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## Population genetic structure of skipjack tuna *Katsuwonus pelamis* from the Indian coast using sequence analysis of the mitochondrial DNA D-loop region

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Genetic structure of skipjack tuna *Katsuwonus pelamis* from the Indian region was investigated using sequence data of mitochondrial DNA (mtDNA) D-loop region. A total of 315 individuals were sampled from six major fishing grounds around the east and west coasts of India including the Andaman (Port Blair) and Lakshadweep (Minicoy) Islands. Nucleotide and gene diversities were high in all the sample collections. Significant genetic heterogeneity was observed for the mtDNA sequence data among sites ( $\phi_{ST} = 0.0273$ ,  $P < 0.001$ ). Analysis of molecular variance (AMOVA) showed significant genetic variation among four groups ( $\phi_{CT} = 0.0261$ ,  $P < 0.05$ ) which was also supported by spatial AMOVA results. The null hypothesis of single panmictic population of *K. pelamis* along the Indian coast can thus be rejected. Phylogenetic analysis of the mtDNA sequence data showed the presence of four clades of *K. pelamis* in the Indian waters. There was no clear pattern, however, of haplotypes and geographic location among samples. The results of this study suggest the occurrence of four genetically differentiated groups of *K. pelamis* across the coastal waters of India.

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Key words: control region; genetic diversity; haplotypes; heterogeneity; population expansion.

### INTRODUCTION

The tunas, belonging to the family Scombridae, comprise a highly valued group of fishes which are marketed fresh, frozen and canned. They are tropical and subtropical in their distribution in the world oceans (Collette & Nauen, 1983). The Food and Agriculture Organization of the United Nations (FAO) has been continuously constituting commissions of enquiry to go into questions of the extent of availability of resources and whether they are being exploited within safe limits without endangering the stocks of species under concern.

The Indian Ocean is one of the most productive areas in the world for tuna fishing (Anganuzzi *et al.*, 1996). In the mainland of India, tuna fishing is carried out by small-scale mechanized and traditional vessels operating in waters up to c. 80 m deep all along the coastline (Somvanshi *et al.*, 2000). The tuna fishery is

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becoming increasingly important because coastal nations are attempting to expand it into offshore and deep-sea ranges and also because of the growing purse seine fishery in the north-west Indian Ocean which is close to the exclusive economic zones of India, Maldives and Sri Lanka.

Skipjack tuna *Katsuwonus pelamis* (L. 1758) comprises about half of the world production of tuna, and is the number one species in every ocean in terms of catch (Majkowski, 2007). Fujino *et al.* (1981) proposed that *K. pelamis* originated in the Indian Ocean, and by dispersal to other oceans together with reproductive isolation, they formed a total of four subpopulations located in the Indian, Atlantic, western Pacific and central-eastern Pacific Oceans. Despite its highly migratory nature, a series of observations such as electrophoretic analysis of serum naphthyl esterase, tag recovery and size distribution of fish schools provided evidence of three genetically distinct subpopulations in the central-eastern Pacific Ocean (Fujino, 1996). This clearly contrasts with the evidence of genetic homogeneity between Atlantic and Pacific Ocean populations (Graves *et al.*, 1984; Ely *et al.*, 2005). As *K. pelamis* often travel in large schools, the potential exists for significant gene flow around the Cape of Good Hope (Graves *et al.*, 1984).

Fundamental to sound management of the commercially important stocks of *K. pelamis* of any ocean is the identification of the number, distribution and degree of reproductive independence of any subpopulation of the species that might occur. Advances in molecular genetic methods have allowed the analysis of large sample sets with higher sensitivity, consequently detecting small to large signals of population subdivision in several species (Rosel & Block, 1995; Chow *et al.*, 1997). Genetic studies of the Pacific population of *K. pelamis* suggest that there is some mixing of fish across the Pacific Ocean, but for management purposes the stocks in the western Pacific Ocean have been considered by most scientists to be independent of those in the eastern Pacific Ocean (Joseph, 2003). Tagging data, showing limited movement of *K. pelamis* from the eastern Pacific to the western Pacific Ocean, support the same conclusion (Joseph, 2003). Simultaneous occurrence of *K. pelamis* schools from genetically distinct subpopulations in a region could be during their extended duration of feeding migration (Fujino, 1996). *Katsuwonus pelamis* are treated as separate stocks in the eastern and western Atlantic Ocean, although there is no conclusive evidence concerning the stock of this species. About 85% of the catch is taken from the eastern Atlantic Ocean, and the rest is taken off Brazil (Joseph, 2003). *Katsuwonus pelamis* in the Indian Ocean are considered to comprise a single stock, so that any management and conservation measures enacted would have to apply over the entire ocean (De Leiva & Majkowski, 2005). It is critical, however, that this assumption be evaluated, as even low levels of stock structure can have important implications for fisheries management (Allendorf *et al.*, 1987). In a recent study of *K. pelamis* (Dammannagoda *et al.*, 2011), two coexisting divergent clades have been revealed in the north-western Indian Ocean around Sri Lankan waters. Tagging studies also indicate that there is little exchange of *K. pelamis* between the Maldives and the rest of the Indian Ocean, but these results are not conclusive (Anderson *et al.*, 1996).

The study of variation present in the mitochondrial DNA (mtDNA) offers an opportunity to unravel several aspects of the population structure of fish species due to its simple mode of transmission avoiding recombination, high mutation rate and predominantly maternal inheritance (Graves *et al.*, 1984; Hoolihan *et al.*, 2004).

mtDNA has been extensively used in vertebrate species, at the restriction fragment length polymorphism (RFLP) level (Chow & Ushiyama, 1995). On many occasions, however, the analyses of DNA sequences are used to complement the RFLP data due to excessive homoplasy observed in the RFLP haplotypes (Alvarado Bremer *et al.*, 1998). Also, the amplification and direct sequencing of highly polymorphic regions of mtDNA provide a potentially rich source of variation at the nucleotide level for determining the molecular population structure within species and the phylogeny of intraspecific lineages (Wenink *et al.*, 1993). In the vertebrate species, most of the variation in the mitochondrial genome is confined to the non-coding control (D-loop) region (Meyer, 1993). Although significant heterogeneity in the distribution of haplotypes have been reported among the *K. pelamis* samples from one locality of the east coast of India (Pondicherry) and the Japanese coast (Menezes *et al.*, 2006) using RFLP data, no *K. pelamis* samples from other localities of the Indian region have been investigated so far. Thus, this study attempts to investigate the genetic structure of six *K. pelamis* samples from the west and east coasts of India representing the main fishing zones along the Indian coast using mitochondrial control region sequence data.

## MATERIALS AND METHODS

Adult *K. pelamis* samples were collected from local trawlers operating at close range from six localities: Veraval (V), Kochi (K), Minicoy (M), Pondicherry (P), Port Blair (PB), Vizag (VI) (Vishakhapatnam) (Table I and Fig. 1.) The samples were preserved in ethanol prior to analysis. Total genomic DNA was extracted from fin clips using the standard Tris, NaCl, ethylenediaminetetraacetic acid and sodium dodecyl sulphate (TNES) urea phenol–chloroform protocol (Asahida *et al.*, 1996).

A fragment of *c.* 500 bp containing the first half of the control region was amplified based on primer set designed from GenBank sequences of *Auxis thazard* (Lacépède 1800) (accession number NC005318). The primer sequences were as follows: 5'-CCGGACGTCGGAGGTTAA AAT-3' (forward) and 5'-AGGAACCAAATGCCAGGAATA-3' (reverse). These primers bind to a part of the threonine transfer RNA (tRNA<sup>Th</sup>) (numbers 16 403–16 423) and a middle portion of the D-loop region (numbers 369–390). The procedures for PCR amplification are described in the study of Menezes *et al.* (2006).

The amplified samples of all the 315 individuals from six localities (Table I) were sequenced at the Bioserve Biotechnologies (India) Pvt. Ltd ([www.bioserveindia.com](http://www.bioserveindia.com)). A few individuals were initially sequenced in both directions and as forward and reverse sequences were perfectly complementary, remaining individuals were then sequenced only in the forward direction. Representative sequences have been deposited in GenBank, with accession numbers JF752020–JF752334.

mtDNA sequences were edited in BioEdit version 7.0.1 (Hall, 1999) and aligned with programme CLUSTAL-W using MEGA5 software (Kumar *et al.*, 2008). Population genetic analysis was performed using the software programme Arlequin version 3.11 (Excoffier *et al.*, 2005) with 1000 permutations as implemented in Arlequin. This programme was used to calculate values of gene diversity ( $H_d$ ), nucleotide diversity ( $\pi$ ), nucleotide composition, estimates of relative population size ( $\theta$ ), relative time since population expansion ( $\tau$ ), Tajima's  $D$  (Tajima, 1989) and Fu's  $F_S$  (Fu, 1997) (indicators of recent founding and rapid expansion of populations), within sampling areas. Significant  $D$ -value can be due to factors such as population expansion, bottlenecks or heterogeneity of mutation rates (Tajima, 1989). Fu's  $F_S$ -test compares the number of haplotypes observed with number of haplotypes expected in a random sample under the assumption of an infinite sites model without recombination (Fu, 1997). Fu (1997) found that  $F_S$  is sensitive to population demographic expansion, which generally leads to large negative  $F_S$  values. Ramos-Onsins & Rozas (2002) showed that Fu's  $F_S$  test outperforms other tests in detecting population growth for large sample sizes. Tau ( $\tau$ ) is a relative measure of time since population expansion, but can also be used to estimate

TABLE I. Descriptions for *Katsuwonus pelamis* samples used in this study and genetic diversity indices based on mitochondrial control region sequence data: number of individuals ( $n$ ), gene diversity ( $H_d$ ), nucleotide diversity ( $\pi$ ), number of transitions ( $s$ ), number of transversions ( $v$ ) and GenBank accession numbers for samples from each locality

Collection sites	Location	Date	$n$	$H_d$	$\pi$	$s$	$v$	Gen Bank accession numbers
Veraval (V)	20° 53' N; 70° 26' E	October 2007	37	1.0	0.114	190	124	JF752020–JF752056
Kochi (K)	9° 58' N; 76° 17' E	February 2008	46	1.0	0.115	198	79	JF752057–JF752102
Minicoy (M)	8° 10' N; 73° 00' E	November 2005	50	1.0	0.101	201	83	JF752103–JF752152
Pondicherry (P)	11° 56' N; 79° 53' E	July 2008	48	1.0	0.105	163	59	JF752153–JF752200
Port Blair (PB)	11° 41' N; 92° 43' E	July 2008	59	1.0	0.131	218	176	JF752201–JF752259
Vizag (VI) (Vishakhapatnam)	17° 42' N; 83° 20' E	February 2009	75	1.0	0.113	224	97	JF752260–JF752334
All			315	1.0	0.116	353	256	JF752020–JF752334

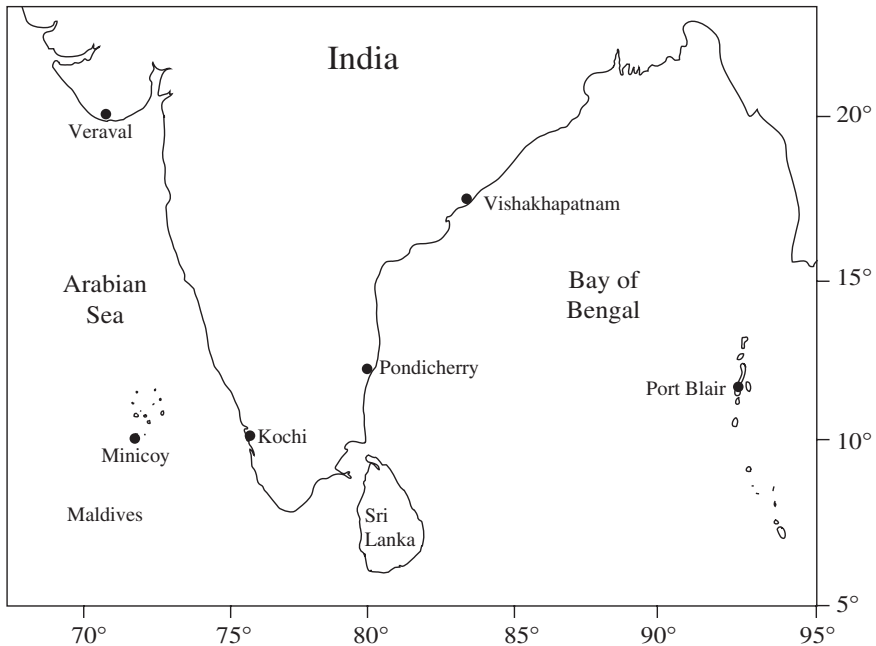


FIG. 1. Map showing sampling areas (●) of *Katsuwonus pelamis* used in this study (see Table I).

the actual time ( $T$ ) since a population expansion by  $T = \tau (2\mu)^{-1}$  (where  $\mu$  is equal to the mutation rate per site per generation). In this study, the mutation rate of  $3.6 \times 10^{-8}$  mutations per site and year was applied for the control region sequence of *K. pelamis* as this rate has been reported for the mtDNA control region in teleosts (Donaldson & Wilson, 1999). Arlequin was used to estimate Harpending's raggedness index ( $r_H$ ; Harpending, 1994) and the sum of squared deviations (SSD) between observed and expected mismatch distributions, which both test whether the sequence data deviates significantly from the expectations of a population expansion model.

To test for genetic differentiation among sampling areas (populations),  $\phi_{ST}$  values were calculated (Excoffier *et al.*, 1992) which includes information on mitochondrial haplotype frequency (Weir & Cockerham, 1984) and genetic distance (Tamura & Nei, 1993). The significance level of  $\phi_{ST}$  was tested using 1000 permutations, and the Bonferroni (Rice, 1989) method was used for multiple comparisons.

Using  $\phi$  statistics, analysis of molecular variance (AMOVA) was performed to examine the amount of genetic variability partitioned within and among *K. pelamis* populations (Excoffier *et al.*, 1992). The entire sample collection was tested for genetic differentiation to examine the null hypothesis of panmixia among collections. Hierarchical AMOVA analysis was undertaken to examine the amount of genetic variability partitioned among groups, among populations within groups and within populations. Spatial population structure was also examined by estimating genetic differentiation between all pairs of sample collections (pair-wise  $\phi_{ST}$  analysis). Significance of pair-wise comparisons was tested using permutation process as given above.

Spatial structure was further investigated *via* spatial analysis of molecular variance (SAMOVA) (Dupanloup *et al.*, 2002) to identify groups of sample sites that are most similar and that are geographically meaningful. SAMOVA uses the statistics derived from an AMOVA and incorporates geographical information on sampling sites with a simulated annealing approach to maximize the  $\phi_{CT}$  among groups of populations as well as identifying possible genetic barriers between them, without predefining populations (Dupanloup *et al.*, 2002).

Thus, SAMOVA defines groups of samples that are geographically homogeneous and also maximally differentiated from each other (Dupanloup *et al.*, 2002).

Only the first 30 numbers of *K. pelamis* individual sequences from each locality were subjected to the neighbour-joining (NJ) method (Saitou & Nei, 1987) of phylogenetic inference. NJ analyses were carried out with the software programme MEGA 5 (Tamura *et al.*, 2011). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The analysis involved a total of 180 nucleotide sequences. All positions containing gaps and missing data were eliminated. Site information along with the *K. pelamis* individual number has been incorporated into the network to illustrate the distribution of haplotypes. Statistical confidence was tested using 1000 bootstrap replications (Felsenstein, 1985). Only nodes of bootstrap support of >50% are shown in the NJ tree.

## RESULTS

mtDNA sequence data of 315 individuals from six locations produced an alignment of 580 bp fragment with a total of 492 polymorphic sites. The control region was 64.1% A + T rich which is consistent with the bias reported in other vertebrates (Alvarado Bremer *et al.*, 1997). The nucleotide content averaged 21.08% C, 28.87% T, 35.23% A and 14.82% G with only very slight deviation among the six samples. The haplotypes were unique to each individual. Transitions for *K. pelamis* control region outnumbered transversions in all the six sample collections (Table I). Empirical evidence has shown that transitions are favoured over transversions in coding and non-coding portions of mtDNA (Moritz *et al.*, 1987). Both the nucleotide diversities (ranging from 0.101 in Minicoy to 0.131 in Port Blair) and gene diversities (1.0) (Table I) were very high. These values are slightly higher than those reported for *K. pelamis* from the Atlantic and Pacific Oceans (Ely *et al.*, 2005). Large differences in  $\theta_0$  and  $\theta_1$  were observed within all collections, suggesting rapid population expansion (Table II). Fu's  $F_S$  (-23.96) statistics were negative and significant ( $P < 0.001$ ), also suggesting a sudden population expansion, which is supported by non-significant sum-of-squares deviation and  $r_H$ . Tajima's  $D$  test, however, was non-significant for all samples. Non-significant values in  $D$  test combined with a

TABLE II. *Katsuwonus pelamis*: statistical tests of neutrality, and demographic parameters estimates for mitochondrial control region data, tau ( $\tau$ ), theta at time 0 and 1 ( $\theta_0$  and  $\theta_1$ ), Tajima's  $D$  test, Fu's  $F_S$  values, Harpending's raggedness index ( $r_H$ ) and sum-of-squared differences (SSD). (Sites shown in Table I and Fig. 1)

Site	$\tau$	$\theta_0$	$\theta_1$	Tajima's $D$	Fu $F_S$	$r_H$	SSD
V	60.197	30.196	99999	-0.575	-8.94*	0.003	0.002
K	66.096	33.975	99999	0.569	-12.85*	0.002	0.001
M	66.551	31.581	99999	-0.013	-15.10***	0.002	0.001
P	77.420	59.080	99999	0.993	-12.64**	0.002	0.001
PB	70.115	45.990	99999	-0.495	-17.87***	0.001	0.001
VI	62.961	32.391	99999	0.565	-24.62***	0.001	0.001
All	69.144	36.624	99999	-0.493	-23.96***	0.000	0.000

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



TABLE III. *Katsuwonus pelamis*: results of analysis of molecular variance (AMOVA) testing genetic structuring based on mitochondrial control region sequence data (sites as in Table I)

Structure tested (sampling sites)	Variance	Variation (%)	$\phi$ Statistics	<i>P</i> -values
One group (V, K, M, P, PB, VI)				
Among populations	0.9269	2.74	$\phi_{ST} = 0.0273$	<0.001
Within populations	32.9371	97.26		
Four groups (V, M), (K), (P), (VI, PB)				
Among groups	0.8875	2.61	$\phi_{CT} = 0.0261$	<0.05
Among populations within groups	0.1756	0.52	$\phi_{SC} = 0.0053$	<0.05
Within populations	32.9371	96.87	$\phi_{ST} = 0.0313$	<0.001

significant  $F_S$  have been interpreted by Fu (1997) as evidence of the absence of background selection, therefore supporting indications of demographic expansions. The value of  $\tau$  was 69.1445 for the total sample, which corresponds to a time of founding of *K. pelamis* in the Indian region of 1 920 681 years [1 672 146 (Veraval) to 2 150 553 (Pondicherry) years before present (BP), 95% C.I.] before present.

Hierarchical analysis that incorporates Tamura and Nei's genetic distance in AMOVA is summarized in Table III. Across the total sample collection, there was significant genetic heterogeneity among the six sampling locations ( $\phi_{ST} = 0.0273$ ,  $P < 0.001$ ). In addition, hierarchical analysis of molecular variance showed significant differentiation among the four groups ( $\phi_{CT} = 0.0273$ ,  $P < 0.05$ ) (Table III). Spatial genetic variation among *K. pelamis* sampling sites was tested at higher resolution using pair-wise  $\phi_{ST}$  analysis. Pair-wise  $\phi_{ST}$  estimates for the entire collection are shown in Table IV. Thirteen pair-wise comparisons of sequence data were significant ( $P < 0.05$ ). After sequential Bonferroni correction (Rice, 1989) ( $P < 0.01$ ,  $0.05/15$  for 15 tests), however, only nine comparisons (V:K, V:P, V:VI, K:M, K:PB, K:VI, M:P, M:VI, P:PB) remained significant (Table IV).

Phylogenetic analysis of the samples revealed four clades of *K. pelamis* in the Indian waters (Fig. 2). There was no clear pattern, however, of haplotypes with respect to geographical location. As a consequence, SAMOVA was employed for the total *K. pelamis* sample to assess whether genetically homogeneous *K. pelamis* groups were present across the study area.

TABLE IV. *Katsuwonus pelamis*: matrix of pair-wise  $\phi_{ST}$  values (below diagonal) and associated *P*-values (above diagonal) based on mitochondrial control region sequence data (sites as in Table I)

Samples	V (1)	K (2)	M (3)	P (4)	PB (5)	VI (6)
1	—	0.0000**	0.22070	0.0000**	0.02344	0.0000**
2	0.07222	—	0.0000**	0.04004	0.0000**	0.0000**
3	0.00314	0.06172	—	0.0000**	0.11523	0.00098**
4	0.07201	0.00923	0.06167	—	0.0000**	0.00586
5	0.01036	0.03923	0.00459	0.02909	—	0.03711
6	0.02776	0.02682	0.02058	0.01307	0.00641	—

\*\*Significant after Bonferroni correction ( $P < 0.01$ ).

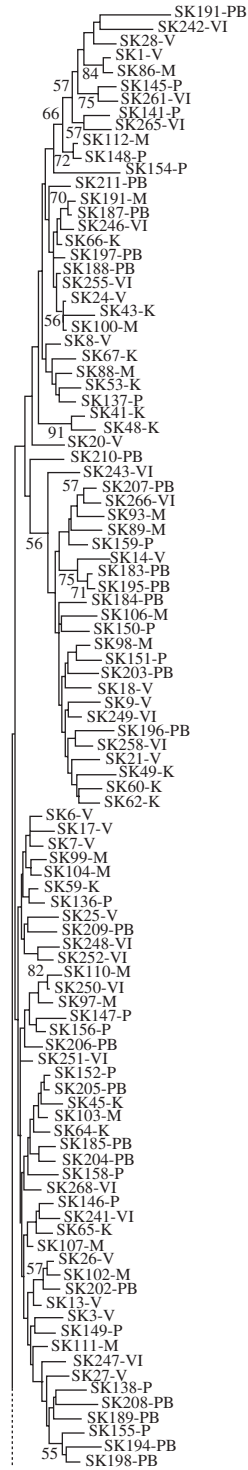


FIG. 2. Continued.



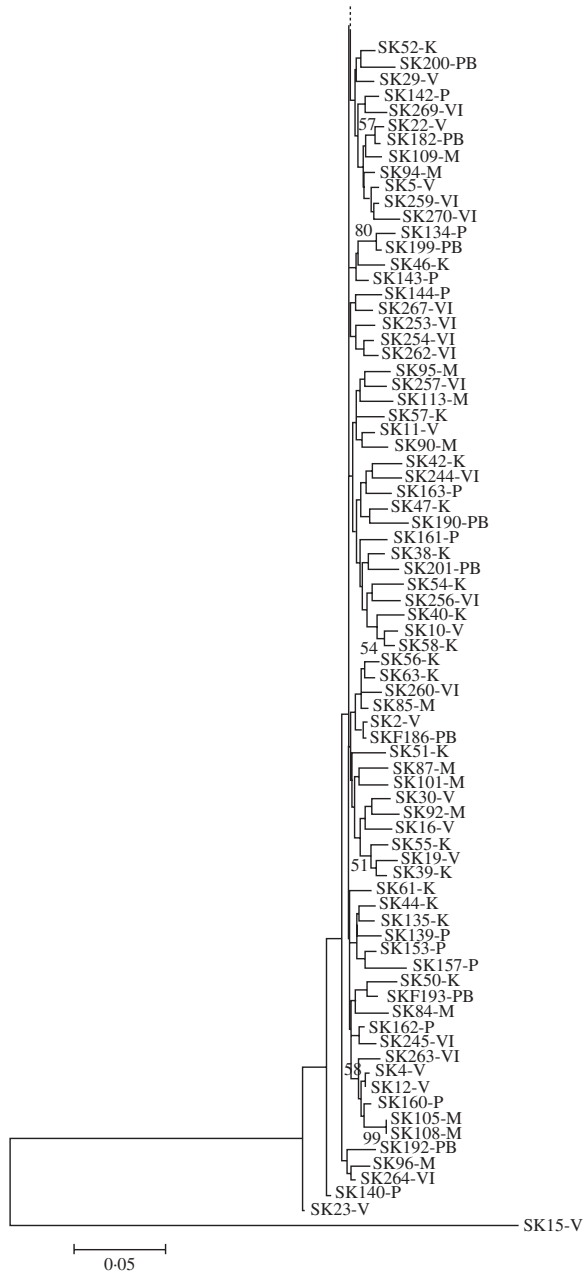


FIG. 2. Neighbour-joining tree of the control region of mtDNA sequences of *Katsuwonus pelamis* (SK) from six different localities of the Indian coast: Veraval (V, 1-30), Kochi (K, 38-67), Minicoy (M, 84-113), Pondicherry (P, 134-163), Port Blair (PB, 182-211), Vizag (VI, 241-270);  $n = 180$ . Bootstrap values after 1000 replications are shown above nodes.

SAMOVA indicated four genetically differentiated ( $\phi_{CT} = 0.0269$ ,  $P < 0.05$ ) *K. pelamis* population groups (Table V). Specifically, the analysis recognized (V, M), (K), (P) and (VI, PB) as discrete populations.

TABLE V. *Katsuwonus pelamis*: population structure based on mtDNA control region differentiation in spatial analysis of molecular variance (SAMOVA). The row in bold type indicates the geographically meaningful groups with maximum genetic differentiation (sites as in Table I)

Number of groups	Structure	Variation among groups	Variation (%)	$\phi_{CT}$	<i>P</i> -values
2	(V, M, PB, VI), (K, P)	0.8684	2.40	0.0239	>0.05
3	(P), (K), (V, M, PB, VI)	0.9479	2.62	0.0262	>0.05
4	<b>(K), (PB, VI), (V, M), (P)</b>	<b>0.9676</b>	<b>2.69</b>	<b>0.0269</b>	<b>&lt;0.05</b>
5	(PB, VI), (V), (P), (K), (M)	1.0339	2.88	0.0288	>0.05

## DISCUSSION

This is the first genetic evaluation of *K. pelamis* samples from the Indian region using mtDNA sequence data. The analysis of population structure of *K. pelamis* rejects the null hypothesis of a single panmictic population in the Indian region. This is supported by significant amount of genetic differentiation in both pooled AMOVA and hierarchical AMOVA.

Hierarchical AMOVA of mtDNA D-loop sequences showed significant genetic heterogeneity among the four groups analysed. These results were further corroborated by SAMOVA analysis which recognized Veraval and Minicoy sample as one group or clade, Kochi as the second group, Pondicherry sample as the third group and Vizag with Port Blair sample as the fourth group. In addition, evidence for fine geographical scale genetic heterogeneity was confirmed by pair-wise analysis of mtDNA data.

The observed heterogeneity among collections of *K. pelamis* from the Indian region could be the result of sampling error, or alternatively biological and physical processes that promote reproductive isolation. Small sample sizes limit the statistical power of an AMOVA, but it would most likely result in a failure to detect population structure (type I error) rather than falsely indicating the presence of population structure. Further, recent genetic studies of *K. pelamis* (Dammannagoda *et al.*, 2011) using both mtDNA and nucleolar (n)DNA microsatellite data also show strong evidence for spatial genetic heterogeneity around Sri Lanka and adjacent areas in the north-western Indian Ocean. Multiple highly divergent intraspecific mtDNA lineages both within and among ocean basins have also been reported for other tuna and billfish species (Martinez *et al.*, 2006). The observed mtDNA heterogeneity in these studies and in this study may have been caused by common vicariance events during Pleistocene glacial maxima that resulted in isolation of populations by reduction in availability of tropical marine habitats due to decreases in ocean water temperature (Alvarado Bremer *et al.*, 2005; Martinez *et al.*, 2006; Dammannagoda *et al.*, 2011). Further, potential unidirectional gene flow of formerly allopatric populations during interglacial periods providing secondary contacts may have resulted in asymmetrical distribution of clades (Alvarado Bremer *et al.*, 2005).

Phylogenetic analysis of samples further revealed four different clades of *K. pelamis* in the Indian waters. The NJ tree, however, indicates that there is no

clear pattern of haplotypes and geographic location among samples. The asymmetrical pattern of distribution of haplotypes in these clades has been hypothesized to be the result of secondary contact and interbreeding between populations subsequent to their geographical isolation for a long period.

High levels of haplotype and nucleotide diversity were observed for *K. pelamis* in this study. These values were slightly higher than those reported for *K. pelamis* from Atlantic and Pacific Oceans (Ely *et al.*, 2005). Several explanations have been proposed for the maintenance of high haplotypic diversity within populations, including large population sizes, environmental heterogeneity and life-history traits that favour rapid population increase (Nei, 1987). It has generally been thought that large population sizes are responsible for extraordinarily high levels of genetic diversity in marine fishes. *Katsuwonus pelamis* is widely distributed in the Indian Ocean, indicating that large population sizes may account for the high levels of haplotypic diversity observed in this study.

Migrations of *K. pelamis* have been examined in the Pacific Ocean. Several migratory routes have been reported, and there are possibly 14 or more groups (not necessarily separate subpopulations) in the Pacific Ocean (Matsumoto, 1974, 1975). Further, Hilborn & Sibert (1988) suggested that a large number of *K. pelamis* do not demonstrate the long-distance movement but, rather, remain around the tropical spawning area, not migrating towards the northern and southern temperate regions. Probable mechanisms for subpopulation differentiation in *K. pelamis* have been proposed on the basis of the observations conducted as well as on some assumptions as follows: (1) isolation by distance and homing behaviour and (2) geophysical characteristics of ocean and ocean currents (Fujino, 1996). Genetic differentiation among Atlantic and Indo-Pacific Ocean populations has also been observed for other widely distributed pelagic fishes such as yellowfin tuna *Thunnus albacares* (Bonnaterre 1788) using allozymes and mtDNA haplotypes (Ward *et al.*, 1997) and for istiophorid billfishes using mtDNA RFLP analysis (Graves & McDowell, 1995). Features of the marine environment that limit gene flow are often large in scale, such as current systems, major changes in temperature or salinity or physical barriers such as the tips of continents or land masses (Finnerty & Block, 1992; Chow & Ushiyama, 1995).

*Katsuwonus pelamis* are the target of the largest tuna fishery in the Indian Ocean and based on fishery data currently managed as a single stock. The observation of genetic heterogeneity among *K. pelamis* samples in the Indian region suggests the existence of genetically independent groups or clades in the Indian region. There are indications that the waters around Minicoy are breeding areas for *K. pelamis* as larvae and female fish with mature ovaries have been collected in this region (Jones & Silas, 1963; Raju, 1963). The possibility of multiple spawning has also been indicated which is supported by the occurrence of more than a single entry of small fish into the fishery (Raju, 1963). Off the west coast of Sri Lanka also, spent female *K. pelamis* have been observed (Sivasubramanian, 1985).

SAMOVA tests of mtDNA sequence data support the presence of four groups, one around the south-west Indian peninsular (Kochi), the other around south-east of the Indian peninsular (Pondicherry), a third in the Arabian Sea (around the Veraval - Minicoy) area and a fourth in the Bay of Bengal (around Vizag-Port Blair) region. Isolation by distance was not observed, and strong philopatry and homing to specific natal spawning grounds would be needed in order to maintain the genetic differentiation observed. Genetic structuring observed in this study would mean that there

is little or no current interpopulation gene flow, as this would preclude populations from showing genetic differentiation.

In addition to providing insight into population structure, genetics also allow for an examination of the evolutionary history of *K. pelamis* in the Indian region. In this study (Table II), rapid historical population expansion is supported by the large differences between  $\theta_0$  and  $\theta_1$  of 36.62 and 99 999, respectively, and also by Fu's  $F_S$  statistics which was negative and significant. Tajima's  $D$  test, however, was non-significant which suggests that the effective size of *K. pelamis* population has been large and stable for a long period (Ely *et al.*, 2005). Based on the estimated values of  $\tau$ , the average time since the demographic expansion of these populations in the Indian region ranged from *c.* 1 672 146 to 2 150 553 years BP. The tau value ( $\tau = 69.14$ ) observed in this study was much higher than that observed for the Brazil *K. pelamis* population ( $\tau = 28.38$ ; Ely *et al.*, 2005). This higher  $\tau$  value could be due to the origin of *K. pelamis* in the Indian Ocean as proposed (Fujino *et al.*, 1981) and thus also being the oldest population.

Coastal upwelling along the coasts of Somalia, Arabia and the Indian subcontinent during south-west monsoon period leads to high primary and secondary productivity (Wyrtki, 1973; Qasim, 1982). High productivity will lead to large schools of bait fishes on which the *K. pelamis* feed. *Katsuwonus pelamis*, which is medium-sized, long-lived and capable of extensive movement, can seek out a convivial habitat and exploit almost the total area of ocean productivity. Thus, although the four genetically different populations of *K. pelamis* observed in this study may spawn in different areas of the Indian Ocean, they may physically mix during their extended duration of feeding migration as observed by Fujino (1996). Another explanation for physical mixture of the *K. pelamis* clades at sampling sites includes differential passive transport of larvae belonging to the different clades to fishing grounds by monsoonal currents in the Indian Ocean (Dammannagoda *et al.*, 2011).

The presence of genetically distinct populations of *K. pelamis* in the Indian region raises important management considerations. Further work is, however, needed to confirm the natal homing of these four *K. pelamis* populations to identify potential spawning sites in the Indian Ocean.

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