RP1S7 – A Possible Marker for Sturgeon Hybrids Identification

Gina – Oana Popa[1, ‡], Maria Samu[1, ‡], Ramona Nechifor[1], Andreea Dudu[1, *], Sergiu Emil Georgescu[1]

[1]*University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology, 91-95 Splaiul Independenței, 050095, Bucharest, Romania*

‡The first two authors contributed equally to this work

Abstract

The *Acipenseridae* family includes sturgeon species that have the ability to easily generate interspecies hybrids in both natural and aquaculture conditions. For the differentiation between hybrids and pure species, mitochondrial markers have been used, but they have not proven effective. Nuclear markers, such as the *RP1S7* marker, encoding the S7 ribosomal protein, show to be more effective in achieving this differentiation. The purpose of this study was to evaluate the capacity of this nuclear marker in discriminating between pure sturgeon species and their interspecies hybrids. DNA samples were isolated from fin fragments. The *RP1S7* marker was amplified by PCR and the obtained amplicons were analysed by agarose gel electrophoresis and sequencing. In order to determine the accuracy of the primers and to validate this marker, the sequences were compared with similar ones from GenBank. The resulting electrophoretic profiles showed differences that can be used to distinguish between pure sturgeon species and their interspecies hybrids**.** Therefore, the *RP1S7* marker can be included in a panel of DNA markers for molecular identification of sturgeon species and their hybrids.

Keywords: sturgeons, hybridization, nuclear markers, *RP1S7*.

1. Introduction

Sturgeons are included in the *Acipenseridae* family (order *Acipenseriformes*), and they are considered authentic "living fossils", representing one of the ancient groups of fish [1]. The overexploitation of sturgeon populations, the uncontrolled restocking activities and the incorrect labelling of possible sturgeon hybrids as pure species have led to a decline of this class of fishes in terms of their ecological status [2].

Due to a polyploid state and to the high similarity regarding their karyotype and genome structure, sturgeons are capable of interspecies hybridization, especially when their breeding sites overlap in time and space [3]. This phenomenon is more abundant in aquaculture compared to wildlife [4].

Regarding the molecular markers used for identifying various sturgeon species and their interspecific hybrids, the mitochondrial ones proved to be ineffective in the differentiation process between hybrids and the maternal species [5]. However, nuclear markers present characteristics, such as codominant mendelian inheritance, and have a greater power of distinction in terms of molecular diagnostic.

Furthermore, due to specific characteristics of introns, namely higher degree of polymorphism and less nucleotide sequence, they can represent a basis for the development of nuclear markers [6].

Such a nuclear marker is the first intron of the S7 ribosomal protein gene. In vertebrates, this gene

* Corresponding author: Dudu Andreea, Phone +40213181575/112, Fax +40213181575/101, Email: tn_andreea@yahoo.com

contains 7 exons and 6 introns, and it is found in a single functional nuclear copy [7]. To amplify this structure, universal primers were designed using the exon sequences to amplify the flanking intron regions [6]. By coupling this marker with other nuclear or mitochondrial markers, various phylogenetic or genetic diversity studies have been performed in various organisms such as catfish species (*Pseudoplatystoma* sp.) [8], darter (*Nothonotus* sp.) [9], anchovies (*Coilia nasus*) [10], etc.

This study aims to verify whether the *RP1S7* marker is useful in distinguishing between pure sturgeon species and their corresponding hybrids.

2. Materials and methods

Sampling and DNA extraction

The biological material was represented by fin clips from four pure sturgeon species (*Acipenser ruthenus*, *A. gueldenstaedtii*, *A. stellatus* and *Huso huso*) and five interspecies hybrids: bester (♀ *H. huso* and ♂ *A. ruthenus*), sterbe (♀ *A. ruthenus* and ♂ *H. huso*), hybrid 203 and 204 (♀ *H. huso* and ♂ *A gueldenstaedtii*), and best beluga, which is a second-generation hybrid (♀ *H. huso* and ♂ *bester*),

The DNA extraction was done using the phenol-chloroform protocol [11] and the concentration and quality of genomic DNA were spectrophotometrically assessed.

PCR amplification

To amplify the intron 1 of the RPS7 gene, a single pair of primers was used, namely the forward S7RPEx1F primer 5'-TGGCCTCTTCCTTGGCCGTC-3' and the S7RPEx1R reverse primer 5'-AACTCGTCTGGCTTTTCGCC-3' [6]. The amplification program was: denaturation at 95°C for 2 minutes, 35 cycles of: denaturation at 95°C for 30 seconds, annealing at 49°C for 30 seconds and extension at 72°C for one minute, with final extension at 72°C for 60 minutes. The PCR reactions were carried out using a Verity PCR System (Applied Biosystems) in a final volume of 25 µl with 1X PCR Buffer, 1.5 mM of MgCl2, 200 µM of each nucleotide, 0.32 pmol/ µl of each primer, 0.5 units of AmpliTaq DNA polymerase, nuclease free water and 50 ng of DNA template.

The amplicons were analyzed by agarose gel electrophoresis.

Sequencing

The sequences of the first intron of the S7 ribosomal protein gene for each sturgeon individual were obtained using BigDye Terminator v3.1 Kit (Applied Biosystems), followed by BigDye XTerminator Purification Kit (Applied Biosystems) and the samples were loaded on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

Data analysis

The analysis of the nucleotide sequences was performed to confirm that the pair of primers amplified the region of interest. The Bioedit program [12] was used to view and to edit the sequences, while the alignment was done through the ClustalW option of the same software. Supplementary, a BLAST analysis was done on GenBank in order to validate the molecular marker nucleotide sequence.

3. Results and discussion

Following the agarose gel electrophoresis using the universal primers, a series of bands were observed, (figure 1). Regarding the pure species, the presence of a different pattern of electrophoretic bands was observed. Thus, the *A. ruthenus*, *A. gueldenstaedtii* and *A. stellatus* pure species have two specific electrophoretic bands at different lengths, namely 700 and 900 pb, while in the case of *H. huso*, the 900 bp band is missing. Also, for the three *Acipenser* species where the second band appears, there are small differences in the exact length in base pairs of the band. For example, *A. ruthenus* species presents a slightly higher band compared to *A. gueldenstaedtii*.

These differences related to the bands profile are also observed for the interspecies hybrids. The presence of the two bands in four of the five analyzed hybrids: bester (♀ *H. huso* and ♂ *A. ruthenus*), sterbe (♀ *A. ruthenus* and ♂ *H. huso*), hybrid 203 (♀ *H. huso* and ♂ *A gueldenstaedtii*) and 204 (♀ *A. ruthenus* and ♂ *A. gueldenstaedtii*) can be noticed. Regarding the fifth hybrid, best beluga, which is a second-generation hybrid (♀ *H. huso* and ♂ bester), the second 900 bp band is missing, aspect by which it can be differentiated from the other hybrids (especially from the bester hybrid, where the identification based on the mitochondrial markers is ineffective). Where it appears, it is observed that the second band

present in hybrids has small differences in length compared to the band found in pure species. For example, 203 hybrid has the second band slightly higher than paternal species, and in the case of the sterbe hybrid, the second band has a slightly smaller length than paternal species. Following the sequencing of the S7 ribosomal protein gene, sequences of the 700 bp fragment were obtained and it was confirmed that the universal primer pair amplified the region of interest and the results are conclusive. Thus, the resulting sequences were aligned using the ClustalW option in BioEdit with two other sequences taken from the GenBank database [13] (figure 2). BLAST analysis provides information about the similarity that occurs between two of the S7 ribosomal protein gene sequences obtained in the laboratory. Following the alignment of our sequences with two reference sequences downloaded from GenBank, it can be observed that the four sequences are similar regarding composition and sequence of nucleotides. The sequence KF771107 is derived from *A. transmontanus* and has an "Ident" score of 93% (458 nucleotides out of 493 are similar) with our *A. stellatus* sequence. The CQ352283 sequence from *A. baerii* shows a lower "Ident" score (88%) with our *A. gueldenstaedtii* sequence. Also following the alignment of the four sequences, we can identify several single nucleotide polymorphisms, especially in the sequence corresponding to *A. baerii*, but deletions and insertions of up to 18 bp as well. The sequence analysis is hampered due to the fact that in the GenBank database there are no other S7 ribosomal protein gene sequences from other surgeons or other closely related species.

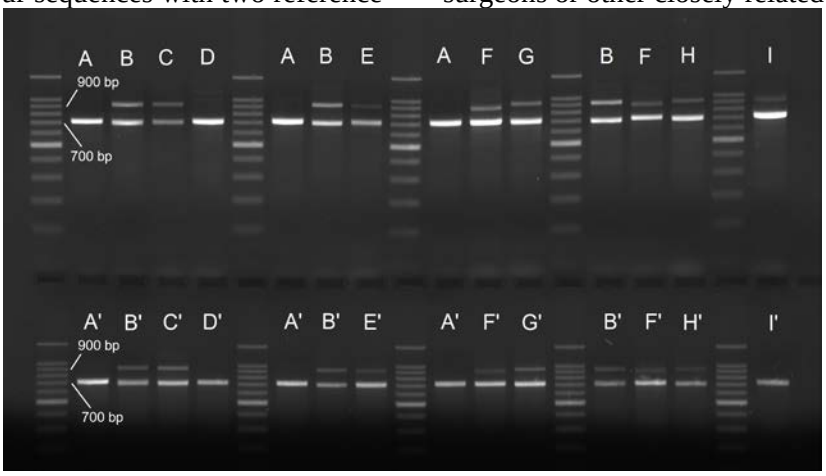

Figure 1. Electrophoretic profile of the S7 ribosomal protein gene using universal primers for 4 pure sturgeon species and 5 interspecific hybrids. A, A' – *H.huso*; B, B' – *A. ruthenus*; C, C' – bester; D, D' – best beluga; E, E' – sterbe; F, F' – *A. gueldenstaedtii*; G, G' – 203; H, H' – 204; I, I' – *A. stellatus*. Fragments sizes were determined with 100 bp. Promega DNA Ladder.

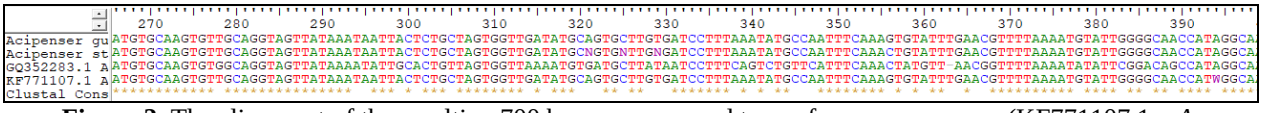

Figure 2. The alignment of the resulting 700 bp sequences and two reference sequences (KF771107.1 – *A. transmontanus*; CQ352283.1 – *A. baerii*)

4. Conclusions

The *RP1S7* nuclear marker has proven useful in discriminating between pure sturgeon species, but also between pure species and their hybrids. The bands that follow from the agarose gel electrophoresis are conclusive for this purpose, although the differences that appear are quite small. A major difference is found in the best beluga hybrid where a single band appears, which can predict that the second-generation hybrids do not have a band profile according to the mendelian model. Therefore, the nuclear markers prove to be more effective in differentiating between pure sturgeon species and their interspecies hybrids as compared to the mitochondrial ones.

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