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Glugea heraldi n. sp. (Microsporida, Glugeidae) from the seahorse *Hippocampus erectus* Perry

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Abstract. A new species of Microsporida, *Glugea heraldi*, is described from the seahorse *Hippocampus erectus*. Measurements of fixed spores and notes on the histopathology of parasitized tissue are given.

Introduction

Species of the genus *Glugea* Thélohan, 1892, are widespread intracellular parasites of freshwater and marine teleosts (Kudo 1924; Lom & Laird 1976). To date, 26 species (Sprague 1977) have been recorded from teleosts.

The seahorse *Hippocampus erectus* Perry from the Atlantic, has been known to be parasitized by a microsporidium for over two decades. Tissue response of the host to the parasite results in large 'parasite-host cell complexes' (xenomas) in the skin. Throughout the literature, the microsporidium has been referred to occasionally as a parasite of *H. erectus*, but apparently has never been named. While vague references have been made to the protozoan simply as 'an undetermined type of *Glugea*' (Reichenbach-Klinke & Elkan 1965), Herald & Rakowicz (1951) commented on the microsporidium as an undetermined species of *Glugea*. They also quoted Johnstone, a San Francisco pathologist who observed the parasite in specimens of *H. erectus*. The present author believes that the Herald & Rakowicz paper was the first documentation and description of clinical signs associated with the disease. Bellomy (1969) referred to it as *Glugea* and further remarked that she believed it to be *Glugea acuta* Thélohan from the European pipefish *Syngnathus acus* L. Thélohan (1895) originally reported the parasite from two European pipefishes, *S. acus* and *Nerophis* (*Entelerus*) *acquoreus* (L.). Lom (personal communication) noted that *G. acuta* has not been reported since the original description. Lom (1972) examined spores of the parasite from *H. erectus* and referred to them as *Nosema* sp.

Materials and methods

Histological sections and gross specimens of parasitized *H. erectus* were recently

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discovered in the Aquarium's parasitology collection and used in this study. The slides are the original ones prepared for study by Johnstone for Herald & Rakowicz.

All infected fish were fixed by Johnstone in 10% formalin. From this material additional histological sections were prepared by the author after decalcification and stained with Harris haematoxylin and eosin, and Giemsa. Cysts were also lanced and smears prepared for phase microscopy. Additional smears were dried and stained prior to examination with fast green and Giemsa.

Additional histopathological slides were obtained for study from the Smithsonian's Registry of Tumors in Lower Animals (RTLA 42). Spore measurements were made with a calibrated ocular micrometer, and were based on ten or more spores selected at random.

Glugea heraldi n. sp. and Nosema sp. Lom, 1972

Host. Hippocampus erectus Perry, 1810.
Locality. Florida, U.S.A.
Infection site. Subcutaneous connective tissue.
Type slides. CAS Invertebrate Zoology, 00715; USNM Collection, 24548.
Diagnosis. Disporous; mature spores oval; vacuole in posterior portion of spore; polaroplast anterior, under light microscopy appears as two paired clear areas; parasite produces xenoma complexes in connective tissue; parasite of *H. erectus*.

Description

Spores (Figs 1 and 2)

Oval, refractive in unstained specimens; fairly consistent in shape; conspicuous oval vacuole in the broader posterior end of the spore; the vacuole almost fills one-half of the total spore volume. Two smaller paired circular clear areas (polaroplast) present at anterior end of spore. Greyish, crescent-shaped sporoplasm between polaroplast and posterior vacuole. Preserved spore size averages 4·0 μm (3·6–4·5) in length and 2·0 μm (1·8–2·3) in width.

Vegetative stages

Developmental stages were observed to some degree in the cysts, although most cysts were highly advanced with mature spores predominating. The following account should thus be held tentative. Sections stained with H & E show distinct masses of mature spores (Fig. 3), which stained light to dark purple and a peripheral area which was eosinophilic. Some vegetative stages were observed peripherally in the cysts, though a continuous series was not seen (Fig. 4). Some early sporonts were noted, approximately 5–6 μm in length, and binucleate. The sporoblasts seen were 7–8 μm in length and 3–3·5 μm in width (Fig. 5).

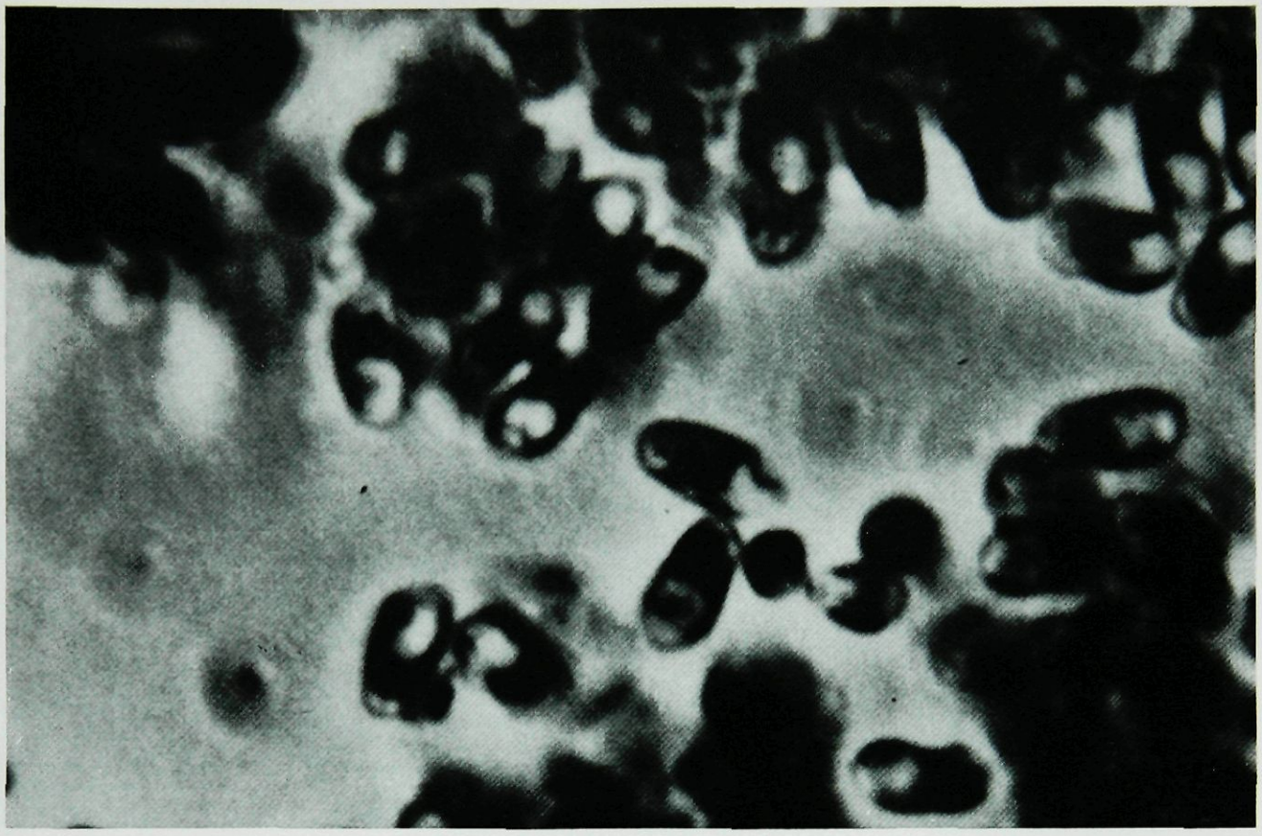


Figure 1. Phase photomicrograph of mature spores of *Glugea heraldi* (×1200).

Cysts

Characteristic, generally non-pigmented glugeal cysts formed within host connective tissue. Grossly, visible as spherical white to cream-coloured cysts ranging from 100 to 800 μm in diameter (Fig. 6). They usually occur singly; they may be grouped, but some may be coalesced. Distributed grossly on all body regions with the exception of the fins.

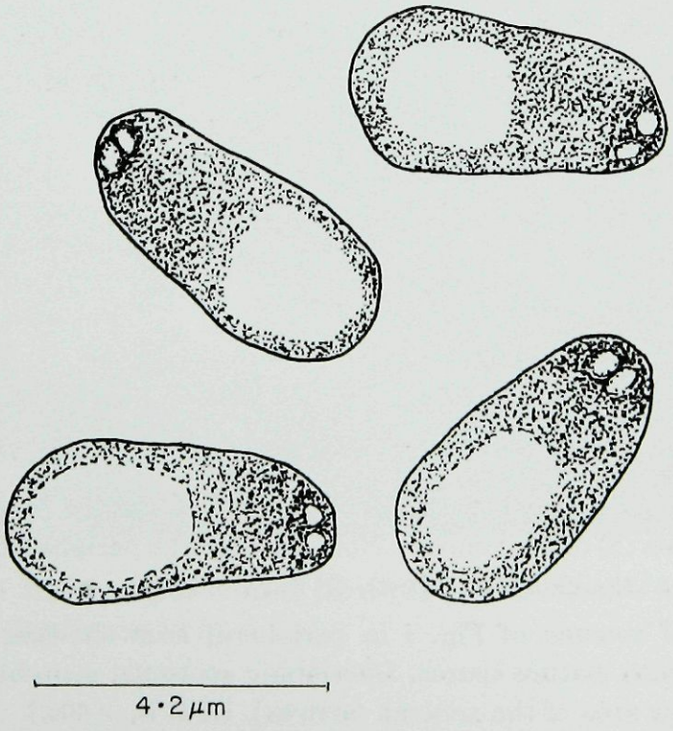
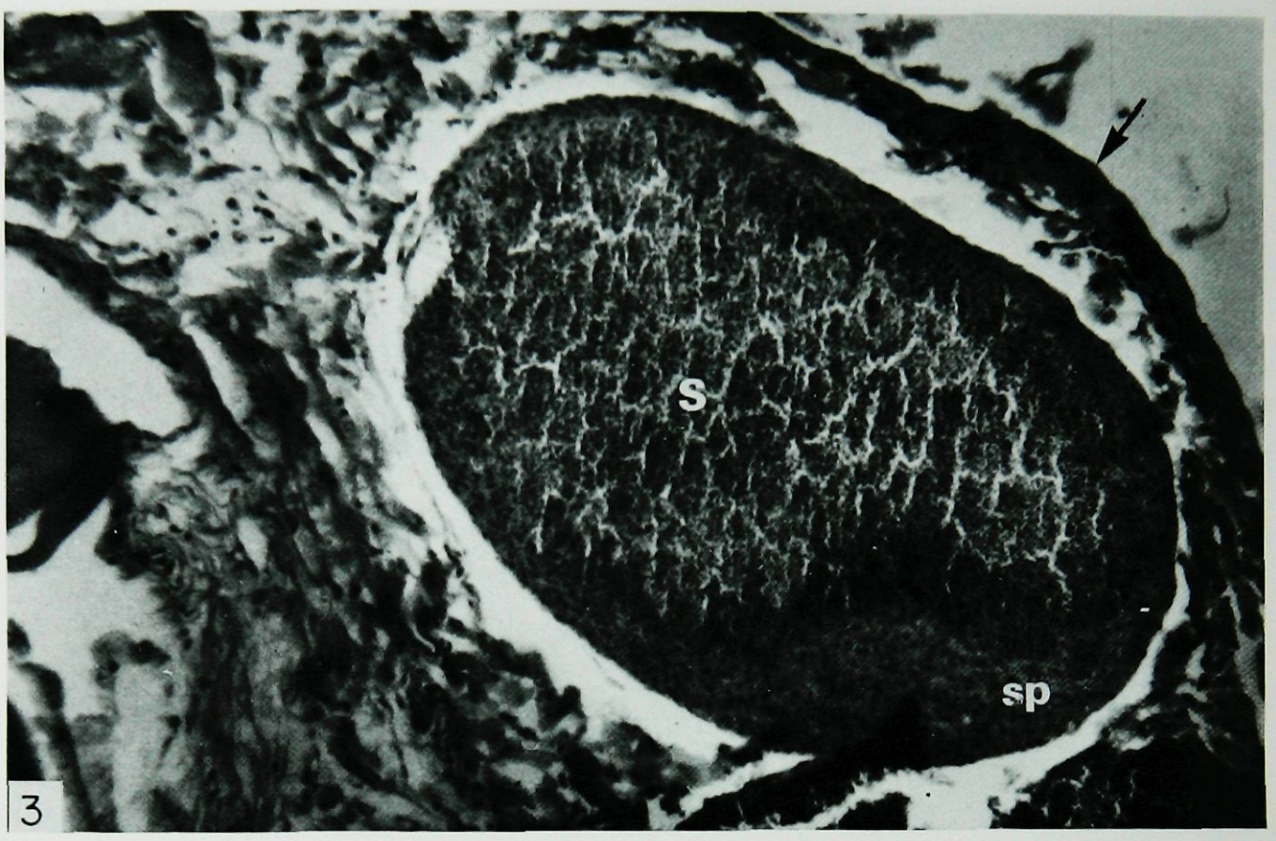


Figure 2. Freehand sketch of mature spores as seen under light microscopy.



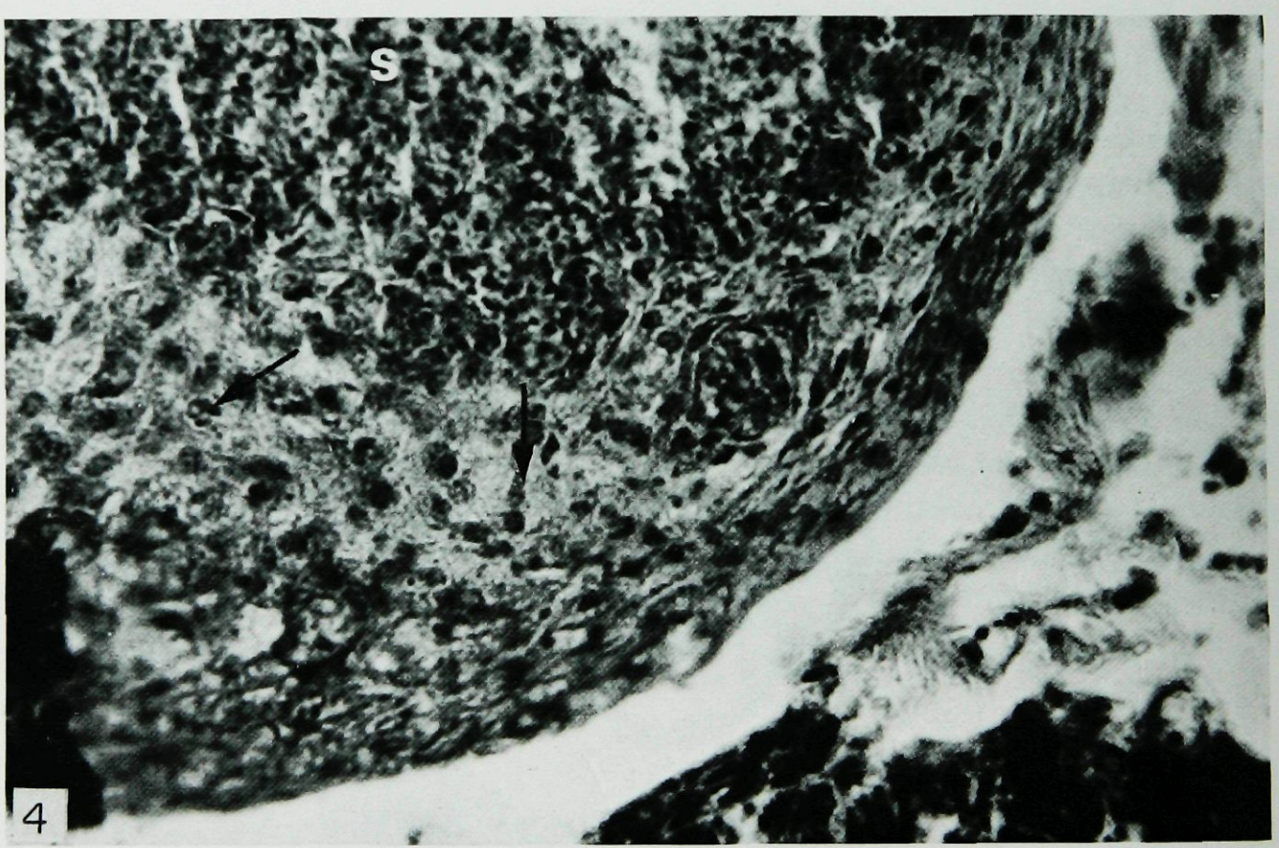


Figure 3. Subepithelial xenoma complex within connective tissue of *H. erectus*. Mature spores are concentrated in central area (S) of the xenoma. Note eosinophilic peripheral area of sporogony (sp) and hypertrophied epithelium of the capsule (arrow). (H & E, ×100.)

Figure 4. Enlargement of xenoma of Fig. 4 in peripheral area showing early spore development. Central area (S) is filled with mature spores. Developing sporonts, sporoblasts, and other stages can occasionally be seen in this area of the xenoma (arrows). (H & E, ×400.)

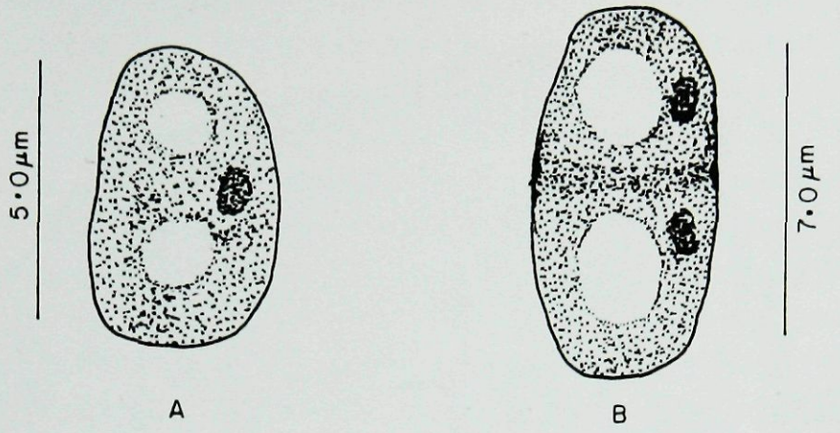


Figure 5. Freehand sketch of: (A) early sporont and (B) sporoblast of *Glugea heraldi*.

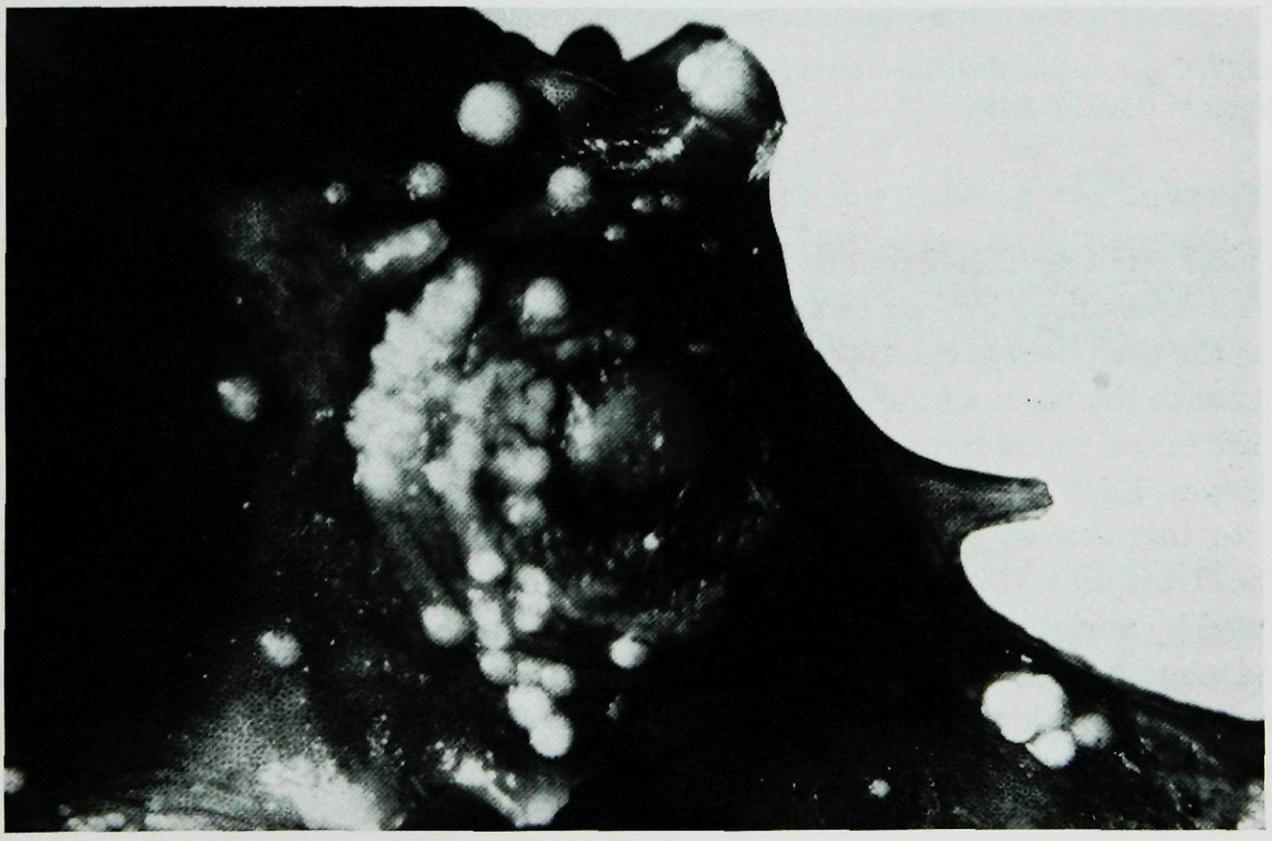


Figure 6. Head region of *Hippocampus erectus* parasitized with *Glugea heraldi*. Note the numerous coalesced xenomas on the host's eye (×8).

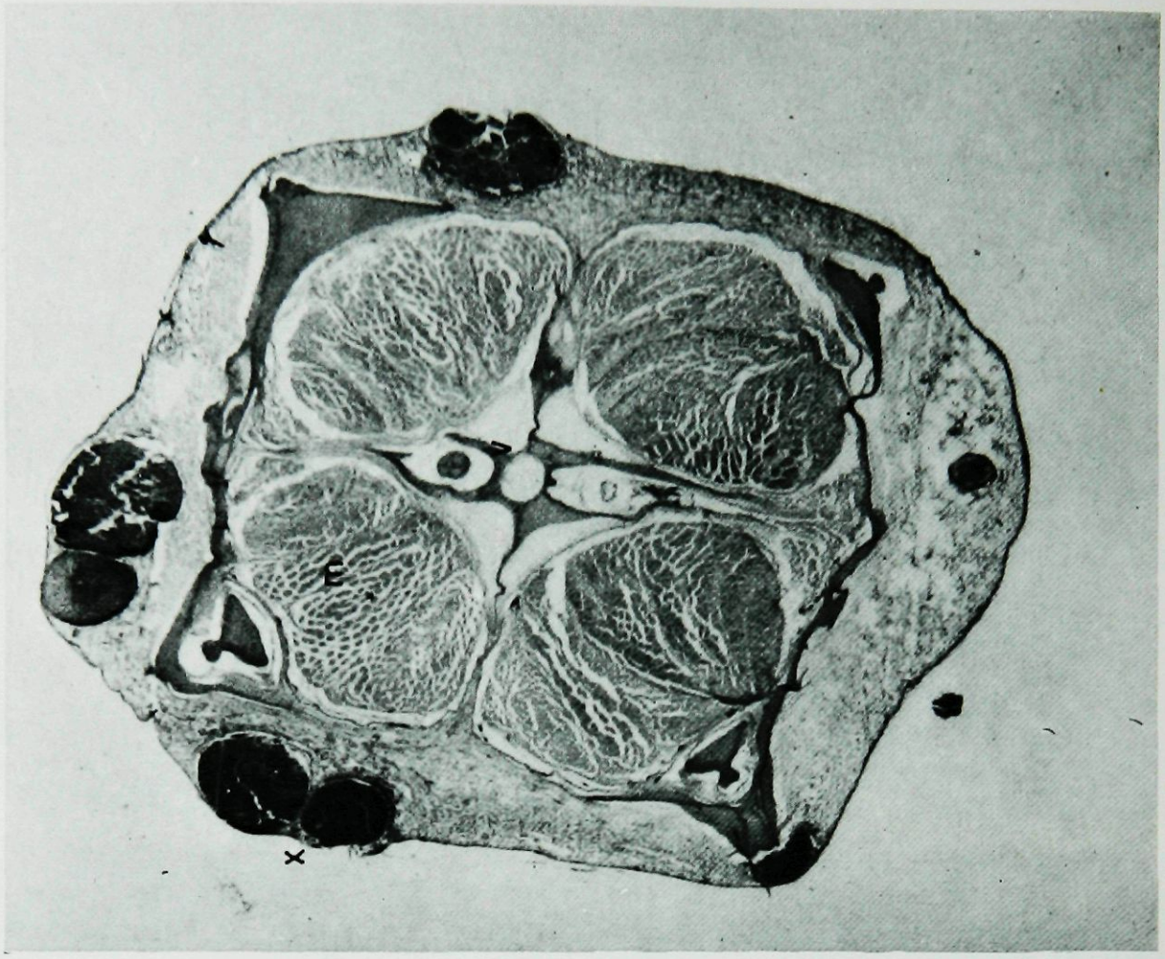


Figure 7. Cross section through infected tissue from tail of *H. erectus*. V, vertebrae; M, skeletal muscle; X, xenomas (H & E, ×10).

Pathology

A typical cross-section through infected tissue (Fig. 7) of the tail shows the vertebral column surrounded by four large symmetrically oriented bundles of skeletal muscle. Along the periphery of each section, located immediately beneath the pigmented layer adjacent to the basal epithelium, are randomly scattered single and multiple foci. The foci are large, round to oval, expansive and basophilic, ranging in diameter from 250 to 750 μm. Each focus (cyst) is composed of large numbers of tightly packed small oval to tear-shaped spores with dark stained sporoplasm. Each cyst (xenoma) is enclosed within a thin, lightly eosinophilic fibrous capsule. The capsules are surrounded by variable numbers of inflammatory cells, the majority of which are large round mononuclear macrophages with dark eccentrically placed nuclei and a variable vacuolated pinkish cytoplasm containing areas of yellowish-brown pigment. Heterophils are randomly scattered among these cells.

Discussion

G. heraldi is placed in the genus *Glugea* on the basis of unpaired nuclei, and the production of characteristic cell hypertrophy tumours, or xenomas (Sprague 1969; Sprague & Vernick 1971).

Considering marine and brackish species which invade connective tissue and muscle, mature spores of *Glugea heraldi* are smaller than *G. punctifera*, *G. acuta*, *G. depressa* and *G. intestinalis*. They are larger than *G. destruens* and *G. stephani* but are comparable in size to *G. cordis* and *G. hertwigii* which mainly infect subcutaneous connective tissue; however, *G. cordis* is a European species. *G. hertwigii* and *G. anomala* resemble *G. heraldi*, with the spores comparable in size but with differences in morphology. The spores of *G. hertwigii* and *G. anomala* both tend to be more elliptical, while *G. heraldi* tends to be consistently broader at the posterior end of the spore. *G. heraldi* has readily distinguishable paired clear areas (polaroplast) at the anterior end of the spore, while the paired appearance is absent in both *G. hertwigii* and *G. anomala*. In addition, *G. anomala* has a large posterior vacuole and a smaller anterior one.

The species is distinguished on the basis of spore size, shape, infection site, and geographical location of the host, *H. erectus*. The parasite may be specific to the host species.

Etymology

The species is named in honour of the late Dr Earl S. Herald, past director of the Steinhart Aquarium, San Francisco, CA.

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