Development and characterization of 57 SNP markers in *Misgurnus anguillicaudatus*

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Abstract

The dojo loach *Misgurnus anguillicaudatus* is an endemic freshwater species to Asia. The effective conservation and molecular-aided selection of *M. anguillicaudatus* have been limited without sufficient molecular markers. In this study, 112 novel single nucleotide polymorphisms (SNPs) were screened based on 2b-RAD sequencing database, and 57 SNP markers were developed and characterized by genotyping 40 individuals using SNaPshot method. The observed heterozygosity (H_o) ranged from 0.025 to 0.675, while the expected heterozygosity (H_e) varied from 0.025 to 0.500. The minor allele frequency (MAF) ranged from 0.013 to 0.500. Among these SNPs, 18 loci were found to deviate significantly from the Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.05$). The first set of SNP markers developed from *M. anguillicaudatus* will provide valuable information in further population genetic analysis and natural resource conservation.

Background

The dojo loach *Misgurnus anguillicaudatus*, a member of the family Cobitidae, is an endemic freshwater species in Asia and is widely distributed along the eastern coasts of the Asian continent (Li et al. 2008; Zeng et al. 2012). For delicious meat and traditional Chinese medicine values, the dojo loach has become one of the most important economic fish in several East Asian countries. In addition to their edible value, loach is regarded as an excellent animal model to explain the biological origin and evolutionary significance of polyploidization because of its complex ploidy composition (Zhou et al. 2014; Zhong et al. 2019). Despite its economic importance, dojo loaches for human consumption are mostly obtained through the exploitation of wild populations. In recent years natural populations have declined dramatically because of over-exploitation and habitat destruction. Therefore, it is urgent to perform population genetic investigation on *M. anguillicaudatus* to protect the natural resources.

The research of genetic markers in *M. anguillicaudatus* has mainly concentrated on mitochondrial DNA and microsatellite marker at present (Zhang et al. 2013; Fujimoto et al. 2017; Lee et al. 2018). Owing to their wide genomic distribution and high level of polymorphism, single nucleotide polymorphisms (SNPs) have gradually become one of the most important DNA markers for genetic studies (Vignal et al. 2002; Seeb et al. 2011). Unfortunately, limited reports are available for *M. anguillicaudatus* on SNPs. To better understand the genetic background and enrich the molecular markers, we firstly developed a set of SNP markers by SNaPshot technique in *M. anguillicaudatus*.

A total of 36,916 putative SNPs were identified using 2b-RAD genotyping method, and 112 novel SNP markers were randomly selected and evaluated to test polymorphic. Forty caudal fin samples of diploid *M. anguillicaudatus* were randomly collected from Qingyuan City, Guangdong Province, China. Genomic DNA was extracted from tissue samples using the HiPure Mollusc DNA Mini Kit (Magen, China) according to the manufacturer's instruction. Each PCR reaction (10 μ l total volume) contained 1 μ l Primer Mix, 5 μ l PCR Mix, 1 μ l DNA, and 3 μ l ddH₂O. The PCR parameters were as follows: 5 min denaturation at 95 °C, 35 cycles of 20 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C, and an extension step of 3 min at 72 °C. Next, the purification and extension of the PCR products were performed using the SNaPshot technique of ABI Company. Finally, genotyping was conducted using ABI 3730XL DNA Analyzer (Applied Biosystems). For validated loci, statistics including MAF , H_o and H_e were calculated in GenAlex v6.5. The polymorphism information content (PIC) and deviations from the Hardy–Weinberg equilibrium (HWE) were estimated using GenePop 3.2.

Of the 112 potential SNP markers, 57 loci were found to be polymorphic and bi-allelic. The H_o and H_e varied from 0.025 to 0.675 and 0.025 to 0.500, respectively. The MAF ranged from 0.013 to 0.500. The PIC ranged from 0.024 to 0.375 (Table1). Among these SNPs, 18 loci showed significant deviations from the Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.05$). To our knowledge, this is the first development of SNP markers in *M. anguillicaudatus*, and these novel markers offer a valuable tool for future studies of population conservation and natural resource conservation in this species.

Declarations

Conflicts of interest

The authors declare no competing financial interests.

Funding information

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Authors' contribution

Conceived and designed the experiments: GC ML. Performed the experiments, analyzed the genetic data: GH. Contributed reagents/materials/analysis tools: FG ZL JC. Wrote the paper: GH ML. All authors read, revised, and approved the manuscript.

Data Availability

The data used to support the findings of this study are available from the article and its supplementary information.

Ethics approval(Ethics)

All experimental procedures for loach handling were approved by the Animal Care and Ethics Committee of the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Consent to Participate (Ethics)

This study was conducted at Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences. The institution and all authors agreed to participate in this research.

Consent to Publish (Ethics)

The Author confirms:

- that the work described has not been published before;
- that it is not under consideration for publication elsewhere;
- that its publication has been approved by all co-authors if any;
- that its publication has been approved explicitly by the responsible authorities at the institution where the work is carried out.

The authors agree to publication in the journal *Conservation Genetics Resources*.

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Tables

Table 1 Characteristics of SNP markers in *Misgurnus anguillicaudatus*

Prime ID	Primer sequence(5' - 3')	Size (bp)	SNP type	Ne	Ho	He	PIC	MAF	PHW
SNP4	F: ACTCTTTGAGGTTGACCCAG R: TTTGCGGGATGCTACATCTG E: GAAGGAGCCACGGTTAAATC	280	G/A	1.220	0.200	0.180	0.164	0.100	1.00
SNP9	F: GGGATCATCTGAAAGACTCG R: CATAACTGCCCTATCATCTG E: gactAACCTACTGTTCTCCCATATA	278	C/A	1.140	0.079	0.123	0.116	0.066	0.13
SNP10	F: GTGAATTACAGTGCTGACGG R: TGTTGGCACGTTCAACAGTC E: gactAACCTACTGTTCTCCCATATA	279	G/C	1.280	0.200	0.219	0.195	0.125	0.47
SNP14	F: CACAAGCATAGGACTCTGAC R: AGGCAGTTTAGTAGGTGTTG E: tgactgactgactATCCCAACTTTGTTACCGCCA	292	C/A	1.342	0.150	0.255	0.222	0.150	0.02
SNP15	F: GTGCTATGCTGTACTGTCTG R: CATCTCTTCTGCACTAGGTC E: actgactgactgactGGAAAGTTCAGCCTGAACCAG	276	C/A	1.105	0.100	0.095	0.090	0.050	1.00
SNP23	F: TGTCCCTTTAAGCTTCCCTG R: CACTTTGTGTTTGACCCCTG E: gactgactgactgactAAATATTCAAACATACCTCCATACGG	298	G/A	1.105	0.100	0.095	0.090	0.050	1.00
SNP29	F: GCATTGGATTGCATCTGGTG R: GCTCTGTGGTTTATAAAGGG E: actgactgactgactgactgactGATGGCCACAGAACTCCAGG	278	A/C	1.753	0.375	0.430	0.337	0.300	0.30
SNP30	F: CACAGTAAGGTTAGAGGGAG R: TAGGTAAAGAAGCGCAGCAG E: ctgactgactgactgactgactgactGTATGTACCCACCTCCTCTTC	280	C/T	1.471	0.200	0.320	0.269	0.200	0.02
SNP37	F: TGCCATGTTCTTCCGGTTTG R: CACCCGAAAGTGAACACATC E: actgactgactgactgactgactgactgactgactCTGTAATGAGTGTGATCTCT	284	G/T	1.161	0.100	0.139	0.129	0.075	0.18
SNP42	F: TGGATAGAGTGTGTGTATCG R: TCACCCATCAAGCCCAATG E: gactgactgactgactgactgactgactgactgactTGTGTGTATCGATAAATACTGTAAT	279	G/A	1.250	0.175	0.200	0.180	0.113	0.39
SNP48	F: ACTCTGCAATCTTGGCCATC R: ATGACGACGATTGGGATGAC E: ctgactgactgactgactgactgactgactgactgactAACCTGAACCGATTCTCCAGCTT	282	G/A	1.809	0.675	0.447	0.347	0.338	1.00
SNP50	F: TGTTTACTGTGGCGCTGATG R: TTCACCTTTGGGCTTTGGGAG E: gactgactgactgactgactgactgactgactgactgactGACCCCAACCCTCCCTCCGC	280	C/T	1.503	0.175	0.335	0.312	0.188	1.00
SNP60	F: ATAGCACCTTTTCAGGAACCC R: TTCTGGTACAGCACACAAGG E: gactgactgactgactgactgactgactgactgactgactCCTGTACAGGACACACAATT	279	C/A	1.632	0.275	0.387	0.279	0.225	0.06
SNP65	F: GTGTGGGAGAAGAACGTATG R: AGCGAAAGTGAGACAGAGAG E: gactgactgactgactTGGATGAGAGAGTGCATGAGTCG	279	C/T	1.584	0.231	0.369	0.301	0.204	0.02
SNP71	F: ACTCCCTGGAGTTAAAGCTG R: TGATCCCAGCACAAATAGGAG E: CACTGGAGGTGCTTGCAAAA	278	C/G	1.105	0.100	0.095	0.090	0.050	0.77

Table 1 (continued)

Prime ID	Primer sequence(5' - 3')	Size (bp)	SNP type	Ne	Ho	He	PIC	MAF	PH
SNP72	F: AGCACTTCCTTTGTGCTACG R: CACTGTCATGTCTGATAGCC E: gactAGGGATACAGGAGTGCGCGT	280	G/A	1.026	0.025	0.025	0.024	0.013	1.0
SNP73	F: TAATGGCACGGGAAGTGAAG R: AGCCTTGcAGAGAGATTTGG E: gactgactGAGAGATTTGGAGGAATAGT	280	G/A	1.220	0.100	0.180	0.164	0.100	0.0
SNP74	F: TTCACCTGCTGCATGTCTTC R: GTGAAACTCAGAGGAGACAG E: actgactgactAGGAGAGAGAAGAGGAGATTG	272	C/T	1.980	0.400	0.495	0.372	0.450	0.1
SNP75	F: TTAGAAGCCCTGCACTTGTCTC R: CTCGAAGAAGAGTCTACAGG E: tgactgactgactgactTGATAAATCTTCAAACTTCAAC	275	A/G	1.133	0.125	0.117	0.110	0.063	0.7
SNP76	F: CATTGTTCTGTAGGCAGACC R: GCTGTTTGGCATGGTTTTTG E: tgactgactgactgactgactATTTTAACGTACCCAGGAAAAT	280	T/G	1.956	0.400	0.489	0.369	0.450	0.2
SNP77	F: TTGAGGCCCTTTCTATGGAC R: TTTGCCGAGCTTATCCTGTG E: gactgactgactgactgactgactTGTCTGAGGGAGATGCACTG	280	G/T	1.809	0.275	0.447	0.347	0.338	0.0
SNP78	F: TGAATACACCGAACTCTGGG R: CACTATCAAGCTGATCTCCG E: ctgactgactgactgactgactgactCATAGGAGTCTGTGTCCGAGTT	288	A/G	1.025	0.025	0.025	0.024	0.013	1.0
SNP79	F: TCTGTGGTGTGTTTTTCCC R: TTGGTGGAAAAAGGCACCAG E: gactgactgactgactgactgactgactTAGGGGGAGCTCTCGTCTAC	281	C/T	1.105	0.050	0.095	0.090	0.050	0.0
SNP80	F: TGACATCAGCAACCACAGAC R: TTTGCCGTATGGGACAGTTG E: actgactgactgactgactgactgactgactTTTGGTCTGGAGAAGGAAGCG	280	G/T	1.051	0.050	0.049	0.048	0.025	0.9
SNP81	F: TTCTGTTAGACGAGATGCCG R: AACTCTTGGCTCATGCGTAG E: gactgactgactgactgactgactgactgactgactCTGTTTAATATCGACTGATATTC	280	T/A	1.374	0.175	0.272	0.235	0.163	0.0
SNP82	F: CCAAATGTGATGGGTCTGTC R: TGCTGCTTGACTCTCAGATG E: ctgactgactgactTTAAGTAAGCCTGTCCAGAAAA	274	T/A	1.632	0.275	0.387	0.312	0.263	0.0
SNP83	F: GCATTTTTGCAATATCCCCCA R: GTCTGTGTGAGGGTGGCTTT E: CACAAAATAATTGACCCTAGCTCCA	486	T/C	1.977	0.107	0.494	0.3720	0.446	0.0
SNP84	F: TTATCAGACACCAACATCCGAAGT R: AAAGCACACTACTAGCCACACA E: ctTTCTAGATTCCGGGAGTGATTGTA	227	T/G	1.576	0.333	0.366	0.299	0.241	0.6
SNP85	F: TCTGTGCCCATCATATCGGC R: CTGAAAGACCTGAGGCTGGG E: ctgactgactgactAAAAGCCACAACGCCACCTCC	254	A/G	1.308	0.182	0.236	0.2080	0.136	0.2

Table 1 (continued)

Prime ID	Primer sequence(5' - 3')	Size (bp)	SNP type	Ne	Ho	He	PIC	MAF	P _{HM}
SNP86	F: CGAGCGAGCGAATGTTTGTGAA R: TGGTTTAGCCATTCTTCTGTGTTGG E: actgactgactgactCTACGACTAGGCCTCCCTCCCT	315	A/G	1.576	0.333	0.366	0.299	0.241	0.64
SNP87	F: CGGTGTGAAGAGCGTCCATA R: CTCTTCTGCCTTGTCCACGT E: tgactgactgactTTGTAGGGGGAGTCTGTGTACAAGG	393	T/A	1.800	0.296	0.444	0.3460	0.333	0.08
SNP88	F: TGGCTTTAAGAATGTCTGAGTGTGT R: GCTCAATAGCATGACGGAGGTG E: tgactgactgactgactgactAAACTATTTACTAGATCTCCTCT	245	A/G	1.906	0.556	0.475	0.362	0.389	0.38
SNP89	F: AGCCAATGCTGCCATTCCATACAA R: GATCGGTTTCTGTGCCAGGACAC E: ctgactgactgactgactgactAAAAGTCTCCACGTATACTCCATCACT	263	G/A	1.957	0.333	0.489	0.3690	0.426	0.09
SNP90	F: TGTGTGCTCTCTGTGTATGTGTTCA R: TCAGTGCTGTTAGGTATTGTGGGTA E: tgactgactgactgactgactGTGATTTTTGTTTCTATTCTTATAAC	331	A/T	1.385	0.037	0.278	0.239	0.167	0.00
SNP91	F: TTAGGCTGGCTACATTGTCCATACG R: AGCGGAGAGCGACTGTCACT E: actgactgactgactgactgactgactgactgactAGAACACATCCTCTCCAGCATG	217	A/T	1.839	0.259	0.456	0.3520	0.352	0.02
SNP92	F: TTGTCCGCAGTGCTAAGCAGTTA R: CGCTTTACCAAGTGTCCCGCTAA E: tgactgactgactgactgactgactgactgactgactCAGCATAGGTATGTTATTCTTAAACATC	334	T/C	1.989	0.556	0.497	0.374	0.463	0.54
SNP93	F: GCAAGTGGTGGAGGTGAAAGTGT R: CGGATGCTTCCCTGGTATGTTGAA E: TATTTAAAGCTCTTACCTCCTCTCC	284	T/C	1.839	0.333	0.456	0.3520	0.352	0.16
SNP94	F: TGGCAGTGCTCAAACCTCAA R: GAAGGATGGCCACAGTGTCA E: actCAAACCTCACTTGATGACTTCGAATT	314	T/G	1.800	0.444	0.444	0.346	0.333	1.00
SNP95	F: ATCCACTCCGATGCTGATGTAAGAT R: GTGAGACCAGGCAGAAATGAAAGC E: ctgactgactGCGATGACTCAGCCTCCAAGTGT	231	C/A	1.246	0.074	0.198	0.1780	0.111	0.00
SNP96	F: TCTCAGTTACACAAAGACGCACACA R: TCAGTGAGCAAGGCATCTTAGACAA E: tgactgactgactgactCTACGCAAAACAAACACACACAC	280	A/T	1.715	0.222	0.417	0.330	0.296	0.01
SNP97	F: GCCTTTCCTCTTCCCGCTAATC R: GATCACATCGCCGTGTACGGTAAT E: actgactgactgactgactAGATCACAAAACTCCCGCTAC	294	T/C	1.759	0.259	0.431	0.3380	0.315	0.03
SNP98	F: GCCGCCACTGAGCAATATTACG R: TTGGACTGCAACACTGCATTCA E: tgactgactgactgactgactTAAGACTTGTTTAATTTCCAAACC	336	G/A	1.989	0.185	0.497	0.374	0.463	0.00
SNP99	F: TCATTGGACCTTGCTCTTTCAAAGC R: GTGTCACATCAAACATTACGCTGA E: gactgactgactgactgactgactgactgactgactAGTGTCCACTGGCTCTCCATT	327	T/A	1.839	0.259	0.456	0.3520	0.352	0.02
SNP100	F: GACTCAGACCATCACTCCACCTACA R: TCCACAGAACCTGTCACTCAATGC E: actgactgactgactgactgactgactgactgactAGCAGGTGAACAACACAGCCAC	312	T/A	1.576	0.407	0.366	0.299	0.241	0.55

Table 1 (continued)

Prime ID	Primer sequence(5' - 3')	Size (bp)	SNP type	Ne	Ho	He	PIC	MAF	P _{HW}
SNP101	F: CCGTTACTGCCACCTTAGTTATTG R: ATGTTTGACGCAGATGTTGTTGATC E: tgactgactgactgactgactgactgactTGTATGGAAAGGAGATGTTGTTCTCTGT	316	C/A	1.874	0.667	0.466	0.358	0.370	0.02
SNP102	F: AGACATCTTTTGGCACATGTG R: TTATGTGCTGTGCTGGGACC E: gactgactgactgactgactgactgactgactgactgactTGGCACATGTGCAGCTTAAAC	317	G/C	1.957	0.259	0.489	0.3690	0.426	0.01
SNP103	F: TTCTGGACCAGCAGGACTTCATTG R: GGTGATTCTGACTGACAGGTGAGA E: AAATGAAATTGGTCAGGAATGCTCC	289	C/T	1.989	0.630	0.497	0.374	0.463	0.16
SNP104	F: GCAACCTGACAAGCCATACTTTCCA R: ACCGTCCATCCTGATGTAAGTGTGTA E: tAAGACTAAACAACATACTTGTCTCCT	336	C/T	1.843	0.125	0.457	0.3530	0.354	0.00
SNP105	F: AGCCCGGTCTGGTTCGTTATTGA R: AGCCCTTACTCGATGTGCATTTGAT E: tgactCATTCAAATGTTGAAATATGAGACAGGC	283	G/T	2.000	0.333	0.500	0.375	0.500	0.08
SNP106	F: TGCTAGTGTGATAACTGCTTGTAGA R: CCGTTGCTGGATATGGCTGTTG E: actgactgactgactGCAGTCGGAAGCTTGGACAGC	286	A/T	1.839	0.556	0.456	0.3520	0.352	0.25
SNP107	F: TTGTGTAGCCAGTTTCTCAGCGAAT R: AAGCATGAACATAAAGCAGGTGGGT E: actgactgactgactAAAAGGTTTACTGTAACCTCCATCCCA	215	T/C	1.715	0.296	0.417	0.330	0.296	0.13
SNP108	F: AGCTGATTCCAGACAAATACATACG R: GTGATAACAACCGGATGCCTATACA E: ctgactgactgactgactAAAAGAAATAGCTACTCACCTCCCA	334	G/A	1.989	0.407	0.497	0.3740	0.481	0.34
SNP109	F: TGATATGGTACTCCTGCTTGGTAGG R: GTAACGGGTCGTGACGTTTGTC E: ctgactgactgactgactgactgactTCCACATCAATGGAGACGTGGTT	292	G/A	1.874	0.370	0.466	0.358	0.370	0.28
SNP110	F: TTATGGCTATGGAGGCTGTTGAATT R: TTGGTATAGTTGTTACTCTGTGGT E: tgactgactgactgactgactgactTACCTTAGCAAAAAGAGCTCGGC	350	A/G	1.906	0.407	0.475	0.3620	0.389	0.45
SNP111	F: AGCTGGGTTAGACTTCTAAGCACAT R: TTATCGAGCGAAGTTTACGTTTGGT E: ctgactgactgactgactgactgactgactCAGCTGCCTGCGATGAAAACACA	334	A/T	1.874	0.444	0.466	0.358	0.370	0.80
SNP112	F: ACATTGCATCATGGAACGGTGTG R: GGACTCACTCGGAGACCAGGAA E: gactgactgactgactgactgactgactgactgactgactCGGACGGAAATGAGGTACAGG	311	G/C	1.976	0.444	0.494	0.3720	0.444	0.60

Sequences and additional SNP information can be found in Supplementary Material.

Primers for each locus including a forward primer (F), a reverse primer (R) and an extension primer (E), *Ho* observed heterozygosity, *He* expected heterozygosity, *PIC* polymorphism information content, MAF minor allele frequency, *P_{HW}* *P*-values for Hardy–Weinberg equilibrium corrected for multiple comparisons using the false discovery rate.

* Significantly deviated from Hardy-Weinberg equilibrium ($P < 0.05$), ** Significantly deviated from Hardy-Weinberg equilibrium ($P < 0.01$), *** Significantly deviated from Hardy-Weinberg equilibrium ($P < 0.001$).

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