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## ***Apopellia endiviifolia* (Dicks.) Nebel & D.Quandt confirmed in North America**

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**Abstract.** Specimens morphologically identified as the liverwort *Apopellia endiviifolia* have been collected in Quebec, Canada, for over 20 years. Their identity as this species is herein confirmed by DNA sequence data, and the species range is extended to the United States, in Maine and Vermont.

**Keywords.** Liverwort, Marchantiophyta, DNA sequencing, North America, range extension.

### INTRODUCTION

Until the late 20<sup>th</sup> century, the liverwort *Pellia endiviifolia* (Dicks.) Dumort. was widely reported from North America, often under the older, illegitimate name *P. fabroniana* Raddi. After extensive field work spanning 35 years, and after studying specimens of "*P. endiviifolia*" from both Europe and the eastern half of North America, Schuster (1981) noted that all of the eastern North American plants lacked the "autumnal proliferations so characteristic of European plants". He also reported, among other characters, that their spores were much larger than those of European plants, and concluded that all eastern North American material so far identified as *Pellia endiviifolia* should be referred to a new species, *Pellia megaspora* R.M.Schust. This conclusion was reaffirmed in Volume V of *Hepaticae and Anthocerotae of North America* (Schuster, 1992).

Schuster (1992) did recognize specimens from the western half of North America as *Pellia endiviifolia* (including subsp. *alpicola* R.M. Schust.). However, when Schütz et al. (2016) elevated *Pellia* subg. *Apopellia* to generic rank, with three recognized species, *Apopellia endiviifolia* (Dicks.) Nebel & D.Quandt, *A. megaspora* (R.M. Schust.) Nebel & D.Quandt, and *A. alpicola* (R.M. Schust. ex L. Söderstr., A. Hagborg & von Konrat) Nebel & D.Quandt, they considered all North American populations to be either *A. alpicola* or *A. megaspora*.

In the meantime, Faubert et al. (2012) reported several specimens of *Pellia endiviifolia* sensu stricto from Quebec, Canada, with characteristic apical propagules. The inclusion of both *P. endiviifolia* and *P. megaspora* in a local flora (Faubert, 2012) prompted the discovery of additional occurrences of *P. endiviifolia* from Quebec (Leclerc, 2013). As of today, at least 26 occurrences are known from the province, mainly from the Quebec City area (Faubert et al., 2014+).

However, the above reports of *Pellia endiviifolia* sensu stricto were largely ignored outside of Quebec, and the species, now named *Apopellia endiviifolia*, was excluded from the North American flora by Schütz et al. (2016), Stotler & Crandall-Stotler (2017), as well as Konstantinova et al. (2023), based on the segregation of western populations as *A. alpicola*.

This paper confirms the occurrence of *Apopellia endiviifolia* in northeastern North America and extends the verified range of the species to Maine and Vermont.

### METHODS

Ten samples morphologically assigned to *Apopellia endiviifolia* were collected from Canada (Quebec) and the United States (Maine, Vermont). DNA extractions were made from air-dried herbarium specimens, with the extractions, PCR amplification and sequencing commonly-used for plant DNA barcoding markers (plastid markers *rbcL*, *psbA-trnH*, *trnL-trnF*; nuclear ribosomal marker ITS2) following standard protocols (Forrest and Hart, 2023; Nishii et al., 2025). Reads were checked against the NCBI database GenBank, using BLASTn. Specimen data and the sequence reads were also uploaded to the Barcode of Life database (Ratnasingham et

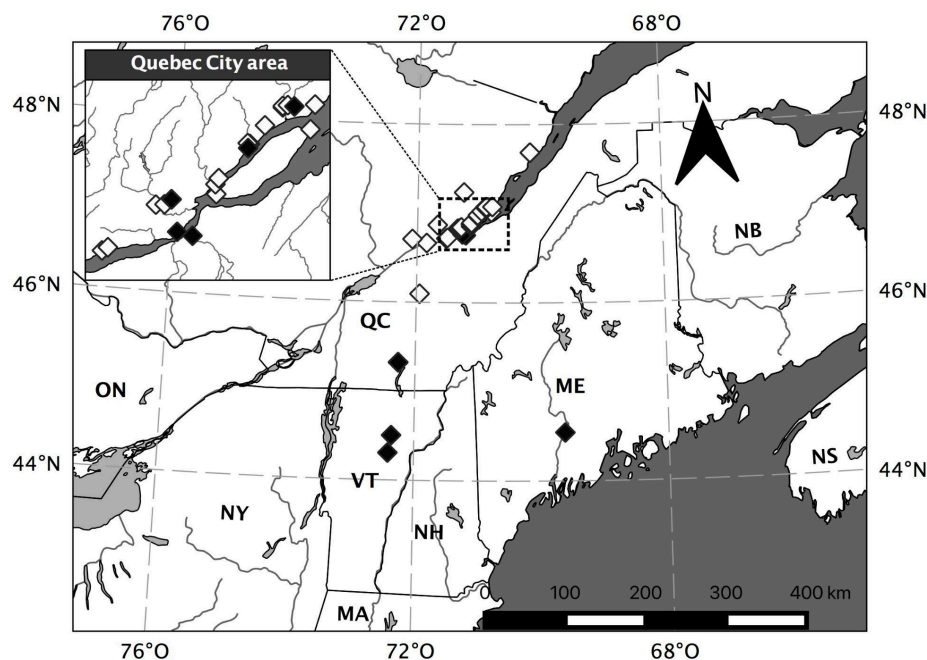
al., 2024; BOLD DS-PELLIA), along with DNA barcode data for 15 European accessions of *A. endiviifolia*. This database is openly accessible.

## RESULTS AND DISCUSSION

The ten North American accessions of *Apopellia endiviifolia* that were sampled had identical sequences for all four markers (*rbcL*, *psbA-trnH*, *trnL-trnF*, ITS2). An NCBI BLASTn search of GenBank shows that the sequences are a 100% match to several other accessions identified as *A. endiviifolia*; they are also a very close match (with 1 base pair difference in *rbcL*) to the sequences for 14 of the 15 European accessions in the BOLD DS-PELLIA dataset (the exception is a collection from Latvia that matches “species A”, or “water form”, from Sawicki et al., 2021). Of the three reportedly cryptic species described within *A. endiviifolia*, the North American accessions match the lineage that has been labelled “EU” (Schütz et al., 2016), “typical form” or “species B” (Sawicki et al., 2021).

In contrast, compared with GenBank sequences for *Apopellia megaspora* submitted by Konstantinova et al. (2023) and Schütz et al. (2016), our *trnL-trnF* sequences are a maximum 94% match (33 variable positions over 550 bases), and our ITS2 sequences are a maximum 88% match (36 variable positions over 310 bases). Compared with GenBank accessions of *Apopellia alpicola*, our *trnL-trnF* sequences are a maximum 94% match (23 variable positions over 526 bases), and our ITS2 sequences are a maximum 92% match (29 variable positions over 386 bases).

Based first on morphological evidence, but also here on DNA sequence data, *Apopellia endiviifolia* is confirmed for North America, and reported here as new to the United States. It is not possible to say whether this stems from a recent introduction to North America, as the DNA barcode markers we have used show no, or little, within-lineage variation between accessions from widely separated collections - e.g., the *trnL* locus used in Konstantinova et al. (2023) shows no genetic variation within the *Apopellia endiviifolia* “typical form” between plants collected in Russia, Greece, Germany, Italy, India, North America etc., while the ITS2 locus shows no variation between plants collected in Russia, Scotland and North America. It is clear that the species has been present in Quebec since at least 2003 (*Claude Roy 2003-10-10*, QFA0503857, det. M. Favreau 2024). The species has been collected in the United States only since 2021. The known distribution of *Apopellia endiviifolia* in North America is shown in Figure 1.



**Figure 1.** Map of southeastern Canada and northeastern United States, showing the known distribution of *Apopellia endiviifolia* in North America. Black diamonds represent specimens sampled for DNA sequencing, while open diamonds represent other specimens morphologically assigned to *A. endiviifolia*. Two of the sampled specimens were from the same locality and are represented by the same diamond.

Morphologically, North American material of *Apopellia endiviifolia* agrees with European material in all respects, including spores that are smaller than those of *A. megaspora*, male organs scattered in individual pustules along the thallus midrib (as opposed to aggregated in an apical disk in *A. megaspora*), anthocyanin pigments frequently present (never present in *A. megaspora*), and the regular production of dichotomously branching terminal propagules (never produced by either *A. megaspora* or *A. alpicola*). However, while in Europe those propagules are only observed toward the end of the growing season, in Quebec they are visible in specimens collected throughout the season, including in a sample collected in early April.

Most of the North American populations of *Apopellia endiviifolia* were found on calcareous substrates or at least in areas where such conditions are frequent; this type of habitat is characteristic of both *A. endiviifolia* and *A. megaspora*, while *A. alpicola* is known to prefer neutral or slightly acidic substrates (Konstantinova et al., 2023). Also, several of the Quebec populations occur in disturbed man-made habitats, such as a footpath or a stone quarry, which is characteristic of *Apopellia endiviifolia* but rarely the case in either *A. megaspora* or *A. alpicola*.

**Specimens sampled for DNA sequencing – U.S.A., MAINE:** Robert S. Capers 3772, CONN (lat/long: 44.54535, -69.62633; Habitat: rocky shore of Kennebec River. Calcareous seep. On soil at base of shrub and in cracks of rock); **VERMONT:** Matthew J. Peters 23-182a, UVMVT252946 (lat/long: 44.50413, -72.35623; Habitat: on thin silt layer on river bank cobbles and boulders); 23-216a, UVMVT252954 (lat/long: 44.30748, -72.40737; Habitat: on recently flood-scoured banks); **CANADA, QUEBEC:** Annie St-Louis ASTL2021-108, QFA0651703 (lat/long: 46.758, -71.213; Habitat: river bank); Mélodie Paquet MP-2023-CP-01, QFA0651756 (lat/long: 46.767, -71.267; Habitat: in a lawn, growing with a pleurocarp moss; very shady humid site); Stéphane Leclerc STL-0813, QFA0651759 (lat/long: 46.845, -71.288; Habitat: on bare clay soil on the edge of a stream, in the flood zone. Willow forest); STL-0812, QFA0651758 (lat/long: 46.972, -71.019; Habitat: on bare clay soil on the edge of a stream, in the flood zone); STL-0811, QFA0651757 (lat/long: 47.073, -70.855; Habitat: at the bottom of a seeping limestone wall, on the edge of a stream); Marc Favreau JLAMF-3563, QFA0651755 (lat/long: 45.322, -72.269; Habitat: in the gravel of a path, in a young forest of *Betula*, with saplings of *Fagus grandifolia* and other deciduous trees); JLAMF-3564, QFA0651754 (lat/long: 45.322, -72.269; Habitat: in the gravel of a path, in a young forest of *Betula*, with saplings of *Fagus grandifolia* and other deciduous trees).

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