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Development of novel microsatellite loci and analyses of genetic diversity in the endangered *Tanakia somjinensis*

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ABSTRACT

Somjin bitterling (*Tanakia somjinensis*), an endemic cyprinid on the Korean Peninsula, is critically endangered with only a few small populations found in limited areas in a single drainage, raising concerns that this species has likely suffered low levels of genetic variability. In the current study, 23 polymorphic microsatellite markers were developed using Illumina paired-end sequencing for quantification of the genetic diversity in this species and to determine their amplification efficiency in other bitterling species. A total of 50 somjin bitterlings collected from two localities were genotyped using these 23 loci. This species showed a remarkably high level of genetic variability, with an average number of alleles per locus of 17.30 and mean observed and expected heterozygosity values of 0.758 and 0.802, respectively. Our relatedness analyses for all pairs of individuals clearly indicated that the two somjin bitterling populations were completely outbred. No signature of drastic demographic decline was detected using our analytical methods. Many of our loci were validated as successfully transferable within and between genera and could potentially be used for genetic and demographic studies in other bitterling species.

1. Introduction

Bitterlings, small cyprinids belonging to the Acheilognathinae, are composed of approximately 70 recorded species and subspecies (Nelson, 2006; Kitamura et al., 2012). The geographic range of bitterlings stretches from West Europe to Southeast Asia (Kim et al., 2014), however the majority of species are found in slow-flowing water systems in East Asia including China, Japan, and Korea (Nelson, 2006; Kitamura, 2007). Bitterlings have an unusual reproductive strategy; a female deposits her eggs into the mantle cavity of freshwater unionid or margaritiferid mussels, and a male (or males) discharge(s) its (their) sperms into the inhalent current of the mussels (Smith et al., 2004). The fertilized eggs grow for approximately 4 weeks inside the mussels before leaving their hosts to begin their independent life in the water (Duyvené de Wit, 1955). Many bitterling

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species represent major components in midstream communities throughout much of their distribution range, however some are currently regarded as threatened and on the brink of extinction (Kubota and Watanabe, 2003; Jeon and Suk, 2014).

Somjin bitterling, *Tanakia somjinensis* (Kim and Kim, 1991), which is endemic to the Korean Peninsula, is classified as 'critically endangered' under the Protection Act of Wild Fauna and Flora by the Ministry of Environment, South Korea. Only a few small populations of somjin bitterling are found in limited areas in the Somjin River, a major river running south through the Korean Peninsula (Kim and Kim, 1991; Yang, 2004), raising concerns that this species has likely suffered low levels of genetic variability and may be more susceptible to environmental disturbance than more widespread species. Given the increasing probability of habitat alteration and extinction due to growing human activities and urbanization in South Korea, comprehensive genetic analyses of this species are necessary to examine the genetic diversity and demographic status. However, no genetic examination of this species has been performed at the population level so far.

In this study, for the first time 23 novel and polymorphic microsatellite markers were developed using Illumina paired-end sequencing specifically for somjin bitterling. Microsatellite loci are inherited in a co-dominant manner, selectively neutral, and often hypervaiable (Abdelkrim et al., 2009), and can therefore provide a suitable solution for evaluating the degree of genetic variability and the pattern of genetic structuring of endangered species with only a small census size throughout the whole distribution. The microsatellite markers developed in this study were used (1) to understand the level of genetic diversity and the pattern of population structure in somjin bitterling, and (2) to determine the efficiency of amplification in other bitterling species.

2. Materials and methods

2.1. Sample collection

Samples of Korean species that were nationally designated as endangered were collected under permission from the local environmental office in the Ministry of Environment, South Korea (protocol 2015-02, -32 and -36). Somjin bitterlings were collected at two locations from the Somjin River (Gwanchon, $N = 25$; Dongbok, $N = 25$; Fig. 1). Based on the collection procedures prescribed in the permission, the tissue samples for population genetic analyses and cross-species amplification tests were obtained by taking a 1 × 1 mm fin clip from the caudal fin of each individual. Following removal of the tissue sample, all individuals were released back to the spots where they were originally collected. Eight other bitterling species belonging to the genera were used in the examinations of cross-species amplification with the markers developed in this study (Fig. 1); *Tanakia koreensis* and *Tanakia lanceolata* from the Tamjin River (South Korea); *Tanakia latimarginata*, *Rhodeus*

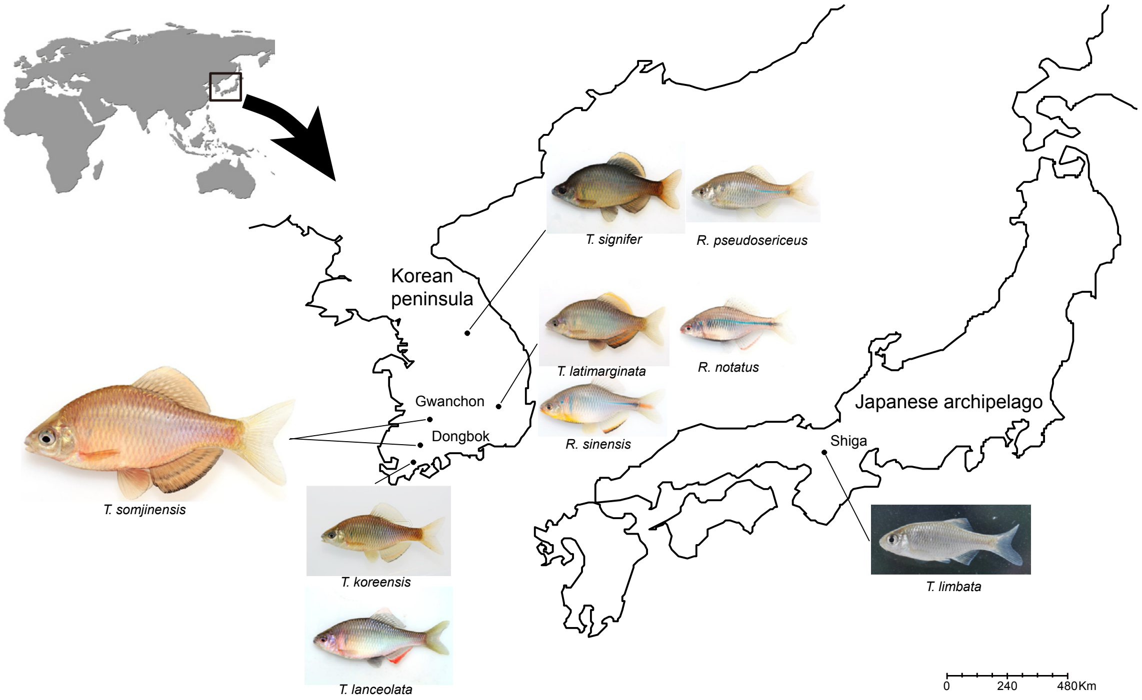

Fig. 1. Locations of the sampling sites in this study. Somjin bitterlings were collected from two locations in the Somjin River, South Korea; Gwanchon (Imsil-gun, Jeollabuk-do) and Dongbok (Hwansun-gun, Jeollanam-do). Sampling locations of eight other bitterling species used in cross-species amplification were also indicated on the map. The collection sites (drainages) are detailed in Materials and methods.