Mitochondrial DNA Control Region Revealed a Single Genetic Stock Structure of *Scomberomorus commerson* Lacepede(1800) in the Northern Tanzania Coastal Waters

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Abstract

The present study used mitochondrial DNA control region to investigate the genetic stock structure and phylogenetic relationship of 38 individuals of Spanish mackerel *Scomberomorus commerson* from the two localities in the northern Tanzania coastal waters. The study revealed that the Spanish mackerel were characterized by high levels of mitochondrial DNA genetic diversity at both haplotypes and nucleotide levels, indicative of large population size. The AMOVA results (F_{ST} = 0.0011) were statically low, indicating lack of genetic differentiation between populations (p = 0.925). Furthermore, AMOVA analysis showed that 99.50% of the total molecular variance was distributed within the populations and 0.5% distributed between populations. The Median-Joining network revealed a star-like median network; indicative of similar evolutionary history for the collected samples and existence of a recent historical population expansion. The present study recommends a single stock model for management of Spanish mackerel in the northern coastal waters of Tanzania. However, considering the migratory nature of this species, a co-management between coastal nations with further studies on the genetic stock structure covering large geographical areas is recommended if sustainable exploitation is to be achieved.

Key words: Spanish mackerel, Tanzania, genetic stock structure, phylogenetic relationship, mitochondrial DNA

1.0 Introduction

`Knowledge of population genetic structure and diversity in marine fishes are crucial in understanding genetic differentiation and historical demographic changes. Defining the genetic stock structure is important for developing fisheries management policies but the information are absent for the Spanish mackerel *Scomberomors commerson* in Tanzania, Western Indian Ocean. Knowledge of population genetic structure and diversity in marine fishes are crucial in understanding genetic differentiation and historical demographic changes. In the marine environment, large pelagic fishes were previously thought to lack or exhibit little genetic differentiation in their distribution ranges (Theisen *et al*. 2008). This is because of their ability to migrate across inter-ocean distances, broadcast spawning, large population sizes, high fecundity, production of numerous pelagic larvae (Nakamura 1985; Theisen *et al*. 2008) and assumed homogeneity of pelagic habitat (Fauvelot and Borsa 2011). However, some studies showed evidence of barriers to gene flow in the marine environments. These include oceanic fronts, oligotrophy, salinity, temperature and predation (Palumbi 1994; Graves 1998), spawning asynchrony among populations, retention of eggs and larvae, and adult homing behavior (Taylor and Hellberg 2003). Thus, exception to the rule of little geographic differentiation in pelagic fishes across oceans deserves analysis.

One exception is the Spanish mackerel *Scomberomorus commerson* Lacepède (1800) and other marine fishes (Palumbi 1994; Sulaiman and Ovenden 2010). Buckworth *et al*. (2007) and Sulaiman and Ovenden (2010) observed genetic differentiation of Spanish mackerel in the northern and western Australian and in the Wallace's Line, respectively. The existence of a fine scale genetic differentiation for pelagic fishes was also reported across oceans for Indian scad mackerel (Perrin and Borsa 2001; Rohfritsch and Borsa 2005) and swordfish (Lu *et al*. (2006). According to FAO (1983), important fishing zones for Narrow-barred Spanish mackerel include FAO Fishing Areas 51, 57 and 71. Such regions include the Western Indian Ocean (WIO) region countries, which are currently facing threats of environmental degradation and declines in biodiversity (Berg *et al*. 2002; Rigway and Sampayo 2005). Given that Spanish mackerel spends a substantial portion of their life cycle along the edge of the continental shelf particularly during the spawning aggregation phase, it requires special conservation attention. If proper conservation attention is not undertaken for the fish, considerable threats including

overexploitation, habitat degradation and pollution near coastal migration routes and spawning areas as reported in other studies (e.g. Collette and Russo 1984; Takahashi 2011) may lead to future disappearance of the fish. As effective management strategies are critical in fisheries management, scientific research leading to the identification of genetic unit of exploited fish population (Stepien 1995; Shaklee and Currens 2003) is important.

It is noted that genetics analysis of non-coding control region of the mitochondrial DNA (mtDNA) is sensitive and reliable in determining population stock of fish species (Graves *et al*. 1984; Hoolihan *et al*. 2006). Mitochondrial DNA analyses have been used by Hoolihan *et al*. (2006); van Herwerden *et al*. (2006) and Ovenden and Street (2007) to successfully delineate the stock structure of Spanish mackerel in other areas of the world. Genetically distinct stocks were suggested between populations in northern Australia, Papua New Guinea, Fiji, off Queensland, western Timor and Southeast Asia nations (Shaklee in Buckworth *et al*. 2007; Ovenden and Street 2007; Sulaiman and Ovenden 2010). On the other hand, no genetic differences were detected at the same locus, among samples from the Persian Gulf and the Oman Sea (Hoolihan *et al*. 2006).

Although a number of studies on the population genetic structure and demographic history of Narrow-barred Spanish mackerel have been conducted in other regions (Hoolihan *et al*. 2006; Shaklee in Buckworth *et al*. 2007; Ovenden and Street 2007; Sulaiman and Ovenden 2010), to the best of my knowledge no study has yet been conducted on the Tanzania coastal waters. Thus, the present study aimed to determine (i) genetic diversity (ii) genetic stock structure, and (iii) genealogical relationship of Spanish mackerel populations along the northern Tanzania coastal waters. In particular, a null hypothesis of single panmictic stock of Spanish mackerel across northern Tanzania coastal waters was tested using mtDNA control region sequences.

2.0 Methodology

2.1 Study areas

The present study was conducted in the two coastal sites; Dar es Salaam and Pangani along the northern coastal waters of Tanzania (Figure 1). The coastal waters of Tanzania are characterized with seasonal variations in water circulation connected with the periods of northeast monsoon (NEM) and

southeast monsoon (SEM) seasons. Along this coast, the NEM occurs from November to March and the SEM commences from April to October (Newell 1957; Mahongo *et al*. 2012). The SEM is usually associated with strong current speeds of up to 2 m/s whereas NEM is associated with weaker current speed that is less than 0.5 m/s (Newell 1957).

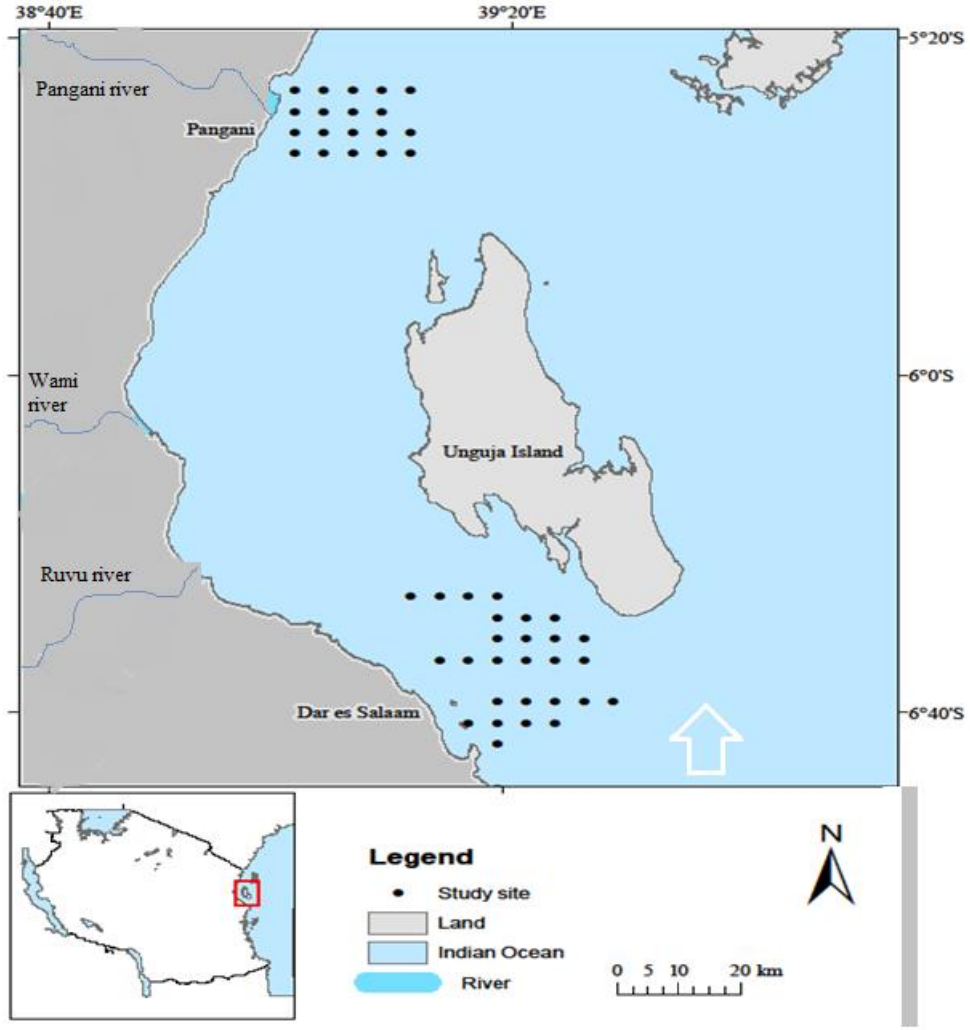

Figure 1: Map of Tanzania coastal waters showing the study sites.

2.2 DNA Extraction, amplification and sequencing

Genomic DNA isolation from the 38 muscle tissues were conducted using the standard TNES-Phenol-Chloroform protocol according Miller *et al*. (1988) and Asahida *et al*. (1996). The extracted Genomic DNA were preserved at -20^{0}C before PCR analysis. A 475 bp fragment containing the first half of

mtDNA control region was subjected to PCR amplification using the primer set and method described by Ovenden *et al*. (2002). The primer set used in the study of the Spanish mackerel were as follows: Pro889U20 (5′CCWCTAACTCCCAAAGCTAG3′) and TDKD1291L21 (5′CCTGAAATAGGAACCAAATGC3′). The reaction mixture contained 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 200 mM of each dNTP, 0.5 mM of each oligonucleotide, 1.25 units of Taq DNA polymerase, and 20 ng of template DNA in a 50.0 µl reaction. Amplification of the samples was performed in a Progene (Techne Cambridge Ltd) thermal cycler set for an initial denaturation temperature of 95 ^{0}C for 5 minutes followed by 35 cycles of 94 ^{0}C for 15 seconds, 55 ^{0}C for 24 seconds, 72 ^{0}C for 30 seconds, and a final extension of 72 ^{0}C for 5 minutes. 5 ml of the amplified products were treated with 1% agarose, 1% synergel gel electrophoresis for 45 minutes, and then stained with ethidium bromide and finally visualized by UV trans-illuminator (Plate 1). The PCR products were purified and sequencing were carried out using 6 Applied Biosystems 3730xl sequencer following manufacturer protocol. Sequences were submitted to the GeneBank and given accession number KU236393-KU236430.

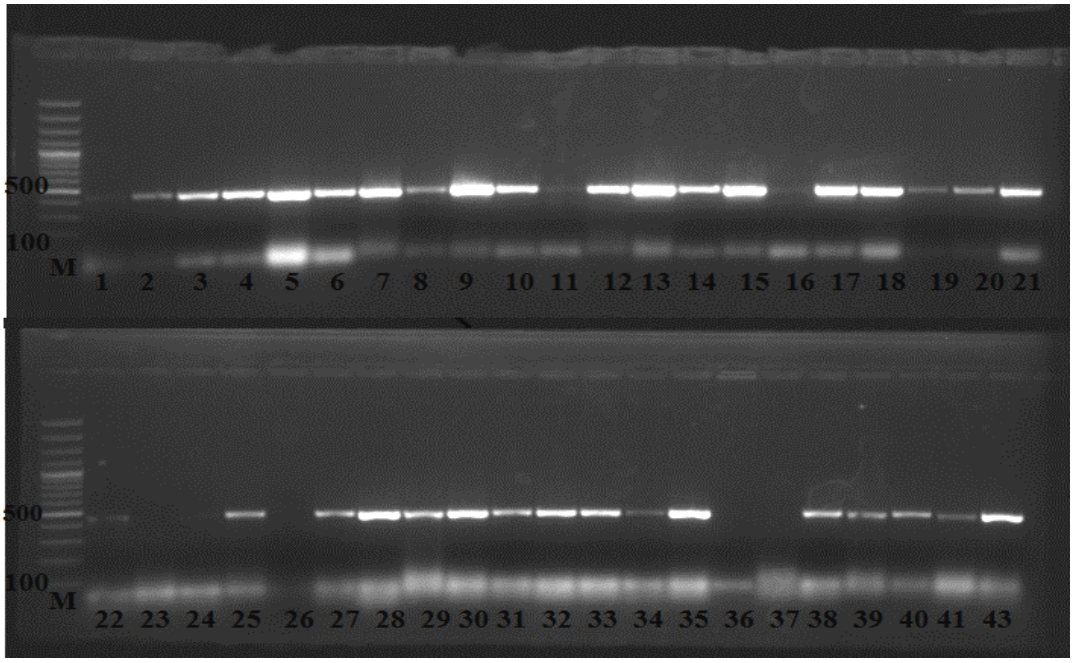

Plate 1: 475 bp PCR amplified product of the Spanish mackerel. M is the 100 bp DNA marker and 1-43 is the amplified DNA product.

2.3 Data Analysis

DNA sequences were assembled and edited with Genious version 5 and adjusted manually as needed. Alignment was carried out using the ClustalW algorithm (Thompson *et al*. 1994) implemented in MEGA 6.0.6 (Tamura *et al*. 2013). Genetic variations defined by haplotype (Hd) and nucleotide (Pi) diversities, number of polymorphic/variable sites, number of parsimony sites and average number of pair wise nucleotide differences (Nei 1987) were calculated using DnaSP 5.10.01 computer program (Rozas *et al*. 2003).

The extent of population genetic differentiation between and within sampling sites was analysed by calculating the inbreeding coefficient F_{ST} using Arlequin 3.11 (Excoffier *et al*. 2005). Hierarchial AMOVA was used to assess variation between sample populations (F_{ST}); variation between sample populations within regions (F_{SC}) and variation between regions for sample populations (F_{CT}) according to Wright (1969) and Nei (1987). DnaSP 5.10.01 (Rozas *et al.* 2010) was used to estimate the nearest-neighbour statistic, *Snn* (Hudson 2000), which is a measure of how often the 'nearest neighbours' (in sequence space) are from the same locality (in geographical space). *Snn* varies from 0 to 1 whereby when values under 0.5 are obtained the population is assumed to be in '*panmixia*' state and values closer to 1 indicate that populations are differentiated. Evolutionary relationships between haplotype variants were obtained by constructing a haplotype network using Network 4.5.1 software, which applies the Median- Joining algorithm (Bandelt *et al*. 1999). A maximum likelihood (ML) phylogenic tree of the sequences was constructed in MEGA 6.0.6 to further assess the genealogical relationship between sequence of the present study and those collected elsewhere. Sequences of the individual species from Wallace Line region (South China Sea, Gulf of Thailand, East China Sea, Andaman Sea, Straits of Malacca, Sulu Sea, Timor Sea and seas surrounding northern Australia), Australia, Indonesia (Java Sea, Bali) and West Papua and New Caledonia were downloaded from the from the GeneBank. Other sequences for ROPME Sea Area (Arabian Gulf, Iran, Ras Khaimahiso, Bahrain, Kuwait, Abu Dhabi, Dibba area, Gulf of Oman and Arabian Sea) were downloaded from the GeneBank (http://www.ncbi.nlm.nih.gov/).

3.0 Results

3.1 Genetic Variation

The sequenced fragments of 475 bp produced a 275 bp after gap exclusion. A total of 41 variable sites constituting 37 parsimony informative sites, 4 singleton variable sites and 221 conserved sites were determined (Figure 2 and Table 1). Haplotype diversity (Hd), which indicates the probability that any two randomly chosen haplotypes are distinct from one another, was high 0.934±0.002 (Table 1) between localities. The nucleotide diversities (Pi) between sampling localities were estimated at 0.028±0.042 (Table 1) and were considered to be higher. Higher nucleotide and haplotype diversities were also observed within each sampling locality (Table 1).

Further analysis of the genetic variations indicates that six haplotypes were shared between localities, while only one haplotype was shared by specific individuals within the Dar es Salaam site (Table 2). This is an indicative of the existence of large number of haplotypes that are specific and/or endemic to Dar es Salaam and Pangani populations (Table 2). The Dar es Salaam samples comprised of seven specific haplotypes that were not shared within the population and only one haplotype (Hap_8) was shared by two individuals within the geographical edge (Table 2). By contrast, Pangani samples formed six specific haplotypes all of which indicated no sharing within the locality (Table 2).

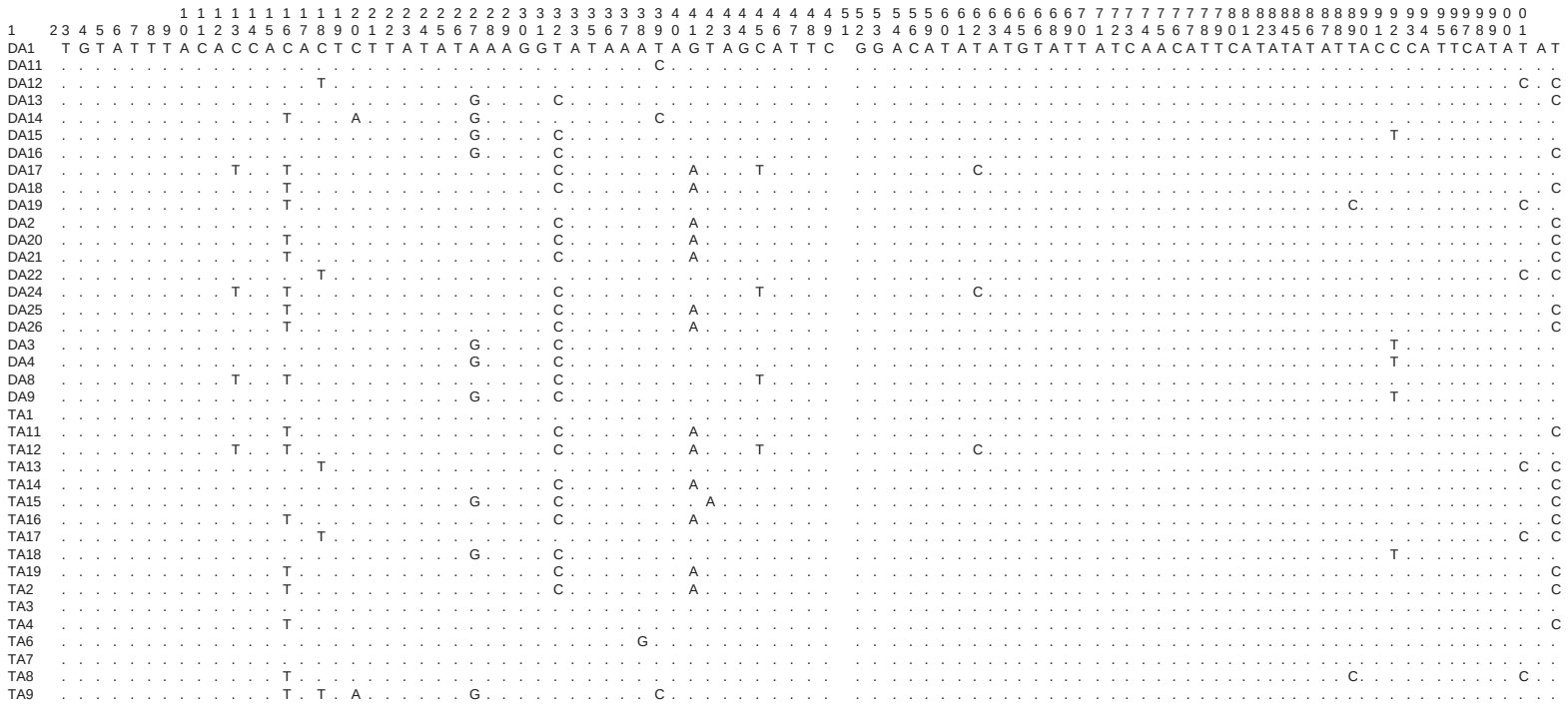

Figure 2: Polymorphic/segregating sites (41) for 38 mtDNA control region sequences from the Spanish mackerel collected along northern coastal waters of Tanzania. DA and TA indicate sequences from Dar es Salaam and Pangani sites respectively.

Table 1: Genetic diversity of Spanish mackerel along the northern Tanzania coastal waters.

Parameters	Within population		Between populations
	Dar es Salaam (1)	Pangani (2)	
Number of sequences	21	17	38
Number of haplotypes (h)	14	12	20
Haplotype diversity (Hd)	0.947±0.031	0.941±0.043	0.934±0.002
Number of polymorphic sites	38	40	41
Average number of nucleotide differences (k)	11.59±0.302	10.926	11.169
Nucleotide diversity, Pi(1)	0.028±0.148	0.027±0.143	0.028±0.042
Nucleotide diversity with Jukes and Cantor, Pi(1)JC	0.035±0.012	0.032±0.043	
Number of observed transitions	34	35	
Number of observed indels	0	0	
Number of observed sites with transitions	34	35	
Number of observed transversions	5	6	
Number of observed sites with transversions	5	6	
Number of substitutions	39	41	
Number of observed sites with substitutions	38		

Table 2: Haplotype inferences of the Spanish mackerel along the northern Tanzania coastal waters.

		Haplotype									
Location		Hp1*	Hp2*	Hp3	Hp4*	Hp5	Hp6	Hp7*	Hp8**	Hp9	Hp10
Dar es Salaam	Hap.freq.	1	1	1	3	1	1	2	2	1	1
	Rel.freq.	0.047	0.047	0.047	0.14	0.047	0.047	0.095	0.095	0.047	0.047
Pangani	Hap.freq.	2	1	0	1	0	0	2	0	0	0
	Rel.freq.	0.117	0.0588	0.027	0	0	0.117	0	0	0	0.108
		Hp11*	Hp12*	Hp13	Hp14	Hp15	Hp16	Hp17	Hp18	Hp19	Hp20
Dar es Salaam	Hap.freq.	4	1	1	1	0	0	0	0	0	0
	Rel.freq.	0.19	0.047	0.047	0.047						
Pangani	Hap.freq.	4	1	0	0	1	1	1	1	1	1
	Rel.freq.	0.058	0	0	0	0.027	0.027	0.027	0.027	0.027	0.027

Hp = Haplotype; *Hp shared between population: Hp1, Hp2, Hp4, Hp7, Hp 11 and Hp12, **Hp shared within population

3.2 Population Genetic Structure and Differentiation

The AMOVA results are presented in Table 3. The results from the F_{ST} pairwise comparison were as low as 0.0011 (p = 0.925), indicating that there was no significant genetic differentiation between populations. This in turn suggests that the Spanish mackerel population along the northern Tanzania coastal waters is characterized by species migration and mixing of individuals between the two study sites. The result demonstrates that a null hypothesis of a 'single panmictic population' of the Spanish mackerel along the northern coastal waters of Tanzania cannot be rejected. The analysis of spatial genetic structure between sites irrespective of time did not show significant genetic differentiation in Dar es Salaam (F_{CT} = 0.013, p = 0.057) and in Pangani (F_{CT} = 0.018, p = 0.065) populations. In addition, there was no genetic differentiation (F_{SC} = 0.021, p = 0.351) between and within the southeast and northeast and southeast monsoon season, suggesting that the population structure was stable at least during the 16 months of the study sampling duration. Furthermore, AMOVA analysis showed that 99.50% of the total molecular variance was distributed within the populations and only 0.5% was distributed between the populations (Table 3).

Table 3: Analysis of molecular variation (AMOVA) for samples of Spanish mackerel collected in the northern Tanzania coastal waters.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	F_{ST}	p
Between population	1	0.284	0.01Va	0.5	0.0011	0.925
Within population	36	17.006	0.473Vb	99.5		
Total	**37**	**17.289**	**0.483**	**100**		

3.3 Phylogenetic Relationships

The genealogical relationship was assessed using sequences and haplotypes. The maximum likelihood (ML) tree for the sequences from Tanzania indicates long genealogical branches (Figure 3a). Individuals were spread in both geographical localities which is an indicative of a single mixing clade of the Spanish mackerel within study areas. On the other hand, sequences from the ROPME Sea Area (Arabian Gulf, Iran, Ras Khaimahiso, Bahrain, Kuwait, Abu Dhabi, Dibba area, Gulf of Oman and Arabian Sea) formed single clade with sequences obtained from Tanzania (*i.e.* Clade 1). This suggests that the different sequences had similar evolutionary history. However, sequences from Wallace Line region (South China Sea, Gulf of Thailand, East China Sea, Andaman Sea, Straits of Malacca, Sulu Sea and Timor Sea) indicated a separate genealogical relationship from that of Tanzania and ROPME Seas Area and formed separate clade (Clade 2). Further analysis on the evolutionary relationship of the Spanish mackerel among haplotypes using a median joining (MJ) network tree revealed that the haplotype network (Figure 4) supported the topology of the phylogenic tree (Figure 3). The MJ network based on nucleotide divergences indicated that most haplotypes were closely related with the dominant haplotypes (i.e. Hap_11) as the centre of radiation (Figure 4). Most of the haplotypes varied from each other by one to two mutational steps from Hap_11. The star-like median network not only suggests similar evolutionary history but also existence of a recent historic population expansion (Figure 4); where the expansion in this case may consist of range expansion and demographic expansion.

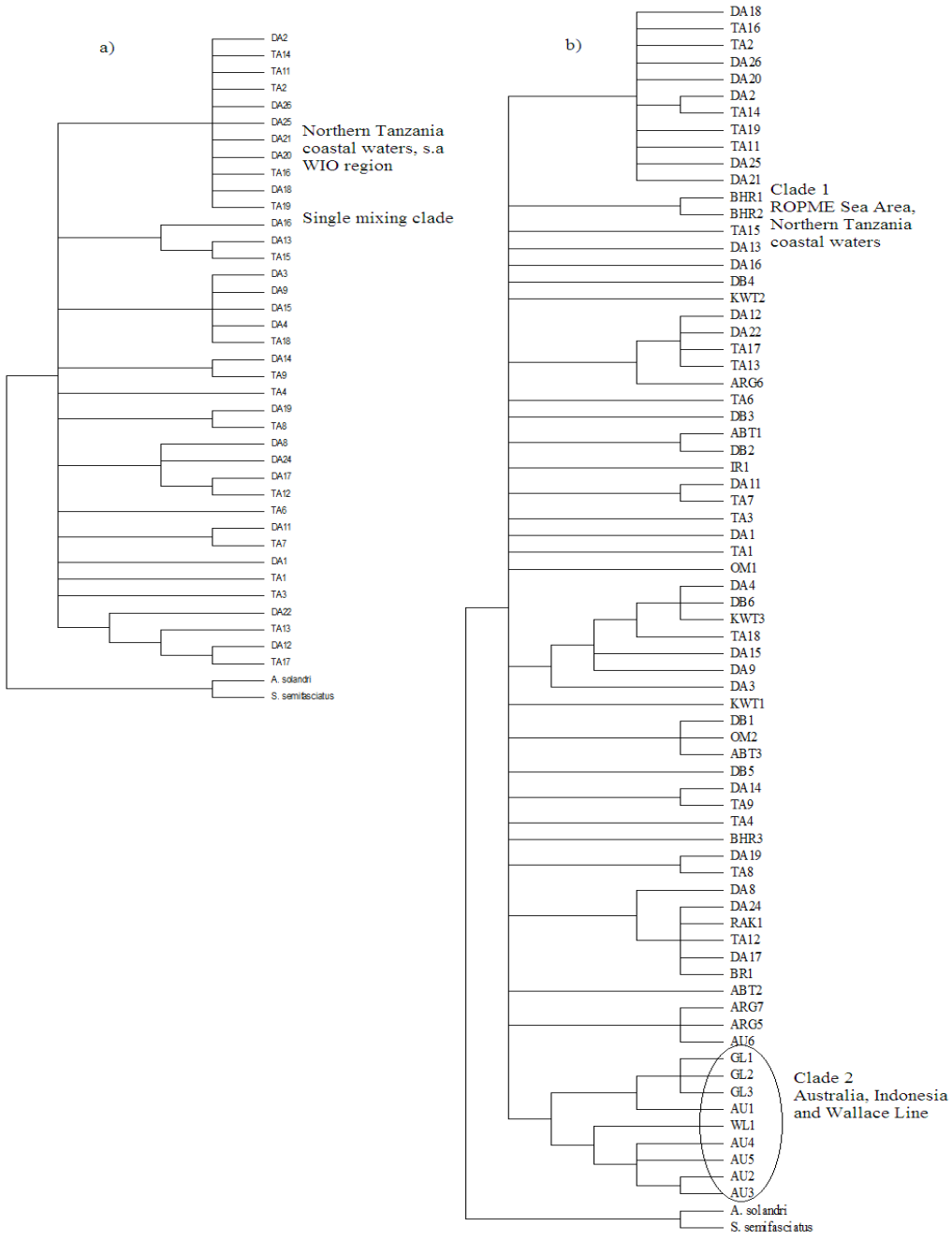

Figure 1: Maximum likelihood phylogenetic relationship of Spanish mackerel from (a) Tanzania and (b) Tanzania and other areas of the globe. Nodal bootstrap support is displayed where nodal support is ≥70%. Scale represents the proportion of polymorphic sites between sequences.

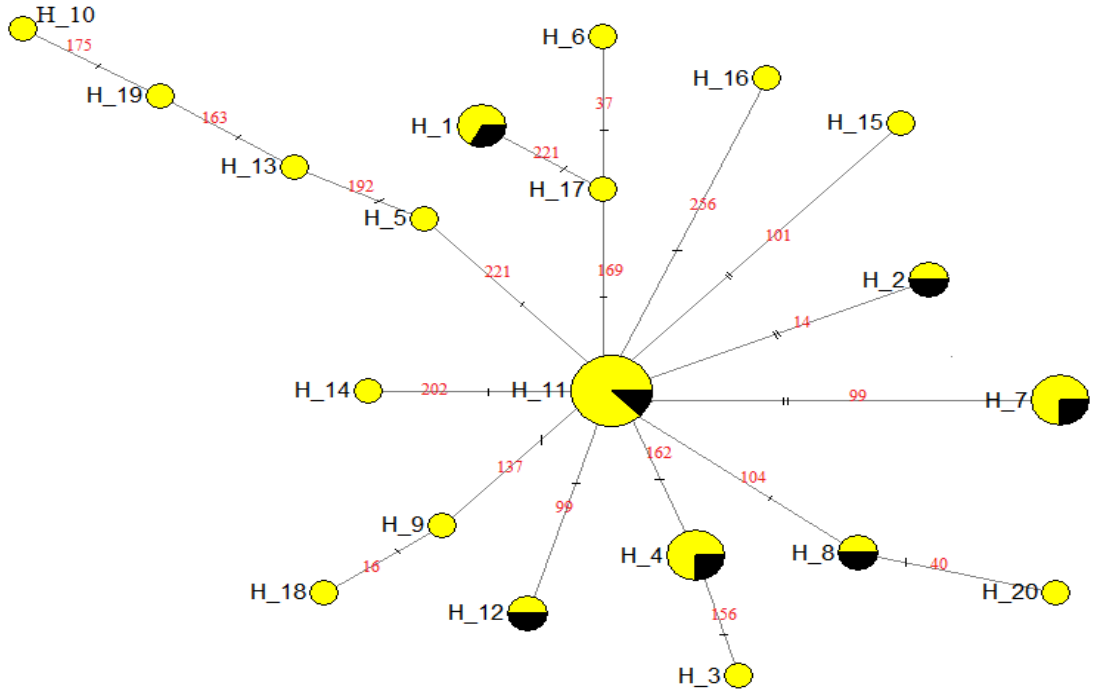

Figure 2: Median-Joining network (MJ) showing genealogical relationships among mtDNA D-loop gene haplotypes of Spanish mackerel. Numbers crossing the lines represent the sites of nucleotide substitutions; circle areas depict proportions of haplotype. Circles with yellow and black colors indicate haplotype shared between populations. In the shared haplotypes, yellow represent percentage shared by samples from Dar es Salaam while black represents percentage from Pangani.

4.0 Discussion

4.1 Genetic Diversity

The present study revealed that the Spanish mackerel at the investigated study areas were characterized by high levels of mtDNA genetic diversity at both haplotypes and nucleotide levels. The high genetic diversities provide an evidence of the presence of several unique haplotypes of Spanish mackerel along the northern parts of the Tanzania coastal waters. The observed high haplotype diversity within population may be attributed to the large population size, environmental heterogeneity and life history traits that favor rapid population growth (Nei 1987). The results are consistent with the theoretical mtDNA haplotype diversity expectations that are based on large population sizes (Avise 1998; Hausser and Ward 1998). Studies by Grant and Bowen (1998) reported that such high levels of genetic diversity at both haplotypes and nucleotides levels could either be associated with a long evolutionary history in a large stable population, or with secondary contact between different lineages. The genetic diversity

of Spanish mackerel observed in the present study is comparable to those observed in several recent studies by Sulaiman and Ovenden (2010) along Wallace's Line and Ovenden and Street (2007) in Australia.

4.2 Genetic Differentiation and Phylogenetic Relationship

The findings of the present study indicate that, Spanish mackerel along the northern coastal waters of Tanzania comprises of a single mixing population of the same genealogical relationship. The observed low levels of F_{ST} values found between localities indicated high connectivity which facilitates genetic homogeneity over the geographic range. The observed higher levels of genetic diversity and a single genetic stock detected at a small spatial scale is in agreement with the assumption that the Spanish mackerel along the northern Tanzania coastal waters is characterized by a large population size that is not very sensitive to genetic drift. The genetic homogeneity for large marine pelagic fishes is a common phenomenon. This is due to their highly migratory nature, very wide reproductive areas and extensive egg and larval dispersal through ocean currents (Hoolihan *et al*. 2006). However, coastal pelagic fishes have shown a stronger evidence of geographic subdivisions than oceanic species (Crosetti *et al*. 1994; Rossi *et al*. 1998). This is probably attributed to their inshore habitat requirements, shorter migration distances, and vulnerability to climatic fluctuations among other factors. Unfortunately, Spanish mackerel samples collected during this study did not indicate any degree of genetic differentiation at both temporal and spatial scales. The study is in conformity with the one conducted by Hoolihan *et al*. (2006) in the ROPME Sea Area but different from that of Sulaiman and Ovenden (2010) in the Wallace Line. This may perhaps be due to the absence of noticeable barriers to dispersal between sampling localities, which reduces heterogeneity among populations. Other factors contributing to the lack of genetic structure include high levels of gene flow, large effective population size and/or the presence of shared ancestral polymorphisms due to recent population divergence. Sex-biased dispersal is another factor that could explain the lack of genetic structure (Avise 2004). MtDNA is an indicator of long-term female dispersal history because it is maternally inherited (Sudath 2007). As a result, no genetic differentiation between populations is expected for species in which females disperse widely.

Low genetic differentiation in Spanish mackerel reported by the present study conforms to the characteristics of high vagility and admixture to the members of the genus *Scomberomorus* within their respective regions (Buonaccorsi *et al*. 1999). Furthermore,

lack of genetic heterogeneity between regions reflects reciprocal gene flow, which is consistent with a single intermingling genetic stock as reported in *S. cavalla* from the western Atlantic and Gulf of Mexico. The findings of the present study are different from the findings of previous studies by Shaklee *et al*. (1990) and Ovenden and Street (2007) which supported the existence of a distinct stock of Spanish mackerel from the east coast and from Northeast Australia. On the other hand, phylogenetic analysis of mtDNA control region sequences showed no obvious phylogeographic pattern separating the sampling sites of Spanish mackerel.

The present results suggest that Spanish mackerel that migrate to and reside in the northern Tanzania coastal waters have the same genealogical history. The presence of a single clade on the other hand is probably due to frequent contact and interbreeding between populations. Another factor that could account for the observed physical mixture of the Spanish mackerel at the investigated area includes the high rate of migration and differential passive transport of larvae to the fishing grounds by the monsoonal currents. The observed sequences formed a single clade with samples from the ROPME Sea Area, which is consistent with the hypothesis that the Spanish mackerel dwelling along the northern Tanzania coastal waters has the same evolutionary history and possibly existence of gene flow between sites. By contrast the fact that the sequences from Wallace Line region, Australia, Indonesia (Java Sea, Bali), West Papua and New Caledonia formed a separate clade with those from Tanzania and ROPME Sea Area, indicates that the sequences have different genealogical histories. Genetic divergence between these populations could possibly be attributed to geographical distances as well as reproductive isolation, which in turn have led to the failure in maintaining the gene flow.

4.3 Conclusion and recommendation

The present study revealed higher genetic diversity of the Spanish mackerel between and within studied populations along the northern Tanzanian coastal waters. The study further revealed a single mixing population of Spanish mackerel in the northern Tanzania coastal waters, which in turn calls for a single stock model for management purpose. However, considering the migratory nature of this species, a co-management between coastal nations is appropriate if sustainable exploitation is to be achieved.

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