Ecology of cryptic invasions: latitudinal segregation among *Watersipora* (Bryozoa) species

Joshua A. Mackie1,*, John A. Darling2 & Jonathan B. Geller1

1Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039, USA, 2National Exposure Research Laboratory, US Environmental Protection Agency, 109 T. W. Alexander Drive, Research Triangle Park, NC 27711, USA.

*Watersipora* is an invasive genus of bryozoans, easily dispersed by fouled vessels. We examined Cytochrome c oxidase subunit I haplotypes from introduced populations on the US Pacific coastline to investigate geographic segregation of species and/or haplotypes. In California, the *W. subtorquata* group fell into three major sub-groups: *W. subtorquata* clades A and B, and *W. “new sp.”*. *W. subtorquata* clades A and B were common in southern California south of Point Conception, a recognized biogeographic boundary, whereas further north, *W. subtorquata* clade A and *W. n. sp.* were frequent. The southern California region also had colonies of a morphologically distinct species, *W. arcuata*, also found in southern Australia and Hawaii; COI variation indicates a common ancestral source(s) in these introductions. The distribution of *Watersipora*-complex lineages on different coastlines is shown to be temperature correlated. Accordingly, pre-existing temperature-based adaptations may play a key role in determining invasion patterns.

Genetic studies have widely supported the appearance of ‘cryptic’ evolutionary divergence at multiple levels of the taxonomic hierarchy, (e.g. multiple species existing within taxa traditionally considered to be single species1–3). The non-native establishment of such diversity is typically referred to as ‘cryptic invasion’4, and these phenomena often reflect unrecognized multiple introduction events. Given the unsettled taxonomic state and the high rate of transport by anthropogenic vectors for these taxa, it is not surprising that processes underlying marine invasions remain poorly understood4–6. For example, there are few cases in which one could confidently determine from vector history alone, in the absence of genetic information, whether the invasive spread of an organism is the result of propagules from one area or multiple areas4.

The existence of cryptic genetic diversity suggests the possibility that corresponding ecological differences could be important for patterns of invasion5. Limited examples suggest that invasion potential among such lineages can vary widely, presumably reflecting intrinsic ecological differences. For example, mussels in the genus *Mytilus* include three cryptic species with different environmental tolerances, of which the warmer water species *M. galloprovincialis* has proven an aggressive invader8,9. Similarly, lineages of the barnacle *Balanus glandula* from warmer southern areas of the native range in North America invaded similarly warm coasts of Argentina, while lineages from colder northern areas were found in similarly cold areas in Japan10. However, for most marine cases where cryptic invasion has been demonstrated, little is known of the ecological differences among the cryptic lineages.

The bryozoan genus *Watersipora* Neviani, 1895 is a promising system to investigate the importance of cryptic invasions. The Bryozoa are a phylum of encrusting animals that have become common in fouling communities the world over due to a predisposition for human-mediated transport11,12, either in ballast water13 or on ships’ hulls14. Species of *Watersipora* are among the most invasive Bryozoa. Once released, populations of *Watersipora* grow explosively due to lateral growth of established colonies and settlement of short-lived larvae that may be retained near parents owing to short dispersal durations (generally <24 h)15,16. Introduced *Watersipora* have become a major space occupier on natural and anthropogenic substrata in protected bays in many areas17,18. In communities experimentally polluted with copper ions (an active agent in antifouling paint)19,20 or subjected to heat-wave conditions21, invasive *Watersipora* species have settled and occupied space more successfully than most indigenous organisms, suggesting adaptations favoring the rapid spread of these species.

Cryptic diversity in *Watersipora* appears rampant and is incompletely resolved19 genetic analysis is therefore critical to resolving introduction patterns. As currently understood, the genus consists of *W. arcuata* Banta, 1969, a species with distally recurved tentacle apertures22 and supported by monophyly of mitochondrial Cytochrome c
oxidase subunit I (COI) sequences, and a group of genetically distinc
t clades we refer to as the W. subtorquata-complex that share a
proximally pointed sinus in the lophophore aperture (henceforth
“sinusoidal”). This latter group has a tortured taxonomic history that
has been described as a taxonomic “can of worms”24. The taxonomic
difficulty associated with this complex is largely because the genus
lacks the spines, avicularia, and external oviscells used for taxonomic
diagnosis in most other bryozaans.

The nominal species Watersipora subtorquata (d’Orbigny, 1852) is
a widespread invader in cool-temperate areas globally. However, a
divergent lineage (15% Kimura 2-parameter nucleotide divergence
in COI) from the previously known W. subtorquata-complex was
found as a single colony collected in California (this clade was
referred to as W. “new sp.”25) and in subsequent sampling in California26. Thus, it appears at present that at least two cryptic
species comprise the W. subtorquata-complex, and that genetic
analysis is necessary to discover their patterns of distribution. While the
type specimen locale of W. subtorquata is Rio De Janeiro, Brazil,
native ranges of W. subtorquata-complex species are not known
due to uncertainties of taxonomy and dispersal history, which may
include undocumented movement by vessels. A third species, W.
subovoidea (Ryland et al., 2009), which may be invasive in tropical
areas, was recently re-described and formally separated from the W.
sinuosus-complex following confirmation of genetic divergence in COI26. Ryland et al.26 postulate that W. subovoidea may be native
to the Mediterranean, however origin is uncertain, and the
IndoPacific presents another possible source for this species group
according to suggested morphological affinity27.

Recognition of introductions of Watersipora began when it was
realized that the species now referred to as W. arcuata and thought to
be native to the tropical eastern Pacific28, had invaded Australian
coastline. In fact, by the mid 20th century, W. arcuata was extremely
common around Australian harbors, indicating hull-fouling in
spread29,30, but the arrival time can only be approximated to within
1889–1940 due to a dearth of intervening surveys29. An introduction
of Watersipora arcuata to New Zealand occurred around 195730
from which time populations spread to become common on the
north and south islands31. It is thought W. arcuata invaded in southern
California, near Los Angeles around 196128. Colonies of a sinus-
oid species form, referred to generally as W. arcuata, were
recognized as invading in Australia in the 1970s and in New
Zealand and California in the 1980s31,32. On the three landmasses,
some regional displacement of W. arcuata was seen as colonies of
sinusoid morph entered areas occupied by W. arcuata27,28. Indicating
that the rapid propagation of sinusoid Watersipora colonies occur-
ing along California coastline did not originate from simply one
introduction, an initial study using COI sequence comparison
located a “subtorquata” haplotype occurring in Australia and else-
where, along with an example of COI sequence that was divergent (in
Monterey Bay California), this genetic group being referred to as the
“new sp.” COI phylogroup21.

Our first objective was to determine the distribution of the cryptic
species noted above in California, and whether intensive sampling of
these communities would indicate a likelihood that the number of
sources in introductions is greater still than recognized. The genetic
(COI) analysis was used secondly to investigate whether Watersipora
species were non-randomly distributed with respect to sea surface
temperature (SST) in the California region and globally. By contrast-
ing multiple invasions of multiple species, our analysis suggests that
different temperature-related physiological mechanisms may be
important drivers of the invasive distributional patterns of the
Watersipora lineages.

Results

We generated 361 sequences (Table 1) with median length of 510
nucleotides and an average base content of A, 31.3%; T, 33.3%; C,
18.5%; G, 16.9%. Amino acid translations had no stop codons.
Tajima’s D statistic22 was a mean of −0.1795, ranging generally
between 0 and 2 (Table 2). Despite slight negativity (as is typical22),
few populations showed significant deviation from the simulated D
null distribution, with the exception of some southern Californian
W. subtorquata population samples, as discussed below.

In California we found divergent COI clades that correspond to
previously recognized Watersipora arcuata, W. n. sp. and W.
subtorquata phylogroups. Sequences were also included in the Bayesian
tree (Figure 1) from colonies identified as W. edmonsondi (on coral,
n=2, Kane‘ohe Bay, Hawaii) and W. subovoidea colonies from souther-
ern Florida, Brazil, and tropical Australia (Table 1). Monophyletic
groups differed by average net divergence of 18.5% (Kimura-2
parameter model); the lowest divergence was observed between
W. subtorquata and W. edmonsondi, 12.3%; greatest divergence
was observed between W. arcuata and W. n. sp. (24.0%). Watersipora
subtorquata sequences differing by a net divergence of 2.8% formed
clades we refer to as W. subtorquata clades A and B (Figure 1).

W. subtorquata clade A was abundant in southern California and
in central California – Moss Landing, San Francisco and Tomales Bay
(Figure 2). Clade B was common in southern California and was
established at Humboldt Bay, northern California, where it was
found in specimens collected in 2002 along with specimens of n.
sp. clade. To increase our understanding of clade composition of
Humboldt Bay, California we then determined COI phylogroup
using a multiplex assay, in which five PCR primers each mismatched
at its 3’ end to one or two A, B, or new sp sequence populations,
generating phylogroup-specific fragment lengths viewed on agarose
gels26. W. n. sp. was dominant at Humboldt Bay (92.67%), with clade
A (3.84%) and clade B (3.49%) being both present at lower frequency
(Figure 2).

W. subtorquata clade A has been the most common group
sampled at a global scale to date (Figure 3). The most common clade
A sequence in California (referred to as haplotype WS123) is also
common in southern Australia, New Zealand, and Europe
(Figure 3B). The second most frequent haplotype of clade A (WS3)
occur also in southern Australia and South Korea (Figure 1). Clade B
variation consisted of one haplotype characterized previously20
(GenBank accession: AY647167) and a second haplotype repre-
sented by two colonies collected at Long Beach (near Los Angeles),
and a third unique sequence represented by a single colony sampled
previously in China22 (collected on seaweed at Qingdao Huiquan
Beach, Qingdao, GenBank accession: EU365892) (Figure 3B).

The W. n. sp. haplotypes, consisting of one common sequence and
several less frequent, related haplotypes (Figure 3C) were common
in many California sites. The southernmost finding of W. n. sp. was
Oxnard, central California, which is within the region defined by the
16°C long term mean SST isotherm (Figure 2). W. n. sp. was the one
group found at Bodega Bay, Morro Bay, and Bremerton. Clade A and
n. sp. occurred at similar frequencies in the area of San Francisco and
Monterey Bay.

The five COI phylogroups that we recognized occur in statistically
distinguishable SST regimes according to a partial Mantel test that
included all five groups (P < 0.0001). From lower to higher SST,
these were W. n. sp. (median=11.8°C, 95% confidence interval
10.9–15.1°C, n=111), W. subtorquata clade A (15.1°C, 14.0–17.3°C, n=212), W. arcuata (16.3°C, 15.1–25.7°C, n=82), W.
sinuosus clade B (17.1°C, 11.8–18.2°C, n=29), and W. sub-
ovoidea (26.0°C, 25.0–26.3°C, n=33). Partial Mantel test compar-
sions of SST for pairs of phylogroups produced significant r
coefficients at P-values between 0.020 and 0.001 (statistically signifi-
cant at an unadjusted α value of 0.05); the weakest correlation (r =
0.1008) occurred in comparison of W. arcuata and W. subtorquata
clade A distributions (P = 0.0127).

Population pairwise ΦST measures were generally higher in the W.
sinuosus clade A, B and arcuata samples than among n. sp.
| Region | Site or Area name | Coordinates* (lat., long.) | mean SST (°C) | COI phylogroup | N | Source (if another study) |
|--------|-------------------|----------------------------|---------------|----------------|---|--------------------------|
| w. US  | Bremerton         | 47.5798, −122.6321          | 10.0          | n. sp.        | 12|                          |
| w. US  | Bodega Bay        | 38.00, −123.00              | 11.3          | n. sp.        | 1 |                          |
| w. US  | Bodega Bay Harbor | 38.3295, −123.0562          | 11.3          | n. sp.        | 16|                          |
| w. US  | Humboldt Harbor   | 40.8074, −124.1635          | 11.8          | B             | 11|                          |
| w. US  | Humboldt Harbor   | 40.8074, −124.1635          | 11.8          | n. sp.        | 33|                          |
| Europe | Plymouth          | 50.30, −4.14                | 13.0          | A             | 1 |                          |
| Europe | Guernsey          | 49.50, −2.58                | 13.0          | A             | 2 |                          |
| Europe | St. Jacut         | 48.60, −2.15                | 13.0          | A             | 4 |                          |
| w. US  | Moss Landing Harbor | 36.8051, −121.7852      | 13.0          | A             | 25|                          |
| w. US  | Moss Landing Harbor | 36.8051, −121.7852      | 13.0          | n. sp.        | 12|                          |
| w. US  | Morro Bay         | 35.37, −120.86              | 13.3          | n. sp.        | 1 |                          |
| w. US  | Morro Bay         | 35.3707, −120.8585          | 13.3          | n. sp.        | 14|                          |
| w. US  | San Francisco Bay | 37.91, −122.35              | 13.5          | n. sp.        | 1 |                          |
| w. US  | San Francisco, Richmond | 37.9130, −122.3503 | 13.5 | A | | 25 |
| w. US  | San Francisco, Richmond | 37.9130, −122.3503 | 13.5 | B | | 1 |
| w. US  | San Francisco, Richmond | 37.9130, −122.3503 | 13.5 | n. sp. | | 1 |
| w. US  | San Francisco, Oakland | 37.8102, −122.3230 | 13.5 | A | | 1 |
| w. US  | San Francisco, Oakland | 37.7845, −122.2676 | 13.5 | A | | 4 |
| Australia | Hobart          | −43.00, 147.28             | 14.0          | A             | 4 |                          |
| Europe | Wellington       | −41.00, 174.78             | 14.2          | A             | 4 |                          |
| w. US  | Tomales Bay       | 38.1991, −122.9220          | 14.3          | A             | 17|                          |
| w. US  | Ventura          | 34.17, −119.23              | 15.1          | B             | 1 |                          |
| Australia | Melbourne     | −38.00, 144.82             | 15.1          | A             | 7 |                          |
| w. US  | Channel Islands Harbor | 34.1666, −119.2250     | 15.1          | A             | 13|                          |
| w. US  | Channel Islands Harbor | 34.1666, −119.2250     | 15.1          | n. sp.        | 1 |                          |
| w. US  | Channel Islands Harbor | 34.1666, −119.2250     | 15.1          | B             | 1 |                          |
| w. US  | Port Hueneme      | 34.1532, −119.2095          | 15.1          | arcuata       | 3 |                          |
| w. US  | Port Hueneme      | 34.1532, −119.2095          | 15.1          | A             | 2 |                          |
| w. US  | Port Hueneme      | 34.1532, −119.2095          | 15.1          | n. sp.        | 14|                          |
| w. US  | Marina Del Rey    | 33.9702, −118.4496          | 15.1          | arcuata       | 8 |                          |
| w. US  | Marina Del Rey    | 33.9702, −118.4496          | 15.1          | A             | 3 |                          |
| w. US  | Santa Barbara     | 34.4067, −119.6890          | 16.0          | arcuata       | 19|                          |
| w. US  | Santa Barbara     | 34.4067, −119.6890          | 16.0          | n. sp.        | 1 |                          |
| Australia | Adelaide    | −34.50, 138.53              | 16.3          | arcuata       | 12|                          |
| Australia | Adelaide    | −34.50, 138.53              | 16.3          | A             | 1 |                          |
| w. US  | Oceanside        | 33.21, −117.40              | 17.1          | arcuata       | 2 |                          |
| w. US  | Oceanside, Shelter Island | 32.71, −117.23 | 17.1 | A | | 1 |
| w. US  | Oceanside        | 33.2121, −117.3954          | 17.1          | arcuata       | 1 |                          |
| w. US  | Oceanside        | 33.2121, −117.3954          | 17.1          | A             | 12|                          |
| w. US  | Oceanside        | 33.2121, −117.3954          | 17.1          | B             | 3 |                          |
| w. US  | Mission Bay      | 32.7671, −117.2362          | 17.1          | A             | 18|                          |
| w. US  | Mission Bay      | 32.7671, −117.2362          | 17.1          | B             | 1 |                          |
| w. US  | Long Beach Harbor | 33.7655, −118.2528         | 17.3          | A             | 17|                          |
| w. US  | Long Beach Harbor | 33.7655, −118.2528         | 17.3          | B             | 4 |                          |
| w. US  | Huntington Harbor | 33.7175, −118.0658         | 17.3          | A             | 17|                          |
| w. US  | Dana Point Harbor | 33.4591, −117.6992         | 17.4          | A             | 8 |                          |
| w. US  | Dana Point Harbor | 33.4591, −117.6992         | 17.4          | B             | 5 |                          |
| w. US  | Dana Point, Tijuana Est. | 33.4614, −117.7146 | 17.5 | A | | 3 |
| w. US  | Newport          | 33.6199, −117.8943          | 18.2          | A             | 14|                          |
| w. US  | Newport          | 33.6199, −117.8943          | 18.2          | B             | 1 |                          |
| n. Asia | Korea, Namhae Sangju | 34.71, 127.99          | 20.0          | A             | 1 |                          |
| n. Asia | Korea, Namhae Sangju | 34.71, 127.99          | 20.0          | n. sp.        | 1 |                          |
| Australia | Sydney      | −33.87, 151.21             | 20.3          | arcuata       | 17|                          |
| Australia | Sydney      | −33.87, 151.21             | 20.3          | A             | 6 |                          |
| Australia | Perth       | −31.93, 115.83             | 20.5          | arcuata       | 10|                          |
| Australia | Perth       | −31.93, 115.83             | 20.5          | A             | 3 |                          |
| n. Asia | Qingdao       | 36.054, 120.38              | 23.0          | B             | 13|                          |
| e. US  | Florida        | 27.20, −80.22              | 25.0          | subovoidea    | 4 |                          |
| Europe | O’ahu          | 21.00, −157.87             | 25.7          | arcuata       | 12|                          |
| Australia | Dampier    | −20.66, 116.71             | 26.0          | subovoidea    | 4 |                          |
| Brazil | Rio De Janeiro | −23.81, −45.43             | 26.0          | subovoidea    | 14|                          |
| Australia | Cairns     | −16.88, 145.80             | 26.5          | subovoidea    | 1 |                          |

* Coordinates given to four decimal places refer to sampling site from present study. Coordinates to two places refer to regional/locality information derived from other sources.
Table 2 | Summary of population diversity and pairwise $\Phi_{ST}$ measures for *Watersipora arcuata*, subtorquata, and 'new sp'. COI sequences (length: 489 base pairs)

| Diversity indices | $\Phi_{ST}$ |
|-------------------|-------------|
|                   |            |
| **A. Watersipora arcuata** | |
|                   |            |
| n                 | K  | S  | S/n | $\pi$ | D  | Santa Barbara |
|                   |    |    |     |      |    |               |
| Santa Barbara     | 19 | 8  | 18  | 0.9474 | 0.0168±0.0094 | 0.5843 | Santa Barb. |
|                  | 7  | 4  | 14  | 2.0000 | 0.0123±0.0079 | 1.3759 | Msr. Del R. |

| **B. Watersipora subtorquata** | |
| n     | K  | S  | S/n | $\pi$ | D  | Ans-NZ  | Tomales Bay | San Fran. Bay | Elkhorn Sl. | Oxnard | Long B. | Hunt. Harb. | Dana Point | Newport | Ocean. |
|-------|----|----|-----|------|----|----------|-------------|---------------|-------------|---------|---------|-----------|------------|----------|--------|
| **A. Watersipora arcuata** | |
|                   |            |
| n                 | K  | S  | S/n | $\pi$ | D  | Santa Barbara |
|                   |    |    |     |      |    |               |
| Santa Barbara     | 19 | 8  | 18  | 0.9474 | 0.0168±0.0094 | 0.5843 | Santa Barb. |
|                  | 7  | 4  | 14  | 2.0000 | 0.0123±0.0079 | 1.3759 | Msr. Del R. |

| **B. Watersipora subtorquata** | |
| n     | K  | S  | S/n | $\pi$ | D  | Ans-NZ  | Tomales Bay | San Fran. Bay | Elkhorn Sl. | Oxnard | Long B. | Hunt. Harb. | Dana Point | Newport | Ocean. |
|-------|----|----|-----|------|----|----------|-------------|---------------|-------------|---------|---------|-----------|------------|----------|--------|
| **A. Watersipora arcuata** | |
|                   |            |
| n                 | K  | S  | S/n | $\pi$ | D  | Santa Barbara |
|                   |    |    |     |      |    |               |
| Santa Barbara     | 19 | 8  | 18  | 0.9474 | 0.0168±0.0094 | 0.5843 | Santa Barb. |
|                  | 7  | 4  | 14  | 2.0000 | 0.0123±0.0079 | 1.3759 | Msr. Del R. |

| **B. Watersipora subtorquata** | |
| n     | K  | S  | S/n | $\pi$ | D  | Ans-NZ  | Tomales Bay | San Fran. Bay | Elkhorn Sl. | Oxnard | Long B. | Hunt. Harb. | Dana Point | Newport | Ocean. |
|-------|----|----|-----|------|----|----------|-------------|---------------|-------------|---------|---------|-----------|------------|----------|--------|
| **A. Watersipora arcuata** | |
|                   |            |
| n                 | K  | S  | S/n | $\pi$ | D  | Santa Barbara |
|                   |    |    |     |      |    |               |
| Santa Barbara     | 19 | 8  | 18  | 0.9474 | 0.0168±0.0094 | 0.5843 | Santa Barb. |
|                  | 7  | 4  | 14  | 2.0000 | 0.0123±0.0079 | 1.3759 | Msr. Del R. |

| **B. Watersipora subtorquata** | |
| n     | K  | S  | S/n | $\pi$ | D  | Ans-NZ  | Tomales Bay | San Fran. Bay | Elkhorn Sl. | Oxnard | Long B. | Hunt. Harb. | Dana Point | Newport | Ocean. |
|-------|----|----|-----|------|----|----------|-------------|---------------|-------------|---------|---------|-----------|------------|----------|--------|
| **A. Watersipora arcuata** | |
|                   |            |
| n                 | K  | S  | S/n | $\pi$ | D  | Santa Barbara |
|                   |    |    |     |      |    |               |
| Santa Barbara     | 19 | 8  | 18  | 0.9474 | 0.0168±0.0094 | 0.5843 | Santa Barb. |
|                  | 7  | 4  | 14  | 2.0000 | 0.0123±0.0079 | 1.3759 | Msr. Del R. |

| **B. Watersipora subtorquata** | |
| n     | K  | S  | S/n | $\pi$ | D  | Ans-NZ  | Tomales Bay | San Fran. Bay | Elkhorn Sl. | Oxnard | Long B. | Hunt. Harb. | Dana Point | Newport | Ocean. |
|-------|----|----|-----|------|----|----------|-------------|---------------|-------------|---------|---------|-----------|------------|----------|--------|
Spatial differentiation was evident within the W. subtorquata COI clade complex found on the California coastline. An AMOVA supported differentiation of northern and southern populations (from Santa Barbara southward) (\( \Phi_{CT} = 0.1068, P = 0.0215 \)); these populations were also structured in COI nucleotide variation at local scale (\( \Phi_{ST} = 0.1625, P < 0.0001, \Phi_{SC} = 0.06229, P = 0.0401 \)). It is noted that southern Californian populations have D statistic deviations that may be due to the sampling of relatively long branches (clade A and B). Newport and Mission Bay (sites 14 and 16, Figure 2) had D statistic values of < 2.0, which were statistically significant (\( P_{D_{obs}} > D_{exp} < 0.05 \)); this appears to reflect unbalanced frequencies of haplotypes separated on divergent branches. In contrast, Dana Point (\( D = +2.388, P_{D_{obs}} > D_{exp} = 0.9989, \)) and Oceanside (\( D = 0.5516, ns \)) had positive D measures, reflecting the more even spread of clades A and B in these samples.

The zooid area-to-operculum area ratio (Figure 4A) distinguished Watersipora subovoidea colonies as a homogeneous grouping from other sinusoidal colonies consisting of subtorquata and n. sp. COI clade colonies (ANCOVA: \( F_{1,97} = 59.83, P < 0.0001 \)). An ANCOVA, comparing morphometric ratio slopes did not support a difference between W. subtorquata (clade A or B) and W. n. sp. populations (\( F_{1,70} = 0.18, P = 0.6727 \)). In considering the invasive populations (Table 2). Given the fact that the W. n. sp. had relatively low nucleotide diversity, COI is likely insufficiently sensitive for detecting post-introduction genetic isolation if present. However, there was significant differentiation (\( \Phi_{ST} \)) occurring between the Morro Bay sample and other populations (pairwise comparisons of \( P < 0.05 \)), but no other significant differentiation.

Ten Watersipora arcuata haplotypes were found in California (Figure 3A). Two (previously designated h1 and h8, Mackie et al. 2006) were identical to haplotypes previously found in Australia, and three (h5, h6, and h7) identical to haplotypes known from both Australia and O’ahu, Hawaii. The COI variation of W. arcuata showed structuring at the scale of collection sites (\( \Phi_{ST} = 0.4459, P < 0.0001 \)) and collection areas (i.e., within O’ahu, California, Perth or Adelaide; \( \Phi_{SC} = 0.6285, P < 0.0001 \)). There was, however, no differentiation observed in comparing the regional sampling areas of O’ahu, California, Perth or Adelaide. In fact, the inter-area variance component was negative (\( \Phi_{CT} = -0.4912 \)), indicating a spatially-dispersed but locally-structured pattern.

**Figure 1 |** Bayesian tree of COI sequences. Posterior probabilities (> 0.5) are shown at nodes. Squares at branch tips, within four phylogroups – Watersipora arcuata, W. subtorquata clades A and B, and W. n. sp. – indicate an introduced haplotype on the Californian coast. Haplotypes found in other areas are indicated: Washington state (Pacific US); AU, Australia; Fl, Florida (Atlantic, US); BR, Brazil; Ha, Hawaii (O’ahu); NZ, New Zealand (Wellington); UK (southern England and Channel Islands); FR, France. Watersipora colonies have uniform zooids. Typical dimensions were determined from multiple colonies in different COI groups, providing a stereotyped zooid appearance (see Figure 4).

**Figure 2 |** Distribution of Watersipora COI phylogroups in the California region, (regional mean annual sea surface temperature indicated). Colonies were collected from 2002–2011. Circles to the left of corresponding site numbers are previously reported COI samples25. Circles are sized in proportion to the number of colonies collected at each site, except as indicated for Humboldt Bay where phylogroups of a larger number of colonies were determined using multiplex PCR25. Each circle represents a different sample location or year of sampling. Number-only labels: Port Hueneme (8), Channel Island Dock, Oxnard (9), Marina del Rey (10), Long Beach Harbor (11), Huntington Harbor (12), Dava Point (13), Newport Harbor (14), Oceanside (15), Mission Bay (16).
Continuous straight lines represent connections with confidence below this limit.

**Figure 3 | Parsimony networks describing relationships of COI haplotypes of Watersipora, introduced to California and other areas.**

A. **W. arcuata**

Key:
- * inferred haplotype
  - Australia
  - Hawaii
  - California

B. **W. subtorquata**

S. Korea  s. Australia  s. Australia  New Zealand  England/France

Clade A

n = 81

Clade B

5

7

13

EU365892, China

AY647167

(n = 2, Long Beach, CA)

C. **W. new sp.**

S. Korea

n = 75

California, Washington

California

Discussion

On the basis of phylogenetic inference using the COI locus, the previously described Watersipora subtorquata-complex represents two cryptic species, *W. subtorquata* and *W. n. sp.*, consistent with previous reports. According to the present sampling, *W. subtorquata* can be further divided into two genetically shallow groups – clade A, which has haplotypes recognized in Europe and Australasia, and clade B, found to be common in southern California and present at much lower frequency in northern California. Other investigations show that these three known COI clades of the *W. subtorquata*-complex also occur in the Asian western Pacific. The absence of reports of sinusoidal *Watersipora* in Californian waters prior to the 1980s probably indicates the true absence of such forms given the intensity of study there. Since then, according to COI data, there have been multiple introductions from separate sources. The introduced *Watersipora* of the Californian region is more diverse than that of Australasia, and COI variation apparent in California reflects different thermal tolerances of the *W. n. sp.* and *W. subtorquata* complex source populations, different sets of introductions to these areas, or both.

*Watersipora* species as a whole were absent from the US Pacific coastline until the 1960s and absent from the fossil record in that region. *Watersipora arcuata* was the first watersiporid to be recognized on the coastline, appearing in southern California around 1963. Soule and Soule then reported a sinusoid species as invading in Los Angeles Harbors and marinas in 1982–3, a period of unusually warm water due to El Niño. We have no reason to suspect that these specimens were not the sinusoidal *Watersipora subtorquata*-complex described here. Examination of collections of the late Dorothy and John Soule at the Santa Barbara Natural History Museum unfortunately did not locate the material referred to in their report (Mackie, pers. obs., July, 2011). Banta suggested *W. arcuata* was native to the tropics or sub-tropics of the eastern Pacific. Evidence of the native source of *Watersipora subtorquata* clade A and clade B or *W. n. sp.* is lacking, as is a precise timing of arrival in California.

Early collections of colonies that best fit the morphological description of *W. arcuata* were made in the Galapagos Islands and the Pacific Mexican coast, including Baja California and Gulf of California, prior to the 1930s. By 1940, *W. arcuata* was a common fouling species in the Gulf of California but was not yet known on the US Pacific coastline. Two hypotheses have been proposed to explain the appearance of *W. arcuata* in California in the 1960s. Soule proposed that larval dispersal or dispersal of colonies on drift material from warmer Pacific areas into California could occur during El Niño events, when currents from southern areas extend further north than usual. Banta and Carlton each, however, favored a scenario of introduction of *W. arcuata* to California from Australasia through ship fouling, in part based on negative evidence, specifically the lack of *W. arcuata* in extant fouling community records or the fossil record of California. Positive circumstantial evidence includes the observation that Australasia has been a donor of a number of marine invasive species to the US Pacific region.

Given the lack of segregation of COI genetic variation among widespread areas – Australia, California, and Hawaii – the arrival of *W. arcuata* colonizers through shipping from common sources is supported, as opposed to regionally independent introductions.
Defining the routes of introductions around the globe based on COI is not possible though given the observed distribution of genetic variation at that locus. Inferring direction and chronology of these invasions genetically will likely rely on the use of multiple loci providing finer spatial resolution to the distribution of genetic variation.

Watersipora species ranges have undergone remarkable shifts with human assistance. Given such widespread global introductions and realizing that ranges are rapidly dynamic, source populations are perhaps traceable now only by searching for remnant phylogeographic patterns. Collection records of *W. arcuata* and *W. subtorquata* indicate that species range boundaries have changed rapidly. In the 1980s *W. subtorquata* replaced *W. arcuata* in southeastern Australia and New Zealand, specifically in cooler areas of these landmasses\(^\text{17,31}\). In the areas of *subtorquata* introduction, *W. arcuata* had been present since its introduction sometime between the late 1800s and 1940 in Australia\(^\text{26}\) and around 1957 in New Zealand\(^\text{30}\). These taxonomic records indicate that species of Watersipora may compete with one another for resources (with space presumably being one of the most important) in the human-modified fouling niche. Further population separation on the basis of COI supports widespread species-replacement interactions.

Figure 4 | (A) Averaged orifice area versus zooid area (log\(_{10}\) versus log\(_{10}\)) for populations distinguishable as four COI clades. As shown previously\(^\text{26}\) the plotted relationship discriminates the recognized species *W. subtorquata* and *W. subovoidea*, however as evident here, it does not discriminate the *subtorquata* (A or B) and *n.* sp. phylogroups. Inset: cartoon of zooids of averaged proportions, by COI phylogroup. (B) Plot showing means and SE (vertical bars) of zooid length at different sites on US west coast, indicating a decrease in length toward warmer localities. Trend lines were calculated by least-squares regression.

Prior studies of Bryozoa have suggested genetically divergent species are recognizable by morphological divergence\(^\text{43,44}\), but our analysis suggests that this conclusion is not universal. Measurements of subsamples of *W. subtorquata* clades A and B and *W. n.* sp. in California showed these to be homogeneous in their zooid geometry (Figure 4A) and they were not definitively sorted by color (unpublished data). Colonies of *W. n.* sp. varied from flat, encrusting forms to large (30 cm) multi-colony ball-shaped forms. Although diagnostic morphological criteria may yet be discovered, currently none seem present that would be practical for rapid identification, supporting recent and widespread introductions of a species suited to tropical conditions. While genetic analysis of the type specimens or neotypes has not been conducted, a *W. subtorquata-W. subovoidea* delineation is supported on the basis of both morphometric and COI comparison\(^\text{26}\). The *W. subtorquata* holotype (Gunabara Bay, Brazil) was described by d’Orbigny from material collected in 1837. Given this historical record, it was surprising that recent collections in the Rio De Janeiro and Sao Paulo regions have revealed only *W. subovoidea*. Similarly Ramalho et al.\(^\text{42}\) reported collections of *Watersipora* matching *W. subovoidea* morphologically at multiple localities spanning a wide range of surfaces and pollutant levels. Thus, there is reason to suspect *W. subovoidea* has displaced *W. subtorquata* in its native locale or at least an area where it was common in the 1800s, and there is clearly a need to relate collections from different points to the diversity patterns now suggested by genetics.

In the present study we confirmed that *W. subovoidea* (matching northern Australian and Brazilian populations morphologically) also occurs in Florida, US. COI sequences show ≤1% divergence among *W. subovoidea* in Brazil, Florida and Australian populations, supporting recent and widespread introductions of a species suited to tropical conditions. While genetic analysis of the type specimens or neotypes has not been conducted, a *W. subtorquata-W. subovoidea* delineation is supported on the basis of both morphometric and COI comparison\(^\text{26}\). The *W. subtorquata* holotype (Gunabara Bay, Brazil) was described by d’Orbigny from material collected in 1837. Given this historical record, it was surprising that recent collections in the Rio De Janeiro and Sao Paulo regions have revealed only *W. subovoidea*. Similarly Ramalho et al.\(^\text{42}\) reported collections of *Watersipora* matching *W. subovoidea* morphologically at multiple localities spanning a wide range of surfaces and pollutant levels. Thus, there is reason to suspect *W. subovoidea* has displaced *W. subtorquata* in its native locale or at least an area where it was common in the 1800s, and there is clearly a need to relate collections from different points to the diversity patterns now suggested by genetics.

Prior studies of Bryozoa have suggested genetically divergent species are recognizable by morphological divergence\(^\text{43,44}\), but our analysis suggests that this conclusion is not universal. Measurements of subsamples of *W. subtorquata* clades A and B and *W. n.* sp. in California showed these to be homogeneous in their zooid geometry (Figure 4A) and they were not definitively sorted by color (unpublished data). Colonies of *W. n.* sp. varied from flat, encrusting forms to large (30 cm) multi-colony ball-shaped forms. Although diagnostic morphological criteria may yet be discovered, currently none seem present that would be practical for rapid identification,
and genetic analysis will be necessary for continued work on *Watersipora*.

The lack of morphological distinctiveness of *W. subtorquata* and *W. n. sp.* COI clades reflects an absence of obvious skeletal characters (a situation that is exacerbated by the relatively featureless zooid morphology of the genus), or wholesale hybridization leading to a continuous range of morphologies. Hybridization is a factor affecting identification and ecological responses in introductions of the *Mytilus* species complex, for example6. No introgression of mitochondrial DNA has been found in colonies of the *W. arcuata* and *W. subtorquata* morphologies (arcuate and sinusoidal orifice form respectively), which supports the standpoint that *Watersipora* COI clade groups are not randomly interbreeding. Assessment of genetic variation at microsatellite and other nuclear DNA loci is being used to test for admixture among COI clade populations.

Zooids were as much as 25% longer in northern California as compared to southern Californian colonies. The phylogroup itself showed no consistent relationship to zooid length, with rather, colony populations of multiple groups exhibiting latitude related zooid length (Figure 4). Size-latitude trends arguably still require extended documentation in invertebrates generally; in mollusks a recent meta-study has however indicated size trends to be common, with the direction of the relationship being variable41. The direction of the zooid size to temperature relationship seen across these recently introduced *Watersipora* populations is consistent with studies of other *Watersipora* spanning the Galapagos archipelago53 and in other bryozoans where there is an inverse relationship between zooid size and temperature42–44. Phenotypic plasticity is a possible explanation (as seen in one study of a limpet in which shell variation - larger size in cold - was explainable by water temperature rather than genetic variance45). *Drosophila* wing-trait62 on the other hand provide an example of post-introduction variation responding in a clinal selective gradient. There are sharp differences in mean size are recognized following introductions in a number of marine metazoans44. Perhaps promisingly, *Watersipora* provide a useful system in which to assess the heritable/plastic components of zooid size, along with net overall growth, and reproductive characteristics, determining whether these variously influence or respond to observed range expansions.

The boundary presented in part by the cold California and warmer Davis current systems allows the California coast to be used as a sensitive test of the role of temperature in differentiating introduction processes. Examination of COI variation occurring in *Watersipora* revealed significant north-south separation of haplotype frequencies in California. This separation coincides with Point Conception, an area with a high turnover of ranges and phyleogeographic breaks in native taxa61. All *W. arcuata* occurrence was to the south of Point Conception (and n. sp., conversely, has not been found far south of Point Conception). The range of *W. arcuata* however did expand briefly in a northward direction in 1982 and 1983, an El Niño period, such that the species was found in Monterey Bay, northern California62 where it has not been reported subsequently. This separation coincides with Point Conception, an area with a high turnover of ranges and phyleogeographic breaks in native taxa61. All *W. arcuata* occurrence was to the south of Point Conception (and n. sp., conversely, has not been found far south of Point Conception). The range of *W. arcuata* however did expand briefly in a northward direction in 1982 and 1983, an El Niño period, such that the species was found in Monterey Bay, northern California62 where it has not been reported subsequently. This particular observation is notable for indicating the likely sensitivity of the ranges to temperature. Our study, and others examining genotypic variance (e.g.18,23–25), suggest genetically related invading populations have temperature related fitness which determines organismal or genotype-level range limits at least in early stages of introduction. Average sea-surface temperatures of 14°C-20°C unite the southern Californian and some Australian localities, where *W. arcuata* and *W. subtorquata* were found together. The situation is analogous to invasions of two monophyletic *Caulerpa* (Chlorophyceae) groups63–65, which also appear to be established in southern Australia, Mediterranean areas and southern Californian regions, but not northern California.

While the COI phylogroup-SST correlation is derived from relatively few global locales, it is possible to define widespread introductions of the five *Watersipora* COI groups by different temperature-zone envelopes, an indication that intrinsic differences in temperature-related fitness structure patterns of spread. With this background information, direct hypothesis testing can be used to determine whether phenotypic differences as opposed to vector transport patterns alone dictate introduction success or local densities of *Watersipora* species. The COI variation of California (which is greater than that observed in Australia) and haplotype distribution pattern provides a useful framework for common-environment experiments to test for physiological restrictions to ranges. The association of different species and COI clades of *Watersipora* with particular temperature zones suggests a global assortment of lineages into similar temperature zones, in other words, natural selection acting on existing variation in parallel in different areas.

While the invasion success of *Watersipora* populations is geographically limited by evolved ecological tolerances, the species in the genus as a whole have cumulatively extremely broad potential for global spread, with a collection of traits (including a high tolerance of copper-based antifouling paint that is apparent in larvae and colonies) which assists in colonization of painted hulls on ships that may further transport colonies. Perhaps because growth rate and reproductive potential are intrinsically connected in modular organisms, temperature modulated growth rate (temperature-related fitness) may prove more useful than other general hypotheses commonly put forward to explain the high invasive capacity of certain introduced species, such as the ability to escape specialist predators and pathogens52–54 or propugule pressure55,56. Clearly, modular fouling organisms warrant attention as a group of organisms sensitively indicating community changes in response to environmental change.

**Methods**

**Collections.** Colonies were collected between 2005 and 2010 from docks and floats, predominantly, throughout California, and additional specimens were obtained from fouling panels in San Francisco Bay, and Humboldt Harbor (collections made in the period of 2002–2005), and field collections from Washington State (Bremerton), Florida, and Brazil (Tables 1 and 2). We generated COI sequence for 361 colonies. Sequences and collection information were lodged in GenBank (accession numbers: JQ715456–JQ715577). We included previously reported sequences23,25,26,36,37 in analyses.

**PCR and sequencing.** Colonies were preserved in 85–95% ethanol. Fragments (which were generally <2 cm across) were sorted into individual colonies with independent ancestry. DNA was extracted by QiaGen DNAeasy Tissue protocol. DNA for sequencing was obtained by amplification of 710 base pairs using LCO1490 and HCO2198 primers63, followed by re-amplification of this product in a second PCR with LCO1490 and a bryozoan-specific primer BRY-HCOI-2161, effectively increasing product yield66. PCR was carried out using GoTaq® DNA polymerase and 2x Buffer, with 3.0 mM Mg2+ ion, at an annealing temperature of 40°C. Products were isolated using QaGen Quickspin columns and sequenced in both directions by BigDye® di-deoxy terminators.

**Sequence analysis.** Sequence chromatograms were read using Codon Code Aligner® software, aligned using MEGA464, and collated to haplotypes using DNA Collapser V. 1 (http://www.hirc.au.de/fabos). A Bayesian analysis (using a flat prior distribution and a General Time Reversible model of nucleotide substitution including a Gamma distribution substitution rate parameter) was used to construct a tree. Nucleotide substitution model parameters were determined using ModelTest46, and the tree constructed using MrBayes46. Posterior probabilities at nodes were calculated using three parallel Metropolis Coupled Markov Chains, searching for 2 million generations. The Bayesian analysis produced a robust topology with posterior support for major clades of 0.94 or higher, at which level there was topological agreement with a parsimony tree found by heuristic search in PAUP*47 (data not shown).

Haplotype relationships within *Watersipora arcuata*, *W. subtorquata* and the *W. n. sp.* clades were evaluated using median joining parsimony networks68. Sequence lengths used in comparisons were determined by the minimum lengths of sequence data available in GenBank: a 388-nucleotide segment of *W. arcuata* COI sequences was compared, including samples from southern Australia (Perth, Adelaide, Sydney areas) and O’ahu, Hawaii7 (*Watersipora subtorquata* and *W. n. sp.* clade networks were constructed using a 489-nucleotide segment: AMOVA (Analysis of Molecular Variance)43 was used to quantify COI sequence variation partitioned among broad geographic regions for both *W. arcuata* (regions included Hawaii, California, and Australia) and *W. subtorquata* (including the Australasian region of southern
Australian and New Zealand regions and Point Conception. Permutation tests (5000 replicates) were used to evaluate AMOVA coefficient significance.  

Mean sea surface temperature (SST) approximation. Local average SST was approximated using year-long measurements spanning 2002–2011. For most US sites, SST was obtained from a monitoring buoy located within 50 km of the sampling location via NOAA National Oceanographic Data Center Coastal temperature tables (http://www.nodc.noaa.gov/dwst/cwtp/cpc.html). Measurements were also obtained using the NASA satellite (Aqua) Moderate Resolution Imaging Spectroradiometer (MODIS) thermal map data archive. Temperatures were resolved to 1°C unit of accuracy using the dominant pixel-record within 50x50 km squares positioned offshore to sampling site. We verified, as elsewhere, that MODIS and buoy-recorded mean SSTs were generally within 1°C.  

Analysis of SST data. The 95% confidence interval of the median sea surface temperature experienced by major COI clades was estimated by bootstrapping (resampling populations of 20 individuals for 1000 replicates). To test the null expectation of no correlation between temperature and clade, Mantel tests were conducted correcting for spatial distance using a partial matrix. Pairwise SSTs and decadal grid coordinates were converted to Euclidean distances. Clades were encoded for spatial scale, and analyzed using Image J imaging software. We recorded five zooid dimensions: zooid length (Lz), zooid width (Wz), frontal shield area (FSA), orifice length (Lo), orifice width (Wo). We tested for a difference between W. n. sp. and other W. subtorquata-complex COI phlyogroups, using zooid area and tentacular orifice area (as W) as covariates via ANCOVA. Log transformations of areas were used, and regressions met the assumption of homogeneity of variances.  

Zooid-dimension comparisons for COI clades of sinuousid Watersipora. The recognized species Watersipora subovoidea and the W. subtorquata-complex can be distinguished by zooid proportions: for a given frontal shield area, the tentacular orifice is smaller in W. subovoidea. In the current study, a subset of the sinuousid colonies analyzed by COI were photographed at 20X magnification using a dissecting scope, and analyzed using Image J imaging software. We recorded five zooid dimensions: zooid length (Lz), zooid width at maximum (Wz), orifice width (Wo), orifice length (Lo). We tested for a difference between W. n. sp. and other W. subtorquata-complex COI phlyogroups, using zooid area and tentacular orifice area (as W) as covariates via ANCOVA. Log transformations of areas were used, and regressions met the assumption of homogeneity of variances.  

1. Knowlton, N. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24, 189–216 (1993).  
2. Carlton, J. T. Biological invasions of cryptic species. Ecology 77, 1653–1655 (1996).  
3. Bickford, D. et al. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22, 148–155 (2006).  
4. Geller, J. B., Darling, J. A. & Mackie, J. A. Genetic perspectives on marine biological invasions. Annu. Rev. Mar. Sci. 3, 367–393 (2010).  
5. Carlton, J. T. Pattern, process and prediction in marine invasion ecology. Biol. Conserv. 78, 97–106 (1996).  
6. Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J. & Hines, A. H. Invasion of coastal marine communities in North America: Apparent Patterns, Processes, and Biases. Annu. Rev. Ecol. Syst. 31, 481–531 (2000).  
7. Wonham, M. J. & Carlton, J. T. Trends in marine biological invasions at local and regional scales: the Northeast Pacific Ocean as a model system. Biol. Invasions 7, 369–392 (2005).  
8. Wonham, M. J. Mini-review: Distribution of the Mediterranean mussel Mytilus galloprovincialis (Bivalvia : Mytilidae) and hybrids in the Northeast Pacific. J. Shellfish Res. 23, 535–543 (2004).  
9. Braby, C. E. & Somero, G. N. Ecological gradients and relative abundance of native (Mytilus trossulus) and invasive (Mytilus galloprovincialis) blue mussels in the California hybrid zone. Mar. Biol. 148, 1249–1262 (2006).  
10. Geller, J. B., Soros, K., Eado, R., Palumbi, S. R. & Schwiderski, E. Sources of invasions of a northeastern Pacific acorn barnacle, Balanus glandula, in Japan and Argentina. Mar. Ecol. Prog. Ser. 358, 211–218 (2008).  
11. Watts, P. C., Thorpe, J. P. & Taylor, P. D. Natural and anthropogenic dispersal mechanisms in the marine environment: a study using cheliotile Bryozoa. Philos. Trans. R. Soc. Lond. B 353, 653–664 (1998).  
12. Barnes, D. Biodiversity: Invasions by marine life on plastic debris. Nature 416, 808–809 (2002).  
13. Carlton, J. T. & Geller, J. B. Ecological roulette: the global transport of nonindigenous marine organisms. Science 261, 78–82 (1993).  
14. Carlton, J. T. & Hodder, J. Biogeography and dispersal of coastal marine organisms: exploring uncharted territories on a replica of a 16th-century sailing vessel. Mar. Biol. 121, 721–730 (1995).  
15. Lynch, W. F. The behavior and metamorphosis of the larva of Bugula neritina (Linnaeus): experimental modification of the length of the free-swimming period and the responses of the larva to light and gravity. Biol. Bull. 92, 115–150 (1947).  
16. Walsey, B. The settling and some experimental reactions of a byronian larva, Watersipora cucullata (Busk). Aust. J. Mar. Freshw. Res. 9, 362–371 (1958).  
17. Keough, M. J. & Ross, J. In Marine Biological Invasions of Port Phillip Bay Victoria (eds Hewitt, C. L. M., Campbell, R. E. & Thresher, R. R. B. Martin) 9–11 (CSIRO Marine Research, 1999).  
18. Rodriguez, L. F. & Ibarra-Olondo, S. E. Cover and colonization of commercial oyster (Crasostrea gigas) shells by fouling organisms in San Quintin Bay, Mexico. J. Shellfish Res. 27, 337–343 (2008).  
19. Cohen, A. N. & Carlton, J. T. Nonindigenous aquatic species in a United States estuary: a case study of the biological invasions of the San Francisco bay and delta. (Aquatic Nuisance Species Taskforce, 1995).  
20. Dufourn, K. A., Glasby, T. M. & Johnston, E. L. Differential effects of tributyltin and copper antifoulsants on recruitment of non-indigenous species. Biofouling 24, 23–33 (2008).  
21. Sorte, C. J. B., Williams, S. L. & Zerebecki, R. A. Ocean warming increases threat of invasive species in a marine fouling community. Ecology 91, 2198–2204 (2010).  
22. Banta, W. C. Watersipora arcuata, a new species in the suborde – cucculata-nigra complex (Bryozoa, Cheilostomatida) in the West South Continental shelf and slope. Memoirs of the New Zealand Oceanographic Institute 97, 1–158 (1989).  
23. Anderson, C. M. & Haygood, M. G. A-Proteobacterial symbionts of marine bryozoans in the genus Watersipora. Appl. Environ. Microbiol. 73, 303–311 (2007).  
24. Ryland, J. S., De Blauwe, H., Lord, R. & Mackie, J. A. Recent discoveries of alien bryozoans (Bryozoa) in Western Europe, with redescriptions of species. Zootaxa 2093, 43–59 (2009).  
25. Anderson, F. E. Distribution of marine invertebrates by ships. Aust. J. Mar. Freshw. Res. 4, 303–316 (1953).  
26. Sun, M. DNA Barcode Examination of Bryozoa (Class: Gymnolaemata) in Korean Sea. Korean J. Syst. Zool. 27, 159–163 (2011).  
27. Soule, J. D. Results of the Canadian-American Museum of Natural History expedition to western Mexico. Am. Mus. Novit. 2053, 1–66 (1961).  
28. Osburn, R. C. Bryozoa of the Pacific Coast of America. Part 2, Cheilostomata--Ascopora. Allan Hancock Pac. Exped. 14(2), 271–612 (1952).  
29. Carlton, J. T. Patterns of transoceanic marine biological invasions in the Pacific Ocean. Bull. Mar. Sci. 41, 452–465 (1987).  
30. Ramalho, V. L., Muricy, G. & Taylor, P. D. Taxonomic revision of some lepaliomorph cheilostome bryozoans (Bryozoa: Lepaliomorpha) from Rio de Janeiro State, Brazil. J. of Nat. Hist. 45, 767–798 (2011).  
31. Jackson, J. B. C. & Crootels, A. H. Evolutionary significance of morphospecies: a test with the Cheilostomata bryozoan. Science 248, 579–583 (1990).  
32. Mackie, J. A., Keough, M. J., Norman, J. A. & Christidis, L. in Bryozoa studies 2001, Proceedings of the Twelfth International Bryozoology Association Conference (eds P. N. Wyse Jackson, C. J. Butler, & M. E. Spencer-Jones) 199–206 (Balkema, 2002).
63. Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Ecol. Lett.* 6, 700–705 (2003).

64. Kumar, S., Tamura, K. & Nei, M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform.* 9, 297–300 (2008).

65. Posada, D. & Crandall, K. A. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818 (1998).

66. Ronquist, F. & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574 (2003).

67. PAUP*: Phylogenetic analysis using parsimony (*and other methods*) v. 4 (Sinauer, Sunderland, 2000).

68. Clement, M., Posada, D. & Crandall, K. A. TCS: a computer program to estimate gene genealogies. *Mol. Evol.* 9, 1657–1660 (2000).

69. Excoffier, L., Smouse, P. & Quattro, J. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Applications to human mitochondrial DNA restriction data. *Genetics* 131, 479–491 (1992).

70. Excoffier, L., Laval, G. & Schneider, S. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1, 47–50 (2005).

71. Feldman, G. C. & McClain, C. R. *Aqua MODIS sea surface temperature (11 μ nighttime)*, Ocean Color Web, eds. Kuring, N., Bailey, S. W., Franz, B. F., Meister, G., Wedell, P. J., Eplee, R. E. NASA Goddard Space Flight Center. http://oceancolor.gsfc.nasa.gov/cgi/id (April 30 2012).

72. Haines, S. L., Jedlovce, G. J. & Lazarus, S. M. A MODIS Sea Surface Temperature Composite for Regional Applications. *IEEE T. Geosci. Remote* 45, 2919–2927 (2007).

73. Smouse, P. E., Long, J. C. & Sokal, R. R. Multiple regression and correlation extension of the Mantel test of matrix correspondence. *Syst. Zool.* 35, 627–632 (1986).

74. Goslee, S. C. & Urban, D. L. The ecodist package for dissimilarity-based analysis of ecological data. *J. Stat. Software* 22, 1–19 (2007).

75. Legendre, P. & Legendre, L. *Numerical Ecology, 2nd English Edition.* (Elsevier Science BV, 1998).

76. Abramoff, M. D., Magalhaes, P. J. & Ram, S. J. Image processing with ImageJ. *Biophotonics Int.* 11, 36–42 (2004).

Acknowledgements

This work was supported by the California Department of Fish and Game (grant to Geller). J. Mackie was assisted by the California State University Council on Ocean Affairs, Science and Technology (COAST) consortium and National Science Foundation (NSF award #1061693) support. We thank M. Ashe, S. Foss, and M. Sowby at California Department of Fish and Game for encouragement and project guidance. R. Fairey and his group at the Marine Pollution Studies Laboratory at Moss Landing Marine Laboratories provided the majority of specimens used in this project. Several individuals kindly provided additional samples: A. Cohen, G. Jenkins, L. McCann, A. Migotto, G. Ruiz, and J. Winston, or sequence information, Yong Jin Won, or provided general assistance: R. Blackwell, S. Bros, S. Craig, D. Gerhinger, E. Jensen, A. Láruson, K. Messer, G. Schroeder. Though this work has been reviewed by the U.S. Environmental Protection Agency and cleared for publication, it may not necessarily reflect official Agency policy.

Author contributions

All authors contributed to experiment design, experiments, manuscript text, and reviewed the manuscript. Mackie prepared figures.

Additional information

Competing financial interests: The authors declare no competing financial interests.

License: This work is licensed under a Creative Commons Attribution 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by/3.0/

How to cite this article: Mackie, J.A., Darling, J.A. & Geller, J.B. Ecology of cryptic invasions: latitudinal segregation among *Watersipora* (Bryozoa) species. *Sci. Rep.* 2, 871; DOI:10.1038/srep00871 (2012).