Mitochondrial DNA sequence-based phylogenetic relationship of *Trichiurus lepturus* (Perciformes: Trichiuridae) from the Persian Gulf

Tamadoni Jahromi, S.[1*]; Mohd Noor, S. A.[2]; Pirian, K.[3]; Dehghani, R.[4]; Nazemi, M.[1] and Khazaali, A.[5]

[1]Department of Genetic, Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Abass, Iran; [2]Department of Biology, School of Biological Sciences, University Science Malaysia (USM), Penang, Malaysia; [3]Department of Biotechnology, School of Agricultural Sciences, Bu-Alisina University, Hamadan, Iran; [4]Department of Biology, Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Abass, Iran; [5]Young Researchers and Elite Club, Bandar Abass Branch, Islamic Azad University, Bandar Abass, Iran

[*]***Correspondence:*** *S. Tamadoni Jahromi, Department of Genetic, Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Abass, Iran. E-mail: stamadoni@yahoo.com*

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Summary

In this study, mitochondrial DNA analysis using 16S ribosomal DNA (rDNA) was performed to investigate the phylogeny relationship of *Trichiurus lepturus* in the Persian Gulf compared to the other investigated area. The amplification of 16S rDNA resulted in a product of 600 bp in all samples. The results showed that the isolated strain belongs to *T. lepturus* showing 42 divergence sites among the same reported partial sequences of 16S rRNA gene from the other area (West Atlantic and Indo-Pacific area). Phylogeny results showed that all 18 haplotypes of the species clustered into five clades with reasonably high bootstrap support of values (>64%). Overall, the tree topology for both phylogenetic and phenetic trees for 16S rDNA was similar. Both trees exposed two major clusters, one wholly containing the haplotypes of the *T. lepturus* species belonging to Indo-Pacific area with two major sister groups including Persian Gulf specimen and the other cleared the Western Atlantic and Japan individuals clustered in another distinct clade supporting the differentiation between the two areas. Phylogenic relationship observed between the Persian Gulf and the other Indo-Pacific Individuals suggested homogeneity between two mentioned areas.

Key words: 16S rDNA, Mitochondrial DNA, Persian Gulf, Phylogeny, *Trichiurus lepturus*

Introduction

The Persian Gulf is a semi-enclosed sea located between the Arabian Peninsula and Iran ,with an area of 260,000 km^2, the Persian Gulf has a mean depth of 35 m and a maximum depth >100 m (UNEP, 1999). It is separated from the Gulf of Oman and the open ocean throughout the Straits of Hormuz. Due to the diverse environmental situations, a wide variety of marine life can be found in the Persian Gulf.

Fig. 1: *Trichiurus lepturus*

The large head cutlass fish, *Trichiurus lepturus* (Fig. 1), is a bentho-pelagic species cached along continental shores and islands in tropical and temperate seas, generally between 45°S and 60°N (Nakamura and Parin, 1993; Randall, 1995). This species is categorized as a coastal species cached in warm waters around the world (Raeisi *et al*., 2011).

The recorded worldwide catch of this species was 1345911 tonnes in 2009 (FAO, 2009).

Cutlass fish are ravenous predators (Martins and Haimovici, 1996; Bitter and Benneditto, 2009) reported in the Indian Ocean, particularly the Persian Gulf. The most abundant species of cutlass fish in the Persian Gulf is *T. lepturus*. Based on recent investigations, the cutlass fish fisheries of the Persian Gulf are the most financially stable of the fisheries located in the Persian Gulf (Raeisi *et al*., 2011). Large population of this species has been associated with low catches of important commercial shrimps, such as *Penaeus semisulcatus*, *Metapenaeus stebbingi*, and *Metapenaeus affinis*, by southern Iranian fishermen which showed that the cutlass fish can be considered to have a significant impact on the shrimp stock (Raeisi *et al*., 2011). Despite the availability of considerable information on special parameters and distribution of this species in the Persian Gulf, there are no confirmed reports in genetic differences for the mentioned species in our territorial waters. Mitochondrial DNA analysis using conserved genes such as

16S ribosomal DNA (rDNA) is a very useful tool for molecular taxonomic studies and is a frequently used marker in genetic studies of terrestrial and marine vertebrates, especially at the genus and family levels (Allard *et al*., 1992; Milinkovitch *et al*., 1993). In addition, the 16S rRNA gene for which the substitution rate is halfthat of the protein-coding gene (Brown *et al*., 1982) is utilized to better identify species (Bourdy *et al*., 2003; Lam and Morton, 2003). In this study, we analyzed partial sequence of 16S rDNA gene from morphologically identified *T. Lepturus* from the Persian Gulf and compared it to that of *T. lepturus* and also *T. Japonicas* (reported from both Western Atlantic and Indo-Pacific waters) in order to verify the validity of mentioned species.

Materials and Methods

Sampling was carried out (10 sample) from Hengam island offshore (Hormozstrait, Persian Gulf, Fig. 2) and transported to the laboratory using alcohol 96%.

Fig. 2: Hengam Island

Total genomic DNA extraction

Total genomic DNA was extracted from 0.1-0.2 g of frozen muscle tissue mixed with 600 µL DNA extraction buffer (10 mM Tris-HCl, pH = 7.5, 100 mM EDTA, 1 µg/ml proteinase K (Sinagene, Iran) and 0.05 µg/ml RNase Rosch, Germany) and subsequently ground using a mortar and pestle. The homogenate was incubated for 12 h at 55°C using thermo mixer (Eppendorf, Germany). DNA was extracted twice with phenol/chloroform/ isoamyl alcohol (25:24:1) followed by extraction with an equal volume of chloroform/isoamyl alcohol (24:1), and precipitated in 100% cold ethanol (Sambrook *et al*., 1989).

Agarose gels (1% weight/volume) were used to fractionate high molecular weight DNA and for observation of the quality of extracted DNA. DNA quantity was estimated by spectrophotometer using a Biophotometer (Eppendorf). DNA quantity was determined by measuring the concentration of DNA by its optical density absorption at 260 nm and 280 nm. The reading ratio at 260 nm and 280 nm provides an estimation of the quality of DNA with respect to the contamination that absorbs UV (e.g. protein contaminants). Pure DNA has an OD_{260}/OD_{280} ratio of 1.8-2.00.

PCR amplification and DNA sequencing

Polymerase chain reaction (PCR) for amplification of 16S rDNA was conducted using universal primers L2510 (5′-GCC TGT TTA ACA AAA ACA T-3′) and H3059 (5′-CGG TCT GAA CTC AGA TCA CGT-3′) (Miya and Nishida, 1996). The PCR amplification was carried out in a standard 25 or 50 µL reaction volume with 2 µL of total genomic DNA, 1 pmol per µL of each diluted primer, 2.5 mM $MgCl_2$ (optimized from 1.5 mM up to 3.0 mM), 2 mM dNTPs (Promega, USA), and 5 U/µL Taq DNA polymerase (Promega). Amplification was performed in a PTC-200 Peltier Thermal Cycler, (MJ Research, Watertown, MA) with a profile of precycle denaturation at 94°C for 4.5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C (optimized annealing temperature), 1 min at 72°C (extension temperature), and a final extension of 5 min at 72°C. Amplified DNA was purified by using a Spin Clean Gel Extraction kit (Rosch, Germany) (Fig. 3).

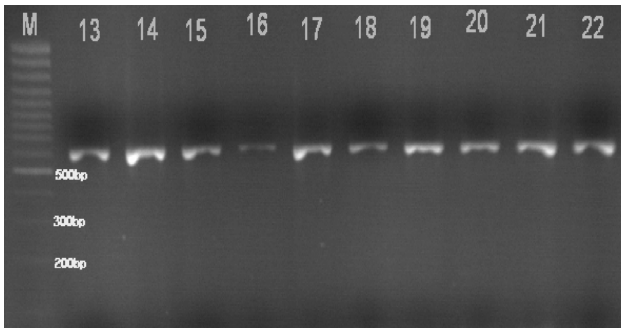

Fig. 3: PCR products of 16S rDNA gene fragment of *T. lepturus*

Ten samples were sent to service provider, (Metabion, Germany) for direct sequencing in both directions. Resulting PCR products were purified using ethanol precipitation and run using Automatic Sequencer (3730xl Applied Biosystem, Foster City, CA).

After editing the sequences, nucleotide BLAST (Basic Local Alignment Search Tool) was performed using NCBI blast main page and recorded (Access number KC307770). The sequences obtained from each sample were aligned and corrected from any ambiguities and assembled using Bio edit program (Hall, 2005). Trees were generated using maximum parsimony (MP), a character-based algorithm and Neighbor-Joining (NJ), a distance-based algorithm for phenetic analysis. The distance matrix option of MEGA4 (Tamura *et al*., 2007) was used to calculate genetic distance according to the Kimura 2-parameter model of sequence evolution (Kimura, 1980). For character-based method, trees were constructed using MP. Maximum parsimony tree was constructed with TBR (Tree Bisection and Reconnection) branch swapping and 10 random taxon addition replicates under a heuristic search, saving no more than

100 equally parsimonious trees per replicate. To estimate branch support on the recovered topology, non parametric bootstrap values were assessed with 1000 bootstrap pseudo-replicates. Similarly a NJ tree (Saito and Nei, 1987) was constructed based on pair-wise genetic distance using the Kimura 2-Parameter algorithm.

Results

Phylogenetic analysis

Phylogenetic trees were constructed based on the final consensus sequence of 495 characters. For 16S rRNA gene, 18 haplotypes including one haplotype for Persian Gulf were clustered into two major groups on both trees with reasonably high bootstrap support of values (Figs. 4 and 5). A bootstrap value of 70% or more is generally considered a robustly supported node which reflected slight homoplasy in the samples.

Overall the tree topology for both phylogenetic and phenetic trees for 16S rDNA was similar. Both trees exposed 2 major clusters, one wholly containing the haplotypes of the *T. lepturus* species belonging to Indo-Pacific area with two major sister groups including Persian Gulf haplotype and the other cleared the Western Atlantic and Japan individuals clustered in another distinct clade supporting the differentiation between the two areas.

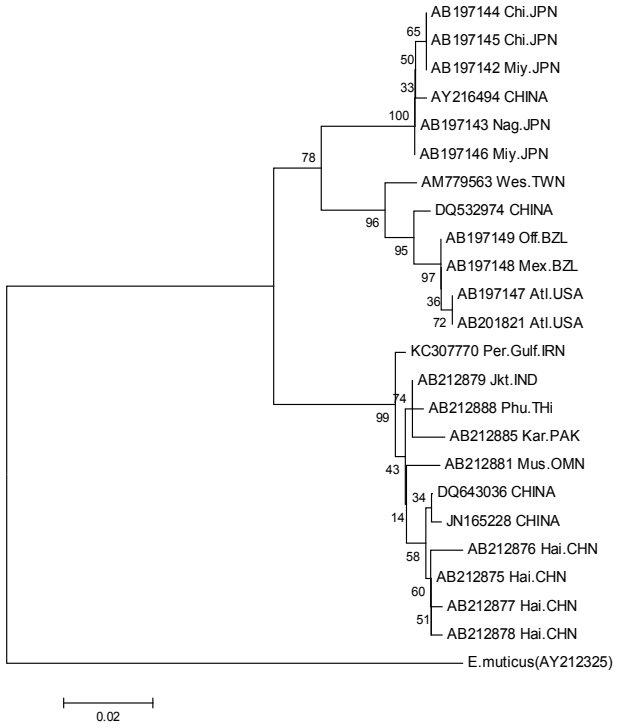

Fig. 4: Neighbor-Joining tree based on genetic distance analysis of 16S rDNA sequences showing the genetic relationships of *T. lepturus* species. *E. muticus* was used as out group. Scale shown refers to genetic distance based on nucleotide substitutions. Numbers at branching points are bootstrap support. The sample of Persian Gulf is shown by Access number KC307770

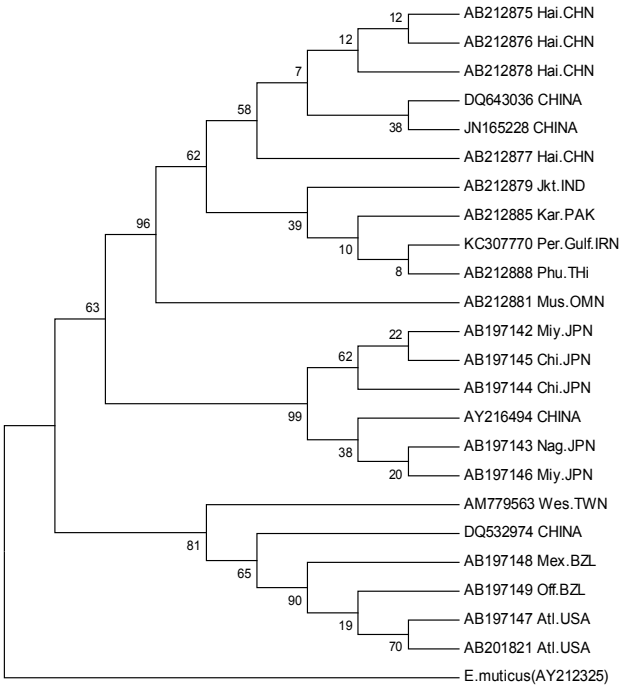

Fig. 5: Maximum parsimony tree based 16S rDNA sequences showing the relationships within *T. lepturus*. Individuals with accession numbers were sequences retrieved from GenBank

Discussion

Mitochondrial DNA analysis provided a good resolution of genetic divergence among the populations. There is an indication of wide scale geographical differentiation between the individuals cached from Indo-Pacific including the Persian Gulf individuals which clustered in one clade compared with the West Atlantic individuals which constructed the other clade with two sister groups and a common ancestor with the Indo-Pacific individuals. However, within the Indo-Pacific individuals, no spatial clustering was observed. In this study, we showed that the varieties of distances among haplotypes of *T. lepturus* were quite high (0.002-0.081), between Pakistan and USA Atlantic individuals. However, those within the Western Atlantic haplotypes alone were only 0.000-0.002. On the other hand, those between Indo-Pacific, Oman Sea (Iran) and Western Atlantic *T. lepturus* were 0.066-0.074 (Table 1).

Previous morphological studies also showed that the Indo-Pacific population of *T. lepturus* differed from the Western Atlantic population (Tokimura *et al*., 1995; Yamada *et al*., 1995; Nakabo, 2002; Kimura and Matsuura, 2003).

Also, the amplification of 16S rDNA resulted in a product of 600 base pairs in all samples analyzed. The aligned sequences resulted in a matrix of 495 unambiguous characters (Fig. 6). The obtained results showed that the isolated strain belongs to *T. lepturus*, indicating 42 divergence sites among the same reported of partial sequences of 16S rDNA gene from the other area (West Atlantic and Indo-Pacific area). Within the major West Atlantic individuals, there were two

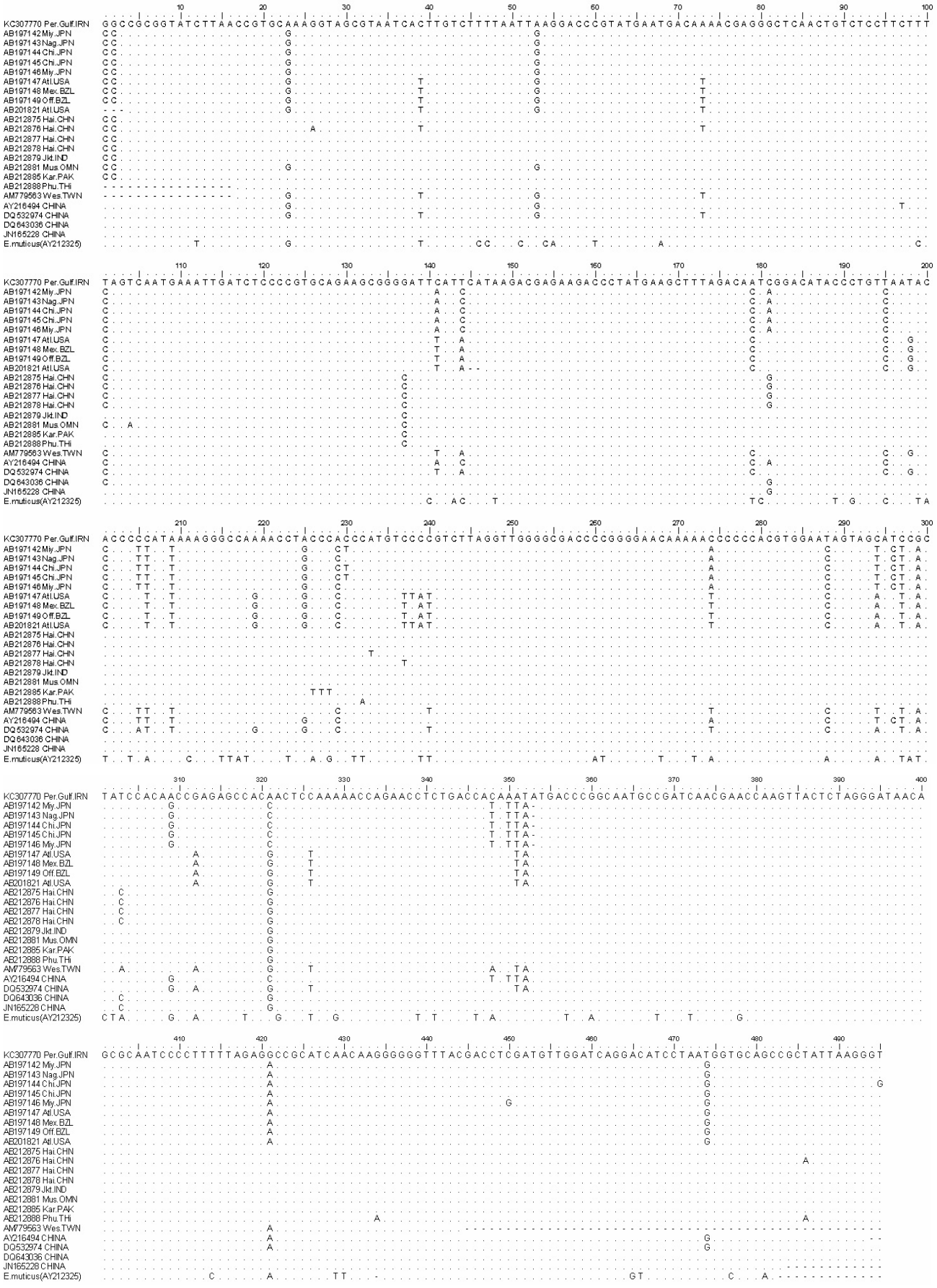

Fig. 6: Aligned sequences of 16S rDNA of 24 taxa including *T. lepturus* from West Atlantic, Indo-Pacific and Persian Gulf. Details of taxa are given in Table 1

Table 1: Pairwise genetic distance based on analysis of aligned 16S rDNA sequences of *T. lepturus* compared to the other individuals reported from the other areas (West Atlantic and Indo-Pacific area) and the outgroup (*E. muticus*), revealed from partial 16S rRNA gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. KC307770 Per.GULF.IRAN																								
2. AB197142 Miy.JPN	0.00																							
3. AB197143 Nag.JPN	0.06	0																						
4. AB197144 Chi.JPN	0.07	0	0																					
5. AB197145 Chi.JPN	0.07	0	0	0																				
6. AB197146 Miy.JPN	0.66	0	0	0	0																			
7. AB197147 Atl.USA	0.07	0.06	0.05	0.06	0.06	0.05																		
8. AB197148 Mex.BZL	0.07	0.05	0.05	0.05	0.05	0.07	0																	
9. AB197149 Off.BZL	0.07	0.05	0.05	0.05	0.05	0.05	0	0																
10. AB201821 Atl.USA	0.07	0.06	0.05	0.06	0.06	0.05	0	0	0															
11. AB212875 Hai.CHN	0.01	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.07	0.08														
12. AB212876 Hai.CHN	0.02	0.08	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.01													
13. AB212877 Hai.CHN	0.02	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0	0.01												
14. AB212878 Hai.CHN	0.02	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0	0.01	0.01											
15. AB212879 Jkt.IND	0.01	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.01	0.02	0.01	0.01										
16. AB212881 Mus.OMN	0.02	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.01	0.02	0.02	0.02	0.01									
17. AB212885 Kar.PAK	0.01	0.08	0.07	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.01	0.02	0.02	0.02	0.01	0.02								
18. AB212888 Phu.THI	0.01	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.08	0.01	0.02	0.01	0.01	0	0.01	0.01							
19. AM779563 Wes.TWN	0.07	0.05	0.04	0.05	0.05	0.04	0.02	0.02	0.02	0.22	0.07	0.07	0.07	0.07	0.07	0.06	0.08	0.07						
20. AY216494 CHINA	0.07	0.01	0	0.01	0.01	0	0.06	0.05	0.05	0.06	0.07	0.08	0.07	0.07	0.07	0.06	0.08	0.07	0.05					
21. DQ643036 CHINA	0.07	0.05	0.04	0.05	0.05	0.04	0.01	0.01	0.01	0.01	0.07	0.07	0.08	0.08	0.07	0.07	0.08	0.07	0.02	0.05				
22. DQ643036 CHINA	0.01	0.07	0.06	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.07	0.07	0.07			
23. JN165228 CHINA	0.01	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.08	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.07	0.07	0.07	0		
24. *E. MUTICUS* (AY212325)	0.19	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.19	0.2	0.2	0.2	0.19	0.2	0.19	0.2	0.19	0

subclusters which distinctly grouped the Brazilian and American individuals from the other samples belonging to Japan and China individuals. Such structuring was not observed within the Persian Gulf and the other Indo-Pacific individuals, suggesting homogeneity between the populations. However, at a narrower geographical scale (including Persian Gulf and Oman Sea) the structuring is not elucidated. This could be due to the small number of investigated individuals and the limited amount of information. However, if the present data achieved is a true reflection of the genetic composition of studied species, then several conclusions can be suggested. The first one is that the Persian Gulf and Oman Sea individuals are homogeneous within the range investigated. Therefore, the most likely description for this study is that the populations share a common gene pool. If this is accurate, the Indo-Pacific populations, especially the Persian Gulf and Oman Sea population could be managed as a single management or evolutionary unit. However, further study needs to be performed to verify the homogeneity of the *T. lepturus* populations. On the other hand, some investigations have been carried out to study the effect of environmental conditions including species composition, currents and oceanographic conditions on the distribution of *T. lepturus* populations (Baik and Park, 1986; Dekun and Cungen, 1987; Martins and Haimovici, 1996; Meriem *et al*., 2011; Raeisi *et al*., 2011). Several currents generated in the Oman Sea influence the movement of broodstocks or larvae towards the Straits of Hormoz and Persian Gulf as well as affecting the gene flow especially between the Oman sae and Persian Gulf (Reynolds, 1993, Fig. 7).

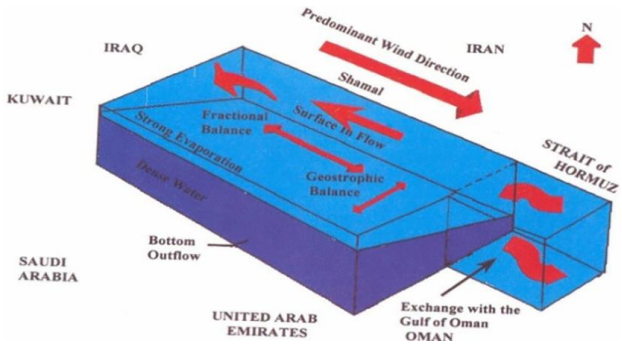

Fig. 7: Marine currents in the Persian Gulf (Reynolds, 1993)

Also mangrove forests are a feature of the intertidal zone of the tropical and subtropical coastlines of the world. They represent a rich and diverse living resource (Duke, 1992) and are made up of a number of different types of habitats. So these forests could be considered as another factor to increase gene flow within the Persian Gulf and the other Indo-Pacific individuals.

This study illustrates the utility of molecular studies as an effective tool for the identification and taxonomy of *T. lepturus* from the natural resources to obtain reliable data.

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References

Allard, MW; Miyamoto, MM; Jareche, L; Kraus, F and Tennant, MR (1992). DNA systematics and evolution of the artiodactyls family Bovidae. Proc. Natl. Acad. Sci. USA., 89: 3972-3976.

Baik, CI and Park, JH (1986). Relationship between oceanographic condition and catch of the hairtail, *Trichiurus lepturus* Linn C from the Stow Net. Bull. Fish Res. Dev. Agency. 39: 29-41.

Bourdy, P; Heurtebise, S and Lapegue, S (2003). Mitochondrial and nuclear DNA sequence variation of presumed *Crassostrea gigas* and *Crassostrea angulata* specimens: a new oyster species in Hong Kong. Aquaculture. 228: 15-25.

Brown, WM; Prager, EM; Wang, A and Wilson, AC (1982). Mitochondrial DNA sequences of primates: tempo and mode of evolution. J. Mol. Evol., 18: 225-239.

Dekun, Z and Cungen, Y (1987). The relation of the environment of fishing ground with the occurrence of hairtail in winter off the middle part of Zhejiang. 1. Fish. China. 11: 195-204.

Duke, NC (1992). Mangrove floristics and biogeography. PP: 63-100. In: Robertson, AI and Alongi, DM (Eds.), *Tropical mangrove ecosystems, coastal and estuarine studies series*. American Geophysical Union, Washington, D.C., P: 329.

FAO (2009). *Fishery statistics yearbook. Catches and landings*. Vol. 91, FAO, Rome. P: 206.

Hall, BG (2004). *Phylogenetics trees made easy: a how-to manual for molecular biologists*, Sunderland, Massachusetts, Sinauer Associates. P: 443.

Kimura, M (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16: 111-120.

Kimura, S and Matsuura, K (2003). *Fishes of Bitung, northern tip of Sulawesi, Indonesia*. Tokyo, Ocean Research Institute, University of Tokyo. P: vi+244.

Lam, K and Morton, B (2003). Mitochondrial DNA and morphological identification of a new species of *Crassostrea* (Bivalvia: Ostreidae) cultured for centuries in the Pearl River Delta, Hong Kong, China. Aquaculture. 228: 1-13.

Martins, AS and Haimovici, M (1996). Distribution, abundance and biological interactions of the cutlassfish *Trichiurus lepturus* in the southern Brazil subtropical convergence ecosystem. Fish. Res., 30: 217-227.

Meriem, SB; Al-Marzouqi, A; Al-Mamry, J and Al-Mazroui, A (2011). Stock assessment and potential management of *Trichiurus lepturus* fisheries in the Arabian Sea, Oman. J. Fish Aquat. Sci., 6: 212-224.

Milinkovitch, MC; Orti, G and Meyer, A (1993). Revised phylogeny of whates suggested by mitochondrial ribosomal DNA sequences. Nature. 361: 346-348.

Miya, M and Nishida, M (1996). Molecular phylogenetic perspective on the evolution of the deep-sea fish genus *Cyclothone* (Stomiiformes: Gonostomatidae). Ichthyol. Res., 43: 375-398.

Nakabo, T (2002). Family Trichiuridae. In: Nakabo, T (Ed.), *Fishes of Japan with pictorial keys to the species*. Tokyo, Tokai University Press. PP: 1344-1345.

Nakamura, I and Parin, NV (1993). FAO species catalogue. Vol. 15. Snake mackerels and cutlassfishes of the world (Families Gempylidae and Trichiuridae). An annotated andillustrated catalogue of the snake mackerels, shocks, escolars, gemfishes, sack fishes, domine, oilfish, cutlassfishes, scabbardfishes, hair-tails, and frostfishes known todate. Fisheries Synopsis No. 125, Vol. 15, FAO, Rome, P: 136.

Raeisi, H; Hosseini, SA; Paighambari, SY; Taghavi, SAA and Davoodi, R (2011). Species composition and depth variation of cutlassfish (*Trichiurus lepturus* L. 1785) trawl by catch in the fishing grounds of Bushehr waters. Persian Gulf. 10: 17610-17619.

Randall, JE (1995). *Coastal fishes of Oman*. Bathurst, New South Wales, Australia, Crawford House Publishing Pty Ltd., P: 439.

Reynolds, RM (1993). Physical oceanography of the Gulf, Strait of Hormuz, and the Gulf of Oman: results from the Mitchell Expedition. Marine Poll. Bull., 27: 35-60.

Sambrook, J; Fritsch, EF and Maniatis, T (1989). *Molecular cloning: a laboratory manual*. 2nd Edn., Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press. P: 1622.

Tamura, K; Dudley, J; Nei, M and Kumar, S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA software). Version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Tokimura, M; Yamada, U and Irie, T (1995). Comments on taxonomy and distributions of the species of *Trichiurus* in the East China and Yellow Seas and adjacent waters. Seikaku Suisan Kenkyusho News. 80: 12-14.

UNEP (United Nations Environment Programme) (1999). *Overview on land-based sources and activities affecting the marine environment in the ROPME Sea area*. UNEP/GPA Coordination Office & ROPME. P: 127.

Yamada, U; Shirai, S; Irie, T; Tokimura, M; Deng, S; Zheng, Y; Li, CS; Kim, YU and Kim, YS (1995). *Names and illustrations of fishes from the East China Sea and the Yellow Sea*. Tokyo, Overseas Fishery Cooperation Foundation. P: xi+288.