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# An unusual ascospore shape and a new species, *Umbilicaria nodulospora* (Umbilicariaceae), from California and Oregon

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**ABSTRACT.** We describe *Umbilicaria nodulospora*, a distinctive new species occurring on steep rock faces on old lava flows in California and Oregon. The species ia unusual among lichenized ascomycetes in consistently having one or two shallowly bulging knobs at one end of each ascospore, suggesting a T, Y, or L shape; otherwise they are consistent with the appearance of ascospores in most *Umbilicaria* species. The species is readily distinguished in the field by a grayish tone to the brown upper surface, in combination with a thickly rhizinate lower surface. Distinctiveness of the species was supported by analysis of the ITS and LSU regions of nrDNA. No close relative or sister taxon was found. *Umbilicaria nodulospora* is so far known only from geologically recent flood basalts in central Oregon to northeastern California.

**KEYWORDS.** California, ITS, Lecanorales, lichenized ascomycetes, lichenized fungi, lichen systematics, LSU, Oregon, rDNA, *Umbilicariaceae*, western North America.

Despite Llano's (1950) thorough monograph of the Umbilicariaceae, closer study has revealed a number of new species or the need to resurrect previously synonymized species (Poelt & Nash 1993; Davydov et al. 2010; McCune & Curtis 2012). Continued collecting in western North America has revealed some problems with the current taxonomy. One of those problems came to our attention as two small specimens from Lava Beds National Monument in northeastern California. Further study and collecting showed this to be a previously unrecognized species. Furthermore, it revealed an ascospore shape that is unusual within lichenized fungi. The purpose of this paper is to describe this new species, its unusual spores, and to use DNA sequence data to evaluate the relationship between this species and other species in the Umbilicariaceae.

## MATERIALS AND METHODS

***Specimen sampling.*** We studied collections of *Umbilicaria* from many herbaria, but no collection had specimens of the target species. Instead we obtained sufficient material by collecting in likely areas.

All specimens were examined for spores. Hand sections of apothecia were suspended in water and studied by standard light microscopy. A selection representing various geographic

locations and with minor variations in thallus form were extracted for DNA. Secondary substances were analyzed by thin-layer chromatography (TLC) using solvents A and C of Culberson (1972). Fragments of specimens were extracted in acetone at room temperature, spotted on aluminum-backed silica gel plates (Merck 5554/7 Silica gel 60 $F_{254}$), lightly brushed with 10% $H_2SO_4$, and gently charred in an oven at 100$^{o}$C.

***DNA extraction and PCR amplification***. We chose to analyze nuclear internal transcribed spacer (ITS) and large subunit (LSU) rDNA regions in keeping with others working on species-level problems in *Umbilicaria* (Davydov et al. 2010; Hestmark et al. 2011; Ivanova et al. 1999; Krzewicka et al. 2009). Total DNA was extracted from ~ 100 mg of homogenized (FastPrep-24, MP Biomedicals, Inc) fresh, frozen and herbarium material following the Fast DNA® Spin Kit (MP Biomedicals, Inc.) protocol. Two µL of total DNA extract was used as template in PCR reactions of 30 µL final volume. Each reaction included 15 µl of Dream Taq Green PCR Master Mix (2x; Thermo Scientific Inc.), 12.8 µl of nuclease-free $H_2O$, and 1.2 µl of each primer. Primers (final concentration: 400 nM) used for amplifying were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) for ITS, and LROR and LR6 (Vilgalys & Hester 1990) for LSU. The reactions were run with the following parameters for ITS: initial denaturation at 94°C for 2 min, 34 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 1 min 20 sec. For amplification of LSU, the parameters were as follows: initial denaturation at 94°C for 2 min, 34 cycles of denaturation at 94°C for 45 sec, annealing at 53°C for 45 sec, and extension at 72°C for 2 min. Ten µL of PCR product from each reaction was analyzed for purity by electrophoresis through a 0.8% agarose gel with GelRed stain (Biotium, Inc.) in water.

PCR product from each reaction was processed for sequencing as follows: Individual PCR gel bands were cut out of the gel and purified following the QIAquick gel extraction kit protocol (Qiagen, Inc.). Two µl of purified PCR product was added to 8.8 µL $H_2O$ and 1.2 µL sequencing primer (final concentration: 100 nM), and then sequenced by the Center for Genome Research and Biocomputing (Oregon State University). In addition to the amplification primers, the following primers were used for sequencing: ITS2 and ITS3 (White et al. 1990). Staden version 1.6.0 (SourceForge.net) was used to quality check the raw sequence, align the four sequencing runs per PCR product, and generate a consensus sequence for phylogenetic analyses.

Not all attempts yielded good sequences, so we ended up with four ITS and three LSU sequences of the new species.

***Phylogenetic analyses.*** We inferred phylogenetic relationships from sequences of rDNA from 5 specimens of the putative new species, along with 22 *Umbilicaria* specimens represented in GenBank (**Table 1**). We also included *Boreoplaca ultrifrigida*, *Hypocenomyce scalaris*, and *Ophioparma ventosa* as outgroups (**Table 1**) following Hestmark et al. (2011). We interpret the accession of *Umbilicaria kraschenninnikovii* in GenBank as *U. polaris* (Schol.) Zahlbr., based on a revised understanding of this group (Davydov et al. 2011). ITS and LSU sequences selected from GenBank represented species across *Umbilicaria*. Sequences were aligned with Geneious alignment using default settings (cost matrix: 65%, gap open penalty 12, gap extend penalty 3). The ends of the alignments were then trimmed to a nearly equal number of sites for all sequences. Alignment gaps were treated as missing data.

Phylogenetic trees were obtained by maximum likelihood analysis of the ITS and partial LSU data, using the GTR (general time-reversible) model and Genious defaults, except we used the "BEST" (slower) topology search, using the PhyML (Guindon et al. 2010) plug-in to Geneious 5.4.3 (Drummond et al. 2011). Statistical support for branches was evaluated with 1000 bootstrap resamplings. We compare the results for ITS and LSU by constructing phylogenetic trees for each subset of the data. The resulting trees were then visually compared for conflicts (taxa found in two distinct clades, each with more than 70% bootstrap support). Excluding the outgroups, the alignments had 155 variable sites of 504 in the ITS and 106 of 812 in the LSU.

## RESULTS

We describe a new species, *Umbilicaria nodulospora,* to accommodate distinctive specimens from northern California and central Oregon. The species is distinctive in gross morphology (Fig. 1-2), ascospore morphology (Fig. 3), and in ITS and LSU sequences (Fig. 4), as described below.

***Ascospore morphology.*** *Umbilicaria nodulospora* has distinctive ascospore morphology (**Fig. 1**). We know of this spore shape in lichenized ascomycetes only in *U. calvescens* group from South America. Most ascospores of *U. nodulospora* are asymmetrical at one end, owing to a shallow

lateral bulge or nodule. This nodule can be seen on most free spores on a microscope slide, but it is difficult to see when the spores are still in the ascus. Also, depending on the angle of repose of the spore on the microscope slide, the bulge can be nearly invisible in a small fraction (ca. 10-20%) of the spores.

***Phylogenetic reconstruction.*** *Umbilicaria nodulospora* was readily distinguished from other *Umbilicaria* species by their ITS and LSU sequences. Although these sequences could be concatenated and analyzed in a single tree, we chose to keep them separate to help clarify the minimum effort needed to detect these species (e.g. by barcoding). Phylogenetic reconstruction for both ITS (**Fig. 4A**) and LSU (**Fig. 4B**) showed the species to be distinct and forming a well-supported monophyletic group. However, relationships among *U. nodulospora* and other species are obscure, because the backbone of the trees within *Umbilicaria* had little support, and no sister group emerged for *U. nodulospora*.

**THE SPECIES**

**Umbilicaria nodulospora** McCune, Di Meglio & M. J. Curtis, sp. nov. (Figs. 1-3)

TYPE: U.S.A.: California: Modoc Co., Lava Beds National Monument, just below east-facing lip of Mammoth Crater on its west side, 41.69200° N 121.54623° W, 1618 m, on volcanic rock, *McCune 32546*. (holotype, OSC; isotypes UC, US).

***Description.*** Thallus umbilicate, monophyllous (Fig. 1A) to more often dividing as it expands into larger polyphyllous colonies (Fig. 1B); upper surface brown to gray brown, faintly to distinctly grayish pruinose (Fig. 2), often grayer near the umbo, matte to slightly shiny, mostly 1–2(3) cm diam, about 0.2 mm thick excluding the rhizines; upper surface smooth to broadly areolate and reticulately cracked, the cracks often deep so that the thallus readily divides into part thalli; submarginal areas occasionally minutely perforate (hold up to light); margins entire to irregularly lacerate or lobulate; lower surface brown to black but apparently lacking thalloconidia, smooth to finely papillose or verrucose, usually developing a dense mat of parallel or tangled rhizines over part or all of the lower surface (Fig. 1D, Fig. 2), the mat about 1–2 mm thick, often interspersed with brown to black trabeculae, sometimes patches apparent without rhizines or trabeculae and thus exposing the papillose lower cortex; upper cortex with superficial POL- (i.e. dark under polarized light) necrotic layer, 0–10 µm thick; cortex 7–12 µm thick,

brown and POL- above, hyaline and POL+ below; medulla about 100 µm thick overall, POL-, the algal layer about 60–70 µm, the lower part hyaline to gray and densely crystalline, rather compact; lower cortex very thick, paraplectenchymatous but the cells thick walled and with narrow lumina, the lowermost layer brown; rhizines mostly 25–115 µm diam and 1–4 mm long, cylindrical or sometimes flattened and grading into trabeculae, brown to black, sometimes tan; apothecia sessile, black, gyrose, to 1.2(2.0) mm diam, initially angular to stellate (Fig. 1C), with age protruding more and becoming convex and roundish in outline; ascospores simple, hyaline, 8 per ascus, ± ellipsoidal but usually with one or two blunt shallowly bulging knobs at one end and forming a Y, T, or L shape, (9.5)10.5–13.0(17.8) × (5.9)6.2–7.5(8.8) µm (**Fig. 3**); pycnidia occasional, embedded in thalline swellings and with a dark ostiole; spermatia bacilliform to narrowly ellipsoid, minute, 3.3–4.3 × 1.0–1.4 µm; thallus containing gyrophoric acid and related compounds by TLC. Photobiont chlorococcoid.

***Distribution and habitat***. So far the species is known only from central Oregon to northeastern California (Fig. 5). In all cases it has been found on steeply sloping surfaces of relatively recent basalt lava flows (i.e. 2,000–12,000 ybp). In most cases it has been found on relatively cool aspects, for example the north-facing lip of collapsed lava tubes.

***Selected specimens examined*** (McCune specimens in OSC; Sheehy specimens at Lava Beds National Monument). U.S.A. CALIFORNIA: Modoc Co., just outside SE corner of Lava Beds National Monument, 41.6922° N 121.4466° W, 1314 m, *McCune 32518, 32519*. Siskiyou Co., Lava Beds National Monument, east of East Sand Butte, 1316 m, 41.6820° N 121.3722° W, *McCune 32507, 32510*; 0.5 km ENE of Skull Cave, *McCune 32530*; near entrance to Boulevard Cave, 1429 m, *McCune 32543*; Devils Homestead upper parking, 1240 m, *Sheehy LABE-36*; Hill Road at fire danger sign, 1240 m, *Sheehy LABE-62*; Merrill Cave Road,1295 m, *Sheehy LABE-3l* and *32*; near summit of Schonchin Butte, 1615 m, *McCune 32536, 32539, 32540*; near bottom of Mammoth Crater, 1547 m, *McCune 32551*; Tickner Road, 1 mile east of Medicine Lake Road, 41.68695° N 121.5326° W, 1592 m, *Sheehy 774.* OREGON. Lake Co., lava flow by Cougar Mountain, 43.41086° N 120.87568° W, 1371 m, *McCune 34466*; W edge of Four Craters Lava Field, 43.37427° N 120.68981° W, 1426 m, *McCune 34506*; east side of East Lava Field, 43.43548° N 120.70260° W, 1371 m, *McCune 34487*.

## DISCUSSION

***Ascospore morphology.*** We found *Umbilicaria nodulospora* to have an ascospore shape unusual among ascomycetes, lichenized or not. One end of the spore typically has one or two shallow bulges suggesting a T, L, or Y shape. Although we initially thought these might represent a developmental aberration, every specimen examined from each of the fifteen known localities for the species showed this spore morphology. Thus, the spore morphology is consistent within the species and cannot be considered an aberration. Ascospores from all other fertile species of *Umbilicaria* known from western North America have also been examined, revealing no other examples of nodulose spores.

The only other description of this kind of ascospore that we have found is Frey (1949), who described and illustrated similar spores in *U. krempelhuberi* Müll. Arg., now considered part of *U. calvescens* Nyl. by Hestmark et al .(2011). Llano (1950) described the spores of *U. krempelhuberi* as "spherical to ellipsoid, or irregular and somewhat constricted medially... peculiar constriction occasionally displayed by some spores, suggestive of a triangle or heart-shape." He described the spores of *U. calvescens* as "ellipsoid to irregular or somewhat constricted medially, becoming brown, muriform." While the spores of *U. nodulospora* are very similar to those of *U. krempelhuberi*, both in size and shape, we have seen no tendency for the spores of *U. nodulospora* to become septate, muriform, or brown as Llano reported for *U. calvescens*. Furthermore Llano's spore measurements for *U. calvescens* are distinctly larger than we observed for *U. nodulospora* (though Nylander's measurements from *U. calvescens* are similar to ours *U. nodulospora*; see Table 15, p. 180 in Llano (1950)). Hestmark et al. (2011) considered *U. krempelhuberi* to be a synonym of *U. calvescens*, but did not address either the unusual spore shapes or differences in spores between *U. krempelhuberi* and *U. calvescens.*

The nodulose spore shape appears gradually in spore development, with spores initially oval, becoming slightly peanut shaped when immature (Fig. 3A, row 1, left side), then finally variously shaped at maturity. The nodulose end of the spore appears is commonly oriented toward the base of the ascus (Fig. 3B), but spores were also seen variously oriented in the ascus.

We considered but rejected two alternative hypotheses for the nature of these spores: one that the spores represent a hymenial parasitic ascomycete, or that the spores represent mitotically produced macroconidia. We rejected the parasite hypothesis, because apothecia were collected from fifteen sites and every single apothecium examined had nodulose spores. We considered it highly unlikely that a hymenial parasite is uniformly present in its host. We rejected the

mitospore hypothesis because the nodulose spores clearly occur in groups of eight within asci. Asci with spores were always seen in conjunction with the spores loosened by sectioning.

Any adaptive or ecological significance of this spore shape remains obscure; nor do we presume that the spore shape is necessarily adaptive. It seems plausible, however, that the nodulose shape affects the mechanics of spore discharge from asci. The knobby spores might impede spore release, perhaps as a mechanism to influence the environmental conditions sufficient for spore discharge, for example desensitizing a moisture-based trigger.

***Phylogenetic relationships.*** *Umbilicaria nodulospora* is a distinctive species in field appearance, habitat, spore shape, and DNA sequences. Specimens of *U. nodulospora* formed a well-supported monophyletic group in phylogenetic trees (Fig. 4). The relationship of this species to other *Umbilicaria* species is, however, obscure, since no support for a sister taxon was found (Fig.4).

The phylogenetic reconstruction based on ITS and LSU offered no clues to the origin of this species, since the species connects directly with the poorly resolved backbone of *Umbilicaria*. Morphologically the species shares some characters with *U. torrefacta*, such as smaller ascospores than most *Umbilicaria* species, occasional presence of trabeculae on the lower surface, and occasionally perforations in the thallus near the margins. Neither of the last two characters are as strongly developed as in *U. torrefacta*. Other rhizinate *Umbilicaria* occur in western North America, but so far we have not found them cohabiting with *U. nodulospora*, with the exception of those species with a distinctly gray upper surface in the *U. vellea* group.

While *U. nodulospora* is morphological similar to the South American *U. calvescens* group, some differences are apparent. The lower surface of *U. krempelhuberi* is pale buff to brown and has sparse to few rhizines, while *U. nodulospora* is brown to black below and thickly rhizinate. Typical *U. calvescens* differs from *U. nodulospora* in lacking rhizines, but *U. calvescens* var. *hypomelaena* is black below and rhizinate, while *U. calvescens* var. *subvellea* is densely rhizinate, similar to *U. nodulospora*, but pale to brown in color. All of these variants were included in the concept of *U. calvescens* by Hestmark et al. (2011), because they lacked phylogenetic support based on ITS, nuLSU, and mitSSU.

We hypothesize that *U. nodulospora* is most closely related to the *U. calvescens* group, based on morphological similarity and the occurrence of this unusual spore type, although this hypothesis lacks bootstrap support. Phylogenetic reconstructions from both the ITS and LSU

regions showed *U. nodulospora* to be distinct from the *U. calvescens* group; furthermore we did not find support for a sister relationship.

The apparent restriction of *U. nodulospora* to lava flows is unusual, but perhaps reflects our scattered lichenological exploration of western North America. Many species frequent basalt, as opposed to other kinds of rock, but we know of no species in this region that have been demonstrated to have an obligate requirement for basalt as a substrate.

Similarly the species poses a distributional puzzle. So far the species is known only from central Oregon and extreme northeastern California, a very narrow range considering the ease with which it can be found in this area. Narrow endemics are seldom seen with lichens, much less in species frequenting subcontinental or continental climates. Is this species a narrow geographic endemic? Is it truly restricted to lava flows? The only resolution to these puzzles is more extensive searching for the species, which we hope this paper will stimulate.


## ACKNOWLEDGMENTS

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**Table 1.** Voucher information for the species sampled and the associated GenBank accession numbers for ITS and nuLSU. All new sequences are vouchered in OSC unless otherwise specified. The term "morph" indicates recognizable morphological variants of uncertain taxonomic value.

| | | GenBank Number | |
|---|---|---|---|
| Species | Voucher | ITS | LSU |
| *U. nodulospora* | California, *McCune 32521* | --- | xxxxxx |
| *U. nodulospora* | California, *McCune 32526* | xxxxxx | --- |
| *U. nodulospora* -- holotype | California, *McCune 32546* | xxxxxx | xxxxxx |
| *U. nodulospora*-- isotype | California, *McCune 32546* | xxxxxx | xxxxxx |
| *U. nodulospora* | Oregon, *McCune 34506* | xxxxxx | --- |
| Previously published sequences | | | |
| *U. angulata* Tuck. | Oregon, *McCune 30050* | JQ764746 | JQ764756 |
| *U. angulata* | Oregon, *McCune 30483* | JQ764734 | --- |
| *U. angulata* | Oregon, *McCune 31231* | JQ764738 | JQ764747 |
| *U. calvescens* Nyl. | Argentina, *Hestmark 09002* | HM161506 | HM161601 |
| *U. calvescens* | Argentina, *Hestmark 09003* | HM161507 | HM161602 |
| *U. calvescens* | Argentina, *Hestmark 09005* | HM161508 | HM161604 |
| *U. calvescens* | Peru, *Hestmark 05060B* | HM161558 | HM161516 |
| *U. calvescens* | Chile, *Hestmark 98025* | HM161559 | HM161517 |
| *U. calvescens* var. *subvellea* Nyl. | Peru, *Hestmark 05083B* | HM161485 | HM161546 |
| *U. calvescens* var. *subvellea* | Peru, *Hestmark 05061B* | HM161460 | HM161519 |
| *U. calvescens* var. *hypomelaena* Nyl. | Bolivia, *Hestmark 05019B* | HM161463 | HM161524 |
| *U. calvescens* var. *hypomelaena* | Peru, *Hestmark 05084B* | HM161486 | HM161547 |
| *U. cinereorufescens* (Schaer.) Frey | Bolivia, *Hestmark 05010B* | HM161503 | HM161598 |
| *U. cinereorufescens* | Ecuador, *Hestmark 094079* | HM161511 | HM161605 |
| *U. crustulosa* (Ach.) Lamy | Norway, *Hestmark 09017* | HM161496 | HM161590 |
| *U. decussata* (Vill.) Zahlbr. | Antarctica, *Ott 2007* | AY603122 | AY603113 |
| *U. haplocarpa* Nyl. | Bolivia, *Hestmark 05110B* | HM161467 | HM161528 |
| *U. haplocarpa* | Bolivia, *Hestmark 05052B* | HM161487 | HM161537 |
| *U. havaasii* Llano | Oregon, *McCune 31230* | JQ764739 | JQ764748 |
| *U. hirsuta* (Sw. ex Westr.) Ach. | Norway, *Hestmark 09015* | HM161494 | HM161588 |
| *U. hirsuta* | Norway, *Hestmark 09016* | HM161495 | HM161589 |
| *U. phaea* Tuck. | California, *McCune 30358* | JQ764736 | JQ764755 |
| *U. phaea* | California, *McCune 30442* | JQ764741 | JQ764751 |
| *U. phaea* | Oregon, *McCune 30545* | JQ764733 | --- |
| *U. polaris* (Schol.) Zahlbr. (as *U. krascheninnikovii*) | Antarctica, *Lumbsch 19046a* | AY603134 | AY603118 |
| *U. polyrrhiza* (L.) Fr. | Oregon, *McCune 30484* | JQ764737 | JQ764749 |
| *U. semitensis* Tuck. morph 1 | Oregon, *McCune 30049* | JQ764742 | JQ764757 |
| *U. semitensis* morph 1 | California, *McCune 30407* | JQ764735 | JQ764753 |
| *U. semitensis* morph 2 | California, *McCune 30410* | JQ764745 | JQ764754 |
| *U. semitensis* morph 3 | California, *McCune 30432* | JQ764743 | JQ764752 |
| *U. torrefacta* (Lightf.) Schrad. | Oregon, *McCune 30482* | JQ764744 | JQ764750 |
| *U. torrefacta* | unknown, Brunauer et al. | DQ660906 | --- |
| Outgroup: | | | |
| *Boreoplaca ultrifrigida* Timdal | Russia, *Haugan & Timdal YAK03/84* | HM161512 | DQ986797 |

| | | | |
|---|---|---|---|
| *Hypocenomyce scalaris* (Ach. ex Lilj.) M. Choisy | ----, *Amtoft 47763* | DQ782852 | DQ782914 |
| *Ophioparma ventosa* (L.) Norman | Norway, *Bjelland 60* | AY011013 | AY853380 |

**Figure 1.** *Umbilicaria nodulospora*. **A.** Habit in situ, moist, monophyllous example (*McCune 32546,* type; photo by M. Hutten). **B.** Habit in situ, dry, of polyphyllous colony in situ (*McCune 32519*). **C.** Detail of upper surface showing young apothecia (*Sheehy LABE31*). **C.** Detail of lower surface showing rhizines, trabeculae, and verrucose bare patches (*Sheehy LABE36*).

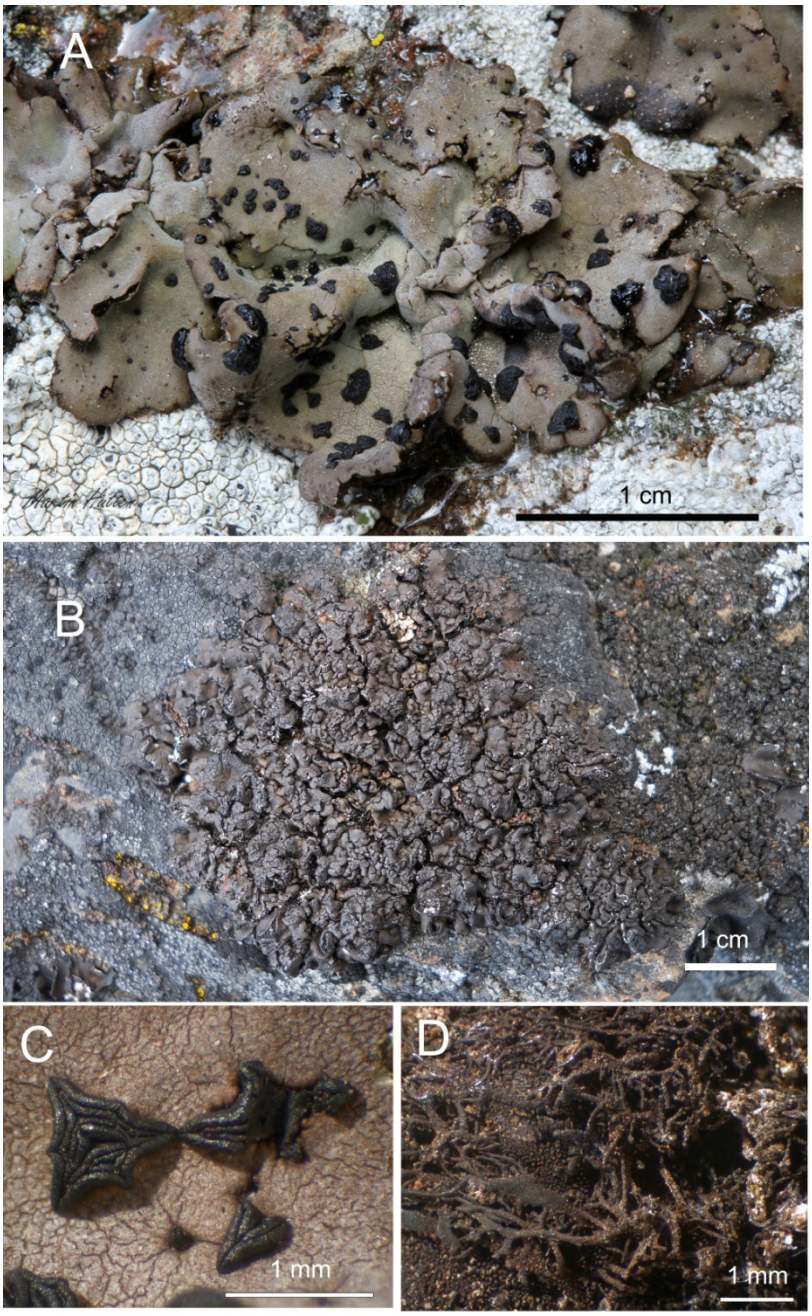

**Figure 2.** Variation in upper and lower surfaces of *Umbilicaria nodulospora*. **A.** Nearly epruinose examples (*McCune 34506*). **B.** Pruinose example (*Sheehy LABE31*).

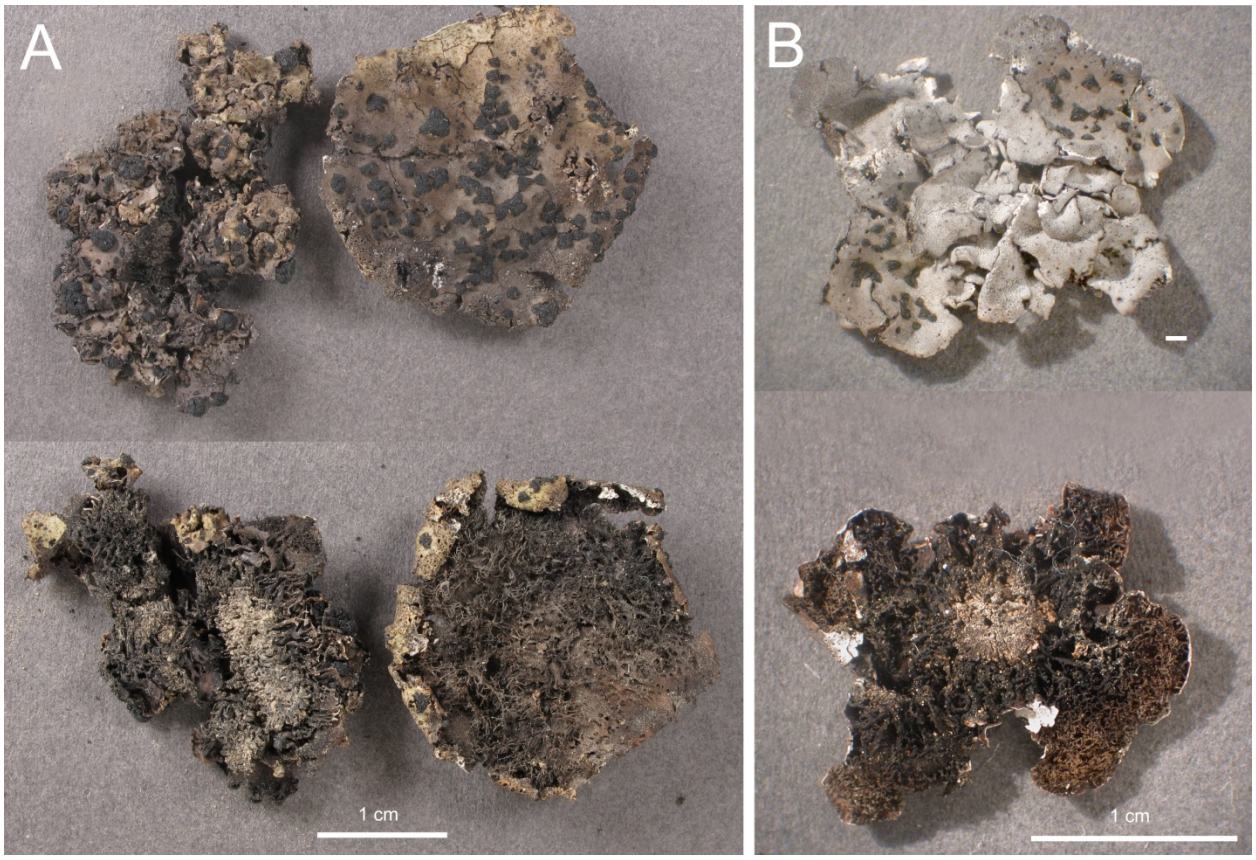

**Figure 3**. Ascospores and asci from *Umbilicaria nodulospora*. A. Spores selected at random from *Sheehy 774*, *McCune 32546*, and *Sheehy LABE-36*. Four spores on the left of 774 are smaller immature spores. B. Hymenium and dark brown incurved exciple, showing spores in ascus in IKI, *Sheehy 774.*

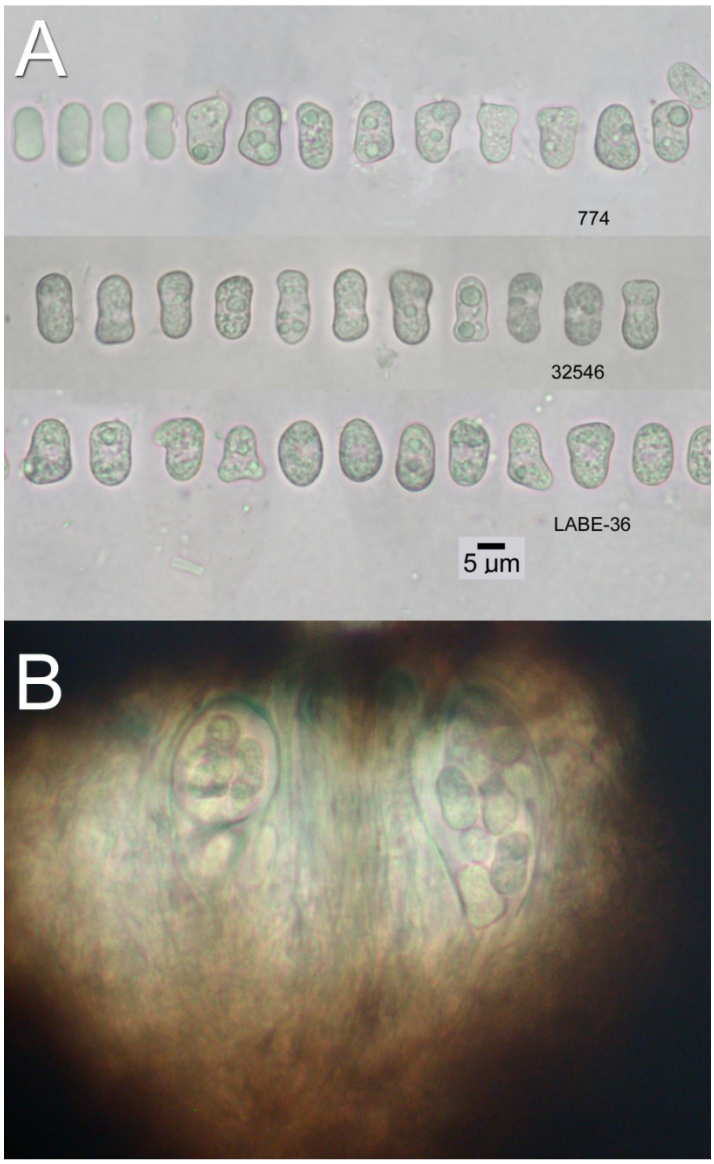

**Figure 4**. Most likely phylogenetic relationships among sampled *Umbilicaria* species inferred from ITS or nuLSU sequences and based on rooting with *Boreoplaca*, *Hypocenomyce,* and *Ophioparma* chosen as outgroups. **A.** Tree from ITS data. **B.** Inferences from LSU data. Bootstrap percentages above 80% are shown above branches; branch lengths are based on estimated number of substitutions per site assuming a GTR model of substitution. Countries of origin for *U. calvescens* var. *calvescens* are given because of geographically-related variation: A = Argentina, C = Chile, P = Peru.

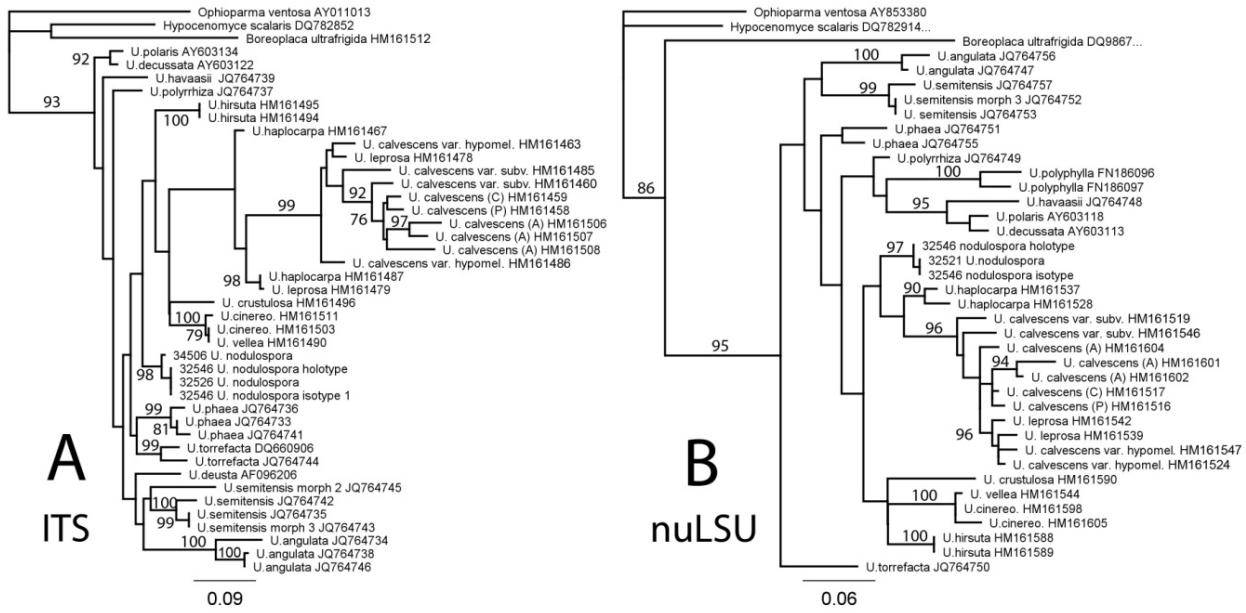

**Figure 5**. Distribution of *Umbilicaria nodulospora*. **A.** All known sites. **B.** Detail showing the cluster of sites in and near Lava Beds National Monument, California.