Isolation and characterization of microsatellite loci in the Persian sturgeon (*Acipenser persicus*, Borodine, 1897) and cross-species amplification in four commercial sturgeons from the Caspian Sea

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Abstract

In order to have a sustainable management on Persian sturgeon as a highly commercial species in the South Caspian Sea, we need to identify its population structure and the level as well as its conservation status in their natural habitat. To develop a conservation program for this all Caspian Sea' sturgeon species it requires knowledge of its genetic diversity using reliable molecular marker to study population genetic structure. For these purposes, an enriched library was prepared based on a modified biotin-capture method. Approximately 1800 positive clones were screened for microsatellites in an *Acipenser persicus* genomic library. Of these 350 positively hybridizing clones were sequenced, and 81 clones were identified as having microsatellites with adequate flanking regions. We developed and tested 68 microsatellite primer pairs for Persian sturgeon. Out of 68 primer pairs developed, 11 pairs resulted in poor or no amplification, 13 were ambiguous, 6 were monomorphic, 20 were tetrasomic and 18 were octosomic in Persian sturgeon. While none of the markers showed disomic inheritance in Persian sturgeon and Russian sturgeon (*A. gueldenstaedtii*). Several of the markers appeared useful for studies stellate sturgeon (*A. stellatus*), ship sturgeon (*A.nudiventris*) and beluga (*Huso huso*). Nearly all the polymorphic pattern for ship, stellate and beluga displayed the simple banding patterns characteristic of disomic loci, while those for Russian sturgeon displayed banding patterns characteristic of tetraploid or higher polyploid levels. These markers may prove useful in a variety of future sturgeon population genetic studies in the Caspian Sea.

Keywords: Persian sturgeon, *Acipenser persicus*, Caspian Sea, Microsatellite, Population genetic

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Introduction

The Persian sturgeon is an anadromous species living in the Caspian Sea, but it mainly inhabits in the southern Caspian region along the Iranian coast. Persian sturgeon enters the rivers for spawning, mainly the Sefid-Rud, Tajan and Gorganrud rivers in Iran and Kura river in Azerbaijan, less -the Volga, Ural, Samur, Terek, Lenkoranka and Astara rivers (Berg, 1948). Its population, after collapse in 1970's, has risen in the 1990 decade and comprises the largest proportion of the total Iranian sturgeon commercial catch in recent years (Pikitch et al., 2005; Moghim et al., 2006). While in 1980-s its catch did not exceed 5% of the total sturgeon catches at the Volga and the Ural rivers, the share of this species decreased to 0.03%, in the year of 2000 (Khodorevskaya et al., 2000). Persian sturgeon is listed as a critically endangered species by the International Union for Nature Conservation (IUCN 2011), due to continued overexploitation, illegal catch spawning habitat loss and pollution.

Persian sturgeon stocks are recovered mainly by artificial propagation and Iranian Fisheries, release millions of 3-5 g fingerlings to the adjacent rivers of Caspian Sea annually (Abdolhay and Baradaran Tahori, 2006; Moghim et al., 2006). The sustainable management and conservation plan of this unique species requires knowledge of its genetic structure and levels of each stock in its natural habitat. Several population genetic studies were conducted on five sturgeon species in the Caspian Sea using microsatellite markers (Pourkazemi, 2007; Safari et al., 2008; Noruzi et al., 2008; Khoshkholgh et al., 2008).

Cross-species amplification using microsatellite primers of *Scaphirhynchus* were applied in the Persian sturgeon by Moghim et al., (2009) but none of the loci exhibited disomic inheritance. While microsatellites are expensive to develop initially, because of the higher degree of statistical power associated with codominant markers -microsatellite loci were developed for the Persian sturgeon to find disomic loci. The objective of the present research was to develop the Persian sturgeon specific microsatellite primers, and compare its application on other four sturgeon species in the Caspian Sea.

Materials and methods

An enriched library was prepared following a modification of the protocols of Hamilton et al., (1999) and Glenn et al., (2000) as described in Heist et al., (2003). Total genomic DNA from a single Persian sturgeon was digested with *RsaI*. Complementary linkers for use as polymerase chain reaction (PCR) primer sites were designed to contain an *Rsa*I site when double-stranded (Linker-F: 5'-CTAAGGCCTTGATCGCAGAAGC-3'; phosphorylated Linker-R: 5'-pGCTTCTGCGATCAAGGCCTTAGAA AA-3') and ligated to genomic DNA fragments. Biotinylated $(GT)_{15}$, $(GA)_{15}$, $(GATA)_5$ and $(GACA)_5$ probes were hybridized to linker-ligated DNA fragments and microsatellite containing DNA was selectively retained by binding biotinylated DNA fragments to streptavidin coated MagneSphere¨ paramagnetic particles (Promega, Madison, WI, USA). Microsatellite-containing fragments were then amplified using PCR reactions containing approximately 10 ng microsatellite-enriched genomic DNA and 1× PCR buffer (200 mM KCl, 100 mM Tris), 200 µm of each dNTP, 2 mM $MgCl_2$, 1 µm Linker-F as primer, and 2 units Taq DNA polymerase. PCR amplifications consisted of 94 °C for 5 min, followed by 40 cycles of 94 °C for 45 s, 62 °C for 1 min, and 72 °C for 1 min using an Quanta Biotec master cycler gradient thermocycler (Quanta Biotech Ltd, Surrey, United Kingdom). The PCR product was ligated

into a pUC19 cloning vector and used to transform DH5α competent cells (Invitrogen, Carlsbad, Ca, USA). Colonies were transferred to a nylon membrane and probed with ^{32}P labeled $(GT)_{15}$, $(GA)_{15}$, $(GATA)_5$ and $(GACA)_5$. We isolated plasmid DNA from positive colonies using the Wizard miniprep kit (Promega). The positive clones were sequenced using M13 (F and R) universal sequence primers. Plasmid DNA was isolated from positive clones and sequenced with the ABI PRISM BigDye terminator cycle sequencing ready reaction kit using an ABI 377 automated sequencer (PE Applied Biosystems, Weiterstadt, Germany). Approximately 1800 positive clones were screened for microsatellites in an *Acipenser persicus* genomic library. Of these 350 positively hybridizing clones were sequenced, and 81 clones were identified as having microsatellites with adequate flanking regions. In total 68 microsatellite PCR primers were designed after omitting 13 clones with the same sequences. Microsatellite PCR primers were designed using the Primer3 (http://www.genome.wi.mit.edu/cgi-in/primer/primer3.cgi) or the MacVector (Oxford Molecular) software package. These loci were tested in Persian sturgeon (n=12) to identify optimal annealing temperatures and to determine if disomic polymorphic products could be reliably amplified. Additional individuals (n=24) from different populations were used to confirm the ploidy status.

Amplification was performed using a gradient thermocycler at annealing temperatures ranging from 52 °C to 64 °C. The ten microlitre PCRs reactions containing approximately 1-10 ng genomic DNA, 0.1 units Taq DNA polymerase, 0.5 mM of each primer, 200 mM of each dNTP, 2 mM $MgCl_2$, and 1× PCR buffer. Amplification consisted of a 5 min denaturing step at 95 °C, 40 cycles of 95 °C for 30 s, 56 - 64 °C for 30 s, and 72 °C for 30 s, followed by a single five-minute extension step at 72 °C. PCR products were suspended 1:1 in 98% formamide/loading dye, denatured at 95°C for 5 min, and separated in a 6% denaturing polyacrylamide gels on a BIO-RAD gel sequencer running at 70 W for 45 - 60 min and visualized via Silver staining (An et al., 2009). Allele sizes were estimated using a 50-bp ladder molecular size standard (Invitrogen).

Amplification results were characterized as monomorphic if a single band of the same size was observed in all individuals, disomic if one or two bands were seen in every individual, tetrasomic if some individuals exhibited three or four bands, octosomic if more than four bands were observed in some individuals, weak if products were too faint to resolve, and ambiguous if banding patters were too complex for us to interpret.

All primer pairs (except Ape-01 to Ape-18) were tested for cross-species amplification efficiency with four sturgeon species of the Caspian Sea, under the same PCR conditions used for Persian sturgeon including; the Stellate sturgeon, Russian sturgeon, Ship sturgeon and Beluga. Six individuals from each species were screened for polymorphism at these loci.

Results

In total 68 microsatellites PCR primers were designed after omitting 13 clones with the same sequences. Out of 68 primer pairs developed, 10 resulted in poor or no amplification, 13 were ambiguous; six of loci that amplified successfully were monomorphic, 21 were tetrasomic and 18 were octosomic in Persian sturgeon. None of the loci exhibited disomic inheritance (Figure 1). Locus name, clone size, GenBank accession number, repeat motif, PCR annealing temperature, and primer sequences are listed for these loci in Table 1.

Table 1: Characterization of 68 microsatellite loci in Persian sturgeon (*Acipenser persicus*), including repeat motifs, primer sequences and GenBank accession numbers, and cross-amplification in Russian (*A. gueldenstaedtii*), stellatus (*A. stellatus*), ship (*A. nudiventris*) sturgeon and beluga (*Huso huso*).

Prime	Repeat type and length	primer sequences (5' to 3')	GenBank accession no.	Product size (bp)	*A. persicus*	*A. gueldenstaedtii*	*A. stellatus*	*A. nudiventris*	*H. huso*
Ape-01	$(CAGA)_{14}$	F:CAATGTCACAAACACACACAGCG R:TTTCTCTCCAGTTCGTCAGATGC	JF773767	171	tetrasomic				
Ape_02	$(GT)_{13}$	F:CAAACATACCGTTCTGTGGGAC R:CGTCCTGCTGAAGAAGGTAAATATC	JF773768	123	octosomic				
Ape_03	$(CAGA)_{14}$	F:CAATGTCACAAACACACACAGCG R:GCAGAAAAACCAGCCCACAGTC	JF773769	141	tetrasomic				
Ape_04	$(CA)_{10}$	F:GATAAAGGCACGACGCTACAACTAC R:CATCTCAACCTGACAAATACCGTG	JF773770	119	octosomic				
Ape_05	$(CAGA)_{6}$	F:ACTGAACCATTGGAGTATTGAGGC R:ACAGTAAACGCACACCAACAAGG	JF773771	137	tetrasomic				
Ape_06	$(CAGA)_{15}$	F:AAACCTTCAGAGAGAGAGGGAGCG R:GCAGAAAAACCAGCCCACAGTC	JF773772	239	octosomic				
Ape_07	$(CT)_{12}$	F:CACAATTCACAGTCAGGGCTGTC R:TGCCACAATTCACAGTCAGGG	JF773773	253	ambiguous				
Ape_08	$(CT)_{41}$	F: AGCCCCTGTGTCTGTCTGTTTG R:GGAAATTCTTTGGTGTGTGTGGG	JF773774	164	ambiguous				
Ape_09	$(CT)_{35}$	F:GATCAGCTCCAGTTTGCAGTGC R:GGAGATAGATTCGTTCTGCCAAGTC	JF773775	299	ambiguous				
Ape_10	$(CAGA)_{13}$	F:AGGGAGCGACAAACTTACTCCTG R:GCAGAAGCACAGCAATGTGAAATC	JF773776	275	octosomic				
Ape_11	$(CAGA)_{7}$	F:AACCATTGGAGTATTGAGGCACTG R:ACAGTAAACGCACACCAACAAGG	JF773777	133	octosomic				
Ape_12	$(CT)_{13}$	F:GCCTTCAACATTCTCCTTATTGAGG R:CGTTACGAAAACAAGTGTTCTTGCC	JF773778	112	octosomic				
Ape_13	$(CTGT)_{13}$	F:TCGCAGAAAAACCAGCCCAC R:AAACCTTCAGAGAGAGAGGGAGCG	JF773779	233	octosomic				
Ape_14	$(GA)_{22}$	F:ATTTCGTGTCTGTCCTTAATTGGTG R:GTAAATCTCACAATGTCCGTGGC	JF773780	164	tetrasomic				
Ape_15	$(CT)_{64}$	F:TTCCTGTTGCCAGACATTTTAACAC R:TCCTTAATTGGTGAAATTCATACCG	JF773781	175	no amplify				
Ape_16	$(GA)_{13}$	F:AATGGAGAGAGAGAGAGAGGGAGTG R:AAGTCTTACAAAACCCGTGGTGG	JF773782	230	tetrasomic				
Ape_17	$(CTGT)_{15}$	F:TCGCAGAAAAACCAGCCCAC R:GCATTTCGGAGAAACCTTCAGAG	JF773783	248	octosomic				
Ape_18	$(GA)_{14}$	F:CGCAGAAGCACTAAAAGTCAAAGTC	JF773784	202	tetrasomic				

		R:GGAAGATTTCAGAGAGCAGCACTC							
Ape-19	$(CA)_{14}$	F:GGGGTTAGAAAGCACAGATGA R:CAAGGTGGCACAGTGGACTA	EU483155	172	octosomic	octosomic	ambiguous s	disomic	disomic
Ape_20	$(GACA)_5$	F:CACTGCCTGCTGCCTAAAAC R:ACTGTGGGGCTCTGTCTGTC	EU531732	176	tetrasomic	tetrasomic	disomic	mono	disomic
Ape_21	$(GACA)_5$	F:GGAGACAGACGAGGGAGAGA R:ATTCGGGACGTGAGACACAT	EU531733	397	tetrasomic	weak	weak	weak	ambiguous
Ape_22	$(GTCT)_{14}$	F:CAGAAAAACCAGCCCACAGT R:GAGAGAGAGGGAGCGACAAA	EU531734	245	octosomic	octosomic	disomic	mono	disomic
Ape_23	$(CA)_{25}$	F:CCTGCCACACCTACACAGAC R:GCGCATGCCTACAACAATTT	JF781300	177	no amplify	ambiguous	disomic	ambiguous	ambiguous
Ape_24	$(CA)_{14}$	F:TGAACACAAAACACGGGACA R:TAAGGCCTTGATCGCAGAAG	EU531735	237	no amplify	ambiguous	disomic	ambiguous	ambiguous
Ape_25	$(GAGAG)_5$	F:CCCGTGTCTGTCTGTCTGTTT R:ATCTCAGCCAGGAAGAACGA	EU531736	159	tetrasomic	tetrasomic	disomic	disomic	mono
Ape_26	$(GA)_{38}$	F:GAGAGAGAGGGAGCGACAAA R:CAGAAAAACCAGCCCACAGT	EU531737	225	tetrasomic	tetrasomic	mono	mono	disomic
Ape_27	$(GA)_{38}$	F:AACGAGTCCATGCTGGAGAG R:CCCCGTGTCTGTTTGTTTGT	EU531738	171	tetrasomic	tetrasomic	disomic	disomic	mono
Ape_28	$(CTGT)_{10}$	F:CTCAGACCCGTGAGACACAA R:GCATTTCGGAGAAACCTTCA	EU531739	192	tetrasomic	no amply	disomic	no amply	disomic
Ape_29	$(GT)_{15}$	F:TGAACACAAAACACGGGACA R:CGCACACACACGCACATA	EU531740	215	mono	tetrasomic	mono	disomic	ambiguous
Ape_30	$(GT)_{11}$	F:AGGGCTACCTCCAGCTGTGT R:TCGCTCCTCAGACTCTGGAC	EU531741	172	tetrasomic	tetrasomic	disomic	disomic	ambiguous
Ape_31	$(CT)_{26}$	F:GCCCCTGTGTCTGTCTGTTT R:CGTGTGTGAGCGAGATAGGA	EU531742	189	no amplify	_	mono	_	mono
Ape_32	$(GACA)_{15}$	F:CAAAGAGAGAGGGAGCGACA R:CAGAAAAACCAGCCCACAGT	EU531743	227	octosomic	octosomic	disomic	mono	disomic
Ape_33	$(CTAT)_9$	F:TGCTGATCTAACCATTTCTTTGC R:AAGGCACACCATCTTTGTCC	EU531744	190	tetrasomic	tetrasomic	disomic	disomic	disomic
Ape_34	$(CA)_{10}$	F:CCACCACCCTCCCACAATA R:GGGCAAATTGACTGCTTGAT	EU531745	162	mono	_	mono	_	mono
Ape-35	(GACA)6	F: ACTGCCTGCTGCCTAAAACA R: CTAAGGCCTTGATCGCAGAA	JF740087	231	ambiguous	mono	disomic	mono	disomic
Ape-36	(CTGT)5	F: TAGCACTGGGAACAGAAGCA R: AAAGCTCCAACACATGGACA	JF740088	240	no amplify	ambiguous	disomic	ambiguous	no amplify
Ape-38	(GTCT)6	F: GTGCGTGTGTGTGTGTGTGT R: GTGTGACAGTGAAGCGGAGA	JF773785	352	no amplify	tetrasomic	mono	disomic	mono
Ape-39	(GA)36	F:GGAAGGGGAGAGAGAGAACG R: GCGCTGTATTGTGGTGACTG	JF773786	269	tetrasomic	ambiguous	mono	ambiguous	mono
Ape-40	(CA)18	F: CCGCAAACACACATACGC R: GCGCTCTCGTAGACTGTGC	JF773787	250	ambiguous	ambiguous	disomic	ambiguous	disomic
Ape-42	(CT)18	F: CGTGCCCACTGTTTTACCTT R: TTGGATTCTAGGACGGTTGG	JF773788	254	no amplify	no amplify	no amplify	no amplify	no amplify
Ape-43	(CT)25	F: GCCCCTGTGTCTGTCTGTTT R: GCATGTCTTTTTCCAAAGTGAA	JF773789	180	no amplify	ambiguous	no amplify	ambiguous	no amplify

Ape-46	(GA)27	F: TGTGCCACAATTCACAGTCA R: CAGAGAGAGTCAGCGGGTCT	JF773790	245	octosomic	octosomic	no amplify	disomic	mono
Ape-47	(GA)34	F: ATCTCAGCCAGGAAGAACGA R: GCCCCTGTGTCTGTCTGTTT	JF773791	180	octosomic	tetrasomic	disomic	disomic	disomic
Ape-48	(GA)32	F: TGTGCCACAATTCACAGTCA R: CCACGTTTATTAACCCAAATCAA	JF773792	201	ambiguous	no amplify	no amplify	no amplify	no amplify
Ape-49	(GA)38	F: ATCTCAGCCAGGAAGAACGA R: GCCCCTGTGTCTGTCTGTTT	JF773793	188	octosomic	tetrasomic	disomic	disomic	no amplify
Ape-50	(CA)24	F: CCTGCTGCTGTATAAACTATGGA R: CGGACTGTGTGTCTGTCTGTC	JF773794	249	mono	mono	disomic	mono	mono
Ape-51	$(GA)_{18}G2(GA)_{19}$	F: ATCTCAGCCAGGAAGAACGA R: CCCGTGTCTGTCTGTCTGTTT	JF773795	189	tetrasomic	tetrasomic	disomic	disomic	mono
Ape-52	(CAGA)6	F:CACTGCCTGCTGCCTAAAAC R: TATTAACCCATCGGCTCCAC	JF773796	151	no amplify	no amplify	mono	no amplify	mono
Ape-53	(CA)14	F: CGCACACACACGCACATA R: ACGGCACTATACGCCAAAAT	JF773797	196	ambiguous	weak	weak	weak	weak
Ape-55	(GA)25	F: ATCTCAGCCAGGAAGAACGA R: CCCGTGTCTGTCTGTCTGTTT	JF773798	165	tetrasomic	tetrasomic	disomic	disomic	mono
Ape-56	(CA)11	F: TCGTCCTGCTGAAGAAGGTAA R: CGTTCTGTGGGACAGTGAGA	JF773799	146	tetrasomic	octosomic	ambiguous	tetrasomic	ambiguous
Ape-57	(CA)15	F: CCATGCACACGCACTAGTTT R: ATTGTCATGCCCGTTTCAGT	JF773800	218	mono	_	no amplify	_	no amplify
Ape-58	(CA)28	F: GGACTCCAGAGACAGTGCAA R: GGACACGCATAGGTGCTTCT	JF773801	155	ambiguous	ambiguous	disomic	ambiguous	disomic
Ape-59	(CA)11	F: CGTCCTGCTCAAGAAGGTAAA R: CGTCCTGCTCAAGAAGGTAAA	JF773802	110	no amplify	no amplify	no amplify	no amplify	no amplify
Ape-60	(CT)25	F: TTCAGGGATCCTGTCTCCAG R: GGGGAGCAGTCACAAAGAGT	JF773803	231	ambiguous	mono	no amplify	no amplify	no amplify
Ape-62	$(CA)5[(C_2)(CA)_2]4$	F: GACTTCGCCTACAGCAGCTC R: TAGGAACCGGACACGCATAG	JF773804	385	octosomic	tetrasomic	disomic	disomic	disomic
Ape-63	(GGCA)6	F: GCACTTTGTTCAGGCAGACA R: GACAGGAGGAAATGCTGGAA	JF773805	360	mono	tetrasomic	weak	mono	disomic
Ape-64	(CAGA)12	F: GAGAGAGGGAGCGACAAACTT R: TAGCTGAGTGGGTGTGGATG	JF773806	213	mono	mono	disomic	mono	weak
Ape-65	(GA)17CA (CAGA)9(GA)6	F: TTGAACCTTCCACATCCTGA R: CCCAAGGACCTACAGTCTGC	JF773807	154	ambiguous	ambiguous	disomic	ambiguous	weak
Ape-66	(GTCT)14	F: CAGAAAAACCAGCCCACAGT R: GAGAGAGAGGGAGCGACAAA	JF773808	225	ambiguous	octosomic	disomic	mono	disomic
Ape-68	(GACA)5	F: AGTTCGCACTGTAGGGATTCA R:TTCGCAATTAAGGTTAAAAAGACA	JF773809	300	ambiguous	mono	weak	disomic	disomic
Ape-70	(CA)11	F: AGTGACCCCTCTCTCCCACT R: GTCAGGGTCAGGGTCTGTGT	JF773810	166	tetrasomic	mono	mono	mono	mono
Ape-71	(GACA)15	F: GAGAGAGAGGGAGCGACAAA R: CAGAAAAACCAGCCCACAGT	JF773811	296	octosomic	tetrasomic	mono	mono	-
Ape-73	(GACA)7G2(CAGA)6	F: GAGAGAGAGGGAGCGACAAA R: CAGAAAAACCAGCCCACAGT	JF773812	221	octosomic	octosomic	disomic	mono	no amplify

Ape-76	(GACA)15	F: GAGAGAGAGGGAGCGACAAA R: CAGAAAAACCAGCCCACAGT	JF773813	225	octosomic	tetrasomic	disomic	disomic	disomic
Ape-77	(GA)28	F: ATCTCAGCCAGGAAGAACGA R: CCCGTGTCTGTCTGTCTGTTT	JF773814	171	tetrasomic	disomic	disomic	disomic	ambiguous
Ape-78	(CAGA)6	F: CACTGCCTGCTGCCTAAAAC R: TATTAACCCATCGGCTCCAC	JF773815	151	tetrasomic	tetrasomic	disomic	disomic	disomic
Ape-80	(CTGT)14	F: GGGGTTCAGGAGGCTTTCTA R: GCACTTTGTTCAGGCAGACA	JF773816	228	ambiguous	disomic	_	mono	mono
Ape-81	(GA)28	F: GGTTCCAATGTATCAGGCAAA R: GCCGAGCAGCTCCATTAG	JF773817	152	tetrasomic	_	ambiguous	_	ambiguous

Because no loci exhibited disomic inheritance in Persian sturgeon, standard tests for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium could not be determined. Fifty two microsatellite primer pairs developed for Persian sturgeon were tested to generate polymorphic genetic markers for four Caspian Sea sturgeon species. In Russian sturgeon, forty six loci were screened in initial screening of samples. Only 32 (83%) of these primer pairs amplified successfully. Of these, 25 loci (54%) were found to be polymorphic in Russian sturgeon. Seven loci were monomorphic while eight loci failed to amplify. Of the 25 polymorphic loci identified, 18 loci were tetrasomic while seven loci were octosomic.

Of the 49 microsatellite loci that were tested in Stellate sturgeon, 39 loci (84%) amplified successfully of which 27 (69%) were polymorphic and seven loci (18%) were monomorphic. All polymorphic loci exhibited disomic banding patterns in stellate sturgeon. Ten loci failed to produce any bands. Forty six loci were tested for cross-species amplification in ship sturgeon. Thirty nine loci (85%) amplified successfully producing 18 polymorphic loci (39%), 13 loci were monomorphic and 8 loci failed to produce any bands. In addition, ambiguous bands were produced at eight loci. All polymorphic loci exhibited disomic banding patterns in Ship sturgeon.

Forty nine loci were screened in Beluga samples. Only 29 loci (83%) amplified successfully. 18 loci (37%) were polymorphic. 11 loci (24%) were monomorphic while 8 loci failed to amplify. All polymorphic loci showed disomic banding patterns. Thus all loci that amplified successfully and that were shown to be polymorphic in ship, stellate and beluga sturgeon species showed simple banding patterns characteristic of disomic loci, while those for Russian sturgeon(like Persian sturgeon) displayed banding patterns characteristic of tetraploid or higher polyploid karyotypes. Examples of electrophoretic banding patterns at polymorphic loci in the four sturgeon species are presented in Figure 2. Detailed results of cross-species amplification efficiency of the SSR primer pairs developed for Persian sturgeon tested on four Caspian Sea sturgeon species are presented Table 1. Due to the polysomic nature of these loci and the small sample sizes screened in each species, it was considered not possible to test for conformation to hardy-Weinberg equilibrium or heterozygosity per locus. These data will require a more extensive study of larger populations per species.

Discussion

Traditionally, microsatellite marker are developed by extensive screening for microsatellite containing clones through repetitive hybridizations of a repeat motif probe to a large number of random clones (Rassmann et al., 1991). Such an isolation strategy resulted in low rate of the number of positive clones (containing microsatellites) detection. This traditional method usually that can be obtained by means of ranges from 12% to less than 0.04% (Zane et al., 2002).

Using modified protocols of Hamilton et al., (1999) and Glenn et al., (2000) to construct and clone genomic libraries increased proportions of inserts that contained tandem repeat arrays. Thus, a greater number of microsatellite repeat regions detected, sequenced and subsequently used to design species-specific flanking primers for microsatellite amplification. This technique reduced the time and effort as well as cost required for microsatellite isolation from Persian sturgeon. To date there has been no species specific microsatellite primers developed for the Caspian Sea sturgeon species and this is the first report for Persian sturgeon.

Developing microsatellite markers for sturgeon species can be challenging particularly in species that have experienced multiple polyploid events (i.e., 4n, 8n and 16n species) for example, Welsh and May (2006) found only nine reliable disomic microsatellites among 254 primer pairs tested in lake sturgeon (*A. fulvescens*), a species with the same ploidy level as Persian sturgeon.

Amplification results for Persian sturgeon and cross-species amplifications in four Caspian Sea sturgeon species were consistent with the reported ploidy levels of each species. Ship, Stellate and Beluga sturgeon are considered to be functional diploids (2n= 120), while Persian and Russian sturgeon are considered to be functional tetraploids (2n= 240) that are undergoing rediploidization (Ludwig et al., 2001; Fontana, 2002, Fontana et. al. 2008).

While none of the markers that amplified in Persian sturgeon were disomic, they may still prove to be useful as dominant markers (e.g. Israel et al., 2009) for this species. Several markers appear to show codominant inheritance patterns in ship, stellate, and beluga sturgeon and may prove useful in a variety of future population genetic applications, ranging from stock assessment to mapping of quantitative trait loci in culture stocks. Testing more individuals and fine tuning optimization of PCR reactions, is likely to identify new alleles at polymorphic loci, as well as the possibility of detecting polymorphisms in loci that were recorded as being monomorphic in small test populations here. Results of these studies suggested that SSR DNA markers developed for Persian sturgeon were candidates for application in other sturgeon species in the Caspian Sea. This proved to be the case and suggests a high level of sequence homology among related species in the Caspian Sea, a result that is consistent with the results from studies on other sturgeon species (May et al. 1997; McQuown et al., 2000).

To eliminate the inherent difficulties associated with tetrasomic loci, future Persian sturgeon genetic marker development required identifying nuclear microsatellite loci that are disomic.

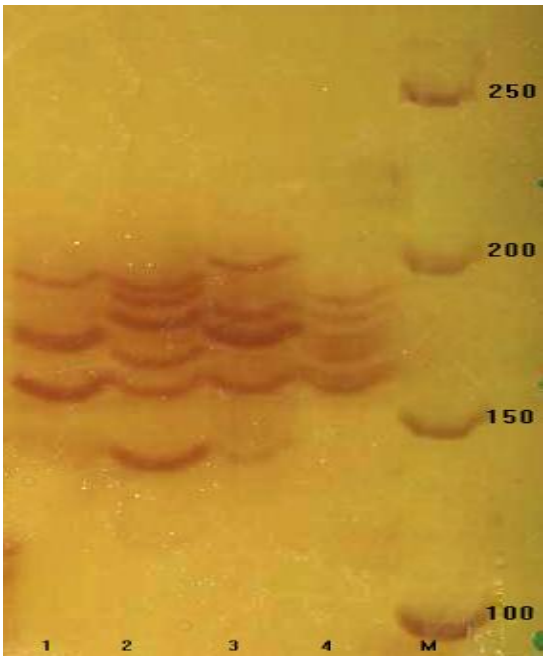
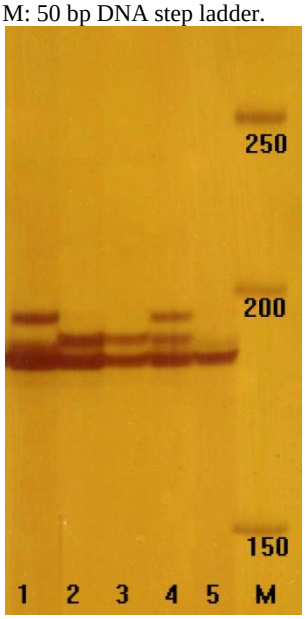
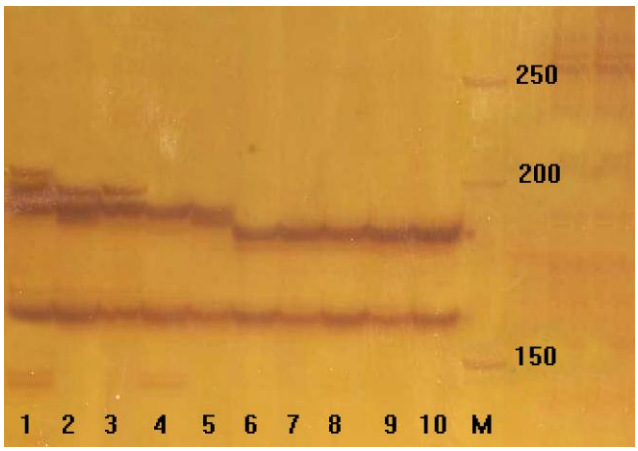


Figure 1: Electrophoretic banding pattern for locus Ape_19 in Persian sturgeon that exhibited octosomic inheritance. Relative allele's density would correspond to gene doses

Lane M: 50 bp DNA step ladder.



1-A: Persian sturgeon



1- B: Russian (lanes1-5) and Ship (lanes 6-10) Sturgeon.

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Figure 2: Electrophoretic banding pattern for locus Ape_20 in Persian (A), Russian (B: 1-5) and ship sturgeon (B: 6-10). This locus exhibited tetrasomy in Persian and Russian sturgeon but was monomorphic inheritance in ship sturgeon. Lane M: 50 bp DNA step ladder

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