



SHORT REPORT

Genetic homogeneity in longtail tuna *Thunnus tonggol* (Bleeker, 1851) from the northwest coast of India inferred from direct sequencing analysis of the mitochondrial DNA D-loop region

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Abstract

Longtail tuna *Thunnus tonggol* is a neritic species of the family Scombridae, having a confined coastal distribution to tropical and temperate waters of the Indo-Pacific region. In the present study, the population structure of longtail tuna was examined using sequence analysis of the mtDNA control region from two geographically distinct locations along the northwest coast of India. A 500 bp segment of the D-loop region was sequenced and analysed for 98 longtail tuna individuals. Analysis of population differentiation showed no significant genetic differentiation between the two sampling localities ($\Phi_{ST} = 0.005$, $P = 0.200$). Results were further corroborated by a non-significant nearest-neighbour statistic ($S_{nn} = 0.489$, $P = 0.549$). Phylogeographic analysis revealed two haplogroups, but there was no obvious phylogeographic pattern separating the samples of the two localities. The present study suggests a single panmictic population of longtail tuna in the northwest coastal waters of India.

Key words: D-loop, Indian waters, longtail tuna, mtDNA, population structure

Introduction

Longtail tuna (LTT) *Thunnus tonggol* (Bleeker, 1851) is among the economically important species of tuna and is the second smallest among the eight species in the genus *Thunnus*. They have an exclusive coastal distribution in the Indo-Pacific region between 47°N and 33°S (Froese & Pauly 2011). Although they are reported all along the Indian coast, fishery data indicate that 93% of catches are concentrated along the west coast of India (Abdussamad et al. 2012). The abundance increases towards higher latitudes, with 83% of the catches found along the northwest coastal waters of India (Abdussamad et al. 2012). LTT constitutes more than 50% of all tuna landings at Veraval (Mohanraj et al. 2007) and approximately 60% at Ratnagiri (Silas et al. 1985), making these two regions the major contributors of LTT along the Indian coast.

Globally, there is a substantial increase in LTT catches from 100,000 tonnes in 1985 to 233,830 tonnes in 2009 (FAO 2012). In Indian waters, LTT is next only to little tuna *Euthynnus affinis* (Cantor, 1849) in terms of annual catch and contributes around 15% among the neritic tunas (Vijayakumaran & Varghese 2010). LTT constitutes an important artisanal and subsistence fishery in the Indo-Pacific region and is considered an important sport-fish in Australian waters, owing to their relatively large size and fighting ability (Griffiths et al. 2007, 2009). The growing commercial and recreational importance of this species, the enhanced exploitation rate and lack of data on stock structure warrant the need for an extensive study to investigate the population stock structure of LTT. Determination of population structure is essential to support resource recovery and to aid in delineating and monitoring populations for fishery management (Roldan et al. 2000). Failure

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to detect population units may lead to local overfishing and ultimately to a severe decline. Molecular genetic techniques offer the ability to identify and delineate fish stock structure where it may not be apparent from phenotypic or behavioural characteristics (Shaklee & Currens 2003).

Genetic markers, in particular mtDNA, have become effective tools for the assessment of intraspecific genetic variation and to identify populations (Menezes et al. 2012). The nucleotide sequences of the mtDNA region have been used for evaluating demographic, genetic and phylogeographic patterns in natural populations (Kumar et al. 2012a,b).

The present work, albeit focused on Indian waters, represents the first genetic study to investigate population substructuring of LTT, a highly valuable and exploited tuna species in the Indo-Pacific. As such, the main aim of the present work was to examine the level of genetic differentiation and the evolutionary history of LTT in northwestern Indian waters, in order to help establish the basis for accurate conservation management and sustainable harvesting.

Materials and methods

Sample collection

LTT ($n = 98$) fin clip samples were collected from two main fishing zones, Veraval (VE; 20.9°N 70.37°E) and Ratnagiri (RA; 16.98°N 73.3°E) along the northwest coast of India (Table I). The samples were preserved in absolute ethanol and brought back to the laboratory for further processing.

Molecular techniques

Extraction of genomic DNA was performed using a TNES-Urea-Phenol Chloroform protocol as described by Asahida et al. (1996) and the DNA was stored at 4°C until further processing. A 500 bp fragment of the first half of the mtDNA control region, including the D-loop, was amplified using the primer set designed by and the procedure followed by Menezes et al. (2006). PCR products were purified using a PCR Cleanup Kit (Axygen Biosciences, California, USA).

Table I. Estimates of genetic diversity within populations and for the overall sample for mtDNA D-loop sequence data: number of samples (n); number of haplotypes (nh); haplotype diversity (h) and nucleotide diversity (π).

Samples	n	nh	$h \pm SD$	$\pi \pm SD$
Veraval	49	48	0.999 \pm 0.0044	0.207 \pm 0.102
Ratnagiri	49	47	0.997 \pm 0.0046	0.167 \pm 0.083
All	98	95	0.998 \pm 0.0045	0.187 \pm 0.093

All samples were sequenced in the forward direction with the same primer as used for PCR amplification. Sequences were obtained at the DNA sequencing facility of CSIR-NIO, Goa, India, using the Big Dye Terminator cycle sequencing kit (V3.1, Applied Biosystems, USA) and analysed using a 3130xl Genetic Analyzer (Applied Biosystems, USA). Sequences have been deposited in GenBank, with accession numbers KC313300–KC313393.

Data analyses

The mtDNA sequences were edited in BioEdit 7.0.1 (Hall 1999) and aligned with ClustalW in MEGA5 (Tamura et al. 2011). Arlequin 3.11 (Excoffier et al. 2005) was used to calculate haplotype diversity (h) and nucleotide diversity (π) within sampling localities and for the overall sample. The null hypothesis of population panmixia was tested using several statistical methods. Pairwise Φ_{ST} (Excoffier et al. 1992) was calculated in Arlequin 3.11 using the Tamura & Nei (1993) nucleotide substitution model selected by Modeltest 3.06 (Posada & Crandall 1998). Significance of Φ_{ST} was tested with 1000 permutations for pairwise comparison. Also, an exact test of differentiation of haplotypes among sampling localities was performed in Arlequin 3.11 with statistical significance assessed after 10,000 Markov steps. Furthermore, to measure the extent of population differentiation by testing if the sequences with low divergence are geographically proximate, the nearest-neighbour statistic (S_{nn} ; Hudson 2000) was calculated in DnaSP 4.0 (Rozas et al. 2003). S_{nn} measures how often closely matched sequences are from the same geographical locality. The significance of S_{nn} was tested by using 1000 permutations.

The phylogeographic relationships among haplotypes were examined with a reconstructed network in NETWORK 4.611, using the median joining algorithm (Bandelt et al. 1999).

The evolutionary and demographic history of LTT was examined following Kumar et al. (2012a). A mismatch distribution was determined in Arlequin 3.11. Harpending's (1994) raggedness index (Hr) and sum of squared deviations (SSD) were calculated to test whether the sequence data deviated significantly from the expectations of a population expansion model. Parameters of the mismatch distribution were used to estimate relative population size and the time since population expansion (using mutation rate $\mu = 3.6 \times 10^{-8}$ mutations/site/year). Tajima's (1989) D test and Fu's (1997) F test were carried out to examine whether samples from the two regions were at genetic equilibrium.

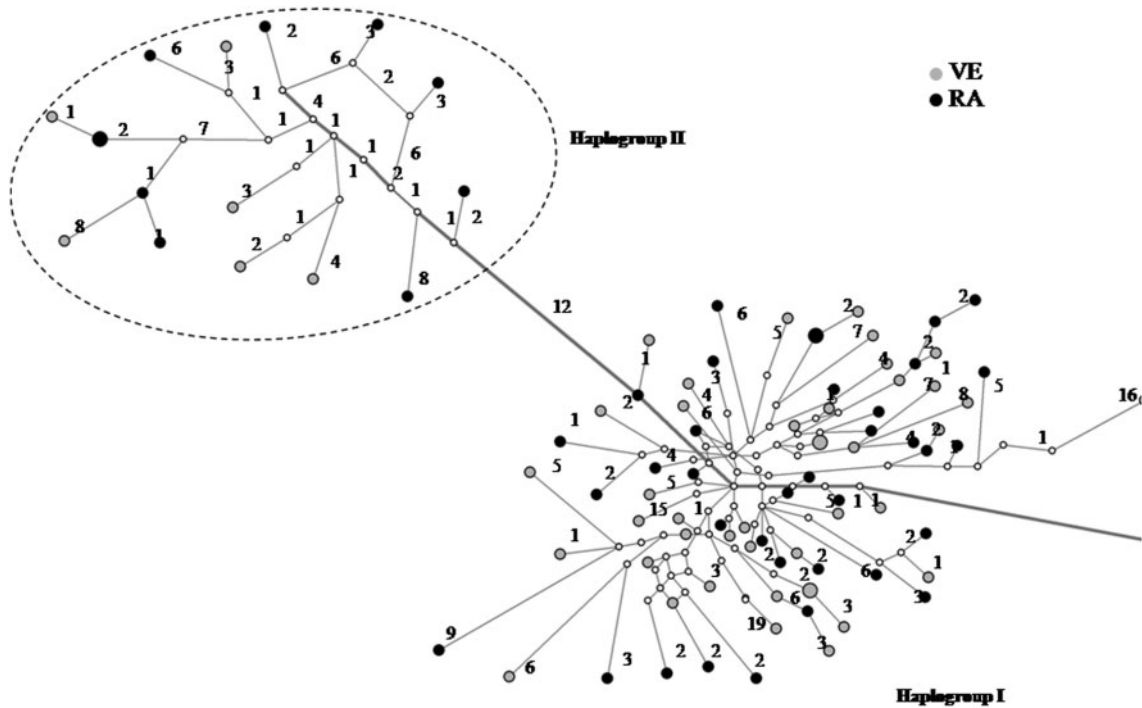


Figure 1. Median-joining network of mtDNA haplotypes of LTT.

Results

Population substructuring and phylogeographic pattern

Sequence analysis of the D-loop region revealed 122 polymorphic sites (87 parsimony-informative sites) defining 95 haplotypes, of which 92 were found in a single individual only. The overall haplotype diversity (h) and nucleotide diversity (π) was 0.998 and 0.187, respectively (Table I). No significant genetic differentiation was found between the two sampling localities as revealed by pairwise Φ_{ST} (0.005, $P = 0.200$), the exact test of population differentiation ($P = 0.119$) and S_{nn} (0.489, $P = 0.549$).

The median joining network of D-loop haplotypes revealed two haplogroups (Figure 1). No common haplotypes were found between sampling sites, with almost all individuals being represented by a private haplotype. Overall, haplotypes were separated by 1 to 19 mutational steps.

Evolutionary history of LTT

As no significant population differentiation was found between sampling sites, the mismatch distribution analysis was performed for the overall data set. The mismatch distribution of pairwise differences among individuals in LTT was found to be bimodal (Figure 2). The results obtained did not allow the rejection of a null hypothesis of population expansion. For the overall sample, there were large differences between θ_0 (population before

expansion) and θ_1 (population after expansion) of 24.16 and 99,999, respectively. The tau (τ) value was 21.36, which reflects the time of founding of LTT in the Indian region 593,334 years ago. Fu's F_s (-24.19 ; $P < 0.001$) statistic was significant while Tajima's D (-0.76), H_{ri} (0.002) and SSD (0.004) were non-significant for the overall sample.

Discussion

The mtDNA D-loop region sequence data exhibited little divergence between the two sampling localities, suggesting the existence of a single panmictic population of LTT around the northwest coast of India. These findings are supported by the low,

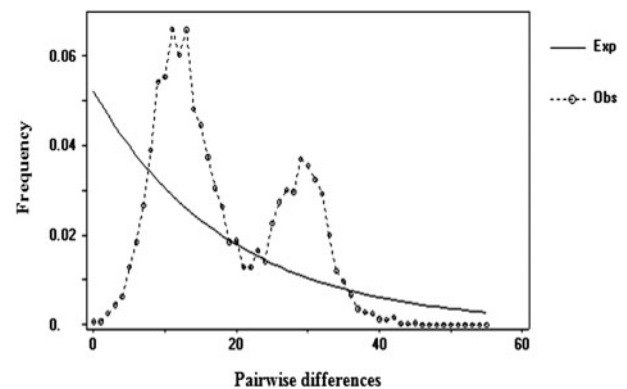


Figure 2. Mismatch distributions of all samples of the LTT mtDNA D-loop region.

non-significant pairwise Φ_{ST} , the exact test of population differentiation and by the value and non-significance of S_{nn} . The present study revealed high haplotypic diversity within two sampling localities, which may be attributed to large population size, environmental heterogeneity and/or life history traits that favour a rapid increase in population size (Nei 1987). Large population sizes are responsible for extraordinary high haplotypic diversity in marine fishes (Avice 2000). Tunas are characterized historically by high effective population sizes and, consequently, high genetic variability (Durand et al. 2005). The high haplotypic diversity observed in the present study is in accordance with the values observed for other tuna species in Indian waters (Kumar et al. 2012a,b; Menezes et al. 2012).

It has been proposed that marine fishes may be classified into four categories based on different combinations of high and low values for haplotype diversity (h) and nucleotide diversity (π) of mtDNA sequences (Grant & Bowen 1998). The present study revealed high h and π values (Table I), which categorize LTT in the fourth category, which indicates large stable populations with a long evolutionary history or a secondary contact between differentiated lineages (Grant & Bowen 1998). Also, the majority of haplotypes appeared only once in the present analysis, which is a common characteristic of scombrid fishes (Zardoya et al. 2004). The findings of the present study are also supported by studies carried out on other coastal tuna species. Based on mtDNA sequence analyses, it has been reported that there is no population structure in little tuna (*Euthynnus affinis*) among five regions across Taiwanese waters (Chiou & Lee 2004) and among eight regions across Indian waters (Kumar et al. 2012a).

It was an intention of this study to examine population structure, but no evidence of geographic structure was found. The reconstruction of phylogeographic relationships between haplotypes retrieved two different haplogroups. However, no clear geographical pattern was observed, with haplotypes of the two sampling sites equally distributed between haplogroups. In several other tuna species, such as *Thunnus obesus* (Lowe, 1839) (Chiang et al. 2006) and *E. affinis* (Kumar et al. 2012a), the heterogeneous distribution of two highly divergent sets of mtDNA lineages has been reported. A possible explanation for the presence of two divergent mtDNA clades within a species in these studies is secondary contact between populations subsequent to their geographical isolation for a long period (Durand et al. 2005). However, that does not seem to be the case in LTT, as no geographical association was found between sampling sites and haplotype distribution.

The present study also aimed at revealing the evolutionary history of LTT in Indian waters by incorporating several statistical analyses as well as the mismatch distribution itself. If a population deviates from neutral expectations, Tajima's D generally provides a low but significant value. When there is excessive mutation, Fu's F_s tends to be negative, indicating deviation caused by population expansion and/or selection (Su et al. 2001). Non-significant values in D tests, combined with significant F_s s, have been interpreted by Fu (1997) as evidence of the absence of background selection, but indicating a demographic expansion instead. The Fu's F_s and Tajima's D results in the present study suggest a demographic expansion of LTT in the past.

Similarly, the large difference observed between θ_0 and θ_1 also suggests rapid population expansion of LTT in the past which supports the high genetic diversity observed in the present study. Corresponding to a τ value of 21.36, the estimated time for population expansion is 593,334 years ago. The overall τ value observed for LTT is much higher than that of other neritic tuna species such as *E. affinis* (Kumar et al. 2012a) and *Auxis thazard* (Lacepède 1800) (Kumar et al. 2012b) in the Indian region. This corresponds to LTT being an older population in the region or, alternatively, having undergone the earliest expansion and maintaining a large population since then.

The non-significant sum of squared deviations (SSD) and Harpending's raggedness index (H_{ri}) were also consistent in supporting the occurrence of a population expansion of LTT in Indian waters. The mismatch distribution is generally unimodal in a population that has gone through sudden demographic expansion while it is bimodal or multimodal in samples drawn from populations with a demographic equilibrium (Slatkin & Hudson 1991; Rogers & Harpending 1992). The bimodal mismatch distribution in LTT conforms to this pattern, suggesting sampling localities are at demographic equilibrium.

The inability to detect population structure in LTT may stem from the fact that the control region is a fast-evolving marker, which, combined with historically high effective population sizes, may impair detection of subtle population structure (as it is too variable). Therefore, slower-evolving markers such as mtDNA cytochrome b (Farias et al. 2001) or COI (Carvalho et al. 2012), or nuclear S7 (Near & Cheng 2008) may be a better approach to investigating population substructuring in such species. Also, although microsatellites have the potential to recover hidden population structure, their effectiveness in species with high population sizes is debatable (Wan et al. 2004).

Nevertheless, the present study has provided new knowledge about the genetic structure of LTT in northwest Indian waters, although much still needs to be investigated to confirm the homogeneity of the LTT population in these waters. Future research involving more sampling localities, the use of additional markers and physical tagging to find migration patterns of this species in Indian waters would help to improve its conservation and management.

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