Microsatellite markers for multiple *Pocillopora* genetic lineages offer new insights about coral populations

Yuichi Nakajima\(^1\), Patricia H. Wepfer\(^2\), Shohei Suzuki\(^2\), Yuna Zayasu\(^3\), Chuya Shinzato\(^3,4\), Noriyuki Satoh\(^3\) & Satoshi Mitarai\(^1\)

Population genetics of the coral genus *Pocillopora* have been more intensively studied than those of any other reef-building taxon. However, recent investigations have revealed that the current morphological classification is inadequate to represent genetic lineages. In this study, we isolated and characterized novel microsatellite loci from morphological *Pocillopora meandrina* (Type 1) and *Pocillopora acuta* (Type 5). Furthermore, we characterized previously reported microsatellite loci. A total of 27 loci (13 novel loci) proved useful for population genetic analyses at two sites in the Ryukyu Archipelago, in the northwestern Pacific. Clonal diversity differed in each genetic lineage. Genetic structure suggested by microsatellites corresponded to clusters in a phylogenetic tree constructed from a mitochondrial open reading frame (mtORF). In addition, we found an unknown mitochondrial haplotype of this mtORF. These microsatellite loci will be useful for studies of connectivity and genetic diversity of *Pocillopora* populations, and will also support coral reef conservation.

The genus *Pocillopora*, especially *Pocillopora damicornis*, is the most studied reef-building coral in terms of population genetics\(^1\)–\(^9\). This genus includes both broadcast-spawning and brooding species\(^9\) and it is distributed from the Indian Ocean to the East Pacific. In *Pocillopora*, 17 species are currently recognized in Veron\(^11\) and in Coral of the World (http://coral.aims.gov.au/), while 22 species are registered in the World Register of Marine Species (WoRMS) (http://www.marinespecies.org/), as of March 2017. However, based on gross morphology, the number of *Pocillopora* species may be overestimated\(^12\), because recent genetic studies have suggested that the traditional classification may not correspond to genetic species\(^10,12–15\). Nevertheless, 16 or more genetic lineages of *Pocillopora* have been considered as putative species based on haplotypes of a mitochondrial DNA region\(^10,12,15\) and genetic structure analyzed using microsatellite markers\(^16,17\).

Approximately 400 reef-building coral species inhabit the Ryukyu Archipelago\(^16\), Japan. A recent phylogenetic approach confirmed six lineages of *Pocillopora* in the northwestern Pacific\(^12\). *Pocillopora* corals have been damaged by various global and local anthropogenic disturbances, and coral populations have not yet recovered from repeated mass bleaching events since 1998 at reefs in the Ryukyu Archipelago\(^17\). Population genetics studies using genetic markers make it possible to distinguish invisible inter- and intraspecific relationships among populations. These studies have estimated genetic diversity, differentiation among locations, reproductive characteristics, and species delimitations for various organisms, including corals. Microsatellites, composed of tandemly repeated regions of nucleotides are highly polymorphic among individuals and chromosomes. Next-generation sequencing technology has facilitated development of microsatellite loci, which have recently been developed for reef building corals\(^18,19\).

In this study, using next-generation sequencing, we developed cross-lineage microsatellite loci in order to facilitate population genetic analyses for multiple lineages of *Pocillopora* in the Ryukyu Archipelago. Although

\(^1\)Marine Biophysics Unit, Okinawa Institute of Science and Technology Graduate University, Tancha 1919-1, Onna, Okinawa, 904-0495, Japan. \(^2\)Okinawa Marine Science Support Section, Okinawa Institute of Science and Technology Graduate University, Tancha 1919-1, Onna, Okinawa, 904-0495, Japan. \(^3\)Marine Genomics Unit, Okinawa Institute of Science and Technology Graduate University, Tancha 1919-1, Onna, Okinawa, 904-0495, Japan. \(^4\)Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwanoha 5-1-5, Kashiwa, Chiba, 277-8564, Japan. Correspondence and requests for materials should be addressed to Y.N. (email: yuichi.nakajima@oist.jp)
Table 1. Characteristics of novel microsatellite loci developed for *Pocillopora* in this study: locus name, repeat motif, forward and reverse primer sequences, and GenBank accession number for each lineage used for isolation of genomic DNA.

| Locus | Repeat motif (Type 1/Type 5) | Forward primer sequence (5’-3’) | Reverse primer sequence (5’-3’) | Accession No. (Type 1/Type 5) |
|-------|-------------------------------|---------------------------------|---------------------------------|-----------------------------|
| Psp_01 | (AAGT)2AAGC(AAGT)4N22(TAGA)11/(AAGT)2AAGC(AAGT)9N22(TAGA)10 | TCTTCCTAATCCACTGACTGC | U19-CTTGGATGGCGATGTAAAT | LC222418/LC222431 |
| Psp_02 | (GACCC)9/(GACCC)6 | CTCTGCTGGAATCTCCCTTC | U19-AGGCTACGCGCCAAGTAGT | LC222419/LC222432 |
| Psp_10 | (TGAG)11(TGAG)3/(TGAG)2TAGA(CAG)2(TGAG)2(TGGG)4 | AGGCCAGACACAATATGTG | U19-CGGTTGTTGGCCTAGGAG | LC222420/LC222433 |
| Psp_16 | (AAAAAC)8/(AAAAAC)5 | CACACGTTGAGTAGGAATGC | U19-AAGGAAACTCGGAAACCAGC | LC222421/LC222434 |
| Psp_18 | (TGTA)12/(TGTA)9 | ACCATTTTGGACGAAAGGA | U19-ATAGGCGTATTAGCGGCTGCT | LC222422/LC222435 |
| Psp_23 | (TTTTG)8/(TTTTG)5 | TTTCGTACCAAAATCCAGGC | U19-CGGATTATCGAAAGTTTG | LC222423/LC222436 |
| Psp_29 | (TTTTT)5/(TTTTT)2 | AAGCACGCAATTCAGCCTAT | U19-AGCCTAAGAGCGAATCGAG | LC222424/LC222437 |
| Psp_32 | (CTAT)12/(CTAT)7 | CCCGTGCTGAGTAAGATGC | U19-ACTTCTCCGCCCAGATATAA | LC222425/LC222438 |
| Psp_33 | (AATC)10/(AATC)6 | CCATTTCCGAGATCTCTTC | U19-AGGCTGTCGCCCAGATATAA | LC222426/LC222439 |
| Psp_35 | (CTAT)3CTAC(CTAT)3/CTAT3/(CTAT)16 | TGGTTGATGTCGTTGGGAAA | GCGTATATCGAAATGTT | LC222427/LC222440 |
| Psp_39 | (AAGTG)8/(AAGTG)4 | TCTTCACGACACAGGAGCA | U19-AGGCTGTCGCCCAGATATAA | LC222428/LC222441 |
| Psp_41 | (ATTT)10/(ATTT)3CTAC(ATT)3 | U19-CGCCACAGAAGAAAATTTGT | TTCACACACCGAAGATAGGAGC | LC222429/LC222442 |
| Psp_48 | (CTTT)11/(CTTT)4 | TGAAATTACGAGAGAATGGGCA | U19-GTTTTCCGATGGGCTT | LC222430/LC222443 |

Microsatellites have already been developed in *Pocillopora*, we developed additional markers to widen the choice of markers for population genetics analyses between and within lineages. We developed 13 additional microsatellite markers from morphological *Pocillopora meandrina* and *Pocillopora acuta* colonies. In addition, we validated and characterized microsatellites previously reported from *Pocillopora* for populations in the Ryukyu Archipelago. Those were developed by Magalon et al.20, Magalon et al. unpublished, and Starger et al.21 and recently, more loci have been isolated by Pinzón & LaJeunesse23 and Torda et al.3. Gorospe & Karl22 re-designed primers for two loci, PV7 from Magalon et al.20 and Pd3-010 from Starger et al.21 and designated them as Pd2-AB79 and Pd3-EF65, respectively. Using all of these validated microsatellite markers, we genetically characterized populations from multiple lineages of *Pocillopora* from Ueno and Yoshino, Miyako Island, in the Ryukyu Archipelago. 

Results and Discussion

We successfully merged single sequences (2,523,457 reads comprising 1,034,311,164 bp for *P. meandrina* and 7,021,083 reads comprising 2,056,539,596 bp for *P. acuta*) from sequence data derived from genomic DNA. These were used for microsatellite detection and primer design. MISA and Primer3 were employed to identify 1,597 non-overlapping loci from 2,523,457 reads comprising 1,034,311,164 bp for *P. meandrina* and 7,021,083 reads comprising 2,056,539,596 bp for *P. acuta*) from sequence data derived from genomic DNA. These were used for microsatellite detection and primer design. MISA and Primer3 were employed to identify 1,597 non-overlapping loci from 2,523,457 reads comprising 1,034,311,164 bp for *P. meandrina* and 7,021,083 reads comprising 2,056,539,596 bp for *P. acuta* from sequence data derived from genomic DNA. These were used for microsatellite detection and primer design. MISA and Primer3 were employed to identify 1,597 non-overlapping loci from 2,523,457 reads comprising 1,034,311,164 bp for *P. meandrina* and 7,021,083 reads comprising 2,056,539,596 bp for *P. acuta*. 

We confirmed that 13 non-overlapping loci could produce sufficient PCR amplification in all genetic lineages, based on mitochondrial haplotypes derived from fragment analysis (Table S1). The mean ID range from 3.3e-26 to 1.5e-20 (Table 3); therefore, tandem use of these loci allows identification of new loci, PV7 and Pd3-010 from Starger et al.21 and designated them as Pd2-AB79 and Pd3-EF65, respectively. Using all of these validated microsatellite markers, we genetically characterized populations from multiple lineages of *Pocillopora* from Ueno and Yoshino, Miyako Island, in the Ryukyu Archipelago.
Table 2. Characteristics of previously developed microsatellite loci for *Pocillopora*: locus name, forward and reverse primer sequences, and GenBank accession number. *We could not confirm the proper amplification for reverse primer sequences, and GenBank accession number.

| Locus | Forward primer sequence (5′-3′) | Reverse primer sequence (5′-3′) | Accession No. | Reference |
|-------|--------------------------------|--------------------------------|---------------|-----------|
| Pd2-001 | U19-CAGACTTGTCGGAGTAATGGCA | TTTTGTATATATCTGGGTGACAACTATGCA | DQ684672 | Starger et al. |
| Pd3-002* | U19-ATCCGAGATCTAGCAGGAAGC | CAAAGACGCTATGACAAATGCAA | DQ684673 | Starger et al. |
| Pd3-003* | U19-CTTCTCCGTTTGTGTGACC | TCTGCTATATCTGGGTACAGCA | DQ684674 | Starger et al. |
| Pd3-004 | U19-ACCACTTTGACTTACGGAAGC | GCAATGGAATATATATGTCGAGA | DQ684675 | Starger et al. |
| Pd3-005 | AGTGGAGGTGTGACTGACG | TGTCTTTTTTGTGTTACAGCA | EF120462 | Torda et al. |
| Pd3-006 | U19-CTCCTATGACGTAATGGCA | TTTTGTATATATCTGGGTGACAACTATGCA | DQ684676 | Starger et al. |
| Pd3-007 | U19-AGAACTTGTCGGAATGAAAGC | TTTTGTATATATCTGGGTGACAACTATGCA | EF120464 | Torda et al. |
| Pd3-008 | U19-CTTCTCCGTTTGTGTGACC | TCTGCTATATCTGGGTACAGCA | EF120465 | Torda et al. |
| Pd3-009 | U19-CCATCGCTTGCTCAGCTTACG | ATCCACCTAAATTCTCACTGCTTACAGCA | EF120466 | Torda et al. |
| Pd3-010* | U19-CAGACTTGTCGGAGTAATGGCA | TTTTGTATATATCTGGGTGACAACTATGCA | EF120467 | Torda et al. |
| PV2 | U19-CCAGGACCCATTATATCCTCC | TGCAGTGTTCACTTGTGGATACCTG | AY397777 | Magalon et al. |
| PV3* | U19-TGAACAGATGGATGCAGA | AACCAGAATATATATGTCGAGA | AY397778 | Magalon et al. (unpublished) |
| PV5* | U19-GTCATCACCGAAATATATGTCGAGA | GAAATGCTCTTCGATTTATGTCGAGA | AY397779 | Magalon et al. |
| PV6* | U19-CTTCTCCGTTTGTGTGACC | TCTGCTATATCTGGGTACAGCA | EF120468 | Torda et al. |
| PV7 | U19-GAGATGGGATTGGTACTGACG | GTATCTTTCGCTCAGTGGTACTG | AY397780 | Magalon et al. |
| Pd2-AB79 (from PV7) | GGAGATGGGATTGGTACTGACG | GTATCTTTCGCTCAGTGGTACTG | AB214379 | Gorospe & Karl |
| Pd3-EF65 (from Pd3-010) | U19-CTTCTCCGTTTGTGTGACC | TCTGCTATATCTGGGTACAGCA | EF120465 | Torda et al. |
| Pd4 | U19-ACCACTTTGACTTACGGAAGC | GCAATGGAATATATATGTCGAGA | EF120466 | Torda et al. |
| Pd11 | U19-GAGATGGGATTGGTACTGACG | GTATCTTTCGCTCAGTGGTACTG | AY397780 | Magalon et al. |
| Pd13 | U19-CTTCTCCGTTTGTGTGACC | TCTGCTATATCTGGGTACAGCA | EF120467 | Torda et al. |
| Poc40 | U19-TTAATATATGGTGTGATGC | CTCGAATGGAATATATATGTCGAGA | Not found | Pinzón & Lajeunesse |

Table 3. The number of samples (N), the number of multilocus genotypes (G), the number of multilocus lineages (N_MLL), the mean number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, and the deviation index from Hardy-Weinberg equilibrium (FIS) across 27 loci for each lineage or site. The probability of identity (P_ID) for 27 loci was determined with GenAlEx. For lineages or sites with low numbers of MLLs, N_A, H_O, H_E, and FIS were not calculated in this table. Values of N_A, H_O, H_E, and FIS for each locus are shown in Supplementary Table S1. PV7 was removed from this analysis because it was identical to Pd2-AB79.

| Site | Lineage | N | G | N_MLL | N_A | H_O | H_E | FIS | P_ID |
|------|---------|---|---|-------|-----|-----|-----|-----|------|
| Ueno | Type 3 | 1 | 1 | 1 | 5.67 | 0.530 | 0.595 | 0.107 | 1.5e-20 |
| | Type 5 | 29 | 16 | 10 | 4.48 | 0.524 | 0.584 | 0.103 | 3.3e-25 |
| Yoshino | Type 1 (ITS2 type T) | 11 | 11 | 11 | 6.07 | 0.530 | 0.595 | 0.107 | 3.3e-25 |
| | Type 1 (ITS2 type C) | 11 | 11 | 11 | 5.85 | 0.519 | 0.615 | 0.170 | 1.8e-23 |
| | Type 3 | 20 | 20 | 20 | 7.82 | 0.539 | 0.648 | 0.172 | 3.3e-20 |
| | Type 4 | 1 | 1 | 1 | 5.67 | 0.526 | 0.596 | 0.106 | 3.0e-21 |
| | Type 5 | 10 | 8 | 8 | 4.67 | 0.526 | 0.596 | 0.106 | 3.0e-21 |
| | Type 8 | 1 | 1 | 1 | 5.67 | 0.526 | 0.596 | 0.106 | 3.0e-21 |
| Unknown | 6 | 5 | 3 | — | — | — | — | — |

fragment analysis, excluding scoring errors. Also, polymorphism of zooxanthella loci was confirmed in the same multilocus lineage (MLL). These loci will facilitate combining multiple loci for effective multiplex PCR.

Mitochondrial data showed two haplotype groups (Types 3 and 5) at the Ueno site, and haplotype groups of mtORF (Types 1, 3, 4, 5, 8, and unknown) at the Yoshino site. Furthermore, Type 1 was separated into two haplotype groups based upon ITS2. At least six lineages of *Pocillopora* inhabit the northwestern Pacific. This study confirmed that all of these, plus one more, occur in the Ryukyu Archipelago. One unknown haplotype sequence was related to a sequence from *Pocillopora brevicornis* (GenBank accession No. KR919858, Mayfield et al. unpublished; see also Schmidt-Roach et al.11 about *Pocillopora brevicornis*), which differed by 5 bp. A maximum likelihood phylogenetic tree based on mtORF haplotypes was constructed (Fig. 1).

Genetic structure among genetic lineages separated on the basis of mtORF and based on 27 microsatellite loci (Fig. 2), supported the results of Pinzón et al.10. Using the method of Evanno et al.24 to determine the most
probable number of genetic populations ($K$), the largest peak of $\Delta K$ was $K = 3$ (Fig. 2). Otherwise, $\Delta K$ values at $K = 5$ and $K = 8$ also showed small peaks; therefore, we showed genetic population structure for values of $K$ ranging from 3 to 8 (Fig. 2). Genetic structure among Type 1 mtORFs was reflected in different ITS2 haplotypes, as reported by Pinzón et al.10. We defined ITS2 types T and C within mtORF Type 1, based on their haplotype groups (see registered sequences: Genbank accession No. LC222452–LC222453). Values in parentheses indicate the number of colonies collected from two sites at Miyako Island. Values in the tree indicate maximum likelihood bootstrapping (over 75%). Cluster colors correspond to genetic lineages reported by Schmidt-Roach et al.12.

Figure 1. Maximum likelihood phylogenetic tree based on the mtORF comparing the variety of haplotypes with the variety of morphological types of Pocillopora. Immense diversity is evident even within lineages in the Ryukyu Archipelago. Boldface indicates haplotypes that were isolated during this study (Genbank accession No. LC222444–LC222452). Values in parentheses indicate the number of colonies collected from two sites at Miyako Island. Values in the tree indicate maximum likelihood bootstrapping (over 75%). Cluster colors correspond to genetic lineages reported by Schmidt-Roach et al.12.

Methods

Next-generation sequencing and isolation of microsatellites for two Pocillopora lineages. We collected branches from the morphological species, P. meandrina and P. acuta, along the western coast of Okinawa Island in the Ryukyu Archipelago, a subtropical area located in the northwestern Pacific Ocean, Japan. These belonged to mitochondrial open reading frame (mtORF) Type 1 and Type 5 (see Results and Discussion).
Figure 2. Population genetic assessment using STRUCTURE suggests five probable genetic clusters within just the pair of sites we surveyed. These clusters reflect primarily the mtORF (including ITS2) types. The Evanno method was used to decide the probable number of genetic clusters. (A) Each bar plot shows the possible membership of genetic clusters for each MLL. Those ranged from three to eight ($K = 3$ to $K = 8$), with $K = 5$ being optimal. G: MLLs of samples collected from the western coast of Okinawa Island, used for Illumina sequencing, U: MLLs of samples collected at Ueno, Miyako Island. Samples of other MLLs were collected at Yoshino, Miyako Island. (B) The model criterion of choice to detect the most probable number of genetic clusters ($K = 1$ to $K = 10$) across 10 iterations per $K$ (the assumed number of clusters). Graphs of (B) mean log probability (Ln $P(D)$) and (C) $\Delta K$ values based on the rate of change in Ln $P$ (D) between successive $K$ values ($K = 2$ to $K = 9$) for detecting the most probable number of $K$.

Specimens were preserved in ethanol and genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen) following the standard protocol. We sequenced 250-bp paired-end reads using a MiSeq sequencer (Illumina) according to the manufacturer’s instructions. After merging single-end sequences using PEAR ver. 0.9.826, to isolate reads of 100 bp or more having repeat sequences of $P$. meandrina (Type 1), we used MISA (http://pgrc.ipk-gatersleben.de/misa/) to detect simple sequence repeats and to design PCR primers using Primer3 (http://pgrc.ipk-gatersleben.de/misa/primer3.html). We searched $P$. acuta (Type 5) using BLAST+ ver. 2.3.0. We confirmed that sequence regions, including microsatellites, were conserved between the two lineages.

Collection of coral samples, sequencing of mtORF, and phylogenetic analysis of $Pocillopora$. To confirm validation and polymorphism of microsatellite loci, branches from 90 $Pocillopora$ colonies were collected at Ueno (30 colonies; 24°43’09” N, 125°20’27” E; < 1 m depth, by walking along the seashore) and Yoshino (60 colonies; 24°44’55” N, 125°26’45” E; < 18 m depth, by SCUBA diving) at Miyako Island at the Ryukyu Archipelago, Japan. Genomic DNA was extracted from preserved coral branches as described above. Mitochondrial haplotypes were confirmed by sequencing the mtORF region. PCR reaction mixtures comprised: (10 μL) containing < 30 ng/μL template DNA, AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific), and the primers (final concentration: 2 μM for each primer) for mtORF: FATP6.1 (5’-TTTGGGSATAGTTGTCTG-3’) and RORF (5’-SCCAATATGTTAAACASCAGATCAG-3’)29. The PCR protocol consisted of 94 °C for 1 min, followed by 40 cycles at 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 75 s, with a final extension at 72 °C for 5 min. After reaction of Exonuclease I (Takara) and Shrimp Alkaline Phosphatase (Takara) to clean up PCR products, sequencing was the same as for mtORF.

mtORF sequences obtained were aligned with others from previous studies including unpublished work of Mayfield et al., using MUSCLE in MEGA ver. 6.06. The molecular evolution model was selected by MEGA, and the best model was determined to be the HKY (Hasegawa-Kishino-Yano) model with a gamma distribution of rate variation across sites (+G). Rapid bootstrap analysis employing the maximum likelihood method for phylogenetic analysis was carried out using MEGA with the model using 1000 bootstrap replicates. The phylogenetic tree was drawn in FigTree ver. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

The nuclear ribosomal internal transcribed spacer 2 (ITS2) was used for identification of less differentiated genetic lineages within Type 1, 2, 4, 5. PCR reaction mixtures comprised: (10 μL) containing < 30 ng/μL template DNA, AmpliTaq Gold 360 Master Mix, and primers (final concentration: 2 μM for each primer) of ITS2: ITSc2-5 (5’-AGCCAGCTGCATAAGTATG-3’) and R28S1 (5’-GCTGCAATCCAAACAACCC-3’)29. Conditions for PCR and sequencing were the same as for mtORF.

Genotyping of $Pocillopora$ using novel and known microsatellite loci. Together with novel microsatellite loci developed here, we characterized loci for $Pocillopora$ previously developed by Magalon et al.20.
Magalon et al. (unpublished), Starger et al., Pinzón & LaJeunesse, Torda et al., and two loci modified by Gorospe & Karl. For scoring of microsatellite genotypes of each colony, the PCR reaction mixture (5 μL) contained template DNA (<30 ng/μL), AmpliTaq Gold 360 Master Mix, and three primers for each locus: a non-tailed reverse primer (0.5 μM), a forward primer with a sequence tail of U19 (5′-GGTTTTCGCCGTCACGACG-3′) (0.5 μM), and a U19 primer (0.5 μM) fluorescently labeled with FAM, VIC, NED, or PET. The PCR protocol consisted of 95°C for 9 min, followed by 35 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. We furthermore conducted PCR using microsatellites for zooxanthellae, C1.02, SymC_3-02, SymC_3-03, and D1Sym114 within the same MLL to confirm that the microsatellites were derived from nuclear loci, since different symbiont genotypes have been detected from the same MLL in Pocillopora. PCR conditions were the same as for microsatellites from colonies, except for the annealing temperature (50°C for 4 zooxanthellae microsatellites). PCR products were analyzed using an ABI 3730 capillary DNA sequencer (Thermo Fisher Scientific) with the GeneScan 600 LIZ size standard (Thermo Fisher Scientific) to identify genotypes by the length of the amplicon. Fragment size and intensity were confirmed using Geneious ver. 9.0.4 (Biomatters).

Data analyses to characterize microsatellites and populations. The concept of multilocus lineage (MLL) was employed to avoid underestimating clonality due to genotyping errors and somatic mutations. If genotypes of ≤3 loci differed and all other loci coincided, a colony was considered to be clonal, derived by asexual reproduction. Either a scoring error or a somatic mutation was assumed to be responsible for the variable locus/loci. The number of different loci was calculated using GenAlEx ver. 6.501. When different genotypes were determined to belong to the same MLL, the most common genotype was assigned. However, if the most common genotype could not be determined (e.g., if two genotypes occurred in equal numbers), the genotype was set to zero. We also calculated Pch to estimate the possibility of coincidence of multilocus genotypes by sexual reproduction, using GenClone ver. 2.0. After removing clonal replicates from the data set, the number of alleles, values of observed and expected heterozygosity (Hs and He, respectively), and deviation index (Fis) from Hardy-Weinberg equilibrium were evaluated for each locus and lineage using GenAlEx. Lineages with few MLLs were not used for calculation of these genetic indices. The probability of identity (Pd) was also calculated using GenAlEx to estimate the resolution of loci for each lineage/site. Linkage disequilibrium was estimated for each lineage using Genepop on the Web.

Genetic structure based on Bayesian clustering was inferred using STRUCTURE ver. 2.3.4. A burn-in period of 100,000 followed by 1,000,000 Markov chain Monte Carlo (MCMC) replications was used for population clustering without LOPCRIOR (prior sampling location information) model under the admixture model and assuming independent allele frequencies. After calculation of the mean log probability, Ln P(D), to estimate the most probable number of genetic clusters, the number of clusters was determined using the method of Evanno et al., as implemented in STRUCTURE HARVESTER. Run data were merged using CLUMPK.

References
1. Stoddart, J. A. Genetic differentiation amongst populations of the coral Pocillopora damicornis off southwestern Australia. Coral Reefs 3, 149–156 (1984).
2. Stoddart, J. A. Genetic structure within populations of the coral Pocillopora damicornis. Mar. Biol. 81, 19–30 (1984).
3. Adjeroud, M. & Tsuchiya, M. Genetic variation and clonal structure in the scleractinian coral Pocillopora damicornis in the Ryukyu Archipelago, southern Japan. Mar. Biol. 134, 753–760 (1999).
4. Magalon, H., Adjeroud, M. & Veilleux, P. Patterns of genetic variation do not correlate with geographical distance in the reef-building coral Pocillopora meandrina in the South Pacific. Mol. Ecol. 14, 1861–1868 (2005).
5. Ridgway, T., Riginos, C., Davis, J. & Hoegh-Guldberg, O. Genetic connectivity patterns of Pocillopora verrucosa in southern African Marine Protected Areas. Mar. Ecol. Prog. Ser. 354, 161–168 (2008).
6. Combosch, D. J. & Vollmer, S. V. Population genetics of an ecosystem-defining reef coral Pocillopora damicornis in the Tropical Eastern Pacific. PLoS One 6, e21200 (2011).
7. Torda, G., Lundgren, P., Willis, B. L. & van Oppen, M. J. H. Genetic assignment of recruits reveals short- and long-distance larval dispersal in Pocillopora damicornis on the Great Barrier Reef. Mol. Ecol. 22, 5821–5834 (2013).
8. Torda, G., Schmidt-Roach, S., Peplow, L. M., Lundgren, P. & van Oppen, M. J. H. A rapid genetic assay for the identification of the most common Pocillopora damicornis genetic lineages on the Great Barrier Reef. PLoS One 8, e58447 (2013).
9. Noreen, A. M. E., Schmidt-Roach, S., Harrison, P. L. & van Oppen, M. J. H. Diverse associations among coral host haplotypes and algal endosymbionts may drive adaptation at genetically peripheral and ecologically marginal locations. J. Biogeogr. 42, 1639–1650 (2015).
10. Pinzón, J. H. et al. Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (Pocillopora, Scleractinia). J. Biogeogr. 40, 1595–1608 (2013).
11. Veron, J. Corals of the World. Australian Institute of Marine Science, Townsville, Queensland, Australia (2000).
12. Schmidt-Roach, S., Miller, K. J., Lundgren, P. & Andreakis, N. With eyes wide open: a revision of species within and closely related to the Pocillopora damicornis species complex (Scleractinia; Pocilloporidae) using morphology and genetics. Zool. J. Linn. Soc. 170, 1–33 (2014).
13. Pinzón, J. H. & LaJeunesse, T. C. Species delimitation of common reef corals in the genus Pocillopora using nucleotide sequence phylogenies, population genetics and symbiosis ecology. Mol. Ecol. 20, 311–325 (2011).
14. Schmidt-Roach, S. et al. Assessing hidden species diversity in the coral Pocillopora damicornis from Eastern Australia. Coral Reefs 32, 161–172 (2013).
15. Gélin, P., Postaire, B., Fauvetot, C. & Magalon, H. Reevaluating species number, distribution and endemism of the coral genus Pocillopora Lamarck, 1816 using species delimitation methods and microsatellites. Mol. Phylogenet. Evol. 109, 430–446 (2017).
16. The Japanese Coral Reef Society & Ministry of the Environment. Coral Reefs of Japan. Ministry of the Environment, Tokyo, Japan (2004).
17. van Woestijne, R., Sakai, K., Ganase, A. & Loya, Y. Revisiting the winners and the losers a decade after coral bleaching. Mar. Ecol. Prog. Ser. 434, 67–76 (2011).
18. Shinzato, C. et al. Development of novel, cross-species microsatellite markers for Acropora corals using next-generation sequencing technology. Front. Mar. Sci. 1, 11 (2014).
19. Nakajima, Y., Shiznato, C., Satoh, N. & Mitarai, S. Novel polymorphic microsatellite markers reveal genetic differentiation between two sympatric types of Galaxea fascicularis. PLoS One 10, e0130176 (2015).

20. Magalon, H., Samadi, S., Richard, M., Aderjoud, M. & Veuille, M. Development of coral and zooxanthella-specific microsatellites in three species of Pocillopora (Cnidaria, Scleractinia) from French Polynesia. Mol. Ecol. Notes 4, 206–208 (2004).

21. Starger, C. J., Yeoh, S. S. R., Dai, C.-F., Baker, A. C. & DeSalle, R. Ten polymorphic STR loci in the cosmopolitan reef coral. Pocillopora damicornis. Mol. Ecol. Resour. 8, 619–621 (2008).

22. Gorospe, K. D. & Karl, S. A. Genetic relatedness does not retain spatial pattern across multiple spatial scales: dispersal and colonization in the coral, Pocillopora damicornis. Mol. Ecol. 22, 3721–3736 (2013).

23. Kitano, Y., Nagai, S., Ueno, M. & Yasuda, N. Most Pocillopora damicornis around Yaeyama Islands are Pocillopora acuta according to mitochondrial ORF sequences. Galaxea 17, 21–22 (2015).

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

25. Nakajima, Y. et al. Elucidating the multiple genetic lineages and population genetic structure of the brooding coral Seriatopora (Scleractinia: Pocilloporidae) in the Ryuku Archipelago. Coral Reefs 36, 415–426 (2017).

26. Zhang, J., Kobert, L., Flouri, T. & Stamatakis, A. PEAK: a fast and accurate Illumina Paired-End read merge. Bioinformatics 30, 614–620 (2014).

27. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. & Madden, T. L. BLAST+: architecture and applications. BMC Bioinformatics 10, 421 (2009).

28. Plot, J.-F. & Tillier, S. 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 (2007).

29. Plot, J.-F., Magalon, H., Craudau, C., Couloux, A. & Tillier, S. Patterns of genetic structure among Hawaiian corals of the genus Pocillopora yield clusters of individuals that are compatible with morphology. C. R. Biol. 331, 239–247 (2008).

30. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729 (2013).

31. Schuelke, M. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18, 233–234 (2000).

32. Bay, L. K., Howells, E. J. & van Oppen, M. J. H. Isolation, characterisation and cross amplification of thirteen microsatellite loci for coral endo-symbiotic dinoflagellates (Symbiodinium clade C). Conserv. Genet. Resour. 1, 199–203 (2009).

33. Howells, E., van Oppen, M. J. H. & Willis, B. High genetic differentiation and cross-shelf patterns of genetic diversity among great barrier reef populations of Symbiodinium. Coral Reefs 28, 215–225 (2009).

34. Pettay, D. T. & Lajeunesse, T. C. Microsatellite loci for assessing genetic diversity, dispersal and clonality of coral symbionts in ‘stress-tolerant’ clade D Symbiodinium. Mol. Ecol. Resour. 9, 1022–1025 (2009).

35. Pettay, D. T., Wham, D. C., Pinzón, J. H. & Lajeunesse, T. C. Genotypic diversity and spatial–temporal distribution of Symbiodinium clones in an abundant reef coral. Mol. Ecol. 20, 5197–5212 (2011).

36. Arnaud-Haond, S., Duarte, C. M., Alberto, F. & Serrão, E. A. Standardizing methods to address clonality in population studies. Mol. Ecol. 16, 5115–5139 (2007).

37. Peakall, R. & Smouse, P. E. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295 (2006).

38. Arnaud-Haond, S. et al. Vicariance patterns in the Mediterranean Sea: east-west cleavage and low dispersal in the endemc seagrass Posidonia oceanica. J. Biogeogr. 34, 963–976 (2007).

39. Nakajima, Y., Zayasu, Y., Shiznato, C., Satoh, N. & Mitarai, S. Genetic differentiation and connectivity of morphological types of the broadcast-spawning coral Galaxea fascicularis in the Nansei Islands, Japan. J. Evol. Mol. Ecol. 6, 1457–1469 (2016).

40. Arnaud-Haond, S. & Belkhir, K. GENCLONE: a computer program to analyze genotypic data, test for clonality and describe spatial clonal organization. Mol. Ecol. Notes 7, 15–17 (2007).

41. Raymond, M. & Rousset, F. Genepop (version 1.2): Population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249 (1995).

42. Rousset, F. Genepop’007: A complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106 (2008).

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

44. Falush, D., Stephens, M. & Pritchard, J. K. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587 (2003).

45. Erla, D. A. & vonHofdlt, B. M. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361 (2012).

46. Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A. & Mayrose, I. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15, 1179–1191 (2015).

Acknowledgements
This study was financially supported by a Japan Society for the Promotion of Science (JSPS) KAKENHI grant (25840152 and 16H05621 to Y.N. and 26290065 to C.S.) and by the Okinawa Institute of Science and Technology Graduate University. We thank Dr. Steven D. Aird (Okinawa Institute of Science and Technology Graduate University) for editing the manuscript. Collection of all corals for this study was approved by Okinawa Prefecture, Japan (permission number: 26–62).

Author Contributions
Y.N. and S.M. conceived and designed the experiments. Y.N., P.H.W., S.S., and Y.Z. collected samples. Y.N. performed the experiments and analyzed the data. C.S. and N.S. contributed regents and analyical tools. Y.N. wrote the manuscript, and P.H.W. and S.M. edited it.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-06776-x

Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or
