

Research Article

The Pacific bivalve *Anomia peruviana* in the Atlantic: a recent invasion across the Panama Canal ?

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Abstract

The Peruvian jingle shell, *Anomia peruviana* d'Orbigny, 1846 is native to the Eastern Pacific including Panama. During recent surveys of Panama's marine fauna using settlement plates, we discovered *A. peruviana* in Limon Bay, near the Atlantic entrance of the Panama Canal. We confirmed our initial morphological identifications using partial sequences of the COI barcode locus. All *Anomia* individuals collected on the settlement plates from Atlantic and Pacific Panama were confirmed to be *A. peruviana*, which is genetically distinct from the native Atlantic *A. simplex*. We suspect *A. peruviana* was transported through the Canal from the Pacific to the Atlantic attached as hull fouling on vessels or recreational boats. Salinity tolerance experiments in the laboratory showed that all individuals in the seawater control survived while 25% survived a 12-hour exposure to freshwater from Gatun Lake, confirming that some *A. peruviana* individuals can survive even the estimated maximum transit of up to 12 hours through the Panama Canal.

Key words: bivalve; introduced species; *Anomia peruviana*; *Anomia simplex*; geminate species; salinity tolerance; Panama Canal

Introduction

Panama has played an important role in global maritime commerce for over 500 years and its role further expanded after the opening of the Panama Canal in 1914. Over 12,000 ships transit the Canal annually, carrying about 5% of the world's cargo, and the Canal receives twice as many vessel arrivals as the largest ports in the US (Ruiz et al. 2009). The Panama Canal is a potential corridor for the movement of marine species between the Pacific and Atlantic Oceans. Although Gatun Lake, a large artificial freshwater lake that forms a significant part of the Canal, serves as a barrier for most marine species (Cohen 2006; Ruiz et al. 2009), some organisms can survive the passage through the Canal, either unaided, or associated with fouling on ships or in

ballast water (Rubinoff and Rubinoff 1969; McCosker and Dawson 1975; Davidson et al. 2008). Some marine organisms, such as bivalves that can seal their valves during adverse conditions, may be particularly predisposed to survive short-term exposure to freshwater during vessel transits (Galtsoff 1964; Sundaram and Shafee 1989; Heilmayer et al. 2008).

The Peruvian jingle shell, *Anomia peruviana* d'Orbigny, 1846 is native to the eastern Pacific Ocean and ranges from southern California to Peru and the Galapagos Islands. It occurs from the intertidal zone to a depth of 110 m (Coan and Valentich-Scott 2012). The shell has 3 subequal muscle scars, small to medium-sized byssal foramen, and is white, yellow, orange, or green in color (Coan and Valentich-Scott 2012). The species has been reported from the Bay of Panama in several past inventories (Zetek 1918;

Table 1. Sites where settlement plates were deployed, number of plates analyzed in parentheses, and presence of *Anomia* sp. at each site over time. N/A = site not sampled in that year.

Site	Coordinates	Year of collection/ <i>Anomia</i> Presence				
		2008	2009	2010	2011	2012
<i>Panama Pacific</i>		(140)	(0)	(20)	(15)	(45)
STRI dock	08°55.050 N/79°31.962 W	No	N/A	Yes	Yes	Yes
Flamenco Marina	08°54.774 N/79°31.322 W	No	N/A	No	N/A	Yes
Balboa Yachtclub	08°55.040 N/79°32.092 W	No	N/A	N/A	N/A	N/A
ACP Diablo	08°57.925 N/79°34.365 W	No	N/A	N/A	N/A	N/A
Taboguilla	08°48.432 N/79°31.414 W	No	N/A	N/A	N/A	N/A
Rodman	08°57.048 N/79°34.398 W	No	N/A	N/A	N/A	N/A
Balboa Port	08°57.412 N/79°33.865 W	No	N/A	N/A	N/A	Yes
Vacamonte	08°51.747 N/79°40.116 W	No	N/A	N/A	N/A	N/A
<i>Panama Atlantic</i>		(0)	(148)	(64)	(5)	(45)
Shelter Bay	09°22.106 N/79°57.054 W	N/A	Yes	Yes	Yes	Yes
Colon CT	09°22.370 N/79°53.105 W	N/A	No	No	N/A	Yes
Manzanillo IT	09°21.445 N/79°52.970 W	N/A	No	N/A	N/A	N/A
Panama Ports	09°20.944 N/79°54.160 W	N/A	No	N/A	N/A	N/A
ACP Davies	09°17.512 N/79°55.142 W	N/A	No	N/A	N/A	Yes

ANAM 2002), but never from the Atlantic side of Panama. While currently recognized as a valid species (Huber 2013), it is morphologically similar to the Atlantic jingle *Anomia simplex* d'Orbigny, 1853, and no attempts have been made to distinguish the two morphologically (see Coan and Valentich-Scott 2012), creating some uncertainty about species identity.

During our recent surveys of fouling organisms near the two entrances to the Panama Canal, we found *A. peruviana* growing on settlement plates deployed on the Atlantic coast of Panama. Due to the morphological similarity between this species and the native *A. simplex*, we conducted genetic comparisons to confirm the identity of *A. peruviana*. To assess whether this species could have invaded the Atlantic through the Panama Canal on the hulls of vessels, we conducted salinity-tolerance experiments in the laboratory to simulate conditions they would likely experience during vessel transits.

Methods

Field sampling

As part of a large-scale survey to examine invasions of marine organisms between the Pacific and Atlantic sides of the Panama Canal, we analyzed and identified sessile invertebrates that colonized settlement plates, which serve as standardized sampling units. PVC plates (14 × 14 × 0.4 cm) were mounted on bricks using cable ties and suspended horizontally (face down) from docks in selected ports and marinas. Within each site, the plates were deployed at randomly

selected locations and suspended about 1 m below mean low water level. Plates were submerged for three months, then retrieved and the colonizing organisms identified to species level whenever possible. Voucher specimens were retained for further morphological and genetic analyses. Between 2008 and 2012, 482 plates were deployed across 5 sites in Limon Bay in the Atlantic and 8 sites in the Pacific near the Panama Canal (Table 1).

To test survival of sessile marine invertebrates when exposed to low salinity, simulating a transit through the Panama Canal, we used 12 plates from Shelter Bay Marina on the Atlantic side (09°22'N, 79°57'W) for salinity tolerance experiments as described below. While these experiments were designed to test the salinity tolerance of multiple taxa on the plates, only the results for *A. peruviana* are reported here.

Genetic analysis

DNA was isolated from *Anomia* specimens found on settlement plates on both sides of the Canal in 2011 and 2012. On the Pacific side, all samples (n = 5) were collected from plates found on the STRI dock (2011 and 2012; Table 1). This site is separated by a narrow causeway from the entrance to the Panama Canal. Samples (n = 4) were obtained from three sites on the Atlantic side: Shelter Bay Marina (2011 and 2012), Colon Container Terminal (2012), and Embarcadero Davis (2012). As little genetic data exist for *Anomia* spp., we also obtained two specimens of *A. peruviana* from Chumical Point near Veracruz (09°53'7.20"N, 79°38'31.67"W), approximately twelve kilometers

from the Pacific entrance to the Canal, and several samples of *Anomia simplex* from Florida, USA (26°56'28.13"N, 80°04'01.85"W) for comparison with the individuals from the settlement plates.

Foot, adductor muscle and mantle tissue samples were preserved and stored in 95% ethanol until DNA was extracted using a modified CTAB protocol (Miura et al. 2012) and resuspended in 50 µl TE buffer. We targeted a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene using the primer pair jgLCO1490/jgHCO2198 (Geller et al. 2013). Amplifications used GoTaq Green Mastermix (Promega) with 1.25% DMSO, 5 mg BSA, 25 pmol of each primer, and 1 µl of DNA extract in 25 µl PCR reactions. Amplification conditions included an initial step at 94°C for one min, followed by 35 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min 30 sec, with a final extension step of 72°C for 7 min. Products were visualized on an agarose gel, and excised bands cleaned using Gelase enzyme. Samples were sequenced on an ABI 3700 sequencer with BigDye chemistry (Applied BioSystems) and aligned using Sequencher 5.0 (GeneCodes). MEGA 5.2.2 (Tamura et al. 2011) was used to calculate Kimura two-parameter (K2P) genetic distances and construct a neighbor joining tree. Bootstrap analysis was performed with 1000 replicates. Sequences have been deposited in GenBank with accession numbers KF850686 - KF850703.

Salinity tolerance experiment

Anomia individuals on settlement plates (see above) were used to test their tolerance to freshwater exposure, simulating transit through the Canal. As the experiment was not designed exclusively for *Anomia*, the plates contained different numbers of individuals, ranging from one to five. We conducted the experiments over two consecutive days in September 2010. Each day, six plates were randomly selected from the field and transported to the laboratory in coolers containing aerated seawater. The plates were photographed and the number and condition (dead/alive, broken, attached or loose) of all *Anomia* individuals was determined. In total, there were 30 live *Anomia* on all plates combined. Fourteen individuals were randomly assigned to the control treatment and 16 to the freshwater treatment. Water for the freshwater treatment was collected from Gatun Lake (salinity 0.1) close to the Gatun Locks in Colón, and seawater for controls

(salinity 31) from the Galeta Marine Laboratory (09°24'07.77"N, 79°51'36.96"W).

At the beginning of each experimental trial, individual plates were transferred into separate aerated 10L plastic containers at 29°C containing either freshwater or seawater. Plates were exposed for 12 hours to simulate the estimated maximum transit time through the Panama Canal. After exposure, the plates were returned to containers containing seawater. Following one hour of acclimation, we counted the number of living *Anomia* on each plate. Individuals were considered dead if their valves were agape and did not close after three minutes of repeated physical stimuli (prodding).

Results

Individuals tentatively identified as *A. peruviana* were initially found on settlement plates at Shelter Bay Marina in the Bay of Limón near the Atlantic entrance of the Panama Canal in December 2009, and were continuously documented at this site in all subsequent surveys (Table 1). The species was not present on settlement plates from any Atlantic localities before December 2009. *Anomia peruviana* was present on Pacific-coast plates from STRI dock in 2010, and at all Pacific-coast sites surveyed in 2012.

Genetic analysis

We sequenced nine *Anomia* individuals found on settlement plates from the two entrances of the Panama Canal (5 Pacific and 4 Atlantic), two individuals from Panama representing *A. peruviana*, and ten individuals from Florida representing *A. simplex*. The aligned amplified product was 624 bp in length and while mixed peaks were not seen in the chromatograms, three stop codons were observed (two in both species, one in *A. simplex* only), suggesting that the amplification product could be a nuclear COI-like pseudogene rather than mitochondrial COI (but see Plazzi and Passamonti 2010 for further discussion). *Anomia* individuals on all settlement plates were closely related and formed a distinct clade with the two *A. peruviana* specimens in our neighbor joining tree (Figure 2). This cluster is clearly separated from *A. simplex*, which formed a monophyletic group of two clades. The K2P within-group pairwise distance between the samples collected on settlement plates and known *A. peruviana* was 0.009 ± 0.002 , whereas the distance between *A. peruviana* and *A. simplex* was

Figure 1. *Anomia peruviana* on a settlement plate from Shelter Bay Marina, Limon Bay near the Atlantic entrance to the Panama Canal (Photo: J. Canning-Clode).

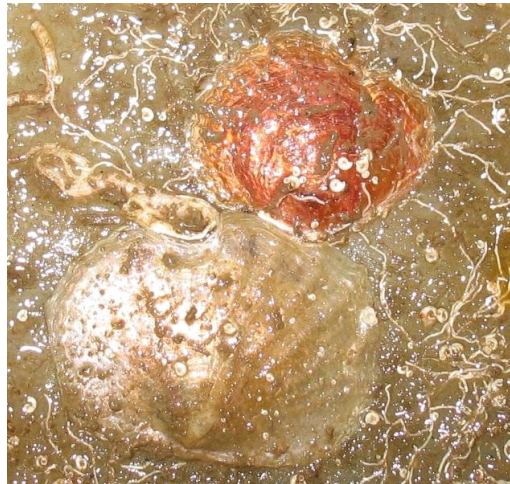
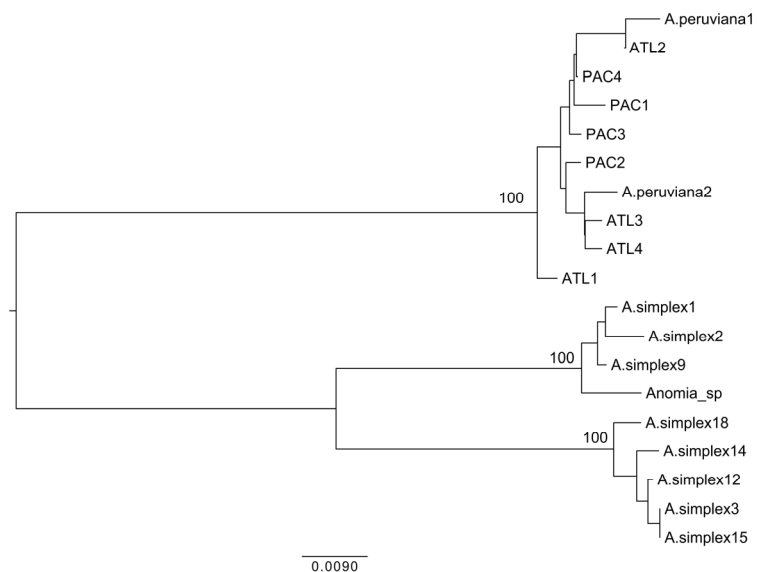


Figure 2. Unrooted neighbor joining bootstrap consensus tree of *Anomia* COI-like sequences. Samples PAC1-4 were collected from settlement plates on the Pacific side of the Canal and samples ATL1-4 from the Atlantic side. *Anomia peruviana* samples 1 and 2 were collected near Veracruz, Panama. All *A. simplex* were collected in Jupiter, FL, USA. The *Anomia* sp. sequence was originally collected in Woods Hole, MA (Genbank # GQ166573; Plazzi and Passamonti 2010). Numbers above branches correspond to bootstrap support estimates based on 1000 replicates.



0.175 ± 0.018 . The two groups within *A. simplex*, one of which includes a COI-like *Anomia* sequence from GenBank that was collected at Woods Hole, MA (Plazzi and Passamonti 2010), had a pairwise difference of 0.085 ± 0.013 .

Salinity tolerance experiment

All 14 *Anomia* individuals in seawater control survived but only 25% of the 16 individuals survived the exposure to freshwater. Although we did not record the individual size of the bivalves, all *Anomia* on settlement plates were a maximum of 3 months old and between 2 and 4 cm maximum length.

Discussion

Our study provides compelling evidence for the establishment of *A. peruviana* on the Atlantic side of the Panama Canal. We found individuals on our settlement plates in the Atlantic over four consecutive years and their presence on plates expanded to new sites during that time interval. All *Anomia* individuals sequenced from plates on both sides of the Canal were closely related to each other and to *A. peruviana* collected elsewhere in Panama. Further, ten of the eleven *A. peruviana* individuals possess unique haplotypes indicating high genetic diversity in the species and the

likelihood of multiple introductions of at least four mitochondrial haplotype lineages to the Atlantic side of the Canal. These lineages are highly distinct from the Atlantic *A. simplex*. The genetic distance between *A. peruviana* and *A. simplex* is comparable to distances of geminate pairs of bivalve species across the Isthmus of Panama (12.9–27.5% mean K2P distance; Lessios 2008). Interestingly, within Atlantic *A. simplex*, two genetically distinct groups were recovered; individuals from both clades co-occur with no obvious distinguishing morphological differences, indicating that the species may comprise a cryptic complex warranting further study. Broader comparisons across the ranges of both species and with other close relatives are necessary to draw further conclusions on the species' phylogeny, origins, and invasion patterns.

Several studies, beginning with Hildebrand (1939) in the 1930's, have evaluated the Panama Canal as a potential passageway for marine species (Rubinoff 1965; Rubinoff and Rubinoff 1969; see additional references in Cohen 2006). It is surprising that only a few species have passed successfully through the Panama Canal and established populations on the opposite side (Roy and Sponer 2002; Robertson et al. 2009). While it is likely other species have passed through the Canal and have yet to be reported, it is interesting to consider why there have not been more obvious invasions through such a short corridor. Davidson et al. (2008) demonstrated that passage through the Panama Canal did not limit the survival of invertebrates in fouling communities (particularly barnacles) on obsolete vessels. Similarly, we suspect that *A. peruviana* may have crossed the Panama Canal via hull fouling on vessels. The jingle shell is commonly found in fouling communities and is known to occur on ship hulls (Coan and Valentich-Scott 2012).

Our experimental results suggest that, under laboratory conditions, some *A. peruviana* are capable of surviving the estimated maximum transit of up to 12 hours through the Panama Canal. Although survivorship is certainly influenced by the animal's condition before exposure and perhaps also by age and/or shell size, 25% of treated animals survived the exposure to freshwater. An important caveat to this finding is that this experiment was part of a broader study examining the survival potential of fouling organisms during transit through the Panama Canal. Thus, the rapid mortality of other fouling organisms (e.g. sponges, tunicates) could have decreased water quality in the experimental

containers, confounding the effects of survivorship to freshwater exposure alone. Also, the length of exposure to freshwater in our experiments was equal to the maximum transit time estimated by the Panama Canal Authority (Autoridad del Canal de Panama 2012). Thus, while our experiments demonstrate the potential for surviving transits on vessels, they likely provide a conservative estimate of survivorship. Unfortunately, information on the ecology of *A. peruviana* is scarce and there is no published data describing the ability of this species to survive at lower salinities or their subsequent performance, in terms of long-term survivorship, growth and reproduction. Nonetheless, by tightly sealing their valves, it appears that individuals of this species may survive prolonged exposure to unfavorable conditions, as common for other bivalves (e.g. Galtsoff 1964; Sundaram and Shafee 1989; Heilmayer et al. 2008).

Hull fouling is not the only possible means of introduction for *A. peruviana* to the Atlantic coast of Panama. Planktonic larvae of the species also could have been transported in ballast water. Little is known about reproductive patterns in *A. peruviana*, but studies on other *Anomia* sp. show that they are pulse spawners with planktotrophic larvae that can remain in the water column for several weeks (Chanley and Andrews 1971 – *A. simplex*, Bramanti et al. 2003 – *Anomia ephippium*). In our study, ten of the eleven *A. peruviana* individuals that we sequenced showed unique sequences suggesting high genetic diversity in the species as well as multiple introductions of at least four mitochondrial haplotype lineages to the Atlantic side of the Canal. This high diversity could also be explained by transportation of larval propagules from other localities of *A. peruviana* to the Canal area. We do not have data on the amount of ballast discharge associated with traffic transiting the Canal. However, regulations of the Panama Canal Authority require that vessels transiting the Canal should have sufficient ballast to permit safe handling during transit (Autoridad del Canal de Panama 2012). It is therefore possible that ships take in ballast water before transiting. In general, we would expect ballast intake before and ballast discharge after a Canal transit to be infrequent, as this would usually be associated with cargo operations or port arrivals. However, even infrequent events may result in introductions, and we cannot overlook this possibility, even though vessel biofouling seems a more likely mechanism of introduction.

Despite nearly 100 years of operation, it is interesting that relatively few marine species

have passed through the Panama Canal and established populations on the other side. This suggests that Gatun Lake, the freshwater portion of the Panama Canal, serves as an effective barrier for most marine species. However, it is also likely that there are other introduced and cryptogenic species that have not yet been detected and reported.

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