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Hydnum atlanticum, a new species from Eastern North America

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Abstract: A new species of *Hydnum* subgenus *Rufescentia* is described based on collections made in Canada (New Brunswick, Newfoundland and Labrador) and the USA (New York). The new species is found in conifer dominated forests (e.g. *Abies*, *Picea*) and occurs in bryophyte-covered (*Sphagnum*, *Bazzania*) soil. It differs from the ecologically similar *H. quebecense* in the duller brown colors of the basidiomes, the smaller basidiospores and the basidia predominantly with three or four sterigmata. Phylogenetic analysis of the ITS region place *H. subconnatum* and *H. oregonense* as the closest relatives of *H. atlanticum*, but these taxa differ in the larger basidiospores, number of sterigmata per basidium, caespitose growth and/or geographic distribution.

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INTRODUCTION

Hydnum is a widespread genus of ectomycorrhizal fungi commonly known as “hedgehog mushrooms” and collected as edibles in many parts of the world. Phylogenetic work in Europe, Asia and North America has revealed considerably higher levels of species diversity than initially estimated based on morphological characters. While European names (e.g. *H. repandum*, *H. rufescens*) have been widely applied to species in other geographic areas, phylogenetic data have shown that most species are endemic at the continental level (Grebenc *et al.* 2009, Olariaga *et al.* 2012, Vizzini *et al.* 2013, Feng *et al.* 2016, Niskanen *et al.* 2018, Swenie *et al.* 2018, Cao *et al.* 2021, Sugawara *et al.* 2022a, b).

The diversity of the genus in Eastern North America has been the focus of a recent monographic study (Swenie *et al.* 2018) that recognized and described 17 phylogenetic species occurring in the area. One of the species included in this monograph was not formally described, as only one collection was available at the time of publication, and was given the provisional code “*Hydnum* sp. AS30”. During a one-year intensive study of the diversity of *Hydnum* in the province of New Brunswick in Canada, 32 collections of this taxon were sequenced, and the species is formally described here as *Hydnum atlanticum* sp. nov.

MATERIALS & METHODS

Collections and morphological analyses

Collections of *Hydnum* studied here were made during weekly field work carried out in New Brunswick, between July and

November 2021, with the exception of the NY and NL collections of *H. atlanticum*, that were studied by one of us (R. Swenie) from fungarium material. Specimens were photographed in the field and annotations were made before drying. Color codes used in the description are from Kornerup & Wanscher (1978). Microscopic study of the New Brunswick collections was carried out using an Olympus BH-2 compound microscope. Study of additional collections followed Swenie *et al.* (2018). Basidiospores and other microscopic features were observed and measured from dried tissue mounted in water or a mixture of KOH and Congo Red. The notation [412/33] indicates the total number of basidiospores measured, and the number of collections studied.

DNA extraction, amplification and sequencing

For DNA extraction small fragments of dried basidiomes were used. Molecular work was carried out at ALVALAB (<http://www.alvalab.es/>) using standard methods for the study of basidiomycete fungi (e.g. Justo & Hibbett 2011). The following primer combinations were used for amplification and sequencing: ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993) for ITS; HEF1F and HEF1R (Feng *et al.* 2016) for part of the translation elongation factor 1-alpha (*TEF1-α*); and RPB2-6F2 and RPB2-7R2 (Matheny *et al.* 2007, Tanaka *et al.* 2020) for the RNA polymerase II second largest subunit (*RPB2*). Raw data were edited and assembled in ChromasPro v. 2.1.10 (Technelysium).

Phylogenetic analyses

The ITS dataset included 132 new sequences generated from New Brunswick collections and reference sequences for other species of *Hydnum*, mostly from the studies of Swenie *et al.*

(2018) and Niskanen *et al.* (2018), with a particular focus on subgenus *Rufescentia*. The dataset included 180 *Hydnum* sequences and two *Sistotrema* sequences as outgroups.

Sequences were aligned using MAFFT v. 7 under the FFT-NS-i strategy (Katoh *et al.* 2019). The alignment was inspected and manually corrected in AliView v. 1.28 (Larsson 2014). Two phylogenetic analyses were run: (i) maximum likelihood (ML) analyses using RAxML v. 8.2.10 (Stamatakis 2014) under a GTRGAMMAI substitution model with 1 000 bootstrap (BS) replicates; (ii) Bayesian inference (BI) analyses using MrBayes v. 3.2.7 (Ronquist *et al.* 2012) for 10 M generations under a GTRGAMMAI model with four chains, and trees sampled every 1 000 generations. The burn-in phase was set to 2.5 M generations, and this value was confirmed to be adequate by checking the graphic representation of the likelihood scores of the sampled trees, the standard deviation of split frequencies (values < 0.01), and the potential scale reduction factor (values close to 1). All analyses were run using resources at the CIPRES Science Gateway (Miller *et al.* 2010). All phylogenetic trees were initially visualized using FigTree v. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Species-level clades represented by multiple sequences were collapsed to facilitate graphical representation. Trees were exported from FigTree as SVG files and edited in Adobe Illustrator v. 27.3.1 for final presentation.

RESULTS

Molecular phylogeny

The topologies of the best tree from the ML analysis and the consensus tree for the BI analysis are essentially identical, and there is no strongly supported conflict between both, so only the ML tree is presented here (Fig. 1) with support values from both analyses. The ITS phylogeny recovers *Hydnum atlanticum* as a well-supported clade in subgenus *Rufescentia*, subsection *Tenuiformia* (Fig. 1). The grouping of *H. subconnatum*, *H. oregonense* and *H. atlanticum* is also well-supported.

In our phylogeny the subgenera recognized in Niskanen *et al.* (2018) and Swenie *et al.* (2018) are also recovered (*Hydnum*, *Alba*, *Pallida* and *Rufescentia*). The subsections *Tenuiformia*, *Mulsicoloria* and *Rufescentia* are not recovered as part of a single group, *viz.* section *Rufescentia* in the phylogeny of Niskanen *et al.* (2018). In the analyses performed here these three groups are well-supported, but they appear intermixed with other lineages in subgenus *Rufescentia*: /Ovoideispora, sect. *Magnorufescentia*, and taxa of uncertain placement within the subgenus.

Taxonomy

Hydnum atlanticum Justo, A. Hood & Swenie, *sp. nov.* MycoBank MB 847209. Figs 2, 3.

Etymology: *atlanticum*, for its distribution in Atlantic North America.

Typus: **Canada**, New Brunswick, Saint John County, Little Salmon River Protected Natural Area, Dustin Brook access trail to Fundy Footpath (45.500016, -65.277409), on bryophyte-covered soil in conifer dominated forest (*Picea*, *Abies*) with some scattered *Betula*, 1 Sep. 2021, A. Justo, AJ1597 (**holotype** NBM-F-009401).

Pileus 20–77 mm wide, convex to plano-convex, becoming depressed at center in mature specimens, often distinctly umbilicate; surface smooth, or minutely squamulose-fibrillose, hygrophanous, often distinctly zonate; predominantly brown, with rather muted dull tones (6C8, 6C7, 6C6, 6C5, 6D8, 5C8, 5C7), rarely with more vivid bright brown yellow or brown orange tones (6B8, 6B7, 5B8); margin incurved and entire when young, becoming wavy to eroded in mature specimens, sometimes slowly staining orange after touch or damage. Spines crowded, acute, sometimes becoming flattened, adnate to slightly decurrent, pale cream to pale brown, up to 7–15 mm long in mature specimens. *Stipe* 24–67 mm × 4–15(–20) mm, cylindrical or slightly widening towards the base, central, sometimes fused in groups of two or three specimens; surface smooth, white, with orange staining when bruised or in damaged places; often with spines at the apex. Flesh white, very slowly becoming cream to pale orange after cut. *Smell* pleasant but not very distinct. *Taste* pleasant, sometimes with a nutty aftertaste, not bitter. *Basidiospores* [412/33] (6.0–)6.5–8.0(–8.6) × 5.4–7.6(–8.0) μm, Q = 1.00–1.17 (1.41), Q_{av} = 1.08–1.13; avl × avw = 7.2–7.7 × 6.5–6.9 μm, mostly globose to subglobose, some broadly ellipsoid, very rarely ellipsoid, smooth, hyaline in KOH. *Basidia* 37–55 × 7.6–10.5 μm, narrowly clavate or suburniform, with (2–)3–4 sterigmata. *Pileipellis* an interwoven cutis, hyphae smooth, cylindrical, thin-walled, hyaline, mostly 5–8 μm wide. *Clamp-connections* common in all tissues.

Distribution: Canada (New Brunswick, Newfoundland and Labrador) and USA (New York).

Ecology: Fruiting gregariously, in patches of 3–10 basidiomes. In conifer dominated forests. All New Brunswick collections were made under *Abies balsamea*, *Picea glauca* and/or *Picea* spp., sometimes with *Betula* and *Pinus banksiana* in the mix; in humid, bryophyte-covered areas, often including *Sphagnum* spp. but also *Bazzania trilobata*. The NY collection was made under *Tsuga* and *Larix* in a bog area in *Sphagnum*-covered soil. The Labrador collection was made under *Alnus*, *Pinus*, and *Picea* and was recorded as “on soil” without specific mention of the presence of moss. July–October.

Additional collections examined: **Canada**, New Brunswick, Queens County, Grand Lake Protected Natural Area, off Pondstream Road (45.9448, -66.11180), in somewhat dense, young stand of *Abies balsamea*, 17 Aug. 2021, A.W. Hood, AH90 (NBM-F-009413); *ibid.*, (45.94503, -66.11151), AH91 (NBM-F-009414); *ibid.*, (45.94514, -66.11031), under *Abies balsamea*, A. Justo, AJ1534, (NBM-F-009418); *ibid.*, (45.94514, -66.11031), under *A. balsamea*, AJ1535 (NBM-F-009419); New Brunswick, Saint John County, City of Saint John, Black Beach trail (45.15611, -66.22657), under *A. balsamea* and *Picea glauca*, 21 Oct. 2021, A.W. Hood, AH183 (NBM-F-009410); *ibid.*, (45.16483, -66.22390), AH198 (NBM-F-009411); *ibid.*, (45.16245, -66.22726), AH199 (NBM-F-009412); New Brunswick, Saint John County, Fundy Footpath, Rapidly Brook access trail (45.485833, -65.261667), under *Picea* and *A. balsamea*, 25 Aug. 2021, A. Justo, AJ1566 (NBM-F-009422); *ibid.*, (45.48461, -65.26389), under *P. glauca*, A.W. Hood, AH97 (NBM-F-009415); New Brunswick, Saint John County, Fundy Trail Parkway, McLeod Brook Falls Loop trail (45.49555, -65.30972), under *Abies*, *Picea* with some *Betula*, 22 Aug. 2021, A. Justo, AJ1557 (NBM-F-009420); *ibid.*, A. Justo, AJ1558 (NBM-F-009421); *ibid.*, (45.495556, -65.309722), 1 Sep. 2021, A. Justo, AJ1624 (NBM-F-009428); *ibid.*, (45.49557, -65.30446), under *P. glauca* near stream, 1 Sep. 2021, A.W. Hood, AH124 (NBM-F-009407); New Brunswick, Saint John County, Fundy Trail Parkway, Road to Walton Glen Reception Center (45.490564, -65.311361), under *Abies* and *Picea*, 25 Sep. 2021, A. Justo, AJ1732 (NBM-F-009429); New Brunswick, Saint John County, Fundy

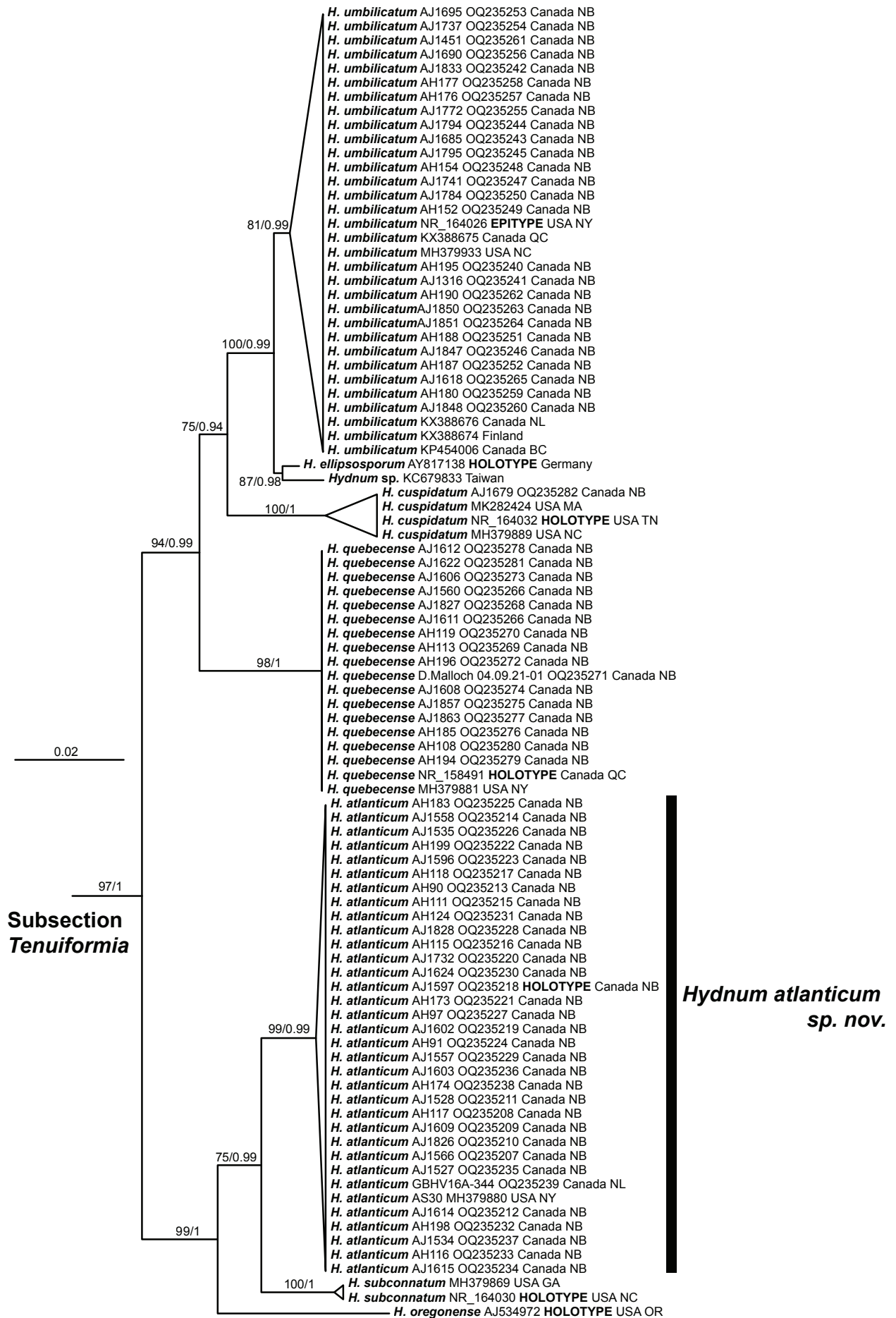


Fig. 1. Best tree from the ML analysis of the ITS dataset. Bootstrap support values from the ML analysis (BS \geq 70 %) and Posterior Probabilities from the BI analysis (PP \geq 0.90) are given on or below the branches. The first part of the tree is a detailed view of the top subsection from the second part of the tree.

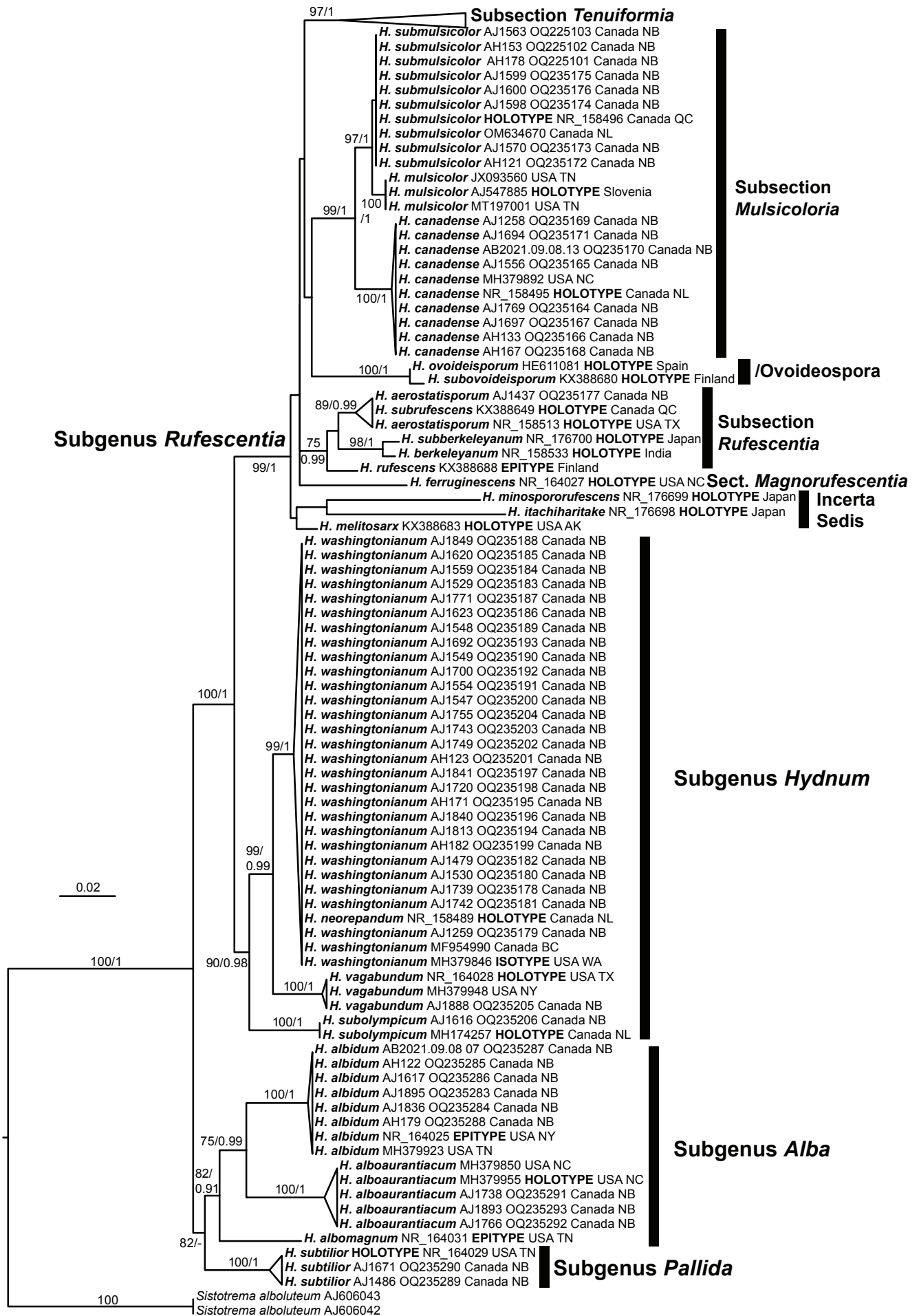


Fig. 1. (Continued).



Fig. 2. *Hydnum atlanticum* basidiomes *in situ*. **A.** Collection AJ1597 (Holotype). **B.** Collection AJ1534. **C.** Collection AJ1603. **D.** Collection AH90. **E.** Collection AJ1615. **F.** Collection AJ1558. Scale bars = 1 cm.

Trail Parkway, Walton Glen Gorge trail (45.489694, -65.307883), upland forest on sandy soil, mostly *Picea* and *Pinus banksiana*, with scattered *Abies* and *Betula*, 13 Oct. 2021, A. Justo, AJ1826 (NBM-F-009430); *ibid.*, AJ1828 (NBM-F-009431); *ibid.*, (45.49213, -65.30287), under *A.*

balsamea and *P. glauca*, A.W. Hood, AH173 (NBM-F-009408); *ibid.*, (45.49210, -65.30271), under *Abies balsamea* and *P. glauca*, A.W. Hood, AH174 (NBM-F-009409); New Brunswick, Saint John County, Little Salmon River Protected Natural Area, Dustin Brook access trail to



Fig. 3. *Hydnum atlanticum* (holotype AJ1597). **A.** Basidium. **B.** Basidiospores. Scale bars = 10 μ m.

Fundy Footpath (45.500016, -65.277409), in conifer dominated forest (*Picea*, *Abies*), with some scattered *Betula*, on moss covered soil, 1 Sep. 2021, A. Justo, AJ1596 (NBM-F-009423); *ibid.*, AJ1602 (NBM-F-009424); *ibid.*, AJ1603 (NBM-F-009443); *ibid.*, AJ1609 (NBM-F-009425); AJ1614 (NBM-F-009426); *ibid.*, AJ1615 (NBM-F-009427); *ibid.*, (45.49910, -65.2774), under *A. balsamea*, *Betula papyrifera* and *P. glauca*, in very damp mossy area, A.W. Hood, AH111 (NBM-F-009402); *ibid.*, (45.49494, -65.27791), under *P. glauca* and *B. papyrifera*, A.W. Hood, AH117 (NBM-F-009405); *ibid.*, (45.49799, -65.27793), under *P. glauca*, with ferns along trail, A. W. Hood, AH116 (NBM-F-009404); *ibid.*, (45.49489, -65.27769), under *P. glauca*, *B. papyrifera* and *A. balsamea*, A.W. Hood, AH118 (NBM-F-009406); *ibid.*, (45.49923, -65.27658), under *P. glauca*, A.W. Hood, AH115 (NBM-F-009403); New Brunswick, Sunbury County, Grand Lake Protected Natural area, Coy Road (45.97962, -66.18163), under *A. balsamea*, 17 Aug. 2021, A. Justo, AJ1527 (NBM-F-009416); *ibid.*, AJ1528 (NBM-F-009417); Newfoundland and Labrador, Labrador, Division No. 10, Goose Bay, Tranquility Trail (53.533461, -60.1395), on soil under *Alnus*, *Pinus*, *Picea*, 10 Sep. 2016, E. Michelin, GBHV16A-344 (TENN-F-078322). **USA**, New York, Hamilton County, Raquette Lake, Silver Beach Bog, *Sphagnum* substrate under *Tsuga*, *Larix*, 550 m a.s.l., 28 Jul. 1982, A. Sabol, AS30 (CORT 007356).

DISCUSSION

Hydnum atlanticum was initially recorded in Swenie et al. (2018) as “*Hydnum* sp. AS30”, but it was not formally described as only one collection was available at the time. In the present paper

we study 33 recent Canadian collections of this taxon, 32 from New Brunswick and one from Newfoundland and Labrador, and formally describe the species. The New Brunswick collections were part of an intensive 1-year study of *Hydnum* in the province, where a total of 162 collections of *Hydnum* were made, and subsequently 132 collections were selected for sequencing. All newly generated ITS sequences are included in the phylogeny presented here (Fig. 1), including three species newly recorded in Canada as part of this study: *Hydnum alboaurantiacum*, *H. cuspidatum* and *H. vagabundum*.

The external morphology of *H. atlanticum* is very similar to other species in subgenus *Rufescentia*, with small to medium basidiomes and brown to orange brown or yellow brown colors. Colors of the basidiomes tend to be a bit more dull brown than other species of this subgenus, but slightly more brightly colored basidiomes can occur. *Hydnum atlanticum* has only been collected so far in humid, moss-covered areas. It shares this character with *H. quebecense*, which occurs in the same habitats, often at the same time as *H. atlanticum*. In the field *H. quebecense* has brighter, more intense orange brown or reddish brown colors. The difference in color of the basidiomes was clear enough in the field that specimens of *H. atlanticum* and *H. quebecense* were recognized as different species whenever they were collected together in the same area. Microscopically, basidiospore size and number of sterigmata per basidium separate the species well: *H. atlanticum* has smaller basidiospores (av. 7.2–7.7 \times 6.5–6.9 μ m), and predominantly three or four sterigmata per basidium, while *H. quebecense*

has larger basidiospores (av. $8.4\text{--}8.9 \times 7.8\text{--}8.1 \mu\text{m}$; cf. Niskanen *et al.* 2018 and Swenie *et al.* 2018), and predominantly two sterigmata per basidium. Color differences might not be clear or consistent enough to separate *H. atlanticum* from other species in subgenus *Rufescentia*. In these cases, habitat can offer important clues for identification, with *H. atlanticum* fruiting in humid, bryophyte-covered soil in conifer-dominated forests. As with other taxa in subgenus *Rufescentia*, sequence data might be needed for confident identification.

Based on the ITS phylogeny presented here (Fig. 1), *H. atlanticum* belongs in subgenus *Rufescentia*, section *Rufescentia* characterized by small to medium basidiomes with deeply colored (brown, orange) pilei and globose to ellipsoid basidiospores (Niskanen *et al.* 2018). Phylogenetically, *H. atlanticum* is recovered as part of subsection *Tenuiformia* together with *H. umbilicatum*, *H. elliposporum*, *H. cuspidatum*, *H. quebecense*, *H. subconnatum* and *H. oregonense* (Fig. 1). Niskanen *et al.* (2018) characterized subsection *Tenuiformia* morphologically by the combination of small to medium basidiomes (pileus 20–45 mm), non-decurrent spines and rather large basidiospores that are ovoid-subglobose (av. $> 8.5 \times 7.5 \mu\text{m}$) or ellipsoid (av. $10.0 \times 6.6 \mu\text{m}$). *Hydnum atlanticum* deviates from this morphological characterization by the larger basidiomes (pileus up to 77 mm), often subdecurrent spines, and smaller basidiospores (av. $7.2\text{--}7.7 \times 6.5\text{--}6.9 \mu\text{m}$). Subsection *Tenuiformia* does receive good phylogenetic support in the ITS analysis.

According to the ITS phylogeny the closest relatives of *H. atlanticum* are *H. oregonense* and *H. subconnatum* (Fig. 1). *Hydnum oregonense* differs by the slightly smaller basidiomes (up to 45 mm), more yellow-orange tones in the pileus, bigger basidiospores (av. $9.5 \times 9.0 \mu\text{m}$), and predominantly 2-spored basidia (Niskanen *et al.* 2018). This species occurs only in Western North America associated with conifers (*Pinus*, *Tsuga*, *Pseudotsuga*). *Hydnum subconnatum* differs from *H. atlanticum* by the often caespitose basidiomes, the bigger basidiospores (av. $8.9 \times 8.5 \mu\text{m}$), and the habitat in hardwood dominated forests of the Southeastern and Midatlantic USA (North Carolina, Tennessee, Georgia, Pennsylvania). We did not collect *H. subconnatum* as part of our 1-year survey of the New Brunswick species, but during the preparation of this article one collection of this species has been confirmed in New Brunswick (<https://www.inaturalist.org/observations/140315127>). The NB collection, a first record for Canada, was collected in a section of an urban park dominated by old *Tsuga canadensis* trees, with some *Fagus* in the mix.

In addition to ITS sequences, we selected 4 collections of *H. atlanticum*, including the holotype, to generate partial *TEF1- α* and *rpb2* sequences (GenBank OQ236551–OQ236558). We did not perform a phylogenetic analysis based on these loci, as the sampling across *Hydnum* is much more limited than for ITS. We did BLAST our newly generated sequences to search for possible matches in GenBank, but no match was found for either loci. The closest match for the *TEF1- α* sequences of *Hydnum atlanticum* is *H. elliposporum* (GenBank KU612773; 97.4 % sequence similarity, 13 individual nucleotide differences). The closest match for the *rpb2* sequences of *Hydnum atlanticum* is *H. umbilicatum* (GenBank LC717869; 98.4 % sequence similarity, nine individual nucleotide differences).

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Conflict of interest: The authors declare that there is no conflict of interest.

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