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Stages of development, from first cleavage to hatching, of an *Echinoderes* (Phylum Kinorhyncha: Class Cyclorhagida)

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Abstract: The first, second and third cleavages of an egg of *Echinoderes kozloffi* are essentially equal. Subsequent cleavages lead to the production of an embryo with three layers of cells. One layer, consisting of 10 cells, separates a rosette-like arrangement of 8 cells from a less symmetrical layer of about 16 cells. Soon there are two contiguous rosette-like layers of 8 cells, two middle layers of 10 cells, and numerous cells in two other layers on the opposite side. After this, rapid divisions result in the formation of an apparently solid embryo that appears not to have a blastopore or conspicuous internal cavity. A dense central mass of cells destined to give rise to most of the digestive tract becomes distinct, however, and is interpreted to consist of endoderm; at this stage the embryo is therefore considered to be a stereogastrula. Embryonic mesoderm has not been identified. After the embryo elongates and bends on itself, the rectum is visible. The formation of the oral cone and the rest of segment 1 of the fully developed juvenile takes place in the inverted position and has not been observed. What is known of early development of *Echinoderes* is compared briefly with that of other invertebrates with similar larval or adult characters.

Résumé : *Stades de développement, de la première segmentation à l'éclosion, chez un* Echinoderes *(Phylum Kinorhyncha : Classe Cyclorhagida).* L'embryologie d'*Echinoderes kozloffi* commence avec trois segmentations égales. Les segmentations suivantes forment trois couches de cellules. La couche du milieu, avec 10 cellules, sépare 8 cellules arrangées en forme de rosette d'une couche de seize cellules environ ; l'arrangement de ces dernières est moins symétrique que celui de la rosette. Les trois couches sont doublées, formant six étages. Le résultat des segmentations rapides qui suivent est un embryon apparemment solide dans lequel ni blastopore, ni cavité interne n'ont été reconnus. Une masse centrale compacte qui engendre la plupart du tube digestif est interprétée comme étant l'endoderme ; à ce stade l'embryon est considéré donc comme une stéréogastrula. Le mésoderme embryonique n'a pas été identifié. Après que l'embryon s'allonge et se plie, le rectum est visible. Le développement du cône oral et d'autres structures du segment 1 du juvénile se situe à l'opposé et n'a pas été étudié. L'embryogenèse d'*Echinoderes* est comparée brièvement à celle d'autres invertébrés présentant des caractères adultes ou larvaires semblables.

Keywords: *Echinoderes kozloffi* ● Kinorhyncha ● Development ● Cleavage ● Hatching

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Introduction

The hatching of a juvenile *Echinoderes*, with eleven of the thirteen segments characteristic of an adult, was described thirty-five years ago (Kozloff, 1972). This study refuted the claim of Nyholm (1947a & b) that the life history included a free-living larval stage, with three-segments, that gradually became a juvenile with eleven segments. No studies have been published, however, on the early embryonic development of any kinorhynch, so I was compelled to revisit the species that is abundant in certain habitats near Friday Harbor and that was named *E. kozloffi* by Higgins (1977).

Material and methods

All adult *Echinoderes* for this research were collected at the type locality of *E. kozloffi*: an inlet of North Bay, San Juan Island, Washington, about 2 km south of the town of Friday Harbor. The kinorhynch is abundant in the upper 5 mm of sediment above a mixture of sand, mud, small stones, and shells. At a tide level of about -0.3 m, the surface sediment is directly above the black layer, largely deprived of oxygen; at lower levels, down to at least -0.6 m, where the substratum is more effectively oxygenated, the black layer becomes progressively deeper. Typically, the upper 5 mm of sediment contains, in addition to *Echinoderes*, abundant diatoms, foraminiferans, ciliates, testate amoebae, small polychaetes, harpacticoid copepods, ostracodes, *Leptochelia dubia* (Kroyer, 1857) (Tanaidacea), *Monocorophium acherusicum* (Costa, 1857) (Amphipoda), and *Transennella tantilla* (Gould, 1853) (Mollusca, Bivalvia). Certain of these are probably important for the success of *Echinoderes*, for they make tubes of mucus and/or clay particles that hold the sediment together. *Echinoderes* burrows through the sediment, feeding on small particles of detritus, which is rich in bacteria, and also on diatoms, which are mostly small species of *Nitzschia* and *Navicula*. Diatoms are perhaps required for normal growth and reproduction, but do not often form a large part of the material visible in the gut. At the type locality, *Echinoderes* is reproductive throughout the year, even January, when the temperature of the seawater may be as low as 8°C, and when the surface sediment, exposed during low tides, is perhaps even colder.

After a small amount of recently collected surface sediment is spread out thinly in a Petri dish of seawater, stirred vigorously with the tip of a finger in order to break up the cohesive material, and examined with a dissecting microscope at a magnification of about 20X, at least a few *Echinoderes* are usually found. For obtaining numerous individuals, however, it is better to place sediment to a depth of 2-3 cm in plastic dishes with screen "windows" and submerge them in a laboratory aquarium through which seawater is flowing. Within about 48 hours, most of the kinorhynchs buried in the sediment will have risen, along with associated organisms, close to the surface, so the concentration of kinorhynchs in the top layer will be considerably higher than in undisturbed sediment. In general, however, preparations in which the sediment is as deep as 3 cm may begin to deteriorate within a few days owing to increasing anoxia. Even in those that appear to be in good condition after two weeks, females with enlarged oocytes are usually scarce.

Finding eggs or embryos of *Echinoderes* in natural sediment may not be impossible, but it is not likely to be successful because this rich deposit has so many distracting components that make the search unproductive. Furthermore, a female typically lays only one egg at a time, and in the process she coats it with a two-layered ball of detritus, making it difficult to recognize within the already opaque sediment.

Attempts to encourage females to oviposit in various mixtures of diluted natural sediment in seawater containing different concentrations of agar or methyl cellulose did not work and were abandoned. Natural sediment passed through a Nitex screen with a mesh size of 35 µm proved to be more satisfactory because the small particles can be separated easily from the the balls of compacted detritus that surround eggs of *Echinoderes*. To about 0.5 ml of seawater in a small glass dish (diameter 17 mm), about 0.1 ml of screened particles of sediment and several females showing at least moderately large oocytes were placed, and usually also a few males were added, in case not all of the females had already been inseminated.

Usually, the assembled kinorhynchs make the modified sediment more cohesive and, within a day or two, consolidate it into a mat or something resembling a haystack. If some of the sediment is dispersed in a small dish of seawater and examined with a dissecting microscope at a magnification of about 20X, any ball-shaped masses about 260-300 µm in diameter are likely to contain an egg or embryo. The balls, when viewed in transmitted light, are nearly opaque and consist of two essentially separate layers. A micropipette with a bore barely larger than the mass as a whole is generally effective for taking up and repeatedly squirting out an egg or embryo in order to remove the relatively loose outer layer (Fig. 1A), revealing a more dense inner layer (Fig. 1B) about 90 to 100 µm in diameter (it is not always perfectly spherical) that contains an egg or embryo. When the egg or embryo is at an early stage of development, the tight inner layer is tenacious and difficult to remove. Older embryos are easier to clean, suggesting that the coating is gradually softened by microbial activity in time for the escape of the juvenile. By

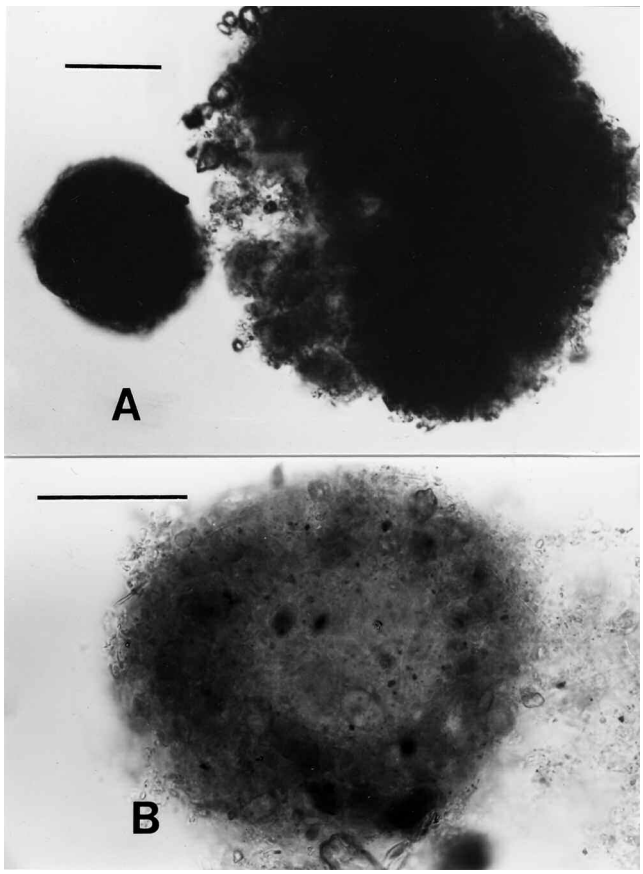

Figure 1. *Echinoderes kozloffi*, photomicrographs. **A.** Ball-like mass of sediment (right) from which a smaller mass enclosing an egg (left) has been forced out. Scale bar = 50 µm. **B.** Egg made visible by allowing the coverglass to press more forcefully on the mass of sediment enclosing it. Scale bar = 50 µm.

Figure 1. *Echinoderes kozloffi*, photomicrographies. **A.** Une masse presque sphérique de sédiment (à droite) de laquelle une masse plus petite (à gauche), qui renferme un oeuf, a été exprimée. Echelle = 50 µm. **B.** Oeuf rendu visible en laissant la lamelle appuyée plus fortement sur la masse du sédiment qui le renferme. Echelle = 50 µm.

placing the smaller mass in a drop of seawater on a slide, covering it with a small (10-12 mm) coverglass, and watching it at a magnification of 100X while moving the coverglass and/or withdrawing small amounts of water with a piece of absorbent paper, the cohesive coating of small particles enclosing the egg or embryo can be observed to weaken. Some seawater must quickly be added to the preparation so that the egg or embryo can be transferred to a small dish of seawater and cleaned with the aid of a pipette. Unfortunately, too-quick compression of the layer of cemented particles often leads to rupture of the egg or embryo it encloses. Furthermore, some eggs that appear to be normal do not develop at all, suggesting that they have been damaged by being compressed or agitated too much.

Thin coverglasses about 10-12 mm in diameter floated on drops of seawater can safely be manipulated by touch, or by blowing on them, to change the orientation of a specimen while it is being studied. Care must be taken, however, not to allow a preparation to evaporate to the extent that salinity of the water is increased. To remove a specimen from the preparation, and to transfer it to a small dish in which it can be kept for later study, it is usually necessary to add some seawater and slide the coverglass around in order to bring the specimen to the edge so that it can be drawn up into a micropipette.

Ideally, one might expect to find one or more recently laid eggs within a few hours after placing some female *Echinoderes* into a dish with screened sediment. This is not likely to happen. The kinorhynchs seem to need time to work the sediment into a more satisfactory medium for feeding and egg-laying, and perhaps also to mate, if they have not already mated. A few eggs or embryos have been found near the end of the second 24 hours, but 48-hour or older preparations have been much more productive.

The seawater temperature in the laboratory aquarium during this study, which was continued through all seasons of two years, ranged from 9 to 13°C. During microscopic and photomicrographic work, however, preparations were exposed temporarily to air temperatures as high as 20°C.

The developmental stages illustrated in this paper have been chosen from several sequences. This was necessary because the development of some embryos could not be observed in its entirety due to loss or damage, and because photomicrographs of some sequences show certain stages more favorably than others. To understand how some tissues and organs differentiate will almost certainly require examination of serial sections. Nevertheless, the results of this study will provide a general review of the embryology and perhaps encourage other zoologists to add details to this account, using either an *Echinoderes* or species of other genera.

The terminology for structures that appear during late stages of development follows that used for mature kinorhynchs by Kristensen and Higgins (1991).

Results

An egg or embryo of *Echinoderes*, after having been forcibly separated from the sediment that a female had deposited around it, is enclosed within a transparent, colorless envelope that has a thickness of about 1 µm. The smallest envelopes containing early stages of development were spherical and 54 µm in diameter; the largest were ovoid and measured 72 by 60 µm. The shape of the envelope does not change during early stages of development, but it becomes proportionately longer later on, when the embryo elongates.

Oviposition has not been observed, but one female that had been for 48 hours in a preparation of modified sediment, and then transferred to a dish of clean seawater, had an egg attached to her ventral side close to the posterior end. She was trying to coat the egg with detritus, but there was almost no particulate material in the dish. As soon as a small amount of sediment was added, she quickly applied some of it to the egg. Then, by doubling-up her body, alternately everting and inverting the head (segment 1) and neck (segment 2) and secreting sticky material, she added more particles and used the scalids to consolidate and smooth the mass. In about 20 minutes, the egg was covered, but not as thoroughly as those deposited under more nearly normal circumstances, and the female separated herself from it. Unfortunately, when this egg was drawn up into a pipette and then squirted out to remove some of the sediment coating it, it ruptured. Perhaps the envelope surrounding an egg that has just been laid is especially delicate.

Five eggs that had not already undergone the first cleavage were found, but only two of these developed. The other three appeared to be normal and did not deteriorate for several days, so failure of development to take place was assumed to have been caused by mechanical disturbance of the eggs while they were being separated from the sediment tightly deposited around them.

Polar bodies, although expected to be present, were not observed on any of the eggs or early embryos. If these could be found, they would probably be helpful in determining the anterior-to-posterior orientation of the embryos.

In addition to the two eggs that did undergo the first and subsequent cleavages, seven 4-cell embryos were found. Four of these were followed through all or almost all of their development. The development of the other three was followed until the juveniles hatched, but only earlier stages, up to four or five days old, were studied intensively because information on later stages could easily be obtained from other embryos, some of which were well beyond the 8-cell stage when they were found.

In the smaller of two eggs that had not yet undergone the first division (Fig. 2A), the nucleus was barely evident because of the dense concentration of lipid inclusions in the cytoplasm. Cleavage was completed (Fig. 2B) in about 40 minutes from the time the two daughter nuclei were distinct, about 2 hours from the time the egg was found. The 4-cell stage (Fig. 2C) was reached after another 2 hours, and the the 8-cell stage in an additional 3 hours. This embryo was monitored at frequent intervals up to the stage at which the juvenile worm hatched in 9 days, and several later stages in its development will be described here. The first cleavage of a larger egg (Fig. 2D) was finished in about an hour after this egg, in which the nucleus or nuclei were obscure, was found. The 4-cell and 8-cell stages, reached respectively, in about two hours and an additional two and a half hours, are shown in Fig. 2E & F.

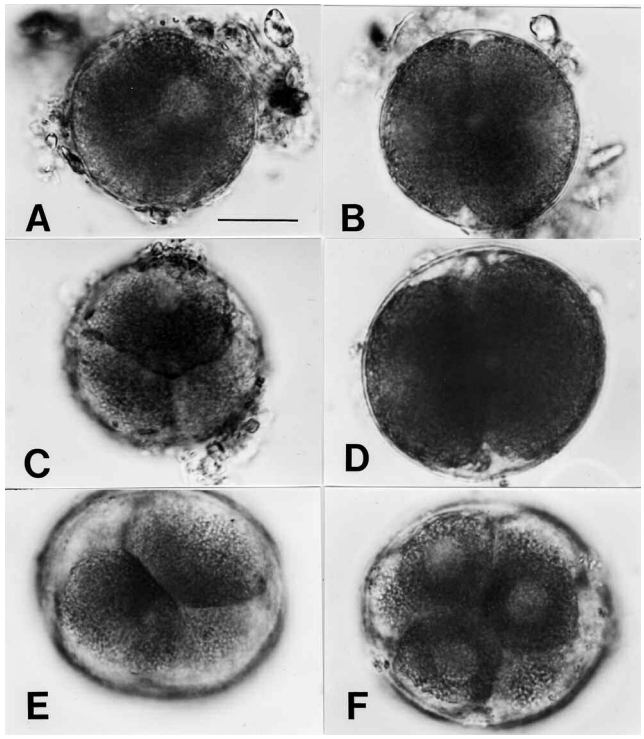

Figure 2. *Echinoderes kozloffi*, photomicrographs. Scale bar = 20 µm. **A.** Small fertile egg. **B.** Same egg after completion of the first cleavage, 40 minutes after the nucleus had divided. Part of one cell is tilted under the other. **C.** Four-cell stage of the same embryo (one of the cells is not visible). **D.** Two-cell stage derived from a larger egg, 50 minutes after the egg was found. **E.** Four-cell stage of the same embryo. **F.** Eight-cell stage.

Figure 2. *Echinoderes kozloffi*, photomicrographies. Echelle = 20 µm. **A**. Oeuf petit fertile. **B.** Même oeuf après la première segmentation, achevée 40 minutes après la division du noyau. Une portion d'une cellule est inclinée au-dessous de l'autre. **C.** Même embryon avec 4 cellules (une cellule n'est pas visible). **D.** Embryon de 2 cellules dérivé d'un oeuf plus grand, achevé 50 minutes après que l'oeuf a été trouvé. **E.** Même embryon avec 4 cellules. **F.** Embryon de 8 cellules.

The products of early cleavages are nearly or fully equal. In the case of two of the several 4-celled embryos whose development was followed, however, one or two cells of the first four were slightly ahead of or behind the others in dividing, so for a time there appeared to be six or seven cells. Similar irregularities were occasionally observed in succeeding stages.

An embryo interpreted to consist of about 18 cells - eight in one layer separating four (Fig. 3A) from six or seven on the opposite side - was reached in about seven and a half hours by the embryo whose 2-cell stage is shown in Fig. 2B (Thus the total elapsed time after completion of the first cleavage was approximately 12 hours, 30 minutes). Six

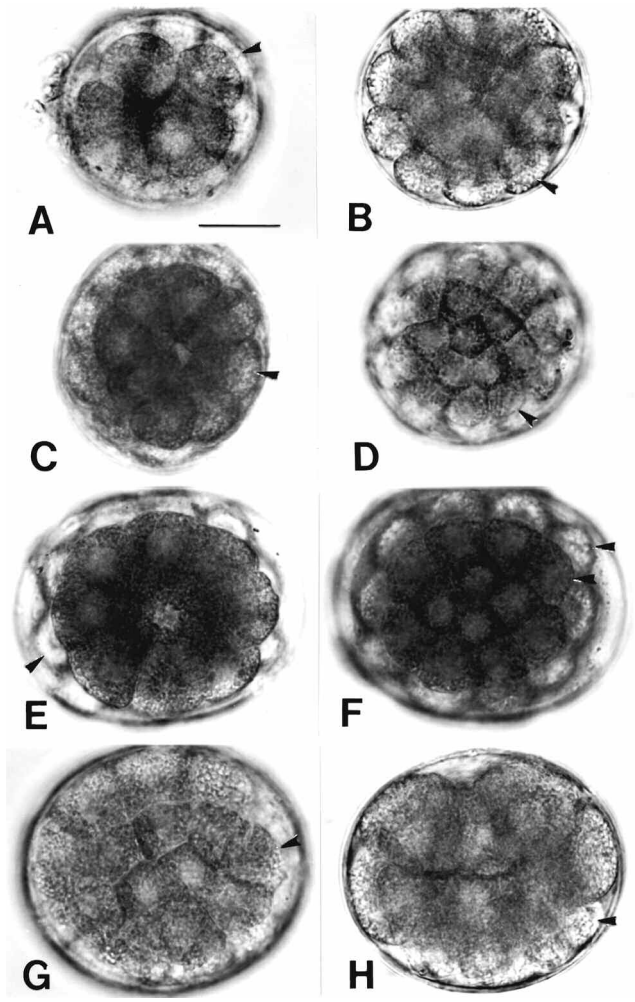

Figure 3. *Echinoderes kozloffi*, photomicrographs. Scale bar = 20 µm. **A.** Embryo with a layer of 8 cells (arrowhead) separating 4 larger cells from about 7 smaller cells, the latter not visible. **B.** Same embryo, slightly later, with 3 layers of cells, only the middle layer of 10 cells (arrowhead) in focus. **C.** Same embryo, only the 8 cells forming the rosette-like layer (arrowhead) in sharp focus. **D.** Same embryo rotated 180°, showing about 14 cells (arrowhead) on the side opposite that consisting of a rosette-like layer; the total number of cells in the embryo is thus about 32. **E.** Another embryo, larger than the preceding, showing a rosette-like layer of 8 cells above a layer of 10 cells (arrowhead). **F.** Same embryo rotated 180°, showing 12 cells (arrowhead) on the side opposite that with the rosette-like layer; cells of the middle layer (arrowhead) are also distinct. **G.** Embryo of about the same age as that of F, but showing cells (arrowhead) on the side opposite the rosettelike-layer arranged in a less symmetrical pattern. **H.** Same embryo, rotated 90°, showing cells of the rosette-like layer (uppermost), middle layer, and opposite side (arrowhead).

Figure 3. *Echinoderes kozloffi*, photomicrographies. Echelle = 20 µm. **A.** Embryon avec une couche de 8 cellules (flèche) qui sépare 4 plus grandes cellules d'environ 7 plus petites cellules, ces dernières non visibles. **B.** Même embryon avec trois couches de cellules, seule la couche de 10 cellules au milieu (flèche) est mise au point. **C.** Même embryon, seulement les cellules en rosette (flèche) mises au point. **D.** Même embryon, pivoté 180°, montrant à peu près 14 cellules (flèche) sur le côté opposé de celle en rosette ; le nombre total des cellule est d'environ 32. **E**. Un autre embryon, plus grand que le précédent, montrant la couche en rosette de 8 cellules et celle de 10 cellules au milieu (flèche). **F.** Même embryon pivoté 180° et comparable à celui de D, montrant 12 cellules environ (flèche) sur le côté opposé à celui en rosette ; les cellules de la couche duu milieu sont aussi visibles (flèche). **G.** Embryon du même âge à peu près que F, mais avec les cellules (flèche) du côté opposé à celui en rosette, arrangées moins symétriquement. **H.** Même embryon, pivoté 90°, montrant (en haut) la couche de cellules en rosette, celle du milieu, et (en bas) les cellules du côté opposé (flèche).

hours and 30 minutes later, a more distinctive stage, observed during development of this and several other embryos, was attained. This consisted of a layer of 10 cells (Fig. 3B) separating a rosette-like layer of eight cells (Fig. 3C) from a layer of about 16 smaller cells (Fig. 3D). That the cells on the side opposite the rosette-like layer are rounded and arranged more symmetrically, at least for a time, is suggested by the embryo estimated to be between 18 and 22 hours old) shown in Fig. 3E & F. Another embryo between 18 and 22 hours old (Fig. 3G), however, shows these cells already arranged more compactly in much the same way as in Fig. 3D. This embryo was pivoted 90° to show the three layers of cells in a profile view (Fig. 3H).

An embryo at a stage comparable to that shown in Fig. 3B, C & D, after about four additional hours, reached a stage (Fig. 4A) in which there were two rosette-like layers of 8 cells, two layers of 10 cells derived from the middle layer, and numerous cells, in two layers, on the opposite side. A similar embryo is shown in Fig. 4B. The two 10-cell layers have broader diameters than the layers next to them, which are broader than the ones on opposite sides of the embryo. The general shape of the embryo up to this stage is therefore nearly spherical.

None of the multicellular embryos described in the two preceding paragraphs showed any evidence of an internal cavity. The only spaces visible for a time were the small ones in the centers of the rosette-like layers, and it was not certain, when there were two such layers, that the spaces were aligned to form a continuous opening.

After the cells of the six-layer stage proliferate, the layered structure of the embryo is obliterated. For a short time, however, cells apparently derived from one or both rosette-like layers can be recognized, together with a small central space (Fig. 4C). On the opposite side of the embryo,

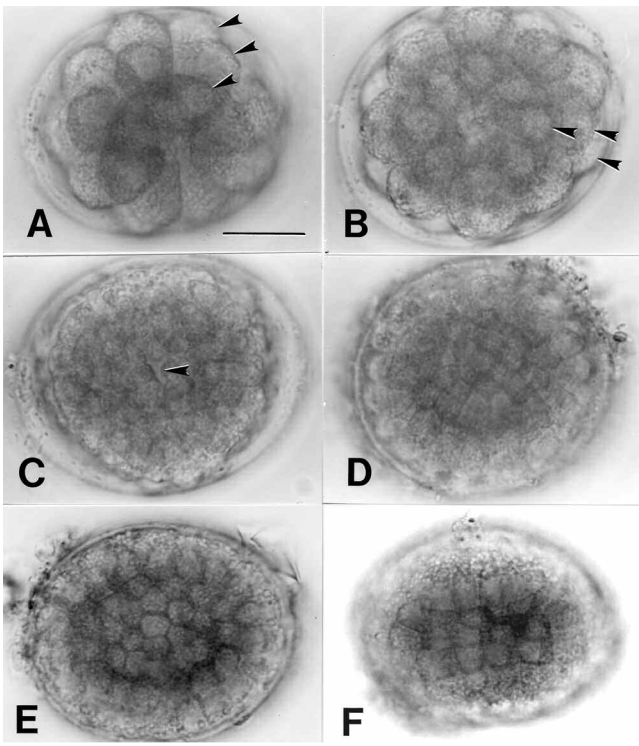

Figure 4. *Echinoderes kozloffi*, photomicrographs. Scale bar = 20 µm. **A.** Embryo shown in Fig. 3B-D, now with two rosette-like layers of 8 cells, two layers of 10 cells each, and two layers of smaller cells on the opposite side; only the nearest three of the six layers are visible (arrowheads). **B.** Another embryo at about the same stage; the three nearest layers are visible (arrowheads). **C.** Embryo after considerable multiplication of cells has taken place, showing derivatives of the rosette-like layers around a small aperture (arrowhead). **D.** Same embryo, opposite side. **E.** Stereogastrula, optical section. **F.** Same embryo, surface view, showing cells of the epidermal layer arranged in a pavement-like pattern.

Figure 4. *Echinoderes kozloffi*, photomicrographies. Echelle = 20 µm. **A.** Embryon montré à la Fig. 3B-D, maintenant avec deux étages des cellules en rosette, deux de dix cellules, et deux à l'autre côté ; seulement les trois étages plus proches sont visibles (flèches). **B.** Un autre embryon du même stade, à peu près ; les trois étages plus proches sont visibles (flèches). **C.** Embryon après multiplication considérable des cellules, montrant les cellules derivées des rosettes autour une ouverture petite (flèche). **D.** Même embryon, côté opposé. **E.** Stéréogastrula, section optique. **F.** Même embryon, vue de surface, montrant les cellules épidermiques arrangées en pavé.

cells with angular outlines (Fig. 4D) are almost certainly comparable to those shown in Fig. 3D & G. Otherwise, there are no obvious landmarks until about 40 to 48 hours after the first cleavage, when a central mass becomes conspicuous (Fig. 4E). The only cells external to the inner mass appear to be those forming the surface layer, which are compactly organized in a pavement-like configuration (Fig. 4F). The central mass is interpreted to be endoderm, because it persists and at least much of it differentiates into the gut. The entire embryo at this stage is therefore viewed as a stereogastrula. Putative mesodermal cells could conceivably be present in the inner mass, but at this stage one cannot predict which cells will differentiate into tissues that are traditionally viewed as being of mesodermal origin.

By the end of about 4 days of total elapsed time, embryos become slightly elongated and their surfaces, in profile, typically appear to be irregular, with some of the small cells of the surface layer projecting out much farther than others (Fig. 5A). When a portion of the epidermal layer is in focus, however, the pavement-like pattern of cells observed in slightly earlier embryos is still obvious. If any internal cavities are present, these have not been observed.

On day 5 the embryo starts to bend on itself (Fig. 5B). After bending has brought the posterior tip of the body to a nearly right angle with respect to the anterior part, the rectum (hindgut) can be identified (Fig. 5C). Soon the process of bending is completed, and by the sixth day the posterior, tapering part of the future juvenile is tucked neatly under the larger anterior portion (Fig. 5D) and muscular activity in the anterior portion of the worm is observed.

During changes that take place between the sixth or seventh to ninth or tenth days, the placids of the neck (Fig. 5E) and the scalids of the head (Fig. 5E & F) become distinct. At least some of the 9 trunk segments become distinct during late stages in development of the juvenile (Fig. 5F & G). Cuticular outgrowths (spines and pectinate fringes) are visible (Fig. 5H) about a day before hatching, but these, along with nerve cords, ganglia, and muscles, are easier to see after the juvenile escapes from the envelope in which it has developed. In order to emerge, the worm needs only to straighten its body and protrude its head, thereby rupturing the envelope, and crawl out (Kozloff, 1972). Feeding on particulate matter begins immediately.

Discussion

The most distinctive stages in the early development of *Echinoderes* are those following the 8-cell stage, when the cells divide in such a way that they become arranged in layers. It is possible that the rosette-like layer or layers on one side and the cells on the other side of the middle layer or layers of the embryo will eventually define the anterior and posterior ends of a juvenile worm. The stage shown in Fig. 4C, in which a slight opening at the surface is surrounded by numerous cells derived from one or both rosette-like layers, was observed only once, and the fate of the cells in question was not traced. That these cells could sink into the embryo, forming the central mass of what I

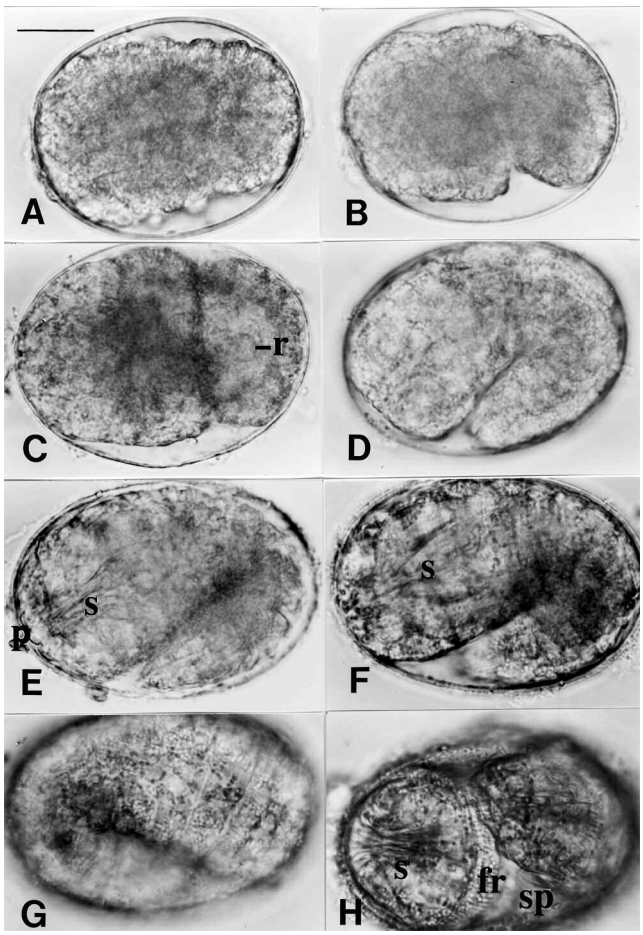

Figure 5. *Echinoderes kozloffi*, photomicrographs. Scale = 20 µm. **A.** Embryo elongated. **B.** Same embryo, lateral view, the posterior portion bending under the anterior portion. **C.** Slightly later stage, ventral view, the posterior portion bent at a right angle to the anterior portion, the hindgut visible. **D.** Same embryo, later stage, lateral view, the bending completed. **E.** Another embryo, slightly older, lateral view, showing developing placids and scalids. **F.** Same embryo, slighty later, lateral view, showing scalids and outlines of some trunk segments. **G.** Same embryo, nearly dorsal view, some trunk segments recognizable. **H.** Juvenile worm ready to hatch, ventral view, showing pectinate fringes, some spines, and some protruded scalids. **fr** = pectinate fringes; **p** = placids; **r** = rectum; **s** = scalids; **sp** = spine.

Figure 5. *Echinoderes kozloffi*, photomicrographies. Echelle = 20 µm. **A.** Embryon allongé. **B.** Même embryon, vue latérale, dont la portion postérieure a commencé de se courber contre la portion antérieure. **C.** Même embryon un peu plus avancé, vue ventrale, la portion postérieure courbée contre la portion antérieure, le rectum visible. **D.** Stade plus avancé, vue latérale, la courbure achevée. **E.** Stade encore plus avancé, vue latérale. **F.** Même embryon, un peu plus avancé, montrant les scalides et contours des segments. **G.** Même embryon, vue presque dorsale, quelques segments visibles. **H.** Jeune prêt à l'éclosion, vue ventrale, montrant les franges en forme de peigne, quelques épines, et quelques scalides saillants. **fr** = franges en forme de peigne ; **p** = placides ; **r** = rectum ; **s** = scalides; **sp** = épine.

have interpreted to be a stereogastrula, is another possibility. Such an endoderm-forming event would be a process of gastrulation.

The surface cells of an embryo at the stereogastrula stage certainly define the epidermis of later stages, and presumably give rise to the cuticularized epithelia of the foregut and hindgut, but these structures do not become evident until late stages in the development of the juvenile worm. The nervous system and cuticularized structures of the reproductive system are probably also ectodermal derivatives. Although the development of all or most of the midgut can be traced to the dense central mass of a stereogastrula, as an embryo elongates and becomes wormlike, then bends on itself, the opacity of the body interferes with observing details of the formation of this part of the alimentary tract. The origin of muscle, non-cuticularized components of the reproductive system, and other structures traditionally viewed as derivatives of mesoderm has not been studied. Mesoderm has not, in fact, even been identified. Perhaps it is formed by delamination from ectoderm, or is somehow associated with the central mass of the stereogastrula.

With respect to what has been said here about the embryology of *Echinoderes*, it is logical to ask: are there strong similarities between its development and that of other invertebrates which, at one stage or another in the life cycle, are characterized by a pseudocoel, a cuticularized body covering, and an oral cone-spiny proboscis complex? Kinorhynchs, priapulids, loriciferans, and nematomorphs form this assemblage, which Malakhov and Adrianov (1995) placed in their "phylum" Cephalorhyncha. These authors presented brief but clear summaries of what was known then about morphology and embryonic development of priapulids and nematomorphs, enlarging appreciably on the review of Malakhov (1994). Earlier accounts of priapulid and nematomorph (gordiacean) development are those, respectively, of Dawydoff (1959) and Dorier (1965). No studies of development of loriciferans have been published. In priapulids, essentially radial cleavage leads first to a hollow blastula; a gastrula is formed by invagination or by inbudding of cells that form a more or less solid columnar structure somewhat comparable to an archenteron. In one account cited by Malakhov and Adrianov, mesoderm originates from near the blastopore; in another, it originates from the archenteron-like structure and fills up much of the space around it so that a parenchymula is formed. Among Nematomorpha, in one species of *Gordius* cleavage is radial and the blastula is said to become a parenchymula by delamination of mesentoderm; invaginations establish the hindgut and foregut, forming the primordia of the introvert and hindgut. In another species of *Gordius*, and also in species of *Paragordius* and *Gordionus*, the pattern of development is

only slightly different. During development of *Echinoderes*, as reported in this paper, no stages closely comparable to those of priapulids and nematomorphs have been observed.

Nematode embryology has received much attention. The most comprehensive comparative studies are those of Nigon (1965) and Malakhov (1994); a brief but helpful recent account is that of Schierenberg (1997). In at least some nematodes, the quartet of cells formed by first two cleavages resembles the first quartet of *Echinoderes*, and after an embryonic nematode has reached the stage where it has been bent on itself and is close to hatching, it looks much like a *Echinoderes* that is on its way to becoming a fully-developed juvenile. In the patterns of development that have been described for nematodes, however, there is little else that resembles what happens in *Echinoderes.*

Some important aspects of the embryology of *Echinoderes* and other kinorhynchs - the origin and fate of mesoderm, the formation of the pseudocoel, and development of muscles and structures concerned with reproduction, among others - will probably be revealed only by study of sections. If an egg or embryo can be persuaded to develop normally after it has been removed from the envelope enclosing it, this will undoubtedly make study of cell lineage much easier. To those who will try to improve on what I have been privileged to observe, I give my best wishes for success.

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