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Short Communication

Isolation and characterization of 19 polymorphic microsatellite loci from the topmouth gudgeon, *Pseudorasbora parva*

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ABSTRACT. The Asiatic topmouth gudgeon, *Pseudorasbora parva*, is recognized as one of the most invasive fish species in many countries outside of Asia. We isolated and characterized 19 microsatellite loci from *P. parva*. The polymorphism of these 19 loci was tested on 40 individuals of *P. parva* sampled from a wild population located in Ezhou, Hubei province of China. The loci had 5 to 11 alleles, with a mean of 7.7 at each locus; 11 loci conformed to Hardy-Weinberg equilibrium. The expected and observed heterozygosities ranged from 0.237 to 0.973 and from 0.647 to 0.914, respectively. All microsatellite loci were in linkage equilibrium. These microsatellite markers are

potentially useful for the assessment of population genetic structure during invasion and dispersal of *P. parva* in new habitats.

Key words: *Pseudorasbora parva*; Invasive species; Microsatellite; Polymorphism

INTRODUCTION

The Asian topmouth gudgeon (*Pseudorasbora parva*) is a small cyprinid that is widely distributed in the littoral zones of freshwater habitats throughout China, Japan and Korea (Okuda et al., 1996; Priyadarshana et al., 2001). Aspects of the life history of this species that predispose it to settle in new aquatic basins include a wide tolerance of environmental conditions, reaching sexual maturity in the first year of life, batch spawning and nest guarding (Sunardi et al., 2005; Prinder et al., 2005; Kapusta et al., 2008).

By the end of the 2000s, in slightly over 40 years, *P. parva* has colonized almost all of Europe, proceeding rapidly from east to west (Witkowski, 2011). Most probably it was introduced in European countries with stocking material of herbivorous fishes that were imported from China for several decades, and is now considered to be an effective invasive species in Europe (Bianco, 1988). It not only feeds on juvenile stages of many valuable native fish species, but also competes for food with farmed fish species (Sunardi et al., 2005; Žitnan and Holčik, 1976). Most importantly it consumes larger species of planktonic crustaceans, which results in an increase in the quantity of phytoplankton, and further increases eutrophication of water bodies (Gozlan et al., 2005). Besides, being a vector of infectious diseases (*Spherotecum destruens*, among others), it constitutes a serious threat to both native and farmed fishes in Europe (Britton et al., 2010). Most studies on *P. parva* have focused on invasion, behavior and toxicology, while the details of their genetic characteristics have been rarely reported. In the current study, we have isolated and characterized a batch of novel polymorphic microsatellites from the genome of *P. parva*, which are potentially helpful in the study of its population genetic structure during invasion and fast dispersal after invasion.

MATERIAL AND METHODS

DNA used for microsatellite-enriched libraries was extracted by traditional proteinase-K digestion and phenol-chloroform protocol with RNase treatment from caudal fin of *P. parva*. Approximately 200 ng extracted DNA was completely digested using the restriction enzyme *Hae*III (BioLabs) and then ligated to adapters (5'-CTC TTG CTT GAA TTC GGA CTA-3'/5'-TAG TCC GAA TTC AAG CAA GAG CAC A-3') by T4 DNA ligase (BioLabs) in a 20-µL volume at 16°C for 10 h. The digestion-ligation mixture was diluted (1:10) and directly amplified using an adapter-specific primer (5'-CTC TTG CTT GAA TTC GGA CTA-3'). The polymerase chain reaction (PCR) amplification program was conducted as follows in a 20-µL volume: a predenaturation at 94°C for 10 min; 30 cycles including denaturation at 94°C for 30 s, annealing at 52°C for 30 s and elongation at 72°C for 45 s, and a final extension at 7°C for 10 min. The PCR products were hybridized with 400 pmol of 5'-biotinylated $(AC)_8$ or $(CT)_8$ oligonucleotide probe, by denaturing DNA for 10 min at 95°C, and then incubated at 55°C for 30 min.

The hybridized DNA fragments were enriched by Streptavidin MagneSphere Paramagnetic Particles (Promega). The fragments were amplified for 30 cycles with an adapter-specific primer and the same program mentioned above was used. The amplified DNA fragments were isolated and purified by AxyPreDNAGel Extraction Kit (AXYGEN). Then, the purified DNA fragments were ligated into the pMD™ 18-T vector (TaKaRa) and transformed into competent *Escherichia coli* DH5α cells. Grown overnight on LB solid medium with ampicillin, 221 positive clones were picked up and confirmed by PCR.

After PCR confirmation, 29 positive clones were sequenced and contained simple sequence repeats. Sequences were analyzed for the repeat region using the Tandem Repeats Finder software (Benson, 1999). The PRIMER 3 software (Rozen and Skaletsky, 2000) was then used to design 29 pairs of primers flanking the repeat regions of interest. PCR conditions were optimized for each pair of primers with a gradient PCR at 40-60°C range of annealing temperatures. Each reaction contained about 20 ng DNA, 2.0 mM $MgCl_2$, 0.4 mM dNTPs, 0.5 µM each primer, 1X Taq buffer, and 0.5 U Taq polymerase (Fermentas). PCR was conducted with the following programs: initial denaturation at 94°C for 10 min; 30 cycles at 94°C for 30 s, annealing at a locus-specific temperature (Table 1) for 30 s, elongation at 72°C for 45 s, and a final extension at 72°C for 10 min.

The polymorphism test was performed on 40 individuals of *P. parva* sampled from a wild population located in Ezhou, Hubei province of China. Electrophoresis for the amplified products was conducted on 8% nondenaturation polyacrylamide gel and visualized by silver staining. Alleles were identified by pUC18/*msp*I (TIANGEN). The number of alleles, the observed heterozygosity (H_O), the expected heterozygosity (H_E), Hardy-Weinberg equilibrium (*HWE*), and pairwise tests for linkage disequilibrium were performed using the POPGENE 32 computer program. Significance criteria were adjusted for the number of simultaneous tests using Bonferroni's correction.

RESULTS AND DISCUSSION

In total, among the 29 microsatellite primers, 19 primer pairs produced polymorphic DNA products, 10 primer pairs were amplified without any result. The number of alleles at the 19 polymorphic microsatellite loci ranged from 5 to 11 with a mean of 7.7. H_E and H_O ranged from 0.237 to 0.973 and from 0.647 to 0.914, with an average of 0.758 and 0.792, respectively (Table 1). Significant deviations from Hardy-Weinberg equilibrium were observed in 11 loci ($P > 0.05$) as shown in Table 1. No significant linkage disequilibrium was detected between any pair of loci.

Nineteen microsatellites isolated in the present study were highly polymorphic, and they could potentially be useful for further studies of population genetics. They could provide clues to assess the genetic diversity and genetic structure of invasive populations of *P. parva*, and serve to reveal changes through space and time in comparison to native populations, which could lead to the identification of general features of the invasive process of this species.

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Table 1. Characterization of 19 polymorphic microsatellite markers isolated from *Pseudorasbora parva*.

	Core motif	Accession No.	Primer sequence (5'-3')	*Ta* (°C)	Size range (bp)	*Na*	H_E	H_O	P_{HWE}
MS01	$(GT)_5(GA)_{23}$	JF418943	F: AATAACAGCCGCGAGCC R: GACCGTTTGACCGATGG	54	135-209	11	0.700	0.914	0.126
MS02	$(CT)_{27}$	JF418944	F: ATCAAACCAGCATCCAC R: TACCCATCCGGCACA	50	222-380	7	0.864	0.667	0.004*
MS03	$(CT)_{10}\ldots(CT)_6$	JF418945	F: TTCTACCGGAAGCGTGT R: GGCCTGGCAACCAAA	52	147-182	8	0.839	0.859	0.000*
MS05	$(CA)_{32}$	JF418947	F: CCTTTAACCCATTTATTCC R: CCCTAAACCTACCCATCA	50	309-487	8	0.818	0.647	0.060
MS07	$(GT)_{44}$	JF418949	F: AGCCTGATCACTATACGCA R: CCATACAAGAACTGACCCAC	52	388-496	10	0.860	0.813	0.138
MS08	$(GA)_{28}$	JF418950	F: GAATTCGGAGTACCAGGAC R: TCATTTATGGCATCAGTTTT	51	277-344	10	0.894	0.912	0.065
MS09	$(TC)_{30}$	JF418951	F: GGCGACCTTAAATTGCTTG R: TGACCCTGTTTGGTTGGAA	56	285-327	7	0.733	0.864	0.002*
MS11	$(CT)_{33}$	JF418953	F: GTCTTAGTGTCATTGTGAGTTT R: GAATCTGTGCAGGACTGTC	49	228-284	9	0.722	0.865	0.390
MS12	$(AG)_5$	JF418954	F: ATCTGAGTCGCTTCTGA R: ATGCCCAAGGCTAA	43	161-179	5	0.243	0.748	0.000*
MS13	$(AC)_{37}$	JF418955	F: AGATCGGCATCTTTTAGCG R: CTGTGGAGGTTGTTGAGGT	55	163-188	6	0.833	0.796	0.002*
MS15	$(AC)_{24}$	JF418957	F: CAAGTGTCCAGTCGTC R: CGCAGGTGCAGG	40	186-218	8	0.973	0.816	0.001*
MS17	$(CA)_{18}\ldots(CA)_7$	JF418959	F: AATCTGTGCTCTTGCTTG R: ATATCAGAGGGCGTTCC	49	154-242	9	0.896	0.666	0.293
MS19	$(CA)_{11}\ldots(CA)_6$	JF418959	F: CACGAGATCAAACATCAT R: AATTCAAGCAAGAGCAC	49	101-146	6	0.514	0.752	0.000*
MS20	$(AC)_{19}\ldots(CA)_{14}$	JF418960	F: TCATGCGCAGTGAGGAGT R: TTGGAATTTGTGATCGGC	54	126-182	9	0.877	0.750	0.361
MS22	$(CA)_{10}$	JF418961	F: GAGCCAGATACCACAGCAC R: CACATAAACAGGCCCACA	52	128-175	9	0.878	0.875	0.489
MS24	$(CA)_{21}\ldots(CA)_7$	JF418963	F: TGGGTAGGTTTAGGGTG R: CTTTAGTCCCATCAGCAG	47	116-150	5	0.677	0.731	0.076
MS25	$(CA)_{21}$	JF418964	F: TAGGATGATAGTGTTGATTATTTACC R: TGTAATCAGAAACCACGCC	54	159-178	5	0.952	0.728	0.027*
MS28	$(CA)_6\ldots(CA)_9$	JF418967	F: TAGGGGCAGGGA R: AAACCACATAAACAGGC	44	241-301	9	0.237	0.864	0.166
MS29	$(AC)_6\ldots(CA)_5\ldots(CA)_{48}\ldots(AC)_{10}$	JF418967	F: ATCCGAAATCCTTGTC R: CCCCTAAACCTACCC	43	125-147	6	0.894	0.777	0.412

Ta (°C) = annealing temperature; *Na* = observed number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; P_{HWE} = P values for exact tests for Hardy-Weinberg equilibrium (HWE); *Significant deviation from HWE after Bonferroni's correction ($P < 0.05$).

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