Isolation And Characterization Of 45 SNP Markers In Triplophysa Tenuis

Ya Liu (liuya2343@163.com)
Sichuan Academy of Agricultural Science https://orcid.org/0000-0002-2744-9049
Yeyu Chen
Sichuan Academy of Agricultural Science
Jiansheng Lai
Sichuan Academy of Agricultural Science
Hongyu Ke
Sichuan Academy of Agricultural Science
Zhongmeng Zhao
Sichuan Academy of Agricultural Science
Han Zhao
Sichuan Academy of Agricultural Science
Xiaoyun Wu
Sichuan Academy of Agricultural Science
Jian Zhou
Sichuan Academy of Agricultural Science
Qiang Li
Sichuan Academy of Agricultural Science

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Abstract

Triplophysa tenuis is an endemic species to China, which mainly distributed in Xinjiang and Gansu province. Effective conservation and management of this species is limited by insufficient molecular markers. In the present study, we reported the isolation and characterization of 45 SNP markers in *T. tenuis*. The minor allele frequency ranged from 0.046 to 0.500, and the observed and expected heterozygosities ranged from 0.061 to 0.667 and 0.088 to 0.508, respectively. Polymorphic information content ranged from 0.083 to 0.375. Among these SNPs, three loci showed significant departures from the Hardy–Weinberg equilibrium. The novel polymorphic SNPs will be helpful for the future study on genetic management and population conservation for this species.

Main Text

Triplophysa tenuis is distributed both in the Bosten lake and Tarim river systems in the south of Xinjiang and in the Shule river and Ruoshui river in the Hexi corridor of Gansu province (Zhu 1989). Although *T. tenuis* has not yet been classified as an endangered species, the fish species in Gansu province showed an obvious decreasing trend, especially the degradation of endemic fish (Wang et al. 2015). At present, the reports on *T. tenuis* are mainly in the aspect of biological characteristics (Chen et al. 2017, Yao et al. 2018). There are very few reports about molecular markers in this species. Genetic markers that are currently available for *T. tenuis* have mostly focused on mitochondrial DNA (Wang et al. 2015, Wang et al. 2016). Single-nucleotide polymorphism (SNP) markers are useful in population genetic studies because of their co-dominance, high levels of polymorphism, low cost and wide distribution (Vignal et al. 2002, Wang et al. 2015, Blanc-Jolivet et al. 2017). In the present study, we developed and characterized 45 SNP markers for the first time in *T. tenuis*. It is expected that these results will contribute to population conservation, genetic management and construction of genetic linkage map for this species.

In this study, restriction-site associated DNA sequencing (RAD-seq) was used to isolate and characterize SNP markers. A total of eighty potential SNP loci were selected for primer design using Primer 5.0. Fin clips of *T. tenuis* were collected from 33 individuals of the Shule river (Gansu, China). Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Doyle 1987) and was stored at -20°C.

PCR amplifications were performed in 30 μL volumes containing 1 μL of genomic DNA, 14 μL of Premix Taq (2×Taq Plus MasterMix, CWBIO), 1 μL of each gene primer and 13 μL of PCR-grade water. The PCR programme was 94°C for 5 min, then 32 cycles at 94°C for 30 s, annealing for 30 s (for annealing temperatures of each primer pair, Table 1), 72°C for 45 s, and one cycle of 72°C for 7 min for the final extension. Amplification products were sequenced using Sanger technology. The sequenced fragments were aligned using Vector NTI 10.3.0 (Invitrogen, Carlsbad, CA, USA) and the genotypes per locus were determined by BioEdit according to the peaks of each base. The minor allele frequency (MAF), observed heterozygosities (Ho), expected heterozygosities (He), polymorphism information content (PIC) and P value representing the deviations from Hardy–Weinberg equilibrium (HWE) were calculated using Cervus 3.0 (Kalinowski et al. 2007).

A total of 45 loci were found to be polymorphic and showed bi-allelic in 33 individuals of *T. tenuis.* The minor allele frequency ranged from 0.046 to 0.500 (Table 1). The observed heterozygosity varied from 0.061 to 0.667, while the expected heterozygosity ranged from 0.088 to 0.508. Polymorphic information content ranged from 0.083 to 0.375. Only three loci showed significant deviations from the HWE after Bonferroni correction. As far as we know, this is the first report of SNP identification in *T. tenuis*, which will be valuable tool for population conservation in this species.

Table 1
Characterization of 45 SNPs in T. tenuis

Primer ID	Primer sequences (5'-3')	Fragment size (bp)	Locus ID	SNP type	SNP position	T_a (°C)	MAF	Ho	He	PIC	HWEP
TtSNP1	F:TCACAGACGTACATTTCCCATA	248	TtSNP1-1	C/T	128	48	0.152	0.182	0.261	0.224	NS
	R:ATTCAAACACTGAGTGCTTTTT		TtSNP1-2	A/G	147		0.273	0.242	0.403	0.318	NS
TtSNP2	F:GCCTGTTTGTGTGTTTAACCTG	356	TtSNP2-1	C/G	177	51	0.182	0.303	0.302	0.253	NS
	R:ctagaaacctgccagattcct		TtSNP2-2	A/T	197		0.197	0.333	0.321	0.266	NS
			TtSNP2-3	G/T	253		0.485	0.667	0.507	0.375	NS
TtSNP3	F:CAATAGAAGTCAATGGGGATCG	340	TtSNP3-1	C/G	186	50	0.106	0.212	0.193	0.172	NS
	R:ACAGACCACATAGATCATCCAT		TtSNP3-2	C/T	187		0.318	0.394	0.441	0.340	NS
			TtSNP3-3	A/G	256		0.303	0.424	0.429	0.333	NS
			TtSNP3-4	A/T	309		0.318	0.455	0.441	0.340	NS
			TtSNP3-5	C/T	314		0.121	0.182	0.216	0.190	NS
TtSNP4	F:GCTTTCCTGTAGCTCAGTTGTA	371	TtSNP4-1	A/G	92	51	0.394	0.364	0.485	0.363	NS
	R:ATAAGGGACTTTATTGCGTTCATGC		TtSNP4-2	A/T	93		0.424	0.364	0.496	0.369	NS
			TtSNP4-3	G/T	162		0.091	0.061	0.168	0.152	*
			TtSNP4-4	A/T	183		0.364	0.424	0.470	0.356	NS
			TtSNP4-5	C/T	313		0.394	0.606	0.485	0.363	NS
			TtSNP4-6	C/T	322		0.136	0.273	0.239	0.208	NS
			TtSNP4-7	A/T	326		0.379	0.576	0.478	0.360	NS
TtSNP5	F:TTCCTAACCAGGTCAAAAGTAA	319	TtSNP5-1	A/G	103	48	0.273	0.121	0.403	0.318	**
	R:AGCTTTGAAGCATTTACAGTCA		TtSNP5-2	A/G	155		0.485	0.364	0.507	0.375	NS
			TtSNP5-3	A/G	251		0.046	0.091	0.088	0.083	NS
			TtSNP5-4	A/T	278		0.227	0.333	0.357	0.290	NS
			TtSNP5-5	A/G	285		0.197	0.333	0.321	0.266	NS
TtSNP6	F:CCCCATCCATTCAGCATCTCT	251	TtSNP6-1	C/G	212	51	0.212	0.303	0.339	0.278	NS
	R:CTATTGCTAATTCGAGAGGGTG										
TtSNP7	F:CAAAATGAAACCAAATCCTGCC	329	TtSNP7-1	A/G	254	49	0.091	0.182	0.168	0.152	NS
	R:GAGGCAAATAAATTAGACCACAAAT		TtSNP7-2	A/T	281		0.349	0.515	0.461	0.351	NS
TtSNP8	F:GTCGTACCCCAAACAGTTCTG	324	TtSNP8-1	A/G	122	51	0.167	0.212	0.282	0.239	NS
	R:AAGATAAGCAGAGAGAACAACAC		TtSNP8-2	C/T	142		0.409	0.576	0.491	0.367	NS
			TtSNP8-3	C/T	153		0.303	0.364	0.429	0.333	NS
			TtSNP8-4	A/T	156		0.061	0.121	0.116	0.107	NS
			TtSNP8-5	C/T	193		0.333	0.364	0.451	0.346	NS
			TtSNP8-6	C/T	197		0.303	0.364	0.429	0.333	NS
			TtSNP8-7	A/G	224		0.091	0.182	0.168	0.152	NS
TtSNP9	F:CTTACCTCCTACTTCCTCG	339	TtSNP9-1	C/T	157	49	0.061	0.061	0.116	0.107	NS
	R:TGTCCTGAAGTTTTGAGCGAA		TtSNP9-2	C/T	172		0.273	0.424	0.403	0.318	NS
			TtSNP9-3	C/G	175		0.046	0.091	0.088	0.083	NS
			TtSNP9-4	G/T	183		0.364	0.364	0.470	0.356	NS
			TtSNP9-5	A/T	185		0.136	0.152	0.239	0.208	NS
			TtSNP9-6	A/T	255		0.106	0.091	0.193	0.172	NS

Primer ID	Primer sequences (5'-3')	Fragment size (bp)	Locus ID	SNP type	SNP position	T_a (°C)	MAF	Ho	He	PIC	HWEP
TtSNP10	F:CTACACACTGTTTACCTGGCC	383	TtSNP10-1	A/T	126	53	0.091	0.182	0.168	0.152	NS
	R:TAATCAGGGGCACTGTGGAAG		TtSNP10-2	A/C	132		0.197	0.091	0.321	0.266	**
			TtSNP10-3	C/T	195		0.152	0.182	0.261	0.224	NS
			TtSNP10-4	C/T	196		0.424	0.545	0.496	0.369	NS
			TtSNP10-5	A/C	229		0.152	0.182	0.261	0.224	NS
			TtSNP10-6	A/T	276		0.197	0.091	0.321	0.266	**
TtSNP11	F:TGTATTTGCTTATTTGACACTCAA	273	TtSNP11-1	A/T	218	48	0.500	0.636	0.508	0.375	NS
	R:TTCTGATGGACTGAGAGATAGA										

MAF minor allele frequency, Ho observed heterozygosity, He expected heterozygosity, PIC polymorphism information content, HWEP results for Hardy–Weinberg Equilibrium text, NS non-significant

*P < 0.05 **P < 0.01

Declarations

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Competing Interests

The authors do not have any conflict of interest.

Availability of data and material

The data and material that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable

Authors' contribution

Ya Liu conceived and designed the experiments. Ya Liu wrote the manuscript. Yeyu Chen revised the manuscript. Jiansheng Lai, Xiaoyun Wu and Qiang Li performed the experiments. Hongyu Ke, Zhongmeng Zhao, Han Zhao and Jian Zhou provide a source of this species. Yeyu Chen and Qiang Li provided formal analysis. All authors read and approved the final manuscript.

Ethics approval

All fish handling and experimental procedures were approved by the Animal Care and Use Committee of the Fishery Institute of the Sichuan Academy of Agricultural Sciences, and all animal collection and use protocols were carried out in accordance with the guidelines and regulations for the care and use of laboratory animals at the Fishery Institute of the Sichuan Academy of Agricultural Sciences.

Consent to participate

The authors provide consent to participate.

Consent to publication

The authors provide consent for publication.

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