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BRIEF NOTE

Development of genome-wide microsatellite resources in a commercially important mussel species (Mytilus chilensis)

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Source description: The Chilean blue mussel (Mytilus chilensis) is one of the most cultivated and marketed marine bivalves of the Mytilus genus. It is one of the top five mollusc species produced worldwide and is widely appreciated for its nutritional value and use in prepared food. In spite of its commercial importance, genetic resources for this species are limited. Here, we describe 16 polymorphic microsatellite markers. Pyrosequencing using a 454 GS Junior instrument yielded 9976 sequences with an average read length of 384 bp (Accession No. SAMN04158627) and allowed for the design of primers for 115 in silico microsatellite loci (Table S1). Eighty-nine microsatellite loci were tested for amplification in M. chilensis (n = 15), 17 of which were validated.

PCR conditions and genotyping: Microsatellite genotyping was performed using the M13-tailed primer method. PCR amplifications were carried out in 15-μl reactions containing 1× manufacturer’s PCR buffer, 1.5–3.2 mM of MgCl₂, 0.2 mM of dNTPs, 0.16 μM of primer reverse, 0.16 μM of fluorescent-labelled universal M13 primer (6-FAM, VIC, NED or PET), 0.04 μM of primer forward with M13 tail, 0.1 U of Taq polymerase (Thermo Scientific®) and 40–60 ng of genomic DNA. Thermal cycling parameters were initial denaturation at 95 °C for 2 min followed by 30 cycles of denaturation at 95 °C for 30 s, primer-specific annealing temperature for 30 s (Table S2), extension at 72 °C for 30 s, then eight additional cycles at 94 °C (30 s), 53 °C (45 s) to bind M13-labelled primer and 72 °C (45 s) with a final extension at 72 °C for 12 min. Amplicons labelled with different fluorescent dyes were pooled in one reaction tube and run on an ABI-3130XL genetic analyser with LIZ GS-500 as an internal size standard (Applied Biosystems®). Alleles were scored with PEAK SCANNER v1.0 (Applied Biosystems®) and checked for possible genotyping errors with MICROCHECKER v2.2.3. Genetic diversity was assessed using CERVUS v3.0.5. Excluding the monomorphic Mch07-Uch marker, observed (Hₒ) and expected (Hₑ) heterozygosities ranged from 0 to 0.60 and from 0.26 to 0.95 respectively (Table S2). The number of alleles by locus (Nₐ) and polymorphism information content (PIC) ranged from 2 to 17 and from 0.062 to 0.915 respectively (Table S2).

We also tested the amplification of these markers in another eight mussel species: Mytilus edulis (n = 16), Mytilus galloprovincialis (n = 16), Mytilus californianus (n = 7), Mytilus trissulus (n = 8), Choromytilus chorus (n = 8), Perumytilus purpuratus (n = 8), Semimytilus algosus (n = 8) and Aulacomya atra (n = 8). The success rate ranged from 13 markers (M. edulis) to zero microsatellites (S. algosus) (Table S3).

We conclude that the markers we have developed show sufficient polymorphism for application in population genetic studies and paternity/maternal exclusion analysis.

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References

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite loci identified in silico within the Mytilus chilensis 454 sequencing.

Table S2 Primer sequences and characteristics for 17 microsatellite loci validated for Mytilus chilensis.

Table S3 Number of alleles detected in the cross-amplification of microsatellite markers derived from the Chilean blue mussel in other mussel species. No amplification was observed for Semimytilus algosus.