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THE PARASITIC COELENTERATE, *POLYPODIUM HYDRIFORME* USSOV, FROM THE EGGS OF THE AMERICAN ACIPENSERIFORM *POLYODON SPATHULA*

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ABSTRACT: No significant differences in macro- and micromorphology were found between the parasitic stolon and free-living polyps of *Polypodium* sp. obtained from infected eggs of the North American acipenseriform fish *Polyodon spathula* and corresponding developmental stages of *Polypodium hydriforme* Ussov, parasitic in the Volga sterlet (*Acipenser ruthenus*). Therefore, both the American and the European forms of *Polypodium* belong to the species *P. hydriforme* Ussov.

For a century, the coelenterate *Polypodium hydriforme* Ussov, parasitic in the eggs of acipenserid fishes, was considered a "purely Russian" animal. It was discovered in 1871 by Ovsjannikov in the eggs of the Volga sterlet, (*Acipenser ruthenus* L.), and for more than 70 years thereafter, it appeared to be confined to that host species and that river. Only after the beginning of extensive ichthyoparasitological research in the USSR was it found in other hosts and other watersheds (Dogiel, 1940). During the last 40 years, *P. hydriforme* has been found in practically all rivers of the European part of the USSR inhabited by Acipenseridae, and also in the tributaries of the Aral Sea and in the Amur River of the Far East. All acipenserid species occurring in the USSR except the Siberian sterlet (*A. ruthenus ruthenus natio marsiglii* Brandt), the Siberian sturgeon (*A. baeri* Brandt), and the Atlantic sturgeon (*A. sturio* L.), proved to be hosts of *P. hydriforme*. It has been found in *Acipenser ruthenus* L. (the European sterlet), *A. güldenstädti* Brandt (the Russian sturgeon), *A. stellatus* Pallas (the sevrjuga), *A. nudiventris* Lovetzky (the ship), *A. schrenki* Brandt (the Amur sturgeon), *Huso huso* (L.) (the beluga), and *H. dauricus* (Georgi) (the kaluga) (Dogiel, 1940; Markov and Trussov, 1966; Svirsky, 1967). Outside the USSR, *P. hydriforme* was

found in Romania (Bogatu, 1961) and in Iran (Nechat and Mokhayer, 1974).

A discovery in 1970 of *Polypodium* on the American continent in the eggs of the sturgeon *Acipenser fulvescens* inhabiting the Great Lakes (Hoffman et al., 1974), was therefore a distinct surprise. Thereafter (in 1973), *Polypodium* also was found in the eggs of several specimens of the American acipenseriform *Polyodon spathula*, a representative of the family Polyodontidae which belongs, with the family Acipenseridae, to the order Acipenseriformes (Suppes and Meyer, 1975). Thus, the range of the hosts of *Polypodium* has exceeded the Acipenseridae, and its area, the Eurasian continent. We describe here the American form of *Polypodium* from *Polyodon*, compare it with the typical *P. hydriforme*, and provide evidence that the American form is probably the same species.

MATERIALS AND METHODS

Our material consisted of both *Polypodium*-infected and healthy eggs of *Polyodon spathula*, obtained from 8 females caught in March, April, and May 1973 and October 1974 in the Osage River, Missouri. The infected eggs in 5 females caught in March and April belonged to the older generation of oocytes. These were nearly mature and corresponded to the Stage IV–V of Nedoshivin's (1928) scale. Two females, caught April 22 and May 11, were already in the post-spawning period and contained no *Polypodium*; one female, caught in October 1974 and infected with *Polypodium*, was at Stage IV of oocyte development (eggs due to be spawned next spring). The oocytes were fixed either with 10% formalin or with Gilson's fluid. Some of the oocytes were infected with the microsporidian *Pleistophora sulci* Rasin 1936, 1949 (Sprague and Vavra, 1977) (*Cocconema sulci*) in addition to *Polypodium*.

Free-living polyps, raised from stolons infecting the oocytes were cultivated in Petri dishes and fixed with 10% formalin at various ages (from their emergence out of the oocytes through a period of 40 days of free life).

For histological investigation, some formalin-fixed infected eggs and free-living polyps were re-fixed with Zenker's and Bouin's fluids and embedded in paraffin. Sections (7 μm thick) were stained with Heidenhain's "azan," iron hematoxylin, Mayer's hemalum, or by the Feulgen technique. Formalin-fixed material was also used to prepare whole mounts of both stolon and polyps, which were mounted in glycerin-gelatin.

RESULTS

Unambiguous determination of the specific identity of the American *Polypodium* proved difficult because only some stages of its development were present in our material. Moreover, no species criteria distinct from generic ones exist for *Polypodium*, the genus always having been monotypic. Therefore, we present here a detailed description of the available developmental stages of the American *Polypodium*, in order to compare them with the respective stages of *P. hydriforme* from the type host, the Volga sterlet (Raikova, 1958, 1960, 1961, 1973).

The parasitic stages of *Polypodium* found in our material were represented by two-layered stolons inside oocytes of both Stages IV (October) and IV–V (March–April). In Stage IV–V oocytes, the stolon occupies the entire periperal part of an oocyte, the yolk and the nucleus being shifted to its center (Fig. 1). The two germ layers of the stolon are inverted. The entoderm is the outermost layer, facing the surrounding yolk, and the ectoderm lines the inner cavity of the stolon, which contains the tentacles. An additional dense layer covers the parasite, but not continuously (Fig. 2); it contains elongated nuclei and inclusions of ingested yolk particles. This incomplete layer certainly corresponds to remnants of a syncytic envelope, the capsule, peculiar to *Polypodium*, which isolates the developing stolon from the yolk, assumes the function of yolk digestion, and degenerates when the stolon is mature (Raikova, 1960). The nucleus of the infected oocyte remains visible; it is strongly lobulated (Figs. 1, 3), and has a dense, almost fibrous karyoplasm and several vacuolized, frequently fused nucleoli. Usually no chromosomes are visible in the nucleus. The envelopes of the infected egg—especial-

ly the zona radiata and the follicular enve-lope—grow thinner (Figs. 1, 2).

The entodermal cells (i.e., cells of the outer layer of the stolon) are flagellated, but the flagella appear poorly preserved after formalin fixation. At places lacking the enveloping capsule, the entodermal cells contain yolk particles, which they apparently ingested (Fig. 4). Groups of protein-secreting glandular cells also occur in the entoderm. They are usually in folds of the stolon (Fig. 5) and therefore contact neither the capsule nor the yolk.

The ectodermal (inner layer) cells (Fig. 6) have small nuclei located in their basal ends, large vacuoles occupying the cells' middle parts, and an apical cytoplasm of reticular appearance. In Volga *Polypodium*, the meshes of this reticulum contained granules of acid mucopolysaccharide, which formed together an apical layer of the ectoderm (Raikova, 1960). It seems that these granules were not preserved in the American material with the fixatives used (Fig. 6). The ectodermal epithelial cells are underlain by muscle cells, which are independent and not muscle parts of epithelio-muscular cells (Figs. 6, 7).

Sections through infected Stage IV–V eggs clearly show tentacles inside the stolon (Figs. 1, 3). These are everted—i.e., their ectoderm is inside and their entoderm is outside. The tentacular ectoderm contains numerous cnidocysts of various size classes. Both walking tentacles and sensory tentacles can be seen—the walking tentacles containing only large (10 μm in diameter) and sometimes small (5 μm) cnidocysts, and the sensory tentacles containing medium-sized (7 μm) and small cnidocysts (Figs. 6–9). The ectoderm is underlain with muscle cells, whereas the entoderm forms a dense axial rod in each tentacle (Figs. 1, 3, 7–9). In some sections this rod seems to have an inner lumen (Figs. 7, 8), but in most sections it is solid.

The earliest of the parasitic stages encountered in the present study came from the eggs of a Stage IV female caught in October. These were stolons with inverted germ layers, as just described, but with continuous capsules around them and with a smaller number of tentacles inside them. Also, mature cnidocysts were far fewer in the ectoderm of the tentacles. The latest stages, found in late April, inhabited eggs ready to be spawned; their capsule had, on the contrary, almost disappeared,
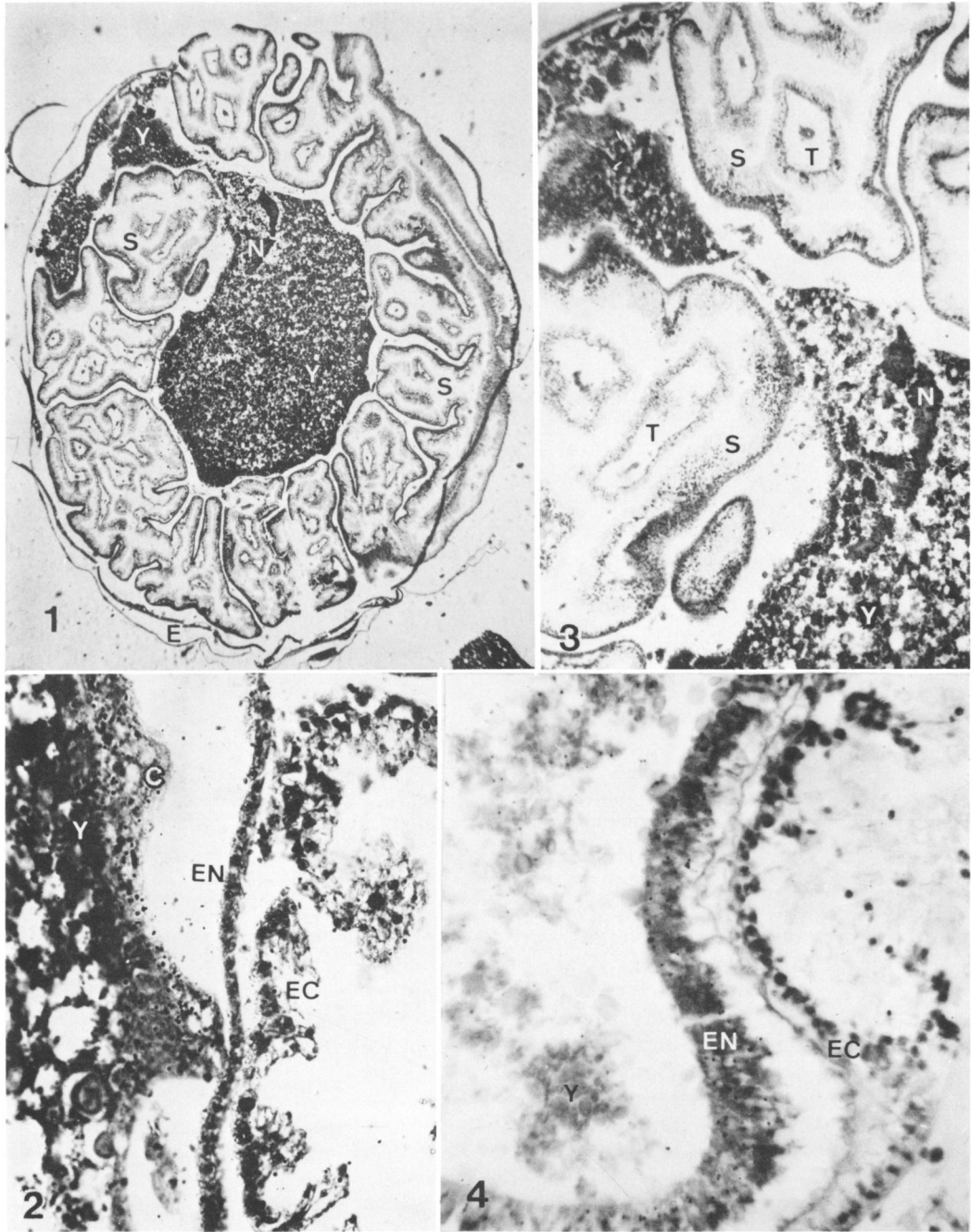
FIGURES 1–4.   Sections of infected eggs of *Polyodon spathula* containing stolons of *Polypodium*. **1.** Orientation photomicrograph showing egg envelopes (E), yolk (Y), oocyte nucleus (N), and the stolon (S). ×30. **2.** Wall of the stolon with ectoderm (EC) inside and entoderm (EN) outside, showing remnants of the capsule (C) separating the stolon from the yolk (Y). ×280. **3.** Top left part of Figure 1, showing oocyte nucleus (N), yolk (Y), and parts of the stolon (S) with internal tentacles (T). ×83. **4.** Stolon wall lacking a capsule, with entodermal (EN) cells directly ingesting yolk (Y); EC, ectoderm. ×330. Staining: 1 and 3, azan; 2 and 4, hemalum.
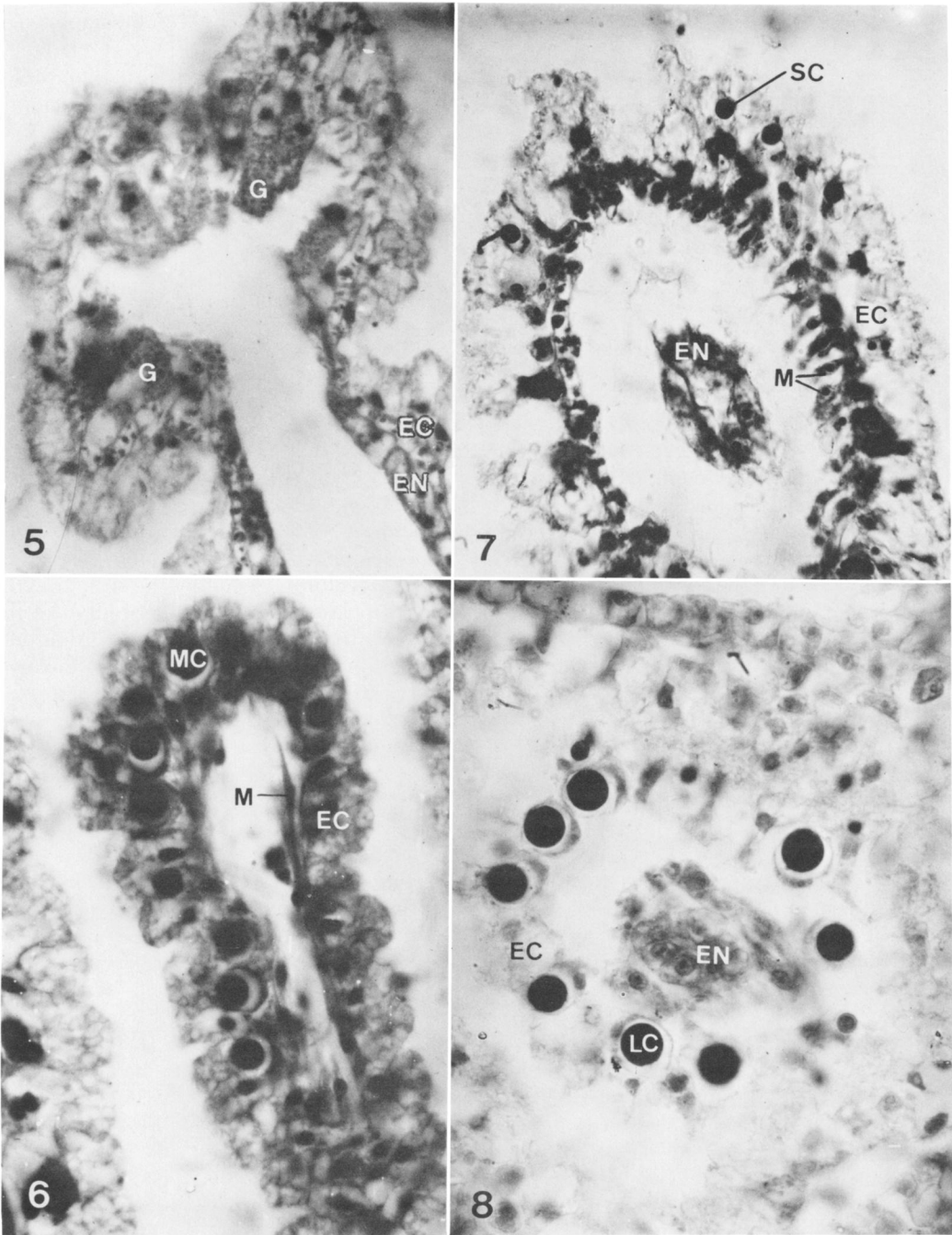
FIGURES 5–8. Details of the structure of the parasite's stolon. **5.** A fold of the stolon wall showing glandular cells (G) in its entoderm (EN); EC, ectoderm. ×1,800. **6.** Oblique section of a sensory tentacle containing medium-sized cnidocysts (MC) in its ectoderm (EC), and muscle cells (M) beneath it. ×700. **7.** Cross section of a sensory tentacle near its base, showing small cnidocysts (SC) in its ectoderm (EC), muscle cells (M), and entodermal axis (EN). ×630. **8.** Cross section of a walking tentacle near its base, showing entoderm (EN) and large cnidocysts (LC) in the ectoderm (EC). ×630. Staining: 5 and 7, iron hematoxylin; 6 and 8, hemalum.

and the entodermal cells of the stolon were full of yolk (Fig. 10).

The following forms have been encountered among the free-living polyps (grown in Petri dishes) after they had hatched from infected eggs.

The stolon becomes evaginated with its ectoderm outside while still in the egg. Very soon after its emergence into water, the stolon fragments into a number of 24-tentacled polyps, each about 1 mm long (tentacles excluded), and lacking mouth. The histological organization of such polyps is simple. Their entoderm is not yet differentiated into regions and consists of cells replete with yolk, which makes the nuclei inconspicuous (Fig. 11). The ectoderm of the tentacle tips contains cnidocytes with mature cnidocysts, whereas that of the tentacle bases contain cnidoblasts with immature cnidocysts.

During the first and second days of the free life, 12-tentacled polyps with primordia of 12 new tentacles also have been observed, which indicated longitudinal division of the polyps coupled with neoformation of tentacles, exactly as it is known to occur in the Volga material (Raikova, 1961). Also some six-tentacled specimens were seen. The histology of all these forms is exactly the same as that of newly formed 24-tentacled polyps.

Within 48 hours after hatching, some specimens with formed mouths appear. Simultaneously, their entoderm begins to differentiate into a buccal and a gastral region. The cells of the buccal region usually contain no yolk particles, whereas the gastral region cells still have yolk inclusions in their cytoplasm. Therefore, the cells of the buccal region appear narrower and higher than those in the gastral region (Fig. 12). Eight days after hatching, the gastral region entodermal cells also lack yolk inclusions; all the yolk captured in the gastral cavity during evagination of the stolon thus seems to be digested by this time (Fig. 13).

On the 15th day of free life, all the polyps investigated had mouths, but their entoderm contained only some pigment granules coming from the egg. No yolk was present. Most polyps had 12 tentacles and some tentacular primordia. On the 17th day, the polyps were still in good condition, but because they were unfed (Suppes and Meyer, 1975), they soon began to degenerate. On the 21st day, only

degenerating specimens with incomplete sets of tentacles (3, 4, 7, 10, etc.) were present in the fixed samples; some lacked a mouth. But the polyps continued to live, and some normal 12-tentacled animals with mouths, and even dividing ones, were observed in the fixed samples as late as the 24th and the 30th days. The last sample (40 days after hatching) contained degenerating specimens only.

DISCUSSION

The gross morphological and histological organization of the available parasitic stages of the American *Polypodium*, as well as the structure, mode of division, histology, and behavior of its free-living polyps—at least at early stages of their life—exactly correspond to the respective stages of the life cycle of the Volga *Polypodium* from the sterlet (*Acipenser ruthenus*), which is the type form of the species *P. hydriforme*. All anatomical and histological features, including the structure, the size, the classes, and the distribution of the cnidocysts, are the same in the American *Polypodium* and in *P. hydriforme* (Raikova, 1960, 1961); in the type form of *P. hydriforme*, the larger cnidocysts are 10 μm, the medium, 7 μm, and the small ones, 4–5 μm. Also similar in both forms is the correspondence of the stages of parasite development to the stages of the host's oocyte development and maturation. In both, the polypodia have the same adaptations to intracellular parasitism, in the form of inversion of the germ layers of the stolon and formation of a capsule around the parasite. Even the reaction of the host oocyte to the presence of a parasite is similar and involves fusion of the nucleoli into one or two large vacuolized bodies and partial reduction of the oocyte's envelopes (cf. Raikova, 1963). Both the American and the Russian free-living polyps lack sensory organs. Finally, some of the eggs of *Polyodon spathula* were infected with the microsporidian, *Pleistophora sulci*, the same as in the European acipenserids.

Thus, a comparison of the available developmental stages of the American *Polypodium* from *Polyodon spathula* with those of the Volga *Polypodium* from *Acipenser ruthenus* indicates with a high degree of probability, that both forms can be attributed to a single species, *Polypodium hydriforme* Ussov. However, a final verification of this determination will be possible only when other stages
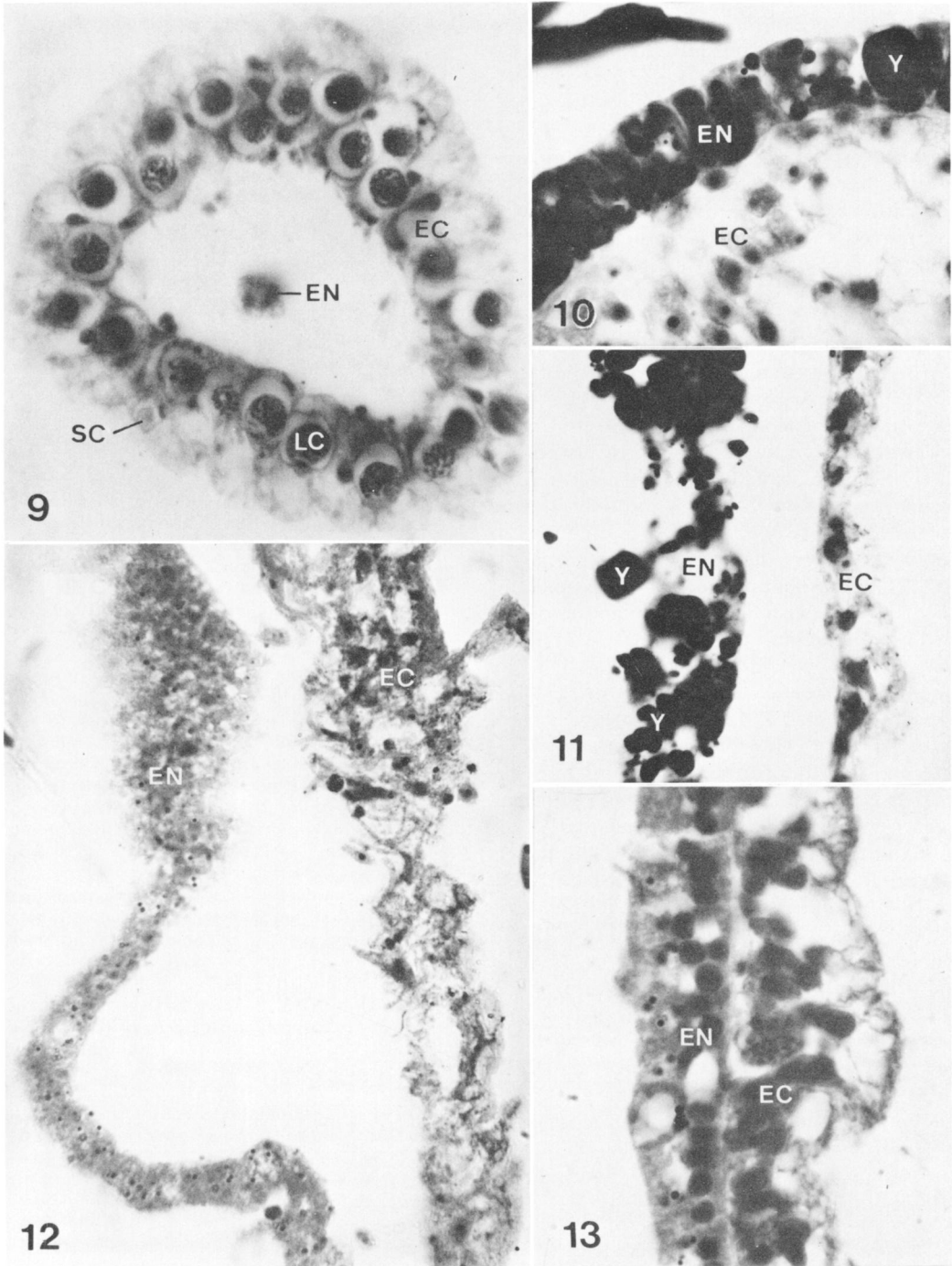
FIGURES 9–13.   Structural details of the stolon and of free-living polyps. **9.** Cross section of a walking tentacle near its end, showing large (LC) and small (SC) cnidocysts in its ectoderm (EC), and a solid entodermal axis (EN). ×760. **10.** Stolon's wall showing entoderm (EN) full of ingested yolk (Y); EC, ectoderm. ×760. **11.** Body wall of a 48-hour-old polyp in its gastral region, the entoderm (EN) contains much yolk (Y); EC, ectoderm. ×540. **12.** Same, but in the mouth region; the entoderm (EN) is free of yolk; EC, ectoderm. ×540. **13.** Body wall of an 8-day-old polyp; EN, entoderm, EC, ectoderm. ×800. Staining: 9, hemalum; 10, azan; 11–13, iron hematoxylin.

of the life cycle of the American *Polypodium* are investigated—especially the sexually mature polyps.

If we accept, provisionally, that the American *Polypodium* is the same species, *P. hydriforme*, as the Eurasian one, a question arises whether it existed there ever since the separation of the respective continents, or was introduced into America artificially. Potentially, *Polypodium* may be inadvertently introduced into a new water basin when either adult fish or eggs of Acipenseridae are transported into it (Dogiel, 1940; Raikova, 1958). We are not aware of any records of the transportation of living sturgeons or their eggs from Eurasian countries to the American continent, and would be grateful to hear from any person having such information. In the meantime, it seems reasonable to accept the first alternative, i.e., that *P. hydriforme* is indigenous in American waters, as it is in Eurasian ones. If so, *P. hydriforme* appears to be very ancient. It must have existed before separation of the two continents and changed little ever since in the two now widely separated parts of its range. This seems the more surprising because no species of Acipenseriformes is common to both America and Eurasia. The species stability of the parasite thus must be greater than that of its hosts. The eggs of *Polyodon* were lightly infected, ca. 0.01% although there was a high incidence of infected fish (up to 88%, according to Suppes and Meyer, 1975). This incidence was higher than that for the Russian sterlet (78%; Raikova, 1958). Consequently, it is possible that in America the principal host of *Polypodium hydriforme* is *Polyodon spathula* and not *Acipenser fulvescens*, of which only one infected specimen had thus far been found (Hoffman et al., 1974). In any case, a further investigation of the morphology and the systematic position of polypodia from various species and genera of Acipenseridae and Polyodontidae is likely to be productive and interesting.

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