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Isolation and characterisation of polymorphic microsatellite markers in the progeny of nine mating groups of snakeheads

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ABSTRACT

Nine mating groups of snakeheads (*Channa maculata* and *Channa argus*) have been successfully bred in our laboratory. During the course of this research, five polymorphic microsatellite loci were initially screened out from the nine populations, which were isolated and characterised. A total of 360 individuals from the nine populations have been genotyped and forty three alleles were found. The number of observed and effective alleles ranged from 6 to 12 and from 3.8907 to 7.4083 per locus. Average numbers of alleles and effective alleles were 8.60 and 5.03, respectively. Average number of observed alleles in each population varied from 2.2 to 4.4, while the mean number of effective alleles varied from 1.78 to 3.10. Average observed heterozygosity (H_O) and expected heterozygosity (H_E) in the nine populations ranged from 0.1600 to 0.4700 and from 0.4082 to 0.6490, respectively. Polymorphism information content (PIC) varied from 0.3351 to 0.5778. Among the forty five population loci, thirty eight showed significant deviation from the Hardy-Weinberg equilibrium. These results demonstrated great significance for the evaluation and conservation of new species and also provided the basic material for predicting heterosis as well as for breeding of new varieties with economic value.

Keywords: Genetic variety, Mating groups, Microsatellite markers, Snakehead

Introduction

The snakeheads *Channa maculata* and *Channa argus* have high proportions of meat, few bone spurs and have high nutritional as well as medicinal value (Nie *et al*., 2002; Zhang *et al*., 2004) and are extremely popular in domestic and international markets. *C. argus* is mainly fed on chilled fish scraps in aquaculture, resulting in high input costs, high disease incidence and serious environmental pollution. While *C. maculata* has drawbacks such as poor disease resistance, low survival rate, small market size, low yields and poor economic benefits, as was gradually revealed in culture (Zhu *et al*., 2001; Zou *et al*., 2011). In addition, due to overfishing (Ma *et al*., 1999), germplasm resources have been continuously degraded in recent years and the species has become much rarer in the wild. As a new species for culture, the first generation hybrids (*C. argus* (♂) × *C. maculata* (♀)) showed better traits than its parents and is widely farmed in China (Zou and Zhuo, 2011). In our study, nine mating groups of snakeheads (*C. maculata* and *C. argus*) were generated and nine combinations of crosses were successfully bred.

Information on the genetic characteristics of the species is essential for conservation and protection of genetic resources (Li *et al*., 2007; Peng *et al*., 2009). As highly variable nuclear genetic markers, microsatellites are co-dominantly inherited in a Mendelian fashion and have been increasingly used in aquaculture in recent years. These studies relate to the investigation of genetic diversity among different groups as a consequence of different breeding strategies and parentage assignment (O'Connell and Wright, 1997; Shikano and Taniguch, 2002; Muths *et al*., 2009; Zhou *et al*., 2012; Sekar *et al*., 2014). Genetic diversity has gradually become one of the most important aspects involved in the management of fishery resources and selective breeding programs for aquaculture (Beaumont and Hoare, 2003; Dunham, 2011). Previously, very few genetic diversity studies have focused on snakehead groups (Li *et al*., 2006; King and Johnson, 2011; Zhuo *et al*., 2012). In the present study, five pairs of polymorphic microsatellite markers were initially screened (Wen and Sun, 2010; King and Johnson, 2011), to evaluate the genetic diversity of the nine crosses of snakeheads to elucidate molecular genetic diversity which could lay the foundation for prediction of heterosis and selection of new strains of fast growing, disease resistant fishes which could yield increased economic returns. The results of the study could aid in breeding programmes to simultaneously identify and characterise the progeny from the mating groups.

Materials and methods

Sample collection and DNA isolation

The nine mating snakehead groups (Table 1) were cultured in different ponds with hybrid snakehead breeding bases in Zhongshan City of Guangdong Province, China in 2012. Forty samples were collected randomly in each group after 3 months of culture. Fin clippings and muscle tissue samples from live fish were collected and immediately soaked in 95% ethanol and then stored at -20°C. Total genomic DNA was extracted from the tissues using a standard extraction kit (DNeasy tissue kit, Baitaike Biotech Co., Ltd, China) and electrophoresed on 1% ethidium bromide stained agarose gels for the purpose of evaluating quality of the extracted DNA.

Table 1. Details of the combinations of purebreds and their crosses

♂→♀↓	*C. maculata* ♂	*C. argus* ♂	F_1 of *C. maculata* X *C. argus* ♂
C. maculata ♀	*C. maculata* purebreds (Group 1)	*C. argus* ♂ X *C. maculata* ♀ (Group 2)	F_1 ♂ X *C. maculata* ♀ (Group 3)
C. argus ♀	*C. maculata* ♂ X *C. argus* ♀ (Group 4)	*C. argus* purebreds (Group 5)	F_1 ♂ X *C. argus* ♀(Group 6)
F_1 of *C. maculata* X *C. argus* ♀	F_1 ♀ X *C. maculata* ♂ (Group 7)	F_1 ♀ X *C. argus* ♂(Group 8)	F_2 generation (Group 9)

Five pairs of microsatellite primers for northern snakehead *Channa argus* (Wen and Sun, 2010; King and Johnson, 2011) (Table 2) were used. The PCR amplification reactions were carried out using a 25 ml reaction volume, containing 1.0 U of Exta q DNA polymerase, 10× PCR buffer (TaKaRa Biotechnology Co., Ltd, Dalian, China), 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, 100 ng DNA template, and 0.4 mM of each primer. PCR was performed in a PE 9700 thermocycler (PerkinElmer Co. Ltd., USA). Thermal cycling conditions for each locus comprised of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, an annealing temperature at 60°C for 40 sec (WL-8 and WL-28) and at 55°C for 40 sec (CarC6, CarD108 and CarD121), followed by an extension temperature of 72°C for 1 min and then a final extension of 72°C for 10 min to ensure complete adenylation of PCR products. The specific annealing temperature of each primer set is given in Table 2. The PCR products were analysed by electrophoresis on 8% non-denaturing polyacrylamide gels using a 1×TBE buffer in the gel and reservoirs at 200 V for 2-3 h in accordance with allele size and were visualised by silver staining. DNA Marker DL 500 (Takara, Dalian, China) was used to determine the allele sizes. Fragment sizes were analysed with the ROX-500 size standard using GeneMapper (Applied Biosystems).

Genetic variation analysis

Genetic variations of the progeny from nine mating groups was evaluated by: the number of alleles per locus (Na), number of effective alleles (Ne), observed heterozygosity (H_O) and expected heterozygosity (H_E) for each cross at each locus. The fixation index (F_{is}) values were calculated directly from microsatellite phenotypes that were computed from the microsatellite genotype data using the software GENEPOP version 3.1 (Raymond and Rouset, 1995). The expected frequency of null alleles was estimated using the equation of Brookfield (1996), $r = (H_E - H_O) / (1 + H_E)$. Polymorphic information content (PIC) of the progeny from nine mating groups at the 5 microsatellite loci were determined using the software PIC_Calc 0.6. POPGENE program for assessment of the Hardy-Weinberg equilibrium (HWE) at each locus for each mating group was tested by the Markov-chain method for exact probability testing.

Results

Genetic variability in microsatellite loci

All five microsatellite markers, WL-8, WL-28, CarC 6, CarD 108 and CarD 121, were observed to have high levels of polymorphism in the nine crosses (Table 3). A total of 360 individual samples from the progeny of nine mating groups revealed 43 alleles while the average number of alleles was 8.6 (specific number ranged from 6 to 12) per locus. Number of effective alleles was 5.0356 (specific number ranged from 3.8907 to 7.4083) per locus. Average number of observed alleles in

Table 2. Characteristics of microsatellite markers used

Locus	Primer sequence	Size (bps)	Annealing Temp. (℃)	$MgCl_2$ (mM)	References
WL-8	F:CTTCCCAGTTCTCCTTGCAG R:CAGAAGGGCCTTACTTGAGC	159-226	60	1.5	Wen and Sun (2010)
WL-28	F:TGCTTCCCTGTAGTCCACCT R:GTGAAAATGGCACAGAACGA	168-198	60	1.5	Wen and Sun,(2010)
CarC6	F:TTCCAAGTCTGTAACCTCGTG R:GCTCTGTCTTTAAACCCCTATG	216–244	55	1.5	King and Johnson (2011)
CarD108	F:GTCTTGCTAAGGGCAGAGAT R:TTAAACCGGAATTTACTGACTG	106–156	55	1.5	King and Johnson (2011)
CarD121	F:GAGTAATCGCCTTTGATTATGG R:ATACAATCATCAGTATGAACAATGG	221–269	55	1.5	King and Johnson (2011)

Table 3. Genetic analyses of five microsatellite loci in nine combinations

Group no.	Microsatellite locus					Mean
	WL-8	WL-28	CarC6	CarD108	CarD121	
F_{is} values of each Group in five locus						
1	0.2933	0.0179	-0.0811	0.5506	1.0000	0.3561
2	1.0000	-0.5866	-0.5504	0.9319	0.9147	0.3419
3	0.7419	0.2875	-0.2800	0.8728	1.0000	0.5244
4	0.5676	-0.0643	-0.4090	0.5767	0.9050	0.3152
5	0.1198	-0.1111	-0.1803	-0.2652	-0.2121	-0.1298
6	0.8519	0.6234	0.5157	0.8462	0.7723	0.7219
7	1.0000	-0.1133	1.0000	0.7878	****	0.6686
8	0.7339	0.1647	0.5960	0.6132	0.4366	0.5089
9	1.0000	-0.6293	1.0000	0.5842	****	0.4887
Mean of all Groups						
Na	12	6	8	8	9	8.6
Ne	7.4083	3.8907	4.0369	5.8075	4.0349	5.0356
H_O	0.1889	0.5611	0.3944	0.2389	0.1111	0.2989
H_E	0.8674	0.7450	0.7544	0.8301	0.7543	0.7902
r	0.3633	0.1105	0.2052	0.3230	0.3666	0.2732
F_{is}	0.7816	0.2448	0.4757	0.7114	0.8523	0.6132

Na = number of observed alleles; Ne = Number of effective alleles; H_O = Observed heterozygosity; H_E = Expected heterozygosity; r, Null allele frequency estimated as r = (He-Ho) / (1+He); F_{is} = Fixation index

each group varied from 2.20 to 4.40, and the mean number of effective alleles ranged from 1.78 to 3.10. Meanwhile, the average polymorphism information content (PIC) value ranged from 0.3401 to 0.6017 per locus, and the average PIC value in each group varied from 0.3351 to 0.5778 (Table 4). Among the nine groups, the lowest mean number of alleles (2.2) was observed in crosses of Group 9 [progeny of Hybrid snakehead (♀) × Hybrid snakehead (♂)], while the highest mean number of alleles (11.4) was found in crosses of Group 3 [progeny of *C. maculata* (♀) × Hybrid snakehead (♂)].

Table 4. Polymorphism information content (PIC) of the nine populations at the 5 microsatellite loci

Locus	G1	G2	G3	G4	G5	G6	G7	G8	G9	Mean
WL-8	0.6500	0.5350	0.7386	0.6318	0.4537	0.6116	0.5594	0.4686	0.3515	0.5556
WL-28	0.4992	0.5201	0.4415	0.4911	0.1638	0.5897	0.5546	0.4811	0.5391	0.4756
CarC6	0.1291	0.5897	0.4277	0.5622	0.3593	0.4634	0.3648	0.3724	0.2688	0.3930
CarD108	0.7479	0.6853	0.7541	0.6629	0.4380	0.5962	0.4327	0.5819	0.5161	0.6017
CarD121	0.3750	0.5063	0.5270	0.4309	0.3725	0.5997	0.0000	0.2499	0.0000	0.3401
Mean	0.4802	0.5673	0.5778	0.5558	0.3575	0.5720	0.3820	0.4308	0.3351	0.4732

Average observed heterozygosity (H_O) per locus was 0.2989 and ranged from 0.1111 (CarD121) to 0.5611 (WL-28), while the expected heterozygosity (H_E) was 0.7902 which ranged from 0.7450 (WL-28) to 0.8674 (WL-8). The expected frequency of null allele (r) was 0.2732 and ranged from 0.1105 (WL-28) to 0.3666 (CarD121). Within groups, the lowest mean observed heterozygosity was found in the crosses of Group 7 [Progeny of Hybrid snakehead (♀) × *C. maculata* (♂)] population (0.1600), while the highest value (0.4700) was in the purebred Group 5 [progeny of *C. argus* (♀) × *C. argus* (♂)]. The lowest average expected heterozygosity was found in the Group 9 crosses (0.4082). The highest value was in the crosses of Group 6 [progeny of *C. argus* (♀) × Hybrid snakehead (♂)) (0.8460].

Hardy-Weinberg equilibrium test

Of the forty five Hardy-Weinberg equilibrium (HWE) tests, only 5 were in HWE ($p>0.05$): CarC6 in progeny of Groups 1, 3 and 5, WL-28 in Group 5, and CorD121 in Group 5. Thirty eight tests were significant ($p<0.05$), and only WL-28 in Groups 1 and 2 and CarC6 in Group 2 were found to be in HWE ($0.01 < p < 0.05$). Others significantly deviated from HWE ($p<0.01$). Based on average F_{is} values (Table 3), data showed that the most significant deviations indicated deficiency of heterozygotes. It can be seen that the pattern of heterozygote deficiency was most pronounced in the Group 6 cross (F_{is}= 0.7219). Heterozygote deficiencies also appeared in the crosses of Groups 1, 2, 3, 4, 6, 7, 8 and 9 populations (0.3152-0.7219). The progeny of Group 5 showed minimum heterozygote deficits (-0.1298).

Discussion

Applicability of the standard reaction system microsatellite primers

For most species, the microsatellite loci point must be first isolated, and proper microsatellite primers must

be designed while using microsatellite markers. Due to the sequence conservation of nuclear microsatellites, the microsatellite primer is versatilised (Zane *et al*., 2002). *C. argus* and *C. maclata* belong to the same genus progeny and their micro-flanking sequence might have a certain homology. Studies have found that, using 47 carp satellite primers for a grass carp albino mutants analysis, 23 pairs of primers can be used for grass carp genotyping (David *et al*., 2001), which is close to 50%. Carp microsatellite primers have been utilised for the detection of the genetic diversity of wheatfish (Lin and Luo, 2003; Ma *et al*., 2007), and hence it was felt feasible to use the microsatellite primers of *C. argus* for PCR amplification of the progeny from the nine mating groups. The premise was the determination of a fit of the PCR reaction system under the conditions of the order to filter out the stable amplified specific bands with regard to microsatellite primers. Based on this, the higher denaturation temperature of the specific amplification obtained in the course of the experiment could greatly reduce the non-specific binding between primer and template, thereby improving the specificity of the PCR amplification.

Analysis of genetic diversity among populations of the nine mating groups

The values for genetic diversity observed increased with increase in the number of alleles detected. From progeny of Group 1 to 9, the numbers of alleles detected in each group based on the five microsatellite loci were 18, 20, 22, 18, 14, 21, 13, 16 and 11. Average numbers of alleles per locus were observed to be 3.6, 4, 4.4, 3.6, 2.8, 4.2, 2.6, 3.2 and 2.2. Average effective numbers of alleles were 2.67, 2.77, 3.1, 2.77, 1.78, 2.78, 2.05, 2.13 and 1.88. These values were higher in Group 3 than in other combinations, indicating that the allelic diversity of the progeny of the F1 backcrossed to the female parent was higher compared to the other combinations. The levels of genetic diversity within Groups 5 and 9 were the lowest among the nine groups. We found that they were more susceptible to the bacterial pathogen, *Aeromonas hydrophila* during culture and the survival rate was extremely low during fingerling stage (Chen *et al*., 2012; Zeng *et al*., 2013). It therefore appears that they are genetically inferior. Simultaneously, the average level of heterozygosity in groups reflects the degree of consistency of the genetic population. In this study, order of the mean observed heterozygosity value of the nine groups from highest to least was: Group 5 (0.47) > 4 (0.43) > 2 (0.41) > 1 (0.31) > 3 (0.25) = 9 (0.25) > 8 (0.24) > 6 (0.17) > 7 (0.16), and the mean expected heterozygosity from highest to least was: Group 6 (0.6490) > 2 (0.6449) > 4 (0.6423) > 3 (0.6413) > 1 (0.5508) > 8 (0.5149) > 7 (0.4544) > 5 (0.4269) > 9 (0.4082). Compared to the other combinations, the expected heterozygosity was higher in Groups 2, 3, 4 and 6, indicating their higher genetic diversity. Among them, the average gene heterozygosity of Groups 2 and 4 was relatively high. This meant that the rich genetic polymorphism in the two groups could be further exploited within breeding groups in order to improve uniformity and production performance, which is similar to the results of previous studies (Lin and Luo, 2003; Zhou *et al*., 2004; Liao *et al*., 2005; Ma *et al*., 2007). Polymorphic information content is a good indicator of the degree of polymorphism. Polymorphic information content in the present study for each group of the five microsatellite loci was between 0.34 and 0.58. Generally, when PIC > 0.5, it indicates a high level of polymorphism at that locus. When PIC ranges between 0.25 and 0.5, the locus reveals moderate polymorphism and when PIC is < 0.25, the locus reveals low polymorphism (Moreno *et al*., 2006). Four (Groups 2, 3, 4 and 6) of the nine groups in the five microsatellite primers demonstrated average PIC that was slightly greater than 0.5 and were thus highly polymorphic, indicating that the overall level of PIC of the four groups was high. Others demonstrated moderate polymorphism. While primer CarD121 presented a monomorphic locus in Groups 7 and 9, showing only one kind of length, no variation was found between these two populations, indicating that this locus was unsuitable for these two groups. However, it revealed significant length differences in other crosses. The average PIC of the five microsatellite primers in the nine groups was 0.3401 ~ 0.6017. The primers WL-8 and CarD108 were highly polymorphic, while others showed moderate polymorphism. As seen from the above analysis, the Groups 2, 3, 4, and 6 revealed higher polymorphism compared to the other groups, indicating that the genetic heterozygosity was greater in these groups suggesting their suitability for further breeding.

This study is the first attempt in elucidating genetic profile of the nine mating groups of snakeheads, which provided great significant insights on the evaluation and conservation of new species. Microsatellite markers revealed high level of genetic diversity among crosses of the nine mating groups. The performance of the crosses also revealed presence of heterosis which can be exploited for increasing the performance traits in snakeheads.

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References

Beaumont, R. A. and Hoare, K. 2003. *Biotechnology and genetics in fisheries and aquaculture*. Black-well Publishing, Oxford.

Brookfield, J. F. Y. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol. Ecol*., 5: 453-455.

Chen, Y. F., Liang, R. S., Zhuo, X. L., Wu, X. T. and Zou, J. X. 2012. Isolation and characterisation of *Aeromonas schubertii* from diseased snakehead, *Channa maculata* (Lacepede). *J. Fish Dis.,* 35: 421-430.

David, L., Rajasekaran, P., Fang, J., Hillel, J. and Lavi, U. 2001. Polymorphism in ormamental and common carp strains (*Cyprinus carpio* L.) as revealed by AFLP analysis and a new set of microsatellite markers. *Mol. Genet. Genomics*, 266: 353-362.

Dunham, R. A. 2011. *Aquaculture and fisheries biotechnology genetic approaches*. CABI Publishing, Oxfordshire. 115 pp.

King, T. L. and Johnson, R. L. 2011. Novel tetra-nucleotide microsatellite DNA markers for assessing the evolutionary genetics and demographics of Northern Snakehead (*Channa argus*) invading North America. *Conserv. Genet. Resour.,* 3(1): 1-4.

Li, D. Y., Kang, D. H., Yin, Q. Q., Sun, X. W. and Liang, L. Q. 2007. Microsatellite DNA marker analysis of genetic diversity in wild common carp (*Cyprinus carpio L.*) populations. *J. Genet. Genom.*, 34(11): 984-993.

Li, X., Musikasinthorn, P. and Kumazawa, Y. 2006. Molecular phylogenetic analyses of snakeheads (Perciformes: Channidae) using mitochondrial DNA sequences. *Ichthyol. Res.,* 53(2): 148.

Liao, X. L., Yu, X. M., Tan, D. Q. and Tong, J. G. 2005. Microsatellite DNA analysis of genetic diversity of grass carp in Yangtze rriver system. *Acta Hydrobiol. Sin.,* 29(2): 113-118.

Lin, K. D. and Luo, C. 2003. Preliminary study on applicability of microsatellite primers developed from common carp for genomic analysis of grass carp. *Acta Laser Biol. Sin.,* 12(2): 121-127.

Ma, H., Yue, Y., Yu, Y., Guo, J. and Wang, H. 2007. Applicability of microsatellite primers developed from carp in *Pseudorasbora parva*. *Acta Hydrobiol. Sin.,* 31(2): 278-281.

Ma, T. W., Xie, C. X. and Gong, S. J. 1999. Studies on population dynamics and maximum sustainable yield of Chinese snakehead *Channa argus*, in the Liangzi Lake. *Acta Hydrobiol. Sin.,* 23(6): 591-598.

Moreno, V. R., Grisolia, A. B., Campagnari, F., Milazzotto, M., Adania, C. H., Garcia, J. F. and Souza, E. B. 2006. Genetic variability of *Herpailurus yagouaroundi*, *Puman concolor* and *Panthera onca* (Mammalia, Felidae) studied using Felis catus microsatellites. *Genet. Mol. Biol.,* 29(2): 290-292.

Muths, D., Grewe, P., Jean, C. and Bourjea, J. 2009. Genetic population structure of the swordfish (*Xiphias gladius*) in the south-west Indian Ocean: Sex-biased differentiation, congruency between markers and its incidence in a way of stock assessment. *Fish. Res.,* 97(3): 263-269.

Nie, G. X., Fu, Y. R., Zhang, J. and Wei, X. J. 2002. The analysis of muscle nutrients of *Channa argus*. *Freshw. Fish.,* 32(2): 46-47.

O'Connell, M. and Wright, J. M. 1997. Microsatellite DNA in fishes. *Rev. Fish Biol. Fish.*, 7: 331-363.

Peng, S. M., Shi, Z. H., Wang, W., Zhao, F. and Zhang, H. 2009. Genetic diversity of silver pomfret (*Pampus argenteus*) populations from the China Sea based on mitochondrial DNA control region sequences. *Biochem. Syst. Ecol.,* 37: 626-632.

Raymond, M. and Rousset, F. 1995. GENEPOP (versionb1.2): population genetic software for exact tests and ecumenicism. *Heredity*, 86: 248-249.

Sekar, M., Absar, A., Suresh, E., Srinivasa, R. P., Kathirvelpandian, A., Aparna, C. and Gopal, K. 2014. Genetic diversity among three Indian populations of black tiger shrimp (*Penaeus monodon* Fabricious, 1798) using microsatellite DNA markers. *Indian J. Fish.,* 61(3): 45-51.

Shikano, T. and Taniguchi, N. 2002. Relationships between genetic variation measured by microsatellite DNA markers and a fitness-related trait in the guppy (*Poecilia reticulata*). *Aquaculture*, 209 (1-4): 77-90.

Wen, X. X. and Sun, X. W. 2010. Isolation and screening of microsatellite markers from Argus snakehead fish *Channa argus*. *J. Dalian Ocean Univ.*, 26(3): 260-264.

Zane, L., Bargelloni, L. and Patamello, T. 2002. Strategies for mierosatellite isolation: a review. *Mol. Ecol.*, 11(1): 11-16.

Zeng, Y. D., Wang, C., Zhou, A. G. and Zou, J. X. 2013. Comparative study on the blood physiological and biochemical indexes of snakehead with nine mating groups. *Freshw. Fish*., 43(04): 20-24.

Zhang, J. W., Tu, N. and Qi, X. X. 2004. The artificial propagation and fry rearing of *Channa argus. Jiangxi Fish. Sci. Technol.,* 1: 32-33.

Zhou, J., Wu, Q., Wang, Z. and Ye, Y. 2004. Genetic variation analysis within and among six varieties of common carp (*Cyprinus carpio L.*) in China using microsatellite markers. *Russian J. Genet.,* 40(10): 1144-1148.

Zhu, L., Xie, N., Zhu, S. R., Guo, S. R., Feng, X. Y. and Li, Y. S. 2011. Progress in crossbreeding of *Channa argus* and *Channa maculata* and biological study on their first filial generation. *Hunan Agr. Sci.,* 7: 134-136.

Zhuo, X. L., Liang, R. S., Chen, Y. F., Huang, G. J., Yu, D. H. and Zou, J. X. 2012. Genetic characterisation of northern snakehead (*Channa argus*) populations in China using microsatellite markers. *Biochem. Syst. Ecol.,* 43: 25-31.

Zou, J. X. and Zhou, X. L. 2011. The breeding and its industrial development overview of hybrid snakehead. *Curr. Fish.,* 1: 54-57.

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