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New species and reports of Cuphophyllus from northern North America compared with related Eurasian species

Andrus Voitk, Irja Saar, D. Jean Lodge, David Boertmann, Shannon M. Berch, and Ellen Larsson

ABSTRACT
This study describes four gray or brown species of Cuphophyllus (Hygrophoraceae, Agaricales), two of them new species, restricted to arctic-alpine and northern boreal zones of North America, and relates them morphologically and phylogenetically using multigene and nuc rDNA internal transcribed spacer ITS1-5.8S-ITS (ITS barcode) analyses to their similar, known counterparts. Cuphophyllus cinerellus, epitypified here, is shown to be a pan-palaearctic species with sequence-confirmed collections from Fennoscandia and easternmost Asia. Occupying a similar habitat in the Nearctic is its sister species, the morphologically similar but novel C. esteriae, so far known only from eastern North America, including Greenland. Sister to the C. cinerellus–C. esteriae lineage, and known only from boreal raised Sphagnum bogs in Newfoundland, is a new medium-sized light cinereous brown species, C. lamarum. It has a yellow stipe but is phylogenetically distant from the yellow-stiped European C. flavipes and its North American sister species, Hygrophorus pseudopallidus. As cryptic speciation was discovered within C. flavipes, we lecto- and epitypify the name and transfer H. pseudopallidus to Cuphophyllus based on ITS analysis of the holotype. We also transfer the small European Hygrocybe comosa to Cuphophyllus based on morphology. Cuphophyllus hygrocyboides is reported from North America with the first sequence-confirmed collections from arctic-alpine British Columbia and Greenland. In addition, sequencing the holotype of C. subviolaceus identifies it as the sister species to the putative C. lacmus. Both species seem to have an intercontinental distribution. In total, we add new sequences to GenBank from 37 Cuphophyllus collections, including the holotypes of C. hygrocyboides and C. subviolaceus, the two new epitypes, and the two novel species.

INTRODUCTION
Cuphophyllus (Donk) Bon is a globally distributed genus of Hygrophoraceae Lotzy with species common in diverse ecosystems of the Northern and Southern Hemispheres. Most species in the genus have broad distribution ranges and occur from the nemoral to the arctic-alpine zone (Hesler and Smith 1963; Bon 1985; Borgen and Arnolds 2004; Boertmann 2010). Members of the genus have been documented from boreal, temperate, and tropical regions of North America (Hesler and Smith 1963), but the few arctic-alpine species, well known in Europe (Kühner 1977), have so far not been described in North American texts or monographs.

Cuphophyllus species produce mostly clitocyboid, rarely mycenoid, basidiomata with thick decurrent lamellae and a white spore print. Microscopically, they have interwoven (or rarely almost subregular) lamellar trama, with or without a regular or subregular central strand; smooth, hyaline, inamyloid basidiospores; very long basidia relative to spore length (usually 7–8, rarely 5–6 times spore length); and basal clamps on the basidia (Lodge et al. 2013). The typically interwoven lamellar trama, together with the long basidium-to-spore length ratios, forms the most reliable characters for separating Cuphophyllus from other white-spored agaric genera. In a systematic review of Hygrophoraceae, Lodge et al. (2013) noted that Cuphophyllus occupied an early-diverging phylogenetic position in the family, but the exact placement relative to Cantharocybe H.E. Bigelow & A.H. Sm. and Ampulloclitocybe Redhead, Lutzoni, Moncalvo & Vilgalys was not resolved.

Species of Cuphophyllus have a biotrophic mode of nutrition, but the nature of the fungus-plant association is largely unknown (Seitzman et al. 2011; Halbwachs...
et al. 2018). *Cuphophyllus virgineus* (Wulfen) Kovalenko was shown to be a root endophyte of *Plantago lanceolata* and vertically transmitted via seeds (Tello et al. 2014). Other plant associates have not been identified conclusively (Halbwachs et al. 2018).

This study focuses on gray and brown new species and new records of *Cuphophyllus* from arctic-alpine regions and *Sphagnum* bogs of northern regions of North America (Canada, Greenland) and compares them with closely related species from elsewhere in the Northern Hemisphere.

**MATERIALS AND METHODS**

*Selection of species.*—Investigating a new species of *Cuphophyllus* in the Canadian province of Newfoundland and Labrador (NL) led to the discovery that it is closely related to the arctic-alpine *C. cinerellus* (Kühner) Bon. This study was initially designed to investigate *C. cinerellus* in eastern Canada and Europe and define its relationship to the new species. During these investigations, we learned of another arctic-alpine species in western Canada (British Columbia) that resembled *C. hygrocyboides* (Kühner) Bon. We enlarged our study to include both arctic-alpine species and their relatives in Canada and compare these with similar species from North America and Europe. We provide a key to the gray, gray-brown, violet-brown, and orange-brown *Cuphophyllus* species in the Northern Hemisphere but have excluded *C. radiatus* (Arnolds) Bon because it is a taxonomic synonym of *C. flavipes* (Britzelm.) Bon (Boertmann 2010). We also excluded *Hygrocybe cinerea* (Pers.) P.D. Orton & Watling and *C. berkeleyi* (P.D. Orton & Watling) Bon because they are poorly circumscribed and *Hygrocybus pallidus* Peck because it is an ambiguous species (Hesler and Smith 1963; Boertmann 2010). Note also that the name *C. lacmus* (Schumach.) Bon, a species with a whitish to cream buff stipe base, was misapplied by Hesler and Smith (1963) to a European taxon with a yellow stipe base that is currently recognized as *C. flavipes* (Boertmann 2010).

*Collections and descriptions.*—TABLE 1 summarizes data of collections from which new sequences were generated for this study. Additional details about these collections can be found in SUPPLEMENTARY TABLE 1. Specimens were collected using routine field techniques and air dried with heat <20 C. Macroscopic descriptions are based on in situ specimens. Extreme (outlier) measurements are enclosed in parentheses and represent the uppermost and lowermost quartiles based on frequency.

Microscopic examinations were carried out on squash mounts in 2% KOH of both fresh and dried specimens, as well as thin sections of the pileipellis. Basidiospore sizes reported in the descriptions were from multiple observers. A separate single-observer spore size study was carried out on sequence-confirmed specimens of *C. cinerellus*, *C. esteriae*, sp. nov., and *C. lamarum*, sp. nov. Spore length measurements of the two macroscopically most similar species, *C. cinerellus* and *C. esteriae*, were analyzed statistically using the Student’s *t*-test and Fisher’s test with Yates’ correction. The latter test was also used to analyze the significance of differences in basidial length of these two species.

*Molecular methods.*—DNA extraction and sequencing was carried out at Tartu University (TU) and at the University of Gothenburg (GB). At TU, DNA was extracted with a lysis procedure in 10× Reaction Buffer B (0.8 M Tris-HCl, 0.2 M (NH₄)₂SO₄, 0.2% w/v Tween-20; Solis Biodyne, Tartu, Estonia), including proteinase K (0.5 mg/mL; Thermo Fisher Scientific, Waltham, Massachusetts) and incubated overnight at 56 C. The High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) was used for older specimens following the protocol of the manufacturer. Polymerase chain reaction (PCR) amplification of nuc rDNA internal transcribed spacer ITS1-5.8S-ITS (ITS barcode) was performed with primers ITSOF/LB-W or ITSOF/ITS4B (Tedersoo et al. 2008) using 5× HOT FIREPol Blend Master Mix Ready to Load (with 10 mM MgCl₂; Solis BioDyne) with 0.5 μM of each primer and 1–3 μL of DNA solution. Further PCR amplification and purification protocols follow those described in Saar and Voitk (2015). Sequences were generated by Macrogen Europe (Amsterdam, Netherlands) using primers ITS5 and ITS4 (White et al. 1990). Sequences were inspected and assembled using Sequencher 5.4 (Gene Codes, Ann Arbor, Michigan). DNA sequences, including collection data (TABLE 1), were submitted to GenBank and the PlutoF cloud database (Abarenkov et al. 2010), accessible through public Web output UNITE (http://unite.ut.ee; Kõljalg et al. 2013).

At GB, DNA extractions, PCR, and sequencing of the ITS and nuc rDNA 28S (28S) regions were performed as described in Larsson et al. (2018). For the most variable region of the second largest subunit of RNA polymerase II (*rpb2*), PCR was carried out with primers rRPB2-5F and rRPB2-7.1R as described in Lui et al. (1999) and Matheny (2005) and sequencing with...
primers fRPB2-5F, fRPB2-6F, and bRPB2-7R (Liu et al. 1999; Matheny 2005). Sequences were edited and assembled using Sequencher 5.1. For the multigene data set, sequences of ITS, 28S, and rpb2 of *Cuphophyllus* and *Ampulloclitocybe clavipes* (Pers.) Redhead, Lutzoni, Moncalvo & Vilgalys (outgroup) were taken from Lodge et al. (2013). These were aligned together with the newly generated ITS, 28S, and rpb2 sequences of species of this study. Alignments of the three-gene regions were performed separately using the L-INS-i strategy implemented in MAFFT 7.017 (Katoh and Standley 2013) before concatenation of the data. The final alignment was adjusted using AliView 1.17.1 (Larsson 2014). Most of the characters from the ITS1 and ITS2 regions were excluded from the analysis due to ambiguous alignment.

For inference of phylogenetic relationships of the multilocus data set, heuristic searches for the most parsimonious trees were performed using PAUP* under the maximum parsimony (MP) criterion (Swofford 2003). All transformations were considered unordered and equally weighted. Variable regions with ambiguous alignment were excluded, and gaps were treated as missing data. Heuristic searches with 1000 random-addition sequence replicates and tree bisection-reconnection (TBR) branch swapping were performed. Relative robustness of clades was assessed by the bootstrap (BS) method using 1000 heuristic search replicates with 100 random taxon addition sequence replicates and TBR branch swapping. BS values >70% were considered to be evidence of strong support.

A Bayesian inference (BI) analysis was also carried out in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2012) with a best-fit model of nucleotide evolution supplied separately for each gene region by MrModeltest 2.2 (Nylander 2004). The protein-coding rpb2 gene was not subjected to partitioning of the third base in each codon. Eight default-setting Metropolis-coupled Markov chain Monte Carlo (MCMCMC) chains were run for 5 million generations, with trees sampled every 2500 generations and an initial burn-in of 500 samples (25%). The MCMC analysis converged well in advance of the burn-in threshold, and chain mixing was found to be satisfactory as assessed by using Tracer 1.5 (Drummond et al. 2012). After discarding the
trees prior to the burn-in threshold, a 50% majority-rule consensus phylogram and posterior probabilities were computed from the remaining trees. Posterior probabilities >0.95 were considered to be evidence of strong support. The multilocus data alignment and MP tree files were submitted to TreeBASE (submission ID 25254).

The ITS data set was compiled based on results from the multilocus analysis and data presented in Lodge et al. (2013). In addition to the newly generated sequences, data of C. canescens and C. basidiosus were included. ITS sequence data of eight collections of the Cuphophyllus pratensis (Schaeff.) Bon complex were taken from GenBank and used for rooting of trees. The ITS of the target species in this study were compared with those in GenBank (Clark et al. 2016) and the UNITE database (Kõljalg et al. 2013) to seek additional available sequence data of our target and closely related taxa. Fourteen additional sequences of C. colemannianus (A. Bloxam) Bon, C. flavipes, C. lacmus, C. subviolaceus, and the holotype of H. pseudopallidus Hesler and A.H. Sm. were found and added to the data set. Alignment and phylogenetic analyses were performed as described above except for the BS analysis, which was performed using 1000 heuristic search replicates with 10 random taxon addition sequence replicates and TBR branch swapping, saving 100 trees in each replicate. The ITS alignment and MP tree files have been submitted to TreeBASE (submission ID 25255).

RESULTS

ITS sequence data of 37 specimens of Cuphophyllus cinerellus, C. flavipes, C. hygrocyboïdes, C. lacmus, and C. subviolaceus (Peck) Bon from Europe and North America were newly generated for this study, including the holotype of C. hygrocyboïdes (Kühner 69-319, G) and C. subviolaceus (NYSF-3099). The sequences have been deposited in GenBank (MK547061–MK547083, MK573930–MK573938, MK836089, MN326457, MN453872–MN453873; see TABLE 1) and also in the UNITE database (UDB numbers; see SUPPLEMENTARY TABLE 1). In addition, 12 28S and 10 rpb2 sequences were generated for one or two representative specimens of the species examined in this study to place them in a multigene analysis. These sequences have been deposited in GenBank (MN430909–MN430920, MN534321, MN556847–MN556855; see TABLE 1).

The concatenated multilocus data set consisted of 28 taxa and 3569 sites. After exclusion of ambiguously aligned positions, mainly from ITS1 and ITS2 regions, the second intron region of the rpb2, and the beginning and the end of the separate gene regions, 2606 sites remained for analysis. Of these, 2045 were constant, 132 were variable but parsimony uninformative, and 429 were parsimony informative. The MP analysis yielded six equally most parsimonious trees (length = 1031 steps, consistency index [CI] = 0.6811, and retention index [RI] = 0.8113). One of these trees is presented in FIG. 1. MP bootstrap analysis recovered C. esteriae (74%), C. lamarum (100%), and C. cinerellus (87%) in a strongly supported clade (100%) (FIG. 1). Sequences of the C. flavipes clade emerged as the sister clade to the C. esteriae–C. lamarum–C. cinerellus clade with strong support (100%). There was low support across the backbone of this tree, but the C. formicatus clade (100%) emerged as an early-divergent lineage in Cuphophyllus but without strong support. The other included taxa formed a weekly supported clade with no supported internal structure, except for the C. esteriae–C. lamarum–C. cinerellus and C. flavipes clades. The nucleotide evolution model K80+I+G was used for the 5.8S gene, GTR+I+G was used for the 28S gene, and K80+I+G was used for rpb2 in the BI analysis, which recovered the same topology and terminal clades as the MP bootstrap analysis.

The aligned ITS-only data set consisted of 61 sequences and 873 sites. After exclusion of ambiguously aligned regions, mainly from the beginning and the end of the data set, 838 positions remained for analysis. Of these, 422 were constant, 27 were variable but parsimony uninformative, and 389 were parsimony informative. The MP analysis yielded 432 equally most parsimonious trees (length = 900 steps, CI = 0.7244, and RI = 0.9574). One of these trees is presented in FIG. 2. Bootstrap analysis recovered 10 weakly to strongly supported clades within the ingroup corresponding to C. flavipes (99%), C. pseudopallidus (64%), C. cf. flavipes (72%), C. cinerellus (89%), C. esteriae (92%), C. lamarum (100%), C. colemannianus (100%), C. lacmus (93%), C. subviolaceus (83%), and C. hygrocyboïdes (100%). The calculated strict consensus tree recovered the same clades as the BS tree (not shown). The nucleotide evolution model GTR+G was used for the ITS1 spacer, JC was used for the 5.8S gene, and HKY+I+G was used for the ITS2 spacer in the BI analysis, which recovered the same major clades as in the BS analysis.

Sequence data of Cuphophyllus hygrocyboïdes emerged in a moderately supported clade together with C. colemannianus, C. lacmus, and C. subviolaceus. Sequences of a British Columbian collection of C. hygrocyboïdes occurred within a clade with the type of C. hygrocyboïdes from the French Alps and one Greenlandic and one Fennoscandian specimen, confirming an intercontinental distribution range for this species. Sequence data of C. lacmus and
**Figure 1.** A phylogram from the multigene MP analysis of combined 5.8S, 28S, and rpb2 sequence data of Cuphophyllus, showing the phylogenetic position of the arctic-alpine species treated in this study. Bootstrap values and Bayesian posterior probabilities are indicated on branches. Clades discussed in the text are indicated with bars and species epithets.

*C. subviolaceus* split in two supported clades, where the sequence of the holotype of *C. subviolaceus*, together with material cited as *Hygrophorus subviolaceus* in Hesler and Smith (1963; Matheny and Wolfenbarger, unpublished), fell within one of the clades, thereby fixing the clade name; the other is putatively named *C. lacmus* and represented by two collections from Denmark and British Columbia.

*Cuphophyllus cinerellus* formed a well-supported species-level lineage of several Fennoscandian collections and one collection from the Kamchatka Peninsula in the Russian Far East. Sister to it was the moderately supported new species, *C. esteriae*, with one collection from Greenland and eight from Newfoundland and Labrador. Sister to these two was the new species, *C. lamarum*, so far only known from *Sphagnum* bogs in the boreal region of Newfoundland and Labrador. Collections identified as the European species *C. flavipes* form two independent lineages (*C. flavipes* on one hand and *C. cf. flavipes* on the other), suggesting occurrence of cryptic speciation in this group. In addition, the ITS of the holotype of *C. canescens* split in two supported clades, where the sequence of the holotype of *C. subviolaceus*, together with material cited as *Hygrophorus subviolaceus* in Hesler and Smith (1963; Matheny and Wolfenbarger, unpublished), fell within one of the clades, thereby fixing the clade name; the other is putatively named *C. lacmus* and represented by two collections from Denmark and British Columbia.
Figure 2. Phylogram showing the phylogenetic relationships among arctic-alpine species of *Cuphophyllus* treated in this study based on ITS sequence data. Bootstrap values and Bayesian posterior probabilities are indicated on branches. Clades discussed in the text are indicated with bars and species epithets. Sequences originating from type specimens are marked in bold.
H. pseudopallidus from North America (HQ185706; Matheny and Wolfenbarger, unpublished) was nearly identical to the ITS (KF291044; Lodge et al. 2013) of a Japanese specimen originally identified as C. flavipes; these two sequences form a monophyletic group but with low support. However, the low support value in the analyses may be due to partly missing data and a few ambiguous sites. Based on the result, we transfer H. pseudopallidus, now known from both eastern North America and Japan, to Cuphophyllus.

Spore size ranges for C. cinerellus, C. esteriae, and C. lamarum showed such overlap that the values were judged to be unhelpful for identification (see TAXONOMY). Single-observer spore measurements showed that the range in spore size of C. esteriae was greater than for C. cinerellus, the latter falling entirely within the range of the former (SUPPLEMENTARY FIG. 1). The average spore size measurements tended to cluster for each species but in very close proximity with the average size of C. cinerellus, slightly greater than that of C. esteriae. Statistical analysis with the Student’s t-test showed that the observed differences in spore length were highly significant (P = 0.0001), and the difference between the occurrence of spores longer than 8 µm between the two species (19% for C. esteriae and 45% for C. cinerellus) was also significant (Fisher’s test with Yates’ correction, P = 0.0004). The difference in basidial size between the two species was much more obvious: most basidia of C. cinerellus were longer than 40 µm, whereas we did not find any basidia of C. esteriae longer than 40 µm; this difference was highly significant (Fisher’s test with Yates’ correction, P = 0.0045). A summary of morphological characters, habitat, and ecology used to identify and discriminate between the newly described species and similar species in Cuphophyllus is presented in SUPPLEMENTARY TABLE 2.

TAXONOMY

Cuphophyllus cinerellus (Kühner) Bon, Doc Mycol 14-(56):10. 1985. FIGS. 3A, 4A

MycoBank MBT389216


Pileus (5–)10–30(–35) mm wide, plano-convex with somewhat involute margin, applanate in age with depressed center, rarely slightly umbonate, often with crenate margin; surface when young transluently striate half way to the center, dry, first smooth, later often becoming finely scaly especially at the center; fuscous to gray-brown, pale grayish brown with age, hygrophanous, becoming pale grayish buff upon drying, often with a lilac tint. Lamellae short to deeply decurrent, arcuate to triangular, furcate, pale gray to grayish brown, sometimes with a slight lilac tint. Stipe 10–45 × 2–6 mm, more or less equal and/or tapering toward the base, dry, slightly fibrillose; pale gray to grayish buff, often with a yellow tone in the lower third, sometimes with a whitish removable covering. Context concolorous, without a distinct odor or taste.

Basidiospores (6.5–)7.5–8(–9.5) × 5–6(–6.5) µm, average 8.0 × 5.4 µm, smooth, broadly elliptic to elliptic, occasionally approaching subglobose or pip-shaped, thin-walled, hyaline, white in deposit, Q = 1.20–2.00, average 1.50 (101 spores, 3 basidiocarps, 3 collections). Basidia 32–54 × 6.5–13.5 µm, 4-spored, 2-spored observed in some collections, clavate, hyaline. Basidioles numerous 25–40 × 3–5 µm, cylindrical to narrowly clavate. Cystidia absent. Lamellar trama subregular to slightly interwoven, made up cylindrical cells 40–75 µm long × 7–20 µm wide. Pileipellis a dense cutis of narrowly cylindric hyphae 3–7 µm wide. Clamp connections in all tissues.

Ecology and distribution: In arctic-alpine zones, usually on nutrient poor soils, in dry lichen alpine heath with Empetrum nigrum, Vaccinium, and Betula nana, also in moist Sphagnum and Polytrichum bogs among Carex and Vaccinium. Presumably biotrophic with moss and/or some other paludine plant associate, occurring Jun–Sep, primarily known from northern Fennoscandia, also from the Kamchatka Peninsula.


Commentary: The Eurasian distribution, longer and wider basidia, and variation in ITS sequences distinguish it from the very similar C. esteriae described below. The small size of the basidiomes, the often finely scaly pileus, and arctic-alpine habitat distinguish it from the more brownish and lighter-colored C. lamarum and species in the C. lacmus and C. flavipes complexes. This species has more pleomorphic spores than the other species studied, although size range is closer than for others.
Cuphophyllus comosus (Bas & Arnolds) Lodge, Boertm. & E. Larss., comb. nov.  
Mycobank MB833466  
≡ Hygrocybe comosa Bas & Arnolds, Persoonia 19:251. 2007 (basionym).  
Commentary: Hygrocybe comosa resembles C. cinerellus and C. esteriae and is clearly by morphology a species that belongs in Cuphophyllus by virtue of the thick subdecurrent lamellae; hyaline, inamyloid, ovoid to ellipsoid basidiospores; basidium-to-basidiospore length ratios of 5.2–6.2; and irregular arrangement of subcylindrical hyphae in the lamellar trama (Bas and Arnolds 2007). Although this taxon was not specifically included in this study of arctic-alpine

Figure 4. Microscopic drawings of the species described. A. *Cuphophyllus cinerellus* (epitype, EL30-16). B. *Cuphophyllus esteriae* (TCAV-09). C. *Cuphophyllus lamarum* (holotype, 10.07.13.av01). D. *Cuphophyllus hygrocyboides* (Berch0283). E. *Cuphophyllus flavipes* (epitype, G/122-00). Bar = 10 µm; same scale for all drawings.
species, we have included it for comparison based on morphology in the key. We therefore here make the combination in Cuphophyllus.

*Cuphophyllus esteriae* Voitk, I. Saar & E. Larss., sp. nov.  
MycoBank MB833018


Etymology: esteriae (L.) honors Esteri Ohenoja, who has made a lasting contribution to the interest in and knowledge of arctic-alpine mycota in Newfoundland and Labrador. She was the first to collect and show us this species, thought at the time to be *C. cinerellus*. Diagnosis: A small Cuphophyllus from arctic-alpine barrens, very similar to *C. cinerellus* and less so to *C. lacmus* or *C. subviolaceus*. Differing from *C. cinerellus* by the Nearctic distribution and smaller basidium, and from *C. lacmus* and *C. subviolaceus* by the smaller basidiole size and habitat. The sequence divergence between *C. esteriae* and *C. cinerellus* is 4 substitutions and two 3 bp and one 1 bp insertion/deletion events in the ITS1 region and 3 substitutions and one 3 bp and two 1 bp insertion/deletion events in the ITS2 region.

Pileus 4–32 mm wide, domed, often developing a shallow central depression, edges curved down becoming plane, occasionally even shallowly funnel-shaped, margin somewhat crenulate with age; surface lubricious when wet, at times becoming scurfy with age, drying pale violaceous gray. Lamellae arced to decurved, grey to dark grayish brown with violet tones, drying pale violaceous gray. Lamellae arced to decurved, distance, not forked, usually only one tier of lamellae, increasing cross-veining in age; light to dark brownish violaceous gray, edges slightly lighter than the faces. Stipe 6–28 × 1.5–4.5 mm, cylindrical, usually straight; surface lightly fibrillose, glabrescent with age; light brownish gray but may be somewhat yellowish near the base. Context whitish, odor unremarkable.

Basidiospores (6–)6.5–7.5(–10.5) × (4–)5–6(–6.5) µm, average 7.6 × 5.3 µm, broadly elliptical, elliptical and occasionally subglobose or pip-shaped, hyaline, white in deposit, Q = 1.00–1.80, average 1.40 (190 spores, 9 basidiomes, 9 collections). Basidia 22–40 × 6–8 µm, 4-spored, 4-spored observed <10%, clavate, hyaline. Basidioles numerous. Cystidia absent. Lamellar trama subreglar to slightly interwoven, made up cylindrical cells 40–75 × 5–15 µm. Pileipellis a dense cutis with hyphae 4–8 µm wide, but in places upright or trichodermal in appearance. Clamp connections in all tissues.

Ecology and distribution: In heath or arctic-alpine pioneer soil, often with *Sphagnum* or other moss, in small groups of one to four separate basidiomes. Presumably biotrophic with mosses and/or some other paludine plant associates, Jun–Sep, so far known from eastern North America (Greenland, Newfoundland and Labrador).

Other specimens examined: CANADA. NEWFOUNDLAND AND LABRADOR: Labrador, Battle Island (52.273698°N, 55.585155°W; 32 m asl), in moist pioneer soil with mosses including *Sphagnum*, 18 Aug 2008, Andrus Voitk FNL Coll BH-096 (DAOM867479; TU117605; GenBank ITS = MK547064; UNITE = UDB034650); Labrador, Great Caribou Island (52.269026°N, 55.591865°W; 38 m asl), in barren heath among *Sphagnum*, 21 Aug 2008, M. Burzynski FNL Coll BH-097 (DAOM867480, TU117606; GenBank ITS = MK547065; UNITE = UDB034651); Labrador, Great Caribou Island (52.269026°N, 55.591865°W; 38 m asl), in barren heath on pioneer soil, 20 Aug 2018, A. Marceau FNL Coll CEMV-03 (DAOM867482, TU117660; GenBank ITS = MK547080; UNITE = UDB038329); ibid., in boggy area among *Sphagnum* on barren heath, 20 Aug 2018, M. Voitk FNL Coll CEMV-03 (DAOM867482, TU117660; GenBank ITS = MK547080; UNITE = UDB038329); ibid., in moss on barren heath, 21 Aug 2018, A. Voitk FNL Coll TCAV-01 (DAOM867483, TU117661); ibid., 43 m asl, in pioneer soil among *Rhamoctrum lanuginosum* on barren heath, 21 Aug 2018, A. Voitk FNL Coll CEMV-03 (DAOM867482, TU117660; GenBank ITS = MK547080; UNITE = UDB038330); ibid., in bog among *Sphagnum*, 21 Aug 2018, A. Voitk FNL Coll CEMV-03 (DAOM867485, TU117665; GenBank ITS = MK547082; UNITE = UDB038331).

Commentary: The shorter and narrower basidia, ITS sequence differences, and the North American distribution distinguish it from the very similar *C. cinerellus*. *Cuphophyllus esteriae* differs from *C. lamarum* by the arctic-alpine habitat, smaller basidiomes, darker pileus, more violet coloration, absent or only occasional pale yellow tint on the stipe base, somewhat longer spores, and ITS sequence data. Although found at times in *Sphagnum*, the habitat is entirely different from that of *C. lamarum* (described below). Like *C. cinerellus*, *C. esteriae* is one of the early macrofungi to colonize pioneer soil, appearing after lichens and bryophytes (including *Sphagnum* in wetter areas), together with some *Vaccinium* and other ericaceous plants.


≡ Hygrophorus flavipes Britzel., Hymenomyc Södbayern 810. 1891.  

≡ Camarophyllus flavipes (Britzel.) Clémençon, Beih Z Mykol 4:55. 1982.  


Pileus 18–39 mm wide, convex, smooth, lubricous (with adhered plant material), pale silver gray to brownish violet, translucently striate, hygrophanous. Lamellae deeply decurrent, arcuate, intervenose, pale gray. Stipe 60–100 × 35–55 mm, cylindrical, somewhat twisted, longitudinally fibrillose, pale gray, marbled and with yellow base. Context pale gray fibrous. Odor and taste, none. 

Basidiospores smooth, to broadly elliptic, a few pip-shaped, (5–)6–7.5–8 × (4.5–)5–6(–7) μm, average 6.8 × 5.8 μm (a single spore was 8 μm long and probably derived from a 2-spored basidium), Q = 1.02–1.47, average Q = 1.20 (10 spores, 1 basidiocarp, 1 collection). Basidia 40–58 × 6.5–8 μm, 4-spored, only very few 2-spored observed. Basidioles numerous, club-shaped, a few capitulate, 19–36 × 3–4 μm. Pileipellis a thin (20–40 μm) ixocutis from a small and young basidiomes, a zebra pattern (incrustad pigment) on cells in subpellis not seen. Lamellar trama interwoven with cylindrical and branched noninflated cells, 3–7.5 μm wide. Lamellar edge fertile. Clamps abundant in all tissues.  


Commentary: This is a widespread but rare European species, so far not known from North America. The yellow stipe base, fibrillose stipe, and lack of an odor differentiate it from the similar *C. lacmus*. Differs from the North American *C. lamarum* by the darker pileus, lamellae, stipe and context, habitat, and ITS sequence data. Differs from *C. cinerellus* by the size, smooth lubricous pileus surface, yellow stem base, and ITS sequence data.  


Pileus 10–30 mm wide, pulvinate when young, flattened to somewhat depressed with age, dry on older fruiting bodies, slightly sticky when young; surface hygrophanous, edge variably translucently striate, bright to dark orange-brown. Lamellae decurrent, subdistant, rather thick, intervenose, pale orange-brown. Stipe 20–40 × 2–4 mm, subcylindrical or slightly tapering downward, straight or slightly sinuous, dry and longitudinally fibrillose at least in age, pale orange-brown (paler than pileus). Context concolorous, odor unremarkable. 

Basidiospores (6–)7–9(–10) × 4.5–5.5 μm, smooth, ellipsoid to ovoid, some with slightly concave side in side view, white in deposit (15 spores, 2 basidiomata 1 collection). Basidia 50–72 × 5.5–7 μm, 4-spored or occasionally 2-spored. Basidioles numerous, cylindrical to narrowly clavate, 30–58 × 2.5–4 μm. Pileipellis a cutis, hyphae 1.5–2.5 μm wide. Stipitipellis a cutis with hyphae 2–3 μm broad. Lamellar trama tightly interwoven and difficult to split in squash preparations. Clamps abundant in all tissues.  

Ecology and distribution: On ground in alpine zone with moss and *Salix stolonifera* Coville, single or in groups of separate basidiomes. Likely circummaleolar, known from the Alps, the Pyrenees, northern Fennoscandia, Greenland (Borgen and Boertmann 2008; Boertmann 2010), and British Columbia. Specimens examined: CANADA. BRITISH COLUMBIA: North of Smithers, near Motase Peak in the Scintine Range of the Skeena Mountains (56°8′ 46.9″N, 127°12′33.0″W; 1805 m asl), 26 Jul 2016, S. Berch 0283 (DAVFP). FRANCE. SAVOIE: Parc National de la Vanoise, Région de Pralognan, 14 Sep 1969, R. Kühner 69-319 (HOLOTYPE G00052005). SWEDEN. TORNE LAPPMARK: Jukkasjärvi, Latnjavagge, 22 Aug 2013, E. Larsson EL118-15 (GB-0156992).
Commentary: To our knowledge, *C. hygrocyboides* is the only such species of *Cuphophyllus* in the underexplored Canadian arctic-alpine habitat. In Fennoscandia, the multi-specific *C. pratenis* has also been identified in high arctic regions. The bright orange-brown color and smaller size distinguish *C. hygrocyboides* from the darker brown *C. colemanianus*, which also has a northern distribution but is not found in exposed barrens. This is the first sequenced-confirmed report of *C. hygrocyboides* from North America.

*Cuphophyllus lamarum* Voitk, Boertm. & I. Saar, sp. nov. Figs. 3D–E, 4C
MycoBank MB833019


Etymology: *lamarum* (L.) of the bogs, in reference to the preferred habitat.

Diagnosis: A spagnichionic North American *Cuphophyllus* with a light brown pileus and varying amounts of violaceous hues, yellow stipe, larger than *C. esteriae*. Sequence divergence between *C. esteriae* and *C. lamarum* is 23 substitutions and one 45 bp, one 9 bp, one 5 bp, one 3 bp, and two 1 bp insertion/deletion events in the ITS1 region and 20 substitutions, one 11 bp, and one 3 bp, and two 1 bp insertion/deletion events in the ITS2 region.

Pileus 20–55 mm wide, round, domed, becoming almost plane with age, edge somewhat in-rolled well into maturity; surface dry to minimally lubricious, opaque, but indistinctly translucently striate at edge, hygrophanous; light to moderately tan, with light grayish violet hues, more yellowish over disc and gray-violet over periphery, drying cream-colored. Lamellae decurrent, distant, not forked, with two tiers of lamellulae and increasing cross-veining in age, whitish gray. Stipe 25–70 × 4–10 mm, uniform, even, often slightly curved; surface dry, light straw-colored, with increasing yellowish tones toward base. Flesh cream-colored; staining orange in injured areas in one of three specimens; smell unremarkable. Basidiospores (5.5–)6.5–7(–9) × (4.5–)5–6.5(–7) µm, smooth, subglobose, broadly elliptic, to pip-shaped, occasionally cylindrical, hyaline, white in deposit, Q = (1.10–)1.20–1.40(–1.80), average 1.30 (70 spores, 4 basidiocarps, 3 collections). Basidia 38–78 × 6–9 µm, 4-spored, clavate. Basidioles simple and numerous. Cystidia absent but tramal cells protruding at lamella edges may resemble cystidia (observed in one basidiome). Lamellar trama interwoven, cells 2.5–8 µm wide. Pileipellis a cutis with a few gelatinized hyphae on top, hyphae 1.5–5.5 µm wide. Clamp connections in all tissues.

Ecology and distribution: In small groups on raised *Sphagnum* bogs in western Newfoundland, presumably biotrophic with *Sphagnum* and/or some other paludine photobiont, Jul, at least a month before other species of the genus in this region.

Specimens examined: CANADA. NEWFOUNDLAND AND LABRADOR: Route 401 to Howley (49.212693°N, 57.072087°W; 98 m asl), in raised *Sphagnum* bog, 28 Jul 2010, A. Voitk 10.07.28.av01 (DAOM867487, TU117562); ibid., in raised bog within 500 m of the former, 20 Jul 2018, T. Booth 18.07.20.av01 (DAOM867488, TU117667).

Commentary: Other species of *Cuphophyllus* (e.g., *C. esteriae, C. cinerellus*) may be found with or near *Sphagnum*, in addition to other bryophytes, but to our knowledge *C. lamarum* is the only species of *Cuphophyllus* seemingly limited to raised *Sphagnum* bogs. However, because our experience is limited to three collections, this conclusion may be premature. Similarly, we have documented an apparent orange staining reaction to injury in one of three collections (Fig. 3E). Without more experience, the significance of this finding is unclear. *Cuphophyllus lamarum* differs from its close relatives, *C. cinerellus and C. esteriae*, by the unique habitat, boreal distribution, larger basidiome size, tannish pileus color, more yellow at the base of the stipe, statistically shorter spores, and ITS sequence data. The species is similar in size and shape to species in the *C. flavipes* and *C. lacmus-subviolaceus* complexes. The yellow stipe separates it from the latter complex, and the unique habitat in raised *Sphagnum* bogs from both. The North American distribution separates it from Eurasian species. The European *C. flavipes* also has a yellow stipe base but differs from *C. lamarum* by a lubricious pileus surface and darker pileus, lamellae, and stipe. In addition to the habitat and yellow stipe base, *C. lamarum* differs from the North American *C. subviolaceus* common in the same region by a more tan to brown (rather than gray) pileus and earlier phenology.


Commentary: Phylogenetic analyses including the ITS sequence data of the holotype of *H. pseudopallidus* show
that it belongs in Cuphophyllus and is closely related to C. flavipes. This is also incongruence with morphology. We therefore make the combination to Cuphophyllus.

KEY TO GRAY AND BROWN NORTHERN SPECIES OF CUPHOPHYLLUS FROM NORTH AMERICA AND EURASIA

1. Basidiomes predominantly warm brown, bay, tan, cream, or orangish brown ........................................... 2
1'. Basidiomes predominantly gray or violaceous . 2
2. Stipe yellow at least near the base . . C. lamarum
2'. Stipe not yellow .......................................................... 3
3. Pileus <30 mm wide, brownish orange, orange; arctic-alpine ................................................. C. hygrocyboides
3'. Pileus often >30 mm wide, brown; primarily in temperate climate zone but also known from arctic-alpine habitats ............. C. colemanianus
4. Stipe base yellow .......................................................... C. flavipes complex (including C. pseudopallidus)
4'. Stipe base colored like rest of stem, not yellow 5
5. Pileus often >30 mm wide, smooth, moist to greasy ...................... C. lacmus-subviolaceus complex
5'. Pileus often <30 mm wide, dry or viscid, smooth or scaly toward the center ......................................................... 6
6. Pileus conico-convex when young, retaining a papilla with erect squamules ............ C. comosus
6'. Pileus convex to plano-convex when young, scales minute on disc .................................................. 7
7. In Eurasia, most basidia >40 µm long .................. C. cinerellus
7'. In North America, all basidia ≤40 µm long ....... C. esteriae

DISCUSSION

Cuphophyllus comprises a few cosmopolitan species interspersed among several parochial species, similar to several other fungal genera, e.g., Morchella Dill. ex Pers. (Richard et al. 2015) and Cantharellus Adans. ex Fr. (Buyck et al. 2014). The arctic-alpine species we review show examples of each: C. hygrocyboides forms a single clade with only minor intraspecific variation, containing specimens from western North America, Greenland, Fennoscandia, and central Europe. By contrast, the clade containing C. cinerellus seems to be limited to Eurasia. Macroscopically similar collections from eastern North America (Greenland, Newfoundland, and continental Labrador) form a homogenous, distinct, and well-supported sister clade of C. cinerellus, the new species C. esteriae.

We did not expect to find two cryptic species identified by the same name and initially debated among ourselves about the validity of or need for considering C. cinerellus and C. esteriae as separate species, in view of their macroscopic similarity and phylogenetic proximity. More critical reviews produced convincing evidence to support their separation: (i) they occupy different biogeographic regions separated by the Bering Strait on one side and the North Atlantic Ocean on the other, both significant barriers to genetic mixing; (ii) their molecular divergence has high statistical support, evidence that this is due to factors other than chance, whereas (iii) within their clades they (particularly C. esteriae) are homogeneous; (iv) the minimum distance to the closest UNITE “species hypothesis” (SH) is a 2.5% difference in basepair sequences, a value generally accepted as a valid indicator of species difference (https://unite.ut.ee); (v) the statistically (if not practically) significant difference in spore size; and (vi) the significant and readily perceptible difference in the size of their basidia.

When introducing new taxa, the onus is on the author to ensure that the species has not been described before. We have separated the new North American species C. esteriae from the similar European C. cinerellus. Cuphophyllus comosus, a rare small species as yet not sequenced, found once among mosses in peat bogs of the French Juras (Bas and Arnolds 2007), shares cinereous coloring with C. esteriae and C. cinerellus but differs from these species by the European distribution, smaller basidiome size, more slender habitus, greater stipe/pileus ratio, distinctly papillate pileus, large conical squamules on the papilla, and strongly interwoven lamellar trama.

The vast majority of species of the current known Cuphophyllus species in North America were described in a monograph on Hygrophorus sensu lato by Hesler and Smith (1963). Several of their species (e.g., H. rainierensis, H. nordmanensis, H. burgdorfsensis) were described from higher elevations than our highest, C. esteriae, but none came from similar barren arctic-alpine habitats with pioneer soil. In addition, all the northern and/or higher-altitude species documented by Hesler and Smith (1963) were recorded from forests or grasslands, habitats where C. esteriae does not occur. Moreover, the three mentioned species also deviate by a viscid pileus. Cuphophyllus esteriae is distinguished from C. recurvatus (Peck) Leubeuf, described from upstate New York and also reported from Florida, by the arctic-alpine habitat, grayish in contrast to brown pileus, absence of gelatinized hyphae in the pileipellis, and narrower spores than those measured in Peck’s type of C. recurvatus by S. A. Cantrell (unpublished data; 7–11 × 5.5–8 µm). In addition to characters listed in the
individual descriptions, both C. esteriae and C. cinerellus differ from the other species treated in this work, as well as other members of the C. lacmus-subviolaceus and C. flavipes complexes, by the arctic-alpine distribution, smaller basidiome size, and ITS sequences. The lack of previous North American descriptions of species of Cuphophyllus from truly arctic-alpine habitats also ensures that C. hygrocyboides was not described on this continent before its 1977 description in Europe.

We have separated the new species C. lamarum from species in the C. flavipes complex (C. flavipes and an undescribed species in Europe and C. pseudopallidus from North America and Japan). Apart from the occasional pale yellow stipe of the smaller arctic-alpine species discussed above, there are no other species of Cuphophyllus with a yellow stipe described from Europe, although the epithet cinereus has been misapplied to such species on occasion (e.g., Helser and Smith 1963). Apart from C. pseudopallidus, Helser and Smith (1963) described no other species of the current Cuphophyllus with a yellow stipe, and we are not aware of other such species descriptions from North America. Also, the lack of other species of Cuphophyllus from raised Sphagnnum bogs confirms that C. lamarum has not been described before under another name.

Most North American species are not well known. The few descriptions available, other than the protologs, often conflict with the latter or seem otherwise misapplied. A full discussion of them requires a modern review including molecular studies of the C. lacmus-subviolaceus complex and other appropriate groups, something outside the scope of this study. Regarding C. pseudopallidus and C. subviolaceus, we thus key out these to a species complex. Where comparisons are made or these species used in the keys, they are interpreted from their respective protologs.

Our key to the gray, brown, purplish brown, and orange-brown Cuphophyllus species in the Northern Hemisphere excludes C. radiatus, as it is a taxonomic synonym of C. flavipes (Boertmann 2010). We also exclude treatment of Hygrocybe cinerea (Pers.) P.D. Orton & Watling, C. berkeleyi (P.D. Orton & Watling) Bon, and Hygrophorus pallidus Peck because they are not well-circumscribed species (Helser and Smith 1963; Boertmann 2010).

We resolve the relationship of C. subviolaceus as a sister species to the putative C. lacmus by generating ITS sequence data from the type specimen of C. subviolaceus and thereby fix the name. The two species have been regarded as conspecific by many authors (e.g., Boertmann 2010), but we show that there are two species and that C. subviolaceus is phylogenetically distinct from the putative C. lacmus. Based on FIG. 2, we can see that both C. subviolaceus and the putative C. lacmus have an intercontinental distribution range. Because of the morphological similarities and confusion in naming, a more extensive sampling is needed to better characterize the species in this complex; however, this was not a target for this study.

Before our report, there were no public DNA deposits of either C. cinerellus or C. hygrocyboides available. Although our study contributes to the knowledge of these arctic-alpine species, it constitutes a preliminary report only. Because these regions are undersurveyed in North America, knowledge of the distribution of the species on this continent remains poor. We hope that this report may provide both background and stimulus for other workers to pursue the mycota of our vast northern regions. Tangentially, we also note that sequences taken from GenBank to construct our tree and our unpublished data revealed that likely other epithets also harbor cryptic species. Although pursuing these species is beyond the scope of the present study, our preliminary enquiries suggest that this is a fruitful area for future study.

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### SUPPLEMENTAL TABLE 2. TABULAR KEY TO DESCRIBED AND SELECTED SIMILAR \textit{CUPHOPHYLLUS} SPECIES

<table>
<thead>
<tr>
<th>Character</th>
<th>Species</th>
<th>cinerellus</th>
<th>esteriae</th>
<th>lamarum</th>
<th>flavipes/ pseudopallidus</th>
<th>lacmus/ subviolaceus</th>
<th>hygrocyboides</th>
<th>colemanianus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap</td>
<td>diameter &gt; 30 mm$^1$</td>
<td>rare</td>
<td>rare</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>never</td>
<td>common</td>
</tr>
<tr>
<td>color</td>
<td>cinereous violet-gray</td>
<td>cinereous violet-gray</td>
<td>light grayish brown</td>
<td>cinereous violet-gray</td>
<td>cinereous violet-gray</td>
<td>bay to light brown</td>
<td>bay brown</td>
<td></td>
</tr>
<tr>
<td>Lamella edge (micro)</td>
<td>smooth</td>
<td>smooth</td>
<td>protruding hyphae</td>
<td>smooth</td>
<td>smooth</td>
<td>smooth</td>
<td>smooth</td>
<td></td>
</tr>
<tr>
<td>Stipe color</td>
<td>whitish light gray</td>
<td>base occasionally light yellow</td>
<td>yellowish, incr. toward base</td>
<td>yellow, incr. toward base</td>
<td>white to light grayish</td>
<td>brownish</td>
<td>white</td>
<td></td>
</tr>
<tr>
<td>Flesh staining$^2$</td>
<td>none</td>
<td>none</td>
<td>orange$^2$</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>Spore size (μm)</td>
<td>range</td>
<td>6.5–9.5 × 4.0–6.5</td>
<td>6.0–10.5 × 4.0–6.5</td>
<td>5.5–9.0 × 4.0–6.5</td>
<td>5.5–8.0 × 5.0–6.5$^1$</td>
<td>6.0–8.5 × 4.0–6.5$^4$</td>
<td>6.0–8.5 × 4–5$^4$</td>
<td>6.0–10.0 × 4.0–7.0$^4$</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>8.0 × 5.4</td>
<td>7.5 × 5.3</td>
<td>7.0 × 5.2</td>
<td>6.8 × 5.8</td>
<td>not calculated</td>
<td>7.6 × 4.4$^5$</td>
<td>not calculated</td>
</tr>
<tr>
<td>Basidium size (μm)</td>
<td>range</td>
<td>32–54 × 6.5–13.5</td>
<td>22–40 × 6.0–8.0</td>
<td>38–78 × 6.0–9.0</td>
<td>40–58 × 6.5–8.0</td>
<td>39–58 × 6.0–7.0$^4$</td>
<td>50–72 × 5.5–7.0</td>
<td>49–60 × 7.0–8.0$^4$</td>
</tr>
<tr>
<td>Habitat</td>
<td>general</td>
<td>arctic-alpine</td>
<td>arctic-alpine</td>
<td>boreal</td>
<td>boreal to alpine</td>
<td>boreal to alpine</td>
<td>arctic-alpine</td>
<td>boreal to arctic-alpine</td>
</tr>
<tr>
<td>specific</td>
<td>tundra, bog, pioneer soil</td>
<td>tundra, bog, pioneer soil</td>
<td>bog</td>
<td>woodland, grassland</td>
<td>woodland, grassland</td>
<td>tundra, bog, pioneer soil</td>
<td>woodland, grassland</td>
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<tr>
<td>------------------------------</td>
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<td>---------------------</td>
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</tr>
<tr>
<td>Photobiont associate&lt;sup&gt;6&lt;/sup&gt;</td>
<td>bryophytes</td>
<td>bryophytes</td>
<td><em>Sphagnum</em></td>
<td>herbaceous, coniferous, ericaceous</td>
<td>herbaceous, coniferous, ericaceous</td>
<td>herbaceous bryophytes</td>
<td>herbaceous, ericaceous?</td>
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</tr>
<tr>
<td>Fruiting time</td>
<td>n/a</td>
<td>Jul-Sep</td>
<td>Jul</td>
<td>n/a</td>
<td>Aug-Oct</td>
<td>n/a</td>
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<tr>
<td>Newfoundland</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Distribution</td>
<td>Eurasia</td>
<td>North America&lt;sup&gt;7&lt;/sup&gt;</td>
<td>so far, only known from NL</td>
<td>Eurasia</td>
<td>Eurasia, North America</td>
<td>Fennoscandia, central Europe</td>
<td>North America</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>majority of large (fully developed) caps  
<sup>2</sup>experience limited to three collections; staining seen with only one  
<sup>3</sup>epitype measurements  
<sup>4</sup>quoted from Boertmann 2010 (including extremes)  
<sup>5</sup>Canadian collection values only  
<sup>6</sup>assumed from adjacent plants  
<sup>7</sup>so far only confirmed from eastern Newfoundland
<table>
<thead>
<tr>
<th>GB or (UNITE nr) Collecting nr</th>
<th>DATE</th>
<th>Male Herbarium</th>
<th>Copy herbarium</th>
<th>NAME</th>
<th>COUNTRY/Province</th>
<th>LOCATION</th>
<th>Lat N</th>
<th>Long E</th>
<th>elev (m a s l)</th>
<th>COLLECTOR</th>
<th>HABITAT</th>
<th>SUBSTRATE</th>
<th>POTENTIAL PHOTOBIOTS</th>
<th>COMMENTS</th>
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<td>DACHM87478</td>
<td>T2117663</td>
<td>Cephalopodium extirpale</td>
<td>CANADA, NL</td>
<td>L'Anse Amour, Labrador</td>
<td>51.4612</td>
<td>-58.8587</td>
<td>21</td>
<td>Maria Votik</td>
<td>big</td>
<td>Sphagnum</td>
<td>moss, Sphagnum</td>
<td>HCOTYPE</td>
<td>Y</td>
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<tr>
<td>MK547054 LD0030450</td>
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<td>DACHM87478</td>
<td>T2117665</td>
<td>Cephalopodium extirpale</td>
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<td>Battle Island, Labrador</td>
<td>52.2700</td>
<td>-55.5844</td>
<td>32</td>
<td>Andre Voith</td>
<td>heath, prey pond edges</td>
<td>peat</td>
<td>HCOTYPE</td>
<td>Y</td>
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<td>Cephalopodium extirpale</td>
<td>CANADA, NL</td>
<td>Great Gulfou Island, Labrador</td>
<td>52.7733</td>
<td>-55.9921</td>
<td>38</td>
<td>Michael Burgess</td>
<td>heath, barrens</td>
<td>Sphagnum</td>
<td>HCOTYPE</td>
<td>Y</td>
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<td>Great Gulfou Island, Labrador</td>
<td>52.7733</td>
<td>-55.9921</td>
<td>38</td>
<td>Anne Macree</td>
<td>heath, barrens</td>
<td>pioneer soil</td>
<td>HCOTYPE</td>
<td>Y</td>
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<td>Great Gulfou Island, Labrador</td>
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Supplemental Figure 1. Comparison of spore measurements of sequence-identified specimens of *C. cinerellus* (range in smaller inner oval and average measurements represented by triangles) and *C. esteriae* (range in larger outer oval and average measurements represented by circles). Length on x-axis and width on y-axis are measured in µm. Note that the complete overlap of one species by the other make measurements unhelpful for identification, but that average measurements from individual specimens tend to cluster somewhat apart. Difference in spore size was statistically highly significant (*P* = 0.0001).