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Rapid Communication

Who's lurking in your lagoon? First occurrence of the invasive hydrozoan *Moerisia* sp. (Cnidaria: Hydrozoa) in New Jersey, USA

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Abstract

Coastal estuaries represent areas of high biological invasions by virtue of their economic importance as ports. We report on the first occurrence of the non-native hydrozoan *Moerisia* sp. in coastal New Jersey, USA. Through the use of artificial settling plates, several diminutive, unknown cnidarian polyps were isolated. Initial morphological assessment indicated that two of the unknown polyps were keyed to *Moerisia*. We then used universal cnidarian primers to amplify and sequence the 16S rDNA mitochondrial locus for molecular identification. Upon evaluation and editing of sequences, two of the unknown polyps were identified as belonging to a group of unresolved *Moerisia* sp. taxa (> 99% homology). Additionally, polyps of *Chrysaora* and *Aequorea* were also identified from settling plates. The presence of *Moerisia* sp. in Barnegat Bay is the second recent documentation of an invasive hydrozoan in New Jersey and suggests that there may be other undescribed hydrozoans in this region that have yet to be been recognized, especially those with cryptic benthic life history phases.

Key words: *Aequorea*, *Chrysaora*, Mid-Atlantic, 16S rDNA, DNA barcoding

Introduction

Changes to marine communities are often driven by complex anthropogenic stressors such as eutrophication, coastline modification, and urbanization. In particular, coastal development related to shipping and commerce presents opportunities for non-native species to be transported to new locations and become established (see de Castro et al. 2017). Many gelatinous zooplankton have life histories which allow them to successfully invade and remain undetected (Wintzer et al. 2011b). Cnidarians are well documented to have invaded marine systems globally (Graham and Bayha 2008; Bayha and Graham 2014), because they have the ability to rapidly increase their population size through high levels of asexual reproduction (Brotz et al. 2012). Cnidarians also have a higher tolerance to hypoxia (Miller and Graham 2012), leading to their ability to thrive in impaired coastal systems. The bipartite life history of many small cnidarians makes it extremely difficult to identify invasions when the critical life history stage is the polyp. Only through thorough investigations of potential polyp habitats can the diminutive polyp stages be collected and identified. Additionally, the presence of many invasive species which are small or cryptic often go undetected, since their presence and impact on the invaded community is minimal or they are indistinguishable from native species (Miglietta and Lessios 2009; Muha et al. 2017).

Settling plates are an effective tool for detecting the presence of invasive species in aquatic systems (Marraffini et al. 2017). Unfortunately, the limited space available for colonization results in plates often being populated by organisms with high reproductive output. However, frequent short-term sampling can

minimize space monopolization and allow for the detection of cryptic species. Many cnidarian species have high reproductive rates, yet the ability to locate polyps within a system remains difficult even with the use of settling plates. This may be due to the small size and morphological variability displayed by many cnidarian polyps (Graham and Bayha 2008). As a result, diminutive species present on settling plates may be misidentified as native or already established invasive species. Therefore, fouling organisms like hydrozoans and other diminutive cnidarian species are ideal candidates for molecular identification, as genetic variability is often the easiest and most accurate way to distinguish cryptic organisms (Meek et al. 2013; Chiaverano et al. 2016; Bayha et al. 2017).

Within the literature there is speculation that the *Moerisia* species are not fully described and they need both taxonomic and molecular resolution (Rees and Gershwin 2000); as such much of the information about the species occurs at the genus level. Species within this genus are known to tolerate a wide variety of environmental conditions including wide salinity and temperature ranges (Rees and Gershwin 2000). *Moerisia inkermanica* (Paltschikowa-Ostroumova, 1925) is a small hydrozoan, believed to be native to the Black Sea (Rees and Gershwin 2000; Gravili et al. 2013). This species is a known invasive with documented occurrences in the North Eastern Atlantic, North Sea, Mediterranean Sea, Southern Atlantic, and Indo-Pacific (Mills and Rees 2000). Like many cnidarians, *M. inkermanica* has a complex life history that involves both a benthic polyp phase and a planktonic phase with free-swimming medusa. As it shares characteristics with other species in the genus, discrepancies of identification are bound to occur in areas where it or sister species have invaded. Consequently, the uncertainty about taxonomic identification among species within the genus has led many scientists to reduce taxonomic certainty to the genus level (Mills and Rees 2000). In some cases, the morphological features of individuals have been unresolved as matching type specimens, with the unresolved individuals being clumped as *Moerisia* sp. (Wintzer et al. 2011a). *Moerisia lyonsi* is another member of the genus which has been described from the mid-Atlantic region of the United States (Calder 1971; Ma and Purcell 2005), but genetic matches from more recent Chesapeake specimens match those of *Moerisia* sp. from San Francisco, CA (Meek et al. 2013). Consequently, the uncertainty in taxonomic resolution, coupled with wide-ranging invasive qualities of *Moerisia*, lead to the potential of numerous invasion pathways for this genus. The objective of this research was to detect and identify unknown hydrozoan polyps from settling plates collected in New Jersey.

Methods

Barnegat Bay, New Jersey (39.910911N; −74.139141W) is a shallow estuary in the Mid-Atlantic region of the United States. Long barrier islands protect the bay from direct oceanic forces, but strong semi-diurnal tides help keep the bay well mixed. To assess the settlement and distribution of cnidarian polyps, settling plates consisting of oyster shells and vinyl bulkhead material were deployed at two week intervals in Barnegat Bay during July and August 2014. These plates were initially used to assess the spatial and temporal distribution of recruiting *Chrysaora chesapeakei (*formerly *quinquecirrha*, Bayha et al. 2017) polyps within this region (Soranno 2014). However, the recent identification of the invasive hydrozoan *Gonionemus vertens* in New Jersey estuaries (Gaynor et al. 2016), prompted a reevaluation of these preserved settling plates to see if any polyps of *G. vertens* or other hydrozoan polyps were present.

In 2017, ethanol preserved plates were re-examined for cnidarian polyps. Plates from August 2014 yielded nine unidentified polyps, which were isolated for morphological and molecular evaluation. These isolated polyps were examined and photographed for morphological comparisons, prior to DNA extraction. Morphological identification included close examination of external features, including number and arrangement of tentacles, shape of the hydranth, and location of medusa buds and keyed using Boillon et al. (2004) and Schuchert (2010). As polyps were preserved, tentacles were generally contracted, but medusa buds were present at the margins of the polyp (Figure 1). These polyps had between 7–9 moniliform tentacles, and an extended hyposome strongly resembling the *Moerisia inkermanica* polyps described in Schuchert (2010). After visual inspection, isolated polyps were placed into sterile 1.7 ml microcentrifuge tubes with 100 µl of sterile 5% (w/v) Chelex® 100 mesh particle size 150–300 µM (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547) in 50 mM Tris base (pH 11).

DNA extraction was performed in accordance with the methods of Walsh et al. (1991), modified for optimization. Samples were boiled (100 °C) in a water bath for 10 minutes, vortexed for 30 seconds, and then cooled on ice for 2 minutes. Samples were then vortexed again for 30 seconds. After the second round of vortexing, samples were centrifuged at 14,000 × g for 10 minutes and the supernatant removed and stored at −20 °C. PCR was carried out in 20 µl reactions using ChoiceTaq Master Mix (2X) (Denville Scientific, Denville, New Jersey, USA). Universal Cnidarian primers designed for 16S rDNA (originally described by Bridge et al. 1992), Forward (UCF)

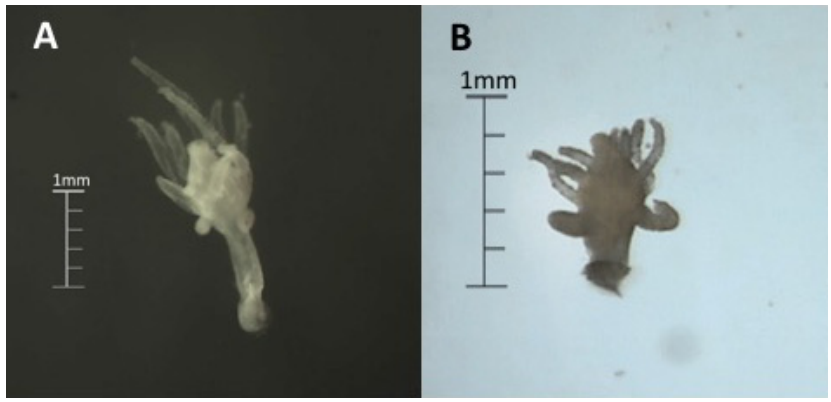

Figure 1. Variable morphology of polyps removed from settling plates in Barnegat Bay, New Jersey identified as *Moerisia* sp. A) Polyp (MG575535) with extended tentacle, showing similar morphology to Fig. 58 (*Moerisia inkermanica* Paltschikowa-Ostroumowa, 1925), Drawing E from Schuchert (2010). B) Polyp (MG577736) with contracted tentacles, resembling the morphology depicted in Fig. 58 (*Moerisia inkermanica* Paltschikowa-Ostroumowa, 1925), Drawing D from Schuchert (2010). Photos by Robert Meredith and John Gaynor.

5'-TCGACTGTTTACCAAAAACATAGC-3'and Reverse (UCR1) 5'-RCGGAATGAACTCAAATCR TGTAWG-3', modified by Restaino (2013) to include three degeneracies, which increase the number of cnidarian species amplified by these primers, were used to characterize each unidentified polyp sample.

PCR was performed in a Veriti™ 96 Well thermal cycler (Applied Biosystems Inc.) according the parameters described in Gaynor et al. (2016). In addition to experimental samples, both positive and negative (no template) controls were also run during each PCR trial. Following PCR, 10 µl of PCR products were run on 1% (w/v) agarose gels to confirm reaction success and verify the size of the amplicon produced. Sanger-dideoxy sequencing was then performed in-house on successful reactions. Sequencing products were generated for both the forward and reverse strands, using the same primers that produced the PCR amplicons. Sequencing was completed on an ABI 3130 Genetic Analyzer using BigDye Terminator Ready Reaction Mix V3.1 following the manufacturer's protocol. Forward and reverse sequence data were aligned and edited using 4Peaks (http://nucleobytes.com/4peaks/index.html). Edited sequences were searched against known sequences in Genbank using BLAST (Altschul et al. 1990). Once taxonomic identifications were verified, DNA sequences were entered into GenBank.

Results

Of the nine polyps isolated, six produced PCR amplicons sufficient for DNA sequencing. Analysis of the DNA sequences in Genbank BLAST revealed that none of the polyps isolated from the settling plates were those of *G. vertens*. However, sequence data generated from these polyps indicates that three cnidarian genera were present in the samples: *Chrysaora, Moerisia,* and *Aequorea.* Additionally, the morphological assessment indicated that two polyps were characteristics of *Moerisia,* but did not display morphological features allowing for complete discrimination to the species level. Two polyps were identified as *Chrysaora chesapeakei* (formerly *quinquecirrha*), with edited sequences matching at 99% with 595/596 identities (Accession #: MG575537) to GU300724.2 and 99% to MF141718.1 with 573/575 identities. The second *C. chesapeakei* polyp (Accession #: MG575538) matched at 100% to multiple *C. chesapeakei* sequences including MF141661.1 and MF141660.1 both with 584/584 identities. Two polyps (Accession #s: MG575539 and MG575540) showed high levels of homology to species in the genus *Aequorea*. Sequences generated from these individuals both match at 96% (533/555 identities) to JQ716017.1. Lastly, two polyps (Accession #s: MG575535 and MG5735536) showed high sequence homology with several samples identified as *Moerisia* sp. and *Moerisia inkermanica*. Both samples showed 99–100% homology to all *Moerisia* sp. and *Moerisia inkermanica* 16S mitochondrial rDNA sequences available in Genbank (Table 1).

Discussion

The identification and location of cnidarian polyps is an important component to understanding the life-history and distribution of these organisms. However, the cryptic nature of many hydrozoan and scyphozoan polyps leads to difficulty in locating their benthic phases (Wintzer et al. 2011b). Additionally, some hydrozoans have highly variable benthic forms, which can make identification based on morphology alone problematic (Cartwright and Nawrocki 2010). The presence of invasive *Moerisia* sp. from our settling plates suggests that the distribution of this species may truly be cosmopolitan, as it has been documented in coastal areas around the world (Bayha and Graham

Table 1. Homology comparisons of the two *Moerisia* sp. polyps (MG575535, MG575536) to *Moerisia inkermanica* and *Moerisia* sp. 16S mitochondrial rDNA sequences in Genbank. * = noted as a voucher specimen in Genbank. ** = Location surmised from GenBank data, although specific location is not provided in the records.

Location	GenBank Accession #	Species Identified	MG575535 Match	Homology	MG575536 Match	Homology
Brazil	KT266626*	*Moerisia inkermanica*	99%	573/575	99%	571/573
California	EU876555	*Moerisia* sp.	99%	573/575	99%	571/573
California	KX355402	*Moerisia* sp.	100%	568/568	99%	565/596
California	AY512534	*Moerisia* sp.	99%	511/512	99%	506/508
China**	KF962500*	*Moerisia inkermanica*	99%	496/500	99%	491/496
Virginia	MH166775 MH166776 MH166777 MH166778	*Moerisia* sp.	100%	567/567	99%	562/563

2014). It also supports the concept that human use and modification of marine systems has led to the increased distribution of non-native species, particularly in areas that are highly populated or occur along shipping routes (Purcell et al. 2007; Graham and Bayha 2008; Rodriguez et al. 2014; de Castro et al. 2017). The presence of multiple *Moerisia* sp. polyps on these settling plates suggests that this hydrozoan has an established population within Barnegat Bay, New Jersey. While this water body does not host any significant ports that include international shipping, it is approximately 75 km from the Port of New York and New Jersey. This commercial port is the largest on the East Coast of the United States and the third largest in the country. Under some wind conditions, the outflow (i.e., Hudson River plume) from this harbor travels along the coast of New Jersey and directly past Barnegat Bay (Choi and Wilkin 2007) providing a potential mechanism for transport of invasive species from ships and recreational watercraft visiting the port.

Interestingly, the *M. inkermanica* invasion in Brazil was documented in close succession to other invasive hydrozoans including *G. vertens* and *Blackfordia virginica* (Nogueira and de Oliveira 2006; Rodriguez et al. 2014) and *G. vertens* was first identified in New Jersey in 2016 (Gaynor et al. 2016). This suggests that the mechanisms by which these species are transported globally may be linked (Bayha and Graham 2014). Remarkably, the sequence data generated from the polyps identified as *Moerisia* sp. show a great deal of homology to *M. inkermanica* sequences from Brazilian populations (Maronna et al. 2016), but also show remarkable identity for *Moerisia* sp. from California and the Chesapeake Bay (Table 1). Given the proximity of Barnegat Bay, New Jersey to the Chesapeake Bay, it is quite possible that *Moerisia* sp. may have been introduced into New Jersey from the Chesapeake area or other invasion pathways (e.g. New York Harbor) could have seeded the Mid-Atlantic region of the United States. While these polyps show a strong genetic match to the *Moerisia* sp. from California and the Chesapeake (Table 1), morphologically the polyps more closely resemble those of *M. inkermanica.* Since *M. lyonsi,* is the only identified *Moerisia* species currently described in the Mid-Atlantic region, one possibility is that *M. lyonsi* and *M. inkermanica* maybe not be completely independent species, but rather show a great deal of morphological plasticity complicating their identification. This is supported by the homology of *M. lyonsi* and *Moerisia* sp. at the ITS1 loci described in Meek et al. (2013) and agrees with our 16S data (Table 1). One puzzling and unresolved question from our work are the two polyps identified as members of the genus *Aequorea*. They do not share significant homology with the two endemic species of *Aequorea (A. forskalea* and *A. macrodactyla*) and their closest match is with *A. australis*, native to the Pacific Ocean. This may suggest yet another undescribed species in this region or potentially an additional invasive species requiring further investigation.

The discovery and record of *Moerisia* sp. occurrence in Barnegat Bay is the second recent documentation of an invasive hydrozoan in New Jersey waters. These recent identifications suggest that there may be other undescribed hydrozoans in this region that have yet to be recognized, due to their cryptic and diminutive size. Additionally, hydrozoan species have been historically neglected when describing organisms from a particular region, creating a likelihood that there are many species present that have not been described. The recruitment of *Moerisia sp.* polyps to settling plates reinforces the strength and utility of this technique for monitoring fouling organisms, especially those with cryptic benthic life history phases and supports the need for more long-term research of hydrozoan species and other gelatinous zooplankton.

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