Development of 55 novel polymorphic microsatellite loci for the critically endangered L. (Actinopterygii: Perciformes: Percidae) and cross-species amplification in five other percids

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SHORT COMMUNICATION

Development of 55 novel polymorphic microsatellite loci for the critically endangered *Zingel asper* L. (Actinopterygii: Perciformes: Percidae) and cross-species amplification in five other percids

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Abstract By combining biotin-enrichment protocol and next generation pyrosequencing, through 454 GS-FLX Titanium technology, 55 polymorphic microsatellites loci with perfect motif were isolated from the Rhone streber (*Zingel asper*), a critically endangered European fish species. Eight multiplex PCR kits were optimised in order to genotype a total of 58 polymorphic loci, including three previously published loci. The level of genetic diversity was assessed for 68 *Z. asper*, 30 *Sander lucioperca*, 33 *Perca fluviatilis* and four *Gymnocephalus schraetzer* individuals. Amplification success was also assessed on *Romanichthys valsanicola* and *Zingel streber* using single individuals. These markers will be useful to investigate the population structure of the highly fragmented Rhone streber. They represent a powerful tool for conservation issues and evolutionary approaches of this endemic species. Moreover, part of our markers demonstrated applicability to other percid species, allowing for potential applications to fisheries and aquaculture management.

Keywords Percidae · STRs · Enriched library · Pyrosequencing · QDD · Conservation genetics

Introduction

The Rhone streber, *Zingel asper* (Linnaeus, 1758) [Actinopterygii: Perciformes: Percidae], is defined as a critically endangered species by the International Union for Conservation of Nature. Since the beginning of the twentieth century, *Z. asper* declined by about 80% of its initial distribution range as a result of combined anthropogenic

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factors: habitat fragmentation, hydraulic disturbance and pollution. This species is now restricted to a few disconnected populations while it was formerly present in the whole Rhone River drainage (Mari et al. 2002). Whether this geographic shrinkage and habitat fragmentations caused a loss of genetic diversity and genetic drift within isolated populations remains unclear (but see Laroche and Durand 2004). Since conservation strategies rely on the understanding of population structure and dynamics, analysing the genetic structure of the Rhone streber with fine-scale molecular markers such as microsatellites would provide useful information. The development of microsatellite markers would allow for delineating the evolutionary history of the species and evaluating the genetic diversity in both evolutionary and conservation frameworks (as initiated in Danancher et al. 2008).

In this study, we detail the development of 58 polymorphic microsatellites for the Rhone streber (including 55 novel markers with perfect motif) using a combination of biotin-enrichment and 454 pyrosequencing technology. We also test for the applicability of these markers on five other percid species (*Sander lucioperca*, *Perca fluviatilis*, *Gymnocephalus schraetzer*, *Romanichthys valsanicola* and *Zingel streber*).

Material and methods

Caudal fin samples were collected from 68 individuals of *Z. asper* in Durance River (southeastern France; $n=36$) and Beaume River (southeastern France; $n=32$). Additionally, individuals from five other Percidae species were collected: 30 *S. lucioperca* individuals (Rhone delta, southeastern France), 33 *P. fluviatilis* (Durance catchment), one *R. valsanicola* (Valsan River, Romania), one *Z. streber* and four *G. schraetzer* (Danube drainage).

Microsatellites were isolated following a biotin-enrichment protocol adapted from Kijas et al. (1994) at Genoscreen (Lille, France). DNA was extracted from samples, digested with *Rsa*I (Fermentas) and then ligated to standard oligonucleotide adapters as described elsewhere (Dubut et al. 2009). Three biotin-labelled oligonucleotides—$(TG)_{10}$, $(TC)_{13}$ and $(AAC)_7A$—were independently hybridised to the ligated DNA at 56°C for 20 min after initial denaturation of the ligation. The enrichment step was completed using Dynabeads (Invitrogen) and standard procedure. The resulting enriched DNA was amplified using standard adapters, with 25 cycles (20 s at 95°C, 20 s at 60°C and 1 min 30 s at 72°C) and a final elongation step of 30 min at 72°C. The amplicons were immediately purified using QIAquick PCR Purification Kit (QIAGEN). Each purified enriched library was used in the GS-FLX 454 Titanium (Roche Applied Science) library preparation following the manufacturer's protocols at Genoscreen (Lille, France).

Emulsion PCR was carried out at a ratio of 1 copy per bead and subsequently disrupted using isopropanol. Beads containing amplified DNA fragments were enriched and recovered for sequencing to finally provide 50,000 to 70,000 enriched beads for each library. The recovered ssDNA beads were packed onto 1/16 region of a 70×75-mm Titanium PicoTiter plate and sequenced with 200 cycles. Sample preparation and analytical processing such as base calling were performed at Genoscreen (Lille, France) using the manufacturer's protocol for Titanium series.

The selection of sequences for primer design was done with the programme QDD (Meglécz et al. 2010). Enrichment adaptors were removed from sequences. Sequences longer than 80 bp and containing perfect microsatellite motif of at least five repetitions for any microsatellite motif of 2–6 bp were selected for further analyses. Sequence similarities were computed through an 'all against all' BLAST (Altschul et al. 1997; *e* value 1E−40) in which microsatellite motifs were soft masked. Sequences for which pairwise similarity along the flanking regions was over 95% were grouped into contigs, and a 2/3 majority rule consensus sequence was created from each contig. Sequences which had significant BLAST hit to other sequences with an overall similarity among the flanking region bellow 95% were discarded to avoid potential intragenomic multicopy sequences. All unique sequences (with no BLAST hit to any other '454' reads) and consensus sequences were checked for the presence of short repetitions in the flanking regions. PCR primers were designed using QDD with the following criteria: (a) target microsatellite displayed at least five repetitions, (b) length of PCR product was between 100 and 300 bp, (c) flanking regions contained at most one mononucleotide stretch of four bases and two repetitions of any di-hexa base pair motifs and (d) annealing temperatures of primers pairs were optimised for falling between 57°C and 63°C. Among the 241 sequences for which primer pairs could be designed, we selected 181 sequences by discarding 60 sequences with only five or six repeats.

Amplifications were performed for each of the 181 primers pairs using reagents and protocols described previously (Dubut et al. 2009). The 105 novel primer pairs displaying specific PCR products on agarose gel electrophoresis were retained for further analyses. At this stage, primers pairs of three previously described microsatellite loci (Svi18: Borer et al. 1999; SviL8 and SviL9: Wirth et al. 1999) were included into the protocol. All the 108 loci were then amplified separately using forward primers labelled with fluorescent dyes 6-FAM (Eurogentec), PET, NED or VIC (Applied Biosystems). Visualisation of the amplicons was conducted on an ABI 3130XL Genetic Analyzer

Table 1 Microsatellite loci and multiplex PCR conditions

Locus (GenBank ID)	Fluorescent dye	Primer sequence (5′–3′)	Core motif	Multiplex PCR	
				Kit	Primers concentration (nM)
Svi18 (G36964)	NED	F: GATCTGTAAACTCCAGCGTG R: CTTAAGCTGCTCAGCATCCAGG	$(AC)_{11}$	1	400
SviL8 (AF144741)	VIC	F: GCTTATACGTCGTTCTTATG R: ATGGAGAAGCAAGTTGAG	$(TG)_{22}$	1	150
SviL9 (AF144742)	6-FAM	F: TACTGTTCACTTATCTATCC R: TGTATGTGTGTGTGTTCATGT	$(CA)_{18}AA(CA)_3A(AC)_4$	1	400
Za022 (HM622293)	VIC	F: AGTCGATGCTTGATGCTGTG R: GAGAAGGAAGGAGAAAGAGATGA	$(CT)_{10}$	1	50
Za097 (HM622309)	PET	F: TATTGAACGAAGAGCTGCGG R: TCGGGTTATTTAACCGGCTC	$(GA)_8$	1	100
Za118 (HM622315)	NED	F: TTGACACATATCATGAACCACTACA R: GACCGCTGGTTCTTCTTCAG	$(GTT)_9$	1	50
Za190 (HM622332)	PET	F: CATTCCATTTTGTTGGCTCA R: GCTTTGGAAAGAAACGATGG	$(TGA)_{10}$	1	100
Za215 (HM622339)	6-FAM	F: ACGCATCTGTCCACCACTG R: CCTCCCCACTGGATAGGAAT	$(AC)_7$	1	100
Za143 (HM622318)	VIC	F: TAAGTGTTAAAATTGTAGCCCATTTC R: GGACATTTCTAAGTGACCCCA	$(CA)_{12}$	2	250
Za176 (HM622327)	PET	F: AGTGGTTACTTGTGGAGCGG R: GATTTCCGATTACCTGGAACA	$(CTT)_8$	2	250
Za179 (HM622329)	NED	F: ATTTCCCATTGCGGGATTA R: GGATTCTTGCATGCTTTGGT	$(TCT)_9$	2	50
Za186 (HM622331)	6-FAM	F: CAGCTTGCAGTGAAAGACCA R: TGGAGAGTGGATGGATTTACAG	$(AC)_{10}$	2	250
Za207 (HM622337)	VIC	F: GGATTCCAGAAGCAAAAGAGG R: TGGGACAAGGCTTTAACCAC	$(GT)_{13}$	2	100
Za209 (HM622338)	NED	F: CATCACTGTCCAGCAAAAGC R: ATGTGAATTTCCCTTGTGGG	$(GAA)_8$	2	40
Za222 (HM622341)	NED	F: TCTCTGTCACAGGAGGTCCA R: ATTACCGGTTTGAAGGGCTG	$(AAGGAG)_5$	2	75
Za040 (HM622299)	PET	F: TTTGTGATATTCCACTCTGAAAGC R: TGGCATAATTCAGCCATCAA	$(CT)_{11}$	3	100
Za107 (HM622313)	NED	F: CCTGTACACGTTATTCCCCA R: CTTCAGCACTGTATTGAGGGG	$(GA)_{17}$	3	100
Za113 (HM622314)	PET	F: ACCACGCACAATCACTCGTA R: CCTGGCTTTACCCAGAAACA	$(CA)_{11}$	3	100
Za144 (HM622319)	NED	F: GCCCACAATAGCACCGTAAT R: TTTGTGAATGTGAGTGAGAGTCAG	$(AC)_8$	3	50
Za161 (HM622322)	VIC	F: AACCAATTTCAGTCCATTCTTCA R: CAAAGAAACAAAACAACTGTTCC	$(TG)_9$	3	250
Za164 (HM622323)	6-FAM	F: TTCATGCAAGTTTGCCGTT R: TGGTTGCCAATCACTGATCTA	$(GAA)_5$	3	150
Za165 (HM622324)	VIC	F: GTTGTCTCAAATCACGCACG R: TTGTCTGGCAACAATAGGTGA	$(TCT)_8$	3	50
Za200 (HM622335)	6-FAM	F: GTTAGCGTGCCAAGTTGATG R: GTAGGTGGCGGTATGCATTT	$(TTC)_{11}$	3	100
Za030 (HM622296)	NED	F: TTTGTAGGGTGTTTAAAGTTTGC R: ATTTTCTGCAGTGGTCCCAG	$(GA)_9$	4	50
Za032 (HM622297)	VIC	F: GAGATGACATGCCTTGCCTC R: CACAAACTAAAGACCAACAAACCA	$(TG)_{10}$	4	150

Table 1 (continued)

Locus (GenBank ID)	Fluorescent dye	Primer sequence (5′–3′)	Core motif	Multiplex PCR	
				Kit	Primers concentration (nM)
Za078 (HM622303)	PET	F: ATTATGACGCGTATCCCACG R: AAAAGGAAGATGTGCACAGTGA	$(GA)_9$	4	50
Za091 (HM622306)	NED	F: TTCTGATTTAGCCCTGCCTG R: GTAGGCAGAAATCCTGCACC	$(TG)_5$	4	50
Za094 (HM622307)	6-FAM	F: CAGACACAAACAACACAACTGG R: CAACACTGTATGGTCATAAATAGCAA	$(GATA)_{11}$	4	250
Za096 (HM622308)	6-FAM	F: AAGATTTTGGTGTTGGCGAG R: TACTTGTCCTTTCCGGGTTG	$(GA)_{17}$	4	300
Za106 (HM622312)	PET	F: ATTACGTTTGGGGAAGAGGC R: AGTTGCTTTCATTTCCCTGC	$(AG)_8$	4	75
Za121 (HM622316)	PET	F: CAAAGTCATGAACGAGCTGC R: AGCCAGGACCACTCTGTGAG	$(CT)_9$	4	150
Za241 (HM622344)	NED	F: ATTTCAATGTGGTGCAAATTAGC R: TGCGCACATACATGCATAG	$(GT)_{10}$	4	100
Za006 (HM622290)	6-FAM	F: TGGTGGCACAAATCTCCATA R: CCTGTAATGATGGCTTGAGGA	$(AG)_7$	5	100
Za038 (HM622298)	NED	F: TGAATCGCTGCTCTTTCTCA R: TATGCAATTACATCGGAGCG	$(AC)_{11}$	5	100
Za069 (HM622300)	VIC	F: AATTCTAAGACAATGTTTCTTTCATCT R: CACATCAATGGTTGCTTCTTG	$(TG)_8$	5	75
Za072 (HM622301)	VIC	F: TTTAAACAAATACACTCAGAGGGA R: TCCCCTATCTCTTCTTTATTTCACC	$(GA)_8$	5	150
Za102 (HM622311)	NED	F: CACCAGCGACAAACAAACAC R: GCAAACTGTTCCAGCTCCTC	$(TC)_5$	5	40
Za138 (HM622317)	PET	F: TTCTTTATACAAGAGGAATAGTTGCAG R: TTTTTGTGATTGTGCTATTTTAAAGG	$(AC)_8$	5	200
Za169 (HM622325)	PET	F: CCCACTGTGTCATCGTCATC R: GAAGAAAATCATAAATGACAACTGC	$(CTT)_6$	5	50
Za170 (HM622326)	6-FAM	F: GGAACACGTCTGTTTTGCTG R: CTTGCAACAGCATCAGTGGT	$(CTT)_{10}$	5	50
Za194 (HM622333)	VIC	F: AGTGCTGCTTCTGTGGAGGT R: CAGCCTGTGCTGGGAATAAT	$(TGG)_5$	5	50
Za011 (HM622291)	VIC	F: CCATCAAATCACATAGCTGGA R: TCTTTGCTTGCGTCACCTAA	$(AG)_8$	6	50
Za019 (HM622292)	PET	F: CTTAGCCAGACGTTGCCTGT R: AATTTGTTTGCGTCTGGTCA	$(AC)_7$	6	100
Za086 (HM622304)	6-FAM	F: CAATGCCACTGGGCTAAATC R: GCTGTCTGGATAATGTTTCAACT	$(AG)_{10}$	6	600
Za154 (HM622320)	NED	F: GCCATGTTCTTTATTCACTTGC R: CCAACGAGCCTTCTCTGAAC	$(GTT)_7$	6	50
Za199 (HM622334)	PET	F: CCTTCCCCTCAAAAGCATGT R: AGGAAATGGAAAGGGAATGC	$(TCT)_{13}$	6	100
Za221 (HM622340)	6-FAM	F: CATTTCACCGCATTCAGTTG R: AAAACCACGAAATACAGCGG	$(TCT)_8$	6	100
Za024 (HM622294)	VIC	F: TGAACCTCCCTATCCCCTCT R: TCTTTTCCACAGCAGGAAGC	$(AC)_7$	7	40
Za087 (HM622305)	6-FAM	F: TCCCTGTCGTCAAAGACCTC R: GACGTCAATGGACTGTTCCC	$(AC)_9$	7	150
Za101 (HM622310)	PET	F: ATCAAGGTCCAGAGGCAAGA R: AACAGATGGTCCTCTGGCTG	$(AG)_{10}$	7	200

Table 1 (continued)

Locus (GenBank ID)	Fluorescent dye	Primer sequence (5′–3′)	Core motif	Multiplex PCR	
				Kit	Primers concentration (nM)
Za157 (HM622321)	NED	F: TCAGACTTGGAGAAGGAGGG R: TCAGTCTCTTTCATCGCTGC	$(GAA)_7$	7	40
Za181 (HM622330)	6-FAM	F: TGGACTTTTCACCACAGCAG R: GGAGGAGGTGGTCAAAGAGA	$(TCA)_6$	7	100
Za203 (HM622336)	PET	F: AGCATCCGGTTCTTTACCTG R: TGGCTGGTATTGAGTGGTAGG	$(AC)_8$	7	100
Za029 (HM622295)	NED	F: TCCTGCTCTAACAGGTGCAA R: CAGACGCACTGATCACCAAT	$(AG)_7$	8	75
Za077 (HM622302)	6-FAM	F: TCTAGCCACCAAATGACCA R: TTTCACCATCTACGTTTTGTTCTT	$(GT)_8$	8	500
Za178 (HM622328)	PET	F: AGGAGGCAGTAATGCAATGT R: GCACTGATGTAAGTGCAATAGGC	$(AAG)_7$	8	100
Za237 (HM622342)	6-FAM	F: ATCTCAAGTCATGGGGCATC R: GGTCCTCTGGTGCAGCTATAA	$(CA)_{10}$	8	150
Za239 (HM622343)	NED	F: CGAAGTATATTCAGATAGACCCT R: CTGTCTTTGTCATAAACTTAACTTACTC	$(GA)_{10}$	8	100

(Applied Biosystems). Alleles sizes were scored against the GeneScan-500 LIZ® Size Standard (Applied Biosystems), and genotypes were obtained using GeneMapper® 3.7 (Applied Biosystems). A total of 58 primers pairs associated with an unambiguous genotype profile in *Z. asper* were selected and combined into eight PCR multiplex kits (Table 1). The multiplex kits were further tested on six percid species (including *Z. asper*).

GENEPOP 4.0 (Rousset 2008) was used to (a) test Hardy–Weinberg (HW) equilibrium, (b) estimate the heterozygosity for all loci and populations and (c) test for linkage disequilibrium (LD) among loci within populations.

Results

Concerning *Z. asper*, the number of alleles per locus ranged from 2 to 25, and expected heterozygosity values ranged from 0.03 to 0.95 (Table 2). After FDR correction (modified as proposed by Benjamini and Hochberg 1995), all but two loci (SviL8 and Za038 in the Durance sample, with significant excess of homozygotes) displayed HW equilibrium (Table 2), and significant LD among loci was detected between two loci (Za207 and Za239) in the Beaume sample only.

As for *S. lucioperca* and *P. fluviatilis*, the amplification revealed successful for 47 and 35 loci, respectively (Table 2). Eighteen loci were polymorphic in *S. lucioperca* (although polymorphic, SviL9 was disregarded as homozygotes with null allele were detected), and 14 were polymorphic in *P. fluviatilis*. All polymorphic loci did not depart from HW equilibrium (except Za072 in *S. lucioperca*), and no LD was detected, except for one pair of loci (Za121 vs. Za144 in *S. lucioperca*). In *S. lucioperca*, the number of alleles per polymorphic locus ranged from 2 to 9, and expected heterozygosity values ranged from 0.32 to 0.85. In *P. fluviatilis*, the number of alleles per polymorphic locus ranged from 2 to 14, and expected heterozygosity values ranged from 0.06 to 0.88 (Table 2). Additionally, cross-species amplifications were successful in 53.4% cases for *G. schraetzer*, in 69.0% cases for *R. valsanicola* and in 70.7% cases for *Z. streber* (Table 2).

Discussion

The combination of biotin-enrichment protocol and next generation pyrosequencing, through 454 GS-FLX Titanium technology allowed the fast development of polymorphic markers for the threatened *Z. asper*. The 55 new markers developed here (along with three previously published markers) constitute a useful tool for achieving detailed information on the genetic structure of this species and investigating its evolutionary history. Furthermore, it will assist in the development of comprehensive long-term management and protection plans. In this context, normalisation of the PCR conditions and multiplexing make faster and cost effective the genotyping of the 58 loci. Moreover, all the novel 55 markers display perfect microsatellite motif. This makes them easily usable not only for

Table 2 Levels of variability and amplifiability of 58 microsatellite loci in *Z. asper* and five other percid species

Locus	*Zingel asper*			*Sander lucioperca* (*n*=30)		*Perca fluviatilis* (*n*=33)		*Gymnocephalus schraetzer* (*n*=4)	*Romanichthys valsanicola* (*n*=1)	*Zingel streber* (*n*=1)
	Total size range (Na)	H_o/H_e Durance River (*n*=36)	H_o/H_e Beaume River (*n*=32)	Total size range (Na)	H_o/H_e	Total size range (Na)	H_o/H_e	Total size range (Na)	Total size range (Na)	Total size range (Na)
Svi18	172–237 (25)	0.94/0.95	0.88/0.86	153–182 (9)	0.73/0.79	164–172 (4)	0.55/0.54	–	–	196 (1)
SviL8	118–156 (12)	0.58/0.84[a]	0.25/0.31	–	nd	–	nd	–	–	127–133 (2)
SviL9	202–228 (15)	0.75/0.88	0.84/0.79	221–224 (3)[c]	nd	198–207 (6)	0.55/0.74	236–263 (2)	202 (1)	–
Za006	282–284 (2)	0.06/0.05	0.06/0.06	280 (1)	nd	281 (1)	nd	274 (1)	282 (1)	–
Za011	116–118 (2)	0.06/0.05	0.38/0.35	–	nd	126–143 (4)	0.18/0.22	–	114 (1)	118 (1)
Za019	117–121 (3)	0.53/0.59	0.59/0.48	121 (1)	nd	113 (1)	nd	121–130 (5)	112 (1)	117–119 (2)
Za022	206–210 (3)	0.50/0.53	0.41/0.33	202 (1)	nd	227 (1)	nd	219 (1)	200 (1)	208–212 (2)
Za024	104–106 (2)	0.06/0.05	0.22/0.20	127–139 (4)	0.47/0.43	120–124 (2)	0.21/0.28	129–191 (6)	103 (1)	108 (1)
Za029	122–130 (4)	0.53/0.45	0.28/0.32	124 (1)	nd	126 (1)	nd	–	127 (1)	124 (1)
Za030	235–268 (5)	0.42/0.53	0.53/0.49	267 (1)	nd	–	nd	–	268 (1)	266–282 (2)
Za032	105–167 (14)	0.33/0.35	0.75/0.75	–	nd	–	nd	–	–	–
Za038	105–110 (4)	0.50/0.69[a]	0.66/0.50	107–130 (6)	0.80/0.77	–	nd	–	–	105 (1)
Za040	103–111 (5)	0.39/0.44	0.34/0.33	111–113 (2)	0.53/0.50	–	nd	103 (1)	121 (1)	–
Za069	126–128 (2)	0.08/0.08	nd[b]	122 (1)	nd	122 (1)	nd	124 (1)	122 (1)	122 (1)
Za072	98–100 (2)	0.22/0.20	nd[b]	99–106 (3)	0.23/0.60[a]	–	nd	–	105–107 (2)	–
Za077	101–116 (5)	0.28/0.29	0.56/0.51	107 (1)[c]	nd	–	nd	–	101–105 (2)	101–107 (2)
Za078	107–111 (3)	0.36/0.40	0.44/0.35	108 (1)	nd	114 (1)	nd	–	–	–
Za086	143–187 (15)	0.92/0.87	0.78/0.77	198 (1)	nd	–	nd	–	–	199–205 (2)
Za087	124–128 (3)	0.06/0.05	0.19/0.18	–	nd	–	nd	167–184 (5)	–	–
Za091	137–143 (2)	0.31/0.30	0.06/0.06	143 (1)	nd	143 (1)	nd	146 (1)	143 (1)	137–145 (2)
Za094	203–262 (16)	0.92/0.91	0.75/0.77	203–207 (2)	0.57/0.50	–	nd	236–268 (6)	251–267 (2)	235–242 (2)
Za096	111–168 (13)	0.33/0.45	0.25/0.37	106 (1)	nd	112 (1)	nd	–	109 (1)	–
Za097	99–103 (3)	0.25/0.22	0.44/0.46	–	nd	–	nd	–	99 (1)	105 (1)
Za101	180–197 (6)	0.67/0.74	0.75/0.73	–	nd	–	nd	190–192 (2)	192 (1)	189 (1)
Za102	188–196 (2)	nd[b]	0.13/0.12	188 (1)	nd	149–194 (14)	0.88/0.88	181 (1)	188 (1)	188 (1)
Za106	131–143 (4)	0.39/0.46	0.44/0.46	141 (1)	nd	–	nd	142 (1)	132 (1)	–
Za107	92–110 (6)	0.61/0.72	0.47/0.47	–	nd	–	nd	–	96 (1)	90–102 (2)
Za113	153–171 (5)	0.50/0.56	0.34/0.34	169–229 (9)	0.70/0.85	135–155 (4)	0.67/0.69	–	151–194 (2)	201–216 (2)
Za118	80–96 (4)	0.39/0.41	0.25/0.26	90 (1)	nd	86 (1)	nd	89 (1)	90–93 (2)	90 (1)
Za121	205–265 (9)	0.64/0.65	0.69/0.63	227–235 (4)	0.63/0.60	228–240 (4)	0.64/0.58	245–308 (6)	266 (1)	229 (1)
Za138	111–120 (6)	0.56/0.74	0.59/0.50	135–148 (5)	0.27/0.43	–	nd	–	–	109 (1)

Za143	94–113 (10)	0.83/0.72	0.72/0.82	–	nd	–	nd	–	–	–
Za144	186–190 (3)	0.56/0.50	0.59/0.59	199–228 (8)	0.70/0.80	218–226 (4)	0.18/0.28	211–230 (6)	–	–
Za154	110–119 (4)	0.78/0.72	0.56/0.74	107 (1)	nd	107 (1)	nd	105 (1)	113 (1)	110 (1)
Za157	106–111 (3)	0.42/0.41	0.16/0.25	94 (1)	nd	94 (1)	nd	105–110 (2)	108 (1)	–
Za161	104–110 (4)	0.50/0.63	0.28/0.33	100 (1)	nd	117–121 (2)	0.09/0.14	103 (1)	–	96 (1)
Za164	170–173 (2)	0.31/0.42	0.16/0.29	155 (1)	nd	170–173 (2)	0.52/0.49	167 (1)	173 (1)	170 (1)
Za165	132–177 (7)	0.39/0.41	0.72/0.72	135–148 (3)	0.43/0.37	–	nd	–	155–161 (2)	183–186 (2)
Za169	157–163 (3)	0.06/0.06	nd[b]	157 (1)	nd	160 (1)	nd	–	–	157 (1)
Za170	146–186 (12)	0.75/0.78	0.75/0.85	136 (1)	nd	138 (1)	nd	156–175 (4)	136 (1)	186–199 (2)
Za176	103–115 (5)	0.42/0.47	0.66/0.62	125 (1)	nd	129–132 (2)	0.36/0.37	106 (1)	104 (1)	115 (1)
Za178	193–196 (2)	0.19/0.39	0.34/0.45	189 (1)	nd	–	nd	–	178 (1)	189 (1)
Za179	146–174 (9)	0.69/0.68	0.19/0.18	171–196 (4)	0.37/0.35	215 (1)	nd	212–217 (2)	192 (1)	180 (1)
Za181	187–199 (4)	0.61/0.52	0.38/0.43	184 (1)	nd	193 (1)	nd	–	190–193 (2)	187–190 (2)
Za186	158–236 (21)	0.94/0.93	0.72/0.76	191–199 (3)	0.33/0.32	192–211 (6)	0.55/0.56	–	159–178 (2)	160–209 (2)
Za190	136–177 (11)	0.75/0.79	0.53/0.64	153–168 (3)	0.60/0.64	–	nd	176 (1)	–	183–189 (2)
Za194	175–181 (3)	0.28/0.25	0.28/0.33	175 (1)	nd	154 (1)	nd	173 (1)	–	181 (1)
Za199	183–201 (7)	0.64/0.63	0.59/0.58	201–234 (7)	0.67/0.74	249–277 (8)	0.58/0.74	171 (1)	228 (1)	–
Za200	106–127 (7)	0.81/0.73	0.16/0.18	–	nd	–	nd	–	–	–
Za203	119–121 (2)	0.17/0.15	0.31/0.35	117 (1)	nd	115 (1)	nd	115 (1)	117 (1)	117 (1)
Za207	174–195 (5)	0.50/0.42	0.38/0.37	222–237 (5)	0.67/0.64	–	nd	255–288 (4)	–	–
Za209	91–106 (5)	0.64/0.63	0.34/0.33	103 (1)	nd	–	nd	–	–	100–103 (2)
Za215	92–98 (2)	0.50/0.48	0.25/0.22	92 (1)	nd	100 (1)	nd	120–134 (3)	102 (1)	109–122 (2)
Za221	291–297 (3)	0.53/0.54	0.69/0.67	–	nd	–	nd	–	294 (1)	291 (1)
Za222	211–228 (5)	0.53/0.56	0.50/0.48	200–217 (4)	0.53/0.53	215 (1)	nd	212–217 (2)	217 (1)	211–217 (2)
Za237	136–142 (3)	0.22/0.21	0.03/0.03	171–178 (5)	0.57/0.54	128–136 (3)	0.06/0.06	–	138 (1)	–
Za239	99–101 (2)	0.72/0.51	0.31/0.41	–	nd	100 (1)	nd	–	–	–
Za241	94–100 (3)	0.56/0.58	0.72/0.63	92 (1)	nd	85 (1)	nd	95 (1)	89 (1)	96 (1)

Na number of alleles per locus, H_o observed heterozygosity, H_e expected heterozygosity, *nd* not determined, – no amplification

[a] Locus deviating from Hardy–Weinberg equilibrium

[b] Locus found monomorphic in this population

[c] Homozygotes with null allele were detected

frequency based analyses but also in a coalescent theory framework for demographic inference.

Furthermore, to our knowledge, only a limited number of microsatellites (<15 markers) were developed for *S. lucioperca* (Kohlman and Kersten 2008) and *P. fluviatilis* (Yang et al. 2009) to date. This set of microsatellites therefore provides an additional tool to researchers and practitioners in need of genetic markers for investigating the genetic structure and evolutionary history of wild populations and for fishery and aquaculture management. Finally, the success in cross-species amplification for the other percid species highlights the potential usefulness of the developed markers for a broader range of evolutionary, conservation and management studies in percids.

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Ethical standards The authors declare that the experiments described in this paper comply with the current French laws.

Conflict of interest The authors declare that they have no conflict of interest.

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