Biogeography of planktonic and coral-associated microorganisms across the Hawaiian Archipelago

Jennifer L. Salerno1,2,∗, Brian W. Bowen1 and Michael S. Rappé1

1Hawaii Institute of Marine Biology, School of Ocean and Earth Science and Technology, University of Hawaii at Manoa, Kaneohe, HI 96744, USA and 2Department of Biology, University of Hawaii at Manoa, Honolulu, HI 96822, USA

∗Corresponding author: Department of Environmental Science and Policy, George Mason University, 10900 University Boulevard, Manassas, VA 20110, USA. Tel: (703) 993-4467; E-mail: jsalerno@gmu.edu

One sentence summary: The biogeographical distribution of bacterial communities associated with Porites lobata corals in Hawaii is influenced by geographical distance and environmental factors and parallels the host distribution.

ABSTRACT

Factors driving the distribution of marine microorganisms are widely debated and poorly understood. Recent studies show that free-living marine microbes exhibit geographical patterns indicative of limited dispersal. In contrast, host-associated microbes face a different set of dispersal challenges, and hosts may function as habitat ‘islands’ for resident microbial populations. Here, we examine the biogeographical distributions of planktonic and adjacent coral-associated bacterial communities across the Hawaiian Archipelago, Johnston Atoll (∼1400 km southwest of Hawaii) and American Samoa in the Pacific Ocean and investigate the potential underlying processes driving observed patterns. Statistical analyses of bacterial community structure, determined using a small-subunit ribosomal RNA gene-based approach, showed that bacterioplankton and coral-associated bacterial communities were distinct, and correlated with geographical distance between sites. In addition, biogeographical patterns of bacterial associates paralleled those of their host coral Porites lobata, highlighting the specificity of these associations and the impact that host dispersal may have on bacterial biogeography. Planktonic and coral-associated bacterial communities from distant Johnston Atoll were shown to be connected with communities from the center of the Hawaiian Archipelago, a pattern previously observed in fish and invertebrates. No significant correlations were detected with habitat type, temperature or depth. However, non-distance-based geographical groupings were detected, indicating that, in addition to dispersal, unidentified environmental factors also affected the distributions of bacterial communities investigated here.

Keywords: microorganisms; bacteria; coral; Porites, diversity; biogeography

INTRODUCTION

The principles that govern microbial distributions are widely debated and poorly understood (Martiny et al. 2006; Hanson et al. 2012). For the past century, the Baas-Becking hypothesis that ‘everything is everywhere; but the environment selects’ has predominated among microbiologists (Baas-Becking 1934). Under this assumption, microorganisms disperse freely, unhindered by geographical boundaries, settling and thriving after encountering a favorable environment (de Wit and Bouvier 2006). However, technical advances in molecular biology have given microbiologists a more detailed picture of microbial diversity, indicating that microorganisms are not as mobile or ubiquitous as previously believed and that many display biogeographical

Marine microbial ecologists face the dual challenges of demonstrating biogeographical variation in microbial assemblages and identifying the underlying processes driving microbial community diversity. Environmental conditions shape existing variation; however, historical events may also affect present-day patterns of microbial biodiversity. For example, dispersal barriers and past climatic conditions can lead to genetic divergence and subsequently, variations in biogeographical distributions (Cox and Moore 2010). Marine biogeographical provinces, or areas where biotic assemblages reflect historical events, have been identified for macroorganisms (Udvardy 1975; Briggs and Bowen 2012). However, the existence of such provinces for microorganisms has only recently been addressed, with biogeographical patterns emerging among a wide range of microbes (Rappé et al. 2000; Martiny et al. 2006; Pommier et al. 2007; Ramette and Tiedje 2007; Casteleyn et al. 2010; Lowe et al. 2012).

Contemporary microbial biogeography has focused on free-living microorganisms (e.g. Hellweger, van Sebille and Fredrick 2014), but microbes associated with host organisms are also of great interest from a biogeographical perspective due to their important contributions to microbial diversity (Taylor et al. 2004). Eukaryotic hosts may function as habitat ‘islands’ (MacArthur and Wilson 1967), allowing allopatric divergence to occur in microbial populations living on physically separated hosts (Taylor et al. 2005). Previous studies have shown that many symbiotic microorganisms display patterns of genetic and morphological diversity paralleling that of their host organism (Distel, Felbeck and Cavanaugh 1994; Funk et al. 2000).

It is well established that corals and seawater harbor distinct bacterial assemblages (Rohwer et al. 2001, 2002; Rosenberg et al. 2007); however, relatively little is known about the fidelity of these associations over a range of spatial scales (Rohwer et al. 2002; Littman et al. 2009; Morrow et al. 2012). Corals are known to host a diversity of bacteria in their surface mucous layer, epithelial tissue and skeleton that are proposed to serve a variety of key functions (Rohwer et al. 2001; Lesser et al. 2004; Ritchie 2006). Much like the photosynthetic zooxanthellae that reef-building corals depend on for survival, some of these microbes may be performing life-sustaining processes for the coral host, and yet others may become pathogenic under certain conditions (Patterson et al. 2002; Lesser et al. 2007; Rosenberg et al. 2007; Sussman et al. 2008; Richardson et al. 2009). To identify which bacteria are consistently associated with a particular species of coral versus those that are simply transient, it is essential to first understand the temporal and spatial variability of bacterial consortia.

Here, we characterize the diversity and biogeography of bacteria associated with reef-building Porites lobata corals and adjacent seawater across the Hawaiian Archipelago, Johnston Atoll and American Samoa in the South Pacific Ocean. The Hawaiian Archipelago is an excellent platform for biogeographical analyses, providing a linear array of reefs and atolls spanning 2600 km across the North Pacific. Genetic diversity in host P. lobata conforms to a pattern of isolation by distance across the Hawaiian Archipelago (Polato et al. 2010). If bacteria form specific associations with P. lobata, we might expect their population genetic structures to be concordant with that of their coral host. Johnston Atoll is the closest coral reef habitat to Hawaii, lying ∼1400 km southwest of Oahu. It is part of the Hawaiian biogeographical province due to close faunal affinities, and long postulated to be a stepping stone for colonization of marine fauna into Hawaii (Maragos and Jokiel 1986; Kosaki et al. 1991). For that reason, it is crucial to understanding the origins of marine biota at the isolated archipelago of Hawaii. Porites lobata genotypes with migrant ancestry have been detected in the Hawaiian Archipelago and Johnston Atoll, indicating that larval exchange occurs in both directions, and that connectivity between these populations is maintained over evolutionary, if not ecological, time scales (Polato et al. 2010). American Samoa lies ∼4200 km southwest of Oahu, on the other side of east–west equatorial currents that may limit dispersal. Hence, it provides an appropriate yardstick for quantifying differences in faunal composition across Hawaii, as assessed here with terminal-restriction fragment length polymorphisms (T-RFLPs) of the small subunit ribosomal RNA (SSU rRNA) gene.

Recent studies have revealed that bacterioplankton also exhibit biogeographical patterns over various taxonomic and spatial scales (Garcia-Martinez and Rodriguez-Valera 2000; Riemann and Middelboe 2002; Pinhasi et al. 2003; Schwabach and Fuhrman 2005; though see Hellweger, van Sebille and Fredrick 2014). In this study, bacterioplankton distributions were compared with those of coral-associated bacteria to examine biogeographical differences between free-living and coral-associated assemblages and to elucidate the factors that contribute to their underlying biogeographical distributions.

**METHODS**

**Sample collection**

The hermatypic lobe coral *P. lobata* (Dana 1846) was targeted for bacterial community assessment due to its widespread Indo-Pacific distribution. The genus *Porites* has a circumtropical distribution and is among the most widely distributed coral genera in terms of both geography and habitats (Veron and Stafford-Smith 2000). *Porites* corals provide most of the framework for reefs in the Hawaiian Archipelago (Maragos 1977) and many reefs worldwide (Veron and Stafford-Smith 2000). These corals can be found as encrusting colonies in high wave areas and as large mounds in wave-protected areas.

Coral and seawater samples were collected from sites across the Hawaiian Archipelago, Johnston Atoll (∼1327 km southwest of Oahu) and American Samoa (∼4195 km southwest of Oahu) (Fig. 1; Table 1; Table S1, Supporting Information). The majority of samples collected in the Hawaiian Archipelago were taken within the Papahānaumokuākea Marine National Monument (PMNM) in the northwestern portion of the Hawaiian island chain during two research cruises in September/October 2005 (HI-05-07) and May/June 2006 (HI-06-07). Access to the uninhabited PMNM is extremely limited, so collection regimes were defined by the cruise schedule of the NOAA research vessel. Locations, dates and depths of samples are provided in Table 1. Distances between Hawaiian samples ranged from approximately 0.001 to 1500 km. Samples were collected from different reef habitat types (Table S1, Supporting Information).

Coral colonies judged as non-diseased by visual inspection were sampled with a stainless steel chisel to remove three pieces of coral 1–2 cm in diameter that consisted of the mucous layer, coral tissue and skeleton. The three subsamples from each colony were put into separate sterile bags (Whirl-pak; Nasco, Fort Atkinson, WI). Seawater was removed from bags at depth, samples were placed on ice, transported back to the ship and frozen at −35 °C. Temperature and depth below sea surface were recorded at each site.

One liter of seawater was collected adjacent to coral heads, placed on ice and filtered through a series of 25 mm diameter,
DNA extraction and T-RFLP of bacterial SSU rRNA genes

Coral samples were thawed on ice and a flame-sterilized core borer was used to remove a 6-mm diameter, 6-mm deep core from each subsample. The three cores from each colony were placed together into a sterile bag containing 2 mL of 0.2-μm nominal pore-sized GF/A glass microfiber filters (Whatman International Ltd, Piscataway, NJ) and 13 mm diameter, 0.2-μm pore-sized polystyrene membrane filters (Supor-200, Pall Corp., East Hills, NY). Filters were stored frozen in 250 μL of DNA lysis buffer (20 mM Tris·HCl pH 8.0, 2 mM EDTA pH 8.0, 1.2% v/v Triton X100) (Suzuki et al. 2001). All samples were transported to the Hawaii Institute of Marine Biology and stored at −80°C until processed for genetic analysis.

Genomic DNA was extracted from coral and seawater samples using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) with an extended physical lysis (vortexing) step of 30 min (Salerno et al. 2011). Total genomic DNA yield was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen Corp., Carlsbad, CA, USA) and SpectraMax M2 plate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

In preparation for terminal-restriction fragment length polymorphism (T-RFLP) analysis (Liu et al. 1997), SSU rRNA fragments were amplified via the polymerase chain reaction (PCR) from 69 ± 34 ng of genomic DNA per sample. The 50 μL PCR reactions included 1X MasterTaq reaction buffer (5 PRIME, Inc., Gaithersburg, MD), 2.25 mM Mg2+, 0.5X TaqMaster reaction enhancer (5 PRIME, Inc.), 0.2 mM each of the fluorescently labeled general bacterial SSU rRNA gene oligonucleotide primer 27F-B-FAM (5′-FAM-AGRGTTYGATYMTGGCTCAG-3′) and universal SSU rRNA gene oligonucleotide primer 1492R (5′-GGYTACCTTGTAGGACTT-3′; Lane 1991), 0.2 mM of each deoxynucleotide (Promega, Madison, WI), 2.5 units of MasterTaq DNA polymerase (Eppendorf, Hamburg, Germany) and sterile water. PCR reactions were carried out in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) using the following ‘touchdown’ protocol: 3 min at 95°C, 30 cycles of 30 s at 95°C, 1 min at 65°C (decreasing by 0.5°C per cycle with end temperature of 50°C) and 2 min at 72°C. Reactions were concluded with 10 cycles of 30 s at 95°C, 1 min at 50°C and 2 min at 72°C, and 1 cycle of 30 s at 95°C, 1 min at 50°C and 20 min at 72°C (Don et al. 1991; Salerno et al. 2011; Morrow et al. 2012). Template-free PCR reactions were used as negative controls.

T-RFLP analysis was chosen as a cost-effective alternative to sequencing to initially assess the natural variability of coral-associated bacterial community structure across a range of spatial scales. As in the case of all culture-independent molecular methods used to assess microbial diversity, T-RFLP has notable limitations. In particular, different microorganisms within a population may share the same terminal-restriction fragment (T-RF) length, resulting in an underestimation of community diversity (Liu et al. 1997). However, despite the lower number of operational taxonomic units (OTUs) recovered using T-RFLP versus next-generation sequencing methods, T-RFLP analysis has a similar capacity to discern statistically robust biological patterns based on geographical distance and to correlate environmental variables with bacterial community structure (Winter, Matthews and Suttle 2013; van Dorst et al. 2014). Fluorescently labeled PCR amplicons were purified using the QIAquick Multiwell PCR Purification System (Qiagen Inc., Valencia, CA), and approximately 100 ng of each purified amplicon was digested in a 10-μL reaction containing 5 units of Haell restriction endonuclease (Promega). After a 6-h incubation at 37°C, digests were purified via gel filtration chromatography using the Millipore MultiScreen Assay System (Millipore Corp., Billerica, MA) paired with Sephadex G-50 Superfine (GE Healthcare, Piscataway, NJ). Purified products were adjusted to a final concentration of 30 ng μL−1 and separated on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Size and relative abundance of T-RFs was determined using GeneMapper software (Applied Biosystems) and...
Table 1. Summary of planktonic (n = 46) and coral-associated (n = 112) microbial communities sampled in this study. The total number of discrete seawater and coral samples and their depth of origin in meters (depth) for each location (decimal degrees) are shown.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Site ID</th>
<th>Habitat</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Seawater</th>
<th>Coral</th>
<th>Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>French Frigate Shoals (FFS)</td>
<td>23</td>
<td>Back reef</td>
<td>23.8657</td>
<td>−166.23965</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33</td>
<td>Patch reef</td>
<td>27.7853333</td>
<td>−175.8231667</td>
<td>1</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H6</td>
<td>Fore reef</td>
<td>23.8805833</td>
<td>−166.27375</td>
<td>1</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R46</td>
<td>Fringing reef</td>
<td>23.7694667</td>
<td>−166.2133333</td>
<td>1</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Kure Atoll (KU)</td>
<td>17</td>
<td>Back reef</td>
<td>28.4316</td>
<td>−178.3659333</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R36</td>
<td>Back reef</td>
<td>28.42062</td>
<td>−178.3715</td>
<td>1</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R33</td>
<td>Fore reef</td>
<td>28.41632</td>
<td>−178.378166667</td>
<td>1</td>
<td>–</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Maro Reef (MA)</td>
<td>22</td>
<td>Back reef</td>
<td>25.3785667</td>
<td>−170.5674667</td>
<td>1</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Patch reef</td>
<td>25.3982</td>
<td>−170.5739833</td>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R12</td>
<td>Patch reef</td>
<td>25.4711167</td>
<td>−170.6421667</td>
<td>1</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Midway Atoll (MI)</td>
<td>R7</td>
<td>Fore reef</td>
<td>28.196517</td>
<td>−177.374917</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Fore reef</td>
<td>28.197383</td>
<td>−177.346083</td>
<td>–</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R3</td>
<td>Fore reef</td>
<td>28.1903333</td>
<td>−177.3995333</td>
<td>1</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Pearl and Hermes Atoll (PE)</td>
<td>R32</td>
<td>Back reef</td>
<td>27.8391666667</td>
<td>−175.7530333</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26</td>
<td>Back reef</td>
<td>27.9579</td>
<td>−175.8022</td>
<td>–</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33</td>
<td>Fore reef</td>
<td>27.7853333</td>
<td>−175.8231667</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R26</td>
<td>Fore reef</td>
<td>27.7859</td>
<td>−175.7832167</td>
<td>1</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R39</td>
<td>Fore reef</td>
<td>27.9406167</td>
<td>−175.8617333</td>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2006</td>
<td>French Frigate Shoals (FFS)</td>
<td>23</td>
<td>Back reef</td>
<td>23.8657</td>
<td>−166.23965</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33</td>
<td>Patch reef</td>
<td>23.8357</td>
<td>−166.2658667</td>
<td>–</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>Patch reef</td>
<td>23.8496833</td>
<td>−166.2974333</td>
<td>–</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37</td>
<td>Patch reef</td>
<td>23.6743</td>
<td>−166.168</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R29</td>
<td>Patch reef</td>
<td>23.67885</td>
<td>−166.1458333</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>Patch reef</td>
<td>23.725383333</td>
<td>−166.275383333</td>
<td>1</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R16</td>
<td>Patch reef</td>
<td>23.850983333</td>
<td>−166.3296</td>
<td>1</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H6</td>
<td>Fore reef</td>
<td>23.8805833</td>
<td>−166.27375</td>
<td>1</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Kaneohe Bay, Oahu (KB)</td>
<td>SBE</td>
<td>Patch reef</td>
<td>21.4190166667</td>
<td>−157.7807833</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CB</td>
<td>Patch reef</td>
<td>21.45705</td>
<td>−157.81135</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KB</td>
<td>Patch reef</td>
<td>9.7038333</td>
<td>−35.5963333</td>
<td>–</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Nihoa (NI)</td>
<td>North side</td>
<td>Fringing reef</td>
<td>23.0582667</td>
<td>−161.92855</td>
<td>1</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS-1</td>
<td>Fringing reef</td>
<td>23.0582667</td>
<td>−161.92855</td>
<td>2</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS-2</td>
<td>Fringing reef</td>
<td>23.0582667</td>
<td>−161.9290333</td>
<td>4</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Gardner Atoll (GA)</td>
<td>7</td>
<td>Fringing reef</td>
<td>25.0000833</td>
<td>−169.98855</td>
<td>1</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>American Samoa (AS)</td>
<td>Ofu, pool 300</td>
<td>Back reef</td>
<td>−14.183555</td>
<td>−169.660622</td>
<td>5</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ofu, pool 400</td>
<td>Back reef</td>
<td>−14.178312</td>
<td>−169.653368</td>
<td>2</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tutuila, Fagaitua Bay</td>
<td>Back reef</td>
<td>−14.27632</td>
<td>−170.614843</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ofu, Sili fore reef</td>
<td>Fore reef</td>
<td>−14.163042</td>
<td>−169.61118</td>
<td>1</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Johnston Atoll (JA)</td>
<td>JOH-5P</td>
<td>Back reef</td>
<td>16.77415</td>
<td>−169.470167</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JOH-23</td>
<td>Back reef</td>
<td>16.7473</td>
<td>−169.5093167</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>Back reef</td>
<td>16.776916667</td>
<td>−169.4900333</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JOH-6P</td>
<td>Back reef</td>
<td>16.718516667</td>
<td>−169.537166667</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JA-HIMB3</td>
<td>Back reef</td>
<td>16.742933333</td>
<td>−169.537166667</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JOH-20</td>
<td>Fringing reef</td>
<td>16.761166667</td>
<td>−169.537166667</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JOH-22</td>
<td>Fore reef</td>
<td>16.7643</td>
<td>−169.525583333</td>
<td>1</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JOH-1AP</td>
<td>Fore reef</td>
<td>16.7680333</td>
<td>−169.4618333</td>
<td>1</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

OTUs were defined as fragments between 50 and 600 base pairs (bp) in length. Fragment lengths were rounded to the nearest integer value, aligned and visually checked for errors. A variable percentage threshold method (Osborne et al. 2006) was used to normalize samples. Finally, peaks were transformed into relative abundance units by dividing integrated peak areas by the total peak area for an individual sample.

SSU rDNA clone libraries

For one P. lobata sample taken from Kaneohe Bay on the island of Oahu in 2006, bacterial SSU rRNA fragments were PCR-amplified using the same primers and protocol used to generate T-RFLP community profiles, with the exception that an unlabeled 7F-B primer was used. The PCR product was purified using the QIAquick Gel Extraction Kit (Qiagen Inc.) and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). The amplified product was cloned using the pGem-T Easy system (Promega), and resulting clones were sequenced on an ABI 3730XL capillary-based DNA sequencer (Applied Biosystems). Sequences were hand-curated for quality, and vector sequences were removed using Sequencer 4.7 software (Gene Codes Corp., Ann Arbor, MI). Chimeric sequences were identified using the CHECK_CHIMERA software of the Ribosomal Database Project.
RESULTS

Overall, 355 unique T-RFs were detected among planktonic and coral-associated bacterial community samples collected in 2005 and 2006 (n = 158). A total of 141 and 344 T-RFs, with an average of 14 ± 5 and 19 ± 14 T-RFs per sample, were detected for seawater and corals, respectively. SIMPROF and ANOSIM analyses indicated that the structure of seawater versus coral-associated bacterial communities was significantly different (Global R = 0.425, P = 0.001). SIMPER analysis showed that the average similarity within seawater samples was 35.1%, while average similarity within coral samples was 13.4%. Average dissimilarity between seawater and coral samples was 92.6%. An NMDS ordination provided a ‘best-fit’ 2D graphical representation of the Bray–Curtis dissimilarity values calculated from abundance data (Fig. 2). The stress value for this 2D ordination is >0.20, indicating the difficulty in compressing these data into two dimensions and the limitation of this plot to accurately represent relationships between these samples (Kruskal 1964). The 35% similarity threshold from the cluster analysis has been overlaid onto the graph to aid in interpretation of the relationships between samples.

Planktonic community structure

Planktonic samples collected in 2005 and 2006 were analyzed separately since replicate data could not be obtained from each island/atoll for both years and therefore, the ability to detect interannual differences was limited (Table 1). SIMPROF and ANOSIM analyses of planktonic microbial communities did not significantly differ between sites within an individual island/atoll (Global R = 0.388, P = 0.002). PERMANOVA analysis confirmed this significant difference (Pseudo-F = 10.637, P = 0.0001), and further revealed that planktonic bacterial communities did not significantly differ between sites within an individual island/atoll (Pseudo-F = 3.924, P = 0.074). Pairwise ANOSIM tests revealed significant differences between Kure Atoll and Maro Reef, Pearl and Hermes Atoll and Maro Reef, and Pearl and Hermes Atoll and French Frigate Shoals (Table S2, Supporting Information), while SIMPER analysis revealed that the average similarity of planktonic bacterial communities within and between islands/atolls ranged from 46%–83% and 35%–63%, respectively (Table S3, Supporting Information). An NMDS ordination of all seawater samples from 2005 graphically illustrated differences in bacterial community structure between individual samples from different islands/atolls based on Bray–Curtis dissimilarities (Fig. 3A).

SIMPROF and ANOSIM analyses of planktonic microbial communities collected in 2006 (n = 26; Table 1) were essentially concordant with 2005 data, but included the outlying sites at Johnston Atoll and American Samoa. Planktonic bacterial communities within Hawaii differed significantly between different islands/atolls (Global R = 0.219, P = 0.007). PERMANOVA analysis also revealed significant island/atoll differences (Pseudo-F = 2.6772, P = 0.0296). As before, bacterial communities did not significantly differ between sites within each island/atoll (Pseudo-F = 1.071, P = 0.452). Pairwise ANOSIM test values revealed that American Samoa was significantly different from French Frigate Shoals, Kaneohe Bay (Oahu) and Johnston Atoll, and that Kaneohe Bay was significantly different from French Frigate Shoals and Johnston Atoll (Table S4, Supporting Information). SIMPER analysis revealed that the average similarity of planktonic bacterial communities within and between islands/atolls ranged from 21%–70% and 10%–48%, respectively (Table S5, Supporting Information).
Information). An NMDS ordination of all seawater samples from 2006 graphically illustrated differences in bacterial community structure between individual samples from different islands/atolls based on Bray–Curtis dissimilarities (Fig. 3B).

**Coral-associated bacterial community structure**

Coral samples collected in 2005 and 2006 were analyzed separately since replicates could not be obtained from each island/atoll in each year (Table 1). Overall, we detected 329 T-RFs from coral samples collected in 2005 (n = 76), 185 of which were only found in corals collected in 2005. Of the 329 T-RFs, 65% were present in 1%–5% of the samples, 19% were present in 5%–10% of samples, 11% were present in 10%–20% of the samples, 4% were present in 20%–40% of the samples and 2% were present in >40% of samples. T-RF 257 was the most prevalent ribotype, found in 61% of samples and comprising an average abundance of 6 ± 5% SD of the bacterial community per sample. T-RF 257 is likely related to the Xanthomonadales, JTB255 marine benthic group based on clone library sequencing. The next most prevalent T-RFs were 291 (possibly related to Rhizobiales, Phyllobacteriaceae – 55%; 8 ± 7% SD) and 191 (53%; 6 ± 6% SD). The most abundant T-RFs were not necessarily the most prevalent T-RFs in coral samples. For example, T-RF 94 was the most abundant ribotype, comprising an average abundance of 42% ± 5% SD of the bacterial community per sample, but only present in 1% of coral samples collected in 2005.

SIMPROF and ANOSIM analyses of coral-associated bacterial community samples collected in 2005 (n = 76) revealed that bacterial communities differed significantly between islands/atolls (Global R = 0.317, P = 0.001). PERMANOVA analysis confirmed these island/atoll differences (Pseudo-F = 2.6048, P = 0.0001) and, unlike planktonic communities, also revealed significant differences between sites within an individual island/atoll (Pseudo-F = 1.261, P = 0.002). However, post-hoc pairwise comparisons did not yield significant differences between sites within an individual island/atoll (Salerno 2013). Pairwise ANOSIM test values between different islands/atolls revealed that Maro Reef and Necker Island were significantly different from each other, as well as from all of the other islands/atolls sampled in 2005 (Table S2, Supporting Information). French Frigate Shoals was also significantly different from Midway Atoll (Table S2, Supporting Information). SIMPER analysis revealed that the average similarity of coral-associated bacterial communities within and between islands/atolls ranged from 9%–20% and 7%–12%, respectively (Table S3, Supporting Information). An NMDS ordination of all coral samples from 2005 graphically illustrated differences in bacterial community structure between different islands/atolls based on Bray–Curtis dissimilarity values (Fig. 4A). The stress value for this 2D ordination is >0.20, indicating the limitation of this plot to accurately represent...
Figure 3. (A) NMDS ordination of planktonic bacterial communities sampled in 2005 (n = 20), based on Bray–Curtis dissimilarities calculated from the square root transformation of T-RFLP relative abundance data. The location of sample collection is indicated by closed circles (Kure Atoll), closed squares (Midway Atoll), closed triangles (Pearl and Hermes Atoll), open circles (Maro Reef), open squares (French Frigate Shoals) and open triangles (Necker Island). The superimposed dashed line delineates sample clusters at the 60% similarity threshold from the cluster analysis dendrogram plot. (B) NMDS ordination of planktonic bacterial communities sampled in 2006 (n = 26), based on Bray–Curtis dissimilarities calculated from the square root transformation of T-RFLP relative abundance data. The location of sample collection is indicated by closed circles (American Samoa), closed squares (French Frigate Shoals), closed triangles (Nihoa), open circles (Kaneohe Bay) and open squares (Johnston Atoll). The superimposed dashed line delineates sample clusters at the 40% similarity threshold from the cluster analysis dendrogram plot.
Figure 4. (A) NMDS ordination of coral-associated bacterial communities sampled in 2005 \( (n = 76) \), based on Bray–Curtis dissimilarities calculated from the square root transformation of T-RFLP relative abundance data. The location of sample collection is indicated by closed circles (Kure Atoll), closed squares (Midway Atoll), closed triangles (Pearl and Hermes Atoll), open circles (Maro Reef), open squares (French Frigate Shoals) and open triangles (Necker Island). The superimposed dashed line delineates sample clusters at the 30% similarity threshold from the cluster analysis dendrogram plot. (B) NMDS ordination of coral-associated bacterial communities sampled in 2006 \( (n = 36) \), based on Bray–Curtis dissimilarities calculated from the square root transformation of T-RFLP relative abundance data. The location of sample collection is indicated by closed circles (Gardner Pinnacles), closed squares (French Frigate Shoals), closed triangles (Nihoa), open circles (Kaneohe Bay) and open squares (Johnston Atoll). The superimposed dashed line delineates sample clusters at the 30% similarity threshold from the cluster analysis dendrogram plot.
relationships between these samples (Kruskal 1964). Therefore, the 35% similarity threshold from the cluster analysis has been overlaid onto the graph to aid in interpretation of the relationships between samples.

In 2005, replicate samples were obtained from four different habitat types within French Frigate Shoals atoll: coastal fringing, patch reef, back reef and fore reef (n = 17; Table 1). SIMPROF and ANOSIM analyses of coral-associated bacterial communities from French Frigate Shoals showed that bacterial communities did not differ significantly based on habitat type (Global R = 0.06, P = 0.254).

A total of 159 T-RFs were detected in coral samples collected in 2006 (n = 36), 15 of which were only found in corals collected in 2006. Of the 159 T-RFs, 39% were present in 1%–5% of the samples, 30% were present in 5%–10% of samples, 16% were present in 10%–20% of the samples, 12% were present in 20%–40% of the samples, and 3% were present in 40% of samples. T-RF 57 was the most prevalent ribotype, found in 75% of samples and comprising an average abundance of 13 ± 13% SD of the bacterial community. T-RFs 66 (50%; 7 ± 6% SD) and 191 (42%; 4 ± 3% SD) were the next most prevalent across samples. T-RF 67 was the most abundant ribotype, comprising an average of 52% ± 9% SD of the coral-associated bacterial community with the only present in 3% of samples. Notably, T-RF 324 was relatively absent (22 ± 11% SD) and prevalent (28% of samples) in coral samples collected in 2006.

SIMPROF and ANOSIM analyses of coral-associated microbial community samples collected in 2006 (n = 36; Table 1) were concordant with 2005 results, revealing significant differences between islands/atolls (Global R = 0.317, P = 0.001). PERMANOVA tests also indicated significant differences between islands/atolls (Pseudo-F = 1.9426, P = 0.0002) and between sites within individual islands/atolls (Pseudo-F = 1.342, P = 0.0382). However, similar to 2005 samples, post-hoc pairwise comparisons did not support significant differences between sites within individual islands/atolls (Salerno 2013). ANOSIM pairwise test values between islands/atolls (Table S4, Supporting Information) indicated that French Frigate Shoals significantly differed from Gardner Pinnacles, Kanehoe Bay and Johnston Atoll. In addition to French Frigate Shoals, Kanehoe Bay also differed significantly from Nihoa and Johnston Atoll. SIMPER analysis revealed that the average similarity of coral-associated bacterial communities within and between islands/atolls ranged from 15–29% and 3–18%, respectively (Table S5, Supporting Information). AN NMDS ordination of all coral samples from 2006 graphically illustrates differences in coral-associated bacterial community structure between islands/atolls based on Bray-Curtis dissimilarities (Fig. 4B).

The SSU rRNA gene clone library from a single coral-associated bacterial community sample taken from Kanehoe Bay in 2006 resulted in 93 clone sequences (Table S6, Supporting Information). Of these, 23 were singletons, while nine lineages consisted of two clones each. The four lineages that consisted of >2 clones include taxa within the Acinetobacter and Enzoonichon genera of Gammaproteobactaria (20 and 5 clones, respectively), the genus Sphingobium of Alphaproteobacteria (5 clones) and the family Entomoplasmataceae of Tenericutes (20 clones). These four lineages contain predicted T-RFs of 253, 39, 227 and 278 bp (Table S6, Supporting Information). While the 39 bp T-RF falls below the 50 bp threshold used for the T-RFLP analysis, the other three predicted T-RFs (253, 227 and 278) were correlated to major peaks of 251, 225 and 276 bp, respectively in the T-RFLP electropherogram from the same sample. This offset between predicted and actual T-RFs is typical (e.g. Aprill et al. 2009; Yeo et al. 2013).

**Correlations with temperature, depth and geographical distance**

BEST analyses did not reveal significant correlations between temperature or depth with bacterioplankton community structure for 2005 or 2006 (data not shown). Similarly, the analysis did not indicate a significant correlation between temperature or depth with coral-associated bacterial community structure for 2005, but did show a significant correlation for both variables with community structure in 2006 (ρ = 0.129, P = 0.04).

RELATE analyses revealed no significant relationship between Bray-Curtis dissimilarities and geographical distances for planktonic bacterial communities sampled in 2005 (ρ = 0.106, P = 0.161). For planktonic communities sampled in 2006, RELATE analyses indicated a significant relationship between Bray–Curtis dissimilarities and geographical distances (ρ = 0.133, P = 0.028). Similarly, RELATE analyses revealed a significant relationship between Bray–Curtis dissimilarities and geographical distances for coral-associated bacterial communities sampled in 2005 (ρ = 0.16, P = 0.001) and 2006 (ρ = 0.12, P = 0.015).

**DISCUSSION**

**Differences between planktonic and coral-associated bacterial communities**

Our findings reveal that P. lobata corals harbor distinct bacterial communities from their surrounding seawater environment concordant with earlier observations and on a larger geographic scale than previously documented for these associations (Rohwer et al. 2001, 2002; Rosenberg et al. 2007). The significant dissimilarity between the two sample types, along with the observation that coral-associated communities were more variable than their planktonic counterparts at both the inter- and intra-island/atoll scale, supports the idea that coral colonies provide complex and heterogeneous microhabitats for microbes (Ainsworth, Thurber and Gates 2010), enabling them to form unique associations with coral host organisms (Knowlton and Rohwer 2003). Our observations indicate that, while both types of microbial communities exhibit biogeography, their distributions are shaped by different factors.

In addition to differences in habitat variability between coral and seawater environments, dispersal mechanisms may also contribute to observed differences between planktonic and coral-associated communities. Planktonic microorganisms have potentially unlimited dispersal due to their high densities, small sizes and absence of barriers, but this viewpoint is controversial (Finlay 2002; Martiny et al. 2006). In contrast, association with an invertebrate host may limit the dispersal capabilities of coral-associated bacteria (Taylor et al. 2005). Determining the role of dispersal in coral-associated communities is further complicated by the range of possible host-bacteria associations available, the specificity of these associations and the mode of transmission throughout the host lifecycle (e.g. obligate symbionts vs facultatively associated or transient microorganisms; mobile larvae vs sessile adult stages; vertical vs horizontal transmission). The underlying nature of these relationships undoubtedly impacts the distribution of bacterial communities, and identifying the mode of transmission in these symbioses remains an
unresolved priority (Dale and Moran 2006; Sharp et al. 2010; Sharp, Distel and Paul 2012; Hester et al. 2016). If corals take up bacteria from the environment during their free-swimming larval stage, we might expect a homogenous bacterial community over broad geographical scales (Laue and Nelson 1997; Nelson and Fisher 2000; Harmer et al. 2008). However, if larvae are brooded or if offspring initiate associations with locally adapted bacteria after settlement (Lema, Bourne and Willis 2014), we might expect more geographically structured communities. Similarly, vertical or maternal transmission would result in relatively structured populations shaped by the dispersal of the host coral (Peek et al. 1998; Hosokawa et al. 2006; Hester et al. 2016).

Our study species, P. lobata, is a gonochoristic broadcast spawner that obtains its symbiotic zooxanthellae through vertical transmission (Fadlallah 1983). Because of this built-in nutritional source, the species is thought to have a relatively long planktonic larval duration and, therefore, high dispersal potential (Glynn et al. 1991). Polato et al. (2010) found that P. lobata corals, collected on the same research cruises and from the same islands/atolls investigated here, conformed to a pattern of isolation by distance across the Hawaiian Archipelago and were strongly isolated from Johnston Atoll. Similarly, we detected a significant correlation between geographical distance and coral-associated bacterial community dissimilarity and found that Johnston Atoll differed significantly from some, but not all islands/atolls within the Hawaiian Archipelago. These parallel findings indicate a close relationship between P. lobata corals and their bacterial associates, consistent with vertical transmission. However, the ratio of truly symbiotic resident bacteria versus transient ‘visitor’ bacteria is unknown for corals and this is likely to weaken correlations between bacterial community structure and host population structure (Ritchie 2006; Hester et al. 2016).

The majority of T-RFs were present in only a small percentage of coral samples and we did not find a single T-RF that was present in 100% of coral samples. However, a small percentage of T-RFs were present in >40% of coral samples and a few were noticeably dominant across samples in terms of prevalence. Also, T-RFs that were the most dominant in terms of relative abundance were not necessarily prevalent in samples. These findings are similar to those of Hester et al. (2016) in which corals were observed to have a high-diversity group of stable symbionts (but with no single bacterial phylotype strictly associated with a single host species) and a low-diversity community of sporadic symbionts whose abundance varied significantly across individual colonies of the same species.

For comparison, Speck and Donachie (2012) constructed 16S rRNA gene clone libraries from the mucus of five P. lobata coral colonies in Hawaii (along a 150 m transect) and found only modest OTU replication. They also determined that bacterial communities were only ~8.4% similar among P. lobata colonies. A single OTU from the genus Endozoicomonas in the Gammaproteobacteria, closely related to a clone group we recovered here, was present in all coral mucus clone libraries, including another Porites species sampled in the study, and has previously been associated with other coral species (e.g., P. astreoides: Rohwer et al. 2002; Acropora hyacinthus and Stylophora pistillata: Kvennefors et al. 2010; Montipora aequisetulcata: Yang et al. 2010; S. pistillata: Bayer et al. 2013; Eunicea verrucosa: Ransome et al. 2014; P. lutea: Hester et al. 2016). Further characterizing and categorizing the range of microorganisms that are consistently (vs transiently) associated with this coral species may help resolve the biogeography of these communities on a finer scale.

Geographical isolation driving biogeography

The two questions central to understanding biogeography, as it pertains to microorganisms, are as follows: (1) Do microbial communities differ by location (i.e. exhibit spatial biogeography) and (2) Is their spatial distribution due to contemporary environmental factors (e.g. temperature, salinity, nutrients), historical events (e.g. dispersal barriers, past major ecological events) or a combination of both (Martiny et al. 2006)? Here, we document that planktonic and coral-associated bacterial communities in the Hawaiian Archipelago, Johnston Atoll and American Samoa exhibit distance-based biogeographical structure. Thus, geographical isolation, possibly due to dispersal limitation may, in part, be driving observed divergences (Martiny et al. 2011). Yet we detected several island/atoll groupings that were not distance based (i.e. similar microbial communities, but geographically distant), indicating that environmental variables are also influencing the distributions of bacterial communities.

Environmental factors driving biogeography

Identifying the environmental factors driving biogeography is challenging due to the sheer number of environmental variables, the technical challenges of measuring said variables and the limited body of knowledge concerning which ones, or combinations thereof, have a significant effect on marine microorganisms. In this study, we were able to test for correlations between temperature or depth and planktonic or coral-associated bacterial community structure and did not observe any significant relationships, with the exception of coral samples from 2006. However, temperature data were collected at a single point in time and it is possible that temperature is an important variable that we could not quantify with the available data (Littman, Bourne and Willis 2010). Notably, coral-associated bacterial communities did not differ among fringing, patch, back and fore reef habitats at French Frigate Shoals. However, these results should be interpreted with caution since data were only obtained for a single location in a single year. Future studies incorporating increased replication of sampled habitats and more comprehensive measurements of local oceanographic and environmental conditions over relevant time scales would help to elucidate the environmental variables that may be shaping the biogeography of coral-associated bacterial communities.

Evidence for biogeographical provinces

Johnston Atoll is the only island outside of the Hawaiian Archipelago that is considered to be part of the Hawaiian biogeographical province based on faunal similarities (Randall 1998; Briggs and Bowen 2012), and may be a stepping stone for colonization into the archipelago by marine organisms (Maragos and Jokiel 1986). Kobayashi (2006) identified potential transport corridors connecting Johnston with the middle portion of the Hawaiian Archipelago in the vicinity of French Frigate Shoals. Recent genetic analyses of reef fishes provide additional support for this corridor (Leray et al. 2010; Gaither et al. 2011; Andrews et al. 2014). Acropora cytherea table corals also exhibit geographical distributions concurrent with the presence of a dispersal corridor (Kosaki et al. 2013).

Our observations for seawater and coral-associated bacterial communities revealed similar connections to Johnston Atoll. Planktonic bacterial communities from Johnston Atoll were significantly different from those at the lower end of the
archipelago (Kaneohe Bay, Oahu), but not from the middle portion (French Frigate Shoals and Nihoa). Coral-associated bacterial communities from Johnston Atoll were also significantly different from those at the lower end of the archipelago (Kaneohe Bay, Oahu), but not from the middle portion (Gardner Pinnacles and Nihoa), with the exception of French Frigate Shoals. Population genetic analysis of P. lobata revealed little gene flow between Johnston Atoll and the Hawaiian Islands, but evidence of rare dispersal events between the two populations was observed (Polato et al. 2010).

The observed biogeographical patterns of seawater and coral-associated bacterial communities in this study provide evidence that both free-living and host-associated microbes are subjected to similar processes that influence the biogeography of marine macrofauna. These concordant connections, spanning protists, invertebrate corals and vertebrates have strong implications for the conservation of Hawaiian biodiversity. As a mid-oceanic archipelago, all life in Hawaii is descended from colonists, and so the oceanic corridors into Hawaii have paramount importance in maintaining biodiversity.

SUPPLEMENTARY DATA
Supplementary data are available at FEMSEC online.

ACKNOWLEDGEMENTS
The authors thank the Captain and the crew of the NOAA R/V Hi’ialakai and Randall Kosaki and the staff of the Papahanaumokuakea Marine National Monument for their assistance with permitting, research cruise logistics, and dive operations. We thank E. Hambleton for assistance with sample processing, and J. Becker, D. Hayakawa, M. Miller, T. Campbell, S. Yeo, A. Apprill, A. Eiler and other members of the Rappé laboratory for support.

AUTHOR CONTRIBUTIONS
JLS and MSR designed the study. JLS and MSR contributed funding support and materials to the project. All fieldwork, sample collection, laboratory work, and data analysis were conducted by JLS in the laboratory of MSR. JLS wrote the manuscript and MSR and BWB assisted with the interpretation and edited the manuscript.

FUNDING
This work was supported by the National Oceanographic and Atmospheric Administration [NOAA NMSP MOA #2005-008/66882] and the National Science Foundation [Grant OCE-0928806 to M.S.R.].

Conflict of interest. None declared.

REFERENCES
Baas-Becking LGM. Geobiologie van Inleiding Tot de Milieukunde. Den Haag, the Netherlands: W.P. Van Stockum and Zoon, 1934, 263.


