ORIGINAL ARTICLE

Yellow tails in the Red Sea: phylogeography of the Indo-Pacific goatfish *Mulloidichthys flavolineatus* reveals isolation in peripheral provinces and cryptic evolutionary lineages

Iria Fernandez-Silva[1,2,3]*, John E. Randall[4], Richard R. Coleman[1], Joseph D. DiBattista[5,6], Luiz A. Rocha[2], James D. Reimer[7], Carl G. Meyer[1] and Brian W. Bowen[1]

[1]*Hawai'i Institute of Marine Biology, University of Hawai'i, Kāne'ohe, HI 96744, USA,* [2]*Section of Ichthyology, California Academy of Sciences, San Francisco, CA 94118, USA,* [3]*Department of Biochemistry, Genetics and Immunology, University of Vigo, Vigo, Spain,* [4]*Bishop Museum, Honolulu, HI 96817, USA,* [5]*Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal 23955, Saudi Arabia,* [6]*Department of Environment and Agriculture, Curtin University, PO Box U1987, Perth, WA 6845, Australia,* [7]*Molecular Invertebrate Systematics and Ecology Laboratory, Graduate School of Engineering and Science, University of the Ryukyus, Okinawa 903-0213, Japan*

ABSTRACT

Aim Broadly distributed reef fishes tend to have high gene flow mediated by a pelagic larval phase. Here, we survey a reef-associated fish distributed across half the tropical oceans, from the Red Sea to the central Pacific. Our goal is to determine whether genetic structure of the broadly distributed Yellowstripe Goatfish (*Mulloidichthys flavolineatus*) is defined by biogeographical barriers, or facilitated via larval dispersal.

Location Red Sea, Indian Ocean, Pacific Ocean

Methods Specimens were obtained at 19 locations from the Red Sea to Hawai'i. Genetic data include mtDNA cytochrome *b* ($n = 217$) and 12 microsatellite loci ($n = 185$). Analysis of molecular variance (AMOVA), STRUC-TURE, a parsimony network and coalescence analyses were used to resolve recent population history and connectivity.

Results Population structure was significant (mtDNA $\phi_{ST} = 0.68$, $P < 0.001$; microsatellite $F_{ST} = 0.08$, $P < 0.001$), but mostly driven by samples from the North-western (NW) Indian Ocean (including the Red Sea) and Hawai'i. There was little population structure across the Indian Ocean to the central Pacific. Hawai'i was distinguished as an isolated population (mtDNA $\phi_{ST} = 0.03$–0.08, $P = $ n.s.; microsatellites $F_{ST} = 0.05$–0.10, $P < 0.001$). Specimens from the NW Indian Ocean clustered as a distinct phylogenetic lineage that diverged approximately 493 ka ($d = 1.7\%$), which indicates that these fish persisted in isolation through several Pleistocene glacial cycles.

Main conclusions These data reinforce the emerging themes that: (1) phylogeographical breaks within species often coincide with biogeographical breaks based on species distributions, and (2) populations on the periphery of the range (NW Indian Ocean and Hawai'i) are isolated and may be evolutionary incubators producing new species.

Keywords
cytochrome *b*, fish, glacial refugia, Hawai'i, marine biogeography, microsatellites, mtDNA, population structure, Red Sea, vicariance

*Correspondence: Iria Fernandez-Silva, Section of Ichthyology, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA.
E-mail: iriafernandezsilva@gmail.com*

INTRODUCTION

Broadly distributed reef fishes tend to show high connectivity and gene flow mediated by a pelagic larval phase that can last from weeks to months. However, geographical and oceano-graphical features, as well as ecological gradients, can act as barriers to gene flow, and as such they often set the boundaries of biogeographical provinces. Their persistence through tectonic movements and glacial cycles has an enormous influence on the evolution of marine life (Rocha *et al.*, 2007).

The Indo-Pacific includes the largest continuous biogeographical province on Earth. Here, the tropical marine habitats are characterized by many wide-ranging species, and it is estimated that more than 1500 fish species have distributional ranges extending more than 10,000,000 km^2 (Allen, 2008; Briggs & Bowen, 2012). Over such a vast geographical scale, knowledge of the spatial distribution of genetic variation is particularly useful to place the bounds over which populations are likely to be demographically linked (Lowe & Allendorf, 2010) or evolutionarily independent (Rocha *et al.*, 2007). For example, genetic studies have revealed cryptic lineages within a single nominal species that led to subsequent recognition of sister taxa (DiBattista *et al.*, 2011). In other cases, species recognized on the basis of distinct morphology or coloration show no genetic differences (DiBattista *et al.*, 2012). The proper alignment of species boundaries is fundamental for effective management of marine resources, as well as to identify areas of elevated endemism and biodiversity hotspots.

Here, we contribute a phylogeographical study of a tropical shore fish, the Yellowstripe Goatfish, *Mulloidichthys flavolineatus* (Lacepède, 1801) (Mullidae). This species is distributed from the Red Sea and East Africa to the Hawaiian, Marquesan and Pitcairn Islands, north to the Ryukyus and Bonin Islands and south to Montague Island in New South Wales (Randall, 2002). Where present, *M. flavolineatus* tends to be common and frequently observed in sandy areas around coral reefs (1–76 m; Randall, 2002). They are valued food and an important catch for many coastal communities. Despite this economical and ecological importance, a comprehensive assessment of intraspecific variability and dispersal in *M. flavolineatus* has not been conducted.

We obtained samples from almost the entire distribution of *M. flavolineatus* and generated data from mitochondrial DNA (mtDNA) sequences and nuclear microsatellites to investigate phylogeographical patterns. In doing so, we discovered the presence of a cryptic lineage endemic to the Red Sea and Arabian Sea. The geographical distribution of this cryptic lineage follows the biogeographical delineation of the North-western (NW) Indian Ocean province (Kulbicki *et al.*, 2013; but see Briggs & Bowen, 2012). We also demonstrate genetic isolation of the Hawaiian population. With these results, we discuss the evolutionary mechanisms that promote elevated endemism in the NW Indian Ocean and Hawai'i, and the role of peripheral provinces as sources of evolutionary innovation.

MATERIALS AND METHODS

Collections and molecular analyses

Genomic DNA was extracted following Meeker *et al.* (2007) from *M. flavolineatus* fin clips collected at 19 locations in the Red Sea, Arabian Sea, Indian Ocean and Pacific Ocean (Fig. 1a,b, Table 1). A fragment of the mtDNA cytochrome *b* gene (cyt *b*) (Meyer, 1993) was sequenced as described in

Appendix S1 in Supporting Information. We aligned, edited and trimmed the sequences to a common length using GEN-EIOUS PRO 6.0 (Biomatters, Ltd, Auckland, NZ). All haplotypes were identified and deposited in GenBank (accession numbers: KT210899–KT210948) and Dryad (http://www.datadryad.org/).

For locations with $n > 5$ we applied 12 previously described microsatellite loci (Fernandez-Silva *et al.*, 2013) as described in Appendix S1. Briefly, microsatellite loci were amplified by polymerase chain reaction following the M13-tailed primer method (Fernandez-Silva *et al.*, 2010), and resolved using an ABI 3130XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). Due to logistic constraints, we were unable to genotype the samples from the Gulf of Aqaba/Eilat ($n = 12$) with microsatellites.

Population genetic analyses

Ten populations (191 individuals) had samples sizes greater than five and were thus suitable for population genetic analyses; however, we included all (217 individuals) for the construction of the mtDNA haplotype network outlined below. ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010) was used to calculate mtDNA haplotype (h) (Nei, 1987) and nucleotide (π) diversity (Nei & Li, 1979) and to investigate population structure at various geographical scales. To test for genetic partitioning, we applied an analysis of molecular variance (AMOVA) based on Φ_{ST}, an analogue of Wright's F_{ST} that incorporates a model of sequence evolution (Weir & Cockerham, 1984; Excoffier *et al.*, 1992). As the specimens from the NW Indian Ocean were phylogenetically distinct, we used the following groupings: (1) within the Red Sea to Arabian Sea, including the Gulf of Aqaba/Eilat, Saudi Arabian Red Sea, Djibouti and Oman (hereafter referred to as the NW Indian province), (2) within the group formed by all remaining sampling sites in the Indian Ocean and Pacific Ocean [Madagascar, Ryukyus, Cook Islands, Line Islands, Johnston Atoll and Hawai'i (hereafter referred to as the Indo-Pacific region)], and (3) between these two groups. We applied the K80 model (Kimura, 1980) allowing rate variation among sites (Gamma parameter $a = 0.07$) as indicated by JMODELTEST 2.1.5 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). We used nonparametric permutation procedures (10,000 iterations) to test the significance of the variance components for each hierarchical comparison and applied the modified false discovery rate method (Narum, 2006) to correct for multiple comparisons. We also calculated Jost's D_{est}, an absolute measure of differentiation between sites that is not affected by within-population diversity (Jost, 2008), with the program SMOGD 1.2.5 (Crawford, 2010).

An unrooted network was constructed using NETWORK 4.6.0.0 and NETWORK PUBLISHER 1.3.0.0 (http://www.Fluxus-engineering.com/network_terms.htm), applying the median-joining and maximum-parsimony options. We calculated corrected average divergence between mitochondrial lineages
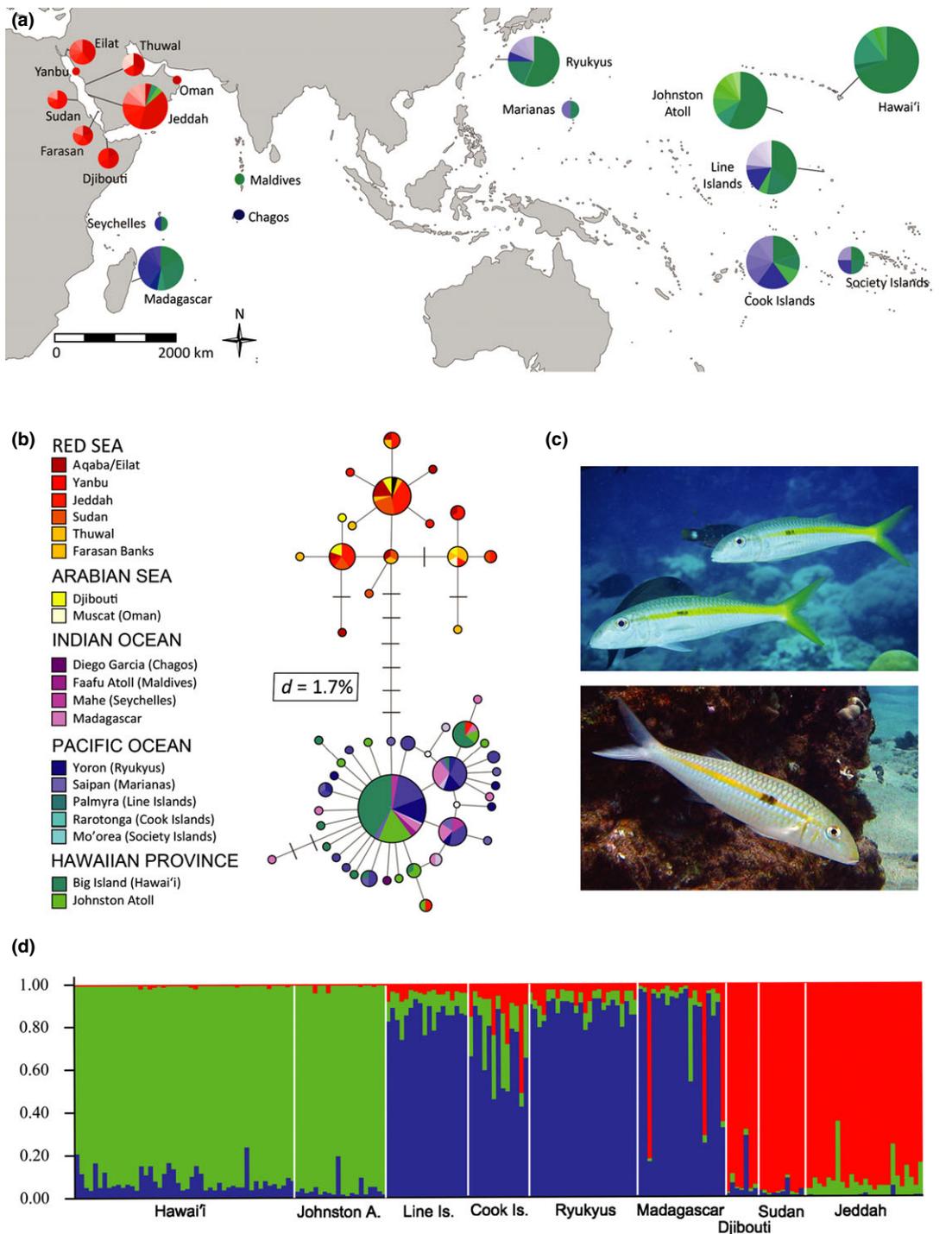
Figure 1 (a) Map of the study area depicting proportion of haplotypes sampled at each site based on 715 bp of mitochondrial cytochrome *b* sequence data (*n* = 217) from *Mulloidichthys flavolineatus* sampled across the Red Sea, Arabian Sea, Indian Ocean and Pacific Ocean. Each pie chart represents a sampling site, with the size proportional to sample size. Across sampling sites, each colour represents a unique haplotype. Haplotypes of the North-western Indian lineage are represented in red, haplotypes of the Indo-Pacific lineage are represented in green and blue. (b) Median-joining network. Each circle represents a haplotype, and its size is proportional to its total frequency. Branches separated by black crossbars represent a single nucleotide change, whereas open circles indicate unsampled haplotypes; colours denote collection location as indicated by the embedded key. The network depicts two distinct clades separated by seven mutational steps (corrected sequence divergence, *d* = 1.7%; Kimura, 1980). (c) The image on the top, taken in Thuwal Reef in the Saudi Arabian Red Sea, depicts specimens of the North-western Indian lineage with yellow caudal fins. On the bottom is an image taken on Hawai'i, of a goatfish of the Indo-Pacific lineage with a white/grey caudal fin. (Photo credits: M. Royer and T. Sinclair-Taylor). (d) Assignment probabilities of *M. flavolineatus* individuals to *K* = 3 clusters based on 12 microsatellite loci, using the program STRUCTURE, with individuals arranged geographically (west to east) along the *x*-axis.

Table 1 Molecular diversity indices for populations of *Mulliodichthys flavolineatus* sampled at sites throughout the Red Sea, the Arabian Sea, the Indian Ocean and the Pacific Ocean. Sample location, number of individuals (n), average number of alleles per locus (n_a), the multi-locus average effective number of alleles (eff_allele), observed heterozygosity (H_O), and expected heterozygosity (H_E) in the microsatellite data set are listed by location; number of individuals (n), number of haplotypes (n_h), haplotype diversity (h) and nucleotide diversity (π) in the cytochrome b (cyt b) data set are also listed.

	Sites			cyt b				Microsatellites				
	Ocean/Sea	Lat.	Long.	n	n_h	h	π	n	n_a	eff_allele	H_O	H_E
Greater Indo-Pacific Region												
Big Island (Hawai'i)	Pacific	19.71	−155.45	44	9	0.52	0.002	47	5.92	3.02	0.58	0.61
Johnston Atoll	Pacific	16.73	−169.53	21	8	0.68	0.003	21	5.08	2.79	0.53	0.58
Palmyra (Line Islands)	Pacific	5.87	−162.08	34	10	0.83	0.002	18	6.25	3.82	0.68	0.70
Rarotonga (Cook Islands)	Pacific	−21.23	−159.78	10	8	0.96	0.004	13	5.75	4.15	0.65	0.73
Yoron (Ryukyus)	Pacific	27.05	128.43	16	6	0.68	0.002	24	6.67	3.80	0.62	0.68
Mo'orea (Society Islands)	Pacific	−17.53	−149.83	4	4	1.00	0.007	–	–	–	–	–
Saipan (Marianas)	Pacific	15.18	145.75	2	2	1.00	0.004	–	–	–	–	–
Madagascar	Indian	−25.32	46.17	17	9	0.88	0.006	19	6.42	3.65	0.57	0.68
Mahé (Seychelles)	Indian	−4.68	55.48	4	2	0.67	0.001	–	–	–	–	–
Diego Garcia (Chagos)	Indian	−7.31	72.41	1	1	–	–	–	–	–	–	–
Faafu Atoll (Maldives)	Indian	3.06	72.88	2	1	–	–	–	–	–	–	–
North-western Indian Province												
Muscat (Oman)	Arabian	23.79	58.59	1	1	–	–	–	–	–	–	–
Djibouti	Arabian	11.75	43.21	6	4	0.87	0.005	7	4.67	3.30	0.63	0.64
Farasan Banks	Red	16.75	41.49	5	5	1.00	0.004	–	–	–	–	–
Sudan	Red	21.02	37.13	10	5	0.76	0.005	10	5.58	3.70	0.57	0.65
Jeddah	Red	21.49	39.11	23	10	0.83	0.005	26	6.25	3.61	0.61	0.66
Thuwal	Red	22.33	38.85	5	4	0.90	0.005	–	–	–	–	–
Yanbu	Red	24.07	38.02	2	2	1.00	0.004	–	–	–	–	–
Gulf of Aqaba/Eilat	Red	29.53	35.94	10	7	0.87	0.004	–	–	–	–	–

(d) with MEGA6 (Tamura *et al.*, 2013), assuming the K80 model and Gamma parameter $a = 0.07$.

We applied the Bayesian Markov chain Monte Carlo (MCMC) approach implemented in BEAST 1.7.5 (Drummond *et al.*, 2012) to estimate coalescence times in *M. flavolineatus*. The analysis was conducted under the HKY model of mutation (allowing for rate variation among sites) with a strict clock, using a rate of change of 0.65% per Myr, which is the average reported for six trans-isthmian geminate reef fishes that are assumed to have diverged as a consequence of the closure of the Isthmus of Panama 3.0 Ma (Lessios, 2008). We also repeated the analyses assuming a faster (1.0% per Myr) molecular clock (Bowen *et al.*, 2001). We computed three independent runs (10^7 generations, after 10^6 burn-in chains) and combined the continuous parameter values sampled every 10^3 generations using the program TRACER 1.6 (http://tree.bio.ed.ac.uk/software/tracer/).

To assess deviations from neutral mutation-drift equilibrium, we estimated Fu's F_S and Tajima's D with ARLEQUIN (99,999 permutations). To further investigate the historical demography of *M. flavolineatus* we calculated mismatch distributions (Schneider & Excoffier, 1999) and Harpending's raggedness index (Rogers & Harpending, 1992). Two individuals sampled in Jeddah were excluded from the historical demography analyses because they belonged to the Indo-Pacific lineage rather than the NW Indian Ocean lineage (see Results).

Microsatellite diversity was calculated as observed (H_O) and expected (H_E) heterozygosity per locus and per sampling location using GENODIVE 2.0 (Meirmans & Van Tienderen, 2004). We calculated the effective number of alleles as $Eff\_allele = 1/(1-H_E)$. Deviations from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium were assessed with ARLEQUIN using default parameters. The fixation index F_{ST} (Excoffier *et al.*, 1992) was calculated with GENODIVE, along with standardized versions G'_{ST} (Nei, 1987) and G''_{ST} (Hedrick, 2005), that account for within-population diversity (H_S) to facilitate comparisons across loci. We also calculated per locus values of Jost's $D_{est.}$. We applied the method in LOSITAN 1.0.0 (Antao *et al.*, 2008) to identify outlier loci that have excessively high or low F_{ST} values compared to neutral expectations.

We analysed genetic structure with microsatellites assuming the infinite allele model under the AMOVA framework using ARLEQUIN with 99,999 permutations, with F_{ST} equivalent to Weir & Cockerham's θ (Weir & Cockerham, 1984). To ensure that observed patterns of genetic structure (or lack thereof) were not driven by the effect of individual loci, we re-calculated indexes of population differentiation after sequential removal of loci and compared the results. Population genetic structure across sampling locations was further evaluated using a Bayesian clustering analysis implemented in STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). This method groups multi-locus genotypes to minimize deviations from

Hardy–Weinberg and linkage equilibrium. We applied a model of admixture and correlated allele frequencies, and included the geographical origin of genotypes as a prior. We determined the most likely number of clusters (K) in the data set by comparing the mean probability of the data (Ln P (D)) over five replicate runs (400,000 iterations after a burn-in of 100,000 repetitions) for each value of $K = 1$ to $K = 5$. The method described by Evanno *et al.* (2005) produced similar results.

To test for isolation-by-distance (IBD), we conducted Mantel tests using linearized F_{ST} [$F_{ST}/(1-F_{ST})$] and the natural log of Euclidean distance among all sample sites (Rousset, 1997) in ARLEQUIN using the cyt b and microsatellite data sets independently. To account for the fact that IBD can be confounded by hierarchical genetic structure (Meirmans, 2012), we tested IBD separately in the NW Indian Ocean and remaining Indo-Pacific regions (excluding Johnston Atoll and Hawai'i).

RESULTS

Molecular diversity: mtDNA

We resolved 715 bp of the cyt b gene from 217 individuals (Fig. 1a,b, Table 1). Across all sites, haplotype diversity (h) was high, ranging from 0.52 to 0.96, with a mean of 0.80 (when populations with more than five individuals were considered; Table 1). In contrast, nucleotide diversity (π) was relatively low, ranging from 0.002 to 0.007, with a mean of 0.004. The haplotype network (Fig. 1b) revealed a genetic and geographical partition of the goatfish into two clusters, one composed exclusively of haplotypes from the NW Indian Ocean province and another cluster composed of all the haplotypes from the remaining Indo-Pacific region plus two unique haplotypes from Jeddah (Saudi Arabia). The NW Indian sample ($n = 59$) included 17 polymorphic sites defining 16 haplotypes. The most common haplotype (39%) was detected at every site. In the remaining Indo-Pacific ($n = 158$), we detected 40 polymorphic sites defining 35 haplotypes, and the most common haplotype (46%) was observed in all sites with $n > 2$. Although in this region a great proportion of haplotypes are shared across sites, shifts in haplotype frequencies indicate isolation of the Hawaiian province (Hawai'i and Johnston Atoll).

Pairwise mismatch distributions did not show significant deviation from the simulated demographic expansion null model in any of the regions tested (Table 2), although Harpending's raggedness index was marginally significant in the Indo-Pacific region when the samples from the Hawaiian province were excluded ($r = 0.086$, $P = 0.04$). Significant values of Fu's F_S and Tajima's D support a scenario of past demographic expansion across all regions (Fu's $F_S = -28.55$ to -6.90, $P < 0.01$; Tajima's $D = -1.24$ to -2.32, $P < 0.09$). Both the NW Indian and Indo-Pacific clades had starburst patterns in the haplotype networks (Fig. 1b), supporting the conclusion of recent population expansion. We verified that

the pattern held when we constructed separate haplotype networks with the Hawaiian province and remainder of the Indo-Pacific (results not shown).

The corrected average pairwise sequence divergence between lineages was $d = 1.7\%$ (Kimura, 1980), much higher than the genetic divergence within each cluster (NW Indian: 0.4%; Indo-Pacific: 0.3%). Assuming a 0.65% per Myr, coalescence time for *M. flavolineatus* was approximately 494 ka [95% highest posterior density intervals (HPD) of 307 and 706 ka]. Coalescence time was 313 ka (95% HPD = 192–447 ka) when assuming a faster (1.0% per Myr) molecular clock. Coalescence times of the NW Indian and Indo-Pacific lineages were estimated at 265 ka (95% HPD = 154–391 ka) and 298 ka (95% HPD = 187–434 ka), respectively, assuming 0.65% per Myr.

Molecular diversity: microsatellites

We genotyped 185 individuals at 12 microsatellite loci. Number of alleles ranged from 4.7 to 6.7 (multi-locus average) across the nine populations surveyed (Table 1). Expected heterozygosity (H_e) ranged from 0.58 to 0.73, while observed heterozygosity (H_o) exhibited a slightly narrower range, from 0.53 to 0.68 (Table 1). After applying a false discovery rate correction, we found that only one of 108 tests of HWE (Hawai'i, locus mflssr034, $P < 0.001$) and 8 of 594 tests for linkage disequilibrium (with biases towards mflssr026 and mflssr033) were significant at 95% confidence.

Two loci had comparatively higher F'_{ST} and G'_{ST} values than the multi-locus average (mflssr034 and mflssr037, Table S1 in Appendix S2), indicating the presence of geographically restricted alleles, possibly owing to positive directional selection acting upon these or linked loci.

Population structure

We detected a sharp genetic subdivision between the NW Indian populations and the remainder of Indo-Pacific as indicated by the AMOVA analyses (Table 3). This break explained most of the variability in mtDNA ($\Phi_{CT} = 0.91$, $P = 0.01$); the variance explained by among populations within regions was negligible in comparison ($\Phi_{SC} = -0.0004$, $P = 0.56$). Although AMOVA tests with microsatellite data also support this break ($F_{CT} = 0.10$, $P = 0.01$), the degree of genetic variation among populations within regions was still high ($F_{SC} = 0.05$, $P < 0.001$), indicating additional genetic sub-structuring.

Consistently, all pairwise Φ_{ST} or F_{ST} values in population comparisons between NW Indian and Indo-Pacific regions were significant and much higher than within these regions (Table 4). Standardized F'_{ST} and G'_{ST} and true population differentiation (D_{est}) indexes followed the same pattern (see Tables S2 and S3 in Appendix S2). Locus mflssr034 showed marked segregation of alleles between the NW Indian province and Indo-Pacific regions ($F_{ST} = 0.26$–0.56, mean $F_{ST} = 0.42$; within the Indo-Pacific: $F_{ST} = 0$–0.01, mean

Table 2 Tests of neutrality and demographic stability for *Mulloidichthys flavolineatus* sampled at sites described in Table 1 throughout the North-western Indian and Indo-Pacific regions. Significant values of Fu's F_S ($P < 0.05$), Tajima's D ($P < 0.02$) and Harpending's raggedness index r ($P < 0.05$) are indicated in bold.

Geographical region	Fu's F	P-value	Tajima's D	P-value	Raggedness index (r)	P-value
North-western Indian province	**−6.90**	**0.004**	−1.24	0.092	0.015	0.973
Indo-Pacific (all sites included)	**−28.55**	**< 0.001**	**−2.32**	**< 0.001**	0.057	0.253
Indo-Pacific (excluding Hawai'i & Johnston A.)	**−21.63**	**< 0.001**	**−2.18**	**0.001**	**0.086**	**0.040**
Hawaiian province (Hawai'i & Johnston A.)	**−10.85**	**< 0.001**	**−1.84**	**0.011**	0.092	0.739

Table 3 Genetic structuring (analysis of molecular variance, AMOVA) of *Mulloidichthys flavolineatus* sampled at sites throughout the North-western Indian province (Madagascar, Djibouti, Sudan and Saudi Arabia) and the greater Indo-Pacific region (Hawai'i, Johnston Atoll, Line Islands, Cook Islands, Ryukyus and Madagascar) based on 715 bp of mtDNA cyt *b* sequence and 12 microsatellite loci. Analyses were conducted (1) with all populations divided into two (North-western Indian province and remaining Indo-Pacific) or three separate groups (North-western Indian province, Hawaiian province and remaining Indo-Pacific) to assess the relationship between these regions; (2) excluding populations in the North-western Indian province. Φ_{CT}: region variance component relative to total variance; Φ_{SC}: between-populations-within-regions variance component divided by the sum of itself and within-population variance; Φ_{ST}: population variance component relative to the total variance. Significant values ($P < 0.05$) are indicated in bold.

		Among groups			Among populations within groups			Among populations	
		% variation	Φ_{CT}	P-value	% variation	Φ_{SC}	P-value	Φ_{ST}	P-value
All populations included:									
NW Indian vs. Indo-Pacific	*mtDNA*	90.83	**0.91**	**0.01**	0.00	0.00	0.55	**0.91**	**< 0.001**
(including Hawai'i+Johnston A.)	*Msats (all loci)*	9.66	**0.10**	**0.01**	4.29	**0.05**	**< 0.001**	**0.14**	**< 0.001**
Hawai'i vs. rest Indo-Pacific vs.	*mtDNA*	84.01	**0.84**	**0.01**	−0.12	−0.01	0.78	**0.84**	**< 0.001**
NW Indian	*Msats (all loci)*	8.46	**0.08**	**0.001**	2.20	**0.02**	**< 0.001**	**0.11**	**< 0.001**
NW Indian vs. Madagascar vs.	*mtDNA*	72.24	**0.72**	**0.006**	−0.25	−0.01	0.90	**0.72**	**< 0.001**
(Cook Is.+Line Is.+Ryukyus) vs. (Hawai'i+Johnston A.)	*Msats (all loci)*	8.17	**0.08**	**0.001**	−0.39	0.00	0.96	**0.08**	**< 0.001**
North-western Indian province excluded:									
(Hawai'i+Johnston A.) vs. Cook Is. vs.	*mtDNA*	2.99	0.03	0.07	−0.17	0.00	0.53	0.03	0.07
(Line Is.+Ryukyus+Madagascar)	*Msats (excluding ssr037)*	4.93	**0.05**	**0.04**	0.22	0.00	0.41	**0.05**	**< 0.001**
(Hawai'i+Johnston A.) vs. Cook Is. vs.	*mtDNA*	4.82	**0.05**	**0.02**	−2.15	−0.02	0.88	0.03	0.07
Line Is. vs. (Ryukyus+Madagascar)	*Msats (excluding ssr037)*	4.05	0.04	0.13	0.35	0.00	0.25	**0.04**	**< 0.001**
(Hawai'i+Johnston A.) vs. (Cook Is.+Line Is.+Ryukyus) vs. Madagascar	*mtDNA*	3.86	0.04	0.07	−0.01	−0.01	0.60	0.03	0.07
	Msats (excluding ssr037)	3.88	0.04	0.07	0.02	0.00	0.63	**0.04**	**< 0.001**
(Hawai'i+Johnston A.) vs. (Cook Is.+Line Is.+Ryukyus+Madagascar)	*mtDNA*	95.63	0.04	0.07	0.62	0.00	0.38	0.04	0.01
	Msats (excluding ssr037)	5.17	0.05	0.07	−0.16	0.00	0.85	**0.05**	**< 0.001**

$F_{ST} = 0.03$; within the North-western Indian province: $F_{ST} = 0$; Tables S4 and S5 in Appendix S2).

Within each geographical region, population pairwise tests provide insights into genetic sub-structuring when differentiation is present, subtle or absent (Table 4). Samples from Hawai'i and Johnston Atoll showed genetic differentiation from all other Indo-Pacific locations (microsatellites: $F_{ST} = 0.04$ to 0.10, $P < 0.001$; mtDNA: pairwise $\Phi_{ST} = 0$–0.10, n.s.). In comparisons among the Line Islands, the Cook Islands and Madagascar, F_{ST} values were significant in four of six tests with microsatellite loci only, but no comparisons were significant when locus mflssr037 was removed from the

analyses (Table 4 and Table S4 in Appendix S2). We found no significant genetic differentiation in comparisons within the NW Indian province or between Hawai'i and Johnston Atoll. Results from the various AMOVA analyses were consistent with these findings (Table 3). However, we cannot exclude the possibility that increasing sample size in the NW Indian province may enhance statistical power and detect genetic differentiation within this region. Tests for IBD were not significant for *M. flavolineatus*.

Bayesian clustering analyses implemented in STRUCTURE identified the mean estimated probability of the data as being highest when samples are grouped into three clusters ($K = 3$)

Table 4 Tests of differentiation among population pairs of *Mulloidichthys flavolineatus* sampled at sites throughout the Red Sea, the Arabian Sea, the Indian Ocean and the Pacific Ocean based on mitochondrial cytochrome *b* (cyt *b*) gene sequences (below diagonal) and 12 microsatellite loci (above diagonal). Mitochondrial cyt *b* pairwise Φ_{ST} estimates are indicated, whereas conventional F_{ST} values are indicated for microsatellites. Bold denotes values that were significant in the tests of population differentiation after false discovery rate correction (corrected $\alpha_{99.0\%} = 0.002$). Multilocus F_{ST} values after removal of locus mflssr037 from the data set are indicated in parentheses (above the diagonal). Only populations with samples sizes $n > 5$ were included in the analyses, and no microsatellite data for the Gulf of Aqaba/Eilat sample were available.

	Hawai'i	Johnston Atoll	Line Is.	Cook Is.	Ryukyus	Madagascar	Djibouti	Sudan	Jeddah
Big Island (Hawai'i)	0	−0.003 (−0.005)	**0.053 (0.056)**	**0.038 (0.043)**	**0.097 (0.046)**	**0.074 (0.063)**	**0.187 (0.191)**	**0.196 (0.181)**	**0.135 (0.132)**
Johnston Atoll	−0.002	0	**0.054 (0.060)**	**0.056 (0.062)**	**0.102 (0.050)**	**0.097 (0.072)**	**0.201 (0.209)**	**0.214 (0.196)**	**0.148 (0.146)**
Palmyra (Line Is.)	0.032	0.030	0	−0.005 (−0.004)	0.050 (−0.001)	0.037 (0.005)	**0.100 (0.103)**	**0.113 (0.088)**	**0.086 (0.080)**
Rarotonga (Cook Is.)	0.082	0.057	−0.011	0	0.050 (0.002)	0.021 (−0.001)	**0.089 (0.088)**	**0.096 (0.071)**	**0.077 (0.072)**
Yoron (Ryukyus)	0.037	0.036	−0.016	−0.007	0	0.063 (0.010)	**0.154 (0.117)**	**0.136 (0.095)**	**0.116 (0.079)**
Madagascar	0.063	0.058	0.030	0.007	−0.022	0	**0.135 (0.100)**	**0.111 (0.076)**	**0.096 (0.074)**
Djibouti	**0.923**	**0.900**	**0.896**	**0.866**	**0.902**	**0.856**	0	0.015 (−0.005)	0.006 (0.004)
Sudan	**0.921**	**0.899**	**0.896**	**0.872**	**0.900**	**0.863**	−0.045	0	0.001 (−0.003)
Jeddah	**0.820**	**0.766**	**0.793**	**0.723**	**0.752**	**0.747**	−0.026	−0.031	0
Gulf of Aqaba/Eilat	**0.913**	**0.886**	**0.887**	**0.853**	**0.884**	**0.851**	−0.033	−0.069	−0.026

Corrected values of alpha (after false discovery rate correction): $\alpha_{95.0\%} = 0.01$, $\alpha_{99.0\%} = 0.002$, $\alpha_{99.9\%} = 0.0002$

(Fig. 1d, Fig. S1 in Appendix S2). All individuals from Hawai'i and Johnson Atoll were assigned to Bayesian Cluster 1 (BC1), all individuals from the Line Islands, the Ryukyus and Cook Islands were assigned to BC2, and all individuals from Djibouti, Oman and Jeddah were assigned to BC3. The sample from Madagascar was predominantly assigned to BC2, but three individuals were assigned to BC3. Notably, the two individuals from Jeddah with mtDNA haplotypes characteristic of the Indo-Pacific cyt *b* clade (see Fig. 1a,b) grouped with individuals from the NW Indian province in BC3 based on microsatellite genotypes (Fig. 1d). In most cases individuals were assigned with high confidence to one of three clusters (> 95% assigned with > 80% probability), with a few exceptions (Cook Islands).

DISCUSSION

The goatfish *M. flavolineatus* shows significant levels of genetic structure that indicate an evolutionary partition (and possible taxonomic distinction) between the NW Indian province and the greater Indo-Pacific regions. We also detected a significant, but more recent, population genetic partition between Hawai'i and our sample locations in the Indian and Pacific Oceans (Tables 3 & 4, Fig. 1).

It is remarkable that the population from the NW Indian province maintained separation from Indian Ocean counterparts despite contemporary connection between these water masses (Nuryanto & Kochzius, 2009; DiBattista *et al.*, 2013). The isolation of Red Sea marine fauna is well documented, with many endemic reef fishes (13%), annelids (13%), arthropods (10%), tunicates (17%), echinoderms (8%), molluscs (6%) and corals (6%) (DiBattista *et al.*, in press a). Several evolutionary processes may enhance this isolation. Sea-level lowering during Pleistocene glacial periods affected the exchange of water through the narrow (18 km) and shallow (137 m) Strait of Bab al Mandab (Siddall *et al.*, 2003), which may have acted as a barrier to larval transport. Reduction in water exchange led to increased residence times of Red Sea water, which resulted in salinity levels up to 50 psu and harsh conditions for survival (Fenton *et al.*, 2000). In addition, cold-water upwelling off the north-east African and southern Arabian coasts, together with a 2200 km stretch of coast between southern Somalia and Djibouti that lacks true coral reefs, may reinforce the isolation of the NW Indian province from the greater Indian Ocean (Kemp, 1998).

The two mitochondrial lineages (NW Indian and Indo-Pacific) became isolated from each other approximately 493 ka based on a molecular clock of 0.65% per Myr (Lessios, 2008). These lineages remained highly isolated as indicated by the absence of admixture detected with microsatellite markers. The time of divergence precedes the most recent desiccation event in the Red Sea (~19 ka), as well as most salinity crises of the Pleistocene (Siddall *et al.*, 2003). This timing indicates that NW Indian populations of *M. flavolineatus* did not go extinct with each ice age, a finding that reinforces the emerging view that some NW Indian

reef fauna survived multiple regional salinity crises associated with Pleistocene sea-level changes (DiBattista *et al.*, 2013; Hodge *et al.*, 2014).

Whether the North-western Indian lineage persisted just outside of the Red Sea or refugia existed within the Red Sea is debatable (DiBattista *et al.*, in press b). Persistence in refugia outside of the Red Sea in the Gulf of Aden (Djibouti or mainland Yemen) is compatible with our results, given that the cold-water upwelling off the north-east African and southern Arabian coasts may account for the isolation of the NW Indian and Indo-Pacific lineages, although the historical continuity of this upwelling is poorly characterized (Rampen *et al.*, 2008). Alternatively, persistence through Pleistocene glacial ages in the Red Sea and later colonization of the Gulf of Aden and Omani coast is feasible. Support for Red Sea refugia comes from fishes endemic to the northern Red Sea, for example, *Sillago suezensis* (Sillaginidae), *Atherinomorus forskalii* (Atherinidae), *Upeneus davidaromi* (Mullidae) and *Diplogrammus gruveli* (Callionymidae) (Golani, 2001; Tikochinski *et al.*, 2013; Randall *et al.*, in press). It may be that species that persisted in the Red Sea during these harsh periods have broader habitat requirements, and therefore are less reliant on corals and associated habitats. In considering habitat during low sea-level stands, *M. flavolineatus* prefers sandy, shallow substrates, including lagoons without coral reefs. Thus, a third possibility is that the Yellowstripe Goatfish persisted in the sediment-loaded southern Red Sea and that its habitat was connected with the Gulf of Aden during low sea-level stands.

Locus mflssr034 (or loci linked to it) shows strong differentiation between the NW Indian province and greater Indo-Pacific regions (see Table S5 in Appendix S2), a genomic signature that invokes the possibility that selection and local adaptation played a role in lineage divergence (Willette *et al.*, 2014). Given the history of dramatic changes in the physical and biotic environment in the region, the possibility of divergence along ecological boundaries is compelling (Rocha *et al.*, 2005). Hence, we favour an evolutionary scenario of parapatric speciation, where vicariance due to biogeographical barriers is reinforced by divergent adaptation in response to habitat differences.

Taxonomic considerations and endemism in the Red Sea

MtDNA divergence between NW Indian and Indo-Pacific *M. flavolineatus* is higher than the genetic distance among four of the other seven species pairs in the genus (*M. vanicolensis*, *M. dentatus*, *M. martinicus* and *M. mimicus*) (Results not shown). Furthermore, high levels of mitochondrial and nuclear differentiation are concordant with differences in morphology (see Table S6 in Appendix S3) and coloration of the caudal fin (Fig. 1c). In light of these observations, we are conducting additional taxonomic work to determine whether a revision of *M. flavolineatus* is appropriate (I. Fernandez-Silva *et al.*, in prep.).

The discovery of an endemic goatfish lineage in the NW Indian province is not unprecedented. Among 84 described species of goatfishes (family Mullidae), 17 are found in the Red Sea, four of which are endemic (Randall *et al.*, in press): *Upeneus pori*, *U. davidaromi*, *U. niebuhri* and *Parupeneus forskali*. Our study exemplifies how phylogeographical analyses provide a framework for uncovering cryptic evolutionary lineages with relevance to marine conservation. Because there has been little genetic work on widespread taxa that range across the Red Sea and other biogeographical provinces (reviewed by Berumen *et al.*, 2013), endemism estimates for the Red Sea (or NW Indian province) are likely to increase (DiBattista *et al.*, in press b).

The isolation of Hawai'i

For the Indo-Pacific lineage, our combined analyses of mtDNA and microsatellite markers revealed population level differentiation between *M. flavolineatus* in Hawai'i and the remainder of the Indo-Pacific (Tables 3 & 4, Fig. 1). Hawai'i is the most isolated province on the Pacific plate (Hourigan & Reese, 1987; Randall, 2007), with 25% endemism in marine fishes, 25% endemism in red algae and 20% endemism in molluscs (Briggs & Bowen, 2012, and references therein). Furthermore, many studies have documented the genetic isolation of Hawaiian populations (Planes & Fauvelot, 2002; DiBattista *et al.*, 2011; Gaither *et al.*, 2011). The coalescence time of the Indo-Pacific populations (~287 ka) indicates that the separation of the Hawaiian province was more recent than the split between the NW Indian and Indo-Pacific lineages (~497 ka). The isolation of the Hawaiian populations of *M. flavolineatus* coincides with the divergence time of many Hawaiian endemics (< 1 Ma, Hodge *et al.*, 2014).

Our study indicates genetic alignment of Johnston Atoll with Hawai'i, a finding congruent with previous work that has documented a high degree of genetic connectivity between these two locations (DiBattista *et al.*, 2011; Andrews *et al.*, 2014), and a strong component of Hawaiian endemics in the fish assemblages of Johnston Atoll (Gosline, 1955). Furthermore, simulations of larval transport from Johnston Atoll to Hawai'i have shown potential connectivity between the two regions (Kobayashi, 2006). Together with a genetic signature of population expansion in this province (Table 2), our results indicate that the Hawaiian populations originated from rare dispersal events from the South Pacific (possibly via Johnston Atoll; Craig *et al.*, 2010). An alternative colonization route from the West Pacific via the warm Kuroshio Current is also possible (Pyle, 1999; Bird *et al.*, 2011).

Outside of Hawai'i, the Indo-Pacific lineage of *M. flavolineatus* demonstrates no obvious geographical patterns of genetic differentiation. We also do not detect an Indian Ocean versus Pacific Ocean split (Tables 3 & 4, Fig. 1) as observed in other widespread taxa (Gaither & Rocha, 2013). Mitochondrial haplotypes are shared among localities separated by more than

15,000 km (Fig. 1a,b), demonstrating the high dispersal capabilities of *M. flavolineatus*. This dispersal capability is also evident in its wide distribution – *M. flavolineatus* has colonized throughout the tropical Indian and Pacific oceans, except the Eastern Pacific and Arabian/Persian Gulf. An extended pelagic larval duration of 60 days (Luiz *et al.*, 2013) and schooling behaviour from larval stages (Leis & Carson-Ewart, 1998) to adulthood are some of the life history traits that may enhance the capacity of *M. flavolineatus* to disperse and establish new populations (Leis, 2006; Luiz *et al.*, 2013).

Peripheral populations as sources of evolutionary innovation

Although previous work found no genetic structure in *M. flavolineatus* (Lessios & Robertson, 2013), by including a larger sample size, broader geographical sampling and more molecular markers, our study revealed isolation of peripheral populations in the NW Indian Ocean and Hawai'i, and the genetic continuity of populations in the core Indo-Pacific. These results are relevant to the long-standing debate over whether the Indo-Pacific biodiversity hotspot (i.e. the Coral Triangle) is a centre of speciation versus a centre of accumulation and/or overlap (Bowen *et al.*, 2013). With this study we contribute another example (Nuryanto & Kochzius, 2009; DiBattista *et al.*, 2011, 2013; Hodge *et al.*, 2012; Tikochinski *et al.*, 2013) that illustrates how coral reefs at the periphery of the Indo-Pacific are sources of evolutionary innovation, contributing to global marine biodiversity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary methods.
Appendix S2 Supplementary Figure S1 and Tables S1–S5.
Appendix S3 Supplementary Table S6.

BIOSKETCH

The authors' research is focused on the evolutionary processes that generate marine biodiversity with the ultimate goal of informing marine conservation. They conduct phylogeographical surveys of marine species in the tropical Indo-Pacific to test models of speciation, hybridization and dispersal.

Author contributions: I.F.S., B.W.B. and L.A.R. conceived the project; I.F.S. and J.D.D. assembled the tissue collection with contributions from all the authors; I.F.S. produced and analysed the genetic data, with contributions from R.R.C.; J.E.R. conducted the morphological analyses; B.W.B., L.A.R. and J.D.R. provided lab space and funding support; I.F.S. led the writing, with contributions from all the authors.

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