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*Current address: School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland.

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Corresponding author:
Craig Stephen Wilding;
Email: c.s.wilding@ljmu.ac.uk

First record of the sea anemone *Actinia equina* (Cnidaria: Anthozoa) on the Mid-Atlantic coast of the United States

Diederik Boonman[1], Craig Stephen Wilding[2,*], James T. Carlton[3] and Jason E. Adolf[1]

[1]School of Science, Monmouth University, West Long Branch, NJ 07764, USA; [2]School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool L3 3AF, UK and [3]Coastal and Ocean Studies Program, Williams College-Mystic Seaport, Mystic, CT 06355, USA

Abstract

Members of the genus *Actinia* are familiar members of rocky shore communities across much of the world. However, to date, no *Actinia* species have been reported from the North American continent. Here, we report *Actinia* from an approximately 22 km length of the New Jersey, US shoreline, where it was first discovered in 2021. Morphology and DNA barcoding data (mitochondrial *cytochrome c oxidase I* and nuclear internal transcribed spacer) indicate that these populations are *Actinia equina*. The presence of these populations close to major ports in New Jersey, New York, and Philadelphia suggests a probable introduction from shipping activities.

Introduction

Sea anemone invasions appear to be increasingly common around the world (Glon *et al*., 2020; Gimenez and Brante, 2021; Gimenez *et al*., 2022). In recent years, for example, the South American anemone *Phymactis papillosa* (Lesson, 1830) has invaded Europe (Pereira *et al*., 2022), a European *Sagartia* species has invaded South Africa (Robinson and Swart, 2015), the Asian *Diadumene lineata* (Verrill, 1869) has continued to spread to become one of the most widespread anemones worldwide (González Muñoz *et al*., 2023), and the southeastern United States anemone *Aiptasiogeton eruptaurantia* (Field, 1949) has arrived in southern New England (Pederson *et al*., 2021). To these records we now add the invasion on the Atlantic coast of North America of the European sea anemone *Actinia equina* Linnaeus, 1758, whose distribution is currently held to be from northern and western Europe to the Mediterranean (Perrin *et al*., 1999). *Actinia equina* is a common species on rocky shorelines throughout its distribution, being found across the intertidal zone from the high-water neap level down to the upper subtidal (Manuel, 1988). Although *A. equina* has been reported to be gonochoristic, whether it reproduces sexually remains moot (Perrin *et al*., 1999; Wilding *et al*., 2020). *Actinia equina* certainly does reproduce through somatic embryogenesis, with clonal individuals internally brooded until release from the parental coelenteron. Although not all individuals are brooding at any one time, those that are typically have up to 20 clonal individuals in their coelenteron and in rare cases more than this (Brace and Quicke, 1986). Consequently, this species has the capacity to rapidly colonize suitable habitat through the release of these clones.

Here, we present data on *Actinia* found recently at multiple sites on man-made rocky structures of the beaches of Monmouth County, New Jersey, on the Mid-Atlantic Coast of the United States. The anemones found match the characteristic *A. equina* morphology, and we confirm identification through DNA barcoding.

Materials and methods

Specimens were examined and collected in 2023 at low tide from man-made rocky groynes on the New Jersey shoreline. Photographs of anemones *in situ* were taken at Roosevelt Ave beach in the town of Deal (latitude 40.2578306, longitude −73.9871418; site A on Figure 1). Twenty-six anemones were collected at Philips Ave beach (latitude 40.2532037, longitude −73.988791; site B in Figure 1), also in Deal, and four anemones were collected from Ocean Grove, Neptune Township (latitude 40.2103212, longitude −74.0020381; site C in Figure 1). To facilitate further laboratory studies, anemones were carefully peeled from rocks and transported individually in glass jars with seawater to a laboratory at Monmouth University.

In addition to our observations reported here, we considered observations of *Actinia equina* reported on iNaturalist (https://www.inaturalist.org/).

Four specimens collected from Ocean Grove (site C) were analysed through barcoding of both the standard mitochondrial barcoding locus *cytochrome c oxidase I* (*CoI*) (Hebert *et al*., 2003) and the nuclear rDNA–internal transcribed spacer (ITS) cluster for which *ITS1*

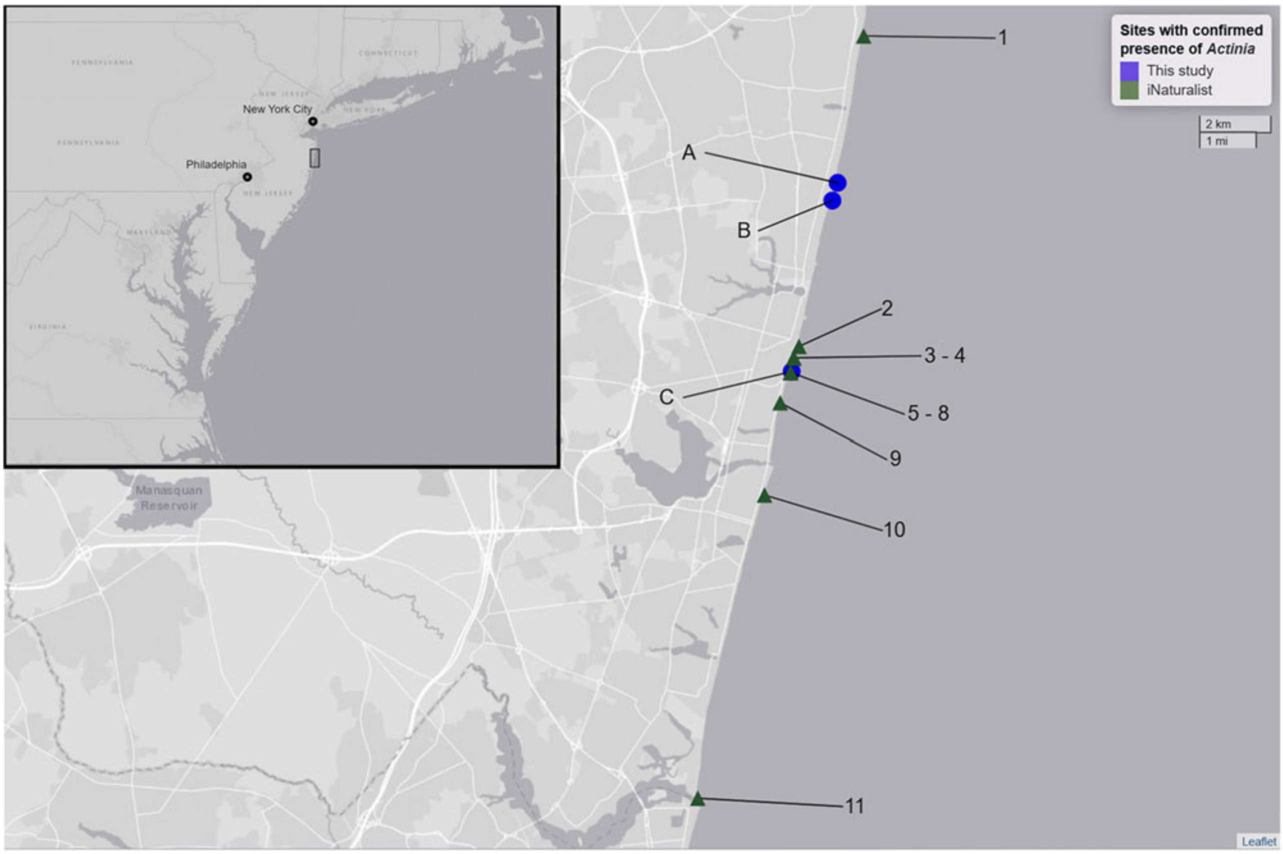

Figure 1. Map of sampling sites alongside iNaturalist records for *Actinia equina*. Sites A, B, C: sites sampled for this study. (A) Roosevelt Avenue, Deal, New Jersey; (B) Phillips Avenue, Deal NJ; (C) Ocean Grove, Neptune Township, NJ. Sites 1–11: iNaturalist records (iNaturalist observation number given). (1) 176354922 (Long Branch Beach, August 2023); (2) 162967884 (Ocean Grove, May 2023); (3) 132054972 (Ocean Grove, May 2022); (4) 121145126 (Ocean Grove, June 2022); (5) 179718648 (Ocean Grove, August 2023); (6) 125436946 (Bradley Beach, July 2022); (7) 127216915 (Ocean Grove, July 2022); (8) 163414767 (Ocean Grove, May 2023); (9) 92531365 (Bradley Beach, August 2021); (10) 129471163 (Belmar, August 2022); (11) 186565245 (Manasquan Inlet, Manasquan, October 2023). For a global picture of the distribution of *A. equina*, see https://www.inaturalist.org/taxa/130085-Actinia-equina.

has been recommended as an nDNA barcode (Santamaria *et al.*, 2017). DNA was extracted using the extraction technique described in Wilding *et al.* (2020). We then amplified and sequenced the mitochondrial *CoI* locus using the protocol of Wilding and Weedall (2019) with the primers of Folmer *et al.* (1994) and the rDNA locus containing *ITS1* and *ITS2* following Reimer *et al.* (2007).

To examine whether these anemones contained clonal individuals, ten anemones were stressed with gentle agitation and irrigation of the anemone's coelenteron to extract clones. Purged clones were observed under a microscope and counted per individual.

Results

Distribution and habitat

Anemones were observed by DB and JEA at three sites in 2023 on the New Jersey shoreline (Figure 1). Animals were found near the base of the seaward end of groynes, in a zone typically associated with blue mussel (*Mytilus edulis*) beds and surrounded by macroalgae (*Ulva* spp.). They were observed on both high-shore open rock and under rocks on the lower shore, although most of them are found in the latter habitat. Anemones were frequently seen in dense aggregations wherein individuals were immediately adjacent to and often in contact with each other (F–J in Figure 2).

On iNaturalist (https://www.inaturalist.org/) we found 11 records (1–11 in Figure 1) where descriptions and photographs match those of *Actinia equina*. The northernmost record is from Long Branch Beach (latitude 40.2946367, longitude −73.9783467; site 1 in Figure 1) found in August 2023; the southernmost record is from Manasquan Inlet, Manasquan (latitude 40.1029159, longitude −74.03307; site 11 in Figure 1) found in October 2023. Six of the records are centred around Ocean Grove. The earliest record is from August 2021, when the anemones were first discovered in Bradley Beach (latitude 40.2023959, longitude −74.0059152; site 9 in Figure 1).

As of July 2024, no records of *A. equina* have been reported on iNaturalist from neighbouring states (New York, Delaware, and Pennsylvania).

Morphology

Anemones collected from the New Jersey shoreline had a pale green colouration in the column with a blue limbus and grey pedal disc. Tentacles had a pale green/yellow colour with blue tips (Figure 2). Individual animals have typical sizes of 10–40 mm, the upper end of which is similar to the maximum size of 40–50 mm disc width reported previously for *A. equina* (Davenport *et al.*, 2011; Carling *et al.*, 2019).

Following gentle agitation and irrigation of the coelenteron of ten individuals, 3.16 (±1 SEM) clonal individuals were released per individual (range 0–10).

Genetic analysis

All four samples genetically analysed had a *CoI* haplotype found previously in *A. equina* (GenBank accession number MH636618) and a rDNA–ITS haplotype also found previously in *A. equina*

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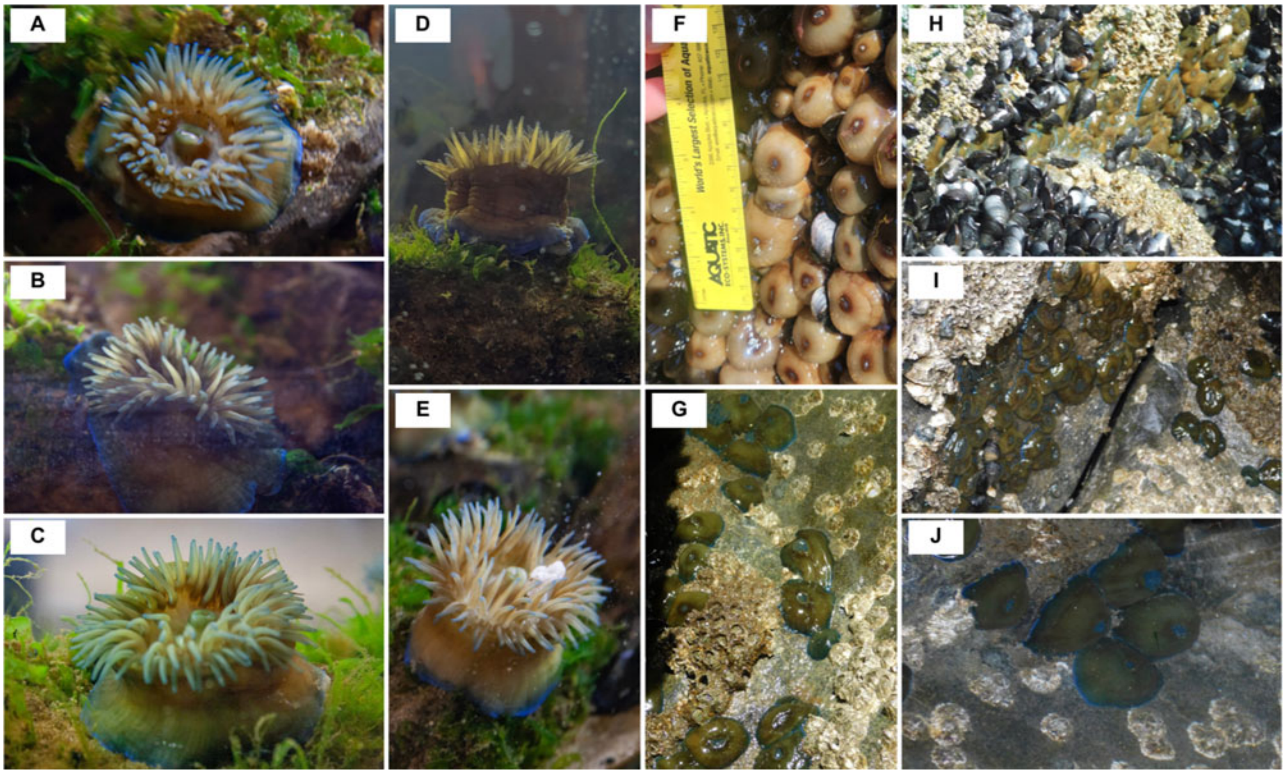

Figure 2. Images of individual *Actinia* specimens with extended tentacles in observation tank (A–E). Note the blue limbus on these individuals. Images of anemone aggregations on rocky groynes (F–J). Image (F) captured in Ocean Grove, (G–J) on Philips Ave, Deal.

(GenBank accession number PP349608). Haplotypes were identical across all the samples studied.

Discussion

Actinia equina is here reported from rocky structures on the New Jersey shore. The limited geographical extent of sites where *Actinia* has been observed (approximately 22 km) and the recent records (only since 2021) suggest this anemone may be a relatively recent introduction. The mitochondrial DNA *CoI* and nuclear ITS haplotypes identified in these samples are identical to those found in other *A. equina* from widespread geographic locations (CSW, personal observation) providing DNA barcoding confirmation of species identity.

Wherever *A. equina* occurs it can be patchily distributed or occur in aggregations. What appears to be unusual about the New Jersey populations is the higher density aggregations with animals abutting each other. Anemones at these sites are aggregated in multiple clusters, some on the order of 1 m^2. Although other species of intertidal anemones which reproduce asexually form tightly packed clonal aggregates (Francis, 1988), aggregations of *A. equina* on European shores are invariably less dense (e.g. Figure 3 of Brace (1990) for an example).

Although individuals can be more aggregated during the winter (Brace, 1990), this does not explain the tightly packed distribution seen in the New Jersey populations. Sea anemones, including *A. equina* (Brace and Pavey, 1978; Brace, 1990) are by nature territorial (Williams, 1991; Turner *et al*., 2003), protecting their territory through aggressive fighting encounters in which nematocysts, chiefly those of the acrorhagi, are used in combat. Fine-scale distribution is mediated by this aggression which ensures individuals can compete for space through territorial disputes. However, relative aggression levels in these territorial disputes varies dependent upon relationships between individuals. Where this aggression can be considerable towards non-relatives, they are more tolerant of, and less aggressive towards, neighbouring clonemates (Ottaway, 1978; Brace *et al*., 1979; Ayre, 1982, 1983) in *A. equina* and *Actinia tenebrosa*, both of which have been confirmed to reproduce through asexual viviparity through genetic analysis of brooded individuals (Black and Johnson, 1979; Orr *et al*., 1982). For *A. tenebrosa*, Ayre (1983) describes densely clumped clusters of adult anemones in which disproportionately more neighbouring adults were genotypically identical. Anemones also exhibit size-related hierarchies of aggression (Brace and Pavey, 1978). In the populations described here, the aggregations are formed of anemones of relatively uniform size. A combination of size homogeneity and clonal expansion from an introduction of one or few individuals may explain the extremely tight clustering of individuals seen in these populations which appears to be different from that seen in their typical range.

The studied locations are approximately 25 km from the entrance to New York Harbour, one of the largest ports in the United States, and around 150 km from the port of Philadelphia. Ocean plumes from New York/New Jersey flow as a constrained coastal current southwards along the New Jersey shore (Choi and Wilkin, 2007), towards the area in which *A. equina* was found. We thus suggest that shipping from Europe is the most probable vector for the introduction of this anemone to the American coast. Sea anemones are known from both vessel hull fouling and from ballast water (see Glon *et al*., 2020). Although there are questions as to whether *A. equina* produces planula larvae (Perrin *et al*., 1999; Wilding *et al*., 2020) which could be transported in ballast water, small anemones could be taken into ballast tanks and ballasted cargo holds attached to bits of debris. We also note that *Actinia* have long been popular aquarium animals (Friese, 1972; Bellomy, 1975).

We suggest that given the small scale of these populations of anemones, they likely represent a relatively recent introduction and are composed either completely or largely of clones of a single or a few immigrant animals. We suggest three reasons for this

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conclusion. First, these animals contain clonal offspring, and, at least in the congeneric *A. tenebrosa*, settlement and recruitment to new sites is largely through asexual clonal proliferation (Ayre, 1983). The ability to reproduce asexually is particularly important for invasion success in anemones (Glon *et al.*, 2020). The homogeneity of body size within the aggregations fits with a single introduction with subsequent spreading through release of clones. Ayre (1983) showed that in populations of *A. tenebrosa*, single clones can dominate an established habitat. Although there is little comparable data for *A. equina* colonizing new habitats, Brace and Quicke (1986) examined colonization and resettlement by *A. equina* of experimentally cleared surfaces. However, such experimentation differs from the situation described here in that neighbouring territory in their study had established long-term, genotypically diverse, adult *A. equina* populations which could move into the newly cleared substrate. Second, the concentrated aggregation with little spacing between individuals is consistent with clonality since these individuals must be less aggressive to clonemates to tolerate this close proximity. Third, the haplotypes at both loci (mitochondrial and nuclear) were the same across all four samples strongly consistent with a clonal origin (although we recognize the caveat of having studied just $N = 4$).

Integrative approaches combining both morphological and molecular studies are vital for understanding Cnidarian diversity (Yap *et al.*, 2023). Here, we have combined these to identify *Actinia* specimens from New Jersey. However, further sampling is recommended to determine the geographic extent of *A. equina* on the US Atlantic coast in order to determine whether these small-scale stable populations are spreading. Recent work has developed environmental DNA technologies for the identification of sea anemones around New Jersey shores (Lockwood *et al.*, 2023) and integrating this technique into future surveys would be prudent.

Data. The authors confirm that the data supporting the findings of this study are available within the article.

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Author contributions. D. B. and J. E. A. examined specimens *in situ* and collected specimens for laboratory observations. C. S. W. undertook molecular analysis. All authors contributed to writing the manuscript.

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