Thesis for the Degree of Master of Science

# Population structure of the ice goby *Leucopsarion petersii* (Gobiidae, Teleostei) from the Northwest Pacific revealed by morphological and molecular markers



by

Yi Jung Kim

Department of Marine Biology

The Graduate School

Pukyong National University

February 2022

# Population structure of the ice goby *Leucopsarion petersii* (Gobiidae, Teleostei) from the Northwest Pacific revealed by morphological and molecular markers

# [형태 및 분자 마커로 밝힌 북서태평양 사백어(*Leucopsarion petersii*)의 집단 구조]

Advisor: Prof. Jin Koo Kim

by

Yi Jung Kim

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in Department of Marine Biology, The Graduate School,

Pukyong National University

February 2022

# Population structure of the ice goby *Leucopsarion petersii* (Gobiidae, Teleostei) from the Northwest Pacific revealed by morphological and molecular markers

A dissertation

by

Yi Jung Kim

Approved by:

(Chairman) Dr. Saang Yoon Hyun

(Member) Dr. Eun Mi Kim

(Member) Dr. Jin Koo Kim

February 25, 2022

# Contents

# 형태 및 분자 마커로 밝힌 북서태평양 사백어(*Leucopsarion petersii*)의 집단 구조

김 이 정

부경대학교 대학원 해양생물학과

## 요 약

망둑어과(Gobiidae)에 속하는 사백어(*Leucopsarion petersii*)는 한반도 및 일본 열도 연안에만 분포하는 고유종이다. 한반도 주변의 해역을 포함한 북서태평양은 플라이스토세에 해수면의 반복적인 상승과 하강으로 집단유전학적 연구에 적합한 지역이다. 본 연구에서는 사백어의 생활사 특성에 근거하여 남해에 서식하는 사백어가 형태 또는 유전적으로 구분 가능한 집단 구조를 형성할 가능성이 높다고 가정했다. 이를 증명하기 위해, 남해 4개 지역(부산, 거제, 통영, 완도)에서 채집한 사백어 120개체를 대상으로 형태학적 특성과 미토콘드리아 DNA *cytb* 및 핵 DNA *myh6* 분자 마커를 이용하여 남해 집단 내에서, 그리고 일본에서 서식하는 사백어와 비교 분석을 수행했다.

남해에 서식하는 사백어 사이에는 계수 형질에 유의한 차이가 없었고, 계측 형질은 지역과 성별에 따라 차이가 있었다. 계측 형질을 대상으로 한 정준판별분석 결과, 남해 동부(부산, 통영, 거제), 완도, 일본 마이즈루만 세 그룹으로 명확하게 구분되었다.

분자 마커를 기반으로 한 일배체형 네트워크는 네 지역이 동일한 유전자 풀을

공유함을 보여주었으나, 미토콘드리아 DNA 분석에서 유전적 분화 정도($\Phi_{ST}$)를 분석한 결과, 완도와 거제 및 통영은 0.060 및 0.041 ($P < 0.05$)로 미약한 분화가 나타났다. 남해 및 일본 서해 집단 사이에 약한 유전적 분화가 확인되었으나($\Phi_{ST} = 0.084$; $P < 0.05$), AMOVA (Analysis of molecular variance)에서 남해와 일본 서해 집단 사이에 명확하게 구분 가능한 집단 구조를 형성하지 않는다고 나타났다. 상기 두 집단은 일본 태평양 집단과는 매우 유의한 차이를 보였다. 핵 DNA 분자 마커 분석에서 남해 및 일본 서해 집단이 유전적으로 동일한 집단에 속하며, 일본 태평양 집단과는 유의한 차이가 있었다. 쓰시마 난류는 남해에서 일본 서해로 사백어 분산을 촉진하여 유전자 흐름을 유발하고 있으나, 완도 사백어는 완도 주변의 복잡한 해양학적 조건으로 인해 분산에 제약을 받는 것으로 추정된다.

개체군통계학적 역사 분석 결과, 남해와 일본 서해 집단의 사백어는 마지막 간빙기에 급격한 개체군 규모 증가를 경험했다고 확인되었다. 보다 자세한 유효 개체군 규모 변화를 확인하기 위한 Bayesian skyline plot 분석에서 상기 두 집단의 사백어는 플라이스토세 간빙기(MIS 5 및 7)에 개체군 규모 확대 사건을 두 번 경험했다고 나타났으며, 이는 빙하기 이후 서식지 확대나 현재와 유사하거나 더 좋은 기후 조건 등의 이유로 개체군 규모가 급격하게 증가한 것으로 추정된다. 북서태평양에 서식하는 사백어의 최근 분화 역사를 명확히 하기 위해서는 보다 민감한 microsatellite DNA 마커를 기반으로 한 추가 연구가 필요하다.

# Ⅰ. Introduction

The marine environment around the Korean Peninsula was segregated from the surrounding seas due to closure of the Korea Strait caused by climate change during the Quaternary glacial-interglacial cycle (Yoo et al. 2017). During the glacial period, cold fresh water flowing into the East Sea formed an inapposite habitat for numerous marine organisms (Gorbarenko and Southon 2000). Geographic segregation due to these environmental changes promoted genetic divergence among populations (Akihito et al. 2008; Kokita and Nohara 2011; He et al. 2015; Aguilar et al. 2019; Kato et al. 2020). Thus, historical events in the marine paleoenvironment likely had a considerable impact on the population genetic structure of marine and anadromous fish in the waters surrounding the Korean Peninsula. Recent population genetic studies have shown that species in the South and Yellow Seas of Korea are genetically similar to, but distinct from, those of the East Sea (Kim et al. 2017a; Jang et al. 2019), strongly supporting this hypothesis.

The ice goby *Leucopsarion petersii* (Pisces: Gobiidae) is an anadromous fish that ascends into river mouths to reproduce and is endemic to the shallow coastal areas of the Korean Peninsula and Japanese Archipelago. Individuals live for a year or less, and adults die after reproducing in the spring. Larvae hatching from adhesive eggs descend into the sea after approximately 3 days, where they live

in eelgrass near the river mouths (Matsui 1986). This life history may promote lineage diversification or population differentiation due to limited dispersal and gene flow. Moreover, habitat adaptation likely reflects local geographic variations in various oceanographic characteristics (Kim et al. 2008). The ice goby is divided into two morphologically and genetically distinct lineages: the East Sea and Pacific Ocean lineages (Kokita and Nohara 2011). Therefore, we hypothesized that ice gobies in the Korean Peninsula have a different population structure based on past ocean climate oscillations or current ocean conditions. Molecular analyses using mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) were performed to understand the population structure caused by historical ocean climate oscillations, and morphological analyses were used to determine population structure based on contemporary ocean conditions. mtDNA does not undergo recombination due to maternal inheritance and is used extensively to clarify the population structure and to estimate evolutionary history due to its high evolutionary rate (Brown et al. 1982; Wilson et al. 1985; Harrison 1989). A rapid morphological response to environmental changes results from morphological variability and is widely used to estimate management units (Kinsey et al. 1994; Begg et al. 1999; Murta 2000). In this study, we used molecular and morphological methods to assess the population structure of ice gobies in the Korean Peninsula and the impact of Pleistocene climate oscillations on the evolution of marine organisms.

# Ⅱ. Materials and methods

## 1. Sampling and DNA analysis

We collected 30 specimens from each locality of ice gobies that ascend to rivers for reproduction in the South Sea of Korea (Busan, Apr 2009; Geoje, Mar 2020; Tongyeong, Apr 2019; Wando, Mar 2021). Analysis was performed including haplotypes reported by Kokita and Nohara (2011) (Fig. 1 and Table 1). These specimens collected in this study were immediately preserved in 99.5% ethanol, and genomic DNA was extracted from the right eyeball or pectoral fin using the AccuPrep® Genomic DNA Extraction Kit (BIONEER, Republic of Korea) following the manufacturer's protocol. A fragment (827 bp) of the mitochondrial cytochrome b gene (*cytb*) was amplified using Gludg-L (Palumbi et al. 1991) and H15915 (Irwin et al. 1991). Because mtDNA *cytb* was frequently utilized for phylogeographic and divergence time of Gobiidae, it was used in this study (e.g., Harada et al. 2002; Sota et al. 2005; Kokita and Nohara 2011). In addition, a partial locus (528 bp) of the protein-coding nuclear gene 'myosin heavy chain 6 (*myh6*)' was amplified using a primer pair (myh6_F459 and myh6_R1325; Li et al. 2007). The nDNA *myh6* gene encodes the alpha heavy chain subunit of cardiac myosin. This nDNA marker was chosen by can identify deep divergence more conservatively than the mtDNA *cytb* region, and

avoid potential problems (e.g., Ballard and Whitlock 2004; Hurst and Jiggins 2005) that occur when used only the mtDNA marker. Thermal conditions were: initial denaturation at 95°C for 3 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C (for *cytb*), 52°C (for *myh6*) for 45 s, and 72°C for 1.5 min (for *cytb*), 45 s (for *myh6*); and 72°C for 7 min. The nucleotide sequence was obtained using BigDye (R) Terminator v3.1 cycle sequencing kits (Applied Biosystems, USA) on an ABI PRISM 3730XL analyzer (96 capillary type). The phylogeographic congruence between obtained mtDNA and nDNA data was examined and compared with the results of previous study. All sequences were aligned using Clustal Omega (Sievers and Higgins 2018) in Geneious Prime 2021.0.3 (https://www.geneious.com) and trimmed to the same length. The accession numbers for the sequences obtained in this study are the next: MZ403810–403929 (mtDNA *cytb*) and MZ403930–404049 (nDNA *myh6*).

Table 1. *Leucopsarion petersii* sampling sites, haplotype numbers (*Nh*), and nucleotide diversity ($\pi$) of each local population. See localities 1–4 are from this study; Kokita and Nohara (2011) for localities 5–38

| | | Mitochondrial DNA *cytb* | | Nuclear DNA *myh6* | |
|---|---|---|---|---|---|
| ID | Location | *Nh* | $\pi$ | *Nh* | $\pi$ |
| 1 | Busan, Korea | 25 | 0.0054 ± 0.0030 | 3 | 0.0010 ± 0.0009 |
| 2 | Geoje, Korea | 18 | 0.0036 ± 0.0021 | 3 | 0.0006 ± 0.0007 |
| 3 | Tongyeong, Korea | 24 | 0.0043 ± 0.0025 | 3 | 0.0007 ± 0.0007 |
| 4 | Wando, Korea | 13 | 0.0056 ± 0.0031 | 2 | 0.0002 ± 0.0004 |
| 5 | Aomori, Japan | 5 | 0.0046 ± 0.0032 | 2 | 0.0004 ± 0.0006 |
| 6 | Ajigasawa, Japan | 4 | 0.0017 ± 0.0014 | 2 | 0.0006 ± 0.0008 |
| 7 | Murakami, Japan | 5 | 0.0029 ± 0.0022 | 2 | 0.0018 ± 0.0015 |
| 8 | Sado, Japan | 4 | 0.0019 ± 0.0016 | 1 | - |
| 9 | Anamizu, Japan | 3 | 0.0019 ± 0.0016 | 2 | 0.0018 ± 0.0015 |
| 10 | Tsuruga, Japan | 4 | 0.0024 ± 0.0019 | 3 | 0.0017 ± 0.0015 |
| 11 | Obama, Japan | 3 | 0.0019 ± 0.0016 | 2 | 0.0007 ± 0.0008 |
| 12 | Kumihama, Japan | 4 | 0.0034 ± 0.0025 | 2 | 0.0004 ± 0.0006 |
| 13 | Oki, Japan | 3 | 0.0022 ± 0.0017 | 3 | 0.0008 ± 0.0009 |
| 14 | Hagi, Japan | 4 | 0.0036 ± 0.0026 | 2 | 0.0004 ± 0.0006 |
| 15 | Karatsu, Japan | 5 | 0.0053 ± 0.0037 | 3 | 0.0011 ± 0.0011 |
| 16 | Tsushima, Japan | 5 | 0.0036 ± 0.0026 | 2 | 0.0007 ± 0.0008 |
| 17 | Saza, Japan | 4 | 0.0039 ± 0.0028 | 3 | 0.0013 ± 0.0012 |
| 18 | Goto, Japan | 3 | 0.0012 ± 0.0011 | 2 | 0.0006 ± 0.0009 |
| 19 | Shinwa, Japan | 4 | 0.0048 ± 0.0034 | 2 | 0.0013 ± 0.0012 |
| 20 | Kadokawa, Japan | 5 | 0.0082 ± 0.0055 | 3 | 0.0011 ± 0.0011 |
| 21 | Saiki, Japan | 5 | 0.0131 ± 0.0084 | 3 | 0.0011 ± 0.0011 |
| 22 | Iwakuni, Japan | 5 | 0.0039 ± 0.0028 | 2 | 0.0007 ± 0.0008 |
| 23 | Higashihiroshima, Japan | 5 | 0.0094 ± 0.0062 | 3 | 0.0017 ± 0.0015 |
| 24 | Uwajima, Japan | 4 | 0.0041 ± 0.0029 | 3 | 0.0017 ± 0.0015 |

Table 1. Continued

| | | Mitochondrial DNA *cytb* | | Nuclear DNA *myh6* | |
|---|---|---|---|---|---|
| ID | Location | *Nh* | π | *Nh* | π |
| 25 | Shimanto, Japan | 5 | 0.0082 ± 0.0055 | 2 | 0.0007 ± 0.0008 |
| 26 | Higashikagawa, Japan | 5 | 0.0120 ± 0.0077 | 3 | 0.0020 ± 0.0016 |
| 27 | Anan, Japan | 5 | 0.0123 ± 0.0080 | 2 | 0.0038 ± 0.0046 |
| 28 | Yuasa, Japan | 5 | 0.0099 ± 0.0065 | 1 | - |
| 29 | Nachikatsuura, Japan | 5 | 0.0092 ± 0.0060 | 3 | 0.0014 ± 0.0013 |
| 30 | Shima, Japan | 5 | 0.0080 ± 0.0053 | 2 | 0.0011 ± 0.0011 |
| 31 | Shimizu, Japan | 5 | 0.0048 ± 0.0034 | 2 | 0.0009 ± 0.0009 |
| 32 | Futtsu, Japan | 4 | 0.0019 ± 0.0016 | 3 | 0.0016 ± 0.0014 |
| 33 | Hitachi, Japan | 5 | 0.0133 ± 0.0085 | 1 | - |
| 34 | Namie, Japan | 5 | 0.0128 ± 0.0082 | 1 | - |
| 35 | Natori, Japan | 2 | 0.0005 ± 0.0006 | 2 | 0.0004 ± 0.0006 |
| 36 | Utatsu, Japan | 1 | - | 1 | - |
| 37 | Rikuzentakata, Japan | 3 | 0.0015 ± 0.0013 | 2 | 0.0007 ± 0.0008 |
| 38 | Busan, Korea[a] | 4 | 0.0085 ± 0.0056 | 3 | 0.0011 ± 0.0011 |

[a]Local population ID 38 data from Kokita and Nohara (2011)

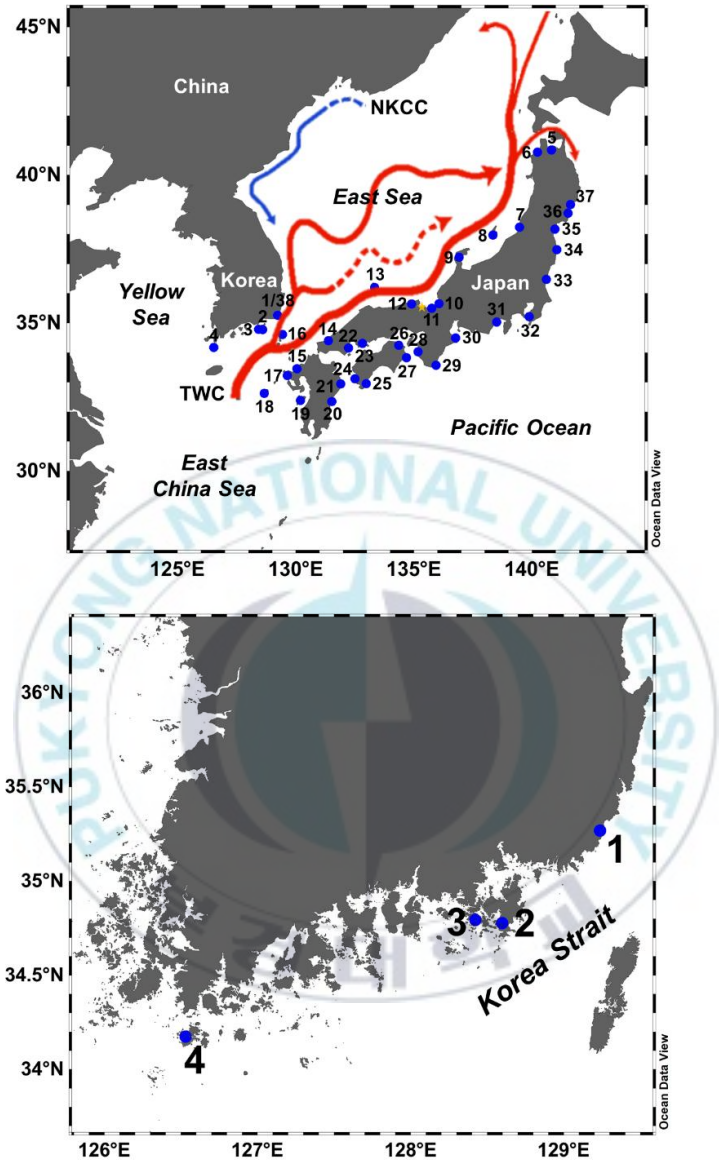


Fig. 1. Distribution of sampling locations for *L. petersii* on the Korean Peninsula and Japanese Archipelago. Numbers are as in Table 1. The star shows the location of Maizuru Bay. Routes of ocean currents around the Northwest Pacific are shown; TWC: Tsushima Warm Current, NKCC: North Korea Cold Current. See localities 1–4 are from this study; Kokita and Nohara (2011) for localities 5–38. Map drawn using Ocean Data View (Schlitzer, 2017)

## 2. Morphological analyses

To analyze the phenotypic difference of ice goby in the South Sea of Korea, except for some damaged individuals, X-rays (CMB-2; SOFTEX, Japan) were taken on samples from 4 localities, and the number of vertebrae was counted including urostyle. Referring to Matsui (1986), 17 morphometric characters were measured to the nearest 0.01 mm using digital vernier calipers (Fig. 2). Sex was determined by Arakawa et al. (1999) (47 males and 72 females). We loaned the Maizuru Bay specimens (FAKU 132998; location see fig.1) from Kyoto University and analyzed morphometric characteristics (7 females and 7 males).

Using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., USA), statistical analyses were performed on differences in standard length (SL) and vertebrae number by sex or local population. The SL according to the local populations of each sex was analyzed severally considering sexual dimorphism (Matsui 1986). Differences in SL and vertebrae number according to sex were confirmed by t-test, one-way ANOVA, and Scheffé's post-hoc. The number of vertebrae per local population did not satisfy the normality assumption, so the Kruskal-Wallis H test was performed. All of the above-mentioned statistical analyses were performed only on ice goby in the South Sea of Korea. We performed a canonical discriminant analysis (CDA) to identify morphological

differences between the individuals from the five locations including Maizuru Bay. The morphometric data used were log-transformed to remove sexual dimorphism and size effect. In the analysis, to satisfy the normality assumption, the local population was grouped with Busan, Tongyeong, and Geoje vs. Wando vs. Maizuru Bay.

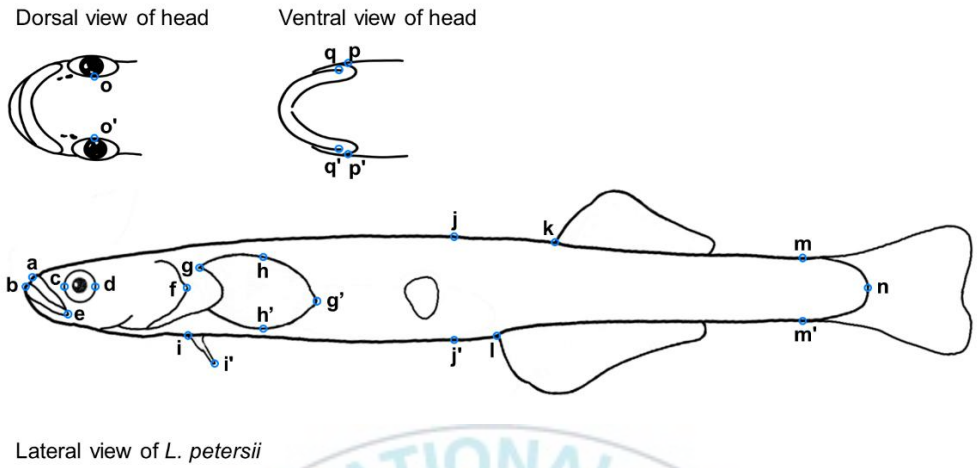


Fig. 2. The measurements of *L. petersii*: a–n, Standard length (SL); a–f, Head length (HD); j–j', Body Depth (BD); a–k, Predorsal fin length (PDL); a–l, Preanus length (PAL); m–m', Caudal peduncle depth (CPD); g–g', Pectoral fin length (PtL); h–h', Pectoral fin width (PtW); i–i', Pelvic fin length (PvL); a–c, Snout length (SNL); c–d, Eye diameter (ED); d–f, Post-orbital length (PoL); o–o', Inter-orbital distance (IoD); a–e: Upper jaw length (UJL); b–e, Lower jaw length (LJL); p–p', Upper jaw width (UJW); q–q', Lower jaw width (LJW)

## 3. Genetic diversity and population structure analyses

All sequences were assigned to haplotypes using DnaSP Version 6.12.03 (Rozas et al. 2017). The number of haplotypes (*Nh*), polymorphic sites (*P*), haplotype (*h*), and nucleotide diversity ($\pi$) were evaluated for each location using Arlequin Version 3.5.2.2 (Excoffier and Lischer 2010). Genetic differentiation between local populations was evaluated through pairwise $\Phi_{ST}$ with 1000 permutations, using Arlequin. In addition, population structure of the ice goby was assessed by analysis of molecular variance (AMOVA; Excoffier et al. 1992) with 10000 permutations. Subclade B (H15, H32, H54, H58, H60, H62, H93, H94, H95) and zones where secondary contact between the East Sea and Pacific Ocean lineages (IDs 21, 22, 23, 24, 26, 27, 28, 33, 34, 35; estimated by Kokita and Nohara 2011), were excluded from analysis (see Fig. 4 and Table 2). The relationship between haplotypes was reconstructed of the Minimum spanning network (Rohlf 1973) analysis in Arlequin. The network was visualized with PopART Version 1.7 (Leigh and Bryant 2015).

Table 2. List of haplotypes assigned per sampling site. Japanese individuals (local population IDs 5–38) use only haplotype data in this study, and the number of individuals is not specified. Bold mtDNA *cytb* haplotypes were found in more than two local populations within the South Sea of Korea

| | Haplotypes names | |
|---|---|---|
| ID | Mitochondrial DNA *cytb* | Nuclear DNA *myh6* |
| 1 | **H1**(2), H2(1), H3(1), **H4**(1), H5(1), H6(2), H7(1), H8(1), H9(1), H10(1), H11(1), H12(1), **H13**(2), H14(1), H15(1), H16(1), **H17**(1), **H18**(1), H19(1), **H20**(1), **H21**(2), H22(2), H23(1), **H24**(1), **H32**(1) | N1(21), N2(6), N3(3) |
| 2 | **H1**(13), **H4**(1), H25(1), H26(1), H27(1), H28(1), H29(1), H30(1), H31(1), **H32**(1), H33(1), H34(1), **H35**(1), H36(1), H37(1), **H38**(1), **H39**(1), H40(1) | N1(25), N2(4), N3(1) |
| 3 | **H1**(5), **H13**(2), **H18**(1), **H20**(1), **H21**(1), **H24**(1), **H35**(1), **H38**(1), H41(1), H42(1), H43(1), H44(1), H45(1), H46(1), H47(2), H48(1), H49(1), H50(1), H51(1), H52(1), H53(1), H54(1), H55(1), H56(1) | N1(25), N3(4), N4(1) |
| 4 | **H1**(4), **H4**(1), **H13**(2), **H17**(1), **H20**(3), **H21**(10), **H39**(1), H57(1), H58(3), H59(1), H60(1), H61(1), H62(1) | N1(28), N2(2) |
| 5 | H63, H64, H65, H66, H67 | N1, N5 |
| 6 | H13, H68, H69, H70 | N1, N5 |
| 7 | H13, H50, H68, H71, H72 | N1, N6 |
| 8 | H13, H68, H73, H74 | N1 |
| 9 | H13, H67, H75 | N1, N6 |
| 10 | H1, H50, H76, H77 | N1, N5, N6 |
| 11 | H13, H67, H78 | N1, N5 |
| 12 | H13, H50, H79, H80 | N1, N5 |
| 13 | H13, H50, H81 | N1, N7, N8 |
| 14 | H13, H16, H82, H83 | N1, N8 |
| 15 | H13, H40, H84, H85, H86 | N1, N7, N9 |
| 16 | H1, H87, H88, H89, H90 | N1, N5 |
| 17 | H40, H50, H91, H92 | N1, N5, N9 |
| 18 | H93, H94, H95 | N1, N5 |

Table 2. Continued

| | Haplotypes names | |
|---|---|---|
| ID | Mitochondrial DNA *cytb* | Nuclear DNA *myh6* |
| 19 | H96, H97, H98, H99 | N1, N6 |
| 20 | H122, H123, H124, H125, H126 | N10, N11 |
| 21 | H13, H127, H128, H129, H130 | N1, N6, N8 |
| 22 | H13, H16, H100, H101, H102 | N1, N5 |
| 23 | H13, H103, H104, H105, H131 | N1, N2, N6 |
| 24 | H40, H106, H107, H108 | N1, N6, N8 |
| 25 | H132, H133, H134, H135, H136 | N10, N12 |
| 26 | H92, H109, H110, H111, H137 | N1, N6, N7 |
| 27 | H112, H131, H138, H139, H140 | N1, N6, N10, N11 |
| 28 | H13, H16, H103, H113, H141 | N1, N11, N12 |
| 29 | H142, H143, H144, H145, H146 | N10, N11, N12 |
| 30 | H147, H148, H149, H150, H151 | N10, N11 |
| 31 | H152, H153, H154, H155, H156 | N10, N11 |
| 32 | H152, H157, H158, H159 | N10, N12, N13 |
| 33 | H70, H114, H115, H160, H161 | N1, N10, N11, N12, N13, N14 |
| 34 | H115, H116, H117, H162, H163 | N1, N10, N11, N12, N13, N14 |
| 35 | H13, H117 | N1, N5 |
| 36 | H117 | N1 |
| 37 | H117, H118, H119 | N1, N5 |
| 38 | H1, H32, H120, H121 | N1, N2, N5 |

## 4. Estimate of demographic history

The demographic history of ice goby was analyzed to Mismatch distributions (MMD; Rogers and Harpending 1992) using Arlequin where goodness of fit between the observed and expected data was verified by the Sum of Squared Differences (SSD) and the Harpending raggedness index (Hri). Also, Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) values were assessed to estimate whether the population size change. All analyses performed bootstrap resampling (1000 replicates). The time of expansion (t) was estimated as Tau ($\tau$) = $2\mu kt$, in which k is the number of nucleotides analyzed and $\mu$ is the substitution rate per nucleotide ($1.10 \times 10^{-8}$ or $1.35 \times 10^{-8}$), and a generation time of 1 year applying to the estimated evolution rate. Estimates for the rate of mitochondrial *cytb* evolution were used 2.2% per Myr (genus *Gymnogobius*; Harada et al. 2002) and 2.7% per Myr (genus *Gymnogobius*; Sota et al. 2005). Because the evolution rate of the nuclear *myh6* gene has not been estimated in Gobiidae, the analysis was performed only with mitochondrial *cytb* gene data.

The Bayesian Skyline Plots (BSPs) were implemented as coalescent models to estimate the most recent common ancestry (TMRCA) and effective population size changes. The BSP analysis was performed on BEAST version 1.10.4 (Suchard et al. 2018) and visualized in Tracer version 1.7.1 (Rambut et al. 2018). The analysis was performed sampling every 1000th generation, during 60

million generations. Burn-in the first 10% of the samples, and the analysis was performed independently for each evolutionary rate described above. As the substitution model used in the analysis, GTR+I+G, the nucleotide substitution model most suitable for the data, was selected based on the Akaike Information Criterion (AIC) in MrModeltest Version 2.4 (Nylander 2004). Other model parameters used default priors. Effective Sample Size (ESS) was above 200 in all analyses.

# Ⅲ. Results

## 1. Morphological analyses

The mean standard length (SL) ± SD of ice gobies in the South Sea of Korea was 44.9 ± 1.9 mm for females and 40.6 ± 1.9 mm for males, with females being significantly larger ($t_{117}$ = 12.124, $P < 0.001$ t-test). However, there was no significant difference in the number of vertebrae according to sex (females, 34.9 ± 0.5; males 34.8 ± 0.5; $t_{115}$ = 1.075, $P > 0.28$). ANOVA confirmed significant differences in SL among the Busan, Geoje, Tongyeong, and Wando populations [females: 46.0 ± 1.1, 45.0 ± 1.4, 45.4 ± 2.1, and 43.3 ± 1.7 mm ($F_{3,68}$ = 8.873, $P < 0.001$); males: 41.1±1.6, 40.2 ± 1.6, 41.7 ± 1.6, and 39.1 ± 2.0 mm ($F_{3,43}$ = 5.385, $P < 0.01$), respectively]. Using Scheffé's post-hoc test, females differed between the Wando population and Busan, Geoje, or Tongyeong population, and males differed between the Tongyeong and Wando populations (Table 3). The number of vertebrae (Busan: 34.9 ± 0.4; Geoje: 35.0 ± 0.5; Tongyeong: 34.9 ± 0.6; Wando: 34.8 ± 0.5) did not differ significantly among the local populations ($P > 0.54$).

The CDA generated two canonical discriminant functions; Function 1 contributed 67.3% of the total variance (eigenvalue = 3.915), and Function 2 contributed 32.7% (eigenvalue = 1.903). The reclassification rate was 100% in

all three groups. All canonical discriminant functions tend to be inconsistent in the order of magnitude of the standardized canonical discriminant function coefficients and the absolute values of the discriminant loadings in the structural matrix due to multicollinearity (Table 4). Therefore, we judged discriminant loading with priority in discriminant power. Function 1 in the order of post-orbital length (PoL), SL, caudal peduncle depth (CPD), and predorsal fin length (PDL); and for Function 2, Upper jaw length (UJL), Lower jaw length (LJL), and Inter-orbital distance (IoD) showed the largest absolute values (Table 4). The CDA results showed that the three groups are clearly separated by two functions (Fig. 3). For Function 1, Busan, Geoje, and Tongyeong vs. Wando, and Maizuru Bay, and Function 2, Wando vs. Maizuru Bay were segregated. One Wando individual overlapped with the Busan, Geoje, and Tongyeong group.

Table 3. Scheffé's post-hoc test of ANOVA for comparison of average standard length by localities

| Sex | Location | | Mean difference | *P* value |
|---|---|---|---|---|
| Female | Busan | Geoje | 1.035 | 0.328 |
| | | Tongyeong | 0.597 | 0.775 |
| | | Wando | 2.739* | 0.000 |
| | Geoje | Tongyeong | -0.438 | 0.880 |
| | | Wando | 1.704* | 0.023 |
| | Tongyeong | Wando | 2.142* | 0.003 |
| Male | Busan | Geoje | 0.861 | 0.694 |
| | | Tongyeong | -0.696 | 0.789 |
| | | Wando | 1.935 | 0.057 |
| | Geoje | Tongyeong | -1.557 | 0.220 |
| | | Wando | 1.074 | 0.540 |
| | Tongyeong | Wando | 2.631* | 0.006 |

*$P < 0.05$

Table 4. Structure matrix and **s**tandardized canonical coefficients based on 17 morphometric characters**.** Bold values have absolute values ≥ 0.3 in the structural matrix and, larger absolute values in the standardized canonical coefficients

| | Structure matrix | | Standardized canonical coefficients | |
|---|---|---|---|---|
| Measurement | Function 1 | Function 2 | Function 1 | Function 2 |
| Post-orbital length | **0.338** | 0.088 | 0.465 | 0.472 |
| Standard length | **0.327** | -0.138 | 0.787 | -0.779 |
| Caudal peduncle depth | **0.326** | -0.110 | 0.919 | -0.414 |
| Predorsal fin length | **0.316** | -0.100 | 0.884 | -0.017 |
| Preanus length | 0.252 | -0.038 | **-0.965** | **1.807** |
| Pectoral fin width | 0.213 | 0.160 | 0.337 | 0.327 |
| Head length | 0.185 | -0.063 | -0.587 | -0.056 |
| Upper jaw width | 0.172 | 0.096 | **2.388** | -0.105 |
| Pelvic fin length | 0.106 | 0.028 | 0.224 | 0.048 |
| Lower jaw width | 0.102 | 0.081 | **-2.558** | **0.989** |
| Upper jaw length | 0.285 | **-0.433** | **1.123** | **-1.442** |
| Lower jaw length | 0.177 | **-0.325** | -0.696 | 0.491 |
| Inter-orbital distance | -0.027 | **-0.305** | -0.495 | -0.463 |
| Eye diameter | 0.069 | -0.172 | -0.040 | -0.178 |
| Pectoral fin length | 0.066 | -0.161 | -0.277 | -0.343 |
| Snout length | 0.065 | -0.160 | -0.222 | -0.423 |
| Body depth | 0.077 | -0.156 | -0.785 | 0.096 |

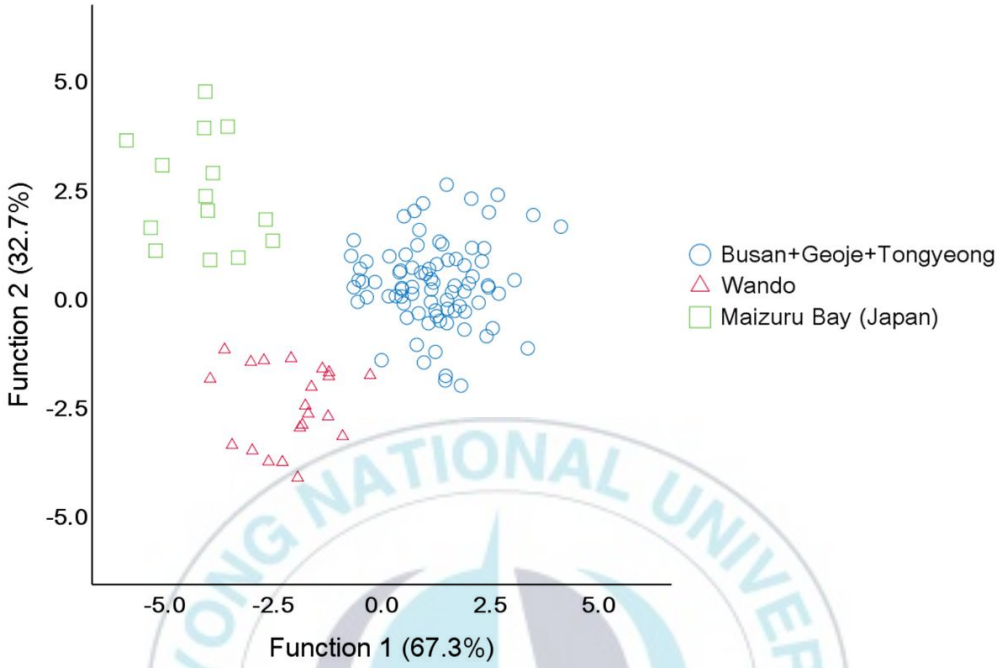


Fig. 3. Plots of canonical discriminant scores on the first and second canonical functions based on 17 morphometric characters

## 2. Genetic diversity and population genetic structure

Analysis of 827 bp of the mtDNA *cytb* gene in 120 specimens collected from the South Sea (Busan, Geoje, Tongyeong, and Wando) revealed 62 haplotypes (Table 2), indicating high haplotype diversity (h) (Table 5). The haplotype network formed a star-like structure in which singleton haplotypes and some rarer haplotypes were derived from a common haplotype by a difference of one or more bases (Fig. 4). H1 was shared by two haplotypes found in the Tsuruga, Tsushima, and Busan populations (IDs 10, 16, and 38; see Table 1) analyzed in Kokita and Nohara (2011), and only 12 haplotypes were found in more than two local populations within the South Sea (see Table 2). Two subclades were identified in the haplotype network. Subclade A contained haplotypes from northern Kyushu and the Seto Inland Sea close to the Korean Peninsula (IDs 15, 17, 22, 23, 24, 26, 28 and 38; see Table 1), including one individual each from Busan and Tongyeong and two from Geoje. Subclade B included the Goto and Busan haplotypes (IDs 18 and 38; see Table 1) analyzed in Kokita and Nohara (2011), five individuals from Wando, two from Busan, and one each from Geoje and Tongyeong (see Fig. 4). Pairwise $\Phi_{ST}$ values comparing the genetic differentiation of the South Sea populations did not differ significantly, ranging from −0.014 to 0.005 in the eastern part of the South Sea (Busan, Geoje, and Tongyeong), while Wando vs. Geoje and Tongyeong showed very weak genetic

differentiation ($\Phi_{ST}$ = 0.060 and 0.041; $P < 0.05$) (Table 6). Weak genetic differentiation was observed between the South Seas of Korea and West Sea of Japan populations ($\Phi_{ST}$ = 0.084; $P < 0.05$). Nevertheless, in AMOVA, it is considered more reasonable to divide into two groups (the South Sea of Korea and the West Sea of Japan populations vs. the Pacific Ocean population) rather than three groups (the South Sea of Korea vs. the West Sea of Japan populations vs. the Pacific Ocean population) (Tables 7 and 8). The South Sea of Korea and West Sea of Japan populations differed significantly from the Pacific Ocean population (Table 7).

For nDNA *myh6*, only four haplotypes were identified at two polymorphic sites, indicating low haplotype and nucleotide diversities (Table 5). In this study, 99 individuals from the South Sea of Korea and a haplotype found in all populations in the West Sea of Japan were assigned to N1. N2 shared a haplotype reported only in the Higashihiroshima and Busan populations (IDs 23 and 38; see Table 1) with that of the West Sea of Japan population in Kokita and Nohara (2011) (Fig. 5). A star-like structure from which a haplotype is derived by a single base difference was formed. The South Sea of Korea and West Sea of Japan populations showed one fixed difference (C/A SNP at position 229) from the Pacific Ocean population, as in Kokita and Nohara (2011). Pairwise $\Phi_{ST}$ values between each pair of local populations in the South Sea of Korea ranged from −0.031 to 0.050, indicating no genetic difference

(Table 6). However, the South Sea of Korea and West Sea of Japan populations differed significantly from the Pacific Ocean population (Table 7).

Table 5. Genetic diversity of the Korean Peninsula based on the mtDNA *cytb* and nDNA *myh6* sequences. n number of specimens, *Nh* number of haplotypes, *P* number of polymorphic sites, *h* haplotype diversity

| | Mitochondrial DNA *cytb* | | | | Nuclear DNA *myh6* | | | |
|---|---|---|---|---|---|---|---|---|
| Location | n | *Nh* | *P* | *h* | n | *Nh* | *P* | *h* |
| Busan | 30 | 25 | 38 | 0.9885 ± 0.0114 | 30 | 3 | 2 | 0.4759 ± 0.0914 |
| Geoje | 30 | 18 | 29 | 0.8207 ± 0.0737 | 30 | 3 | 2 | 0.2966 ± 0.0989 |
| Tongyeong | 30 | 24 | 37 | 0.9724 ± 0.0209 | 30 | 3 | 2 | 0.2966 ± 0.0989 |
| Wando | 30 | 13 | 21 | 0.8667 ± 0.0483 | 30 | 2 | 1 | 0.1287 ± 0.0792 |

Table 6. Pairwise Φst values for mtDNA *cytb* (below the diagonal) and nDNA *myh6* (above the diagonal). Negative values indicate excess heterozygotes and are estimated the zero

| | Busan | Geoje | Tongyeong | Wando |
|---|---|---|---|---|
| Busan | | -0.012 | -0.021 | 0.050 |
| Geoje | 0.005 | | -0.031 | -0.078 |
| Tongyeong | -0.009 | -0.014 | | 0.013 |
| Wando | 0.026 | 0.060* | 0.041* | |

*$P$ <0.05

Table 7. Analysis of molecular variance (AMOVA) of population structure in *L. petersii*. Statistical probabilities derived from 10000 permutations. Groups were allocated as the South Sea of Korea and West Sea of Japan populations vs. the Pacific Ocean population. Populations correspond to each location

| Locus | Source of variation | d.f. | *F* statistic | % Variation |
|---|---|---|---|---|
| *cytb* | Among groups ($F_{CT}$) | 1 | 0.731* | 73.08 |
| | Among populations within groups ($F_{SC}$) | 25 | 0.120* | 3.24 |
| | Within populations ($F_{ST}$) | 197 | 0.763* | 23.68 |
| | Total | 223 | | |
| *myh6* | Among groups ($F_{CT}$) | 1 | 0.701* | 70.09 |
| | Among populations within groups ($F_{SC}$) | 26 | 0.125* | 3.75 |
| | Within populations ($F_{ST}$) | 332 | 0.738* | 26.17 |
| | Total | 359 | | |

*$P$ <0.001

Table 8. Analysis of molecular variance (AMOVA) of population structure in *L. petersii*. Statistical probabilities derived from 10000 permutations. Groups were allocated as the South Sea of Korea vs. the West Sea of Japan populations vs. the Pacific Ocean population. Populations correspond to each location

| Locus | Source of variation | d.f. | *F* statistic | % Variation |
|---|---|---|---|---|
| *cytb* | Among groups ($F_{CT}$) | 2 | 0.546* | 54.59 |
| | Among populations within groups ($F_{SC}$) | 24 | 0.072* | 3.25 |
| | Within populations ($F_{ST}$) | 197 | 0.578* | 42.15 |
| | Total | 223 | | |
| *myh6* | Among groups ($F_{CT}$) | 2 | 0.522* | 52.15 |
| | Among populations within groups ($F_{SC}$) | 25 | 0.112* | 5.38 |
| | Within populations ($F_{ST}$) | 332 | 0.575* | 42.47 |
| | Total | 359 | | |

*$P$ <0.001

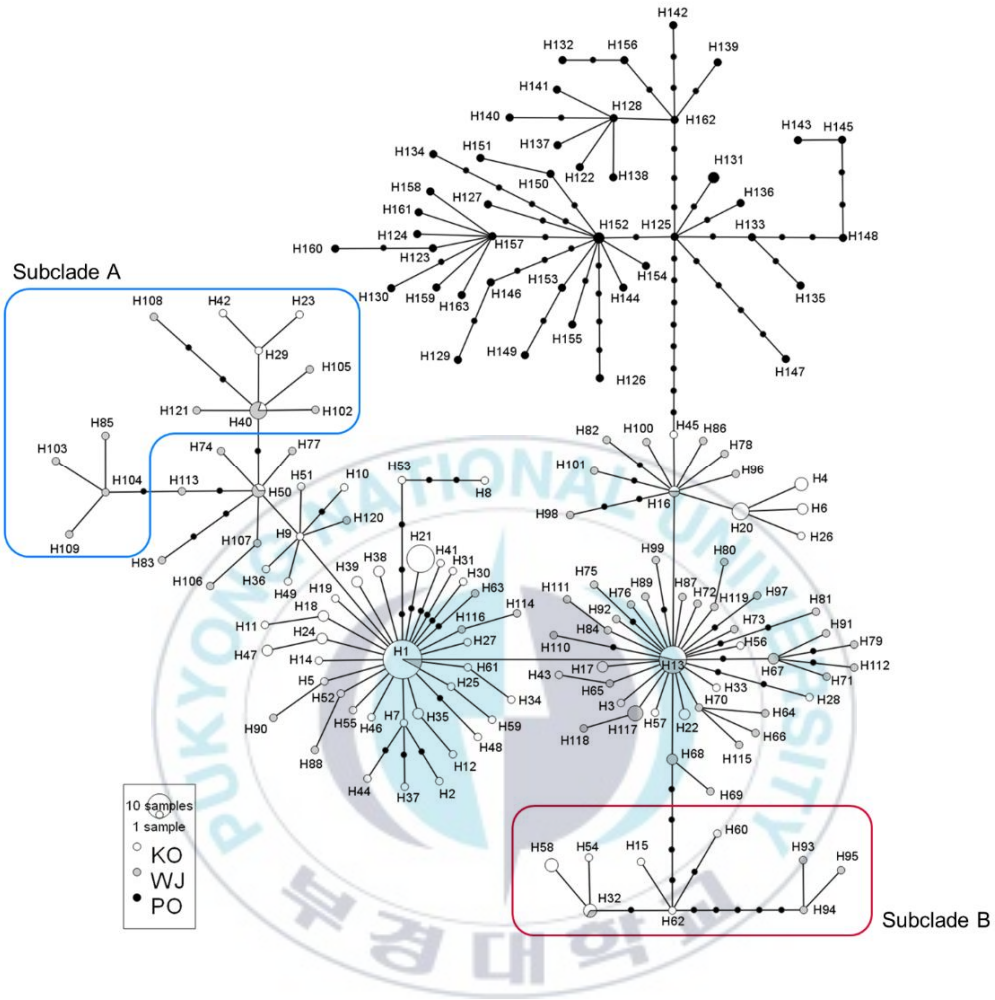


Fig. 4. Minimum spanning networks of the mtDNA *cytb*. KO South Sea of Korea, WJ West Sea of Japan, PO Pacific Ocean haplotypes. Subclade A (H23, H29, H40, H42, H85, H102, H103, H104, H105, H108, H109, H113, H121) and Subclade B (H15, H32, H54, H58, H60, H62, H93, H94, H95)

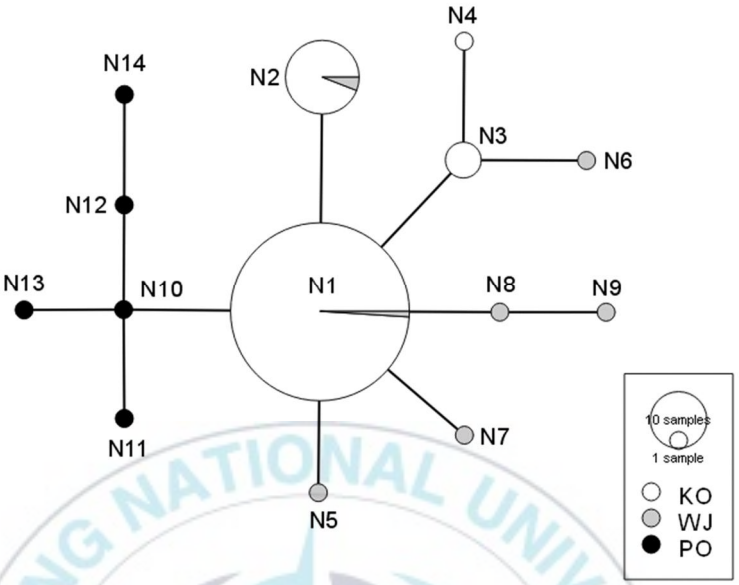


Fig. 5. Minimum spanning networks of the nDNA myh6. KO South Sea of Korea, WJ West Sea of Japan, PO Pacific Ocean haplotypes

## 3. Demographic history

The neutrality test yielded significant negative values for Tajima's *D* and Fu's *Fs*, and unimodal curves indicating a recent extreme population expansion were observed in the MMD analysis (Fig. 6 and Table 9). The SSD test and Harpending raggedness index showed that the MMD results were consistent with the distribution expected in the recent rapid population expansion model. The population expansion time estimated using the parameter τ was 100,000–120,000 years ago (0.10–0.12 Mya). Estimating the most recent common ancestor and more detailed effective population size changes using BSP, the most recent common ancestor of the South Sea of Korea and West Sea of Japan populations was from 0.619 Mya (95% CI 0.349–0.927) using 2.2% per Myr, and from 0.505 Mya (95% CI 0.283–0.755) using 2.7% per Myr. The effective size of the South Sea of Korea and West Sea of Japan populations expanded moderately 0.20–0.22 Mya, with extreme expansion 0.10–0.12 Mya. After 0.05–0.07 Mya, the effective population size did not change significantly (Fig. 7).

Table 9. Mismatch distribution analysis and neutrality test of the South Sea of Korea and West Sea of Japan populations

| Neutrality tests | | Mismatch distribution | | | | |
|---|---|---|---|---|---|---|
| Tajima's *D* | Fu's *Fs* | Hri | SSD | τ (95% CI) | t (Mya) (rate: $1.10\times10^{-8}$) (95% CI) | t (Mya) (rate: $1.35\times10^{-8}$) (95% CI) |
| -2.358* | -25.43* | 0.020 | 0.002 | 2.21 (1.39–5.79) | 0.12 (0.08–0.32) | 0.10 (0.06–0.26) |

*$P$ <0.001

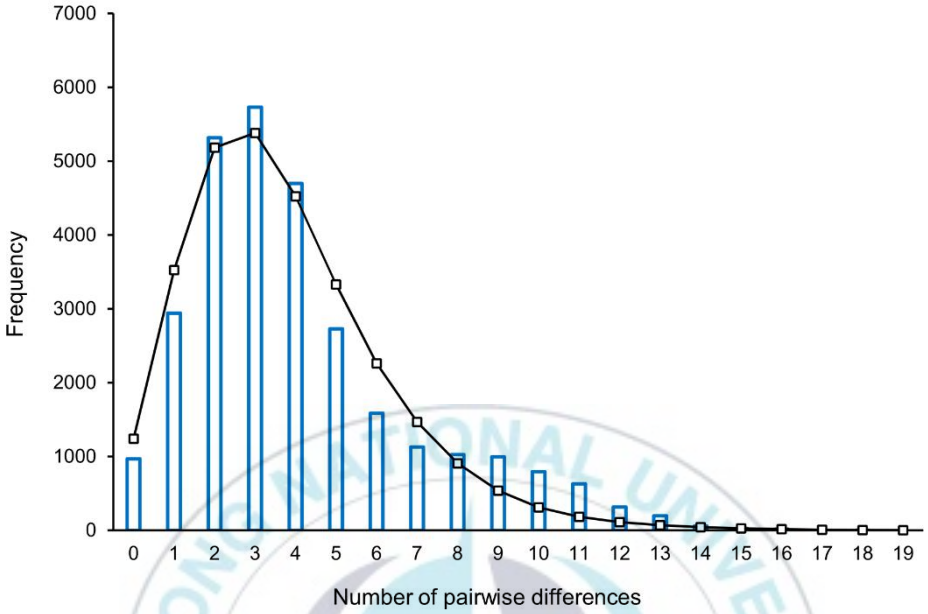


Fig. 6. Mismatch distribution analysis of the South Sea of Korea and West Sea of Japan populations

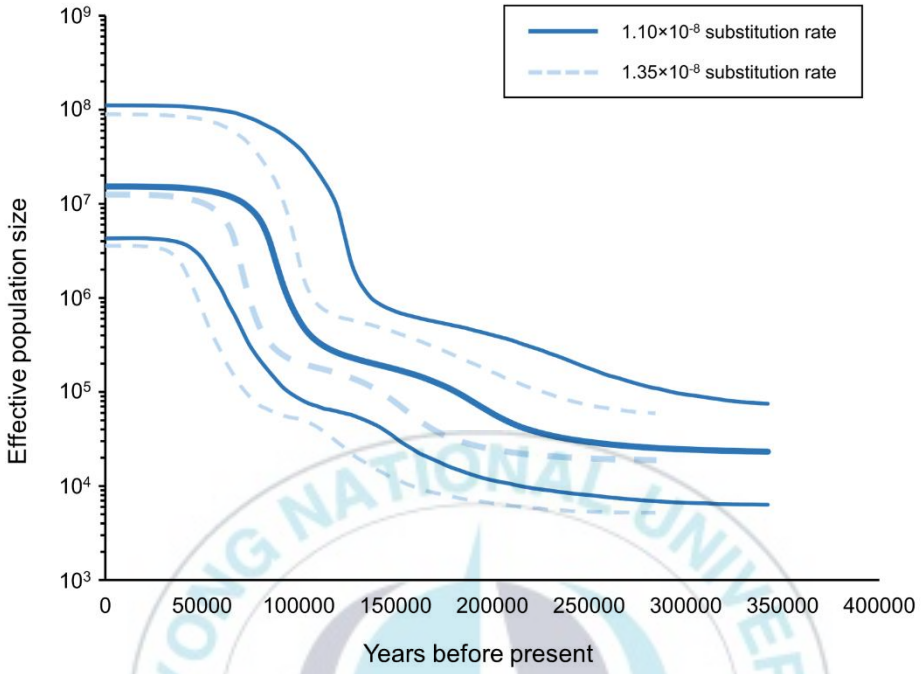


Fig. 7. Bayesian skyline plots for the South Sea of Korea and West Sea of Japan populations. The central bold lines indicate the median value for the effective population size and the narrow lines point to the 95% upper and lower confidence limits (95% HPD)

# Ⅳ. Discussion

This study performed phenotypic and molecular analyses of 120 ice gobies collected from four localities in the South Sea of Korea (Busan, Geoje, Tongyeong, and Wando). In phenotype analysis, meristic characters were similar, but significant differences in morphometric characters. According to Matsui (1986), the SL difference between the sexes is due to sexual dimorphism. The SL of females differed significantly between Wando and the other three local populations (Busan, Geoje, and Tongyeong) and in males between Wando and Tongyeong only.

Ice gobies feed mainly on copepods and amphipods before ascending upstream (Matsui 1986). The southeastern part of the Korean Peninsula (Busan, Geoje, and Tongyeong) has very high primary productivity compared with Wando, due to expansion of the North Korean Cold Current and the influence of the Nakdong River (Jeong et al. 2013; Kim et al. 2017b; Park et al. 2020). The numbers of copepods and amphipods are positively correlated with the chlorophyll *a* concentration (Kang et al. 2018). The average chlorophyll *a* concentration from spring to summer over the past decade was lower in Wando (1.28 μg/L in the surface layer and 1.31 μg/L in the bottom layer) than in Busan (2.37 and 1.88 μg/L, respectively), Geoje (2.88 and 2.67 μg/L), and Tongyeong (1.99 and 1.87 μg/L) (Marine Environment Information System,

https://www.meis.go.kr/). The productivity in the eastern part of the South Sea is higher from spring to summer than in the western part (Jeong et al. 2004; Seo et al. 2018). As a result, the difference in SL in these localities is likely driven by environmental factors, such as the chlorophyll *a* concentration.

The CDA results showed separation into three groups (Busan, Geoje, and Tongyeong vs. Wando vs. Maizuru Bay). The main characteristics that separating the three groups are PoL, SL, CPD, and PDL. Wando ice goby is separated from Maizuru Bay ice goby by UJL, LJL, and IoD. The reclassification rate is 100%, and according to the criteria (> 85%) presented by Silva (2003), the three groups seem to have different morphological adaptation. Morphometric characters are known to be more susceptible to environmental factors than meristic characters (Lindsey 1988; Swain et al. 2001). Therefore, our ANOVA and CDA results support the fact that the phenotype is short-term and more useful for detecting variations caused by environmental factors, suggested by Grant and Utter (1984). However, although the SL and number of vertebrae are associated traits (Lindsey 1975), we did not find differences in vertebrae number among the study localities. According to Hirase et al. (2020), the SL of the ice goby is regulated by the NPY gene, and the vertebrae number can be explained genetically by determining which East Sea and Pacific Ocean populations share the same nuclear genotype. More detailed field studies of habitat conditions with analyses of NPY and nuclear genotypes are needed to

identify the factors causing the differences in morphometric characters among the study populations.

The high haplotype and low nucleotide diversities of the mtDNA *cytb* region in the South Sea of Korea populations are related to the recent rapid population expansion following bottleneck during the founder events. This is supported by the significant negative value of Fu's *Fs*, indicating an excess of new mutations following a rapid expansion in population size. nDNA diversity is higher immediately following population expansion, whereas mtDNA diversity is higher after the population recovers from a bottleneck. As populations expand, the mitochondrial genome evolves faster than nuclear loci encoding proteins, resulting in accumulation of mutations (Grant and Bowen 1998). Therefore, the nDNA *myh6* region may show relatively lower genetic diversity compared with mtDNA *cytb*.

While we hypothesized that a high degree of differentiation according to locality exists due to the unique life cycle of the ice goby, we found that ice gobies in Busan, Geoje, and Tongyeong are genetically close to those in northern Kyushu and the Seto Inland Sea (pairwise $\Phi_{ST}$ 0.049–0.091). The Tsushima Warm Current is thought to be responsible for the dispersion of gobies and other fish, which has promoted gene flow in various fish species in the Northwest Pacific (e.g., Liu et al. 2006; Nohara et al. 2010; Yu et al. 2016; Gwak and Roy 2021). The spatial scale of this dispersion varies between species. Dispersion also expands to the Pacific Ocean (through the Tsugaru Strait;

Akihito et al. 2008, Kokita and Nohara 2011, Hirase and Ikeda 2014) or Hokkaido (Okazaki et al. 2020) or spread to both (Hirase et al. 2012). In comparison, the Wando population, which is relatively far from the Japanese Archipelago, had a pairwise $\Phi_{ST}$ of 0.117 with the West Sea of Japan population. This is a relatively high value, indicating moderate genetic differences (Hartl and Clark 1997). Therefore, considering the $\Phi_{ST}$ values (0.026–0.060 for *cytb*) between the populations of Wando and Busan, Tongyeong, or Geoje, gene flow likely occurs in the South Sea of Korea populations due to the dispersion of juveniles. However, the complex oceanographic conditions near Wando population acted as a weak barrier to gene flow with the West Sea of Japan population. The tide-induced residual current flows eastward near Wando (the western part of the South Sea) during spring tide but stagnates or flows westward during neap tide. In particular, during the early life stage (spring to summer) of ice goby, the tidal-induced residual currents tend to mainly flow westward due to the prevailing southeasterly wind (Kim and Bae 2011). Similar results were seen in inshore hagfish (*Eptatretus burgeri*) (Song et al. 2020). In addition, among the South Sea of Korea populations, the neutrality test did not support rapid expansion of population size in the Wando population only; compared with the other local populations, the genetic diversity of the Wando population was low, and haplotype H21 appeared almost exclusively in Wando. Wando has low salinity/temperature maintained by the influx of fresh water from China and the East China Sea Coastal Water during the last glacial period

(Kim et al. 2020). Because ice gobies live in shallow coastal and river mouth waters (Matsui 1986), low salinity may not harm them as much. Thus, since retrograde travel in the Tsushima Warm Current is difficult, it is thought that the local Wando population maintained its population as a refugia, unlike the South Sea of Korea populations, which would have spent the last glacial period on the continental shelf on the eastern side of the Korea Strait, similar to the West Sea of Japan populations, and would have been exposed to seawater (Kokita and Nohara 2011). This presumably gave rise to the weak genetic differences between the Wando and other local populations. The Tsushima Warm Current likely had a substantial influence on marine organisms in the South Sea of Korea, previously and currently. However, mtDNA markers may be less useful for explaining recent differentiation, whereas microsatellites reveal population genetic structures due to their high polymorphism (O'Connell and Wright 1997; Toews and Belsford 2012). Therefore, further studies using more sensitive microsatellite markers are needed.

The historical analysis of the South Sea of Korea and West Sea of Japan populations confirmed extreme expansion of the effective population size. The BSP analysis showed a slight population expansion at 0.20–0.22 Mya, coinciding with the end of the little ice age (Marine isotope stages (MIS) 7a or 7b) within the Aveley interglacial period (MIS 7) (Dutton et al. 2009; Columbu et al. 2019), when post-glacial expansion of many species populations occurred (Hewitt 2004). Common haplotypes (H1 and H13) at the centers of the star-like

haplotype networks can be regarded as ancestral haplotypes (Castelloe and Templeton 1994). During MIS 7a or 7b, individuals represented by this haplotype spread to other locations, including the southern Korean Peninsula. There was rapid population expansion at 0.10–0.12 Mya, which coincided with MIS 5c or 5e within the last interglacial period. This rapid population expansion can be attributed to habitat expansion and improved climate conditions (Ohshima 1990; Dutton and Lambeck 2012; Kohfeld and Chase 2017). These findings were consistent with the results of MMD and neutrality tests, with a star-like haplotype network in which rare haplotype/singletons have one or more base differences from a common haplotype, supporting the rapid population expansion.

In conclusion, this study for the first time revealed morphological and molecular variations in 120 ice gobies collected from four localities in the South Sea of Korea (Busan, Geoje, Tongyeong, and Wando), together with the comparison of Japanese populations. The CDA results based on 17 morphometric characters indicated that ice gobies were divided into the eastern part of the South Sea of Korea (Busan, Geoje, and Tongyeong) vs. the western part of the South Sea of Korea (Wando) vs. the West Sea of Japan (Maizuru Bay), although there was no difference in meristic characters, such as the number of vertebrae. The genetic analysis confirmed that all four South Sea populations had the same gene pool despite their weak genetic differentiation between the eastern and western populations, slightly differing from the

morphological analysis. To resolve the discordance, further studies using more sensitive markers such as microsatellites are needed.

# Ⅴ. References


Aguilar C, Miller MJ, Loaiza JR et al (2019) Tempo and mode of allopatric divergence in the weakly electric fish *Sternopygus dariensis* in the Isthmus of Panama. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-55336-y

Akihito, Fumihito A, Ikeda Y et al (2008) Evolution of Pacific Ocean and the Sea of Japan populations of the gobiid species, *Pterogobius elapoides* and *Pterogobius zonoleucus*, based on molecular and morphological analyses. Gene 427:7–18. https://doi.org/10.1016/j.gene.2008.09.026

Arakawa T, Kanno Y, Akiyama N et al (1999) Stages of Embryonic Development of the Ice Goby (Shiro-uo), *Leucopsarion petersii*. Zoolog Sci 16:761–773. https://doi.org/10.2108/zsj.16.761

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. Mol Ecol 13:729–744. https://doi.org/10.1046/J.1365-294X.2003.02063.X

Begg GA, Hare JA, Sheehan DD (1999) The role of life history parameters as indicators of stock structure. Fish Res 43:141–163. https://doi.org/10.1016/S0165-7836(99)00071-5

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequences of primates: Tempo and mode of evolution. J Mol Evol 18:225–239. https://doi.org/10.1007/BF01734101

Castelloe J, Templeton AR (1994) Root probabilities for intraspecific gene trees under neutral coalescent theory. Mol Phylogenet Evol 3:102–113. https://doi.org/10.1006/mpev.1994.1013

Columbu A, Spötl C, De Waele J et al (2019) A long record of MIS 7 and MIS 5 climate and environment from a western Mediterranean speleothem (SW Sardinia, Italy). Quat Sci Rev 220:230–243. https://doi.org/10.1016/j.quascirev.2019.07.023

Dutton A, Bard E, Antonioli F et al (2009) Phasing and amplitude of sea-level and climate change during the penultimate interglacial. Nat Geosci 2:355–359. https://doi.org/10.1038/ngeo470

Dutton A, Lambeck K (2012) Ice volume and sea level during the last interglacial. Science 337:216–219. https://doi.org/10.1126/science.1205749

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to

human mitochondrial DNA restriction data. Genetics 131:479–491. https://doi.org/10.1093/genetics/131.2.479

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925. https://doi.org/10.1093/genetics/147.2.915

Gorbarenko SA, Southon JR (2000) Detailed Japan Sea paleoceanography during the last 25 kyr: Constraints from AMS dating and $\delta^{18}O$ of planktonic foraminifera. Palaeogeogr Palaeoclimatol Palaeoecol 156:177–193. https://doi.org/10.1016/S0031-0182(99)00137-6

Grant WS, Utter FM (1984) Biochemical population genetics of pacific herring (*Clupea pallasi*). Can J Fish Aquat Sci 41:856–864. https://doi.org/10.1139/f84-102

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and

anchovies and lessons for conservation. J Hered 89:415–426. https://doi.org/10.1093/jhered/89.5.415

Gwak WS, Roy A (2021) Genetic diversity and population structure of Pacific herring *Clupea pallasii* in the Northeast Asia inferred from mtDNA marker. Ecol Genet Genomics 18:100076. https://doi.org/10.1016/j.egg.2020.100076

Harada S, Jeon SR, Kinoshita I et al (2002) Phylogenetic relationships of four species of floating gobies (*Gymnogobius*) as inferred from partial mitochondrial cytochrome b gene sequences. Ichthyol Res 49:324–332. https://doi.org/10.1007/s102280200048

Harrison RG (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. Trends Ecol Evol 4:6–11. https://doi.org/10.1016/0169-5347(89)90006-2

Hartl DL, Clark AG (1997). Principles of population genetics. Sinauer Associates, Sunderland

He L, Mukai T, Hou Chu K et al (2015) Biogeographical role of the Kuroshio Current in the amphibious mudskipper *Periophthalmus modestus* indicated by mitochondrial DNA data. Sci Rep 5:1–12. https://doi.org/10.1038/srep15645

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Phil Trans R Soc Lond B 359:183–195. http://doi.org/10.1098/rstb.2003.1388

Hirase S, Ikeda M, Kanno M, Kijima A (2012) Phylogeography of the intertidal goby *Chaenogobius annularis* associated with paleoenvironmental changes around the Japanese Archipelago. Mar Ecol Prog Ser 450:167–179. https://doi.org/10.3354/MEPS09584

Hirase S, Ikeda M (2014) Divergence of mitochondrial DNA lineage of the rocky intertidal goby *Chaenogobius gulosus* around the Japanese Archipelago: Reference to multiple Pleistocene isolation events in the Sea of Japan. Mar Biol 161:565–574. https://doi.org/10.1007/s00227-013-2359-5

Hirase S, Kokita T, Nagano AJ, Kikuchi K (2020) Genomic and phenotypic consequences of two independent secondary contact zones between allopatric lineages of the anadromous ice goby *Leucopsarion petersii*. Heredity (Edinb) 124:223–235. https://doi.org/10.1038/s41437-019-0239-6

Hurst GD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of

inherited symbionts. Proc R Soc B Biol Sci 272:1525–1534. https://doi.org/10.1098/RSPB.2005.3056

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome b gene of mammals. J Mol Evol 32:128–144. https://doi.org/10.1007/BF02515385

Jang SH, Lee JW, Kim JK (2019) Molecular and Morphometric Variations in the Sea Raven, *Hemitripterus villosus* from Korea, with Its Implication on Fisheries Management. Ocean Sci J 54:419–433. https://doi.org/10.1007/s12601-019-0021-y

Jeong DH, Shin HH, Jung SW, Lim Dl (2013) Variations and Characters of Water Quality during Flood and Dry Seasons in the Eastern Coast of South Sea, Korea. Environ Biol Res 31:19–36. https://doi.org/10.11626/kjeb.2013.31.1.019

Jeong SJ, Yu OH, Suh HL (2004) Seasonal Variation and Feeding Habits of Amphipods Inhabiting Zostera marina Beds in Gwangyang Bay, Korea. Korean J Fish Aquat Sci 37:122–128. https://doi.org/10.5657/kfas.2004.37.2.122

Kang HS, Seo MH, Yang YS et al (2018) Zooplankton and Neustonic Microplastics in the Surface Layer of Yeosu Coastal Areas. Environ Biol Res 36:11–20. https://doi.org/10.11626/kjeb.2018.36.1.011

Kato S, Arakaki S, Kikuchi K, Hirase S (2020) Complex phylogeographic patterns in the intertidal goby *Chaenogobius annularis* around Kyushu Island as a boundary zone of three different seas. Ichthyol Res 68:86–100. https://doi.org/10.1007/s10228-020-00772-4

Kim DS, Bae SW (2011) A Study on the Transport of Anchovy *Engraulis japornicus* Egg-larvae in the South Sea of Korea. J Environ Sci Int 20:1403–1415. https://doi.org/10.5322/jes.2011.20.11.1403

Kim HC, Son S, Kim YH et al (2017b) Remote sensing and water quality indicators in the Korean West coast: Spatio-temporal structures of MODIS-derived chlorophyll-a and total suspended solids. Mar Pollut Bull 121:425–434. https://doi.org/10.1016/j.marpolbul.2017.05.026

Kim JK, Park JH, Kim YS et al (2008) Geographic variations in Pacific sand eels *Ammodytes personatus* (Ammodytidae) from Korea and Japan using multivariate morphometric analysis. J Ichthyol 48:904–910. https://doi.org/10.1134/S003294520810007X

Kim JK, Bae SE, Lee SJ, Yoon MG (2017a) New insight into hybridization and unidirectional introgression between *Ammodytes* japonicus and *Ammodytes heian* (Trachiniformes, Ammodytidae). PLoS One 12:e0178001. https://doi.org/10.1371/journal.pone.0178001

Kim YM, Yi SH, Jun CP et al (2020) New findings on palynofacies characteristics of semi-enclosed deep-sea environments in the East Sea over 2 million years. Sci Rep 10:1–10. https://doi.org/10.1038/s41598-020-73493-3

Kinsey ST, Orsoy T, Bert TM, Mahmoudi B (1994) Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. Mar Biol 118:309–317. https://doi.org/10.1007/BF00349798

Kohfeld KE, Chase Z (2017) Temporal evolution of mechanisms controlling ocean carbon uptake during the last glacial cycle. Earth Planet Sci Lett 472:206–215. https://doi.org/10.1016/j.epsl.2017.05.015

Kokita T, Nohara K (2011) Phylogeography and historical demography of the anadromous fish *Leucopsarion petersii* in relation to geological history and oceanography around the Japanese Archipelago. Mol Ecol 20:143–164. https://doi.org/10.1111/j.1365-294X.2010.04920.x

Leigh JW, Bryant D (2015) POPART: Full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410

Li C, Ortí G, Zhang G, Lu G (2007) A practical approach to phylogenomics: The phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evol Biol 7:44. https://doi.org/10.1186/1471-2148-7-44

Lindsey CC (1975) Pleomerism, the Widespread Tendency Among Related Fish Species for Vertebral Number to be Correlated with Maximum Body Length. J Fish Res Board Canada 32:2453–2469. https://doi.org/10.1139/f75-283

Lindsey CC (1988) Factors controlling meristic variation. In: Hoar WS, Randall DJ (eds) Fish Physiology, Academic Press, San Diego, pp 197–274

Liu JX, Gao TX, Yokogawa K, Zhang YP (2006) Differential population structuring and demographic history of two closely related fish species, Japanese sea bass (*Lateolabrax japonicus*) and spotted sea bass (*Lateolabrax maculatus*) in Northwestern Pacific. Mol Phylogenet Evol 39:799–811. https://doi.org/10.1016/j.ympev.2006.01.009

Matsui S (1986) Studies on the ecology and the propagation of the ice goby, *Leucopsarion petersi* Hilgendorf. J Fac Agric 40:135–174. https://doi.org/10.5109/1936951

Murta A (2000) Morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic: implications for stock identification. ICES J Mar Sci 57:1240–1248. https://doi.org/10.1006/jmsc.2000.0810

Nohara K, Takeuchi H, Tsuzaki T et al (2010) Genetic variability and stock structure of red tilefish *Branchiostegus japonicus* inferred from mtDNA sequence analysis. Fish Sci 76:75–81. https://doi.org/10.1007/s12562-009-0188-8

Nylander JAA (2004) MrModeltest Version 2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University, Uppsala

O'Connell M, Wright JM (1997) Microsatellite DNA in fishes. Rev Fish Biol Fish 7:331–363. https://doi.org/10.1023/A:1018443912945

Ohshima K (1990) The history of straits around the Japanese Islands in the Late-Quaternary. Quat Res 29:193–208. https://doi.org/10.4116/jaqua.29.193

Okazaki T, Stevenson DE, Kai Y et al (2020) Genetic population structure and demographic history of a pelagic lumpsucker, *Aptocyclus ventricosus*. Environ Biol Fishes 103:283–289. https://doi.org/10.1007/s10641-020-00955-y

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR. University of Hawaii, Honolulu

Park JE, Park KA, Kang CK, Kim GB (2020) Satellite-Observed Chlorophyll-a Concentration Variability and Its Relation to Physical Environmental Changes in the East Sea (Japan Sea) from 2003 to 2015. Estuaries and Coasts 43:630–645. https://doi.org/10.1007/s12237-019-00671-6

Rambaut A, Drummond AJ, Xie D et al (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol 67:901–904. https://doi.org/10.1093/sysbio/syy032

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569. https://doi.org/10.1093/oxfordjournals.molbev.a040727

Rohlf JF (1973) Algorithm 76. Hierarchical clustering using the minimum spanning tree. Comput J 16:93–95.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC et al (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Mol Biol Evol 34:3299–3302. https://doi.org/10.1093/molbev/msx248

Schlitzer R (2017) Ocean Data View, http://odv.awi.de.

Seo MH, Choi SY, Park EO et al (2018) Species Diversity of Planktonic Copepods and Distribution Characteristics of Its Major Species in Coastal Waters of Korea. Environ Biol Res 36:525–537. https://doi.org/10.11626/kjeb.2018.36.4.525

Sievers F, Higgins DG (2018) Clustal Omega for making accurate alignments of many protein sequences. Protein Sci 27:135–145. https://doi.org/10.1002/pro.3290

Silva A (2003) Morphometric variation among sardine (*Sardina pilchardus*) populations from the northeastern Atlantic and the western Mediterranean. ICES J Mar Sci 60:1352–1360. https://doi.org/10.1016/S1054-3139(03)00141-3

Song YS, Bae SE, Kang JH et al (2020) Cryptic diversity in the inshore hagfish, *Eptatretus burgeri* (Myxinidae, Pisces) from the northwest Pacific. Mitochondrial DNA Part B Resour 5:3428–3432. https://doi.org/10.1080/23802359.2020.1823256

Sota T, Mukai T, Shinozaki T et al (2005) Genetic differentiation of the gobies *Gymnogobius castaneus* and *G. taranetzi* in the region surrounding the Sea of Japan as inferred from a mitochondrial gene genealogy. Zoolog Sci 22:87–93. https://doi.org/10.2108/zsj.22.87

Suchard MA, Lemey P, Baele G et al (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol 4:vey016. https://doi.org/10.1093/ve/vey016

Swain DP, Frank KT, Maillet G (2001) Delineating stocks of Atlantic cod (*Gadus morhua*) in the Gulf of St Lawrence and Cabot Strait areas using vertebral number. ICES J Mar Sci 58:253–269. https://doi.org/10.1006/JMSC.2000.1007

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595. https://doi.org/10.1093/genetics/123.3.585

Toews DPL, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. 21:3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

Wilson AC, Cann RL, Carr SM et al (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biol J Linn Soc 26:375–400. https://doi.org/10.1111/j.1095-8312.1985.tb02048.x

Yoo DG, Kim KJ, Lee GS et al (2017) Seismic stratigraphic reconstruction of late Pleistocene lowstand wedges on the shelf margin and trough region of the Korea Strait: a review. Geosci J 21:933–949. https://doi.org/10.1007/s12303-017-0043-3

Yu HJ, Kai Y, Kim JK (2016) Genetic diversity and population structure of *Hyporhamphus sajori* (Beloniformes: Hemiramphidae) inferred from mtDNA control region and msDNA markers. J Fish Biol 89:2607–2624. https://doi.org/10.1111/jfb.13152