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# Isolation and characterization of novel microsatellite loci in *Nibea albiflora*

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**ABSTRACT.** Eleven novel microsatellite loci were isolated from a $(CA)_{10}$-enriched genomic DNA library of *Nibea albiflora*. The characteristics of these microsatellites were determined in a sample of 48 *N. albiflora* individuals. The number of alleles at the 11 microsatellite loci ranged from 5 to 25, with an average of 13.5 per locus. The observed and expected heterozygosities varied from 0.583 to 0.917 and from 0.568 to 0.964, respectively. Eight of the 11 microsatellite loci conformed to the Hardy-Weinberg equilibrium. No significant linkage disequilibrium was found among all 11 loci. These polymorphic microsatellites will be useful for population genetic analyses of *N. albiflora*.

**Key words:** *Nibea albiflora*; Microsatellite; Polymorphism

 

## INTRODUCTION

*Nibea albiflora* is a coastal fish belonging to the family Sciaenidae in the order Perciformes (Zhu et al., 1963). This species is mainly distributed from the South China Sea to the coastal waters of Japan and Korea (Takita, 1974). *N. albiflora* is an economically important fisheries and aquaculture species in China. However, in recent years, the wild resource of *N. albiflora* has decreased sharply as a result of overfishing and water pollution. For developing rational strategies to protect the genetic resources and to utilize the valuable resources sustainably, it is critical to analyze the genetic variation in *N. albiflora* to reveal its genetic background. To date, studies on the population structure of *N. albiflora* have only been carried out using amplified fragment length polymorphism and mitochondrial DNA analyses (Han et al., 2006, 2008).

Microsatellite markers have been widely used in genetics and ecology studies, such as on genetic identification, parentage, and population variability, and are considered one of the best genetic markers (Liu and Cordes, 2004). Previously, 29 microsatellite markers were developed in *N. albiflora* (Xing et al., 2009; Ma et al., 2011). However, these loci were not enough for developing a large-scale population genetic study on *N. albiflora*. To find more species-specific markers with high polymorphism, herein, we constructed DNA-enriched libraries for the dinucleotide microsatellite (CA repeats), and 11 novel polymorphic microsatellite loci were isolated from it.

## MATERIAL AND METHODS

Genomic DNA was extracted from the fin clips of one individual fish by using a previously described method (Yue and Orban, 2005). Microsatellite loci were isolated and cloned according to the protocol described by Yue et al. (2000). A partial genomic DNA library enriched for CA repeats was constructed using the enrichment technique. DNA was digested with *Rsa*I, and the resulting fragments were separated on a 1.0% agarose gel. Fragments of 250 to 1000 bp were excised, purified, and ligated with 20 pmol 21- and 25-mer oligo adaptors (Fischer and Bachmann, 1998). The CA repeats were enriched from the ligated DNA using biotinylated probes $(CA)_{10}$ and streptavidin-coated magnetic beads (Dynal). The magnetic beads-captured DNA containing the microsatellites were eluted. The eluted fragments were amplified using 21-mer as primers and then ligated to pGEM-T-vector (Promega) according to the manufacturer protocol. The ligation products were transformed into XL1-Blue competent cells (Strategene). Colony polymerase chain reaction (PCR) products were sequenced in both the 5' and 3' directions on an ABI3100 sequencer (Applied Biosystems) using a BigDye sequencing kit (Applied Biosystems). Primers were designed for 11 microsatellites in the regions flanking the repeats using the software Primer 3.0 (Rozen and Skaletsky, 2000). One of each primer pair was labeled with FAM, NED, PET, or VIC fluorescent dye.

To characterize the isolated microsatellites, we genotyped 48 individual fish collected from the waters of Zhoushan Coast (122°30′N, 30°08′E) in the East China Sea. PCR amplification was carried out in a 25-µL reaction mixture including 10 pmol each primer set; 100 µM dNTPs; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 1 U *Taq*

 

polymerase (TaKaRa); and 40 ng template DNA. The PCR profile consisted of an initial denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 30 s, annealing temperature for 45 s, and 72°C for 1 min; and finally one cycle of 72°C for 7 min. Following amplification, the PCR products were mixed with 5 µL sequencing dye and heated for 5 min at 95°C. The PCR products were separated by electrophoresis on an ABI3730xl DNA sequencer (Applied Biosystems). The number of alleles and observed ($H_O$) and expected ($H_E$) heterozygosities were calculated using the software Arlequin 3.01 (Excoffier et al., 2005). Conformance of genotypic proportions to Hardy-Weinberg equilibrium was tested using exact tests in the software Genepop 4.1 (Raymond and Rousset, 1995). Probability values were determined based on 10,000 dememorizations, 1000 batches, and 10,000 iterations per batch.

## RESULTS AND DISCUSSION

The characteristics of the 11 primer pairs that achieved successful amplification of a scorable PCR product are present in Table 1. The observed numbers of alleles ranged from 5 to 25, with an average of 13.5 alleles per locus. The $H_O$ values ranged from 0.583 to 0.917 and the $H_E$ values from 0.568 to 0.964. The average $H_O$ and $H_E$ values were 0.754 and 0.832, respectively. Significant deviations from Hardy-Weinberg equilibrium were found at three microsatellite loci (Nibea08, Nibea10, and Nibea11). No evidence of linkage disequilibrium was observed by the linkage disequilibrium test for each pair of loci. In conclusion, these 11 microsatellite markers will be useful for the development of parentage, population genetics, and genome mapping studies in *N. albiflora*.

**Table 1.** Characteristics of 11 microsatellite markers developed for *Nibea albiflora*.

| Locus | Repeat motif | Primer sequence 5'-3' (label) | Ta (°C) | $N_A$ | Allele-size range | $H_O$ | $H_E$ | $P_{HW}$ |
|---|---|---|---|---|---|---|---|---|
| Nibea01 | $(TG)_{10}$ | F: CTTGGTTTTAGGTATCCAGGCTCT (FAM)<br>R: TGCCCACTCACCTGTGTCATT | 55 | 10 | 212-244 | 0.708 | 0.757 | 0.437 |
| Nibea02 | $(CT)_{25}$ | F: ACAGAACACATCCAAACAAAGG(VIC)<br>R: AACCAAAAGAAAGGAGCGAC | 51 | 25 | 207-254 | 0.917 | 0.940 | 0.338 |
| Nibea03 | $(GT)_9$ | F: TGTCACACGCAGGGTAATG(NED)<br>R: TCAGGCAGCCGCAGTAT | 52 | 9 | 157-215 | 0.667 | 0.817 | 0.055 |
| Nibea04 | $(GA)_{15}$ | F: AAGACGGTATGCCAGGGTT(PET)<br>R: GTTCAGGAGCAAAAAAATAAGAGA | 51 | 20 | 228-306 | 0.833 | 0.901 | 0.069 |
| Nibea05 | $(CT)_{10}$ | F: CAAAGGTTAGCCCCAAATCTG(VIC)<br>R: CGCACAATAAGCAATCACATAC | 55 | 6 | 201-219 | 0.750 | 0.716 | 0.910 |
| Nibea06 | $(CA)_{10}$ | F: GCACACCTGACCGTGAACA(NED)<br>R: GAAGAGAACAACCTGGTAATGAACT | 54 | 15 | 180-217 | 0.875 | 0.899 | 0.139 |
| Nibea07 | $(AG)_{13}$ | F: GAAGAAGTGAGAGAAAAGGGG(PET)<br>R: ATTATGTGAAAGTGCTGCGAG | 53 | 8 | 191-207 | 0.833 | 0.840 | 0.926 |
| Nibea08 | $(GACA)_{23}$ | F: AAATGTTTGGCTGTGCTATGA(FAM)<br>R: ATTGTGTGGCTGTTTTGTGTTA | 51 | 22 | 236-306 | 0.667 | 0.964 | 0.000 |
| Nibea09 | $(TC)_{26}$ | F: TGCCTTTTGGAAATCAGCCT (VIC)<br>R: ACATCTCCCTAACAGTCGCATAATA | 51 | 18 | 262-303 | 0.875 | 0.936 | 0.724 |
| Nibea10 | $(AC)_{11}$ | F: TCTTTGCTTTCTCTCGGTGA (NED)<br>R: GGATGTGGTTTTGGTGTGTC | 51 | 10 | 191-203 | 0.583 | 0.816 | 0.003 |
| Nibea11 | $(TG)_{10}$ | F: GAGTGTCCTTGTGTGTTTATTCATCTG(PET)<br>R: AGCACCTGCCGCTCTCTGTA | 55 | 5 | 113-197 | 0.583 | 0.568 | 0.042 |

Ta = annealing temperature; $N_A$ = number of alleles; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; $P_{HW}$ = probability of departure from Hardy-Weinberg equilibrium.

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