[UCI]I804:21028-200000011882

Thesis for Master Degree

Population genetic variation of rock bream (*Oplegnathus fasciatus* Temminick & Schlegel, 1884) revealed by mtDNA (COI) sequence in Korea and China

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October 2017

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본 논문을 박현석의 이학석사 학위논문으로 인준함

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2017 년 10 월 30 일

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Abstract

The rock bream, *Oplegnathus fasciatus*, is a common rocky reef game fish in East Asia and recently becomes an aquaculture species. Although being commercial and economically important species, the population genetic structure remains poorly understood. In the current study, one hundred and sixty-three specimens have been collected from 6 localities along the coastal waters of Korea and China including Kangnung (KN; East Sea), Goseong (GS; Korea Strait), Heuksando (HS; Yellow Sea), Ieodo (ID; Ieodo Ocean Research Station of Korea), Jiaonan (JN; Yellow Sea), and Zhoushan (ZS; East China Sea near China/Taiwan) to analyze population genetic variation of the species using mitochondrial DNA COI sequences. A total of 34 polymorphic sites were detected and 30 haplotypes were defined. The genetic diversity of *O. fasciatus* shows a pattern of low level of nucleotide diversity (0.04±0.003) and high level of haplotype diversity (0.83±0.02). The individuals from Zhoushan (ZS) in the East China Sea near China/Taiwan presented the highest genetic diversity in terms of nucleotide diversity (0.009±0.005), haplotype diversity (0.81±0.06), and average pairwise nucleotide differences (5.65±2.79) among the six groups. Two major genealogical clades were identified in the 30 haplotypes through Bayesian and statistical parsimony analyses, and the net genetic distance between two clades were 0.018±0.004 nucleotide difference per site. The analyses of the AMOVA, *Fst*, and the exact *p* test yielded a significant population differentiation between the Zhoushan group and the rest of the populations. Neutrality tests and mismatch distribution analyses indicated recent population expansion of the species inhabiting the coastal waters of

Korea and China approximately 133-183 kyr before present. The results suggest that the East Asian rock bream was divided into the north and south populations having the mouth of the Yangtze river as a boundary and has experienced demographic expansion at the late Pleistocene. Such population genetic structure needs to be considered in the establishment of the stock management strategies for this fisheries species.

KEYWORDS: mitochondrial COI; *Oplegnathus fasciatus*; genetic diversity; population structure; East Asia

미토콘드리아 COI 분자마커를 이용한 한국과 중국 돌돔의 군집구조 및 유동관계 확립

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요약

돌돔(영명, rock bream; 학명, *Oplegnathus fasciatus*)은 동아시아 온대해역의 암초지대에 광범위하게 서식한다. 이 종은 주로 낚시 물고기로 알려져 있으며 최근에 양식에 대한관심이 증가되고 또 이루어지고 있다. 경제적 그리고 상업적으로 중요한 종임에도 불구하고 집단 유전학적 구조에 대한 이해가 부족하다. 본 연구에서는 집단 유전학적 구조를 연구하기 위하여 강릉 (KN; 동해), 고성 (GS; 한국해협), 흑산도 (HS; 황해), 이어도 (ID; 한국의 이어도 해양과학기지), 지아오난 (JN; 황해), 그리고 조유샨 (ZS; 중국과 대만 근처의 동중국해)를 포함한 6개의 한국과 중국 연안에서 160개의 표본이 수집되었다. 연구에 사용된 유전자 마커는 미토콘드리아 DNA의 COI (Cytochrome C Oxidase I) 부위였다. 총 34개 부위에서 유전자 변형이 일어났으며 30개의 하플로타입이 정의 되었다. *O. faciatus*의 유전적 다양성을 측정한 결과 낮은 수준의 뉴클레오티드 다양성 (0.04±0.003)과 높은 수준의 하플로타입 다양성 (0.91±0.005)을 보였다. 채집된 6개 지역 중 중국과 대만 근처 연안의 조우샨 (ZS)에서 유래된 군집들은 뉴클리오티드 다양성 (0.0009±0.005), 하플로타입 다양성 (0.81±0.06), 그리고 평균 페어 와이즈 뉴클레오티드 다양성 (5.65±2.79)의 가장 높은 수치를 기록했다. Bayesian과 통계학 적 분석을 통하여 30개의 하플로타입에서 두 개의 주요 계통 클레이드가 확인되었으며 두 클레이드 간의 합산된 유전적 거리는 각 사이트 당 0.018±0.004 뉴클리오티드 차이를 나타내었다. AMOVA (Analysis of Molecular Variance), *Fst* (군집 내 변이율), 그리고 exact *p* 테스트는 조우샨 군집과 나머지 군집들 간에 significant한 군집 차별화를 보여주었다. Neutrality 테스트와 mismatch distribution을 분석한 결과 약 133,000-183,000년 전에 한국과 중국 연안 해역에 서식하는 돌돔 종의 recent population expansion이 일어났을 것이라고 추정된다. 본 연구의 결과에 의하면 돌돔은 양자강의 돌출부를

경계로 남쪽의 조우샨 지역의 군집과 북쪽의 나머지 지역들의 군집들로 나뉘어져있으며 홍적세 후기에 집단 팽창이 일어난 것으로 예측된다. 본 연구를 통하여 얻은 집단 유전학적 구조의 정보는 돌돔을 포함한 암초지대에 서식하는 어류자원들에 대한 관리 전략 수립에 중요한 지침을 제공할 수 있다.

KEYWORDS: mitochondrial COI 미토콘드리아 시토크롬c 산화효소; *Oplegnathus fasciatus* 돌돔; genetic diversity 유전적 다양성; population structure 집단 구조; East Asia 동아시아

Chapter 1. Introduction

1.1. Background

1.1.1 Role of Population Genetic Diversity and Structure

Population genetic diversity and genetic structure has increasingly become a valuable resource when it comes to ecological and biological conservation as well as speciation. The genetic drift and gene flow determines population genetic structure and population genetic diversity which provides guidelines for the sustainable marine resource utilization and for the ecosystem conservation and management (Mora et al., 2007). Wide variety of species from marine to terrestrial could displays different genetic structure and diversity within the same species in the geographically divided area (Opazo et al., 2008). Understanding the population genetic structure is crucial before making any management and conservation policies in order to prevent overfishing and overhunting.

1.1.2. Characteristics of Rock Bream, *Oplegnathus fasciatus*

The rock bream, *Oplegnathus fasciatus* (Temminick & Schelegel, 1844), belongs to the family Oplegnathidae and inhabits the coastal waters of Korea, Japan, China and Hawaii (Xiao et al., 2008; Schembri et al., 2010). The rock breams are commonly found in wide range rocky reef area around depth of 1-10m (Schembri et al., 2010; Mundy, 2005). Previous studies have revealed the optimal temperature for the 2 to 8 month old rock bream to be 28.6℃ and 24.7℃ as they grew older. Generally, 8 month or older rock bream prefer a narrow range of 25℃ to 26℃ (Tsuchida & Tabata, 1997). The tolerance for salinity goes down as low as 15psu (practical salinity unit) and their optimal salinity is 25-35psu (Vitas et al., 2016). The egg spawning season is known to be May to July in the East Sea and Yellow Sea and April to June in Taiwan (Xiao et al., 2013; Saran, 1990). The hatched rock bream juveniles adheres to algae in proximity and drift for approximately 30 days, along the currents before settling down on a shallow substrate (Xiao et al., 2013). The pattern of juvenile transportation has been observed in the ichthyofaunal ecology in Japan Tohoku area during May to June, attached to the drifting seaweed (Safran, 1990). Such characteristics of long distant migration accompanied with long pelagic period commonly yields low genetic differentiation and genetic homogeny.

1.2. Preceding Studies and Purpose

1.2.1. Preceding Studies

The rock bream is locally called “Dol-Dom” in South Korea and its meat is considered a delicacy for all around the country, served as sashimi or dried filefish fillet. Besides being served as a food, they have commercially important values for being a game fish and aquaculture subject. There has been a recent development of the artificial breeding technique (Quan & Xiao, 2007) to increase the rock bream resources in Korea, Japan, China, and Hawaii. Also the assessment has been made for the economic potential of offshore aquaculture of the *O. fasciatus* (Lipton &kim, 2007). Utilizing the breeding methods, large quantities of cultured fried have been distributed around the coastal waters of the South Korea including Jeju Island.

A moderate amount of previous researches were performed on *O. fasciatus* which includes ecology (Mundy., 2005), morphology (Mundy.,2005), disease (Yoshikoshi & Inoue, 1990; Jung & Oh, 2000; Lee at al., 2004; Cho et al., 2006), development (Chang et al., 2015; Liu et al., 2008., 2008), aquaculture (Quan & Xiao, 2007; Lipton & Kim, 2007; White et al., 1995; Lim & Lee, 2009), and nutrition (Liu et al., 2008; Wang et al., 2003; Nam et al., 2005; Shan et al., 2008). Despite the scientific breakthroughs in various research areas, studies for the genetic variation and population genetic structure for rock bream are at primitive stage. The literature survey indicated that no significant population genetic structure in the coastal waters of South Korea were identified with SSR (Simple Sequence Repeat) molecular marker (Sun et al., 2011; An & Hong, 2008). However, Xiao (2016) have been able to identify the differences in population genetic structure of rock bream inhabiting in two geographically divided area in the coastal area of China utilizing the mtDNA (mitochondrial DNA) COI (cytochrome c oxidase subunit 1) marker, northern region (Jiaonan) and southern region (Zhoushan and Taiwan). Considering the fact that COI gene was able to identify the two regional difference, increasing the quantity of samples and sampling localies would yield more promising results.

1.2.2. Purpose

The lack of understanding on spatial pattern and population genetic structure of the valuable fish species have been hitherto lead to overfishing and sometimes extinction of the species. Obtaining a reliable genetic information can not only be used for stock conservation and establishment of the artificial breeding plan but also for evaluating the genetic impact of the released artificial seeds on the wild stock. The current study aims to understand population genetic structure of East Asian ocean including Yellow Sea, East China Sea, Korea Strait, and East Sea in order to efficiently conserve and manage rock bream population.

The population genetics study of O. fasciatus carried out by Xiao et al. (2006) was limited by its sample size and location. The study can be further improved by increased sample size and the range of the sampling sites. In this study, we included additional 121 samples from four additional localities including Yellow Sea, East China Sea, Korea Strait, and East Sea and conducted a comprehensive population genetic analysis for the Seast Asia rock bream with a combined data set in order to understand its population structure and thus contribute to sustainable management of the fish stock. Because of the promising result from Xiao et al. (2016) which yielded sufficient level of variation that distinguished the populations, the same mtDNA COI region was used to carry out comprehensive study including the Korea and China rock breams.

Chapter 2. Materials and Methods

2.1. Materials and Sequencing

2.1.1. Sample Collection

A total of 121 samples of mature wild rock bream were collected: 59 from Kangnung (KN; East Sea) in October 2015, 14 from Goseong (GS; Korea Strait) in September 2015, 30 from Heuksando (HS; Yellow Sea) in August 2015, and 18 from Ieodo (ID; East China Sea) in July 2015 of which breeding seasons are included during the range of investigation (Fig 1, Table 1). The additional 42 mtDNA COI sequences data of Chinese rock bream were downloaded from GenBank (accession numbers: KM668597.1-KM668612.1): 12 from Jiaonan (JN; Yellow Sea) and 30 from Zhoushan (ZS; East China Sea). These 2 localities can also be found in the Fig. 1.

The collected specimens were preserved in 99% ethanol on site and transferred to laboratory from those sites. Each of the specimens were tagged with collection date and incubated in -20℃ freezer for the DNA extraction.

Table 1. Sampling and sequence information for *O. fasciatus* used in this study

Sampling location	Sample size	Collection date	Sequence length	Gene type
Kangnung(KN) - East Sea(Sea of Japan)	59	October, 2015	642	Mitochondrial DNA(COI region)
Goseong(GS) – Korea Strait	14	September, 2015	642	Mitochondrial DNA(COI region)
Heuksando(HS) - Yellow Sea	30	August, 2015	642	Mitochondrial DNA(COI region)
Ieodo(ID) - East China Sea	18	July, 2014	642	Mitochondrial DNA(COI region)
Jiaonan(JN) - Yellow Sea	12	(Downloaded from GenBank)[α]	642	Mitochondrial DNA(COI region)
Zhoushan(ZS) - East China Sea	30	(Downloaded from GenBank)[β]	642	Mitochondrial DNA(COI region)

α: GenBank accesssion number - KM668612.1 to KM668605.1 & KM668599.1 to KM668859.1; **β**: GenBank accession number – KM668604.1 to KM668600.1; Sample size: number of individuals collected per site; Sequence length: number of nucleotide obtained by sequencing specific region of mtDNA COI region

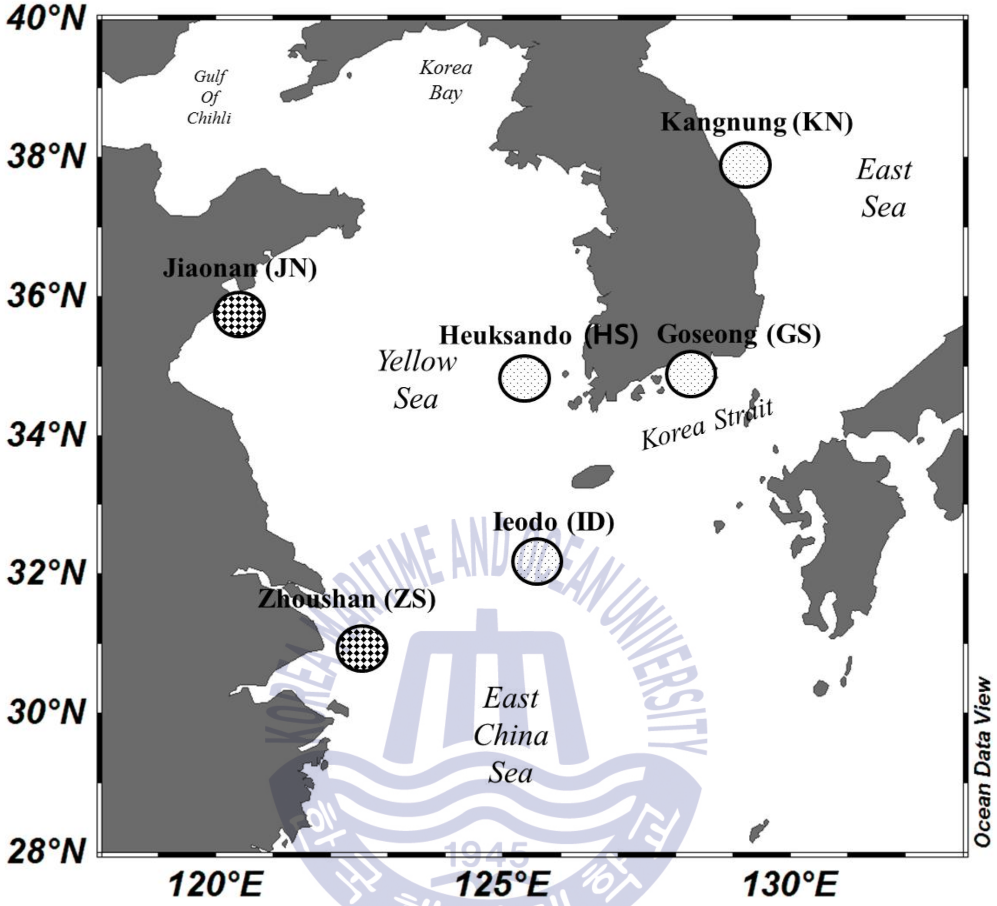

Fig. 1. Sampling localities of O. fasciatus: Kangnung (KN), Goseong (GS), Heuksando (HS), Ieodo (ID), Jiaonan (JN), and Zhoushan (ZS)

GACCCTGCAGGAGGAGGAGACCCCATCCTTTACCAACACCTCTTCTGATTTTTCG
GACATCCGGAAGTCTATATCCTGATTCTTCCAGGGTTTGGTATAATTTCACACATC
GTTGCTTACTACTCTGGTAAAAAAGAACCCTTCGGCTATATGGGCATGGTCTGAG
CTATGATGGCAATTGGCCTTCTAGGATTTATTGTTTGAGCCCACCATATGTTCACA
GTTGGAATGGATGTTGACACACGCGCCTACTTTACATCCGCCACTATAATTATTGC
AATCCCCACAGGTGTAAAAGTCTTTAGCTGACTTGCAACCCTTCATGGAGGAGCA
ATCAAATGAGAAACCCCTCTTCTCTGAGCCCTCGGCTTCATTTTCCTTTTCACAGT
AGGGGGTCTAACGGGCATTGTCCTAGCCAACTCCTCCCTAGATATCGTCCTACAT
GACACATACTACGTAGTAGCACATTTCCATTACGTTCTCTCTATAGGAGCCGTATT
TGCCATCGTTGCAGCCTTTGTACATTGATTCCCCCTATTTACAGGCTACACCCTCC
ACAGCACTTGAACAAAAATCCACTTCGGAATTATGTTTATTGGGGTAAACCTTACC
TTTTTCCCTCAACACTTCCTAGGGCTAGCC

Fig. 6. The typical mtDNA COI sequence obtained from sequencing

2.1.2. DNA Extraction

For the genomic DNA extraction, muscle tissue, caudal and pectoral fin, liver tissue, and gill tissue were removed using sterilized blade and forceps. The tissue isolation was performed on top of sterile aluminum foil placed on clean bench setting. The obtained tissues of 25mg were placed in 1.5ml microcentrifuge tube along PBS (Phosphate Burffer Solution) and centrifuge at 4000rpm for 2 min. PBS was removed using 1000㎕ pipette and 180㎕ of ATL buffer and 20㎕ of proteinase K provided by 'DNeasy blood and tissue kit' (Quiagen, Germany) were added. The tissue was grinded into fine particle using micro pestle. The solution were then incubated in a heat block set to 56℃ for 4-24 hours depending on the tissue mass. The lysed tissue were filtered through series of filters (DNeasy Mini spin column) in order to extract DNA: centrifuge was performed at 8000rpm for 1min with isolated DNA solution, flow-through was discarded and centrifuge was performed at 8000rpm for 1min with 500㎕ AW buffer added, flow-through was discarded and centrifuge was performed at 14,000rpm for 3min to dry the membrane. Lastly, the DNeasy Mini spin column was placed in 1.5ml microcentrifuge tube and centrifuged at 8000rpm for 1min with 200㎕ AE buffer added. The isolated DNA was checked for its purity and quantity using Nanodrop machine. The isolated DNA were stored in a -20℃ freezer for further use.

2.1.3 PCR Amplification

Prier to PCR (Polymerase Chain Reaction), mixture was prepared to match 19㎕ volume in which 1㎕ of DNA will be added: 10㎕ of 2X*TOPsimple*^{TM} [*n*Taq-Tenuto DNA polymerase (0.2 unit/㎕), *n*Taq-Tenuto buffer (containing 4 mMMG^2), dNTP mixture (2X 0.4 mM each), stabilizer, dyes (Xylene cyanol, Orange G)], 0.5㎕ of forward primer sequence (New COIS: 5’-TCGCTGCTGGCATCACCATGCTCC-3’), 0.5㎕ of reverse primer sequences (New COIR: 5’-TCTGGGTAGTCTGAATACCGTCG-3’), and 8㎕ of DW (Deionized Water). 1㎕ of DNA from each specimen was added to the mixture making it a 20㎕.

PCR amplification was performed under following condition: preheating at 95℃ for 2 min, followed by 5 cycles of denaturation (95℃, 30sec), annealing (55-50℃, 40sec, -1℃ per cycle), elongation (72℃, 1min), and 35 cycles of denaturation (95℃, 30 sec), annealing (50℃, 40sec), elongation (72℃, 1 min), and terminated with final extension at 72℃ for 5min on a PCR Thermal Cycler (TaKaRa Bio Inc, Japan).

2.1.4. Electrophoresis

The amplified PCR products were tested using electrophoresis method: Place the 2% agarose gel into the electrophoresis chamber and fill it up with the 1xTAE (Tris-acetate-EDTA) until it over flows the top of the gel. Then load the 2㎕ PCR product into the gel openings and run for 20 min under 100V current. When the run has been completed, place the gel in 30㎕ EtBr and

300ml DW mixture for 15min under the ventilated hood. Finally, rinse the gel with DW and take a photo under UV (Ultraviolet) light.

2.1.5. Sequencing

Depending on the electrophoresis results, the PCR products were sequenced by Macrogen’s (http://www.macrogen.com) single primer extension service using 3730xl DNA analyzer (Thermo Fisher Scientific Inc, USA).

The forward and reverse sequence of the PCR product data comes as an ab1 format which could be edited using Geneious 8.1.6 (Kearse et al., 2012). First, the forward sequence and reverse sequence were aligned with 65% similarity. Second, the forward and reverse primer regions are removed. Lastly, any missing gaps or ambiguous nucleotide were removed. The resulting consensus sequence with 95-100% quality were ran through NCBI data base to identify whether it is the right species or not.

2.2. Data Analysis

2.2.1. Haplotypes

Haplotypes were obtain using the program MEGA (Molecular Evolutionary Genetic Analysis) (Kumar et al., 2001) and TCS version 1.18 (Clement et al., 2000). The consensus sequences for each specimen's mtDNA COI gene produced from Geneious 8.1.6 has been aligned and transferred to MEGA where more detailed analysis could be performed. From MEGA, the alignment of consensus sequences were edited to .nex format suited for the TCS 1.18 program. The .nex file has been ran through TCS 1.18 which automatically calculates the phylogenetic distances among the sequences, generating a phylogenetic tree and list of haplotypes. The haplotypes were arranged based on its locality and all the haplotypes were deposited into the GENBANK (accession numbers: MF773986-MF774015).

2.2.2 Molecular diversity

Molcular diversity indicts are composed of the number of polymorphic sites (S), number of transitions (Ti), number of transversions (Tv), nucleotide diversity (π) (Nei, 1987), haplotype diversity (h), and pairwise differences (K) (Tajima, 1983). These values were calculated with ARLEQUIN ver. 3.1 (Exofficer, 2015) by imputing the haplotype sequence data arranged in .arp format.

2.2.3. Population genetic structure

Population genetic structure and differentiation among localities were evaluated with conventional F_{ST} (the genetic variability among population) and

Φ_{CT} (the genetic variability among groups) values obtained from AMOVA (hierarchical analysis of molecular variance) test. The AMOVA was set to standard computation for haplotypes (locus by locus AMOVA with 10,000 permutations).The exact test of population differentiation was set to 100,000 steps in Markov chain and 1,000 bootstrap replication.

2.2.4. Phylogenetic Relationships Among Haplotypes

The phylogenetic relationships among haplotypes were estimated using Markov chain Monte Carlo (MCMC) methods to calculate the distribution of posterior probability of model parameters utilizing the program MrBayes (Huelsenbeck et al., 2001). Based on BIC (Bayesian Information Criterion) score calculated with MODELTEST (Posada & Crandall) implemented in MEGA program which provides the gamma shape parameter for the rate heterogeneity among sites and best fits of nucleotide substitution, the K2P+G (0.06) (model with a proportion of invariable sites and gamma-distributed rate variation) was selected for the evolutionary model parameter. The analysis preferences set to neighbor-joining tree, maximum likelihood for the statistical method, and complete deletion of gaps and missing data.

2.2.5. Minimum Spanning Network

The minimum spanning network/tree (MST) of genetic relationship among COI haplotypes for *O. fasciatus* was produced based on previously calculated statistical parsimony method from TCS 1.18 (Clement et al., 2000), a java computer program which performs cladistics analysis of phenotypic associations with haplotypes. Unpolished cladogram produced by TCS 1.18 program was modified using Network 5.0.0.0 (Fluxus Technology, http://www.fluxus-engineering.com) program by adding a pie chart and other graphical features relevant to the number of haplotypes and thier geographical distribution.

2.2.6. Demographic History

The demographic pattern of rock bream haplotypes were analyzed with neutrality statistics inferred from Tajima’s *D* (Tajima, 1989) and Fu’s *Fs* (Fu, 1997). These values were calculated by simulating the haplotypes 10,000 times with original definition and mismatch distribution (Rogers & Harpending, 1992) with 1,000 permutation of bootstrap replication. Based on neutral mutations and population equilibrium hypothesis, the significance of the value was determined by randomly regenerated samples. The negative values of Fu’s *Fs* and Tajima’s *D* are indicative of a recent demographic expansion. The equation $\tau=2ut$ was used to calculate the the historical demographic expansion time: τ (tau) is an onset period of population expansion expressed as mutational time, u is the mutation rate of the whole sequence, and t is the time since expansion. The current study employes molecular clack of 1.98-2.73% per million years. The same molecular clock has been used for mtDNA COI fragment in previous study developed by precision research for marine fish (Xiao et al., 2016).

Chapter 3. Results

3.1. Intraspecific Genetic Diversity of *O. fasciatus*

A total of 163 mtDNA COI sequences with length of 642 base pairs were analyzed in this study of which 121 sequences from Korea (KN, GS, HS, ID) and 42 from China (JN, ZS). All mtDNA COI sequences have been successfully edited utilizing Geneious 8.1.6 program as well as obtaining haplotypes using MEGA and TCS. A sum of 34 variable sites were identified within the mtDNA COI sequences composed of 20 transitions and 14 transversions (Table 2 & Table 3). A total of 30 haplotypes were defined by the 34 variable sites based on mtDNA COI fragment (Table 2). The numbers of haplotypes per each geographic location follows 16 in Kangnung (KN), 4 in Goseong (GS), 8 in Heuksando (HS), 6 in Ieodo (ID), 5 in Jiaonan (JN), and 11 in Zhoushan (ZS).

10 out of 30 haplotypes (33.3%) were shared in more than one localities. The most frequent haplotypes, H01 and H04 which consist of 60 sequences and 25 sequences respectively out of the total 163 sequences, are observed in all the localities but ZS. All eleven ZS haplotypes are specific to the sample without being shared with any other samples. Moreover, six haplotypes from KN, two from Hs, and one from JN are specific to the corresponding localities. The nucleotide substitutions in the ZS specific haplotypes reside mostly in the first one-third of the COI fragment while the substitutions in the other haplotypes are rather evenly spread (Table 3).

Table 2. Genetic diversity based on the mtDNA COI of *O. fasciatus* populations within six localities

Samples (Wild)	N_s	N_h	N_h/N_s	**Substitutions [ti + tv]**	h	π	S	k
KN_{korea}	59	16	0.27	14 [10+4]	0.80 ± 0.04	0.003 ± 0.002	14	1.65 ± 0.99
GS_{Korea}	14	4	0.29	4 [2+2]	0.58 ± 0.14	0.001 ± 0.001	4	0.93 ± 0.68
HS_{Korea}	30	8	0.27	8 [6+2]	0.73 ± 0.07	0.003 ± 0.002	8	1.63 ± 0.99
ID_{korea}	18	6	0.33	5 [3+2]	0.75 ± 0.07	0.002 ± 0.002	5	1.41 ± 0.90
JN_{China}	12	5	0.42	5 [4+1]	0.74 ± 0.12	0.003 ± 0.002	5	1.96 ± 1.20
ZS_{China}	30	11	0.37	21 [9+12]	0.81 ± 0.06	0.009 ± 0.005	21	5.65 ± 2.79
Combined	163	30	0.18	34 [19+15]	0.83 ± 0.02	0.004 ± 0.003	34	2.77 ± 1.48

N_s: ♯ of Sequences, N_h: ♯ of haplotypes, Substitutions: transitions (ti) + transversions(tv), h: haplotype diversity, π: nucleotide diversity, S: ♯ of polymorphic sites, K: average pairwise differences among haplotypes

Table 3. Nucleotide substitutions in the 642 base pair region of 30 mtDNA COI haplotypes from 6 localities from Korea and China.

Haplotypes\ Nucleotide position	21	22	25	36	42	45	52	54	69	76	111	116	120	123	125	147	162	174	184	187	192	201	204	216	239	294	330	396	397	426	486	534	621	633
H01	C	C	A	A	C	C	T	C	G	C	C	C	C	C	C	C	C	G	C	C	A	T	A	G	C	A	A	T	C	C	T	A	T	A
H02	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	G	·	·	G
H03	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	T	G	·	·	·
H04	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	G	·	·	·
H05	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	T	G	·	·	·
H06	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·
H07	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	T	·	·	·	·	T	G	·	·	·
H08	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·
H09	G	T	·	·	·	G	C	T	·	·	G	T	T	A	T	·	A	A	G	A	·	·	·	·	T	·	·	·	·	T	G	·	·	·
H10	·	T	·	·	T	G	·	T	G	G	·	·	·	·	·	·	A	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H11	·	T	·	·	T	G	·	T	G	G	·	·	·	·	·	·	A	A	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H12	G	T	·	·	T	G	·	T	G	G	·	·	T	·	T	A	A	A	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H13	·	T	·	·	T	G	·	·	G	G	·	·	·	·	·	A	A	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H14	·	T	·	·	·	G	·	·	G	·	·	·	T	·	·	·	A	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H15	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	T	G	·	·	·
H16	G	T	·	·	T	G	·	T	G	·	·	T	T	·	·	A	A	A	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H17	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·
H18	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H19	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·
H20	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·
H21	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·
H22	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·
H23	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·
H24	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·
H25	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G
H26	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·
H27	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·
H28	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·
H29	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
H30	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

Intrapopulation diversity values of mtDNA COI fragment for *O. fasciatus* are shown in Table 2. Low levels of nucleotide diversity (π=0.004±0.003) and high levels of haplotype diversity (*h*=0.83±0.005) has been identified. The population genetic diversity of rock bream appeared much higher on the East China Sea (0.009±0.005) compared to those in Yellow Sea and East Sea (KN, GS, HS, ID, JN; π=0.001~0.003) according to the result (Table 2). It is notable that the Ieodo (ID) sample presents almost identical level of low genetic diversity as the Yellow Sea and East Sea samples dispite its geographical proximity to Zhoushan (ZS).

3.2. Phylogenetic Relationships of Haplotypes

In order to obtain phylogenetic relationship of 30 COI haplotypes, MCMC (Markov Chain Monte Carlo) methods were used to estimate the posterior probability of model parameters using the Kimura 2-parameter + G (0.06) obtained from the MODEL TEST implemented in MEGA by inputting the values in MrBayes program (Fig.2). The calculation yielded two mjor clades: clade A (23 haplotypes with 155 individuals) and clade B (7 haplotypes with 8 individuals limited to ZS). Clade A is again divided into two subclades a1 and a2: a1 includes two haplotypes with 6 individuals from Kangnung (KN) and Ieodo (ID) and a2 includes 6 haplotypes with 48 individuals from all sites. The time of genetic differentiation/separation was calculated between the clades based the molecular clock 1.98%-2.73% per million year by precision research: 183-252 kyr ($nd_{A/B}$=0.010) between clade A and clade B, 91-126 kyr

($nd_{a1/a2}$=0.005) between subclade a1 and a2. Subclade a1 and a2 appeared to be much recently diverged compared to clad A and B.

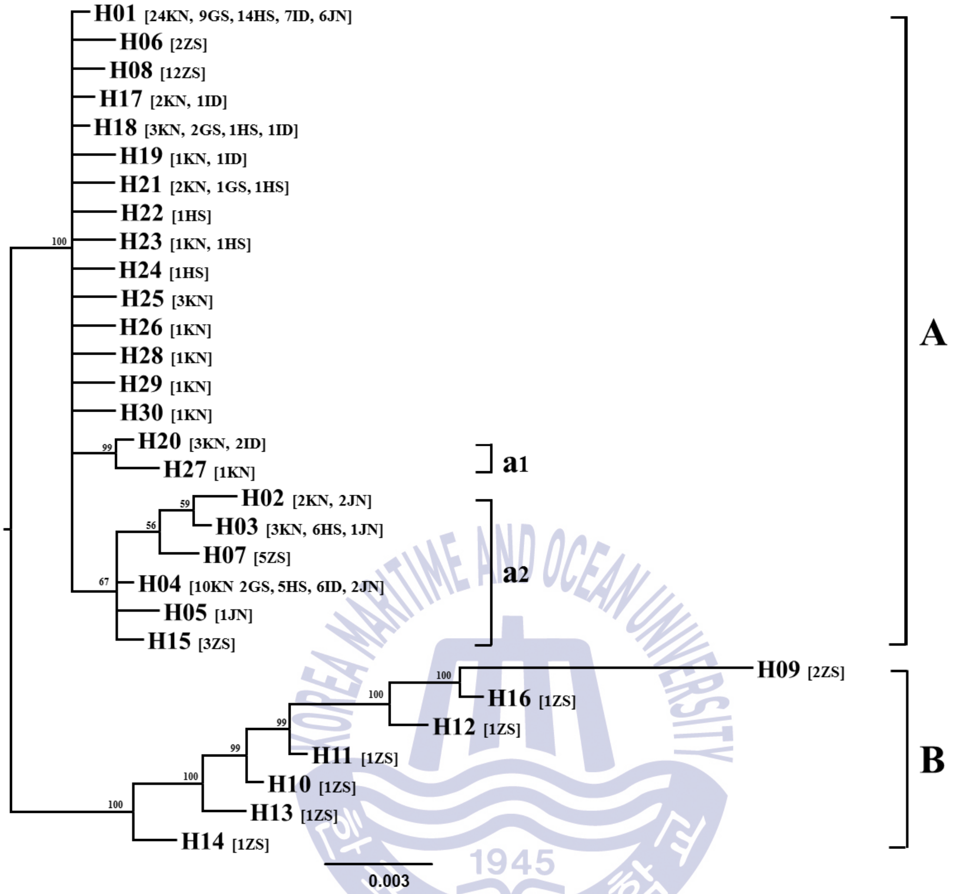

Fig. 2. Bayesian tree of the *O. fasciatus* COI haplotypes based on K2P + G (0.06) substitution model. The posterior probabilities values are indicated on the nodes. The scale bar shows a sequence divergence of 0.003 nucleotide difference per site. A and B represent the two major clades with a and b representing the two subclades within clade A.

The structure of minimum spanning network/tree (MST) displayed a star-shaped network with a long tail of ZS haplotypes attached (Fig 3). Most of the haplotypes are distant from the main haplotypes, H01 and H04, by one or two nucleotide while ZS specific haplotypes comprising the long tail are no less than six to eight nucleotides apart from H01. The other four ZS specific haplotypes, H06, H07, H08, and H15 are closely associated with the main haplotypes as most of the other haplotypes. It is rather clear that haplotypes from Kangnung (KN), Goseong (GS), Heauksando (HS), Ieodo (ID), and Jiaonan (JN) has undergone extensive gene flow, sharing haplotypes without forming any significant geographical cluster. The majority of the tree is clumped by only one or two nucleotide differences indicating low genetic variation. The only divergence that has been observed is the independent ZS unique haplotypes that reaches out as far as 25 nucleotide difference.

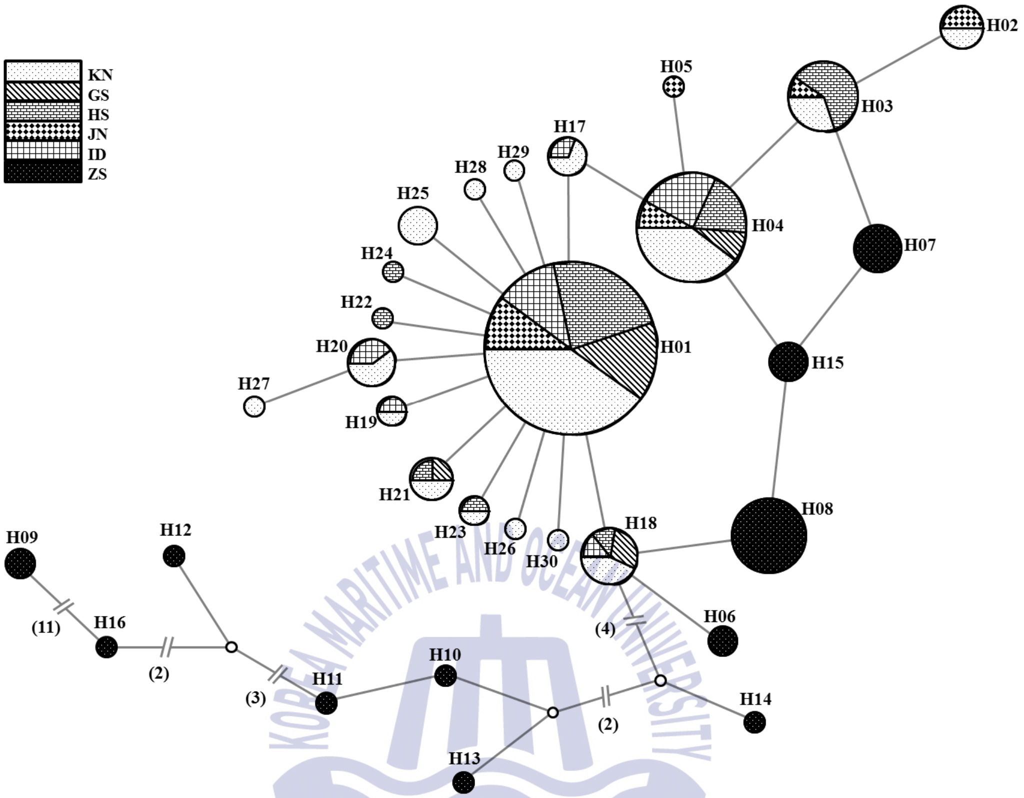

Fig. 3. Minimum spanning haplotype network based on *O. fasciatus* COI sequences. Each line between haplotypes represents a single nucleotide substitution and the white dots represent hypothetical haplotypes. Size of the samples and localities are indicated within each haplotype circles. The numbers written within bracket represents the hidden mutational steps with hypothetical haplotypes in each.

All in all, the haplotype frequency presented extensive gene flow between haplotypes between all other localities except for the ZS haplotypes (H06-H16) (Table 4). The haplotype H01 and H04 formed the two largest circles on MST diagram which includes haplotypes from KN, GS, HS, ID, and JN (Fig. 3). The main haplotypes, H01 and H04, contain 60 sequences and 25 sequences respectively which come from all localities but ZS (Fig. 3 & Table 4). Interestingly ZS haplotypes did not share with haplotypes from all other localities while other haplotypes being mixed with at least one other haplotype from different localities (table 4). Few other unique haplotypes includes H25 to H30 from GS, H22 and H24 from HS, and H05 from JN. In the figure 4, it clearly shows that distribution of unique ZS haplotypes are restricted to Zhoushan location only, whereas KN, GS, HS, ID, and JN were all intertwined with at least 4 other haplotypes around every other locality. The major haplotypes H01, H03, H04, and H18 dominates the coastal waters of Korea, especially H01 and H04 which includes KN, GS, HS, ID, and JN.

Table 4. Haplotype frequency of *O. fasciatus* COI haplotypes from Korea and China

Haplotype	KN_{Korea}	GS_{Korea}	HS_{korea}	ID_{korea}	JN_{China}	ZS_{china}	Total
H 01	24	9	14	7	6	·	60
H 02	2	·	·	·	2	·	4
H 03	3	·	6	·	1	·	10
H 04	10	2	5	6	2	·	25
H 05	·	·	·	·	1	·	1
H 06	·	·	·	·	·	2	2
H 07	·	·	·	·	·	5	5
H 08	·	·	·	·	·	12	12
H 09	·	·	·	·	·	2	2
H 10	·	·	·	·	·	1	1
H 11	·	·	·	·	·	1	1
H 12	·	·	·	·	·	1	1
H 13	·	·	·	·	·	1	1
H 14	·	·	·	·	·	1	1
H 15	·	·	·	·	·	3	3
H 16	·	·	·	·	·	1	1
H 17	2	·	·	1	·	·	3
H 18	3	2	1	1	·	·	7
H 19	1	·	·	1	·	·	2
H 20	3	·	·	2	·	·	5
H 21	2	1	1	·	·	·	4
H 22	·	·	1	·	·	·	1
H 23	1	·	1	·	·	·	2
H 24	·	·	1	·	·	·	1
H 25	3	·	·	·	·	·	3
H 26	1	·	·	·	·	·	1
H 27	1	·	·	·	·	·	1
H 28	1	·	·	·	·	·	1
H 29	1	·	·	·	·	·	1
H 30	1	·	·	·	·	·	1
Total	59	14	30	18	12	30	163

3.3. Population Genetic Structure and Historical Demography

The evaluation of population genetic structure and differentiation among localities of *O. fasciatus* population using conventional F_{ST} and hierachical analysis of molecular variance Φ_{CT} (variation among groups) as well as global p test has revealed the highest and significant F_{ST} (variation among population within group) value in Zhoushan (ZS; East China Sea) of 0.23(p=0.00) while other localities have shown (-0.021~0.032, p=0.18~0.78) (Table 5). The AMOVA test have also shown the highest value of 0.22 ($p>0.05$) although insignificant: KN+GS+HS+ID+JN vs ZS = 0.22 ($p>0.05$); JN+ZS vs KN+GS+HS+ID=0.09 ($p>0.05$); KN+GS vs ZS+JN+HS+ID=-0.04 ($p>0.05$); KN+GS vs JN+HS vs ID+ZS = 0.01 ($p>0.05$).

Historical demography of *O. fasciatus* have shown high probability of recent demographical expansion in all the localities excluding Zhoushan. The nutrality test stataistics of Tajima's *D* and Fu's *Fs* have shown significantly negative values in all localities combined and without ZS {[(D=-1.57, $p<0.03$), (Fs=-15.83, $p<0.001$)], [(D=-1.34, $p<0.07$), (Fs=-10.44, $p<0.001$)]} (Table 6). Negative values of Tajima's D and Fu's *Fs* are related with demographic expansion. These neutrality test results suggest a history of recent demographical expansion in all the localities except Zhoushan (Table 6) in which Tajima's D and Fu's *Fs* are 0.23 (p=0.65) and 0.20 (p=0.57). In terms of a whole population, on the other hand, the combined data sets indicate that the East Asia rock bream has most likely experienced recent demographic expansion.

Additionally, the mismatch distribution analysis from the combined and no ZS populations corroborate the demographic history of sudden population expansion. The frequency of distribution of pairwise nucleotide differences observed from the sample sequences show almost the same pattern as the expected distribution under a sudden expansion model (Fig. 5 A and B). The goodness-of-fit test firms such agreement between the observed and the expected mismatch distributions: the sum of squared difference (*SSD*) and Harpending’s raggedness index (*Hri*) are 0.005 (p=0.39) and 0.029 (p=0.74) for the combined dataset and 0.001 (p=0.89) and 0.20 (p=0.97) for the no ZS dataset. The result of population genetic structure and historical demography suggests that the demographic expansion in Zhoushan region is highly unlikely. The time of the expansion were calculated with τ=2ut equation where τ is the onset time of population expansion expressed as mutational time and u is the mutation rate of the whole sequence. The times of population expasions are estimated for the combined and no ZS populations: The whole East Asia rock bream population (KN+GS+HS+ID+JN+ZS=2.33) (τ=2.33) to be 133-183 kyr and population spanning the norhtern East China Sea, Yellow Sea, Korea Strait and East Sea (KN+GS+HS+ID+JN=2.05) (τ=2.05) to be 117-161 kyr before present, when molecular clock of 1.98-2.73% per million year (Xiao et al. 2016) is applied.

Table 5. Pairwise values (below diagonal) and P values (above diagonal) permuting haplotypes among the population of *O. fasciatus*

Gene	Population	KN	GS	HS	ID	JN	ZS
COI	KN	-	0.29	0.41	0.60	0.78	0.00
	GS	0.007	-	0.28	0.18	0.46	0.00
	HS	-0.001	0.012	-	0.20	0.64	0.00
	ID	-0.010	0.032	0.021	-	0.38	0.00
	JN	-0.021	-0.010	-0.021	-0.001	-	0.00
	ZS	0.192*	0.286*	0.226*	0.212*	0.217*	-

KN: Kangnung, East Sea/Sea of Japan; GS: Goseong, Korea Strait; HS: Heukando, Yellow Sea; ID: Ieodo, East China Sea; JN: Jiaonan, Yellow Sea; ZS: Zhoushan, East China Sea; *: value with significant P value

Table 6. Tajima’s D and Fu’s Fs statistic, and mismatch distribution for *O. fasciatus*.

Population	**Tajima’s *D***		**Fu’s F_s**		**Mismatch distribution**			**Goodness-of-fit test**			
	D	***P***	F_s	***P***	τ	θ_0	θ_1	***SSD***	***P***	***Hri***	***P***
KN	-1.34	0.08	-9.46	0.00	1.76	0.00	24.05	0.000	0.98	0.035	0.74
GS	-0.85	0.24	-0.57	0.29	1.48	0.01	2.01	0.002	0.84	0.049	0.96
HS	-0.59	0.31	-2.02	0.12	2.74	0.01	3.585	0.011	0.51	0.046	0.80
ID	-0.11	0.49	-1.44	0.13	1.86	0.00	10.38	0.009	0.43	0.061	0.76
JN	0.70	0.78	-0.26	0.42	3.39	0.00	5.15	0.025	0.42	0.092	0.59
ZS	0.23	0.65	0.20	0.57	14.06	0.00	5.31	0.029	0.63	0.059	0.46
No ZS	-1.34	0.07	-10.44	0.00	2.05	0.00	5.03	0.001	0.89	0.020	0.97
Combined	-1.57	0.03	-15.83	0.00	2.33	0.00	9.00	0.005	0.39	0.029	0.74

τ : mutational time unites for the onset of population expansion; θ_0 and θ_1: mutational parameters of population size before and after the population expansion; *SSD*: sum of squared difference; *Hri*: Harpending’s raggedness index

Chapter 4. Discussion

4.1. Population Genetic Structure of East Asian Rock Bream

The population genetic analysis of East Asian rock bream *O. fasciatus* with mtDNA COI sequences reveals that at least two distinct genetic units exist: one in the southern East China Sea below the Yangtze River mouth (Zhoushan) and the other in the northern East China Sea, Yellow Sea, Korea Strait and East Sea. Separation of the two populations might have occurred approximately 330~455kyBP according to the net genetic distance ($nd_{A/B}$=0.018) between the clades in phylogenetic tree (Fig.2) and the molecular clock of 1.98~2.73%/MY for marine fish (Xiao et al. 2016).

The current study revealed extensive gene flow and homeogeneity of *O. fasciatus* population in five regions of coastal waters in Korea and China [Kangnung (KN), Goseong (GS), Heuksando (HS), Ieodo (ID), and Jiaonon (JN)] excluding Zhoushan (ZS) populations. The conventional *F-Statistics* and AMOVA as well as the Bayesian tree and the minimum spanning network of haplotypes confirm such genetic distinction between the two population, suggesting that extensive gene flow and genetic homogeneity have occurred among the northern samples but the gene flow has been limited between the northern and southern populations. All of the pairwise FST are very low among the five locality samples in the northern population (FST=0.000~0.32, p=0.18~0.78) while they are significantly high between the five samples from the northern population and the ZS samples from the southern population (FST=0.192~0.286, $p<0.01$) (Table 2). The AMOVA test values of ϕ_{CT} an indicative of variation among groups also become highest (ϕ_{CT}=0.22, p=0.16) when the samples are grouped into two, the northern (ID+HS+JN+GS+KN) and the southern (ZS) sample groups. The Bayesian tree of the COI haplotypes presents two distinct clades with posterior probability of approximately 100% (Fig.2), one of which is composed of only the seven ZS specific haplotypes. In the minimum spanning network of haplotypes, the seven haplotypes comprise a long tail separated from the main haplotypes by no less than six to eight nucleotides though all the other haplotypes are closely associated with the main haplotypes apart by only one or two nucleotides (Fig. 3).

The population genetic structure of rock bream is distinct from other pelagic fishes including Japanese anchovy (*Engraulis japonicus*) (Liu et al., 2006), Japanese Spanish mackerel (*Scomberomorus niphonius*) (Shui et al., 2008), yellow drum (*Nibea albiflora*) (Su et al. 2009) and small yellow croaker (*Larimichthys polyactic*) (Han et al. 2009). The Japanese anchovy and Japanese Spanish mackerel did not show any population genetic structure in the region spanning the East China Sea and the Yellow Sea, suggesting their panmictic population in the region (Liu et al., 2006; Shui et al., 2008). On contrary, yellow drum displayed population genetic structure along the Chinese coastal waters from Qingdao in the north to Zhuhai in the south (Xu et al., 2009). Two overwintering aggregation each in the Yellow Sea and the East China Sea is suggested in the small yellow croaker which demonstrated cryptic adaptive population structure and exchange of individual through spawning migration (Han et al. 2009).

The East Asian rock bream *O. fasciatus* appears to have population boundary at Yangtze River mouth in the middle of the East China Sea. In other words, the nother population of the rock bream is separated from the southern population by the Yangtze River mouth and its plume. In the current study, Zhoushan (ZS) and Ieodo (ID) samples belong to two different populations despite being in a geographical proximity within East China Sea. Zhoushan belongs to southern population and Ieodo belongs to the northern population. The two samples did not even share a single haplotype.

This bags a question of why these two populations are showing such distinction. We suggest the hydrological disturbance caused by ocean currents and the Yangtze River plume which creates a physical barrier to the larval dispersal of rock bream. The Yangtze River plume cause freshwater characteristics and flow over the surface the East China Sea, extending southeast or northeast during spring to summer times (Beardsley et al. 1985). From June to early September, the plume extends northeast in the coastal water and then moves eastward over 400 km offshore across the northern East China Sea (Lie et al., 2003). Such characteristics of Yangtze River plume would block the larval dispersal of rock bream during the breading season before settling on a shallow substrate as an adult phase. A similar pattern of population genetic structure is observed in a common limpet *Cellana toreuma* inhabiting the China coast which shows northern and southern separation of population at the Yangtze River mouth (Dong et al., 2012). It is also reported that the abundance of fish eggs and larvae becomes influenced by the change water salinity and temperature due to the Yangtze River runoff (Jiang et al., 2006). Additionally, the large discontinuity by the fresh water outflow of Amazon River in tropical Atlantic coast has affected the phylogeographical pattern of reef associated fishes such as *Acanthurus bahinaus* (Rocha et al. 2002).

The possible cause of the genetic homogeneity and low variation in the northern population of rock bream over the vast range of ocean can be the high larval dispersal range and period (30 days) following the ocean current. It is common that marine species with long larval period displays low genetic differentiation (Ward et al. 1994). The rock bream is known to live in the coastal rocky shores at its sedentary adult, however, the species spends a long pelagic period as a larvae and fry utilizing drifting seaweeds as a transportation vehicle (An and Hong, 2008; Xiao et al., 2013). The larvae and fries appear during April and June in East China Sea and May to July in the Yellow Sea (Xiao et al., 2013) and affected by major warm currents including Kuroshio, Tsushima, and Yellow Sea in the East Asian ocean. Similar results have been shown in the Japanese flounder. Its larvae is reported to travel more than 600 Km by the Tsushima Current during 30-50 days. Other marine species such as red tilefish *Branchiostegus japonicus* (Nohara et al., 2010), fat greenling *Hexagrammos otakii* (Habib et al., 2011) and spotty belly greenling *Hexagrammos agrammus* (Habib et al., 2015) also have sedentary adult phases with long range of larval dispersal as well as little population genetic structure in the Korean coastal waters. On the other hand, distinct genetic lineages and genetically structured populations are shown between Yellow Sea and East Sea populations in the species of gizzard shad *Konosirus punctatus* (Myoung and Kim, 2014) and sand lances *Ammodytes japonicus* and *Ammodytes Heian* (Kim et al, 2017) using control region of the mitochondrial DNA and microsatellite as a genetic marker. It is possible that subtle genetically separable unites exists within the southern and northern population when more variable genetic markers are used for population analysis. Therefore, it would be safe to assume dispersal range of the rock bream larvae is more than enough to cover the entire coastal waters of Korea and China including Yellow Sea, East Sea, Korea Strait, and East China Sea. This characteristics of larval dispersal could easily be the contributing factor for the genetic homogeny observed in the current study. Also the large quantities of the cultured larvae released in Korean coastal waters may have caused genetic contamination and premature migration resulting in the panmixia between the wild population and the hatchery-released fish (White et al., 1995).

4.2. Demographical History of The Rock Bream

Population bottle neck and sudden demographic expansion of approximately 133~183 kyr before present (D=-1.57, p<0.03; Fs=-15.83, p<0.001; τ =2.33) and the northern population by itself approximately 117~161 kyr before present (D =-1.34, p=0.07; Fs=-10.44, p<0.001; τ =2.05) during the late Pleistocene in the

East Asian rock bream has been indicated through neutrality test statistics, Tajima’s *D* and Fu’s *Fs*, and the mismatch distribution analysis (Fig. 5 & Table 6). This demographic history is reflected in the genetic diversity of the species, a pattern of low level of nucleotide diversity (π = 0.004±0.003) and high level of haplotype diversity (*h* = 0.83±0.02) (Table 2). The low nucleotide diversity and high haplotype diversity within the population of our rock bream study suggests large population size resulted from sudden demographic expansion (Nei, 1987) as the rate of stochastic loss slows down retaining more haplotypes than loss by genetic drift (Avis et al., 1984). These molecular diversity indicts also suggests that the rock bream population has likely undergone sudden demographic expansion from a small effective population size. Additionally, the starburst network shape of the minimum spannning tree excluding the long tail of seven Zhoushan exclusive haplotypes (Fig. 3) supports the sudden expansion of the rock bream populations.

The calculated time of expansion (117-161kyr) which belongs to the Pleistocene period which is characterized by shifts in temperature, salinity, and sea level of the ocean caused by glaciation and deglaciation cycles separated by approximately 100,000 year interval (Imbri et al., 1992; Bond et al., 1997; Petit et al., 1999). This factor would have had great impact on the rock bream population: First, fluctuation in sea level in some case has dropped by 120-140m below the present level which exposed the shallow water habitats. The rock bream inhibits the shallow water habitats of coastal rocky zones and coral reef between the depth of 1-10m and would have had great impact as the ocean floor get exposed. Second, the sea temperature fluctuation would have had lead the *O. fasciatus* to migrate to warmer region, drastic reduction in population size, and possible extinction as the suitable water temperature of adult rock bream is know to be 25℃ to 26℃ (Tsuchida & Tabata, 1997). Third, the fluctuation in salinity caused by repeated glaciation and deglaciation would have had great impact on rock bream species. The salinity tolerance for this species is known as 15psu and optimal at 25-35psu (Vitas et al., 2016). Drop and rise of the salinity below 15psu or above 35psu would cause the region to be not habitable. Therefore, during the Pleistocene period, the *O. fasciatus* population inhabiting the collected area, would have been eradicated, displaced, recolonized, and undergone a rapid population expansion afterwards (Hewitt, 2000; Lambeck et al., 2002; Liu et al., 2006). In Korean waters, fat greenling *Hexagrammos otakii* is known to have experienced the sudden demographic expansion at approximately 91~327 kyr before present (Habib et al., 2011) and spotty belly greenling *Hexagrammos agrammus* as approximately 141~406 kyr before present (Habib et al., 2015), similar to rock bream *Oplegnathus fasciatus*.

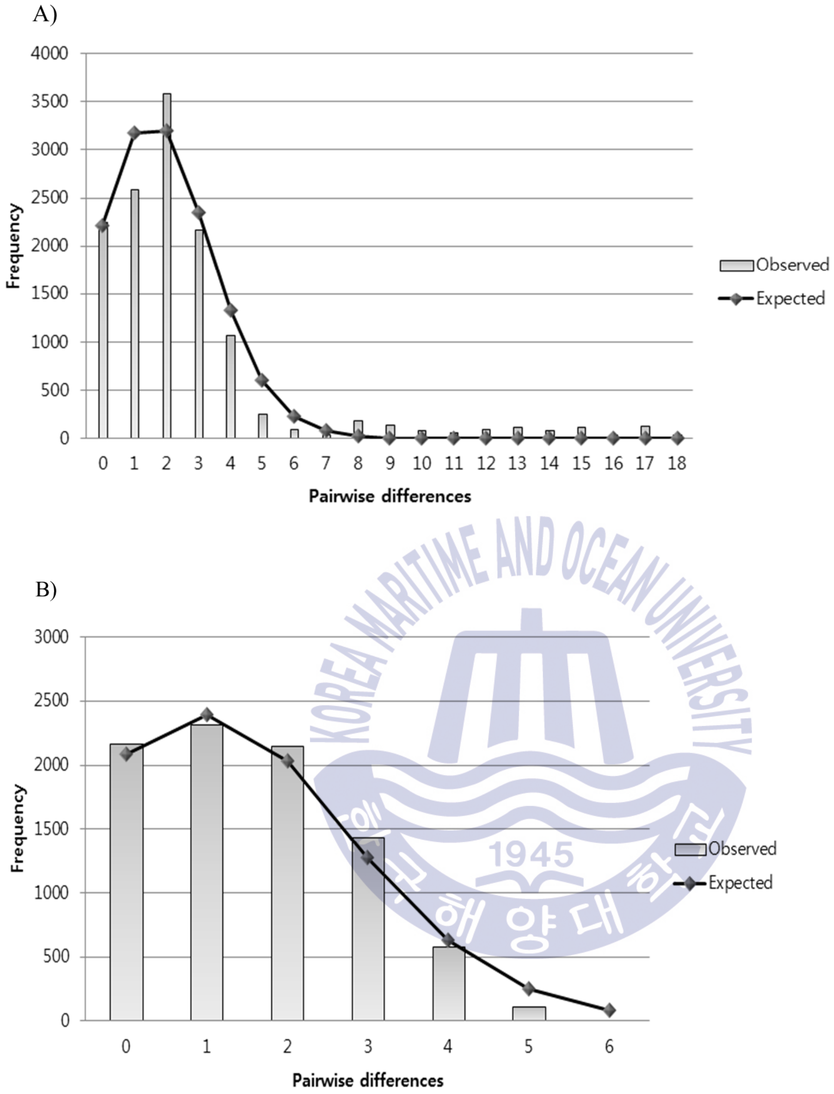

Fig. 5. Mismatch distribution of pairwise sequence differences among *O. fasciatus* COI sequences. The x-axis represents the number of pairwise differences among haplotypes and the y-axis represents the observed (bar) and expansion model (line) frequencies: A) mismatch distribution of the sequences. B) mismatch distribution of the sequences except the ZS sample sequences. The line graph represents an expected mismatch distribution under a sudden expansion model.

Zhoushan population, on the other hand, has shown distinction with the rest of the *O. fasciatus* population in several ways: First, the Zhoushan (ZS) population has not likely experienced sudden population expansion based on the neutrality [Tagima's *D* (0.23, *p*=0.65), Fu's *F* (0.65, *p*=0.20)] test values and the disconformity in the expansion model test between measured and expected values of mismatch distribution. Second, Zhoushan unique populations are distinguished in the MST diagram as a tail sticking out of large cluster (Fig. 3) and did not share any localities nor haplotypes shown in haplotype frequency Table 4. It is clearly shown in the Figure 4 that ZS specific population does not disperse to other localities. The MST diagram resulted in a shallow tree with exception of ZS haplotypes represented in block circles. All other haplotypes were mixed with at least one other haplotype or location connected by one or two nucleotide difference. In contrast Zhoushan haplotypes such as H08-H14 and H16 have nucleotide difference ranging from 5 to 25 with the furthest being H09 (Fig. 3). Although, the ZS haplotypes H06-H08, and H15 have shown only one to two nucleotide difference from the main cluster, they still retained only the ZS population. The haplotype frequency (Table 4) have also shown clear separation between ZS haplotypes (H06-H16) from the rest (H01-H05, H17-H30) where all the haplotypes from KN, GS, HS, ID, and JN localities were included in H01 and H04. Lastly, geological distribution of haplotypes (Fig. 4) has presented single large cluster of ZS haplotypes found only in its original collection region while other haplotypes from all the other localities were extensively mixed. Further investigation is needed in order to understand this phenomenon, especially by adding samples from Taiwan region to clarify the division between haplotypes of interest. Third, the Bayesian tree of the COI haplotypes displayed two distinct clades A and B (Fig. 2): Clade A were composed of haplotype from all the localities whereas clade B were composed of only the Zhoushan haplotypes with net genetic distance of 0.018±0.004. The calculated genetic divergence time between these two clades were to be 366-505kyr before present which could be interpreted as majority of the Zhoushan specific haplotypes diverged much earlier during the Pleistocene period as well as being genetically distinct from the rest of the population. Furthermore, the conventional *Fst* statistics have shown the distinction of Zhoushan (ZS) population from the rest of the population by exhibiting highly significant value(0.792-0.286, $p=0.00$) in terms of variation among population. A highest F_{CT} (differentiation among groups) values (0.222, $p>0.05$) as well as F_{SC} (Variation among population with group) value (0.22, $p=0.50$) has been observed when grouped between Zhoushan (ZS) and the rest (ZS, KN+GS+JN+HS+ID) by hierarchical analysis of molecular variance (AMOVA) although insignificant.

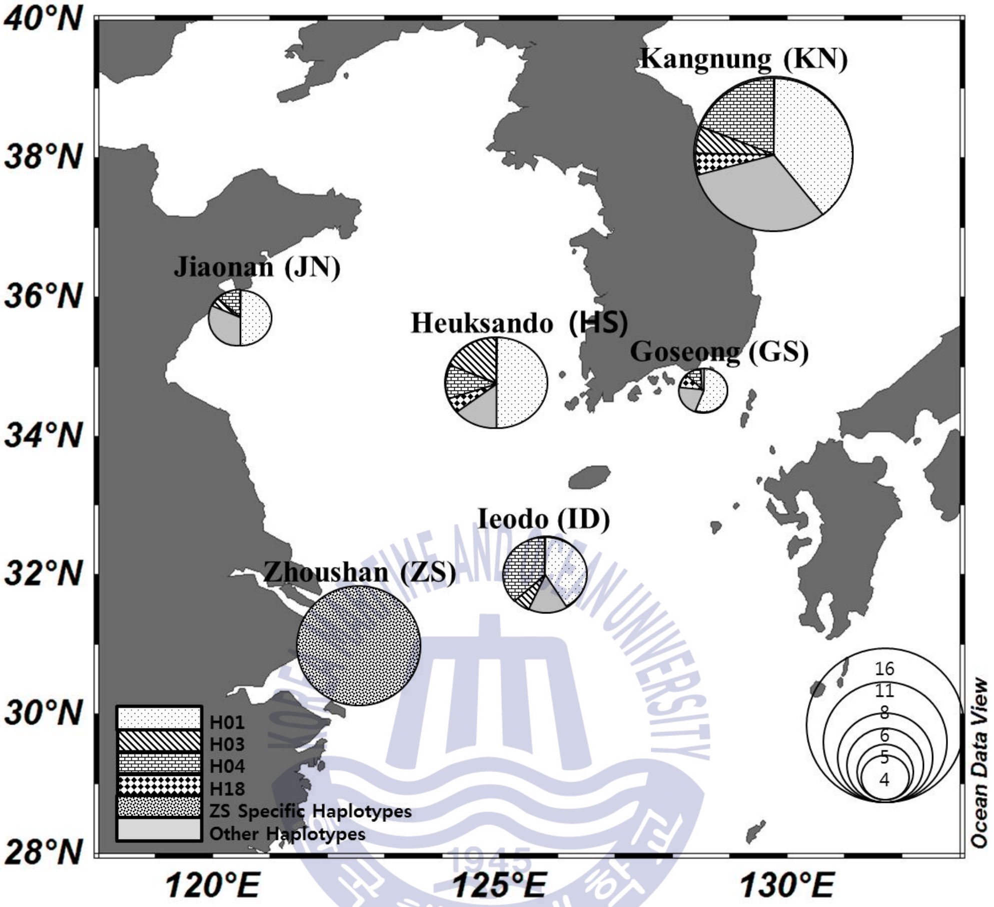

Fig. 4. Biogeographical distribution of four major haplotypes such as H01, H03, H04, and H18 as well as of the ZS specific haplotypes are mapped at each sample collection locality as a pie diagram with different shade of patterns. The size of the circle is proportional to the amount of the samples. The ZS sample contains only its locality specific haplotypes while the other locality samples all share the same major haplotypes.

Chapter 5. Conclusion

The outcome from the current study demonstrated genetic variability and the population genetic structure of the wild rock bream population inhabiting the coastal waters of Korea and China. This information is crucial for any large-scale stock enhancement program to be executed. The genetic distinction of the southern population in the southern East China Sea (Zhoushan, ZS) from the northern population in the northern East China Sea (Ieodo, ID), Yellow Sea (Heuksando, HS; Jiaonan, JN), Korea Strait (Goseong, GS), and East Sea (Kangnung, KN) needs to be considered when the stock management strategy such as release of artificial seeds and conservation of genetic wild population is implemented. Additionally, our study could provide more efficient guidelines of aquaculture not only for the government sponsored execution of large-scale stock enhancement programs. That is, even the stock from Zhoushan and the one from Ieodo to be treated as separate ones and different fisheries regulations be applied accordingly. Further research should be carried out such as using other genetic markers [SSR (Simple Sequence Repeat), CytB (Cytochrome B)] in order to provide more accurate, clear, and detailed information which has proven successful in previous studies (Xu et al., 2009). Therefore, utilizing various markers along with increased and balanced number of samples are required. Finally, investigation is needed on geological association of haplotypes between Zhoushan (ZS) area by adding more samples and possibly from Taiwan coastal area to verify not only the difference between other localities but also the effect of Yangtze River plume.

Acknowledgements

This research was supported by the Ocean Science, Technology school (OST-school) and Korea Institute of Ocean Science and Technology (KIOST, PO01292), the National Natural Science Fund of China (41506170,31672672, 41276166), Qingdao National Laboratory For Marine Science and Technology (2015ASKJ02, 2015ASKJ02-03-03), STS project (KFZD-SW-106, ZSSD-019), Shandong Province agricultural seed project (2014-2017), the STS (No. ZSSD-019), Modern Agro-industry Technology Research System (nycytx-50), Qingdao Source Innovation Program (17-1-1-57-jch), Marine Fishery Institute of Zhejiang Province, Key Laboratory of Mariculturre & Enhancement of Zhejiang Province (2016KF002) and the Guangdong Provincial Key Laboratory of Fishery Ecology and Environment (LFE-2015-8)

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