Meridianelia, a new genus in the Elixiaceae (Ascomycota) from Tasmania

Gintaras KANTVILAS and H. Thorsten LUMBSCH

Abstract: *Meridianelia maccarthyana* Kantvilas & Lumbsch, gen. et sp. nov., is described, based on collections from Tasmania. This taxon represents only the second member of the family *Elixiaceae*. It is compared to *Elixia flexella* (Ach.) Lumbsch and to the superficially similar genus *Trapelia*. The family *Elixiaceae* is redefined briefly to account for the inclusion of a second genus.

Key words: lichens, flora of Australia, phylogenetic analysis, Lecanoromycetes, Elixia, Trapelia

Introduction

During the course of the ongoing project to document the lichen flora of the island of Tasmania, a curious epiphytic species was collected in subalpine woodland. This lichen displayed an unusual combination of characters: hemiangiocarpous ascomata, a reduced, cupulate excipulum, delicate, branched and anastomosing paraphyses, eight-spored, lecanoralean asci, and simple, hyaline ascospores. Whereas some of these attributes are also found in the genus Trapelia M. Choisy, others suggested alternative relationships within the Agyriinae in the sense of Lumbsch (1997). The systematic position of this lichen was ultimately resolved using DNA sequence data that supported our earlier anatomical observations, particularly those pertaining to ascus structure, which had hinted at links with the monotypic family, Elixiaceae. We describe this lichen as a new monotypic genus, Meridianelia, within this unique family of lichenized fungi.

The *Elixiaceae* was erected by Lumbsch (1997) to accommodate the single species *Elixia flexella* (Ach.) Lumbsch. The relation-

ships of this lichen to other seemingly related groups, such as the families Agyriaceae (including Trapeliaceae), Anamylopsoraceae and Schaereriaceae, were elucidated in detail therein (Lumbsch op. cit.). These families have subsequently undergone considerable scrutiny using molecular data. The Agyriaceae was shown to be unrelated to the Trapeliaceae, but grouped with the Pertusariales (Lumbsch et al. 2007b). The placement of the Anamylopsoraceae needs further studies, but preliminary studies suggest it may not be closely related to the *Trapeliaceae* either (Lumbsch et al. 2001). The Schaereriaceae is also shown to be not closely related to the Trapeliaceae, but again, the relationships remain uncertain (Wedin et al. 2005). In fact, the *Elixiaceae* appears to be closest to the Umbilicariaceae (Lumbsch et al. 2004; Wedin et al. 2005).

Materials and Methods

Morphology, anatomy and chemistry

The study is based chiefly on collections housed in the Tasmanian Herbarium (HO) and the Field Museum (F). Anatomical and morphological observations were undertaken using light microscopy, with thin hand-cut sections mounted in water, 10% KOH (K), lactophenol Cotton Blue and Lugol's iodine, either with prereatment with KOH (indicated as K/I) or without (I). Ascospore measurements are based on at least 100 observations and are presented in the format: smallest measurement—mean—largest measurement; single outlying values are given in parentheses. Nomenclature of

G. Kantvilas: Tasmanian Herbarium, Private Bag 4, Hobart, Tasmania 7001, Australia. Email: gkantvilas@tmag.tas.gov.au

H. T. Lumbsch: The Field Museum, Department of Botany, 1400 S Lake Shore Drive, Chicago, IL 60605, USA.

asci follows Lumbsch (1997). Nomenclature of apothecial pigments follows Meyer & Printzen (2000), although we used concentrated acids for N (HNO $_3$) and H (HCl), whereas Meyer & Printzen used 50% N and 10–20% H. Chemical constituents were identified by thin layer chromatography (Culberson 1972; Culberson et al. 1981; Culberson & Johnson 1982; Elix & Ernst-Russell 1993).

Molecular methods

Sequence data of 70 species were assembled using sequences of the mitochondrial small subunit rDNA (Table 1) with a newly obtained sequence extracted from the isotype of Meridianelia maccarthyana [Genbank no: F[763185]. Three Umbilicaria species were used as the out-group since the genus has a basal placement in Lecanoromycetes in previous studies (e.g., Lumbsch et al. 2007a,c). Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the instructions of the manufacturer. Dilutions $(10^{-1} \text{ up to } 10^{-2})$ 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, 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 dH₂O, 2 μl of 3 M NaOAc and 50 μl of 95% EtOH, before being 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

The alignment was carried out using Clustal W (Thompson et al. 1994). Ambiguously aligned regions were removed manually. The alignment was analysed by maximum parsimony (MP) and a Bayesian approach (B/MCMC). 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 & 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 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 (i.e., the first 2000 trees) were deleted as the 'burn in' of the chain. The log-likelihood scores of sample points were plotted 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 & 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).

Phylogenetic analyses

The new sequences of Meridianelia maccarthyana were aligned with sequences obtained from GenBank as listed in Table 1. A matrix of 715 unambiguously aligned nucleotide position characters was produced; 327 characters in the alignment were constant. Maximum parsimony analysis yielded 68 most parsimonious trees (1928 steps long). The strict consensus tree 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 = -9628.635 (0.15), base frequencies $\pi(A) =$ $0.333 (0.00046), \pi(C) = 0.139 (0.00025),$ $\pi(G) = 0.204 \ (0.00026), \ \pi(T) = 0.324$ (0.00017), the gamma shape parameter alpha = 0.776 (0.0065), and p(invar) = 0.285 (0.00029).

Since the topologies of the MP and B/MCMC analyses did not show any strongly supported conflicts, only the 50% majority-rule consensus tree of Bayesian tree sampling is shown (Fig. 1). Those nodes that received strong support (i.e. $PP \ge 0.95$ in B/MCMC analysis and MP bootstrap $\ge 70\%$) in both the MP and Bayesian analyses are in bold as shown in Figure 1. In the

Table 1. Sequences obtained from Genbank for the study. Family placement after Eriksson (2006).

Name	Family	nuLSU
Ainoa geochroa	Agyriaceae	DQ871006
A. mooreana	Agyriaceae	AY212828
Arctomia delicatula	Arctomiaceae	AY853355
A. teretiuscula	Arctomiaceae	DQ007346
Arthrorhaphis citrinella	Arthrorhaphidaceae	AY853356
Aspicilia caesiocinerea	Hymeneliaceae	DQ780303
A. cinerea	Hymeneliaceae	DQ780304
Baeomyces placophyllus	Baeomycetaceae	AF356658
B. rufus	Baeomycetaceae	DQ871008
Boreoplaca ultrafrigida	Unclassified Lecanoromycetes	AY853360
Caloplaca flavorubescens	Teloschistaceae	AY300831
Cladonia rangiferina	Cladoniaceae	AY300832
Coccotrema cucurbitula	Coccotremataceae	AF274092
C. pocillarium	Coccotremataceae	AF274093
Elixia flexella	Elixiaceae	AY853368
Euxia Jiexeua Everniopsis trulla	Parmeliaceae	EF108290
Gregorella humida	Arctomiaceae	AY853378
Gregoreua numuaa Ionaspis lacustris	Arctomiaceae Hymeneliaceae	AY853371
ionaspis iacusiris Hypocenomyce scalaris	Hymeneuaceae Lecideaceae	AY853373
	Lecineaceae Lecanoraceae	
Lecanora hybocarpa L. paramerae		EF105421
1	Lecanoraceae Lobariaceae	EF105422
Lobaria pulmonaria Lobothallia radiosa		AF183934
	Hymeneliaceae	DQ780306
Loxospora lecanoriformis	Loxosporaceae	EF252278
L. ochrophaea	Loxosporaceae Pertusariaceae	DQ871009
Ochrolechia androgyna		AY300846
O. oregonensis	Pertusariaceae	DQ780308
O. pallescens	Pertusariaceae	DQ780310
O. parella	Pertusariaceae	AF274097
O. turneri	Pertusariaceae	AY568002
Ophioparma ventosa	Ophioparmaceae	AY853380
Orceolina antarctica	Agyriaceae	AF274115
O. kerguelensis	Agyriaceae	AY212830
Peltigera aphthosa	Peltigeraceae	AF286759
Pertusaria albescens	Pertusariaceae	AF329176
P. corallina	Pertusariaceae	AY300850
P. corallophora	Pertusariaceae	DQ780316
P. gibberosa	Pertusariaceae	DQ780322
P. hemisphaerica	Pertusariaceae	AF381556
P. lactea	Pertusariaceae	AF381557
P. leioplaca	Pertusariaceae	AY300852
P. mesotropa	Pertusariaceae	DQ780325
P. ophthalmiza	Pertusariaceae	AY568006
P. pertusa	Pertusariaceae	AF279300
P. subventosa	Pertusariaceae	AY300854
P. velata	Pertusariaceae	AY300855
Placopsis cribellans	Agyriaceae	DQ871010
P. gelida	Agyriaceae	AY212836
P. santessonii	Agyriaceae	AY212845
Placynthiella icmalea	Agyriaceae	AY212846
Protoparmelia badia	Parmeliaceae	DQ871011
Protothelenella corrosa	Protothelenellaceae	AY607734
P. sphinctrinoidella	Protothelenellaceae	AY607735
Rhizocarpon sphaerosporum	Rhizocarpaceae	AY853390

Table 1. Continued

Name	Family	nuLSU
Rimularia psephota	Agyriaceae	DQ871012
Schaereria corticola	Agyriaceae	AY300859
Sporastatia polyspora	Catillariaceae	AY640968
S. testudinea	Catillariaceae	AY640969
Thamnolia vermicularis	Icmadophilaceae	AY853395
Trapelia chiodectonoides	Agyriaceae	AY212847
T. glebosa	Agyriaceae	AF274103
Trapeliopsis flexuosa	Agyriaceae	AF274118
T. granulosa	Agyriaceae	AF274119
T. percrenata	Agyriaceae	AF279302
Tremolecia atrata	Hymeneliaceae	AY853397
Umbilicaria crustulosa	Umbilicariaceae	AY300869
U. decussata	Umbilicariaceae	AY603113
U. hyperborea	Umbilicariaceae	AY853399
Wawea fruticulosa	Arctomiaceae	DQ007347

majority-rule consensus tree, *Meridianelia* maccarthyana clusters strongly supported with *Elixia flexella* (MP-bootstrap support 99%, B/MCMC posterior probability 1·0) and hence supports placement of the new taxon in the *Elixiaceae* as circumscribed by Lumbsch (1997). Members of the Trapeliaceae sensu Lumbsch et al. (2007b) form a strongly supported group within Ostropomycetidae. The strongly supported topology of the other parts of the phylogenetic tree is in agreement with previously published phylogenies (Lumbsch et al. 2007a, b) and is not discussed further here.

Taxonomy

Meridianelia Kantvilas & Lumbsch gen. nov.

Mycobank: MB 513000

Genus fungorum lichenisatorum, ad *Elixiaceas* pertinens, thallo crustaceo, algas chlorococcaleas continenti. Apothecia hemiangiocarpia, excipulo cupulato, prosoplectenchymato, reducto, gelatina hymenialia nonamyloidea, ascis octosporis, *Elixia*-typis, paraphysibus subtilibus, ramosis anastomosantibusque, ascosporis simplicibus, non-halonatis, hyalinis. Pycnidia ignota.

Typus: Meridianelia maccarthyana Kantvilas & Lumbsch.

Thallus crustose, ecorticate. Photobiont chlorococcoid.

Ascomata apothecia, hemiangiocarpous. Excipulum cupulate, prosoplectenchymatous, very reduced. Hymenial gel nonamyloid. Paraphyses very delicate, branched and anastomosing, non-capitate. Asci 8-spored, of the Elixia-type. Ascospores simple, non-halonate, thin-walled. Pycnidia not seen.

Etymology. The generic name is derived from the Latin *meridianus* (meaning 'southern'), in reference to the geographic distribution of the taxon, and also infers the similarity of this lichen to members of the genus *Trapelia* M. Choisy.

The genus contains the single species, *M. maccarthyana* Kantvilas & Lumbsch.

Meridianelia maccarthyana Kantvilas & Lumbsch sp. nov.

Mycobank: MB 513001

Thallus crustosus, ecorticatus, sordide griseo-albidus, aliquando sorediatus, acidum protolichesterinicum continens. Apothecia adnata vel leviter immersa, 0·3–1 mm lata, disco concavo, plano vel undulato, dense albidopruinosa. Paraphyses intricatae, 0·8–1 µm crassae, apicibus non capitatis. Asci elongato-clavati, 130–170 µm longi, 17–25 µm lati. Ascosporae uniseriatae, globosae vel subglobosae vel ovato-ellipsoideae, 14–23(–26) µm longae, 9–20 µm latae.

Typus: Australia, Tasmania, 1 km SE of Lake Fenton, 42°41'S 146°38'E, 950 m altitude, on trunk of *Eucalyptus coccifera* in subalpine woodland,

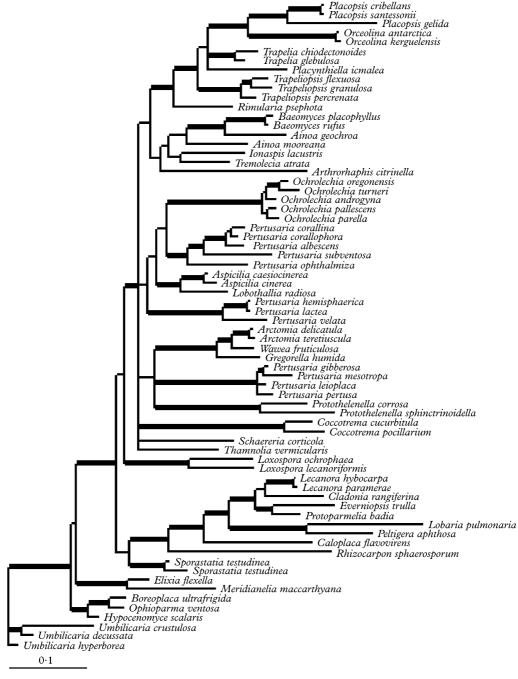


Fig. 1. Phylogeny of Lecanoromycetes as inferred from a mt SSU rDNA sequence analysis to investigate the phylogenetic placement of *Meridianelia maccarthyana*. This is a 50% majority-rule consensus tree based on 38 000 trees from a B/MCMC tree sampling procedure. Branches with posterior probabilities equal to or above 0.95 and MP bootstrap support values above 70% are indicated by wide internodes.

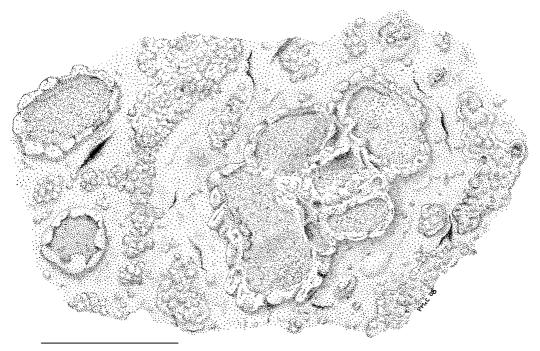


Fig. 2. Meridianelia maccarthyana, habitus (holotype). Scale = 1 mm.

17 December 2003, G. Kantvilas 752/03 (HO—holotypus; F—isotypus).

Figs (2, 3A–C)

Thallus crustose, not delimited, forming spreading colonies to 10-20 cm wide, dull greyish white, somewhat glossy, ecorticate, to 120-200 µm thick, smooth at first, becoming verruculose to tuberculate, with individual verrucae 0.5-1 mm wide, at length becoming abraded, eroded or ± coarsely sorediate; soredia generally aggregated in irregularly roundish, erose soralia to c. 1 mm wide, occasionally becoming diffuse and spreading, concolorous with the thallus or occasionally tinged greenish grey, inspersed with angular-rhomboidal crystals to 20 μm wide that fluoresce in polarized light and do not dissolve in KOH. Photobiont a unicellular green alga with individual cells globose to broadly ellipsoid, $9-20 \times 9-16 \mu m$, generally clustered in fascicles within the thallus.

Apothecia erupting from abraded thalline warts, broadly adnate to somewhat im-

mersed in the thallus, roundish to irregularly rhomboidal or angular, 0.3-1 mm wide, generally clustered in groups of 2-3(-5), each usually orientated obliquely to the thallus surface and to each other; disc at first deeply concave, then plane to undulate, dark grey, thickly whitish pruinose; 'thalline margin' ragged, incomplete, comprised of abraded, adhering remnants of thallus; proper margin barely evident. Excipulum in section cupulate, Trapelia-like, very reduced, to c. 10 µm thick, pale greyish green, K± olive, composed of poorly differentiated, intertwined, prosoplectenchymatous hyphae to c. 1 µm thick. Hypothecium colourless, 40–60 μm thick, poorly differentiated from the hymenium, sparsely inspersed with scattered oil droplets. Hymenium colourless, 160-200 µm thick, separating readily in water and KOH, likewise inspersed, I+ and K/I+ blue, with the reaction confined to the asci, with a grey-green, K± olive epihymenial layer 30-50 µm thick, composed of the entangled uppermost portions of the paraphyses, inspersed with amorphous crystalline

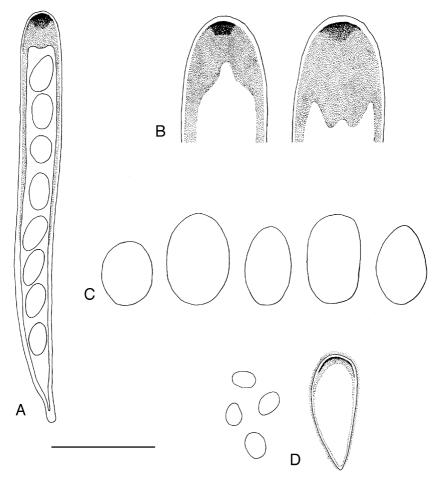


Fig. 3. Asci (with amyloid parts stippled) and ascospores. A–C, Meridianelia maccarthyana; D, Elixia flexella. Scale: $A=40~\mu m;~B-D=20~\mu m.$

material insoluble in KOH. Asci 8-spored, $130-170 \times 17-25 \,\mu\text{m}$, elongate-clavate with a very long attenuate 'tail', approximating the *Elixia*-type: outer wall K/I+ blue; tholus well-developed, K/I+ pale blue, with an intensely K/I+ blue cap or 'plug' at the apex; ascoplasm generally truncate or concave at the apex, occasionally with a short, beak-like ocular chamber. *Paraphyses* very delicate, highly branched, anastomosing and entangled, $0.8-1 \,\mu\text{m}$ thick; apices tapered. Ascospores uniseriate in the ascus, thin-walled, non-halonate, globose to subglobose to ovate-ellipsoid, $14-18\cdot1-23(-26) \times 9-13\cdot4-20 \,\mu\text{m}$ [length: width ratio $1-1\cdot4-1\cdot8(-2)$],

often deformed by mutual pressure when within the ascus, typically with a single, large vacuole.

Pycnidia not found.

Chemistry. Protolichesterinic acid; thallus K-, KC-, C-, P-, UV-.

Etymology. The specific epithet honours our friend and colleague, Dr Patrick McCarthy, who prepared the habit illustration and has made very significant contributions to the knowledge of lichens, especially in Australasia.

Distribution and ecology. The new species has been recorded from open subalpine woodland dominated by Eucalyptus coccifera Hook. f. growing on a dolerite boulder field. In this vegetation, the understorey is generally sparse and comprises scattered low shrubs, chiefly Nothofagus gunnii (Hook.f.) Oerst. and Olearia pinifolia (Hook.f.) Benth., or patches of Bauera rubioides Andrews intermixed with species of Epacridaceae and Proteaceae. Meridianelia maccarthyana forms conspicuous white patches up to 20 cm wide on the lowermost 1–2 metres of the eucalypt trunks as well as on larger individuals of Olearia that have very loose, papery bark. Although a generally lichen-rich environment, particularly with respect to saxicolous and terricolous species, these corticolous habitats are not highly colonized by lichens, and species associated with the new taxon are relatively few. These include Mycoblastus campbellianus (Nyl.) Zahlbr., M. coniophorellus (Elix & A.W. Archer) Kantvilas & Elix, Ochrolechia sp., Pertusaria jamesii Kantvilas, P. pertractata Stirt., Pseudoramonia richeae Kantvilas & Vězda, Ramboldia stuartii (Hampe) Kantvilas & Elix, Trapeliopsis granulosa (Hoffm.) Lumbsch and Usnea oncodes Stirt. On the eucalypts, below this 'Meridianelia-zone', there is a basal stocking of bryophytes and species of Cladia and Cladonia, whereas above, the eucalypt bark is generally too unstable for epiphytes. At a second locality, the new taxon was found in subalpine eucalypt woodland at the margins of a Nothofagus cunninghamii (Hook.) Oerst.-dominated cool temperate rainforest.

Meridianelia occurs most frequently in sunny conditions, being restricted to trees where the understorey is most sparse; it is absent where the undershrubs form a closed cover. It is curious that the new lichen has been encountered so infrequently. The vegetation community in which it has been found is not uncommon in Tasmania, nor is the lichen itself uncommon at the localities where it has been recorded. Further field observations are clearly required to better understand its ecological requirements.

Remarks. This new species is a most enigmatic lichen. Its spreading, white thallus is reminiscent of such unrelated taxa as Pertusaria or Ochrolechia, both of which occur in the same or similar habitats. The oftenclustered, pruinose ascomata, arising from ruptured thalline warts, are not unlike those seen in certain species of *Phlyctis*, which is likewise unrelated. Only with careful study of the asci do the relationships to Elixia become evident. The asci of Meridianelia have a prominent, weakly amyloid tholus with an intensely amyloid cap that sometimes extends down into the tholus as a 'plug' (Fig. 3A & B). The asci of *Elixia* are very short and compact and rather more difficult to observe, but display essentially the same salient features (Fig. 3D).

The differences between *Elixia* and *Meridi*anelia are stark, leaving no doubt that a new genus for the latter is justified. Superficially the two taxa are completely dissimilar, with Elixia having a highly reduced to inapparent thallus and often rather angular to almost lirelliform apothecia to only c. 0.4 mm wide. The excipulum of *Elixia* is well-developed and opaque dark brown, not unlike that seen in Rimularia. The hymenial gel is hemiamyloid in Elixia, whereas in Meridianelia, the amyloid reaction is confined to the asci. The paraphyses in Elixia are also robust and apically brown-pigmented. Furthermore, the ascospores of *Elixia* are rather small (8.5-13) $\times 4.5-6.5 \mu m$), whereas those of Meridianelia are comparatively large (Fig. 3C & D).

The genus shows the strongest superficial links with Trapelia, which also has hemiangiocarpous apothecia, surrounded by a 'margin' of adhering thallus fragments, delicate, branched and anastomosing paraphyses, a highly reduced excipulum of prosoplectenchymatous hyphae, and uniseriate, oftenvacuolate ascospores. The grey-green apothecial pigment in the new taxon approximates caesiocinerea-green of Meyer & Printzen (2000); this has also been recorded in Trapeliopsis, Xylographa and a range of other, unrelated taxa. Furthermore, the shape of the asci is very *Trapelia*-like, being very elongate-clavate, and with the apex of the ascoplasm being generally concave or

2009

truncate. Only after staining with Lugol's solution do the fundamental structural differences between the asci of the two genera become evident.

The addition of a further genus to the *Elixiaceae* necessitates a recircumscription of the family. Accordingly, the *Elixiaceae* is defined as: lichenized Ascomyctes with the thallus crustose, containing a coccoid, green photobiont; ascomata apothecia, hemiangiocarpous, with excipulum cupulate, prosoplectenchymatous, often reduced; asci 8-spored, of the *Elixia*-type; ascospores simple, hyaline, non-halonate, thin walled; pycnidia unknown; chemistry: aliphatic acids or nil.

Specimens examined. Australia: Tasmania: 1 km SE of Lake Fenton, 42°41′S 146°38′E, 950 m alt., 2003, G. Kantvilas 752/03 (HO); Lake Fenton Hut, 42°41′S 146°38′E, 1010 m alt., 2008, G. Kantvilas 345/08 (HO); Hartz Mountains Road, 43°12′S 146°46′E, 730 m, 2008, G. Kantvilas 376/08 (HO) (all specimens sterile only); track to Lake Nicholls, 42°40′S 146°39′E, 1000 m, 2008, G. Kantvilas 353/08 (BM, HO).

Selected comparative material examined. Elixia flexella (Ach.) Lumbsch. **Great Britain:** Scotland: **V.C. 105**, West Ross: Kinlochewe, Beinn Eighe NNR, 150–200 m, 2001, B. J. Coppins 19753 & A. M. Coppins (HO).—**Austria:** Salisburgia, Hohe Tauern, 960 m, 1986, R. Türk (A. Vězda: Lich. Sel. Exsicc. 2101) (HO).

We thank Dr Patrick McCarthy for providing the habit drawing of the new lichen, and Professor Jack Elix for confirming the chemical composition. 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. Dr Jean Jarman prepared the line drawings for publication.

REFERENCES

- Culberson, C. F. (1972) Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* 72: 113–125.
- Culberson, C. F., Culberson, W. L. & Johnson, A. (1981) A standardized TLC analysis of β-orcinol depsidones. *Bryologist* 84: 16–29.
- Culberson, C. F. & Johnson, A. (1982) Substitution of methyl tert.-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. Journal of Chromatography 238: 483–487.
- Elix, J. A. & Ernst-Russell, K. D. (1993) A Catalogue of Standardized Thin Layer Chromatographic Data and

- Biosynthetic Relationships for Lichen Substances, 2nd edn. Canberra: Australian National University.
- 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.
- Lumbsch, H. T. (1997) Systematic studies in the suborder Agyriinae (Lecanorales). *Journal of the Hattori Botanical Laboratory* **83:** 1–73.
- Lumbsch, H. T., Schmitt, I., Döring, H & Wedin, M. (2001) ITS sequence data suggest variability of ascus types and support ontogenetic characters as phylogenetic discriminators in the Agyriales (Ascomycota). Mycological Research 105: 265–274.
- Lumbsch, H. T., Palice, Z., Wiklund, E., Ekman, S. & Wedin, M. (2004) Supraordinal phylogenetic relationships of Lecanoromycetes based on Bayesian analysis of combined nuclear and mitochondrial sequences. *Molecular Phylogenetics and Evolution* 31: 822–832.
- Lumbsch, H. T., Schmitt, I., Lücking, R., Wiklund, E. & Wedin, M. (2007a) The phylogenetic placement of Ostropales within Lecanoromycetes (Ascomycota) revisited. *Mycological Research* 111: 257– 267.
- Lumbsch, H. T., Schmitt, I., Mangold, A. & Wedin, M. (2007b) Ascus types are phylogenetically misleading in Trapeliaceae and Agyriaceae (Ostropomycetidae, Ascomycota). Mycological Research 111: 1133–1141.
- Lumbsch, H. T., Archer, A. W. & Elix, J. A. (2007c) A new species of *Loxospora* (lichenized Ascomycota: *Sarrameanaceae*) from Australia. *Lichenologist* 39: 509–517.
- Meyer, B. & Printzen, C. (2000) Proposal for the standardized nomenclature and characterization of insoluble lichen pigments. *Lichenologist* 32: 571– 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
- Swofford, D. L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sunderland, Mass: Sinauer Associates.
- 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. *Mycologi*cal 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.

Accepted for publication 3 March 2009