frontiers in
MARINE SCIENCE

Marine Molecular Biology and Ecology

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Journal Name:	Frontiers in Marine Science
ISSN:	2296-7745
Article type:	Original Research Article
Received on:	16 Aug 2014
Accepted on:	26 Oct 2014
Provisional PDF published on:	26 Oct 2014
www.frontiersin.org:	www.frontiersin.org
Citation:	Baums IB, Durante MD, Laing AA, Feingold J, Smith T, Bruckner A and Monteiro J(2014) Marginal coral populations: the densest known aggregation of Pocillopora in the Galápagos Archipelago is of asexual origin. *Front. Mar. Sci.* 1:59. doi:10.3389/fmars.2014.00059

Marginal coral populations: the densest known aggregation of *Pocillopora* in the Galápagos Archipelago is of asexual origin

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Keywords: Coral, asexual reproduction, clones, ENSO, El Niño-Southern Oscillation, *Symbiodinium*, Galápagos Islands, fragmentation

Abstract

Coral populations at distributional margins frequently experience suboptimal and variable conditions. Recurrent El Niño-Southern Oscillation (ENSO) warming events have caused extensive mortality of reef-building corals in the Eastern Pacific, and particularly impacted branching pocilloporid corals in the Galápagos Islands. *Pocillopora* spp. were previously more common and formed incipient reefs at several locations in the Archipelago but now occur as scattered colonies. Here, we report an unusually concentrated aggregation of colonies and evaluate their current genetic diversity. In particular we focus on a large population of 1614 live *Pocillopora* colonies found in a volcanic lagoon along the southern shore of Isabela Island. Forty seven colonies were sampled, primarily using a spatially explicit sampling design, and all colonies belonged to *Pocillopora* mitochondrial *open reading frame* lineage type *3a*. Typing of additional *Pocillopora* samples (n = 40) from three other islands indicated that this stand is the only known representative of type *3a* in the Galápagos Islands. The Isabela *Pocillopora* type *3a* colonies harbored *Symbiodinium ITS-2* clade *C1d*. Multilocus genotyping (n = 6 microsatellites) capable of resolving individual clones indicated that this stand is monogenotypic and thus the high density of colonies is a result of asexual reproduction, likely via fragmentation. Colony size distribution, while imperfect, suggested the stand regrew from remnant colonies that survived the 1997/98 ENSO event but may postdate the 1982/83 ENSO. The community of *Pocillopora* colonies at Isabela is of particular ecological value due to its high density and support of associated organisms such as fish and benthic invertebrates. The Galapagos *Pocillopora* corals will continue to provide insights into the genetic structure and population dynamics of marginal coral populations.

1. Introduction

Many reef building corals occur over large geographic ranges and experience suboptimal and variable conditions especially at their distribution margins. Hence, marginal populations can provide unique insights into how corals might respond to climate change (Guinotte et al., 2003;Lirman and

Manzello, 2009;Hennige et al., 2010;Goodkin et al., 2011). For example, coral communities in the tropical eastern Pacific (TEP) already experience seasonal cold upwelling, El Nino Southern Oscillation warm events and reduced aragonite saturation states (Glynn and Colgan, 1992;Fong and Glynn, 2000).

The Galápagos Islands harbor some of the most vibrant coral communities in the remote Tropical Eastern Pacific. The center of the archipelago is located 1,000 km offshore from the equatorial South American coastline and 1,200 km away from the more diverse central Pacific coral communities. Recent analyses show that the offshore islands are well connected with coral populations along the Central American coast (Pinzón and LaJeunesse, 2011;Baums et al., 2012). Coral communities in the Galápagos Islands have experienced large scale bleaching events killing 97-100% of colonies during the 1982/83 El Niño-Southern Oscillation (ENSO) event (Glynn, 1988). Recent (primarily 1982/83 and 1997/98) ENSO events left a legacy of depressed coral populations (Glynn, 2003). Whereas *Porites* mostly recovered at the northern-most reefs at Darwin Island, *Pocillopora* density is still lower than prior to the ENSO events (Glynn et al., 2009). Even more limited recovery of *Pocillopora* has occurred in the central and southern Archipelago (Feingold and Glynn, 2014).

Branching corals in the genus *Pocillopora* form ecologically important reef structures throughout the tropical eastern Pacific (TEP). *Pocillopora* is the primary constructor of modern reefs in the Eastern Pacific (Toth et al., 2012) and provides habitat for associated reef species in this low-diversity coral system (Glynn, 2004). In the Galápagos Islands, pocilloporid reef structures were known within the shallow basin of the nearly submerged volcanic cone, Devil's Crown, Floreana (Glynn and Wellington, 1983). Also, aggregations of colonies that formed incipient reefs were observed within semi-enclosed lava pools at Punta Espinosa, Fernandina Island, and well-developed communities occurred on the islands of San Cristobal, Española and Darwin (Glynn, 1994;2003;Glynn et al., 2009). However, these structures were lost due to impacts associated with the 1982-83 El Niño-Southern Oscillation (ENSO) event and subsequent bio-erosion. In all previously studied research sites in the archipelago, *Pocillopora* now occurs only as isolated, scattered colonies. One such recovering population of scattered *Pocillopora* is now present at the former reef site in Devil's Crown (Feingold and Glynn, 2014), but no live colonies have been noted in the lava rock pools of Punta Espinosa (Glynn, 2003). Recently, high densities of *Pocillopora* colonies were observed in the Concha y Perla Lagoon on the southern coast of Isabela Island (M Schmale, personal communication). Here, we set out to characterize the genetic diversity of the corals and their associated *Symbiodinium* dinoflagellates in this isolated yet highly dense population of *Pocillopora* and compare it to other *Pocillopora* collections from throughout the Galápagos Islands.

Pocillopora species designations were traditionally based on morphological characteristics and 8 or 9 (Hickman, 2008) separate species were identified within the Galápagos Islands. However, within the genus *Pocillopora* there is little correlation between morphology and species designation in the TEP. Only three evolutionary divergent lineages were found based on mitochondrial sequencing phylogenies and Bayesian clustering analysis (Flot et al., 2008;Pinzón and LaJeunesse, 2011). The mismatch between genetic data and traditional species designations based on morphology calls into question previously published species distributions and occurrences of *Pocillopora* in the TEP and elsewhere (Combosch and Vollmer, 2011;Pinzón et al., 2013;Schmidt-Roach et al., 2013). A re-evaluation of *Pocillopora* species distribution in the TEP is thus necessary especially in light of recent large-scale disturbances during El Niño Southern Oscillation (ENSO) events that can cause local extirpations (Glynn and Deweerdt, 1991;Toth et al., 2012). Here, we employ genetic markers

to determine species and clonal diversity of *Pocillopora* and their dinoflagellate symbionts at Isabela Island and throughout the Galápagos Archipelago.

Size frequency distributions of colonies can provide insights into the recovery process from large scale disturbance events such as ENSO. However, correlating age and size is complicated in fragmenting corals such as *Pocillopora damicornis.* In addition to asexual reproduction via fragmentation, *P. damicornis* can produce asexual (ameiotic) (Yeoh and Dai, 2010) as well as sexual planula larvae leading to populations of mixed asexual and sexual origin, e.g. in the Western Australia, Panama, Hawaii and the Ryukyu Islands (Adjeroud & Tsuchiya 1999; Richmond 1987; Stoddart 1984; Whitaker 2006). In contrast, on the Great Barrier Reef and Lord Howe Island reef, sexual reproduction dominates (Ayre et al. 1997; Ayre & Miller 2004; Benzie et al. 1995; Miller & Ayre 2004). Sexual reproduction in eastern Pacific pocilloporids occurs via spawning of female and male gametes into the water column where fertilization occurs (Glynn et al., 1991). Larvae can spend considerable time in the plankton and are already inoculated with *Symbiodinium*, their dinoflagellate symbionts (Richmond, 1987). *Pocillopora* colonies thus may achieve high population densities via either sexual or asexual reproduction. Fingerprinting with high-resolution genetic markers allows for identification of asexually produced colonies (Coffroth and Lasker, 1998;Baums et al., 2006), and in combination with size frequency distributions of colonies can provide insights into population growth and recovery processes.

While asexual reproduction allows for population expansion, it does not allow genetic recombination and, thus, only preserves existing genotypic variation rather than increasing it. Considerable variability in genotypic evenness and richness on small spatial scales is common in corals, ranging from minimal clonal replication to reefs dominated by just one genet (Hunter, 1993;Ayre and Hughes, 2000;Miller and Ayre, 2004;Baums et al., 2006;Sherman et al., 2006). Often asexual reproduction is common at the edges of a species range where sexual partners may be absent (Baums, 2008;Silvertown, 2008). Asexual reproduction allows genets to persist potentially indefinitely in the absence of a sexual partner. Locally well adapted coral clones may thus extend the range of a species (Boulay et al., 2014). Little is known about the contribution of asexual versus sexual reproduction to population maintenance in *Pocillopora* corals in the Galápagos. Surveys of *Pocillopora* clonal structure in the SW Gulf of California, Mexico revealed that a site with little physical disturbance were dominated by a large clone whereas more disturbed sites had a higher occurrence of sexual recruits (Pinzón et al., 2012).

Here, we extend previous efforts (Combosch and Vollmer, 2011;Pinzón and LaJeunesse, 2011;Cunning et al., 2013;Pinzón et al., 2013) to evaluate the genetic diversity and population structure of *Pocillopora* in the Eastern Pacific at the geographic margins of this genus' range. By applying multilocus genotyping methods we discovered that the high density stand of *Pocillopora* corals at Isabela Islands was monogenotypic and aimed to determine whether this clone was a recent colonizer or a survivor of the large-scale ENSO events in 1982/83 and 1997/98. The community of *Pocillopora* colonies at Isabela is of particular ecological value due to its unique presence in the archipelago and support of associated organisms such as fish and benthic invertebrates. Its proximity to the population center of Puerto Villamil gives this ecological oasis high touristic appeal and consequently high economic value.

2. Materials and methods

2.1. Sample collection and DNA Extraction

2.1.1. Species diversity survey

Pocillopora corals were collected during the Global Reef Expedition onboard the M/V Golden Shadow to the Galápagos Islands in 2012. Forty colonies (Table 1) were sampled from across the Galápagos Islands, 6 from Darwin (01.67603° N, 091.99481° W), 24 from Marchena (00.30779° N, 090.40228° W), and 10 from Wolf (01.3856° N, 091.8146° W). Further, three neighboring aggregations of *Pocillopora* colonies were sampled on Isabela Island during the same cruise in 2012 (Table 2). They were located in 2-3m depth just east of the tourist area of Concha y Perla lagoon at 00.96294° S, 090.95600° W. The colonies were found in a volcanic lagoon separated by a basalt sill into a small and large basin. A small sample was clipped from the tips of colonies using bone cutters and the colonies were photographed. Samples were preserved in ethanol and extracted using the DNeasy tissue kit (Qiagen) according to the manufacturer's instruction; however, extraction time in the lysis buffer was extended to 12 hrs.

2.1.2. Clonal structure in the Concha y Perla lagoon

The three *Pocillopora* aggregations in the Isabela volcanic lagoon were sampled for clonal structure following the sampling design of Baums et al. (2006). Briefly, coral branch tips (n = 41) were collected haphazardly in 5m radius circular plots for a total of 4 plots within the volcanic pools on Isabela Island (Figure 1). Plots 3 and 4 were located in the same aggregation. Coordinates had a precision of 5° of arc and of 0.5m along strike. Using a compass and a measuring tape secured to the center point of the circle, colonies were located by a team of SCUBA divers and mapped. The center of the plot was diver selected to maximize colony density and therefore sampling feasibility. An additional 6 colonies were sampled from areas outside of the four plots. A total of 47 branch tips from individual colonies were collected and preserved in 95% non-denatured ethanol. Samples were extracted for Genomic DNA using the DNeasy tissue kit (Qiagen) as above.

2.2. Colony size measurements and percent mortality

The extent of each of the three *Pocillopora* aggregations was outlined using a handheld GPS while snorkeling around the perimeter of each. A series of photographic images were obtained over the complete area of the coral aggregations in the Concha y Perla Lagoon. A Nikon D5100 with a Nikon 10-24 mm lens and Ikelite waterproof housing and a housed Canon G12 camera were used without flash units. These images were taken as perpendicular as possible to the substrate, rather than strictly vertically, and care was taken to not overlap or repeat sections of the aggregation. A 1-meter stick with graduated millimeter increments was used for scale and included in each image. Images were obtained only in areas with live colonies.

Coral Point Count with Excel extensions (CPCe) was used to measure the circumference of the colonies contained within each image (Kohler and Gill, 2006). The 2-D projection of each colony was outlined around the perimeter to calculate planar surface area. These data do not provide measurements of the actual 3-dimensional tissue area, only the planar (2-D) surface area. Measurements were made of individual colonies and fragments. For colonies with partial mortality two measurements were made, the total area and the portion that had died. Adjacent colonies were discriminated from each other by growth pattern, tissue color, and other distinctive patterns. These boundaries would be clear in some cases, but in others close consideration of which way the coral was growing or how they were connected helped determine boundaries. Fragments were

distinguished in a similar fashion. A fragment would normally be clearly unattached from the aggregation and typically much smaller in size and laying on the benthic substrata. Some fragments showed partial mortality, but this was not discriminated. Instead a single measurement of the total planar surface area of each fragment was made. Dead areas were determined mostly by pigment differences from live tissue and the presence of turf algae on the skeleton.

2.3. Colony age estimation

Area estimates from colony sizes were used with published data on *Pocillopora* spp. growth rates to estimate age ranges of the colonies in the pool and to assess if any of the colonies were older than the 1982-83 and 1997-98 El Niño disturbances. The area of each colony was converted to colony radii assuming a circular colony shape with the formula

$$\sqrt{(Area/\pi)}$$

Age was estimated as the radius divided by the linear extension rate (cm year^{-1}). Linear extension rates were estimated at 2.24 cm year^{-1} and were derived from measurements for pocilloporids (*P. damicornis* and *P. elegans*) from the Galápagos Islands based on Glynn et al. (1979). These estimates are lower than the mean linear extension rates from all studies conducted on pocilloporids in the eastern Pacific [mean = 3.31 cm yr^{-1} ± 0.24 SEM, n=11 studies, colony range 2.13 - 7.56; see table 2 in Manzello (2010)]. Estimation of ages from colony sizes is made difficult by processes that allow colony fission or fusion (Hughes, 1984). Assuming that fission (fragmentation) is the more important process, then linear extension likely overestimates colony growth rates from a group of colonies because it is usually measured as pristine growth (i.e., damaged colonies were excluded, Glynn et al., 1979) and, thus, underestimates age. Therefore, these age estimates are likely conservative.

2.4. Polymerase Chain Reaction (PCR) amplification of the mitochondrial *open reading frame of unknown function*

The mitochondrial *open reading frame of unknown function* (ORF) was amplified with the FATP6.1 and the RORF primers (Flot and Tillier, 2007;Flot et al., 2008). This was done for a subset of samples; 4 from inside the volcanic pools and all 40 from the islands of Darwin, Wolf, and Marchena. Amplified products were sequenced on the ABI Hitachi 3730XL genetic analyzer. DNA sequence chromatograms were reviewed and edited using CodonCode Aligner (CodonCode Corporation, Centerville, MA). Sequences (GenBank Accession #s: KM610241-KM610280, Supplementary Table 1) were aligned using ClustalW (Thompson et al., 1994) and neighbor-joining phylogenetic trees were constructed for the mitochondrial ORF using MEGA (Kumar et al., 2001). Trees (Figure 2) were generated using the Bootstrap method with 500 replications and the p-distance model. A representative of each previously-described *Pocillopora* mitochondrial lineage type (sensu Pinzon and LaJeunesse (2011) was included in the phylogenetic analysis: four unique haplotypes (GenBank Accession #s: HQ378758–HQ378761) from the Eastern Pacific and 16 from the Indo-Pacific (GenBank Accession #s: JX994072- JX994088) were included for the phylogenetic tree.

2.5. Host microsatellite genotyping

Pocillopora colonies were genotyped using six published microsatellite loci: Pd3-002, Pd3-005, Pd2-006, Pd2-007, Pd3-008 and Pd3-009 (Starger et al., 2008, Supplement 1). Single-plex reactions consisted of: 1X Taq polymerase buffer, 2.5 mM magnesium chloride, 0.5 mg/mL Bovine Serum Albumin (BSA), 0.2 mM of dNTPs, 0.15 µM forward primers, 0.15 µM reverse primers, 0.5U/µL Taq polymerase and 1 µL of DNA (concentrations ranged from 37ng/µL to 240ng/µL). PCR products were visualized using an ABI3730 (Applied Biosystems) automated DNA sequencer with an internal size standard (Gene Scan 500-Liz, Applied Biosystems) for accurate sizing. Electropherograms were analyzed using GeneMapper Software 5.0 (Applied Biosystems). These 6 markers should have enough power to accurately distinguish between closely related genotypes and those produced by asexual reproduction [probability of identity = $4.2 * 10^{-6}$; (Waits et al., 2001)].

2.6. Denaturing-gradient gel electrophoresis (DGGE) and minicircle analysis

A denaturing-gradient gel electrophoresis (DGGE) was used to analyze the Internal Transcribed Spacer 2 (ITS2) of nuclear ribosomal RNA genes (LaJeunesse, 2001) for a total of 16 samples, 4 from each plot in the volcanic pools. The PCR was conducted using the forward primer, ‘‘ITSintfor2’’ (LaJeunesse and Trench, 2000), which anneals to a ‘‘*Symbiodinium*-conserved’’ region in the middle of the 5.8S ribosomal gene and an ITS-reverse universal primer modified with a 39-bp GC clamp (LaJeunesse and Trench, 2000). Samples and a ladder containing a mix of C1, D1a, and B1 were loaded onto an 8% polyacrylamide denaturing gradient gel (45%–80% urea-formamide gradient; 100% consists of 7 mol L21 urea and 40% deionized formamide) and separated by electrophoresis for 15 h at 115 V at a constant temperature of 60°C (LaJeunesse, 2002). The gel was stained with Sybr Green (Molecular Probes) for 25 min according to the manufacturer’s specifications and photographed (Figure 3). Comparison of the samples with the ladder indicated that all samples contained *ITS-2* Clade *C1*. To determine the ITS2-subclade, the noncoding region of the *psbA* minicircle, an element in the chloroplast genome that allows high resolution comparisons among *Symbiodinium* clades, was sequenced on the Applied Biosystems 3730XL using the primers miniC-F and miniC-Rev and protocol as specified by Moore et al. (2003).

3. Results

3.1. Microsatellite analysis reveals only one genet in Isabela’s lava pools

Using 6 microsatellite markers, multi-locus genotypes were determined for 47 colonies from within the lava pools on Isabella and 40 samples haphazardly collected from Darwin, Marchena and Wolf Islands (Table 1). All 47 colonies sampled from within the volcanic pools of Isabela Island were of the same multi locus genotype (Table 1), that is they were all clonemates of the same genet (PD100, Figure 1). In contrast, the maximum number of clonemates per genet was seven (genet PD107) for any of the samples collected from Darwin, Marchena and Wolf (Table 1). However, note that sampling of colonies outside of Isabela occurred over a larger area within each site than sampling within the lava pools. Greater spatial dispersion of sampled colonies could lead to less genetic similarity. Within each of the four plots, about 10 % of colonies were genotyped (Table 2).

3.2. Typing of the host’s mitochondrial open reading frame

Four colonies belonging to genet PD100 from within the lava pools at Isabela Island were typed for the ORF *of unknown function* of the host’s mitochondria and found to be of lineage 3a (Figure 2). In addition to the lava pool samples, 40 of the 42 samples randomly collected throughout the Galápagos

Islands including Marchena, Wolf and Darwin Islands, successfully amplified for the mitochondrial lineage and were found to be of type 1a.

3.3. DGGE reveals genet PD100 harbors *Symbiodinium* ITS2-clade C1d

Internal transcribed spacer 2-DGGE analysis of 16 samples belonging to genet PD100 from the volcanic pools at Isabela identified *Symbiodinium* ITS-2 clade *C1* as the major symbiont in all samples (Table 1, Figure 3). No other ITS-2 clades appeared to be present at detectable levels. Sequencing of the non-coding *psbA* region of the minicircle of two of the samples from within the volcanic pools further resolved the identified *Symbiodinium* ITS-2 type as sublcade *C1d* (Table 1).

3.4. Colony size measurements and percent mortality

The three aggregations of *Pocillopora* colonies in the Concha y Perla lava pools occupied areas of 53 m^2, 104 m^2, and 291 m^2. These aggregations contained a total of 1,614 colonies at a density of 3.6 colonies m^{-2} (Table 3). There was a total of 43.5 m^2 of overall colony area (planar view of live tissue and dead skeleton), of which 40.3 m^2 was live coral tissue. The average live tissue area of each colony was 249.8 cm^2. Of the total colony surface area, 92.7% was live tissue. In addition, 263 fragments were observed, indicating that asexual reproduction was occurring

3.5. Age estimates

An estimate of colony ages based on southern Galápagos *Pocillopora* spp. growth rate averages of Glynn et al. [(1979), 2.24 cm $year^{-1}$] gave a mean colony age of 3.59 years ± 2.05 SD. The range was 1.68-3.59 years when using the average growth rate of all 11 ETP studies. The three largest colonies found within the three aggregations had estimated ages of 14, 15, and 18 years using the Glynn et al. growth rates. When assuming minimum ages based on the fastest eastern Pacific growth rate from the Gulf of Papagayo, Costa Rica [4.78 cm $year^{-1}$; (Manzello, 2010)] the three largest colonies were 7, 7, and 8 years old.

4. Discussion

The Galápagos Islands harbor some of the most vibrant coral communities in the Tropical Eastern Pacific. Here, we showed that the densest known *Pocillopora* population in the entire Galápagos Archipelago was the result of asexual reproduction. We cannot say for certain whether this clone is a survivor of the 1982/83 ENSO or a later arrival but preliminary age estimates from colony sizes indicate that the birth of the clone may predate the 1997/98 ENSO event. The three largest colonies found within the three aggregations had estimated ages of 14, 15, and 18 years, suggesting a conservative estimated recruitment date of at or just before the 1997-98 El Niño, whereas the remaining 1,611 colonies were estimated to be younger than the 1997-98 El Niño. If only three colonies survived 1997-98, they were probably remnants from a larger population. This bottleneck makes it impossible to determine if the clone survived through the 1982-83 El Niño in the volcanic pool or recruited afterwards from more distant locations.

4.1. Mitochondrial markers define two distinct lineages in the Galápagos archipelago

Pocillopora damicornis is a small branching coral (Figure 1) that forms dense stands in shallow reefs throughout the Pacific (Goreau, 1959). Morphological identification is a challenge (Combosch et al., 2008;Souter, 2010) but sequencing of the mitochondrial *open reading frame of unknown function* (ORF) allows for designation of distinct lineages (Flot et al., 2008;Souter et al., 2009;Pinzón and LaJeunesse, 2011;Pinzón et al., 2013). Three types (Type 1 – 3) can be distinguished genetically that appear to be broadcast spawners (Toonen unpubl. data, Pinzón and LaJeunesse, 2011). An additional four types (4 – 7) appear to be brooders (Pinzón, 2011). Type 3 and 5 are prevalent throughout the Pacific. Co-occurrence of types might reconcile observations of broadcast spawning and brooding in colonies identified as *Pocillopora damicornis* from the same reef (Ward, 1992). Both brooding and broadcasting types are hermaphroditic (Sier and Olive, 1994;Kruger and Schleyer, 1998).

From inside the volcanic pools at Isabela Island, all samples typed for the mt-ORF were found to be of lineage 3a (Figure 2) making the Isabela Island genet the only known representative of this lineage in the Galápagos Archipelago albeit sampling has not been exhaustive thus far. In Panama, type 3a is commonly found on reefs in Taboga and Uraba. Pinzon and LaJeunesse (2011) also found three *Pocillopora* colonies of type 3b in the Galápagos; 1 on Marchena Island and 2 on Darwin Island. The remainder of the *Pocillopora* colonies analyzed by Pinzon and LaJeunesse (n = 19, 2011) and here (n = 38, Table 1) from throughout the Galápagos Island were of type 1a. Lineages 3a and 3b are only separated by 2 nucleotide changes whereas types 3 and 1 are separated by 14 nucleotide differences (Pinzón and LaJeunesse, 2011). It is not known if mitochondrial lineage types 3a and 3b are sexually compatible (i.e. if they represent different species), however type 3b appears to be rare in the Eastern Pacific (Cunning et al., 2013;Pinzón et al., 2013). Therefore, it is possible that the Isabela colonies represent a founder or remnant genet.

4.2. Population dynamics of marginal coral populations

Populations at the edges of a species' range may only receive sporadic immigrants from more central populations. The "abundant center" model makes specific predictions about the demographic properties and genetic diversity of marginal populations (Antonovics, 1976;Brussard, 1984;Lawton, 1993;Hoffmann and Blows, 1994;Lesica and Allendorf, 1995;Vucetich and Waite, 2003) such as those in the tropical Eastern Pacific, Japan and the Red Sea. Evidence for the model has been equivocal in terrestrial and marine systems (reviewed in Sagarin and Gaines, 2002;Eckert et al., 2008) and we do not directly test its validity here. However, according to the hypothesis, physical isolation is expected to increase and population size is expected to decrease with increasing distance from the geographic center of a species' range (reviewed in Sagarin and Gaines, 2002;Eckert et al., 2008). If gene flow is correlated with distance, differentiation will be higher among peripheral populations than central populations ones, and so enhance the probability of inbreeding and the loss of allelic diversity in marginal populations. Because corals can reproduce locally by asexual means, reduced gene flow into marginal populations can result in increased clonality (i.e. decreased genotypic diversity).

Because successful fertilization of gametes is dependent on the distance among adults in broadcast spawning organisms (Levitan, 1992), marginal populations frequently experience Allee effects (Eckert, 2002;Baums et al., 2006). In species capable of asexual reproduction and/or self-fertilization, a rare migrant to a novel environment can successfully establish high local population densities via fragmentation and local recruitment of selfed larvae even in the absence of other sexual partners (Eckert, 2002). Such genetically depauperate populations can persist for extended periods of time

until additional migrants arrive. In the Eastern Pacific, ENSO events change current patterns sometimes bringing migrants to locations where these species are not normally found (Glynn and Ault, 2000). Often the species fail to establish due to a lack of mates and other stochastic factors. Because of the lack of genetic diversity, such populations are vulnerable to disease outbreaks, and they carry an extinction debt (Honnay and Bossuyt, 2005).

Conversely, marginal conditions combined with reduced gene flow can lead to evolution of locally adapted genotypes in edge populations (Bell and Gonzalez, 2011). Asymmetrical gene flow from the center to the margins (driven by the higher densities in the center) can offset the loss of genetic diversity on the edges (Kirkpatrick and Barton, 1997) and improve fitness (Sexton et al., 2011) but also swamp locally adapted genotypes (Haldane, 1956;Case and Taper, 2000). Given this complexity, it remains unknown whether marginal coral populations retain enough functional genetic diversity to adapt to changing conditions and if those adaptations are shared among populations.

Dispersal of type 3a larvae from other TEP locations to Isabela may occur in the future. This assessment is supported by limited data on gene flow and connectivity in corals across the TEP. Of the *Pocillopora* types, Type 1a is the only one with sufficient samples sizes across the region to allow for population-level analysis. Structure results, utilizing seven microsatellite markers, suggested limited partitioning, however F_{st} and R_{st} calcuations were not significant, indicating panmixia within this region which includes the Mexican mainland, Revillagigedo Island, Clipperton Atoll, the Galápagos and Panama (Pinzón and LaJeunesse, 2011). *Porites lobata* was similarly well connected throughout the TEP (Baums et al., 2012). A more comprehensive assessment of coral gene flow patterns within the TEP across a range of species is needed to determine routes of successful larval dispersal within the region (Lessios and Baums, in prep).

4.3. The densest known community of Pocillopora in the Galápagos archipelago formed asexually

Initial establishment of the *Pocillopora* community in Concha y Perla lagoon could have been via sexually or asexually produced (ameiotic) planula larvae that settled on available basalt substrata. Once established at the study site, the high density of the Isabela *Pocillopora* aggregations resulted from asexual reproduction, either via fragmentation or ameiotic larvae (Table 1, Figure 1). While we cannot say for certain, the data indicate that fragmentation is the dominant reproductive process generating the high population density. Accordingly, a high number of fragments were observed within the lava pools (Table 3). Large fragments have a higher chance of survival (Lirman, 2000) so dispersal is limited but over time genets can extend over 10s of meters (Lasker, 1990;Baums et al., 2006;Foster et al., 2007;Pinzón et al., 2012).

Asexually produced propagules of *Pocillopora* are not always the result of fragmentation. *Pocillopora* and other coral species release ameiotic planulae as evidenced by having multilocus genotypes identical to their mothers' (Stoddart, 1983;Stoddart et al., 1988;Brazeau et al., 1998;Sherman et al., 2006;Yeoh and Dai, 2010). Ameiotic planulae have, theoretically, the same dispersal potential as their sexually produced counterparts and thus could be transported further than fragments (Stoddart, 1983). Several clones of the coral *P. damicornis* were found distributed over 8 reefs in Hawaii (Stoddart, 1983) and over 800 km in Australia (Whitaker, 2006). However, we did not find evidence of genet PD100 outside of the larva pools despite searching habitat around Isabela that previously had been settled by *Pocillopora*. Had we found PD100 elsewhere, this would have indicated that the clone produced ameiotic planulae with dispersal potential. The pools are flushed daily – the tidal flow is quite strong so that larvae should have been able to disperse outside the pool.

However, larvae may not find suitable habitat easily in the southern Galápagos due to low temperatures and unfavorable alkalinity (Manzello, 2010). Nevertheless, there is a chance that further searches may yet reveal evidence of PD100 outside the pools.

4.4. Symbiodinium

The three mt-DNA lineages of *Pocillopora* in the Tropical Eastern Pacific identified by Pinzon and LaJeunesse (2011) associate primarily with one or two *Symbiodinium* ITS-2 clade types. *Pocillopora* mt-DNA Lineage 1a was found to harbor both *Symbiodinium* C1b-c and *S. glynni* (clade D) whereas *Pocillopora* mt-DNA Lineage type 3 contained only *Symbiodinium* C1d (LaJeunesse et al., 2008;Pinzón and LaJeunesse, 2011). Analysis of a larger dataset from the Eastern Pacific subsequently also discovered *Symbiodinium* clade D in *Pocillopora* lineage 3 (Cunning et al., 2013). Nevertheless, all 16 tested *Pocillopora* mt-DNA Lineage type 3a samples from within the volcanic pools at Isabela harbored only *Symbiodinium* ITS-2 clade C1d.

The uniformity of the host genet-*Symbiodinium* association in the lava pools at the subclade level is not surprising (Thornhill et al., 2014). Analysis of *Symbiodinium* ITS-2 clade C1d from within the Isabela pools with multiple microsatellite markers may reveal additional subcladal genetic and thereby, perhaps, functional diversity (Howells et al., 2012). However, in other coral species with extensive asexual reproduction, colonies usually associate with just one clonal strain of *Symbiodinium* (Andras et al., 2011;Andras et al., 2012;Baums et al., 2014) and clonemates of the same host genet often harbor the same clonal strain of *Symbiodinium* (Baums et al., 2014).

4.5. Conservation implications

The clone of *Pocillopora* mtORF type 3a in the lava pools of Concha y Perla is the only known representative of its type in the Galápagos. While local density is quite high, the low genotypic diversity may limit the evolutionary potential to selfing and somatic mutations (Van Oppen et al., 2011). No evidence of selfing was found within the pools as that would have generated distinct albeit similar genotypes rather than identical ones. We are quite confident in the conclusion that all sampled colonies were the result of asexual reproduction due to the high number of microsatellite markers used which results in high power to distinguish between closely related and identical genotypes. We cannot exclude the possibility that additional sampling may have detected other *Pocillopora* genotypes, however the chances seem remote. Moreover, all tested colonies only harbored one ITS-2 clade type, *Symbiodinium* ITS-2 clade C1d. This apparent absence of genetic diversity makes the Isabela population vulnerable to infectious disease outbreaks and environmental perturbations. While other corals are rare in the pool, the pool is heavily visited by snorkelers who generally have travelled to other areas of the Archipelago and may serve as disease vectors. Physical contact via fins is one way to spread infectious coral diseases (Williams and Miller, 2005). Rinsing of snorkel gear in a mild bleach solution is one way to reduce the risk of introducing an infectious disease. The population should be monitored for arrival of new, genetically diverse recruits.

5. Acknowledgement

The data presented here represent one component of a larger assessment of coral reefs undertaken by the Khaled bin Sultan Living Oceans Foundation and their partners during the Global Reef Expedition. Samples were collected and exported with appropriate permissions from the Galápagos National Park (Permiso de investigacion cientifoca pc-07-12, No. 0059922, issued 28/05/2012), and logistical support was provided by the Charles Darwin Research Station. Special thanks to Peter W

Glynn for his leadership during this expedition. Thanks also to the other expedition members and Francesca Fourney who helped process coral population data. Funding was provided by NSF grant OCE 0928764 to IBB and an Undergraduate Discovery grant from the PSU Eberly College of Science to BAL and IBB.

6. References

Andras, J.P., Kirk, N.L., and Drew Harvell, C. (2011). Range-wide population genetic structure of *Symbiodinium* associated with the Caribbean Sea fan coral, *Gorgonia ventalina*. *Molecular Ecology* 20**,** 2525-2542.

Andras, J.P., Rypien, K.L., and Harvell, C.D. (2012). Range-wide population genetic structure of the Caribbean sea fan coral, *Gorgonia ventalina*. *Molecular Ecology***,** online early.

Antonovics, J. (1976). Nature of limits to natuarl selection. *Annals of the Missouri Botanical Garden* 63**,** 224-247.

Ayre, D.J., and Hughes, T.P. (2000). Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution* 54**,** 1590-1605.

Baums, I.B. (2008). A restoration genetics guide for coral reef conservation. *Molecular Ecology* 17**,** 2796-2811.

Baums, I.B., Boulay, J., Polato, N.R., and Hellberg, M.E. (2012). No gene flow across the Eastern Pacific Barrier in the reef-building coral *Porites lobata*. *Molecular Ecology* 21**,** 5418-5433.

Baums, I.B., Devlin-Durante, M., and Lajeunesse, T.C. (2014). New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *Cover article. Molecular Ecology* online early.

Baums, I.B., Miller, M.W., and Hellberg, M.E. (2006). Geographic variation in clonal structure in a reef building Caribbean coral, *Acropora palmata*. *Ecological Monographs* 76**,** 503-519.

Bell, G., and Gonzalez, A. (2011). Adaptation and Evolutionary Rescue in Metapopulations Experiencing Environmental Deterioration. *Science* 332**,** 1327-1330.

Boulay, J.N., Hellberg, M.E., Cortés, J., and Baums, I.B. (2014). Unrecognized coral species diversity masks differences in functional ecology. *Proceedings of the Royal Society B: Biological Sciences* 281.

Brazeau, D.A., Gleason, D.F., and Morgan, M.E. (1998). Self-fertilization in brooding hermaphroditic Caribbean corals: Evidence from molecular markers. *Journal of Experimental Marine Biology and Ecology* 231**,** 225-238.

Brussard, P.F. (1984). Geographical patterns and environmental gradients - the central-marginal model in *Drosophila* revisited. *Annual Review of Ecology and Systematics* 15**,** 25-64.

Case, T.J., and Taper, M.L. (2000). Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *American Naturalist* 155**,** 583-605.

Coffroth, M.A., and Lasker, H.R. (1998). Population structure of a clonal gorgonian coral: The interplay between clonal reproduction and disturbance. *Evolution* 52**,** 379-393.

Combosch, D.J., Guzman, H.M., Schuhmacher, H., and Vollmer, S.V. (2008). Interspecific hybridization and restricted trans-Pacific gene flow in the Tropical Eastern Pacific *Pocillopora*. *Molecular Ecology* 17**,** 1304-1312.

Combosch, D.J., and Vollmer, S.V. (2011). Population Genetics of an Ecosystem-Defining Reef Coral *Pocillopora damicornis* in the Tropical Eastern Pacific. *PLoS ONE* 6**,** e21200.

Cunning, R., Glynn, P.W., and Baker, A.C. (2013). Flexible associations between *Pocillopora* corals and *Symbiodinium* limit utility of symbiosis ecology in defining species. *Coral Reefs***,** 1-7.

Eckert, C.G. (2002). The loss of sex in clonal plants. *Evolutionary Ecology* 15**,** 501-520.

Eckert, C.G., Samis, K.E., and Lougheed, S.C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* 17**,** 1170-1188.

Feingold, J.S., and Glynn, P.W. (2014). "Coral Research in the Galápagos Islands, Ecuador.," in *The Galapagos Marine Reserve. A Dynamic Social-Ecological System.,* eds. J. Denkinger & L. Vinueza. Springer), 3-22.

Flot, J.F., Magalon, H., Cruaud, C., Couloux, A., and Tillier, S. (2008). Patterns of genetic structure among Hawaiian corals of the genus *Pocillopora* yield clusters of individuals that are compatible with morphology. *Comptes Rendus Biologies* 331**,** 239-247.

Flot, J.F., and Tillier, S. (2007). The mitochondrial genome of *Pocillopora* (Cnidaria : Scleractinia) contains two variable regions: The putative D-loop and a novel ORF of unknown function. *Gene* 401**,** 80-87.

Fong, P., and Glynn, P.W. (2000). A regional model to predict coral population dynamics in response to El Nino-Southern Oscillation. *Ecological Applications* 10**,** 842-854.

Foster, N.L., Baums, I.B., and Mumby, P.J. (2007). Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. *Journal of Animal Ecology* 76**,** 384-391.

Glynn, P.W. (1988). El-Niño Southern Oscillation 1982-1983 - Nearshore population, community, and ecoystem responses. *Annual Review of Ecology and Systematics* 19**,** U309.

Glynn, P.W. (1994). State of Coral-Reefs in the Galapagos-Island - Natural Vs Anthropogenic Impacts. *Marine Pollution Bulletin* 29**,** 131-140.

Glynn, P.W. (2003). "Coral communities and coral reefs of Ecuador," in *Latin America Coral Reefs,* ed. J. Cortes. (Amsterdam: Elsevier), 449-472.

Glynn, P.W. (2004). High complexity food webs in low-diversity eastern Pacific reef-coral communities. *Ecosystems* 7**,** 358-367.

Glynn, P.W., and Ault, J.S. (2000). A biogeographic analysis and review of the far eastern Pacific coral reef region. *Coral Reefs* 19**,** 1-23.

Glynn, P.W., and Colgan, M.W. (1992). Sporadic disturbances in fluctuating coral-reef environments - El Nino and coral-reef development in the eastern Pacific. *American Zoologist* 32**,** 707-718.

Glynn, P.W., and Deweerdt, W.H. (1991). Elimination of 2 Reef-Building Hydrocorals Following the 1982-83 El-Nino Warming Event. *Science* 253**,** 69-71.

Glynn, P.W., Gassman, N.J., Eakin, C.M., Cortes, J., Smith, D.B., and Guzman, H.M. (1991). Reef Coral Reproduction in the Eastern Pacific - Costa-Rica, Panama, and Galapagos-Islands (Ecuador) .1. Pocilloporidae. *Marine Biology* 109**,** 355-368.

Glynn, P.W., Riegl, B., Correa, A.M.S., and Baums, I.B. (2009). Rapid recovery of a coral reef at Darwin Island, Galapagos Islands. *Galapagos Research* 66**,** 6-13.

Glynn, P.W., and Wellington, G.M. (1983). *Corals and coral reefs of the Galápagos Islands.* Berkeley, CA: University of California Press.

Glynn, P.W., Wellington, G.M., and Birkeland, C. (1979). Coral reef growth in Galapagos - limitation by sea-urchins. *Science* 203**,** 47-49.

Goodkin, N.F., Switzer, A.D., Mccorry, D., Devantier, L., True, J.D., Hughen, K.A., Angeline, N., and Yang, T.T. (2011). Coral communities of Hong Kong: long-lived corals in a marginal reef environment. *Marine Ecology-Progress Series* 426**,** 185-196.

Goreau, T.F. (1959). The ecology of Jamaican coral reefs: Species composition and zonation. *Ecology* 40**,** 67-90.

Guinotte, J.M., Buddemeier, R.W., and Kleypas, J.A. (2003). Future coral reef habitat marginality: temporal and spatial effects of climate change in the Pacific basin. *Coral Reefs* 22**,** 551-558.

Haldane, J.B.S. (1956). The relation between density regulation and natural selection. *Proceedings of the Royal Society of London Series B-Biological Sciences* 145**,** 306-308.

Hennige, S.J., Smith, D.J., Walsh, S.J., Mcginley, M.P., Warner, M.E., and Suggett, D.J. (2010). Acclimation and adaptation of scleractinian coral communities along environmental gradients within an Indonesian reef system. *Journal of Experimental Marine Biology and Ecology* 391**,** 143-152.

Hickman, C.P. (2008). *A field guide to corals and other radiates of Galápagos.* Lexington, VA: Sugar Spring Press.

513 Hoffmann, A.A., and Blows, M.W. (1994). Species borders - ecological and evolutionary perspectives. *Trends*
514 *in Ecology & Evolution* 9**,** 223-227.
515 Honnay, O., and Bossuyt, B. (2005). Prolonged clonal growth: escape route or route to extinction? *Oikos* 108**,**
516 427-432.
517 Howells, E.J., Beltran, V.H., Larsen, N.W., Bay, L.K., Willis, B.L., and Van Oppen, M.J.H. (2012). Coral thermal
518 tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change* 2**,** 116-120.
519 Hughes, T.P. (1984). Population dynamics based on individual size rather than age: A general model with a
520 reef coral example. *American Naturalist* 123**,** 778-795.
521 Hunter, C.L. (1993). Genotypic variation and clonal structure in coral populations with different disturbance
522 histories. *Evolution* 47**,** 1213-1228.
523 Kirkpatrick, M., and Barton, N.H. (1997). Evolution of a species' range. *American Naturalist* 150**,** 1-23.
524 Kohler, K.E., and Gill, S.M. (2006). Coral Point Count with Excel extensions (CPCe): A Visual Basic program for
525 the determination of coral and substrate coverage using random point count methodology.
526 *Computers & Geosciences* 32**,** 1259-1269.
527 Kruger, A., and Schleyer, M.H. (1998). Sexual reproduction in the coral *Pocillopora verrucosa* (Cnidaria :
528 Scleractinia) in KwaZulu-Natal, South Africa. *Marine Biology* 132**,** 703-710.
529 Kumar, S., Tamura, K., Jakobsen, I.B., and Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis
530 software. *Bioinformatics* 17**,** 1244-1245.
531 Lajeunesse, T.C. (2001). Investigating the biodiversity, ecology, and phylogeny of endosymbiotic
532 dinoflagellates in the genus *Symbiodinium* using the its region: In search of a "species" level marker.
533 *Journal of Phycology* 37**,** 866-880.
534 Lajeunesse, T.C. (2002). Diversity and community structure of symbiotic dinoflagellates from Caribbean coral
535 reefs. *Marine Biology* 141**,** 387-400.
536 Lajeunesse, T.C., Bonilla, H.R., Warner, M.E., Wills, M., Schmidt, G.W., and Fitt, W.K. (2008). Specificity and
537 stability in high latitude eastern Pacific coral-algal symbioses. *Limnology and Oceanography* 53**,** 719-
538 727.
539 Lajeunesse, T.C., and Trench, R.K. (2000). Biogeography of two species of *Symbiodinium* (Freudenthal)
540 inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biological Bulletin* 199**,**
541 126-134.
542 Lasker, H.R. (1990). Clonal propagation and population-dynamics of a gorgonian coral. *Ecology* 71**,** 1578-
543 1589.
544 Lawton, J.H. (1993). Range, population abundance and conservation. *Trends in Ecology & Evolution* 8**,** 409-
545 413.
546 Lesica, P., and Allendorf, F.W. (1995). When are peripheral populations valuable for conservation?
547 *Conservation Biology* 9**,** 753-760.
548 Lessios, H., and Baums, I.B. (in prep). "Gene flow in coral reef organisms of the tropical eastern Pacific," in
549 *Coral Reefs of the Eastern Pacific,* eds. P.W. Glynn, D. Manzello & I. Enochs. Springer).
550 Levitan, D.R. (1992). Community Structure in Times Past - Influence of Human Fishing Pressure on Algal
551 Urchin Interactions. *Ecology* 73**,** 1597-1605.
552 Lirman, D. (2000). Lesion regeneration in the branching coral Acropora palmata: effects of colonization,
553 colony size, lesion size, and lesion shape. *Marine Ecology-Progress Series* 197**,** 209-215.
554 Lirman, D., and Manzello, D. (2009). Patterns of resistance and resilience of the stress-tolerant coral
555 *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. *Journal of Experimental*
556 *Marine Biology and Ecology* 369**,** 72-77.
557 Manzello, D.P. (2010). Coral growth with thermal stress and ocean acidification: lessons from the eastern
558 tropical Pacific. *Coral Reefs* 29**,** 749-758.
559 Miller, K.J., and Ayre, D.J. (2004). The role of sexual and asexual reproduction in structuring high latitude
560 populations of the reef coral *Pocillopora damicornis*. *Heredity* 92**,** 557-568.

Moore, R.B., Ferguson, K.M., Loh, W.K.W., Hoegh-Guldberg, C., and Carter, D.A. (2003). Highly organized structure in the non-coding region of the psbA minicircle from clade C *Symbiodinium*. *International Journal of Systematic and Evolutionary Microbiology* 53**,** 1725-1734.

Pinzón, J. (2011). *The evolution of animal-microbe mutualisms.* PhD, The Pennsylvania State University.

Pinzón, J., Reyes-Bonilla, H., Baums, I., and Lajeunesse, T. (2012). Contrasting clonal structure among *Pocillopora* (Scleractinia) communities at two environmentally distinct sites in the Gulf of California. *Coral Reefs* 3**,** 765-777.

Pinzón, J.H., and Lajeunesse, T.C. (2011). Species delimitation of common reef corals in the genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular Ecology* 20**,** 311-325.

Pinzón, J.H., Sampayo, E., Cox, E., Chauka, L.J., Chen, C.A., Voolstra, C.R., and Lajeunesse, T.C. (2013). Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia). *Journal of Biogeography***,** n/a-n/a.

Richmond, R.H. (1987). Energetics, Competence, and Long-Distance Dispersal of Planula Larvae of the Coral *Pocillopora damicornis*. *Marine Biology* 93**,** 527-533.

Sagarin, R.D., and Gaines, S.D. (2002). The 'abundant centre' distribution: to what extent is it a biogeographical rule? *Ecology Letters* 5**,** 137-147.

Schmidt-Roach, S., Lundgren, P., Miller, K.J., Gerlach, G., Noreen, A.M.E., and Andreakis, N. (2013). Assessing hidden species diversity in the coral *Pocillopora damicornis* from Eastern Australia. *Coral Reefs* 32**,** 161-172.

Sexton, J.P., Strauss, S.Y., and Rice, K.J. (2011). Gene flow increases fitness at the warm edge of a species' range. *Proceedings of the National Academy of Sciences*.

Sherman, C.D.H., Ayre, D.J., and Miller, K.J. (2006). Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. *Coral Reefs* 25**,** 7-18.

Sier, C.J.S., and Olive, P.J.W. (1994). Reproduction and reproductive variability in the coral *Pocillopora verrucosa* from the Republic of Maldives. *Marine Biology* 118**,** 713-722.

Silvertown, J. (2008). The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences* 169**,** 157-168.

Souter, P. (2010). Hidden genetic diversity in a key model species of coral. *Marine Biology* 157**,** 875-885.

Souter, P., Henriksson, O., Olsson, N., and Grahn, M. (2009). Patterns of genetic structuring in the coral Pocillopora damicornis on reefs in East Africa. *BMC Ecology* 9**,** 19.

Starger, C.J., Yeoh, S.S.R., Dai, C.-F., Baker, A.C., and Desalle, R.O.B. (2008). Ten polymorphic STR loci in the cosmopolitan reef coral, *Pocillopora damicornis*. *Molecular Ecology Resources* 8**,** 619-621.

Stoddart, J.A. (1983). Asexual production of planulae in the coral *Pocillopora damicornis*. *Marine Biology* 76**,** 279-284.

Stoddart, J.A., Babcock, R.C., and Heyward, A.J. (1988). Self-fertilization and maternal enzymes in the planulae of the coral *Goniastrea favulus*. *Marine Biology* 99**,** 489-494.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22**,** 4673-4680.

Thornhill, D.J., Lewis, A.M., Wham, D.C., and Lajeunesse, T.C. (2014). Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution* 68**,** 352-367.

Toth, L.T., Aronson, R.B., Vollmer, S.V., Hobbs, J.W., Urrego, D.H., Cheng, H., Enochs, I.C., Combosch, D.J., Van Woesik, R., and Macintyre, I.G. (2012). ENSO Drove 2500-Year Collapse of Eastern Pacific Coral Reefs. *Science* 337**,** 81-84.

Van Oppen, M.J.H., Souter, P., Howells, E.J., Heyward, A., and Berkelmans, R. (2011). Novel Genetic Diversity Through Somatic Mutations: Fuel for Adaptation of Reef Corals? *Diversity* 3**,** 405-423.

610 Vucetich, J.A., and Waite, T.A. (2003). Spatial patterns of demography and genetic processes across the
611 species' range: Null hypotheses for landscape conservation genetics. *Conservation Genetics* 4**,** 639-
612 645.
613 Waits, L.P., Luikart, G., and Taberlet, P. (2001). Estimating the probability of identity among genotypes in
614 natural populations: cautions and guidelines. *Molecular Ecology* 10**,** 249-256.
615 Ward, S. (1992). Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora*
616 *damicornis*. *Marine Biology* 112**,** 641-646.
617 Whitaker, K. (2006). Genetic evidence for mixed modes of reproduction in the coral *Pocillopora damicornis*
618 and its effect on population structure. *Marine Ecology-Progress Series* 306**,** 115-124.
619 Williams, D.E., and Miller, M.W. (2005). Coral disease outbreak: pattern, prevalence and transmission in
620 Acropora cervicornis. *Marine Ecology-Progress Series* 301**,** 119-128.
621 Yeoh, S.R., and Dai, C.F. (2010). The production of sexual and asexual larvae within single broods of the
622 scleractinian coral, *Pocillopora damicornis*. *Marine Biology* 157**,** 351-359.

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7. Figure legends

Figure 1 *Pocillopora* colonies were sampled in four polar plots within the volcanic pools at Concha Y Perla, Isabela Island, Galápagos. All colonies shared the same host multilocus genotype (indicated by the symbol shape) and harbored *Symbiodinium* ITS-2 clade C1d (indicated by fill color of the symbol). The host genet assigned to the *Pocillopora* mtDNA-*ORF of unknown function* lineage *3a*. Polar plots: radial axis in m, angular axis in degrees.

Figure 2 Neighbor-joining phylogenetic tree of the *Pocillopora* mtDNA *open reading frame of unknown function*. Each genet (names begin with letters PD) was included once in this dataset. Each genet name includes its geographic location as the last two letters, with "DA"=Darwin, "MR" = Marchena, "WO" = Wolf, "IS" = Isabela. The number of times a genet was observed is indicated in parentheses. Genet PD 119 failed to amplify for this marker. The topology of the tree matches the one published by Pinzon et al. (2013), however Type 4 clusters with Type 5 here rather than with Types 3 and 7. Pinzon et al. reported clustering of Type 4 with Type 5 in their STRUCTURE analysis. Gene Bank accession numbers: KM610241-KM610280.

Figure 3 Internal transcribed spacer 2-DGGE analysis of 16 samples belonging to genet PD100 from the volcanic pools at Isabela identified *Symbiodinium* ITS-2 sublcade C1d as the major symbiont in all samples. Second to last lane from the right is the size standard (mixture of clades D1, B1, and C1).

Table 1 *Pocillopora* colonies collected at Darwin, Isabela, Marchena and Wolf Islands, Galápagos Islands. Given are the number of colonies genotyped (Msat - ramets) and the number of unique multi-locus genotypes identified at 6 microsatellite loci (Msat – genets). Mitochondrial lineage of the host was determined via sequencing of the MtDNA *open reading frame of unknown function* (2 samples failed). The *ITS-2* region (16 samples) and the *pbs minicircle* (4 samples) were sequenced to identify the *Symbiodinium* lineage associated with genet PD100.

Island	Host					Symbiont	
	Msat		MtDNA			ITS2 and psb	
	Genets	Ramets	1A	3A	Failed	C1d	C1d
Darwin		6	6				
	PD108	1	1			NA	
	PD114	1	1			NA	
	PD116	2	1			NA	
	PD117	1	1			NA	
	PD118	1	1			NA	
Isabela		47		4			
	PD100	47		4		16	4
Marchena		24	22		2		
	Failed	2	1		1	NA	
	PD101	3	1			NA	
	PD103	1	1			NA	
	PD105	1	1			NA	
	PD107	7	1			NA	
	PD111	2	1			NA	
	PD112	4	1			NA	
	PD115	3	1			NA	
	PD119	1			1	NA	
Wolf		10	10				
	PD102	2	1			NA	
	PD104	2	1			NA	
	PD106	1	1			NA	
	PD109	1	1			NA	
	PD110	2	1			NA	
	PD113	2	1			NA	
Total	20	87					

Table 2 *Pocillopora* colonies in the Concha y Perla lagoon on Isabela Island, Galápagos Islands were sampled (n = 41) in four plots of 5 m diameter. All colonies were counted within a 3m diameter circle only. Based on those counts, the proportion of colonies sampled was estimated. An additional 6 samples were obtained from outside the four plots. Stdev = standard deviation.

	Total # of colonies sampled within 5m	# of colonies within 3m	# of sampled colonies within 3m	Prop of colonies sampled within 3m
Plot 1	11	75	8	0.11
Plot 2	10	92	9	0.10
Plot 3	10	153	10	0.07
Plot 4	10	73	7	0.10
Total	41	393	34	
Average	10.25	98.25	8.50	0.09
Stdev	0.50	37.48	1.29	0.02

664 **Table 3** *Pocillopora* colony and fragment size measurements
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Planar Surface Area (cm²)

	Fragment	Total Colony	Live Area	Dead Area
Mean	40.8	269.5	249.8	96.4
Standard Deviation	45.3	325.0	305.3	105.7
Minimum	1.7	1.6	1.6	0.7
Maximum	322.9	4915.9	4448.5	547.1
Count	263	1614	1614	330

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Figure 1.JPEG

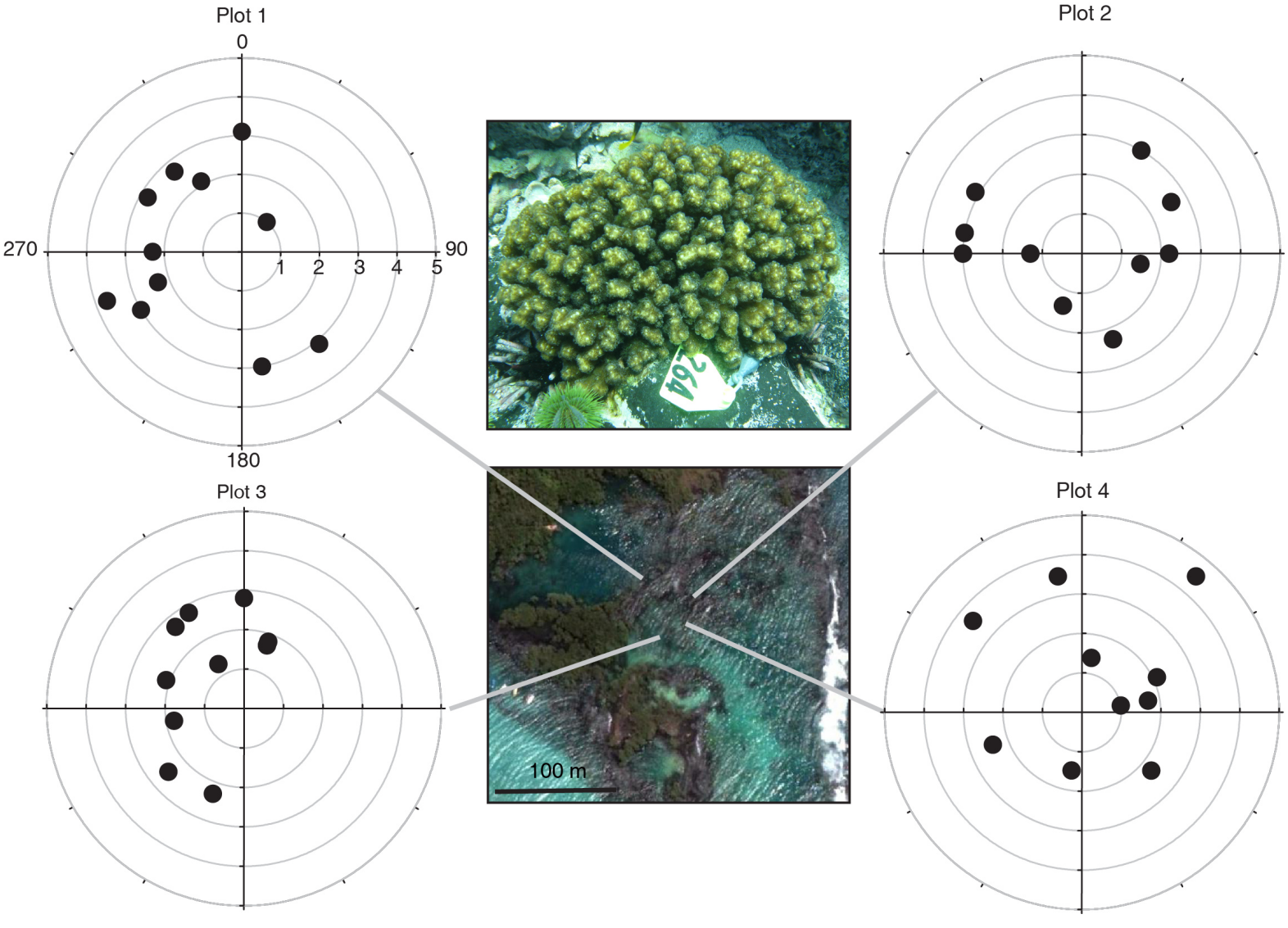

Figure 2.JPEG

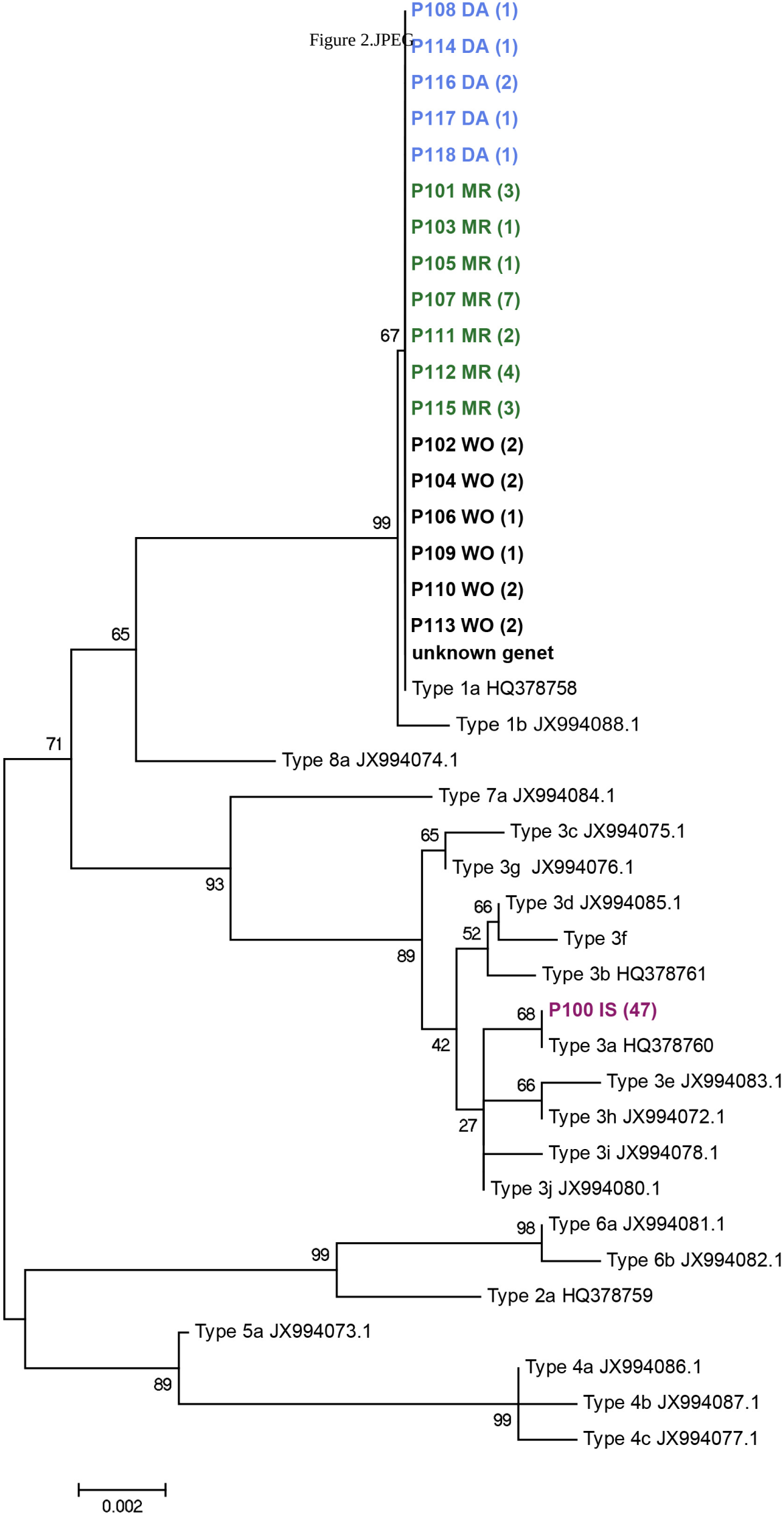

Figure 3.JPEG