

# Two new species in the *Umbilicaria torrefacta* group from Alaska and the Pacific Northwest of North America

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Field studies in Alaska, Oregon, and Washington revealed unusual morphotypes that appeared similar to *U. torrefacta* in many ways, but differed in their tough, multilayered growth form. DNA sequencing and thin-layer chromatography confirmed our suspicion that these do not belong with *U. torrefacta*. Two new species are described, *Umbilicaria dura* and *U. multistrata*.

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## Introduction

Although *Umbilicaria* is one of the better known lichen genera in North America (Llano 1950), new taxa have been described or old ones resurrected (Poelt & Nash 1993, McCune & Curtis 2012, McCune et al. 2014), ranges and species concepts are being clarified (Løfall & Timdal 2005, Davydov et al. 2011, Kofranek & McCune 2008), and the broad phylogenetic structure of the genus has been revealed (Davydov et al. 2017), yet several problem groups remain. One of those was mentioned by Krog (1968, p. 84) in her treatment of *Umbilicaria torrefacta* from Alaska. She wrote, “Some specimens from high mountains of the interior had a very thick, compact, polyphyllous appearance, the underside with wide umbilicus and prominent lamellae. Chemically these specimens differed in producing umbilicarin in addition to gyrophoric acid.” Specimens recently collected in Alaska, Oregon, and Washington resembled that description, providing an opportunity to evaluate the problem using DNA sequences combined with thin-layer chromatography.

*Umbilicaria torrefacta* (Lightf.) Schrader is one of the most easily recognized species in its genus throughout the northern Hemisphere, with an areolate upper surface on which the areoles meet in contorted lines like skull sutures, a minutely perforate thallus, and a mixture of pale rhizines and trabeculae or lamellae on the lower surface. The species was recently typified by Hestmark (2017). *Umbilicaria torrefacta* is common on rock in western North America, occurring from low elevations to alpine sites.

A handful of specimens from western North America depart from the norm for *U. torrefacta* in accordance with Krog’s observations. These have a thick, rigid thallus with a black lower surface that is thickly covered with multiple layers of dark brown to black plates and rhizines. Similar to *U. torrefacta* they are usually fertile, with small spores for the genus, and have an areolate upper surface. Very often these specimens were found in an atypical microhabitat for *U. torrefacta*, occurring in semi-sheltered spots on montane to alpine ridges, while *U. torrefacta*

frequents sites that are either exposed or with diffuse light (“open shade”, e.g., N-facing talus). These observations suggested that more than one species was involved, so I searched more intensively in the field and in herbaria for this morphology, then evaluated differences in morphological, chemical, and genetic characters.

## Material and Methods

Most specimens studied were gathered in the field. Locations were recorded using the WGS84 datum. Few specimens of the putative new species were found in herbaria, except for the specimens (O) mentioned by Krog (1968). Specimens were studied with a combination of microscopy, thin-layer chromatography (TLC), and, for recently collected material, DNA sequencing. In general, TLC protocols followed the methods of Culberson and Kristinsson (1970), Culberson (1972), and the later modification by Culberson and Johnson (1982). All three solvent systems were used (A, B' and C), but primarily solvents A and B' as these yielded good separation of the most common major substances (gyrophoric, umbilicic, and crustinic acids). Umbilicic acid appears as a large spot just below (and partially overlapping with) gyrophoric acid in A and a spot with long tail just below norstictic acid in B'. Crustinic acid is found at about the same  $R_f$  as gyrophoric acid in A, but is very low (Culberson zone 2) in B'.

DNA sequences were obtained for selected critical specimens, along with a representative sampling of *Umbilicaria torrefacta* in North America (Table 1). As context I also included other *Umbilicaria* species that frequent the same region. I chose to analyze the nuclear ITS rDNA and LSU regions because of their general utility and frequent application for species-level problems in *Umbilicaria* (Davydov et al. 2010, Hestmark et al. 2011, Ivanova et al. 1999, Krzewicka et al. 2009).

DNA was extracted with the REDExtract-N-Amp Plant PCR kit by Sigma-Aldrich. The procedure used was according to the manufacturers' instructions except that 1/6<sup>th</sup> of the suggested amount of reagent was used for each sample: 15  $\mu$ L extraction solution was added and incubated at 95°C for 10 minutes and finalized by adding 15  $\mu$ L dilution solution. In some cases we used FastDNA<sup>TM</sup> SPIN KIT (MP Biomedicals). The manufacturers' instructions were followed with the plant tissue CLS-VF and PPS cell lysis solutions used. In both cases DNA extracts were refrigerated at 4°C.

Amplification protocols are in McCune & Curtis (2012). Primers for amplifying nuclear ITS were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and primers for LSU were LROR and LR6 (Vilgalys & Hester 1990). In addition to the amplification primers, the following primers were sometimes used for sequencing: ITS2 and ITS3 for the ITS, and LR3 and LR5 for the LSU. PCR products were viewed via gel electrophoresis and successful samples were then cleaned using ExoSAP-IT<sup>TM</sup> Affymetrix 78200. The protocol was modified to use 3  $\mu$ L PCR product and 1  $\mu$ L ExoSAP-IT reagent. These were processed according to manufacturer's protocol 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  $\mu$ L forward primer (as above), 7.7  $\mu$ L nuclease free H<sub>2</sub>O, and 1.9  $\mu$ L 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>, Kears et al., 2012) was used to quality check the raw sequences, align the two or more reads per sample, and generate a consensus sequence for phylogenetic analyses.

We inferred phylogenetic relationships from new sequences from 28 specimens along with numerous pre-existing sequences from GenBank (Table 1). For the latter, I took ITS and nuLSU sequences for all closely related species, with special attention to *Umbilicaria torrefacta*,

**Table 1.** Voucher information for GenBank accessions of *Umbilicaria*. New accessions are in bold face. Abbreviations: “unk.loc.” = unknown location.

| Species                      | Voucher                                | nuITS           | nuLSU           |
|------------------------------|----------------------------------------|-----------------|-----------------|
| <i>Umbilicaria americana</i> | USA: Idaho, <i>McCune 37451</i>        | <b>MH302509</b> | –               |
| <i>U. angulata</i>           | USA: Oregon, <i>McCune 30050</i>       | JQ764746        | JQ764756        |
| <i>U. angulata</i>           | USA: Oregon, <i>McCune 30483</i>       | JQ764734        | –               |
| <i>U. angulata</i>           | USA: Oregon, <i>McCune 31231</i>       | JQ764738        | JQ764747        |
| <i>U. angulata</i>           | USA: Oregon, <i>McCune 37312</i>       | <b>MH302510</b> | –               |
| <i>U. antarctica</i>         | Antarctica, <i>Ott 2001</i>            | AY603123        | AY603107        |
| <i>U. antarctica</i>         | Antarctica, <i>Ott 2002</i>            | AY603124        | AY603108        |
| <i>U. aprina</i>             | Bolivia, <i>Hestmark 05007B</i>        | HM161483        | HM161597        |
| <i>U. aprina</i>             | Bolivia, <i>Hestmark 05030B</i>        | HM161454        | HM161514        |
| <i>U. arctica</i>            | Norway, <i>Hestmark 04201</i>          | HM161454        | DQ986772        |
| <i>U. arctica</i>            | USA: Alaska, <i>McCune 32940</i>       | <b>MH302511</b> | <b>MH290346</b> |
| <i>U. calvescens</i>         | Argentina, <i>Hestmark 09005</i>       | HM161508        | HM161604        |
| <i>U. calvescens</i>         | Chile, <i>Hestmark 98025</i>           | HM161459        | HM161517        |
| <i>U. cinereorufescens</i>   | Ecuador, <i>Hestmark 094079</i>        | HM161511        | HM161605        |
| <i>U. crustulosa</i>         | Norway, <i>Hestmark 2002</i>           | HM161498        | HM161593        |
| <i>U. crustulosa</i>         | Norway, <i>Hestmark 9017</i>           | HM161496        | HM161590        |
| <i>U. cylindrica</i>         | Greenland, <i>Ivanova 10</i>           | AF096199        | –               |
| <i>U. cylindrica</i>         | Ukraine, <i>Ivanova 48</i>             | AF096209        | –               |
| <i>U. decussata</i>          | Antarctica, <i>Ott 2007</i>            | AY603122        | AY603113        |
| <i>U. decussata</i>          | Bolivia, <i>Hestmark 05003B</i>        | –               | HM161596        |
| <i>U. dendrophora</i>        | Bolivia, <i>Hestmark 5021</i>          | HM161504        | HM161599        |
| <i>U. dendrophora</i>        | Argentina, <i>Hestmark 9001</i>        | HM161505        | HM161600        |
| <i>U. deusta</i>             | Norway, <i>Feige 31</i>                | AF096206        | –               |
| <i>U. dura</i>               | USA: Alaska, <i>McCune 34977</i>       | <b>MH302512</b> | <b>MH290352</b> |
| <i>U. dura</i>               | USA: Oregon, <i>McCune 35928</i>       | <b>MH302514</b> | <b>MH290353</b> |
| <i>U. dura</i>               | USA: Alaska, <i>McCune 35008</i>       | <b>MH302513</b> | –               |
| <i>U. flocculosa</i>         | unk.loc., <i>Guo smll007</i>           | AF297670        | –               |
| <i>U. grisea</i>             | Sweden, <i>Hestmark 9013</i>           | HM161493        | HM161587        |
| <i>U. grisea</i>             | Spain, <i>Hestmark 87001</i>           | HM161500        | HM161595        |
| <i>U. haplocarpa</i>         | Bolivia, <i>Hestmark 5052B</i>         | HM161487        | HM161537        |
| <i>U. haplocarpa</i>         | Bolivia, <i>Hestmark 5110B</i>         | HM161467        | HM161528        |
| <i>U. havaasii</i>           | Oregon, <i>McCune 31230</i>            | JQ764739        | JQ764748        |
| <i>U. hirsuta</i>            | USA: S Dakota, <i>Zimmerman 14-001</i> | <b>MH302515</b> | –               |
| <i>U. hirsuta</i>            | USA: Oregon, <i>Sheehy 909</i>         | <b>MH302516</b> | –               |
| <i>U. hirsuta</i>            | Norway, <i>Hestmark 9015</i>           | HM161494        | HM161588        |
| <i>U. hirsuta</i>            | Norway, <i>Hestmark 9016</i>           | HM161495        | HM161589        |
| <i>U. hyperborea</i>         | Austria, <i>Feige 33</i>               | AF096216        | –               |

|                                  |                                            |                  |                 |
|----------------------------------|--------------------------------------------|------------------|-----------------|
| <i>U. hyperborea</i>             | USA: Alaska, <i>McCune 35477</i>           | <b>MH302518*</b> | <b>MH290350</b> |
| <i>U. hyperborea</i>             | USA: Alaska, <i>McCune 30048</i>           | <b>MH302517</b>  | <b>MH290351</b> |
| <i>U. lambii</i>                 | USA: Washington, <i>Haldeman 2565</i>      | <b>MH302519</b>  | –               |
| <i>U. leiocarpa</i>              | Switzerland, <i>Feige 34</i>               | AF096211         | –               |
| <i>U. leprosa</i>                | Bolivia, <i>Hestmark 5004B</i>             | HM161478         | HM161539        |
| <i>U. leprosa</i>                | Bolivia, <i>Hestmark 5039B</i>             | HM161479         | HM161542        |
| <i>U. lyngei</i>                 | USA: Alaska, <i>McCune 34982</i>           | <b>MH302520</b>  | –               |
| <i>U. lyngei</i>                 | China, <i>Guo smll006</i>                  | AF297669         | –               |
| <i>U. mammulata</i>              | USA: N Carolina, <i>Lutzoni 03.07.04-5</i> | DQ782851         | DQ782912        |
| <i>U. mammulata</i>              | unk.loc., <i>Guo smll002</i>               | AF284448         | –               |
| <i>U. muehlenbergii</i>          | Siberia, <i>Ivanova 37</i>                 | AF096204         | –               |
| <i>U. muehlenbergii</i>          | USA: Wisconsin, <i>Yahr 4246</i>           | KT232215         | AY640977        |
| <i>U. muehlenbergii</i>          | unk.loc., <i>Wang 20085035</i>             | FJ854372         | –               |
| <i>U. multistrata</i>            | USA: Alaska, <i>McCune 34957</i>           | <b>MH302521</b>  | –               |
| <i>U. multistrata</i>            | USA: Alaska, <i>McCune 34957 re-extr.</i>  | <b>MH302522</b>  | –               |
| <i>U. multistrata</i>            | USA: Alaska, <i>McCune 36465 apo.</i>      | <b>MH302523</b>  | –               |
| <i>U. multistrata</i>            | USA: Alaska, <i>McCune 36465 thallus</i>   | <b>MH302524</b>  | –               |
| <i>U. nodulospora</i>            | USA: California, <i>McCune 32521</i>       | –                | KJ40722         |
| <i>U. nodulospora</i>            | USA: California, <i>McCune 32526</i>       | KJ40719          | –               |
| <i>U. nodulospora</i> , holotype | USA: California, <i>McCune 32546</i>       | KJ40718          | KJ40721         |
| <i>U. nylanderiana</i>           | Bolivia, <i>Hestmark 05009B</i>            | HM161488         | HM161543        |
| <i>U. nylanderiana</i>           | Ecuador, <i>Hestmark 94200</i>             | HM161489         | HM161549        |
| <i>U. phaea</i>                  | USA: California, <i>McCune 30358</i>       | JQ764736         | JQ764755        |
| <i>U. phaea</i>                  | USA: California, <i>McCune 30442</i>       | JQ764741         | JQ764751        |
| <i>U. polaris</i>                | Antarctica, <i>Lumbsch 19046a</i>          | AY603134         | AY603118        |
| <i>U. polaris</i>                | USA: Oregon, <i>McCune 36800</i>           | –                | <b>MH290355</b> |
| <i>U. polyphylla</i>             | Poland, <i>Krzewicka 3046</i>              | FN185975         | FN186096        |
| <i>U. polyphylla</i>             | Spain, <i>Krzewicka 3296</i>               | FN185976         | FN186097        |
| <i>U. polyrrhiza</i>             | USA: Oregon, <i>McCune 30484</i>           | JQ764737         | JQ764749        |
| <i>U. proboscidea</i>            | USA: Alaska, <i>McCune 35388</i>           | <b>MH302526</b>  | –               |
| <i>U. proboscidea</i>            | USA: Alaska, <i>McCune 34948</i>           | <b>MH302525</b>  | –               |
| <i>U. pustulata</i>              | Norway, <i>Hestmark 03202</i>              | HM161456         | DQ883690        |
| <i>U. rigida</i>                 | Norway, <i>Hestmark 04202</i>              | HM161455         | –               |
| <i>U. rigida</i>                 | USA: Alaska, <i>McCune 35009</i>           | <b>MH302527</b>  | <b>MH290347</b> |
| <i>U. rigida</i>                 | USA: Alaska, <i>McCune 35010</i>           | <b>MH302528</b>  | <b>MH290348</b> |
| <i>U. semitensis</i>             | USA: California, <i>McCune 30049</i>       | JQ764742         | JQ764757        |
| <i>U. semitensis</i>             | USA: California, <i>McCune 30410</i>       | JQ764745         | JQ764754        |
| <i>U. torrefacta</i>             | USA: Oregon, <i>McCune 30482</i>           | JQ764744         | JQ764750        |
| <i>U. torrefacta</i> (typical)   | USA: Oregon, <i>McCune 30546</i>           | <b>MH302529</b>  | –               |

|                                         |                                   |                 |                 |
|-----------------------------------------|-----------------------------------|-----------------|-----------------|
| <i>U. torrefacta</i> (multistrata-like) | USA: Oregon, <i>McCune 31621</i>  | <b>MH302530</b> | –               |
| <i>U. torrefacta</i> (black below)      | USA: Oregon, <i>McCune 31723</i>  | <b>MH302531</b> | <b>MH290343</b> |
| <i>U. torrefacta</i> (typical)          | USA: Montana, <i>McCune 31999</i> | <b>MH302532</b> | <b>MH290344</b> |
| <i>U. torrefacta</i> (typical)          | USA: Oregon, <i>McCune 32491</i>  | <b>MH302533</b> | <b>MH290345</b> |
| <i>U. torrefacta</i> (typical)          | USA: Alaska, <i>McCune 35097</i>  | <b>MH302534</b> | <b>MH290349</b> |
| <i>U. torrefacta</i> (multistrata-like) | USA: Oregon, <i>McCune 36203</i>  | <b>MH302535</b> | <b>MH290354</b> |
| <i>U. torrefacta</i> (multistrata-like) | USA: Alaska, <i>McCune 36873</i>  | –               | <b>MH290356</b> |
| <i>U. torrefacta</i>                    | unk.loc., <i>Brunauer gbM25</i>   | DQ660906        | –               |
| <i>U. umbilicarioides</i>               | unk.loc., <i>Ivanova s.n.</i>     | AF096210        | –               |
| <i>U. umbilicarioides</i>               | Antarctica, <i>Friedl 4(23)</i>   | AJ431606        | –               |
| <i>U. vellea</i>                        | Bolivia, <i>Hestmark 5038B</i>    | HM161490        | –               |
| <i>U. vellea</i>                        | China, <i>Guo sml005</i>          | AF297668        | –               |
| <i>U. virginis</i>                      | unk.loc., <i>Guo sml010</i>       | AF297673        | –               |

supplemented by additional sequences from elsewhere in *Umbilicaria*, including many of the commonly co-occurring species. This resulted in a total of 86 sequences for ITS and 55 for LSU. Sequences were aligned with MAFFT in Geneious using default settings (Auto algorithm selection, gap open penalty 1.53, offset 0.123). The ends of our new sequences were trimmed to match those from GenBank, introns removed, then concatenated into a single alignment. Phylogenetic trees were obtained by maximum likelihood analysis of the combined alignment using the GTR (general time-reversible) model with Geneious defaults, optimizing topology, length, and rate with an NNI search using the PhyML (Guindon et al. 2010) plugin to Geneious. Statistical support for branches was evaluated with 1000 bootstrap pseudoreplications.

The ITS alignment had 536 bases with 51 % (274) variable positions. The LSU sequences were highly variable in length and many contained an intron near the downstream end, so this alignment was trimmed at the start of the intron. An additional intron was embedded in the LSU in a few specimens and was removed. After trimming the remaining LSU alignment had 1108 bases with 19 % (206) variable positions. Although nuLSU in many groups is considered much less useful at the species level than the ITS, I found both the ITS and nuLSU to be very informative in separating species.

## Results

The ITS barcoding locus along with nuLSU yielded good resolution at the species level, though as expected, the backbone was poorly resolved (Fig. 1). Maximum likelihood phylogenetic reconstruction based on the concatenated alignments revealed three supported clades resembling *U. torrefacta*. Similar results were obtained for ITS alone, demonstrating that it is sufficient to distinguish the three clades (PhyML bootstrap support: 100, 98, and 96 % for *multistrata*, *dura*, and *torrefacta* clades respectively). Two of those clades corresponded to a morphology exclusively with thick multilayered structure, while one clade contained typical *U. torrefacta*. Each of these



**Figure 1.** Most likely phylogenetic relationships among *Umbilicaria* species inferred from ITS and nuLSU sequences and based on rooting with *Umbilicaria pustulata* as an outgroup. Bootstrap percentages above 75% are indicated by black dots; red lines highlight new species; magenta box indicates *U. torrefacta* s. str.; branch lengths are based on the estimated number of substitutions per site assuming a GTR model of substitution using PhyML. Species names are followed by collection numbers, where known. GenBank numbers for sequences are given in Table 1.

three clades is distinct in lichen substances, and to some extent in morphology, as summarized below:

1. *U. torrefacta* s. str.: Gyrophoric (major), lecanoric (minor or trace), usually with crustinic acid (major), and accessory norstictic acid (minor or trace). Lower surface cream, pale brown, or light brown, rarely blackening, with a monolayer of trabeculae and rhizines. Thallus thin. Widespread and common in the northern Hemisphere.
2. *U. dura* sp. nov.: Gyrophoric acid (major), lecanoric (minor or trace). Lower surface dark brown to black, with one to several layers of trabeculae and rhizines. Thallus thick. Alaska and Cascade Range of Oregon and Washington.
3. *U. multistrata* sp. nov.: Gyrophoric acid (major), lecanoric acid (trace), and umbilicatic acid (major). Lower surface black, with one to several layers of trabeculae and rhizines. Thallus thick. Alaska.

Because each of the clades numbered above is distinguishable morphologically and/or chemically, new taxa are created here to accommodate all three clades at the species level. Note, however, that the first clade includes a few specimens of *U. torrefacta* with a black lower surface, but otherwise typical of *U. torrefacta* in chemistry, spores, and morphology.

## Taxonomy

***Umbilicaria dura* McCune, sp. nov.** (Fig. 2)

MycoBank: MB823121

**Diagnosis:** Similar to *Umbilicaria torrefacta* but with multilayered dark brown to black plates on the lower surface, thallus lacking umbilicatic acid, containing gyrophoric acid; spores 7.5–9.5 (12.8) × 4.5–6.3 (7.0) µm.

**Type:** U.S.A. Oregon: Lane County, Cascade Range, Browder Ridge, open rocky ridgetop with andesite outcrops, 44.3852°N 122.1261°W, 1607 m, on rock, 2015-09-04, McCune 35928 (OSC, holotype).

**Etymology:** The species is named for the tough, hard thallus.

**Description:** Thallus umbilicate, monophyllous (Fig. 2); upper surface brownish gray, brown, or very dark brown, not discolored near the umbo, matte to shiny, epruinose, mostly 1–4 cm diam. and 2–10 mm thick; margins entire to irregularly incised, slightly to strongly rolled under; upper surface initially smooth, soon areolate (similar to *U. torrefacta*), developing coarse folds, imperforate to moderately perforate with pinholes (hold up to light); lower surface black or dark brown, lacking thalloconidia, verrucose, usually developing a dense mat of multiple levels of black or dark brown parallel plates (trabeculae) and rhizines over part or all of the lower surface (Fig. 2B), the mat to about 5 mm thick, rarely with patches not covered by trabeculae or rhizines and thus exposing the verrucose lower cortex; rhizines few to abundant, cylindrical to flattened, dark brown to black, to 3 mm long; upper cortex with a thin superficial POL– (i.e. dark under polarized light) necrotic layer, 0–12 µm thick; cortex 12–25 µm thick, dark brown and POL– in a thin layer above, hyaline and POL+ throughout the remainder; medulla about 75–130 µm thick, POL+, containing gray crystals, extending 15–57 µm below the algal layer; algal layer 40–60 µm thick; lower cortex very thick (50–140 µm), paraplectenchymatous but the cells thick walled, gelatinized,

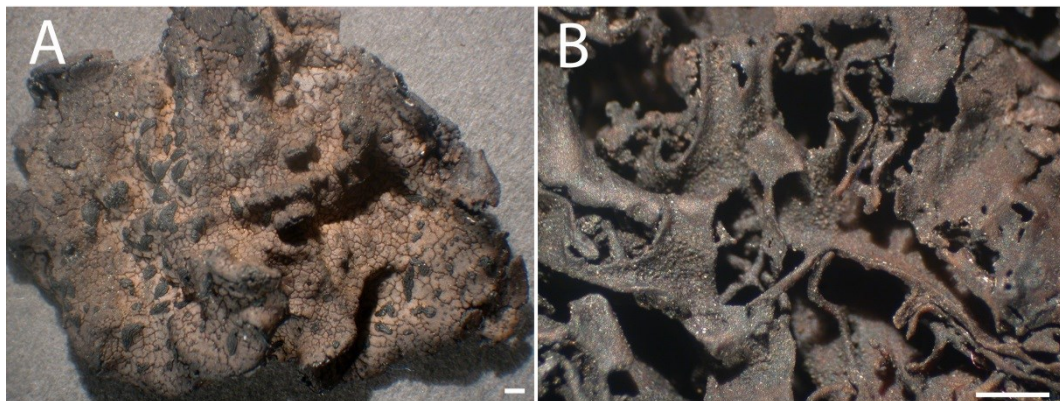


**Figure 2.** *Umbilicaria dura* (McCune 23132). A. Upper surface. B. Trabeculae on lower surface. Scale bars 1 mm.

and with small, rounded lumina and no visible intercellular spaces, the lowermost layer dark brown.

Apothecia subsessile to sessile, black, gyrose, to about 4 mm diam, initially elliptic, becoming angular to stellate, then compound and forming dense aggregations. Ascospores simple, hyaline, 8 per ascus, ellipsoidal,  $7.4\text{--}9.4$  ( $12.8$ )  $\times$   $4.5\text{--}6.3$  ( $7.0$ )  $\mu\text{m}$ .

Pycnidia common, slightly protruding from the thallus, with a dark ostiole and dark wall in section; spermatia bacilliform,  $2.6\text{--}3.4 \times 0.8\text{--}1.2$   $\mu\text{m}$ .

*Chemistry:* Thallus containing gyrophoric acid (major) and lecanoric acid (trace).

*Photobiont:* Chlorococcoid.

*Substrate:* Noncalcareous rock.

*Distribution and habitat:* Alaska to subalpine of Cascade Range in Oregon. On somewhat sheltered to exposed surfaces of noncalcareous rock.

*Additional specimens examined:* **U.S.A. Alaska:** Lake Clark National Park, slopes and flats above Turquoise Lake, alpine tundra with noncalcareous boulders and outcrops,  $60.8075^{\circ}\text{N}$   $153.9948^{\circ}\text{W}$ , 990 m, on rock, McCune 34977, 35008 (OSC, ALA). **Oregon:** Lane County, McKenzie Pass, basalt lava flow with widely scattered *Pinus*, *Abies*, and *Tsuga mertensiana*,  $44.25^{\circ}\text{N}$   $121.833^{\circ}\text{W}$ , July 1996, McCune 23132 (OSC). **Washington:** Skamania County, volcanic spur on NW slope of Mount Adams, near Killen Cr. Trail, rocky ridge with dwarf *Abies* - *Tsuga* - *Pinus albicaulis*, on rock, August 1999, McCune 24736 (OSC).

***Umbilicaria multistrata* McCune sp. nov.** (Fig. 3)

MycoBank: MB823122

*Diagnosis:* Similar to *Umbilicaria torrefacta* but with multilayered black plates on the lower surface, forming a thick thallus; thallus containing umbilicaric acid (major) in addition to gyrophoric acid; spores ( $8.8$ )  $9.0\text{--}11.0$  ( $11.5$ )  $\times$   $4.5\text{--}7.0$  ( $10.0$ )  $\mu\text{m}$ .

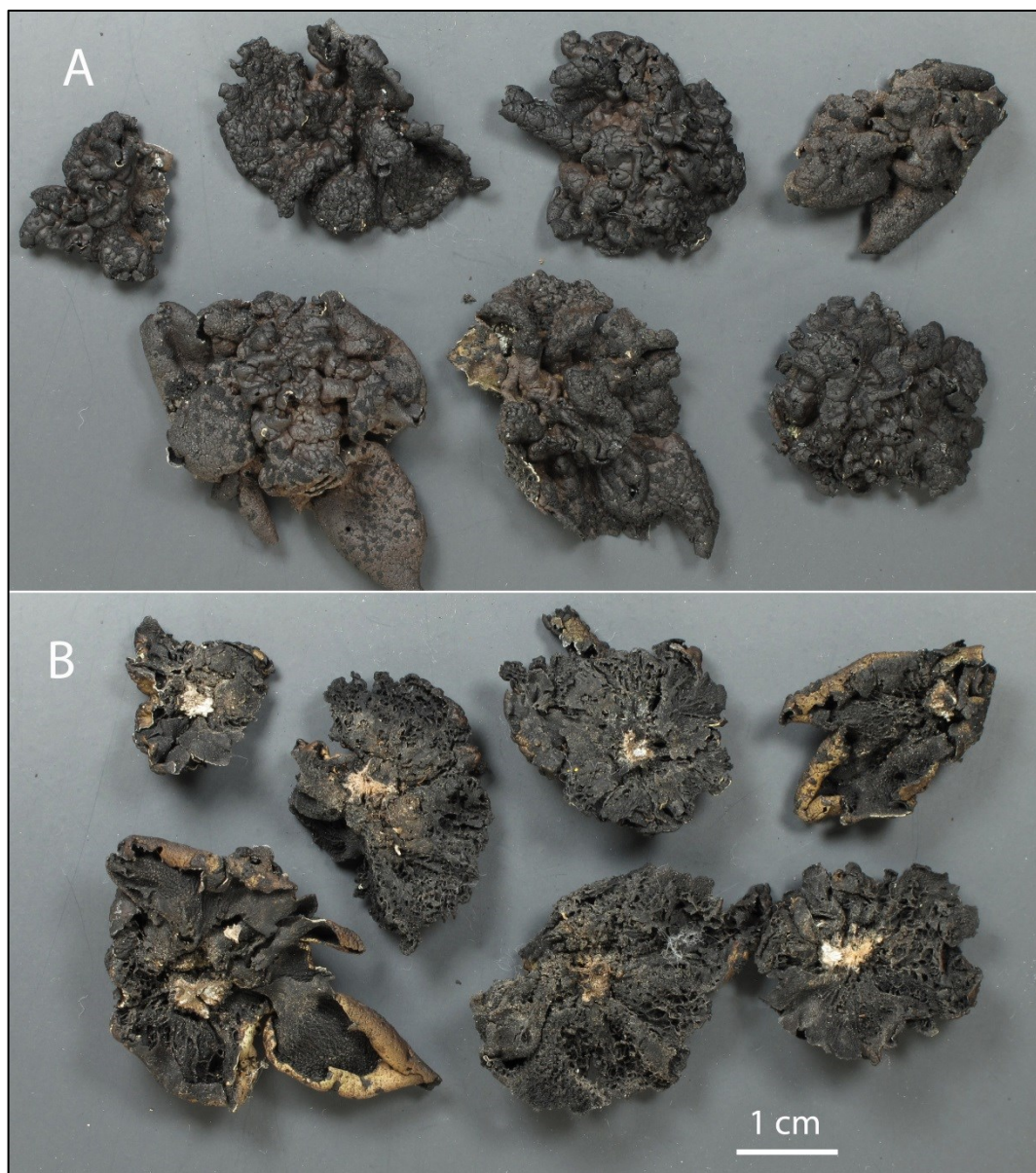


**Figure 3.** *Umbilicaria multistrata* type specimen. A. Upper surfaces. B. Lower surfaces.

**Type:** U.S.A. Alaska: Lake Clark National Park, slopes and flats above Turquoise Lake, alpine tundra with noncalcareous boulders and outcrops, 60.8075°N 153.9948°W, 990 m, on rock, 2014-07-10, McCune 34857 (OSC, holotype; ALA, isotype).

**Etymology:** The species is named for the multilayered plates on the lower surface.

*Description:* Thallus umbilicate, monophyllous (Fig. 3); upper surface brown to very dark brown, epruinose, not discolored near the umbo, matte to shiny, mostly 1–3 cm diam. and 1–5 mm thick; margins entire to irregularly incised, slightly to strongly rolled under; upper surface initially smooth, soon areolate (similar to *U. torrefacta*), developing coarse folds, mostly imperforate but submarginal areas with occasional pinholes (hold up to light); lower surface black, lacking thalloconidia, verrucose, usually developing a dense mat of multiple levels of parallel plates (trabeculae) over part or all of the lower surface (Fig. 3B), with or without rhizines, the mat mostly about 1–2 mm thick, sometimes with patches not covered by trabeculae and thus exposing the verrucose lower cortex; rhizines highly variable, few or none to abundant, black to 2 mm long, cylindrical to flattened and intergrading with trabeculae; upper cortex in places with a very thin superficial POL– necrotic layer, 0–4 µm thick; cortex 15–20 µm thick, dark brown and POL– in a thin layer above, hyaline and POL+ throughout the remainder; medulla about 35–85 µm thick overall, POL+, containing hyaline to brownish crystals, extending 12–60 µm below the algal layer; algal layer diffuse, not well delimited, about 25 µm thick; lower cortex very thick (60–160 µm), paraplectenchymatous but the cells thick walled, gelatinized, and with very narrow lumina and no visible intercellular spaces, the lowermost layer dark brown.

Apothecia partially sunken and nearly level in the thallus to sessile, black, gyrose, to about 2 mm diam, initially elliptical, becoming angular to stellate, becoming compound, sometimes roundish in outline. Ascospores simple, hyaline, 8 per ascus, ellipsoidal, 8.8–11.0 (11.5) × (4.5) 5.4–7.0 (10.0) µm.

Pycnidia common, immersed, even with the upper surface or slightly protruding, with a dark ostiole and dark wall in section; spermatia not seen.

*Photobiont:* Chlorococcoid.

*Chemistry:* Thallus containing gyrophoric acid (major), lecanoric acid (trace), and umbilicic acid (major).

*Substrate:* Noncalcareous rock.

*Distribution and habitat:* So far the species is known only from Alaska. Most commonly it is on steeply sloping, somewhat sheltered surfaces of noncalcareous rock.

*Additional specimens examined:* U.S.A. Alaska: Alaska Range District: Denali Highway: Mile 28, mountain ridge N of highway, 1957-07-24, Krog (O L-127877); Bering Strait District: Seward Peninsula: Rainbow Creek, 671 m, 1957-08-06, Krog 4099 (O); Kenai Peninsula: Kenai Fjords National Park, Harding Icefield Trail, lower slope, 227 m, McCune 36465 (OSC); Valdez-Cordova Borough, above Thompson Pass, east of Valdez, 1043 m, Aug 2010, McCune 30830 (OSC; rhizinate, hardly trabeculate morph).

## Discussion

*Umbilicaria dura* and *U. multistrata* are even more similar in morphology to each other than to *U. torrefacta*; in fact, on morphological grounds I originally considered *U. dura* and *U. multistrata* to be a single species, but was forced to reconsider that conclusion by the phylogenetic reconstruction and differences in secondary metabolites and geographic range, which suggest they should be treated as separate species. Although subtle differences in coloration and spores can be used in routine identification (see key below), either TLC or DNA sequence data are needed for conclusive identification of difficult specimens.

While typical *U. torrefacta* is easily distinguished from *U. dura* and *U. multistrata* by its pale lower surface and thin thallus, *U. torrefacta* can rarely be similar in appearance to the other two species in having a moderately thick thallus and black lower surface. These forms of *U. torrefacta* differ from *U. multistrata* in that the fissures developing in the upper surface become perforate near the thallus edge (hold up to light), while *U. multistrata* is only sparsely perforate. The habitat of these multistrata-like specimens of *U. torrefacta* is normally exposed rather than sheltered or semi-sheltered rock. Like other forms of *U. torrefacta*, the thallus chemistry of those with a black lower surface is highly variable but usually includes crustinic acid, occasionally with norstictic acid or gyrophoric acid. Neither crustinic acid nor norstictic acids are present in either *U. dura* or *U. multistrata*, although gyrophoric acid is present in all three species.

In contrast with most descriptions of *U. torrefacta*, it is clear that *U. torrefacta* can occasionally have a thallus with a single layer of dark brown to black trabeculae and dark brown to black lower surface, while being otherwise typical in morphology. Such specimens are embedded in the typical *U. torrefacta* clade and do not appear to merit taxonomic recognition.

A rare North American species of the coastal mountains, *U. lambii*, resembles *U. dura* and *U. multistrata* in its thickly, multilayered lower surface. *Umbilicaria lambii* is, however, distinctive in being squamulose to almost crustose and bearing a smooth, unfissured disk.

So far *U. dura* and *U. multistrata* are known only from western North America. It seems likely, however, that one or both of these will eventually be found in oceanic areas of the Nordic countries and/or Far East Russia. Although I have not yet encountered these in herbaria, apart from Krog's Alaskan material in O, it seems possible that more specimens may be found filed under *U. torrefacta* or undetermined specimens of *Umbilicaria*.

### Key to *Umbilicaria torrefacta* and similar species in northwestern North America

1. Spores mostly >13 µm long; platy structures mostly near the umbilicus; margins seldom perforate; upper surface smooth and continuous, not areolate-segmented; lower surface black; upper surface brown but often with a gray to purplish cast; apothecia ± angular, common; lower surface with cylindrical to flat, tangled, elongate, sometimes sparse, black rhizines; lower cortex verrucose or papillose, black; spores (13) 15–20 (22) × 8.7–11.5 (13.5) µm; thallus containing gyrophoric acid (major), lecanoric acid (minor), and zeorin (minor); Alaska to California ..... *U. angulata*
1. Spores small, mostly <13 µm long; platy structures continuous or scattered over the lower surface, but often concentrated toward the umbilicus; margins imperforate to heavily perforate (hold up to light) and dissected; upper surface areolate-segmented; lower surface black to brown, tan, or cream ..... 2
2. Lower surface beige to light brown or dark brown, rarely black; trabeculae mostly in a single layer; thallus almost always abundantly but minutely perforate toward the margins; thallus usually containing crustinic acid (major) in addition to gyrophoric acid, never with umbilicic acid; spores (7.5)8–12(14.5) × (4)6.5–8(9.5) µm; widespread ..... *U. torrefacta*
2. Lower surface black or brownish black; trabeculae in one to more often multiple layers; thallus imperforate to somewhat perforate ..... 3
3. Thallus thin to moderately thick, fissures developing in the upper surface that become perforate near the thallus edge (hold up to light); habitat normally exposed rock; spores 8.8–11.5 (15) × 4.6–7.7 µm; thallus chemistry highly variable but usually with crustinic acid, occasionally with norstictic acid or gyrophoric acid ..... rare form of *U. torrefacta*

3. Thallus thick, tough, imperforate to moderately perforate; habitat semi-sheltered rock faces . . 4
4. Spores mostly  $>9.5 \mu\text{m}$  long; thallus containing umbilicatic acid (major) in addition to gyrophoric acid; spores  $(8.8) 9.0\text{--}11.0 (11.5) \times 4.5\text{--}7.0 (10.0) \mu\text{m}$ ; so far known only from Alaska . . . . . *U. multistrata*
4. Spores mostly  $< 9.5 \mu\text{m}$ ; thallus lacking umbilicatic acid, containing gyrophoric acid; spores  $7.5\text{--}9.5 (12.8) \times 4.5\text{--}6.3 (7.0) \mu\text{m}$ ; sw Alaska to Washington and Oregon Cascade Range . . . . . *U. dura*

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