Original Article

Population genetic status of endangered whitespotted whipray *Maculabatis gerrardi* (Gray, 1851) in Tanzania

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Abstract

The whitespotted whipray *Maculabatis gerrardi* is exploited in Tanzania for its meat, skin and cartilage, and is classified as an endangered species by the IUCN. A mitochondrial COI gene fragment from 105 *M. gerrardi* individuals obtained from four unprotected and one protected marine area in Tanzania was used to determine the present genetic diversity, demographics, and effective population size of whiprays. Lower levels of nucleotide and haplotype diversity and mean mutational effective population size were apparent in unprotected than in protected areas. Analysis of molecular variance (AMOVA) identified a significant genetic difference between sub-populations of *M. gerrardi* and hierarchical AMOVA identified separate genetic stocks, indicative of high levels of philopatry or individual sedentarity in *M. gerrardi*. The importance of marine protected areas to conserve genetic diversity of whiprays is highlighted.

Keywords: genetic diversity, effective population size, genetic monitoring, elasmobranch, gene flow

Introduction

Rays are economically important to the people living along the coastline in terms of food security, nutrition, source of income and employment opportunities (Barrowclift *et al.*, 2017). Unfortunately, their numbers are dwindling and they are more likely to be overfished due to their overall body shape and benthic habits, which make them vulnerable to capture in net and trawl operations (Schluessel *et al.*, 2010). Rays of various sizes from Tanzania's coastline are typically caught for dried filets and liver oils, and their stomachs are cleaned, boiled, and turned into soup(Dulvy *et al.*, 2014). Elasmobranchs generally display slow growth, late maturity and low fecundity which causes a low rate of increase of the species (Compagno *et al.*, 1990; Dulvy *et al.*, 2014). Rays have been receiving less attention when compared to other elasmobranches (sharks); this makes this group more threatened and currently their extinction risk is substantially higher than most other vertebrates with only one-third of them being considered safe (Jorgensen *et al.*, 2022).

Maculabatis gerrardi formerly known as *Himantura gerrardi*, dwells in shallow waters and feeds on a diverse range of species, most notably crustaceans, followed by polychaetes and teleosts (Rastgoo *et al.*, 2018).They are ovoviparous, bearing live young developed from eggs retained within the mother's body, implying that dispersal occurs through juvenile or adult migration (Borsa *et al.*, 2012).The biological productivity of sharpnose stingray, *M. gerrardi*is constrained. Nonetheless, the meat is regarded as being of high quality, their skins are used to make leather and it is consumed locally and traded globally.The International Union for Conservation of Nature (IUCN) categorizes *M. gerrardi*s pecies under the endangered red list

due to overfishing through bycatch in trawl, gillnet, and longline fisheries.The *M. gerrardi* population is believed to have decreased by 50–79% over the last 75 years as a result of overexploitation (Sherman, 2020).

There have been a number of initiatives to conserve and manage marine species, including the creation of marine protected areas (MPAs). In order to safeguard stocks of marine species, particularly fish and their habitats, the Marine Parks and Reserves Unit in Tanzania was established under the Marine Parks and Reserves (MPRU) Act 29 of 1994.The MPAs serve as a refuge for fish communities, increasing their diversity and density. Extractive resource usage is permitted within the park but is more restricted than in the nearby areas where fishing is typically practiced (Kamukuru *et al.*, 2004). However, different marine species are still threatened by various factors that include habitat degradation, overfishing, bycatch and climate change (Trathan *et al.*, 2015).

Fisheries experts should be able to use techniques that can help to precisely identify fish populations or units in order to manage and conserve marine species. Also, they must be capable of monitoring and reporting on migration trends and effective population sizes. By doing this, they can protect important areas and influence action plans. For example, the data can be applied for creation of fishing quotas and spatiotemporal closures. In order to monitor and evaluate the stocking structure and improve the protection and conservation of diverse marine species, several researchers have been employing various approaches. Nevertheless, the majority of marine monitoring and evaluation is based on conventional methods that are time consuming and provide only a partial picture of the ecological situation. The advancement of genetic technologies has the potential to fundamentally alter how marine life is conserved and protected. For instance, determining effective population sizes, introgression across species, and gene flow among populations all benefit from the knowledge of genetic diversity at neutral loci (Van Oppen and Coleman, 2022). The design of MPA networks may be improved by using data on neutral and adaptive genetic diversity in recovery plans for threatened species. Genetic and genomic approaches can be employed in the context of conservation and restoration to direct and improve conventional conservation measures as well as to design more modern aided evolution techniques (Van Oppen *et al.*, 2015). The use of genetic information has facilitated the detection of genetically diverse groups, the measuring of genetic connectivity and the identification of risks related to inbreeding and demographic change (Ekblom and Wolf, 2014). Unfortunately, genetic data has not been extensively used in management plans for a number of marine species, including rays. Understanding genetic connectivity and genetic diversity are important for effective conservation and management of rays (O'Dwyer *et al.*, 2021). There are very few genetic studies available for the Western Indian Ocean (WIO) coastline, making it impossible to draw broad generalizations regarding the patterns of connection of marine fish species (Muths *et al.*, 2012). The consequences of the decline in the populations of marine fish species on genetic diversity have drawn increasing attention (Domingues *et al.*, 2018). There is an urgent need for more population genetic studies to report on ray species since only few studies describe the genetic diversity of rays (Domingues *et al.*, 2018). Currently, only 10 % of ray species have been investigated in terms of their population genetic structure, genetic diversity and demographic history (Marion *et al.*, 2014). Despite the considerable dispersal capacity of some marine animals with large body sizes, recent research has demonstrated that elasmobranch species in the Indo-Pacific have high levels of genetic differentiation (Arlyza *et al.*, 2013). This can be a sign that certain elasmobranch species need particular management strategies. The objective of this study is to employ molecular genetic methods to support the management and conservation of *M. gerrardi* through population monitoring.

Materials and methods

Sampling

Maculabatis gerrardi samples were collected from five different locations along Tanzania's coastline. The five locations were the Deep Sea-Tanga, Kivukoni-Dar es Salaam, Bweju-Mafia, Bandarini-Mtwara, and Malindi-Unguja (Fig. 1). Bweju-Mafia was selected as the control location since it is an MPA. A sample of *M. gerrardi* fin tissue was cut and immediately stored in 99.9% ethanol.

From its border with Kenya (4°49'S) to its border with Mozambique (10°28'S), Tanzania's coastline is more than 1424 km long (URT 2015). The East African Coast Current (EACC), which always moves in a northerly direction, is the main ocean current in this area. The other ocean currents that influence the gene flow of marine species in the area are the eastward-movingSouth Equatorial Countercurrent (SECC) and the westward-moving South Equatorial Current (SEC) (Fig. 1).

DNA extraction

DNA was extracted from about 25mg of tissue. Total DNA was recovered for mtDNA analysis following the instructions of the tissue DNA extraction kits (ZYMO Research Inc, California, USA). Extracted DNAwas kept in the freezer for further investigation. The quality of the DNA extracts was evaluated using agarose gel electrophoresis.

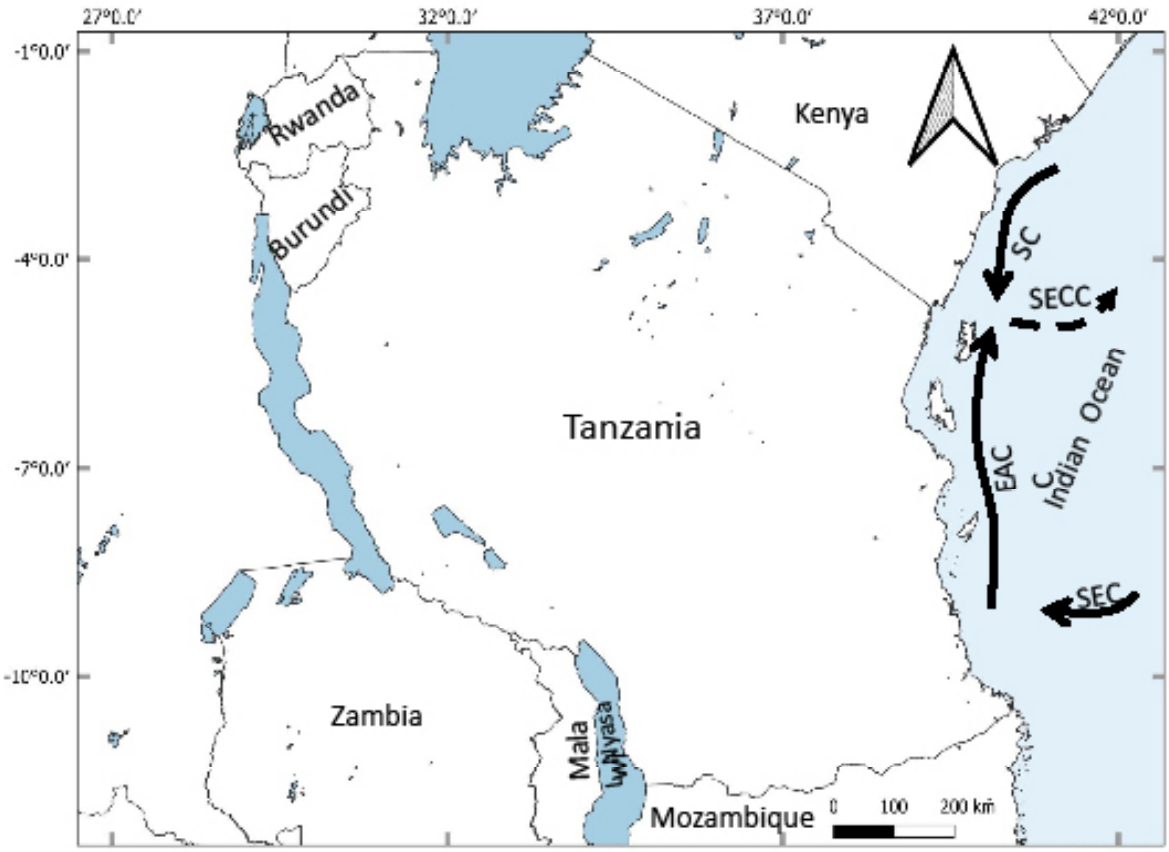

Figure 1. Sampling area of *Maculabatis gerrardi* along the Tanzania coastline (White circles indicate sampling sites). Black arrows signify the Somali Current (SC), East African Coast Current (EACC), South Equatorial Countercurrent (SECC) and South Equatorial Current (SEC).

Amplification of mtDNA and sequencing

A fragment of the COI gene was amplified using the primers Fish F1: 5'- TCAACCAACCACAAAGATTGGCAC-3' and Fish R1: 5'-TAGACTTCTGGGTGGCCAAATCA-3' (Ward *et al*., 2005). A BIORAD T100 thermocycler was used for polymerase chain reaction (PCR). The reaction was carried out in a 35 µl volume with 11.7 µl RNase-free water, 17.5 µl Mastermix, 1.75 µl BSA, 0.3µM of each primer, and 2 µl DNA templates. Initial denaturation was set at 94 ˚C for 5 minutes, followed by 35 cycles of denaturation at 94 ˚C for 40 seconds, annealing at 54 ˚C for 45 seconds, extension at 72 ˚C for 1 minute, and final extension at 72 ˚C for 15 minutes. PCR products were electrophoresed using 0.8g agarose gels.Using an ABI 3770XL automated sequencer (Applied Biosystems, Foster City, USA), 105 samples were successfullysequenced using the primer FishF1 and FishR2.

Mitochondrial DNA (mtDNA) analysis

The sequences were initially edited using the Chromaspro v. 1.5 (Technelysium) programme, and then the species identity was verified by comparing the edited sequences to the sequences present in GenBank using the BLAST programme. Squint Alignment Editor v. 1.02 was used to determine the frequency of stop codons, which indicate sequencing mistakes or pseudogenes (Goode and Rodrigo, 2007). CLUSTAL W implemented in the software MEGA 6 was used to do multiple alignments of the sequences (Thompson *et al.*, 1994; Tamura *et al.*, 2013). The sequences were collapsed intohaplotypes using the online FaBox 1.41 Collapse programme. The statistical parsimony approach, as implemented in the software PopArt, was used to investigate the genetic relationship between

the samples from the five landing sites(Leigh and Bryant, 2015). Arlequin v.3.5.2.2 was used to calculate nucleotide and haplotype diversity (Excoffier and Lischer, 2010).The same programme was used to determine the historical demographics as well as the neutrality indicators, Tajima's D test, and Fu's Fs (Tajima, 1989; Rogers and Harpending, 1992, Rogers, 1995; Fu, 1997). Fu's Fs is sensitive in detecting demographic expansion and genetic hitchhiking, whereas Tajima's D test is effective in detecting selective sweeps (Fu, 1997). Analysis of molecular variance was used to investigate the differences between populations (AMOVA) (Excoffier *et al.*, 1992). Pairwise Fst values were calculated among sample sites using the same method, and Bonferroni correction was applied to the significance P-values sequentially.The spatial analysis of molecular variance (SAMOVA 2.0) strategy was used to identify populations that are geographically homogeneous and maximum dissimilar from one another(Dupanloup *et al.*, 2002). K values ranged from 2 to 3, with the initial condition set to 400. The configuration with the highest variance among clusters (Fct) was deemed to be the best population grouping.

In order to determine whether population genetic structure was present in the dataset, hierarchical AMOVA was carried out using the findings of SAMOVA, pairwise Fst-values and haplotype network. The mtDNA sequence data were estimated using the MIGRATE v.3.116 programme. The programme was used to estimate the boundaries Θ ($2Nef\mu$) where *Nef* stands for effective population size of females and μ for the mutation rate per generation per locus(Bradic *et al.*, 2012). The Bayesian search strategy and Brownian mutation model was applied. The Metropolis sampling algorithm was used to sample from the prior distributions and generate posterior distributions. The exponential prior distribution was used to estimate Θ (range = 0–50) and M (range = 0–400). The initial runs were carried out in three replicates, each of which included a single lengthy chain with 50,000 recorded steps and 50 increments. Burn-in was 100,000 and the sampling parameter value was 2,500,000.The final runs were a single, lengthy chain of 50,000 recorded steps, 50 increments, four replicates, 2,500,000 sampled parameter values, and a burn-in value of 100,000. A static heating scheme with four chains and temperatures set at 10, 4, 2, and 1 was used.

Results

Haplotype and nucleotide diversities

The overall nucleotide and haplotype diversity values for populations were 0.00301 ± 0.0019 and 0.7714 ± 0.0403 respectively. High value nucleotide diversity 0.0045 ± 0.0028 as well as high haplotype diversity 0.889 ± 0.0640 were recorded in Mafia while the lowest nucleotide diversity 0.0009 ± 0.0009 and haplotype diversity 0.2842 ± 0.1284 were recorded in Unguja (Fig. 2).The alignment of 105 sequences with 628 bp length was obtained. The sequences were deposited at the GenBank (accession numbers OQ455827 - OQ455931). A total number of 27 haplotypes were identified from COI sequences. Among the 27 haplotypes detected, 16.19% were haplotypes seen only once in a group of samples. The dominant

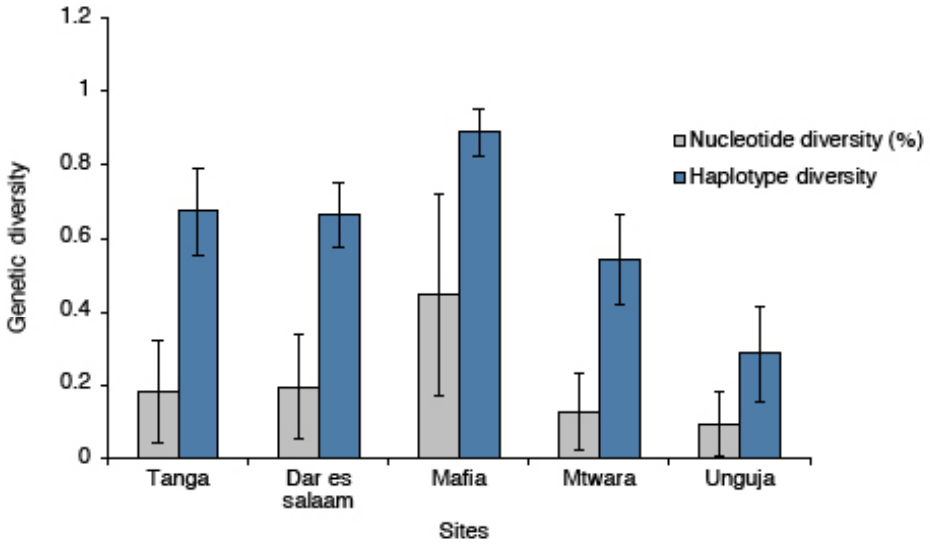

Figure 2. Nucleotide and haplotype diversities of *Maculabatis gerrardi* along the coastline of Tanzania in the Western Indian Ocean.

Table 1. Number of haplotype distributions for each sampled landing site. Abbreviations: N = number of individuals; Nh = number of haplotypes.

Sites	Code	N	Haplotype distribution																											Nh
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Tanga	Ta	19	11			2			1	1														1	1	1	1			8
Dar es salaam	Da	30	17	1	1	5	2	1	1	1	1																			9
Mafia	Ma	18										1	6	2	1	1	1	2	1	1	1	1								11
Mtwara	Mt	18	3			1	2																12							4
Unguja	Un	20	17																						1			1	1	4
	Total	105	48	1	1	8	4	1	2	2	1	1	6	2	1	1	1	2	1	1	1	1	12	1	2	1	1	1	1	27

haplotype (h1) accounted for 46% of all individuals and was present in four populations out of five, except the Mafia population, whereas 17 individuals were from Dar, 17 from Unguja, 11 from Tanga and 3 from Mtwara. The second most frequent haplotype (h21) had12 individuals all from Mtwara. The third frequently occurring haplotype (h4) had 8 individuals of which 5 were from Dar, 2 from Tanga and 1 from Mtwara. The fourth most frequently occurring haplotype (h11) had 6 individuals all from Mafia (Table. 1).

Population genetic structure

The genetic differentiation across all groups was significant (Fst = 0.248, P < 0.001). All pairwise AMOVA that involved samples from Mafia and Mtwara demonstrated significant genetic differentiation after sequential Bonferroni correction (Table 2). The haplotypes seen only once in a group of samples occurred in high frequencies with a starlike network (Fig. 3).

Three groups of genetic subpopulation clustering were identified by SAMOVA. The three best groups

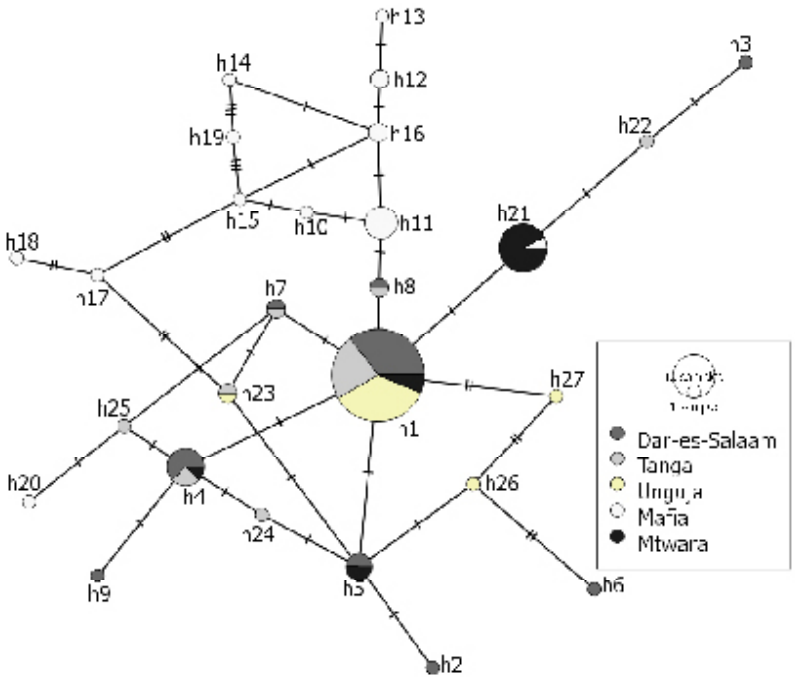

Figure 3. Minimum spinning network indicating haplotype distribution of *Maculabatis gerrardi* based on cytochrome oxidase subunit I sequences. The central haplotype network indicates percentage of haplotypes from all sampled subpopulations except Mafia. The central haplotype represents 48 individuals. The size of the other circles corresponds to the number of individuals as indicated at the bottom right side of the haplotype network.

Table 2. Pairwise F_{st} values of *Maculabatis gerrardi* subpopulations from the Tanzanian coastline based on cytochrome oxidase subunit I sequences (for site codes see Table 1).

CODES	TA	DAR	MA	MT	UN
TA	0.00000				
DAR	-0.0251	0.00000			
MA	0.2202*	0.2334*	0.00000		
MT	0.3224*	0.3159*	0.2843*	0.00000	
UN	0.0548	0.0757	0.4215*	0.5232*	0.00000

Statistically significant differences of adjusted P-values after sequential Bonferroni ($p < .001$) are indicated by *

of subpopulations found by SAMOVA are the Mafia subpopulation, the Mtwara subpopulation, and the Tanga, Dar es Salaam, and Unguja subpopulations (Fct = 0.494).The Hierarchical AMOVA revealed that the Mafia subpopulation was genetically different from Tanga, Dar es Salaam, Mtwara and Unguja populations (Fct = 0. 0.241, $p < 0.001$). The Mtwara subpopulation was also genetically different from Tanga, Dar es Salaam and Unguja subpopulations (Fct = 0.211, $P < 0.05$).

The average mean mutation-scaled effective population size recorded was 0.027 ± 0.0027. The estimate of effective population size indicated the highest mean mutation-scaled effective population size for samples collected at Mafia (0.0091) and the lowest for the samples collected from Unguja Island (0.0027) (Table 3).

Mismatch distribution and demographic history

All populations of *Maculabatis gerrardi* showed negative Fu's Fs-values, with samples from Tanga, Dar es Salaam and Mafia being significantly different from zero while Mtwara and Unguja had non-significant values. All populations from five landing sites showed non-significant negative Tajima's D-values, with the exception of samples from Dar es Salaam which had significant negative D-values. The population of Tanga and Dar es Salaam had non-significant values for the SSD and Raggedness index analysis while the population of Mafia, Mtwara and Unguja showed significant values for SSD and Raggedness index analysis (Table 4). Mismatch distribution analysis displayeda unimodal distribution with a poorly pronounced nature of the curve (Fig. 4).

Discussion

Haplotype and nucleotide diversities

The present study's analysis of COI sequences reveals that *M. gerrardi* along the Tanzanian coastline has low to moderate nucleotide and high haplotype diversities as well as the occurrence of an excess of private haplotypes. In comparison to the results of the current study, a recent investigation on the queen mackerel revealed very similar nucleotide diversity but reduced haplotype diversity (Rumisha *et al.*, 2023). The majority of the mitochondrial COI gene investigations that have been carried outin the WIO to date have revealed low to moderate genetic and haplotype diversities (Nehemia and Kochzius, 2017; Nehemia *et al.*, 2017). The high haplotype diversity and low nucleotide diversity is an indication of a genetic bottleneck caused by stochastic extinction of the majority of haplotypes and population growth (Alves *et al.* 2001). So, the results of this study may suggest that the *M. gerrardi* population has experienced a bottleneck that has led to stochastic extinction of most haplotypes, followed by population expansion. The genetic diversity found at Mafia was high compared to that obtained at other sites. In comparison to other locations, Mafia sites also had a large number of haplotypes and higher

Table 3. Estimated effective population size using the programme MIGRATE (mean, 2.5% and 97.5 % confidence interval) in *Maculabatis gerrardi* from the Tanzanian coast on the Western Indian Ocean. Θ: mutation-scaled effective population size (for site codes see Table 1).

	Θ		
Codes	mean	2.5%	97.5%
TA	0.0062	0.0093	0.0127
DA	0.0064	0.0021	0.0178
MA	0.0091	0.0021	0.0178
MT	0.0027	0.0000	0.0065
UN	0.0029	0.0000	0.0065

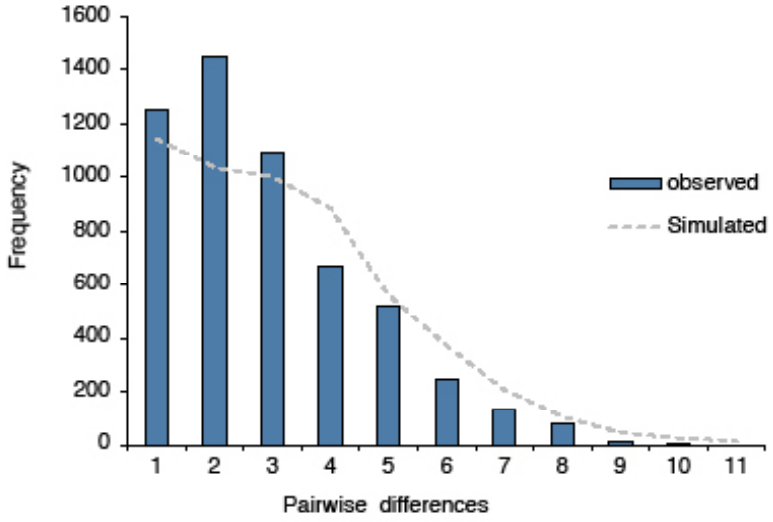

Figure 4. The observed (bars) and expected (dotted line) mismatch distributions of COI sequences for *Maculabatis gerrardi* under the sudden expansion model.

effective population size. Mafia Island's higher genetic diversity may be related to the larger effective population size that has been found for this subgroup. A positive correlation between effective population size and genetic diversity has been observed (Tringali and Bert, 1998; Rieman and Allendorf, 2001). However, there are other aspects, like biological, ecological, and evolutionary history traits of the species, which should not be disregarded, that are also connected to genetic diversity (Petit-Marty *et al.*, 2022).

The mafia sample collection site is located in aMPA with fewer human activities. Mafia Island's high genetic diversity might be a sign that aMPA could be useful for managing and conserving marine resources. A gear restriction and fishing exclusion zone management system is used to regulate the fishery in the Mafia MPA catch of site. Because of restrictions on the minimum mesh size for nets and the minimum size of the individual fish collected, this management strategy tends to reduce thefisherman's catch. Fish that can be fished must be mature and have spawned at least once. It might also be a sign that the Mafia subpopulation is more resilient to environmental changes than other subpopulations and is therefore more stable. Genetic diversity increases species' ability to adapt to environmental changes and increases population resilience (Faulks *et al.*, 2011). The findings might also point to a restriction on Mafia interactions with other subpopulations along Tanzania's coastline.

Population genetic structure

The Analysis of Molecular Variance (AMOVA) indicates significant genetic differences among the subpopulations of *M. gerrardi* studied. SAMOVA and hierarchical AMOVA supported existence of genetic groupings of populations along the Tanzanian coast. These findings are also supported by pairwise AMOVA and the haplotype network. The research conducted in the Coral Triangle region revealed the presence of population genetic groups in stingrays, *N. kuhlii* species, which are distributed in a parapatric manner as a result of sedentarity or philopatry (Arlyza *et al.*, 2013). The population genetic groupings revealed in

Table 4. Neutrality tests and mismatch distribution for cytochrome oxidase subunit I sequences in *Maculabatis gerrardi* from the Tanzanian coastline (for site codes see Table 1).

Codes	Fu's Fs	p	Tajima's D	p	SSD	p	Raggedness index	p
TA	-4.562	0.000	-1.076	0.165	0.011	0.400	0.064	0.550
DA	-4.353	0.005	-1.806	0.017	0.005	0.400	0.060	0.490
MA	-4.827	0.004	-0.136	0.500	0.000	0.000	0.000	0.000
MT	-0.650	0.275	-0.257	0.437	0.000	0.000	0.000	0.000
UN	-1.237	0.069	-1.408	0.066	0.159	0.000	0.668	0.000

the present study may be due to the sedentarity or philopatry of *M. gerrardi*, as the stingray species share some behaviors. The influence of ocean currents and eddies and isolation by distance (IBD) can help to describe the genetic structuring of marine species (White *et al.*, 2010; Nehemia *et al.*, 2019). Most research carried out along Tanzania's coast demonstrates that there is significant gene flow among the subpopulations of most marine species (Nehemia *et al.*, 2019; Bugota and Rumisha, 2023; Rumisha *et al.*, 2023). But most of these studies concentrated on species that have a pelagic larval phase. Fish species whose eggs develop in females until they reach the juvenile stage have less potential for geneflow, whereas those with a pelagic larval phase have greater potential (Mitton *et al*., 1989). The genetic connectivity observed in *M. gerrardi* between the subpopulations of Dar es Salaam, Tanga, and Unguja may be attributed to the East African Coastal Current (EACC), which runs northward between the coasts of Tanzania and Kenya. It is possible that this current is distributing juveniles of this species among these subpopulations (Fig. 1). The limited interaction between the Mafia subpopulation and other subpopulations may indicate that the Mafia MPA is unlikely to sustain the species' existing mtDNA genetic diversity or act as an effective source population (Schwanck *et al.*, 2023). MPAs are predicted to increase genetic diversity, but to be effective, gene flow between protected and non-protected areas is required (Allendorf *et al.*, 2008).

Mismatch distribution and demographic history

All samples from five landing sites of Tanga, Dar es Salaam, Mafia, Mtwara and Unguja showed negative Fu's Fs-values, with samples from Tanga, Dar es Salaam and Mafia being significantly different from zero. All the five landing sites show populations having non-significant but negative Tajima's D values, perhaps supporting the hypothesis of neutrality of the COI marker. In addition to that, all sampled populations have significant negative Fu's Fs values, suggesting rapid population expansion. According to Fu (1997) Fu's Fs has been regarded as more powerful than Tajima's D in signifying traces of past population expansion. The presence of a star-like topology in the haplotype network, which may also reflect population bottlenecks and the recolonization of new habitats after the sea level rose, supports the idea of population growth as does the unimodal distribution for mismatch analyses observed. Similar observations have been made for a wide range of species in the region (Kochzius and Nuryanto, 2008; Nehemia *et al.*, 2017; Rumisha *et al.*, 2023).

Conclusion

The results of this study show that the endangered species *M .gerrardi* has low to high nucleotide and haplotype diversity, and a high effective population size for samples from MPAs. However, three genetic group subpopulations of *M. gerrardi* were discovered on the Tanzanian coastline, which may indicate that there is less chance of geneflow in fish species whose eggs grow in females until they reach the juvenile stage. Despite being an endangered species, the high genetic diversity and large effective population size at Mafia Island may be indicative of the effectiveness of the MPA in protecting, conserving, and managing marine species. It is recommended that countries take initiatives to curb destructive fishing and expand MPAs. The results of this study will broaden the body of knowledge that may be used to make marine management decisions in the WIO by increasing the information that is now available in the area. Future research should take into account the use of nuclear loci since the maternal genetic mtDNA markers have been criticized for only reflecting one locus and reflecting only the maternal population. In order to improve marine species conservation, future research studies should focus on additional aspects that influence the genetic diversity and gene flow of marine species, such as environmental factors, selection efficiency, and mutation rate.

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