

Research Note

Calyptospora funduli* (Apicomplexa, Calyptosporidae) in the Liver of the Gulf Toadfish, *Opsanus beta

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ABSTRACT: Oocysts of the apicomplexan protozoan *Calyptospora funduli* were found in the liver of a gulf toadfish (*Opsanus beta*). The infected specimen was 1 of 54 (1.9%) toadfish livers examined histologically. The paraffin-embedded specimen containing the infection as well as similar material from *Fundulus similis* were processed for scanning electron microscopical (SEM) examination to view diagnostic surface features of *C. funduli* sporocysts. SEM examination confirmed sporopodia and a thin veil surrounding each of the 4 sporocysts per oocyst. Although a single case, the toadfish infection expands the broad host specificity of *C. funduli* to include a host other than an atheriniform fish species.

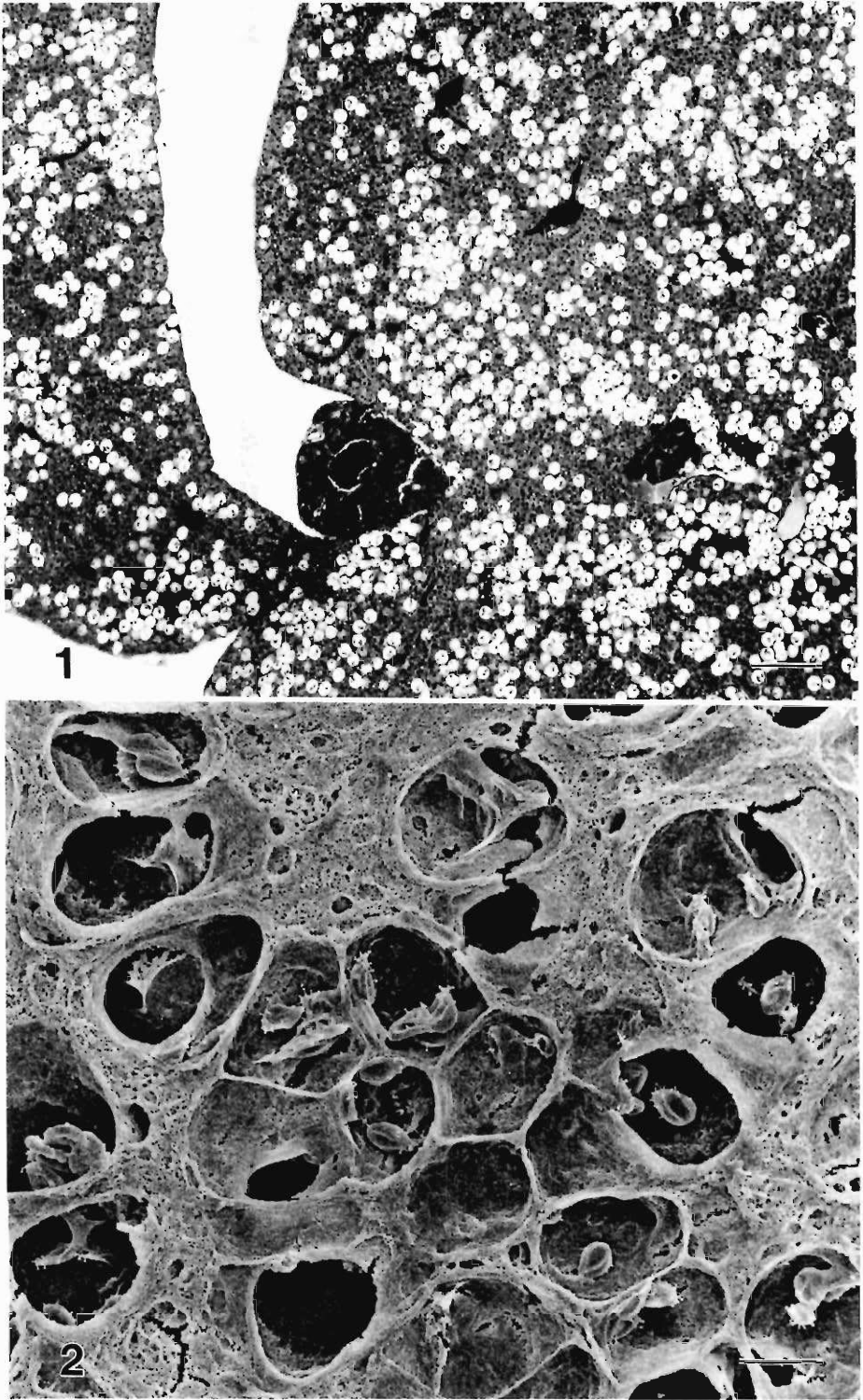
KEY WORDS: Protozoa, Coccidia, *Calyptospora funduli*, fish, liver, host specificity, scanning electron microscopy.

Sporocysts of coccidian species in the genus *Calyptospora* Overstreet, Hawkins, and Fournie, 1984, lack a Stieda body and are enclosed in 2 incompletely separated valves, and each is surrounded by a membranous veil that is supported by projections from the sporocyst wall. Members of the genus appear to require an invertebrate intermediate host (Fournie and Overstreet, 1983; Overstreet et al., 1984). The genus includes 4 described species: *C. funduli* (Duszynski, Solangi, and Overstreet, 1979), *C. empristica* Fournie, Hawkins, and Overstreet, 1985, *C. serrasalmi* Cheung, Nigrelli, and Ruggieri, 1985, and *C. tucunarensis* Békési and Molnár, 1991. All infect mainly liver parenchymal cells. Both *Calyptospora funduli* (Overstreet et al., 1984) and *C. empristica* (Fournie et al., 1985) commonly infect estuarine and freshwater killifishes of the genus *Fundulus* in North America, and in freshwater of Brazil, *C. serrasalmi* infects the black piranha (*Serrasalmus niger*) (Cheung et al., 1985) and *C. tucunarensis* infects the tucunare (*Cichla ocellaris*) (Békési and Molnár, 1991). Although piscine coccidians are generally expected to have

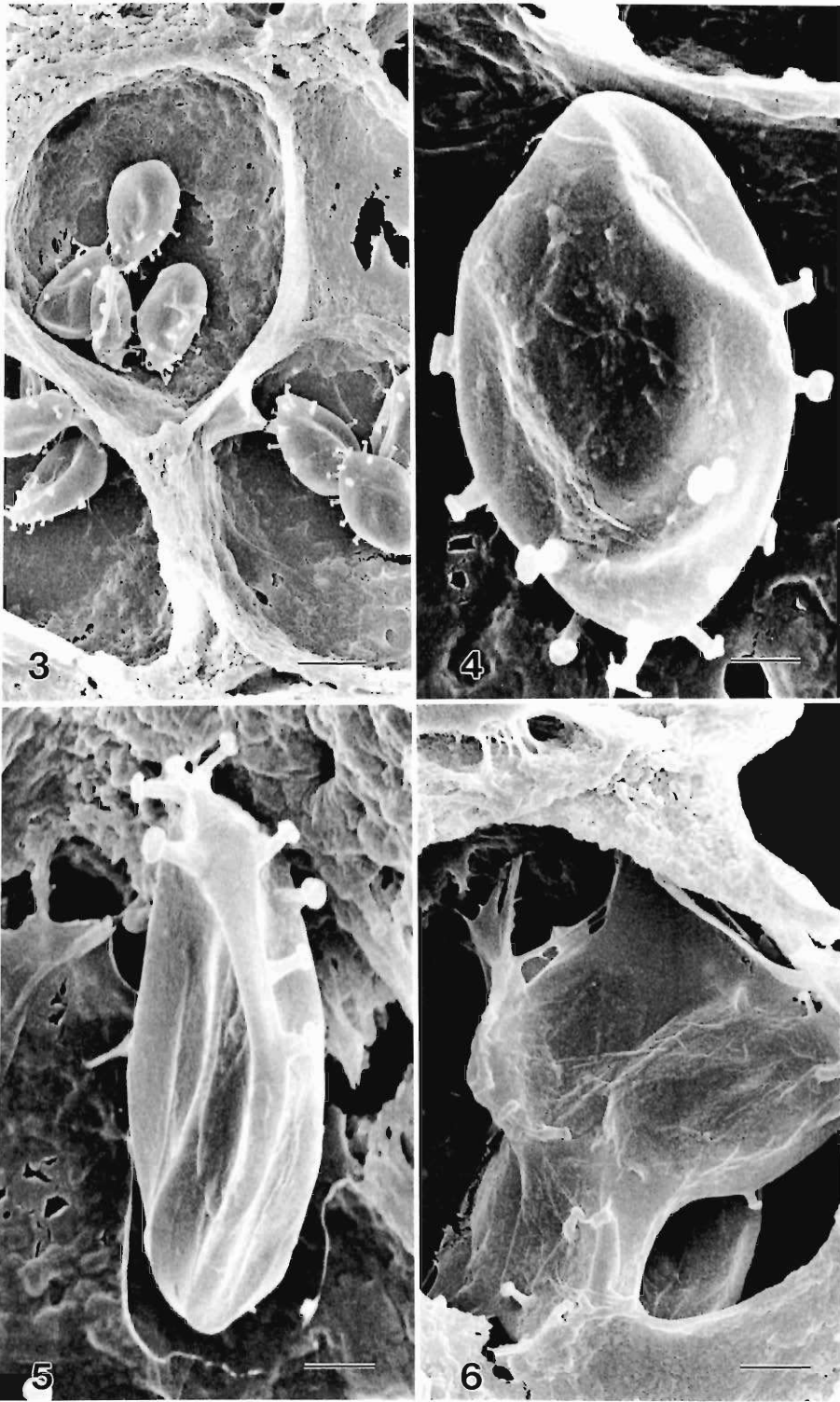
a narrow host specificity, *Calyptospora funduli* naturally infects at least 6 estuarine species of atheriniform fishes (Fournie and Overstreet, 1982). Experimental infectivity studies on *C. funduli* confirm a rather broad host specificity within atheriniform fishes (Fournie and Overstreet, unpubl.). Here we report the occurrence of *C. funduli* infecting the liver of a gulf toadfish, *Opsanus beta* (Goode and Bean, 1879), from Mississippi.

Toadfish were captured by trawling from waters of the Mississippi Sound near Ocean Springs, Mississippi (30°24'N, 88°51'W). Specimens were brought alive to the laboratory, where they were anesthetized in 0.1% MS-222 (tricaine methanesulfonate) and examined for external lesions. Liver, kidney, spleen, and a gill arch from each specimen were removed, fixed in Lillie's fixative (formalin, picric acid, and formic acid), and embedded in paraffin. Paraffin sections were cut, placed on glass slides, and stained with hematoxylin and eosin. After the infection was detected by light microscopy, paraffin sections approximately 10 μm thick were cut and processed for examination by scanning electron microscopy (SEM) following modifications of techniques described by Oshel (1985) and Felgenhauer (1987). The paraffin sections were placed on round glass coverslips, deparaffinized in Shandon xylene substitute (Shandon Inc., Pittsburgh, Pennsylvania), rinsed in 100% ethanol, air-dried, and sputter-coated with gold-palladium. The coated coverslips were mounted on aluminum stubs with double-faced adhesive tape and examined with a JEOL T-330 scanning electron microscope. For comparison, a paraffin block containing sporulated oocysts of *C. funduli* from the liver of the longnose killifish (*Fundulus similis*) was processed similarly.

A single toadfish specimen from a total of 54



Figures 1, 2. Micrographs of paraffin-embedded material of *Calyptospora funduli* from liver of toadfish *Opsanus beta*. 1. Hematoxylin-and-eosin-stained section showing oocysts replacing much of the liver parenchyma. $\times 125$. Bar = $80\ \mu\text{m}$. 2. SEM-prepared material showing scattered oocysts. $\times 600$. Bar = $20.0\ \mu\text{m}$.



Figures 3–6. SEM micrographs of *Calyptospora funduli* from liver of toadfish *Opsanus beta*. 3. Three oocysts containing sporocysts of which 2 of them exhibiting 4 sporocysts are visible. $\times 2,000$. Bar = $5.0 \mu\text{m}$. 4. Sporocyst showing sporopodia arranged along the lateral margins and clustered at the posterior end. $\times 10,000$. Bar = $1.0 \mu\text{m}$. 5. Lateral view of a partially collapsed sporocyst. Posterior end (arrowhead). $\times 10,000$. Bar = $1.0 \mu\text{m}$. 6. Oocyst with sporocysts obscured by sporocyst veils. $\times 5,000$. Bar = $2.0 \mu\text{m}$.

toadfish specimens from which livers were examined histologically was infected with *Calyptospora funduli*. The infected specimen was collected from offshore waters of about 25‰ salinity near Horn Island, approximately 18 km from the mainland. The toadfish was a juvenile, 85 mm in total length and weighed 9 g.

Examination of paraffin sections revealed oocysts that were about 20 μm in diameter occurring singly or in clusters and replacing more than 75% of the liver parenchyma (Fig. 1). Exocrine pancreatic cells did not appear to be infected. There was no evidence of a host inflammatory response. All oocysts examined were sporulated. Oocysts contained 4 ovoid sporocysts (about $8-9 \times 2-3 \mu\text{m}$). A Stieda body could not be resolved in the sporocysts, although there was a dense structure in the sporocyst wall at the posterior end of the sporocyst. Projections (sporopodia) of the sporocyst wall supported a thin membranous veil that was attached at the anterior end of the sporocyst. Each sporocyst had 2 elongated sporozoites that were partly coiled together. Examination by SEM confirmed the heavy infection (Fig. 2). Oocysts and the enclosed sporocysts were well preserved but not the surrounding host tissues. The oocyst wall appeared thin in places where it could be seen between adjacent oocysts. Sporocysts were more pointed at the posterior than at the anterior end (Fig. 3). The sporocyst wall was smooth except where it gave rise to sporopodia. Sporopodia were about 1 μm long and knobbed at the distal end (Fig. 4). Because we could count 10 or 11 sporopodia on one side in an SEM specimen, we estimated that each sporocyst had about 20 sporopodia. The sporopodia appeared numerous along an anterior-posterior line and were especially numerous at the posterior (pointed) end. Figure 5 shows this line interpreted as the site of an underlying suture in a sporocyst that was partially collapsed. In Figure 6, 4 sporocysts are obscured by what appear to be remnants of the membranous veil. Examination by SEM revealed no morphological differences between *Calyptospora funduli* from the liver of a longnose killifish and the organism in the toadfish.

The method of SEM examination of paraffin-embedded material utilized in this study yielded considerable ultrastructural detail of this coccidian. The coccidian in the toadfish liver appeared to be *Calyptospora funduli*, and this was confirmed by comparing similarly prepared material of *C. funduli* in *Fundulus similis*. The infection

in the toadfish, even though rare, appeared to be well tolerated because the parasite was fully developed and there was no evidence of degenerating stages of the parasite or a host inflammatory response.

Most eimerian coccidians have a strong host affinity and rarely do they naturally infect more than 1 genus (Long and Joyner, 1984). *Calyptospora funduli*, however, has a broad host specificity, and experimental infections can be produced in several species. With the exception of the batrachoidiform toadfish, all the hosts, whether natural or experimental, belong to the order Atheriniformes, primarily the Cyprinodontidae (Fournie and Overstreet, unpubl.). Possibly, the infected toadfish represents an abnormally susceptible individual rather than the result of feeding behavior. This would have to be investigated by experimental transmission studies using laboratory-reared toadfish. The coccidian requires a palaemonid shrimp intermediate host (Fournie and Overstreet, 1983), and the toadfish readily feeds on those grass shrimps (R. W. Heard and R.M.O., pers. obs.). A relatively high percentage of grass shrimp in enzootic areas have *C. funduli* infections (Solangi and Overstreet, 1980; Fournie and Overstreet, 1983). Consequently, if the toadfish were a susceptible species, the prevalence of infected individuals should have been higher.

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Research Note

Sarcocystis felis in Captive Cheetahs (*Acinonyx jubatus*)

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ABSTRACT: *Sarcocystis felis* was detected in the musculature of 7 of 10 cheetahs (*Acinonyx jubatus*) from a captive breeding colony in Winston, Oregon. This is the first report of *Sarcocystis felis* from cheetahs.

KEY WORDS: *Sarcocystis felis*, cheetah, *Acinonyx jubatus*.

Species of the genus *Sarcocystis* have a predator-prey cycle consisting of a definitive carnivore (predator) host and intermediate herbivore (prey) host. In the intermediate host, schizonts or muscle sarcocysts are the result of asexual reproduction, and in the definitive carnivore host, sexual reproduction occurs in intestinal cells, with oocysts or sporocysts passed in feces (Dubey et al., 1989). Carnivores infrequently develop sarcocysts in muscles or function as intermediate hosts. Definitive hosts have not been identified for *Sarcocystis* spp. with sarcocysts in carnivore muscles. In North America, sarcocysts identified as *Sarcocystis felis* Dubey, Hamir, Kirkpatrick, Todd, and Rupprecht, 1992, have been reported from bobcats (*Felis rufus*), domestic cats (*Felis domesticus*), Florida panther (*Felis concolor coryi*), and cougar (*Felis concolor*) (Kluge, 1967; Kirkpatrick et al., 1986; Everitt et al., 1987; Edwards et al., 1988; Fiori and Lowndes, 1988; Hill et al., 1988; Greiner et al., 1989; Anderson et al.,

1992; Dubey et al., 1992). This report documents *S. felis* in the musculature of captive cheetahs (*Acinonyx jubatus*) from a wildlife facility in Winston, Oregon.

All cheetahs were part of a captive breeding program at Wildlife Safari, Winston, Oregon. All animals had been born in the United States, ranged in age from 5 to 14 yr, and had been in captivity all of their lives. Muscle biopsy specimens from 8 cheetahs, 1 male and 7 females, were obtained from the biceps femoris after administration of lidocaine. Necropsy specimens of biceps femoris were collected from 2 additional male cheetahs. Tissues were fixed in 10% buffered formalin, sectioned at 5 μ m, and stained with hematoxylin and eosin. Tissues were examined microscopically ($\times 400$), and sarcocysts were counted within a 1-cm² marked section of randomly chosen tissue.

Additional muscle specimens were processed for electron microscopy by methods described previously (Foreyt, 1989) and viewed with a transmission electron microscope (Hitachi H600, Hitachi, Santa Clara, California 95044).

Sarcocysts of *S. felis* were detected in 7 of 10 cheetahs (Fig. 1). Mean size of 48 sectioned sarcocysts was 251 \times 121 μ m (range, 64-997 \times 49-