

Genetic Distances and Variations of Three Clupeid Species Determined by PCR Technique

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ABSTRACT : In this study, seven oligonucleotides primers were shown to generate the shared loci, specific loci, unique shared loci to each species and shared loci by the three species which could be obviously calculated. Euclidean genetic distances within- and between-species were also calculated by complete linkage method with the sustenance of the hierarchical dendrogram program Systat version 13. The genomic DNA isolated from herring (*Clupea pallasii*), Korean anchovy (*Coilia nasus*) and large-eyed herring (*Harengula zunashi*), respectively, in the Yellow Sea, were amplified several times by PCR reaction. The hierarchical dendrogram shows three chief branches: cluster 1 (PALLASII 01, 02, 03, 04, 06 and 07), cluster 2 (NASUS 08, 09, 10, 11, 12, 13 and 14), and cluster 3 (ZUNASHI 15, 16, 17, 18, 19, 20, 21 and PALLASII 05). In three clupeid species, the shortest genetic distance displaying significant molecular difference was between individual PALLASII no. 03 and PALLASII no. 02 (0.018). Individual no. 06 of PALLASII was most distantly related to NASUS no. 11 (genetic distance = 0.318). Individuals from herring (*C. pallasii*) species (0.920) exhibited higher bandsharing values than did individuals from Korean anchovy (*C. nasus*) species (0.872) ($P < 0.05$). As a result, this PCR analysis generated on the genetic data displayed that the herring (*C. pallasii*) species was widely separated from Korean anchovy (*C. nasus*) species. Reversely, individuals of Korean anchovy (*C. nasus*) species were a little closely related to those of large-eyed herring (*H. zunashi*) species.

Key words : Clupeid, *Clupea nasus*, *Clupea pallasii*, Euclidean genetic distance, *Harengula zunashi*

INTRODUCTION

The authors have investigated three species of Clupeidae such as herring (*Clupea pallasii*), Korean anchovy (*Coilia nasus*) and large-eyed herring (*Harengula zunashi*), belonging to the order Clupeiformes. The fish is also indigenous to some parts of the southern regions of the Yellow Sea. Fishes are the most popular marine products in Korea because of their taste and nutritional value, and Koreans consume them in large quantities. Especially, Korean anchovy

and large-eyed herring are widely distributed in the entirety of brackish-water habitats and seawater areas of the Yellow Sea in the Korean Peninsula, as well as in several areas in China. However, in spite of their economic and scientific consequences, a little information currently exist regarding the taxonomical (Kim et al., 2001), physical (An et al., 2013) and nutritional (Heu et al., 2012) levels only of clupeid species in Korea. During the last two decades, environmental contamination and various environmental disruptions by industries and city sewage, have threatened

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the coastal fisheries, and then reduction of individual number of this fish is an increasing trend in the 2000s. This study attempt is to elucidate the genetic distances and variations within and between herring, Korean anchovy and large-eyed herring species from the Yellow Sea of the Korean Peninsula.

MATERIALS AND METHODS

Muscle tissues were obtained separately from individuals of three clupeid species such as herring (*C. pallasii*), Korean anchovy (*C. nasus*) and large-eyed herring (*H. zunashi*) from the Yellow Sea of the Korean Peninsula, respectively. Three species of fish muscle was collected in sterile tubes, placed on ice immediately, and stored under refrigeration until needed. Genomic DNA was extracted and purified under the conditions described previously (Yoon & Kim, 2004; Song & Yoon, 2013). The DNA pellets were incubation-dried for 2 hrs, held at -40°C until analysis, and then dissolved in the TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). The concentrations of the extracted genomic DNA samples were estimated based on the absorbance at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK). PCR analysis was performed on DNA samples extracted from a total of 21 individuals using seven decamer primers. Seven oligonucleotides primers (BION-07, BION-10, BION-11, BION-18, BION-25, BION-27 and BION-40; Bioneer Co. Daejeon, Korea) were displayed to generate the shared loci, specific loci, unique shared loci to each species and shared loci by the three species which could be obviously scored. The degree of variability was calculated by use of the Dice coefficient (F), which is given by the formula: $F = 2 \text{ nab} / (\text{na} + \text{nb})$, where nab is the number of bands shared between the samples a and b, na is the total number of bands for sample a and nb is the total number of bands for sample b (Jeffreys & Morton, 1987; Yoke-Kqueen & Radu, 2006). Euclidean genetic distances within- and between-species

were also calculated by complete linkage method with the support of the hierarchical dendrogram program Systat version 13 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The genomic DNA isolated from herring (*C. pallasii*), Korean anchovy (*C. nasus*) and large-eyed herring (*H. zunashi*) in the Yellow Sea, respectively, were amplified several times by PCR reaction, as shown in Fig. 1. Simi-

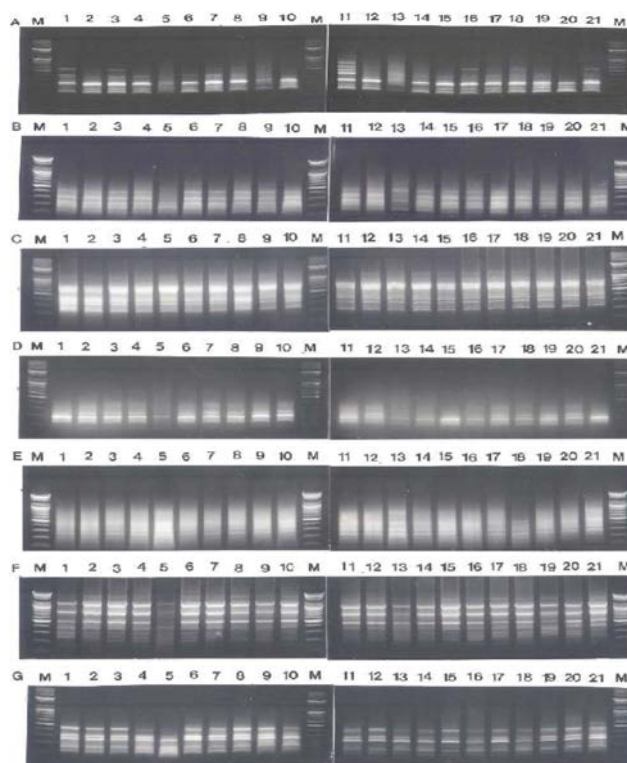


Fig. 1. PCR-based electrophoretic profiles of *C. pallasii* (lane 1-7), *C. nasus* (lane 8-14) and *H. zunashi* (lane 15-21), respectively, from the Yellow Sea of the Korean Peninsula, amplified by oligonucleotides primers BION-07 (A), BION-10 (B), BION-11 (C), BION-18 (D), BION-25 (E), BION-27 (F) and BION-40 (G). Each lane shows different individual DNA samples used. Amplified products were electrophoresed on a 1.4% agarose gel and detected by staining with ethidium bromide. 100 bp ladder markers were used as a DNA molecular size marker (M).

larity matrix including bandsharing values (BS) and genetic differences was calculated using Nei and Li's index of the similarity of clupeid individuals from the Yellow Sea of the Korean Peninsula, respectively. The band-sharing value between individuals no. 02 and no. 03 was 0.976 within the herring species (*C. pallasii*), which was the highest value identified among the three species (unpublished data). In the present study, averagely, an oligonucleotides primer generated 40.3 amplified products per primer in the herring species, 37.9 in Korean anchovy species and 39.3 in large-eyed herring species, as demonstrated in Table 1. Here, the seven oligonucleotides primers BION-07, BION-10, BION-11, BION-18, BION-25, BION-27 and BION-40 were used to generate the unique shared loci to each species and shared loci by the three species, as illustrated in Table 2. 259 unique shared loci to each species, with an

average of 37 per primer, were observed in the herring species, and 217 loci, with an average of 31 per primer, were observed in the Korean anchovy species. As regards average bandsharing value (BS) results, individuals from herring (*C. pallasii*) species (0.920) exhibited higher bandsharing values than did individuals from Korean anchovy (*C. nasus*) species (0.872) ($P < 0.05$), as illustrated in Table 3. DNA fragments attained by seven decamer primers ranged in size from 50 bp to 2,200 bp in the three clupeid species, as displayed in Fig. 2. The higher fragment sizes ($>1,000$ bp) are much more observed in the herring species. Many researchers studied the sizes of DNA fragments in the PCR profiles of barramundi (*Lates calcarifer*) (Partis & Wells, 1996), four species of the Mullidae family (Mamuris et al., 1999), the eastern Pacific yellowfin tuna (*Thunnus albacares*) (Diaz-Jaimes & Uribe-Alcocer, 2003), five species

Table 1. The number of average loci per lane and specific loci by PCR inquiry using 7 oligonucleotides primers in herring (*C. pallasii*), Korean anchovy (*C. nasus*) and large-eyed herring (*H. zunashi*) species

Item	No. of average loci per lane			No. of specific loci		
	CP	CN	HZ	CP	CN	HZ
Primer						
BION-07	7.14 (50)	8.14 (57)	8.57 (60)	1	8	4
BION-10	5.57 (39)	4.71 (33)	5.29 (37)	18	26	16
BION-11	3.86 (27)	3.14 (22)	3.14 (22)	6	1	8
BION-18	7.57 (53)	6 (42)	7 (49)	11	21	0
BION-25	6.71 (47)	7.43 (52)	7 (49)	12	10	0
BION-27	4.86 (34)	4.57 (32)	5.57 (39)	6	4	4
BION-40	4.57 (32)	3.86 (27)	2.71 (19)	11	11	5
Total no.	40.3 (282)	37.9 (265)	39.3 (275)	65	81	37
Average no. per primer	40.3	37.9	39.3	9.3	11.6	5.3

CP: *Clupea pallasii*, CN: *Coilia nasus*, HZ: *Harengula zunashi*

Table 2. The number of unique loci to each species and number of shared loci by the three species generated by PCR analysis using 7 oligonucleotides primers in herring (*C. pallasii*), Korean anchovy (*C. nasus*) and large-eyed herring (*H. zunashi*) species, respectively

Item	No. of unique loci to each species			No. of shared loci by the three species (7 individuals per species)
	CP	CN	HZ	
BION - 07	49	49	56	126
BION - 10	42	28	42	63
BION - 11	42	42	35	63
BION - 18	42	21	49	63
BION - 25	35	35	49	84
BION - 27	28	28	14	21
BION - 40	21	14	14	42
Total no.	259	217	259	462
Average no. per primer	37	31	37	66

CP: *Clupea pallasii*, CN: *Coilia nasus*, HZ: *Harengula zunashi*

Table 3. Multiple comparisons of average bandsharing values among herring (*C. pallasii*), Korean anchovy (*C. nasus*) and large-eyed herring (*H. zunashi*) species, respectively, were generated according to the band-sharing values

Species	CP	CN	HZ
CP	0.920±0.081 ^a	0.763±0.048 ^{cd}	0.770±0.055 ^{cd}
CN	-	0.872±0.091 ^b	0.704±0.054 ^e
HZ	-	-	0.890±0.090 ^b

CP: *Clupea pallasii*, CN: *Coilia nasus*, HZ: *Harengula zunashi*

^{a-c}: Values with different superscript are significantly different, $P < 0.05$

Each value is a result of three different experiments.

of Korean catfish (*Silurus asotus*) (Yoon & Kim, 2004), venus clam (*Gomphina aequilatera*) (Kim et al., 2006) and the turtle leg (*Pollicipes mitella*) (Song & Yoon, 2013).

The hierarchical dendrogram indicates three main branches: cluster 1 (PALLASII 01, 02, 03, 04, 06 and 07), cluster 2 (NASUS 08, 09, 10, 11, 12, 13 and 14), and cluster 3 (ZUNASHI 15, 16, 17, 18, 19, 20, 21 and PALLASII 05) (Fig. 3). The genetic distance among the three clupeid

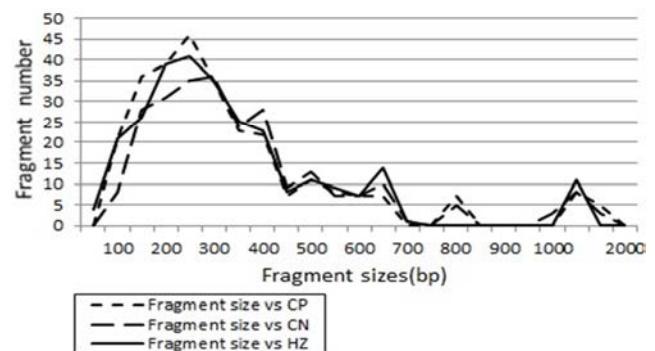


Fig. 2. Distribution of fragment sizes of herring (*C. pallasii*), Korean anchovy (*C. nasus*) and large-eyed herring (*H. zunashi*) species, respectively. Solid lines: HZ species. Dotted line: CP species. Thick dotted lines: CN species. The fragment numbers in each size interval have been computed from the pooled fragments obtained with all the primers. The higher fragment sizes (>1,000 bp) are much more observed in the HZ species.

species ranged from 0.018 to 0.318. In three clupeid species, the shortest genetic distance displaying significant molecular difference was between individual PALLASII no. 03 and PALLASII no. 02 (0.018). In the long run, individual no. 06 of the PALLASII was most distantly related to NASUS

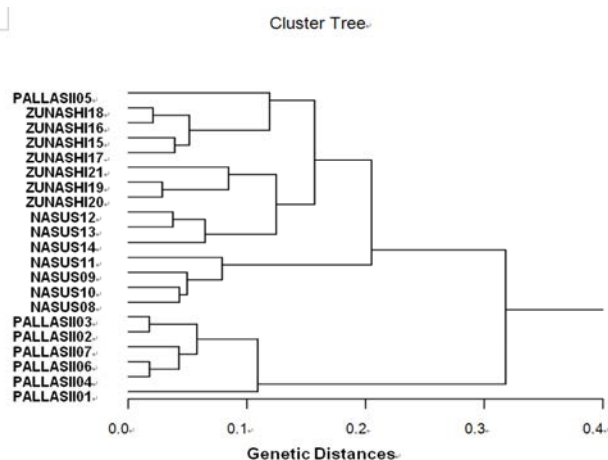


Fig. 3. Hierarchical dendrogram of genetic distances, accomplished from three clupeid species. The related construction among different individuals from herring (PALLASII), Korean anchovy (NASUS) and large-eyed herring (ZUNASHI) species, respectively, was created according to the bandsharing values and similarity matrix.

no. 11 (genetic distance = 0.318). DNA fragments identified in this study may be useful as a DNA marker. The values of the pairwise comparisons of unbiased genetic distance between the populations of the Indian major carp (*Catla catla*) from the combined data for the four primers, ranged from 0.025 to 0.052 (Islam et al., 2005). They reported that the Padma and the Jamuna populations were separated from each other with the lowest genetic distance ($D = 0.025$). The genetic distance between the Indian Ocean lobster and the Korean slipper lobster species ranged between 0.040 and 0.612 (Park et al., 2005). They suggested that this genetic technique could be used to discriminate between different river populations of major carp.

Consequently, PCR analysis generated on the genetic data displayed that the herring (*C. pallasii*) species was widely separated from Korean anchovy (*C. nasus*) species. Reversely, individuals of Korean anchovy species were properly closely related to those of large-eyed herring species, as shown in the hierarchical dendrogram of genetic distances. As stated above, the potential of genetic analysis

to identify diagnostic markers for the identification of three clupeid species has been demonstrated (McCormack et al., 2000). Generally speaking, this PCR analysis method has been applied to identify specific markers particular to line, species and geographical population, as well as genetic diversity/polymorphism in diverse species of organisms (McCormack et al., 2000; Kim et al., 2006; Yoon & Kim, 2004).

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