

## FIRST RECORD OF ENTODINIOMORPH CILIATES IN A CARNIVORE, THE MANED WOLF (*CHRYSOCYON BRACHYURUS*), FROM BRAZIL

Carly Vynne, and John M. Kinsella, Ph.D.

- 1** *Abstract:* The entodiniomorph ciliates (Ciliophora: Entodiniomorpha) are endosymbiotes widely found in the intestines of herbivorous mammals. These commensals commonly occur in the Artiodactyla and Perissodactyla and have also been described in the Proboscidea, Primates, Rodentia, and Diprotodontia. This study reports the first finding of a ciliate in a member of order Carnivora, the maned wolf (*Chrysocyon brachyurus*). Fecal samples from wild and captive maned wolves were screened using ethyl acetate sedimentation. Prevalence in fecal samples collected from free-ranging maned wolves in Brazil was 40% (6 of 15). Fecal samples from two of four captive individuals from the St. Louis Zoo also had the same species of ciliate. The largely frugivorous diet of the maned wolf likely explains the occurrence of these normally herbivore-associated endosymbiotes in a carnivore.

*Key words:* Carnivora, *Chrysocyon brachyurus*, ciliate, endosymbiote, entodiniomorph, maned wolf.

### BRIEF COMMUNICATION

The entodiniomorph ciliates (Ciliophora: Entodiniomorpha) are endosymbiotes widely found in the intestines of herbivorous mammals. They are regarded as most likely commensal, with little value or harm to the welfare of their hosts.<sup>2</sup> The great majority of species are found in the rumens of hosts of the orders Artiodactyla and Perissodactyla, but several species have been described from elephants (Proboscidea); gorillas and chimpanzees (Primates); and capybaras and guinea pigs (Rodentia).<sup>2</sup> Recently, a new family of entodiniomorphs was described from Australian marsupials (Diprotodontia).<sup>4</sup> To date, no species have been recorded from the order Carnivora.

The maned wolf (*Chrysocyon brachyurus*) is the largest canid in South America and is associated with the grassland and scrub habitats of Bolivia, Paraguay, Argentina, and Brazil.<sup>3</sup> The maned wolf is a CITES Appendix II species and is considered “near threatened” by IUCN (<http://www.redlist.org>) and “endangered” by the U.S. Fish and Wildlife Service (<http://endangered.fws.gov>). Population trends and status of the maned wolf are unknown, and there is little information about how extensive agricultural conversion throughout their range is affecting their population.<sup>10</sup> Road kills are a significant source of

mortality outside of protected areas, and disease is a major concern in the conservation of this species.<sup>3</sup>

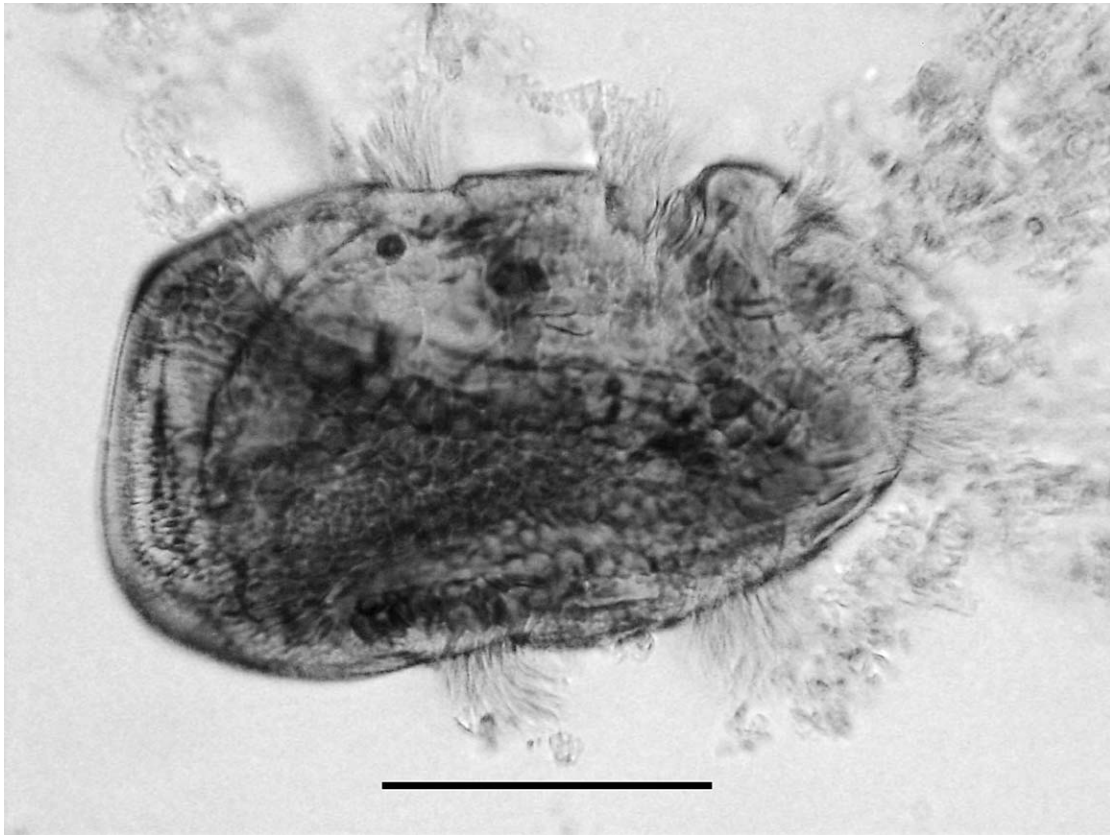
Individuals are strictly territorial against like-sexed adults and are usually seen alone. The species is facultatively monogamous, and males and females form long-term pair-bonds.<sup>6</sup> Maned wolves are omnivores and, in addition to vertebrates and invertebrates, consume large quantities of fruit. Frugivory in the maned wolf is extensive, with studies showing occurrence of at least one fruit species in 57–86% of all scats encountered.<sup>1,8</sup> One particular fruit, *Solanum lycocarpum*, has been shown to occur in up to 90% of scats during certain times of the year and is considered the single most important food item in the diet of maned wolves.<sup>5,6</sup>

Fecal samples from wild maned wolves were located in and around Emas National Park, in the extreme southwest portion of Goiás State, Brazil (18°19'S, 52°45'W). Samples were detected by using specially trained scat detector dogs from the University of Washington's Center for Conservation Biology.<sup>11</sup> Samples were collected in a plastic bag, mixed with a gloved hand, and divided for DNA, hormone, parasite, and diet analysis. Only samples determined to be less than 2 day old (i.e., sample was moist-fresh and/or structurally intact; about 20% of encountered samples) were collected for fecal parasite analysis. A plum-sized portion of the fecal sample was placed in a 40-ml screw-top vial and preserved in a 10% formalin solution. Samples were stored at room temperature until shipment to the United States for analysis.

Wild-collected samples were confirmed to be from maned wolves by DNA fragment analysis.

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**Figure 1.** Entodiniomorph ciliate of the Family Cycloposthiidae from feces of a maned wolf. Scale bar = 50  $\mu\text{m}$ .

DNA extraction and analysis were performed using protocols previously demonstrated to be effective by our laboratory.<sup>12</sup> Bands amplifying at expected base-pair length were compared with known controls of maned wolves and other sympatric carnivores. Fifteen samples from wild maned wolves were randomly selected for protist screening. In addition, one fecal sample from each of four captive maned wolf individuals from the St. Louis Zoo was examined.

Fecal samples were mixed in water, filtered through two layers of cheesecloth, and concentrated using ethyl acetate sedimentation. The sediment plug was mixed with Lugol's iodine, and two drops were placed on a slide and examined under a light microscope. Two slides were examined for each sample.

Entodiniomorph ciliates (Fig. 1) were found in 6 of 15 (40%) of the wild-collected samples. Since it seemed a possibility that the ciliates had been ingested from prey, fecal samples were obtained from four maned wolf individuals at the St. Louis Zoo. Two of the four wolves were infected with

what appeared to be the same species of ciliate, indicating that it is indeed a parasite of this host. The organisms possessed an adoral ring of cilia and two symmetric pairs of synciliary tufts on the dorsal and ventral surfaces, placing them in the Family Cycloposthiidae, but their morphology did not match any known genus or species. They ranged from 105–133  $\mu\text{m}$  in length and 50–58  $\mu\text{m}$  in width.

Although the main radiation of the Entodiniomorphida has been in artiodactyls and perissodactyls, these ciliates have also been reported in hosts as disparate as gorillas, elephants, and capybaras. Most genera are specific to one order of hosts, but on rare occasions, the same genus is found in ecologic associates (e.g., *Prototapirella* spp. in African elephants and gorillas).<sup>2</sup> That the maned wolf is the first member of order Carnivora known to host a normally herbivore-associated ciliate is likely due to the extreme omnivory of this species. The diet of the maned wolf is unique among large-bodied carnivores for its high level of fruit consumption. While the

energetics of this relationship have yet to be studied, it is likely that the physiology of the gastrointestinal tract of the wolf has some similarities to that of the herbivores in which these commensal ciliates are typically found.

The prevalence of 40–50% of affected individuals may be an underestimate. From studies in other hosts, it is expected that the prevalence would be near universal.<sup>7,9</sup> It is possible that not enough samples were collected and that ciliate prevalence was variable because of the consistency of the fecal samples. Samples of this omnivore are variable and included contents ranging from nearly all prey hair and bone remains to large quantities of partially digested fruit. Maned wolf fecal samples are large in size (our 15 wild samples varied from 26–38 cm in diameter), and it is possible that by partitioning the sample for protist screening, portions of the fecal sample containing the ciliates may not have been preserved and thus were not observed in this study. Since entodiniomorphs do not have an encysted stage, viability of the trophozoites outside the host may also be a factor. DNA studies are underway to determine the phylogenetic relationships of these ciliates.

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#### LITERATURE CITED

1. Bueno, A. D., and J. C. Motta. 2004. Food habits of two syntopic canids, the maned wolf (*Chrysocyon brachyurus*) and the crab-eating fox (*Cerdocyon thous*), in southeastern Brazil. *Rev. Chil. Hist. Nat.* 77: 5–14.
2. Corliss, J. O. 1979. *The Ciliated Protozoa: Characterization, Classification and Guide to the Literature*. Pergamon Press, New York, New York. 455 pp.
3. Deem, S. L., and L. H. Emmons. 2005. Exposure of free-ranging maned wolves (*Chrysocyon brachyurus*) to infectious and parasitic disease agents in the Noel Kempff Mercado National Park, Bolivia. *J. Zoo Wildl. Med.* 36: 192–197.
4. Dehority, B. A. 1996. A new family of entodiniomorph protozoa from the marsupial forestomach, with descriptions of a new genus and five new species. *J. Eukaryot. Microbiol.* 43: 285–295.
5. Dietz, J. M. 1984. Ecology and social organization of the maned wolf (*Chrysocyon brachyurus*). *Smithson. Contrib. Zool.* 392: 1–51.
6. Eisenberg, J. F., and K. H. Redford. 1999. *Mammals of the Neotropics: The Central Neotropics*. Univ. of Chicago Press, Chicago, Illinois.
7. Freeman, A., J. Kinsella, C. Cipolletta, S. L. Deem, and W. Karesh. 2004. Endoparasites of western lowland gorillas (*Gorilla gorilla gorilla*) at Bai Hokou, Central African Republic. *J. Wildl. Dis.* 40: 775–781.
8. Jácomo, A. T. d. A., L. Silveira, J. Alexandre, and F. Diniz-Filho. 2004. Niche separation between the maned-wolf (*Chrysocyon brachyurus*), crab-eating fox (*Dusicyon thous*) and the hoary-fox (*Dusicyon vetulus*) in the Cerrado of Central Brazil. *J. Zool.* 262: 99–106.
9. Kinsella, J. M., S. L. Deem, S. Blake, and A. S. Freeman. 2004. Endoparasites of African forest elephants (*Loxodonta africana cyclotis*) from the Republic of Congo and Central African Republic. *Comp. Parasitol.* 71: 104–110.
10. Rodden, M., F. Rodrigues, and S. Bestelmeyer. 2004. Maned wolf *Chrysocyon brachyurus*. In: Sillero-Zubiri, C., M. Hoffman, and D. W. McDonald (eds.). *Canids: Foxes, Wolves, Jackals and Dogs, Status Survey and Conservation Action Plan*, ed. IUCN/SSC Canid Specialist Group, Gland, Switzerland. P. 43.
11. Wasser, S. K. 2008. Lucky dogs: rescued from the pound, single-minded dogs sniff out the scat of endangered animals, trumping more technical tracking methods. *Nat. Hist.* 117: 48–53.
12. Wasser, S. K., B. Davenport, E. R. Ramage, K. E. Hunt, M. Parker, C. Clarke, and G. Stenhouse. 2004. Scat detection dogs in wildlife research and management: applications to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. *Can. J. Zool.* 82: 475–492.

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*Authors Queries*

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