Genetic diversity and contemporary population genetic structure of *Avicennia marina* from Mozambique

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Highlights

- *Avicennia marina* species-specific microsatellites were used to determine genetic variation and its distribution along the Mozambique coast
- High levels of allele diversity and medium to low levels of inbreeding was observed for all the sampling localities
- The results suggested high connectivity and high levels of gene flow for *A. marina* along the coast of Mozambique
- Currents and eddies within the Mozambique Channel are responsible for the pattern of genetic variation of *A. marina*.

Abstract

The aim of this study was to determine the genetic diversity and population genetic structure of *Avicennia marina*, an important mangrove tree species with a wide distribution. Samples were collected from four sampling localities, characterised by different environmental conditions, situated along the coast of Mozambique within southern Africa: Pemba-Metuge, Bons Sinais Estuary, Costa do Sol and Inhaca Island. We used five species-specific polymorphic microsatellite loci for screening 20 samples from each of the sampling localities. The overall level of polymorphism observed was relatively high with 3–22 alleles detected at each locus. AMOVA showed the highest level of variation, 96.6 %, was located within populations. Pairwise F_{ST} comparisons were non-significant between sampling localities. No isolation by distance was detected. Structure results suggested the presence of one population after independent clustering of all samples. The results showed high levels of gene flow for *A. marina* along the coast of Mozambique and suggested the absence of barriers to gene flow. The results and information from this study will inform strategies for the conservation of *A. marina*. We discuss the importance of preserving the mangrove forest,

supported by our own result, by managing and reducing anthropogenic activities that can damage the genetic diversity within these localities that are under high pressure due to urban growth. This will positively contribute to how and where effort and resources should be focused on for its conservation and sustainable management and utilisation.

Keywords: Mangrove; Microsatellites; Gene flow; Mozambique channel

1. Introduction

Mangrove forests are plant communities, occurring within the intertidal zone of tropical and sub-tropical coastlines. They are productive ecosystems with important ecological and economic value. Mangroves house a vast range of biodiversity within its complex structure. They also protect the coastline by reducing impacts of natural catastrophes. They serve as breeding and nursery habitat for a wide variety of fish species. Mangroves also serve as a source of firewood, wood for charcoal production and poles for building houses (Maguire et al., 2000; Giri et al., 2011; Triest et al., 2018). Mangroves have high levels of above and below ground biomass that store carbon, making them globally significant carbon reserves. Mangroves, together with their associated soils, could sequester an estimated 22.8 million metric tons of carbon per year (Giri et al., 2011; Stringer et al., 2015). Despite the high ecological and economic value of mangroves, they are under severe pressure mostly due to anthropogenic activities. It is therefore essential to protect mangrove ecosystems (Maguire et al., 2000; Giri et al., 2011).

Globally, Mozambique is ranked 13th in terms of mangrove coverage, with a surface area of 318,851 ha, forming approximately 2.3 % of the global mangrove forest area (Giri et al., 2011). Mangroves are distributed along most of the Mozambican coast. The northern region, located between the River Rovuma and Angoche, has several islands (mostly at Quirimbas archipelago) that provide protection to mangroves and coral reefs. Here, the coastal plain is narrow, the rivers are non-tidal and the mangrove forest is mostly confined to the river mouths. The northern coast has well established mangrove creeks in Lumbo, Mecufi, Ibo Island and the mainland areas north of Pemba City (Barbosa et al., 2001). The central region located between the Angoche and Save River has wide and well established mangroves due to the alluvium and freshwater discharge that is obtained from 18 rivers running into the Indian Ocean. The estuaries that receive discharge from the big rivers such as the Zambezi, Púngue, Buzi and Save are all in this central region. The mangroves of the Zambezi delta extend inland for approximately 50 km. These mangroves are continuous from areas in the south to Quelimane City covering approximately 180 km of coastline and represents close to 50 % of the Mozambican mangroves (Barbosa et al., 2001). The southern region, from the Save River southward to Ponta do Ouro on the Mozambique/South Africa border, has a wide area of mangroves in the Morrumbene Estuary, Inhambane Bay, Maputo Bay and Inhaca Island. Maputo Bay with its four main rivers flowing into the bay is one of the main mangrove areas in southern Mozambique. At Inhaca Island, mangroves cover approximately 50 % of the coastline (Barbosa et al., 2001).

Avicennia marina (Forssk.) Vierh. is the dominant mangrove species in Mozambique (Bandeira et al., 2009). Its wide distribution has been associated with its high fecundity and its capacity to develop and reproduce across a wide range of climate, salinity, and tidal conditions. This suggests that the species has considerable growth plasticity (Arnaud-Haond et al., 2006; Almahasheer et al., 2016). As pioneer tree, *A. marina* usually occurs at seaward edges of mangrove forests where the salinity ranges from seasonally freshwater to

hypersaline conditions. On the landward side of a mangrove forest the salinity can reach up to 80 PSU (Practical Salinity Unit), often caused by a lower frequency of tidal inundation, where this is characteristic for the higher topographic areas (Nguyen et al., 2014). The origin of *A. marina* is believed to be in Australia where the earliest pollen fossil records have been recorded (Arnaud-Haond et al., 2006). It is thought that *A. marina* migration between Australia and Southern Africa potentially happened *via* an island archipelago which included the Indian subcontinent during the Late Cretaceous (Duke, 1995). Its distribution takes place by the cryptoviviparous water-dispersed propagules that is typical for *Avicennia* (Tomlinson, 1986). The dispersal and gene flow of *A. marina*, similar to other mangrove species, is predominantly explained by the dispersion of pollen, by wind or insects, over short distances at a within-site level, and long distance water dispersed of propagules. Restricted pollen and non-random propagule dispersal may result in fine-scale genetic structure (Hasan et al., 2018; Triest et al., 2018). Reduced reproductive success in small mangrove stands is thought to reflect a pollen specific limitation since the deposition of pollen on the stigma of flowers, within these smaller stands, are significantly reduced when compared to large mangrove stands (Hermansen et al., 2017).

Sea dispersal of mangrove propagules has been recorded for distances exceeding 3000 km, during periods exceeding 2.5 months. Propagules in eddy-driven marine currents may delay arrival at suitable sites for establishment and can be in transit for up to 100 days. The estimated diameter of circular movements range between 80 km and 200 km, which matches an estimated circular dispersal distance of 250–630 km, respectively (Tonné et al., 2017; Van der Stocken and Menemenlis, 2017). Van der Stocken and Menemenlis (2017) showed that trajectories connected locations on both sides of the Mozambique Channel through their simulation model that estimated the dispersal trajectories of propagules.

Main barriers that could influence the distribution of mangrove species in Mozambique include the southward anticyclonic eddies circulation present in the Mozambique Channel, the geomorphology of the coastline, effect of climate variation, reduction of fresh water flow due to the construction of dams, and anthropogenic overexploitation (Barbosa et al., 2001; Hancke et al., 2014). Anthropogenic threats to mangroves include urban development, direct conversion of mangrove forests for other land uses, aquaculture and overexploitation of timber as well as indirect effects such as sea level rise and rainfall patterns. These factors contribute to the reduction and fragmentation of mangrove areas, influencing the landscape connectivity and causing isolation, reduced gene flow, increasing endogamy and consequently leading to the reduction of genetic diversity. This will in return affect the ability of mangroves tree species to respond to new environmental conditions (Maguire et al., 2000; Kahrood et al., 2008; Pautasso, 2009).

Microsatellite markers have previously been used for studying the population genetic structure of *A. marina*. They have revealed reduced levels of genetic diversity and high inbreeding. Differences in connectivity range from high levels of genetic differentiation to moderate levels of gene flow in populations of *A. marina*. These patterns were suggested to be caused by environmental and ecological factors, local demographic conditions, consequence of historical sporadic arrival of founders and propagule dispersal properties, especially the dispersal limitations due to coastal geomorphology (Maguire et al., 2000; Arnaud-Haond et al., 2006; Kahrood et al., 2008; Zolgharnein et al., 2010; De Ryck et al., 2016; Manurung et al., 2017; Do et al., 2019).

According to our knowledge, there are no published studies and/or existing information about the genetic structure of mangroves from Mozambique. The present study, therefore, aimed to describe the genetic variation and the population genetic structure of *A. marina* from Mozambique. It is hypothesized that, since there is an absence of physical barriers for obstructing sea dispersal of mangrove propagules along the Mozambique Channel, that *A. marina* will show no contemporary geographical population genetic structuring. In general, this study will contribute to the genetic information available for mangroves and specifically for mangroves from Mozambique. This study may also in future contribute to how and where more effort has to be focused to ensure the sustainable utilisation, management and conservation of *A. marina* populations specifically in the regions investigated here, but possibly also in other countries where *A. marina* can be found.

2. Materials and methods

2.1. Sampling localities and sample collection

Four sampling localities were chosen for this study due to differences in the geomorphology and the dynamics of the local conditions where the mangrove forests are located. Costa do Sol and Pemba-Metuge mangroves are in general more influenced by bay characteristics, Bons Sinais Estuary mangrove is more an estuarine mangrove and Inhaca Island is a mangrove situated on an island where it is also influenced by the nearby bay with some swamp water (Barbosa et al., 2001; Bandeira et al., 2009; Amade et al., 2019). In addition, the zonation of the different mangrove forests considered here is distinct among the sampling localities. In the northern part of Mozambique, at Pemba-Metuge, the fringe part of mangroves is predominantly occupied by *Sonneratia alba*. The opposite is seen in southern Mozambican mangroves, at Costa do Sol and Inhaca Island, where the fringe zone of the mangrove is only occupied by *A. marina*. Anthropogenic disturbance of these mangroves also varies among the sampling localities. Costa do Sol is more affected by urban growth while at Pemba-Metuge, Bons Sinais Estuary and Inhaca Island the mangroves are more exposed to intensive selective harvesting (Amade et al., 2019). Pemba-Metuge is located at Cabo Delgado province on the North coast of Mozambique (Fig. 1). The mangrove forest in this locality is developed structurally (Table 1) (Amade et al., 2019). These mangroves at Pemba-Metuge are impacted through harvesting of wood for construction, firewood and charcoal production. Pemba-Metuge is situated approximately 842 km from Bons Sinais Estuary (Amade et al., 2019).

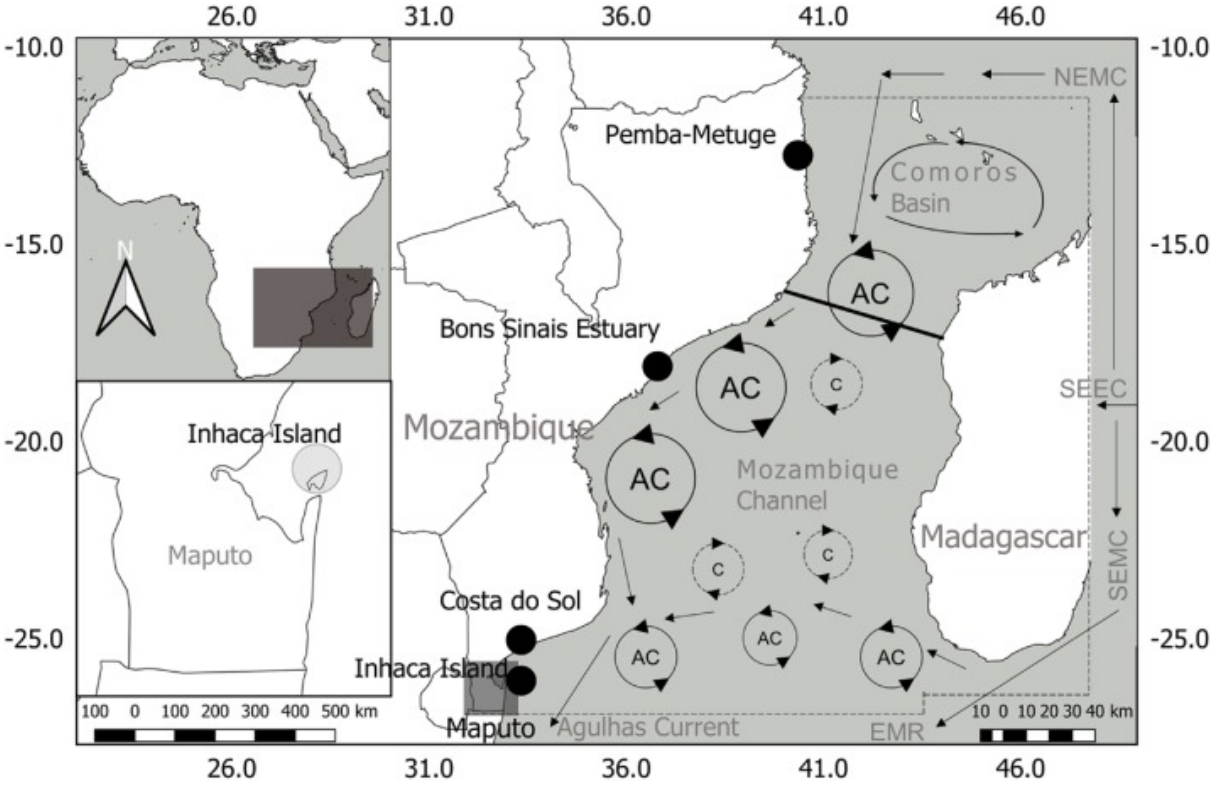

Fig. 1. Geographical locations of the four sampling localities, Pemba-Metuge, Bons Sinais Estuary, Costa do Sol and Inhaca Island, along the Mozambique coast where 20 samples per locality were collected. Anticyclonic eddies (AC), Cyclonic eddies (C), Southeast Equatorial Current (SEEC), North East Madagascar Current (NEMC), South East Madagascar Current (SEMC), East Madagascar Retroflection (EMR), extracted from (Hancke et al., 2014) (QGIS v.3.2.3, http://qgis.osgeo.org).

Table 1. Summary of mangrove forest attributes for sampling localities extracted from Amade et al. (2019).

Study sites	***Pemba-Metuge***	***Bons Sinais Estuary***	***Costa do Sol***	***Inhaca Island***
Location	Lat. 12° 58' 39.9'' S Long. 40° 24' 44.3'' E	Lat. 18° 01' 51.0" S Long. 36° 51' 15.2" E	Lat. 25° 55' 01.0'' S Long. 32° 38' 24.1'' E	Lat. 26° 02' 33.0'' S Long. 33° 54' 44.4'' E
Number of tree species	4	7	3	4
Mean tree height (m)	2.5	3.3	2.3	3.9
Tree basal area (m^2 ha^{-1})	50.7	51.8	24.2	10.3
Tree density of (trees ha^{-1})	3,580	2,680	1,790	1,966

Bons Sinais Estuary is situated within the Zambezia province in the centre of Mozambique (Fig. 1). The mangrove forest is well developed (Table 1) (Amade et al., 2019). This mangrove area is affected by pollutants from the port of Quelimane and domestic wastewater from the city that is discharged directly into the mangrove forest. Industrial and aquaculture

activities as well as harvesting of wood for firewood, construction poles and local production of charcoal also directly impacts the mangroves in this area. Bons Sinais Estuary is located approximately 1231 km of Costa do Sol (Amade et al., 2019). It is located within Maputo Bay, 7 km north of Maputo City (part of Maputo province), in the south of Mozambique (Fig. 1). The Costa do Sol mangroves are not structurally complex and are currently in the process of regeneration due to severe previous degradation (Table 1). The mangroves at this location are greatly affected by urbanization, pollution from the sewage of the city, harvesting for firewood and past aquaculture activities that changed the topography of some parts of the mangrove stand. This also changed the natural flow of water within the mangrove resulting in the restriction of the tide to reach certain parts. The Costa do Sol mangrove is situated at approximately 33 km from Inhaca Island (Amade et al., 2019). It is situated within the Maputo Bay area, 32 km east of Maputo City (Fig. 1). The mangrove forest is well developed (Table 1). Within this area mangroves are harvested for firewood and construction poles (Bandeira et al., 2009).

Leaf samples were collected at Pemba-Metuge, Bons Sinais Estuary, Costa do Sol and Inhaca Island. A total of 80 leaf samples, twenty per sampling locality, were collected at random in the four localities and stored at - 20 °C until further use (Maguire et al., 2000, 2002). Random selection sites were spaced far enough to avoid sampling clones of the same tree.

2.2. Genomic DNA extraction

Genomic DNA was extracted using a Zymo scientific plant/seed kit (Zymo Research Corporation), according to the manufacturer's instructions. The development of the microsatellite primers used in the present study has been discussed by Amade (2019). The 5′ end of the forward primers were labelled with different fluorescent colours. Atto dyes that are activated fluorescently are alternatives to common fluorophores like VIC, NED, and Pet (Amade, 2019). PCR amplifications were prepared in 20 µL volumes containing 25 ng genomic DNA, 1 unit of EconoTaq PLUS 2X Master Mix (Lucigen Corporation), 10 µM of the forward primer, 10 µM of the reverse primer according to the multiplex combinations (Amade, 2019). The PCR conditions were as follows: 95 °C for 2 min, followed by 20 cycles of 95 °C for 30 s, 50°–60 °C for 1 min and 72 °C for 2 min, and a final extension step at 72 °C for 20 min. Fragment amplification was performed in a Thermal Cycler 2720 (Applied Biosystems). Fragments were analysed on an ABI3500XL genetic analyser with a 50 cm capillary, using POP7 polymer (Thermo Fisher Scientific) and allele sizes were scored using the GeneMapper 5 software (Thermo Fisher Scientific) comparing the fragment sizes to the LIZ size standard (Applied Biosystems).

2.3. Data analysis

MICROCHECKER v.2.2.3. (Van Oosterhout et al., 2004) was used to investigate each locus for the potential presence of null alleles, allelic dropout and scoring errors. Linkage disequilibrium between loci across populations was tested in GENEPOP v.4.6 (Rousset, 2008) using a Markov Chain with 1000 iteration for dememorization, 100 batches and 1000 iterations per batch. Loci that showed linkage were further investigated to assess if alleles were linked by using GENETIX (Belkhir et al., 2004). Significance levels for multiple tests performed were tested using the false discovery rate (Benjamini and Hochberg, 2015). Locus by locus analyses were conducted by combining all samples as one population. Observed (*H*o) and expected (*H*e) heterozygosity were calculated for each locus using GenAlEx (Peakall and Smouse, 2012) and GENEPOP v.4.6 (Rousset, 2008). The observed number of

alleles per locus (A_N), allelic richness (A_R) and the inbreeding coefficient (F_{IS}) were determined using FSTAT 2.9.3. The lowest number of individuals considered for calculating allelic richness for all individuals (80) as one population was 62, and that for each population from each sampling locality was 13 (Goudet, 1995). Deviation from Hardy-Weinberg equilibrium (HWE) was assessed for each locus across the populations using GENEPOP v.4.6 (Rousset, 2008). The exact *p*-values of HWE tests were estimated using a Markov Chain with the parameters set as 1000 iteration for dememorization, 100 batches and 1000 iterations per batch. A global test of heterozygote deficiency was also performed using the same set of Markov Chain parameters. For each sampling locality the number of samples analysed (N), the mean number of alleles per locus (N_A), private alleles (P_A), observed heterozygosity (H_O), expected heterozygosity (H_E) and the unbiased expected heterozygosity (uH_E) were determined and calculated using GenAlEx (Peakall and Smouse, 2012).

Evaluation of the resolution power, estimating the lowest possible F_{ST} value for detecting population differentiation for the set of microsatellite loci used, were calculated using Powsim v.4.1 (Ryman and Palm, 2006). AMOVA and pairwise population differentiation between samples based on Wright's standardized variance in allelic frequencies (F_{ST}) was calculated using Arlequin v.3.5. (Excoffier and Lischer, 2010).

Bayesian clustering analysis was carried out using Structure v.2.3.4. (Pritchard et al., 2000), following the admixture model. The number of possible populations (K) tested varied from $K = 1$ to $K = 10$ with a burn-in length of 100 000, and 1 000 000 Markov Chain Monte Carlo (MCMC) repeats with 20 iterations. Results were collated using Structure Harvester (Earl and vonHoldt, 2012) and visualized using Excel. Selection of the optimal K-value was based on both the log-likelihood value closest to zero and the ΔK parameter (Evanno et al., 2005) as implemented in Structure Harvester v.0.6.94 (Earl and vonHoldt, 2012). A Mantel test of association between pairwise genetic $(F_{ST})/(1-F_{ST})$ (Rousset, 1997) and geographic distances were conducted in GenAlEx v.6.5 (Peakall and Smouse, 2012) to test for isolation by distance (Wright, 1943).

3. Results

3.1. Genetic diversity

Six species-specific microsatellite markers developed by Amade (2019) were used for the genetic diversity screening of all 80 extracted DNA samples. Appendix A (Figs. A1–A64) contains images that represent all the alleles scored for the different loci. Amplification success was high in general. Scoring results obtained for each of the loci were evaluated separately. No large allelic dropout was observed for any of the loci. Signs of stuttering was observed for locus Am23. Null were present at locus Am01, Am17 and Am23. Allele frequencies were corrected to take this into account. Linkage disequilibrium was present at one pairwise comparison, between locus Am01 and locus Am02. Further investigation led to the exclusion of locus Am01 and a final total of five loci were used for the final analyses.

Summary statistics were calculated for the five polymorphic microsatellite loci overall the sampling localities ($N = 80$). The overall level of polymorphism detected in five loci was high with a total of 48 alleles and a mean of 10 alleles per locus (Table 2). The number of alleles per locus per population ranged between 17 and 26 (Table 3). Loci Am19 presented the highest allelic richness at 19.9. The highest observed heterozygosity (Ho) was 0.436 for Am19 and the highest expected heterozygosity and unbiased expected heterozygosity was the

highest for Am23 at 0.539 and 0.543, respectively. The lowest inbreeding coefficient was observed for locus Am19 at 0.098. Significant deviation from Hardy-Weinberg equilibrium (HWE) was detected for all loci (Table 2).

Table 2. Summary statistics for five polymorphic microsatellite loci overall sampling localities ($N = 80$). The mean number of samples analysed (N), a number of alleles identified (N_A), allelic richness (Ar), observed heterozygosity (H_O), expected heterozygosity (H_E), unbiased expected heterozygosity (uH_E) and inbreeding coefficient (F_{IS}) values are given for each locus. Values in bold represent significant deviations from Hardy-Weinberg equilibrium.

Locus	*N*	N_A	*Ar*	H_O	H_E	uH_E	Fi_S
Am02	80	5.0	4.5	0.263	0.300	0.302	**0.131**
Am03	80	4.0	3.9	0.025	0.073	0.074	**0.662**
Am17	64	3.0	3.0	0.250	0.361	0.364	**0.315**
Am19	78	22.0	19.9	0.436	0.480	0.483	**0.098**
Am23	62	14.0	14.0	0.226	0.539	0.543	**0.586**

Table 3. Summary statistics for each sampling locality with the mean number of samples analysed (N), mean number of alleles per locus (N_A), allelic richness (Ar), the number of private alleles (P_A), observed heterozygosity (H_O), expected heterozygosity (H_E), unbiased expected heterozygosity (uH_E) and inbreeding coefficient (F_{IS}). Values in bold represent significant deviations from Hardy-Weinberg equilibrium.

Sampling locality	*N*	N_A	*Ar*	P_A	H_O	H_E	uH_E	Fi_S
Pemba-Metuge	19	17.0	15.8	5.0	0.143	0.248	0.256	**0.448**
Bons Sinais Estuary	18	25.0	21.9	5.0	0.284	0.400	0.413	**0.318**
Costa do Sol	18	26.0	22.3	11.0	0.260	0.333	0.343	**0.248**
Inhaca Island	19	19.0	16.8	3.0	0.296	0.353	0.363	**0.189**

Focussing on the sampling localities, Costa do Sol presented the highest number of alleles (26) and highest allelic richness (22.3). The number of private alleles was the highest for Costa do Sol at 11. The highest observed heterozygosity was found at Inhaca Island, 0.296. The highest expected heterozygosity and unbiased expected heterozygosity was calculated for Bons Sinais Estuary at 0.400 and 0.413, respectively. The lowest inbreeding coefficient was calculated for Inhaca Island at 0.189 (Table 3).

The power analysis using Powsim showed that the five microsatellite loci used would be able to detect genetic differentiation with Fst ≥ 0,019 at a 0.05 significance level (Ryman and Palm, 2006).

3.2. Population genetic structure

Pairwise F_{ST} comparisons were not significant for all pairwise comparisons, except for the comparison between Pemba-Metuge and Costa do Sol ($Fst = 0.198$, $p = 0.035$). A standard AMOVA for the 4 populations (without a hierarchy of regions) showed that 83.8 % of the variation was located within individuals and only 48 % among populations. AMOVA was performed for all possible groupings of the sampling localities and the grouping (Pemba-Metuge + Costa do Sol + Inhaca Island) as one + (Bons Sinais Estuary) alone presented the highest variation within populations at 96.6.

The independent clustering of all samples, into $K = 1$ to $K = 10$ potential populations, supported the presence of only one population. The ln$K$ value was closest to zero for $K = 1, 2$

and 3 (Fig. 2) and the STRUCTURE bar plot (Fig. 3) supported the presence of only one genetic grouping ($K = 1$). The statistical correlation between genetic and geographic distances among populations (Mantel test) showed no significant isolation by distance with $R^2 = 0.244$, $p = 0.164$.

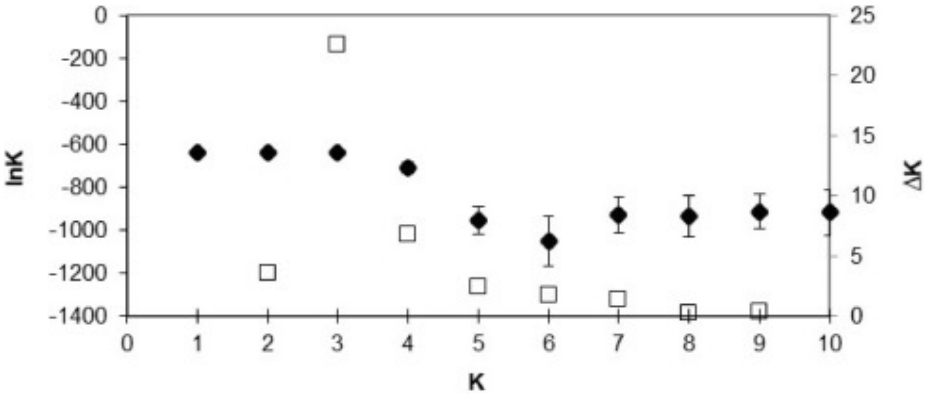

Fig. 2. Structure graph for $K = 1$ up to $K = 10$ with mean Ln likelihood values (ln$K$ ± SD) indicated on the primary Y-axis (lnK, data points indicated with diamonds, whiskers represent minimum and maximum Ln likelihood values). The secondary Y-axis represents the rate of change in the log probability of data between successive K values (Δk, data points indicated with squares).

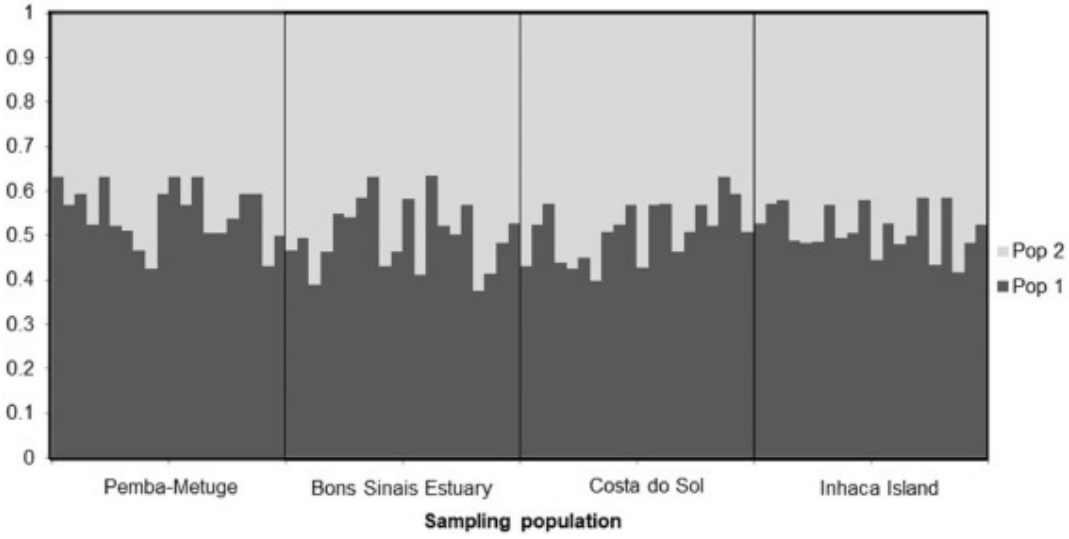

Fig. 3. Structure bar plot for *A. marina* with $K = 3$ for all sampling localities. The colours represent the proportion of ancestry for each sampled individual to one of the two inferred genetic groups.

4. Discussion

The aim of the present study was to determine the genetic diversity and population genetic structure of *A. marina* from Mozambique. A total of 80 samples, 20 per sampling locality, were collected at Pemba-Metuge, Bons Sinais Estuary, Costa do Sol and Inhaca Island. We hypothesised that, due to an absence of physical barriers for preventing dispersal of mangrove propagules along the Mozambique Channel, gene flow along the Mozambique coast would be high. Data obtained from five species-specific polymorphic microsatellite loci were used for the population genetic screening.

From the ten loci selected for the population screening, six were excluded from the analyses. Costa do Sol presented the highest number of alleles at 26 and the highest allelic richness at 22.3. The allelic richness highlights the number of alleles and their frequencies and is affected by migration rates and demographic parameters (Greenbaum et al., 2015). High allelic richness as well as the high number of private alleles observed at Costa do Sol may be due to the dominance of *A. marina* trees (80 %) in this sampling locality (Amade et al., 2019). This may indicate that a high diverse gene pool for this species has been established in this locality and it is most likely maintained over time.

Pemba-Metuge showed low genetic diversity for *A. marina*. A reduced population size is known to affect the genetic diversity and it can clearly be observed in the reduced heterozygosity observed here (Greenbaum et al., 2015). An overall heterozygosity deficit was observed for Pemba-Metuge with the presence of null alleles and inbreeding. The presence of null alleles was detected at some microsatellite loci and this could lead to the observation of a high frequency of homozygote for those loci in this sampling population (Dakin and Avise, 2004). The levels of inbreeding in the present study could have been influenced by the number of samples analysed, the actual population size of the mangrove in the study site, the mating system, suitable habitat for seedling establishment and possible isolation due to environmental barriers (De Ryck et al., 2016; Triest et al., 2018). The high frequency of harvested *A. marina* trees in Pemba-Metuge, with a mean density of dead stumps of 66.7 stems. ha^{-1} (Bandeira et al., 2009), most likely resulted in a reduction in the overall population size of *A. marina*. The harvesting could also have contributed to the altering of the landscape and fragmentation of the population, which in turn may have caused the loss of genetic diversity and an increase in inbreeding (Pautasso, 2009; Castillo-Cárdenas and Toro-Perea, 2012). The fringe section of the mangrove forest in the northern part of Mozambique, where Pemba-Metuge is located, is predominantly occupied by *S. alba*. This reduces the density of *A. marina* trees in this part of the forest. This could be the reason for *A. marina* being restricted to the upward side of the mangrove forest, and in this part of the forest there is frequently a restricted tidal inundation due to its high topography. The restricted tidal movements play a role in limiting the regular exchange of nutrients, limiting the dispersal of propagules, and decreases the possibility of local pollen flow through insect or wind pollination. Habitat fragmentation and the reduction of the population size of mangroves has been shown to result in a loss of genetic diversity. This is mainly due to diminished reproductive success caused by the disruption of pollinators, the limitation of pollen dispersal and the large effect of inbreeding that tends to be more pronounced in small populations (Maguire et al., 2002; Hermansen et al., 2017). Similar low levels of observed heterozygosity were also observed by De Ryck et al. (2016), and they concluded that the high level of heterozygote deficiency in populations of *A. marina* from Kenya, Tanzania and South Africa could most likely be because of past bottleneck events. They suggested that for some South African populations, the bottleneck events could be caused by the subsequent die-off of a substantial part of the mangrove populations due to the recurrent opening and closing of river mouths. These aspects have been shown to have an impact on the overall genetic structure of mangroves (De Ryck et al., 2016).

Private alleles are alleles that are found only in a single sampling locality among all the sampling localities (Szpiech and Rosenberg, 2011). The number of private alleles detected were highest at Costa do Sol. A possible explanation for this high number of private alleles is that there is high connectivity and exchange of propagules among Costa do Sol and other *A. marina* populations around the Maputo Bay area that contains unique diversity. On the north-western part of Maputo Bay, the Incomati Estuary can be found that includes three main

islands Benguelene, Xefina Grande and Xefina Pequena, and on the western side of Maputo Bay is the Espirito Santo Estuary (Amade, 2008; Macamo et al., 2015). Maputo Bay forms one of the three points (referred to as the Delagoa bight), where high current speed and directional stability of the Mozambique Channel have been identified. This is where all these mangrove populations are located. Thus, the anti-cyclonic eddies may play an important role in propagule circulation among these mangrove populations (Hancke et al., 2014).

No significant population differentiation was detected using the five microsatellite loci used in this study. The power analysis showed that the set of microsatellite loci used in this study would be able to detect genetic differentiation at an $F_{ST} \geq 0.019$ at a 0.05 significance level. The only significant pairwise F_{ST} comparison was between Pemba-Metuge and Costa do Sol. This overall non-significant F_{ST} values between the sampling localities highlight the genetic similarity between the sampling localities. The sampling localities are most likely connected by the different currents formed off the Mozambican coast.

Results from STRUCTURE indicated the presence of only one population after independent clustering of all samples. Overall results in the present study suggest high levels of gene flow for *A. marina* along the coast of Mozambique. The present pattern is perhaps influenced by the dynamics of the currents within the Mozambique Channel and the absence of known physical barriers obstructing the dispersal of *A. marina* propagules. The ocean currents play an important role in the connectivity and the resulting genetic structure of *A. marina* populations (Triest et al., 2018; Do et al., 2019).

The Mozambique Current is a non-continuous southwards flowing current dominated by trans-, intermittent-, and passing mesoscale eddies (Halo et al., 2014; Hancke et al., 2014). Within the Mozambique Channel, there is a general anti-cyclonic circulation with three areas of high current speed and directional stability: (1) North of the channel narrows; (2) along the Inhambane shelf; and (3) south of Delagoa bight (Hancke et al., 2014). The anticyclonic eddies are generated at the northern tip of Madagascar, propagate across the Comoros basin and subsequently into the Mozambique Channel. Also, cyclonic eddies are formed along the northwest coast of Madagascar and the majority of these dissipate within the Comoros basin (Collins et al., 2016). The origin of the eddies and direction of the currents suggest that potential exchange of mangrove propagules between the Madagascar and Mozambican populations could be possible in the northern part closest to the sampling locality Pemba-Metuge. *Avicennia marina* mainly reproduces sexually and the offspring are distributed through the production of water buoyant propagules, that undergo long distance dispersal by the sea and estuarine currents, and are mainly affected by tides, wind and oceanic circulation (Clarke, 1993; Van der Stocken and Menemenlis, 2017).

Avicennia marina propagules most likely disperse according to the same pattern of circulation as the eddies that can be found close to the sampling localities. This will strengthen the connectivity among the populations of *A. marina* and is probably responsible for eliminating any potential of isolation by distance among these sampling localities. The circular movements of the eddies, potentially moving propagules along, can reach an estimated circular dispersal distance of up to 630 km (Van der Stocken and Menemenlis, 2017). This movement of the anti-cyclonic eddies are characterised to be in a southward direction, but there is strong evidence for a northward movement due to the circular characteristics. This will allow the exchange of propagules between sampling localities. This could thus create the possibility of gene flow of *A. marina* to take place in both directions

along the Mozambique coastal shelf, consistent with the high level of gene flow observed in this study.

We observed similar patterns in our study as was detected for *Avicennia officinalis* in Sundarbans (Bangladesh) where a low level of population differentiation and a high level of connectivity among populations were identified. It was suggested that this was due to a combination of abiotic environmental factors, such as river and ocean currents, tides and wind action (Hasan et al., 2018). The opposite was seen by Ochoa-Zavala et al. (2020), that found two probable barriers to gene flow related to ocean features in populations of *Avicennia germinans* along Mexico's Atlantic and Pacific coasts. The first barrier suggested was associated with surface water circulation at the mouth of the Gulf of California and the second, observed as the genetic discontinuity located at the gulf of Tehuantepec, comprised of numerous oceanic gyres and fronts that developed with a periodical change of direction and strength. This prevented gene flow between the tropical south Pacific and the rest of the Pacific Coast population by transporting propagules offshore into the open ocean.

5. Conclusions

This study showed high levels of allele and genotypic diversity within populations of *A. marina* from Mozambique. High gene flow was observed between all the sampling localities and this is most likely due to the currents and eddies within the Mozambique Channel, capable of distributing propagules in a northern as well as a southern direction along the Mozambique coast. This implies that currents within the Western Indian Ocean region will have a significant influence on the population genetic structure of mangroves in general, especially along the African coast. Extending this study further along the African coast, as well as including Madagascar, will most likely show that *A. marina* mangroves north and south of the Mozambique coast are genetically similar.

The effective management and conservation of mangroves will lead to a healthy state of the forests and the maintenance of the genetic diversity within these areas. Sustainable utilisation and exploitation strategies will have to be developed for mangroves impacted by anthropogenic activities, specifically within these study localities included in the current study. This will have to include the careful management of anthropogenic activities that threaten mangroves and strategies of promoting the natural regeneration of species by avoiding activities that could reduce the size of the mangrove populations.

The Costa do Sol mangrove forest is under very high anthropogenic pressure due to urban growth and associated activities. Surprisingly, this mangrove forest that is dominated by *A. marina* trees, showed a high potential of regeneration even though a high number of dead stumps were recorded previously. The Costa do Sol mangroves showed signs of being in the process of recovering with a possibility of developing into a forest with high structural complexity. This locality also contained the highest genetic diversity of *A. marina*. With minimal anthropogenic interference or a clear management strategy, this locality would be ideal for allowing the regeneration of *A. marina* mangroves and the recovery of the population, thereby preserving the high genetic diversity and the gene flow within the area and the gene flow to other areas. Therefore, it would be equally convenient and ideal to create an *in situ* conservation area for *A. marina* at Costa do Sol in order to preserve the high genetic diversity for the species and promote good regeneration of the forest. The management strategy could also potentially include the reconstruction of the natural

hydrodynamic system and the topography of the area by closing the previous aquaculture ponds in order to reconnect the isolated populations within the area.

Future studies should include a more comprehensive study approach where an increase in the number of polymorphic loci used is combined with an increase in the number of samples per sampling site as well as an increase in the number of sampling localities spanning the distributional range of the species. This will eliminate the possibility of not detecting any low level structuring that might be present. The potential sampling localities should also be extended to include countries where *A. marina* occur, along the coasts of Africa, Madagascar, the Arabian Gulf throughout Asia to China and Japan, to the south-western Pacific, New Zealand and Australia. This would allow for a more comprehensive inference regarding the pattern of genetic structure, population differentiation and gene flow along the distributional range of *A. marina*.

Author statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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