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Isolation and characterization of fifteen microsatellite loci for the giant clam *Hippopus hippopus* (family Tridacnidae)

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Abstract

Fifteen polymorphic microsatellite markers were developed for *Hippopus hippopus* in order to assess the effectiveness of population replenishment within MPAs in New Caledonia. Number of alleles varied from 2 to 11 per locus, observed and expected heterozygosities ranged from 0.300 to 0.866 and 0.495 to 0.858 respectively. Significant deviations from HWE were detected in two loci. Cross-amplifications were tested in four other species of Tridacnidae.

Keywords

Hippopus hippopus; Tridacnidae; microsatellite loci; Marine Protected Area; paternity analyses; New Caledonia

Giant clams are an important though declining resource for the Indo-Pacific countries. Because of the overexploitation for its meat and shell, *Hippopus hippopus* (Linné 1758) is already extinct from several island countries and is listed on the IUCN Red List of Threatened Species since 2004. Alerted by recent stock declines in New Caledonia, in agreement and following local communities wish, World Wildlife Fund with the support of the Department of Fisheries and Aquaculture of the Northern Province initiated and coordinated a restocking

event of *Hippopus hippopus* in September 2009 within the co-managed Marine Protected Areas (MPAs) of the North-East Lagoon: Hyabé/LeJao (Pouébo) and Yeega (Hienghène). Microsatellite loci were developed to provide genetic tools for assessing the effectiveness of this replenishment and estimating spatial scales of larvae dispersal of this species from these MPAs.

Approximately 20 ng of genomic DNA was isolated from muscle tissue of one individual conserved in 80% EtOH. Size-selected fragments from genomic DNA were enriched for SSR content by using magnetic streptavidin beads and biotin-labeled CT and GT repeat oligonucleotides. The SSR-enriched library was analyzed on a Roche 454 platform using the GS FLX Titanium reagents. From the 28'526 reads, 1'689 contained a microsatellite insert with a tetra- or a trinucleotide of at least 6 repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 4141 reads and 72 were tested for polymorphism on 8 individuals. Genomic DNA was isolated from mantel biopsies using DNeasy Blood and Tissue Kit (Qiagen). PCR reactions were performed using Type-It Microsatellite (Qiagen) in two distinct multiplexes of 5µl final volume containing 1X Master Mix, 0.5X of Q-solution, 0.1µM of each primer (fluorescent-labeled forward primer 6-FAM, PET, NED or VIC) and 50 to 150ng of DNA template (Table 1). PCRs were conducted in GeneAMP PCR System 9700 (Applied Biosystems) with 5min at 94°C, 28 cycles at 95°C for 30s, 57°C for 90s and 72°C for 30s, and a final step at 60°C for 30min. Fluorescent PCR fragments were visualized on an ABI 3130XL Genetic Analyser (Applied Biosystems) with GS-500-LIZ (Applied Biosystems). Alleles were sized using the program GeneMapper® (Applied Biosystems).

Using GENETIX (Belkhir et al. 2002), between 2 and 11 alleles (mean = 7.54) were observed per locus among 30 individuals from Hyabé/LéJao (Pouébo) MPA, with expected heterozygosity values ranging from 0.495 to 0.858 (Table 1). No significant genotypic linkage disequilibrium among loci was found using GENEPOP (Raymond and Rousset 1995). Significant deviations from Hardy-Weinberg equilibrium were observed at Hiphip_13326 and Hiphip_14220 after Bonferroni corrections. Null alleles were suggested at these two loci by MICRO-CHECKER (Van Oosterhout et al. 2004) and confirmed by the presence of null homozygotes.

Cross-priming was tested on 8 individuals of *Tridacna crocea* (from New Caledonia), *T. maxima* (from La Réunion, Juan de Nova and Glorieuses, Indian Ocean), *T. squamosa* (from Juan de Nova and Glorieuses) and *T. derasa* (from New Caledonia). Using the same PCR conditions as above, but an annealing temperature of 52°C, the test resulted in 12 loci amplifying in *T. crocea*; 5 loci amplifying in *T. maxima*; 2 loci amplifying in *T. squamosa*; and 8 loci amplifying in *T.derasa* (Table 2).

The 15 new microsatellite loci will be helpful tools for connectivity studies on *H. hippopus* populations and to investigate genetic stocks for fisheries management of this endangered species.

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