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Spatial Structure and Genetic Variation of a Mangrove Species (*Avicennia marina* (Forssk.) Vierh) in the Farasan Archipelago

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Abstract: *Avicennia marina* (Forssk.) Vierh is distributed in patches along the Farasan archipelago coast and is the most common mangrove species in the Red Sea. However, to date, no studies have been directed towards understanding its genetic variation in the Farasan archipelago. In this investigation, genetic variations within and among natural populations of *Avicennia marina* in the Farasan archipelago were studied using 15 microsatellite markers. The study found 142 alleles on 15 loci in nine populations. The observed (H_o) and expected (H_e) heterozygosity values were 0.351 and 0.391, respectively, which are much lower than those of earlier studies on *A. marina* in the Arabian Gulf. An inbreeding effect from self-pollination might explain its heterozygote deficiency. Population genetic differentiation ($F_{ST} = 0.301$) was similar to other mangrove species. Our findings suggest that the sea current direction and coastal geomorphology might affect genetic dispersal of *A. marina*. The more isolated populations with fewer connections by sea currents exhibited lower genetic variation and differentiation between populations. The genetic clustering of populations fell into three main groups—Group 1 (populations of Farasan Alkabir Island), Group 2 (populations of Sajid Island), and Group 3 (mix of one population of Farasan Alkabir Island and a population of Zifaf Island). More genetic variation and less genetic differentiation occurred when the population was not isolated and had a direct connection with sea currents. Both of these factors contributed to limited propagule dispersal and produced significant structures among the population. It is expected that the results of this research will be useful in determining policy and species-conservation strategies and in the rehabilitation of *A. marina* mangrove stands on the Farasan islands in an effort to save this significant natural resource.

Keywords: *Avicennia marina*; genetic variation; conservation; the Farasan archipelago; genetic structure

1. Introduction

Mangroves are trees or shrubs found in coastal areas, lagoons, estuaries, and deltas. They form the main vegetation in tidal and saline wetlands, in turn providing habitats for many other species [1]. They are present in the tropics [2], and grow in mud with a flow of freshwater bringing nutrients [3]. The most-extensive mangrove areas are situated in Asia (42%), Africa (20%), North and Central America (15%), Oceania (12%), and finally South America (11%) [4]. They play a vital role in marine life and fisheries by providing food and shelter for a large and varied group of marine organisms, including fish and shellfish [5], and protect coastal areas from storms and sea level rises [6]. Globally, many mangrove forests have been converted to productive lands for agriculture and aquaculture [7]. Estimates of loss range from 35 to 86% [4,8] for the last two decades. Approximately only 6.9% of

the world's mangrove areas are protected under the International Union for Conservation of Nature (IUCN) program [8]. These mangrove communities are vulnerable to threats, mainly due to human impact through coastal construction, industrial pollution, littering, a loss of water quality, and the development of fisheries [9–12]. In addition, in some areas, natural disasters, such as earthquakes, tsunamis, coastal erosion [13,14], and climate change [15,16], threaten mangroves.

The first record of mangroves in the Red Sea dates to 323 BC [17,18]. Mangroves are not continuously distributed in the Red Sea and show evidence of being at the edge of their environmental range, being stunted in comparison to other deltaic and estuary areas in the world [19]. This is mainly due to a high salinity, reaching around 40‰, as well as poor soil textures and very high seawater temperatures (32 °C) [19,20]. A study conducted by Saifullah (1997) revealed that the conditions in the southern part of the Red Sea are more favorable for mangroves than the northern parts because of higher nutrient concentrations, more rainfall, many runnels, and a lower salinity due to the connection to the Indian ocean and the water flow from the Gulf of Aden into the Red Sea [21]. Although large stands of mangroves are found along the Red Sea mainland coast, the Farasan archipelago, which is an uplifted fossil coral reef in the southern part of the Arabian coast of the Red Sea [22–24], only has around 36.15 km² of mangroves [25]. The Farasan archipelago is characterized by a high humidity, high mean annual temperatures, and low rainfall [26]. Two species of mangrove occur on these islands: *Avicennia marina* and the less common *Rhizophora mucronata* [27,28].

This paper focuses on *Avicennia marina* (Avicenniaceae) in the Farasan archipelago because it is the dominant mangrove species and because it can reproduce and thrive across a wide range of climatic, saline, and tidal conditions [29,30]. It has several mechanisms leading to a high tolerance of high salinity levels, reaching 70‰ for populations of *Avicennia marina* in the Arabian Gulf [31]. The scientific literature on *A. marina* found on the Farasan islands is limited in coverage of the distribution and coastal ecology. The distribution size of *A. marina* in the archipelago ranges from stunted bushes, usually growing on the inner fringes of the stand, to well-developed trees reaching up to 4 m in height. This species has been reported on three major islands, including Farasan Al-Kabir, Sajid, and Zifaf (Figure 1); the largest stands occur within the Port of Farasan Alkabir Island [32,33].

Figure 1. Geographical locations of the nine natural populations of *Avicennia marina* in the Farasan archipelago.

A. marina is important for the people of the islands and there is a strategy for its conservation in the natural environment [11]. However, the loss and fragmentation of *A. marina* are enormous at a regional level. Camel grazing has been reported to be a major problem causing the degradation of *A. marina*, excluding only a few stands growing on Zifaf Island [12]. Cutting has led to a reduction in the number of trees [34]. Mass mortality of *A. marina* trees has been caused by the construction of a new soil dam,

leading to dry-up, particularly for one stand of *A. marina* on Farasan Alkabir Island. This modification in the topography of the coastal area diverts tidal water away from *A. marina*, reducing the area of this species inundated by tidal water in the upper reaches of the stands [12].

The pollution of *A. marina* areas is largely confined to domestic solid waste, such as polythene bags and bottles, plastic, and metal cans, which are disposed of in large quantities by tourists visiting places near *A. marina* populations [35]. This practice may have a serious impact by covering the young seedlings and pneumatophores, and blocking tidal channels [36]. The industrial/commercial and artisanal fisheries sectors and coastal urbanization are likely to increase [37]. Many changes are expected on land, and a large part of southeast Farasan Alkabir has been earmarked for a naval base. This development could affect terrestrial and marine wildlife in the area [36].

To provide an integrated management plan for the species, the use of genetic-based methods to measure the diversity and structure among and within populations is fundamental [38]. For *A. marina* worldwide, genetic diversity has been studied using allozymes [39], random amplified polymorphic DNA (RAPD) [40–42], amplified fragment length polymorphisms (AFLPs) [43,44], and microsatellites (SSRs). SSRs have proved appropriate for conservation studies focused on estimating population sizes, population structures, genetic variation, genetic drift, and inbreeding [45]. SSRs have been widely used to study the population-level variation of *A. marina* in South Africa and Oceania [46–49]. In addition, the population structure of *A. marina* has been studied by many researchers and two scenarios have been found. First, some researchers have suggested that there are no discrete boundaries between different parts of the range with allele frequencies changing gradually without any discontinuities, and with little genetic structure [50,51]. This scenario is a rare case in mangrove forests; however, it is common in major forest trees [52,53]. Others have reported propagule dispersal limited by barriers of sea movement and/or isolation by distance causing highly genetic structures between populations [54,55]. Table 1 presents summaries of the findings of these studies on *A. marina*.

Only one study has focused on *A. marina* in the Red Sea [47], which concluded that the loss of genetic diversity in the northern Red Sea populations could be attributed to limited gene flow and increased genetic drift arising from a patchy distribution. This study suggested that the Red Sea plants have a distinct genetic composition in comparison to other regions of the Indo-Pacific Ocean [47]. To date, the extent and patterns of genetic diversity in natural *A. marina* populations in the Farasan archipelago are unknown.

Table 1. Summary of the findings from genetic variation and genetic structure studies on *A. marina*.

Study Area	No. of Pop	Marker	No. Loci	No. Alleles	Genetic Diversity	Fst	Genetic Structure	Data Analysis	Study Source
Pichavaram, Bhitarkanika Goa, Calicut	10	RAPD	14	132	27.47%	26.5%	Discrete subpopulations, 77% differential among populations.	Multivariate statistics package	[40]
Worldwide	18	Allozymes	8	26	High level 0–0.217	Low level 0.384	Discrete subpopulations, gene flow among populations was relatively low, except where populations were geographically continuous.	Biosys-1	[39]
India	10	RAPD	14	172	76.7%	-	-	Nei's index, UPGMA tree	[41]
Worldwide closet site = 500 km	14	SSRs	3	70	0.407	0.384	Discrete sub-populations with inbreeding at the edge.	AMOVA- Mantel tests- Genepop	[54]
Australia	6	SSRS AFLPs	3 3	52 918 b	0.78 0.193	-	-	GeeneAIEEX (AMOVA-Mantel test)	[43]
Local scale: Sydney	9	Isozyme	22	83	-	-	Isolation by distance with inbreeding in each estuary.	AMOVA, TWINSpan	[56]
Vietnam	6	AFLAP SSRS	3 5	232 21	0.086 0.210	0.262 0.338	Discrete sub-populations with inbreeding at all sites.	Popgene 3.2, PHYLIP	[44]
Southeast Asia	12	SSRs	7	118	0.15–0.79	0.01–0.67	Discrete sub-populations with reduced diversity at range edges.	Bottleneck 1.2.02, Genetix.	[55]
Iran	3	SSRs	3	14	0.451 to 0.667	0.03–0.05	Reduced level of genetic variation was found in the central population, indicating a strong genetic structure among the other population with a large area and less exploitation.	Popgene 3.2, UPGMA tree	[57]
Arabian Gulf	4	SSRs	5	4 to 4.6	0.782 to 0.960	0.044	Low genetic differentiation among the populations.	FSTAT, UPGMA tree by TFGA Ver. 1.3	[48]
East coast of India	3	RAPD	10	388	0.2274 ± 0.1122	-	-	PopGene	[42]
North Red Sea coast	3	Est SSRs	5	3–6	0.5–0.7	-	-	GeeneAIEEX Version 6.4	[47]
Indonesia	3	SSRs	4	14	0.54–0.6	0.002–0.09	Low level of genetic variation in heavily polluted area.	PopGene, AMOVA by GeneAIEEX Ver. 6.4, UPGMA tree by NTSys	[49]

Sexual reproduction in *A. marina* is conducted through flies and bees. The production of one-seeded fruits may be due to maternal resource limitations or the maternal regulation of seed sets. Each fruit contains one seed, and it grows and matures within 4 weeks [58]. In all these species, the single seed is not germinated directly to produce offspring but remains within the fruit, while still on the maternal parent. This is a specific distinguishing feature of “crypto-viviparous” mangroves [39]. The propagules are flattened ovoids that are small and light, floating on the surface of the water, and the entire embryo is buoyant after detachment from the maternal parent [39]. When the seedlings settle, a radicle penetrates the sediment before the cotyledons unfold. The first formal leaves appear one month after germination and the second pair after one to two months [59].

Propagule dispersal is an important ecological factor in understanding the distribution of mangrove populations and patterns of genetic diversity. The high density of the propagules of *A. marina* is not found in other mangrove species. Due to their high density, they are not affected by wind action [60]. Knowledge of dispersal distances and directions is essential, as it allows an assessment and prediction of the chance that propagules reach and colonize remote habitat fragments. Flowers appear all year round, but propagule maturation occurs during July–August in the northern hemisphere [50,61]. It has been suggested that the dispersal of *A. marina* is strongly affected by sea currents [50]. In addition to the effect of sea currents on the genetic structure of *A. marina*, some barriers exist, such as land barriers in fragmented habitats, the distance between populations [44,56], the geomorphological coast line of the islands (Pavlopoulos et al., 2018), and sea level change. Although there are limited studies on the impacts of climate change on mangroves within the Middle East, a recent study by [62] estimated the recent relative sea level rise (SLR) to be 3.3 mm/year in the Gulf of Aden and the Red Sea. This may threaten the mangroves, as [63] have suggested that up to 96% of coastal wetlands including mangroves are likely to be lost from the region because of the sea level rise.

Here, microsatellite markers, which were developed for *A. marina* by [46], were used to: (1) assess the genetic diversity of the mangrove species *A. marina* in the densest area of the Farasan archipelago, (2) identify levels of inbreeding in populations of *A. marina* found on the Farasan islands, and (3) investigate the genetic structure of populations of *A. marina* found on the Farasan islands.

2. Material and Methods

2.1. Study Area

A. marina leaves were collected from nine populations across the Farasan archipelago, during three field studies, between 2015 and 2016 (Table 2). Populations were sampled based on the accessibility of different habitats (Figure 2) and ensuring 5 m between samples to prevent sampling ramets. The number of individuals collected ranged from six to 33 per population, based on the accessibility of samples.

Table 2. *A. marina* populations tested (name and code), latitude and longitude of the location, island area, island habitat, and inhabited status.

Island's Populations	Habitat	Inhabited Area	Code	Latitude	Longitude
Farasan Al-Kabir	Sand	Yes	Pop1	16.708608	42.177948
Farasan Al-Kabir	Sand	Yes	Pop2	16.752721	42.065627
Farasan Al-Kabir	Rocky	Yes	Pop3	16.704969	42.172678
Farasan Al-Kabir	Rocky	Yes	Pop4	16.804166	42.069108
Farasan Al-Kabir	Sand	Yes	Pop5	16.747602	42.000543
Sajid	Sand	Yes	Pop6	16.756755	42.00426
Sajid	Sand	Yes	Pop7	16.856417	41.968098
Sajid	Sand	Yes	Pop8	16.857648	41.981981
Zifaf	Rocky	No	Pop9	16.733156	41.745078

Figure 2. Different habitats of *A. marina* in the Farasan archipelago. (A) coral sand on Farasan Alkabir Island, (B) sand on Sajid Island, (C) rocky habitat on Farasan Alkabir Island, (D) propagule of *A. marina*, and (E) the flower of *A. marina*.

Avicennia marina populations on the Farasan islands occur in discontinuous stretches along the coast in suitable environments interspersed by unsuitable habitats, such as long sandy beaches and rocky shores. The island's name and size, sampling locations, and coordinates were recorded using a global positioning system (GPS) and are given in Table 2. Permission for sampling was obtained from the Saudi wildlife authority in the Kingdom of Saudi Arabia because these islands are registered as protected areas due to the presence of endemic Arabian gazelle.

2.2. DNA Extraction

Leaves were dried in silica gel prior to extraction. The total genome of DNA was extracted from each individual of *A. marina* using a modified CTAB protocol, following the method of [64]. The final DNA was suspended in 50 μ L of TE buffer. DNA extractions were visualized on 0.7% agarose gels in 1 $\times$ TAE buffer, in order to confirm the size and concentration of the extracted DNA, using Hyper Ladder™ 1kb (Bioline Reagents Ltd., London, UK) as a marker, and gels were illuminated by T: Genius. The concentration and quality were determined with NanoDrop Lite (Thermo Fisher Scientific Inc., Waltham, MA, USA). DNA was diluted to 5 ng/ μ L for polymerase chain reaction (PCR) amplification. After dilution, the stock DNA solutions were stored in the freezer at -20°C .

2.3. DNA Amplification by PCR

Microsatellite primer sequences (16 pairs) published by [46] were applied to *A. marina* samples. Initial primer optimization and screening resulted in the selection of 15 of the original 16 published primer sets for this study. Primer M76 was discarded due to its failure to prime to the samples. The 193 *A. marina* individuals were genotyped by PCR with 15 polymorphic microsatellite markers, as described in [48,54]. Primers were labeled with fluorescent dyes (6FAM, VIC, PET, and NED) for suspend amplification. The 1 $\times$ PCR buffer contained 6 μ L BioMix™, 2.6 μ L water, 0.2 μ L of each primer, and 10–50 ng genomic DNA and yielded a total volume of 10 μ L.

The conditions for amplifying the loci (M3, M32, M34, M40, M47, M62, M64, M73, M75, M81, M98, and M85) were one activation cycle at 94°C for 3 min, followed by the samples being incubated

for 30 cycles of 94 °C for 30 s, 60 °C for 35 s, and 72 °C for 1 min. Reactions were completed by incubating samples at 72 °C for 5 min and holding them at 4 °C. For loci (M13, M27, and M49), the conditions were one activation cycle at 94 °C for 3 min, and then 35 amplification cycles as follows: 94 °C for 40 s, 56 °C for 40 s, and 72 °C for 30 s, ending with one extension cycle at 72 °C for 4 min. The microsatellite fragments were separated on 2% agarose gels in 1× TAE buffer. The allele size was estimated using Hyper Ladder™ 100 bp (100 to 1000 bp; Bioline Reagents Ltd., London, UK) as a marker. Amplified products were run on an ABI 3031xl automated sequencer with the GeneScan-600 LIZ size standard. Visualization and scoring were carried out using Geneious R11 [65].

2.4. Basic Genetic Parameters: Diversity and Differentiation

After genotyping, the Hardy–Weinberg equilibrium (HWE) was tested using the software popgene 32 [66]. Linkage disequilibrium (LD) between all microsatellite loci pairs was estimated for all accessions using *poppr* in Rstudio [67]. The number of permutations performed for the LD analysis was 999.

GenAlEx v6.5 software [68], was used to determine the observed heterozygosity (H_O), expected heterozygosity (H_E), number of alleles for each population, allelic richness (AR), and average of inbreeding coefficient F_{IS} and F_{ST} across all loci [69] to assess the population differentiation.

Population differentiation was measured between population pairs across all loci using *Arlequin* 3.5 [70].

2.5. Genetic Structure Across the Farasan Islands

A simple Mantel test was carried out to determine the relationship between the geographic distance and genetic distance of populations with 10,000 permutations. The genetic differentiation between population pairs was calculated as an F_{ST} computed with GenAlEx v6.5 [69].

AMOVA was carried out to examine the hierarchical partitioning of genetic variance, and was performed with GenAlEx v6.5 with 10,000 permutations. As a complementary visualization of the genetic structure, principal coordinate analysis (PCoA) was conducted for *A. marina* accessions using GenAlEx v6.5 software. The analysis computed a matrix of mean genotypic distance values between all pairs of individuals. A scatter diagram was plotted according to the eigenvalues along the first two principal coordinate axes, which accounted for most of the variation. Discriminant analysis of principal components (DAPC) was used to identify and describe genetic clusters. These analyses were done using the adegenet 2.00 package [71] and the visualization function for DAPC by ‘find.clusters’ [72]. A model-based clustering program Structure v2.3.4 was used to infer the population structure of all accessions in *A. marina* and the ΔK method was used with the Structure harvester v0.6.93 to identify the optimal K value following [73]. The length of the Burnin Period was 10,000 and the number of MCMC Reps after Burnin was 50,000. The ancestry model was a no admixture model and to ensure that the run was long enough, we obtained 20 different runs. The Unweighted Pair Group Method with Arithmetic (UPGMA) phylogram was clustered with the function *hclust*. Murtagh et al. [74] in Rstudio to estimate the genetic distance among accessions.

2.6. The Red Sea Movement

To generate the sea surface circulation, the data were collected from [75–78], and the Earth observatory EOS at NASA Goddard Space Flight Center. Circulation patterns were compared to the genetic clusters and clusters were identified by STRUCTURE on the map bb QGIS 12.18.16 [79].

3. Results

3.1. Basic Parameters: Genetic Diversity and Variation

Polymerase chain reaction optimization and presentation resulted in a range of 15 simple sequence repeat (SSR) published primers. The observed heterozygosity was slightly lower than the expected heterozygosity in all primers and populations. All the markers were polymorphic except M62 and a

total of 142 alleles were detected across the 15 primers (Table 3). The number of alleles per locus varied among markers, ranging from 2 (M62) to 16 (M3, M47, and M81). Major allele frequencies per locus ranged from 1.043 (M62) to 4.153 (M40) (Table 4). Primers M13, M27, and M62 might not be amplified in some individuals, which were preserved as missing data. Missing data comprised 0.06% of the entire data set for *A. marina*. According to the result of LD, alleles are linked across loci with $p < 0.001$ overall P_{ic} percent from 93.33% (pop1 and pop3) to 73.33% (pop8 and pop9). Five loci were monomorphic in some populations (M62 in all populations except Pop 2, M98 in Pop4 and Pop9, M27 in Pop2, M64 in Pop7, and M85 in Pop2 and Pop9). Genetic diversity index values ranged from 0.422 (Pop8) to 1.040 (Pop1), indicating low genetic diversity in Sajid and Zifaf Island populations whereas the Farasan Alkabir Island population indicated moderate genetic diversity (Table 4). Hardy–Weinberg Equilibrium (HWE) departures were tested. All of populations had significant departures from HWE, which corresponded to their significant levels of inbreeding. In each case, departures from HWE were due to homozygote excess. Expected heterozygosity (H_E) ranged from 0.500 (Pop1) to 0.252 (Pop8), based on populations analysis. The average number of alleles was from 5.733 (Pop1) to 2.067 (Pop8), indicating populations with a mean of 3.467 affected alleles. The number of alleles detected across the nine populations ranged from 86 (Pop1) to 31 (Pop8) (Table 3). The value of F (inbreeding coefficient) was negative in Pop3 from Farasan Alkabir Island and Pop9 from Zifaf Island, implying a considerable degree of outbreeding, whereas positive F in the other populations showed a considerable degree of inbreeding. Pairwise F_{ST} values showed significant differentiation between the two populations and among the pairs of all subpopulations ranging from 0.886 to 0.004 (Figure 3), which indicated that the populations and the subpopulations were significantly different. Pop3 and Pop8 were more differentiated from each other according to the F_{ST} value (0.886), followed by Pop3 and Pop6 ($F_{ST} = 0.866$). The AMOVA for the nine populations revealed that 55% ($p < 0.001$) of the genetic variation is found within populations, whereas 45% ($p < 0.001$) of the genetic variation is found among populations.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8
Pop1								
Pop2	0.574							
Pop3	0.061	0.546						
Pop4	0.038	0.588	0.061					
Pop5	0.016	0.547	0.078	0.04				
Pop6	0.771	0.34	0.866	0.774	0.728			
Pop7	0.773	0.362	0.835	0.796	0.739	0.088		
Pop8	0.802	0.332	0.886	0.823	0.755	0.039	0.121	
Pop9	0.742	0.004	0.69	0.753	0.691	0.339	0.355	0.315

Figure 3. Heatmap of pairwise F_{ST} values estimated from microsatellite data between all populations. All values are significant at the $p < 0.001$ level, a dark color indicates the minimum value of genetic variation, and a light color indicates the maximum value of genetic variation between populations. For abbreviations of localities see Table 1.

Table 3. Simple Sequence Repeat (SSR) primers screened for SSR-polymerase chain reaction (PCR) in *A. marina*: primer sequence, repetitive sequence, range size (bp), total number of alleles per primer over all localities (A), observed heterozygosity (Ho), expected heterozygosity (He), gene flow (Nm), and F-statistic (Fis, Fit, and Fst).

Locus	Primer Sequence	Tm (°C)	Repetitive Sequence	Range Size (bp)	A	Ho	He	Nm	Fis	Fit	Fst
M13	F:CAATGGTGATTCTCCAAAATTGCTTTG R:TGGTGAATAGATGACAGTAAGGATCAGCC	56	(AT)10(GT)12	175–205	12	0.777	0.627	0.889	−0.238	0.033	0.219
M3	F:GGTTCCTGCAAGTATGTCAACACCCTC R:ACCTCGATTCTCCCCGAATGC	60	(TG)15	183–200	16	0.694	0.709	0.965	0.021	0.222	0.206
M27	F:GGTGGAGTTTCAGTTCATCGTTTCG R:CCGCAGTGGGGTTCATCAAAC	56	(CCG)8	103–108	5	0.383	0.280	1.666	−0.370	−0.191	0.131
M32	F:TGTGAACTTTGCTTCAGAGTCTCGAAGATG R:AGTCAAATGGAGCCTCATTCCTCCG	60	(AC)14	156–177	11	0.417	0.370	2.252	−0.126	−0.014	0.100
M34	F:TCTGCTGTTGCTGTTGTTGTTGATGC R:TGGTGTTGAAGACTAATCATGTGTTTCGC	60	(GCT)14	189–194	4	0.262	0.215	0.147	−0.218	0.550	0.630
M40	F:CCCATAGATGACGGCAATCTTATGATCC R:ACCATCCAAAATAAAATAAATCTCCCTCCC	60	(AG)32	140–168	14	0.609	0.682	1.284	0.106	0.252	0.163
M47	F:TGACACCAAGGGAAATCAACATGCC R:GAACCTAGCGACCAATAGATCATCTCTGG	60	(CA)13	180–210	16	0.333	0.665	0.813	0.499	0.617	0.235
M49	F:TTTCCTCACGACAGACTAGAAACCACC R:CAATAAACTTGGATAAAGGCAACTCCGAC	56	(TG)16	176–180	5	0.098	0.297	0.572	0.671	0.771	0.304
M62	F:TTGAGGAAAACATGGGACTTTCCTCG R:GTGGGAGTAGCCGCATAGAGTCACG	60	(CGC)8	227–229	2	0.000	0.031	0.017	1.000	1.000	0.938
M64	F:CAAACCCTACCAATCAGAACAATTCAAGC R:CGATATTTGGCTAATCCACTCTGCTGACTG	60	(CAG)8	148–158	7	0.395	0.330	0.192	−0.199	0.479	0.565
M73	F:TTCCACAATCACTTGACCCTCGTCC R:TCTTCACAGGTCTCTCTGCCCTG	60	(TG)15	167–172	12	0.184	0.276	0.180	0.334	0.721	0.582
M75	F:TCCATAATCAAACAACCTCGACAACGAAATC R:TCTTCTCTCCCTATTCCAAACTGGCTTG	60	(TG)14	208–210	9	0.412	0.574	1.688	0.281	0.374	0.129
M81	F:GAATGATGATCGGATGTTGCTACTCCTG R:CAATCCCAAAGCCCCAAAATAATCC	60	(CA)9(CT)1	154–167	16	0.292	0.429	0.351	0.318	0.602	0.416
M85	F:TGACAGAGGTTTAGAGACATGGAGGGTGAG R:TGCCTCCACATTCACCACACTGC	60	(GGC)8	105–118	9	0.201	0.224	2.251	0.104	0.193	0.100
M98	F:CCCAAACCTCGTTACGATGGATGACTTC R:CTTACAGTTGCGGTAAAATGAGACGTGC	60	(CGG)8	211–220	4	0.212	0.186	2.077	−0.139	−0.017	0.107

Table 4. Descriptive statistics of the overall loci for each population of *A. marina*: number of individuals (N), number of alleles (A), average number of alleles per locus (Na), percentage of polymorphic loci (P_{ic}, %), number of effective alleles (Ne), observed heterozygosity (H_o), expected heterozygosity (H_E), Shannon's information index (I), and fixation index (F).

Population	N	A	Na	P _{ic}	Ne	H _o	H _E	I	F
Pop1	33	86	5.733	93.33%	2.759	0.434	0.500	1.040	0.114
Pop2	6	48	3.200	86.67%	2.236	0.356	0.421	0.791	0.190
Pop3	11	55	3.667	93.33%	2.412	0.461	0.459	0.878	−0.047
Pop4	19	58	3.867	86.67%	2.398	0.344	0.437	0.841	0.169
Pop5	32	61	4.067	93.33%	2.402	0.404	0.462	0.888	0.084
Pop6	50	54	3.600	86.67%	2.045	0.320	0.389	0.740	0.098
Pop7	20	40	2.667	80.00%	1.679	0.273	0.292	0.535	0.028
Pop8	10	31	2.067	73.33%	1.470	0.253	0.252	0.422	0.005
Pop9	12	35	2.333	73.33%	1.820	0.317	0.325	0.557	−0.041
Total/Mean	193	52	3.467	85.19%	2.136	0.351	0.393	0.743	0.070

3.2. Population Structure and Cluster Analysis Across the Farasan Islands

Significant genetic divergence between populations from different islands was detected in *A. marina* populations, with the exception of Pop2 on Farasan Alkabir Island, which was most closely linked to the population of Zifaf Island. Six different genetic analyses detected *A. marina* populations in the Farasan archipelago: Mantel tests revealed a weak positive correlation between the genetic distance matrix and the geographic distance matrix among the islands (Figure 4) ($R^2 = 0.0778$; $p < 0.054$).

Figure 4. Mantel's test for the correlation between Fst genetic distance and geographic distance (km) ($R^2 = 0.0508$; $p < 0.05$).

The PCoA (Figure 5), DAPC (Figure 6), and structure analysis showed the distinct clustering of individuals based on the origin of the island, and the first two principal component axes cumulatively accounted for 90% of the total variance in *A. marina* accessions on the different islands, including Group 1 *A. marina* on Farasan Alkabir Island (Pop1, Pop3, Pop4, and Pop5), Group 2 *A. marina* on Sajid Island (Pop6, Pop7, and Pop8), and Group 3 accessions from Farasan Alkabir Island (Pop2) and *A. marina* from Zifaf Island (Pop9).

The results from the structure harvester analysis indicated a high peak for the number of clusters when the K value was 2.5–3. This meant that the optimal value of K should have been K = 3. A UPGMA (Figure 7) tree of all 193 accessions was constructed based on Nei's genetic distance and all the accessions were assigned to the three main clusters. The clustering of accessions in the UPGMA tree was generally in agreement with the population structure identified by the structure analyses.

Figure 5. Principal coordinate analysis (PCoA) of the microsatellite diversity among the 193 accessions of *A. marina*. Samples are color coded according to their membership in the nine populations.

Figure 6. Discriminant analysis of principal components (DAPC) among nine natural populations of *A. marina* across the Farasan archipelago. Eigenvalues are presented here.

Figure 7. UPGMA tree of *A. marina* from nine natural populations along the Farasan archipelago, red color includes individuals from Farasan Alkabar Island (Pop1, Pop3, and Pop4), blue color includes individuals from Sajid Island (Pop5, Pop6, and Pop7), and green color includes individuals from Zifaf Island and Farasan Alkabar Island (Pop2 and Pop9).

3.3. Red Sea Current Surface Simulation

The Red Sea current flows in a southeast direction from the Indian Ocean to the northwest direction (January) and there are strong north-west and east-west current drifts during the maturation period of *A. marina* in the winter season. In contrast, during the summer season (June), the maps of surface circulation climatology indicate that the current direction is from the north to south direction and the flow through the northern part is stronger than that in the southern part of the islands. The genetic link detected between pop9 from Zifaf Island and Pop2 of Farasan Alkabir Island was congruent with Red Sea current patterns on the northern side of the islands (Figure 8).

Figure 8. Maps depicting the genetic structure among populations of *Avicennia marina* and the sea surface circulations in the Farasan archipelago. (A,B) Surface current climatology from the model is shown for (A) the summer season (B) and winter season, where the number indicates the current speed in m/s. (C) Structure bar plots showing the assignment of individuals to three distinct genetic clusters (K = 3).

4. Discussion

In this investigation, *A. marina* from the Farasan archipelago was studied to understand the genetic variation and the pattern of population structure of this ecologically- and economically-dominant

mangrove tree species. This study included 193 individuals and 15 SSR loci, and is more comprehensive and representative in comparison with previous microsatellite studies of *A. marina* because the number of samples and SSR loci were low in these studies [46–48,57]. This study showed that the lack of polymorphism of some loci could be associated with shorter repeats (GGC) 8 and Tri or combined repeats type, which were more frequently monomorphic than long repeats, and dinucleotide repeats (TG) 15 [80]. Departures from HWE of a similar magnitude were also reported in *A. marina* in Vietnam [44] and *Avicennia germinans* and *Rhizophora mangle* in the Caribbean and the Pacific estuaries of Panama [81].

In the analyses of allelic diversity per locus, the average allele diversity found here was 9.4, showing a slightly high level of allelic diversity compared to the values detected previously for *A. marina* samples from the Australia's Northern Territory (6.7) [43,46,48]. This is possibly because these previous studies used less than half of the SSR loci and small-sized populations. The allelic diversity in this study showed a lower level of allelic diversity than [46], who investigated six different Australian states and territories (17.3).

The SSRs were more informative for understanding population genetic diversity when the studies used a greater number of loci and increased the number of samples. A comparative analysis of allelic diversity across the populations, focused on the observed and expected heterozygosity, revealed that *A. marina* populations display moderate genetic variation on Farasan Alkabir Island, which is consistent with the study of *Avicennia marina* worldwide (H_e 0.494; Maguire et al., 2000) and north of the Red Sea [47]. The low genetic variation on Zifaf and Sajid islands was also revealed for *A. marina* in Vietnam (H_e 0.322; Le et al., 2003); Australia, especially the northern population [43]; and the east coast of India [48]. In comparison with *A. marina* populations in the Arabian Gulf [42], the Red Sea populations show a lower genetic diversity, because of the harsh environmental conditions in the Red Sea. Authors of previous studies suggested that a reduction in the heterozygosity level may be due to repeated bottlenecks and founder effects [82,83], which are associated with the geoclimatic history in the Red Sea and genetic drift in the small isolated populations. This could explain the pattern of *A. marina* populations in the Farasan archipelago. The historical Red Sea level fluctuations due to past climatic changes [84] would further reduce the long-term effective population size, in addition to distinct genetic compositions, which would lead to low genetic diversity within populations. Besides the sea level fluctuation factor, the patchy distribution of *A. marina* populations along the coast of the Farasan archipelago, with a complicated geomorphological coastline [85], prevents genetic exchanges among sampled populations, as demonstrated in the study on the north Red Sea by [47]. The pattern of isolated populations increases the inbreeding coefficient, which is the most reasonable explanation for heterozygote deficiency. Moreover, strong inbreeding and high self-fertilization have been reported as common characteristics among mangrove species *Avicennia germinans* and *Rhizophora mangle* [81].

The estimation of F_{ST} showed high genetic differentiation among populations (0.322). The value was similar to those in populations of *A. marina* worldwide [8,42] and in Vietnam populations [44]. This indicates that the gene flow is limited between populations within the archipelago. As *A. marina* pollen dispersal can only occur within populations or between populations, propagule dispersal is the main factor for the genetic connectivity in *A. marina* populations. In this study, the dispersal of *A. marina* propagules was limited by sea currents and the gene flow has shown the populations which are separated by a few tens of kilometers [54,86]. Therefore, it is clear that *A. marina* in the Farasan archipelago exhibit significant genetic differentiation among populations. The hierarchical analysis of molecular variance (AMOVA) also showed that most of the variation was partitioned among populations and within individuals in the total population. There was little variation among individuals within populations. From these analyses, discrete subpopulation patterns are likely for *A. marina* which agrees with [40,44,54,57].

The results of Bayesian clustering showed that the genetic structure of *A. marina* was affected by many factors, such as the isolation by distance, geomorphological coastline, and direction of sea currents (Figure 8A,B). The isolation by distance has weak effects on the genetic structure, as indicated

by the Mantel test, which displayed a weak correlation between the genetic and geographic distance. For example, populations 2 and 9 have the greatest geographic distance (49.28 km); however, they share the same genetic group. In this case, migration could have been driven by high-speed currents during winter seasons. However, population 5 displays a distance of only 1.460 m over the water from the nearest population, which is population 6; these two populations exhibited significant differentiation between each other and each population in a different genetic group [54,57].

The geomorphological coastline plays important roles in the distribution pattern of populations in the Farasan archipelago and has a strong effect on the limited dispersal ability of propagules. The populations that are isolated by land and with less interconnected sea currents have smaller gene flow, as shown among populations between islands. In contrast, a higher level of genetic exchange occurred when the population location was directly connected by the sea currents and there were no land barriers, such as the populations of each island on the same coast [44].

There is a correlation between the genetic structure and the sea circulation patterns in the archipelago, preventing propagule exchange between Farasan Alkabir populations and Sajid Island populations. Further evidence for sea circulation in the northern part of the archipelago revealed the mixing of some individuals from Farasan Alkabir Island and Zifaf Island (Figure 4C). This similar congruence between the genetic structure and bifurcating sea currents was also found in *A. marina* populations in southeast Asia [55] and Vietnam [44]. Hence, the explicit inclusion of sea circulation patterns in phylogeographical studies of coastal (even if it is a straight coast) and marine organisms is valuable in understanding complex genetic structures.

5. Conclusions

Populations of *A. marina* are threatened by complicated geomorphological coastlines, human-induced pressure, urbanization, wood harvesting, grazing, and tourism sector development. This could lead to a loss of genetic diversity through increased population fragmentation of *A. marina* in the Farasan archipelago. The use of microsatellites to assess the genetic diversity of natural populations can support the improved conservation of *A. marina* in the Farasan archipelago, which may be one of the most important issues facing the future of mangrove forestry practices. *A. marina* on Sajid and Zifaf Islands displayed a lower genetic diversity than populations on Farasan Alkabir Island. This suggests that sufficient genetic variation exists for the selection of superior individuals or populations to advance the genetic improvement program. These results contribute to knowledge of the population structure in the Farasan archipelago, as consequences of restricted gene dispersal. High levels of genetic structure on local scales can be detected here due to the coastal geomorphology, climate change, and the direction of sea currents. Therefore, they are likely to be of interest for conservation strategies and breeding or genetic-improvement programs not only based on preserving large areas, but also smaller and separate ones, in order to encompass the different genetic patterns found between the islands.

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