Received Date : 02-Jan-2015
Revised Date : 03-Oct-2015
Accepted Date : 19-Oct-2015
Article type : Special Issue

For the virtual issue, "Red Sea and Western Indian Ocean Biogeography"

Original Article

LRH: E. Waldrop *et al*.

RRH: Phylogeography of *Corallochaetodon* butterflyfishes

Phylogeography, population structure and evolution of coral-eating butterflyfishes (Family Chaetodontidae, genus *Chaetodon*, subgenus *Corallochaetodon*)

Ellen Waldrop[1], Jean-Paul A. Hobbs[2], John E. Randall[3], Joseph D. DiBattista[2,4], Luiz A. Rocha[5], Randall K. Kosaki[6], Michael L. Berumen[4] and Brian W. Bowen[1*]

[1]*Hawai'i Institute of Marine Biology, Kane'ohe, HI 96744, USA,* [2]*Department of Environment and Agriculture, Curtin University, PO BOX U1987, Perth, WA 6845, Australia,* [3]*Bishop Museum, Honolulu, HI 96817, USA,* [4]*Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Thuwal 23955, Saudi Arabia,* [5]*Section of Ichthyology, California Academy of Sciences, San Francisco, CA 94118, USA,* [6]*Papahānaumokuākea Marine National Monument, NOAA/Daniel K. Inouye Regional Center, Honolulu, HI 96818, USA*

*Correspondence: Brian W. Bowen, Hawai'i Institute of Marine Biology, P.O. Box 1346, Kane'ohe, HI, 96744, USA.
E-mail: bbowen@hawaii.edu

Word count: 6975

ABSTRACT

Aim This study compares the phylogeography, population structure and evolution of four butterflyfish species in *Chaetodon* subgenus *Corallochaetodon*, with two widespread species (Indian Ocean – *C. trifasciatus* and Pacific Ocean - *C. lunulatus*), and two species that are largely restricted to the Red Sea (*C. austriacus*) and northwestern (NW) Indian Ocean (*C. melapterus*). Through extensive geographical coverage of these taxa, we seek to resolve patterns of genetic diversity within and between closely-related butterflyfish species in order to illuminate biogeographical and evolutionary processes.

Location Red Sea, Indian Ocean and Pacific Ocean.

Methods A total of 632 individuals from 24 locations throughout the geographical ranges of all four members of the subgenus *Corallochaetodon* were sequenced using a 605 bp fragment (cytochrome *b*) of mtDNA. In addition, 10 microsatellite loci were used to assess population structure in the two widespread species.

Results Phylogenetic reconstruction indicates that the Pacific Ocean *C. lunulatus* diverged from the Indian Ocean *C. trifasciatus* approximately 3 million years ago, while *C. melapterus* and *C. austriacus* comprise a cluster of shared haplotypes derived from *C. trifasciatus* within the last 0.75 Myr. The Pacific *C. lunulatus* had significant population structure at peripheral locations on the eastern edge of its range (French Polynesia, Johnston Atoll, Hawai‘i), and a strong break between two ecoregions of the Hawaiian Archipelago. The Indian Ocean *C. trifasciatus* showed significant structure only at the Chagos Archipelago in the central Indian Ocean, and the two range-restricted species showed no population structure but evidence of recent population expansion.

Main conclusions Patterns of endemism and genetic diversity in *Corallochaetodon* butterflyfishes have been shaped by 1) Plio-Pleistocene sea level changes that facilitated evolutionary divergences at biogeographical barriers between Indian and Pacific Oceans, and the Indian Ocean and Red Sea, and 2) semi-permeable oceanographic and ecological barriers working on a shorter timescale. The evolution of range-restricted species (Red Sea and NW Indian Ocean) and isolated populations (Hawai‘i) at

peripheral biogeographic provinces indicates that these areas are evolutionary incubators for reef fishes.

Keywords

biogeography, *Chaetodon austriacus, Chaetodon lunulatus, Chaetodon melapterus, Chaetodon trifasciatus*, microsatellites, mtDNA, reef fish, speciation**INTRODUCTION**

How do new species with high dispersal potential arise in an aquatic medium? The Indo-Pacific reef fishes have two biogeographic traits that inform this issue. First, the biodiversity of fishes and other coral-associated species peaks at the central Indo-Australian Archipelago, where Indian and Pacific Ocean faunas overlap (Blum, 1989; Gaither & Rocha, 2013). Second, the highest endemism is in peripheral regions at the ends of the range, including the Red Sea and Hawai‘i (Randall, 1998). Evidence supporting genetic differentiation in peripheral biogeographical regions comes from both peripheral locations, which are the western and eastern limits for numerous Indo-Pacific species (DiBattista *et al.*, 2013; Eble *et al.*, 2015). Phylogeographical studies indicate that new species are arising in both the peripheral regions and the biodiversity centre (Bowen *et al.*, 2013). However, few studies have focused on diversification in the Red Sea and northwestern (NW) Indian Ocean.

The well-resolved phylogeny of butterflyfishes (family Chaetodontidae), has made this group an appropriate model for understanding the evolution of reef fishes (Fessler & Westneat, 2007; Cowman & Bellwood, 2013; Hodge *et al*., 2014). Butterflyfishes embody the primary biogeographic patterns outlined above, with greatest diversity in the Indo-Australian Archipelago and highest endemism in peripheral areas. The Red Sea and adjacent Gulf of Aden has 32% endemism in butterflyfishes, compared to 13% in Hawai‘i and < 10% elsewhere in the Indo-Pacific (Randall, 2007; DiBattista *et al.*, in review). Understanding how the highest levels of endemism arose far from the center of diversity remains an enigma. Biogeographical barriers at these locations may have created isolated populations or endemic species depending on the divergence time (Briggs & Bowen, 2013).

Among butterflyfishes, the subgenus *Corallochaetodon* contains four corallivorous species that have mostly parapatric distributions with narrow areas of overlap on the range edges (Fig. 1). *Chaetodon lunulatus* Quoy & Gaimard, 1824 occurs throughout the Pacific Ocean from Hawai‘i and the Tuamotu Islands westward to Indonesia and the eastern Indian Ocean (Christmas Island), while *Chaetodon trifasciatus* Park, 1797 is distributed in the Indian Ocean from Indonesia and Christmas Island to East

Africa, but is not known from the Red Sea (Allen *et al*., 1998). *C. lunulatus* and *C. trifasciatus* may be Indian-Pacific Ocean sister species that diverged during Plio-Pleistocene sea level changes that created the transient Sunda Shelf Barrier (Hsu *et al*., 2007). *Chaetodon melapterus* Guichenot, 1863 is restricted to the Arabian Gulf, Gulf of Oman, Gulf of Aden and the southern Red Sea, while *Chaetodon austriacus* Rüppell, 1836 occurs predominantly in the northern and central Red Sea (Zekeria *et al.*, 2005), with rare records in the southern Red Sea and adjacent Arabian Sea (DiBattista *et al.*, in review). It is unknown if the two range-restricted species (*C. melapterus* and *C. austriacus*) arose independently, and whether they evolved from the widespread Indian Ocean species *C. trifasciatus*, as current geographic distributions would indicate. Thus the subgenus *Corallochaetodon* provides the opportunity to determine how the speciation of butterflyfishes in peripheral locations (*C. melapterus* and *C. austriacus*) compares to that in the center of diversity (*C. lunulatus* and *C. trifasciatus*).

This study is motivated by four primary questions. First, what is the evolutionary history of the subgenus *Corallochaetodon*? Second, what are the geographical patterns of genetic diversity within and between species? Third, what is the population structure (as revealed by mtDNA) of all four species across their geographical ranges? Fourth, what is the fine-scale population structure (as revealed by microsatellite DNA) in the two widespread species (*C. lunulatus* and *C. trifaciatus*), and is there evidence of peripheral speciation? These genetic patterns can illuminate the origins of marine biodiversity, and the measures that would conserve building blocks of future biodiversity.

MATERIALS AND METHODS

Sample Collection

Tissue (fin clips or gill filament) were obtained from specimens collected using polespears whilst SCUBA diving at 24 locations across the Indo-Pacific (including the Red Sea) from 2005 to 2013 (*C. lunulatus N* = 603, *C. trifasciatus N* = 143, *C. melapterus N* = 95, *C. austriacus N* = 30) (Table 1). *Chaetodon lunulatus* was intensively sampled in the Hawaiian Archipelago to assess connectivity across this 2600 km island chain. All tissues were preserved in a saturated salt DMSO solution (Seutin *et al*., 1991). DNA was extracted using a "HotSHOT" protocol (Meeker *et al*., 2007), and aliquots were stored at -20 ˚C.

Mitochondrial DNA Sequencing

A 605 base pair (bp) segment of mtDNA cytochrome *b* (cyt *b*) gene was resolved for all specimens. Details of the PCR methodology are available in Appendix S1 and Waldrop (2014). The cyt *b* data comprises a single locus but offers the advantage of haploid inheritance, lack of recombination, comparison to existing studies and availability of universal primers for efficient production of sequence data. Unique mtDNA cyt *b* haplotypes are deposited in GenBank under accession numbers KP241594 to KP241672.

Phylogenetic relationships

Phylogenetic relationships were examined among the four species by constructing neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) trees from the cyt *b* haplotypes of all individuals (PAUP*, Swofford, 2003, implemented in Geneious Pro 6.0.6, Drummond *et al.*, 2010, and MEGA 5.2.2, Tamura *et al*., 2011). Bootstrap support values were calculated using default settings with 10,000 replicates in both packages. A single *Chaetodon vagabundus* Linnaeus, 1758 sample (Genbank accession numbers: JF458006) was used to root trees. For simplicity, a subset of unique haplotypes was used to create the final tree. An unrooted network of haplotypes was also assembled using a median-joining algorithm and default settings in NETWORK 4.5.1.0 (Bandelt *et al*., 1999). Molecular clock rate is provisionally estimated at 2% per Myr (between lineages) for the cyt *b* gene (Bowen *et al*., 2001; Reece *et al*., 2011). Evolutionary distances among lineages were calculated with the Tamura-Nei model and 1,000 bootstrap replicates in MEGA.

Population structure for mtDNA

An Akaike information criterion (AIC) test in jModelTest 2.1.3 (Posada, 2008) was used to determine the best nucleotide substitution model for each species. The HKY model (Hasegawa *et al*., 1985) was selected for *C. lunulatus*, *C. trifasciatus* and *C. austriacus*, and TrN+G (Tamura & Nei, 1993) was selected for *C. melapterus*. The TrN+G is the only one of these models available in Arlequin 3.5.1.3 (Excoffier *et al*., 2005) analytical software and was selected for all phylogeographical inferences. Arlequin was used to calculate haplotype (*h)* and nucleotide diversity *(π)*, Fu's *Fs* test of neutrality (Fu, 1997) and to apply an analysis of molecular variance (AMOVA; Excoffier *et al*., 1992) to test for patterns of population structure; tests were run for each species separately. Samples with $N < 5$ were excluded from all population-level analyses and pooled into their respective larger sampling locations to provide adequate statistical power. Hawaiian specimens of *C. lunulatus* were subdivided into the Main Hawaiian Islands (MHI, high islands) and Northwestern Hawaiian Islands (NWHI, low islands

and atolls) to test for genetic structure within the archipelago. *C. trifasciatus* specimens from the eastern Indian Ocean (Cocos-Keeling Islands and adjacent Christmas Island) were pooled to increase statistical power as they were indistinguishable in preliminary analyses.

Population structure - microsatellites

Microsatellite primers were designed for *C. lunulatus* by Lawton *et al.* (2010; 2011). Here the widespread *C. lunulatus* and *C. trifasciatus* were genotyped at 10 loci (Table S1.1 in Appendix S1). The range-restricted *C. melapterus* and *C. austriacus* were not genotyped because large samples were not available, finances were limited and cross-species applications can be complicated by allele dropout, homoplasy and other problems (see Selkoe & Toonen, 2006). Details of PCR amplifications are available in Appendix S1 and Waldrop (2014). Initially specimens from Hawai‘i were separated into individual sampling locations by island. However mtDNA data revealed a genetic break between the MHI and NWHI concordant with a multi-species connectivity study (Toonen *et al.*, 2011). For subsequent analyses, Hawai‘i was partitioned into two groups; MHI and NWHI. However, a full comparison among Hawaiian sample sites is provided in Table S2.1 in Appendix S2.

For each locus the mean number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, departure from Hardy-Weinberg proportions (HWE) and linkage disequilibrium (LD) were assessed with GENEPOP 4.2 (Raymond & Rousset, 1995). MICRO-CHECKER 2.2.3 was used to identify null alleles and excessive stutter peaks (van Oosterhout *et al*., 2004), and significance levels for multiple comparisons were adjusted using the sequential Bonferonni correction. GENODIVE 2.0b23 (Meirmans & Tienderen, 2004) was used to estimate population structure for each species. STRUCTURE 2.3.4 was used to assign individuals to distinct genetic clusters (populations) without presumption of predefined geographical locations (Pritchard *et al*., 2000). The most likely number of clusters was identified based on the probability of $K = 1$ to $K = 12$ or $K = 1$ to $K = 4$ for *C. lunulatus* and *C. trifasciatus*, respectively. Analyses were repeated five times and averaged. Each replicate run consisted of 1,000,000 MCMC repetitions, a burn-in of 10,000 iterations and assumed correlated allele frequencies with admixed populations (as per DiBattista *et al*., 2012). STRUCTURE HARVESTER 0.6.93 was used to determine most likely value of *K* following Evanno *et al*. (2005) to visualize likelihood values and the number of groups that best fit the data (Earl & von Holdt, 2012).

RESULTS

Phylogenetic relationships

The authors recognize the limitations of a single-locus phylogeny, and so here we provide the mtDNA results as an initial hypothesis of relationships among the four species. All tree-building methods used to analyze the mtDNA cyt *b* fragment (605 bp) produced nearly identical tree topologies with bootstrap support values for species level relationships of 80 to 100% (Fig. 2). The primary feature of this phylogeny is a bifurcation with $d = 0.06$ sequence divergence between Pacific Ocean *C. lunulatus* and the Indian Ocean *C. trifasciatus*. The two range-restricted species, *C. melapterus* and *C. austriacus*, are more closely related to the Indian Ocean species ($d = 0.015$). However, they did not form monophyletic groups, and share the most common haplotype (Fig. 2). The relationship within the subgenus *Corallochaetodon* is apparent in the parsimony network (Fig. 3), where Pacific Ocean *C. lunulatus* and Indian Ocean *C. trifasciatus* are separated by 28 diagnostic nucleotide substitutions, and the *C. melapterus–C. austriacus* cluster is separated from *C. trifasciatus* by three diagnostic nucleotide substitutions.

Genetic diversity

Haplotype diversity within each species was moderate to high (*C. lunulatus* $h = 0.45$ to 0.87; *C. trifasciatus* $h = 0.67$ to 0.80; *C. melapterus* $h = 0.63$ to 0.78; *C. austriacus* $h = 0.84$ to 0.87; Table 1). For the species with the largest geographic range (*C. lunulatus*), haplotype diversity was highest at the peripheral location on the western edge of its range (Christmas Island), and was generally lowest at peripheral locations on the eastern edge of its range (Johnston Atoll, Main Hawaiian Islands - MHI, Northwestern Hawaiian Islands - NWHI). For *C. trifasciatus*, haplotype diversities are similar across the range. In the two range-restricted species (*C. melapterus*, and *C. austriacus*), haplotype diversity was lower at one sampled location (Table 1). Nucleotide diversity was low for all species (*C. lunulatus* $\pi = 0.001$ to 0.005; *C. trifasciatus* $\pi = 0.001$ to 0.088; *C. melapterus* $\pi = 0.000$ to 0.001; *C. austriacus* $\pi = 0.000$ to 0.002; Table 1), indicating a cluster of closely-related haplotypes within each species.

For the two widespread species, only one of the 17 sample locations was significant for Fu's *Fs* (*C. trifasciatus* at Diego Garcia). For the two range-restricted species, tests for Fu's *Fs* could only be conducted on samples from five locations and all produced significant negative values: *C. melapterus* at Maskali, Obock and Oman; *C. austriacus* at Jazirat Baraqan and Yanbu (Table 1).

Population structure (mtDNA)

Significant population structure was observed in *C. lunulatus* (overall Φ_{ST} = 0.27; P < 0.001). In comparisons among sample locations, 30 out of 78 pairwise comparisons were statistically significant (P < 0.05; Table 2). Five locations accounted for all the significant comparisons: Fiji with 6 out of 12 significant comparisons, Johnston Atoll with 3 out of 12 significant comparisons, Mo‘orea (French Polynesia) with 12 out of 12 significant comparisons, MHI with 5 out of 12 significant comparisons and the NWHI with 12 out of 12 significant comparisons (Table 2). Within the Hawaiian Archipelago, there were 13 out of 28 significant comparisons among sample locations (Table S2.1 in Appendix S2). All of the significant comparisons were among the three southernmost sampled locations (Hawai‘i Island, O‘ahu and French Frigate Shoals) and the most northern sample location (Kure Atoll).

No significant structure overall or significant pairwise comparisons were detected among four locations in *C. trifasciatus* (Φ_{ST} = 0.01; P = 0.50), four locations in *C. melapterus* (Φ_{ST} = 0.01; P = 0.16), or three locations in *C. austriacus* (Φ_{ST} = 0.04; P = 0.21) (Table 3). However, *C. melapterus* and *C. austriacus* were significantly isolated at a population level (Φ_{ST} = 0.06; P = 0.001). Notably, we did not sample *C. melapterus* in the Arabian Gulf and along the Somalian coastline due to logistical limitations; additional sampling in these regions could change conclusions about population structure.

Population structure (msatDNA) within *C. lunulatus* and *C. trifasciatus*

Significant population structure was also detected for *C. lunulatus* using msatDNA (F_{ST} = 0.05, P = 0.001). The msatDNA results were similar to that of mtDNA with most of the significant pairwise comparisons involving locations on the eastern edge of the geographic range: Johnston Atoll, Mo‘orea, MHI and the NWHI. Microsatellite allele frequencies were significantly different in 49 out of 91 comparisons for *C. lunulatus* (Table 4; see also Table S2.1 in Appendix S2).

For *C. lunulatus*, STRUCTURE identified mean probabilities as being highest at K = 3 (Fig. 4), which was verified using STRUCTURE HARVESTER (Fig. S2.1 in Appendix S2). One widespread population spanned locations from the western range edge (Christmas Island) eastward to Kiribati in the central Pacific Ocean. The second population was comprised predominately of individuals from isolated locations on the eastern range edge: Johnston Atoll, MHI and the NWHI. The third population was largely restricted to the NWHI.

The msatDNA data revealed low but significant population structure for *C. trifasciatus* (F_{ST} = 0.003, *P* = 0.03). Microsatellite allele frequencies were significantly different in three out of six comparisons (Table 5), between Diego Garcia and all the other sampled locations (Seychelles, Christmas Island and Indonesia). Microsatellite statistics for each location and both species are provided in Table S2.2 in Appendix S2. STRUCTURE identified mean probabilities as being highest at *K* = 2 (Fig. 5), which was consistent with the results from STRUCTURE HARVESTER (Fig. S2.2 in Appendix S2), indicating isolation of Diego Garcia but no distinction of samples from the east (Christmas Island, Indonesia) and west (Seychelles) of this remote location in the Chagos Archipelago. Overall, there was no consistent evidence for departure from HWE, linkage disequilibrium or null alleles across all sampled locations in both species.

DISCUSSION

Phylogenetic relationships

The primary phylogenetic feature of the subgenus *Corallochaetodon* is mtDNA sequence divergence of *d* = 0.06 between Indian Ocean *C. trifasciatus* and Pacific *C. lunulatus*. Based on the conventional molecular clock of 2% per Myr, this corresponds to approximately 3 Myr of separation (Table S2.3 in Appendix S2) (consistent with Hsu *et al*., 2007; Bellwood *et al*., 2010), which is close to the onset of modern glacial cycles at 2.6 to 2.8 Ma (Dwyer *et al*., 1995; Williams *et al*., 1997). The shallow Sunda Shelf is exposed during glacial periods with low sea levels, forming land bridges through the Indonesian Archipelago that restricted exchange between the Indian and Pacific Oceans (Randall, 1998; Rocha *et al*., 2007). This indicates that transient allopatry may have a role in the formation of this species pair, a process that is apparent (or suspected) in other Indian-Pacific species pairs (Gaither & Rocha, 2013).

A divergence time of approximately 3 Myr for *C. trifasciatus* and *C. lunulatus* falls within the range of divergence times (0.3 – 6.6 Myr) for other Indian and Pacific sister species of reef fishes (Gaither & Rocha, 2013). However, divergence times in other Indian and Pacific Ocean butterflyfish sister species tend to be less (0.3 – 1.4 Myr) (Fessler & Westneat 2007; Hsu *et al.*, 2007; Bellwood *et al*., 2010; DiBattista *et al*., 2012). Variation in divergence times may be due to a number of factors including: 1) potential differences in mutation rates; 2) the intermittency of the Sunda Shelf Barrier during the Pleistocene due to repeated glacial cycles (i.e. different species pairs diverged at different low sea level

281 stands); and 3) the conditions determining secondary contact and reproductive isolation affected
282 species differently.
283
284 The range-restricted *C. austriacus* and *C. melapterus* share a common haplotype, and are closely
285 affiliated with *C. trifasciatus* (d = 0.015). The divergence between *C. trifasciatus* and the range-
286 restricted species is approximately 0.75 Myr (Table S2.3 in Appendix S2), which corresponds with
287 Pleistocene sea level changes that repeatedly isolated the Red Sea region from the Indian Ocean (Fig.
288 1; Blum, 1989; DiBattista *et al.*, 2013). Furthermore, strong upwelling in the NW Indian Ocean (off the
289 southern Oman coast) may facilitate allopatric divergence between species from the Indian Ocean (e.g.
290 *C. trifasciatus*) and Red Sea to Arabian Gulf region (*C. austriacus* and *C. melapterus*).
291
292 While the monophyly of *C. austriacus* and *C. melapterus* could not be corroborated, these two putative
293 species are genetically distinct at a population level (Φ_{ST} = 0.06; P = 0.001) indicating either early
294 stages of speciation or distinct colour morphs separated by habitat discontinuities. This finding should
295 be interpreted in light of the relatively recent origins of reef faunas inhabiting the Red Sea (DiBattista
296 *et al*., 2013) and Arabian Gulf (Sheppard *et al*., 2010). Estimated time since divergence is
297 approximately 50 kyr, and was likely initiated by vicariant isolation at the Strait of Bab al Mandab (at
298 the mouth of the Red Sea – Fig. 1). This barrier flooded about 20 ka, and *C. austriacus* and *C.*
299 *melapterus* now have limited contact in the southern Red Sea (Randall, 1994), a region characterised
300 by changes in environmental conditions (e.g. salinity, temperature, nutrients: Kemp, 1998; Sheppard,
301 1998) that are reflected in the fish community (Roberts *et al*., 1992; DiBattista *et al*., in review). Given
302 that *C. austriacus* and *C. melapterus* inhabit different environmental conditions on either side of this
303 area, successful colonisation across this potential barrier may be limited, thereby facilitating
304 divergence. When the two species come into contact, differences in colouration and assortative mating
305 may maintain reproductive isolation (McMillan *et al*., 1999).
306
307 The distribution of all four sister species overlap at their range edges, at (or adjacent to)
308 biogeographical barriers (Fig. 1). In the eastern Indian Ocean, cohabitation and a breakdown in
309 assortative mating between *C. lunulatus* and *C. trifasciatus* at Christmas Island has led to hybridisation
310 (Hobbs *et al*., 2009; Montanari *et al*., 2014); however, there has only been limited and localised
311 introgression between the species. In the western Indian Ocean, *C. trifasciatus* and *C. melapterus*
312 hybridise at Socotra, with some evidence of introgression beyond this hybrid zone in Djibouti

(DiBattista *et al*., 2015). In the southern Red Sea, *C. austriacus* and *C. melapterus* cohabit and potentially hybridise (Randall, 1994; Kuiter, 2002), but the former is considered rare in this understudied region (Righton e*t al.,* 1996). This pattern of decreasing hybridisation and introgression with increasing divergence time is consistent with other butterflyfish studies (Montanari *et al*., 2014). Overall, it appears that Plio-Pleistocene sea level changes have facilitated allopatric speciation in both the butterflyfish centers of diversity (Indonesia) and peripheral areas (Red Sea). Secondary contact and hybridisation could erode species boundaries (Coleman *et al.,* 2014); however, abrupt differences in environmental conditions across areas of secondary contact could facilitate evolutionary divergence.

Genetic diversity

Although the geographical ranges of the four species in the subgenus *Corallochaetodon* vary by an order of magnitude, there was no obvious relationship between haplotype diversity and range size. Terrestrial studies commonly find low haplotype diversity in range-restricted endemics (Frankham, 1998). However, endemic reef fishes can have population sizes numbering in the millions (Hobbs *et al*., 2011) and this may explain why they have haplotype diversities similar to widespread species (Eble *et al.,* 2009; Hobbs *et al.,* 2013; Delrieu-Trottin *et al.,* 2014). Excluding the Arabian Gulf, where atypical conditions have resulted in an unusually low abundance and diversity of butterflyfishes (Pratchett *et al*., 2013), *C. austriacus* and *C. melapterus* are the most common butterflyfish species in their respective ranges (Berumen & Hobbs, unpub. data). Therefore, the large population sizes of the range-restricted *C. austriacus* and *C. melapterus* would help generate and maintain high haplotype diversity. Nearly all the populations of the two restricted-range species had significant negative Fu's *Fs* values. Therefore, it appears that *C. austriacus* and *C. melapterus* have undergone recent population expansion.

Population structure - mtDNA

Data from the wide-ranging *C. lunulatus* indicates strong population structure, whereas the sister species *C. trifasciatus* showed significant genetic structure only at Diego Garcia (Chagos Archipelago). Data from the two range-restricted species, *C. austriacus* and *C. melapterus*, detected no population structure based on our approach, which may indicate that each represents a single panmictic population. This can be explained by their limited distributions in the NW Indian Ocean, with no apparent biogeographical barriers within each range.

345 *Corallochaetodon* mtDNA sequence data revealed that range size was not related to genetic population
346 structure, which is a proxy for realised dispersal ability (Eble *et al.*, 2009). The widespread *C. lunulatus*
347 showed significant population structure at eastern peripheral locations, consistent with known
348 distributional barriers (Blum, 1989; Hsu *et al.*, 2007). The distinction of the Mo'orea population of *C.*
349 *lunulatus* (Lawton *et al*., 2011; this study) is concordant with other Pacific Ocean species and may be
350 caused by isolating oceanographic currents (Gaither *et al.*, 2010; Eble *et al*., 2011). The isolation of
351 Johnston Atoll indicates that the pelagic larval duration (~35 days: Soeparno *et al*., 2012) of *C.*
352 *lunulatus* is insufficient to make the 40 to 50 day transit to the nearest reef (Hawaiian Archipelago)
353 (Kobayashi, 2006).
354
355 Population differentiation between Hawai'i and other Pacific locations has been reported in many other
356 reef fishes (Leray *et al*., 2010; DiBattista *et al*., 2011; Gaither *et al*., 2011; Szabo *et al*., 2014;
357 Fernandez-Silva *et al*., in press). The recurrent trend of genetic distinctness in this region can be
358 attributed to three factors: (1) isolation due to location and oceanographic currents, (2) dispersal
359 characteristics of the fishes and (3) adaptation to environmental conditions in Hawai'i (Hourigan &
360 Reese, 1987). Widespread reef fishes usually exhibit genetic homogeneity within the Hawaiian
361 archipelago (Craig *et al*., 2007; Eble *et al*., 2009; Gaither *et al*., 2010, 2011; Reece *et al*., 2011;
362 DiBattista *et al*., 2011, 2012; Ludt *et al*., 2012); however, the genetic differentiation of *C. lunulatus*
363 across the archipelago (between the low islands of the NWHI and the high volcanic islands of the MHI)
364 is more typical of endemic reef fishes and invertebrates (Eble *et al*., 2009; Craig *et al*., 2010; Toonen *et*
365 *al*. 2011).
366
367 **Population structure – msatDNA**
368 Investigation of fine-scale population structure in the two widespread species using msatDNA revealed
369 patterns similar to the mtDNA with *C. trifasciatus* exhibiting low structure, whereas *C. lunulatus* had
370 more pronounced structure. For *C. trifasciatus*, the msatDNA differed from mtDNA results in one
371 point –the former support the genetic isolation of Diego Garcia (Chagos Archipelago) in the central
372 Indian Ocean. The population genetic separation of Chagos has been observed in other reef fauna
373 (Gaither *et al*., 2010; Eble *et al*., 2011; Vogler *et al*., 2012) and may be related to seasonal monsoon-
374 driven currents that switch direction between easterly and westerly, possibly limiting larval dispersal to
375 this location (Sheppard *et al*., 2012).
376

MsatDNA analyses for *C. lunulatus* were consistent with the mtDNA results in indicating divergent populations at peripheral locations on the eastern range edge: Mo‘orea, Johnston Atoll, MHI and NWHI. The majority of the geographic range of *C. lunulatus* is comprised of relatively close islands and reefs throughout the Central-West Pacific; however, the large distance and prevailing currents work against colonisation of Hawai‘i and French Polynesia, thus explaining the genetic distinctness of populations at these peripheral locations (Hourigan & Reese, 1987; Gaither *et al*., 2010). This isolation is the starting point for peripheral speciation, explaining why Hawai‘i has one of the highest levels of reef fish endemism in the world (Randall, 2007).

An interesting outcome for *C. lunulatus* is the population separation between the high islands of the MHI and the low islands and atolls of the NWHI; *C. lunulatus* is the first widespread reef fish to show strong population structure across the Hawaiian Archipelago. Part of the explanation may be habitat preference: this species uses sheltered, coral-rich areas and the lack of this habitat between MHI and NWHI may explain the genetic break. Indeed, at the MHI region adjacent to this break (Kaua‘i), previous transect data (unpub. data) and our own efforts indicate a near absence of *C. lunulatus*. Another part of the explanation may include Johnston Atoll to the south. Johnston has long been postulated to be a gateway into Hawai‘i (Hourigan & Reece, 1987), and STRUCTURE analysis shows an affiliation between Johnston and the MHI, to the exclusion of the NWHI (Fig. 4). This invokes the possibility that Hawai‘i was colonized twice, possibly from different sources.

Conclusion

We conclude that Plio-Pleistocene sea level changes have influenced speciation at both the center of diversity and peripheral areas for butterflyfishes of the subgenus *Corallochaetodon*. Evolutionary divergence among *Corallochaetodon* species may have been initiated along the intermittent biogeographical barriers between Indian and Pacific Oceans, and between the Indian Ocean and Red Sea. Phylogenetic analyses revealed that the two species restricted to the Red Sea to Arabian Sea region are indistinguishable at cyt *b*. Genetic diversity decreases from west to east for the widespread *C. lunulatus*, but there are no patterns for the other three species. The two range-restricted species appear to have undergone recent population expansion and exhibit no population structure, while the widespread Indian Ocean species (*C. trifasciatus*) showed little population structure, which is likely attributed to variable local conditions (e.g. seasonal monsoon currents). Peripheral populations on the eastern range edge of the widespread Pacific species *C. lunulatus* were genetically distinct from

populations in the center of the range. The recent evolution of *C. melapterus* and *C. austriacus* in the Red Sea to Arabian Sea region, and genetic distinctness of peripheral populations of the widespread *C. lunulatus,* indicate that such peripheral marine habitats can be engines of biodiversity (Bowen *et al*., 2013). Thus peripheral speciation (through isolation and vicariant events) would help explain why the Red Sea and Hawai‘i, at opposite extremes of the Indo-Pacific ranges, are endemic hotspots for reef fishes.

ACKNOWLEDGMENTS

For assistance with field work and collections, we thank Alexander Alfonso, Senifa Annadale, Kim Anderson, Paul H. Barber, W.K. Chan, Howard Choat, Richard Coleman, Pat and Laura Colin, Greg Concepcion, Joshua Copus, Matthew Craig, Toby Daly-Engel, Nancy Daschbach, Joshua A. Drew, John L. Earle, Jeff Eble, Kevin Flanagan, Michelle Gaither, Brian Greene, Matthew Iacchei, Stephen Karl, Jonathan Mee, Carl Meyer, Darren Okimoto, Yannis Papastamatiou, David Pence, Mark Priest, Jon Puritz, Richard Pyle, Joshua Reece, D. Ross Robertson, Nick Russo, Jennifer Schultz, Charles Sheppard, Derek Skillings, Derek Smith, Zoltan Szabo, Sue Taei, Kim Tenggardjaja, Tukabu Teroroko, Robert Thorn, Allen Tom, Bill Walsh, Christie Wilcox, Ivor Williams, Jill Zamzow and the officers and crew of the NOAA ship Hi‘ialakai. For logistic support and advice, we thank Robert Toonen, Robert Thomson, Stephen Karl, Jo-Ann Leong, David Pence, Eric Mason at Dream Divers, Nicolas Prévot at Dolphin Divers and the crew of the M/V Deli in Djibouti, the Ministry of Agriculture and Fisheries in Oman including Abdul Karim, Jason Jones, Narineh Nazarian, Iria Fernandez-Silva, Zac Forsman, Jennier McIlwain and Amy Eggers. For institutional support, we thank the Papahānaumokuākea Marine National Monument, University of Hawai‘i Diving Safety Program, Hawai‘i Department of Land and Natural Resources, the KAUST Coastal and Marine Resources Core Lab and Amr Gusti, Conservation International, Coral Reef Research Foundation, Phoenix Island Protected Area, Dept. of the Environment (Australia), Government of Kiribati, Administration of the British Indian Ocean Territories, Dept. of Marine and Wildlife Resources (American Samoa), Government of Fiji and the Chiefs and people of Wagamimi, Tavewa, and Yasawas villages, Government of French Polynesia and U.S. Fish and Wildlife Service (Johnston Atoll). We thank Zoltan Szabo, the Center for Genomics, Proteomics, and Bioinformatics at the University of Hawai’i at Manoa and the KAUST Bioscience Core Facility for their assistance with DNA sequencing. Thanks to Keoki Stender for sharing photographs. We thank two anonymous reviewers for valuable comments that improved the manuscript. This project was funded by the National Science Foundation Grant OCE-

0929031 to B.W.B., Elizabeth Alison Kay Endowed Award, University of Hawai‘i Graduate Student Organization, NOAA National Marine Sanctuaries Program MOA No. 2005-008/66882 to R.J. Toonen, National Geographic Society Grant 9024-11 to J.D.D. and KAUST Office of Competitive Research Funds (OCRF) under Award No. CRG-1-2012-BER-002 and baseline research funds to M.L.B.

LITERATURE CITED

Allen, G.R., Steene, R.C. & Allen, M. (1998) *A Guide to Angelfishes & Butterflyfishes*. Odyssey Publishing/Tropical Reef Research, Perth.

Bandelt, H.J., Forster, P., & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Bellwood, D.R., Klanten, S., Cowman, P.F., Pratchett, M.S., Konow, N. & van Herwerden, L. (2010) Evolutionary history of the butterflyfishes (f: Chaetodontidae) and the rise of coral feeding fishes. *Journal of Evolutionary Biology*, **23**, 335-349.

Blum, S.D. (1989) Biogeography of the Chaetodontidae: an analysis of allopatry among closely related species. *Environmental Biology of Fishes*, **25**, 9-31.

Bowen, B.W., Bass, A.L., Rocha, L.A., Grant, W.S. & Robertson, D.R. (2001) Phylogeography of the trumpetfishes (*Aulostomus*): Ring species complex on a global scale. *Evolution*, **55**, 1029-1039.

Bowen, B.W., Rocha, L.A., Toonen, R.J., Karl, S.A. & The ToBo Laboratory. (2013) The origins of tropical marine biodiversity. *Trends in Ecology & Evolution*, **28**, 359-366.

Briggs, J.C. & Bowen, B.W. (2013) Evolutionary patterns: Marine shelf habitat. *Journal of Biogeography*, **40**, 1023–1035. doi:10.1111/jbi.12082.

Coleman, R.R., Gaither, M.R., Kimokeo, B., Stanton, F.G., Bowen, B.W. & Toonen, R.J. (2014) Large-scale introduction of the Indo-Pacific damselfish *Abudefduf vaigiensis* into Hawai'i promotes genetic swamping of the endemic congener *A. abdominalis*. *Molecular Ecology*, **23**, 5552–5565.

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.

Craig, M.T., Eble, J.A. & Bowen, B.W. (2010) Origins, ages, and population histories: Comparative phylogeography of endemic Hawaiian butterflyfishes (genus *Chaetodon*). *Journal of Biogeography*, **37**, 2125 – 2136.

Delrieu-Trottin, E., Maynard, J., Planes, S. (2014) Endemic and widespread coral reef fishes have similar mitochondrial genetic diversity. *Proceeding of the Royal Society B: Biological Sciences*, **281**. doi: 10.1098/rspb.2014.1068.

DiBattista, J.D., Wilcox, C., Craig, M.T., Rocha, L.A. & Bowen, B.W. (2011) Phylogeography of the Pacific Blueline Surgeonfish *Acanthurus nigroris* reveals a cryptic species in the Hawaiian Archipelago. *Journal of Marine Biology*, Article ID 839134.

DiBattista, J.D., Rocha, L.A., Craig, M.T. Feldheim, K.A. & Bowen, B.W. (2012) Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *Journal of Heredity*, **103**, 617–629.

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., Rocha, L.A., Hobbs, J-P.A., He, S., Priest, M.A., Sinclair-Taylor, T.H., Bowen, B.W. & Berumen. M.L. (2015) When biogeographic provinces collide: Hybridization at the crossroads of three marine biogeographic provinces in the Arabian Sea. *Journal of Biogeography*, Online early

DiBattista J.D., Roberts, M, Baird, A.H., et al. (in review) A review of contemporary patterns of endemism for shallow water reef fauna in the Red Sea. *Journal of Biogeography*.

Drummond, A.J., Ashton, V., Buxton, V., Cheung, M., Cooper, V., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S. Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. (2010) Geneious version 5.4, Available from http://www.geneious.com.

Dwyer, G.S., Cronin, T.M., Baker, P.A., Raymo, M.E., Buzas, J.S. & Correge, T. (1995) North Atlantic deepwater temperature change during late Pliocene and late Quaternary climatic cycles. *Science*, **270**, 1347–1351.

Earl, D.A., & von Holdt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359-361.

Eble, J.A., Toonen, R.J. & Bowen, B.W. (2009) Endemism and dispersal: comparative phylogeography of three surgeonfishes across the Hawaiian Archipelago. *Marine Biology*, **156**, 689-698.

Eble, J.A., Rocha, L.A., Craig, M.T. & Bowen, B.W. (2011) Not all larvae stay close to home: Long-distance dispersal in Indo-Pacific reef fishes, with a focus on the Brown Surgeonfish (*Acanthurus nigrofuscus*). *Journal of Marine Biology*, Article ID 518516.

Eble, J.A., Bowen, B.W. & Bernardi. G. (2015) Phylogeography of coral reef fishes. *Ecology of Fishes on Coral Reefs* (ed. C. Mora). Pp. 64 – 75. University of Hawaii Press, Honolulu

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131, 479–491.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fernandez-Silva, I., Randall, J.E., Coleman, R.R., DiBattista, J.D., Rocha, L.A., Reimer, J.D., Meyer, C.G. & Bowen, B.W. 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* In press.

Fessler, J.L. & Westneat, M.W. (2007) Molecular phylogenetics of the butterflyfishes (Chaetodontidae): Taxonomy and biogeography of a global coral reef fish family. *Molecular Phylogenetics and Evolution*, **45**, 50–68.

Frankham, R. (1998) Inbreeding and extinction: island populations. *Conservation Biology*, **12**, 665–675.

Fu, Y.X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. *Genetics*, **147**, 915-925.

Gaither, M.R., Toonen, R.J., Robertson, D.R., Planes, S. & Bowen, B.W. (2010) Genetic evaluation of marine biogeographical 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., Rocha, L.A., Newman, S.J., Gomez, J.A., van Herwerden, L. & Craig, M.T. (2011) 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 & 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. doi:10.1111/jbi.12126.

Hasegawa, M., Kishino, H. & Yano, T. (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160-174.

Hobbs, J.-P.A., Frisch, A.J., Allen, G.R. & van Herwerden, L. (2009) Marine hybrid hotspot at Indo-Pacific biogeographic border. *Biology Letters*, **5**, 258–61.

Hobbs, J.-P.A., Jones, G.P. & Munday, P.L. (2011) Extinction risk in endemic marine fishes. *Conservation Biology*, **25**, 1053–1055. doi:10.1111/j.1523-1739.2011.01698.x.

Hobbs, J.-P.A., van Herwerden, L., Jerry, D.R., Jones, G.P. & Munday, P.L. (2013) High genetic diversity in geographically remote populations of endemic and widespread coral reef angelfishes (genus: Centropyge). *Diversity*, **5**, 39-50.

Hodge, J.R., van Herwerden. L. & Bellwood. D.R. (2014) Temporal evolution of coral reef fishes: global patterns and disparity in isolated locations. *Journal of Biogeography*, **41**, 2115–2127.

Hourigan, T.F. & Reese, E.S. (1987) Mid-ocean isolation and the evolution of Hawaiian reef fishes. *Trends in Ecology and Evolution*, **2**, 187-191.

Hsu, K.C., Chen, J.P. & Shao, K.T. (2007) Molecular phylogeny of *Chaetodon* (Teleostei: Chaetodontidae) in the Indo-West Pacific: evolution in geminate species pairs and species groups. *The Raffles Bulletin of Zoology Supplement* **14**, 77-86.

Kemp, J.M. (1998) Zoogeography of the coral reef fishes of the Socotra Archipelago. *Journal of Biogeography*, **25**, 919-933.

Kobayashi, D.R. (2006) Colonization of the Hawaiian Archipelago via Johnston Atoll: a characterization of oceanographic transport corridors for pelagic larvae using computer simulation. *Coral Reefs*, **25**, 407-417.

Kuiter, R.H. (2002) Butterflyfishes, Bannerfishes and their Relatives. A comprehensive Guide to Chaetodontidae and Microcanthidae. The Marine Fish Families Series, TMC Publishing, Chorleywood, UK.

Lawton, R.J., Bay, L.K. & Pratchett, M.S. (2010) Isolation and characterization of 29 microsatellite loci for studies of population connectivity in the butterflyfishes *Chaetodon trifascialis* and *Chaetodon lunulatus*. *Conservation Genetics Resources*, **2**, 209-213.

Lawton, R.J., Messmer, V., Pratchett, M.S. & Bay, L.K. (2011) High gene flow across large geographic scales reduces extinction risk for a highly specialised coral feeding butterflyfish. *Molecular Ecology*, **20**, 3584-3598.

Leray, M., Beldade, R., Holbrook, S.J., Schmitt, R.J., Planes, S. & Bernardi, G. 2010. Allopatric divergence and speciation in coral reef fish: the three-spot *Dascyllus, Dascyllus trimaculatus*, species complex. *Evolution*, **64**, 1218–1230.

Ludt, W.B., Bernal, M., Bowen, B.W. & Rocha, L.A. (2012) Living in the past: phylogeography and population histories of Indo-Pacific wrasses (genus *Halichoeres*) in shallow lagoons versus outer reef slopes. PLoS ONE, 7, e38042.

McMillan, W.O., Weight, L.A. & Palumbi, S.R. (1999) Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution*, **53**, 247-260.

Meeker, N.D., Hutchinson, S.A., Ho, L. & Trede, N.S. (2007) Method for isolation of PCR-ready genomic DNA from zebrafish tissues. *BioTechniques*, **43**, 610-614.

Meirmans, P.G. & van Tienderen, P.H. (2004) GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes 4: 92-794.

Montanari, S.R., van Herwerden, L., Pratchett, M.S., Hobbs, J.P.A & Fugedi, A. (2012) Reef fish hybridization: lessons learnt from butterflyfishes (genus *Chaetodon*). *Ecology and Evolution*, **2**, 310-328.

Montanari, S.R., Hobbs, J-P.A., Pratchett, M.S., Bay, L.K. & van Herwerden, L. (2014) Does genetic distance between parental species influence outcomes of hybridisation among coral reef butterflyfishes? *Molecular Ecology*, **23**, 2757-2770.

Posada, D. (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 253-1256.

Pratchett, M.S., Hoey, A.S., Feary, D.A., Bauman, A.G., Burt, J.A. & Riegl, B.M. (2013) Functional composition of *Chaetodon* butterflyfishes at a peripheral and extreme coral reef location, the Persian Gulf. *Marine Pollution Bulletin*, **72**, 333–341.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Randall, J.E. (1994) Twenty-two new records of fishes from the Red Sea. *Fauna Saudi Arabia*, **14**, 259–275.

Randall, J.E. (1998) Zoography of shore fishes of the Indo-Pacific region. *Zoological Studies*, **37**, 227-268.

Randall, J.E. (2007) *Reef and Shore Fishes of the Hawaiian Islands*. University of Hawaii Press, Honolulu.

Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.

Reece, J.S., Bowen, B.W. & Larson, A.F. (2011) Long larval duration in moray eels (Muraenidae) ensures ocean-wide connectivity despite differences in adult niche breadth. *Marine Ecology Progress Series*, **437**, 269–277.

660 Righton, D., Kemp, J. & Ormond, R. (1996) Biogeography, community structure and diversity of Red
661 Sea and western Indian Ocean butterflyfishes. *Journal of the Marine Biological Association of the*
662 *United Kingdom*, **76,** 223–228.
663
664 Roberts, C.M., Shepherd, A.R.D. & Ormond, R.F.G. (1992) Large scale variation in assemblage
665 structure of Red Sea butterflyfishes and angelfishes. *Journal of Biogeography*, **19**, 239-250.
666
667 Rocha, L.A., Craig, M.T. & Bowen, B.W. (2007) Phylogeography and the conservation genetics of
668 coral reef fishes. *Coral Reefs*, **26**, 501-512.
669
670 Selkoe, K.A. & Toonen, R.J. (2006) Microsatellites for ecologists: a practical guide to using and
671 evaluating microsatellite markers. *Ecology Letters*, **9**, 615-629.
672
673 Seutin, G., White, B.N. & Boag, P.T. (1991) Preservation of avian blood and tissue samples for DNA
674 analyses. *Canadian Journal of Zoology*, **69**, 82-90.
675
676 Sheppard, C.R.C. (1998) Biodiversity patterns in Indian Ocean corals, and effects of taxonomic error in
677 data. *Biodiversity and Conservation*, **7**, 847-868.
678
679 Sheppard, C., Al-Husiani, M., Al-Jamali, F. & *et al*. (2010) The Gulf: A young sea in decline. *Marine*
680 *Pollution Bulletin*, **60**, 13–38.
681
682 Sheppard, C., Ateweberhan, M., Bowen, B.W. *et al*. (2012) Reefs and islands of the Chagos
683 Archipelago, Indian Ocean: Why it is the world's largest no-take marine protected area. Aquatic
684 Conservation: Marine and Freshwater Ecosystems, **22**, 232-261.
685
686 Soeparno, Nakamura, Y., Shibuno, T. & Yamaoka, K. (2012) Relationship between pelagic larval
687 duration and abundance of tropical fishes on temperate coasts of Japan. *Journal of Fish Biology*, **80**,
688 346–357.
689
690 Swofford, D.L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).
691 Version 4. Sinauer Associates, Sunderland, Massachusetts.

692

693 Szabo, Z., Snelgrove, B., Craig, M.T., Rocha, L.A. & Bowen, B.W. 2014. Phylogeography of the
694 Manybar Goatfish, *Parupeneus multifasciatus* reveals moderate structure between the Central and
695 North Pacific and a cryptic endemic species in the Marquesas. *Bulletin of Marine Science*, **90**, 493 –
696 512.

697

698 Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region
699 of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

700

701 Tamura, K., Peterson, D., Peterson, N., Stecher, G. , Nei, M. & Kumar, S. (2011) MEGA5: Molecular
702 evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum
703 parsimony methods. Molecular Biology and Evolution, **28**, 2731-2739.

704

705 Toonen, R.J., Andrews, K.R., Baums, I.B., *et al*. (2011) Defining boundaries for ecosystem-based
706 management: a multispecies case study of marine connectivity across the Hawaiian archipelago.
707 *Journal of Marine Biology*, Article ID 460173.

708

709 van Oosterhout, C., Hutchinson, W.F., Willis, D.P.M. & Shipley, P. (2004) MICRO-CHECKER:
710 software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology*
711 *Notes*, **4**, 535–538.

712

713 Vogler, C., Benzie, J.A.H., Tenggardjaja, K., Ambariyanto, Barber, P.H. & Wörheide, G. (2012)
714 Phylogeography of the crown-of-thorns starfish in the Indian Ocean. *Coral Reefs*, **32**, 515–525.

715

716 Waldrop, E. 2014. Phylogeography and Evolution of Butterflyfishes in the Subgenus
717 Corallochaetodon: *Chaetodon lunulatus, Chaaetodon trifasciatus, Chaetodon austriacus, Chaetodon*
718 *melapterus*. Thesis, University of Hawaii, Honolulu

719

720 Williams, D.F., Peck, J., Karabanov, E.B., Prokopenko, A.A., Kravchinsky, V., King, J. & Kuzmin,
721 M.I. (1997) Lake Baikal record of continental climate response to orbital insolation during the past five
722 million years. *Science*, **278**, 1114-1117.

723

Zekeria, Z.A., Afeworki, Y. & Videler, J.J. (2005) The distribution patterns of Red Sea Chaetodontid assemblages. Aquatic Conservation: *Marine and Freshwater Ecosystems*, **15**, S71–S76.

SUPPORTING INFORMATION

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

Appendix S1: Additional materials & methods

Appendix S2: Supporting tables & figures

BIOSKETCH

Ellen Waldrop conducted this research as a M.Sc. thesis project at the University of Hawai‘i. The authors are interested in the origins of marine biodiversity and the prudent management of evolutionary lineages.

Author contributions: B.W.B. initiated the research; E.W., J.-P.A.H., J.D.D., L.A.R., R.K.K., M.L.B. and B.W.B. conducted field expeditions and sampling; E.W and J.D.D. provided genetic data; E.W. analysed the data; E.W., B.W.B., J.P.H. and J.D.D contributed to the writing; and all authors commented on the final draft.

Editor: Michelle Gaither

Table 1. Sample size and molecular diversity indices for *Chaetodon lunulatus*, *C. trifasciatus*, *C. melapterus* and *C. austriacus* based on mtDNA cytochrome *b* sequence data (significant Fu's *Fs* values are in bold, $P < 0.02$). For *C. trifasciatus*, specimens from the eastern Indian Ocean (Cocos-Keeling Islands and adjacent Christmas Island) were pooled to increase statistical power as they were indistinguishable in preliminary analyses.

Location	*N*	Number of Haplotypes	Haplotype diversity (*h* ± SD)	Nucleotide diversity (π ± SD)	Fu's *Fs*
C. lunulatus					
Christmas Island	6	4	0.867 +/- 0.129	0.005 +/- 0.004	0.24
American Samoa	15	5	0.714 +/- 0.081	0.005 +/- 0.003	1.40
Fiji	30	10	0.602 +/- 0.104	0.004 +/- 0.003	-1.92
Kanton Island	15	5	0.695 +/- 0.109	0.004 +/- 0.003	0.95
Marshall Islands	29	8	0.727 +/- 0.057	0.005 +/- 0.003	0.91
Mo‘orea	32	8	0.669 +/- 0.086	0.005 +/- 0.003	-0.04
Okinawa	8	4	0.643 +/- 0.184	0.004 +/- 0.003	0.73
Pohnpei	30	10	0.782 +/- 0.065	0.005 +/- 0.003	-0.57
Kiribati	22	3	0.589 +/- 0.066	0.004 +/- 0.003	4.63
Palau	26	2	0.471 +/- 0.063	0.004 +/- 0.002	6.68
Johnston Atoll	31	2	0.516 +/- 0.024	0.004 +/- 0.003	7.63
MHI	33	2	0.504 +/- 0.034	0.004 +/- 0.003	7.64
NWHI	161	13	0.452 +/- 0.048	0.001 +/- 0.001	-0.51
C. trifasciatus					
Diego Garcia	29	8	0.672 +/- 0.074	0.001 +/- 0.001	**-4.538**
Seychelles	21	9	0.795 +/- 0.077	0.088 +/- 0.044	9.843
Christmas Island	14	7	0.802 +/- 0.094	0.010 +/- 0.006	0.959
Indonesia	5	3	0.700 +/- 0.218	0.002 +/- 0.002	0.061
C. melapterus					
Maskali	17	5	0.353 +/- 0.353	0.001 +/- 0.001	**-2.527**
Obock	29	7	0.778 +/- 0.584	0.001 +/- 0.001	**-3.754**
Bay of Ghoubbet	15	1	0.000 +/- 0.000	0.000 +/- 0.000	na

Oman	34	9	0.631	+/-	0.507	0.001	+/-	0.001	**-7.615**
C. austriacus									
Al Lith	10	2	0.200	+/-	0.154	0.000	+/-	0.000	na
Jazirat Baraqan	10	6	0.844	+/-	0.103	0.002	+/-	0.002	**-3.127**
Yanbu	10	7	0.866	+/-	0.107	0.001	+/-	0.001	**-1.404**

749

Table 2. Matrix of population pairwise Φ_{ST} values (above diagonal) and associated *P* values (below diagonal) based on 605 bp of mtDNA cytochrome *b* sequence data from *Chaetodon lunulatus*. Significant *P* values are indicated in bold ($P < 0.05$). All negative Φ_{ST} values were adjusted to 0.

Location	Christmas Island	American Samoa	Fiji	Kanton Island	Marshall Island	Mo‘orea	Okinawa	Pohnpei	Kiribati	Palau	Johnston Atoll	MHI	NWHI
Christmas Island	–	0	0.097	0.012	0	**0.284**	0.107	0	0	0.084	0.006	0.003	**0.597**
American Samoa	0.568	–	**0.105**	0.095	0	**0.286**	**0.074**	0	0	0.040	0	0	**0.507**
Fiji	0.108	**0.036**	–	0	**0.086**	**0.478**	0	0.024	0.022	0.000	**0.083**	**0.162**	**0.114**
Kanton Island	0.333	0.081	0.477	–	0.079	**0.470**	0	0	0.031	0.040	0.105	**0.178**	**0.245**
Marshall Islands	0.414	0.973	**0.036**	0.099	–	**0.307**	0.050	0	0	0.023	0	0	**0.431**
Mo‘orea	**0.036**	**<0.001**	**0.000**	**0.000**	**0.000**	–	**0.463**	**0.370**	**0.371**	**0.431**	**0.342**	**0.298**	**0.757**
Okinawa	0.234	**0.036**	0.847	0.387	0.189	**<0.001**	–	0.008	0	0	0.037	0.125	**0.099**
Pohnpei	0.658	0.387	0.144	0.423	0.369	**<0.001**	0.252	–	0	0.010	0.016	**0.055**	**0.332**
Kiribati	0.324	0.514	0.126	0.216	0.640	**<0.001**	0.306	0.667	–	0	0	0.017	**0.335**
Palau	0.252	0.198	0.324	0.126	0.234	**<0.001**	0.396	0.207	0.559	–	0.003	0.068	**0.228**
Johnston Atoll	0.324	0.450	**0.018**	0.063	0.577	**<0.001**	0.108	0.189	0.631	0.432	–	0	**0.405**
MHI	0.279	0.550	**0.009**	**0.018**	0.423	**<0.001**	0.099	**0.045**	0.342	0.108	0.622	–	**0.509**
NWHI	**0.009**	**<0.001**	**0.009**	**<0.001**	**<0.001**	**<0.001**	**0.018**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	–

753

754 **Table 3.** Matrix of population pairwise Φ_{ST} values (above diagonal) and associated *P* values (below
755 diagonal) based on 605 bp of mtDNA cytochrome *b* sequence data from *Chaetodon trifasciatus*, *C.*
756 *melapterus* and *C. austriacus*. All negative Φ_{ST} values were adjusted to 0.

C. trifasciatus				
Location	Diego Garcia	Seychelles	Christmas Island	Indonesia
Diego Garcia	–	0.014	0.027	0
Seychelles	0.268	–	0	0
Christmas Island	0.238	0.961	–	0
Indonesia	0.483	0.769	0.678	–
C. melapterus				
Location	Maskali	Obock	Bay of Ghoubbet	Oman
Maskali	–	0.030	0	0.001
Obock	0.108	–	0.022	0.007
Bay of Ghoubbet	0.991	0.270	–	0
Oman	0.459	0.288	0.667	–
C. austriacus				
Location	Al Lith	Jazirat Baraqan	Yanbu	
Al Lith	–	0.095	0.028	
Jazirat Baraqan	0.207	–	0	
Yanbu	0.491	0.573	–	

757

758 **Table 4.** Matrix of population pairwise F_{ST} values (above diagonal) and associated P values (below diagonal) based on microsatellite
759 genotypes for *Chaetodon lunulatus*. Significant P values are highlighted in bold ($P < 0.05$). All negative F_{ST} values were adjusted to 0.

Location	Christmas Island	Indonesia	American Samoa	Fiji	Kanton Island	Marshall Islands	Mo'orea	Okinawa	Pohnpei	Kiribati	Palau	Johnston Atoll	MHI	NWHI
Christmas Island	—	0	0.003	0.001	0.012	0.006	**0.041**	0.010	0.006	0	0.011	**0.084**	**0.032**	**0.090**
Indonesia	0.498	—	0.007	0.002	0.001	0	**0.030**	0.002	0.0	0	0	**0.079**	**0.024**	**0.078**
American Samoa	0.378	0.067	—	**0.009**	0.002	0.006	**0.027**	0.012	**0.010**	0	0.007	**0.082**	**0.037**	**0.075**
Fiji	0.396	0.267	**0.036**	—	0.002	0.002	**0.030**	0.007	0.005	0.000	**0.007**	**0.088**	**0.030**	**0.089**
Kanton Island	0.124	0.411	0.322	0.260	—	0	**0.023**	0.003	0.001	0	0.004	**0.087**	**0.035**	**0.076**
Marshall Islands	0.217	0.706	0.067	0.150	0.772	—	**0.029**	0.005	0.000	0.000	0.002	**0.084**	**0.030**	**0.079**
Mo'orea	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	—	0.056	0.032	0.024	0.029	**0.087**	**0.058**	**0.095**
Okinawa	0.203	0.300	0.089	0.093	0.331	0.116	**<0.001**	—	0.005	0.007	0.005	**0.096**	**0.034**	**0.082**
Pohnpei	0.232	0.676	**0.022**	0.071	0.361	0.531	**<0.001**	0.151	—	0	0.000	**0.085**	**0.029**	**0.081**
Kiribati	0.497	0.744	0.602	0.443	0.779	0.394	**<0.001**	0.109	0.773	—	0	**0.076**	**0.023**	**0.067**
Palau	0.128	0.779	0.072	**0.017**	0.154	0.203	**<0.001**	0.140	0.441	0.554	—	**0.080**	**0.023**	**0.078**
Johnston	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	—	**0.051**	**0.038**
MHI	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	—	**0.053**
NWHI	**<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**	—

760

761 **Table 5.** Matrix of population pairwise F_{ST} values (above diagonal) and associated *P* values (below
762 diagonal) based on microsatellite genotypes for *Chaetodon trifasciatus*. Significant *P* values are
763 highlighted in bold ($P < 0.05$). All negative F_{ST} values were adjusted to 0.

Location	Diego Garcia	Seychelles	Christmas Island	Indonesia
Diego Garcia	–	0.005	0.006	0.012
Seychelles	**0.047**	–	0	0
Christmas Island	**0.013**	0.742	–	0.001
Indonesia	**0.018**	0.496	0.350	–

764

765 **TITLES AND LEGENDS TO FIGURES**

766

767 **Figure 1.** Distribution map of *Chaetodon* subgen. *corallochaetodon* (redrawn from Blum, 1989).
768 *Chaetodon lunulatus* (blue, widespread Pacific Ocean), *C. trifasciatus* (red, widespread Indian Ocean),
769 *C. austricaus* (green, largely restricted to the northern and central Red Sea; but see DiBattista *et al.*, in
770 review) and *C. melapterus* (yellow, restricted to the southern Red Sea through the Arabian Gulf). The
771 known geographic range of each species is outlined with a dotted line and solid pink lines represent
772 known marine biogeographic barriers (Hsu *et al*., 2007) that influence the genetic partitions and
773 evolution of *corallochaetodon.* Sample locations are shown with species-specific coloured symbols and
774 numbers that correspond to the following location names: 1. Jazirat Baraqan, 2. Yanbu, 3. Al Lith, 4.
775 Obock, 5. Bay of Ghoubbet, 6. Maskali, 7. Oman, 8. Seychelles, 9. Diego Garcia, 10. Cocos (Keeling)
776 Islands, 11. Christmas Island, 12. Indonesia, 13. Okinawa, 14. Palau, 15. Pohnpei, 16. Marshall Islands,
777 17. Fiji, 18. American Samoa, 19. Kanton Island, 20. Kiribati, 21. Mo‘orea, 22. Johnston Atoll, 23.
778 Main Hawaiian Islands, 24. Northwestern Hawaiian Islands. Sample sizes for each location are
779 presented in Table 1. Photo Credits: L.A. Rocha for *C. austriacus*, T. Sinclair-Taylor for *C. lunulatus*,
780 *C. trifasciatus*, and *C. melapterus*.

781

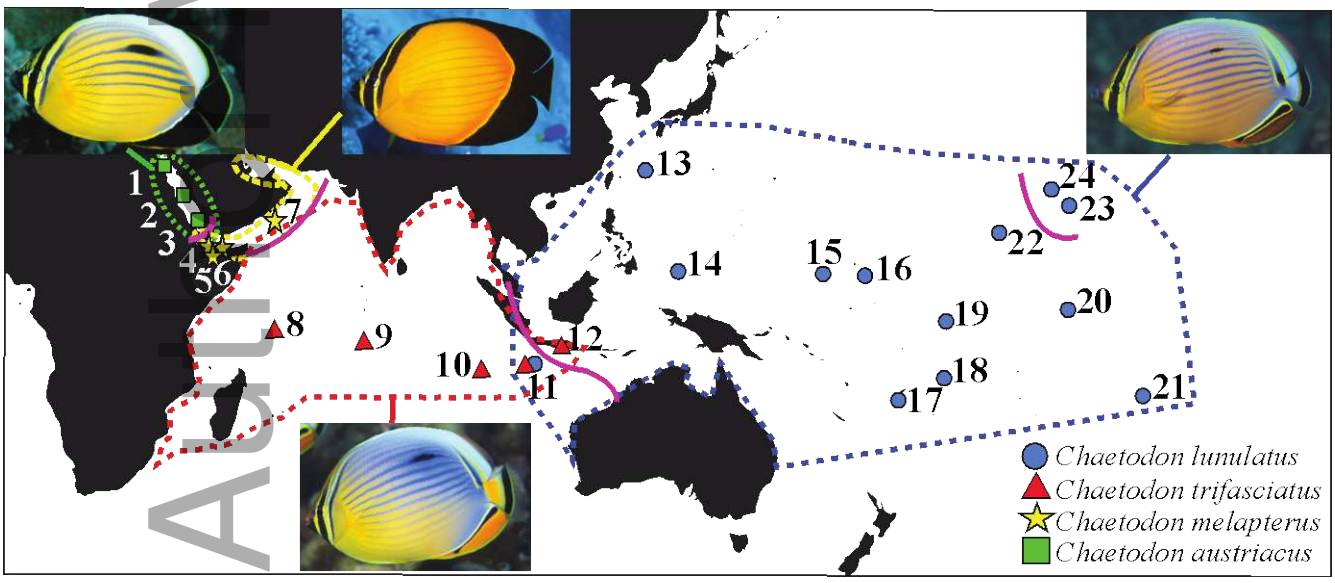
782 **Figure 2.** Neighbour-joining tree based on mtDNA cytochrome *b* sequences, highlighting the
783 relationship between sister species in *Chaetodon* subgenus *corallochaetodon* (bootstrap values shown
784 based on 1000 replicates). For simplicity, only a representative subset of specimens is shown.
785 Maximum-likelihood and maximum-parsimony trees yielded the same topology among species.

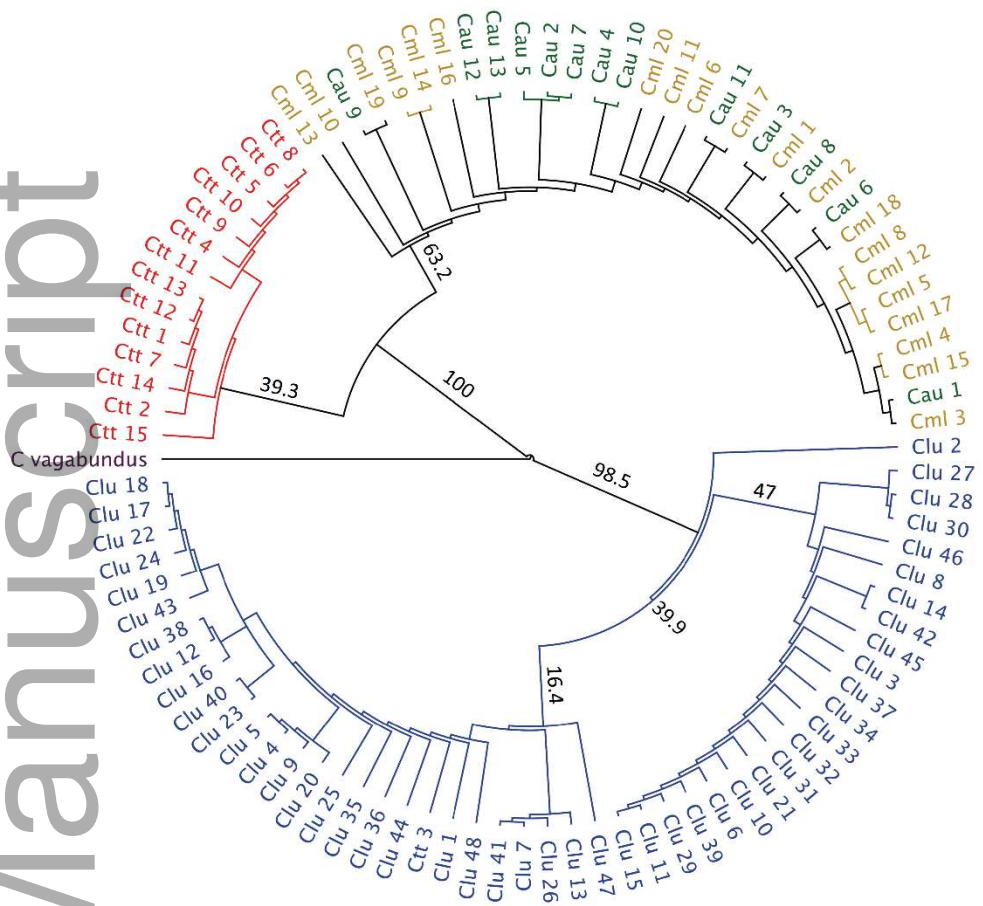
Chaetodon vagabundus is used as an outgroup (Genbank accession number JF458006). Abbreviations: *C. lunulatus* = Clu, *C. trifasciatus* = Ctt, *C. melapterus* = Cml and *C. austriacus* = Cau.

Figure 3. Statistical parsimony network for *Chaetodon lunulatus* (pink, purple, blue shades), *C. trifasciatus* (green shades), *C. melapterus* (yellow and orange) and *C. austriacus* (red) based on mtDNA cytochrome *b* sequences. The area of each circle is proportional to the abundance of the respective haplotype: small circles indicate rare or unique haplotypes and the largest circle indicate the most common haplotype observed in 286 sampled individuals. Black bars and black branches represent a single mutation (unless otherwise noted) and colours indicate haplotype sampling location (see key).

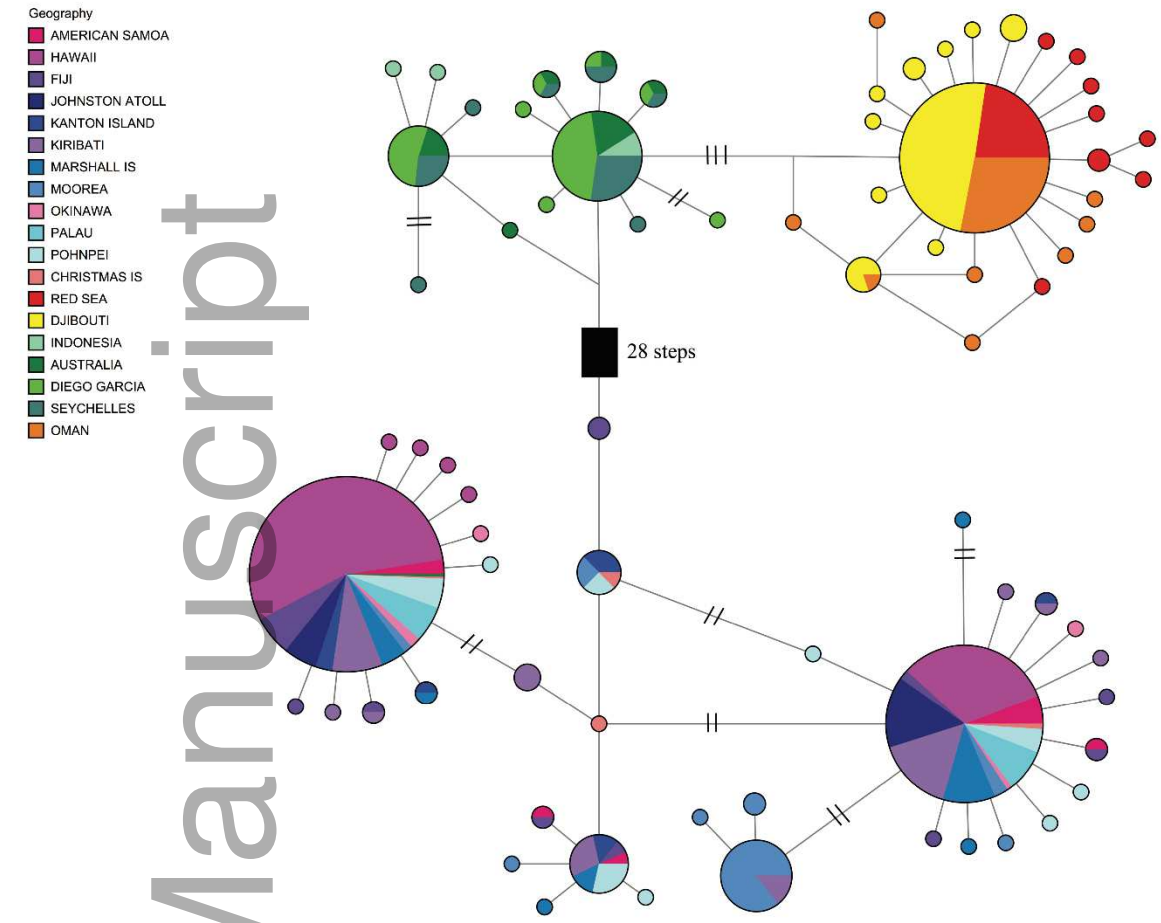
Figure 4. STRUCTURE bar plot for *Chaetodon lunulatus* showing the highest mean probability of *K* = 3. Locations: 1. Christmas Island, 2. Indonesia, 3. Palau, 4. Okinawa, 5. Pohnpei, 6. Marshall Islands, 7. Fiji, 8. American Samoa, 9. Mo'orea, 10. Kanton Island, 11. Kiribati, 12. Johnston Atoll, 13. MHI, 14. NWHI.

Figure 5. STRUCTURE bar plot for *Chaetodon trifasciatus*, showing the highest mean probability of *K* = 2. Locations: 1. Diego Garcia, 2. Seychelles, 3. Christmas Island, 4. Indonesia.

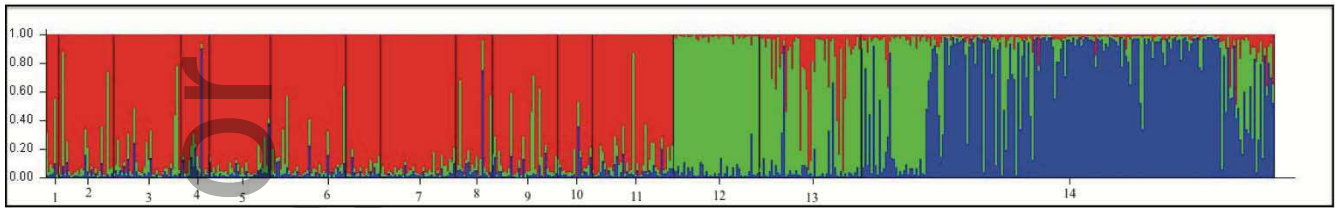




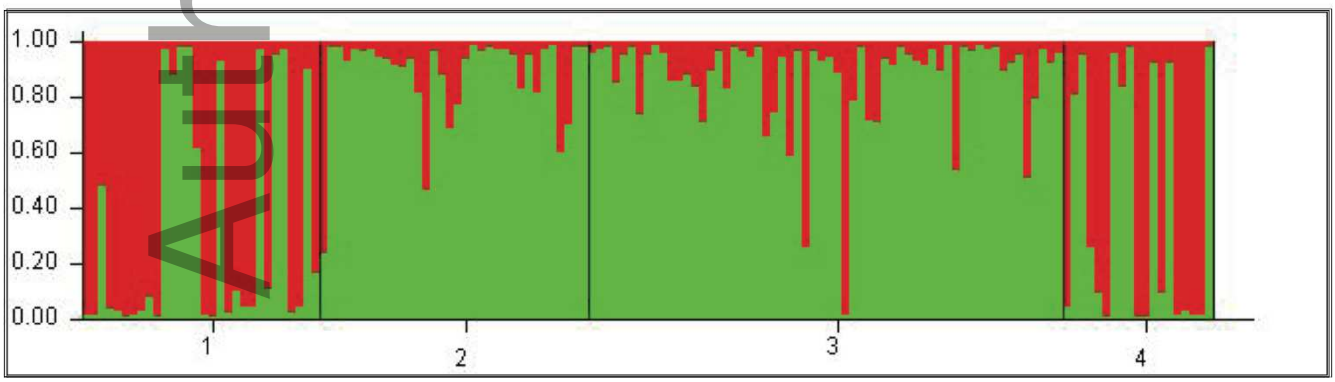
806
807



808
809



810



811