

NOTE

Occurrence of a microsporean with characteristics of *Glugea anomala* in ornamental fish of the family Cyprinodontidae

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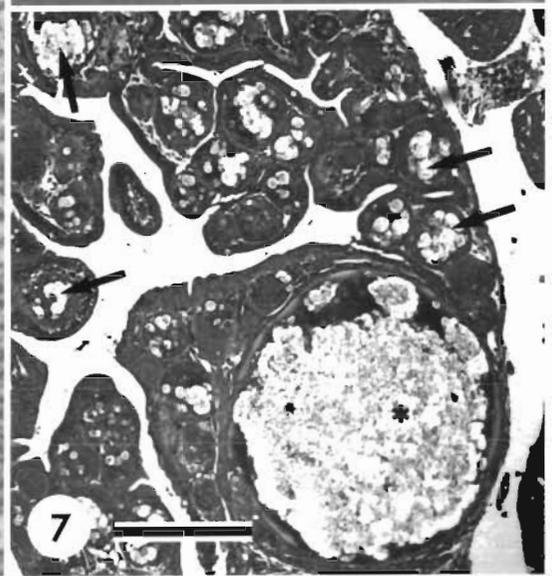
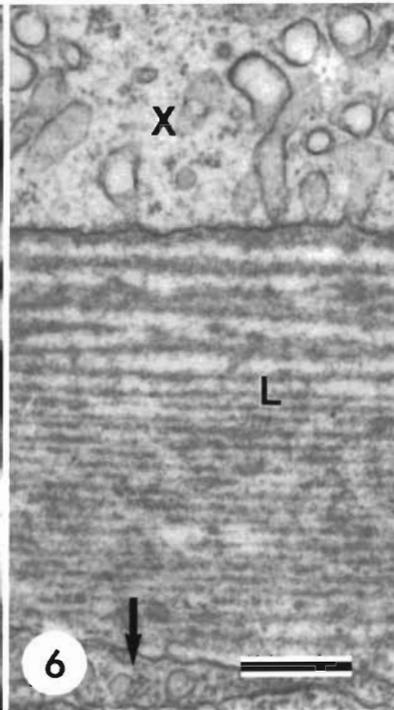
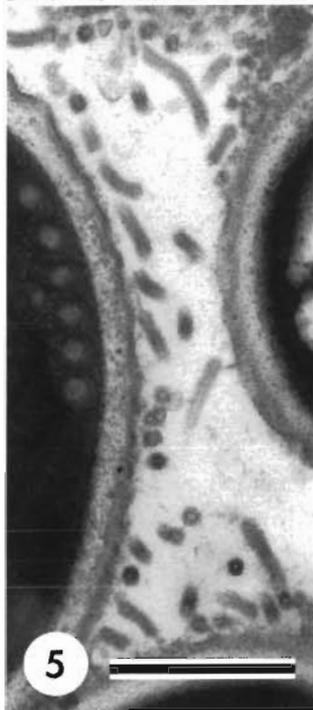
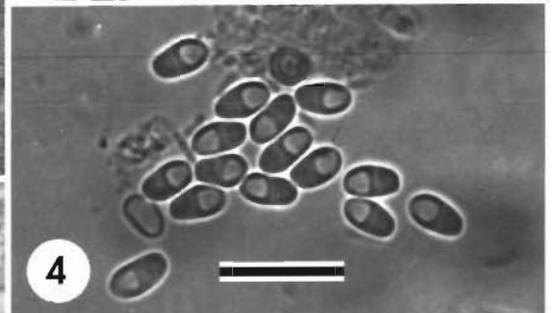
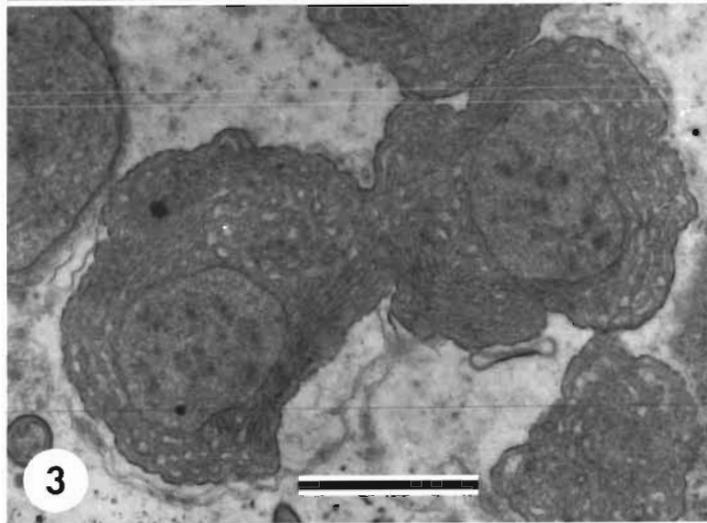
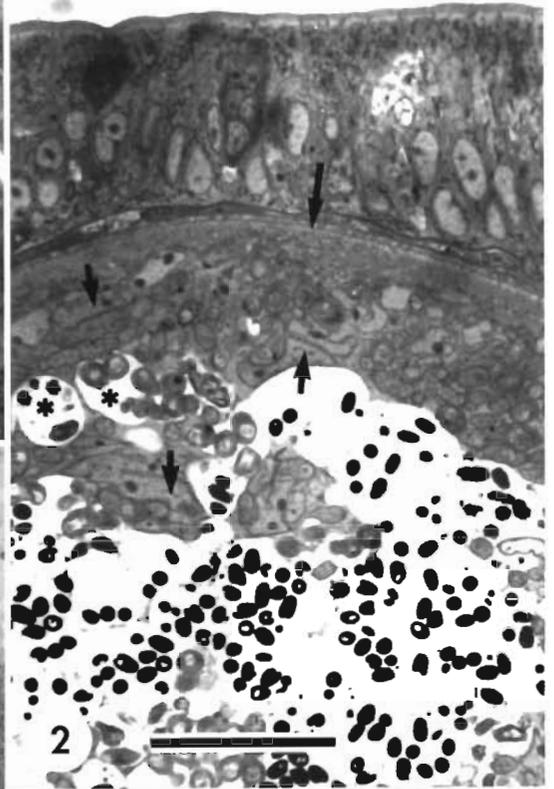
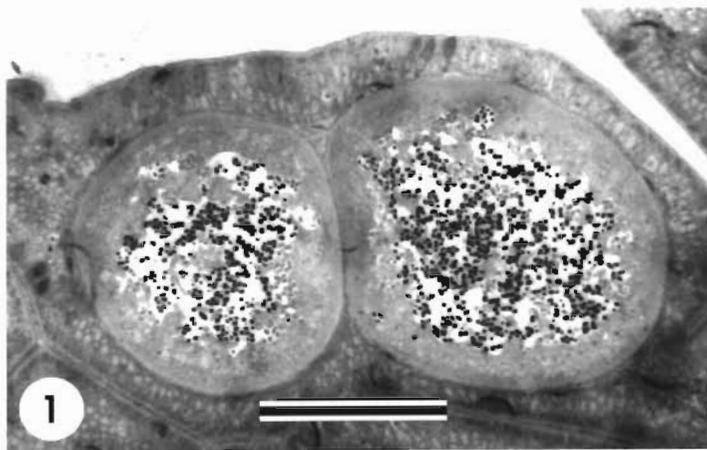
ABSTRACT: Ornamental fishes of the species *Nothobranchius eggersi* and *N. korthausae* (Cyprinodontidae) were infected by a microsporean of the genus *Glugea*. In various body organs, primarily in the intestinal wall, it elicited the formation of xenomas with a structure typical of *Glugea anomala* (Moniez, 1887). In fact, the shape and size of spores coincided with *G. anomala* and there was no significant difference in ultrastructural features of either the xenoma, its developmental stages, or mature spores. However, because the cyprinodontid hosts are from a different family and there was no known contact with the original hosts of *G. anomala*, the identification of the present parasite as this species cannot be confirmed and requires further study. Microsporean infection was also found in *Fundulopanchax filamentosus* and *Cynolebias nigripinnis*, and there are indications that it might be identical with that of *Nothobranchius*.

KEY WORDS: *Glugea* · Microsporea · Ultrastructure · Ornamental fishes · *Nothobranchius* · *Cynolebias* · *Fundulopanchax*

Four species of killifishes (Cyprinodontidae: Cypriniformes) i.e. *Nothobranchius korthausae* Meinken, 1973 and *N. eggersi* Seegers, 1982 (region of origin East Africa), *Fundulopanchax (Aphyosemion) filamentosus* Meinken, 1933 (region of origin southwest Africa) and *Cynolebias nigripinnis* Regan, 1912 (region of origin La Plata and Paraná in South America), kept as ornamental fish in Michigan and New York, USA, were found to harbour microsporidian infections manifested by formation of rather voluminous xenomas. The xenoma structure assigns them to the genus *Glugea* Thélohan, 1891. Thus far, 13 species of *Glugea* having a similar type of large xenoma with peripheral fragments of host cell nucleus have been recorded from freshwater and marine fishes. Several other authors (see e.g. Canning & Lom 1986) have reported what appear to be similar parasites, but descriptions are inadequate for identification at the species level. In this note, we present briefly our observations on the killifish *Glugea*.

Materials and methods. The precise origin of the infected fishes is not certain. Hobbyists in the USA reported receiving infected fishes from Germany and Canada. These killifishes are commonly shipped throughout the world making it difficult to determine the precise source of infection. Thus the fish which developed the infection had been propagated in captivity. Fresh spores were observed and photographed. Tissue with xenomas was fixed in 10% neutral buffered formalin and histological sections were stained with hematoxylin and eosin or Brown and Brenn Gram's stain. For transmission electron microscopy, isolated xenomas were fixed in McDowell and Trump's fixative (McDowell & Trump 1976), postfixed in 1% OsO₄ in 0.10 M phosphate buffer (pH 7.3), dehydrated through a series of graded ethanols, transferred to 100% acetone, and embedded in Spurr's resin. Thin sections were cut at 80 nm on a Reichert Ultracut-E microtome, stained with methanolic uranyl acetate and Reynold's lead citrate, and then viewed on a Phillips 410 LS transmission electron microscope.

Results. Microsporea from *Nothobranchius* spp.: When the disease appeared in tanks of *Nothobranchius* spp., it almost invariably affected virtually all individuals, resulting in 100% mortality (R. Goldstein & K. Doering pers. comm.). The xenomas mostly affected intestine, but also occurred in spleen, kidney, peritoneal cavity, subcutaneously and in the eye. Grossly, groups of xenomas often formed white masses up to 5 mm in diameter in various tissues. Individual xenomas ranged from about 25 to 850 µm; in sectioned and stained material, the diameter of a mature xenoma was 250 to 750 µm. Mature xenomas resembled those of *Glugea anomala* (Moniez, 1887); the laminated xenoma wall covered the peripheral cytoplasm with a mass of merogony stages (Fig. 1) and separate fragments of host cell nucleus; more centrally were the



sporophorous vesicles with maturing sporoblasts and spores; the center had a mass of mature spores released from their sporophorous vesicle walls.

Fresh spores (Fig. 4) were elongated, ovoid, and 2.6 (2.4 to 2.8) × 4.9 (4.5 to 5.5) μm in size (n = 20). A large posterior vacuole occupied the broader posterior half, reaching sometimes up to mid-spore length; the anterior border of the vacuole was slightly convex.

Transmission electron microscopy: The laminae of the xenoma wall — in fact the shed cell coat layers — were indistinct and closely apposed (Fig. 6); there were proportionately more laminae in larger xenomas. The xenoma plasmalemma gave rise to numerous pinocytotic vesicles which lined the periphery of the xenoma. More centrally were merogony stages (spherical and uninucleate to cylindrical and multinucleate) with closely adhering cisternae of host endoplasmic reticulum (Fig. 2). Most meronts had stacks of smooth endoplasmic reticulum, but in some, the cisternae were largely dilated, appearing as many vesicles filled with amorphous, lucent material.

Sporogony began as described by Canning et al. (1982). There was a blister-like detachment of the thin sporophorous vesicle membrane from the sporont surface, with a thick surface coat deposited on the sporont plasmalemma. Later, division of the sporont produced sporoblast mother cells, which initially retained a discontinuous, opaque, surface coat (Fig. 3). Sporoblasts were produced by sporoblast mother cells and later matured into spores.

In immature spores, the exospore surface had finger-like villousities; as the spore gradually matured, the exospore became more even, but had numerous narrow tubules (Fig. 5). The tubules had about 40 nm in diameter and had an inner core lined with fuzzy material. The inner core was filled with an indistinct matrix. The tubules were already formed when the early sporophorous vesicles had dividing sporonts.

In mature spores, the polaroplast had an opaque anterior end consisting of compact lamellae, and a posterior end composed of tubular sacs. There were 11 to 16 turns (mode = 14) of the isofilar polar tube. Depending on the level of section, the anchoring disc appeared either apical or slightly lateral. The tubules often adhered closely to the surface of the exospore layer of the spore wall.

The host cell nucleus was broken into numerous, peripheral fragments. In the xenoma cytoplasm, chains of small globules were sandwiched between endoplasmic reticulum membranes ('annulate lamellae'; Sprague & Vernick 1968).

Microsporea from Fundulopanchax filamentosus:

The only material available for study was specimens processed for electron microscopy.

The xenoma structure was similar to that of the preceding hosts, except for the presence of electron-lucent regions in the xenoma cytoplasm, that were as large as sporophorous vesicles. The electron-lucent regions contained dense, concentric membranes within a finely granular matrix. These areas were surrounded by a meshwork of tubules and vesicles, the same as those beneath the xenoma plasmalemma in the pinocytotic zone.

The merogony and sporogony stages were identical to those in *Nothobranchius* spp. Younger meronts had cytoplasm replete with lucent vesicles. Sporogony plasmodium cleaved into sporoblast mother cells, which gave rise by binary fission to sporoblasts. Mature spores were identical to those in *Nothobranchius*; the isofilar polar tube had 11 to 14 coils (mode = 13).

Microsporea from Cynolebias nigripinnis: Spores appeared similar to both preceding populations, but could not be studied in detail, since only histological material was available. There were numerous xenomas pervading the intestinal wall (Fig. 7) as well as liver, mesentery and kidney. They revealed the same structure — fragmented host cell nuclei and developmental stages of the parasite at the periphery of the xenoma and a mass of mature spores in its center.

Discussion. An exact comparison of our parasites with existing microsporean species can only be done with the *Nothobranchius* spp. pathogen where both live and fixed material was available. The ultrastructural features of the *Fundulopanchax filamentosus* parasite suggest that it might be the same species; the histological material from *Cynolebias nigripinnis* is less conclusive. According to some ornamental fish hobbyists, a (single?) species of microsporidian can infect all 3 genera, in the genus *Nothobranchius* also the species *N. rubripinnis*, the infection occurring at various aquaria in North America, and diagnosed at Belle Isle

Figs. 1 to 7. *Glugea* sp. from *Nothobranchius* spp. (Figs. 1 to 6) and *Cynolebias nigripinnis* (Fig. 7). Fig. 1. Maturing xenomas located beneath the intestinal epithelium; semithin section, toluidine blue; scale bar = 100 μm. Fig. 2. Peripheral part of a mature xenoma with fragments of host cell nuclei (short arrows) and developmental stages of *Glugea*. Long arrow points to the xenoma wall; asterisks mark the sporophorous vesicles; semithin section; scale bar = 30 μm. Fig. 3. Sporoblast mother cell cleaving to produce sporoblasts; scale bar = 2 μm. Fig. 4. Fresh spores; scale bar = 10 μm. Fig. 5. Tubular structures within the parasitophorous vesicle space, scale bar = 0.5 μm. Fig. 6. Part of the xenoma wall. X: periphery of the xenoma; L: laminar layer; arrow points to the adhering host cell; scale bar = 0.5 μm. Fig. 7. Intestinal wall replete with small, maturing xenomas (arrows) and a mature xenoma (asterisk); semithin section; scale bar = 200 μm

Aquarium in Detroit, MI, USA (Karl Doering pers. comm.). There have been claims that losses of whole tanks of fish occurred, meaning that the parasite can be a serious pathogen. This is a good reason to try to clarify its identity and assess its host specificity.

There is no doubt that all our cases are caused by *Glugea* species, that form large xenomas typified as having a peripheral cytoplasmic layer with parasite developmental stages and fragments of the host cell nucleus. The pattern of merogonial development, formation of sporoblasts via sporoblast mother cells, origin of the sporophorous vesicle membrane, spore structure, and presence of annulate lamellae (Sprague & Vernick 1968) in the xenoma cytoplasm, are characteristic of this commonly reported *Glugea* group [*G. anomala* (Canning et al. 1982); *G. weissenbergi* (Sprague & Vernick 1968); *G. stephani* (Jensen & Wellings 1972, Takvorian & Cali 1983, Bekhti 1984); *G. atherinae* (Berrebi 1979); *G. plecoglossi* (Takahashi & Egusa 1977, Canning & Lom 1986)]. The presence of tubules in developing sporophorous vesicles is common in *Glugea* species, and also occurs in many other genera of microsporidia (e.g. in *Systemotrema alba* tubules also have a central core lined with fuzzy material; Larsson 1988). However, the adherence of tubules to the exospore surface has not been reported in *Glugea*.

As to the species identity, our *Glugea* has spores under light microscopy that are indistinguishable from *G. anomala* (Moniez, 1887) Gurley, 1893 from sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius*. The size range of *G. anomala* spores as given by Canning & Lom (1983) is $1.9\text{--}2.7 \times 3\text{--}5.6 \mu\text{m}$ [it may be extended to $1.9\text{--}2.8 \times 3\text{--}6 \mu\text{m}$ to include Voronin's (1974) data on *G. gasterostei*, an obvious junior synonym of *G. anomala*], and can easily accommodate the size of the *Nothobranchius* spp. parasite. The number of polar tube coils in *G. anomala* (about 12 to 14) also agrees with the present finding. No other *Glugea* species is so similar; other species with similarly shaped spores (e.g. *Glugea luciopercae* Dogel & Bykhovski, 1939) have a different xenoma structure or infect marine hosts. Some fish microsporeae are not very host specific; e.g. *G. stephani* (Hagenmüller, 1899) Woodcock, 1904 infects many species of marine flatfish and *Pleistophora hypheosobryconis* Schäperclaus, 1941 is known from 4 families of freshwater hosts (Canning & Lom 1986). *G. anomala* was recorded several times from fishes taxonomically very distant from its type hosts, the sticklebacks. These reports (e.g. Laird 1956 identified as *G. anomala* his findings from Malaysian fishes *Aeoliscus strigatus* and *Scatophagus argus*) hardly gave more than spore dimensions. However, because of structural identity, we can assume that the *Nothobranchius* parasite might be *G. anomala*. Nevertheless, because the hosts belong to a different family

and there was no proven contact with sticklebacks (the documented hosts of *G. anomala*) such a conclusion might be premature, unless at least cross-infection experiments are in favour of such identity. It seems that microsporidia from *Fundulopanchax filamentosus* and *Cynolebias nigripinnis* might be identical with those of *Nothobranchius*. Nothing can be said on the possible kinship of the microsporidia infecting *Cyprinodon variegatus*, since there is no detailed description of the parasite in the report by Majeed et al. (1985).

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