

Genome-wide population structure of a threatened coral with a disjunct distribution: genetic differentiation among peripheral populations

Supisara Pipithkul

The University of Tokyo

Hirofumi Yokochi

NPO Shiosai 21

Taisei Kikuchi

The University of Tokyo

Shelby E. McIlroy

The Chinese University of Hong Kong

Akifumi Shimura

University of Miyazaki

Hironobu Fukami

University of Miyazaki

Nina Yasuda

27yasuda@e.ecc.u-tokyo.ac.jp

The University of Tokyo

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Abstract

Peripheral coral populations may provide valuable insights into lineage diversification and persistence under marginal environmental conditions, yet their evolutionary divergence and conservation significance remain poorly understood. *Acropora pruinosa* is a temperate coral with a disjunct distribution range, officially reported only from Hong Kong and temperate Japan, and is closely related to the widely distributed *A. aff. divaricata*, but their genome-wide relationship has not been tested. Here we used genome-wide SNP data from Japanese *A. pruinosa*, Hong Kong *A. pruinosa*, and Japanese *A. aff. divaricata* to examine genomic differentiation, lineage relationships, and conservation implications. Genome-wide SNP analyses separated *A. pruinosa* from *A. aff. divaricata*, despite previous evidence of close genetic and complex morphological relationships. SNP-based species tree inference recovered Hong Kong *A. pruinosa* as an early-diverging sampled lineage within *A. pruinosa*, while STRUCTURE and DAPC also showed genetic differentiation between *A. pruinosa* lineages. The AMOVA and pairwise F_{ST} analyses showed contrasting patterns between two species. Hong Kong *A. pruinosa* also showed intermediate gross morphology, whereas both Hong Kong and Japanese *A. pruinosa* showed higher clonal fractions than *A. aff. divaricata*. These findings support genomic differentiation between *A. pruinosa* and *A. aff. divaricata*. The genetic distinctiveness of Hong Kong, eastern Japanese, and western Japanese *A. pruinosa* suggests that these lineages should be considered separately in conservation planning. Overall, this study clarifies lineage relationships and regional genetic uniqueness in a threatened temperate coral and highlights the conservation importance of peripheral coral populations occurring in marginal habitats.

Introduction

The distribution and diversity of reef-building corals are primarily centered in tropical regions (DeVantier and Turak 2017), but marginal temperate regions, where species face environmental constraints, may promote lineage divergence at the periphery of species distributions (Budd and Pandolfi 2010). For species in the genus *Acropora*, whose diversity is mostly concentrated in the central Indo-Pacific (Wallace and Rosen 2006), seasonally low temperatures and geographically discontinuous habitats in temperate areas can reduce genetic connectivity with tropical populations and promote genetic differentiation (Nakabayashi et al. 2019; Fifer et al. 2022). Such conditions may drive potentially adaptive divergence by favoring traits that enhance persistence at peripheral habitats, including greater tolerance to low temperatures, altered morphology, and increased dependence on clonal propagation (Baums et al. 2014; Abrego et al. 2021). Therefore, peripheral populations provide useful systems for improving our understanding of the potential for adaptive evolution in coral species.

Acropora pruinosa is a temperate reef-building species with an arborescent colony form and is presumed to be endemic to Hong Kong and temperate Japan (Veron 1992). This species shows relatively high tolerance to low temperature (down to 13°C) compared to other *Acropora* spp., being a dominant species in the 34°N area in Japan, which is the latitudinal extreme of reef-coral distributions (Higuchi et al. 2015). Although located at a lower latitude, this species also occurs in Hong Kong where winter sea surface temperatures (SSTs) can be as low as those in some Japanese temperate areas; SSTs at Bluff Island, Hong Kong, range from 14 to 31°C, while it is about 13.5 to 26.6°C in Iki and Tsushima Island in the 33–34°N area (Goodkin et al. 2011; Yamano et al. 2001) in Japan. In addition to an arborescent colony form observed in both Japanese and Hong Kong populations, arborescent-tabular colonies have also been recorded from Hong Kong waters (The Hong Kong Biodiversity Information Hub 2025). This morphological variation resembles *Acropora aff. divaricata* (formerly arborescent-tabular *A. solitaryensis*) (Chow et al. 2025; Fukami et al. 2021), a widely distributed tropical Indo-Pacific species that is closely related to *A. pruinosa*. Despite differences in colony forms, natural spawning time, and distribution range, previous genetic and cross-fertilization experiment showed that *A. aff. divaricata* and *A. pruinosa* are genetically and morphologically closely related; the result showed that *A. cf. divaricata* with arborescent-tabular colony form can cross-fertilize with *A. pruinosa* in the cross-fertilization experiment, these two species also cannot be genetically distinguished by the mitochondrial control region, and their septal development of radial corallites are quite similar (Suzuki and Fukami 2012). Based on these findings, Suzuki and Fukami (2012) hypothesized that "*A. pruinosa* and/or *A. aff. divaricata* speciated not in tropical regions but in peripheral zones of coral distribution". Together, these characteristics make *A. pruinosa* a suitable system in which peripheral areas of distribution, environmental marginality, and morphological variation can be considered together.

Despite sharing genetic and micro-morphological similarity, as well as the resemblance of Hong Kong *A. pruinosa* and *A. aff. divaricata*, no study has compared Japanese *A. pruinosa*, Hong Kong *A. pruinosa*, and Japanese *A. aff. divaricata* within a single genome-wide SNP framework. In addition, a previous genetic study using microsatellite markers revealed two hidden lineages of *A. pruinosa* in western and eastern temperate Japan, suggesting that two lineages may have arisen from the past climate refugia and should be conserved separately (Pipithkul et al. 2021). Examining the genetic relationships among these populations and closely related species may improve our understanding of how coral lineages diversify in peripheral habitats with marginal environments. Such knowledge is also relevant for conservation management, particularly because *A. pruinosa* is currently threatened and listed in the Japanese Red List (Ministry of Environment 2017).

This study aimed to clarify lineage boundaries and population structure of *A. pruinosa* in its marginal environments at the periphery using genome-wide SNP data. The two main questions of this study were: (1) whether *A. pruinosa* is genomically distinct from the closely related *A. aff. divaricata*, and (2) whether geographically disjunct *A. pruinosa* populations represent differentiated lineages that may require separate consideration in conservation management.

Materials and Methods

Coral sampling and DNA extraction

A total of 379 coral fragments were collected by SCUBA diving and genotyped in this study, comprising 133 colonies of Japanese *A. pruinosa* from 14 sites, 20 colonies of Hong Kong *A. pruinosa* from 2 sites, and 226 colonies of Japanese *A. aff. divaricata* from 9 sites (Fig. 1; Table 1). Each fragment was collected from a separate colony, and sampled colonies were spatially separated to avoid repeated sampling of the same genet. The samples were preserved using different methods before DNA extraction; fragments of Japanese *A. pruinosa* were preserved in CHAOS solution, Hong Kong *A. pruinosa* samples were

received as frozen coral skeleton branch fragments and stored frozen until DNA extraction, and fragments of Japanese *A. aff. divaricata* were preserved in 99.5% ethanol. The DNA extraction of Japanese *A. pruinosa* was conducted following the method by Fukami et al. (2004), from CHAOS solutions of each sample, and DNA from the Hong Kong *A. pruinosa* sample was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manual of the kit. Japanese *A. aff. divaricata* samples' DNA was extracted using the hot alkaline solution method (Meeker et al. 2017), then using ethanol precipitation to clean and purify the DNA (Richards et al. 2018). We also took underwater photos of Japanese *A. pruinosa* and Hong Kong *A. pruinosa* while sampling and used the photos to compare their gross morphology as well.

Coral samples in Japanese temperate waters were collected by SCUBA diving in accordance with local procedures and with the consent or cooperation of relevant local fisheries cooperatives, research institutions, or local collaborators. A special collection permit was obtained for Shizuoka Prefecture (Permit No. Toku 21-21). Where special collection permits were not required at the time of sampling, collections were conducted with local consent or institutional cooperation, including sampling in Chiba, Wakayama, Kochi, Tokushima, Nagasaki, and Kumamoto. Sampling in Nagasaki was conducted with local consent and notification to the Japan Coast Guard.

Table 1. List of sampling sites, population label and their lineage (Eastern or Western), sample dates, location coordinates, sample size, number of genets, genotypic richness (G/N: number of genets divided by total sampled colonies) and clonal fraction (1 - G/N).

Population sites	Label / lineage	Sampling dates	Location		size (n)	number of genets	G/N	Clonal frac
		Year/Month	latitude (N°)	longitude (E°)				
<i>Acropora pruinosa</i> (Japanese)								
Tsushima (Futagose), Nagasaki	TS / W	2010/09	34°25'02"	129°16'12"	11	7	0.636	0.364
Tsushima (Otaura), Nagasaki	TU / W	2010/09	34°16'26"	129°19'56"	15	6	0.4	0.60
Iki (Hongu), Nagasaki	I / W	2010/09	33°49'23"	129°40'28"	13	10	0.769	0.231
Iki (Kouze), Nagasaki	IK, IKN / W	2010/09	33°46'09"	129°39'17"	7	7	1	0.0
Amakusa (Haruhae), Kumamoto	A, AMA / W	2009/10	32°10'50"	130°01'15"	11	10	0.909	0.091
Mishidomari, Kochi	KO / E	2009/08	32°46'41"	132°43'55"	8	5	0.625	0.375
Takegashima, Tokushima	T / E	2009/09	33°32'33"	134°18'58"	10	5	0.5	0.50
Takatomi, Wakayama	W / E	2009/05	33°28'57"	135°45'30"	13	6	0.462	0.538
Fukuro Bay, Wakayama	WT / E	2009/06	33°28'34"	135°46'22"	4	2	0.5	0.50
Kuzura, Shizuoka	Ku / E	2009/08	35°01'23"	138°52'08"	5	3	0.6	0.40
Enashi, Shizuoka	E / E	2009/08	35°01'25"	138°48'00"	6	5	0.833	0.167
Tago, Shizuoka	G / E	2010/07	34°48'23"	138°45'39"	10	8	0.8	0.20
Nakagi, Shizuoka	N / E	2009/10	34°36'26"	138°49'25"	9	8	0.899	0.111
Tateyama, Chiba	C / E	2009/08	34°59'30"	139°49'34"	11	6	0.545	0.455
<i>Acropora pruinosa</i> (Hong Kong)								
Bluff Island, Hong Kong	HKB	2021/12	22°19'26"	114°21'11"	10	5	0.500	0.500
Port Shelter, Hong Kong	HKP	2022/01	22°19'39"	114°17'33"	10	8	0.8	0.20
<i>Acropora</i> aff. <i>divaricata</i> (formerly arborescent-tabular <i>A. solitaryensis</i>)								
Goto island, Nagasaki	AADGAS	2016/11	32°34'21"	128°53'48"	27	24	0.889	0.111
Tsushima, Nagasaki	AADTUS	2017/11	34°25'25"	129°16'00"	10	8	0.889	0.111
Amakusa, Kumamoto	AADAmak	2009/09	32°33'16"	129°59'59"	46	38	0.826	0.174
Miyazaki	AADMYO	2017/12	31°31'3"	131°24'40"	4	4	1.000	0.000
Tatsukuchi, Kochi	AADKouch	2017/05	32°45'49"	132°51'38"	8	8	1.000	0.000
Yokonami, Kochi	AADKOS	2017/05	33°24'09"	133°24'29"	27	20	0.741	0.259
Wakayama	AADWAS	2016/06	33°28'45"	135°44'44"	41	36	0.878	0.122
Shikine-jima, Tokyo	AADZAS	2016/09	34°19'00"	139°12'36"	50	47	0.940	0.06
Tateyama, Chiba	AADTYA	2018/10	34°59'27"	139°49'4"	13	13	1.000	0.0

Construction of the MIG-seq library

In this study, we prepared MIG-seq libraries following Suyama and Matsuki (2015). MIG-seq is a method for detecting genome-wide SNPs in various inter-simple sequence repeat (ISSR) regions, which requires two PCR steps to amplify multiple anonymous non-repetitive regions from ISSRs (Gupta et al. 1994; Zietkiewicz, Rafalski and Labuda 1994). To construct the MIG-seq libraries of each sample, eight primer pairs of multiplexes tailed ISSR primers (MIG-seq primer set 1; Suyama and Matsuki 2015) in the 1st PCR step, then tailed the 1st PCR product with common and indexed primers in the 2nd PCR step. After two PCR steps, 350-800 bp size fragments are isolated on the gel, purified and then used for sequencing.

We used Multiplex PCR Assay Kit Ver.2 (TaKaRa Bio Inc. Otsu, Shiga, Japan) with primer mix of 8 primer pairs for the 1st PCR step. In total 7 µl of reaction volume (6 µl PCR cocktail with 1 µl of DNA extract) of each sample was processed in a thermal cycler with the following setting: initial denaturation at 94°C for 1 min, followed by 25-30 cycles of 94°C for 30s, 38°C for 1 min, 72°C for 1 min, and finish with the final extension at 72°C for 10 mins. The 1st PCR products quality was checked with gel electrophoresis. We used Prime STAR GXL DNA polymerase (TaKaRa Bio Inc. Otsu, Shiga, Japan) for the 2nd PCR step. In total 12 µl of reaction volume of each sample were processed in a thermal cycler with following setting: 21 cycles of 98°C for 10 secs, 54°C for 15 secs, and 68°C for 1 min. All second-PCR products with different indices were pooled into a single mixture and run on an agarose gel with TAE buffer, then DNA fragments with a size range of 350-800 bp were extracted from excised gel. DNA libraries of each sample were sequenced using MiSeq (sequencing control software v2.0.12, Illumina) with the MiSeq Reagent v3 150 cycle kit (Illumina).

MIG-seq read processing and mapping

Low quality reads and primer sequences were filtered out from the raw data with the FASTX-toolkit version 0.0.14 (fastq_quality_filter) (Gordon and Hannon 2012) with the following setting of fastq_quality_filter: -Q 33 -q 30 -p 40. Adapter sequences from both the 5' end (GTCAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC) and the 3' end (CAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAC), were removed by Cutadapt version 1.13 (Martin 2011) with an error rate of 0.05 (-e 0.05). Reads shorter than 80 bp were discarded.

GSTACKS program in Stacks v. 2.2 (Catchen et al. 2013; Rochette and Catchen 2017) was used to genotype all individuals in this study. For genotyping, trimmed sequence data were mapped against an in-house unpublished reference genome assembly of *Acropora aff. divaricata* using Burrows-Wheeler Aligner (BWA) program (Li and Durbin 2009), after PCR duplicates were removed by retaining only one read pair from duplicate sets. The results were stored as SAM files and converted into BAM files using SAMtools software (Li et al. 2009). POPULATIONS software in Stacks v. 2.2 (Catchen et al. 2013) was used to filter loci according to the -R setting, which specified the minimum proportion of individuals across all populations required to retain a locus. SNP datasets generated under different -R thresholds (ranging from 0.5 to 0.7), were used as inputs of other analyses in this study as described below. The minimum minor allele frequency required to process a nucleotide site at a locus (min_maf) was assigned as 0.01, and the maximum observed heterozygosity required to process a nucleotide site at a locus (max_obs_het) was assigned as 0.99 in this study. All MIG-seq sequences obtained in this study were deposited in the DNA Data Bank of Japan (DDBJ accession number: PRJDB2202)

Datasets retaining all SNPs per locus were used for putative clone mate detection, whereas datasets generated with the *-write-single-snp* option were used for other downstream analyses to reduce linkage among markers.

Putative clone mates' detection

Putative clone mates of *A. pruinosa* and *A. aff. divaricata* were identified by manually inspecting the distribution of pairwise relatedness values calculated using Lynch and Ritland estimator (Lynch and Ritland 1999). Separate SNP datasets were generated for *A. pruinosa* including both Japanese and Hong Kong samples and for *A. aff. divaricata* using the -R 0.7 setting in the POPULATIONS software in Stacks v. 2.2 and were used as inputs to detect putative clone mates within each species. For each species-specific dataset, all SNPs per locus were retained to maximize the resolution of pairwise relatedness estimates. In total, 787 SNP loci were retained from 153 *A. pruinosa* samples (Online Resource 1), and 3,317 SNP loci were retained from 226 *A. aff. divaricata* samples (Online Resource 2).

We calculated pairwise relatedness values using GenAlex ver. 6.503 (Peakall and Smouse 2005; Peakall and Smouse 2012). Putative clone mates were defined as sample pairs with pairwise relatedness values greater than 0.4, based on observed distribution of pairwise relatedness values generated by the Lynch and Ritland estimator. Because the estimator values ranged from -0.5 to 0.5 in this analysis, values above 0.4 were treated as highly related pairs. The same threshold was applied to both species for consistency, and to avoid introducing group-specific filtering rules. For Japanese *A. pruinosa*, putative clone mate assignments were further compared with putative clone estimates based on nine microsatellite markers from Pipithkul et al. (2021) as well as unpublished raw microsatellite data for samples that included some missing data, because Japanese *A. pruinosa* samples used in this study overlapped with those in Pipithkul et al. (2021). In the microsatellite dataset, individuals were considered putative clones only when they shared identical genotypes across all loci, using a strict zero-mismatch criterion; individuals differing at one or more microsatellite loci were treated as genetically distinct. Genotypic richness (G/N) was calculated as the number of genets divided by the total number of sampled colonies, and clonal fraction was calculated as one minus G/N (Table 1).

For each putative clone group identified from SNP data, one representative sample was retained as a genet for downstream analyses. After putative clone-mate detection, 101 *A. pruinosa* genets and 198 *A. aff. divaricata* genets were identified (Table 1). Because sample sizes were uneven both between species and among populations of *A. aff. divaricata*, representative samples of this species were subsampled using a population-stratified approach to reduce overrepresentation of populations with large sample sizes. Within each population, samples with lower proportions of missing data were preferentially retained to improve SNP data completeness for downstream analyses.

After subsampling, 102 *A. aff. divaricata* genets were retained, resulting in a final balanced dataset of 201 samples, including 99 *A. pruinosa* genets, excluding population WT, and 102 *A. aff. divaricata* genets. MIG-seq data processing, including read mapping and SNP calling, was repeated for this balanced dataset before downstream analyses. Population WT retained only two genets after putative clone mate detection and was excluded from the balanced dataset. This exclusion did not remove geographic coverage of the area because WT was located close to population W.

SNP-based species tree inference

Bayesian inference of species trees using SNP data was conducted using SNAPPER (Stoltz et al. 2021) implemented in BEAST 2.7.7 (Bouckaert et al. 2019) under the multispecies coalescent model. The input dataset consisted of the putative clones-filtered balanced dataset of 201 ingroup samples, with one additional *Acropora* cf. *glauca* sample collected from Gotou Island, Nagasaki, included as an outgroup to root the species tree. This input dataset was generated using the -R 0.7 setting and the “--write-single-snp” option to ensure independence among SNP loci. In total, 530 SNP loci were retained for this analysis. A variant call format (VCF) file containing 530 SNP loci was generated and converted into a binary NEXUS format file using PGDSpider2 version 2.1.1.5 (Lischer and Excoffier 2012). The binary NEXUS file was then imported into BEAUti v.2.7.8 (Bouckaert et al. 2019) and converted into XML format for species tree inference using SNAPPER.

Analyses were run for 10,000,000 Markov chain Monte Carlo (MCMC) iterations, after discarding the first 10% of samples as burn-in, the maximum clade credibility tree was summarized using TreeAnnotator v2.7.7 (Bouckaert et al. 2019) and visualized using FigTree v1.4.4 (Rambaut 2018). In this analysis, samples were assigned to five taxon labels: AAD, HK, APE, APW, and outgroup, corresponding to *A. aff. divaricata*, Hong Kong *A. pruinosa*, the eastern lineage of Japanese *A. pruinosa* (Populations: KO, T, W, Ku, E, G, N, C), the western lineage of Japanese *A. pruinosa* (Populations: TS, TU, I, IK, A/AMA), and *A. cf. glauca*, respectively. Convergence of the runs, and the effective sample sizes of the parameters, were confirmed using Tracer v1.7.2 (Rambaut et al. 2018).

Population structure analysis

STRUCTURE software (Pritchard et al. 2000; Falush et al. 2003, Falush et al. 2007; Hubisz et al. 2009) was used to investigate the population structure. The putative clones-filtered balanced dataset of 201 samples was used to generate the STRUCTURE format input file. This dataset was generated using the -R 0.7 setting and the “--write-single-snp” option, resulting in 535 SNP loci retained for this analysis. An admixture model was used with LOCPRIOR option, which was used to incorporate sampling locality information as prior information, to assist the detection of population structure. The following settings were used to assign each sample to a predefined number of hypothetical cluster (K): a burn-in of 100,000 chains followed by 100,000 Markov Chain Monte Carlo (MCMC) replications. Ten independent runs for each K = 1 to 7 were performed to confirm convergence among runs. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to estimate delta K and CLUMPAK (Kopelman et al. 2015) was used to summarize and visualize the coefficient of ancestry for each sample across 10 replicate runs for the inferred K values.

To further investigate population structure within *A. pruinosa*, a second balanced dataset containing only 99 *A. pruinosa* samples after putative clone filtering was used to generate another STRUCTURE format input file. The dataset was generated using the same filtering settings and option described above, resulting in 376 SNP loci retained. Ten independent runs for each K = 1 to 4 were performed with following settings: a burn-in of 100,000 chains followed by 100,000 MCMC replications. Both STRUCTURE HARVESTER and CLUMPAK were also used to evaluate the clustering results, summarize replicate runs, and visualize individual ancestry coefficients.

Genetic differentiation among predefined groups

Discriminant analysis of principal components (DAPC) was performed to summarize and visualize genetic differentiation among predefined groups. The same putative clones-filtered balanced SNP dataset used for STRUCTURE analysis, including 535 SNP loci, was used for this analysis. STRUCTURE format file was also used as an input in this study. DAPC combines principal component analysis and discriminant analysis, using principal components as uncorrelated input variables for discriminant analysis (Miller, Cullingham and Peery 2020).

Before DAPC analysis, R package POPPR (Kamvar et al. 2014; Kamvar et al. 2015) was used for cross-validation to find the appropriate number of principal components retained for the subsequent DAPC analysis. DAPC was then performed using the R package *adegenet* (Jombart 2008). The four predefined groups of this study (*A. aff. divaricata*, Hong Kong *A. pruinosa*, eastern Japanese *A. pruinosa*, and western Japanese *A. pruinosa*) were used directly as the grouping factor for DAPC. Therefore, DAPC was intended to summarize and visualize genetic differentiation among focal biological groups, rather than to infer the number of genetic clusters independently. The *scatter* function was used to visualize the DAPC results.

Genetic structure and population connectivity

To assess genetic structure and connectivity within each taxon, we performed analysis of molecular variance (AMOVA) separately for *A. aff. divaricata* and *A. pruinosa*. AMOVA was used to partition genetic variation among populations, among individuals within populations, and within individuals. To further identify which population pairs contributed to the observed structure, pairwise FST was calculated among populations within each species.

19 populations with at least five genets were used as inputs for both analyses, separately for each taxon: HK_Bluff, HK_Port, TS, TU, AMA, I, IK, W, N, G and C for *A. pruinosa*, and AADAmak, AADGAs, AADKOS, AADKouch, AADTUS, AADTYA, AADWAS and AADZAS for *A. aff. divaricata*. Bluff Island, Hong Kong with 5 samples was included as an exception because this population represents a key population for one predefined management unit. Species-specific population maps were used separately for SNP filtering within each taxon with the -R 0.7 and “--write-single-snp” setting. The resulting SNP numbers were 402 SNPs for *A. pruinosa* and 1104 SNPs for *A. aff. divaricata*. AMOVA and pairwise FST were calculated using GenAlEx ver. 6.503 (Peakall and Smouse

2005; Peakall and Smouse 2012) with the distance-based option. P-values for AMOVA and pairwise F_{ST} were generated using 9,999 permutations, and Bonferroni corrections were applied to pairwise F_{ST} P-values.

Results

MIG-seq mapping analysis

Across 380 individuals, including *A. aff. divaricata*, Hong Kong *A. pruinosa*, the eastern lineage of Japanese *A. pruinosa*, the western lineage of Japanese *A. pruinosa*, and one *A. cf. glauca* outgroup sample, sequencing produced 59,200,023 raw reads, with an average of 155,789 reads per sample. After quality filtering and adapter trimming, 30,329,029 reads remained, with an average of 79,813 reads per sample. After two-step filtering, 2,681,097 reads remained, with an average of 7,055 reads per individual (Online Resource 3).

Mapping rates varied among lineages. *A. aff. divaricata* showed the highest mean mapping rate (88.97%) and the largest number of covered sites (619,688 sites). Hong Kong *A. pruinosa* lineage showed an intermediate to high mean mapping rate of 84.52%, while the eastern and western Japanese *A. pruinosa* lineages showed lower mean mapping rates of 73.38% and 77.78%, respectively. The details regarding mapping rates, mapped reads, total reads, read depth, and number of covered sites of each sample of each lineage is available in Online Resource 4.

Clonal structure and clonal fraction

Pairwise relatedness distributions were examined separately for *A. pruinosa* and *A. aff. divaricata*. In both species, the distributions showed an upper tail of highly related sample pairs above 0.4. Based on this distribution, sample pairs above this threshold were treated as putative clone mates, although SNP markers cannot identify clone mates with absolute certainty (Online Resources 5 and 6). Sensitivity analyses using alternative relatedness thresholds showed that the estimated number of genets changed only slightly at similar thresholds (> 0.35 and > 0.45), indicating that the overall estimate of putative clonality was relatively robust to threshold choice. The threshold of > 0.4 was retained to reduce the potential influence of highly related individuals or putative clone mates on linkage disequilibrium and other downstream analyses. Comparisons of the number of genets estimated under different relatedness thresholds are presented in Online Resource 7.

Using this criterion, *A. aff. divaricata* showed a low clonal fraction, whereas both Japanese and Hong Kong *A. pruinosa* showed higher clonal fractions. The clonal fraction of *A. aff. divaricata* ranged from 0 (Miyazaki; Tatsukuchi, Kochi; Tateyama, Chiba) to 0.259 (Yokonami, Kochi), with a mean of 0.09. In contrast, the clonal fraction of Japanese *A. pruinosa* ranged from 0 (Iki, Kouze, Nagasaki) to 0.538 (Takatomi, Wakayama), with a mean of 0.366. The clonal fractions of Hong Kong *A. pruinosa* at Bluff Island and Port Shelter were 0.50 and 0.20, respectively, with a mean of 0.35 (Table 1).

SNP-based species tree inference supports genetic differentiation among focal lineages

The species tree was inferred using 530 SNP loci (-R 0.7, "--write-single-snp", including the outgroup) with adequate effective sample sizes (ESS) for all parameters (> 750) (Online Resource 8). The species tree included four predefined clades together with *Acropora cf. glauca* collected in Gotou Island (Nagasaki) as an outgroup (Fig. 2). The tree topology showed that *A. aff. divaricata* (AAD) was placed outside the sampled *A. pruinosa* lineages. Within sampled *A. pruinosa*, the Hong Kong clade (HK) was recovered as sister to the eastern (APE) and the western Japanese *A. pruinosa* clade (APW). The APE–APW node and the HK–Japanese *A. pruinosa* node were strongly supported, with posterior probabilities of 0.9999 for both nodes. By contrast, the deeper node separating AAD from all *A. pruinosa* lineages showed low posterior support (posterior = 0.2398). All samples included in this species tree are provided in Online Resource 9.

Population structure between species and their genetic distribution

The STRUCTURE analysis of 535 SNP (-R 0.7, "--write-single-snp") identified the strongest hierarchical signal at $K = 2$, which showed a clear separation of *A. aff. divaricata* and *A. pruinosa* (Fig. 3a). This result should be interpreted as hierarchical population structure rather than independent evidence of separated species. Further analysis with *A. pruinosa* dataset identified $K = 2$ ($\Delta K = 39.86$) as the primary genetic structure with STRUCTURE HARVESTER, although a similarly high ΔK was observed at $K = 3$ ($\Delta K = 36.46$). At $K = 2$, seven of ten replicate runs were assigned to the major clustering mode, whereas $K = 3$ produced two equally frequent modes (five runs each) (Fig 3b). At $K = 3$, the major mode showed genetic differentiation corresponding to the three geographic groups: Hong Kong, eastern Japanese, and western Japanese *A. pruinosa* (Fig 3c). Summary statistics used to evaluate the number of genetic clusters, including mean $\text{LnP}(K)$, standard deviation of $\text{LnP}(K)$, $\text{Ln}'(K)$, $|\text{Ln}''(K)|$, and ΔK , are provided in Online Resource 10.

The DAPC result from the same SNP dataset was used to summarize genetic differentiation among four predefined groups. Cross-validation identified 34 as the number of PCs achieving the highest mean success. This value was therefore retained for the analysis, accounting for 64.78% of the variance in the dataset (Online Resource 11). Because DAPC was performed using predefined groups, we interpreted the ordination as a visualization of genetic differentiation among focal groups rather than as an independent clustering analysis. The ordination mainly separated samples into three main clusters including *A. aff. divaricata* cluster, Japanese *A. pruinosa* cluster, and Hong Kong *A. pruinosa* cluster. The ordination showed that Japanese *A. pruinosa* cluster occupied a broader area of the ordination space than the other two clusters (Fig. 4). The predefined group labels used for DAPC are provided in Online Resource 12.

Genetic structure and connectivity

AMOVA revealed statistically significant genetic structure among populations in both species, but the degree of population differentiation differed between them. In *A. aff. divaricata*, genetic structure among the eight populations around temperate Japan was relatively weak but significant ($F_{ST} = 0.082$). In

contrast, *A. pruinosa* showed substantially stronger population structure with a larger proportion of genetic variation explained by differences among populations ($F_{ST} = 0.252$).

Pairwise F_{ST} values of *A. aff. divaricata* ranged from 0.0231 ($P < 0.01$, between AADWAS and AADZAS) to 0.2118 ($P < 0.01$, between AADTYA and AADZAS) and that of *A. pruinosa* ranged from 0 after conversion of a negative estimate (uncorrected $F_{ST} = -0.016$) (not statistically significant, between HK_Bluff and HK_Port) to 0.4780 ($P < 0.01$, between W and C). Overall, pairwise F_{ST} values were higher among *A. pruinosa* populations than among *A. aff. divaricata* populations, consistent with the stronger population structure detected by AMOVA.

Gross morphological observations

In Japan, *A. aff. divaricata* was identified based on gross morphology characterized by horizontally fused branches (tabular-arborescent form), whereas *A. pruinosa* was identified based on branches that are not fused (arborescent form) (Fukami et al. 2021: Fig. 1, Supplementary Fig. 3). This morphology-based identification agreed with the genetic analyses in this study. Among the Hong Kong samples, three colonies of *A. pruinosa* had branches that were not fused, 12 colonies had *A. aff. divaricata*-like, partially fused branches, and five colonies showed intermediate gross morphology.

Table 2. Analysis of molecular variance (AMOVA) results of *A. aff. divaricata* and *A. pruinosa*

Source	df	Sum of squares	Mean square	Estimated variance	%	F_{ST}
<i>Acropora aff. divaricata</i>						
Among Pops	7	4620.526	660.075	14.870	8%	0.08
Among Indiv.	90	27155.846	301.732	134.527	74%	
Within Indiv.	98	3202.500	32.679	32.679	18%	
Total	195	34978.872		182.075	100%	
<i>Acropora pruinosa</i>						
Among Pops	10	3939.366	393.937	19.865	25%	0.25
Among Indiv.	70	7187.251	102.675	43.785	56%	
Within Indiv.	81	1223.500	15.105	15.105	19%	
Total	161	12350.117		78.755	100%	

F_{ST} values are reported for the among-population component. Statistical significance was assessed using permutation tests with 9,999 permutations in Gen

Table 3. Pairwise F_{ST} values for eight *A. aff. divaricata* populations and eleven *A. pruinosa* populations

Populations	AADAmak	AADGAs	AADKOS	AADKouch	AADTUS	AADTYA	AADWAS	AADZAS
AADAmak		0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**
AADGAs	0.0574		0.0001**	0.0001**	0.0001**	0.0001**	0.0002**	0.0001**
AADKOS	0.1079	0.0773		0.0001**	0.0001**	0.0001**	0.0001**	0.0001**
AADKouch	0.1173	0.1190	0.0931		0.0002**	0.0001**	0.0001**	0.0001**
AADTUS	0.1389	0.1521	0.1197	0.0776		0.0001**	0.0001**	0.0001**
AADTYA	0.1900	0.1735	0.1329	0.1359	0.1385		0.0001**	0.0001**
AADWAS	0.0444	0.0266	0.0903	0.1322	0.1664	0.1897		0.0001**
AADZAS	0.0529	0.0351	0.1076	0.1564	0.1870	0.2118	0.0231	

Lineage	Populations	HK_Bluff	HK_Port	TS	TU	AMA	I	IK	W	N	G	C
HK	HK_Bluff		0.4875	0.0001**	0.0001**	0.0001**	0.0001**	0.0003*	0.0001**	0.0001**	0.0003*	0.0001
HK	HK_Port	0.0000		0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001
W	TS	0.2232	0.2523		0.0002*	0.0002*	0.0001**	0.0009*	0.0001**	0.0001**	0.0001**	0.0001
W	TU	0.2950	0.3140	0.1616		0.0001**	0.0001**	0.0021	0.0001**	0.0001**	0.0001**	0.0001
W	AMA	0.2113	0.2302	0.0953	0.1776		0.0001**	0.0052	0.0001**	0.0001**	0.0001**	0.0001
W	I	0.2829	0.2917	0.1717	0.2223	0.1353		0.0006*	0.0001**	0.0001**	0.0001**	0.0001
W	IK	0.2478	0.2773	0.1377	0.1707	0.0654	0.1357		0.0001**	0.0001**	0.0002*	0.0001
E	W	0.3518	0.3382	0.3495	0.4499	0.2957	0.3785	0.3563		0.0001**	0.0001**	0.0001
E	N	0.3334	0.3096	0.2817	0.3762	0.2663	0.3206	0.3354	0.4535		0.0001**	0.0001
E	G	0.1983	0.2255	0.1547	0.2269	0.1147	0.2266	0.1429	0.3115	0.2546		0.0001
E	C	0.2978	0.3072	0.2999	0.4165	0.2700	0.3712	0.3402	0.4780	0.4091	0.2852	

Values below the diagonal indicate pairwise F_{ST} values, and values above the diagonal indicate Bonferroni-corrected P -values for each pairwise population comparison. Statistical significance was assessed at $\alpha = 0.05$. Asterisks indicate significance after Bonferroni correction: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

The negative F_{ST} value (-0.016) between HK_Bluff and HK_Port was converted to zero.

Discussion

This study addressed species and lineage boundaries in peripheral coral populations using *Acropora pruinosa*, a temperate coral species with a disjunct distribution, as a focal system. Genome-wide SNP analyses of Japanese *A. pruinosa*, Hong Kong *A. pruinosa*, and Japanese *A. aff. divaricata* revealed genetic boundaries between closely related species and among geographically separated lineages. Together with evidence from gross morphological observations and putative clonality, these findings indicate that *A. pruinosa* requires conservation attention and provide information relevant to the management of marginal coral populations.

Relationship between Acropora pruinosa and Acropora aff. divaricata

This study revealed the genetic relationship between the highly threatened temperate coral species, *A. pruinosa* and the closely related *A. aff. divaricata*, covering the Japanese and Hong Kong distribution sites of *A. pruinosa* reported by Veron (1992). Using genome-wide SNP data, we separated *A. pruinosa* from *A. aff. divaricata* (Fig. 2, 3a, 4), which were previously reported to be difficult to distinguish and to share mitochondrial and nuclear haplotypes (Suzuki and Fukami 2012).

Although the species tree placed *A. aff. divaricata* outside the sampled *A. pruinosa* lineages, the deeper node separating *A. aff. divaricata* from *A. pruinosa* showed low posterior support and should therefore be interpreted cautiously. Within sampled *A. pruinosa* in this study, several lines of evidence suggest Hong Kong *A. pruinosa* as an early-diverging lineage. The species tree recovered Hong Kong *A. pruinosa* as sister to Japanese *A. pruinosa* (Fig. 2), the clonal fraction estimated from putative clonality of Hong Kong *A. pruinosa* was also similar to that of Japanese *A. pruinosa* (Table 1), and the gross morphology of Hong Kong *A. pruinosa* appeared intermediate between Japanese *A. aff. divaricata* and Japanese *A. pruinosa*. However, this topology does not directly demonstrate that Hong Kong *A. pruinosa* is ancestral to Japanese *A. pruinosa*. It only indicates that Hong Kong *A. pruinosa* may be an early-diverging sampled lineage within *A. pruinosa*, and further study would be required to evaluate its ancestral status.

The two taxa also showed contrasting patterns of population genetic structure. Population differentiation was stronger in sampled *A. pruinosa* than in *A. aff. divaricata*, as indicated by both AMOVA and pairwise F_{ST} analyses (Tables 2 and 3). These contrasting patterns may reflect differences in population connectivity or reproductive processes between the two species.

In addition, as all samples were mapped to an *A. aff. divaricata* reference genome, reference bias may have affected comparisons between the two species (Online Resource 4). Consistent with this possibility, *A. aff. divaricata* showed the highest mean mapping rate and the largest number of covered sites. Therefore, comparative analyses involving absolute SNP recovery or missing-data patterns should be interpreted cautiously.

Gross morphological variations

Based on underwater photographs, Hong Kong *A. pruinosa* populations showed high morphological variation. Although Japanese *A. aff. divaricata* and Japanese *A. pruinosa* are generally distinguishable by colony morphology, most Hong Kong *A. pruinosa* collected in this study showed gross morphology more similar to Japanese *A. aff. divaricata* than to Japanese *A. pruinosa*, with several morphologies intermediate between the typical Japanese forms. Despite this substantial gross morphological variation, no obvious genetic differences among morphotypes were detected using genome-wide SNP markers. However, it remains unclear why Hong Kong *A. pruinosa* retains these intermediate morphologies.

During the sampling conducted for this study, we also became aware that *A. pruinosa* had been confused with *Acropora tumida* (Nishihara and Veron 1995) in Hong Kong, possibly because of the morphological and colony-form variation of Hong Kong *A. pruinosa*. This suggests that differences in coral identification methods and criteria may cause confusion among regions. Further detailed examination is needed to confirm the morphological characteristics of Hong Kong *A. pruinosa*.

We also noted that the species that is regarded as *A. aff. divaricata* in Hong Kong, may correspond more closely to *Acropora cf. glauca* under Japanese criteria of species identification. *A. cf. glauca* is previously treated as *A. cf. solitaryensis* (formerly referred to as 'A. solitaryensis' with plate-like colony form) that has been shown to be genetically and morphologically distinct from Japanese *A. aff. divaricata* with other colony forms (Suzuki and Fukami 2012; Chow et al. 2025) and later named as *Acropora cf. glauca* in Sugihara et al. (2015) (Fukami et al. 2021). Therefore, the presence of true *A. aff. divaricata* in Hong Kong remains uncertain and requires further taxonomic confirmation.

Putative clonality

While SNP datasets with missing data cannot confirm clone mates with absolute certainty, high putative clonality was observed in both Japanese and Hong Kong *A. pruinosa*, while low putative clonality was observed in *A. aff. divaricata*. Lower coral density in marginal habitats may contribute to lower success rate of sexual reproduction and thus asexual propagation may play an important role for sustaining such populations. In addition, suboptimal conditions for coral growth in temperate areas may increase the relative dependence on asexual propagation in *A. pruinosa*, possibly contributing to local population persistence.

In clonal plants, more frequent asexual reproduction is observed in peripheral habitats, mostly because of reduced sexual reproduction (Silvertown 2008). Likewise, at least in the Northern Hemisphere, asexual reproduction tends to occur more frequently in marginal, resource-poor environments (Peck et al. 1998). Another possible factor that may contribute to a greater contribution of asexual propagation is that coral species with arborescent growth forms such as *A. pruinosa* are more structurally fragile than species with tabular growth forms, potentially facilitating fragmentation and asexual propagation (Highsmith 1982).

Evolutionary implications of peripheral population structure

Based on all findings from SNP markers in this study, including species tree, genetic structure, and distribution, peripheral populations may have played an important role in the divergence of *A. pruinosa*. The inferred species tree placed the Hong Kong *A. pruinosa* lineage as an early-diverging sampled lineage within *A. pruinosa*, while STRUCTURE and DAPC suggested genetic differentiation between Hong Kong and Japanese *A. pruinosa*. Furthermore, the intermediate gross morphology and high estimated putative clonality of Hong Kong *A. pruinosa* suggest that peripheral areas of distribution with marginal environments may have contributed to lineage differentiation and to traits that may have facilitated persistence under marginal environments.

Here, we propose a hypothesis of a possible evolutionary scenario in which *A. pruinosa* may have diverged from an *A. aff. divaricata*-like ancestor through persistence in marginal cold-water habitats in Hong Kong waters. Under this hypothesis, Hong Kong *A. pruinosa* may represent an early-diverging sampled lineage that persisted under cold-water conditions (Higuchi et al. 2015) and may be more dependent on asexual reproduction. *A. pruinosa* then expanded toward temperate Japan during past climatic fluctuations. Other habitats or local populations along this migration route may have been lost in intervening warmer regions, contributing to the present disjunct distribution of *A. pruinosa* between Hong Kong and temperate Japan. This scenario is also compatible with the refugia hypothesis proposed by Pipithkul et al. (2021), in which past climatic changes may have contributed to the divergence of eastern and western Japanese *A. pruinosa* lineages in temperate Japan.

The reason why Hong Kong *A. pruinosa* retains intermediate colony morphologies remains unclear. These morphologies may represent retained variation, local morphological plasticity, or response to selective pressures that differ from those experienced by Japanese *A. pruinosa*. Because the existence of true *A. aff. divaricata* in Hong Kong remains uncertain, these *A. aff. divaricata*-like forms should be interpreted cautiously.

Furthermore, this hypothesis remains hypothetical, and this study does not directly estimate migration routes, divergence times, or the genomic basis of adaptation to cold-water environments. A broader sampling of *A. aff. divaricata* and *A. pruinosa*, together with explicit demographic modelling and genomic tests of evolutionary adaptation, will be needed to evaluate the hypothesized evolutionary scenario.

Conservation implications for threatened species, *Acropora pruinosa*

The genetic differentiation between Hong Kong and Japanese *A. pruinosa*, and between the two genetic lineages within temperate Japan, is consistent with the threatened status and disjunct distribution of *A. pruinosa*. Although FST estimates may have been influenced by small sample sizes, FST values between the two Hong Kong populations were relatively lower than those observed between Hong Kong and Japanese population pairs. The present study also suggests that FST values for *A. pruinosa* are relatively higher than those reported for other acroporid corals, including the closely related *A. aff. divaricata*, *A. digitifera*, and *A. hyacinthus* (Nakajima et al. 2010; Nakabayashi et al. 2019). The combination of higher estimated population differentiation and observed putative clonality suggests that geographically disjunct distributions may contribute to greater genetic differentiation among populations. Therefore, the present results indicate that Hong Kong *A. pruinosa* and Japanese *A. pruinosa* should be considered separate management units for conservation planning.

Conclusion

This study revealed the genetic relationship between *A. pruinosa* and *A. aff. divaricata* in Japan and Hong Kong using genome-wide SNP data. Despite their close relationship and the intermediate gross morphology observed in marginal Hong Kong populations, *A. aff. divaricata* and *A. pruinosa* retained genomic differentiation as distinct taxa. Based on these findings, we propose a hypothesis that *A. pruinosa* may have diverged from an *A. aff. divaricata*-like ancestor

in peripheral areas of species distribution, where marginal environmental conditions may have contributed to lineage divergence. Further studies are needed to test this hypothesis. This study also provided important implications for conservation, as Hong Kong, eastern Japanese, and western Japanese populations of *A. pruinosa* appear to represent distinct genetic lineages. Overall, these findings provide insight into the evolutionary potential of coral species in peripheral areas of coral distribution, while also providing information relevant to conservation management under future climate change.

Declarations

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Competing interests

The authors declare no competing interests.

Ethics approval

No human participants were involved. Coral samples were collected in accordance with local procedures and permissions, including Shizuoka Prefecture Permit No. Toku 21-21 where a special collection permit was required. Collections in other localities were conducted with local consent or institutional cooperation as described in the Materials and Methods.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

MIG-seq sequence data generated in this study have been deposited in the DNA Data Bank of Japan under accession number PRJDB2202. Supplementary datasets and analysis summaries are provided as Online Resources 1-12.

Code availability

Custom scripts and analysis files are available from the corresponding author upon reasonable request.

Author contributions

N.Y. conceived and designed the study. S.P. performed laboratory work, analyzed the data, and drafted the manuscript. H.Y., T.K., S.E.M., A.S., H.F., and N.Y. contributed to sampling, materials, analysis, interpretation, and manuscript revision. All authors read and approved the final manuscript.

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Figures

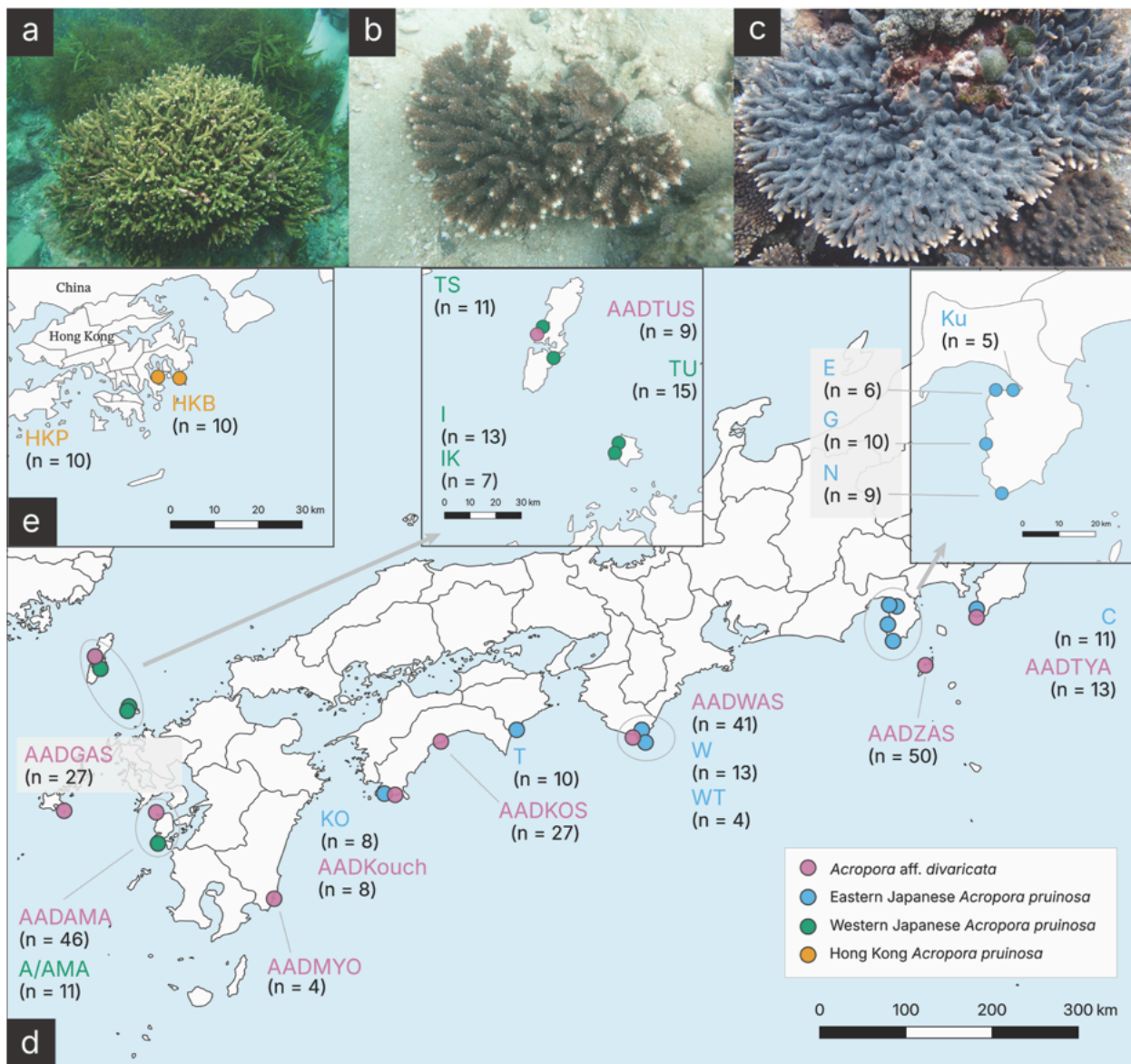


Figure 1

Representative colony photographs and sampling localities used in this study (d and e). Each color indicates predefined management units: *Acropora aff. divaricata* (Photo c), Eastern and Western Japanese *Acropora pruinosa* (Photo a), and Hong Kong *A. pruinosa* (Photo b). Values indicate the number of sampled colonies (n) and inferred genets in each locality (g). Symbols and labels were slightly displaced where necessary for clarity

Species tree inferred from 530 SNPs

AAD - *A. aff. divaricata*

APE - Eastern Japanese *A. pruinosa*

APW - Western Japanese *A. pruinosa*

HK - Hong Kong *A. pruinosa*

outgroup - *A. cf. glauca*

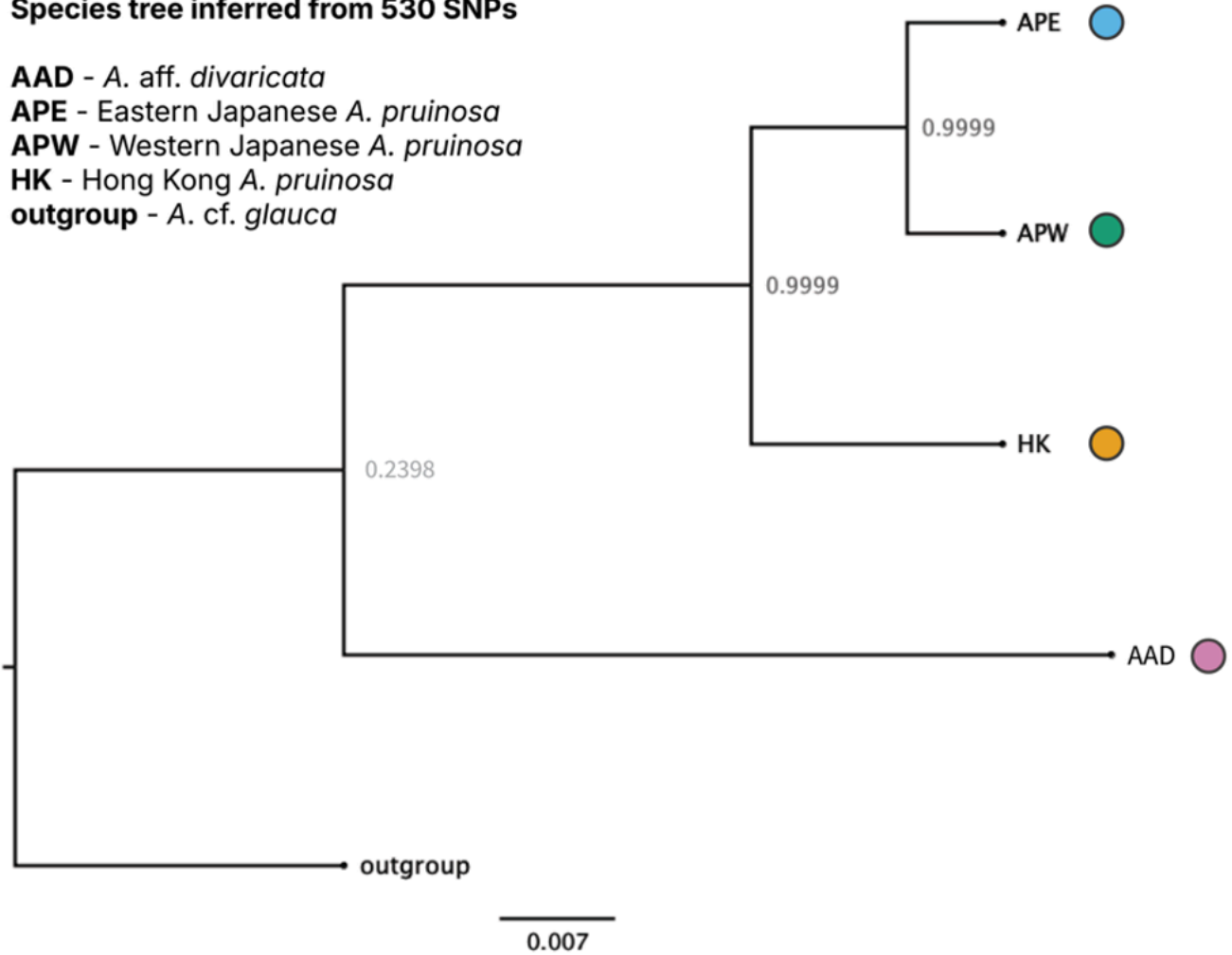
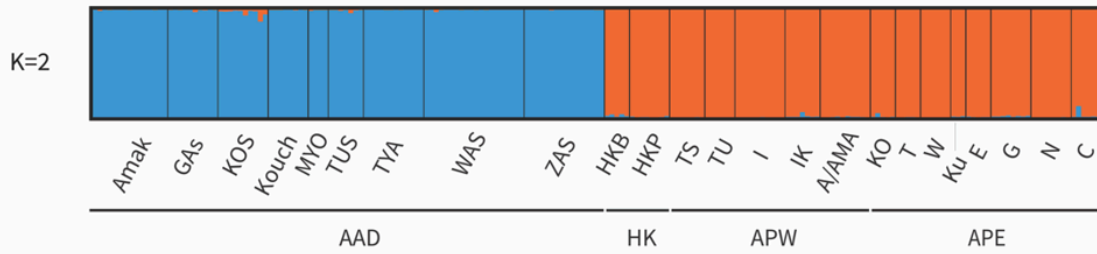


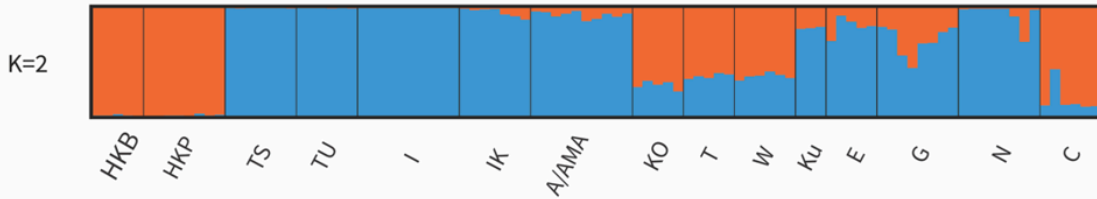
Figure 2

SNP-based species tree inferred from 530 SNPs using SNAPPER. The tree includes four predefined focal lineages: *A. aff. divaricata*, Hong Kong *A. pruinosa*, Japanese eastern and western *A. pruinosa*. One *A. cf. glauca* was used as an outgroup to root the tree. Numbers beside nodes indicate posterior probabilities. Colors correspond to management units shown in Fig. 1

(3a)



(3b)



(3c)

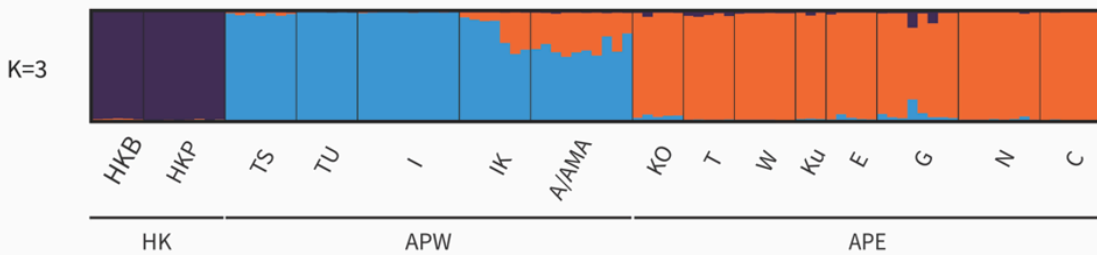


Figure 3

Bayesian clustering analysis of *Acropora* populations inferred using STRUCTURE. (a) STRUCTURE bar plot for the combined dataset of *A. aff. divaricata* (AAD) and *A. pruinosa* (AP) at K = 2. (b–c) STRUCTURE bar plots for the *A. pruinosa*-only dataset at K = 2 and K = 3, respectively. Individuals are grouped by sampling locality, with black vertical lines separating populations.

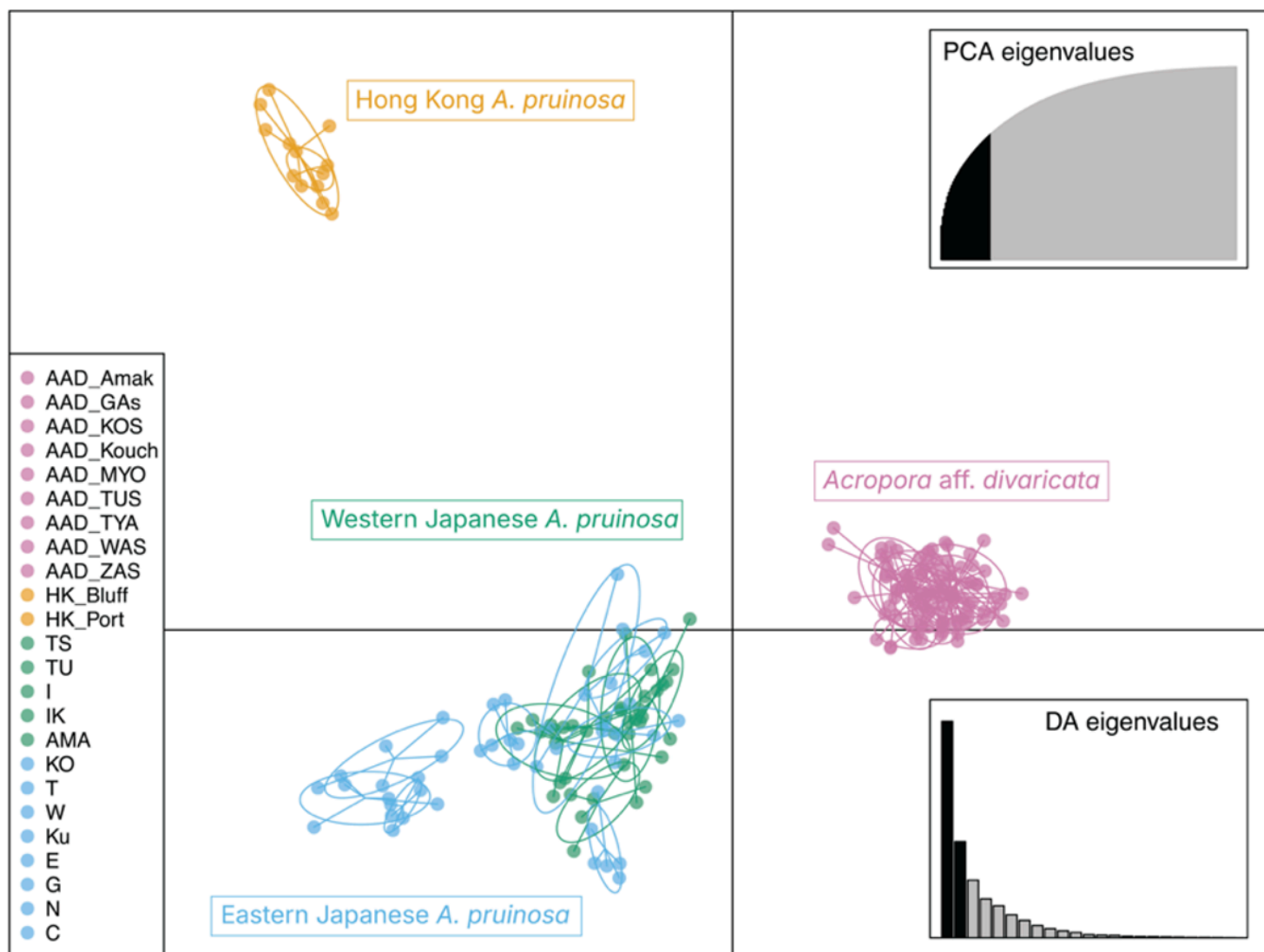


Figure 4

Discriminant analysis of principal components (DAPC) based on 535 SNPs. DAPC was performed using predefined population groups and visualizes genetic differentiation among them. Colors indicate management units shown in Fig. 1. Insets show eigenvalues for the retained PCA and DA axes

Supplementary Files

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