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# Molecular Evidence of the Existence of Two Sibling Species within the Echinothurioid Echinoid *Asthenosoma ijimai* from Japanese Waters

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**ABSTRACT**—The echinoid, *Asthenosoma ijimai* belonging to the order Echinothurioida from Japanese waters shows the geographical variation in morphological and ecological characters. The echinothurioid from Ryukyu Islands in southern Japan is clearly different from that of Sagami Bay and Suruga Bay in the middle part of Japan at non-molecular level. Their phylogenetic and taxonomic relationships were studied at the molecular level by allozyme analysis. The results demonstrated that the two echinothurioids from Ryukyu Islands and Sagami Bay do not share gene pools with each other, and they were fixed for different alleles at five genetic loci (*Mdh*, *G6pd*, *Po*, *Alk-3* and *Est-7*) in a total of 23 enzyme genetic loci scored. This indicates no gene flow between the two echinothurioids, and is a molecular evidence for that they are reproductively isolated and genetically distinct species. The Nei's genetic distance ( $D=0.181$ ) between the two were significantly higher than those between conspecific local populations, and comparable to those between closely related species in many other animals containing echinoderms. The present molecular data are well consistent with the non-molecular evidence from morphology, developmental biology and ecology. Putting these data together, we propose that the two echinothurioids should be classified as two sibling species of the genus *Asthenosoma* and would like to give the following scientific names: the echinothurioid species from Sagami Bay is *Asthenosoma ijimai* and that from Ryukyu Islands is *A. ijimai R.*

**Key words:** echinoid, Echinothurioida, *Asthenosoma ijimai*, *Asthenosoma sp.*, genetic differentiation

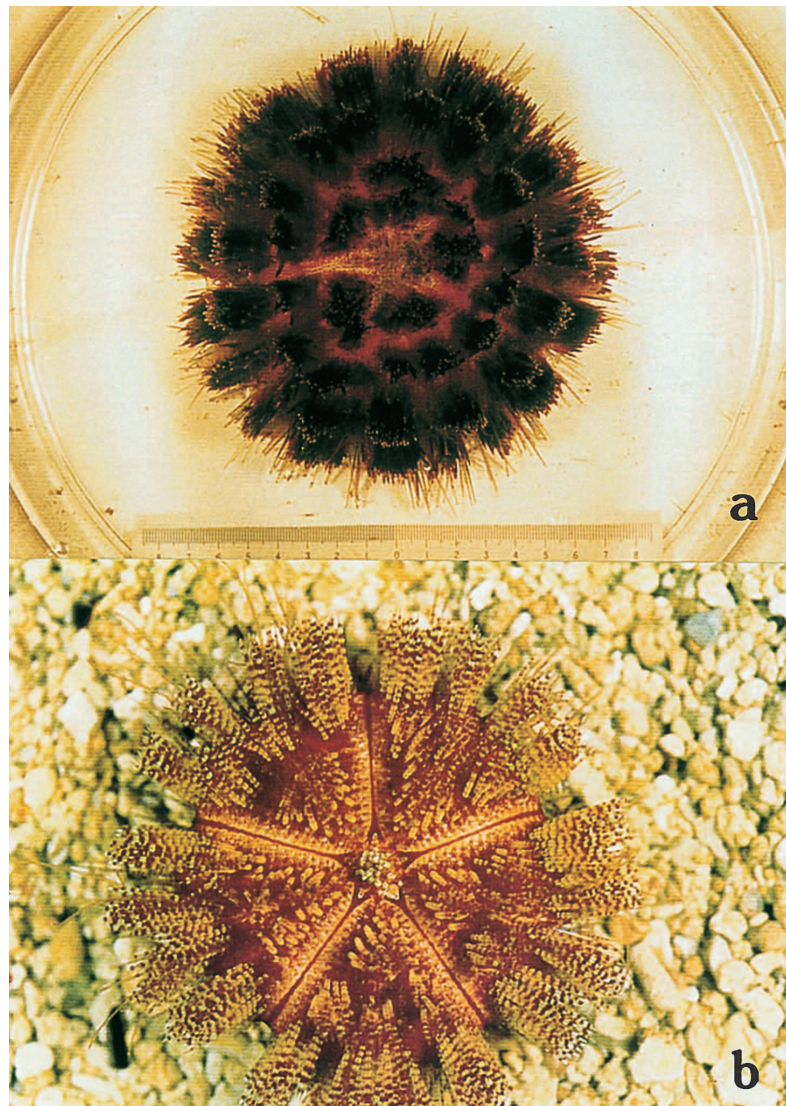
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## INTRODUCTION

The echinoid fauna is very abundant in Japanese waters and there are many Japanese endemic species (Shigei, 1974). The order Echinothurioida is considered to be the most ancestral echinoids in the Euechinoids from the morphological and palaeontological viewpoints and famous to be one of the poison echinoids characterized by having the soft shell (Shigei, 1974). Therefore, the scuba divers have been afraid of these echinoids. At present, the order Echinothurioida contains about 45 species in the world sea

and many species are from deep-sea (about 200 m depth) (Shigei, 1974). From the Sagami Bay and Suruga Bay in middle parts of Japan, some Japanese endemic echinothurioid species are found: *Asthenosoma ijimai*, *Araosoma owstoni*, *Hapalosoma gemmiferum* and so on. Recently, one of the present authors (SA) suggested that *A. ijimai* from Ryukyu Islands is different from *A. ijimai* from Sagami Bay and Suruga Bay in morphological, developmental and ecological viewpoint and called that as *Asthenosoma sp.* (Amemiya and Uehara, 1999) (Fig. 1). Although the morphological, developmental and ecological differences between the two echinothurioids have been reported (Amemiya and Emler, 1992; Amemiya *et al.*, 1980; Amemiya and Tsuchiya, 1979; Amemiya and Uehara, 1999), the molecular phyloge-

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**Fig. 1.** The two echinothurioids of the family Echinothuriidae from Japanese waters. a: *Asthenosoma ijimai* from Sagami Bay (dorsal view) , b: *Asthenosoma sp.* from Ryukyu Islands (oral view). This color photographs of the two echinothurioids were taken by Amemiya, S. The scale is shown in the upper color photograph of *A. ijimai*.

netic and taxonomic study would be desired for the phylogenetic and taxonomic identification of these two echinothurioids. Namely, the problem whether these two echinothurioids are simply intraspecific variation or two distinct, separate and sibling species differentiated genetically is important in the field of echinoid taxonomy. One of the present authors (NM) reported the molecular phylogenetic studies of various echinoids from Japanese waters (Matsuoka, 1985, 1987, 1989, 1993; Matsuoka and Hatanaka, 1991; Matsuoka and Inamori, 1996, 1999; Matsuoka and Suzuki, 1989; Minokawa *et al.*, 2003).

In this paper, we report the results of the molecular phylogenetic study by allozyme analysis on the two morphologically indistinguishable echinothurioids (*Asthenosoma ijimai* and *A. sp.*) from Japanese waters. These data would provide useful information on the phylogeny, evolution and taxonomy in the order Echinothurioida including the sole family,

Echinothurioid.

## MATERIALS AND METHODS

The two local populations of the echinothurioid, *Asthenosoma ijimai* Yoshiwara, 1897 were collected from Sagami Bay near at the Misaki Marine Biological Station of the University of Tokyo and Sesokojima near at the Sesoko Marine Science Station of University of the Ryukyus by scuba diving. The number of individuals examined in this allozyme study was 12 for *A. ijimai* and 12 for *A. sp.* The enzymes analyzed in the present allozyme study were the followings 10 enzymes: glucose-6-phosphate dehydrogenase (G6PD), malate dehydrogenase (MDH), nothing dehydrogenase (NDH), octanol dehydrogenase (ODH), superoxide dismutase (SOD), phosphoglucosomerase (PGI), leucine amino peptidase (LAP), peroxidase (PO), alkaline phosphatase (ALK) and esterase (EST). The allozyme analysis was conducted as described in Matsuoka and Hatanaka (1991).

## RESULTS AND DISCUSSION

## Genetic variation in two echinothurioids

Twenty three enzyme genetic loci were scored in this allozyme study. The allele frequencies at 23 enzyme genetic loci were shown in Table 1. As evident from Table 1, 17 loci were polymorphic, but six genetic loci (*Mdh*, *Odh*, *Sod-2*, *Lap-1*, *Lap-5* and *Est-1*) were monomorphic in two echinothurioids. From the allele frequency data in Table 1, the number of alleles per loci (*A*), the proportion of polymorphic loci (*P*) and the expected average heterozygosity (*H*) were calculated. *A. ijimai* showed the high polymorphism ( $A=1.74$ ,  $P=65.2\%$  and  $H=22.4\%$ ) and *A. sp.* also showed the similar high genetic variation ( $A=2.00$ ,  $P=65.2\%$  and  $H=27.7\%$ ). These values were much higher than those of many other echinoid species from shallow water reported previously (Matsuoka and Asano, 2003). Nei (1983) indicated the positive correlation between the degree of genetic variation and population size. Matsuoka and Asano (2003) and Matsuoka *et al.* (1992) reported that echinoderms (echinoids and asteroids) from shallow water show relatively low genetic variation, but echinoderms from deep sea had relatively high polymorphism. One of the present authors (NM) suggested that the population size of echinoderms from shallow water is relatively small, and thus they would not maintain high genetic variation within populations, but as the population size of echinoderms from deep sea is much larger they would maintain higher genetic variation. Judging from the present allozyme data obtained in two echinothurioids, the population size of *A. ijimai* and *A. sp.* might be relatively larger than those of other shallow water echinoids.

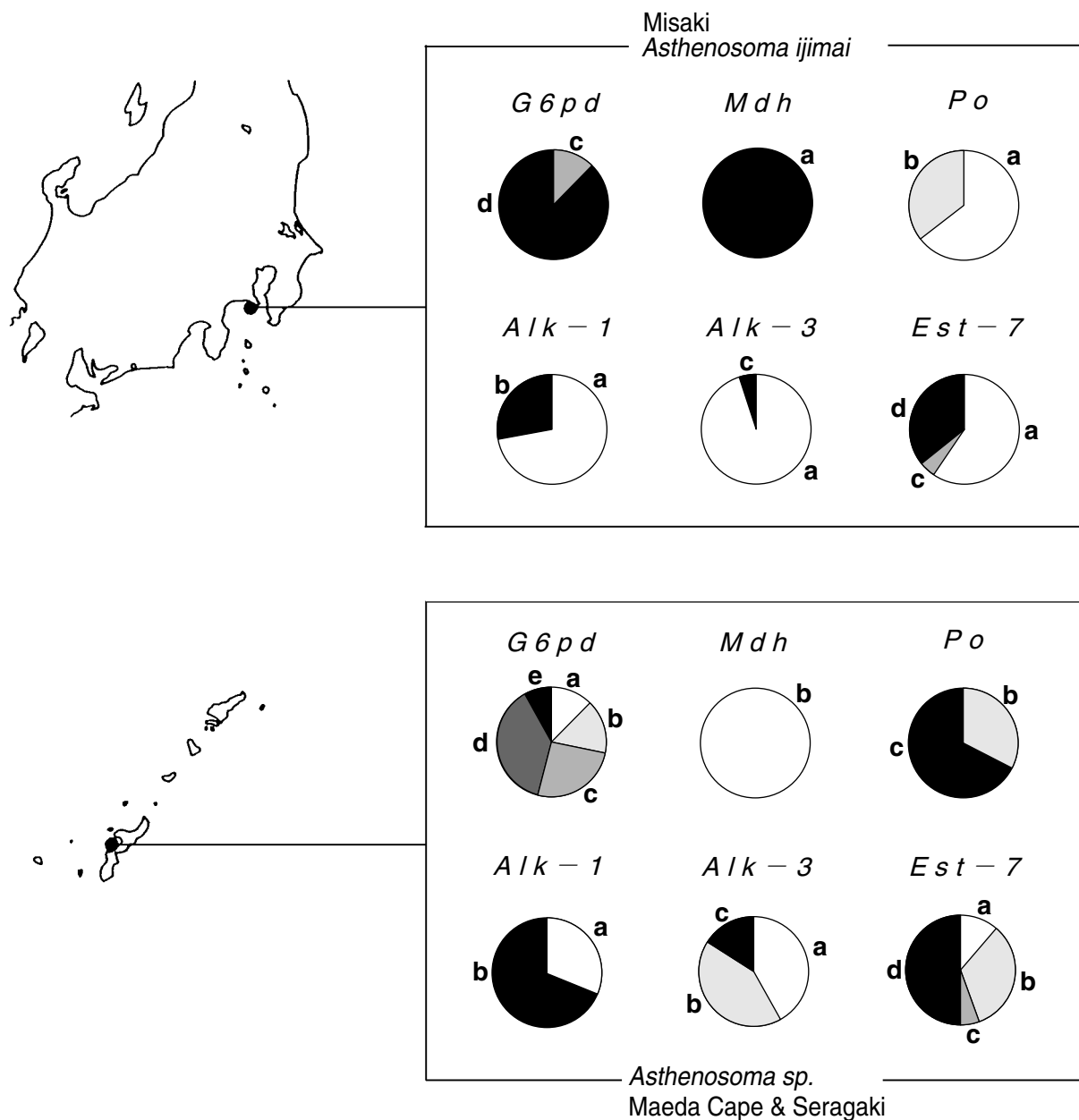
## Genetic differentiation between the two echinothurioids

The two echinothurioids, *A. ijimai* and *A. sp.* (Fig. 1) showed the different allelic compositions in several genetic loci as evident from Table 1: they do not share gene pools to each other. Namely, they were fixed for different alleles at *Mdh* genetic locus and *A. sp.* had three diagnostic alleles (*G6pd-a*, *G6pd-b* and *G6pd-e*) that were not observed in *A. ijimai*. In *Po* locus, they had the respective diagnostic alleles (*Po-a* in *A. ijimai* and *Po-c* in *A. sp.*). Further, *Alk-3* and *Alk-7* were also diagnostic genetic loci. These diagnostic genetic loci are useful molecular characters for distinguishing the two morphologically similar echinothurioids. Fig. 2 shows the circular graph of the comparison of allele frequencies in six genetic loci (*G6pd*, *Mdh*, *Po*, *Alk-1*, *Alk-3* and *Est-7*) between two echinothurioids, *A. ijimai* from Misaki and *A. sp.* from Ryukyu Islands. This figure indicates the genetic difference in allelic composition and allele frequencies between two echinothurioids, and it does not show the geographical cline of alleles between conspecific local populations. According to a number of biochemical systematic studies on a variety of animal taxa, distinct species which are reproductively isolated are typically fixed for different alleles at the same locus. The present allozyme data (Table

**Table 1.** Allele frequencies at 23 genetic loci coding for 10 different enzymes in two echinothurioids of the genus *Asthenosoma*.

Locus	Allele	<i>Ai</i>	<i>Asp</i>
<i>G6pd</i>	a	–	0.12
	b	–	0.17
	c	0.12	0.25
	d	0.88	0.38
	e	–	0.08
<i>Mdh</i>	a	1.00	–
	b	–	1.00
<i>Ndh</i>	a	0.04	0.09
	b	0.54	0.41
	c	0.42	0.50
<i>Odh</i>	a	1.00	1.00
	b	0.96	1.00
<i>Sod-1</i>	a	0.96	1.00
	b	0.04	–
<i>Sod-2</i>	a	1.00	1.00
	b	0.73	0.96
<i>Pgi</i>	a	0.73	0.96
	b	0.27	0.04
<i>Lap-1</i>	a	1.00	1.00
	b	0.50	0.67
<i>Lap-2</i>	a	0.50	0.33
	b	–	0.67
<i>Lap-3</i>	a	1.00	0.33
	b	0.75	1.00
<i>Lap-4</i>	a	0.75	1.00
	b	0.25	–
<i>Lap-5</i>	a	1.00	1.00
	b	0.64	–
<i>Po</i>	a	0.64	–
	b	0.36	0.33
	c	–	0.67
<i>Alk-1</i>	a	0.72	0.31
	b	0.28	0.69
<i>Alk-2</i>	a	0.37	0.50
	b	0.63	0.50
<i>Alk-3</i>	a	0.95	0.42
	b	–	0.42
	c	0.05	0.16
<i>Est-1</i>	a	1.00	1.00
	b	0.27	0.42
<i>Est-2</i>	a	0.27	0.42
	b	0.73	0.58
<i>Est-3</i>	a	0.88	0.86
	b	0.12	0.14
<i>Est-4</i>	a	0.25	0.09
	b	0.75	0.86
<i>Est-5</i>	a	–	0.08
	b	1.00	0.92
<i>Est-6</i>	a	0.08	0.23
	b	0.92	0.77
<i>Est-7</i>	a	0.59	0.11
	b	–	0.33
	c	0.05	0.06
	d	0.36	0.50

Alleles are correspondingly lettered from "a", this being the allele of the lowest mobility. *Ai*=*Asthenosoma ijimai*; *Asp*=*A. sp.*



**Fig. 2.** The circular graph showing the comparison of allele frequencies in six diagnostic genetic loci between two echinothurioids, *A. ijimai* from Misaki and *A. sp.* from Ryukyu Islands.

1 and Fig. 2) clearly show that there is the genetic difference between the two echinothurioids, *A. ijimai* and *A. sp.* (Fig. 1). The present allozyme study provided a molecular evidence for that they are genetically distinct and separate species.

To estimate the degree of genetic differentiation between two echinothurioids, the genetic distance ( $D$ ) and genetic identity ( $I$ ) were calculated by the method of Nei (1972) from the allele frequency data (Table 1). As a result, we obtained the data of  $D=0.181$  and  $I=0.834$ . When compared with the values in various animals, the  $D$  and  $I$  values between two echinothurioids were higher than those (ca.  $I=0.9\sim 1.0$ ) observed between conspecific local populations and comparable to those (ca.  $I=0.8\sim 0.9$ ) between closely

related and sibling species (Nei, 1987). Matsuoka and Hatanaka (1991) reported in the previous allozyme study that the tropical echinoid, *Echinometra mathaei* from Ryukyu Islands consists of four different sibling species. The present  $D$  and  $I$  values were comparable to those (ca.  $I=0.8\sim 0.9$ ) among four sibling species of *Echinometra mathaei*. Judging from the genetic distance data, it is speculated that *A. ijimai* and *A. sp.* from Japanese waters are two closely related or sibling species. One of the present authors (SA) reported that *A. sp.* was different from *A. ijimai* in the following morphological, developmental and ecological characters (Amemiya and Emlet, 1992; Amemiya *et al.*, 1980; Amemiya and Tsuchiya, 1979; Amemiya and Uehara,

1999). Namely, *A. sp.* had smaller body size than that of *A. ijimai* and was distinct from *A. ijimai* in the color of oral spines and body color in external morphological viewpoint (Fig. 1). Further, from the comparative developmental study, it was showed that *A. sp.* was distinct from *A. ijimai* in the size of egg and sperm head and the character of fertilization membrane. In addition, the ecological observation indicated that *A. ijimai* lives on the rocks, but *A. sp.* in the dark borrows of rocks. Thus, the collection of *A. sp.* by scuba diving is more difficult than *A. ijimai*. These non-molecular data are well consistent with the present allozyme study. Nishida and Lucas (1988) reported on the genetic differentiation among many local populations of the starfish, *Acanthaster planci* based on allozyme study. Their study showed that the genetic differentiation among local populations of the starfish was a little because of the very long migration distance (about 10,000km) of larva. In contrast with the starfish, *A. ijimai* metamorphoses after about five days from fertilization and the young adults lives on rocks after about two weeks. This developmental evidence might suggest that the migration distance of larva is much limited in *A. ijimai* and thus the degree of gene diffusion of the echinoid is considerably low. From these data, we speculate that the genetic differentiation among local populations of the echinothurioid would be more promoted by such factors.

In conclusion, we propose that the two echinothurioids should be classified as two separate and sibling species of the genus *Asthenosoma*. Further, we would like to give the following scientific names: the echinothurioid species from Sagami Bay is *Asthenosoma ijimai* and that from Ryukyu Islands is *Asthenosoma ijimai R.*

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