Short Communication

Development of 17 Novel Cross-Species Microsatellite Markers for Dabry's Sturgeon (*Acipenser dabryanus*) from Chinese Sturgeon (*Acipenser sinensis*) via Next-Generation Sequencing

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A B S T R A C T

In this study, we report 17 novel cross-species microsatellite markers for Dabry's sturgeon via next-generation sequencing from Chinese sturgeon (*Acipenser sinensis*). The 17 microsatellites were polymorphic with 3 to 6 alleles per locus and the total number of alleles is 80. The mean expected heterozygosity (H_E, 0.506 to 0.781), observed heterozygosity (H_O, 0.542 to 1), Hardy-Weinberg departure value (d, -0.042 to 0.427), polymorphic information content (*PIC*, 0.482 to 0.753) and the Shannon-Wiener Diversity Indices (*H'*, 0.827 to 1.565) of all the 17 polymorphic loci indicated a high level of informativeness. Those markers will be useful tool to study the genetic diversity of Dabry's sturgeon.

D abry's sturgeon (*Acipenser dabryanus*), inhabited the upper and middle sections of the Yangtze River, is a kind of endemic species in China. Dabry's sturgeon was commercial fisheries before the 1980s. However, the number of Dabry's sturgeon wild population declined drastically due to environmental pollution, such as overfishing, and habitat alteration and destruction (Zhuang *et al.*, 1997). Nowadays, Dabry's sturgeon is listed as Critical Endangered species in the Red List of Threatened Species (http://www.iucnredlist.org). A lot of efforts, including artificial releasing, re-stocking of wild juveniles, controlled reproduction and national nature reserve, have been done to save this species (Zhang *et al.*, 2011). Nevertheless, the status of Dabry's sturgeon in the Yangtze River are still further worsened as habitat degradations and poaching. Therefore, Dabry's sturgeon has been intensively breeding in artificial condition for conservation purposes.

Dabry's sturgeon and Chinese sturgeon *Acipenser sinensis* are only two species of acipenserids found in the Yangtze River, China. The two species are so similar that they are not scientifically distinguished until the middle of the 19th century. There was some taxonomic confusion between Chinese sturgeon and Dabry's sturgeon until the 1960s. Species identification of Chinese sturgeon and Dabry's sturgeon is mainly based in body color, gill raker number, skin roughness between ganoid scales and length. It is accept that Chinese sturgeon is an anadromous species that inhabits the Yangtze River, the East China and Yellow Seas, while Dabry's sturgeon is a freshwater species restricted to the Yangtze River (Zhuang *et al.*, 1997). Dabry's sturgeon has been characterized as a landlocked ecotype of Chinese sturgeon in the Yangtze River basin.

Microsatellite plays an important role in population genetics research, and has been of great utility in conservation and management of biological resources (Chistiakov *et al.*, 2006). To better address the conservation status of Dabry's sturgeon in the Yangtze River, a management project based on genetic is being used to define the efficiency of artificial releasing. Development

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microsatellite markers are helpful to make a protective strategy of Dabry's sturgeon (Zhu *et al.*, 2005). A lot of microsatellite markers have been developed for Dabry's sturgeon in previous studies (Que *et al.*, 2014; Zeng *et al.*, 2013; Zhang *et al.*, 2013). However the loci in previous studies only provided little data about genetic analysis, especially polymorphic information content (*PIC*) of microsatellites markers. Besides, the traditional method of the microsatellite marker developing was time-consuming, costly, laborious and often inefficient (Zane *et al.*, 2002). Microsatellite is conserved among closely related species and may be useful to clarify interspecific genetic relationships between recently diverged taxa (Dawson *et al.*, 2010). However, the microsatellite developed for one

species may not always be useful for others because of their high mutation rate. The mutations at primer binding sites may lead to disruptions within tandemly repeated elements that can reduce the level of observed polymorphism in the non-target species. The development of microsatellite should be closely related to maximize utility and offset the costs. Therefore, it has become fairly standard practice to test the newly developed microsatellite loci for cross-species utility (Huang *et al.*, 2014; Wang et al., 2018). In this study, we report 17 novel cross-species microsatellite markers for Dabry's sturgeon from Chinese sturgeon via next-generation sequencing, and those markers will be useful for the research of genetic management, genetic analysis and supportive breeding evaluation.

Table I.- Characterization of 17 microsatellite loci in Dabry's sturgeon.

Loci	GenBank accession No.	Repeat motif(s)	Primer sequence (5'-3')	Tm (°C)
ZHX70	MH667327	$(TATC)_{11}$	F:GGCATTATAGACCCCTGTCGG R:ACAGCTGGGGAGGAACAGTA	60
ZHX80	MH667328	$(ATAA)_{10}$	F:AGTGTTGTGAATAGGACCCAAAGT R:GCATTTTGCTGTACAGACCTTGA	60
ZHX91	MH667329	$(TTTA)_{10}$	F:TTGGCGATCCGATCACCAAA R:TGCCATTTGACTCAACTGTGC	60
ZHX76	MH667330	$(TATC)_{14}$	F:GCGTTCACTGAGTCAATGCA R:CTGGACAGAGAACAGATAGCGT	59
Z15	MH667331	$(ATAG)_{14}$	F:AGCTAGCAACTGAAGCCCTG R:ACAGCTGCAGCACACTTTTG	60
Z120	MH667332	$(TCTT)_{13}$	F:CTGTGTCTTCTGCTCCTGGT R:GCATGTCAGGGCCGGTATTA	59
Z125	MH667333	$(AGAT)_{12}$	F:CTGGCTAAGGTCATGGCCAA R:CAAACTTTATTTGGAAAATTGCACAAT	58
Z153	MH667334	$(TCTT)_{13}$	F:TGGACTGACAACACTGCTCC R:ACAGAGCACGTACAGCCAAA	60
Z166	MH667335	$(ATCT)_{15}$	F:GTGTTTAGAATGTTTAAACACTGAGGA R:TGGCCTCAAGTTCAAGCACA	60
Z167	MH667336	$(GAAA)_{13}$	F:GCACGAGAGAGACAGGACAA R:CAGGTTGAAAGTGCTGGTGC	59
Z175	MH667337	$(AGAA)_{12}$	F:GACGCGCTCTCTGCAATTTC R:TCTCACCTCAATTCTCGTGAGT	59
Z181	MH667338	$(ACTA)_{10}$	F:TTCGGTAACAAGACGCTGCT R:AGGGAAATACCTGTACATTTAAGAGA	58
Z182	MH667339	$(AGAT)_{10}$	F:TGCAACTGGGTTCACTATGCA R:GATCATGCCCTGGCTTCTGA	60
Z189	MH667340	$(TGTC)_{10}$	F:TGGTTCCTCCCAGTCCTCAA R:GTGAGGAGCAGACTGGACTG	60
Z193	MH667341	$(TCTT)_{8}$	F:GGGCCGTTCTGCTTTTTGAG R:CCCCGTGTAGGACACTAACG	60
Z199	MH667342	$(TCTT)_{13}$	F:GACGTTTGGAGCGTGGAAAC R:TGGCATTTACAGCATAAACTAAACCT	60
Z219	MH667343	$(AAAG)_{9}$	F:GTCCAGGGCAGCATCTACTG R:CTCAAACGCACAACCAGCAA	60

Tm, annealing temperature.

Materials and methods

The RNA was extracted from the fin of Chinese sturgeon using Trizol (Invitrogen, USA), according to the manufacturer protocol. RNase-free DNase I was used to remove DNA from the RNA and the total RNA was evaluated by 1% agarose gel electrophoresis. The cDNA library was created by using approximately 5 μg RNA and was sequenced on an Illumina Hiseq2000. The sequences were selected by constrained to perfect repeat motifs of 4 bp from the cDNA library by the Microsatellite Identification tool (MISA: http://pgrc.ipkgatersleben.de/misa/). All the selected sequences were then used to design microsatellite primers using Primer Premier 5.0 software (http://www.premierbiosoft.com/primer design/).

We collected 24 individuals of Dabry's sturgeon from the Chinese Sturgeon Research Institute in Yichang City, China. The total DNA was extracted from the fin of Dabry's sturgeon by the method of rapid salt-extraction (Aljanabi and Martinez, 1997). Polymerase chain reaction (PCR) was carried out using the DNA as template. The recommended cycling conditions of PCR is 3 min at 94°C; 35 (30s at 94°C, 30s at annealing temperature; 30s at 72°C); 10 min at 72C. The PCR was carried out in a 25-ul reaction volume, containing 0.25μM for each primer, 1×PCR buffer (*TaKaRa*), 1.5 mM MgCl$_2$, 50-100 ng genomic DNA and 0.25 U *Taq* DNA polymerase (*TaKaRa*). The PCR products were visualized on 10% polyacrylamide gel stained (PAGE gel) with silver staining. The pBR332 DNA/MspI molecular marker (*TaKaRa*) was used as a standard to identify size of alleles.

The statistics of the observed heterozygosity (H_O), the mean expected heterozygosity (H_E) and Shannon-Weiner Diversity Indices (*H'*) for every loci were performed using software ATetra1.2 (Puyvelde *et al.*, 2010). The Hardy-Weinberg departure value (*d*) and the polymorphic information content (*PIC*) were performed according to formula:

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

$$d = \frac{(H_o - H_e)}{H_e}$$

Where, P$_i$ and P$_j$ is the frequency of I and J allele in the group.

All fish handling and experimental procedures in this study were approved by the Chinese Sturgeon Research Institute, the China Three Gorges Corporation, and the Hubei Key Laboratory of Three Gorges Project for Conservation of Fishes.

Results

In this study, a total of 96 sequences were found and were selected to designing microsatellite primer. The 96 sequences that tested in Dabry's sturgeon were consistent with Chinese sturgeon. Among the 96 pairs of microsatellites primers, 27 pairs failed to produce any PCR product, 52 pairs were produced low quality results, and 17 pairs showed polymorphism (Table I). The percentage of amplification success was 17.7%. The percentage of errors by replicating 5 samples was 0. Using the 17 microsatellite markers, we can identify each individuals of all the 24 Dabry's sturgeon. The 17 microsatellites sequences were polymorphic with 3 to 6 alleles per locus and the total number of alleles is 80. The mean expected heterozygosity (H_E) and observed heterozygosity (H_O) ranged from 0.506 to 0.781, from 0.542 to 1, respectively. The Hardy-Weinberg departure value (*d*, -0.042 to 0.427), polymorphic information content (*PIC*, 0.482 to 0.753) and the Shannon-Wiener Diversity Indices (*H'*, 0.827 to1.565) of all the 17 polymorphic loci indicated a high level of informativeness (Table II).

Table II.- Genetic diversity for 17 microsatellite markers in Dabry's sturgeon.

Loci	H_E	H_O	*d*	*PIC*	*H'*	*Na*	Allele range
ZHX70	0.701	1	0.427	0.653	1.266	4	220-252
ZHX80	0.781	1	0.28	0.753	1.565	5	173-217
ZHX91	0.772	0.958	0.241	0.75	1.542	5	202-242
ZHX76	0.746	1	0.34	0.724	1.494	6	224-300
Z15	0.743	1	0.346	0.725	1.505	6	206-238
Z120	0.781	1	0.28	0.749	1.562	5	160-188
Z125	0.705	0.792	0.123	0.672	1.274	4	240-272
Z153	0.724	1	0.381	0.676	1.331	4	222-250
Z166	0.774	1	0.292	0.745	1.545	5	232-260
Z167	0.609	0.583	-0.042	0.532	1	4	218-262
Z175	0.726	1	0.377	0.683	1.333	4	186-226
Z181	0.722	1	0.385	0.683	1.328	4	190-246
Z182	0.768	1	0.302	0.739	1.529	5	172-200
Z189	0.709	0.917	0.293	0.666	1.347	5	250-310
Z193	0.722	0.958	0.327	0.698	1.392	5	170-210
Z199	0.681	0.917	0.346	0.664	1.34	6	225-253
Z219	0.506	0.542	0.07	0.482	0.827	3	200-280

H_E, mean expected heterozygosity; H_O, observed heterozygosity; *d*, Hardy-Weinberg departure value; *PIC*, polymorphic information content; *H'*, Shannon–Wiener Diversity Indices; *Na*, number of alleles in per locus.

Discussion

In this study, 17 novel cross-species microsatellite markers were developed for Dabry's sturgeon from Chinese sturgeon via next-generation sequencing for the first time. All the 17 microsatellite markers showed a high polymorphism (*PIC*>0.5), except Z219. No microsatellite marker showed significant deviation from the Hardy-

Weinberg departure value ($d < 0.05$) except Z167, indicating that they were polymorphism. Little genetic analysis was performed in previous microsatellites studies of Dabry's sturgeon (Que *et al.*, 2014; Zeng *et al.*, 2013; Zhang *et al.*, 2013). For example, the calculation of H_O, d and *PIC* were totally or partially missing in these studies. Considering that H_O, d and *PIC* are indicative of population genetic variation, microsatellite markers without those information are hard to determine whether they are suitable for population analysis. The high estimates of *PIC* of the microsatellites in this study substantiated the suitability of the markers to applications such as genetic diversity study, linkage-mapping programs in Dabry's sturgeon. Compared to traditional method, we developed a cheaper and faster way to identify microsatellite loci. This method can be used as a reference for relative studies in other species.

The number of alleles per microsatellite locus developed in Que *et al.* (2014) study, Zeng *et al.* (2013) study, Zhang *et al.* (2013) study is 1 to 11, 4 to 14 and 3 to 13, respectively. The number of alleles per microsatellite locus in this study was less than the previous studies, with the value of 3 to 6. Maybe the samples used in this study were consisted sib individuals, or the microsatellites were cross-amplified. The mean expected heterozygosity of microsatellites in this study (0.506 to 0.781) is similar to the microsatellites developed by Zeng *et al.* (2013) (0.487 to 0.874) and is better than the microsatellites developed by Que *et al.* (2014) (0.127 to 0.828) and Zhang *et al.* (2013) (0.303 to 0.806). The mean expected heterozygosity in this study may be higher than the fact due to the fact that the number of alleles per microsatellite locus in this study is less than others. Only 17 out of 96 microsatellites showed polymorphism, and the rest microsatellites produced low quality results or even nothing. We sequenced all the microsatellites in this study by Dabry's sturgeon DNA. Although those microsatellites were developed based on cDNA sequences of Chinese sturgeon, they can also be used to analysis the genetic of Dabry's sturgeon, proving that the relationship between Chinese sturgeon and Dabry's sturgeon is very close. In this study, all loci in each sample have no more than four alleles, which is consistent with two previous studies (Que *et al.*, 2014; Zeng *et al.*, 2013). Those microsatellite markers in this paper will be useful tool to study the population structure and genetic diversity of Dabry's sturgeon.

Ethical consideration

The experiments were performed in accordance with relevant institutional and national guidelines and regulations for the care and use of laboratory animals.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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