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***Ammothea hilgendorfi* (Pycnogonida: Ammotheidae)
Associated with a Sea-Star, *Coscinasterias acutispina*
(Echinodermata: Asteroidea), from
Sagami Bay, Japan**

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Pycnogonids of the species *Ammothea hilgendorfi* (Böhm, 1879) were found attached to sea-stars, *Coscinasterias acutispina* (Stimpson, 1882), from the intertidal zone of Hayama, Sagami Bay, Japan. The sea-stars were collected throughout the year, but the pycnogonids were found only between March and August, mostly in April. The pycnogonids attached to the body surface of the sea-stars mainly by clinging to spines of the aboral and lateral sides of the arms. Developmental stages of the pycnogonids attached to the sea-stars comprised the four final juvenile stages as well as adults, but earlier juvenile stages were not found.

Key Words: sea spider, asteroid, echinoderm, association, developmental stage, juvenile, seasonal occurrence, Japan.

Introduction

Many associations of pycnogonids with other marine animals have been recorded by many authors; Arnaud and Bamber (1987) provide a review. Associations with cnidarians, annelids, mollusks, and echinoderms have been reported so far. Some of them are considered as ectosymbiotic associations, while pycnogonids that damage their host are considered as parasites.

Pycnogonid-echinoderm associations, such as with echinoids, crinoids, and asteroids, have appeared in the literature, but only as simple observations during animal collections. Losina-Losinsky (1961) reported that *Decachela dogieli* Losina-Losinsky, 1961 was found attached to the asteroid *Pteraster* sp. Stock (1981) reported that *Pycnosomia asterophila* Stock, 1981 occurred on the oral side of the sea-star *Calliaster corynetes* Fisher, 1913. The relationships between these pycnogonids and asteroids were considered as ectocommensal. We present here another example of a pycnogonid-echinoderm association, between *Ammothea hilgendorfi* (Böhm, 1879) and *Coscinasterias acutispina* (Stimpson, 1882).

Ammothea hilgendorfi is a widely-distributed pycnogonid that is common at the shore in Japan (Nakamura 1987). This pycnogonid has been so far known to

be free-living (Nakamura and Sekiguchi 1983) or associated with the holothurian *Holothuria (Selenkothuria) moebii* (Ludwig, 1883) (Ohshima 1927a). During a study of the population of the sea-star *Coscinasterias acutispina* in the intertidal zone of Hayama, Sagami Bay, Japan (Fujita 1999), adults and juveniles of *A. hilgendorfi* were found attaching to the body surface of the sea-stars. In this paper, we report the association between the pycnogonid and the asteroid and its seasonal occurrence.

Materials and Methods

Coscinasterias acutispina was collected from the intertidal rocky shore of Shibasaki, Hayama, from May, 1996, to April, 1999, mostly once a month (see Fujita 1999 for details). Specimens were anesthetized with isotonic $MgCl_2$ solution and then fixed with 10% buffered seawater formalin, and later kept in 70% alcohol.

Pycnogonids found attached to the sea-stars were separated from the hosts with forceps and identified. They were observed under a binocular microscope and the body size of each (length from chelifore insertion to tip of fourth lateral process, and width across second lateral processes) was measured. Developmental stages of the associated pycnogonids, *Ammothea hilgendorfi*, were determined by the number of segments of the ovigerous legs (ovigers) as indicated in the "Results" section. The juvenile stages were designated as "-4 stage" to "-1 stage" as explained in "Results".

Ammothea hilgendorfi were also collected by dredging at Nabeta Bay, Shimoda, Shizuoka Prefecture, to examine the developmental stages. These stages were confirmed by culturing some specimens individually and observing their molting.

Results

Recognition of developmental stages of *Ammothea hilgendorfi* by culturing

Adult and free-swimming juveniles of *Ammothea hilgendorfi* from Nabeta Bay were cultured individually in the laboratory. All the juveniles had four pairs of walking legs (four-leg-pair stage). Among the juveniles, four stages with ovigerous legs that differ in segment number were recognized (Fig. 1). Juveniles cultured individually in Petri dishes at room temperature underwent one molt in vitro but no more (confirmed by noting the presence of never more than one molted cuticle in a dish). An increase in the number of oviger segments following the molt was confirmed by comparison of the transformed individual and the molted cuticle. The four stages may be characterized as follows: the youngest juvenile of the four-leg-pair stage has only bud-like protrusions of the ovigers, the next-stage juvenile has rod-like ovigers with no segmentation, the third stage has four-segmented ovigers, and the fourth stage has seven-segmented ovigers. This most advanced juvenile requires one more molt to become an adult with 10-segmented ovigers. We henceforth refer to the fourth-stage juvenile as the "-1 stage" juvenile because it is one stage before the adult. In the same manner, juveniles with four-segmented ovigers are designated as "-2 stage", juveniles with one-segmented ovigers are "-3 stage",

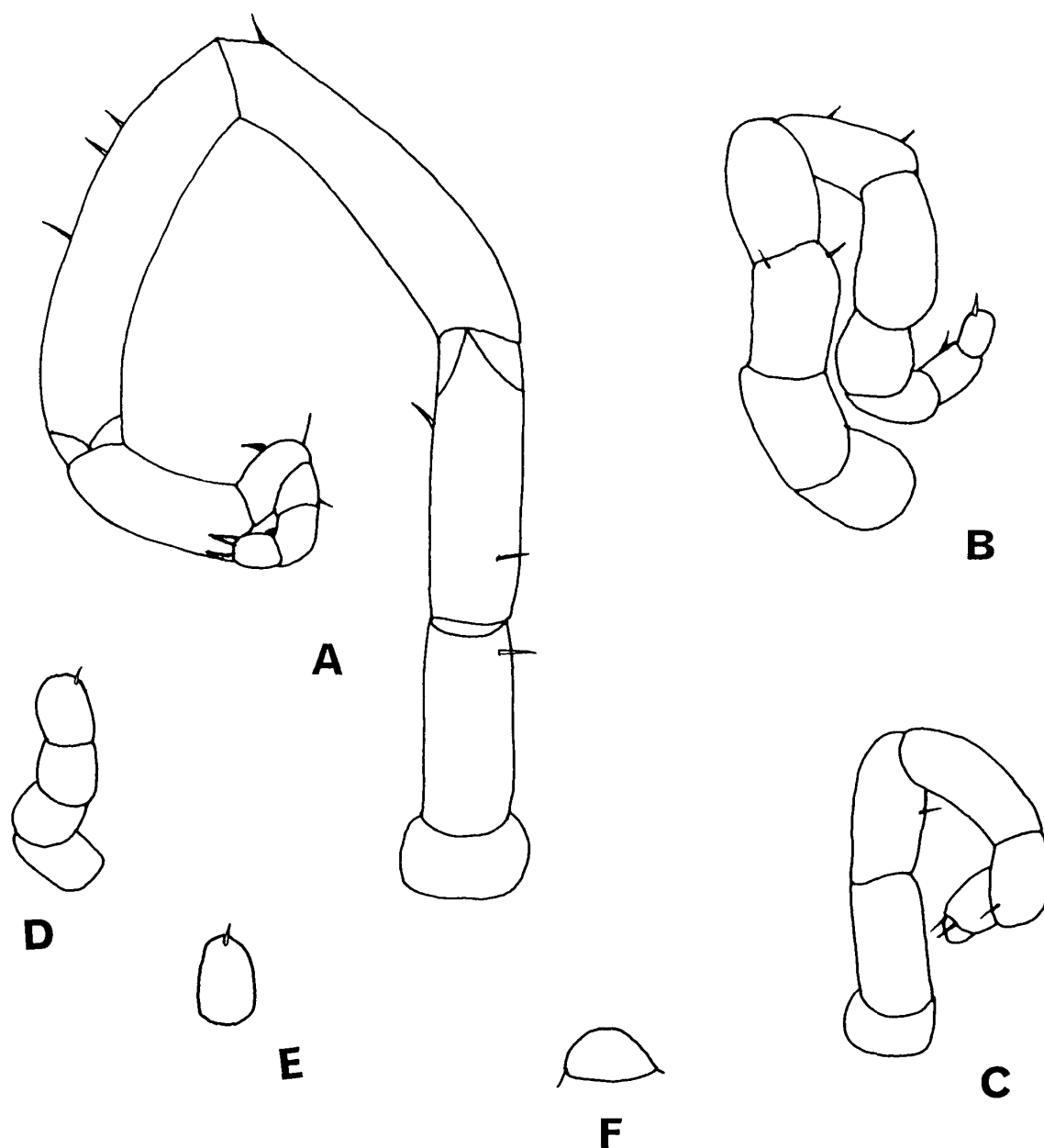


Fig. 1. Oviger of *Ammothea hilgendorfi* in each available developmental stage. Juvenile specimens were collected at Shimoda. A, Adult male; B, adult female; C, -1 stage juvenile; D, -2 stage juvenile; E, -3 stage juvenile; F, -4 stage juvenile. Scale 0.2 mm for A-E, 0.4 mm for F.

and those with bud-like ovigers are “-4 stage” juveniles. The morphology of juveniles did not change from stage to stage except for the number of oviger segments; however, through their development, the body size of juveniles increased.

Population of *Ammothea hilgendorfi* associated with sea-stars

In all, 32 individuals of *Ammothea hilgendorfi* were isolated from 2,884 examined *Coscinasterias acutispina* (Table 1). No other species of pycnogonid was found associated with this species of sea-star in the present study. In most cases, one pyc-

Table 1. Occurrence of *Ammothea hilgendorfi* associated with *Coscinasterias acutispina* in each month.

Month*	Number of pycnogonids	Number of sea-stars	Mean abundance (%)**
January	0	79	0
February	0	234	0
March	4	385	1.0
April ^a	19	222	8.6
May	5	242	2.1
June	1	291	0.3
July	2	203	1.0
August	1	195	0.5
September	0	303	0
October	0	326	0
November	0	196	0
December	0	196	0
Total	32	2884	0.7

* The specimens were collected almost monthly from May, 1996, to April, 1999.

** Mean number of pycnogonids per examined sea-star.

^a One sea-star in this month bore two pycnogonids; all other pycnogonids occurred singly.

nogonid was found on one sea-star, but in only one case (see Table 1), two pycnogonids were associated with one sea-star. The pycnogonids appeared to be clinging to the spines of the sea-stars (Fig. 2). They held on by grasping the host sea-star's spines by their legs, and many of them remained on the hosts even after narcotization and fixation. Among the 32 pycnogonids, 17 (53%) were attached to the aboral surface of an arm, four (13%) to the lateral side of an arm, two (6%) to the oral submarginal side of an arm, four (13%) to the oral side of an arm, and one (3%) near the mouth. The remaining four animals had become detached from the host sea-stars at the time of fixation. There was no indication that the associated pycnogonids caused damage to the sea-stars.

The observed pycnogonids associated with sea-stars showed a seasonal variability in their occurrence (Table 1). Pycnogonids were found only between March and August, and the highest mean abundance (8.6%) was found in April when 19 pycnogonids were recovered from 222 sea-stars. From September to February, no pycnogonid was found associated with any sea-star.

The developmental stages found among the collected pycnogonids comprised -4 stage to -1 stage juveniles and adults. No younger individuals were observed. Juveniles of the four stages showed no morphological differences except for oviger development and body size. Table 2 shows the distribution of developmental stages at each sampling. No clear pattern of seasonality between March and August is apparent, but the data are too few for a meaningful statistical analysis of this point. Body length and width are shown in Fig. 3. The ranges of body length and width within each developmental stage overlapped between the stages, but mean length and width increased with development from 1.0 to 2.1 mm and from 0.6 to 1.3 mm, respectively.

Table 2. Developmental stages of *Ammothea hilgendorfi* associated with *Coscinasterias acutispina*.

Date*	Number of pycnogonids	Developmental stage**				
		-4	-3	-2	-1	Adult (M/F) ¹
1 May 1996	1	0	0	0	0	1/0
2 July 1996	1	0	0	0	1	0/0
16 Aug. 1996	1	1	0	0	0	0/0
9 Apr. 1997	5	0	3	0	2	0/0
9 May 1997	4	2	1	1	0	0/0
3 July 1997	1	0	1	0	0	0/0
31 Mar. 1998	4	1	0	0	2	0/1
29 Apr. 1998	5	0	3	1	1	0/0
26 June 1998	1	0	0	0	0	0/1
18 Apr. 1999	9	2	1	1	4	0/1
Total	32	6	9	3	10	1/3

* No pycnogonids were found in months without data.

** Juvenile stages are referred to as -4, -3, -2, and -1, from younger to older.

¹ Numbers of males (M) and females (F) are shown for adults.

Discussion

A part of the developmental sequence of *Ammothea hilgendorfi* juveniles was confirmed and described for the first time in this study. Developmental stages reported for *Achelia alaskensis* (Cole, 1904) by Okuda (1940) show similar changes in ovigerous leg segmentation to the pattern we found in *Ammothea hilgendorfi*. How-

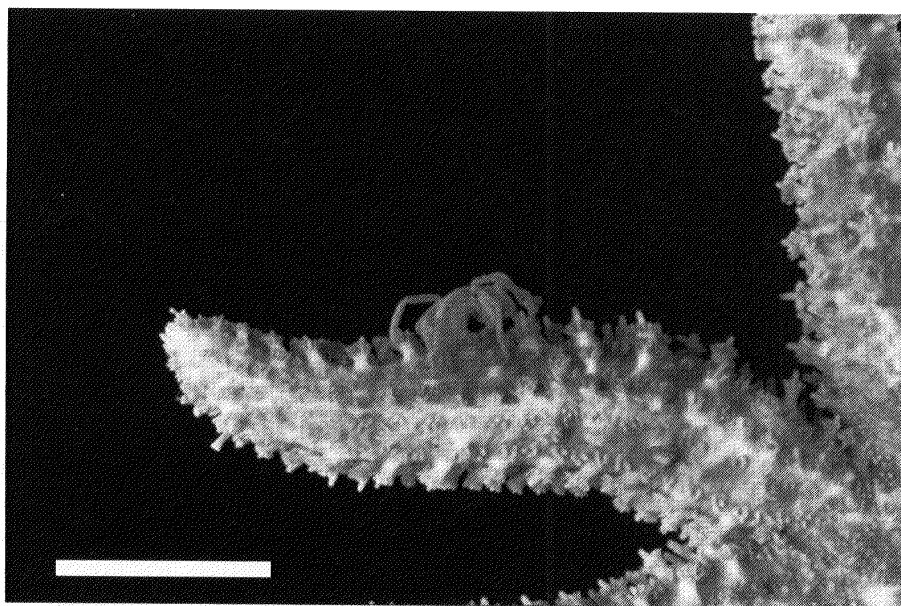


Fig. 2. *Ammothea hilgendorfi* associated with *Coscinasterias acutispina*. The pycnogonid is an adult female (collected on 31 March 1998). Scale 10 mm.

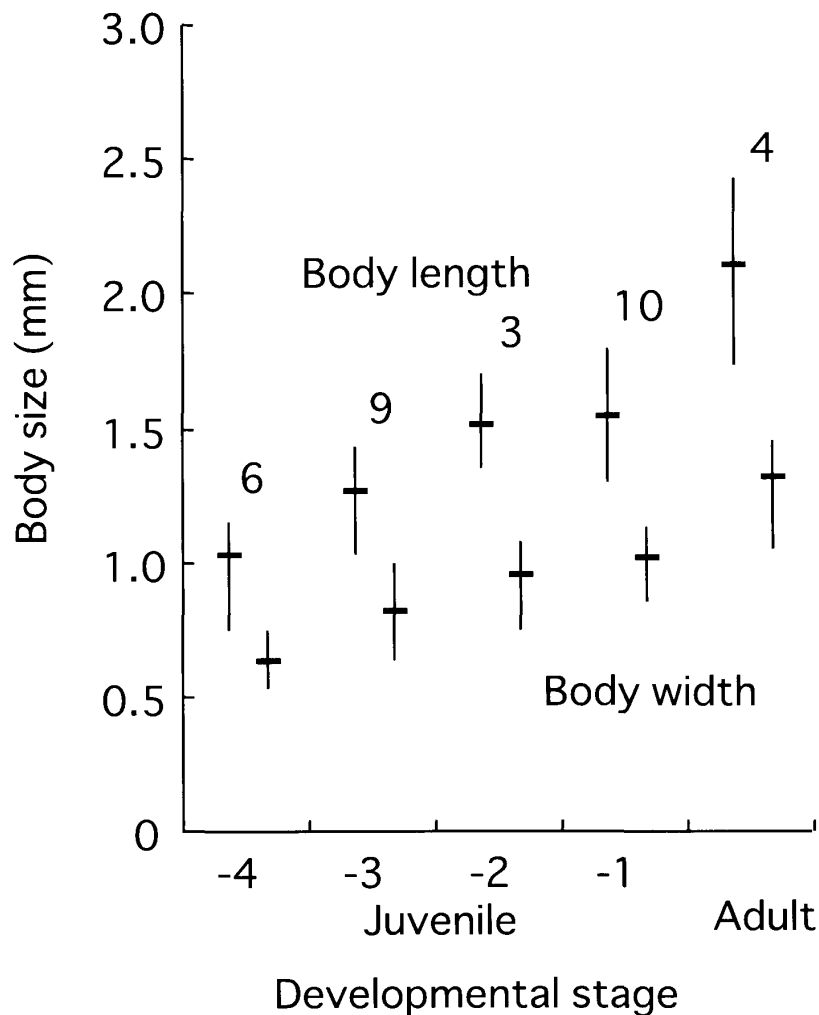


Fig. 3. Body size of *Ammothea hilgendorfi* associated with *Coscinasterias acutispina*. Mean and range of body length and body width are shown for each developmental stage. Numerals near bars denote numbers of specimens of each stage.

ever, the development of the ovigers is different in *Propallene longiceps* (Böhm, 1879) (Nakamura 1981). In *P. longiceps*, juveniles with no-segmented ovigers become juveniles with 10-segmented ovigers after a single molt. No additional change in the number of oviger segments is seen in further three molts to the adult.

Ammothea hilgendorfi was found on *Coscinasterias acutispina* from the rocky intertidal zone at Hayama, Japan. *Coscinasterias acutispina* and another sea-star, *Asterina pectinifera* (Müller and Troschel, 1842), are the dominant asteroid species in this area, but no *Ammothea hilgendorfi* has been noticed associated with *Asterina pectinifera* (Fujita unpubl. data). *Coscinasterias acutispina* has large spines each with a cluster of pedicellariae on the aboral and marginal plates (Fig. 2), and such a body surface may serve both for easier attachment of *A. hilgendorfi* to the asteroid and for more effective use of the sea-star as a refuge. *Pycnosoma strongylocentroti* Losina-Losinski, 1933 was reported attached firmly to spines of sea-urchins by their chelae (Losina-Losinsky 1933), not by their legs as in the present case.

Ammothea hilgendorfi was considered an ectoparasite of a holothurian (Ar-

naud and Bamber 1987), although Ohshima (1927a) did not mention any feeding activity of *A. hilgendorfi*. The association of *A. hilgendorfi* with *C. acutispina* appears to be ectocommensal because no indication of damage to the sea-star was observed. While half of the observed *A. hilgendorfi* were found attached to the host's aboral side in the present study, most specimens of *Anoplodactylus ophiurophilus* Stock, 1979 associated with ophiuroids were found attached to the oral side of their host, where they might possibly feed on tube-feet (Sloan 1979). Another pycnogonid associated with an asteroid, *Pycnosomia asterophila* Stock, 1981, attaches to the oral side of its host sea-star, but no feeding on the sea-star was observed (Stock 1981).

The occurrence of the pycnogonids showed a clear seasonal variability; they occurred mostly in spring, and not at all in fall and winter (Table 2). This phenomenon may be related to the breeding season of the pycnogonid, although reproduction of *Ammothea hilgendorfi* has not been studied. Ohshima (1927a) found only immature pycnogonids attached to holothurians and suggested that they detach from the hosts to become free-living when they become mature. In the present study, only four adults (one male and three females) were collected. All the females had eggs in their ovaries. The male carried no eggs on its ovigers. Most of the pycnogonids (87%) were immature (Table 2). This all suggests that juveniles detach from the sea-stars when they become mature.

In most cases, one pycnogonid was collected from one sea-star. Only one case was observed of a sea-star with two pycnogonids. Juveniles younger than the "–4 stage" were never found; they have not ever been recorded at all. In the case of another pycnogonid, *Nymphonella tapetis* Ohshima, 1927, only juveniles are found living in bivalve shells in spring and no larva or free-living mature adult has been collected (Ohshima 1927b).

Two associated animals have been known for *Coscinasterias acutispina* until now. The eulimid gastropod *Apicalia habei* Warén, 1981 is an ectoparasite (Hori 2000) and the ascothoracidan crustacean *Dendrogaster okadai* (Yosii, 1931) is an endoparasite (Fujita *et al.* 2001). Association of pycnogonids with this sea-star was reported for the first time in this paper.

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