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OF WILD FAUNA AND FLORA

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MOLECULAR GENETIC IDENTIFICATION OF BESTER STRAINS [*H. HUSO, A. RUTHENUS*, SIN. A. NIKOLJUKINII]

1. The Annex to this document has been submitted by the Management Authority of the Russian Federation.

Ministry of Agriculture of Russian Federation

Federal Agency for Fisheries of Russian Federation

Russian Federal Research Institute of Fisheries and Oceanography (VNIRO)

The CITES Scientific Authority on Sturgeon Species in the Russian Federation

Molecular genetic identification of Bester strains (H. huso x A. ruthenus, sin. A. nikoljukinii)

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Summary: Prerequisites for molecular identification of origin of different hybrid forms of sturgeon fishes is determination of the parental species. Species of maternal ascend can be determined by mitochondrial DNA analysis. Additionally, available nuclear DNA markers are scored. Parental species is determined by nuclear markers alone. Quantitative ratio of paternal and maternal markers in the fish allows determination of the hybridization events, leading to formation of particular hybrid breed. Our data indicates, that all three officially registered breeds of bester ("Burtsevskaya", "Vnirovskaya", and "Aksayskaya") can be described by specific DNA markers, in addition to the phenotypic characters, and can be used for identification of breeds, individuals, and commercial products (caviar, filet) derived from this farmed fish.

INTRODUCTION

Origin of new breed of sturgeon fish, known as bester (Nikolyukin, Timofeeva, 1953) roots up to the last century. By work of professor Nikoljukinii, highly productive hybrids of beluga *H. Huso (♀)* and sterlet *A. ruthenus (♂)* was created. Further experiments for selfish propagation of these fish (Burtsev, 1969, Burtsev et al., 1987), as well as reciprocal hybridization with both parental species, gave rise of three breeds. Because of different schemes of hybridizations leading to its development, these breeds have different proportions of genetic material from beluga and sterlet, maintained in downstream generations. Not discussing validity of species rank designation for these hybrids (*A. nikoljukinii*), we will focus on possibility of molecular identification of origin of pure hybrids as well as backcrosses.

There are three bester breeds that are protected by patents:

Breed "Burtsevskaya". Nuclear genomic ratio of beluga and sterlet is 50%, maternal species – beluga, paternal – sterlet, mtDNA derived from beluga.

Breed "Vnirovskaya". Reciprocal hybrid bester × beluga. Maternal origin – bester, paternal – beluga (BSB), or maternal – beluga, paternal – bester (BBS). mtDNA from beluga, beluga/sterlet genomic ratio – 75/25.

Breed "Aksayskaya". Reciprocal hybrid bester × sterlet. Maternal origin – sterlet, paternal – bester (BSB), mtDNA from sterlet, beluga/sterlet genomic ratio – 25/75.

Because of different origin, molecular identification requires both, mtDNA and nuclear DNA analysis.

RESULTS AND DISCUSSION

First approach for molecular identification of beluga (♀) and sterlet (♂) hybrids was done by RAPD (Random Amplified Polymorphic DNA). Method based on one-primer PCR amplification of DNA fragments, flanked by short (10 b.p.) primer sites. Visualization of RAPD PCR presented on figs. 1 and 2.

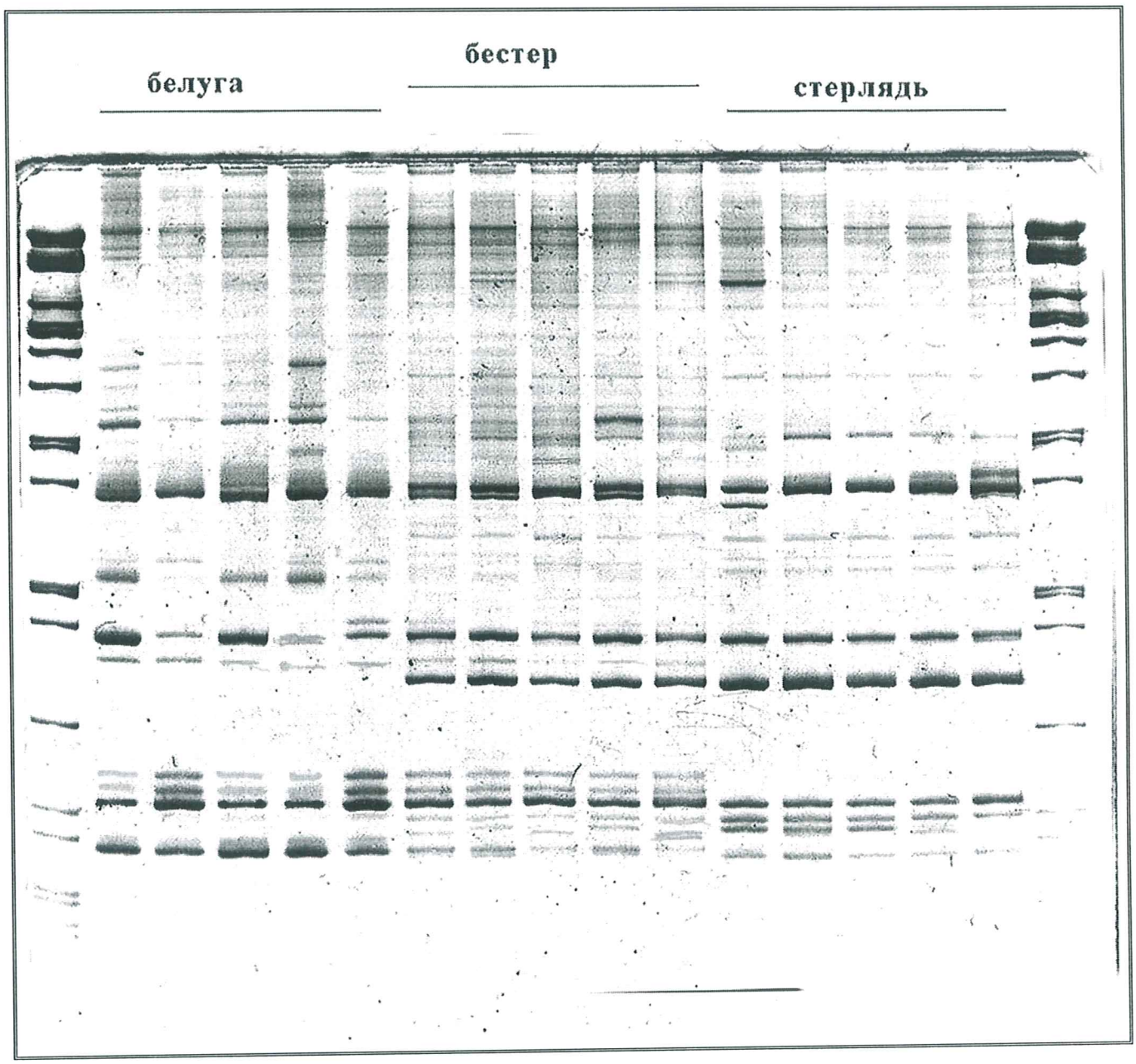

Fig. 1. A07 primer RAPD pattern of genomic DNA of beluga (Caspian population), sterlet (Volga population) and its hybrid (bester). First and last slots – nuclear ladder λ/PstI.

On the Fig1 you can see, that species-specific bands in hybrid genome are present in equal ratio.

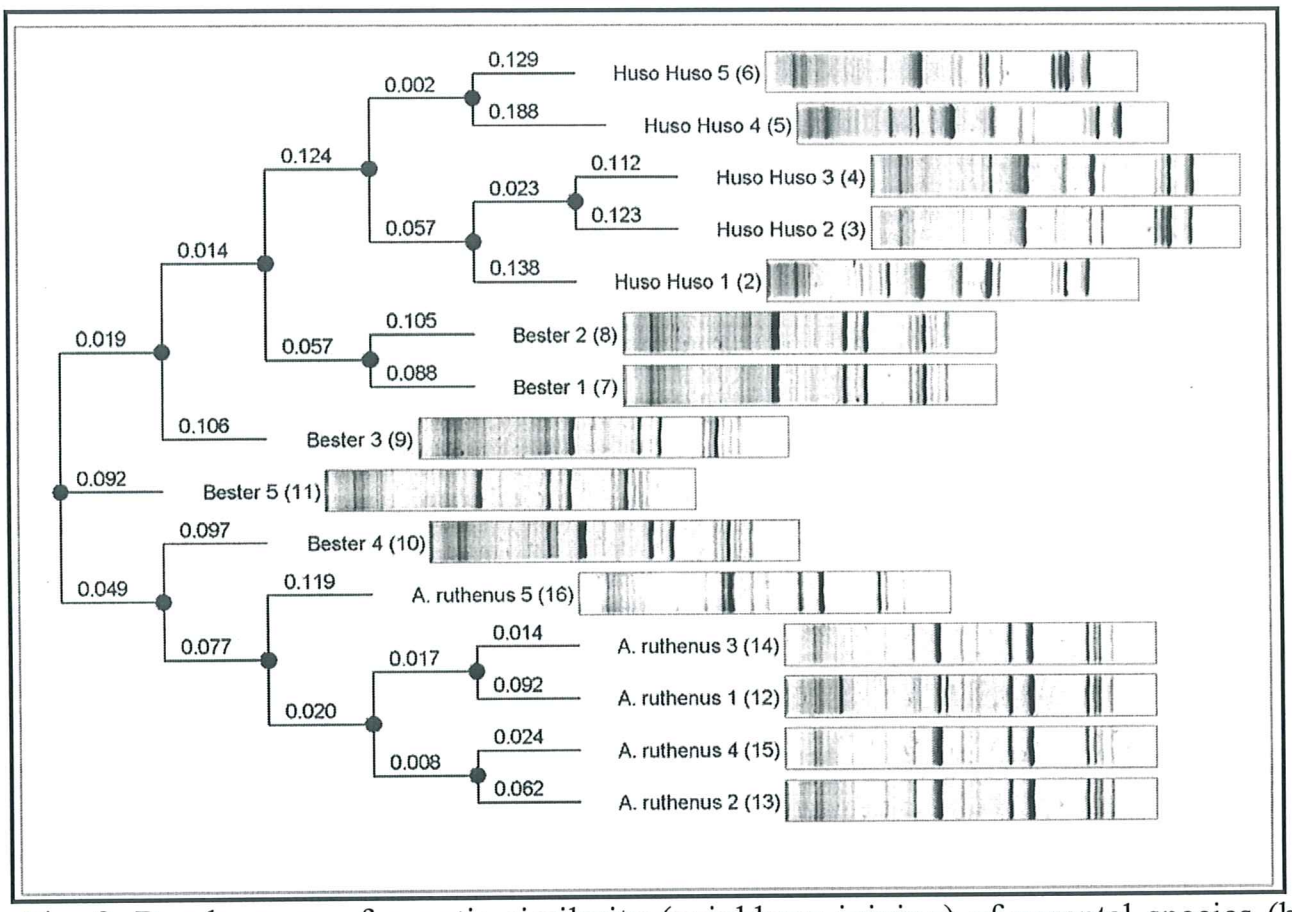

Fig. 2. Dendrogram of genetic similarity (neighbour-joining) of parental species (beluga, sterlet) and its hybrids (bester).

On the NJ dendrogram (Fig. 2) bester has intermediate and ancestral position between beluga and sterlet clusters, because posses characters, presented in both parental species.

Specimens of bester used in RAPD analysis belong to the F3 generation of selfing crosses. Equal presence of parental genetic markers indicate the integration of genomes without segregation.

Nuclear marker analysis of bester reveals its hybrid origin. However, to determine the maternal ascent (beluga or sterlet), identification of mitochondrial DNA is required. On the Fig. 3 displayed mtDNA analysis of different bester breed with beluga-specific PCR primers

Bel U (5'TTATTACTAACCTCCTCTCC3') and

Bel L 5'CCCAACTAACATTAGGATG3').

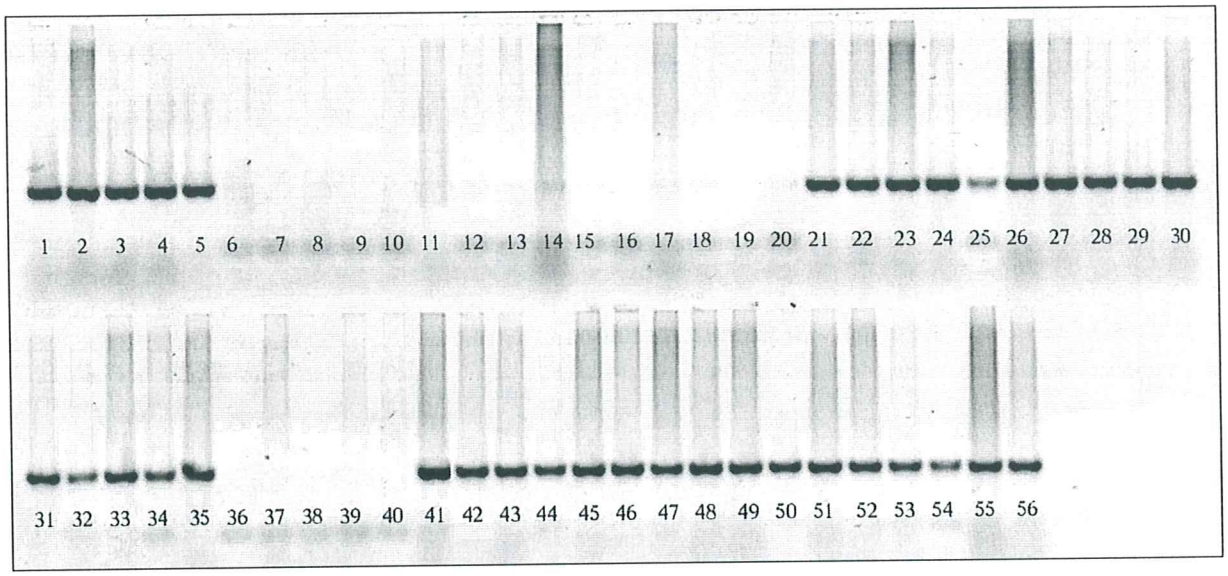

Fig. 3. Agarose electrophoresis of amplified mtDNA fragments (CytB).
DNA extracted from finclip samples.
Lines 1-5, 31-35 - beluga (H.huso, specimens# 205-209; 115-119)
Lines 6-10, 36-40 sterlet (*A. ruthenus*, specimens# 130-134, 164-168)
Lines 11-20 Bester, breed "Aksayskaya" (*A. ruthenus*×*Bester*)
Lines 21-30 bester breed "Burtsevskaya" (*Bester*×*Bester*)
Lines 41-56 bester breed "Vnirovskaya" (*H. huso*×*Bester, Bester*×*H. huso*).

Positive PCR reaction with beluga-specific primer in breed "Burtsevskaya" and breed "Vnirovskaya" indicates presense of beluga mtDNA and that maternal species was beluga. Absence of beluga-specific signal in breed "Aksayskaya" indicates that mtDNA derived from sterlet which was a maternal species for this breed.

Because of different origin, proportion of nuclear markers in bester breeds is different allelic frequencies of for several microsatellite loci were determined. On the Fig. 4 presented microsatellite polymorphism analysis of locus Afug51 of different bester breeds.

The number of allelic variants in each individual never exceed two, which corresponds with diploid pattern of inheritance in beluga and sterlet and hypothesis of autotetraploidisation of its hybrids can be ruled out.

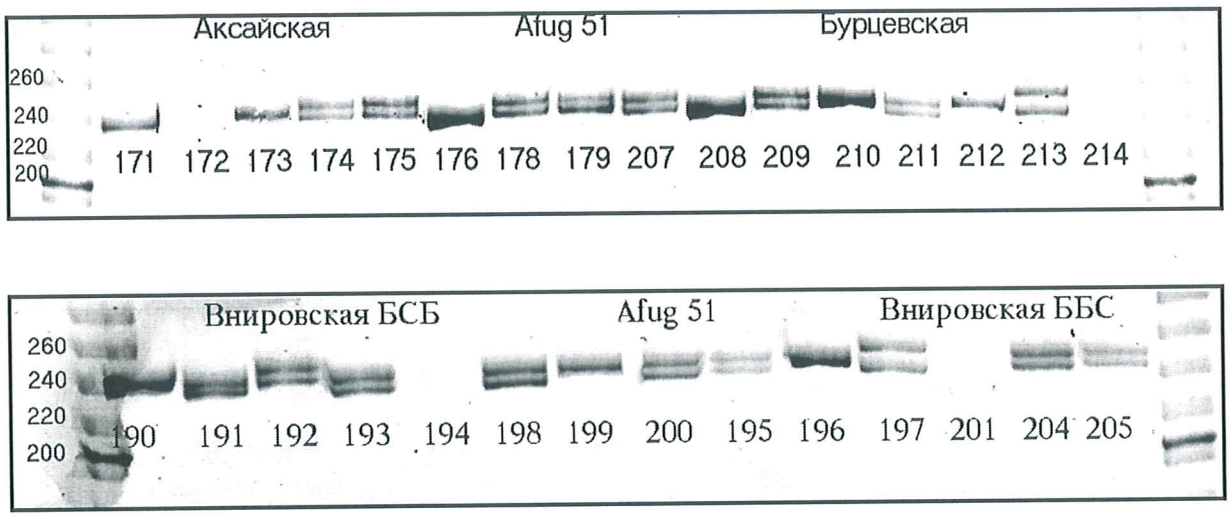

Fig. 4. Allelic variation of microsatellite locus Afug51 of three breed of bester, "Aksayskaya", "Burtsevskaya", and "Vnirovskaya" (variants BSB and BBS). The firsrt and the last lines – molecular size markers.

Allelic distribution of Afug51 locus in bester breeds illustrated on Fig. 5.

Fig. 5. Proportion of Afug51 alleles (A - blue, B - magenta, C - yellow) in different bester breeds.

Breed "Burtsevskaya" (classic hybrids) possess alleles A and B, with relatively low frequency of allele C. Analysis of reciprocal hybrids reveal that alleles B and C belongs to beluga, alleles A and B are characteristic to sterlet. Therefore, locus Afug51 can be used for diagnostic purposes in bester breed identification.

This data supported by analysis of another highly polymorphic microsatellite locus, Afug41. Patterns of allelic distribution in different bester breed present in Fig. 6.

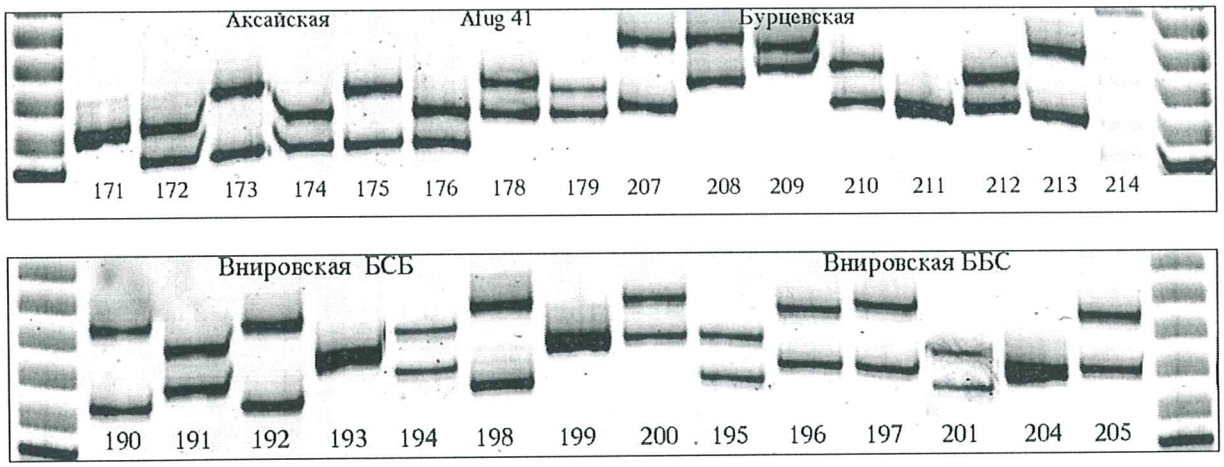

Fig. 6. Allelic content of microsatellite locus Afug41 of three bester breeds. "Aksayskaya", "Burtsevskaya", and "Vnirovskaya" (variants BSB and BBS). The firsrt and the last lines – molecular size markers

More variable allelic content allows to reveal the input of parental genomes in each particular bester breeds in better resolution. Some alleles presumably can be found in both parental species.

For example, allele "D" obviously is common in beluga and in sterlet, because both reciprocal hybrids (breeds "Aksayskaya"and "Vnirovskaya") have higher frequency of this allele compare with selfish breed (breed "Burtsevskaya"). Allele "B" is characteristic of sterlet, while allele "F" derived from beluga genome. Presence of allele "A" in breed "Aksayskaya" and allele "C" in breed "Vnirovskaya" can be explained by founder effect, because DNA samples from exact specimens – founders of the breeds are not available (Fig. 7).

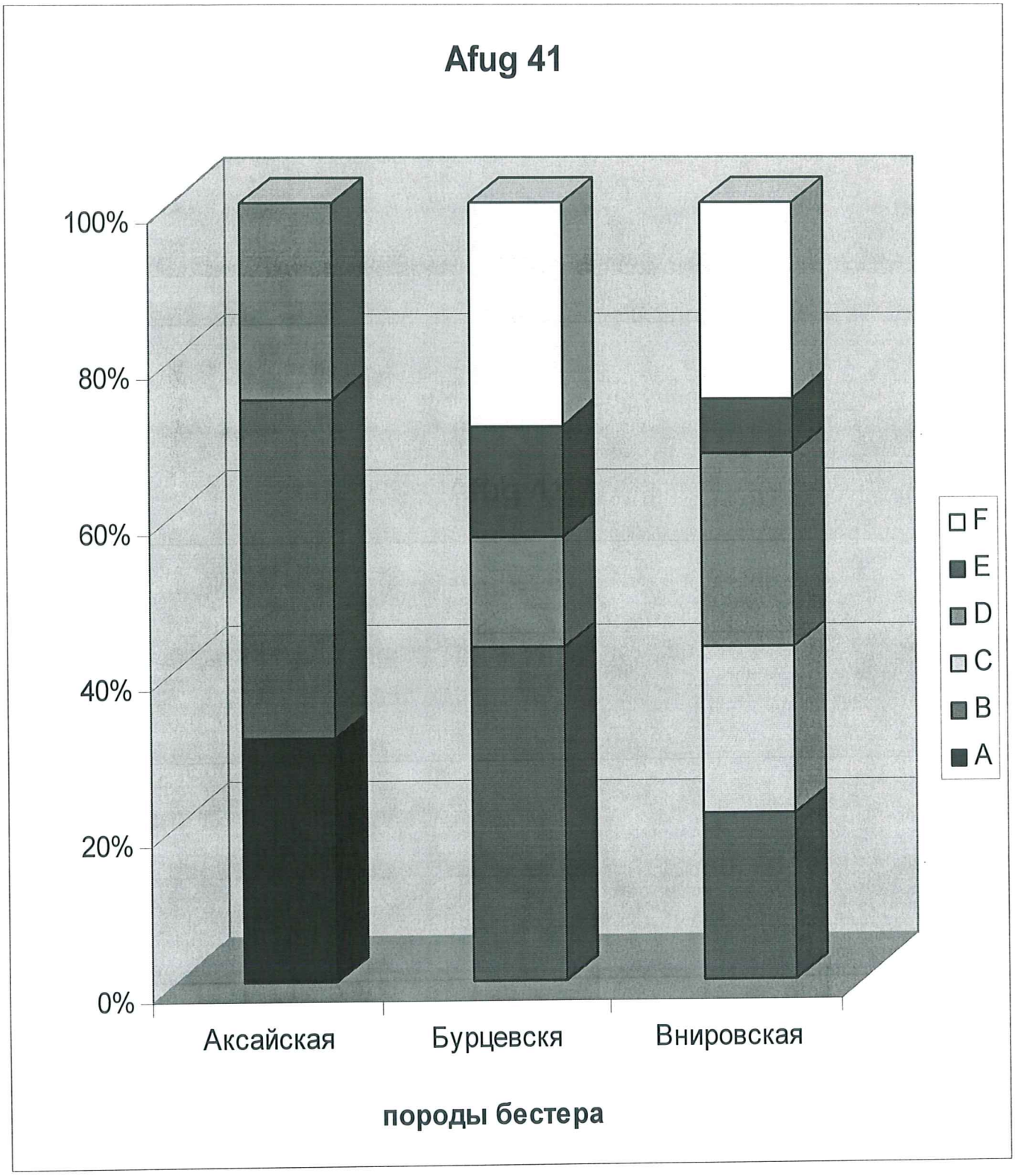

Fig. 7. Allelic frequency of locus Afug41 in bester breeds. Six alleles found in this locus (A-F) are designated by different colors.

Significant decrease of allele :"B" frequency in breed "Vnirovskaya" compare with breed "Aksayskaya" can be considered as a important diagnostic marker.

It is important to correspond the molecular identification with phenotypic diagnostic characters of fish studied. On Figs. 8-10 present a characteristic structures of mouth region all three bester breeds. Morphology of rostrum, lip shape and pretrematic (pre-gills) folds are clearly distinct.

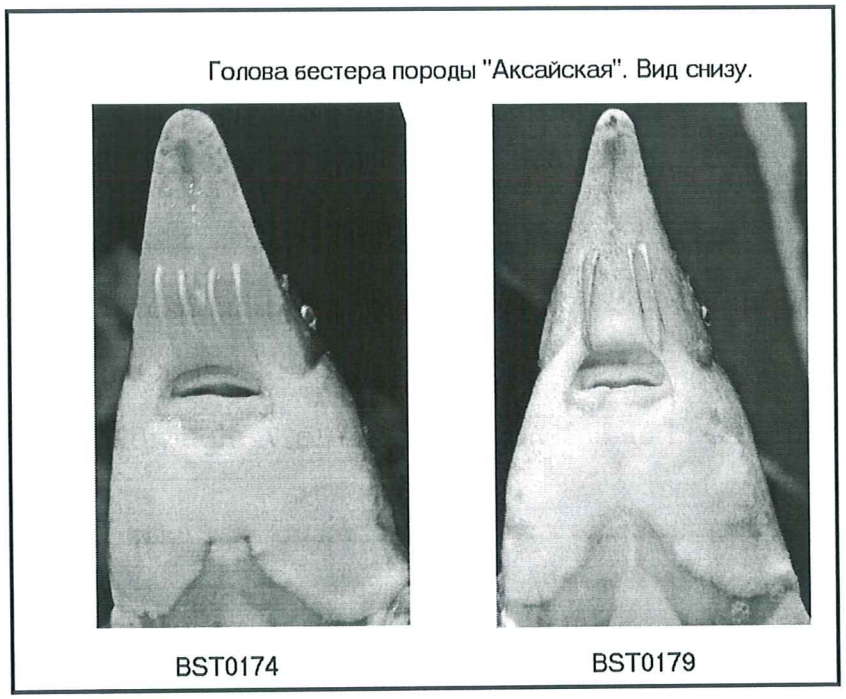

Fig. 8. Breed "Aksayskaya". Sterlet-like mouthparts with variable shape of rostrum.

On this photo clearly seen parental features – foliaceous (flattened) tentacles, elongated snout, and relatively small mouth opening.

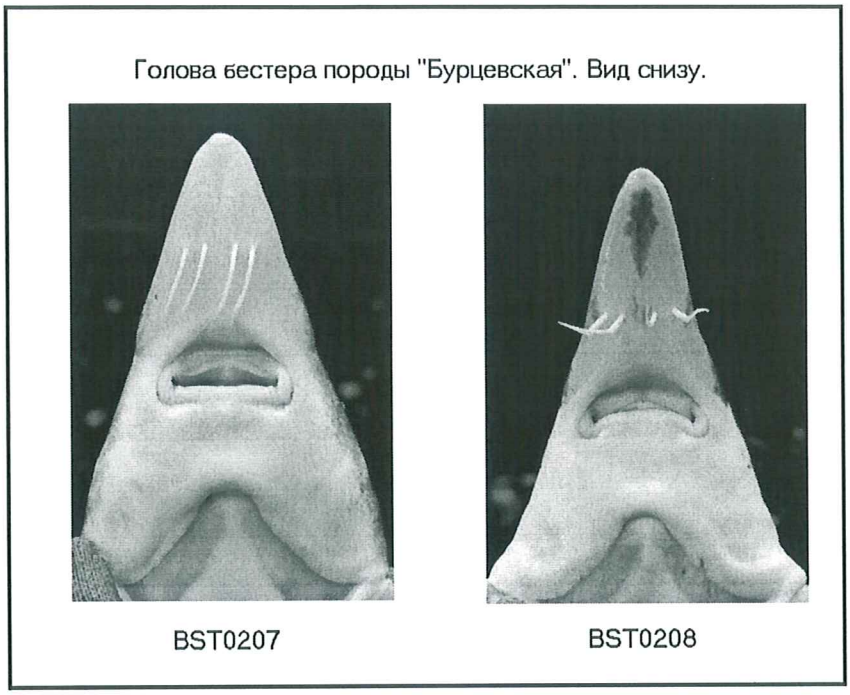

Fig. 9. Characteristic view of rostrum of "classical" hybrid (beluga (♀) and sterlet (♂) breed "Vnirovskaya").

Notice beluga-shaped pretrematic fold and enlarged moth opening.

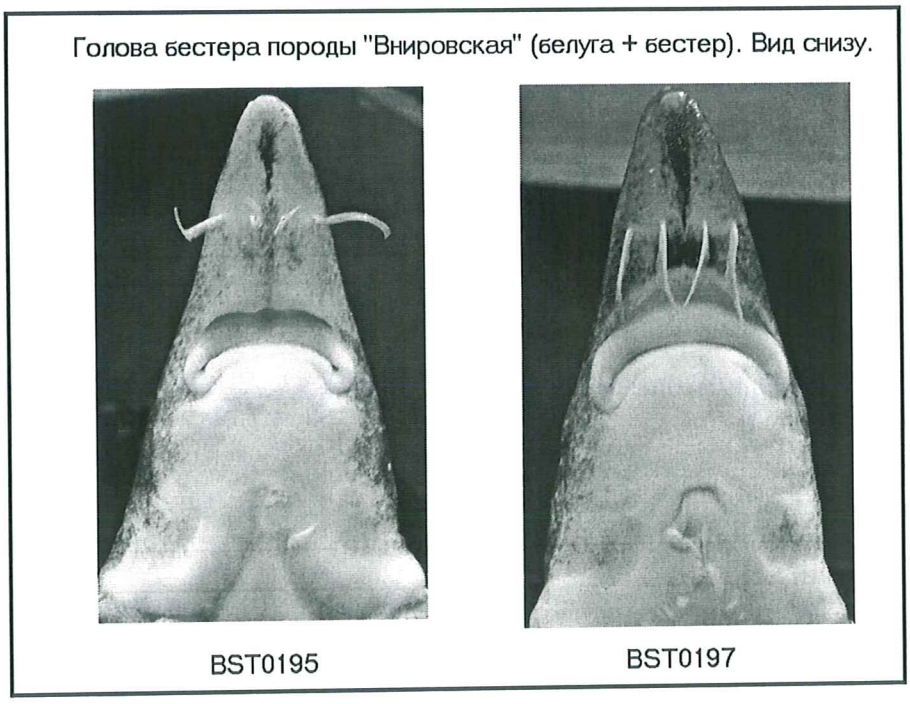

Fig. 10. Characteristic view of rostrum, tentacles, and mouth region in reciprocal hybrid breed "Vnirovskaya".

This photo illustrate that rostrum, tentacles, and mouth region morphology is very similar to its maternal species - beluga.

CONCLUSIONS

All existing at present time hybrid sturgeon breeds, developed by direct or reciprocal hybridization between beluga and sterlet, can be positively identified by molecular analysis.

In particular:

1. **bester as a hybrid, and artificially maintained hybrid breed "Burtsevskaya" can be identified by nuclear DNA analysis. Maternal origin (beluga or sterlet) revealed by mitochondrial DNA analysis.**
2. **breed "Aksayskaya", in contrast to breeds "Burtsevskaya" and "Vnirovskaya", do not posses beluga-type mtDNA.**
3. **breed "Vnirovskaya" is defined by increased frequency of allele "B" of microsatellite locus Afug51 and decreased frequency of allele "B" of locus Afug41.**
4. **Obtained molecular genetic markers can be used in identification of commercial products (caviar, fillet, etc.), while traditional morphological characters such as head shape can be used in live fish identification only.**

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