Application of ITS1 and ITS2 for population genetic studies of sturgeons (Acipenseridae)

Guzel Davletshina [1,2] (guzel@mcb.nsc.ru), Sergey Kliver [1], Dmitry Prokopov [1], Elena Interesova [3], Vladimir Trifonov [1,4]

1 - IMCB SB RAS, Novosibirsk, Russia;
2 - ICG SB RAS, Novosibirsk, Russia;
3 - TSU, Tomsk, Russia;
4 - NSU, Novosibirsk, Russia

The order Acipenseridae is a very interesting group for evolutionary genetics: all species have unique morphology, inter-specific hybrids are widely occurring and there are variations between species in ploidy levels. Most acipenserids are endangered due to poaching and special efforts are required for the maintenance of natural populations. The genetic studies of acipenserids are still limited, although these are needed for successful farming. ITS (Internal transcribed spacer)– is the DNA spacer located between the small subunit and large subunit rRNA genes (fig.1). The genes encoding ribosomal RNAs are repeated several hundred times and tandemly arranged, thus new generation sequencing is the method of choice for estimation the SNP repertoire of an organism. Usually, ITS1 and ITS2 are used as nuclear phylogenetic markers to study the relationships between highly diverged taxonomic groups. Despite high interest to differentiate sturgeon species and their hybrids, acipenserid ITS1 and ITS2 sequences are missing in the GenBank depository, and most sturgeon population studies are performed using mitochondrial markers. Here we study the structure of ITS1 and ITS2 in several sturgeon species and demonstrate efficiency of these nuclear markers for species identification and interspecific hybrids confirmation.

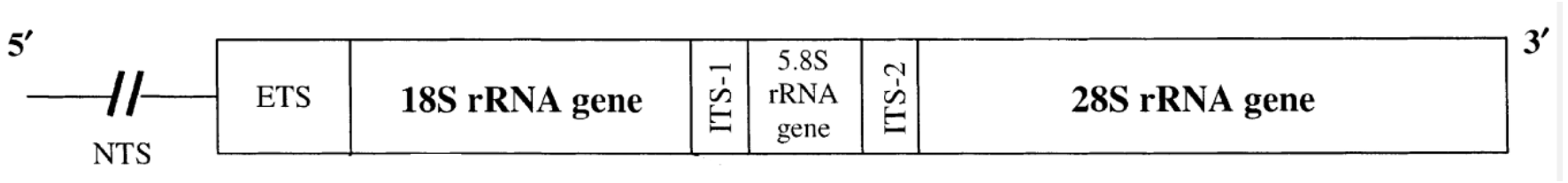

Figure 1. The structure of the rRNA gene cluster

Results

- We generated consensus sequences of ITS1 and ITS2 for four sturgeon species: *Acipenser baerii*, *A.ruthenus*, *A. gueldenstaedtii* ,and *A. nudiventris*. These consensus were identical for 120 chromosomal species (*A.ruthenus* and *A. nudiventris)*, as well as for 240-chromosomal species (*A. gueldenstaedtii* and *A. baerii).* We found six single nucleotide substitutions differentiating these two sturgeon groups (Table 1).

Table 1. S ingle nucleotide substitutions characteristic of the consensus of some species of sturgeon

Position	**SNP**	
ITS1	ABAE, AGUE*	ARU,ANU*
1934	G	A
2454	T	C
ITS2	ABAE, AGUE*	ARU,ANU*
2898	T	C
2917	C	T
3065	T	C
3103	T	C

*ABAE – *Acipenser baerii,* AGUE – *A. gueldenstaedtii*
ARU – *A. ruthenus,* ANU – *A. nudiventris*

Results

We sequenced ITS1 and ITS2 of putative interspecific hybrids between *A.baerii* and *A.ruthenus*, and found that indeed there were characteristic SNPs (in ITS1 and ITS2) for both species. Based on NGS data analysis, we demonstrated that *A.ruthenus* specific SNPs were represented in around 33% reads, while *A.baerii* specific SNPs were found in 67%, which corresponds to first generation hybrids subgenome ratio (as Siberian sturgeon genome is twice as large as sterlet genome) (Table 2).

Table 2. The mean frequency of variant occurrence in the reads for all samples characteristic of ITS1 and ITS2 SNPs in two species of sturgeons and their hybrids.

Species, number of individuals		***A. baerii*, n=7**		***A. ruthenus*, n=11**		***A.baerii*+*A.ruthenus*, n=3**	
number of chromosomes		240		120		120+60	
ITS1	1934	**G (0,974)**	A (0,025)	G (0,005)	**A (0,995)**	G (0,668)	A (0,332)
	2454	**T (0,974)**	C (0,025)	T (0,021)	**C (0,979)**	T (0,682)	C (0,318)
ITS2	2898	**T (0,747)**	C (0,253)	T (0,002)	**C (0,998)**	T (0,481)	C (0,519)
	2917	**C (0,972)**	T (0,028)	C (0,002)	**T (0,998)**	C (0,655)	T (0,345)
	3065	**T (0,952)**	C (0,048)	T (0,002)	**C (0,998)**	T (0,579)	C (0,421)
	3103	**T (0,970)**	C (0,030)	T (0,042)	**C (0,958)**	T (0,625)	C (0,375)

*-reference allels for *A.baerii* and *A.ruthenus* are in bold. Allels from both parental species were detected in hybrids

Conclusions

- ITS sequences can be applied as a convenient nuclear marker for species and hybrid identification in Acipenseridae.

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