Genetic diversity and population structure of razor clam *Sinonovacula constricta* in Ariake Bay, Japan, revealed using RAD-Seq SNP markers.

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Abstract:

The razor clam *Sinonovacula constricta* is a commercially important bivalve in Japan. The current distribution of this species in Japan is limited to Ariake Bay, where the fishery stock is declining. It is necessary to understand the genetic population structure of this species in order to restore the fishery stock while preserving the genetic diversity of the clam. Here, we report for the first time the genetic population structure of *S. constricta* in Ariake Bay, Japan. Paired-end restriction site-associated DNA sequencing (RAD-Seq) analyzed samples of *S. constricta* collected from seven mudflats located along Ariake Bay. Two different genetic populations exist in Ariake Bay, one inhabiting wild habitats and the other inhabiting the transplanted area of artificial seedlings. Our results suggest that genetic differentiation occurred between these two populations (F_{st} value = 0.052), and a high level of genetic differentiation is maintained between the two groups. In the future, the two genetically distinct populations need to be available as fishery resources, while taking into account their conservation and hybridization status.

Key words: Aquaculture, Bivalve, Seedlings**,** SNP markers, Stock enhancement, RAD-Seq

1. Introduction

The technique of artificially producing seedlings and releasing them into fishing grounds is an effective method of aquaculture enhancement. This technique has been implemented with a variety of fishery species (e.g., finfish, Fushimi, 2001; Masuda and Tsukamoto, 1998; flounder, Sekino et al., 2005; shellfish, Arnold, 2001; Castell et al., 1996; McCormick et al., 1994; Zohar et al., 2008). In artificial seed production, large numbers of seedlings are often produced by a few broodstocks and released into natural seawater, which reduces the genetic diversity of wild populations in the field. Therefore, when seedlings are produced and released into natural seawater, there is a need to understand the genetic structure within the wild population in order to preserve genetic diversity.

The razor clam *Sinonovacula constricta* (Lamarck, 1818) is an important economic species in East Asian coastal waters (Funge-Smith et al., 2012; Mao et al., 2019; Ran et al., 2017). Shell records suggest that *S. constricta* was once distributed in tidal flats in western Japan (Suzuki et al., 2006), but its current distribution is limited to

44 the mudflats around Ariake Bay located on the west coast of Kyusu, Japan. According
45 to a report by Ministry of the Environment, Japan (2006), the annual catch of *S.*
46 *constricta* in Ariake Bay often recorded more than 1000 tons per year from the 1900s to
47 the early the 1920s, but it decreased to less than 500 tons in most years from the latter
48 half of the 1920s to the latter half of the 1980s; no catches have been made since 1994.
49 In order to restore the *S. constricta* resources, the Saga Prefectural Ariake Fisheries
50 Research and Development Center has developed seed production techniques (Ito et al.,
51 2001; Ohkuma et al., 2004), and since 1988, artificial seedlings of *S. constricta* have
52 been released into the transplanting areas established in natural seawaters (Tsukuda et
53 al., 2017). However, the genetic population structure of *S. constricta* within Ariake Bay
54 has not been examined.

55 In recent years, technological innovations have made the genome-wide analysis
56 of non-model organisms more widely available, and these techniques are useful for
57 examining the genetic population structure of non-model aquatic organisms (Holman et
58 al., 2017; Li and Wang, 2017; Robledo et al., 2018; You et al., 2020). In the present

59 study, to clarify the genetic population structure of *S. constricta* in Ariake Bay, we
60 conducted field sampling surveys at 11 mudflats located along the bay in 2019 and used
61 restriction-site-associated DNA sequencing (RAD-Seq) to analyze population genetics
62 and phylogenetics. We propose guidelines concerning the optimal seed production and
63 seed release methods for the conservation of the genetic diversity of *S. constricta* in
64 Japan.
65

66 **2. Materials and methods**

67 *2.1. Sampling and genomic DNA extraction*

68 Sampling of the razor clam *S. constricta* was conducted at 11 mudflats located
69 along Ariake Bay (Fig. 1). Information on the sampling day and habitat types at each
70 site is shown in Table 1. Habitats are divided into two types: transplanted area of
71 artificial seedlings, and wild habitat. As mentioned above, artificial seedlings of *S.*
72 *constricta* have been released into the transplanting areas established in natural
73 seawaters since 1988. Therefore, artificially produced individuals live within the

74 transplanted area, and the wild habitat would be populated by natural individuals and

75 offspring of seedlings from the transplanted areas. At each site, razor clams were

76 explored by hand and their abundance was assessed using the catch per unit effort index

77 (CPUE), calculated as the number of clam individuals collected divided by the number

78 of explorers and sampling time (i.e., ind. person^{-1} hour^{-1}). The samples were stored at

79 -80°C until genomic DNA extraction. In addition to the collected individuals, the oldest

80 seedlings (produced in 2005, n = 2) stored at the Saga Prefectural Ariake Fisheries

81 Research and Development Center, were included in the RAD-Seq analysis for

82 comparison with seedlings produced in the past. Genomic DNA was extracted from

83 finely chopped foot tissue of the specimens using DNAs-ici!-F (RIZO, Tsukuba, Japan),

84 and the concentration of the DNA was assessed using a Qubit 3.0 Fluorometer (Thermo

85 Fisher Scientific).

86

87 *2.2. RDA-Seq analysis*

88 The preparation for *the Eco*RI RAD-Seq library has been described previously
89 (Kato et al., 2020), based on the established method (Etter et al., 2011). The library was
90 sequenced with 150 bp paired-end reads in one lane of an Illumina HiSeq X (Illumina,
91 San Diego, CA, USA). The RAD-Seq data were analyzed using the Stacks program
92 (version 2.52) (Rochette and Catchen, 2017). The data were quality filtered using the
93 process_radtags program in the Stacks package (-r -c -q, - adapter_1, adapter_2, and
94 adapter_mm2). Filtered sequences were registered in the DRA (DRA accession number:
95 DRA010922). The data were aligned with the reference genome of *S. constricta* (Ran et
96 al., 2019; NCBI/DDBJ BioProject number: PRJNA508451) using BWA-MEM (version
97 0.7.17) (Li, 2013). Aligned data were analyzed using the ref_map.pl script of the Stacks
98 package.
99
100 *2.3. Population genetics analysis*
101 Principal component analysis (PCA) was conducted based on a variant call
102 format (VCF) file using the SNPrelate program (v.1.18.1) (Zheng et al., 2012). The

103 VCF file was created by the populations program of the Stacks package

104 (--rm-pcr-duplicates, -R 0.7, --write-single-snp, --min-maf 0.05) (Danecek et al., 2011).

105 The admixture analysis was conducted based on the PLINK file using ADMIXTURE (v.

106 1.3.0) (Alexander et al., 2009). The PLINK file was created with the same Stacks

107 populations program that created the VCF file (Purcell et al., 2007). The genetic

108 diversity of razor clams among and within populations was assessed using AMOVA F_{st}

109 values obtained from 'populations.fst_files', which were calculated by the Stacks

110 populations program. To visualize the distribution of all data points, pairwise F_{st} values

111 between individuals in each population are shown in boxplot with beeswarm using R

112 (package: 'beeswarm', Eklund, 2016).

113

114 *2.4. Phylogenetic tree analysis*

115 Phylogenetic tree analysis was performed using two different approaches: the

116 maximum likelihood (ML) method and SVDquartets analysis. Multiple alignments

117 within the cluster were obtained using the Stacks package populations program

118 mentioned earlier. The phylogenetic tree based on the ML method was constructed

119 using the RAxML program (version 8.2.12) (Stamatakis, 2014) (-f = a, -x = 12 345, -p =

120 12 345, -N (bootstrap value) = 1000, and -m = GTRGAMMA). The SVDquartets

121 analysis based on the coalescent model was computed as implemented in PAUP ver.

122 4.0a 167 using multiple alignments (Chifman and Kubatko, 2014; Swofford, 2002). The

123 number of bootstrap analyses replicates was 1000. In each analysis, the oldest samples

124 (i.e., sample ID, 2005.1; 2005.2), were used as the root.

125

126 **3. Results**

127 *3.1. Sampling data*

128 We collected 28 razor clams from seven of the 11 mudflats and found no razor

129 clams on the remaining four mudflats (Table 1). The mean CPUE of the seven mudflats

130 where individuals could be collected was 3.2 (± 3.0 SD) ind. person^{-1} hour^{-1}, and the

131 range of CPUE at each site was 0.3 to 8.5 ind. person^{-1} h $^{-1}$. The shell length of the

132 collected individuals was 39.7 to 94.6 mm, implying that they contained specimens

133 from 1 to 5 years old. The mean shell length of individuals collected from the
134 transplanted area was 86.0 (± 4.5 SD) mm for the 3-year-old seedlings produced in
135 2016 (i.e., sample ID: 2016.1-2016.7) and 92.9 (± 1.1 SD) mm for the 5-year-old
136 seedlings produced in 2014 (i.e., sample ID: 2014.1-2014.5).
137
138 *3.2. RAD-Seq data*
139 We used 30 individuals for RAD-Seq analysis, including 16 individuals
140 collected from wild habitats, 12 individuals collected from the transplanted area of
141 artificial seedlings, and 2 individuals stored at the Saga Prefectural Ariake Fisheries
142 Research and Development Center (Table 1). In all, 401 109 332 raw reads were
143 generated from the HiSeq X run, and 249 040 001 reads were retained after discarding
144 reads with low quality and adapters. The final dataset included 137 246 loci, with an
145 average of 679.38 bp (SE ± 0.18) of genotyped sites per locus.
146
147 *3.3. Population genetics structure*

148 The genetic relationships among sampling sites were clearly divided into
149 individuals collected from wild habitat and those from the transplanted area of artificial
150 seedlings along the axis of PC1 (Fig. 2 (a)). The PC2 axis represents the differences
151 within each habitat type. In the PCA plot, the coordinates of the transplanted samples
152 were more widely scattered than those of the wild samples were, showing more diverse
153 genetic relationships among transplanted samples. There were no distinct genetic
154 relationships among generations, and the generation with a shell length of 75 to 90 mm
155 showed the most genetic diversity (Fig. 2(b)). The results of admixture analysis also
156 showed a significant genetic difference between the individuals collected from the wild
157 versus those collected from the transplanted areas of artificial seedlings (Fig. 3). The F_{st}
158 value between populations consisting of individuals collected from the wild and the
159 transplanted areas was 0.052. Owing to the calculation of the pairwise F_{st} values for
160 each individual within the two populations, there was no significant difference in the
161 mean values between these populations (Fig. 4). The pairwise F_{st} values within the

162 population were more varied in the transplanted area (from 0.44 to 0.64) than in the

163 wild habitat (from 0.53 to 0.56).

164

165 *3.4. Phylogenetic tree analysis*

166 ML tree and SVDquartets analysis of RAD-Seq data showed that the

167 phylogenies were highly consistent with each other (Fig. 5(a) and (b)). The obtained

168 phylogenetic trees were clearly divided into individuals collected from the wild habitat

169 and those collected from the transplanted area of artificial seedlings, with the exception

170 of one sample (i.e., sample ID: 2016.7).

171

172 **4. Discussion**

173 *4.1. Genetic population structure of S. constricta in the Ariake Bay, Japan*

174 All our analyses of the RAD-Seq data showed that individuals collected from

175 wild habitats were genetically different from those collected from sites where artificial

176 seedlings were transplanted. Only one sample collected from the area transplanted in

177 2016 (sample ID: 2016.7) showed different results from other seedlings collected in the
178 same area, and belonged to a population consisting of individuals collected from the
179 wild habitat (Fig. 1(a), Fig. 3, 4). Since this individual had a distinctly larger shell
180 length than other 3-year-old seedlings in the same area, and its size was the same as that
181 of 5-year-old seedlings (Table 1), it was likely a wild individual inhabiting the
182 transplanted area prior to the 2016 transplantation. Therefore, we consider that there
183 were two genetically distinct populations in Ariake Bay in 2019.

184 The reason for the differences between these two populations is the different
185 parental origins of the seedlings. The parental origins can be divided into the Nanaura
186 mudflat and the Kashima mudflat for a number of reasons. Artificial seedlings of *S.*
187 *constricta* have been released by the Saga Prefectural Ariake Fisheries Research and
188 Development Center into transplanting areas established in several mudflats along the
189 coast of Ariake Bay. The seedlings collected from the transplanted area in this study
190 (Takori_b and Takori_c) originated from parents at the Nanaura mudflat (hereinafter
191 referred to as Nanaura lineage population), since the parents were collected from the

192 Nanaura mudflat before 2010 and released into the transplanting areas of the Takori
193 mudflat. In contrast, individuals collected from the wild habitat of Kashima, Nanaura,
194 and Takori mudflats are likely to have originated from parents of Kashima mudflat
195 (hereinafter referred to as the Kashima lineage population). In 2017 and 2018, the
196 seedlings produced by parents collected from Kashima mudflat were released into new
197 transplanting areas at Takori and Nanaura mudflats due to declining populations in wild
198 and transplanted areas in these mudflats. Therefore, the 1- to 2-year old individuals
199 collected in wild habitats in 2019 were likely the offspring of the transplanted Kashima
200 seedlings in 2017 and 2018.

201 Artificial transplantation of aquatic organisms has been reported to alter the
202 geographic genetic structure of wild populations (Arthington, 1991; Habtemariam et al.,
203 2015; Williams and Orth, 1998; Yamakawa and Imai, 2013), including that of *S.*
204 *constricta,* in this case along the Chinese coast (Niu et al., 2012). Anthropogenic
205 transplantation likely strongly influenced the distribution of the genetic population
206 structure of *S. constricta* in Japan. From the dataset of this study, it is unclear why the

207 origins of the parents differed between the Kashima mudflat and the Nanaura mudflat,

208 which are in close proximity to each other. In future analyses, comparisons with

209 ancestral samples from the coast of the continent of China may lead to further

210 understanding of the phylogenetic relationship between these two populations.

211

212 *4.2. Genetic differentiation among and within populations*

213 For artificial seed production and release of seedlings, it is necessary to

214 understand the distribution of populations with different genetic structures in natural

215 seawater. Therefore, it is necessary to select broodstocks and release areas according to

216 the degree of genetic differentiation among and within populations. F_{st} values are

217 widely used as a measure of the degree of genetic differentiation among and within

218 populations (DeFaveri et al., 2012; Jeffery et al., 2017; Kato et al., 2020). According to

219 Niu et al. (2012), the F_{st} value among populations of *S. constricta* at 10 sites along the

220 coast of mainland China is 0.044. The F_{st} value between the two populations in the

221 present study was 0.052, suggesting that a similar level of genetic differentiation
222 occurred between the two populations in Ariake Bay, Japan.

223 For these two populations, the genetic differentiation within each population
224 was maintained at a high level, and its variation was greater within the population
225 consisting of individuals collected from the transplanted area (Fig. 4). There are two
226 possible reasons why the variation in the degree of genetic differentiation within
227 populations differed between the two populations. The first is that the degree of
228 variation in genetic differentiation in these populations originally may have been
229 different. Second, the methods of seedling production may lead to greater variation of
230 genetic differentiation within populations in the transplanted area. In seedling
231 production, broodstocks were randomly selected with closely related individuals
232 collected from the same transplanted area or distantly related individuals collected from
233 different transplanted areas. After stimulating the mature individuals to spawn, 20 to 30
234 individuals were placed in the same tank to produce fertilized eggs. Thus, the degree of

genetic differentiation may have been more varied in the transplanted populations than in the wild habitat population.

4.3. Guidelines concerning the best seed production and seed release methods

The current abundance of *S. constricta* is estimated to be very low based on the CPUE results of this study (Table 1). These results suggest that artificial seed production and release of seedlings could be an effective strategy for the recovery of *S. constricta* abundance in Ariake Bay, Japan. The present study suggested genetic differentiation between the two populations of *S. constricta* in Ariake Bay, and a high level of genetic differentiation within each population was maintained. Therefore, maintaining the two populations without interbreeding seems to be an appropriate conservation strategy. As of 2019, the Kashima lineage population is widely distributed in wild habitats, whereas the Nanaura lineage population is limited to the Takori mudflats. In order to avoid hybridization with the Kashima lineage population, the

249 release of seedlings of the Nanaura lineage population should continue to be restricted

250 to the mudflats within the current cove, with a transplanting area.

251 To produce seedlings to slow down the decline in genetic diversity within a

252 population, mathematical models have evaluated five strategies: I) avoidance of line

253 breeding, II) selective use of wild-born individuals, III) line breeding, IV) avoidance of

254 artificial breeding of males, and V) selective use of wild-born males (Yokota et al.,

255 2003). Among them, strategy II has been considered the most effective. The best way to

256 produce seedlings of the Kashima lineage population would be to follow strategy II; all

257 parental individuals would be randomly collected from wild habitats and previously

258 produced seedlings would not be used as parents. In contrast, the Nanaura lineage

259 population has already been maintained by a similar approach to strategy I; all parental

260 individuals will be randomly collected from transplanted areas without excluding

261 released seedlings. This approach gradually reduces the genetic diversity within the

262 population. Indeed, lower pairwise F_{st} values were detected in the transplanted

263 population (Fig. 4). Therefore, in the Nanaura lineage population, countermeasures

264 should be taken to increase the number of parents used for seed production or to avoid
265 using the produced seedlings as parents of the next generation.
266 RAD-Seq analysis is an inexpensive way to identify populations, but it requires
267 a lot of manipulation and time. Several DNA markers, such as SSRs, SNPs, and InDels,
268 have been developed for simple and inexpensive identification of populations, and these
269 techniques are useful for monitoring the genetic hybridization of each population in a
270 short period of time (Liu and Cordes, 2004; Maqsood and Ahmad, 2017). For example,
271 PCR and agarose gel electrophoresis alone can detect short indels (Noda et al., 2020).
272 Monitoring the interbreeding status of the two genetically distinct populations and the
273 genetic differentiation within each population is important to enhance the stock of *S.*
274 *constricta* in Japan by producing and releasing seedlings, while preserving genetic
275 diversity.
276
277 **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19, 1655–1664. Available online: https://pubmed.ncbi.nlm.nih.gov/19648217/.

293 Arnold, W.S., 2001. Bivalve enhancement and restoration strategies in Florida, USA.
294 Hydrobiologia 465, 7–19. https://doi.org/10.1023/A:1014596909319.
295 Arthington, A.H., 1991. Ecological and genetic impacts of introduced and translocated
296 freshwater fishes in Australia. Can. J. Fish. Aquat. Sci. 48, 33–43.
297 https://doi.org/10.1139/f91-302.
298 Castell, L.L., Naviti, W., Nguyen, F., 1996. Detectability of cryptic juvenile *Trochus*
299 *niloticus* Linnaeus in stock enhancement experiments. Aquaculture 144, 91–101.
300 https://doi.org/10.1016/S0044-8486(96)01320-8.
301 Chifman, J., Kubatko, L., 2014. Quartet inference from SNP data under the coalescent
302 model. Bioinformatics 30, 3317–3324.
303 https://doi.org/10.1093/bioinformatics/btu530.
304 Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A.,
305 Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R.,
306 1000 Genomes Project Analysis Group, 2011. The variantcall format and VCFtools.
307 Bioinformatics 27, 2156–2158. http://doi.org/10.1093/bioinformatics/btr330.

308 DeFaveri, J., Shikano, T., Ghani, N.I.A., Merilä, J., 2012. Contrasting population
309 structures in two sympatric fishes in the Baltic Sea basin. Mar. Biol. 159, 1659–
310 1672. https://doi.org/10.1007/s00227-012-1951-4.
311 Eklund, A., 2016. R package beeswarm: the bee swarm plot, an alternative to stripchart.
312 Available online: https://cran.r-project.org/web/packages/beeswarm/beeswarm.pdf.
313 Etter, P.D., Bassham, S., Hohenlohe, P.A., Johnson, E.A., Cresko, W.A., 2011. SNP
314 discovery and genotyping for evolutionary genetics using RAD sequencing, in:
315 Molecular methods for evolutionary genetics. Methods Mol Biol. Humana Press
316 772, 157–178. https://doi.org/10.1007/978-1-61779-228-1_9.
317 Funge-Smith, S., Briggs, M., Miao, W., 2012. Regional Overview of Fisheries and
318 Aquaculture in Asia and the Pacific 2012. RAP Publication (FAO).
319 Fushimi, H., 2001. Production of juvenile marine finfish for stock enhancement in
320 Japan. Aquaculture 200, 33–53. https://doi.org/10.1016/S0044-8486(01)00693-7.
321 Habtemariam, B.T., Arias, A., García-Vázquez, E., Borrell, Y.J., 2015. Impacts of
322 supplementation aquaculture on the genetic diversity of wild *Ruditapes decussatus*

323 from northern Spain. Aquacult. Environ. Interact. 6, 241–254.
324 https://doi.org/10.3354/aei00128.
325 Holman, L.E., Garcia de la serrana, D., Onoufriou, A., Hillestad, B., Johnston, I.A.,
326 2017. A workflow used to design low density SNP panels for parentage assignment
327 and traceability in aquaculture species and its validation in Atlantic salmon.
328 Aquaculture 476, 59–64. https://doi.org/10.1016/j.aquaculture.2017.04.001.
329 Ito, S., Eguchi, T., Kawahara, I., 2001. Rearing experiment on planktonic larvae of the
330 JackKnife clam, *Sinonovacula constricta*. Boll. Saga Prefect. Ariake Fish. Res. Dev.
331 Cent. 20, 49–53. Available online:
332 https://agriknowledge.affrc.go.jp/RN/2010813185.pdf.
333 Jeffery, N.W., DiBacco, C., Van Wyngaarden, M., Hamilton, L. C., Stanley, R.R.,
334 Bernier, R., FitzGerald, J., Matheson, K., McKenzie, C.H., Ravindran, P.N., Beiko,
335 R. Bradbury, I.R., 2017. RAD sequencing reveals genomewide divergence between
336 independent invasions of the European green crab (*Carcinus maenas*) in the
337 Northwest Atlantic. Ecol. Evol. 7, 2513–2524. https://doi.org/10.1002/ece3.2872.

338 Kato, D., Suzuki, H., Tsuruta, A., Maeda, J., Hayashi, Y., Arima, K., Ito, Y., Nagano,
339 Y., 2020. Evaluation of the population structure and phylogeography of the
340 Japanese Genji firefly, *Luciola cruciata*, at the nuclear DNA level using RAD-Seq
341 analysis. Sci. Rep. 10, 1533. https://doi.org/10.1038/s41598-020-58324-9.
342 Li, H., 2013. Aligning sequence reads, clone sequences and assembly contigs with
343 BWA-MEM. arXiv preprint arXiv:1303.3997. Available online:
344 https://arxiv.org/abs/1303.3997.
345 Li, Y.H., Wang, H.P., 2017. Advances of genotyping-by-sequencing in fisheries and
346 aquaculture. Rev. Fish Biol. Fish. 27, 535–559.
347 https://doi.org/10.1007/s11160-017-9473-2.
348 Liu, Z.J., Cordes, J.F., 2004. DNA marker technologies and their applications in
349 aquaculture genetics. Aquaculture 238, 1–37.
350 https://doi.org/10.1016/j.aquaculture.2004.05.027.
351 Mao, Y., Lin, F., Fang, J., Fang, J., Li, J., Du, M., 2019. Chapter 4. Bivalve production
352 in China, in: Goods and Services of Marine Bivalves. Springer, Cham, pp. 51–72.

Maqsood, H.M., Ahmad, S.M., 2017. Advances in molecular markers and their applications in aquaculture and fisheries. Genet. Aquat. Org. 1, 27–41. Available online: http://www.genaqua.org/uploads/pdf_1.pdf.

Masuda, R., Tsukamoto, K., 1998. Stock enhancement in Japan: review and perspective. Bull. Mar. Sci. 62, 337–358. Available online: https://www.ingentaconnect.com/content/umrsmas/bullmar/1998/00000062/00000002/art00005.

McCormick, T.B., Herbinson, K., Mill, T.S., Altick, J., 1994. A review of abalone seeding, possible significance and a new seeding device. Bull. Mar. Sci. 55, 680–693. Available online: https://www.ingentaconnect.com/content/umrsmas/bullmar/1994/00000055/f00200002/art00035.

Ministry of the Environment, Japan., 2006. Committee Report. Ministry of the Environment, Bay, A., Sea, Y. Comprehensive Survey Evaluation Committee, 52 pp. Available online: https://www.env.go.jp/council/20ari-yatsu/rep061221/all.pdf.

368 Niu, D.H., Feng, B.B., Liu, D.B., Zhong, Y.M., Shen, H.D., Li, J.L., 2012. Significant
369 genetic differentiation among ten populations of the razor clam *Sinonovacula*
370 *constricta* along the coast of china revealed by a microsatellite analysis. Zool. Stud.
371 51, 406–414. Available online: http://zoolstud.sinica.edu.tw/Journals/51.3/406.pdf.
372 Noda, T., Daiou, K., Mihara, T., Nagano, Y., 2020. Development of indel markers for
373 the selection of *Satsuma mandarin* (*Citrus unshiu* Marc.) hybrids that can be used
374 for low-cost genotyping with agarose gels. Euphytica 216, 1–13.
375 https://doi.org/10.1007/s10681-020-02654-2.
376 Ohkuma, H., Ymagachi, T., Kawahara, I., Eguchi, T., Ito, S., 2004. A study on the
377 development of techniques for mass production of seeds of jackknife clam,
378 *Sinonovacula constricta*. Boll. Saga Prefect. Ariake Fish. Res. Dev. Cent. 22, 47–
379 54. Available online: https://agriknowledge.affrc.go.jp/RN/2030813210.pdf.
380 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D.,
381 Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a

382 tool set for whole-genome association and population-based linkage analyses. Am.
383 J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795.
384 Ran, Z., Chen, H., Ran, Y., Yu, S., Li, S., Xu, J., Liao, K., Yu, X., Zhong, Y., Ye, M.,
385 Yan, X., 2017. Fatty acid and sterol changes in razor clam *Sinonovacula constricta*
386 (Lamarck 1818) reared at different salinities. Aquaculture 473, 493–500.
387 https://doi.org/10.1016/j.aquaculture.2017.03.017.
388 Ran, Z., Li, Z., Yan, X., Liao, K., Kong, F., Zhang, L., Cao, J., Zhou, C., Zhu, P., He, S.,
389 Huang, W., Xu, J., 2019. Chromosome level genome assembly of the razor clam
390 *Sinonovacula constricta* (Lamarck, 1818). Mol. Ecol. Resour. 19, 1647–1658.
391 https://doi.org/10.1111/1755-0998.13086.
392 Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., Houston, R., 2018.
393 Applications of genotyping by sequencing in aquaculture breeding and genetics.
394 Rev. Aquac 10, 670–682. https://doi.org/10.1111/raq.12193.
395 Rochette, N.C., Catchen, J.M., 2017. Deriving genotypes from RAD-seq short-read data
396 using Stacks. Nat. Protoc. 12, 2640–2659. https://doi.org/10.1038/nprot.2017.123.

397 Sekino, M., Saitoh, K., Yamada, T., Hara, M., Yamashita, Y., 2005. Genetic tagging of
398 released Japanese flounder (*Paralichthys olivaceus*) based on polymorphic DNA
399 markers. Aquaculture 244, 49–61.
400 https://doi.org/10.1016/j.aquaculture.2004.11.006.
401 Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and
402 post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
403 https://doi.org/10.1093/bioinformatics/btu033.
404 Suzuki, T., Inoue, K., Ozawa, T., 2006. Environmental degradation in Ise and Mikawa
405 Bays after 1960's as viewed from intertidal molluscan community. Boll. Nagoya
406 Univ. Museum 22, 31–64. http://doi.org/10.18999/bulnum.022.04.
407 Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other
408 methods). Version 4. Sinauer Assoc. Sunderland, Massachusetts.
409 Tsukuda, M., Kanzaki, H., Tsujo, K., Fukumoto, T., Umeda, T., Aramaki, H., Ito, S.,
410 2017. Variation in the Distribution of the JackKnife Clam, *Sinonovacula constricta*,
411 on the Muddy Tidal flat of Ariake Sound off Saga Prefecture. Boll. Saga Prefect.

Ariake Fish. Res. Dev. Cent. 28, 47–49. Available online: https://agriknowledge.affrc.go.jp/RN/2010925685.pdf.

Williams, S.L., Orth, R.J., 1998. Genetic diversity and structure of natural and transplanted eelgrass populations in the Chesapeake and Chincoteague Bays. Estuaries 21, 118–128. https://doi.org/10.2307/1352551.

Yamakawa, A.Y., Imai, H., 2013. PCR-RFLP typing reveals a new invasion of Taiwanese Meretrix (Bivalvia: Veneridae) to Japan. Aquat. Invas. 8, 407–415. http://doi.org/10.3391/ai.2013.8.4.04.

Yokota, M., Harada, Y., Iizuka, M., 2003. Genetic drift in a hatchery and the maintenance of genetic diversity in hatchery-wild systems. Sci 69, 101–109. https://doi.org/10.1046/j.1444-2906.2003.00593.x.

You, X., Shan, X., Shi, Q., 2020. Research advances in the genomics and applications for molecular breeding of aquaculture animals. Aquaculture 526. https://doi.org/10.1016/j.aquaculture.2020.735357,

426 Zheng, X., Levine, D., Shen, J., Gogarten, S.M., Laurie, C., Weir, B.S., 2012. A
427 high-performance computing toolset for relatedness and principal component
428 analysis of SNP data. Bioinformatics 28, 3326–3328.
429 https://doi.org/10.1093/bioinformatics/bts606.
430 Zohar, Y., Hines, A.H., Zmora, O., Johnson, E.G., Lipcius, R.N., Seitz, R.D., Eggleston,
431 D.B., Place, A.R., Schott, E.J., Stubblefield, J.D., Chung, J.S., 2008. The
432 Chesapeake Bay blue crab (*Callinectes sapidus*): A multidisciplinary approach to
433 responsible stock replenishment. Rev. Fish. Sci. 16, 24–34.
434 https://doi.org/10.1080/10641260701681623.
435

436 Table 1. Sampling infromation for *Sinonovacula constricta* in the Ariake Bay, Japan

Sampling site	Sampling day	Habitat type	CPUE* (ind. person^{-1} h^{-1})	Sample ID	Shell length (mm)
Kashima_a	Nov. 11, 2019	Wild	0.3	Kashima_a.1	39.7
Kashima_b	Oct. 11, 2019	Wild	1.3	Kashima_b.1	46.6
				Kashima_b.2	45.2
				Kashima_b.3	54.7
				Kashima_b.4	54.0
Kashima_c	Nov. 19, 2019	Wild	4.0	Kashima_c.1	44.7
				Kashima_c.2	51.4
				Kashima_c.3	48.1
				Kashima_c.4	67.0
	Oct. 23, 2019	Wild	N/A†	Kashima_c.5	70.2
Kashima_d	Nov. 11, 2019	Wild	0.0	–	–
Nanaura_a	Nov. 13, 2019	Wild	0.7	Nanaura_a.1	47.0
				Nanaura_a.2	53.9
Tara_a	Nov.13, 2019	Wild	0.0	–	–
Takori_a	Nov. 15, 2019	Wild	1.8	Takori_a.1	75.0
				Takori_a.2	55.7
				Takori_a.3	81.7
				Takori_a.4	94.6
Takori_b	Nov. 15, 2019	Tranplanted area‡	5.5	2014.1	92.8
				2014.2	94.8
				2014.3	92.1
				2014.4	91.9
				2014.5	92.9
Takori_c	Nov. 15, 2019	Tranplanted area‡	8.5	2016.1	88.4
				2016.2	87.0
				2016.3	83.7
				2016.4	82.6
				2016.5	86.7
				2016.6	79.7
				2016.7	93.6

Higashiyoka_a	Nov. 12, 2019	Wild	0.0	–	–
Higashiyoka_b	Oct. 18, 2019	Wild	0.0	–	–
N/A†	Dec. 10, 2006	Tranplanted area‡	N/A†	2005.1	66.7
				2005.2	74.7

*: Catch per unit effort (individual person^{-1} hour^{-1})

†: Not available

‡: Transplanted area of airtficial seedlings

437

Figures

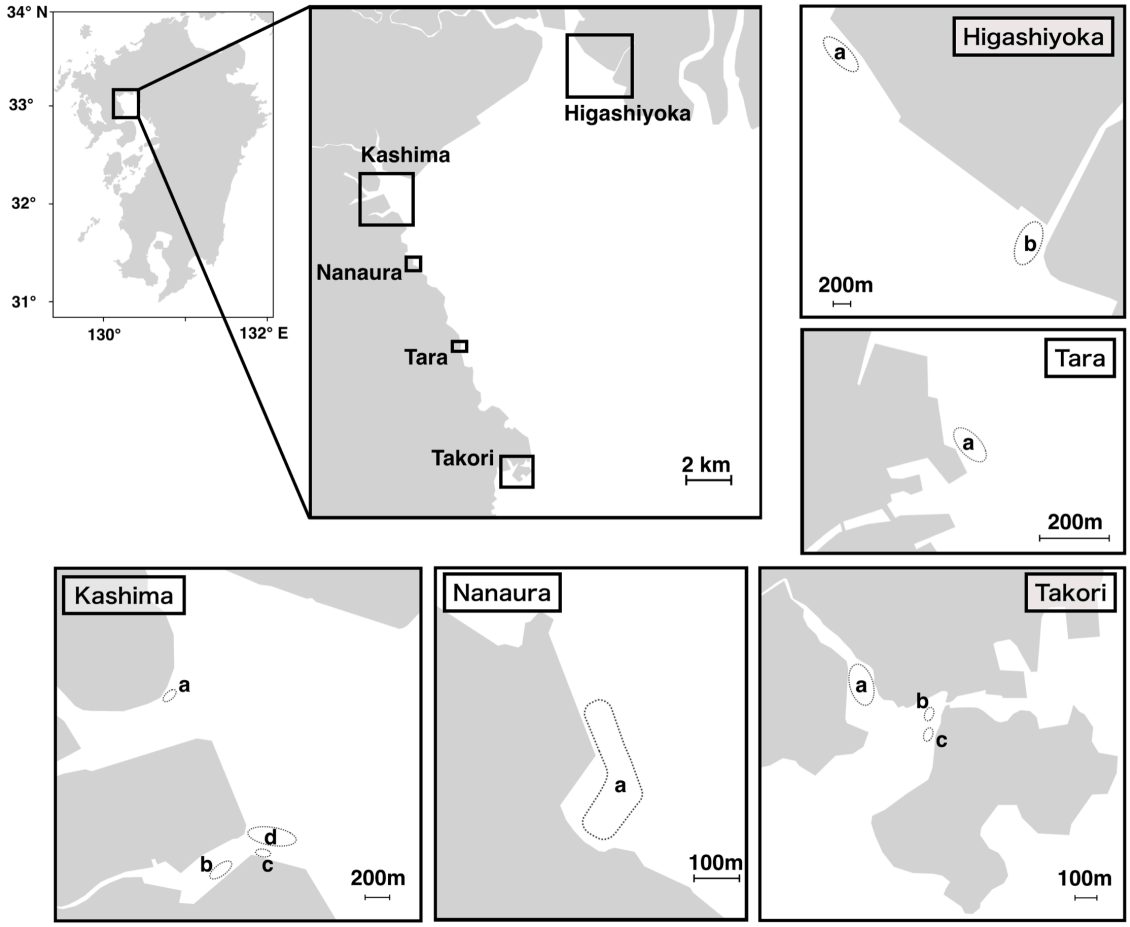


Fig. 1. Sampling sites. The dotted line shows the area explored.

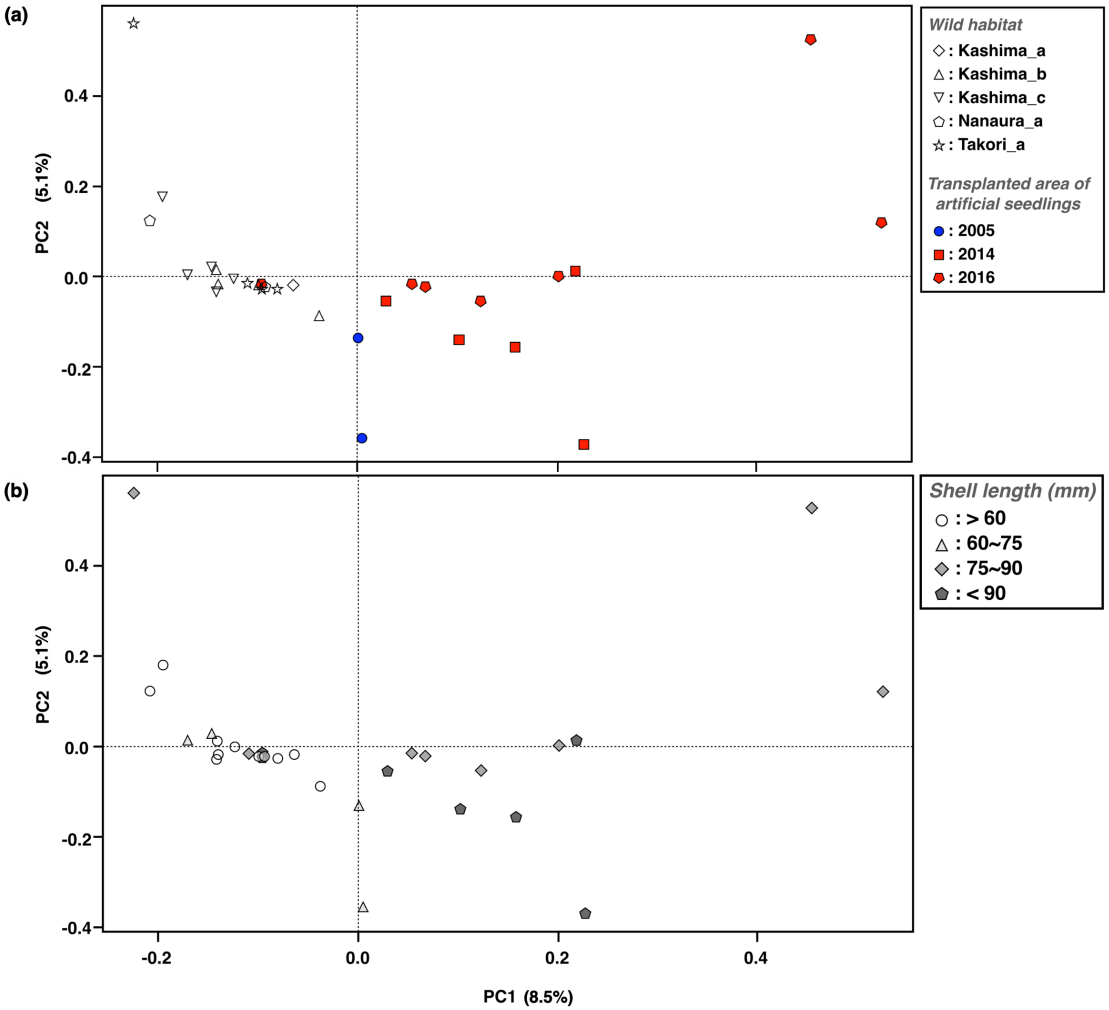


Fig. 2. Biplots of principal component analysis. (a) Showing by sampling areas. (b) Showing by generations.

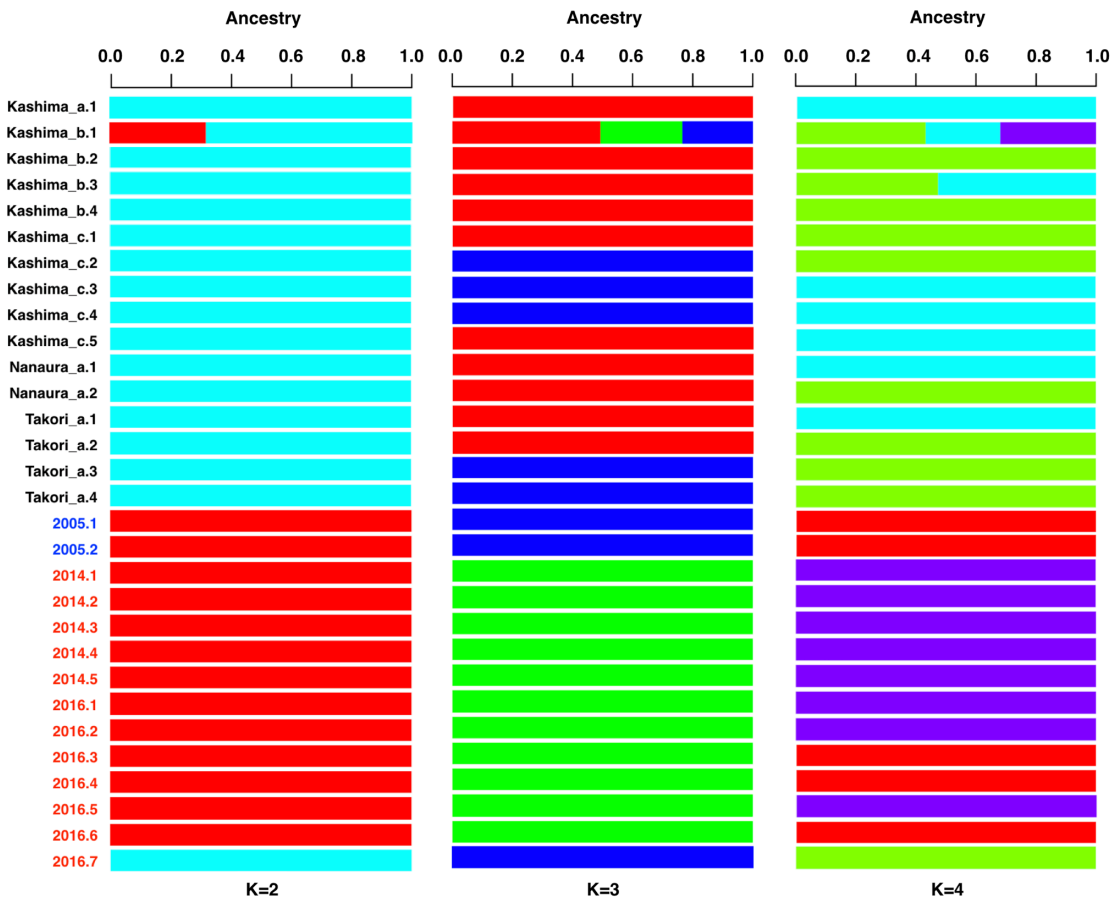
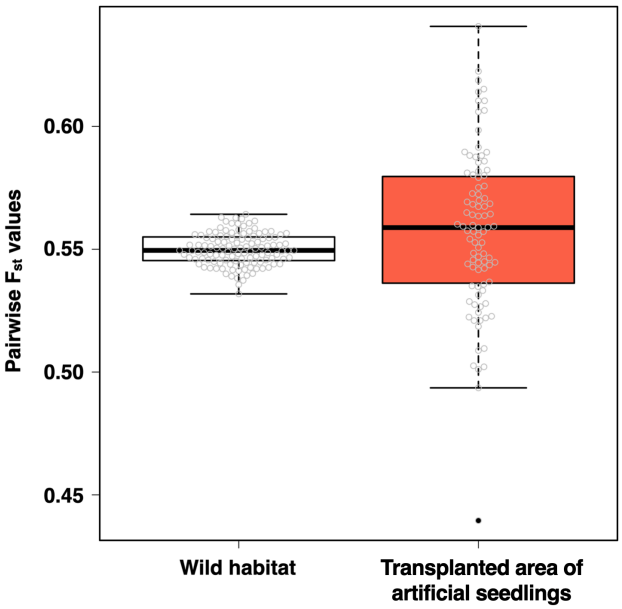


Fig. 3. Admixture analysis of *S. constricta* in the Ariake Bay, Japan.

448



449 **Fig. 4.** Pairwise F_{st} values between individuals in each population.

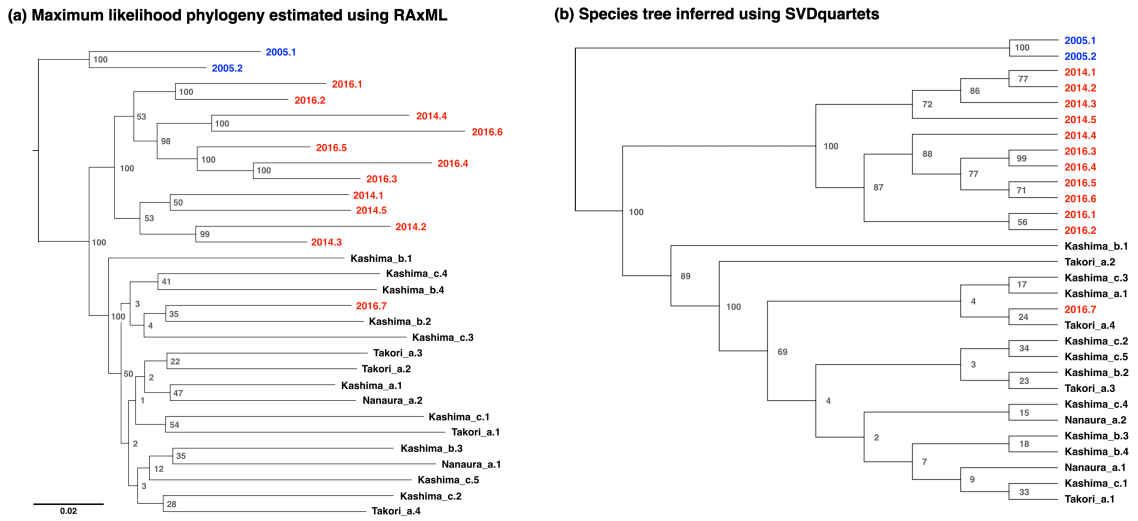


Fig. 5. Phylogenetic trees of *S. constricta* inferred from RAD-Seq dataset (a) Maximum likelihood phylogeny estimated using RAxML. (b) Species tree inferred using SVDquartets. Numbers at the nodes indicate bootstrap values (1000 replicates).