Journal of Applied Biology and Biotechnology Vol. 3 (01), pp. 024-029, Jan-Feb, 2015
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2015.3105

Analysis of genetic population structure of an endangered Serranid fish species in the South Korean waters: a bioinformatic simulation.

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ARTICLE INFO

Article history:
Received on: 17/01/2015
Revised on: 03/02/2015
Accepted on: 15/02/2015
Available online: 27/02/2015

Key words:
Epinephelus akaara, CO1, East China Sea, GenBank, population genetics.

ABSTRACT

Groupers (Order: Perciformes, Family: Serranidae) are of the most economically important fishes in the world to both fisheries and aquaculture sectors. Several species are now classified as endangered. Red-spotted, Hong Kong grouper, *Epinephelus akaara*, is a grouper that provides high economic values for the markets in Hong Kong and Japan. This species falls under the International Union for Conservation of the Nature (IUCN) Red List of endangered species. In order to perform a bioinformatic simulating analysis for the genetic population structure of this species in the South Korean waters, more specifically in Namhae island, 73 nucleotide sequences of cytochrome oxidase subunit 1 (CO1) were retrieved from the GenBank database. Number of haplotypes, polymorphic sites, and the interrelationships between haplotypes were all determined. The results indicated the main haplotype lineages in the area of study. Also, signs of recent population expansion could be detected, alongside with identifying some low frequency haplotypes that may have originated as a result of adaptation to the conditions at this area. This study resulted in partitioning of *E. akaara* population in the Namhae island into several units of interest for conservation.

1. INTRODUCTION

Species belonging to the family Serranidae represent the most important group of commercial interest for aquaculture and fisheries in the world. It consists of a complex of species collectively named ¨groupers¨, inhabiting tropical and subtropical areas in the world, commonly in relation to coral reefs and rocky bottoms. Groupers are known as protogynous hermaphrodites, sexually maturing first as females, and 3-12 years later resorbing their ovarian tissues and developing testicular tissues instead to become functional males.

Their high economic importance and their unique sexual behavior motivated many studies aiming to investigate their genetic diversity, improve growth, and prevent severe diseases [1-7]. The red-spotted grouper *Epinephelus akaara* is a considerably important economic fish in the Western Pacific, especially in Hong Kong and Japan. Its easy reproduction in captivity increases the future possibilities for aquaculture, but the high mortality rates of larvae make it necessary to optimize its in-hatchery production. The global population of *E. akaraa* has declined by approximately 63 % over the last 21 years due to high fishing pressure. Also, seed capture from the wild is suffering severe decline, putting then the wild populations of the fish in an endangered state [8]. Its fishing efforts are the maximum in Japan, Taiwan, Republic of Korea and southern China. In Hong Kong, it is the most expensive of all groupers available in the market. The great economic importance of this species and its endangered state produced a vast body of research, covering most aspects of *E. akaara* biology and ecology. Broodstock management and larval rearing techniques are extensively studied in Japan [1]. Sperm cryopreservation for purposes of in-hatchery propagation and conservation could be experimentally achieved and enhanced by several conditions [9]. Moreover, immunity and its molecular bases was a target of extensive research in this species due to the great vulnerability of its hatchery-reared larvae to the fish Nodavirus of the genus *Betanodavirus* [10]. DNA barcoding, the term that is applied nowadays to the mitochondrial cytochrome c oxidase sub-unit I (CO1), provides an efficient method for biodiversity assessment as it meets the need for fast, efficient and reliable species identification at this time of climate change and massive habitat destruction. DNA barcoding also has the power to connect different life stages such as eggs, larvae and adults. As such, it can link hundreds of years of taxonomic, ecological, faunistic and ethological studies [11,12,13]. This study aims to study the available data about *E. akaara* in certain area in the Eastern China Sea in order to determine the degree of variability within the population there, as well as to test the effect of some geological events on the relationships between this population and the ones found in other areas in the China Sea.

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2. MATERIALS AND METHODS

73 *Epinephelus akaara* cytochrome oxidase 1 gene (CO1) nucleotide sequences were retrieved from the GenBank database, all are available in the website http://www.ncbi.nlm.nih.gov/nuccore/?term=epinephelus+akaara+COI. These sequences were solely belonging to the waters of Namhae island in South Korea, from where they were previously sequenced and submitted to GenBank database [14]. The sequences were first aligned using the program clustalX 2.1 [15]. Later on, they were uploaded to the program MEGA6 [16] and aligned using ClustalW [17] in order to calculate the pairwise distances within the population. The alignment was then uploaded to DNAsp 5.0 Software [18] in order to determine the existing haplotypes. The obtained haplotypes were uploaded to the program Network 4.6.1.2 [19] in order to draw median-joining haplotypes network and further demonstrate their inter-relationships. The software ARLEQUIN 3.5.1.1 [20] was then applied in order to estimate the D test statistic of Tjima [21] and the Fs statistic of Fu [22], whose negative values result from the excess of low-frequency haplotypes that arise from selection or rapid population growth [21,23]. Recent population expansions as detected by the increasing diversity of haplotypes in a given population and the homogenous patterns of pairwise differences among them were inferred from calculating the index of raggedness, r [24] and R2 parameter [25], using DNAsp software.

The aligned sequences were up-loaded to MEGA6 software. Best DNA substitution model was determined by the ModelTest procedure. Based on this, a neighbor-joining phylogenetic tree was constructed. 1,000 bootstraps were used to enhance the quality of the test.

3. RESULTS

Alignment of the 73 nucleotide sequences for *E. akaara* CO1 resulted in total common length between all sequences of 633 base-long. Alignment is shown in Figure 1. In this common CO1 zone, 17 polymorhic sites were found (Fig. 1). Pairwise distances were very low, never exceeding 0.0096. Uploading these sequences into the program DNAsp 5.0 resulted in merging the 73 sequences in 18 different haplotypes.

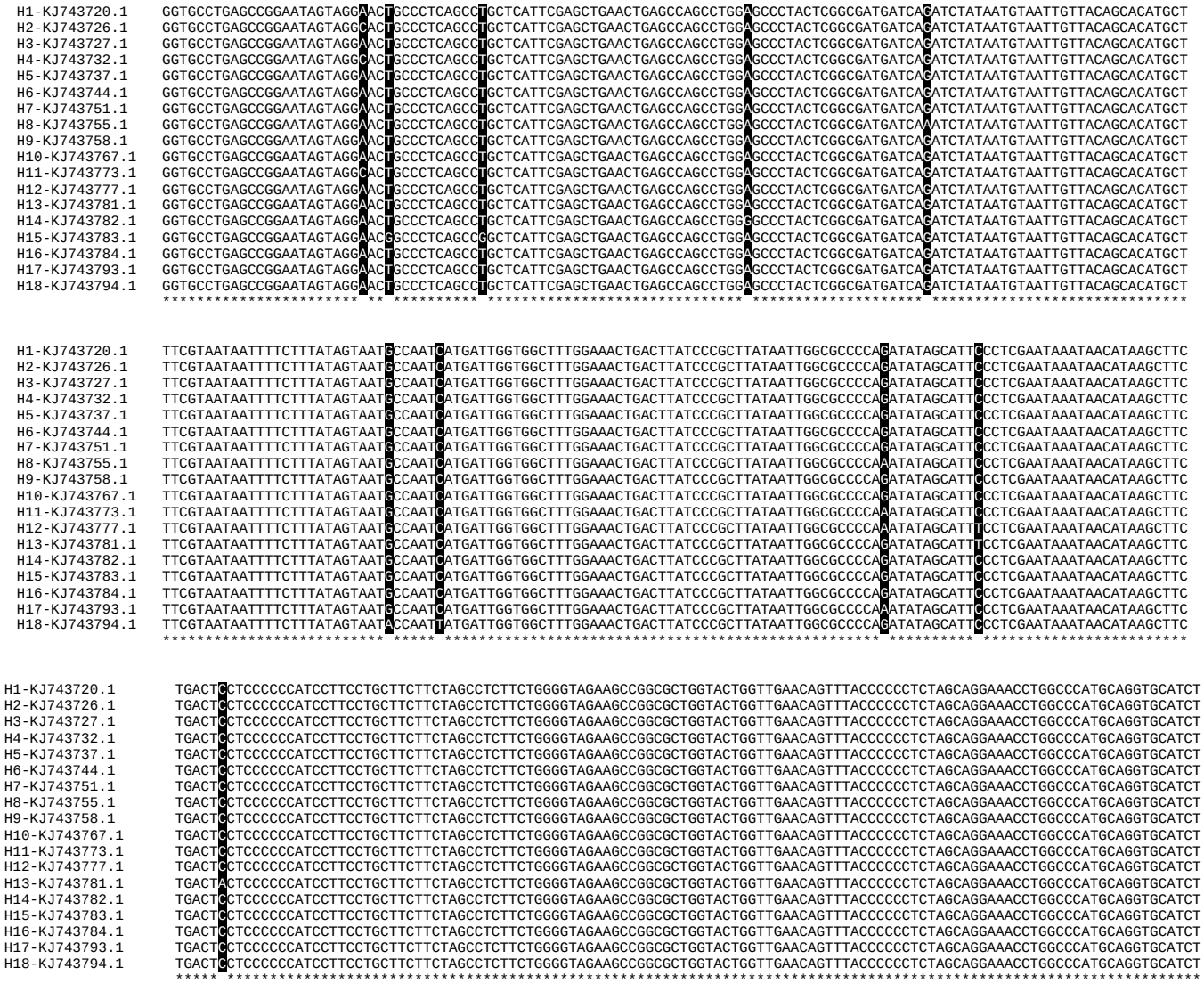

Fig. 1: Continued….

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H3-KJ743727.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCCGCCACCTCTCAGTATCAAACACCA
H10-KJ743767.1      GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCA
H2-KJ743726.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H11-KJ743773.1      GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H4-KJ743732.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAGACACCC
H5-KJ743737.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAGACACCC
H6-KJ743744.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAGACACCC
H9-KJ743758.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAGACACCC
H7-KJ743751.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAGACACCA
H1-KJ743720.1       GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H16-KJ743784.1      GTAGACCTAACTATTTTTCCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H14-KJ743782.1      GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H18-KJ743794.1      GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H15-KJ743783.1      GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H13-KJ743781.1      GTAGACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H8-KJ743755.1       GTAAACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H12-KJ743777.1      GTAAACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCC
H17-KJ743793.1      GTAAACCTAACTATTTTCTCCCTACATCTTGCAGGGATTTCATCAATTCTAGGGGCAATTAACTTCATTACAACCATCATCAACATGAAACCCCCGCCACCTCTCAGTATCAAACACCA
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H1-KJ743720.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H2-KJ743726.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H3-KJ743727.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H4-KJ743732.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H5-KJ743737.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H6-KJ743744.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGATCCG
H7-KJ743751.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H8-KJ743755.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTCTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H9-KJ743758.1       TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTCTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H10-KJ743767.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAAATCGTAACCTCAACACTACTTTCTTTGACCCG
H11-KJ743773.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H12-KJ743777.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H13-KJ743781.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H14-KJ743782.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H15-KJ743783.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H16-KJ743784.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
H17-KJ743793.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAAATCGTAACCTCAACACTACTTTCTTTGACCCG
H18-KJ743794.1      TTGTTTGTATGGGCTGTCCTAATTACAGCGGTGCTACTGCTCCTGTCCCTCCCTGTTCTTGCCGCCGGCATTACAATACTTCTAACAGATCGTAACCTCAACACTACTTTCTTTGACCCG
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H1-KJ743720.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H2-KJ743726.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H3-KJ743727.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H4-KJ743732.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H5-KJ743737.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H6-KJ743744.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H7-KJ743751.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H8-KJ743755.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H9-KJ743758.1       GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H10-KJ743767.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H11-KJ743773.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H12-KJ743777.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H13-KJ743781.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H14-KJ743782.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H15-KJ743783.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H16-KJ743784.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H17-KJ743793.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
H18-KJ743794.1      GCCGGAGGGGGAGATCCTATCCTTTACCAACAC
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Fig. 1: Clustal X alignment for *E. akaara* CO1 sequence haplotypes (H1-H18). White letters over black background mark the polymorphic nucleotide sites.

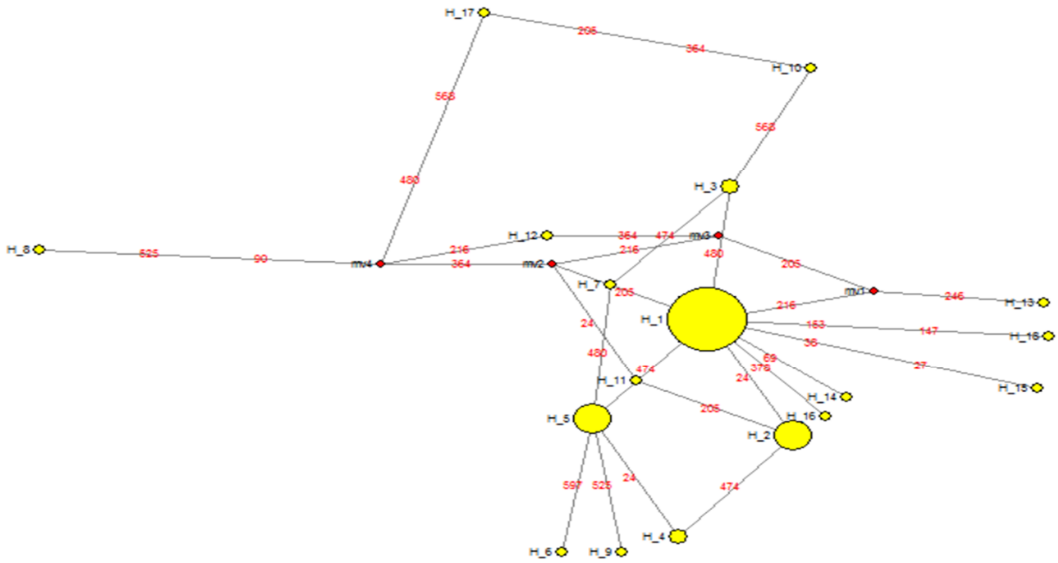

Fig. 2: Median joining network constructed for different haplotypes of *E. akaara* in Namhae island. Size of each yellow circle is proportional to the number of individuals belonging to a given haplotypes represented by the circle. Haplotypes 1 (H1), 2, 5 are shown as greater circles. Other low-number and singletone haplotypes are shown as the minor circles.

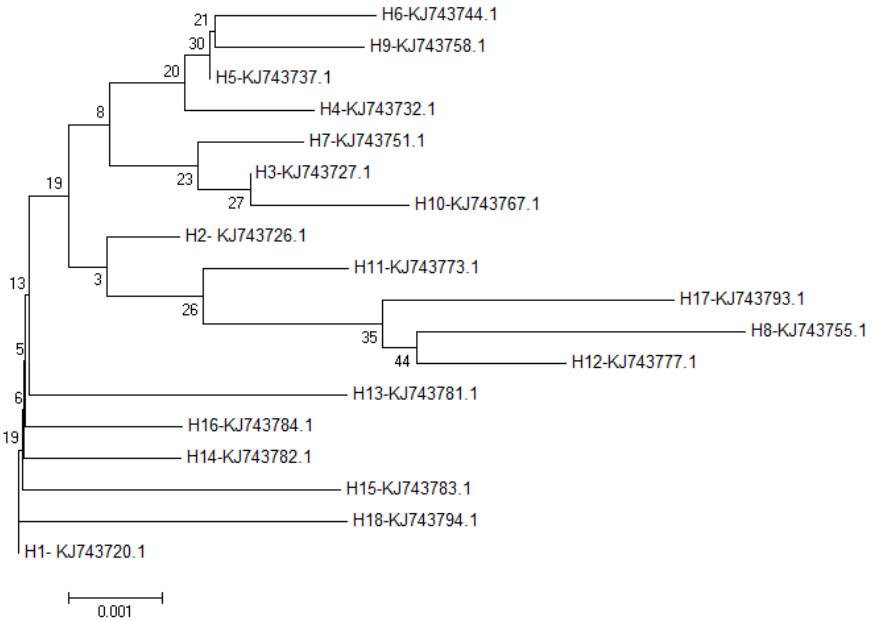

Fig. 3: Neighbor-Joining tree constructed between the 18 haplotypes found for *E. akaara* CO1 gene, rooted to the ancestral haplotype H1.

Haplotype 1 (H1) included 38 sequences with the following GenBank accession numbers: *KJ743720.1, KJ743721.1, KJ743722.1, KJ743728.1, KJ743730.1, KJ743731.1, KJ743733.1, KJ743734.1, KJ743735.1, KJ743736.1, KJ743738.1, KJ743739.1, KJ743741.1, KJ743742.1, KJ743743.1, KJ743745.1, KJ743748.1, KJ743749.1, KJ743750.1, KJ743753.1, KJ743759.1, KJ743760.1, KJ743766.1, KJ743768.1, KJ743771.1, KJ743775.1, KJ743776.1, KJ743778.1, KJ743780.1, KJ743785.1, KJ743786.1, KJ743787.1, KJ743790.1, KJ743789.1, KJ743791.1, KJ743792.1, KJ743795.1, KJ743796.1*. Haplotype 2 (H2) included the sequences with the 9 following accession numbers: *KJ743726.1, KJ743729.1, KJ743747.1, KJ743756.1, KJ743761.1, KJ743765.1, KJ743772.1, KJ743774.1, KJ743788.1***.** Haplotype 5 (H5) included the 9 sequences with the following accession numbers: *KJ743737.1, KJ743740.1, KJ743754.1, KJ743757.1, KJ743762.1, KJ743763.1, KJ743764.1, KJ743769.1, KJ743770.1*. Haplotype 3 (H3) included the 2 sequences with the following accession numbers: *KJ743727.1, KJ743779.1*. Haplotype 4 (H4) included the 2 sequences with the following accession numbers: *KJ743732.1, KJ743752.1***.** Haplotypes from 6 to 18 (H6-H18) were only represented by singletone sequences, belonging to the accession numbers: *KJ743744.1, KJ743751.1, KJ743755.1, KJ743758.1, KJ743767.1, KJ743773.1, KJ743777.1, KJ743781.1, KJ743782.1, KJ743783.1, KJ743784.1, KJ743793.1, KJ743794.1.*

Haplotypes Median Joining Network drawn by the program Network 4.6.1.2 showed an intricate system with a star-like distribution around the haplotype 1 (H1), which can be then considered as the main lineages for *E. akaara* in Namhae waters. Two more haplotypes (H2, H5) are also well-established and form bases for radiation for the other haplotypes less commonly distributed in the area (Figure 2).

Demographic parameters used indicated all a recent expansion. Significantly negative D value of Tajima (-1.83923, p= 0.00900) and Fs of Fu (-14.05508, p= 0.0000) were found. Furthermore, mismatch analyses suggest a strong possibility of recent population expansion as inferred from the non-significant raggedness (0.81000) and the low R2 value (0.04225). Finally, the genealogical relationships between the 17 obtained haplotypes are shown in the neighbor-joining tree presented in Figure 3.

4. DISCUSSION

Despite the threats *E. akaara* is facing in its natural habitats, this species in Namhae waters seem to be well-adapted and with marks of recent expansion. The presence of several haplotypes provides a special importance for the stock of this species in that area due to the presence of several conservation units. Trials of broodstock development should take into account this haplotypes diversity for keeping the integrity of their natural equilibrium. The relatedness between the main haplotypes (H1) and the other haplotypes with lower frequencies may indicate some local adaptation of in the area.

Structuring of *E. akaara* populations in China Sea is an attractive topic, despite receiving very little attention from the population genetics viewpoint. Higher nucleotide diversities were found in *E. akaara* in Northern populations of the China Sea than in Southern populations [26]. The areas covered by the previous study [26] were to the South of Namhae island from which the samples of *E. akaara*, to which the sequences in the current study belong, were obtained [14]. However, the low nucleotide diversity index (π=0.00208) for *E. akaara* in Namhae island, coupled to the low raggedness values found in this study among COI sequences may refer to that the effect of the low seawater level in the last

glacial maximum (130 meters below the level of today) not only affected *E. akaara* in the continental shelf of East and South China sea, but also extended to Namhae region. This possibly leads to a conclusion that this species suffered an old bottle-nick effect followed by a recent extension. No similar studies for *E. akaara* in this region were found, except the one mentioned before [26], that applied the sequence of the mitochondrial control region for their investigation. However, South and Eastern China Sea represented a key area for fish population genetic studies, using different mitochondrial markers in several species. Several studies pointed to the impacts of climatic and seawater levels oscillations in the Pleistocene in East and South Asian seas. Populations of crimson snapper *Lutjanus erythropterus* in China Sea had possibly experienced some bottleneck effect followed by population expansion since the late Pleistocene [27]. The mud crab *Scylla paramamosain* populations thriving along the Chinese coast also seem to have similar bottleneck and recent population expansion [28]. However, no signs of population bottlenecks were found in the pelagic, migratory species found in the same area, the mackerel *Scomber japonicus*, despite being a target for excessive fishing and habitat destruction [29]. Another school-forming fish, the Fourfinger threadfin, *Eleutheronema tetradactylum*, did not show any signs for recent population expansion [30]. Therefore, marine species living in the East Asia responded differentially to the lowering of sea levels during the Pleistocene, with the effect is more profound over reef-dwelling fish species. The high market popularity of the groupers in the international markets and the sincere trials for optimization of their aquaculture requires more work for characterization of different lineages and genealogy, especially for detection of species and individuals with good degree of adaptation to the environment where they will be introduced. From the ecological viewpoint, characterization of different units of conservation is a crucial subject, especially when a species facing population declining and a plausible risk of extinction is concerned.

5. ACKNOWLEDGMENT

The author would like to appreciate his deep thanks to Doctros. S.H. Han, Y.D. Lee, H.J. Baek, H.S. Oh, and C.H. Noh for making the sequences of *E. akaara* CO1 gene available in the GenBank database, what was fundamental for compliance of this study. Also, I would like to appreciate the sincere efforts of the anonymous reviewers and editors of the *Journal of Applied Biology & Biotechnology* in enhancing the quality of this work by their suggestions.

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How to cite this article:

Khaled Mohammed-Geba. Analysis of genetic population structure of an endangered Serranid fish species in the South Korean waters: a bioinformatic simulation. J App Biol Biotech, 2015; 3 (01): 024-029.