Development of microsatellite markers for the kelp grouper *Epinephelus bruneus* by 454 pyrosequencing and transfer to related species

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ABSTRACT. The kelp or longtooth grouper (*Epinephelus bruneus*), which inhabits Eastern Asia, is the most economically important of 11 grouper species that inhabit the Southern Sea near Jeju Island in Korea. This species is listed as vulnerable by the International Union for the Conservation of Nature and Natural Resources because of a rapid decrease in its resources. We developed microsatellite markers for *E. bruneus* using the pyrosequencing technique for applications in resource management and aquaculture. In addition, we tested the cross-species transferability of the microsatellite markers in four species belonging to the *Epinephelus* genus. Among 66,452 simple sequence repeats, 64 loci containing more than eight CA or TG repeats were randomly selected for primer synthesis; 45 primer sets (75.0%) produced polymerase chain reaction (PCR) products of 100-
300 bp and were selected as candidates. After primary testing with four *E. bruneus* fish, 28 polymorphic loci were selected as the final microsatellite markers, and 23 sets showing clear amplification of polymorphic loci were used to analyze 71 fish. These loci have allele numbers ranging from 2 to 23. Null alleles were detected at three loci, and three loci showed an excess of homozygotes in the Hardy-Weinberg equilibrium test. Of the three species used for cross-species transfer of these markers, *Epinephelus moara* showed the highest transferability (92.9%) and polymorphism (67.9%), followed by *Epinephelus fuscoguttatus* (75.0 and 67.9%, respectively) and *Epinephelus septemfasciatus* (57.1 and 46.4%, respectively). These results suggested that these microsatellite loci should be valuable tools for population genetic studies of the species *Epinephelus*.

**Key words:** Kelp grouper; Pyrosequencing; Cross-species; Transferability