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Lambiella arenosa, a new species from the coastal Oregon dunes

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ABSTRACT. The *Lambiella caeca* group of North America is a poorly known group of diminutive epiphytes with black lecideine apothecia occurring on conifer bark. We describe a new species in this group, *L. arenosa*, which occurs on *Pinus contorta* on the dunes of the Oregon coast. Both *Lambiella caeca* and *L. arenosa* share a black disk with raised, thin, persistent, often flexuose, black margins, an internally dark exciple that is thin and even, a thin, usually dark brown epithecium, and branched paraphyses that are moniliform in the upper part with K. The easiest characters to differentiate them are the smaller spores (averaging 8–11 μm long) and more conspicuous thallus of *L. caeca*, in contrast to somewhat larger spores (averaging over 11 μm long) and endosubstratal thallus in *L. arenosa*. While the known ranges of *L. arenosa* and *L. caeca* s.str. do not overlap (hyperoceanic West Coast for *L. arenosa*, boreal for *L. caeca* s.str.), a third group of specimens is problematic. Currently, we lack the data to establish whether previously sequenced members of the *L. caeca* group from western North America belong to *L. caeca*, *L. arenosa*, or additional undescribed species.

KEYWORDS. *Lambiella caeca*, lichen, North America, Trapeliaceae, Xylographaceae.



Hertel (1984) described the genus *Lambiella* based on an I+ blue medulla, a disk lacking gyri and umbo, and less reticulate paraphyses than in *Rimularia*. See Hertel & Rambold (1990) for further discussion of *Rimularia* and related genera. Since then the genus concepts for *Lambiella* and *Rimularia* were refined based on phylogenetic reconstructions (Resl et al. 2015; Spribille et al. 2014). As a result, *Lambiella* was placed in the Xylographaceae, while *Rimularia* was placed in the Trapeliaceae. Not all species of *Rimularia* s.l. have been included in molecular sampling, but the current evidence suggests that the presence of a thin, vertical, non-amyloid tube in the ascus tip is characteristic of *Rimularia* s.str., while that tube is absent in *Lambiella* (Resl et al. 2015).

While most species of *Lambiella* are saxicolous, *L. caeca* (J. Lowe) Resl & T. Sprib. (= *Rimularia caeca* (J. Lowe) Rambold & Printzen) is a fairly common epiphytic species in boreal and Great Lakes regions of northeastern North America. Meanwhile, an undescribed species of uncertain generic affinity,

with similarities to *L. caeca*, but differing in a number of morphological and anatomical characters, was found by the first author at numerous sites on the dune sheets of the Oregon coast.

A third group of specimens from interior western North America (Sierra Nevada and Rocky Mountains) is represented by five sequences in GenBank and assigned to *Lambiella caeca* in previous work (Resl et al. 2015; Spribille et al. 2014). This material has not been compared to *L. caeca* in the literature, yet it is the only material within the species complex for which prior DNA sequences exist in GenBank.

Full resolution of this species group would require anatomical and molecular studies of all three groups of specimens. This is beyond the scope of the present paper, where our purpose is to provide a name for the species that is common on coastal dunes in Oregon, and that is clearly distinct from *L. caeca* in eastern North America.

MATERIALS AND METHODS

We used standard microscopy and chemical spot tests (McCune 2017). Apothecial anatomy and

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Table 1. Voucher information for new GenBank accessions.

Species	Voucher	ITS	mtSSU
<i>Lambiella arenosa</i>	Oregon, Douglas Co., McCune 29714	MB464548	MB464550
<i>Lambiella arenosa</i>	Oregon, Tillamook Co., McCune 30459	MB464549	MB464551

measurements were studied with bright-field microscopy on freehand sections in water, with and without iodine (IKI) staining.

DNA extraction and PCR amplification. We generated molecular data for two ribosomal loci: the internal transcribed spacer (ITS) and the mitochondrial small subunit (mtSSU) rDNA. To obtain fungal sequences, we used apothecia for extracting the total DNA with the Prepease DNA Isolation Kit (USB, Cleveland, OH, U.S.A.), following the leaf extraction protocol. Amplification of the targeted loci used the Ready-To-Go PCR Beads (GE Healthcare) protocol. We prepared 25 μ l PCR samples by adding 23 μ l of sterile water, 1 μ l of each primer at 10 μ M concentration, and 0.5 μ l template DNA to the tubes containing the beads. The primers used and the PCR settings were described by Zhao et al. (2016). We purified PCR products with Exo SAP-IT (USB, Cleveland, Ohio, USA), following the manufacturer's instructions. Sequencing was performed using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, U.S.A.) with the same primers as for amplification. Products were then run on an ABI 3730 automated sequencer according to established protocols (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics at the Field Museum, Chicago, IL, U.S.A.

Phylogenetic analyses. To explore relationships of the putative new species with other taxa, we built phylogenetic trees from sequences of ITS and mtSSU. Taxon sampling included two representatives of the putative new coastal species, five existing sequences in the *Lambiella caeca* group from GenBank, all from interior western North America, and a representative selection of other species of *Lambiella* (Table 1). Sequences were aligned with Geneious alignment using default settings (cost matrix: 65%, gap open penalty 12, gap extend penalty 3), then adjusted manually. The ends of the alignments were then trimmed to a nearly equal number of sites for all sequences, including all of ITS1, 5.8S, and ITS2, except for two shorter sequences of "*L. caeca*" in GenBank (354 and 438 bases). Alignment gaps were treated as missing data.

Phylogenetic trees were obtained by maximum likelihood analysis of the ITS data, using the GTR (general time-reversible) model and Geneious defaults, except we used the "BEST" (slower) topology search and three substitution rate categories, using the PhyML (Guindon et al. 2010) plug-in to Geneious 5.4.3 (Drummond et al. 2011). Very similar topologies and support were obtained with Bayesian, maximum parsimony, and RAxML (not shown). Statistical support for branches was evaluated with 1000 bootstrap resamplings. The mtSSU sequences had insufficient variation with the *L. caeca* complex to be useful, and are not discussed further. Excluding *Rimularia*, the alignment in *Lambiella* had 200 variable sites of 571 in the ITS.

RESULTS AND DISCUSSION

Three groups of specimens are known for the *Lambiella caeca* group in North America: (1) *L. caeca* s.str. from northeastern North America; (2) "*L. caeca*" from areas in western North America outside the coastal dunes and represented by sequences in GenBank; those collections include three Printzen collections from the Yosemite National Park area in California, and two Spribille collections, one from British Columbia, and one from western Montana; and (3) a common species on the coastal dunes of Oregon, described here as *L. arenosa*.

We considered three alternative hypotheses:

- H_A. All three groups belong to a single species, *L. caeca*.
- H_B. Specimen groups 2 and 3 belong to a single species distinct from *L. caeca* s.str.
- H_C. The specimen groups represent 3 or more species, *L. caeca*, *L. arenosa* and one or more additional species from western North America.

We rejected H_A on morphological and anatomical grounds, as summarized in Table 2. Although we lack DNA sequence data for *L. caeca* s.str., a combination of thallus and apothecial characters readily distinguish *L. caeca* from *L. arenosa*. Both *Lambiella caeca* and *L. arenosa* occur on conifer bark, share a black disk with raised, thin, persistent, often

Table 2. Comparison of *Lambiella caeca* and *L. arenosa*.

	<i>Lambiella caeca</i>	<i>Lambiella arenosa</i>
Thallus	distinct; thinly areolate to thickly verrucose-areolate	indistinct; mostly endosubstratal or forming a whitish stain
Photobiont	chlorococcoid	<i>Trentepohlia</i> ?
Spore size	7.5–11.5(13.5) × 4.5–7.5 μm	11.0–16.2 × 6.2–7.7 μm
Spore shape	ellipsoid	ellipsoid to oblong
Exciple pigment (upper part)	dark brown or dark yellowish brown, K– or K+ dull violet; occasionally inner part POL+	dark greenish brown to greenish black; always K– and POL–
Epithecium	dark brown, POL–, often K+ dull violet	dark brown, rarely greenish black, POL– or more often POL+, always K–
Hymenium pigment	hyaline to pale olive brown, K–, K+ weak violet, or with K+ pale violet streaks	hyaline, K–
Distribution	boreal and northeastern U.S.	dunes on immediate coast of Oregon

flexuose, black margin, an internally dark exciple that is rather thin and even, a thin, usually dark brown epithecium, and branched paraphyses that become more clearly moniliform in the upper part with K. They differ, however in a number of characters (**Table 2**). The easiest characters to differentiate them are the smaller spores (averaging 8–11 μm long) and more conspicuous thallus of *L. caeca*, in contrast to somewhat larger spores (averaging over 11 μm long) and endosubstratal thallus in *L. arenosa*. With our

current knowledge, they can also be distinguished by location, because their ranges do not overlap (hyperoceanic West Coast for *L. arenosa*, boreal for *L. caeca*). This may change, however, with improved sampling in this group.

While the photobiont of *L. caeca* is readily demonstrated to be chlorococcoid, the photobiont of *L. arenosa* is challenging to determine. *Lambiella arenosa* is commonly associated with free-living *Trentepohlia* and *Trentepohlia*-containing crustose lichens, such as *Opegrapha* s.l., as well as chlorococcoid algae-containing species, such as *Pertusaria glaucomela* (Tuck.) Nyl. Although we have not seen a well-developed algal layer in *L. arenosa*, in the material we have examined the species is most closely associated with small groups of *Trentepohlia* just below the peeling outer bark layers.

We do not have the data for all three specimen groups that would be required to conclusively select between H_B and H_C. In other words, the relationships between *L. arenosa* and the samples of the existing sequences in GenBank of the *L. caeca* group are unresolved. Our two ITS sequences for *L. arenosa* (group 3) are monophyletic, but do not show reciprocal monophyly with the specimens of group 2 (**Fig. 1**). Morphological and anatomical studies of the Printzen and Spribille specimens are needed, as are sequences of *L. caeca* from eastern North America.

Lambiella arenosa McCune & Lumbsch, *sp. nov.*

Fig. 2

MYCOBANK MB 821117.

Similar to Lambiella caeca but with thallus barely apparent, epithecium and exciple K–, and spores somewhat larger, 11.0–16.2 × 6.2–7.7 μm.

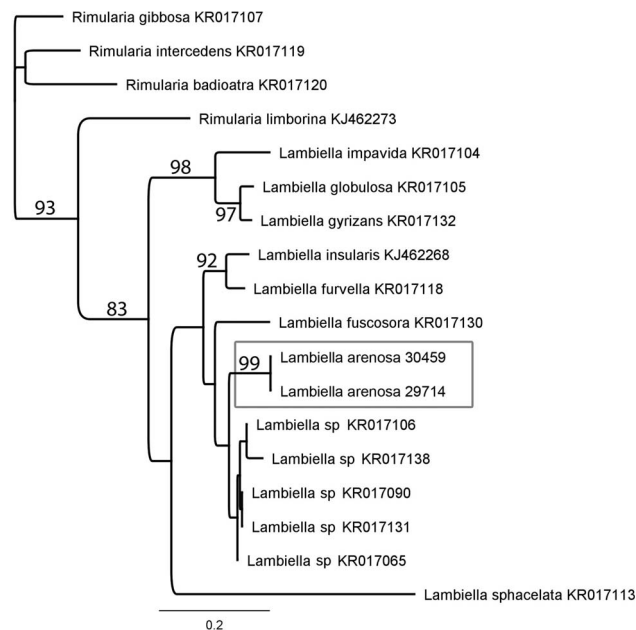


Figure 1. Most likely phylogenetic relationships among sampled *Lambiella* species inferred from ITS sequences and based on rooting with *Rimularia* as an outgroup. Bootstrap percentages above 75% are shown; branch lengths are based on the estimated number of substitutions per site assuming a GTR model of substitution. GenBank accession numbers are given for previously published sequences. GenBank numbers for new sequences are given in Table 1.

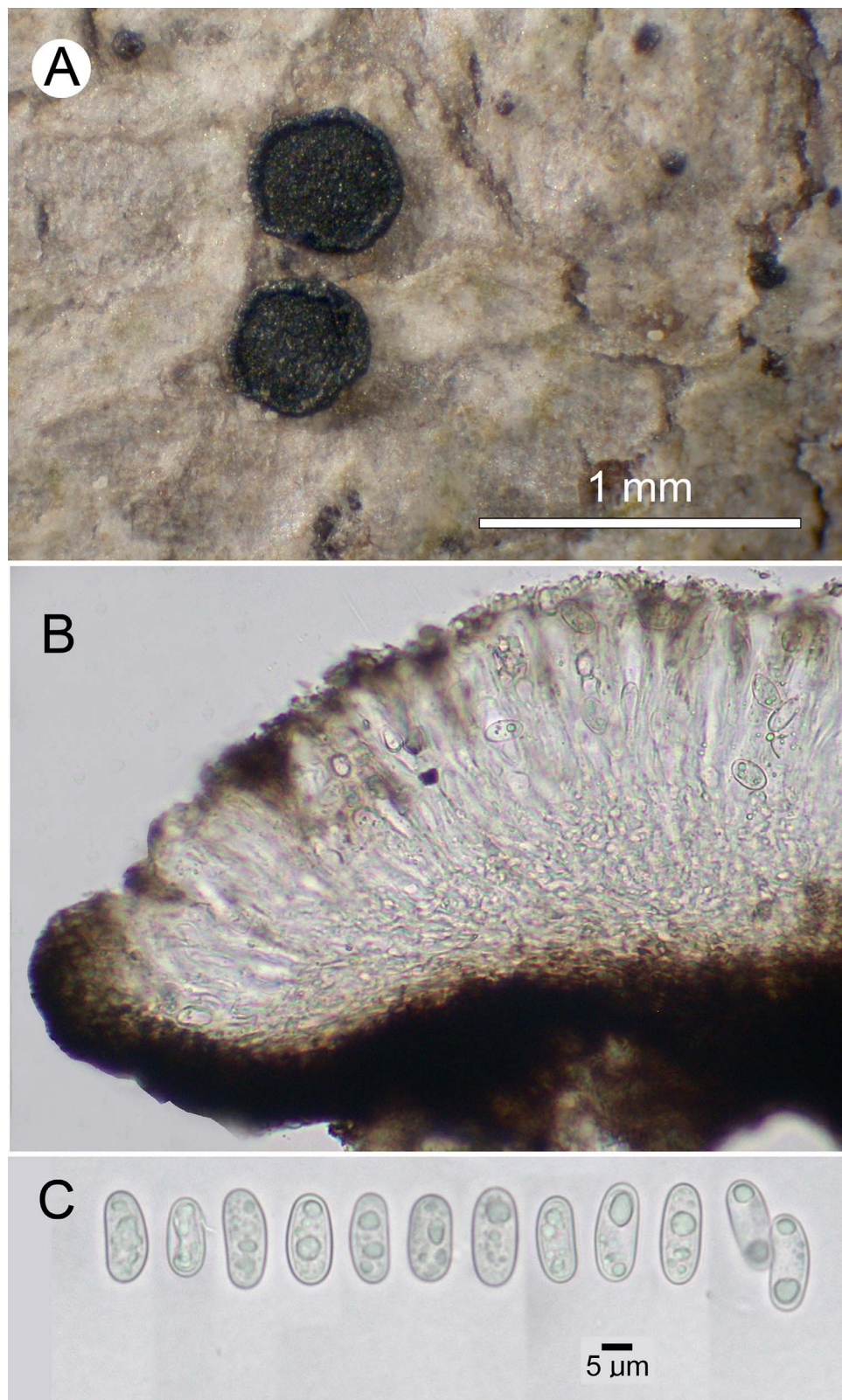


Figure 2. *Lambiella arenosa*. A. Thallus and apothecia (35919). B. Apothecial section in water (26697). C. Ascospores (32405).

TYPE: U.S.A. OREGON: Coos Co., Eel Creek dunes, island in wet depression, 43.5892°N, 124.1995°W, 6 m, on *Pinus contorta* bark, 30 Dec 2012, McCune 32405 (holotype: osc; isotype: f).

Description. Thallus crustose, mostly endosubstratal, forming a smooth whitish stain or not apparent; prothallus not evident; soredia and isidia lacking; apothecia mostly 0.2–0.7 mm diam, black, lecideine, circular to wavy in outline, with a raised, black, thin, persistent margin; disk nearly flat, when degenerate persisting as cupulate shells; proper exciple thin (25–30(40) μm wide), dark greenish brown at upper edge, dark brown below hypothecium, POL–; epithecium thin, brown to brown black, sometimes with an olive tinge, K– or yellowish brown, N–, POL– or POL+ in clumps or a continuous layer; hypothecium thin, hyaline or faintly grayish above grading to brown below and merging imperceptibly with the exciple; subhymenium indistinct, about 10–13 μm thick; hymenium hyaline, 70–80 μm thick, not interspersed, I+ deep blue, K–; paraphyses thin (lower portions 1–1.5 μm wide), branched and anastomosed, moniliform in the upper part and expanded to about 3 μm wide, especially in K, with hyaline or brown tips; asci thick walled, I+ blue, the tip lacking a non-amyloid tube; ascospores nonseptate, hyaline, ellipsoid to oblong, 11.0–16.2 \times 6.2–7.7 μm , lacking a halo, 8/ascus; pycnidia black; spermatia not seen (pycnidia barren).

Chemistry. No lichen substances detected; spot tests negative.

Photobiont. Uncertain, apparently *Trentepohlia* in some cases, but a definite lichenized thallus is often difficult to discern, and the species grows intermixed with other crustose chlorococcoid- and *Trentepohlia*-containing lichens and freeliving *Trentepohlia*.

Etymology. The epithet “*arenosa*” indicates the association of this species with sandy habitats in the dune sheets of coastal Oregon.

Substrate. On conifer bark and cones; so far known only from *Pinus contorta*.

Habitat. Coastal dunes and deflation plains.

Distribution. So far known only from the immediate coast of Oregon.

Additional specimens examined. *Lambiella arenosa*. All collections were by McCune from bark on branches of *Pinus contorta* unless otherwise indicat-



Figure 3. Typical habitat of *Lambiella arenosa* on the Oregon dunes. The species is most often found on *Pinus contorta* with low, living branches, as in the mid ground of this photo, where *Pinus* occurs mixed with ericaceous shrubs. The foreground has patches of bare sand, grasses, and mats of *Cladonia* and mosses. Older emergent *Pinus contorta* are in the distance.

ed; specimens in osc. U.S.A. OREGON: Coos Co., Horsfall Dunes, 43.4800°N, 124.2500°W, 7 m, on cone of *Pinus contorta*, June 1988, 18108. Douglas Co., Three Mile Lake, N end, 43.7744°N, 124.1670°W, 6 m, Aug 2008, 29714. Lane Co., Alder Creek Campground, west of, 44.0659°N, 124.1036°W, 29 m, 27 Aug 2015, 35919; near base of North Jetty at Florence, 44.0167°N, 124.1283°W, 6 m, Dec 2002, 26697. Lincoln Co., Beaver Creek, 44.5167°N, 124.0500°W, 4 m, Nov 1990, 18682. Tillamook Co., SW side of Whalen Island, 45.2744°N, 123.9526°W, 7 m, 30 Dec 2009, 30459.

Lambiella caeca. Representative specimens of *L. caeca* s.str. used for comparison (all from MIN). CANADA. NEW BRUNSWICK: Chance Harbor, 35 km SW of St. John, 6 July 1981, Wetmore 43403. U.S.A. MAINE: Hancock Co., Acadia National Park, Mt. Desert Island, 5 July 1983, Sullivan 1302. MICHIGAN: Alger Co., Pictured Rocks National Lakeshore, 0.5 miles S of Twelvemile Beach Campground, on *Pinus banksiana*, 13 July 1987, Wetmore 58951. MINNESOTA: St. Louis Co., Voyageurs National Park, SW of Namakan Narrows, on *Pinus banksiana*, July 1979, Wetmore 39400. WISCONSIN: Douglas Co., Brule River State Forest, North Country Trail, barrens S of abandoned airstrip, on a *Pinus banksiana* cone, 18 July 2009, Wetmore 97974.

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