Hidden genetic history of the Japanese sand dollar *Peronella* (Echinoidea: Laganidae) revealed by nuclear intron sequences

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21 **Abstract**

22 The marine environment around Japan experienced significant changes during
23 the Cenozoic Era. In this study, we report findings suggesting that this dynamic
24 history left behind traces in the genome of the Japanese sand dollar species
25 *Peronella japonica* and *P. rubra*. Although mitochondrial Cytochrome C Oxidase I
26 sequences did not indicate fragmentation of the current local populations of *P.*
27 *japonica* around Japan, two different types of intron sequence were found in the
28 *Alx1* locus. We inferred that past fragmentation of the populations account for the
29 presence of two types of nuclear sequences as alleles in the *Alx1* intron of *P.*
30 *japonica*. It is likely that the split populations have intermixed in recent times;
31 hence, we did not detect polymorphisms in the sequences reflecting the current
32 localization of the species. In addition, we found two allelic sequences of the*Alx1*
33 intron in the sister species *P. rubra*. The divergence times of the two types of *Alx1*
34 intron sequences were estimated at approximately 14.9 and 4.0 million years ago
35 for *P. japonica* and *P. rubra*, respectively. Our study indicates that information
36 from the intron sequences of nuclear genes can enhance our understanding of
37 past genetic events in organisms.

38

39 Key words: Alx1, intron sequence, biogeography, *Peronella*, sea urchin

40

41 **Introduction**

42 Geographic history has a profound influence on the genetic structure of
43 organisms, and the intensity of the influence is dependent on their life history
44 (Avise 2000; Cowen and Sponaugle 2009). Populations of limited dispersal ability
45 are more likely to be fragmented due to geographic changes and are thus more
46 likely to record past geographic conditions in their genetic structure (Bohonak
47 1999; Cowen and Sponaugle 2009). In marine benthic metazoans, dispersal
48 potential is largely associated with early life history, i.e., type of larval
49 development (Cowen and Sponaugle 2009). It has been shown that the modes of
50 early life history have a significant influence even on the genomic evolution of
51 metazoans (Romiguier et al. 2014).

52 Sea urchins belong to a group of marine animals whose genetic evolution
53 as well as their life history has been extensively studied. Although most species of
54 sea urchin spend the planktotrophic larval stage as pluteus, some species skip the
55 planktotrophic stage and develop directly into juveniles (Strathmann 1978). This
56 process of direct development leads to restrictions in the gene flow (Hart 2002).
57 *Heliocidaris erythrogramma* is a sea urchin species that undergoes direct
58 development. This species has been shown to have a relatively fragmented
59 genetic population structure compared with the closely related species *H.*
60 *tuberculata*, which develops through planktotrophic pluteus larvae(McMillan et
61 al. 1992).

62 The Japanese sand dollar *Peronella japonica* is widely distributed in
63 shallow water along the Japanese coastline. The fertilized eggs of this species

develop into pluteus larvae with clearly elongated larval arms supported by the skeleton (Okazaki and Dan 1954). However, the digestive system does not differentiate properly in the larval body; they are thus lecithotropic, and the larval stage lasts only a few days before metamorphosis(Okazaki and Dan 1954). Therefore, this species has relatively limited dispersal ability, and its genetic structure is likely to be more prone to effects from changes in the marine environment.

The marine environment around Japan experienced dynamic changes during the Cenozoic Era. The main islands of Japan and the Ryukyu Archipelago were subjected to complex vertical and horizontal movement, as well as sea level changes caused by climate transitions, and these diastrophisms led to temporary fluctuations in the area of exposed land (Kizaki and Oshiro 1977, 1980; Ujiie 1990; Ota 1998). The East China and Japan Seas were established and subsequently collapsed in these geological movements, and subsequent bifurcations of warm ocean currents, such as the Kuroshio and Tsushima Currents, were established with the emergence of the two seas (e.g., Kizaki and Oshiro 1977; Chinzei 1986). These environmental changes would be expected to have affected the distributions of sea urchin populations.

In this study, we examined variations in mitochondrial and nuclear genes and elucidated phylogenetic relationships to determine whether geographic events along the Japanese coastline left behind traces in the sequence variations in *Peronella japonica* and its relatives. We also discuss the relationships of the paleogeographic and paleoenvironmental conditions during the Cenozoic Era

87 with the established phylogeny.

88

89

Materials and methods

DNA extraction, PCR amplification, and sequencing

Tissues were obtained from 26 and 14 individuals of *Peronella japonica* and *P. rubra,* respectively, taken from a total of five localities along the coastline around Japan (Table 1). We also obtained DNA from two clypeasterid species, *Astriclypeus manni* and *Clypeaster japonicus* belonging to other families of Clypeasteroida.

Genomic DNA was extracted from the gonads or sperm of *P. japonica* and from the mouth of *P. rubra, A. manni* and *C. japonicus* using a DNeasy tissue kit (Qiagen) following the manufacturer's protocol.

We initially analyzed COI haplotypes from 17 specimens of *P. japonica*. Polymerase chain reaction (PCR) amplification of the partial cytochrome c oxidase I (COI) gene was performed using Ex Taq DNA polymerase (Takara Bio, Inc., Tokyo, Japan) and echinoderm COI universal primers (Hoarearu and Boissin 2010) under the following conditions: 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR products were treated with ExoSAP-IT (Affymetrix) prior to the sequencing reactions. Sequencing reactions using a BigDye(r) Terminator v3.1 cycle sequencing kit (Applied Biosystems) and sequencing were performed by Eurofins Genomics (Tokyo) using an ABI 3730XL DNA analyzer (Applied Biosystems). The sequences were deposited in GenBank, the European Molecular Biology Laboratory (EMBL), and the DNA Data Bank of Japan (DDBJ) under the Accession Numbers provided in Table 1.

113 Amplification of the fourth intron of *Alx1* was performed with a Prime
114 Star GXL (Takara Bio, Inc.) using primers designed in the fourth and fifth exons
115 of *P. japonica Alx1* (Forward: 5'-GTTTCAAAAACAGAAGGGCAAAAT-3', Reverse:
116 5'-AGTCAAACTCCCTCCTCAGGTT-3'). The intron position was inferred by
117 comparison with the genome sequence of *Strongylocentrotus purpuratus*
118 *Alx1*(Sea Urchin Genome Sequencing Consortium 2006). The same set of primers
119 were used for amplification of the fourth intron of *P. rubra Alx1*. The amplified
120 DNA was sequenced as described for COI. Several additional primers were
121 designed for sequencing the full length of the *Alx1* intron. In order to analyze
122 haplotype distribution, additional specimens were analyzed for *Alx1* intron.
123
124 Phylogenetic analyses and estimation of divergence time
125 The alignments for two nuclear genes were determined based on the
126 maximum nucleotide similarity using a MEGA6 (Tamura et al. 2013) and MAFFT
127 v. 6.864 (Katoh and Toh 2008). Pairwise base differences were calculated using
128 PAUP* 4.0 beta 10 software (Swofford 2003).
129 To determine the phylogenetic relationships among COI haplotypes, we
130 performed phylogenetic analyses using the maximum likelihood (ML) and
131 Bayesian inference (BI) methods. Model selection for ML and BI analyses was
132 performed using the Akaike information criterion (AIC) in jModeltest v. 0.1.1
133 (Posada 2008). For ML and BI analyses, we also performed a partitioning scheme
134 following recent studies (e.g., (Brandley et al. 2005; Wiens et al. 2010). The
135 schemes used for the data were non-partition and three-partition strategies by

136 codon position. These strategies were assessed using ML implemented in
137 Treefinder, v. October 2008 (Jobb 2008). The three-partition strategy was selected
138 as optimal for the COI gene. In this strategy, SYM + I, F81 + G, and GTR + I + G
139 were selected as the best models for the first, second, and third positions,
140 respectively.

141 ML analysis was performed using Treefinder under the models selected
142 in the above process. The confidence of the branches in the ML was determined
143 using bootstrapping (Felsenstein 1985) with 1,000 replicates in Treefinder. Tree
144 topologies with bootstrap proportions of ≥70% were regarded as sufficiently
145 resolved nodes (Huelsenbeck and Hills 1993; Shaffer et al. 1997).

146 BI using the Markov chain Monte Carlo (MCMC) technique was also
147 performed using MrBayes 3.2 software (Ronquist et al. 2012). We initiated four
148 independent analyses with a random starting tree that ran for 10 million
149 generations. We used the program Tracer 1.5 (Rambaut and Drummond 2007) to
150 determine when the log likelihood of sampled trees reached stationary
151 distribution. Because apparent stationarity of the MCMC runs was reached at no
152 later than one million generations, we conservatively discarded the first 2.5
153 million generations from each run as burn-in, and sampled one of every 100
154 generations from the remaining 8 million generations to calculate the posterior
155 probability for each branch in the Bayesian tree. Bayesian posterior probabilities
156 (BPPs) ≥0.95 were considered significant support (Larget and Simon 1999)
157 (Huelsenbeck et al. 2001).

158 BEAST 1.8.0 (Drummond et al. 2012) was used with a relaxed clock

159 model and with a lognormal distribution and Yule process to obtain Bayesian
160 estimates of the timing of diversification events. The Hasegawa, Kishino, and
161 Yano (HKY) model (Hasegawa et al. 1985) was selected as the best model for both
162 noncoding sequences using jModeltest. The program ran for 10 million
163 generations, with sampling occurring every 1,000 generations for each analysis,
164 assuming the calibration point between *P. japonica* and *P. rubra* as 45.6 Myr BP
165 (see above). A burn-in of 20% was applied to obtain the node age estimates using
166 TreeAnnotator 1.8.0 (Drummond et al. 2012).
167
168

Results and Discussion

Mitochondrial DNA sequences did not resolve the genetic structure of *P. japonica*

We examined the partial COI sequences from a total of 17 individuals of *P. japonica* from five localities around Japan and obtained 10 haplotypes (Table 1, Fig. 1A, E). *P. japonica* distributes from the main island of Japan (Honshu) to Kyushu and Okinawa. We analyzed these sequences in three species of clypeasterid with that of *Strongylocentrotus purpuratus* as outgroups. In total, we analysed COI sequences from four species of Clypeasteroida (*P. japonica*, *P. rubra*, *Clypeaster japonicas*, *Astriclypeus manni*) and one Camarodonta (*Strongylocentrotus purpuratus*; Jacobs et al. 1988). The sequence differences within species were relatively low (0–1.9%, 0–13 bp), whereas the sequence differences among the four echinoid species ranged from 12.4 to 20.9% (84–141 bp).

Figure 2 shows the ML derived from 645 bp of mitochondrial COI gene. The BI tree (not shown) was almost identical to the ML tree. Monophyly was supported for the genus *Peronella* as well as *P. japonica* and *P. rubra* by high bootstrap values. However, the intraspecific variations within *P. japonica* were low. The populations of this species on the Japanese coastline seem to be slightly fragmented either due to recent divergences or because they are panmixed assemblages. Therefore, we focused on another genetic marker with a higher substitution rate and examined the intron sequences of the nuclear encoding gene *Alx1* in to investigate these two explanations for the divergence.

Two types of intron sequences in the nuclear genes of *P. japonica*

We inferred the exon–intron structure by referring to the genomic sequence of another species of sea urchin, *Strogyrocentrotus purpuratus* (Sea Urchin Genome Sequencing Consortium 2006). Using the *Alx1* sequence from Notojima specimens, we designed primers to amplify the fourth intron of *Alx1*. Unexpectedly, although a single PCR product was obtained from some specimens, PCR products of two different sizes were obtained from several specimens. Because two paralogs of Alx genes (*Alx1* and *Alx4*) were isolated from *S. purpuratus* (Koga et al. 2016), we examined the sequence of amplified products and confirmed that both came from *alx1* and not from *alx4* (Fig. 3). We sequenced the PCR products and found that the fourth intron of *Alx1* from *P. japonica* can be classified into two types: L(ong)-type and S(hort)-type (Table 1; Acc No. LC374910 for L-type (about 1.9kb) and LC374909 for S-type (about 1.5kb)). The sequences of the two types were alignable, although several gap inserts were observed (Fig. 3, SFig. 1). Overall, L-type and S-type showed 93.9 % sequence identity, excluding indels.

Heterozygous individuals were found in all sampling localities except Okinawa, where only L-type was observed. The haplotype distribution is summarized in Table 1. Except for Okinawa individuals, there appeared to be a uniform distribution of two haplotypes along the coastline of Japan.

Evolutionary origin of the two types of intron sequences

Because *P. rubra*, a sister species of *P. japonica*, is distributed throughout

215 the Japanese coastline, we reasoned that the occurrence of two intron haplotypes
216 in *P. japonica* may be due to introgression between the two species. These two
217 species can be distinguished by the morphology of periproct (Fig. 1A-D). To test
218 this hypothesis, we investigated the intron sequences in *P. rubra*, and found that
219 *P. rubra* also possess two types of sequence (Fig. 3; Acc. No. LC374913 for A-type
220 (about 1.5 kb) and LC374912 for B-type (about 1.9 kb); minor variation was
221 observed in B-type (Fig. S1; LC374911). However, the two types of sequence in *P.*
222 *rubra* were quite different from those in *P. japonica*. In contrast, the intraspecies
223 sequence similarity was higher, indicating that the two haplotypes were
224 generated via distinct genetic events in the two species (Fig. 3, 4). These
225 observations are clearly inconsistent with the idea that the two haplotypes in *P.*
226 *japonica* are due to an introgression event in *P. rubra*. Our observations of two
227 haplotypes in the intron sequences of *P. japonica* suggest that both species
228 experienced genetic separation into two distinct populations, followed by
229 interbreeding of the populations. In addition, *P. rubra* experience a similar, but
230 independent genetic separation followed by interbreeding.
231
232 Dating the generation of two types of intron sequences
233 To elucidate the geographic events corresponding to the genetic
234 separation, we estimated a divergence time between the two genotypes observed
235 in *Alx1*.
236 First, we estimated the divergence time between *P. japonica* and *P. rubra*
237 based on COI dataset. The divergence times between *Strongylocentrotus* and

238 *Peronera* were estimated at 200 Myr BP from fossil evidence (Smith et al. 2006).
239 This date was set as a calibration point for the separation of the two genera in the
240 analysis of the COI phylogeny. We obtained 45.6 Myr BP between *P. japonica* and
241 *P. rubra*. Therefore, we tentatively used this value as a calibration point between
242 the two species in the phylogeny of *Alx1*, as there were no reliable fossil data
243 directly applicable to the divergence between them.
244 Using intron sequences of the gene, initial divergences were determined
245 as 14.9 Myr BP and 4.0 Myr BP for *P. japonica* and *P. rubra*, respectively (Fig. 4).
246 The deduced divergence time of the *P. japonica* populations (14.9 Myr BP) may
247 have been influenced by the emergence and expansion of the Japan Sea in the
248 middle Miocene (ca. 15–14 Mya; Chinzei, 1986; but see Tamaki and Kobayashi
249 1988). However, fossils of *Peronella* species have not been recorded in sediments
250 of that age around the Japan Sea, whereas there are several descriptions from
251 around the East China Sea (e.g., Hayasaka and Morishita 1947; Nisiyama 1968).
252 Rather, the geographic history of the East China Sea appears more
253 compatible with the diversification of *P. japonica*. The East China Sea emerged
254 during the early Cenozoic (ca. 66 Mya: Kizaki and Oshiro 1977, 1980). After its
255 emergence, the East China Sea's connections with the Pacific Ocean were
256 interrupted by the Ryukyu Cordillera, although it is arguable whether the sea
257 was completely separated from the Pacific or was connected through straits (Ota
258 1998). The differentiation of two genotypes in *P. japonica* (14.9 Myr BP) appears
259 to have been affected by the opening of one or more straits and corresponding
260 inflows of warm ocean currents during the Miocene (23.0–5.3 Mya). In the early

261 Miocene (ca. 20 Myr BP), the East China Sea was connected with the Pacific
262 Ocean through straits, whereas the earlier sea was closed by the Ryukyu Arc in
263 the late Miocene (ca. 10 Myr BP) (Kizaki and Oshiro 1977, 1980). The ancestor of
264 this species is assumed to have originated in a relatively low-latitude marine
265 environment, somewhere on the subtropical coast of East Asia. It is probable that
266 the species invaded the East China Sea from the Pacific Ocean in the early
267 Miocene and then diversified genetically due to the isolation of the East China
268 Sea in the late Miocene. Therefore, the present coexistence of two nuclear
269 genotypes and the loss of variation in the mitochondrial haplotypes on the
270 Japanese coastline appear to be attributable to recent dispersals and re-mixing
271 by two strong ocean currents flowing from the southwest to northeast after the
272 insularization of the Ryukyu Archipelago and establishment of the Tsushima
273 Strait. This conclusion is based solely on the variation in a single gene of a single
274 genus, and a genome-wide examination of the nuclear intron sequences is
275 required to determine a more precise divergence time for the genotype splitting of
276 *Peronella*,. In addition, to infer the possible relationship between the population
277 splitting and paleogeography, the nuclear intron sequences should be analyzed in
278 several other marine species.
279 Mitochondrial DNA sequences and microsatellite sequences have been
280 used in most population genetic studies, and these markers have provided
281 abundant information on the genetic structure and evolutionary history of
282 organisms. Our study suggests that the nuclear intron sequence has the potential
283 to reveal the genetic history that cannot be identified from mitochondrial or

284 microsatellite sequences. Because mitochondrial DNA behaves as a single locus
285 and is inherited maternally, evidence of interbreeding can be easily erased. In
286 addition, the rapid evolution of microsatellites may hide the genetic record of past
287 genetic isolation followed by intermixing, similar to the events inferred in this
288 study. In this sense, it is notable that two distinct types of intron were found in
289 two species of *Peronella*. Additional cases of hidden genetic isolation might be
290 found by examining other nuclear intron sequences.
291
292 **Conclusions**
293 Although mitochondrial DNA sequences did not resolve the genetic
294 structures of *Peronella* japonica along the Japanese coastline, we unexpectedly
295 found two different alleles in the intron sequences of nuclear genes. The presence
296 of the two alleles suggests that genetic isolation occurred in the past and that the
297 two present-day populations are intermixed. Similar genetic isolation was also
298 indicated for *P. rubra*. We suggest that partial separation of the East China Sea
299 from the Pacific Ocean led to the split of the *Peronella* populations and that the
300 subsequent rise in sea level caused intermixing of the split populations. Our
301 study indicates that information from the intron sequences of nuclear genes can
302 enhance our understanding of past genetic events in organisms.
303

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305

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406 **Legends for Figures**

407 **Figure 1. Morphology of *P. japonica* and *P. rubra.***

408 *P. japonica*; Apical view (A) and periproct necked (B). *P. rubura*; Apical view (C)
409 and pericroct covered with spines (arrows) (D). bars, A and C = 1 cm; B and C = 1
410 mm. (D) Sampling locations.

411

412 **Figure 2. Phylogenetic tree constructed from mitochondrial COI sequences.**

413 Bootstrap proportions larger than 70% are shown above nodes. The tree was
414 made by ML method, and SYM + I, F81 + G, and GTR + I + G were selected as the
415 best models for the first, second, and third positions, respectively.

416

417 **Figure 3. Partial alignment of *Alx1* intron sequences.** (A, B) 5' and 3' end of the
418 fourth intron of *Alx1* is shown. Sequences identical to *P. japonica Alx1* mRNA are
419 shaded in black. Sequences identical to *P. japonica* intron S-type are shaded in
420 grey. (C) Diagram of exon-intron structure of Alx1 from S. purpuratus genome.

421

422 **Figure 4. Calibration of divergence time of *Alx1* intron alleles.** The divergence
423 time of the intron alleles were calculated referring the divergence time of *P.*
424 *japonica* and *P. rubra* estimated based on COI sequences.

425

426

427

428

429

Table1

Table 1. Samplimg localities and genotypes

specimens	Speceis	locality	sex	mtCOI Acc. No	Alx1_genotype
P_jponica_MjM1	*Peronella japonica*	Mukaijima	Male	LC374893	LL
P_jponica_MjM2	*Peronella japonica*	Mukaijima	Male	LC374894	LL
P_jponica_MjM3	*Peronella japonica*	Mukaijima	Male	LC374895	LS
P_jponica_MjF1	*Peronella japonica*	Mukaijima	Female		LS
P_jponica_NtF1	*Peronella japonica*	Notojima	Female	LC374887	LS*
P_jponica_NtM1	*Peronella japonica*	Notojima	Male	LC374896	LS*
P_jponica_NtM2	*Peronella japonica*	Notojima	Male	LC374897	LL
P_jponica_OkF3	*Peronella japonica*	Okinawa (Bis	Female	LC374898	LL*
P_jponica_OkF4	*Peronella japonica*	Okinawa (Bis	Female	LC374899	LL
P_jponica_OkM1	*Peronella japonica*	Okinawa (Bis	Male	LC374888	LL*
P_jponica_OkM2	*Peronella japonica*	Okinawa (Bis	Male	LC374900	LL
P_jponica_OkM8	*Peronella japonica*	Okinawa (Bis	Male	LC374901	LL*
P_jponica_OkF2	*Peronella japonica*	Okinawa (Bis	Female		LL
P_jponica_OkF5	*Peronella japonica*	Okinawa (Bise	Female		LL
P_jponica_OkF6	*Peronella japonica*	Okinawa (Bise	Female		LL
P_jponica_OkF8	*Peronella japonica*	Okinawa (Bise	Female		LL*
P_jponica_OkM3	*Peronella japonica*	Okinawa (Bis	Male		LL
P_jponica_OkM4	*Peronella japonica*	Okinawa (Bis	Male		LL*
P_jponica_SmF1	*Peronella japonica*	Shimoda	Female	LC374889	SS
P_jponica_SmM1	*Peronella japonica*	Shimoda	Male	LC374902	LL
P_jponica_SmM2	*Peronella japonica*	Shimoda	Male	LC374890	LL
P_jponica_TyF1	*Peronella japonica*	Tateyama	Female	LC374903	LL*
P_jponica_TyM2	*Peronella japonica*	Tateyama	Male	LC374891	LL
P_jponica_TyM3	*Peronella japonica*	Tateyama	Male	LC374892	LS
P_jponica_TyF3	*Peronella japonica*	Tateyama	Female		SS
P_jponica_TyM1	*Peronella japonica*	Tateyama	Male		LS
P_rubra_Ty223	*Peronella rubra*	Tateyama	-	LC374904	AB
P_rubra_Ty229	*Peronella rubra*	Tateyama	-	LC374905	BB
P_rubra_Ty232	*Peronella rubra*	Tateyama	-	LC374906	
P_rubra_Ty229	*Peronella rubra*	Tateyama	-		BB
P_rubra_Ty230	*Peronella rubra*	Tateyama	-		AB
P_rubra_Ty253	*Peronella rubra*	Tateyama	-		BB
P_rubra_Ty254	*Peronella rubra*	Tateyama	-		AA
P_rubra_Ty255	*Peronella rubra*	Tateyama	-		AA
P_rubra_Ty256	*Peronella rubra*	Tateyama	-		BB
P_rubra_Ty257	*Peronella rubra*	Tateyama	-		AA
P_rubra_Ty258	*Peronella rubra*	Tateyama	-		BB
P_rubra_Ty259	*Peronella rubra*	Tateyama	-		AB
P_rubra_Ty260	*Peronella rubra*	Tateyama	-		AA
P_rubra_Ty261	*Peronella rubra*	Tateyama	-		AA
C_japonicus	*Clypeaster japonicus*	Tateyama	-	LC374908	
A_manni	*Astriclypeus manni*	Tateyama	-	LC374907	

-: sex not determined

*: genotype examined by analysis amplicon size after performing gel electrophoresis on PCR products

Figure1

Click here to download high resolution image

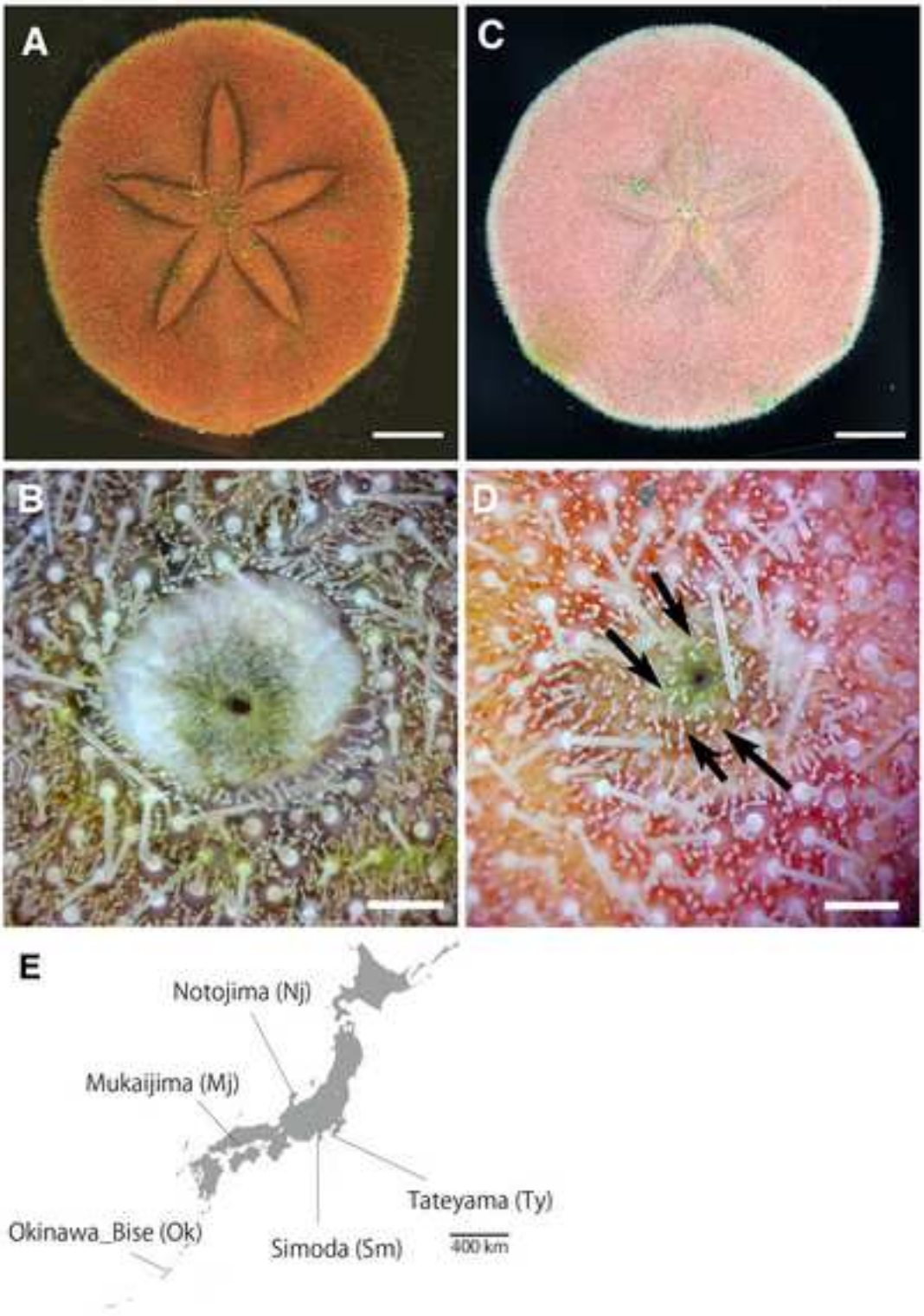


Figure2

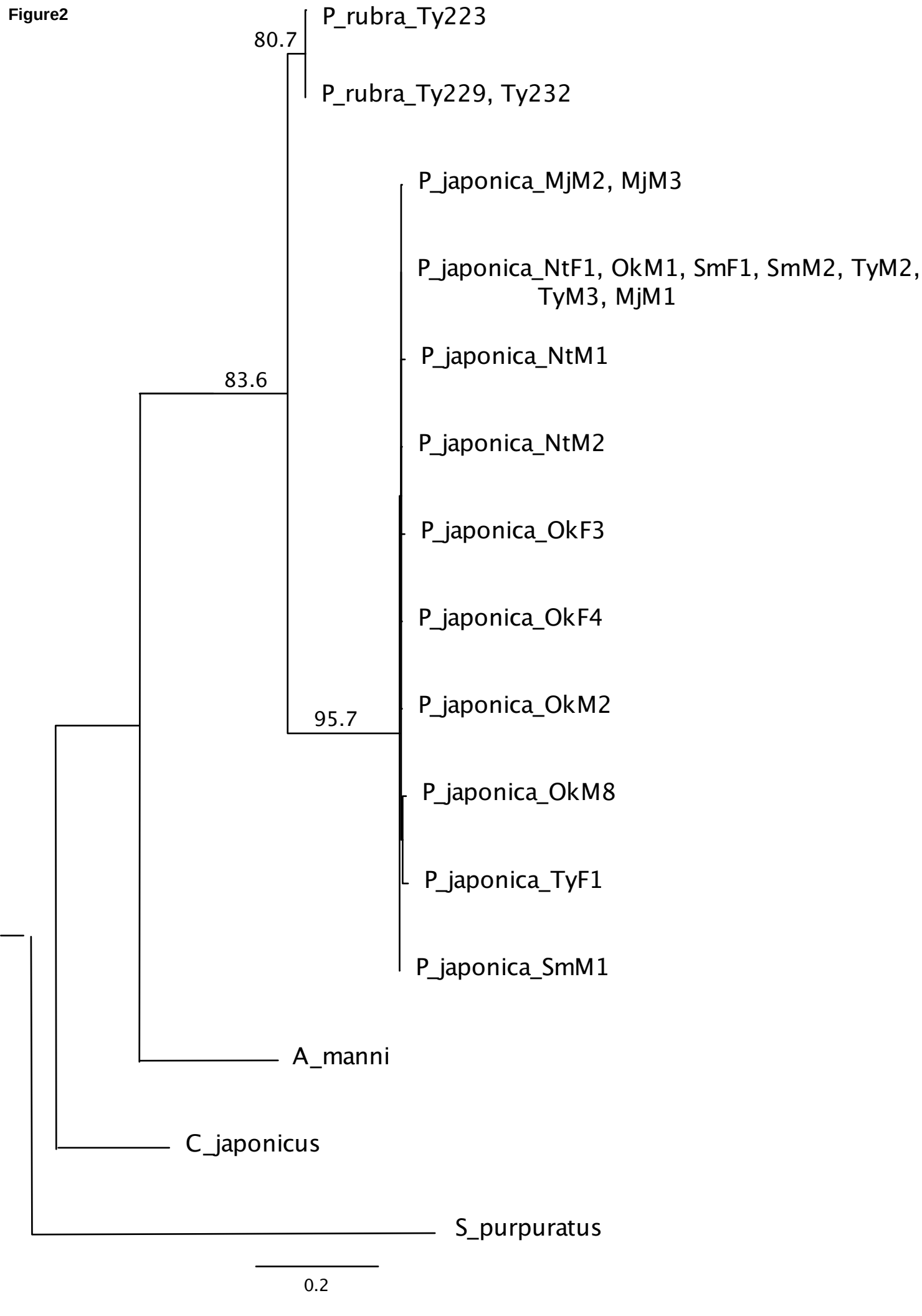

P_rubra_Ty223
80.7
P_rubra_Ty229, Ty232
P_japonica_MjM2, MjM3
P_japonica_NtF1, OkM1, SmF1, SmM2, TyM2, TyM3, MjM1
P_japonica_NtM1
83.6
P_japonica_NtM2
P_japonica_OkF3
P_japonica_OkF4
P_japonica_OkM2
95.7
P_japonica_OkM8
P_japonica_TyF1
P_japonica_SmM1
A_manni
C_japonicus
S_purpuratus
0.2

Figure3

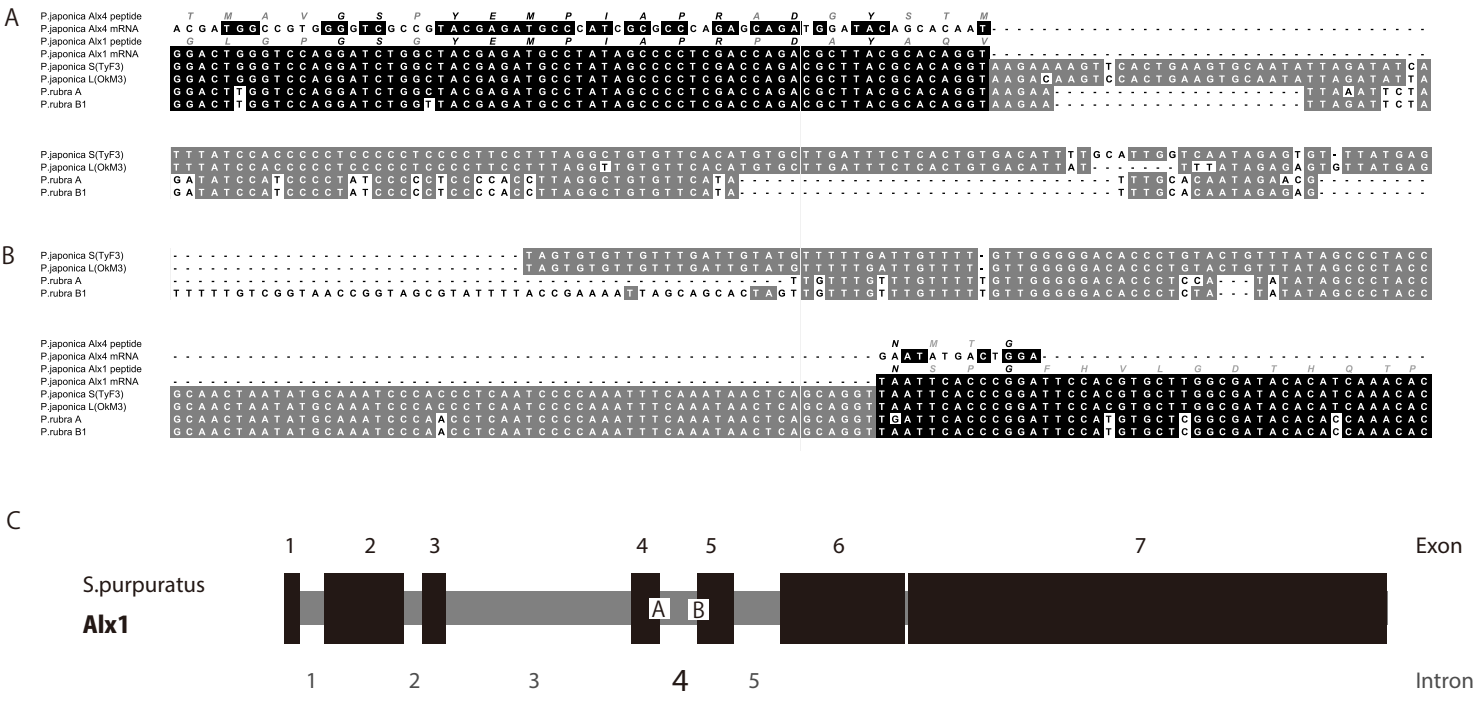
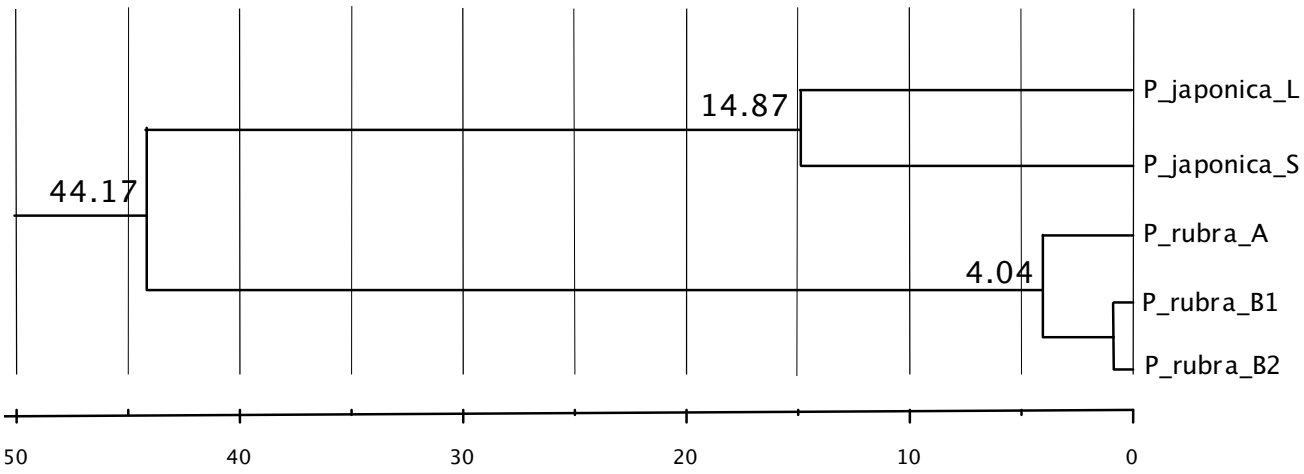

A
P.japonica Alx4 peptide
P.japonica Alx4 mRNA
P.japonica Alx1 peptide
P.japonica Alx1 mRNA
P.japonica S(TyF3)
P.japonica L(OkM3)
P.rubra A
P.rubra B1
B
C
1 2 3 4 5 6 7 Exon
S.purpuratus
Alx1
A B
1 2 3 4 5 Intron

Figure4


14.87
44.17
4.04
P_japonica_L
P_japonica_S
P_rubra_A
P_rubra_B1
P_rubra_B2
50
40
30
20
10
0

Supplementary material for on-line publication only

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