Genetic connectivity of octocoral populations in South and Western Hong Kong water: implications to conservation management of deep-water community
(MEEF2022008)

Completion Report

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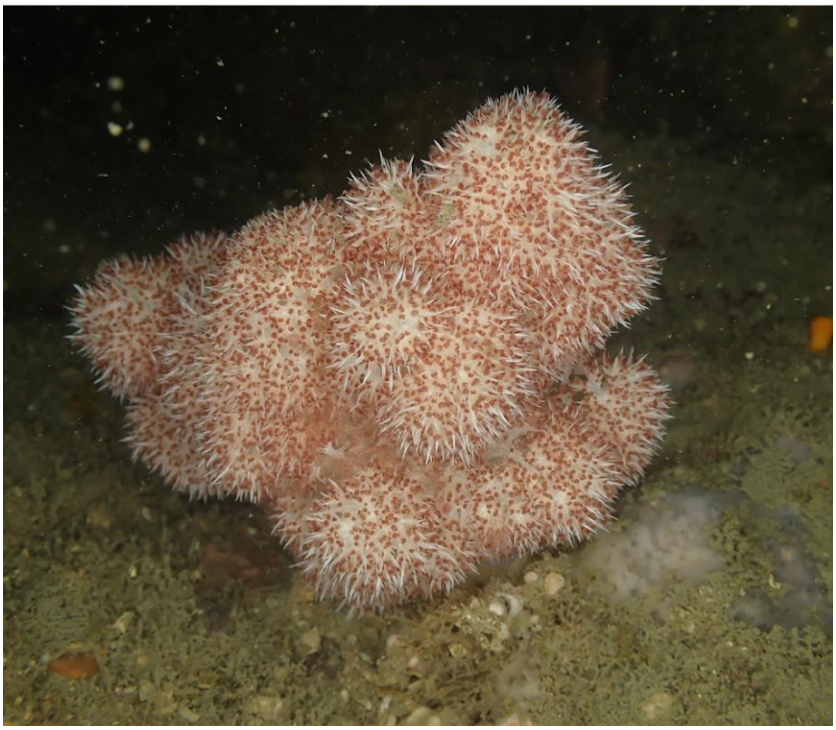

Genetic connectivity of octocoral populations in South and Western Hong Kong water: implications to conservation management of deep-water community (MEEF2022008)

Executive Summary of the project

The objective of this study was to investigate the population genetic structure of octocoral communities in Hong Kong, with a focus on two targeted genera: the alcyoniid soft coral *Dendronephthya* and the gorgonian coral *Echinomuricea*. Samples of *Dendronephthya* and *Echinomuricea* were collected along an East to West gradient and divided into three geographic regions: East, South, and West. A total of 52 samples of *Dendronephthya* and 47 samples of *Echinomuricea spinifera* were analyzed for this study. Among the 52 *Dendronephthya* samples, 27 were identified as *Dendronephthya gigantea*, while 25 were identified as *Dendronephthya spinifera*. Spatial partitioning of distribution was observed between *Dendronephthya* and *Echinomuricea* individuals in the western waters. *Dendronephthya* was found in shallower water and favour boulder substratum, whereas *Echinomuricea* could settle on more silty substratum. For the analysis of population genetic structure, a whole-genome resequencing approach was employed to generate a genome-wide dataset of single nucleotide polymorphisms (SNPs) by mapping sequencing reads from individual samples to their respective reference genome. A significant number of loci were identified for subsequent population genetic analyses. Maximum likelihood phylogenomic estimation, principal component analysis on variance-standardized relationship matrix, admixture analysis, and F_{ST} statistics were used to assess and compare the population genetic structure among regions in Hong Kong. The results revealed that *Dendronephthya gigantea*, *Dendronephthya spinifera* and *Echinomuricea spinifera* studied here, each species in Hong Kong represent a panmictic population with high level of connectivity and genetic exchange across locations. This genetic connectivity suggests that these octocoral species possess the potential to recolonize and recover from disturbances or localized population declines. However, pairwise comparison of F_{ST} values indicated a moderate degree of genetic differentiation for *Dendronephthya spinifera* in Steep Island and *Echinomuricea spinifera* in Waglan Island compared to other sites. These findings suggested the potential presence of subpopulations for octocorals in Hong Kong at Steep Island and Waglan Island. Consequently, careful consideration is necessary for future conservation or development projects in the vicinity of these two islands.

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I. Introduction

I.1 Octocorals in Hong Kong

Hong Kong is located on the eastern side of the Pearl River Delta, and its western waters are heavily influenced by freshwater and sediment discharge from the surrounding area. Coupled with the oceanic currents, the relatively turbid environment makes it a less favourable habitat for scleractinian corals that rely on photosynthetic zooxanthellae for food. Consequently, azooxanthellate octocoral communities, which do not depend on photosynthetic symbionts, dominate the habitat in deeper waters of western Hong Kong, particularly in natural rocky or sandy areas.While sporadic studies have been conducted on octocoral communities in Hong Kong over the past 30 years, these investigations have been limited in scope and primarily focused on the eastern and southern waters. Nonetheless, these studies have recorded a total of 67 species of octocorals (Fabricius & McCorry, 2006; Lam & Morton, 2008; Ang et al., 2010, unpublished AFCD report).

The octocoral communities in Hong Kong waters play a crucial role in the mesophotic zone by providing shelter and acting as a food resource for a diverse range of marine organisms (Lau et al., 2019). Various marine organisms utilize octocorals as shelters, such as feather stars and sea cucumbers, which attach themselves to the octocorals to take advantage of the enhanced exposure to water currents (Buhl-Mortensen & Mortensen, 2005). Octocorals also serve as symbiotic hosts for numerous other invertebrates, including gorgonian shrimps and brittle stars that inhabit gorgonian sea fans, spindle cowries and marine gastropods that attach themselves to soft corals, and porcelain crabs that reside between the vanes of sea pens (Morton, 1988). This indicates that octocoral communities play a vital role in supporting marine invertebrates, particularly in the deeper waters of Hong Kong. Consequently, it is crucial to enhance our understanding of the biology and ecology of these organisms in the western and southern waters of Hong Kong to effectively conserve these deep-water

I.2 Importance of population genetics study

Understanding the larval dispersal patterns and population genetic connectivity of octocoral species in Hong Kong is of utmost importance for future conservation and management efforts. Genetic diversity plays a crucial role in the resilience and long-term survival of species, particularly in degrading environments. Conservation biologists and the scientific community are increasingly recognizing the significance of preserving genetic diversity. Information on the distribution and level of genetic diversity serves as essential criteria for designating marine protected areas and selecting appropriate individuals for breeding and restoration programs.

Connectivity and larval dispersal among octocoral populations facilitate the exchange of individuals, thereby maintaining genetic diversity within a population. Larval dispersal is particularly important for the natural recovery of previously depleted populations by replenishing them with new recruits. Understanding population genetic structure can also reveal hierarchical levels of connectivity, aiding in the understanding of source-sink dynamics among populations. This knowledge is crucial for designating Marine Protected Areas (MPAs) that protect larval sources. Additionally, there have been proposed and implemented translocations of octocoral

populations during the development near Lantau and the construction of the 3rd runway. Assessing genetic similarity is vital in determining appropriate translocation sites to avoid maladaptation and genetic contamination. Therefore, assessing the genetic structure among populations is fundamental in targeting the preservation of genetic diversity and the integrity of populations.

However, population genetic studies of ecologically important species, such as scleractinian corals and octocorals along the South China coast, are scarce and limited in the Hong Kong region (Ng & Morton, 2003). With advancements in technology, next-generation sequencing (NGS) techniques provide high-throughput genetic data, enabling the assessment of genome-wide SNPs for population genomics studies. For instance, a study on *Acropora* coral populations in Okinawa successfully determined their natural recovery after a mass bleaching event. Scientists were interested in identifying the sources of larval supply supporting population recovery, which could provide valuable information on the factors contributing to coral reef resilience and recovery. Shinzato et al. (2015) adopted Whole Genome Resequencing (WGR) techniques to assess the population genomics of *Acropora* coral in the Ryukyu Archipelago. The results indicated that the recent recovery of Okinawa corals was driven by self-recruitment from southern Okinawa. Therefore, WGR could provide a comprehensive understanding of the genetic structure of Hong Kong's scleractinian coral communities and their level of connectivity with neighboring populations.

I.3 Previous study on corals in Hong Kong

The recent genetic analyses conducted on population connectivity of several scleractinian coral species in Hong Kong, supported by the Environmental Conservation Fund (ECF), have revealed surprising findings. Despite the close geographic proximity between Hong Kong and Shenzhen, very limited gene flow was observed in all three studied coral species. The high genetic differentiation and distinct clusters observed in individual assignment tests indicate a low level of migration between the two sites. This finding may partially explain the low recruitment rate in Hong Kong, as there is a lack of larval supply from neighbouring coral communities in Shenzhen. These findings highlight the importance of considering population genetic structure between Hong Kong and neighboring regions, as well as within Hong Kong, in future conservation strategies.

To preserve the genetic diversity within the metapopulation, it is recommended to implement conservation measures for each community. Additionally, it is crucial to avoid the introduction of coral fragments across populations to maintain genetic purity within local coral populations. By taking these factors into account, conservation efforts can effectively preserve the genetic diversity of coral populations in Hong Kong and ensure their long-term viability.

I.4 Expected outcomes and deliverables

The octocoral community in Hong Kong can be found in the western, southern, and eastern waters due to their higher tolerance to a wider range of conditions, including lower salinity and turbid water in the western region. However, it is anticipated that there may be population genetic differentiation among the octocoral populations in these different regions. The variations in hydrology, water chemistry, and habitat conditions among the three regions may contribute to such differentiation, similar to what has been observed in scleractinian corals. If proven to be true, it will have major conservation implication as we will have to manage different populations individually to preserve the genetic diversity and larval supply source populations to achieve a sustainable long-term conservation of the octocoral communities in Hong Kong.

Unfortunately, there is a lack of knowledge regarding the distribution of genetic diversity and gene flow patterns within Hong Kong and elsewhere in the Asia Pacific region for octocoral species. To address these knowledge gaps, the current study aims to employ state-of-the-art genomic techniques to investigate the genetic diversity distribution and population connectivity among Hong Kong's octocoral communities using two common octocoral genera, *Dendronephthya* and *Echinomuricea*, as model systems. This information will be valuable in identifying the dynamics of sinks and sources between the Hong Kong octocoral populations, providing insights for future conservation and management efforts. It can inform decisions regarding the designation of marine parks, special areas, and potential translocation of octocoral individuals or populations.

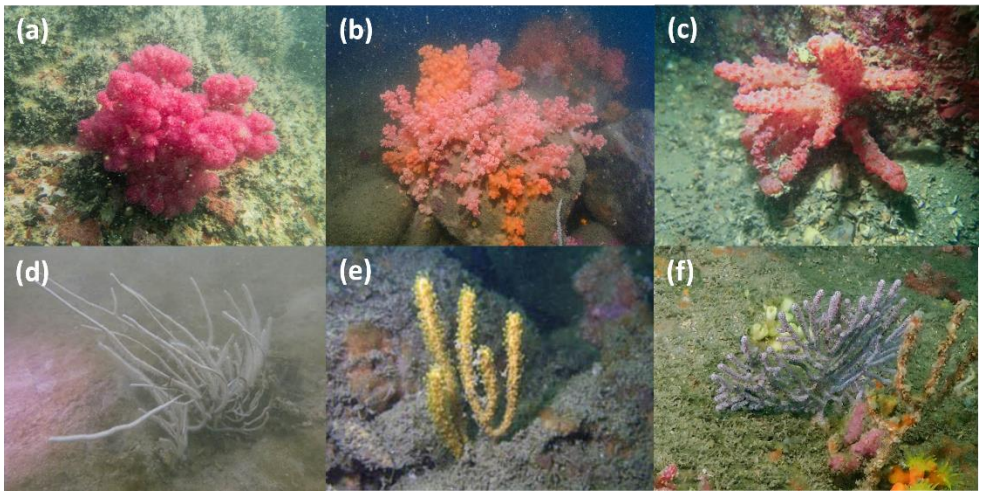


Figure 1: Some of the common octocorals recorded in Hong Kong waters. (a) *Dendronephthya* sp., (b) *Scleronephthya* sp., (c) *Nephthyigorgia* sp., (d) *Echinomuricea* sp., (e) *Euplexaura* sp., (f) *Menella* sp.

II. Methodology

II.1 Sample Collection

The study focuses on three species from two genera of octocoral within the Order Malacalcyonacea: *Dendronephthya gigantea* (Verrill, 1864) and *Dendronephthya spinifera* (Holm, 1895) (Nephtheidae) representing alcyoniid soft corals and *Echinomuricea spinifera* Nutting, 1910 (Paramuriceidae) representing gorgonian corals. To collect the octocoral samples, 27 sites were chosen along a west-to-east gradient in Hong Kong waters. These sites were grouped into West (site 1-14), South (site 15-22), and East (site 23-27) regions (Figure 2). The West and South groups were separated by the East Lamma Channel, while the South and East groups were separated by the Tathong Channel.

The collection of octocoral specimens was conducted through scuba diving. Approximately 4 cm sections of branch from the octocoral colonies were sampled. Prior to collection, live photos of the specimens were taken using an Olympus TG-6 underwater camera, and the depth of collection was recorded. The collected coral fragments were preserved in 95% ethanol and transported to the laboratory for genomic DNA extraction.

To validate the species identity of the collected samples, macromorphology and sclerite structures were examined. A small section of tissue was dissolved using 5% bleach, and the resulting solution was used to examine the sclerite structures under a compound microscope. The description of each genus and species followed the works of Kükenthal (1905), Thomson & Mackinnon (1910), and Fabricius & Alderslade (2001). This approach provided a systematic approach to sample collection and species identification, ensuring the accuracy and reliability of the subsequent genetic analyses on the selected octocoral genera and species.

II.2 DNA Extraction and genome re-sequencing

After preserving the collected samples, tissue sections were carefully picked using sterilized forceps. Genomic DNA extraction was performed using the Qiagen DNeasy Blood & Tissue Kit (Germany), following the protocol provided by the manufacturer. The extraction process included RNase treatment to minimize the presence of RNA in the subsequent library preparation for sequencing. To assess the quality of the extracted genomic DNA, agarose gel electrophoresis was conducted to examine the integrity of the DNA. Additionally, the Thermo Fisher Scientific Nanodrop Microvolume Spectrophotometer (USA) was used to estimate the concentration of DNA. Qualified genomic DNA samples from the octocoral specimens were then sent to commercial biotechnology company, Novogene (China) for sequencing. The genomic DNA was subjected to de novo library preparation, with a 350 bp insert size, for each individual genome. Sequencing was conducted using the Illumina Novaseq 6000 platform, generating 150 bp paired-end reads.

II.3 SNP identification and detection

Adaptor sequence from raw reads were trimmed by Trimmomatics 0.32 (Bolger & Lohse 2014). The cleaned reads from each individual were mapped to the most closely related reference genome using BWA 0.5.9 (Li & Durbin 2009). Specifically, reads from *Dendronephthya* samples were

mapped to the *Dendronephthya gigantea* genome, while reads from *Echinomuricea* samples were mapped to the *Paramuricea clavata* genome.

To process the mapped reads, SAMtools 0.1.18 (Li et al. 2009) was used to remove unmapped reads and duplicated reads. Local realignments were then improved using the MarkDuplicates function of the Genome Analysis Toolkit 4.1.8.1 (GATK4) (McKenna et al. 2010). The realigned BAM files were further processed by GATK4 with default settings for initial variant site identification. Consensus calls were filtered to include only no-missing-call single nucleotide polymorphisms (SNPs) and used as known sites for base quality recalibration with GATK4 using default settings. The resulting recalibrated BAM files were used as input for variant calling with GATK4.

The SNP dataset for further analysis included only independent and neutral (non-adaptive) SNPs. The resulting variant matrices were filtered using VCFtools 0.1.16 (Danecek et al. 2011) based on specific criteria. These criteria included sites with a mean depth greater than 10 (--min-meanDP 10), no missing data (--max-missing 0), and a minimum distance of 300 between each site (--thin 300). To create pruned datasets, sites exhibiting linkage disequilibrium (LD) were pruned. Pairwise linkage disequilibrium and Hardy-Weinberg equilibrium (HWE) calculations were performed using Plink 1.9 (Purcell et al. 2007). SNPs showing extended LD, defined as a correlated pair of SNPs ($r2 = 0.2$) within a 100 kb window, and extensive deviation ($p < 0.001$) from HWE, were excluded from the dataset to avoid artifacts due to LD. A SNP window size (--K) of 1,000 was adopted for these calculations. This comprehensive series of data processing steps ensures the generation of a high-quality SNP dataset suitable for subsequent population genetic and connectivity analyses of the octocoral species under study.

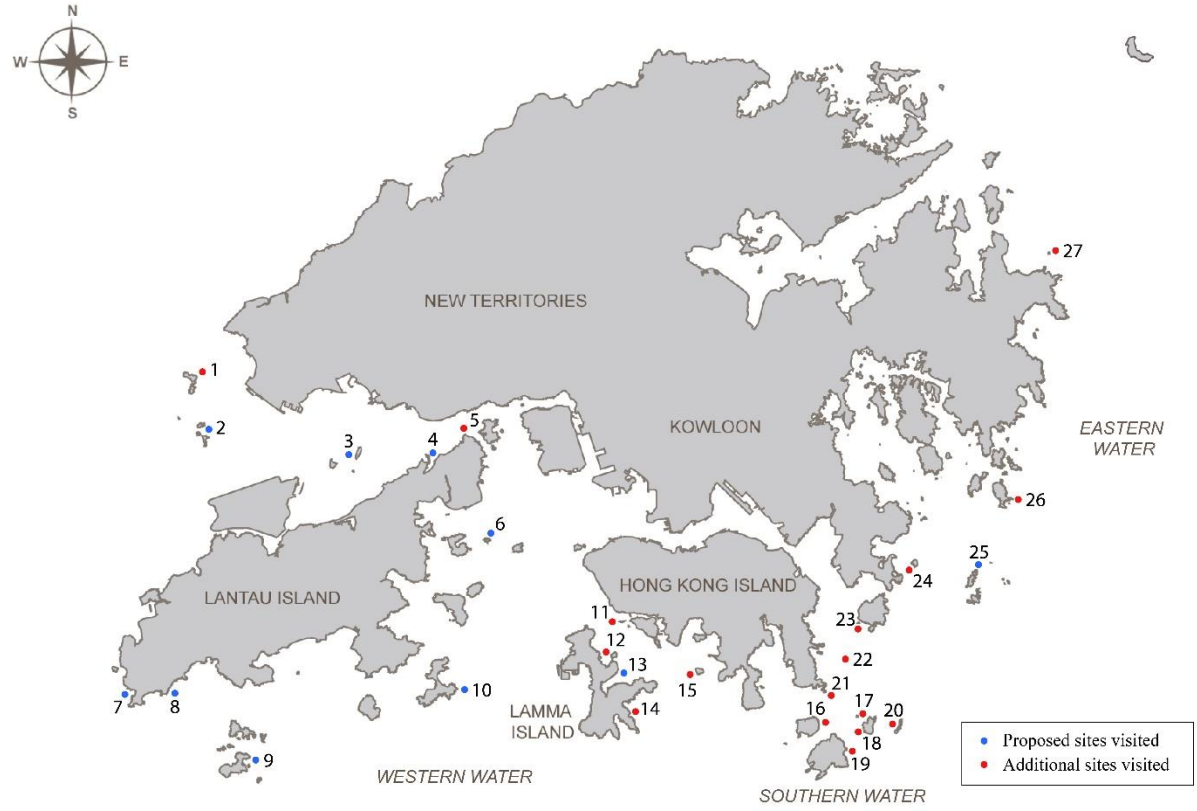
II.4 Population Genetic Structure Analysis

To investigate the population genetic structure of *Dendronephthya* and *Echinomuricea* samples, several analyses were conducted. Firstly, the phylogenetic relationships among the samples were estimated using the maximum-likelihood (ML) method. The genome-wide SNP datasets of *Dendronephthya* and *Echinomuricea* samples were converted from VCF files to the required PHYLIP format using vcf2phylip 2.0 (Ortiz 2019) for phylogenomic analysis. These datasets were then analyzed in RAxML 8.2.10 (Stamatakis 2014) using the GTR + GAMMA substitution model for ML phylogenetic inference. The branch supports of the resulting tree was evaluted by 100 rapid bootstrap replicates, and the final trees were visualized using iTol 6.6 (Letunic & Bork 2021).

Secondly, principal component analysis (PCA) was conducted to summarize the distribution of genotypic variation across the *Dendronephthya* and *Echinomuricea* samples. This analysis was performed using the pruned datasets, and a variance-standardized relationship matrix calculated by Plink 1.9 (Chang et al., 2015) under default settings. The PCA plots were visualized using the R package "tidyverse".

Thirdly, admixture analysis was conducted to quantify the spatial distribution of genomic variation within each sample. Admixture proportions of individuals were estimated using ADMIXTURE v.1.3.0 (Alexander et al., 2009) based on the pruned datasets. This analysis assumes that the genome of each individual is a mixture of genetic loci inherited from *K* sources, where *K* represents

the assumed number of clusters or sources. The study considered *K* values ranging from 1 to 5, and the cross-validation (CV) error was estimated and plotted for each *K* value to determine the best number of clusters with the lowest CV error. Finally, F_{ST} analysis was performed to estimate gene flow between regions and populations. Pairwise F_{ST} values, which represent genetic distances among populations, were calculated for bi-allelic sites using vcftools 0.1.12b (Danecek et al. 2011). These analyses provide insights into the population genetic structure, genetic relationships, spatial distribution of genomic variation, and gene flow patterns within and between the *Dendronephthya* and *Echinomuricea* samples in the study.



No.	Sites	No.	Sites	No.	Sites	No.	Sites	No.	Sites
1	Lung Kwu Chau	7	Southwest Lantau Marine Park (West)	11	Magazine Island	17	Fury Rock	23	Nga Ying Pai
2	Sha Chau	8	Southwest Lantau Marine Park (East)	12	Luk Chau	18	Sung Kong	24	Steep Island
3	East Brother	9	Tai A Chau	13	Picnic Bay	19	Po Toi Island	25	North Ninepin
4	Yam O Wan	10	Cheung Chau	14	Tung O Wan	20	Waglan Island	26	Basalt Island
5	Ma Wan			15	Round Island	21	Cape D'Aguilar	27	Wong Mau Chau
6	Siu Kau Yi Chau			16	Beaufort Island	22	Bokhara Rock		

Figure 2: Map of Hong Kong waters showing 27 collection sites in this study. Sites proposed in the original proposal were marked in blue, and additional sites were marked in red. Sites were categorized into three regions: West (site 1-14), South (site 15-22) and East (site 23-27)

III. Result & Discussion

III.1 Ecological Survey of *Dendronephthya* and *Echinomuricea*

III.1.1 Collection of octocorals

In this study, a total of 52 *Dendronephthya* and 47 *Echinomuricea* samples were collected from 27 different sites. It was observed that the abundance of *Dendronephthya* and *Echinomuricea* was extremely low in western waters, contrary to previous studies on octocoral communities in Hong Kong (Ang et al., 2010, unpublished AFCD report). In fact, no *Dendronephthya* or *Echinomuricea* specimens were observed or collected in most of the proposed sites in western waters. As a result, additional sites in southern and eastern waters were visited to collect samples. The distribution of *Dendronephthya* and *Echinomuricea* was illustrated in Figure 3 and number of collected samples was listed in Table 1 & 2. Two species of *Dendronephthya* were collected: *Dendronephthya gigantea* (Verrill, 1864) and *Dendronephthya spinifera* (Holm, 1895) (Figure 4). Additional specimens of *Dendronephthya spinifera* were also included in the subsequent analysis.

During the sample collection process, it was noted that an octocoral genus *Euplexaura* is closely resembled the targeted species *Echinomuricea spinifera* in field morphology, particularly in newly settled colonies where the branching pattern was not yet developed for identification. Furthermore, *Euplexaura* sp. was widely distributed in western waters (Table 3), leading to confusion in the collection of *Echinomuricea spinifera*. To verify their identity, *ex situ* examinations of the micromorphology of the sclerite structures were conducted. *in situ* field photos, *ex situ* specimen photos, and microscopic photos of sclerites for the morphological comparison of the two genera were illustrated in Figure 5. Sclerites of *Echinomuricea* are characterized by a single, long spine arising from a warty base, forming the thornscales of the calyces (Fabricius & Alderslade 2001). In contrast, the sclerites in the conenchyme of *Euplexaura* are oval or sub-spheroidal in shape, usually densely covered with large complex warts (Fabricius & Alderslade 2001).

In the proposed sites where *Dendronephthya* and *Echinomuricea* were absent, other octocoral species observed in the field were recorded (Table 3). The diversity of octocorals in these sites (species abundance = 7) was significantly lower compared to records in Hong Kong. Western water in Hong Kong is heavily influenced by freshwater discharge from the Pearl River, resulting in hyposalinity and heavy sedimentation, which is unfavorable for octocorals. Only resilient octocoral species could settle in these sites. *Euplexaura* was widely distributed among these six sites, suggesting that it is highly resilient in turbid and hyposaline environments. Among the six sites, Yam O Wan (site 4) had the highest species richness (S = 7). Yam O Wan is located in Northern Lantau, adjacent to Urmston Road Fairway. Field observations indicated that the substrate in Yam O Bay had a lesser degree of siltation compared to the other sites mentioned above. The neighboring site, Ma Wan (site 5), which is also situated alongside Urmston Road Fairway, had relatively less siltation and higher species richness. The strong current from the Urmston Road Fairway could help remove the silt in Yam O Wan, and the less silted substrate could provide a suitable environment for the settlement of a greater variety of octocoral species, contributing to the higher species richness observed in Yam O Wan.

III.1.2 Spatial distribution of octocorals

For the two species of *Dendronephthya* collected in this study, the occurrence and abundance of both *Dendronephthya gigantea* and *Dendronephthya spinifera* among sites in this study were listed in Table 2. The spatial difference between two species was tested by Chi-squared test,

$$\chi 2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

where O is the observed frequency and E is the expected frequency of occurrence of two species individuals in sites. The *p*-values for both Chi-squared testes by regions and by sites are greater than 0.05. There was no significant difference in the spatial distribution for *Dendronephthya gigantea* and *Dendronephthya spinifera* on local and regional scale, suggesting that these two species had shown sympatry in Hong Kong waters.

On the other hand, for two genera in this study *Dendronephthya* and *Echinomuricea*, there was little overlapping of their geographical distribution. Most collections from each site were exclusive to one genus and only collected samples from both genera in Waglan Island (Figure 4). The Chi-squared test also confirmed the disparity in geographical distribution for two genera with a *p*-value < 0.0001 (Table 5), suggesting that two genera favour different environments and occupy different niches in Hong Kong waters.

III.1.3 Bathymetric distribution of octocorals

The depths at which *Dendronephthya* and *Echinomuricea* collected were recorded, and both genera exhibited a wide range of distribution along the depth gradient. Dendronephthya specimens were found at depths ranging from 6.9 - 22.3 m, with a mean depth of 14.1 m. Similarly, *Echinomuricea* specimens were collected at depths ranging from 8.4 - 21.5 m, with a mean depth of 14.2 m. However, the distribution of *Dendronephthya* along the depth varied across regions. In this study, *Dendronephthya* sampled from western waters occupied significantly shallower depths compared to *Dendronephthya* in southern ($p \leq 0.0001$) and eastern waters ($p \leq 0.0001$) (Figure 6). On the other hand, there were no significant differences in depth distribution for *Echinomuricea* across regions.

The water quality in Hong Kong waters undergoes changes along a gradient, ranging from western estuarine conditions influenced by the Pearl River to eastern oceanic conditions facing the South China Sea. The sediment carried by the Pearl River leads to heavy silt deposition in the western region of Hong Kong waters, resulting in vertical compression of *Dendronephthya* distribution in this turbid setting. In sediment dynamics, shallower water is less prone to silt deposition due to a phenomenon known as "tidal sweeping" (Morgan et al., 2020). High-velocity currents generated by diurnal tidal flows resuspend fine particles and remove sediments from shallower water. Tidal sweeping action helps maintain ecological stability of shallow-water corals in Singapore, which also experiences heavy sedimentation (Maxwell, 1968; Cacciapaglia and van Woesik, 2015). The difference in depth distribution of *Dendronephthya* in western waters suggests that *Dendronephthya* is more susceptible to silt deposition compared

to *Echinomuricea*. In the turbid waters of the western region, *Dendronephthya* prefers occupying shallow water that is maintained by tidal sweeping. On the other hand, *Echinomuricea* has shown higher resistance to silt deposition. The depth distribution of *Echinomuricea* did not significantly differ among regions, despite the more turbid conditions in western waters. In this study, *Echinomuricea* was observed in silty substrates where other octocorals were rarely found (Figure 4). *Echinomuricea* has the ability to settle and survive in heavily silted environments in the deep western waters.

Table 1: A table showing the number of *Dendronephthya* and *Echinomuricea* samples collected in each site.

Regions		Sites	Abbr.	*Dendronephthya*	*Echinomuricea*
W	1	Lung Kwu Chau	LKC	0	0
	2	Sha Chau	SC	0	0
	3	East Brother	EB	0	0
	4	Yam O Wan	YO	0	0
	5	Ma Wan	MW	5	0
	6	Siu Kau Yi Chau	SKY	5	0
	7	Southwest Lantau Marine Park (West)	MPW	0	0
	8	Southwest Lantau Marine Park (East)	MPE	0	0
	9	Tai A Chau	TAC	2	0
	10	Cheung Chau	CC	1	0
	11	Magazine Island	MI	0	5
	12	Luk Chau	LC	0	6
	13	Picnic Bay	PB	0	7
	14	Tung O Wan	TO	6	0
S	15	Round Island	RI	0	5
	16	Beaufort Island	BI	0	0
	17	Fury Rock	FR	2	0
	18	Sung Kong	SK	2	0
	19	Po Toi Island	PT	5	0
	20	Waglan Island	WL	7	6
	21	Cape D'Aguilar	CA	2	0
E	22	Bokhara Rock	BR	6	0
	23	Nga Ying Pai	NYP	0	12
	24	Steep Island	STI	4	1
	25	North Ninepin	NP	5	0
	26	Basalt Island	BS	0	0
	27	Wong Mau Chau	WMC	0	3
			Total	52	47

Table 2: A table showing the number of *Dendronephthya gigantea* and *Dendronephthya spinifera* samples collected in each site.

Regions	Sites	Abbr.	*D. gigantea*	*D. spinifera*
W	Ma Wan	MW	2	3
	Siu Kau Yi Chau	SKY	3	2
	Tai A Chau	TAC	2	0
	Cheung Chau	CC	0	1
	Tung O Wan	TO	6	0
S	Fury Rock	FR	1	1
	Sung Kong	SK	0	2
	Po Toi Island	PT	3	2
	Waglan Island	WL	2	5
	Cape D'Aguilar	CA	2	0
E	Bokhara Rock	BR	3	3
	Steep Island	STI	1	3
	North Ninepin	NP	2	3
		Total	27	25

Table 3: A table showing the octocoral species observed in proposed sites without *Dendronephthya* and *Echinomuricea*. SW LMP is the abbreviation for Southwest Lantau Marine Park

Order	Family	Species	LKC	SC	EB	YO	MPW	MPE
Scleralcyonacea	Veretillidae	*Cavernularia* sp.	✓					
	Virgulariidae	*Virgularia* sp.	✓					
	Ellisellidae	*Junceella* sp.					✓	✓
		Dichotella gemmacea				✓		
Malacalcyonacea	Euplexauridae	*Euplexaura* sp.	✓	✓		✓	✓	✓
	Paramuriceidae	*Echinogorgia* sp.				✓		
		Menella sp.				✓		✓
		Species Richness	3	1	0	4	2	3

Table 4: Contingency Table of the abundance of *Dendronephthya gigantea* and *Dendronephthya spinifera* from the sites in this study with their respective Chi-squared test table: (a) Chi-squared test by sites; (b) Chi-squared test by regions

a)

Regions	West					South					East		
Sites	MW	SKY	TAC	CC	TO	FR	SK	PT	WL	CA	BR	STI	NP
Dendronephthya gigantea	2	3	2	0	6	1	0	3	2	2	3	1	2
Dendronephthya spinifera	3	2	0	1	0	1	2	2	5	0	3	3	3

$\chi 2$	16.0
d.f.	12
p -value	0.190

b)

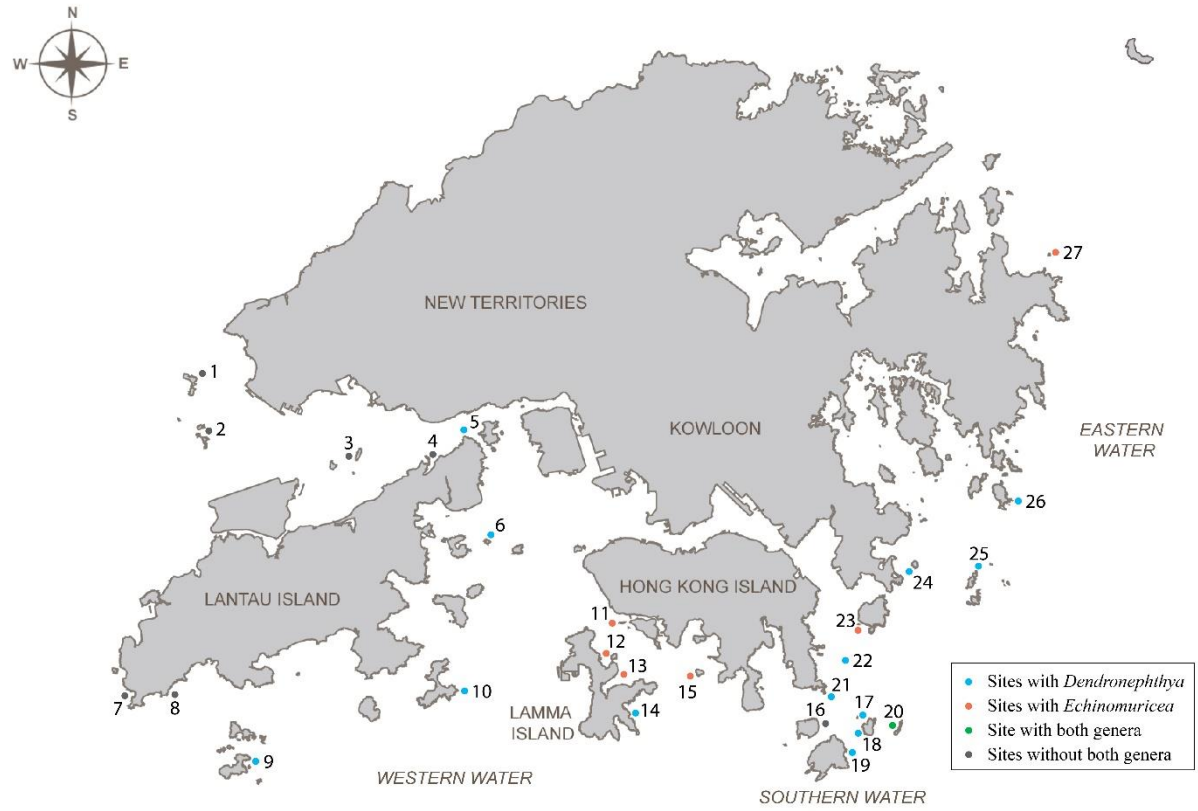
Regions	West	South	East
Dendronephthya gigantea	13	8	6
Dendronephthya spinifera	6	10	9

$\chi 2$	3.33
d.f.	2
p -value	0.19

Table 5: Contingency Table of the abundance of *Dendronephthya* and *Echinomuricea* from the sites in this study with the Chi-squared test table

Regions	West								South						East				
Sites	MW	SKY	TAC	CC	MI	LC	PB	TO	RI	FR	SK	PT	WL	CA	BR	NYP	STI	NP	WMC
Dendronephthya	5	5	2	1	0	0	0	6	0	2	2	5	7	2	6	0	4	5	0
Echinomuricea	0	0	0	0	5	7	7	0	5	0	0	0	6	0	0	12	1	0	3

$\chi 2$	81.8
d.f.	18
p -value	< 0.0001



No.	Sites	No.	Sites	No.	Sites	No.	Sites	No.	Sites
1	Lung Kwu Chau	7	Southwest Lantau Marine Park (West)	11	Magazine Island	17	Fury Rock	23	Nga Ying Pai
2	Sha Chau	8	Southwest Lantau Marine Park (East)	12	Luk Chau	18	Sung Kong	24	Steep Island
3	East Brother	9	Tai A Chau	13	Picnic Bay	19	Po Toi Island	25	North Ninepin
4	Yam O Wan	10	Cheung Chau	14	Tung O Wan	20	Waglan Island	26	Basalt Island
5	Ma Wan			15	Round Island	21	Cape D'Aguilar	27	Wong Mau Chau
6	Siu Kau Yi Chau			16	Beaufort Island	22	Bokhara Rock		

Figure 3: Map of Hong Kong waters showing distribution of *Dendronephthya* and *Echinomuricea*. Sites without both genera were marked in grey, sites with both genera were marked with green, sites with only *Echinomuricea* were marked with orange and sites with only *Dendronephthya* were marked in blue.

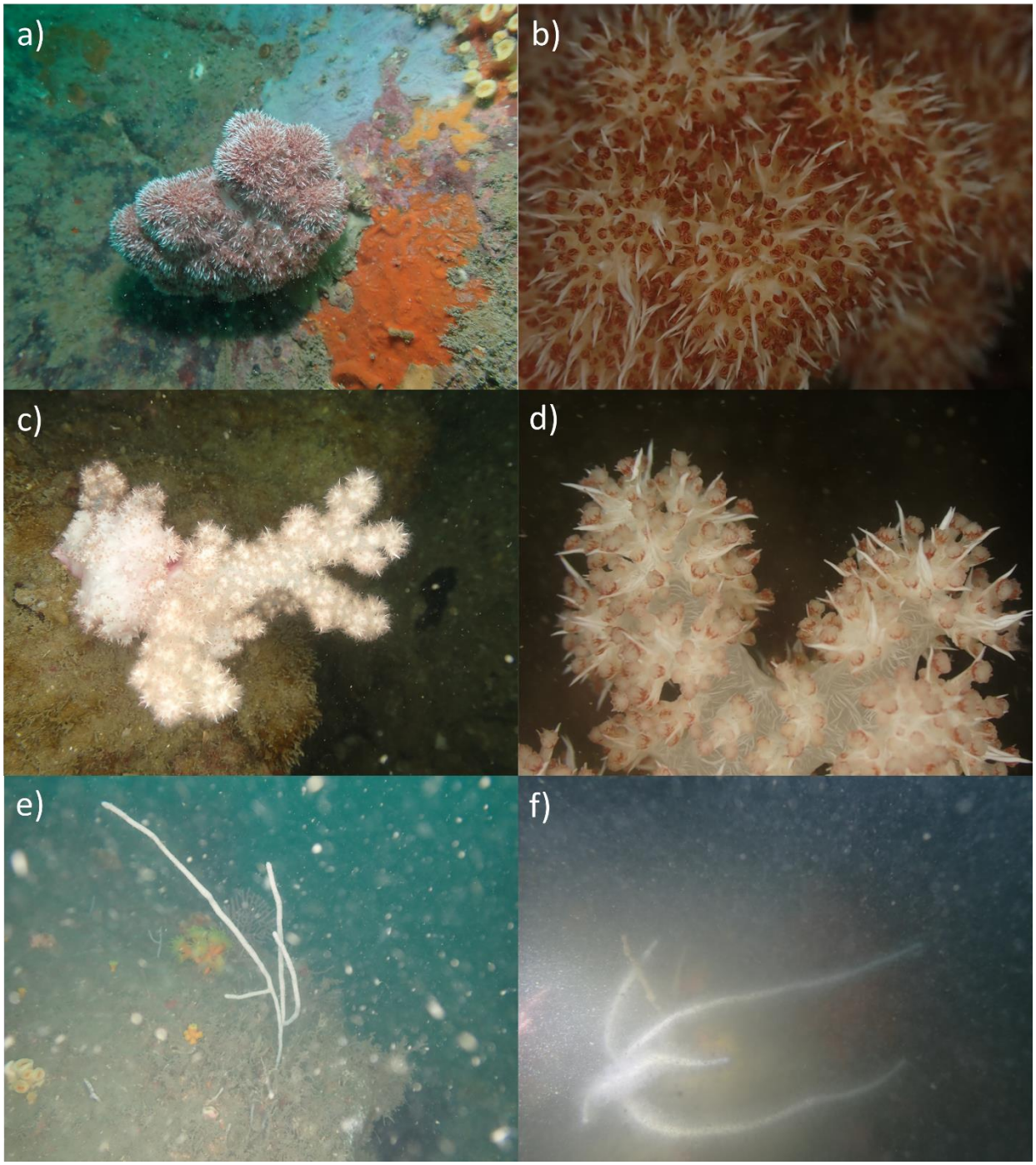


Figure 4: Field photo of three target species in this study: *Dendronephthya gigantaea* (a - b), *Dendronephthya spinifera* (c – d) and *Echinomuricea spinifera* (e -f). Field photo showing whole corallum (a, c) and close up photo showing polyps (b, d) of *Dendronephthya*. Field photos showing *Echinomuricea spinifera* on boulder (e) and silty (f) substrata.

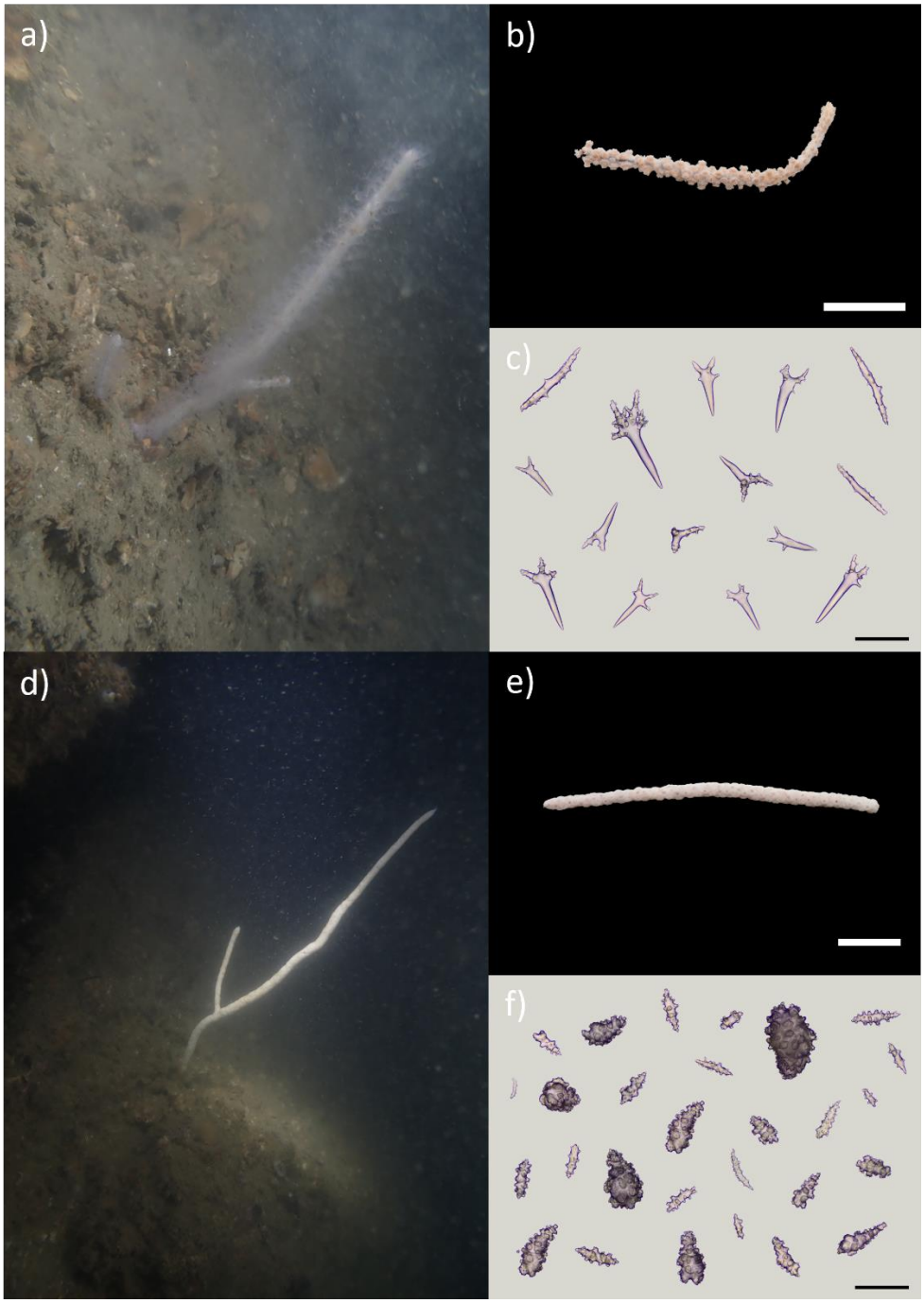


Figure 5: Photos of *Echinomuricea spinifera* (a – c) and *Euplexaura* sp (d – f). Field photo showing whole corallum (a, d), specimen photo of collected samples (b, e) and sclerite morphology under compound microscope (100X) (c, f).

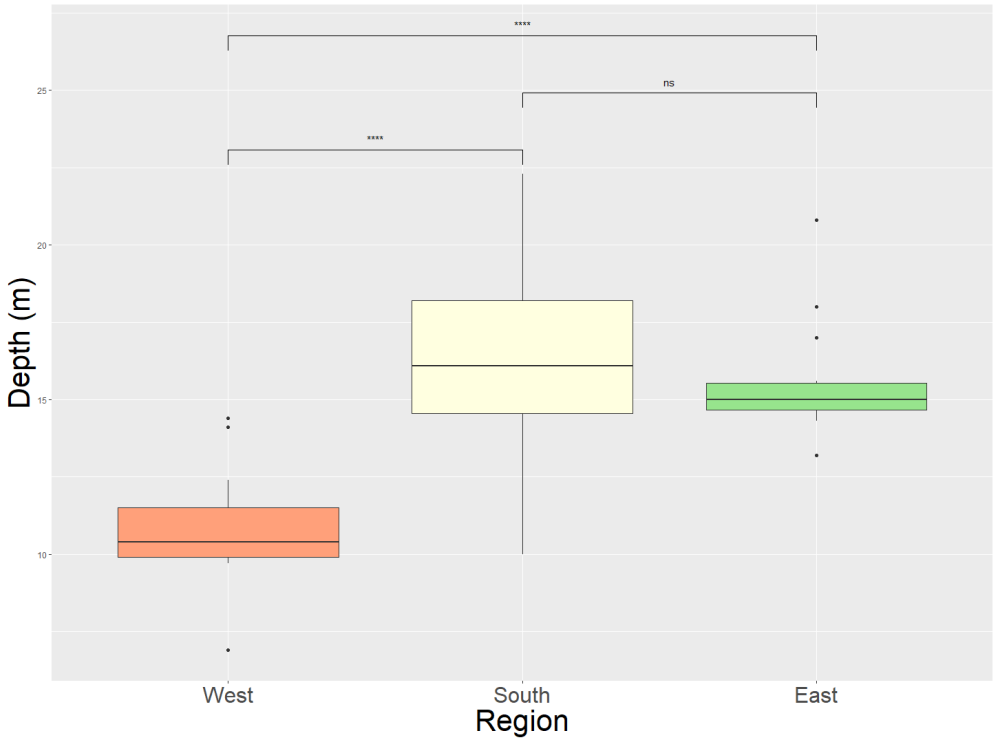


Figure 6a: Boxplot showing sampling depth of *Dendronephthya* samples in West, South, East region in Hong Kong with pairwise *t*-test comparing means among region (Asterisk indicating significance levels: **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; ns $p \geq 0.05$).

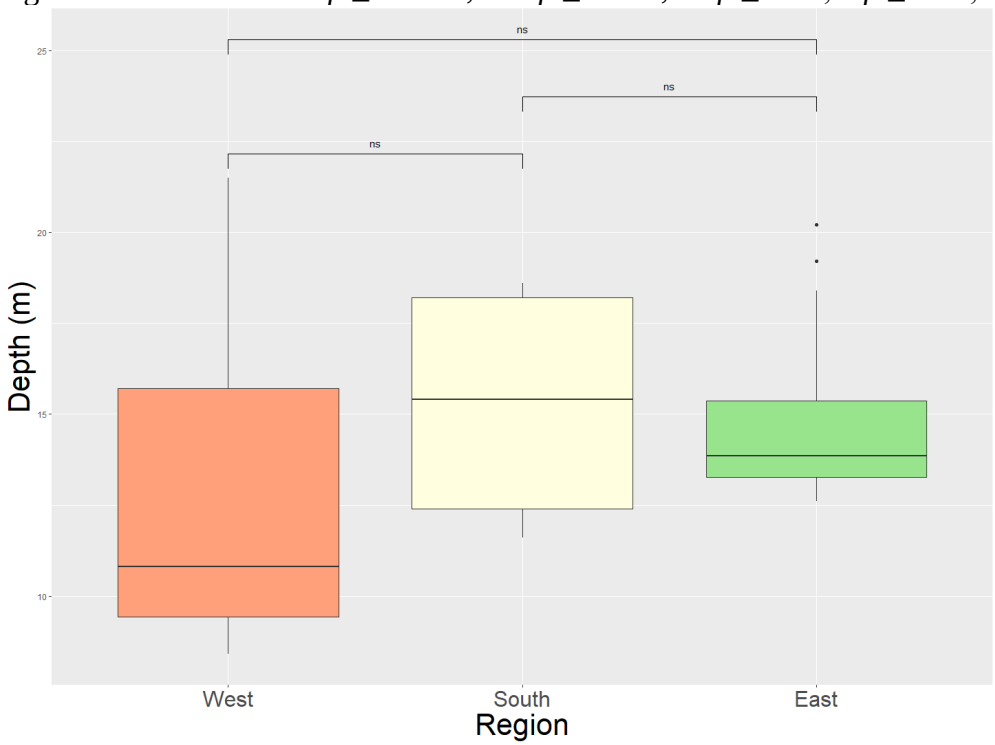


Figure 6b: Boxplot showing sampling depth of *Echinomuricea* samples in West, South, East region in Hong Kong with pairwise *t*-test comparing means among region (Asterisk indicating significance levels: **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; ns $p \geq 0.05$).

III.2 Population Genetic Structure of *Dendronephthya* and *Echinomuricea*

III.2.1 Bioinformatics data statistics

In the analysis of *Dendronephthya*, the raw reads from 52 *Dendronephthya* samples were aligned to the reference genome of *Dendronephthya gigantea*, and the mapping quality for each sample was listed in Table 6. The mapped read depth of the *Dendronephthya* samples ranged from 16.07 to 33.00, with an average of 24.87-fold coverage. A total of 32,732,341 SNPs were initially called using GATK v4.1.8.1 and 539,835 SNPs were retained after filtering with VCFtools. SNPs displaying linkage disequilibrium were further pruned using Plink, resulting in a pruned dataset containing 65,342 SNPs. The variant matrix of *Dendronephthya* samples was then separated into *Dendronephthya gigantea* and *Dendronephthya spinifera* for subsequent population genetic analyses of each species. For the *Dendronephthya gigantea* variant matrix, 594,343 SNPs remained after VCFtools filtering, and after pruning for linkage disequilibrium, 58,637 SNPs were retained. In the case of *Dendronephthya spinifera*, 407,410 SNPs passed the VCFtools filtering, and after linkage disequilibrium pruning, 40,075 SNPs were retained for analysis.

The raw reads from 46 *Echinomuricea spinifera* samples were aligned to the reference genome of *Paramuricea clavata*, and the mapping quality for each sample was listed in Table 7. The mapped read depth of the *Echinomuricea spinifera* samples ranged from 3.88 to 18.96, with an average coverage of 5.84-fold. A total of 3,706,915 SNPs were called using GATK v4.1.8.1, and after filtering with VCFtools, 72,458 SNPs remained. Pruning for linkage disequilibrium using Plink resulted in a pruned dataset containing 30,050 SNPs. Although the *Echinomuricea spinifera* samples in this study had fewer variants passing the SNP filtering process compared to the *Dendronephthya* samples due to their phylogenetic distance from the reference genome (*Paramuricea clavata*), a substantial number of SNPs were still available for downstream analyses.

III.2.2 Delineation of *Dendronephthya* species

In this study, two species of *Dendronephthya*, namely *Dendronephthya gigantea* (Verrill, 1864) and *Dendronephthya spinifera* (Holm, 1895), were sampled. Phylogenomic analyses were conducted to determine the distinctiveness of these two species. A maximum-likelihood (ML) tree was constructed using the genome-wide SNP dataset derived from all 52 *Dendronephthya* samples collected. The ML tree revealed two reciprocally monophyletic clades, indicating the separation of the two species (Figure 7). To further confirm the clustering of the two species, a principal component analysis (PCA) plot was generated using a variance-standardized relationship matrix (Figure 8). The samples representing each species were clearly separated along the PC1 axis, which accounted for 37% of the variance, a significantly higher contribution compared to PC2 (3.65%). The high percentage of variance suggested that the differentiation between the two species contributed the most to the genetic variations among the samples.

To investigate the potential hybridization between the *Dendronephthya* species in Hong Kong, an admixture analysis was conducted (Figure 9). The analysis aimed to examine whether there was any mixing of genetic ancestry between the two species. The cross-validation error value

was lowest when $K = 2$, indicating that the samples were most likely derived from two distinct ancestries corresponding to the two collected *Dendronephthya* species. The admixture plot for $K = 2$ revealed no evidence of admixture between the two species across the samples. Thus, no hybridization was observed between *Dendronephthya gigantea* and *Dendronephthya spinifera* in this study. Based on these results, the genome-wide SNP dataset of the *Dendronephthya* samples was separated according to their respective species for subsequent population genetic structure analyses.

III.2.3 Population structure analysis of *Dendronephthya gigantea*

In the study focusing on *Dendronephthya gigantea*, a subset of samples from different regions in Hong Kong waters was used to construct a maximum-likelihood (ML) tree to assess the phylogenomic relationships among them (Figure 10). However, no discernible clustering pattern based on the regions of sampling was observed, indicating limited genetic differentiation among *Dendronephthya gigantea* populations from each region. To further confirm the genetic homogeneity among *Dendronephthya gigantea* samples from different regions, a PCA plot using the variance-standardized relationship matrix was generated (Figure 11). The PCA plot supported the finding that the genetic contents of *Dendronephthya gigantea* were similar regardless of the sampling region, reinforcing the lack of genetic differentiation. An admixture analysis was performed to explore the potential ancestral components and population structure of *Dendronephthya gigantea* in Hong Kong (Figure 12). The cross-validation error value was lowest when $K = 1$, indicating that the samples were most likely derived from a single ancestry. The admixture analysis suggested that *Dendronephthya gigantea* in Hong Kong originated from a single population.

Pairwise comparisons of F_{st} values were calculated to estimate gene flow and population differentiation among sites and regions (Figure 13). The Fst values were generally low, ranging from 0 to 0.0304, with a mean of 0.00360. According to Hartl and Clark (1997), these values indicate little genetic differentiation ($F_{st} < 0.05$). The low Fst values suggested a high degree of gene flow among populations, resulting in a high level of genetic homogeneity among *Dendronephthya gigantea* in Hong Kong.

III.2.4 Population structure analysis of *Dendronephthya spinifera*

A ML tree was constructed for a subset of *Dendronephthya spinifera* samples to assess the phylogenomic relationships among them in different regions of Hong Kong waters (Figure 14). Similar to *Dendronephthya gigantea*, no distinct clustering pattern based on the regions of sampling was observed, indicating little genetic differentiation among *Dendronephthya spinifera* individuals from each region. The PCA plot using the variance-standardized relationship matrix also showed the lumping of samples without differentiation based on regions, indicating high homogeneity of their genetic contents among samples from different regions (Figure 15). An admixture analysis was performed to explore the potential ancestral components and population structure of *Dendronephthya spinifera* in Hong Kong (Figure 16). The cross-validation error value was lowest when $K = 1$, suggesting that the samples were most likely

derived from a single ancestry. The admixture analysis indicated that *Dendronephthya spinifera* in Hong Kong originated from a single population.

The F_{st} values among sites and regions ranged from 0 to 0.162, with a mean of 0.0194 (Figure 17). Most pairwise F_{st} values were low, indicating little genetic differentiation among sites. However, the pairwise F_{st} values between Steep Island (site 24) and other sites were significantly higher than the values between other sites. The F_{st} values ranged from 0.0713 to 0.162, with a mean of 0.0911, which falls into the category of moderate genetic differentiation ($0.05 < F_{st} < 0.15$) according to Hartl and Clark (1997). This suggests the presence of a potential subpopulation of *Dendronephthya spinifera* located in Steep Island.

III.2.5 Population structure analysis of *Echinomuricea spinifera*

Similar to the *Dendronephthya* species analyzed, no discernible clustering pattern based on the regions of sampling was observed in the ML tree on the *Echinomuricea spinifera* (Figure 18), indicating little genetic differentiation among *Echinomuricea spinifera* individuals from each region. The PCA plot for *Echinomuricea spinifera* samples (Figure 19) supported the ML phylogenomic analysis, showing that the samples from different regions were not separated from each other. This suggests high genetic homogeneity among *Echinomuricea spinifera* individuals across different regions in Hong Kong. An admixture analysis was conducted to explore the potential ancestral components and population structure of *Echinomuricea spinifera* in Hong Kong (Figure 20). The cross-validation error value was lowest when $K = 1$, indicating a high likelihood that the samples originated from a single ancestry. The admixture analysis further supported the absence of population structure within Hong Kong waters for *Echinomuricea spinifera*, suggesting a single population origin.

The F_{st} values among sites and regions were generally low, ranging from 0 to 0.0211, with a mean of 0.00414 (Figure 21). These values indicate little genetic differentiation among sites, consistent with high genetic homogeneity in *Dendronephthya* samples. However, there were slightly higher pairwise F_{st} values between Waglan Island (site 20) and other sites, as well as a higher value for the Steep Island-Picnic Bay pair. These elevated F_{st} values suggest the possibility of potential subpopulations of *Echinomuricea spinifera* in Waglan Island and Steep Island.

III.2.6 Population structure of octocorals in Hong Kong

Overall, the results indicate that there is limited genetic differentiation and a high level of gene flow among *Dendronephthya gigantea*, *Dendronephthya spinifera* and *Echinomuricea spinifera* populations in different regions of Hong Kong. The samples showed genetic homogeneity in ML phylogenomic estimation and PCA plot on the variance-standardized relationship matrix. In addition, admixture analysis indicated a single population origin for three octocoral species in this study, suggesting a lack of population structure within Hong Kong waters for octocorals in general. However, the pairwise comparison of F_{st} values highlighted a potential subpopulation of *Dendronephthya spinifera* and *Echinomuricea spinifera* in Steep Island and *Echinomuricea spinifera* in Waglan Island, with moderate genetic differentiation compared to other sites.

It is worth noting that this study focused on the genetic analysis of octocorals and their population structure. The observed similarities in population genetic structure between *Dendronephthya* and *Echinomuricea*, despite differences in their reproductive modes, suggest a potential common pattern of connectivity and genetic exchange among octocorals in Hong Kong. Further research could investigate the octocoral community structure and hydrology around Steep Island and Waglan Island to better understand the factors shaping the population structure of *Dendronephthya spinifera* in Steep Island and *Echinomuricea spinifera* in Waglan Island.

Table 6: A Table showing the mapping quality of the *Dendronephthya* samples

Sample ID	Mapped Reads (%)	Average Depth	S.D.	Mapping Quality Score
DNMW01	91.67	24.48	3241.02	16.19
DNMW02	92.04	25.53	4047.42	16.24
DNMW03	92.67	31.42	112.53	21.45
DNMW04	91.69	25.24	2741.70	16.12
DNMW05	97.29	32.55	147.98	21.62
DNSKY01	96.12	26.51	100.14	21.18
DNSKY02	88.34	16.45	131.57	15.94
DNSKY03	88.16	17.79	141.01	15.84
DNSKY04	96.11	26.50	117.18	20.68
DNSKY05	94.89	24.07	88.66	20.59
DNTAC01	96.2	26.97	109.86	20.64
DNTAC02	96.35	27.26	102.24	20.64
DNCC01	89.25	18.40	135.39	15.4
DNTO01	96.2	28.04	110.85	20.85
DNTO02	95.61	32.32	98.84	20.67
DNTO03	83.97	22.66	61.79	21.28
DNTO04	95.24	27.85	75.62	20.59
DNTO05	96.53	25.01	87.17	21.26
DNTO06	95.83	26.87	103.29	20.75
DNFR01	96.21	28.08	131.23	20.46
DNFR02	88.09	21.79	182.65	15.23
DNSK01	88.76	20.16	126.01	16.02
DNSK02	87.51	21.58	145.35	15.93
DNPT01	96.56	33.00	159.72	21.24
DNPT02	96.61	24.42	86.22	21.31
DNPT03	88.08	23.15	153.84	15.84
DNPT04	89.03	16.40	114.79	15.87
DNPT05	97.34	32.02	124.65	21.8
DNWL01	94.27	21.31	154.22	17.37
DNWL02	92.12	25.83	3995.95	16.15
DNWL03	93.84	22.57	9326.81	15.97
DNWL04	88.88	18.65	137.06	15.93
DNWL05	93.29	24.40	8304.89	16.01
DNWL06	90.92	28.30	3335.31	16.21
DNWL07	96.43	28.30	136.78	21.15
DNWL08	96.15	30.24	115.76	20.89
DNCA01	95.31	26.83	104.72	20.26
DNCA02	93.07	25.98	124.71	20.92
DNBR01	92.01	23.72	4833.87	16.01
DNBR02	96.52	32.02	170.44	21.36
DNBR03	96.49	31.88	143.89	22.11
DNBR04	91.64	24.05	2995.52	16.38
DNBR05	91.01	25.54	2887.85	16
DNBR06	93.73	29.28	273.29	17.53
DNBR07	97.15	31.52	234.81	21.43
DNSTI01	93.55	25.70	110.74	21.1
DNSTI02	87.81	16.45	119.56	15.98
DNSTI03	87.71	16.74	140.27	15.59
DNSTI04	88.25	16.59	143.21	15.89
DNNP01	96.62	26.58	92.88	21.31
DNNP02	93.11	27.93	93.36	21.1
DNNP03	87.75	16.07	145.04	15.77
DNNP04	87.56	17.74	146.71	15.9
DNNP05	88.95	20.06	139.39	16.66
DNBS01	92.33	27.08	145.51	16.46

Table 7: A Table showing the mapping quality of the *Echinomuricea* samples.

Sample ID	Mapped Reads (%)	Average Depth	S.D.	Mapping Quality Score
ECMI01	63.78	5.66	162.37	14.37
ECMI02	61.33	5.97	234.33	13.18
ECMI03	61.55	5.49	254.08	13.03
ECMI04	59.48	5.55	154.35	13.01
ECMI05	60.83	5.63	231.12	12.90
ECLC01	60.78	5.64	231.30	12.94
ECLC02	60.47	5.67	158.42	13.01
ECLC03	54.75	4.12	49.28	13.20
ECLC04	56.04	4.35	55.85	13.28
ECLC05	50.11	18.96	193.19	13.45
ECLC06	51.94	10.94	126.51	13.39
ECLC07	52.05	15.22	174.48	13.52
ECPB01	52.91	3.88	54.10	12.91
ECPB02	54.69	4.11	54.30	13.13
ECPB03	55.17	4.83	59.21	13.19
ECPB04	53.42	4.03	49.63	13.41
ECPB05	55.18	4.35	53.31	13.23
ECPB06	54.9	4.29	52.19	13.13
ECPB07	55.63	4.64	51.77	13.22
ECRI01	54.57	4.14	57.60	13.19
ECRI02	54.94	6.12	69.17	13.92
ECRI03	56.08	4.23	54.37	13.37
ECRI04	55.53	4.35	49.67	13.31
ECRI05	55.72	4.27	56.10	13.27
ECWL01	62.95	5.88	121.43	13.65
ECWL02	64.2	5.57	125.25	13.63
ECWL03	62.33	5.67	168.31	13.43
ECWL04	63.11	5.63	121.37	13.52
ECWL05	62.3	6.90	126.06	13.55
ECWL06	62.59	5.63	137.45	13.38
ECNYP01	62.46	5.66	120.65	12.95
ECNYP02	57.68	4.95	118.84	13.36
ECNYP03	62.48	5.87	137.51	12.79
ECNYP04	58.72	7.20	180.92	13.35
ECNYP05	50.18	4.53	102.76	13.20
ECNYP06	62.89	5.62	115.43	13.03
ECNYP07	64.25	5.98	133.74	13.15
ECNYP08	63.43	5.64	115.14	13.07
ECNYP09	63.69	5.73	116.21	12.96
ECNYP10	62.13	5.44	129.52	12.97
ECNYP11	63.72	5.64	142.14	12.78
ECNYP12	64.09	6.75	186.82	12.97
ECSTI01	57.72	4.62	57.79	12.79
ECWMC01	55.02	4.29	52.94	13.06
ECWMC02	57.82	4.33	57.16	13.71
ECWMC03	54.7	4.66	54.76	13.67

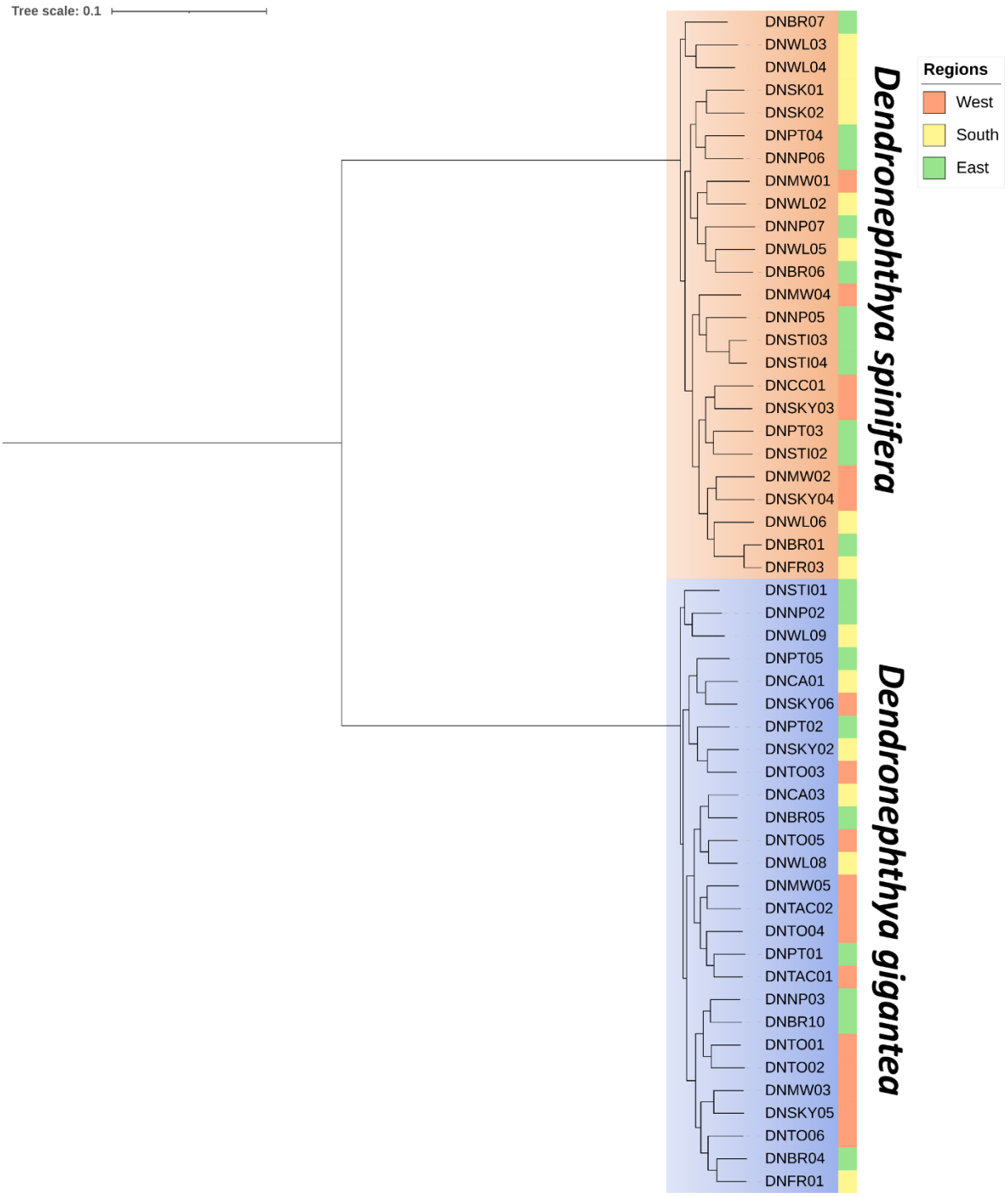


Figure 7: A Maximum likelihood (ML) phylogenetic tree of *Dendronephthya* samples dataset estimated with RAxML v.8.2.10 using dataset of 539,835 SNPs. Branch support is based on ML bootstrap analyses ≥ 90. Regions from which the samples were collected were denoted by the colour strips at the leaves. Red: West; Yellow: South; Green: East

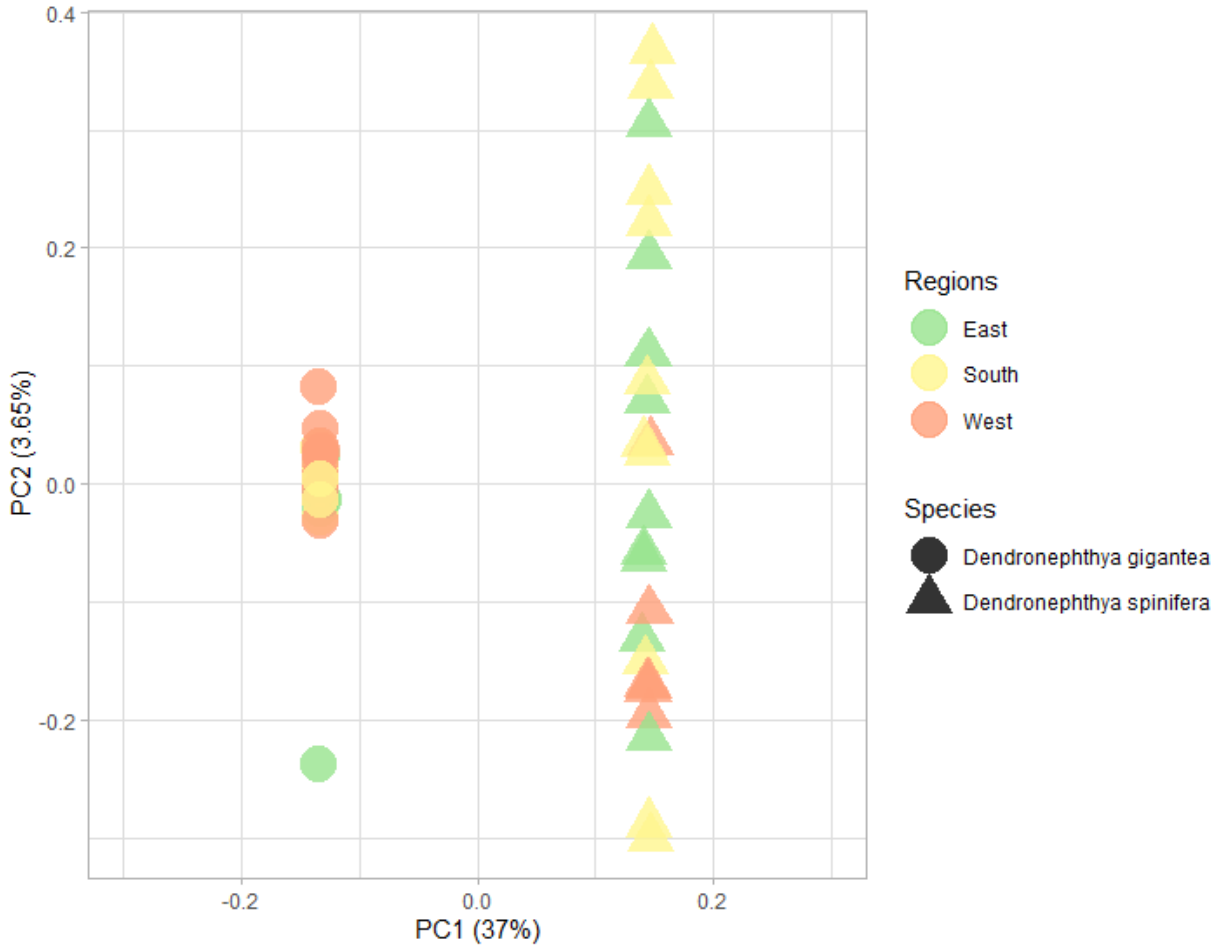
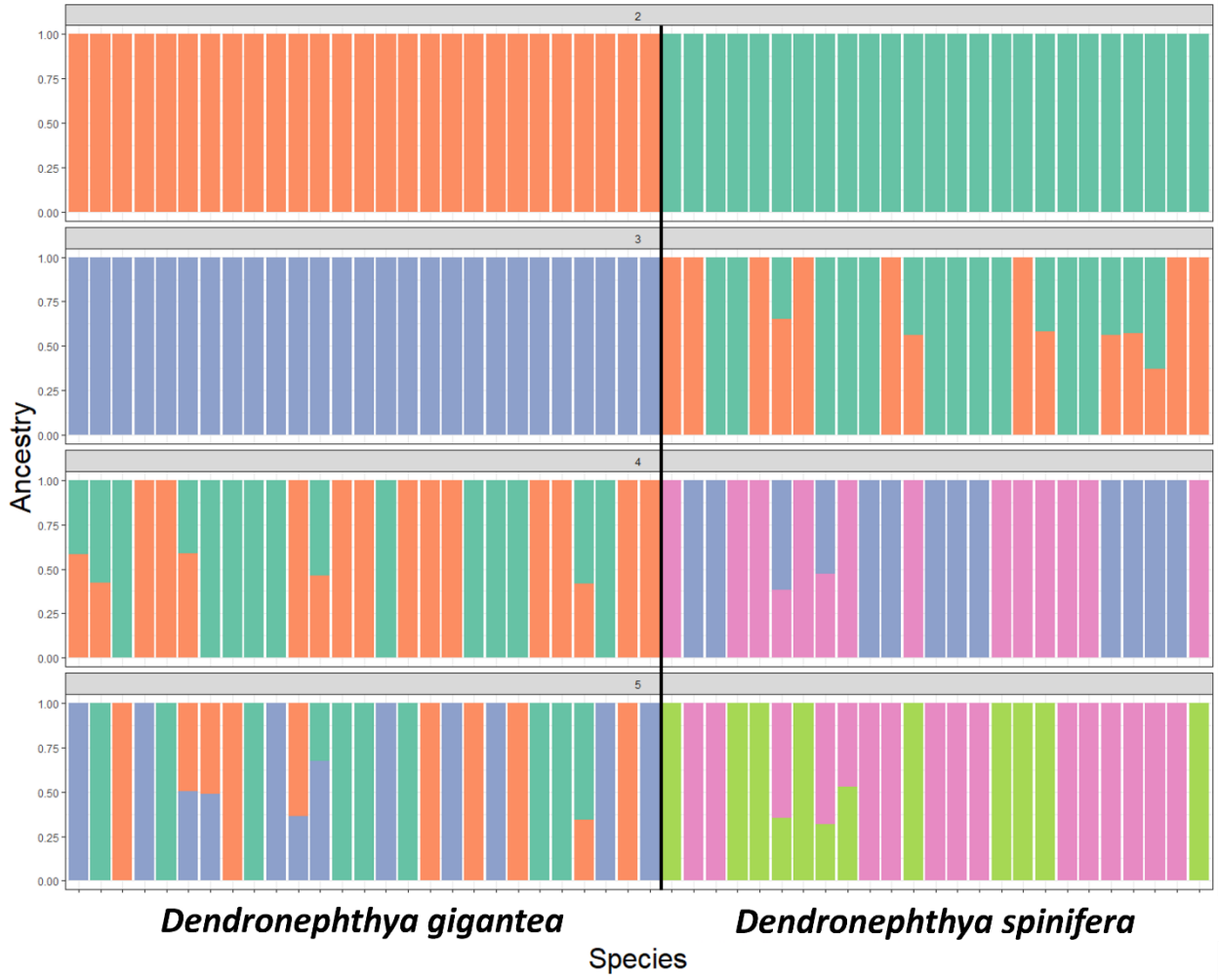


Figure 8: A principal component analysis (PCA) plot of variance-standardized relationship matrix calculated from *Dendronephthya* of 65,342 unlinked SNPs. Regions from which the samples were collected were denoted by the colours. Red: West; Yellow: South; Green: East. Two species of *Dendroephthya* were denoted by shapes. Circle: *Dendronephthya gigantea*; Triangle: *Dendronephthya spinifera*.

a)



b)

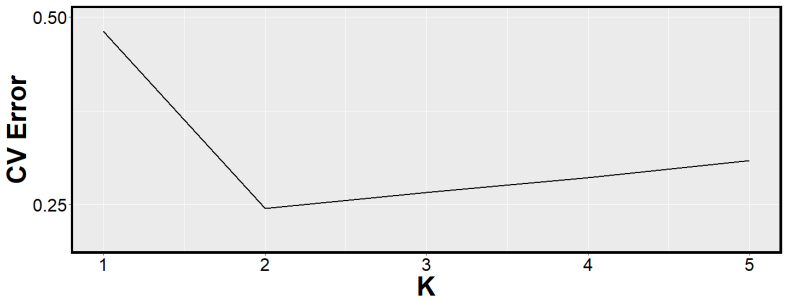


Figure 9: a) An admixture plot of *Dendronephthya* showing the potential proportion of ancestry from number of *K* (1 - 5) population(s) using 65,342 unlinked SNPs from pruned dataset. Samples are grouped by their species identity. b) A cross-validation (CV) error plot for the *Dendronephthya* dataset where *K* range from 1 to 5

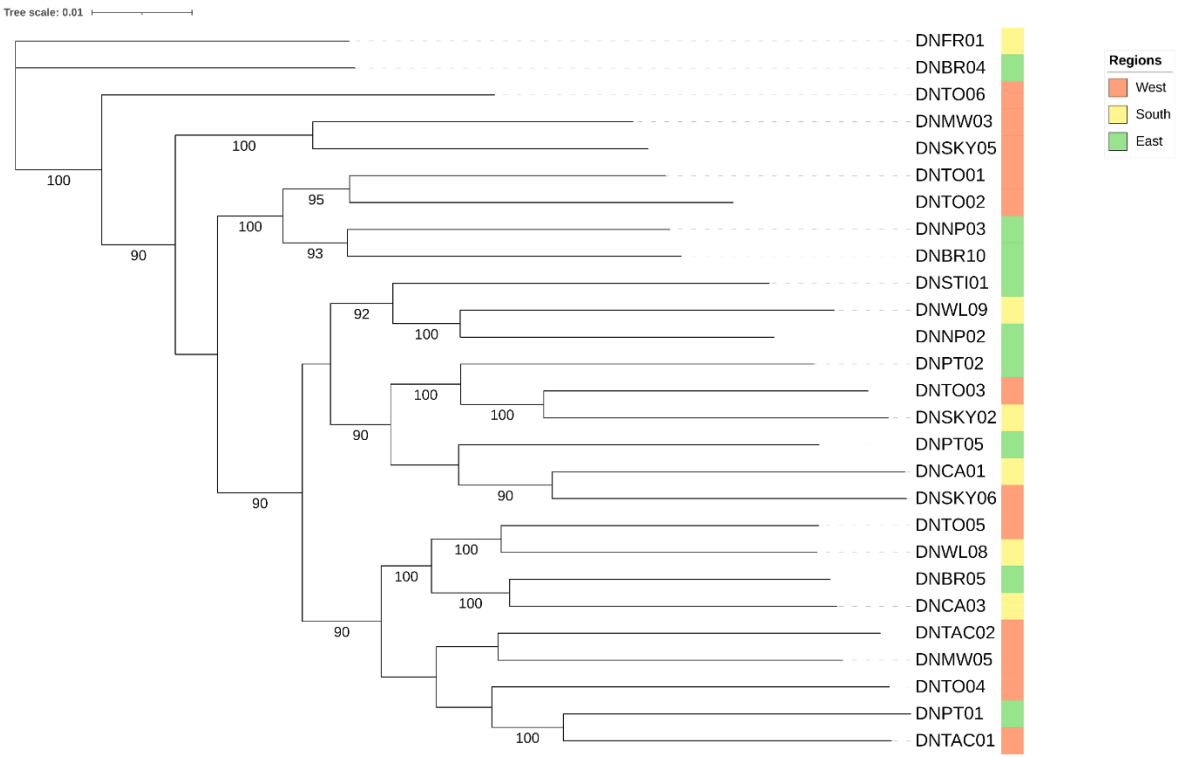


Figure 10: A Maximum likelihood (ML) phylogenetic tree of *Dendronephthya gigantea* samples dataset estimated with RAxML v.8.2.10 using 594,343 SNPs. Branch support is based on ML bootstrap analyses ≥ 90. Regions from which the samples were collected were denoted by the colour strips at the leaves. Red: West; Yellow: South; Green: East

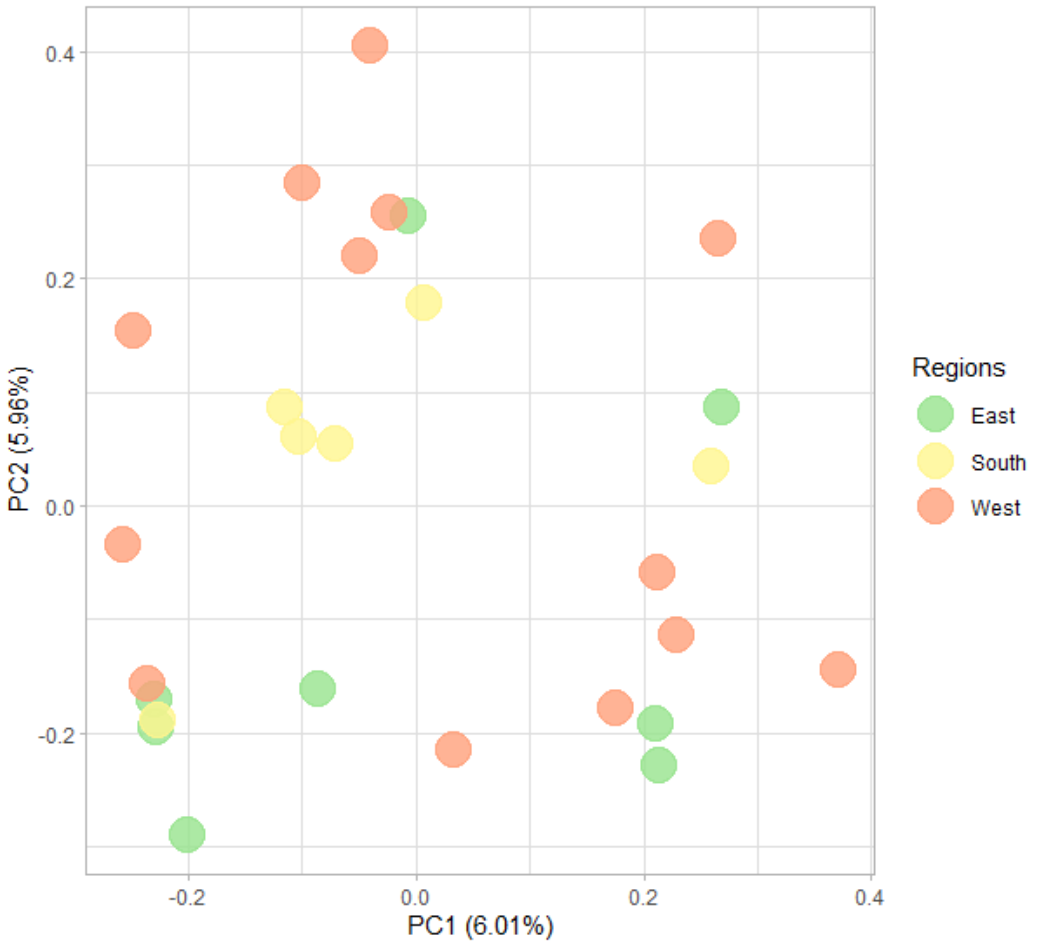
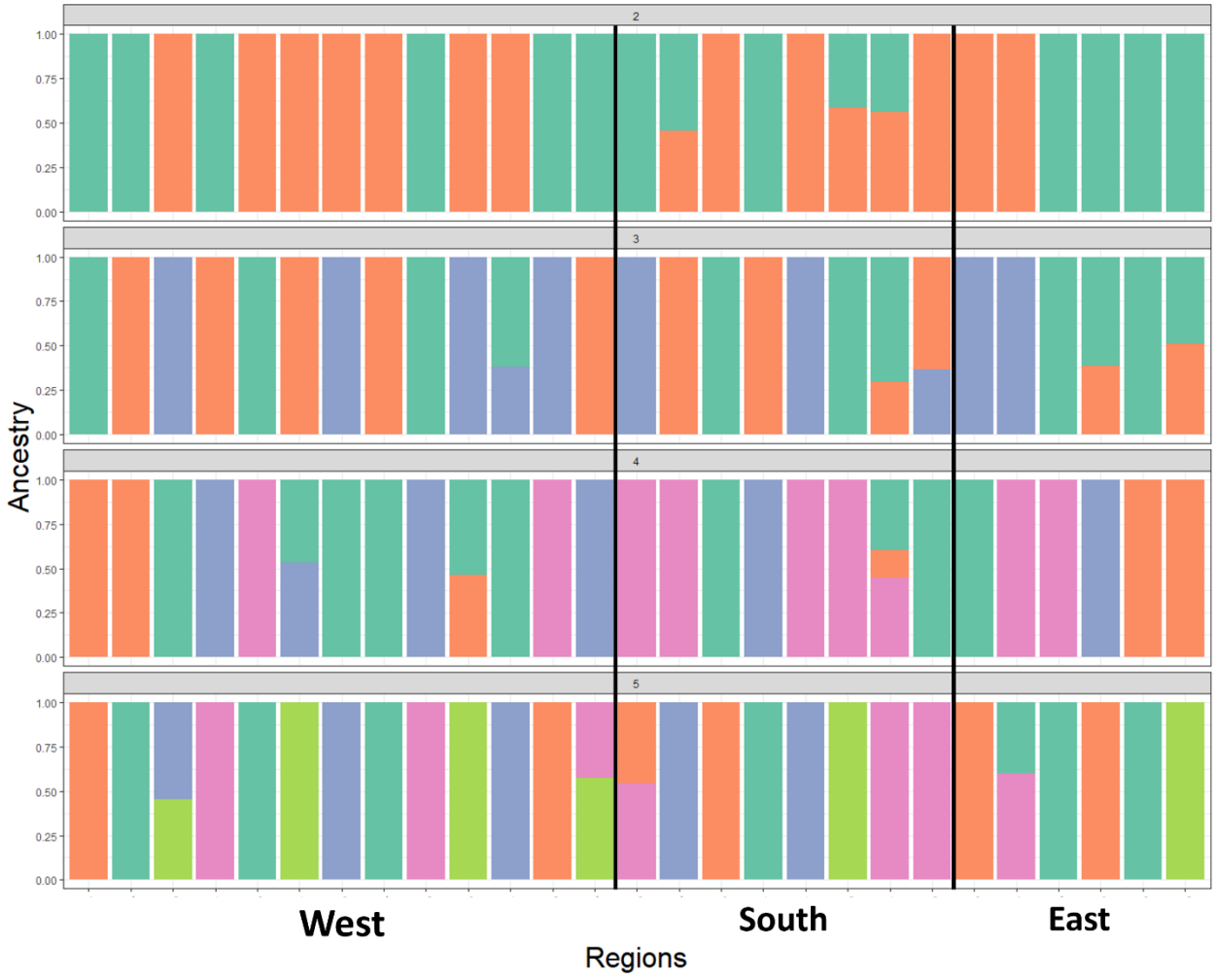
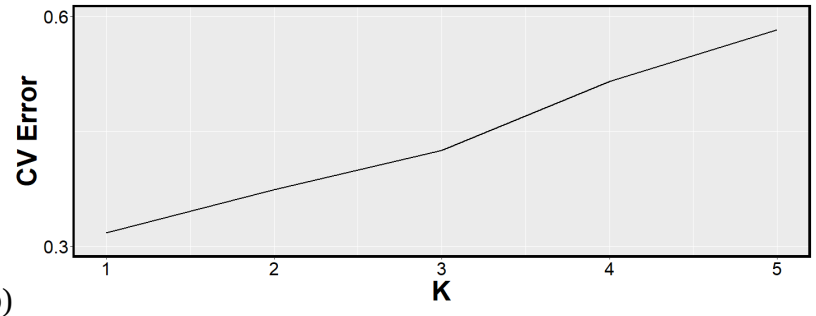


Figure 11: A principal component analysis (PCA) plot of variance-standardized relationship matrix calculated from *Dendronephthya gigantea* of 58,637 unlinked SNPs. Regions from which the samples were collected were denoted by the colours. Red: West; Yellow: South; Green: East.

a)





b)

Figure 12: a) An admixture plot of *Dendronephthya gigantea* showing the potential proportion of ancestry from number of *K* (1 - 5) population(s) using 58,637 unlinked SNPs from pruned dataset. Samples are grouped by the regions of sampling. b) A cross-validation (CV) error plot for the *Dendronephthya gigantea* dataset where *K* range from 1 to 5

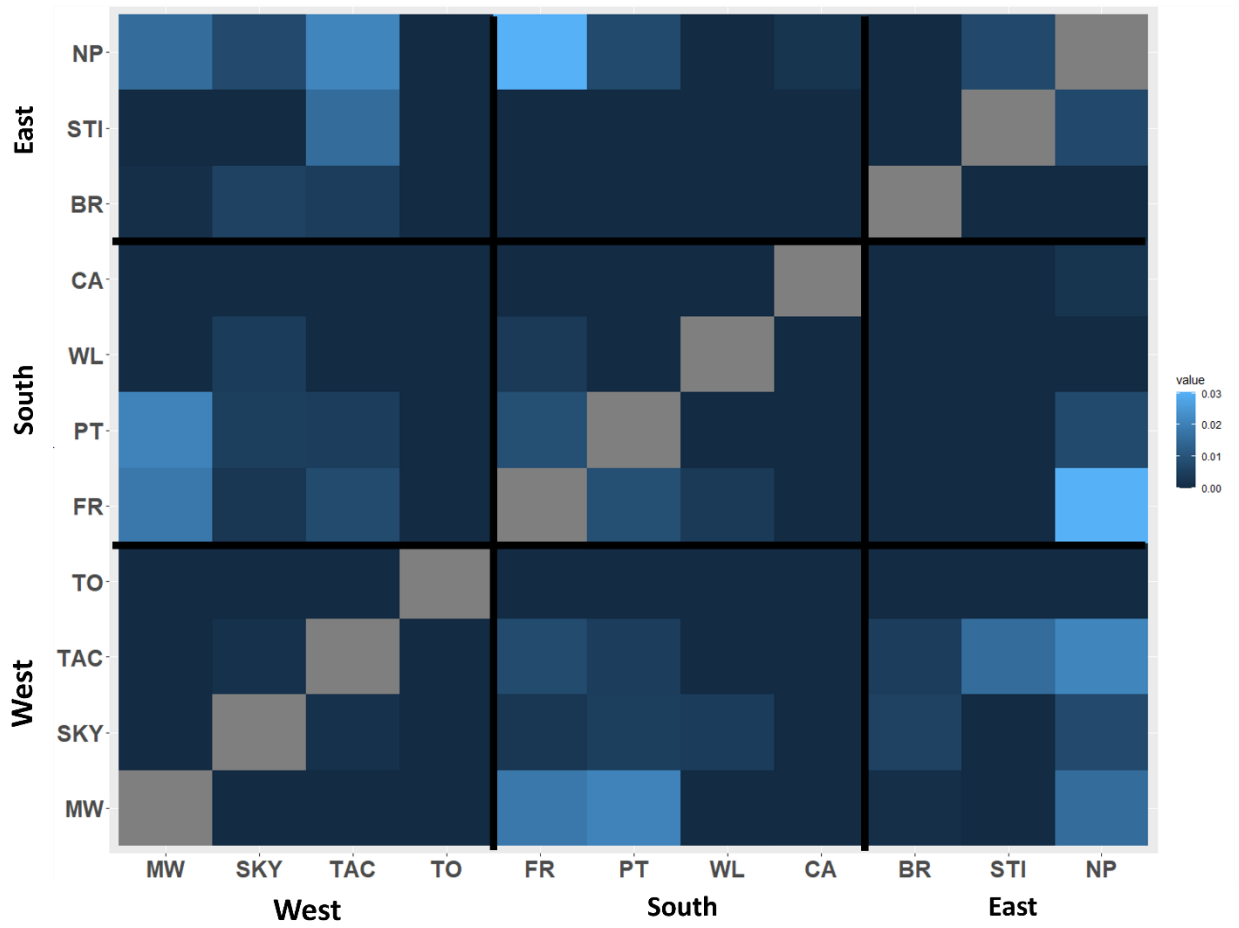


Figure 13: A heatmap showing the pairwise comparison of F_{st} value of *Dendronephthya gigantea* samples among sites of collections using 58,637 unlinked SNPs from pruned dataset. Sites are grouped by their regions.

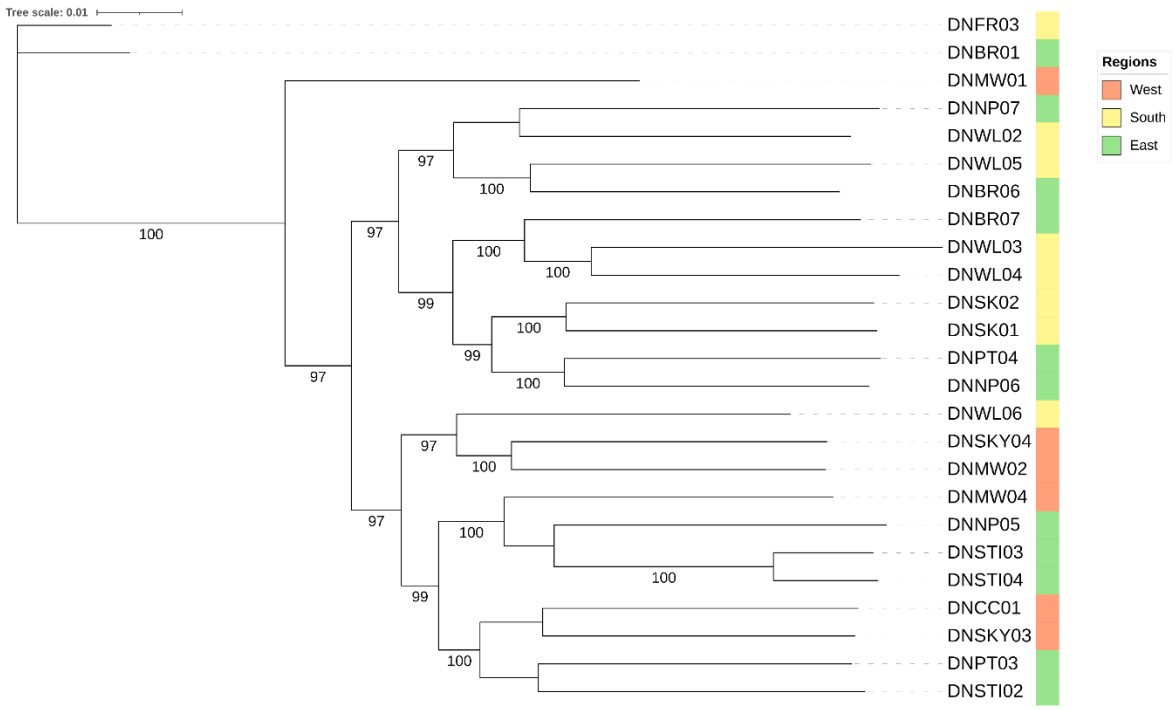


Figure 14: A Maximum likelihood (ML) phylogenetic tree of *Dendronephthya gigantea* samples dataset estimated with RAxML v.8.2.10 using 594,343 SNPs. Branch support is based on ML bootstrap analyses ≥ 90. Regions from which the samples were collected were denoted by the colour strips at the leaves. Red: West; Yellow: South; Green: East

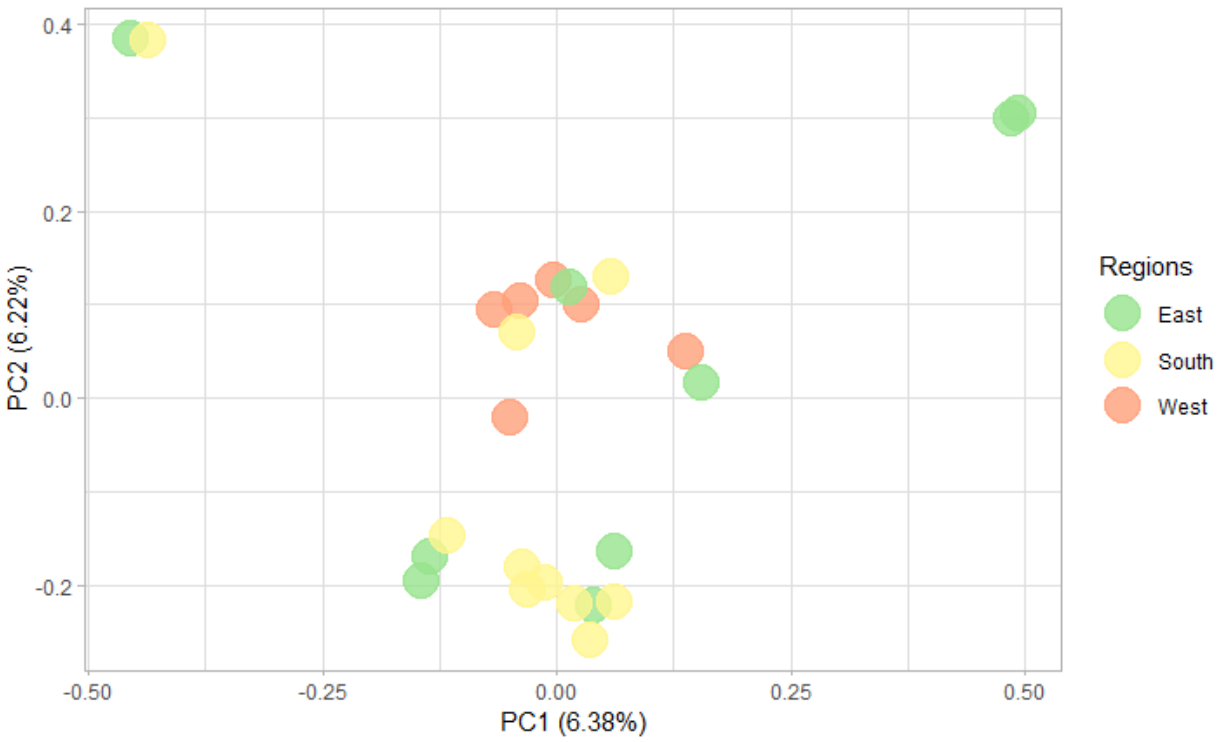


Figure 15: A principal component analysis (PCA) plot of variance-standardized relationship matrix calculated from *Dendronephthya spinifera* of 40,075 unlinked SNPs. Regions from which the samples were collected were denoted by the colours. Red: West; Yellow: South; Green: East.

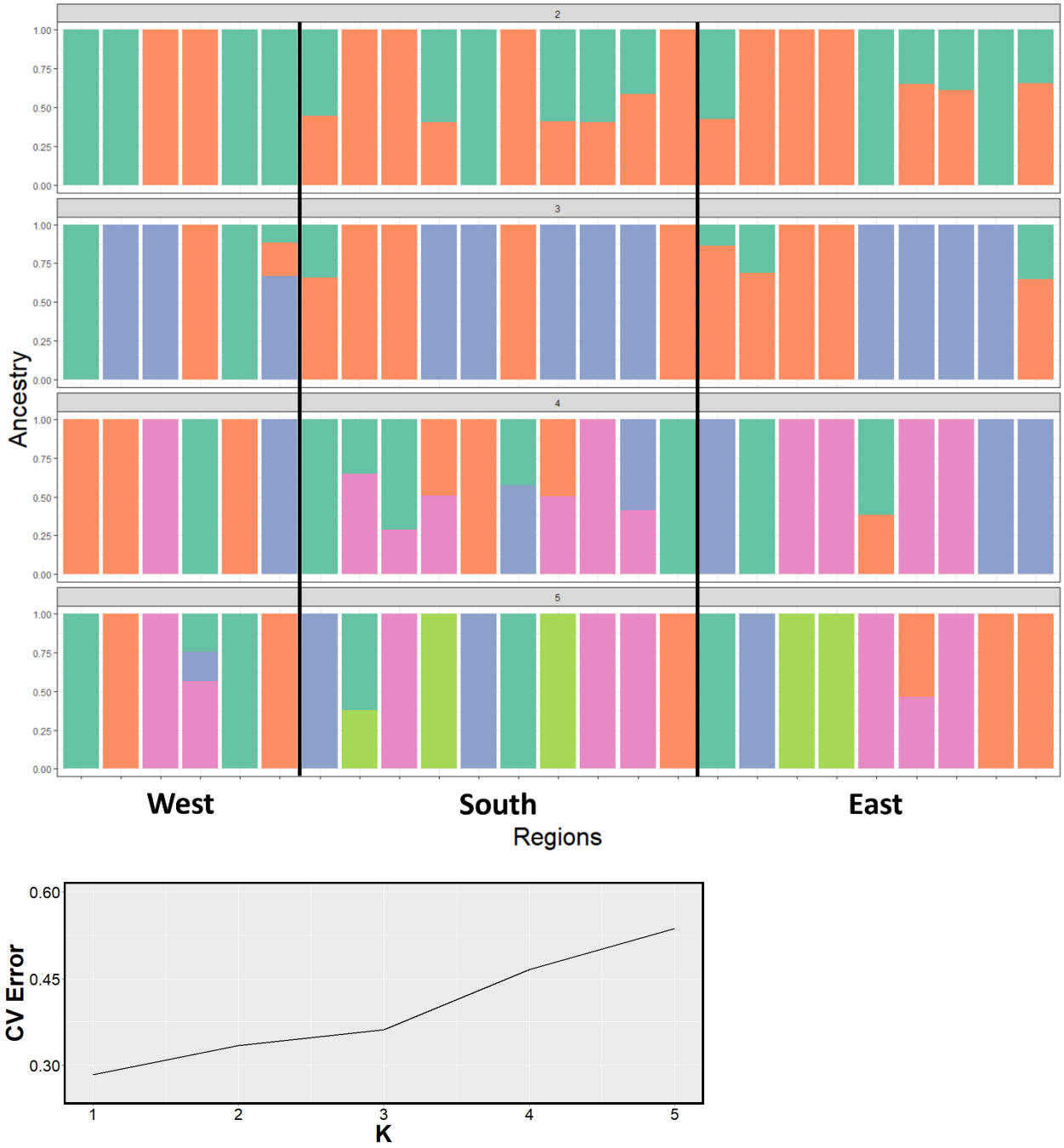


Figure 16: a) An admixture plot of *Dendronephthya spinifera* showing the potential proportion of ancestry from number of *K* (1 - 5) population(s) using 40,075 unlinked SNPs from pruned dataset. Samples are grouped by the regions of sampling. b) A cross-validation (CV) error plot for the *Dendronephthya spinifera* dataset where *K* range from 1 to 5

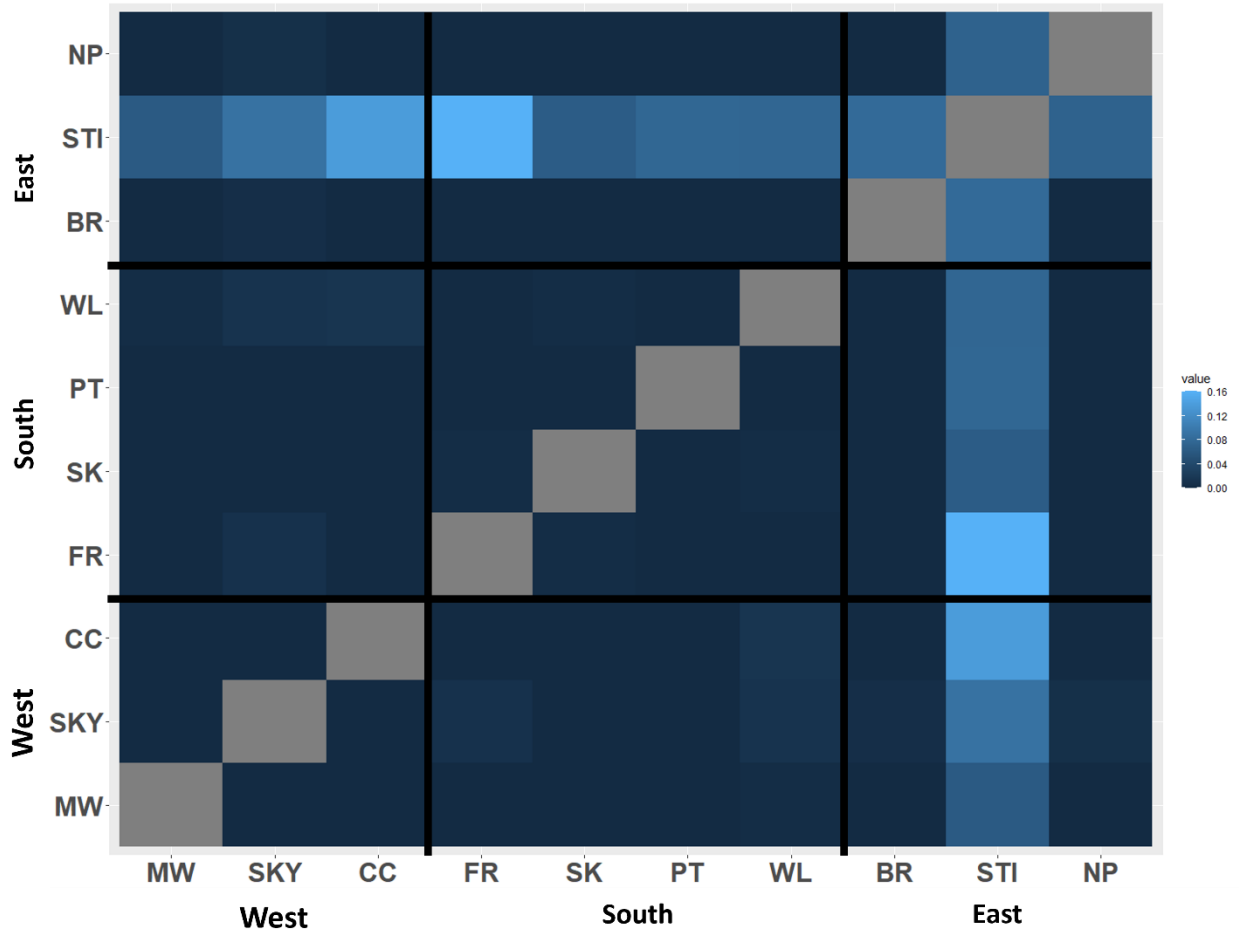


Figure 17: A heatmap showing the pairwise comparison of F_{st} value of *Dendronephthya spinifera* samples among sites of collections using 40,075 unlinked SNPs from pruned dataset. Sites are grouped by their regions.

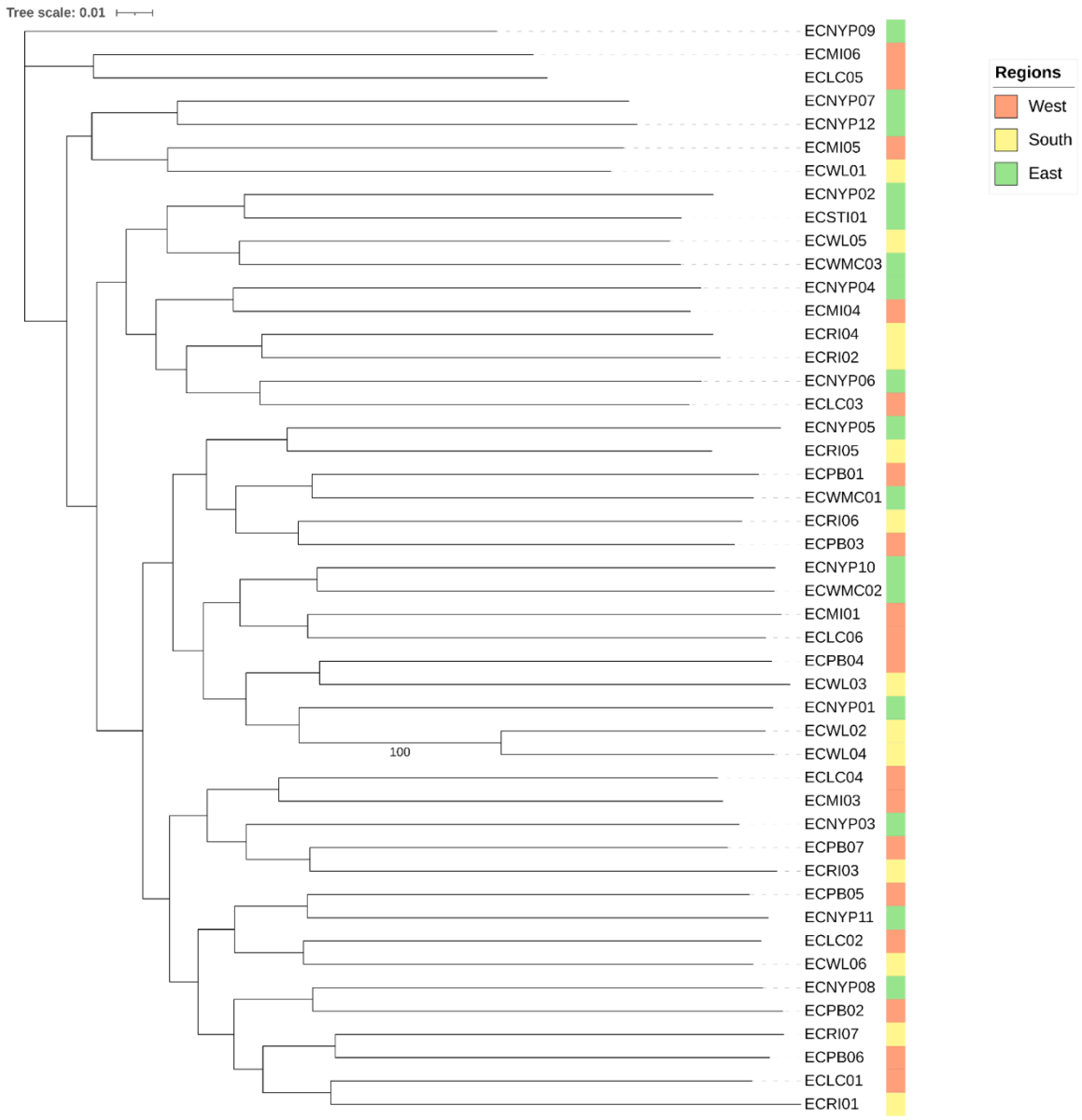


Figure 18: A Maximum likelihood (ML) phylogenetic tree of *Echinomuricea* samples dataset estimated with RAxML v.8.2.10 using of 71,888 SNPs. Branch support is based on ML bootstrap analyses ≥ 90. Regions from which the samples were collected were denoted by the colour strips at the leaves. Red: West; Yellow: South; Green: East

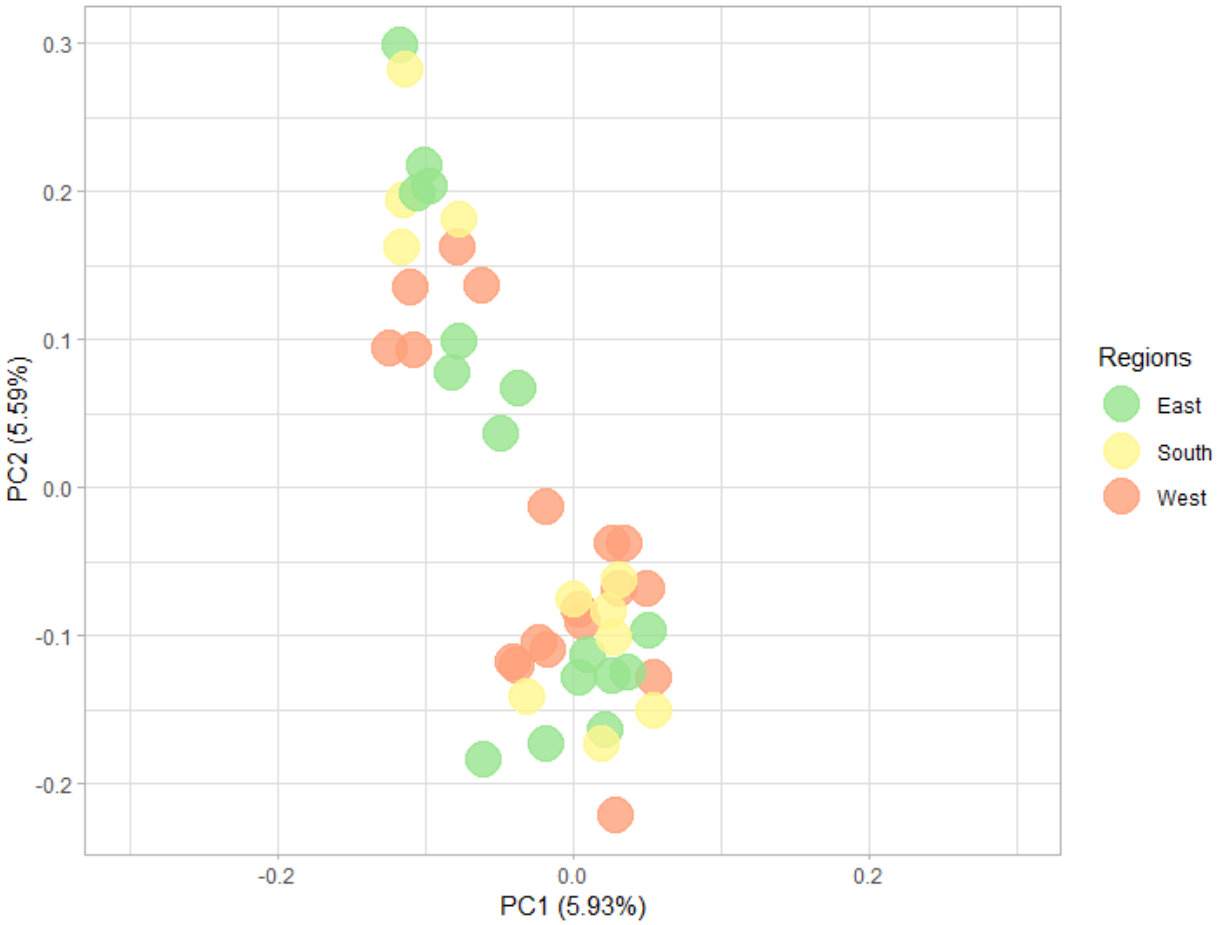
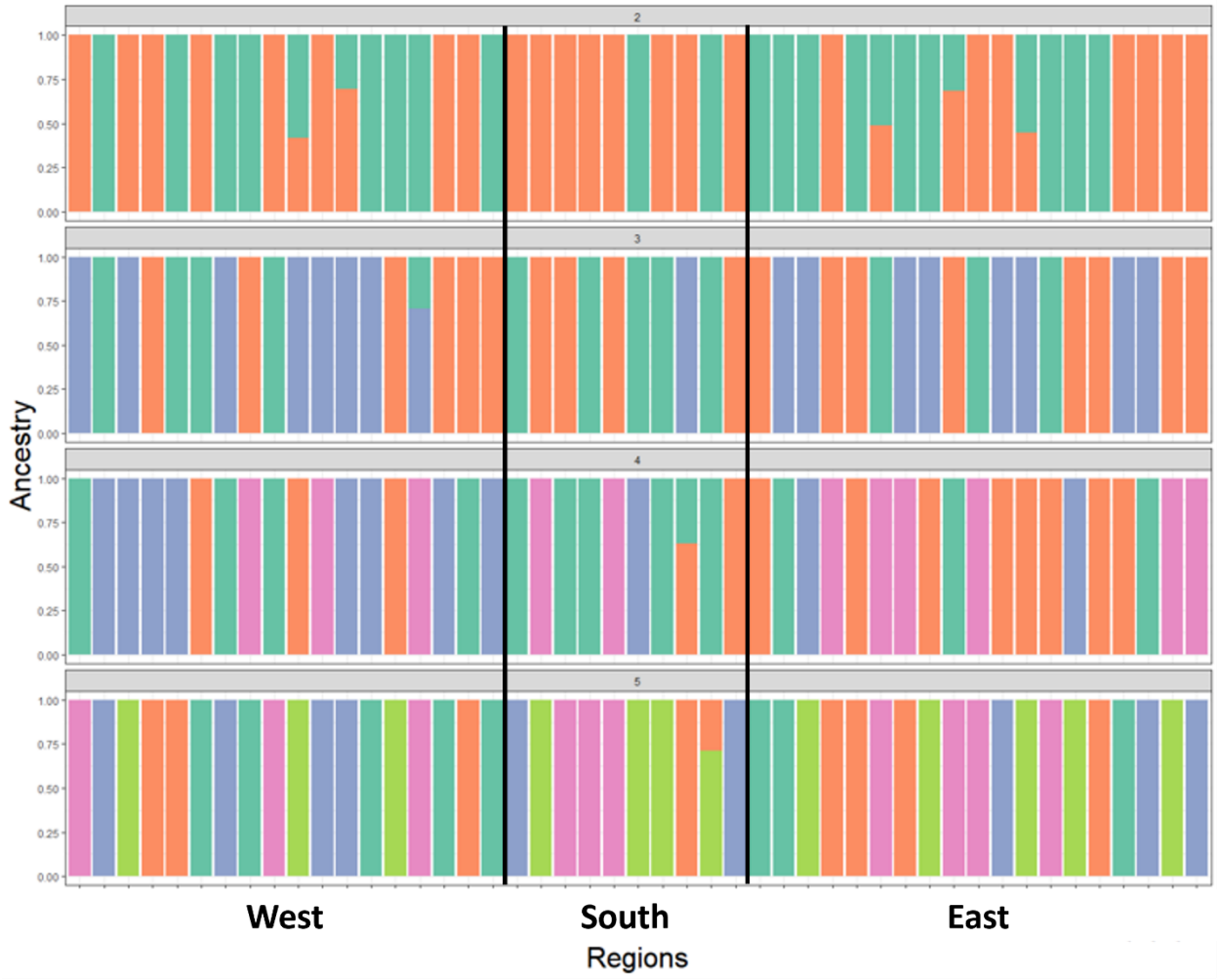


Figure 19: A principal component analysis (PCA) plot of variance-standardized relationship matrix calculated from *Echinomuricea spinifera* of 30,050 unlinked SNPs. Regions from which the samples were collected were denoted by the colours. Red: West; Yellow: South; Green: East.

a)



b)

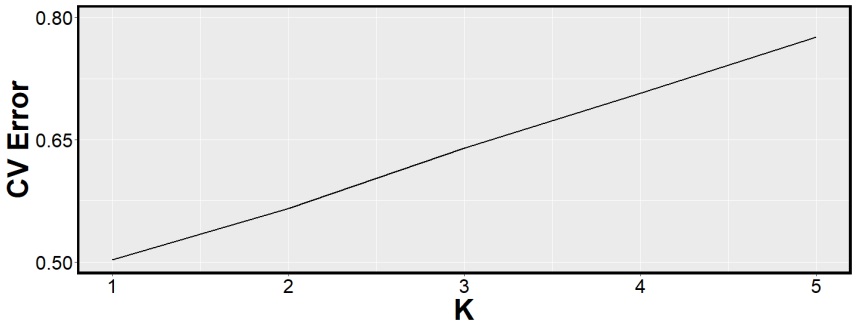


Figure 20: a) An admixture plot of *Echinomuricea spinifera* showing the potential proportion of ancestry from number of *K* (1 - 5) population(s) using 30,050 unlinked SNPs from pruned dataset. Samples are grouped by the regions of sampling. b) A cross-validation (CV) error plot for the *Echinomuricea spinifera* dataset where *K* range from 1 to 5

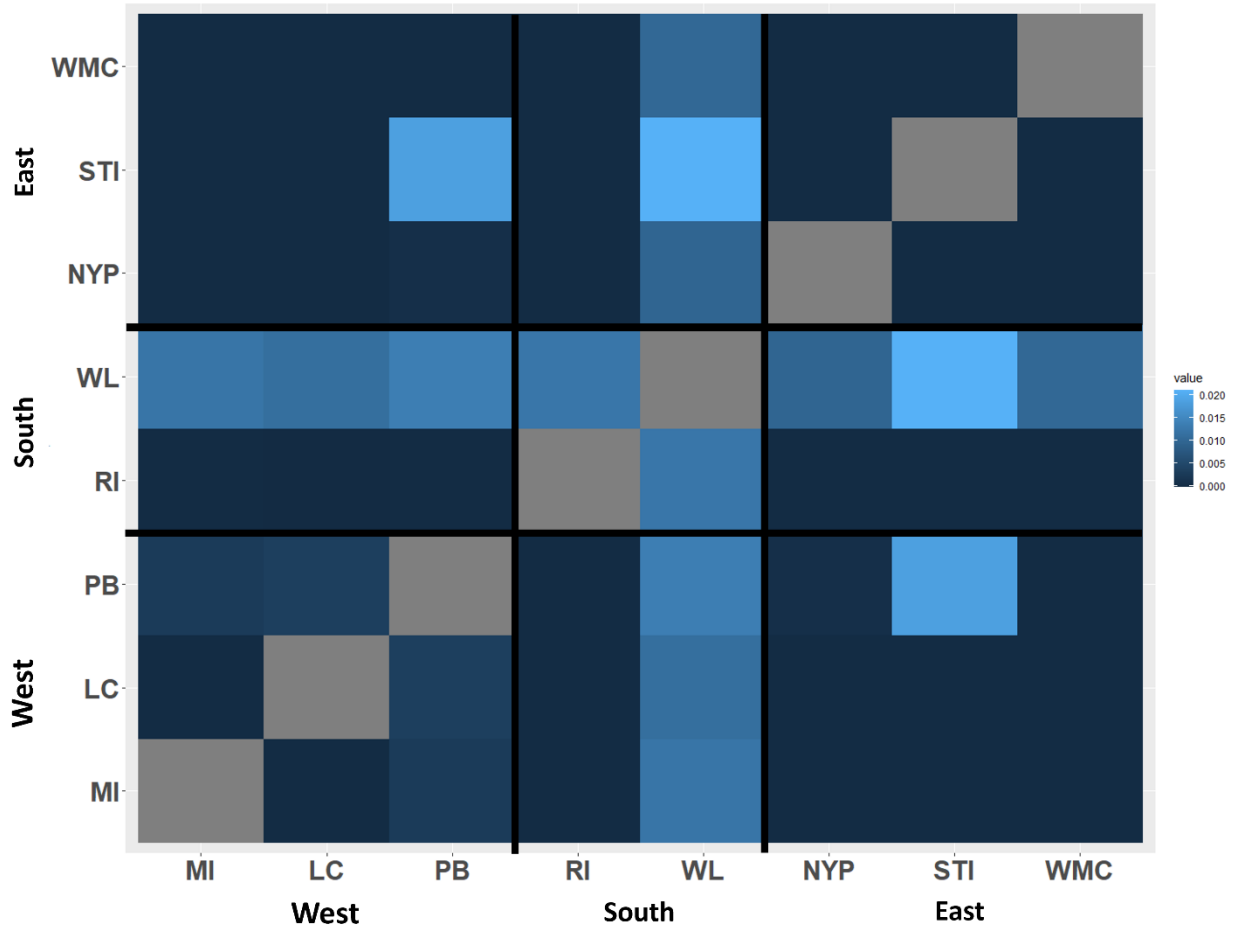


Figure 21: A heatmap showing the pairwise comparison of F_{st} value of *Echinomuricea spinifera* samples among sites of collections using 30,050 unlinked SNPs from pruned dataset. Sites are grouped by their regions.

IV. Summary

In this study, alcyoniid soft corals *Dendronephthya* and gorgonian coral *Echinomuricea* were sampled across the West, South, and East regions of Hong Kong waters. These two genera exhibited a partitioning in spatial distribution, with *Dendronephthya* preferring boulder substrates and shallower depths in the western waters, while *Echinomuricea* could occupy silty substrates across various depths, including areas experiencing heavy sedimentation from the Pearl River in the west. Furthermore, we also found lower octocoral diversity and coverage then previously documented. Further ecological study should be conducted to investigate the octocoral diversity more comprehensively in Hong Kong.

For the analysis of population genetic structure, whole-genome resequencing (WGR) was employed as a powerful tool to achieve high-resolution genome-wide SNP analysis, enabling an in-depth and holistic investigation of population genetics. The results indicated that the populations from each species of *Dendronephthya* and *Echinomuricea* in Hong Kong represent a panmictic population with high level of connectivity and genetic exchange across locations. This genetic connectivity suggests that these octocoral species possess the potential to recolonize and recover from disturbances or localized population declines. Despite these positive findings, it is important to note the potential decline in octocoral coverage observed in many sites compared to early report. This decline raises concerns about the natural resilience of these populations in the face of ongoing threats and environmental changes. However, the observed genetic connectivity and dispersal capabilities suggest that, if appropriate conservation measures are implemented, the populations may have the potential to recover and restore their abundance.

Therefore, to ensure the long-term sustainability and conservation of *Dendronephthya* and *Echinomuricea* populations, it is important to consider the following recommendations for population and conservation management:

1. **Conservation management of octocoral populations in Hong Kong**:

Although high level of gene flow and dispersal is observed, we also noticed potential decline in species diversity and abundance of octocoral in HK waters. To maintain resilience in octocoral species facing potential population declines, effective conservation management strategies should be implemented. Measures should address causes of decline, such as habitat degradation and pollution, through habitat protection, would be needed. Genetic management strategies, including genetic monitoring and promoting connectivity, enhance adaptive potential. Monitoring genetic diversity and population structure helps identify declines and assess conservation actions. Facilitating gene flow through habitat restoration and assisted gene flow initiatives maintains genetic diversity and resilience. By combining habitat protection, stakeholder engagement, public awareness, and genetic management, we can ensure the long-term survival and ecological importance of octocoral species.

2. **Proper planning and selection of translocation location:**

Translocation of octocorals should be approached with careful planning and selection to maximize success and minimize risks. Consider the following aspects:
i/ Habitat Suitability: When conducting translocation projects, it is crucial to consider the environmental parameters of the recipient areas, as the physical environment can significantly

influence the settlement and survivorship of translocated colonies. Some octocorals are susceptible to different environmental stressors. For instance, we observed habitat segregation in the three octocoral species studied here, with *Dendronephthya* species be susceptible to silty and turbid environments. Therefore, areas with boulder substrates and strong current flow are suggested as suitable recipient areas for translocation projects. Proper planning and management of optimal recipient areas are essential for the success and effectiveness of translocation projects. Thorough site assessments should be conducted to ensure the presence of adequate food resources and protection from potential stressors.

ii/ Selection of individuals with similar genetic composition: by selecting individuals that share similar genetic profiles, we can minimize the risk of disrupting local adaptation and maintain the genetic integrity of the recipient population. This approach helps ensure that the translocated individuals are well-suited to the environmental conditions of the target location, increasing their chances of successful establishment and integration into the existing population. In the case of octocorals, where low genetic differences are observed among populations, it becomes feasible to use individuals from multiple sources to minimize disturbance to existing population and maximize the genetic diversity of translocating individuals.

iii/ Monitoring and adaptive management: Implement a robust monitoring program to assess the success of translocations, including survival rates, reproductive success, and genetic integration. This information will inform adaptive management strategies and allow for adjustments if necessary.

3. Future studies on octocoral communities in Steep Island and Waglan Island:

Steep Island and Waglan Island have exhibited exceptionally high genetic differentiation compared to other sites, making them of special interest for conservation and scientific purposes. It is recommended to conduct extensive studies to better understand the baseline characteristics of these two sites. For example, an intensive survey could be conducted to assess the octocoral community composition, and hydrological studies could be conducted to investigate potential factors leading to the isolation and differentiation of octocoral populations in Steep Island and Waglan Island.

The progress against the work schedule

Summary of proposed work plan	Summary of final report
Target sites, sample collection and identification of octocorals	
• Visit **10 study sites** and collect samples for the two target species—*Dendronephthya gigantea* (Soft coral) *and Echinomuricea spinifera* (Gorgonia)	• Visited **27 sites**, of which 10 is in the proposed list and 17 other sites not included in the original proposal (due to prevailing wind conditions resulting in strong current and high turbidity in original sites)—Figure 2. • Collected a total of 99 samples for the two target genera— 52 individuals of *Dendronephthya* (Soft coral) and 47 individuals of *Echinomuricea spinifera* (Gorgonia)—Table 1.
DNA Extraction and genome re-sequencing	
• Genomic DNA of the coral tissue will be extracted. • Genomic DNA of coral samples will be sent to commercial biotechnology company for high throughput sequencing.	• Completed as per proposed work plan for all samples collected.
SNP identification and detection & Genetic diversity and population structuring analyses	
• Bioinformatic analyses of the obtained sequence data. • Population genetic structure will be investigated based on the sequence data using various analytical tool (e.g. PCA, F_{ST} and admixture analyses)	• Bioinformatic analyses were conducted on all sequenced samples. • Population genetic analyses completed.

Evaluation of the project effectiveness in achieving the proposed objectives as well as the impact (benefits) of the project

Objective 1: To investigate the population genetic structure of two octocoral species in the Pearl River estuary (PRE), covering the western and southern waters of Hong Kong, using low coverage whole genome sequence data.

100% completed.

By sequencing nearly 100 individuals of octocoral from the western, southern, and newly included eastern waters, we have uncovered valuable insights into the population genetics of these species. Our findings indicate a high level of connectivity among the three regions, regardless of the distinct hydrology and water gradients they possess. This suggests that octocoral populations in the PRE region exhibit extensive gene flow, indicating a panmictic population structure and high levels of genetic exchange. These results contribute to our understanding of the overall connectivity and genetic dynamics of octocoral populations in the study area.

Objective 2: To delineate genetic diversity and larval dispersal pattern among octocoral communities in Hong Kong waters using *Dendronephthya gigantea* (Soft coral) and *Echinomuricea spinifera* (Gorgonia) as model system that provide background information for future conservation planning and strategy.

100% completed.

The two octocoral genera studied in Hong Kong waters exhibit a notable pattern of high gene flow, indicating a robust capacity for population recovery and offering flexibility for future translocation projects. This finding is encouraging for the conservation of these species. However, it is important to note that certain locations, such as Waglan and Steep Island, appear to be distinct from the rest of the populations. These unique populations warrant further investigation and conservation efforts to ensure their preservation. By focusing conservation efforts on these specific locations, we can better understand the factors contributing to their distinctiveness and implement targeted measures to safeguard their genetic diversity and ecological significance.

Benefits:

1. Database for the diversity, composition and distribution of octocoral species in Hong Kong western water for future monitoring and conservation programme.
   100% completed. We have surveyed and sampled more sites than proposed and found significant differences in spatial and bathymetric distribution of octocoral among sites. These can provide the baseline data for the community structure of octocoral in various locations.
2. Discovery genetic diversity distribution and any endemic/distinct population of octocoral in Hong Kong that will increase public awareness to HK marine biodiversity in the western waters.
   100% completed. We found that Steep Island and Waglan Island have exhibited exceptionally high genetic differentiation compared to other sites, making them of special interest for conservation and scientif/ic purposes.
3. Database for connectivity and dispersal range for local octocoral species will be made available and used as baseline information in future conservation planning and management purpose.
   100% completed. We found high level connectivity and dispersal capacity within Hong Kong for the three octocoral species analyzed here, suggesting that if appropriate conservation measures are implemented, the populations may have the capacity to sustain themselves.

4. Photos will be published as species photo catalogues and/or simple field guide of octocoral communities in HK western and southern waters. The photos will be presented on the website of the applicant's lab and Agricultural, Fisheries and Conservation Department (AFCD), as well as Facebook/Instagram account for the applicant's lab that can be accessed and visualized by general public beyond academics.
   100% completed. We have obtained images for many HK octocoral species both in their natural habitat underwater and close up photos taken in laboratory (see Figure 4 & 5 for examples). Some of the images were presented in the report and internet, while the others will be displayed in near future.

List of completed list of completed activities against the proposed Work Schedule

Proposed schedule	**Completed activities**
Recruitment of project staff	A junior research assistant was recruited on Oct 2022
Field sampling	Octocoral samples were collected from 27 different sites during the project period.
Laboratory work (including but not limited to photography, identification, DNA sequencing etc.)	- Field and lab photo for the target species and some other octocoral species encountered during the trip were taken (see Figure 1, 4 & 5) - The octocoral species were identified through field observation and sclerite morphology (Figure 4 & 5). - A total of 52 *Dendronephthya* and 47 *Echinomuricea* samples were successfully sequenced.
Data analyses	- Sequenced raw reads from high throughput sequencing were mapped to the reference genomes and then the single nucleotide polymorphisms (SNPs) among individual were identified. - Genetic diversity within population and differentiation among populations were inferred from the SNP data.
Preparation of deliverables	The photo and species distribution map of octocoral map was compiled
Preparation of interim and final progress reports	A progress report was submitted at Jan, 2023 and a final report was also prepared and submit here.
Dissemination of project findings (i.e. journal publication/ sharing session with public)	- A scientific journal manuscript is under preparation for submission. - The distribution map and photo of the octocoral species will be displayed in the website of the applicant for dissemination.

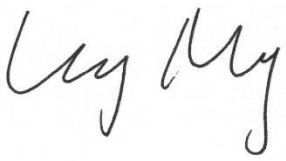
Staff attendance record and staff recruitment record are not disclosed due to confidentiality reasons.

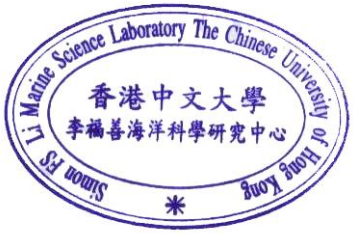
Declaration

"I hereby irrevocably declare to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that all the dataset and information included in the completion report has been properly referenced, and necessary authorisation has been obtained in respect of information owned by third parties."

Name of Principal investigator: TSANG Ling Ming

Signature of Principal investigator:





Disclaimer

"Any opinions, findings, conclusions or recommendations expressed in this report do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee."

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