Microsatellite loci were characterized in walking catfish random clones from a small genomic library using a (GT)$_{15}$ probe. Of 2,841 recombinant plasmids, 173 clones were sequenced and 41 of which contained microsatellites. Most of *Clarias macrocephalus* microsatellites isolated in this study contained dinucleotide core sequence with perfect repeats (61.36%). Primers for DNA amplification using PCR were designed and synthesized for 23 loci. Twelve loci were polymorphic.

Genetic variation of *C. macrocephalus* collected from 5 natural populations in northern Thailand (Nakorn Sawan, Sukhothai, Pisanulok-Bangrakam, Pisanulok-Watbot and Uttaradit) were assessed using five microsatellite loci (*Cma-5*, *Cma-8*, *Cma-13*, *Cma-17* and *Cma-20*). High levels of polymorphism were observed across all populations with an average of 8.4 to 13.6 alleles per locus, 6.0-8.5 average effective number of alleles per locus and average heterozygosity at all loci 0.64 to 0.76. Genotype frequencies of all populations except Nakorn Sawan deviated from Hardy-Weinberg equilibrium. $F_{st}$ value of 0.0637 indicated moderate genetic diversity among populations. A UPGMA dendrogram derived from Nei's unbiased genetic distance divided the populations into 2 groups correspond to geographical distances; Sukhothai-Pisanulok (Bangrakam)-Nakorn Sawan and Pisanulok (Watbot)-Uttaradit.
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