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A new species of *Diploschistes* (*Lecanoromycetes*) occurring on *Stereocaulon* from the Pacific Northwest of North America

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Abstract. We describe *Diploschistes stereocaulorum* as a new species of lichenized fungi growing on phyllocladia and stalks of *Stereocaulon* in the Pacific Northwest of North America. The larger ascospores of this species, the absence of a noticeable thallus, and the ITS barcode locus distinguish it from *D. muscorum*, which has been reported as growing on *Stereocaulon*, but has smaller ascospores and a distinct thallus. In phylogenetic reconstruction, the new species is sister to *D. diacapsis*, albeit without support.

Key words: *Diploschistes*, lichenized fungi, new species, parasitic lichens, *Stereocaulon*

Introduction

A number of crustose lichens are parasitic on other lichens, especially in the genera *Acarospora* A. Massal., *Caloplaca* Th. Fr. and *Rhizocarpon* Ramond ex DC. (Poelt & Doppelbaur 1956; Poelt 1958; Poelt & Steiner 1971; Poelt 1990). Within the genus *Diploschistes* Norman, three species are known to be parasitic, at least facultatively: *D. caesioplumbeus* (Nyl.) Vain. on *Lecanora gangaleoides* Nyl. (Hawksworth 1982), *D. euganeus* (A. Massal.) J. Steiner on *Ochrolechia parella* (L.) A. Massal., and *D. muscorum* (Scop.) R. Sant., which frequently parasitizes different *Cladonia* species, but is also rarely found on species of the genus *Stereocaulon* Hoffm. (Lumbsch 1989). The latter species has two different populations largely, but not exclusively, restricted to either the northern or southern Hemisphere respectively, distinguished by the number of ascospores in the asci (four vs. eight), which are currently recognized as different subspecies (Lumbsch 1987). While studying specimens of *Stereocaulon sterile* (Savicz) Lamb ex Krog from Oregon, USA, we noticed odd growths of apothecia appearing to be *Diploschistes*. Study of apothecial sections demonstrated that to be true; furthermore, the thallus was poorly developed and the ascospores were unusually large for *D. muscorum* previously reported as growing on *Stereocaulon* (Zhurbenko 2010). Therefore, the new species, *D. stereocaulorum*, is introduced as new.

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Materials and methods

DNA extraction and PCR amplification

We extracted DNA sequences from two recently collected specimens of the putative new species. We chose to analyze the barcode marker for fungi, the nuclear internal transcribed spacer (ITS) (Schoch et al. 2012).

For DNA isolation, a 1 mm^3 sample of apothecia of each specimen was selected. Samples were first eluted in a 1.5 mL Eppendorf tube with 50 µL of 99.7% acetone for 10 min. The acetone was then removed by pipette and the remaining sample air dried for 10 min.

DNA was extracted with various methods, but primarily using the RED Extract-N-Amp Plant PCR kit by Sigma-Aldrich and substituting DreamTaq Green (2×, Thermo Scientific Inc.) for REDtaq. The procedure used was according to the manufacturers' instructions: 20 µL extraction solution was added and incubated at 95°C for 10 minutes and finalized by adding 20 µL dilution solution. We amplified ITS with primers ITS1F and ITS4 (White et al. 1990).

PCR reaction conditions were initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 45 seconds, and extension at 72°C for 45 sec, followed by an elongation cycle for 5 min at 72°C. We viewed PCR products with gel electrophoresis and successful samples were then cleaned using ExoSAP-IT™ Affymetrix 78200 following the manufacturer's protocol. These were processed using thermocycler incubation of 37°C for 15 min followed by 80°C for 15 min. Cleaned PCR products were prepared for sequencing by combining 2.4 µL

forward primer (as above), 7.8 µL nuclease free H_2O, and 1.8 µL ExoSAP-IT product, then sequenced with forward and reverse reads (Eurofins MWG Operon Inc., Kentucky, USA).

Geneious version 10.0.9 (http://www.geneious.com, (Kearse et al. 2012) was used to check quality of the raw sequences, align the two reads per sample, and generate a consensus sequence for phylogenetic analyses.

The new sequences were aligned with 36 sequences downloaded from Genbank. Sequences were aligned using GENEIOUS Pro 9.1.8 with MAFFT using the auto option (Katoh & Toh 2005; Katoh et al. 2009). The program Gblocks v. 0.91b (Talavera & Castresana 2007) was used to delimit and remove ambiguous nucleotide positions from the final alignment using the online web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), implementing the options for a less stringent selection of ambiguous nucleotide positions. The final alignment was subjected to a maximum likelihood search with RAxML 8.2.0 (Stamatakis 2014) using the GTRgamma model, and non-parametric bootstrapping using 1000 replicates. A midpoint rooted tree was chosen using the program FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

The bootstrap support values for the tree topology are generally low, which is to be expected with a single marker phylogeny. In addition, we used Gblocks to eliminate all ambiguous sites. However, the samples of each species formed monophyletic groups (Figure 1). The newly collected samples growing over *Stereocaulon* were only distantly related to *D. muscorum*, but clustered with *D. diacapsis* (Ach.) Lumbsch, albeit without bootstrap support, hence supporting that this is a species that is distinct from *D. muscorum* (Figure 1). Additionally, the phylogenetic data support the micromorphological evidence suggesting that these specimens do not belong to *D. muscorum*, but represent an undescribed species, which is closer to *D. diacapsis*. Consequently, a new species is proposed below.

Diploschistes stereocaulorum Lumbsch, C. Morris & McCune, sp. nov. (Fig. 2)

MycoBank MB 849632

Diagnosis: Species similar to *D. muscorum*, but distinguished by having larger ascospores and a poorly developed thallus.

Type: USA, Oregon, Lane County, Horse Rock Ridge, 44.2996, –122.8824, 823 m, open, rocky west-facing ridge, on

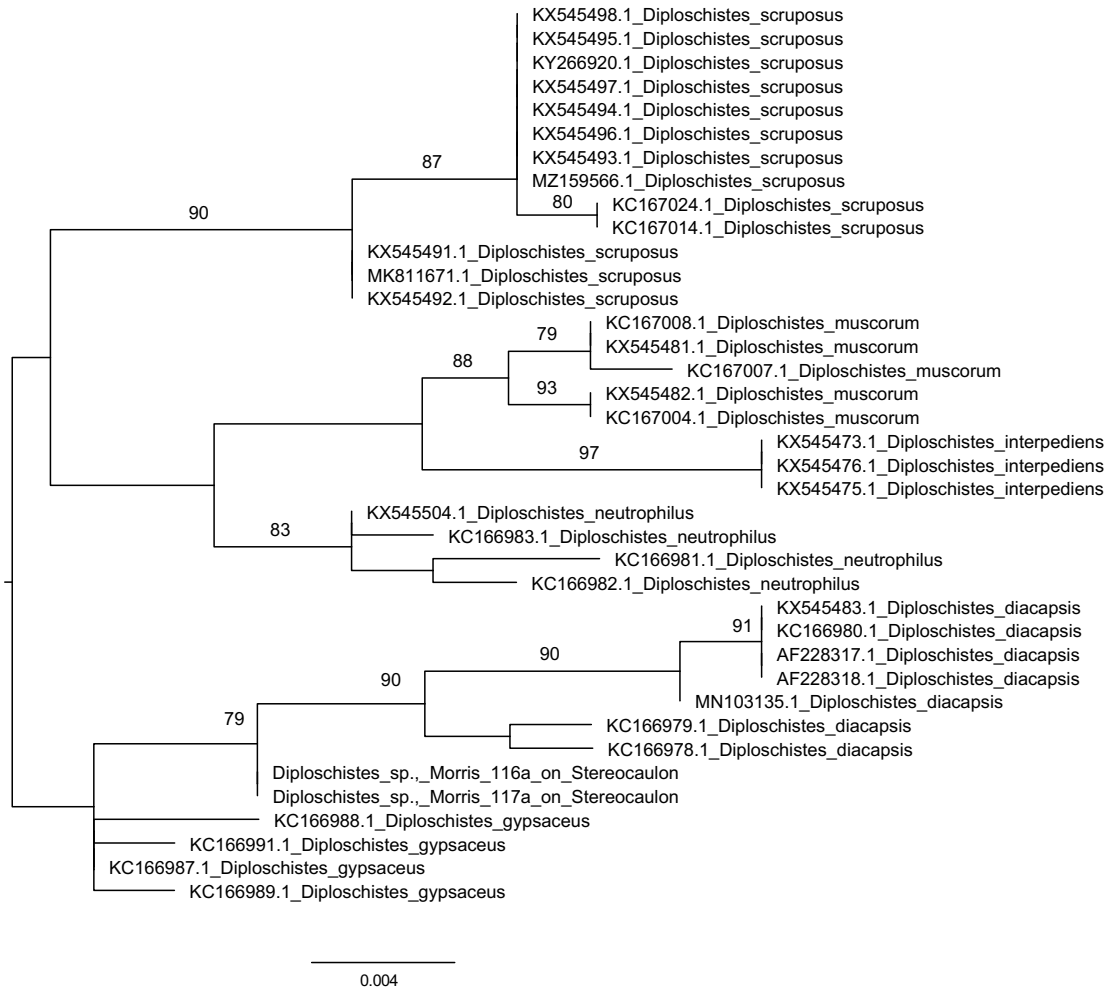

Figure 1. Maximum likelihood phylogenetic reconstruction of the nrITS barcode locus for *Diploschistes* species. Bootstrap support values equal to or above 75% are indicated at the branches. This is a mid-point rooted tree.

Stereocaulon sterile on basalt, rock face just down from the cave, with *Lepraria gracilescens* and *Lepraria neglecta* s.l., 1 May 2022, C. Morris 116a (OSC – holotype! GenBank OR365611).

Description. Thallus not apparent, the margins of the apothecia blending seamlessly with the thallus of the host.

Apothecia solitary, partially sunken to subsessile on phyllocladia of *Stereocaulon*, urceolate, to 0.8 mm diam, the margin whitish to pale gray on the outer part and grayish and ± radially striate to cracked on the inner part; disk dark gray-black; proper exciple POL–, brown to dark brown, partly green black, 50–70 µm thick, pseudoparenchymatous; thalline exciple thick, POL+, K+ orangish diffusion, excipular cortex C+ pink, KC+ pink; epithecium blue-green-black, POL+ or sparsely POL+; hymenium hyaline, not inspersed, about 130–140 µm thick; paraphyses 1–1.5 µm thick, simple; subhymenium hyaline, about 15–20 µm thick, hypothecium thin, brown to dark brown, POL–; asci cylindrical, apically thickened, 90–120 × 20–30 µm, non-amyloid: ascospores brown, muriform, 4/ascus, (29)33–46 × 15–21 µm, median 42 × 18 µm.

Pycnidia not seen.

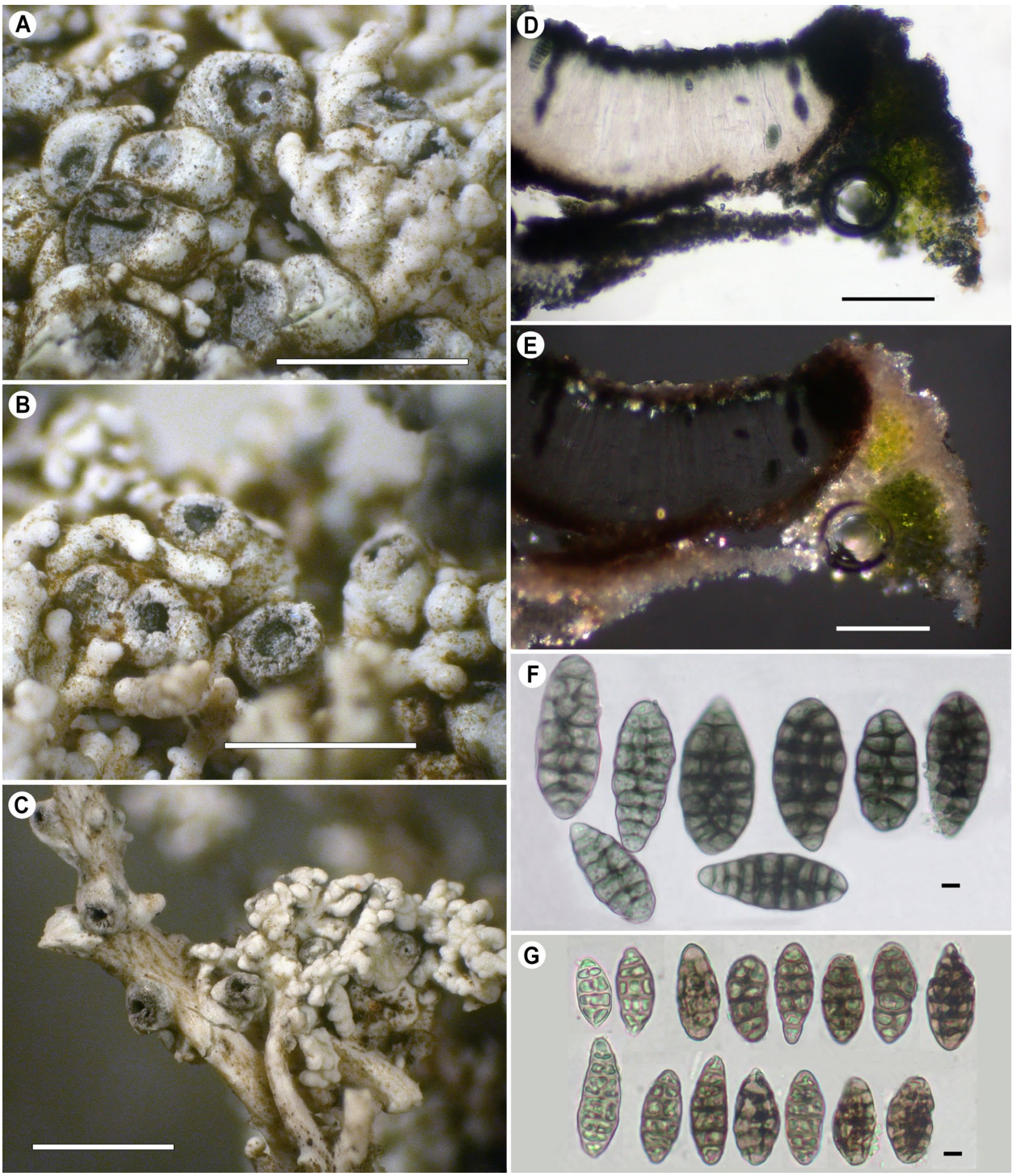

Figure 2. *Diploschistes stereocaulorum*. A–B – habit, Morris 116a; C – habit, Morris 117a; D – apothecial section, Morris 116a; E – same as D but under polarized light; F – ascospores of Morris 116a; G – comparison ascospores from *Diploschistes muscorum* at same scale, McCune 6718 (USA, Montana, Lake County). Scales: A–C = 1 mm; D–E = 0.1 mm; F–G = 5 µm.

Secondary metabolites. Given the almost nonexistent thallus, too little material was available for TLC, but we hypothesize the presence of diploschistesic and/or lecanoric acid based on the C reactions in the apothecia and their common occurrence in *Diploschistes* (Lumbsch 1989).

Distribution and ecology. Currently, the species is only known from two localities in the Pacific Northwest of North America where it grows on *Stereocaulon* species.

Etymology. The epithet refers to the fact that the new species grows on *Stereocaulon* species.

Notes. This new species differs morphologically in having larger ascospores and a very reduced thallus from *D. muscorum.* Another similar taxon is *D. muscorum* ssp. *bartlettii* Lumbsch, which occurs in the southern Hemisphere and differs in having smaller ascospores and containing eight spores per ascus (Lumbsch 1987). The other two parasitic species of *Diploschistes* are saxicolous and have perithecioid ascomata and, hence, readily distinguished from the new species (Lumbsch 1989). Other terricolous species include *D. diacapsis* (Ach.) Lumbsch and *D. thunbergianus* (Ach.) Lumbsch & Vězda. The latter is restricted to the southern Hemisphere and differs in having smaller ascospores (Lumbsch 1993). The cosmopolitan *D. diacapsis* also has smaller and narrower ascospores and a thick, verruculose to bullate thallus (Lumbsch 1988).

A phylogenetic reconstruction inferred from ITS barcode marker sequences supports that this new species is not closely related to *D. muscorum.* The *Diploschistes* apothecia are growing on top of *Stereocaulon* phyllocladia and stalks without the formation of an obvious thallus. The positive C reaction, unknown in *Stereocaulon*, but common in *Diploschistes*, indicates that the exciple consists of *Diploschistes* and its enclosed algae, rather than *Stereocaulon* tissue.

The identity of the algae associated with the new species is unknown. While juvenile parasitism was traditionally interpreted as a way to gain a photobiont from other lichens (Poelt & Doppelbaur 1956; Poelt 1958), it was shown that *Diploschistes* species contain *Trebouxia* photobionts (Friedl & Gärtner 1988), whereas *Stereocaulon* contains *Asterochloris*, *Chloroidium*, and *Vulcanochloris* species (Škaloud & Peksa 2010; Peksa & Škaloud 2011; Vančurová et al. 2017). A study focused on the algal photobiont of *D. muscorum* showed that the photobiont is switched from *Asterochloris* in early stages to *Trebouxia* during the life cycle of this parasitic lichen (Friedl 1987).

From the two Oregon samples, we attempted to sequence both the *Diploschistes* and the *Stereocaulon* from an uninfected portion. While one of those *Stereocaulon* fragments revealed a typical ITS sequence for *S. sterile*, the other amplified the *Diploschistes*, demonstrating that this species can occur as a non-apparent endolichenic fungus.

Zhurbenko's (2010) compendium of lichenicolous fungi on *Stereocaulon* lists many instances of *D. muscorum* on *Stereocaulon*, most from Russia. Unfortunately, these samples are currently unavailable for study. However, it cannot be ruled out that at least some of these records belong to the new species. The Russian material should be re-examined for ascospore size. In addition, the association of *D. muscorum* and *Stereocaulon* has also been reported from Europe (Lumbsch 1989). We re-examined the material that was previously identified as *D. muscorum* from Europe and can confirm that those indeed belong to *D. muscorum* having smaller ascospores than the new species. Hence, two *Diploschistes* species can occur on *Stereocaulon.*

Additional specimens examined. CANADA. British Columbia, Quadra Island, near summit of South Peak of Chinese Mountain, 50.1426, 125.2631, 316 m, mossy rock outcrops of quartz amygdaloid greenstone and andesite, N to E facing, with broken forest, on *Stereocaulon sterile* on greenstone, 21 June 2018, B. McCune 37683b (OSC). USA. Oregon, Lane County, Horse Rock Ridge, open, rocky ridge on thickly moss-covered bank, 44.2997, –122.8818, 823 m, on *Stereocaulon sterile* on basalt, 1 May 2022, C. Morris 117a (OSC; GenBank OR365612).

Specimens of *D. muscorum* on *Stereocaulon* examined. SWEDEN. Lule Lappmark, Porjus par., Muddus Nat. Park, on E side of Muddusälvern below footbridge N of Muddusfallet, 66°49′N, 20°10′E, parasitic on thallus of *Stereocaulon* sp., 23 June 1977, B. J. Coppins & L. Tibell (E). NORWAY. Prov. Oppland, Grimsdalen in Rondane, E of Dombas near Grimsdalshytta. Treeless, weakly calcareous rocky slope, 1000 m, leg. "Stud. biol. Rheno-Tran in itinere", 2 Aug 1975 (L).

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