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Abstract:	A novel coccidian species was discovered in the prostate of an Antechinus flavipes (yellow-footed antechinus) in South Australia, during the period of post-mating male antechinus immunosuppression and mortality. This novel coccidian is unusual because it develops extra-intestinally and sporulates endogenously within the prostate gland of its mammalian host. Histological examination of prostatic tissue revealed dense aggregations of spherical and thin-walled tetrasporocystic, dizoic sporulated coccidian oocysts within tubular lumina, with unsporulated oocysts and gamogonic stages within the cytoplasm of glandular epithelial cells. This coccidian was observed occurring concurrently with dasyurid herpesvirus 1 infection of the antechinus' prostate. Eimeria-specific 18S small subunit ribosomal DNA PCR amplification was used to obtain a partial 18S rDNA nucleotide sequence from the antechinus coccidian. Bayesian phylogenetic analysis based on 18S rDNA gene sequences revealed that the novel coccidian clusters with reptile-host coccidians, forming an ancestral basal lineage of the eimeriid clade. The species has been named Eimeria taggarti n. sp., based on both sporulated oocyst morphology and molecular characterization. It is suspected that E. taggarti is sexually transmitted via excretion of sporulated oocysts and/or free sporocysts with prostatic secretions in semen.

EIMERIA TAGGARTI* N. SP., A NOVEL COCCIDIAN (APICOMPLEXA: EIMERIORINA) IN THE PROSTATE OF AN *ANTECHINUS FLAVIPES

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ABSTRACT: A novel coccidian species was discovered in the prostate of an *Antechinus flavipes* (yellow-footed antechinus) in South Australia during the period of post-mating male antechinus immunosuppression and mortality. This novel coccidian is unusual because it develops extra-intestinally and sporulates endogenously within the prostate gland of its mammalian host. Histological examination of prostatic tissue revealed dense aggregations of spherical and thin-walled tetrasporocystic, dizoic sporulated coccidian oocysts within tubular lumina, with unsporulated oocysts and gamogonic stages within the cytoplasm of glandular epithelial cells. This coccidian was observed occurring concurrently with dasyurid gammaherpesvirus 1 infection of the antechinus' prostate. *Eimeria*-specific *18S* small subunit ribosomal DNA PCR amplification was used to obtain a partial *18S* rDNA nucleotide sequence from the antechinus coccidian. Bayesian phylogenetic analysis based on *18S* rDNA gene sequences revealed that the novel coccidian clusters with reptile-host coccidians, forming an ancestral basal lineage of the eimeriid clade. The species has been named *Eimeria taggarti* n. sp., based on both sporulated oocyst morphology and molecular characterization. It is suspected that *E. taggarti* is sexually

transmitted via excretion of sporulated oocysts and/or free sporocysts with prostatic secretions in semen.

This report describes a novel eimeriid coccidian species discovered in the prostate of an *Antechinus flavipes*. This new parasite is unusual because it develops extra-intestinally and sporulates endogenously within the prostate gland of its mammalian host, whereas eimeriid coccidians infecting homeothermic vertebrates most commonly develop within their host's gastrointestinal tract and sporulate in the external environment. This novel parasite's life cycle also provides an interesting example of niche specialisation within a semelparous host, by proliferating in an enlarged reproductive gland of the immunosuppressed male antechinus – presumably to ensure sexual transmission prior to its host's post-reproductive mortality.

Antechinus are small insectivorous Australian marsupials of the family Dasyuridae. The life history of all species within the genus *Antechinus* features a semelparous pattern of reproduction: a 2-wk mating period in late winter, followed by the stress-related synchronous annual die-off of the entire male population at approximately 11.5 mo of age (Barker et al., 1978; Braithwaite and Lee, 1979; Lee et al., 1982; Bradley, 2003; Tyndale-Biscoe, 2005; Naylor et al., 2008; Ladds, 2009). A number of pathogens and parasites take advantage of this natural phenomenon of annual glucocorticoid-mediated immunosuppression of male hosts, including a gammaherpesvirus that targets the prostate of male antechinus (Amery-Gale et al., 2014).

Among other potential pathogens which might also take advantage of the enlarged reproductive organs of immunosuppressed male hosts during their breeding season are eimeriid coccidians. Coccidia infecting homeothermic vertebrates most commonly complete their endogenous development within the cytoplasm of

enterocytes (Garner et al., 2006), with resistant oocysts generally being shed in the feces of their host to sporulate in the external environment (Overstreet, 1981; Ogedengbe et al., 2011). However, some eimeriid coccidian species have adapted their life cycles to include development in extra-intestinal tissues (Mugridge et al., 1999), and parenteral development of eimeriid parasites in homeotherms may be more prevalent than commonly thought (Novilla et al., 1981). During the course of investigations into herpesvirus infection in a male *Antechinus flavipes* (Marsupialia, Dasyuridae) (Amery-Gale et al., 2014), a novel coccidian undergoing endogenous sporulation was unexpectedly observed in histological sections of the prostate.

MATERIALS AND METHODS

Specimen collection and examination

An adult male *A. flavipes* was captured toward the end of the antechinus breeding period in August 2011 using an Elliott trap (Elliott Scientific, Upwey, Victoria, Australia) from a free-ranging population in Inman Valley, Fleurieu Peninsula, South Australia, Australia (35°28’S, 138°29’E). Following euthanasia, samples of liver, lung, kidney, spleen and prostate were collected and stored at -20 C. Heart, lung, kidneys, adrenal glands, spleen, liver, pancreas, bladder, diaphragm, hind leg skeletal muscle, brain, skin punch biopsies, testes, penis, prostate and bulbourethral glands were fixed in 10% phosphate-buffered formalin.

Formalin fixed tissues were processed and stained with hematoxylin and eosin for histological examination. Measurements of 50 sporulated oocysts and 30 sporocysts were obtained using a calibrated ocular micrometer and an oil-immersion 100 × objective with an Olympus BH2 light microscope (Olympus Corporation, Tokyo, Japan) equipped with Nomarski interference-contrast optics (Carl Zeiss Microscopy, Jena, Germany). Measurements are presented herein in µm as means

followed parenthetically by range and standard deviation. Ultrathin sections of fixed prostatic tissue were stained with uranyl acetate and Reynold's lead citrate for examination using a CM10 transmission electron microscope (Philips Electron Optics, Eindhoven, Netherlands). Large intestinal contents and feces that had been stored in potassium dichromate (for 7 mo) were screened for the presence of coccidian oocysts using flotation over saturated zinc sulphate and light microscopy. Histological slides and formalin fixed tissues have been deposited in the Queensland Museum, Brisbane, Queensland, Australia (QM syntype registration numbers G466192-3, voucher tissues G466194).

DNA extraction, PCR amplification and sequencing

A small piece of prostatic tissue (approximately 125 mg) that had been previously homogenized for herpesvirus testing (Amery-Gale et al., 2014) was further pulverized manually with phosphate-buffered saline using a sterile mortar and pestle. Genomic DNA was then extracted using the DNeasy Tissue Kit spin-column protocol for animal tissues (Qiagen, Hilden, Germany), incorporating oocyst and sporocyst wall lysis by incubation with proteinase K at 56 C for 2 hr.

Nuclear *18S* small subunit ribosomal DNA (*18S SSU* rDNA) was polymerase chain reaction (PCR) amplified using the *Eimeria*-specific primers 18SF1 and 18SR2 (Zhao and Duszynski, 2001). The 25 µL reaction mixtures contained 1.25 U Go*Taq* Flexi DNA polymerase (Promega, Madison, Wisconsin), 5 µL 5x Green Go*Taq* Flexi Buffer, 50 µM each deoxynucleotide, 2.5 mM $MgCl_2$, 1 µM of each primer (18SF1 and 18SR2; GeneWorks, Adelaide, Australia), 5 µL DNA template (DNA extracted from the prostatic tissue sample) and MiliQ H_2O to volume. PCR amplification was performed under the following thermocycling conditions: initial denaturation at 95 C in a 4 min precycle; then denaturation at 93 C for 45 sec, primer annealing at 63 C for

45 sec, and elongation at 72 C for 90 sec for 35 cycles; then final extension at 72 C for 7 min to allow complete elongation.

PCR products (approximately 1,500 bp in length) were visualized using 1.5% agarose gel electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, California), run in Tris-borate-EDTA buffer (89 mM Tris–HCl, 89 mM boric acid, 2 mM ethylenediaminetetraacetic acid) at 100 V for 2 hr. Amplicons were purified from PCR solution using the UltraClean PCR Clean-Up DNA Purification Kit (Mo Bio Laboratories, Carlsbad, California), and DNA quantities were estimated using a spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). PCR products were sequenced in both directions using BigDye Terminator version 3.1 chemistry (Applied Biosystems, Carlsbad, California) with both forward and reverse amplification primers (18SF1 and 18SR2).

Phylogenetic analysis

GENEious Bioinformatics software (Biomatters Ltd., Auckland, New Zealand) was used to align obtained sequences, which were compared with publicly available sequences in the GenBank database (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/genbank/) using the nucleotide BLASTN online algorithm. GENEious was used to align the consensus sequence with the following 59 homologous partial *18S* rRNA gene sequences selected from GenBank to represent the major lineages of related coccidian parasites: *Acroeimeria* cf. *tarentolae* KR360731, *Acroeimeria sceloporis* KR360735, *Babesia microti* AB032434, *Besnoitia besnoiti* AF109678, *Caryospora bigenetica* AF060975, *Choleoeimeria gallotiae* KR360728, *Choleoeimeria scincorum* KR360730, *Choleoeimeria wiegmanniana* KR360733, *Cyclospora cayetanensis* AF111183, *Cyclospora colobi* AF111186, *Cyclospora papionis* AF111187, *Eimeria alabamensis* AF291427,

Eimeria albigulae AF307880, *Eimeria antrozoi* AF307876, *Eimeria arizonensis* AF307878, *Eimeria arnyi* AY613853, *Eimeria bovis* U77084, *Eimeria chaetodipi* AF339489, *Eimeria dipodomysis* AF339490, *Eimeria eutropidis* KR360729, *Eimeria falciformis* AF080614, *Eimeria gruis* AB544336, *Eimeria langebarteli* AF311640, *Eimeria leucopi* AF339491, *Eimeria macropodis* JQ392575, *Eimeria mitis* U67118, *Eimeria mivati* U76748, *Eimeria necatrix* U67119, *Eimeria nieschulzi* U40263, *Eimeria onychomysis* AF307879, *Eimeria papillata* AF311641, *Eimeria peromysci* AF339492, *Eimeria quokka* KF225636, *Eimeria ranae* EU717219, *Eimeria reedi* AF311642, *Eimeria reichenowi* AB544308, *Eimeria rioarribaensis* AF307877, *Eimeria separata* AF311643, *Eimeria setonicis* KF225639, *Eimeria steinhausi* KR360732, *Eimeria taggarti* KX130898, *Eimeria telekii* AF246717, *Eimeria tenella* U67121, *Eimeria tokayae* KR360734, *Eimeria trichosuri* FJ829320, *Eimeria tropidura* AF324217, *Frenkelia microti* AF009244, *Goussia janae* AY043206, *Goussia neglecta* FJ009242, *Goussia noelleri* FJ009241, *Hammondia hammondi* AF096498, *Hyaloklossia lieberkuehni* AF298623, *Cystoisospora felis* L76471, *Isospora gryphoni* AF080613, *Isospora robini* AF080612, *Lankesterella minima* AF080611, *Neospora caninum* U17346, *Sarcocystis mucosa* AF109679, *Theileria parva* AF013418, *Toxoplasma gondii* U12138. A Bayesian posterior probability phylogenetic tree was generated from this alignment using MrBayes software using a GTR substitution model with a chain length of 1,000,000, a burn-in length of 100,000 and a subsampling frequency of 200 (Huelsenbeck and Ronquist, 2001). Two piroplasms (*B. microti* and *T. parva*) were used as outgroup taxa based on the recommendations of Morrison et al. (2004).

DESCRIPTION

***Eimeria taggarti* n. sp.**

(Figs. 1-5)

Description of oocyst (n = 50): Sporulated oocysts spheroidal and thin-walled, containing 4 dizoic sporocysts; maximum diameter 15.6 (14.3-16.9, 0.63); smooth, flexible oocyst wall often deformed or tightly moulded to enclosed sporocysts.

Description of sporocysts (n = 30): Ellipsoidal; length 8.95 (7.5-10.1, 0.72); width 7.0 (6.2-7.8, 0.43); sporocyst L/W ratio = 1.3:1; prominent sporocyst residuum; no Stieda, substieda or parastieda bodies observed; 2 banana-shaped sporozoites arranged in parallel.

Endogenous development: Dense aggregations of numerous sporulating and fully sporulated tetrasporocystic oocysts and free sporocysts located extracellularly within prostatic tubular lumina, with unsporulated oocysts, zygotes, macrogametocytes and microgametocytes developing intracytoplasmically within prostatic glandular epithelial cells, localized above the host cell nucleus. The aggregations of sporogonic and gamogonic stages cause distortion and compression of the epithelia of infected prostatic tubules, with intracytoplasmic endogenous stages occupying most of the volume of infected host cells.

Taxonomic summary

Type host: Antechinus flavipes flavipes Waterhouse, 1838 (Marsupialia, Dasyuridae; yellow-footed antechinus).

Type locality: Inman Valley, Fleurieu Peninsula, South Australia, Australia (35°28’290’’S, 138°29’288’’E).

Site of infection: Prostate.

Type material deposited: Syntype histological slides in QM G466192, G466193. Symbiotype host tissues including prostate in QM G466194, and a photovoucher of the host has been linked to this registration number in the Queensland Museum

database.

Etymology: The specific epithet is given in honor of Dr. David A. Taggart, for his contributions to the fields of marsupial reproduction and conservation, and for his mentoring of the first author.

GenBank™ accession number for partial 18S rDNA nucleotide sequence: KX130898.

Remarks

Eimeria taggarti was observed concurrently and often in close association with dasyurid gammaherpesvirus 1 infection of the *A. flavipes* prostate gland. No evidence of coccidian infection was found in any other tissue examined histologically, and no coccidian oocysts were found by flotation of either large intestinal contents or feces. As the oocysts are not shed in feces, *E. taggarti* has been described from histological sections of the prostate.

The *Eimeria*-specific *18S* rDNA PCR robustly amplified the targeted DNA extracted from the *A. flavipes* prostatic tissue. When the 1,086 bp consensus nucleotide sequence obtained by alignment of both strands of this PCR product was compared by homology sequence searching against the GenBank database using nucleotide BLAST, the first 100 matches were to the *18S* rRNA genes of coccidia within the family Eimeriidae. The closest matches were to the partial *18S* rRNA gene sequences of the reptile-hosted coccidians *E. eutropidis*, *E. tokayae*, *A.* cf. *tarentolae*, *C. scincorum* and *A. sceloporis* (Megía-Palma et al., 2015), with 96-97% nucleotide identity. All of these reptile-hosted coccidians have bivalved sporocysts lacking Stieda bodies, and all are described from fecal samples collected from lizards (Megía-Palma et al., 2015). In the Bayesian posterior probability phylogenetic tree generated in this study, *E. taggarti* was found to cluster with these reptile-hosted coccidians (Fig. 6), forming a basal lineage of the family Eimeriidae ancestral to the clade

comprising all Stieda body-bearing eimeriid coccidia (Morrison et al., 2004; Jirků et al., 2009b). *Eimeria taggarti* has been placed within *Eimeria* pending future revision of Eimeriidae, as some currently recognized coccidian groups are not natural evolutionary clusters (Morrison et al., 2004). On BLAST analysis the closest marsupial-hosted coccidian to *E. taggarti* in the GenBank database is the partial *18S* rRNA gene sequence of *E. trichosuri* from the mountain brushtail possum (*Trichosurus cunninghami*) (Power et al., 2009), with 94% nucleotide identity.

DISCUSSION

Coccidian parasites (Apicomplexa: Eimeriorina) are traditionally classified and identified based primarily on host range and taxonomy; life cycle; and morphological characteristics of sporulated oocysts (the exogenous stage), primarily the number of sporocysts within each oocyst, the number of sporozoites per sporocyst, and oocyst size and shape (Barta et al., 1997; Jirků et al., 2002; Hill et al., 2012). Sporulated oocysts of *Eimeria* species contain 4 sporocysts, each enclosing 2 sporozoites (tetrasporocystic, dizoic oocyst morphology) (Mugridge et al., 1999; Jirků et al., 2009b; Power et al., 2009; Ogedengbe et al., 2011; Megía-Palma et al., 2015). However, the phylogenetic pattern within Eimeriorina based on molecular data from *18S SSU* rDNA gene sequences shows a strong correlation with sporocyst excystation structures (the mode of release of sporozoites from sporocysts) rather than the phenotypic features of sporulated oocysts traditionally used for the generic classification of coccidia (Modrý et al., 2001; Jirků et al., 2009b). Several authors have suggested that traditional classification of Apicomplexa based on sporulated oocyst morphology is artificial and limited, creating a confusing incongruity between the current classification system and accepted molecular phylogenetic analyses (Modrý et al., 2001; Jirků et al. 2002).

Eimeriid coccidia are typically intestinal oocyst-forming species with fecal-oral transmission, primarily homoxenous, and have sporozoites that excyst through a dissolving complex of Steida and substeida bodies in the sporocyst wall (Barta, 2001; Jirků et al., 2002). Extra-intestinal coccidian infections are more prevalent in poikilothermic hosts than in homeotherms (Novilla et al., 1981; Overstreet, 1981; Dyková et al., 1983; Athanassopoulou-Raptopoulou and Vlemmas, 1986), and eimeriid coccidia infecting poikilotherms commonly undergo endogenous sporulation (Dyková and Lom, 1981; Overstreet, 1981). Endogenous sporulation occurs in all extra-intestinal fish-inhabiting coccidian species and Paperna (1995) suggested that in situ sporulation may be an inevitable consequence of an extra-intestinal location. Oocysts from amphibian, reptile, fish and aquatic invertebrate coccidians are sometimes very thin-walled and fragile (Duszynski and Wilber, 1997). The genera *Goussia* (both intestinal and extra-intestinal coccidia parasitizing aquatic poikilotherm vertebrates – fish, amphibians and a crocodilian), *Choleoeimeria* (biliary coccidia infecting reptiles with ellipsoidal oocysts) and *Acroeimeria* (intestinal coccidia infecting reptiles with spheroidal oocysts) within Eimeriorina exhibit thin-walled tetrasporocystic, dizoic oocysts that usually complete sporulation endogenously and excyst using a curved longitudinal suture dividing the bivalved sporocyst wall (which lacks a Stieda body) (Dyková and Lom, 1981; Abollo et al., 2001; Jirků et al., 2002, 2009a; Jirků and Modrý, 2006; El-Mansy, 2008; Jirků et al., 2009a; Megía-Palma et al., 2015).

Parenteral coccidia have also been reported in the uterus of impala (*Aepyceros melampus*), the uterus and placenta of hippopotamus (*Hippopotamus amphibius*), the uterus and male genital tract of golden hamsters (*Mesocricetus auratus*), the mammary glands of long-clawed shrews (*Sorex unguiculatus*), the liver of several

mammal species including rabbits (*Oryctolagus cuniculus*), mink (*Mustela lutreola),* pigs (*Sus scrofa*) and chamois (*Rupicapra rupicapra)* and the lymph nodes of sheep (*Ovis aries*) and goats (*Capra hircus),* such that extra-intestinal development of coccidia in mammals is no longer considered unusual (Desser, 1978; Novilla et al., 1981; Hrudka et al., 1983; Duszynski and Marquardt, 2003). *Eimeria neitzi* completes its life cycle in the uterus of the impala, inhabiting the distal portions of uterine glands and adjacent surface epithelium, with oocysts sporulating endogenously while still inside host cells (but in most cases with no serious deleterious effects on reproduction) (McCully et al., 1970). Unsporulated oocysts have been found in the epididymal fluid of a wapiti (*Cervus canadensis nelsoni*), with the coccidian developing in the upper seminal ducts and tail of the ductus epididymidis, making venereal transmission a possibility (Hrudka et al., 1983).

Amongst Australian mammals, disseminated coccidiosis has been described in short-beaked echidnas (Monotremata; *Tachyglossus aculeatus*), with unsporulated oocysts and gamonts within alveoli in the lungs, granulomas in the liver and intestinal epithelial cells, and schizonts and merozoites in the liver, lungs, heart, kidneys, spleen and intestines (Dubey and Hartley, 1993). Protistans in the extraintestinal tissues of the echidnas were unidentified, but appeared structurally similar to the intestinal coccidia of echidnas: *Eimeria tachyglossi*, *Eimeria echidnae* and *Octosporella hystrix* (Dubey and Hartley, 1993). Hepatic-intestinal coccidiosis with coccidial cholangitis and schizonts within granulomas in the liver has been reported in several macropodid marsupials including tammar wallabies (*Macropus eugenii*), Parma wallabies (*Macropus parma*) and black-striped wallabies (*Macropus dorsalis*), with schizonts also found within the mesenteric lymph nodes in black-striped wallabies (Ladds, 2009). Hepatic involvement is common in tammar wallabies with coccidiosis, with

schizogony in the liver, free merozoites and gametogony in the bile ducts and oocysts released into bile (Ladds, 2009). Coccidian gamonts have also been reported within the walls and lumina of blood vessels in the liver and intestine of a young eastern barred bandicoot (*Perameles gunnii*) – another marsupial (Ladds, 2009).

Eimeria taggarti is distinguished using both sporulated oocyst morphology and molecular sequence data. This study obtained a partial *18S* rDNA sequence of a novel eimeriid species from a previously unsampled host family, hence providing a potential link that can be used to further elucidate coccidian phylogeny and clarify the evolutionary relationships of these parasites in more detail. This new sequence enhances the taxonomic resolution of the poorly studied marsupial eimeriids, although *E. taggarti* clusters separately from the marsupial clade in the phylogenetic tree generated in this study (Fig. 6).

Phylogenetic analysis based on *18S* rRNA gene sequences places *E. taggarti* within the eimeriid clade, but in a cluster with the coccidians of reptiles. The reptilian-hosted coccidia of the genus *Choleoeimeria* undergo endogenous development within biliary epithelial cells and then sporulate in the host's gall bladder, while *Acroeimeria* spp. undergo their endogenous development epicytoplasmically in the microvillous zone of the intestinal epithelium and then sporulate exogenously (Paperna and Landsberg, 1989; Megía-Palma et al., 2015). Eimeriid species parasitizing lizards that undergo their endogenous development intracytoplasmically in the intestinal epithelium and have spheroidal oocysts have retained the generic name *Eimeria*, albeit incertae sedis (Megía-Palma et al., 2015). In the phylogenetic analyses of Megía-Palma et al. (2015), the evolutionary origin of these *Eimeria*-like species isolated from reptiles was confirmed to be independent from that of eimeriids infecting mammals and birds, as originally proposed by

Paperna and Landsberg (1989) based on the morphological and developmental characteristics of their oocysts (Megía-Palma et al., 2015). The ellipsoidal oocysts of *Choleoeimeria* spp. and more spheroidal oocysts of the genus *Acroeimeria* both contain 4 sporocysts lacking Stieda bodies that instead excyst via a bivalvate suture (Megía-Palma et al., 2015). No Stieda bodies were observed in the spherical endogenously sporulating oocysts of *E. taggarti* in the histological sections of prostatic tissue from their mammalian *A. flavipes* host, but the presence of a longitudinal suture dividing a bivalved sporocyst wall could not be confirmed or refuted by examination of the material available in this study. The phylogenetic analyses of this study did, however, place a mammalian-host eimeriid parasite with a cluster of reptile-hosted coccidians with bivalved sporocysts lacking Stieda bodies, in a clade that was previously thought to be reptile-specific (Megía-Palma et al., 2015).

The infected male *A. flavipes* host was collected in August 2011 during the post-mating period of synchronous annual male immunosuppression and mortality. Infected prostatic tubules often exhibited epithelial cell hyperplasia, but no evidence of inflammatory cell infiltration or any other inflammatory response was observed, despite heavy infections within individual tubules, consistent with a profoundly immunosuppressed host. It is likely that *E. taggarti* is sexually transmitted via excretion of sporulated oocysts and/or free sporocysts with prostatic secretions in semen. The site of infection in female antechinus is unknown, as are almost all details of the life cycle of this novel parasite, including how transmission between host generations occurs. Generations of mature male antechinus are separated by several months, eliminating the possibility of horizontal transmission of the parasite between generations of male hosts. However transmission within a single generation of antechinus could occur during their prolonged and polygamous mating (with

intromission lasting up to 12 hr at a time), when sperm from multiple males is stored in the isthmus of the female's oviduct for up to 2 wk prior to ovulation, providing an opportunity for host oocyte and zygote/embryo exposure at this site (Taggart and Temple-Smith, 1991; Shimmin et al., 2000). Coccidia from terrestrial vertebrates generally have thick, resistant oocyst walls which have evolved to persist in the external environment (Dyková and Lom, 1981; Duszynski and Wilber, 1997; Zhao et al., 2001; Hill et al., 2012), whereas the coccidian found in the prostate of *A. flavipes* appears to have a thin, flexible oocyst wall that is probably less resistant to desiccation than typical eimeriid oocysts from mammals. This makes persistence of infectious oocysts in the environment for several months between generations of male hosts unlikely, although the existence of a vector, intermediate or paratenic host cannot be ruled out. One possibility is that sporulated oocysts are ingested by invertebrates feeding on the decomposing carcasses of male antechinus following their post-mating mortality and that these invertebrates then become the prey of female antechinus, resulting in ingestion of the oocysts/sporocysts by the females. Whether transmission is venereal or via invertebrate paratenic hosts, the parasite is probably vertically transmitted via female antechinus to be maintained in its definitive host population despite the absence of the prostate gland (the site of sexual reproduction and completion of the coccidian life cycle) for part of the year. It may be that the parasite is transmitted in utero resulting in congenital infection of offspring as has been demonstrated for *T. gondii* (Hide et al., 2009).

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Figure 1. Prostate of *Antechinus flavipes*, H&E x 200, showing sporulating oocysts of *Eimeria taggarti* n. sp. within tubular lumina (outlined by solid lines), basophilic intranuclear dasyurid gammaherpesvirus 1 inclusions within cytomegalic glandular epithelial cells (outlined by dashed lines), and normal prostatic epithelium (in the lower right-hand corner).

Figure 2. Unsporulated *Eimeria taggarti* oocysts within prostatic epithelial cells and fully sporulated oocysts within the lumen of prostatic tubules of *Antechinus flavipes* H&E x 400. Zygotes, macrogametocytes and microgametocytes of *E. taggarti* are

also present within glandular epithelial cells. The zygotes and unsporulated oocysts are composed largely of a prominent nucleus with a distinct nucleolus, while the rare microgametocytes (arrow) are characterized by multiple peripherally arranged nuclei, and the macrogametocytes feature basophilic stippling.

Figure 3. Gamonts and tetrasporous, dizoic oocysts of *Eimeria taggarti* within a prostatic tubule of *Antechinus flavipes*, H&E x 400. Macrogametocytes (arrows) with their prominent basophilic stippling can be observed around the periphery of the tubule, while sporulated oocysts occupy the majority of space within the tubule lumen.

Figure 4. Transmission electron micrograph (x 16,000) of apicomplexan sporocysts containing 2 sporozoites in the prostate of *Antechinus flavipes*. The nucleus and apical complex (arrow) of the sporozoite to the left are clearly visible.

Figure 5. Gamonts and unsporulated oocysts of *Eimeria taggarti* within prostatic epithelial cells of *Antechinus flavipes*, H&E x 400. Nuclei of infected host cells appear deformed and compressed by the intracytoplasmic unsporulated oocysts and zygotes (arrows), which take up almost the entire host cell volume. Overt proliferation of glandular epithelial cells of an infected prostatic tubule is apparent in the top left-hand corner of this photomicrograph.

Figure 6. Phylogenetic tree for coccidia based on Bayesian posterior probability inferred from the alignment of partial *18S* rRNA gene sequences for 59 representative taxa and *Eimeria taggarti*. Posterior probabilities are indicated at branch points, and the class, infraclass or order of the definitive hosts of species within the family Eimeriidae are indicated to the right. *Eimeria taggarti* and its host species are highlighted in bold.

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Figure

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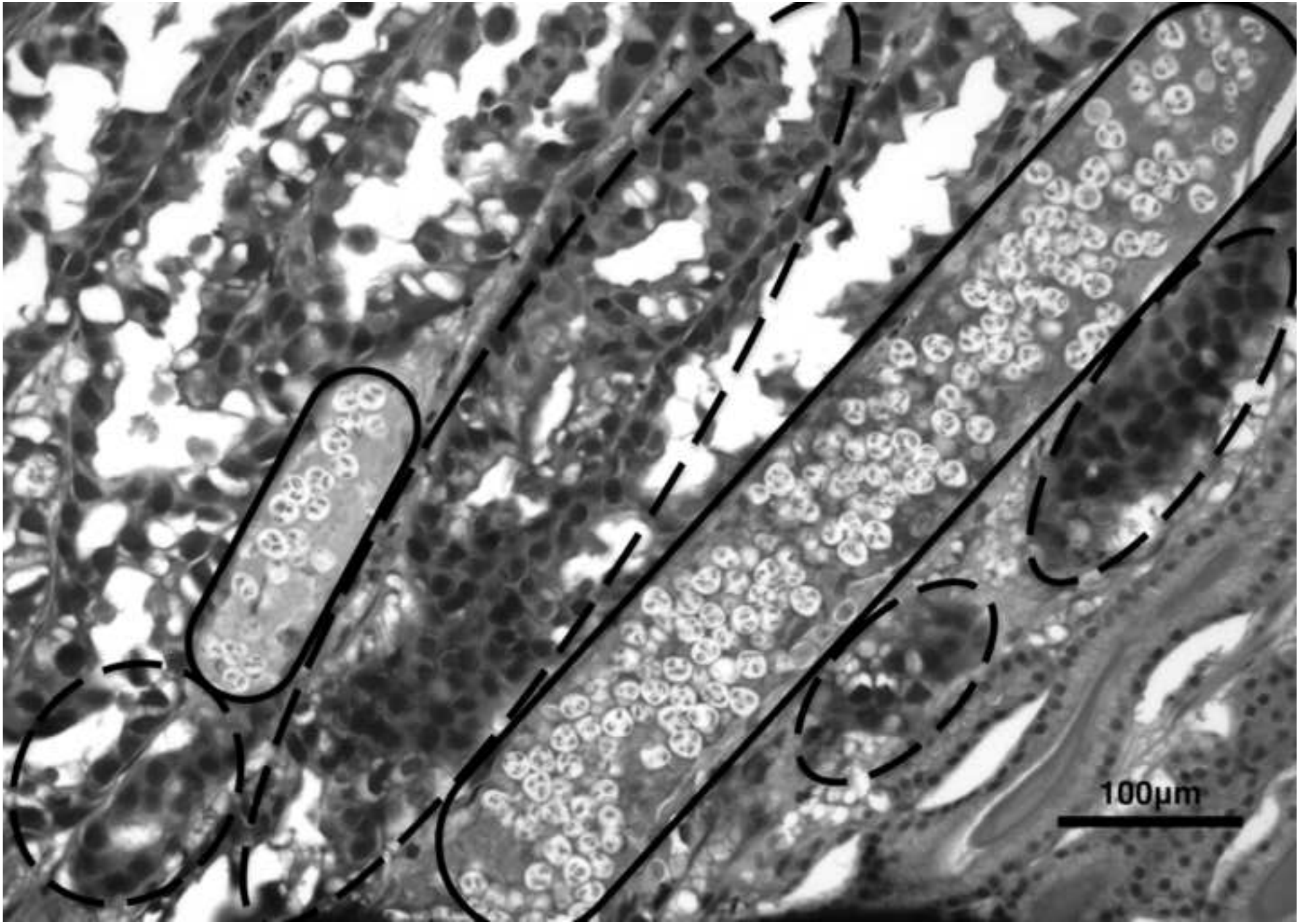

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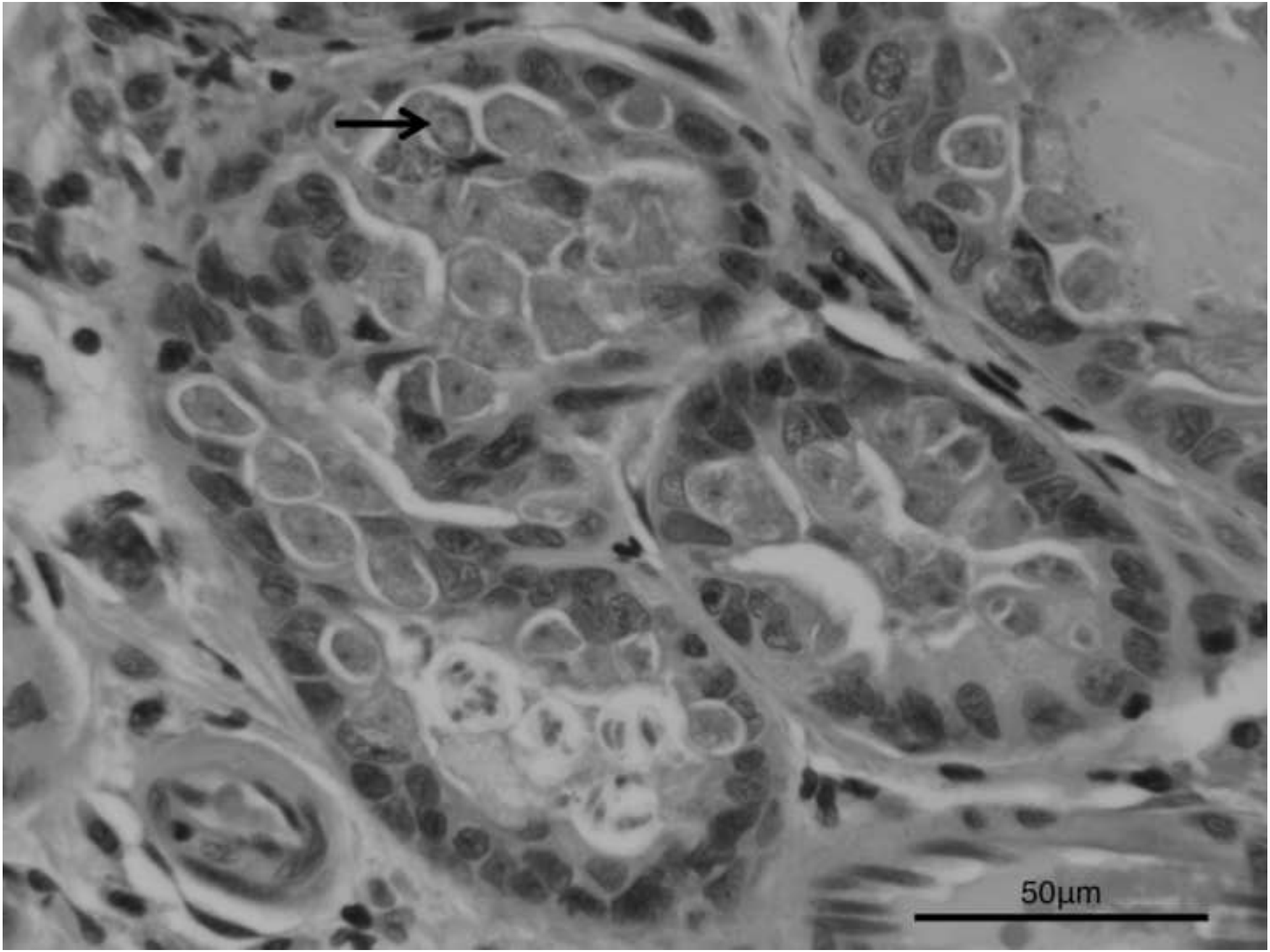

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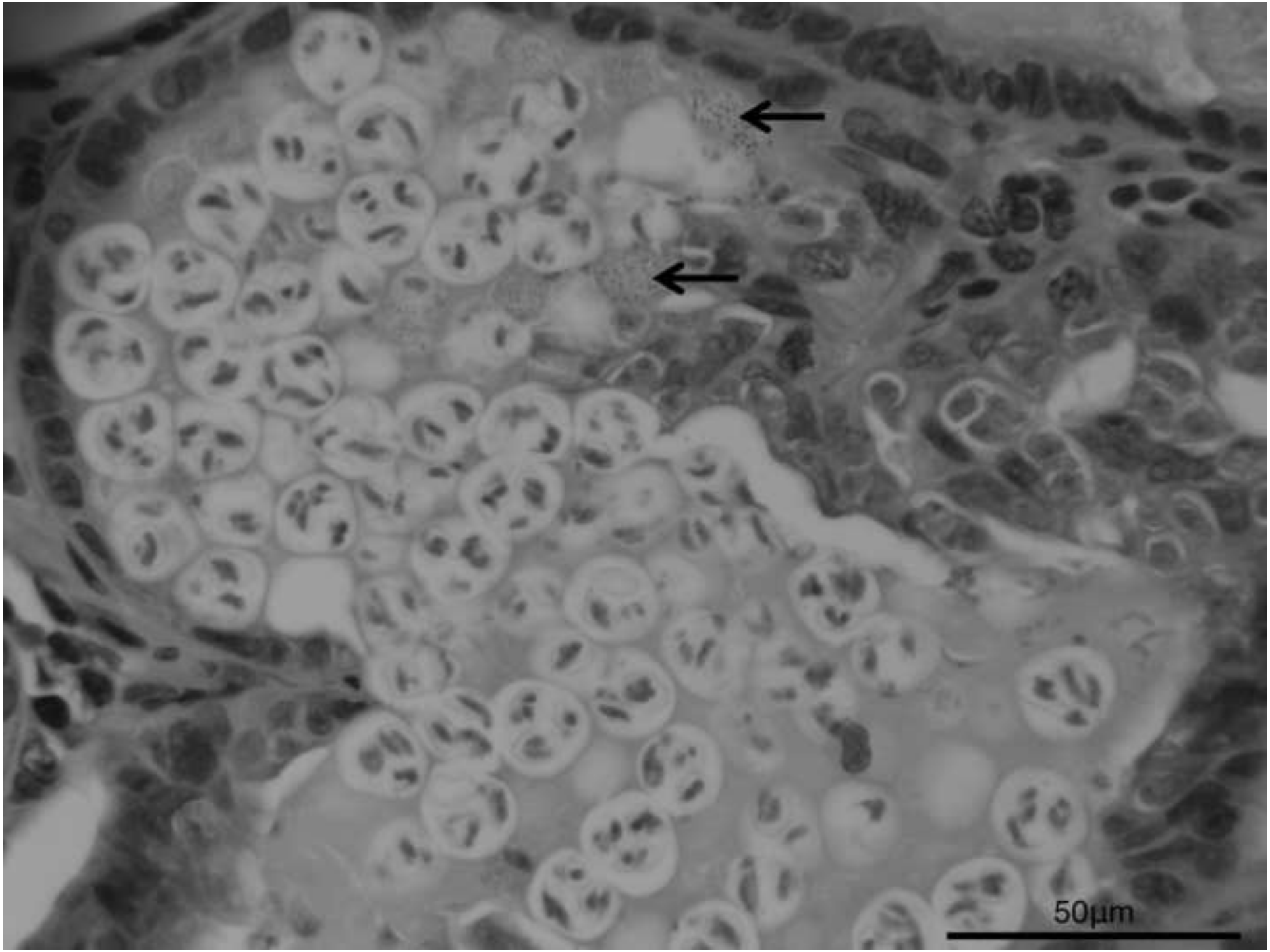

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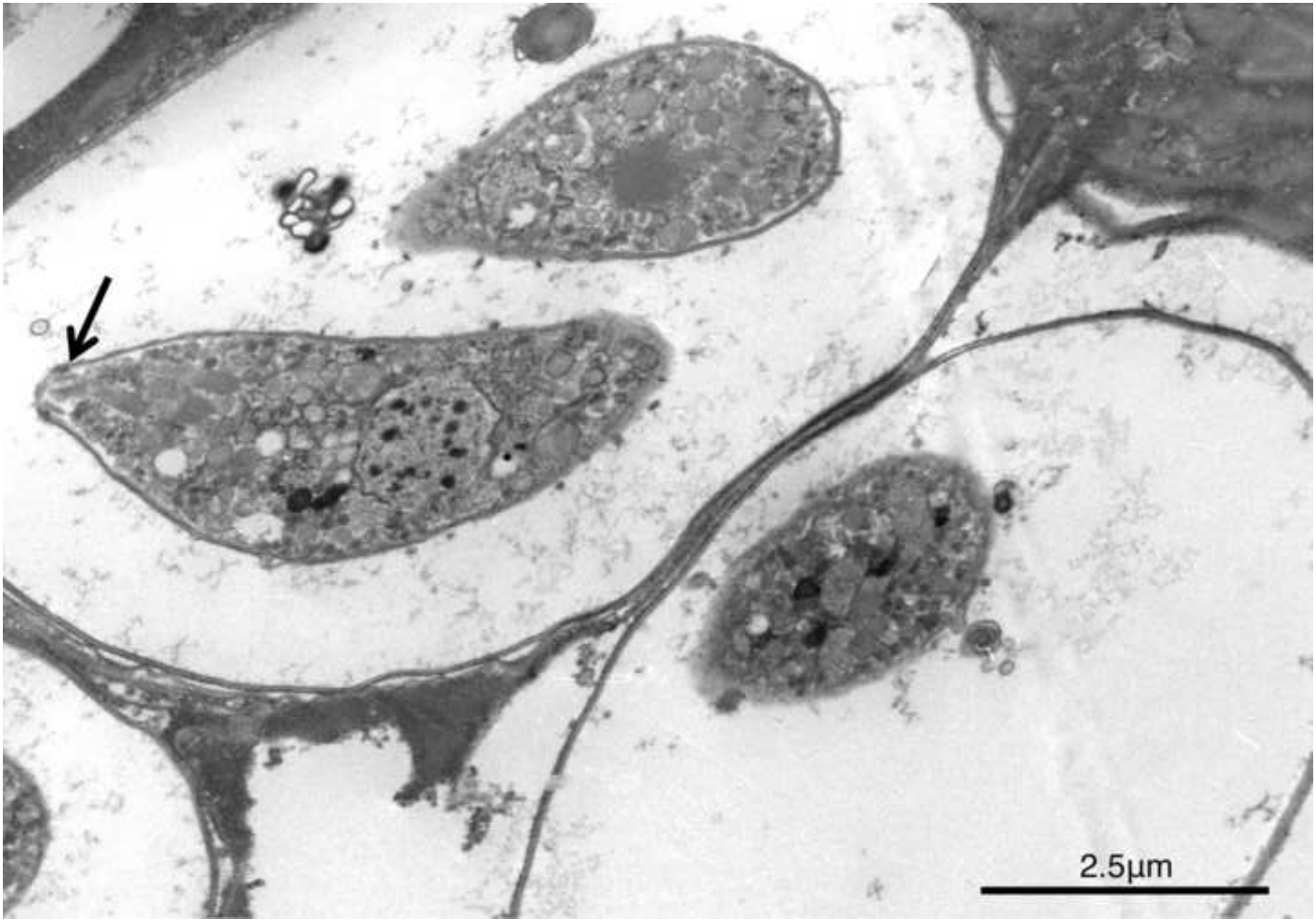

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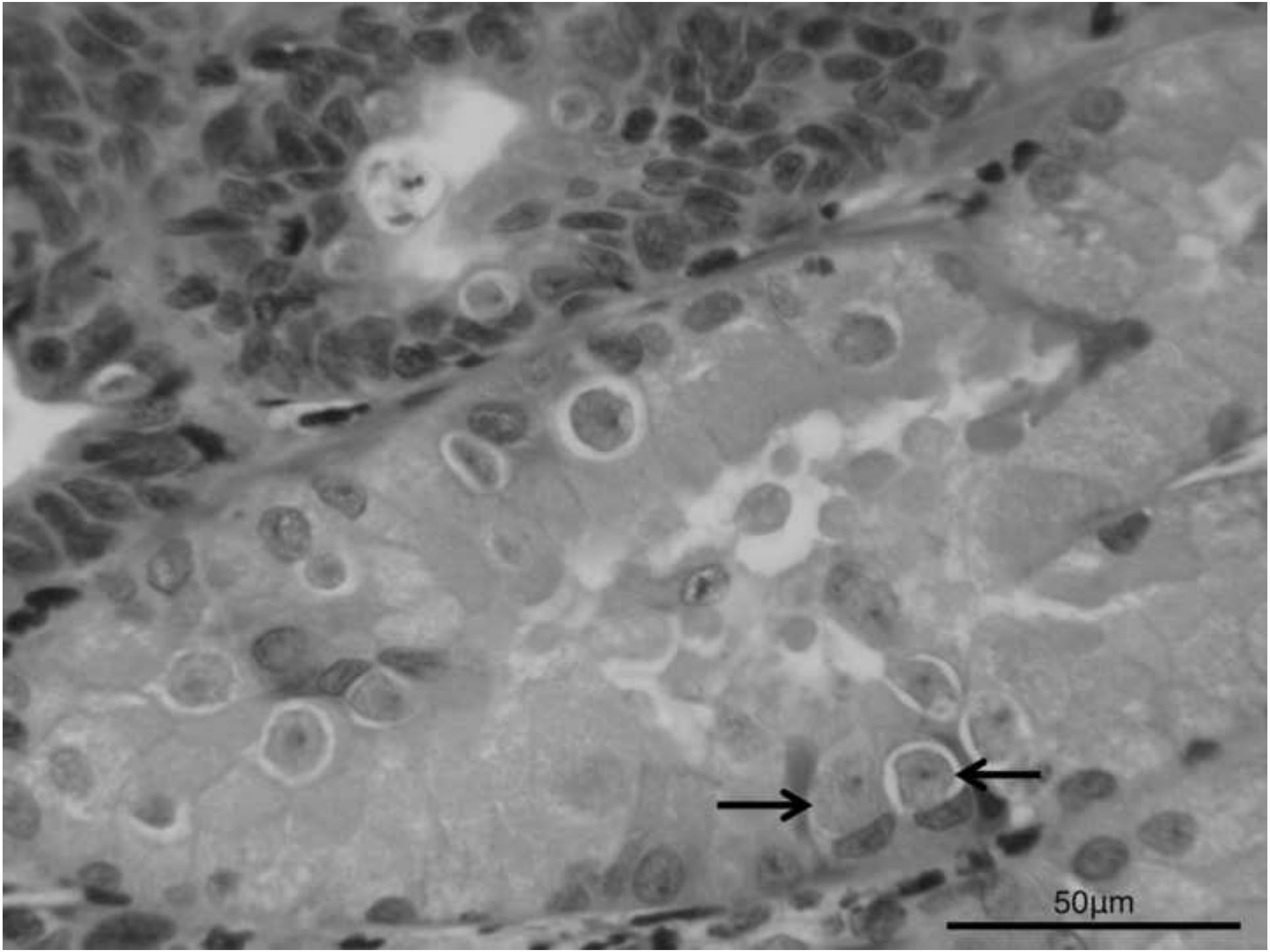

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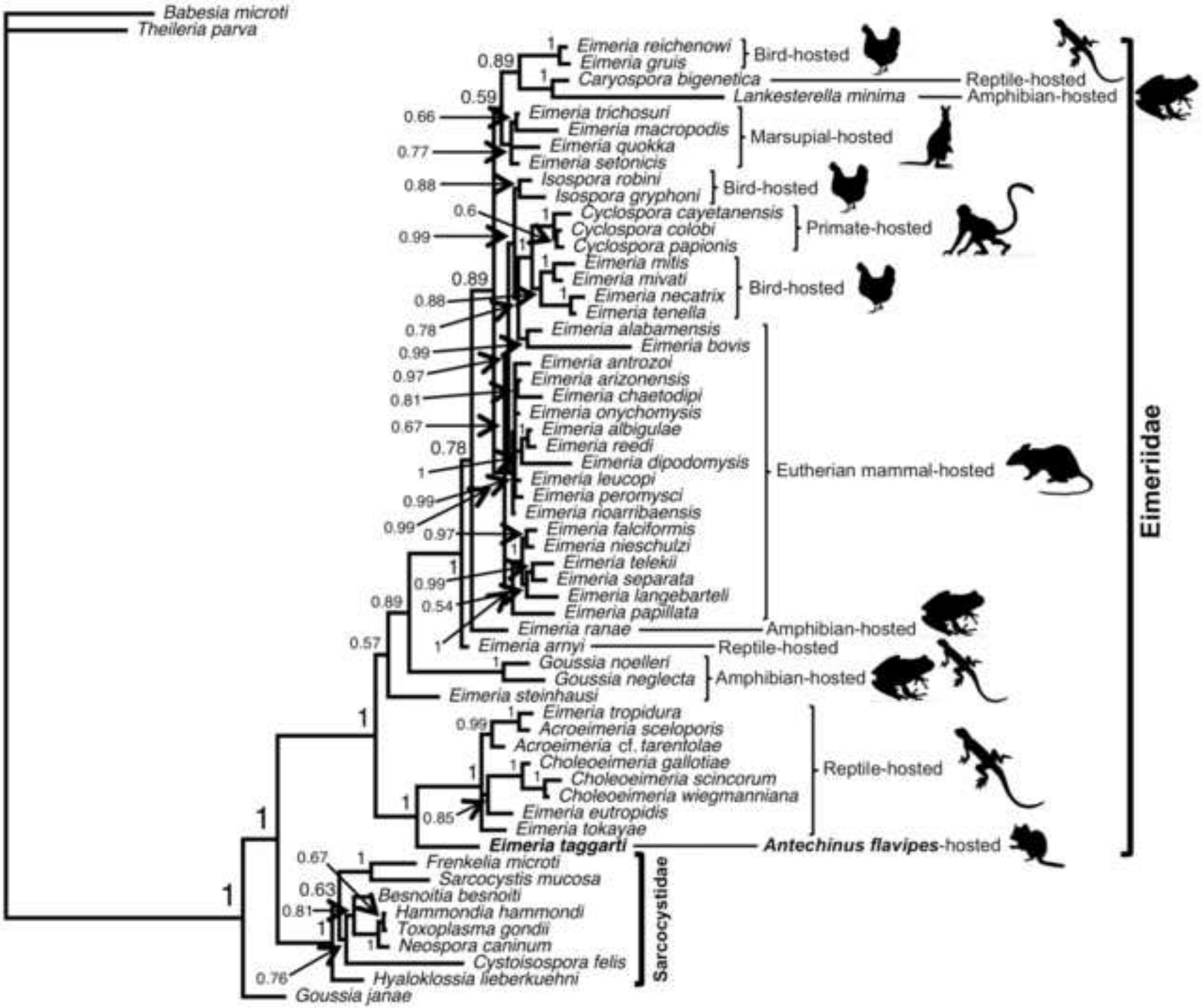

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