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VERSITA

Morphologic characterization of *Sarcocystis* sp. sarcocysts from the Buffon's macaw (*Ara ambigua*)

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Abstract

A species of *Sarcocystis* is reported from two naturally infected Buffon's macaws (*Ara ambigua*) from Costa Rica. Only mature sarcocysts, measuring up to 950 µm in length and up to 75 µm in width, were observed. By light microscopy the sarcocyst wall was thin (< 1 µm thick) and smooth. The villar protrusions on the sarcocyst wall were up to 4.0 µm long and up to 0.6 µm wide; they were folded over the sarcocyst wall giving a thin-walled appearance. The microtubules in villar protrusions were smooth and confined to villar protrusions. Bradyzoites in sections were 4.0–5.9 × 0.8–1.8 µm in size. Structurally, sarcocysts from the macaw appeared different from sarcocysts of other avian species. This is the first report of *Sarcocystis* infection in this host.

Key words

Buffon's macaw, *Ara ambigua*, *Sarcocystis*, sarcocysts, coccidia, Costa Rica

Introduction

Species of *Sarcocystis* are apicomplexan parasites characterized by life-cycles requiring 2 hosts, a predator species and its prey (Dubey *et al*. 1989). Herbivores (prey) and carnivores (predator) serve, respectively, as the intermediate and definitive hosts. The definitive host becomes infected by ingesting the asexual stage (sarcocyst) encysted in the tissues (muscles) of the intermediate host. The sexual cycle occurs only in the carnivore host and it is restricted to the intestinal lamina propria. Typically, species of *Sarcocystis* exclusively parasitize a single intermediate host species. Although more than 100 species of *Sarcocystis* have been described, the life-cycles of only a few are known completely (Dubey *et al*. 1989).

Little is known of the species of *Sarcocystis* in birds, but two avian species have been well characterized: *Sarcocystis rileyi* cycles between ducks (*Anas clypeata*, the intermediate host) and the skunk (*Mephitis mephitis*, the definitive host); *Sarcocystis falcatula* employs passerine birds as intermediate hosts and the opossum (*Didelphis virginianus*) as the definitive host (Cawthorn *et al*. 1981; Box *et al*. 1984; Dubey *et al*. 1989, 2003). Two new species, *S. ramphastosi* and *S. sulfuratusi* were reported from the keel-billed toucan (*Ramphastos sulfuratus*) from South Africa by Dubey *et al*. (2004). Recently, we reported on the morphologic, biologic, and molecular characteristics of a macroscopic species of *Sarcocystis* in the African grey parrot *(Psittacus erithacus)* from Costa Rica (Dubey *et al*. 2006). Here, we report on the morphology of a species of *Sarcocystis* identified for the first time in the Buffon's macaw (*Ara ambigua*), also from Costa Rica.

Materials and methods

Two dead birds from an avian rescue, reproduction, and reintroduction facility, Amigos de las Aves, Rio Segundo de Alajuela, Costa Rica were submitted to the Departamento de Patología, Escuela Medicina Veterinaria, Universidad Nacional Autonoma, Costa Rica for diagnosis. Bird No. 1 (male, four-year-old) had died on August 9, 2000 and bird No. 2 (male, two-year-old) had died on September 10, 2004. Both birds were examined at necropsy and their tissues were fixed in 10% buffered neutral formalin. Routine histologic examination was performed on paraffin-embedded sections (5 µm) stained with hematoxylin and eosin (H and E). Paraffin blocks containing sarcocysts were sent to the Animal Parasitic Diseases Laboratory, United States Department of Agriculture, Beltsville, MD for further evaluation. For transmission elec-

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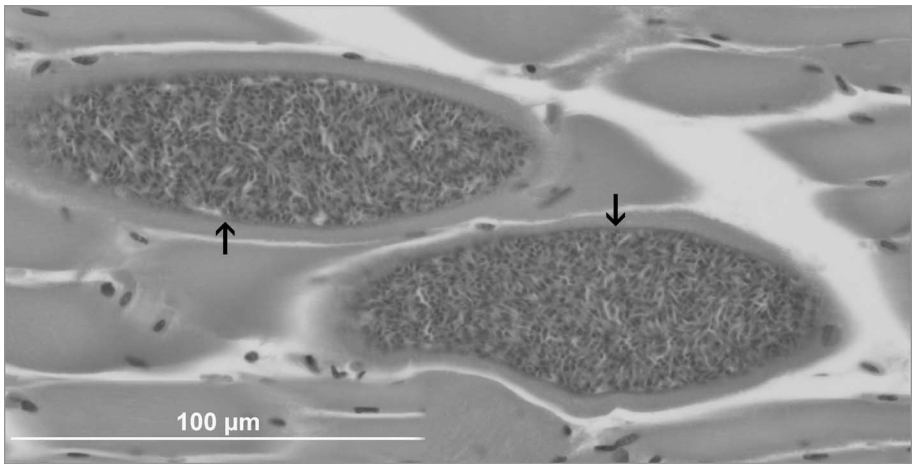

Fig. 1. Sarcocysts of *Sarcocystis* sp. in skeletal muscles of the naturally-infected macaw No. 2. Note thin sarcocyst walls (arrows); H and E

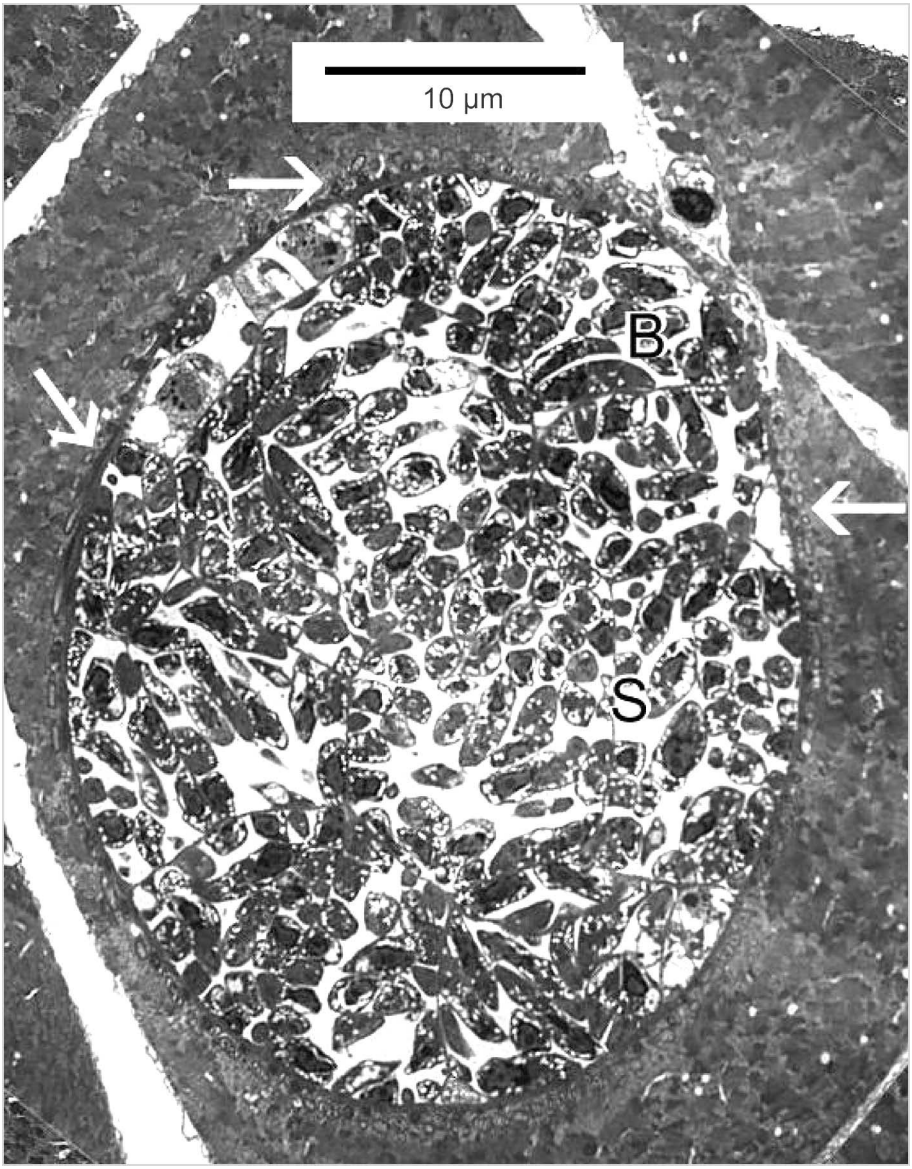

Fig. 2. TEM of a *Sarcocystis* sp. sarcocyst No. 1 from macaw No. 1. Note variability in structure of the villar protrusions (arrows) on the sarcocyst wall. Also note septa (S) dividing the sarcocysts into compartments that contain numerous bradyzoites (B)

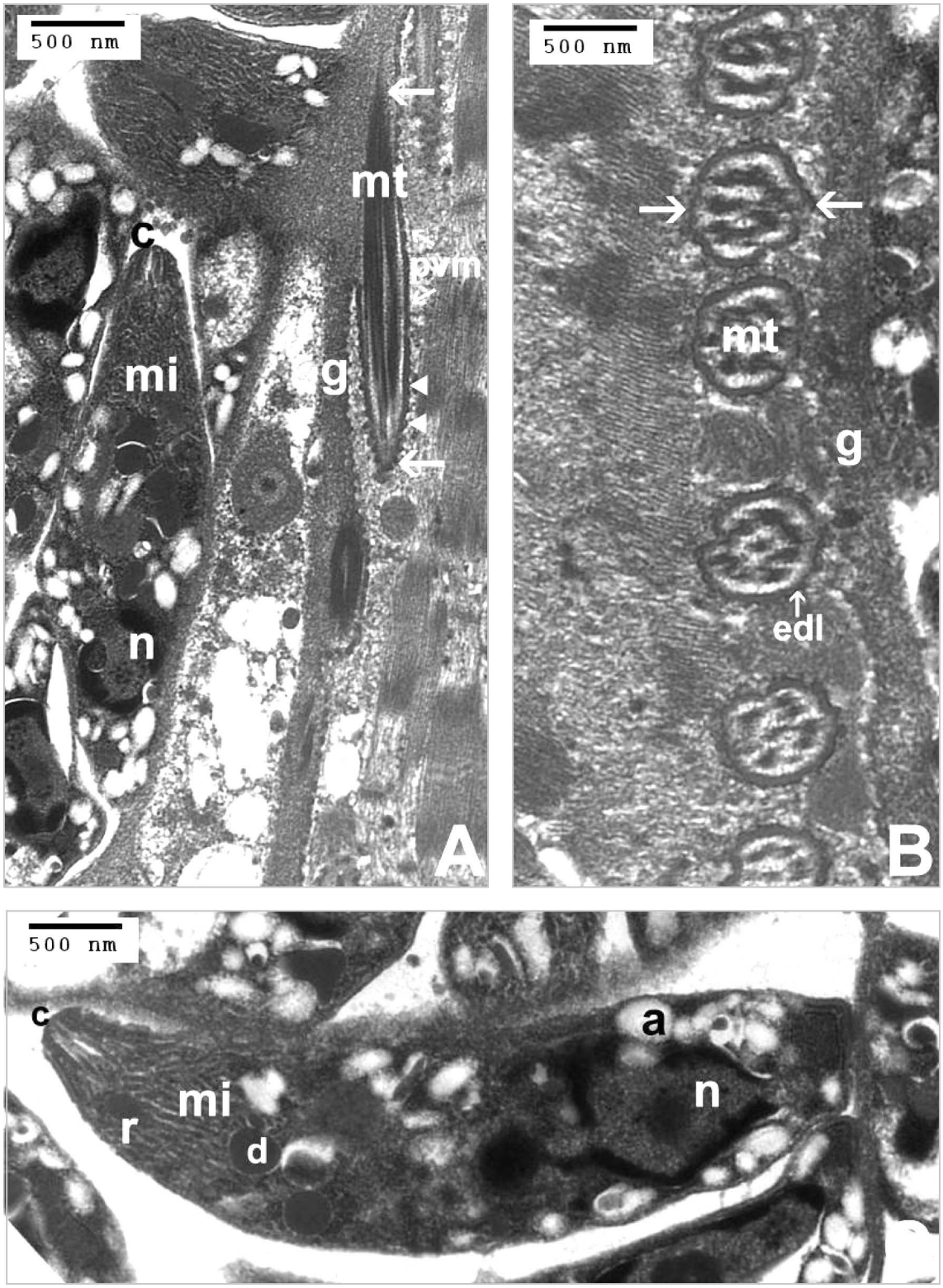

Fig. 3. TEM of a sarcocyst wall of *Sarcocystis* sp. in sarcocyst No.1 from macaw No. 1. **A**. Note longitudinal section of a villar protrusion (arrows), smooth ground substance (g), and longitudinal section of a bradyzoite. The villar protrusion has a wavy parasitophorous vacuolar membrane (pvm) that has undulations (arrowheads). The villar protrusion has numerous microtubules (mt) that are dense at the base of the villus. The mt do not extend into the ground substance. Also note the conoid (c), micronemes (mi), and the posteriorly located nucleus (n) of a bradyzoite. **B**. Villar protrusions cut in cross-section (arrows). Note electron-dense layer (edl) lining the parasitophorous vacuolar membrane and smooth microtubules, and ground substance. **C.** Longitudinal section of a bradyzoite containing a conoid, numerous micronemes, a rhoptry (r), dense granules (d), amylopectin (a), and a nucleus

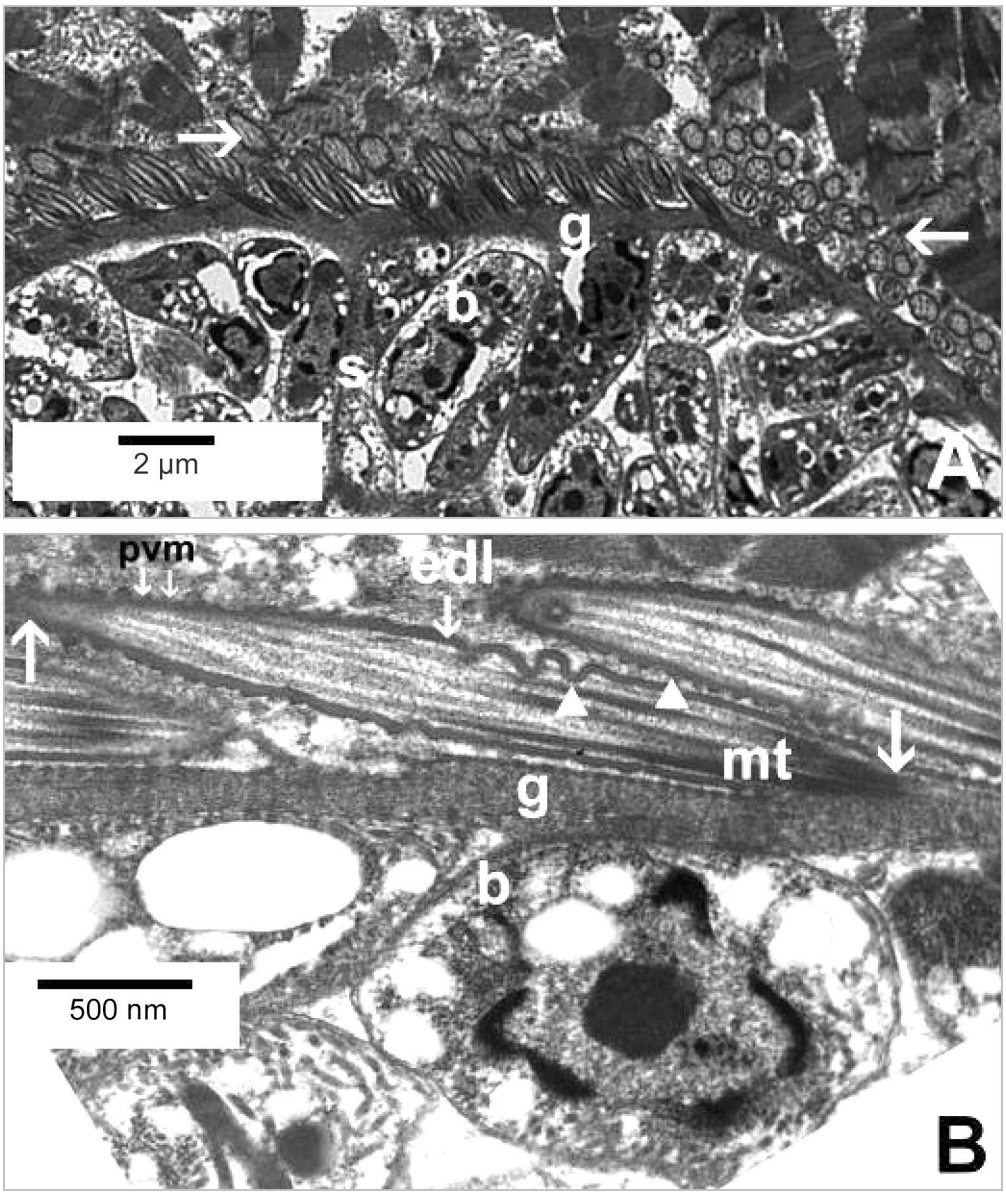

Fig. 4A. TEM of *Sarcocystis* sp. sarcocyst No. 3 from macaw No. 2. showing villar protrusions cut at different angles (arrows) giving a variable appearance of the cyst wall. Note smooth ground substance (g), numerous bradyzoites (b), and septum (s). **B.** TEM of sarcocyst wall of sarcocyst No. 4 from macaw No. 2. The parasitophorous vacuolar membrane (pvm) is convoluted and has indentations and protrusions (arrowheads) and is lined by an electron-dense layer (edl). The villar protrusions contain microtubules (mt) that are sparse at the tip and more dense at the base and do not extend into the ground substance. Also note a bradyzoite adjacent to the ground substance

tron microscopy (TEM), deparaffinized muscle tissue was processed and examined as reported previously (Dubey *et al*. 2006).

Results

Both birds died of causes unrelated to *Sarcocystis* infection. Microscopic sarcocysts were found in sections of muscles. Only a few sarcocysts were found in skeletal muscles of bird No. 1. Numerous sarcocysts were present in skeletal muscles of bird No. 2; a few sarcocysts were also seen in the heart. All sarcocysts were microscopic (Fig. 1). In 5 µm sections stained with H and E, the sarcocyst wall appeared thin (< 1 µm) and smooth. The sarcocyst interior was divided into compartments by septa. Sarcocysts measured up to 75 µm wide and up to 950 µm long. There was no host reaction around sarcocysts.

Sections of nine (two from bird No. 1 and seven from bird No. 2) sarcocysts were examined ultrastructurally (Figs 2–5). The sarcocyst wall contained conspicuous villar protrusions which were sloping, one side often being longer than the other. The outer layer of the sarcocyst, the parasitophorous vacuo-

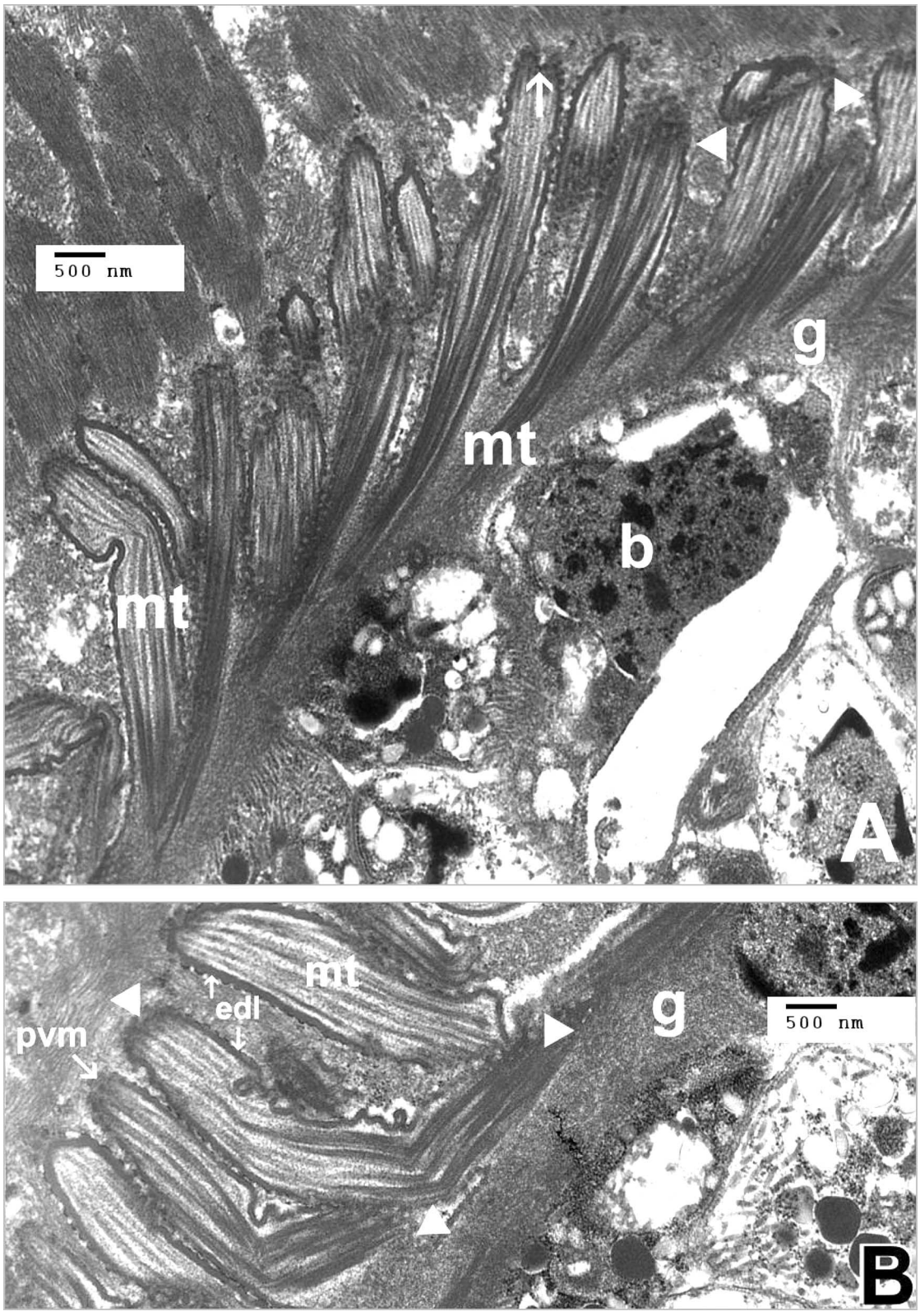

Fig. 5. TEM of cyst wall of sarcocyst No. 5 from macaw No. 2. Note longitudinally cut villus protrusions. **A.** Note sloping villar protrusions with a wavy parasitophorous vaculoar membrane. Terminal end of one villus appears to be bifid (arrow) and the tip of another villus is bent over (arrowheads). The microtubules (mt) do not extend into the ground substance (g). A bradyzoite (b) is present juxtaposed with the ground substance. **B.** Note one villus is bent at an angle (arrowheads). Also note wavy parasitophorous vacuolar membrane (pvm) lined by edl. The villar tips are not tapered. The microtubules do not extend into the ground substance

lar membrane, was wavy in outline and undulated (Figs 3–5). The pvm was lined by a 50–70 nm thick electron-dense layer (edl), thickest at the undulated areas projecting from the parasitophorous vacuolar membrane (Fig. 4B). These undulations occurred at irregular distances and were present on both sides of the parasitophorous vacuolar membrane (Fig. 4B). The villar protrusions were spaced at irregular intervals (Fig. 2). They appeared narrow at the tip and wider in the middle, and narrow at the base. The inner (or closed) ends of the villar protrusions were approximately half the length of the open ends. The villar protrusions were up to 3.9 µm long and up to 0.6 µm wide. They contained smooth microtubules (mt) that converged towards the base of the villar protrusions and that were more electron-dense at the base than at the tip of the villar protrusions (Figs 3–5). Microtubules were restricted to villar protrusions. The ground substance (g) appeared smooth and continued into the sarcocyst as septa. The ground substance layer measured up to 0.3 µm in width.

All sacrocysts were mature and contained fully formed bradyzoites. Longitudinally cut bradyzoites measured 4.0–5.9 × 0.8–1.8 µm (n = 20). Bradyzoites contained a conoid, micronemes, 1–2 rhoptries per section, subpellicular microtubules, and a posteriorly located nucleus (Fig. 3). Micronemes were numerous and located in the anterior third of the bradyzoite; most were arranged longitudinally. The nucleus was located in the posterior half of the parasite. Amylopectin granules were present in the posterior quarter of the bradyzoites.

Specimens deposited: Histological sections stained with H and E and toluidine blue from both birds were deposited in the United States National Parasite Collection (USNPC Nos. 099085 and 099086) United States Department of Agriculture, Beltsville, Maryland 20705, USA.

Discussion

Complete life-cycles of *Sarcocystis* are known for only a few species of animals, mostly those in livestock (Dubey *et al*. 1989). Most *Sarcocystis* species have been named based on their intermediate host occurrence and their structure. Among all diagnostic morphological criteria, the structure of the sarcocyst wall is most valuable for differentiating those species that share a given host. Dubey *et al*. (1989) and Dubey and Odening (2001) recognized 35 types of sarcocyst walls based on their structure. The sarcocyst wall of the parasites occurring in macaw was structurally distinct from those of *S. rileyi*, *S. falcatula* and *S. ramphastosi*. The sarcocyst wall in *S. rileyi* corresponds to type 21, characterized by cauliflower-like anastomosing villar protrusions (Dubey *et al*. 1989, 2003). By contrast, the sarcocyst wall in *S. falcatula* is thick, striated, with finger-like villar protrusions (Drouin and Mahrt 1980; Box *et al*. 1984; Dubey *et al*. 2000, 2001a, b, c; Luznar *et al*. 2001). The sarcocysts of *S. ramphastosi* are macroscopic and divided into two distinct zones, whereas the species in the macaw is microscopic. Genetic studies, using available primers, did not reveal clear cut differences among avian *Sarcocystis* species (Dubey *et al.* 2006).

The sarcocysts in macaw most closely resemble *S. sulfuratusi*, however, the villar protrusions in *S. sulfuratusi* have distinctive microtubules that extend halfway into the ground substance and that are more electron-dense in the ground substance layer than in the villar protrusions (Dubey *et al.* 2004). Additionally, the bradyzoites from macaw sarcocysts appear shorter than those in *S. sulfuratusi* and the micronemes in the bradyzoites are more numerous in this macaw parasite than they are in *S. sulfuratusi*. Whether these differences may have stemmed in part from fixation artifacts cannot be known with certainty, underscoring the need for additional comparative analyses.

Analysis of molecular variation among sarcocysts in avian species, including sarcocysts from a grey parrot from Costa Rica suggests that opossums of the New World genus *Didelphis* may transmit avian species in Costa Rica (Dubey *et al*. 2006). Alternatively, the hawks and falcons that naturally prey upon the parrots could be considered as candidate definitive hosts for this newly recognized parasite in the macaw and the African grey parrot.

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