*The Lichenologist* **42**(4): 365–371 (2010) 
doi:10.1017/S0024282910000058

# A new species of *Elixia* (*Umbilicariales*) from Greece

**Toby SPRIBILLE and H. Thorsten LUMBSCH**

**Abstract:** *Elixia cretica* T. Sprib. & Lumbsch is described as a new species from the mountains of western Crete. The second member of the previously monotypic genus and only the third member of the family *Elixiaceae*, it is distinguished by its surficial thallus, larger ascospores and corticolous habit. Molecular phylogenetic analysis based on a sequence of mitochondrial small subunit DNA confirms the position of the new species. We also report *E. flexella* from New Hampshire (USA) as new to eastern North America.

**Key words:** Ascomycota, *Elixiaceae*, Lecanoromycetes, phylogenetic analysis

## Introduction

During field work in 2004 on Crete, the first author discovered a bizarre lichen with lirelloid apothecia growing on the bark of *Pinus brutia*, and subsequently showed the specimen to several colleagues. The lichen initially defied any attempt at identification, but eventually it became clear that it had affinities to the genus *Elixia*. In 2008 we obtained DNA from the sample and were able to test this hypothesis and confirm that the lichen is, indeed, an *Elixia*, only the second species known in the genus and the third species in the family *Elixiaceae* (after the Tasmanian *Meridianelia maccarthyana*; see Kantvilas & Lumbsch 2009). We delayed describing the taxon as new until now, in the hopes of acquiring new or more substantial material, since all of our knowledge of this lichen is based on a single collection. However, in our view three considerations have come to outweigh our hesitation to publish: 1) the species is clear-cut morphologically and there is no overlap with related taxa, 2) DNA evidence confirms our hypothesis and 3) one may wait a long time to obtain more material of rare taxa, and in the meantime conservationists and lichenologists could make use of the data to search for more or ensure protection of known habitats. We therefore describe the taxon here as new and hope that doing so encourages lichenologists to search for and find more.

T. Spribille: Institute of Plant Sciences, University of Graz, Holteigasse 6, A-8010 Graz, Austria. Email: toby.spribille@uni-graz.at
H. T. Lumbsch: The Field Museum, Department of Botany, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA.

## Material and Methods

Lichen specimens were investigated using material mounted in water or Lugol's iodine (I) and examined using a Zeiss Axioskop microscope fitted with an AxioCam MRc5 digital camera. Ascospore measurements are given as smallest measurement–(mean)–largest measurement.

*Selected additional material studied*. *Elixia flexella* (Ach.) Lumbsch. **Austria:** *Kärnten*: Nationalpark Hohe Tauern, 1988, *Hafellner* 28652 (GZU); Salzburg, Hüttwinkl Tal S von Rauris, 1978, *Hafellner* 2851 (GZU).—**Italy:** *Veneto*: Passo Tre Croci, 1985, *Mayrhofer* 8304 (GZU).—**Romania:** Vályе Valeriaszka, Retezat, 08 viii 1872, *Lojka* (GZU).—**USA:** *New Hampshire*: Jefferson Notch, next to parking area, 2009, *T. Spribille* 31212 & *V. Wagner* (GZU, NY).

### Molecular Methods

Sequence data of 64 species were assembled using sequences of the mitochondrial small subunit rDNA (Table 1) with a newly obtained sequence extracted from the holotype of *Elixia cretica* [GenBank no: GQ892058] using apothecial material. The taxon sampling includes different clades of Lecanoromycetes and taxa that were previously believed to be close to *Elixia* or recently shown to be close to this genus (Lumbsch 1997; Wedin *et al.* 2005; Miądlikowska *et al.* 2006). Attempts

TABLE 1. *Species and GenBank sequences included in this study. Family placement follows Lumbsch & Huhndorf (2009).*

| Name | Family | mtSSU |
|---|---|---|
| *Ainoa geochroa* | *Trapeliaceae* | DQ871015 |
| *A. mooreana* | *Trapeliaceae* | AY212850 |
| *Arctomia delicatula* | *Arctomiaceae* | AY853307 |
| *A. teretiuscula* | *Arctomiaceae* | DQ007349 |
| *Aspicilia caesiocinerea* | *Megasporaceae* | DQ780271 |
| *A. cinerea* | *Megasporaceae* | DQ780272 |
| *Baeomyces placophyllus* | *Baeomycetaceae* | AY300878 |
| *B. rufus* | *Baeomycetaceae* | DQ871016 |
| *Boreoplaca ultrafrigida* | Unclassified Lecanoromycetes | AY853312 |
| *Caloplaca flavorubescens* | *Teloschistaceae* | AY143403 |
| *Cladonia rangiferina* | *Cladoniaceae* | AY300881 |
| ***Elixia cretica*** | ***Elixiaceae*** | **GQ892058** |
| *E. flexella* | *Elixiaceae* | AY853320 |
| *Everniopsis trulla* | *Parmeliaceae* | EF108289 |
| *Gregorella humida* | *Arctomiaceae* | AY853329 |
| *Ionaspsis lacustris* | *Hymeneliaceae* | AY853323 |
| *Hypocenomyce scalaris* | *Ophioparmaceae* | AY853325 |
| *Lecanora hybocarpa* | *Lecanoraceae* | EF105417 |
| *L. paramerae* | *Lecanoraceae* | EF105418 |
| *Lobothallia radiosa* | *Megasporaceae* | DQ780274 |
| *Loxospora ochrophaea* | *Sarrameanaceae* | DQ871017 |
| *Meridianelia maccarthyana* | *Elixiaceae* | FJ763185 |
| *Ochrolechia androgyna* | *Ochrolechiaceae* | AY300897 |
| *O. oregonensis* | *Ochrolechiaceae* | DQ780276 |
| *O. pallescens* | *Ochrolechiaceae* | DQ780277 |
| *O. parella* | *Ochrolechiaceae* | AF320173 |
| *O. turneri* | *Ochrolechiaceae* | AY567982 |
| *Ophioparma ventosa* | *Ophioparmaceae* | AY853331 |
| *Orceolina antarctica* | *Trapeliaceae* | AY212852 |
| *O. kerguelensis* | *Trapeliaceae* | AF381561 |
| *Pertusaria albescens* | *Pertusariaceae* | AF329175 |
| *P. corallina* | *Pertusariaceae* | AY300901 |
| *P. corallophora* | *Pertusariaceae* | DQ780285 |
| *P. gibberosa* | *Pertusariaceae* | DQ780289 |
| *P. hemisphaerica* | *Pertusariaceae* | AF381563 |
| *P. lactea* | *Pertusariaceae* | AF381564 |
| *P. leioplaca* | *Pertusariaceae* | AY300903 |
| *P. mesotropa* | *Pertusariaceae* | DQ780292 |
| *P. ophthalmiza* | *Pertusariaceae* | AY567993 |
| *P. pertusa* | *Pertusariaceae* | AF381565 |
| *P. subventosa* | *Pertusariaceae* | AY300905 |
| *P. velata* | *Pertusariaceae* | AY300906 |
| *Placopsis cribellans* | *Trapeliaceae* | DQ871018 |
| *P. gelida* | *Trapeliaceae* | AY212859 |
| *P. santessonii* | *Trapeliaceae* | AY212867 |
| *Placynthiella icmalea* | *Trapeliaceae* | AY212870 |
| *Protoparmelia badia* | *Parmeliaceae* | AF351179 |
| *Rhizocarpon sphaerosporum* | *Rhizocarpaceae* | AY853340 |
| *Rimularia psephota* | *Trapeliaceae* | DQ871019 |
| *Schaereria corticola* | *Schaereriaceae* | AY300909 |
| *Sporastatia polyspora* | *Catillariaceae* | AY584724 |
| *S. testudinea* | *Catillariaceae* | AY584725 |
| *Thamnolia vermicularis* | *Icmadophilaceae* | AY853345 |
| *Trapelia chiodectonoides* | *Trapeliaceae* | AY212873 |

TABLE 1. *Continued*

| Name | Family | mtSSU |
|---|---|---|
| *T. placodioides* | *Trapeliaceae* | AF431962 |
| *Trapeliopsis flexuosa* | *Trapeliaceae* | AY212875 |
| *T. granulosa* | *Trapeliaceae* | AF381561 |
| *T. percrenata* | *Trapeliaceae* | AY212876 |
| *Tremolecia atrata* | *Hymeneliaceae* | AY853347 |
| *Umbilicaria crustulosa* | *Umbilicariaceae* | AY300919 |
| *U. decussata* | *Umbilicariaceae* | DQ871021 |
| *U. hyperborea* | *Umbilicariaceae* | AY853349 |
| *Wawea fruticulosa* | *Arctomiaceae* | DQ871023 |

to obtain DNA sequences of the new species for additional genes, such as ITS and nu LSU rDNA, were unsuccessful. Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the instructions of the manufacturer. Dilutions ($10^{-1}$ up to $10^{-2}$) of DNA were used for PCR amplifications. Primers for amplification were: mr SSU1 (Zoller *et al.* 1999) and MSU 7 (Zhou & Stanosz 2001). The 25 µl PCR reactions contained 2·5 µl buffer, 2·5 µl dNTP mix, 1 µl of each primer (10 µM), 5 µl BSA, 2 µl Taq (0·5 U/µl), 2 µl genomic DNA extract and 9 µl distilled water. Thermal cycling parameters were: initial denaturation for 3 min at 95 °C, followed by 30 cycles of 1 min at 95 °C, 1 min at 52 °C, 1 min at 73 °C, and a final elongation for 7 min at 73 °C. Amplification products were viewed on 1% agarose gels stained with ethidium bromide and subsequently purified using the QIAquick PCR Purification Kit (Qiagen). Fragments were sequenced using the Big Dye Terminator reaction kit (ABI PRISM, Applied Biosystems). Sequencing and PCR amplifications were performed using the same sets of primers. Cycle sequencing was executed with the following program: 25 cycles of 95 °C for 30 sec, 48 °C for 15 sec, 60 °C for 4 min. Sequenced products were precipitated with 10 µl of sterile $dH_2O$, 2 µl of 3 M NaOAc, and 50 µl of 95% EtOH before they were loaded on an ABI 3100 (Applied Biosystems) automatic sequencer. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and manually adjusted.

### Sequence Alignments and Phylogenetic Analysis

Alignments were done using Clustal W (Thompson *et al.* 1994). Ambiguously aligned regions were removed manually. The alignments were analysed by maximum likelihood (ML) and a Bayesian approach (B/MCMC). Maximum likelihood analyses were performed using the program GARLI (Zwickl 2006) employing the general time reversible model of nucleotide substitution (Rodriguez *et al.* 1990) including estimation of invariant sites, assuming a discrete gamma distribution with six rate categories. Bootstrapping (Felsenstein 1985) was performed based on 2000 replicates. The B/MCMC analyses were conducted using the MrBayes 3.1.1 program (Huelsenbeck & Ronquist 2001). The analyses were performed assuming the general time reversible model of nucleotide substitution (Rodriguez *et al.* 1990) including estimation of invariant sites, assuming a discrete gamma distribution with six rate categories. A run with 8 000 000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file. The first 400 000 generations (i.e. the first 4000 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 check whether stationarity was achieved after the first 400 000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist 2001). Additionally, we used AWTY (Nylander *et al.* 2007) to compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining 152 000 trees (76 000 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 ML and posterior probabilities $\geq$ 0·95 were considered as strongly supported. Phylogenetic trees were visualized using the program Treeview (Page 1996).

## Results and Discussion

### Phylogenetic analyses

The new sequence of *Elixia cretica* was aligned with sequences obtained from GenBank as listed in Table 1. A matrix of 715 unambiguously aligned nucleotide position characters was produced; 315 characters in the alignment were constant. ML analysis yielded a maximum likelihood tree that did not contradict the Bayesian tree

topology. In the B/MCMC analysis of the combined data set, the likelihood parameters in the sample had the following mean (Variance): LnL = −9238·206 (0·19), base frequencies π(A) = 0·332 (0·00023), π(C) = 0·146 (0·00021), π(G) = 0·206 (0·00024), π(T) = 0·316 (0·00026), rate matrix r(AC) = 0·113 (0·009), r(AG) = 0·28 (0·0008), r(AT) = 0·132 (0·0004), r(CG) = 0·005 (0·0001), r(CT) = 0·354 (0·0004), r(GT) = 0·007 (0·0001), the gamma shape parameter alpha = 0·773 (0·0076), and p(invar) = 0·32 (0·00151).

Since the topologies of the ML and B/MCMC analyses did not show any strongly supported conflicts, only the tree of the ML analysis is shown (Fig. 1). *Elixia cretica* clusters strongly supported with *E. flexella* (ML-bootstrap support 99%, B/MCMC posterior probability 1·0); the two *Elixia* species form a strongly supported sister-group with *Meridianelia maccarthyana* and this thus supports placement of the new taxon in *Elixiaceae*. The supported nodes in the topology of the other parts of the phylogenetic tree are in agreement with previously published phylogenies (Lumbsch *et al.* 2007*a*, *b*) and are consequently not discussed further here.

## Taxonomy

### Elixia cretica T. Sprib. & Lumbsch sp. nov.

Mycobank No. MB 515175

*Elixiae flexellae* similis sed thallo bene evoluto, apotheciis magnis, ascosporis magnis et hymeniis non-euamyloideis differt.

Typus: Greece, Kriti, Chania Prefecture, Levka Ori, just W of Agii Theodori, *c.* 3 km SW of Omalos, between mountains Mavri Kimite and Tourli, 35°19.5′N, 23°52.2′E, on bark of *Pinus brutia* near base of tree, 1125 m, 10 June 2004, *T. Spribille* 13340 (GZU—holotypus; B—isotypus).

(Fig. 2B–D)

*Thallus* crustose, areolate to cracked areolate or ±confluent-rimose in places, thin, not >50 μm thick; areoles 0·4–0·5(–0·6) mm, brownish to greenish in sheltered bark cracks, matt; *soredia* and *isidia* absent. *Photobiont* chlorococcoid, cells to 10–11 μm.

*Ascomata* lirelloid apothecia, young apothecia relatively simple lirellae, elongate with two to three ends, disc initially concealed, developed later into larger apothecia with exposed discs and up to seven points on star-shaped ascomatum; *apothecia* at their widest ranging from 0·5–1·4 mm diam.; margin jet black, matt, protruding above disc, often appearing inrolled, creases developed at apothecial corners; disc dark brown but lighter than the margin, matt. *Excipulum* in section 15–20 μm wide, darkly pigmented to near carbonaceous, of paraplectenchymatous cells; hypothecium occupying a narrow zone between excipulum and hymenium, of light brown or reddish brown paraplectenchymatous cells, to 10 μm thick; *hymenium* basically colourless, 55–60 μm deep, of conglutinated paraphyses not easily separable in water, deep rusty red in I but blue after pretreatment with KOH; *paraphyses* septate, unbranched, 1·5–2·0 μm wide, with strongly expanded tips to 3·0–5·0 μm, the tips with dark brown pigment inclusions that combine with diffuse brownish pigment to form an epihymenium; *asci* cylindrical to clavate, 34–40 × 10–13 μm, I+ blue after KOH, with light amyloid tissue throughout but darker staining regions located around the apex and in a narrow zone below the inner upper wall of the tholus ('*Elixia* type' *sensu* Lumbsch 1997, see Fig. 2D); *ascospores* 8 per ascus, ellipsoid, simple, colourless or faintly light brown in water, 7·0–(8·2)–10·0 × 3·2–(3·7)–4·5 μm.

*Pycnidia* not seen.

*Chemistry*. No substances detected by thin layer chromatography; all thallus spot tests negative.

*Distribution*. Currently known only from the type locality on the island of Crete (Greece).

*Comments*. *Elixia cretica* differs from the only other species known in the genus, *E. flexella* (Fig. 2A), in possessing a well-developed surficial thallus (Fig. 2B), which

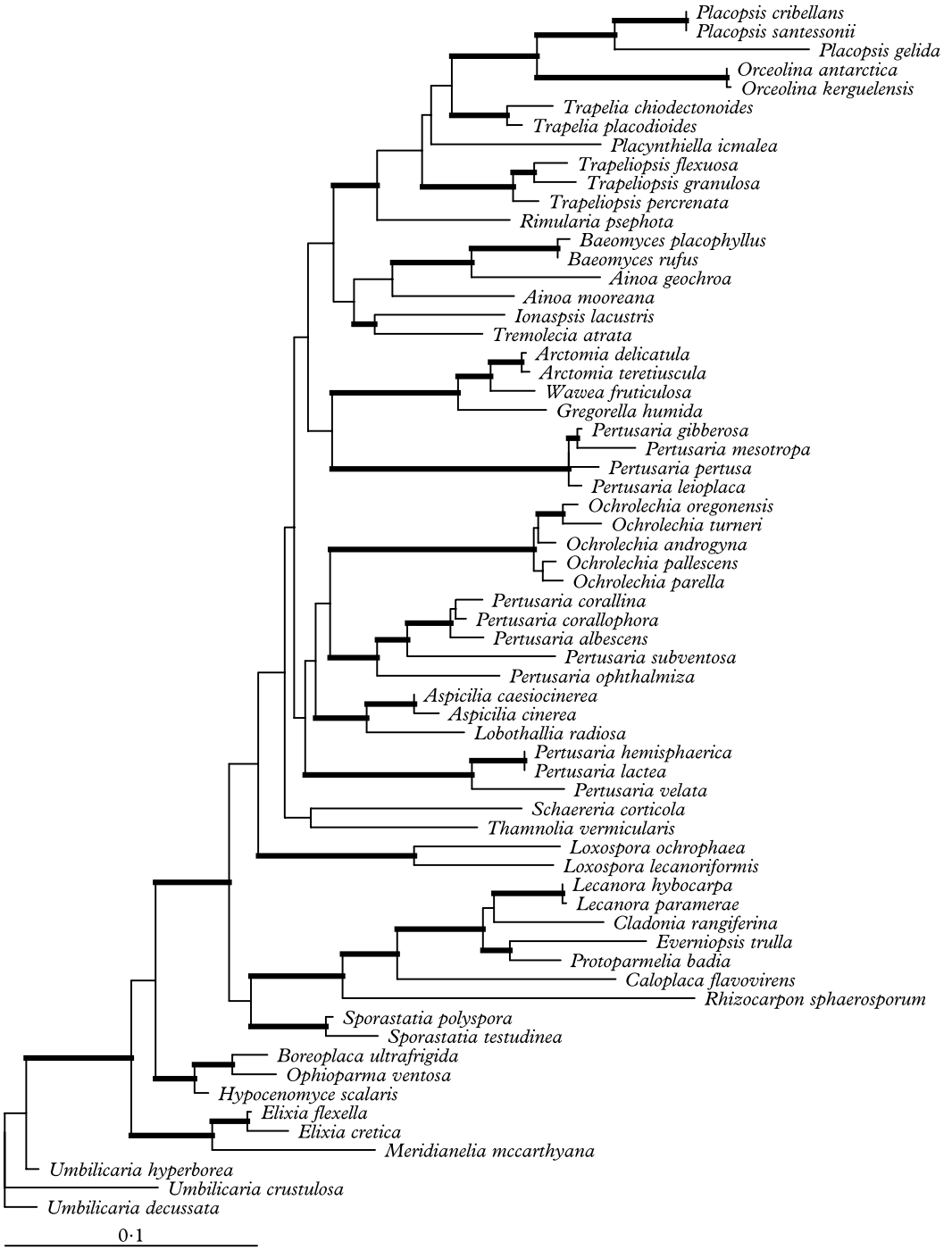


FIG. 1. Phylogeny of Lecanoromycetes as inferred from a mtSSU rDNA analysis to investigate the phylogenetic placement of *Elixia cretica*. Maximum likelihood tree obtained using the program GARLI. Branches with posterior probabilities equal to or above 0·95 and ML bootstrap support values above 70% are indicated by wide internodes.

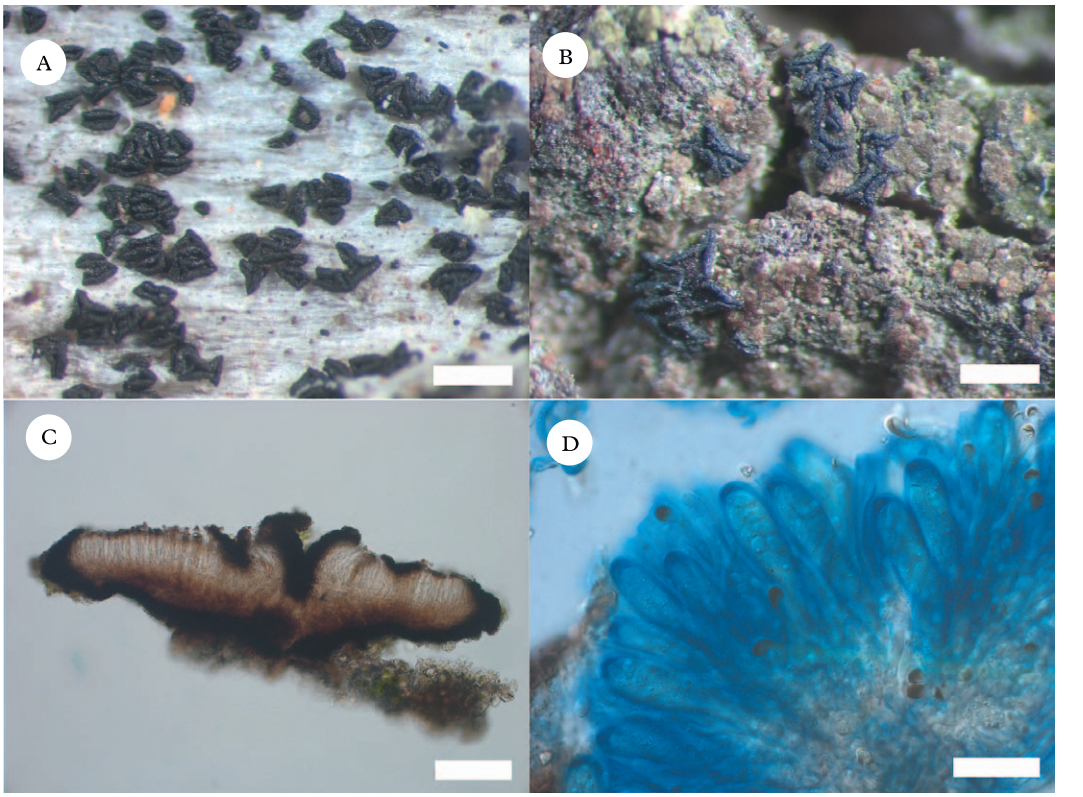


FIG. 2. *Elixia* species. A, *E. flexella*, habit (Austria, *Hafellner* 28652, GZU). B–D, *E. cretica* (holotype); B, habit; C, section of apothecium in water; D, asci and paraphyses in Lugol's after pretreatment with KOH. Scale: A & B = 1 mm; C = 100 µm; D =10 µm.

somewhat recalls that of some *Hypocenomyce* species. Anatomical details of the apothecia of *E. flexella* have been described by Lumbsch (1997) and Coppins (1992, as *Ptychographa*) and likewise differ on several counts from our new species. Apothecia tend to be substantially larger in *E. cretica* than in *E. flexella* (0·5–1·4 mm versus 0·2–0·5 mm; no apothecia > 0·5 mm have been seen in *E. flexella*) and develop a free, expanded disc, whereas in many specimens of *E. flexella* the disc remains concealed and larger apothecia instead develop a substantial umbo or become strongly gyrose. Interestingly, the hymenium in *E. cretica* reacts I+ rusty red (hemiamyloid) if not pre-treated with K, whereas in *E. flexella* it reacts blue (euamyloid). The ascospores of *E. cretica* are also longer than those in *E. flexella* (7–10 µm in *cretica* versus 5–8 µm in *flexella*) and paraphysis tips more expanded (to 5 µm in *E. cretica* versus usually < 3 µm in *E. flexella*).

The two species of *Elixia* are also ecologically and geographically distinct. *Elixia flexella* is a temperate-boreal species; it is known from scattered records in Europe, Asia and North America (Spribille & Björk 2008), with the first record for eastern North America reported here (see 'selected additional material studied' above). It appears that all but one of these (a record from Idaho, USA.) are from wood, and many are associated with calicioid lichens such as *Calicium glaucellum* or *C. trabinellum*. The only collection of *E. cretica* to date was from bark of *Pinus brutia*, where it was closely associated with *Caloplaca herbidella*.

This study was supported financially by a start up fund of the Field Museum to HTL and a Field Museum scholarship to TS. The newly obtained DNA sequence was generated in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum. Fabian Ernemann (Chicago) assisted with laboratory work.

## References

Coppins, B. J. (1992). *Ptychographa*. In *Lichen Flora of Great Britain and Ireland* (O. W. Purvis, B. J. Coppins, D. L. Hawksworth, P. W. James & D. M. Moore, eds): 514. London: Natural History Museum.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

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

Kantvilas, G. & Lumbsch, H. T. (2009) *Meridianelia*, a new genus in the *Elixiaceae* (Ascomycota) from Tasmania. *Lichenologist* **41:** 261–270.

Lumbsch, H. T. (1997) Systematic studies in the suborder Agyriineae (Lecanorales). *Journal of the Hattori Botanical Laboratory* **83:** 1–73.

Lumbsch, H. T. & Huhndorf, S. (2009) Outline of Ascomycota – 2009. *Persoonia* **23:** in press.

Lumbsch, H. T., Schmitt, I., Lücking, R., Wiklund, E. & Wedin, M. (2007*a*) The phylogenetic placement of Ostropales within Lecanoromycetes (Ascomycota) revisited. *Mycological Research* **111:** 257–267.

Lumbsch, H. T., Archer, A. W. & Elix, J. A. (2007*b*) A new species of *Loxospora* (lichenized Ascomycota: Sarrameanaceae) from Australia. *Lichenologist* **39:** 509–517.

Miądlikowska, J., Kauff, F., Hofstetter, V., Fraker, E., Grube, M., Hafellner, J., Reeb, V., Hodkinson, B. P., Kukwa, M., Lücking, R. *et al.* (2006) New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* **98:** 1088–1103.

Nylander, J. A. A., Wilgenbusch, J. C., Warren, D. L. & Swofford, D. L. (2007) AWTY (Are We There Yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24:** 581–583.

Page, R. D. M. (1996) Treeview: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12:** 357–358.

Rodriguez, F., Oliver, J. F., Martín, A. & Medina, J. R. (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

Spribille, T. & Björk, C. R. (2008) New records and range extensions in the North American lignicolous lichen flora. *Mycotaxon* **105:** 455–468.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Wedin, M., Wiklund, E., Crewe, A., Döring, H., Ekman, S., Nyberg, Å., Schmitt, I & Lumbsch, H.T. (2005) Phylogenetic relationships of Lecanoromycetes (Ascomycota) as revealed by analyses of mtSSU and nLSU rDNA sequence data. *Mycological Research* **109:** 159–172.

Zhou, S. & Stanosz, G. R. (2001) Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* **105:** 1033–1044.

Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* **31:** 511–516.

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* 08 *January* 2010