Aquat. Living Resour. 2018, 31, 22

https://doi.org/10.1051/alr/2018011

Aquatic Living Resources
Available online at:
www.alr-journal.org

Research Article

Population structure of Spanish mackerel *Scomberomorus commerson* (Lacepede 1800) in the Northern Indian Ocean determined using microatellite markers

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Received 27 December 2017 / Accepted 2 July 2018

Handling Editor: Carlos Saavedra

Abstract – Narrow barred Spanish mackerel, *Scomberomorus commerson* (Lacepede, 1800) is one of the economically important marine fish species in the Northern Indian Ocean. Genetic stock structuring is reported in *S. commerson* from Arabian Peninsula, Indo-West Pacific, Northern Australian, Southeast Asia and Northern Australia. The present study utilized microsatellite markers to elucidate the population structure of *S. commerson* (Lacepede, 1800) in the Arabian Sea and Bay of Bengal. A total of 12 polymorphic microsatellite loci were amplified in 250 samples collected from five different geographic locations. Low F_{ST} values (0.0023–0.027), AMOVA, PCoA and the Bayesian analysis of genetic structure indicated unit stock of the species in Indian waters. Bottleneck analysis using Wilcoxon signed rank tests and Mode shift test indicated lack of recent bottleneck events across populations of *S. commerson*. The findings of the present study could be used in managing the commercially important species, *S. commerson* as a unit stock in Arabian Sea and Bay of Bengal of Northern Indian Ocean and reinforces the need for regional cooperation on fisheries management.

Keywords: *Scomberomorus commerson* / microsatellite-genetic stocks / Arabian Sea / Bay of Bengal

1 Introduction

The narrow-barred Spanish mackerel *Scomberomorus commerson* (Lacepede, 1800) is an epipelagic predator and one of the most important and highly valued commercial species distributed throughout the Indo-West Pacific. In India, it is distributed along the Arabian Sea, including Lakshadweep Islands and Bay of Bengal including Andaman Islands. The species thrive predominantly in shallow coastal waters associated with continental shelves and the adults undertake extensive seasonal long-shore migrations (Collette and Russo, 1984). Juveniles are highly dependent on estuaries and foreshores as nursery and feeding areas (McPherson, 1981). The capture production of this species in the Indian Ocean, where India being the second largest producer is with 1 54 723 tonnes (FAO, 2014). Spanish mackerel are known to aggregate on reefs in large numbers to spawn. The nature of *S. commerson* fishery is that a relatively large proportion of the catch is taken from a particular area/a stock where they aggregate to spawn (Welch et al., 2002). Large quantities of immature fish are caught before reaching their sexual maturity (Devaraj, 1983; Welch et al., 2002; Jayabalan et al., 2011). As per IUCN conservation categorization, *S. commerson* is under 'Near Threatened' category. As *S. commerson* is a commercially important species, it is essential to utilize the fishery in a sustainable manner for which information on genetic stock structure is vital. The migratory nature, high commercial demand and the increased fishing pressures on the resources emphasize the need for stock identification of this species which can help to achieve a sustainable management.

Genetic differentiation observed among marine populations is often low due to combined effect of large spawning populations that may limit genetic drift effects, the apparent absence of physical barriers to dispersal for the species in their environment and the presence of highly dispersive life history stages that contribute to increased gene flow among populations (Allendorf and Luikart, 2009). However, distinct population structuring is reported in some species, which is usually explained by a complex interaction of ecological,

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Table 1. Collection locations of *S. commerson* in the Arabian Sea and Bay of Bengal.

Sl.No	Location (code)	Geographical coordinates	Sample size
1	Veraval, Gujarat (GU)	20.90° N, 70.37° E	50
2	Mangalore, Karnataka(KA)	12.87° N, 74.88° E	50
3	Kochi, Kerala (KE)	9.97° N, 76.28° E	50
4	Chennai, Tamil Nadu(TN)	13.08° N, 80.27° E	50
5	Visakhapatnam, Andhra Pradesh (AP)	17.69° N, 83.21° E	50

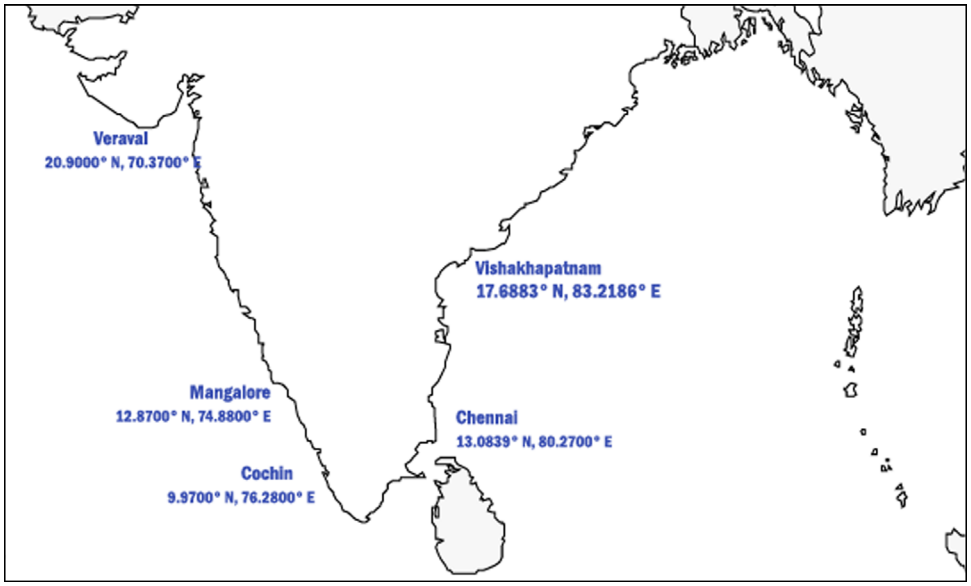

Fig. 1. Map depicting the sampling location of *S. commerson.*

demographic, behavioural, genetic, oceanographic, climatic and tectonic processes (Keeney et al., 2005). The concept of the stock/population structure is crucial for sustainable fishery management. Molecular analyses have been used successfully to document the stock structure of *S. commerson* across various regions (Hoolihan et al., 2006; Van Herwerden et al., 2006; Ovenden and Street, 2007). Earlier, the genetic stock structure of *S. commerson* distributed along the Indian waters was studied using mitochondrial DNA sequences (Vineesh et al., 2016, 2017). The findings of these studies suggested a panmictic structure. Non recombining mt DNA evolves more slowly and is generally valued for evolutionary and demographic studies. However, molecular studies using polymorphic microsatellite markers are supposed to reveal evidence of fine scale, often complex, levels of genetic structuring among marine species. Hence the present study was undertaken to reveal the genetic stock structure of *S. commerson* in the Indian region of northern Indian Ocean including Arabian Sea and Bay of Bengal using microsatellite markers.

2 Material and methods

2.1 Sample collection and DNA extraction

Sampling for *S. commerson* was carried out in five different geographic locations along the Indian coast (Veraval, Mangalore and Kochi in the Arabian Sea; Chennai and Vishakhapatnam in the Bay of Bengal) (Tab. 1; Fig. 1), during May 2013 to July 2016. The sampling area covers the majority of the geographic range of *S. commerson* in the Indian region (~7500 Km) of the northern Indian Ocean. Total genomic DNA was isolated from muscle/fin tissue using Qiagen DNeasy Blood and Tissue kitin two hours time. Quality and quantity of DNA was ascertained as per the standard requirement for molecular analysis.

2.2 Microsatellite loci amplification and data analysis

A total of 250 individuals from each of the five sampled populationswere used for the microsatellite analysis. 12 microsatellite loci were selected for stock structure study in *S. commerson* from published resources (Appendix 1). PCR reactions were carried out in Veriti^{TM} 96-Well Thermal Cycler (Applied Biosystems, USA) employing the 6-FAM labelled microsatellite primers. Amplifications were performed using standard protocols, in 25 μL reaction mixture by using Takara master mix (EmeraldAmp GT PCR Master Mix). The reaction mixture was pre-heated at 94 °C for 5 min followed by 25 cycles (94 °C for 30 s, annealing temperature depending upon the Tm value of primer (50–60 °C; Appendix 1) and final extension at 72 °C for 2 min. The alleles were separated using capillary electrophoresis on an ABI Prism 3730 genetic analyzer (Applied Biosystems). Electrophoregram obtained in the form of .fsa files were analyzed using GeneMapper

software (version 4.0; Applied Biosystems, California, USA) to generate genotype calls for each marker and verified manually for each sample. Fragment sizing were carried out using the default analysis settings in the software, by comparison with the internal size standard ROX 400 or LIZ 500. Random subset of the samples (~10%) were rescored for a second time by manual observation and compared with the previous results to ensure reproducibility of genotyping and allele calling.

2.3 Genetic variability

The presence of null alleles, stutters, small allele dominance, large allele drop out and other genotyping errors were checked using Micro Checker (Van Oosterhout et al., 2004). GENEPOP 4.0 (Raymond and Rousset, 1995) was used to test linkage disequilibrium by means of the log likelihood ratio statistic (G-test), with dememorization number = 10,000, number of batches = 1,000, number of iterations per batch = 10 000. Genetic variation within samples was characterized using numbers of alleles (N_A), allelic richness (AR), gene diversity (GD), observed heterozygosity (H_O) and expected heterozygosity (H_E) using FSTAT 2.9.3 (Goudet, 1995). GENEPOP 4.0 software (Raymond and Rousset, 1995) was used to check for deviations from Hardy Weinberg equilibrium (HWE), at each locus and within geographic samples by means of exact probability tests of heterozygote deficiency. Exact *P*-tests for conformity to Hardy-Weinberg Equilibrium (probability and score test) were performed by the Markov Chain method with parameters, dememorization = 10000; batches = 1000 and iterations per batch = 10000 and based upon a null hypothesis of random union of gametes. The significant criteria were adjusted for the number of simultaneous tests using the sequential Bonferroni technique (Rice, 1989). Deviations from HWE were measured using F_{IS}, calculated according to Weir and Cockerham (1984) and tested for significance by 10 000 permutations in FSTAT 2.9.3. The presence of null alleles and the bias they caused on genetic diversity among the five populations (F_{ST}) (Weir and Cockerham, 1984) were evaluated using FreeNA (Chapuis and Estoup, 2007).

2.4 Population genetic structure

Genetic differentiation was quantified by using pairwise F_{ST} and R_{ST} estimators, with associated significance evaluated by 10 000 permutations using Genepop 4.0. Locus wise F_{ST} was also calculated using GenAlex 6.5 (Peakall and Smouse, 2012). Initially, one way AMOVA (Analysis of Molecular Variance) taking all five populations into consideration were performed in Arlequin ver 3.5 (Excoffier et al., 1992). Hierarchical AMOVA was also performed to test for significant genetic variation among groups of populations of Arabian Sea and Bay of Bengal. Apart from this other multiple approaches were also used to examine the genetic differentiation and structuring in *S. commerson* populations. The Bayesian analysis using STRUCTURE 2.3.4 software (Pritchard et al., 2000) was used to study population clusters. Model runs had a burn-in of 200000 followed by 400000 Markov chain Monte Carlo (MCMC) steps, for 1 to 5 assumed clusters (K) each simulated for 5 iterations, and allele frequencies correlated. STRUCTURE outputs were post-processed in the web versions of STRUCTURE HARVESTER (Earl and von Holdt, 2012), which estimated the optimal number of K using Evanno's delta-K method (Evanno et al., 2005). Principal Component Analysis (PCoA) was conducted on individual multilocus genotypes using GenAlEx 6.5 with the standardized covariance method. The correlation between F_{ST} and geographical distance between populations was assessed with the mantel test statistic R^2 calculated in GenAlex6.5.

2.5 Bottleneck analysis

To assess whether *S. commerson* populations have undergone a recent reduction in genetic diversity, the BOTTLENECK Version 1.2.02 software (Cornuet and Luikart, 1996) was used implementing the following mutation models: Infinite Alleles Model (IAM), Stepwise Mutation Model (SMM), and Two-Phase Model (TPM). The significance of the genetic diversity excess (HE > Heq) was evaluated using the Wilcoxon test, because it is more consistent when used for less than 20 loci (Piry et al., 1999), based on 10 000 replications. In addition, the graphical mode-shift test was incorporated to detect shifts from the normal L-shaped distribution of allele frequencies that are expected at equilibrium (Luikart et al., 1998).

3 Results

3.1 Genetic variability

No significant linkage disequilibrium was detected between different genotypes at each of the different loci for any populations. Therefore independent assortment was assumed and all the loci were considered for analysis. None of the loci showed large allele drop out or stuttering. A total of 318 alleles were detected across the 12 loci. The total number of alleles per locus ranged from 11 (J10Sc and 90RTE) to 45 (SCA30) with a mean of 26.5 alleles for all populations. The allele size ranged from 108 to 450 bp size. The average number of allelic richness between populations ranged from 7.16 (J10Sc) to 29.72 (SCA30) and the average gene diversity varied between populations 0.477–0.947. The genetic variability parameters of five populations of *S. commerson* distributed along Indian waters is given in Appendix 2. The observed and expected heterozygosities per locus ranged from 0.38 (J10Sc) to 0.92 (SCA30), and from 0.37(J10Sc) to 0.94 (SCA30), respectively. Significant deviation from Hardy Weinberg expectations were observed after applying sequential Bonferroni correction only in 18 cases out of 60. Departures were primarily related to excess of homozygosity at locus SCA8 and SCA30. The frequency of null alleles estimated by FreeNA across all loci was < 5.0%. No populations were deviating significantly from HWE by global tests across all loci.

3.2 Population genetic structure

Lack of population structuring in *S. commerson* in the northern Indian Ocean including Arabian Sea and Bay of Bengal was supported by our AMOVA analysis. The estimates

Table 2. Pair-wise F_{ST} (below diagonal) and R_{ST} (above diagonal) values among five locations of *S. commerson* with 12 microsatellite loci.

	Veraval	Mangalore	Kochi	Chennai	Vishakhapatnam
Veraval	0	0.1403	0.0446	0.049*,**	0.1484*,**
Mangalore	0.02241	0	0.0168	0.0207	0.000
Kochi	0.01929	0.00224	0	−0.0058	0.0188
Chennai	0.03981*,**	0.01929	0.01417	0	0.0337
Vishakhapatnam	0.02776*,**	0.0099	0.01280	0.0164	0

* Level of significance, $P < 0.05$.
** $P < 0.01$.

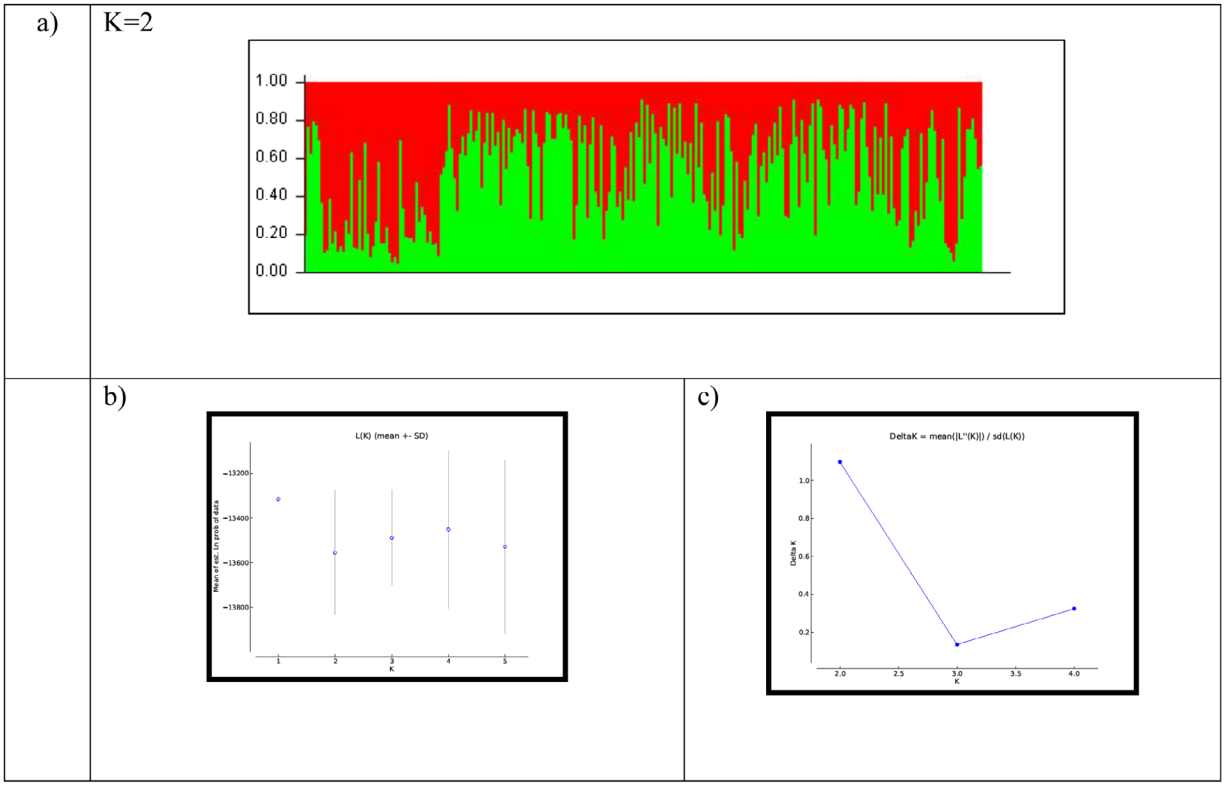

Fig. 2. Bayesian clustering of *S. commerson* individuals from STRUCTURE analysis. (a) Barplots for $K = 2$. Each individual is represented by a vertical bar partitioned into coloured sub-bars whose lengths are proportional to its estimated probability of membership for the K clusters. (b) Plot of the mean of estimated "log probability of data" for each value of K. (c) Delta K of Evanno's method based on the rate of change in the log probability of data.

of genetic differentiation, F_{ST} ranged from 0.009 for the locus SCA44 to 0.053 for the locus 90RTE, with a mean of 0.023. Pairwise F_{ST} ranged from 0.0023 (between Mangalore and Kochi) to 0.0398 (between Veraval and Chennai). In marine fin fish species, the low levels of genetic differentiation commonly observed among wild populations have usually been attributed to historically large population sizes. Pairwise F_{ST} were low and insignificant, except at two comparisons (0.002–0.019, $P > 0.01$, 0.05 and 0.027–0.039, $P < 0.01$, 0.05) and indicated high levels of connectivity among locations across Indian waters (Tab. 2). Low genetic difference was noted among samples from Veraval in the Arabian waters to samples from Bay of Bengal, while no significant difference was noted among rest of the populations. Although null alleles were detected at two loci (SCA8 and SCA30), their frequencies estimated at each locus was <5%.The F_{ST} value after the null allele correction was 0.023, the same as the original value without correction (= 0.02), suggesting that biases, due to null alleles, in genetic structure analysis would be limited. In addition to F_{ST}, population differentiation was estimated using pairwise and overall R_{ST} (Slatkin, 1995) based on the differences in repeat numbers (allele sizes) in microsatellite loci which were also not significant at $P > 0.01$, except at two comparisons (Tab. 2).The Bayesian analysis of genetic structure performed in STRUCTURE 2.3.3 showed a single cluster among the samples using the Delta K estimator (Fig. 2). In line with the F_{ST} results, whatever the k tested, STRUCTURE and STRUCTURE HARVESTER pictured a lack of structure (Fig. 2). The AMOVA based on microsatellite data analysis rejected the five populations sub-structuring

Table 3. One way AMOVA based on microsatellite markers in *S. commerson* populations.

Source of variation	*df*	Sum of squares	Variance components	Percentage variation
Among populations	4	54.550	0.08479	1.78
Among individuals within populations	245	1263.78	0.47714	10.01
Within individuals	250	1051	4.20400	88.21
Total	499	2369.33	4.76593	

Table 4. Hierarchical Analysis of Amova Variance (AMOVA) based on microsatellite markers in *S. commerson* populations, F_{ST}: 0.023, $P > 0.01$

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	18.607	0.0276	0.5779
Among populations within groups	35.943	0.06823	1.42827
Among individuals within populations	1263.78	0.47714	9.98838
Within individuals	1051	4.204	88.00544
Total	2369.33	4.76593	

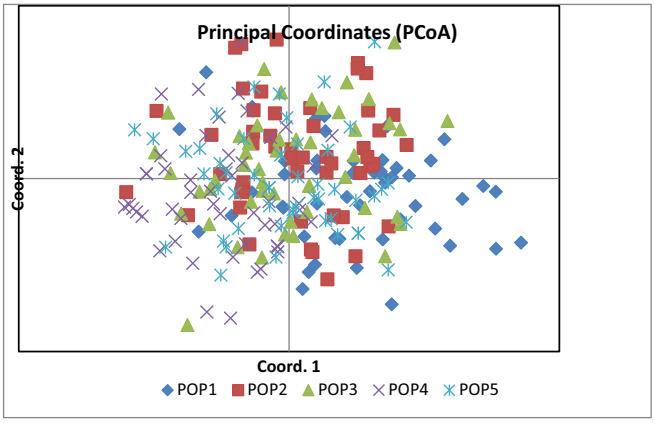

Fig. 3. Two-dimensional plot of the Principal Coordinates Analysis (PCoA) of *S. commerson* performed using GenAlEx.

hypotheses. F-statistic-based AMOVA analysis suggested there was little population genetic differentiation among populations, with inter-population variation contributing less than 2% of the total variation (Tab. 3). Hierarchical AMOVA was also performed to analyze if any genetic variance exists among groups of populations of Arabian Sea and Bay of Bengal which indicated only 0.58% variation observed among the groups (Tab. 4). Most of the variance was found within populations and not between groups indicating panmixia among *S. commerson* populations in the region. The fixation index among groups (F_{CT}) was not significant ($F_{CT} = 0.005$, $P > 0.01$). The result of AMOVA is further confirmed by the findings of PCoA. Considering the percentage of variation explained by the first three axes 20.43%, 17.49% and 16.87% respectively and all the individuals overlap along all the coordinates (Fig. 3). The estimates of the effective number of migrants per generation (Nm) ranged between 4.48 and 28.68 with an average of 14.6. The lowest value of gene flow was found between populations Veraval and Vishakhapatnam and the highest value was found between Kochi and Mangalore. Mantle regression tests also showed only marginal significance in the pattern of isolation by distance across the geographical range ($P = 0.02$, $R^2 = 0.506$).

3.3 Bottleneck analysis

Bottleneck analysis using the Two Phase Model (TPM) yielded 90% probability ranged from 0.97 to 1. Wilcoxon signed rank tests values were also not significant. No significant heterozygotes excess was detected under the infinite alleles model (IAM), two-phase model (TPM), and the stepwise mutation model (SMM). These results were consistent with the normal L-shaped distribution of allele frequency, indicating no genetic bottleneck in any of the five populations in the recent past.

4 Discussion

The genetic variability estimates for *S. commerson* using microsatellite loci was moderately high. Mean H_o value of *S. commmerson* from Indian region is 0.77 and H_e is 0.76 comparable with the reports of van Herwerden et al. (2006) in the Arabian coasts. In the present study, significant deviations from Hardy Weinberg equilibrium were observed at two loci SCA8 and SCA30. Estimated frequencies of null alleles were considered insignificant for analysis purposes. The observed high allelic richness and high mean heterozygosity values estimated in the present study could be attributed to low selection pressure of *S. commerson* in Indian waters. We investigated the genetic diversity of *S. commerson* in the Indian region by sequencing and analysing partial sequence information of mt DNA control region and ATPase 6/8 region (Vineesh et al., 2016, 2017). High haplotype diversity was recorded in the study, indicating larger population size of the species in this region.

Marine species often have low levels of genetic differentiation, because few migrants per generation are sufficient to eliminate genetic evidence of stock structure. Moreover, marine species generally have high fecundities and dispersal abilities. In the present study, the genetic structure of *S. commerson* populations from the Arabian Sea and Bay of Bengal, in the Northern Indian Ocean, was studied using microsatellite markers and several analytic approaches such as F_{ST}, AMOVA, Bayesian clustering and Principal Component Analysis. In the earlier studies, mitochondrial marker was not capable enough to differentiate the genetic stocks in the Indian region, thereby molecular studies using polymorphic microsatellite markers were used to reveal evidence of fine scale, often complex, levels of genetic structuring among this marine species. Our results have revealed a weak differentiation of populations ($F_{ST} = 0.023$, $P > 0.01$) and a general lack of stock structuring. Being pelagic and oceanodramous, the species is known to undertake lengthy long-shore migrations, but permanent resident populations also seem to exist. They spawn in reef slopes and edges, and form spawning aggregations in specific areas (Collette, 2001). Depending on temperature regime, the spawning season may vary accordingly. In the southern coast of India, Devaraj (1983) determined *S. commerson* engage in three distinct spawning periods spread across January to September. Though there is limited information on the movements of the species in this region, some reports indicate the mode of spawning migration. Moore et al. (2003) observed about 5% of the *S. commerson* were supposed to have undertaken long distance movements independent of their co-habitants. Earlier studies using parasite analysis also indicated that adult *S. commerson* was relatively sedentary, but egg/larvae exhibits migration (Claereboudt et al., 2005). Genetic homogeneity of the species could have resulted from intermixing at early life history stages due to advection of eggs or larvae by water currents combined with spawning migration of the species in the region. The Western Indian Coastal Current flows toward the equator during the Indian summer monsoon (May–September) and polewards during the winter monsoon (November–February). The seasonally reversing boundary currents along the Indian coast play a critical role in connecting the Arabian Sea and the Bay of Bengal basins (Shankar, 2002). The reversal of current pattern which coincides with the spawning period of the species and the extended larval duration of 2–4 weeks might have contributed to the increased larval dispersal across the region. When the continuous dispersal capacity of larvae is coupled with extensive movements of adults, might be sufficient to result in genetically homogenous populations of the species in Indian waters.

It can be proposed that the lack of inter-sea differentiation in *S. commerson*, may be due to insufficient time since the beginning of population expansion to allow for the populations to get differentiated (Pogson et al., 1995; Pampoulie et al., 2008). The general absence of noticeable barriers to dispersal in the marine environment of Indian waters also might have significantly reduced heterogeneity among populations, often making it difficult to differentiate discrete populations. Other suggestions for a low F_{ST} are large effective population sizes (Ward et al., 1994) and selective sweeps (Arnason, 2004). In addition, the generally higher level of genetic variation (gene diversity, H) in marine organisms may limit the absolute level of F_{ST} (Hedrick, 1999). The study could able to reveal low values of F_{ST} indicating lack of structuring in *S. commerson* in the region. Large scale panmixia is not fully supported in this study, due to the occurrence of very few significant pairwise F_{ST} values (with only two values left after correction for multiple tests), allow us to accept the hypothesis of subtle pattern of very low differentiation, though difficult to conclude with the low statistical power of our data set. The F_{ST} estimates based on microsatellite, support a limitation of geneflow between groups of species sampled in the Arabian Sea (Veraval) and the ones caught in Bay of Bengal (Chennai and Visakhapatnam). However pairwise F_{ST} among other locations were found to be statistically insignificant. The interpretation in terms of population structure among the population distributed in Indian region, support the need for more robust analysis to ascertain the existence of differentiated genetic stocks. Ovenden and Street (2007) reported the presence of a distinct genetic stock on the east coast and a single stock on the northern and western Australian coastline. Single stock of *S. commerson* was found in the Gulf of Oman and Arabian Sea (Hoolihan et al., 2006) and in addition, a separate stock was identified to a particular locality in the Arabian Sea due to unique oceanographic and behavioural changes rather than explicitly by geographic affinity (Van Herwerden et al., 2006). Considerable genetic differences were also suggested to exist between populations in northern Australian and western Timor that were separated from the Australian coastline by only few hundreds of kilometres (Ovenden and Street, 2007). Genetic data generated in the study make an essential contribution to the understanding of past and current processes that have shaped the evolution and genetic structure of *S. commerson,* as well as to conservation and management strategies. This genetic data can be integrated with population dynamic data to establish the stock extent and composition for the effective management of the species. Despite the lack of population structuring of the species in northern Indian Ocean requires no immediate intervention of conservation of the species, wherein genetic stock structuring observed in Indo west Pacific region (Vineesh et al., 2016, 2017) warranting the need for international cooperation among management agencies on a global scale for conserving and management of this valuable resource. The study emphasise the need to understand the genetic stock structure of the species in the entire range of distribution and also to strengthen an international plan of action than a regional plan for management and conservation of this transboundary stocks in the context of ecosystem based fisheries management.

5 Conclusion

The genetic stock structure of *S. commerson* across its distributional range, specifically in the Indian region of northern Indian Ocean including Arabian Sea and Bay of Bengal using microsatellite markers was revealed. The findings of the present study could be used in the management of this valuable species as a unit stock in Arabian Sea and Bay of Bengal of Northern Indian Ocean and reinforces the need for international cooperation among management agencies on a global scale, for the conservation and management of the species. An in-depth genome analysis using more markers is

suggested to confirm the genetic homogeneity or distribution of different genetic stocks of the species in this region. Our findings also suggest that the populations of *S. commerson* in the Indian waters are genetically stable as they do not present evidence of genetic bottle necks.

Acknowledgment. We gratefully acknowledge the support by the Indian Council of Agricultural Research, New Delhi. We sincerely thank the anonymous reviewers for their critical suggestions which helped to improve the manuscript.

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Cite this article as: Radhakrishnan DP, Vineesh N, Kathirvelpandian A, Basheer VS, Gopalakrishnan A. 2018. Population structure of Spanish mackerel *Scomberomorus commerson* (Lacepede 1800) in the Northern Indian Ocean determined using microatellite markers. *Aquat. Living Resour.* 31: 22

Appendices

Table A1. Details of polymorphic microsatellite loci used for stock structure analysis in *S. commerson.*

Sl No	Locus	Primer sequence	Repeat motif	Ta (°C)	No: of alleles	Size range
1	F6Sc	F- TGATGAGGCTGAAAGACTGAC R- AGGTAGTGACCAACGCTCC	$(TGTC)_8$	55	26	144–244
2	J10Sc	F- CACAGTCCTCTGGACCAAAC R- TCGTTGGAAACGGTCGCTGT	$(GATT)_{10}$	54	12	168–292
3	J43Sc	F- TGATCTAATCAATGGGAGAGG R- TGCTCACATGTGCAAGCAAT	$(TG)_4CC(TG)_{15}$	52	21	128–282
4	L42Sc	F- ATGGCAACGGCGAGATTAAGG R- TCCAGAACAGCAGCAGTTTCC	$(CA)11AA(CA)_3$	55	43	158–388
5	90RTE	F: ATGCTGTCCACTTCCTCCAGC R: TTTCTCAAACTCCTGCCCTTCC	(CA)4 CTCATA $(CA)_{17}$	57	11	164–210
6	Sa2657	F-TGTCAGAGATGTAGCACATACGG R-AGCATTATCTGGTGCTGTAAGGA	$(CG)_8$ $(AG)_6$	54	37	140–450
7	Sa2770	F-AGAAATGAAAAGGGCTTTAAGGA R-ACTGAGCTGCTTAAAATGCAAAA	$(AC)_{15}$ $(CCT)_4$	55	24	142–234
8	SCA8	F- CAGCTGTTCATTCCCATAGCCCA R- ATGAAGGAACAATGAGCCTCCAGC	(CA)n	53	36	120–218
9	SCA30	F- TGGCTGTCGGTCACTCTGCCTC R- ACACACACGGGTACACACAGGG	(CA)n	57	48	108–202
10	SCA37	F-GCG CCGTGACTTTTTATTGCTC R-CAACAATTAGTCGCAGCCCTAG	$(GT)_{10}$	55	38	128–234
11	SCA44	F- ATGGCCAAATGGCACATAATCA R-GGGCAGCTCCATGGGTCTGAGT	$(TCTG)_8$	53	16	156–240
12	SM3	F: GAAGGAGGAGGAGGAGCTGT R: GTTTCTTGGTCAGTCTGCCGG	$(CA)_{24}$	56	20	122–196

Table A2. Summary statistics of genetic variability parameters of microsatellite loci.

Population		F6Sc	J10Sc	J43Sc	L42Sc	90RTE	Sa2657	Sa2770	SCA8	SCA30	SCA37	SCA44	SM3
Veraval	Na	15	9	14	28	8	13	18	23	19	21	11	14
	Ne	5.04	2.51	3.71	12.59	3.82	5.50	8.68	8.73	11.49	3.69	5.54	3.97
	Ho	0.78	0.48	0.56	0.88	0.70	0.64	0.78	0.62	0.46	0.74	0.90	0.72
	He	0.80	0.60	0.73	0.92	0.74	0.82	0.88	0.88	0.91	0.73	0.82	0.75
	F	0.037	0.212	0.243	0.054	0.062	0.227	0.128	0.309	0.504	-0.005	-0.088	0.048
	Signif	**	***	ns	*	ns	***	***	***	***	***	ns	ns
	P	0.01	0.00	0.94	0.02	0.09	0.00	0.00	0.00	0.00	0.00	0.06	0.50
	GD	0.81	0.609	0.74	0.93	0.746	0.828	0.895	0.897	0.927	0.736	0.827	0.756
	AR	15	9	14	28	8	13	18	23	19	21	11	14
Mangalore	Na	13	6	13	24	8	14	12	23	32	19	12	14
	Ne	4.31	1.90	5.27	8.36	3.27	3.94	3.60	8.50	17.30	3.02	5.73	6.28
	Ho	0.74	0.50	0.76	0.80	0.68	0.70	0.70	0.66	0.78	0.56	0.88	0.82
	He	0.77	0.47	0.81	0.88	0.69	0.75	0.72	0.88	0.94	0.67	0.82	0.84
	F	0.046	−0.043	0.072	0.101	0.03	0.072	0.041	0.261	0.182	0.172	−0.056	0.035
	Signif	***	NS	***	NS	NS	NS	NS	***	NS	***	NS	NS
	P	0.00	0.98	0.00	0.20	0.26	1.00	0.84	0.00	0.59	0.00	0.95	0.97
	GD	0.776	0.48	0.819	0.89	0.701	0.754	0.73	0.894	0.953	0.676	0.833	0.85
	AR	13	6	13	24	8	14	12	23	32	19	12	14
Cochin	Na	10	6	9	23	8	15	12	21	29	20	9	14
	Ne	3.75	1.60	4.36	9.11	3.71	5.73	3.87	13.26	17.86	3.21	5.32	6.26
	Ho	0.74	0.38	0.82	0.78	0.74	0.84	0.56	0.68	0.80	0.44	0.82	0.70
	He	0.73	0.37	0.77	0.89	0.73	0.83	0.74	0.92	0.94	0.69	0.81	0.84
	F	0.001	−0.002	−0.054	0.134	−0.003	−0.008	0.255	0.274	0.162	0.37	0	0.177

Table A2. (continued).

Population		F6Sc	J10Sc	J43Sc	L42Sc	90RTE	Sa2657	Sa2770	SCA8	SCA30	SCA37	SCA44	SM3
	Signif	NS	NS	NS	NS	NS	NS	***	***	**	***	NS	NS
	P	0.28	0.78	0.93	0.34	0.90	0.99	0.00	0.00	0.00	0.00	0.99	0.20
	GD	0.741	0.379	0.778	0.9	0.738	0.834	0.751	0.937	0.955	0.698	0.82	0.85
	AR	10	6	9	23	8	15	12	21	29	20	9	14
	Na	11	6	9	21	8	11	12	24	30	19	8	13
	Ne	4.36	1.65	4.88	6.71	1.74	4.65	3.38	10.06	16.34	2.81	5.14	8.00
	Ho	0.82	0.42	0.78	0.74	0.52	0.48	0.60	0.68	0.82	0.52	0.90	0.76
	He	0.77	0.39	0.79	0.85	0.43	0.78	0.71	0.90	0.94	0.64	0.81	0.87
Chennai	F	−0.054	−0.058	0.029	0.14	−0.209	0.397	0.158	0.254	0.136	0.203	−0.107	0.141
	Signif	*	NS	NS	***	NS	NS	***	***	NS	***	NS	***
	P	0.03	0.91	0.35	0.00	1.00	0.08	0.00	0.00	0.14	0.00	0.81	0.00
	GD	0.778	0.397	0.803	0.861	0.43	0.796	0.713	0.912	0.95	0.652	0.813	0.885