
A hidden basidiolichen rediscovered: *Omphalina oreades* is a separate species in the genus *Lichenomphalia* (Basidiomycota: Agaricales: Hygrophoraceae)

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Abstract: Molecular studies have shown the type collection of *Omphalina oreades* to be conspecific with a small brown basidiolichen from the Appalachian range in Newfoundland, both with 4-spored basidia. Two sequences deposited in GenBank, originally identified as *O. grisella*, fell in the same clade. Sequences of the type collection of *Omphalina grisella*, with 2-spored basidia, formed a sister clade together with two GenBank deposits, one identified as *O. grisella* and the other as *Omphalina velutina*. *Omphalina oreades* is recombinant here as *Lichenomphalia oreades* comb. nov., and the species redescribed and illustrated. Sequences of the internal transcribed spacer regions of nuclear ribosomal DNA (ITS rDNA) from the algae associated with two collections of *L. oreades* fell within a highly supported clade with members of an undetermined species of *Coccomyxa*. The most abundant algal ribosomal large subunit sequence from granules at the base of a different collection matched GenBank sequences identified as *Chloroidium ellipsoideum*, which is probably either a free-living algal species or a partner to a species of *Trapeliopsis*. The second most abundant sequence matched *Coccomyxa subellipsoidea* and is the most likely photobiont of *L. oreades*. Further studies are required to elucidate the relationship of *L. velutina* to these taxa.

Key words: ITS barcoding, Illumina sequencing, New Hampshire, phylogeny, typification

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Introduction

Lichenomphalia Redhead *et al.* is a small genus of lichenized Basidiomycota in the Agaricales, family Hygrophoraceae (Lodge *et al.* 2014). This group, briefly known as *Botrydina* Bréb. (Redhead & Kuyper 1987) and *Phytoconis* Bory (Redhead & Kuyper 1988), was separated from the genus *Omphalina* (Bull.) Quél.

based on its lichenized habit by Redhead (1984) and Kuyper (1986), subsequently supported by molecular studies (Redhead *et al.* 2002). *Lichenomphalia* currently includes 13 accepted species (Index Fungorum Partnership 2016), forming the only agaricoid lichenized fungal genus. The recognition of lichenized species in this genus and their separation from similar, non-lichenized taxa is

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not always straightforward, particularly for those taxa that produce inconspicuous, granular algal thalli, which has led to confusion about the biological nature of material forming the basis for certain species. Even more confusing has been, and still is, the separation of small brown agaricoid basidiolichen species from each other because species boundaries in the group are unclear.

The present study was prompted by the discovery of a small, brown agaric basidiolichen on a barren mountain top in Newfoundland in 2012, for which various names were initially available. Table 1 lists the species that we have been able to find which might fit that description. They come from three centuries and three continents, and have either primarily 2- or 4-spored basidia. Type specimens are available for all but one species, *Lichenomphalia velutina*, for which no type or authentic material is known and the original illustration by Quélet (1886) has been chosen as lectotype (Jørgensen & Ryman 1989). In addition to these taxa, Singer & Cléménçon (1972) considered *Omphalina rustica* (Fr.) Quélet to be an earlier epithet for the allegedly 2-spored *L. velutina* but *O. rustica* is currently interpreted as a non-lichenized species of *Arrhenia* Fr. (Redhead *et al.* 2002). Reviewing these taxa, we considered it likely that our species might fit with *Omphalina oreades* Singer because both are 4-spored species from the Appalachian Range in North America. Described from Mt. Washington by Singer (1948), *O. oreades* was collected again from the same site by Bigelow (1968), who treated it in *Clitocybe* (Fr.) Staude as *C. albimontana* Bigelow because Singer's epithet, *oreades*, was being used in *Clitocybe* by *C. oreades* Murrill. Both Singer and Bigelow noted 4-spored basidia and an absence of clamp connections; subsequently Bigelow (1983) noted the presence of algal granules representing the lichen thallus.

The primary aim of this study was to use the ITS rDNA fungal barcoding locus (Schoch *et al.* 2012) to determine whether the Newfoundland species indeed belonged to the genus *Lichenomphalia* and, by analyzing appropriate type specimens, whether it was *O. oreades*, another previously described

species, or represented a new taxon. Secondary aims were to identify its photobiont and provide an illustrated circumscription of the taxon.

Material and Methods

Specimens were photographed *in situ* and collected from Mt. Ignoble near Humber Village, above the Humber River, and Big Level in the Long Range Mountains, Gros Morne National Park, both parts of the Appalachian range in Newfoundland. Macroscopic descriptions were based on fresh material. Specimens were air dried, without the use of additional heat. Microscopic examination was carried out on fresh and dried material and spores measured from both spore print and squash mount using a Zeiss 392560 microscope with Apo 100/1.25 at $\times 1000$ magnification (oil immersion), mounting the material in 2% KOH. Basidiospores were measured to 0.5 μm precision expressed as range and *mean*. Recorded measurements deviating from 0.5 μm increments are due to calculation of a lens correction factor. Specimens from Duke University were examined for basidial sterigma count in ammoniacal Congo Red using a Zeiss photomicroscope with phase contrast Apo 63/1.4 at $\times 630$ magnification (oil immersion); unfortunately, only one of three specimens sequenced (Lutzoni 1997; Zoller & Lutzoni 2003) had any remaining hymenial tissue for examination.

New sequences of the internal transcribed spacers of ribosomal DNA (ITS rDNA) from selected sporocarps of the target fungus and related species were generated following the protocol used for extraction and sequencing of *Lepidostromataceae* (Hodkinson *et al.* 2013; Sulzbacher *et al.* 2016). Additional sequences, including from type material of published names, were generated using previously published techniques (Saar & Voitek 2015). Algal sequences were obtained by subjecting algal granules on soil near the base of five collections to either CTAB DNA isolation following Grube *et al.* (1995) or a bead-beating protocol with a GeneJET Plant Genomic DNA Purification Kit (Fermentas Life Sciences). PCR of the ITS rDNA followed Beiggi & Piercey-Normore (2007) and DNA sequencing followed Doering & Piercey-Normore (2009). For community genomic DNA (algal cells and substratum), PCR and Illumina sequencing of the D1 variable domain of the 26S rDNA, and processing of the raw sequence data, we followed the protocols of Asemaninejad *et al.* (2016).

To illustrate the placement of the target species within the genus *Lichenomphalia* and to determine its closest relative, the newly generated ITS rDNA sequences were aligned with selected sequences representing the genus *Lichenomphalia* s. lat., using *L. umbellifera* (L.) Redhead *et al.* as internal outgroup following previous studies (Table 2, Fig. 1). Sequences were arranged into a multiple sequence alignment (MSA) using BioEdit 7.09 (Hall 1999) and automatically pre-aligned with MAFFT 7.294 using the “-auto” option (Katoh *et al.* 2009). Since ITS sequences are difficult to align within

TABLE 1. *Brown agaricoid basidiolichen species described in the literature.*

Basionym	Author & year	Current name	Transferring authority & year	Type location	No. of sterigmata	Type specimen
<i>Omphalia velutina</i>	Quélet 1886	<i>Lichenomphalia velutina</i>	Redhead <i>et al.</i> 2002	FRANCE, Jura	undescribed	None; lectotype Pl. XII, Fig. 1 in Quélet 1886
<i>O. grisella</i> *	Karsten 1889	<i>Lichenomphalia grisella</i>	Redhead <i>et al.</i> 2002	FINLAND, Etelä-Häme	2	H 6042076
<i>Clitocybe payettensis</i>	Bigelow 1983	<i>Clitocybe payettensis</i>	n/a	USA, Colorado	2	MICH 5594
<i>C. borealis</i>	Bigelow 1983	<i>Clitocybe kemptoniae</i>	Bigelow 1985	USA, Alaska	2	MICH 10127
<i>Omphalina meridionalis</i>	Contu & La Rocca 1999	<i>Lichenomphalia meridionalis</i>	Moreau & Courtecuisse 2008 (in Courtecuisse 2008)	ITALY, Sardinia	2	IB 1999/0879
<i>O. oreades</i> *	Singer 1948	<i>Lichenomphalia oreades</i>	This paper	USA, NH (Mt. Washington)	4	FH W 45, Bar code 00543609
<i>O. defibulata</i>	Singer 1952	<i>Omphalina defibulata</i>	n/a	ARGENTINA, Tierra del Fuego	4	LIL M3 55
<i>O. subalpina</i>	Horak 1960	<i>Omphalina subalpina</i>	n/a	AUSTRIA, Ötztal	4	Herbario Horak
<i>O. pararustica</i>	Cléménçon 1982	<i>Lichenomphalia pararustica</i>	Elborne 2008	SWITZERLAND, Jura	4	LAU (HC 81/36)
<i>Clitocybe solumophila</i>	Bigelow 1983	<i>Clitocybe solumophila</i>	n/a	USA, California	4	MICH 10211
<i>Lichenomphalia cinereispinula</i>	Neville & Fouchier 2009	<i>Lichenomphalia cinereispinula</i>	n/a	FRANCE, Provence	4	Herbier F. FOUCHIER FF93074

Species are grouped by basidial sterigma number and then oldest to most recent description.

* Species used in this study.

TABLE 2. *New sequences from the brown agaricoid basidiolichen species used in this study.*

Name	Location	Collector	Collecting number	Date	Herbarium number*	GenBank/ UNITE no
<i>Omphalia grisella</i> TYPE	FINLAND, Etelä-Häme	<i>PA Karsten</i>	<i>PA Karsten</i> 3244	28 Aug. 1889	H 6042076	LT592287 UDB024271
<i>Omphalina oreades</i> TYPE	USA, NH, Mt. Washington	<i>R Singer</i>	<i>Singer</i> FH 45	4 Aug. 1945	FH 00543609	LT592282 UDB024406
<i>Lichenomphalia oreades</i>	Humber Village, Mt. Ignoble*	<i>A Voitek</i>	12.10.06.av01	6 Oct. 2012	DAOM 706314 TU 117231	LT592283 UDB024574
<i>L. oreades</i> with <i>Coccomyxa</i> sp.	Humber Village, Mt. Ignoble*	<i>A Voitek</i>	15.10.25.av01	25 Oct. 2015	DAOM 706315 TU 117232	LT592284 UDB024575
<i>L. oreades</i>	Big Level, GMNP**	<i>A Voitek</i>	FNL GM15a-300	24 Sept. 2015	DAOM 706316 TU 117233	LT592285 UDB024576
<i>L. oreades</i> with <i>Coccomyxa</i> sp.	Big Level, GMNP**	<i>M Voitek</i>	FNL GM15a-305	24 Sept. 2015	DAOM 706317 TU 117234	LT592286 UDB024577
OTU [†] 0 <i>Lichenomphalia oreades</i>	Humber Village, Mt. Ignoble*	<i>A Voitek</i>	13.11.07.av01	7 Nov. 2013	UWO 13.11.07.av01	KX383953
OTU 2 <i>Lichenomphalia umbellifera</i>						KX383954
OTU 4 <i>Sebacina</i> sp.						KX383955
OTU 3 <i>Chloroidium ellipsoideum</i>						KX383956
OTU 15 <i>Coccomyxa</i> sp.						KX383957
OTU 5 <i>Coccomyxa subellipsoidea</i>						KX383958
<i>Lichenomphalia oreades</i>	Humber Village, Mt. Ignoble*	<i>A Voitek</i>	13.09.30.av01	30 Sept. 2013	Lost in the mail [‡]	KY495790
<i>L. oreades</i>	Humber Village, Mt. Ignoble*	<i>A Voitek</i>	13.10.14.av01	14 Oct. 2013	Lost in the mail [‡]	KY495791

* CANADA, Newfoundland and Labrador; 48°59'57"N, 57°45'04"W.

** CANADA, Newfoundland and Labrador, Gros Morne National Park; 49°43'38"N, 57°48'30"W.

[†] Operational taxonomic unit (OTU) designated in analysis of sequences derived from sample substratum using Illumina MiSeq.

[‡] These two specimens were unicates sent by AV to the Field Museum for DNA extraction and sequencing and upon return to AV were lost in the mail. We included the sequences here, because the DNA extracts are available and the sequences match those of the other specimens of this taxon collected in the same area.

Lichenomphalia s. lat., the unaligned sequences were also subjected to assessment of alignment ambiguity through the Guidance web server (Penn *et al.* 2010a, b), and columns with less than 95% alignment reliability were removed. This was done for two datasets: the entire set of *Lichenomphalia* s. lat. sequences (49 operational taxonomic units (OTUs)), and a subset representing a supported clade including the target species with the addition of *L. grisella* (P. Karst.) Redhead *et al.*, *L. hudsoniana* (H. S. Jenn.) Redhead *et al.* (internal outgroup) and *L. velutina* (15 OTUs).

The final alignments were subjected to a maximum likelihood search using RAxML 8.2.0 (Stamatakis 2006) with the GTR+Gamma model, with parametric bootstrapping using 500 replicates. For algal sequences, BLAST searches in NCBI GenBank, alignments and phylogenetic analysis were conducted using MEGA6 (Tamura *et al.* 2013).

New collections used in this study were deposited in DAOM, FH, TU and UWO (fungaria codes as per Index Herbariorum, Thiers (2016)); raw Illumina sequence data were deposited in GenBank's Sequence Read Archive, Accession number SRP076466; assembled sequences were deposited in GenBank and some duplicates in UNITE (Table 2).

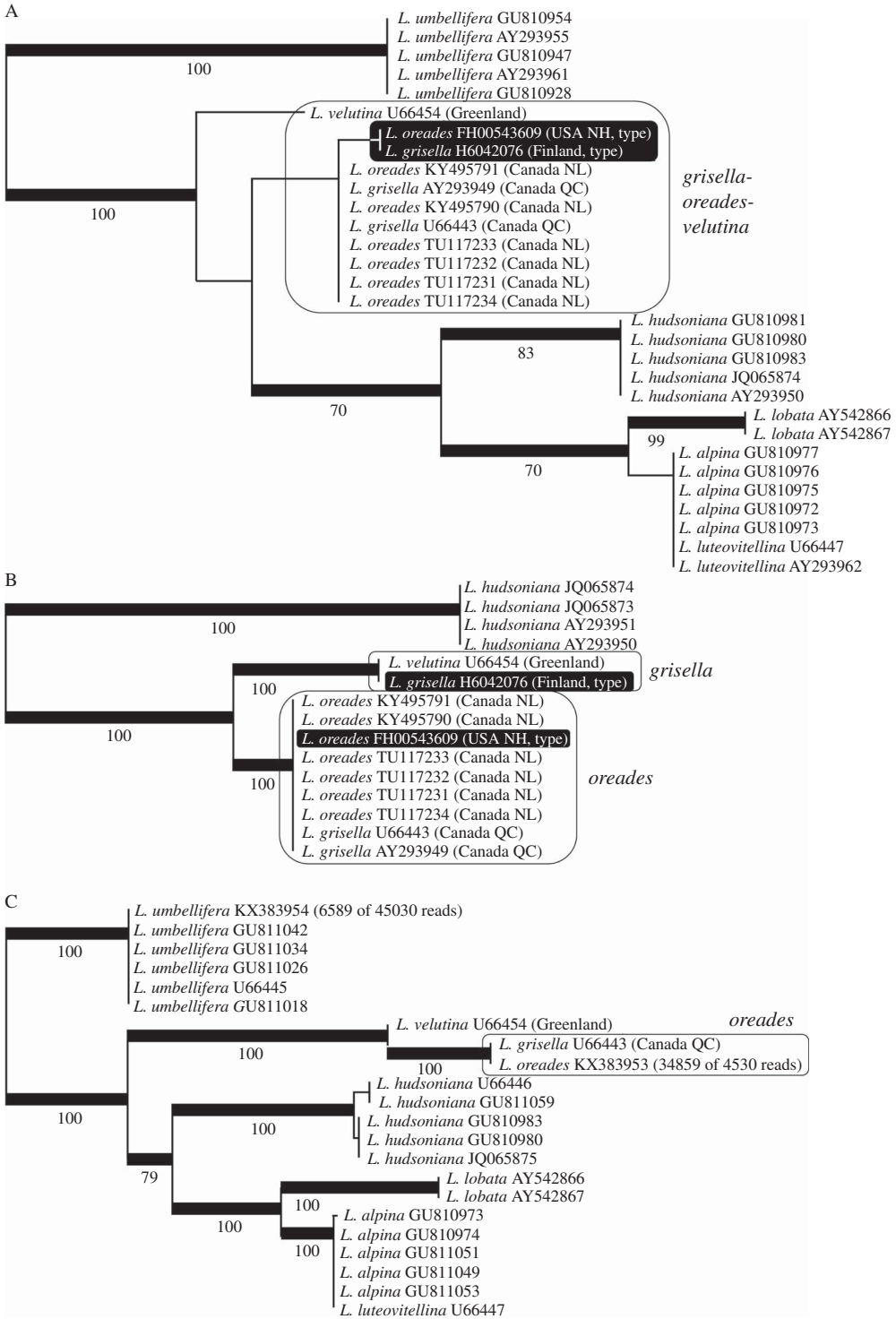
Results

Macroscopically, the separate descriptions of *Omphalina oreades* by Singer (1948) and Bigelow (1968) matched the Newfoundland collections. Microscopic examination of tissues and measurements of basidiospores and hymenial components revealed Singer's type of *O. oreades* to be identical to these collections. On sequencing, the Newfoundland collections matched two GenBank deposits labelled *Omphalina grisella* from Schefferville, Québec. This identification did not seem felicitous because since its description (Karsten 1889), the type material of *O. grisella* has been shown to have 2-spored basidia (confirmed by us) whereas our collections had four spores. Sequenced specimens originally identified as *O. grisella* (Lutzoni 930822-6 = U66443 and 930822-5 = AY293949) (Lutzoni 1997; Zoller & Lutzoni 2003) were clampless but had no hymenium remaining to confirm the number of sterigmata per basidium. However, contemporary collections also identified by Lutzoni as *O. grisella* (Lutzoni 930825-3 and 930816-9) had 4-spored basidia and were otherwise identical to our specimens of *O. oreades*. In contrast, specimens originally

identified as *O. velutina* (Lutzoni 930812-1 = U66454 and 930805-1) (Lutzoni 1997) were clampless, had 2-spored basidia, and were identical to the holotype of *O. grisella*.

Phylogenetically, based on analysis of the complete *Lichenomphalia* s. lat. dataset of ITS sequences with ambiguously aligned columns removed, the type of *O. oreades* clusters with the type of *L. grisella* (Fig. 1A); in the reduced dataset, it clusters with strong support with the specimens from Newfoundland, whereas the type of *L. grisella* clusters with a sequence labelled *L. velutina* from Greenland (Fig. 1B). This topological discrepancy came about because the removal of ambiguously aligned columns in the complete dataset left only 37% (313 of 847) of the original alignment columns. Among the removed columns were all columns that, while ambiguously aligned across all species of *Lichenomphalia*, are diagnostic to delimit species in the *L. oreades*-*L. grisella* clade. On the other hand, a single, non-ambiguous column was retained that exhibited variable polymorphism which produced two artefactual clusters in this clade. In contrast, for the reduced dataset with a much lower level of alignment ambiguity, 63% (478 of 761) of the original alignment columns remained, including the columns that contain the phylogenetic signal required to properly separate the two species. The most abundant fungal 26S rDNA sequence recovered by Illumina sequencing of community DNA from soil and the algal crust at the base of one fruiting body of *O. oreades* (13.11.07.av01) yielded a match to the same sequence in GenBank identified as *L. grisella*, from Schefferville, Quebec (Fig. 1C). Also present were sequences matching multiple GenBank accessions identified as *L. umbellifera*, as well as an unidentified species of *Sebacina* Tul. & C. Tul. (not shown).

The only publicly available sequence of putative *Arrhenia rustica*, deposited without phylogenetic analysis (Geml *et al.* 2012), did, indeed, fall within *Arrhenia*, not *Lichenomphalia*. As our phylogeny (Fig. 1) is limited to *Lichenomphalia*, this placement is not shown here. Because *O. oreades* falls into *Lichenomphalia* and is not conspecific with any known



species of that genus, its correct name should be *L. oreades*. Below we make the transfer, redescribe and illustrate the species, and explore the identity of its photobiont.

Unfortunately, the herbarium holding the type collection of *L. pararustica*, Musée et Jardins Botaniques Cantonaux (LAU), did not respond to our loan requests. Bigelow's collections of an unresolved 2-spored species from Washington State could not be located in NY, MICH, the online records of the Consortium of Pacific Northwest Herbaria (2016), or Mycology Collections Portal (2016).

***Lichenomphalia oreades* (Singer)
Voitk, Thorn & I. Saar comb. nov.**

Mycobank No.: MB 819711

Basionym: *Omphalina oreades* Singer, *Pap. Mich. Acad. Sci. Arts Lett.* 32: 123 (1948). (FH00543609—holotype!).

Homotypic synonym: *Clitocybe albimontana* H. E. Bigelow, in Bigelow & Barr, *Rhodora* 68: 178 (1966).

Synonyms: *Omphalina grisella* sensu Lutzoni (1997) non (P. Karst.) M. M. Moser, *Lichenomphalia grisella* sensu Redhead et al. (2002) non (P. Karst.) Redhead, Lutzoni, Moncalvo & Vilgalys.

(Fig. 2)

General. A small, brown, agaric, lichenized basidiomycete, with 4-spored basidia and a granular thallus, apparently confined to alpine, arctic and subarctic habitats, currently known only from the northern Appalachian range of North America.

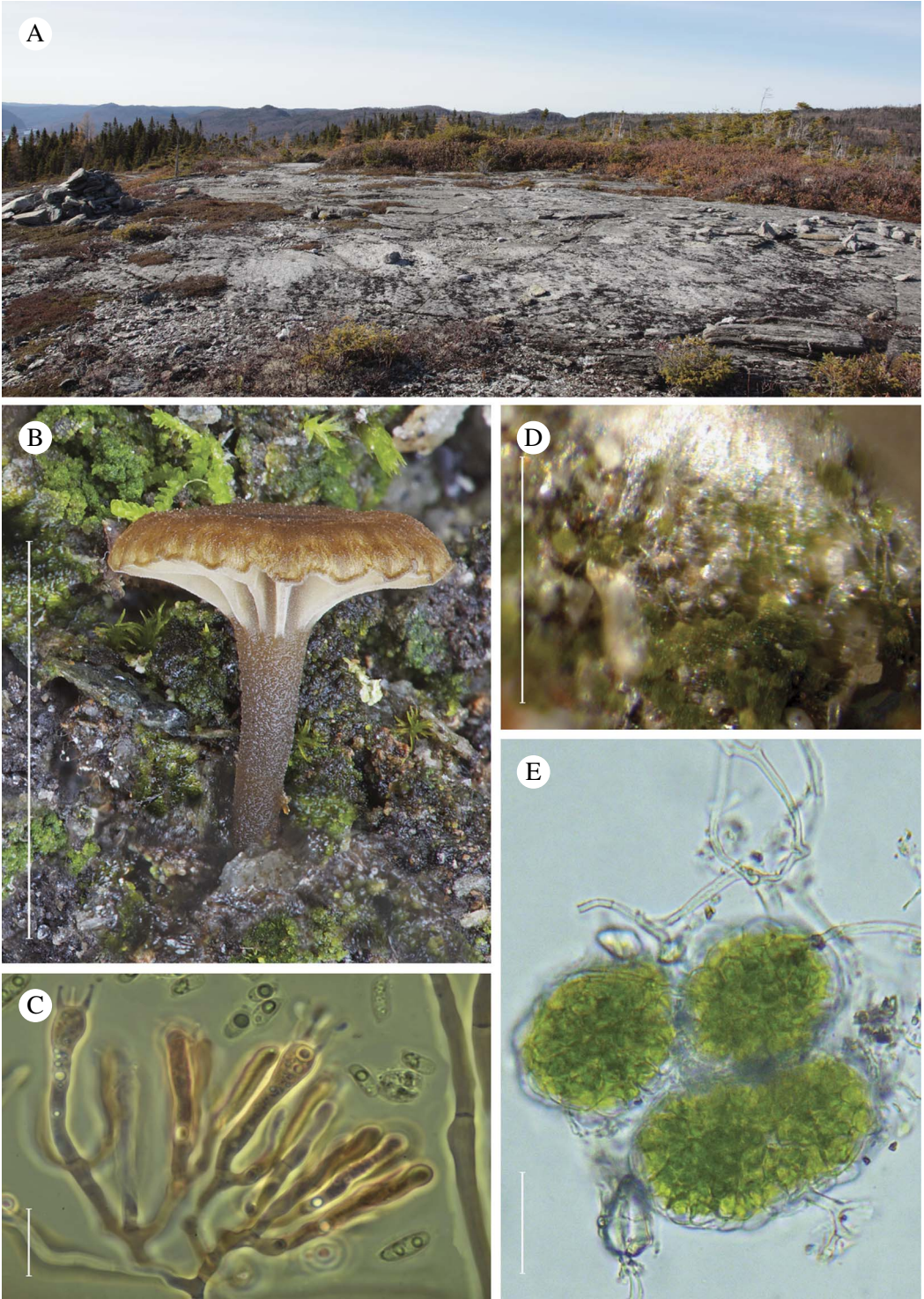
Fruiting body macroscopic characters. Stem length usually greater than cap diam. *Pileus* 2–9 mm wide, edges initially inrolled, then concave, plane at maturity with a shallow

central depression, radially ribbed over the gills, with increasingly crenate margin, no veil, surface smooth but minutely pebbly, hygrophanous, translucent when moist, beige to tan and dark brown radial bands alternate over the gills. *Lamellae* moderately to distantly spaced, moderately decurrent, some lamellulae, occasional forking, low cross-veining in advanced age, edges smooth, buff cream. *Stipe* 0.5–2.0 × 4–11 mm, even to slightly tapered downwards, no ring or ring zone, variably but markedly pruinose, becoming glabrous, base often slightly curved and covered by white tomentum intimately associated with the lichen thallus in soil, concolorous with cap. *Flesh* light, odour unremarkable. *Spore print* white.

Fruiting body microscopic characters. *Basidiospores* (10 collections, 17 fruit bodies, 274 spores) 6.7–8.5–10.1 × 2.9–4.3–5.8 μm, $Q_{ave} = 2.0$; 1–2 droplets, prominent apiculus; *basidia* 6–8 × 20–35 μm, 4 sterigmata (0–20%, 2 sterigmata), vacuolar; no cystidia; no clamp connections; *lamellar trama* of interwoven to sub-parallel, broad, thin-walled, branching, clampless hyphae; *pileipellis* a cutis, some incrustated brown pigment in its hyphae; *stipitipellis* of parallel, cylindrical hyphae 3–6 μm broad, with bundles of erect, tubular, thin-walled end cells projecting at a right angle up to 100 μm from stem; *true caulocystidia* absent.

Thallus morphology. The thallus is of the *Botrydina* type (Poelt & Oberwinkler 1964; Oberwinkler 2012), a granular mass of lichenized algal globules on the substratum around the base of the fungal fruiting body (Fig. 2B), attached to visible hyphae

FIG. 1. Maximum likelihood phylogenetic trees of the *Lichenomphalia* dataset, identified by GenBank number, and with location indicated for studied collections. Branches with bootstrap values $\geq 70\%$ are thickened and support values are given below the branch. Type specimens with black background. A, maximum likelihood tree for ITS, all OTU's, internally rooted in *L. umbellifera* inferred with 534 ambiguously aligned columns (out of 847) removed which also removed segments required for proper species identification within the *grisella-oreades-velutina* complex, resulting in false clustering. B, maximum likelihood tree for an ITS subset of the *Lichenomphalia* dataset after removal of 283 ambiguously aligned columns (out of 761) leaving a reduced dataset with most diagnostic columns retained and with the added phylogenetic signal for species identification, the 2-spored type of *Omphalia grisella* from Finland clusters in a clade with a collection identified as *Omphalina velutina*, from Greenland; the 4-spored Newfoundland collections, together with the 4-spored holotype of *O. oreades* from New Hampshire and two specimens from Québec identified as *Omphalia grisella*, form a clade of their own on a highly supported sister arm. C, maximum likelihood tree for the nuLSU dataset, including Illumina environmental sequence data for *L. oreades*.



at the base of the stem (Fig. 2D); globules 20–100 µm diam. Larger globules composed of conglomerates of smaller globules. Globules are enveloped by flat, adpressed hyphal cells, and are interconnected by a hyphal matrix (Fig. 2E); enveloping cells angular, 4–9 µm diam. and 2–4 µm thick. Interconnecting hyphae thin-walled, 1.5–3.0 µm in width, in agreement with the findings of Redhead & Kuyper (1987). Thread-like hyphal cells seem to communicate directly with a network of flattened cells coursing through the globules, forming septa that compartmentalize the algal aggregates into smaller units, permitting intimate contact between individual algal cells and the hyphal network.

Photobiont (Fig. 3). The algal sequences from soil and the algal crust at the base of one fruiting body of *L. oreades* (13.11.07.av01) are shown in the 26S rDNA phylogeny (Fig. 3A). The most abundant sequence matches GenBank collections identified as *Chloroidium ellipsoideum* (Gerneck) Darienko *et al.*, and the second matches *Coccomyxa subellipsoidea* E. Acton; a third, unidentified species of *Coccomyxa* Schmidle was also present in low numbers. Algal ITS rDNA sequences from another collection (15.10.25.av01) are shown in Fig. 3B; the two sequences recovered fall within a highly supported clade with members of an undetermined *Coccomyxa* sp.

Ecology, distribution, phenology. Alpine to subalpine and arctic habitat, solitary to gregarious, exposed in sandy cracks and crevasses of bare rock, or on turf, among lichen, moss, liverwort and ericaceous plants, but often away from vascular plants. *Lichenomphalia umbellifera*, *Clavaria argillacea* Pers., and *Arrhenia obscurata* (D. A. Reid) Redhead *et al.* were noted in the proximity of the Mt. Ignoble collections.

Reported in July and August on Mt. Washington in Newfoundland, the species appears most abundantly in September and October, rarely at the end of July. It recovers from freezing to sporulate during the thaw but seemingly is unable to revive after drying. To date, it is genetically confirmed only from the northern Appalachian range in North America.

Additional specimens examined (see Table 2 for specimens examined from which new sequences were generated for this study). **Canada:** Newfoundland and Labrador: Humber Village, Mt. Ignoble, 48°59'57"N, 57°45'04"W, 277 m, pioneer soil in rock cracks on barren mountaintop, 2012, *A. Voitek* 12.10.31.av02 (FH 00458214); *ibid.*, 2013, *A. Voitek* 13.07.24.av01 (DAOM 706318); *ibid.*, 2013, *A. Voitek* 13.08.31.av02 (DAOM 706322); *ibid.*, 2013, *A. Voitek* 13.10.09.av02 (DAOM 706319); *ibid.*, 2015, *A. Voitek* 15.09.08.av03 (FH 00458215); Gros Morne National Park, Big Level, 49°43'38"N, 57°48'30"W, 716 m, turf among ericaceous plants, 2015, *M. Voitek*, coll. Foray Newfoundland & Labrador (FNL) GM15A-280 (FH 00458216); *ibid.*, 2015, *M. Voitek*, coll. FNL GM15A-299 (DAOM 706319); *ibid.*, 2015, *A. Voitek*, coll. FNL GM15A-303 (DAOM 706319); *ibid.*, 2015, *M. Voitek*, coll. FNL GM15A-306 (FH 00458217). **Québec:** Saguenay Co., Schefferville, 54°8'N, 66°8'W (approx.), c. 521 m, on soil among young mosses, 1993, *F. Lutzoni* 930822-5 (DUKE 0011662, as *L. grisella*); *ibid.*, 1993 *F. Lutzoni* 930822-6 (DUKE 0011663, as *L. grisella*); north of Kuujuarapik, east of the mine, 55°3'N, 77°7'W (approx.), c. 15 m, terricolous in the large spaces between polygons of *Cladina stellaris*, 1993, *F. Lutzoni* 930825-3 (DUKE 0011665, as *L. grisella*).—**Greenland:** Nuuk, near the cement factory, 64°2'N, 51°7'W (approx.), c. 90 m, on disturbed soil on a small tussock, 1993, *F. Lutzoni* 930816-9 (DUKE 0011660, as *L. grisella*).

Lichenomphalia grisella (P. Karst.) Redhead *et al.* **Finland:** Etelä-Häme, Mustiala, near Myllyperä manor, 60°8'N, 23°8'E, 110 m (approx.), dry, gravelly places, 1889, *P. Karsten* 3244 (Holotype!), (H6042076).—**Greenland:** Disko Island: Arctic Station at Godhavn, 69°15'17"N, 53°31'10"W, 39 m (approx.), terrace soil, 1993, *F. Lutzoni* 930812-1 (DUKE 0011658, as *L. velutina*).—**Iceland:** Myvatn Lake, on soil at edge of sulphurous crater, 65°7'N, 16°9'W, 288 m (approx.), 1993, *F. Lutzoni* 930805-1 (DUKE 0011658, as *L. velutina*).

FIG. 2. *Lichenomphalia oreades*. A, habitat, Mt. Ignoble near Humber Village, Newfoundland (NL), mountain top below tree line (287 m a.s.l.) showing a mixture of bare rock and heath; *L. oreades* fruits in granitic silt found in cracks in the rock. B, *L. oreades* *in situ*, Mt. Ignoble, NL, 6 October 2012 (12.10.06.av01); note the copious thallus granules on the ground. C, basidia and spores, mounted in 10% ammonia and dyed with Congo Red SDS; note the lack of clamp connections, 4-spored basidia and elongated, vacuolar spores. D, base of stipe (light-coloured structure at upper end) of a rehydrated specimen; note green algal granules intimately associated with fine mycelia emanating from the stipe. E, detail of thallus structure; note tile-like appearance of enveloping mycelium, clear and flat at the periphery in cross-section. Scales: B = 10 mm; C = 10 µm; D = 100 mm; E = 20 µm.

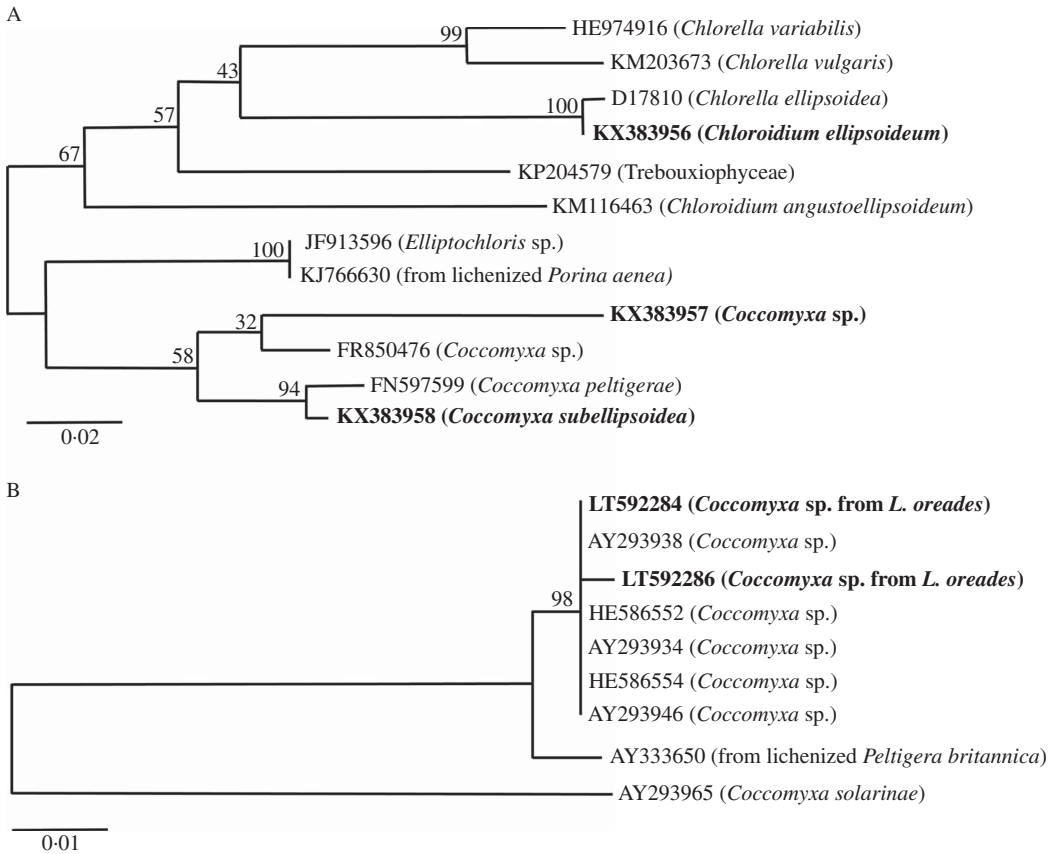


FIG. 3. Maximum likelihood phylogeny of green algal sequences obtained from soil samples. Samples from this study in bold. Bootstrap values from 300 resamplings shown above nodes. A, 26S rDNA, based on an alignment of 248 nucleotides with three indels (1–3 nt each) and LogL = -1010.57. B, ITS rDNA, based on an alignment of 500 nucleotides with eight indels (1–2 nt each) and LogL = -886.14. The basal position of the alga from *P. britannica* is consistent with the findings of Lohtander *et al.* (2003).

Discussion

Basidial sterigma count

All Newfoundland collections of *Lichenomphalia oreades* from two different sites, as well as the type collection from a third, were 4-spored, whereas the type of *L. grisella* was 2-spored, as was the collection from Greenland that fell in the *L. grisella* clade. The differences were statistically significant (Chi-squared; $P = 0.02$) but apply only to the populations studied, and do not exclude the possibility that other populations of either species have different sterigma counts. For example, Bigelow (1983) reported a 2-spored brown basidiolichen from

Rainier National Park in Washington State, USA, which he considered conspecific with *Clitocybe albimontana* (a synonym of *L. oreades*). Unfortunately, we were unable to locate the collection and are therefore unable to determine whether it is this species or another. Whether designated primarily 2- or 4-spored, most of the species in Table 1 are noted to have some basidia with the other count. In our 4-spored collections, the incidence of 2-spored basidia varied from 0–20%. Unpublished data from our other ongoing investigations include an example of 2-spored and 4-spored collections of a species of *Arrhenia* from different regions, occupying the same

phylogenetic clade. The limited samplings here do not exclude a similar possibility, and many specimens from several different regions are needed before we can conclude that the sterigma count is a globally reliable character for separating these species, which is work well beyond the scope of the present investigation.

Relationship to *Lichenomphalia grisella*

The only brown basidiolichen described before *Omphalina oreades* for which type material is available is *Omphalia grisella*, currently known as *Lichenomphalia grisella*. Our molecular type studies have demonstrated that the two are sister species, providing a sound foundation for application of the name *L. grisella*. They seem to be macroscopically indistinguishable but *L. grisella* has 2-spored basidia, whereas *L. oreades* has 4-spored basidia. The type locality of *L. grisella* is a gravelly clearing in the lowland boreal forest, whereas that of *L. oreades* is an arcto-alpine setting; the habitat differences may be circumstantial rather than differentiating characters.

Relationship to *Lichenomphalia velutina*

The only small, brown basidiolichen, described before either *Lichenomphalia grisella* or *L. oreades*, is *Omphalina velutina*, currently *L. velutina*. Although the protologue did not mention associated lichen granules, Quélet's (1886) illustration, which was selected as the lectotype by Jørgensen & Ryman (1989), fits well with either species. To date, the only sequences of *L. oreades* come from the Appalachian range of eastern North America. We were unable to obtain type material of the 4-spored European *L. pararustica* for examination or analysis, and there are no rDNA sequences of "*L. velutina*" from Europe. Therefore, we have no geographical evidence to suggest that *L. oreades* is a later synonym for *L. velutina*. Significantly, the protologue of *L. velutina* does not describe the number of sterigmata (Quélet 1886) and there is no other type material for such determination.

Parenthetically, the same paucity of information hinders synonymizing *L. velutina* with *L. grisella*. The current interpretation of *L. velutina* as a European 2-spored species (Elborne 2008, 2012) is based on recent collections rather than the type specimen. Its synonymization with *L. grisella* (Redhead & Kuyper 1987; Barrasa & Rico 2001) began prior to sequence-based phylogenies linked to basidial sterigma count. For example, as recently as 1995, Kuyper synonymized the alpine *Omphalina velutina* (which he considered a 2-spored species) with the lowland *Omphalia grisella* (a 2-spored species), the alpine *Omphalina oreades/Clitocybe albimontana* (a 4-spored species) and the alpine *Omphalina pararustica* (another 4-spored species). The type location for *L. velutina* in the European Alps is virtually identical to that of the 4-spored *L. pararustica*, and the type locality for the 4-spored *Omphalina subalpina* is also nearby, which makes it quite possible that all are conspecific 4-spored species. This alpine setting differs from the type locality of *L. grisella*, although that does not necessarily exclude *L. grisella* from consideration. Indeed, Favre (1955) identified as *Omphalia grisella* a 2-spored collection from the same region as Quélet's *Omphalina velutina*. Therefore, even if molecular studies provide a different interpretation to Favre's collection, he has documented that 2-spored brown basidiolichens are also native to the Alps, in addition to the previously named 4-spored ones. Much more study of material from the European Alps in addition to a much wider geographical sampling and, ideally, study of the types listed in Table 1 would be required before a decision about epitypifying *L. velutina* can be made; again, work which is well beyond the scope of this study. Since we lack information to circumscribe *L. velutina*, we recommend the use of *L. oreades* for collections phylogenetically conspecific with the species described by Singer, and *L. grisella* for those conspecific with that described by Karsten. The number of additional species within the complex and the placement of *L. velutina* and other taxa named in Table 1 remains to be determined.

Lichenomphalia oreades and its peers

Lichenomphalia oreades is the fourteenth accepted species in *Lichenomphalia*. It is the fourth agaricoid basidiolichen to be recorded for the Canadian province of Newfoundland and Labrador (NL). The others are *L. alpina* (Britzelm.) Redhead *et al.*, *L. hudsoniana* and *L. umbellifera* (Voitk 2006). *Lichenomphalia oreades* is distinguished from the others, whose fruiting bodies are partly or entirely yellow, by its brown coloration. *Lichenomphalia hudsoniana* is further distinguished by its discoid to leafy algal thallus (“*Coriscium* type”) and white stem, *L. alpina* by its entirely yellow colour (cap, gills and stem) and the most common species, *L. umbellifera*, by its tan to ochraceous cap (fading to whitish) and stipe with a purplish or brownish apex. Of the four, only *L. umbellifera* is found in lowland woods as well as on arcto-alpine turfmland. *Lichenomphalia oreades* can be distinguished from *L. grisella* (not yet known from North America with certainty) by its 4-spored basidia, and from similar brown *Arrhenia* species sharing the same habitat by the presence of a granular lichen thallus, as well as the absence of clamp connections. In addition to the four agaricoid lichenomphalias, we know of three clavarioid basidiolichens in the province: *Multiclavula corynoides* (Pers.) R. H. Petersen, *Multiclavula mucida* (Pers.) R. H. Petersen and *Multiclavula vernalis* (Schwein.) R. H. Petersen. In NL, the first is found on exposed, damp, roadside, sandy soil, the second on dead or rotten wood in lowland forests, and the last in moist arcto-alpine pioneer soils (Voitk 2017). To date, foliose, non-stipitate basidiolichens have not been identified in the province.

In addition to its turfy habitat, *L. oreades* is the only basidiolichen species we have observed fruiting in cracks of bare rock, as on Mt. Ignoble. *Lichenomphalia umbellifera*, which fruits only on turfy areas on Mt. Ignoble, can be found from May onwards. In turfy areas of Mt. Washington *L. oreades* has been reported in July, but in the cracks of bare rock on Mt. Ignoble its earliest appearance is the beginning of August. Mt. Ignoble is a burn bald (bare rock exposed

after a forest fire *c.* 85 years ago) below the tree line that has failed to recover because of loss of topsoil and constant exposure to wind, rain and sun. We suspect that the fruiting time is later in an otherwise milder climate because the exposed rock face, kept clear of insulating snow in the winter, does not provide the protection against frost that a turfy heathland might and consequently the mycelium dies each winter, making fruiting dependent on new growth of last year’s spores. Only a life strategy depending on a mutualistic association allows this species to survive in such a harsh setting.

Photobiont

We are unable to identify the algal partner of *L. oreades* with certainty. The most common alga recovered, *Chloroidium ellipsoideum*, has been described as the dominant member of the free-living algal crust, frequently recorded on roofing tiles, plaster, concrete, soil and rocks, in the absence of symbiotic basidiolichens (Darienکو *et al.* 2010). It has also been reported (as *Chlorella ellipsoidea*) by Lumbsch & Schmitt (2001) as the closest match to sequences derived from the inconspicuous, granulose lichens *Trapeliopsis granulosa* (Hoffm.) Lumbsch and *T. flexuosa* (Fr.) Coppins & P. James, which could be expected in the same habitat. However, because the photobiont was not cultured and the morphology not examined, this relationship remains unconfirmed. Therefore, despite its abundance and propinquity to *L. oreades* in our setting, *C. ellipsoideum* may not be the partner of *L. oreades* but either a free-living algal species on the thallus or surrounding soil, or the partner of an undetected species of *Trapeliopsis* Hertel & Gotth. Schneid. The second most frequent alga recovered, *Coccomyxa subellipsoidea*, has been identified as the symbiont of both *Lichenomphalia umbellifera* and *L. oreades*, the latter identified as *Omphalina grisella* (Zoller & Lutzoni 2003; Malavasi *et al.* 2016), and is most likely the true symbiont here. A number of *Lichenomphalia* species, particularly *L. oreades*, are frequently found fertile on quite barren rocky substrata and these might

explain records of “epilithic” isolates of *Coccomyxa subellipsoidea* that cluster with the remaining sequences that are shown as symbiotic (lichenized) in Figure 6B of Malavasi *et al.* (2016). Those “epilithic” isolates may well have been on barren substrata together with their *Lichenomphalia* mycobiont which went undetected because it was not fruiting.

Comment

Our aim was to identify the 4-spored species collected in NL. We can state confidently that it is *Lichenomphalia oreades* and that it differs from its sister species, *L. grisella*. Although somewhat less certain, we have a reasonable idea about the identity of its symbiont. In the course of our studies, we learned that from a molecular standpoint “*Omphalina velutina*” *sensu* Lutzoni (1997) is conspecific with the type of “*O. grisella*” while “*O. grisella*” *sensu* Lutzoni (1997) is conspecific with “*O. oreades*”; their molecular signatures were the basis for Redhead *et al.* (2002) to recognize two taxa. However, without considerably more study we can only speculate about the relationship of these two small, brown, agaricoid basidiolichens to other similar taxa in Table 1. We suspect that molecular type studies will show that there are not 11 such species globally but probably two to four, and that several of these species will prove to be conspecific with each other. We also suspect that species in the Northern Hemisphere have a circumpolar distribution, as reported by Geml *et al.* (2012), and are not limited to North America or Eurasia. Furthermore, we suspect that the sterigma count in this group will be shown to have taxonomic significance. Lastly, we suspect that after appropriate study *L. velutina* may either be declared an ambiguous name and formally proposed for rejection, or may be re-interpreted and arbitrarily epitypified as one of the known circumpolar species.

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