

Phylogenetic Relationships of Four Families of the Asteroidea based on Allozyme

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Introduction

Echinoderms are attractive invertebrates at the evolutionary viewpoint. From some non-molecular and molecular evidence, echinoderms are closely related to vertebrates, and thus they are on the key position of animal evolution. The evidence is as follows: (1) The vertebrates and echinoderms have skeleton system. (2) The developmental biology showed that they belong to Deuterostomia. (3) They have hexose-6-phosphate dehydrogenase (H6PD) of glucose-6-phosphate dehydrogenase (G6PD) isozyme which there does not exist in invertebrates except echinoderms (Matsuoka and Hori, 1980). (4) The asteroids have strong trypsin-like digestive enzyme which is homologous to trypsin of vertebrates (Hori *et al.*, 1977). (5) The metamorphosis in echinoids is induced by thyroid hormones which are found in vertebrates (Chino *et al.*, 1994; Saito *et al.*, 1998). (6) The molecular phylogenetic tree constructed by the sequence analysis of some proteins demonstrated the close affinity between echinoderms and vertebrates (Miyata, 1994).

I have been investigating the phylogeny of the class Echinoidea (echinoids), which is one of the major groups of the phylum Echinodermata, by using allozyme analysis and immunological method (eg., Matsuoka, 1986, 1987; Matsuoka and Hatanaka, 1991; Matsuoka and Suzuki, 1989; Matsuoka *et al.*, 2004). Another large group of Echinodermata is the class Asteroidea (asteroids). Although the taxonomy of asteroids has been extensively studied from morphological and paleontological standpoint, there are disagreements between taxonomists, and thus several taxonomic systems have been proposed (Hayashi, 1940; Spencer and Wright, 1966; Clark and Downey, 1992). Namely, many unresolved problems concerning the phylogeny and taxonomy of asteroids still remain. In parallel with the molecular phylogenetic studies of echinoids, I reported the molecular phylogeny of some asteroid groups (Matsuoka *et al.*, 1994; Matsuoka and Hatanaka, 1998; Matsuoka and Asano, 1999, 2003). Although the molecular phylogenetic studies of asteroids at species and genus level have been carried out by some workers, the interfamilial relationships at higher taxonomic rank have not yet been resolved at molecular level until now. As a first step to clarify the problem, I adopted the following four representative and common families as target taxa: the families Asterinidae, Acanthasteridae, Solasteridae and Echinasteridae. One of the purpose of this study is the molecular evolution of traditional and/or classical taxonomic system based on morphology: the old taxonomic system (Hayashi, 1940; Spencer and Wright, 1966) included these four families into the same order Spinulosida, but the new taxonomic system which was proposed by Clark and Downey (1992) classified these four families into three different orders: the two families, Asterinidae and Acanthasteridae are members of the order Valvatida, Echinasteridae is that of the order Spinulosida, and Solasteridae is that of the order Velatida. The difference of two taxonomic systems

is due to the different estimation of the morphological characters adopted in taxonomy. Other purpose that I chose these four families is to examine the phylogenetic heterogeneity of the family Asterinidae which was suggested by some workers. From morphological viewpoint, the distinction of arm and disk is unclear and the arrangement of skeleton is irregular in the members of the Asterinidae in contrast with other families. Furthermore, immunological study using antibody against hexokinase (HK) suggested that the family Asterinidae is heterogenous lineage (Mochizuki and Hori, 1980). To clarify the phylogenetic relationships among these four families, I adopted the following four typical and common species from each family: *Asterina pectinifera* from Asterinidae, *Acanthaster planci* from Acanthasteridae, *Echinaster luzonicus* from Echinasteridae, and *Solaster dawsoni* from Solasteridae (Fig. 1). *Asterina pectinifera* is much common species and one of the typical seashore animals in Japanese waters, and has often been used in developmental biology, physiology or biochemistry as experimental animal. The two species (*A. planci* and *E. luzonicus*) are found in shallow water of subtropical seas of Ryukyu Islands (Okinawa) in Japan. It is famous that *A. planci* is the poisonous asteroid with many sharp spines and destroys the coral reef by eating the polyps of corals. *Echinaster luzonicus* has hard body and shows the extensive body color variation. *Solaster dawsoni* is often found in northern seas of Japan and it is one of ferocious asteroids that the species eats the asteroids and the edible scallop in Mutsu Bay of Aomori Pref. of northern Japan.

In this paper, I report the allozyme study of the phylogenetic and taxonomic relationships among the four families (Asterinidae, Acanthasteridae, Echinasteridae and Solasteridae), and the molecular evaluation of taxonomic systems of asteroids.

Materials and Methods

The starfish, *Asterina pectinifera* was collected from Asamushi of Mutsu Bay in Aomori Pref. by snorkeling. *Solaster dawsoni* was collected from Yokohama-machi of Mutsu Bay by starmop-method which fishermen have been using in the extermination of asteroids to protect the scallop. *Acanthaster planci* and *Echinoaster luzonicus* were collected from Seragaki in Onnamura of Okinawa Pref. by scuba diving and snorkeling. Immediately after collection, the pyloric caeca were cut off from the living specimens and frozen in dry ice, and stored at -40°C until use. The number of individuals used in allozyme analysis was 12 for *A. pectinifera*, 10 for *S. dawsoni*, 3 for *A. planci* and 12 for *E. luzonicus*. As *A. planci* is poisonous and dangerous starfish, it was much difficult to collect many individuals and thus the number of specimens was small. Allozyme analysis was performed on 7.5% polyacrylamide gel by the method described in Matsuoka and Hatanaka (1991): About 0.2g pyloric caeca was individually homogenized with 3 vols. of 20mM phosphate buffer containing 0.1M KCl and 1mM EDTA (pH 7.0) by using Potter-Elvehjem type homogenizer in an ice water bath. The tissue extract was centrifuged at 10,000 rpm at 5min and the clear supernatant was used for protein electrophoresis. Electrode buffer was Glycine-tris buffer, pH 8.3. After electrophoresis, allozyme variation of the following 11 different enzymes was analyzed: malate dehydrogenase (MDH), nothing dehydrogenase (NDH), xanthine dehydrogenase (XDH), glucose-6-phosphate isomerase (GPI), hexokinase (HK), superoxide dismutase (SOD), aspartate aminotransferase (AAT), alkaline phosphatase (ALK), peroxidase (PO), esterase (EST) and leucine amino peptidase (LAP). Allozyme analysis and stain recipes of enzymes were performed as described in Matsuoka and Hatanaka (1991).

Results and Discussion

From allozyme variation in 11 enzymes, 39 genetic loci were detected. Of 39 genetic loci, the

Table 1. Genetic identities (above diagonal) and genetic distances (below diagonal) among four asteroids.

Species	1	2	3	4
1. <i>Asterina pectinifera</i>	-	0.405	0.385	0.405
2. <i>Solaster dawsoni</i>	0.904	-	0.513	0.442
3. <i>Acanthaster planci</i>	0.955	0.667	-	0.417
4. <i>Echinaster luzonicus</i>	0.904	0.816	0.875	-

Genetic identities and genetic distances were calculated by the method of Nei (1972).

following 17 loci were polymorphic (*Mdh-2*, *Hk*, *Po-3*, *Po-4*, *Sod-4*, *Sod-6*, *Est-1*, *Est-2*, *Est-3*, *Est-4*, *Est-6*, *Alk-4*, *Alk-8*, *Alk-9*, *Lap-1*, *Lap-2* and *Lap-6*). The remaining 22 loci were monomorphic.

To quantify the degree of genetic differentiation among four species, the genetic identity (*I*) and genetic distance (*D*) between each species were calculated by the method of Nei (1972) from the allele frequencies data in 39 genetic loci. Table 1 shows the matrices of *I* and *D* values between all pairs of species. The highest *I* value ($I=0.513$) was found between *Acanthaster planci* and *Solaster dawsoni*. Figure 1 shows the molecular phylogenetic tree for the four families which was constructed from the Nei's genetic distance matrix of Table 1 by UPGMA clustering method of Sneath and Sokal (1974). The molecular phylogenetic tree indicated the followings:

- (1) Of the four families, Acanthasteridae and Solasteridae are most closely related to each other ($D=0.667$).
- (2) Echinasteridae is more closely related to the cluster of Acanthasteridae and Solasteridae than Asterinidae.
- (3) The Asterinidae is the most distant family of the four families. The genetic distance between Asterinidae and the cluster of other three families was $D=0.921$.

The allozyme results are well consistent with the morphological evidence: As evident from Fig. 1,

- (1) *A. planci* (Acanthasteridae) and *S. dawsoni* (Solasteridae) have multi-arms (10~14) in contrast with five arms of many asteroids.
- (2) They are similar to each other in external morphology and have large body size. On the other hand, *A. pectinifera* (Asterinidae) and *E. luzonicus* (Echinasteridae) have small body size.
- (3) The spines on lateral ambulacral plates are long and sharp in the two families, Acanthasteridae and Solasteridae.
- (4) The distinction between arm and disk is unclear in *A. pectinifera* (Asterinidae), though in other three families it is clear.
- (5) The arrangement of skeletal plates is regular in *A. pectinifera* (Asterinidae), but the other three species have irregular arrangement.

The morphological evidence described above is well consistent with the present molecular phylogenetic study.

I would like to compare the present allozyme results to taxonomic systems. In spite of many morphological studies, taxonomy of asteroids remains to be extremely controversial and several different taxonomic systems are proposed. Hayashi (1940) and Spencer and Wright (1966) classified the four families (Asterinidae, Acanthasteridae, Solasteridae and Echinasteridae) into the order Spinulosida. On the other hand, Clark and Downey (1992) proposed new taxonomic system and established many orders. According to their taxonomic system, the four families studied here are classified into the following three different orders:

- Asterinidae, Acanthasteridae: Valvatida
- Echinasteridae: Spinulosida
- Solasteridae: Velatida

The present allozyme study did not support the taxonomic system of Clark and Downey (1992)

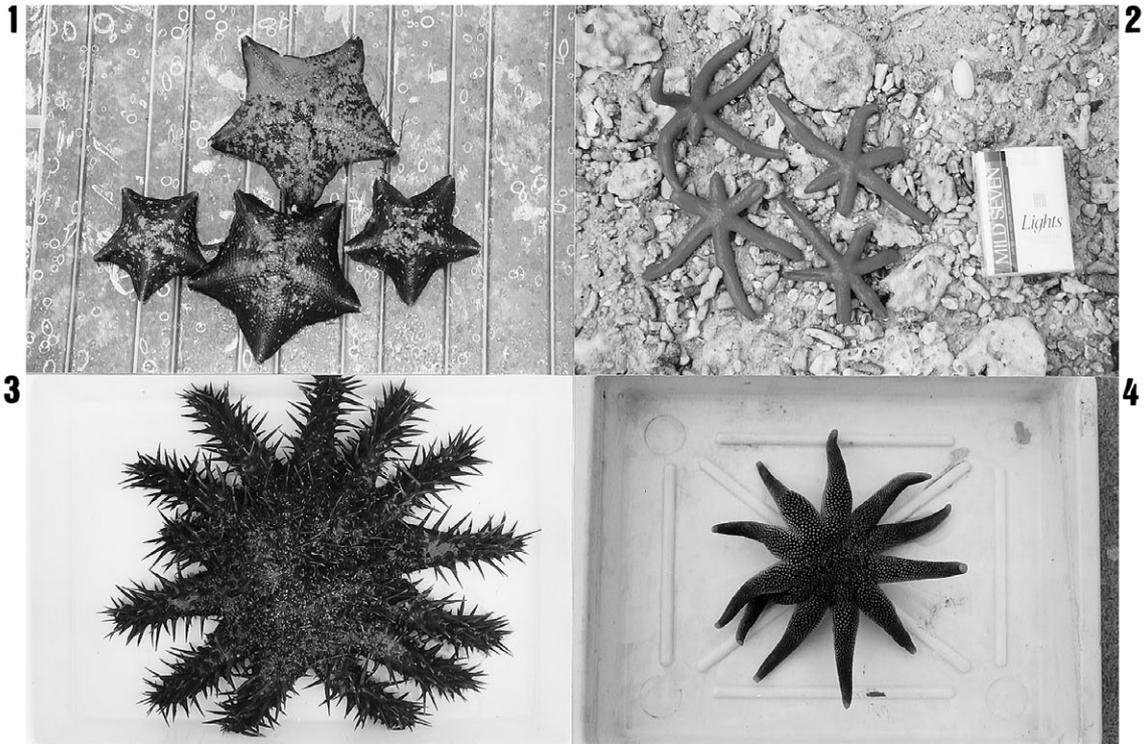


Fig. 1. Four typical species of four families of Asteroidea adopted in the present allozyme study.
1=*Asterina pectinifera*, 2=*Echinoaster luzonicus*, 3=*Acanthaster planci*, 4=*Solaster dawsoni*.

which is widely adopted by many workers. The present author considers that they established many taxonomic groups (orders), and thus that it is complicated taxonomic system. The phylogenetic relationships among four families (Fig. 2) and genetic distance data (Table 1) showing that the genetic distances among four families are comparable to those between different families or genera of the same order in many other animals (Thorpe, 1980) support the taxonomic system proposed by Hayashi (1940) and Spencer and Wright (1966).

Ferguson (1980) reported that the allozyme analysis is much effective in the distinction of morphologically very similar species (sibling species) or in the estimation of genetic relationships at species level, and also that it is effective for elucidating relationships among different genera and families at higher taxonomic ranks. We reported the allozyme study on the distinction of four sibling species of the echinoid, *Echinometra mathaei* (Matsuoka and Hatanaka, 1991) or of two sibling species of the echioid, *Asthenosoma ijimai* (Matsuoka *et al.*, 2004). Although the molecular phylogenetic study of different families is the first attempt, it would provide some useful information to asteroid taxonomy.

It is interesting to compare the present allozyme study with other molecular phylogenetic studies. Mochizuki and Hori (1980) examined the phylogenetic relationships among 22 asteroid species belonging to different orders by the immunological technique of the enzyme inhibition method using antibody against hexokinase purified from *Asterina pectinifera*. The immunological method was also used in the phylogenetic study of echinoids by the present author and provided some useful information into echinoid phylogeny (Matsuoka, 1986). Mochizuki and Hori (1980) found that *Asterina pectinifera* of the family Asterinidae was considerable different lineage from other asteroids, and they suggested that *Asterina pectinifera* belongs to divergent lineage from other asteroids. The present allozyme study suggesting the heterogeneous lineage of Asterinidae is well consistent with the immunological results of Mochizuki and Hori (1980). More recently, Matsubara *et al.* (2004) reported the molecular phylogenetic study of various asteroids by nuclear and mitochondrial DNA sequence analysis. They

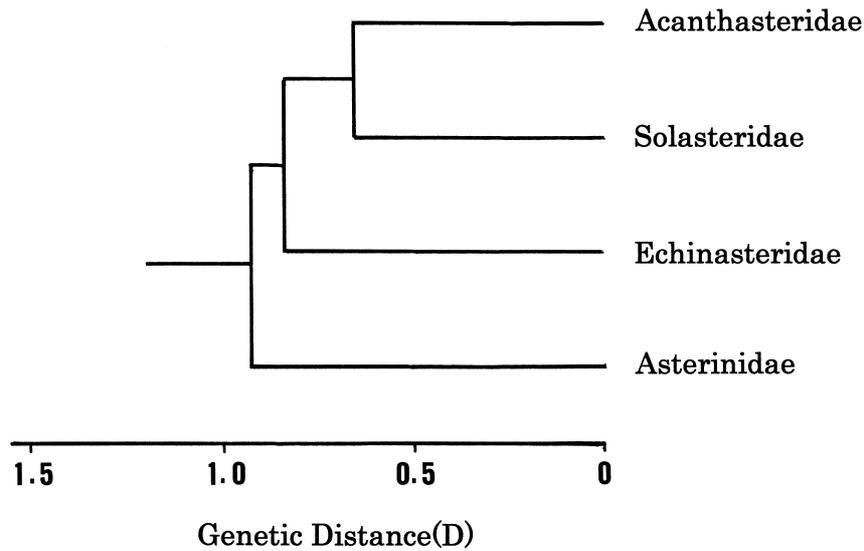


Fig. 2. Molecular phylogenetic tree of four families of the Asteroidea based on allozyme analysis. It was constructed from Nei's genetic distances by the UPGMA clustering method.

adopted three molecular parameters of the nuclear 18S rDNA, mitochondrial 12S rDNA and 16S rDNA. As a result, they suggested the close relationship between genus *Asterina* and family Solasteridae belonging to the different orders. As evident from Fig. 2, the present allozyme study was inconsistent with their molecular phylogenetic study.

In conclusion, the present allozyme study supports the old taxonomic system by Hayashi (1940) and Spencer and Wright (1966) which did not establish many taxonomic groups.

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Abstract

The phylogenetic relationships among four families (Asterinidae, Acanthasteridae, Solasteridae and Echinasteridae) of the Asteroidea were investigated by allozyme analysis of 11 different enzymes. As typical species from each family, the following four common species were adopted: *Asterina pectinifera* from Asterinidae, *Acanthaster planci* from Acanthasteridae, *Solaster dawsoni* from Solasteridae, and *Echinaster luzonicus* from Echinasteridae. From allozyme variation in 11 enzymes, 39 genetic loci were detected and Nei's genetic distances among four families were calculated from allele frequencies data in 39 genetic loci. The molecular phylogenetic tree for four families indicated that Acanthasteridae and Solasteridae are most closely related to each other, and that Asterinidae is most distant family. The allozyme results are well consistent with morphological evidence. Furthermore, the genetic distance values among four families were comparable to those between different families or genera of the same order in other animals. The present allozyme study supports the taxonomic system of Hayashi (1940) and Spencer and Wright (1964) that classified these four families into one same order Spinulosida, but

were inconsistent with that of Clark and Downey (1992) which established many orders. The present allozyme result suggesting that Asterinidae might be heterogeneous lineage was well consistent with the immunological study by Mochizuki and Hori (1977).

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アロザイム変異からみたヒトデ類 4 科の系統類縁関係

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棘皮動物ヒトデ類の系統分類学は議論が多く、研究者間で見解の相違が大きい。特に上位の分類ランク（科・目）間の系統関係はほとんど解明されていないのが現状である。本研究では、有棘目に属するヒトデ類 4 科（イトマキヒトデ科、オニヒトデ科、ニチリンヒトデ科、ルソンヒトデ科）の系統類縁関係をアロザイム分析により調査した。4 科の代表種として、それぞれの科より普通種であるイトマキヒトデ、オニヒトデ、ドーソンニチリンヒトデ、ルソンヒトデを用いた。11 酵素のアロザイム分析から 39 酵素遺伝子座が検出された。各酵素遺伝子座における対立遺伝子頻度から、Nei (1972) の方法により 4 種間の遺伝的距離を算出し、UPGMA 法を用いて 4 科の分子系統樹を作成した。その結果、(1) 多腕の体型を持つオニヒトデ科とニチリンヒトデ科が最も近縁である、(2) このクラスターに近縁な科はルソンヒトデ科

である、(3) イトマキヒトデ科は系統的に最も遠く、イトマキヒトデ科の系統的異質性が示唆される、(4) 4 科間の遺伝的距離は、他動物の同目の別科・別属間で観察される数値と同程度である。これらの結果は、ヒトデ類を多くの目に細分類し、本研究で調べた 4 科を 3 目に分類して、イトマキヒトデ科とオニヒトデ科が近縁であると主張した Clark and Downey (1992) の分類体系とは全く一致せず、4 科を系統的に近縁なグループと考えて 1 つの同じ目（有棘目: Spinulosida）に分類した Hayashi (1940) や Spencer and Wright (1966) の分類体系を強く支持する。またイトマキヒトデ科の系統的異質性を示唆した今回の結果は、Mochizuki and Hori (1980) の糖代謝酵素ヘキソキナーゼの抗体を用いた酵素活性阻害法による免疫学的研究ともよく一致する。

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