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Author(s)	Aoki, Yuh; Nakajima, Souta; Nakamura, Futoshi
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1	Genetic structure and diversity of amphidromous sculpin in Shiretoko, a mountainous peninsula
2	in Japan
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4	Yuh Aoki [*] , Souta Nakajima ^{*,#} , Futoshi Nakamura
5	
6	Laboratory of Ecosystem Management, Graduate School of Agriculture, Hokkaido University, Kita-ku
7	N9W9, Sapporo, Hokkaido 060-8589, Japan
8	
9	* Contributed equally
10	# Corresponding author
11	Present address:
12	Watershed Restoration Team, Water Environment Research Group,
13	Public Works Research Institute, Minamihara 1-6, Tsukuba, Ibaraki 305-8516, Japan
14	Email: n.souta891@gmail.com, Phone: +81-29-879-6775
15	ORCID: https://orcid.org/0000-0003-3701-5428
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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

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

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

2017). Considering that diadromous fish usually exhibit high mobility and connectivity between
neighboring tributaries, it will be necessary to identify the group of strongly connected populations
(subpopulation structure) and to evaluate the genetic diversity of each subpopulation unit. In areas
where check dams are often placed near the mouth of rivers to prevent coastal residential areas from
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.

Given the need to understand the current status of diadromous sculpin populations where the habitat is decreasing, we conducted a population genetic study of *C. hangiongensis* in the Shiretoko 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 °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 µl reaction containing 5.0 µl of 2× Type-it Multiplex
- 119 PCR Master Mix, 1.0 µl of primer mix with 0.2 µM of each of forward and reverse primer, 2.0 µl of

120	RNase-free water, and 2.0 µl 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 Cgo1016, 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 (Δ 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 176N is the effective population size and μ is the mutation rate per generation), prior_THETA_max = 10,177prior_GSM_min = 0.1 (GSM is the generalized stepwise mutation model for microsatellites),178prior_GSM_max = 0.8, prior_T_max = 250 (maximum time, measured in number of generations),179prior_MUTRATE_min = 0.0001 (MUTRATE is the μ value; μ for microsatellites generally ranges180from 10⁻⁴ to 10⁻³ [Estoup and Angers 1998; Yue et al. 2007]), prior_MUTATE_max = 0.0010, and the181repeat size for each locus was specified (2 for all). All other options and priors were set to default182values.

183

184 **Results**

185 Summary statistics

186 $F_{\rm IS}$ 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, ΔK showed the highest value at K = 2, and LnP(D) increased until K =

198 4 (Fig. 1B). The population structure at K = 2 was quite clear, and each cluster corresponded with

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

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 $H_{\rm E}$ (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 $F_{\rm ST}$ 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*

246Genetic 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.

In contrast to the level of genetic diversity, BOTTLENECK and demographic analysis consistently showed that only the west subpopulation experienced recent bottlenecks (Fig. 2; Table 4). While genetic diversity often maintains equilibrium at low levels, experiencing a bottleneck indicates that genetic diversity is in decline and population viability is threatened (Teixeira and Huber 2021). In this case, although current genetic diversity in western populations is still high, it may be in the 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 DIYABCskylineplot 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, 274understanding 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

impacts, the conservation priority would be rather higher in the west region which has experienced arecent 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. 297 298 Acknowledgments 299 We are grateful to Takahiro Nobetsu and Taihei Yamada for providing us with local information. We 300 also thank Kaiji Suzuki for his help in the field sampling and Uchu Yamakawa for his advice on ocean 301 currents. This study was supported by JSPS KAKENHI Grant Number JP21H03647. 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.

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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	

Tables

Pop ID	Name of river	Latitude (N°)	Longitude (E°)	Ν	AR	$H_{ m E}$	$F_{\rm IS}$
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
Рорб	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

Table 1 Sampling localities and genetic diversity of each population.

462 N, number of individuals; AR, allelic richness; $H_{\rm E}$, expected heterozygosity; $F_{\rm IS}$, fixation index

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

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

465 ^a Loci amplified but not included in the dataset

467 **Table 3** Pairwise F_{ST} (below diagonal) and F'_{ST} (above diagonal) matrices among the nine populations

		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
Рорб	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	

468 of C. hangiongensis.

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.

	Mutation model					
Subpopulation	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 (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



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 $N_{\rm e}$.
- 491



