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Research Article

Parentage Determination of Chinese Hooksnout Carp (*Opsariichthys bidens*) Based on Microsatellite DNA Markers

Jianhui Ge,[1,2] **Xiaojun Xu**,[1] **Jindong Ren**,[1] **Xiaoming Shen**,[3] **Jin Yu**,[4] and **Bao Lou**[1]

[1]*State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Institute of Hydrobiology, Zhejiang Academy of Agricultural Sciences, Hangzhou, China*
[2]*College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo, China*
[3]*Deqing Haoyuan Aquatic Seed Industry Co., Ltd., Huzhou, China*
[4]*Longyou Aquaculture Development Center, Agricultural and Rural Bureau of Longyou County, Quzhou, China*

Correspondence should be addressed to Jin Yu; 5653135@qq.com and Bao Lou; loubao6577@163.com

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In this study, a panel of 14 microsatellite markers was screened to determine the parentage of 144 progenies in 12 Chinese hooksnout carp (*Opsariichthys bidens*) full-sib families. The combined exclusion probabilities for 14 loci were 97.6% and 99.9%, respectively, when there was no available parent information or only one parent information was provided. Simulation analysis demonstrated that the power of five loci to exclude false parents exceeded 99.0%, while that of eight loci reached 99.9% based on allele frequency data obtained from full-sib families (168 individuals). Moreover, the cumulative assignment success rate reached 100% with known parental and filial information when utilizing 13 or more loci, even in cases where no parent information was available. The results showed that this set of microsatellite markers proved to be a reliable and efficient tool for parentage determination of *O. bidens*.

1. Introduction

Chinese hooksnout carp (*Opsariichthys bidens*), belonging to the family Cyprinidae of order Cypriniformes, is an endemic minnow with a wide distribution in lakes, rivers, mountains, and streams throughout East Asia [1, 2]. For the superior taste, fast growth, and high economic value, *O. bidens* has become an emerging commercial fish species. Previous studies on *O. bidens* were mainly focused on artificial propagation [3], reproductive biology [4–6], and flesh nutrition content [7]. The natural resources of *O. bidens* have dramatically decreased in the past decades due to river damming, water environment pollution, and overfishing. With the expansion of *O. bidens* culture, breeders began to pay attention to the selective breeding work. Family selection has been proven to be an efficient breeding technique in aquatic species breeding practice (*Oreochromis niloticus*, *Cyprinus carpio*, *Megalobrama amblycephala*) [8–10].

Traditional population selection programs relied on phenotypic selection; inbreeding would be inevitable, which led to the reduction of genetic diversity [11]. However, the credible kinship information was essential to reduce the negative effects caused by inbreeding in population selection. When implementing family selection, it was necessary to maintain the offspring of each family in a separate pool or pond until they reached a sufficient size for physical tagging. The approach, therefore, required significant labor and space resources. Moreover, there were some environmental differences between fish ponds, which caused some discrepancies in the estimation of genetic parameters related to breeding [12].

Fortunately, due to the rapid advancements in sequencing technologies [13], highly polymorphic microsatellite markers have been developed for numerous aquaculture species, such as *Pinctada fucata*, pearl oyster (*Pinctada maxima*), and black amur bream (*Megalobrama terminalis*) [14–16]. Because of its high density, genome-wide distribution, and codominance, microsatellite has been widely used in the survey of genetic diversity and population structure [17, 18]. For its high polymorphism, microsatellite has been used as a valuable molecular tool for parentage determination and pedigree tracing

Table 1: Characteristics of the 14 polymorphic microsatellite markers of *O. bidens*.

Primer code	GenBank accession no.	Repeat motif	Primer sequence (5'–3')	T_a (°C)	Allele size range (bp)	Chromosome
2Chr25	OQ689677	(TG)40	F: TACCAACCAAAATGCCAACC R: GCTCCAAGACATTTCTGTGC	60	171–241	25
2Chr19	OQ689678	(TG)34	F: ATTCATTCGAAATCGGCACC R: ACCTTATCCCAGAGACCCAT	58	129–203	19
3Chr15	OQ689685	(TAT)13	F: TCGGCCTAACACCATAGATG R: GGGTCCATTCATCTAACCCT	56	246–258	15
3Chr25	OQ689687	(TAT)19	F: GGCTTGACTTGTTGCATGAC R: GCTCATCAATGGTGCTTACC	56	242–266	25
3Chr14	OQ689684	(AAT)12	F: TTAAGGTCGGGTTGTTAGGC R: GGGTATATCTGAAGGCCCAG	56	223–235	14
3Chr3	OQ689679	(TAT)12	F: GGTTACTCGACATTTCGCTG R: CAAACTCACGTGGCTTCATT	56	236–245	3
3Chr7	OQ689682	(AAT)15	F: GTCAAGAAGAGGGAGCACAT R: GCCTTTGCTATTAGTTATGGGA	58	231–246	7
3Chr6-1	OQ689681	(TTG)16	F: ACATCCAGAGGGCAATCATT R: AGATCAGGCACACTCTCAAA	58	134–158	6
3Chr23-1	OQ689686	(ATT)15	F: GAGTCTGACAGTCATCCAGA R: AACCTGGATCAGTGATGCTC	58	236–251	23
3Chr34-1	OQ689690	(TAA)11	F: TGGTGACCATGAAACGTTTG R: GGCACGTTGCTATGAATGTT	58	242–251	34
3Chr26	OQ689688	(ATT)15	F: ATTGGTCTTGGTTCTCAGCT R: TCGCCTCCTCACTACAATTC	56	234–249	26
3Chr4-1	OQ689680	(GAT)12	F: GCTGCTGTCCTCTAGAGTTT R: CAGTCAGAGAGCGATGTCTT	58	233–239	4
3Chr28-1	OQ689689	(AAT)12	F: CTAGCAGAGGACTATGGCAG R: CTCTTCAGGGTTTGGGCTAT	58	152–173	28
3Chr11	OQ689683	(TAA)10	F: CAGCAGACCGAAAAGAAAGC R: ACACAGGTGGAAACATGTCT	56	241–253	11

Notes: T_a, annealing temperature.

[19–25]. So far, no parentage determination of *O. bidens* has been reported. The development of a series of polymorphic microsatellite markers for *O. bidens* has been accomplished by our team recently [26]. The purpose of this study was to evaluate the efficiency of microsatellite markers in parentage determination of *O. bidens*.

2. Materials and Methods

2.1. Sample Collection. All *O. bidens* fish were sourced exclusively from Deqing Haoyuan Fish Farm in Zhejiang Province. Twelve full-sib families were established through artificial insemination, utilizing 12 sires and 12 dams. Subsequently, newly hatched fries were sampled from each family. Parental tissue samples were collected from tail fins. All samples were preserved in 100% ethanol for DNA extraction.

2.2. DNA Extraction and Microsatellite Analysis. The genomic DNA was extracted from newly hatched fries and tail fins of parents using the TIANamp Micro DNA Kit (TIANGEN, Beijing, China), following the manufacturer's instructions. Extracted DNA was diluted in distilled water with a final concentration of 50 ng/μL. The selection of 14 microsatellite loci was made for the purpose of conducting polymerase chain reactions (PCRs) (polymorphism information content (PIC) > 0.7 in loci characterization) (Table 1). Seven loci (3Chr15, 3Chr25, 3Chr14, 3Chr3, 3Chr7, 3Chr26, and 3Chr11) were developed previously [26] and seven new loci (2Chr25, 2Chr19, 3Chr6-1, 3Chr23-1, 3Chr34-1, 3Chr4-1, and 3Chr28-1) were developed in this study. PCR amplification was carried out in a final volume of 20 μL, comprising approximately 50 ng of DNA template, 2 × Hieff PCR Master Mix (YEASEN, Shanghai, China), forward (labeled with FAM or HEX), and reverse primers at a concentration of 3 μM each. PCR conditions were as follows: an initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 20 s, annealing at the optimal temperature for 20 s, elongation at 72°C for 40 s, and a final extension at 72°C for 5 min. PCR products were separated using an ABI 3730 XL Genetic Analyzer (Applied Biosystems), and GS-500 was employed as an internal size standard. Fragment sizes were determined utilizing GeneMarker v2.2.0 (SoftGenetics, State College, PA, USA).

2.3. Data Analysis. The number of alleles (N_A), observed (H_O), and expected (H_E) heterozygosity, PIC, frequency of

Table 2: Genetic diversity of 14 microsatellite markers in 168 *O. bidens* individuals.

Locus	N_A	H_O	H_E	PIC	Excl-1	Excl-2	HWE	F (null)
2Chr25	12	0.935	0.87	0.854	0.584	0.739	**	−0.0425
2Chr19	6	0.821	0.765	0.731	0.377	0.558	NS	−0.038
3Chr15	6	0.869	0.748	0.699	0.325	0.501	*	−0.0756
3Chr25	7	0.75	0.708	0.657	0.296	0.467	NS	−0.0305
3Chr14	4	0.595	0.682	0.617	0.247	0.406	NS	0.0701
3Chr3	4	0.708	0.679	0.609	0.239	0.394	NS	−0.0247
3Chr7	5	0.565	0.592	0.509	0.177	0.306	NS	0.0167
3Chr6-1	4	0.506	0.537	0.498	0.16	0.27	NS	0.0448
3Chr23-1	4	0.488	0.53	0.485	0.155	0.318	NS	0.0608
3Chr34-1	4	0.536	0.566	0.469	0.149	0.304	NS	0.0286
3Chr26	3	0.565	0.532	0.427	0.141	0.234	NS	−0.0339
3Chr4-1	2	0.381	0.499	0.374	0.124	0.187	*	0.1326
3Chr28-1	8	0.321	0.33	0.308	0.057	0.177	NS	0.0155
3Chr11	4	0.256	0.288	0.269	0.042	0.15	ND	0.0727

Notes: N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F (Null), frequency of null allele; PIC, polymorphism information content; HWE, Hardy–Weinberg equilibrium; NS, no significance; ND, not determined; **, significant deviation at $P < 0.01$; *, significant deviation at $P < 0.05$.

null alleles (F (null)), and probability of Hardy–Weinberg equilibrium (HWE) were computed by Cervus 3.0 software [27]. The exclusion probabilities for each locus, when the genotype of one parent was known and when neither parent was known, were referred to as Excl-2 and Excl-1, respectively. The feasibility and confidence of parentage determination were assessed by conducting a simulation program using Cervus 3.0 software, with the following parameters: 10,000 replication cycles, a pool of 24 candidate parents, 100% of the candidate parents sampled and genotyped, and a default typing error rate (1%).

To validate the theoretical estimation through simulations, a set of 12 full-sib families genotyped at 14 microsatellite loci were utilized for parentage determination, incorporating known parental and filial information. Parentage determination of these 12 families was assessed using the likelihood-based approach implemented in CERVUS 3.0 software.

3. Results

3.1. Computer Simulations. In total, 144 progenies and 24 parents, with each full-sib family of *O. bidens* containing 12 offspring, were genotyped using 14 microsatellite markers (Table 1). The PIC ranged from 0.269 to 0.854, exhibiting an average value of 0.536. The number of alleles (N_A) ranged from 2 to 12, exhibiting an average of 5.2 alleles per locus. The observed (H_O) and expected (H_E) heterozygosity ranged from 0.256 to 0.935 and 0.288 to 0.870, respectively. The informative value of seven loci was found to be high (PIC > 0.5), while the remaining seven loci exhibited a moderate level of informativeness ($0.25 < \text{PIC} < 0.5$) (Table 2).

Summary statistics for the 14 microsatellite loci utilized in the simulation study were organized in Table 2 based on their decreasing order of PIC values. The theoretical probability of parentage exclusion per locus ranged from 0.042 to 0.584 in the absence of any parent information (Excl-1),

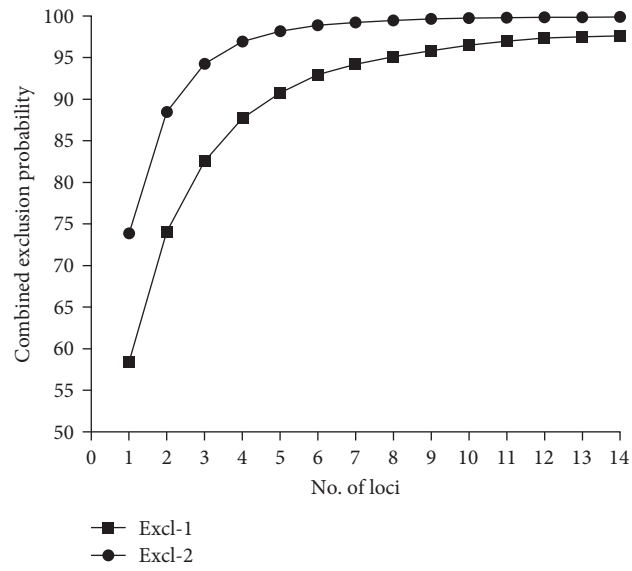

Figure 1: Combined probabilities of exclusion were calculated for over 14 polymorphic loci when both (Excl-1) and one parent were unknown (Excl-2).

while it varied between 0.150 and 0.739 when one parent information was available (Excl-2) (Table 2). Furthermore, the combined exclusion values of 14 loci exceeded 97.0% both for Excl-1 and Excl-2 (Figure 1). Simulation analysis revealed that a minimum of five loci were necessary to accurately assign more than 99.0% of the progenies to both parents. The utilization of eight loci resulted in a remarkable assignment success rate of 99.9%, demonstrating its robust capability for parentage determination.

3.2. Parentage Identification with Known Parental and Filial Information. To ensure ease of allele scoring error, 144 offspring were genotyped by the 14 microsatellite loci alongside

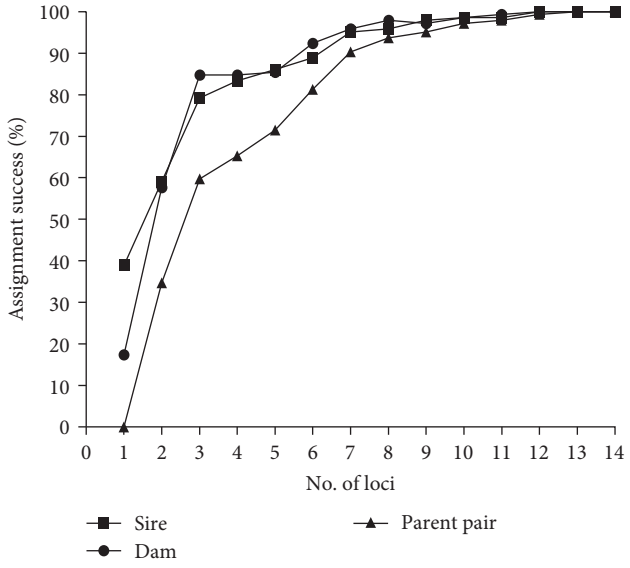

FIGURE 2: Assignment success of progenies to correct sire, dam, and parent pair based on parent and offspring genotypes.

24 parents. The utilization of the authentic dataset resulted in a successful assignment rate of 95% for progenies to their respective parent pairs, employing nine microsatellite markers. Moreover, we tested the exclusionary power of 100% to sire, 100% to dam, and 100% to parent pair when 13 or more microsatellite loci were applied (Figure 2).

4. Discussion

In the process of selective breeding for aquaculture species, it was extremely important to maintain correct pedigree relationships. Generally, physical tags such as visible implant elastomer and passive integrated transponder have been applied to identify different families in the breeding of lobsters (*Homarus gammarus* L.), prawns (*Macrobrachium rosenbergii*), and European eels (*Anguilla anguilla*) [28–31]. The physical tags, however, had several limitations, including a restricted tagging size, labor-intensive processes, and a certain rate of tag loss [32, 33]. Using microsatellite for parentage determination could overcome the limitations of traditional physical tags. Due to their high polymorphism and codominance, microsatellite markers have been widely utilized in parentage determination of aquaculture species, such as *Siniperca chuatsi*, *Siniperca scherzeri*, *Odontobutis potamophlia*, *Scylla paramamosain*, *Fenneropenaeus chinensis*, *Penaeus monodon*, *Exopalaemon carinicauda*, and *P. fucata* [14, 19, 21, 34–38].

Although microsatellites are abundant and widely distributed in the genome, developing a highly efficient microsatellite marker set for parentage determination still requires great efforts. Microsatellite loci for parentage determination were selected according to the following criteria: high polymorphism and no stutter band. In general, di-nucleotide loci had higher polymorphism than tri-nucleotide and larger repeat motif loci [39]. However, di-nucleotide loci were more susceptible to generate stutter bands due to their PCR slippage products than larger repeat motif loci [40, 41]. To avoid the appearance of stutter bands, tri-nucleotide loci were the major part of this study. In addition, two highly polymorphic di-nucleotide loci without stutter bands were used to improve the efficiency of parentage determination in this study.

Computer simulation was necessary to evaluate the application of parentage determination with microsatellites. In this study, considerable differences between computer simulation and real parentage determination were observed. This situation was also found in the studies of other aquatic species, such as mandarin fish (*S. chuatsi*), mud crab (*S. paramamosain*), and black tiger shrimp (*P. monodon*) [34, 36, 37]. The reasons of this phenomenon were as follows: First, the presence of null alleles was considered to be a substantial factor affecting the accuracy of parentage determination [32, 38]. In the present study, 3 of 14 microsatellites had null allele frequency exceeding 5%. The presence of null alleles with a frequency estimate exceeding 5% was harmful to the accuracy of parentage determination [42]. Second, the error in the genotyping process was another important factor. Capillary electrophoresis was a conventional microsatellite detection method. The generation of Joule heat in capillary electrophoresis led to uneven temperature gradients and local viscosity changes, which could affect the genotyping accuracy [43].

In previous studies, 94% of 260 offspring were accurately assigned to their correct parent pairs with nine loci in mandarin fish, *S. chuatsi* [34]. In golden mandarin fish, *S. scherzeri*, 110 of 120 offspring were accurately assigned to their correct parent pairs using seven microsatellite markers [19]. When seven informative microsatellite loci were used, the correct parentage of assignments' success was more than 80.0% in *Procambarus clarkii* [44]. The markers for parentage determination were considered applicable when discrimination power was more than 80% [45]. In this study, a high accuracy rate of 90.28% was achieved in the assignment of offspring using seven microsatellite markers, and the assignment success rate reached 100% when using 13 or more microsatellite markers in 12 full-sib families. The results presented in this study demonstrate that this set of 14 microsatellites serves as a reliable and efficient tool for parentage determination in the selective breeding programs of *O. bidens*.

5. Conclusions

In this study, 14 polymorphic microsatellite markers were selected for parentage determination of *O. bidens*. Overall, 100% assignment success was achieved in 12 full-sib families when 14 microsatellite markers were used. This study provided a reliable and efficient tool for parentage determination in the selective breeding programs of *O. bidens*.

Data Availability

The data used to support the findings of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jianhui Ge and Xiaojun Xu conducted the experiment. Jindong Ren conducted the data analysis. Xiaojun Xu and Bao Lou designed and supervised the project. Jin Yu and Xiaoming Shen prepared and collected experimental samples. Jianhui Ge wrote the manuscript. All authors read and approved the final manuscript. Jianhui Ge and Xiaojun Xu contributed equally to this work as co-first authors.

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