

The logo for the Chilean Jack Mackerel Workshop is a dark blue rectangular box with rounded corners. Inside the box, the text "Chilean Jack Mackerel Workshop" is written in a white, sans-serif font, centered and arranged in two lines.

Genetic variation on mtDNA Cytb sequence of three populations of Chilean jack mackerel, *Trachurus murphyi* from the Southern Pacific

Min ZHANG • Yong-jiu XU • Cheng-hui WANG

Min Zhang(✉)¹· Yongjiu Xu¹· Chenghui Wang²

E-mail: mzhang@shfu.edu.cn

(1. College of Marine Science and Technology, Shanghai Fisheries University, Shanghai 200090, China)

(2. College of Aqua-life Science, Shanghai Fisheries University, Shanghai 200090, China)

Abstract: Mitochondrial cytb gene sequences of Chilean Jack mackerel (*Trachurus murphyi*) with 730bp were examined, the samples were collected from the Southeast Pacific beyond Chile's EEZ in 2006 (OEZ2006) and 2007 (OEZ2007), and collected inside of Chile's EEZ in 2006 (IEZ2006). The observation shown that 11 haplotypes were found in 39 analyzed samples and only one haplotype was shared among three populations, which has 23 polymorphic sites in analyzed 730 bp sequence. 3 and 22 polymorphic sites were detected in OEZ2006 and OEZ2007 respectively, and no sequence variation was found in IEZ2006. The haplotype diversity ratios were 0.3309, 0.6405 and 0.0000, and nucleotide diversity ratios were 0.0005, 0.0043 and 0.0000 in OEZ2006, OEZ2007 and IEZ2006 respectively. The results from AMOVA analysis indicated that no significant genetic divergence among three populations. NJ tree also showed that all individuals from three populations clustered into one clade. It might be concluded that the three fish stocks of Chilean Jack mackerel inside and outside of EEZ in the Southern Pacific could be one population.

Keywords: Chilean jack mackerel; EEZ; Mitochondrial Cytb gene; Southern Pacific.

Introduction

Chilean jack mackerel (*Trachurus murphyi*) belongs to *Chordata*, *Osteichthyes*, *Perciformes*, *Carangidae*, *Trachurus*, which inhabits in the Southern Pacific Ocean as a highly migratory pelagic species. Researchers have centered majority efforts on the ecological and biological studies of the species, nevertheless, the taxonomic and phylogenetic relationships within this taxon remain controversial (Zhang Min et al.,2005; Arcos, D.F, et al.,2001; Miao Shengci,2000; Ben Salem,1995). Russian scientists, based on their surveys in 1980's, distinguished two sub-species existing in the Southern Pacific ocean (Tang Xiaoman). The molecular markers and DNA sequencing have been taken as good tools to classify the taxonomy and phylogenetic relationships of species. Mitochondrial DNA (mtDNA) is the genetic material that exists outside the nucleus in eukaryotic cells. Cyt *b* gene has a moderate evolutionary rate and a clear evolutionary pattern that is suitable for the studies on the phylogenetic evolution at the intra- and inter-specific levels (Astorga, M., 1998; Barraclough, T.G. Nee, S., 2001). Thus the analyses of genetic structure and genetic taxonomic status have been taken as the fundamental measures to recognize the genetic differentiation between populations for facilitating the fisheries resources conservation and management.

According to the cytb sequence analysis on the three populations of jack mackerel (totally 46 specimens) sampled beyond the pacific ocean outside Chile in Nov. 2006 and 2007, respectively. This study discussed genetic variation of the three jack mackerel populations beyond Chile, the aims was to support the sustainable exploitation of the jack mackerel resources.

1. Materials and methods

1.1. Sampling

46 samples of Chilean jack mackerel were collected as three groups. The first group with 22 samples and the second groups with 20 samples were collected by the fishing vessel KaiLi and KaiXin of Shanghai KaiChuang Deed-sea Fisheries Com.Ltd. outside EEZ(Exclusive Economic Zone) in November of 2006 and 2007(Tab.1), marked as OEZ2006 and OEZ2007 respectively.

The third group of 4 samples was bought in the Chile coast water beyond Valparaiso port(Chile) in November of 2006 which marked as IEZ2006. The samples of OEZ2006 and OEZ2007 were stored at -18°C and brought to the laboratory in Shanghai Fisheries University. The samples of IEZ2006 were kept in vacuum situation and sent to SFU laboratory. The weight of samples ranged from 200g to 300g, with the flock length from 20 to 30cm. The tail fins were removed and preserved in 95% ethanol and stored at -20° C for the DNA examination.

Tab1.Geographical origin and GeneBank Accession No. for mt DNA gene sequences of *Trachurus* species sequenced in this study

Species	Sampling area		Locality	GeneBank
	Latitude	Longitude		Accession No
				Cytb
<i>T.murphyi</i> OEZ2006	32°30'00"S~34°30'00"S	94°30'00"~95°30'00"W	fishing ground	-
<i>T.murphyi</i> OEZ2007	34°30'00"S~35°30'00"S	92°30'00"~94°30'00"W	fishing ground	AY526538
<i>T.murphyi</i> IEZ2006	33°02'00"S	71°39'00"W	Valparaiso port	-

1.2. DNA extraction, PCR amplification and sequencing

The total genomic DNA from the fins was extracted using a proteinase K and phenol-chloroform procedure. The quantity and quality of the extracted DNA were estimated on 1% agarose gels stained with ethidium bromide (EB). The polymerase chain reaction (PCR) was used to amplify the mitochondrial part Cytb fragment. The primers for the part Cytb fragment were as follows: Cytb-F : 5'-TCC GTA AA(G)A CCC ACC CCA T-3' and Cytb-R : 5'-AAC TGG TAT GCC GCC AAT TC-3'. These primers were designed from the reported mtDNA sequences of the *Trachurus* (Leyla Cárdenas et al., 2005; GenBank Accession No. AY526539). PCR was performed on an Eppendorf Thermal Cycler in a reaction mixture of 50µl containing 2 units Taq DNA polymerase (2µL) (Tiangen products, China), 5µl PCR buffer solution (Tiangen products, China), 2µl template DNA (50ng/µl), 2µl dNTP (0.1 mmol·L⁻¹ each), 4µl primers (0.2 µM each),

and 35µl distilled water. The amplification conditions were 94°C for 5 min. This was followed by 30 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, and a final extension was done at 72°C for 5 min. The verification of successful PCR amplification was assessed by agarose gel electrophoresis. The PCR products of the part Cytb were purified and directly sequenced using the PCR primers. All specimens were sequenced on an Applied Biosystems ABI 3730 DNA sequencer.

1.3. Sequences alignment and data analyses

The DNA sequences were processed by means of the BioEdit software (Hall, 1998), aligned by CLUSTAL W version 1.83 (Thompson, 1994), and checked via ocular inspection. The aligned sequences were used to analyze the population structure and genetic variation by using Arlequin version 3.01 (Excoffier et al. 2005). The genetic diversity was obtained by estimating nucleotide diversity (π) and Haplotype diversity (h) for the mtDNA using Tajima's (1983) and Nei's (1987) methods. The pairwise fixation index (F_{ST}) was employed to test the genetic differentiation between populations. The phylogenetic tree was constructed by the neighboring-joining method using MEGA 4 software (reference). The analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented in Arlequin, was used to assess the population structure of the *Trachurus murphyi*. The neutrality tests of Tajima's D (Tajima, 1989), Fu and Li's D (Fu and Li, 1993) on the total number of segregating sites were performed in Arlequin version 3.01.

2. Results

2.1. Sequence variation

The totally 46 individuals from three populations were collected, of which 39 specimens 'genomic DNA were sequenced for the mtDNA Cytb genes. 17 samples from group OEZ2006, 18 from OEZ2007 and 4 from IEZ2006. The unambiguous lengths of the Cytb segment were 730 bp. The PCR amplified profiles of partial individuals are shown in Fig.1. The Cytb

fragment from OEZ2006 population contained 3 variable sites and 22 variable sites are found in OEZ2007 group, while none variable sites were found in IEZ2006 population.

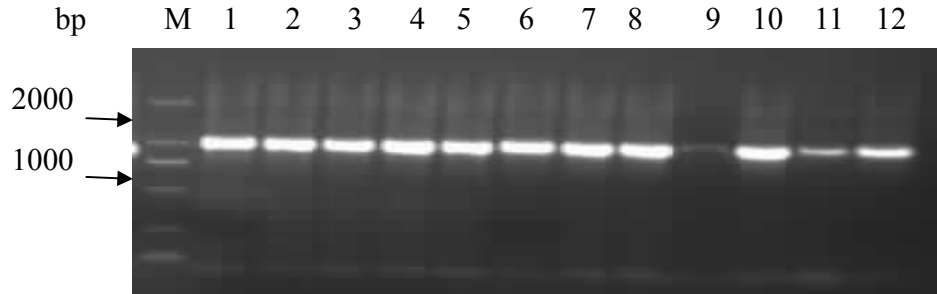


Fig. 1 PCR amplification results of mitochondrial DNA *Cytb* gene in some individuals

Table 2 Haplotype and nucleotide diversity, neutrality tests in three populations of *Trachurus murphyi*
(mean \pm SD)

Populations	Haplotype diversity(<i>h</i>)	Nucleotide diversity(π)	Tajima's <i>D</i>	Fu's <i>test</i>
OEZ2006	0.3309 \pm 0.1426	0.0005 \pm 0.005	-1.7057*	-1.7057*
OEZ2007	0.6405 \pm 0.1300	0.0043 \pm 0.0026	-2.3761**	-2.3761**
IEZ2006	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.0000	0.0000

2.2. Population structure and genetic diversity

A total of 11 haplotypes were detected in all individuals, and 4 haplotypes were found in OEZ2006, 8 haplotypes in OEZ2007 and 1 haplotypes in OEZ2006. Only one haplotype (H1) was shared in the three populations. It was clear that the haplotype H1 was both the most widespread (all populations) and the most common in the *Cytb* sequence (Table 1).

The genetic diversity information in the mtDNA sequences of all populations were given in Table 2. The haplotype diversity(*h*) and nucleotide diversity(π) in the OEZ2007 population was the highest, with the value 0.6405 and 0.0043, respectively, while the haplotype diversity (*h*) and

nucleotide diversity (π) in the IEZ2006 were zero because of no variable sites in it (Table 2). The neutrality test from Tajima's D and Fu's test showed significant findings from the OEZ2006 and OEZ2007 populations, indicating recent population expansion would had been occurred in them.

Tab.1 Absolute frequency of haplotypes from three populations of *Trachurus murphyi*

Haplotypes	OEZ2006	OEZ2007	IEZ2006
Hap-1	0.824	0.611	1.000
Hap-2	0.0588		
Hap-3	0.0588		
Hap-4	0.0588		
Hap-5		0.0556	
Hap-6		0.0556	
Hap-7		0.0556	
Hap-8		0.0556	
Hap-9		0.0556	
Hap-10		0.0556	
Hap-11		0.0556	

2.3. Geographic differentiation

The results of Analysis of Molecular Variation (AMOVA) (Excoffier *et al.*(1992)) revealed that 102.42% of the variance was from intra-populations. The variance of intera-populations and inter-dividuals affected the total variance of jack mackerel significantly (Table 3). The genetic differentiation among the three populations was not significant. The pairwise (OEZ2006-OEZ2007, OEZ2006-IEZ2006, OEZ2007-IEZ2006) F_{ST} values between all populations were 0.0117, -0.1357 and -0.1241, indicating that most of the pairwise comparisons were not significant (Table 3 and Table 4). The IEZ2006 population had the largest F_{ST} values within population as compared to the other populations.

Table 3 AMOVA analysis of mtDNA Cytb sequences in three populations**of *Trachurus murphyi***

sources of variation	df	sum of squares	variance components	percentage of variance	global divergence(Φ_{st})
Among populations	2	1.206	-0.0196	-2.42	-0.0242
Within populations	36	29.708	0.8269	102.42	
Total	38	30.974	0.8073	100%	

The genetic distance in pairwise comparison among the three populations (OEZ2006-OEZ2007, OEZ2006-IEZ2006 and OEZ2007-IEZ2006) were 0.0019, 0.0002, 0.0017, respectively, indicating the genetic distance between OEZ2006-OEZ2007 was the highest. Within populations, the genetic distance from populations OEZ2006, OEZ2007 and IEZ2006 were 0.0005, 0.0034 and 0.0000, respectively.

Tab.4 Genetic distance (below diagnose) and F_{st} value (above diagnose)**between populations**

	OEZ2006	OEZ2007	IEZ2006
OEZ2006		0.0117	-0.1357
OEZ2007	0.0019		-0.1241
IEZ2006	0.0002	0.0017	

2.4. Evolutionary relationships

Using Chilean jack mackerel (*Trachurus murphyi*) (GenBank Accession No. AY526539) as an outgroup and by means of the Neighbour-Joining (NJ) method, all sequences consistently displayed that all haplotypes or specimens from the three populations were mixed with one another and were clustered into one big group (Fig.2). Only one specimen from OEZ2006

(remarked as 2006(10)) was differentiate from of the cluster. This indicated that most specimens from the outside of EEZ were closer to coastal specimens than to the other from the outside EEZ specimens.

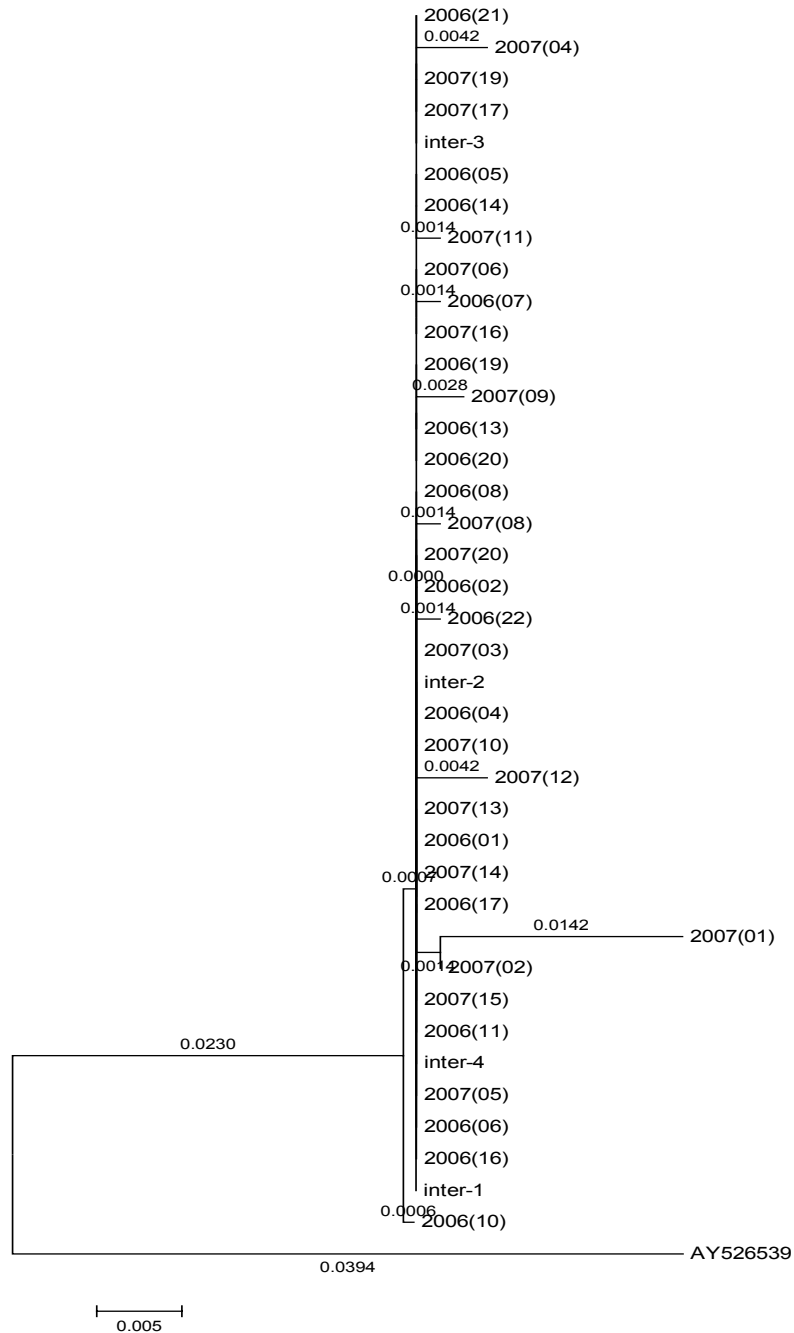


Fig 2 Phylogenetic tree among individuals of *Trachurus murphyi* based on NJ method

2006: OEZ2006; 2007: OEZ2007; inter: IEZ2006;

Discussion

Genetic variability and structure

This population would be affected by founder effects or genetic bottle-necks, it would be resulted from small samples. There is a preliminary view that, genetic diversity is lower in coastal populations than in deep-sea ones. In this study, the genetic variability, expressed as haplotype and nucleotide diversity, in the deep-sea populations is higher than that in the coastal populations. One reason might be the intensive fishing effort and aquaculture in offshore water of Chile, which might have impact on the diversity. The other reason would be the small sample chosen. The IEZ2006 population only contained 4 samples in the offshore Chile, and obviously the genetic diversity of the group is extremely lower, considering the quantity. For this reason, it was not excluded that the collected samples of IEZ2006 population were the off-spring of parent from the same population migrating in the outer-sea, which makes the gene sequence the same. It's essential to collect more samples to emphasize on the further research about the genetic relationship in the future.

Genetic differentiation

As to the taxonomy status of Chilean jack mackerel, studies have been conducted on genetic relationship in *Trachurus*. Russian scientists, proposed that in the high seas, there existed two subspecies, including coastal subspecies and the oceanic subspecies. The two subspecies communities have mutual connection, but the degree of interaction between them was still unknown (Tang Xiaoman, 1989). This is in agreement with the results of the work of Evseenko et al. However, Serra, J.R. et al pointed out that Chilean jack mackerel was a single self-sustained population, which includes the oceanic fraction off central-south Chile, exhibiting a strong seasonal migration pattern (Serra et al. 1991, Sergio et al. 2004), showing an offshore migration towards the reproductive oceanic habitat in early spring, extending along the southeastern Pacific and an onshore migration during the summer related to coastal food availability. The preliminary research showed that, Chilean jack mackerel is highly migrated. The populations distributed

inside and outside the EEZ are mutually interacted each other (Zhang Min et al., 2005). The complicated distributing construction of Chilean jack mackerel was influenced by the effects of region frontal zone and ocean current (Evseenko, S.A. et al., 1987; Xu Yongjiu, Zhang Min., 2007). Using isozyme markers, Stepien & Rosenblatt (1996) found little genetic divergence between populations of jack mackerel from the Pacific along North and South America coasts, concluding that the taxa belonged to the same species. Leyla Cardenas., et al (2005) addressed phylogenetic relationships in the genus *Trachurus* using cytochrome b gene and D-loop sequences, and distinguished five groups in *Trachurus*. Through research, he found no significant differentiation between the populations from New Zealand and Chile coastal waters. The New Zealand (*Trachurus murphyi* GeneBank Accession No.AY526539) population and two Chile populations (*Trachurus murphyi* GeneBank Accession No.AY526537 and AY526538) belong to one group.

This study revealed that populations outside and inside the EEZ didn't form obvious bunch groups according to the spatial distribution, and they are extremely possible to be the identical communities from the same population.

Acknowledgements

The authors would like to thank our colleagues from Key Laboratory of Aquatic Genetic Resources and Aquaculture Ecosystem, Ministry of Agriculture for their advices. It is also appreciated that graduate students Song Xiao et al. in the lab help us the DNA extraction, the PCR experiment. We also thank the chief captain of the fishing fleet from Shanghai KaiChuang Deep-sea Fisheries Com.Ltd. for their assistances and data collection during the experiment. This work was supported by Ministry of Agriculture funds of China under program of Fishery Exploration in High seas in 2006 and Shanghai Leading Academic Discipline Project (Project No.T1101)

References

- Arcos, D.F., L. Cubillos & S. Núñez. The jack mackerel fishery and El Niño 1997-1998 effects off Chile. *Progress and Oceanography*, 2001,49:597-617.
- Astorga, M., Galleguillos, R., Genetic divergence of jack mackerel of genus *Trachurus* from northwestern and southeastern Pacific. *Rev. Biol. Mar. Oceanogr.* 1998,33,155-161.
- Barracough, T.G.Nee, S. Phylogenetic and speciation. *Trends Ecol. Evol.* 2001, 16, 391-399.
- Evseenko, S.A. On the reproduction of the Peruvian jack mackerel, *Trachurus symmetricus murphyi* (Nichols), in the southern part of the Pacific Ocean. *Voprosy Ikhtiologii*, 1987,27(2):264-273.
- Hall TA.. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acid Symp Ser*, 1998, 41: 95–98.
- Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment[J]. *Briefings in Bioinformatics*, 2004, 5:150-163.
- Leyla Cardenas,Cristian E. Hernandez, Elie Poulin, Antonios Magoulas,Irv KornWeld,F.Patricio Ojeda., Origin, diversification, and historical biogeography of the genus *Trachurus* (Perciforms: Carangidae).*Molecular Phylogenetics and Evolution.* 2005,35,496-507.
- Miao Shengci. Analysis on the availability for the exploratory fishing of Chilean jack mackerel (*Trachurus murphyi*) in the open sea of the southeast Pacific. *Deep-sea Fisheries*, 2000,(3) :19-26.

- Ramos-Onsins SE, Rozas J. Statistical Properties of New Neutrality Tests Against Population Growth. *Mol Biol Evol*, 2002,19: 2092–2100.
- Schneider S, Roessli D, Excoffier L. Arlequin., A software for population genetics data analysis. Vers.3.100. Genetics and Biometry Lab, Dept. of Anthropology, Univ. of Geneva. 2005:26-103.
- Serra, J.R., Important life history aspects of the Chilean jack mackerel, *Trachurus symmetricus murphyi*. *Investigación Pesquera (Chile)* .1991, 36, 67-83.
- Stepien,C.A. & Rosenblatt,R.H., Genetic divergence in antitropical pelagic marine fishes (*Trachurus*, *Merluccius* ,and *Scomber*) between North and south America. *Copeia* 1996,586-598.
- Tang Xiaoman. The fisheries resources and status of jack mackerel (*Trachurus*) in Pacific Ocean. *Deep-sea Fisheries [J]*.1989,2.65-69.
- Tajima F. Evolutionary relationship of DNA sequences in finite populations [J]. *Genetics*, 1983, 105:437-460.
- Thompson J D, Higgins D G, Gibson T J. CLUSTAL W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice [J]. *Nucl Acids Res*, 1994, 22:4673-4680.
- Xu Yongjiu, Zhang Min. Distribution of plankton on *Trachurus murphyi* fishing grounds of the Southeast Pacific and its relationship with fishing grounds. *Marine Fisheries [J]*. 2007,

29(4):289-295.

Zhang Min, Zou Xiaorong, Ji Xinghui et al. Discussion on exploratory fishing of Chilean jack mackerel (*Trachurus murphyi*) in the open sea of the southeast Pacific and prospect of its commercial exploitation. Journal of Fisheries of China [J]. 2005, 29(3):386-391.