EMBRYONIC DEVELOPMENT, EARLY INSTAR MORPHOLOGY, AND BEHAVIOR OF *TORTOPUS INCERTUS* (EPHEMEROPTERA: POLYMITARCIDAE)[1]

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ABSTRACT

The chorion of the egg of *Tortopus incertus* (Traver) was studied with the scanning electron microscope. Embryonic development at various temperatures was observed externally. Anatrepsis was blocked at 13.65° ± 1° C. Eggs kept at 19.45° ± 1° and 23.0° ± 1° C hatched after 33 and 41 days respectively. Mandibular tusks were present in the fully developed embryos. The morphology of the first and second nymphal instars was given; the cleaning and burrowing behavior of the first nymphal instars were described. First nymphal instars have a decided preference for burrowing in mud substrate over sand.

Nymphs of the burrowing mayfly *Tortopus incertus* (Traver) occur in interconnecting U-shaped burrows in clay banks of many streams and rivers in the Nearctic region. Scott, Berner and Hirsch (1959) studied the nymphal biology of this species. This paper reports the study of the various embryonic stages of *T. incertus* as seen externally through the chorion, the effect of different temperatures on embryogenesis, and the morphology and behavior of the early postembryonic instars.

METHODS

We collected eggs for this study from female imagos 21 and 28 August 1971, from the Apalachicola River near Bristol on Hwy 20, Liberty County, Florida. Female imagos collected at mercury vapor lamp deposited their eggs into cups of river water. Comparatively fewer male imagos appeared at the light trap, and it is possible that some females were unmated. The actual mating behavior of *T. incertus* is unknown at the present time. We transferred the eggs into watch glasses filled with distilled water and kept the eggs at 3 different temperatures: 13.65° ± 1° C, 19.45° ± 1° C, and 23.0° ± 1° C. The water was changed daily, and the eggs were observed daily for the first 15 days of the study and then at 2 day intervals. We dissected the eggs from their adhesive coverings in 80% alcohol and transferred them directly into a drop of xylene-free balsam dissolved in cellosolve (ethylene glycol mono ethyl ether). Staining of the embryo is unnecessary since it has a reddish-brown coloration that readily allows differentiation. Some early stages of embryogeny cannot be studied by external observation, but a major part of the embryonic development can be seen as the chorion is relatively transparent.

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RESULTS AND DISCUSSION

The egg of *T. incertus* is bowl-shaped; it has the appearance of "a sphere which has had one side pushed in." (Koss 1968). Each female imago produces 700-1,200 eggs, depending upon her size, and eggs range in size from 0.45-0.50 mm diam. An adhesive coat covers each egg, and the chorion is transparent and evenly punctated. Fig. 1 and 2 are scanning electron micrographs of the chorion taken at magnifications of 4000X and 15,000X respectively. Each pit has an average diameter of about 3μ, and the area in the pit is highly corrugated.

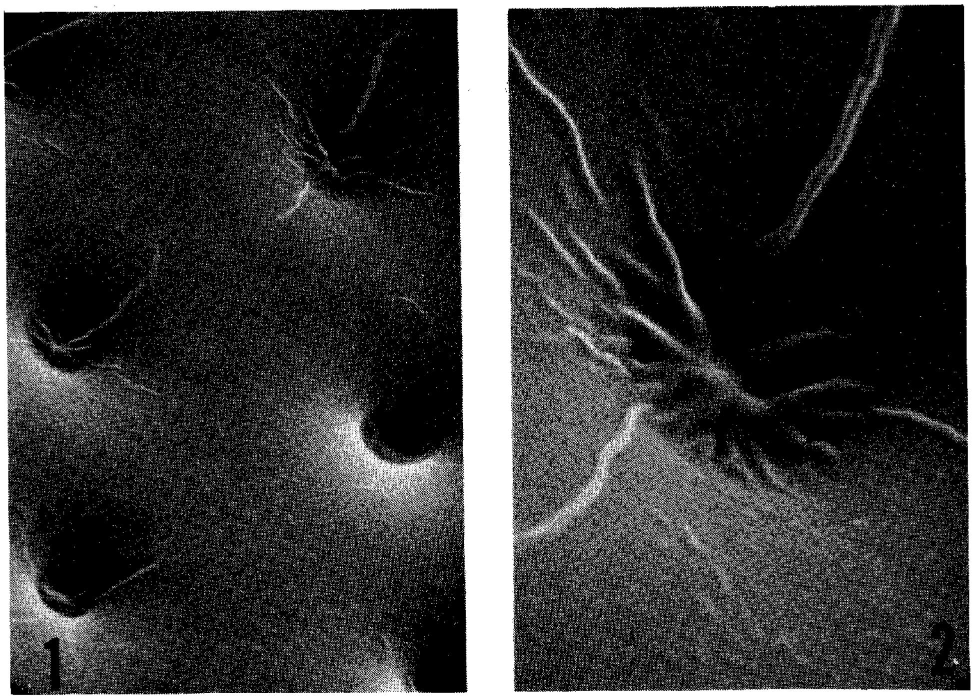

Fig. 1-2. Scanning electron micrographs of the chorion of *T. incertus*. 1—4000 ×; 2—15,000 ×.

Eggs of *T. incertus* have a highly variable rate of development; therefore, the ages (in days) given are only approximated. The following description applies to those eggs that were kept at the temperature of 19.45° ± 1° C. At the beginning of development the egg appeared to be a mass of yolky substance enclosed in the chorion. On about the 8th day, large yolk cells could be observed externally. Since no sectioning was made, it was not possible to observe the formation of the blastoderm and the differentiation of the ventral plate. It is possible that by the 8th day the ventral plate was already formed, as the development of an opening on the posterior surface of the cell mass leading into a cavity (the amniotic pore and cavity) occurred on the 12th day. Embryogeny of *T. incertus* is of the invaginated type, and has been similarly reported for *Ephemera strigata* Eaton [Ephemeridae] by Ando and Kawana (1956), and for *Baetis vernus* Curtis and *B. rhodani* (Pictet) [Baetidae] by Bohle (1969). In this type of development, the germ band is invaginated into the yolk except in the protocephalic region. Anatrepsis or the segmentation of

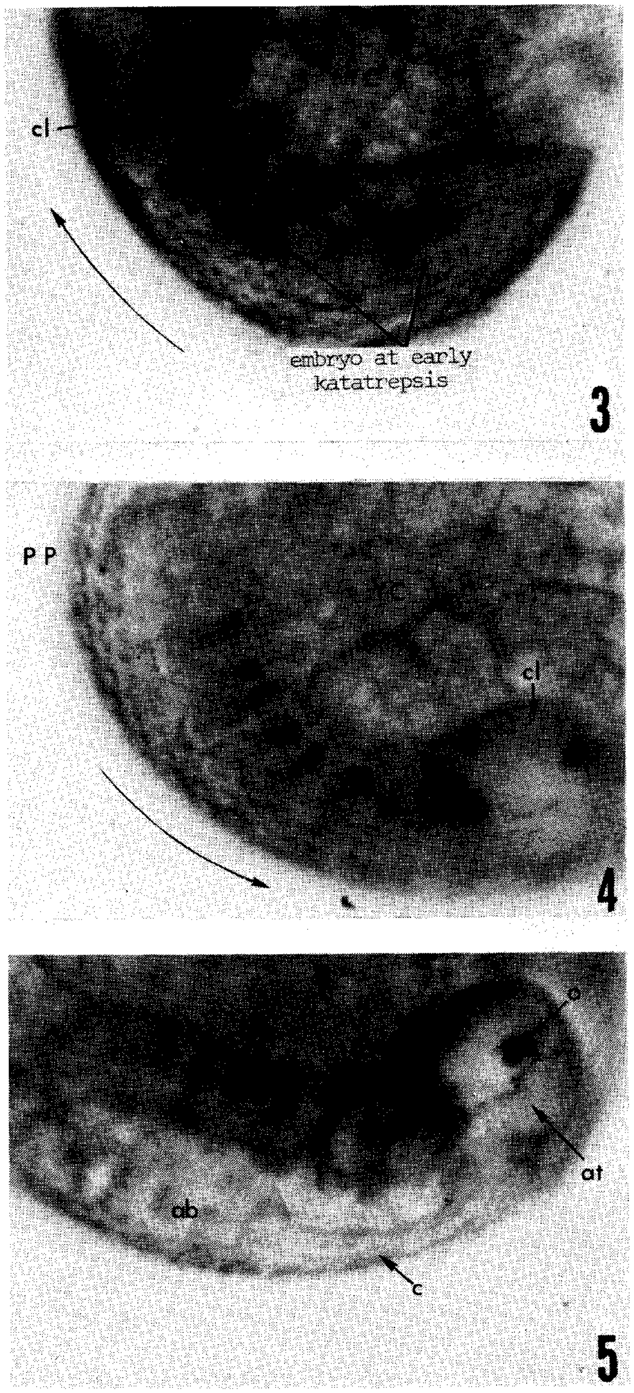

Fig. 3-5. Photographs of *T. incertus* embryo (arrow indicates the direction in which the embryo is moving). 3—Early katatrepsis; 4—late katatrepsis; 5—post-katatrepsis. PP = posterior pole, YC = yolk cell, ab = abdominal segment, at = antenna, c = cercus, cl = cephalic lobe, o = ocellus, th = thoracic segment.

the germ band proceeded inside the yolk and occurred between the 12th and the 15th day. At the terminal stage of anatrepsis, the cephalic lobe and the antennary segment protruded out of the yolk cells at the posterior pole of the egg. Following anatrepsis the embryo entered the katatrepsis stage. This involved the evagination of the embryo from the yolk mass and revolution of the embryo from the posterior pole to the anterior pole (Fig. 3, 4). Most of the embryos completed katatrepsis between the 15th and the 21st day of development (Fig. 5). Once the embryo had reorientated, it began to grow, and the various gnathocephalic and thoracic appendages became fully differentiated. At the same time the amount of yolky material gradually decreased. On about the 27th to 29th day most of the embryos were well developed (Fig. 6). Anteriorly, the head was well differentiated and the antennae, rudiments of the compound eyes, and 3 ocelli were visible. The ocelli were larger than the undeveloped compound eyes, whitish at the lower portions, and black at the upper portions. Beneath the median ocellus the egg burster could be recognized easily by its brownish color. The mandibular tusks were present at this stage; they were 0.11-0.13 mm long and about 0.03 mm wide at the base. In the thoracic region, the legs were developed with smooth and curved tarsal claws that measured 0.11-0.12 mm, and the abdominal segments and the 3 terminal filaments were developed.

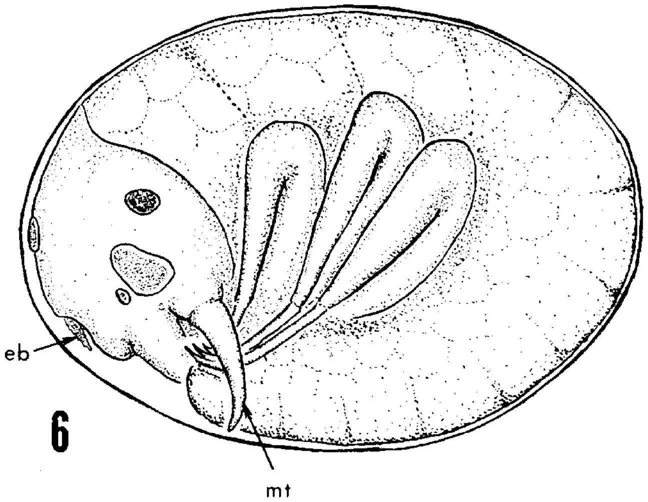

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Fig. 6. A 28th day embryo of *T. incertus* (eb = egg burster, mt = mandibular tusk).

In 2 separate experiments at the same temperature (19.45° ± 1° C) the eggs began to hatch on the 33rd day. We did not observe eclosion. In a fully developed egg a preformed line of weakness in the form of a semicircle was visible at the anterior end (Fig. 7). Eclosion probably occurred by breaking this

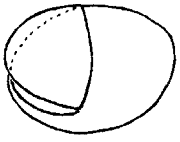

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Fig. 7. Egg shell of *T. incertus* after eclosion.

line of weakness with the help of the egg burster. We mounted a prelarval cast skin, which is a fine embryonic cuticle enclosing the embryo, on a slide, in water, and studied it under a phase-contrast microscope. The structure of the egg burster is shown in Fig. 8, 9, and 10. It is a knife-like structure about 36μ long. There are 10 lateral projections along the sides and it has a V-shaped cross section at the larger apical end. The cutting edge of the egg burster is highly serrated.

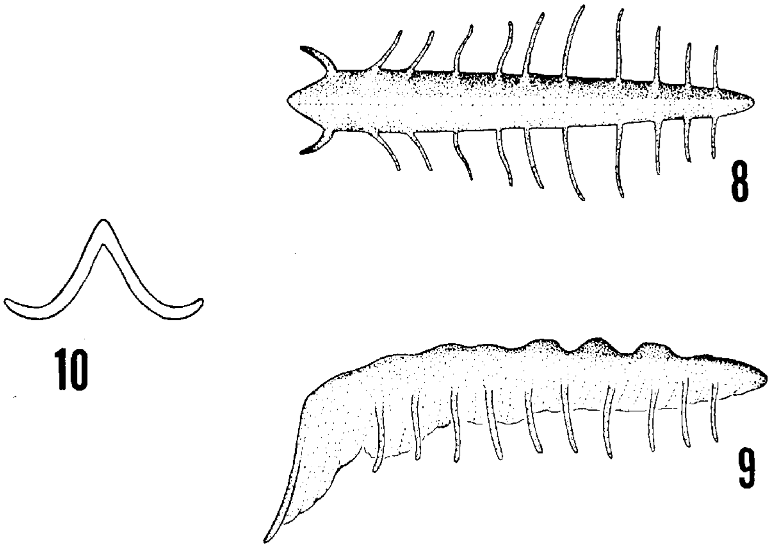

Fig. 8-10. Structure of the egg burster. 8—Anterior view; 9—lateral view; 10—apical cross section, enlarged twice.

Eggs kept at 13.65° ± 1° C did not undergo anatrepsis, and the amniotic pore and cavity were not observed. The low temperature blocked morphogenesis and caused quiescence. In 2 experiments, eggs kept in this temperature for 20 and 40 days were subsequently transferred to a water bath of higher temperature (19.45° ± 1° C), where they continued to undergo normal development and later hatched to healthy first nymphal instars. Eggs kept at the room temperature (23.0° ± 1° C) developed more slowly than those at 19.45° ± 1° C. At this temperature morphogensis was slightly retarded. The first instars eclosed after 41 days of incubation.

Following are descriptions of the first and second instar nymphs of *T. incertus.*

First Nymphal Instars. (Fig. 11-13, 15): Body campodeiform, length 0.98-1.01 mm; width of head 0.16-0.18 mm. Head (Fig. 15): prognathous, opaque white, a little triangular, slightly concave medially. Compound eyes black, relatively undeveloped. Three ocelli present, black. Antennae (Fig. 15): a little longer than head, 5 segmented, segment 3 equal to segments 1 and 2 combined. Mandibular tusks present (Fig. 15); length 0.18 mm, maximum width 0.05 mm, anterior inner margin with 2 hairs. Thorax: opaque white, 3 thoracic segments rectangular, length of meso and metathorax equal, length of prothoracic segment shorter. Legs (Fig. 11-13): opaque, all with long, moderately curved tarsal claws, without denticles. Fore tarsi comparatively

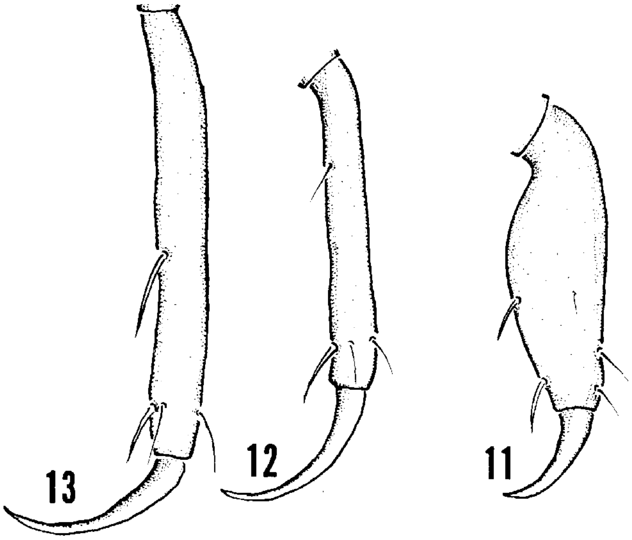

Fig. 11-13. Tarsi and tarsal claws of the first instar nymph. 11—Fore; 12—middle; 13—hind.

dilated. Abdomen: 10 visible segments, gills absent. Three caudal filaments, 5 visible segments, median filament longer than cerci.

Second Nymphal Instars. (Fig. 14, 16): Body campodeiform, length 1.3-1.4 mm; width of head 0.21-0.23 mm. Head (Fig. 16): opaque white; more square. Compound eyes and ocelli similar to first instars. Antennae (Fig. 16): a little longer than head, 6 segmented. Mandibular tusks (Fig. 16): longer than first instars, length 0.28 mm, maximum width 0.13 mm. Thorax similar to first instars. Legs similar to first instars except fore tarsi (Fig. 14). Abdomen: abdominal gills 2-7 present, filamentous, length 0.08-0.10 mm. Caudal filaments longer than first instars, 6 segmented.

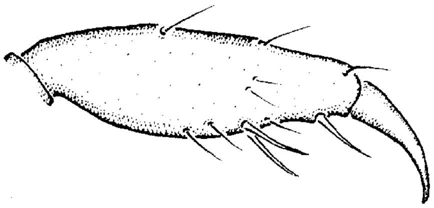

Fig. 14. Fore tarsus and tarsal claw of the second instar nymph.

The early appearance of the mandibular tusks in *T. incertus* as compared to other burrowing mayflies, is of particular interest. Neave (1932) found that the mandibular tusks were absent in the first nymphal instars of *Hexagenia limbata occulata* Walker (Ephemeridae) and that the mandibular tusks began to develop in *Ephemera*-species when they reached a body length of 2.50 mm. Ide (1935a) showed that the first nymphal instars of *Ephemera simulans* Walker did not have mandibular tusks. From Ide's illustrations, the mandibular tusks were seen only as tubercles in the 7th instars. Similarly, Ide (1935b) reported the absence of mandibular tusks for the first nymphal instars

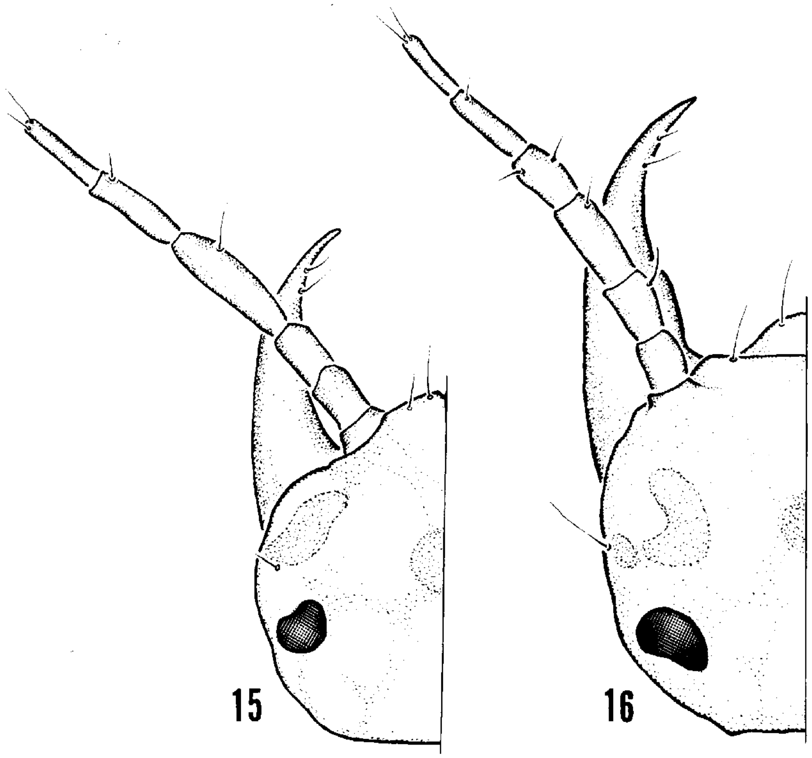

Fig. 15-16. Dorsal view of the nymphal head (left half). 15—First instar; 16—second instar.

of *Ephoron leukon* Williamson (Polymitarcidae) and *Potamanthus rufus* Argo (Potamanthide). Ando & Kawana (1956) in their study of the embryology of *Ephemera strigata* also provided a description of the first and second instar nymphs, and again the mandibular tusks were absent. Britt (1962) reported the absence of mandibular tusks in the first and second instars of *Ephoron album* (Say) but in the fifth instars, the mandibular tusks extended 0.067 mm beyond the rostrum. In the present study we observed that the mandibular tusks were already formed in the late embryo; they measured 0.18 mm and 0.28 mm in length in the first and second instars respectively. From an evolutionary standpoint, the early development of the mandibular tusks is regarded as embryonization. Observations made on the behavior of the first instars showed that the early appearance of the mandibular tusks are of great use to the nymphs in performing their cleaning, digging, and burrowing.

First and second instars are active swimmers, and we frequently observed them cleaning their caudal filaments. While lying on their backs they caught the basal portion of the caudal filaments between their mandibular tusks, then they straightened out pulling the filaments through the mouthparts. When nymphs were placed in a petri dish with fine mud from their natural habitat, the significance of this cleaning behavior became evident. Unless frequently cleaned, the caudal filaments entangled in a slime-like film on the mud substrate which impeded swimming activity.

When provided with a fine mud substrate, these early instars burrowed into it. The fore legs dug into the mud while the mandibular tusks pushed the mud away. Digging ceased when the entire body minus the caudal filaments was buried.

TABLE 1. PERFORMANCE OF FIRST INSTAR *T. incertus* IN THE CHOICE CHAMBER*.

Trial No.	Number of nymphs used	Number of nymphs recovered from mud	Number of nymphs recovered from sand	Nymphs on side of chamber
1	20	14	4	2
2	14	12	2	0
3	14	8	6	0
Total	48	34	12	2

*Substrate preference, based on Mann-Whitney U Test, was statistically significant, $p = 0.05$.

To determine substrate preference, we made a small choice chamber (4 cm diam) by spreading a thin layer of mud 1 cm thick in half of the petri dish and a layer of sand (particle size 380-490μ) in the other half. First instars were introduced into the middle of the chamber, left for 20-25 min, and recovered. In each trial, fresh nymphs were used. The results of this experiment is represented in Table 1. It can be seen that a greater percentage of nymphs were recovered from the mud substrate; therefore, the first instar nymphs appear to have a preference for the mud substrate.

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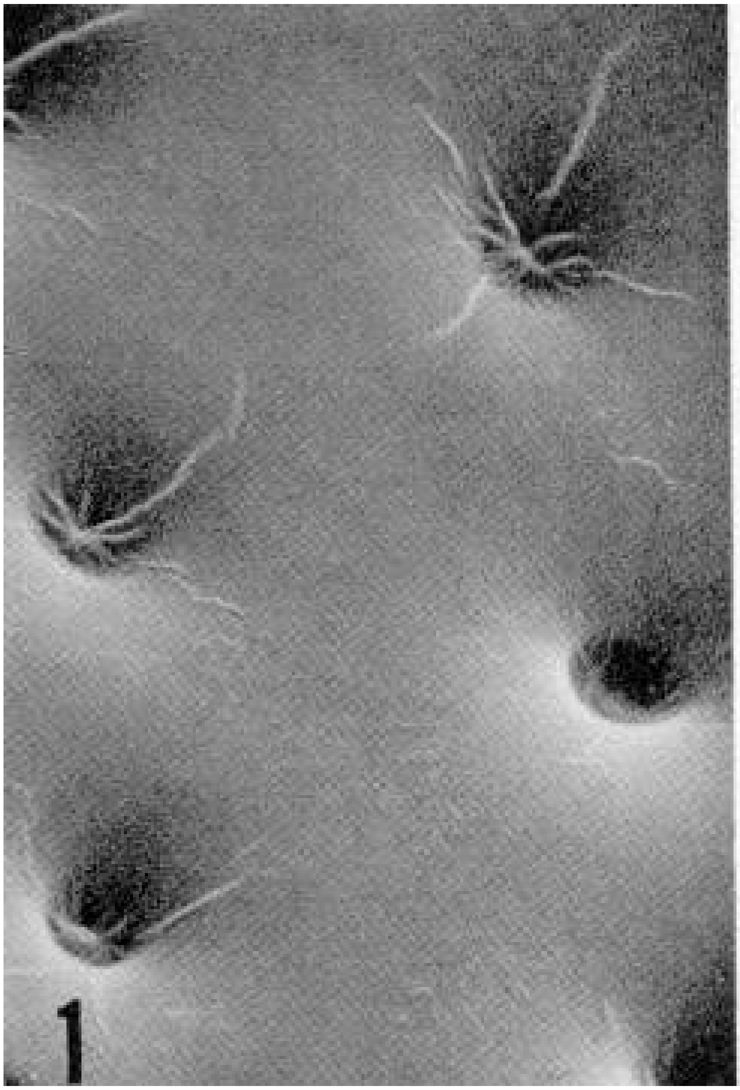

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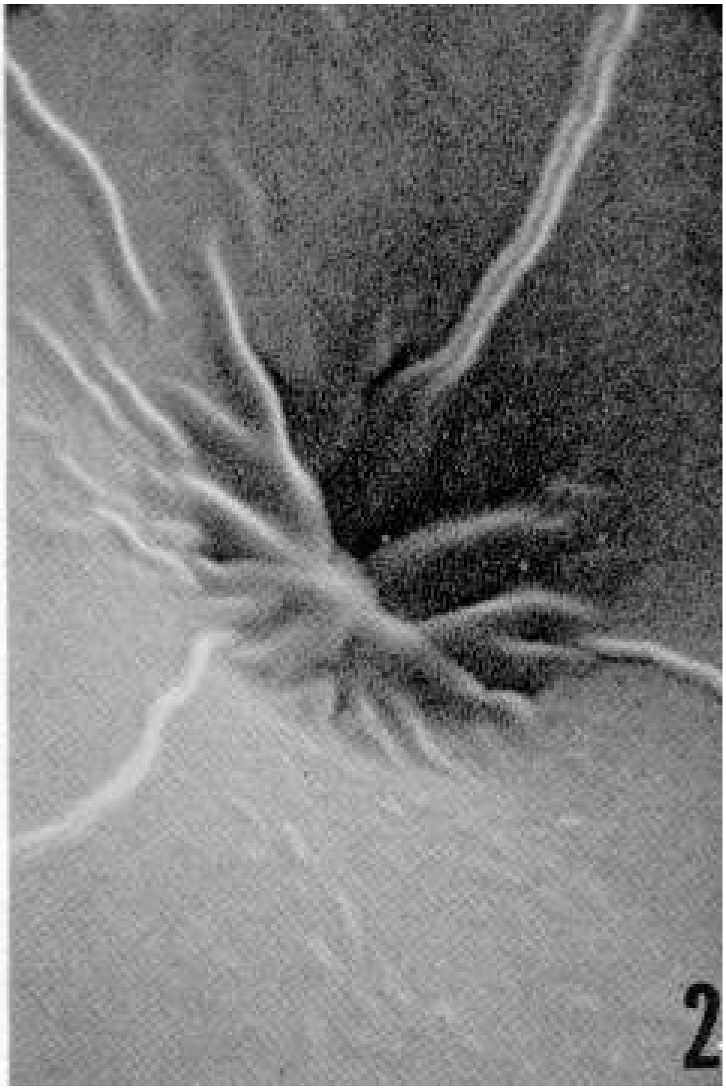

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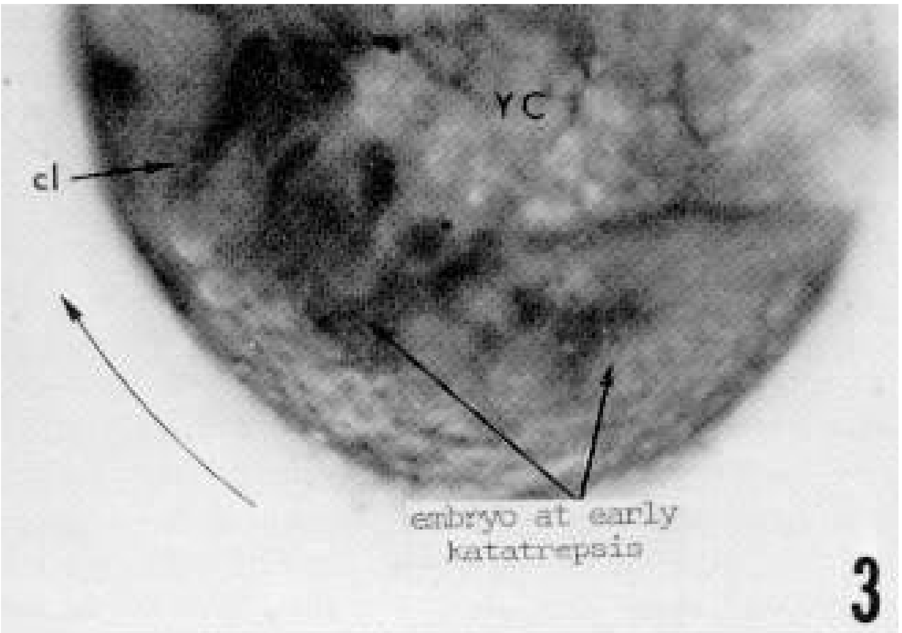

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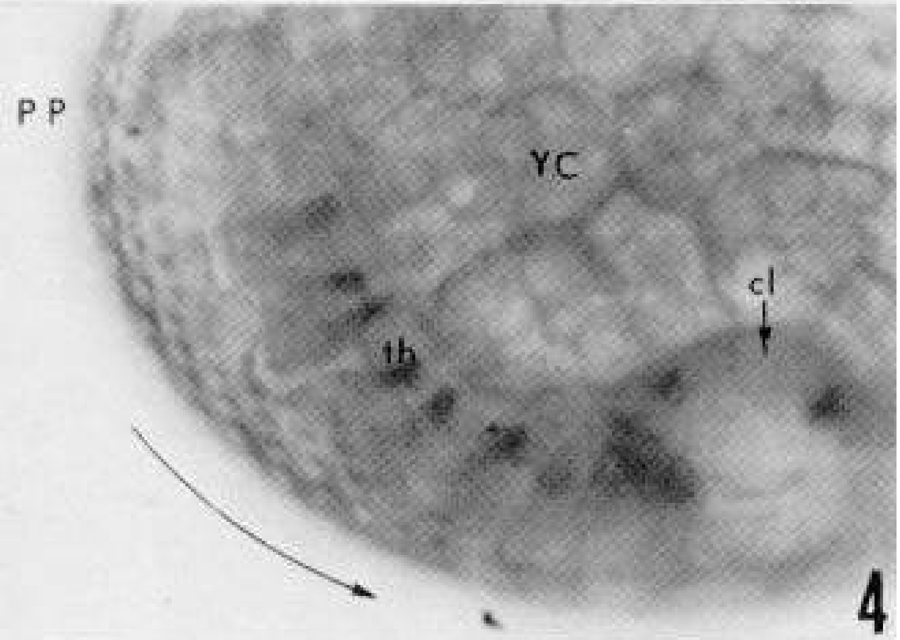

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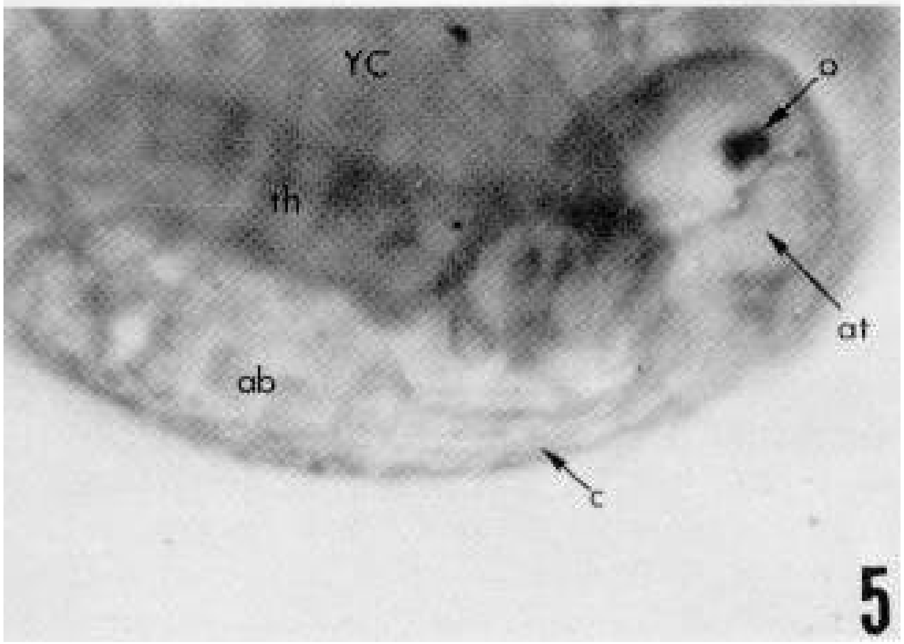

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