

Vectored introductions of marine endosymbiotic dinoflagellates into Hawaii

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Abstract Endosymbiotic dinoflagellates belonging to the genus *Symbiodinium* associate with a diverse range of marine invertebrate hosts and also exist free-living in the ocean. The genus is divided into eight lineages (clades A–H), which contain multiple subclade types that show geographic and host specificity. It is commonly known that free-living dinoflagellates can and have been introduced to new geographic locations, primarily through shipping ballast water. In this study we sequenced the ITS2 region of *Symbiodinium* found in symbiosis with the coral *Acropora cytherea* in the Northwestern Hawaiian Islands Marine National Monument and from shipping ballast water. Identification of an unusual symbiont in *Acropora cytherea* and an analysis of the distribution of this symbiont suggests an introduction to Hawaii vectored by the scyphozoan host, *Cassiopea* sp. *Symbiodinium* were also detected in shipping ballast water. This work confirms that marine invertebrate endosymbionts can be introduced to new geographic locations vectored by animal hosts or the ballast water of ships.

Keywords Ballast water · *Cassiopea* · Coral · Dinoflagellate · Invasive · *Symbiodinium*

Introduction

Dinoflagellates belonging to the genus *Symbiodinium* are symbiotic with a wide range of marine invertebrates and protists including hard and soft coral, anemones, hydroids, zoanthids, giant clams, jellyfish, and foraminifera (reviewed in Stat et al. 2006). These predominately intracellular symbiotic dinoflagellates are photosynthetic and provide the host invertebrate with fixed carbon in return for inorganic waste metabolites and an environmental niche free from predators (Muscatine 1967; Muscatine and Porter 1977). *Symbiodinium* can also exist free-living within the ocean environment providing aposymbiotic hosts with a pool of potential symbionts for infection (Carlos 1999; Coffroth et al. 2006; Gou et al. 2003).

The genus *Symbiodinium* is divided into eight lineages (clades A–H) based on nuclear rDNA, which contain many subclade types defined using higher resolution markers such as the ITS2 (Carlos 1999; LaJeunesse 2001; LaJeunesse and Trench 2000; Pawlowski et al. 2001; Pochon et al. 2004; Pochon et al. 2001; Rowan and Powers 1991). There are several *Symbiodinium* ITS2 subclade types that have a global distribution (e.g. C1 and C3) and are termed generalists for their ability to form symbioses with a wide range of hosts (LaJeunesse 2005). However, the majority of identified ITS2 types are specialists found at specific geographic locations in symbioses with particular hosts (LaJeunesse 2005; LaJeunesse et al. 2004). For example, the coral genus *Porites*

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associates with ITS2 type C60 in the Indo-Pacific and C9 in the Atlantic (LaJeunesse 2005). These phylogeographic patterns in *Symbiodinium* types are thought to reflect geographic barriers and adaptive radiation to local environments (LaJeunesse 2005).

It is well known that dinoflagellate cysts can be transported in the ballast water of marine vessels and on floating debris and that both represent avenues for the geographic spread of these single celled eukaryotes (Blackburn et al. 2001; Carlton and Geller 1993; Chu et al. 1997; Dickman and Zhang 1999; Hallegraeff 1998; Hallegraeff and Bolch 1992; Lilly et al. 2002; Locke et al. 1993; Marangoni et al. 2001; Maso and Garces 2006; Maso et al. 2003; Rigby et al. 1993; Ruiz et al. 1997; Williams et al. 1988; Yoshida et al. 1996; Zhang and Dickman 1999). The Global Ballast Water Management Program has implemented guidelines to reduce the transfer and spread of alien species, which includes an open water exchange protocol to flush ballast water as far from shore as possible (<http://globallast.imo.org/index.asp?>). Several studies have shown however, that dinoflagellates can persist in ship hulls post-ballast water exchange (Dickman and Zhang 1999; Zhang and Dickman 1999).

In this study, we examine whether symbiotic dinoflagellates within the genus *Symbiodinium* can be introduced to new geographic locations by animal vectors or in the ballast water of shipping vessels.

Materials and methods

Colonies of *Acropora cytherea* (Fig. 1a) were sampled from French Frigate Shoals within the Northwestern Hawaiian Islands Marine National Monument during May 2006. Approximately 2 cm² of coral was removed from each colony with a hammer and chisel and stored at -20°C in DMSO preservation buffer (Seutin et al. 1991). Water samples were supplied from the ballast of Matson[®] shipping vessels entering and leaving Honolulu harbor, Hawaii, from various port locations in the Pacific.

DNA from the stored coral samples was extracted using a modified CTAB protocol (Dempster et al. 1999). Briefly, the tissue was incubated in 500 µl of CTAB buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% (wt/vol) CTAB, 2% (vol/vol) 2-mercaptoethanol, 1% (wt/vol) PVP, pH 8.0) at

65°C for 30 min. An equal amount of phenol:chloroform:isoamyl alcohol (25:24:1) was added, the contents of the tube mixed, and centrifuged at 13,000 rpm for 20 min. The aqueous phase was removed and an equal volume of chloroform:isoamyl alcohol (24:1) added, thoroughly mixed, and centrifuged at 13,000 rpm again for 20 min. The DNA was precipitated from the resulting aqueous phase by the addition of 1/2 volume of 5 M NaCl and an equal volume of isopropanol and incubated for 1 hour at -80°C. The DNA was collected by centrifugation at 13,000 rpm for 30 min, washed twice with 70% ethanol, and resuspended in sterile water. For ballast water samples, the water was passed through filter paper with a 5 µm pore size, the filter paper directly placed into the CTAB lysis buffer, and the protocol followed as above. The ballast water samples were further cleaned and purified by processing through the DNeasy Plant Mini Kit (Qiagen) from step 3 following the manufacturer's instructions.

The ITS2 region of the nuclear rDNA was amplified from DNAs extracted from *Symbiodinium* using the primers ITSintfor2 and the conserved reverse primer lacking the GC clamp (LaJeunesse 2002). PCR products were purified using the QIAquick[®] PCR Purification Kit (Qiagen), ligated into the pCR[®]II-TOPO vector (Invitrogen) and cloned into One Shot[®] Top 10' F competent cells. Plasmid DNA was extracted using the GeneJet[™] Plasmid miniprep kit (Fermentas Life Sciences), and five clones from each PCR reaction from coral samples and three clones from each PCR from ballast water samples, were cycle-sequenced using BigDye Terminators (PerkinElmer) on an ABI-3100 automated sequencer at the Hawaiian Institute of Marine Biology. Sequences were inspected using MacVector[®] 8.0.2. and *Symbiodinium* identity assigned using BLAST comparison against published sequences deposited in Genbank.

The Genbank database and available literature were searched to identify hosts that harbor the *Symbiodinium* ITS2 types identified in this study and explore their geographic distributions.

Results and discussion

Acropora cytherea were found to harbor *Symbiodinium* belonging to clade A and C (Fig. 2). Clade A

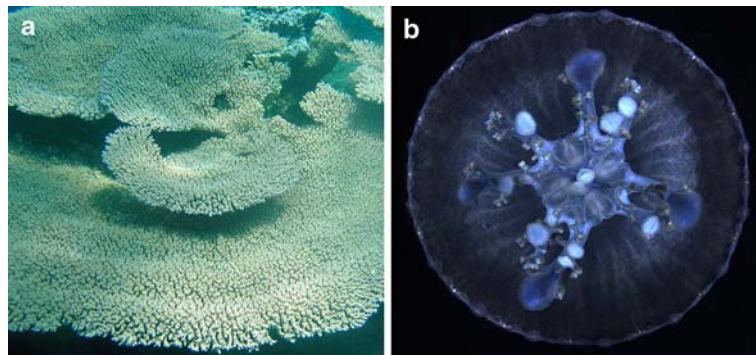


Fig. 1 (a) The coral *Acropora cytherea* symbiotic with *Symbiodinium* sp. from French Frigate Shoals, Northwestern Hawaiian Islands Marine National Monument (photo courtesy

of Jen Salerno and Anderson Mayfield); (b) The upside jellyfish *Cassiopea* sp. symbiotic with *Symbiodinium* (photo courtesy of Mahealani Kaneshiro-Pineiro)

Symbiodinium was found in four *A. cytherea* colonies while clade C was found in two *A. cytherea* colonies. One colony of *A. cytherea* harbored both clades A and C. *Symbiodinium* ITS2 subclade A1 was detected in four *A. cytherea* colonies, while the remaining clade A clones did not match any *Symbiodinium* sequences in Genbank and have been scored equally as a subclade A type (EF656428-EF656432). *Symbiodinium* types belonging to clade C were identified as C1c and C27, as well as additional new sequences that have been scored equally as a subclade C type (EF656433-EF656434). *Symbiodinium* closely related to ITS2 subclade D1a (EF656435) was identified in ballast water samples originating from Changjiang Kou, Shanghai Anchorage.

Symbiodinium ITS2 A1 is a rare symbiont type that has only been identified from animals or cultures originating from the jellyfish *Cassiopea* sp. from the

Caribbean (LaJeunesse 2001; Santos et al. 2002), the Red Sea (LaJeunesse 2001), and Hawaii (Santos et al. 2002), the reef-building corals *Acropora* sp. and *Stylophora pistillata* from the Red Sea (LaJeunesse 2001; Pochon et al. 2001), the hydroid *Millepora* sp. from the Red Sea (Pochon et al. 2001), the zoanthid *Zoanthus vietnamensis* from Japan (Reimer et al. 2007), and the gastropod *Pteraeolidia ianthida* from Japan (Ishikura et al. 2004). Conversely, the clade C *Symbiodinium* C1c and C27 are only found in coral from the Pacific (LaJeunesse 2005). The diverse geographic range of *Symbiodinium* ITS2 A1 could be interpreted as A1 having a global distribution. However, we suggest that the presence of *Symbiodinium* ITS2 A1 in Hawaii reflects an introduction to a new geographic location. The only host in Hawaii previously known to harbor A1 symbionts is the jellyfish *Cassiopea* sp (Fig. 1b). This scyphozoan is

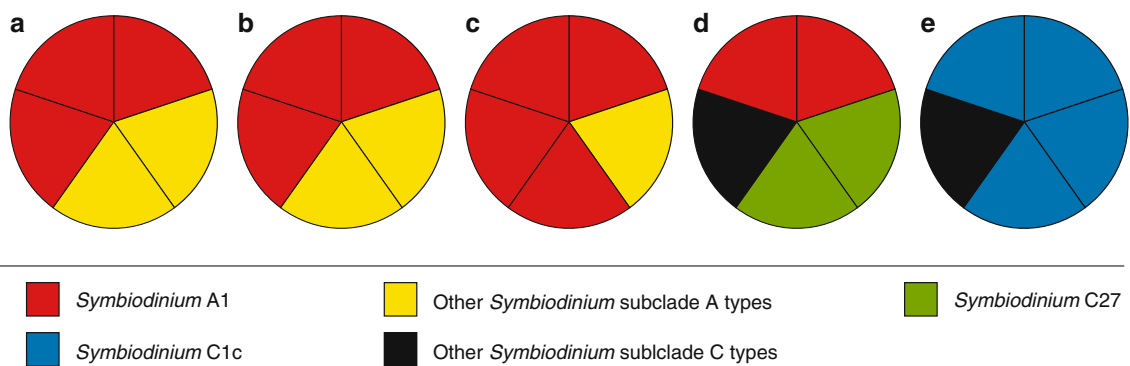


Fig. 2 Pie graphs represent *Symbiodinium* types harbored by the five colonies (a–e) of *A. cytherea* sampled from the Northwestern Hawaiian Islands Marine National Monument.

Each pie graph segment represents a *Symbiodinium* ITS2 clone sequence from that colony

not native to Hawaiian waters but has been introduced at least twice, once from the Indo-Pacific and once from the Atlantic Caribbean and/or the Red Sea (Holland et al. 2004). Interestingly, *Cassiopea* sp. from both the Red Sea and the Caribbean harbor *Symbiodinium* ITS2 A1 (LaJeunesse 2001; Santos et al. 2002). These data suggest that A1 has been vectored into Hawaii via *Cassiopea* sp. from the Red Sea and/or Atlantic Caribbean and has spread in its distribution and formed a symbiotic association with *A. cytherea* in the Northwestern Hawaiian Island Marine National Monument.

The presence of *Symbiodinium* belonging to clade D in ship ballast water suggests that either free-living or symbiotic (in small animal hosts such as invertebrate larvae or Foraminiferan hosts) *Symbiodinium* can be transported via this process. Whether endosymbiotic dinoflagellates are viable at the end of the voyage will likely depend on the duration in the ballast tanks of shipping vessels, which are void of sunlight (Carlton and Geller 1993; Chu et al. 1997; Hallegraeff and Bolch 1992; Locke et al. 1993; Rigby et al. 1993; Ruiz et al. 1997; Williams et al. 1988; Yoshida et al. 1996). In this context, several studies have shown that dinoflagellates persist after ballast water exchange (Dickman and Zhang 1999; Locke et al. 1993) and potentially increases the geographic range of these taxa.

This study suggests that patterns in the biogeography and phylogeography of *Symbiodinium* can be influenced by recent introductions that obscure the natural ecology of these endosymbiotic dinoflagellates. Although the long term consequences are unknown, the introduction of *Symbiodinium* sp. to new geographic locations vectored by animal hosts or through shipping ballast water may provide the opportunity for new host-symbiont assemblages to occur. This may be advantageous to animal hosts, as increasing the pool of symbiont types with potentially different physiological properties may allow hosts to persist under a greater range of environmental conditions, i.e. The Adaptive Bleaching Hypothesis (Buddemeier and Fautin 1993). Conversely, these new assemblages may reduce the fitness of animal hosts rendering them more susceptible to coral bleaching, disease, and mortality. Identifying and defining the extent of these introductions and the capacity of corals to form new associations with the introduced *Symbiodinium* is fundamental to

understanding the forces governing the health of coral reef ecosystems and is an important future research direction.

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