

**Rapid Communication****First record of the Asian *Antithamnion sparsum* Tokida, 1932 (Ceramiales, Rhodophyta) in Nova Scotia, Canada**

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**OPEN ACCESS****Abstract**

We report the first record of a non-native species of *Antithamnion* detected on the eastern shore of Nova Scotia, Canada and assign these collections to the Asian *Antithamnion sparsum* based on available morphological and molecular data. Two collections of this non-native species were made at three sites in the subtidal (10 m) in August 2021, and it was observed as a dominant subtidal (10 m) turf at an additional four sites from April to August 2022. We describe here morphological traits that match the original type description of *A. sparsum* and generated *rbcL* 3P data (851 bp) for two 2021 collections which closely match a record in Genbank from South Korea that is likely *A. sparsum* (99.9% identity). These collections represent the first occurrence of *A. sparsum* in the Northwest Atlantic.

**Key words:** invasive species, northwest Atlantic, cryptic species, non-indigenous species, Ceramiaceae

**Introduction**

Members of the genus *Antithamnion* Nägeli, 1847 are generally diminutive, fine red uniseriate filaments defined by a combination of pinnate branch habit, the form and position of gland cell bearing branches, the length (in cells) of tetrasporangial branches and the position of indeterminate laterals relative to determinate branches (Kim et al. 2008). Members of the genus *Antithamnion* reportedly grow (in culture) at temperatures of 15–19 °C and reproduce between 16–19 °C, undergoing a typical triphasic life history characterized by a carposporophyte, tetrasporophyte and dioecious gametophyte stages (Boo and Lee 1983). The taxonomic history of this genus, and of *Antithamnion sparsum* Tokida, 1932 in particular, is confused (Lindstrom and Gabrielson 1989; Kim et al. 2008) owing to the morphological similarities within members of *Antithamnion* and between sister genera (particularly *Antithamnionella* Lyle, 1922 (Wollaston 1968)).

Only two species of *Antithamnion* are reported from the northwest Atlantic. *Antithamnion cruciatum* Nägeli, 1847 is reportedly a late summer annual distributed along the entire eastern coast of North America from

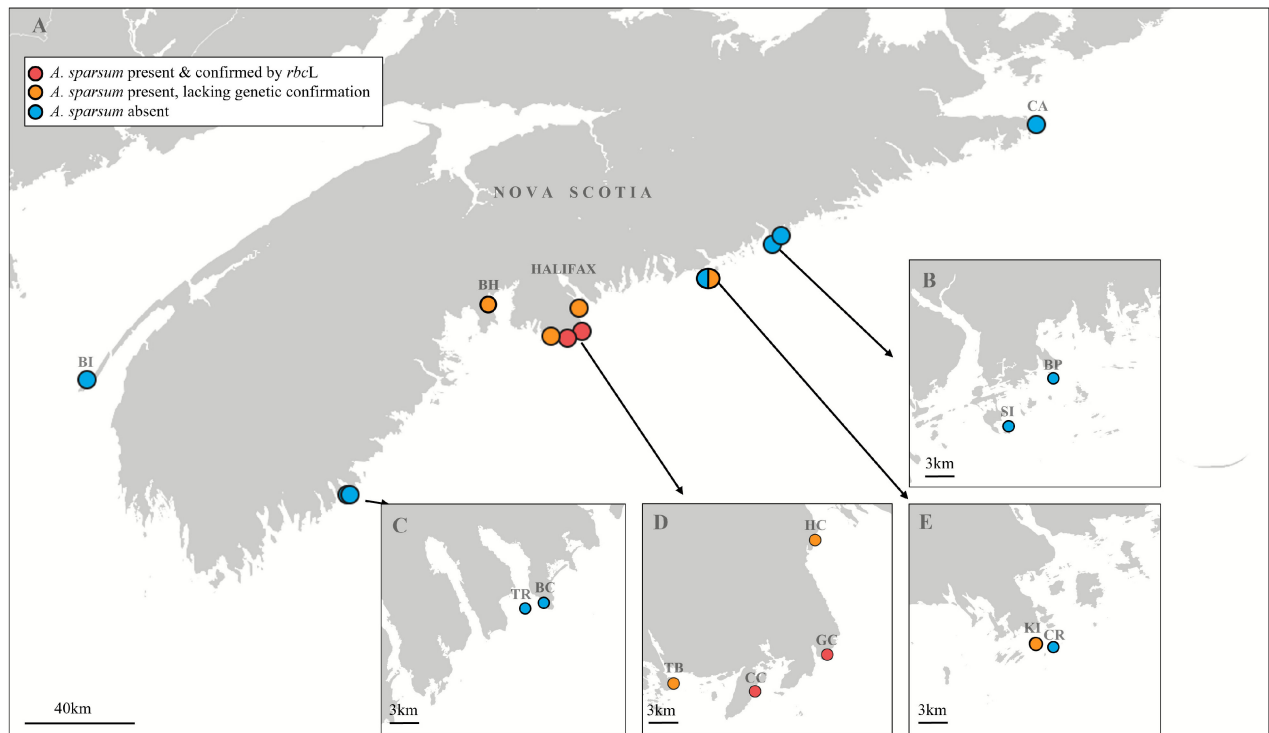
Newfoundland to Florida, U.S.A (Mathieson and Dawes 2017) and *A. hubbsii* E.Y Dawson, 1962, an aseasonal annual for which Cape Cod, Massachusetts, U.S.A is reportedly the northern range limit. To date, there are three genetically verified Canadian collections of *A. cruciatum* and no genetically verified Canadian records of *A. hubbsii* (Saunders 2022). Specimens collected from the eastern shore of Nova Scotia, Canada, in 2021 are morphologically distinct from both *A. cruciatum* and *A. hubbsii* and instead match the type description of *A. sparsum*.

*Antithamnion sparsum* was originally described from Sakhalin, Japan (Tokida 1932) and later reported as an introduced species in Ireland (as *Antithamnion densum*) by Guiry and Maggs (1991). Those authors noted in their collections a branching habit typical of *A. sparsum* and not *A. densum*—unpaired indeterminate laterals arising every third or fourth branch—suggesting their collections are more appropriately assigned to *A. sparsum*. These Irish collections were found subtidally at depths of 5–10 m growing epiphytically on *Meredithia microphylla* (J. Agardh) J. Agardh, 1892 at a highly wave-exposed site. Moreover, the authors noted that while some of their collections bore tetrasporangia, no mature male or female structures were observed and highlighted similar behavior in *Antithamnion cruciatum* at its northern range-limit in Newfoundland (Whittick and Hooper 1977), suggesting that monosporogenesis may be rare in antithamnioid algae near their northern range limits. While habitat and reproduction (or lack thereof) of these Irish collections is well-documented, the impacts of that introduction remain understudied and therefore the strength of *Antithamnion sparsum* as an invasive species remains unknown. At least one close relative has successfully invaded elsewhere and may be an adequate proxy; *Antithamnion nipponicum* (Yamada and Inagaki 1935) was introduced to the lagoon of Venice in 1994 and as of 2011 the wet weight of this species was estimated to be roughly three tonnes across the entire lagoon (432 km<sup>2</sup>) (Sfriso et al. 2020), ranking 14<sup>th</sup> of 29 non-indigenous species by biomass in the lagoon.

We report here on the first occurrence of a species of *Antithamnion* and putatively assign it to *Antithamnion sparsum* based on available morphological and molecular data. If this identification is correct, this record represents a range expansion of this species and the first record of *A. sparsum* in Nova Scotia, and in the Northwest Atlantic.

## Material and methods

Subtidal surveys to document the presence and abundance of algal species in the rocky subtidal (3–10 m depth) were conducted at six sites on the Atlantic coast of Nova Scotia, Canada in August and September 2021. Surveys consisted of SCUBA divers collecting all seaweeds in eight 0.125 m<sup>2</sup> quadrats along a “deep” (approx. 8–10 m) and “shallow” (approx. 3–5 m) transect (50 m) at each site. Sites sampled included Crystal Crescent Beach



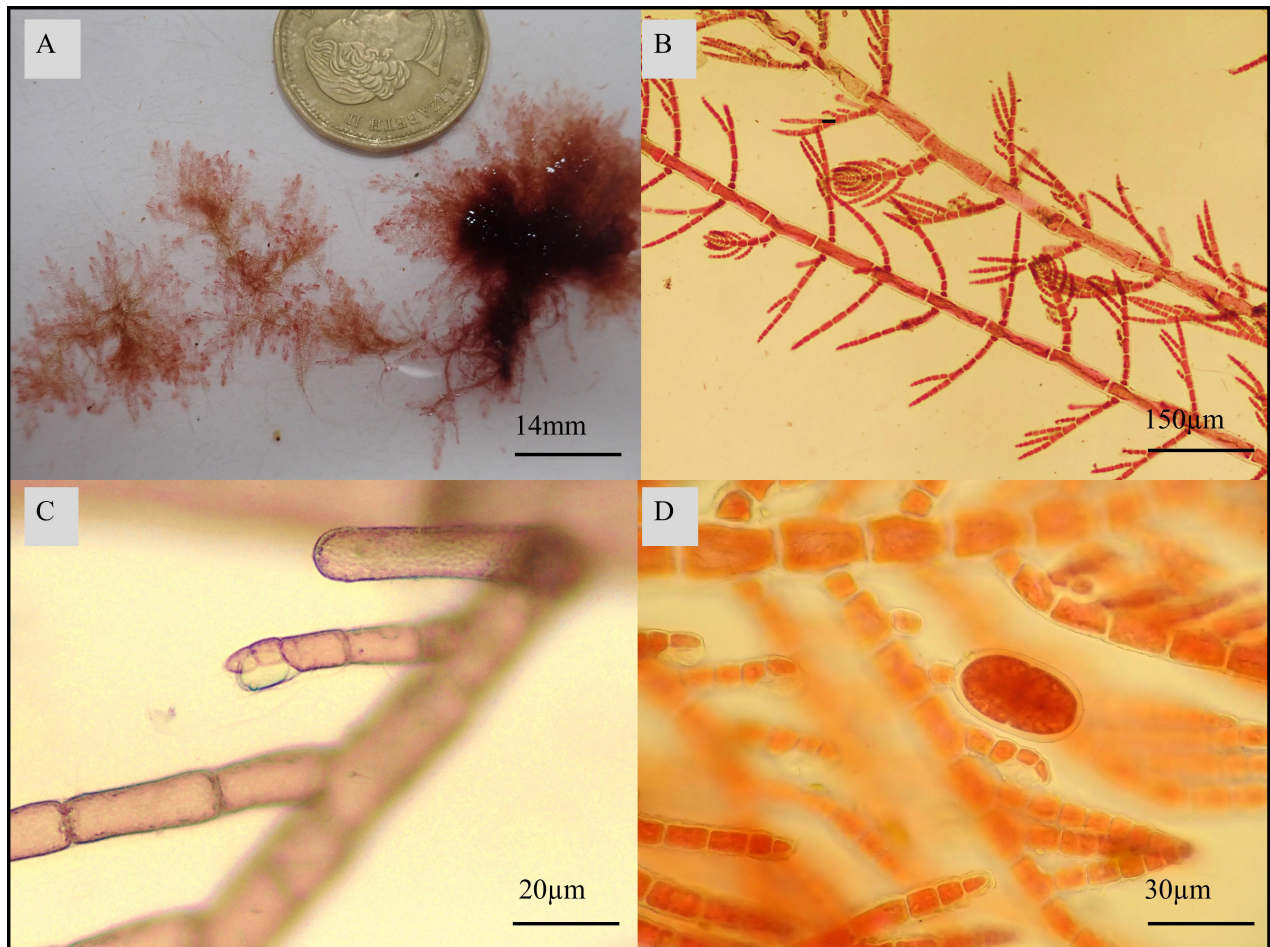
**Figure 1.** Map of site locations. BH: Birchy Head, BI: Brier Island (Garbary et al. 2019), BP: Beaver Point, SI: Sober Island, TR: Thomas Raddall (Miller and Wong 2010), BC: Boyd's Cove (Miller and Wong 2010), CA: Canso, CC: Crystal Crescent, GC: Gill Cove, KI: Key Island, CR: Chain Rocks, TB: Terence Bay, HC: Herring Cove (Supplementary material Table S1).

(44.456; -63.61567), Gill Cove (44.4843; -63.53373 ), Key Island (44.69991; -62.82885), Chain Rocks (44.6977; -62.80729), Sober Island (44.83324; -62.4491), and Beaver Point (44.86681; -62.39813) (Figure 1). Between March and May 2022 we also performed haphazard visual surveys for this species at Terence Bay (44.4619; -63.7081), Herring Cove Provincial Park (44.5774; -63.5482) and Birchy Head (44.5685; -64.0363).

Samples were stored at  $-20\text{ }^{\circ}\text{C}$  prior to further processing. Upon discovery of the species in quadrat samples, a  $1\text{ cm}^2$  piece of material was taken from each plant and dried in silica for subsequent DNA extraction at the Center for Marine Algal Research following Saunders and McDevit (2012). The *rbcL* region was amplified using primers F57 and *rbcLrevNew* following thermocycler protocols outlined in Saunders and Moore (2013) and the 3P end was sequenced using *rbcLrevNew* at Génome Québec on an Applied Biosystem 3730xl DNA Analyzer. Raw data were edited to a final length of 851 bp in Geneious R8.1 (BioMatters Inc., San Diego) and compared against available sequences in BOLD (Barcode of Life Data System; Ratnasingham and Hebert 2007) and Genbank to confirm identifications. Edited sequence data were deposited in Genbank (Genbank Accession OP600458 and OP600459).

## Results

Our collections are fine red filaments to 3 cm in height with an overall distichous branching habit and paired opposite branches (Figure 2). Indeterminate laterals appear three to five nodes and lack a paired opposite



**Figure 2.** Morphology of collected specimens of *Antithamnion sparsum*. Macroscopic view (A), typical branching habit (B) including indeterminate laterals with suppressed opposite branch, gland cell on short, specialized stalk (C), and a tetrasporangium borne on a 1-celled pedicel (D).

determinate lateral. Determinate branches are oppositely pinnate, adaxially secund and bear gland cells on branchlets ~ 4 cells in length, these gland cells always covering two parent cells. A single tetrasporangium was observed in our collections, borne on a single-celled stalk. No male or female reproductive structures were observed. Our collections are compared against features of the type descriptions of *Antithamnion cruciatum*, *A. hubbsii*, and other morphologically similar *Antithamnion* taxa in Table 1 (Howe 1914; Kim 2008; Kylin 1925; Nägeli 1847; Saunders 2022; Wollaston 1968). Morphologically our two collections are a good match for either *A. sparsum* or *A. defectum* Kylin, 1925 and distinct from the local species *A. cruciatum*, which branches in a decussate rather than distichous pattern. The distichous branching of our new collections clearly distinguishes it from the more local *A. cruciatum* and unpaired indeterminate laterals suggest either *A. sparsum* and *A. defectum*, which themselves form a cryptic complex (Kim et al. 2008). Morphologically, the key distinguishing features of our collections are only a match to this *A. sparsum/defectum* complex (Table 1).

We successfully generated 851 bp of *rbcL* data for two collections, one each from Crystal Crescent beach and Gill Cove from 8–10 m depth, and have

**Table 1.** Distinguishing characteristics of local and morphologically similar *Antithamnion* spp., as well as *Antithamnionella spirographidis*.

Species	Gland cells	Indeterminate laterals	Pinnae	Branching plane	Tetrasporangia
New collections, assigned to <i>Antithamnion sparsum</i> Tokida, 1932	On pinnae, covering 2 cells	Unpaired	Adaxially pectinate	Distichous	1-cell pedicel
<i>Antithamnion cruciatum</i> Nägeli, 1847	On pinnae, covering 2 cells	Paired w/ determinate lateral	Branched, decussate	Decussate	1-cell pedicel or sessile, tetrahedral
<i>Antithamnion defectum</i> Kylin, 1925	On pinnae, covering 2 cells	Unpaired	Branched, pectinate	Distichous	Pedicellate, undefined pedicel length
<i>Antithamnion densum</i> Howe, 1914	On pinnae, covering 2 cells	Paired w/ determinate lateral	Adaxially pectinate	Distichous	1-cell pedicel
<i>Antithamnion hubsii</i> Dawson, 1963	Borne on pinnae, adaxial, covering 2 cells	Paired	Pinnate	Distichous	NA
<i>Antithamnion sparsum</i> Tokida, 1932	On pinnae, covering 2 cells	Unpaired	Adaxially pectinate	Distichous	1-cell pedicel, rarely sessile or 2-celled
<i>Antithamnionella spirographidis</i> Wollaston, 1968	On primary branches, partly covering 1 cell	Unpaired	Simple	Distichous	Sessile

collections lacking molecular data from Terence Bay, Herring Cove Provincial Park, Birchy Head and Key Island which nonetheless are morphologically identical to sequenced collections. Both sites are highly exposed to the south, southeast, and east (max fetch distance > 1,000 km), representing the dominant direction from which storms approach and pass the Atlantic coast of Nova Scotia (Hart and Evans 2001). Our sequences share 100% identity with each other over 851 bp. The closest match to our collections available in GenBank is assigned to *Antithamnionella miharai* (Tokida) Itono, 1977 (GQ252485.1), a synonym of *A. spirographidis* (Schiffner) E.M. Wollaston, 1968 (Lindstrom and Gabrielson 1989) and differs from our sequences in five ambiguous base calls, all at highly conserved sites. This *A. spirographidis* collection is ~ 12% different from other *Antithamnionella* spp. but only 2–5% different from other *Antithamnion* spp., suggesting this collection was misidentified and should be assigned to *Antithamnion*. After removing the ambiguous base calls from this collection it differs from our new collections by a single base pair (99.9% identity) and otherwise groups nearest to *A. sparsum* collected in South Korea (JN089392; 98.8% identity; this latter sequence shares none of the ambiguities of the former *A. miharai* sequence and appears to be of good quality). Given that the *A. miharai* sequence was collected in South Korea, relatively near to the type locality of *A. sparsum* (Sakhalin, Japan), it seems likely that this sequence, as well as our own, belongs to *Antithamnion sparsum*.

## Discussion

Considerable taxonomic confusion exists between *Antithamnion sparsum* (type locality: Sakhalin, Japan), *A. defectum* (type locality: Washington, USA) and *A. densum* (Suhr) M.Howe, 1914 (type locality: Peru), confusion which must be resolved, at least in part, before inferences can be made regarding this new occurrence, its origin and identity. A collection matching the type description of *A. sparsum* has been previously reported in Ireland (Guiry and Maggs 1991, as *A. densum*). The authors of that study followed a review of Antithamniae (Athanasias 1990) and considered *A. sparsum*,

*A. defectum* and *A. densum* conspecific, however the type descriptions of *A. defectum* and *A. densum* refer to unpaired indeterminate laterals (see Figure 2B) while the indeterminate branches of *A. densum* have an opposite, determinate branch. The three species are then morphologically distinct and these Irish collections are a match to *A. sparsum* and *A. defectum* but not to *A. densum* (Table 1). The status of these two species as separate entities has been debated (Kim 2008; Boo and Lee 1983), but for now *A. defectum* and *A. sparsum* remain separate species for which available genetic data is sparse. Our collections are a good match (99.9% over 851 bp) to a sequence likely misidentified as *Antithamnionella miharai* in GenBank, but which was collected near the type locality of *A. sparsum* (Sakhalin, Japan) and likely belongs to this group rather than *Antithamnionella*. This collection, as well as our own, is nearest to *A. sparsum*, and so we assign our collections to this species.

Several recent surveys of the Nova Scotia marine flora have been published, however none to date include the sequence data or anatomical notes of their collections necessary to confirm the presence of *Antithamnion sparsum*. Surveys of Brier Island (Garbary et al. 2019), Canso & Boyd's Cove (Miller and Wong 2010) have not reported any species of *Antithamnion*, while others have reported *A. cruciatum* but not *A. sparsum* (Mathieson and Dawes 2011), and others yet have reported only red turf (Vandermeulen 2018, though the author does note that *Antithamnion* spp. may be among the turf-forming species at their sites). Collections matching the type description of *A. sparsum* have also been reported from the Northeastern Atlantic (Guiry and Maggs 1991, as *A. densum*), it therefore seems likely that *A. sparsum* crossed the Atlantic as a hull foulant from Europe, as was the case with *Codium fragile* (Suringar) Hariot, 1889 (Carlton and Scanlon in Bird 1993) and *Dasyisiphonia japonica* (Yendo) H.-S.Kim, 2012 (Schneider 2010). It is possible that *A. sparsum* will subsequently invade the Gulf of Saint Lawrence through human-mediated vectors, similar to *Bonnemaisonia hamifera* Hariot, 1891 and *Codium fragile* (Watanabe and Scheibling 2010; Johnson et al. 2012).

In field surveys in 2022, *Antithamnion sparsum* was exceedingly common at Birchy Head as a dense epiphytic turf. Though the persistence of this dense *A. sparsum* turf through the year remains unknown, it was abundant as early as mid-April, coinciding with the critical period of sporophyte recruitment of *Alaria esculenta* (Linnaeus) Greville, 1830 (Birkett et al. 1998), *Laminaria digitata* (Hudson) J.V. Lamouroux, 1813 (Martins et al. 2017) and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl and G.W. Saunders, 2006 (Andersen et al. 2011). Ecological effects of *Antithamnion sparsum* as an invasive species are not well studied, however turf communities are associated with kelp forest decline and silt accumulation caused by turf and invasive communities inhibit the recruitment of new

kelp gametophytes (Filbee-Dexter 2016; Connell and Russell 2010). This new introduction could therefore contribute to, or accelerate, the documented regime shift from kelp forest to turf habitat already occurring globally (Pessarrodonna et al. 2021) and accelerate the community-level shift from a native-dominated to an invasive macroalgal flora (Dijkstra et al. 2017). We report here the first occurrence of a new *Antithamnion* species in Nova Scotia, Canada and assign it to *Antithamnion sparsum* based on available morphological and molecular data. If this identity remains correct, these collections represent the latest in a series of introductions from the Northeast Atlantic. Further monitoring will be necessary to track this species' ecological impacts and its potential for impact on native floral and faunal communities.

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### Authors' contribution

CB, KK: research conceptualization; CB, KK: sample design and methodology; CB, KK: investigation and data collection; CB: data analysis and interpretation; KK: ethics approval; KK: funding provision; CB: writing – original draft; CB, KK: writing – review and editing.

### Ethics and permits

All research reported in this article was covered under permit number M-22-04, Maritime Aquatic Invasive Species Monitoring; Rapid Assessment and Research Project Support Through the Collection of Benthic and Pelagic Specimens. Ethics approval was not required.

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## Web sites, online databases and software

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Records of *Antithamnion sparsum* in Nova Scotia, Canada.

This material is available as part of online article from:

[http://www.reabic.net/journals/bir/2023/Supplements/BIR\\_2023\\_Brooks\\_Krumhansl\\_etal\\_SupplementaryMaterial.xlsx](http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Brooks_Krumhansl_etal_SupplementaryMaterial.xlsx)