



SPECIES IDENTIFICATION OF HYBRID PUFFERFISH BETWEEN *TAKIFUGU RUBRIPES* AND *TAKIFUGU PORPHYREUS*

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Abstract

Pufferfish contain tetrodotoxin and toxicity varies among species. In Japan, the edible species and the portions are described in 'The Guideline for Eating Pufferfish' issued by the Ministry of Health, Labour, and Welfare. In this guideline, species identification is the most important index for ensuring food safety. However, natural hybrid pufferfish individuals have frequently been collected along Japan's coast in recent years. They are eliminated from the market because of being unidentified species and having unknown toxicity. Difficulties with morphological authentication mean that it is necessary to establish new molecular biological methods to identify parental species of hybrid pufferfish. In this study, we focused on hybrid individuals between *Takifugu rubripes* and *T. porphyreus*. Total cellular DNA was extracted from the muscle of artificial hybrids, parents of artificial hybrids (*T. rubripes* and *T. porphyreus*), and natural hybrids. Maternal species were identified using nucleotide sequences of 16S rRNA and cytochrome *b* regions. Identities of the hybrid individuals whose mothers were *T. rubripes* or *T. porphyreus* were 100% matched with the sequences in the pufferfish database, respectively. Paternal species were identified using a microsatellite marker composed of GAAAG repeats. PCR products derived from *T. rubripes* (194–209 bp) and *T. porphyreus* (125–145 bp) were obtained from all specimens. The results suggested that the method combining use of mitochondrial DNA and the microsatellite marker is applicable to identify hybrids between *T. rubripes* and *T. porphyreus*.

Keywords: Hybrid pufferfish, species identification, microsatellite marker, mitochondrial DNA