# New North American Records for *Ascocoryne turficola* (Ascomycota: Helotiales)

Britt A. Bunyard,[1*] Zheng Wang,[2] David Malloch,[3] Stephen Clayden,[3] and Andrus Voitk[4]

**Abstract**

*Ascocoryne turficola* (Boud.) Korf, one of the rarest fungi in Europe, is reported from North America. This interesting ascomycete was collected in 2007 from boggy localities in eastern and western Newfoundland. Morphological descriptions and ecological notes for this mysterious fungus, as well as its placement within the Helotiales, are discussed.

KEY WORDS: Ascomycetes, *Coryne*, mushrooms, Newfoundland fungi, *Sarcoleotia*

## Introduction

This communication reports the occurrence of *Ascocoryne turficola* (Boud.) Korf in three disparate areas of Newfoundland over one season. *Ascocoryne turficola* is an uncommon fungus, recorded from the Far East and Europe. In Europe it is considered one of their rarest fungal species (Stasinska and Sotek, 2004; Watling et al., 2001; Dennis, 1968) with few recorded sightings; in most European countries where it has been recorded, it is listed in that country's Red Book. *Ascocoryne turficola* was first noted in France where Boudier (1905) published the first description of the species. It later was recorded in Switzerland (Favre, 1948) and Great Britain (Dennis, 1968; 1971). The first reports of *A. turficola* from Norway and Sweden came in 1972 (Eckblad and Torkelsen, 1972), the Falkland and Shetland Islands in 2001 (Watling et al., 2001), and Poland in 2004 (Stasinska and Sotek, 2004). The fungus also is known from a handful of localities in Finland, Denmark, Germany, and Czech Republic, but on the whole, the precise geographical distribution has not been delineated (Stasinska and Sotek, 2004). Furthermore, little about the ecology of *A. turficola* is understood. The purpose of this paper is to confirm the presence of *A. turficola* in North America as well as to present morphological and ecological notes for this enigmatic ascomycete fungus.

[1]W184 N12633 Fond du Lac Ave., Germantown, WI 53022, USA; [2]Department of Ecology & Evolutionary Biology, Yale University, Osborne Memorial Labs, 165 Prospect Street, PO Box 208106, New Haven, CT 06520, USA; [3]New Brunswick Museum, Natural Sciences Department, 277 Douglas Ave., Saint John, New Brunswick E2K 1E5, Canada; [4]Foray Newfoundland and Labrador, PO Box 2312, RR #1, Corner Brook, NL A2H 2N2, Canada. *Corresponding author, bbunyard@wi.rr.com.

## History

The organism was first described by Boudier (1905) and was possibly not found again until it was collected in 1935 by Jules Favre (Favre, 1948). Favre described five collections he had made between 1935 and 1944 in the same Jura Mountains explored by Boudier. Four of these resembled Boudier's *C. turficola,* while one collection differed somewhat; this he accepted as natural variation within the general spectrum of a species.

Groves and Wilson (1967) reviewed the genus *Coryne* and concluded that the generic name was typified by an asexual form (an anamorph or "imperfect state") and was therefore not available for sexually reproducing ("perfect") fungi like *C. turficola.* For the sexual forms of fungi described in the genus *Coryne,* they proposed a new genus, *Ascocoryne,* to which they transferred *Coryne sarcoides* as the type species (Groves and Wilson, 1967). Subsequent name transfers and changes by future workers were to follow.

Dennis (1971) cited some British material collected in *Sphagnum* sp. that he identified as *C. turficola* (Dennis, 1968). Dennis's drawings differed somewhat from those of Boudier and Favre and his specimens had smaller spores and had no apothecial gelatinous layer. Because his specimens lacked the gelatinous tissue characteristic of the genus *Ascocoryne*, he transferred the species to *Sarcoleotia,* a genus characterized by the lack of gelatinous tissue (Dennis, 1971).

The same year saw publication of a different transfer for this ascomycete. In the course of preparing a key to the genera of Discomycetes Korf (1971a), made several transfers and rearrangements to reconcile groupings with current knowledge. Because Boudier had described an ascomycete sporophore with gelatinous tissue, and because the new *Ascocoryne* was a genus characterized by a gelatinous layer below the hymenium, Korf transferred *C. turficola* to the genus *Ascocoryne*.

## North American Records

At the time of our finds, we were not aware of previous records of this mushroom in North America. Exhaustive searches of books, periodicals, the Web and extensive personal enquiries yielded four encounters with this taxon in North America. Torbjørn Borgen collected it ca. 1980 near Paamiut, Greenland, among *Sphagnum* sp. in a bog based on gneiss (Borgen, personal communication). Bessette, Bessette, and Fischer include it in the key of cup and

Figure 1. The first specimen of *Ascocoryne turficola* collected in Newfoundland on June 15, 2007, near Junction Pond among *Sphagnum squarrosum*.

saucer fungi in their book of eastern North American mushrooms (Bessette et al., 1997). Alan Bessette said this was done because of a record of this taxon in the University of Maine Herbarium (personal communication). Indeed, Maine records show that it was collected among *Sphagnum* sp., on September 29, 1984, from Marble Fen in Maine by Richard Homola, who identified it as *Ascocoryne turficola* (Boudier) Dennis. This identification has been confirmed by one of the authors (DM) from photographs of the specimen made available by the University of Maine Herbarium. The last encounter is a record in the Cornell Plant Pathology Herbarium, collected in 1975 growing on *Ulmus americana* and identified as *Ascocoryne turficola* (Boudier) Korf by Richard Korf. In light of it reportedly growing on elm, Korf suspects it may well be a misidentification, but was unable to check as the specimen was unavailable (personal communication).

## Materials and Methods

The first Newfoundland collection, a single immature sporocarp (Fig. 1), was collected by one of the authors (SC) on June 15, 2007, near Junction Pond in a peaty depression among *Sphagnum squarrosum* in an area apparently opened up by blowdowns on a ridge of balsam fir (*Abies balsamea*), yellow birch (*Betula alleghaniensis*), mountain white birch (*B. cordifolia*), and mountain woodfern (*Dryopteris campyloptera*), and small adjoining or intermixed boggy areas with scattered balsam fir and black spruce (*Picea mariana*). The specimen was identified by another of the authors (DM) as *S. turficola*. The second Newfoundland collection, two sporocarps (Fig. 2), were found by Maria Voitk on September 21, 2007, on the Western Brook Pond trail, at the edge of a large raised bog (Fig. 3), in *Sphagnum capillifolium* var. *tenellum*, among a moderate growth of sedges (*Carex* sp.). This site is about 400 km removed from the site of the first discovery (Fig. 4). The trail was made with crushed limestone and represents an alkaline zone in an otherwise

Figure 2. The second Newfoundland collection was found on September 21, 2007, along the Western Brook Pond trail among *Sphagnum capillifolium* var. *tenellum* and sedges (*Carex* sp.).

acid bog. The specimen was identified (by DM) from a photograph as *S. turficola*. The third Newfoundland collection consists of one collection by one of the authors (BB) and two collections by Kenny

Figure 3. Boggy habitat of Western Brook Pond trail.


Figure 4. Map of Newfoundland, Canada. Red dots are 2007 collection sites for *Ascocoryne turficola* and are approximately 400 km apart.

Tuach at FORAY NEWFOUNDLAND & LABRADOR on September 29, 2007 (Fig. 5), in a bog near the administrative buildings of the Brother Brennan Environmental Education Centre, a site about 50 km from the first discovery. All were confirmed to be *S. turficola*. One of Tuach's collections is from the same population as BB's; the other may be the same or from a site a few meters away. The fourth Newfoundland collection was taken from the same site by one of the authors (AV) on October 11, 2007 (Fig. 6). For more information, see supplemental information online at www.fungimag.com.

### DNA Sequence Analysis

The last three Newfoundland collections underwent molecular DNA analysis to examine the genetic similarity to European isolates. For generating DNA sequence data of two ribosomal DNA region, the ITS and LSU-rDNA, two primer pairs ITS1F/4 (White et al., 1990) and LR0R/5 (Vilgalys and Hester, 1990) were used (see Table 1).

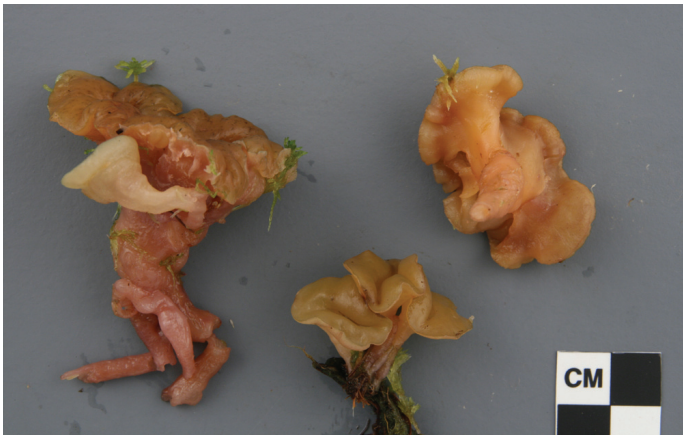


Figure 5. The third Newfoundland collection was made on September 29, 2007, in a bog near the administrative buildings of the Brother Brennan Environmental Education Centre, a site about 50 km from the first discovery.

Figure 6. The fourth Newfoundland collection was taken on October 11, 2007, from the same location as that in Fig. 5.

**Table 1.** PCR primers sequences used for the amplification of *A. turficola* rDNA (after White et al., 1990) and the genomic region amplified (LSU = gene for large subunit of the ribosome; ITS = internal transcribed spacer region of DNA between two ribosomal RNA genes).

| **Primers** | **Sequences 5′–3′** | Gene |
|---|---|---|
| LR0R | ACCCGCTGAACTTAAGC | LSU-rDNA |
| LR5 | TCCTGAGGGAAACTTCG | LSU-rDNA |
| ITS1 | TCCGTAGGTGAACCTGCGG | ITS |
| ITS4 | TCCTCCGCTTATTGATATGC | ITS |
| **Primers** | **Sequences 5′-3′** | Gene |
| LR0R | ACCCGCTGAACTTAAGC | LSU-rDNA |
| LR5 | TCCTGAGGGAAACTTCG | LSU-rDNA |
| ITS1 | TCCGTAGGTGAACCTGCGG | ITS |
| ITS4 | TCCTCCGCTTATTGATATGC | ITS |

### Results

***Ascocoryne turficola* (Boudier) Korf** 1971. *Phytologia* 21(4): 201–7.

=*Sarcoleotia turficola* [Boudier] Dennis 1971. *Kew Bulletin* 25: 335–74.

=*Ombrophila turficola* (Boud.) Svrcek 1957. *Ceská Mykologie* 11: 32–41.

=*Coryne turficola* Boudier 1905. *Bullelltin de Societe Mycologie de France* 21: 71.

**Apothecia** 0.6–2.5 mm wide, round, flat, translucent, with sharp and distinct margin, becoming irregular and wavy in age, gelatinous, pale olive green in color, becoming vinaceous, brown

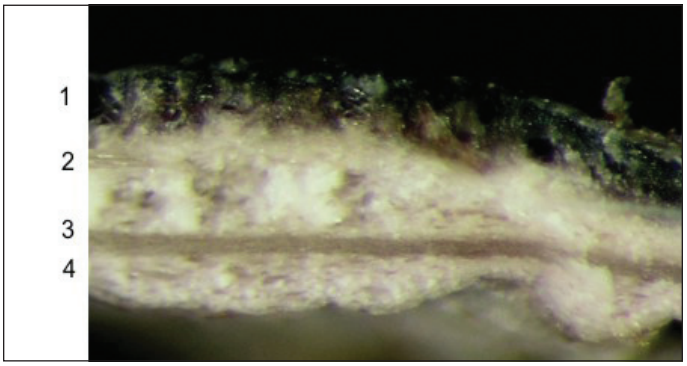


Figure 7. Dry cross-section of *A. turficola* (from collection 4). The numbers refer to the various layers: 1) hymenium (asci and paraphyses), 2) inner medullary excipulum, 3) outer medullary excipulum, and 4 ectal excipulum. The medullary excipulum is composed of two layers, the outer gelatinous zone (Layer 3) and the inner non-gelatinous zone (Layer 2) that is similar in appearance to the ectal excipulum.

or tan in advanced age. **Stipe** 20–45 mm long, 4–8 mm wide at apex, straight, becoming curved and wavy in advanced age, tapering evenly to 1 mm at base, translucent, gelatinous, smooth, whitish to tan in young specimens, darkening to vinaceous or brown in age, gentle vinaceous shading of variable intensity toward the base, may reach to near the cap in some specimens. **Medullary excipulum** composed of two layers: an inner zone of non-gelatinized hyphae (Fig. 7-2) and an outer zone of gelatinized hyphae that is compact and firm when dried but becomes considerably expanded when fresh or remoistened (Fig. 7-3). Outer zone composed to parallel to interwoven hyphae with cells 17–45 X 4–9 µm and with weakly to strongly gelatinized walls, with hyphae becoming irregularly arched and coiled when moistened, with some highly refractile non-gelatinized hyphae intermixed with the gelatinous ones (Fig. 8). Inner zone similar in gross appearance to the ectal excipulum, composed of non-gelatinized interwoven hyphae

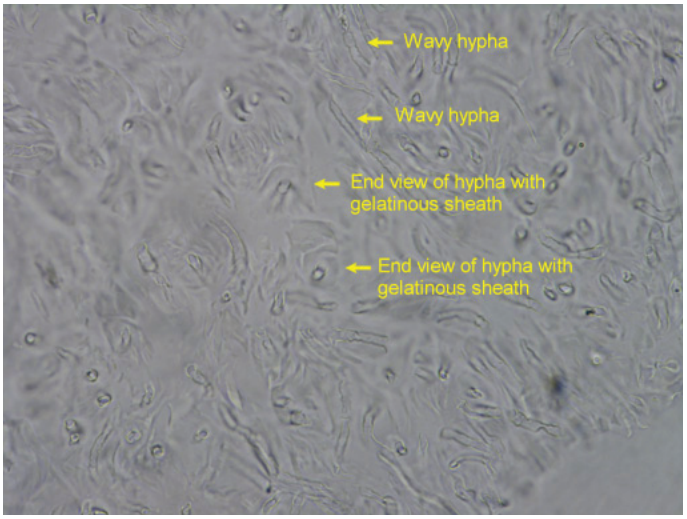


Figure 8. Hyphae with gelatinized walls are shown. Note the appearance of irregularly arched and coiled hyphae.

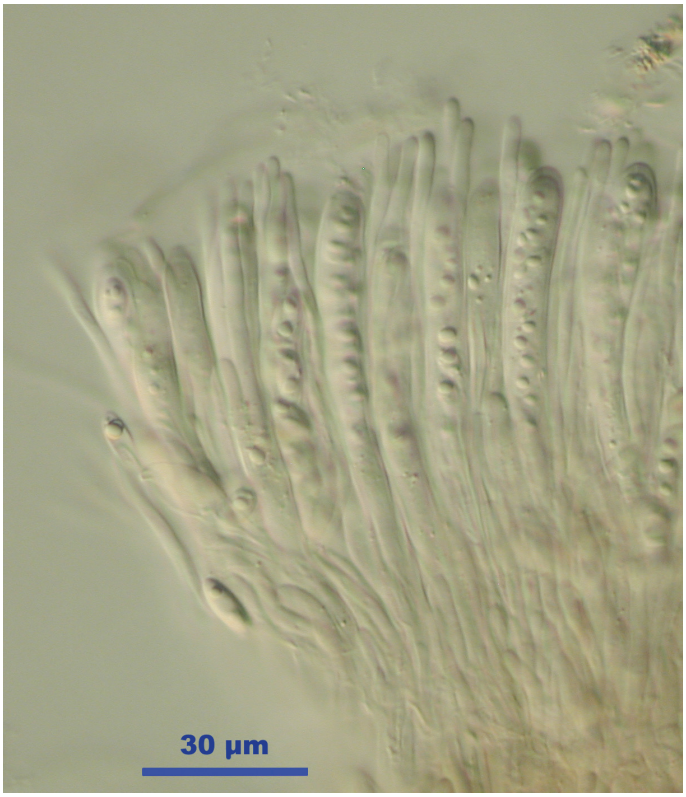


Figure 9. Asci and paraphyses of SPC WP440, collection 1 from 15 June 2007. The asci are immature but the paraphyses show up well.

with cells measuring 20–35 X 4–10 µm. **Ectal excipulum** 250–300 µm thick composed of parallel to slightly interwoven hyphae with individual cells nearly cylindrical to ellipsoidal and measuring 20–60 X 5–18 µm in diameter (Fig. 7-4). **Subhymenium** brown in Melzer's Solution, composed of a very compact mass of interwoven hyphae 2.5–4.8 µm in diameter, with some hyphae having dextrinoid material on their walls. **Paraphyses** cylindrical, mostly unbranched, septate, 1.4–2.4 µm in diameter (Fig. 9). **Asci** arising from croziers, cylindrical to very narrowly clavate, thin-walled, with a prominent apical cushion containing an elongated pore, with the lumen often prominently depressed below the apical cushion, 90–120 X 6.4–10.0 µm. Apical apparatus of the asci consisting of a cylindrical pore 2.4–3.2 µm long embedded in a thickened matrix, with pore strongly amyloid in Melzer's and Lugol's solution with or without 5% KOH pretreatment (Fig. 10). **Ascospores** uniseriate or more rarely sub-uniseriate in the ascus, ellipsoidal at first but later becoming more tapered toward the poles and finally fusoid, hyaline, smooth, unicellular at first but often with one or even two septa at maturity or upon germination, 8.9–16.4 X 3.6–5.6 µm (median(n=47)=12.86 ± 2.35 X 4.63 ± 0.42), Q=1.92–3.80 (Median(n=47)=2.78 ± 0.47) (Table 2), germinating by means of germ tubes produced at one or both poles (Fig. 11). **Habitat:** Singly or often clusters fused at the base, invariably among a variety of *Sphagnum* spp., usually intimately associated with stem of *Sphagnum* sp. at base, in open bog or edge of forest, with a variety of *Carex* spp.

Parsimony analyses using ITS sequence data in PAUP* 4.0b (Swofford, 1999) produced a 100% match with unidentified environmental samples of A. turficola from Europe, showing only minor geographic variation (Fig. 12). They fit completely within the Ascocoryne clade. When compared with previously analyzed taxa, the Newfoundland specimens are grouped among the Ascocoryne clade within the Helotiales (see supplemental materials online).

## Discussion

*Ascocoryne turficola* is an enigmatic ascomycete. A number of questions regarding this species persist. For example, little is known of its ecology, besides the fact that this species has been found exclusively in peat bogs. All other known species of *Ascocoryne* (including the cosmopolitan *A. sarcoides*, see Fig. 13) grow on wood. Therefore, it is possible that *A. turficola* finds subsphagnic wood bits in the peat to rot (all specimens demonstrate a very long tapering, "rootlike" base). Stasinska and Sotek (2004) reported it from the stems of sedges (*Carex rostrata*) in a *Sphagnum* bog in Poland. Other bog inhabiting fungi are known to be wood rotters. For example, *Pholiota astragalina* and *P. scamba* (Fig. 14) both grow in deep moss but have a connection with small pieces of wood (personal observations). Of course it is also possible that *A. turficola* does not utilize wood at all. Environmental (soil) samples from the United Kingdom yielded base sequences identified as those of *A. turficola* (Wang et al., 2006b), suggesting that substrates other than wood may have been colonized. In fact, even though *A. sarcoides* and other species of *Ascocoryne* occur exclusively on wood, there may not be any experimental evidence to show that they actually digest its components. Future collectors of this species would benefit our understanding of this fungus by searching for evidence of its nutritional activities.

Yet another question regarding *A. turficola* is whether it is a recent arrival to North America or has it merely gone unnoticed for a long time. Likewise, its occurrence in Europe is widespread although it is rarely recorded. Watling et al. (2001) determined that because of its conspicuous shape and color it would be more frequently reported, were it not so scarce. However, Eckblad and Torkelsen (1972) argued that it is likely to be fairly common but often overlooked as mycologists rarely investigate bogs.

If *A. turficola* has been in the New World for a long time, was it present before the breakup of Gondwanaland or is it the result of long distance dispersal? Little is known about the distribution

**Table 2.** Ascospore sizes of *A. turficola* of collections reported previously from Europe and herein from Newfoundland. Median measurements and standard deviations are shown, where available; Q value is the ratio of mean length to width of elongate spores, thus spores ellipsoidal or ovoid when Q < 2, and spores ellipsoidal-oblong, fusoid, cylindrical, etc. when Q > 2.

| Collection | Length µm (Mean; std deviation) | Width µm (Mean; std deviation) | Q µm (Mean; std deviation) |
|---|---|---|---|
| Boudier[1] | 14.8 – 18.8 | 4.1 – 4.9 | |
| Favre[2] | 15 – 18 | 5.6 – 6.6 | |
| Cheype[3] | 15 – 18 | 5.5 – 6.0 | |
| Moreau[4] | 11.5 – 17.5 | 4.2 – 5.5 | |
| Dennis[5] | 12 – 14 | 4 – 5 | |
| Kirk[6] | 10.8 – 16.4 | 4.0 – 5.2 | |
| Dissing[7] | 15 – 20 | 3.6 – 5.6 | |
| Ohenoja[8] | 12 – 13 | 4.5 – 5.0 | |
| NL, all colls.[9] | 8.9 – 16.4 | 3.6 – 5.6 | |
| NL coll. 1[10] | 8.9 – 10.9 (9.728; 0.640) | 3.6 – 4.8 (4.368; 0.376) | (2.246; 0.282) |
| NL coll. 2[11] | 9.2 – 13.2 (11.564; 3.741) | 4.0 – 5.2 (4.58; 1.405) | (2.536; 0.375) |
| NL coll. 3[12] | 13.6 – 16.0 (14.440; 1.111) | 4.0 – 5.2 (4.520; 0.434) | (3.215; 0.338) |
| NL coll. 4[13] | 12.0 – 16.4 (14.397; 1.261) | 4.0 – 5.6 (4.738; 0.432) | (3.052; 0.274) |

1. Original values from Boudier (1905), corrected for magnification (see Brummelen, 1985).
2. Favre,1955.
3. Sourced from website http://jlcheype.free.fr/.
4. Personal communication, Pierre-Arthur Moreau.
5. Dennis, 1971.
6. Collected from Europe and measured by one of the current authors (DM), data not published.
7. Dissing, 1992.
8. Collected from northern Finland and Norway, kept in University of Oulu Herbarium; personal communication, E. Ohenoja.
9. n=54.
10. n=10.
11. n=11.
12. n=10.
13. n=10.

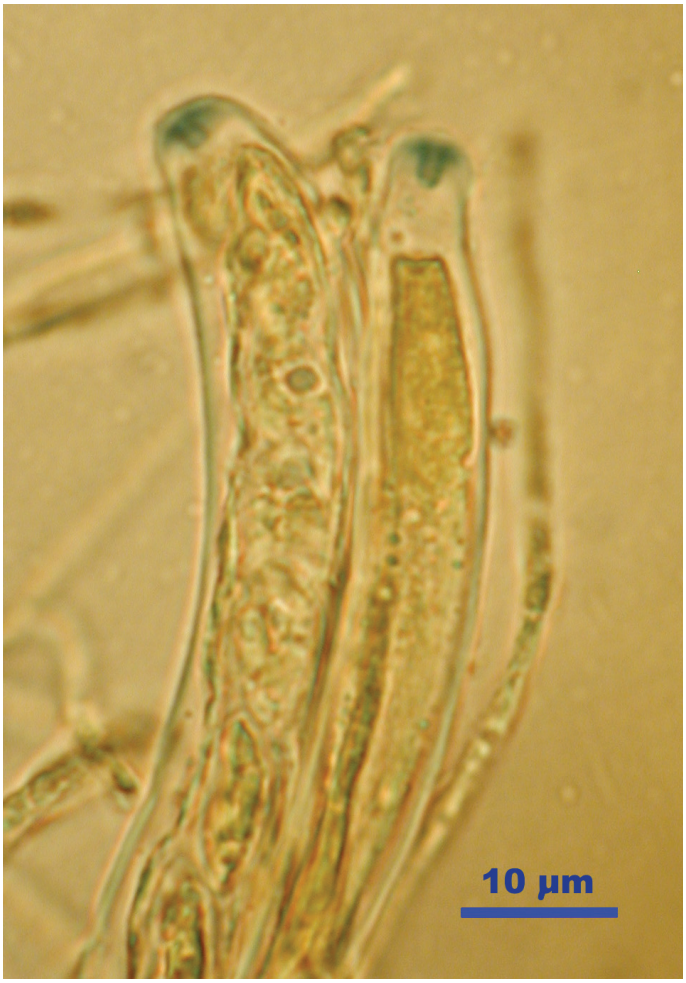


Figure 10. Asci mounted in Lugol's solution without KOH pretreatment. Collection 3 of 29 September 2007.

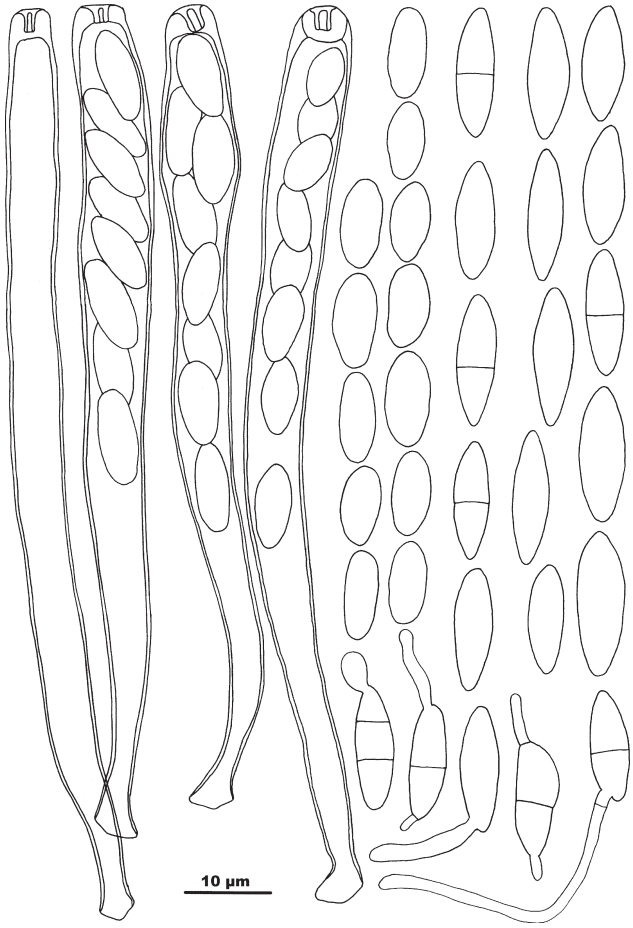


Figure 11. Four asci from collection 1. The ellipsoidal ascospores to the right of the asci also are from same the material and are probably immature. The rest of the spores, including the germinating ones, are from the September and October collections and are mature.

of this species which is not surprising as mycologists have been slow to determine the distribution patterns, within evolutionary contexts, for fungi, in general (Lumbsch et al., 2008). With the accumulation of DNA sequence information we are now beginning to see the emergence of a brand new field of research (phylogeography) to answer such questions. It is probably safe to assume that *A. turficola* is a recent arrival to Newfoundland as most of the soil was eroded away (taking the original flora and fauna with it) during the last glacial period, which ended around 7,000 years ago (South, 1983). Likewise, Newfoundland has always been the first landfall for organisms (including humans) traveling from the Old World to the New World.

While very similar to European isolates, our collections were not morphologically identical. Ascospores of our specimens were not as large as those reported by Boudier. Brummelen has reported that the scaling of Boudier's microscope introduced a magnification factor, requiring that all measurements be multiplied by 0.82 to correct them (Brummelen, 1985). We have used this correction in our comparisons (Table 2). When this is done, Boudier's match those of Favre, but both remain somewhat larger than ours. Table 2 shows the sizes and ranges of spores of *A. turficola* from published and unpublished observations, as well as from our four collections. Likewise, the mean spore size for each of our collections is shown. With one exception, spores of collections from the European Alps (Boudier, Favre, Cheype) seem to be larger than those from elsewhere. If consistent, this would be opposite to reports for many mushrooms, where smaller spores are noted from southern latitudes (Redhead and Ginns, 1980; Steyaert, 1975). However, the report by Dissing in Nordic Macromycetes (Dissing, 1992) clearly breaks this relationship. Although Dissing does not report the location of his long-spored specimens, since the book encompasses mushrooms of Scandinavia, they must be considerably north of the Alps.

Therefore, another explanation for spore size discrepancy must be sought. Our collections represent mushrooms at different stages of maturity. Collection 1 is a single immature specimen, with almost all spores still within the asci; the few free ones were just at the mouths of the asci. Not surprisingly, these were the

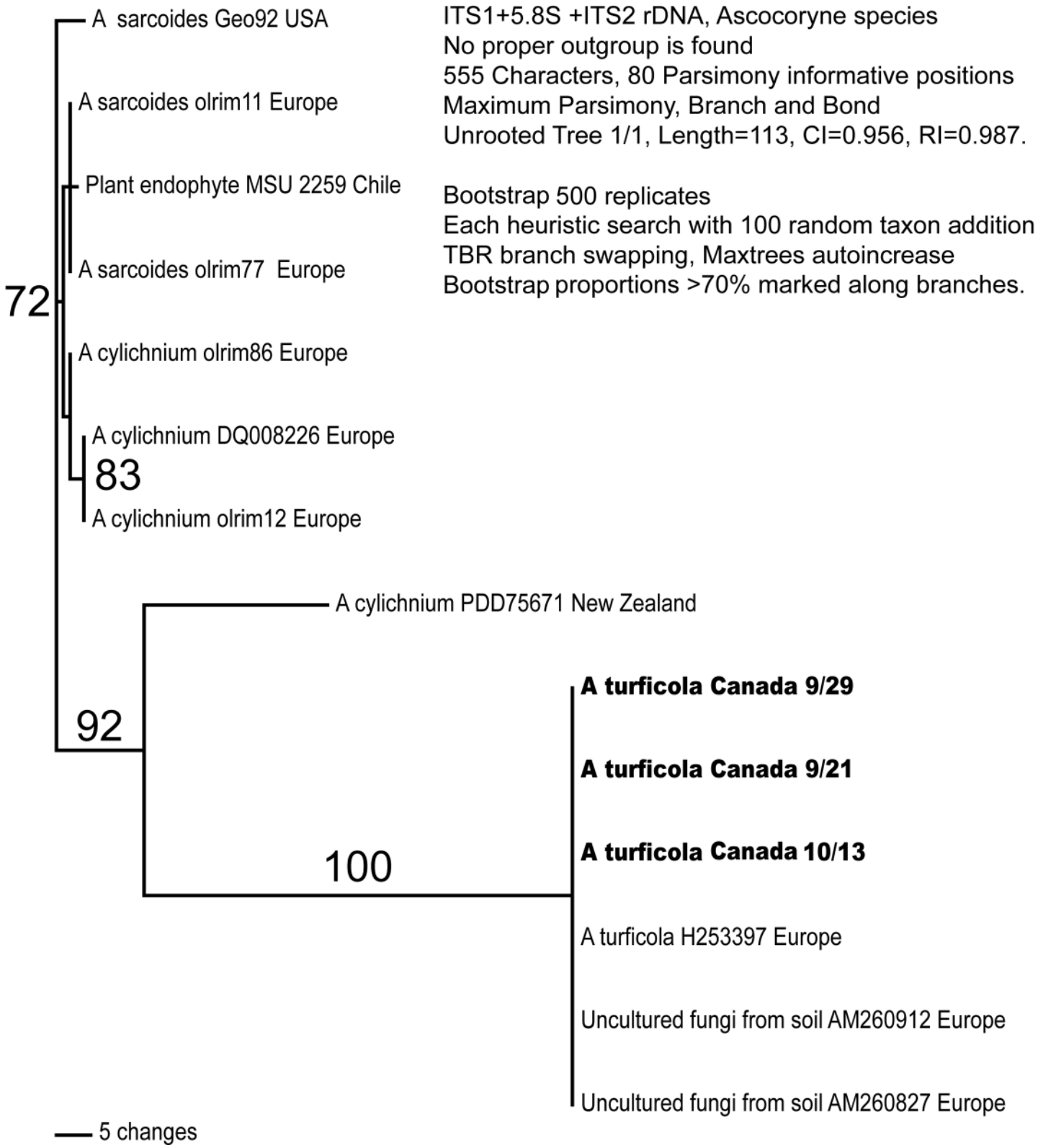


Figure 12. ITS phylogeny of *Ascocoryne* species.

Figure 13. The cosmopolitan *Ascocoryne sarcoides* grows on wood.

smallest spores we encountered. Collection 2 consisted of one juvenile and one young adult specimen. The spores were considerably larger. Collections 3 and 4 were from the same population, two weeks apart, toward the end of the season. As expected, they contained several overmature specimens. Spores from these collections were the largest. Maturity of the spores was also shown by the appearance of septation and evidence of germination in some cases. Seemingly contrary to the trend, the median spore size of Collection 4, the latest in the season, was slightly smaller than that of Collection 3. However, as seen in the photograph (Fig. 6), Collection 4 contained more immature specimens than Collection 3. If the range of size is examined (Table 2), it is evident that Collection 4 contained the largest spores of all four.

Because our collections had a good match with the DNA of European specimens, we can be reasonably assured that we are dealing with the same species. We also had an opportunity to examine one of the European specimens used for the DNA studies (Kirk specimen). Our review of reported spore sizes suggests that there is a wide size range for spores of *A. turficola*. The review of our own collections suggests that most of this variation relates to the age of the spores. Spores from our more mature and overmature specimens matched the range reported from the Alps. Therefore, some circumspection in interpreting spore size for this mushroom is advised.

### Coryne, Sarcoleotia *or* Ascocoryne*?*

As discussed above, the genus *Coryne* is now applied only to non-sexually reproducing fungi and is unavailable for Ascomycetes. However, the names *Sarcoleotia* Imai and *Ascocoryne* Groves and Wilson are both available for Ascomycetes and have been used to accommodate *C. turficola*. The only morphological feature that has been used to resolve this problem is the presence or absence of a gelatinous excipular layer within the apothecia. While most previous authors have described *Coryne turficola* as having gelatinous apothecial tissues Dennis (1971) reported the British collections to lack these features, and on that basis assigned it to *Sarcoleotia* as *S. turficola* Dennis. The Newfoundland material is so similar to the specimens illustrated by Boudier that we have no reason to doubt their conspecificity and, in accordance with Boudier's description, ours have well-defined gelatinous tissues. We can only speculate that Dennis examined poorly dried or atypical representatives of *C. turficola* or, perhaps more likely, had another species altogether. Only an examination of Dennis' material will settle this question. Thus we are confident that our material fits the *morphological* concept of *C. turficola* as understood by all authors other than Dennis. Morphologically, it also fits within the genus *Ascocoryne*.

To quote Hsü Yu, "Names are only the guests of reality." The organism remains the same and always has a gelatinous layer below the apothecium, no matter what we call it. When we claim to reassign it on the basis of "new" genetic evidence, we should remember that the evidence is not new, only our awareness of it. Studying the genetic relationships of the Helotiales, Wang and coworkers (2006 a; b) discovered that the genus *Sarcoleotia* sensu Schumacher and Sivertsen (1987) was made up of two separate genetic groups (Fig. 9). One group (the Geoglossum clade) includes *S. globosa,* the type species of *Sarcoleotia*, which shares genetic, morphological and ecological similarity with the so-called earth-tongue fungi, *Geoglossum* and *Trichoglossum.* A second group (the Ascocoryne clade) contains species having genetic similarity with the small

Figure 14. Besides *A. turficola*, other bog inhabiting fungi are known to be wood rotters. *Pholiota astragalina* (top) and *P. scamba* (bottom) both grow in deep *Sphagnum* moss but have a connection with small pieces of wood.

genus *Ascocoryne* and includes *C. turficola*. Thus the genetic evidence supports inclusion of *C. turficola* in *Ascocoryne* and not *Sarcoleotia*.

## Appeal

We have done our best to capture every record of this taxon from North America. Nevertheless, it is possible that despite our best efforts some records may have been missed. If any reader is aware of a North American record of this mushroom, please notify the Editor of FUNGI, <bbunyard@wi.rr.com>, so that we may complete our records. One of the advantages of electronic publication is that while the printed version remains as is, the electronic version of this article, available for download from the FUNGI Web site, <www.fungimagazine.com>, can be amended constantly to reflect new information. We also request that having been alerted to the existence of this beautiful bog denizen, readers who find it, please notify the Editor.

## Acknowledgments

During the search for information about *Ascocoryne turficola,* the authors have engaged several authorities, who gave unstintingly of their time. We wish to thank those, who added illumination or insight, allowing us to piece together the bits to form a picture: Seanna Annis, Alan Bessette, David Boertmann, Torbjørn Borgen, David Fischer, Gro Gulden, Kathie Hodge, Henning Knudsen, Richard Korf, Thomas Læssøe, Pierre-Arthur Moreau, Esteri Ohenoja, Scott Redhead, Brian Spooner, Markus Thormann, Jan Vesterholt, and Roy Watling.

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