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SHORT COMMUNICATION

Hints for panmixia in *Scomberomorus commerson* in Indian waters revealed by mitochondrial ATPase 6 and 8 genes

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Abstract

Scomberomorus commerson is an economically important migratory fish distributed worldwide. The genetic stock structure of *S. commerson* distributed along the Indian waters was identified using mitochondrial ATPase 6 and 8 genes. A total of 842 bp sequence of ATPase 6/8 genes obtained in this study revealed 23 haplotypes with mean low nucleotide diversity and high haplotype diversity. Co-efficient of genetic differentiation (F_{ST}) values obtained for pair wise populations were low and non-significant with an overall value of −0.02074. The high haplotype and low nucleotide diversity values together with mismatch distribution analysis suggested a history of genetic bottleneck events or founder effect, with subsequent population expansion in *S. commerson.* The findings of the present study indicated the panmixia nature of the species which can be managed as a unit stock in Indian waters.

Keywords

Genetic differentiation, mitochondrial ATPase 6/8 genes, *Scomberomorus commerson*

History

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Introduction

Narrow barred Spanish mackerel, *Scomberomorus commerson* of family Scombridae (Lacepède, 1800), is one of the economically important marine fish species in Indian waters. The species possess high demand in domestic market and preferred commodity in the export market. This species is distributed across Red Sea, Indo-West Pacific, and the Mediterranean Sea (Eschmeyer, 2015). Although its commercial landing has been recorded as 27,680 tonnes during last year (CMFRI, 2014) in Indian waters, the species experiences increased pressure in the Indian Ocean mainly due to overexploitation. It is also important to note that the species is categorized under threatened category in conservation assessment (Collette et al., 2011). The apparent fidelity of this species to the particular region is a matter of concern as overfishing in these areas can lead to localized depletion, as no solid information about the stock structure and the total catch of *S. commerson* available in the region. At present, there is no quantitative stock assessment is available for this species in the entire Indian Ocean. Due to lack of fishery data for several gears, only preliminary stock indicators can be used to assess the exploitation status of the species in this region (IOTC, 2013).

Understanding the genetic stock structure is vital for effective and sustainable long-standing management of fishery resources. Molecular genetics tools offer the ability to recognize and delineate fish stock structure where it may not be accrued through phenotypic or behavioral characteristics. Generally, maternally inherited mitochondrial genes are widely used for population genetic structure studies in fish species (McGlashan & Hughes, 2000). ATPase genes of mtDNA are moderately fast evolving and is very much useful in detecting population structure, levels of connectivity, and influence of historical processes in fish species (McGlashan & Hughes, 2001; Ovenden & Street, 2003). Mitochondrial ATPase 6/8 gene has been successfully used to reveal the genetic stock structure across different groups of fish species (Divya et al., 2015; Kathirvelpandian et al., 2014; Rupesh et al., 2014). The objective of the present study is to reveal the genetic stock structure of *S. commerson* along the Indian coast using variable mitochondrial ATPase 6/8 gene.

Materials and methods

A total of 87 tissue samples of *S. commerson* were collected from five different geographical locations along Indian coast [Mangaluru (12.8700 °N, 74.8800 °E), Kochi (9.9700 °N, 76.2800 °E), and Veraval (20.9000 °N, 70.3700 °E) in the west coast; Chennai (13.0839 °N, 80.2700 °E) and Vishakhapatnam (17.6883 °N, 83.2186 °E) in the east coast]. Total genomic DNA was isolated from muscle tissue/fins using DNeasy Blood and Tissue Kit (Qiagen, Limburg, Netherlands). DNA concentration was estimated by optical density (OD) reading using a spectrophotometer (SPECORD 205, Qiagen, Limburg, Netherlands) set at 260 nm.

ATPase 6/8 genes were amplified by PCR (Applied Biosystems, Foster City, CA) using universal primers ATP8 2L8331: 5′-AAAGCRTYRGCCTTTTAAGC-3′ and COIII 2H9236: 5′-GTTAGTGGTCAKGGGCTTGGRTC-3′ (http://nmg.si.edu/bermlab.htm). Amplification was conducted in 25 µl volume containing 2.5 µl of 10 × PCR buffer (100 mM Tris, pH 8.8, 500 mM KCl, 25 mM $MgCl_2$, 0.8% (v/v) (Fermentas, Hanover, MD), and 1.5 units of Taq DNA polymerase (Fermentas, Hanover, MD), 200 µM of each dNTPs (dATPs, dCTPs, dGTP, and dTTPs) (Fermentas, Hanover, MD), 20 pmol of

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each primer and 20 ng of genomic DNA. The thermal conditions used to amplify ATPase gene consisted of an initial denaturation of 95 °C for 5 min; followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were sequenced bi-directionally using ABI 3730 capillary sequencer (Fermentas, Hanover, MD) at sequencing facility.

The raw DNA sequences were edited using the software BioEdit sequence alignment editor version 7.0.5.2 (Abbott Molecular, Carlsbad, CA) (Hall, 1999). DnaSP 5.0 (Abbott Molecular, Carlsbad, CA) (Librado & Rozas, 2009) was used to calculate the haplotype frequency and nucleotide diversity (π) and haplotype diversity (h) among samples. Pair-wise F_{ST} values were calculated for overall as well as between samples collected from different locations using the software ARLEQUIN ver 3.0 (Abbott Molecular, Carlsbad, CA). Pair-wise sequence divergence among populations according to the Kimura two-parameter model (Kimura, 1980) and the number and the rate of transitions/transversions, and the NJ tree using pair-wise F_{ST} were calculated using the program MEGA version 6.0 (MEGA Inc., Englewood, NJ) (Tamura et al., 2013).

Results

A total of 842 bp sequence of ATPase 6/8 gene was obtained in 87 individuals from samples of five geographic locations along Indian coast. Two fragments, 168 bp fragment of ATPase 8 and 684 bp of ATPase 6 were used together for detecting the genetic variation in *S. commerson*. An overlapping region (10 bp) between two genes was found in the region of 159–168 bp. A total of 22 variable positions with 23 haplotypes including 9 parsimony informative sites and 13 singleton variable sites were observed. The average frequencies of four nucleotides for all the samples of *S. commerson* were A: 27.37%; T: 27.37%; C: 32.15%, G: 13.12%; nucleotide sequences of ATPase6/8 were A + T rich (54.74%) with transition transversion bias (R) of 12.02. All the haplotypes were submitted to NCBI GenBank (KP281779–KP281801). More haplotypes were found in Chennai population (11) followed by Kochi and Vishakhapatnam (7). Veraval and Mangaluru populations contained the least number of haplotypes (6). Four haplotypes (H20–H23) were specific to Vishakhapatnam; seven were specific to Chennai (H13–H19); H8 and H10 were specific to Kochi; H11 and H12 were specific to Mangaluru and H1, H4, and H5 were specific to Veraval. These location-specific haplotypes can be used for the traceability of *S. commerson* (Table 1).

The mean nucleotide diversity for all the samples from five locations of *S.commerson* was found to be 0.0021, whereas haplotype diversity was recorded as 0.809 (variance 0.00102 ± 0.032) (Table 2). Pair-wise F_{ST} values were calculated which did not show any differentiation between five populations of *S. commerson.* The overall F_{ST} value was also found to be -0.02074. Fu's Fs statistic and Tajima's D values were found to be -19.263 and -1.77158, respectively (Table 2). Significantly negative D value of Tajima and F value of Fu in all the population as a whole indicates a deviation from the expectations of the mutation-drift equilibrium, and indicated population expansion. Mismatch distribution analysis was performed with ATPase 6/8 genes for all the samples collected from five different locations. The histogram was found to be unimodal ($p < 0.05$) showing that samples of all the locations has passed through a demographic expansion after recent bottleneck event. In addition, non-significant raggedness index (RI = 0.06762, $p = 0.594$) and the SSD values, sum of square deviations (SSD = 0.01174, $p = 0.482$), hold up the null hypotheses of population expansion for all samples of *S. commerson* across different collection sites (Table 2).

Table 1. Relative haplotype frequencies of five populations of *Scomberomorous commerson*.

Haplotype/location	Veraval	Kochi	Mangaluru	Chennai	Vishakhapatnam
H1	0.111	0	0	0	0
H2	0.444	0.273	0.4	0.462	0.263
H3	0.111	0.273	0.2	0.115	0.211
H4	0.111	0	0	0	0
H5	0.111	0	0	0	0
H6	0.111	0.273	0.1	0.0385	0.211
H7	0	0.0455	0.1	0	0
H8	0	0.0455	0	0	0
H9	0	0.0455	0	0.0385	0
H10	0	0.0455	0	0	0
H11	0	0	0.1	0	0
H12	0	0	0.1	0	0
H13	0	0	0	0.0385	0
H14	0	0	0	0.0385	0
H15	0	0	0	0.0769	0
H16	0	0	0	0.0385	0
H17	0	0	0	0.0385	0
H18	0	0	0	0.0385	0
H19	0	0	0	0.0769	0
H20	0	0	0	0	0.0526
H21	0	0	0	0	0.0526
H22	0	0	0	0	0.0526
H23	0	0	0	0	0.0526

Discussion

Mitochondrial DNA is only a small part of the whole genome but showing hyper variability and can be used for population studies (Fuchs et al., 2008). Mitochondrial DNA are successfully used for population studies in other Scombroid fishes (Buonaccorsi et al., 2001; Shui et al., 2009). A 10 bp overlapping sequence observed in this study is similar to those reported in some other fishes like American Cichlid *Channa marulius* (Habib et al., 2012), *Coilia dussumieri* (Kathirvelpandian et al., 2014), and *Pampus argenteus* (Divya et al., 2015). Most nucleotide variations were resulted from transitions (Ts: 92.32) followed by transversions (Tv: 7.68). Similar results were reported in *Channa marulius* (Habib et al., 2012) and *Coilia dussumieri* (Kathirvelpandian et al., 2014).

Statistically significant low F_{ST} values showing the weak genetic differentiation of *S. commerson* between the locations may be attributed to higher larval and adult stage dispersal together with the non-existence of a physical barrier for movement between ocean basins. In oceans, the coastal current is continuously changing and this phenomenon may be resulting in the exchange of larvae and finally in low population genetic differentiation (Mandal et al., 2012). This low differentiation is also indicating that high level of gene flow is there between Bay of Bengal and Arabian Sea for these highly migratory fishes. The concept is well suited with the nature of long shore migration of the species which abode in coastal waters at depth range 15–200 m (Randall, 1995). In the same manner, Abedi et al. (2012) revealed a single stock in Persian Gulf using microsatellite markers.

According to Grant & Bowen (1998), high h and low π are interpreted as population bottleneck followed by rapid population growth and accumulation of mutations. The present finding suggests the scenario with high haplotype with low nucleotide diversity. Similar results were obtained in *Pampus argenteus* (Divya et al., 2015) and *Coilia dussumieri* (Kathirvelpandian et al., 2014). The observed high haplotype diversity and low nucleotide diversity are interpreted as occurrence of population bottleneck followed by rapid population expansion due to

Table 2. Intra-population haplotype diversities, nucleotide diversities, and demographic parameters for ATPase 6/8 gene from five different locations of *Scomberomorous commerson*.

Location	No: of polymorphic sites	Haplotype diversity (Hd)	Nucleotide diversity	Tajima *D*	Fu's Fs statistic	Raggedness index (*p* value)	SSD (PSSD)
Veraval	6	0.833	0.00224	−0.6299	−2.329	0.07716 (0.71)	0.016 (0.510)
Kochi	7	0.805	0.00209	−0.27110	−1.410	0.06276 (0.49)	0.01530 (0.24)
Mangaluru	6	0.844	0.00179	−1.1894	−2.605	0.09136 (0.58)	0.00551 (0.74)
Chennai	10	0.782	0.00231	−1.1386	−4.576	0.04839 (0.67)	0.01357 (0.42)
Vishakapatnam	9	0.842	0.00214	−1.0484	−2.718	0.04839 (0.52)	0.00768 (0.51)

accumulation of new mutations. The concept is further strengthened with negative and significant Tajima *D* value which implies that there were more nucleotide site variants than would be expected under neutral model of evolution. The unimodal mismatch frequency distribution pattern for overall populations also accorded well the predicted distribution under a model of population expansion. All put together, necessitates the need for devising appropriate management measures for conservation and sustainable utilization of the populations of commercially important *S. commerson* in Indian waters. Moreover, the non-significant values for SSD and raggedness index values also indicated that the values do not depart from that expected under the model of expansion. Recent decline of catch compared with the previous year (CMFRI, 2013, 2014) and classification of the species threatened category in conservation assessment (Collette et al., 2011) supports the above observation. Management measures to be devised for conservation and sustainable utilization of the commercially important fish species.

In conclusion, the analysis of *S. commerson* using mitochondrial ATPase 6/8 genes revealed low level of genetic differentiation between samples collected from east and west coast of India. The findings of the present study can be used in the management of this valuable species as a unit stock in Indian waters.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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