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Two New Species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the Brain of a South American Knife Fish, *Eigemannia virescens* (V.)

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Two New Species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the Brain of a South American Knife Fish, *Eigemannia virescens* (V.)

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ABSTRACT. Two new species of Myxozoa from the brain of the green knife fish *Eigemannia virescens* are described: *Myxobolus inaequus* sp. n. has an unusually large spore body and extremely unequal polar capsules, and *Henneguya theca* sp. n. has an attenuated spore encased in a sheath not previously described in other Myxozoa. Only spores of the two species were observed, and infections caused no obvious pathological changes in the brain.

NUMEROUS descriptions of myxosporidans from fishes of the USA, Europe, and Asia have been reported but little is known of them from South American fresh waters. Species have been described from Brazilian freshwater fishes (3–7, 9, 15, 16, 19) but none have been reported from the green knife fish *Eigemannia virescens* (V.), of the family Sternopygidae. This common aquarium fish is used for neurophysiological research at Scripps Institution of Oceanography, University of California, San Diego, California. Reported here are the descriptions of *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of *Eigemannia virescens* held at Scripps Institution of Oceanography.

MATERIALS AND METHODS

From September 1981 to October 1982, 27 green knife fish, *Eigemannia virescens,* imported from Brazil between 1980 and 1982 and maintained at Scripps Institution of Oceanography, were examined. Fish were received alive, sacrificed, and wet mounts of brain and visceral organs were prepared as described by Minchew (14) or preserved in 10% (v/v) buffered formalin. Measurements of spores were made by using an oil immersion objective and a calibrated ocular micrometer, and drawings were made with the aid of a camera lucida. Lugol's iodine was used to demonstrate iodinophilous vacuoles in fresh material, and smears were stained in Gomori's trichrome (8). Formalin-fixed infected brains were embedded in paraffin and sectioned at 5 μm. The sections were stained with Mayer's hematoxylin and eosin, periodic acid Schiff (PAS), or Giemsa (8).

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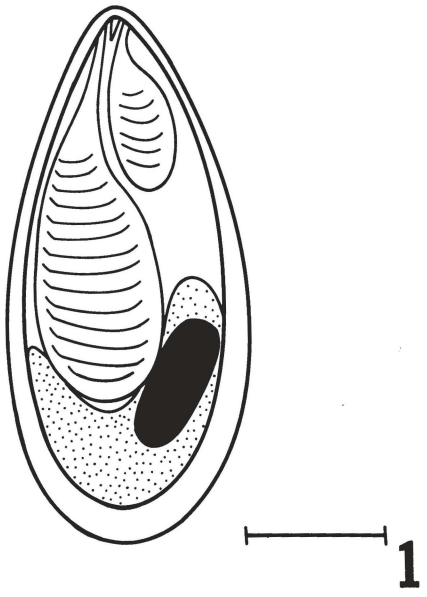


Fig. 1. Spore of *Myxobolus inaequus*. Bar = 5 μm.

DESCRIPTIONS AND DISCUSSION

Spores of *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. were found in the brains in 24 (89%) of the 27 *Eigemannia virescens* examined. Spores of only *M. inaequus* sp. n. were present in nine samples (33%); spores of only *H. theca* sp. n. were present in 12 samples (44%), and a mixed infection occurred in three samples (11%). No myxosporidans were apparent in other organs.

Myxobolus inaequus sp. n.
(Figs. 1–3B)

Host. Eigemannia virescens (V.) family: Sternopygidae.

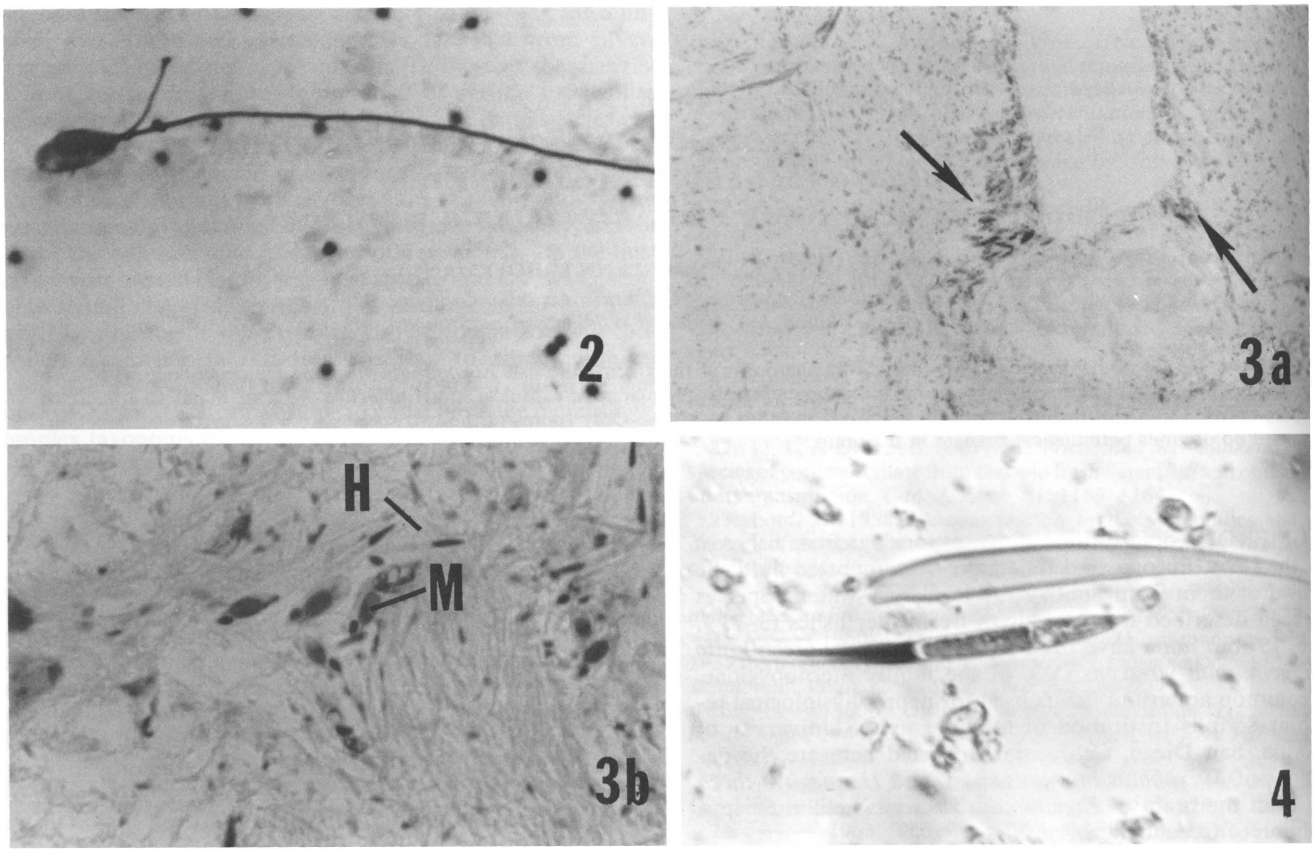
Locality. Northern South America, held at Scripps Institution of Oceanography.

Habitat. Brain, spores occur in small nests, most commonly in medulla around ventricle IV above median longitudinal fasciculus; occasional spores in cerebellum.

Description. Trophozoites not found. Spore: pyriform, shell valve smooth, sutural ridge in plane of polar capsules, symmetric without articulations or thickenings. Intercapsular process small and rod-shaped. Polar capsules extremely unequal pyriform, elongated anteriorly. Sporoplasm encloses a small nucleus and a glycogen vacuole, observed only in PAS-stained paraffin sections.

Characteristics of spore (n = 30). Length 19.8 (15.6–22) μm, width 8.6 (7.8–9.3) μm, thickness 8.0 (7.7–8.5) μm. Polar capsules (larger): length 11.8 (9.4–13) μm, width 3.6 (3.1–3.9) μm; (smaller): length 4.8 (3.9–5.5) μm. Polar filament lengths (n = 4): larger, 191 μm, smaller 22 μm.

Remarks. Spore characteristics of *Myxobolus inaequus* sp. n. agree with those described for the genus (10) but this species



Figs. 2–4. 2. Spore of *M. inaequus* with extruded polar filaments (Gomori's trichrome). ×400. **3a, b.** Section of *Eigemannia virescens* brain with "nests" of spores (arrows) of *M. inaequus* and *Henneguya theca* (Giemsa). 3a, ×100, 3b, ×400. H = *H. theca* spore and M = *M. inaequus* spore. **4.** Free spore and empty sheath of *H. theca* (Lugol's iodine-stained wet-mount). ×1000.

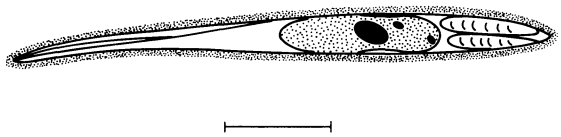


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Fig. 5. Spore of *Henneguya theca* sp. n. Stippled area represents sheath. Bar = 10 μm.

differs from all others due to its large spore size, extremely unequal polar capsules, and site in the host. *Myxobolus inaequus* sp. n. is larger than all other *Myxobolus* spp., except *M. gigas, M. magnasherus,* and *M. ovoidalis* but differs from these in its pyriform shape and extremely unequal polar capsules. Certain *Myxobolus* spp. such as *M. anioscapsularis, M. toyami, M. dispar* and *M. pseudodispar* have unequal polar capsules (2), but the spores of all of these are considerably smaller than those of *M. inaequus* sp. n. and do not infect nervous tissue. As shown in Fig. 3, no obvious tissue reactions were observed to be associated with spore "nests."

The genera *Myxobolus* and *Myxosoma* appear closely related and are separated by the presence of an iodinophilous vacuole in the former (17). This vacuole is sometimes difficult to demonstrate (18) and may not be present in all spores (11). Therefore it has been suggested that the genus *Myxosoma* be abolished and its species be transferred to the genus *Myxobolus* (18). Though the vacuole was not evident in wet mounts of *Myxobolus inaequus* stained in Lugol's iodine, this species is placed in this genus because a PAS-postive vacuole, corresponding to the iodinophilous vacuole, was clearly evident in stained sections.

Henneguya theca sp. n.
(Figs. 3A–5)

Host. Eigemannia virescens (V.) family: Sternopygidae.

Locality. Northern South America, held at Scripps Institution of Oceanography.

Habitat. Brain, spores occur in small nests most commonly in medulla around ventricle IV above median longitudinal fasciculus; occasional spores in cerebellum.

Description. Trophozoites not found. Spore attenuated, anterior convex, spherical in front view, valves smooth without a clear sutural ridge, tapering to two pointed tails. Polar capsules slightly unequal, attenuated anteriorly. Sporoplasm encloses two small nuclei and an iodinophilous vacuole. Individual spores encased in a tight-fitting sheath that conforms to the shape of the spore as shown in Fig. 5. Anterior of sheath compressed laterally; anterior appears convex when viewed dorsally and truncate when viewed in the plane of the polar capsules. Spores emerge from the anterior of sheath when pressure is applied in wet-mount preparations.

Characteristics of the spore (n = 30). Spore free from sheath including tails; length 48.0 (40.6–52.6) μm, width 3.5 (3.0–4.1) μm. Polar capsules (larger): length 11.1 (9.8–12.5) μm, width 1.4 (1.0–1.6) μm; (smaller): length 10.4 (8.7–11.7) μm, width 1.4 (1.0–1.6) μm. Tail length 23.2 (20.3–24.2) μm. Sheath only: length 50.8 (43.7–55.4) μm, width 5.78 (5.5–6.6) μm. Polar filaments (n = 20, Giemsa-stained), 19.9 (17.1–23.4) μm.

Remarks. Henneguya theca sp. n. is placed in this genus on spore characteristics as described by Kudo (1966) but differs from all other species in that genus in having a sheath that conforms closely to the shape of the spore and in its unique host and site in the host. No *Henneguya* spp. have been described from nervous tissues; the spore is considerably more attenuated and is larger than others in this genus from South American freshwater fish (9). As in *Myxobolus inaequus* sp. n., no obvious tissue reaction was observed associated with "nests" of spores in the brain.

The sheath structure that surrounds spores of *Henneguya theca* sp. n. has not been described in Myxozoa. Unlike spores of many myxosporidans, which are encased in a loose, amorphous, gelatinous envelope that is not visible in bright field or phase contrast examinations (13), the sheath described here conforms closely to the spore shape and is clearly visible in wet-mount preparations.

Pathology. The pathology associated with brain infections by these species was similar to that described by Bond (1) for *Myxobolus subtectalis* in that even in heavy infections little host response was observed, which is typical of myxosporidan infections (12). The infections were also similar to those caused by *Myxobolus kisutchi* from salmon brains (21), in which no cysts or vegetative stages were found in the brain. The "nests" of the spores that we describe are probably comparable to the cysts of *M. kisutchi* described by Wyatt (20). Though spores were not specifically associated with blood vessels in the knife fish, the immature stages may be carried to the brain from other organs via the circulatory system.

All knife fish examined had been in captivity in the USA for at least one week and some had been maintained for over a year before examination. Therefore the possibility that the fish were infected in the USA exists; however, in a subsequent study 12 green knife fish hatched and raised in captivity were found to be free of myxosporidans, indicating that the fish become infected in their native waters.

Further studies of myxosporidans from the brain of *Eigemannia virescens* are warranted. The identification and description of vegetative stages remain to be done, as well as electron microscopy of spores of *Henneguya theca* sp. n. to elucidate the structure of the sheath that surrounds them.

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