The Lichenologist **43**(4): 345–356 (2011) © British Lichen Society, 2011
doi:10.1017/S002428291100020X

Molecular and morphological data place *Blarneya* in *Tylophoron* (*Arthoniaceae*)

Damien ERTZ, Frank BUNGARTZ, Paul DIEDERICH and Leif TIBELL

Abstract: Based on morphological, anatomical, chemical, ecological and molecular evidence, *Blarneya* is synonymized here with *Tylophoron*. The molecular phylogeny derived from sequences obtained from sporodochia of *Blarneya* places this genus, described to accommodate an anamorphic lichen with white cushion-shaped sporodochia, within *Tylophoron*. This conclusion is further supported by the discovery of *Tylophoron*-type ascomata emerging directly from thalli with *Blarneya*-type sporodochia and producing identical hyaline conidia. In one specimen pycnidia were also observed. This represents a surprising variety of morphologically different conidiomata. A different anamorphic type was previously reported from *Tylophoron*, and this is confirmed here by molecular analysis for *T. moderatum*: besides thalli with ascomata this species has anamorphic thalli with an irregularly delimited brown sporodochial felt and brown conidia. Ascomata are not known from these entirely anamorphic thalli, whereas they do occur infrequently in *Tylophoron* species with *Blarneya*-type sporodochia. A key to all currently accepted species of *Tylophoron* is provided. In addition to the corticolous *Tylophoron hibernicum*, confined to humid forests, two saxicolous species with *Blarneya*-type sporodochia are described here as new: *T. galapagoense*, known only from Galapagos, differs from *T. hibernicum* by a thicker, more compact, beige rather than white, more strongly C+ red thallus, growing below sheltered rock overhangs in dry forests; *T. stalactiticum* has a C− thallus with stipitate, white, C+ red sporodochia; the species is known only from a single locality in Tenerife, on a large slope with volcanic boulders.

Introduction

The genus *Blarneya* was described by Hawksworth *et al*. (1979) for a sterile, corticolous lichen with a pinkish white thallus and conspicuous white sporodochia. The thalli were considered to be initially lichenicolous on different species of *Roccellaceae* and on *Belonia caudata*, eventually becoming autonomous after killing the host mycobiont and taking over its photobiont (*Trentepohlia*). The species was described from Ireland and later discovered in SW England and Wales, the Western Pyrenees (France and Spain) and Macaronesia (Canary Islands and Madeira) (Etayo 1989, 1992, 1998; Chambers & Purvis 2009).

Recent field work by the authors has led to the discovery of additional localities of *Blarneya* in the Pyrenees, the Canary Islands, continental Africa (Democratic Republic of Congo), the USA (Florida and Hawaiian Islands) and the Galapagos Islands (Ecuador). Several saxicolous populations were discovered, differing slightly from the corticolous ones, suggesting that more than one species might be involved, implying that *Blarneya* could no longer be accepted as a monotypic genus. Among the material examined, we also encountered specimens that were apparently fertile with *Tylophoron*-like ascomata. This observation led us to wonder if the sporodochial thalli called *Blarneya* might be an anamorph of *Tylophoron*, or if these specimens were just sterile *Blarneya* overgrowing fertile thalli of *Tylophoron*. The genus *Tylophoron* has recently been shown to

D. Ertz: National Botanic Garden, Department Bryophytes-Thallophytes (BT), Domein van Bouchout, B-1860 Meise, Belgium. Email: damien.ertz@br.fgov.be
F. Bungartz: Charles Darwin Foundation, Puerto Ayora, Santa Cruz Island, Galapagos; mailing address: Av. 6 de Diciembre N 36-109 y Pasaje California, caja postal 17-01-3891, Quito, Ecuador.
P. Diederich: Musée national d'histoire naturelle, 25 rue Munster, L-2160 Luxembourg, Luxembourg.
L. Tibell: Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18 D, SE-752 36 Uppsala, Sweden.

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

be a calicioid member of the *Arthoniales* using molecular data (Lumbsch *et al*. 2009), while *Blarneya* is considered as an anamorphic ascomycete of uncertain position referred by some authors to the *Arthoniales* based on the chemistry and the method of conidiogenesis (e.g., Chambers & Purvis 2009).

Therefore, we carefully studied all available specimens both macroscopically and microscopically; we analyzed their chemistry by TLC or, in the case of small thalli, by spot test reactions. In several cases we were able to culture specimens from conidia of recently collected material and we were able to sequence LSU rDNA from eight specimens, including four saxicolous ones. The results conclusively demonstrate that *Blarneya* is an anamorph of *Tylophoron* and, further, allow us to describe two new *Tylophoron* species with *Blarneya*-type sporodochia.

Material and Methods

Morphology

Sections were investigated microscopically on material mounted in water and 5% KOH. Measurements of asci and ascospores all refer to material examined in water; conidia were measured in KOH, which dissolves surrounding crystals. Ascospores and conidia measurements are indicated as (minimum–) $\bar{x}$-σ_x – $\bar{x}$+σ_x (–maximum) followed by the number of measurements (*n*); the length/breadth ratios of the ascospores and conidia are indicated as l/b. For other characters, the minimum and the maximum values are given. The thallus colour always refers to herbarium specimens.

Thin-layer chromatography (TLC) of acetone extracts of almost all specimens was performed in solvent systems C, EA and G (Orange *et al*. 2001).

Taxon sampling and cultures

Cultures were isolated from ascospores or conidia of freshly collected material on malt-yeast extract medium following Yoshimura *et al*. (2002). We obtained cultures from three specimens of *Blarneya* for this study (*Ertz* 10880, 11546, 11794). All cultures were obtained from conidia. When cultures were not available, well-preserved and freshly collected lichen specimens lacking any visible symptoms of fungal infection were used for DNA isolation. We obtained 9 new sequences of *Arthoniales* for this study from France, the Democratic Republic of Congo, the Galapagos Islands (Ecuador), the Hawaiian Islands (USA) and the Canary Islands (Spain). For the phylogenetic analyses, 27 sequences were retrieved from GenBank in addition to our own sequences (Table 1). Three outgroup species were chosen based on Lutzoni *et al*. (2004): *Curvularia brachyspora* (Dothideomycetes), *Seynesia erumpens* (Sordariomycetes) and *Cudonia circinans* (Leotiomycetes).

Molecular data

Genomic DNA was isolated from mycobiont cultures or from lichen specimens using the Puregene Genomic DNA Purification Kit (GENTRA Systems, Minnesota), following the manufacturer's Plant Tissue extraction protocol. Amplification reactions were prepared for a 50 µl final volume containing 5 µl 10x Taq Buffer (Roche), 2·5 µl of each of the 20 µM primers, 1 µl of 10 mg ml^{-1} bovin serum albumin (Ambion # 2616), 1 µl of 25 mM $MgCl_2$, 1·25 U Taq DNA polymerase (Roche) and 1 µl of template genomic DNA. PCR was performed on Peltier Thermal Cyclers PTC-100 or PTC-150 (MJ Research). A targeted fragment of about 1 kb at the 5′end of the nuclear LSU rDNA was amplified using primers LR0R (Rehner & Samuels 1994), LIC15R (Miądlikowska *et al*. 2002) with LR5 or LR6 (Vilgalys & Hester 1990). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). The yield of the PCRs was verified by running the products on a 1% agarose gel using ethidium bromide. Both strands were sequenced directly using BigDye terminators (Applied Biosystems) and the amplification primers. Additional primers for sequencing were used: LR3R and LR3 (Vilgalys & Hester 1990; Vilgalys' website, http://www.botany.duke.edu/fungi/mycolab). Sequence fragments were assembled with Sequencher version 4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to BLAST searches to verify their closest relatives and to detect potential contaminations.

Phylogenetic analyses

NucLSU sequences were aligned manually using MacClade 4.05 (Maddison & Maddison 2002). Ambiguous aligned regions and introns representing a total of 224 bp were delimited manually and excluded from the analyses.

Maximum Parsimony (MP) analysis was performed in PAUP*4.0b10 (Swofford 2002) on the nucLSU matrix of 778 unambiguously aligned characters including 296 variable characters, of which 211 are parsimony-informative. The nucLSU matrix was subjected to a symmetric step matrix constructed with the STMatrix 2.2 MacOS Program (François Lutzoni & Stefan Zoller, Department of Biology, Duke University). Heuristic searches were used with 1000 random addition sequence replicates, MaxTrees set to autoincrease, tree-bisection-reconnection (TBR) branch swapping, MulTrees option in effect and gaps treated as a fifth character state. The MP analysis yielded one most parsimonious tree (1436·05 steps, CI = 0·5413, RI = 0·7213). Bootstrap values (MP-bs) were obtained from 1000 replicates with 3 random addition sequences (all other parameters identical to the original MP search).

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

TABLE 1. *Specimens and DNA sequences used in this study, with their respective voucher information. GenBank accession numbers in bold refer to sequences (9) generated by this project. All other sequences (27 GenBank accession numbers) were obtained directly from GenBank*

Name	Voucher	Substratum	nucLSU GenBank number
Arthonia anglica	Rwanda, *D. Ertz* 7775 (BR)	bark	EU704084
A. didyma	Belgium, *D. Ertz* 7587 (BR)	bark	EU704083
A. dispersa	UPSC2583	bark	AY571381
A. zwackhii	Canary Islands, *D. Ertz* 10928 (BR)	bark	HQ454514
Arthothelium galapagoense	Galapagos Islands, *D. Ertz* 11654 (BR)	rock	HQ454515
Arthothelium galapagoense	Galapagos Islands, *D. Ertz* 11790 (BR)	rock	HQ454516
Chiodecton natalense	Zambia, *D. Ertz* 6576 (BR)	bark	EU704085
Cryptothecia candida	Gabon, *D. Ertz* 9260 (BR)	leaf	HQ454520
Cudonia circinans	JP232, AFTOL-ID353	–	AF279379
Curvularia brachyspora	ATCC58872, ATCC12330	–	AF279380
Dirina catalinariae	Galapagos Islands, *A. Tehler* 8726 (S)	rock	EF081387
Enterographa crassa	France, *D. Ertz* 5041 (BR)	bark	EU704088
Lecanactis abietina	Belgium, *D. Ertz* 5068 (DUKE)	bark	AY548812
Opegrapha celtidicola	Portugal, *P. Diederich* 16053 (BR)	bark	EU704094
O. filicina	Rwanda, *D. Ertz* 7994 (BR)	leaf	EU704095
O. longissima	Florida, *D. Ertz* 9155 (BR)	bark	EU704097
O. ochrocheila	Luxembourg, *D. Ertz* 7519 (BR)	bark	EU704100
O. varia	France, *D. Ertz* 7570 (BR)	bark	EU704103
O. vermicellifera	Belgium, *D. Ertz* 7562 (BR)	bark	EU704105
O. viridis	Luxembourg, *D. Ertz* 7619 (BR)	bark	EU704106
O. vulgata	Belgium, *D. Ertz* 7564 (BR)	bark	EU704108
Roccella fuciformis	Tenerife, *P. Diederich* 15572 (DUKE)	rock	AY584654
Schismatomma pericleum	*A. Tehler* 7701 (S)	bark	AF279408
Seynesia erumpens	SMH1291 (F)	–	AF279410
Tylophoron crassiusculum	Costa Rica, *R. Lücking* 18006 (F)	bark	EU670258
T. galapagoense	Galapagos Islands, *F. Bungartz* 8749 (CDS)	rock	**JF295078**
T. galapagoense	Galapagos Islands, *F. Bungartz* 8750 (CDS)	rock	**JF295079**
T. galapagoense	Galapagos Islands, *D. Ertz* 11794 (BR)	rock	**JF295080**
T. hibernicum	Hawaiian Islands, *C. Smith* T904L (UPS)	bark	**JF295081**
T. hibernicum	Hawaiian Islands, *C. Smith* T905L (UPS)	bark	**JF295082**
T. hibernicum	Galapagos Islands, *D. Ertz* 11546 (BR)	bark	**JF295083**
T. hibernicum	France, *P. Diederich* 16335 (herb. P. Diederich)	bark	**JF295084**
T. moderatum	Costa Rica, *R. Lücking* 15081a (F)	bark	EU670256
T. moderatum	RD Congo, *D. Ertz* 14504 (BR)	bark	**JF295085**
T. protrudens	Kenya, *T. Lumbsch* 19557 *et al.* (F)	bark	EU670257
T. stalactiticum	Canary Islands, *D. Ertz* 10880 (BR)	rock	**JF295086**

The best-fit model of DNA evolution was chosen using the Akaike information criterion (AIC) as implemented in Modeltest v. 3.06 (Posada & Crandall 1998). Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Analyses were run under the GTR model of nucleotide substitution including a proportion of invariable sites and a discrete gamma distribution with six rate categories. Two parallel MCMCMC runs were performed each using four independent chains and 10 000 000 generations, sampling trees every 1000th generation. The proportion of burn-in trees sampled before reaching equilibrium was estimated using TRACER v.1.5 (Rambaut & Drummond 2007) by plotting the log-likelihood values of the sample points against generation time. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the last 9000 of the 10 000 trees sampled.

The Maximum Likelihood (ML) analysis was performed using GARLI (Zwickl 2006, v.0.951 for OS X) with default settings, and a single most likely tree was produced (–lnL = 5048·749961). Maximum Likelihood bootstrap values (ML-bs) were derived from 1000 bootstrap replicates using default settings.

The MP tree and the ML tree did not contradict the Bayesian tree topology for the strongly supported

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

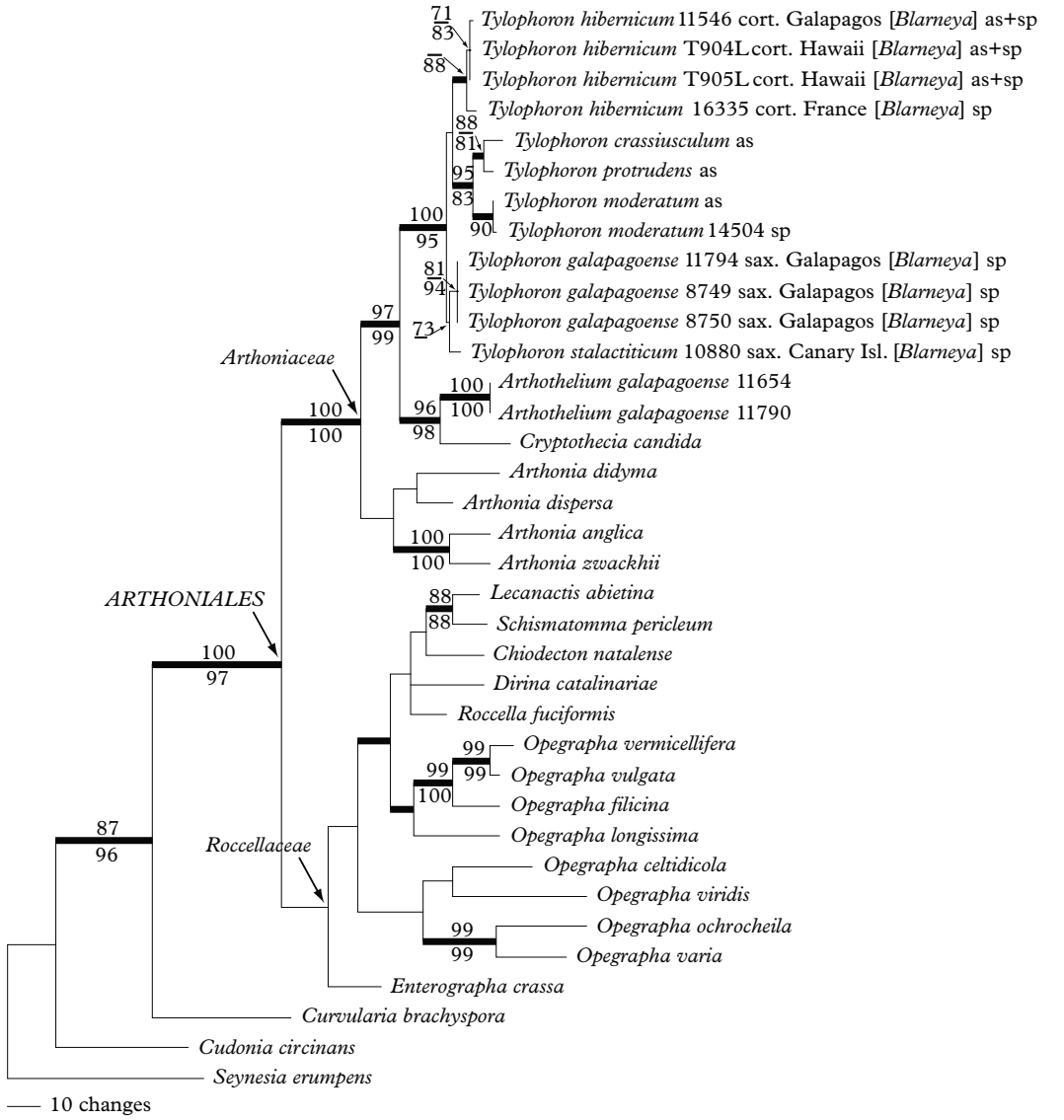


FIG. 1. One locus (nucLSU) 50% majority-rule consensus tree produced by the Bayesian analysis and representing the proposed phylogenetic relationships among 33 specimens of *Arthoniales*. Internal branches with a PP $\geq$ 95% are considered strongly supported and represented by thicker lines. MP-bs values $\geq$ 70 are shown above, and ML-bs values $\geq$ 70 are shown below, internal branches. Collecting numbers of authors following the species names act as specimen and sequence identifiers. *Tylophoron* specimens with *Blarneya*-like sporodochia are followed by the name *Blarneya* within brackets, those with ascomata are followed by 'as', those with sporodochia are followed by 'sp', those with ascomata and sporodochia are followed by 'as+sp'.

branches and hence only the majority rule consensus tree of the Bayesian analysis is shown here, with the branch supports of the two other analyses (Fig. 1). PP $\geq$ 95%, ML-bs $\geq$ 70% and MP-bs $\geq$ 70% were considered to be significant.

Results and Discussion

The most striking result of the phylogenetic analysis is the position of *Blarneya* within the

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

Arthoniaceae and its very close relationship with *Tylophoron* in its usual delimitation (Lumbsch *et al*. 2009). The discovery of *Tylophoron*-like ascomata emerging from the surface of thalli with *Blarneya*-like sporodochia further supports this result (Fig. 2D & E). As *Blarneya* is considered to be initially a lichenicolous fungus, eventually lichenized (Hawksworth *et al*. 1979), it is futile to argue, on morphological observations alone, that these ascomata were not produced by *Tylophoron* thalli invaded by *Blarneya*. However, all sequences of *Blarneya* used here for the phylogenetic analysis were obtained directly from *Blarneya*-like sporodochia. Moreover, three sequences are based on culture isolates from *Blarneya*-like conidia: specimens *Ertz* 10880 from Tenerife, and *Ertz* 11546 and 11794 from the Galapagos Islands. In addition, one sequence comes from the Pyrenees (*Diederich* 16335) and one from Tenerife (*Ertz* 10880), where the genus *Tylophoron* is not known. For all these reasons, we can exclude any contamination with *Tylophoron* for all sequences obtained from the '*Blarneya*' material studied here. Instead we can conclude that the ascomata observed on the sporodochial thalli belong to these thalli.

Regarding their very close phylogenetic relationship, we consider *Blarneya* a synonym of *Tylophoron* and therefore combine *B. hibernica* into *Tylophoron* (see taxonomy below). Ascomata and ascospores are morphologically and anatomically identical, which also supports this new synonymy. Several morphological differences nevertheless exist between the anamorphic states of *Tylophoron hibernicum* and the previously accepted *Tylophoron* species, in particular *T. moderatum*. In *T. hibernicum* the sporodochia are always pale and usually form well-delimited cushions (Fig. 2C & D), whereas the anamorph of *T. moderatum* is characterized by a dark brown felt of sporodochia that are not distinctly delimited and often fuse to form irregular aggregates (Fig. 2F). The conidia in *T. hibernicum* are hyaline, simple or 1-septate (Fig. 3E & F); they are dark brown and always aseptate in *T. moderatum* (Fig. 3I). These distinct morphological and anatomical differences of the anamorphic thalli would suggest distinguishing two different genera with almost identical ascomata and ascospores. However, molecular evidence clearly does not support this split. All sequences obtained from thalli with *Blarneya*-type sporodochia are included within the same, strongly supported clade as *Tylophoron*. They do not form a separate monophyletic group. The molecular data support a single genus, and the differences between anamorphs must therefore be regarded as significant only at species level.

In this context it is particularly interesting that thalli with brown, felty sporodochia have so far been confirmed with certainty only for *Tylophoron moderatum*. It appears that this species forms two distinct types of thalli: either exclusively with apothecia (the teleomorph), or only producing conidia arranged in brown, irregularly delimited, felty sporodochia (the anamorph, Fig. 2F). Unlike the *Tylophoron* species that form *Blarneya*-type sporodochia (*T. hibernicum* and *T. galapagoense*), the brown anamorphs of *T. moderatum* have never been found with ascomata. These exclusively anamorphic thalli have been ascribed to *T. moderatum* based on similarities in their thallus structure, chemistry, ecology and features of axenic cultures of the mycobiont obtained from ascospores and conidia, respectively (Tibell 1996). Field observations also suggested that both types of thalli exist in *Tylophoron crassiusculum*, but no sporodochial thalli are known from *T. protrudens* (but see notes under the description of *T. hibernicum*). Here, we have sequenced one anamorphic thallus of *Tylophoron* from Africa producing dark brown and aseptate conidia (*Ertz* 14504). Our phylogenetic tree shows that this thallus clearly belongs to *Tylophoron moderatum*, thus providing evidence for the existence of an anamorphic thallus characterized by a brown, diffusely delimited sporodochial felt.

In our phylogenetic tree (Fig. 1), the clade including *Tylophoron* is strongly supported but poorly resolved. The nucLSU sequences alone are certainly not sufficient for resolving the species circumscription, as it is too conserved. However, it must be noted that the

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

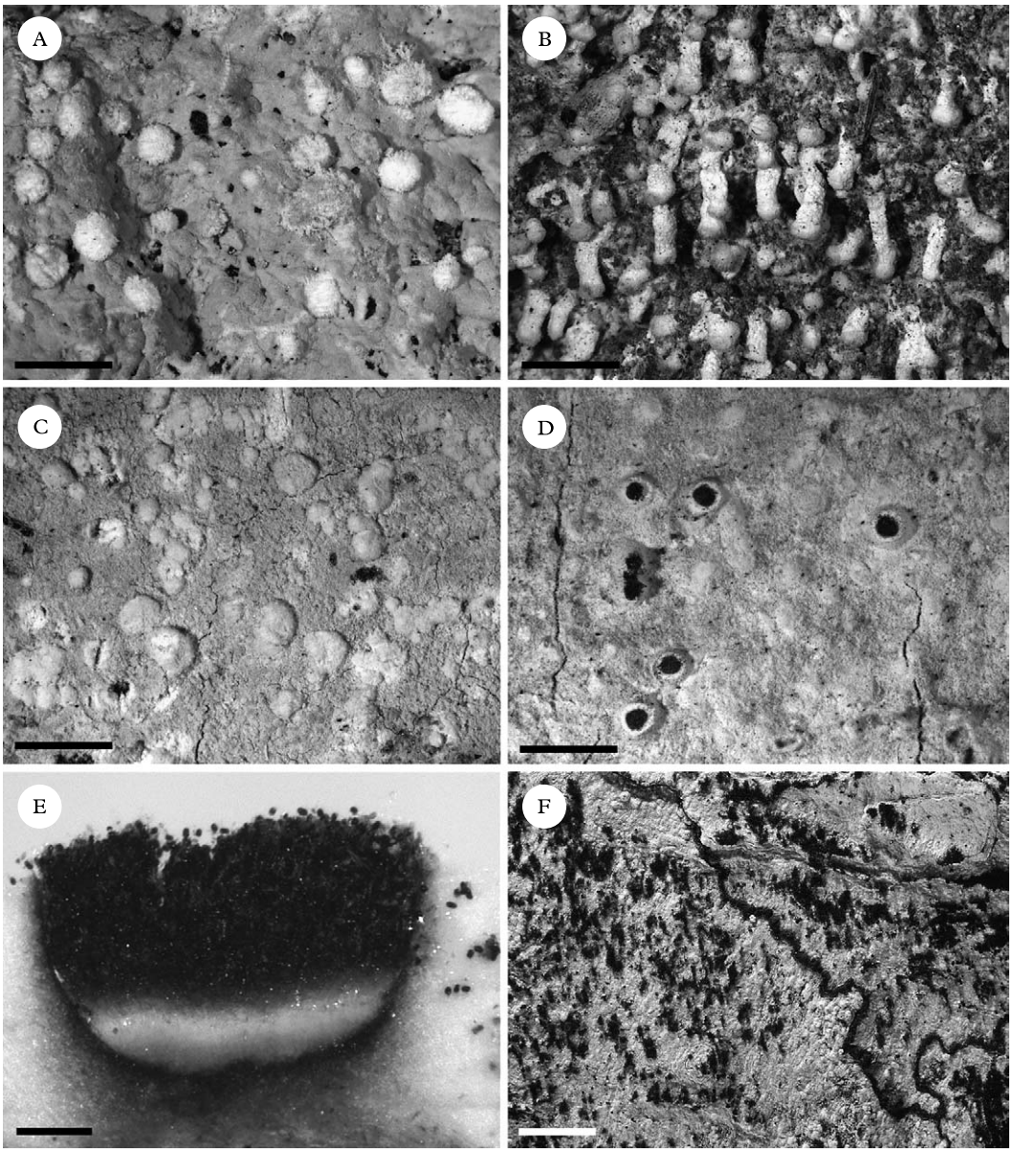


FIG. 2. *Tylophoron* species with *Blarneya*-type sporodochia. A, *T. galapagoense*, thallus and sporodochia (*Bungartz* 8750); B, *T. stalactiticum*, thallus with sporodochia present at the tip of the hanging stipes (*Ertz* 10880 *& Diederich*); C–E, *T. hibernicum*; C, thallus and sporodochia (*Diederich* 16335); D, thallus with ascomata and sporodochia (*Smith* T904); E, cross section through an ascoma (*Smith* T904); F, *Tylophoron moderatum*, anamorphic thallus from the Galapagos Islands (*Bungartz* 7109), the pale white thallus is covered by an irregular felt of brownish sporodochia. Scales: A–D = 2 mm, E = 0·1 mm, F = 5 mm.

corticolous specimens of *Tylophoron* with *Blarneya*-like sporodochia group together with strong support (PP=100, ML-bs=88 but MP-bs=68), just like the saxicolous ones, but here with weak support (PP=78, ML-bs=68 and MP-bs=73). Interestingly,

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

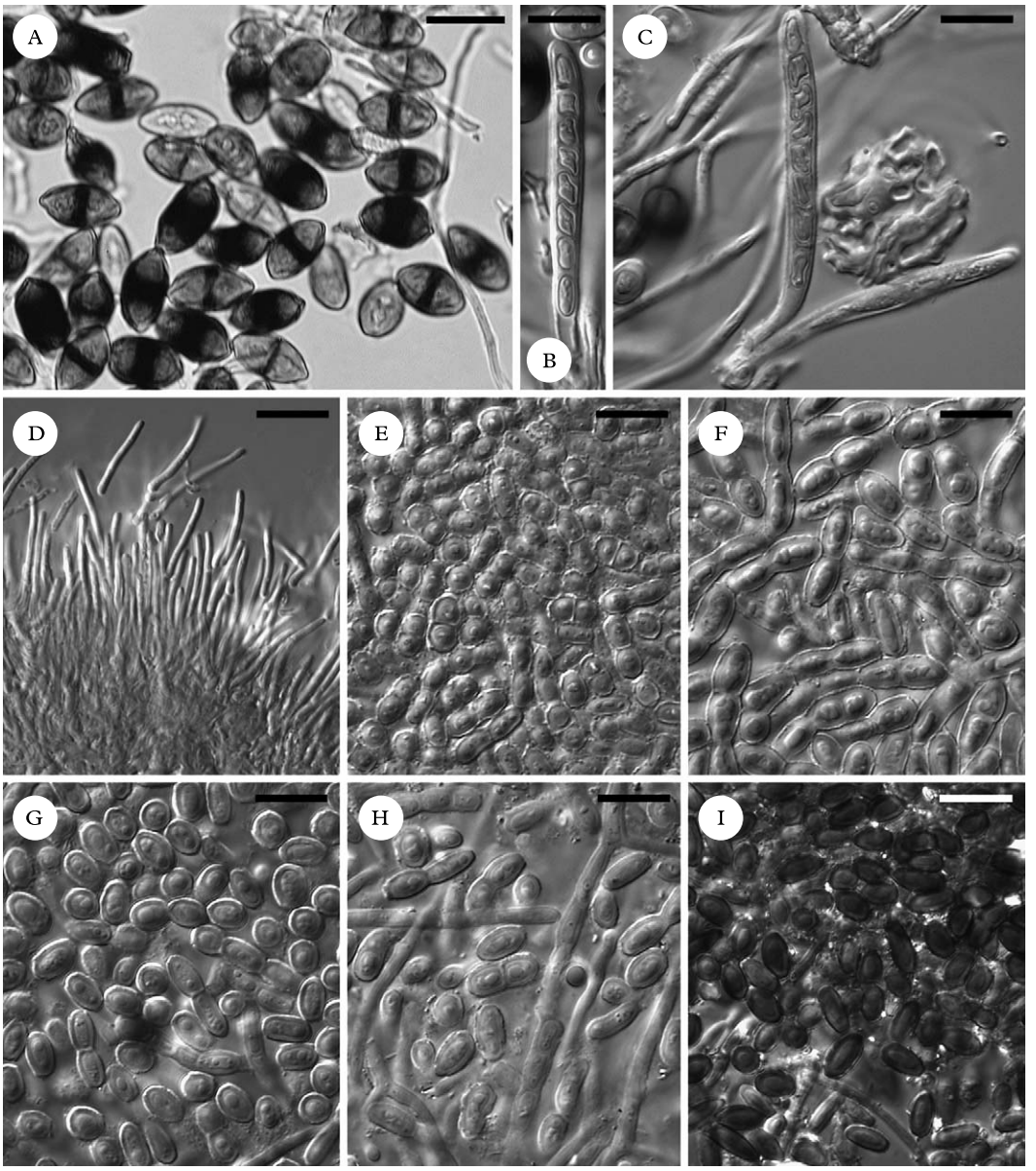


FIG. 3. *Tylophoron* species with sporodochia (A–C in water; D–I in KOH). A–F, *T. hibernicum* (A, *Ertz* 11546; B–E, *Smith* T904; F, *Diederich* 16335); A, brown, ellipsoid, one-septate ascospores, the spores are typically slightly pointed and more strongly pigmented around the septum; B–C, asci and paraphyses; D, pycnoconidia; E–F, sporodochial conidia; G, *T. galapagoense* (*Bungartz* 8750), sporodochial conidia; H, *T. stalactiticum*, sporodochial conidia (holotype); I, *T. moderatum*, sporodochial conidia (*Ertz* 14504). Scales: A–I = 10 μm.

the corticolous specimen from the Galapagos Islands groups with the other corticolous specimens from Hawaii and the Pyrenees, but not with the saxicolous specimens from the Galapagos Islands as would have been expected with a single species. In Galapagos, the saxicolous specimens generally grow in sheltered, shaded sites, under rock

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

overhangs, but they are generally found in dry forests, whereas the corticolous specimen has been collected in humid forests in the highlands. Based on morphological, chemical, ecological and molecular data, we decided to describe two new species and to restrict the name *Tylophoron hibernicum* to the corticolous specimens.

Taxonomy

Key to all currently accepted species of *Tylophoron*

1 Thallus with sporodochia, sometimes also with ascomata 2
Thallus lacking sporodochia, with numerous ascomata 5

2(1) Thallus with an irregular, not distinctly delimited felt of dark brown sporodochia, lacking ascomata. . . . . **T. moderatum**
Thallus with cushions of clearly delimited, white to cream, rarely pinkish, stipitate or sessile sporodochia, sometimes also with ascomata 3

3(2) Thallus thick, compact, of densely packed hyphae, with a smooth surface, distinctly creamy white to beige, thick, C+ strongly red; saxicolous . . . **T. galapagoense**
Thallus thin, of loosely interwoven hyphae with a felty surface, bright to pale greyish or bluish white, rarely cream coloured (prolonged herbarium storage?), C− or C+ weakly red 4

4(3) Thallus C−, with hanging, stipitate sporodochia; saxicolous . . . **T. stalactiticum**
Thallus C+ weakly red, with sessile sporodochia; corticolous . . . **T. hibernicum**

5(1) Thallus C+ red; ascomata 0·6–1·2 mm diam. . . . . **T. protrudens**
Thallus C−; ascomata 0·3–0·8 mm diam. 6

6(5) Thallus corticate, excipulum laterally 160–180 μm thick. . . . . **T. gibsonii**
Thallus ecorticate 7

7(6) Lateral part of excipulum 55–100 μm thick, of richly branched and interwoven hyphae . . . . . **T. crassiusculum**
Lateral part of excipulum 15–25 μm thick, of sparsely branched, periclinally arranged hyphae . . . . . **T. moderatum**

The Taxa

Tylophoron Nyl. ex Stizenb.

Ber. Thät. St. Gallen Naturwiss. Ges. **1861–1862:** 177 (1862); type: *Tylophoron protrudens* Nyl.

Syn. nov. *Blarneya* D. Hawksw., Coppins & P. James, *Bot. J. Linn. Soc.* **79:** 358 (1979); type: *Blarneya hibernica* D. Hawksw., Coppins & P. James. Originally monotypic.

Tylophoron galapagoense Bungartz, Ertz, Diederich & Tibell sp. nov.

MycoBank No: MB 519736

A *Tylophorone hibernico* thallo leviore et crassiore cremeo differt.

Typus: Ecuador, Galapagos Islands, Isabela, Volcán Darwin, south-western slope, above Tagus Cove, 724 m, transition zone, SW-exposed lava flow of weathered AA-lava with scarce vegetation (*Macraea laricifolia*, *Dodonaea viscosa*, …), on rock, overhang, 2007, *D. Ertz* 11794 (CDS 37153—holotypus; BR, hb. Diederich—isotypi).

(Figs 2A, 3G)

Thallus crustose, superficial, more or less smooth, creamy white to pale brownish, ecorticate, thin, up to 0·5 mm thick; hyphae irregularly branched, hyaline, mainly 2–2·5 μm diam., forming numerous convex to subglobose sporodochia 0·5–1 mm diam., which are similar in colour, but paler than the thallus. Sporodochial conidia hyaline, smooth to slightly roughened, mostly simple, more rarely 1-septate, with rounded or

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

truncate apices, spherical, ellipsoid or rarely oblong, simple conidia (4·5–)5–6·5(–9) × (3–)3·5–4·5(–5·5) µm, l/b ratio 1·1–1·9 (n = 60), 1-septate conidia (7–)8–11(–15) × (3–)3–4·5(–6) µm, l/b ratio 2–3·2 (n = 70). *Prothallus* whitish cream, byssoid, 0·5–1 mm wide. Photobiont *Trentepohlia*.

Ascomata densely covering the thallus of one specimen (*Aptroot* 65477), but all mazaedia composed of degenerated ascospores and brownish hyphae, no intact ascospores found.

Pycnidia unknown.

Chemistry. Thallus C+ red, K−, KC+ red, P−, UV−. Sporodochia C+ red, K+ yellow, KC+ red, P−, UV−. TLC: lecanoric acid in all specimens examined. In addition, a pale orange substance in daylight after heating in *Ertz* 11794.

Notes. The new species is distinguished from *T. hibernicum* by a smoother and thicker, more compact thallus, which is always distinctly cream coloured. Furthermore, the C+ red reaction of the thallus is always stronger than in *T. hibernicum*. In some specimens of *T. galapagoense* (e.g., *Bungartz* 8749 and 8750) the majority of conidia are simple (Fig. 3G), in contrast to *T. hibernicum* in which one-septate conidia usually dominate (Fig. 3F). However, in other specimens of both species, a mixture of one-septate and simple conidia occurs. Overall, even within sporodochia that predominantly produce one-septate conidia, a few simple conidia can typically be found, and vice versa. Because one-septate conidia easily fracture, it is difficult to assess if the formation of simple vs. one-septate ones follows a different conidiogenesis. Specimens that produce mostly simple conidia generally have conidia with a more spherical shape, whereas simple conidia derived secondarily from septate ones that broke apart, are typically more elongate, with an oval to ellipsoid, not spherical shape. Overall we could not find any clear correlation between these differences and a particular species, and the predominance of simple vs. one-septate, spherical vs. elongate conidia may perhaps be related more to environmental factors than to genetic factors.

Tylophoron galapagoense is currently known only from the Galapagos, on five different islands, where all specimens have been found growing on rock below shaded overhangs or in crevices, from the coast through the dry lowland forests to the transition zone. However, in Santiago the species has also been found in the highlands, where it is known from hidden crevices of the dry, N- NE-exposed steep crater rim of Cerro Gavilan. On Volcán Darwin, where the type specimen was collected, the western slopes of the volcano lie within the rain-shadow of Fernandina. Here the vegetation is generally dry and the transition zone reaches unusually high altitudes. Whereas on the opposite sides of the island humid forests abound, the dry lava flows on the western slopes are characterized by low, transition zone shrubs. In contrast, the corticolous *T. hibernicum*, although occasionally found in the transition zone, appears more common in the humid forests of the Galapagos highlands.

Additional selected specimens examined. **Ecuador:** *Galapagos Islands*: Santa Cruz Island: along dirt road to Mina Granillo Rojo, off the main road to the channel, 583 m, transition zone, dry deciduous forest with basalt outcrops in between on N-slope of island, on sheltered rock face, 2007, *D. Ertz* 11576 (BR); *ibid*., *F. Bungartz* 7113 (CDS 37598); Mina Granillo Rojo, on the N-side of the island, above the mine, 633 m, transition zone, lava outcrop in open forest, ENE-exposed overhang (70°–80°) of basalt boulder in rock outcrop, on volcanic rock, 2010, *F. Bungartz* 8749 (CDS 44629); *ibid*., *F. Bungartz* 8750 (CDS 44630). Isabela Island: Volcán Alcedo, along the trail going up the E-slope, basalt rubble field to the SE-side of the trail and the barranco, 530 m, dry zone, basalt rubble field with scattered vegetation, 2006, *A. Aptroot* 64943 (CDS 31522); Volcán Sierra Negra, Cerro Orchilla, *c*. 4 km W of Puerto Villamil, 56 m, dry zone, S exposed slope of hill, open *Bursera graveolens* forest, SE-exposed overhang of basalt outcrop, 2008, *Bungartz* 8449 (CDS 41095). Pinta Island, SW-part of the island, along trail going up the SW slope to Las Pampas on the western saddle, 316 m, transition zone, open forest, top of basalt boulder, 2008, *Nugra* 564 (CDS 38942). Santiago Island, summit of Cerro Gavilan, inner N- and NE-exposed crater rim, 840 m, humid zone N- and NE-exposed, steep basalt cliffs of crater rim with ferns growing in crevices, 2006, *A. Aptroo*t 65760 (CDS 32352). Pinzón Island, along the trail going up from Playa Escondida, N- to W-facing cliff above a crater, 318 m, dry transition zone with *Cordia lutea*, *Croton scouleri*, and at the bottom of the cliff

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

also *Scalesia baurii* ssp. *baurii*, 2006, *A. Aptroot* 64030 (CDS 30591).

Tylophoron hibernicum (D. Hawksw., Coppins & P. James) Ertz, Diederich, Bungartz & Tibell comb. nov.

MycoBank No: MB 519738

Basionym: *Blarneya hibernica* D. Hawksw., Coppins & P. James, *Bot. J. Linn. Soc.* **79:** 358 (1979); type: Ireland, Co. Kerry, Killarney, Eagles Nest, on *Cresponea premnea* in dry recess of ancient *Quercus*, 1966, *P. W. James* (K!—holotype).

(Figs. 2C & D, 3A–F)

Thallus crustose, superficial, felty, pale greyish or rarely creamy or bluish white, ecorticate, thin, up to 0·3 mm thick; hyphae irregularly branched, hyaline, mainly 1·5–2 µm diam., forming numerous convex to subglobose sporodochia 0·4–1 mm diam. which are white, cream or sometimes with a pinkish or orange tinge, usually fading to white in the herbarium. *Sporodochial conidia* hyaline, smooth to slightly roughened, 0–1-septate, with rounded or truncate apices, simple conidia ellipsoid to oblong, rarely spherical, (4–)4·5–6·5(–8) × (3–)3–4(–5), l/b ratio 1·3–1·9 (n = 60), 1–septate conidia often distinctly constricted at the septum, (6–)7–11·5(–15) × (3–)3·5–4·5(–5) µm, l/b ratio 1·9–2·9 (n = 70) (for a detailed description of the conidiogenesis see Hawksworth *et al.* 1979). *Prothallus* brown, byssoid, 1–4(–5) mm wide. Photobiont *Trentepohlia*.

Ascomata very rare, sessile, short cylindrical to conical, 0·5–1 mm diam., 0·4–0·6 mm high (excluding the mass of ascospores), 1–1·5(–2) times as wide as high. Thalline margin well-developed, *c.* 50–60 µm wide, approx. half the height of the ascomata, densely inspersed with minute colourless crystals dissolving in K (observed in polarized light), of hyaline hyphae 1·5–2 µm diam. *Excipulum* 10–25 µm laterally, sometimes thinner or absent below, of dark brown, sparingly branched and interwoven hyphae, 1·5–2 µm diam. Mazaedium well-developed, black, sometimes only slightly projecting over the thalline margin but often extending further. *Paraphyses* branched, anastomosing, 1·5 µm wide. *Asci* dissolving at early stages, cylindrical, with eight uniseriate ascospores, 35–45 × 4–5 µm. *Ascospores* 1-septate, dark brown, with a heavily pigmented band around the central part, ellipsoid, sometimes constricted at the septum, occasionally with slightly pointed ends, wall thick, (9–)10·5–13·5(–17) × (5·5–)6·5–8·0(–9) µm, l/b ratio 1·4–1·8 (n = 110).

Pycnidia rare (only observed in *Smith* T904), present at the margin of the thallus, immersed, visible as brownish spots of *c.* 0·15–0·3 mm, surrounded by a white thalline margin of *c.* 50–80 µm thick; pycnidial wall very reduced; pycnoconidia filiform, straight or slightly curved, hyaline, 9–14 × 1 µm.

Chemistry. Thallus (incl. margin of ascomata) C+ weakly red, K−, KC+ red, P−, UV− or UV+ orange. Sporodochia C+ red, K+ yellow, KC+ red, P−, UV− or UV+ orange. TLC: lecanoric acid in all specimens examined; in addition, lichexanthone is present in *Ertz* 11546, 14915 and 14898, the last two having an obvious UV+ orange thallus; trace of a fatty acid in *Diederich* 16335. Hawksworth *et al.* (1979) did TLC of two specimens of *Blarneya hibernica* when describing that species: the holotype overgrowing *Cresponea premnea* (sub *Lecanactis premnea*) and a second specimen overgrowing *Lecanactis abietina*. They detected lecanoric and schizopeltic acids as major substances, and in addition a xanthone, a fatty acid, and unknown substances fluorescing ice blue and yellow in ultra-violet light. No schizopeltic acid could be detected in our specimens. The holotype of *Blarneya hibernica* is extremely reduced (3 sporodochia on *Cresponea premnea*) and therefore does not allow re-examination by TLC. As the second specimen examined by Hawksworth *et al.* (1979) grows on *Lecanactis abietina*, a lichen producing schizopeltic acid in large amounts, a contamination of schizopeltic acid from the host is very likely. Therefore, we conclude that the detection of schizopeltic acid in *Blarneya hibernica* is likely to be the result of contamination from other lichen thalli.

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

Notes. Hawksworth *et al*. (1979) suggested that *Tylophoron hibernicum* starts as a lichenicolous species overgrowing other lichens, taking over their algae, and eventually developing an independent thallus. All specimens collected by us have large thalli, and therefore we could not confirm that they started growth as a lichenicolous fungus. *Tylophoron hibernicum* differs from other known *Tylophoron* species by wider ascospores and, when ascomata are lacking, by a thin, bright to pale greyish or bluish white thallus with a felty surface.

It must be noted here that the New York Botanical Garden Herbarium has published on its website (http://sciweb.nybg.org/science2/hcol/lena/index.asp) a photograph of a specimen (*Lendemer* 15891) collected in Florida, USA, with *Blarneya*-like sporodochia (but without ascomata) under the name *Tylophoron protrudens*; the specimen obviously belongs to *Tylophoron hibernicum* according to the results of our study. It seems that the colleagues in NY had no doubt that their material belongs to *Tylophoron* but they did not make the 'connection' with what Europeans have called *Blarneya*.

Fertile specimens examined (*with ascomata and sporodochia*). **Ecuador:** *Galapagos Islands*: Santa Cruz Island, *c*. 1 km N of Bellavista along dirt road to Media Luna, 143 m, humid zone, farm area, coffee plantation with *Cedrella odorata* trees and one large *Ceiba pentandra* tree, on trunk of *C. odorata*, 2007, *D. Ertz* 11546 (BR).—**USA:** *Hawaiian Islands*: Kailua, east side of Olomana mauka op Kalanianaole Hwy, near Waimanalo Quarry, on dead *Acacia confusa*, 2008, *C. Smith* T904, T905 (UPS).

Sterile specimens examined (*with sporodochia only*). **France:** *Pyrénées-Atlantiques*: forêt de Sare between Sare and Col de Lizarrieta, on *Quercus*, 2006, *P. Diederich* 16335 (hb. Diederich).—**Spain:** *Navarra*: 20 km E of San Sebastian, col d'Ibardin, on *Quercus* in old forest in valley, 1991, *P. Diederich* 9761, 9762 & *J. Etayo* (hb. Diederich).—**Democratic Republic of Congo:** *Equateur Prov*.: Lisala, quelques km en aval de Lisala sur la rive droite du fleuve Congo, 350 m, forêt secondaire dense sur sol sec à hydromorphe, sur gros tronc, 2009, *D. Ertz* 14133 (BR). *Orientale Prov*.: Kisangani, réserve forestière de Masako située dans une boucle de la rivière Tshopo à environ 14 km au NNE de Kisangani, gros tronc de *Gilbertiodendron*, 2009, *D. Ertz* 14898 (BR); *ibid*., gros tronc près de la station de recherche, *D. Ertz* 14915 (BR).—**USA:** *Florida*: Everglades National Park, along the road from Florida city to Flamingo, Mahogany Hammock trail, 7 m, big trunk in a small patch of forest, 2005, *D. Ertz* 9074 (BR).—**Ecuador:** *Galapagos Islands*: Santa Cruz Island: abandoned farm along the northern part of the loop road from Bellavista to Garrapatero, 143 m, humid zone, overgrown farm area with introduced trees, trunk of *Persea americana* (*c*. 40 cm in diam.), 2006, *F. Bungartz* 3703 (CDS 27558); along the road from Bellavista to Baltra, between Bellavista and Santa Rosa, close to the road, 571 m, humid zone, agricultural area, farmland with an alley of large *Cedrela odorata* trees, trunk of *Syzygium* sp. (*c*. 65 cm diam.), 2006, *F. Bungartz* 3931 (CDS 27813); *ibid*., trunk of *Cedrela*, *A. Aptroot* 64494 (CDS 31066); on farm N of Bellavista, 250 m, humid zone, agricultural area, trunk of *Psidium*, 2005, *A. Aptroot* 63329 (CDS 30073); along road from Bellavista to El Garrapatero, *c*. 4 km W from the campsite of the National Park, 159 m, dry zone, deciduous dry lowland forest, trunk of *Erythrina velutina* (*c*. 25 cm diam.), 2006, *F. Bungartz* 3571 (CDS 27370). San Cristóbal Island, trail to Ochoa, at the border of the National Park, *c*. 1 km N of El Progreso, 297 m, transition zone, agricultural area, farm with *Citrus* sp., *Inga schimpfii*, *Coffea arabica* and *Ciruela* trees, trunk of *Inga schimpfii*, 2008, *F. Bungartz* 8638 (CDS 41284).

Tylophoron stalactiticum Ertz & Diederich sp. nov.

MycoBank No: MB 519737

A *Tylophorone hibernico* sporodochiis stipitatis pendentibus et thallo C− differt.

Typus: Canary Islands, Tenerife, S of Garachico, W of San Juan del Reparo, 565 m, volcanic rock, 2007, *D. Ertz* 10880 & *P. Diederich* (BR—holotypus; hb. Diederich—isotypus).

(Figs. 2B, 3H)

Thallus crustose, superficial, felty, white, ecorticate; hyphae irregularly branched, hyaline, mainly 1·5–2 µm diam., forming numerous convex to subglobose, pale cream sporodochia 0·4–1 mm diam., produced on white, elongate, straight, simple or exceptionally bifurcate, hanging stipes with a rough surface, 0–1·5 mm long and 0·4–0·6 mm diam. Sporodochial conidia hyaline, smooth, 0–1-septate, with rounded or truncate apices, simple conidia (4·5–)5·5–7·5(–8·5) × (3–)3–4(–4·5) µm (n = 50), 1-septate conidia (7–)8·5–12·5(–15) × (3–)3–4(–4·5) µm (n = 60). *Prothallus* whitish, byssoid, 0·5–1 mm wide. Photobiont *Trentepohlia*.

Ascomata and *pycnidia* unknown.

Chemistry. Thallus and stipes of sporodochia C−, K−, KC−, P−, UV−. Sporodochia

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press

C+ red, K+ yellowish, KC+ red, P−, UV−. TLC: lecanoric acid, *cf*. roccellic acid and a colourless substance becoming pale olivaceous or brownish, UV+ green after heating.

Etymology. The stipitate, hanging sporodochia strongly resemble stalactites.

Notes. The new species is easily distinguished from all other known *Tylophoron* species with *Blarneya*-type sporodochia by the stipitate sporodochia and the white, felty C− thallus. It is known only from the type locality on underhangs of volcanic rocks in Tenerife (Canary Islands).

We particularly want to thank Emmanuël Sérusiaux for his critical and useful comments on the manuscript, as well as Paul Kirk and the keeper of the herbarium K for their effort to locate the holotype of *Tylophoron hibernicum*. The first author thanks the University of Kisangani, the Royal Museum for Central Africa and the Royal Belgian Institute of Natural Sciences for support during field work in the Democratic Republic of the Congo in 2009. We thank the Galapagos National Park, especially the technical director Washington Tapia for specimen export permits and continued support of the Galapagos Lichen Inventory. Frauke Ziemmeck (Charles Darwin Foundation) helped with collecting, data entry and curating of Galapagos material. André Aptroot contributed important Galapagos collections of '*Blarneya*'. This publication is contribution number 2033 of the Charles Darwin Foundation for the Galapagos Islands. The Galapagos Lichen Inventory received funding from the National Geographic Society (Grant #8162-2007), The Paul and Bay Foundations, Basler Stiftung, and the Erwin-Warth Stiftung. The first author received funding from the Fonds National de la Recherche Scientifique, Belgium (FNRS).

References

Chambers, S. P. & Purvis, O. W. (2009) *Blarneya* D. Hawksw., Coppins & P. James (1980). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley, eds): 219–220. London: Natural History Museum Publications & British Lichen Society.

Etayo, J. (1989) Flora liquénica epífita del Robledal de Ibardin (Navarra, España). *Anales del Jardín Botánico de Madrid* **46:** 323–332.

Etayo, J. (1992) Adiciones al catálogo de líquenes de la isla de Madeira (Portugal). *Lazaroa* **13:** 179–181.

Etayo, J. (1998) Aportación a la flora liquénica de las Islas Canarias. IV. Líquenes epifitos de La Gomera (Islas Canarias). *Tropical Bryology* **14:** 85–107.

Hawksworth, D. L., Coppins, B. J. & James, P. W. (1979) *Blarneya*, a lichenized hyphomycete from southern Ireland. *Botanical Journal of the Linnean Society* **79:** 357–367.

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Lumbsch, H. T., Lücking, R. & Tibell, L. (2009) Molecular data place *Tylophoron* as an additional calicioid genus in the Arthoniales (Ascomycota). *Bibliotheca Lichenologica* **99:** 285–296.

Lutzoni, F., Kauff, F., Cox, J. C., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T. Y., Baloch, E. *et al.* (2004) Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91:** 1446–1480.

Maddison, D. & Maddison, W. (2002) *MacClade version 4.03PPC: Analysis of Phylogeny and Character Evolution*. Sunderland, MA: Sinauer Associates.

Miądlikowska, J., McCune, B., Lutzoni, F. (2002) *Pseudocyphellaria perpetua*, a new lichen from Western North America. *Bryologist* **105:** 1–10.

Orange, A., James, P. W. & White, F. J. (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Posada, D. & Crandall, K. A. (1998) MODELTEST: Testing the model of DNA substitution. *Bioinformatics* (*Oxford, England*) **14:** 817–818.

Rambaut, A. & Drummond, A. J. (2007) *Tracer v1.5*. Available from: http://beast.bio.ed.ac.uk/Tracer.

Rehner, S. A. & Samuels, G. J. (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98:** 625–634.

Ronquist, F. & Huelsenbeck, J. P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Swofford, D. L. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony* (**and Other Methods*). Version 4. Sunderland, MA: Sinauer Associates.

Tibell, L. (1996) The anamorph of *Tylophoron moderatum*. *Mycological Research* **95:** 290–294.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172:** 4238–4246.

Yoshimura, I., Yamamoto, Y., Nakano, T. & Finnie, J. (2002) Isolation and culture of lichen photobionts and mycobionts. In *Protocols in Lichenology: Culturing, Biochemistry, Physiology and Use in Biomonitoring* (I. Kranner, R. P. Beckett & A. K. Varma, eds): 3–33. Berlin: Springer.

Zwickl, D. J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. thesis, The University of Texas at Austin.

Accepted for publication 16 *February* 2011

https://doi.org/10.1017/S002428291100020X Published online by Cambridge University Press