



# Testing dispersal limits in the sea: range-wide phylogeography of the pronghorn spiny lobster *Panulirus penicillatus*

Matthew Iacchei<sup>1,2\*</sup>, Michelle R. Gaither<sup>3</sup>, Brian W. Bowen<sup>1</sup> and Robert J. Toonen<sup>1</sup>

<sup>1</sup>Hawai'i Institute of Marine Biology, Kāne'ohe, HI 96744, USA, <sup>2</sup>Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA, <sup>3</sup>School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK

## ABSTRACT

**Aim** To resolve phylogeographical partitions in the pronghorn spiny lobster, *Panulirus penicillatus*, which has a nine-month pelagic larval phase and the broadest distribution among spiny lobsters. With samples from the Red Sea to the East Pacific, we test genetic partitions across two-thirds of the planet, in a species with one of the longest pelagic phases.

**Location** Red Sea, Indian and Pacific Oceans.

**Methods** A fragment of the mtDNA cytochrome *c* oxidase subunit I gene (COI) was resolved in 774 individuals from 28 locations, plus 55 sequences (10 locations) from public databases. Portions of COI, 12S and 16S mtDNA regions were resolved for phylogenetic analyses on a subset of individuals. Phylogenetic and population-level analyses were used to detect evolutionary partitions and dispersal barriers.

**Results** Significant population structuring was detected (overall  $\Phi_{ST} = 0.310$ ,  $P < 0.001$ ). Samples from the Red Sea and East Pacific Provinces (western and eastern range edges) comprise distinct phylogenetic lineages, divergent at  $d = 1.1\%$  and  $1.8\%$ , respectively, from the Indo-Pacific. Coalescent analyses indicate a Pleistocene isolation for the Red Sea (0.71 Ma) and East Pacific (1.52 Ma). Excluding the Red Sea and East Pacific, structure across the rest of the range was comparatively low, but significant ( $\Phi_{ST} = 0.018$ ,  $P < 0.001$ ).

**Main conclusions** The Red Sea and East Pacific populations are phylogenetically distinct from populations elsewhere in the range. The East Pacific population diverged earliest, and may represent a distinct subspecies. Phylogeographical divisions align with the major Indo-Pacific biogeographical barriers, and with subsets of provincial designations from two frameworks: one based on levels of endemism and one on species composition. Tropical and temperate regions in the Pacific are significantly differentiated, potentially indicating an ecological partition. Isolation at eastern and western peripheral provinces may serve as a starting point for evolutionary diversification within this group.

## Keywords

East Pacific, Indo-West Pacific, larval dispersal, marine biogeography, mtDNA, Palinuridae, Red Sea

\*Correspondence: Matthew Iacchei, Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, 1000 Pope Rd., Honolulu, HI 96822, USA.  
E-mail: iacchei@hawaii.edu

## INTRODUCTION

Among reef-associated invertebrates, the species with the greatest dispersal potential are the spiny lobsters (family Palinuridae). Within the Palinuridae, there are 16 *Panulirus* spp. known in the Indo-Pacific Basin; seven have broad distribu-

tions that extend across multiple biogeographical provinces, and four occur from the Red Sea to the Central Pacific. Of these, the pronghorn lobster, *Panulirus penicillatus* (Olivier, 1791) is the most broadly distributed (George & Main, 1967), occurring across all three major biogeographical barriers in the tropical Indo-Pacific: the break between the Red

Sea and the Indian Ocean (Klausewitz, 1989); the Indo-Pacific Barrier between the Pacific and Indian Oceans (Briggs, 1974); and the East Pacific Barrier (EPB) that comprises the oceanic gap between the Central and East Pacific (Ekman, 1953).

The vast distribution of *P. penicillatus* is thought to be sustained by a phyllosoma larval stage that has been collected in the middle of the Pacific Ocean, 3500–4000 km from the nearest reef habitat (Johnson, 1974). Based on field collections, Johnson (1968) estimated the pelagic larval duration (PLD) of *P. penicillatus* to be > 7–8 months; estimates based on cultured larvae are 8.3–9.4 months (Matsuda *et al.*, 2006). These PLDs are much greater than the estimated 30–40 days required to cross most patches of open water in the Indo-Pacific (Mora *et al.*, 2012), and even surpass the longest estimates (100–155 days) of the time required to cross the EPB (Wyrтки *et al.*, 1981). Thus, the phyllosoma larval stage may provide a mechanism to maintain gene flow across Darwin's 'impassable' marine dispersal barrier for shallow water species (Darwin, 1872), and in turn, would designate *P. penicillatus* a truly 'trans-Pacific' species (Briggs, 1961).

In contrast to expectations based on PLD, recent evidence indicates genetic differentiation between East and West Pacific populations of *P. penicillatus* (Chow *et al.*, 2011), and between Indian Ocean and Red Sea populations (Abdullah *et al.*, 2014). There is also morphological variation across the species range. In the East Pacific, *P. penicillatus* is known as the 'red lobster' (Holthuis & Loesch, 1967; Holthuis, 1991), in contrast to the brownish-yellowish-green to blue-black coloration observed elsewhere (Holthuis, 1991; George, 2005; see Appendix S1 in Supporting Information). The longitudinal stripes on the legs also vary in colour and thickness across the species range (see Appendix S1), as do the posterolateral sternal spines of phyllosoma (McWilliam, 1995), providing additional evidence for cryptic lineages.

Here, we analyse mtDNA sequence data from the Red Sea to the East Pacific to assess genetic partitions across major biogeographical barriers and provincial boundaries for *P. penicillatus* (Fig. 1). Biogeographical provinces can be defined by various criteria, including high levels of endemism (> 10%, Briggs, 1974) or species presence/absence data (Kulbicki *et al.*, 2013). Once defined, provinces provide a framework to test hypotheses concerning the origin, distribution and evolution of species. In the Indo-Pacific region, *P. penicillatus* populations span eight of the Briggs & Bowen (2012) tropical biogeographical provinces, which are delineated by high levels of endemism in both invertebrates and fishes (hereafter called Briggs & Bowen Provinces; Fig. 1a). The *P. penicillatus* distribution also crosses eight of the Kulbicki *et al.* (2013) tropical biogeographical provinces, which are based on dissimilarity among assemblages of reef-associated species (hereafter called Kulbicki Provinces; Fig. 1b). While many species have ranges that overlap adjacent provinces, few species inhabit all of these tropical Indo-Pacific provinces. To our knowledge, this is the first study to evaluate all three major biogeographical divisions within the tropical Indo-Pacific, providing the broadest marine phylogeographical coverage to date (Keyse *et al.*, 2013).

## MATERIALS AND METHODS

### Sample collection

We collected 774 *Panulirus penicillatus* from 28 sites (Fig. 1, Table 1). Lobsters were collected by hand or obtained from fishers. Tissue samples were preserved in salt-saturated 20% DMSO buffer or 95% ethanol, and stored at room temperature. We obtained an additional 55 COI sequences from 10 locations from GenBank for phylogeographical analyses (see Appendix S2).

### DNA extraction, PCR and sequencing

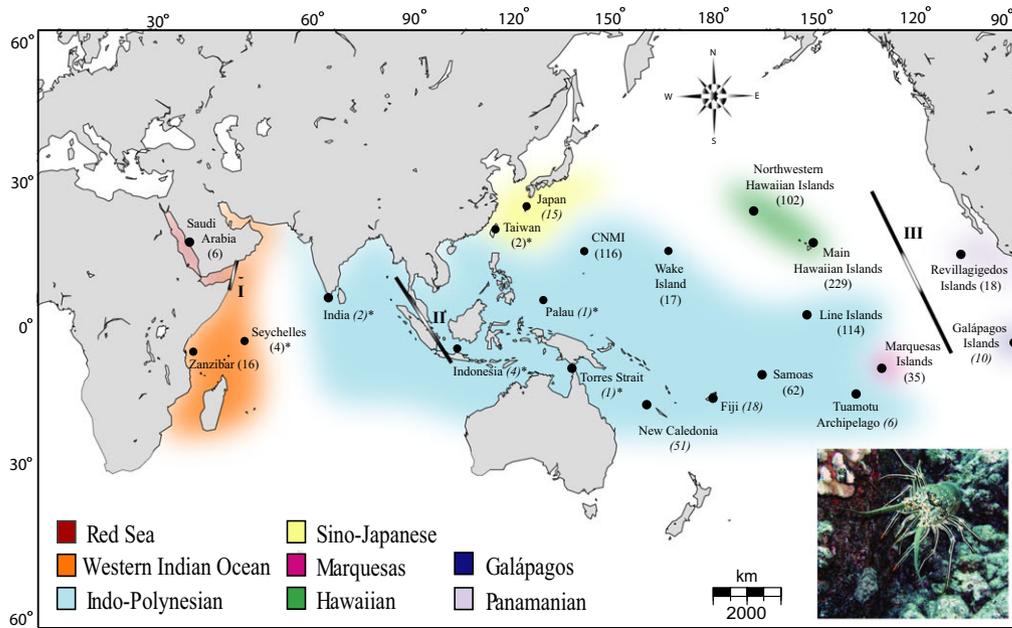
Genomic DNA was isolated using a HotSHOT method (Truett *et al.*, 2000) or a DNeasy Animal Tissue kit (Qiagen Inc., Valencia, CA, USA) following manufacturer instructions. We resolved a 460 bp fragment of COI using species-specific primers (Iacchei *et al.*, 2014; see Appendix S2) for all individuals. We also resolved 522 bp of the 12S and 440 bp of the 16S mtDNA region for a subset of individuals for phylogenetic analyses (see Appendix S2). Polymerase chain reactions (PCRs), sequencing and quality control were performed per Iacchei *et al.* (2014), but with a 50 °C PCR annealing temperature for 12S and 16S. Sequences were edited, aligned and trimmed using GENEIOUS Pro R7 (Biomatters Ltd., Auckland, New Zealand). Unique haplotypes were identified using the Haplotype Collapser and Converter in FaBox 1.35 (<http://users-birc.au.dk/biopv/php/fabox/>), and deposited in GenBank (accession numbers: KT954552 - KT954894).

### Phylogenetic analyses

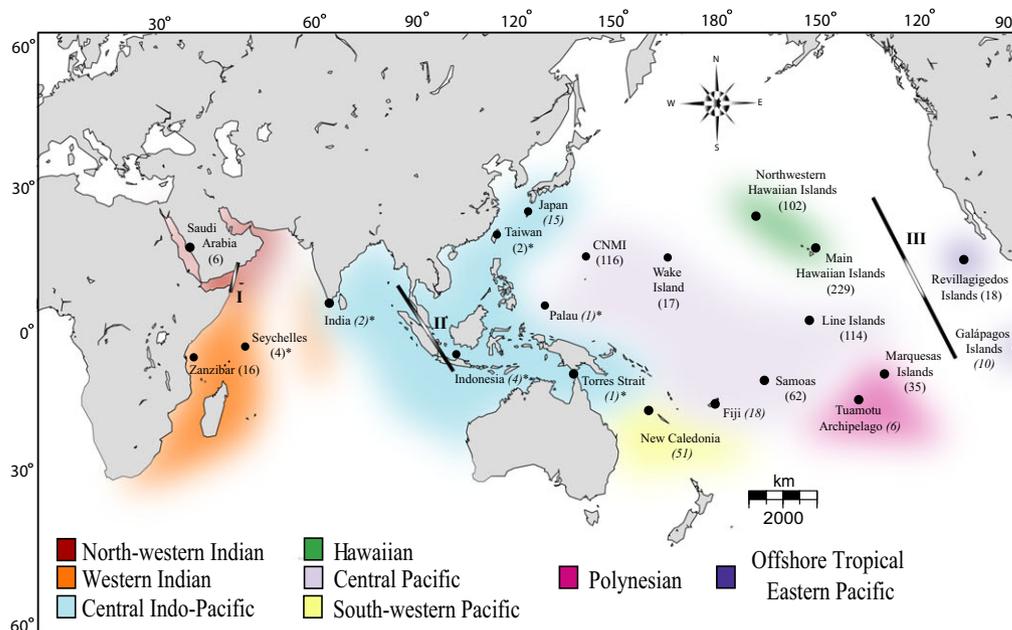
An intra-specific phylogeny was produced for a geographically stratified random subsample of the lobsters ( $N = 24$ ) from Indo-Pacific locations, and all individuals collected from the East Pacific and Red Sea (see Table S4 in Appendix S2). Sequences were aligned and concatenated in GENEIOUS Pro R7. We used PARTITIONFINDER 1.1.1 (Lanfear *et al.*, 2012) to determine the most appropriate model of evolution for each marker (COI, 12S, 16S) in our data set for each analysis (see Appendix S2).

To estimate the time to most recent common ancestor (TMRCA), we used the Bayesian MCMC approach implemented in BEAST 1.7.5 (Drummond & Rambaut, 2007). *Panulirus echinatus* is the most closely related species to *P. penicillatus* (Ptacek *et al.*, 2001), and resides in N.E. Brazil and the Central Atlantic Islands (Holthuis, 1991). Tourinho *et al.* (2012) estimated a divergence of 4.7 Myr based on COI between these two taxa that reside on either side of the Isthmus of Panama. Based on our phylogenetic data set, we found an average COI sequence divergence of 13.1% between these species (range = 12.0–13.9%, estimated using GENEIOUS). Assuming a separation time of 4.7 Myr, we calculated a within lineage divergence rate of 1.39% per Myr (uncorrected). BEAST

(a) Indo-Pacific Provinces - Briggs and Bowen (2012)



(b) Indo-Pacific Provinces - Kulbicki *et al.* (2014)



**Figure 1** Collection locations and sample sizes for *Panulirus penicillatus* across the Indo-Pacific. Colours indicate the biogeographical provinces within which sample sites have been grouped following: (a) Briggs & Bowen (2012), and (b) Kulbicki *et al.* (2013). The three predominant biogeographical barriers in the Indo-Pacific are identified by Roman numerals: I) Red Sea/Indian Ocean Barrier; II) Indo-West Pacific (IWP) Barrier; III) East Pacific Barrier (EPB). Several islands were sampled in some of the island groups: Commonwealth of the Northern Marianas Islands (CNMI: Rota, Saipan, Sarigan Island, Pagan Island), New Caledonia (Chesterfield Islands, New Caledonia), Samoa (Samoa, American Samoa), Line Islands (Palmyra Atoll, Kiritimati), Northwestern Hawaiian Islands (Pearl and Hermes Reef, Lisianski Island, Maro Reef, French Frigate Shoals, Mokumanamana), and Main Hawaiian Islands (Kaua'i, Moloka'i, Lana'i, Maui, Hawai'i). These sample sites were combined into the island groups after determining that there was no significant genetic structure within that group. In parentheses is the total number of individuals sampled for that group. Sample sizes and diversity statistics for each of the islands individually are reported in Table 1. Italicized sample numbers identify sites where some or all of the sequences were obtained from publicly available databases. Locations marked with an asterisk were not included in population level analyses due to low sample size ( $N < 5$ ). Photo credit: Kydd Pollock.

analyses using 4.7 Myr as a calibration point at the basal node of the tree failed to converge, so we used 1.39% as our molecular clock for COI (strict clock) in all subsequent BEAST runs (see Appendix S2). A maximum clade credibility tree was constructed using TREEANNOTATOR 1.7.5.

We constructed a maximum likelihood (ML) tree using RAxML 7.2.6 (Stamatakis, 2006), and rooted trees using *P. echinatus* sequences (see Appendix S2). We created an additional ML tree using RAxML with just COI sequences for all lobsters included in the study ( $N = 829$ ; see Appendix S2). We calculated genetic distance between mitochondrial lineages using Kimura's two-parameter distance model (K2P) as implemented in MEGA 6.06 (Tamura *et al.*, 2013).

### Phylogeographical analyses

ARLEQUIN 3.5 (Excoffier *et al.*, 2010) was used to estimate haplotype ( $h$ ) and nucleotide diversity ( $\pi$ ) for COI, to test for hierarchical population structure using an analysis of molecular variance (AMOVA) and to calculate pairwise  $\Phi_{ST}$  comparisons among all locations with  $N > 5$  (see Appendix S2). We constructed a median-joining network (Bandelt *et al.*, 1999) using NETWORK 4.6.0.0 (<http://www.fluxus-engineering.com/sharenet.htm>) to visualize the frequencies, spatial distributions and relationships among COI haplotypes. We used the ISOLATION-BY-DISTANCE WEB SERVICE 3.23 (Jensen *et al.*, 2005) to test for correlations between genetic and geographical distances across the Western and Central Pacific (see Appendix S2).

## RESULTS

### Phylogenetic structure

Our phylogenetic analyses of three mtDNA loci revealed three divergent lineages with Bayesian posterior probabilities of 1.0 that correspond to the East Pacific (ML bootstrap = 52), Indo-Pacific (ML bootstrap = 97) and Red Sea (ML bootstrap = 71). The mean K2P distance within lineages ranged from  $d = 0.002$ – $0.004$ , while distances between lineages were much deeper;  $d = 0.011$ – $0.020$ . The East Pacific lineage was most distant with  $d = 1.8\%$  (vs. Indo-Pacific) and  $2.0\%$  (vs. Red Sea). The Red Sea and Indo-Pacific lineages were less divergent with  $d = 1.1\%$  (Fig. 3). Coalescent analyses indicate a TMRCA for *P. penicillatus* and *P. echinatus* at 6.89 Ma with highest posterior density (HPD) intervals that are narrower (95% HPD = 3.48–10.82 Ma) and are well within the values found by Tourinho *et al.* (2012) (reported values, 95% HPD = 0.6–11.1 Ma). TMRCA = 1.52 Ma (95% HPD = 0.8–2.31 Ma) for *P. penicillatus* lineages, while the distinct lineages were of roughly equal ages with overlapping confidence intervals [East Pacific, TMRCA = 0.22 Ma (95% HPD = 0.09–0.40 Ma); Indo-Pacific, TMRCA = 0.30 Ma (95% HPD = 0.17–0.46 Ma); Red Sea, TMRCA = 0.25 Ma (95% HPD = 0.10–0.47 Ma)].

### Phylogeographical structure

We resolved 460 bp of COI in 829 individuals from 35 locations (including the 55 GenBank sequences) across the species range. Number of individuals ( $N$ ), haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) for each location are provided in Table 1. Overall nucleotide diversity was low ( $\pi = 0.007$ ) while haplotype diversity was high ( $h = 0.902$ ). We detected 221 haplotypes; 139 (62.9%) were singletons. The most common haplotypes were shared across all Indian and Pacific Ocean sites, but no haplotypes were shared among the Indo-Pacific, East Pacific and the Red Sea (Fig. 2).

Global population structure was  $\Phi_{ST} = 0.310$  ( $P < 0.001$ ); if the divergent Red Sea and East Pacific samples are excluded,  $\Phi_{ST} = 0.018$  ( $P < 0.001$ ). Using the three dominant marine biogeographical barriers within the tropical Indo-Pacific as a priori divisions, we found significant differentiation among the Red Sea, Indian Ocean, Western and Central Pacific Ocean, and East Pacific Ocean ( $\Phi_{CT} = 0.744$ ,  $P < 0.001$ ). When biogeographical provinces were tested, we found significant population structure among the provinces for both the Briggs & Bowen Provinces and Kulbicki Provinces frameworks (Table 2). For samples within the range from the Indo-Pacific Barrier to the East Pacific Barrier,  $\Phi_{ST} = 0.018$  ( $P < 0.001$ ), but the genetic variance across this range was not partitioned significantly among island groups or among provinces of either classification (Table 2); however, the Briggs & Bowen Sino-Japanese Province was genetically distinct ( $\Phi_{CT} = 0.074$ ,  $P = 0.048$ ). There was no pattern of IBD across the Indo-West Pacific (IWP) for any of the combinations tested ( $r = -0.202$ – $0.123$ ;  $P = 0.161$ – $0.976$ ). Across the species' range, 83 of 190 (43.7%) pairwise  $\Phi_{ST}$  comparisons between sampling locations were significant (Table 3), and significant  $\Phi_{ST}$  values ranged from 0.012 to 0.885. After correcting for false discovery rate (FDR), 76 of 83 comparisons remained significant (Table 3).

## DISCUSSION

Our range-wide phylogeographical survey of *Panulirus penicillatus* reveals one of the highest levels of genetic structure detected in spiny lobsters to date (overall  $\Phi_{ST} = 0.310$ ,  $P < 0.001$ ). Despite an estimated 9-month PLD, *P. penicillatus* shows significant genetic differentiation throughout the species range, including phylogenetic-level divergences across two of the three major biogeographical barriers (EPB and Red Sea/Indian Ocean split) at the edges of the species distribution. Across the IWP, genetic structure is comparatively lower, but still significant ( $\Phi_{ST} = 0.018$ ;  $P < 0.001$ ). This pattern is driven by restricted gene flow between the tropical Indo-Pacific and the only warm-temperate region occupied by *P. penicillatus* (Briggs & Bowen's Sino-Japanese Province). However, even within the tropical IWP, a few locations appear relatively isolated [e.g. Fiji, Samoa, Northwestern

**Table 1** Summary statistics for 829 *Panulirus penicillatus* mtDNA cytochrome *c* oxidase subunit I (COI) sequences obtained from 35 sites: total number of individuals sequenced (*N*), haplotype diversity (*h*) and nucleotide diversity ( $\pi$ ). Sites are listed individually, and grouped by the biogeographical provinces as designated by Briggs & Bowen (2012) and by Kulbicki *et al.* (2013). NW Indian = North-western Indian Province; Offshore TEP = Offshore Tropical Eastern Pacific Province. Sites marked with an asterisk include some, if not all sequences from GenBank.

Briggs & Bowen Provinces	Collection site (Abbreviation)	<i>N</i>	<i>h</i>	$\pi$	Kulbicki Provinces
Red Sea	Saudi Arabia (SAUD)	6	0.93	0.004	NW Indian
Western Indian Ocean	Zanzibar (ZANZ)	16	0.93	0.005	Western Indian
	Seychelles Islands (SECH)	4	1.00	0.006	
Sino-Japanese	Taiwan (TAIW)	2	0.00	0.000	Central Indo-Pacific
	Japan (JAPA)*	15	0.97	0.006	
Indo-Polynesian	India (INDI)*	2	1.00	0.007	Central Pacific
	Indonesia (INDO)*	4	1.00	0.005	
	Torres Strait, Australia (TORRE)*	1	1.00	0.000	
	Palau (PALA)*	1	1.00	0.000	
	Rota (ROTA)	26	0.86	0.005	
	Saipan (SAIP)	4	1.00	0.003	
	Sarigan Island (SARI)	36	0.85	0.004	
	Pagan Island (PAGA)	50	0.91	0.005	
	Wake Island (WAKE)	17	0.92	0.006	
	Fiji (FIJI)*	18	0.80	0.003	
	Samoa (SAMO)	37	0.91	0.006	
	American Samoa (ASAM)	25	0.96	0.006	
	Palmyra Atoll (PALM)	51	0.88	0.004	
	Kiritimati (KIRI)	63	0.92	0.005	
	Chesterfield Islands (CHEST)*	18	0.97	0.007	
New Caledonia (NCAL)*	33	0.90	0.005		
Tuamotu Archipelago (TUAM)*	6	0.73	0.004	Polynesian	
Marquesas Hawaiian	Marquesas Islands (MARQ)	35	0.93	0.005	Hawaiian
	Pearl and Hermes Reef (PEAR)	30	0.91	0.006	
	Lisianski Island (LISI)	18	0.96	0.006	
	Maro Reef (MARO)	5	1.00	0.007	
	French Frigate Shoals (FREN)	44	0.90	0.005	
	Mokumanamana (NECK)	5	0.90	0.005	
	Kaua'i (KAUA)	52	0.89	0.005	
	Moloka'i (MOLO)	71	0.80	0.004	
	Lāna'i (LANA)	35	0.85	0.004	
	Maui (MAUI)	24	0.75	0.004	
	Hawai'i (HAWA)	47	0.89	0.005	
	Galápagos	Galápagos Islands (GALA)*	10	0.78	
Panamanian	Revillagigedo Islands, Mexico (REVI)	18	0.84	0.015	

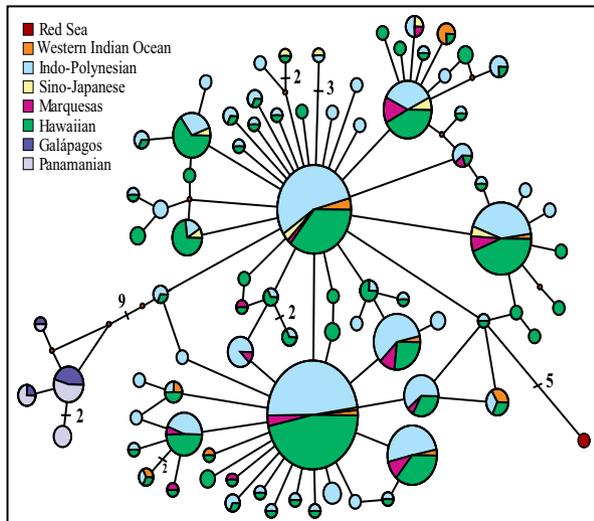
Hawaiian Islands (NWHI); Table 3]. Below, we examine these patterns in light of biogeographical theory and hypotheses regarding the evolution and radiation of this species.

### East Pacific

The EPB was long thought to be an 'impassable' barrier to dispersal for nearshore marine species (Darwin, 1872; Mayr, 1954). Recent molecular data support this hypothesis for both a trans-EPB coral (*Porites lobata*; Baums *et al.*, 2012; Forsman *et al.*, 2015), and even a broadly distributed soldierfish (Craig *et al.*, 2007). However, numerous fish species have been documented on both sides of the EPB (reviewed in Robertson *et al.*, 2004). From the perspective of vicariance biogeography, these species ranges are regarded as relics of pan-Tethyan species that were connected to the Caribbean through the Central American corridor (McCoy & Heck,

1983). Alternatively, these distributions could have been achieved via dispersal events from the IWP (Cortes, 1997). Molecular evidence indicates recent gene flow for at least 16 fishes (Lessios & Robertson, 2006) and an echinoderm (*Echinothrix diadema*; Lessios *et al.*, 1998). The magnitude, timing and direction of gene flow vary among these species, but it is primarily from the Central to East Pacific.

For *P. penicillatus*, our data support a pan-Tethyan progenitor that subsequently diverged during the late Miocene into the Pacific *P. penicillatus* and the Atlantic sister species *P. echinatus*, as proposed by George (2005). Our coalescent analyses date the TMRCA between *P. penicillatus* and *P. echinatus* at 6.89 Ma (HPD = 3.48–10.82 Ma). Our confidence intervals overlap the 4.7 Ma TMRCA estimate of Tourinho *et al.* (2012). During this time, gene flow between populations in the Atlantic and Pacific was likely restricted because trans-oceanic water flow across the



**Figure 2** Median-joining network for *Panulirus penicillatus* mtDNA, constructed using 460 base pairs of cytochrome *c* oxidase subunit I (COI) from 829 individuals in the program NETWORK. Each circle represents a unique haplotype that is proportional in size to the number of individuals with that haplotype. The smallest circle represents two individuals; the largest represents 240 individuals. Haplotypes are colour-coded according to the Briggs & Bowen (2012) biogeographical provinces. All haplotypes are separated by one base pair difference unless labeled with a number greater than one.

Panama Seaway decreased about 6 Ma (Collins *et al.*, 1996), and cessation occurred with the rise of the Isthmus of Panama approximately 2.8 Ma (Coates & Obando, 1996). Coral reef fishes that originated in the East Pacific are generally restricted to that region (Allen & Robertson,

1994; Cowman & Bellwood, 2013). *Panulirus penicillatus*, however, maintained broad connectivity across the Pacific after the closure of the Panama Seaway: the divergence of East and West Pacific lineages did not occur until 1.5 Ma (HPD = 0.89–2.3 Ma).

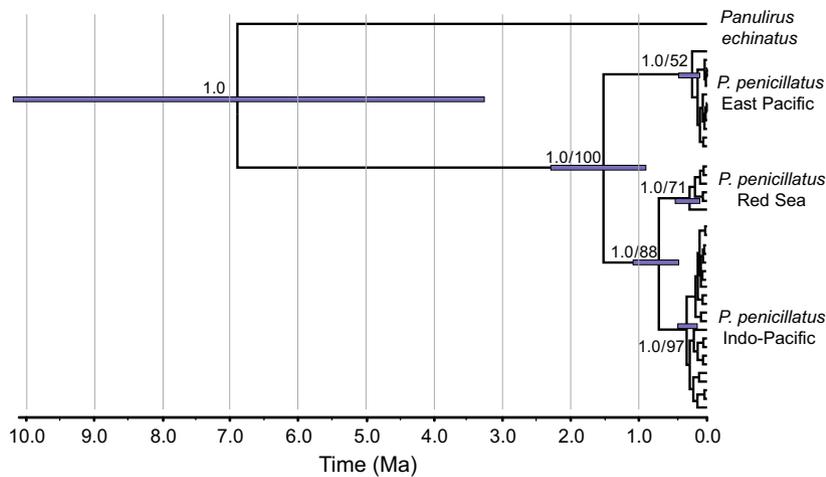
The East Pacific *P. penicillatus* populations remain phylogenetically distinct, sharing no haplotypes with IWP populations ( $d = 1.8\%$ ; Fig. 3). These findings extend the results of Chow *et al.* (2011) who suggested that the EPB is a significant barrier to gene flow for *P. penicillatus*. Here, we add an additional East Pacific sample (Revillagigedo Islands), and show there is no genetic structure between the Revillagigedo Islands and the Galápagos ( $\Phi_{ST} = -0.004$ ;  $P = 0.451$ ). This result fits the Kulbicki Provinces, which group these locations into the Offshore Tropical East Pacific Province, rather than separating them as in the Briggs & Bowen classification (Fig. 1). This also matches the species distribution data within the Panamanian Province: *P. penicillatus* is rarely found east of the offshore islands (Holthuis, 1991). The lack of differentiation between East Pacific locations, combined with the phylogenetic-level divergence and morphological differences between the East Pacific and IWP forms (see Appendix S1) corroborate the evolutionary distinction of the East Pacific form, and add support to the hypothesis of George (2005) that the *P. penicillatus* ‘red’ morph is a distinct taxa, isolated by the EPB. Despite a much longer PLD than species maintaining gene flow across the EPB (Lessios & Robertson, 2006), *P. penicillatus* may have adapted larval retention behaviours similar to other Panuliridae (Jeffs *et al.*, 2005; Butler *et al.*, 2011; Iacchei *et al.*, 2013) to maintain local populations during the changing currents at the beginning of the Pleistocene (as suggested by George, 2005).

**Table 2** AMOVA results showing degrees of freedom (d.f.), sum of squares (SS), variance components (Var),  $\Phi$ -statistics ( $\Phi_{CT/ST}$ ) and *P*-values (*P*) for each biogeographical framework tested for *Panulirus penicillatus*.  $\Phi$ -statistics reported are  $\Phi_{ST}$  for the Rangewide and Indo-Pacific Rangewide comparisons, and  $\Phi_{CT}$  for all others. Bold values indicate significant differences at  $P < 0.050$ . All comparisons remained significant after correcting for false discovery rate ( $P > 0.0476$ ). The major biogeographical barriers, and marine biogeographical provinces of Briggs & Bowen (2012) and Kulbicki *et al.* (2013) are shown in Fig. 1. For the island group analysis, groups could consist of one island, but had to have at least 5 individuals sampled.

Among regions	d.f.	SS	Var	$\Phi_{CT/ST}$	<i>P</i>
Rangewide	26	419.20	0.531	<b>0.310</b>	< <b>0.001</b>
Indo-Pacific Rangewide (without Red Sea and East Pacific clades)	23	40.77	0.021	<b>0.018</b>	< <b>0.001</b>
Major Biogeographical Barriers (Red Sea/Indian Ocean/Western-Central Pacific/East Pacific)	3	377.51	3.499	<b>0.744</b>	< <b>0.001</b>
Briggs & Bowen Provinces: (Red Sea, Western Indian Ocean, Sino-Japanese, Indo-Polynesian, Marquesas, Hawaiian, Galápagos, Panamanian)	7	386.42	0.732	<b>0.379</b>	<b>0.025</b>
Kulbicki Provinces: (North-western Indian Ocean, Western Indian Ocean, Central Indo-Pacific, Central Pacific, South-western Pacific, Hawaiian, Polynesian, Offshore Tropical Eastern Pacific)	7	385.92	0.665	<b>0.356</b>	<b>0.003</b>
Pacific Briggs & Bowen Provinces: (Indo-Polynesian, Sino-Japanese, Marquesas, Hawaiian)	3	6.56	-0.002	-0.001	0.316
Pacific Kulbicki Provinces: (Central Indo-Pacific, Central Pacific, South-western Pacific, Hawaiian, Polynesian)	4	8.10	-0.002	-0.002	0.187
Pacific Island Groups: (Japan, CNMI, Wake Island, New Caledonia and Chesterfield Islands, Fiji, Samoa and American Samoa, Tuamotu Archipelago, Marquesas Islands, Line Islands, Hawaiian Archipelago)	9	16.58	0.007	0.006	0.218

**Table 3** Pairwise population genetic structure results for a 460 bp fragment of the mtDNA cytochrome *c* oxidase subunit I region (COI).  $\Phi_{ST}$  is below the diagonal and *P*-values are above the diagonal. Shaded boxes below the diagonal indicate significant differences at *P* < 0.05. Asterisks indicate comparisons that were no longer significant after correcting for false discovery rate (*P* > 0.015). Sites are grouped by biogeographical region and by island archipelago – only sites where *N* > 5 are included. Regional abbreviations: RS = Red Sea; WIO = Western Indian Ocean. Site Abbreviations (West to East): 1. SAUD = Saudi Arabia; 2. ZANZ = Zanzibar; 3. JAPA = Japan; 4. ROTA = Rota Island; 5. SARI = Sarigan Island; 6. PAGA = Pagan Island; 7. CHES = Chesterfield Islands; 8. NCAL = New Caledonia; 9. WAKE = Wake Island; 10. FIJI = Fiji; 11. SAMO = Samoa; 12. ASAM = American Samoa; 13. PALM = Palmyra Atoll; 14. KIRI = Kiritimati; 15. TUAM = Tuamotu Archipelago; 16. MARQ = Marquesas Islands; 17. NWHI = Northwestern Hawaiian Islands; 18. MHI = Main Eight Hawaiian Islands; 19. GALA = Galápagos Islands; 20. REVI = Revillagigedo Islands.

	RS	WIO	Central and West Pacific																East Pacific	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Red Sea																				
1. SAUD		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001
WIO																				
2. ZANZ	0.739		0.023	0.062	0.026	0.080	0.330	0.057	0.399	0.010	0.522	0.240	0.090	0.331	0.431	0.185	0.329	0.048	< 0.001	< 0.001
3. JAPA	0.714	0.059*		< 0.001	< 0.001	< 0.001	0.003	< 0.001	0.001	< 0.001	0.015	< 0.001	< 0.001	0.001	0.063	< 0.001	0.002	< 0.001	< 0.001	< 0.001
Central and West Pacific																				
4. ROTA	0.763	0.036	0.139		0.213	0.855	0.579	0.945	0.354	0.053	0.133	0.267	0.106	0.502	0.626	0.825	0.159	0.315	< 0.001	< 0.001
5. SARI	0.786	0.049*	0.198	0.007		0.301	0.192	0.181	0.329	0.410	0.009	0.108	0.344	0.051	0.494	0.393	0.005	0.180	< 0.001	< 0.001
6. PAGA	0.744	0.025	0.147	-0.010	0.002		0.398	0.798	0.422	0.205	0.030	0.727	0.272	0.225	0.726	0.796	0.029	0.399	< 0.001	< 0.001
7. CHES	0.699	0.004	0.081	-0.007	0.010	0		0.622	0.857	0.083	0.579	0.343	0.303	0.790	0.917	0.825	0.556	0.255	< 0.001	< 0.001
8. NCAL	0.758	0.036	0.149	-0.017	0.008	-0.008	-0.008		0.288	0.074	0.073	0.222	0.074	0.249	0.585	0.839	0.064	0.252	< 0.001	< 0.001
9. WAKE	0.709	0.001	0.104	0.002	0.003	-0.001	-0.020	0.006		0.149	0.255	0.366	0.516	0.556	0.773	0.645	0.361	0.188	< 0.001	< 0.001
10. FIJI	0.811	0.095	0.268	0.035	0	0.009	0.030	0.028	0.021		0.002	0.143	0.081	0.013	0.080	0.117	0.003	0.046	< 0.001	< 0.001
11. SAMO	0.725	-0.005	0.047	0.013	0.041	0.021*	-0.006	0.018	0.007	0.089		0.103	0.045	0.749	0.851	0.151	0.908	0.036	< 0.001	< 0.001
12. ASAM	0.716	0.011	0.122	0.005	0.014	-0.007	0.003	0.008	0.002	0.019	0.015		0.430	0.286	0.837	0.423	0.133	0.206	< 0.001	< 0.001
13. PALM	0.769	0.026	0.153	0.014	0.001	0.003	0.004	0.015	-0.004	0.025	0.019*	-0.001		0.224	0.620	0.341	0.036	0.294	< 0.001	< 0.001
14. KIRI	0.728	0.003	0.075	-0.002	0.016	0.003	-0.010	0.004	-0.005	0.044	-0.006	0.003	0.004		0.873	0.563	0.900	0.224	< 0.001	< 0.001
15. TUAM	0.787	-0.005	0.063	-0.024	-0.006	-0.029	-0.054	-0.020	-0.037	0.071	-0.045	-0.042	-0.021	-0.044		0.560	0.894	0.764	< 0.001	< 0.001
16. MARQ	0.747	0.015	0.133	-0.012	0	-0.008	-0.015	-0.011	-0.009	0.021	0.010	-0.001	0.001	-0.003	-0.016		0.243	0.286	< 0.001	< 0.001
17. NWHI	0.718	0.003	0.063	0.008	0.031	0.014*	-0.004	0.014	0.001	0.065	-0.008	0.010	0.014*	-0.005	-0.044	0.004		0.006	< 0.001	< 0.001
18. MHI	0.768	0.029*	0.147	0.002	0.005	0	0.006	0.003	0.010	0.027*	0.016*	0.006	0.001	0.002	-0.035	0.002	0.012		< 0.001	< 0.001
East Pacific																				
19. GALA	0.880	0.849	0.835	0.858	0.871	0.848	0.820	0.856	0.827	0.885	0.836	0.836	0.862	0.837	0.868	0.847	0.832	0.863		0.451
20. REVI	0.714	0.722	0.708	0.755	0.782	0.780	0.710	0.767	0.711	0.753	0.756	0.739	0.792	0.780	0.680	0.763	0.792	0.840	-0.004	



**Figure 3** Maximum clade credibility tree for *Panulirus penicillatus* compiled from ten independent Bayesian MCMC analyses implemented in BEAST, and based on three loci: COI, 12S and 16S. Bayesian posterior probabilities (before slash) and bootstrap branch support for maximum likelihood analyses (after slash) are indicated above a cut off of 0.5 and 50, respectively. Node ages are presented as median node heights with 95% HPD intervals represented by bars.

### Red Sea

The Red Sea is connected to the Indian Ocean via the constricted (18 km) and relatively shallow (137 m) Strait of Bāb al-Mandab. Pleistocene glacial cycles repeatedly lowered sea levels in the region, isolating populations due to physical separation at Bāb al-Mandab with potential reinforcement by temperature and salinity changes in the Red Sea (Siddall *et al.*, 2004; DiBattista *et al.*, 2013). This combination of factors is likely responsible for the elevated levels of endemism (Randall, 1994; DiBattista *et al.*, 2015b), as well as the genetic distinction of Red Sea populations of crown-of-thorns seastars (Benzie, 1999), mud crabs (Fratini & Vannini, 2002) and six of eight reef fishes (DiBattista *et al.*, 2013; Fernandez-Silva *et al.*, 2015).

Red Sea *P. penicillatus* are smaller and have slower growth rates relative to *P. penicillatus* in other parts of the IWP, leading to speculation that these populations are distinct (Plaut & Fishelson, 1991). Alternatively, differences in size and growth rates may be due to environmental conditions and/or prey regimes, as has been shown for congener *P. marginatus* and the slipper lobster *Scyllarides squammosus* in Hawai'i (O'Malley *et al.*, 2012).

Our genetic data indicate a phylogenetic separation between IWP and Red Sea *P. penicillatus* ( $d = 1.1\%$ ; Fig. 3), corroborating data from Abdullah *et al.* (2014), and in accordance with the Briggs & Bowen Red Sea province. The isolation of the Red Sea population (0.7 Ma; HPD = 0.42–1.1 Ma) occurred after the divergence between the East Pacific and the IWP *P. penicillatus* populations (1.5 Ma; HPD = 0.89–2.3 Ma), and was likely due to sea level fluctuation in the mid-Pleistocene. This divergence may have been subsequently reinforced by the differences in sizes and growth rates between these populations (Plaut & Fishelson, 1991). Although *P. penicillatus* has been documented throughout the Red Sea as far north as the Gulf of Aquaba (Holthuis, 1968), it remains to be seen whether the Red Sea lineage extends to the adjacent Gulf of Aden, which shares many endemic species with the Red Sea (DiBattista *et al.*,

2015b). Additional collections from the Persian Gulf or the Gulf of Oman are required to evaluate whether this population would encompass the North-western Indian Province (Kulbicki Provinces). Studies of morphology and the nuclear genome may determine if the separation between the Red Sea and the IWP has produced a Red Sea endemic.

### Indian Ocean

The Indo-Pacific Barrier (IPB) is a widely recognized disjunction in marine faunal distributions between the Indian and Pacific Ocean basins (Briggs, 1974). At the height of the Pleistocene glaciation, sea level dropped up to 120 m below present depths, creating a nearly complete land barrier between the two oceans (Voris, 2000; Naish *et al.*, 2009). This loss of shallow water habitat, combined with strong upwelling in the region (Voris, 2000; Naish *et al.*, 2009), created a barrier between ocean basins and reduced population sizes of shallow fauna on both sides of the IPB (Gaither & Rocha, 2013). Signatures of isolation can be seen for a diversity of taxa (Reid *et al.*, 2006; Barber *et al.*, 2011; Gaither *et al.*, 2011a), with over 80% of species surveyed across the region showing significant population structure (Gaither *et al.*, 2010) and few exceptions (e.g. Crandall *et al.*, 2008; Gaither *et al.*, 2010, 2011b).

Here, we find no genetic structure in *P. penicillatus* across the IPB ( $\Phi_{CT} = 0.001$ ,  $P = 0.180$ ). Two of 12 pairwise comparisons between the Indian and Pacific Ocean sites were significant, but not of greater magnitude than comparisons within the tropical Pacific (Table 3). Increased sampling in the Indian Ocean will allow better resolution of genetic connectivity in this region, although our results corroborate those of Abdullah *et al.* (2014), who resolved the mtDNA control region for *P. penicillatus* samples from Indonesia and the Maldives and found no significant pairwise genetic differentiation. The lack of genetic structure across the IPB may be facilitated by adult habitat preference. *Panulirus penicillatus* adults prefer windward surf zones with high wave energy (George, 1974; Pitcher, 1993), and will move into shallow

(< 0.5 m) surge channels on the reef flat to forage at night (Pitcher, 1993). This habitat was likely common in areas of high upwelling in the region, and the affinity for shallow, scoured shorelines may have allowed adult *P. penicillatus* to persist during glacial cycles, and to maintain population connectivity across the IPB.

Both Briggs & Bowen and Kulbicki provincial frameworks set the barrier between the Indian and Pacific Oceans west of the IPB, indicating that both in terms of endemism rates and overall species composition, the West Indian Ocean region is the most distinct from the Pacific. For *P. penicillatus*, we did not detect any significant differentiation between the WIO and the IWP ( $\Phi_{CT} = 0.008$ ,  $P = 0.14$ ), although more sampling will provide further insights. Notably, there may be population subdivision for *P. penicillatus* at the southernmost end of the WIO. Abdullah *et al.* (2014) report significant pairwise genetic differentiation for *P. penicillatus* between southern Madagascar and sites in the Maldives and in Indonesia. The South-west Indian Ocean (SWIO) also hosts divergent lineages of two other spiny lobster species (Gopal *et al.*, 2006; Lavery *et al.*, 2014), and isolated populations within other broadly distributed species (Castelin *et al.*, 2013; Hoareau *et al.*, 2013), likely reinforced by the complex current regimes in the region. Hoareau *et al.* (2013) provide evidence that the SWIO is a hotspot of evolutionary diversification for brittle stars, a pattern that should be evaluated for additional taxa.

### Indo-Pacific

The largest biogeographical region in the *P. penicillatus* distribution is the IWP, which contains three of the eight Briggs & Bowen provinces and five of the eight Kulbicki provinces (Fig. 1). This expanse of coral reefs and island archipelagos extends over half the circumference of the globe, with no oceanic gap greater than 800 km from the westernmost edge at the IPB to the Polynesian Islands in the east (Schultz *et al.*, 2008). Most reefs throughout the region can be connected within the 30–40 day PLD typical of reef fishes and invertebrates (Mora *et al.*, 2012). This hypothesis of highly connected metapopulations of reefs has been corroborated by biophysical dispersal models (e.g. Trembl *et al.*, 2008), population genetic and phylogenetic surveys of reef-associated species (e.g. Craig *et al.*, 2007; Crandall *et al.*, 2008; Gaither *et al.*, 2010, 2011a) and the combination of dispersal models with genetic data (Crandall *et al.*, 2012).

There is significant genetic structure across the IWP for *P. penicillatus* ( $\Phi_{ST} = 0.018$ ;  $P < 0.001$ ); however, this structure is not driven by differences among provinces within either framework (Table 2), among archipelagos (Table 2) or by IBD. For example, *P. penicillatus* at the Marquesas is not significantly differentiated from sites in the Indo-Polynesian or Hawaiian provinces (Table 3), unlike many fish species (Bernardi *et al.*, 2002; Planes & Fauvelot, 2002; Gaither *et al.*, 2010). Rather, there are specific sites that are driving the pattern [the Commonwealth of the Northern Marianas Islands

(CNMI), Fiji, Samoa and the NWHI; Table 3]. The isolation of the most northern atolls, the NWHI and CNMI, matches dispersal pathways predicted by Trembl *et al.* (2008). Similar site-specific, rather than regional patterns of genetic differentiation, have been documented in five fishes (Drew *et al.*, 2008), and two *Panulirus* spp. hypothesized to be panmictic based on long (> 6 month) PLDs (Iacchei *et al.*, 2013, 2014). In isolation, these significant pairwise comparisons may be statistical artifacts, but as more species are surveyed across this geographical range, it will be informative to reassess potential patterns (e.g. Toonen *et al.*, 2011; Selkoe *et al.*, 2014), with a particular emphasis on utilizing new methodologies to identify potential drivers of site-specific population differentiation (e.g. Iacchei *et al.*, 2013; Selkoe *et al.*, 2014; Gaither *et al.*, 2015).

### Sino-Japanese Province

Within the Indo-Pacific, the most genetically distinct *P. penicillatus* population is in Japan and Taiwan, aligning with the Briggs & Bowen provincial classification of this region. The Sino-Japanese Province represents the northernmost range of *P. penicillatus*, and is the only warm-temperate region inhabited by *P. penicillatus*. Few large-scale phylogeographical studies include sampling from this region. The most well studied island in this transition zone, Taiwan, has fish fauna on the north-western coast affiliated with the warm-temperate mainland, but fauna on the south-eastern coast are tropical in origin (Shao *et al.*, 1999). The persistence of tropical reef species, including *P. penicillatus*, likely depends on the magnitude and location of the Kuroshio Current, a northerly flowing, western boundary current that originates near the tropical Philippines and Taiwan (Ujii *et al.*, 2003).

Population genetic separations of this region for *P. penicillatus* ( $\Phi_{ST} = 0.047$ – $0.268$ ) are greater than most significant comparisons within the Indo-Pacific, including sites separated by greater than ten thousand kilometres (Table 3). Our results are concordant with findings of genetic structure for sea cucumbers (Skillings *et al.*, 2011) and yellow tang (Eble *et al.*, 2011), but not soldierfish (Craig *et al.*, 2007). Surveys that have sampled the Sino-Japanese Province and the tropical IWP are limited in number, but the heretofore evidence of restricted gene flow or isolated populations in this temperate/tropical transition zone suggest a fruitful topic for future phylogeographical investigations.

### CONCLUSION

Our genetic survey of the pronghorn spiny lobster *Panulirus penicillatus* indicates that even a species with an extremely long pelagic larval duration (*c.* 9 months), and one of the broadest distributions in the sea, is influenced by the same barriers to gene flow that have facilitated speciation in other taxa. Genetic connectivity for *P. penicillatus* is not impeded by the ephemeral Indo-Pacific Barrier, but is clearly restricted by the Red Sea/Indian Ocean divide, and

most notably by the East Pacific Barrier. We find genetic partitions are aligned with biogeographical provinces from both the Briggs & Bowen and Kulbicki provincial frameworks (Offshore TEP, Sino-Japanese, Red Sea). Notably, our data indicate potential for evolutionary divergence at the periphery of the range, as demonstrated for other marine species across this region (e.g. Malay & Paulay, 2010; Gaither *et al.*, 2011b, 2015; Lavery *et al.*, 2014; DiBattista *et al.*, 2015a,b). In light of this, the partition between the tropical Indo-Pacific and the warm-temperate Sino-Japanese Province may emerge as a general pattern when more taxa are examined.

While many *Panulirus* spp. have broad distributions, approximately half of the Pacific species are archipelagic endemics or regionally restricted (Holthuis, 1991). These species have likely evolved mechanisms to increase local retention within their restricted ranges (reviewed in Jeffs *et al.*, 2005). Here, our data indicate that even the most broadly distributed *P. penicillatus* may have evolved similar mechanisms for larval retention, especially the East Pacific form, which warrants taxonomic recognition as the red form described by George (2005).

## ACKNOWLEDGEMENTS

We appreciate the individuals who facilitated collection of specimens (including DIPnet; NSF-DEB#14-57848), assisted with lab work, and provided thoughtful commentary (see Appendix S3), including the editor and three anonymous reviewers. This work was funded in part by NSF (OCE#06-23678, OCE#09-29031, OCE#12-60169), NMFS, National Marine Sanctuaries NWHICRER-HIMB partnership (MOA-2005-008/6882), NOAA Project R/HE-6, sponsored by UH Sea Grant under NA09OAR4171060 (to MI and RJT) and NA05OAR4171048 (to MRG and BWB) from NOAA Sea Grant (UNIHI-SEAGRANT-JC-11-07), an EPA STAR Fellowship, the Yoshimoto Foundation, Edmondson Research Fund and the Jessie D. Kay Research Grant. MI was funded by NSF-OCE#10-29478 and OCE#13-338959 (to E. Goetze), and NSF-OCE#15-22572 during the writing of this manuscript. This is contribution 1634 from the Hawai'i Institute of Marine Biology, and 9525 from SOEST.

## REFERENCES

- Abdullah, M.F., Muththalib, M., Salama, A.J. & Imai, H. (2014) Genetic isolation among the Northwestern, Southwestern and Central-Eastern Indian Ocean populations of the pronghorn spiny lobster *Panulirus penicillatus*. *International Journal of Molecular Sciences*, **15**, 9242–9254.
- Allen, G.R. & Robertson, D.R. (1994) *Fishes of the Tropical Eastern Pacific*. University of Hawaii Press, Honolulu, HI.
- Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.
- Barber, P.H., Cheng, S., Erdmann, M. & Tenggardjaja, K. (2011) Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. *Crustacean Issues*, **19**, 129–156.
- Baums, I.B., Boulay, J.N., Polato, N.R. & Hellberg, M.E. (2012) No gene flow across the East Pacific Barrier in the reef-building coral *Porites lobata*. *Molecular Ecology*, **21**, 5418–5433.
- Benzie, J.A.H. (1999) Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution*, **53**, 1782–1795.
- Bernardi, G., Holbrook, S.J., Schmitt, R.J., Crane, N.L. & DeMartini, E. (2002) Species boundaries, populations and colour morphs in the coral reef three-spot damselfish (*Dascyllus trimaculatus*) species complex. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 599–605.
- Briggs, J.C. (1961) The East Pacific Barrier and the distribution of marine shore fishes. *Evolution*, **15**, 545–554.
- Briggs, J.C. (1974) *Marine zoogeography*. McGraw-Hill, New York, NY.
- Briggs, J.C. & Bowen, B.W. (2012) A realignment of marine biogeographic provinces with particular reference to fish distributions. *Journal of Biogeography*, **39**, 12–30.
- Butler, M.J., Paris, C.B., Goldstein, J.S., Matsuda, H. & Cowen, R.K. (2011) Behavior constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series*, **422**, 223–237.
- Castelin, M., Feutry, P., Hauteceur, M., Marquet, G., Wowor, D., Zimmermann, G. & Keith, P. (2013) New insight on population genetic connectivity of widespread amphidromous prawn *Macrobrachium lar* (Fabricius, 1798) (Crustacea: Decapoda: Palaemonidae). *Marine Biology*, **160**, 1395–1406.
- Chow, S., Jeffs, A., Miyake, Y. *et al.* (2011) Genetic isolation between the Western and Eastern Pacific populations of pronghorn spiny lobster *Panulirus penicillatus*. *PLoS ONE*, **6**, e29280.
- Coates, A.G. & Obando, J.A. (1996) The geologic evolution of the Central American isthmus. *Evolution and environment in tropical America* (ed. by J. Jackson, A.F. Budd and A.G. Coates), pp. 21–56. University of Chicago Press, Chicago.
- Collins, L.S., Coates, A.G., Berggren, W.A., Aubry, M.P. & Zhang, J. (1996) The late Miocene Panama isthmian strait. *Geology*, **24**, 687–690.
- Cortes, J. (1997) Biology and geology of eastern Pacific coral reefs. *Coral Reefs*, **16**, S39–S46.
- Cowman, P.F. & Bellwood, D.R. (2013) The historical biogeography of coral reef fishes: global patterns of origination and dispersal. *Journal of Biogeography*, **40**, 209–224.
- Craig, M.T., Eble, J.A., Bowen, B.W. & Robertson, D.R. (2007) High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Marine Ecology Progress Series*, **334**, 245–254.
- Crandall, E.D., Frey, M.A., Grosberg, R.K. & Barber, P.H. (2008) Contrasting demographic history and phyloge-

- graphical patterns in two Indo-Pacific gastropods. *Molecular Ecology*, **17**, 611–626.
- Crandall, E.D., Tremblay, E.A. & Barber, P.H. (2012) Coalescent and biophysical models of stepping-stone gene flow in neritid snails. *Molecular Ecology*, **21**, 5579–5598.
- Darwin, C. (1872) *The origin of species by means of natural selection*, 6th edn. Doubleday & Co., Garden City, NY.
- DiBattista, J.D., Berumen, M.L., Gaither, M.R., Rocha, L.A., Eble, J.A., Choat, J.H., Craig, M.T., Skillings, D.J. & Bowen, B.W. (2013) After continents divide: comparative phylogeography of reef fishes from the Red Sea and Indian Ocean. *Journal of Biogeography*, **40**, 1170–1181.
- DiBattista, J.D., Waldrop, E., Rocha, L.A., Craig, M.T., Berumen, M.L. & Bowen, B.W. (2015a) Blinded by the bright: a lack of congruence between colour morphs, phylogeography and taxonomy for a cosmopolitan Indo-Pacific butterflyfish, *Chaetodon auriga*. *Journal of Biogeography*, **42**, 1919–1929.
- DiBattista, J.D., Roberts, M., Baird, A.H. *et al.* (2015b) A review of contemporary patterns of endemism for shallow water reef fauna in the Red Sea. *Journal of Biogeography*. doi:10.1111/jbi.12689.
- Drew, J.A., Allen, G.R., Kaufman, L. & Barber, P.H. (2008) Regional color and genetic differences demonstrate endemism in five putatively cosmopolitan reef fishes. *Conservation Biology*, **22**, 965–975.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214–221.
- Eble, J.A., Toonen, R.J., Sorensen, L.L., Papastamatiou, Y. & Bowen, B.W. (2011) Escaping paradise: larval export from Hawaii in an Indo-Pacific reef fish, the yellow tang (*Zebrafish flavescens*). *Marine Ecology Progress Series*, **428**, 245–258.
- Ekman, S. (1953) *Zoogeography of the sea*. Sidgwick and Jackson, London, UK.
- Excoffier, L., Laval, G. & Schneider, S. (2010) ARLEQUIN version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.
- Fernandez-Silva, I., Randall, J.E., Coleman, R.R., DiBattista, J.D., Rocha, L.A., Reimer, J.D., Meyer, C.G. & Bowen, B.W. (2015) Yellow tails in a Red Sea: phylogeography of the Indo-Pacific goatfish *Mulloidichthys flavolineatus* reveals isolation in peripheral provinces and cryptic evolutionary lineages. *Journal of Biogeography*, **42**, 2402–2413.
- Forsman, Z., Wellington, G.M., Fox, G.E. & Toonen, R.J. (2015) Clues to unraveling the coral species problem: distinguishing species from geographic variation in *Porites* across the Pacific with molecular markers and microskeletal traits. *PeerJ*, **3**, e751.
- Fratini, S. & Vannini, M. (2002) Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology*, **272**, 103–116.
- Gaither, M.R. & Rocha, L.A. (2013) Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: evidence for the centre of overlap hypothesis. *Journal of Biogeography*, **40**, 1638–1648.
- Gaither, M.R., Toonen, R.J., Robertson, R.R., Planes, S. & Bowen, B.W. (2010) Genetic evaluation of marine biogeographic barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). *Journal of Biogeography*, **37**, 133–147.
- Gaither, M.R., Bowen, B.W., Bordenave, T.R., Newman, S.J., Gomez, J.A., van Herwerden, L. & Craig, M.T. (2011a) Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific Barrier with contemporary overlap in the Coral Triangle. *BMC Evolutionary Biology*, **11**, 189.
- Gaither, M.R., Jones, S.A., Kelley, C., Newman, S.J., Sorensen, L. & Bowen, B.W. (2011b) High connectivity in the deepwater snapper *Pristipomoides filamentosus* (Lutjanidae) across the Indo-Pacific with isolation in the Hawaiian Archipelago. *PLoS ONE*, **6**, e28913.
- Gaither, M.R., Bernal, M.A., Coleman, R.R., Bowen, B.W., Jones, S.A., Simison, W.B. & Rocha, L.A. (2015) Genomic signatures of geographic isolation and natural selection in coral reef fishes. *Molecular Ecology*, **24**, 1543–1557.
- George, R.W. (1974) Coral reefs and rock lobster ecology in the Indo-West Pacific region. *Proceedings of the 2nd International Coral Reef Symposium*, **1**, 321–325.
- George, R.W. (2005) Tethys sea fragmentation and speciation of *Panulirus* spiny lobsters. *Crustaceana*, **78**, 1281–1309.
- George, R.W. & Main, A.R. (1967) The evolution of spiny lobsters (Palinuridae): a study of evolution in the marine environment. *Evolution*, **21**, 803–820.
- Gopal, K., Tolley, K.A., Groeneveld, J.C. & Matthee, C.A. (2006) Mitochondrial DNA variation in spiny lobster *Panulirus delagoae* suggests genetically structured populations in the southwestern Indian Ocean. *Marine Ecology Progress Series*, **319**, 191–198.
- Hoareau, T.B., Boissin, E., Paulay, G. & Bruggemann, J.H. (2013) The Southwestern Indian Ocean as a potential marine evolutionary hotspot: perspectives from comparative phylogeography of reef brittle-stars. *Journal of Biogeography*, **40**, 2167–2179.
- Holthuis, L.B. (1968) The Palinuridae and Scyllaridae of the Red Sea. *Zoologische Mededelingen (Leiden)*, **42**, 281–310.
- Holthuis, L.B. (1991) Marine lobsters of the world: an annotated and illustrated catalogue of species of interest to fisheries known to date. *FAO Fisheries Synopsis*, **13**, 1–292 (FAO Species Catalogue, Rome).
- Holthuis, L.B. & Loesch, H. (1967) The lobsters of the Galápagos Islands (Decapoda, Palinuridae). *Crustaceana*, **12**, 214–222.
- Iacchei, M., Ben-Horin, T., Selkoe, K.A., Bird, C.E., García-Rodríguez, F.J. & Toonen, R.J. (2013) Combined analyses of kinship and  $F_{ST}$  suggest potential drivers of chaotic

- genetic patchiness in high gene flow populations. *Molecular Ecology*, **22**, 3476–3494.
- Iacchei, M., O'Malley, J.M. & Toonen, R.J. (2014) After the gold rush: population structure of spiny lobsters in Hawai'i following a fishery closure and the implications for contemporary spatial management. *Bulletin of Marine Science*, **90**, 331–357.
- Jeffs, A.G., Montgomery, J.C. & Tindle, C.T. (2005) How do spiny lobster post-larvae find the coast? *New Zealand Journal of Marine and Freshwater Research*, **39**, 605–617.
- Jensen, J.L., Bohonak, A.J. & Kelley, S.T. (2005) Isolation by distance, web service. *BMC Genetics*, **6**, 13. v.3.23 <http://ibdws.sdsu.edu/>
- Johnson, M.W. (1968) Palinurid phyllosoma from the Hawaiian archipelago (Palinuridae). *Crustaceana*, **S2**, 59–79.
- Johnson, M.W. (1974) On the dispersal of lobster larvae into the East Pacific Barrier (Decapoda, Palinuridea). *Fisheries Bulletin*, **72**, 639–647.
- Keyse, J., Crandall, E.D., Toonen, R.J., Treml, E.A. & Riginos, C. (2013) The scope of published population genetic data for Indo-Pacific marine fauna and future research opportunities. *Bulletin of Marine Science*, **90**, 47–78.
- Klausewitz, W. (1989) Evolutionary history and zoogeography of the Red Sea ichthyofauna. *Fauna of Saudi Arabia*, **10**, 310–337.
- Kulbicki, M., Parravicini, V., Bellwood, D.R., Arias-González, E., Chabanet, P., Floeter, S.R., Friedlander, A., McPherson, J., Myers, R.E., Vigliola, L. & Mouillot, D. (2013) Global biogeography of reef fishes: a hierarchical quantitative delineation of regions. *PLoS ONE*, **8**, e81847.
- Lanfear, R., Calcott, B., Ho, S.Y. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695–1701.
- Lavery, S.D., Farhadi, A., Farahmand, H., Chan, T.Y., Azhdakoshpour, A., Thakur, V. & Jeffs, A.G. (2014) Evolutionary divergence of geographic subspecies within the scalloped spiny lobster *Panulirus homarus* (Linnaeus 1758). *PLoS ONE*, **9**, e97247.
- Lessios, H.A. & Robertson, D.R. (2006) Crossing the impassible: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2201–2208.
- Lessios, H.A., Kessing, B.D. & Robertson, D.R. (1998) Massive gene flow across the world's most potent marine biogeographic barrier. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 583–588.
- Malay, M.C.M.D. & Paulay, G. (2010) Peripatric speciation drives diversification and distributional pattern of reef hermit crabs (Decapoda: Diogenidae: *Calcinus*). *Evolution*, **64**, 634–662.
- Matsuda, H., Takenouchi, T. & Goldstein, J.S. (2006) The complete larval development of the pronghorn spiny lobster *Panulirus penicillatus* (Decapoda: Palinuridae) in culture. *Journal of Crustacean Biology*, **26**, 579–600.
- Mayr, E. (1954) Geographic speciation in tropical echinoids. *Evolution*, **8**, 1–18.
- McCoy, E.D. & Heck, K.L. (1983) Centers of origin revisited. *Paleobiology*, **9**, 17–19.
- McWilliam, P.S. (1995) Evolution in the phyllosoma and puerulus phases of the spiny lobster genus *Panulirus* White. *Journal of Crustacean Biology*, **15**, 542–557.
- Mora, C., Treml, E.A., Roberts, J., Crosby, K., Roy, D. & Tittensor, D.P. (2012) High connectivity among habitats precludes the relationship between dispersal and range size in tropical reef fishes. *Ecography*, **35**, 89–96.
- Naish, T., Powell, R., Levy, R. *et al.* (2009) Obliquity-paced Pliocene West Atlantic ice sheet oscillations. *Nature*, **458**, 322–328.
- O'Malley, J.M., Drazen, J.C., Popp, B.N., Gier, E. & Toonen, R.J. (2012) Spatial variability in growth and prey availability of lobsters in the northwestern Hawaiian Islands. *Marine Ecology Progress Series*, **449**, 211–220.
- Pitcher, C.R. (1993) Spiny Lobster. *Nearshore marine resources of the South Pacific: information for fisheries development and management* (ed. by A. Wright and L. Hill), pp. 539–607. Suva, Institute of Pacific Studies.
- Planes, S. & Fauvelot, C. (2002) Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution*, **56**, 378–399.
- Plaut, I. & Fishelson, L. (1991) Population structure and growth in captivity of the spiny lobster *Panulirus penicillatus* from Dahab, Gulf of Aqaba, Red Sea. *Marine Biology*, **111**, 467–472.
- Ptacek, M.B., Sarver, S.K., Childress, M.J. & Herrnkind, W.F. (2001) Molecular phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). *Marine and Freshwater Research*, **52**, 1037–1047.
- Randall, J.E. (1994) Twenty-two new records of fishes from the Red Sea. *Fauna of Saudi Arabia*, **14**, 259–275.
- Reid, D.G., Lal, K., Mackenzie-Dodds, J., Kaligis, F., Littlewood, D.T.J. & Williams, S.T. (2006) Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography*, **33**, 990–1006.
- Robertson, D.R., Grove, J.S. & McCosker, J.E. (2004) Tropical transpacific shore fishes. *Pacific Science*, **58**, 507–565.
- Schultz, J.K., Feldheim, K.A., Gruber, S.H., Ashley, M.V., McGovern, T.M. & Bowen, B.W. (2008) Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Molecular Ecology*, **17**, 5336–5348.
- Selkoe, K.A., Gaggiotti, O.E., Laboratory, ToBo, Bowen, B.W. & Toonen, R.J. (2014) Emergent patterns of population genetic structure for a coral reef community. *Molecular Ecology*, **23**, 3064–3079.
- Shao, K.T., Chen, J.P. & Wang, S.C. (1999) Biogeography and database of marine fishes in Taiwan waters. *Proceedings of the 5th Indo-Pacific Fish Conference, Noumea, 1997* (ed. by B. Seret and J.Y. Sire), pp. 673–680. Societe Francaise Ichthyologique, Paris.

- Siddall, M., Smeed, D.A., Hemleben, C., Rohling, E.J., Schmeltzer, I. & Peltier, W.R. (2004) Understanding the Red Sea response to sea level. *Earth and Planetary Science Letters*, **225**, 421–434.
- Skillings, D.J., Bird, C.E. & Toonen, R.J. (2011) Gateways to Hawai'i: genetic population structure of the tropical sea cucumber *Holothuria atra*. *Journal of Marine Biology*, **2011**, 783030.
- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, **30**, 2725–2729.
- Toonen, R.J., Andrews, K.R., Baums, I.B. *et al.* (2011) Defining boundaries for ecosystem-based management: a multi-species case study of marine connectivity across the Hawaiian Archipelago. *Journal of Marine Biology*, **2011**, 460173.
- Tourinho, J.L., Solé-Cava, A.M. & Lazoski, C. (2012) Cryptic species within the commercially most important lobster in the tropical Atlantic, the spiny lobster *Panulirus argus*. *Marine Biology*, **159**, 1897–1906.
- Treml, E.A., Halpin, P.N., Urban, D.L. & Pratson, L.F. (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. *Landscape Ecology*, **23**, 19–36.
- Truett, G.E., Mynatt, R.L., Truett, A.A., Walker, J.A. & Warman, M.L. (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). *BioTechniques*, **29**, 52–54.
- Ujiiie, Y., Ujiiie, H., Taira, A., Nakamura, T. & Oguri, K. (2003) Spatial and temporal variability of surface water in the Kuroshio source region, Pacific Ocean, over the past 21,000 years: evidence from planktonic foraminifera. *Marine Micropaleontology*, **49**, 335–364.
- Voris, H.K. (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography*, **27**, 1153–1167.
- Wyrutki, K., Firing, E., Halpern, D., Knox, R., McNally, G.H., Patzert, W.C., Stroup, E.D., Taft, B.A. & Williams, R. (1981) The Hawaii to Tahiti shuttle experiment. *Science*, **211**, 22–28.

## SUPPORTING INFORMATION

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

**Appendix S1** Photographs depicting regional variation in *P. penicillatus* specimens.

**Appendix S2** Sample list, detailed methods, additional analyses.

**Appendix S3** Acknowledgements.

## DATA ACCESSIBILITY

DNA Sequences: GenBank accession numbers for unique COI haplotypes for the full dataset: KT954552 - KT954772. Genbank accession numbers for phylogeny sequences (see Appendix S2): KT954773 - KT954894.

## BIOSKETCH

**Matthew Iacchei** is a post-doctoral researcher at the University of Hawai'i. This data set is part of his PhD dissertation in the Zoology Department at the University of Hawai'i at Mānoa, entitled, "Population genetic and phylogeographic insights into the phyllosomal odyssey", which was completed under the supervision of **Robert J. Toonen** and **Brian W. Bowen**, with assistance and inspiration from **Michelle R. Gaither**.

Author contributions: M.I., M.R.G., B.W.B and R.J.T conceived the ideas for this study, collected samples and contributed to the writing of the paper. M.I. produced the DNA sequence data; M.I. and M.R.G. analysed the data; and M.I. was the primary author.

---

Editor: Gustav Paulay