

Intraspecific phylogeography of the Japanese threadfin bream, *Nemipterus japonicus* (Perciformes: Nemipteridae), from the Persian Gulf and Indo-West Pacific: a preliminary study based on mitochondrial DNA sequence

Farivar S.^{1*}; Jalil-Piran Z.¹; Zarei F.²; Hosseinzadeh Sahafi H.³

Received: September 2015

Accepted: June 2016

Abstract

The Japanese threadfin bream, *Nemipterus japonicus*, the most abundant and crucially economic *Nemipterus* species is widespread throughout the Indo-West Pacific. The species has been studied widely for various aspects but genetic studies are scanty. This preliminary study contributes to the species phylogeography through the study of the genetic diversity and historical demography of *N. japonicus* populations from the Persian Gulf and Indo-West Pacific based on cytochrome c oxidase subunit I (COI) gene sequence. Grouping of the data into phylogenetic trees indicated that the Japanese threadfin bream consists of two reciprocally monophyletic phylogroups with 2.3% net sequence divergence which may qualify as cryptic species: clade I, consists of two subclades (Ia and Ib) occurs in the Persian Gulf and Western India, and clade II, which is restricted to the South China Sea. Historical and demographic hypotheses were raised to explain the observed phylogeographic pattern and population structure. Among the possible key mechanisms, sea level fluctuations driven by glacial episodes of the second half of the Pleistocene Epoch appear to have played an active role in initiating major phylogeographic separation. Apart from presumptive Pleistocene vicariance, a trend of increasing genetic differences with increasing geographic distance (i.e., isolation-by-distance) and regional differences in breeding season were also proposed as possible alternative scenarios. Since the baseline knowledge on the intraspecific genetic diversity and management and evolutionary significant units is the first step before any action to be taken, the basic findings provided by this research are particularly relevant to conservation efforts, fishery management and stock assessment.

Keywords: *Nemipterus Japonicus*, mtDNA, Phylogeography, Persian Gulf, Indo-West Pacific

1-Department of Genetics, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University (GC), Tehran 1983963113, Iran.

2-Department of Animal Sciences, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University (GC), Tehran 1983963113, Iran.

3-Iranian Fisheries Science Research Institute (IFSRI), P.O. Box 14155-6116, Tehran, Iran.

*Corresponding author's Email: s_farivar@sbu.ac.ir

Introduction

The Japanese threadfin bream, *Nemipterus japonicus* (Bloch, 1795), the most abundant and crucially economic *Nemipterus* species is widespread throughout the Indo-West Pacific ranging from East Africa, including the Persian Gulf and Red Sea to the Indo-Malay Archipelago (Fig. 1A). It is a benthic, non-migratory species, very abundant in coastal waters, found on mud and sand bottoms in the 5-80 m depth ranges, usually in schools (Russell, 1990). As a result of faster growth rates in males, females predominate at small sizes and males at large sizes (Lee, 1973). As a demersal carnivore, the diet of this species mainly consists of crustaceans, fishes, molluscs, polychaetes and echinoderms (Salarpouri *et al.*, 2010; Afshari *et al.*, 2013; Manojkumar *et al.*, 2015). This species is trawled in commercial quantities in the Persian Gulf and Oman Sea (Valinassab *et al.*, 2006), the Andaman Sea (Senta and Tan, 1975), as well as in the Bay of Bengal (Krishnamoorthi, 1971), and the South China Sea (Eggleston, 1972; Lee, 1973; Weber and Jothy, 1977). It has been studied widely for various aspects like maturity, reproduction and related aspects (Krishnamoorthi, 1971; Eggleston, 1972; Murty, 1984; Russell, 1990; Bakhsh, 1996; Rajkumar *et al.*, 2003; Kerdgari *et al.*, 2009; Fazeli, 2013), biology and biochemistry (Krishnamoorthi, 1971; Murty, 1984; Murty, 1987; Russell, 1990; Samuel, 1990; Bakhsh, 1996; Chawla *et al.*,

1996; Zacharia, 1998; Raje, 2003; Manojkumar, 2004; Joshi, 2010; Naqash and Nazeer, 2010), and population dynamics (Vivekanandan and James, 1986; Iqbal, 1991; Zacharia, 1998; Rajkumar *et al.*, 2003; Fazeli, 2014; Rezayi *et al.*, 2014), but morphological and genetic studies are scanty. Analysis of morphometric characters have indicated that separate stocks of this species may exist along the east and west coasts of India (Sreekanth *et al.*, 2015). Evaluation of mitochondrial cytochrome b (Cyt b) gene sequence among *N. japonicus* populations along the coast of Peninsular Malaysia by Jamsari *et al.* (2008) and Lim *et al.* (2016) provided evidence for possible occurrence of cryptic species in that region. Analysis of mitochondrial cytochrome c oxidase subunit I (COI) gene sequence among nine *Nemipterus* species by Ravitchandirane *et al.* (2012) revealed that the highest intraspecific K2P distance belongs to *N. Japonicus* (0.069; based on 5 specimens). DNA barcode results based on COI gene for three *N. Japonicus* specimens supported the possible existence of two geographically separated lineages, the Indian Ocean and West Pacific lineages (Ning *et al.*, 2015). On the other hand, the population genetic structure and phylogeographic pattern within the western range of species (consists of the Indian Ocean, Arabian Sea and two evaporative basins, the Red Sea and Persian Gulf) is even more poorly known. However, the early findings

clearly suggest that the diversity of the Japanese threadfin bream in the Indo-West Pacific remains largely unexplored.

Mitochondrial DNA (mtDNA), due to its maternal and non-recombining mode of inheritance and rapid sequence evolution, provides multiple haplotypes that can be ordered phylogenetically within a species (Avise, 2000). Among the mitochondrial genes, a 650 bp fragment from the 5' region of the mitochondrial COI gene, with a strong track record in revealing cryptic species, has been adopted as a global bio-identification system for members of the animal kingdom (Hebert *et al.*, 2004; Ward *et al.*, 2005). Recent studies have successfully proved the efficiency of this gene in diagnosis of threadfin breams (Ravitchandirane *et al.*, 2012) and other marine fishes from the Persian Gulf (Asgharian *et al.*, 2011) and Indian Ocean (Lakra *et al.*, 2011). This study contributes to the species phylogeography through the study of the genetic diversity and historical demography of *N. japonicus* populations from the Persian Gulf and Indo-West Pacific based on original and published COI sequences. Results should provide a primary understanding of the species history, and may have relevance for conservation, stock assessment and fishery management.

Materials and methods

Origin of samples and populations

From June to November 2012 a total number of seventeen specimens were

collected from 8 stations along the northern coast of the Persian Gulf which based on geographical proximity were grouped into two populations (Fig. 1B and Table 1): the Bushehr population (consists of 10 specimens from five sampling stations including the Bushehr waterfront, Bandar Deyr, Bandargah, the Nayband Gulf and the Suru Village coast) and the Hormozgan population (consists of 7 specimens from three sampling stations including Bandar-e Kong, Bandar Abbas and the Salkh Village coast). All specimens were identified as *N. japonicus* based on taxonomic keys and descriptions as referenced in the FAO species catalogue vol. 12 (Russell, 1990). In addition, 14 previously published COI sequences from the South China Sea, Western India (coast of Maharashtra; Lakra *et al.*, 2011) and coast of Bushehr Province (from Nayband National Park; Asgharian *et al.*, 2011) were also retrieved from the National Center for Biotechnology Information (NCBI).

DNA extraction and mtDNA sequencing

DNA was isolated from fin tissue samples by standard Phenol-Chloroform method (Sambrook *et al.*, 1989), and diluted in TE buffer to a final concentration of 50 ng/μL. The COI gene (approximately 655 bp in length) was amplified using a set of primers FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1: 5'-TAGACTTCTGGGTGGCCAAAGAA TCA-3' (Ward *et al.*, 2005).

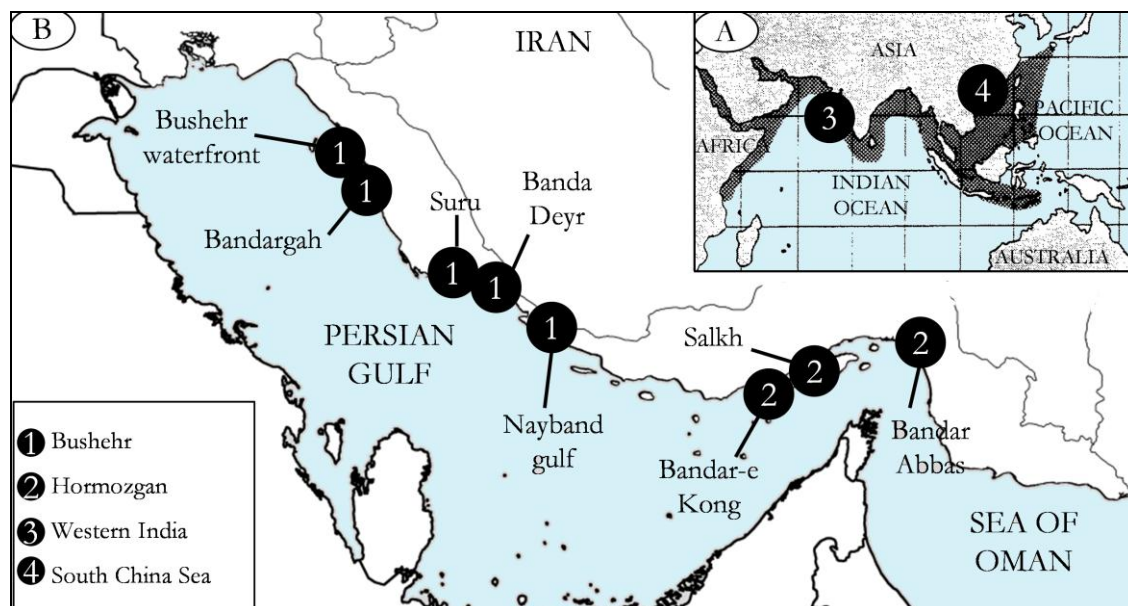


Figure 1: (A) Map of the Indo-West Pacific showing the current range of *Nemipterus Japonicus* (black area; after Russell, 1990). (B) The sample collection sites in the Persian Gulf. Numbers are the same as in Table 1.

Table 1: Sampling localities, geographical coordinates and number of specimens.

Population	No.*	Lat. N	Lon. E	<i>n</i>	GenBank Acc. No.
Bushehr	1	28°17′	51°64′	12	KU739523; KU739524; KU739525; KU739526; KU739527; KU739528; KU739529; KU739530; KU739531; KU739532; HQ149889; HQ149888
Hormozgan	2	26°83′	55°45′	7	KU739533; KU739534; KU739535; KU739536; KU739537; KU739538; KU739539
Western India	3	19°01′	72°78′	5	EF609553; EF609554; EF609555; EF609556; FJ347947
South China Sea	4	22°39′	114°10′	7	KF134003; KF134004; KF134005; EU871686; EU871687; JF493971; JQ681509

* Population number on Fig. 1, *n* = sample size.

The 25 μ L PCR reaction mixes included 16.5 μ L of dH₂O, 2.5 μ L of 10X PCR buffer, 1 μ L of Mg²⁺ (50 mM), 1 μ L of each primer (0.01 mM), 0.5 μ L of each dNTP (0.05 mM), 0.5U of Taq DNA polymerase, and 2 μ L of DNA template. PCR utilized the following thermocycler (Primus 96 advanced Gradient, PeqLab, Germany) parameters: an initial hold at 95°C for 2 min, 35 cycles of 40 sec at 94°C, 1 min at 51°C, 1 min at 72°C, followed in turn by 10 min at 72°C and then held at 4°C.

Products were visualized on 1% agarose gels and the sharp and most intense products were selected for DNA typing. The PCR products were sequenced using an ABI Prism™ 3730 Genetic Analyzer (Applied Biosystems, Foster City, California) by the MacroGen Company, South Korea.

Sequence alignment and data analysis

All newly obtained sequences were deposited in GenBank (Acc. No. KU739523-KU739539). The COI gene

sequences were first aligned with ClustalW procedure implemented in MEGA 5.2 (Kumar *et al.*, 2008), and then manually. The best-fit model of nucleotide substitution for analyses was selected under the corrected Akaike Information Criterion (AICc) with jModelTest 2.1.3 (Posada, 2008). Parameters of genetic diversity and F_{st} distance values among population pairs were calculated using the Arlequin 3.5.1.3 (Excoffier and Lischer, 2010). We used the Median-Joining (MJ) network method to depict the relationships between haplotypes, as implemented in the Network 5.0.0 (Bandelt *et al.*, 1999). To reconstruct the phylogenetic relationships among the COI gene sequences, Neighbor Joining (NJ) and Maximum Likelihood (ML) phylogenetic trees were constructed using the MEGA software. The phylogenetic trees were rooted using homologous sequences of *N. peronii* (GenBank Acc. No. HQ149891 and HQ149890). Using the Arlequin program, changes of effective population size through time were examined by the tests of neutral evolution (i.e. Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) with Arlequin, and Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas, 2002) with DnaSP 5.10 (Librado and Rozas, 2009), and distribution of pairwise differences or mismatch distribution (MMD; Harpending, 1994). The validity of observed MMD was evaluated by the tests of raggedness index (Hri) and the sum of squared

deviations (SSD). We used τ (Tau: time elapsed since the beginning of expansion in mutational units) value provided by MMD analysis to calculate the time since the beginning of expansion. The relationship with the absolute time in years (t), is $t = \tau/2\mu$, where μ is the fragment-specific mutation rate. The Geographic Distance Matrix Generator 1.2.3 (Ersts, 2011) was applied to make a geographic distance matrix among population pairs. To detect putative genomic boundaries, we used a user-friendly geometric method implemented in the Barrier 2.2 (Manni *et al.*, 2004) known as Monmonier's maximum difference (Monmonier, 1973). Complete details on the procedure are given in Manni *et al.* (2004). Then, the statistical significance of the identified boundaries was tested by hierarchical Analysis of Molecular Variance (AMOVA; Excoffier *et al.*, 1992) using the Arlequin package. Applying the Mantel test (using 1000 permutations; Mantel, 1967) with Vegan 2.0 library (Dixon, 2003) and R 3.0.1 package (Ihaka and Gentleman, 1996), we also tested the potential hypothesis for regional subdivision under isolation-by-distance (IBD; Slatkin, 1993).

Results

Genetic diversity

The sequence analysis of a 348 bp fragment of the COI gene detected 44 variable nucleotide sites (26 singleton variables and 18 parsimony informative sites) among 31 specimens from four *N.*

japonicus populations, which defined a total of 17 haplotypes. From these, 15 (88.2%) haplotypes were singletons and only two were shared between populations, one was shared between two adjacent populations (haplotype No. 2 was found in 4 individuals from the Bushehr / Hormozgan populations), and another haplotype was shared between distant populations (haplotype No. 1 was found in 7 individuals from the Bushehr/Western India populations). None of the observed haplotypes in the South China Sea were found elsewhere. Overall haplotype diversity (h) was 0.929 ± 0.02 , ranging from 0.904 ± 0.10 for the South China Sea to 0.700 ± 0.21 for the Western India population (Table 2). The haplotype diversity was nearly similar across the Hormozgan and Bushehr populations (0.878 ± 0.07 and 0.809 ± 0.12 , respectively). Overall nucleotide diversity (π) was 0.0210 ± 0.011 , ranging from 0.0169 ± 0.009 for Bushehr, to 0.0041 ± 0.003 for the South China Sea population. The high haplotype and nucleotide diversities in the Bushehr and Hormozgan populations were clearly evident in the large values of the average number of nucleotide differences (5.86 and 3.97, respectively).

Genealogical relationships and phylogeographic depth

The ML analysis (Fig. 2A), using the Jukes-Cantor (JC69) model (Jukes and Cantor, 1969) of sequence evolution, grouped all the COI sequences into two

reciprocally monophyletic clades with a high statistical support: the western clade (clade I) included all the COI sequences from Bushehr, Hormozgan and the Western India, while the eastern clade (clade II) was restricted to the South China Sea. The NJ tree (not shown) had similar subdivisions. Consistent with the NJ and ML trees, the MJ analysis showed a clear discrimination of two clades by numerous mutational steps (Fig. 2B). The net sequence divergence between these two major matrilineal phylogroups was 2.3%, which under a rate calibration for teleost COI gene sequence (3% per site per million years; Ludt *et al.*, 2012) implies a date of cladal separation about 0.76 Myr ago. Further, the western clade was divided into two sub-clades: sub-clade Ia restricted to the Persian Gulf and Western India, while sub-clade Ib occurs sympatrically with some members of the first sub-clade in the Western India.

Genetic differentiation and population structure

The range of pairwise F_{st} estimates between the Persian Gulf and Western India populations was low to moderate (ranging from 0.140 to 0.374), while the levels of sequence divergence between the South China Sea and other populations were strongly high (ranging from 0.659 to 0.797) (Table 3).

Table 2: Parameters of genetic diversity in the *N. japonicus* populations.

Populations	<i>N</i>	<i>H</i>	<i>s</i>	<i>k</i>	<i>h</i> ± SD	π ± SD
Bushehr	12	7	24	5.86	0.878 ± 0.07	0.0168 ± 0.009
Hormozgan	7	4	14	3.97	0.809 ± 0.12	0.0114 ± 0.007
South China Sea	7	5	4	1.43	0.904 ± 0.10	0.0041 ± 0.003
Western India	5	3	4	2.21	0.700 ± 0.21	0.0063 ± 0.004
Total	31	17	45	7.34	0.929 ± 0.02	0.0210 ± 0.011

n = sample size, *H* = number of haplotypes, *s* = number of polymorphic sites, *k* = the average number of nucleotide differences, *h* = the haplotype diversity, π = the nucleotide diversity, and SD = standard deviation.

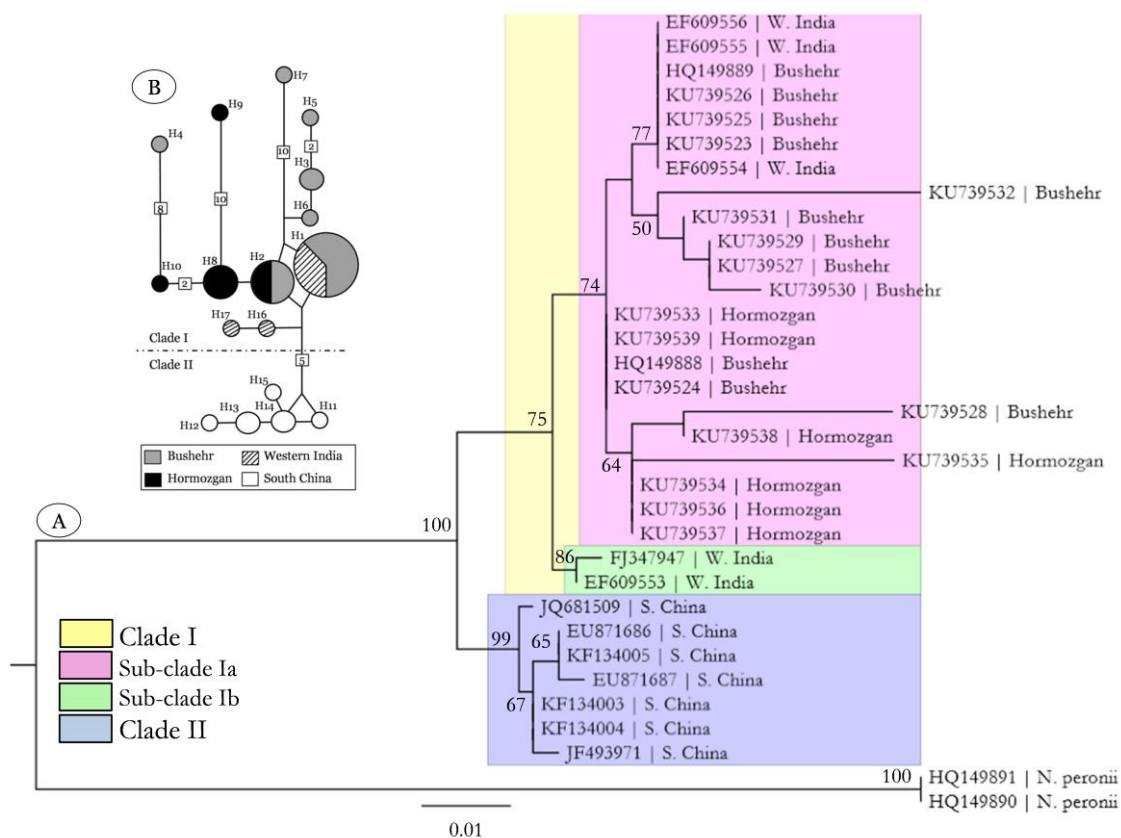


Figure 2: (A) Maximum-Likelihood (ML) tree summarizing relationships between the COI sequences. Bootstrap resampling values are provided at each fork (given only if ≥ 50%). (B) Median-joining (MJ) network describing the relationship among the seventeen COI haplotypes. The circle area is proportional to the frequency of each haplotype and the colors indicate the four different populations.

Because the *N. japonicus* populations are distributed along an east-west axis, we expected geographic isolation to contribute to genetic affinities. Concordantly, an overall significant positive correlation was found between geographical and genetic distances

($r=0.95, p=0.02$). Further, to investigate the level correlation within the range of the western clade, we conducted a partial mantel test without taking into account the South China Sea sample.

Table 3: Estimates of F_{st} among pairs of populations (below the diagonal) and their p -values (above the diagonal; the significance threshold was ≤ 0.05).

Populations	Bushehr	Hormozgan	South China Sea	Western India
Bushehr	0.000	0.0004	0.0001	0.0430
Hormozgan	0.193	0.000	0.0005	0.0009
South China Sea	0.659	0.763	0.000	0.0009
Western India	0.140	0.374	0.797	0.000

The results indicated that the mantel index was still large but statistically non-significant ($r = 0.71$, $p = 0.08$).

Applying the Monmonier maximum difference method (not shown) based on pairwise F_{st} values; a zone with the maximum degree of genetic discontinuity separating the South China Sea sample from all other samples was detected. AMOVA among post-hoc defined regions, as a test to assess the statistical significance of detected discontinuity, confirmed that a significant portion of the mtDNA variation was because of the among groups component ($F_{CT} = 0.561$, 56.14% of total variation; Table 4).

Dynamics of effective population size

An inspection of demographic histories presented the pronounced differences between the western and eastern clades (Table 5; Fig. 3). Recent expansion for

clade II was confirmed by the negative and significant Fu's F_s and positive and significant Ramos-Onsins and Rozas's R_2 values. Further, the mismatch distribution proved close in pattern to theoretical expectation. The time since the beginning of expansion estimated for clade II was approximately 77.7 kyr ago. For clade I, the distribution of pairwise differences wasn't unimodal as expected, an expected signature for a stable population with large long-term effective population size; or, this also might be observed in an admixture sample of individuals from historically sundered populations (Grant and Bowen, 1998). Statistical analyses of the neutrality tests (with the exception of Tajima's D) also supported this molecular-based inference.

Table 4: Hierarchical analysis of molecular variance (AMOVA) according to the geography criterion.

Source of variation	df	Variance components	% variation	Fixation indices
Among groups*	1	3.358	56.14	$F_{CT} = 0.561^{**}$
Among populations within groups	2	0.664	11.10	$F_{SC} = 0.253^{**}$
Within populations	27	1.960	32.76	$F_{ST} = 0.672^{**}$
Total		5.982	100	

* The western group consists of the Bushehr, Hormozgan and Western India populations, and the eastern group consists of the South China Sea population; ** $p < 0.05$; df = degree of freedom.

Table 5: Statistics of demographic expansion and MMD goodness-of-fit tests based on mitochondrial COI sequences for major clades.

Populations	Tajima's D	Fu's Fs	R ₂	Hri (p-value)	SSD (p-value)	τ	t
Clade I	-1.70*	-1.33	0.089	0.032 (0.5)	0.019 (0.4)	-	-
Clade II	-0.59	-2.31**	0.142*	0.231 (0.3)	0.042 (0.3)	1.62	77.7

Hri = Harpending's raggedness index; SSD = sum of squared deviations; τ = tau value (time since expansion in mutational units); t = time since expansion in thousand years (Kyr); *p<0.05; **p<0.02.

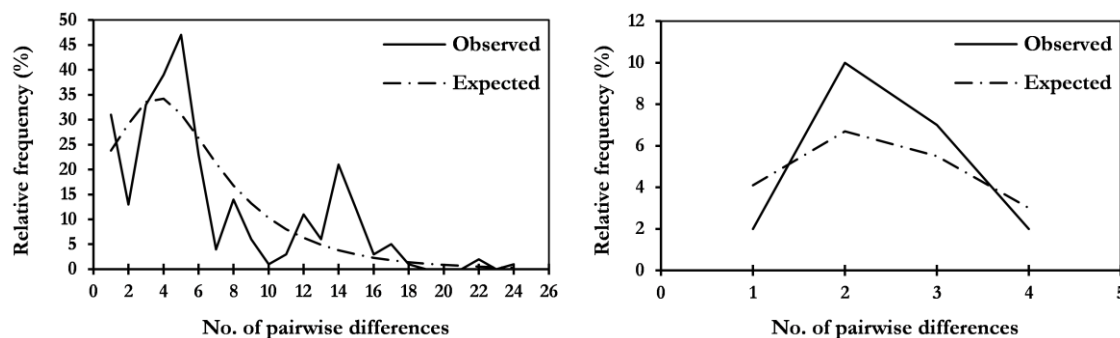


Figure 3: Observed and expected mismatch distributions showing the frequencies of pairwise differences for clade I (left) and clade II (right). X-axis: number of substitutions, Y-axis: is $Ne*\mu$ (effective population size * substitution rate in generation).

Discussion

An evolutionary significant unit (ESU), by definition, is one or a set of conspecific populations with a long-term, distinct evolutionary history (Ryder, 1986). Operational criteria have been suggested for defining intraspecific ESUs. The general recommendation is that ESU must contribute considerably to the overall genetic diversity of a species (Waples, 1991). In this study, based on AMOVA, 56.14% of the total variation observed was mainly between two geographical groups of populations, the western group consists of populations from the Persian Gulf and Western India and the eastern group consists of the South China Sea population. The more detailed suggestion is that ESUs be identified as groups of populations reciprocally monophyletic for mtDNA

haplotypes (Moritz, 1994). Likewise, in the gene trees (ML and NJ), two reciprocally monophyletic phylogroups were split with high amount of statistical support, one contains specimens from the Persian Gulf and Western India and the other one contains specimens from the South China Sea. Redescription of the species based on morphology by Ning *et al.* (2015) concordantly presented diagnostic differences in belly color, with specimens in the South China Sea having a silver belly, while those from the Indian Ocean (Nayband National Park; Asgharian *et al.*, 2011) have a yellow coloration. The pattern represented by phylogenetic trees in this study belongs to the phylogeographic category I (Avise, 2000): deep gene tree with major phylogroups allopatric, which

correspond to populations that have been geographically isolated for a long period. The current data indicates that the main phylogeographic subdivision within *N. japonicus* date to 0.76 Myr ago, namely the mid-Pleistocene. Apparently, series of episodes of low sea level through the second half of the Pleistocene epoch (approximately 2.5 Myr to 11.8 Kyr ago; Hewitt, 2000) appear to have played an active role in initiating major phylogeographic separation within this species. The Indonesian Archipelago constitutes a crucial biogeographic barrier among the Pacific and Indian Ocean provinces (Briggs, 1974). During the Pleistocene, sea level fluctuations of up to 130 m exposed the Sunda and Sahul shelves as dry lands, restricting waterways within the archipelago and sundering populations of many marine organisms into geminates now found in the west Pacific and Indian Ocean (Porter, 1989; Voris, 2000), in conjunction with limited larval exchange among oceans, drift and selection promoted genetic subdivision (Jackson *et al.*, 2014). Apart from biogeographic studies (e.g. McManus, 1985; Woodland, 1986; Springer and Williams, 1990), a key array of evidence supporting Pleistocene vicariance between the Indian Ocean and the west Pacific biotas comes from comparative phylogeography. Numerous examples of pronounced Indo-West Pacific phylogeographic break at the intraspecific level in marine taxa have been reported (reviewed in Carpenter *et*

al., 2011 and Borsa *et al.*, 2015). These cases typify what is implied by Aspect III of genealogical concordance (Avice and Ball, 1990): the concordance in the geography of gene-tree partitions across multiple co-distributed species implicates shared historical biogeographic factors in shaping intraspecific phylogenies (in this case, allopatric divergence driven by Pleistocene sea level fluctuations).

Clade I presented two significant sub-clades; sub-clade Ia belongs to the Persian Gulf and Western India, while sub-clade Ib occurs sympatrically with some members of the sub-clade Ia in the Western India. Theoretically, this outcome could arise in a species with large evolutionary effective population size and high gene flow. Then, some anciently separated lineages might by chance have been retained whereas many intermediate genotypes were lost over time by gradual lineage sorting. Balancing selection also could promote this outcome by favoring long-term evolutionary survival of some haplotype lineages (Avice, 2000). On the other hand, morphometric analysis on *N. japonicus* has indicated that more than one stock is present in the west as well as east coast of India (Sreekanth *et al.*, 2015). Thus, another possible explanation involves secondary admixture between allopatrically evolved populations from the west and east coast of India (Avice, 2000). Hydrological and ecological evidence formally support this molecular-based inference. The recirculation cells at the

western frontier of the Bay of Bengal (Durand *et al.*, 2009), low mean sea surface temperature, presence of freshwater received from the peninsular rivers, weak winds and the continental shelf pattern differentiates the ecology of the Bay of Bengal from that of the Arabian Sea (Jaswal *et al.*, 2012). Further, around the latitude of 10°S, there is a strong hydrographical and chemical front with sharp gradient changes in dissolved oxygen, salinity, nitrate and phosphate content, separating the profile of the Arabian Sea from the Bay of Bengal (Tsang *et al.*, 2012). Accordingly, these would enhance larval retention, resulting in unique genetic composition within the Arabian Sea from the Bay of Bengal. No further evidence for geographical lineage substructure was evident within the range of sub-clade Ia, an indication of high gene flow today between populations across this region with rather tight historical connections. The Persian Gulf, a sedimentary basin with very shallow water of about 30 m in depth, is considered a remnant of the Tethys Sea (Omar and Steckler, 1995). Due to its shallow depth, during the late Pleistocene glacial period when the sea level was lowered, it was completely dry (Lambeck, 1996). It was re-colonized from the Indian Ocean refuge after the connection with the Indian Ocean was reestablished (Tsang *et al.*, 2012).

Studies on the spawning time indicate that spawning season of *N. japonicus* in the South China Sea

(Eggleston, 1972), and the Persian Gulf (Kerdgari *et al.*, 2009; Fazeli, 2013), Western India (Acharya, 1990; Raje, 2003; Manojkumar, 2004; Joshi, 2010), and Red Sea (Bakhsh, 1996) mainly occur during May to October. However, Krishnamoorthi (1971) reported breeding season in the Bay of Bengal expending from November to March. Thus, the genetic subdivision pattern presented in *N. japonicus* may reflect the regional differences in reproductive behavior. On the other hand, a trend of increasing genetic differences with increasing geographic distance was also uncovered, which leaves open the hypothesis that part of the differences between populations might be explained by isolation by distance. However, since the *N. japonicus* populations and two major biogeographic barriers (the Indo-West Pacific barrier and the mainland of India) are distributed along an east-west axis, the exact discrimination between the role of the Pleistocene vicariance events and isolation by distance in shaping the current spatial distribution of genetic lineages is difficult.

The demographic histories of populations are of extreme relevance to phylogeographic patterns over microevolutionary time scales because of their inevitable influence on the structures of gene genealogies (Avise, 2000). An approach to concluding the overall historical demography of a population from gene genealogy data involves examination of two different measures of variation, h and π (Grant

and Bowen, 1998). The high h but low π value for clade II ($h = 0.904 \pm 0.10$; $\pi = 0.0041 \pm 0.003$) indicates rapid population growth from a bottlenecked ancestral population (Grant and Bowen, 1998), provided the time was adequate for recovery of haplotype diversity through mutation but too short for an accumulation of significant sequence differences (Lowe *et al.*, 2009). Concordantly, the distribution of pairwise differences and tests of neutral evolution implied that a late Pleistocene population expansion (approximately 77.7 Kyr ago) may have happened in the South China Sea. Conversely, the high values for h and π in clade I ($h = 0.890 \pm 0.04$; $\pi = 0.0161 \pm 0.008$) are an expected signature for a stable population with large long-term effective populations size; or, they also might be observed in an admixture sample of individuals from historically separated populations (in this case, perhaps allopatrically evolved populations from the west and east coast of India) (Grant and Bowen, 1998). This scenario is further supported by non-significant neutrality tests (with the exception of Tajima's D) and multimodal distribution of pairwise differences. However, we cannot rule out the possibility of retention of ancient lineages and loss of intermediate polymorphisms and / or imperfect sampling.

Our findings clearly indicate the diversity in the Indo-West Pacific remains largely unexplored, even for a coastal species like *N. japonicus* that is

easily encountered. We identified two reciprocally monophyletic phylogroups (or ESUs) in *N. japonicus* which may qualify as cryptic species. Observed pattern of regional subdivision was consistent with the role of Pleistocene vicariance events. However, Pleistocene vicariance is considered only one of the key mechanisms. A trend of increasing genetic differences with increasing geographic distance and regional differences in breeding season were also proposed. Given the limited number of populations and low sample sizes, these results should be considered provisional. However, since the baseline knowledge on the intraspecific diversity and cladal distribution is the first step before any action is to be taken, the basic information provided by this research is particularly germane to conservation efforts, fishery management, and stock assessment.

Acknowledgments

Special thanks go to anonymous reviewers for their helpful comments. The authors acknowledge Shahid Beheshti University for financial support.

References

- Acharya, P., 1990.** Studies on maturity, spawning and fecundity of *Nemipterus japonicus* (Bloch) off Bombay coast. *Journal of the Indian Fisheries Association*, 20(1), 51–57.
- Afshari, M., Valinassab, T., Seifabadi, J. and Kamaly, E., 2013.** Age determination and feeding

- habits of *Nemipterus japonicus* (Bloch, 1791) in the northern Oman Sea. *Iranian Journal of Fisheries Sciences*, 12(2), 248–264.
- Asgharian, H., Sahafi, H.H., Ardalan, A.A., Shekarriz, S. and Elahi, E., 2011.** Cytochrome c oxidase subunit 1 barcode data of fish of the Nayband National Park in the Persian Gulf and analysis using meta-data flag several cryptic species. *Molecular Ecology Resources*, 11(3), 461–472.
- Avise, J.C. and Ball, R.M., 1990.** Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology*, 7(1), 45–67.
- Avise, J.C., 2000.** Phylogeography: The history and formation of species. USA: Harvard University Press, 447P.
- Bakhsh, A.A., 1996.** The biology of thread bream, *Nemipterus japonicus* (Bloch) from the Jizan Region of the Red Sea. *Journal of King Abdulaziz University (Marine Sciences)*, 7(1), 17–189.
- Bandelt, H.J., Forster, P. and Röhl, A., 1999.** Median-Joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37–48.
- Bloch, M. E., 1795.** Naturgeschichte der ausländischen Fische. Berlin: Verfasser, 420P.
- Borsa, P., Durand, J.D., Chen, W.J., Hubert, N., Muths, D., Mou-Tham, G. and Kulbicki, M., 2015.** Comparative phylogeography of the Western Indian Ocean reef fauna. *Acta Oecologica*, 30(1), e15.
- Briggs, J.C., 1974.** Marine zoogeography. New York: McGraw-Hill, 475P.
- Carpenter, K.E., Barber, P.H., Crandall, E.D., Ablan-Lagman, M.C.A., Mahardika, G.N., Manjaji-Matsumoto, B.M., Juinio-Meñez, M.A., Santos, M.D., Starger, C.J. and Toha, A.H.A., 2011.** Comparative phylogeography of the Coral Triangle and implications for marine management. *Journal of Marine Biology*, 2011(396982), 1–14.
- Chawla, S.P., Venugopal, V. and Nair, P.M., 1996.** Gelation of proteins from washed muscle of threadfin bream (*Nemipterus japonicus*) under mild acidic conditions. *Journal of Food Science*, 61(2), 362–367.
- Dixon, P., 2003.** VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, 14(6), 927–930.
- Durand, F., Shankar, D., Birol, F. and Shenoi, S.S.C., 2009.** Spatiotemporal structure of the East India Coastal Current from satellite altimetry. *Journal of Geophysical Research Oceans*, 114(C02013), 1–18.
- Eggleston, D., 1972.** Patterns of biology in the Nemipteridae. *Journal of the Marine Biological Association of India*, 14(1), 357–364.

- Ersts, P. J., 2011.** Geographic distance matrix generator (version 1.2. 3). American Museum of Natural History: Center for Biodiversity and Conservation.
- Excoffier, L., Smouse, P.E. and Quattro, J.M., 1992.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, 131(2), 479–491.
- Excoffier, L. and Lischer, H.E.L., 2010.** Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567.
- Fazeli, F., 2013.** Investigation on spawning season and fecundity of *Nemipterus japonicus* (Bloch, 1791) species in Khuozestan coastal (Northern Persian Gulf). *Journal of Animal Researches (Iranian Journal of Biology)*, 26(2), 186–192.
- Fazeli, F., 2014.** Identification on growth parameters and mortality of *Nemipterus japonicus* (Bloch, 1791) by using length frequency in Khuozestan Coastal (Northern Persian Gulf). *Journal of Animal Researches (Iranian Journal of Biology)*, 27(2), 100–105.
- Fu, Y.X., 1997.** Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147(2), 915–925.
- Grant, W.A.S. and Bowen, B.W., 1998.** Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, 89(5), 415–426.
- Harpending, H.C., 1994.** Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, 66(4), 591–600.
- Hebert, P.D.N., Stoeckle, M.Y., Zemplak, T.S. and Francis, C.M., 2004.** Identification of birds through DNA barcodes. *PLoS Biology*, 2(10), e312.
- Hewitt, G., 2000.** The genetic legacy of the Quaternary ice ages. *Nature*, 405(6789), 907–913.
- Ihaka, R. and Gentleman, R., 1996.** R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, 5(3), 299–314.
- Iqbal, M., 1991.** Population dynamics of *Nemipterus japonicus* from the northern Arabian Sea, Pakistan. *Fishbyte*, 9(1), 16–18.
- Jackson, A.M., Erdmann, M.V., Toha, A.H.A., Stevens, L.A. and Barber, P.H., 2014.** Phylogeography of commercial tuna and mackerel in the Indonesian Archipelago. *Bulletin of Marine Science*, 90(1), 471–492.
- Jamsari, A., Ahmad, A.T., Nor, S.A. M., Lim, H.C. and Nuruddin, A.A., 2008.** Population studies of *Nemipterus japonicus* (Bloch, 1791) along the coast of Peninsular Malaysia based on mitochondrial

- DNA cytochrome b gene. Proceedings of the 5th National Fisheries Symposium. Jabatan Perikanan Malaysia, Kuala Terengganu, pp. 281–288.
- Jaswal, A.K., Singh, V. and Bhambak, S.R., 2012.** Relationship between sea surface temperature and surface air temperature over Arabian Sea, Bay of Bengal and Indian Ocean. *Journal of Indian Geophysical Union*, 16(2), 41–53.
- Joshi, K.K., 2010.** Population dynamics of *Nemipterus japonicus* (Bloch) in the trawling grounds off Cochin. *Indian Journal of Fisheries*, 57(1), 7–12.
- Jukes, T. H. and Cantor, C. R., 1969.** Evolution of protein molecules. In: Munro, H. N. (ed), *Mammalian Protein Metabolism*. Academic Press, New York, pp. 21–132.
- Kerdgari, M., Valinassab, T., Jamili, S., Fatemi, M.R. and Kaymaram, F., 2009.** Reproductive biology of the Japanese threadfin bream, *Nemipteru japonicus*, in the northern of Persian Gulf. *Journal of Fisheries and Aquatic Science*, 4(3), 143–149.
- Krishnamoorthi, B., 1971.** Biology of the threadfin bream, *Nemipterus japonicus* (Bloch). *Indian Journal of Fisheries*, 18(1 and 2), 1–21.
- Kumar, S., Nei, M., Dudley, J. and Tamura, K., 2008.** MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics*, 9(4), 299–306.
- Lakra, W.S., Verma, M.S., Goswami, M., Lal, K.K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K.V., Ward, R.D. and Hebert P., 2011.** DNA barcoding Indian marine fishes. *Molecular Ecology Resources*, 11(1), 60–71.
- Lambeck, K., 1996.** Shoreline reconstructions for the Persian Gulf since the last glacial maximum. *Earth and Planetary Science Letters*, 142(1), 43–57.
- Lee, C.K.C., 1973.** The exploitation of *Nemipterus japonicus* (Bloch) by Hongkong vessels in 1972-73. The Pacific Science Association Special Symposium on Marine Science. Govt. Printer, Hong Kong, pp. 48–52.
- Librado, P. and Rozas, J., 2009.** DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451–1452.
- Lim, H.C., Ahmad, A.T., Nuruddin, A.A. and Mohd Nor, S.A., 2016.** Cytochrome b gene reveals panmixia among Japanese threadfin bream, *Nemipterus japonicus* (Bloch, 1791) populations along the coasts of Peninsular Malaysia and provides evidence of a cryptic species. *Mitochondrial DNA*, 27(1), 575–584.
- Lowe, A., Harris, S. and Ashton, P., 2009.** *Ecological genetics: Design, analysis, and application*. USA: John Wiley and Sons, 432P.
- Ludt, W.B., Bernal, M.A., Bowen, B.W. and Rocha, L.A., 2012.**

- Living in the past: Phylogeography and population histories of Indo-Pacific wrasses (genus *Halichoeres*) in shallow lagoons versus outer reef slopes. *PloS One*, 7(6), e38042.
- Manni, F., Guerard, E. and Heyer, E., 2004.** Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by using Monmonier's algorithm. *Human Biology*, 76(2), 173–190.
- Manojkumar, P.P., 2004.** Some aspects on the biology of *Nemipterus japonicus* (Bloch) from Veraval in Gujarat. *Indian Journal of Fisheries*, 51(2), 185–191.
- Manojkumar, P.P., Pavithran, P.P. and Ramachandran, N.P., 2015.** Food and feeding habits of *Nemipterus japonicus* (Bloch) from Malabar coast, Kerala. *Indian Journal of Fisheries*, 62(1), 64–69.
- Mantel, N., 1967.** The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27(2), 209–220.
- McManus, J.W., 1985.** Marine speciation, tectonics and sea-level changes in southeast Asia. Proceedings of the 5th International Coral Reef Congress. Museum National d'Histoire Naturelle, Tahiti, French Polynesia, pp. 133–138.
- Monmonier, M.S., 1973.** Maximum-difference barriers: An alternative numerical regionalization method. *Geographical Analysis*, 5(3), 245–261.
- Moritz, C., 1994.** Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, 9(10), 373–374.
- Murty, V.S., 1987.** Further studies on the growth and yield per recruit of *Nemipterus japonicus* (Bloch) from the trawling Grounds off Kakinada. *Indian Journal of Fisheries*, 34(3), 265–276.
- Murty, V.S., 1984.** Observations on the fisheries of threadfin breams (Nemipteridae) and on the biology of *Nemipterus japonicus* (bloch) from Kakinada. *Indian Journal of Fisheries*, 31(1), 1–18.
- Naqash, S.Y. and Nazeer, R.A., 2010.** Antioxidant activity of hydrolysates and peptide fractions of *Nemipterus japonicus* and *Exocoetus volitans* muscle. *Journal of Aquatic Food Product Technology*, 19(3 and 4), 180–192.
- Ning, P., Sha, Z., Hebert, P.D.N. and Russell, B., 2015.** The taxonomic status of Japanese threadfin bream *Nemipterus japonicus* (Bloch, 1791)(Perciformes: Nemipteridae) with a redescription of this species from the south china sea based on morphology and DNA barcodes. *Journal of Ocean University of China*, 14(1), 178–184.
- Omar, G.I. and Steckler, M.S., 1995.** Fission track evidence on the initial rifting of the Red Sea: Two pulses, no propagation. *Science*, 270(5240), 1341–1344.
- Porter, S.C., 1989.** Some geological implications of average Quaternary

- glacial conditions. *Quaternary Research*, 32(3), 245–261.
- Posada, D., 2008.** jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, 25(7), 1253–1256.
- Raje, S.G., 2003.** Observations on the biology of *Nemipterus japonicus* (Bloch) from Veraval. *Indian Journal of Fisheries*, 49(4), 433–440.
- Rajkumar, U., Rao, K.N. and Kingsly, H.J., 2003.** Fishery, biology and population dynamics of *Nemipterus japonicus* (Bloch) off Visakhapatnam. *Indian Journal of Fisheries*, 50(3), 319–324.
- Ramos-Onsins, S.E. and Rozas, J., 2002.** Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, 19(12), 2092–2100.
- Ravitchandirane, V., Geetha, V., Ramya, V., Janifer, B., Thangaraj, M., Subburaj, J., Ramanadevi, V. and Ganesan, T., 2012.** Molecular identification and phylogenetic relationships of threadfin breams (Family: Nemipteridae) using mtDNA marker. *Notulae Scientia Biologicae*, 4(2), 13–18.
- Rezayi, S., Peyghambari, S.Y., Reisi, H. and Shabani, M.J., 2014.** Population dynamics of Japanese threadfin bream (*Nemipterus japonicus* Bloch, 1971) in the Persian Gulf waters, Bushehr Province (in Persian). *Aquatic Ecology*, 3(4), 67–75.
- Russell, B.C., 1990.** FAO species catalogue: Nemipterid fishes of the world. An annotated and illustrated catalogue of Nemipterid species known to date. FAO Fisheries synopsis 125, Vol 12, 125P.
- Ryder, O.A., 1986.** Species conservation and systematics: The dilemma of subspecies. *Trends in Ecology and Evolution*, 1(1), 9–10.
- Salarpouri, A., Behzadi, S., Darvishi, M. and Momeni, M., 2010.** A study on feeding habits of *Nemipterus japonicus* (Bloch, 1791) in Persian Gulf, Tonb to Hengam Island waters. *Journal of Aquatic Animal and Fisheries*, 1(3), 40–47.
- Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989.** Molecular cloning. New York: Cold Spring Harbor Laboratory Press, 1626P.
- Samuel, M., 1990.** Biology, age, growth and population dynamics of threadfin bream *Nemipterus japonicus*. *Journal of the Marine Biological Association of India*, 32(1), 66–76.
- Senta, T. and Tan, K., 1975.** Species and size-composition of threadfin snappers in the South China Sea and the Andaman Sea. *Singapore Journal of Primary Industries*, 3(1), 1–11.
- Slatkin, M., 1993.** Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, 47(1), 264–279.
- Springer, V.G. and Williams, J.T., 1990.** Widely distributed Pacific plate endemics and lowered sea-

- level. *Bulletin of Marine Science*, 47(3), 631–640.
- Sreekanth, G.B., Chakraborty, S.K., Jaiswar, A.K., Renjith, R.K., Ratheesh Kumar, S.K.P., Vaisakh, G., Ail, S.S., N. M. Lekshmi and Pazhayamado, D. G., 2015.** Can the *Nemipterus japonicus* stocks along Indian coast be differentiated using morphometric analysis? *Indian Journal of Geo-Marine Sciences*, 44(4), in press.
- Tajima, F., 1989.** Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123(3), 585–595.
- Tsang, L. M., Achituv, Y., Chu, K. H. and Chan, B. K. K., 2012.** Zoogeography of intertidal communities in the West Indian Ocean as determined by ocean circulation systems: Patterns from the *Tetraclita* barnacles. *PloS One*, 7(9), e45120.
- Valinassab, T., Daryanabard, R., Deghani, R. and Pierce, G.J., 2006.** Abundance of demersal fish resources in the Persian Gulf and Oman Sea. *Journal of the Marine Biological Association of the United Kingdom*, 86(6), 1455–1462.
- Vivekanandan, E. and James, D.B., 1986.** Population dynamics of *Nemipterus japonicus* (bloch) In the trawling grounds off Madras. *Indian Journal of Fisheries*, 33(2), 145–154.
- Voris, H.K., 2000.** Maps of pleistocene sea levels in southeast Asia: Shorelines, river systems and time durations. *Journal of Biogeography*, 27(5), 1153–1167.
- Waples, R.S., 1991.** Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. *Marine Fisheries Review*, 53(3), 11–22.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N., 2005.** DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1462), 1847–1857.
- Weber, W. and Jothy, A.A., 1977.** Observations on the fish *Nemipterus* spp. (Family: Nemipteridae) in the coastal waters of East Malaysia. *Archiv fur Fischereiwissenschaft*, 28(2 and 3), 109–122.
- Woodland, D.J., 1986.** Wallace's Line and the distribution of marine inshore fishes. Proceedings of the 2nd International Conference on Indo-Pacific Fishes. Ichthyological Society of Japan, Tokyo, pp. 453–460.
- Zacharia, P., 1998.** Dynamics of the threadfin bream, *Nemipterus japonicus* exploited off Karnataka. *Indian Journal of Fisheries*, 45(3), 265–270.