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Rapid Communication

First occurrence of the invasive hydrozoan *Gonionemus vertens* A. Agassiz, 1862 (Cnidaria: Hydrozoa) in New Jersey, USA

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Abstract

Gonionemus vertens A. Agassiz, 1862 is a small hydrozoan native to the Pacific Ocean. It has become established in the northern and southern Atlantic Ocean as well as the Mediterranean Sea. We report on the first occurrence of this species in estuaries in New Jersey, USA, and confirm species identification through molecular sequence analysis. Given the large number of individuals collected, we contend that this is a successful invasion into this region with established polyps. The remaining question is the vector and source of these newly established populations.

Key words: clinging jellyfish, Mid-Atlantic, 16S rDNA, COI

Introduction

Invasive species are well documented to have significant impacts on communities they have invaded (Thomsen et al. 2015). However, many diminutive species have been transported globally, yet seemingly have few impacts on the systems they have invaded (Hewitt et al. 2004; Gasith et al. 2011). Small hydrozoans in particular have invaded estuaries globally with seemingly no substantial impact. For example, the hydrozoan *Blackfordia virginica* Mayer, 1910, originally thought to be a native of the western mid-Atlantic, originated from the Black Sea and has successfully become established globally (Harrison et al. 2013).

The native range of *Gonionemus vertens* A. Agassiz, 1862, the clinging jellyfish, includes coastal regions of the North Pacific Ocean (Fofonoff et al. 2003). Its common name originates from the behavior of the medusa stage to "cling" to vegetation using modified adhesive tentacle pads. The medusae are predators of small zooplankton and epifaunal organisms that share the same vegetated habitats. Edwards (1976) suggests that two variants of this species exist and exhibit different levels of sting potency, with the more venomous organisms present from the western North Pacific, while those from the eastern North Pacific have less potent stings. This species has now been documented to have invaded the coast of Norway, the Baltic Sea, the Mediterranean Sea, the northwest Atlantic, and the coast of Argentina (Fofonoff et al. 2003, and references within; Schuchert 2016). Govindarajan and Carman (2016) review and document the invasions within the Cape Cod region of Massachusetts and occurrences in other regional New England estuaries. Interestingly, the initial introduction in the late 1800s appears to be of the less venomous form and the potential re-introduction in the 1990s is of the more toxic variety. Here we report on successful invasion into New Jersey's estuaries of the more venomous variety.

Material and methods

The first reported *G. vertens* was collected in the Manasquan River estuary (Figure 1) with subsequent initial samples (n = 5) collected from the Shrewsbury

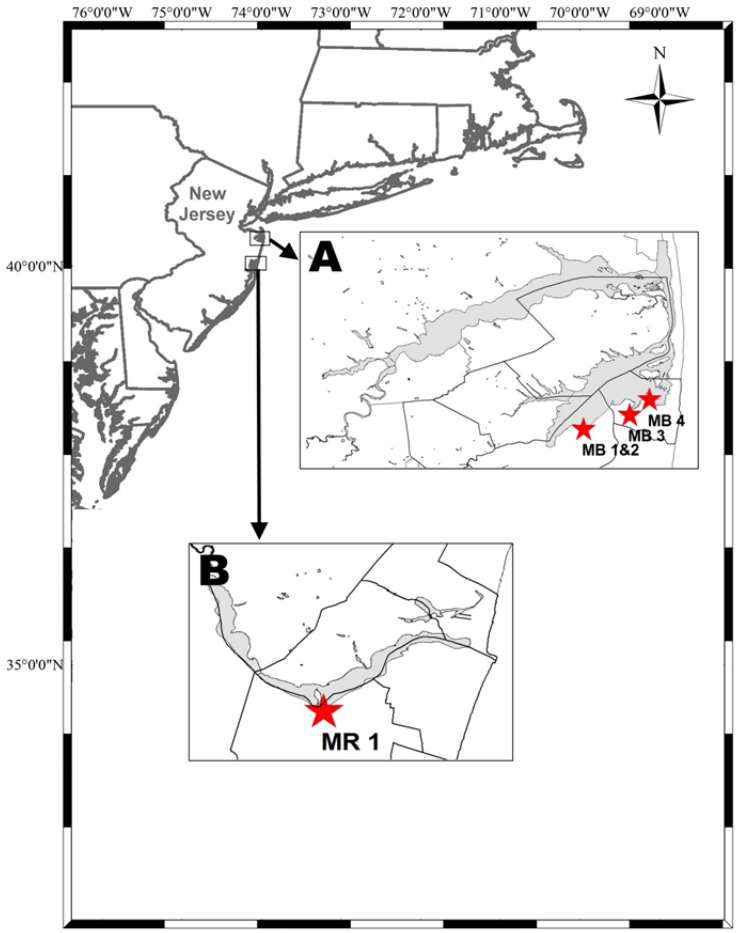

Figure 1. Map of the distribution of *Gonionemus vertens* in New Jersey estuaries, A: Shrewsbury River, B: Manasquan River (see Supplementary material Table S1 for geo-referenced locations).

River estuary). Individuals (e.g., Figure 2) were taken to Montclair State University (Montclair, New Jersey, USA) where tentacles were dissected from these five samples. Tentacles were excised from each individual (4 from each sample) and extracted using a CTAB/NaCl protocol (Winnepennickx et al. 1993) with the following modifications. All extractions were carried out in 500 µL volumes in 1.7 mL microcentrifuge tubes and homogenized by grinding for 30 s with a micropestle (Eppendorf). Homogenized samples were incubated at 60 °C for 60 min, and RNA was digested by incubation with RNase A (Sigma-Aldrich; 10 µg for 30 min at 37 °C) prior to precipitation. DNA was precipitated with 2/3 volume of isopropanol, pelleted in a microfuge (16,100 × g for 10 min at 4 °C), washed twice with ice-cold 70% (v/v) ethanol, briefly dried in a Speed-Vac, and resuspended in 20 µL of TE (10 mM Tris, 0.1 mM EDTA, pH 8). DNA concentrations and $OD_{260/280}$ ratios were determined in a NanoDrop ND-1000.

PCR amplifications were performed using ChoiceTaq Master Mix (Denville Scientific, Denville, New Jersey, USA) according to manufacturer's directions with the exception that we used 20 µL reaction volumes. Primers used for amplification of the 16S rDNA and COI loci are listed in Table 1. PCR was carried out in a ProFlex Thermal Cycler (Applied Biosystems, Inc.) according to the following parameters: 95 °C for 1 min (1×); 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s (30×); 72 °C for 7 min (1×); followed by a hold at 4 °C. Positive and negative template controls were always run and typically 10 µL of each sample was run on a 1% (w/v) agarose gel to assess purity and yield of PCR product. PCR products were subject to direct Sanger dideoxy sequencing on an ABI3130 Genetic Analyzer. Sequencing reactions were carried out using BigDye Terminator Ready Reaction Mix V3.1 according to manufacturer's instructions except that we used 1/16 diluted reactions. Excess unincorporated fluorescent dideoxy

Table 1. List of PCR Primers.

Locus	Primer Sequence (5' to 3')	Reference
16S	TCGACTGTTTACCAAAAACATAGC ACGGAATGAACTCAAATCATGTAAG	Bridge et al. 1995
COI	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994

Figure 2. Image of *Gonionemus vertens* collected in New Jersey. Photograph by Christie Barry.

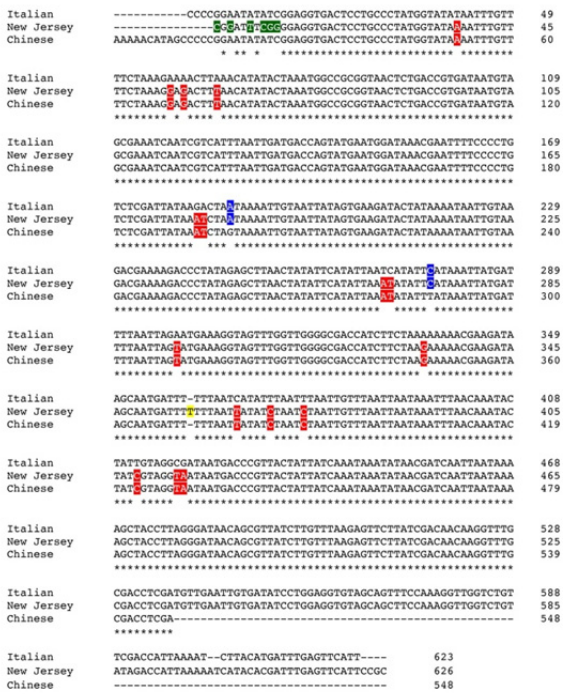

Figure 3. CLUSTAL Omega Alignment of *Gonionemus vertens* 16S rDNA Sequences. Asterisks show identity of all three aligned sequences. Red identifies unique homology between New Jersey and Chinese samples; blue identifies unique homology between New Jersey and Italian samples; green identifies bases unique only to New Jersey samples; and yellow demonstrates the point of insertion of one additional T in a run of 6 T's found in the other two samples. (Genbank Accession numbers for 16S loci are KF962471 (Chinese), EU293976 (Italian), and KX656923 (New Jersey).

nucleotides were removed by Performa Gel DTR Gel Filtration Cartridges (EdgeBio, Gaithersburg, Maryland, USA). Both strands of each product were sequenced using the same primers used to generate the fragment. Raw sequences were edited and aligned using 4 Peaks (http://nucleobytes.com/4peaks/index.html) and CLUSTAL Omega (Sievers et al. 2011; http://www.ebi.ac.uk/Tools/msa/clustalo/), and searched for homology against all known genetic sequences using the BLAST algorithm (Altschul et al. 1990).

Results

Sequencing and bioinformatic analysis of both the 16S ribosomal and the cytochrome oxidase subunit I (COI) loci confirm the identity of our samples as the clinging jellyfish, *Gonionemus vertens*. Figure 3 shows the CLUSTAL Omega alignment of our sample (Genbank KX656923) with the two closest matches in Genbank for the 16S locus: our best match demonstrates a 99.4% homology (521/524 identities) with only 3 SNP's and one gap to a sample collected from the China Sea (Genbank KF962471). Alignment of our COI locus shows a similar strong homology to *Gonionemus vertens* COI as well (97.2% homology; 633/651 identities) at the nucleotide level (data not shown). The fact that both of these loci, which are commonly employed for DNA barcoding of Hydrozoa (Harrison et al. 2013), point to the identification of our samples as *Gonionemus vertens,* strengthens our conclusions. Analysis of both the 16S and COI loci from all five New Jersey samples sequenced to date were identical. The lack of genetic variation in all of these samples, which were isolated from two populations nearly 20 miles distant from each other, suggests that they were the result of the same biological invasion event.

Discussion

The spread of non-native species throughout the worlds' oceans has accelerated due to anthropogenic means, and they may pose significant risks to biodiversity and ecosystem functions (Bax et al. 2003; Thomsen et al. 2015). In particular, several activities have promoted these invasions including aquaculture, bulk transport of cargo, travel, and exploration over the last 500 years (Katsanevakis et al. 2013). Processes which facilitate these invasions include hull fouling, ballast water discharge, and the construction of transport canals (see Hulme 2009). The development of the Panama and Suez Canals has opened pathways between distant ecoregions and their proposed expansions have highlighted the potential for greater invasion (Carman et al. 2011; Muirhead et al. 2015; Galil et al. 2015).

For *G. vertens*, its introduction to the Mediterranean has been speculated, but the prevalence of *G. vertens* invasions in northern Europe (Tambs-Lyche 1964; Bakker 1980), the western North Atlantic (Govindarajan and Carman 2016), and western South Atlantic (Rodriguez et al. 2014) suggest that this species has a great potential for transport and invasion (Schuchert 2016). Based upon our sequence analysis, the *G. vertens* discovered in New Jersey estuaries matches most closely a sample from the China Sea (Figure 3), indicating the more venomous group. This is in alignment with Govindarajan and Carman (2016) assertions. Consequently, the potential avenue for invasion may be a unique introduction to the area, but more likely comes from the Cape Cod, Massachusetts, USA, population; possibly transferred by local currents or vessels (Lavoie et al. 1999). Ultimately, determination of the precise invasion pathway may require a regional population genetics approach that compares genetic distance with physical distance.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Records of the hydrozoan *Gonionemus vertens* from New Jersey, USA.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2016/Supplements/BIR_2016_Gaynor_etal_Supplement.xls