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## Larval Development and Metamorphosis of the Sea Star *Luidia foliolata* (Echinodermata: Asteroidea)

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Spawning of *Luidia foliolata* in Barkley Sound, British Columbia, Canada, occurs in spring. Ova are transparent and about 150  $\mu\text{m}$  in diameter. The first cleavage occurs approximately 3 hr after fertilization at ca. 10°C. The embryo develops into a bipinnaria after a wrinkled blastula stage. Metamorphosis takes place about 4 mo after fertilization, without the larva's passing through a brachiolaria stage. The full-grown bipinnaria is 2.5 mm long and has five pairs of bipinnaria arms. At metamorphosis the larval part is absorbed into the asteroid rudiment. Juveniles are about 730  $\mu\text{m}$  in diameter. They have five arms, each bearing two pairs of tube-feet. The present observations show that *L. foliolata* undergoes a non-brachiolarian type of development, as do all species of *Luidia* previously studied.

**Key Words:** starfish, larval development, metamorphosis, *Luidia foliolata*, wrinkled blastula, bipinnaria, non-brachiolarian development.

### Introduction

Development through metamorphosis has been reported for seven species of *Luidia* (see Table 1). They are *L. ciliaris* (Philippi, 1837), *L. clathrata* (Say, 1825), *L. maculata* Müller and Troschel, 1842, *L. quinaris* v. Martens, 1865, *L. sarsi* Düben and Koren, 1845, *L. savignyi* (Audouin, 1826), and *L. senegalensis* (Lamarck, 1816) (Komatsu *et al.* 1982, 1991, 1994; Mortensen 1938). The bipinnariae of all these species metamorphose without passing through a brachiolarian stage, thus exhibiting a non-brachiolarian type of development (Oguro *et al.* 1976; Oguro 1989). Although Strathmann (1987) briefly noted the development of *L. foliolata* Grube, 1866, little is known about the larvae and metamorphosis of this species.

Luidiid larvae have been considered clearly distinct from those of other asteroid groups (see Discussion). The bipinnariae are large, with well-developed arms and median processes (Mortensen 1913, 1938; Tattersall and Sheppard 1934; Wilson 1978; Bosch *et al.* 1989; Jaekle 1994). Giant bipinnariae that might belong to this family have also been described without identifying the species (Mortensen 1927; Tokioka 1942). On the other hand, Komatsu *et al.* (1991) showed that luidiid bipinnariae are not always large; in comparison with bipinnariae of other asteroid gen-

Table 1. Developmental features of *Luidia* species. Key to abbreviations: Wrinkled blastula; (+) presence, (-) absence. Fate of larval stalk; (\*) both separation and absorption. (#) no data.

Species	Egg diameter ( $\mu\text{m}$ )	Wrinkled blastula	Bipinnaria length (mm)	Number of bipinnaria arms (in pairs)	Number of tube feet in each arm at completion of metamorphosis (in pairs)	Fate of larval stalk	References
<i>Luidia alternata</i>	193	+	#	#	#	#	Komatsu <i>et al.</i> 1991
<i>L. ciliaris</i>	#	+	7	6 or 7	5	absorption	Mortensen 1913, 1938
<i>L. clathrata</i>	168	+	1.8	5	2	absorption	Komatsu <i>et al.</i> 1991
<i>L. foliolata</i>	150	+	2.5	5	2	absorption	Present study
<i>L. maculata</i>	173	+	2.5	5	2	absorption	Komatsu <i>et al.</i> 1994
<i>L. quinaria</i>	124	+	2.5	6	3	absorption	Komatsu <i>et al.</i> 1982
<i>L. sarsi</i>	#	-	30	6 or 7	10	separation*	Mortensen 1938 *Wilson 1978
<i>L. savignyi</i>	#	+	1.5	5	2	absorption	Mortensen 1938
<i>L. senegalensis</i>	197	+	1.5	5	2	absorption	Komatsu <i>et al.</i> 1991

era, these larvae's morphological features vary more among species. In this context, it is important to make a study of the larval development of more species of *Luidia*.

The genus *Luidia* is the sole genus in the family Luidiidae (Hyman 1955; Clark and Downey 1992). This family has been traditionally considered primitive among living asteroids (Fell 1963; Hedde 1967; Jaekle 1994). Blake (1988), however, proposed on the basis of a cladistic analysis that paxillosidans, comprising the Luidiidae, are not primitive, but specialized. In addition, he cited the presence of a brachiolaria in the development of paxillosidans in support of this view, based on the erroneous interpretations offered by Strenger and Erber (1983) and Erber (1985). This situation spotlights the need for thorough studies of the development of luidiid species in order to provide accurate data for phyletic discussions. We undertook this study to clarify the similarities and differences between the development of luidiids and that of related groups.

## Materials and Methods

*Luidia foliolata* occurs on sandy or muddy bottoms. Specimens were collected by dredging in Barkley Sound, Vancouver Island, British Columbia, Canada, from the end of March to the middle of April, 1990. Spawning was induced by intracoelomic injection of 1 to 2 ml of  $10^{-3}$  M 1-methyladenine per individual on March 30, April 16, and April 18, 1990. A dilute sperm suspension was added to the eggs for fertilization. Hatched embryos were kept in covered glass bowls, 80 cm tall and 10 cm in diameter, at the Bamfield Marine Station at about  $10^{\circ}\text{C}$  and then transferred into a tank, 100 cm tall and 150 cm in diameter, after formation of the larval mouth. The sea water in the tank was stirred by a suspended paddle and changed every 3 days. Larvae were fed a combined diet of the algae *Dunaliella teriolecta* Butcher, 1959 and *Isochrysis galbana* Parke, 1971 and the diatom *Skeletonema costatum* (Greville, 1878) (Komatsu *et al.* 1991).

The living embryos and larvae were observed and measured under a light microscope equipped with an ocular micrometer. The skeletal system was examined

with a compound microscope, using either fresh squash preparations or specimens fixed in 70% ethanol and macerated in a 10% aqueous solution of KOH.

## Results

Specimens collected in Barkley Sound could be spawned artificially from the end of March to the middle of April. *Luidia foliolata* is dioecious, with gonads and gonopores arranged serially along both sides of each arm. During the breeding season the gonads extended from the base to near the tip of each arm. Mature testes were milky white and ovaries were pale salmon pink in color.

The mature ova were spherical and transparent, measuring approximately  $150\ \mu\text{m}$  in average diameter ( $n=10$ ,  $\text{min}=144\ \mu\text{m}$ ,  $\text{max}=160\ \mu\text{m}$ ). The fertilization membrane became apparent about 3 min after fertilization. The height of the perivitelline space was approximately  $17\ \mu\text{m}$  100 min later (Fig. 1A). The first and second polar bodies were visible in the perivitelline space. First cleavage occurred on a plane through the animal and vegetal poles approximately 3 hr after fertilization at about  $10^\circ\text{C}$ . Cleavage is total, equal, and radial. The embryos were in the 16-cell stage 7 hr after fertilization. The wrinkled blastula stage lasted about 7 hr, from 19 to 26 hr after fertilization. The surface of the late blastula regained its smoothness. Ciliated coeloblastulae began to rotate within their fertilization membranes 30 hr after fertilization. While they were rotating, gastrulation occurred at the vegetal pole. One hr after the beginning of rotation, hatching took place and early gastrulae became free-swimming larvae. Hatched gastrulae gradually elongated along the archenteric axis. Mesenchyme cells were obvious in the blastocoel of the gastrula 2 days after fertilization. Fifty-two hr after fertilization the archenterons of the gastrulae had expanded blind ends. Gastrulae were  $275\ \mu\text{m}$  long and  $180\ \mu\text{m}$  wide 4 days after fertilization, with a differentiated coelomic pouch on each side of the archenteron's inner end. The mouth opened at the stomodaeum 7 days after fertilization. Two ciliary bands became obvious 8 days after fertilization. The bipinnaria at this stage was  $350\ \mu\text{m}$  long and  $270\ \mu\text{m}$  wide. By this time the archenteron had differentiated a functional, tripartite digestive tract: esophagus, stomach, and intestine. Both of the coelomic pouches had separated from the digestive tract and were situated dorso-laterally in the body between the esophagus and stomach. The left coelomic pouch was  $65\ \mu\text{m}$  long and larger than the right one ( $40\ \mu\text{m}$ ). The hydrocanal from the left coelomic pouch opened dorsally through the hydropore. The bipinnariae were feeding larvae and grew to 1.25 mm in length and  $800\ \mu\text{m}$  in width by 37 days after fertilization. Five pairs of bipinnaria arms were formed at this stage: anterior dorsal, posterior dorsal, posterior lateral, postoral, and preoral. Forty days after fertilization the tips of the right and the left coelomic pouches were in close contact with each other. Bipinnaria reached a length of 1.9 mm and a width of 1.2 mm by 50 days after fertilization.

Metamorphosis took place 4 mo after fertilization without the larvae having passed through a brachiolaria stage (Fig. 1B). Spicules, corresponding to the rudiments of adult skeletal plates, appeared on the posterior part of the bipinnaria (Fig. 1C): one madreporic plate and five terminal plates on the future aboral side and five pairs of oral plates on the future oral side. The largest bipinnaria at this stage was about 2.5 mm in length. The bipinnaria arms were by now well devel-

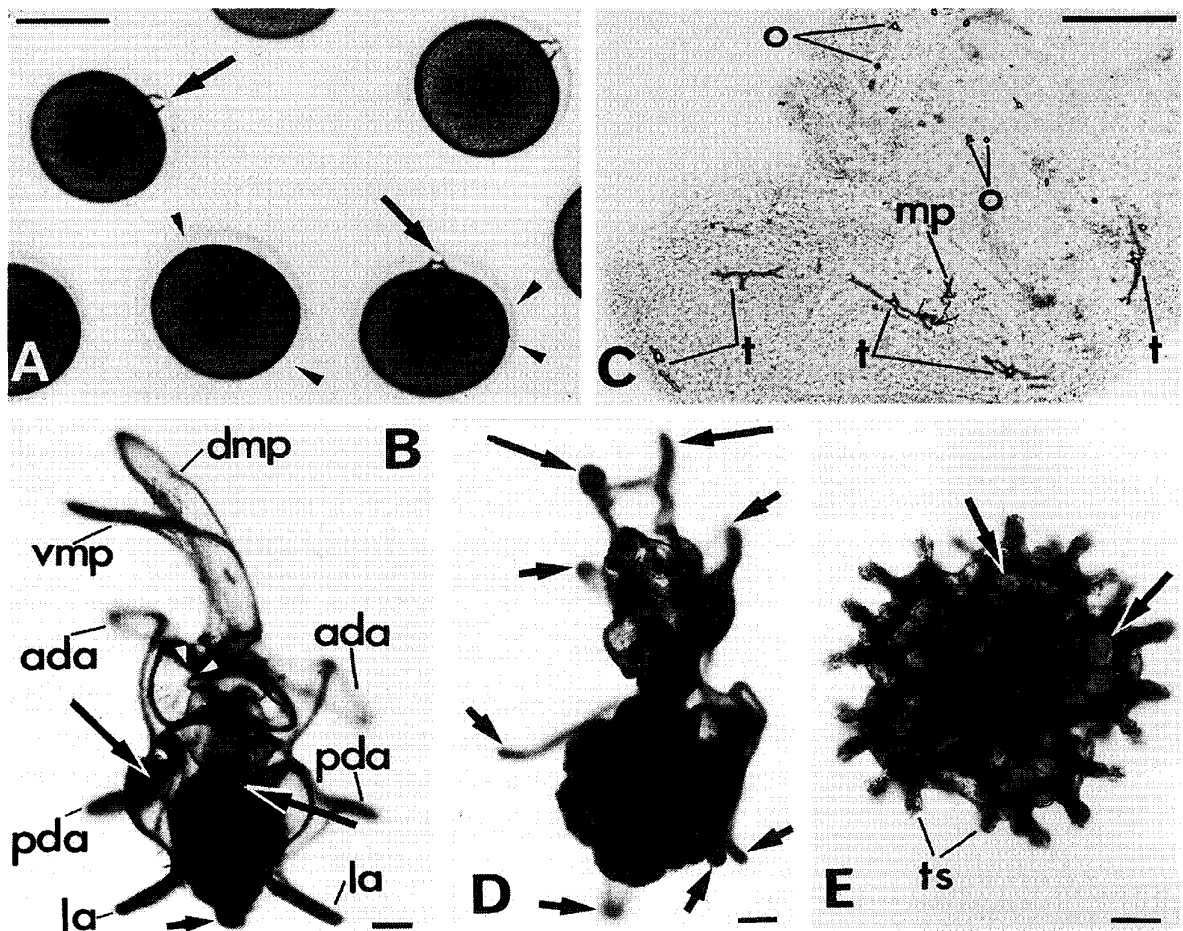


Fig. 1. Development of *Luidia foliolata*. Scale=100  $\mu$ m. A: Fertilized eggs with elevated fertilization membrane (arrowheads) and polar bodies (arrows). B: Bipinnaria fixed in 70% alcohol, 4 months after fertilization, ventral view, with asteroid rudiment (short arrow) on posterior part of body; long arrows and white arrowheads show postoral arms and preoral arms, respectively. Abbreviations: ada, anterior dorsal arm; dmp, dorsal median process; la, lateral arm; pda, posterior dorsal arm; vmp, ventral median process. C: Rudiments of skeletal plates, from squash preparation of a bipinnaria, same stage as shown in B. Abbreviations: mp, madreporic plate; o, oral plate; t, terminal plate. D: Later bipinnaria fixed in 70% alcohol, future aboral view, with shrunken larval body, or stalk; long and short arrows show the median processes and bipinnaria arms, respectively. E: Juvenile, 5 months after fertilization, oral view; arrows show tube-feet. Abbreviation: ts, terminal spine.

oped; in particular, one pair of the lateral arms was about 400  $\mu$ m long. There were two preoral lobes at the anterior end of the bipinnaria, the dorsal and ventral median processes. The ventral surface of the anterior end was surrounded by a ciliary band passing in front of and above the mouth; the frontal field was 1.4 mm long and included a well-developed ventral median process.

The end of metamorphosis was indicated by the reduction of the larval body's anterior portion, the stalk; Fig. 1D shows a metamorphosing bipinnaria with this reduced part. Arm primordia of the sea star were visible already on the posterior part of the body. The stalk was quickly resorbed into the posterior part of the bip-

innaria corresponding to the asteroid rudiment until it completely disappeared. Figure 1E shows a newly metamorphosed juvenile 5 mo after fertilization; it was 750  $\mu\text{m}$  in diameter with two pairs of tube-feet and one terminal tentacle per arm. At this stage, the juvenile moved about using its tube-feet; a red eye-spot was formed at the base of each terminal tentacle; and the adult mouth was apparent.

### Discussion

Although Strathmann (1987) reported that bipinnaria larvae of *Luidia foliolata* do not form brachiolar arms or an adhesive disk, she made no explicit statement concerning possible later larvae. She neither described a brachiolaria stage of the larva nor referred to the development of this species as non-brachiolarian, a term proposed by Oguro *et al.* (1976) to denote a new type of asteroid development. The present study shows that bipinnariae of *L. foliolata* begin metamorphosis without passing through a brachiolaria stage. They exhibit the same non-brachiolarian type of development as has been reported in all other luidiids studied to date.

The developmental features observed in *L. foliolata* are similar to those of 8 other luidiid species (Table 1). The egg in *L. foliolata* is transparent, with an average diameter of about 150  $\mu\text{m}$ , intermediate between the smallest egg size of 124  $\mu\text{m}$  in *L. quinaria* (Komatsu *et al.* 1982) and the largest, 197  $\mu\text{m}$  in *L. senegalensis* (Komatsu *et al.* 1991). Size of egg in asteroids differs with species, ranging from 100  $\mu\text{m}$  in *Acanthaster planci* (Linnaeus, 1758) (Henderson 1969) to 3.0–4.0 mm in *Rhopiella koehleri* Fisher, 1940 (Fisher 1940). Eggs having direct development are generally larger and more opaque than those with indirect development (see Hyman 1955; Chia 1968). It is interesting that the eggs of luidiid species, which undergo non-brachiolarian development, not indirect development, are also less than 200  $\mu\text{m}$  in diameter, a relatively small size for asteroids, and are transparent.

*Luidia foliolata* has a wrinkled blastula like many echinoderms (Chia *et al.* 1993; Cerra and Byrne 1995; Henry *et al.* 1991), although Strathmann (1987) did not mention the presence of this stage. The wrinkled blastula has been reported in all other studied luidiid species except for *L. sarsi* (Table 1); it seems, therefore, that the wrinkled blastula is a common feature of the species of *Luidia*.

In asteroids, the full-grown bipinnaria is generally around 1.0 mm long (Yamaguchi 1973). On the contrary, since bipinnariae of *L. sarsi* and *L. ciliaris* were respectively reported to be ca. 30 and 7 mm long (Mortensen 1927, 1938), luidiid bipinnariae have been considered to be large, as mentioned above. The fully grown bipinnaria of *L. foliolata* at 4 months after fertilization was only about 2.5 mm in length in the present study, which confirms the observation of Komatsu *et al.* (1991) that the bipinnaria of *Luidia* is not always large, depending on species.

An asteroid bipinnaria generally bears five pairs of bipinnaria arms and two anterior median processes. The large bipinnaria of some species of *Luidia* is more complex than this; the median processes are elongate and the arms are long and greater in number. In *L. ciliaris* and *L. sarsi* each of the anterior dorsal arms is divided from the base into two (or three) equally developed branches (Mortensen 1938). Bipinnariae of *L. quinaria* (Komatsu *et al.* 1982) have two pairs of branched anterior dorsal arms and their bipinnaria arms total 12 in number, while the bipinnariae are only 2.5 mm long, much smaller than those of *L. ciliaris* (7 mm) and *L.*

*sarsi* (30 mm). However, the fully grown bipinnaria of *L. foliolata*, which is the same size as that of *L. quinaria*, has only five pairs of bipinnaria arms like *L. clathrata* and *L. senegalensis* (Komatsu *et al.* 1991), even though the arms and median processes of *L. foliolata* are as long as those of *L. quinaria*. Thus, the present observations are in accord with the conclusions proposed by Komatsu *et al.* (1991): the bipinnaria of *Luidia* is not always complex and large in size, and its morphological features vary among the species.

Shortly after the completion of metamorphosis, juveniles of *L. foliolata* bear two pairs of tube-feet in each arm as those of most asteroids do, including *L. clathrata*, *L. savignyi*, and *L. senegalensis* (Mortensen 1938; Komatsu *et al.* 1991). However, among the luidiids there are some exceptions (Table 1): three, five, and more than ten pairs of tube-feet occur in the newly metamorphosed juveniles of *L. quinaria*, *L. ciliaris*, and *L. sarsi*, respectively (Mortensen 1938; Komatsu *et al.* 1982). As mentioned above, the bipinnariae of these three species have more than six pairs of bipinnaria arms. Therefore, it is likely that the occurrence of more than three pairs of tube-feet in just-metamorphosed juveniles is related to the more complex morphology of the bipinnariae of the relevant luidiid species.

The larval stalk of *L. foliolata* is absorbed into the future body of the juvenile during metamorphosis. The same fate of the larval stalk has been reported in six species of *Luidia* (Table 1), but *L. sarsi* casts off the larval stalk from the starfish rudiment (Mortensen 1927, 1938; Tattersall and Sheppard 1934). The bipinnaria of *L. sarsi* is extremely large (30 mm long) compared to those of the other species. In the family Astropectinidae, the fate of the larval stalk of the barrel-shaped larva has been reported in three species (Komatsu 1975, 1982; Komatsu and Nojima 1985): that of *Ctenopleura fisheri* Hayashi, 1957 (1,500  $\mu\text{m}$  long) was either absorbed or it ruptured and collapsed; that of *Astropecten latespinosus* Meissner, 1892 (700  $\mu\text{m}$  long) was absorbed; and the larval stalk of *A. gisselbrechti* Döderlein, 1917 (875  $\mu\text{m}$  long), intermediate in size between those of *C. fisheri* and *A. latespinosus*, was absorbed in most cases. Komatsu (1982) proposed that casting off of the larval stalk is characteristic of large-sized bipinnariae and large-sized barrel-shaped larvae. The fact that the larval stalk of the small, 2.5 mm long bipinnaria of *L. foliolata* was absorbed may support Komatsu's proposal.

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