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Bacterial communities associated with lionfish in their native and invaded ranges

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ABSTRACT: Lionfish, which are native to the Indo-Pacific, were introduced into the Western Atlantic Ocean, where they have become highly successful invaders. Previous work has demonstrated the presence of distinct lionfish populations within both the invaded and native ranges, but little is known about the specificity of the bacterial communities associated with lionfish surfaces. This study was designed to evaluate whether lionfish-associated bacterial communities reflected the geographical distribution patterns of their host. To this end, fin-associated bacterial communities from sites throughout the native and invaded ranges were analyzed. Lionfish were collected from 9 locations in the invaded Western Atlantic Ocean and 8 locations throughout their native Indo-Pacific. The composition of lionfish-associated bacterial communities was examined by terminal restriction fragment length polymorphism analyses following amplification of the bacterial 16S rRNA gene with universal prokaryotic primers 8F and 1392R. Lionfish-associated external bacterial communities were not significantly different between the native and invaded ranges, but differences were found between collection locations within each range. Although bacterial communities on lionfish skin demonstrated a greater similarity within collection site than between collection sites, a core group of bacteria dominated by members of the γ *Proteobacteria* were found in lionfish-associated communities from both ranges. It is likely that lionfish maintain a core group of external bacteria throughout both the native and invaded ranges, but are differentially colonized by other bacteria from their local environments.

KEY WORDS: Core bacterial communities · Host-associated bacteria · Invasive species · Lionfish · *Pterois volitans* · Surface bacterial communities

INTRODUCTION

Lionfish [*Pterois volitans* (Linnaeus) and *Pterois miles* (Bennet)], which are indigenous to the Indo-Pacific, have become invasive throughout the Western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico. Sightings of individual lionfish in the 1980s off the Florida coast preceded the current invasion, which has been shown to detrimentally affect coral reef ecosystems (Albins & Hixon 2008, Albins 2013). Analysis of mitochondrial genetic markers suggested that the original invasive lionfish population was derived from Indonesian lionfish and consisted of at least 3 female *P. volitans* and 1 female *P. miles* (Hamner et al. 2007). In the native range, although *P. volitans* is restricted to Pacific waters and *P. miles* is found only in the Indian Ocean (Kochzius & Blohm 2005, Kulbicki et al. 2012), populations of both species can be found in Indonesia where these oceans meet. In total, the initial invasive lionfish population is thought to have consisted of fewer than 12 individuals, which resulted in a strong founder effect (Hamner et al. 2007, Freshwater et al. 2009). Within the invasive range, Betancur-R et al. (2011) recently showed that a significant

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population break existed between invasive lionfish populations in the Bahamas, Bermuda, and North Carolina (northern region), where haplotype diversity was relatively high, and lionfish in the Cayman Islands, Nicaragua, and Colombia (southern region) that exhibited low genetic diversity.

Out of this finding arose the question of whether their skin-associated bacterial communities also reflected the distribution of the host lionfish population, as a wide variety of host-associated microbial communities have been shown to display patterns of distribution similar to those of their hosts (reviewed in Martiny et al. 2006 and Robinson et al. 2010). The intimate interactions between the host and its microorganisms potentially create specialized niches that free-living microorganisms cannot inhabit (Nemergut et al. 2011). Therefore, the patterns of host-associated microbial communities might reflect the long-term biogeographical patterns of their hosts and are likely more resistant to fluctuations in environmental conditions (e.g. nutrient availability, temperature, salinity) than free-living microbial communities (Pinhassi et al. 2003, Martiny et al. 2006).

Previous research suggested that lionfish likely acquire their skin-associated community from the environment, as lionfish eggs and egg masses are free of bacteria prior to release from the female, limiting the possibility that these communities are vertically transmitted (Stevens & Olson 2013). Although the waters of the invaded range of the lionfish are known to be different than those in their native range, both in abiotic (e.g. temperature, nutrient availability) and biotic factors (microbial community composition; e.g. Yutin et al. 2007, Ma et al. 2009, Jing et al. 2013), the influence of these altered conditions on the microbial colonization of lionfish skin is unknown. Thus, although lionfish share similar diets (small fish and crustaceans) and habitats in both ranges, they are likely exposed to different bacterial communities as potential colonizers. In this study, a molecular fingerprinting technique was employed to examine the dominant members of the skin-associated community in order to assess whether lionfish are colonized by taxonomically similar bacteria regardless of range or whether the biogeographical patterns of lionfish are reflected by the composition of their associated bacteria. Bacterial community composition was also compared to invasive lionfish genetic patterns reported in earlier studies (Hamner et al. 2007, Freshwater et al. 2009, Betancur-R et al. 2011).

MATERIALS AND METHODS

Field collection

Lionfish were collected from 9 locations within the invaded range (Fig. 1): Bermuda (n = 4); Key Largo, USA (n = 10); Gulf of Mexico, USA (n = 10); Bahamas (n = 10); Cayman Islands (n = 10); Belize (n = 10); Panama (n = 10); Honduras (n = 10); and the US Virgin Islands (USVI) (n = 5). SCUBA divers with pole spears captured invasive lionfish, which were euthanized via pithing while still on the spear. Fish were placed into individual WhirlPak® (Nasco) bags until return to the surface where fin clips (approx. 2–4 cm) from the non-venomous pectoral fins were removed and preserved in 1.8 ml of RNAlater® (Ambion). All samples were preserved within 2 h of collection and frozen at −20°C until laboratory analysis.

In the native range, lionfish were collected from 8 locations (Fig. 1): Taiwan (n = 10); Northern Philippines (n = 10); Central Philippines (n = 10); Guam (n = 10); Indonesia (n = 10); Maldives (n = 4); Sri Lanka (n = 9); and Kenya (n = 6). Collection locations span both the Pacific and Indian Oceans; thus, samples include *Pterois volitans* and *P. miles*. SCUBA divers with

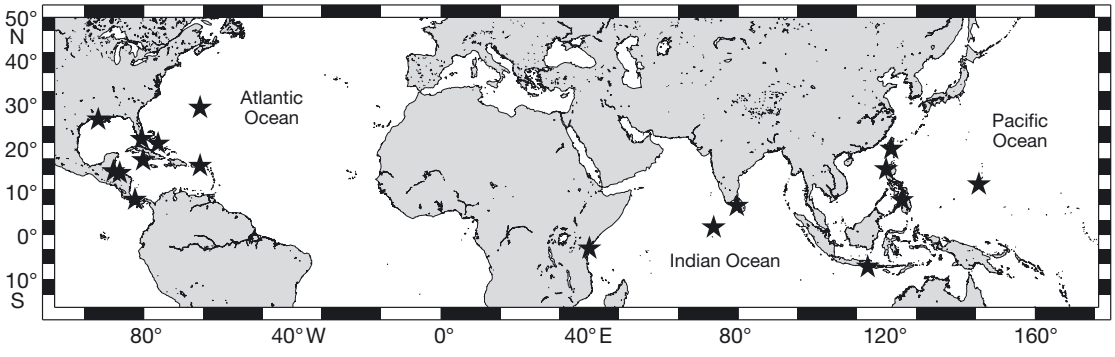

Fig. 1. Lionfish collection locations (black stars) in their native Indian and Pacific Oceans and the invaded Atlantic Ocean, Caribbean Sea, and Gulf of Mexico

hand nets caught lionfish, which were immediately placed in individual WhirlPak® bags (Nasco). Upon surfacing, fin clips were taken as described for the invaded range and the fish were either returned to the water or euthanized and added to the fish collection at the National Museum of Marine Biology and Aquarium (Checheng, Taiwan).

Concurrent with lionfish capture in a subset of locations within both the native and invaded ranges, 500 ml seawater volumes were filtered through 0.2 µm polycarbonate filters (Millepore). Native range collection locations were Indonesia (n = 2), Taiwan (n = 5), and Northern Philippines (n = 1). Invaded range collection locations were Key Largo, USA (n = 3), Honduras (n = 5), and Bahamas (n = 5). Individual filters were preserved in 1.8 ml of RNAlater® and maintained at −20°C until use.

DNA extraction and Polymerase Chain Reaction (PCR) amplification

Total community DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's protocol, with an extended cell lysis step at 37°C with shaking. Universal prokaryotic primers 8F (5′- AGA GTT TGA TCM TGG CTC AG-3′; Edwards et al. 1989) with a fluorescent *S*-hexachlorofluorescein (HEX) label and 1392R (5′- ACG GGC GGT GTG TAC A-3′; Lane 1991) were used to amplify an approximately 1385 bp region of the 16S rRNA gene by PCR. Each reaction was run in triplicate and consisted of 2 U PerfectTaq (5-Prime), 1× PerfectTaq buffer, 1.25 mM $Mg(OAc)_2$, 0.06 mM deoxynucleoside triphosphates, 0.8 µg bovine serum albumin (BSA), 8% formamide, 25 pmol of each primer, and sterile deionized water to a final volume of 100 µl. Reaction conditions were 85°C for 5 min, followed by 30 cycles of 94°C for 45 s, 62°C for 90 s, and 72°C for 90 s, with a final 10 min extension at 72°C. To reduce the formation of chimeras, the temperature ramp speed for all cycles was set at 1°C s^{-1} (Stevens et al. 2013). Amplification products were subjected to electrophoresis on 1.5% agarose gels containing GelRed™ (Biotium) for 70 min at 70 V, and visualized under UV transillumination with a gel imaging system (Fotodyne). Negative reagent controls were run for all reactions. PCR products were individually cleaned using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek) following the manufacturer's protocol, and the triplicate products were pooled prior to terminal restriction fragment length polymorphism (T-RFLP) analysis.

T-RFLP analysis

The concentration of DNA within the pooled PCR products was quantified using a Nano-drop 2000c spectrophotometer (Thermo Scientific). Digestion reactions were carried out using 100 ng of cleaned PCR product, 2 U of the restriction endonuclease *Hae*III (New England BioLabs), 1× enzyme buffer, and sterile deionized water to a total volume of 50 µl. Following an 8 h incubation at 37°C, enzymes were deactivated by a 30 min incubation at 80°C. Digestion products were precipitated overnight in 100% ethanol at −20°C prior to being centrifuged at 16 000 × *g* for 15 min. The resulting pellets were rinsed with 500 µl ice-cold 70% ethanol, centrifuged for another 15 min, and dried in a Jouan RC1022 centrivac (Thermo Scientific). The pellets were resuspended in 10 µl of deionized formamide and 0.5 µl of 6-carboxytetramethylrhodamine size standard (Applied Biosystems) prior to being analysed with an ABI 310 Genetic Analyser with a 50 cm capillary array (Applied Biosystems). Terminal restriction fragment (T-RF) lengths were determined using the Local Southern size-calling algorithm of the Peak Scanner v. 1.0 analysis software (Applied Biosystems).

Data matrices were constructed from peaks above a threshold of 50 fluorescence units, which was considered to be the background level. Peaks smaller than 100 bp and greater than 500 bp were removed from the data set to avoid uncertainties associated with fragment size determination (reviewed in Schütte et al. 2008). With the software program T-REX (Culman et al. 2009), the resulting profiles were binned at a 0.5 clustering threshold prior to statistical analysis. *In silico* digestions of sequenced 16S rRNA genes from clones (Stevens & Olson 2013) and isolates were performed to determine the size of the resulting fragment. Based on results from previous studies (Olson et al. 2014), 2 bp were added to each predicted *Hae*III T-RF.

Statistical analyses

Using PRIMER v. 6 software (Clarke & Gorley 2006), Bray-Curtis similarity matrices were constructed from fourth root transformations of the normalized T-RFLP peak area data from T-REX. Paired *t*-tests were used to compare T-RF richness between invaded and native ranges. A nested 2-way analysis of similarity (ANOSIM) was used to examine the effect of collection location nested within ranges

(native or invaded) on bacterial community composition. Additionally, 1-way ANOSIMs were used to calculate bacterial community differences between collection locations within each range as well as between species of lionfish in their native range. Pairwise comparisons with a Bonferroni correction were made between collection locations within each range. Both p-values and ANOSIM R-values are used to report significance. The R-value provides an estimate of the degree of similarity between samples, with an R-value of 1 indicating that the bacterial communities (evaluated by both presence and abundance) were completely different from each other, whereas an R-value of 0 represents identical communities. Non-metric, multidimensional scaling (MDS) plots provided visualization of bacterial community composition by range and collection location in 2-dimensional space. One-way similarity percentages (SIMPER) were used to determine the contribution of each T-RF to the total community composition within and between collection locations. Shannon diversity and Pielou's evenness indices were calculated for each sample.

RESULTS

Comparison of lionfish bacterial communities to ambient bacterioplankton

Because individual T-RFs can represent multiple bacterial species with a shared restriction endonuclease digestion site, individual peaks are not necessarily representative of individual species but do provide an estimate of taxonomic diversity (e.g. Schütte et al. 2008). However, because different bacterial communities had been previously detected in water from different oceans (Yutin et al. 2007, Ma et al. 2009, Jing et al. 2013), this level of resolution was selected to assess the relative composition of the associated communities. Different methods provide varying levels of resolution; for our purposes, obtaining short next generation sequence reads would not have provided species identification below the phylum level. Instead, we chose to combine T-RFLP analyses with sequencing of the 16S rRNA gene from associated bacteria to provide more specific information about the community. Not surprisingly, the bacterioplankton communities in the water samples from the Indo-Pacific (native range, n = 8) and the western Atlantic (invaded range, n = 13) were significantly different (R = 0.543, p = 0.001). However, the bacterioplankton present in the ambient water were significantly different than the bacterial communities associated with lionfish (R = 0.629, p = 0.001). Pairwise comparisons revealed the lionfish-associated bacterial communities within the invaded range (n = 79) were significantly different from the bacterioplankton in waters from the invaded (R = 0.656, p = 0.001) and native (R = 0.847, p = 0.001) ranges. The bacterial communities on native lionfish (n = 69) were also significantly different from bacterioplankton in the native (R = 0.682, p = 0.001) and invaded (R = 0.481, p = 0.001) range water samples.

Bacterial community comparison between lionfish ranges

The number of T-RFs detected from lionfish in the invaded range (mean ± SD = 79.1 ± 45.7) was not significantly different than in the native range (77.1 ± 46.8; *t*-test p > 0.05), indicating that the richness of lionfish-associated T-RFs was similar between ranges. Bacterial community composition was also not significantly different between the native and invaded ranges (2-way nested ANOSIM; R = 0.067, p > 0.05). Similarly, the bacterial community composition was not significantly different between *Pterois volitans* collected in the Pacific Ocean and *P. miles* collected in the Indian Ocean (ANOSIM, R = 0.084, p > 0.05). However, identification of the distinguishing characters cited by Schultz (1986) between *P. volitans* and *P. miles* in the invaded range was found to be equivocal, resulting in the use of a species complex for both taxa in the Atlantic (Whitfield et al. 2007). Non-metric, multidimensional scaling plots showed no clustering of lionfish-associated bacterial communities by range (Fig. 2).

Bacterial community comparisons between lionfish collection locations

Collection location did have an overall significant effect on the composition of lionfish-associated bacterial communities (invaded range R = 0.427, p = 0.0001; native range R = 0.578, p = 0.0001). Following Bonferroni corrections, 23 of 28 pairwise comparisons between collection locations in the native range were significantly different (Table 1). Similarly, 18 of 36 pairwise comparisons of collection locations in the invaded range were significantly different. In a MDS plot, some clustering of individuals by collection location can be seen (Fig. 3).

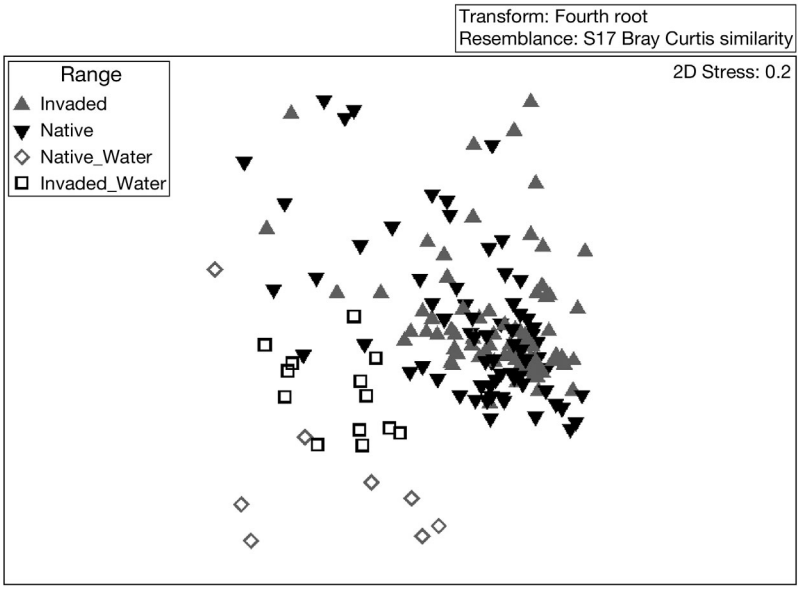

Fig. 2. Multidimensional scaling plot of bacterial communities associated with lionfish in their native (n = 69) and invaded ranges (n = 79) indicates that the communities were not significantly different. Lionfish-associated bacterial communities were significantly different from ambient bacterioplankton in both ranges (native range water, n = 8; invaded range water, n = 13)

Diversity indices indicated a high degree of evenness in lionfish-associated bacterial communities. The Shannon diversity index revealed no significant difference in community diversity between native and invaded ranges (*t*-test, $p > 0.05$), and Pielou's evenness index was above 0.97 for all samples (Table 2). Additionally, SIMPER analyses showed that the greatest contribution of a single

Table 1. Pairwise comparisons based on analysis of similarity of lionfish-associated bacterial communities between collection locations within their native and invaded ranges. *Italics* denote that the comparison was **not** statistically significant after Bonferroni correction. USVI = US Virgin Islands; C. Philippines = Central Philippines; N. Philippines = Northern Philippines

	R-value	p-value
Invaded range		
Belize, Panama	0.536	0.0001
Belize, Key Largo	0.545	0.0001
Belize, Honduras	0.578	0.0001
Belize, Little Cayman	0.577	0.0001
Panama, Gulf of Mexico	0.689	0.0001
Honduras, Gulf of Mexico	0.773	0.0001
Little Cayman, Gulf of Mexico	0.431	0.0001
Bahamas, Gulf of Mexico	0.624	0.0001
Belize, Gulf of Mexico	0.596	0.0002
Key Largo, Gulf of Mexico	0.588	0.0002
Panama, Little Cayman	0.275	0.0002
Key Largo, Bahamas	0.433	0.0003
Honduras, USVI	0.702	0.0003
Key Largo, Honduras	0.269	0.0005
Honduras, Bahamas	0.332	0.0006
Panama, Honduras	0.315	0.0006
Panama, USVI	0.6	0.0007
Belize, Bahamas	0.237	0.001
Bahamas, USVI	*0.584*	*0.002*
Panama, Bermuda	*0.669*	*0.002*
Honduras, Little Cayman	*0.287*	*0.002*
Honduras, Bermuda	*0.748*	*0.002*
Little Cayman, Bahamas	*0.243*	*0.002*
Bahamas, Bermuda	*0.654*	*0.003*
Key Largo, USVI	*0.556*	*0.004*
Key Largo, Bermuda	*0.655*	*0.005*
Belize, USVI	*0.432*	*0.007*
Panama, Bahamas	*0.265*	*0.007*
Belize, Bermuda	*0.44*	*0.024*
Key Largo, Little Cayman	*0.16*	*0.013*
USVI, Gulf of Mexico	*0.334*	*0.019*
Little Cayman, Bermuda	*0.439*	*0.024*
Little Cayman, USVI	*0.327*	*0.028*
Panama, Key Largo	*0.094*	*0.07*
USVI, Bermuda	*–0.038*	*0.516*
Native range		
Guam, C. Philippines	0.761	0.0001
Guam, Indonesia	0.647	0.0001
Guam, Taiwan	0.505	0.0001
Guam, Sri Lanka	0.617	0.0001
N. Philippines, C. Philippines	0.722	0.0001
N. Philippines, Indonesia	0.711	0.0001
N. Philippines, Taiwan	0.601	0.0001
N. Philippines, Sri Lanka	0.651	0.0001
C. Philippines, Indonesia	0.668	0.0001
C. Phillipines, Taiwan	0.74	0.0001
C. Philippines, Sri Lanka	0.705	0.0001
C. Philippines, Kenya	0.663	0.0001
Indonesia, Taiwan	0.432	0.0001
Indonesia, Sri Lanka	0.604	0.0001
Taiwan, Sri Lanka	0.585	0.0001
Taiwan, Kenya	0.76	0.0001
N. Philippines, Kenya	0.73	0.0002
Guam, Kenya	0.713	0.0005
Sri Lanka, Kenya	0.678	0.0006
Guam, N. Philippines	0.35	0.0006
Indonesia, Kenya	0.586	0.001
Sri Lanka, Maldives	0.821	0.001
C. Philippines, Maldives	0.688	0.001
Taiwan, Maldives	*0.642*	*0.002*
N. Philippines, Maldives	*0.745*	*0.002*
Guam, Maldives	*0.724*	*0.003*
Maldives, Kenya	*0.512*	*0.014*
Indonesia, Maldives	*0.431*	*0.016*

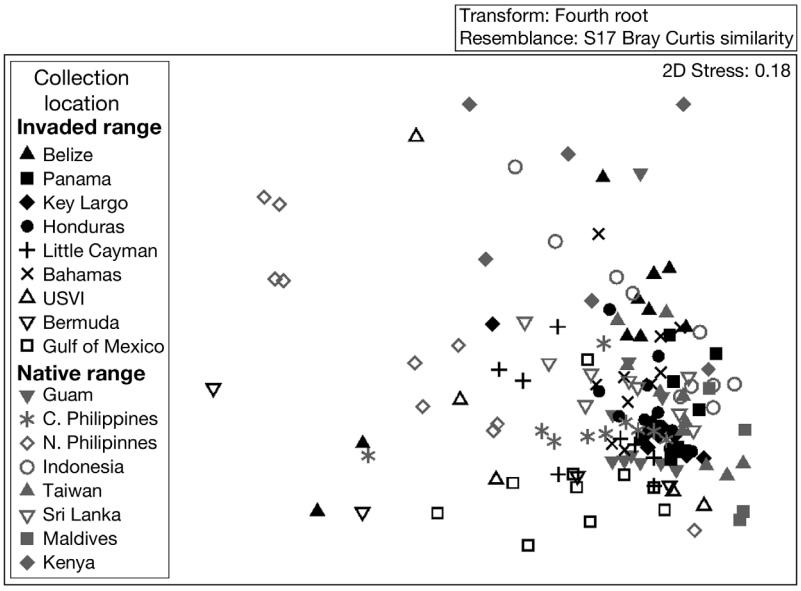

Fig. 3. Multidimensional scaling plot of bacterial communities associated with lionfish from collection locations in their native and invaded ranges revealed significant differences between individual collection locations but not across ranges. USVI = US Virgin Islands, C. Philippines = Central Philippines; N. Philippines = Northern Philippines

Table 2. Diversity indices calculated based on the average number of terminal restriction fragments (T-RF) reported in lionfish-associated bacterial communities from each collection location. Numbers in parentheses indicate standard deviation. USVI = US Virgin Islands, C. Philippines = Central Philippines; N. Philippines = Northern Philippines

Location	T-RF Richness	Pielou's Evenness	Shannon Index
Invaded range			
Belize	38.7 (18.6)	0.994 (0.002)	3.49 (0.599)
Panama	112.8 (27.9)	0.993 (0.002)	4.66 (0.254)
Key Largo	115.4 (41.7)	0.994 (0.001)	4.61 (0.589)
Honduras	86.8 (30.1)	0.992 (0.002)	4.35 (0.424)
Little Cayman	85.8 (42.0)	0.995 (0.003)	4.28 (0.577)
Bahamas	73.5 (21.1)	0.994 (0.002)	4.22 (0.38)
USVI	90.8 (93.8)	0.995 (0.004)	3.97 (1.18)
Bermuda	58.3 (65.1)	0.998 (0.002)	3.37 (1.49)
Gulf of Mexico	77.2 (42.0)	0.998 (0.001)	4.19 (0.604)
Native range			
C. Philippines	38.4 (45.9)	0.992 (0.006)	3.22 (0.881)
Indonesia	82.2 (35.1)	0.991 (0.004)	4.26 (0.523)
Taiwan	116.8 (48.4)	0.987 (0.003)	4.61 (0.48)
Sri Lanka	82.9 (32.5)	0.992 (0.001)	4.30 (0.44)
Maldives	138.5 (43.4)	0.995 (0.001)	4.87 (0.32)
Kenya	33.8 (33.0)	0.989 (0.006)	3.14 (0.877)
Guam	80.2 (33.6)	0.988 (0.005)	4.21 (0.587)
N. Philippines	63.9 (30.7)	0.995 (0.002)	4.01 (0.561)

T-RF to overall community similarity was 17.03% in samples from Kenya (Table S1 in the Supplement at www.int-res.com/articles/suppl/m531p253_supp.pdf). A single T-RF accounted for 15.88% and 15.34% of the similarity present in fish-associated bacterial communities from Central Philippines and Bermuda, respectively. For the remaining 14 collection locations, no single T-RF accounted for more than 7% of community similarity. Thus, in most cases, it does not appear that individual T-RFs were responsible for the majority of the similarity detected in community composition.

Identification of bacterial associates of lionfish

Putative taxonomic identifications of members of the lionfish core bacterial community (>90% presence in all fish samples) were made using data from sequenced 16S rRNA genes obtained from clones (Stevens & Olson 2013) and isolates (J. Stevens unpubl. data). The communities were dominated by members of the γ *Proteobacteria*, with α and β *Proteobacteria* present at lesser abundances. Members of the *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Gemmatimonadetes* were also detected. SIMPER was used to evaluate the T-RFs that were responsible for the observed similarity within the associated bacterial communities. For lionfish in the invaded range, 25 T-RFs accounted for 50% of the observed similarity while 24 T-RFs represented this percentage on lionfish from the native range. Of these T-RFs, 17 were found in both ranges, considered here to be the core community, and included members of both gram positive (*Exiguobacterium*) and gram negative (*Achromobacter*, *Gemmatimonas*, *Pseudomonas*, *Paracoccus*, *Serratia*, and *Vibrio*) genera. While some organisms were more specifically associated with lionfish (e.g. *Gemmatimonas*, *Serratia*), others were also commonly detected in the water column (e.g. *Achromobacter*, *Paracoccus*). Lesser components of the lionfish bacterial community included members of the genera *Amphritea*, *Bacteroides*, *Cetobacterium*, *Clostridium*, *Cobetia*, *Marinomonas*, *Nautella*, *Propionigenium*, *Tenacibaculum*, and *Tha-*

lassomonas. Some T-RFs appeared to be more prevalent in certain oceans (e.g. *Pseudomonas* 200 bp T-RF, *Nautella* 194 bp T-RF, and *Achromobacter* 215 bp T-RF were more common in native range samples) while others were found at low levels in all locations (e.g. water and fish samples; *Marinomonas* 257 bp T-RF, *Amphritea* 325 bp T-RF, *Cobetia* 321 bp T-RF, and *Paracoccus* 193 bp T-RF).

Comparison of bacterial communities and lionfish population structure

In order to compare the distinguishable invasive lionfish populations reported by Betancur-R et al. (2011) with their associated bacterial communities, collection locations were grouped into northern and southern regions and examined in a 1-way ANOSIM. Lionfish collected from Bermuda, Key Largo (USA), and the Bahamas were grouped as the northern region while those collected in Panama, Belize, Honduras, US Virgin Islands, and Little Cayman were grouped as the southern region. Because the Betancur-R et al. (2011) study did not include lionfish specimens from the Gulf of Mexico (as they had not yet been detected at this location), separate analyses were run with the Gulf of Mexico samples included as part of the northern region, as part of the southern region, and as a new region as proposed by Betancur-R et al. (2011). No significant differences in bacterial community structure were identified between lionfish from the northern and southern regions (1-way ANOSIM; $R = -0.039$, $p > 0.05$). Additionally, no significant differences were found when the Gulf of Mexico samples were placed in either the northern or southern region. However, when the Gulf of Mexico was treated as a separate region, significant differences in lionfish-associated bacterial communities were observed between these samples and samples from the northern ($R = 0.336$, $p = 0.003$) and southern ($R = 0.239$, $p = 0.012$) regions.

DISCUSSION

Comparison of skin-associated bacterial communities from lionfish collected within their native Indo-Pacific versus the invaded western Atlantic Ocean did not reflect the biogeography of their hosts. Instead, the results indicated that lionfish retained a similar core bacterial community across species of lionfish and across the native and invaded ranges. However, when individual collection locations were examined using pairwise comparisons, differences in the bacterial communities associated with lionfish were evident. These data suggested that while the core bacterial community was retained, local biotic and/or abiotic influences generated measureable differences in associated bacterial communities.

The maintenance of core bacterial communities is common in host–microbe relationships in the marine environment (e.g. Taylor et al. 2005, Smith et al. 2007, Wilson et al. 2008, Apprill et al. 2011, Larsen et al. 2013), yet local variation in bacterial community composition has been shown to be sufficient to identify host populations (Nguyen et al. 2008, Tatsadjieu et al. 2010). Variation in surface-associated bacterial communities within a fish species is often due to shifts in relative abundances of bacterial taxa rather than to drastic changes in the composition of the assemblage (Horsley 1977, Smith et al. 2007, Wilson et al. 2008, Larsen et al. 2013). For example, in comparisons of the surface bacteria associated with whiting *Merlangius merlangus* and Atlantic cod *Gadus morhua* from geographically separated populations within the same ocean, similar bacterial assemblages were maintained across collection locations (Smith et al. 2007, Wilson et al. 2008). Likewise, lionfish appear to have retained a core bacterial community across ocean basins. Because the bacterial communities associated with lionfish displayed high richness and evenness (Table 2), the differences in composition between fish from particular collection locations were likely driven by slight variations within these highly diverse communities.

Although the average richness of T-RFs on lionfish (80.45 ± 46.2; Table 2) appears to be substantially greater than what was found on individual cod (5.2 ± 2.4 to 8.2 ± 3.0; Wilson et al. 2008) or whiting (17.3 ± 15.9; Smith et al. 2007), the bacterial communities were similarly dominated by members of the γ *Proteobacteria*. Larsen et al. (2013) also reported the dominance of γ *Proteobacteria* in fish surface bacterial assemblages, with distinct communities detected via ribosomal internal spacer analysis on each of the 6 Gulf of Mexico fish species examined. However, the utilization of different fingerprinting methods and/or restriction endonucleases in T-RFLP analyses in these studies must be taken into consideration as it affects the resulting estimates of diversity. Additionally, both the Smith et al. (2007) and Wilson et al. (2008) studies were conducted in temperate oceans, while many of the lionfish in this study were collected in tropical waters. Regardless of the diversity present, fish surface-associated bacterial communities were largely dominated by *Proteobacteria*, mostly

within the γ class, reinforcing results obtained previously in culture-dependent analyses (e.g. Colwell 1962, Horsley 1973, Gilmour et al. 1976, Austin 1983).

As expected from previous studies (e.g. Yutin et al. 2007, Ma et al. 2009, Jing et al. 2013), the bacterioplankton present in water samples from the native and invaded ranges were significantly different. The bacteria present on lionfish surfaces were also significantly different from ambient bacterioplankton, supporting previous molecular-based analyses for lionfish (Stevens & Olson 2013) and other fish species (Jensen et al. 2004, Smith et al. 2007, Larsen et al. 2013). Interestingly, as there is no evidence of vertical transmission of bacteria in lionfish eggs (Stevens & Olson 2013), this suggests that fish were selectively colonized by bacteria present in their environment. The similarity in lionfish bacterial communities across oceans with differing ambient bacterioplankton suggests that lionfish skin is a selective medium supporting a distinctive bacterial assemblage that may be important for providing some benefit to the host fish. Additional studies are needed to examine the potential roles of these bacterial associates.

When pairwise comparisons of lionfish-associated bacterial communities were performed for collection sites within a range, more locations within the native range (82%) were significantly different than locations within the invaded range (50%). Additionally, the locations within the invaded range with significantly different bacterial communities did not reflect the proposed population breaks within the Caribbean Sea (Cowen et al. 2006, Betancur-R et al. 2011). The lack of differentiation of the associated bacterial communities at many of the invasive lionfish collection locations may reflect that the invasive populations arose from a genetically limited founding population and may not have had sufficient time to select for distinct bacterial communities. Also, little is known about the influence of host genetics in the acquisition and maintenance of associated bacteria. Alternately, the often shorter geographic distances between collection sites in the invaded range may be responsible for this observation. However, there were some important exceptions: significant differences were detected for lionfish-associated bacterial communities between 2 collection locations within both the native (Central versus Northern Philippines ~100 km apart; Table 1) and invaded (Belize versus Honduras; <400 km; Table 1), while communities on fish separated by over 5000 km were not significantly different. These results suggest that distance is not a critical factor in regulating the composition of lionfish-associated bacterial communities.

Our data on the composition of the associated bacterial communities were compared to previously reported genetic differences between invasive lionfish populations (Hamner et al. 2007, Betancur-R et al. 2011) that split the invasive lionfish in the Atlantic into 2 populations based on geographic location (Betancur-R et al. 2011). Similarly, geographic location divides *Pterois volitans* from *P. miles* in the native Indo-Pacific region (Kochzius & Blohm 2005, Kulbicki et al. 2012). However, our data did not show similar separation of the bacterial communities, suggesting that factors other than genetic differences within the host contributed to the composition of lionfish-associated bacterial communities. Although the associated bacteria may be acquired from the reef environment, our results found differences in bacterial communities associated with lionfish from the same reef system (e.g. Mesoamerican Reef), indicating that reef is likely not the appropriate scale for assessing the origins of the bacterial communities. Interestingly, the bacterial communities associated with lionfish collected in the Gulf of Mexico were significantly different than those from fish collected in the northern and southern regions of the invaded range. There are several potential interpretations of this result. First, the predictions regarding population breaks made by Betancur-R et al. (2011) may have been correct and the Gulf of Mexico supports a significantly different population of lionfish than the rest of the invaded range; additional population genetic analyses of invasive lionfish, especially from the Gulf of Mexico, should be conducted to confirm this hypothesis. Alternatively, this difference in associated bacteria might be an artifact of having a single sampling location in the Gulf of Mexico. Because collection location had a significant effect on the bacterial communities associated with lionfish, additional locations throughout the Gulf of Mexico need to be sampled.

Lionfish are successful invaders in the Atlantic Ocean, Caribbean Sea, and Gulf of Mexico. Their ability to feed on dozens of species of prey (Morris & Akins 2009), live in estuarine environments (Jud & Layman 2012), and reproduce prolifically (Morris & Whitfield 2009) are thought to contribute to this success. Additionally, the retention of a diverse bacterial community has been linked to disease resistance via pathogen inhibition in other fishes (Olsson et al. 1992, Sugita et al. 2002, Chabrillón et al. 2005). Here we have shown that invasive lionfish share a similar yet highly diverse external bacterial community with lionfish in their native Indo-Pacific region. Investigations into the function of these core bacteria could further explain the success of the lionfish.

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