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Phylogenetic Relationships among Four Echinoids of the Family Cidaridae (Cidaroida) Based on Allozymes

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ABSTRACT—The family Cidaridae of the order Cidaroida from Japanese waters includes four common echinoid species belonging to four different genera: *Stereocidaris japonica*, *Eucidaris metularia*, *Prionocidaris baculosa*, and *Phyllacanthus dubius*. Phylogenetic relationship among the four species were investigated by allozyme analysis. From the allozyme variation in 18 genetic loci, Nei's genetic distances between species were calculated. The genetic distances were higher than those observed between confamilial genera in many other echinoids, but comparable to those between different families. The result suggests that the four cidarids diverged in earlier time from one another and generally have older evolutionary origin than members of families of the orders Echinoida and Diadematoida. A molecular phylogenetic tree for the four cidarids indicated the following: (1) *S. japonica* and *Pr. baculosa* are the most closely related to each other and diverged later. (2) *E. metularia* is more closely related to the cluster of *S. japonica* and *Pr. baculosa* than *Ph. dubius*. (4) *Ph. dubius* is the most distant among four species and diverged first. These allozyme results are discussed through the comparison with other non-molecular evidence.

INTRODUCTION

The echinoid fauna of Japanese waters is remarkably rich and about 160 species have been recorded by Shigei (1986). With such extensive species diversity, taxonomy and phylogeny of the echinoids have been extensively studied from the morphological and/or palaeontological standpoints (Nisiyama, 1966,1968; Shigei, 1974, 1986). However, many unresolved problems still remain for the phylogenetic and evolutionary relationships of the echinoids. For an elucidation of these problems, it would be desirable to actively introduce the molecular approaches which are more analytic and quantitative than the usual morphological methods into the field of echinoid systematics.

In the previous papers, we have reported on the biochemical systematics of echinoids of four different families (Toxopneustidae, Strongylocentrotidae, Echinometridae, and Temnopleuridae) of the order Echinoida (Matsuoka,1980, 1985,1986,1987; Matsuoka and Suzuki, 1989; Matsuoka and Hatanaka, 1991; Matsuoka and Inamori, 1996), two families (Phymosomatidae and Stomopneustidae) of the order Arbacioida (Matsuoka and Nakamura, 1991), and family Diadematidae of the order Diadematoida (Matsuoka, 1989). Through these serial biochemical systematic studies, we could provide reliable and, in some cases, critical information to the echinoid systematics.

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Another evolutionarily attractive echinoid group whose phylogeny has not yet been studied at molecular level is the order Cidaroida. The echinoid taxonomists claim that the order Cidaroida is a primitive echinoid group which diverged earlier, from the morphological standpoint (Shigei, 1974, 1986). The echinoids have a unique morphology when compared with the order Echinoida which is generally thought to be the recent echinoids. For example, they possess a few but thick rod-like primary spines. Furthermore, coronal plate of the test is large,the ambulacral plate is of simple type, the interambulacral plate bears single large tubercle, and the gill slits are absent. These morphological characters have been regarded as primitive by echinoid taxonomists (Shigei, 1974, 1986).

Since the order Cidaroida includes many rare species and deep-sea species, it is much more difficult to collect specimens. In addition, the number of individuals which can be sampled is decreasing rapidly now because of rapid marine pollution. Therefore, the molecular phylogenetic studies of these echinoids have not been carried out for the long time. Fortunately, we recently collected four species of the order: *Stereocidaris japonica* (Döderlein), *Eucidaris metularis* (Lamarck), *Prionocidaris baculosa* (Lamarck), and *Phyllacanthus dubius* Brandt. These echinoids are representative species of the order Cidaroida from Japanese waters. Taxonomically, they belong to the family Cidaridae of the order Cidaroida. The order Cidaroida consists of three families including fossil species alone and two families including extant species. All the living species of the order Cidaroida are

classified into the family Cidaridae except for one species, and thus the family Cidaridae can be considered a representative group of the order Cidaroida (Shigei, 1974, 1986). However, as mentioned above, the number of species of the Cidaridae that are commonly found in Japanese waters are exceedingly scanty, when compared with many other echinoid groups.

More recently, Littlewood and Smith (1995) examined the sequences of 18S ribosomal RNA genes of various echinoids. The aim of their study was to clarify the relationships among different orders of the higher taxonomic rank. In the study, they provided no useful information about the phylogenetic relationships within the order Cidaroida, because they used only two European species, *Cidaris cidaris* and *Eucidaris tribuloides*, from the cidarids. Therefore, until now, the phylogenetic relationships among members of the cidarids have not yet been studied by molecular approaches, and thus the phylogeny within the cidarids still remain uncertain.

In this study, we have attempted an allozyme study to clarify the phylogenetic relationships among four species of the family Cidaridae (Cidaroida) from Japanese waters. This is the first report of biochemical systematics of the cidarids using allozyme analysis.

MATERIALS AND METHODS

Echinoid materials

The four species examined are *Stereocidaris japonica* (Döderlein), *Eucidaris matularia* (Lamarck), *Prionocidaris baculosa* (Lamarck), and *Phyllacanthus dubius* Brandt. *Stereocidaris japonica* (4 specimens) were provided from the Noto Marine Laboratory of Kanazawa University (Ogi, Noto Peninsula, Ishikawa Pref.) and *Pr. baculosa* (4 specimens) from the Seto Marine Biological Laboratory of Kyoto University (Shirahama, Wakayama Pref.). *Eucidaris matularia* (4 specimens) were collected from the coast near the Sesoko Marine Science Center, University of the Ryukyus, Sesoko Island, Okinawa Pref. with SCUBA. *Phyllacanthus dubius* (2 specimens) were collected at the coast of Miyanohama, Chichi-jima, Ogasawara Island, Tokyo, also with SCUBA. Immediately after collection, the whole bodies were frozen on dry ice, and stored at –80°C until use. Before electrophoresis, the guts and gonads were cut off and the extracts of these tissues were prepared as described below.

Enzyme electrophoresis

Electrophoresis was performed on 7.5% polyacrylamide gels as described previously (Matsuoka, 1985): About 0.1 g of gut or gonad was individually homogenized in 3 vols of 20 mM phosphate buffer,pH 7.0, containing 0.1 M KCl and 1 mM EDTA, by using a small polyethylene homogenizer of the Potter-Elvehjem type in an ice water bath. After centrifugation at 12,000 rpm for 3 min at 4°C, 0.05 ml of clear supernatant was used for electrophoretic analyses of enzymes. Electrode buffer was 0.38 M glycine-tris buffer, pH 8.3. After electrophoresis, the gels were stained for the following 11 different enzymes: malate dehydrogenase (MDH), octanol dehydrogenase (ODH), xanthine dehydrogenase (XDH), glucose-6-phosphate isomerase (GPI), phosphoglucomutase (PGM), superoxide dismutase (SOD), peroxidase (PO), alkaline phosphatase (ALK), esterase (EST),amylase (AMY), and leucine amino peptidase (LAP). Two enzymes (MDH and ALK) were assayed with extract of the gonad, and the other nine enzymes with that of the gut. Stain recipes for these enzymes have been described previously (Matsuoka and Hatanaka, 1991; Matsuoka *et al*. 1991).

RESULTS AND DISCUSSION

Eighteen genetic loci were inferred from the allozyme variation observed in the 11 different enzymes. Table 1 shows the allele frequencies at 18 genetic loci. Nine loci (*Xdh*, *Pgm*, *Sod-2*, *Alk-1*, *Alk-2*, *Est-2*, *Est-4*, *Amy-1*, and *Amy-2*) were monomorphic and exhibited a single active band which varied inter-specifically. *Est-3* showed single- and double-banded phenotypes between species, and they were assumed as the products of two different alleles. The remaining eight loci (*Mdh*, *Odh*, *Pgi*, *Sod-1*, *Po*, *Alk-3*, *Est-1*, and *Lap*) were polymorphic in at least one species. In general, the enzymes such as SOD, EST and LAP are highly polymorphic in echinoids or asteroids.

Relationship between the estimation of average heterozygosity (*H*) and sample size has been reported by some workers in the electrophoretic surveys of genetic variation within population (Nei and Roychoudhury, 1974; Nei, 1978; Gorman and Renzi, 1979).Their general conclusion was that even a few individuals are sufficient for estimating *H* value if the number of loci examined is large. Furthermore, Nei (1987) reported

Table 1. Allele frequencies at 18 genetic loci in the four echinoids of the family Cidaridae

Locus	*Sj*	*Em*	*Pd*	*Pd*
Mdh	d (0.33) e (0.67)	c	a (0.75) b (0.25)	a
Odh	c (0.75) d (0.25)	b	c	a
Xdh	b	b	b	a
Pgi	a (0.33) b (0.67)	c	d	d
Pgm	b	a	b	a
Sod-1	a	–	a (0.25) b (0.75)	c
Sod-2	a	a	a	–
Po	c	c	b (0.50) c (0.50)	a
Alk-1	b	a	b	b
Alk-2	c	b	a	a
Alk-3	–	c	b (0.50) c (0.50)	a
Est-1	b	a	c	a (0.50) c (0.50)
Est-2	a	a	a	–
Est-3	b	a	a	b
Est-4	c	c	b	a
Amy-1	b	a	a	a
Amy-2	b	a	–	–
Lap	a	b	a (0.25) c (0.25) d (0.50)	a

Alleles are correspondingly lettered from “a”, this being the allele of the lowest electrophoretic mobility. The value in parentheses represents the frequency of each allele in species. A hypen indicates that the allele was not detected in the locus. In *Mdh* and *Pgi* of *S. japonica* three individuals were used in the allozyme analysis and in other 16 loci four individuals were assayed. *Sj*, *Stereocidaris japonica*; *Em*, *Eucidaris metularia*; *Pb*, *Prionocidaris baculosa*; *Pd*, *Phyllacanthus dubius*.

that 20–30 loci have often been used in allozyme studies and the number of loci is adequate for estimating *H* values. Bearing their findings in mind, we calculated expected average heterozygosity per locus (*H*) in the four species. The *H* values obtained were 7.4% for *S. japonica*, 0% for *E. metularia*, 14.0% for *P. baculosa*, and 3.3% for *Ph. dubius*. We have previously reported the amount of genetic variation within populations of various echinoderm species (Matsuoka *et al.*, 1993). According to it, the *H* value (14.0%) of *P. baculosa* was comparable to those of many other deep-sea echinoderms, while the H values of other three species (*E. metularia* and *Ph. dubius* are shallow water echinoids) were comparable to those of many other shallow water echinoderms.

To quantify the degree of genetic differentiation between the four species, we calculated genetic identity (*I*) and genetic distance (*D*) between two of each species by the method of Nei (1972) from the allele frequencies data in Table 1. Table 2 shows the matrices of *I* and *D* values between all pairs of four species. The *I* and *D* values between four cidarids were in the range of 0.141–0.417, with a mean of 0.289, and in the range of 0.875–1.959, with a mean of 1.315, respectively.

Although the number of individuals used in this study is small, it has been shown that the number of loci used has far more effect on the *D* estimates than the number of individuals sampled (Nei and Roychoudhury, 1974; Nei, 1978; Gorman and Renzi, 1979). Indeed, it has been demonstrated in the study of *Anolis* lizards that the *D* estimates from a sample size of only one or two individuals deviate only slightly from those from a much larger sample size (Gorman and Renzi, 1979). Furthermore, our serial allozyme studies on the phylogeny of echinoderms suggested that in the phylogenetic studies with allozyme analysis at higher taxonomic rank of different genera the number of individuals has hardly any effect to the estimation of the genetic distance and the phylogenetic relationships (Matsuoka, unpublished data).

Table 3 summarizes the *I* and *D* values observed between two confamilial genera of the orders Echinoida and Diadematoida reported previously (Matsuoka, 1987, 1989; Matsuoka and Suzuki, 1989; Matsuoka and Inamori, 1996). Furthermore, Table 4 shows the *I* and *D* values observed between two echinoids of different families. All the data were compiled from our serial biochemical systematics of echinoids by using allozyme analysis (Matsuoka, 1987; Matsuoka and Nakamura, 1991), and thus they can be compared at the equivalent level. As evident from Table 3, the mean *I* and *D* values between 28 pairs of confamilial genera were 0.501 and 0.706, respectively. The present mean *I* and *D* values between 6 pairs of the family Cidaridae were 0.289 and 1.315, respectively, and these values were not comparable to those between confamilial genera in other echinoids. On the other hand, the mean *I* and *D* values between 13 pairs belonging to different families were 0.295 and 1.245, respectively, and these values were comparable to the present values. Namely, the genetic distances between four species of the family Cidaridae are considerably higher (about two times) than those between genera in many other echinoids, and rather equivalent with those between families.

Nei (1975) reported that the genetic distance (*D*) estimated electrophoretically corresponds well with the divergence time (*T*) from the common ancestor, and that T of two taxa can be calculated by $T=5\times10^6 D$ (years). Judging from the findings, it is considered that these four cidarid species diverged at earlier time: the present allozyme study indicated that four cidarids studied here are much more genetically differentiated from one another than members of families of the orders Echinoida and Diadematoida.

Although the higher genetic distances more than $D=1$ were observed in this study, the similar values have also obtained in the allozyme study among seven echinoids belonging to the two different families, Toxopneustidae and Strongylocentrotidae, of the order Echinoida, as mentioned above (Matsuoka, 1987). In the biochemical dendrogram constructed, the seven species of the two families examined were clearly divided into two large clusters corresponding to the two families. Further, the allozyme results including the relationships among taxa at species and genus level were strongly supported by the other biochemical and non-molecular studies including the morphological studies. It indicates that even the higher genetic distances more than $D=1$ can provide reliable information to the estimation of the phylogenetic relationships among taxa (Matsuoka, 1987; Matsuoka *et al.*, 1994). Thus, it is considered that the higher genetic distances among the cidarids obtained in this study have hardly any effect to the estimation of the phylogeny of the cidarids.

To clarify their phylogenetic relationships, the molecular phylogenetic tree for four species was constructed from Nei's genetic distance matrix of Table 2 by using the UPGMA clustering method of Sneath and Sokal (1973). The phylogenetic tree (Fig. 1) indicated the following: (1) *S. japonica* and *Pr. baculosa* are most closely related to each other among the

Table 2. Genetic identities (above diagonal) and genetic distances (below diagonal) between four species of the family Cidaridae

Species	1	2	3	4
1. *Stereocidaris japonica*	–	0.288	0.417	0.175
2. *Eucidaris metularia*	1.245	–	0.358	0.141
3. *Prionocidaris baculosa*	0.875	1.027	–	0.354
4. *Phyllacanthus dubius*	1.743	1.959	1.038	–

Genetic identity (*I*) and genetic distance (*D*) were calculated by the method of Nei (1972).

Table 3. Genetic identity (*I*) and genetic distance (*D*) between two species of confamilial genera in various echinoids

Paris				*I*	*D*
(1) Family Toxopneustidae					
Toxopneustes pileolus	vs	*Tripneustes gratilla*		0.471	0.753
Toxopneustes pileolus	vs	*Pseudoboletia maculata*		0.542	0.612
Tripneustes gratilla	vs	*Pseudoboletia maculata*		0.474	0.747
(2) Family Strongylocentrotidae					
Strongylocentrotus intermedius	vs	*Hemicentrotus pulcherrimus*		0.562	0.576
Strongylocentrotus nudus	vs	*Hemicentrotus pulcherrimus*		0.570	0.562
Strongylocentrotus intermedius	vs	*Pseudocentrotus depressus*		0.472	0.751
Strongylocentrotus nudus	vs	*Pseudocentrotus depressus*		0.418	0.872
(3) Family Echinometridae					
Anthocidaris crassispina	vs	*Echinometra mathaei* (A-type)		0.631	0.460
Anthocidaris crassispina	vs	*Echinostrephus aciculatus*		0.587	0.533
Anthocidaris crassispina	vs	*Echinostrephus molaris*		0.579	0.546
Anthocidaris crassispina	vs	*Heterocentrotus mammillatus*		0.380	0.968
Anthocidaris crassispina	vs	*Colobocentrotus mertensii*		0.487	0.719
Echinometra mathaei (A-type)	vs	*Echinostrephus aciculatus*		0.585	0.536
Echinometra mathaei (A-type)	vs	*Echinostrephus molaris*		0.581	0.543
Echinometra mathaei (A-type)	vs	*Heterocentrotus mammillatus*		0.434	0.835
Echinometra mathaei (A-type)	vs	*Colobocentrotus mertensii*		0.509	0.675
Echinostrephus aciculatus	vs	*Heterocentrotus mammillatus*		0.390	0.942
Echinostrephus aciculatus	vs	*Colobocentrotus mertensii*		0.426	0.853
Echinostrephus molaris	vs	*Heterocentrotus mammillatus*		0.369	0.997
Echinostrephus molaris	vs	*Colobocentrotus mertensii*		0.442	0.816
Heterocentrotus mammillatus	vs	*Colobocentrotus mertensii*		0.476	0.742
(4) Family Temnopleuridae					
Temnopleurus toreumaticus	vs	*Mespilia globulus*		0.447	0.805
Temnopleurus hardwickii	vs	*Mespilia globulus*		0.387	0.949
Temnopleurus reevesii	vs	*Mespilia globulus*		0.426	0.853
(5) Family Diadematidae					
Diadema setosum	vs	*Echinothrix calamaris*		0.635	0.450
Diadema setosum	vs	*Echinothrix diadema*		0.596	0.519
Diadema savignyi	vs	*Echinothrix calamaris*		0.699	0.359
Diadema savignyi	vs	*Echinothrix diadema*		0.456	0.785
			Average	0.501	0.706
(5) Family Cidaridae					
Stereocidaris japonica	vs	*Eucidaris metularia*		0.288	1.245
Stereocidaris japonica	vs	*Prionocidaris baculosa*		0.417	0.875
Stereocidaris japonica	vs	*Phyllacanthus dubius*		0.175	1.743
Eucidaris metularia	vs	*Prionocidaris baculosa*		0.358	1.027
Eucidaris metularia	vs	*Phyllacanthus dubius*		0.141	1.959
Prionocidaris baculosa	vs	*Phyllacanthus dubius*		0.354	1.038
			Average	0.289	1.315

The data except for the Cidaridae were compiled from Matsuoka (1987,1989), Matsuoka and Suzuki (1989) and Matsuoka and Inamori (1996).

four species and diverged later. (2) *E. metularia* is more closely related to the cluster of *S. japonica* and *Pr. baculosa* than *Ph. dubius*. (3) *Ph. dubius* is the most distant among the four species and diverged first.

The molecular phylogenetic tree (Fig.1) shows not only their relationships, but also the sequence of their evolutionary divergences. From the equation of Nei (1975), their divergence times were calculated. According to them, *Ph. dubius* diverged firstly about 8 million years ago (MY). Next, *E. metularia* diverged from the common ancestor of *S. japonica* and *Pr. baculosa* about 6 MY. Lastly, the divergence of *S. japonica* and *Pr. baculosa* occurred about 4 MY. These divergence times are considerably older than those of echinoids belonging to the orders Echinoida and Diadematoida (Matsuoka, 1987, 1989; Matsuoka and Suzuki, 1989; Matsuoka and Inamori, 1996), and it shows their older evolutionary origin.

With respect to the taxonomic relationships among the four cidarids, Shigei (1974) classified the four species into three subfamilies based on the morphology of globiferous pedicellaria: the subfamily Stereocidarinae for *S. japonica*, the

Table. 4. Genetic identities (*I*) and genetic distances (*D*) between two echinoids belonging to different families

Pairs				*I*	*D*
(1) Family Toxopneustidae	vs	Family Strongylocentrotidae			
Toxopneustes pileolus	vs	*Pseudocentrotus depressus*		0.335	1.094
Toxopneustes pileolus	vs	*Strongylocentrotus intermedius*		0.395	0.929
Toxopneustes pileolus	vs	*Strongylocentrotus nudus*		0.314	1.158
Toxopneustes pileolus	vs	*Hemicentrotus pulcherrimus*		0.287	1.248
Tripneustes gratilla	vs	*Pseudocentrotus depressus*		0.314	1.158
Tripneustes gratilla	vs	*Strongylocentrotus intermedius*		0.327	1.118
Tripneustes gratilla	vs	*Strongylocentrotus nudus*		0.222	1.505
Tripneustes gratilla	vs	*Hemicentrotus pulcherrimus*		0.172	1.760
Pseudoboletia maculata	vs	*Pseudocentrotus depressus*		0.288	1.245
Pseudoboletia maculata	vs	*Strongylocentrotus intermedius*		0.348	1.056
Pseudoboletia maculata	vs	*Strongylocentrotus nudus*		0.345	1.064
Pseudoboletia maculata	vs	*Hemicentrotus pulcherrimus*		0.240	1.427
(2) Family Phymosomatidae	vs	Family Stomopneustidae			
Glyptocidaris crenularis	vs	*Stomopneustes variolaris*		0.244	1.417
			Average:	0.295	1.245
(3) Family Cidaridae					
Stereocidaris japonica	vs	*Eucidaris metularia*		0.288	1.245
Stereocidaris japonica	vs	*Prionocidaris baculosa*		0.417	0.875
Stereocidaris japonica	vs	*Phyllacanthus dubius*		0.175	1.743
Eucidaris metularia	vs	*Prionocidaris baculosa*		0.358	1.027
Stereocidaris japonica	vs	*Phyllacanthus dubius*		0.141	1.959
Prionocidaris baculosa	vs	*Phyllacanthus dubius*		0.354	1.038
			Average:	0.289	1.315

The data except for the Cidaridae were compiled from Matsuoka (1987) and Matsuoka and Nakamura (1991).

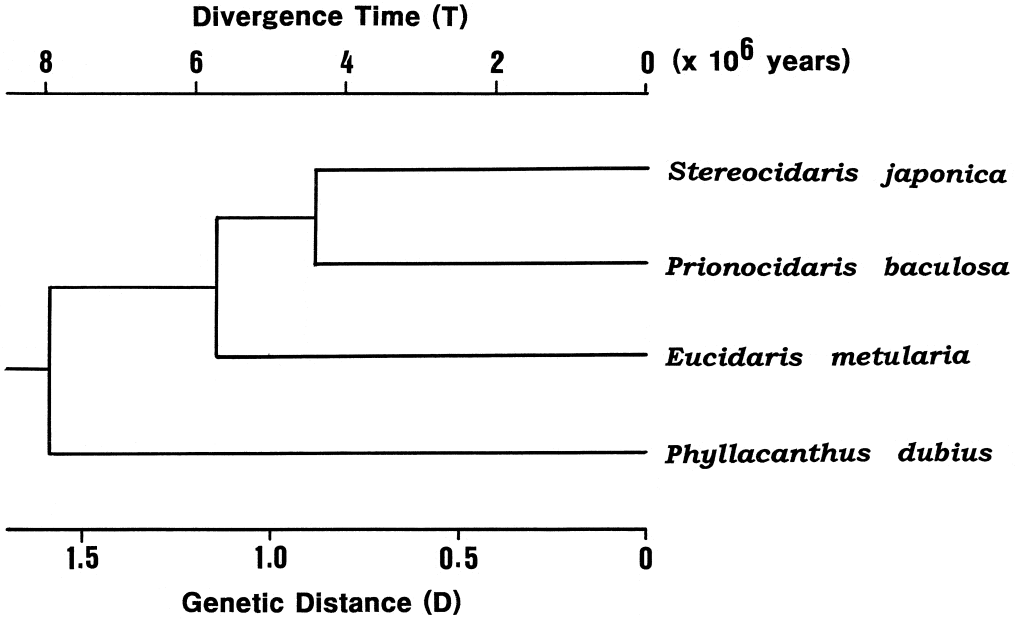

Fig. 1. Molecular phylogenetic tree for the four echinoids of the family Cidaridae (Cidaroida) which was constructed from the Nei's genetic distances (Nei,1972) by using the UPGMA clustering method. The divergence time estimated from the Nei' equation (Nei, 1975) using the genetic distance is also given in the phylogenetic tree.

Cidarinae for *E. metularia*, and the Rhabdocidarinae for *Pr. baculosa* and *Ph. dubius*. In this system, he suggested the close affinity between *Pr. baculosa* and *Ph. dubius*. Thereafter, Shigei (1986) revised his previous subfamily system and proposed the new subfamily system based on the morphology of ambulacral plates and spines besides globiferous pedicellaria. Under the revised system, the present four cidarids are classified into the following three subfamilies: the subfamily Stereocidarinae for *S. japonica*, the Stylocidarinae for *Pr. baculosa* and *E. metularia*, and the Phabdocidarinae for *Ph. dubius*. The present molecular study clearly indicated the close affinity between *Pr. baculosa* and *S. japonica*. Shigei (1976,1986) claimed in his two subfamily systems that *S. japonica* is an isolated species from other members of the family Cidaridae. However, the present allozyme study does not support his view concerning the systematic position of *S. japonica*. On the other hand, Shigei (1986) suggested that *Ph. dubius* may be a phylogenetically distant species from other cidarids, based on the unique morphological structures of peristomial ambulacral plates, secondary tubercles and so on. The present allozyme result strongly supported his view for the systematic position of *Ph. dubius*.

There exists a morphologically very similar echinoid to *Ph. dubius*. The species is *Phyllacanthus imperialis*. In contrast to the fact that *Ph. dubius* is endemic to Ogasawara Islands, *Ph. imperialis* is widely distributed in the Indo-West Pacific Ocean. More recently, Shigei (1994) suggested that these two echinoids may be conspecific from the morphological standpoint. The previous allozyme study demonstrated that the tropical echinoid, *Echinometra mathaei* consists of four distinct species (Matsuoka and Hatanaka, 1991). Therefore, the problem whether *Ph. dubius* and *Ph. imperialis* are the same single species or two distinct species may be clarified by allozyme study. In the near future, if *Ph. imperialis* is collected, we are planning to investigate their taxonomic and genetic relationship by allozyme analysis.

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REFERENCES

Gorman GC, Renzi J (1979) Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979: 242–249

Littlewood DT, Smith AB (1995) A combined morphological and molecular phylogeny for sea urchins (Echinoidea; Echinodermata). Proc R Soc Lond B Biol Sci 347: 213–234

Matsuoka N (1980) Immunological relatedness of sea-urchin glucose-6-phosphate dehydrogenases: Phylogenetic implication. Comp Biochem Physiol 66B: 605–607

Matsuoka N (1985) Biochemical phylogeny of the sea-urchins of the family Toxopneustidae. Comp Biochem Physiol 80B: 767–771

Matsuoka N (1986) Further immunological study on the phylogenetic relationships among sea-urchins of the order Echinoida. Comp Biochem Physiol 84B: 465–468

Matsuoka N (1987) Biochemical study on the taxonomic situation of the sea-urchin, *Pseudocentrotus depressus*. Zool Sci 4: 339–347

Matsuoka N (1989) Biochemical systematics of four sea-urchin species of the family Diadematidae from Japanese waters. Biochem Syst Ecol 17: 423–429

Matsuoka N, Hatanaka T (1991) Molecular evidence for the existence of four sibling species within the sea-urchin, *Echinometra mathaei* in Japanese waters and their evolutionary relationships. Zool Sci 8: 121–133

Matsuoka N, Inamori M (1996) Phylogenetic reelationships of echinoids of the family Temnopleuridae inferred from allozyme variation. Genes Genet Syst 71: 203–209

Matsuoka N, Nakamura Y (1991) Genetic distance and protein polymorphism in two sea-urchin species of the order Arbacioida and implications for their evolution. Comp Biochem Physiol 98B: 21–27

Matsuoka N, Inamori M, Sugawara M (1993) High genetic variability in the starfish *Distolasterias* nipon revealed by enzyme electrophoresis. Comp Biochem Physiol 104B: 75–79

Matsuoka N, Suzuki H (1989) Electrophoretic study on the phylogenetic relationships among six species of sea-urchins of the family Echinometridae found in the Japanese waters. Zool Sci 6: 589–598

Matsuoka N, Yoshida K, Fukuda K, Shigei M (1991) Genetic variation in the starfish *Coscinasterias acutispina*. Comp Biochem Physiol 99B: 893–898

Nei M (1972) Genetic distance between populations. Am Nat 106: 283–292

Nei M (1975) Molecular Population Genetics and Evolution. North-Holland, Amsterdam.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei M, Roychoudhury AK (1974) Sampling variance of heterozygosity and genetic distance. Genetics 76: 379–390

Nisiyama S (1966) The echinoid fauna from Japan and adjacent regions, Part I. Palaeontol Soc Japan Spec Pap No.11

Nisiyama S (1968) The echinoid fauna from Japan and adjacent regions, Part II. Palaeontol Soc Japan Spec Pap No.13

Shigei M (1974) Echinoids. In “Systematic Zoology” Ed by T Uchica, Nakayama-shoten, Tokyo, Vol. 8b, pp 208–332 (in Japanese)

Shigei M (1986) The Sea Urchins of Sagami Bay. Maruzen, Tokyo.

Shigei M (1994) Echinoids. In “Marine organisms in coral reefs” Ed by T Okutani, Yama-to-Keikoku-sha, Tokyo, pp 255–266 (in Japanese)

Sneath PHA, Sokal PR (1973) Numerical Taxonomy. Freeman, San Francisco, CA.

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