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A new species of *Loxospora* (lichenized Ascomycota: *Sarrameanaceae*) from Australia

H. Thorsten LUMBSCH, Alan W. ARCHER and John A. ELIX

Abstract: *Loxospora lecanoriformis* Lumbsch, A. W. Archer & Elix is described from deeply shaded trunks of *Doryphora sassafras* Endl. in temperate rainforest in north-eastern New South Wales. This species is characterized by large, lecanorine apothecia, mostly unbranched paraphyses, non-amyloid asci and large, thin-walled, simple, ellipsoid ascospores. Molecular data has established that this taxon is related to *Loxospora ochrophaea* (Tuck.) R. C. Harris.

Key words: flora of Australia, Lecanoromycetes, phylogenetic analysis

Introduction

In early 2005 a specimen of an anomalous, lecanoriform lichen was collected by one us (JAE) at Mount Hyland in north-eastern New South Wales, Australia. This lichen possesses a unique suite of thalline and apothecial characters: a trebouxioid photobiont; secondary thalline metabolites (2′-*O*-methylperlatolic and perlatolic acids); large, dark brown, lecanorine apothecia; unbranched paraphyses; uniformly thin-walled, non-amyloid asci; and large, simple, broadly ellipsoid, straight or slightly curved (kidney shaped) ascospores with a single, thin wall. This new taxon exhibited characters reminiscent of *Lecanora* Ach., *Pertusaria* DC. and *Loxospora* A. Massal., but at first sight did not fit comfortably into any of these genera. Thus it lacked a dome-like, uniformly amyloid tholus, spiralled, indistinctly septate or simple, kidney-shaped ascospores of *Loxospora* and did not contain thamnolic acid. Likewise, it lacked either a *Lecanora*-type or *Pertusaria*-type ascus but did possess lecanorine apothecia and large, simple, ellipsoid ascospores. This prompted us to undertake a molecular investigation as detailed below, which indicated that this new taxon should be accommodated in the *Sarrameanaceae*.

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Material and Methods

The morphology of the lichen specimens was examined using a Zeiss Stemi 2000C stereomicroscope, and the anatomy, conidia and ascospores were studied using a Zeiss Axiolab compound microscope. Hand-cut sections and squash preparations were examined in water and in 10% KOH; asci were also examined in Lugol's iodine. Chemical constituents were identified by thin layer chromatography (Culberson 1972; Culberson *et al.* 1981; Culberson & Johnson 1982; Elix & Ernst-Russell 1993), high performance liquid chromatography (Elix *et al.* 2003) and by comparison with authentic samples.

Molecular methods

Sequence data of 98 species were assembled using sequences of nuclear large and mitochondrial small subunit rDNA (Table 1) with two newly obtained sequences extracted from the isotype of *Loxospora lecanoriformis* [Genbank nos: EF525279 (for the mtSSU) and EF25278 (for nuLSU)]. We included *Cladosporium* sp. and *Myriangium duriaei* (Dothideomycetes) as outgroup. Total DNA was extracted from herbarium specimens 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

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

Species	Family	Genbank Accession Number	
		nuLSU	mtSSU
Acarospora schleicheri	*Acarosporaceae*	AY853353	AY853305
Ainoa geochroa	*Agyriaceae*	DQ871006	DQ871015
A. mooreana	*Agyriaceae*	AY212828	AY212850
Arctomia delicatula	*Arctomiaceae*	AY853355	AY853307
A. teretiuscula	*Arctomiaceae*	DQ007346	DQ007349
Arthroraphis citrinella	*Arthroraphidaceae*	AY853356	AY853308
Aspicilia caesiocinerea	*Hymeneliaceae*	DQ780303	DQ780271
A. cinerea	*Hymeneliaceae*	DQ780304	DQ780272
A. izcoana	*Hymeneliaceae*	AY853359	AY853311
Baeomyces placophyllus	*Baeomycetaceae*	AF356658	AY300878
B. rufus	*Baeomycetaceae*	DQ871008	DQ871016
Boreoplaca ultrafrigida	*Unclassified Lecanoromycetes*	AY853360	AY853312
Caloplaca flavorubescens	*Teloschistaceae*	AY300831	AY143403
Candelaria concolor	*Lecanoraceae*	EF436461	EF436460
Candelariella aurella	*Lecanoraceae*	AY853361	AY853313
Cladonia rangiferina	*Cladoniaceae*	AY300832	AY300881
Cladosporium sp.	–	AY016367	AY571386
Coccotrema cucurbitula	*Coccotremataceae*	AF274092	AF329161
C. pocillarium	*Coccotremataceae*	AF274093	AF329166
Diploschistes cinereocaesius	*Thelotremataceae*	AY300835	AY300885
D. scruposus	*Thelotremataceae*	AF279389	AY584692
Elixia flexella	*Elixiaceae*	AY853368	AY853320
Evernia prunastri	*Parmeliaceae*	AF107562	AF351162
Everniopsis trulla	*Parmeliaceae*	EF108290	EF108289
Flavoparmelia caperata	*Parmeliaceae*	AY578922	AF351163
Graphis scripta	*Graphidaceae*	AY853370	AY853322
Gregorella humida	*Arctomiaceae*	AY853378	AY853329
Hymenelia lacustris	*Hymeneliaceae*	AY853371	AY853323
Hypocenomyce scalaris	*Lecideaceae*	AY853373	AY853325
Hypogymnia physodes	*Parmeliaceae*	AY756338	AY756400
Lecanora hybocarpa	*Lecanoraceae*	EF105421	EF105417
L. paramerae	*Lecanoraceae*	EF105422	EF105418
L. sulphurea	*Lecanoraceae*	EF105423	EF105419
Lepraria usnica	*Stereocaulaceae*	AY300843	AY300894
Lobaria pulmonaria	*Lobariaceae*	AF183934	AF069541
Lobothallia radiosa	*Hymeneliaceae*	DQ780306	DQ780274
Loxospora ochrophaea	*Loxosporaceae*	DQ871009	DQ871017
Myriangium duriaei	*Myriangiaceae*	AY016365	AY571389
Ochrolechia androgyna	*Pertusariaceae*	AY300846	AY300897
O. oregonensis	*Pertusariaceae*	DQ780308	DQ780276
O. pallescens	*Pertusariaceae*	DQ780310	DQ780277
O. parella	*Pertusariaceae*	AF274097	AF320173
O. turneri	*Pertusariaceae*	AY568002	AY567982
Ophioparma ventosa	*Ophioparmaceae*	AY853380	AY853331
Orceolina antarctica	*Agyriaceae*	AF274115	AY212852
O. kerguelensis	*Agyriaceae*	AY212830	AF381561
Peltigera aphthosa	*Peltigeraceae*	AF286759	AY340515
Pertusaria albescens	*Pertusariaceae*	AF329176	AF329175
P. amara	*Pertusariaceae*	AF274101	AY300900
P. coccodes	*Pertusariaceae*	AF279295	AY567984
P. corallina	*Pertusariaceae*	AY300850	AY300901
P. corallophora	*Pertusariaceae*	DQ780316	DQ780285
P. coronata	*Pertusariaceae*	AY300851	AY300902

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TABLE 1. *Continued*

Species	Family	Genbank Accession Number	
		nuLSU	mtSSU
Pertusaria gibberosa	*Pertusariaceae*	DQ780322	DQ780289
P. hemisphaerica	*Pertusariaceae*	AF381556	AF381563
P. lactea	*Pertusariaceae*	AF381557	AF381564
P. lecanina	*Pertusariaceae*	AF279296	AY567991
P. leioplaca	*Pertusariaceae*	AY300852	AY300903
P. mammosa	*Pertusariaceae*	AY212831	AY212854
P. mesotropa	*Pertusariaceae*	DQ780325	DQ780292
P. ophthalmiza	*Pertusariaceae*	AY568006	AY567993
P. panyrga	*Pertusariaceae*	DQ780327	AY567994
P. pertusa	*Pertusariaceae*	AF279300	AF381565
P. plittiana	*Pertusariaceae*	DQ780328	DQ780294
P. scaberula	*Pertusariaceae*	AF274099	AF431959
P. subventosa	*Pertusariaceae*	AY300854	AY300905
P. velata	*Pertusariaceae*	AY300855	AY300906
Placopsis cribellans	*Agyriaceae*	DQ871010	DQ871018
P. gelida	*Agyriaceae*	AY212836	AY212859
P. santessonii	*Agyriaceae*	AY212845	AY212867
Placynthiella icmalea	*Agyriaceae*	AY212846	AY212870
Protoparmelia badia	*Parmeliaceae*	DQ871011	AF351179
Protothelenella corrosa	*Protothelenellaceae*	AY607734	AY607746
P. sphinctrinoidella	*Protothelenellaceae*	AY607735	AY607747
Pseudevernia furfuracea	*Parmeliaceae*	AY607826	AF351181
Rhizocarpon sphaerosporum	*Rhizocarpaceae*	AY853390	AY853340
Rimularia psephota	*Agyriaceae*	DQ871012	DQ871019
Schaereria corticola	*Agyriaceae*	AY300859	AY300909
Sporastatia polyspora	*Catillariaceae*	AY640968	AY584724
S. testudinea	*Catillariaceae*	AY640969	AY584725
Staurothele fissa	*Verrucariaceae*	DQ329028	DQ329003
S. rufa	*Verrucariaceae*	DQ329029	DQ329004
Stictis urceolatum	*Stictidaceae*	AY300833	AY300882
Thamnolia vermicularis	*Icmadophilaceae*	AY853395	AY853345
Thelotrema subtile	*Thelotremataceae*	DQ871013	DQ871020
T. suecicum	*Thelotremataceae*	AY300867	AY300917
Trapelia chiodectonoides	*Agyriaceae*	AY212847	AY212873
T. placodioides	*Agyriaceae*	AF274103	AF431962
Trapeliopsis flexuosa	*Agyriaceae*	AF274118	AY212875
T. granulosa	*Agyriaceae*	AF274119	AF381561
T. percrenata	*Agyriaceae*	AF279302	AY212876
Tremolecia atrata	*Hymeneliaceae*	AY853397	AY853347
Umbilicaria crustulosa	*Umbilicariaceae*	AY300869	AY300919
U. decussata	*Umbilicariaceae*	AY603113	DQ871021
U. hyperborea	*Umbilicariaceae*	AY853399	AY853349
Usnea antarctica	*Parmeliaceae*	EF116569	EF116571
U. trachycarpa	*Parmeliaceae*	EF116570	EF116572
Wawea fruticulosa	*Arctomiaceae*	DQ007347	DQ871023

amplifications. Primers for amplification were: a) nu-LSU-0155-5′ (Döring *et al.* 2000) and nu-LSU-1125-3′ (=LR6) (Vilgalys & Hester 1990) for the nuclear LSU rDNA, and b) mr SSU1 (Zoller *et al.* 1999) and MSU 7 (Zhou & Stanosz 2001) for the mitochondrial SSU rDNA. 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

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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 they were loaded on an ABI 3100 (Applied Biosystems) automatic sequencer. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and adjusted manually.

Sequence alignments and phylogenetic analysis

Alignments for the single data sets were done using Clustal W (Thompson *et al.* 1994) Ambiguously aligned regions were removed manually. The alignments were analysed by maximum parsimony (MP) and a Bayesian approach (B/MCMC). To test for potential conflict, parsimony bootstrap analyses were performed on each individual data set, and 70% 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 & 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. The data set was portioned into two parts (nu LSU, mt SSU). Each partition was allowed to have its own model parameters and 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 (i.e. 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://tree.bio.ed.ac.uk/software/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 $\geq$70% under MP 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

Two new sequences of *Loxospora lecanoriformis* were aligned with sequences obtained from GenBank as listed in Table 1. A matrix of 1438 unambiguously aligned nucleotide position characters was produced; 590 characters in the alignment were constant. Insertions present in some taxa were removed from the alignment. The MP-bootstrap support method for testing data sets for incongruence indicated no strongly supported conflict (data not shown); hence a combined analysis was performed. Maximum parsimony analysis of the combined data set yielded two most parsimonious trees (5168 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 values (variance): LnL= − 26477·621 (0·45), base frequencies $\pi(A)_{\{all\}}$=0·279 (0·00026), $\pi(C)_{\{all\}}$=0·184 (0·00024), $\pi(G)_{\{all\}}$=0·263 (0·00026), $\pi(T)_{\{all\}}$=0·273 (0·00024), rate matrix $r(AC)_{\{all\}}$=0·085 (0·002), $r(AG)_{\{all\}}$= 0·215 (0·0004), $r(AT)_{\{all\}}$=0·119 (0·0002), $r(CG)_{\{all\}}$=0·074 (0·0002), $r(CT)_{\{all\}}$= 0·442 (0·0005), $r(GT)_{\{all\}}$=0·065 (0·0002), the gamma shape parameter $alpha_{\{mtSSU\}}$ =0·499 (0·0055), $alpha_{\{nuLSU\}}$=0·763 (0·0075),and $p(invar)_{\{all\}}$=0·232 (0·00031).

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$\geq$0·95 in B/MCMC analysis and MP bootstrap $\geq$70%) in both the MP and Bayesian analyses are shown in bold in Figure 1. In the majority-rule consensus tree, *Loxospora lecanoriformis* clusters strongly supported with *L. ochrophaea* (MP-bootstrap support 100%, B/MCMC posterior probability 1·0) and hence supports placement of the new taxon

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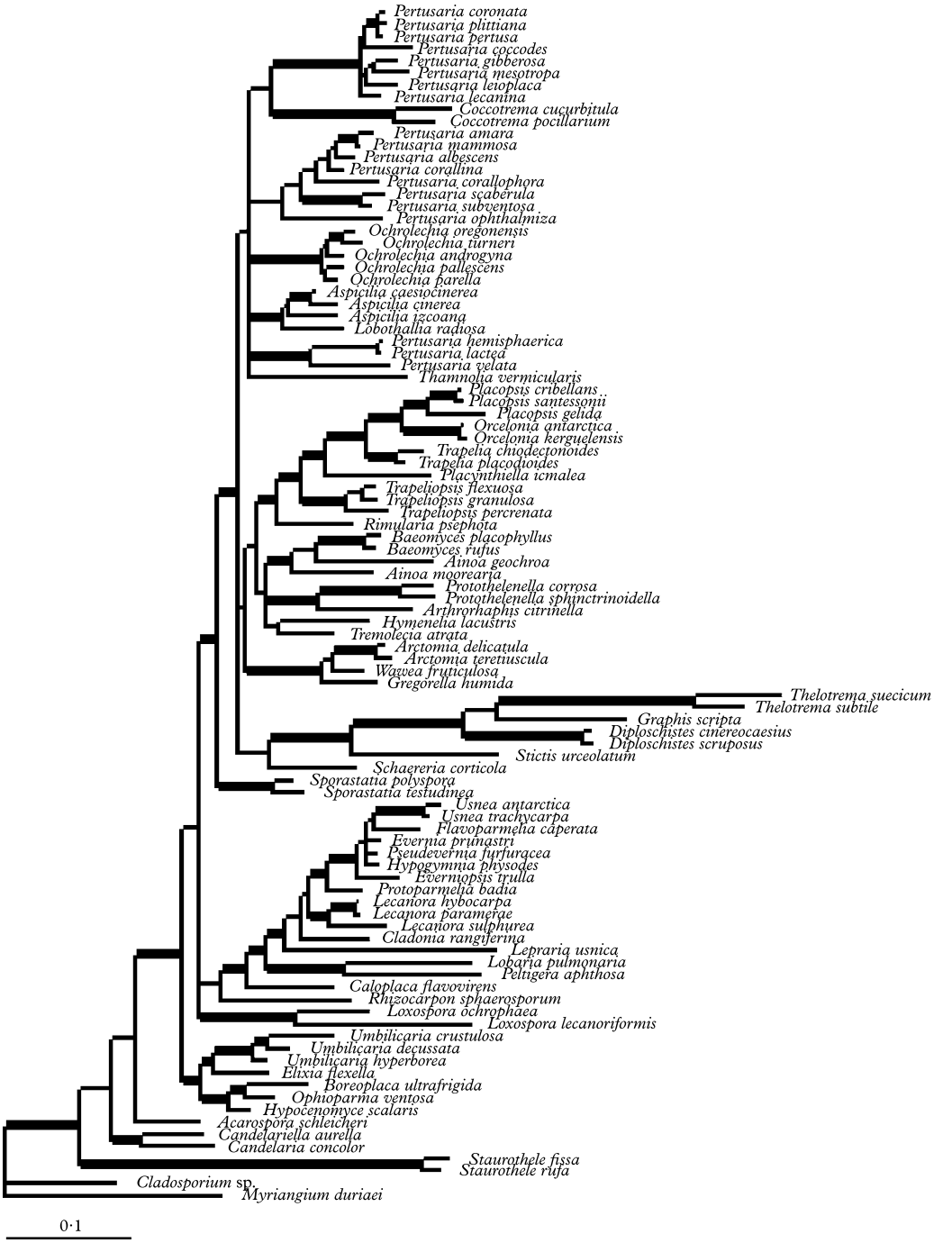

FIG. 1. Phylogeny of *Lecanoromycetes* as inferred from a three gene-partition analysis to investigate the phylogenetic placement of *Loxospora lecanoriformis*. This is a 50% majority-rule consensus tree based on 38 000 trees from a B/MCMC tree sampling procedure. Branches with posterior probabilities equal to or above 0·95 and MP bootstrap support values above 70% are indicated by wide internodes.

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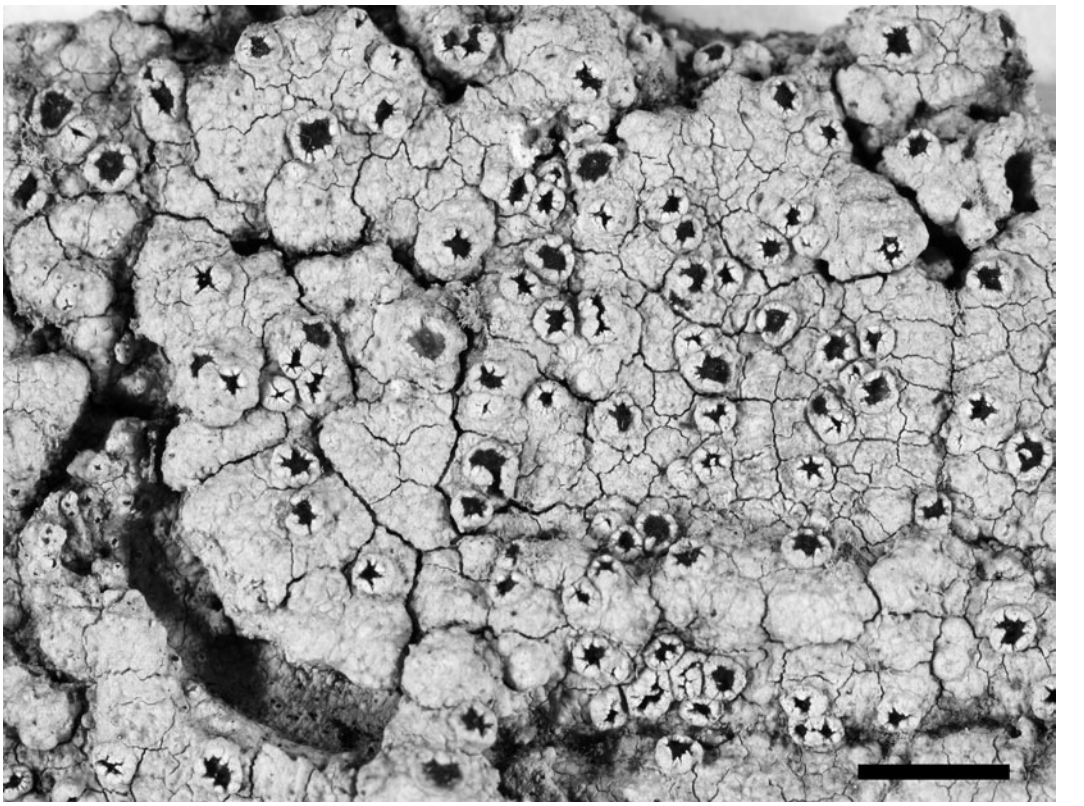

FIG. 2. *Loxospora lecanoriformis* (holotype in CANB). Scale=5 mm.

in *Sarrameanaceae*. The topology of the other parts of the phylogenetic tree is in agreement with a previously published phylogeny (Lumbsch *et al.* 2007) and is not discussed further here.

Taxonomy

Loxospora lecanoriformis Lumbsch, A. W. Archer & Elix, sp. nov.

Loxosporae cyamidiae similis sed ab hac specie ascis non-amyloideis, ascosporis maioribus et acido 2′-*O*-methylperlatolico et acido perlatolico continente differt

Typus: Australia. New South Wales, Mt Hyland Nature Reserve, 20 km N of Hernani, 30°10′44″S, 152°25′19″E, 1340 m, on fallen *Doryphora sassafras* in rainforest, 30 April 2005, *J. A. Elix* 36561 (CANB—holotypus; F, NSW—isotypi).

(Figs 2 & 3)

Thallus corticolous, pale grey-green to olive-grey, rather thick, surface smooth and dull but becoming verrucose and cracked, lacking isidia and soredia.

Apothecia lecanorine, 0·8–1·5 mm diam., numerous, sessile, concave; thalline margin rather ragged and scabrid when young, then entire, dentate, persistent, often flexuose, raised and inrolled, 0·2–0·5 mm thick; disc dark reddish brown to black, epruinose, ± flat. *Excipulum* with numerous spherical algal cells to 8 µm diam., inspersed with a few red-brown granules (K−) and large agglomerations of colourless crystals. *Epihymenium* 12–20 µm tall, composed of dense red-brown granules, K−. *Hymenium* colourless, 350–500 µm tall, inspersed with infrequent oil droplets to 12 µm diam., K−, I−, KI− or slightly greenish blue.

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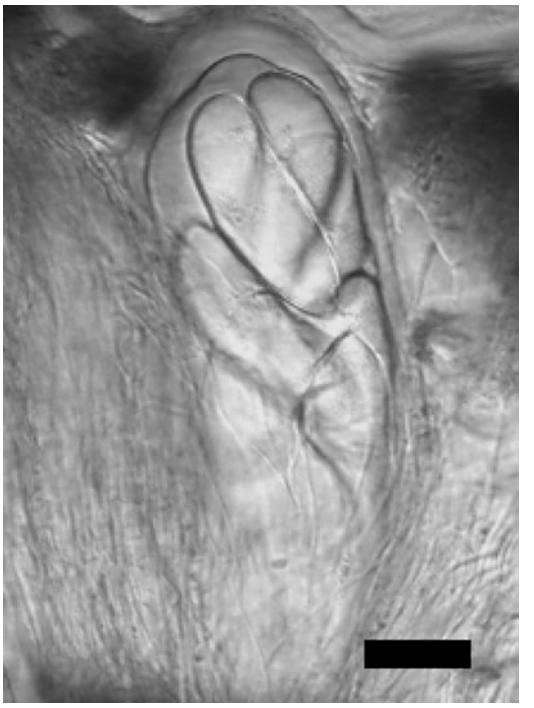

FIG. 3. *Loxospora lecanoriformis*, ascus in KI (isotype in F). Scale = 25 μm.

Paraphyses simple, unbranched, with colourless apices. *Hypothecium* 50–75 μm tall, colourless or pale yellow-brown, K − . *Asci* claviform to obovate, 135–145 × 40–50 μm, I − , KI+ slightly blue-green, damaged asci amyloid. *Ascospores* 6–8 per ascus, broadly ellipsoid, straight or slightly bent, 50–65 × 18–22 μm, with a single thin wall, *c.* 1 μm thick.

Pycnidia perithecioid, immersed in elevated thalline warts, visible as minute black dots, *c.* 0·05 mm wide. *Conidiospores* are formed according to Vobis-Type IV (Vobis 1980). *Conidia* bacilliform, 3–4·5 × 0·5–0·7 μm.

Chemistry. Cortex K − , C − , KC − , P − ; UV·, medulla K − , C − , KC − , P − ; UV+ white; containing 2′-*O*-methylperlatolic acid (major) and perlatolic acid (minor or trace).

Etymology. The specific epithet derives from the superficial similarity of this lichen to robust *Lecanora* species.

Remarks. This new species is characterized by a crustose thallus, a trebouxioid photobiont, lecanorine apothecia with a dark reddish brown to black disc, unbranched paraphyses, non-amyloid asci, large, simple, broadly ellipsoid ascospores with a single thin wall, bacilliform conidia and the presence of the *para*-orcinol depsides, 2′-*O*-methylperlatolic and perlatolic acids. Its affinities have been deduced from a molecular investigation (see above). The family *Loxosporaceae* was segregated from *Haematommataceae* by Staiger & Kalb (1995), based on differences in ascus-type, paraphyses, epihymenial and conidiomatal pigments, and secondary metabolites. However, the *Loxosporaceae* was placed into synonymy with the *Sarrameanaceae* (Kantvilas 2000, 2004) based on morphological evidence, and this was subsequently accepted by Lumbsch & Huhndorf (2007). Based on morphology, the *Sarrameanaceae* was believed to be close to *Haematommataceae* and *Ophioparmaceae* (Kantvilas 2004), a hypothesis that was not supported by molecular analyses. In fact molecular studies placed the *Sarrameanaceae* outside of the *Lecanorales* sensu stricto (Miądlikowska *et al.* 2007; Lumbsch *et al.* 2007). The family had an unresolved position in the *Ostropomycetidae* (Miądlikowska *et al.* 2007) or a basal position to both the *Lecanoromycetidae* and *Ostropomycetidae* (Lumbsch *et al.* 2007). Species of the *Sarrameanaceae* are characterized morphologically by asci with a well-developed, dome-like, uniformly amyloid tholus lacking a masse axiale (Hafellner 1984; Staiger & Kalb 1995), spiralled, simple or indistinctly septate, hyaline, non-halonate ascospores, and simple or sparingly branched paraphyses (Kantvilas 2000).

In overall morphology *Loxospora lecanoriformis* resembles *L. cyamidia* (Stirt.) Kantvilas, but the latter can be distinguished by the densely, grey-pruinose apothecial discs, the smaller (20–36 × 6–11 μm vs. 50–65 × 18–22 μm), broadly fusiform, curved ascospores and by the presence of thamnolic acid. The new species differs in several characters (including non-amyloid asci, Fig. 3) from other *Loxospora* species and hence it may represent

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an undescribed genus. However, we refrain from describing a new genus within the *Sarrameanaceae* at this point, until additional molecular data from this family become available. These are necessary in order to allow us to evaluate the systematic value of these characters. Ascoma development has been used as an additional character for the circumscription of *Loxospora* (Brodo & Henssen 1995; Kantvilas 2000). While the genus *Sarrameana* has gymnocarpous ascomata, the ascomata of *Loxospora* are hemiangiocarpous. Hemiangiocarpous ascomata were observed in the new species.

Distribution and habitat. This lichen is known only from the type locality in the Northern Highlands of New South Wales where it occurs on the bark of *Doryphora sassafras*. Cool temperate rainforest dominated by *Doryphora sassafras* and *Cryptocarya foveolata* C. T.White & W. D.Francis clothes the three separate peaks that make up Mt Hyland, which at 1400 m is one of the highest points on the New England Plateau. Common associated species include *Haematomma infuscum* (Stirt. ex F. M. Bailey) R. W. Rogers, *Hypogymnia pulverata* (Nyl.) Elix, *Hypotrachyna osseoalba* (Vain.) Y. S. Park & Hale, *Lecanora achroa* Nyl., *Parmelia erumpens* Kurok., *Pertusaria erythrella* Müll. Arg., *P. neotriconica* Elix & A. W. Archer, *P. scaberula* A. W. Archer and *Usnea dasaea* Stirt.

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