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MICROSATELLITE LETTERS

EST-based microsatellites for northern pike (*Esox lucius*) and cross-amplification across all *Esox* species

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Abstract Northern pike experience a global decline of populations and a better understanding of the species' population genetic diversity and structure is needed for proper conservation and management. We developed 17 novel microsatellite markers in North American northern pike, *Esox lucius*, and tested cross-amplification on European populations as well as all five other *Esox* species. One marker deviated significantly from Hardy–Weinberg equilibrium and no linkage disequilibrium among all loci pairs was observed. A mean of 6.88 alleles per locus was found (between 2 and 23 alleles) and mean expected heterozygozity was 0.49 (range 0.033–0.950). All loci were successfully amplified on *E. lucius* from North America and Europe and *E. reichertii*. Between eight and 11 loci were successfully amplified in other *Esox* species and four out of 17 loci were successfully cross-amplified on all species.

Keywords *Esox lucius* · Northern pike · EST-linked microsatellite · Primers · PCR

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Primer note

Northern pike (*Esox lucius*) is a ubiquitous fish in the northern hemisphere's freshwater bodies and is heavily exploited by recreational and commercial fisheries. Many stocks are also impacted by habitat alterations (Casselman and Lewis 1996). Northern pike populations have been shown to decline globally (e.g. Casselman and Lewis 1996; Smith et al. 2007). A better understanding of the species' population genetic diversity and structure is needed if we are to achieve proper conservation and management. Towards this end we developed novel polymorphic microsatellite markers for this species.

We developed 17 pairs of primers from EST sequences available from the cGRASP EST database (Table 1). Based on blast searches (Blast2Go software; Conesa et al. 2005), only EL21 was annotated and corresponded to the *Danio rerio* solute carrier organic anion transporter family member 2b1 gene. Microsatellite markers were amplified on 30 *E. lucius* individuals captured from three locations in St. Lawrence River in North America (46°24′2″°N, −72°22′8″°W; 45°22′11″°N, −73°46′19″°W; 44°25′16″°N, −75°51′55″°W). Cross-amplification was tested on all five *Esox* species [*E. lucius* from Europe ($n = 20$), *E. reichertii* ($n = 17$) from Eurasia, *E. americanus americanus* ($n = 10$), *E. americanus vermiculatus* ($n = 10$), *E. masquinongy* ($n = 10$) and *E. niger* ($n = 10$) from North America]. PCR cycles started with a 3 min denaturation step at 95 °C, followed by 35 cycles with 30 s of denaturation at 95 °C, 30 s of hybridization at 56 or 60 °C and 30 s of elongation at 72 °C, and were completed with a final 30 min elongation step at 60 °C. Electrophoresis was completed using an Applied Biosystems 3130xl Genetic Analyzer and DATA COLLECTION v3.1.1. Genotypes were resolved using GENEMAPPER v.4.1. Number of alleles per locus (N_A),

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Table 1 Microsatellite markers characteristics

Locus	Marker set	Repeat motif	Primer sequence 5′–3′	Fluorescent color	T_A (°C)	Concentration in PCR reaction (pg/µl)
EL01	E	AG	F: CAGCACTATCCAAAGGCGTAG	VIC	60	0.18
			R: TCTTCCACCGTTATCATCACA			
EL02	A	AC	F: ACACATGCGTCTACAAGCACA	VIC	60	0.18
			R: CCTCCCTGGCATACGTCTTAC			
EL03	A	CA	F: GGCGAGTTAGGAGCTCAGGTA	VIC	60	0.12
			R: TGGGGAGCGATGTGTATGTA			
EL05	A	AC	F: GGAAATGGGGAGTCTCCTGTA	NED	60	0.08
			R: TGCAAACAGTCCTTTTGGAAG			
EL09	D	CA	F: TTTAATGACAATGCCCACTGC	6-FAM	60	0.18
			R: ACTCCGCATGAGAGAGACAGA			
EL10	D	AC	F: CACCTGTCCTCACTTTGATTG	NED	60	0.18
			R: GTGGTTCCATGTGTGTCTCAG			
EL12	A	CA	F: TGGGGTTGTACACAAACTTTCTC	6-FAM	60	0.18
			R: TAGCCCAATCATTACCCTTGG			
EL15	B	CGCA	F: TGAAAAGCAGTGTTGCATCTG	NED	60	0.11
			R: TTCTGTCCATCCATCTCCATC			
EL16	E	TATG	F: GGGTGAAGATTCCCATAAAGC	6-FAM	60	0.18
			R: AGTGTTGGTGACGTTGACTCC			
EL17	B	AC	F: GTGCTTTTGGGTACAATCAATG	NED	60	0.23
			R: TTTCACCAAATACCTGGTCTCA			
EL19	E	GT	F: GAGGTTCAAAAGGAAGGGTTG	NED	60	0.18
			R: AAGAACACTTCACCGCCATAA			
EL20	A	AC	F: CCAAAGCCAGTTGATGCTAAG	6-FAM	60	0.18
			R: CTCAAACTGTGCCTCACACAA			
EL21	D	AG	F: GAGGAGTGCACAGAGAACAGG	VIC	60	0.18
			R: CCAAAACTGGTGAACACAACC			
EL22	B	CT	F: ACATTCATTCGGAGCAGTCAG	6-FAM	60	0.33
			R: CCCATGCCAATGAAGATATTG			
EL23	A	GA	F: GTGCGTTCGTCTCTCATCTTC	VIC	60	0.14
			R: GATCTGTCCATCCTTCCTGTG			
EL26	C	AG	F: ACAGGGATATGTCATGGTGGA	6-FAM	60	0.18
			R: CCAAAACTGGTGAACACAACC			
EL27	B	TAC	F: AATGCTTCTGAATGCTTGCAC	6-FAM	60	0.08
			R: CAAAGGATAGACAGCAAAGCAA			
EL28	C	CT	F: TGTAGGTGCACTGTGGAGATG	VIC	60	0.18
			R: ACGCACACAAAGGGAATAGTG			

T_A annealing temperature

expected and observed heterozygozity (H_E, H_O), allele range size, F_{is}, linkage disequilibrium (LD) tests and Hardy–Weinberg equilibrium (HWE) tests (on the three northern pike populations combined), were computed with GENEPOP v.4.2 (Raymond and Rousset 1995). For multiple tests, Bonferroni corrections were applied (Rice 1989).

For North American *E. lucius*, allele sizes ranged between 111 and 376 bp (Table 2). Only one significant deviation from HWE (Table 2) was observed for EL16 among the three populations. The average level of genetic variation was moderate (mean $N_A = 6.88$, mean $H_E = 0.49$, mean $H_O = 0.51$) and highly variable across loci (N_A ranged between two and 23 alleles, H_E and H_O ranged between 0.033 and 0.950 and 0.033 and 0.967, respectively (Table 2). F_{is} values varied between −0.06 and 0.13, except for EL16, which revealed a strong deficit in heterozygotes (0.65) (Table 2) and most likely presents null alleles. All loci were successfully amplified in both *E. lucius* from North America and Europe and *E. reichertii* from Eurasia (Table 2). Among the other species, the

Table 2 Microsatellite markers characterization

Locus	*E. lucius* (North America, $N = 30$)							Cross-amplification							
								E. lucius (Europe, $N = 20$)				*E. americanus americanus* ($N = 10$)			
	Quality	Allele size range (bp)	N_A	H_E	H_O	F_{is}	*P*-HWE	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)
EL01	+++	317–327	5	0.6283	0.6333	−0.01	0.4444	++	3	0.3778	335–343	−	−	−	−
EL02	+++	197–211	6	0.5113	0.5000	0.02	0.3771	+++	7	0.8256	183–207	+++	2	0.1895	176–182
EL03	+++	126–142	7	0.4763	0.5000	−0.05	0.5985	+++	6	0.6308	118–134	+++	1	0	120
EL05	+++	176–192	7	0.6090	0.6000	0.02	0.7941	+++	6	0.7026	164–192	+	4	0.2600	178–190
EL09	+++	120–134	11	0.8576	0.8333	0.03	0.8118	+++	8	0.7013	134–150	+++	2	0.3462	114–115
EL10	+++	188–192	2	0.0333	0.0333	0.00	NA	+++	5	0.5030	202–214	++	1	0	164
EL12	+++	148–162	6	0.4215	0.4000	0.05	0.7208	+++	3	0.1845	144–166	+	3	0.2429	154–158
EL15	+++	111–131	6	0.4503	0.4000	0.11	0.2235	+++	2	0.4662	115–123	++	3	0.2556	115–123
EL16	+++	317–341	5	0.5921	0.9667	−0.65	0.0000	+++	2	0.4824	347–351	−	−	−	−
EL17	+++	329–341	4	0.3452	0.3333	0.03	0.7253	+++	8	0.7905	329–249	+	1	0	337
EL19	+++	178–186	4	0.2678	0.2333	0.13	0.4944	+++	4	0.4526	180–192	−	−	−	−
EL20	+++	308–376	23	0.9503	0.9667	−0.02	0.7249	+++	13	0.9282	320–360	−	−	−	−
EL21	+++	227–253	7	0.3537	0.3667	−0.04	0.6268	+++	5	0.6081	241–257	−	−	−	−
EL22	+++	295–303	4	0.4311	0.5000	−0.16	0.7637	+++	5	0.6654	267–297	++	7	0.5364	259–301
EL23	+++	246–267	8	0.6616	0.7000	−0.06	0.4514	+++	7	0.6814	275–290	−	−	−	−
EL27	+++	152–173	7	0.4746	0.4667	0.02	0.7074	+++	6	0.6689	158–173	+++	2	0.3368	155–167
EL28	+++	256–270	5	0.1898	0.1667	0.12	0.2208	+++	9	0.7859	282–326	+++	1	0	260

Locus	Cross-amplification															
	E. americanus vermiculatus ($N = 10$)				*E. masquinongy* ($N = 10$)				*E. niger* ($N = 10$)				*E. reichertii* ($N = 17$)			
	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)
EL01	−	−	−	−	−	−	−	−	−	−	−	−	++	1	0	329
EL02	+++	3	0.5316	180–184	−	−	−	−	+	1	0	164	+	1	0	171
EL03	+++	1	0	118	+++	11	0.8737	145–175	++	2	0.4765	112–114	+	2	0.0588	114–116
EL05	−	−	−	−	+	1	0	190	−	−	−	−	+	5	0.2017	170–190
EL09	++	3	0.4059	114–116	−	−	−	−	++	5	0.5667	114–168	+++	5	0.6863	114–122
EL10	−	−	−	−	−	−	−	−	++	1	0	164	+++	1	0	190
EL12	+	4	0.2000	150–160	−	−	−	−	+	2	NA	158–160	+	3	0.1597	140–150
EL15	+	1	0	123	−	−	−	−	−	−	−	−	+	2	0.1711	111–115

Table 2 continued

Locus	Cross-amplification														
	E. americanus vermiculatus ($N = 10$)				*E. masquinongy* ($N = 10$)				*E. niger* ($N = 10$)				*E. reichertii* ($N = 17$)			
	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)	Quality	N_A	H_E	Allele size range (bp)
EL16	–	–	–	–	–	–	–	–	–	–	–	–	++	2	0.2125	285–351
EL17	+	1	0	337	+	3	0.2857	337–343	–	–	–	–	+++	1	0	303
EL19	–	–	–	–	++	4	0.5882	170–186	–	–	–	–	++	6	0.6836	192–212
EL20	+	2	NA	345–347	–	–	–	–	–	–	–	–	+	2	0.1261	315–317
EL21	–	–	–	–	++	2	0.1000	221–223	–	–	–	–	+++	1	0	239
EL22	+++	2	0.1000	265–285	++	3	0.5533	297–301	++	5	0.4455	259–301	++	1	0	289
EL23	–	–	–	–	–	–	–	–	–	–	–	–	+	1	0	250
EL27	+++	3	0.5684	161–167	+++	2	0.1895	148–151	++	3	0.5333	141–158	+++	2	0.2585	130–136
EL28	+++	1	0	250	+	3	0.2857	302–308	+	2	0.1714	252–256	++	1	0	264

N_A number of alleles, H_O/H_E observed/expected heterozygozity

Amplification success: +++ 100 %, ++ ≥50 %, 50 % < + > 0 %, – 0 %

number of successfully amplified loci varied between eight and 11 and the quality of amplification was lower than observed in *E. lucius* and *E. reichertii*. Four out of 17 loci were successfully cross-amplified on all *Esox* species for the tested PCR conditions (Table 2). Given the scarcity of microsatellite markers currently available for *Esox* species, these new polymorphic microsatellite markers should be very useful to assist the study of lowly polymorphic *E. lucius* populations as well as other *Esox* species across the world.

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