PD - 26

# Genetic Analysis of Long Snout Bullhead, *Leiocassis dumerili*, Using Mitochondrial Cytochrome C Oxidase I and 12S rRNA Genes

**Lei Peng[1], Wan-Ok Lee[2], Jae Hyun Im[1], Soo Jin Lee[1], Yoon Kwon Nam[1] and Dong Soo Kim[1]**

[1]Department of Aquaculture, Pukyong National University, Busan 608-737, Korea
[2]Chongpyong Inland Fisheries Research Institute, National Fisheries Research and Development Agency, Chongpyong, 477-810, Korea

## Introduction

Recently, the low survival rates in breeding of Long Snout Bullhead, *Leiocassis dumerili*, suggest that inbreeding depression may lead to a generalized loss of fitness. In general, fish farmers use only a few individuals as broodstock. This practice may lead to the erosion of genetic diversity of the stocks. Nucleotide sequencing data are one of the most appropriate tools to detect genetic variation and DNA sequencing of the cytochrome region of the mtDNA is very specific and at one base solution allows the differentiation at the individual level. In addition, other ports of the mtDNA such as the rRNA genes have been shown to be useful for determining relationships at different taxonomic levels.

In this study, the genetic relationships between individuals and generations of long snout bullhead, *Leiocassis dumerili*, have been analyzed by sequences of mitochondrial cytochrome subunit I gene and 12S ribosomal RNA regions to estimate the genetic variation. Also, due to the very limited sequence data of Bagridae species, the results obtained in present study would provide basic information for further research.

## Materials and Methods

Twenty adult and 25 F1 offsprings were sampled. Genomic DNA was prepared by using conventional SDS/proteinase K method. Total RNA was prepared from pituitary, liver and brain using Tripure reagent (Roche). mRNA was isolated form total RAN using mRNA isolation Kit (Roche). cDNA library was constructed. The synthesized cDNA was subjected to size fraction and ligated to Uni-Zap XR vector. Plasmid containing cDNA insert was randomly selected and purified and subjected to sequencing reaction following the procedure used by Nam and Kim (2002). After confirmation of the cytochrome C oxidase subunit I sequence, primers were constructed. The primers used in amplification

were universal primers for 12S rRNA mtDNA segments. Amplified DNA was purified with Gel Purification Kit (Bioneer) and applied to sequencing reaction.

```
1:5'-gcgcgtctcagagcctaattcaaaggacactctatttgttttttactgctaaatccaccttcagacatttgttt
2:5'------------agcctaattcaaaggacactctatttgttttttactgctaaatccaccttcagacatttgttt
1:cagtatgtcattcgtaatattctatatgtatagaaaatgtagcccatttcttcccacttcgtacgctacacctcgac
2:cagtatgtcattcgtaatattctatatgtatagaaaatgtagcccatttcttcccacttcgtacgctacacctcgac
1:ctgacgtttttgggcgggcccattttgcttactcttggaccttcacagggtaagctgacgacggcggtatataggcg
2:ctgacgtttttgggcgggcccattttgcttgctcttggaccttcacagggtaagctgacgacggcggtatataggcg
1:gggaaaacaagaagtggtgaggtttaacgggggttatcggttctagaacaggctcctctaggtgggtctgagacacc
2:gggaaaacaagaagtggtgaggtttaacgggggttatcggttctagaacaggctcctctaggtgggtctgagacacc
1:gccaagtcctttgggttttaagctgtgctcgtagtacccgggcggatgtttgtgtaaatatacatctaggtttatag
2:gccaagtcctttgggttttaagctgtgctcgtagtacccgggcggatgtttgtgtaaatatacatctaggtttatag
1:ctaagcatagtcccctatctaatc-3'
2:ct----------------------3'
```

Fig. Nucleicacid sequences obtained and aligned for 12S ribosomal RNA.

## Results and summary

Our primary goals of the present experiment were to assess the genetic diversity of individuals of the long snout bullhead imported form China and those F1 groups produced in Korea through comparison of 12S rRNA gene sequences and cytochrome C oxidase subunit I sequences. Nucleotide sequence of the bullhead was determined in the 406-bp segment of the 12S rRNA gene. Among 45 samples analyzed, this 406-bp segment provided only 1 variable site, which is a G-C transversion. In addition, one gap was found in some sequences. A slightly A-T nucleotide bias was presented in this gene, about 54.4%. To construct the primers for cytochrome c oxidase subunit I of bullhead, cDNA library was constructed. No variable site was found in the 513-bp segment of cytochrome C oxidase subunit I. These results reveal that there is no genetic divergence among samples, though the bullheads were imported from two different localities in China. For genetic improvement of this species, broodstock should be imported from other localities or other genetic methods should be applied.

## References

Nam Y.K. and D.S. Kim. 2002. Screening of potential stress-responsive and immune-related genes by expressed sequence tags in mud loach (*Misgurnus mizolepis*). J.Fish. Pathol., 15(2), 83-92.