

A new species of *Eimeria* (Apicomplexa, Eimeriidae) from the weedy sea dragon *Phyllopteryx taeniolatus* (Osteichthyes: Syngnathidae)

Steve J. Upton^{1,*}, M. Andrew Stamper², Andrea L. Osborn¹, Sonia L. Mumford¹,
Laura Zwick³, Michael J. Kinsel³, Robin M. Overstreet⁴

¹Division of Biology, Ackert Hall, Kansas State University, Manhattan, Kansas 66506, USA

²New England Aquarium, Central Wharf, Boston, Massachusetts 02110, USA

³Laboratory of Veterinary Diagnostic Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois 61802, USA

⁴The University of Southern Mississippi, Gulf Coast Research Laboratory, PO Box 7000, Ocean Springs, Mississippi 39566, USA

ABSTRACT: A new species of intestinal coccidian is described from the weedy or common sea dragon *Phyllopteryx taeniolatus* housed at the New England Aquarium in Boston and at the Shedd Aquarium in Chicago, USA. Live oocysts of *Eimeria phyllopterycis* sp. n. are spherical, 30.9 (28.0–34.4) μm , with a thin, single-layered wall. Both a micropyle and oocyst residuum are absent and a large polar granule is sometimes present. Sporocysts are ellipsoidal and elongate, 24.3×10.4 ($23.4\text{--}25.6 \times 9.2\text{--}11.2$) μm , with Stieda and substieda bodies; shape index (length/width) 2.33 (2.14–2.70). A sporocyst residuum is present, consisting of numerous granules of various sizes. Sporozoites each possess 3 refractile bodies. Preliminary evidence suggests that the coccidian may affect the health of sea dragons; however, it could not be determined whether this parasite caused significant morbidity or mortality.

KEY WORDS: Apicomplexa · Coccidia · *Eimeria phyllopterycis* · Pathology · *Phyllopteryx taeniolatus* · Sea dragon

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INTRODUCTION

The common, or weedy, sea dragon *Phyllopteryx taeniolatus* is a pipefish endemic to waters off southern Australia, where it occurs commonly among algal beds and rock reefs (Dawson 1985) from Port Stephens in New South Wales to Geraldton in western Australia. This also includes waters around the island of Tasmania (Hutchins & Swainston 1986). Although now a protected species, its pregnant males were previously caught in the wild and the eggs or offspring allowed to develop and then sold to various aquaria worldwide. Sea dragons in most aquaria are derived from such lineages.

Sea dragons from the same southern Australian supplier housed at both the New England Aquarium in Boston and Shedd Aquarium in Chicago, USA, became ill or died, and their health status was investigated. Microscopic examination of the intestinal contents of 2 fish revealed coccidian oocysts, and histological sections from the other dead or moribund fish revealed light to heavy infections of the same coccidian. The protozoan was found to represent a previously unreported species which is described herein.

MATERIALS AND METHODS

Two specimens of the sea dragon *Phyllopteryx taeniolatus*, housed at the New England Aquarium in Boston, became anorectic and growth became stunted.

*E-mail: coccidia@ksu.edu

Fecal samples were collected from each sea aragon, placed individually into vials containing seawater, and examined by light microscopy for the presence of parasites. Samples were found to contain coccidial oocysts, which were then shipped to Kansas State University for further diagnosis. Oocysts were examined in wet mount preparations directly by Nomarski interference contrast microscopy, photographed, and measured using a calibrated ocular micrometer. Live oocysts were 7 d old when measured, and measurements in 'Description' are reported in micrometers (μm) followed by the ranges in parentheses.

Four additional fish from the Shedd Aquarium revealed infections with the same coccidian. Histological sections from 2 of these fish were independently sent to the Gulf Coast Research Laboratory for identification. Paraffin sections for light microscopy were stained with hematoxylin and eosin.

DESCRIPTION

Eimeria phyllopterycis sp. n. (Figs. 1 to 10)

Viable oocysts spherical, 30.9 (28.0–34.4) ($n = 20$) with thin, membrane-like, single-layered wall; outer wall 0.6–0.8 thick; micropyle and oocyst residuum absent; polar granule present in about $\frac{1}{3}$ of oocysts, 2.0–3.0 \times 3.0–4.0; oocysts in histological sections ca 26–30 in diameter; sporocysts ellipsoidal and elongate, 24.3 \times 10.4 (23.4–25.6 \times 9.2–11.2) ($n = 15$), with single-layered wall 0.8–1.0 thick; shape index 2.33 (2.14–2.70); sporocysts in sectioned material ca 19–20 \times 8; Stieda body present, consisting as thinning of sporocyst wall with distinct pore; substieda body present, large, homogenous; sporocyst residuum present, 11.3 \times 6.7 (8.8–16.0 \times 4.2–8.0) ($n = 15$), consisting as hundreds of globules of various sizes; sporozoites elongate, 26.0–31.0 \times 3.0–3.5 *in situ*, often with transverse striations anteriorly; each sporozoite with spherical anterior refractile body, 2.2 (2.0–2.8) ($n = 15$), an ellipsoidal middle refractile body 7.1 \times 3.0 (4.8–12.0 \times 2.6–3.4) ($n = 10$), and a large, posterior refractile body 12.6 \times 3.2 (9.6–14.4 \times 3.0–3.4) ($n = 10$); nucleus located between the anterior and middle refractile bodies.

Synonym: *Eimeria phyllopteryx* Osborn, Stamper, Reimschuessel, Greenwell, Zwick, and Kinsel 1999, *nomen nudum*. Osborn et al. (1999) originally named this species but provided no morphologic data on the oocysts or other developmental stages, thus leaving this coccidian as a *nomen nudum*.

Type host: *Phyllopteryx taeniolatus* (Lacépède, 1804) Swainson, 1839 (common or weedy sea dragon) (Syngnathidae).

Geographic locality: Oocysts described from the fish originated at the New England Aquarium, Boston. Histological sections were obtained from fish housed at the Shedd Aquarium, Chicago. Sea dragons at both aquaria were originally purchased from the Dallas World Aquarium in Texas; the original stock animals presumably came from marine waters around southern Australia.

Sporulation: Endogenous. All oocysts were passed fully sporulated.

Etymology: The specific epithet reflects the host genus; it is a noun in the genitive case.

Type specimens: Phototypes have been deposited in the US parasite collection, Beltsville, MD, as USNPC No. 89748.

Remarks: Histological sections revealed abundant gamogony and sporogony, which occurred primarily in the columnar epithelial cells of the small intestine (Fig. 5). However, stages were also abundant in the lamina propria (Figs. 5 to 8). Meronts were not clearly defined in any of the sectioned material, indicating that the parasite was probably in the terminal phase of development. Microgametocytes were not discernable in any of the sections, whereas macrogametes were numerous (Figs. 6 to 8). Macrogametes had a vesicular cytoplasm and contained a large, homogeneous globule which gradually increased in size as the cytoplasm enlarged (Figs. 6 to 8). This globule was also retained by the zygote (Figs. 7 to 8). Wall-forming bodies could be seen along the periphery of many

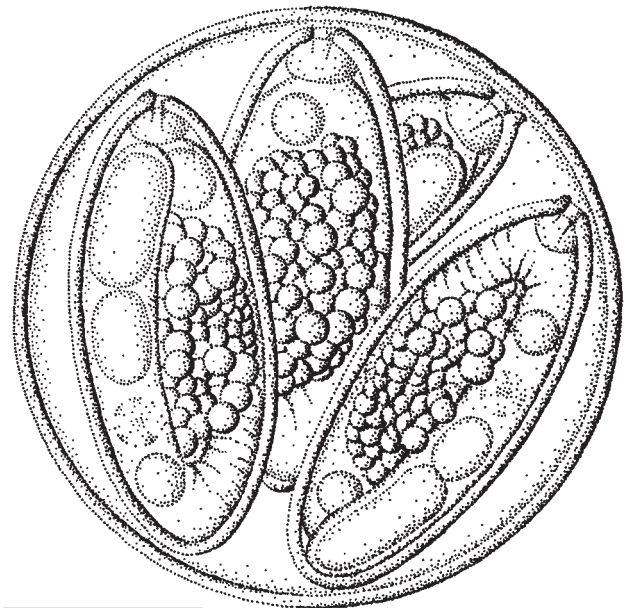
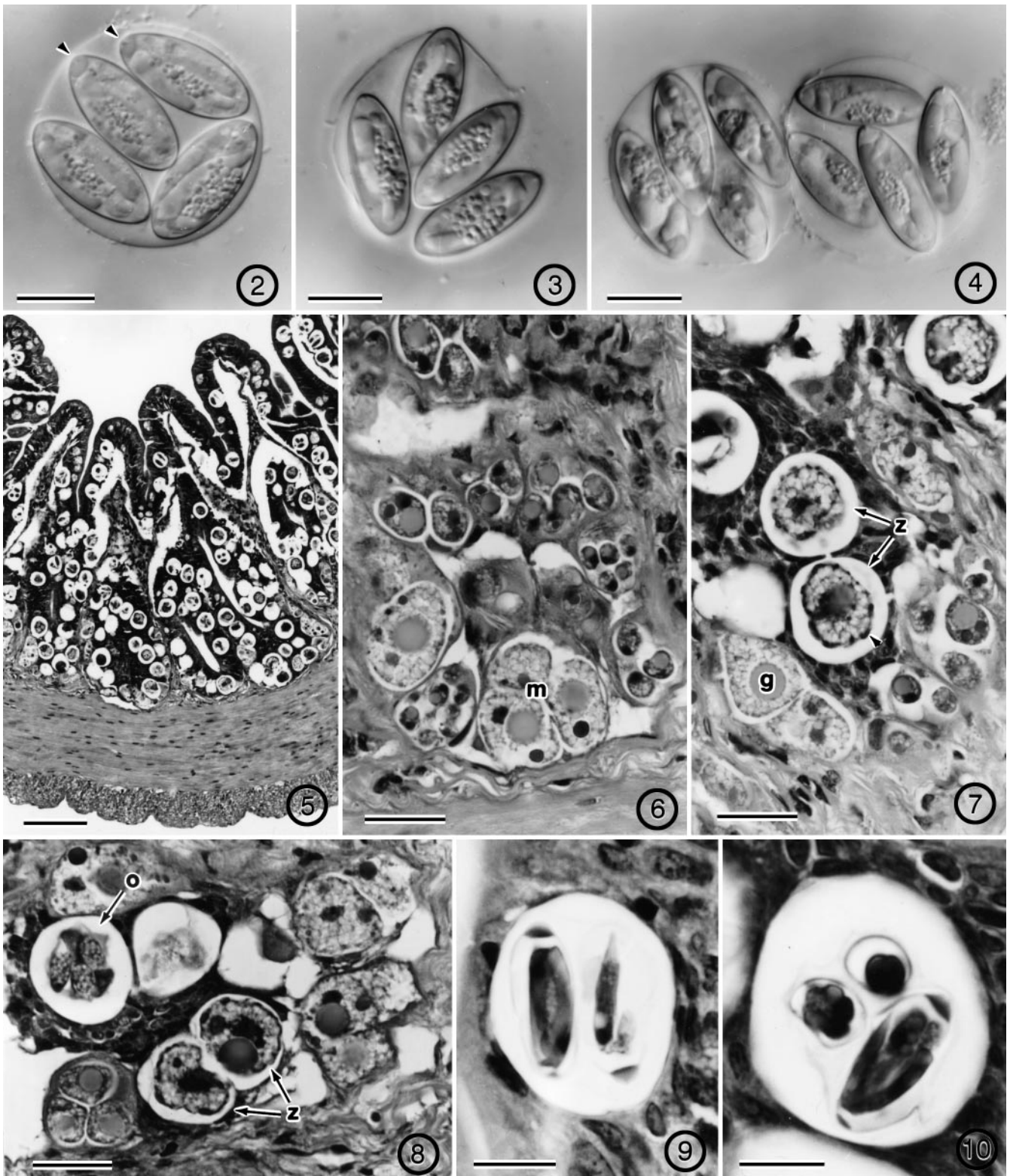


Fig. 1. Line drawing of the sporulated oocyst of *Eimeria phyllopterycis* sp. n. Scale bar = 10 μm



Figs. 2 to 10. Nomarski interference contrast (NIC) and brightfield (BM) microscopy of *Eimeria phyllopterycis* sp. n. from *Phyllopteryx taeniolatus*. Figs. 2–4. NIC photomicrographs showing viable sporulated oocysts of *E. phyllopterycis* sp. n. from intestinal contents. Arrowheads (Fig. 2) point to Stieda body/substieda body complex. Scale bars = 10 μ m. Figs. 5–10. BM photomicrographs of developmental stages from histological sections through infected intestinal tissue. Hematoxylin and eosin. Fig. 5. Low magnification showing extent of intestinal involvement. Note absence of extensive inflammatory response. Scale bar = 100 μ m. Fig. 6. Developing stages, predominately macrogamonts (m). Scale bar = 20 μ m. Fig. 7. Two zygotes (z) early during oocyst wall formation. Note presence of wall forming bodies (i.e. arrowhead) being distributed under plasma membrane, and homogenous globule (g). Scale bar = 20 μ m. Fig. 8. Slightly later stage of oocyst wall formation (z) and sporulating oocyst (o). Scale bar = 20 μ m. Figs. 9 & 10. Fully sporulated oocysts with cross sections through sporocysts. Scale bars = 10 μ m

zygotes (Figs. 7 & 8), providing the only visual evidence that fertilization had occurred. Developing sporozoites initially had a vesicular cytoplasm (Fig. 8), but this later became less vesicular as the sporozoites matured (Figs. 9 & 10).

DISCUSSION

Syngnathidae is sometimes placed in its own order, Syngnathiformes, whereas others consider the taxon a suborder of Gasterosteiformes (Robins et al. 1991). This family consists primarily of pipefish, centriscids, trumpetfish, coronetfish, and seahorses. To date, only a single coccidian species has been described from any member of the Syngnathidae. However, oocysts of *Eimeria syngnathi* Yakimoff and Gousseff, 1936, from the black-line pipefish *Syngnathus nigrolineatus* are ellipsoidal rather than spherical, measuring $28.7 \times 20.6 \mu\text{m}$, possess an oocyst residuum unlike the form we describe here, and have smaller sporocysts that measure $10.6\text{--}14.4 \times 7.7\text{--}9.2 \mu\text{m}$ (Yakimoff & Gousseff 1936). There are currently 5 named species of coccidia in the order Gasterosteiformes, and all occur in fish in the Gasterosteidae (sticklebacks). *Eimeria aculeati* Jastrzebski, 1984, *E. gasterostei* (Thélohan, 1890) Doflein, 1909, and *E. zarnowskii* Jastrzebski, 1982, have all been reported from *Gasterosteus aculeatus*; *E. haneki* Molnar and Fernando, 1974 occurs in *Culaea inconstans*, and *E. pungitii* Molnar and Hanek, 1974 parasitizes *Pungitius pungitius*. None of these coccidia can be confused with the form described herein as they all have considerably smaller oocysts and sporocysts (Thélohan 1890a,b, Molnar & Fernando 1974, Molnar & Hanek 1974, Jastrzebski 1982, 1984).

We are uncertain whether *Eimeria phyllopterycis* is a normal parasite of the sea dragon; however, ample opportunity exists for transmission by conspecifics to occur. The fish are reared by the male in brood patches along the body and are exposed to the environment constantly during gestation. After birth, they are then separated from the male but are eventually shipped to other aquaria where they are placed with other sea dragons. However, it is also possible that other closely related species of fish may represent the primary host for the parasite. Most fish coccidia appear to be genus specific, but cross-transmission between distantly related hosts has occasionally been reported. For example, the heteroxenous coccidian *Calyptospora funduli* not only infects members of the genus *Fundulus*, but on occasion other genera of fish. These other piscine hosts not only involve the closely related orders Antheriniformes and Cyprinodontiformes, but also the batrachoidiform toadfish *Opsanus beta* (Fournie & Overstreet 1993, Oliveira et al. 1993).

It is also unknown whether *Eimeria phyllopterycis* was the sole cause of mortality in any of the infected fish. Osborn et al. (1999) reported that the parasite occupied up to 80% of the epithelial cells in the distal one-third of the small intestine of 2 fish that died of unknown causes at the New England Aquarium. However, the coccidian could not be directly linked as the cause of mortality. In the case of the 4 fish from Chicago, death was attributed to acute bacterial sepsis accompanied by renal necrosis in 1 fish, severe proliferative branchitis in another, and emaciation reflecting prolonged anorexia or debilitation in the others. Like the fish from New England, much of the intestinal tissue was replaced with parasite developmental stages probably contributing to intestinal dysfunction and decreased health. Compression of infected intestinal cells, as well as distortion of normal villous and crypt architecture, was apparent in all fish. However, little sign of host response or inflammation was noted. It is possible that the coccidian causes little pathology when environmental conditions are optimal, but becomes a significant pathogen when conditions deteriorate. Such is the case for *Calyptospora funduli* in the liver and pancreas of killifish when exposed to decreased temperatures (Overstreet 1993).

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