Biochemical Systematics and Ecology 66 (2016) 326–330

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Development and characterization of 26 polymorphic microsatellite markers in *Lateolabrax maculatus* and cross-species amplification for the phylogenetically related taxa

Hao-Ran Zhang, Su-Fang Niu, Ren-Xie Wu*, Yun Zhai, Le-Tian Tian

College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, PR China

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 13 May 2016
Accepted 14 May 2016
Available online 26 May 2016

Keywords:
Lateolabrax maculatus
Polynucleotide-repeat microsatellites
The high-throughput sequencing technology
Cross-amplification

ABSTRACT

The spotted sea bass *Lateolabrax maculatus*, a commercially important species of fishery resource in the Northwestern Pacific, has become a popular cultured fish species in China. Although the microsatellite markers of *L. maculatus* have been isolated, they mainly focused on dinucleotide-repeat loci. In this study, we developed 26 polynucleotide-repeat microsatellite loci for *L. maculatus* through high-throughput DNA sequencing technology. The number of alleles per locus ranged from 3 to 19. Observed and expected heterozygosities varied from 0.188 to 0.906 and 0.175 to 0.938, respectively. One locus showed significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction. These 26 novel microsatellite markers will serve as useful tools which benefit population genetics studies and conservation management of *L. maculatus*. In addition, cross-species amplification tests showed that the transferability of 6 loci was positive across 10 species of two phylogenetically related families Latidae and Serranidae, and 3 loci were successful amplified in at least 5 species. The transferable loci would be helpful for the detection of genetic relationship and divergence between the tested taxa.

1. Introduction

Lateolabrax is a genus of commercially important fishes in the Northwestern Pacific, belonging to the family Moronidae (Teleostei, Percoidei) (Nelson, 2006), which contains three species *Lateolabrax maculatus, Lateolabrax japonicus* and *Lateolabrax latus* (Katayama, 1957; Yokogawa and Seki, 1995). Among them, the most popular species is *L. maculatus*, with a wide distribution in Pacific Asia including the borders of Vietnam, China and Korea (Yokogawa and Seki, 1995). However, its natural resource has been significantly decreasing over the last 30 years due to overfishing (Jiang et al., 2009; An et al., 2013). Moreover, the large scale aquaculture and resource enhancement of *L. maculatus* in China may have significant effects on its population genetic (Taniguchi, 2003; Niu et al., 2015). Thus, it is required to investigate the genetic background and make an effective conservation strategy for *L. maculatus*.

As a co-dominant molecular marker, microsatellite has shown remarkable value for population genetics, stock discrimination, genome mapping and parentage studies on account of the high levels of polymorphism (O'Connell and Wright, 1997; Shao et al., 2008). The majority of microsatellites isolated from *L. maculatus* and its closely related species *L. japonicus* were

* Corresponding author.
E-mail address: wurenxie@163.com (R.-X. Wu).

http://dx.doi.org/10.1016/j.bse.2016.05.008
0305-1978/

dinucleotide-repeat loci (Shao et al., 2009; Zhao et al., 2011). However, dinucleotide-repeat microsatellites often tend to produce stutter bands affecting the efficiency and accuracy of allele assignment (Hearne et al., 1992; Diwan and Cregan, 1997). Therefore, development of polynucleotide-repeat microsatellite markers is urgently needed for population genetic studies and resource conservation of *L. maculatus*.

In the present study, we developed 26 novel polynucleotide-repeat microsatellites (including tetra-, penta- and hexa-nucleotide repeats) for *L. maculatus* through high-throughput sequencing technology. Furthermore, we tested the cross-species amplification of these markers in 10 phylogenetically related species from two families Latidae and Serranidae. These markers may serve as valuable tools to conduct genetic resource and parentage studies for *L. maculatus*, and also to unveil phylogenetic problems for its related taxa.

2. Materials and methods

2.1. DNA sequencing and microsatellite discovery

One specimen of *L. maculatus* was collected from Maoming Coast of Guangdong Province, the South China Sea, and muscular tissue was preserved in 95% alcohol for DNA extraction. Extracted DNA was sequenced through high-throughput DNA sequencing technology on Illumina Hiseq 2000 System (Illumina Inc., San Diego, CA). This sequencing run yielded over 132.78 M reads, with a sequencing depth of 20.55×. The potential microsatellite loci were searched for simple sequence repeats (SSRs) with lssr_finder, using a minimum search criterion of 22 bp. About 200 loci were selected randomly, of which 54 with enough flanking sequence were used for primer design using Primer Premier 5.0 (Premier Biosoft International).

2.2. SSR markers screening

Each pair of primers was tested on 32 specimens of *L. maculatus* collected from Zhanjiang Harbor of Guangdong Province, the South China Sea. Total genomic DNA was extracted from muscle according to Sambrook and Russell (1989). The PCR reactions (15 μL) contained 1 × PCR buffer [100 mM Tris-HCl (pH 8.8, 25 °C), 500 mM KCl, 0.8% (v/v) Nonidet P40, 15 mM $MgCl_2$], 1 μL of extracted DNA (10–50 ng), 0.3 μL each primer (10 μM), 0.3 μL dNTP mixture (10 mM), and 1.7 U *Taq* DNA polymerase (Sangon Biotech Co., Shanghai). After initial denaturation at 95 °C for 4 min, a touchdown PCR protocol was followed for 25 cycles of 95 °C for 30 s, 62–52 °C for 30s (decreasing 1 °C per cycle for the first 10 cycles), and 72 °C for 30 s. Final extension was at 72 °C for 10 min. By electrophoresis on an 8% polyacrylamide gel, 26 of 54 loci were successfully amplified and shown to be polymorphic. These 26 microsatellite loci sequences were submitted to the NCBI (GenBank accession numbers: KU596542–KU596567, Table 1).

2.3. PCR amplification and genotyping

An M13 tail (5′-AGGGTTTTCCCAGTCACG-3′ or 5′-GAGCGGATAACAATTTCACAC- 3′) was added to the 5'end of each forward primer of these polymorphic loci. The M13 universal primer with the same sequence to the M13 tail was labeled (Generay Biotech Co., Shanghai) with FAM or HEX at its 5′ end. Three-primer PCR amplification conditions of above 32 specimens were slightly revised on the basis of the protocol described by Boutin-Ganache et al. (2001) and Schuelke (2000). Briefly, the PCR reactions (15 μL) containing 1 × PCR buffer, 1 μL of extracted DNA (10–50 ng), 0.3 μL forward primer (10 μM) with M13 tail, 0.1 μL labeled M13 universal primer (10 μM), 0.3 μL reverse primer (10 μM), 0.3 μL dNTP mixture (10 mM), and 1.7 U *Taq* DNA polymerase. PCR programs were started with a denaturation step for 5 min at 95 °C, followed by 25 cycles at 95 °C for 30 s, 30 s at the locus-specific annealing temperature (50–60 °C), 30 s at 72 °C, and then given a final elongation for 10 min at 72 °C. All sets of PCR included a negative control reaction tube in which all reagents were included, except the template DNA. The genotyping of PCR products were performed in Shanghai Generay Biotech Company using the Applied Biosystems 3730 DNA Analyzer and GeneMapper® software version 4.0.

2.4. Microsatellite cross-species amplification

Cross-species amplifications of all microsatellite loci were tested in 10 species from two families (Table 2) using the same amplification conditions described above. The PCR products were visualized on 8% polyacrylamide gel. The loci with at least one band of the expected size were considered transferable.

2.5. Data analysis

The number of alleles (N_a), polymorphism information content (PIC), and observed (H_o) and expected (H_e) heterozygosities for each locus were calculated by the program Cervus 3.0.7 (Kalinowski et al., 2007). Hardy-Weinberg equilibrium (HWE) was estimated using GenePop 4.3 (Rousset, 2008).