Sporormiella tela, a New Species of Pleosporales from Dung of Geese

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Abstract - A new species of *Sporormiella*, *S. tela*, so far only found on dung of Anseriformes, is described and illustrated. The species has a thick mycelial weft covering the pseudothecia. In addition to a collection on *Branta canadensis* (Canada Goose) dung from New York, 2 collections on goose dung from Iceland, and 1 each on *Branta leucopsis* (Barnacle Goose) dung from Norway and Canada Goose dung from Germany, 3 close-matching sequences have been identified from environmental samples from Estonia, China (Tibet), and India (Kashmir).

Introduction

Sporormiella is a widespread genus of mainly coprophilous fungi in the Pleosporales. Many species have 4-celled ascospores in bitunicate asci, but others have ascospores with 5 or more cells. There has been no monographic treatment since that of Ahmed and Cain (1972), which included 63 coprophilous species. Others have since been described, but the exact number of species in the genus is not clear, as some taxonomists consider *Sporormiella* to be synonymous with *Preussia* (Kruys and Wedin 2009), and some of Ahmed and Cain's species are currently classified in *Preussia* (Index Fungorum 2023, Lytvynenko et al. 2022). Richardson (2004) reported 2 collections with atypically large, tough pseudothecia and 7–9-celled ascospores on goose dung from Iceland, after incubation in a damp chamber. Those collections remained unidentified until 2023, when M.J. Richardson was contacted by S. Jakob, who had found a very similar fungus on dung of *Branta canadensis* (L.) (Canada Goose) collected from a park in New York, and incubated in a moist chamber. Subsequently it was found on dung of *Branta leucopsis* (Bechstein) (Barnacle Goose) collected by M.J. Richardson in Svalbard, Norway in July 2023, with an identical sequence to the holotype collection. A fifth collection was obtained from a collection of dung of Canada Goose from Bavaria, Germany.

That fungus is described here as a new *Sporormiella* species.

Methods

We incubated samples that provided the holotype in a Petri dish kept moist by regular misting and inspected them at intervals with an Olympus SZ40 stereoscopic microscope (x 6.7–40) with halogen lighting (Tokyo, Japan). We conducted the microscopic observations with a Motic Panthera C2 compound microscope (Hong

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Manuscript Editor: Richard Baird

Kong) and photographed with a Canon 5D Mark II camera (Tokyo, Japan). We measured 30 mature ascospores of the holotype in water and incubated other samples in moist chambers. Herbarium abbreviations follow Thiers (2023).

DNA extraction, amplification and sequencing

We extracted DNA from dry pseudothecia following a modified version of the protocol developed by Osmundson et al. (2012). PCR reactions (Mullis and Faloona 1987) included 30 cycles at annealing temperature of 52 °C. We used the primers ITS1-F KYO2-ITS4 (Toju et al. 2012) and LR0R-LR5 (Cubeta et al. 1991, Vilgalys and Hester 1990) for the sequencing of the ITS and 28S regions of nuclear ribosomal DNA, respectively. We checked PCR products on 2% agarose gels and sequenced products of positive reactions with the above primers. We checked the chromatograms and corrected them manually using the free software SnapGene 6.2.1 (Dotmatics, Boston, MA).

We retrieved all sequences of Sporormiaceae from the National Center for Biotechnology Information (NCBI; Sayers et al. 2022) GenBank (Sayers et al. 2020), filtered to include only those of the nrITS and nrLSU region, and organized by specimen voucher, culture collection, strain number, isolate, and isolation source. After these fields were reconciled, we removed duplicate records (same region from the same source), keeping the longest sequence. We preliminarily analyzed the ITS sequences of *Sporormiella tela* by BLAST (Altschul et al. 1990) in GenBank and UNITE (Abarenkov et al. 2023) databases to identify environmental sequences similar to the target, which were also included in the dataset. We added large subunit sequences of 2 species of Pleosporales from other families (*Stemphylium vesicarium* (Wallr.) E.G. Simmons and *Trichophoma cylindrospora* Magaña-Dueñas, Cano & Stchigel) to serve as outgroups. We extracted nuclear ribosomal ITS and LSU sequences from the resulting dataset (see Supplementary File 1, available online at http://www.eaglehill.us/NENAonline/suppl-files/n31-1-N2056-Richardson-s1 and, for BioOne subscribers, at https://www.doi.org/10.1656/N2056.s1) and aligned them separately with MAFFT (Katoh and Standley 2013) in Unipro UGENE version 49 (Okonechnikov et al. 2012) using default parameters. We manually trimmed flanking regions and identified and removed ambiguously alignable regions in the ITS alignment with Gblocks version 0.91b (Castresana 2000) using default parameters except we reduced "minimum length of a block" to "5" and set "allow gap positions" to "all".

We concatenated the resultant alignments using MEGA11 (Tamura et al. 2021) and estimated a preliminary partitioned maximum-likelihood phylogeny using raxmlHPC-PTHREADS-SSE3 (Stamatakis 2014) in raxml GUI version 2.0.10 (Edler et al. 2021) and the GTR+GAMMA+i substitution model (Abadi et al. 2019). We visualized the resultant phylogeny in FigTree version 1.4.4 (Rambaut 2023). We reexamined sequences placed in spurious positions in the phylogeny (i.e., clustering with the outgroup away from other supposedly contaxic sequences or on very long branches) and excluded those we determined to be the result of misidentification, contamination, or poor sequence quality. After excluding these sequences, we created a concatenated alignment as above (see Supplementary File 2, available

online at http://www.eaglehill.us/NENAonline/suppl-files/n31-1-N2056-Richardson-s2 and, for BioOne subscribers, at https://www.doi.org/10.1656/N2056.s2) and reran phylogenetic analysis except the "autoMRE" option was selected for bootstrap support estimation. We collapsed and annotated major clades for clarity. An unedited tree is provided (see Supplementary File 3, available online at http://www.eaglehill.us/NENAonline/suppl-files/n31-1-N2056-Richardson-s3 and, for BioOne subscribers, at https://www.doi.org/10.1656/N2056.s3).

Results

The description is based on observations and measurements of the holotype and 4 paratypes.

Sporormiella tela S. Jakob and M.J. Richardson sp. nov. (Figs. 1A–C, 2A–E) MycoBank: MB852020

Etymology: from *tela*—Latin for web or membrane, referring to the pseudothecial covering.

Pseudothecia 500–900 µm diameter, dark, globose, superficial but immersed in a dense mycelial weft so dense that there was no indication that it would contain a pseudothecium, sometimes with spikes of hyphae <450 µm (Fig. 1D), with a hard peridium, thick-walled, more or less coriaceous, subglobose, brown-black, glabrous once removed from the mycelial weft, occasional setae at the base appear to be anchor hairs. Neck 70–120 µm high and 120–150 µm wide, ostiolate. *Asci* bitunicate, mostly 8-ascospored, clavate, 300–350 µm × 30–36 µm, biseriate to triseriate, tapering to a stalk below, non-amyloid. *Pseudoparaphyses* abundant**,** slightly exceeding the asci, cylindrical, moniliform, 12–13 µm wide, sometimes with an attenuated apex. *Ascospores* at first hyaline, then yellowish/greenish and finally dark brown, variously 7–12-celled, not necessarily all the same number in an ascus, straight or slightly curved, fusiform, circular in cross section, surrounded by a gelatinous sheath, (58) 70–89 (96) µm × (7) 7.5–11.5 (14) µm, Q = (5.5) 7–8 (8.5). More than half of ascospores are 8-celled, with 9- and 10-celled ascospores less frequent and 7-celled, 11-celled, and 12-celled ascospores rare. Overall ascospore size is relatively uniform despite variations in the number of cells. Ascospore cell shape, length, and breadth are highly variable, especially for the middle cells; larger and often longer cells are mostly found in ascospores with lower cell number (6-celled to 8-celled). Component cells are generally deeply and transversely septate, giving the ascospores a bead-like appearance. End cells conical and rounded at the tip. Germ slits diagonal.

Holotype: United States of America: Van Cortlandt Park, New York. On dung of *Branta canadensis* (Canada Goose), collected on grassy field, 40°53'24"N, 73°53'24"W, 9 m a.s.l., leg. S. Jakob, 26 February 2023. Once incubated, the first mature specimens appeared on 24 March, 2023 and in subsequent months. (GenBank: OR226391 [ITS] and OR226402 [LSU]).

Paratypes: Germany: Coburg, Bavaria. On the dung of Canada Goose, 50°21'7.0128"N, 11°1'22.7994"E, 310 m a.s.l., 23 January, 2023, leg. Peter Püwert; Norway: Svalbard. On *Branta leucopsis* (Barnacle Goose) dung on sparse coastal

vegetation, 78°14'58"N, 15°29'59"E, 3 m a.s.l., 27 July 2023. leg. M.J. Richardson, MJR 1/23 (in Herb. NY). (GenBank OR710299 [ITS]); Iceland: Eyjan, Jökulsárgljúfur National Park, Asbyrgi. On goose dung on *Salix*/ericaceous heath. 66°1'12"N, 16°30'0"W, 50 m a.s.l., 18 July 2002, leg. M.J. Richardson, MJR 34/02 (in Herb. AMNH); Eldgjá. On goose dung on river gravel vegetation. 63°57'36"N, 18°37'12"W, 450 m a.s.l., 23 July 2002, leg. M.J. Richardson, MJR 48/02 (in Herb. AMNH). (GenBank OR226393).

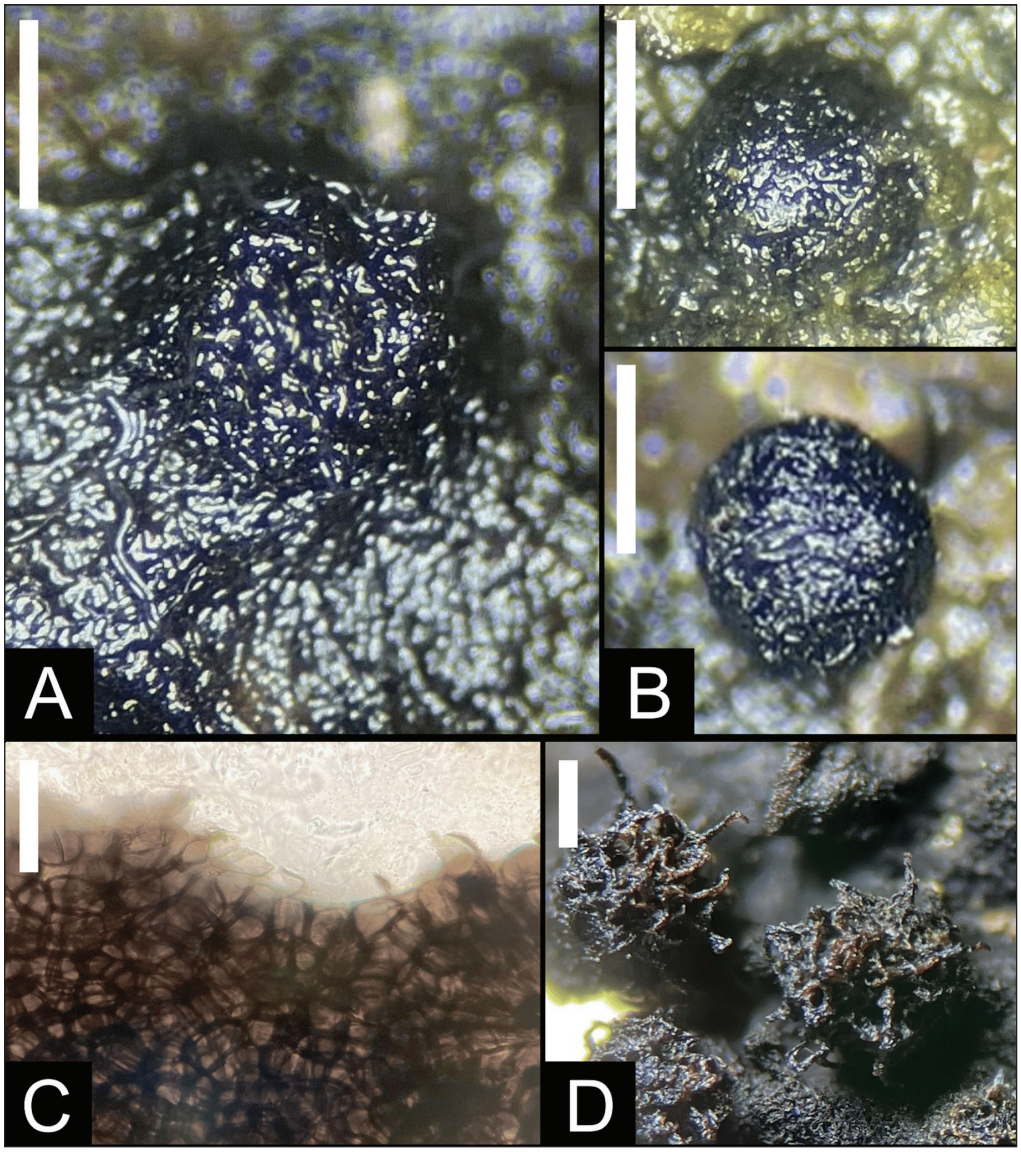

Figure 1. Images of *Sporormiella tela* holotype: (A) pseudothecia under mycelial weft, scale bar = 500 µm; (B) underside of mycelial weft with exposed pseudothecia and, below, pseudothecium separated from mycelial weft, scale bar = 500 µm; and (C) detail of exoperidium, scale bar = 50 µm. (D) Norwegian paratype: pseudothecial covering with appendages, scale bar = 500 µm.

Other occurrences from GenBank or UNITE sequence data-bases. China: Tibet: A 99.19% match from an environmental sample (MF971559) from Golmud, Haixi Mongol and Tibetan Autonomous Prefecture, Qinghai, China, 34°15'36"N, 92°30'0"E, altitude 4600 m (Qin et al. 2017); Estonia: A 100% match to sequence

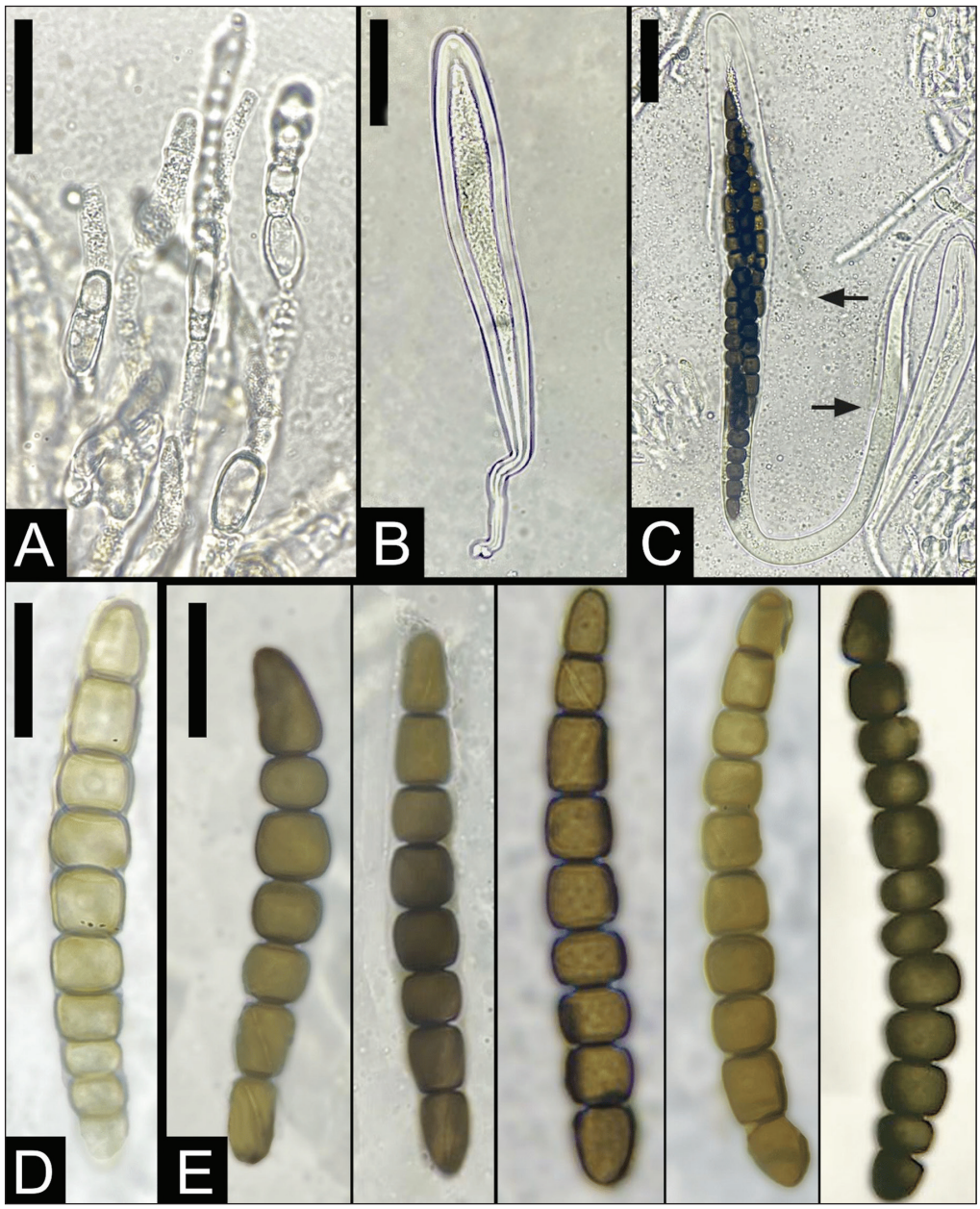

Figure 2. Images of *Sporormiella tela* holotype: (A) pseudoparaphyses; (B) immature ascus; (C) asci, one immature and one after rupture of the outer wall, with the break points indicated by the arrows; note the long, slender, tapering lower part; (D) immature ascospore; and (E) mature ascospores, showing examples of 7- to 12-celled spores. Scale bars: A–C = 50 µm, D–E = 20 µm.

in the UNITE database (UDB0389211) from a soil sample from Tartu, 58°21'40"N, 26°39'54"E, altitude 67 m (Tedersoo et al. 2014); India: Kashmir: A 96.2% match in the UNITE database (UDB07557686) from a soil sample (PMID: 25430773) from the Himalayan region in Jammu and Kashmir, India, 34°48'14"N, 77°6'27"E, altitude 3041 m (Tedersoo et al. 2014).

Phylogenetic analysis

The resultant concatenated alignment was 1729 positions long (485 ITS sites and 1244 LSU sites) and was composed of 412 ITS and 162 LSU sequences for a total of 432 records. While the backbone of the phylogeny received very low support, major clades previously identified by Kruys and Wedin (2009) were recovered with moderate to high bootstrap support except that the *Sporormiella intermedia* (Auersw.) S.I. Ahmed and Cain ex Kobayasi clade was recovered as polyphyletic (Fig. 3). *Sporormiella tela* is recovered within or sister (depending on clade definition) to the *Sporormiella vexans* (Auersw.) S.I. Ahmed and Cain clade of Kruys and Wedin (2009), but without strong support. *Sporormiella tela* is recovered in a strongly supported monophyletic group along with closely related environmental sequences. Interestingly, genera included in the family but not represented in the aforementioned phylogenetic analysis of the family clustered with the outgroups rather than Sporormiaceae sensu stricto (S*particola* Phukhams., Ariyaw., Camporesi and K.D. Hyde, *Sporormurispora* Wanas., Bulgakov, Gafforov and K.D. Hyde, *Trichophoma* Magaña-Dueñas, Cano and Stchigel, and *Xenomonodictys* Hern.-Restr., Karimi, Alizadeh and T. Ghanbary) but exclusion from the family is not strongly supported.

Discussion

Sporormiella commutata (Niessl) Ahmed and Cain was considered as a possible identity of these collections, but it is described as having small pseudothecia 235–273 µm diam., with no mention of their firmness or mycelial weft, asci 145–200 × 18.5–23 µm, and ascospores 7–9-celled, 50–65 µm × 8–10.5 µm. (Ahmed and Cain 1972:437) reported them to be "usually nine-celled, occasionally seven- or eight-celled", while Lytvynenko et al. (2022) found them to be "usually 8-celled, occasionally 7- and 9-celled". The differences in these 4 features would suggest it is not *S. commutata*.

There are many *Sporormiella* species that are basically 8-ascospored (some with some variation), but none that share the same suite of characters as *S. tela*: *Sporormiella pascua* Niessl, *S. bipartis* Cain, *S. octonalis* S.I. Ahmed and Cain, *S. ontariensis* Cain, *S. corynespora* Niessl, *S. schadospora* S.I. Ahmed and Cain, *S. subticinensis* (Mouton) Dugan and R.G. Roberts, *S. minipascua* S.I. Ahmed and Cain, *S. octomera* (Auersw.) S.I. Ahmed and Cain, *S. platymera* S.I. Ahmed and Cain, *S. insignis* (Niessl) S.I. Ahmed and Cain, *S. splendens* (Cain) S.I. Ahmed and Cain, *S. decamera* S.I. Ahmed and Cain, *S. calomera* S.I. Ahmed and Cain, *S. herculea* (Ellis and Everh.) S.I. Ahmed and Cain, *Preussia alpina* N. Lundq. and Kruys, *P. octocylindrospora* N. Lundq. and Kruys, and *P. octosymmetrica* Chalange and Vigneron.

The phylogenetic reconstruction based on the nrLSU and nrITS regions places *S. tela* in a unique lineage relative to *Sporormiella* and *Preussia* environmental samples. The new species is recovered as a sister lineage to the *Sporormiella vexans*

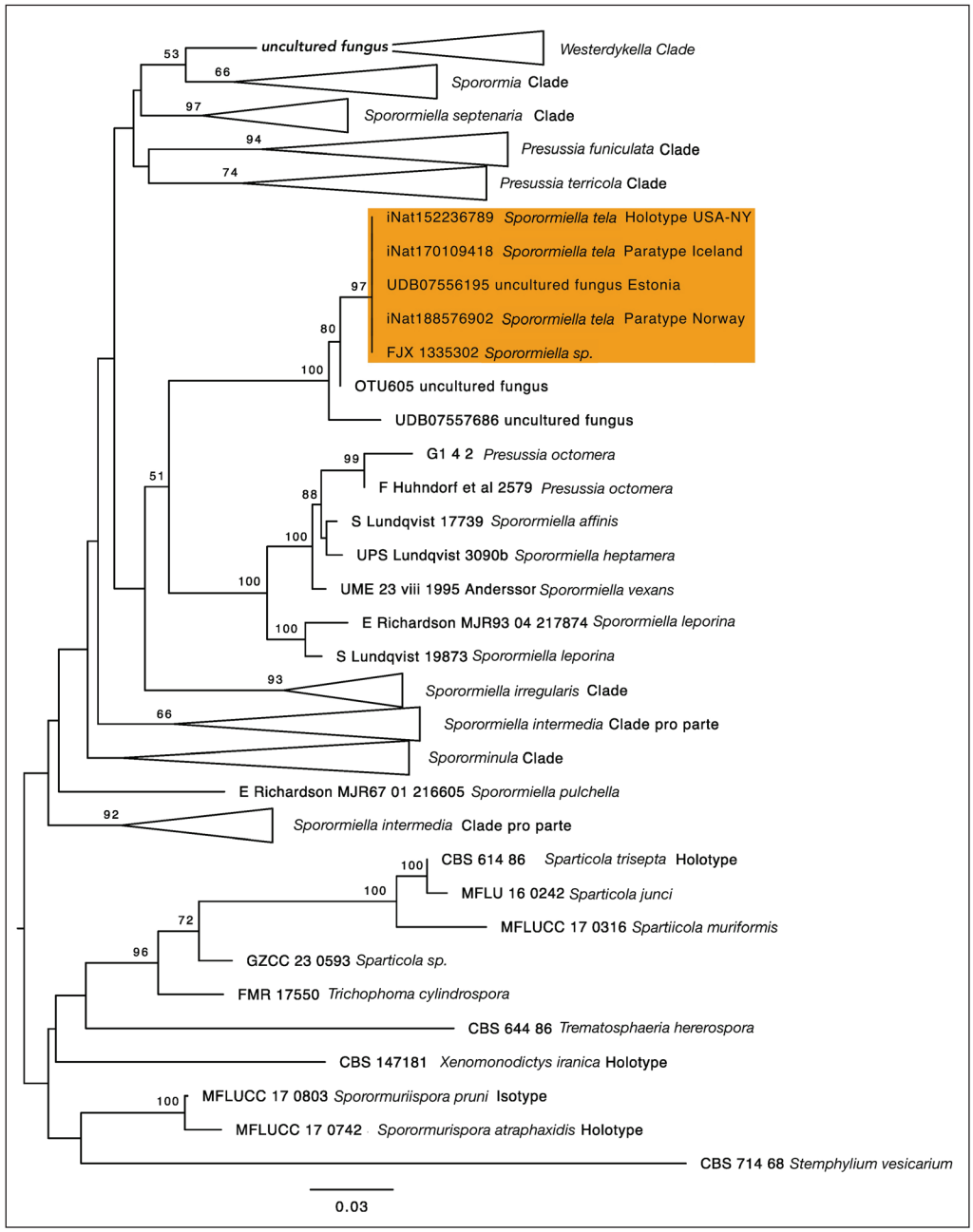

Figure 3. A maximum-likelihood phylogenetic reconstruction of the Sporormiaceae based on a partitioned analysis of the nrITS and nrLSU regions. Major clades are collapsed and annotated as in Kruys and Wedin (2009) except for the *Sporormiella vexans* clade. Bootstrap support values greater than 50 are displayed. *Sporormiella tela* is highlighted in orange.

clade (with low support) but may represent a new clade in the Sporormiaceae along with the closely related environmental samples.

Although the ITS sequence of the Norwegian sample (OR710299) was a 100% match with that of the holotype, there was some variation, expressed as filamentous appendages on the upper part of the pseudothecial covering (Fig. 1D), and a lower tendency of the ascospores to vary in the number of cells (the majority were 8-celled and there were very few 7-, 9- and 10-celled ascospores). Other observations suggest that, despite the close sequence similarity, *S. tela* can show a range of morphological variation in different collections.

Sporormiella tela exhibits some similarity with *Preussia dubia*, with subglobose glabrous pseudothecia and a thick, almost black, membranous peridium, but it also exhibits significant differences; *Preussia dubia* is 4-celled, 38–45 µm x 8–9 µm. *S. irregularis* is even more different, with a thin, yellowish peridium and 4-celled ascospores that measure 50–58 µm x 10–13.5 µm.

The environmental ITS sequences in GenBank and UNITE that are a >99% match are either from high altitude (China/Tibetan Plateau, 4600 m) or relatively high latitude (Estonia, 58ºN). This possible preference for higher latitudes or altitudes fits with the collections from Iceland and Svalbard (Norway). The holotype was found on the dung of a species whose summer range is arctic or subarctic, but whose winter range stretches as far south as Mexico. This preference for colder climates for at least part of the year suggests a potential explanation for the mycelial weft as a strategy for temperature control. This trait has been reported before. Dissing (1989) described 4 species of *Ascobolus* and *Saccobolus* from Greenland. Richardson (2007), from a study of presence/absence records from over 1000 dung samples from various parts of the world, reported that *A. immersus* Pers. and *S. depauperatus* (Berk. and Broome) Rehm occurred significantly more frequently at lower latitudes, while *A. albidus* P. Crouan and H. Crouan, *A. stictoideus* Speg. and *S. versicolor* (P. Karst.) P. Karst. were significantly more frequent at higher latitudes.

As noted in the introduction, some taxonomists consider *Sporormiella* to be synonymous with *Preussia* (Kruys and Wedin 2009), and that more loci should be sequenced for all species of *Preussia* and *Sporormiella* for which living material is available in order to make a polyphasic study of both genera to know the delimitation of both genera and the taxonomic placement of all species belonging to them. At the time of writing, an insufficient number of protein-coding gene sequences (e.g., rpb1, tef1, rpb2) are available in public databases to resolve the generic boundaries in this family. We prefer to introduce *S. tela* as a new species of *Sporormiella* due to its morphological characteristics until the boundaries of both genera are resolved.

Canada Geese occur in Canada, the United States, Iceland, and northwest Europe, but the species has not been recorded from the Tibetan Plateau in China, though other geese (e.g., *Anser indicus* (Latham) [Bar-headed Goose]) have (GBIF Secretariat 2023). Thus, it appears that *S. tela* could be found on the dung of other birds.

Acknowledgments

The authors thank Yasmina Marin Felix for advice on the usefulness of sequencing in this study, and Peter Püwert, Björn Wergen, Gyða Eyjólfsdóttir, Åsa Kruys, Sven Heinz, and Norbert Heine for their advice and support.

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