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Sphaeropezia leucocheila sp. nov. (Stictidaceae): a liverwort pathogen from New Zealand

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Abstract

Sphaeropezia leucocheila is described as a new species from New Zealand. Known from a single specimen, its fruiting bodies were consistently associated with a patch of dead and dying leaves in a liverwort colony that included several species of Lepidoziaceae. A phylogenetic analysis places this fungus in *Sphaeropezia*, a genus that includes several putative parasites of liverworts and mosses from the Northern Hemisphere.

Keywords: Lepidoziaceae, Odontotremataceae, Ostropales, phylogeny, taxonomy

Introduction

Sphaeropezia includes ostropalean fungi with dark-walled, deeply urceolate apothecia mostly erumpent at maturity, living as saprotrophs on wood or herbaceous material, or as putative parasites of bryophytes or lichens (Baloch et al. 2013). Several of the species currently accepted in the genus *Sphaeropezia* were previously regarded as members of the morphologically and ecologically similar Odontotremataceae. A phylogenetic study by Baloch et al. (2013) showed Odontotremataceae as previously accepted was polyphyletic, and they placed *Sphaeropezia* in the Stictidaceae. In the same paper they transferred the bryophyte-associated species that had been described in *Bryodiscus* to *Sphaeropezia*. Following the Baloch et al. (2013) revision, the genus *Sphaeropezia* currently includes 21 species, most from north-temperate regions. Three of these, originally described in the genus *Bryodiscus*, are considered to be parasites of mosses (*S. arctoalpina* and *S. grimmiae*) or liverworts (*S. hepaticarum*).

In this paper we report a *Sphaeropezia* liverwort parasite from the Southern Hemisphere. A molecular phylogeny clearly places this fungus in *Sphaeropezia*, and the morphology is also consistent with the genus.

Methods

Ascomata were initially examined from a fresh collection. Individual ascomata were sectioned at about 15 µm using a freezing microtome, with sections mounted in lactic acid. Dried specimens were rehydrated in 3% KOH, and hymenial elements were examined in Melzer's reagent. The specimen has been deposited in the New Zealand Fungarium (PDD).

For DNA extraction, three fresh ascomata were placed in separate Eppendorf tubes and DNA was extracted using REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich, USA) following the manufacturer's protocol except that the ascomata were ground in 30 µL extraction solution with a plastic pestle. Amplification primers for LSU were LR0R and LR5 (Vilgalys & Hester 1990, Bunyard *et al.* 1994), and for mtSSU they were mtSSU1-KL and mtSSU2-KL (Lohtander *et al.* 2002).

For the phylogeny, sequences from the two genes were concatenated and combined with concatenated LSU and mtSSU sequences from the analysis in Baloch *et al.* (2013) (Table 1). The sequences were aligned using MAFFT,

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as implemented in Geneious R10 (https://www.geneious.com); ML analyses were performed with RAxML 8.2.11 using the GTR model and the BFGS method to optimize GTR rate parameters (Stamatakis 2014), as implemented in Geneious, with support estimated from 1000 bootstrap replications.

TABLE 1. Specimens and GenBank accession numbers of sequences used for the phylogeny in Figure 1. All except *S. leucocheila* are used in the analyses in Baloch *et al.* 2013.

Species	Voucher	Country, Collector	mtSSU	LSU
Carestiella socia	UPS (mtSSU Wedin 7194 and LSU Gilenstam 2437a)	Sweden, M. Wedin and G. Gilenstam	JX266155	AY661682
Coccomycetella richardsonii	S (Baloch SW068)	Sweden, E. Baloch	HM244737	HM244761
Coenogonium luteum	AFTOL-ID 352 and ASU (Ryan 31430)	USA	AY584699	AF279387
Coenogonium pineti	—	Germany, Z. Palice	AY300884	AY300834
Cryptodiscus foveolaris	S (Baloch SW168)	Sweden, E. Baloch	FJ904695	FJ904673
Cryptodiscus pallidus	S (Baloch SW174)	Sweden, E. Baloch	FJ904702	FJ904680
Cryptodiscus pini	S (Baloch & Arup SW175	Sweden, E. Baloch and U. Arup	FJ904706	FJ904684
Graphis scripta	UPS (Wedin 6476)	Sweden, M. Wedin	AY853322	AY853370
Gyalecta flotowii	UPS (Svensson 679)	Sweden, M. Svensson	HM244740	HM244764
Gyalecta jenensis	AFTOL-ID 361	USA, F. Lutzoni	AY584705	AF279391
Odontotrema phacidiellum	UPS (Gilenstam 2625)	Sweden, G. Gilenstam	HM244748	HM244769
Odontotrema phacidioides	S (Palice 11440)	Morocco, Z. Palice	HM244749	HM244770
Orceolina kerguelensis	C (Poulsen 456)	Îles Kerguelen, R. S. Poulsen	AY212853	AF274116
Ostropa barbara	S (Wedin & Baloch SW071)	Sweden, M. Wedin and E. Baloch	HM244752	HM244773
Placopsis perrugosa	AFTOL-ID 383	USA, Streimann	AY584716	AF356660
Porina aenea	S (Arup & Baloch SW154)	Sweden, U. Arup and E. Baloch	HM244754	—
Porina lectissima	S (Arup & Baloch SW152)	Sweden, U. Arup and E. Baloch	HM244756	HM244774
Rhexophiale rhexoblephara	—	Sweden, Z. Palice	AY853341	AY853391
Sagiolechia protuberans	S (Nordin 5893)	Sweden, A. Nordin	HM244757	HM244775
Schizoxylon albescens	UPS (Gilenstam 2696a)	Sweden, G. Gilenstam	DQ401142	DQ401144
Sphaeropezia arctoalpina	S (Baloch SW057)	Sweden, E. Baloch	HM244736	HM244760
Sphaeropezia capreae	UPS (Gilenstam 2633a)	Sweden, G. Gilenstam	HM244751	HM244772
Sphaeropezia diffindens	S (Baloch SW020)	Sweden, E. Baloch	HM244747	—
Sphaeropezia leucocheila	PDD 98299	New Zealand, M. Renner	MK547101	MK547099
Sphaeropezia lyckselensis	S (Gilenstam 2651)	Sweden, G. Gilenstam	JX266156	JX266158
Sphaeropezia mycoblasti	S (Wedin 8509 & Westberg)	Sweden, E. Baloch and Westberg	JX266157	JX266159
Sphaeropezia ochrolechiae	UPS (Wedin 6729)	Sweden, M. Wedin	—	JX266160
Stictis radiata	AFTOL-ID 398	USA, J. Platt	AY584727	AF356663
Thelotrema lepadinum	—	Slovenia, U. Arup	DQ431957	DQ431924

Results

Phylogeny

The DNA sequences from each separate ascoma from PDD 98299 were identical, so confirming that chance, contaminating fungi from the surface of the ascomata were not being accidentally amplified. The topology of the phylogenetic tree (Fig. 1), based on LSU and mtSSU, matches that from Baloch *et al.* (2013); their analysis also used RPB2. The genus *Sphaeropezia* is strongly supported as a monophyletic clade within the Stictidaceae *sensu* Baloch *et al.* (2013). The new species described here, *S. leucocheila*, is basal in this clade.

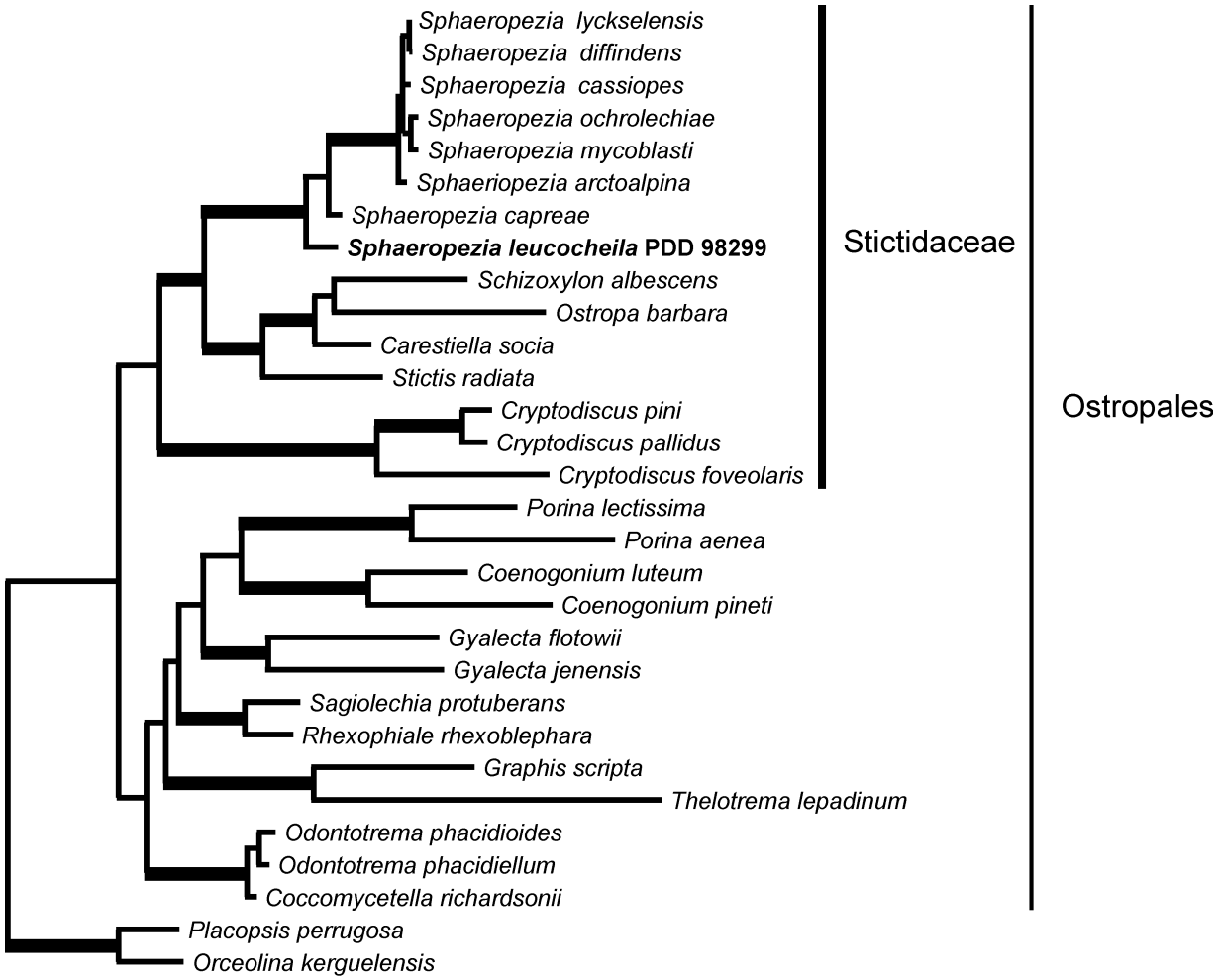

FIGURE 1. RAxML tree based on concatenated partial large subunit and partial mitochondrial small subunit sequences from selected Ostropales, based on Baloch *et al.* (2013). *Placopsis* and *Orceolina* (Baeomycetales) selected as the outgroup. Edges with bootstrap values greater than 90% are indicated with thick lines. GenBank accession numbers of the sequences used are given in Table 1.

Taxonomy

Sphaeropezia leucocheila P.R. Johnst. & M.A.M. Renner *sp. nov*.

Registration number: IF556315

Holotype. New Zealand: Nelson, Moutere Valley, Ellis River, on Lepidoziaceae (*Lepidozia* and *Neolepidozia* spp.), coll. M. Renner, 16 Sept. 2009 (PDD 98299).

Diagnosis. Differs from the liverwort-inhabiting *S. hepaticarum* by its smaller, 1-septate ascospores.

Etymology. leuco- (gk) = white and cheilus (gk) = lipped, referring to the white margin of the ascomatal opening.

Description. Ascomata develop on the surface of dead and dying host leaves that form a small patch amongst otherwise symptomless leaves. Ascomata form on a pad of tightly packed fungal hyphae with thick, brown walls, which develops on, or just below, the surface of the host tissue. Fungal hyphae with pale brown, thin walls pack the adjacent host cells. Ascomata globose-urceolate, up to about 0.3 mm diam.; a ring of white tissue surrounds the wide, round apical pore, otherwise the ascomatal wall is uniformly dark grey when young; as the ascomata expand the wall develops a patchy appearance with a network-like pattern of darker patches on a pale grey background. Hymenium dark silvery-grey. In vertical section ascomatal wall 20–30 µm thick, comprising tangled hyphae 2–3 µm diam., some with thick, very dark walls, others with pale brown walls, the paler cells forming more or less triangular patches along the wall. The wall near the ascomatal opening is up to 80 µm thick, lined with short-cylindric, unbranched,

periphysoids with thin, pale walls. Hymenial gel J+ blue with Melzers's reagent + KOH. Paraphyses 1.5 µm diam., undifferentiated at apex. Asci 50–55 × 6–8 µm, cylindric, with characteristic ostropalean thickening at the rounded apex, 8-spored. Ascospores 8–11.5 × 2–3 µm (average 9.6 × 2.7 µm) oblong-elliptic, ends rounded, walls hyaline, (0–) 1-septate.

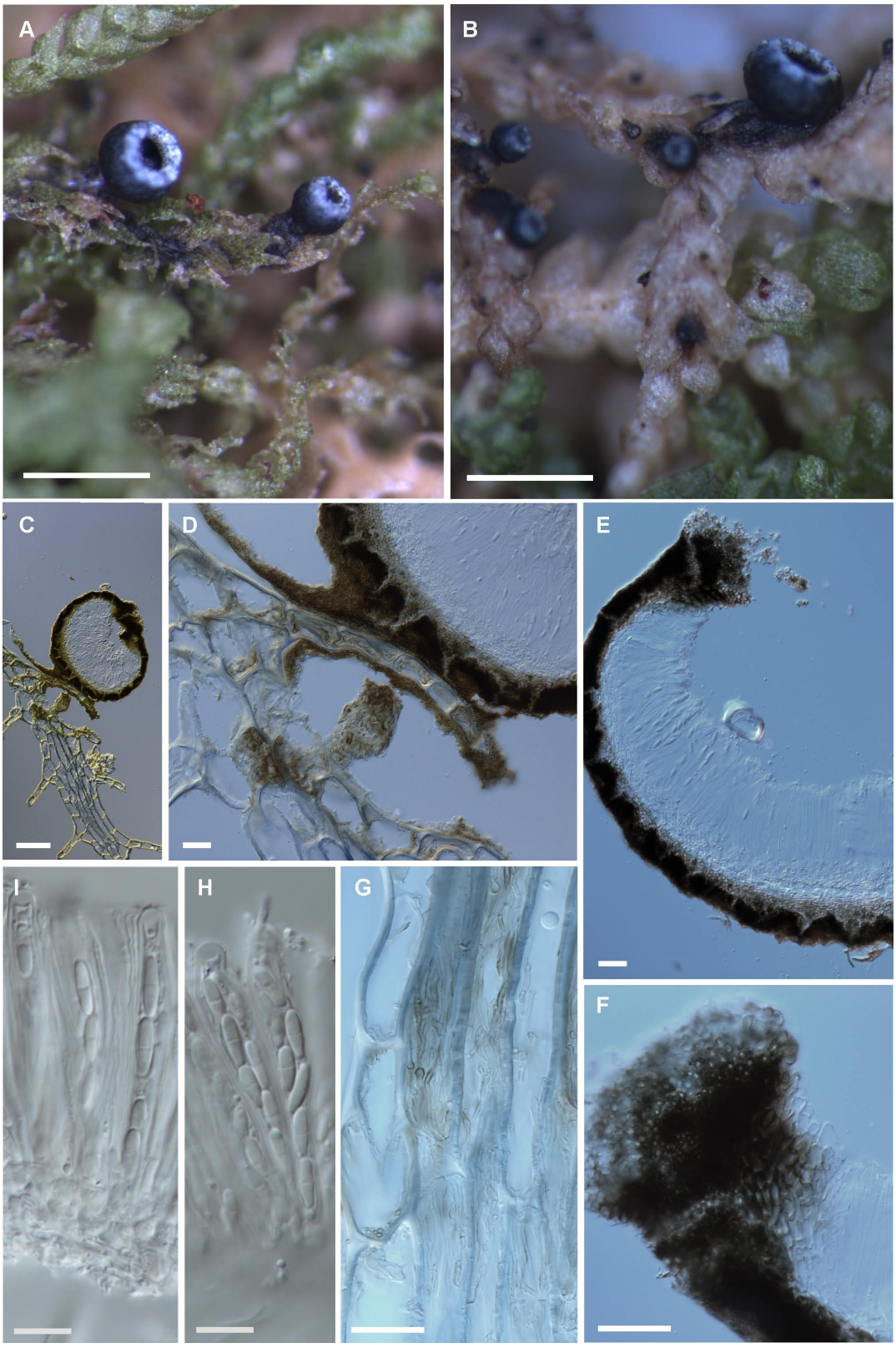

FIGURE 2. *Sphaeropezia leucophila* (holotype, PDD 98299). **A–B**, Fresh ascomata showing dead patches of host tissue. **C**, Ascoma in vertical section. **D**, detail of base of ascoma in vertical section, showing pad of fungal tissue at base of ascoma. **E**, Ascomatal wall in vertical section. **F**, detail of ascomatal wall and periphysoids near ascomatal opening. **G**, fungal hyphae within host cells adjacent to ascoma. **H–I**, asci and ascospores. Scale bars A–B = 0.5 mm; C = 100 µm; D–G = 20 µm; H–I = 10 µm.

Discussion

Sphaeropezia leucocheila has been found only once. In that specimen, fruiting bodies of the fungus were consistently associated with a patch of dead or dying leaves in a liverwort colony that included the intermixed dead leaves of several species of liverworts from the family Lepidoziaceae. As *Sphaeropezia* includes several putative parasites of liverworts and mosses from the Northern Hemisphere, it is likely that S. *leucocheila* may also be a pathogen. *Sphaeropezia hepaticarum*, a putative pathogen from liverworts and mosses from Austria and Sweden, is morphologically distinct, with much larger, multi-septate ascospores (Sherwood 1987, Döbbeler & Poelt 1974, as *Bryodiscus hepaticarum*). Similar to *S. hepaticarum*, *S. leucocheila* is not specialised to a single host species or genus.

Like the other *Sphaeropezia* species that had originally been placed in the genus *Bryodiscus*, *S. leucocheila* has ascomata that develop more or less superficially on the host leaves, forming on a dense pad of mostly superficial fungal tissue, which is connected to less dense fungal hyphae within the cells of the diseased host leaves.

Although a small fungus, *S. leucocheila* was associated with a distinctive, dead patch within a liverwort colony. One of the authors (Renner) has carried out an extensive survey of liverworts in New Zealand, and only once saw a combination of these kinds of symptoms with *Sphaeropezia* fruiting bodies. Although best characterized for now as Data Deficient, further surveys targeting patches of liverworts with similar symptoms may show *S. leucocheila* to be one of New Zealand's 'naturally uncommon' fungi (Townsend *et al.* 2008).

Acknowledgements

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