Journal of Biogeography (J. Biogeogr.) (2013) **40**, 1595–1608

Journal of Biogeography

ORIGINAL ARTICLE

Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia)

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ABSTRACT

Aim Using high-resolution genetic markers on samples gathered from across their wide distributional range, we endeavoured to delimit species diversity in reef-building *Pocillopora* corals. They are common, ecologically important, and widespread throughout the Indo-Pacific, but their phenotypic plasticity in response to environmental conditions and their nearly featureless microskeletal structures confound taxonomic assignments and limit an understanding of their ecology and evolution.

Location Indo-Pacific, Red Sea, Arabian/Persian Gulf.

Methods Sequence analysis of nuclear ribosomal (internal transcribed spacer 2, ITS2) and mitochondrial (open reading frame) loci were combined with population genetic data (seven microsatellite loci) for *Pocillopora* samples collected throughout the Indo-Pacific, Red Sea and Arabian Gulf, in order to assess the evolutionary divergence, reproductive isolation, frequency of hybridization and geographical distributions of the genus.

Results Between five and eight genetically distinct lineages comparable to species were identified with minimal or no hybridization between them. Colony morphology was generally incongruent with genetics across the full range of sampling, and the total number of species is apparently consistent with lower estimates from competing morphologically based hypotheses (about seven or eight taxa). The most commonly occurring genetic lineages were widely distributed and exhibited high dispersal and gene flow, factors that have probably minimized allopatric speciation. Uniquely among scleractinian genera, this genus contains a monophyletic group of broadcast spawners that evolved recently from an ancestral brooder.

Main conclusions The delineation of species diversity guided by genetics fundamentally advances our understanding of *Pocillopora* geographical distributions, ecology and evolution. Because traditional diagnostic features of colony and branch morphology are proving to be of limited utility, the identification of *Pocillopora* species for future ecological and experimental work should rely on genetic characters that will improve research and aid in conservation strategies for these and other reef-building corals, including the detection of real and mistaken endemic populations.

Keywords

Biogeography, life history evolution, molecular systematics, phylogenetics, *Pocillopora*, reef corals, species diversity, species recognition.

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doi:10.1111/jbi.12110

INTRODUCTION

For centuries, comparative morphology was foremost in the classification and identification of species. The recent evaluation of genetic traits has led to important changes in systematics and taxonomy in ways that have improved our understanding of ecological and evolutionary patterns and processes (Sites & Marshall, 2004). Now, high-resolution population genetic data are increasingly used to generate objective independent evidence for delimiting species under the precepts of the Biological Species Concept (Hey & Pinho, 2012).

The global decline of reef-building corals resulting from anthropogenic impacts at local and global scales has made it increasingly urgent to obtain accurate data on the ecological responses of coral populations to these stressors (Pandolfi *et al.*, 2011). Research is, however, potentially hindered by the poor resolution of species that is confounded by substantial morphological variation, phenotypic plasticity and hybridization. Many researchers now rely more on genetic data when delimiting species and deducing their evolutionary relationships, rather than strictly employing morphological features (Lopez *et al.*, 1999; Medina *et al.*, 1999; Fukami *et al.*, 2004; Benzoni *et al.*, 2007, 2010; Stefani *et al.*, 2008; Eytan *et al.*, 2009; Forsman *et al.*, 2009, 2010; Huang *et al.*, 2011; Pinzón & LaJeunesse, 2011). Most of these genetic studies advocate significant reorganization in the systematics of scleractinians at all taxonomic ranks (Budd *et al.*, 2010, 2012; Kitahara *et al.*, 2010; Kerr *et al.*, 2011).

The use of genetics in recognizing reproductively isolated separately evolving populations, or species, is critical for ongoing research into coral biology and efforts to conserve their diversity and abundance. *Pocillopora* is among the few coral genera for which multiple genetic markers are available to examine genetic isolation and species boundaries. It occurs in shallow-water habitats throughout the Indo-Pacific and the Red Sea, where colony aggregations provide important habitat for numerous fish and invertebrate species (Veron & Pichon, 1976; Veron, 2000). These corals are also common in high-latitude habitats and/or high-sediment environments, where conditions are suboptimal for coral reef development. In some regions or habitats, such as in the Eastern Tropical Pacific, *Pocillopora* colonies are abundant and dominate the benthic landscape down to depths of 5–6 m (Cortés, 1997; Glynn & Ault, 2000). Despite being widely distributed, *Pocillopora* species are among the most susceptible reef-building corals to rapid climate change (McClanahan, 2004), and so are often the subject of ecological and physiological studies (Stoddart & Black, 1985; Ayre & Miller, 2004; Pinzón *et al.*, 2012).

Seventeen morphospecies of *Pocillopora* are listed and described by Veron (2000), but Cairns (1999) recognized only seven of these [*P. capitata* Verrill, 1864; *P. damicornis* (Linnaeus, 1758); *P. elegans* Dana, 1846; *P. eydouxi* Edwards & Haime, 1860; *P. meandrina* Dana, 1846; *P. verrucosa* (Ellis & Solander, 1786); and *P. woodjonesi* Vaugan, 1918]. Traditional classification within this group is based on skeletal features, including colony (macroskeletal) appearance, density and shape of verrucae, and the few available microskeletal (e.g. calice diameter) features (Veron & Pichon, 1976; Cantera *et al.*, 1989; Budd, 1990), but determining which of these most accurately delimits species is a matter of dispute (Budd, 1990).

Recent genetic evidence calls into question the accuracy of morphology in delimiting species boundaries in *Pocillopora* (Flot *et al.*, 2010; Souter, 2010; Pinzón & LaJeunesse, 2011; Schmidt-Roach *et al.*, 2012). This work uses a genetic approach to delimit reproductively isolated independently evolving lineages through the analysis of mitochondrial (ORF, the open reading frame encoding a putative protein of unknown function and the second most variable region of the mitochondrial genome: Flot & Tillier, 2007) and nuclear ribosomal internal transcribed spacer 2 (ITS2) sequences obtained from various morphospecies collected throughout the Indo-Pacific, Red Sea and Arabian (Persian) Gulf. Seven microsatellite markers were employed to test for reproductive isolation among and genetic connectivity within phylogenetically discrete lineages (types) co-occurring (sympatric) in populations sampled over large geographical ranges.

MATERIALS AND METHODS

Field collections

Pocillopora colonies were collected at multiple sites throughout much of this group's distribution (Fig. 1, and Appendix S1 in Supporting Information). Data previously acquired from the tropical and subtropical Eastern Pacific (Pinzón & LaJeunesse, 2011) are based on collections from Mexico (Gulf of California, Banderas Bay, Revillagigedo Islands and Oaxaca), Clipperton Atoll, Panama and the Galápagos Islands (Fig. 1). Identification of morphospecies followed the descriptions and photographs of colonies in Veron (2000, 2002) and was verified by local experts when possible. Some colonies were photographed before sampling using Canon A570 or G12 PowerShot digital cameras in underwater housings. Morphologies that could not be assigned to a particular morphotype were recorded as *Pocillopora* sp. Samples consisted of small fragments (< 1 cm long) collected using bone clippers. Fragments were fixed in ethanol or 20% DMSO (dimethyl sulfoxide) salt solution (Seutin *et al.*, 1991) and stored at –20 °C.

DNA extractions

Genomic DNA extractions were performed on the samples using a non-toxic protocol modified from LaJeunesse *et al.* (2003). A small piece of skeleton and tissue was combined with glass beads (Ceroglass, Columbia, SC, USA) and 600 μL of a cell lysis solution [0.2 M Tris, 2 mM EDTA (ethylenediaminetetraacetic acid), 0.7% SDS (sodium dodecyl sulfate), pH 7.6], and shaken at high frequency in a BioSpec beadbeater. The extract was then incubated with proteinase K

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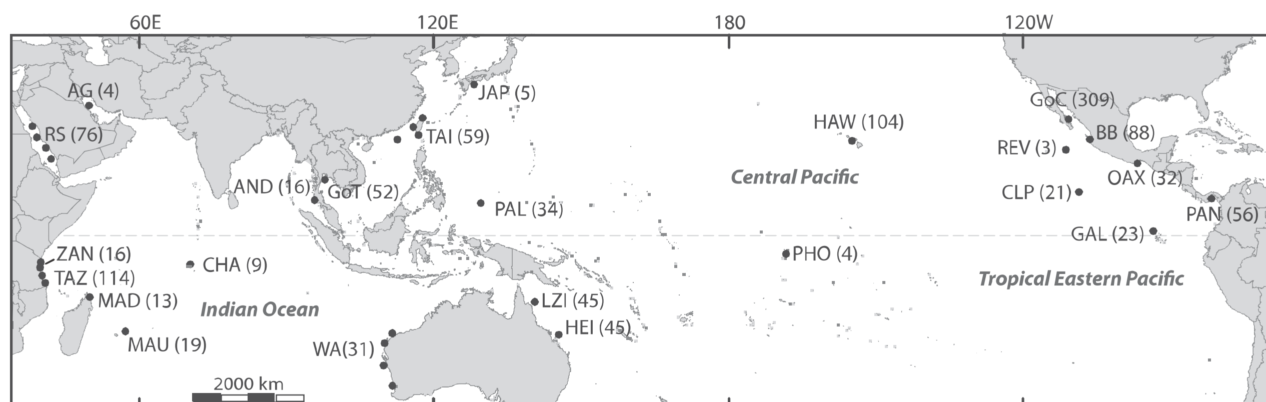

Figure 1 Geographical locations across the Indo-Pacific, Red Sea and Arabian Gulf where species of *Pocillopora* were collected. The total number of colonies sampled at each location is indicated in parentheses. Abbreviations: TAN, Tanzania; ZAN, Zanzibar; RS, Red Sea; AG, Arabian Gulf; MAD, Madagascar; MAU, Mauritius Island; CHA, Chagos; WA, Western Australia; AND, Andaman Sea; GoT, Gulf of Thailand; TAI, Taiwan; JAP, Japan; PAL, Palau; LZI, Lizard Island; HEI, Heron Island; PHO, Phoenix Islands; HAW, Hawaii; CLP, Clipperton Atoll; GoC, Gulf of California; REV, Revillagigedo Islands; BB, Banderas Bay; OAX, Oaxaca; GAL, Galápagos Islands; PAN, Panama.

(20 mg mL^{-1}) at 65 °C for 1 h. After incubation, proteins were precipitated from solution using 9 M ammonium acetate and the sample was stored at –20 °C overnight. The frozen extract was centrifuged (10,000 *g* for 15 min), the supernatant removed and placed into a new tube, and the DNA precipitated from solution with 100% isopropanol, and centrifuged (10,000 *g* for 5 min). The DNA pellet was washed with 70% ethanol, air-dried, re-suspended in 75 μL of distilled water and stored at –20 °C.

PCR amplification and sequencing

The mitochondrial ORF was amplified with the primers FATP6.1 (5′-TTTGGGSATTCGTTTAGCAG-3′) and RORF (5′-SCCAATATGTTAAACASCATGTCA-3′) (Flot *et al.*, 2008). For a subset of samples, the entire ITS2, as well as a portion of the 5.8S, and large subunit rRNA (LSU) genes of the nuclear ribosomal array were amplified using the Scler5.8Sbforward and ITSrev primers (LaJeunesse & Pinzón, 2007). Amplified products were sequenced with the forward primer using BigDye 3.1 terminator mix (Applied Biosystems, Grand Island, NY, USA) following the manufacturer's protocol using an ABI Hitachi 3730xl Genetic Analyzer (Applied Biosystems).

DNA sequence chromatograms were reviewed and edited using Geneious Pro 5.0 (Drummond *et al.*, 2009). Sequences were aligned by eye or using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) with a gap-opening penalty of 15 and extension penalty of 6 (Thompson *et al.*, 1994), and the resulting alignments were exported into paup* 4.0b10 (Swofford, 2000). Separate unrooted phylogenies were constructed for the mitochondrial ORF and ITS2. Heuristic search and bootstrapping (1000 replicates) were performed using the maximum parsimony, neighbour-joining and maximum likelihood methods. In addition, a Bayesian analysis was run in MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001), using the HKG85 substitution model with a chain length of 1,100,000 and the first 100,000 removed (the burn-in).

Microsatellite genotyping and population structure analysis

In addition to the molecular markers, seven microsatellite loci already known to differentiate species and populations of *Pocillopora* (Starger *et al.*, 2008; Pinzón & LaJeunesse, 2011) were used to determine allelic diversity in the genus. Microsatellites were amplified as described in Pinzón & LaJeunesse (2011). Each microsatellite locus, with a labelled primer, was amplified in a separate PCR (an initial denaturation step at 94 °C for 2 min, followed by 31 cycles of 94 °C for 15 s, annealing for 15 s and 72 °C for 30 s, with a final extension at 72 °C for 5 min), then pairs of products from loci with different fluorescent labels were mixed and loaded on an ABI Hitachi 3730xl, using LIZ500 as a size standard. Allele sizes were scored in the software GeneMarker (Softgenetics, State College, PA, USA).

Microsatellite data were used to establish population/species differentiation with the Bayesian model implemented in structure 2.3.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003; Hubisz *et al.*, 2009) using 806 complete multilocus genotypes (MLGs). The analyses in structure assumed admixture, correlated allele frequencies and a location prior. Simulations included five iterations for each K value (K = 1 to 10), with a burn-in value of 100,000 and 1,000,000 chain length. The most probable number of genetically homogeneous groups (K) was determined following the ΔK statistical procedure (Evanno *et al.*, 2005) as implemented in structure harvester (Earl, 2009). Graphics were generated with distruct (Rosenberg, 2004) after aligning all multiple runs for each K with clumpp (Jakobsson & Rosenberg, 2007). Probability tests were performed to detect deviations from

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Hardy–Weinberg equilibrium in phylogenetic groups/lineages using GENEPOP (Raymond & Rousset, 1995; Rousset, 2008). The degree and significance of genetic differentiation (R_{ST} and F_{ST}; Meirmans & Hedrick, 2011) among genotypes between and within lineages, or types, that were defined by DNA sequence analysis were further examined using GENALEX 6.4 (Peakall & Smouse, 2006).

NEWHYBRIDS (v1.1Beta3; Anderson & Thompson, 2002) was used to test for recent hybrid colonies (e.g. generation F1 or F2) between genetically defined *Pocillopora* clusters, globally (across locations) and in sympatry (clusters within the same location). NEWHYBRIDS uses a Markov chain Monte Carlo (MCMC) procedure to distinguish individuals belonging to three categories: pure (Species 1 and Species 2), hybrids (e.g. F1) and backcrosses (e.g. Species 1 × F1). Analyses were run without prior or any individual-specific assumptions, with a 50,000 burn-in value and 500,000 sweeps.

RESULTS

Nucleotide sequences and phylogenetic analyses

A total of 21 distinct haplotypes in the mitochondrial ORF were recovered from 906 samples of various *Pocillopora* morphospecies collected throughout the Indo-Pacific, Red Sea and Arabian/Persian Gulf (Appendix S1). Eight well-differentiated lineages or groups were resolved with strong statistical support using maximum parsimony. Haplotypes that differed by more than five or six base changes were assigned to types 1 to 8 and colour-coded. Closely related variants were discriminated with superscripted letters (Fig. 2). In the unrooted phylogeny, the proportion of each haplotype is represented by different-sized circles (Fig. 2; GenBank accession numbers HQ378559–HQ378561 and JX994072–JX994088).

A phylogeny based on directly sequenced ITS2 from a subset of the samples described above (n = 131) was largely concordant with ORF data (Fig. 3a; GenBank accession numbers HQ378552–HQ378557 and KC015015–KC015037), but several ORF lineages were unresolved, including types 3, 4 and 7, which shared similar and in some cases identical ITS2 sequences (ITS2 of type 8 could not be amplified). Some phylogeographical patterning was observed, as certain ITS2 ribotypes (and ORF haplotypes) occurred only in a particular region or location (Fig. 3b).

Among specimens identified as ORF type 1, ITS2 ribotypes were placed into two groupings. Samples with similar and well-differentiated ITS2 ribotypes were designated as type 1.

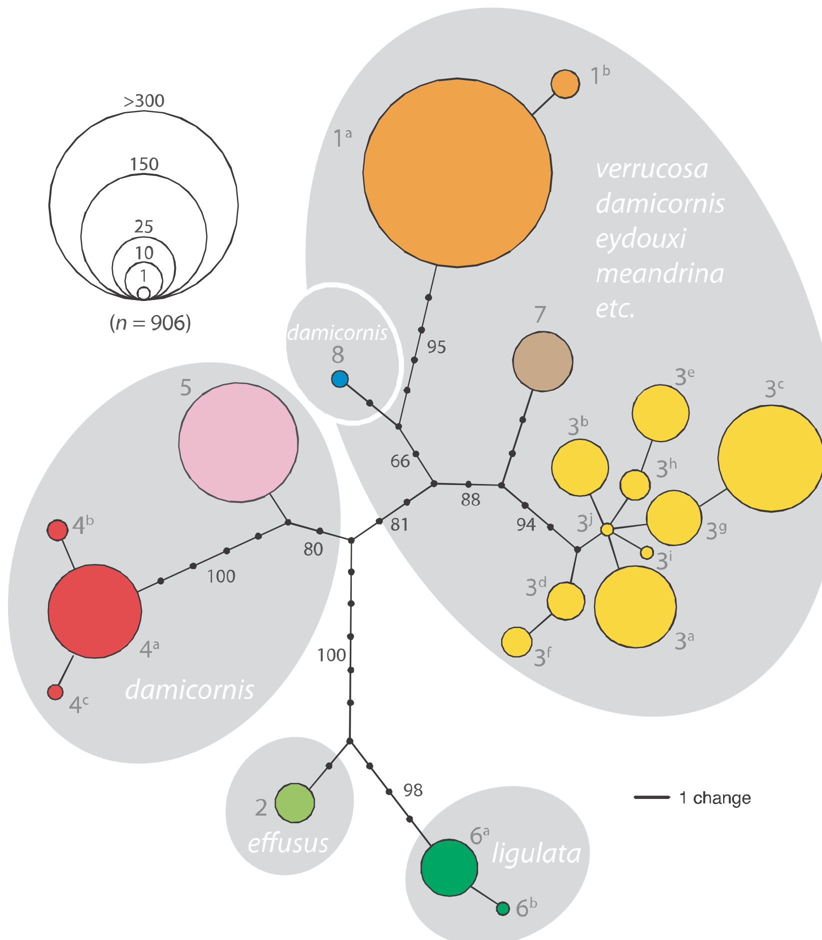

Figure 2 Unrooted parsimony tree redrawn to include the relative number of each mitochondrial open reading frame haplotype found (circle size) and number of nucleotide substitutions that differentiate each haplotype recovered from *Pocillopora* morphospecies surveyed across the Indo-Pacific, Red Sea and Arabian Gulf. The grey shading and white species labels indicate the morphospecies corresponding to a particular genetic lineage. Types 4 and 5 consistently appeared '*damicornis*-like', while various morphospecies comprised types 1 and 3. Numbers adjacent to branches are bootstrap values based on 1000 replicate parsimony analyses. GenBank accession numbers are HQ378559–HQ378561 and JX994072–JX994088.

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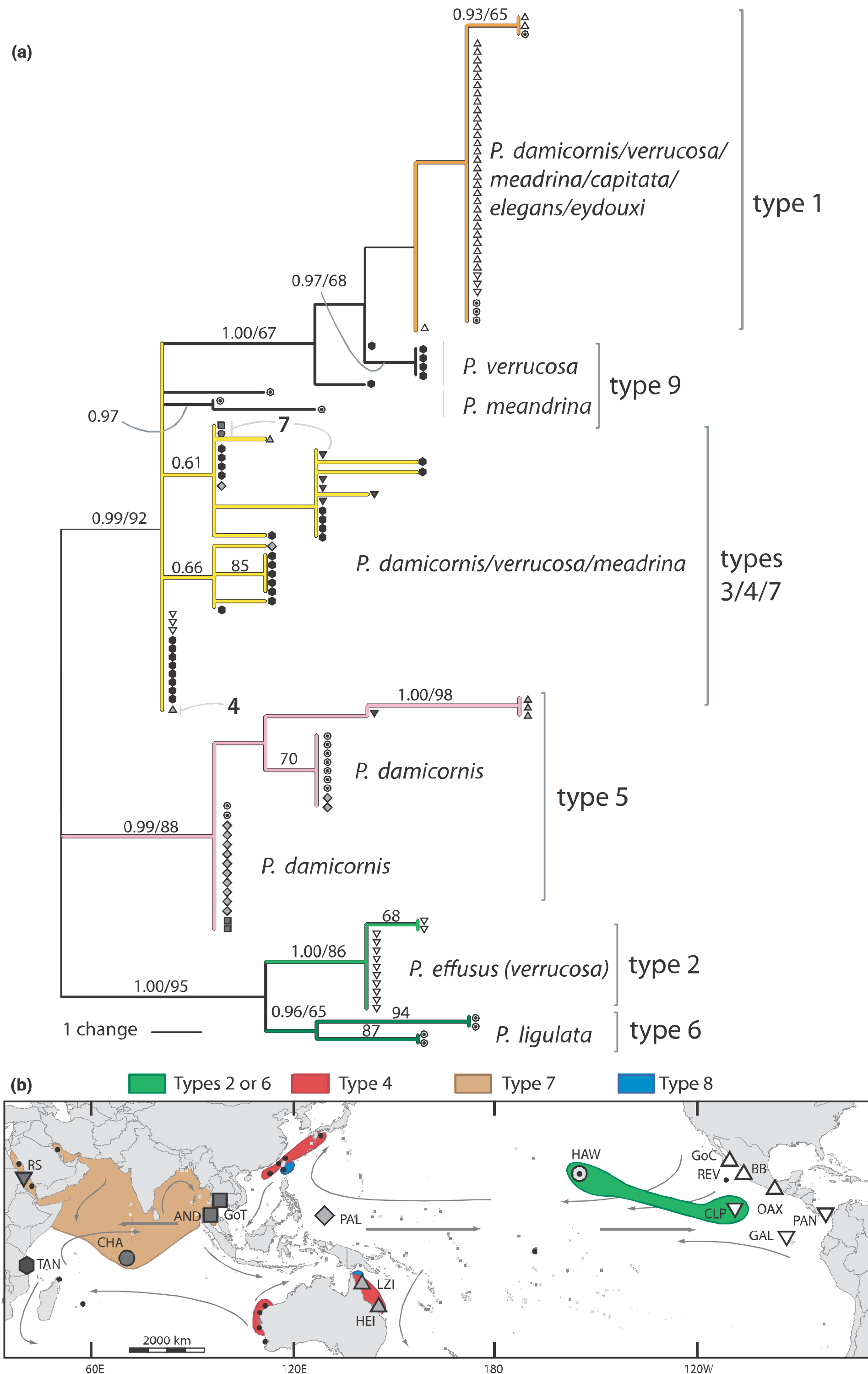

Figure 3 Phylogenetic analysis of internal transcribed spacer 2 (ITS2) ribosomal DNA and geographical distributions observed for rare or less common *Pocillopora* types. (a) Maximum parsimony trees of ITS2 sequence data were concordant with mitochondrial open reading frame haplotypes, but ribotypes of 3, 4 and 7 were unresolved. Symbols adjacent to branch termini correspond to each sample's geographical origin in panel (b). Morphospecies representative of each ribotype grouping are labelled. Bayesian posterior probabilities and maximum parsimony bootstrap values (> 70%) based on 1000 iterations are labelled next to statistically supported branches. The classification of type 9 as an independent lineage was based primarily on microsatellite data. (b) Map with colour shadings depicting the known geographical distributions of what appear to be geographically restricted (regionally endemic) *Pocillopora* types. Arrows indicate the direction of major sea-surface currents that potentially influence dispersal and isolation (Briggs, 1974). For abbreviations, see the legend of Fig. 1. GenBank accession numbers are HQ378552–HQ378557 and KC015015–KC015037.

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The second group was designated as type 9 and was characterized by sequences that were poorly differentiated from the ribotypes corresponding to types 3, 4 and 7 (Fig. 3a). This separation was based on our microsatellite analysis (see below), which showed that individuals assigned as type 1 or type 9 belong to widely distributed overlapping populations that are reproductively isolated from each other.

Microsatellite multilocus genotypes

Reciprocal monophyly between nuclear and mitochondrial DNA sequence phylogenies indicated that concordant lineages within each phylogeny represented genetically isolated groupings, or discrete species. We tested this by analysing genotype data from microsatellite markers. Multilocus genotypes were obtained from a large subset of samples (Fig. 1) to identify populations comprising isolated gene pools. Bayesian clustering using STRUCTURE conducted on the entire data set of genotypes (n = 806) assembled from seven variable loci conservatively delimited at least five but no more than seven genetically cohesive Indo-Pacific populations based on differences in allele diversity and frequency (Fig. 4a, Appendix S2). Genetic subdivision was detected between populations of widely distributed *Pocillopora* located in the Indian and Pacific Oceans, respectively (Fig. 4b, and see Appendix S2a for F_{ST} and R_{ST} values and their statistical significance for types 3 and 5). In Hawaii, the STRUCTURE analysis of population genetic data identified five (K = 5) reproductively isolated lineages corresponding to the morphospecies *P. damicornis*, *P. meandrina*, *P. eydouxi*, *P. molokensis* and *P. ligulata* (Fig. 5). Our calculation of F_{ST} and R_{ST} showed further statistical support for these being natural groupings (Appendix S2b). Some (10 out of 103; 9.7%) genotypes exhibited admixture, indicating the possibility that higher than normal hybridization occurs in this Central Pacific location.

In contrast to Hawaii, the Gulf of California contained a single genetic lineage, type 1, whose colonies were *damicornis*-, *eydouxi*-, *meandrina*-, *verrucosa*- and *capitata*-like in morphology (Fig. 5a,b). No apparent population subdivision was detected between type 1 populations in Hawaii, which appear strictly as *P. eydouxi*, and in the Eastern Pacific, indicating that some connectivity exists between these Central and Eastern Pacific populations (Fig. 5c).

Hybridization

NEWHYBRIDS pairwise analyses showed low or non-existent levels of genetic exchange among sympatric populations involving types 1, 3, 5 and 9 (results not shown). Evidence for recent hybridization was inconsequential at many locations, but some admixture was identified in populations from the Eastern Tropical Pacific (Pinzón & LaJeunesse, 2011) and Hawaii. Our Indo-Pacific-wide comparison of multilocus genotypes revealed a low proportion of admixed genotypes classified as F2 hybrids (5 out of 630; c. 1%). Hybridization was corroborated by a mitochondrial ORF sequence from a colony in Hawaii that exhibited double peaks in the chromatogram corresponding to base substitutions diagnostic of types 5 and 6, respectively (PCR and sequencing were repeated for verification). However, this sequence mosaic was found in only 1 of 906 samples (*c.* 0.1%).

DISCUSSION

The ecological and economic significance of reef-building corals and concerns over their susceptibility to the adverse effects of rapid climate warming have motivated numerous studies on coral diversity, biogeography, physiology (and gene expression), ecology and evolution. However, the quality of these investigations, the conclusions drawn, and their inter-compatibility are dependent on the accurate delimitation of the species under investigation.

Genetic data delimit species boundaries

While nucleotide and allelic data consistently partitioned *Pocillopora* into similar groupings, they differed in resolution. For example, sibling lineages defined by mitochondrial ORF data were not always distinguished by microsatellite analyses. Individuals with mitochondrial ORF haplotypes $3^{h–j}$ and 7 consist of a single genetically homogeneous population in the Red Sea and Arabian Gulf (Fig. 4a). These additional genetic data suggest that type 7 is a divergent sequence variant found in the species lineage (or metapopulation) comprising all type 3 ORF haplotypes (Fig. 2). Nucleotide sequence divergence in both ITS2 and mitochondrial ORF resolved types 2 and 6 as separately evolving lineages, but microsatellite genotyping did not. These may still represent distinct species, with the lack of resolution possibly attributable to low sample numbers, the sharing of conserved microsatellite alleles, and/or poor statistical resolution when analysed in a data set that includes large numbers of more distinct genotypes (Fig. 4a). Indeed, types 4 and 5 are resolved as distinct in analyses using STRUCTURE when the genotypes from all other *Pocillopora* are removed. The development and application of additional, more variable, microsatellite loci will increase the sensitivity of this population genetic approach and may further resolve genetic discontinuities between species (see the discussion below).

When estimated either by genetic or by morphological evidence, the species diversity of *Pocillopora* is low relative to that of other widespread genera (e.g. *Montipora*, *Acropora*). This may be explained in part by life history traits that limit opportunities for interspecific recombination (Kinzie, 1996). Genetic 'experimentation' through hybridization is believed to be important in the diversification of mass-spawning *Acropora* (Willis *et al.*, 2006). However, large temporal differences in spawning (sometimes separated by days or months) minimize chances for sexual recombination among *Pocillopora* (Kinzie, 1996). Genetic cohesion was maintained in populations of distinct *Pocillopora* types at various Indo-Pacific locations, suggesting that recent hybridization and

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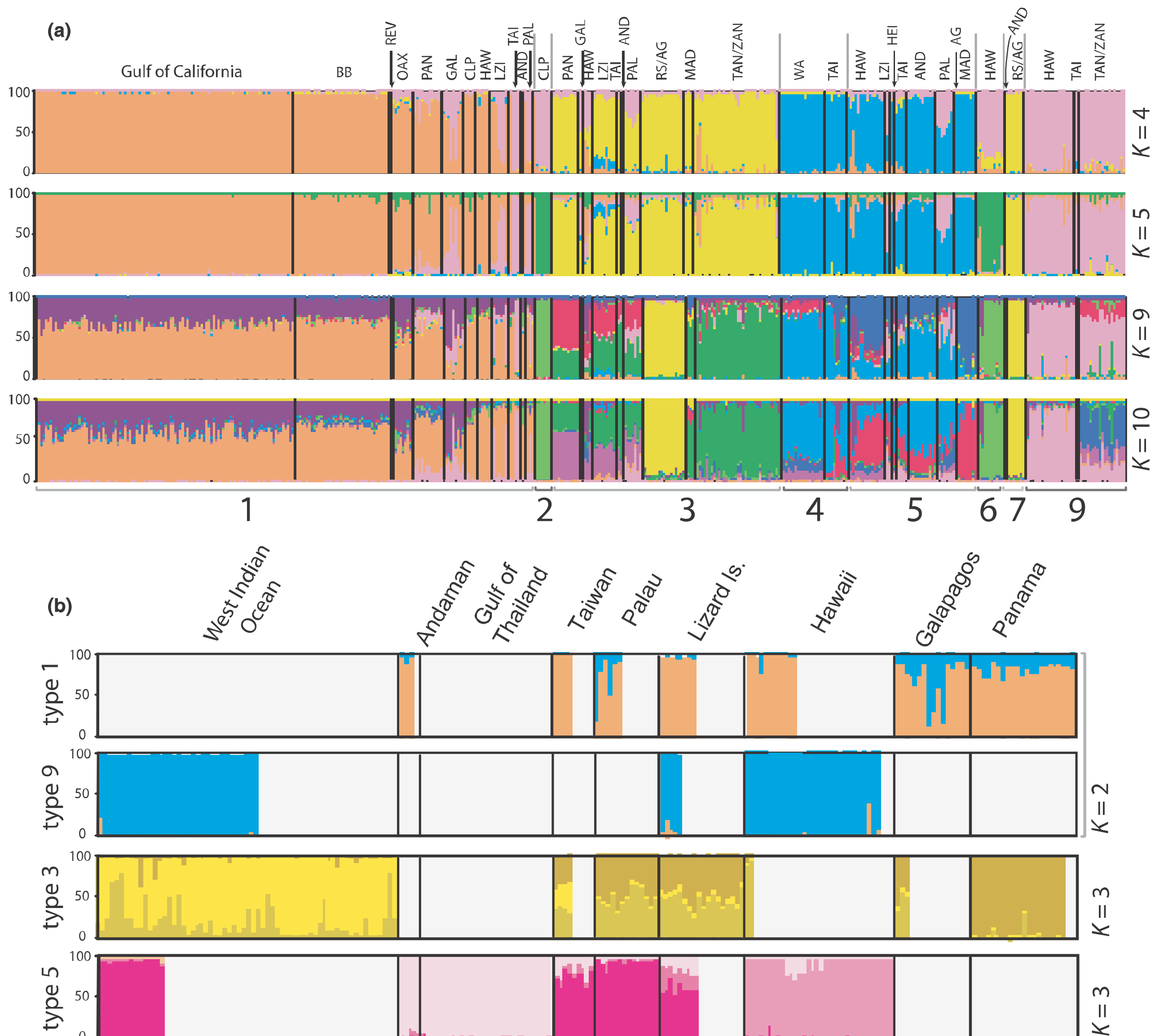

Figure 4 Analysis of population genetic structure based on allelic data across seven microsatellite loci obtained from Indo-Pacific *Pocillopora*. (a) Clustering using the software STRUCTURE of multilocus genotypes from 806 *Pocillopora* colonies collected throughout the Indo-Pacific and examined assuming 1 to 10 populations, respectively (only K = 4, 5, 9 and 10 are shown). Five genetically isolated populations (K = 5) appear optimal for these data. Mitochondrial open reading frame haplotypes initially designated as type 1 were partitioned into two genetically differentiated populations (F_{ST} = 0.224, P < 0.01; n = 806), motivating our designation of a ninth type. Analyses at higher K values resolved populations around the Arabian Peninsula as genetically distinct. Each coloured bar represents the multilocus genotype of an individual and its statistical assignment to a particular population. (b) A preliminary analysis of fine-scale genetic structure calculated for individual genotypes constituting a distinct phylogenetic lineage, or type, acquired from populations across the Indo-Pacific. Allelic compositions were similar between most populations, indicating connectivity over large geographical distances. Some substructure was often detected between populations from different ocean basins (for F_{ST} and R_{ST} values, see Table S2). These differences were considerably less than the genetic differentiation observed between types. The white empty spaces in the graphical representation of (b) indicate where genotypic data were limited or unavailable owing to differences in distribution, sample number, rarity and/or the absence of certain *Pocillopora* types from each region.

introgression in most regions is insignificant (Fig. 4a,b). While some individuals may represent hybrids, interspecific recombination does not appear to break down the genetic differentiation that persists between types over thousands of kilometres (Fig. 4b; Ladner & Palumbi, 2012).

How concordant are morphospecies and genetic lineages?

Flot *et al.* (2008) observed that *Pocillopora* morphospecies around Hawaii corresponded well to genetically differentiated

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lineages (Fig. 5a). For example, canonical colonies of *P. damicornis* had corresponding sequences of nuclear and mitochondrial DNA markers that were distinct from those of all other morphospecies sampled. However, Pinzón & LaJeunesse (2011) found only three reproductively isolated lineages among seven *Pocillopora* morphospecies in the Eastern Tropical Pacific and designated then simply as types 1, 2 and 3 (Fig. 5b). Type 1 was the most common and widespread, consisting of colonies with classical '*damicornis*', '*verrucosa*', '*capitata*', '*meandrina*' and '*eydouxi*' morphologies (Fig. 5c; Pinzón & LaJeunesse, 2011). The broad morphological appearance, or 'character release', exhibited by type 1 in the Gulf of California may relate to relaxed competition for resources (Grant, 1972). This possibility remains speculative, however, until genomic approaches are developed to examine the developmental basis of branching pattern and thickness, and the relative size, shape and density of verrucae (Ball *et al.*, 2002). Such future analyses might show that certain traits currently diagnostic of *Pocillopora* morphospecies are coded by sets of co-localized genes that, when separated in the genome by disruptive selection and/or drift, contribute to greater inter-individual variation (allelic variation).

The frequent discordance between morphology and genetics over broad geographical scales could lead to incorrect inferences about connectivity and hybridization. For example, colonies of various type 3 ORF haplotypes appeared '*damicornis*-like' in certain regions, while in other places they were predominantly '*verrucosa*-like' (Fig. 3a). In the western Indian Ocean, Souter identified two lineages attributed to *P. damicornis* (i.e. types '*NF*' and '*F*', which correspond to types 3 and 5, respectively). We found that type 3 also appears as the morphospecies *P. verrucosa* in Tanzania (and in Pacific Panama, where it also exhibits both phenotypes), but because Souter (2010) never collected other morphospecies, this morphological–genetic discordance was missed. In eastern Australia, Schmidt-Roach *et al.* (2012) discovered that various ecomorphs, or morphotypes, of *P. damicornis* morphospecies consisted of several distinct genetic lineages, or species. Designating according to morphotype (*sensu* Veron & Pichon, 1976) and using Greek letters, they described at least three to five genetic groups that correspond to many of the genetic types described in this study: α = type 4; β and δ = type 5; γ = type 3; ε = type 8. Unsuspecting researchers who gathered population genetic data from colonies that appear to be the same species would introduce error in statistical analyses, as the combination of independent gene pools collected in different ratios across different sites may result in perceived population structure and hybridization where none really exists (Magalon *et al.*, 2005; Combosch *et al.*, 2008; Combosch & Vollmer, 2011; J.H. Pinzón, unpublished data).

The 'global perspective' provided by these findings also indicates that morphospecies once thought to be endemic are instead morphological variants of a widespread species. In our analyses, the protected species *P. molokensis* Quelch, 1886 is not endemic to Hawaii, but instead belongs to type 3 (see below). However, *P. ligulata* Dana, 1846 (type 6) was found only in Hawaii and may indeed be a rare endemic, but is still similar to type 2 from the Clipperton Atoll. These findings indicate that, while colony morphology is probably suitable for species recognition at some locations, reliance on morphology may confound scientific conclusions and mislead conservation strategies in many regions across the Indo-Pacific. The general concordance of various types of genetic data indicates that *Pocillopora* species, without further consideration of morphological characters, are definable under precepts of the Phylogenetic and Biological Species Concept (de Queiroz, 2007). Therefore, genetic evidence should be gathered to examine the identity of morphospecies as a standard part of ecological research and conservation. In the future, morphological features may be found to correspond to genetics-based species hypotheses (e.g. Fukami *et al.*, 2004), but, ultimately, a taxonomic revision informed foremost by genetic evidence is needed for the entire genus.

Widely distributed and regionally endemic 'species'

When ascribed to a particular genetic lineage, each *Pocillopora* type differed in its range of distribution (Fig. 3b). Prevailing circulation patterns within each ocean basin appeared to have minimal influence on dispersal, gene flow and population structure among widespread types, or 'species'. Allelic compositions in the populations of types 1, 3, 5 and 9 were usually homogeneous over thousands of kilometres, suggesting that these types are capable of successful long-distance dispersal (Fig. 4a,b). For example, the Eastern Pacific Barrier had no measurable effect on the genetic structure and distribution of types 1 and 3 (Fig. 4b). Recent population genetic analysis on *Porites lobata* found populations in the Eastern Pacific to be strongly isolated from populations in the Central Pacific and Hawaii (Baums *et al.*, 2012). Further investigations on other scleractinians from distant populations, such as those widely separated by the Eastern Pacific Barrier, are required to diagnose whether unusually high gene flow is unique to *Pocillopora*. Indeed, the brooded larvae of *P. damicornis* are exceptionally long-lived in experimental aquaria (Richmond, 1987), and these genetic patterns provide further evidence of a long planktonic residence time. High connectivity that persists over time ultimately minimizes the potential for allopatric speciation, and may further explain why species diversity in *Pocillopora* is disproportionately low relative to other widespread, ecologically common coral genera (see the discussion above).

Continental landmasses restrict major ocean currents, limit connectivity, and maintain a major zoogeographical break between oceans (Briggs, 1974; Benzie, 1999). *Pocillopora* type 3 was found in all of the major biogeographical provinces where colonies of this genus are known to occur (Appendices S1 & S3). However, many distinct ORF haplotypes and ITS2 ribotypes in this group (Figs 2 & 3a, Appendix S3) were restricted geographically to locations in the Indian or the Pacific Ocean, consistent with dissimilar allele frequencies between popula-

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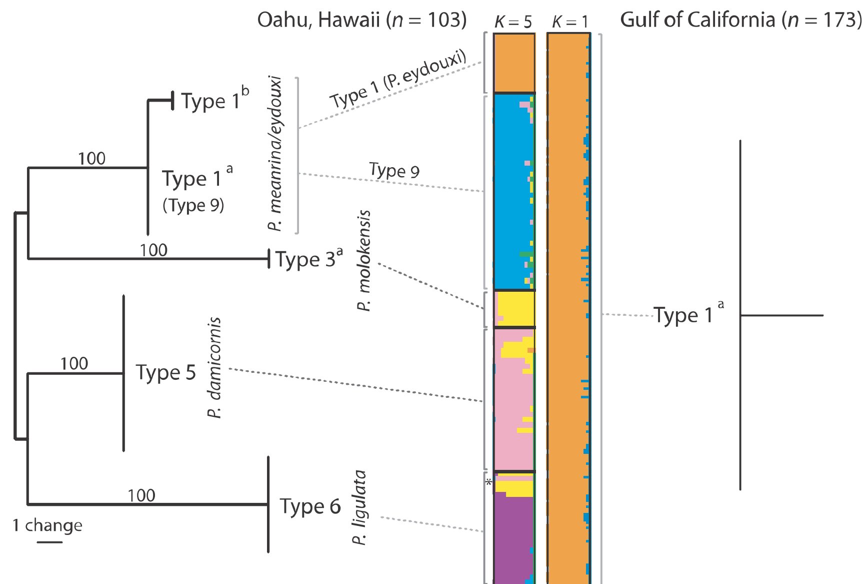

Figure 5 Comparison of mitochondrial open reading frame sequence phylogenies and genetic structure (based on microsatellite analysis) among *Pocillopora* morphospecies found in the Central Pacific (O'ahu, Hawaii) and the Eastern Tropical Pacific (Gulf of California). (a) Morphospecies from Hawaii displaying typical examples of colony morphology for (i) *P. eydouxi*, (ii) *P. molokensis*, (iii) *P. meandrina*, (iv) *P. damicornis*, (v) *P. ligulata* and (vi) close-up of branch morphology of *P. ligulata*. (b) Morphospecies found in the Gulf of California, which include (i) *P. damicornis*, (ii) *P. verrucosa*, (iii) *P. meandrina*, (iv) *P. elegans* and (v) *P. capitata*, comprising an undifferentiated reproductive population (Pinzón & LaJeunesse, 2011). (c) Maximum parsimony reconstructions (open reading frame) and population genetic analyses showing the contrast in the genetic differentiation of five morphospecies found in Hawaii (left) and a monotypic lineage found in the Gulf of California (right), where a single haplotype sequence was recovered from 173 colonies comprising six morphospecies sampled from the Gulf (Pinzón & LaJeunesse, 2011). Type 1 found in the Gulf and throughout the Eastern Tropical Pacific is homologous and genetically undifferentiated from the Hawaiian morphospecies *P. eydouxi*.

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tions in separate ocean basins (Fig. 4b, Appendices S1 & S3). Populations from sites around the Arabian Peninsula were genetically unique, perhaps owing to factors influencing the high endemism observed in this region (e.g. haplotypes 3^h, 3^i and 3^j were exclusive to the Red Sea; Hughes *et al.*, 2002).

Several other genetic lineages exhibited limited distributional ranges. Of these, types 2 and 6 were the most divergent lineages that exhibited narrow and possibly endemic distributions. Type 2 was found at Clipperton Atoll and most samples correspond to the morphospecies *P. effusus* Veron, 2002 described in Glynn *et al.* (1996). Type 6 (*P. ligulata*) was found only in O'ahu, Hawaii, and was differentiated from type 2 by both the ORF and ITS2; however, these sibling lineages were not separated by microsatellite data (see discussion above). Type 8 was identified in several samples from Orchid Island, adjacent to the Kuroshio Current near Taiwan (Fig. 3b). A single colony of this type was identified by Schmidt-Roach *et al.* (2012) on the northern outer Great Barrier Reef of Australia, indicating that its distribution extends to both hemispheres, but it is possibly rare. The divergence of this mitochondrial haplotype was sufficient for it to be recognized as a distinct entity, but additional genetic data are needed to determine whether this represents a distinct species lineage.

Finally, type 4 (type α *sensu* Schmidt-Roach *et al.*, 2012) was commonly identified from sites along the east and west coasts of Australia and at several locations around Taiwan and Japan in the north-west subtropical Pacific (Fig. 3b). Schmidt-Roach *et al.* (2012) found type 4 and a related lineage to be common at the highest latitudes in eastern Australia and Lord Howe Island. While this lineage probably extends to the Philippines and Indonesia, our sampling suggests that it is rare or non-existent in the Central and Eastern Pacific and western Indian Ocean regions. From existing geographical data, it appears to exist over a wide latitudinal range and is particularly common in cold-water environments. More frequent geographical sampling and analyses are necessary to determine the extent of regional distributions and how sea-surface currents, temperature and/or light differently affect the dispersal and gene flow of this and other *Pocillopora* across the Indo-Pacific.

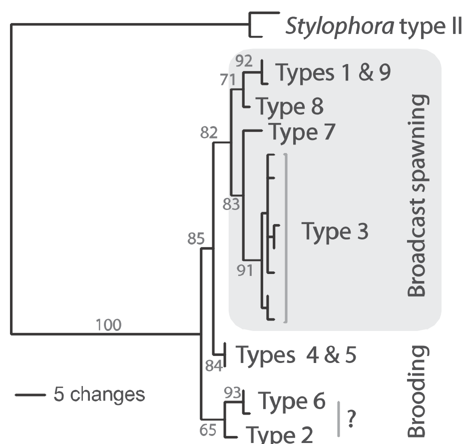

Figure 6 The proposed evolution of broadcast spawning among species of *Pocillopora* based on a rooted mitochondrial open reading frame phylogeny (maximum parsimony; 382 aligned bases). Haplotypes from the large branch morphology type II of *Stylophora* sp. in the Red Sea were used as outgroups (*sensu* Stefani *et al.*, 2011). Maximum parsimony bootstrap values based on 1000 iterations for statistically well-supported branches (> 65%) are provided.

The evolution of broadcast spawners from a brooding family

Scleractinians reproduce by broadcasting eggs into the water column or brooding fertilized embryos and later releasing well-developed larvae (Richmond & Hunter, 1990; Kerr *et al.*, 2011). Brooding is a general feature of the pocilloporid genera *Stylophora*, *Seriatopora* and *Madracis*, but not *Pocillopora* (Richmond & Hunter, 1990; Vermeij *et al.*, 2004). Based on reports in the literature and our phylogenetic analyses, a monophyletic lineage within *Pocillopora*, comprising types 1, 3, 7, 8 and 9, broadcast spawns (Fig. 6). The morphospecies *P. damicornis* from the Eastern Tropical Pacific and eastern Australia (Glynn *et al.*, 1991; Ward, 1992; Chavez-Romo & Reyes-Bonilla, 2007), *P. eydouxi* and *P. meandrina* from Hawaii (Kinzie, 1996), and *P. verrucosa* from the Red Sea (Fadlallah, 1985; Shlesinger *et al.*, 1998) broadcast spawn. These morphologies probably correspond to types 1, 3, 7 and 9 in these regions (Appendix S1). Therefore, broadcast spawning probably evolved in *Pocillopora* from an ancestral brooder (Stolarski *et al.*, 2011). Whereas brooding is generally thought to have evolved frequently from broadcasting ancestors, the state transition of broadcasting evolving from brooding is rare among scleractinians (Shlesinger *et al.*, 1998; Kerr *et al.*, 2011).

The ecological and evolutionary consequences of broadcast spawning versus brooding are considerable. The group of broadcast spawning *Pocillopora* exhibit higher genetic connectivity, are more widespread, and globally more abundant (Fig. 4b; Graham *et al.*, 2008; Harrison, 2011). The brooders, types 4 and 5, appear exclusively as the canonical morphotype *P. damicornis* throughout their distributions (Fig. 2). Whether this lack of morphological variation relates to their mode of reproduction requires further research. They release large larvae with the capacity for long-range dispersal into the water column (Richmond, 1987). However, genetic and distributional evidence indicate that their dispersal and gene flow are restricted relative to types 1, 3 and 9. Widely distributed populations of type 5 exhibited more genetic structure than populations of type 3 across the same geographical range (Fig 4b; higher F_{ST} values, Appendix S2). No information on the reproductive modes of types 2 and 6 was available, but their phylogenetic placement and narrow geographical distribution suggest that they are brooders. The genus *Pocillopora* offers an ideal system with which to further examine how genetic, ecological and evolutionary

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processes relate to reproductive 'strategies' in sedentary clonal animals.

ACKNOWLEDGEMENTS

The authors acknowledge the help of many collaborators in acquiring permits and collecting samples, including A.L. Cupul-Magaña, A. López-Pérez, M. Walther, P. Medina-Rosas, K. Kaiser, M. Torchin, C. Schloeder, W.K. Fitt, D. Kemp, R. Silverstein, A.C. Baker, N. Phongsuwan, D.O. Obura, R. Rojan, L. Tonk, S. Keshavmurthy, O. Hoegh-Guldberg, D. Wham, C. Roder, J. Schnetzer and N. Ichim-Moreno. Frank Stanton provided images of *Pocillopora* colonies from Hawaii. We also would like to acknowledge the valuable comments of Christine Maggs and three anonymous referees. Coral samples were collected and exported with appropriate collection and CITES export permits from participating countries. This research was funded in part by the Pennsylvania State University, the National Science Foundation (IOB 544854), the IOC-UNESCO-World Bank Targeted Working Group on Coral Bleaching, an Alfred P. Sloan Scholarship to J.P., a National Science Council and Academia Sinica Thematic Grant, Taiwan to C.A.C., and a KAUST Academic Excellence Alliance (AEA) award to C.R.V.

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SUPPORTING INFORMATION

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

Appendix S1 The mitochondrial open reading frame diversity found in collections of *Pocillopora* from throughout the study area.

Appendix S2 Pairwise calculations of R_{ST} and F_{ST} among *Pocillopora* populations of type 3 and 5 (a) and among types found in O'ahu, Hawaii (b).

Appendix S3 Biogeographical distributions of type 3 open reading frame haplotypes of *Pocillopora*.

DATA ACCESSIBILITY

Sample list including locations, ORF and ITS2 designations, microsatellite allelic data, and STRUCTURE input files: DRYAD entry doi:10.5061/dryad.jd512.

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BIOSKETCH

Jorge H. Pinzón is currently a National Science Fellowship postdoctoral fellow working at the University of Texas Arlington. His research examines processes important in the evolution and persistence of species diversity, and how global climate change is affecting these processes. He uses genetic approaches to independently examine species boundaries and to explore to what extent morphology is a reliable metric for species recognition.

Author contributions: J.P. and T.C.L. conceived the ideas; J.P., E.S., E.C., L.J.C., C.A.C., C.R.V. and T.C.L provided samples and collected data; J.P. analysed the data; and J.P. and T.C.L. led the writing.

Editor: Christine Maggs

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