BioInvasions Records (2013) Volume 2, Issue 3: 185–189
doi: http://dx.doi.org/10.3391/bir.2013.2.3.02

Open Access

Rapid Communication

A survey of Sam Orr's Pond (New Brunswick, Canada) uncovers the invasive green alga *Codium fragile* (Chlorophyta) and the orange-striped green anemone *Diadumene lineata* (Cnidaria), first records for the Bay of Fundy and Canada, respectively

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Received: 8 March 2013 / Accepted: 19 April 2013 / Published online: 3 May 2013

Handling editor: Melisa Wong

Abstract

A DNA-barcode-based bio-inventory of macroalgae and macro-invertebrates in Sam Orr's Pond, New Brunswick, was completed as part of the Canadian Barcode of Life Network's efforts to generate a comprehensive comparative DNA database (BOLD) for rapid species identification. During an early sampling episode the invasive species *Codium fragile* was identified and subsequently confirmed with *tufA* sequence data – the first record of this species in the Bay of Fundy. An unidentified sea anemone was also collected, but COI-5P data failed to find a match in current databases (BOLD and GenBank). Subsequent morphological identification resulted in the first Canadian records for *Diadumene lineata*. Our discoveries highlight the importance of ongoing field monitoring, trained taxonomic expertise and comprehensive DNA barcode libraries in global efforts to track the spread of introduced species.

Key words: *Codium fragile*; COI-5P; *Diadumene lineate*; DNA barcode; invasive; range extension; Sam Orr's Pond; *tuf*A

Introduction

Sam Orr's Pond (45.162 latitude, -67.045 longitude; Figure 1 A) is located twelve kilometers northeast of St. Andrews in Passamaquoddy Bay, an inlet on the New Brunswick side of the Bay of Fundy. The pond was likely formed by glacial ice action and is divided into two regions – an upper basin of ~16 acres and a lower (seaward) basin of ~3.5 acres (Mortimer and Downer 1961). Uplifting of the land or subsidence of the sea floor has occurred during recent geologic history separating the upper and lower basins by a sill. This separation limits the flow of saltwater into the upper basin to periods of high spring tides (Mortimer and Downer 1961). Freshwater enters Sam Orr's Pond from precipitation, surface drainage from the surrounding hills and from McTaggart Brook, which flows into the west side of the upper basin (Mortimer and Downer 1961).

Sam Orr's Pond first attracted serious attention from the biological community in 1951 with the discovery of the quahog *Mercenaria mercenaria* (Linnaeus), which occurs at only one other locality in the Bay of Fundy. This discovery led to a full biotic study of Sam Orr's Pond from the summer of 1957 through 1959 (Mortimer and Downer 1961). These observations established a solid baseline against which changes in flora and fauna can be recognized.

In light of its interesting geological history, proximity to the University of New Brunswick, established diversity baseline and defined boundaries, Sam Orr's Pond was considered a suitable location for a DNA-based bio-inventory by students as part of the Canadian Barcode of Life Network. We initiated this survey using the cytochrome *c* oxidase I gene (COI-5P) to assess macro-invertebrate life in the pond (Hebert et al. 2003), as well as for the red and brown seaweeds (Saunders 2005; McDevit and Saunders 2009), and the plastid elongation factor Tu gene (*tufA*) for the green seaweeds (Saunders and Kucera 2010). In addition to the expected macroflora and fauna, two additional species were uncovered, which interestingly did not rely on molecular data for their identification as introductions to this unique habitat.

Methods

Samples with their respective collection data are listed in Table 1 [only for *Codium fragile* (Suringar) Hariot and *Diadumene lineata* (Verrill)]. For algal collections, each specimen included a voucher pressed dry on herbarium paper and a corresponding subsample dried in a vial with silica gel for subsequent molecular analyses. Invertebrates were stored individually in vials with 95% ethanol, these serving as both voucher and for DNA analyses. Total DNA was extracted with a protocol modified from Saunders (1993) (instead of the final agarose gel cleaning procedure, the DNA was purified with the Wizard® DNA Clean-Up System, Promega Corp., Madison, WI). The plastid marker *tufA* was PCR-amplified for our green algal collections as outlined in Saunders and Kucera (2010), while COI-5P amplification followed Saunders and McDevit (2012) with the respective PCR primers used for each sample listed with that record on BOLD (http://www.boldsystems.org) and GenBank (Table 1). Sequencing used the PE Applied Biosystems Big Dye (v 3.0) kit following the manufacturer's protocol (ABI, Foster City, CA). Forward and reverse sequence reads from the respective PCR primers were edited and aligned using Sequencher™4.2 (Gene Codes Corporation, Ann Arbor, MI, USA). Resulting sequences were searched for matches at BOLD and GenBank.

Results and discussion

In addition to collecting and generating barcode data for many of the expected species reported from Sam Orr's Pond, we uncovered two species that are known to be invasive in nearby waters and which were not reported in the earlier survey of Mortimer and Downer (1961) viz. the green alga *Codium fragile* and the orange-striped green anemone *Diadumene lineata* (both discussed below). Of lesser note, but nonetheless a change since the baseline report of Mortimer and Downer, we also collected the common Atlantic slippersnail *Crepidula fornicata* (Linnaeus) and the European green crab *Carcinus maenas* (Linnaeus) at this location (data not shown). We identified substantially more macroalgal species than listed by Mortimer and Downer, including unattached populations of the red algae *Coccotylus brodiei* (Turner) Kützing and *Polyides rotundus* (Hudson) Greville, but only the green alga *Ulva laetivirens* Areschoug is of note here being possibly introduced from Australia (Kirkendale et al. 2013).

Codium fragile (Suringar) Hariot

Codium fragile is a large, dichotomously branched green alga native to the coasts of Japan that has spread throughout the world's oceans in the last century (Trowbridge 1998). It was first documented growing along the east coast of North America in 1957 (Bouck and Morgan 1957) and has since become a problem species, spreading along the coast into the Maritime Provinces of Canada where it can grow at high densities (Bégin and Scheibling 2003). The distinctive morphology (Figure 1B) left no doubt as to the identity of these individuals, the *tufA* data only confirming that the Sam Orr's Pond populations were genetically identical to others in the Maritimes. To our knowledge this is the first record of this species from the Bay of Fundy. We first found this species in Sam Orr's Pond in 2008 and during our scuba exploration in 2009 and 2010 it dominated the lower basin forming extensive meadows. Since that time, the number of individuals has dropped off precipitously and we collected very few individuals in 2011 and 2012. The decline may have to do with the temperature tolerance of *C. fragile*, which is typically considered a warm water species (Hanisak 1979; Trowbridge 1998). However, growth and reproduction of this alga has been reported to occur at temperatures as low as 12^{o}C and some individuals survived temperatures of -2^{o}C (Chapman 1999).

Fragments of *Codium fragile* can be buoyant (Trowbridge 1999) and likely floated from populations in the Gulf of Maine into the lower basin of Sam Orr's Pond during high tide. The fragments would not be easily flushed from this basin once introduced presenting ample opportunity to establish resident individuals. Vegetative propagation through the formation of buds and or fragmentation, as well as parthenogenic development of female gametes (Prince and Trowbridge 2004), could rapidly populate the lower basin. Once established in the

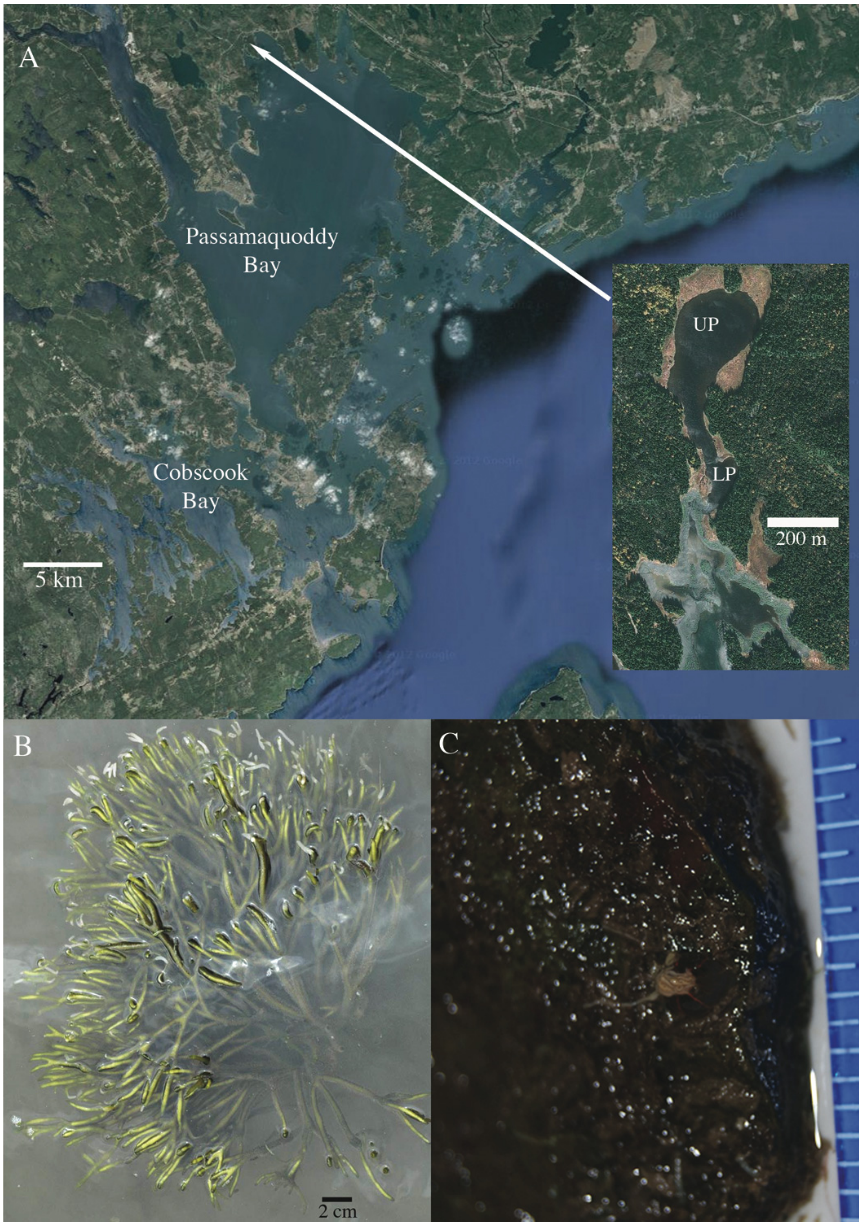

Figure 1. A. Map of Cobscook Bay, Maine, and Passamaquoddy Bay, New Brunswick, with an insert magnification of the upper (UP) and lower (LP) basins of Sam Orr's Pond (maps.google.ca). **B**. *Codium fragile* from Sam Orr's Pond (photo by GWS). **C**. *Diadumene lineata* from Sam Orr's Pond (ruler in mm divisions; photo by NH).

Table 1. Vouchered collections of *Codium fragile* and *Diadumene lineata* from Sam Orr's Pond and GenBank accessions for *tufA* and COI-5P, respectively. LB and UB indicate lower and upper basins (Figure 1 A), respectively.

Name	BOLD voucher	Habitat	Date	GenBank
Codium fragile	ULVA744-09	Subtidal (0.25 m) on cobble, UB	23.7.08	No data
	ULVA745-09	Subtidal (0.25 m) on shell, UB	23.7.08	KC191578
	ULVA954-10	Subtidal (1 m) on cobble, LB	14.10.09	No data
Diadumene lineata	BIBOF039-10	Subtidal (1 m) on cobble, UB	14.10.09	HM884243
	BIBOF040-10	Subtidal (1 m) on cobble, UB	14.10.09	HM884244
	BIBOF041-10	Subtidal (1 m) on cobble, UB	14.10.09	HM884245

lower basin a subsequent spread to the upper basin would have occurred during the highest tides.

Diadumene lineata (Verrill)

Whereas most of the collected invertebrates generated DNA barcodes with matches in BOLD and GenBank (data not shown) for species formerly reported from Sam Orr's Pond (Mortimer and Downer 1961), three of our 2009 collections from the upper basin had identical, but novel, COI-5P sequences. The closest match, 97%, was to a genetic cluster containing individuals assigned to both *D. lineata* and *D. leucolena* (Verill) most likely indicating some uncertainty regarding the identity of the specimens for these records in BOLD (hybridization between these species may also account for this confusion). Morphological identification resulted in our collections being considered *Diadumene lineata*, the orange-striped green anemone (Figure 1 C) – the distinctive orange strips up the column a clear defining feature of this species (Gerhard Pohle personal communication).

The orange-striped green anemone is native to the coasts of Japan, but has been documented in many locations owing to anthropogenic transport via shipping (Gollasch and Riemann-Zurneck 1996). For the northeast coast of North America this species was first collected from New Haven, Connecticut, in 1892 (Cohen 2011). Although reported from the neighboring state of Maine for decades (Shick 1976) and indeed recently from Cobscook Bay adjacent the Canadian-United States border (Trott 2004), we believe that this is the first report from Canadian waters. Outside of the native range, reproduction by longitudinal fission appears to be the sole means of propagation, which results in relatively large monoclonal populations in a local area over a short period of time (Shick 1976; Ting and Gellar 2000). Individuals of *D. lineata* in Sam Orr's Pond were found attached to small rocks at depths of about 1m and were very abundant. Although we will never know for certain, the presence of *D. lineata* in Sam Orr's Pond may have been facilitated by *Codium fragile,* which notoriously floats away with its substrate including living shellfish gaining it the common name "oyster thief" (Wassman and Ramus 1973). This mode of introduction is likely given that North American populations of *D. lineata* do not produce planula larvae, limiting their ability to disperse and suggesting that substrate carrying attached individuals were transported directly to Sam Orr's Pond. This raises the concern that the invasion of *C. fragile* into northeastern ecosystems could have provided a vector to facilitate the introduction and spread of other invasive species.

Conclusions

Ironically, we set out to complete a survey of macroalgae and macro-invertebrates in Sam Orr's Pond using genetic tools, which in the end were either not necessary or not useful for our two most significant finds.

In the "not necessary" category was our discovery of *Codium fragile*. Being a macroalgal laboratory, we identified this species immediately on discovery in the field; the molecular data only serving to confirm that this population is genetically consistent with invasive *Codium* along the northeastern coast of North America. However, had it not been for our involvement in building the BOLD library, this population may have gone unreported. Fortunately, at least for this population of *Codium*, that would not have been the case. Sam Orr's Pond is now included in the Nature Trust of New Brunswick's Caughey-Taylor Reserve and, as such, is frequented by dedicated citizen naturalists who independently noticed masses of 'white spaghetti' along the pond's edge around the time we made our discovery. These collections were sent to our lab and identified as dead *Codium fragile*.

In the "not useful" category was our discovery *Diadumene lineata* for which our COI-5P data failed to find matches in BOLD and GenBank. This highlights an obvious reality – bio-inventory surveys based on molecular tools are only as good as the comparative libraries that are available. Invertebrate taxonomists at the Atlantic Reference Centre, St. Andrews, generously confirmed our identification.

Clearly the early and accurate discovery of introduced species to an area requires comprehensive genetic databases, trained taxonomic experts able to confirm the results of the previous or give identification in lieu of comparative genetic data, and dedicated naturalists (professional and amateur) consistently frequenting an area to become familiar enough with its native flora and fauna to notice when something is different.

Acknowledgements

Sarah Hamsher, Melissa Lang and Dan McDevit are thanked for assistance with fieldwork, as is Tanya Moore for molecular assistance. Dr. Gerhard Pohle of the Atlantic Reference Centre confirmed invertebrate identifications. We thank the Nature Trust of New Brunswick for their interest in our work and for granting collection approval to work in this unique habitat. Craig Schneider and an anonymous reviewer provided constructive reviews that greatly improved this manuscript. This research was supported through funding to GWS from the Canadian Barcode of Life Network from Genome Canada through the Ontario Genomics Institute, Natural Sciences and Engineering Research Council of Canada and other sponsors listed at www.BOLNET.ca. Additional support to GWS was provided by the Canada Research Chair Program, the Canada Foundation for Innovation and the New Brunswick Innovation Foundation.

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