Pakistan J. Zool., vol. 52(3), pp 1177-1179, 2020 DOI: https://dx.doi.org/10.17582/journal.pjz/20170909190927

Short Communication

Specific PCR-based Marker for Identification of Common Carp 'Hainan' in China

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Article Information
Received 09 September 2017
Revised 15 April 2018
Accepted 02 April 2019
Available online 16 March 2020

Authors' Contribution
MYL, ZZ and YG helped in the experimental work and wrote the manuscript. PL, SL and JX conducted experimental work. ARK helped in statistical analysis. JL and YZ designed the project and experiments.

Key words
Common carp, Genetic diversity, Hainan population, PCR, Specific molecular marker

ABSTRACT

The common carp (*Cyprinus carpio* L.) is one of the most important aquaculture species in the world. In China, due to the geographical isolation of Hainan island from the mainland, the genetic diversity in Hainan island is different to that in the mainland. One of fifty sequences isolated from SINE element was polymorphic, including six haplotypes with an average sequence divergence of 11.03% (SE = 0.78%). Four haplotypes (MF177501-MF177504) were shared in these four population. However, two specific haplotypes (MF177499-MF177500) were only observed in Hainan population. To simplify procedure and improve effectiveness of identifying the Hainan population, one specific PCR-based marker acted as INDEL marker was developed and then 120 samples were used as templates for PCR validation. The specific marker will facilitate the assessment of genetic diversity and selective breeding programs for Hainan common carp.

Common carp (*Cyprinus carpio* L.) is one of the most widely distributed fish species in the world. Common carp had plenty of varieties in China. Because of high environment tolerance of this species, it widely distributes from northeast to southeast in China (Barus *et al.,* 2001). Hainan Island is isolated from the mainland, locating (Hall, 1998). Landscape features in Hainan act as barriers to dispersal for some taxa (Zhang *et al.,* 2010). Thus, the knowledge of the genetic diversity of common carp in this island was poor. Therefore, likely contains undocumented genetic diversity for some species which is suspected but not confirmed, molecular data and dating may serve to clarify patterns of variation and identify the probable drivers of these patterns (Blair *et al.,* 2013).

Materials and methods

We sequenced the genome of common carp and identified 6,0436 copies putative SINE retrotransposons (Xu *et al.,* 2014). The sequences with shortest divergence time were selected based on result of the pairwise sequence divergence evaluated by the Ka/Ks calculator (https://code.google.com/p/kaks-calculator/wiki/KaKs_Calculator). A total of fifty primers were designed, but only one pair primer had positive result for identifying Hainan common carp form other populations.

In the present study, a total of 120 fin samples of common carp were collected from four wild populations in Heilongjiang province, Songhua river (N 45° 52', E 126° 41'), Jilin province, Yueliang lake (N 45° 43', E 123° 59'), Jiangsu province, Changjiang river (N 32° 02', E 120° 43') and Hainan province, Nandu river (N 19° 43', E 109° 59'), respectively. Common carp samples were stored in 95% ethanol for DNA analysis. Total genomic DNA was extracted using a modified phenol–chloroform method (Sambrook and Russell 2001). DNA amplification and sequencing for the SINE polymorphic sequence and specific sequence for Hainan population: SL1-F: AACGACGACACATGGACATCAAGGAACC), SL1-R: GTTCAGTCGGTCAGTATTGGA; T1-F: AACGACGACACATGGACATCAA;

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0030-9923/2020/0003-1177 $ 9.00/0

T1-R: GTTCAGTCGGTCAGTATTGGA.

PCR amplification for the two sequences was the same and carried out in a final volume of 20 µL containing 10 µL Premix Taq (containing 0.5 UExTaq, 0.4 mM dNTPs, 4 mM EX Taq buffer), 1 µL each primer (0.4 pmol), 1 µL template DNA (~30 ng DNA), and 7 µL double-distilled water. Thermal cycling conditions were as follows: 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, the optimal annealing temperature 57°C for 30 s, extension at 72°C for 40 s, and a final extension step for 10 min at 72°C. Finally, PCR products were sequenced with an ABI 3730XL DNA analyzer (Applied Biosystems), and sequencing results were analyzed by Sequencer 5.1 (Gene Codes Corporation). DNA sequences were edited using BioEdit (Hall, 1999) and aligned by ClustalW (Thompson *et al.,* 1994) with default parameters. The haplotypes of sequence were checked manually and verified using the software DnaSP 5.0 (Librado and Rozas, 2009). Haplotype diversity (hˆ) (Nei, 1987) and nucleotide diversity (π) were calculated with DnaSP v.5.0 (Librado and Rozas, 2009). Tajima's D (Tajima, 1989) was estimated by the coalescent as implemented in DnaSP v. 5.0 with 10,000 replications and used to assess evidence for population range expansion, under which negative values are expected (Aris-Brosou and Excoffier, 1996). Genetic distances among different haplotypes of CR were estimated through the software MEGA 5.2 using Kimura 2-parameter (K2p) distance model. Mismatch analyses and raggedness indices were calculated in Arlequin 3.0 (Schneider *et al.,* 2000) and significance was assessed with 1000 bootstrap replicates.

Results and discussion

Firstly, we used the primer T1-F and T1-R to amplify 40 individuals from four carp populations, and these sequences fragment (603bp) produced six unique haplotypes (GeneBank Accessios: MF177499-MF177504) with an average sequence divergence of 11.03% (SE = 0.78%). Two specific haplotypes were only found in Hainan population and four haplotypes were shared in these four populations. Overall, haplotype diversity was mid-range while nucleotide diversity was low (Table I). Pair wise genetic distance among these four lineages were very significantly different based on the sequence (0.0102-0.02760).

We developed one new PCR-based marker (SL1) to identify the Hainan population in further based on the two specific haplotypes observed in Hainan population. The primers had positive product in four populations (a total of 90 individuals) and negative product in Hainan population (30 individuals) (Supplementary Fig. 1). In conclusion, we identified Specific PCR-based marker that will facilitate the evaluation of genetic resources and assist selective breeding programs for Hainan common carp.

Table I. parameters of the sequence polymorphisms.

	N	n	π	hˆ	Tajima's D
HN	10	6	0.0701	0.096	-1.575
YLH	10	4	0.0813	0.0992	-0.331
CJ	10	4	0.0924	0.08690	-0.223
HLJ	10	4	0.0785	0.0774	-0.346

N, number of individuals per region; n, Number of haplotypes; π, Nucleotide diversity; hˆ, haplotype diversity; HN, Hainan region; YLH, JILIN region; CJ, JIANGSU region; HLJ, HEILONGJIAGN region.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20170909190927

Acknowledgment

This study was supported by National Natural Science Foundation of China (31672644) and the Special Scientific Research Funds for Central Non-profit Institutes, Chinese Academy of Fishery Sciences (2018B004 and 2018HY-ZD0207).

Statement of conflict of interest

The author declares that they have no conflict of interests.

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