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Research Article

First report of genetic data from two invasive *Watersipora* (Bryozoa) species in the central California coast rocky intertidal

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Abstract

Several taxa forming a species complex within the bryozoan genus *Watersipora* are successful global marine invaders that are common in marine fouling communities in bays and harbors. This species complex has recently been recorded spreading to natural intertidal and subtidal habitats on the central California outer coast near the San Francisco Bay, suggesting that its invasive potential is greater than previously believed. To determine the species and clades present in these outer coast populations, and their relationship to populations in nearby bays and harbors, we produced COI sequences for a set of new *Watersipora* specimens sampled from four rocky intertidal sites, for which there is currently no published genetic data. *Watersipora subtorquata* and *Watersipora* new species were both identified at rocky intertidal sites. All haplotypes identified in this analysis have previously been reported in California harbors and marinas, suggesting that intertidal populations may be sourced from existing populations in harbors, rather than representing independent invasions. Field observations of intertidal populations support *Watersipora*'s year-round persistence at rocky intertidal sites, which has significant implications for invasion dynamics and invasion management strategies. Continued sequencing of outer coast *Watersipora* samples will be crucial to further characterize the genetic diversity and structure of these coastal populations and describe the dynamics of *Watersipora*'s outer coast spread.

Key words: cryptic species, introduced species, San Francisco Bay, marine invertebrates, cytochrome oxidase I

Introduction

Several clades of the bryozoan genus *Watersipora* form an invasive cryptic species complex (previously regarded as one species, *Watersipora subtorquata* d'Orbigny, 1852) that has been implicated in the disruption and homogenization of diverse native assemblages (Needles and Wendt 2013) and is difficult to control, perhaps in part due to its tolerance of copper-based antifouling paints (Floerl et al. 2004; Piola and Johnston 2006). *Watersipora* has limited dispersal potential due to possessing non-feeding brooded larvae, with a planktonic larval duration of generally less than eight hours (Marshall and Keough 2003). Its global distribution from an uncertain native range has been linked to dispersal on ship hull fouling (Ryland et al. 2009; Mackie et al. 2012). It has recently been observed colonizing natural rocky intertidal and subtidal habitats on the central California outer coast (GGNPB 2014; Lonhart 2012; Zabin et al. 2018), suggesting that its invasive potential is even greater than previously believed.

In many marine systems, the highest species diversity and population densities of invasive species are typically found in estuaries and high-traffic human-modified areas such as jetties, docks, harbors, and marinas (hereafter referred to collectively as harbors), while outer coast habitats may remain comparatively free of invasive species (Preisler et al. 2009; Ruiz et al. 1997; Wasson et al. 2005), with some well-known exceptions. Examples of outer coast invaders in other regions include *Undaria pinnatifida* and *Balanus glandula* in Argentina (Orensanz et al. 2002; Schwindt 2007), *Mytilus galloprovincialis* (Grant and Cherry 1985; Bownes and McQuaid 2006; Hockey and Schurink 1992; Ma et al. 2021) and *Semimytilus patagonicus* (previously *S. algosus*; de Greef et al. 2013; Ma et al. 2020) in South Africa, *Sargassum muticum* in Europe and on the Pacific coast of the United States (Britton-Simmons 2004), *Anemonia alicemartinae* in Chile and Peru (Glon et al. 2020), and *Istiblennius meleagris* in the Mediterranean (Rothman et al. 2020). Diverse invasions, including by colonial invertebrates, have also been recorded on rocky coasts in New England (McIntyre et al. 2013) and California (Wasson et al. 2005; Zabin et al. 2018), and in the intertidal and subtidal reefs of Hawaii (DeFelice et al. 2001; Zabin et al. 2013). On the whole, however, marine invasions affect estuaries and harbors to a greater extent than rocky outer coasts. The disproportionate invasion of estuarine habitats relative to adjacent outer coast rocky intertidal habitats is seen even in central California near the San Francisco Bay (Zabin et al. 2018), which is documented as one of the most heavily invaded estuaries in the world (Cohen and Carlton 1995; Foss 2009). It is also very unusual for marine invasives to successfully make the transition between harbor habitats and the open coast rocky intertidal, with only a handful of harbor-to-coast range expansions by aggressive invaders such as *Undaria pinnatifida* (Zabin et al. 2009) and *Terebrasabella heterouncinata* (Kuris and Culver 1999) being reported from the intertidal of the California coast. This difference may be driven by numerous factors, including different physical environments and a greater supply of invasive organisms to harbors, as well as increased habitat alteration and greater ecosystem disruption, which may decrease native competition and make habitats more vulnerable to invasion (Bando 2006; Byers 2002; Piola and Johnston 2007).

Watersipora spp., however, appears to have successfully made this critical transition from harbor to coast. Already common in marinas and harbors along the California coast from San Diego to Humboldt Bay (Mackie et al. 2012), it has also been recorded at several rocky intertidal habitats along the open coast outside San Francisco Bay, and in subtidal habitats in Monterey Bay (Zabin et al. 2018). Furthermore, it is the only recorded invasive at several of these sites, and at other sites is seen with significantly higher frequency and abundance than other invasives (Zabin et al. 2018, M. Duncan *pers. observ*.). Colonization of several intertidal and subtidal reef sites and offshore oil platforms in the Santa Barbara Channel by a watersiporid has also recently been reported (Page et al. 2019a, b). Page et al. (2019a, b) identified the species as *Watersipora subatra,* but this has not yet been validated with molecular data and there remains some taxonomic uncertainty within this genus, so *W. subtorquata* species complex clades may also be involved. The wide distribution of the *W. subtorquata* species complex (hereafter referred to as *Watersipora*), and its success in habitats that are otherwise not significantly invaded, make it an organism of high potential concern. These instances of "coastal escape" demonstrate this clade's capacity for wider colonization of relatively uninvaded marine habitats, including local, state, and federal Marine Protected Areas.

Watersipora is a colonial encrusting invertebrate animal that grows on both natural and anthropogenic hard substrate, such as rocky cobble and dock pilings, where it competes for space with other encrusting organisms including algae, sponges, tunicates, mollusks, and other bryozoans. Competition from encrusting species like *Watersipora* threatens native species and contributes to the homogenization of marine ecosystems that have historically been richly diverse (McKinney and Lockwood 1999). One dramatic example of this can be seen in Morro Bay, a smaller estuary approximately 200 miles south of San Francisco Bay, where *Watersipora* has almost entirely replaced *Mytilus* mussels as the estuary's foundation species, potentially precipitated by climate change and predation pressure on mussels from the recently-reintroduced Southern Sea Otter (*Enhydra lutris nereis*) (Needles and Wendt 2013). Members of *Watersipora* are difficult to distinguish by anatomical characteristics but can be identified by analysis of sequences from the mitochondrial Cytochrome oxidase subunit I (COI) gene (Mackie et al. 2012). Previous research described this species complex consisting of three closely-related clades (*W. subtorquata* clade A, *W. subtorquata* clade B, and *Watersipora* new species), and limited the California ranges of these three clades to estuarine environments (Korcheck 2015; Wostenberg 2015; Mackie et al. 2012). This work showed latitudinal segregation of some clades, potentially driven by differences in temperature tolerance. However, these sequences were generated before *Watersipora* was known to be present in the rocky intertidal and had only recently been observed in the subtidal in Monterey Bay. Specimens were sampled only from harbors and marinas, and thus the genetic composition of outer coast rocky intertidal populations remained undescribed.

To address this lack of data, we have begun sampling and generating COI sequences from all the outer coast sites from which *Watersipora* is known. Here we report our first set of 30 sequences from the immediate San Francisco Bay area, 21 of which are from four outer coast rocky intertidal sites associated with the San Francisco Bay outflow. These represent a first report of the sequencing and species identification of California outer coast samples, and the first installment of larger efforts to genetically characterize outer coast populations. We also report the presence or apparent absence of *Watersipora* invasion in an array of outer coast sites flanking the San Francisco Bay outflow as determined by our own observations as well as observations from citizen scientists, including citizen scientist data recorded during broader ecological monitoring projects managed by the National Park Service or in collaboration with San Francisco State University. Several field observations of *Watersipora*'s ecological interactions and invasive capacity that are relevant to potential future management initiatives are also presented.

Materials and methods

Specimen Collection

Specimens were collected from eight sites in the San Francisco Bay area: Mile Rock, Point Bonita, Muir Beach, Slide Ranch, Hyde Street Pier, Pillar Point Harbor, and Spud Point Marina and Porto Bodega Marina in Bodega Harbor (Table 1, Figure 1). Samples were taken between March 2014 and June 2019 (Table 2). Four of these sites (Mile Rock, Point Bonita, Muir Beach, Slide Ranch) are outer coast rocky intertidal habitats. The remaining four sites are harbors, one (Hyde Street Pier) within the San Francisco Bay, and three (Spud Point Marina, Porto Bodega Marina and Pillar Point Harbor) outside of San Francisco Bay. Both Spud Point Marina and Porto Bodega Marina are located within Bodega Bay, a small coastal inlet, whereas Pillar Point Harbor is located on the coastline with a seawall that has a direct outlet to the open ocean. Small sections of live colonies were sampled (5 mm^2 to 50 mm^2, depending on colony size), and preserved in 95% molecular-grade ethanol. At rocky intertidal sites, specimens were taken from the low intertidal zone on tides of –0.7 feet or lower. Harbor samples were taken from piers, floating dock structures, and pre-existing settlement plates.

Monitoring surveys to assess presence, abundance, and qualitative observations of colony color, size, and apparent health (physical integrity of colony and damage, presence and extent of black and grey patches, color uniformity) were regularly conducted at six of the sites where *Watersipora* is present (Mile Rock, Point Bonita, Muir Beach, Slide Ranch, Hyde Street Pier, Pillar Point Harbor; Table 1, Figure 1) as well as at three other rocky intertidal sites (Mussel Rock, Rockaway Beach, and Pigeon Point) where *Watersipora* presence has not yet been observed. These surveys were conducted from spring 2019 to spring 2020 at harbor sites, spring 2019 to

Table 1. Sampling and monitoring survey study sites. Site numbering corresponds to numbering on site map in Figure 1. Harbor sites are indicated in grey shading, and intertidal sites that were monitored but where *Watersipora* was not observed are indicated with italics. Sites indicated with an asterisk (*) were monitored by citizen scientist volunteers. Pillar Point (IBSPP 2021), Drakes Bay Oyster Company (AN Cohen pers. comm., Elliott-Fisk et al. 2005), and the mouth of Drakes Estero (J Goddard email to JT Carlton 22 Jul 1995, Cohen and Carlton 1995) are included from external sources (**). At sites where *Watersipora* is present, we note the first published record to the best of our knowledge; where no published record yet exists, or where observations within our lab pre-date the earliest known published record, the date of our first observation or collection is indicated. Sites within Bodega Harbor and San Francisco Bay list the first record in that bay as a whole, due to lack of early records at specific sites and potential rapid transmission within contained bays.

Study Site	Latitude	Longitude	Site Type	First *Watersipora* Record
[1] Bodega Harbor - Spud Point Marina (Sonoma County)	38.3294	−123.0575	Harbor (coastal)	Bodega Harbor, 1994 (Cohen & Carlton 1995)
[2] Bodega Harbor - Porto Bodega (Sonoma County)	38.3341	−123.0513	Harbor (coastal)	Bodega Harbor, 1994 (Cohen & Carlton 1995)
** [3] Drakes Bay Oyster Company (Marin County)	38.0827	−122.9325	Harbor (coastal)	2004 (Elliott-Fisk et al. 2005)
[4] Hyde St. Pier (San Francisco)	37.8100	−122.4225	Harbor (SF Bay)	San Francisco Bay, 1992 (Cohen & Carlton 1995)
[5] Pillar Point Harbor (San Mateo County)	37.5019	−122.4822	Harbor (coastal)	1993 (Cohen & Carlton 1995)
** [6] Drakes Estero (Marin County)	38.0338	−122.9291	Rocky Intertidal	4/24/1984 (J Goddard pers. comm. to JT Carlton 1995)
[7] Slide Ranch (Marin County)	37.8738	−122.6005	Rocky Intertidal	3/28/2014 (NPS BioBlitz)
[8] Muir Beach (Marin County)	37.8588	−122.5805	Rocky Intertidal	12/5/2014 (CS Cohen lab)
[9] Point Bonita (Marin County)	37.8186	−122.5294	Rocky Intertidal	3/28/2014 (NPS BioBlitz)
[10] Mile Rock (San Francisco)	37.7872	−122.5069	Rocky Intertidal	12/16/2014 (CS Cohen lab)
* [11] McClures Beach (Marin County)	_38.1838_	_−122.9663_	_Rocky Intertidal_	_none observed, July 2019 - present (B Schriock & C Hunt)_
* [12] Santa Maria Beach (Marin County)	_38.0122_	_−122.8500_	_Rocky Intertidal_	_none observed, 2018 (K Khtikian)_
* [13] Chimney Rock (Marin County)	_37.9902_	_−122.9638_	_Rocky Intertidal_	_none observed, 2018(K Khtikian)_
* [14] Bolinas Point at Duxbury Reef (Marin County)	_37.9036_	_−122.7269_	_Rocky Intertidal_	_none observed, 2018 - present (K Khtikian)_
[15] Mussel Rock (San Mateo County)	_37.6663_	_−122.4961_	_Rocky Intertidal_	_none observed, 1/20/2019 - 3/7/2021 (CS Cohen lab)_
[16] Rockaway Beach (San Mateo County)	_37.6080_	_−122.5008_	_Rocky Intertidal_	_none observed, 1/10/2019 - 2/27/2021 (CS Cohen lab)_
** [17] Pillar Point Reef (San Mateo County)	_37.4938_	_−122.4969_	_Rocky Intertidal_	_none observed, 4/1/2008 - present (IBSPP)_
[18] Pigeon Point (San Mateo County)	_37.1850_	_−122.3975_	_Rocky Intertidal_	_none observed, 1/11/2019 - present (CS Cohen lab)_

fall 2021 at Pigeon Point, and spring 2019 to spring 2021 at other intertidal sites. Invaded sites were surveyed seasonally (≥ 4 times a year) and uninvaded sites were surveyed at least twice a year. Surveys involved 1 to 3 researchers and consisted of close observation of the site for 0.5–2 hours. Intertidal surveys were performed while walking in a haphazard pattern through the low intertidal zone, focusing on cobble and boulders where *Watersipora* is typically found (as described below in results). At harbor sites, submerged dock surfaces and structures were surveyed within 5 to 10 boat slips, depending on marina size, distributed haphazardly across each site. Salinity and water temperature readings were taken on all surveys (unpublished data available on request). Monitoring of Rockaway Beach was also supported by observations from the Rockaway Ocean Conservation Stewards citizen science monitoring project, coordinated by M. Hubbell, which conducts biodiversity surveys on low tides approximately once a month.

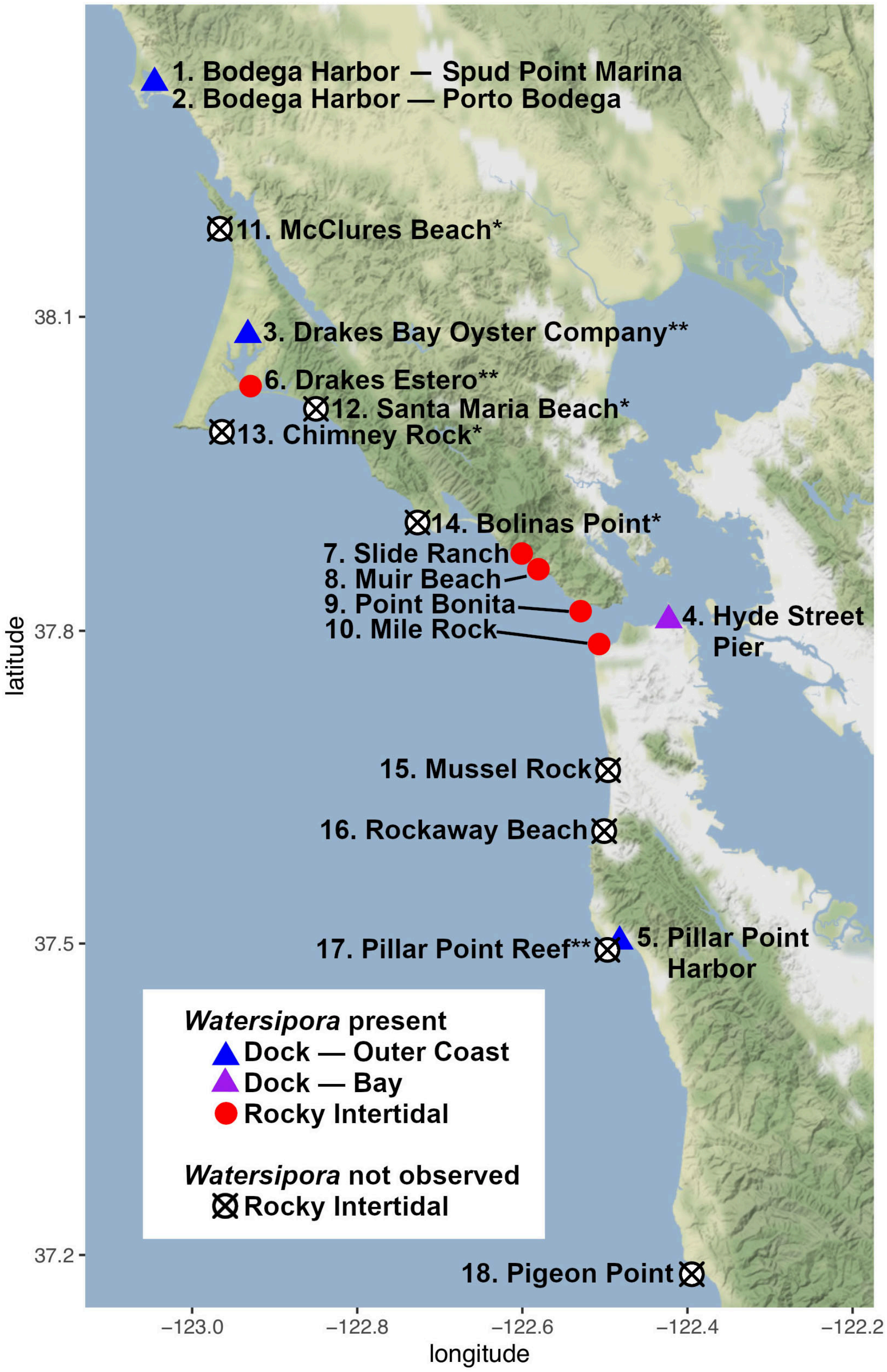

Figure 1. Sampling and survey sites in central California. Sites indicated with an asterisk (*) were monitored by citizen scientist volunteers, supplementary to other surveying activities. Three additional sites of interest (**) are included from other sources: Pillar Point Reef, where extensive citizen science intertidal surveys from 2008 to present are documented as public records on the iNaturalist website (IBSPP 2021); Drakes Bay Oyster Company (AN Cohen *pers. comm.*, Elliott-Fisk et al. 2005); and the mouth of Drakes Estero (J Goddard email to JT Carlton 22 Jul 1995, Cohen and Carlton 1995). Rockaway Beach was collaboratively surveyed by both the Cohen lab and the Rockaway Ocean Conservation Stewards citizen science monitoring project. Map was generated in R using the ggmap package (Kahle and Wickham 2015).

Several other sites within Point Reyes National Seashore were surveyed for *Watersipora* by citizen scientists during other intertidal censusing activities. Santa Maria Beach and Bolinas Point at Duxbury Reef observations

Table 2. Summary of specimens analyzed. Phylogroup and haplotype identity are matched with GenBank accession numbers for previously-published sequences. Specimen indicated with an asterisk (*) contains a sequencing ambiguity at a diagnostic SNP: it is currently included under the single *Watersipora* new species haplotype comprising all other *Watersipora* new species specimens, pending resequencing.

Site Type	Study Site	Sample Date	Clade		n	GenBank Match
Harbor	Bodega Harbor - Spud Point Marina	4/14/2017	*W.* new species		1	JQ715473
	Bodega Harbor - Porto Bodega	4/14/2017	*W.* new species		1	
	Hyde Street Pier	4/19/2019	*W.* new species		1	
	Pillar Point Harbor	5/19/2019	*W.* new species		6	
Rocky Intertidal	Muir Beach	2/18/2015	*W.* new species		1	
	Mile Rock	11/25/2018	*W.* new species		8	
		2/18/2019	*W.* new species		2	
		2/14/2018	*W.* new species		1	
	Point Bonita	6/16/2019	*W.* new species		2	
		3/28/2014	*W.* new species*		1	
		6/16/2019	*W. subtorquata*	clade A, haplotype 1	1	JQ715476
		6/16/2019	*W. subtorquata*	clade A, haplotype 2	1	JQ715472
		6/16/2019	*W. subtorquata*	clade A, haplotype 3	2	JQ715521
	Slide Ranch	9/27/2015	*W. subtorquata*		1	
		12/4/2018	*W. subtorquata*	clade A, haplotype 4	1	JQ715479

were made during Multi-Agency Rocky Intertidal Network (MARINe) surveys (Kent Khtikian *pers. observ.*), and observations at McClures Beach were made during volunteer sea star population monitoring for the Cohen lab (Estuary and Ocean Science Center; Elizabeth Schriock and Carol Hunt *pers. observ.*). Chimney Rock was monitored on a more informal basis during tagging and censusing of elephant seals (Kent Khtikian *pers. observ.*). Although *Watersipora* was not the primary target of these activities and survey methods varied, the contributing volunteers are experienced citizen scientists with extensive field experience and proficiency with various intertidal survey methods, who are familiar with identifying local invertebrate species and *Watersipora* specifically. These sites are included as community volunteer observations (Table 1).

Molecular Methods

Molecular analysis was performed on 30 samples of *Watersipora* collected between 2014 and 2019. Total genomic DNA was extracted from approximately 5 mm^2 tissue using a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel) and a ~ 700-bp fragment of COI was amplified. Samples collected between late 2018 and 2019 were amplified with LCO 1490M 5'GGT-CTA-CTA-ATC-ACA-AAG-AYA-THG-G 3' and HCO 2198M 5'TAA-ACT-TCA-GGG-TGA-CCA-AAR-AAY-CA 3' (courtesy Stephen Palumbi Lab, Stanford University) following protocols in Melroy et al. (2017). Primers were modified from the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). Samples collected from 2014 to early 2018 were amplified using protocols and custom primers BryCOIH2161 (5'-TGTTGGTATAGAATAGGATC-3') and BryCOIL1548 (5'-CATAACAG GAAGAGGTTTAAG-3') developed by Mackie et al. (2006). Sequences were produced at the San Francisco State Estuary and Ocean Science Center genetics facility on an ABI 3130 Genetic Analyzer, or through the Elim Biopharm (Hayward, CA) DNA sequencing service on an ABI 3730 Genetic Analyzer. Sequences were aligned using MUSCLE 3.8.425 (Edgar 2004) in Geneious 11.0.5 and trimmed to a 489 bp section to match sequences published in GenBank by Mackie et al. (2012). Clade and haplotype identity of each sample was initially determined by comparing COI sequences to the Mackie et al. sequences, and then validated with a maximum-likelihood tree constructed in Mega 10.1.8.

Results

Collections and Surveys

Watersipora was consistently found at the six invaded sites that were regularly surveyed by the Cohen lab (Mile Rock, Point Bonita, Muir Beach, Slide Ranch, Hyde Street Pier, Pillar Point Harbor; Table 1, Figure 1). Although we were able to locate colonies on every sampling or monitoring survey at all these sites, abundance varied dramatically throughout the year, and was typically highest during the summer dry season and lowest after the winter rains. No new *Watersipora* presence was detected by our surveys at Mussel Rock, Rockaway Beach, or Pigeon Point, nor at Rockaway Beach by the Rockaway Ocean Conservation Stewards (Table 1). *Watersipora* was also not observed by community volunteers at McClures Beach at Point Reyes National Seashore from July 2019 to present (Elizabeth Schriock and Carol Hunt *pers. observ.*), at Santa Maria Beach and Bolinas Point at Duxbury Reef in 2018 (Kent Khtikian *pers. observ.*), or at Chimney Rock from 2018 to present (Kent Khtikian *pers. observ.*).

In the rocky intertidal, colonies were almost exclusively found in cobble beds and on boulder and rock wall surfaces that receive some protection from direct wave action (e.g., in crevices, under overhangs, sheltered by larger seaward boulders) and limited sand scouring, particularly at sites such as Mile Rock that generally experience strong currents and high wave action. Colonies were observed across the mid-to-low intertidal zone, and the highest abundance was observed in the low intertidal, often in association with other mid-to-low intertidal organisms including the sea anemone *Anthopleura elegantissima,* the turban snail *Tegula funebralis,* and various species of encrusting tunicates, encrusting sponge, and coralline algae. Direct competition for space (via organisms overgrowing each other) was frequently observed with both encrusting tunicate and encrusting sponge species, and occasionally with native bryozoans. *Watersipora* was also often found in association with the reef-building tube worm *Phragmatopoma californica* (sandcastle worm). *Watersipora* overgew both live and dead *P. californica* colonies, even where bare rock was available, and *Watersipora* was observed overgrowing at least one *P. californica* colony at every site where *Watersipora* and *P. californica* co-occur. *Phragmatopoma californica* reef structures were often overgrown by numerous *Watersipora* colonies, with *Watersipora* growth on individual reef structures ranging from several small colonies, up to dozens of small colonies and multiple larger patches of mature colonies. At harbor sites, *Watersipora* was observed almost universally in association or competition with invasive tunicate species, particularly the solitary tunicate *Ciona intestinalis* and the colonials *Didemnum vexillum, Botryllus schlosseri, Diplosoma listerianum*, and *Botrylloides* spp. (Figure 2C, D).

Consistent with previous descriptions (Mackie 2003; Lonhart 2012), *Watersipora* was observed showing a range of foliose and encrusting forms (Figure 2), and colors ranging from bright orange-red to almost solid black. Qualitative observation suggests that bright-colored and foliose morphologies are more common in harbors than in the intertidal, but a range of morphologies can be found in both habitat types.

An additional observation regarding dispersal potential was made on settlement plates at Pillar Point Harbor: on two separate settlement plates, multiple fragments of adult *Watersipora* colonies had drifted onto the plates after being transported amongst loose sediment and debris. In several instances, these colony fragments were still alive and feeding, and had been partially adhered to the plate by colonial tunicates or mucus from tube-building organisms (Figure 3). No adult colony fragments have been observed re-attaching to new substrates with their own growth, but these observations do demonstrate that fragments of adult *Watersipora* colonies can be transported to new areas and survive.

Clade Identification and Molecular Results

Of the 30 samples successfully sequenced, 6 were identified as *Watersipora subtorquata* clade A, representing 4 different haplotypes, and 24 were identified as a single clade of *Watersipora* n. sp. (Table 2) by comparison with NCBI records. All of the harbor samples that were sequenced were identified as *Watersipora* n. sp., while outer coast samples included both *Watersipora* n. sp. and *W. subtorquata* clade A. No specimens of the less-common *W. subtorquata* clade B (Mackie et al. 2012) were found in either the outer coast or harbor samples. No novel haplotypes were identified: all haplotypes have been previously reported from harbor sites by Mackie et al. (2012) and published in GenBank. However, one haplotype of *W. subtorquata* (clade A, haplotype 4; Table 2) has only previously been reported from sites in southern California. The earliest sample taken from Point Bonita may represent a second *Watersipora* n. sp. haplotype (also previously recorded by Mackie et al.) due to a sequencing ambiguity at a diagnostic SNP. However, this cannot be confirmed without resequencing, so for the purposes of this report, this sample is not regarded as a distinct haplotype. The maximum-likelihood tree generated in Mega is consistent with the tree produced by Mackie et al. (2012).

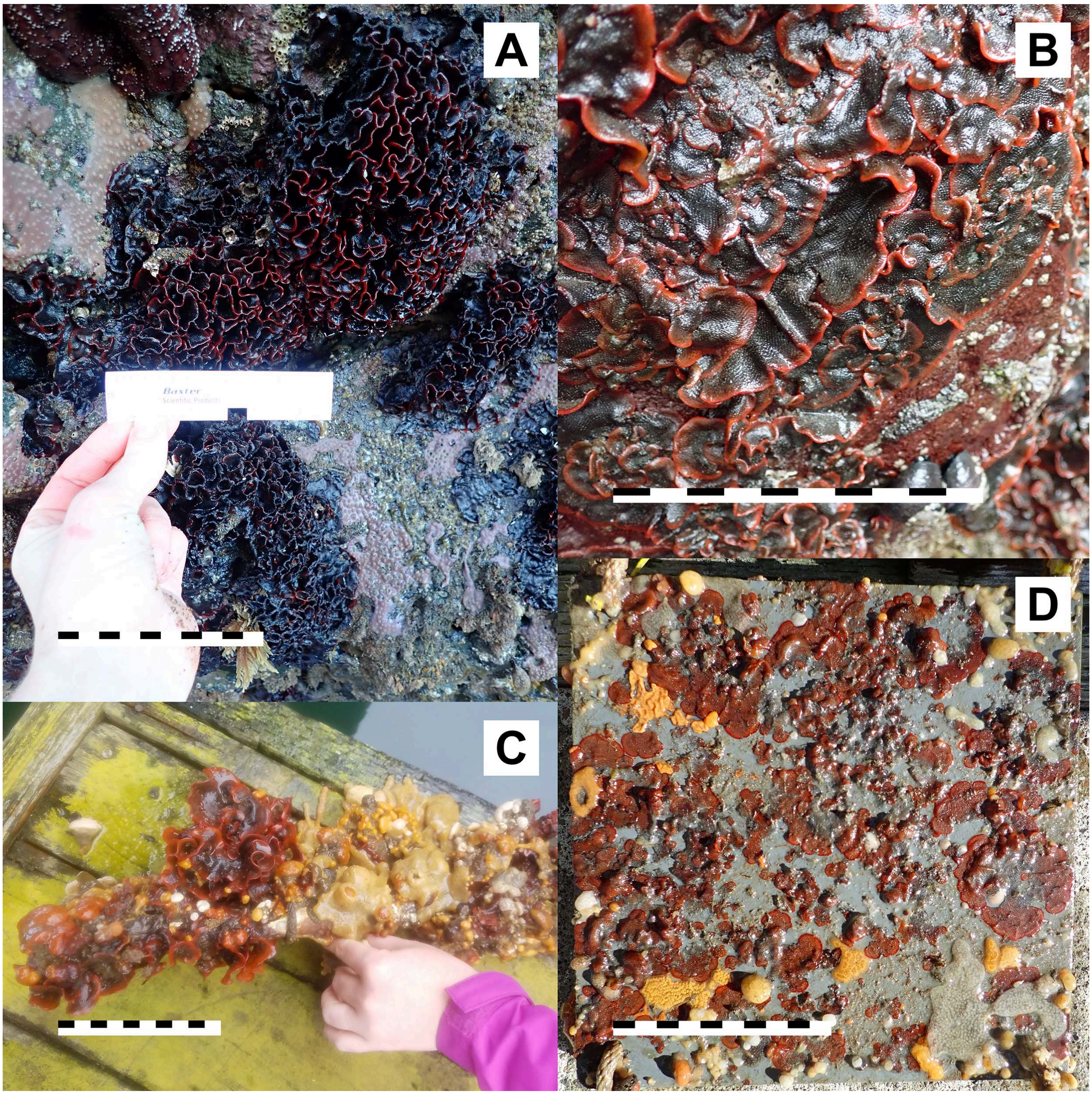

Figure 2. A range of *Watersipora* spp. morphologies and colors observed in the rocky intertidal and in harbors in the San Francisco Bay area. A: A large mass of dense, foliose colonies growing on the shore-facing side of a large boulder in the rocky intertidal. (site: Mile Rock Beach, San Francisco, California. January 9, 2020). B: A rock surface in the intertidal covered with encrusting colonies, showing a primarily dark color with bright red growth margins. (site: Slide Ranch, Marin County, California. June 18, 2019). C: Dock debris colonized by numerous invasives, including large foliose “heads” of *Watersipora*. (site: Pillar Point Harbor, San Mateo County, California. May 19, 2019). D: A dock settlement plate heavily colonized by bright red, encrusting *Watersipora*. (site: Pillar Point Harbor, San Mateo County, California. January 11, 2019). All scale bars represent 10 cm. Photographs by M. Duncan.

Discussion

This study presents the first genetic data establishing that the invasive species *Watersipora subtorquata* and *Watersipora* new species are both found in rocky intertidal habitats on the outer coast of central California, near the highly invaded San Francisco Bay. Although the outer coast tends to experience overall cooler water temperatures than the San Francisco Bay, and previous research has shown geographic stratification by temperature (Mackie et al. 2012) and temperature-mediated differences in growth rates

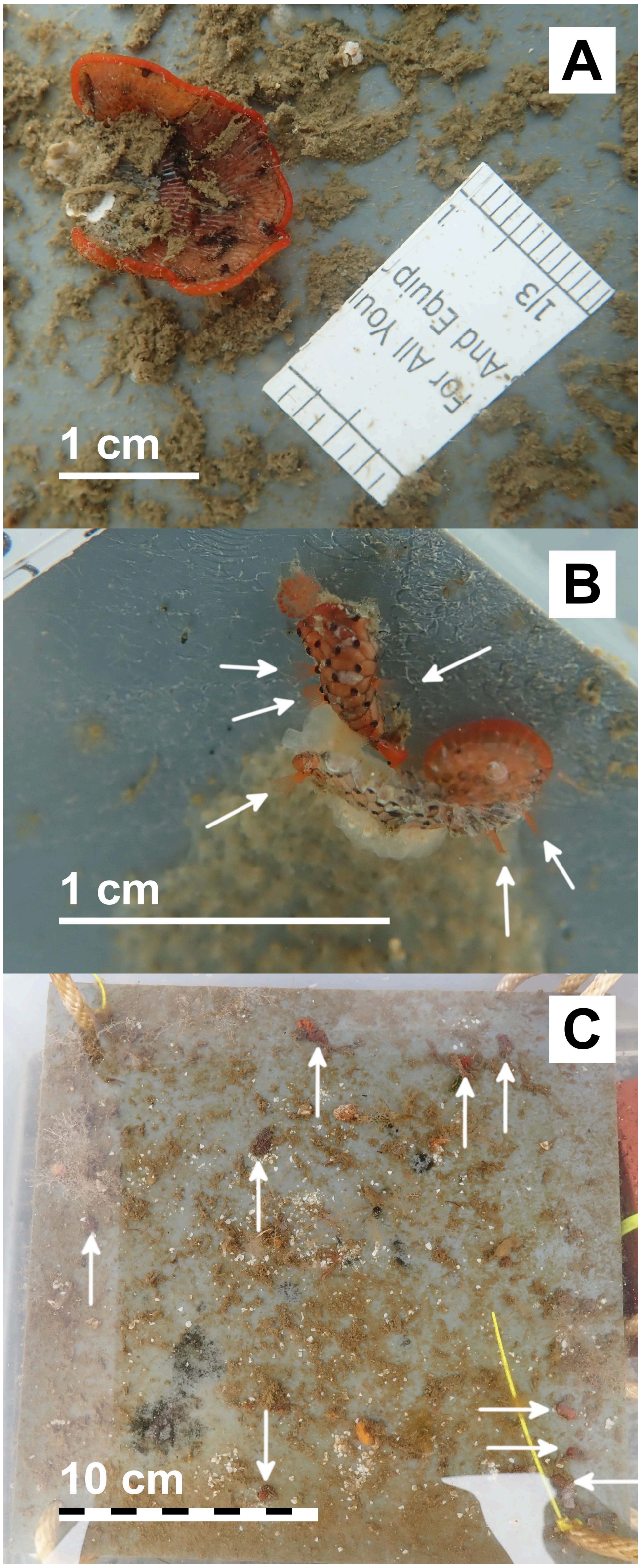

Figure 3. Live fragments of adult *Watersipora* colonies observed on settlement plates after fragmentation and transport. All plates were placed at Pillar Point Harbor (San Mateo County, California) at depths of 1 meter or 2.5 meters. Plates were deployed on July 5, 2019; fragment observations were recorded on July 19, 2019. A: A fragment partially adhered to the plate by mud and mucus debris from tube-building organisms. B: A small fragment adhered by association with *Diplosoma* sp. Extended lophophores are indicated by white arrows, demonstrating that the re-adhered colony is alive and actively feeding. C: A settlement plate covered in loose debris, including several small fragments of adult *Watersipora* colonies (indicated by white arrows). Photographs by M. Duncan.

and reproductive success between *W. subtorquata* and *Watersipora* n. sp. (Korcheck 2015), both species are capable of establishing and surviving in outer coast rocky intertidal habitats. Additionally, at the four outer coast intertidal sites we monitored, *Watersipora* was persistent year-round from late 2018 to present, suggesting that this species is a highly successful invader once established. The continuous presence of adult colonies indicates that outer coast intertidal populations are likely self-sustaining from highly localized (within-site) recruitment and do not require constant larval input from other sources (e.g., San Francisco Bay or from small traveling watercraft) to persist, though further genetic analysis is needed to determine how gene flow affects diversity in these populations.

We did not identify any haplotypes in outer coast intertidal populations that had not previously been sampled from harbors by Mackie et al. (2012), so our data do not provide any evidence for independent sourcing of these outer coast intertidal populations. It is plausible that most invasive populations were originally established by larvae or propagule fragments transported to outer coast sites from nearby harbors and marinas, as would be expected for an organism with short larval duration. In addition to our sites around the mouth of San Francisco Bay, populations from both rocky intertidal and harbor habitat types have been observed in the past at Point Reyes National Seashore within Drakes Estero, where *Watersipora* was found on rocky substrate just inside the mouth of the estuary (J. Goddard email to J.T. Carlton 22 July 1995, Cohen and Carlton 1995) as well as on oyster lines at Drakes Bay Oyster Farm in the Schooner Bay arm of the upper estuary (A.N. Cohen *pers. comm.*; Elliott-Fisk et al. 2005). Modeling of *Watersipora* larval dispersal in the Santa Barbara Channel of Southern California also supports predominantly local transmission (Page et al. 2019a), and previous studies have suggested local small vessel traffic as a major vector for invasive species in and around the San Francisco Bay (Davidson et al. 2010; Wasson et al. 2001). Testing patterns of dispersal between habitat types with a larger set of high-resolution genetic data is critical for invasive management strategy. If outer coast populations are predominantly locally sourced and self-sustaining, site-by-site eradication has the potential to be an effective method of controlling the spread of *Watersipora* in intertidal habitats. Cleared sites are unlikely to be recolonized by distant propagule sources, and removal may be paired with management and monitoring in nearby marinas and other high-traffic, heavily-colonized habitats to reduce the chances of repopulation by local propagule sources.

However, our surveys demonstrate that proximity to established marina or bay populations is not the only factor driving the spread to rocky intertidal habitats. All of the intertidal study sites where we consistently observed significant *Watersipora* populations are located in the mouth of the San Francisco Bay (Mile Rock, Point Bonita) or north of it (Mile Rock, Slide Ranch). *Watersipora* was never observed on the outer coast rocky intertidal at the sites located an equivalent distance to the south of the mouth of the Bay (Mussel Rock, Rockaway Beach), nor are we aware of any other reports of *Watersipora* in the rocky intertidal anywhere between the San Francisco Bay and Monterey Bay. Notably, Pillar Point Harbor is a major coastal marina south of the bay that is significantly invaded by *Watersipora* and experiences high traffic from small commercial fishing and recreational vessels (Zabin et al. 2009), but *Watersipora* has not yet been observed in immediately adjacent rocky intertidal habitats, despite a long-running and intensive citizen science initiative to catalogue local native and invasive species at Pillar Point, Half Moon Bay (IBSPP 2021; Alison Young and Rebecca Johnson *pers. comms.*; Table 1). Potential variables affecting local larval and colony transport (e.g., water currents, vessel traffic patterns, adherence to hull cleaning regulations at individual marinas) or habitat suitability (competition with native species, protection from wave action, local freshwater inputs, other sources of disturbance, etc.) may influence which rocky intertidal sites are most susceptible to invasion.

Two incidental field observations are also of note: *Watersipora*'s frequent association with *Phragmatopoma californica* in the intertidal, and the transport of live adult fragments at Pillar Point. *Phragmatopoma californica* may have a facilitative relationship with *Watersipora*, possibly by providing physical structure that is attractive or protective to new *Watersipora* recruits, or because the tube structures are an inhospitable substrate for other encrusting species that *Watersipora* would otherwise compete with. This association is potentially useful in field monitoring of *Watersipora* populations in the rocky intertidal, particularly in presence/absence surveys, because *P. californica* colonies can be targeted as likely hosts of *Watersipora* settlement. However, worm reefs may only act as seasonal or ephemeral habitat due to their susceptibility to removal in winter storms (Barry 1989), which would limit their utility as indicators or site-specific “landmarks” in long-term *Watersipora* monitoring. Additionally, although *Watersipora* colonies tend to be loosely anchored to the *P. californica* tube substrate and easy to remove, the surface of the reefs is highly irregular and the tubes themselves can be fragile. Established *Watersipora* colonies often cannot be removed without damage to the worm tubes underneath. The interactions between *P. californica* reefs and *Watersipora* should be explored further to facilitate strategic *Watersipora* eradication and control efforts.

The rafting and facilitated reattachment of adult fragments on settlement plates, although only observed on two plates in one marina, is significant because it demonstrates the possibility of colony fragmentation as a mode of propagation. The viability of unanchored adult colonies has precedent in observations of *Watersipora* colonies in Monterey Bay (Aiken 2014) and colonies of another cheilostome bryozoan, *Schizoporella errata,* in south San Francisco Bay (Zabin et al. 2010), surviving as “underwater tumbleweeds” in soft sediment environments. It is still unclear whether displaced *Watersipora* fragments are capable of self-adherence to new substrates or whether they would continue to depend on partial overgrowth by other encrusting organisms, but self-adherence is plausible if fragments are undisturbed for long enough because encrusting bryozoans produce bioadhesive substances as they grow (Soule and Soule 1977). Further study is needed to determine the frequency of fragment propagation, as well as the viability and reproductive capacity of both loose and “resettled” fragments. If evidence suggests that fragmentation may be a significant propagation route, any removal and control strategies must take disposal and destruction of removed colonies into account. California Department of Fish and Wildlife regulations require consideration of the issue of dispersing fragments of invasive species that may persist and reproduce.

The genetic data and field observations reported here emphasize the need for further population genetic analysis of coastal *Watersipora*. Successful colonization of open-coast intertidal habitats is both an unusual and troubling feature of this species. Without intervention, it is likely that *Watersipora* will continue to spread and damage delicate and increasingly stressed coastal ecosystems. A deeper understanding of the invasive dynamics of these populations, especially their genetic composition and diversity, will contribute to developing effective management and mitigation strategies. These initial data contribute to an ongoing effort to characterize the population genetics of the *Watersipora* invasion of California’s outer coast.

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Authors' contribution

MD: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft; BC: investigation and data collection; KM: sample design and methodology, investigation and data collection; JS: investigation and data collection; MH: investigation and data collection; ES: investigation and data collection; CH: investigation and data collection; WKK: investigation and data collection; CSC: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – review and editing.

Ethics and permits

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