Elucidating the multiple genetic lineages and population genetic structure of the brooding coral *Seriatopora* (Scleractinia: Pocilloporidae) in the Ryukyu Archipelago

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Abstract

The elucidation of species diversity and connectivity is essential for conserving coral reef communities and for understanding the characteristics of coral populations. To assess the species diversity, intraspecific genetic diversity, and genetic differentiation among populations of the brooding coral *Seriatopora* spp., we conducted phylogenetic and population genetic analyses using a mitochondrial DNA control region and microsatellites at ten sites in the Ryukyu Archipelago, Japan. At least three genetic lineages of *Seriatopora* (Seriatopora-A, -B, and -C) were detected in our specimens. We collected colonies morphologically similar to *Seriatopora hystrix*, but these may have included multiple, genetically distinct species. Although sexual reproduction maintains the populations of all the genetic lineages, Seriatopora-A and -C had lower genetic diversity than Seriatopora-B. We detected significant genetic differentiation in Seriatopora-B among the three populations as follows: pairwise F_{ST} = 0.064–0.116 (all P = 0.001), pairwise G_{ST} = 0.107–0.209 (all P = 0.001). Additionally, only one migrant from an unsampled population was genetically identified within Seriatopora-B. Because the peak of the settlement of *Seriatopora* larvae is within 1 d and almost all larvae are settled within 5 d of spawning, our observations may be related to a low dispersal ability. Populations of *Seriatopora* in the Ryukyu Archipelago will

probably not recover unless there is substantial new recruitment from distant populations.

Introduction

Reef-building corals are important marine organisms in tropical and subtropical reef areas because they constitute the framework of the ecosystem and enhance the productivity and diversity of the coral-reef community. In addition, coral species diversity helps to maintain the functionality and resilience of coral-reef communities to environmental stresses (e.g., Hughes et al. 2003; Richards and Hobbs 2014). However, stony corals are threatened by climate change and anthropogenic disturbances at both local and global scales (e.g., Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007). Accurate identification of coral species is essential to monitor, conserve, and manage coral populations (e.g., Bickford et al. 2007). However, in some cases, species definitions that are based solely on colony morphological traits have been misleading, and morphological homoplasy conceals the cryptic evolutionary relationships (Richards et al. 2013, 2016 and references therein). Because traditional morphological species definitions have been problematic in corals, genetic tools have more recently proven to be useful in discriminating between morphologically similar species (e.g., Nakajima et al. 2012, 2016; Pinzón et al. 2013; Warner et al. 2015; Suzuki et al. 2016).

Population genetic approaches provide potential for distinguishing inter- and intraspecific relationships more clearly even when the relationships are impacted by recent speciation and/or hybridization. This is because changes in allele frequencies within a population are expected to occur at an ecological timescale, which can be properly assessed by genetic markers (e.g., Nakajima et al. 2012).

Mitochondrial loci and polymorphic nuclear microsatellite loci provide fundamental information about species boundaries and intraspecific genetic diversity and differentiation. Mitochondrial DNA generally shows a higher mutation rate than nuclear DNA and is suitable as a genetic marker for phylogeny and species identification in animals (e.g., Avise et al. 1987; Hebert et al. 2003). However, low mutation rates in coral species make this analysis inappropriate (van Oppen et al. 1999, 2001; Fukami et al. 2000). Recent studies have elucidated species boundaries in the family Pocilloporidae. Species within the genus *Pocillopora* were delineated using mitochondrial loci, an open reading frame with an unknown function and a control region, and a nuclear internal transcribed spacer 2 (ITS2) region. These loci suggested a mismatch between genetic clusters and species definitions based on colony morphological traits (e.g., Pinzón and LaJeunesse 2011; Pinzón et al. 2013; Schmidt-Roach et al. 2013, 2014). Furthermore, species within the genus *Stylophora* were

divided into three clusters using mitochondrial loci or ITS2, and into four clusters using ITS1, and these were morphologically more variable than previously thought (Flot et al. 2011). *Seriatopora* collected from Okinawa, New Caledonia, and the Philippines consisted of four genetic clusters on a phylogenetic tree constructed based on the sequences of mitochondrial loci, D-loop, a control region between *atp*8 and *cox*1, along with the control region between *atp*6 and *nad*4 (Flot et al. 2008). Furthermore, *Seriatopora hystrix* was classified into four genetic lineages, including cryptic species, on the Great Barrier Reef (GBR) (Warner et al. 2015). Therefore, it can be difficult to estimate the species diversity within the family Pocilloporidae based on traditional morphological descriptions. Clarification of the genetic structure of sympatric populations using nuclear multilocus microsatellites is a robust method for estimating species diversity (*Pocillopora*: Pinzón et al. 2013; *Seriatopora*: Warner et al. 2015).

If genetic tools detect genetic homogeneity that is maintained within a species through genetic exchanges, populations could be genetically differentiated due to geographical and oceanographic barriers and population fluctuations, including genetic drift and bottlenecks that are caused by historical events (e.g., Bay et al. 2008). Microsatellites can be used to describe the genetic connectivity among populations and species. Genetic connectivity

among populations within species is maintained by larval transport via oceanographic systems such as sea currents (e.g., Pineda et al. 2007). The Kuroshio Current is a strong oceanographic current that flows southwest to northeast along the Ryukyu Archipelago of Japan. This current is considered a major factor in the expansion and maintenance of coral reefs and reef-dwelling organisms around the islands (Nishihira and Veron 1995). The Ryukyu Archipelago is a subtropical area in the northwestern Pacific and includes the northern limit for most tropical coral species (Nishihira and Veron 1995). It is composed of multiple stepping-stone, isolated islands and a well-developed reef system. In the reef system, distances between islands range from tens to several hundreds of kilometers in most cases, and the area of suitable coral reef habitat is small compared to regions such as the GBR. Thus, we predict that larval recruitment among the isolated reefs is rare, especially in brooding corals, but occurs occasionally over long periods owing to the Kuroshio Current and its branches.

Seriatopora is mainly distributed on tropical and sub-tropical reefs in the western Indo-Pacific region (Veron 2000). Nishihira and Veron (1995) described two species of *Seriatopora* (*S. hystrix* and *S. caliendrum*) that inhabit the Ryukyu Archipelago. Veron (2000) and the Corals of the World website (http://coral.aims.gov.au/) suggest that four

morphological species of *Seriatopora* are probably distributed in the Ryukyu Archipelago: *S. hystrix*, *S. caliendrum*, *S. guttata*, and *S. stellata*. The Japanese Coral Reef Society and Ministry of the Environment (2004) reported that three species of *Seriatopora* (*S. hystrix*, *S. caliendrum*, and *S. stellata*) inhabit the area, but *S. stellata* is only in the Miyako and Yaeyama regions of the Ryukyu Archipelago.

The extent of coral recovery after a mass bleaching event is highly variable among species. The population recovery of *S. hystrix* is very slow in the Ryukyu Archipelago and it is considered a long-term loser species that does not recover after a mass bleaching event (e.g., Loya et al. 2001; van Woesik et al. 2011). We assess multiple genetic lineages of *Seriatopora* and the genetic structure and connectivity among sites using microsatellite markers to understand the historical interspecific delimitation, intraspecific population dynamics including reproductive strategies, and recent migration patterns. In addition, we discuss the potential for recovery of *Seriatopora* corals along the Ryukyu Archipelago.

Materials and methods

Sampling and genomic DNA extraction

We collected *Seriatopora* samples from ten sampling sites in four regions of the

Ryukyu Archipelago (Fig. 1, ESM Table S1). While at least three morphological species of *Seriatopora* appear to be distributed in the Ryukyu Archipelago, we collected colonies with a *S. hystrix* morphology and avoided colonies with a different morphology, which were rare in the sampling areas. A small piece of branch was preserved in 99.5% ethanol and transferred to the laboratory. Total DNA was extracted from the ethanol-preserved tissue pellets using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) or the AquaPure Genomic DNA Isolation Kit (BioLad, Hercules, CA, USA) according to the manufacturer's protocols.

Sequencing of the mitochondrial putative control region

As the most comparable sequence with other publications, the mitochondrial putative control region (mtCR) between *atp6* and *nad4* was amplified by polymerase chain reaction (PCR) using Ex Taq DNA polymerase (Takara, Kusatsu, Shiga, Japan) with the primers SerCtl-F1 (5 -GTCTGCTCACATTAATTTAT-3) and SerCtl-R1 (5 -AGAGATCGAACTAAGAGTCG-3) (Bongaerts et al. 2010). These primers were designed from the mitochondrial genome of *S. hystrix* (Bongaerts et al. 2010; and see Chen et al. 2008; GenBank EF633600). The mtCR was defined as a control region in Chen et al. (2008), but almost all the sequences can be translated into an open reading frame (ORF) of a hypothetical protein-encoding gene (Flot et al. 2008). The location corresponds to a novel

mitochondrial ORF that is next to *atp6* in the case of *Pocillopora,* as shown by Flot and Tillier (2007). The PCR conditions were as follows: 40 cycles of 30 s at 94°C, 1 min at 56°C, 1 min at 72°C, followed by a final extension for 5 min at 72°C, carried out in a total reaction volume of 10 L. For direct sequencing, each PCR product was treated with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol. The primers used for the sequencing reactions were the same as those for the PCR amplification. The products were sequenced using an automated capillary-based DNA sequencer, the ABI 3130*xl* Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Phylogenetic analyses for mtCR haplotypes

Tandem repeat sections in the intergenic spacer were identified using Tandem Repeat Finder ver. 4.09 (Benson 1999); the alignment parameters for mismatches and indels were three and five, respectively. If two or more patterns were detected in a haplotype, the pattern with the larger score was adopted. We used maximum likelihood (ML) and Bayesian inference (BI) analyses to construct a phylogenetic tree of the generated mtCR haplotypes, with the 23 *Seriatopora* sequences classified according to Chen et al. (2008), Flot et al. (2008), Bongaerts et al. (2010), and Warner et al. (2015), and one *Pocillopora* sequence identified from Flot and Tillier (2007) as the outgroup. These sequences were aligned using

MUSCLE on MEGA, ver. 6.0 (Tamura et al. 2013). The consensus sequences, including repeat motifs, ranged from 513 bp to 741 bp of the mtCR. Prior to analysis, we searched for the best-fit model of evolution using MEGA, and the HKY (Hasegawa–Kishino–Yano) +G (Gamma distributed) model was selected. The robustness of each ML tree was tested by bootstrapping (1,000 replicates) under the condition of complete deletion of gaps. Furthermore, this model was applied for the BI analysis using MrBayes, ver. 3.2.5 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Two isolated runs were conducted under the following conditions: four Markov chain Monte Carlo (MCMC) iterations, 50,000,000 generations, a print frequency of 10,000, a sample frequency of 100 generations, and a 0.25 burn-in fraction.

Scoring of microsatellite genotypes

We attempted to use nine microsatellite loci with amplification in our specimens of *Seriatopora*. Seven loci characterized by Underwood et al. (2006) were effective for our genetic analysis (ESM Table S2). Of the two remaining loci, Sh3-008 (Underwood et al. 2006) showed no amplification for specimens from some sites, and Sh4.28 (Maier et al. 2001) contained very few heterozygotes. The reaction mixture (5 μL) contained template DNA (<100 ng), AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific), and three

primers for each locus. The primers were a non-tailed forward primer (0.5 μM), a reverse primer with a U19 sequence tail (0.5 μM), and a U19 (5 -GGTTTTCCCAGTCACGACG-3) primer (0.5 μM) fluorescently labeled with FAM, VIC, or NED. Amplifications for all microsatellite loci were carried out under the following conditions: 95°C for 9 min, followed by 35 cycles at 95°C for 30 s, 54°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products amplified with an internal size standard, GeneScan 600 LIZ, (Thermo Fisher Scientific) were analyzed using an ABI 3130*xl* Genetic Analyzer and GeneMapper ver. 3.7 (Thermo Fisher Scientific).

Estimating the genetic groupings using microsatellites

We inferred the genetic structure of *Seriatopora* through Bayesian clustering of microsatellites using STRUCTURE, ver. 2.3.4 (Pritchard et al. 2000). First, we analyzed the genetic structure with the sampling location information. A burn-in period of 100,000 iterations followed by 1,000,000 MCMC replications was used for population clustering without LOCPRIOR model under the admixture model and correlated allele frequencies were assumed (Falush et al. 2003). Individuals were assumed to be drawn exclusively from the genetic *K* clusters and were allowed to have mixed ancestry in the admixture model (Pritchard et al. 2000; Falush et al. 2003). Simulations included 10 iterations, and the number

of assumed subpopulations was 1 to 11. After calculation of the mean log probability, Ln P(D), determination of the number of *K* clusters that best fit the data was conducted based on the highest Δ*K* value using the method of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012). To obtain adequate output panels to unify the colors, merged run data were implemented by CLUMPAK (Kopelman et al. 2015). Second, we analyzed the genetic structure and inferred the number of genetic lineages with the mtCR haplotype information using STRUCTURE. The run conditions of STRUCTURE were the same as the first step analysis except for the sampling location information. Furthermore, discriminant analysis of principal components (DAPC) was conducted in R ver. 3.0.2 (http://www.r-project.org) using the package adegenet, ver. 1.3–9.2 (Jombart et al. 2010), to represent genetic clusters formed by microsatellite genotypes for each mtCR haplotype. This clustering method does not assume Hardy–Weinberg equilibrium (HWE) or linkage equilibrium. All multilocus genotypic data of microsatellites were plotted both with and without six clusters defined a priori based on mtCR haplotypes. A total of 40 principal components were retained, accounting for 97.9 % of the genetic variability.

For multilocus genotypes (including replicated genotypes), we also calculated the probability of a given multilocus genotype occurring *n* times for each population within a

genetic lineage, repeated as a consequence of different recombination events (P_{SEX}, calculated taking into account the F_{IS} estimates in the data set) using GenClone, ver. 2.0 (Arnaud-Haond and Belkhir 2007). We retained the replicated multilocus genotype if two or more multilocus genotypes were the same but occurred by chance because of sexual reproduction (the threshold of the P_{SEX} value is 0.01). Based on the microsatellite genotypes for each genetic lineage and site, clonal diversity was estimated with the following index: $R = (N_{MLG} - 1)/(N - 1)$, where N_{MLG} is the number of retained multilocus genotypes and N is the number of colonies analyzed. We removed duplicated genotypes of clonemates within a population to analyze the genetic differentiation and structure, genetic diversity, and migrants within genetic lineage. Genetic differentiation based on microsatellites was estimated using a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992), partitioning among lineages, among populations within lineages, and within populations, by GenAlEx, ver. 6.501 (Peakall and Smouse 2006).

Population genetic analyses between sites within a genetic lineage

After removal of the duplicate genotypes of clonemates, the genetic structure within each genetic lineage was also inferred using STRUCTURE. The run conditions were the same as previously, except for the number of assumed subpopulations (K = 1 to 6 for each

genetic lineage). Additionally, we ran a LOCPRIOR model because the detailed genetic structure as shown in all lineages was not detected without the LOCPRIOR model (see Results). Only populations with 20 or more retained multilocus microsatellite genotypes within each lineage were used in further analyses. For each suitable population, the number of alleles and values of observed and expected heterozygosity (H_O and H_E, respectively) for each site were evaluated with GenAlEx. We calculated the fixation index (F_{IS}) and the allelic richness for genetic diversity at each site using FSTAT ver. 2.9.3.2 (Goudet 1995). Additionally, the significance of the F_{IS} was tested by randomization using FSTAT. The genetic differentiation index between sites was calculated using GenAlEx. The significance of each pairwise F_{ST} value was tested with 999 permutations. Furthermore, the G-statistic method was used to calculate the pairwise G_{ST} values to account for the small number of populations (Meirmans and Hedrick 2011), and the significance of each value was tested with 999 permutations. Migration patterns over the recent ecological timescale were estimated using GeneClass2 (Piry et al. 2004) and the Bayesian method developed by Rannala and Mountain (1997). We assigned each multilocus genotype to a source site using a 10,000 resampling algorithm. A multilocus genotype with a >99% ($P < 0.01$) confidence interval was excluded from the sampled population, and we then assigned the colony as an immigrant to

the site of highest probability when the confidence of assignment was ≥10% ($P \geq 0.1$). If a given multilocus genotype had low probability with <10% ($P < 0.1$) confidence of assignment for all sites, the origin of this multilocus genotype was considered an immigrant from an unsampled site.

Results

Genetic differentiation and structure among the genetic lineages

Of the 195 colonies of *Seriatopora* collected from ten sites (Fig. 1; ESM Table S1), we succeeded in obtaining mtCR haplotypes (6 haplotypes; Ser-1 to -6) and multilocus genotypes with seven microsatellites in 182 colonies (93.3%). The community composition of individuals from different haplotypes differed among sites (Fig. 1; ESM Table S1). While some sites were dominated by a single haplotype (e.g., sites O-NKJ and K-AKA; see Fig. 1 for site names), others had population mixtures with up to five different haplotypes (e.g., Y-TKT). The haplotype sequences are available in the GenBank database (accession numbers: LC107881–LC107886). The composition of each haplotype group differed greatly among sites (Fig. 1; ESM Table S1). Thirty-five replicated multilocus microsatellite genotypes were detected, and 32 replicated multilocus microsatellite genotypes were detected

considering the mtCR haplotypes. Namely, three multilocus microsatellite genotypes were shared between the Ser-1 and Ser-2 mtCR haplotypes in O-OUR (one multilocus microsatellite genotype was shared between the two Ser-1 and two Ser-2 colonies, one other multilocus genotype was in the five Ser-1 and 1 Ser-2 colonies, and the last multilocus genotype was also in the five Ser-1 and one Ser-2 colonies). Multilocus microsatellite genotypes were not shared between the sites.

For the Bayesian clustering, the Evanno method indicated that the most probable number of genetic populations was three: best $K = 3$ ($\Delta K = 2712.52$ when each site was designated as the population, and $\Delta K = 4632.23$ when each mtCR haplotype was designated as the population). The STRUCTURE analyses suggested that there was no obvious agreement with geographical sites (Fig. 2). For example, multiple clusters were mixed in O-ONA and Y-TKT, and a common cluster was shared between populations from different regions (e.g., O-ONN and K-AKA, K-TKS and Y-IRO). STRUCTURE found at least three distinct genetic lineages among the sampled individuals, and the lineages include different mtCR haplotypes (Seriatopora-A: Ser-1 and Ser-2; Seriatopora-B: Ser-3 and Ser-4; Seriatopora-C: Ser-5 and Ser-6), although the lineages of five colonies in Ser-5 and Ser-6 were not defined (Fig. 2; ESM Table S1). DAPC detected three main clusters when each

haplotype was assigned to each multilocus genotype (ESM Fig. S1). Of the five colonies with lineage undefined by STRUCTURE, four were assigned to Seriatopora-B without prior information of mtCR haplotype and to Seriatopora-C with prior information. A hierarchical AMOVA quantified the extent of the genetic differentiation among the genetic lineages based on STRUCTURE after removal of the five colonies with undefined lineage (total $F_{RT} = 0.349$, $P = 0.001$; Table 1). Thus, we concluded there were at least three genetic lineages of *Seriatopora* in the Ryukyu Archipelago.

On the ML (Fig. 3) and BI (ESM Fig. S2) phylogenetic trees, four haplotypes (Ser-1, Ser-2, Ser-3, and Ser-4) were assigned to the clusters including some haplotypes of *S. caliendrum*, and two (Ser-5 and Ser-6) were assigned to the group including several *S. hystrix* lineages. Within the mtCR analyzed, Ser-2 was identical to the haplotype previously obtained from Okinawa and Taiwan, and had one nucleotide gap compared with the haplotype from the Philippines. Ser-5 is identical to *S. hystrix* HostU from the GBR, which is the same haplotype from Okinawa and Taiwan. In contrast, the other four haplotypes have not been reported before. However, the relationship of the phylogenetic trees defined according to mtCR haplotype was not in agreement with the relationship identified through the genetic structure from microsatellites. All Ser-5 and one Ser-6 were the same clusters by

microsatellites, but were separated on the phylogenetic tree using mtCR, although there were only two samples in Ser-6. In addition, tandem repeats (composed of 51 bp) were observed in the mtCR, and these were separated into three groups based on the type of tandem repeat [Ser-1: 8.5 times; Ser-2, Ser-3, and Ser-4: 4.4 times; Ser-5 and Ser-6: 5.2 times (ESM Fig. S3)], but this pattern was also not completely related to the genetic structure obtained using microsatellites.

Clonal diversity and the population genetic index within the genetic lineages

Although 35 multilocus microsatellite genotypes (32 when considering mtCR haplotypes) were replicated the P_{SEX} value revealed that the number of clonal replicates was low, with only six of the 182 colonies appearing to be derived from asexual reproduction (Table 2). The clonal diversity for each genetic lineage and site was relatively high, especially considering the P_{SEX} value (R = 0.75–1.00) (Table 2).

We retained 176 multilocus genotypes after removing the six duplicate genotypes of clonemates. We calculated the allelic richness (A_R), standardized against 21 multilocus genotypes (i.e., N_{MLG} = 21; lowest number of multilocus genotypes). We also calculated the expected heterozygosity (H_E) at each site and for each genetic lineage to estimate the genetic diversity. The results for Seriatopora-A colonies at O-OUR and Seriatopora-C colonies at

Y-IRO indicated that these populations were maintained under low genetic diversity (Table 3). The genetic differentiation indexes, the pairwise F_{ST} and G_{ST}, were 0.064–0.116 (all P = 0.001) and 0.107–0.209 (all P = 0.001), respectively, among the three sites (with 20 or more retained mutilocus microsatellite genotypes) within Seriatopora-B (ESM Fig. S4). The genetic structure was established for each genetic lineage without the LOCPRIOR model (Seriatopora-A: best K = 2, ΔK = 188.38; Seriatopora-B: best K = 2, ΔK = 35.80; Seriatopora-C: best K = 2, ΔK = 790.50) and with the LOCPRIOR model (Seriatopora-A: best K = 2, ΔK = 284.83; Seriatopora-B: best K = 2, ΔK = 39.54; Seriatopora-C: best K = 2, ΔK = 67.90) (ESM Fig. S4). The migration estimation using GeneClass2 indicated that migration from other sites was rare within the analyzed genetic lineages. Only one migrant was found in Seriatopora-B (to M-IKM from an unsampled site); therefore, the migration direction was not estimated.

Discussion

Population genetic analyses using microsatellite markers revealed that there are at least three distinct genetic lineages and population genetic structure of *Seriatopora* corals in the Ryukyu Archipelago. However, the phylogenetic trees that were based on mtCR

haplotypes did not always correspond to the genetic structure that was derived using microsatellites. The community composition of genetic lineages was variable among the sites, and only Y-TKT included all three genetic lineages in our data set. Considering the P_{SEX} value, the clonal diversity was high in all three genetic lineages, and clonal replicates were found in only six of our 182 genotyped colonies. Therefore, our data suggest that sexual reproduction is the main contributor to population maintenance of *Seriatopora* in the Ryukyu Archipelago. Nevertheless, there was large genetic differentiation among the populations within Seriatopora-B. This suggests that larval recruitment from other populations rarely occurred over the long-term history in the lineage.

Genetic lineages of *Seriatopora* in the Ryukyu Archipelago

We identified and collected all specimens of *Seriatopora* spp. in the Ryukyu Archipelago, but previous studies by Flot et al. (2008) noted that morphological traits do not align with genetic clusters of *Seriatopora*. Warner et al. (2015) suggested the existence of cryptic species within *S. hystrix*; four genetic types of *S. hystrix* were detected on the GBR. Two microsatellites showed no amplification for specimens from some sites, which may also reflect the difference of species. *Seriatopora* has a very limited population size and is endangered in the Ryukyu Archipelago (in our field observation, see also van Woesik et al.

2011). Therefore, it may take considerable time or not be possible to identify more genetic lineages due to the difficulty of collecting samples from only a limited number of populations or colonies even if more minor lineages are distributed in this region.

On the phylogenetic tree, the Ser-1 haplotype of the mtCR, found only at the O-OUR site, appeared to be the most recent common ancestor type for Ser-2, Ser-3, and Ser-4. Tandem repeats in this locus were about twice as long in Ser-1 (8.5 times) as in haplotypes Ser-2, Ser-3, and Ser-4 (4.4 times). However, these mitochondrial groupings revealed by the phylogenetic tree and tandem repeats were not necessarily supported by levels of genetic differentiation and Bayesian clustering using microsatellite genotypes, which suggests that Seriatopora-A is one genetic lineage, comprised of haplotypes Ser-1 and Ser-2. Although Chen et al. (2008) and Flot et al. (2008) investigated and registered the D-loop, a control region between *atp*8 and *cox*1, sequences on GenBank, the number of sequences is fewer than that of the mtCR and therefore it is not possible to use the D-loop to compare haplotypes from multiple regions on the phylogenetic tree. By increasing the number of registered sequences and using a greater number of genetic loci and amino acid sequences, including other nuclear loci, it may be possible to resolve the evolutionary processes and relationships among *Seriatopora* species.

Habitat selection related to depth and geographical location

Although we could not perform statistical analysis along depth gradients due to limited sample sizes, we found that Seriatopora-A appeared to occur mostly at relatively deep sites (>13 m) compared to the distributions of the other two lineages. In particular, one of the two associated haplotypes (Ser-1) was only found at ≥30 m (O- OUR). Our data set indicated that all three genetic lineages of *Seriatopora* inhabit both shallow (2–10 m) and deep (30–32 m) habitats. Flot et al. (2008) also confirmed that all genetically different clusters of *Seriatopora* were distributed in various water depths (Cluster 1: 6.7–40.3 m; Cluster 2: 0.6–34.0 m; Cluster 3: 0.5–30.2 m; Cluster 4: 1.0–34.0 m). Recent studies, however, have suggested that there is strong genetic structuring with depth in *Seriatopora* populations (Bongaerts et al. 2010; van Oppen et al. 2011). Larval habitat selection for settlement among depths is an important factor in determining coral distribution patterns (Baird et al. 2003). Further sampling efforts including expanding or changing the focal area may clarify any potential depth structure among lineages.

Our data did not indicate habitat differentiation between sheltered vs. exposed sites, or among latitudes. However, the community composition of genetic lineages was clearly variable among the sites sampled, although genetic lineages of *Seriatopora* were separated

between sheltered and exposed sites on the GBR, and habitat selection been found to be related to the symbionts hosted (Warner et al. 2015). The geographical structure of the Ryukyu Archipelago consists of a series of isolated reefs that may restrict the genetic connectivity among populations and the homogeneity of community composition of genetic lineages in each population. In addition, there are populations with only one cryptic species in the large habitat of the GBR (Warner et al. 2015). The relationship between species distribution and geographical distance is also somewhat complicated in the Ryukyu Archipelago. For example, while K-TKS and K-AKA are geographically isolated from each other by approximately 6 km, the community composition of the genetic lineage is completely different. Conversely, one haplotype (Ser-2) in Seriatopora-A was shared between O-OUR and Y-TKT, regions that are isolated by approximately 470 km. Historical reef events and other environmental factors likely determine the distributions of the different lineages. For example, Y-TKT is located on Sekisei Reef, which constitutes the largest reef area and is also the only site we sampled that contained corals from all three lineages. Non-random sampling, standardization of site size and sample density, and further standardized demographic, ecological and biological information for each population will be helpful to solve the outstanding questions about habitat selection and population dynamics of

Seriatopora.

Population connectivity and the reproductive system of *Seriatopora*

We found large genetic differentiation among sites in Seriatopora-B. Previous studies have also found a high degree of genetic subdivision among the populations of *S. hystrix* (Ayre and Hughes 2000, 2004; Underwood et al. 2007, 2009; van Oppen et al. 2008; Noreen et al. 2009), which corresponds to the findings of this study. Some previous studies showed complex distribution patterns of multiple genetic clusters unrelated to geographical location (e.g., Noreen et al. 2009), but may not have divided the genetic lineage and may have overestimated the genetic differentiation among sites. Nevertheless, large genetic differentiation might be related to the low dispersal ability of the brooding coral *Seriatopora*. The low dispersal ability is because the larvae settle soon after release from the natal colony, the peak of settlement is within 1 d, and almost all larvae are settled within 5 d of spawning (Atoda 1951; Ayre and Hughes 2000). The range of almost all dispersal in *Seriatopora* will occur at the local habitat scale.

Mean larval duration of *Seriatopora* is much shorter than for broadcast-spawning species such as *Acropora*. The settlement peak of *Acropora* is 5 to 8 d after spawning (Suzuki et al. 2011), and the maximum rearing durations of two *Acropora* species have been found to

be more than 50 d under artificial rearing conditions (Nishikawa and Sakai 2005). However, some brooding corals have been found to have the potential for long distance dispersal with successful settlement competency suggested by both experiment and model predictions (e.g., approximately 100 d in *Pocillopora damicornis*; Richmond 1987). The genetic differentiation of *Acropora* in this region has often been shown to be significant, but lower overall among islands (Nishikawa et al. 2003; Nishikawa and Sakai 2005; Nakajima et al. 2010; Zayasu et al. 2016). Estimates of migration in the present study did not reveal the source population of recruitment. Migration analysis also suggested that larval dispersal of *Seriatopora* occurs mostly within the local site. Nevertheless, gene flow among sites within a genetic lineage might contribute to long-term population persistence because few long-distance migrants are likely between populations of *S. hystrix* (van Oppen et al. 2008, Noreen et al. 2009). Inbreeding by self-recruiting in a restricted geographical range may enhance the fixation of a small number of genotypes because most larval recruitment occurs within 100 m of the natal habitat (Underwood et al. 2007), but oceanographic conditions and high reproductive output may facilitate the occasional dispersal of brooded larvae over 10 km for *S. hystrix* (Underwood et al. 2009).

Clonal diversity in the Ryukyu Archipelago suggests that all three genetic lineages

have been maintained by sexual reproduction. Recruitment by migration of *S. hystrix* on the GBR was mainly by sexually produced larvae (van Oppen et al. 2008). However, some multilocus genotypes were the same among colonies, indicating that populations are maintained with low genetic diversity (Seriatopora-A in O-OUR and Seriatopora-C in Y-IRO). While there is no obvious relationship between genetic diversity by neutral loci and rarity in corals (Richards and van Oppen 2012), populations including these colonies may be endangered due to low genotypic variation because genetic diversity is an important index for evolutionary responses to rapid climate change (Ayre and Hughes 2004). More populations of *Seriatopora* should be surveyed for further estimation of genetic diversity and gene flow within each lineage, especially from the southern region near the center of the geographical distribution including the Coral Triangle. Different reef systems should be examined to understand overall genetic diversity and differentiation and the possibility for migration

When the population size of *Seriatopora* is unusually large and the colonies are also large, large numbers of larvae are produced and larvae that reach other reefs probably help to establish and maintain populations (van Oppen et al. 2008). Reef areas in the Ryukyu Archipelago are fewer and smaller than those in other regions such as the Coral Triangle and GBR, and therefore the population size of *Seriatopora* will not be large compared with

populations on large reefs. This difference in the reef system is likely to dictate the number of migrants between sites. Using individual-based ecological modeling with ecological parameters for a *Seriatopora* population in the Ryukyu Archipelago, constant recruitment or occasional abundant recruitment from other populations was found to be necessary to recover the population after disturbances (Muko et al. 2014). Our population genetic analysis found evidence of only a single migrant in three populations of Seriatopora-B. Without continuous recruitment from other populations, *Seriatopora* in the Ryukyu Archipelago is in danger of extinction locally if the local habitat suffers from disturbances.

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appropriate permissions within the Okinawa prefecture in Japan.

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Figure legends

Fig. 1 Map of the sampling sites, depths, numbers of samples, and number of haplotypes of the mitochondrial control region (mtCR) of *Seriatopora* in the Ryukyu Archipelago. Location data, sample size, and the number of colonies of each genetic lineage are shown in ESM Table S1

Fig. 2 STRUCTURE analysis for 182 microsatellite multilocus genotypes. Analyses for each genetic lineage were conducted with 176 genotypes after removing the duplicate genotypes of clonemates. Probable cluster number, shown as *K*, for each site (**a**) was assumed as three (*K* = 3) based on the method described by Evanno et al. (2005). Probable cluster number *K* for each genetic lineage (**b**) was assumed as *K* = 3 to 6. The arrow shows the undefined lineage detected by this analysis

Fig. 3 Maximum likelihood phylogenetic tree composed of 30 sequences using the mtCR of *Seriatopora* and one sequence of *Pocillopora damicornis* as an outgroup. Values on the branches indicate bootstrap values (%). Twenty-three haplotypes shown by their GenBank accession numbers were cited from Chen et al. (2008), Flot et al. (2008), Bongaerts et al. (2010), and Warner et al. (2015). Sequences from other references were selected to avoid duplication of sequence, region, or publication

Fig. 1

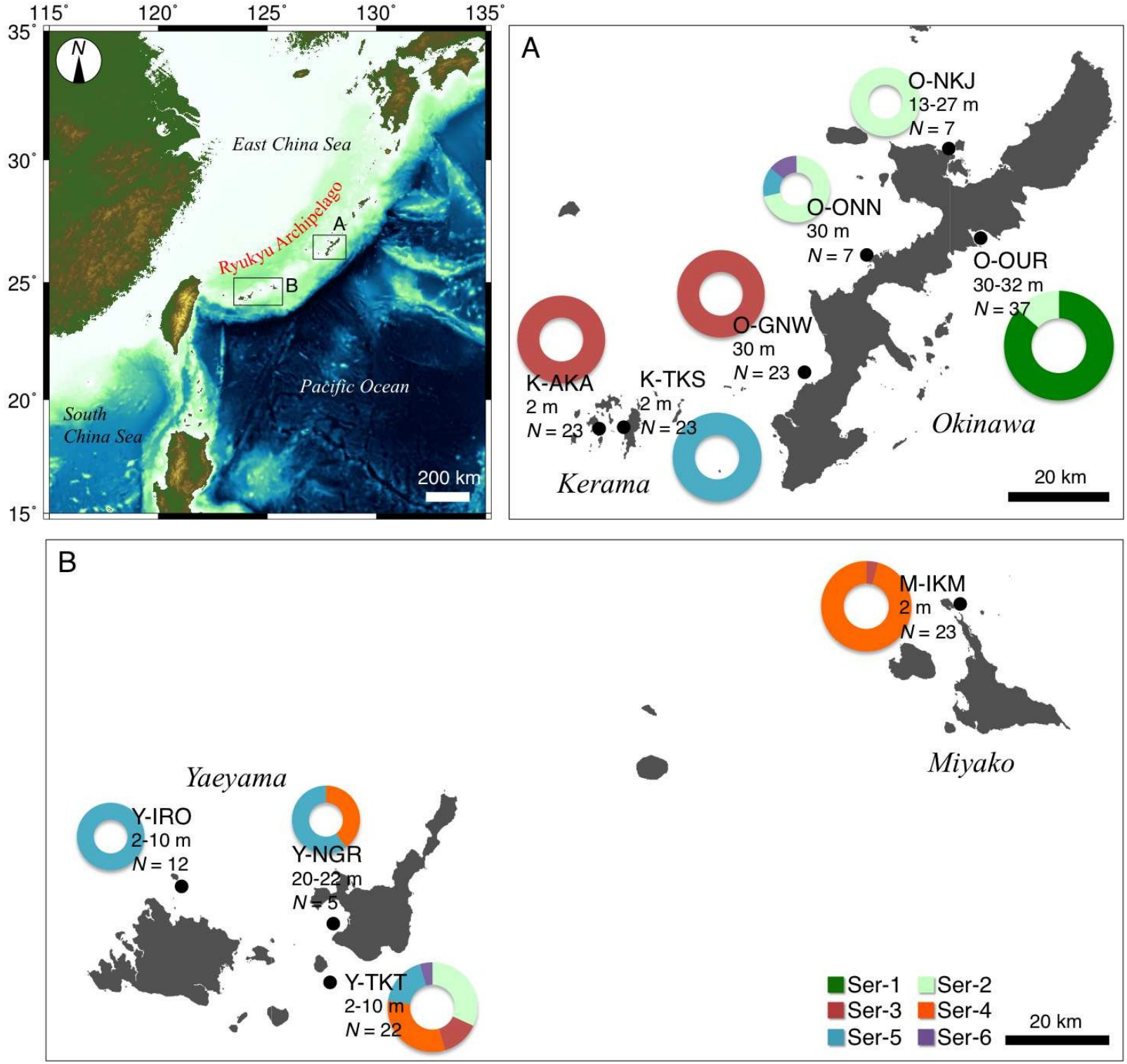

115°
120°
125°
130°
135°
35°
30°
25°
20°
15°
N
East China Sea
Ryukyu Archipelago
A
B
Pacific Ocean
South
China Sea
200 km
A
O-NKJ
13-27 m
N = 7
O-ONN
30 m
N = 7
O-OUR
30-32 m
N = 37
O-GNW
30 m
N = 23
K-AKA
2 m
N = 23
K-TKS
2 m
N = 23
Okinawa
Kerama
20 km
B
M-IKM
2 m
N = 23
Miyako
Yaeyama
Y-IRO
2-10 m
N = 12
Y-NGR
20-22 m
N = 5
Y-TKT
2-10 m
N = 22
Ser-1
Ser-2
Ser-3
Ser-4
Ser-5
Ser-6
20 km

Fig. 2

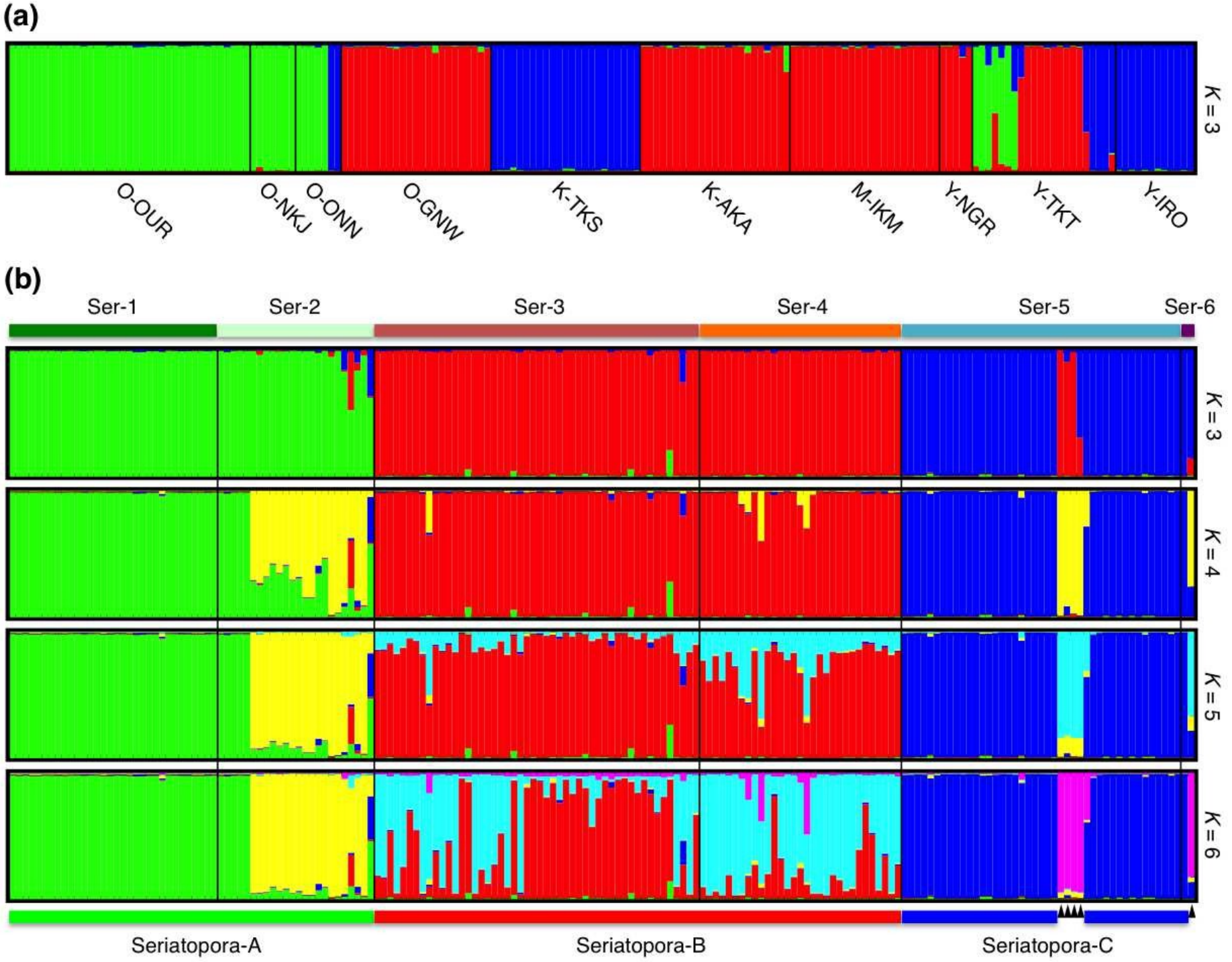
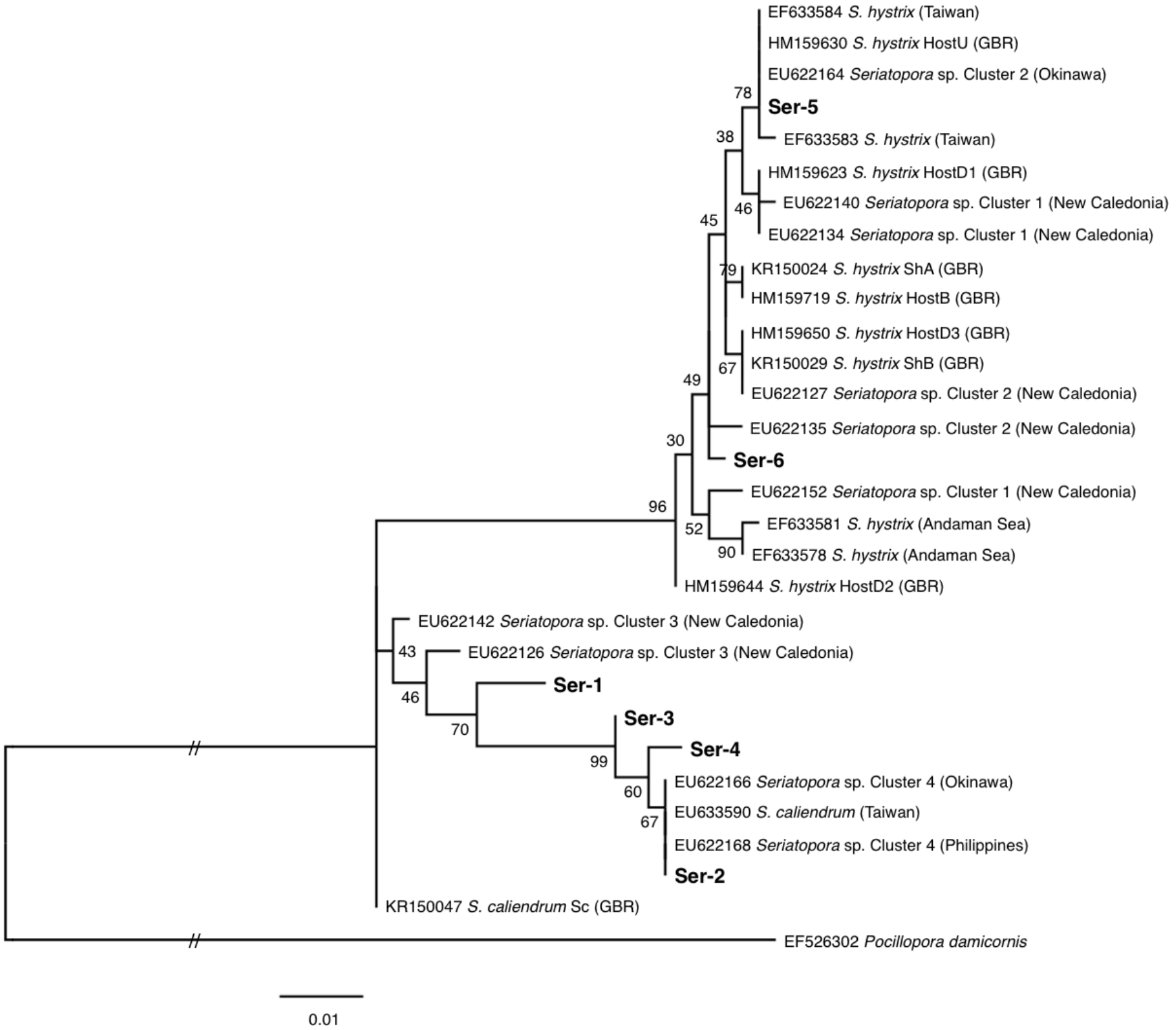

(a)
K = 3
O-OUR
O-NKJ
O-ONN
O-GNW
K-TKS
K-AKA
M-IKM
Y-NGR
Y-TKT
Y-IRO
(b)
Ser-1
Ser-2
Ser-3
Ser-4
Ser-5
Ser-6
K = 3
K = 4
K = 5
K = 6
Seriatopora-A
Seriatopora-B
Seriatopora-C

Fig. 3


EF633584 S. hystrix (Taiwan)
HM159630 S. hystrix HostU (GBR)
EU622164 Seriatopora sp. Cluster 2 (Okinawa)
78
Ser-5
38
EF633583 S. hystrix (Taiwan)
HM159623 S. hystrix HostD1 (GBR)
46
EU622140 Seriatopora sp. Cluster 1 (New Caledonia)
45
EU622134 Seriatopora sp. Cluster 1 (New Caledonia)
79
KR150024 S. hystrix ShA (GBR)
HM159719 S. hystrix HostB (GBR)
HM159650 S. hystrix HostD3 (GBR)
67
KR150029 S. hystrix ShB (GBR)
49
EU622127 Seriatopora sp. Cluster 2 (New Caledonia)
EU622135 Seriatopora sp. Cluster 2 (New Caledonia)
30
Ser-6
EU622152 Seriatopora sp. Cluster 1 (New Caledonia)
96
52
EF633581 S. hystrix (Andaman Sea)
90
EF633578 S. hystrix (Andaman Sea)
HM159644 S. hystrix HostD2 (GBR)
EU622142 Seriatopora sp. Cluster 3 (New Caledonia)
43
EU622126 Seriatopora sp. Cluster 3 (New Caledonia)
46
Ser-1
70
Ser-3
99
Ser-4
60
EU622166 Seriatopora sp. Cluster 4 (Okinawa)
67
EU633590 S. caliendrum (Taiwan)
EU622168 Seriatopora sp. Cluster 4 (Philippines)
Ser-2
KR150047 S. caliendrum Sc (GBR)
EF526302 Pocillopora damicornis
0.01

Hierarchical group	Source	d.f.	SS	Var. (%)	*F*-statistics	*P* value
Lineages-haplotypes (N_{MLG} = 170)	Among lineages	2	197.778	0.794 (35%)	F_{RT} = 0.352	0.001
	Among haplotypes within lineages	2	22.291	0.158 (7%)	F_{SR} = 0.108	0.001
	Within haplotypes	335	436.502	1.303 (58%)	F_{ST} = 0.422	0.001
	Total	339	656.571	2.255 (100%)		
Lineages-sites (N_{MLG} = 171)	Among lineages	2	206.244	0.788 (35%)	F_{RT} = 0.349	0.001
	Among sites within lineages	10	78.473	0.297 (13%)	F_{SR} = 0.202	0.001
	Within sites	341	399.218	1.171 (52%)	F_{ST} = 0.481	0.001
	Total	353	683.935	2.256 (100%)		

Table 1

	Seriatopora-A				Seriatopora-B				Seriatopora-C				Undefined			
Code	*N*	*G*	N_{MLG}	*R*	*N*	*G*	N_{MLG}	*R*	*N*	*G*	N_{MLG}	*R*	*N*	*G*	N_{MLG}	*R*
O-OUR	37	14	35	0.94	-	-	-	-	-	-	-	-	-	-	-	-
O-NKJ	7	7	7	1.00	-	-	-	-	-	-	-	-	-	-	-	-
O-ONN	5	4	4	0.75	-	-	-	-	2	2	2	1.00	-	-	-	-
O-GNW	-	-	-	-	23	22	22	0.95	-	-	-	-	-	-	-	-
K-TKS	-	-	-	-	-	-	-	-	23	21	23	1.00	-	-	-	-
K-AKA	-	-	-	-	23	23	23	1.00	-	-	-	-	-	-	-	-
M-IKM	-	-	-	-	23	21	21	0.91	-	-	-	-	-	-	-	-
Y-NGR	-	-	-	-	2	2	2	1.00	-	-	-	-	3	3	3	1.00
Y-TKT	7	7	7	1.00	10	10	10	1.00	3	3	3	1.00	2	2	2	1.00
Y-IRO	-	-	-	-	-	-	-	-	12	6	12	1.00	-	-	-	-
Total	56	32	53	0.95	81	78	78	0.96	40	32	40	1.00	5	5	5	1.00

Table 2

Genetic lineage	Site	N_{MLG}	A_R	H_O	H_E	F_{IS}
Seriatopora-A	O-OUR	35	1.66	0.151	0.178	0.165*
Seriatopora-B	O-GNW	22	4.81	0.390	0.413	0.079
	K-AKA	23	4.87	0.348	0.421	0.195**
	M-IKM	21	5.00	0.442	0.474	0.091
Seriatopora-C	K-TKS	23	2.92	0.193	0.258	0.275**

Table 3