



Conference Abstract

Development of a set of oligonucleotides for the identification of the Sakhalin sturgeon *Acipenser mikadoi* Hilgendorf, 1892 by PCR

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Abstract

Sturgeons (family Acipenseridae) are valuable commercial fish and aquaculture resources. The *Acipenser* genus includes about 20 species, 12 of which are recorded for the Russian territory. One of the rarest is the Sakhalin sturgeon *A. mikadoi* Hilgendorf, 1892. At present, its population size is significantly decreased and the species is close to extinction. Natural populations of the species have survived in the Tumnin River in Khabarovsk Region and the Viakhtu River in Sakhalin Region. Vihtu River in Sakhalin Region. Due to the small numbers of rare and endangered fish species, noninvasive approaches, including the use of DNA from the environment, appear to be the most suitable for monitoring their species and genetic diversity. The method is well established in this area and has been successfully tested for the monitoring of several species of rare and endangered sturgeons. This paper presents the results of the development of primers specific for the mitochondrial DNA of the species *A. mikadoi*.

A matrix of 15 sequences of complete mitochondrial genomes from 3 sturgeon species whose ranges may overlap - *A. mikadoi* (KX276658), *A. dauricus* (KJ402277) and *A. schrenckii* (MH973728- MH973734, KX276660, KX276659, KF150287, KC905169, KC820796) - was generated. *A. medirostris* (NC_028405), which is genetically closest to the Sakhalin sturgeon, was also included in the analysis. A sliding window algorithm was

applied to the sequence matrix. A graph showing the distribution of divergence values along the mitochondrial genome of sturgeon was plotted based on the results of the analysis Fig. 1.

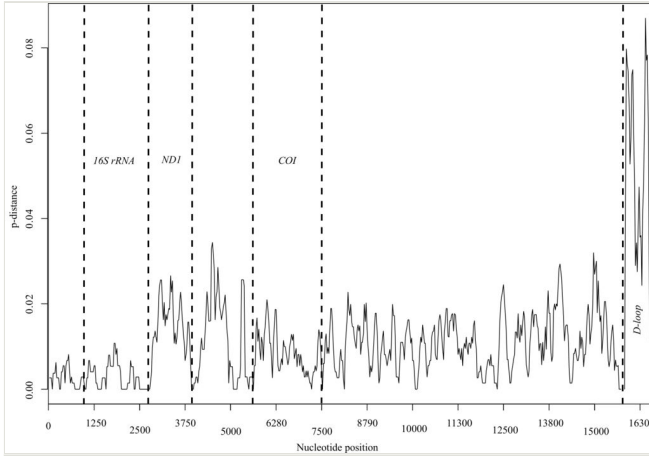


Figure 1. [doi](#)

Figure 1: Distribution of divergence values (p -distance) along 15 mitochondrial genome sequences of 4 sturgeon species. The analysis was performed using a sliding window algorithm. Vertical dashed lines indicate fragment boundaries, which were used to design species-specific primers.

Table 1.

Table 1: Information on pairs of the most appropriate species-specific primers selected by DECIPHER based on several regions of the sturgeon mitochondrial genome. L_{amp} - length of the amplified fragment, bp; T_m - melting temperature of forward and reverse primers, °C; Spn - species detected by Primer-BLAST searches among taxa of the family Acipenseridae (excluding *A. mikadoi*); Spc - number of homologous sequences detected by Primer-BLAST searches among taxa of the class Acipenseridae (excluding *A. mikadoi*)

Name of the pair of primers	Forward and reverse primer sequences, 5'-3' direction	L_{amp}	T_m	Spn	Spc
D-loop_114	CACTAACATGCGATTCCCGATACT / CAGTTGTGAATCCCTACAGTTGTT	114	56.9 / 55.2	<i>A.</i> <i>medirostris</i>	0
COI_219	ACTCGTCCCTAGATATTGTGCTT / GGCCTAGGAAATGTTGGGAAAA	219	55.4 / 57.6	<i>A.</i> <i>medirostris</i>	379
D-loop_I	AAGGCCTGTCTAGAACATTAGTT / CCATTCACTATTCACTCCTCTGGGAG	308	59.46 / 60.97	–	0

Based on matrices of individual genome fragments (*16S rRNA*, *COI*, *ND1*, and *D-loop*), a search for species-specific primers was performed in the DECIPHER program. A Primer-BLAST algorithm was run based on each pair of primers. Taxa Actinopterygii and Acipenseridae were used as references separately in different runs. In addition, primers were checked using local reference sequences of the complete mitochondrial genome of

representatives of the genus *Acipenser*. The results of primer evaluation are summarized in the table Table 1. As a result, a combination of primer pairs developed on the basis of D-loop and COI fragments seems to be the most successful for error-free identification of Sakhalin sturgeon.

Keywords

Sakhalin sturgeon, *Acipenser mikadoi*, primer design

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