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Genetic Diversity of the Slender Shinner (*Pseudopuntungia tenuicorpa*) and Its Conservational Implications

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ABSTRACT The slender shinner (*Pseudopungtungia tenuicorpa*), a tiny freshwater fish of about 8 to 10 cm belonging to Cyprinidae, is an endangered species found only in the Han and Imjin Rivers on the Korean Peninsula. During the breeding season, this species spawns in nests of *Coreoperca herzi*, a predator of this species, or small crevices on rocks. This unique reproductive ecology can make this species more vulnerable to anthropogenic perturbance that can further limit the places to spawn. Here, mtDNA and microsatellite loci were analyzed to identify the genetic diversity and structure of slender shinners and further to provide the basic data necessary for the conservation planning of this species. A total of 28 polymorphic microsatellite markers were developed using Illumina paired-end sequencing, and 67 slender shinners collected from three localities in the Han River were genotyped using these loci. This species showed a remarkably high level of genetic diversity with mean expected heterozygosity of 0.914 and mean allele number per locus of 27.9, and no signature of drastic demographic decline was detected. As a result of our microsatellite analysis, the genetic structure between the two stems of the Han River, North Han and South Han, was prominent. Such a genetic structure was also evident in the sequence analysis of 14 haplotypes obtained from mtDNA control region. Although slender shinners are only found in very limited areas around the world, the genetic structure indicates that there is a block of gene flow among the populations, which should be reviewed in the future if management and restoration of this species is needed.

Key words: Genetic diversity, slender shinner, *Pseudopuntungia tenuicorpa*, Cyprinidae, conservation genetics

INTRODUCTION

More than 40% of all known fish species in the world are found in freshwater ecosystems, such as rivers, swamps and lakes (Valdez and Mandrekar, 2019), which account for less than 1% of the total surface area on this planet. However, the species diversity of freshwater fish has been disappearing at a faster rate than any other vertebrate species over the recent decades (Harrison *et al*., 2018; Reid *et al*., 2019). To reduce the rate at which species in freshwater ecosystems disappear the primary focus should be on the management of endangered species. Understanding the genetic diversity and population structure of the target species is increasingly being suggested by many scientists as the essential first step in establishing its conservation and management strategies (Waples and Gaggiotti, 2006; Funk *et al*., 2012).

The slender shinner (*Pseudopungtungia tenuicorpa*), a tiny freshwater fish of about 8 to 10 cm belonging to Cyprinidae (Cypriniformes), is a rare species found only in the Han and Imjin Rivers on the Korean Peninsula (Jeon and Choi, 1980; Kim *et al*., 2005). This species is legally designated as an endangered species (Class II) under the Protection Act of Wild Fauna and Flora by the Ministry of Environment, South Korea (NIBR, 2012; Kim *et al*., 2016). This species is mostly observed around well-oxygenated minor tributaries with substrates being composed of gravels and small rocks branched from the main stem

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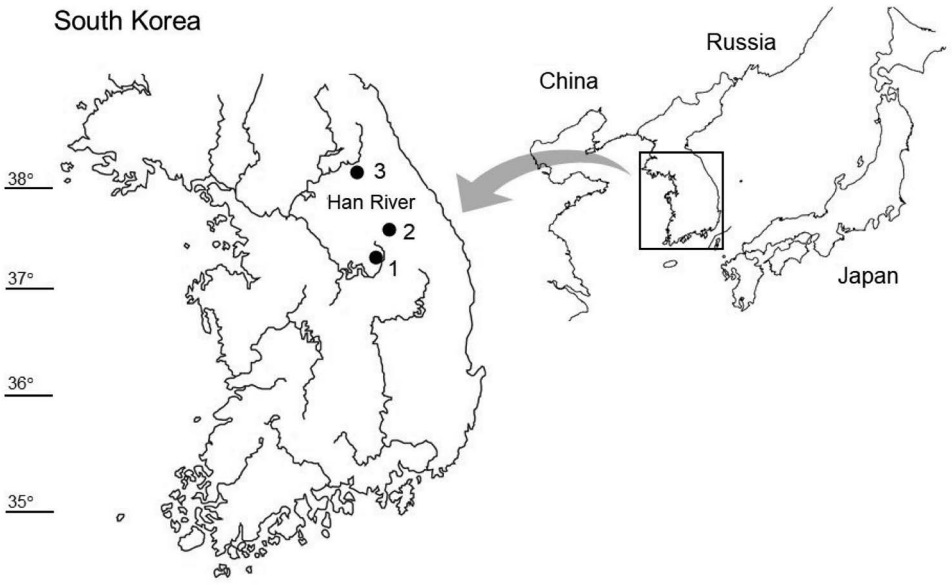

Fig. 1. Locations of the sampling sites in this study. Slender shinners (*Pseudopuntungia tenuicorpa*) were collected from three locations in the Han River, South Korea; 1. Yeongwol (South Han, 37°12′11.4″N 128°25′58.0″E, $N=16$), 2. Jeongseon (South Han, 37°22′04.6″N 128°43′01.3″E, $N=16$) and 3. Hongcheon (North Han, 37°41′51.8″N 127°54′41.8″E, $N=35$).

(Kim *et al*., 2005). It is known that *Pseudopungtungia* species including slender shinners lay eggs in the nest of a paternally caring male of *Coreoperca herzi*, known as a powerful predator in South Korean stream waters (Lee, 2011; Ko *et al*., 2012). When brood parasitism is not sufficiently available, slender shinners may spawn in narrow crevices of rocks (Lee, 2011; Ko *et al*., 2012). This unique reproductive ecology can make this species more vulnerable to anthropogenic perturbance that can further limit the places to spawn.

Genetic research on slender shinners has recently been conducted. In a molecular phylogenetic study using nuclear loci, the slender shinner was found to show closer genetic affinity to *Puntungia herzi* than congeneric *P. nigra* (Kim *et al*., 2013). There are two reports of microsatellite markers that could be used for slender shinners. Yun *et al*. (2013) developed novel microsatellites from *P. herzi* and tested for the cross-species amplification with slender shinners. More recently, cross-species amplification was tested for five species in Gobioninae (*P. nigra*, *Gobiobotia naktongensis*, *G. macrocephala*, *G. brevibarba*, and *Microphysogobio koreensis*) by the microsatellite loci developed newly for slender shinners (Kim *et al*., 2016). The mitochondrial genome sequence and structure of this species was completely characterized (Hwang *et al*., 2014). The egg development and early life history of this species is also well known (Ko *et al*., 2012).

Here, we used mitochondrial and microsatellite loci to analyze this species at the population level. Novel microsatellites were newly developed for this study. Based on these results, two questions were attempted to be addressed. First, are the genetic diversity formed historically in this species well preserved? Second, are there any signatures of genetic structure or isolation among populations? The genetic data obtained in this study can provide the basic information necessary for the conservation and restoration planning of this species in the future.

MATERIALS AND METHODS

1. Sampling

The slender shinner populations were sampled at three localities from the Han River ($N=67$; Fig. 1) under the approval from the local environmental office in the Ministry of Environment, South Korea (protocol # 2016-3 and 2016-5). Although the presence of this species was also known in the Imjin River (Jeon and Choi, 1980; Kim *et al*., 2005), which shares the estuary with the Han River, we have not able to find the populations even after several attempts. Following the collection methods recommended in the approval, the tissue samples for our genetic analyses were gained by removing a 2×2 mm fin clip from the caudal peduncle of each individual. After taking the fin clip, all individuals were released back to the sites where they were collected.

2. Isolation of microsatellites using Illumina paired-end sequencing

Genomic DNA of a slender shinner individual (from Hongcheon) was extracted using a DNeasy Blood and Tissue kit according to the manufacturer's guide (Qiagen, Dusseldorf, Germany). The quality and concentration of DNA was examined using a Qubit 2.0 Fluorometer (Life Technologies, San Diego, CA, USA). DNA was sheared to generate 700 bp fragments through Covaris hydrodynamic shearing processes (Woburn, MA, USA). A sequencing library was constructed using an Illumina Paired-End DNA Sample Preparation Kit (Illumina; San Diego, CA, USA) and was subjected to the pair-end sequencing mode of 100 bp read length in HiSeq2500 platform (Illumina). Base calling was performed using Illumina's Real-Time Analysis (RTA) 1.8.70, and the sequencing reads were processed to filter out adapters and low-quality sequences. The processed sequences were subjected to de novo assembly using the de Brujin graph algorithm and SOAPdenovo2 software package 2.04 under the default settings (Luo *et al*., 2012). RepeatModeler (http://www.repeatmasker.org/; Smith *et al*., 2014) was used for the identification of repetitive DNA sequences. SSR Finder (ftp://ftp.gramene.org/pub/gramene/archives/software/scripts/; Stieneke and Eujayl, 2007) was used for annotation of reads containing simple repeat sequence motifs. Only reads including tandem repeats more than 12 were considered 'positive' and subjected to the primer design implemented in Primer3 (http://fokker.wi.mit.edu/primer3/; Untergasser *et al*., 2012). Finally, sixty primer pairs (40 di- and 20 tetranucleotide repeat motifs) were selected for the testing of amplification and polymorphism.

3. Genomic DNA extraction for population genetic analysis

Genomic DNA was extracted from 67 slender shinners using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the protocol provided by the manufacturer.

4. Microsatellite genotyping

For microsatellite genotyping, the forward primers were fluorescently labeled with either FAM, NED, PET or VIC (Macrogen, Seoul, South Korea). For the PCR amplification, GenePro (BIOER, Hangzhou, PR China) was used with a 10 μL reaction mixture containing 1 μL genomic DNA (10∼50 ng), 1 μL 10X *Taq* buffer, 0.2 μL 0.2 M dNTP solution, 0.2 μL 10 pmole/μL of each primer and 0.03 μL 5 unit/μL Diastar *Taq* DNA polymerase (Solgent, Daejeon, South Korea). Thermal cycling profile was composed of denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 55∼60°C for 30 sec and extension at 72°C for 30 sec and final extension at 72°C for 10 min. PCR products with fluorescence were commercially genotyped (Macrogen) on an ABI3730XL genetic analyzer (Applied Biosystems, Foster City, CA, USA). Alleles were scored using GeneMapper 3.7 (Applied Biosystems). Allele sizes confirmed with Peak Scanner 1.0 (Applied Biosystems) for each individual were organized as an Excel file.

5. Analysis of mitochondrial DNA

Control region was sequenced for mitochondrial analysis using a previously reported primer set (DL1: ACC CCT GGC TCC CAA AGC; DH2: ATC TTA GCA TCT TCA GTG; Liu and Chen, 2003). PCR amplification was performed (annealing at 58°C) in the identical way described in microsatellite genotyping, except that 25 μL reaction mixture was used. The PCR products were purified using Primeprep PCR Purification Kit (GenetBio, Daejeon, South Korea) and were commercially sequenced (Macrogen) using the PCR primers on an ABI 3730XL DNA Analyzer with BigDye terminator system (Applied Biosystems).

6. Statistical analysis of microsatellite data

Convert 1.31 (Glaubitz, 2004) was used to automatically change the microsatellite size data stored in Excel to an input format compatible with each software. Microchecker 2.2.3 (Van Oosterhout *et al*., 2004) was used to test the presence of errors in scoring alleles or large allele dropout for each gene. The level of genetic diversity was shown by a variety of indices, number of alleles per locus (A), allelic richness (A_R), and observed heterozygosity (H_O) and expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}; Weir and Cockerham, 1984) that were calculated using Arlequin 3.5 (Excoffier and Lischer, 2010), FSTAT 2.9.3 (Goudet, 2001) and Genepop 4.2 (Raymond and Rousset, 1995). Fisher's exact test was performed to statistically determine whether genotypic proportions were deviated from the prediction of the Hardy-Weinberg equilibrium (HWE; Guo and Thompson, 1992) in Genepop. Bottleneck 1.2.02 (Piry *et al*., 1999) was used to test whether H_E under the mutation-drift equilibrium significantly exceeds H_E under the HWE. Wilcoxon sign-rank test was used to test heterozygosity excess under the TPM (two phase model) with

Table 1. Summary of 28 polymorphic microsatellite loci developed for the slender shinner (*Pseudopuntungia tenuicorpa*) and the diversity indices obtained from the 67 individuals. Data comprise locus name, number of alleles (A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and genetic divergence indices (F_{ST} and R_{ST}).

Locus	A	A_R	H_O	H_E	F_{IS}	F_{ST}	R_{ST}
Pte002	42	19.84	0.899	0.966	0.074	0.009	0.062
Pte004	34	17.73	0.879	0.960	0.077	0.012	0.227
Pte005	31	12.89	0.698	0.819	0.168	0.022	−0.023
Pte008	20	11.50	0.831	0.896	0.102	0.007	0.013
Pte009	34	18.35	0.909	0.960	0.045	0.020	0.058
Pte011	30	17.66	0.799	0.946	0.160	0.034	0.031
Pte012	36	17.80	0.899	0.952	0.056	0.022	−0.002
Pte013	29	15.94	0.909	0.935	0.022	0.025	−0.007
Pte015	28	17.24	0.764	0.943	0.163	0.036	0.111
Pte017	25	15.54	0.634	0.953	0.343	0.010	−0.028
Pte018	25	13.91	0.422	0.892	0.472	0.083	0.084
Pte019	32	17.32	0.772	0.956	0.186	0.019	0.012
Pte020	31	16.90	0.880	0.940	0.078	0.024	−0.005
Pte021	30	16.62	0.791	0.938	0.177	0.025	0.123
Pte025	28	16.00	0.949	0.919	−0.030	0.025	0.023
Pte026	23	12.91	0.899	0.901	−0.011	0.042	0.009
Pte027	25	16.13	0.453	0.932	0.504	0.039	0.009
Pte028	30	17.75	0.879	0.950	0.076	0.017	−0.012
Pte030	32	16.95	0.596	0.955	0.371	0.016	−0.034
Pte037	24	14.95	0.917	0.931	−0.018	0.031	−0.001
Pte038	25	14.07	0.920	0.918	0.006	0.029	−0.008
Pte045	31	16.94	0.960	0.953	−0.003	0.003	−0.007
Pte046	23	12.00	0.780	0.871	0.112	0.075	−0.024
Pte047	22	11.40	1.000	0.736	−0.358	0.004	0.028
Pte052	32	16.93	0.909	0.932	0.024	0.033	−0.008
Pte056	15	9.43	0.281	0.792	0.622	0.013	−0.018
Pte057	19	11.81	0.827	0.896	0.063	0.030	−0.020
Pte060	24	14.92	0.886	0.933	0.026	0.016	0.012
Average	27.9	15.41	0.798	0.917	0.125	0.026	0.024

a setting of 70% single-step mutations. Bottleneck was also used to examine a mode-shift away from the typical L-shaped distribution of allelic frequencies (Luikart *et al*., 1998).

The level of genetic differentiation among populations was estimated based on the difference in allele frequency (F_{ST}) and variance of allele sizes (number of repeat motif; R_{ST}), while testing the significance using Fisher's exact test after 10,000 permutations implemented in Arlequin. The level of genetic structure was examined in a Bayesian framework implemented in Structure 2.3.4 (Pritchard *et al*., 2000). The most optimal number of genetic clusters was inferred using the delta K method suggested by Evanno *et al*. (2005) implemented in Structure Harvester 0.6.94 (Earl and vonHoldt, 2012). The result of Structure was visualized using CLUMPAK (Clustering Markov Packager Across K; version b) server (Kopelman *et al*., 2015).

7. Analysis of mitochondrial DNA

The sequences of mitochondrial DNA control region obtained were rechecked through BLAST searches and against the inferred reading frame for the corresponding protein in MEGA 6.06 (Tamura *et al*., 2013) and were aligned using Geneious 9.1.8 (Kearse *et al*., 2012). All haplotypes obtained in this study were deposited in GenBank. The level of genetic diversity was shown by the number of haplotypes (h), haplotype diversity (h_d; Nei, 1987), and nucleotide diversity (p; Nei, 1987) calculated using DnaSP 5.10 (Librado and Rozas, 2009). Tajima's D (Tajima,

Table 2. List of three slender shinner (*Pseudopuntungia tenuicorpa*) populations and the diversity estimates obtained from 28 microsatellites (see Table 1). Data comprise population, drainage number of alleles (A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and the signatures of bottleneck (using H_E excess and mode-shift).

Population	Drainage	A	A_R	H_O	H_E	F_{IS}	Bottleneck H_E excess	Bottleneck mode
Hongcheon	North Han	18.8	13.18	0.799	0.902	0.116	NS	L-shaped
Yeongwol	South Han	15.3	14.29	0.799	0.920	0.134	NS	L-shaped
Jeongson	South Han	15.6	14.68	0.796	0.921	0.140	NS	L-shaped
	Mean	16.6	15.41	0.798	0.914	0.130		

Table 3. Summary of pairwise microsatellite genetic differentiation of three slender shinner (*Pseudopuntungia tenuicorpa*) populations. Estimates of F_{ST} appear below the diagonal and estimates of R_{ST} appear above the diagonal.

Population	Drainage	Hongcheon	Yeongwol	Jeongson
Hongcheon	North Han		0.040	0.025
Yeongwol	South Han	0.032		-0.006^{NS}
Jeongson	South Han	0.030	0.020	

All comparisons are significantly different from zero ($P<0.05$) except those denoted by 'NS'.

1989) was quantified to statistically determine whether our haplotype data were deviated from the expectation made under the neutral theory model with constant population size using DnaSP. To examine the pattern of relationships among haplotypes, a haplotype network was reconstructed using TCS 1.263 (Clement *et al*., 2000).

RESULTS

1. Analysis of microsatellite data

When a total of 60 candidate microsatellite loci were tested using 7 individuals, 28 showing a convincing pattern in amplification (data not shown) and clear polymorphism were used in the subsequent population analyses ($N=67$; Table 1). An extensive level of polymorphism was detected at these loci. Based on the results of the Microchecker, our analysis seems to have a very low probability of scoring errors or null alleles (data not shown). The number of alleles (A) per locus ranged from 15 to 42, with an average of 27.9 (Table 1). Allele richness (A_R) ranged from 9.429 to 19.837, with an average of 15.408 (Table 1). Heterozygosity values were also high with mean expected heterozygosity (H_E) of 0.914. Some values of heterozygote observed (H_O) were too low compared to the predicted values (H_E), which could be caused by sampling errors but not by intrapopulation structure, given that F_{IS} was not significantly high on average (Table 1).

All three populations investigated showed similar degrees of genetic diversity (Table 2). No population showed signatures of genetic bottleneck in both tests using H_E excess under the model of mutation-drift equilibrium and mode-shift in allele class distribution (Table 2). Based on the pairwise-F_{ST} and -R_{ST} values, Hongcheon (North Han River) was relatively distinguished from other two populations (South Han River), though the overall values were not that big (Table 3). This pattern was also evident in the results of the Structure (Fig. 2). Delta K analysis showed that $K=2$ was the most optimal (data not shown), and the genetic structure between North and South Han River was very clear (Fig. 2). The structured pattern between North and South was consistent even when the K value was changed (Fig. 2).

2. Analysis of mitochondrial DNA

After the alignment and trimming of the control region, 907 bp was used for the analysis. A total of 12 variable sites were detected, seven of which were parsimoniously informative. A total of 14 haplotypes were obtained (GenBank ACCN#: MT459772 - MT459785), and haplotype diversity and nucleotide diversity were 0.67 and 0.00114, respectively. The level of mitochondrial genetic diversity was higher in the North Han River than in the South Han River (H_d; Table 4). Although the individual numbers analyzed of the South Han populations was relatively small (Table 4), when these two populations were combined and

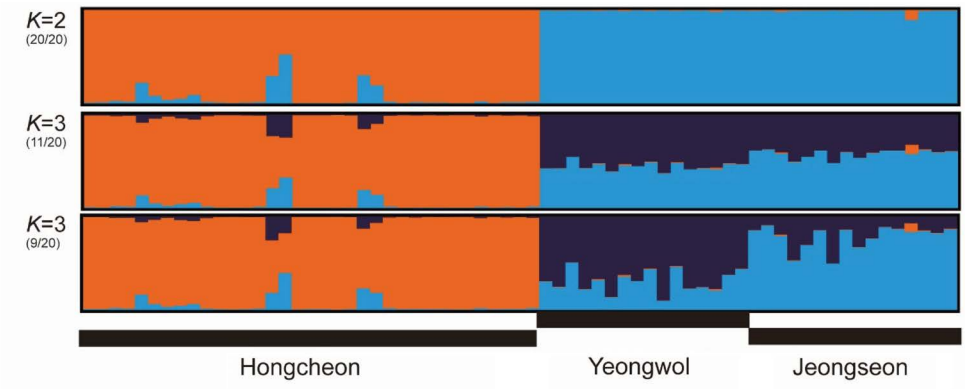

Fig. 2. Population structure of slender shinners (*Pseudopuntungia tenuicorpa*) estimated from microsatellite genotyping. The results of Structure indicated the existence of two genetic clusters, North and South Han ($K=2$ to 3). When $K=3$, two slightly different results emerged, as seen in the structures above (11 cases out of 20) and below (9 cases out of 20).

Table 4. Haplotype frequency distribution and diversity (haplotype diversity (H_d) and nucleotide diversity (p)) of mtDNA control region among three slender shinner (*Pseudopuntungia tenuicorpa*) populations.

Haplotype	ID	Hongcheon	Yeongwol	Jeongseon
1	BH1	17	11	13
2	BH2	2		
3	BH4	3		
4	BH8	1		
5	BH10	3	1	1
6	BH15	1		
7	BH19	1		
8	BH21	1		
9	BH25	4	1	
10	BH31	2		
11	NH1		1	
12	NH10		2	
13	NH26			1
14	NH32			1
N		35	16	16
H_d		0.748	0.533	0.350
p		0.00511	0.02020	0.02183

calculated together, H_d was 0.440, which was not comparable to the North Han River (Hongcheon). Tajima's D value was statistically significant at -2.064 in the South Han River, though negative in the North Han River (-0.556) but not statistically significant. Although all the populations are located in the same water system, only three haplotypes were shared among the populations, and the rest were the private haplotypes to each population (Yeongwol: 2; Jeongseon: 2; Hongcheon: 7; Fig. 3; Table 4).

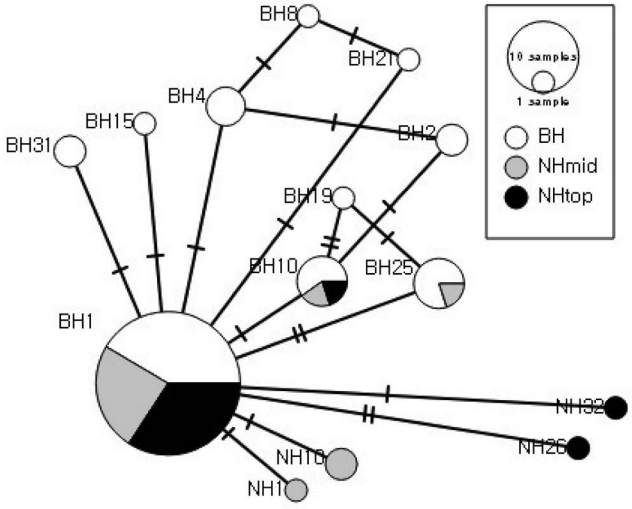

Fig. 3. The unrooted haplotype network generated based on the control region sequences of slender shinners (*Pseudopuntungia tenuicorpa*). The 14 circles indicate haplotypes, and the size of the circles is proportional to the frequency of haplotypes. The names of haplotypes are the same as those deposited in NCBI (BH: Hongcheon (North Han; white); NHmid: Jeongseon (South Han; gray); NHtop: Yeongwol (South Han, black).

DISCUSSION

The slender shinner is a species that is limited to only one water system on the Korean Peninsula in the world and requires special strategic management for the conservation. Although no study has yet been attempted at the population level, the sizes of the populations were expected to be small, due to the limited distribution and not being a species commonly found in the habitat area. It was a surprising that the genetic diversities of all populations analyzed were very high. The microsatellite diversity of this

species was well above the average level of freshwater fish (mean expected heterozygosity (H_E) = 0.54) and can even be seen to be higher than that of marine fish (H_E) = 0.77; DeWoody and Avise, 2000). In addition, no signatures were discovered in our genetic results to suspect genetic bottlenecks. Taken together, it could be said that there has been no contemporary decline in the size of the slender shinner populations.

However, prudence is required to determine the demographic status of a species solely based on microsatellite genetic data for the following reasons. First, microsatellite data do not provide absolute values (Abdelkrim *et al*., 2009). In other words, it is difficult to determine whether the level of heterozygosity or allelic diversity is high or low. It is only possible to compare with existing studies, but, given that it is difficult to obtain the data from a wide range of taxa, statistically stable comparison may not be possible. Second, when selecting microsatellite loci from genomic data, researchers generally prefer to choose the sequences with higher repetition number that are more likely to show higher polymorphism. The microsatellite diversity of a species when using markers developed specifically for that species tends to be higher than when using those characterized for other related species (Abdelkrim *et al*., 2009). Third, there are many cases where there is no correlation between species scarcity and microsatellite diversity. For example, *Tanakia somjinensis*, an endangered bitterling species (Acheilognathidae) that appears only in the Seomjin River on the Korean Peninsula, has a fairly high microsatellite genetic diversity (mean H_O = 0.758; mean H_E = 0.802; Jeon *et al*., 2016). However, *Rhodeus notatus* in the same family showed low relative diversity, although it is a species commonly found throughout the Korean Peninsula (mean H_O = 0.449; mean H_E = 0.504; Won *et al*., 2020).

It is necessary to note the genetic structure among the populations of slender shinners. Pairwise F_{ST} and R_{ST} data showed a relatively strong genetic differentiation between the North and South Han River, which was also prominent in the Bayesian Structure results. This differentiation means that there is a limited gene flow between these two main stems of the Han River. Considering the morphological and ecological nature of this species, which is small and normally found in small tributaries, it is not likely to migrate over a long distance along the main stem of the Han River. The level of gene flow may be one of the most important factors in maintaining the effective population size (DeWoody and Avise, 2000). Thus, from a forward-looking perspective, the genetic structure among the populations can be seen as a trace of factors negatively affecting the conservation of this species.

Our mitochondrial results at least partially support the genetic structure observed in the microsatellite results. Of the 17 haplotypes detected in this study, only 3 were shared between the North and South Han River. Considering the relatively low frequencies, it can be assumed that the private alleles occurred after the historical distribution to the south and north stems. Although not clearly seen in microsatellites, the mitochondrial results show that the North Han is somewhat more diverse than the South Han.

Although our genetic findings may not provide concrete evidence that reflects the current demographic status of the slender shinner, it should be regarded that, given its reproductive ecology (Lee, 2011; Ko *et al*., 2012), this species is very vulnerable to rapid environmental changes caused by external factors. As mentioned earlier, the spawning location of this species is naturally limited, since it spawns in the nests of *Coreoperca herzi* or very small crevices on or between rocks (Lee, 2011; Ko *et al*., 2012). To preserve this species in its natural state, the environment in which *C. herzi* can stably thrive must be maintained, and deformation should not be made in the physical structure of the water bodies and surroundings. Since the Han River passes through large cities and has a high density of surrounding population, it has been (and will continue to be) severely transformed for anthropogenic use, which gives a somewhat gloomy prospect for the conservation of this species. Considering the complex reproductive ecology of this species, a realistically effective policy-based conservation approach is to prevent access to the minimum section required for the breeding of this species. To do this, detailed ecological monitoring of where this species spawns must be prioritized.

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가는돌고기(*Pseudopuntungia tenuicorpa*) 보전을 위한 유전적 다양성 연구

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요 약 : 가는돌고기(*Pseudopungtungia tenuicorpa*)는 8~10 cm 크기의 소형 잉어과 어류로 전 세계에서 한국의 한강 그리고 임진강에만 서식하는 멸종위기종이다. 가는돌고기는 국내 담수의 상위 포식자 중 하나인 꺽지 수컷이 돌보는 수정된 알이 있는 둥지에 탁란(brood parasitism)을 하거나 작은 바위에 생긴 틈에 산란을 하는 생식 행동을 보인다. 이 종의 특이한 생식 생태는 환경 파괴가 극심한 현대 사회에서 산란 장소를 더욱 제한할 가능성이 높아 특별한 관리와 보전 전략이 필요하다. 본 연구에서는 microsatellites와 mtDNA control region 유전자를 이용하여 가는돌고기의 종 보전 관리 전략에 필요한 개체군 수준의 유전적 다양성 등 기초자료를 확보하고자 하였다. 유전체 분석에서 얻어진 28개의 microsatellite 유전자들을 이용하여 한강의 3지역에서 채집된 67개체들의 유전자형을 밝혔다. 본 microsatellite 유전자 분석 결과, 가는돌고기는 일반적으로 알려진 담수어류의 microsatellite 다양성 정도를 훨씬 뛰어 넘는 높은 유전적 다양성을 보여주었고(평균 이형접합자 빈도 예측치 = 0.914; 유전자 당 평균 대립인자 빈도 = 27.9), 개체군 감소나 inbreeding의 흔적은 나타나지 않았다. 그러나 북한강과 남한강 사이의 유전적 분화가 두드러졌다. 이런 유전적 구조는 14개 haplotype이 발견된 mtDNA 분석 결과에서도 유사하게 나타났다. 매우 좁은 지역에 서식하는 고유 멸종위기종에서 유전자 흐름의 제한 가능성이 나타났기 때문에, 장기적 측면에서 개체군들의 크기에 대한 고민이 필요하다. 추후 적응 유전적 분석 결과에서도 유사한 결과가 나타난다면, 북한강과 남한강 개체군들은 별도 관리가 이루어져야 하며, 복원 계획에도 이러한 유전적 구조에 대한 검토가 수반되어야 할 것이다.

찾아보기 낱말 : 유전적 다양성, 가는돌고기, *Pseudopuntungia tenuicorpa*, 잉어과, 보전유전학