

Bigeye tuna (*Thunnus obesus*) in the Indian Ocean are composed of a single panmictic population

Hsin-chieh Chiang^{*1,2}, Chien-chung Hsu¹, Georgiana Cho-Chen Wu², Shui-Kai Chang³ and Hsi-Yuan Yang²

1. Institute of Oceanography, National Taiwan University, Taipei106, Taiwan
2. Institute of Molecular and Cell Biology and Department of Life Science, National Taiwan University, Taipei106, Taiwan
3. Fisheries Agency, Council of Agriculture, Taipei, Taiwan, R.O.C.

Abstract

Population structure of bigeye tuna (*Thunnus obesus*) in the Indian Ocean, Western Pacific Ocean and Eastern Atlantic Ocean were investigated using mitochondrial DNA sequence data. A total of 380 specimens were sampled from four regions in the Indian Ocean (Cocos Islands, Southeastern Indian Ocean, Southwestern Indian Ocean and Seychelles), and one region each from the Atlantic (Guinea) and the Western Pacific Oceans, respectively. The reconstructed neighbor-joining phylogeny based on the first hypervariable region (HVR-1) of the mitochondrial control region sequence data showed that haplotypes from the Indian and the Western Pacific Oceans can be grouped into two clades (Clade I and Clade III) whereas in the Atlantic Ocean, two divergent clades (Clade I and Clade II) coexist. A single stock of bigeye tuna in the Indian Ocean is supported by hierarchical AMOVA tests and pairwise Φ_{ST} analysis. Clade I is the dominant population in the Indian and the Western Pacific Oceans which consists of more than 96% of the specimens and Clade II is a specific group exclusively restricted to the Atlantic Ocean which makes up 77% of its specimens. A new minor Clade, Clade III, which is restricted to the Indian and the Western Pacific Oceans, was recently discovered. However, the extremely small amount of this Clade III within these Oceans deserves extra attention and further investigations to benefit future fishery managements.

Introduction

The bigeye tuna (*Thunnus obesus* Lowe, 1839) is a large pelagic fish widely distributed in tropical and subtropical waters (45°N to 43°S). It is an important commercial species of the world fisheries, and its resources appear to be decreasing due to overfishing in recent years. A better understanding of fish population structure is important to an effective fisheries management. However, our knowledge of the bigeye tuna population structure is still limited (Alvarado-Bremer et al., 1998; Chow et al., 2000; Appleyard et al., 2002; Durand et al., 2005).

In 1998, Alvarado-Bremer et al. examined the mitochondria DNA (mtDNA)

control region of this species from Atlantic, Indian and Pacific Oceans using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay to investigate its population structure. Analyses of frequency distribution of the RFLP haplotypes indicate that while no significant differences were found between the Pacific and Indian Ocean samples, bigeye tuna samples of these waters are genetically distinct from those of the Atlantic Ocean. The existence of two Clades (I and II) of bigeye tuna was found; Clade I exists both in the Atlantic and Indo-Pacific Oceans, and Clade II is mainly limited to the Atlantic Ocean (Alvarado-Bremer et al., 1998). These results were later corroborated by those of PCR-RFLP analyses of two mtDNA segments, the control region and a segment (ATCO) flanking the ATPase and cytochrome oxidase III genes (Chow et al., 2000). It is interesting to note that only two genotypes (α and β) were detected following *RsaI* digestion of the ATCO segment. The α type predominates in the Atlantic samples (i.e., 178 of 244), and all but one of the 195 Indo-Pacific samples are β type. Moreover, bigeye tuna samples of the Indian Ocean were examined for variations at seven microsatellite loci and at the ATCO region by PCR-RFLP analyses (Appleyard et al., 2002). The results indicate that genetic differentiation is non-significant between samples collected from eastern and western Indian Ocean. Recently, the genetic differentiation between the Atlantic and Indo-Pacific mitochondrial lineages was further confirmed by characterization of four nuclear DNA loci (Durand et al., 2005). The results also indicate unidirectional gene flow from Indo-Pacific to Atlantic bigeye tuna populations and their admixture off southern Africa. In 2006, Martínez et al. first used the first hypervariable region (HVR-1) of the mitochondrial control region sequence data to examine the genetic variability of bigeye tuna in the Atlantic Ocean. The results also indicated that two divergent mitochondrial lineages existed in the Atlantic bigeye tuna and suggested present unidirectional gene flow from the Indo-Pacific into the Atlantic Ocean (Martínez et al. 2006). Furthermore, sequence data analysis revealed no existence of Clade II in both the Eastern Pacific Ocean and the Seychelles of the Indian Ocean. Recently, Chiang et al. investigated population structure of bigeye tuna in the Western Pacific Ocean, including the South China Sea, the Philippine Sea and the Western Pacific Ocean using mitochondrial HVR-1 sequencing analysis. The results showed that bigeye tuna over the Western Pacific Ocean constitutes a single panmictic population (Chiang et al. 2006).

According to the results of PCR-RFLP analysis, the particular clade of Atlantic Ocean (Clade II, Alvarado-Bremer et al. 1998; α type, Chow et al. 2000; BET1 haplotype, Appleyard et al. 2002) included few individuals from the Indian Ocean. However, while Martínez et al. (2006) conducted the PCR-sequencing analysis of the Atlantic bigeye tuna population, none of the individuals from the small sample they

collected from Indian Ocean belonged to the major Atlantic clade (Clade II, Martínez et al. 2006). Due to dissimilar outcomes between the bigeye tuna population structure in Indian Ocean derived by PCR-RFLP and PCR-sequencing analyses and putting into consideration that PCR sequencing seems to be the more effective among the two approaches available, we used this procedure in this study to analyze the bigeye tuna's population genetic structure of the whole Indian Ocean as it has not been conducted previously.

In this study, we sampled 380 bigeye tuna specimens from the Indian, Western Pacific and Atlantic Oceans to further investigate and confirm whether any of the bigeye tuna from the Indian Ocean is found in the major Atlantic clade.

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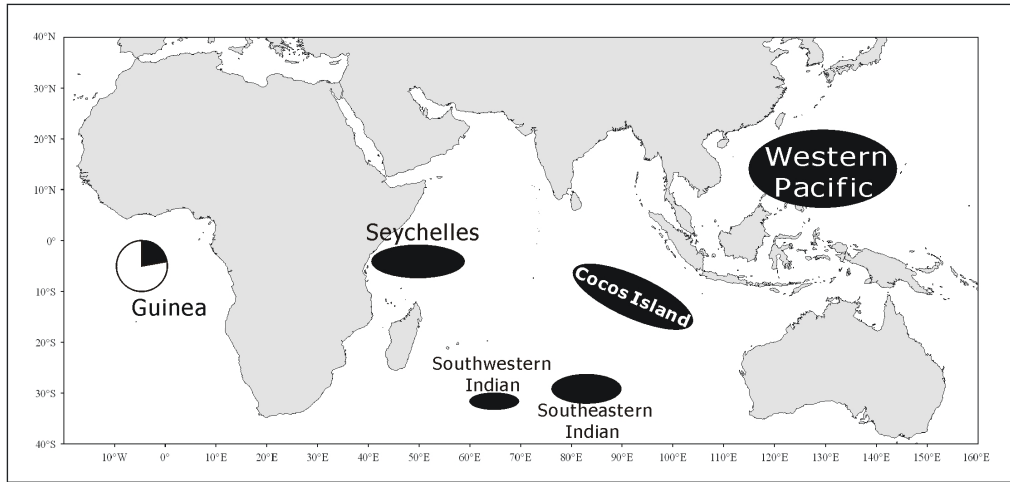


Figure 1. Map showing the bigeye tuna sampling areas under study.

Table 1. Descriptive statistics for the studied *T. obesus* samples.

Population	Location	Date	<i>n</i>	H	S	<i>Hd</i>	<i>k</i>	π	θ
Western Pacific Ocean	115°~144°E; 7°~22°N	May 2000 to Jan 2003	100	96	104	0.999	15.4	0.043	21.4
Cocos Island	80°~104°E; 4°~18°S	Aug 2004	24	23	62	0.996	15.3	0.043	16.6
SE Indian Ocean	76°~90°E; 27°~32°S	Aug 2004	32	31	80	0.998	15.4	0.044	20.1
SW Indian Ocean	60°~70°E; 30°~33°S	Aug 2005	56	48	90	0.998	15.5	0.043	20.9
Seychelles	41°~60°E; 1°~6°S	Jun 2006	111	101	98	0.998	15.4	0.0431	19.9
Guinea	0°~10°W; 0°~10°S	May-Aug 2003	57	56	86	0.999	22.1	0.062	19.3

N, sample size; H, number of haplotypes ; *S*, number of polymorphic sites; *Hd*, haplotypic diversity (Nei, 1987) ; *k*, mean pairwise nucleotide differences (Tajima, 1983) ; π , nucleotide diversity (Nei, 1987) ; θ , expected heterozygosity per site (Watterson, 1975)

Table 2. Genetic structuring of bigeye tuna populations based on mitochondrial control region sequence data

Structure tested	Observed partition		Φ statistics	<i>p</i>
	Variance	% total		
<i>1. One gene pool (Cocos Island, Southeastern Indian Ocean, Southwestern Indian Ocean , Seychelles, Western Pacific and Guinea)</i>				
Among populations	2.12	20.14	$\Phi_{ST}=0.20$	<0.001
Within populations	8.40	79.86		
<i>2. One gene pool Clade I (Cocos Island, Southeastern Indian Ocean, Southwestern Indian Ocean, Seychelles, Western Pacific and Guinea)</i>				
Among populations	0.04	0.49	$\Phi_{ST}=0.005$	0.06
Within populations	7.90	99.51		
<i>3. One gene pool Indian(Cocos Island, Southeastern Indian Ocean, Southwestern Indian Ocean, and Seychelles)</i>				
Among populations	0.05	0.68	$\Phi_{ST}=0.007$	0.06
Within populations	7.80	99.32		

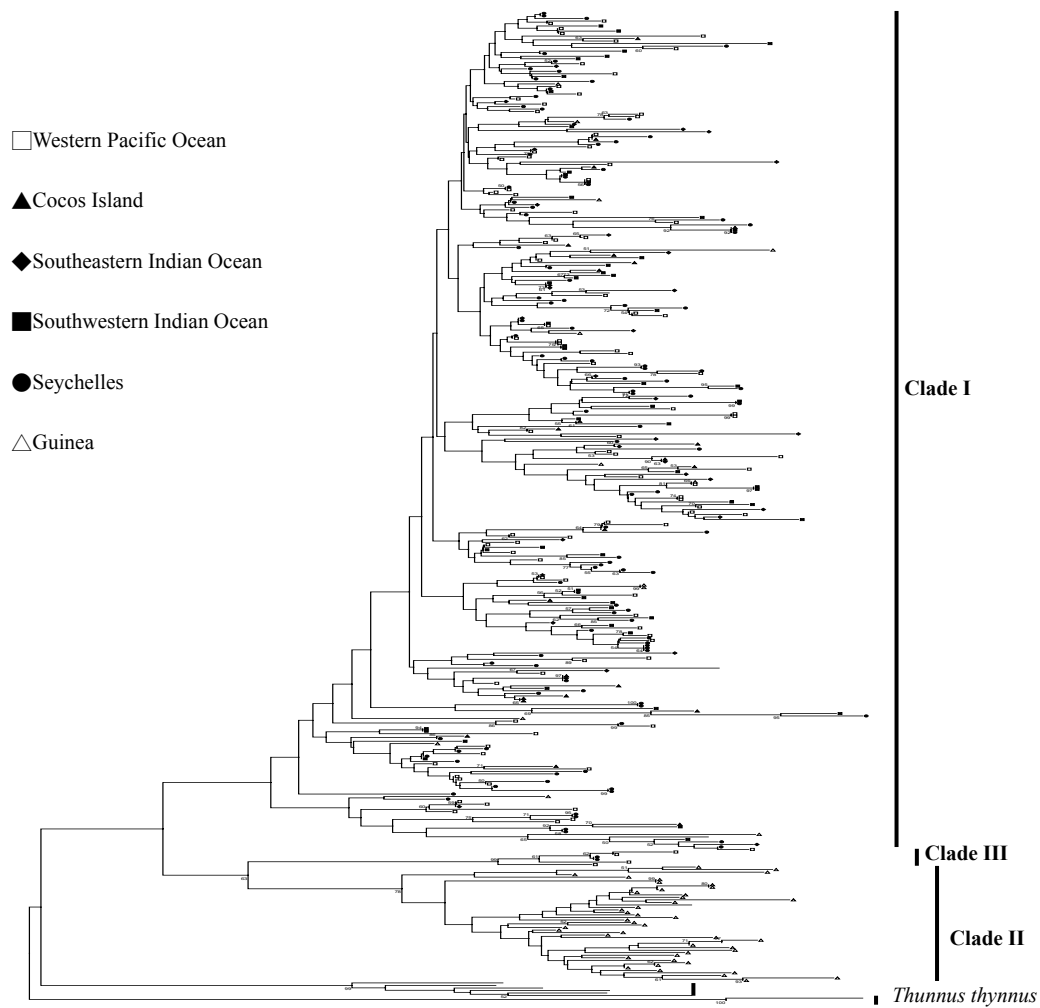


Figure 2. Neighbor-joining tree estimated with the Tamura and Nei model among mtDNA lineages of bigeye tuna. Haplotypes collected from the Western Pacific, Cocos Island, Southeastern Indian, Southwestern Indian, Seychelles and Guinea are shown as white squares, black triangles, black rhombus, black squares, black circles and white triangles, respectively. Numbers at nodes indicate the bootstrap values. Only values >50% are shown.

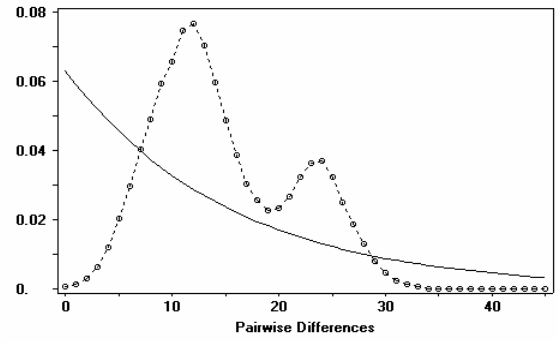
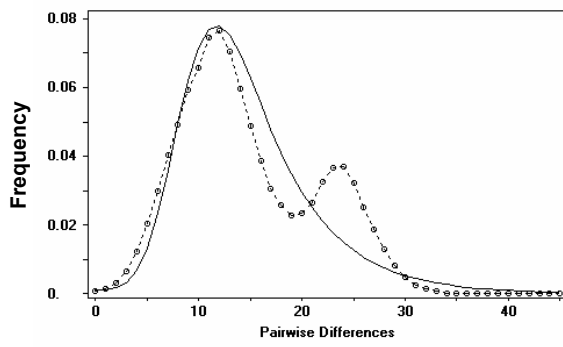
Table 3. Matrix of pairwise Φ_{ST} (below diagonal) values and associated p values (above diagonal) among *T. obesus* phylogroups (Clade I and II) based on mitochondrial control region sequence data

Sampling area	Western Pacific Ocean	Cocos Island	SE Indian Ocean	SW Indian Ocean	Seychelles	Guinea (I / II)
Western Pacific Ocean	—	0.31	0.25	0.15	0.73	0.12/<0.001
Cocos Island	0.002	—	0.07	0.15	0.31	0.17/<0.001
SE Indian Ocean	0.003	0.016	—	0.34	0.03	0.32/<0.001
SW Indian Ocean	0.004	0.009	0.002	—	0.17	0.06/<0.001
Seychelles	0.002	0.003	0.015	0.004	—	0.08/<0.001
Guinea (I / II)	0.017/ 0.586*	0.015/ 0.601*	0.005/ 0.615*	0.026/ 0.609*	0.021/ 0.590*	<0.001

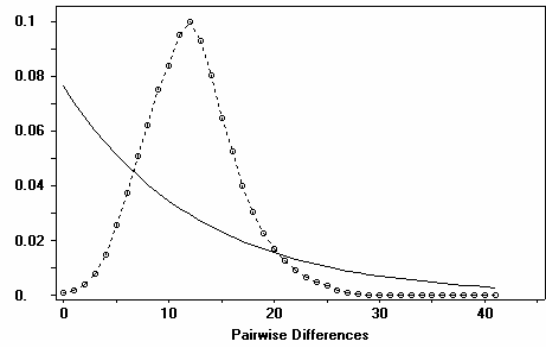
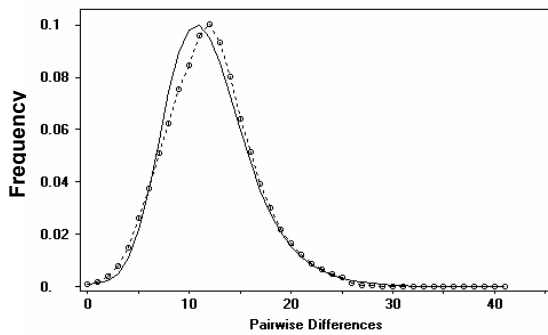
Table 4. Statistical tests of neutrality, and demographic parameters estimates for *T. obesus* entire mitochondrial control region data set, and phylogroups (Clade I and II)

	All samples	Clade I	Clade II	Clade III
<i>Goodness of fit tests</i>				
Tajima D	-1.37	-1.61	-1.04	-0.33
Fu's F_S	-634.18*	-555.47*	-33.48*	0.12
<i>Demographic parameters</i>				
H_{ri}	19.910	0.0009	0.0050	0.4444
SSD	0.0014	0.0007	0.0013	0.1975
S	142	141	64	15
θ_0	5.331	2.655	2.372	1.757
θ_1	166.953	96.797	141.309	4682.5
τ	9.489	9.425	8.768	3.043

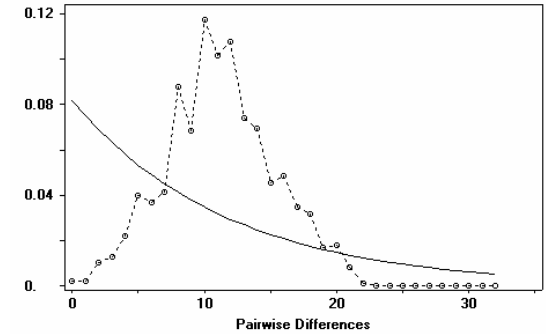
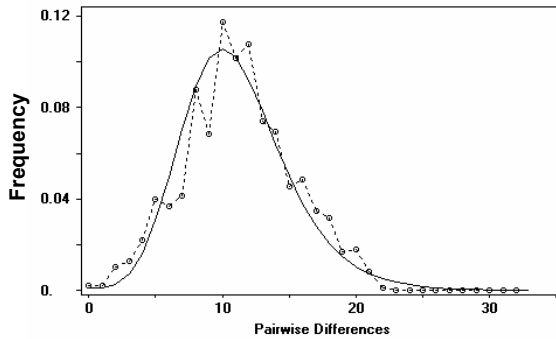
Entire data set (n=380)



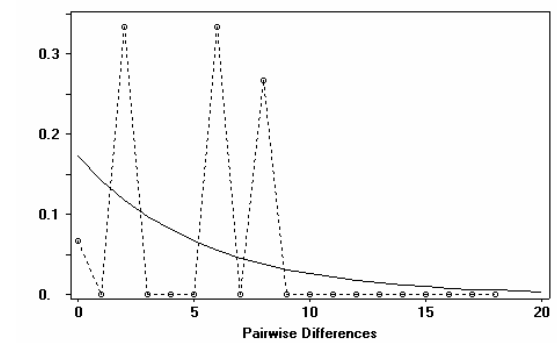
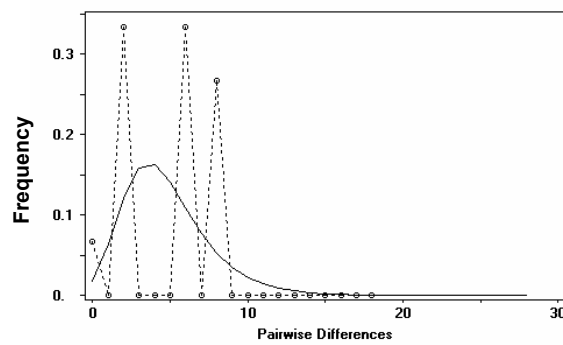
Clade I (n=336)



Clade II (n=44)



Clade III (n=6)



— Growth-decline population model
 --- Observed

— Constant population model
 --- Observed

Figure 3. Observed, growth-decline model, and constant population model mismatch distributions for all pairwise combinations of: the entire mitochondrial control region data set, 380 individuals; Clade I, 330 individuals; Clade II, 44 individuals.