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1 **Genetic structure and diversity of amphidromous sculpin in Shiretoko, a mountainous peninsula**  
2 **in Japan**

3

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16

17 **Abstract**

18 Despite the global crisis facing migratory benthic fishes, conservation genetic knowledge of these  
19 species remains scarce. In this study, we conducted a population genetic analysis using seven  
20 microsatellite loci to obtain basic information for determining conservation units and priorities of  
21 *Cottus hangiongensis* in Shiretoko, a mountainous peninsula where sculpin habitats are thought to be  
22 in decline throughout the region. The genetic structure was clearly divided between west and east  
23 coastal populations, and there was little recent migration between them. The western populations,  
24 which are closer to the center of the species' range, had significantly higher genetic diversity than the  
25 eastern populations. However, a bottleneck analysis and the inference of demographic history using  
26 approximate Bayesian computation showed that only the west group had experienced a significant  
27 recent bottleneck, probably due to recent habitat losses. These results suggest that the western and  
28 eastern populations should be different conservation units and that the western populations should be  
29 prioritized for conservation despite their high genetic diversity. This study contributes to the  
30 conservation genetics of diadromous sculpin and reiterates the importance of analyzing not only the  
31 current levels but also temporal changes in assessing genetic diversity.

32

33 **Keywords:** Diadromous benthic fish, *Cottus hangiongensis*, Bottleneck, Central–marginal hypothesis,  
34 Approximate Bayesian skyline plots, Microsatellite

35

## 36 **Introduction**

37 In a global biodiversity crisis, understanding the genetic structure and diversity of wild populations  
38 prior to planning conservation measures is effective in strategically preventing the loss of species and  
39 intraspecific diversity (Spielman et al. 2004; Hohenlohe et al. 2021). This is because genetic structure  
40 provides the basis for deciding management units based on population connectivity (Moritz 2002), and  
41 genetic diversity is an indicator of effective population size related to population vulnerability and  
42 adaptability to change (Reusch et al. 2005; Willi et al. 2022). Since natural and human interference  
43 with the environment will also change genetic diversity, investigating recent changes in population  
44 size is also considered to be effective for understanding the current health and viability of populations  
45 in a changing environment (Nunziata et al. 2017; Nakajima et al. 2020). Currently, it is required to  
46 investigate genetic structure and diversity in a wide range of organisms to assess extinction risks of  
47 species and genetically defined populations (Laikre et al. 2020).

48 Fishes with diadromous (migration between marine and freshwater) life histories are in rapid  
49 global decline due to river fragmentation and subsequent loss of available habitats (Limburg and  
50 Waldman 2009; Mota et al. 2016). The impact of such habitat loss has been recognized mainly in  
51 salmonids, and habitat restoration through environmental improvement or fishway installation has  
52 been carried out for these species (Roni et al. 2008; Nakamura and Komiyama 2010), but to a lesser  
53 extent for benthic fishes typified by sculpin. The lack of focus on benthic fishes may be due not only  
54 to their low economic and commercial value (Lucek et al. 2018) but also to a lack of understanding of  
55 the vulnerability of populations. In the case of the genus *Cottus* (Teleostei: Cottidae), a highly  
56 diversified sculpin group that comprises more than 60 species worldwide (Goto et al. 2015), although  
57 genetic diversity in species with fluvial life-history has been studied in relation to dams (Hänfling and  
58 Weetman 2006; Junker et al. 2012) or environments (Ruppert et al. 2017; Nakajima et al. 2021), there  
59 are not many studies pertaining to species with diadromous life history. Additionally, a few studies  
60 treating genetic data of diadromous species have usually focused on macroevolutionary processes such  
61 as speciation, and have not assessed and discussed recent changes in genetic diversity (e.g.,  
62 Tsukagoshi et al. 2013; Dennenmoser et al. 2014, 2017; Kanno et al. 2018; but see Baek et al. 2018),  
63 even though benthic fish are particularly susceptible to river-crossing structures (Terui and Miyazaki

64 2017). Considering that diadromous fish usually exhibit high mobility and connectivity between  
65 neighboring tributaries, it will be necessary to identify the group of strongly connected populations  
66 (subpopulation structure) and to evaluate the genetic diversity of each subpopulation unit. In areas  
67 where check dams are often placed near the mouth of rivers to prevent coastal residential areas from  
68 sediment-related disasters, the decline in genetic diversity is expected to be more pronounced.

69 *Cottus hangiongensis* is a diadromous benthic fish that is distributed along the Sea of Japan in  
70 Russia, Korea, and northern Japan (Baek et al. 2018). In Japan, this species is distributed on the Sea of  
71 Japan side from Central Honshu to Hokkaido Island, with a discontinuous distribution on the Sea of  
72 Okhotsk side of Hokkaido and around the Tsugaru Strait (Goto 1981). Korean and Honshu  
73 populations are locally threatened with extinction in part due to river construction pressures and a  
74 decrease in available habitat (National Institute of Biological Resources of Korea 2011; Ministry of  
75 the Environment Government of Japan 2020). Hatched juveniles of this species migrate downstream to  
76 the ocean, and after 4–6 weeks they return to the river to begin their benthic life (i.e., a life history  
77 called "amphidromous" among diadromous; Goto 1981, 1984), while it is still not known whether the  
78 juveniles of *C. hangiongensis* return to the same rivers where they hatched. From the perspective of  
79 conservation, although studies have been conducted on what kind of environment should be improved  
80 to increase suitable habitat (Goto 1981; Kitagawa et al. 2021), research on conservation genetics is  
81 scarce. The only population genetic study of *C. hangiongensis* using microsatellite loci was conducted  
82 by Baek et al. (2018), who evaluated the genetic structure and diversity of this species on the east  
83 coast of Korea and revealed the absence of genetic structure and highly maintained genetic diversity.  
84 However, since this is an example of one region with a relative abundance of rivers where fish can run  
85 up, we may not be able to generalize this pattern. For instance, patterns may be different in regions  
86 where the habitat is decreasing at the regional level. Although it is obvious that the habitat of this  
87 species has been decreasing in recent years in some regions, our knowledge about the current status  
88 and genetic demography of this species is lacking, as it is for other diadromous sculpin species.

89 Given the need to understand the current status of diadromous sculpin populations where the  
90 habitat is decreasing, we conducted a population genetic study of *C. hangiongensis* in the Shiretoko  
91 Peninsula, a mountainous peninsula where the habitat of this species is thought to be shrinking at the

92 regional level. The aims of the present study are (i) to identify the connectivity and substructure of the  
93 *C. hangiongensis* populations in the Shiretoko Peninsula and (ii) to evaluate patterns of genetic  
94 diversity in this species, considering recent temporal changes. This study will provide a basis for  
95 determining conservation units and priorities for this species as a case study in the conservation  
96 genetics of diadromous sculpin whose habitat is decreasing.

97

## 98 **Methods**

### 99 *Sample collection*

100 This study was conducted in the rivers of the Shiretoko Peninsula, which is located at the northeastern  
101 tip of Hokkaido Island (Japan) and protrudes into the Sea of Okhotsk, characterized by a branch of the  
102 Tsushima Current (Fig. 1; Table 1). The Shiretoko Peninsula has a mountainous topography, and most  
103 of the streams empty directly into the ocean with the appearance of mountain streams, so it is not  
104 uncommon for check dams to be installed only a few hundred meters from the sea (Nakamura and  
105 Komiyama 2010); the space available for migratory fishes to inhabit would have been correspondingly  
106 decreased. A total of 165 *Cottus hangiongensis* were caught from nine rivers using electrofishing  
107 (Model 12-B Backpack Electrofisher; Smith-Root, Vancouver, USA) in October 2020. Three of nine  
108 populations were sampled from the Okhotsk (west) side and the other six were from the Nemuro Strait  
109 (east) side. All sampling sites are downstream of the lowest dam of each river, as we could not capture  
110 any individuals above the dams. From the captured fish, small pieces of fin tissue were clipped, placed  
111 in 99.5% ethanol, and stored at  $-20^{\circ}\text{C}$  in the laboratory until DNA extraction.

112

### 113 *DNA extraction, amplification, and sequencing*

114 Total DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Hilden,  
115 Germany). The nine nuclear microsatellite loci (Englbrecht et al. 1999; Nolte et al. 2005), where the 5'  
116 end of each forward primer was labeled with either a HEX, 6-FAM, NED, or PET fluorescent dye,  
117 were amplified in two multiplex sets A and B (Table 2). PCR was conducted using the Type-it  
118 Microsatellite PCR Kit (Qiagen), with each 10.0  $\mu\text{l}$  reaction containing 5.0  $\mu\text{l}$  of 2 $\times$  Type-it Multiplex  
119 PCR Master Mix, 1.0  $\mu\text{l}$  of primer mix with 0.2  $\mu\text{M}$  of each of forward and reverse primer, 2.0  $\mu\text{l}$  of

120 RNase-free water, and 2.0  $\mu$ l of extracted genomic DNA. For multiplex set A, the PCR process  
121 consisted of an initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s,  
122 annealing at 58 °C for 90 s, and extension at 72 °C for 30 s; and a final extension at 60 °C for 30 min.  
123 For multiplex set B, an initial denaturation at 95 °C for 5 min; 27 cycles of denaturation at 95 °C for  
124 30 s, annealing at 57 °C for 90 s, and extension at 72 °C for 30 s; and a final extension at 60 °C for 30  
125 min. Fragment sizes were determined using an ABI PRISM 3130 Genetic Analyzer (Applied  
126 Biosystems, Foster City, USA) in the DNA Sequencing Facility of the Graduate School of Agriculture  
127 (Hokkaido University) and GeneMarker software (SoftGenetics, State College, USA) with GeneScan  
128 500 LIZ dye Size Standard (Applied Biosystems). All samples were scored blindly (i.e., with sample  
129 names removed) and repeated multiple times to ensure accuracy and consistency. The two loci,  
130 Cott207 and Cg01016, did not allow for accurate scoring in multiple samples; these loci were  
131 excluded from the analysis.

132

### 133 *Genetic diversity and differentiation*

134 To evaluate the intrapopulation genetic diversity, allelic richness (El Mousadik and Petit 1996),  
135 expected heterozygosity ( $H_E$ ), and the fixation index ( $F_{IS}$ ) were calculated for each population using  
136 FSTAT 2.9.4 (Goudet 1995). To examine regional trends in genetic diversity, the differences in allelic  
137 richness and  $H_E$  between the western and eastern populations were tested by Welch's t-test. Significant  
138 deviations from Hardy–Weinberg equilibrium (HWE), as indicated by  $F_{IS}$  deviation from zero, were  
139 tested by 1,000 randomizations using FSTAT. Genetic differentiation between populations was  
140 evaluated by calculating global/pairwise  $F_{ST}$  (Weir and Cockerham 1984) and its standardized value,  
141  $F'_{ST}$  (Meirmans and Hedrick 2011), with 9,999 randomizations using GenAlEx 6.5 (Peakall and  
142 Smouse 2012).

143

### 144 *Population structure*

145 The population structure was examined using STRUCTURE 2.3.4 (Pritchard et al. 2000), which  
146 implements a Bayesian clustering method using multi-locus allele frequency data. The STRUCTURE  
147 settings were the admixture and allele frequency correlated model with previous sampling location

148 information (LOCPRIOR; Hubisz et al. 2009). The algorithm was run 20 times for each K from 1 to 9  
149 with a burn-in of 20,000 followed by 30,000 Markov chain Monte Carlo (MCMC) replicates. The  
150 program CLUMPAK (Kopelman et al. 2015) was used to summarize the results and to generate  
151 barplots for each K. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was employed to  
152 calculate the probability of the data (LnP(D); Pritchard et al. 2000), the corresponding standard  
153 deviation, and Evanno's delta K ( $\Delta K$ ; Evanno et al. 2005).

154

#### 155 *Gene flow between subpopulations*

156 Because STRUCTURE divided western and eastern individuals distinctly (see Results), we pooled  
157 individuals of western and eastern populations into their respective population groups (hereafter,  
158 subpopulation) and estimated recent gene flow between the two subpopulations. We used BA3MSAT  
159 (Mussmann et al. 2019), a reconstructed version of BayesAss (Wilson and Rannala 2003) that  
160 estimates dual-direction pairwise migration rates over a few generations, for the two subpopulations.  
161 The setting was 10,000,000 MCMC iterations, including a burn-in period of 5,000,000. The inferred  
162 migration rates with 95% credible intervals (estimates  $\pm 1.96 \times SD$  [SD: standard deviation]) that did  
163 not include zero were regarded as significant migration.

164

#### 165 *Temporal changes in genetic diversity indicated by population demography*

166 To evaluate whether each subpopulation had experienced recent bottlenecks, heterozygosity excess  
167 was tested using Wilcoxon's signed rank test in software BOTTLENECK 1.2.02 (Piry et al. 1999)  
168 with 1,000 iterations. The program was run under the infinite alleles model (IAM), the stepwise  
169 mutation model (SMM), and the 2-phase model (TPM; assuming 70% single step mutations and a  
170 variance among mutation steps of 30). In addition to the bottleneck analysis, we inferred the shape of  
171 the demographic trajectories of each subpopulation using DIYABCskylineplot 1.0.1 (Navascués et al.  
172 2017), which implements a relatively novel approach that introduces appropriate Bayesian  
173 computation (ABC) methods into the model-flexible demographic inferences. We set the following  
174 parameter values for DIYABCskylineplot analysis: num\_of\_points = 100 (number of points to draw  
175 skyline plot), prior\_THETA\_min = 0.1 (THETA is the population size and is measured by  $4N\mu$ , where



176  $N$  is the effective population size and  $\mu$  is the mutation rate per generation), prior\_THETA\_max = 10,  
177 prior\_GSM\_min = 0.1 (GSM is the generalized stepwise mutation model for microsatellites),  
178 prior\_GSM\_max = 0.8, prior\_T\_max = 250 (maximum time, measured in number of generations),  
179 prior\_MUTRATE\_min = 0.0001 (MUTRATE is the  $\mu$  value;  $\mu$  for microsatellites generally ranges  
180 from  $10^{-4}$  to  $10^{-3}$  [Estoup and Angers 1998; Yue et al. 2007]), prior\_MUTATE\_max = 0.0010, and the  
181 repeat size for each locus was specified (2 for all). All other options and priors were set to default  
182 values.

183

## 184 **Results**

### 185 *Summary statistics*

186  $F_{IS}$  showed negative and positive values (ranged from -0.126–0.151) but did not deviate significantly  
187 from zero in all populations, suggesting that HWE could be assumed in all populations. The allelic  
188 richness and  $H_E$  ranged from 1.748–2.420 and 0.287–0.463, respectively (Table 1). Across the nine  
189 populations, the global  $F_{ST}$  value was 0.121, and its standardized value,  $F'_{ST}$ , was 0.206, indicating  
190 moderate population differentiation. Pairwise  $F_{ST}$  and  $F'_{ST}$  values ranged from 0.000–0.284 and  
191 0.000–0.455, respectively (Table 3). The  $F'_{ST}$  values were 0.159–0.455 and significant in all pairs  
192 between populations on different seaward sides, but up to 0.141 between populations on the same side  
193 and were not significant in most pairs, with no differentiation observed in some of the especially close  
194 populations (Pop5, 6, and 7; Pop7 and 8; Pop8 and 9).

195

### 196 *Population structure and gene flow*

197 In the STRUCTURE analysis,  $\Delta K$  showed the highest value at  $K = 2$ , and  $\text{LnP(D)}$  increased until  $K =$   
198 4 (Fig. 1B). The population structure at  $K = 2$  was quite clear, and each cluster corresponded with  
199 actual western and eastern populations (Fig. 1C). In  $K = 4$ , individuals of Pop4 were grouped into  
200 another single cluster, but this cluster was also mixed in with individuals of other eastern populations,  
201 especially individuals of Pop8 and 9. Bayesian estimate of contemporary migration rate from west to  
202 east subpopulations was 0.014 (SD = 0.012) and that from east to west subpopulations was 0.011 (SD  
203 = 0.008), indicating that no significant migration occurred between the two subpopulations.

204

205 *Genetic diversity and demographic histories in west and east subpopulations*

206 From the comparison of genetic diversity, western populations have significantly higher genetic  
207 diversity than eastern populations with respect to both allelic richness ( $p < 0.05$ ; Fig. 2A) and  $H_E$  ( $p <$   
208  $0.001$ ; Fig. 2B). BOTTLENECK software detected a recent population bottleneck owing to significant  
209 heterozygosity excess under all mutation models in the west subpopulation (Table 4), but not for the  
210 east subpopulation. DIYABCskylineplot estimated simple trajectories with overall constant population  
211 sizes, except that the west subpopulation experienced a rapid population contraction from tens of  
212 generations ago (Figs. 2C and 2D).

213

214 **Discussion**

215 *Genetic differentiation and structure*

216 This study revealed the patterns of population structure and genetic diversity of *C. hangiongensis* in  
217 the mountainous Shiretoko Peninsula. Genetic differentiation was low on the same seaward side, and  
218 there was no differentiation in some of the especially close populations (Table 3). This means that the  
219 tributaries on the same side are strongly connected by gene flow and that this species is not  
220 constrained to return to the same river. In the STRUCTURE analysis, Pop4 may appear to be grouped  
221 in a different cluster at high K. This population is a bit distant from the other eastern sampling sites  
222 and has low genetic diversity (Table 1), which may have led to greater genetic drift. However,  
223 individuals of other eastern populations also have a mixture of the cluster found in Pop4, and the  $F_{ST}$   
224 between Pop4 and the majority of eastern populations was not significant; this population should be  
225 considered as being part of the east subpopulation.

226 Contrary to the low genetic differentiation between populations on the same side of the ocean,  
227 there was significant genetic differentiation and little gene flow between the western and eastern  
228 populations. Based on the distribution of *C. hangiongensis*, dispersal of this species is thought to be  
229 related to the Tsushima Current, which flows northward across the Sea of Japan and partly into the Sea  
230 of Okhotsk around the Shiretoko Peninsula (Goto 1981; Fig. 1A). Typically, the genetic differentiation  
231 of coastal fish inhabiting along ocean currents is low even if they are in distant locations (>500 km)

232 because the constitutive individuals are potentially dispersed by ocean currents (Mukai et al. 2009;  
233 Xiao et al. 2011; Yamakawa et al. 2019). In the case of *Cottus* species, although a previous study  
234 pertaining to diadromous populations in a gulf region (no currents) has shown clear genetic variation  
235 between different tributaries (Dennenmoser et al. 2014), Baek et al. (2018), studying the same species  
236 as the present study along the Sea of Japan, reported the absence of genetic structure in a maximum  
237 distance of 140 km between sites, a spatial scale almost identical to that of our study (the maximum  
238 coastal distance is 127 km in the present study). Therefore, a clear genetic structure within a narrow  
239 region may represent an unusual case and the genetic differentiation between the western and eastern  
240 populations may be due to the geographical or environmental conditions of the Shiretoko Peninsula.  
241 For example, genetic exchange with the eastern populations may be less likely to occur because the  
242 majority of the Tsushima Current coming from the west exits to the northeast instead of turning to the  
243 south (east coastal) direction (Takizawa 1982; Fig. 1).

244

#### 245 *Genetic diversity and its temporal change*

246 Genetic diversity varied among the populations (Table 1), and there was a clear pattern of higher  
247 genetic diversity in the western populations than in the eastern populations (Fig. 2). It is known that  
248 wild organisms have higher genetic diversity in populations at the center of their distribution and  
249 lower in the peripheral (edge) populations (central–marginal hypothesis; Micheletti and Storfer 2015;  
250 Takahashi et al. 2016). In *C. hangiongensis*, the center of the distribution is around the Sea of Japan,  
251 and populations on the Sea of Okhotsk coast are considered to have been dispersed and established by  
252 the Tsushima Current (Goto 1981). Thus, the detected patterns of genetic diversity are consistent with  
253 the central–marginal hypothesis.

254 In contrast to the level of genetic diversity, BOTTLENECK and demographic analysis  
255 consistently showed that only the west subpopulation experienced recent bottlenecks (Fig. 2; Table 4).  
256 While genetic diversity often maintains equilibrium at low levels, experiencing a bottleneck indicates  
257 that genetic diversity is in decline and population viability is threatened (Teixeira and Huber 2021). In  
258 this case, although current genetic diversity in western populations is still high, it may be in the  
259 process of decreasing with shrinking population. The rapid population contraction was estimated to

260 have begun tens of generations ago, although the uncertainty of the DIYABC skyline plot somewhat  
261 increases in bottleneck patterns (Navascués et al. 2017). What BOTTLENECK is inferring is also a  
262 recent population shrinkage because this analysis detects heterozygosity excess based on the principle  
263 that allelic diversity is reduced faster than heterozygosity in recently bottlenecked populations. These  
264 results suggest that the population contraction of the west subpopulation is probably related to recent  
265 habitat loss due to anthropogenic impacts. In particular, the rapid installation of check dams in rivers  
266 of the Shiretoko Peninsula began approximately 60 years ago (Takahashi et al. 2005), and the inferred  
267 time in the demographic analysis is consistent with this period if the generation time of the species is  
268 assumed to be 2.5 years based on Goto (1981). However, there is no evidence to indicate that the  
269 habitat loss along the west coastal side was particularly severe than in the east, and it is also possible  
270 that the bottleneck was caused by reduced gene flow from unsampled populations in more central  
271 parts of the species' distribution. To understand the reasons why only the west subpopulation is  
272 declining, it is necessary to elucidate the relationship with the local environment or to sample more  
273 extensively. Since the purpose of this study is to identify patterns of genetic structure and diversity,  
274 understanding the details of its process is left for future work.

275

#### 276 *Implication for conservation genetics*

277 While most applications of conservation genetics are limited to calculating current genetic diversity  
278 and differentiation, examining recent changes in genetic diversity may lead to different conclusions.  
279 With respect to the conservation of the local genetic diversity of *C. hangiongensis* in the Shiretoko  
280 Peninsula, we first found that the western and eastern populations are genetically distinct and should  
281 be considered different management units. We then investigated the genetic diversity of each  
282 subpopulation and found that the genetic diversity of the western populations was significantly higher  
283 than that of the eastern populations. However, the recent bottleneck was more pronounced in the west  
284 subpopulation. In terms of the level of genetic diversity alone, the conservation priority appears to be  
285 higher in the eastern region, but the lower genetic diversity in the eastern populations is probably a  
286 natural state in line with the central–marginal hypothesis. From the perspective of reducing human

287 impacts, the conservation priority would be rather higher in the west region which has experienced a  
288 recent bottleneck.

289         There are a lot of sculpin species in the world, some of which are diadromous species or have  
290 polymorphisms displaying diadromous life history (Goto et al. 2015). Even though diadromous  
291 populations have not been well studied, it should be recognized that there may be unknown  
292 evolutionarily significant units in regions where genetic studies have not yet been conducted.  
293 Additionally, this is probably the first study to actually show evidence of a bottleneck in the genetic  
294 data of diadromous *Cottus* species. We present that other populations that appear to have high genetic  
295 diversity may also have been potentially impacted in this era of global habitat loss for migratory  
296 fishes.

297

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302

#### 303 **References**

- 304 Baek SY, Kang JH, Jo SH, et al (2018) Contrasting life histories contribute to divergent patterns of  
305 genetic diversity and population connectivity in freshwater sculpin fishes. *BMC Evol Biol* 18:1–  
306 14. <https://doi.org/10.1186/s12862-018-1171-8>
- 307 Dennenmoser S, Rogers SM, Vamosi SM (2014) Genetic population structure in prickly sculpin  
308 (*Cottus asper*) reflects isolation-by-environment between two life-history ecotypes. *Biol J Linn*  
309 *Soc* 113:943–957. <https://doi.org/10.1111/bij.12384>
- 310 Dennenmoser S, Vamosi SM, Nolte AW, Rogers SM (2017) Adaptive genomic divergence under high  
311 gene flow between freshwater and brackish-water ecotypes of prickly sculpin (*Cottus asper*)  
312 revealed by Pool-Seq. *Mol Ecol* 26:25–42. <https://doi.org/10.1111/mec.13805>
- 313 Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing  
314 STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4:359–361.

315 <https://doi.org/10.1007/s12686-011-9548-7>

316 El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among  
317 populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theor Appl*  
318 *Genet* 92:832–839. <https://doi.org/10.1007/BF00221895>

319 Englbrecht CC, Largiadèr CR, Hänfling B, Tautz D (1999) Isolation and characterization of  
320 polymorphic microsatellite loci in the European bullhead *Cottus gobio* L. (Osteichthyes) and  
321 their applicability to related taxa. *Mol Ecol* 8:1966–1969. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-294X.1999.00778-6.x)  
322 [294X.1999.00778-6.x](https://doi.org/10.1046/j.1365-294X.1999.00778-6.x)

323 Estoup A, Angers B (1998) Microsatellites and Minisatellites for Molecular Ecology: Theoretical and  
324 Empirical Consideration. In: Carvalho G (ed) *Advances in molecular ecology*. NATO Press,  
325 Amsterdam, Holland, 55–86pp

326 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the  
327 software STRUCTURE: A simulation study. *Mol Ecol* 14:2611–2620.  
328 <https://doi.org/10.1111/j.1365-294X.2005.02553.x>

329 Goto A (1981) Life History and Distribution of a River Sculpin, *Cottus hangiongensis*. *Bull Fac Fish*  
330 *Hokkaido Univ* 32:10–21. (In Japanese with English abstract)

331 Goto A (1984) Comparative ecology of young-of-the-year between two amphidromous species of  
332 *Cottus* in Hokkaido: 1. Upstream migration and growth. *Bull Fac Fish Hokkaido Univ* 35:133–  
333 143

334 Goto A, Yokoyama R, Sideleva VG (2015) Evolutionary diversification in freshwater sculpins  
335 (Cottoidea): a review of two major adaptive radiations. *Environ Biol Fishes* 98:307–335.  
336 <https://doi.org/10.1007/s10641-014-0262-7>

337 Goudet J (1995) FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *J Hered*  
338 86:485–486

339 Hänfling B, Weetman D (2006) Concordant genetic estimators of migration reveal anthropogenically  
340 enhanced source-sink population structure in the river sculpin, *Cottus gobio*. *Genetics* 173:1487–  
341 1501. <https://doi.org/10.1534/genetics.105.054296>

342 Hohenlohe PA, Funk WC, Rajora OP (2021) Population genomics for wildlife conservation and

343 management. *Mol Ecol* 30:62–82. <https://doi.org/10.1111/mec.15720>

344 Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the  
345 assistance of sample group information. *Mol Ecol Resour* 9:1322–1332

346 Junker J, Peter A, Wagner CE, et al (2012) River fragmentation increases localized population genetic  
347 structure and enhances asymmetry of dispersal in bullhead (*Cottus gobio*). *Conserv Genet*  
348 13:545–556. <https://doi.org/10.1007/s10592-011-0306-x>

349 Kanno K, Onikura N, Kurita Y, et al (2018) Morphological, distributional, and genetic characteristics  
350 of *Cottus pollux* in the Kyushu Island, Japan: indication of fluvial and amphidromous life  
351 histories within a single lineage. *Ichthyol Res* 65:462–470. [https://doi.org/10.1007/s10228-018-](https://doi.org/10.1007/s10228-018-0637-4)  
352 [0637-4](https://doi.org/10.1007/s10228-018-0637-4)

353 Kitagawa T, Muraoka K, Nakamura K, Goto A (2021) Environmental preference of juvenile  
354 amphidromous *Cottus* fishes in brackish-water river mouth. *Ecol Civ Eng* 24:27–38.  
355 <https://doi.org/10.3825/ece.21-00002>. (In Japanese)

356 Kopelman NM, Mayzel J, Jakobsson M, et al (2015) Clumpak: A program for identifying clustering  
357 modes and packaging population structure inferences across K. *Mol Ecol Resour* 15:1179–1191.  
358 <https://doi.org/10.1111/1755-0998.12387>

359 Laikre L, Hoban S, Bruford MW, et al (2020) Post-2020 goals overlook genetic diversity. *Science*  
360 367:1083–1085. <https://doi.org/10.1126/science.abb2748>

361 Limburg KE, Waldman JR (2009) Dramatic declines in north Atlantic diadromous fishes. *Bioscience*  
362 59:955–965. <https://doi.org/10.1525/bio.2009.59.11.7>

363 Lucek K, Keller I, Nolte AW, Seehausen O (2018) Distinct colonization waves underlie the  
364 diversification of the freshwater sculpin (*Cottus gobio*) in the Central European Alpine region. *J*  
365 *Evol Biol* 31:1254–1267. <https://doi.org/10.1111/jeb.13339>

366 Meirmans PG, Hedrick PW (2011) Assessing population structure:  $F_{ST}$  and related measures. *Mol*  
367 *Ecol Resour* 11:5–18. <https://doi.org/10.1111/j.1755-0998.2010.02927.x>

368 Micheletti SJ, Storfer A (2015) A test of the central-marginal hypothesis using population genetics and  
369 ecological niche modelling in an endemic salamander (*Ambystoma barbouri*). *Mol Ecol* 24:967–  
370 979. <https://doi.org/10.1111/mec.13083>

371 Ministry of the Environment Government of Japan (2020) Red List of Japan. (In Japanese)

372 Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it.  
373 Syst Biol 51:238–254. <https://doi.org/10.1080/10635150252899752>

374 Mota M, Rochard E, Antunes C (2016) Status of the diadromous fish of the Iberian Peninsula: Past,  
375 Present and Trends. Limnetica 35:1–18. <https://doi.org/10.23818/limn.35.01>

376 Mukai T, Nakamura S, Nishida M (2009) Genetic population structure of a reef goby, *Bathygobius*  
377 *cocosensis*, in the northwestern Pacific. Ichthyol Res 56:380–387.  
378 <https://doi.org/10.1007/s10228-009-0111-4>

379 Mussmann SM, Douglas MR, Chafin TK, Douglas ME (2019) BA3-SNPs: Contemporary migration  
380 reconfigured in BayesAss for next-generation sequence data. Methods Ecol Evol 10:1808–1813.  
381 <https://doi.org/10.1111/2041-210X.13252>

382 Nakajima S, Hirota SK, Matsuo A, et al (2020) Genetic structure and population demography of  
383 white-spotted charr in the upstream watershed of a large dam. Water 12:2406.  
384 <https://doi.org/10.3390/w12092406>

385 Nakajima S, Sueyoshi M, Hirota SK, et al (2021) A strategic sampling design revealed the local  
386 genetic structure of cold-water fluvial sculpin: a focus on groundwater-dependent water  
387 temperature heterogeneity. Heredity 127:413–422. <https://doi.org/10.1038/s41437-021-00468-z>

388 Nakamura F, Komiyama E (2010) A challenge to dam improvement for the protection of both salmon  
389 and human livelihood in Shiretoko, Japan’s third Natural Heritage Site. Landsc Ecol Eng 6:143–  
390 152. <https://doi.org/10.1007/s11355-009-0083-6>

391 National Institute of Biological Resources (2011) Red data book of endangered fishes in Korea.

392 Navascués M, Leblois R, Burgarella C (2017) Demographic inference through approximate-Bayesian-  
393 computation skyline plots. PeerJ 5:e3530. <https://doi.org/10.7717/peerj.3530>

394 Nolte AW, Stemshorn KC, Tautz D (2005) Direct cloning of microsatellite loci from *Cottus gobio*  
395 through a simplified enrichment procedure. Mol Ecol Notes 5:628–636.  
396 <https://doi.org/10.1111/j.1471-8286.2005.01026.x>

397 Nunziata SO, Lance SL, Scott DE, et al (2017) Genomic data detect corresponding signatures of  
398 population size change on an ecological time scale in two salamander species. Mol Ecol



399 26:1060–1074. <https://doi.org/10.1111/mec.13988>

400 Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for  
401 teaching and research-an update. *Bioinformatics* 28:2537–2539.  
402 <https://doi.org/10.1093/bioinformatics/bts460>

403 Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent  
404 reductions in the effective population size using allele frequency data. *J Hered* 90:502–503.  
405 <https://doi.org/10.1093/jhered/90.4.502>

406 Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus  
407 Genotype Data. *Genetics* 155:945–959. <https://doi.org/10.1007/s10681-008-9788-0>

408 Reusch TBH, Ehlers A, Hammerli A, Worm B (2005) Ecosystem recovery after climatic extremes  
409 enhanced by genotypic diversity. *Proc Natl Acad Sci* 102:2826–2831.  
410 <https://doi.org/10.1073/pnas.0500008102>

411 Roni P, Hanson K, Beechie T (2008) Global Review of the Physical and Biological Effectiveness of  
412 Stream Habitat Rehabilitation Techniques. *North Am J Fish Manag* 28:856–890.  
413 <https://doi.org/10.1577/m06-169.1>

414 Ruppert JLW, James PMA, Taylor EB, et al (2017) Riverscape genetic structure of a threatened and  
415 dispersal limited freshwater species, the Rocky Mountain Sculpin (*Cottus* sp.). *Conserv Genet*  
416 18:925–937. <https://doi.org/10.1007/s10592-017-0938-6>

417 Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic  
418 factors impact them. *Proc Natl Acad Sci* 101:15261–15264.  
419 <https://doi.org/10.1073/pnas.0403809101>

420 Takahashi G, Kuwahara T, Yamakawa M (2005) Dams in the Shiretoko Peninsula -Issues in river  
421 management and environmental conservation-. *Japanese J Conserv Ecol* 10:139–149.  
422 [https://doi.org/10.18960/hozen.10.2\\_139](https://doi.org/10.18960/hozen.10.2_139). (In Japanese with English abstract)

423 Takahashi Y, Suyama Y, Matsuki Y, et al (2016) Lack of genetic variation prevents adaptation at the  
424 geographic range margin in a damselfly. *Mol Ecol* 25:4450–4460.  
425 <https://doi.org/10.1111/mec.13782>

426 Takizawa T (1982) Characteristics of the Soya Warm Current in the Okhotsk Sea. *J Oceanogr Soc*

427 Japan 38:281–292

428 Teixeira JC, Huber CD (2021) The inflated significance of neutral genetic diversity in conservation  
429 genetics. *Proc Natl Acad Sci* 118:e2015096118. <https://doi.org/10.1073/pnas.2015096118>

430 Terui A, Miyazaki Y (2017) Combined effects of immigration potential and habitat quality on  
431 diadromous fishes. *Limnology* 18:121–129. <https://doi.org/10.1007/s10201-016-0495-6>

432 Tsukagoshi H, Sakai K, Yamamoto K, Goto A (2013) Non-indigenous amphidromous sculpin *Cottus*  
433 *pollux* small-egg type (Teleostei: Cottidae) detected in rivers entering the Sea of Japan off  
434 Honshu Island, Japan. *Ichthyol Res* 60:93–97. <https://doi.org/10.1007/s10228-012-0316-9>

435 Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population Structure.  
436 *Evolution* 38:1358. <https://doi.org/10.2307/2408641>

437 Willi Y, Kristensen TN, Sgrò CM, et al (2022) Conservation genetics as a management tool: The five  
438 best-supported paradigms to assist the management of threatened species. *Proc Natl Acad Sci*  
439 119:e2105076119. <https://doi.org/10.1073/pnas.2105076119>

440 Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus  
441 genotypes. *Genetics* 163:1177–1191

442 Xiao Y, Zhang Y, Yanagimoto T, et al (2011) Population genetic structure of the point-head flounder,  
443 *Cleisthenes herzensteini*, in the Northwestern Pacific. *Genetica* 139:187–198.  
444 <https://doi.org/10.1007/s10709-010-9536-y>

445 Yamakawa U, Kaneko S, Imai R, et al (2019) Development of microsatellite markers for the  
446 endangered sleeper *Eleotris oxycephala* (Perciformes: Eleotridae). *Genes Genet Syst* 94:219–  
447 224. <https://doi.org/10.1266/ggs.19-00007>

448 Yue GH, David L, Orban L (2007) Mutation rate and pattern of microsatellites in common carp  
449 (*Cyprinus carpio* L.). *Genetica* 129:329–331. <https://doi.org/10.1007/s10709-006-0003-8>

450

#### 451 **Statements & Declarations**

452 Competing Interests: The authors declare no competing interests.

453 Author Contributions: Y.A., S.N., and F.N. conceived and designed the study. Y.A. performed the  
454 field sampling. Y.A. and S.N. performed the laboratory work. S.N. analyzed the data and wrote the  
455 manuscript, with contributions from Y.A. and F.N. All authors read and approved the final manuscript.

456

457 **Data Availability**

458 Genotype data generated in this study is available at Figshare (doi: 10.6084/m9.figshare.17429417).

459

460 **Tables**461 **Table 1** Sampling localities and genetic diversity of each population.

Pop ID	Name of river	Latitude (N°)	Longitude (E°)	N	AR	$H_E$	$F_{IS}$
Pop1	Osyamappu	43.987	144.882	35	2.410	0.463	-0.025
Pop2	Kanayama	43.992	144.886	17	2.392	0.425	0.151
Pop3	Opekepu	44.027	144.928	18	2.420	0.452	-0.124
Pop4	Rusa	44.139	145.265	20	1.748	0.287	0.139
Pop5	Matsunori	43.987	145.162	22	2.051	0.371	-0.126
Pop6	Tachikariusu	43.968	145.142	9	2.127	0.342	-0.051
Pop7	Chashibetsu	43.902	145.102	11	2.343	0.375	0.091
Pop8	Ponrikushibetsu	43.881	145.096	9	2.221	0.327	-0.067
Pop9	Rikusibetu	43.878	145.097	24	1.993	0.343	-0.015

462 N, number of individuals; AR, allelic richness;  $H_E$ , expected heterozygosity;  $F_{IS}$ , fixation index

463

464 **Table 2** Characteristics of the nine microsatellite loci used in this study.

Locus	Motif size	Allele size	Multiplex set	Fluorescent dye	References
Cgo18	2	242–250	B	6-FAM	Englbrecht et al. 1999
Cgo22	2	203–213	B	HEX	Englbrecht et al. 1999
Cgo33	2	164–168	B	6-FAM	Englbrecht et al. 1999
Cgo56	2	232–240	A	HEX	Englbrecht et al. 1999
Cgo1016 <sup>a</sup>	2	-	B	PET	Englbrecht et al. 1999
Cgo1114	2	145–163	A	NED	Englbrecht et al. 1999
Cott112	2	166–170	A	HEX	Nolte et al. 2005
Cott138	2	270–274	A	6-FAM	Nolte et al. 2005
Cott207 <sup>a</sup>	2	-	B	NED	Nolte et al. 2005

465 <sup>a</sup> Loci amplified but not included in the dataset

466

467 **Table 3** Pairwise  $F_{ST}$  (below diagonal) and  $F'_{ST}$  (above diagonal) matrices among the nine populations  
 468 of *C. hangiongensis*.

	West			East					
	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9
Pop1		0.035	0.008	0.439	0.310	0.340	0.262	0.308	0.298
Pop2	0.019		0.028	0.455	0.274	0.332	0.224	0.303	0.271
Pop3	0.004	0.016		0.369	0.221	0.259	0.159	0.191	0.197
Pop4	0.252**	0.284**	0.225**		0.163	0.107	0.141	0.094	0.077
Pop5	0.170**	0.162**	0.127**	0.104**		0.000	0.000	0.028	0.058
Pop6	0.181**	0.191**	0.145**	0.068	0.000		0.000	0.013	0.063
Pop7	0.136**	0.125**	0.086**	0.087*	0.000	0.000		0.000	0.019
Pop8	0.173**	0.186**	0.114*	0.063	0.018	0.008	0.000		0.000
Pop9	0.170**	0.167**	0.119**	0.051	0.036	0.039	0.012	0.000	

469 Asterisks in below diagonal denote significant differentiations; \* $p < 0.05$  and \*\* $p < 0.01$  after  
 470 Bonferroni correction  
 471

472 **Table 4** BOTTLENECK results under infinite alleles model (IAM), stepwise mutational model  
 473 (SMM), and 2-phase models (TPM).  $P$ -values are shown.

Subpopulation	Mutation model		
	IAM	TPM	SMM
West	<0.01	<0.01	<0.05
East	0.08	0.50	0.92

474

475

476 **Figure legends**

477 **Fig. 1** Study area and genetic structure. (A) Sampling localities. Labels correspond to population IDs  
 478 (Pop1, 2, ..., 9) in Table 1. The broad-area map shows the location of the Shiretoko Peninsula and  
 479 routes of the Tsushima Current, including its branches. (B) The values of posterior probability of the  
 480 data ( $\text{LnP(D)}$ ) from 20 runs for each value of  $K$  (1–9) and Evanno's delta  $K$  in the STRUCTURE  
 481 analysis. (C) Population structure estimated in STRUCTURE. Barplots display the proportion of the  
 482 membership coefficient in the inferred clusters at  $K = 2$  and 4 for all individuals, and the numbers  
 483 indicate the population IDs.

484

485 **Fig. 2** Comparison of genetic diversity between west and east subpopulations. (A, B) Boxplots  
 486 showing differences in the level of allelic richness (A) and expected heterozygosity (B) between the

487 western and eastern populations. (C, D) Approximate Bayesian skyline plots showing the demographic  
488 history of *C. hangiongensis* for west (C) and east (D) subpopulations. The solid line shows the median  
489 estimates of historical effective population size ( $N_e$ ), and the dashed lines show the 95% highest  
490 posterior density estimates of the  $N_e$ .  
491



