THE D-LOOP SEQUENCE DETERMINATION FROM *CARASSIUS AURATUS GIBELIO* BLOCH. SPECIES

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Key words: mitochondrial DNA, sequence, D-loop, *Carassius auratus gibelio* Bloch.

Abstract: In this experiment was sequenced only the first part of mitochondrial control region (D-loop), that grows from five individuals of *Carassius auratus gibelio* Bloch, because represent a very high variability and could be considered relevant for evolution. The sequenced segment is about 300bp.

INTRODUCTION

The Cyprinidae are one of the most successful families of fish, with more than 2000 species grouped in approximately 340 genera (Banarescu & Coad 1991). *Cyprinid* fishes have received much attention from evolutionary biologists, as they show a wide distribution around the world and occur in almost every freshwater environment.

Our scope was in the first time to determine the sequence of mitochondrial DNA control region (D-loop), because after this we can compare it with the other haplotypes from Gen Bank (that's mean other individuals from the same species, but, with different geographic proveniences), further, it is possible to establish the phylogenetic relationships between *Carassius auratus gibelio* Bloch and other subspecies of the same species.

THE AIM OF INVESTIGATIONS

The principal purpose of this experiment is the establishment of nucleotide sequence for mitochondrial DNA control region (D-loop) from five individuals of *Carassius auratus gibelio* Bloch. The provenience of biological material is S.C.A.E.A. Fishing Farm, and the period of samples harvest was august 2002.

MATERIALS AND METHODS

The mitochondrial DNA extraction and precipitation was made about phenol-clorophorm method.

The amplification for this fragment was effected using universal primers for fishes and a program with a primers annealing temperature of 60^0C. It had been used an reaction volume of 50µl/sampel, respectively 47,5µl reactive and 2,5µl DNA.

Primers: CTRL A: 5`-TTCCACCTCTAACTCCCAAAGCTAG-3` (2µM)
CTRL E: 3`-CATCTGGTTCCTACTTCAGG-3` (2µM)

After the amplification process, the PCR product was checked by migration in electrophoresis gel (figure 3), using a loading buffer and a 100 base pairs molecular marker.

The PCR products purification was made in QIAGEN columns using a protocol given by the same brand.

For the sequence reaction, we used a sequencing kit given by Beckman Coulter. The reaction volume was by 20µl, which mean that 12,5µl is the sequencing cocktail and 7,5µl DNA.

The program used for amplification had a number of 30 replication cycles and a primers annealing temperature of 50^0 C (figure 1)

The sequencing was made using a Beckman Coulter automatic sequencer with 8 capillary for the direct and reverse chains.

The sequences matching was made using the ESEE32 (The Eyeball Sequence Editor) software.

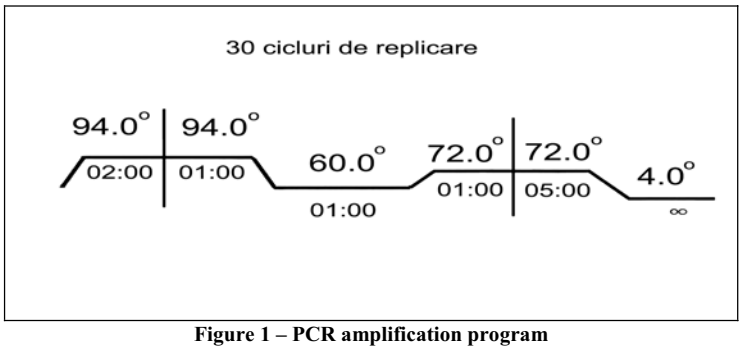

Figure 1 – PCR amplification program

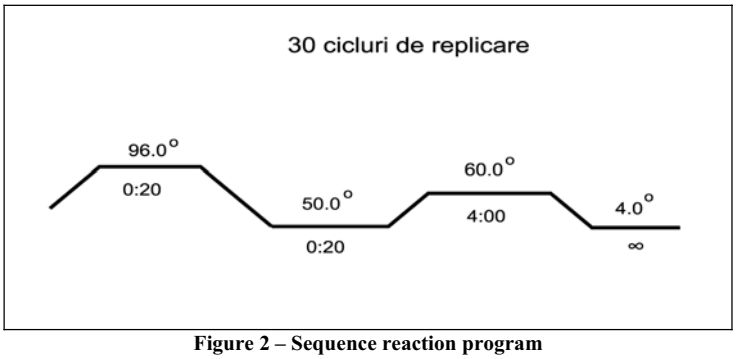

Figure 2 – Sequence reaction program

RESULTS AND DISCUSSIONS

The PCR product was checked by migration in an electrophoresis gel, and in this way, we observed that the PCR product has the expected length - approximately 300 base pairs. For the length determination, it was used a molecular marker (Figure 3, M) of 100bp and a negative control (figure 3, C) for eventually contaminations.

In conformity with table 1, we compared 300 base pairs for five individuals of *Carassius auratus gibelio* Bloch and we observed that these five sequences are identically, except only the position 160. In this position, for three individuals (CaI01D, CaI02D, CaI05D) it is present cytosine, and for two individuals (CaI03D, CaI04D), in this position is thymine.

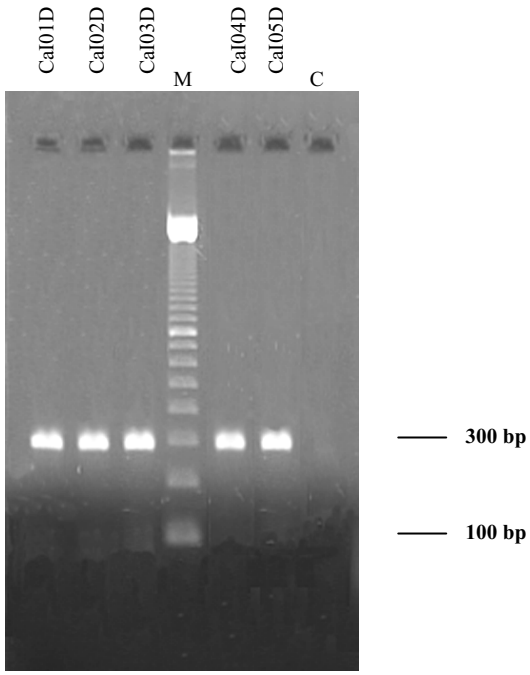

Figure 3 Visualisation of PCR products of D-loop from *Carassius auratus gibelio* Bloch.
M=molecular marker of 100bp, C=negative control

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CaI01D: 1 ttccacctctaactcccaaagctagtacatgtaaattaaggtatacataaagcataatct 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI02D: 1 ttccacctctaactcccaaagctagtacatgtaaattaaggtatacataaagcataatct 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI03D: 1 ttccacctctaactcccaaagctagtacatgtaaattaaggtatacataaagcataatct 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI04D: 1 ttccacctctaactcccaaagctagtacatgtaaattaaggtatacataaagcataatct 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI05D: 1 ttccacctctaactcccaaagctagtacatgtaaattaaggtatacataaagcataatct 60

CaI01D: 61taagactcacaagttaaattattttgacccgggtaatatattattccccaagaaattgtc 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI02D: 61taagactcacaagttaaattattttgacccgggtaatatattattccccaagaaattgtc 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI03D: 61taagactcacaagttaaattattttgacccgggtaatatattattccccaagaaattgtc 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI04D: 61taagactcacaagttaaattattttgacccgggtaatatattattccccaagaaattgtc 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CaI05D: 61taagactcacaagttaaattattttgacccgggtaatatattattccccaagaaattgtc 120
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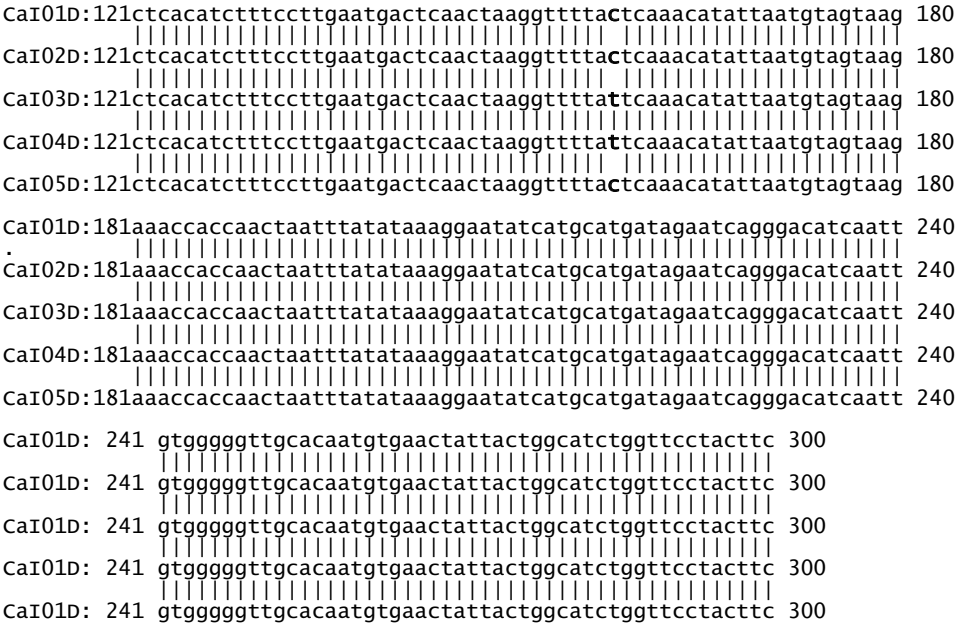

CONCLUSIONS

The length for the first part of D-loop is about 300bp.

The difference between 3 sequences (CaI01D, CaI02D, CaI05D) and the other two (CaI03D, CaI04D), appears because a transition occurred between two pyrimidine bases thymine and cytosine.

The different types of observed sequences are identically for 99,7% of base pairs.

BIBLIOGRAPHY

Ausubel F.M., Brent R., Kingston R.E., Moore D.D., Seidman J.G., Smith J.A., Struhl K., 1995. *Current protocols in molecular biology*, Vol. 1, cap.2 – *Precipitation and analysis of DNA – Phenol extraction and ethanol precipitation of DNA*, p. 2.1.1.- 2.1.3., Edited by John Wiley & Sons, Inc.

Bănărescu, P., Coad, B. W., 1991. *Cyprinids of Eurasia.* In *Cyprinid fishes. Systematics, biology and exploitation*. Ed. I. J. Winfield & J. S. Nelson, pp. 127-155. London: Chapman &Hall.

Zardoya R., Doadrio I., 1998. *Phylogenetic relationships of Iberian cyprinids: systematic and biogeographical implications*, Proc. R. Soc. London, 265, p. 1365-1372.

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