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Genetic Divergence Between Two Forms of the Pulmonate Limpet *Siphonaria japonica* (Gastropoda: Siphonariidae)

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Abstract: Genetic differences between two forms (tall and short forms) of the pulmonate limpet *Siphonaria japonica* were examined using isozymes in order to reveal their identity. Three local samples from the tall form, two local samples from the short form, plus *S. laciniosa* and *S. acmaeoides* samples as outgroups were examined. Following the isozyme analysis, 26 loci within 17 enzymes were available commonly to the two forms. Allelic compositions between the two forms show extreme differences from each other, alleles completely or almost replacing each other at the 18 loci. The genetic distances (*D* values) calculated from the allelic frequencies of the samples indicated considerably high values both among the tall form samples (*D* = 0.0060–0.0126) and short form samples (*D* = 0.0181), inferring high genetic divergence in local populations. The difference in *D* values between the tall and short form samples were between 1.3882–1.5114, figures normally considered significant at the inter-generic level. This difference in values is considerably greater than that between the *D* values of the tall form samples and *S. laciniosa*, and the genetic divergence degree between the two forms is thus greater than that at the inter-specific level in the genus *Siphonaria*. The given results suggest that the tall and short forms of *Siphonaria japonica* are distinct species, and a taxonomic revision is required.

Keywords: *Siphonaria japonica*, genetic divergence, isozyme, hidden species

Introduction

The pulmonate limpet *Siphonaria japonica* (Gastropoda: Siphonariidae) is a common marine species, which inhabits coastal rocky areas around Japan. This species occurs in two forms, that is, individuals with larger and taller shells (Fig. 1A) (hereafter "tall form") and those with smaller and shorter shells (Fig. 1B) ("short form").

The two forms are separable from each other also in the following characteristics: 1) Radial costae originate from the shell apex and terminate at the shell margin without projections in the tall form but terminate with projections in the short form. 2) Thick and thin radial costae range almost alternately in the tall form, but radial costae comprise mostly thick ones in the short form. 3) The shell is thinner and lighter in the tall form (Figs. 1A, 1B). Individuals which have intermediate morphology between the two forms have not been detected so far as the authors have investigated.

Further, the two forms live almost separately, that is, the tall form inhabits the high and mid intertidal zones and the short form inhabits the mid and low intertidal zones and low tide zone. Therefore, shells of the short form are frequently found with attached seaweeds and are abraded because they are mostly submerged. In contrast, shells of the tall form are without seaweeds and

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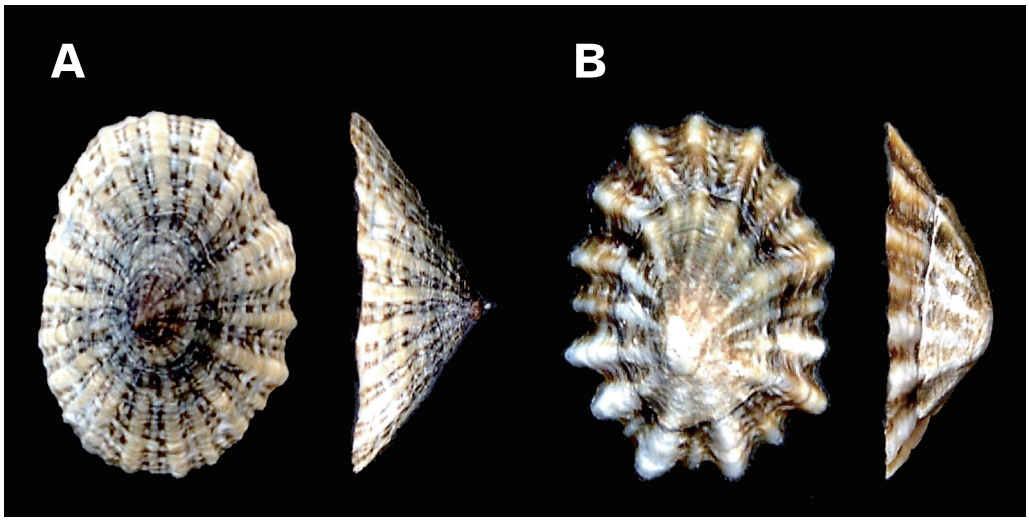

Fig. 1. Dorsal and lateral views of two forms of *Siphonaria japonica*. **A**. Tall form (from Takamatsu, Kagawa, SL 16.7 mm). **B**. Short form (from Kure, Hiroshima, SL 11.5 mm).

Table 1. Data from specimens examined.

Species	Locality of collection	Date	n
Siphonaria japonica			
Tall form (Kagawa)	Yashima, Takamatsu City, Kagawa Pref.	Nov. 12, 1999	20
Tall form (Hiroshima)	Nigata, Kure City, Hiroshima Pref.	July 2, 2000	29
Tall form (Ehime)	Sunokawa, Ainan Town, Ehime Pref.	July 10, 2000	16
Short form (Hiroshima)	Nigata, Kure City, Hiroshima Pref.	July 2, 2000	45
Short form (Ehime)	Sotodomari, Ainan Town, Ehime Pref.	July 9, 2000	18
Siphonaria laciniosa	Iriomote I., Taketomi Town, Okinawa Pref.	Sep. 24, 1999	13
Siphonaria acmaeoides	Hirabae, Ainan Town, Ehime Pref.	July 10, 2000	16

are not abraded because they are exposed to the air at low tide. Although the two forms co-occur in the mid intertidal zone, individuals in that zone are fewer in number and almost restricted to immature examples of both forms. It can thus be regarded that the two forms occupy different niches from each other.

Although the two forms have been regarded as one species, Hamamura (2004) described them and suggested that they represent distinct species. Resolving the identity of the two forms necessitates a genetic examination. Therefore, the present study was performed to reveal genetic divergence between the two forms using isozymes as genetic markers.

Materials and Methods

For *Siphonaria japonica*, three local samples from the tall form and two local samples from the short form were selected to examine. The tall and short forms were classified using the morphological characteristics mentioned in the introduction. In addition, two further congeners, *S. laciniosa* and *S. acmaeoides,* also were examined as outgroups. The sampling data from specimens are shown in Table 1 and Fig. 2. Both forms of *S. japonica* were collected from Kure, Hiroshima (Fig. 2, Table 1), from an identical rocky coast, although they occurred in almost

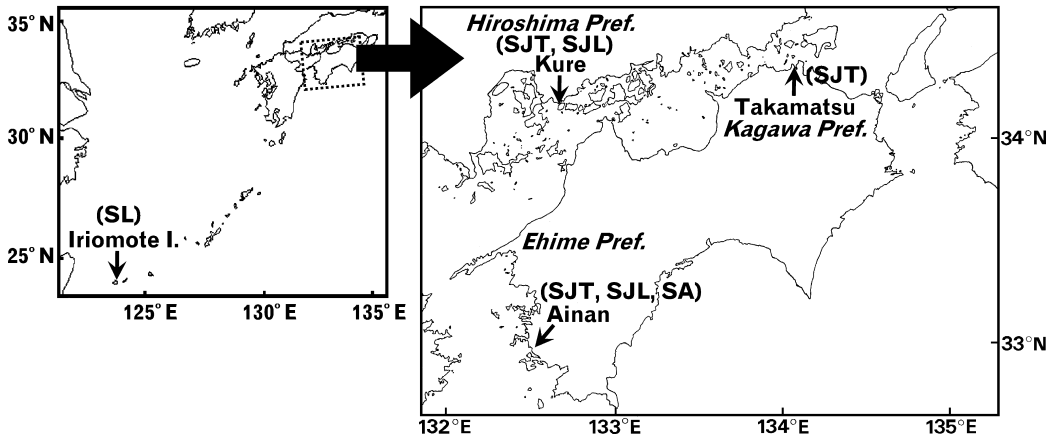

Fig. 2. Localities where specimens were collected. Upper case letters in parentheses represent species or forms collected at the localities (SJT: *Siphonaria japonica* tall form, SJL: *S. japonica* short form, SL: *S. laciniosa*, SA: *S. acmaeoides*).

different tidal zones from each other as mentioned earlier. The specimens were preserved in a freezer at –80°C prior to analysis. Using the methodologies of Yokogawa (1999), electrophoresis was performed to examine isozymes.

The gene nomenclature follows Shaklee *et al*. (1990), and the alleles were symbolized as relative mobility percentages compared with the most dominant alleles (**100* in the anodal zone, **–100* in the cathodal zone) in the tall form of *S. japonica* at each locus. For the polymorphic loci, correspondence to the Hardy-Weinberg equilibrium was tested with GENEPOP software (ver. 4.0.7) (Rousset, 2008).

Genetic distances (*D* values), following Nei (1972), were calculated with the allelic frequencies of the samples. Dendrograms of genetic relationships between the samples were constructed from the genetic distances by using the unweighted pair group method with arithmetic mean (UPGMA), and bootstrap estimates to test reliability of the dendrogram were performed with the Populations genetic software (ver. 1.2.30) (Langella, 2002).

Results

Table 2 summarizes the enzymes and tissues examined and status of locus detection. For the tall and short forms of *Siphonaria japonica*, loci were not always detected identically, but 26 available loci within 17 enzymes were common. Similarly, the other congeners had some peculiarity in locus appearance (Table 2).

Concerning the correspondence to Hardy-Weinberg equilibrium, because no significance was recognized at any loci of any samples, all were regarded as corresponding to the equilibrium.

Table 3 shows allelic frequencies together with values to indicate genetic features of the samples. Although the two forms of *S. japonica* had similarities in the values indicating genetic features, they greatly differed from each other in the allelic frequencies. The alleles have completely replaced each other between the two forms at the *AAT-1**, *ACP-1**, *ACP-2**, *CAT-2**, *FBALD-1**, *IDHP-1**, *MDH-1**, *MDH-2**, *MDH-3**, *MEP** and *PGDH** loci; they almost have completely replaced each other at the *AH**, *CAT-1**, *DIA-1**, *EST-5**, *LAP-2**, *PEPS** and *PGM** loci, and the allelic frequencies of the two forms further differed from each other greatly at the *EST-3** and *MPI-1** loci (Table 3).

Table 2. Enzymes and tissues examined and status of locus detection.

Enzyme or protein name	Enzyme number	Locus	Tissue	Status of locus (A: Available; UA: Unavailable; ND: Not detected)			
				Siphonaria japonica		*S. laciniosa*	*S. acmaeoides*
				Tall form	Short form		
Aspartate aminotransferase	2.6.1.1	*AAT-1**	Foot muscle	A	A	A	A
		*AAT-2**	Foot muscle	A	A	A	A
Acid phosphatase	3.1.3.2	*ACP-1**	Visceral mass	A	A	UA[1]	A
		*ACP-2**	Visceral mass	A	A	A	A
Aconitate hydratase	4.2.1.3	*AH**	Visceral mass	A	A	A	A
Adenosine deamimase	3.5.4.4	*ADA-1**	Visceral mass	A	ND	ND	ND
		*ADA-2**	Visceral mass	A	ND	ND	ND
Alkaline phosphatase	3.1.3.1	*ALP**	Visceral mass	A	A	A	UA[1]
Catalase	1.11.1.6	*CAT-1**	Foot muscle	A	A	A	A
		*CAT-2**	Foot muscle	A	A	A	A
Creatine kinase	2.7.3.2	*CK**	Foot muscle	A	A	A	A
Diaphorase	1.6.–.–	*DIA-1**	Visceral mass	A	A	A	A
		*DIA-2**	Visceral mass	A	ND	A	A
		*DIA-3**	Visceral mass	UA[1]	UA[1]	UA[1]	UA[1]
Esterase	3.1.1.–	*EST-1**	Foot muscle	A	ND	A	ND
		*EST-2**	Foot muscle	A	A	A	A
		*EST-3**	Foot muscle	A	A	A	A
		*EST-4**	Foot muscle	UA[1]	UA[1]	UA[1]	UA[1]
		*EST-5**	Foot muscle	A	A	A	A
Fructose biphosphate aldolase	4.1.2.13	*FBALD**	Foot muscle	A	A	A	A
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*IDHP-1**	Foot muscle	A	A	A	A
		*IDHP-2**	Foot muscle	A	ND	ND	A
Leucine aminopeptidase	3.4.11.1	*LAP-1**	Visceral mass	UA[1]	ND	ND	ND
		*LAP-2**	Visceral mass	A	A	A	A
Malate dehydrogenase	1.1.1.37	*MDH-1**	Foot muscle	A	A	A	A
		*MDH-2**	Foot muscle	A	A	A	A
		*MDH-3**	Foot muscle	A	A	A	A
Malic enzyme ($NADP^+$)	1.1.1.40	*MEP**	Foot muscle	A	A	A	A
Mannose-6-phosphate isomerase	5.3.1.8	*MPI-1**	Foot muscle	A	A	A	A
		*MPI-2**	Foot muscle	UA[1]	UA[1]	UA[1]	A
Peptidase	3.4.–.–	*PEPA**	Visceral mass	A	ND	ND	ND
		*PEPB-1**	Foot muscle	A	A	A	A
		*PEPB-2**	Foot muscle	ND	UA[1]	ND	A
		*PEPD**	Foot muscle	A	A	A	A
		*PEPS**	Foot muscle	A	A	A	A
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Foot muscle	A	A	A	A
Phosphoglucomutase	5.4.2.2	*PGM**	Visceral mass	A	A	UA[2]	A

[1] Electrophoretic bands could not be read owing to their insufficient focusing or thinness.
[2] Target locus could not be identified because multiple band zones appeared.

Table 3. Allelic frequencies together with values to indicate genetic features in two forms of *Siphonaria japonica*, *S. laciniosa* and *S. acmaeoides.*

Locus	Allele	*Siphonaria japonica*					*S. laciniosa*	*S. acmaeoides*
		Tall form			Short form			
		Kagawa	Hiroshima	Ehime	Hiroshima	Ehime		
*AAT-1**	**0*	1.000	1.000	1.000	0	0	0	0
	**–50*	0	0	0	0	0	0.962	1.000
	**–100*	0	0	0	1.000	1.000	0	0
	**–120*	0	0	0	0	0	0.038	0
*AAT-2**	**–35*	0	0	0	0	0	0	1.000
	**–100*	1.000	1.000	1.000	1.000	1.000	1.000	0
*ACP-1**	**510*	0	0	0	0.111	0.031		0
	**460*	0	0	0	0.889	0.969		0
	**100*	0.875	0.875	0.875	0	0		0
	**50*	0.125	0.125	0.125	0	0		0
	**–100*	0	0	0	0	0		–0.156
	**–260*	0	0	0	0	0		0.844
*ACP-2**	**–40*	0	0	0	1.000	1.000	0	0
	**–55*	0	0	0	0	0	0	1.000
	**–85*	0	0	0	0	0	1.000	0
	**–100*	1.000	1.000	1.000	0	0	0	0
*AH**	**100*	0.975	1.000	0.969	0	0	0	0
	**90*	0.025	0	0.031	0.038	0	0	0
	**85*	0	0	0	0.897	1.000	1.000	0
	**75*	0	0	0	0.064	0	0	0.962
	**65*	0	0	0	0	0	0	0.038
*ALP**	**100*	1.000	1.000	1.000	1.000	1.000	1.000	
*CAT-1**	**100*	0.921	0.983	0.906	0	0	0	0.125
	**35*	0.079	0.017	0.094	0.025	0	0	0
	**20*	0	0	0	0	0	0	0.844
	**0*	0	0	0	0.975	1.000	0	0.031
	**–70*	0	0	0	0	0	1.000	0
*CAT-2**	**–70*	0	0.018	0	0	0	0	1.000
	**–100*	0.950	0.982	1.000	0	0	1.000	0
	**–110*	0.050	0	0	0	0	0	0
	**–140*	0	0	0	1.000	1.000	0	0
*CK**	**145*	0	0.069	0	0.275	0	0	0
	**130*	0.025	0.155	0	0.200	0	0	0
	**100*	0.975	0.741	0.969	0.400	0.700	1.000	0
	**85*	0	0	0	0	0	0	1.000
	**70*	0	0.034	0.031	0.113	0.300	0	0
	**50*	0	0	0	0.013	0	0	0
*DIA-1**	**115*	0.029	0	0	0	0	0	0
	**110*	0	0	0	0	0	0.962	1.000
	**100*	0.794	0.971	0.923	0	0	0.038	0
	**85*	0.176	0.029	0.077	0.962	1.000	0	0
	**70*	0	0	0	0.038	0	0	0

Table 3. (Continued).

*EST-2**	**110*	0.088	0.019	0.156	0.022	0	0	0
	**100*	0.794	0.962	0.688	0.922	0.833	0.538	0
	**90*	0.118	0.019	0.156	0.056	0.167	0	0
	**85*	0	0	0	0	0	0.462	0.719
	**80*	0	0	0	0	0	0	0.281
*EST-3**	**140*	0	0.018	0.031	0.011	0	0	0
	**130*	0.175	0.196	0.250	0.716	0.529	0	0
	**120*	0	0	0	0.261	0.412	0	0
	**100*	0.725	0.536	0.469	0	0.059	0	0
	**80*	0.100	0.196	0.250	0.011	0	0.038	0
	**60*	0	0.054	0	0	0	0	0
	**50*	0	0	0	0	0	0	1.000
	**40*	0	0	0	0	0	0.077	0
	**30*	0	0	0	0	0	0.885	0
*EST-5**	*–80*	0	0.018	0.094	0	0	0.077	0.875
	–100	0.875	0.911	0.906	0.039	0	0.769	0.063
	–120	0.075	0.071	0	0.053	0.125	0.154	0.063
	–125	0.025	0	0	0.671	0.875	0	0
	–130	0.025	0	0	0.105	0	0	0
	–150	0	0	0	0.132	0	0	0
*FBALD**	**230*	0	0	0	0.068	0	0	0
	**200*	0	0	0	0.057	0.045	0	1.000
	**150*	0	0	0	0.864	0.955	0	0
	**135*	0	0	0	0.011	0	0	0
	**100*	1.000	1.000	1.000	0	0	1.000	0
*IDHP-1**	**250*	0	0	0	0	0	0	1.000
	**190*	0.225	0.143	0.188	0	0	0	0
	**130*	0	0	0	1.000	1.000	0	0
	**100*	0.775	0.857	0.813	0	0	1.000	0
*LAP-2**	**240*	0	0	0	0	0	0	1.000
	**200*	0	0	0	0.022	0	0.654	0
	**180*	0	0	0	0	0	0.346	0
	**160*	0	0	0	0.833	0.857	0	0
	**130*	0	0	0	0.133	0.143	0	0
	**125*	0.050	0.034	0.031	0.011	0	0	0
	**100*	0.900	0.948	0.906	0	0	0	0
	**75*	0.025	0.017	0.063	0	0	0	0
	**60*	0.025	0	0	0	0	0	0
*MDH-1**	**140*	0	0	0	0.216	0.036	0	0
	**120*	0	0	0	0	0	0	1.000
	**35*	0	0	0	0.784	0.964	0	0
	**0*	0	0	0	0	0	1.000	0
	–100	1.000	1.000	1.000	0	0	0	0
*MDH-2**	**50*	0	0	0	0	0	0	1.000
	–60	0	0	0	0	0	1.000	0
	–80	0	0	0	1.000	1.000	0	0
	–100	1.000	1.000	1.000	0	0	0	0

Table 3. (Continued).

*MDH-3**	*–85	0	0	0	1.000	1.000	0	0
	*–100	1.000	1.000	1.000	0	0	0	0
	*–160	0	0	0	0	0	1.000	1.000
*MEP**	*25	0	0	0	1.000	1.000	0	0
	*–100	1.000	1.000	1.000	0	0	0.938	1.000
	*–130	0	0	0	0	0	0.063	0
*MPI-1**	*160	0	0	0	0.406	0.214	0	0.063
	*150	0	0	0	0	0	0	0.938
	*125	0.025	0	0	0.438	0.786	0	0
	*100	0.725	0.586	0.875	0.125	0	0	0
	*75	0.250	0.414	0.125	0.031	0	0.615	0
	*50	0	0	0	0	0	0.385	0
*PEPB-1**	*120	0.200	0.233	0.094	0.081	0	0.962	0.813
	*100	0.750	0.733	0.906	0.849	0.857	0.038	0.188
	*90	0.050	0.017	0	0.058	0.143	0	0
	*75	0	0.017	0	0.012	0	0	0
*PEPD**	*115	0.050	0.167	0.063	0	0	0	0
	*100	0.875	0.767	0.875	0.988	1.000	0	0.531
	*75	0.075	0.067	0.063	0.013	0	1.000	0.469
*PEPS**	*120	0	0.034	0	0	0	0	0
	*100	0.900	0.845	0.969	0	0	0	0
	*80	0.100	0.121	0	0.148	0	0	0
	*70	0	0	0.031	0.705	0.885	0	0
	*60	0	0	0	0	0	0	0.967
	*40	0	0	0	0	0	0	0.033
	*30	0	0	0	0	0	1.000	0
	*20	0	0	0	0.148	0.115	0	0
*PGDH**	*270	0	0	0	0.081	0.083	0	0
	*215	0	0	0	0	0	0.115	1.000
	*200	0	0	0	0.395	0.208	0	0
	*125	0	0	0	0	0	0.885	0
	*100	0	0	0	0.523	0.708	0	0
	*0	1.000	1.000	1.000	0	0	0	0
*PGM**	*230	0.200	0.054	0.200	1.000	1.000		0
	*100	0.725	0.786	0.800	0	0		0
	*–100	0.075	0.161	0	0	0		0
	*–250	0	0	0	0	0		1.000
Alleles/Locus		1.964	2.000	1.750	2.269	1.560	1.500	1.423
Percentage of	P*	0.571	0.464	0.500	0.500	0.360	0.259	0.259
polymorphic loci	P	0.071	0.143	0.107	0.115	0.120	0.074	0.074
	P+P*	0.643	0.607	0.607	0.615	0.480	0.333	0.333
Average	Ho	0.169	0.158	0.148	0.187	0.121	0.114	0.082
heterozygosity	He	0.161	0.159	0.141	0.197	0.126	0.106	0.086
	Ho/He	1.050	0.992	1.053	0.949	0.959	1.073	0.964

P*: Polymorphism less than 0.95. Ho: Observed heterozygosity.
P: Polymorphism greater than 0.95. He: Expected heterozygosity.

Table 4 exhibits genetic distances (*D* values) between the samples from the tall and short forms based on the 26 loci common to them. The *D* values among the tall form samples and those between the short form samples resulted in 0.0060–0.0126 and 0.0181, respectively (Table 4), both may exceed the local population level (Nei, 1990). While those between the two forms were 1.3882–1.5114 (Table 4). Those values exceed the inter-specific level, being significant at the inter-generic level (Nei, 1990).

Figure 3 is a dendrogram which shows genetic relationships between the samples based on the *D* values shown in Table 4. The two form samples cluster respectively and the two clusters finally connect with each other over a long distance.

The congeners *Siphonaria laciniosa* and *S. acmaeoides* had many divergent loci, both from each other and from the two forms (Table 3). Twenty-three loci (the remainders of which, the *ACP-1**, *ALP** and *PGM** loci, were omitted from the 26 loci) were common to the two congeners and two forms. Subsequently the *D* values were re-calculated based on the 23 loci (Table 5) and the dendrogram was re-constructed (Fig. 4).

Although *S. acmaeoides* was genetically the furthest from the other samples, it was notable that the *D* values between the two forms (1.5140–1.6233) were greater than those between the tall form and *S. laciniosa* (1.0019–1.0490) (Table 5). In the dendrogram, *S. laciniosa* formed a single cluster with the tall form, and that cluster connected with the short form cluster at a genetic distance of about 1.6 (Fig. 4). This implies that the genetic divergence degree between the two forms is greater than that at the inter-specific level in the genus *Siphonaria*.

Table 4. Genetic distances (*D* values) based on 26 loci between two forms of *Siphonaria japonica.*

	Tall form			Short form
	Kagawa	Hiroshima	Ehime	Hiroshima
Tall form				
Kagawa				
Hiroshima	0.0084			
Ehime	0.0060	0.0126		
Short form				
Hiroshima	1.3882	1.4565	1.4082	
Ehime	1.4121	1.5114	1.4250	0.0181

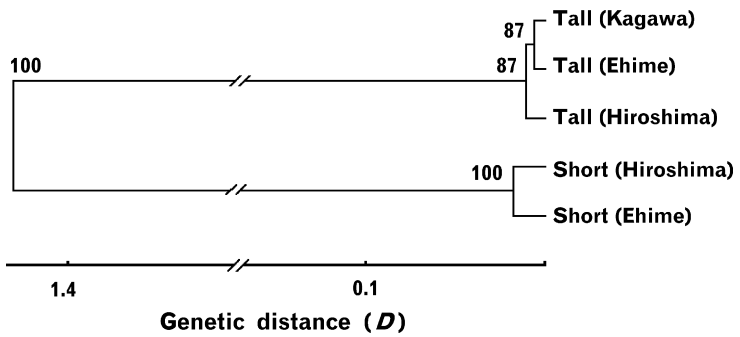

Fig. 3. A dendrogram based on genetic distances (*D* values) calculated from the 26 loci for tall and short forms of *Siphonaria japonica*. Numbers in the dendrogram represent bootstrap probability values based on 1000 replicates.

Table 5. Genetic distances (*D* values) based on 23 loci between tall and short forms of *Siphonaria japonica, S. laciniosa* and *S. acmaeoides.*

	Siphonaria japonica					
	Tall form			Short form		*S. laciniosa*
	Kagawa	Hiroshima	Ehime	Hiroshima	Ehime	
S. japonica						
Tall form						
Kagawa						
Hiroshima	0.0085					
Ehime	0.0065	0.0130				
Short form						
Hiroshima	1.5140	1.5625	1.5345			
Ehime	1.5351	1.6233	1.5603	0.0205		
S. laciniosa	1.0170	1.0019	1.0490	1.8609	1.8409	
S. acmaeoides	2.3039	2.3286	2.3346	3.0369	3.2545	1.2934

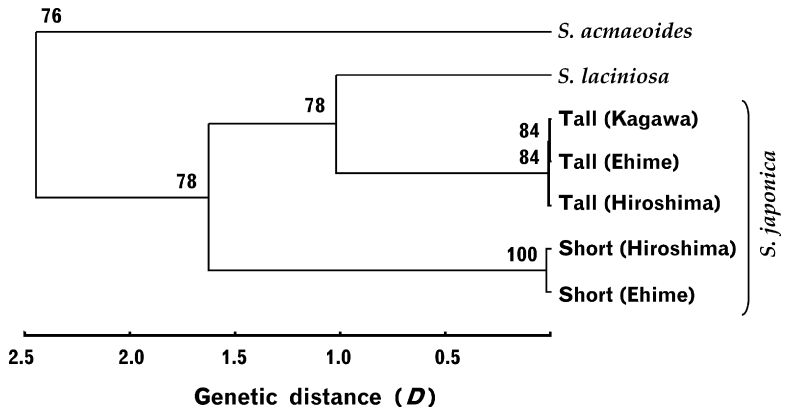

Fig. 4. A dendrogram based on genetic distances (*D* values) calculated from the 23 loci for tall and short forms of *Siphonaria japonica*, *S. laciniosa* and *S. acmaeoides*. Numbers in the dendrogram represent bootstrap probability values based on 1000 replicates.

Discussion

The extreme genetic differences between the tall and short forms of "*Siphonaria japonica*" strongly indicate that they are in fact distinct species. This is also supported by the fact that the genetic divergence degree between the two forms exceeds that of the inter-specific level in the genus *Siphonaria*.

Siphonaria japonica was originally described by Donovan (1825) as *Patella japonica,* with a well drawn illustration. His description and illustration corresponds well with the tall form (Fig. 1A) in morphological characteristics. Therefore Hamamura (2004) referred to the tall form as *S. japonica* and the short form as an unknown but closely related species. Thus, the tall form should be hereafter referred to as *Siphonaria japonica* and the short form should be as *Siphonaria* sp., until formally described.

So far as the authors have determined, there is no literature in which *S.* sp. is figured except

Hamamura (2004). This may be because *S*. sp. inhabits a deeper zone than that of *S*. *japonica*, although they are distributed almost sympatrically. This makes it difficult to find and collect *S*. sp. In addition, it can be mis-identified as *S*. *japonica* because it is very similar in appearance. Therefore, *S*. sp. has been overlooked until now. It is likely to be a new species that should be taxonomically described with detailed morphological examinations.

Further, it may be significant that both *S*. *japonica* and *S*. sp. have considerable intra-specific genetic diversity, which may exceed the local population level (Table 4). Although reports of genetic studies using isozymes on marine gastropods are a few, Fujio *et al.* (1989) examined the genetic distances (*D* values) between local samples of Japanese abalones (*Haliotis discus discus* and *H. d. hannai*). They gave *D* values of 0.0011–0.0029 (average 0.0020) for *H. d. discus* and 0.0016–0.0170 (average 0.0085) for *H. d. hannai*, respectively. Compared with these values, the *D* values between the local samples of *S*. *japonica* and *S*. sp. are both higher, inferring the presence of a mechanism of population isolation in these species. Including this issue, further studies on genetics, morphology and ecology for the two species are necessary.

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References

Donovan, E. 1825. *Patella japonica*, small Japan limpet. *Naturalist's Repository, or Miscellany of Exotic Natural History Exhibiting Rare and Beautiful Specimens of Foreign Birds, Insects, Shells, Quadrupeds, Fish and Marine Productions*. Vol. 3, pl. 79. London.

Fujio, Y., Oniwa, K., Yuzawa, A. & Tabata, K. 1989. Genetic variability and structure in abalone population. *In*: Japan Fisheries Resource Conservation Association (eds.), *Population Analyses of Fishes and Shellfishes by Isozyme*, pp. 419–435. Japan Fisheries Resource Conservation Association, Tokyo. (in Japanese)

Hamamura, Y. 2004. *An Illustrated Guide to Marine Shells of Geinan Region*. vii+220 pp. Ranto Bunka Shinko Zaidan, Kure. (in Japanese)

Langella, O. 2002. Populations, 1.2.28 Copyright (C) 1999, CNRS UPR9034. Available from http://bioinformatics.org/~tryphon/populations/

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283–292.

Nei, M. 1990. *Molecular Evolutionary Genetics* (translated from English into Japanese by Gojobori T. & Saito N.). vii+433 pp., Baifukan, Tokyo. (in Japanese)

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

Shaklee, J. B., Allendorf, F. W., Morizot, D. C. & Whitt, G. S. 1990. Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society* 119: 2–15.

Yokogawa, K. 1999. Morphological and genetic characteristics in two forms of gibbose conch *Gibberulus gibberulus gibbosus*. *Suisanzoshoku* 47: 363–368.

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カラマツガイ 2 型の遺伝的分化

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要　約

カラマツガイ *Siphonaria japonica* は日本各地の海岸の岩礁帯に生息する普通種だが，殻が大きくて殻高が高い型（高頂型）と小型で殻高が低い型（低頂型）の 2 型が存在する。本研究ではこれら 2 型についてアイソザイム系遺伝子による遺伝的差異を調べ，両者の実体を明らかにすることを試みた。用いた材料は，高頂型は香川県高松市産の 20 個体，広島県呉市産の 29 個体，愛媛県愛南町産の 16 個体の 3 標本群，低頂型は広島県呉市産の 45 個体，愛媛県愛南町産の 16 個体の 2 標本群であり，外群として沖縄県西表島産のコウダカカラマツ *S. laciniosa* 13 個体，愛媛県愛南町産のシロカラマツ *S. acmaeoides* 16 個体も合わせて調べた。

アイソザイム分析の結果，高頂型と低頂型に共通して 17 酵素を検出し計 26 遺伝子座を推定したが，両者の遺伝子組成は著しく相違し，18 遺伝子座で対立遺伝子が完全置換かそれに近い状態であった。各標本群の遺伝子頻度から Nei の遺伝的距離（*D* 値）を計算したところ，高頂型間の *D* 値は 0.0060〜0.0126，低頂型間のそれは 0.0181 とかなり大きく，いずれも地域による遺伝的分化が大きかった。一方，高頂型と低頂型の間の *D* 値は 1.3882〜1.5114 となり，一般的な生物の種間の水準を超えて属間の水準に達していた。また 2 型間の *D* 値は高頂型とコウダカカラマツとの間の *D* 値よりも大きく，両者の遺伝的分化はカラマツガイ科既知種の種間水準より大きいことが示された。以上の結果から，カラマツガイの高頂型と低頂型は明らかに別種であるものと思われ，今後の分類学的整理が望まれる。