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Eremithallus costaricensis (Ascomycota: Lichinomycetes: Eremothallales), a new fungal lineage with a novel lichen symbiotic lifestyle discovered in an urban relict forest in Costa Rica

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Abstract

The NSF-funded TICOLICHEN biodiversity inventory in Costa Rica revealed a new fungal lineage with a novel type of lichen symbiosis, here described as Eremothallales ordo novus and Eremothallaceae familia nova, with the single species *Eremithallus costaricensis* genus et species nova, the 'Costa Rican hermit crab lichen'. Instead of forming a proper thallus enclosing the photobiont, the *Trentepohlia* algal cells are located in groups within individual periderm cells of the tree bark, from where they connect to the superficial apothecia of the lichen fungus by hyaline fungal hyphae. Such a mode of lichenization is unknown in other lichenized fungi and raises the question about the mechanics of algal cell positioning during the development of the lichen. Although showing morphological and anatomical similarities with Ostropales, molecular analysis using a three gene approach (mtSSU, nuLSU, RPB1) places the new lichen fungus outside the Lecanoromycetes. Instead, the taxon forms a separate lineage presumably close to Lichinales in the Lichinomycetes. The novel lichen fungus was found in a small forest remnant on the campus of the University of Costa Rica (Leonelo Oviedo Ecological Reserve), founded by the renown Costa Rican ecologist and conservationist Dr Luis Fournier, inmidst the extensive deforested area of the Costa Rican central valley. This underlines the importance of such refugia for conserving biodiversity and, as in this case, even previously unknown evolutionary lineages.

Keywords: Ascomycota, conservation, Leotiomycetes, Lichinomycetes, Ostropales, *Propolidium*, *Phaeographis*, *Trentepohlia*, lichenization, hermit crab, Luis Fournier

1. Introduction

The number of existing fungal species is estimated to be more than one million; yet, less than ten percent have been described (Hawksworth, 1991, 1997, 2001, 2004; Mueller et al., 2004). Fungi display the widest array of lifestyles among living organisms, ranging from saprotrophism to parasitism to symbiosis and even carnivory (Kirk et al., 2001; Mueller et al., 2004), and the lichen symbiosis plays a significant role for fungal diversification, as half of the known Ascomycota are lichenized (Kirk et al., 2001). Most of the undiscovered species occur in the tropics, where the largest fungal and lichen inventory is currently being undertaken in Costa Rica (Mueller and Halling, 1995; Mata et al., 2003; Fernández et al., 2004; Lücking et al., 2004; Rogers et al., 2004), with an estimated total of 8,000 collectable species (excluding those microfungi that have to be cultured for study). During this inventory, we discovered an epiphytic lichen fungus new to science, which displays a unique form of lichenization: instead of producing its own thallus, the algal cells are located intracellularly within the periderm of the tree bark, interconnected by fungal hyphae emerging from beneath the erumpent apothecia. The new fungus uses the periderm cells of the tree bark in a similar way as the hermit crab employs empty shells to protect its body, and we name this new lichen *Eremithallus costaricensis* (the 'Costa Rican hermit crab lichen'). Not only does *Eremithallus* exhibit a unique form of lichenization, but according to our molecular phylogenetic

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analysis falls outside the Lecanoromycetes, representing a previously unknown lineage reminiscent of Ostropales in apothecial morphology, but phylogenetically presumably more related to Lichinomycetes. The order Eremithallales and the family Eremithallaceae are introduced for this new, thus far monospecific lineage.

2. Materials and Methods

Specimens were examined both in fresh (fully hydrated) and dry (air dried) condition, using a LEICA MS 5 stereomicroscope and a ZEISS Axioscop 2 compound microscope. Images of thallus and apothecia were made with a NIKON Coolpix 5400 digital camera connected to the stereomicroscope, while images of anatomical details and ascospores were created from thin sections using a DAGE MTI DC-330 3CCD Color Camera connected to the compound microscope.

New sequence data of nuLSU rDNA, mtSSU rDNA, and RPB1 were obtained from five samples of *Eremithallus costaricensis*. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen). Dilutions (10^{-1} up to 10^{-3}) or undiluted DNA was used for PCR amplifications. Primers for amplification were nu-LSU-0155-5' (Döring et al., 2000), nu-LSU-1432-3' (LR7; Vilgalys and Hester, 1990), and nu-LSU-0948-3' (LR5; http://www.biology.duke.edu/fungi/mycolab/primers.htm) for nuLSU rDNA, mrSSU1, mrSSU2 (Zoller et al., 1999) and MSU 7 (Zhou et al., 2001) for mtSSU rDNA, and gRPB1-A (Stiller and Hall, 1997) and fRPB1-C (Matheny et al., 2002) for RPB1. The 25 µl PCR reactions contained 2.5 µl buffer, 2.5 µl dNTP mix, 2 µl of each primer (20 µM), 5 µl BSA, 2 µl Taq, 2.5 µl genomic DNA extract and 6.5 µl distilled water. Thermal cycling parameters were: initial denaturation for 3 min at 94°C, followed by 34 cycles of 45 sec at 94°C, 1 min at 50°C (mtSSU primers) or 54°C (nuLSU-0155-5'/LR6), 1:30 min at 72°C, and a final elongation for 10 min at 72°C. Amplification products were viewed on 1% agarose gels stained with ethidium bromide and subsequently purified using the QIAquick PCR Purification Kit (Qiagen) or Nucleo Spin DNA purification kit (Macherey-Nagel). PCR amplifications were performed using the following program: initial denaturation for 1 min at 96°C followed by 32 cycles of 96°C for 15 sec, 50°C for 10 sec, and 60°C for 4 min. Bands of the expected size were excised from 1% low melting point agarose TALE gels after electrophoresis. Agarose was digested using GELase (Epicentre Technologies), and purified PCR products were ligated into the 2.1-TOPO cloning vector using TOPO TA cloning kits (Invitrogen). Multiple clones were sequenced from each ligation reaction using vector primers M13F and M13R. Automated sequencing was carried out on an ABI 3730 DNA analyzer (Applied Biosystems).

Sequence fragments were assembled using SeqMan 4.03 (DNASTAR) and manually adjusted. Vector sequences were trimmed off. Of the five samples of *Eremithallus* sequences, three resulted identical to the other two and only the two different samples were used for the analysis. The new sequences were submitted to GenBank as follows (including voucher specimen collection numbers): EU622916 (*Eremithallus costaricensis* Lücking 15683 mtSSU), EU622917 (16273 mtSSU), EU622918 (15683 nuLSU), EU622919 (16273 nuLSU), EU622920 (15683 RPB1), and EU622921 (16273 RPB1).

The sequences were aligned with selected sequences of Lecanoromycetes and other Pezizomycotina from GenBank, with emphasis on Ostropomycetidae, with a larger 2-gene set comprising mtSSU and nuLSU (see Lumbsch et al., 2002, 2007 for further details on taxa and GenBank accession numbers) and a smaller 3-gene subset including also RPB1 (GenBank accession numbers given in Fig. 3). We employed an alignment procedure that uses a linear Hidden Markov Model (HMM) as implemented in the software SAM (Sequence Alignment and Modelling system) (Karplus et al., 1998) for the mitochondrial alignment. Regions that were not aligned with statistical confidence were excluded from the phylogenetic analysis. The nuLSU rDNA was aligned using Clustal X; all ambiguous regions were excluded from the alignments. The alignments were analyzed by maximum parsimony (MP) and a Bayesian approach (B/MCMC) (Huelsenbeck et al., 2001; Larget and Simon, 1999). To test for potential conflict, parsimony bootstrap analyses were performed on each individual data set, and 75% bootstrap consensus trees were examined for conflict (Lutzoni et al., 2004). Maximum parsimony analyses were performed using the program PAUP* (Swofford, 2003). Heuristic searches with 200 random taxon addition replicates were conducted with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein, 1985) was performed based on 2000 replicates with random sequence additions. The B/MCMC analyses were conducted using the MrBayes 3.1.1 program (Huelsenbeck and Ronquist, 2001). The analyses were performed assuming the general time reversible model of nucleotide substitution (Rodriguez et al., 1990), assuming a discrete gamma distribution with six rate categories. The data set was portioned into two parts (nuLSU, mtSSU) and these were allowed to have their own gamma shape parameters (Nylander et al., 2004). No molecular clock was assumed. A run with 4,000,000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file. The first 200,000 generations (the first 2000 trees) were deleted as the "burn in" of the chain. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to ensure that stationarity was achieved after the first 200,000

generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist, 2001). Of the remaining 76,000 trees (38,000 from each of the parallel runs) a majority rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 70% under MP and posterior probabilities ≥0.95 were considered as strongly supported. Phylogenetic trees were visualized using the program TREEVIEW (Page, 1996).

3. Results and Discussion

Eremithallales Lücking and Lumbsch ordo novus
Eremithallaceae Lücking and Lumbsch familia nova

Ascomycota lichenisati. Thallus lichenisatus desunt; cellulae algarum si praesentes in cellulis peridermis corticis arborum habitantes. Apothecia hemiangiocarpia. Hymenium non amyloideum, paraphysis simplicibus compositum. Asci tolis instructi, non amyloidei. Ascosporae elipsoideae, hyalinae, non amyloideae. Typus: *Eremithallus* Lücking, Lumbsch & Umaña.

Eremithallus *Lücking, Lumbsch and L. Umaña genus novum*

Thallus lichenisatus desunt; cellulae algarum ad *Trentepohliam* pertinentes, in cellulis peridermis corticis arborum habitantes, conectatis per hyphas fungorum originem in parte basalis apotheciorum habentibus. Apothecia ex cellulis peridermis corticis arborum erumpentes, cum disco atrofusco et lobulis marginalibus triangularibus ex cellulis peridermis corticis arborum formatis. Excipulum fuscum, sine crystallis. Hymenium non amyloideum, paraphysis simplicibus compositum. Asci elipsoidei, tolis instructi, non amyloidei. Ascosporae 8-nae, elipsoideae, 1-septatae, hyalinae, non amyloideae, parietibus subtiliter plicatae. Typus: *Eremithallus costaricensis* Lücking, Lizano & Chaves.

Eremithallus costaricensis *Lücking, Lizano and Chaves species nova*

Cellulae algarum 8–12 × 4–7 µm diam. Apothecia usque ad 1 mm diam., *Phaeographi lobatae* similia; lobuli marginales glaucescentes. Excipulum 20–30 µm latum. Hymenium 90–120 µm altum. Asci 80–100 × 35–45 µm. Ascosporae 20–30 × 7–8 µm.

Typus. Costa Rica. San José: Leonelo Oviedo Ecological Refuge, campus of the University of Costa Rica, San Pedro, 5 km E of the center of San José, 84° 03' W, 9° 56' N, 1200 m, lower montane moist forest zone; relict forest and regrown secondary forest, shady understory, on bark of unidentified tree; 22 Apr 2003, R. Lücking 16273 (USJ, holotype; CR, F, INBio, isotypes). Same locality, 4 Nov. 2002, R. Lücking 15683 (F, USJ, paratypes); 15 Apr 2003, H.J.M. Sipman 51178e (B, USJ, paratypes), M. Grube 11824 (GZU, USJ, paratypes); 8 Mar 2004, A. Aptroot 60034 (ABL, INB, paratypes).

Thallus superficially delimited by grey color and partially a thin, brown-black prothallus (Fig. 1A–E), but proper thallus structures (cortex, algal layer, medulla) absent; photobiont a species of *Trentepohlia*, cells 8–12 × 4–7 µm, located within the periderm cells of the tree bark and connected to the base of the apothecia by hyaline fungal hyphae (Fig. 1K–O). Apothecia erumpent from periderm, rounded, up to 1 mm diam., with dark grey-brown disc and irregular marginal lobules (remnants of the initially covering periderm; Fig. 1C–E). Proper excipulum 20–30 µm broad, dark brown, lateral towards the hymenium with anastomosing periphysoids and paraphysoids (Fig. 1F–G). Hymenium 90–120 µm high, non-amyloid, composed of unbranched paraphyses; asci ellipsoid, 80–100 × 35–45 µm, non-amyloid and with indistinct tholus (Fig. 1H–J). Ascospores 8 per ascus, ellipsoid, 1-septate, 20–30 × 7–8 µm, hyaline, non-amyloid, with folds in the wall (Fig. 1J). Chemistry: no substances detected by TLC.

Eremithallus costaricensis forms part of a rich, mid-elevation, shade epiphytic lichen community dominated by crustose Graphidaceae, Ramalinaceae, and Coenogoniaceae (Rivas Plata et al., 2006). The new lichen is remarkably similar to the common tropical species, *Phaeographis lobata* (Graphidaceae), which was found at the same site, and the two species can be distinguished with certainty only microscopically. The grey 'thallus' of *Eremithallus* superficially resembles that of other crustose bark lichens, although true thallus structures are absent. The formation of a dark prothallus, composed of pigmented fungal hyphae when bordered by other lichens (Fig. 1A), as well as the formation of a prothallus by other lichens when growing adjacent to *Eremithallus* (Fig. 1B), is a typical feature of long-lived lichenized fungi. The apothecia develop beneath the uppermost layers of the bark periderm, which ruptures forming irregular lobules and eventually expose dark greyish brown discs (Fig. 1C–E). This type of apothecial development is characteristic of many Ostropomycetidae, in particular Stictidaceae, Thelotremataceae, Asterothyriaceae, Graphidaceae, and Gomphillaceae (Henssen and Jahns, 1974; Sherwood, 1977; Henssen et al., 2002; Frisch et al., 2006), but unknown from other Ascomycota except *Propolidium* (see below). Such lichens are often incorrectly called 'endophloedal', but correctly should be termed 'endoperidermal', since they do not actually penetrate the phloem but only the outer bark layers of the periderm.

A section through an apothecium shows the proper dark brown margin or excipulum (Fig. 1F–G), covered by

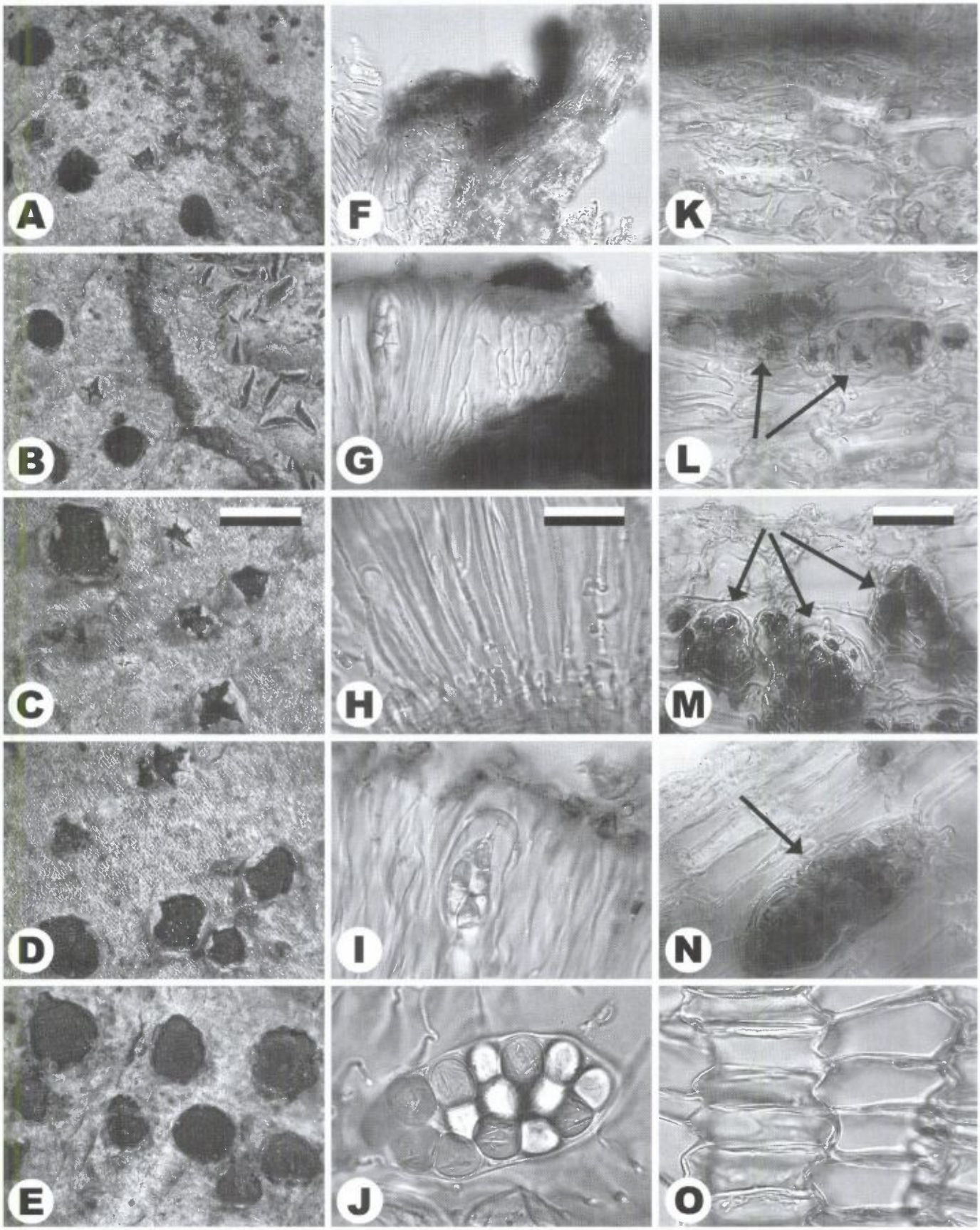

Figure 1. *See legend on the next page.*

several layers of bark periderm including algal cells (Fig. 1F). The innermost part of the excipulum and the lateral parts of the hymenium are formed by hyaline, anastomosing periphysoids and paraphysoids. The hymenium (Fig. 1H–I) is non-amyloid, composed of unbranched paraphyses and produces non-amyloid, ellipsoid asci with indistinct tholus (Fig. 1I–J). While apothecial morphology and anatomy recall Ostropomycetidae, the ellipsoid asci and the folds produced in the walls of the ascospores (Fig. 1J) are more commonly found in the perithecial Chaetothyriomycetidae and Dothideomycetidae (Aptroot, 1991; Del Prado et al., 2006).

The most unique feature of *Eremithallus*, however, is its vegetative organization (Fig. 1K–O). Instead of forming a proper thallus, its algal cells, which on account of their cell morphology and arrangement belong in the genus *Trentepohlia* s.lat. (López-Bautista et al., 2002, 2006), are located in groups within cells of the bark periderm beneath the surface (Fig. 1L–N). The algal groups are connected by hyaline fungal hyphae (Fig. 1L; arrows) that connect to a dense net of fungal hyphae emerging beneath the base of the apothecia (Fig. 1K; arrows). No other thallus structure is formed; the medulla and (pseudo-)cortex typical of most crustose bark lichens are absent from *Eremithallus*. Only the upper layers of the bark periderm are occupied by the lichen; the lower layers are empty (Fig. 1O), demonstrating that the lichen fungus does not connect to the living tissues (phloem) of the tree and hence is not a tree parasite facultatively associated with algal symbionts.

We have studied several other corticolous lichens, particularly endophloedic species, in order to assess the nature of the lichenization in *Eremithallus*. In endophloedic species, e.g. of the lichen families Graphidaceae and Thelotremataceae (Staiger, 2002; Frisch et al., 2006), periderm layers are frequently included in the thallus structure of the lichen, especially the apothecial margins. However, the algal cells never occur initially within the periderm cells; they form an irregular layer above the periderm and partly between splitting periderm layers, and only occasionally, when the lichen thallus breaks up the periderm structure, algal cells do appear within broken periderm cells (see also Frisch et al., 2006). In contrast, in *Eremithallus* the algal cells are located within individual, intact periderm cells several layers deep into the periderm. The organization of the lichenization in *Eremithallus* is thus very different from the usual thallus structure of 'endoperidermal' lichens.

The location of the algal cells of *Eremithallus* within intact, dead cells of the periderm raises a number of interesting biological and developmental questions: How do the algal cells get into the periderm cells? Are they located within the periderm cells before or after initiating symbiotic contact with the fungal hyphae? Why are groups of algal cells only found clustered in a few periderm cells and not in a continuous layer? What is the role of the mycobiont in the spatial distribution of photobiont cells? With the material at hand, all mature thalli, we have not been able to address these questions in any detail, but further studies are on their way including molecular analysis of the photobiont.

Molecular phylogeny of the new lineage

While we initially expected *Eremithallus* to fall within the order Ostropales (Ostropomycetidae), known for its diversity in lichenized and non-lichenized lineages and with similar ascomatal features (Sherwood, 1977; Henssen and Lücking, 2002; Lumbsch et al., 2007), molecular phylogeny of nuLSU, mtSSU, and RPB1 sequences places the new taxon (five samples displaying two slightly different sequence patterns) on a separate clade within Lichinomycetes (Figs. 2, 3). Thus, although the morphology and anatomy of *Eremithallus* suggests affities with Ostropales, molecular phylogenetic analysis using combined nuclear large subunit (nuLSU) and mitochondrial small subunit (mtSSU) rDNA, as well as nuclear RPB1, shows that the new taxon represents a previously unknown lineage within the Ascomycota, outside the Lecanoromycetes. Although Lichinales appear as closest ally, *Eremithallus* differs morphologically, anatomically, and in its biology fundamentally from that order, which include lichenized fungi with well-developed thalli invariably associated with cyanobacterial photobionts, found in semiaquatic habitats, and with very different ascomata development (Henssen, 1963; Schultz et al., 2001). Since the distantly related Lecanoromycetes and its two subclasses Lecanoromycetidae and Ostropomycetidae involve considerable morphological, anatomical, and biological variation among their lineages, we cannot exclude at this point that *Eremithallus* indeed forms part of Lichinomycetes and the two represent extreme morpho-biological variation in that lineage. However, we assume that the found relationship between *Eremithallus* and Lichinales is at best a preliminary result possibly reflecting a spurious relationship, and that further studies including

Figure 1. Morphology and anatomy of *Eremithallus costaricensis*. (A–E) Thalli with developing and mature apothecia, showing prothallus when bordered by other lichens. (F) Apothecial margin showing excipulum and periphysoids. (G) Lateral hymenium showing anastomosing paraphysoids and young ascus with developing ascospores. (H) Basal hymenium showing paraphyses emerging from the subhymenium. (I) Young ascus with developing ascospores showing apical tholus with narrow ocular chamber. (J) Ascus with mature ascospores showing longitudinal wall folds. (K) Fungal hyphae originating from below the apothecial base and penetrating into the upper epidermis to form contact with the symbiotic alga. (L–N) Groups of algal cells (arrows) enclosed in individual epidermis cells (showing association with fungal hyphae in G). (O) Lower epidermis showing completely empty cells lacking both fungal hyphae and algal cells.

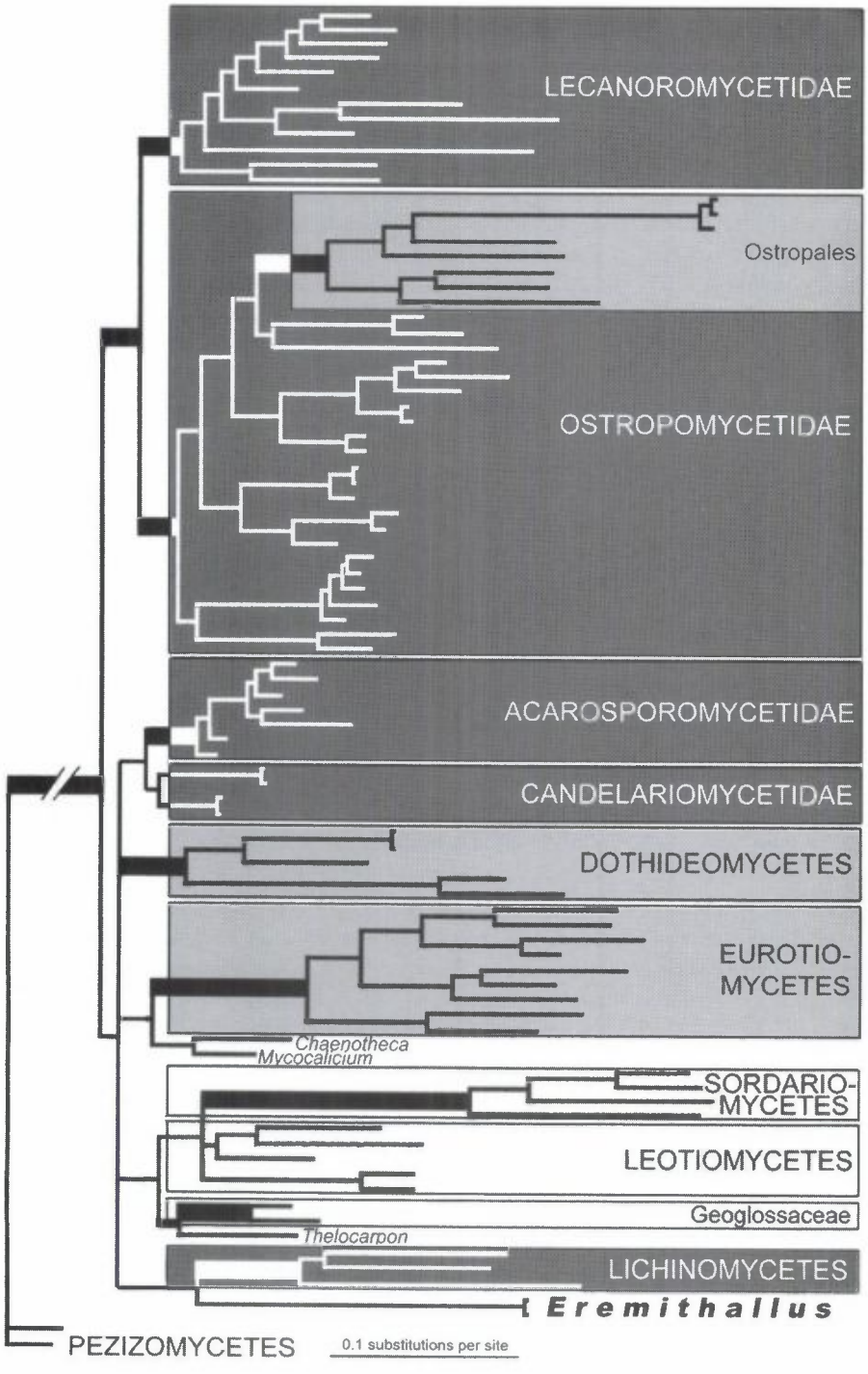

Figure 2. Bayesian analysis of combined mtSSU and nuLSU sequences including *Eremothallus costaricensis* (complete taxon set). Thick lines indicate 100%, moderately thick lines >95% posterior probabilities. Boxes indicate major lineages (dark grey with white lines: chiefly lichenized; white: non-lichenized; light grey: mixed lichenized and non-lichenized). Most of the topology is recovered with strong support in congruence with previous analyses (Lutzoni et al., 2001, 2004; Lumbsch et al., 2002; Liu and Hall, 2004; James et al., 2006; Miadlikowska et al., 2006; Sparafora et al., 2006), except for Acarosporomycetidae and Candelariomycetidae (both elsewhere supported as basal lineages in Lecanoromycetes), and Geoglossaceae (elsewhere sister to Lichinomycetes).

more material especially of Leotiomycetes s.lat. might reveal that *Eremithallus* deserves its own class. We therefore did not employ further testing of the data, since the taxon sampling for these groups of fungi is currently quite insufficient.

Apart from Ostropales, which fall on a distant part of the tree unrelated to *Eremithallus*, we are only aware of one taxon in Ascomycota that is similar to *Eremithallus*, the non-lichenized, saprophytic *Propolidium glaucum* (Sherwood, 1977; Johnston, 2001). *Propolidium* shares the ellipsoid, one-celled ascospores and non-amyloid hymenium and asci with *Eremithallus*, but differs in calcium oxalate depositions in the margin of the apothecia, cylindrical asci, and thick-walled ascospores lacking longitudinal wall folds. In addition, its ascus tips feature an I+ amyloid ring structure. *Propolidium* is currently placed

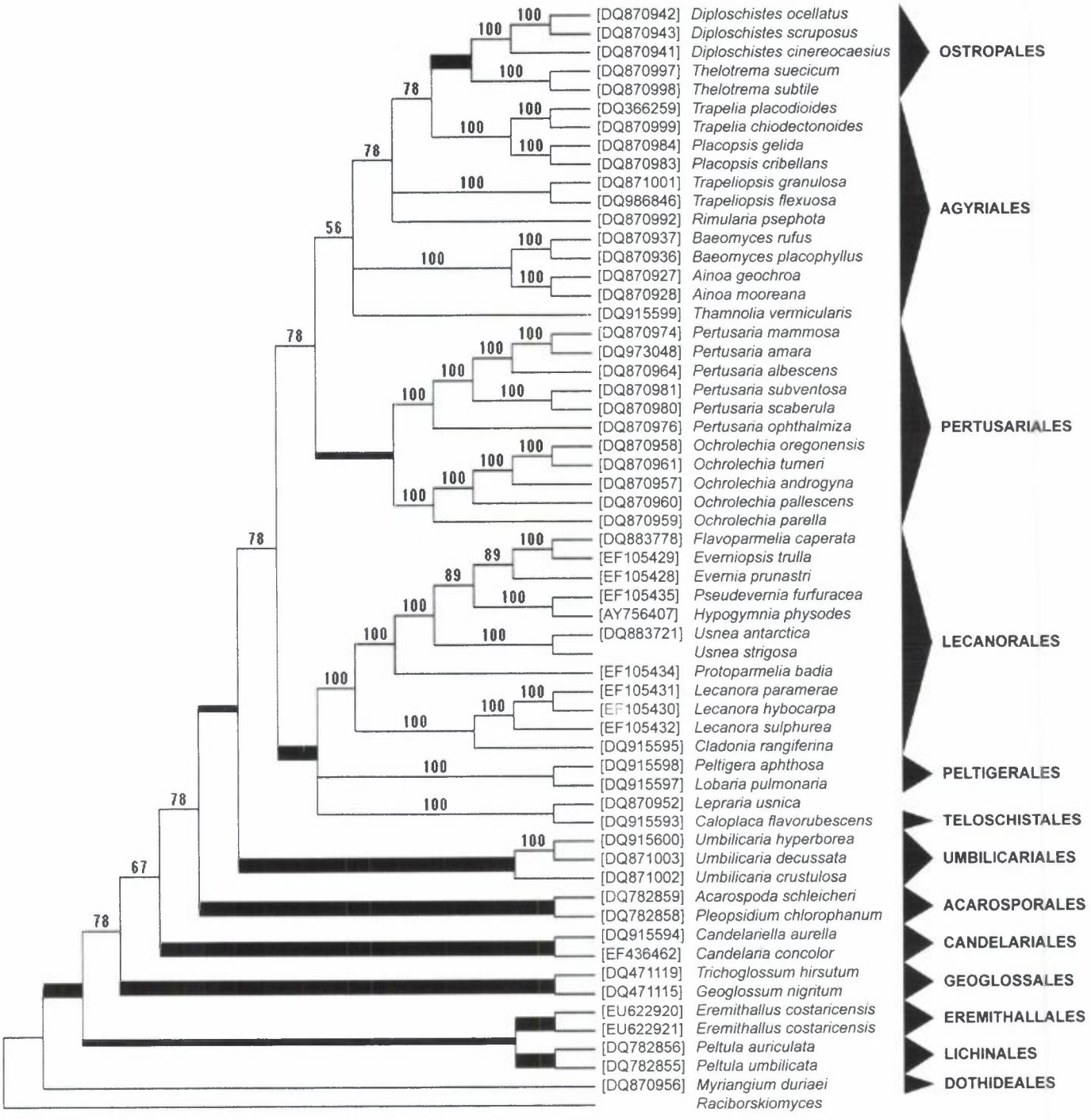

Figure 3. Maximum parsimony analysis of combined mtSSU, nuLSU, and RPB1 sequences (reduced taxon set) including *Eremothallus costaricensis*. Thick lines indicate 90% or higher, moderately thick lines 70% or higher bootstrap support. Major lineages are recovered in congruence with previous analyses (Lutzoni et al., 2001, 2004; Lumbsch et al., 2002; Liu and Hall, 2004; James et al., 2006; Miadlikowska et al., 2006; Sparafora et al., 2006). Number in [brackets] indicate RPB1 accession numbers for each taxon.

in Rhytismatales in Leotiomycetes (Korf, 1973; Johnston, 2001), although its remarkable similarity with ostropalean fungi has been recognized (Sherwood, 1977). Leotiomycetes s.str. comprise exclusively non-lichenized fungi with sessile, superficial apothecia and a different type of hymenium and asci (Korf, 1973; Verkley, 1994; Wang et al., 2006). Leotiomycetes s.lat., on the other hand (Lumbsch and Huhndorf, 2007) are one of the most problematic

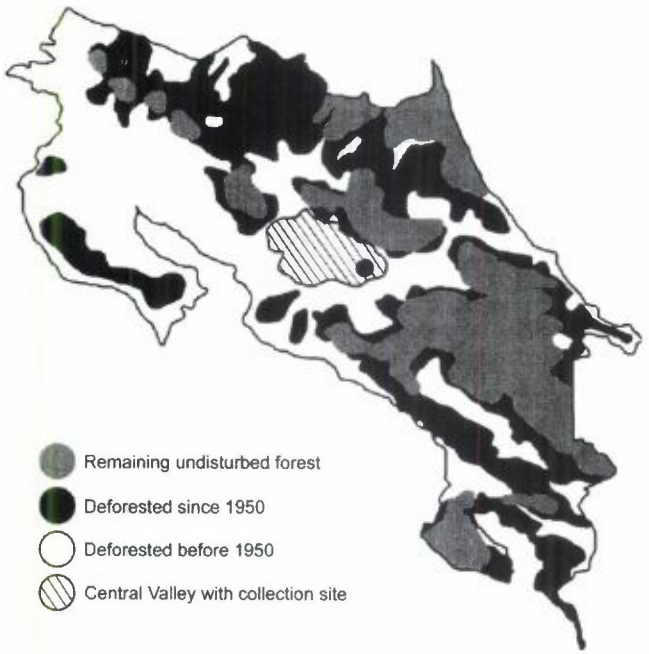

Figure 4. Type locality of *Eremithallus costaricensis*. The new lichen fungus was found in a relict forest (Leonelo Oviedo Ecological Reserve) on the campus of the University of Costa Rica in the capital, San José. The map indicates remaining undisturbed forest and deforested areas in Costa Rica.

classes of Ascomycota, likely to disintegrate into several lineages (Lutzoni et al., 2001, 2004; Lumbsch et al., 2002; Wang et al., 2006). Unfortunately, no molecular data exist for *Propolidium* and many other critical taxa in Leotiomycetes s.lat., but that genus is probably unrelated to either Lecanoromycetes or *Eremithallus*. However, its ostropoid morphology supports the notion that ostropoid fungi have evolved several times independently within Ascomycota, now representing at least three unrelated main lineages: *Propolidium* in the Leotiomycetes s.lat., *Eremithallus* in the Lichinomycetes, and Ostropales in the Lecanoromycetes.

Conservation

The new lichen fungus, *Eremithallus costaricensis*, is known from several collections made during the NSF-funded TICOLICHEN Costa Rican lichen biodiversity inventory (Lücking et al., 2004). All specimens originate from the Leonelo Oviedo Ecological Reserve (Di Stéfano et al., 1996; http://www.biologia.ucr.ac.cr/estaciones.html #LO), a small relict forest on the campus of the University of Costa Rica in the capital, San José (Fig. 3), founded 40 years ago by the renown Costa Rican botanist and conservationist, the late Luis Fournier (Fournier, 1977; Morales, 2002). The surrounding Central Valley, once covered by lush mixed forest, is the most densely populated and most heavily polluted area in the country and now almost completely deforested (Sanchez-Azofeifa et al., 2001; Campbell, 2002; Bonilla-Carrion and Rosero-Bixby, 2004). Ongoing studies indicate that the University campus and reserve shelter many plant, animal, and fungal species now extinct from the surroundings (Di Stéfano et al., 1996; Huber, 1998; Hansson and Nishida, 2002), and our lichen survey detected a number of rare and even new species occurring nowhere else in Costa Rica (Rivas Plata et al., 2006), exemplifying the unique value that this reserve has for conserving the biodiversity of the Central Valley.

The discovery of this novel lichen fungus underlines the role of biotic inventories of understudied regions and little known fungal groups, not only for cataloging the world's biodiversity, but also with regard to the evolution of life on this planet. An increasing number of large-scale molecular phylogenetic studies have changed our understanding of the evolution and classification of Ascomycota (Lutzoni et al., 2001, 2004; Lumbsch et al., 2002; Liu and Hall, 2004; James et al., 2006; Miadlikowska et al., 2006; Sparafora et al., 2006). Yet, many lineages require extensive sampling of tropical taxa to be fully understood. The discovery of *Eremithallus* also demonstrates the importance of habitat protection for the conservation of biodiversity, even if dealing with a tiny relict forest inmidst a densely populated, strongly polluted, and largely deforested area, as exemplified by the Leonelo Oviedo Ecological Reserve in Costa Rica.

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phylogeny and systematics of Ostropalean fungi and lichens. Joey Spataphora and an anonymous reviewer commented on a previous version of this manuscript and helped to improve it considerably.

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