Northward range expansion of three non-native ascidians on the west coast of North America

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Abstract

The solitary ascidians Microcosmus squamiger Michaelsen, 1927, Molgula ficus (MacDonald, 1859), and Styela canopus (Savigny, 1816) have been found beyond their previously known introduced range on the coast of California. The recent appearance in surveys confirms their presence within San Francisco Bay, indicating possible range expansions northward of more than 500 kilometers. All three species have spread across the natural biogeographic barrier of Point Conception, west of Santa Barbara, California. Species identities were confirmed using microscopy, and key distinguishing features were described. These records contribute to the increasing number of non-native species which are expanding their range northwards on the Pacific coast of North America, implicating both human vectors and ocean warming.

Key words: biogeography, climate change, distribution, marine fouling species, tunicate

Introduction

Solitary ascidians are commonly found among marine assemblages fouling artificial and natural substrates. As a consequence, they frequently spread to new locations via human vectors (e.g. vessel biofouling, shellfish transfers, and movement of fouled aquaculture gear and product, oil and gas structures) and often become established as non-native species. Being conspicuous, ascidians are ideal for studies of range expansion, vector strength, invasion dynamics, and management strategies (Zhan et al. 2015; Simkanin et al. 2016). In addition, ascidians are particularly important indicators of invasion due to their short-lived, non-feeding, low-dispersal larval stage; thus the appearance of such species in new regions separated by tens to hundreds of kilometers or more from the closest known populations, exceeding natural dispersal ability, indicates that human-mediated transport has likely occurred (Lambert 2007a). Such is the case for three solitary ascidians recently collected hundreds of kilometers north of their known northern range border on the coast of California: Microcosmus squamiger Michaelsen, 1927, Molgula ficus (MacDonald, 1859), and Styela canopus (Savigny, 1816).

Microcosmus squamiger is native to Australia (Kott 1985) and has established introduced populations on the west coasts of the United States and Mexico, the Mediterranean Sea, the Atlantic coast of Spain, the Canary Islands, and South Africa where it is commonly found on both natural and artificial substrates (Fofonoff et al. 2003; Rius et al. 2008, 2009). In California, M. squamiger was first collected in Alamitos Bay, Long Beach in 1986 (Lambert and Lambert 1998). By 1994, its range extended from San Diego Bay to Santa Barbara Harbor (Lambert and Lambert 1998, 2003). In 2000, individuals were
collected in Ensenada, Baja California, Mexico, (Lambert and Lambert 2003) and were observed in 2002 fouling cultured oysters in Bahía San Quintín, Baja California (Rodríguez and Ibarra-Obando 2008). The introduction of *M. squamiger* in Bahía San Quintín resulted in economic damage to aquaculture operations (Rodríguez and Ibarra-Obando 2008). Competition may occur in other introduced regions as well. For example, *M. squamiger* now outnumbers *Styela canopus*, an early invader in San Diego Bay, and forms single-species patches at some locations, possibly crowding out other species in shallow water communities (Lambert and Lambert 2003).

*Molgula ficus* is also considered native to Australia and has a wide distribution in the Indo-West Pacific region including the Gulf of Thailand, Singapore, and Hong Kong (Kott 1985). In California, *M. ficus* was originally misidentified as the native species *Molgula verrucifera* Ritter and Forsyth, 1917 by P. Kott, but was later correctly identified by its larger size and morphology (Lambert 2007b). Due to this initial misidentification, *M. ficus* was recorded in surveys in Southern California between 1994 and 1997 as the native *M. verrucifera* and therefore not included in reviews by Lambert and Lambert (1998, 2003). Since 1994, *M. ficus* has been found from San Diego Bay north to Channel Islands Harbor, adjacent to Port Hueneme (Lambert 2007b). *Molgula ficus* was collected in San Francisco Bay in Ballena Bay, Alameda in 2005, but it was not considered to be established in the Bay at the time (Lambert 2007b). In Antofagasta Bay, Chile, *M. ficus* was first collected in 1997 and was common on ropes used for scallop aquaculture (Clarke and Castilla 2000; Castilla et al. 2005); however, the impact on culture operations is unclear.

*Styela canopus* was first described from the Red Sea in 1816, and later reported as introduced on both sides of the North Atlantic, the Gulf of Mexico, Ascension Island in the South Atlantic, the NW Pacific, the tropical Indo-Pacific, and temperate waters of Australia (Kott 1998). In the NE Pacific, *S. canopus* was first reported from San Diego Bay, California, in 1972 (Lambert and Lambert 1998). It has since been collected as far north as Alamitos Bay, Long Beach, but is otherwise thought to be rare outside San Diego Bay (Lambert and Lambert 1998). *Styela canopus* is commonly found on ships, buoys, piers, docks, mangroves and coral reefs, although no economic or ecological impacts have been documented for this species (Fofonoff et al. 2003).

We report on the poleward range expansion of these three solitary ascidians, recorded in fouling community surveys of San Francisco Bay during 2011–2016.

### Methods

As part of an ongoing study, marine fouling communities were monitored within San Francisco Bay, California, USA (37°42′30″N; 122°16′49″W) at 15 different sites (marinas and ports) over a six-year (2011–2016) timespan using settlement panels. Settlement panels (hereafter referred to as “panels”) were cut from grey high-impact polyvinyl chloride (PVC) sheets to 14 × 14 cm squares, lightly sanded on one side to optimize attachment conditions, attached to bricks with the settlement surface facing down, and suspended horizontally at one meter depth below docks. Ten replicate panels were deployed per site and remained in the water for three months. Deployment location for each panel was randomly assigned within each site. Panels served as passive collectors for the colonization of marine organisms and provided a standardized method to assess fouling community assemblages at each sampling location. Surveys were conducted during summer (between June and September) to coincide with the season of maximum larval recruitment. Sampling sites were chosen to provide comprehensive coverage of the San Francisco Bay estuary (Figure 1). The location and number of sites surveyed each year varied due to funding sources and permissions from site managers.

Upon retrieval, all macrofauna attached to the plates were identified by taxonomic experts and all taxa that could be identified to species level were assigned an invasion status of native, non-native, or cryptogenic (*sensu* Carlton 1996) using the National Exotic Marine and Estuarine Species Information System (NEMESIS; Fofonoff et al. 2003). Sessile organisms were sorted live, creating individual field vouchers for each morpho-species at each site. These preserved field vouchers were then identified to species (or lowest taxonomic unit) based upon morphological characteristics described below. Additional individual ascidian specimens were collected from one sampling location (Oakland Yacht Club) to supplement samples from panels and provide additional material for morphological examination.

**Identification of Microcosmus squamiger**

*Microcosmus squamiger* is an irregularly-shaped, solitary ascidian up to 5 cm in diameter (Lambert and Lambert 1998) with an often-fouled, purple to reddish, leathery tunic (Kott 1985). The wide-set siphons are directed away from the animal in opposite directions (Figure 2). The internal body is reddish-purple in coloration with siphons lined in red stripes. One primary distinguishable characteristic of *Microcosmus* spp. is
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**Figure 1.** San Francisco Bay with sites sampled during 2011–2016. Bars below the site names indicate the years in which the sites were surveyed (black: survey completed during the year; red: survey completed and novel ascidians found), key to the years is shown in the bottom left. *Microcosmus squamiger* was identified in 2015 and 2016 from Oakland Yacht Club; *Molgula ficus* was identified in 2016 at Oyster Point and Coyote Point; *Styela canopus* was identified in 2015 from Oakland Yacht Club.

The presence of microscopic spines embedded in the siphonal lining (Kott 1985). The spines of *M. squamiger* are short and uniquely cup-shaped (Figure 2E) (Kott 1985; Mastrototaro and Dappiano 2005).

**Identification of Molgula ficus (MacDonald, 1859)**

*Molgula ficus* is a squarish, solitary ascidian with a six-lobed oral siphon and a four-lobed atrial siphon located divergently at the anterior end of the body (Figure 3). The tunic is whitish, thick and very opaque. The species has seven branchial folds per side with predominantly straight stigmata. The gonads have a characteristic fig-shaped form, with the testes encircling the posterior end of the ovary on each side (Lambert 2007b). Attachment of the body most frequently occurs posteriorly, but many individuals often occur in large clusters and the position of the area of attachment may be variable. This species is a free spawner and does not brood its embryos.

*Molgula ficus* can be difficult to distinguish from the previously established *Molgula manhattensis* (De Kay, 1843). Particular characteristics used to distinguish the two species were (1) the tunic: white, thick and very opaque in *M. ficus*, thin and usually translucent in *M. manhattensis*; (2) number of branchial folds per side: seven for *M. ficus* and six for *M. manhattensis*; and (3) the shape of the ovotestes and position of the testis on the ovary: fig-shaped ovotestis in *M. ficus* with the testis at the posterior end of the ovary, and oblong in *M. manhattensis* with the testis arranged along the entire length of the ovary (Van Name 1945; Lambert 2007b).

**Identification of Styela canopus (Savigny, 1816)**

*Styela canopus* is a small (1 to 3 cm) slightly-elongated, solitary ascidian (Figure 4). The tunic is usually tough and pale red to brown in appearance. It has two short, maroon and white-striped siphons that are relatively close together, the oral siphon at the anterior end and the atrial siphon slightly posterior.

Two features that distinguish *S. canopus* from others in the Styelidae family are the morphology of the muscles and gonads. The muscles are well-spaced and longitudinal with the internal organs being visible through the body wall when removed from the tunic (Kott 1985). There are two longitudinally oriented gonads on each side on the body (rarely three). The anterior gonad on the left side extends over the gut loop, while the posterior curves downward into the gut loop (Kott 1985).
**Figure 2.** *Microcosmus squamiger* Michaelsen, 1927 A, B. Tunic removed showing distinct cross-hatching muscle striations of *Microcosmus* spp. (A. Left side; B. Right side); C, D. Opened body through the branchial sac depicting very leathery, thick outer tunic and 8 overlapping branchial folds on each side (C. Left side; D. Right side) Scale Bar A–D = 1 cm. E. Cup-shaped siphonal spines, each about 0.01 mm. Photos A-D by K. Larson. Photo E by G. Lambert.
Results

During the 2015 and 2016 surveys, three novel species were found on panels deployed in San Francisco Bay that were not identified in prior sampling years (2011–2014). This was the first time that Microcosmus squamiger and Styela canopus had been recorded during the annual surveys (Figure 1). The first record for Molgula ficus in San Francisco Bay was 46 specimens collected at Ballena Isle Marina in 2005 (Lambert 2007b).

*Microcosmus squamiger* was identified at Oakland Yacht Club on three of ten panels in 2015 (Figure 2). In addition, one individual that could only be identified to genus level (small specimen resembling *M. squamiger* but not mature) was collected in 2016. Consequently, in October 2016, the authors revisited Oakland Yacht Club. Following an additional search of the docks by one person dockside and one snorkeling, two individuals were collected and confirmed to be *M. squamiger*. A single *Molgula ficus* was collected in 2016 at both Oyster Point and Coyote Point Marinas (Figure 3).

*Styela canopus* was identified in 2015 from Oakland Yacht Club (Figure 4). A single individual could be confirmed as *S. canopus*, while four others were considered likely *S. canopus* (*Styela cf. canopus*) with each individual being found on a different panel. Unfortunately, species identification could not be confirmed for the four specimens because they lacked distinguishing features, were immature, or of poor quality. The specimens collected were, however, distinctly different from the only other species in the genus known to be in or near San Francisco Bay (*Styela clava* Herdman, 1881 and *Styela montereyensis* (Dall, 1872)).

Discussion

These data represent the northernmost records for these three species on the west coast of North America, indicating northward range expansion, breaching the biogeographic regional boundary of Point Conception, California, as was predicted for *Microcosmus squamiger* by Lambert and Lambert (1998). Several features of the records suggest that each species has only recently been introduced to San Francisco Bay and is perhaps not yet fully established: 1) species were found only in later years of annual field surveys; 2) the numbers of individuals were few in each location, with a single individual being sampled on up to five of ten plates deployed; 3) the marine environment of San Francisco Bay is well-studied (See Cohen and Carlton 1995, 1998; Ruiz et al. 1997, 2000, 2011; CDFG 2011).

Figure 3. *Molgula ficus* (MacDonald, 1859) A. External view; B, C. Tunic removed showing internal body wall; B. Left side, gut loop and gonad; C. Right side, gonad and kidney. Scale Bar A–C = 1 cm. Photos by K. Larson.
and no other record of these species from this region is reported. Additionally, these three species were absent from surveys conducted in nearby Morro Bay to the south in 2013 and Bodega Bay to the north in 2012 (Simkanin et al. 2016).

Low abundance of these species at survey locations suggests that we may have detected the onset of the invasion process. The presence of *M. squamiger* during successive years suggests that it may be established in Oakland Yacht Club. *Molgula ficus* was detected at two sites sampled in 2016, both on the opposite side of the Bay from Ballena Isle, where it was first recorded in 2005 (Lambert 2007b) and not recorded during this study. It seems likely that *M. ficus* is at least a transient visitor to the Bay, with a recurring introduction vector, or at most an established but limited population that is occasionally detected in our surveys. There is insufficient evidence for *Styela canopus* to be considered “established” in San Francisco Bay. Its detection in surveys during 2015 provides confirmation of a recent active vector for this species, but its status in the Bay remains unknown.

Commercial vessel traffic via both ballast water and hull fouling is the largest source of marine species transfers worldwide (Ruiz et al. 1997). San Francisco Bay is an important shipping hub, with links to southern California where these species (*M. squamiger, S. canopus, M. ficus*) are already established. Introduction via commercial vessel biofouling (especially via sea chests) is one potential vector for ascidian introductions (Coutts et al. 2003). However, the presence of the species identified in this study within fouling communities of recreational marinas suggests a small vessel vector either in addition to or instead of commercial vessels. This expansion may serve as new hub for these ascidians, providing additional opportunities for continued northward expansion (Ruiz et al. 2011; Ashton et al. 2014) and a local source for spread to nearby smaller bays and harbors via transfer on small boats (Wasson et al. 2001; Davidson et al. 2010; Zabin et al. 2014).

In addition to human-mediated dispersal, changing environmental conditions may have contributed to the timing of the northward expansion. These recent northward range expansions coincide with warmer sea temperatures due to a multiyear marine heat wave along the Pacific coast that led to sea surface temperatures exceeding historical highs by as much as 3 °C during 2014−2016 (Hartmann et al. 2015; Cavole et al. 2016; Greene 2016). This event generated northward shifts of marine species as a response to the warm-water anomaly, including marine invertebrates (Leising et al. 2015). With the combination of projected climate change-induced ocean warming and increased frequency of warm weather events (IPCC 2014), future introductions and poleward range expansions of northern and southern species are predicted. Continued monitoring will help with early detection of new non-native species introductions and increase our understanding of these expansions and their implications.

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