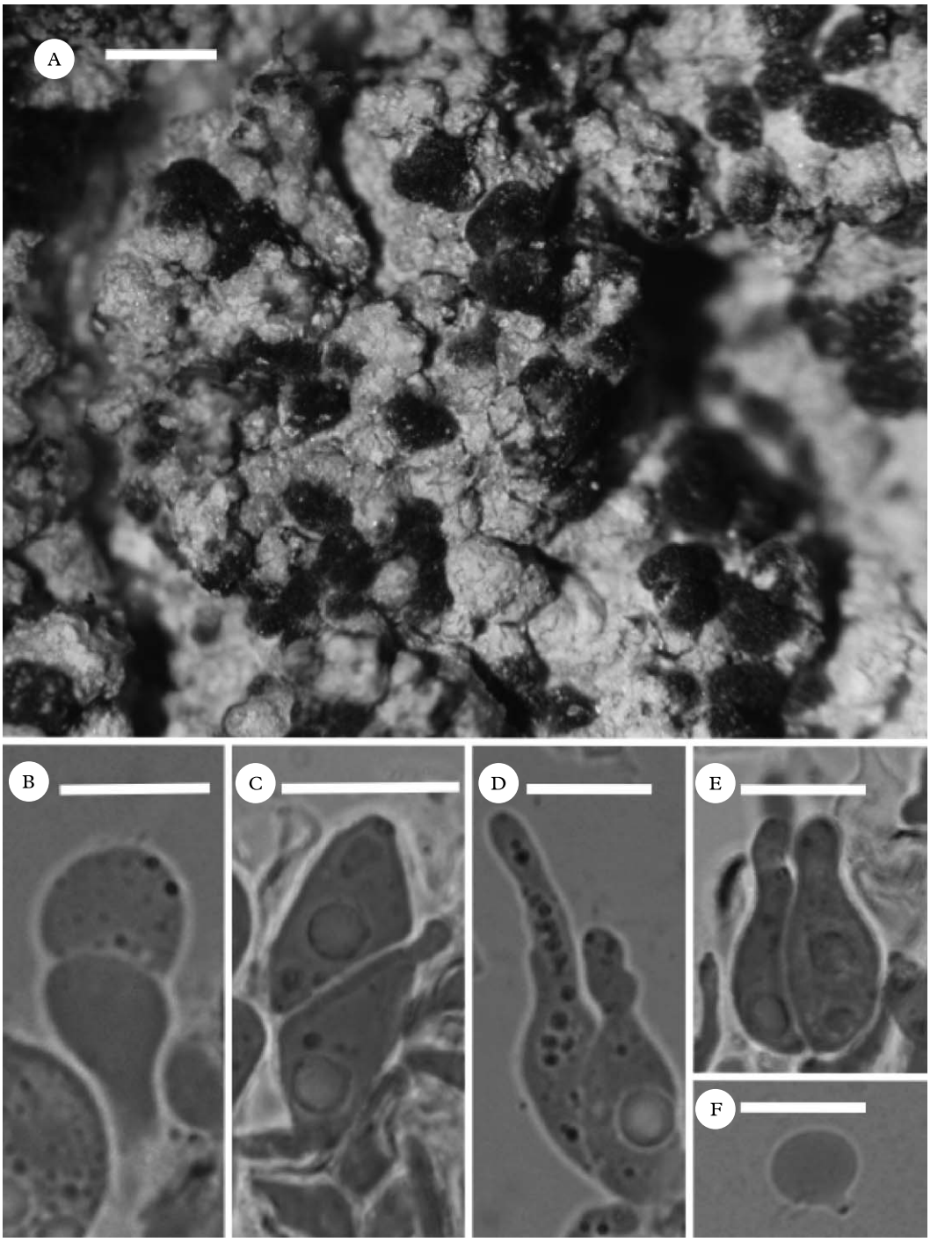

FIG. 2. *Tremella diploschistina*. A, habit; B–E, basidia showing different septation pattern; F, basidiospore. A & B (holotype); C & D (IMI 35641); F (*M. Westberg* 09-452 (S)). Scales: A = 1 mm; B–F = 10 μm.

The combined matrix contained 1212 characters (ITS: 1-300; nLSU: 301-1212), from which 348 unambiguously aligned parsimony informative sites were used in the parsimony analysis. Our maximum parsimony analysis resulted in 2 most parsimonious trees of 694 steps, with CI = 0·622 and RI = 0·773.

When the Bayesian analysis halted after 200 000 generations, the average standard deviation of split frequencies across runs was 0·004, which indicates that the two runs have converged (<0·01). A majority rule consensus tree was constructed from the 2000 trees of the stationary tree sample.

The three specimens of *T. diploschistina* formed a single clade, supported both by parsimony bootstrap (100%) and Bayesian Posterior Probabilities (1.0) (Fig. 3). The *Tremella* species included in our analysis also formed a supported clade (parsimony bootstrap = 100% and BPP = 1·0), including the clade formed by the three specimens of *T. diploschistina*.

Discussion

Tremella diploschistina is distinguished from other known lichenicolous species growing on the host thallus with two-celled basidia with variable septation pattern, by the bigger size of basidia and basidiospores, the presence of hyphidia in the hymenium, and the different host-selection (Table 2). Two other species, *T. psoromicola* and *T. stictae*, form ramified hyphidia similar to those found in *T. diploschistina*, although the hyphidial walls are thicker in the latter species. Also, these two species grow on hosts of *Peltigerales*, and basidia of *T. psoromicola* do not show longitudinal septa. However, *T. psoromicola* was described from a single specimen, and we do not discard the possibility that longitudinal septa might be observed in additional collections. Other morphological traits such as basidium size and basidiospore size are within the ranges of *T. diploschistina*, suggesting a possible relationship to both species. *Tremella stictae*, however, has smaller basidia and basidiospores, and an asteroconidia-producing anamorph has been observed in this species, which is not known in *T. diploschistina*.

As species of *Diploschistes* are widely distributed and common, *T. diploschistina* is probably much more common and widespread than currently known. The genetic similitude between distantly collected specimens from the USA and Sweden, growing on different *Diploschistes* species (Fig. 3), is remarkable. Together with the morphological results, our molecular data further support the establishment of *T. diploschistina* as a well-delimited species, which appears clearly nested within *Tremella* in our molecular study, although the phylogenetic relationship with other *Tremella* species is not supported (Fig. 3). Millanes *et al.* (2011) suggested that species in the *Foliacea* group might represent a distinct genus, if *Tremella* should be split in different taxa, but our new species is clearly not closely related to *T. foliacea* (Fig. 3). Preliminary analyses including *T. diploschistina* in the general phylogeny of the *Tremellales* did not support the phylogenetic relationship of the new species with any other taxa in the group (analysis based on the matrix from Millanes *et al.* (2011); data not shown here).

Only two other lichenicolous *Tremella* species have been described so far on species of *Graphidaceae sensu* Mangold *et al.* (2008) and Rivas-Plata & Lumbsch (2011), viz., *T. phaeographidis* and *T. phaeographinae* (Diederich 1996). However, basidia of *T. phaeographidis* do not show transverse septa, basidiomata are flattened, pale to dark brown or reddish brown, basidia and basidiospores are smaller, and asteroconidia have been observed. *Tremella phaeographinae* forms basidiomata only in the hymenium of the host, which becomes reddish at maturity, the basidia can be 3-celled, the basidiospores are smaller, and blastoconidia have been observed. It would be interesting to test in the future whether, despite their morphological differences, the three *Tremella species* growing on *Graphidaceae*, (i.e. *T. diploschistina*, *T. phaeographinae* and *T. phaeographidis*) could be closely related. Millanes *et al.* (2011) found that several lichenicolous species

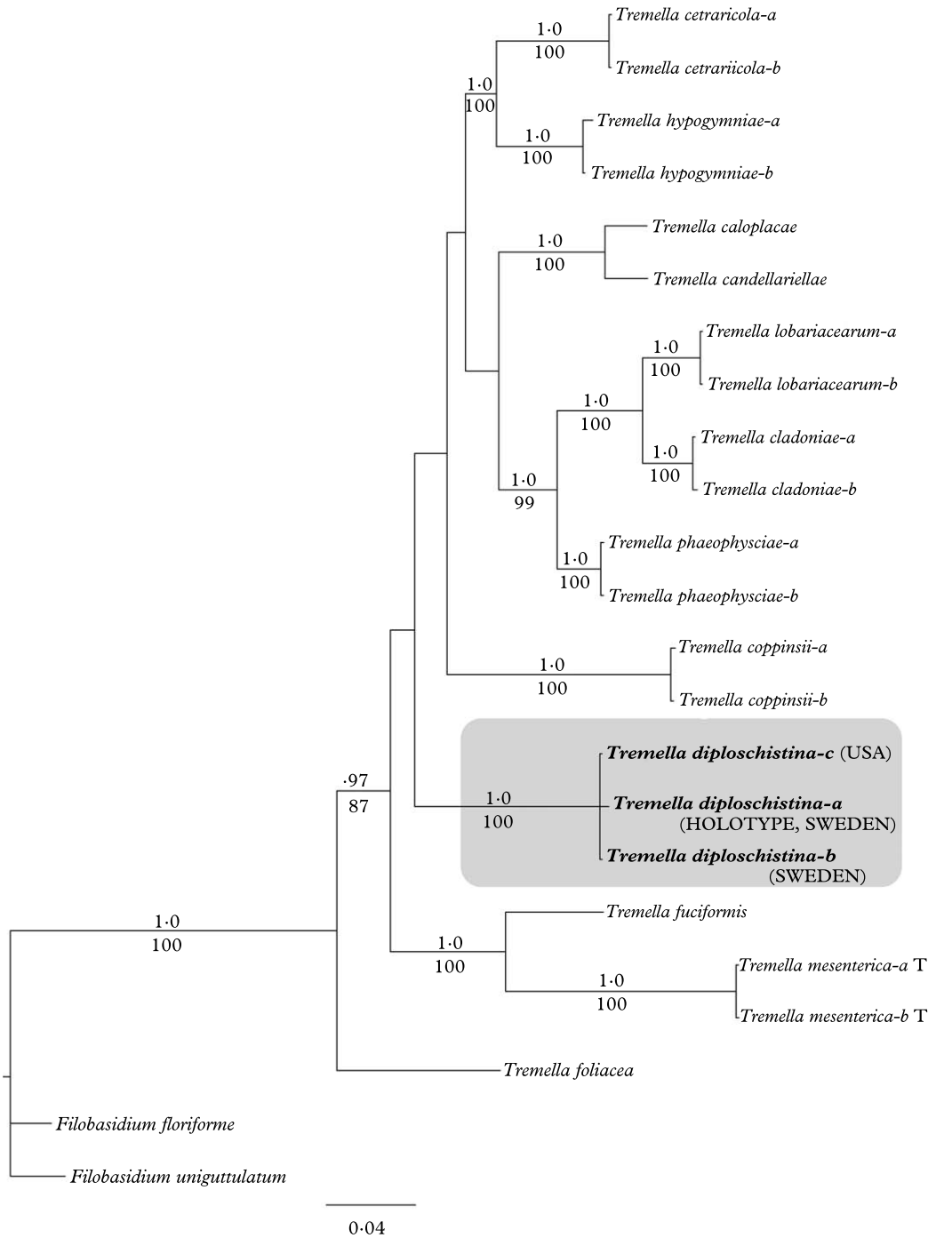

FIG. 3. Fifty per cent majority rule Bayesian consensus tree with average branch lengths from the combined analyses of ITS and nSSU datasets. PP values $\geq$ 0·95, obtained in the Bayesian analysis, are indicated over the branches, and bootstrap values $\geq$70%, obtained in the parsimony analysis, below the branches. Branch lengths are scaled to the expected number of nucleotide substitutions per site. A grey box encloses the clade containing the new species.

TABLE 2. *Morphological and anatomical characters of* Tremella diploschistina, *compared to morphological characters of other* Tremella *species growing on the thallus of the host, and also bearing two celled basidia with variable septation pattern, and to morphological characters of other* Tremella *species growing on* Graphidaceae. *Data of all species except* T. diploschistina *are taken from Diederich (1996, 2007).*

Species	Basidium septation and morphology	Basidium size (mm)	Basidium stalk	Basidiospores size (mm)	Clamps	Galls	Anamorph	Hyphidia	Hosts
T. diploschistina	one longitudinal, oblique or transverse septum	(13–)14–30 (–34)×8–14	yes	6–9×7–10	present	on host thallus; pale yellow, dark brown to black	unknown	present	*Diploschistes muscorum* and *D. scruposus*
T. christiansenii	one longitudinal (exceptionally transverse) septum; cells elongated at maturity	10–18 in diam.; near the septum 10–17 long; elongated cells up to 30 long, 4–11 in diam.	no	9–12×8·5–10·5	not observed	on host thallus; brown to dark brown	unknown	absent	*Physcia stellaris* and *P. tenella*
T. hypocenomycis	one longitudinal (exceptionally transverse) septum; cells elongated at maturity	10–17(–20) in diam.; near the septum 8–14 long; elongated cells up to 24 long, 3·5–10·0 diam.	no	6·5–5·5×5·5–6·5	not observed	on host thallus; dark brown to black	unknown	absent	*Hypocenomyce scalaris*
T. hypogymniae	one longitudinal, oblique or transverse septum	11–16(–20)× 7–12	no	7–10×5·5–7	present	on host thallus; pale to pinkish.	present	absent	*Hypogymnia physodes*
T. lobariacearum	one oblique, rarely longitudinal or transverse septum	14–23×7–11	no	6–10×5–7·5	present	on isidia, soredia, rarely margin or lower surface of the host; pale brown to dark brown or blackish	lunate conidia and asteroconidia	absent	*Lobaria* spp. and *Pseudocyphellaria* spp.
T. macroceratis	one longitudinal, rarely oblique or transverse septum	8·5–12×7–8·5 (when oblique or transverse septum, up to 14 long)	no	5·5–7·5(–8)×(3·5–)4–5·5	present	on host thallus; pale to dark reddish brown	unknown	absent	*Cladonia macroceras*
T. montis-wilhelmii	one transverse, oblique or rarely longitudinal septum	12–17(–22)× (6–)7–9	no	6–7×5–6	not observed	on host thallus; reddish brown	unknown	absent	*Normandina simodense*
T. nephromatis	one longitudinal, oblique or transverse septum	11–20×7·5–10	no	5·5–8×5–6(–7·5)	not observed	on soredia of the host; dark reddish brown	unknown	absent	*Nephroma parile*
T. normandinae	one transverse, oblique or longitudinal septum	(12–)14·5–21 (–24)×8·5–11·5	no	6·5–8·5×6–7	not observed	on host thallus; pale or pinkish brown	unknown	present	*Normandina pulchella*

TABLE 2. *Continued*

Species	Basidium septation and morphology	Basidium size (mm)	Basidium stalk	Basidiospores size (mm)	Clamps	Galls	Anamorph	Hyphidia	Hosts
T. parmeliellae	one transverse, oblique or rarely almost longitudinal septum	14–22×5–9	yes	5–8×4–6	present	on host thallus; pale brownish to black	unknown	absent or rare	*Parmeliella foliicola*
T. phaeographidis	one transverse or oblique septum	16–24×8–12	yes	5·5–7·5×5–6	not observed	on host thallus; pale to dark brown, often reddish brown	asteroconidia	absent	*Phaeographis* spp.
T. phaeographinae	one transverse septum; the lower part sometimes with an additional oblique septum and the upper part often with an additional longitudinal septum.	22–32×9·5–11·0	yes	5·5–7·5×5·0–6·5	present	in the hymenium of the host; first reduced, later superficial reddish to orange- brown	blastoconidia	present	*Phaeographina* sp.
T. psoromicola	one transverse or slightly oblique septum	17–24×8·5–11·5	yes	7–9×6·5–8·0	present	on host thallus; reddish brown	unknown	present (morphology similar to *T. diploschistina*)	*Psoroma* sp.
T. santessonii	one transverse, rarely oblique or longitudinal septum	16–21×8–9	yes	6·5–8·0×5·5–7·0	present	on host thallus; reddish brown to almost black	unknown	absent	*Usnea* spp.
T. stictae	one longitudinal, oblique or transverse septum	10–16×6·0–8·5	no	5·0–7·5×4·0–5·5	present	on margin of host thallus, rarely on lower surface, mainly on isidia; pale brownish to dark brown	asteroconidia	present (morphology similar to *T. diploschistina*)	*Sticta* spp. and *Dendriscocaulon* sp.
T. tuckerae	one longitudinal or rarely oblique or almost transverse septum; cells elongated at maturity	10·5–15·5 in diam.; near the septum 12–17 long; elongated cells up to 30 long, 4·5–7 in diam.	no	(5·5–)7·5–9 (–11)×(4–)6·5–8	present	on host thallus; pale brown to blackish	unknown	absent	*Ramalina sinensis* and *R. cuspidata*
Tremella sp. 5	one transverse, oblique or longitudinal septum	15–19×10–15 (not including stalk)	yes	7×7	not observed	on host thallus; blackish	unknown	absent	*Anaptychia ciliaris*

growing on *Parmeliaceae* (*Biatoropsis usnearum*, *T. cetrariicola*, *T. coppinsii*, *T. everniae*, and *T. hypogymniae*) were all nested within the same monophyletic group, although their micro- and macromorphology were clearly divergent.

It is interesting that *Diploschistes* is the only genus of the '*Thelotrema* clade' of the *Graphidaceae* with a trebouxioid photobiont. Moreover, thalli of most *Diploschistes* species contain orcinol depsides, differing from most thelotremoid taxa, which usually have β-orcinol depsidones (Mangold *et al.* 2009). Little if anything, however, is known on the factors influencing the high host-specificity observed in the lichenicolous *Tremella* species, and it has not been investigated whether the secondary lichen compounds could be involved in this specificity. Also, no kind of interaction between lichenicolous *Tremella* species and the photobiont of their lichenized host has ever been reported, and the interaction is considered to be exclusively mycoparasitic (Grube & de los Ríos 2001). Unfortunately, we could not amplify any DNA from the two *Tremella* species growing on other *Graphidaceae*, in order to test their possible relationship with *T. diploschistina*. Moreover, not all lichenicolous *Tremella* species described have been sequenced yet, and therefore future molecular studies adding more lichenicolous representatives might reveal the phylogenetic relationships of *T. diploschistina* with other lichen-inhabiting taxa in the *Tremellales*.

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