Genetic population structure of shortfin mako *(Isurus oxyrinchus)* inferred from mitochondrial DNA on inter-oceanic scale[1]

Mioko Taguchi

National Research Institute of Far Seas Fisheries, Japan
5-7-1 Orido, Shimizu-ku, Shizuoka 424-8633, JAPAN

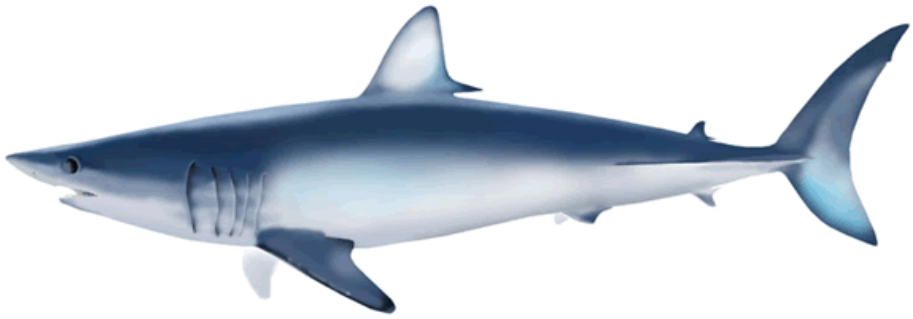
Toru Kitamura

Japan NUS Co., LTD.
Nishi-Shinjuku Kimuraya Bldg., 5F, 7-5-25 Nishi-Shinjuku,
Shinjuku-Ku, Tokyo 160-0023, JAPAN

Kotaro Yokawa

National Research Institute of Far Seas Fisheries, Japan
5-7-1 Orido, Shimizu-ku, Shizuoka 424-8633, JAPAN

Email: tagu305@affrc.go.jp



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Summary

Genetic population structure of shortfin mako on inter-oceanic scale was examined to contribute the stock assessment of shortfin mako in the North Pacific, using a total of 106 partial control region sequences in mitochondrial DNA of shortfin mako, caught in the North Atlantic, eastern and western Indian Ocean, central North Pacific, and eastern and western South Pacific. The spatial analysis of molecular variance estimated the genetic structuring of shortfin mako between the North Atlantic and Pacific Ocean groups with a genetic break in the Indian Ocean, which was also supported by pairwise ϕ_{st} estimates and geographical distribution of haplotypes. Moreover, the observed high haplotype diversities compared to nucleotide, and the star-like genealogy in minimum spanning network were associated with a recent population growth of shortfin mako.

Introduction

The scientific committee of WCPFC that was held in August 2009 requested discussion on feasibility of stock assessment by ISC for key shark species in the North Pacific, especially for shortfin mako and blue shark. In response to this recommendation, ISC held Shark Taskforce group meeting (STFG) on July 2010 at Victoria, Canada, and the STFG agreed the stock assessment of shortfin mako and blue shark in the North Pacific under the leadership of ISC.

Genetic population structure is one of the important information for fishery stock assessment, but that of many pelagic sharks including shortfin mako remains unknown. The genetic variations of shortfin mako in the North Pacific should be evaluated in relation to the genetic population structure on global scale before determination of the management unit within the North Paicific. The genetic population structure was examined using AFLP (Amplified Fragment Length Polymophism) for the mitochondrial DNA (mtDNA) of shortfin mako in the central and western North Atlantic, western South Atlantic, western South Pacific and eastern North Pacific (Heist et al. 1996), which suggested strong differentiation of shortfin mako in the North Atlantic from other oceans however any differentiation were not found among them. Furthermore, the microsatellite analyses didn't find strong evidence of genetic structuring for shortfin mako (Schrey and Heist, 2003), which implied the mitigation of genetic differentiation and genetic structuring through the gene flow of male mako sharks. However, the discussion on genetic relationship between the Pacific and Indian Ocean, giving meaningful information to determine management unit in the Pacific Ocean, is insufficient in the previous studies because of a lack of samples in the Indian Ocean.

The global genetic population structure of shortfin mako was thus reviewed using a total of 106 partial sequences in mitochondrial control region of shortfin mako from not only the North Atlantic, South and North Pacific Ocean but also the Indian Ocean.

Materials and Methods

Sample collection

Tissue samples were obtained from a total of 106 shortfin mako caught in the North and South Pacific, North Atlantic and Indian Ocean (Fig. 1), which were preserved in 99% ethanol at room temperature.

DNA extraction and amplification

Total genomic DNA of 106 mako sharks was extracted from muscle tissues using the DNeasy Tissue Kit (Qiagen). Polymerase chain reaction (PCR) was used to amplify - 400bp of mtDNA control region, using primers which were designed referring to mitochondrial sequences of the gummy shark (GenBank accession number:NC000890), horn shark (GenBank accession number:AJ310141) and smaller spotted catshark (GenBank accession number: Y16067). These two primers were IsurusD-L (5'-GAG TGC TGT CAG AGC ATG AA-3') and IsurusD-H (5'-CAG GTT TAT CTC AGT GTC CC-3'), and the DNA polymerase used was TaKaRa Ex Taq Hot Start Version (TaKaRa). PCR conditions were performed on 1.0μL of DNA extracted from muscle tissues in a 25μL volume containing 2.5μL of 10x PCR buffer, 2.0μL of dNTP (4mM), 2.5μL of each primer (5μM), 0.1μL of 0.5 units of TaKaRa Ex Taq Hot Start Version (TaKaRa), and 14.4μL of sterile distilled water, and thermal cycle profile was as follows: 98°C for 30sec then 30 cycles (denaturation at 98℃ for 10sec, annealing at 55°C for 30sec, and extension at 72°C for 60sec) followed by 72°C for 2min (Palumbi et al. 1991). The control region fragments were sequenced with BigDye Terminator v.3.1 Cycle Sequence Kit (Applied Biosystems) and aligned using the software DNASIS Pro V2.2 (Hitachi Solutions). Gaps and missing data were not observed at all sequences of mako sharks.

Nucleotide sequence analysis of the mtDNA control region

In consideration of geographical distribution of samples, six sub-populations, western Indian Ocean (N=16; No.1-16), eastern Indian Ocean (N=16; No.17-32), central North Pacific (N=39; No.33-71), western South Pacific (N=16; No.72-87), eastern South Pacific (N=10; No.88-97) and the North Atlantic (N=9; No.98-106), were defined for all analyses in the present study (Fig. 1).

Nucleotide and haplotype diversities (Nei 1987) were estimated for each sub-population. Pairwise φ_{ST} values based on the Kimura two-parameter (K2P) model of evolution (Kimura 1980) were calculated to measure the genetic differentiation among sampling sites, using 10,000 random permutations of the original data sets. These analyses were conducted in ARLEQUIN ver. 3.5. (Excoffier and Schneider 2005).

To investigate genetic structuring, a spatial analysis of molecular variance (SAMOVA) was used (Dupanloup et al. 2002). The program SAMOVA can detect genetic boundary without *a priori* definition of population groups by a combination with AMOVA (Excoffier et al. 1992) and geographic information of the sampling sites.

A neighbor-joining (NJ) and Maximum Likelihood (ML) tree were drawn to infer the phylogenetic relationships among individuals with Mega v.5 (Tamura et al. 2007, Tamura et al. 2011) using the genetic distances calculated by K2P model (Kimura 1980). The maximum parsimony method was used in the case of the number of common sites with < 100 or less than one fourth of the total number of sites, otherwise the BIONJ method (Gascuel 1997), an improved version of the NJ algorithm, with distance matrix estimated by the maximum composite likelihood method was used for initial tree(s) for the automatic heuristic search. The reliability of obtained phylogenetic tree was evaluated by 1,000 permutations of bootstrap resampling (Felsenstein 1985).

A minimum spanning network was constructed via the program TCS v1.21 (Clement et al. 2000), with a 95% probability of parsimonious connection limit, to infer the phylogenetic relationships among mitochondrial DNA haplotypes of shotfin mako.

Results and Discussion

A total of 17 variable sites were identified in 106 partial mtDNA control region sequences, defining 27 haplotypes for the analyses (Table 1).

The haplotype (h) and nucleotide (π) diversities of shotfin mako were 0.92 and 0.0070 all over their range, respectively (Table 2). Overall, the haplotype diversities in each sub-population were high compared to the nucleotide diversities observed in the present study (Table. 2).

Inter-oceanic scale

The pairwise φ_{ST} estimates (Table 3) showed clearly genetic differentiations among sub-populations in the North Atlantic and the Pacific Ocean, which was coincident with previous study (Heist et al. 1996). Additionally, shortfin mako in the eastern Indian Ocean showed distinct genetic differentiation from the proximal Pacific Ocean rather than the distant North Atlantic (Table 3). Geographical distribution of haplotypes observed in the present study was shown in Table 1 and Fig. 2. Half of haplotypes observed in the Pacific Ocean, the central North Pacific, eastern and western South Pacific, were ocean specific (Fig. 2 and Table 1). Moreover, the most commonly occurring haplotype, Hap 3, was not found in the eastern Indian Ocean (Fig. 2), whereas most of haplotypes observed in that ocean were shared with other sub-populations, especially with the North Atlantic and western Indian Ocean. The SAMOVA results showed the maximal genetic divergence among groups with two hierarchical groups, three sub-populations in the North Atlantic and Indian Ocean, and three in the Pacific Ocean. AMOVA confirmed the distinct genetic structure, with 18.32% of the total variance ascribed to differences between these two groups (Table 4, P=0.06), was not statistically supported, but the variations within groups and sub-populations were also statistically significant with 3.96% and 77.72% of the total variance (within groups: P<0.001, within populations: P<0.001), respectively (Table 4). The phylogenetic

relationships of sequences was not so different between NJ and ML methods, thus only the dendrogram by ML methods was shown in Fig. 3. Overall, it did not show any local populations completely, although partial clusters composed of sequences in the Pacific Ocean were supported with higher than 60% reliability in bootstrap value (Fig. 3). Minimum spanning network was constructed of two star-like genealogies of haplotypes (Fig. 4), and the observed haplotypes in each sub-population, except in the eastern South Pacific, were distributed in both genealogies.

Given the distinct differentiation between the North Atlantic and Pacific Ocean shown in pairwise φ_{ST} estimates, the observed haplotypes endemic to the Pacific Ocean, and the result of SAMOVA, shortfin mako in the Pacific Ocean should be diverged from the North Atlantic Ocean with a genetic break in the Indian Ocean. This finding was also supported by the phylogenetic tree of sequences for shortfin mako used in the present study. Moreover, the pattern of genetic distance between the Indian Ocean and other oceans contradicting the geographical distance suggested by pairwise φ_{ST} estimates, and a lack of common haplotype observed in the eastern Indian Ocean might arise from the population history of sharks in the Indian Ocean. Furthermore, the star-like genealogies observed in minimum spanning network means understood as a recent population growth of shortfin mako (Avise 2000). This was coincident with the observed high haplotype diversities compared to nucleotide in the present study.

Intra-oceanic scale

The pairwise φ_{ST} estimates did not show any significant differentiations within the Pacific Ocean (Table 3), although genetic divergence was larger between the South and North Pacific than within the South Pacific, which implied some genetic divergence of sharks between the North and South Pacific Ocean. Moreover, the observed haplotypes in the central North Pacific and western South Pacific Ocean were distributed widely in two star-like genealogies of minimum spanning network, whereas all haplotypes in the eastern South Pacific Ocean were distributed in one genealogy only (Fig. 4). This is likely to suggest the younger population history of sharks in the eastern South Pacific than other sub-populations in the Pacific Ocean.

Conclusion and further works

In conclusion, the present mtDNA analysis suggested distinct genetic structuring of shortfin mako between the North Atlantic and Pacific Ocean groups with a genetic break in the Indian Ocean, but the finer location of boundary between them remains unknown. A few hundred of samples in each oceans, especially in the Indian and Pacific Ocean, were needed, which should be evenly collected all over their range through the year. Additionally, some implication of the relationship between the observations and population history of shortfin mako in the Indian Ocean is still disputable. Further phylogeographic analyses with substantial sample size as described above were required for the conclusion.

The genetic structuring within the Pacific Ocean were also implied in this study, but distinct evidence were not found. In further analysis, at least 500 samples (approximately 70 samples in each sub-population) collected from the west, center and east in both hemispheres, especially in the whole South Pacific, eastern North Pacific, and tropical area in both hemispheres, will need to reveal the genetic differentiation and structuring within the Pacific Ocean. It is preferable that those samples are evenly collected from mature sharks on both sexes during mating period, April to September. Additionally, further analysis with other region in mtDNA as well as multilocus analysis in combination with mtDNA and nuclear DNA, which allows tracing both parental demographic traits, are required because they should help understanding the finer genetic population structure of shortfin mako.

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Table 1 Geographical distribution and frequency of haplotypes observed in the present study

Haplotype	WIO	EIO	CNP	WSP	ESP	NA	Total
Hap 1	0	0	0	1	1	0	2
Hap 2	0	0	0	1	0	0	1
Hap 3	2	0	13	3	2	1	21
Hap 4	1	0	1	1	1	0	4
Hap 5	0	0	0	0	1	0	1
Hap 6	0	0	0	0	2	0	2
Hap 7	0	0	2	3	1	0	6
Hap 8	0	0	10	0	0	0	10
Hap 9	0	0	5	0	0	0	5
Hap 10	2	3	1	1	0	0	7
Hap 11	0	1	0	1	1	0	3
Hap 12	0	1	0	0	0	0	1
Hap 13	1	5	0	1	0	3	10
Hap 14	2	1	0	0	0	0	3
Hap 15	2	4	2	2	0	3	13
Hap 16	3	0	0	0	0	0	3
Hap 17	1	1	0	0	0	0	2
Hap 18	1	0	0	1	0	0	2
Hap 19	1	0	0	0	0	0	1
Hap 20	0	0	0	0	0	1	1
Hap 21	0	0	0	0	0	1	1
Hap 22	0	0	2	0	0	0	2
Hap 23	0	0	1	0	0	0	1
Hap 24	0	0	1	0	0	0	1
Hap 25	0	0	1	0	0	0	1
Hap 26	0	0	0	1	0	0	1
Hap 27	0	0	0	0	1	0	1
Total	16	16	39	16	10	9	106

See Fig. 1 for abbreviation of sub-populations

Table 2 Haplotype (h) and nucleotide (π) diversities for each sub-population

Sub-populations	N	h	s.d.	π	s.d.
WIO	16	0.94	0.04	0.0063	0.0041
EIO	16	0.84	0.06	0.0049	0.0033
NP	39	0.82	0.04	0.0058	0.0036
WSP	16	0.94	0.04	0.0082	0.0050
ESP	10	0.96	0.06	0.0057	0.0039
NA	9	0.83	0.10	0.0064	0.0043
Global	106	0.92	0.01	0.0070	0.0041

See Fig. 1 for abbreviation of sub-populations

Table 3 Pairwise ϕ_{st} values among the sub-populations based on 10,000 permutations

Sub-populations	WIO	EIO	CNP	WSP	ESP	NA
WIO						
EIO	0.0819					
CNP	0.0740	**0.2748**				
WSP	-0.0051	**0.1401**	0.0777			
ESP	0.1327	**0.3721**	0.0704	0.0437		
NA	0.0658	-0.0683	**0.2526**	0.1041	**0.3237**	

See Fig. 1 for abbreviation of sub-populations. The statistical significance after sequential Bonferroni correction was shown as bold

Table 4 Results of the AMOVA with sub-population groups inferred from SAMOVA

Source of Variation	d.f.	Variance components	Percentage of variation	Fixation Index
Among groups	1	0.284	18.32	0.183
Among sub-populations within groups	4	0.061	3.96	0.048**
Within sub-populations	100	1.206	77.72	0.223**
Total	105	1.551		

**P<0.0001

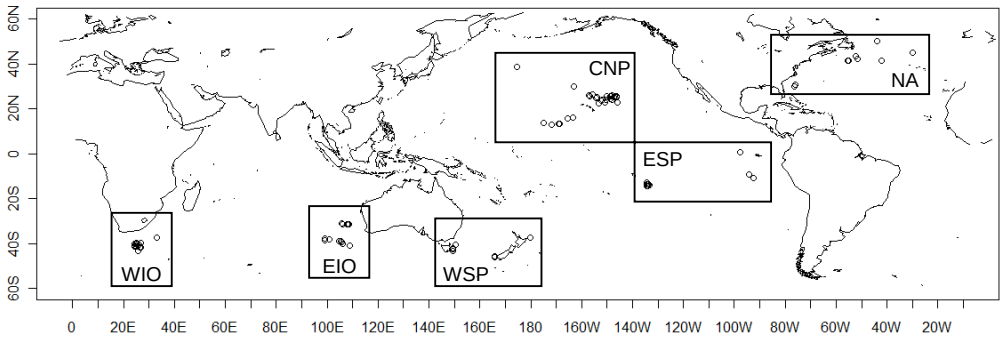

Fig. 1 Sampling locations for shotfin mako used in the present study. The abbreviations of sub-population were given followed: WIO, western Indian Ocean; EIO, eastern Indian Ocean; CNP, central North Pacific; WSP, western South Pacific; ESP, eastern South Pacific; NA, North Atlantic

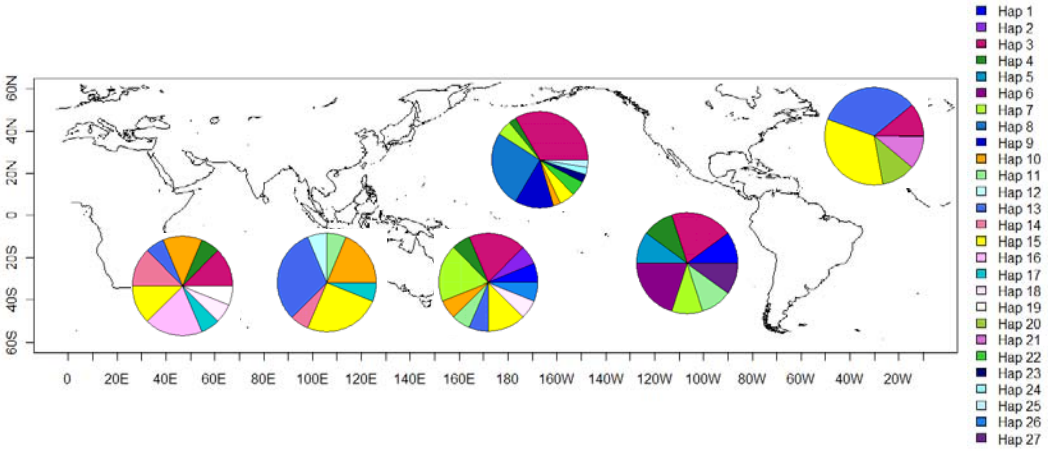

Fig. 2 The frequency and geographical distribution of the observed haplotypes in the present analysis

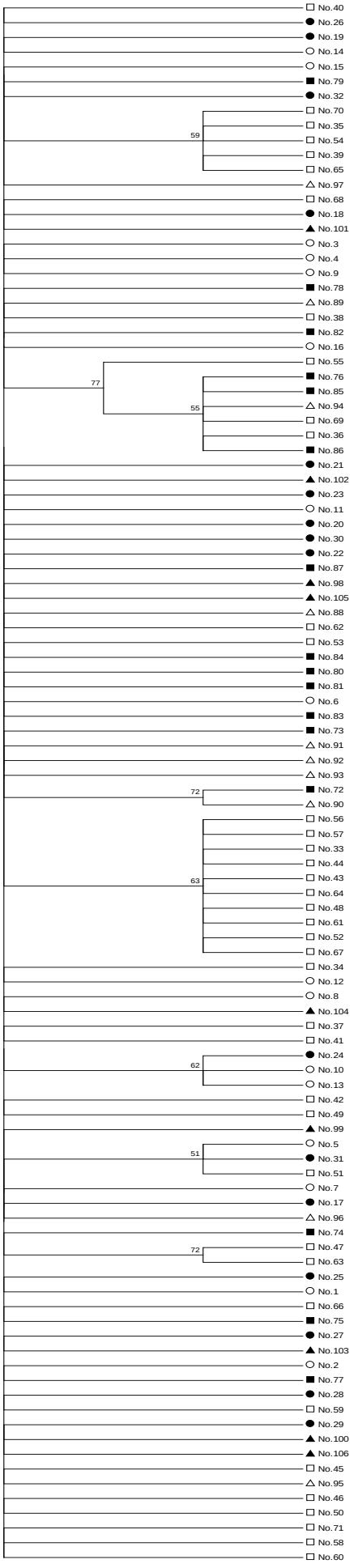

Fig. 3 Phylogenetic relationship among partial sequences of control region in mitochondrial DNA by the bootstrap consensus tree inferred from 1,000 replicates with maximum likelihood method based on Kimura two-parameter model of evolution. Branches corresponding to partitions reproduced in less than 50% bootstrap reliability were collapsed. The numbers above the branch indicated bootstrap reliability. The geographical information of each sequence were given followed; ○: western Indian Ocean, ●: eastern Indian Ocean, □: central North Pacific, ■: western South Pacific, △: eastern South Pacific and ▲ : North Atlantic

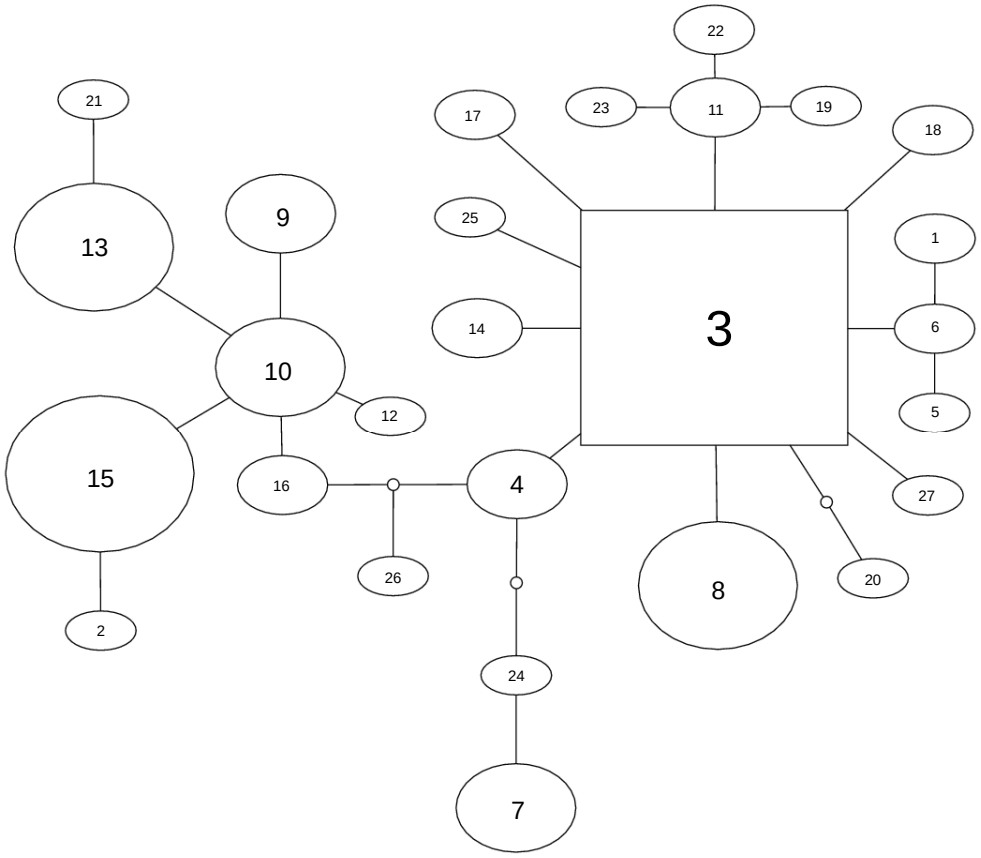

Fig. 4 Minimum spanning network for mtDNA control region haplotypes of the shortfin mako. Each line indicated one mutational step. Circles represent haplotype and the size refers to approximate haplotype abundance. Small open circles indicate intermediate haplotypes not found in the present study