Development of pedicellariae in the pluteus larva of *Lytechinus pictus* (Echinodermata: Echinoidea)

ROBERT D. BURKE[1]
Department of Zoology, University of Maryland, College Park, MD, U.S.A. 20742
Received December 11, 1979

BURKE, R. D. 1980. Development of pedicellariae in the pluteus larva of *Lytechinus pictus* (Echinodermata: Echinoidea). Can. J. Zool. **58**: 1674–1682.

Three tridentate pedicellariae develop in the pluteus larva of *Lytechinus pictus*. Two are located on the right side of the larval body and the third is on the posterior end of the larva. The pedicellariae form from mesenchyme associated with the larval skeleton which becomes enclosed in an invagination of larval epidermis. The mesenchyme within the pedicellaria primordium aggregates into groups of cells that become skeletogenic tissues which secrete the pedicellaria jaws, and smooth and striated muscles. Nerves and sensory cells develop within the epidermis covering the pedicellariae. Pedicellaria formation takes 3 days and occurs about midway through the development of the adult rudiment. During metamorphosis the pedicellariae are shifted to the aboral surface of the juvenile.

Pedicellariae that develop in the larvae are fully operable prior to metamorphosis and do not appear to be released from any rudimentary state of development by metamorphosis. At least 16 echinoid species are reported to form pedicellariae in the larva. The precocious development of these adult structures appears to be dispersed throughout the orders of regular urchins.

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Il y a trois pédicellaires tridentés chez la larve pluteus de *Lytechinus pictus*. Deux se développent du côté droit de la larve et le troisième se trouve à son extrémité postérieure. Les pédicellaires se forment à partir de mésenchyme associé au squelette de la larve, mésenchyme qui se développe dans une invagination de l'épiderme. Le mésenchyme, dans le primordium du pédicellaire, s'accumule en un groupe de cellules, précurseurs destissus squelettogènes qui sécrètent les mâchoires pédicellaires de même que les muscles lisses et striés. Des cellules nerveuses et sensorielles se développent à l'intérieur de l'épiderme qui recouvre les pédicellaires. La formation d'un pédicellaire dure trois jours et se produit à peu près au milieu du développement du rudiment de l'adulte. Durant la métamorphose, les pédicellaires se déplacent vers la surface aborale du jeune animal.

Les pédicellaires qui se développent chez les larves peuvent fonctionner parfaitement avant la métamorphose; ils ne semblent donc pas être, avant la métamorphose, à un stade rudimentaire de développement. Au moins 16 espèces d'échinoïdes possèdent des pédicellaires au stade larvaire. Le développement précoce de ces structures d'adulte semble assez répandu chez tous les ordres d'oursins ordinaires.

[Traduit par le journal]

Introduction

Echinoid development typically includes the formation of a planktonic pluteus larva which undergoes a rapid and radical metamorphosis to become a benthic adult. During the latter half of larval life, adult tissues develop principally in a rudiment on the left side of the larva. The rudiment is everted and the larva resorbed into it during metamorphosis (Hyman 1955 for review). In some species adult structures appear elsewhere on the larval body prior to metamorphosis.

MacBride (1903, 1914, 1918) and von Ubisch (1913) provide the most complete accounts of the development of adult tissues in echinoid larvae. However, neither the origins of adult tissues, nor the relationship between larval and adult tissues prior to and during metamorphosis is well understood.

Pedicellariae are small appendages of the adult, characteristic of the echinoderm classes Echinoidea and Asteroidea (Hyman 1955). Each pedicellaria consists of a head with movable jaws, mounted on a flexible stalk. In echinoids they are dispersed over the external surface between the spines and are thought to function in repelling intruders, deporting unwanted particles, and possibly in catching small animals (Hyman 1955).

MacBride (1903) described briefly the formation of pedicellariae primordia on the right side of the larva of *Echinus esculentus* in a rudiment separate from the adult rudiment. Pedicellariae develop in

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[1]Present address: Smithsonian Institution, Fort Pierce Bureau R.R. 1, Box 194-C, Fort Pierce, FL, U.S.A. 33450.

0008-4301/80/091674-09$01.00/0

the larva of *Lytechinus pictus* in a similar manner and provide an opportunity to examine the origins of specific adult tissues and the degree to which adult tissues differentiate prior to metamorphosis. A brief discussion of the occurrence of pedicellariae in larvae of various echinoid taxa is included.

Materials and methods

Larval culture

Plutei were reared from fertilized eggs using the standard procedures outlined by Strathmann (1968) and Hinegardner (1969). Adult *L. pictus* were obtained from Pacific Biomarine Supply, Venice, California. Spawning was induced by intracoelomic injection of 0.55 *M* KCl. Embryos and larvae developed normally in artificial seawater prepared from synthetic sea salts (Instant Ocean Inc.). Larvae were kept in 4-L glass beakers, stirred with a 4 rpm clock motor, and fed on alternate days a mixture of *Dunaliella salina*, *Monochrysis lutheri*, and *Isochrysis galbana*. Larvae developed best at concentrations of 1 to 2 larvae per millilitre.

Microscopy

Repeated observations of individual, living larvae were made throughout the development of pedicellariae using Hoffman modulation contrast optics on a Nikon inverted microscope and both Kohler critical illumination and Nomarski differential interference contrast optics on a Zeiss compound microscope.

Specimens were fixed for transmission electron microscopy (TEM) for 1 to 2 h in a solution containing 2.5% glutaraldehyde, 0.2 *M* phosphate buffer (Millonig 1961), and 0.14 *M* sodium chloride (Cloney and Florey 1968), and postfixed in 2% osmium tetroxide in 1.25% sodium bicarbonate for 1 h. After a brief rinse in distilled water larvae were dehydrated in increasing concentrations of ethanol, transferred through two changes of propylene oxide, and infiltrated and embedded in Epon according to the methods of Luft (1961). Sections were stained in 50% ethanol saturated with uranyl acetate, and lead hydroxide chelated with sodium citrate (Reynolds 1963), and observed and photographed with a Hitachi HU-12 electron microscope. No attempt was made to decalcify the tissues.

Specimens were prepared for scanning electron microscopy (SEM) by fixing and postfixing as described above for TEM, dehydrated using ethanol exchange, and then transferred through increasing concentrations of amyl acetate. Larvae were critical-point dried (CO_2), coated with evaporated carbon and gold–palladium, and viewed and photographed with an AMR-1000 scanning electron microscope.

Thick sections (0.5 μm) of material prepared for TEM were stained with methylene blue and azure II (Richardson *et al.* 1960), and observed and photographed with a Zeiss Universal compound microscope.

Results

Formation of the pedicellaria primordium

Pedicellaria formation begins in the eight-arm pluteus after the adult rudiment has reached a stage in which the five primary podia are distinct. At this stage the somatocoels enclose the anterior half of the larval stomach. The first pedicellaria forms in a medial position at the posterior end of the larva (Fig. 1). Mesenchyme from the remnant blastocoel of the larva and cells associated with the larval skeleton migrate by means of filopodial extension and contraction to the location of the presumptive pedicellaria (Fig. 2). These cells coalesce and begin secretion of the posterior transverse rod of the larval skeleton. The epidermis directly over this group of cells thickens and invaginates. The invaginated epithelium encapsulates a small group of mesenchymal cells to form a bulb at the base of the invagination (Figs. 3, 4, 5). The mesenchyme within the pedicellaria primordium remains contiguous with the mesenchyme of the posterior transverse rod throughout the formation of the pedicellariae.

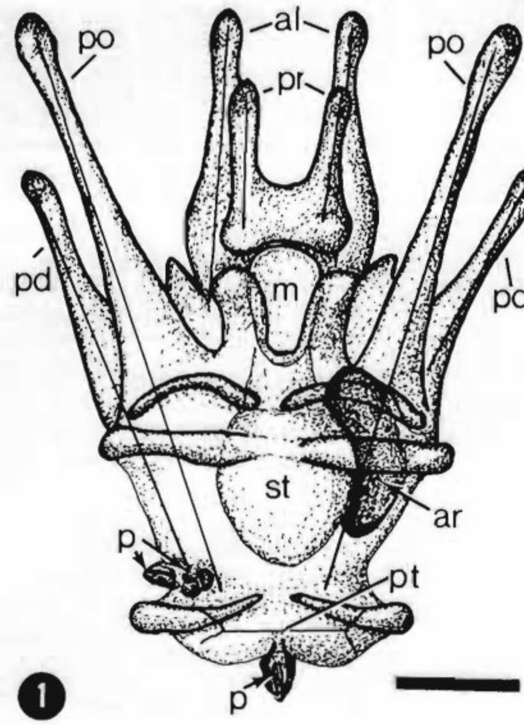

FIG. 1. Eight-arm pluteus of *L. pictus* showing the location of pedicellariae (*p*). Bar = 100 μm. *al*, anterolateral arms; *ar*, adult rudiment; *m*, mouth; *pdm*, posterodorsal arms; *po*, postoral arms; *pr*, preoral arms; *pt*, posterior transverse rod; *st*, stomach.

Two other pedicellaria primordia form by an identical process on the right side of the larval body at the bases of the postoral and posterodorsal rods of the larval skeleton (Fig. 1).

The pedicellaria primordium

Two days after pedicellaria formation has begun, the pedicellaria primordium is 15 to 18 μm in diameter and located within the small vestibule produced by the invagination of the larval epidermis (Figs. 5, 7). A layer of simple cuboidal epithelium comprising cells 5 μm in height and 3 to 4 μm wide surrounds the mesenchyme core (Fig. 6). The epithelial cells are not ciliated, but have an external surface of branched microvilli up to 0.5 μm in length. The cells are attached near their apical surface by maculate adherent-like junctions. A nucleus, 3 to 4 μm in diameter containing scattered patches of electron-dense chromatin and a single nucleolus, is located centrally in each cell. The nucleus appears to be surrounded by a layer of rough endoplasmic reticulum (RER) and mitochondria, as well cisternae of RER are dispersed throughout the cytoplasm.

The mesenchyme cells contained within the pedicellaria primordium are 5 to 8 μm in diameter, spherical, and appear to be unattached (Fig. 6). Mitotic figures were frequently observed in the mesenchyme cells at this stage. Ultrastructurally the mesenchymal cells appear to be relatively unspecialized. The nuclei are centrally located, 3 to 5 μm in diameter, and contain one or two nucleoli. Nuclei are enveloped in a layer of RER, and clumps of free ribosomes, Golgi bodies, and mitochondria are the most prevalent organelles of the cytoplasm.

Cleft stage

Pedicellaria primordia, 25 to 30 μm in diameter, are attached by a 10 μm long stalk to the larva (Figs. 7, 8). A triradiate cleft forms on the external surface separating the three presumptive jaws of the pedicellaria (Fig. 9).

The external epithelium of the developing pedicellaria remains as it was in the previous stage. The mesenchyme has become organized into seven bundles of cells (Fig. 10). Triplicates of two types surround a central core of cells. Three of the bundles extend distally between the clefts; these cells secrete the skeleton of the pedicellaria jaws. The other three bundles of cells, which will become the muscles of the pedicellaria are located proximally between the jaw primordia. The central core of cells extends from the base of the cleft through the stalk to the posterior transverse rod of the larval skeleton.

The skeleton of each jaw of the pedicellaria forms initially as a triradiate spicule. The crystal is secreted into a vacuole contained in a mesenchymal cell (Fig. 12). Several mesenchymal cells, ultrastructurally similar to the mesenchymal cells described above surround the spicule.

At the base of each cleft, extending between the developing jaw skeletons, the adductor and abductor muscles form. The developing muscle cells are elongate, 2 to 3 μm wide and 15 to 18 μm long (Fig. 11). Mitochondria, clumps of free ribosomes, and cisternae of RER are scattered throughout the cytoplasm. Filaments, 5 nm and 10 to 12 nm in diameter, frequently occur in association with clumped ribosomes.

Pedicellariae

The fully formed pedicellariae of the larva of *L. pictus* are typical echinoid tridentate pedicellariae (Hyman 1955) (Figs. 13, 14). The head of each pedicellaria is 25 μm long and 20 μm wide at its base. The stalk of each pedicellaria is 5 to 8 μm in diameter but only 15 μm long. Pedicellariae are

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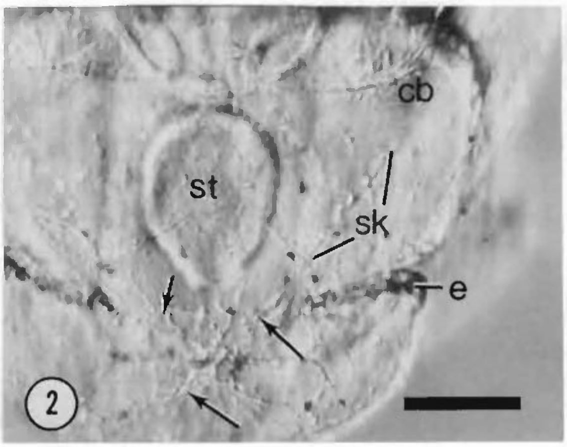
FIG. 2. Hoffman modulation contrast image of the posterior end of an eight-arm pluteus of *L. pictus* during the initial phase of pedicellaria primordium formation. The arrows indicate mesenchyme which is migrating from the postoral and posterodorsal skeletal rods to the location where the pedicellaria primordium will form. Bar = 25 μm. *cb*, ciliary band; *ep*, epaulette; *sk*, skeletal rod; *st*, stomach. FIG. 3. Midsagital section of the pedicellaria primordium. Bar = 20 μm. *ep*, epidermis; *my*, mesenchyme; *st*, stomach. FIGS. 4, 5. SEM images of the pedicellaria primordium showing the invagination (Fig. 4) and the encapsulation (Fig. 5). Bars = 10 μm. FIG. 6. TEM of a cross section of the pedicellaria primordium. Note the mesenchymal cell in mitosis. Bar = 3 μm. *ep*, epithelial cells; *my*, mesenchymal cells.

FIG. 7. Light micrograph of a pedicellaria primordium prior to the formation of the cleft. Bar = 10 μm. *ep*, epidermis; *my*, mesenchyme; *pc*, pigmented cell; *pt*, posterior transverse rod of the larval skeleton; *s*, triradiate spicule. FIG. 8. Midsagital section of pedicellaria primordium at a stage similar to that shown in Fig. 7. Bar = 10 μm. *ep*, epidermis; *my*, mesenchyme; *pc*, pigmented cell. FIG. 9. SEM of the pedicellaria primordium at the cleft stage of its development. Bar = 10 μm. FIG. 10. Cross section of a cleft stage pedicellaria primordium. Bar = 3 μm. *m*, incipient adductor muscles; *s*, skeletogenic cells; *c*, central core of mesenchyme. FIG. 11. TEM of incipient muscle cells within a cleft stage primordium. Bar = 2 μm. *mf*, developing myofilaments; *n*, nucleus. FIG. 12. TEM of the skeletogenic cells in a cleft stage primordium, the skeletal spicule forms in the large vacuole (*sk*) at the center of the micrograph. Bar = 2 μm.

FIG. 13. SEM of a fully formed pedicellaria in the pluteus larva of *L. pictus*. Bar = 10 μm. FIG. 14. Midsagital section of a pedicellaria located on the posterior end of a *L. pictus* larva. Bar = 10 μm. *ab*, abductor muscle; *ad*, adductor muscle; *ep*, epidermis; *h*, head region of pedicellaria; *s*, stalk region of pedicellaria. FIG. 15. TEM of a section cut through both striated adductor muscle and smooth abductor muscle in a fully formed pedicellaria. Bar = 1 μm. *db*, dense bodies; *h*, H-band; *sm*, sarcomere; *sr*, sarcoplasmic reticulum; *t*, transverse tubules; *z*, Z-bands. FIG. 16. TEM of a section showing the insertion of an abductor muscle onto a pedicellaria jaw. An axonal tract, seen here in cross section is associated with the jaw and muscle. Bar = 1 μm. *ax*, axons; *m*, muscle fiber; *sk*, skeleton of the pedicellaria jaw. FIG. 17. TEM of sensory cells that occur on the interior surface of the pedicellariae jaws. Bar = 1 μm. *c*, cilium; *mv*, microvilli; *sr*, striated rootlet.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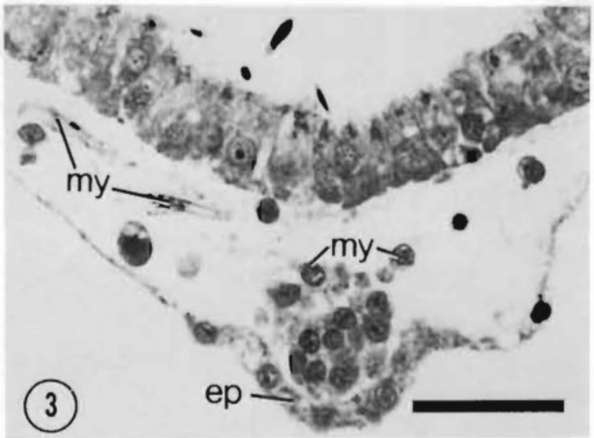
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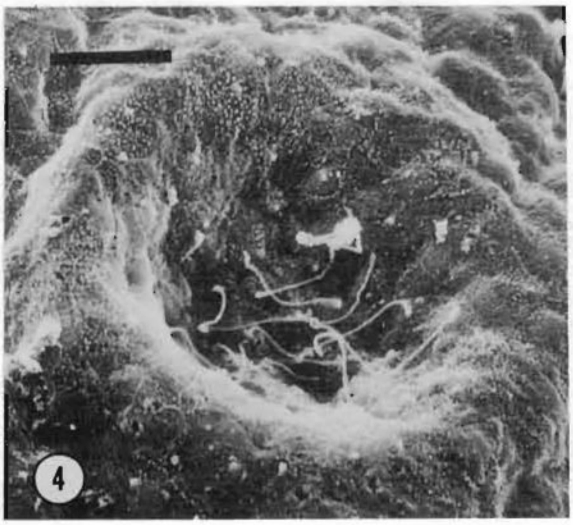
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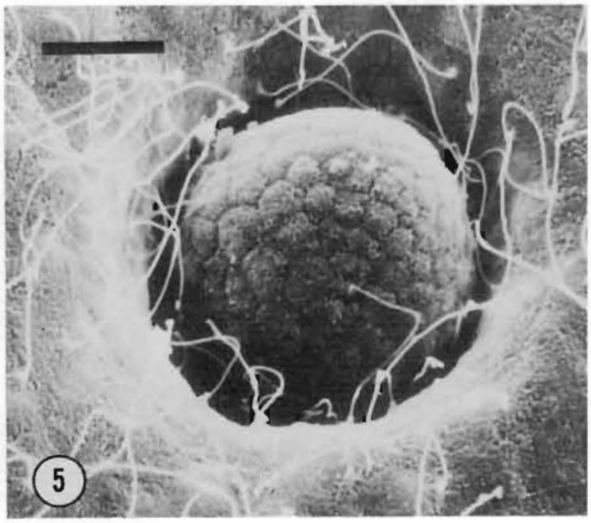
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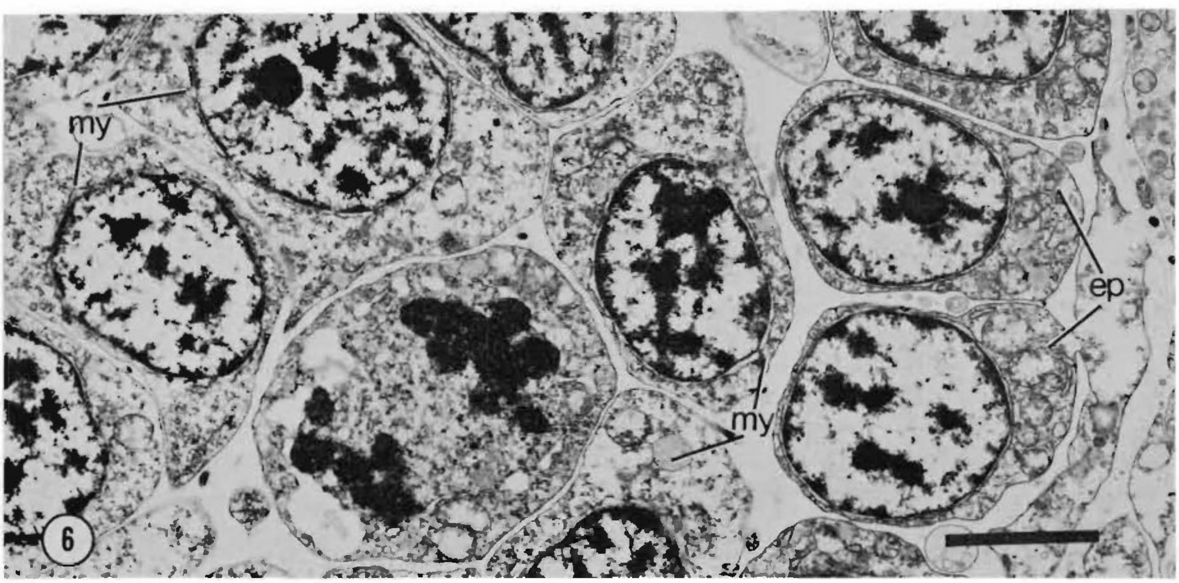
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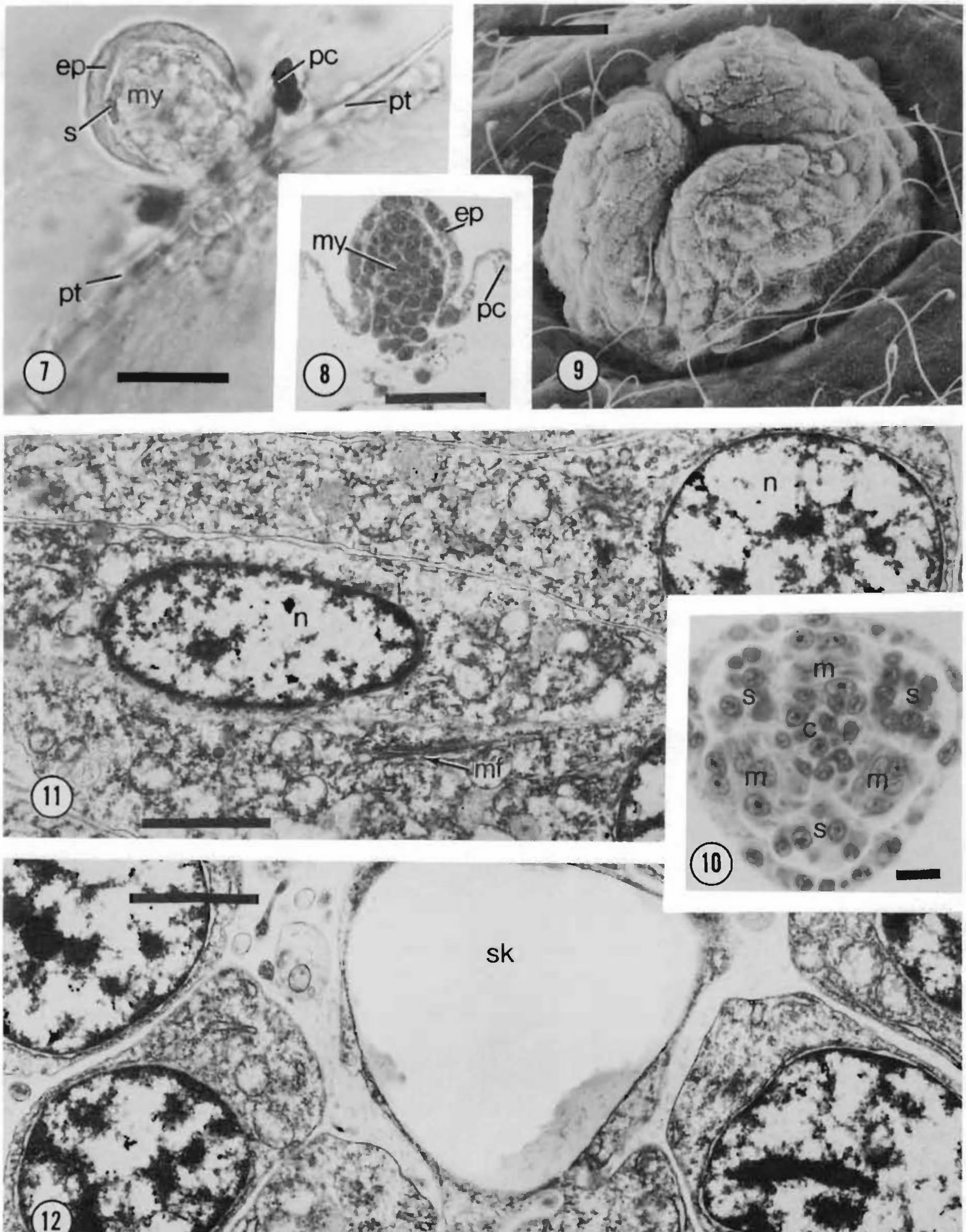
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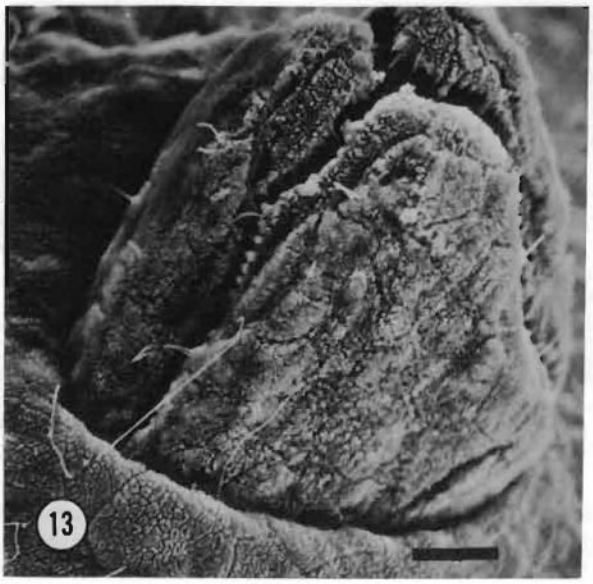
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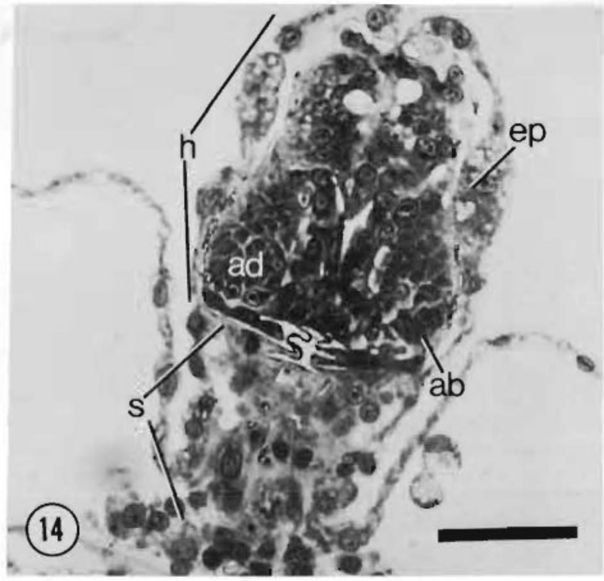
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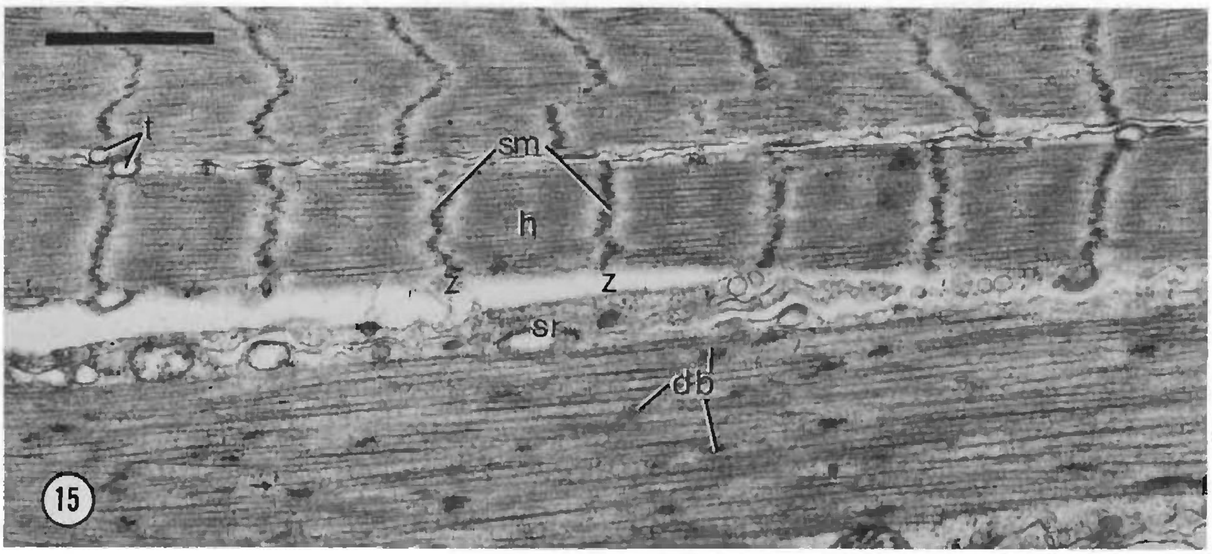
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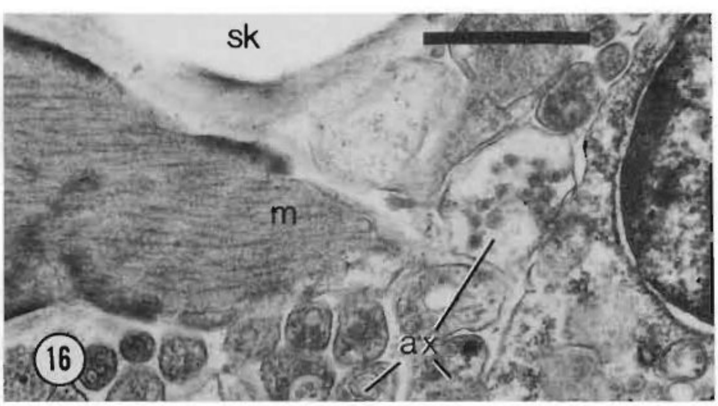
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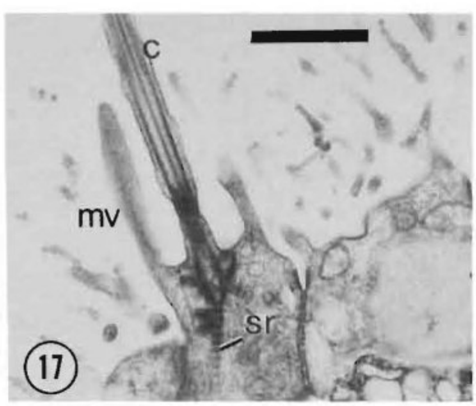
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partially enclosed within the vestibule in which they were formed. The stalk is capable of flexion and the jaws are able to open and close.

Structurally the pedicellariae are the same as those of *Echinus esculentus* described by Cobb (1967*a*, 1967*b*). The skeletons of the pedicellariae jaws are serate-edged and formed into two triangular shaped baskets with two cup-like regions at the base which serve as areas of attachments for the muscles. The skeleton, once formed is no longer enclosed within the skeletogenic mesenchyme (Fig. 16). The muscles are of two types, cross-striated adductor muscles and smooth abductor muscles (Fig. 15). The adductor muscles insert onto the interior surface of the jaw skeleton and comprise fibers 2 to 3 μm wide and up to 10 μm long. Each sarcomere is about 1.3 μm long; there are indistinct H-bands but no M-lines, and a system of T-tubules is associated with the Z-lines. The thick filaments range from 15 to 18 nm in diameter and the thin filaments from 5 to 9 nm. The abductor muscles are proximal to the adductor muscles and insert on the base and exterior surface of the jaw skeleton. The smooth muscle fibers are about 2 μm in diameter and up to 15 μm in length. Thick filaments in the smooth muscle fibers are 15 to 20 nm in diameter and the thin filaments about 5 nm in diameter. Mitochondria and sarcoplasmic reticulum occur along the peripheries of the cells. The thin filaments appear to insert on dense plaques at the ends of the cells (Fig. 16).

As reported by Cobb (1967*a*), tracts of axons course between sensory cells on the surface of each jaw and the muscles at the base of each jaw. Sensory cells occur along the midline of the interior of each jaw, are spindle shaped, and have a single cilium surrounded at its base by a circle of microvilli (Fig. 17). Each sensory cell appears to extend a process into an axonal tract.

The jaws are covered with simple cuboidal and squamous epithelia comprising vacuous cells which have an external surface elaborated into numerous branched microvilli (Figs. 13, 14).

Metamorphosis

During the phase of metamorphosis in which the larval epidermis is retracted, the pedicellariae are drawn to the aboral surface of the juvenile. The three pedicellariae are symmetrically arranged around the aboral pole of the postmetamorphic juvenile and the base of the stalks of the pedicellariae serve as loci for the formation of the genital plates of the adult test.

Discussion

There are several reports of pedicellariae in fully developed echinoplutei (MacBride 1903; Mortensen 1921, 1931, 1937, 1938; Gordon 1926; Hinegardner 1969; Strathmann 1979). However, there are no detailed accounts of pedicellaria development. The description given here agrees with the brief report of the development of the primordium presented by MacBride (1903). Although, he suggested that the primordium forms from an evagination of epidermis, whereas the observations reported here indicate that there is initially an invagination of the epidermis which encapsulates the mesenchyme. Gordon (1926) described in detail the development of the pedicellaria skeleton in *Psammechinus (Echinus) miliaris*.

The complete development of three tridentate pedicellariae occurs within the larva of *L. pictus* prior to metamorphosis. The pedicellariae described here were operable in the larva, and the muscles, nerves, and sensory cells appeared to be ultrastructurally identical to adult pedicellaria as described by Cobb (1967*a*, 1967*b*). Although pedicellariae which develop in the larva are often called anlage or rudiments (Hyman 1955; Czihak 1971), they are not rudimentary in the sense that the adult tissues are not fully differentiated. Histogenesis of pedicellariae, and perhaps several other adult structures (MacBride 1903; R. D. Burke, unpublished observations), appears to be development that occurs independently of metamorphosis; if metamorphosis is considered the relatively rapid transformation of the planktonic pluteus into the benthic juvenile. Larvae may not be fully competent to metamorphose without having first completed the formation of certain adult structures, but metamorphosis does not appear to involve the release of adult structures from an arrested state of development.

The cells which form pedicellariae in the larva of *L. pictus* are derived from two sources: the epidermis which covers the pedicellaria is transformed from the larval epidermis, and the mesenchyme which forms the muscles and jaw skeletons originates with mesenchyme from the remnant blastocoel of the larva. The mesenchyme initially enclosed in the pedicellaria primordium, probably comprises cells that were associated with the larval skeleton and cells with no apparent function that form a network of filiform extensions which spans the blastocoel.

In general, there appear to be three developmental relationships between larval and adult tissues in echinoid development. Some adult structures are transformed directly from larval structures; such as the pedicellaria epidermis which is formed from larval epidermis. As well, Gordon (1926) describes several situations in which adult test grows directly from larval skeleton, and Cameron and Hinegardner (1978) note that the epithelium lining the rudiment vestibule becomes the aboral epidermis of the adult. The second relationship is one in which larval tissues are destroyed and only incorporated into adult tissues as resorbed, dead cells. The destruction of the larval epidermis during metamorphosis and its subsequent resorption into the gut is an example of this form of development (Chia and Burke 1978). In the third situation, cells with no apparent function in the larva, which probably form a reservoir of undifferentiated cells, develop directly into adult tissues. The apparently undifferentiated blastocoelar mesenchyme which forms the connective tissue layers and appendages of the adult body wall (MacBride 1903) is one example, and the encapsulation of blastocoelar mesenchyme into the pedicellaria primordium serves as another (MacBride 1903).

Pedicellariae are reported to develop in the larvae of at least 16 species in both the Perischioechinoidea and the Euechinoidea subclasses (Table 1). However, the character is apparently restricted to orders of regular urchins. Although complete reports of larval development are scanty, within any one family not all the genera, nor all the species in a genus, will develop pedicellariae precociously. In the family Strogylocentrodidae, *Strongylocentrotus franciscanus* is the only species reported to develop pedicellariae in the larva, even though there are detailed accounts of the larvae of several congeners (Strathmann 1979). This observation appears to be contrary to the thesis of Mortensen (1921) that the form of fully developed larvae is the same for all members of an echinoid family. Evidently the development of pedicellariae prior to metamorphosis has arisen, or been preserved, in several instances independently of other familial characters. Pedicellariae development in the larva may correlate with species that settle in environments in which juveniles require the immediate use of these primarily defensive adult appendages.

TABLE 1. Taxonomic distribution of echinoid species reported to form pedicellariae in the larva

	Reference
Subclass: Perischioechinoidea	
Order: Cidaroidea	
Family: Cidaridae	
Eucidaris metularia	Mortensen 1937
Prionocidaris baculosus	Mortensen 1937
Subclass: Euechinoidea	
Order: Diadematoida	
Family: Diadematidae	
Diadema setosum	Mortensen 1937
Order: Arbacioida	
Family: Arbaciidae	
Coelopleurus floridanus	J. Miller, 1979, personal communication
Order: Temnopleuroida	
Family: Toxopneustidae	
Tripneustes gratilla	Mortensen 1937
Tripneustes esculentus	Mortensen 1921
Nudechinus gravieri	Mortensen 1937
Lytechinus variegatus	Mortensen 1921
Lytechinus pictus	Hinegardner 1969
Family: Temnopleuridae	
Mespilia globulus	Mortensen 1921
Order: Echinoida	
Family: Echinometridae	
Echinometra lucunter	Mortensen 1921
Heliocidaris tuberculatus	Mortensen 1921
Heterocentrotus mammillatus	Mortensen 1937
Family: Strongylocentrodidae	
Strongylocentrotus franciscanus	Mortensen 1937
Family: Echinidae	
Echinus esculentus	MacBride 1903
Psammechinus miliaris	Gordon 1926

Acknowledgements

This research was supported by NSF grant No. 77-16262 to Dr. D. B. Bonar. I thank Dr. F. S. Chia, Dr. D. B. Bonar, and J. E. Miller for their helpful comments on the manuscript. Dr. M. E. Rice provided facilities and Mrs. J. J. Jones typed the manuscript.

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