3

4   Original Article

5

6   Testing dispersal limits in the sea: range-wide phylogeography of the pronghorn spiny

7   lobster *Panulirus penicillatus*

8

9   Matthew Iacchei[1,2,*], Michelle R. Gaither[3], Brian W. Bowen[1], Robert J. Toonen[1]

10

11   *[1]Hawaiʻi Institute of Marine Biology, Kāneʻohe, HI 96744, USA, [2]Department of*

12   *Oceanography, School of Ocean and Earth Science and Technology, University of*

13   *Hawaiʻi at Mānoa, Honolulu, HI 96822, USA, [3] School of Biological and Biomedical*

14   *Sciences, Durham University, South Road, Durham DH1 3LE, UK*

15

16   *Correspondence: Matthew Iacchei, Department of Oceanography, School of Ocean and

17   Earth Science and Technology, University of Hawaiʻi at Mānoa, 1000 Pope Rd.,

18   Honolulu, HI, 96822 USA. E-mail: iacchei@hawaii.edu

19

24

25

26

27

28

29

30   **ABSTRACT**

Aim To resolve phylogeographic 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 $\Phi_{CT} = 0.310$, $P < 0.001$). 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 ($\Phi_{CT} = 0.018$, $P < 0.001$).

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 sub-species. Phylogeographic divisions align with the major Indo-Pacific biogeographic 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

INTRODUCTION

Among reef-associated invertebrates, the species with the greatest dispersal potential are the spiny lobsters (genus *Panulirus*). There are 16 *Panulirus* spp. known in the Indo-

Pacific Basin; seven have broad distributions that extend across multiple biogeographic 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 biogeographic barriers in the tropical Indo-Pacific: the break between Red Sea and Indian Ocean (Klausewitz, 1989); the Indo-Pacific Barrier between 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, 3,500-4,000 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 (Wyrtki *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 colouration 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 biogeographic barriers and provincial boundaries for *P. penicillatus* (Fig. 1). Biogeographic 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

98   hypotheses concerning the origin, distribution, and evolution of species. In the Indo-

99   Pacific region, *P. penicillatus* populations span eight of the Briggs & Bowen (2012)

100   tropical biogeographic provinces, which are delineated by high levels of endemism of

101   both invertebrates and fishes (hereafter called Briggs & Bowen Provinces; Fig. 1a). The

102   *P. penicillatus* distribution also crosses eight of the Kulbicki *et al.* (2013) tropical

103   biogeographic provinces, which are based on dissimilarity among assemblages of reef-

104   associated species (hereafter called Kulbicki Provinces; Fig. 1b). While many species

105   have ranges that overlap adjacent provinces, few species inhabit all of these tropical Indo-

106   Pacific provinces. To our knowledge, this is the first study to evaluate all three major

107   biogeographic divisions within the tropical Indo-Pacific, providing the broadest marine

108   phylogeographic coverage to date (Keyse *et al.*, 2013).

109

110   **MATERIALS AND METHODS**

111   **Sample Collection**

112   We collected 774 *Panulirus penicillatus* from 28 sites (Fig. 1, Table 1). Lobsters were

113   collected by hand or obtained from fishers. Tissue samples were preserved in salt-

114   saturated 20% DMSO buffer or 95% ethanol, and stored at room temperature. We

115   obtained an additional 55 COI sequences from 10 locations from GenBank for

116   phylogeographic analyses (see Appendix S2).

117

118   **DNA Extraction, PCR, and Sequencing**

119   Genomic DNA was isolated using a HotSHOT method (Truett *et al.*, 2000) or a DNeasy

120   Animal Tissue kit (Qiagen Inc., Valencia, CA, USA) following manufacturer

121   instructions. We resolved a 460bp fragment of COI using species-specific primers

122   (Iacchei *et al.*, 2014; see Appendix S2) for all individuals. We also resolved 522bp of the

123   12S and 440bp of the 16S mtDNA region for a subset of individuals for phylogenetic

124   analyses (see Appendix S2). Polymerase chain reactions (PCRs), sequencing, and quality

125   control were performed per Iacchei *et al.* (2014), but with a 50°C PCR annealing

126   temperature for 12S and 16S. Sequences were edited, aligned, and trimmed using

127   GENEIOUS Pro R7 (Biomatters Ltd., Auckland, New Zealand). Unique haplotypes were

128   identified using the Haplotype Collapser and Converter in FABOX 1.35 (http://users-

129   birc.au.dk/biopv/php/fabox/), and deposited in GenBank (accession numbers: XXXX-

130   XXXX).

131

Phylogenetic Analyses

An intra-specific phylogeny was produced for a geographically stratified random subsample (*N*=24) from Indo-Pacific locations, and all individuals collected from the East Pacific and Red Sea (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 dataset 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 dataset, 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 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 created 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).

Phylogeographic Analyses

ARLEQUIN 3.5 (Excoffier *et al.*, 2010) was used to estimate haplotype (*h*) and nucleotide diversity (π) for COI, to test for hierarchical population structure using an analysis of molecular variance (AMOVA), and to calculate pairwise Φ_{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

166   relationships among COI haplotypes. We used the ISOLATION-BY-DISTANCE WEB

167   SERVICE 3.23 (Jensen *et al.*, 2005) to test for correlations between genetic and geographic

168   distances across the Western and Central Pacific (see Appendix S2).

169

170   **RESULTS**

171   **Phylogenetic structure**

172   Our phylogenetic analyses of three mtDNA loci revealed three divergent lineages with

173   Bayesian posterior probabilities of 1.0 that correspond to the East Pacific (ML bootstrap

174   = 52), Indo-Pacific (ML bootstrap = 97), and Red Sea (ML bootstrap = 71). The mean

175   K2P distance within lineages ranged from $d = 0.002$–0.004, while distances between

176   lineages were much deeper; $d = 0.011$–0.020. The East Pacific lineage was most distant

177   with $d = 1.8\%$ (vs. Indo-Pacific) and 2.0% (vs. Red Sea). The Red Sea and Indo-Pacific

178   lineages were less divergent with $d = 1.1\%$ (Fig. 3). Coalescent analyses indicate a

179   TMRCA for *P. penicillatus* and *P. echinatus* at 6.89 Ma with highest posterior density

180   (HPD) intervals that are narrower (95% HPD = 3.48-10.82 Ma) and are well within the

181   values found by Tourinho *et al.* (2012) (reported values, 95% HPD = 0.6-11.1 Ma).

182   TMRCA = 1.52 Ma (95% HPD = 0.8-2.31 Ma) for *P. penicillatus* lineages, while the

183   distinct lineages were of roughly equal ages with overlapping confidence intervals [East

184   Pacific, TMRCA = 0.22 Ma (95% HPD = 0.09-0.40 Ma); Indo-Pacific, TMRCA = 0.30

185   Ma (95% HPD = 0.17-0.46 Ma); Red Sea, TMRCA = 0.25 Ma (95% HPD = 0.10-0.47

186   Ma)].

187

188   **Phylogeographic structure**

189   We resolved 460bp of COI in 829 individuals from 35 locations (including the 55

190   GenBank sequences) across the species range. Number of individuals (*N*), haplotype

191   diversity (*h*), and nucleotide diversity (π) for each location are provided in Table 1.

192   Overall nucleotide diversity was low ($\pi = 0.007$) while haplotype diversity was high (*h* =

193   0.902). We detected 221 haplotypes; 139 (62.9%) were singletons. The most common

194   haplotypes were shared across all Indian and Pacific Ocean sites, but no haplotypes were

195   shared among the Indo-Pacific, East Pacific, and the Red Sea (Fig. 2).

196   Global population structure was $\Phi_{ST} = 0.310$ ($P < 0.001$), and $\Phi_{ST} = 0.018$ ($P <$

197   0.001) if the divergent Red Sea and East Pacific samples were excluded. Using the three

198   dominant marine biogeographic barriers within the tropical Indo-Pacific as a priori

199   divisions, we found significant differentiation among the Red Sea, Indian Ocean, Western

200   and Central Pacific Ocean, and East Pacific ($\Phi_{CT} = 0.744$, $P < 0.001$). When

201   biogeographic provinces were tested, we found significant population structure among

202   the provinces for both the Briggs & Bowen Provinces and Kulbicki Provinces

203   frameworks (Table 2). For samples within the range from the Indo-Pacific Barrier to the

204   East Pacific Barrier, $\Phi_{ST} = 0.018$ ($P < 0.001$), but the genetic variance across this range

205   was not partitioned significantly among island groups or among provinces of either

206   classification (Table 2); however, the Briggs & Bowen Sino-Japanese Province was

207   genetically distinct ($\Phi_{CT} = 0.074$, $P = 0.048$). There was no pattern of IBD across the

208   Indo-West Pacific (IWP) for any of the combinations tested (r = -0.202-0.123; $P = 0.161$-

209   0.976). Across the species' range, 83 of 190 (43.7%) of pairwise Φ_{ST} comparisons

210   between sampling locations were significant (Table 3), and significant Φ_{ST} values ranged

211   from 0.012 to 0.885. After correcting for false discovery rate (FDR), 76 of 83

212   comparisons remained significant (Table 3).

213

214   **DISCUSSION**

215   Our range-wide phylogeographic survey of *Panulirus penicillatus* reveals one of the

216   highest levels of genetic structure detected in spiny lobsters to date (overall $\Phi_{ST} = 0.310$,

217    $P < 0.001$). Despite an estimated 9-month PLD, *P. penicillatus* shows significant genetic

218   differentiation throughout the species range, including phylogenetic-level divergences

219   across two of the three major biogeographic barriers (EPB and Red Sea/Indian Ocean

220   split) at the edges of the species distribution. Across the IWP, genetic structure is

221   comparatively lower, but still significant ($\Phi_{ST} = 0.018$; $P < 0.001$). This pattern is driven

222   by restricted gene flow between the tropical Indo-Pacific and the single warm–temperate

223   region occupied by *P. penicillatus* (Briggs & Bowen's Sino-Japanese Province).

224   However, even within the tropical IWP, a few locations appear relatively isolated (e.g.,

225   Fiji, Samoa, Northwestern Hawaiian Islands (NWHI); Table 3). Below, we examine these

226   patterns in light of biogeographic theory and hypotheses regarding the evolution and

227   radiation of this species.

228

229   **East Pacific**

230   The EPB was long thought to be an 'impassable' barrier to dispersal for nearshore marine

231   species (Darwin, 1872; Mayr, 1954). Recent molecular data support this hypothesis for

232   both a trans-EPB coral (*Porites lobata*; Baums *et al.*, 2012; Forsman *et al.*, 2015), and

233 even a broadly distributed soldierfish (Craig *et al*., 2007). However, numerous fish

234 species have been documented on both sides of the EPB (reviewed in Robertson *et al.*,

235 2004). From the perspective of vicariance biogeography, these species ranges are

236 regarded as relics of pan-Tethyan species that were connected to the Caribbean through

237 the Central American corridor (McCoy & Heck, 1983). Alternatively, these distributions

238 could have been achieved via dispersal events from the IWP (Cortes, 1997). Molecular

239 evidence indicates recent gene flow for at least 16 fishes (Lessios & Robertson, 2006)

240 and an echinoderm (*Echinothrix diadema*; Lessios *et al.*, 1998). The magnitude, timing,

241 and direction of gene flow vary among these species, but it is primarily from the Central

242 to East Pacific.

243   For *P. penicillatus*, our data support a pan-Tethyan progenitor that subsequently

244 diverged during the late Miocene into the Pacific *P. penicillatus* and the Atlantic sister

245 species *P. echinatus*, as proposed by George (2005). Our coalescent analyses date the

246 TMRCA between *P. penicillatus* and *P. echinatus* at 6.89 Ma (HPD = 3.48-10.82 Ma).

247 Our confidence intervals overlap the 4.7 Ma TMRCA estimate of Tourinho *et al.* (2012).

248 During this time, gene flow between populations in the Atlantic and Pacific was likely

249 restricted because trans-oceanic water flow across the Panama Seaway decreased about 6

250 Ma (Collins *et al.*, 1996), and cessation occurred with the rise of the Isthmus of Panama

251 approximately 2.8 Ma (Coates & Obando, 1996). Coral reef fishes that originated in the

252 East Pacific are generally restricted to that region (Allen & Robertson 1994; Cowman &

253 Bellwood, 2013). *Panulirus penicillatus*, however, maintained broad connectivity across

254 the Pacific after the closure of the Panama Seaway: the divergence of East and West

255 Pacific lineages did not occur until 1.5 Ma (HPD = 0.89-2.3 Ma).

256   The East Pacific *P. penicillatus* populations remain phylogenetically distinct,

257 sharing no haplotypes with IWP populations ($d = 1.8\%$; Fig. 3). These findings extend

258 the results of Chow *et al.* (2011) who suggested that the EPB is a significant barrier to

259 gene flow for *P. penicillatus*. Here we add an additional East Pacific sample

260 (Revillagigedo Islands), and show there is no genetic structure between the Revillagigedo

261 Islands and the Galápagos ($\Phi_{ST} = -0.004$; $P = 0.451$). This result fits the Kulbicki

262 Provinces, which group these locations into the Offshore Tropical East Pacific Province,

263 rather than separating them as in the Briggs & Bowen classification (Fig. 1). This also

264 matches the species distribution data within the Panamanian Province: *P. penicillatus* is

265 rarely found east of the offshore islands (Holthuis, 1991). The lack of differentiation

266 between East Pacific locations, combined with the phylogenetic-level divergence and

267 morphological differences between the East Pacific and IWP forms (see Appendix S1)

268 corroborate the evolutionary distinction of the East Pacific form, and add support to the

269 hypothesis of George (2005) that the *P. penicillatus* 'red' morph is a distinct taxa,

270 isolated by the EPB. Despite a much longer PLD than species maintaining gene flow

271 across the EPB (Lessios & Robertson, 2006), *P. penicillatus* may have adapted larval

272 retention behaviors similar to other Panuliridae (Jeffs *et al.*, 2005; Butler *et al.*, 2011;

273 Iacchei *et al.*, 2013) to maintain local populations during the changing currents at the

274 beginning of the Pleistocene (as suggested by George, 2005).

275

276 **Red Sea**

277 The Red Sea is connected to the Indian Ocean via the constricted (18 km) and relatively

278 shallow (137 m) Strait of Bāb al-Mandab. Pleistocene glacial cycles repeatedly lowered

279 sea levels in the region, isolating populations due to physical separation at Bāb al-

280 Mandab with potential reinforcement by temperature and salinity changes in the Red Sea

281 (Siddall *et al.*, 2003; DiBattista *et al*., 2013). This combination of factors is likely

282 responsible for the elevated levels of endemism (Randall, 1994; DiBattista *et al*., 2015),

283 as well as the genetic distinction of Red Sea populations of crown-of-thorns seastars

284 (Benzie, 1999), mud crabs (Fratini & Vannini, 2002), and six of eight reef fishes

285 (DiBattista *et al.*, 2013; Fernandez-Silva *et al.*, 2015).

286 Red Sea *P. penicillatus* are smaller and have slower growth rates relative to *P.

287 penicillatus* in other parts of the IWP, leading to speculation that these populations are

288 distinct (Plaut & Fishelson, 1991). Alternatively, differences in size and growth rates may

289 be due to environmental conditions and/or prey regimes, as has been shown for congener

290 *P. marginatus* and the slipper lobster *Scyllarides squammosus* in Hawaiʻi (O'Malley *et

291 al.,* 2012).

292 Our genetic data indicate a phylogenetic separation between IWP and Red Sea *P.

293 penicillatus* (*d* = 1.1%; Fig. 3), corroborating data from Abdullah *et al.* (2014), and in

294 accordance with the Briggs & Bowen Red Sea province. The isolation of the Red Sea

295 population (0.7 Ma; HPD = 0.42-1.1 Ma) occurred after the divergence between the East

296 Pacific and the IWP *P. penicillatus* populations (1.5 Ma; HPD = 0.89-2.3 Ma), and was

297 likely due to sea level fluctuation in the mid-Pleistocene. This divergence may have been

298 subsequently reinforced by the differences in sizes and growth rates between these

299 populations (Plaut & Fishelson, 1991). Although *P. penicillatus* has been documented

300 throughout the Red Sea as far north as the Gulf of Aquaba (Holthuis, 1968), it remains to

301 be seen whether the Red Sea lineage extends to the adjacent Gulf of Aden, which shares

302 many endemic species with the Red Sea (DiBattista *et al*., 2015). Additional collections

303 from the Persian Gulf or the Gulf of Oman are required to evaluate whether this

304 population would encompass the North-western Indian Province (Kulbicki Provinces).

305 Studies of morphology and the nuclear genome may determine if the separation between

306 the Red Sea and the IWP has produced a Red Sea endemic.

307

308 **Indian Ocean**

309 The Indo-Pacific Barrier (IPB) is a widely recognized disjunction in marine faunal

310 distributions between the Indian and Pacific Ocean basins (Briggs, 1974). At the height

311 of the Pleistocene glaciation, sea level dropped up to 120 m below present depths,

312 creating a nearly complete land barrier between the two oceans (Voris, 2000; Naish *et al.*,

313 2009). This loss of shallow water habitat, combined with strong upwelling in the region

314 (Voris, 2000; Naish *et al.*, 2009), created a barrier between ocean basins and reduced

315 population sizes of shallow fauna on both sides of the IPB (Gaither & Rocha, 2013).

316 Signatures of isolation can be seen for a diversity of taxa (Reid *et al.*, 2006; Barber *et al.*,

317 2011; Gaither *et al.,* 2011a), with over 80% of species surveyed across the region

318 showing significant population structure (Gaither *et al.,* 2010), and few exceptions (e.g.,

319 Crandall *et al.*, 2008; Gaither *et al.*, 2010, 2011b).

320   Here, we find no genetic structure in *P. penicillatus* across the IPB ($\Phi_{CT} = 0.001$,

321 $P = 0.180$). Two of 12 pairwise comparisons between the Indian and Pacific Ocean sites

322 were significant, but not of greater magnitude than comparisons within the tropical

323 Pacific (Table 3). Increased sampling in the Indian Ocean will allow better resolution of

324 genetic connectivity in this region, though our results corroborate those of Abdullah *et al.*

325 (2014), who resolved the mtDNA control region for *P. penicillatus* samples from

326 Indonesia and the Maldives and found no significant pairwise genetic differentiation. The

327 lack of genetic structure across the IPB may be facilitated by adult habitat preference.

328 *Panulirus penicillatus* adults prefer windward surf zones with high wave energy (George,

329 1974; Pitcher, 1993), and will move into shallow (< 0.5m) surge channels on the reef flat

330 to forage at night (Pitcher, 1993). This habitat was likely common in areas of high

331 upwelling in the region, and the affinity for shallow, scoured shorelines may have

332 allowed adult *P. penicillatus* to persist during glacial cycles, and to maintain population

333 connectivity across the IPB.

334   Both Briggs & Bowen and Kulbicki province frameworks set the barrier between

335   the Indian and Pacific Oceans west of the IPB, indicating that both in terms of endemism

336   rates and overall species composition, the West Indian Ocean region is the most distinct

337   from the Pacific. For *P. penicillatus*, we did not detect any significant differentiation

338   between the WIO and the IWP (Φ_{CT} = 0.008, P = 0.14), although more sampling will

339   provide further insights. Notably, there may be population subdivision for *P. penicillatus*

340   at the southernmost end of the WIO. Abdullah *et al.* (2014) report significant pairwise

341   genetic differentiation for *P. penicillatus* between southern Madagascar and sites in the

342   Maldives and in Indonesia. The Southwest Indian Ocean (SWIO) also hosts divergent

343   lineages of two other spiny lobster species (Gopal *et al.,* 2006; Lavery *et al.,* 2014), and

344   isolated populations within other broadly distributed species (Castelin *et al.,* 2013;

345   Hoareau *et al.*, 2013), likely reinforced by the complex current regimes in the region.

346   Hoareau *et al.* (2013) provide evidence that the SWIO is a hotspot of evolutionary

347   diversification for brittle stars, a pattern that should be evaluated for additional taxa.

348

349   **Indo-Pacific**

350   The largest biogeographic region in the *P. penicillatus* distribution is the IWP, which

351   contains three of the eight Briggs & Bowen provinces and five of the eight Kulbicki

352   provinces (Fig. 1). This expanse of coral reefs and island archipelagos extends over half

353   the circumference of the globe, with no oceanic gap greater than 800 km from the

354   westernmost edge at the IPB to the Polynesian Islands in the east (Schultz *et al.*, 2008).

355   Most reefs throughout the region can be connected within the 30–40 day PLD typical of

356   reef fishes and invertebrates (Mora *et al.*, 2012). This hypothesis of highly connected

357   metapopulations of reefs has been corroborated by biophysical dispersal models (e.g.,

358   Treml *et al.*, 2008), population genetic and phylogenetic surveys of reef-associated

359   species (e.g., Craig *et al.*, 2007; Crandall *et al.*, 2008; Gaither *et al.*, 2010; Gaither *et al.*,

360   2011a), and the combination of dispersal models with genetic data (Crandall *et al.*, 2012).

361   There is significant genetic structure across the IWP for *P. penicillatus* (Φ_{ST} =

362   0.018; $P < 0.001$); however, this structure is not driven by differences among provinces

363   within either framework (Table 2), among archipelagos (Table 2), or by IBD. For

364   example, *P. penicillatus* at the Marquesas is not significantly differentiated from sites in

365   the Indo-Polynesian or Hawaiian provinces (Table 3), unlike many fish species (Bernardi

366   *et al.*, 2002; Planes & Fauvelot, 2002; Gaither *et al.,* 2010). Rather, there are specific

367   sites that are driving the pattern (the Commonwealth of the Northern Marianas Islands

368   (CNMI), Fiji, Samoa, and the NWHI; Table 3). The isolation of the most northern atolls,

369   the NWHI and CNMI, matches dispersal pathways predicted by Treml *et al.* (2008).

370   Similar site-specific, rather than regional patterns of genetic differentiation, have been

371   documented in five fishes (Drew *et al.*, 2008), and two *Panulirus* spp. hypothesized to be

372   panmictic based on long (>6 month) PLDs (Iacchei *et al.*, 2013, 2014). In isolation, these

373   significant pairwise comparisons may be statistical artifacts, but as more species are

374   surveyed across this geographic range, it will be informative to reassess potential patterns

375   (e.g., Toonen *et al*., 2011; Selkoe *et al.,* 2014), with a particular emphasis on utilizing

376   new methodologies to identify potential drivers of site-specific population differentiation

377   (e.g., Iacchei *et al.* 2013; Selkoe *et al.*, 2014; Gaither *et al.*, 2015).

378

379   **Sino-Japanese Province**

380   Within the Indo-Pacific, the most genetically distinct *P. penicillatus* population is in

381   Japan and Taiwan, aligning with the Briggs & Bowen provincial classification of this

382   region. The Sino-Japanese Province represents the northernmost range of *P. penicillatus*,

383   and is the only warm-temperate region inhabited by *P. penicillatus*. Few large-scale

384   phylogeographic studies include sampling from this region. The most well studied island

385   in this transition zone, Taiwan, has fish fauna on the northwestern coast affiliated with

386   the warm-temperate mainland, but fauna on the southeastern coast are tropical in origin

387   (Shao *et al.*, 1999). The persistence of tropical reef species, including *P. penicillatus,*

388   likely depends on the magnitude and location of the Kuroshio Current, a northerly-

389   flowing, western boundary current that originates near the tropical Philippines and

390   Taiwan (Ujiie *et al.*, 2003).

391   Population genetic separations of this region for *P. penicillatus* ($\Phi_{ST} = 0.047$-

392   0.268) are greater than most significant comparisons within the Indo-Pacific, including

393   sites separated by greater than ten thousand kilometres (Table 3). Our results are

394   concordant with findings of genetic structure for sea cucumbers (Skillings *et al.,* 2011)

395   and yellow tang (Eble *et al.,* 2011), but not soldierfish (Craig *et al.,* 2007). Surveys that

396   have sampled the Sino-Japanese Province and the tropical IWP are limited in number, but

397   the heretofore evidence of restricted gene flow or isolated populations in this

398   temperate/tropical transition zone suggest a fruitful topic for future phylogeographic

399   investigations.

400

401   **Conclusion**

402   Our genetic survey of the pronghorn spiny lobster *Panulirus penicillatus* indicates that

403   even a species with an extremely long pelagic larval duration (~9 months), and one of the

404   broadest distributions in the sea, is influenced by the same barriers to gene flow that have

405   facilitated speciation in other taxa. Genetic connectivity for *P. penicillatus* is only

406   minimally impeded by the ephemeral Indo-Pacific Barrier, but is clearly restricted by the

407   Red Sea/Indian Ocean divide, and most notably by the East Pacific Barrier. We find

408   genetic partitions are aligned with biogeographic provinces from both the Briggs &

409   Bowen and Kulbicki province frameworks (Offshore TEP, Sino-Japanese, Red Sea).

410   Notably, our data indicate potential for evolutionary divergence at the periphery of the

411   range, as demonstrated for other marine species across this region (e.g. Malay & Paulay,

412   2010; Gaither *et al.*, 2011b; Lavery *et al.*, 2014; DiBattista *et al.*, 2015; Gaither *et al.*,

413   2015). In light of this, the partition between the tropical Indo-Pacific and the warm-

414   temperate Sino-Japanese Province may emerge as a general pattern when more taxa are

415   examined.

416   While many *Panulirus* spp. have broad distributions, approximately half of the

417   Pacific species are archipelagic endemics or regionally restricted (Holthuis, 1991). These

418   species have likely evolved mechanisms to increase local retention within their restricted

419   ranges (reviewed in Jeffs *et al.*, 2005). Here, our data indicates that even the most broadly

420   distributed *P. penicillatus* may have evolved similar mechanisms for larval retention,

421   especially the East Pacific form, which warrants taxonomic recognition as the red form

422   described by George (2005).

423

424   **ACKNOWLEDGEMENTS**

435   manuscript. This is contribution 1634 from the Hawaiʻi Institute of Marine Biology, 9525

436   from SOEST, and 2015-XXX from UH Mānoa Biology Department.

437

438   **REFERENCES**

439   Abdullah, M.F., Muththalib, M., Salama, A.J. & Imai, H. (2014) Genetic isolation among

440   the Northwestern, Southwestern and Central-Eastern Indian Ocean populations of

441   the pronghorn spiny lobster *Panulirus penicillatus*. *International Journal of*

442   *Molecular Sciences*, **15**, 9242-9254.

443   Allen, G.R. & Robertson, D.R. 1994. *Fishes of the Tropical Eastern Pacific*. University

444   of Hawaii Press, Honolulu, HI.

445   Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring

446   intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

447   Barber, P.H., Cheng, S., Erdmann, M. & Tenggardjaja, K. (2011) Evolution and

448   conservation of marine biodiversity in the Coral Triangle: insights from

449   stomatopod Crustacea. *Crustacean Issues*, **19**, 129-156.

450   Baums, I.B., Boulay, J.N., Polato, N.R. & Hellberg, M.E. (2012) No gene flow across the

451   East Pacific Barrier in the reef-building coral *Porites lobata. Molecular Ecology,*

452   **21**, 5418–5433.

453   Benzie, J.A.H. (1999) Major genetic differences between crown-of-thorns starfish

454   (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution*, **53**,

455   1782–1795.

456   Bernardi, G., Holbrook, S.J., Schmitt, R.J., Crane, N.L. & DeMartini, E. (2002) Species

457   boundaries, populations and colour morphs in the coral reef three-spot damselfish

458   (*Dascyllus trimaculatus*) species complex. *Proceedings of the Royal Society B:*

459   *Biological Sciences*, **269**, 599–605.

460   Briggs, J.C. (1961) The East Pacific Barrier and the distribution of marine shore fishes.

461   *Evolution*, **15**, 545–554.

462   Briggs, J.C. (1974) *Marine zoogeography*. McGraw-Hill, New York, NY.

463   Briggs, J.C. & Bowen, B.W. (2012) A realignment of marine biogeographic provinces

464   with particular reference to fish distributions. *Journal of Biogeography*, **39**, 12–30.

465   Butler, M.J., Paris, C.B., Goldstein, J.S., Matsuda, H. & Cowen, R.K. (2011) Behavior

466   constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology*

467   *Progress Series*, **422**, 223–237.

468 Castelin, M., Feutry, P., Hautecoeur, M., Marquet, G., Wowor, D., Zimmermann, G. &
469   Keith, P. (2013) New insight on population genetic connectivity of widespread
470   amphidromous prawn *Macrobrachium lar* (Fabricius, 1798) (Crustacea:
471   Decapoda: Palaemonidae). *Marine Biology*, **160**, 1395-1406.

472 Chow S., Jeffs, A., Miyake, *et al*. (2011) Genetic isolation between the Western and
473   Eastern Pacific populations of pronghorn spiny lobster *Panulirus penicillatus*.
474   PLoS ONE, **6**, e29280.

475 Coates, A.G. & Obando, J.A. (1996) The geologic evolution of the Central American
476   isthmus. Evolution and environment in tropical America (ed. by J. Jackson, A.F.
477   Budd and A.G. Coates), pp. 21–56. University of Chicago Press, Chicago.

478 Collins, L.S., Coates, A.G., Berggren, W.A., Aubry, M.P. & Zhang, J. (1996). The late
479   Miocene Panama isthmian strait. *Geology*, **24**, 687–90.

480 Cortes, J. (1997) Biology and geology of eastern Pacific coral reefs. *Coral Reefs*, **16**,
481   S39–S46.

482 Cowman, P.F. & Bellwood, D.R. (2013) The historical biogeography of coral reef fishes:
483   global patterns of origination and dispersal. *Journal of Biogeography*, **40**, 209–
484   224.

485 Craig, M.T., Eble, J.A., Bowen, B.W. & Robertson, D.R. (2007) High genetic
486   connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis*
487   *berndti* (Holocentridae). *Marine Ecology Progress Series*, **334**, 245–254.

488 Crandall, E.D., Frey, M.A., Grosberg, R.K. & Barber, P.H. (2008) Contrasting
489   demographic history and phylogeographical patterns in two Indo-Pacific
490   gastropods. *Molecular Ecology,* **17,** 611–626.

491 Crandall, E.D., Treml, E.A. & Barber, P.H. (2012) Coalescent and biophysical models of
492   stepping-stone gene flow in neritid snails. *Molecular Ecology*, **21**, 5579-5598.

493 Darwin, C. (1872) *The origin of species by means of natural selection*, 6th ed. Doubleday
494   & Co., Garden City, NY.

495 DiBattista, J.D., Berumen, M.L., Gaither, M.R., Rocha, L.A., Eble, J.A., Choat, J.H.,
496   Craig, M.T., Skillings, D.J. & Bowen, B.W. (2013) After continents divide:
497   comparative phylogeography of reef fishes from the Red Sea and Indian Ocean.
498   *Journal of Biogeography*, **40**, 1170–1181.

499 DiBattista, J.D., Waldrop, E., Rocha, L.A., Craig, M.T., Berumen, M.L. & Bowen, B.W.
500   (2015) Blinded by the bright: a lack of congruence between colour morphs,
501   phylogeography and taxonomy for a cosmopolitan Indo-Pacific butterflyfish,

502   *Chaetodon auriga*. *Journal of Biogeography*, doi: 10.1111/jbi.12572.

503 DiBattista J.D., Roberts, M., Baird, A.H., *et al*. (2015) A review of contemporary patterns

504   of endemism in the Red Sea. *Journal of Biogeography*, in press.

505 Drew, J.A., Allen, G.R., Kaufman, L. & Barber, P.H. (2008) Regional color and genetic

506   differences demonstrate endemism in five putatively cosmopolitan reef fishes.

507   *Conservation Biology*, **22**, 965–975.

508 Drummond, A.J., Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by

509   sampling trees. *BMC Evoutionaryl Biology*, **7**, 214–221.

510 Eble, J.A., Toonen, R.J., Sorensen, L.L., Papastamatiou, Y. & Bowen, B.W. (2011)

511   Escaping paradise: larval export from Hawaii in an Indo-Pacific reef fish, the

512   yellow tang (*Zebrasoma flavescens*). *Marine Ecology Progress Series*, **428**, 245–

513   258.

514 Ekman, S. (1953) *Zoogeography of the sea*. Sidgwick and Jackson, London, UK.

515 Excoffier L., Laval G. & Schneider S. (2010) ARLEQUIN version 3.5: A new series of

516   programs to perform population genetics analyses under Linux and Windows.

517   *Molecular Ecology Resources*, **10**, 564–567.

518 Fernandez-Silva I., Randall, J.E., Coleman, R.R., DiBattista, J.D., Rocha, L.A., Reimer,

519   J.D., Meyer, C.G. & Bowen, B.W. (2015) Yellow tails in a Red Sea:

520   Phylogeography of the Indo-Pacific goatfish *Mulloidichthys flavolineatus* reveals

521   isolation in peripheral provinces and cryptic evolutionary lineages. *Journal of*

522   *Biogeography*, in press.

523 Forsman, Z., Wellington, G.M., Fox, G.E. & Toonen RJ. (2015) Clues to unraveling the

524   coral species problem: distinguishing species from geographic variation

525   in *Porites* across the Pacific with molecular markers and microskeletal

526   traits. *PeerJ* 3:e751.

527 Fratini, S. & Vannini, M. (2002) Genetic differentiation in the mud crab *Scylla serrata*

528   (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine*

529   *Biology and Ecology*, **272**, 103–116.

530 Gaither, M.R. & Rocha, L.A. (2013) Origins of species richness in the Indo-Malay-

531   Philippine biodiversity hotspot: evidence for the centre of overlap hypothesis.

532   *Journal of Biogeography*, **40**, 1638–1648.

533 Gaither, M.R., Toonen, R.J., Robertson, R.R., Planes, S. & Bowen, B.W. (2010) Genetic

534   evaluation of marine biogeographic barriers: perspectives from two widespread

535   Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). *Journal of*

536   *Biogeography*, **37**, 133–147.

537   Gaither, M.R., Bowen, B.W., Bordenave, T.R., Newman, S.J., Gomez, J.A., van

538   Herwerden, L. & Craig, M.T. (2011a) Phylogeography of the reef fish

539   *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the

540   Indo-Pacific Barrier with contemporary overlap in the Coral Triangle. *BMC*

541   *Evolutionary Biology*, **11**, 189.

542   Gaither, M.R., Jones, S.A., Kelley, C., Newman, S.J., Sorenson, L. & Bowen, B.W.

543   (2011b) High connectivity in the deepwater snapper *Pristipomoides filamentosus*

544   (Lutjanidae) across the Indo-Pacific with isolation in the Hawaiian Archipelago.

545   *PLoS ONE*, **6**, e28913.

546   Gaither, M.R., Bernal, M.A., Coleman, R.R., Bowen, B.W., Jones, S.A., Simison, W.B.

547   & Rocha, L.A. (2015) Genomic signatures of geographic isolation and natural

548   selection in coral reef fishes. *Molecular Ecology*, **24**, 1543-1557.

549   George, R.W. (1974) Coral reefs and rock lobster ecology in the Indo-West Pacific

550   region. *Proceedings of the 2nd. International Coral Reef Symposium*, **1,** 321-325.

551   George, R.W. (2005) Tethys sea fragmentation and speciation of *Panulirus* spiny lobsters.

552   *Crustaceana*, **78**, 1281–1309.

553   George, R.W. & Main, A.R. (1967) The evolution of spiny lobsters (Palinuridae): a study

554   of evolution in the marine environment. *Evolution*, **21,** 803–820.

555   Gopal, K., Tolley, K.A., Groeneveld, J.C. & Matthee, C.A. (2006) Mitochondrial DNA

556   variation in spiny lobster *Palinurus delagoae* suggests genetically structured

557   populations in the southwestern Indian Ocean. *Marine Ecology Progress*

558   *Series*, **319**, 191-198.

559   Hoareau, T. B.; Boissin, E.; Paulay, G. & Bruggemann, J. H. (2013) The Southwestern

560   Indian Ocean as a potential marine evolutionary hotspot: perspectives from

561   comparative phylogeography of reef brittle-stars. *Journal of Biogeography*,

562   **40**, 2167–2179.

563   Holthuis, L.B. (1968) The Palinuridae and Scyllaridae of the Red Sea. *Zoologische*

564   *Mededelingen (Leiden)*, **42**, 281–310.

565   Holthuis, L.B. (1991) Marine lobsters of the world: an annotated and illustrated catalogue

566   of species of interest to fisheries known to date. *FAO Fisheries Synopsis*, **13**, 1–

567   292. (FAO Species Catalogue, Rome).

568   Holthuis, L.B. & Loesch, H. (1967) The lobsters of the Galápagos Islands (Decapoda,

569   Palinuridae). *Crustaceana*, **12**, 214–222.

570 Iacchei, M., Ben-Horin, T., Selkoe, K.A., Bird, C.E., García-Rodríguez, F.J. & Toonen,
571　　　R.J. (2013) Combined analyses of kinship and F_{ST} suggest potential drivers of
572　　　chaotic genetic patchiness in high gene flow populations. *Molecular Ecology*, **22**,
573　　　3476–3494.

574 Iacchei, M., O'Malley, J.M. & Toonen, R.J. (2014) After the gold rush: population
575　　　structure of spiny lobsters in Hawai'i following a fishery closure and the
576　　　implications for contemporary spatial management. *Bulletin of Marine Science*,
577　　　**90**, 331–357.

578 Jeffs, A.G., Montgomery, J.C. & Tindle, C.T. (2005) How do spiny lobster post-larvae
579　　　find the coast? *New Zealand Journal of Marine and Freshwater Research*, **39**,
580　　　605–617.

581 Jensen, J.L., Bohonak, A.J. & Kelley, S.T. (2005) Isolation by distance, web service.
582　　　*BMC Genetics,* **6**, v.3.23 http://ibdws.sdsu.edu/

583 Johnson, M.W. (1968) Palinurid phyllosoma from the Hawaiian archipelago
584　　　(Palinuridae). *Crustaceana*, **S2**, 59–79.

585 Johnson, M.W. (1974) On the dispersal of lobster larvae into the East Pacific Barrier
586　　　(Decapoda, Palinuridea). *Fisheries Bulletin*, **72**, 639–647.

587 Keyse, J., Crandall, E.D., Toonen, R.J., Treml, E.A. & Riginos, C. (2013) The scope of
588　　　published population genetic data for Indo-Pacific marine fauna and future
589　　　research opportunities. *Bulletin of Marine Science*, **90**, 47–78.

590 Klausewitz, W. (1989) Evolutionary history and zoogeography of the Red Sea
591　　　ichthyofauna. *Fauna of Saudi Arabia*, **10**, 310–337.

592 Kulbicki, M., Parravicini, V., Bellwood, D.R., Arias-Gonzàlez, E., Chabanet, P., Floeter,
593　　　S.R., Friedlander, A., McPherson, J., Myers, R.E., Vigliola, L. & Mouillot, D.
594　　　(2013) Global biogeography of reef fishes: a hierarchical quantitative delineation
595　　　of regions. *PLoS One* 8:e81847 .

596 Lanfear, R., Calcott, B., Ho, S.Y. & Guindon, S. (2012). PartitionFinder: combined
597　　　selection of partitioning schemes and substitution models for phylogenetic
598　　　analyses. *Molecular Biology and Evolution*, **29**, 1695-1701.

599 Lavery, S.D., Farhadi, A., Farahmand, H., Chan, T.Y., Azhdehakoshpour, A., Thakur, V.
600　　　& Jeffs, A.G. (2014) Evolutionary divergence of geographic subspecies within the
601　　　scalloped spiny lobster *Panulirus homarus* (Linnaeus 1758). *PLoS ONE*, **9**,
602　　　e97247.

603 Lessios, H.A. & Robertson, D.R. (2006) Crossing the impassible: genetic connections in

604　　　　　20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society*

605　　　　　*B: Biological Sciences*, **273**, 2201–2208.

606　Lessios, H.A., Kessing, B.D. & Robertson, D.R. (1998) Massive gene flow across the

607　　　　　world's most potent marine biogeographic barrier. *Proceedings of the Royal*

608　　　　　*Society B: Biological Sciences,* **265**, 583–588.

609　Malay, M.C.M.D. & Paulay, G. (2010) Peripatric speciation drives diversification and

610　　　　　distributional pattern of reef hermit crabs (Decapoda: Diogenidae: *Calcinus*).

611　　　　　*Evolution*, **64**, 634-662.

612　Matsuda H., Takenouchi T. & Goldstein J.S. (2006) The complete larval development of

613　　　　　the pronghorn spiny lobster *Panulirus penicillatus* (Decapoda: Palinuridae) in

614　　　　　culture. *Journal of Crustacean Biology,* **26**, 579–600.

615　Mayr, E. (1954) Geographic speciation in tropical echinoids. *Evolution*, **8**, 1-18.

616　McCoy, E.D. & Heck, K.L. (1983) Centers of origin revisited. *Paleobiology*, **9**, 17–19.

617　McWilliam, P.S. (1995) Evolution in the phyllosoma and puerulus phases of the spiny

618　　　　　lobster genus *Panulirus* White. *Journal of Crustacean Biology*, **15,** 542–557.

619　Mora, C., Treml, E.A., Roberts, J., Crosby, K., Roy, D. & Tittensor, D.P. (2012) High

620　　　　　connectivity among habitats precludes the relationship between dispersal and

621　　　　　range size in tropical reef fishes. *Ecography*, **35**, 89–96.

622　Naish, T., Powell, R., Levy, R., *et al.* (2009) Obliquity-paced Pliocene West Atlantic ice

623　　　　　sheet oscillations. *Nature*, **458**, 322–328.

624　O'Malley, J.M., Drazen, J.C., Popp, B.N., Gier, E. & Toonen, R.J. (2012) Spatial

625　　　　　variability in growth and prey availability of lobsters in the northwestern

626　　　　　Hawaiian Islands. *Marine Ecology Progress Series*, **449**, 211–220.

627　Pitcher, C.R. (1993) Spiny Lobster. *Nearshore marine resources of the South Pacific:*

628　　　　　*Information for fisheries development and management* (ed. by A. Wright and L.

629　　　　　Hill), pp. 539-607. Institute of Pacific Studies, Suva.

630　Planes, S. & Fauvelot, C. (2002) Isolation by distance and vicariance drive genetic

631　　　　　structure of a coral reef fish in the Pacific Ocean. *Evolution*, **56**, 378–399.

632　Plaut, I. & Fishelson, L. (1991) Population structure and growth in captivity of the spiny

633　　　　　lobster *Panulirus penicillatus* from Dahab, Gulf of Aqaba, Red Sea. *Marine*

634　　　　　*Biology,* **111**, 467–472.

635　Ptacek, M.B., Sarver, S.K., Childress, M.J., Herrnkind, W.F. (2001) Molecular

636　　　　　phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). *Marine*

637　　　　　*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., ToBo Laboratory, 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., Schmelzer, 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 multispecies 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

672   commercially most important lobster in the tropical Atlantic, the spiny lobster

673   *Panulirus argus*. *Marine Biology*, **159**, 1897-1906.

674   Treml, E.A., Halpin, P.N., Urban, D.L. & Pratson, L.F. (2008) Modeling population

675   connectivity by ocean currents, a graph-theoretic approach for marine

676   conservation. *Landscape Ecology*, **23**, 19–36.

677   Truett, G.E., Mynatt, R.L., Truett, A.A., Walker, J.A. & Warman, M.L. (2000)

678   Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide

679   and Tris (HotSHOT). *BioTechniques*, **29**, 52–54.

680   Ujiie, Y., Ujiie, H., Taira, A., Nakamura, T., Oguri, K. (2003) Spatial and temporal

681   variability of surface water in the Kuroshio source region, Pacific Ocean, over the

682   past 21,000 years: evidence from planktonic foraminifera. *Marine*

683   *Micropaleontology*, **49**, 335–364.

684   Voris, H.K. (2000) Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river

685   systems and time durations. *Journal of Biogeography*, **27**, 1153–1167.

686   Wyrtki, K., Firing, E., Halpern, D., Knox, R., McNally, G.H., Patzert, W.C., Stroup, E.D.,

687   Taft, B.A. & Williams, R. (1981) The Hawaii to Tahiti shuttle experiment.

688   *Science*, **211**, 22–28.

689

SUPPORTING INFORMATION

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

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

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

694   **Appendix S3** Acknowledgements

695

DATA ACCESSIBILITY

697   DNA Sequences: GenBank accession numbers: XXXXX-XXXXX

698   **BIOSKETCH**

699   Matthew Iacchei is a post-doctoral researcher at the University of Hawaiʻi. This dataset is

700   part of his PhD dissertation in the Zoology Department at the University of Hawaiʻi at

701   Mānoa, entitled, "Population genetic and phylogeographic insights into the phyllosomal

702   odyssey", which was completed under the supervision of Robert J. Toonen and Brian W.

703   Bowen, with assistance and inspiration from Michelle R. Gaither.

704

705 Author contributions: M.I., M.R.G, B.W.B, and R.J.T conceived the ideas for this study,

706 collected samples, and contributed to the writing of the paper. M.I. produced the DNA

707 sequence data; M.I. and M.R.G. analysed the data; and M.I. was the primary author.

708

709 Editor: Gustav Paulay

Author Manuscript

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 (π). Sites are listed individually, and grouped by the biogeographic provinces as designated by Briggs and 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)	*N*	*h*	π	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	
	Indonesia (INDO)*	4	1.00	0.005	
	Torres Strait, Australia (TORRE)*	1	1.00	0.000	
	Palau (PALA)*	1	1.00	nc	**Central Pacific**
	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	**South-Western Pacific**
	New Caledonia (NCAL)*	33	0.90	0.005	
	Tuamotu Archipelago (TUAM)*	6	0.73	0.004	**Polynesian**
Marquesas	Marquesas Islands (MARQ)	35	0.93	0.005	
Hawaiian	Pearl and Hermes Reef (PEAR)	30	0.91	0.006	**Hawaiian**
	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	
	Kauaʻi (KAUA)	52	0.89	0.005	
	Molokaʻi (MOLO)	71	0.80	0.004	
	Lānaʻi (LANA)	35	0.85	0.004	
	Maui (MAUI)	24	0.75	0.004	
	Hawaiʻi (HAWA)	47	0.89	0.005	
Galápagos	Galápagos Islands (GALA)*	10	0.78	0.005	**Offshore TEP**
Panamanian	Revillagigedo Islands, Mexico (REVI)	18	0.84	0.015	

Table 2 AMOVA results showing degrees of freedom (df), sum of squares (SS), variance components (Var), Φ-statistics (Φ_{CT}) and *P*-values (*P*) for each biogeographic framework tested for *Panulirus penicillatus*. Bold values indicate significant differences at *P* < 0.050. All comparisons remained significant after correcting for false discovery rate (*P* > 0.0476). The major biogeographic barriers, and marine biogeographic 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	**df**	**SS**	**Var**	$\Phi_{CT/ST}$	*P*
Rangewide	26	419.20	0.531	**0.310**	**<0.001**

Indo-Pacific Rangewide (without Red Sea and East Pacific clades)	23	40.77	0.021	**0.018**	**<0.001**
Major Biogeographic Barriers (Red Sea/Indian Ocean/Western-Central Pacific/East Pacific)	3	377.51	3.499	**0.744**	**<0.001**
Briggs & Bowen Provinces: (Red Sea, Western Indian Ocean, Sino-Japanese, Indo-Polynesian, Marquesas, Hawaiian, Galápagos, Panamanian)	7	386.42	0.732	**0.379**	**0.025**
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	**0.356**	**0.003**
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 a 460bp fragment of the mtDNA cytochrome *c* oxidase subunit I region (COI). Φ_{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 biogeographic 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	0.002	<0.001	<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
	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
Central	**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
and West	**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
Pacific	**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	**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
Pacific	**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 LEGENDS

Figure 1 Collection locations and sample sizes for *Panulirus penicillatus* across the Indo-Pacific. Colours indicate the biogeographic provinces within which sample sites have been grouped following: (a) Briggs and Bowen (2012), and (b) Kulbicki *et al.* (2013). The three predominant biogeographic 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). In parentheses is the total number of individuals sequenced across all of the islands in that island group. Several islands were sampled in some of the island groups: Commonwealth of the Northern Marianas Islands (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 (Kauaʻi, Molokaʻi, Lānaʻi, Maui, Hawaiʻi). These sample sites were combined into the island groups after determining that there was no significant genetic structure within that group. Samples sizes and diversity statistics for each of these 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.

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 the number of individuals that have that haplotype. The smallest circle represents two individuals; the largest represents 240 individuals. Haplotypes are colour-coded according to the Briggs & Bowen (2012) biogeographic provinces where they were found. All haplotypes are separated by one base pair difference unless labeled with a number greater than one.

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

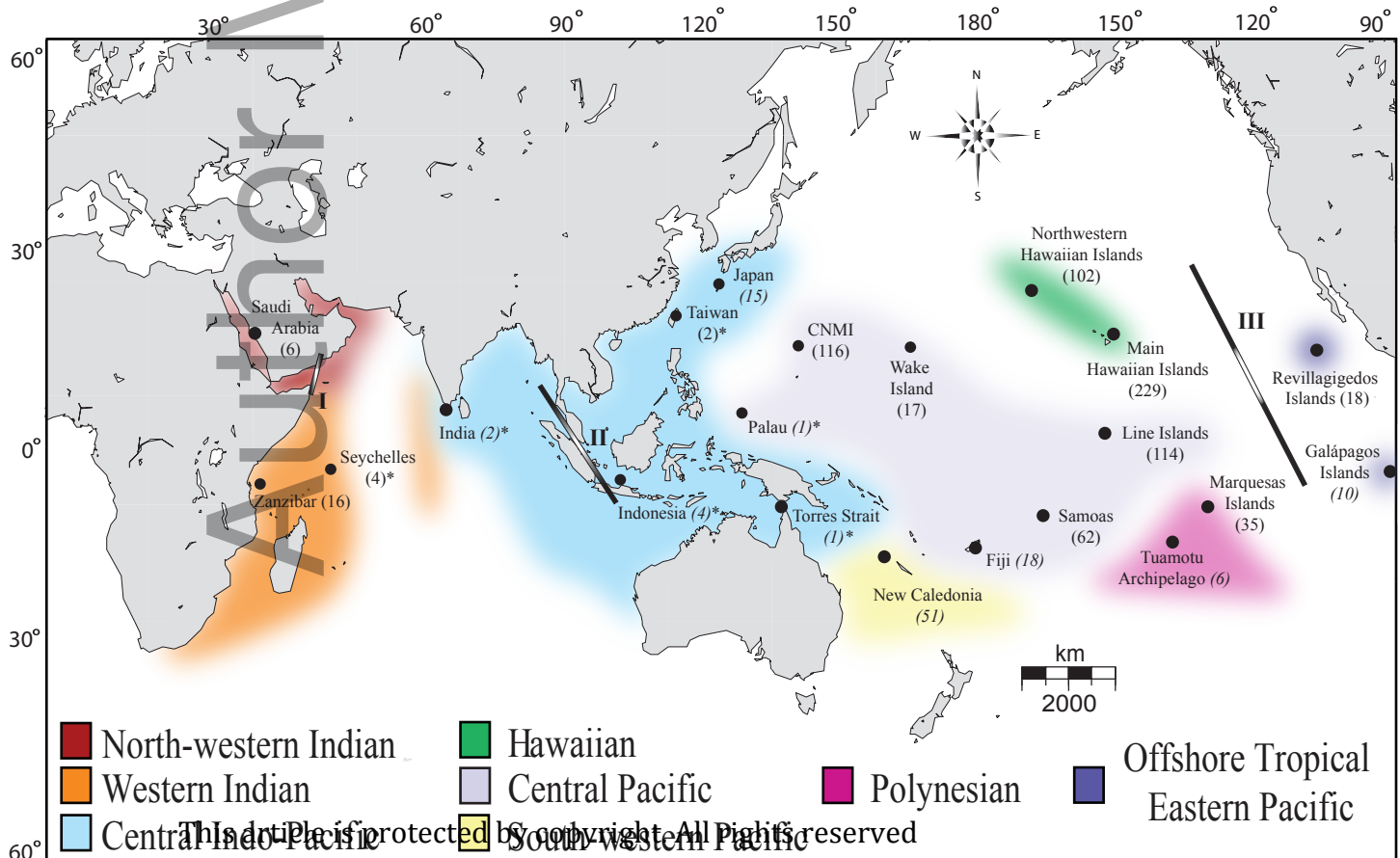slash) and bootstrap branch support for maximum likelihood analyses (after slash) are indicated above a cut off of 0.5 and 50, respectively. Node ages are presented as median node heights with 95% HPD intervals represented by bars.

Author Manuscript

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

Legend:
- Red Sea
- Western Indian Ocean
- Indo-Polynesian
- Sino-Japanese
- Marquesas
- Hawaiian
- Galápagos
- Panamanian

Localities (panel a):
Saudi Arabia (6), Seychelles (4)*, Zanzibar (16), India (2)*, Japan (15), Taiwan (2)*, CNMI (116), Wake Island (17), Palau (1)*, Indonesia (4)*, Torres Strait (1)*, Line Islands (114), Samoas (62), Fiji (18), New Caledonia (51), Tuamotu Archipelago (6), Marquesas Islands (35), Galápagos Islands (10), Revillagigedos Islands (18), Main Hawaiian Islands (229), Northwestern Hawaiian Islands (102)

km 2000

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

Legend:
- North-western Indian
- Western Indian
- Central Indo-Pacific
- Hawaiian
- Central Pacific
- South-western Pacific
- Polynesian
- Offshore Tropical Eastern Pacific

Localities (panel b):
Saudi Arabia (6), Seychelles (4)*, Zanzibar (16), India (2)*, Japan (15), Taiwan (2)*, CNMI (116), Wake Island (17), Palau (1)*, Indonesia (4)*, Torres Strait (1)*, Line Islands (114), Samoas (62), Fiji (18), New Caledonia (51), Tuamotu Archipelago (6), Marquesas Islands (35), Galápagos Islands (10), Revillagigedos Islands (18), Main Hawaiian Islands (229), Northwestern Hawaiian Islands (102)

km 2000

Red Sea
Western Indian Ocean
Indo-Polynesian
Sino-Japanese
Marquesas
Hawaiian
Galápagos
Panamanian

Panulirus echinatus

1.0/52

P. penicillatus East Pacific

1.0

1.0/100

P. penicillatus Red Sea

1.0/71

1.0/88

P. penicillatus Indo-Pacific

1.0/97

| 10.0 | 9.0 | 8.0 | 7.0 | 6.0 | 5.0 | 4.0 | 3.0 | 2.0 | 1.0 | 0.0 |

Time (Ma)