Tremella imshaugiae and *T. tubulosae* (Tremellomycetes, Basidiomycota), two new lichenicolous fungi on *Imshaugia aleurites* and *Hypogymnia tubulosa*

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Abstract. The new *Tremella imshaugiae*, lichenicolous on *Imshaugia aleurites*, is described from Scotland and Maine (USA); it is characterized by small, 0.1–1 mm diam., amber coloured basidiomata not inducing gall-formation, 2–4-celled, mainly longitudinally septate basidia, 15.5–21.5 × 13–16.5 µm, and relatively large, subspherical basidiospores, 6.5–9 × 6.5–8.5 µm; it is sister to *T. diploschistina* in a phylogeny using ITS and nLSU markers. The new *Tremella tubulosae*, lichenicolous on *Hypogymnia tubulosa*, is described from Scotland and Spain; it is characterized by pale to dark brown or blackish basidiomatal galls, 0.2–1.8 mm diam., 2-celled, mainly longitudinally septate basidia, 10–17 × 8–11 µm, and medium-sized, subspherical to ellipsoid basidiospores, 6.5–8 × 5–6.5 µm; it is sister to *T. hypogymniae* in a phylogeny using ITS and nLSU markers.

1. Introduction

The genus *Tremella* Pers. includes over 200 mycoparasitic species, many being strictly host-specific. Diederich (1996) revised the lichenicolous taxa and accepted 46 species, amongst which six were left unnamed. More lichenicolous species have been described in subsequent papers, such as Diederich (2007) or Zamora et al. (2016), and a total number of 60 lichen-inhabiting species were accepted by Diederich et al. (2018). A first phylogenetic study of the lichenicolous species was presented by Millanes et al. (2011).

In this paper we will describe two new lichen-inhabiting species of *Tremella*, one developing amber-coloured basidiomata on the thallus of *Imshaugia aleurites*, and the other inducing conspicuous galls on the thallus of *Hypogymnia tubulosa*. Both hosts are members of the Parmeliaceae.

2. Material and methods

2.1. Molecular studies

In addition to the specimens studied, 34 specimens representing 27 species in the Tremellales were included in the molecular study (Table 1). The sampling included (1) lichenicolous species growing on Parmeliaceae or closely related to the new species in preliminary phylogenetic analyses and

(2) a representation of the main filamentous and yeast families in the Tremellales, including the groups of lichenicolous *Tremella* species distinguished by Millanes et al. (2011). *Phaeotremella foliacea* was used as outgroup following Liu et al. (2016). *Phaeotremella* is the sister group to the rest of Tremellales, and our preliminary analyses showed that the new species were nested in *Tremella*, but not closely related to this genus. Species names, voucher information, and GenBank accession numbers are given in Table 1.

DNA extraction and PCR amplifications were achieved following protocols in Millanes et al. (2012).

Alignments and Bayesian analyses were conducted following Diederich et al. (2019). Likelihood models were selected for each of the four partitions with the corrected Akaike information criterion (AICc) as implemented in jModeltest (Posada 2008). A GTR+Γ model was selected for the ITS1, a K80+I for the 5.8S, a K80 +Γ for the ITS2, and finally a GTR+I+ Γ for the nLSU rDNA. Maximum likelihood analyses were achieved in IQ Tree v. 1.6.12 (Nguyen et al. 2015), using the same partitions and model selection than in the Bayesian analysis. We used edge-linked branch lengths between partitions but separate models between partitions (Chernomor et al. 2016). We assessed node support using standard non-parametric bootstrap with 1000 replicates. Significant support is considered as Bayesian posterior probability (BPP) values ≥ 0.95 in the Bayesian analysis, and bootstrap values ≥ 75% in the ML analysis.

2.2. Morphological examination

Dry herbarium specimens were examined and measured under a binocular microscope Leica MZ 7.5. Macroscopic photographs were done using a Canon 40D camera with a Canon MP-E 65 mm lens or a Nikon BD Plan 10× microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Hand-made sections of basidiomata were studied in a mixture of Phloxine B and 5% KOH. Microscopic photographs were prepared using a Leica DMLB microscope with DIC optics and a Leica EC3 camera. Measurements of basidia and basidiospores are indicated as (min.–)$\overline{X}$-σ_x–$\overline{X}$+σ_x (–max.), followed by the number of measurements (n); the ratio length/width of basidia and basidiospores is indicated as l/w and given in the same way; basidial length designates the distance from the basidial base to the top, but excluding the epibasidia; basidiospores width is measured without the apiculus; in both cases, the 'length' may be shorter than the 'width'; in the diagnoses, measurements are rounded to the nearest 0.5 µm.

3. Results

3.1. Phylogenetic results

We generated 12 new sequences that were aligned together with sequences already available in GenBank (Table 1). The combined matrix contained 1269 characters (ITS1: 1–81; 5.8S: 82–234; ITS2: 235–362; nLSU: 363–1269). BI was halted after 2 300 000 generations, at which time the average standard deviation of split frequencies across runs was below 0.01, which indicates that the three runs had converged. Moreover, the potential scale reduction factor (PSRF) for all models and parameters was below 1.002. A majority rule consensus tree was constructed from the 11500 trees of the stationary tree sample from the Bayesian analysis. The best tree obtained from the ML analyses had a log-likelihood value of -7591.9780. No incongruence was found between the Bayesian and ML trees. Therefore only the Bayesian tree is shown in Fig. 1.

The new species *Tremella tubulosae* is the sister taxon to *T. hypogymniae* Diederich & M. S. Christ. and forms a clade together with other lichenicolous taxa growing on Parmeliaceae, called "Clade III" in Millanes et al. (2011) (Fig. 1). The new species *Tremella imshaugiae* is the sister taxon to *Tremella diploschistina* Millanes, M. Westb., Wedin & Diederich.

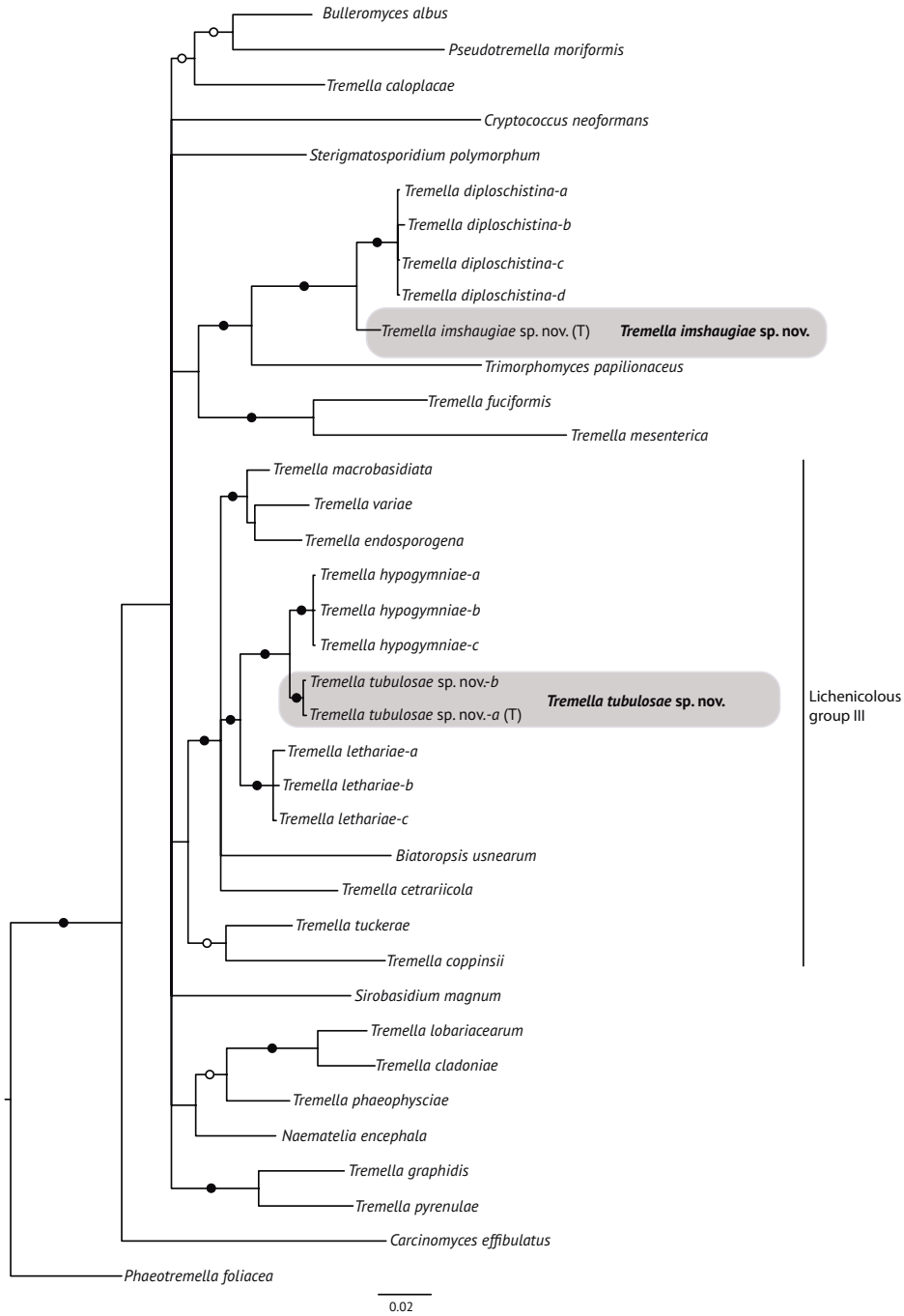


Fig. 1. Fifty per cent majority rule Bayesian consensus tree with average branch lengths from the combined analyses of ITS and nLSU datasets. White dots indicate PP values ≥ 0.95, obtained in the Bayesian analysis. Black dots indicate PP values ≥ 0.95, and bootstrap values ≥ 75%. Branch lengths are scaled to the expected number of nucleotide substitutions per site. The new species *Tremella tubulosae and T. imshaugiae* are enclosed in grey boxes. Type specimens of the new species are indicated with (T). The clade corresponding to "Clade III" in Millanes et al. (2011) is indicated to the right.

Table 1. Sequences newly produced (bold), and sequences downloaded from GenBank, with specimen data or culture references given. Type specimens or strains are indicated with (T).

Species names	Culture	Specimen data	ITS	nLSU
Biatoropsis usnearum		Sweden, Westberg 09-676 (S F264681)	KJ404876	KJ437221
Bulleromyces albus (T)	CBS 501		KY101819	KY106261
Carcinomyces effibulatus (T)		Sweden, Santos (S F40014)	AF444315	AF189842
Cryptococcus neoformans (T)	B-3501A		BR000310	BR000310
Sterigmatosporidium polymorphum (T)	CBS 8088/ IGC 5647		AF444320	AY032662
Naematelia encephala		Sweden, Hjortsberg 500 (S F102416)	JN053481	JN043587
Pseudotremella moriformis (T)	CBS 7810		KY104686	AF075493
Sirobasidium magnum	CBS 8485		JN053497	JN043603
Tremella caloplacae		France, Sérusiaux (S F102489)	JN053469	JN043574
Tremella cetrariicola		Finland, Suija (S F102413)	JN053490	JN043596
Tremella cladoniae		Estonia, Suija 872 (TU 45019)	JN053477	JN043583
Tremella coppinsii		Estonia, Suija 38a (TU 38637)	JN053496	JN043602
Tremella diploschistina-a (T)		Sweden, Westberg 09-400 (S F211875)	JN790586	JN790588
Tremella diploschistina-b		Sweden, Westberg 09-452 (S F211901)	JN790587	JN790590
Tremella diploschistina-c		Sweden, Westberg (S F211910)	MN258553	MN243148
Tremella diploschistina-d		USA, Rosentreter 6836 (IMI 365462)	JN790585	JN790589
Tremella endosporogena (T)		Spain, Zamora (MAF Lich. 19742)	KT334579	KT334591
Tremella fuciformis	CBS 6970		JN053466	JN043571
Tremella graphidis (T)		USA, Common 9434B1 (BR)	MN258557	MN243152
Tremella hypogymniae-a		Sweden, Millanes (S)	**MW115406**	**MW115388**
Tremella hypogymniae-b		Estonia, Suija (TU 39402)	JN053485	JN043591
Tremella hypogymniae-c		Spain, Millanes 464 (S)	**MW115407**	**MW115389**
***Tremella imshaugiae* (T)**		UK, Paul (E 00722344)	**MW115411**	**MW115393**
Tremella lethariae-a		USA, 2014, Spribille	MG774368	-
Tremella lethariae-b		USA, 2014, Spribille	MG774384	-
Tremella lethariae-c		USA, 2010, Miller (E)	**MW115410**	**MW115392**
Tremella lobariacearum (T)		Madeira, Diederich 4935 (S-F102418)	JN053473	JN043579
Tremella macrobasidiata		Portugal, Zamora (MAF Lich. 19755)	KT334583	KT334595
Tremella mesenterica		Sweden, Ryman 9146 (S F102411)	JN053463	JN043568
Tremella phaeophysciae		Luxemb., Diederich 12429 (S F102505)	JN053479	JN043585
Tremella pyrenulae (T)		USA, Common, 9170B (S, isotype)	KR058784	KR058789
***Tremella tubulosae-a* (T)**		UK, Coppins 22916 (E 00722365)	**MW115409**	**MW115391**
Tremella tubulosae-b		Spain, Zamora **(S)**	**MW115408**	**MW115390**
Tremella tuckerae (T)		Mexico, Tucker 37335, SBBG	JN053482	JN043588
Tremella variae (T)		Spain, Pérez-Ortega (MAF Lich. 19748)	KT334587	KT334599
Trimorphomyces papilionaceus	CBS 443.92		AF444483	AF075491
Outgroup				
Phaeotremella foliacea		Sweden, Wiklund 018 (S F102409)	JN053502	JN043609

3.2. Taxonomy

Tremella imshaugiae Diederich, Coppins, R. C. Harris, Millanes & Wedin, sp. nov. (Fig. 2)

MycoBank MB837687

Lichenicolous on the thallus of *Imshaugia aleurites*; characterized by small, 0.1–1 mm diam., amber coloured basidiomata not inducing gall-formation, 2–4-celled, mainly longitudinally septate basidia, 15.5–21.5 × 13–16.5 µm, and relatively large, subspherical basidiospores, 6.5–9 × 6.5–8.5 µm.

Type: UK, Scotland, VC 96, Easterness, Glen Feshie, Allt Fhearnagan, 27(NN)/858 971, 410 m, on *Pinus* twig, on *Imshaugia aleurites*, 3 xi 2013, H. Paul (E 00722344 – holotype).

Basidiomata not gall-inducing, amber coloured, convex, gelatinous, often fusing, 0.1–1 mm diam. *Context hyphae* thin-walled, often with clamp connections, 2–3 µm diam.; haustorial branches present, mother cells subspherical, 2.5–4.5 × 2.5–4 µm, haustorial filament 1–1.5 µm diam. *Hymenium* hyaline, containing numerous probasidia; hyphidia not observed; probasidial initials clavate, proliferations occurring through the basal clamp; fertile hyphae 2.5–5 µm thick. *Basidia*, when mature, 2–4-celled, with longitudinal, rarely oblique septa, slightly constricted at the septa, not stalked, (13.5–)15.7–21.3(–28.0) × (11.3–)12.9–16.5(–20.3) µm, ratio l/w (0.9–)1.0–1.5(–2.0) (n=45); epibasidia subcylindrical, up to at least 32 µm long, 4–7 µm diam. *Basidiospores* subspherical, with a distinct apiculus, (5.7–)6.6–9.0(–10.0) × (5.8–)6.4–8.6(–9.5) µm, ratio l/w (0.9–)0.9–1.1(–1.2) (n=33). *Anamorph* not observed.

Distribution and host. Known from two Scottish localities and from Maine, USA, always on the visibly undamaged thallus of *Imshaugia aleurites*.

Discussion. *Tremella diploschistina*, the sister species in our phylogeny (Table 1), differs by 2-celled basidia and a hymenium containing numerous thick-walled hyphidia (Millanes et al. 2012). Amongst the known lichenicolous *Tremella* species with 2–4-celled, non-stalked basidia with distinct, not gall-inducing, amber coloured basidiomata developing over the host thallus, *T. graphidastrae* Diederich is distinguished by much smaller basidiospores, 5.5–7 × 3.5–5 µm, and smaller basidia, 14.5–17 × 11–12.5 µm; *T. wirthii* Diederich is distinguished by pale to dark greyish brown basidiomata; *T. haematommatis* Diederich has much larger basidia, 15–36 × 11–20 µm; *T. coppinsii* Diederich & Marson is distinguished by reddish ascomata, much smaller basidia, 8–13 × 5–12 µm, and ellipsoid basidiospores, 6–10 × 4–6.5 µm (Diederich 1996). *Tremella nashii* Diederich has pale to dark brown basidiomata and smaller basidiospores, 5–8 × 4–6 µm (Diederich 2007).

Additional specimens examined (all on *Imshaugia aleurites*): **UK**: Scotland, VC 92, South Aberdeen, E of Braemar, Creag Clunie & Lion's Face SSSI, E side of Creag Clunie, valley of Allt na Claise Moire, 37/18.90, 350 m, 9 iv 1999, Coppins 18234 (E 00722368). – **USA**: Maine, Washington Co., Town of Beals, Great Wass Island, Great Wass Island Preserve, 44°28'51" N, 67°35'41" W, coastal conifer forest, 29 vi 2012, Harris 57529 (NY 1595942).

Tremella tubulosae Diederich, Coppins, J. C. Zamora, Millanes & Wedin, sp. nov. (Fig. 3)

MycoBank MB837688

Lichenicolous on the thallus of *Hypogymnia tubulosa*, gall-inducing; characterized by pale to dark brown or blackish basidiomatal galls, 0.2–1.8 mm diam., 2-celled, mainly longitudinally septate basidia, 10–17 × 8–11 µm, and medium-sized, subspherical to ellipsoid basidiospores, 6.5–8 × 5–6.5 µm.

Type: UK, Scotland, VC 92, South Aberdeen, Glen Fenzie, 38(NJ) 318 030, on *Juniperus*, on *Hypogymnia tubulosa*, 1 iv 2008, B. J. Coppins 22916 & C. J. Ellis (E 00722365 – holotype).

Basidiomata inducing the formation of distinct, convex galls on the host thallus, base not constricted, pale to dark brown or blackish, matt, 0.2–1.8 mm diam. *Context hyphae* thin-walled, 2–3 µm diam., clamp connections not observed; haustorial branches present, mother cells subspherical, c. 5 × 4.5 µm, haustorial filament 1–1.5 µm diam. *Hymenium* hyaline, containing numerous probasidia; hyphidia not observed; probasidial initials clavate, proliferations occurring through the basal clamp; fertile hyphae 2–5 µm thick. *Basidia*, when mature, 2-celled, with one longitudinal, rarely oblique or transverse septum, slightly constricted at the septum, not or slightly stalked, (6.9–)10.0–16.9(–23.0) × (7.0–)7.8–10.8(–14.7) µm, ratio l/w (0.8–)1.0–1.9(–2.6) (n=28), rarely with an attenuated stalk-like base; epibasidia subcylindrical, up to at least 40 µm long, 2.5–4.5 µm diam. *Basidiospores* subspherical to ellipsoid, with a distinct apiculus, (5.8–)6.6–7.8(–8.3) × (4.9–)5.2–6.6(–7.9) µm, ratio l/w (0.9–)1.1–1.4(–1.6) (n=35). *Anamorph* not observed.

Distribution and host. Known from three localities in Scotland and one in Spain, always on the thallus of *Hypogymnia tubulosa*, gall-inducing.

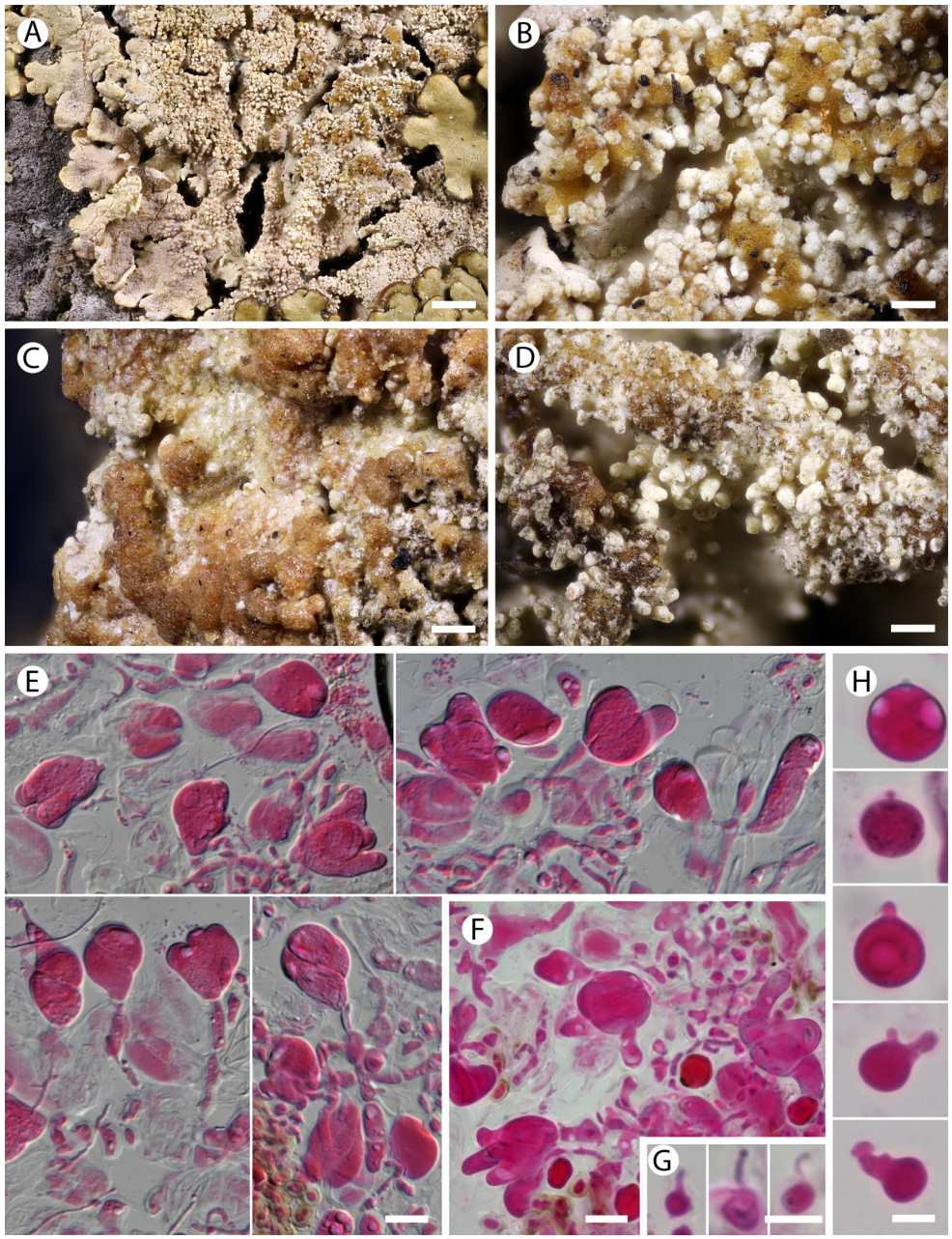


Fig. 2. *Tremella imshaugiae* (A–B, Coppins 18234; C, E–H, holotypus; D, Harris 57529; E–H in a mixture of Phloxine B and 5% KOH). A, Thallus of *Imshaugia aleurites*, with numerous small, amber coloured basidiomata in the upper right part. B–D, Basidiomata at a higher magnification. E–F, Hymenium with basidia. G, Haustorial branches. H, Basidiospores, the two lower ones germinating. Scale bars: A = 1 mm, B–D = 200 µm, E–F = 10 µm, G–H = 5 µm.

Discussion. The new species is phylogenetically distinct and sister to *Tremella hypogymniae*, known from *Hypogymnia physodes* (thallus); that species is distinguished by pale brown or orange to pinkish basidiomatal galls, becoming blackish only in the presence of other lichenicolous fungi, and by slightly larger basidiospores, 7–10 × 5.5–7 µm (Diederich 1996). *Tremella papuana* Diederich, described from *Hypogymnia pseudobitteri-*

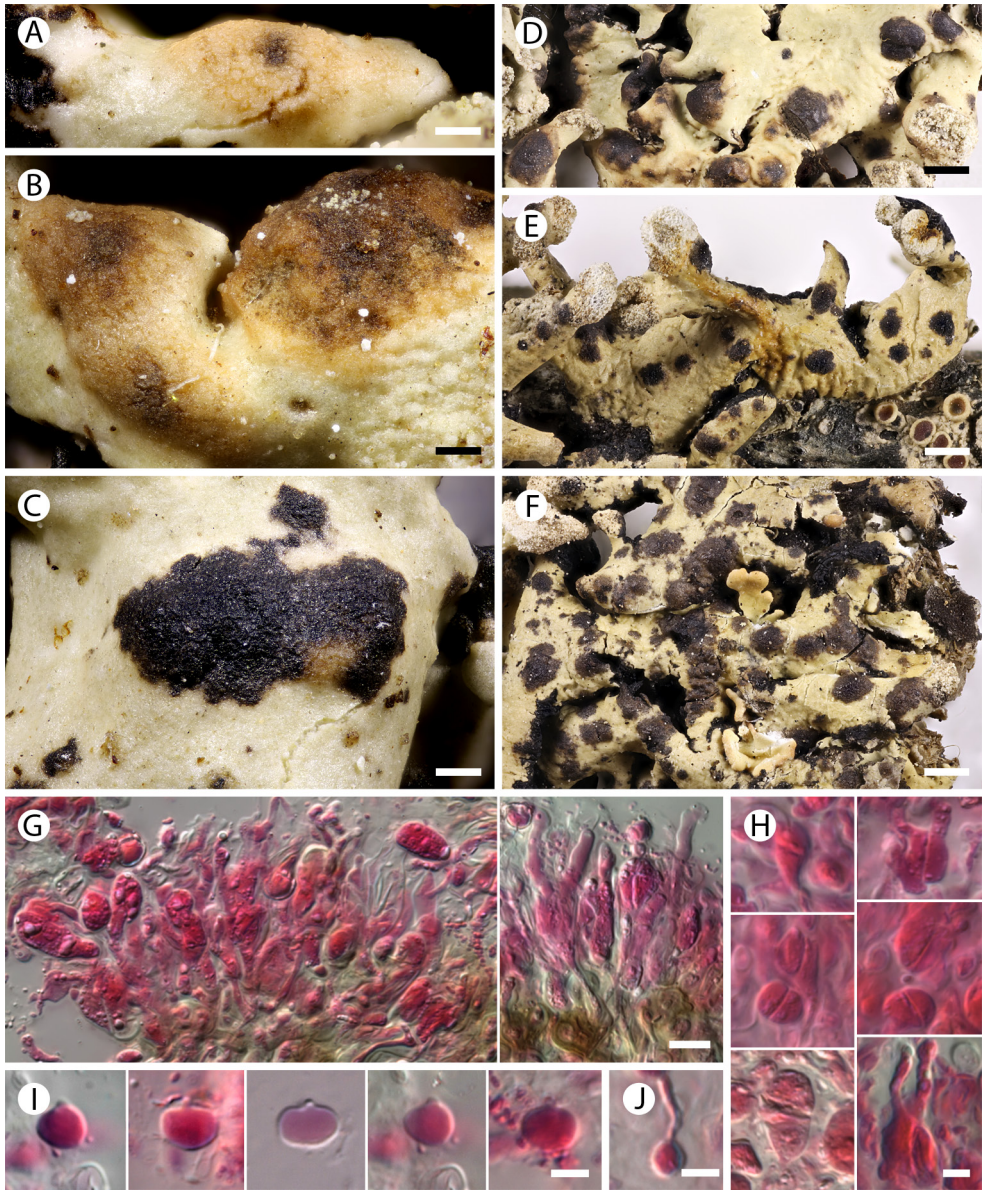


Fig. 3. *Tremella tubulosae* (A–D, G–J, holotypus; E, Coppins 18843; F, Coppins 18906; G–J in a mixture of Phloxine B and 5% KOH). A–C, Development of basidiomatal galls on the thallus of *Hypogymnia tubulosa*. D–F, Mature basidiomata. G, Hymenium with basidia. H, Basidia. I, Basidiospores. J, Haustorial branch. Scale bars: A–C = 200 µm, D–F = 1 mm, G = 10 µm, H–J = 5 µm.

ana, has basidiomata constantly darker in the centre, usually smaller, 0.2–0.5 mm diam., and frequently narrower basidiospores, 3.5–6 µm wide (Diederich 1996).

Amongst the known lichenicolous *Tremella* species with rarely stalked, 2-celled, mainly longitudinally septate basidia, with cells not becoming elongate at maturity, without conspicuous hymenial hyphidia, inducing gall formation on the host thallus, with galls basally not constricted, pale brown when young and dark brown to blackish when old, *Tremella nephromatis* Diederich has distinctly larger, dark reddish brown basidiomata, up to 4 mm diam.; *T. coccocarpiae* Diederich and *T. microcarpa* Diederich have very small basidiomata, < 0.2 mm diam.; *T. macroceratis* Diederich & Hafellner has narrower basidiospores, 4–5.5 µm wide and shiny basidiomata; and *T. montis-wilhelmii* Diederich and *T. normandinae* Diederich have pale to pinkish or reddish brown basidiomatal galls never turning dark brown or black (Diederich 1996).

Additional specimens examined (all on *Hypogymnia tubulosa*): **Spain**: Castilla y León, Segovia, San Ildefonso, next to road CL-601 at Las Siete Revueltas, entrance to Ramal de Vaquerizas, following Venerillo stream, 40º49' N, 4º0' W, on *Pinus sylvestris*, 18 ii 2012, Zamora, Pérez-Ortega & Millanes s.n. (S). – **UK**: Scotland, VC 95, Moray, Culbin Forest, 28/995.630, < 10 m, on pine twigs, 6 v 1999, Coppins 18843 (E 00722360); VC 107, East Sutherland, Loch Fleet NNR, Ferry Links, 28/80.95, < 10 m, on *Calluna*, 10 viii 1999, Coppins 18906 (E 00722345).

Acknowledgments

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