

Development of SNP parentage assignment techniques in the yellowfin seabream *Acanthopagrus latus*

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Abstract

Acanthopagrus latus is an essential aquaculture species on the south coast of China. However, there is a lack of systematic breeding of *A. latus*, which considerably limits the sustainable development of *A. latus*. As a result, genetic improvements are urgently needed to breed new strains of *A. latus* with rapid growth and strong resistance to disease. During selective breeding, it is necessary to estimate the genetic parameters of the target trait, which in turn depends on an accurate disentangled pedigree for the selective population. Therefore, it is necessary to establish the parentage assignment technique for *A. latus*. In this study, 95 individuals selected from their parents and their 14 families were used as experimental material. SNPs were developed by genome re-sequencing, and highly polymorphic SNPs were screened on the basis of optimized filtering parameters. A total of 14 392 738 SNPs were discovered and 205 SNPs were selected for parentage assignment using the CERVUS software. In the model where the gender of the parents is known, the assignment success rate is 98.61% for the male parent, 97.22% for the female parent, and 95.83% for the parent pair. In the model where the gender of the parents is unknown, the assignment success rate is 100% for a single parent and 90.28% for the parent pair. The results of this study were expected to serve as a reference for the breeding of new varieties of *A. latus*.

Key words: *Acanthopagrus latus*, parentage assignment, SNP, Genome re-sequencing

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1 Introduction

The yellowfin seabream *Acanthopagrus latus* is a member of the genus *Acanthopagrus* in the family Sparidae, in the order Perciformes. *Acanthopagrus latus* is being cultivated on a large scale in the coastal areas of the Fujian and Guangdong Provinces of China, bringing considerable economic benefits to local villagers every year (Zheng et al., 2023). However, there is a lack of systematic breeding of *A. latus*, which considerably limits sustainable development of *A. latus* (Zhu et al., 2020; Zheng et al., 2023).

Selective breeding has been shown to be an effective method of breeding improved varieties. However, Gjerdem et al. (2012) estimated that only 10% of aquaculture animals in the world were based on genetically improved populations. An influential reason is the difficulty of obtaining pedigree information for aquatic animals when formulating breeding programs. For example, newly hatched fry are only a few centimetres in size, making it difficult to perform regular physical tagging. Pedigree information is an essential basis for the formulation of breeding programs, evaluation of population genetic parameters, and breeding value (Vandeputte and Haffray, 2014). However, a complete and accurate record of pedigree information has not been established for most Chinese aquaculture varieties. Incorrect pedigree information will reduce the accuracy of genetic evaluation, affect

the progress of breeding original varieties, and cause economic losses to the aquaculture industry (Houston et al., 2020). In view of this, it is of great importance to carry out studies on the analysis of the pedigree of aquatic animals.

In the field of parentage assignment, microsatellites have been the dominant approach for some time. High heterozygosity and abundant polymorphism allow it to assign offspring to the correct parents and establish accurate pedigree relationships. In the early 1990s, microsatellite research entered a golden period of development (Flanagan and Jones, 2019). Scientific researchers have successively developed microsatellite markers for a large number of species, and parentage assignment techniques based on microsatellite markers have gradually matured.

With the development of high-throughput technologies and advances in SNP typing methods, population geneticists and breeders have shifted their goals to more potential SNP markers. Compared to microsatellites, SNP has some advantages, including its suitability for high-throughput genotyping, low genotyping error rate, and the ability to merge and standardize SNP data from different sources (Yue and Xia, 2014). Therefore, more and more aquatic animals estimate pedigree information using SNP markers. For example, in *Cyprinus carpio*, Xu et al. (2017) developed a 48-SNP array based on the Fluidigm genotyping plat-

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form, and successfully assigned 94% of the offspring to the most-likely parental pair, which was consistent with actual pedigree records. To evaluate the highly efficient alternative of the SNP-haplotypes approach for microsatellite loci in inferring parentage, García-Fernández et al. (2018) screened 164 SNPs from gilthead sea bream *Sparus aurata* using direct sequencing as the final panel and genotyped 58 individuals using iPLEX Gold MassARRAY technology. The results showed that SNP-haplotypes resolved the parentage assignment with an accuracy of more than 99%, resulting in 10.5% and 3.5% better accuracy than the set of 58 SNPs and 9 microsatellite loci, respectively. Zhao et al. (2018) generated 58 450 genome-wide SNPs for Florida bass (*Micropterus floridanus*) using a cost-effective genotyping-by-sequencing method and a total of 58 SNPs were shown to assign parents to offspring with 100% accuracy, irrespective of sex and with the presence of full-sib relationships. In addition, the accuracy and assignment power of the SNP panel are better than those of the ten microsatellites genotyped on the same parent and progeny. In Atlantic salmon (*Salmo salar*), a total of 86 468 SNPs were identified from RAD sequencing libraries. Following the application of quality control filters and stringent selection criteria, a panel comprising 94 SNPs balanced across the salmon genome were identified, providing 100% assignment accuracy in known pedigrees (Holman et al., 2017). Hauser et al. (2011) evaluated the assignment power of 80 SNPs ($H_e=0.30$, 80 independent alleles) and 11 microsatellites ($H_e=0.85$, 192 independent alleles) in a wild population of about 400 sockeye salmon (*Oncorhynchus nerka*). The results indicate that assignment success is higher for SNPs than for microsatellites, especially for the parent pair, regardless of the method used. Liu et al. (2016) developed a panel of 95 SNPs for parentage assignment in rainbow trout (*Oncorhynchus mykiss*), and was used to genotype 499 fish with known pedigrees. After analysis, when all 95 SNPs were used, the parentage assignment matches perfectly with the known pedigree. For the data, no parentage assignment using SNP markers was reported in *A. latus*.

In this experiment, 95 individuals of *A. latus*, selected from parents and their families, were used as experimental material. SNP markers with high polymorphisms were developed by genome re-sequencing. The parentage assignment technology of *A. latus* was established based on SNP markers. The results of this study are expected to serve as a reference for the breeding of new varieties and for the evaluation of the effects of *A. latus* enhancement and release.

2 Materials and methods

2.1 Animals

In September 2020, 14 families of *A. latus* were built at Dajing Farm in Longhai City, Fujian Province, China. 95 individuals selected from their parents and their families were used as experimental material (Table 1). The pedigree of each family was recorded.

2.2 SNP discovery and screening for high polymorphism markers

Genomic DNA was extracted from the muscle or fin of *A. latus* using the Genome DNA Extraction Kit (Vazyme, China) according to the manufacturer's instructions. DNA quality was detected using the Agilent 5400 fragment analysis system.

The fAcaLat1.1 (GenBank assembly access: GCA_904848185.1) was used as the reference genome of *A. latus* in this study. The total length of the assembly sequence is 685 Mb, including 215 contigs and 14.88 Mb for contig N50. FastQC was used to assess the basic quality of the sequenced reads and to determine whether

Table 1. Parental combinations and number of samples per family

Family Id	Female parent	Male parent	Number of offspring taken
J01	d01	s01	4
J05	d05	s02	5
J06	d06	s02	5
J09	d09	s03	3
J10	d10	s04	6
J11	d11	s04	4
J17	d17	s07	4
J18	d18	s07	7
J19	d19	s07	5
J20	d20	s08	6
J23	d23	s09	6
J25	d25	s09	5
J26	d26	s10	6
J30	d30	s11	6
Total	14	9	72

er the primer adapters were contaminated. Burrows-Wheeler-Alignment Tool - maximal exact matches (BWA-MEM) was used to compare the reads of each sample with the reference genome. SAMtools was used to filter the unpaired reads in the output file and to avoid invalid tags in further analysis (Li and Durbin, 2009). To obtain high-quality BAM files, all duplicate reads were labeled with Picard software. The GATK 4.0 genome analysis toolkit (Genome Analysis Toolkit) was used for mutation, detection a VCF file was obtained containing all the information from the mutation site.

To obtain a highly polymorphic SNP, the filter parameters were set as follows. (1) It must be a biallelic site. (2) The missing ratio is less than 20%. (3) The minor allele frequency (MAF) is greater than 0.15. (4) The genotype quality is greater than 30. (5) SNPs without polymorphisms in the parent pool are filtered out. (6) SNPs with no polymorphisms in the offspring pool are filtered out. (7) We filter out anomalous loci that do not obey Mendel's law of heredity. (8) Sites that are not on the 24 chromosomes of *A. latus* are filtered out. (9) Sites that do not conform to the Hardy-Weinberg equilibrium are filtered out. Additionally, the aforementioned genome was used for the VCF annotation after QC filtering in an off-line mode to explore the SNP distribution.

2.3 Assignment of parents according to SNP markers

In order to find the best SNP combination for parentage assignment in *A. latus*, i.e., the success rate of parentage assignment is more than 90%, and the number of required SNP was as small as possible, 334 polymorphism SNPs were classified from high to low according to the expected heterozygosity (H_e), and divided into five groups. The corresponding SNP number of five groups was 46 ($H_e > 0.4$), 87 ($H_e > 0.35$), 146 ($H_e > 0.3$), 205 ($H_e > 0.25$), and 334 ($H_e > 0.2$). Parentage assignment was analyzed using CERVUS 3.0.3 software (Kalinowski et al., 2007). The observed heterozygosity, expected heterozygosity and polymorphism information content of the optimal SNP combination were calculated at 95% confidence. The parameters for parentage assignment were set as follows: 10 000 simulated offspring, 99% candidate parent detection rate, 84.36% mean percentage of loci typed, 10% typing error rate, and confidence levels set to 80% and 95%.

The results of the parentage assignment based on SNP markers from the CERVUS software were compared with real pedigree records to determine the success rate of the parentage assignment.

3 Results

3.1 SNP discovery and screening for high polymorphism markers

All qualified samples were sequenced using the dual-terminal 150-bp mode in the Illumina NovaSeq sequencing platform. Altogether, 423.8 Gb of clean data were obtained, and 4.461 Gb of clean data were generated on average for each sample. The Q30 of all samples exceeded 90.25% and the GC content ranged from 42.21% to 43.22% with normal distribution.

The clean data was compared with the reference genome using the BWA software. The size of the reference genome is 685 144 197 bp. The mapping rate ranged from 97.75% to 99.41% for all samples. The average coverage depth of the reference genome (excluding the N region) was 4.24–5.67 \times , and coverage with 1 \times (at least one base coverage) is more than 96.25%. The mapping results are normal and can be used for subsequent mutation detection and correlation analysis.

The SNPs were mined from the 95 *A. latus* resequencing data using the Joint Calling software. Collectively, 14 392 738 SNPs were obtained (Table 2). SNPs are mainly distributed in Intronic, Intergenic, and Others, with 7 161 298 (49.76%), 3 836 766 (26.66%) and 2 040 243 (14.18%), respectively. The remaining SNPs are located in the exonic, splicing, upstream, downstream, and upstream/downstream regions.

A total of 334 SNPs were obtained after optimizing the filter parameters. Observed heterozygosity, expected heterozygosity, and polymorphism information content were calculated using CERVUS3.0.3. The results showed that, with 95% confidence, the average observed heterozygosity for 334 SNPs was 0.56, the average expected heterozygosity was 0.39, and the polymorphism information content was 0.31. These SNPs can be used in the analysis of parentage assignment.

3.2 Analysis of parentage assignment based on SNP markers

In the model where the gender of the parent is known, the identification results for the 72 offspring are shown in Fig. 1. When there were 46 SNPs, the identification success rate was over 65% for the male parent, 47.2% for the female parent and 37.5% for the parent pair. As the number of SNPs increased, the identification success rate also increased. When the number of SNPs was 146, the identification success rate was more than 90% for male, female and parent pair. When the number of SNPs reached 205, the identification success rate exceeded 95% for the male parent, female parent and the parent pair. When the number of SNPs was increased to 334, the identification success rate

was 100% for all of them.

In the model where the gender of the parents is unknown, only the identification success rates of single and parent pair are calculated. Due to the lack of gender information for male parent and female parent, the candidate parents provided by CERVUS can be either male parent or female parent. The results of the parentage assignment for 46, 87, 146, 205 and 334 SNPs are shown in Fig. 2. The success rate of parentage assignment gradually increased with increasing SNP number at 95% confidence level. When the number of SNPs reached 87, the identification success rate for a single parent was more than 90%, and a minimum of 205 SNPs were required to achieve the same level of success for the parent pair.

3.3 Optimal SNP marker selection

The results of the parentage assignment for the five sets of SNPs are shown in Table 3. Both models showed a positive correlation between the identification success rate and the number of SNPs used. The success rate of identification using the same number of SNPs in the model with unknown gender was lower compared to the model with known parent gender, suggesting

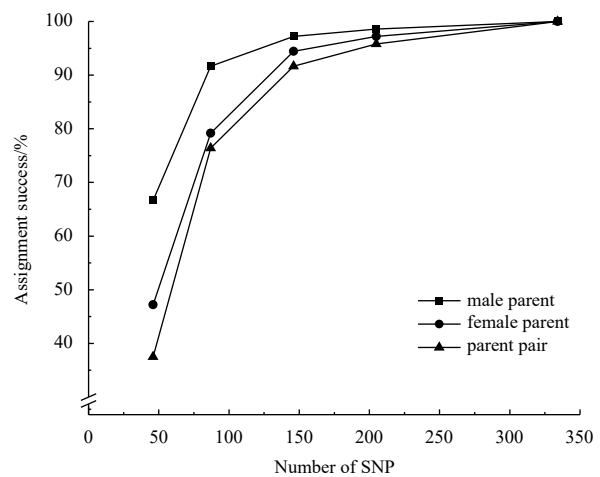


Fig. 1. Success rates of assignment for different numbers of SNPs at the 95% confidence level for the model where the gender of the parents is known.

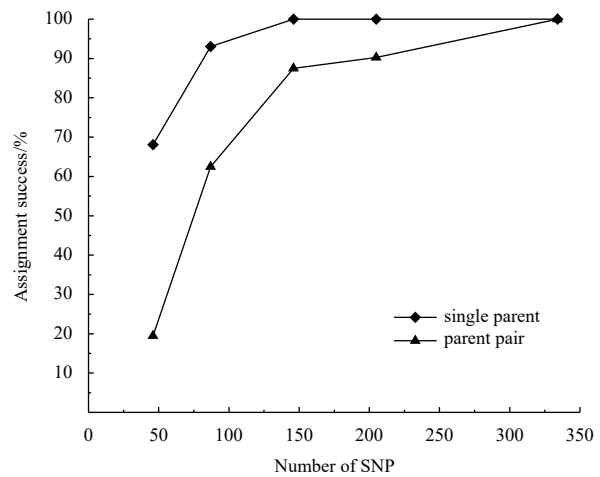


Fig. 2. Success rates of assignment for different numbers of SNPs at the 95% confidence level for the model where the gender of the parents is unknown.

Table 2. SNP annotation information statistics in *A. latus*

Category	Number of SNP	Proportion of total /%
Exonic	579 057	4.02
Exonic Stop gain	2 310	0.02
Exonic Stop loss	247	0.00
Exonic Synonymous	337 298	2.34
Exonic Non-synonymous	239 202	1.66
Intronic	7 161 298	49.76
Splicing	1 915	0.01
Upstream	349 101	2.43
Downstream	342 049	2.38
Upstream/Downstream	82 478	0.57
Intergenic	3 836 766	26.66
Others	2 040 243	14.18
Total	14 392 738	-

Table 3. Parentage assignment results of five sets of SNPs (with 95 % confidence)

Number of SNP	Model of known parental gender			Model of unknown parental gender	
	Assignment success rates of male parent	Assignment success rates of female parent	Assignment success rates of parent pair	Assignment success rates of single parent	Assignment success rates of parent pair
46	66.67%	47.22%	37.50%	68.06%	19.44%
87	91.67%	79.17%	76.39%	93.06%	62.50%
146	97.22%	94.44%	91.67%	100%	87.50%
205	98.61%	97.22%	95.83%	100%	90.28%
334	100%	100%	100%	100%	100%

Notes: Grey shading indicates the best combination of markers.

that the absence of information about the gender of the parents negatively affects the identification of the parents and offspring.

In general, 205 SNP markers were ideal for the identification of parent-offspring of *A. latus*. On the one hand, the number of SNP required was relatively minor and, on the other hand, the identification success rate was strong enough (more than 90%).

4 Discussion

In this study, 205 polymorphic SNP markers were ultimately selected as the optimal combination for the assignment of *A. latus* parentage. The results of parentage assignment under both models showed that the identification success rate increases with the number of SNP markers, confirming that the number of SNPs can compensate for the deficiency in the polymorphic information content of the SNPs. Under the model where the gender of the parents is known, the difficulty of identifying a parent pair is highest, followed by the female parent and then the male parent. Under the model where the gender of the parents is unknown, the difficulty of identifying a parent pair is also higher than that of a single parent. Compared to two models, the success rate for single parent identification is higher in the model where the gender of the parents is unknown than in the model where the gender of the parents is known. This may be due to the increased probability of matching the correct parent due to the absence of gender differentiation under the model where the gender of the parents is unknown (Slate et al., 2000; Kalinowski et al., 2007).

Based on data simulation, Anderson and Garza (2006) predicted that 60–100 SNPs would be sufficient for parentage assignment. This conjecture has also been confirmed in several species. For example, Hauser et al. (2011) successfully carried out parentage assignment in wild sockeye salmon population using 80 SNPs. In *Oncorhynchus mykiss*, 95 SNPs can be used to complete parentage assignment (Abadía-Cardoso et al., 2013; Steele et al., 2013). Tokarska et al. (2009) demonstrated that 50–60 SNPs with the highest heterozygosity were sufficient for parent-offspring identification of the bison. In pigs, 60 SNPs or 80 SNPs were able to match the correct male parent from a large number of candidate boars with 100% accuracy (Harlizius et al., 2011). Similarly, Weinman et al. (2015) found that about 60 SNPs with an average heterozygosity of 0.41 could be used for phylo-genetic identification in bird populations. In this study, 205 SNPs were needed to achieve a success rate of identification of more than 95% when the gender of both parents is known.

In general, the success rate of parentage assignment is influenced by numerous aspects, such as the test population, the number of SNP markers, the content of polymorphic information, the accuracy of the genotyping and the number of parents (Marshall et al., 1998; Walling et al., 2010; Vandeputte and Haf-fray, 2014; Yue and Xia, 2014). In this study, families were constructed using the same batch of fish, for which parent-parent relationships are close and therefore difficult to identify. In addition,

many of the families were half-siblings, and it was more difficult to identify the parents in these families than in full-sibling families. For the above two reasons, the number of SNPs used in this study is relatively large.

5 Conclusions

In this study, genome-wide resequencing was used to develop polymorphic SNP markers, and the parentage assignment technique for *A. latus* is well established based on the constructed family material. The results showed that using 205 SNPs markers, in the model where the gender of the parents was known, the assignment success rate for the male parent, the female parent and the parent pair was greater than 95%; in the model where the gender of the parents was unknown, the assignment success rate was 100% for single parents and 90.28% for the parent pair.

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