American Genetic Association

Journal of Heredity, 2018, 162–175
doi:10.1093/jhered/esx056
Original Article
Advance Access publication 16 June 2017

OXFORD

Original Article

Phylogeography of Lionfishes (*Pterois*) Indicate Taxonomic Over Splitting and Hybrid Origin of the Invasive *Pterois volitans*

Christie L. Wilcox, Hiroyuki Motomura, Mizuki Matsunuma, and Brian W. Bowen

From the Hawai'i Institute of Marine Biology, 46-007 Lilipuna Road, Kane'ohe, HI 96744 (Wilcox and Bowen); Pacific Biosciences Research Center, University of Hawai'i at Mānoa, Honolulu, HI (Wilcox); The Kagoshima University Museum, Kagoshima, Japan (Motomura); and Laboratory of Marine Biology, Faculty of Science, Kochi University, Kochi, Japan (Matsunuma).

Address correspondence to B. W. Bowen at the address above, or e-mail: bbowen@hawaii.edu.

Received January 20, 2017; First decision March 18, 2017; Accepted June 13, 2017.

Corresponding Editor: Jose Lopez

Abstract

The lionfish is an iconic marine fish, and recently renowned for a disastrous introduction into the West Atlantic. Genetic surveys of the putative invaders (*Pterois volitans* and *Pterois miles*) in their natural Indo-Pacific range can illuminate both topics. Previous research indicated that *P. volitans* and *P. miles* are sister species that hybridize in the invasive range, but hybridization in the native range is unknown. Here, we apply mtDNA COI and 2 nuclear introns (S7 RP1 and Gpd2) from 229 lionfish including the 2 invaders and 2 closely-related taxa (44 *P. miles*, 91 *P. volitans*, 31 *Pterois lunulata*, and 63 *Pterois russelii*) from 10 locations in their native ranges. Genetic data are supplemented with key morphological characters: dorsal, anal, and pectoral fin ray counts. We observed 2 lineages (d = 4.07%, 0.89%, and 2.75% at COI, S7 RP1, and Gpd2, respectively) among the 4 putative species: an Indian Ocean lineage represented by *P. miles*, and a Pacific Ocean lineage represented by *P. lunulata* and *P. russelii*. All specimens of the invasive *P. volitans* appear to be hybrids between the Indian Ocean *P. miles* and a Pacific lineage encompassing *P. lunulata*/*russelii*, a conclusion supported by both genetics and morphology. The divergences between Indian and Pacific forms are within the range of species-level partitions in fishes, and we recommend retention of the names *P. miles* and *P. russelii* for Indian and Pacific forms. The hybrid origin of the Atlantic invasion invokes the possibility of heterosis as a contributing factor to invasion success.

Subject areas: Population structure and phylogeography; Conservation genetics and biodiversity
Key words: Atlantic invasion, hybridization, marine speciation, *Pterois lunulata, Pterois russelii, Pterois miles*

The lionfishes (family Scorpaenidae, subfamily Pteroinae) are renowned marine predators, with venomous spines that provide a formidable defense against predation. The Pteroinae currently comprise 27 recognized species in 5 genera: *Pterois* (12 species), *Dendrochirus* (6 species), *Ebosia* (4 species), *Brachypterois* (3 species), and *Parapterois* (2 species) (Kochzius et al. 2003; Allen and Erdmann 2008; Matsunuma and Motomura 2013a; Matsunuma et al. 2013; Matsunuma and Motomura 2015a, 2015b, 2016a, 2016b). Sister species in this group are difficult to distinguish visually and are identified primarily through small variations in meristic

counts (Matsunuma and Motomura 2013b). In most cases, the meristic differences have been corroborated with molecular phylogenetic studies (Kochzius et al. 2003; Freshwater et al. 2009a; Matsunuma and Motomura 2015a, 2015b). In contrast, evidence of hybridization within this group has recently emerged as 2 species, *Pterois miles* and *Pterois volitans* (which were considered synonyms until Schultz 1986 formally split them) are identified in the Atlantic invasion (Morris and Whitfield 2009).

The lionfish have co-evolved in the Indo-Pacific with both predators and prey, so their successful evolutionary innovations are accommodated in reef ecosystems by natural checks and balances. No such balance exists (yet) in the introduced range, and the lionfish invasion in the Atlantic has reached crisis proportions (Schofield 2009; Kulbicki et al. 2012; Rocha et al. 2015; Hixon et al. 2016). Invasive species are considered one of the greatest threats to global biodiversity, because of their potential to directly consume or outcompete native species, alter habitats, and ultimately disrupt ecosystem structure and function (Lovell and Stone 2005; Sutherland et al. 2010). In the western Atlantic Ocean, the explosive population growth of the invasive lionfishes identified as *P. volitans* and *P. miles* has become a region-wide concern (Albins and Hixon 2013). They are now ubiquitous in marine habitats throughout the Caribbean region (Morris 2012), and have recently jumped to Brazil (Ferreira et al. 2015). Effects on native fish communities include a dramatic reduction in native fish recruitment (Albins and Hixon 2008; Albins 2013), steep population declines in prey species (Green et al. 2012; Rocha et al. 2015) and possible phase shifts to algal-dominated reefs (Lesser and Slattery 2011; Albins and Hixon 2013).

The taxonomic distinction of *P. miles* and *P. volitans* is supported by reciprocal monophyly of mtDNA sequences divergent between 4% and 11% depending on the marker (Kochzius et al. 2003; Freshwater et al. 2009a). Mitochondrial barcodes indicate that the invasive lionfishes are predominantly *P. volitans* (>90%), and that there are severe founder effects in both species (Hamner et al. 2007). More specifically, evidence of interbreeding between *P. volitans* and *P. miles* in their invaded range has surfaced, based on ambiguous (or "mixed") meristic characters (Freshwater et al. 2009a). However, confirmation of hybridization has been hindered by a lack of species-specific nuclear markers (Hamner 2005). No investigation of hybridization has occurred in the native ranges of *P. volitans* and *P. miles*, though their ranges overlap at a marine hybridization hotspot near the boundary of the Indian and Pacific Oceans (Hobbs et al. 2009; Gaither and Rocha 2013).

Over 25% of plants and 10% of animal species hybridize naturally (Mallet 2005), but such crosses are believed to be rare in marine fishes (Hubbs 1955), and less than 1% of marine fish species have been reported to hybridize (Montanari et al. 2012). Hybridization is most frequently reported in recently diverged sister species (Mallet 2005; Hobbs and Allen 2014). For reef fishes, in particular, a genetic break greater than 5% divergence in mtDNA cytochrome *b* seems to indicate a higher cost to hybridization, reducing the number of fertile hybrids (Montanari et al. 2014).

Previous investigations of reef fish hybridization have focused on a limited number of families: the surgeonfishes (family Acanthuridae; Marie et al. 2007), butterflyfishes (Chaetodontidae; McMillan et al. 1999; Hobbs et al. 2013), wrasses (Labridae; Yaakub et al. 2006; Yaakub et al. 2007), damselfishes (Pomacentridae; van Herwerden and Doherty 2006; Mullen et al. 2012; Coleman et al. 2014; Gainsford et al. 2015), angelfishes (Pomacanthidae; Pyle and Randall 1994; DiBattista et al. 2012, 2017), and groupers (family Serranidae; van Herwerden et al. 2002, 2006; Frisch and van Herwerden 2006). As a result, hybridization in marine fishes has been underappreciated as an evolutionary phenomenon (Hobbs et al. 2009; Gainsford et al. 2015). Potential hybrids are often identified through color patterns that are intermediate between parental species (e.g., Randall 1956; Pyle & Randall 1994), and subsequently confirmed with genetics (reviewed by Richards and Hobbs 2015). Several instances of hybridization in marine fishes have been accidentally discovered during molecular studies (e.g., McMillan et al. 1999; Kuriiwa et al. 2007; DiBattista et al. 2012), indicating that hybridization often goes undetected, and is therefore underestimated (Mallet 2005), especially in genera with conserved phenotypes or drab coloration (e.g., Henriques et al. 2016). Here, we resolve the phylogeographic structure of the 2 invasive lionfishes, *P. miles* and *P. volitans*, across their native ranges (Supplementary Figure S.1), and test for evidence of hybridization. We include the closely related *Pterois lunulata* and *Pterois russelii*, as these species proved indistinguishable from *P. volitans* across multiple genetic markers. The results prompt a reevaluation of both lionfish taxonomy and the nature of the Atlantic invasion.

Methods

Study Sites and Sampling

A total of 229 lionfish specimens were obtained from 10 locations in their native ranges (Figure 1): 44 *P. miles*, 91 *P. volitans*, 31 *P. lunulata*, and 63 *P. russelii*. Samples were obtained either through collaborations with museums and universities or collected directly using pole spears while SCUBA diving or snorkeling. Species were identified in the field by researchers trained in lionfish morphology, focusing on dorsal, anal, and pectoral fin ray counts and using a photokey designed by the first author, and identified in the laboratories by keys to the lionfish species given in published literature (Poss 1999; Nakabo and Kai 2013) and unpublished data taken by the author M.M. Taxonomic assignments were confirmed with voucher specimens; most specimens have been deposited at the Kagoshima University Museum, Japan. Sample data including collection location/loan information, voucher specimen ID numbers, and morphological information per individual can be found at Dryad doi: 10.5061/dryad.p81m1/2. Tissue samples (fin, muscle, or gill) were stored in ethanol or a saturated salt-DMSO buffer (Amos and Hoelzel 1991). Total genomic DNA was extracted using a hot-shot protocol (Meeker et al. 2007) and frozen in TE buffer stored at –20 °C. In addition, tissue or DNA samples were obtained from 57 lionfishes collected in North Carolina (USA) believed to be near the source of the Atlantic invasion (Freshwater et al. 2009b).

Because no prior studies have amplified nuclear DNA (nDNA) from lionfishes, preliminary PCR amplification and sequencing was performed with 20 primer pairs designed for fish or coelomate introns, using 4 individuals from each species (Hillis and Dixon 1991; Streelman and Karl 1997; Chow and Hazama 1998; Colgan et al. 1998; Hassan et al. 2002; Jarman et al. 2002). Only 2 of the nuclear loci tested—S7 ribosomal protein intron 1 (S7 RP1) and glyceraldehyde-3-phosphate dehydrogenase intron 2 (Gpd2)—amplified and contained polymorphisms, and thus were chosen for subsequent analyses (Supplementary Table S.1). These 2 nuclear loci and a subsection of the mitochondrial cytochrome oxidase gene (COI), using the primers FishF2 and FishR1 from Ward et al. (2005) were amplified in all individuals. Polymerase chain reaction (PCR) mixes were prepared following manufacturer's instructions using MangoMix (Bioline Ltd., London, UK), 0.26 µM concentration of each primer, and 5–50 ng template DNA in 15 µl total volume. Thermal cycling

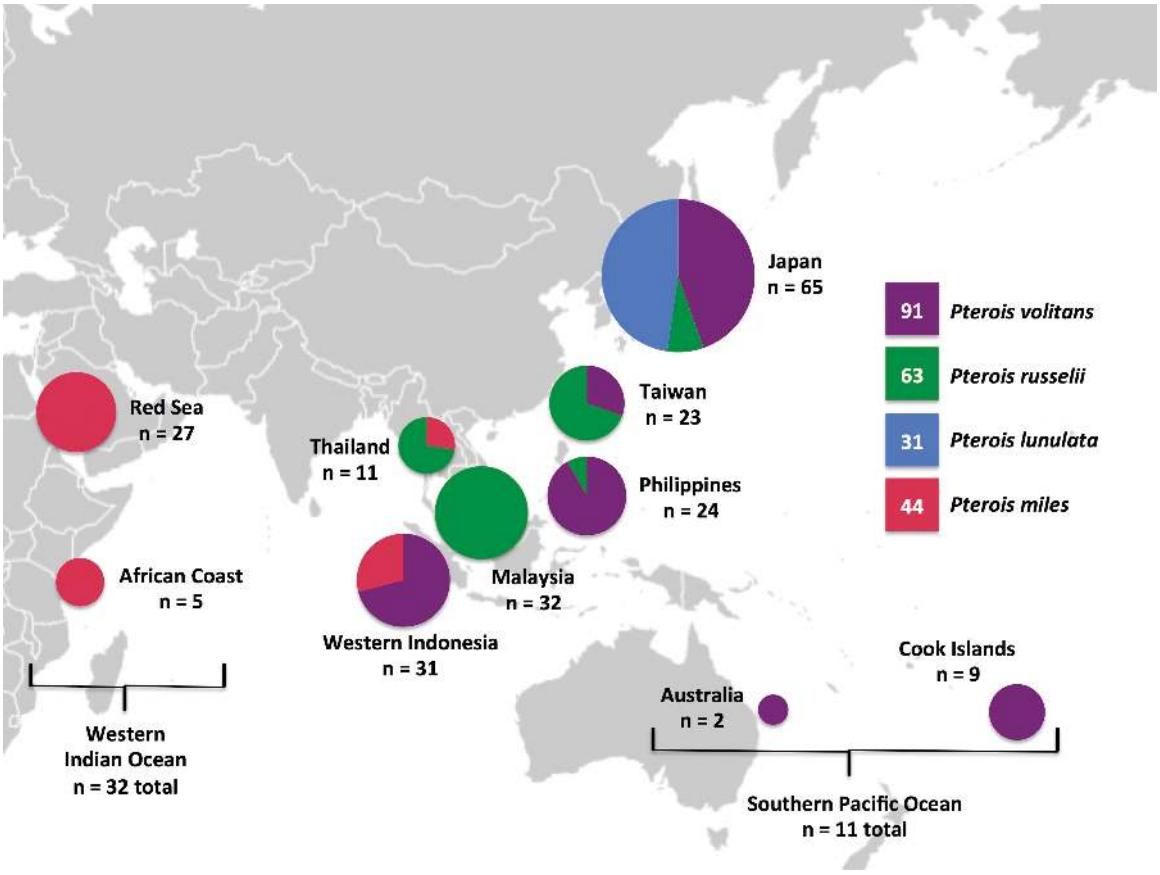

Figure 1. A sampling of *Pterois* throughout the Indo-Pacific. Sample locations, sizes, and proportion of each species sampled at each site. Each circle represents a location, and its size is proportional to the total sample size at that location, while its coloration indicates the proportion of individuals identified based on meristics for each putative species. Because of low numbers, the African Coast (including Kenya and Socotra) was grouped with the Red Sea and East Australia was grouped with the Cook Islands for all population genetic analyses. Sampling was targeted at the putative species, thus the proportion of each species at each site may not represent their true abundance at that site.

conditions consisted of an initial denaturing step at 95 °C for 5 min, then 35 cycles of amplification with 30 s of denaturation at 95 °C, 1 min 30 s of annealing (at the optimum temperature determined for each marker) and 45 s of extension at 72 °C, with a final extension of 30 min at 72 °C. Annealing temperatures were 50° C for COI, 58° C for S7 RP1, and 51° C for Gpd2.

Excess oligonucleotide primers were removed through simultaneous incubation of PCR product with exonuclease I and shrimp alkaline phosphatase (ExoSAP; USB, Cleveland, OH) at 37 °C for 60 min, followed by deactivation at 85 °C for 15 min. All samples were sequenced in the forward direction with fluorescently labeled dye terminators following manufacturer's protocols (BigDye; Applied Biosystems Inc., Foster City, CA, USA) using an ABI 3130XL Genetic Analyzer (Applied Biosystems) at the University of Hawai'i at Mānoa ASGPB Genomics Laboratory. Rare or questionable haplotypes as well as heterozygous individuals were sequenced in both directions. The phase for heterozygotes was resolved by comparison to homozygotes and was unambiguous in all cases. The sequences were aligned, edited, and trimmed to a common length using Geneious Pro version 6.1.8 (Biomatters; available from http://www.geneious.com/). Two observed indels were scored as missing data. Variable sites were visually checked to ensure accuracy, and unique mtDNA COI haplotypes and intron sequences are deposited in GenBank, accession numbers are provided in Data Availability section below.

Genetic Diversity and Partitioning

jModelTest version 1.0.1 (Posada 2008) was used with a corrected Akaike information criterion (AICc) test to determine the best nucleotide substitution model; the models selected were general time reversible (GTR) +I for COI, Hasegawa-Kishino-Yano (HKY) for S7 RP1, and transition model 2 (TIM2) +G for Gpd2. Genetic variation was described as nucleotide diversity (π; equation 10.19 in Nei 1987) and haplotype diversity (h; equation 8.5 in Nei 1987) for each locus at each location using ARLEQUIN version 3.5.1 (Excoffier and Lischer 2010).

Genetic partitioning between species and among populations was assessed with an analysis of molecular variance (AMOVA) as implemented in ARLEQUIN (Excoffier et al. 1992), which generated Φ_{ST} values (a molecular analog of F_{ST} that incorporates sequence divergence among haplotypes). Nonparametric permutation procedures (N = 99 999 iterations) were used to construct null distributions and test the significance of variance components for each hierarchical comparison; significance was tested by permutation and P values adjusted according to the modified false discovery rate method (Narum 2006). For population genetic analyses, the African Coast and the Red Sea were grouped into one population (western Indian Ocean) and eastern Australia and the Cook Islands were grouped into another (southern Pacific Ocean) (Figure 1). Samples with less than 7 individuals were removed from the population-level analyses.

Relationships among haplotypes and species were estimated by constructing unrooted parsimony-based haplotype networks with NETWORK version 4.5.1.0 (available at http://www.fluxus-engineering.com/). Each network was generated using a median joining algorithm with default settings, with indels coded as gaps. These data revealed 2 lineages in the 4 putative species that correspond to Indian and Pacific Oceans, and thus for hybridization analyses, nuclear and mitochondrial haplotypes were identified as either Indian or Pacific.

Hybridization Indices and Hybrid Zone Structure

To visualize the distribution of genotypes (as recommended by Jiggins and Mallet 2000) hybridization indices (*h*-index) were calculated for each individual, putative species, and location. Each allele was scored as a 0 if from the Indian Ocean lineage or as a 1 if from the Pacific Ocean lineage, following the initial approach of Szymura and Barton (1991). Each individual's degree of hybridization was defined as the score across all 3 markers divided by the total allele count (1 allele for the mitochondrial locus and 2 alleles at each nuclear locus); thus an individual with purely Indian Ocean alleles would have a score of 0, while a purely Pacific Ocean individual would have a score of 1. All individuals with scores between 0 and 1 were categorized as hybrids. Mean *h*-indices were calculated for each putative species as well as the total population at each collection location. In addition, alleles for each individual were examined to resolve F1 hybrids versus later hybrids and backcrosses.

Morphological Correlations

Species-distinguishing meristic characters were recorded for 15 specimens of *P. miles*, 84 specimens of *P. volitans*, 32 specimens of *P. lunulata*, and 58 specimens of *P. russelii* from the native range; specifically, dorsal, anal, and pelvic fin ray counts were taken as well as counts of scale rows in longitudinal series and scale rows between the base of the dorsal fin and the lateral line. For invasive range specimens, only dorsal and anal fin ray counts were recorded. Standard descriptive statistics (median and range) for each meristic character were calculated from the raw data. A Kruskal–Wallis test followed by Conover–Iman post hoc test (Conover 1999) corrected for multiple comparisons with the Bonferroni method in XLStat version 2015.5 was used to evaluate differences in fin ray counts between genotypes (Indian, Pacific, or hybrid).

Results

Species Delineation

A total of 472 bp of COI, 611 bp of S7 RP1, and 279 bp of Gpd2 were resolved. Population genetic comparisons (Φ_{ST}) between species show that *P. miles* is strongly differentiated from all other species (Φ_{ST} = 0.849–0.900 in COI), putative *P. volitans* is moderately differentiated from *P. lunulata* and *P. russeli* (Φ_{ST} = 0.367–0.422 in COI), with no significant differentiation between *P. lunulata* and *P. russeli* (Table 1). The nuclear loci S7 RP1, Gpd2 show concordant but somewhat lower differentiation (Table 1). Haplotype networks for all 3 markers reveal that at every locus, the haplotypes are split into 2 lineages; *P. lunulata* and *P. russelii* share haplotypes that are separate from *P. miles* haplotypes, while putative *P. volitans* shares haplotypes with all 3 species (Figure 2). These 2 lineages are geographically partitioned approximately at the boundary of the Indian and Pacific Oceans (Figure 3C), a recognized biogeographic barrier (Briggs and Bowen 2012; Gaither and Rocha 2013). Thus phylogenetic inferences support the genetic (and geographic) partitioning of these 4 putative species into 2 primary lineages, an Indian Ocean cluster (consisting largely of haplotypes from *P. miles* and *P. volitans*), and a Pacific Ocean and eastern Indian Ocean cluster (consisting largely of haplotypes from *P. lunulata*, *P. russelii*, and *P. volitans*). In subsequent passages, we refer to these 2 genetic lineages according to the ocean in which they dominate. The average corrected sequence divergence between the Indian and Pacific lineages was d = 4.07%, 0.89%, and 2.75% at COI, S7 RP1, and Gpd2 respectively, while the divergence within each lineage was much lower. Comparing the AMOVA results from this 2-lineage breakdown revealed that the vast majority of sequence variation is explained by a significant break between these 2 lineages (Φ_{ST} = 0.860, 0.949, and 0.796 for COI, GPD2, and S7, respectively; Table 2). Comparisons of the haplotype and nucleotide diversity between the lineages revealed consistently higher diversity in the Pacific lineage (Table 3), which could be influenced by sampling bias (Figure 1). Migration model testing using a coalescence approach also supported a lack of gene flow between *P. miles* and *P. lunulata/russelii* (with gene flow between both of those and *P. volitans*). The probabilities of the panmictic model and the fully mixed model were negligable when compared with a hybrid scenario model (Supplementary Table S.2). The most probable model estimated that gene flow was orders of magnitude greater from *P. lunulata/russelii* and *P.miles* into *P. volitans* ($Nm_{L/U>V}$ = 705.9, $Nm_{M>V}$ = 2459.1) than from *P. volitans* back into the parent populations ($Nm_{V>L/U}$ = 3.3, $Nm_{V>M}$ = 3.7).

Table 1. Genetic partitioning between putative species

	Pterois miles	*Pterois volitans*	*Pterois lunulata*
Pterois volitans	**0.849**		
	0.139		
	0.142	—	
Pterois lunulata	**0.871**	**0.367**	—
	0.975	**0.618**	
	0.882	**0.640**	
Pterois russelii	**0.900**	**0.422**	0.096
	0.955	**0.687**	0.027
	0.896	**0.688**	0.005

The Φ_{ST} values between species are in the order COI, S7 RP1, Gpd2. All Φ_{ST} values in bold are significant at the level of $P < 0.001$. These data show high and significant structure between *P. miles* and the other species, moderate and significant structure between *P. volitans* and the other species, and no structure between *P. lunulata* and *P. russelii*.

Population Genetic Analyses

Because of the 2 divergent clusters of haplotypes, all population genetic analyses were conducted both on the dataset as a whole and each lineage independently. There was strong population differentiation for all markers across the range when both Pacific and Indian haplotypes were included in the analyses (Φ_{ST} = 0.470–0.547, all significant at $P < 0.001$; Table 2). However, when the 2 lineages were run independently, Φ_{ST} scores were much lower and mostly not significant, indicating that the primary structure is based on the distribution of Indian and Pacific alleles (Table 2). Low but significant population structure was detected in the Pacific lineage with COI and Gpd2, driven by differences between the central Pacific and the northern Pacific, likewise in the Indian lineage with S7, driven by differentiation between the Western Indian Ocean and the Indo-Pacific (Table 2).

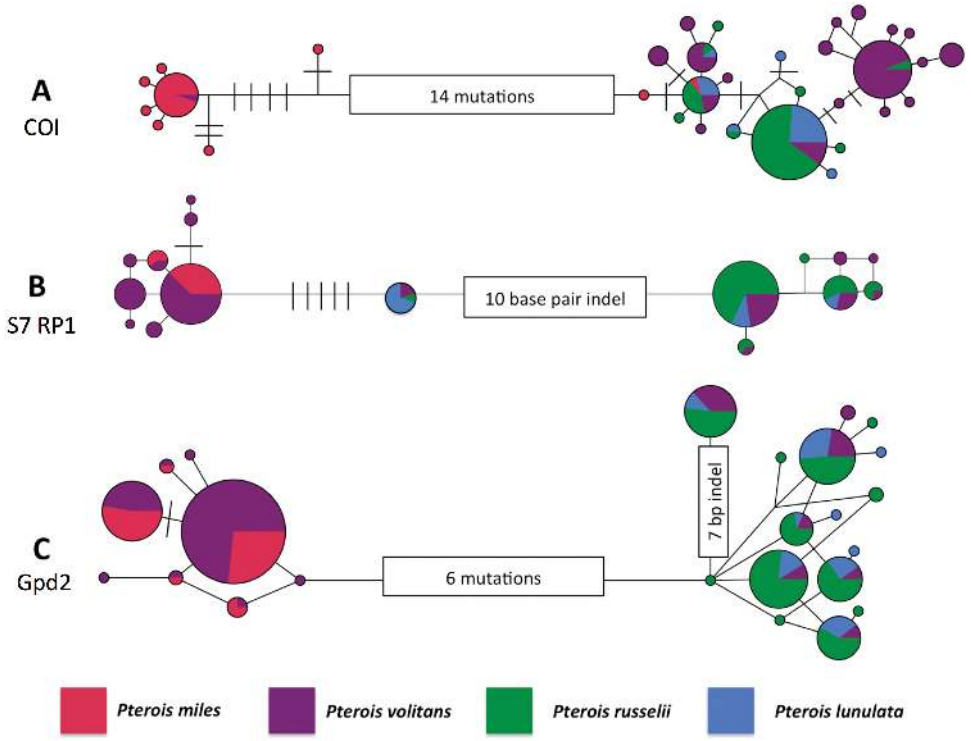

Figure 2. Lionfish haplotype networks by *Pterois*. Median-joining statistical parsimony network based on (**A**) COI, (**B**) S7 RP1, and (**C**) Gpd2 sequence data from *Pterois* across the Indo-Pacific. Each circle represents a haplotype, and its size is proportional to its total frequency. Branches represent a single nucleotide change and crossbars indicate unsampled haplotypes; colors denote species identification as indicated by the embedded key. For all 3 loci, haplotypes split into 2 distinct lineages with corrected sequences divergences of 4.07%, 0.89%, and 2.75%, for COI, S7 RP1, and Gpd2, respectively.

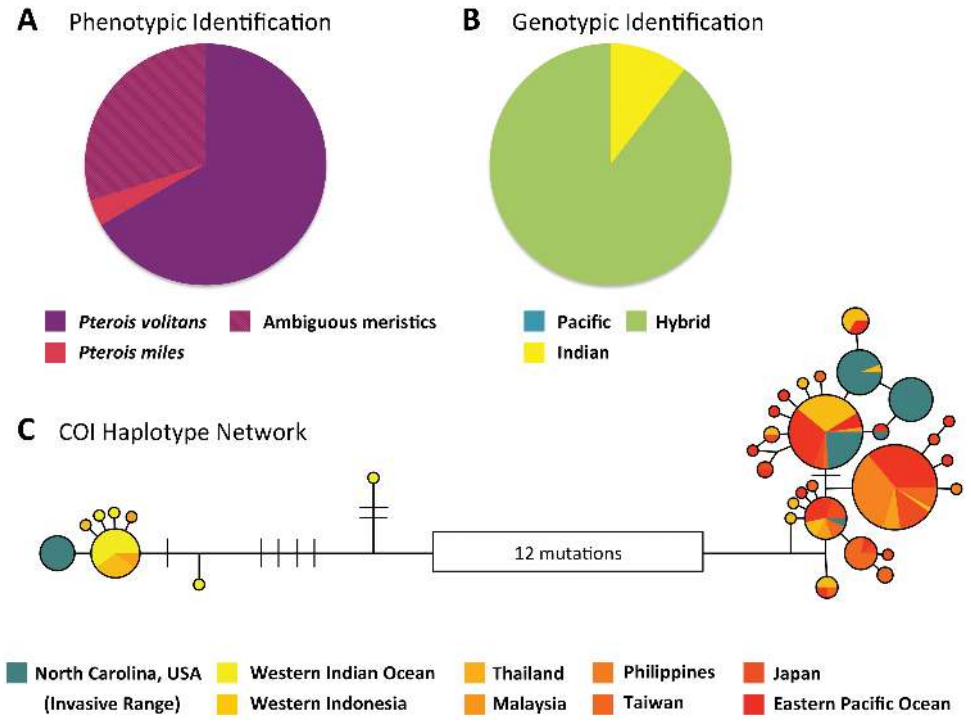

Figure 3. Invasive range lionfish meristics and genetics. (**A**) Phenotypic identification of 57 invasive range lionfishes collected from the coast of North Carolina. (**B**) Genotypic identification of the same lionfishes. (**C**) Median-joining statistical parsimony network based on COI sequence data from *Pterois* across the Indo-Pacific (shades of yellow, orange, and red) as well as invasive samples (blue-green). Each circle represents a haplotype, and its size is proportional to its total frequency. Branches represent a single nucleotide change and crossbars indicate unsampled haplotypes. Multiple haplotypes from the invasive population were not detected across the range of native lionfish sampling locations, suggesting that we did not sample the source population(s) of the invasion. The addition of haplotypes from the native range reduced the primary separation between lineages in the COI network from 14 mutations (in Figure 2) to 12 mutations, due to suspected homoplasy.

Table 2. Global Φ_{ST} values when individuals are grouped by location, species, or lineage

	COI	S7 RP1	GPD2
By location	**0.547**	**0.476**	**0.470**
Indian only	0.134	0.155*	0.008
Pacific only	**0.285**	0.025	0.058*
By putative species	**0.761**	**0.710**	**0.686**
By lineage	**0.860**	**0.949**	**0.796**

Grouping by lineage explains a much greater amount of the genetic variation than the other two. All Φ_{ST} values in bold are significant at the level of $P < 0.001$, all marked with asterisk are only significant to the FDR adjusted level of $P < 0.02$.

Hybridization

Hybridization between putative lionfish taxa was extensive in both prevalence and geographic scope (Supplementary Figure S.2, Figure 4 and Table 4). Not a single F1 hybrid, defined as being heterozygous for Indian and Pacific Ocean alleles across both nuclear markers, was identified in this study, indicating that hybrids are fertile and able to interbreed or backcross to parental lineages.

The 4 putative species had marked differences in the extent and degree of introgression. Less than 10% of individuals identified as *P. miles* or *P. russelii* were scored as hybrids genetically, while 29% of *P. lunulata* and 77% of *P. volitans* individuals were hybrids (Table 4). Around 1% of *P. volitans* individuals contained no Pacific alleles at any locus, appearing identical to *P. miles* across all markers. The mean *h*-indices for each species were consistent with the slight meristic differences that have been used as identifying characteristics for each species (Table 4), with intermediate counts of dorsal and anal fin rays in the species with higher degrees of introgression. Individuals genotypically identified as hybrids had intermediate average dorsal, anal, and pectoral fin ray counts as compared to those with purely Indian or Pacific haplotypes (Table 5). These differences were highly significant (Kruskal–Wallis, $P < 0.0001$), and post hoc multiple comparisons revealed that the hybrid lineage was statistically distinct from both parental lineages for at least one of the 3 characters (Tables 5 and 6).

No hybrids were observed in the western Indian Ocean (*h*-index = 0), but all other locations contained hybrids. The hybridization indices by location ranged from highly introgressed (western Indonesia $N = 31$; *h*-index = 0.22) to predominantly Pacific (Malaysia $N = 32$; *h*-index = 0.99), however, these appeared to be biased by the proportion of each species collected from that location, particularly the proportion of putative *P. volitans*. Introgression reached deep into the Pacific Ocean, with Indian Ocean haplotypes occurring in even the most eastern and northern sampling locations.

Invasive Individuals

Among the 57 specimens from the invasive range, 38 were phenotypically identified as *P. volitans*, 2 were identified as *P. miles*, and 17 had ambiguous meristic characters (Figure 3A). Genotypically, however, 51 possessed hybrid genotypes (Pacific mtDNA, Indian nDNA, *h*-index = 0.20) while 6 had Indian Ocean genotypes (*h*-index = 0) (Figure 3B). None of the invasive range samples possessed wholly Pacific genotypes (*h*-index = 1), and no Pacific nuclear genotypes were detected, indicating that the initial founding population consisted of a mix of Indian and hybrid individuals. In our survey of the native ranges, we feel it is unlikely that we sampled the source population of the Atlantic invasion, as several of the mtDNA haplotypes detected in the Atlantic were not observed in our Indo-Pacific samples (Figure 3C).

Discussion

Decades of debate on whether *P. miles* and *P. volitans* are separate species have been hampered by morphological similarity, incomplete genetic data, and incomplete sampling of *Pterois* lineages. For the recognized lionfishes *P. miles*, *P. volitans*, *P. lunulata*, and *P. russelii*, mitochondrial and nuclear data have revealed no molecular evolutionary divergence or significant population structure between Pacific *P. lunulata* and *P. russelii*, and extensive introgression of the 2 with *P. miles* in the Indian Ocean, which has resulted in hybrid individuals identified as *P. volitans*.

Population Genetic Analyses

Strong population genetic structure was observed across the geographic range of this species complex for all markers (Tables 1 and 2). However, these results are dominated by 2 divergent lineages (corrected divergences $d = 4.07\%$, 0.89%, and 2.75% at COI, S7 RP1, and Gpd2, respectively), corresponding primarily to the Indian and Pacific Ocean (Figures 2 and 3C). Since sampling at each site was nonrandom for these 2 lineages (targeted at putative species rather than a representative geographic sample), the proportion of specimens from each location may not represent their relative abundance. In addition, the pooling of individuals in the western Indian Ocean and the Southern Pacific may have led to underestimated population structure. Thus, the high Φ_{ST} values may overestimate or underestimate the population genetic partitions between locations.

Inferences within each of the 2 primary lineages may more accurately reflect the level of gene flow; these indicate dispersal across large distances in the Indo-Pacific, consistent with previous studies. Kochzius and Blohm (2005) report no population structure for *P. miles* across the length of the Red Sea. The release of *Pterois* eggs within floating mucus balls in addition to the 26-day pelagic larval stage (Fishelson 1975; Imamura and Yabe 1996) likely enhances dispersal capability, a conclusion supported by the rapid spread of the invasive lionfish in the Atlantic (Morris and Whitfield 2009).

Species Delineation

The lack of genetic distinction between Pacific *P. lunulata* and *P. russelii* is not entirely surprising. These 2 putative species have similar morphology (or morphological features) distinguished only by statistical differences in the number of body scales above the lateral line (7–10 vs. 9–12) and the number of scale rows in longitudinal series (60–80 vs. 70–95), white spots on the inner pectoral fin in *P. lunulata*, and statistical differences in pectoral fin length (data not shown). Given the lack of diagnostic differences either genetically or morphologically (Tables 4 and 5), the detection of recent gene flow between the 2 taxa, and the introgression between both and *P. miles*, it is likely that *P. lunulata* and *P. russelii* are members of a single polytypic species. If these species are formally synonymized by future taxonomic investigation, then *P. russelii* (Bennett 1834) would be the senior synonym over *P. lunulata* (Temminck and Schlegel 1843).

Extensive introgression across the Indian-Pacific boundary raises the question of whether the separation between the Indian Ocean *P. miles* and Pacific Ocean *P. lunulata/russelii* warrants species-level distinction. The mtDNA divergences range between ~4% (COI, this study) and ~11% (control region; Kochzius et al. 2003; Kochzius and Blohm 2005), which indicates typical species-level separations

Table 3. Number of haplotypes (mtDNA) or alleles (nDNA), haplotype or allelic diversity, and nucleotide diversity when individuals are grouped by location, species, or lineage

	No. of haplotypes or alleles			Haplotype or allelic diversity (h)			Nucleotide diversity (π)		
	COI	S7	Gpd2	COI	S7	Gpd2	COI	S7	Gpd2
Total	34	53	45	0.83 ± 0.02	0.95 ± 0.01	0.90 ± 0.01	0.02 ± 0.009	0.02 ± 0.009	0.02 ± 0.01
By lineage									
Indian	9	26	15	0.50 ± 0.12	0.87 ± 0.03	0.67 ± 0.03	0.01 ± 0.005	0.0007 ± 0.0007	0.002 ± 0.002
Pacific	25	27	30	0.77 ± 0.03	0.95 ± 0.01	0.95 ± 0.01	0.006 ± 0.004	0.001 ± 0.0009	0.01 ± 0.007
By putative species									
Pterois miles	9	6	8	0.51 ± 0.12	0.51 ± 0.10	0.78 ± 0.03	0.009 ± 0.005	0.0002 ± 0.0003	0.003 ± 0.002
Pterois volitans	19	35	24	0.78 ± 0.05	0.96 ± 0.01	0.72 ± 0.04	0.007 ± 0.004	0.009 ± 0.005	0.01 ± 0.008
Pterois lunulata	6	8	15	0.56 ± 0.11	0.90 ± 0.05	0.91 ± 0.03	0.003 ± 0.002	0.0003 ± 0.0005	0.01 ± 0.006
Pterois russelii	9	24	24	0.44 ± 0.08	0.95 ± 0.01	0.94 ± 0.009	0.003 ± 0.002	0.0006 ± 0.0006	0.01 ± 0.006
By location									
Southern Pacific Ocean	3	4	4	0.71 ± 0.13	0.90 ± 0.16	0.80 ± 0.17	0.003 ± 0.002	0.00 ± 0.00	0.001 ± 0.002
Indonesia	11	14	12	0.78 ± 0.07	0.94 ± 0.02	0.73 ± 0.05	0.02 ± 0.01	0.005 ± 0.003	0.007 ± 0.005
Japan	14	23	20	0.76 ± 0.05	0.97 ± 0.01	0.85 ± 0.03	0.006 ± 0.004	0.02 ± 0.009	0.02 ± 0.011
Malaysia	2	16	17	0.09 ± 0.08	0.95 ± 0.02	0.91 ± 0.02	0.0002 ± 0.0004	0.0007 ± 0.0007	0.01 ± 0.006
Philippines	8	18	14	0.83 ± 0.06	0.97 ± 0.02	0.95 ± 0.02	0.006 ± 0.004	0.01 ± 0.008	0.02 ± 0.010
Taiwan	9	14	14	0.84 ± 0.08	0.95 ± 0.03	0.91 ± 0.03	0.006 ± 0.004	0.02 ± 0.008	0.02 ± 0.01
Thailand	5	5	14	0.82 ± 0.10	0.93 ± 0.12	0.95 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Western Indian Ocean	5	3	5	0.45 ± 0.15	0.31 ± 0.12	0.72 ± 0.03	0.004 ± 0.003	0.00 ± 0.00	0.002 ± 0.002

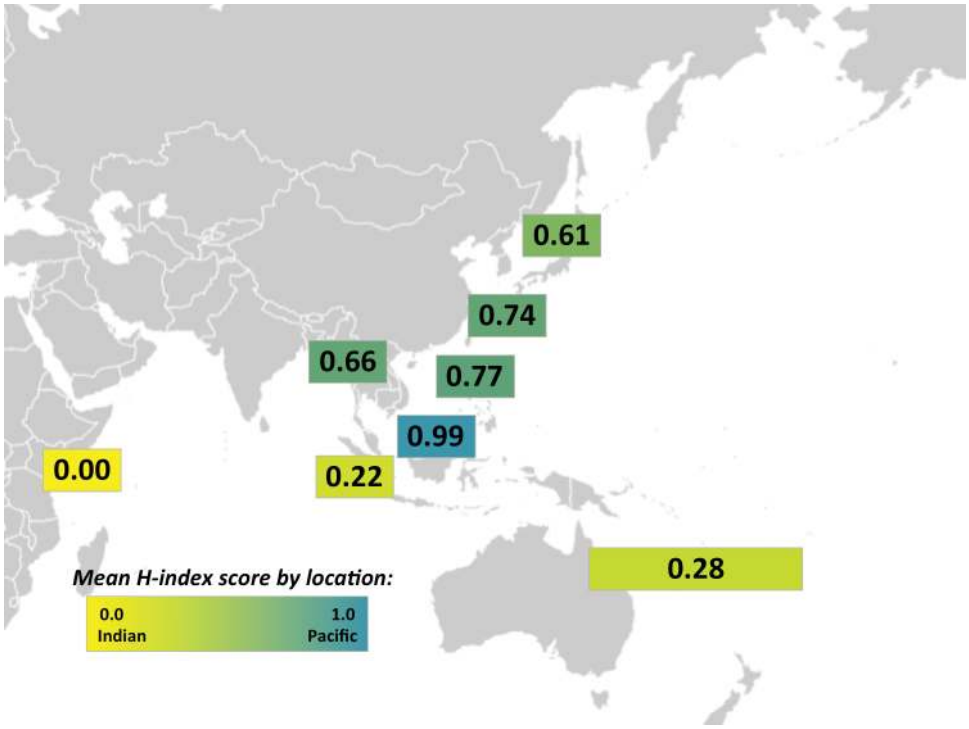

Figure 4. Mean *h*-index score by location. Color denotes the degree of hybridization using the scale bar shown in the figure. Hybridization was detected at all locations outside of the Western Indian Ocean to differing degrees.

Table 4. Comparison of *h*-index, the proportion of pure lineage and hybrid individuals, and meristic characters of the 4 lionfish species

	Pterois miles	*Pterois volitans*	*Pterois lunulata*	*Pterois russelii*
h-index	0.02	0.40	0.92	0.99
Proportion Indian	0.94	0.01	0.00	0.00
Proportion Pacific	0.00	0.22	0.71	0.95
Proportion hybrid	0.06	0.77	0.29	0.05
Dorsal fin rays	9–11[1,2,3]	10–12[1,2,3,6]	10–11[4,5,6]	11–12[3,6]
Anal fin rays	5–6[1,2,3]	5–8[1,2,3,6]	7–8[4,5,6]	7–8[3,6]
Pectoral fin rays	14[2,3]	14[2,3,6]	13–14[4,6]	12–13[3,6]

The known range of meristic counts for each character was taken from multiple sources, as no definitive source exists for all of the 4 species. [1]Schultz (1986), [2]Bennett (1934), [3]Day (1876), [4]Fowler (1903), [5]Günther (1860), and [6]de Beaufort and Briggs (1962).

Table 5. Meristic differences between genetic lineages in this study

	Indian	Hybrid	Pacific
Dorsal fin rays	**10.07** (10–11)[a]	**10.94** (9–11)[b]	**11.02** (10–12)[b]
Anal fin rays	**6.07** (6–7)[a]	**6.99** (6–8)[b]	**7.00** (7)[b]
Pectoral fin rays	**14.00** (14)[a]	**13.77** (13–14)[a]	**12.99** (12–13)[b]

For each genotype, average meristic counts and count range (in parentheses) are given. Kruskal–Wallis tests found highly significant differences between lineages for all 3 meristic characteristics ($P < 0.0001$). Significant pairwise differences between lineages as determined by post hoc Conover—Iman (Bonferroni corrected to $P < 0.0167$) tests as noted using superscript letters a and b; individuals with hybrid genotypes had significantly different dorsal and anal fin ray counts from those with Indian Ocean genotypes and significantly different pectoral fin ray counts from those with Pacific Ocean genotypes.

in marine fishes (Bellwood et al. 2004; Fessler and Westneat 2007; Rocha et al. 2008). Freshwater et al. (2009a) and Kochzius et al. (2003) both concluded that mtDNA divergences supported species level distinction of the Indian Ocean *P. miles* and Pacific Ocean *P. lunulata/russelii*. The COI divergences indicate 2.8–4.2 million years of separation, assuming a molecular clock rate of 1–1.5% per million years as observed in species-pairs calibrated across the Isthmus of Panama (Lessios 2008; Reece et al. 2010). Of course, monophyletic lineages do not invariably indicate distinct species, and can instead result from sampling distant geographic populations of a single species (Bernardi and Crane 1999; McCafferty et al. 2002; Crandall et al. 2008; Kochzius et al. 2009), or from allopatric separation *without* speciation. Outside of the marine realm, there are numerous examples of high mtDNA divergences within species due to long-isolated populations (e.g., Webb et al. 2011; Hogner et al. 2012).

A propensity for ecological specialization by a taxonomic group or species may facilitate speciation during periods of allopatric isolation (Schluter 2009). Conversely, generalists with wide ecological tolerances are less likely to develop adaptations in isolation that prevent remerging upon secondary contact (Webb et al. 2011). Lionfishes are generalist mesopredators able to survive in a

Table 6. Genotypes and fin ray counts

Meristic character	Pacific	Hybrid	Indian
Dorsal fin rays	(87)	(72)	(14)
9	0.00	0.01	0.00
10	0.02	0.03	0.93
11	0.93	0.96	0.07
12	0.05	0.00	0.00
Anal fin rays	(87)	(72)	(14)
6	0.00	0.03	0.93
7	1.00	0.96	0.07
8	0.00	0.01	0.00
Pectoral fin rays	(69)	(44)	(6)
12	0.01	0.00	0.00
13	0.99	0.23	0.00
14	0.00	0.77	1.00

The proportion of individuals in each lineage with the given fin ray counts (*N* in parentheses). Highly significant differences were detected among the treatments for each meristic character (Kruskal–Wallis, $P < 0.0001$). Significant differences as determined by post hoc Conover–Iman comparisons within meristic characters (corrected significance level of $P < 0.0167$, Bonferroni method) are indicated by shading. For dorsal fin rays, post hoc comparisons indicated that the Indian lineage was significantly different from the hybrids and the Pacific lineage ($P < 0.0001$). The difference between the hybrids and the Pacific lineage was not significant ($P = 0.097$). For anal fin rays, the Indian lineage was significantly different from the hybrids and the Pacific lineage ($P < 0.0001$). The difference between the hybrids and the Pacific lineage was not significant ($P = 0.527$). For pectoral fin rays, the Pacific lineage was significantly different from the hybrids and the Indian lineage ($P < 0.0001$) while the difference between the hybrids and the Pacific lineage approached significance ($P = 0.051$).

wide range of salinities and a diversity of habitats (Whitfield et al. 2007; Morris and Whitfield 2009; Albins and Hixon 2013; Côté et al. 2013; Jud et al. 2015). Thus, the range of ecological tolerance in these species may diminish opportunities for allopatric or parapatric speciation.

The geographic distribution of genotypes (Figure 1; Supplementary Figure S.2) is strikingly similar to those summarized by Gaither and Rocha (2013; see also Crandall et al. 2008; Kochzius et al. 2009; Daly-Engel et al. 2012; Bowen et al. 2016)—overlap at the boundary of the Indian and Pacific Oceans with some leakage tilting towards the West Pacific. Gaither et al. (2010) reported Indian Ocean haplotypes of a grouper (*Cephalopholus argus*) at low frequency deep into the Pacific, a pattern also observed in regal angelfishes (*Pygoplites diacanthus*; Coleman et al. 2016) and in this study. Leakage into the Pacific may be facilitated by the eastward-flowing Pacific Equatorial Countercurrent as well as the Kuroshio Current which sweeps into high latitudes east of Japan (Wyrtki and Kendall 1967; Hourigan and Reese 1987). Leakage into the South Pacific may be facilitated by the availability of shallow reef habitat from the Indian/Pacific boundary eastward to the south-central Pacific, with no gap greater than 800 km between shallow-water habitats, a feature notably absent in the Indian Ocean (Schultz et al. 2008).

The geographic pattern documented in this study indicates a period of allopatric separation followed by secondary contact between the Indian and Pacific lineages. In light of these phylogenetic, morphological, and biogeographic considerations, we endorse Freshwater et al. (2009a) and Kochzius et al. (2003) in retaining the names *P. miles* and *P. russelii* for Indian and Pacific forms, albeit with the recognition of introgression across the Indian-Pacific boundary.

Evolutionary Consequences of Hybridization

The role of hybridization in evolutionary diversification is hotly debated, particularly regarding the possibility that intraspecific crosses are an important source of evolutionary novelty (e.g., Seehausen 2004; Gompert et al. 2006; Mallet 2007; Schluter and Conte 2009; Forsman et al. 2017). As noted by Harrison and Larson (2014), introgression allows for new combinations of alleles between somewhat distant parental lineages, with the possibility of generating "transgressive" phenotypes (not seen in either parental species).

At every marker, our *P. volitans* specimens shared haplotypes with at least one of the other species. A majority of the specimens identified as *P. volitans* contained alleles from both the Indian and Pacific Ocean lineages, while 1% had only Indian Ocean alleles (*h*-index = 0). Fewer crosses were discovered with Indian Ocean mitochondrial haplotypes and Pacific nuclear haplotypes. However, this may be a reflection of low sample size for specimens morphologically identified as *P. miles* from the region of overlap, rather than an indication of unidirectional introgression. Among the 12 *P. miles* individuals collected in the Central-Eastern Indian Ocean, 2 were genetically identified as hybrids. Increased sampling of *P. miles* from the Coral Triangle (between Indonesia, Papua New Guinea, and the Philippines) would indicate whether introgression is directional. It is also possible that these data may be explained by ancestral introgression or incomplete lineage sorting rather than current introgression. However, these hypotheses are unlikely given the significant association between introgression and phenotype (Tables 5 and 6), the geographic partitioning of alleles (Supplementary Figure S.2; Toews and Brelsford 2012), and the evidence that introgression is bidirectional (with 17% of *P. miles* from the overlapping region classified as hybrids). We concede that more robust phenotype–genotype associations would be desirable to illustrate this point. A re-evaluation of phenotypic characters and how they correlate to genotypes should be a priority if larger datasets become available.

The extent of introgression detected in this study is rare for marine fishes. No marine fishes with a genetic break of >5% in mtDNA COI are known to hybridize across such a broad geographical area, and such hybrids reported in a more restricted range are usually less fertile than their parents (Montanari et al. 2014). There is no evidence for a cost of hybridization based on the limited inference from phylogeographic data; specifically, the high proportion of hybrids in several locations as well as backcrossed and later generation hybrids indicates that these individuals are successful. It is even possible that these *Pterois* hybrids are more fit than their parental lineages (heterosis; Bartley et al. 2001). The broad distribution of putative *P. volitans* (consisting largely or entirely of hybrids) throughout the Pacific and into the Indian Ocean as far as Sri Lanka (Schultz 1986) supports such a hypothesis, but a robust test would require evaluating the relative fitness of hybrids compared to parental individuals.

There is another intriguing explanation for the pattern of allele distributions in this study: that *P. volitans* is a recent species which arose as the result of hybridization between *P. miles* and *P. russelii*. There are terrestrial examples, like the *Heliconius* butterflies (Pardo-Diaz et al. 2012), where adaptive introgression from one species into another split the latter into 2 distinct species. Such reticulate evolution is not unknown in fish (Seehausen 2004); for example, Dowling and DeMarais (1993) reported "a pervasive influence of hybridization" throughout the evolutionary histories of *Gila* minnows, Stemshorn et al. (2011) observed rapid formation of distinct hybrid lineages upon secondary contact between 2 sculpin (*Cottus*) species, and Cui et al. (2013) showed extensive historical hybridization in

the swordtail fish genus *Xiphophorus*. Notably, these examples are all drawn from freshwater environments, and few marine examples exist. Halldórsdóttir and Árnason (2015) demonstrated a hybrid origin for the (marine) walleye pollock (*Gadus chalcogrammus*) using whole genome sequencing, indicating that this species arose from very recent introgression between Atlantic and Arctic cod. As is the case with hybrids in this study, Halldórsdóttir and Árnason (2015) found that hybrids were intermediate in phenotype between the 2 pure types, a trait which may have allowed them to colonize new habitats and proliferate. Other scholars, though, maintain that hybrid speciation events are rare and unlikely to contribute significantly to biodiversity (Barton 2013; Servedio et al. 2013; Schumer et al. 2014).

New genetic technologies are illuminating the role hybridization plays in the evolution of species, indicating that the model of bifurcating allopatric speciation often does not reflect reality (Feder et al. 2012; DiBattista et al. 2017). The data presented in this study indicate that interspecific introgression may have played a role in the diversification of lionfishes, in addition to known allopatric mechanisms. These data reinforce the emerging theme that hybridization plays a larger role than previously thought in the generation of marine biodiversity (Gardner 1997; Hobbs et al. 2009; Montanari et al. 2012; DiBattista et al. 2015; Gainsford et al. 2015).

Invasive Hybrids

Understanding invasive species in their native ranges is a foundation for addressing the problems caused by invasive populations. Such comparisons can predict the likelihood and extent of invasions (Roman 2006; Gaither et al. 2013a), identify parasites that infect naïve native species (Torchin et al. 2003; Gaither et al. 2013b), and reveal shifts in ecology or behavior that contribute to invasion success (Meyer and Dierking 2011; Gaither et al. 2012).

Perhaps the most relevant conservation finding from this study is that putative *P. volitans*—the predominant invasive lionfish in the Atlantic—is a hybrid between 2 divergent lineages. Of the 57 lionfishes tested from the invasive range, no purely Pacific individuals were detected. While all of our samples were sourced from the same location—North Carolina—range-wide studies indicate that this location is representative of the genetic diversity throughout the range (Betancur et al. 2011; Butterfield et al. 2015). This finding prompts a reconsideration of life history comparisons between native and invasive lionfishes. Studies of purebred lionfish species, such as those made by Darling et al. (2011) of Kenyan *P. miles* or McTee and Grubich (2014) of Red Sea *P. miles*, are certainly informative, but may not be completely applicable to invasive Atlantic hybrids. Natural history studies of the hybrid lionfishes should address differences from parental lineages, including the possibility of heterosis.

Recent studies have found that hybridization can increase invasiveness (Ellstrand and Schierenbeck 2000; Drake 2006; Gaither et al. 2010; Keller and Taylor 2010). Enhanced invasiveness of aquatic hybrids has been particularly well documented for cases where hybridization occurs postintroduction with native counterparts or 2 native but previously allopatric species (Rosenfield et al. 2004; Nolte et al. 2005; Hänfling 2007; Coleman et al. 2014). Further, Halldórsdóttir and Árnason (2015) suggest that the productive and profitable nature of the walleye pollock fishery may be partially due to heterosis. However, the introduction of a hybrid lineage into a novel marine environment is unprecedented to our knowledge.

The hybrid nature of the invasive lionfishes may explain why the severe founder effects identified by Hamner et al. (2007) have no apparent effect on viability, as hybridization can increase genetic diversity in the nuclear genome (Bartley et al. 2001). Furthermore, hybridization in fishes can increase growth rates, environmental and disease tolerances, and overall hardiness (Bartley et al. 2001), thus heterosis may amplify the severity of the lionfish invasion. To evaluate this possibility, identifying the source population(s) for the invasion and studying their biology and ecology should be a priority.

Conclusions

The evolutionary relatedness and population structure of 4 closely related species of *Pterois* were assessed using morphology along with mitochondrial and nuclear DNA sequences. These data demonstrate 2 evolutionary lineages: an Indian Ocean lineage, represented by *P. miles*, and a Pacific Ocean lineage represented by (the dubious *P. lunulata* and) *P. russelii*. Lionfish identified as putative *P. volitans* are hybrids between the sister lineages of *P. miles* and *P. russelii*. The degree and geographic extent of introgression are both vast, indicating 2 species instead of 4, and the possibility of introgressive speciation. The invasive populations of lionfish in the Atlantic Ocean are predominantly or completely composed of these hybrids; further studies of the native hybrid range can enhance the scientific foundations for managing the Atlantic Ocean invasion. These data support the emerging theme that introgressive hybridization may play a larger role than previously suspected in the generation of marine biodiversity. Finally, the competitive advantage conferred to the spinous, venomous lionfish introduced to a naïve environment may be magnified by heterosis, potentially an unprecedented calamity in the annals of invasive species.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

Funding

This research was supported by the National Science Foundation (OCE-1558852 to B.W.B.), a King Abdullah University of Science and Technology (KAUST) Collaborative Research (to M. Berumen), a National Geographic Society (9024-11 to J. DiBattista), and grants from Sigma Xi's Grants-In-Aid of Research, PADI Foundation, and Achievement Rewards for College Scientists Honolulu Chapter (to C.L.W.). This study was supported in part by Japan Society for the Promotion of Science KAKENHI (JP26241027, 24370041, 23580259, and 26450265); the Japan Society for the Promotion of Science Core-to-Core Program, "Research and Education Network on Southeast Asian Coastal Ecosystems"; the "Coastal Area Capability Enhancement in Southeast Asia Project" of the Research Institute for Humanity and Nature, Kyoto, Japan.

Acknowledgments

We thank the Australian Museum, the Biodiversity Research Museum Academia Sinica, and the University of Kansas Biodiversity Research Center for tissue samples used in this study. Philippine samples were collected under a Memorandum of Agreement for joint research made by and among the Department of Agriculture of the Republic of the Philippines (DA), the University of the Philippines Visayas (UPV), the Kagoshima University Museum, the Research Institute for Humanity and Nature, and Tokai University, facilitated by S. L. Sanchez [Bureau of Fisheries and Aquatic Resources (BFAR), DA]. P. J. Alcala (DA) provided a prior informed consent certificate, and I. P. Cabacaba and S. M. S. Nolasco (BFAR, DA) provided a fish specimen export certificate (no. 2016–39812). We thank the staff of the

Office of the Vice-Chancellor for Research and Extension, UPV, and UPV Museum of Natural Sciences, College of Fisheries, UPV for their support of collecting samples in the Philippines. For specimens from the Red Sea, we gratefully acknowledge the logistic support of King Abdullah University of Science and Technology. For support in Socotra, we thank the Environment Protection Authority (EPA), especially Salah Saeed Ahmed, Fouad Naseeb, and Thabet Abdullah Khamis, as well as Ahmed Issa Ali Affrar from Socotra Specialist Tours. For specimen collections, we thank D. Freshwater, J. Morris, R. Coleman, J. Copus, J. DiBattista, D. Pence, R. Whitton, M. Gaither, C. Ka'apu-Lyons, M. Berumen, T. Sinclair-Taylor, L. Rocha, M. Priest, J.-P. Hobbs, P. Ahti, and members of the ToBo Lab. Special thanks to Athline Clark for enduring the painful venom of the lionfish during sampling efforts. We also thank editor Jose Lopez, anonymous reviewers, Giacomo Bernardi, John Horne, and Kira Krend for their thoughtful suggestions which greatly improved this manuscript. This study complied with current laws in the United States and was conducted in accordance with the regulations of the University of Hawai'i Institutional Animal Care and Use Committee (IACUC 09-753-5). This is contribution #1700 from the Hawaii Institute of Marine Biology, and contribution #10077 from the University of Hawaii School of Ocean and Earth Science and Technology.

Data Availability

In accordance with the Journal of Heredity data archiving policy, we have deposited the primary data underlying these analyses as follows:
COI haplotypes: Genbank accessions KT358509–KT358541, S7 haplotypes: Genbank accessions KT358587–KT358639, GPD2 haplotypes: Genbank accessions KT358542–KT358586.
Sample data including collection location/loan information, voucher specimen ID numbers, and morphological information per individual at Dryad doi: 10.5061/dryad.p81m1/2.

References

Albins M. 2013. Effects of invasive Pacific red lionfish *Pterois volitans* versus a native predator on Bahamian coral-reef fish communities. *Biol Invasions*. 15:29–43.

Albins M, Hixon M. 2008. Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Mar Ecol Prog Ser*. 367:233–238.

Albins M, Hixon M. 2013. Worst-case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. *Environ Biol Fish*. 96:1151–1157.

Allen G, Erdmann M. 2008. *Pterois andover*, a new species of scorpionfish (Pisces: Scorpaenidae) from Indonesia and Papua New Guinea. *Aqua*. 13:127–138.

Amos B, Hoelzel A. 1991. Long-term preservation of whale skin for DNA analysis. In: Hoezel AR, editor. *Genetic ecology of whales and dolphins*, International Whaling Commission Special Issue 13. Cambridge: Black Bear Press. p. 99–103.

Bartley D, Rana K, Immink A. 2001. The use of inter-specific hybrids in aquaculture and fisheries. *Rev Fish Biol Fish*. 10:325–337.

Barton NH. 2013. Does hybridization influence speciation? *J Evol Biol*. 26:267–269.

Bellwood DR, van Herwerden L, Konow N. 2004. Evolution and biogeography of marine angelfishes (Pisces: Pomacanthidae). *Mol Phylogenet Evol*. 33:140–155.

Bennett J. 1834. *A selection from the most remarkable and interesting fishes found on the coast of Ceylon*. London: Edward Bull.

Betancur RR, Hines A, Acero PA, Ortí G, Wilbur AE, Freshwater DW. 2011. Reconstructing the lionfish invasion: insights into Greater Caribbean biogeography. *J Biogeogr*. 38:1281–1293.

Bernardi G, Crane N. 1999. Molecular phylogeny of the humbug damselfishes inferred from mtDNA sequences. *J Fish Biol*. 54:1210–1217.

Bowen BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, Toonen RJ, Briggs JC. 2016. Comparative phylogeography of the ocean planet. *Proc Natl Acad Sci USA*. 113:7962–7969.

Briggs J, Bowen B. 2012. A realignment of marine biogeographic provinces with particular reference to fish distributions. *J Biogeogr*. 39:12–30.

Butterfield JSS, Díaz-Ferguson W, Sulliman BR, Saunders JW, Buddo D, Mignucci-Giannoni AA, Searle L, Allen AC, Hunter ME. 2015. Wide-ranging phylogeographic structure of invasive red lionfish in the Western Atlantic and Greater Caribbean. *Mar Biol*. 162:773–781.

Chow S, Hazama K. 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. *Mol Ecol*. 7:1255–1256.

Coleman RR, Eble JA, DiBattista JD, Rocha LA, Randall JE, Berumen ML, Bowen BW. 2016. Regal phylogeography: range-wide survey of the marine angelfish *Pygoplites diacanthus* reveals evolutionary partitions between the Red Sea, Indian Ocean, and Pacific Ocean. *Mol Phylogenet Evol*. 100:243–253.

Coleman RR, Gaither MR, Kimokeo B, Stanton FG, Bowen BW, Toonen RJ. 2014. Large-scale introduction of the Indo-Pacific damselfish *Abudefduf vaigiensis* into Hawai'i promotes genetic swamping of the endemic congener *A. abdominalis*. *Mol Ecol*. 23:5552–5565.

Colgan DJ, McLauchlan GD, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust J Zool*. 46:419–437.

Conover W. 1999. *Practical nonparametric statistics*. 3rd ed. New York (NY): Wiley.

Côté I, Green S, Hixon M. 2013. Predatory fish invaders: insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. *Biol Conserv*. 164:50–61.

Crandall ED, Frey MA, Grosberg RK, Barber PH. 2008. Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. *Mol Ecol*. 17:611–626.

Cui R, Schumer M, Kruesi K, Walter R, Andolfatto P, Rosenthal GG. 2013. Phylogenomics reveals extensive reticulate evolution in *Xiphophorus* fishes. *Evolution*. 67:2166–2179.

Daly-Engel TS, Seraphin KD, Holland KN, Coffey JP, Nance HA, Toonen RJ, Bowen BW. 2012. Global phylogeography with mixed-marker analysis reveals male-mediated dispersal in the endangered scalloped hammerhead shark (*Sphyrna lewini*). *PLoS One*. 7:e29986.

Darling E, Green S, O'Leary J, Côté I. 2011. Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. *Biol Invasions*. 13:2045–2051.

Day F. 1876. *The fishes of India; being a natural history of the fishes known to inhabit the seas and fresh waters of India, Burma, and Ceylon*. London (UK): Bernard Quaritch.

de Beaufort LF, Briggs JC. 1962. *The Fishes of the Indo-Australian Archipelago XI. Scleropparei, Hypostomides, Pediculati, Plectognathi, Opisthomi, Discocephali, Xenopterygii*. Leiden: EJ Brill.

DiBattista JD, Gaither MR, Hobbs JPA, Rocha LA, Bowen BW. 2017. Angelfishes, paper tigers, and the devilish taxonomy of the *Centropyge flavissima* complex. *J Hered*. 107:647–653.

DiBattista JD, Rocha LA, Hobbs J-PA, He S, Priest M, Sinclair-Taylor T, Bowen BW, Berumen ML. 2015. When biogeographic provinces collide: hybridization at the junction of three marine biogeographic provinces in the Arabian Sea. *J Biogeogr*. 42:1601–1614.

DiBattista JD, Waldrop E, Bowen BW, Schultz JK, Gaither MR, Pyle RL, Rocha LA. 2012. Twisted sister species of pygmy angelfishes: discordance between taxonomy, coloration, and phylogenetics. *Coral Reefs*. 31:839–851.

Dowling TE, DeMarais BD. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature*. 362:444–446.

Drake JM. 2006. Heterosis, the catapult effect and establishment success of a colonizing bird. *Biol Lett*. 2:304–307.

Ellstrand N, Schierenbeck K. 2000. Hybridization as a stimulus for the evolution of invasiveness. *Proc Natl Acad Sci USA*. 97:7043–7050.

Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour*. 10:564–567.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*. 131:479–491.

Feder JL, Egan SP, Nosil P. 2012. The genomics of speciation-with-gene-flow. *Trends Genet*. 28:342–350.

Ferreira CE, Luiz OJ, Floeter SR, Lucena MB, Barbosa MC, Rocha CR, Rocha LA. 2015. First record of invasive lionfish (*Pterois volitans*) for the Brazilian coast. *PLoS One*. 10:e0123002.

Fessler JL, Westneat MW. 2007. Molecular phylogenetics of the butterflyfishes (Chaetodontidae): taxonomy and biogeography of a global coral reef fish family. *Mol Phylogenet Evol*. 45:50–68.

Fishelson L. 1975. Ethology and reproduction of pteroid fishes found in the Gulf of Aqaba (Red Sea), especially *Dendrochirus brachypterus* (Cuvier), (Pteroidae, Teleostei). *Pubblicazioni Della Stazione Zoologica di Napoli*. 39:635–656.

Forsman ZH, Knapp ISS, Eaton DAR, Belcaid M, Toonen RJ. 2017. Coral hybridization or polymorphism? Genomic data reveal gene flow between *Porites lobata* and *P. compressa*. *Mol Phylogenet Evol*. 111:132–148.

Fowler H. 1903. Descriptions of several fishes from Zanzibar Island, two of which are new. *Proc Acad Nat Sci Phila*. 55:161–176.

Freshwater D, Hamner R, Parham S, Wilbur A. 2009a. Molecular evidence that the lionfishes *Pterois miles* and *Pterois volitans* are distinct species. *J N C Acad Sci*. 125:39–46.

Freshwater DW, Hines A, Parham S, Wilbur A, Sabaooun M, Woodhead J, Akins L, Purdy B, Whitfield PE, Paris CB. 2009b. Mitochondrial control region sequence analyses indicate dispersal from the US East Coast as the source of the invasive Indo-Pacific lionfish *Pterois volitans* in the Bahamas. *Mar Biol*. 156:1213–1221.

Frisch A, van Herwerden L. 2006. Field and experimental studies of hybridization between coral trouts, *Plectropomus leopardus* and *Plectropomus maculatus* (Serranidae), on the Great Barrier Reef, Australia. *J Fish Biol*. 68:1013–1025.

Gainsford A, van Herwerden L, Jones GP. 2015. Hierarchical behaviour, habitat use and species size differences shape evolutionary outcomes of hybridization in a coral reef fish. *J Evol Biol*. 28:205–222.

Gaither MR, Aeby G, Vignon M, Meguro Y, Rigby M, Runyon C, Toonen RJ, Wood CL, Bowen BW. 2013b. An invasive fish and the time-lagged spread of its parasite across the Hawaiian archipelago. *PLoS One*. 8:e56940.

Gaither M, Bowen B, Toonen R. 2013a. Population structure in the native range predicts the spread of introduced marine species. *Proc Biol Sci*. 280:20130409.

Gaither MR, Bowen BW, Toonen RJ, Planes S, Messmer V, Earle J, Ross Robertson D. 2010. Genetic consequences of introducing allopatric lineages of Bluestriped Snapper (*Lutjanus kasmira*) to Hawaii. *Mol Ecol*. 19:1107–1121.

Gaither M, Rocha L. 2013. Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: evidence for the center of overlap hypothesis. *J Biogeogr*. 40:1638–1648.

Gaither M, Toonen R, Bowen B. 2012. Coming out of the starting blocks: the importance of lag period and stochasticity in shaping patterns of genetic diversity in marine invaders. *Proc Biol Sci*. 279:3948–3957.

Gardner JPA. 1997. Hybridization in the sea. In: Blaxter J, Southward A, editors. *Advances in marine biology*. New York: Academic Press. p. 1–78.

Gompert Z, Fordyce JA, Forister ML, Shapiro AM, Nice CC. 2006. Homoploid hybrid speciation in an extreme habitat. *Science*. 314:1923–1925.

Green SJ, Akins JL, Maljković A, Côté IM. 2012. Invasive lionfish drive Atlantic coral reef fish declines. *PLoS One*. 7:e32596.

Günther A. 1860. *Catalogue of the acanthopterygian fishes in the collection of the British Museum*. 2nd ed. London (UK): Taylor and Francis.

Halldórsdóttir K, Árnason E. 2015. Whole-genome sequencing uncovers cryptic and hybrid species among Atlantic and Pacific cod-fish. *bioRxiv*:034926. doi: 10.1101/034926.

Hamner R. 2005. *Genetic analyses of lionfish: venomous marine predators invasive to the western Atlantic* [Honors thesis]. Wilmington (NC): University of North Carolina.

Hamner R, Freshwater D, Whitfield P. 2007. Mitochondrial cytochrome *b* analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. *J Fish Biol*. 71:214–222.

Hänfling B. 2007. Understanding the establishment success of non-indigenous fishes: lessons from population genetics. *J Fish Biol*. 71:115–135.

Harrison RG, Larson EL. 2014. Hybridization, introgression, and the nature of species boundaries. *J Hered*. 105(Suppl 1):795–809.

Hassan M, Lemaire C, Fauvelot C, Bonhomme F. 2002. Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish. *Mol Ecol Notes*. 2:334–340.

Henriques R, von der Heyden S, Matthee CA. 2016. When homoplasy mimics hybridization: a case study of Cape hakes (*Merluccius capensis* and *M. paradoxus*). *PeerJ*. 4:e1827.

Hillis DM, Dixon MT. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Q Rev Biol*. 66:411–453.

Hixon MA, Green SJ, Albins MA, Akins JL, Morris JA Jr. 2016. Lionfish: a major marine invasion. *Mar Ecol Prog Ser*. 558:162–265.

Hobbs JP, Frisch AJ, Allen GR, Van Herwerden L. 2009. Marine hybrid hotspot at Indo-Pacific biogeographic border. *Biol Lett*. 5:258–261.

Hobbs JPA, Allen GR. 2014. Hybridization among coral reef fishes at Christmas Island and the Cocos (Keeling) Islands. *Raffles Bull Zool Suppl*. 30:220–226.

Hobbs JPA, van Herwerden L, Pratchett MS, Allen GR. 2013. Hybridization among coral reef butterflyfishes. In: Pratchett MS, Berumen ML, Kapoor BG, editors. *Biology of butterflyfish*. Enfield (NH): Science Publishers, Inc.

Hogner S, Laskemoen T, Lifjeld JT, Porkert J, Kleven O, Albayrak T, Kabasakal B, Johnsen A. 2012. Deep sympatric mitochondrial divergence without reproductive isolation in the common redstart *Phoenicurus phoenicurus*. *Ecol Evol*. 2:2974–2988.

Hourigan TF, Reese ES. 1987. Mid-ocean isolation and the evolution of Hawaiian reef fishes. *Trends Ecol Evol*. 2:187–191.

Hubbs CL. 1955. Hybridization between fish species in nature. *Syst Zool*. 4:1–20.

Imamura H, Yabe M. 1996. Larval record of a red firefish, *Pterois volitans*, from northwestern Australia (Pisces: Scorpaeniformes). *Bull Fac Fish Hokkaido Univ*. 47:41–46.

Jarman SN, Ward RD, Elliott NG. 2002. Oligonucleotide primers for PCR amplification of coelomate introns. *Mar Biotechnol (NY)*. 4:347–355.

Jiggins CD, Mallet J. 2000. Bimodal hybrid zones and speciation. *Trends Ecol Evol*. 15:250–255.

Jud ZR, Nichols PK, Layman CA. 2015 Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization. *Environ Biol Fish*. 98:135–143.

Keller SR, Taylor DR. 2010. Genomic admixture increases fitness during a biological invasion. *J Evol Biol*. 23:1720–1731.

Kochzius M, Blohm D. 2005. Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea. *Gene*. 347:295–301.

Kochzius M, Seidel C, Hauschild J, Kirchhoff S, Mester P, Meyer-Wachsmuth I, Nuryanto A, Timm J. 2009. Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*. *Mar Ecol Prog Ser*. 396:211–219.

Kochzius M, Söller R, Khalaf MA, Blohm D. 2003. Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Mol Phylogenet Evol*. 28:396–403.

Kulbicki M, Beets J, Chabanet P, Cure K, Darling E, Floeter SR, Galzin R, Green A, Harmelin-Vivien M, Hixon M, *et al*. 2012. Distributions of Indo-Pacific lionfishes *Pterois* spp. in their native ranges: implications for the Atlantic invasion. *Mar Ecol Prog Ser*. 446:189–205.

Kuriiwa K, Hanzawa N, Yoshino T, Kimura S, Nishida M. 2007. Phylogenetic relationships and natural hybridization in rabbitfishes (Teleostei: Siganidae) inferred from mitochondrial and nuclear DNA analyses. *Mol Phylogenet Evol*. 45:69–80.

Lesser M, Slattery M. 2011. Phase shift to algal dominated communities at mesophotic depths associated with lionfish (*Pterois volitans*) invasion on a Bahamian coral reef. *Biol Invasions*. 13:1855–1868.

Lessios H. 2008. The great American schism: divergence of marine organisms after the rise of the Central American Isthmus. *Annu Rev Ecol Evol Syst*. 39:63–91.

Lovell S, Stone S. 2005. *The economic impacts of aquatic invasive species: a review of the literature*. US Environmental Protection Agency. Working Paper #05-02.

Mallet J. 2005. Hybridization as an invasion of the genome. *Trends Ecol Evol*. 20:229–237.

Mallet J. 2007. Hybrid speciation. *Nature*. 446:279–283.

Marie A, van Herwerden L, Choat J, Hobbs J. 2007. Hybridization of reef fishes at the Indo-Pacific biogeographic barrier: a case study. *Coral Reefs*. 26:841–850.

Matsunuma M, Motomura H. 2013a. A new lionfish of the genus *Dendochirus* (Scorpaenidae: Pteroinae) from the Tuamotu Archipelago, South Pacific Ocean. *Species Divers*. 18:1–7.

Matsunuma M, Motomura H. 2013b. Newly recognized diagnostic characters of the poorly known lionfish *Pterois brevipectoralis* (Scorpaenidae: Pteroinae), with notes on fresh coloration. *Species Divers*. 18:163–173.

Matsunuma M, Motomura H. 2015a. A new species of scorpionfish, *Ebosia saya* (Scorpaenidae: Pteroinae) from the western Indian Ocean and notes on fresh coloration of *Ebosia falcata*. *Ichthyol Res*. 62:293–312.

Matsunuma M, Motomura H. 2015b. *Pterois paucispinula*, a new species of lionfish (Scorpaenidae: Pteroinae) from the western Pacific Ocean. *Ichthyol Res*. 62:327–346.

Matsunuma M, Motomura H. 2016a. A new species of scorpionfish, *Ebosia vespertina* (Scorpaenidae: Pteroinae), from the southwestern Indian Ocean. *Ichthyol Res*. 63:110–120.

Matsunuma M, Motomura H. 2016b. Redescriptions of *Pterois radiata* and *Pterois cincta* (Scorpaenidae: Pteroinae) with notes on geographic morphological variations in *P. radiata*. *Ichthyol Res*. 63:145–172.

Matsunuma M, Sakurai M, Motomura H. 2013. Revision of the Indo-West Pacific genus *Brachypterois* (Scorpaenidae: Pteroinae), with description of a new species from northeastern Australia. *Zootaxa*. 3693:401–440.

McCafferty S, Bermingham E, Quenouille B, Planes S, Hoelzer G, Asoh K. 2002. Historical biogeography and molecular systematics of the Indo-Pacific genus *Dascyllus* (Teleostei: Pomacentridae). *Mol Ecol*. 11:1377–1392.

McMillan WO, Weigt LA, Palumbi SR. 1999. Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution*. 53:247–260.

McTee SA, Grubich JR. 2014. Native densities, distribution, and diurnal activity of Red Sea lionfishes (Scorpaenidae). *Mar Ecol Prog Ser*. 508:223–232.

Meeker ND, Hutchinson SA, Ho L, Trede NS. 2007. Method for isolation of PCR-ready genomic DNA from zebrafish tissues. *Biotechniques*. 43:610.

Meyer AL, Dierking J. 2011. Elevated size and body condition and altered feeding ecology of the grouper *Cephalopholis argus* in non-native habitats. *Mar Ecol Prog Ser*. 439:203–212.

Montanari SR, Hobbs JP, Pratchett MS, Bay LK, Van Herwerden L. 2014. Does genetic distance between parental species influence outcomes of hybridization among coral reef butterflyfishes? *Mol Ecol*. 23:2757–2770.

Montanari SR, van Herwerden L, Pratchett MS, Hobbs JP, Fugedi A. 2012. Reef fish hybridization: lessons learnt from butterflyfishes (genus Chaetodon). *Ecol Evol*. 2:310–328.

Morris JA. 2012. *Invasive lionfish: a guide to control and management*. Marathon (FL): Gulf and Caribbean Fisheries Institute Special Publication Series.

Morris J, Whitfield P. 2009. *Biology, ecology, control and management of the invasive Indo-Pacific lionfish: an updated integrated assessment*. NOAA Technical Memorandum NOS NCCOS 99.

Mullen SP, Little K, Draud M, Brozek J, Itzkowitz M. 2012. Hybridization among Caribbean damselfish species correlates with habitat degradation. *J Exp Mar Biol Ecol*. 416:221–229.

Nakabo T, Kai Y. 2013. Scorpaenidae. Scorpionfishes. In: Nakabo T, editor. *Fishes of Japan with pictorial keys to the species*. 3rd ed. Hadano: Tokai University Press.

Narum S. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conserv Genet*. 7:783–787.

Nei M. 1987. *Molecular evolutionary genetics*. New York (NY): Columbia University Press.

Nolte A, Freyhof J, Stemshorn K, Tautz D. 2005. An invasive lineage of sculpins, *Cottus* sp. (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. *Proc Biol Sci*. 272:2379–2387.

Pardo-Diaz C, Salazar C, Baxter SW, Merot C, Figueiredo-Ready W, Joron M, McMillan WO, Jiggins CD. 2012. Adaptive introgression across species boundaries in *Heliconius* butterflies. *PLoS Genet*. 8:e1002752.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol*. 25:1253–1256.

Poss SG. 1999. Scorpaenidae. Scorpionfishes (also, lionfishes, rockfishes, stingfishes, stonefishes, and waspfishes). In: Carpenter KE, Niem VH, editors. *FAO species identification guide for fisheries purposes. The living marine resources of the western central Pacific. Vol. 4. Bony fishes part 2 (Mugilidae to Carangidae)*. Rome: FAO.

Pyle R, Randall J. 1994. A review of hybridization in marine angelfishes (Perciformes: Pomacanthidae). *Environ Biol Fishes*. 41:127–145.

Randall J. 1956. *Acanthurus rackliffei*, a possible hybrid surgeon fish (*A. achilles* × *A. glaucopareius*) from the Phoenix Islands. *Copeia*. 1956:21–25.

Reece JS, Bowen BW, Smith DG, Larson A. 2010. Molecular phylogenetics of moray eels (Muraenidae) demonstrates multiple origins of a shell-crushing jaw (*Gymnomuraena, Echidna*) and multiple colonizations of the Atlantic Ocean. *Mol Phylogenet Evol*. 57:829–835.

Richards ZT, Hobbs J-P. 2015. Hybridisation on coral reefs and the conservation of evolutionary novelty. *Curr Zool*. 61:132–145.

Rocha LA, Lindeman KC, Rocha CR, Lessios HA. 2008. Historical biogeography and speciation in the reef fish genus *Haemulon* (Teleostei: Haemulidae). *Mol Phylogenet Evol*. 48:918–928.

Rocha LA, Rocha CR, Baldwin CC, Weigt LA, McField M. 2015. Invasive lionfish preying on critically endangered reef fish. *Coral Reefs*. 34:803–806.

Roman J. 2006. Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proc Biol Sci*. 273:2453–2459.

Rosenfield J, Nolasco S, Lindauer S, Sandoval C, Kodric-Brown A. 2004. The role of hybrid vigor in the replacement of *Pecos* pupfish by its hybrids with sheepshead minnow. *Conserv Biol*. 18:1589–1598.

Schluter D. 2009. Evidence for ecological speciation and its alternative. *Science*. 323:737–741.

Schluter D, Conte GL. 2009. Genetics and ecological speciation. *Proc Natl Acad Sci USA*. 106:9955–9962.

Schofield PJ. 2009. Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) in the Western North Atlantic and Caribbean Sea. *Aquat Invasions*. 4:473–479.

Schultz E. 1986. *Pterois volitans* and *Pterois miles*: two valid species. *Copeia*. 1986:686–690.

Schultz JK, Feldheim KA, Gruber SH, Ashley MV, McGovern TM, Bowen BW. 2008. Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Mol Ecol*. 17:5336–5348.

Schumer M, Rosenthal GG, Andolfatto P. 2014. How common is homoploid hybrid speciation? *Evolution*. 68:1553–1560.

Seehausen O. 2004. Hybridization and adaptive radiation. *Trends Ecol Evol*. 19:198–207.

Servedio MR, Hermisson J, van Doorn GS. 2013. Hybridization may rarely promote speciation. *J Evol Biol*. 26:282–285.

Stemshorn KC, Reed FA, Nolte AW, Tautz D. 2011. Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). *Mol Ecol*. 20:1475–1491.

Streelman J, Karl S. 1997. Reconstructing labroid evolution with single-copy nuclear DNA. *Proc Biol Sci*. 264:1011–1020.

Sutherland WJ, Clout M, Côté IM, Daszak P, Depledge MH, Fellman L, Fleishman E, Garthwaite R, Gibbons DW, De Lurio J, *et al*. 2010. A horizon scan of global conservation issues for 2010. *Trends Ecol Evol*. 25:1–7.

Szymura JM, Barton NH. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. *Evolution*. 45:237–261.

Temminck CJ, Schlegel H. 1843. Pisces. Fauna Japonica, sive descriptio animalium quae initinere per Japoniam suscept annis 1823–1830 collegit, notis observationibus et adumbrationibus illustravit P.F. de Siebold. *Pisces, Fauna Japonica*. 1:1–20.

Toews DP, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Mol Ecol*. 21:3907–3930.

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM. 2003. Introduced species and their missing parasites. *Nature*. 421:628–630.

van Herwerden L, Choat JH, Dudgeon CL, Carlos G, Newman SJ, Frisch A, van Oppen M. 2006. Contrasting patterns of genetic structure in two species of the coral trout *Plectropomus* (Serranidae) from east and west Australia: introgressive hybridisation or ancestral polymorphisms. *Mol Phylogenet Evol*. 41:420–435.

van Herwerden L, Davies C, Choat J. 2002. Phylogenetic and evolutionary perspectives of the Indo-Pacific grouper *Plectropomus* species on the Great Barrier Reef. *J Fish Biol*. 60:1591–1596.

van Herwerden L, Doherty PJ. 2006. Contrasting genetic structures across two hybrid zones of a tropical reef fish, *Acanthochromis polyacanthus* (Bleeker 1855). *J Evol Biol*. 19:239–252.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD. 2005. DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci*. 360:1847–1857.

Webb WC, Marzluff JM, Omland KE. 2011. Random interbreeding between cryptic lineages of the Common Raven: evidence for speciation in reverse. *Mol Ecol*. 20:2390–2402.

Whitfield PE, Hare JA, David AW, Harter SL, Munoz RC, Addison CM. 2007. Abundance estimates of the Indo-Pacific lionfish *Pterois volitans/miles* complex in the Western North Atlantic. *Biol Invasions*. 9:53–64.

Wyrtki K, Kendall R. 1967. Transport of the Pacific equatorial countercurrent. *J Geophys Res*. 72:2073–2076.

Yaakub SM, Bellwood DR, Herwerden LV, Walsh FM. 2006. Hybridization in coral reef fishes: introgression and bi-directional gene exchange in *Thalassoma* (family Labridae). *Mol Phylogenet Evol*. 40:84–100.

Yaakub SM, Bellwood DR, van Herwerden L. 2007. A rare hybridization event in two common Caribbean wrasses (genus *Halichoeres*; family Labridae). *Coral Reefs*. 26:597–602.