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Standard Paper

Sinuicella denisonii, a new genus and species in the *Peltigeraceae* from western North America

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Abstract

The new genus *Sinuicella*, an early successional lichen, was found on bare soil in Oregon, USA. The thallus is minute fruticose, grey to nearly black, branching isotomic dichotomous, branches round, 20–90 µm wide in water mount. The cortex is composed of interlocking cells shaped like jigsaw puzzle pieces. Spores are hyaline, 1-septate, 25–40(–50) × 6.5–9(–11) µm. Maximum likelihood phylogenetic analyses on multilocus data sets, first spanning the entire order *Peltigerales* and then restricted to *Peltigeraceae* with extended sampling from *Solorina* and *Peltigera*, revealed the placement of *Sinuicella* outside of currently recognized genera, sister to *Peltigera*, with high support. Based on the phylogenetic, morphological and ecological distinctness of *Sinuicella*, we formally introduce a new genus represented by the single species *S. denisonii*. The cyanobiont of *S. denisonii* is *Nostoc* from phylogroup XL, Clade 2, Subclade 3 based on the *rbcLX* marker.

Key words: lichenized ascomycetes, lichenized fungi, *Nostoc*, Oregon, *Peltigera*, *Peltigerales*, *Solorina*, USA

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Introduction

Sifting through unidentified and partly identified specimens in the Oregon State University herbarium, we found a minutely fruticose cyanolichen on soil that we had not seen before. Under the microscope it was revealed to be a *Nostoc*-containing cyanolichen with cortical cells shaped like jigsaw puzzle pieces, immediately suggesting *Leptogidium contortum* (Henssen) T. Sprib. & Muggia (photograph p. 288, McCune & Geiser 2009). While that species occurs in Oregon on trees, we knew of no occurrences on soil. Furthermore, the photobiont was not *Rhizonema* (Lücking *et al.* 2009; Cornejo *et al.* 2016), as is found in *L. contortum* and *L. dendriscum* (Nyl.) Nyl., but a species of *Nostoc*. Based on the unusual combination of characters and the nearby location, we decided to try to find fresh material for further study.

The presumed collector of the original specimen was William C. Denison, long-time mycologist at Oregon State University, now deceased. Because the collecting location was quite vague, we asked his son, Tom Denison, if he knew anything of his father's explorations in that area. Tom did remember that their family took long hikes following the railroad grade up the Luckiamute River from Hoskins to the town of Valsetz. Valsetz no longer exists, and all that remains of the railroad are sections of the railroad bed where it has not been destroyed by road construction. Currently, the river is paralleled by a major logging road, with a slender riparian zone surrounded by young forests and clear cuts. Because the area is so heavily impacted by logging and road dust, we had only a slight hope of finding fresh material. However, we were pleasantly surprised to find not only a modern location for the unknown lichen, but also *Gregorella humida* (Kullh.) Lumbsch as an ecological associate of the new lichen (McCune & Stone 2020).

Our first attempts at sequencing the ITS region of the new lichen gave surprising results: it blasted closest to *Peltigera venosa* (L.) Hoffm., but examination of an alignment with various *Peltigera* species revealed such large differences within the ITS region that we suspected a sequencing problem. Repeating the extraction and sequence yielded nearly identical results, prompting us to pursue the problem in earnest. The purpose of this paper is to present these results as a new monospecific genus *Sinuicella* (*S. denisonii*) in the *Peltigeraceae*, with affinities to *Solorina* and *Peltigera* but clearly falling outside of both.

Materials and Methods

We studied the original specimens collected in 1969 and the new collections from near Hoskins, Oregon with standard light microscopy. Thin sections and whole thalli were studied in material mounted in water, K/IKI, and IKI. Spores were measured at ×400. Microscopic photographs were taken with a Nikon Coolpix 995 digital camera through an Olympus BX40 microscope, coupled with an Optem 25-70-14-03s to the UVT-1X C-mount on the trinocular head, using magnifications up to

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×400. Photographs under the dissecting microscope were created with the same camera and coupler to the SZ-CTV mount on the trinocular head of an Olympus SZ40 stereo microscope, using magnifications up to ×40 and photo stacking with Helicon Focus 6 (HeliconSoft, Ukraine).

Other than the historical specimen and collections from the new site, we have been unable to locate material elsewhere, either in the field or in regional herbaria. It is likely that the material, particularly if sterile, would have been filed under *Leptogidium* (*Polychidium*) *contortum*, because this species also has a jigsaw cortical structure, is similar in size, and has been of considerable regional interest since its early reports from North America by Brodo (1995) and McCune *et al.* (1997). Over the decades we have studied most of the readily available material of *Leptogidium contortum* in regional herbaria and have not seen any other soil-dwelling material labelled under that name.

Thin-layer chromatography (TLC) was performed on the specimen *D. Stone* 10122 (OSC) using solvents A, B' and C (Culberson & Ammann 1979; Culberson & Johnson 1982; solvent C is the same as solvent 'TA' of Holtan-Hartwig (1993)). Four *Peltigera* specimens (*P. britannica* (Gyelnik) Holt.-Hartw. & Tønsberg, *P. horizontalis* (Hudson) Baumg., *P. malacea* (Ach.) Funck and *P. retifoveata* Vitik.) and three *Solorina* specimens (*S. crocea* (L.) Ach., *S. octospora* (Arnold) Arnold and *S. saccata* (L.) Ach.) were included as the reference samples for comparison.

We extracted DNA from a single specimen (*Stone* 10024) without grinding the thallus using the REDExtract-N-Amp Plant PCR kit by Sigma-Aldrich (St Louis, Missouri, USA). Details of PCR for ITS and LSU follow McCune *et al.* (2019). Briefly, we amplified the fungal ITS region with primers ITS1F and ITS4, and the nuLSU region with primers AL2R and LR6 (Vilgalys & Hester 1990), with PCR annealing at 64 °C for 45 s; the mtSSU region was amplified with primers mrSSU1 and mrSSU3R, with PCR annealing at 53 °C for 1 min. We also amplified one region of the cyanobiont, *rbcLX*, with primers CX and CW (primers and PCR conditions follow O'Brien *et al.* (2005)). PCR products were visualised with gel electrophoresis and successful samples were cleaned using ExoSAP-ITTM Affymetrix 78200, and subsequently sequenced with forward and reverse reads (Eurofins MWG Operon Inc., Kentucky, USA). At least two reads per region were combined into a consensus sequence.

Data matrices and phylogenetic analyses

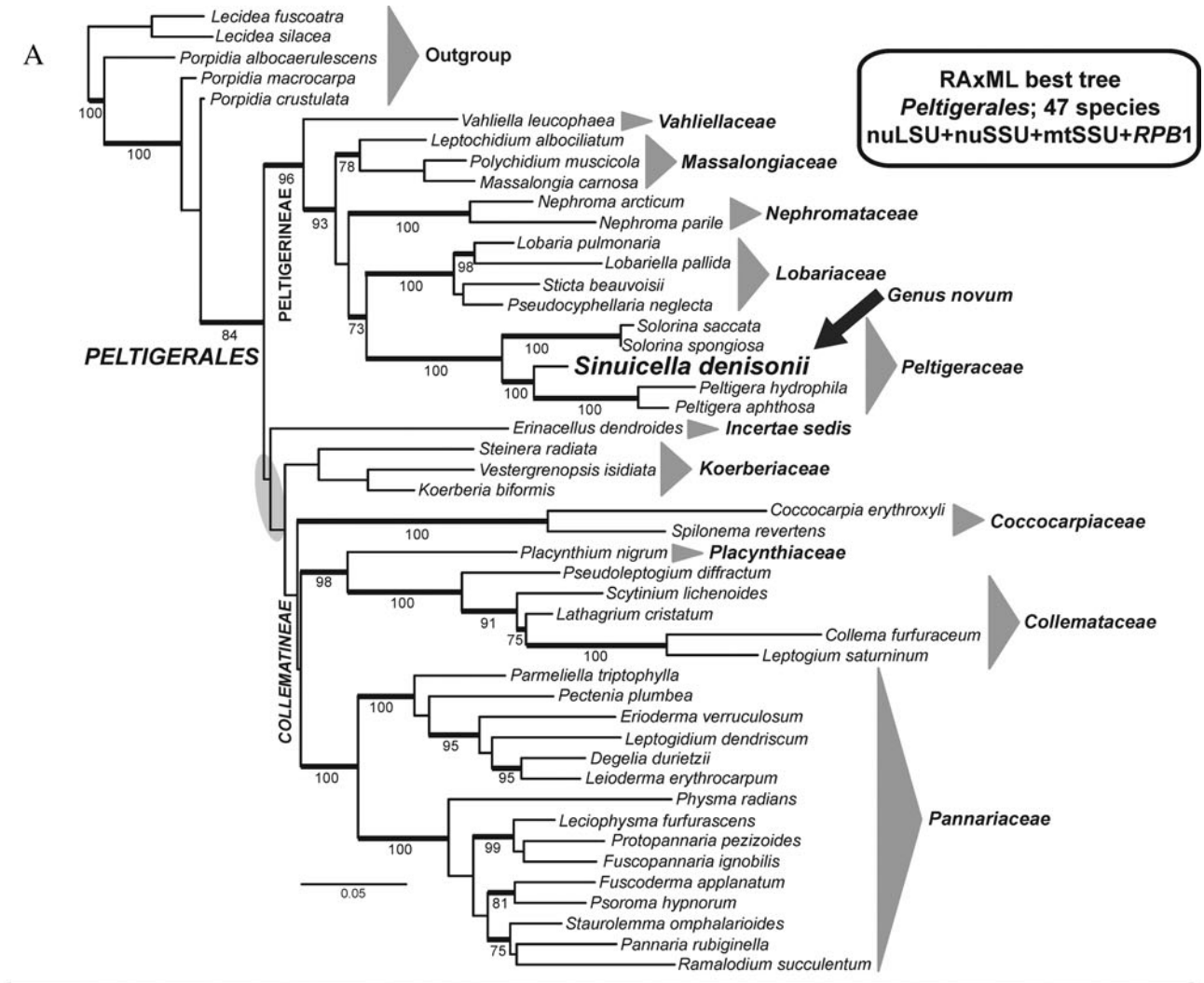
We applied the Evolutionary Placement Algorithm (EPA; Berger & Stamatakis 2011) as implemented in the Tree-Based Alignment Selector toolkit (T-BAS version 2.1, available at http://tbas.hpc.ncsu.edu; Carbone *et al.* 2017, 2019) using the Lecanoromycetes reference tree (Miadlikowska *et al.* 2014*a*; Carbone *et al.* 2019) based on mtSSU and nuLSU sequences separately and combined. For each EPA analysis we used the GTR substitution model (Rodríguez *et al.* 1990) with gamma distribution parameter (GTRGAMMA) and calculated likelihood weights with a placement cut-off distance of 10. Based on the EPA analyses, *Sinuicella denisonii* was consistently shown as sister to the clade representing the genus *Peltigera* (results not shown). This sister relationship was also confirmed by a follow up search of the best tree and bootstrap analyses (1000 replicates) (RAxML 8.2.12; Stamatakis 2006; Stamatakis *et al.* 2008) as implemented in T-BAS v.2.1 via the CIPRES Science Gateway v.3.3 (Miller *et al.* 2010, 2015) based on the multilocus matrix for Lecanoromycetes. The newly added mtSSU and nuLSU sequences were realigned with MAFFT v.7.402 (Katoh & Toh 2010), the GTRGAMMA nucleotide substitution model was calculated, and the backbone constraint on the multifurcating Lecanoromycetes reference tree (where internodes with bootstrap support < 70% were collapsed) was implemented.

Based on the resulting RAxML phylogeny showing a sister relationship between *Sinuicella* and *Peltigera* (with 99% bootstrap support), we selected representatives from each family in the order *Peltigerales* together with two members (*Lecidea* and *Porpidia*) from the closely related order *Lecideales* (Miadlikowska *et al.* 2014*a*) to root the tree. Single-locus alignments for three ribosomal RNA loci, nuSSU, nuLSU and mtSSU, and the protein coding *RPB*1 gene were downloaded from T-BAS v.2.1 (files associated with the Lecanoromycetes reference tree; Carbone *et al.* 2017, 2019). We supplemented these data with six additional taxa from the *Peltigerales* (Spribille *et al.* 2014), which were not present in the Lecanoromycetes reference tree in T-BAS, giving a total of 47 species (see Supplementary Material Table S1, available online). All single-locus alignments were manually adjusted using Mesquite v.3.11 (Maddison & Maddison 2015) with the option 'Nucleotide with AA color' for guiding the *RPB*1 alignment. Ambiguously aligned regions (*sensu* Lutzoni *et al.* 2000) were delimited manually and excluded from subsequent analyses. The combined 4-locus, 47 species data set for the *Peltigerales* includes a single representative from most genera in all 10 families currently recognized in the order (*Coccocarpiaceae*, *Collemataceae*, *Koerberiaceae*, *Lobariaceae*, *Massalongiaceae*, *Nephromataceae*, *Pannariaceae*, *Peltigeraceae*, *Placynthiaceae* and *Vahliellaceae*; Lücking *et al.* 2016). Six of the 47 species had two loci, 18 species had three loci, and 23 species had four loci (Supplementary Material Table S1).

To re-evaluate the phylogenetic placement of *Sinuicella* within *Peltigeraceae* and confirm its sister relationship to *Peltigera*, we used a 4-locus data set that includes ITS, nuLSU, ß-tubulin and *RPB*1, derived from a 7-locus data matrix (Chagnon *et al.* 2019) containing a single representative of every putative *Peltigera* species and selected species of the genus *Solorina*. To this data matrix we added the newly obtained ITS and nuLSU sequences for *Sinuicella denisonii* (*Stone* 10024), individuals of *Solorina* species for which at least one of the four loci was available in GenBank, and newly-generated sequences for two additional *Solorina* specimens. We expanded the outgroup by adding three members of the family *Lobariaceae*. All single-locus alignments were manually adjusted using Mesquite v.3.11 with the option 'Nucleotide with AA color' for guiding the protein-coding alignments. Ambiguously aligned regions (*sensu* Lutzoni *et al.* 2000) were delimited manually and excluded from subsequent analyses. The final 4-locus data set for the *Peltigeraceae* includes 185 taxa, 13 of which are represented by a single locus, 45 by two loci, 45 by three loci, and 82 taxa by four loci (see Supplementary Material Table S2, available online).

To determine the phylogenetic identity of the *Nostoc* cyanobiont from *Sinuicella denisonii* (*Stone* 10024), we added its *rbcLX* sequence to a matrix containing 503 *rbcLX* haplotypes derived from Pardo-De la Hoz *et al.* (2018) and Magain *et al.* (2018). These sequences represent a broad sampling of published symbiotic and free-living *Nostoc* from all currently recognized phylogroups within *Nostoc* Clade 1 and Clade 2, Subclades 1, 2 and 3 (*sensu* Otálora *et al.* 2010; Magain *et al.* 2017*a*, *b*, 2018).

Maximum likelihood analyses using RAxML-HPC2 on XSEDE were performed at the nucleotide level on each data set (4 loci for *Peltigerales*, 4 loci for *Peltigeraceae*, and *rbcLX* for



Nostoc) as implemented on the CIPRES Science Gateway. Optimal tree and bootstrap searches were conducted with the rapid hill-climbing algorithm for 1000 replicates with the GTRGAMMA nucleotide substitution model. Each data set was partitioned into subsets using PartitionFinder2 on XSEDE (Lanfear *et al.* 2017) as implemented on the CIPRES portal, with greedy search and using the AICc (corrected Akaike Information Criterion) for model selection. The 4-locus data set for *Peltigerales* was partitioned into five subsets: mtSSU, nuSSU, nuLSU, *RPB*1-1st + 2nd codon positions, and *RPB*1-3rd codon position + intron. The 4-locus data set for *Peltigeraceae* was partitioned into two subsets: nuLSU + *RPB*1-1st + 2nd + intron + ß-tubulin-1st + 2nd + 3rd codon positions + ITS, and ß-tubulin introns + *RPB*1-3rd codon position. The *rbcLX* data set was partitioned into six subsets: *rbcL*-1st, *rbcL*-2nd, *rbcL*-3rd, *rbcX*-1st, *rbcX*-2nd and *rbcX*-3rd codon positions. Relationships receiving bootstrap support ≥ 70% were considered well supported.

Results

BLAST results (as of 3 May 2020) for *Sinuicella* sequences were inconclusive: the nuLSU and mtSSU sequences showed a low similarity (*c.* 94%, 100% coverage) to multiple species of *Peltigera* from various sections, while the ITS was most similar (91%, 100% coverage) to *Solorina saccata*. All EPA analyses using the Lecanoromycetes reference tree in T-BAS placed *Sinuicella* outside of currently delimited genera, sister to *Peltigera* (results not shown). This phylogenetic affiliation was confirmed by maximum likelihood inferences, first within the broader phylogenetic context of the order *Peltigerales* (Fig. 1A; Supplementary Material Table S1, available online) and then within the narrower context of the family *Peltigeraceae* (Fig. 1B; Supplementary Material Fig. S1, Supplementary Material Table S2, available online). This sister relationship of the genera *Sinuicella* and *Peltigera* was highly supported in both phylogenies (100%; Fig. 1A & B).

The cyanobiont from *Sinuicella denisonii* represents *Nostoc* phylogroup XL (Fig. 1C) within *Nostoc* Clade 2, Subclade 3 (Magain *et al.* 2018). This highly supported phylogroup (79%) includes *Nostoc* associated with various *Peltigera* species from section *Peltigera* collected in Central and South America, and a symbiont of *Anthoceros* sp. (hornwort) from Germany (Fig. 1C; Supplementary Material Fig. S2, available online). The cyanobiont of *Sinuicella denisonii* is the only member of phylogroup XL known from North America. All *rbcLX* sequences in this phylogroup share an identical nucleotide sequence for the spacer region separating *rbcL* from *rbcX*. This spacer of the *rbcLX* locus cannot be aligned across all taxa included in this study and, therefore, was excluded from phylogenetic analyses.

The New Genus and Species

Sinuicella D. F. Stone, McCune & Miadl. gen. nov.

MycoBank No.: MB 836896

Thallus minutely fruticose, isotomic dichotomously branched, branches roundish in section; cortex with cells shaped like interlocking jigsaw puzzle pieces; medulla of isodiametric cells. Apothecia initially globose, expanding with age to a flattened disc, proper exciple minutely tomentose; spores hyaline, 1-septate, broadly fusiform with blunt ends. Photobiont *Nostoc* sp.

Monospecific; refer to species description for details.

Sinuicella denisonii D. F. Stone, McCune & Miadl. sp. nov.

MycoBank No.: MB 836898

Diagnosis as in the genus.

Type: USA, Oregon, Polk County, Wildwood Road, Hoskins to Valsetz, former railroad grade, near bridge, 44.75556°N, 123.5543°W, 172 m, on soil, 13 November 2018, *D. Stone* 10024 (holotype—OSC; isotypes—DUKE, WTU, UPS).

(Figs 2–4)

Thallus minutely fruticose, indeterminate, spreading across bare soil, grey to nearly black (Fig. 2A); branching mostly isotomic, branches round to slightly flattened, 20–90 µm wide in water mount; *prothallus* not apparent; thallus cortex one cell deep, these cells vertically elongate and 15–30 µm high, on the outside surface appearing as interlocking puzzle pieces (Fig. 3B); *medulla* of densely packed cyanobacteria cells among isodiametric, angled hyphal cells up to 25 µm wide; *vegetative propagules* absent; *photobiont Nostoc* (Fig. 3C), phylogroup XL of Magain *et al.* (2018; GB *rbcLX*: MT944984).

Apothecia initiating as a spherical knob with little fruiting surface showing and proper exciple whitish and distinctly tomentose; *disc* expanding with age to 1.5 mm wide, circular when first expanded and becoming irregular in outline, the proper exciple torn and ragged, fruiting surface reddish brown and flat to slightly convex; base narrow at attachment to thallus; apothecial section POL−, K− except K+ pale yellowish brown in exciple and hypothecium; *proper exciple* well developed, radiate (Fig. 4A & D), cells with lumina narrowly elliptic near the upper edge to broadly elliptic to nearly isodiametric and up to 25 µm wide towards the base, cell walls 2.5 µm thick; *hypothecium c.* 90 µm tall overall, two layered, hyaline to pale brownish, the upper

Fig. 1 (see previous page). Phylogenetic placement of *Sinuicella denisonii* in the order *Peltigerales* (A) and the family *Peltigeraceae* (B), and its cyanobiont within *Nostoc* phylogroup XL of *Nostoc* Clade 2, Subclade 3 (C, in grey). A, phylogenetic relationships inferred from maximum likelihood (ML) analyses of a 4-locus data set (nuLSU + nuSSU + mtSSU + *RPB*1) for 42 species representing all recognized families within *Peltigerales* and five outgroup species from *Lecideales*. The two *Lecidea* species were used to root the tree (following Miadlikowska *et al.* 2014*a*). Bootstrap support is provided for the internodes with values ≥ 70%, which are also shown with thick lines. The scale bar represents number of nucleotide substitutions per site. Classification follows Lücking *et al.* (2016). Internodes highlighted in grey represent uncertain placement of *Koerberiaceae* (currently a member of *Peltigerineae*) and *Erinacellus* (currently *incertae sedis* in *Peltigerales*). B, phylogenetic relationships inferred from ML analyses of a 4-locus data set (ITS + nuLSU + ß-tubulin + *RPB*1) for 185 taxa representing *Peltigeraceae* and six outgroup species from the closely related families *Lobariaceae* and *Nephromataceae*. *Nephroma* was used to root the tree (following Miadlikowska *et al.* 2014*a*). Within-section relationships (*sensu* Miadlikowska & Lutzoni 2000) of the genus *Peltigera* were collapsed (see Supplementary Material Fig. S1, available online, for the complete phylogeny). Bootstrap support is provided for the internodes with values > 70%, which are also shown with thick lines. The scale bar represents number of nucleotide substitutions per site. C, *Nostoc* phylogroup XL part of Clade 2, Subclade 3 of the phylogeny that resulted from an ML analysis of the *rbcLX* data set which includes 504 representatives of free-living and symbiotic *Nostoc* (modified data set from Pardo-De la Hoz *et al.* (2018) and Magain *et al.* (2018)). Names refer to the mycobiont with which the *Nostoc* associates. Bootstrap support is provided for the main internodes, and those with values > 70% are shown with thick lines. See Supplementary Material Fig. S2 (available online) for the complete *Nostoc* phylogeny with scale.

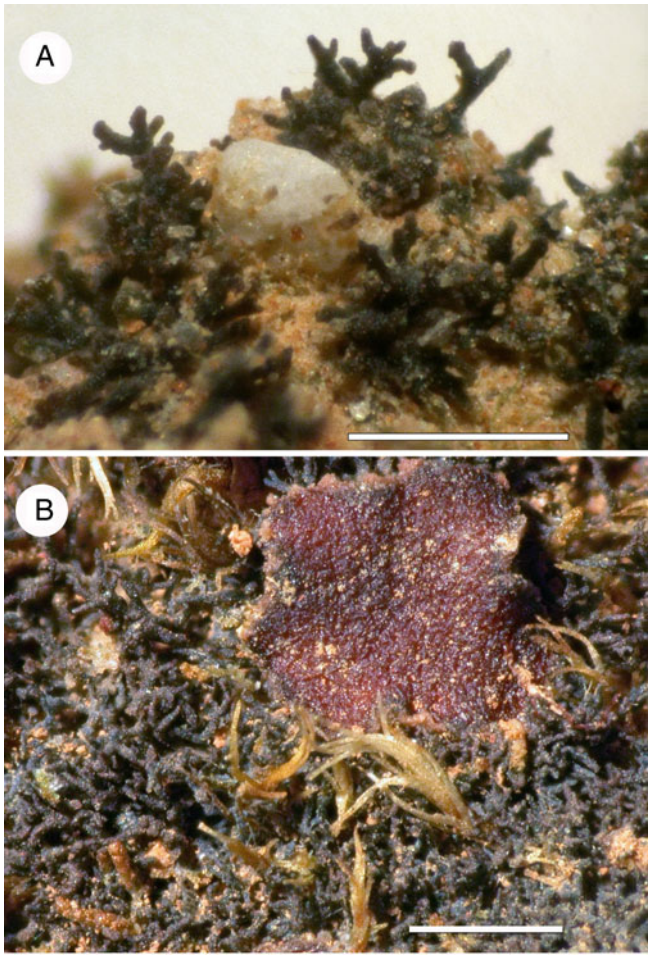


Fig. 2. *Sinuicella denisonii*. A, habit (*Stone* 10024). B, apothecium (OSC 35280). Scales = 1 mm. In colour online.

layer 25–55 µm thick, of randomly oriented hyphae, lower layer 35–40 µm thick, prosoplectenchymatous, of small pale brownish cells, ±gelatinized, the hyphal structure obscure; *hymenium* 150 µm tall; *epihymenium c.* 10 µm tall, pale reddish brown or orangish brown; *paraphyses* simple, cylindrical, 2 µm diam., some to 3 µm at the tips, others not enlarged, without defined coloration at tips but pale reddish brown similar to the epihymenium; *asci* cylindrical, K/IKI+ blue, with a K/IKI+ darker blue annulus (*Peltigera* type; Fig. 4B); *ascospores* at least 4 per ascus (up to 6 observed), hyaline, 1-septate, broadly fusiform with blunt ends, 25.3–45.0 × 6.6–11.0 µm (Fig. 4C; $n = 29$, mean length 35.0 ± 4.6 µm, mean width 8.6 ± 1.2 µm).

Pycnidia not seen.

Chemistry. No secondary metabolites were detected with TLC.

Etymology. The genus name '*Sinuicella*' refers to the cells of the cortex which have curved protrusions in their outlines, similar to interlocking jigsaw puzzle pieces. The epithet '*denisonii*' refers to William C. Denison, long-time mycologist at Oregon State University, now deceased. He was a pioneer in the use of lichens to monitor air quality in the United States.

Ecology and substratum. *Sinuicella denisonii* is so far known from soil in recently disturbed areas along Wildwood Road which follows the Luckiamute River between Fort Hoskins and the former town of Valsetz in the Coast Range of western Oregon. The known elevation range is so far narrow, at *c.* 172 m. It occurs in exposed to somewhat sheltered sites with high diversity and cover of early-successional bryophytes and lichens, but still with patches of bare, compacted, iron-rich reddish mineral soil of the Jory Series. The climate of this area is oceanic. The area receives an average of *c.* 198 cm of precipitation annually, with mean January and July temperatures of 4.9 °C and 18.2 °C, respectively (interpolation by ClimateWNA.com; 1981–2010).

Additional specimens examined. **USA:** *Oregon*: Polk County, collector unknown and presumed to be William Denison, 23 iii 1969, s. n. (OSC 35280); Polk County, Wildwood Road, Hoskins to Valsetz, former railroad grade, near bridge, 44.75556°N, 123.5543°W, 172 m, on soil, with *Gregorella humida*, 2019, *McCune* 38618 (OSC), *D. Stone* 10122 (OSC).

Discussion

Phylogenetic analysis of species across the *Peltigerales* (Fig. 1A) recovered two suborders (Miadlikowska & Lutzoni 2004): highly supported (96%) *Peltigerineae* (encompassing the families *Lobariaceae*, *Massalongiaceae*, *Nephromataceae*, *Peltigeraceae* and *Vahliellaceae*) and weakly supported (below 50%) *Collematineae* (encompassing the families *Coccocarpiaceae*, *Collemataceae*, *Pannariaceae* and *Placynthiaceae*). The delimitation of these suborders is consistent with published phylogenies (e.g. Miadlikowska & Lutzoni 2004; Miadlikowska *et al.* 2006, 2014*a*; Muggia *et al.* 2011; Spribille *et al.* 2014). The phylogenetic placement of *Koerberiaceae*, currently classified in *Peltigerineae* (Spribille & Muggia 2012; Lücking *et al.* 2016) was not recovered (poor support in Fig. 1A) and the affiliation of *Erinacellus*, currently *incertae sedis* in *Peltigerales* (Spribille *et al.* 2014; Lücking *et al.* 2016), remains unsettled (Fig. 1A). Despite differences in taxon sampling, loci sequenced and analyses performed, the existing phylogenies for *Peltigerales* are highly congruent. Most dissimilarities in the phylogenetic relationships are due to the lack of phylogenetic signal in some data sets.

The phylogeny restricted to the family *Peltigeraceae*, which includes all putative species of *Peltigera* and all available representatives of *Solorina* (Fig. 1B; Supplementary Material Fig. S1, available online), is in overall agreement with Chagnon *et al.* (2019). We recovered the relationships among major clades in *Peltigera*, including the monophyly of the sections (*Phlebia*, *Peltidea* and *Chloropeltigera*) encompassing the tri-membered taxa (Miadlikowska *et al.* 2014*b*; Chagnon *et al.* 2019). The only exception was the nested placement of section *Retifoveatae* within section *Peltigera* recovered here, instead of the usual sister relationship between these two (Miadlikowska *et al.* 2014*b*; Chagnon *et al.* 2019); however, this nested relationship is not well supported.

The genus *Solorina* is not monophyletic, but its paraphyly caused by the separate lineage leading to *S. crocea* (see Miadlikowska & Lutzoni 2004) was weakly supported (below 50%). Although our phylogeny has the most extensive sampling of *Solorina* compared to published phylogenies, the majority is represented by less than four loci in the data matrix. Moreover, large portions of the sequences of the two spacers (ITS1 and ITS2) of the ITS locus were hardly alignable across the three genera (*Peltigera*, *Sinuicella* and *Solorina*) of the family *Peltigeraceae* and, therefore, were excluded from phylogenetic analyses.

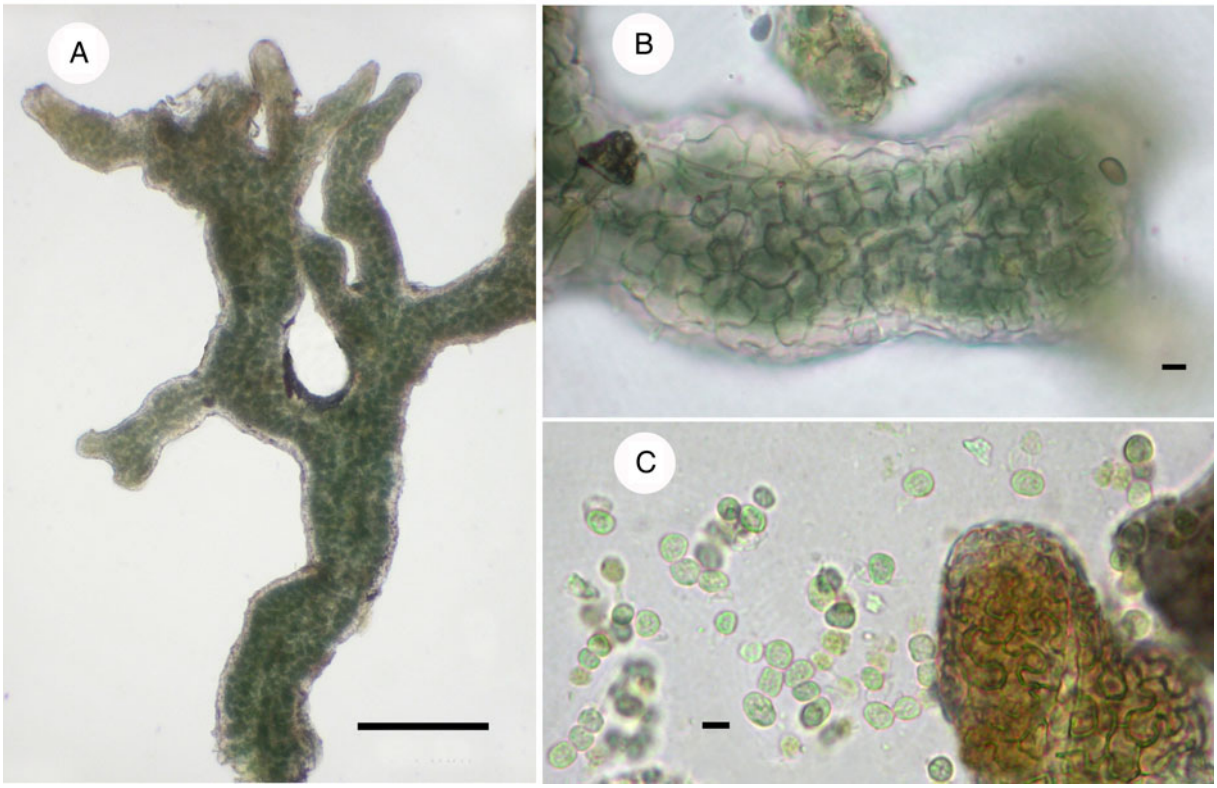


Fig. 3. *Sinuicella denisonii*. A, whole mount of thallus branches (*McCune* 38618). B, surface view of jigsaw puzzle-shaped cortical cells (*McCune* 38618). C, photobiont (*Nostoc*) in squash mount (*Stone* 10024). Scales: A = 100 µm; B & C = 5 µm. In colour online.

Monophyly of *Solorina* and the current delimitation of morphospecies within the genus (e.g. *S. bispora* is not monophyletic in our tree; Fig. 1B) should be re-evaluated based on revised taxon sampling and more extensive sequence data.

The highly supported phylogenetic placement of *Sinuicella* within *Peltigeraceae* (i.e. with *Solorina* and *Peltigera*) (Fig. 1A & B), is corroborated by the pronounced hemiangiocarpous development of the apothecia, which is characteristic of *Peltigera* and *Solorina* (Henssen 1981), and the presence of the *Peltigera*-type ascus apex (Honegger 1978; Bellemère & Letrouit-Galinou 1981) with its distinctive strongly amyloid ring (in *Solorina* the amyloid regions are more elongated).

Ascospore morphology also supports the placement of *Sinuicella* with *Solorina* and *Peltigera*. The ascospores of *Sinuicella* are intermediate between those genera, having the large size, broadly fusiform shape and single septum of most *Solorina* spores, but hyaline as in *Peltigera*, lacking the dark brown pigmentation of *Solorina* spores (Fig. 4C). Note that the asci of all three genera are of the *Peltigera* type with a distinctly dark blue-staining annulus in K/IKI (Fig. 4B).

A character that is atypical of most *Peltigera* and *Solorina* species is the cortex made of a single layer of cells. However, the cortex of aquatic lichen *Peltigera hydrothyria* Miadl. & Lutzoni does have only a single layer of cells, although these cells are not shaped like interlocking puzzle pieces. The shape of these cells is another unusual character of *Sinuicella* (Fig. 3B). Cells similar to these are seen in several other phylogenetically unrelated genera in the Pacific Northwest and are cases of convergent evolution (Muggia *et al.* 2011). *Nodobryoria* species (*Parmeliaceae*, *Lecanorales*) have cells that are similarly shaped but have protruding lumps on the surface formed by short, anastomosed hyphae (Common & Brodo 1995). *Leptogidium* thalli (*Pannariaceae*, *Peltigerales*) are more similar to *Sinuicella* in outward appearance, with a dichotomously branching thallus and interlocking cells forming the nearly smooth cortex (Muggia *et al.* 2011). The convergent evolution of a polychidioid thallus in the two suborders of *Peltigerales*, *Polychidium* (*Massalongiaceae*, *Peltigerineae*) and *Leptogidium* (*Pannariaceae*, *Collematineae*), has already been demonstrated (Muggia *et al.* 2011). Here we demonstrate convergent evolution of both the polychidioid thallus and interlocking cortical cells in phylogenetically unrelated *Leptogidium* (*Collematineae*) and *Sinuicella* (*Peltigeraceae*, *Peltigerineae*).

The identity of the cyanobiont of *Sinuicella denisonii* as the only North American representative of *Nostoc* phylogroup XL (Fig. 1C; Supplementary Material Fig. S2, available online) is puzzling, considering that all other members of that phylogroup were collected from South and Central America and Germany. However, *Nostoc* in phylogroups XXXIX (Magain *et al.* 2018) and XII (Magain *et al.* 2017*a*) are similarly diverse in geographical origin, taxonomic identity of their partner/host, and biology (free-living versus symbiotic). For example, the latter group contains *Peltigera* cyanobionts from Asia (China and the Philippines) as well as *Nostoc* associated with the fungus *Geosiphon pyriforme* (Kütz.) v. Wettstein, the liverwort *Blasia pusilla* L., and the vascular plant *Gunnera manicata* Linden from Germany. Phylogroup XXXIX is not strongly supported in the global *Nostoc* phylogeny and may represent multiple taxonomic units (Magain *et al.* 2018). It is possible that the unusual disjunct geographical pattern observed within phylogroup XL resulted from sampling bias, but homoplasy across very similar *rbcLX* sequences cannot be excluded. Phylogenetic analysis revealed further phylogenetic structure within phylogroup XL (with the cyanobiont of

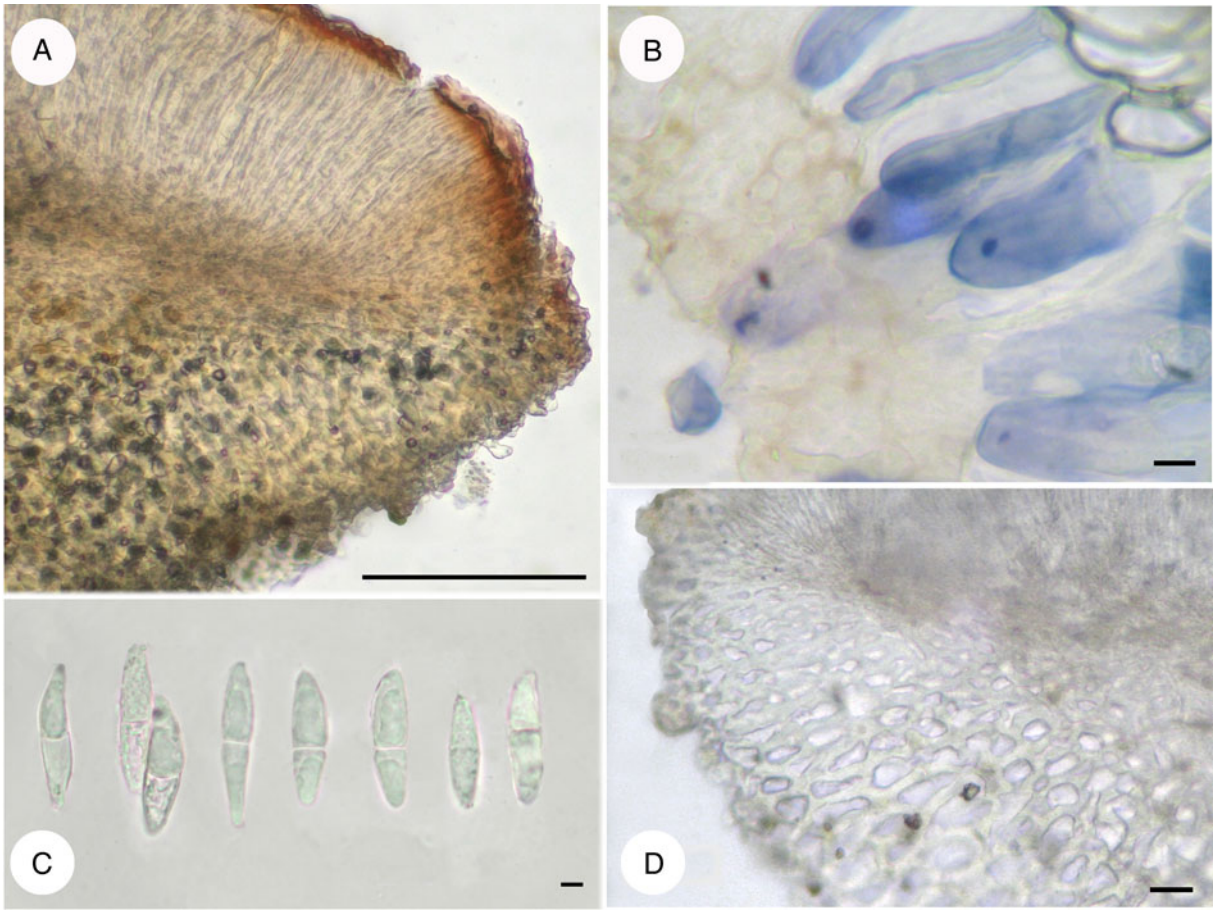


Fig. 4. Apothecial details of *Sinuicella denisonii* (OSC 35280). A, apothecial section in water. B, asci in K followed by IKI. C, ascospores in K. D, proper exciple. Scales: A = 100 µm; B & D = 10 µm; C = 5 µm. In colour online.

Sinuicella denisonii as the sole representative of one of three distinct, well-supported lineages; Fig. 1C). This phylogenetic pattern could indicate that each lineage represents an isolated, independently evolving population of *Nostoc*, rather than a single one with a broad, disjoint distribution. *Sinuicella* co-occurs with numerous *Peltigera* species (including members from section *Peltigera*) and multiple large populations of hornworts (probably *Anthoceros fusiformis* Aust.) and, therefore, all of these host species have the potential to share *Nostoc* phylogroup XL (to be explored in a future study).

The existence of *Sinuicella denisonii* at only one known location leaves us with the problem of having just one sequence of the species as well as a description that reflects a very small population. Two of the authors (BM and DFS) have searched extensively in the Pacific Northwest for small, soil-inhabiting lichens and have not found another site. However, it was originally found in 1969 and later by us at a location close to the original, at least indicating that it is extant and reproducing. Because it looks similar to several other species, including *Leptogidium contortum* and *Polychidium muscicola* (Sw.) Gray, there is a possibility that others have collected and misidentified it. Because it is small and cryptic, we feel that publishing the description will make others aware of its existence and is perhaps the best way to facilitate discovery of other sites.

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