THE LIFE-CYCLE AND ULTRASTRUCTURE OF *SARCOCYSTIS AMEIVAMASTIGODRYASI* N. SP., IN THE LIZARD *AMEIVA AMEIVA* (TEIIDAE) AND THE SNAKE *MASTIGODRYAS BIFOSSATUS* (COLUBRIDAE)[1]

LAINSON R.* & PAPERNA I.**

Summary :

Sarcocysts in muscles of the teiid lizard *Ameiva ameiva* from north Brazil were fed to the colubrid snake *Mastigodryas bifossatus*, the faeces of which had been shown to be devoid of coccidial oocysts or sporocysts. When necropsied 16 days later the snake was shown to have developed a massive intestinal infection of *Sarcocystis*. Mature sporocysts from another, naturally infected *M. bifossatus* were fed to juvenile specimens of *A. ameiva* in which no sarcocysts could be detected in tail muscle biopsies. When examined 30 and 47 days later they had very large numbers of sarcocysts in their tail and tongue muscles. The parasite is given the name of *Sarcocystis ameivamastigodryasi* n. sp. An ultrastructural study has been made of the sarcocyst and of the parasite's sporulation in the *lamina propria* of the snake: the latter provides details of the wall formation process in developing sporocysts. Attempts to infect a specimen of the boid *Boa constrictor constrictor* by feeding it with infected *Ameiva* failed, suggesting that sporocysts previously recorded in genera of the family Boidae may be those of a different species of *Sarcocystis*.

KEY WORDS : *Sarcocystis ameivamastigodryasi* n. sp., *Ameiva ameiva*, *Kentropyx calcarata*, lizards, *Mastigodryas bifossatus*, *Mastigodryas boddaerti*, *Epicrates c. cenchria*, *Boa c. constrictor*, snakes, transmission, Brazil.

Résumé : CYCLE ET ULTRASTRUCTURE DE *SARCOCYSTIS AMEIVAMASTIGODRYASI* N. SP. CHEZ LE LÉZARD *AMEIVA AMEIVA* (TEIIDAE) ET LE SERPENT *MASTIGODRYAS BIFOSSATUS* (COLUBRIDAE)

Des muscles de lézard Ameiva ameiva *du nord du Brésil, infestés par des sarcocystes ont été donnés en nourriture à un serpent* Mastigodryas bifossatus *dont les fèces ne présentaient ni oocystes ni sporocystes de coccidie. Une nécropsie réalisée 16 jours plus tard a révélé une infection intestinale massive à* Sarcocystis *de ce serpent. Des sporocystes matures d'un autre* M. bifossatus *naturellement infecté ont été donnés en nourriture à de jeunes* A. ameiva *chez lesquels aucun sarcocyste n'avait été détecté par biopsie au niveau de la queue. Examinés 30 et 47 jours plus tard, ceux-ci présentaient de nombreux sarcocystes au niveau des muscles de la queue et de la langue. Le parasite est nommé* Sarcocystis ameivamastigodryasi *n. sp. Une étude ultrastructurale du sarcocyste et de la sporulation au niveau de la* lamina propria *a été réalisée. Des tentatives d'infestation d'un boa –* Boa constrictor constrictor *– ont toutes échoué, ce qui laisse supposer que les sporocystes précédemment décrits chez ce serpent sont d'un genre de* Sarcocystis *différent.*

MOTS CLÉS : Sarcocystis ameivamastigodryasi *n. sp.*, Ameiva ameiva, Kentropyx calcarata, *lézards*, Mastigodryas bifossatus, Mastigodryas boddaerti, Epicrates c. cenchria, Boa c. constrictor, *serpents, transmission, Brésil.*

INTRODUCTION

Carnivorous or omnivorous animals acting as definitive hosts of *Sarcocystis* species include mammals such as felids, dogs, foxes, mustelids, procyonids, marsupials, man and other primates, birds of prey, and a variety of snakes (Levine, 1988; Dubey, Speer & Fayer, 1989).

Among the reptiles, tissue-cysts of *Sarcocystis* species have been recorded in at least 13 different lizards, one snake and a single chelonid (Lainson & Shaw, 1971, 1972; Matuschka, 1987; Levine, 1988; Dubey, Speer & Fayer, 1989) but in most cases the definitive hosts of these parasites have yet to be discovered. However, the life-cycles of three species involving lizards as the intermediate (prey) hosts and snakes as the definitive (predator) hosts have been described in the Mediterranean region. *S. podarcicolubris* has a life cycle in snakes of the genus *Coluber* and lacertid lizards, while that of *S. chalcidicolubris* is in these snakes and skinks (Matuschka, 1981, 1987). *S. gongyli* Trinci, 1911 has the snake *Spalerosophis diadema* as the definitive host and the skink *Chalcides o. ocellatus* as the intermediate host (Abdel-Ghaffer *et al.*, 1990).

In the following communication we describe the life-cycle and ultrastructure of a new species of *Sarcocystis* from north Brazil, with an intermediate host in the lizard *Ameiva ameiva* (Linn.) (Teiidae) and a definitive host in the snake *Mastigodryas bifossatus* (Raddi) (Colubridae). This would appear to be the first incrimination of a snake as the definitive host of a reptilian *Sarcocystis* species in the Americas.

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MATERIALS AND METHODS

Twenty specimens of *A. ameiva* were captured, by hand, in open country near Capanema, Pará State, north Brazil (1° 12´ S: 47° 11´ W). The lizards were killed with chloroform and, in a general search for parasites, pieces of muscle from the tail and hind legs were squashed between glass slides and examined under a dissecting microscope for the presence of sarcocysts. When these were present, some were teased out of the surrounding muscle and smears of the ruptured contents air dried, fixed in aqueous Bouin's fluid and stained by a modified Giemsa method (Lainson, 1958). For light-microscope examination, pieces of tail, leg, tongue and heart muscle of the positive lizards were fixed in 10 % neutral buffered formalin. After dehydration in graded ethanol concentrations, some material was embedded in paraffin wax and sections, cut at 4.0-5.0 µm, were stained with haematoxylin and eosin. Other pieces were similarly dehydrated, embedded in glycol-methacrylate medium (GMA, Agar Scientific Ltd., Stansted, UK), and sectioned at 2.0-3.0 µm with a glass knife on a Sorval JB4 microtome. The sections were stained either in Meyer's haemalum-eosin (MH-E), or with Giemsa. Material for transmission electron microscope (TEM) study was fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.1M, pH 7.4) for 24 hours at 4° C, rinsed repeatedly in the same buffer, post-fixed in 1.0 % osmium tetroxide in the same buffer for one hour and, after rinsing in the buffer, dehydrated in graded ethyl alcohol concentrations and embedded in Agar 100 medium (Agar Scientific Ltd., U.K.). Thin sections, cut on a Reichert "Ultracut" microtome with a diamond knife, were stained on grids with uranyl acetate and lead citrate and examined with a Jeol 100CX TEM. Semi-thin sections cut with a glass knife were stained with toluidin blue.

Snakes used in the following experiments were two wild-caught specimens of *M. bifossatus* and a single *B. c. constrictor* bred in captivity at the Museu Emílio Goeldi Zoological Gardens, Belém: they were maintained on a diet of laboratory-bred white mice. Faecal samples were gently triturated in 2 % (w/v) aqueous potassium dichromate solution ($K_2Cr_2O_7$), maintained as thin layers in loosely covered Petri-dishes kept at room temperature (approximately 24° C), and checked for the presence of coccidial oocysts by both direct examination and flotation concentration in zinc sulphate solution (sp. gr. 1.18). Measurements of parasites (in µm, except where otherwise stated) were by normal light microscopy, using a × 100 neofluor objective, × 10 eyepieces and an ocular micrometer: those of the sporocysts are given as means, followed by the range in parentheses and the shape-index (ratio of length/width). Photomicrographs were prepared with a Zeiss "Photomicroscope III" and Kodak "TMX 100" film.

ATTEMPTS TO INFECT THE SNAKES *MASTIGODRYAS BIFOSSATUS* AND *BOA C. CONSTRICTOR*

Faecal samples from one of the wild-caught *M. bifossatus* and the young *Boa c. contrictor* bred in captivity, proved devoid of coccidial oocysts or sporocysts after repeated direct examinations and zinc sulphate flotation. Pieces of muscle containing sarcocysts from a heavily infected *A. ameiva* were force-fed to both snakes, which were killed and examined after16 days and 40 days, respectively. Fresh scrapings from the small intestine were searched for oocysts or sporocysts. Portions of the intestine from the snake necropsied at 16 d.p.i. were fixed for both light-microscope and TEM studies.

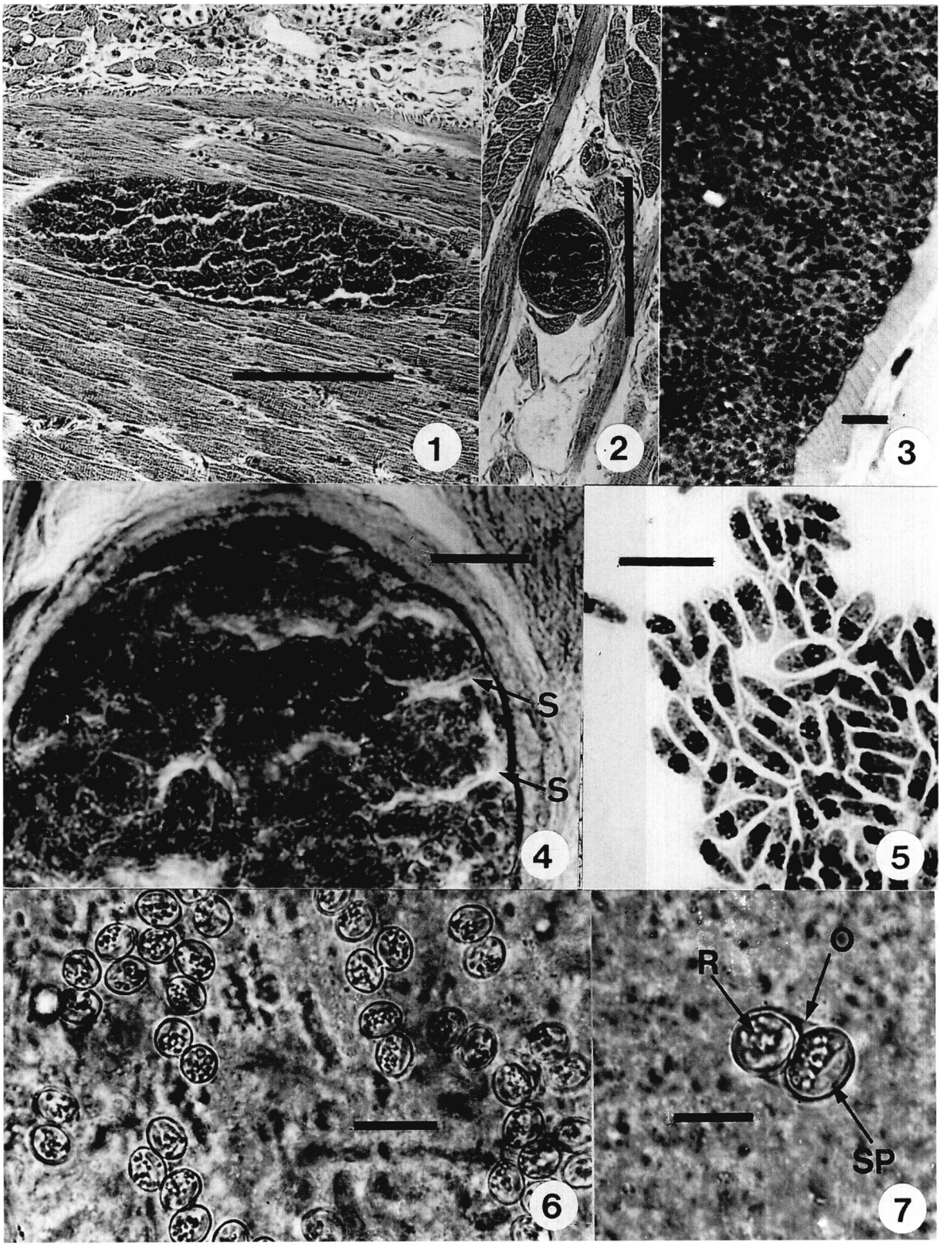
ATTEMPTS TO INFECT THE LIZARD *AMEIVA AMEIVA*

On arrival in the laboratory the second specimen of *M. bifossatus* was found to be passing abundant oocysts and sporocysts, morphologically indistinguishable from those previously seen in *M. boddaerti* several years previously. The snake was killed and scrapings of the small intestine, containing vast numbers of parasites, were accumulated in distilled water and stored in the refrigerator at 4°C. Ten days later, six juvenile *A. ameiva* were obtained from a different study area on the outskirts of Belém, and muscle biopsies were made from the base of the tail of each animal: squash preparations of these revealed no evidence of sarcoscysts. Each lizard was forced-fed with approximately 0.1 ml of the sporocyst suspension from *M. bifossatus*, using a 1.0 ml syringe and a small length of plastic tubing. All six lizards were then housed in a glass aquarium containing sterilized pieces of old house tiles and sand, in which they could make their burrow-like retreats. They were provided with water that had been sterilized by boiling, and a diet of laboratory-bred *Tenebrio* larvae. Thirty days later, one lizard was removed, killed, and examined for the presence of sarcocysts in muscle from the base of the tail, the legs, tongue and heart: three others were examined in the same manner after 47 days. Two of the experimental animals had died and the decomposed remains did not permit a parasitological examination.

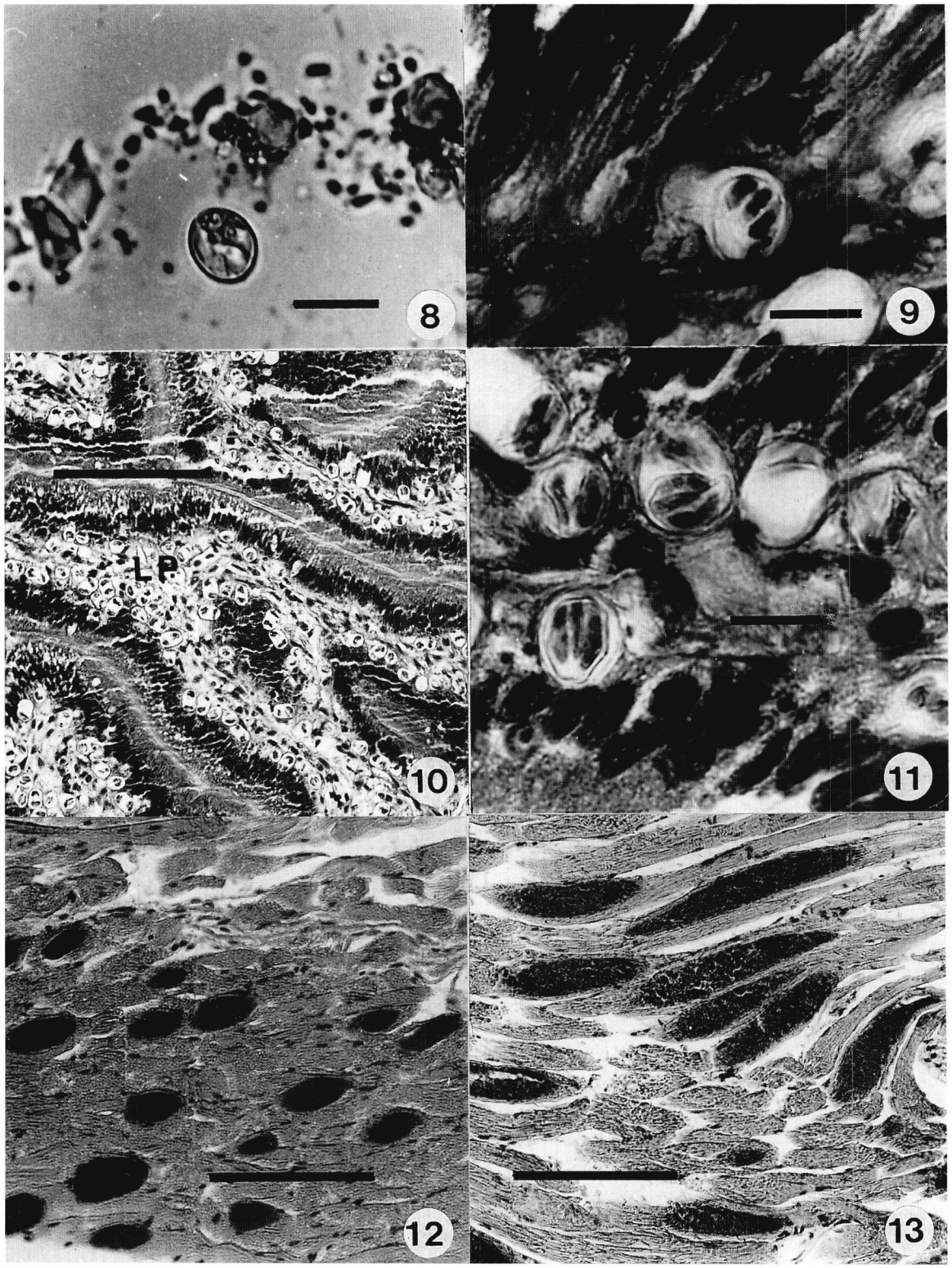
RESULTS

NATURAL *SARCOCYSTIS* INFECTIONS IN THE LIZARD *AMEIVA AMEIVA*: A LIGHT MICROSCOPE STUDY

Sarcocysts, in moderate to high numbers, were found in five of the 20 lizards from Capanema and were visible to the naked eye as delicate white



Figs 1-7. – *Sarcocystis ameivamastigodryasi* n. sp. in the lizard *Ameiva ameiva* and the snake *Mastigodryas bifossatus*. Figs 1-2. Longitudinal and transverse sections of sarcocysts in the tail muscle of *A. ameiva*: natural infection, wax embedded, haematoxylin and eosin staining. Fig. 3. Increased magnification to show smooth nature of the sarcocyst wall: GMA embedded, Meyer's haemalum-eosin staining. Fig. 4. High power view to show the septa arising from the sarcocyst wall and forming loculi containing the bradyzoites. Fig. 5. Bradyzoites as seen in a Giemsa-stained smear of the sarcocyst contents. Fig. 6. Low power view of living oocysts and sporocysts in a scraping from the small intestine of a naturally infected *M. bifossatus*. Fig. 7. High power view of an oocyst. O = distorted oocyst wall; R = sporocyst residuum; S= septa; SP = sporozoite. Bars = 100µm (Figs 1, 2), 10 µm (Figs 3-5, 7), 20 µm (Fig. 6).



Figs 8-13. – *Sarcocystis* species in snakes and lizards. Fig. 8. Sporocyst of an unidentified *Sarcocystis* sp., in the faeces of the snake *Boa c. constrictor*. Fig. 9. Histological section of the small intestine of another boid snake, *Epicrates c. cenchria*, showing a sporocyst, containing its four sporozoites, in the *lamina propria*. Fig. 10. Experimentally induced infection of *S. ameivamastigodryasi* n. sp. in the snake *Mastigodryas bifossatus*: low power view showing huge numbers of oocysts and sporocysts in the *lamina propria* of the small intestine. Fig. 11. High power view of the same material, showing sporozoites within the sectioned sporocysts. Figs 12, 13. Experimentally induced infection of *S. ameivamastigodryasi* n. sp. in *Ameiva ameiva*: low power view of developing sarcocysts in tongue muscle, 30 and 47 days after feeding the lizards with sporocysts from the snake *M. bifossatus*. LP = *lamina propria*. Bars = 10 µ (Figs 8, 9, 11) and 100 µ (Figs 10, 12, 13).

threads. Histological sections confirmed location of the sarcocysts within the muscle fibres: they reached from 1.0-2.0 mm in length and about 80 µm in diameter. In all lizards examined the cysts were most abundant in muscle of the tongue, and none was seen in that of the heart. Seen by light microscopy (Figs 1-3) the sarcocysts were in the form of smooth cylinders, with a wall devoid of any conspicuous invaginations or the "villi" and "spines" described in some sarcocysts of mammalian hosts (Shaw & Lainson, 1969). Internally the cysts were subdivided by septae into numerous loculi (Fig. 4) containing the bradyzoites which, in the stained contents of ruptured cysts, had a mean measurement of 7.2 × 2.5 µm (Fig. 5): metrocytes were not found in smears. No evidence was found of naturally rupturing sarcocysts and these provoked no surrounding cellular reaction or pathological changes.

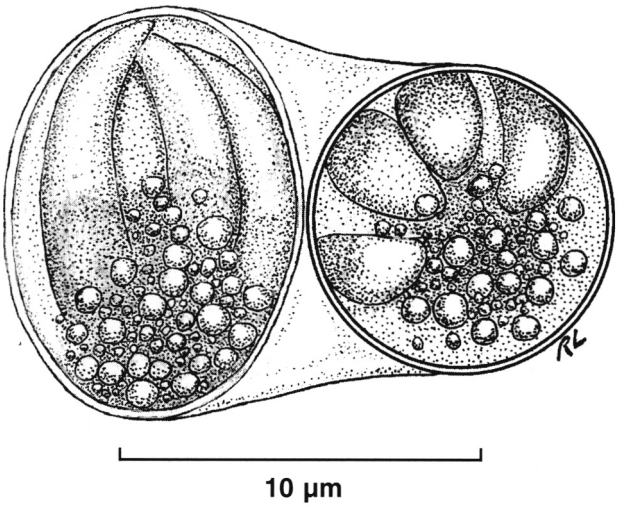


Fig.14. – Line drawing of a living oocyst of *Sarcocystis ameivamastigodryasi* n. sp. from the snake *Mastigodryas bifossatus*.

Natural infections in snakes

A past examination of snakes captured in Pará in 1985 (Lainson, unpublished observations) had revealed mature oocysts and freed sporocysts, considered to be those of *Sarcocystis*, in newly passed faeces of two specimens of *Boa constrictor constrictor* Linn., (Boidae), one *Epicrates cenchria cenchria* Linn., (Boidae) and one *Mastigodryas boddaerti* (Sentzen) (Colubridae). Snakes, therefore, were logical suspects as definitive hosts of the *Sarcocystis* in *Ameiva*, a lizard which is known to be a common prey of these animals (Avila Pires, 1995). Particularly suggestive was the fact that one of the *M. bifossatus* used in the following study was caught while devouring an adult specimen of *A. ameiva*.

In each case, as in most other *Sarcocystis* species, the wall of the oocyst was very thin, rapidly collapsed around the two sporocysts and usually broke down to release them. There was no significant difference in the morphology of the oocysts and sporocysts seen in the two species *M. boddaerti* and *M. bifossatus* (Figs 6, 7, 14), sporocysts of which measured 10.7 × 8.7 (10 × 8.1-11.2 × 9.3) and 10.9 × 8.1 (10 × 7.5-11.2 × 8.7 respectively. Those previously found in two specimens of *Boa c. constrictor* and a single *Epicrates c. cenchria* were slightly smaller, measuring 9.8 × 8.6 (8.7 × 7.5-10 × 8.7) and 9.12 × 8.6 (8.5 × 8.0-10 x 8.8), respectively (Figs 8, 9).

Experimental transmission to the snake *M. bifossatus*

Very large numbers of oocysts and sporocysts were present in scrapings of the epithelial surface of the small intestine of *M. bifossatus* that had been fed 16 days previously with the muscle of *Ameiva* containing sarcocysts, and they were indistinguishable from those seen in the naturally infected snakes, *M. boddaerti* and *M. bifossatus*. In histological sections most of the sporocysts were fully sporulated and already abundant in the *lamina propria* (Figs 10, 11).

No oocysts or sporocysts could be found in a faecal sample collected from the *B. c. constrictor* fed with similar material 21 days previously, or in scrapings from the small intestine when the animal was killed 40 days d.p.i.

Experimental transmission to the lizard *Ameiva ameiva*

All four lizards fed with the suspension of sporocysts from the snake *M. bifossatus* showed a massive infection of sarcocysts with the same morphological features of those seen in natural infections of *A. ameiva*. Their somewhat smaller size (Figs 12, 13) was presumably because they had not yet reached their full size. As in natural infections of this lizard, the tongue muscle was the most heavily parasitised and no sarcocysts could be detected in the heart muscle.

Fine structure of stages in the small intestine of the experimentally infected snake, *M. bifossatus*

The single mature microgamont found (Fig. 17) contained amylopectin granules and residual nuclei and was shedding numerous microgametes (arrows) within the limiting membrane of the parasitophorous vacuole (PV). Its detachment from the host peripheral cytoplasm is apparently a processing artifact.

The walls of oocysts and sporocysts resisted penetration of fixative and/or resin, with a resulting poor qua-

lity for the ultrathin sections and an accumulation of staining residue in the induced abrasions. Possibly due to these processing faults, the contained cytoplasm appears to be very dense, with resulting obscuring of structural details. The greater part of the oocyst is filled with amylopectin granules. The host cell housing the oocyst degenerates and becomes greatly shrunken: its dense, residual cytoplasm contains numerous mitochondria, accompanied by endoplasmic reticulum (ER). It was not possible to say with certainty which of the membranes bounding the PV is formed by the parasite or separated off from the host cell: the oocyst is bounded by a thick, distinctly bilayered wall, superficially coated by a membranous residue. Aggregates of ribosomes suggest the accumulation of dense, rough ER beneath the cell wall (Fig. 21).

Sporocysts remain invested by the homogenous cytoplasmic residue of the enclosing oocyst up to their latest stage of development (Figs 20-25), and the sporulated oocysts occupy host cells which are seemingly fully functional (Figs 21-23). The host-cell cytoplasm along the PV margin beneath the PV wall membrane contains large ER cisternae filled with conspicuous electron dense particles (Figs 21, 22, 24). At the initial stage of sporocyst wall formation, a thick belt of granular substance of medium electron density is formed on the boundry of the future sporocyst. This coincides with an accumulation of heavily electron-dense substance along the edge of the oocyst cytoplasm (Figs 21, 22), and the occurrence of heavy granular aggregates in the developing sporocyst's peripheral cytoplasm (Fig. 22). The outer aggregates disappear and the intracytoplasmic granules become less conspicuous in later stages of the sporocyst wall formation (Figs 23, 24). The thick belt of granular substance subsequently transforms into three unit membranes, with the residue of this substance remaining between the two innermost membranes (Fig. 23). The developing sporocyst wall ultimately consolidates into a thick structure comprised of an inner electron-dense layer and an outer electron-lucent layer with an electron-dense, uneven surface. The space between the wall and the outer, apposed unit membrane contains a homogenous, granular matrix (Figs 24, 25).

Fine structure of the sarcocysts in naturally infected *A. ameiva*

In the material examined by TEM the sarcocysts were evidently mature: they contained mostly bradyzoites and only very few actively dividing metrocytes (Figs 26, 27). The morphology of these two stages was similar to that described in ultrastructural studies on *Sarcocystis* in diverse intermediate hosts (Dubey, Speer & Fayer, 1989). The primary wall of the sarcocyst at the interphase between it and the host muscle sarcoplasm is composed of small anastomosing osmiophilic protrusions of up to 100 nm which alternate with tiny micropyle-like "windows" possessing a single unit membrane (Figs 27-29).

DESCRIPTION

Sarcocystis ameivamastigodryasi n. sp.
(Figs 1-7, 10-29)

Definitive host. The snake *Mastigodryas bifossatus* (Serpentes: Colubridae).

Intermediate host. The lizard *Ameiva ameiva* (Sauria: Teiidae).

Description of the oocyst in the snake M. bifossatus (Figs 6, 7, 14). By light microscopy, mature oocysts with no micropyle, oocyst residuum or polar bodies. Wall single layered, colourless and usually distorted when the two sporocysts are positioned in different planes: characteristic of the genus, it most frequently ruptures to release the sporocysts which are passed, already mature, in the faeces. Sporocysts (30 measured) 10.9 × 8.1 (10 × 7.5 – 11.2 × 8.7), shape-index 1.3 (1.2-1.4), with no Stieda/substiedal bodies. Sporocystic residuum of globules and fine granules.

Description of the sarcocyst in the lizard A. ameiva (Figs 1-5; 26-29). Reaching up to approximately 1-2 mm × 80 µm. By light microscopy, with a smooth, thin wall devoid of spines or villi. By TEM the wall is seen to be composed of small anastomosing osmiophilic protrusions of up to 100 nm which alternate with tiny "windows" of a single-unit membrane. Bradyzoites 7.2 × 2.5, as measured in Giemsa-stained smears, and located in loculi formed by septal extensions of the sarcocyst wall: by TEM, they have the usual morphology of the genus.

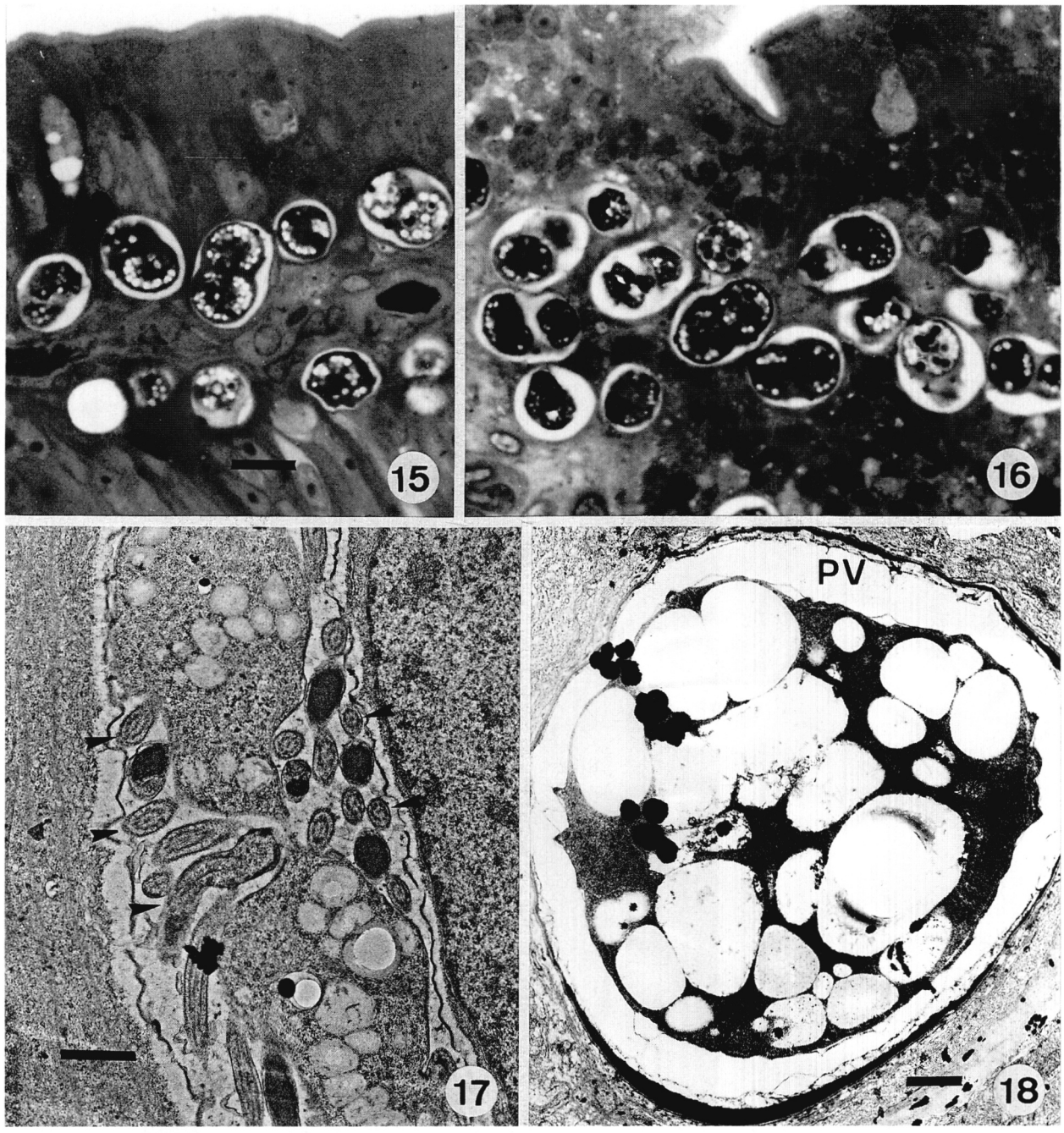
Location in the definitive and intermediate hosts. Gametogony in the epithelial cells of the small intestine of the snake: maturation of the oocysts in the *lamina propria*, with both intact oocysts and (mostly) free, sporulated sporocysts shed in the faeces. Sarcocysts are located within the striated muscle cells of the lizard, predominantly in the tongue. No development seen in the heart muscle.

Type locality. Capanema, Pará State, north Brazil (1° 12′ S: 47° 11′ W).

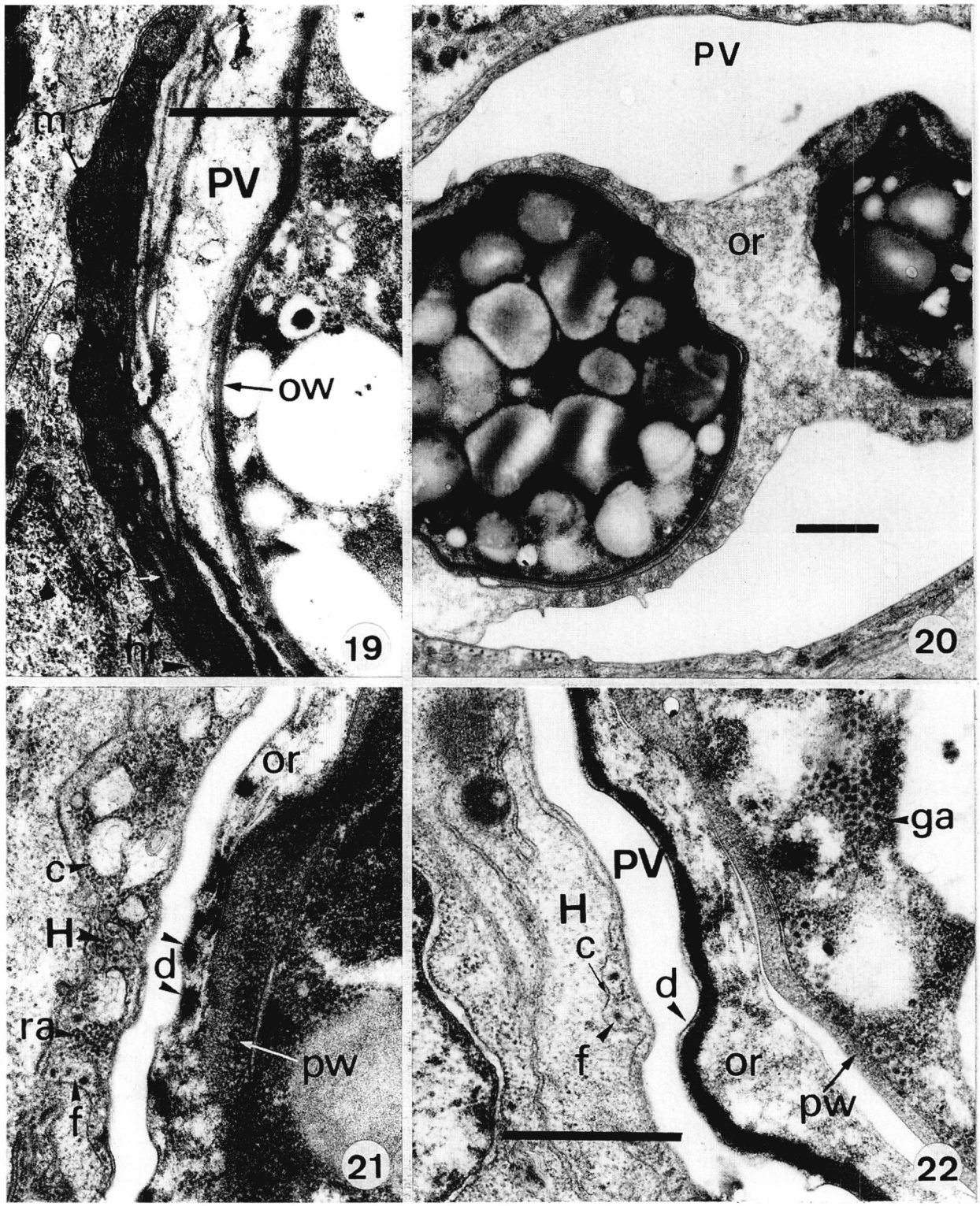
Prevalence. Of 20 *A. ameiva* examined, five were infected (25 %). Prevalence in the snake host uncertain: One of two *M. bifossatus* examined was naturally infected.

Pathology. Neither the snake nor the lizard host appeared to suffer ill effects from the infection.

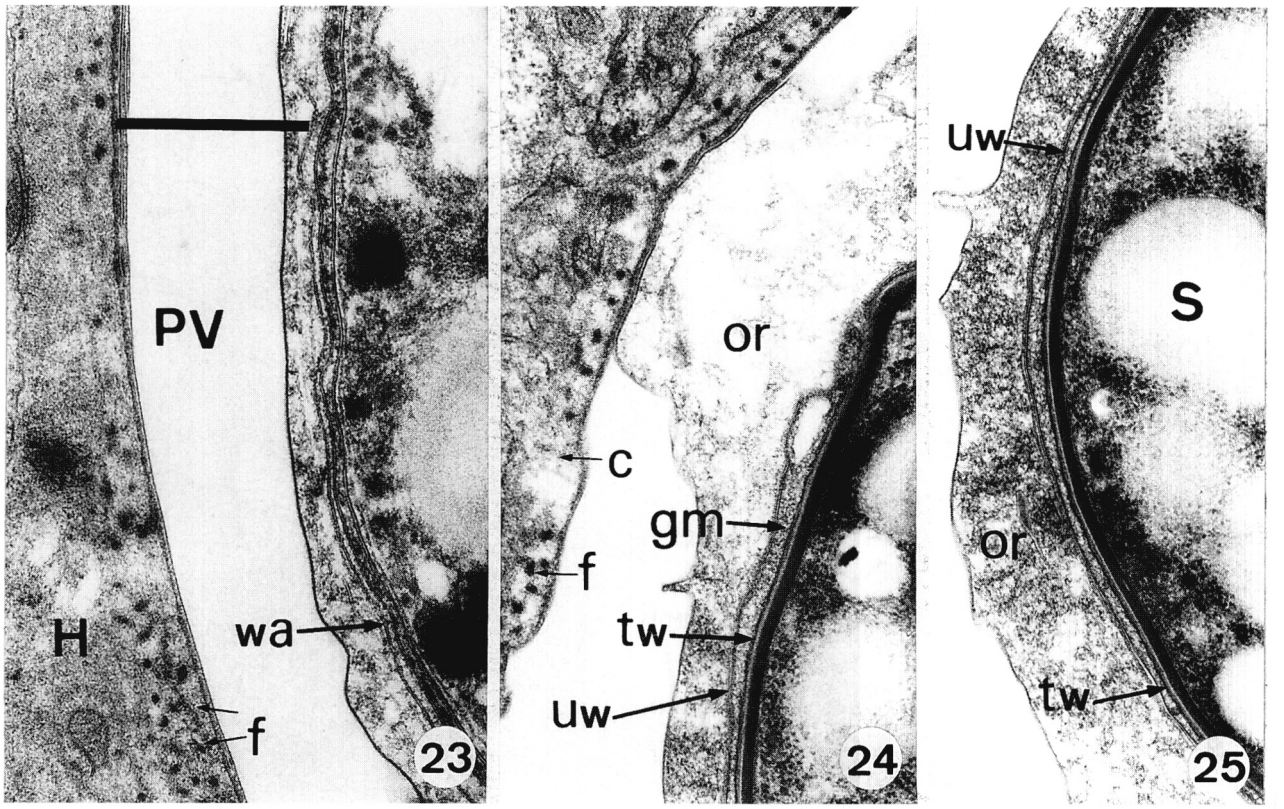
Etymology. The generic names of the lizard and snake hosts are combined to form the specific name of *S. ameivamastigodryasi.*



Figs 15-18. – Experimental infection of *Sarcocystis ameivamastigodryasi* n. sp., in the snake *Mastigodryas bifossatus*. Figs 15, 16. Sporulating oocysts in semi-thin sections from two areas of the small intestine: GMA embedded, toluidine blue staining, Bar = 10 µm. Fig. 17. Microgamont with differentiated microgametes (arrowed): TEM. Bar = 1.0 µm. Fig. 18. Oocyst in a parasitophorous vacuole (PV), with remains of the host cell: TEM. Bar = 1.0 µm.



Figs 19-22. – Experimental infection of *S. ameivamastogodryasi* n. sp., in the snake *M. bifossatus*. Fig. 19. Details of the oocyst wall (OW) and the host cell residue (hr) which is loaded with mitochondria (m) and endoplasmic reticulum (er): TEM. Bar = 1.0 µm. Fig. 20. Sporulated oocyst with its two, walled sporocysts and oocyst residue (or): TEM. Bar = 1.0 µm. Figs 21, 22. Early stages in sporocyst wall formation. The PV is bordered by an active host cell (H) with cisternae (c) containing electron-dense flocculate (f), ER and ribosome aggregates (ra). Note the electron-dense deposit (d) along the edge of the oocyst residue (or). The initial wall structure (pw) is accompanied by granular aggregates (ga): TEM. Bar = 1.0 µm.



Figs 23-25. – Experimental infection of *S. ameivamastigodryasi* n. sp., in the snake *M. bifossatus*. Consolidation of the sporocyst (S) wall. Of the array of membranes (wa) differentiated from the initial wall structure, the inner two fuse into a thick wall (tw). The apposed outer membrane (uw) encloses a granular matrix (gm); or = oocyst residue. Cisternae (c) in the host cell (H) are filled with electron-dense floculate (f): TEM. Bar = 1.0 µm.

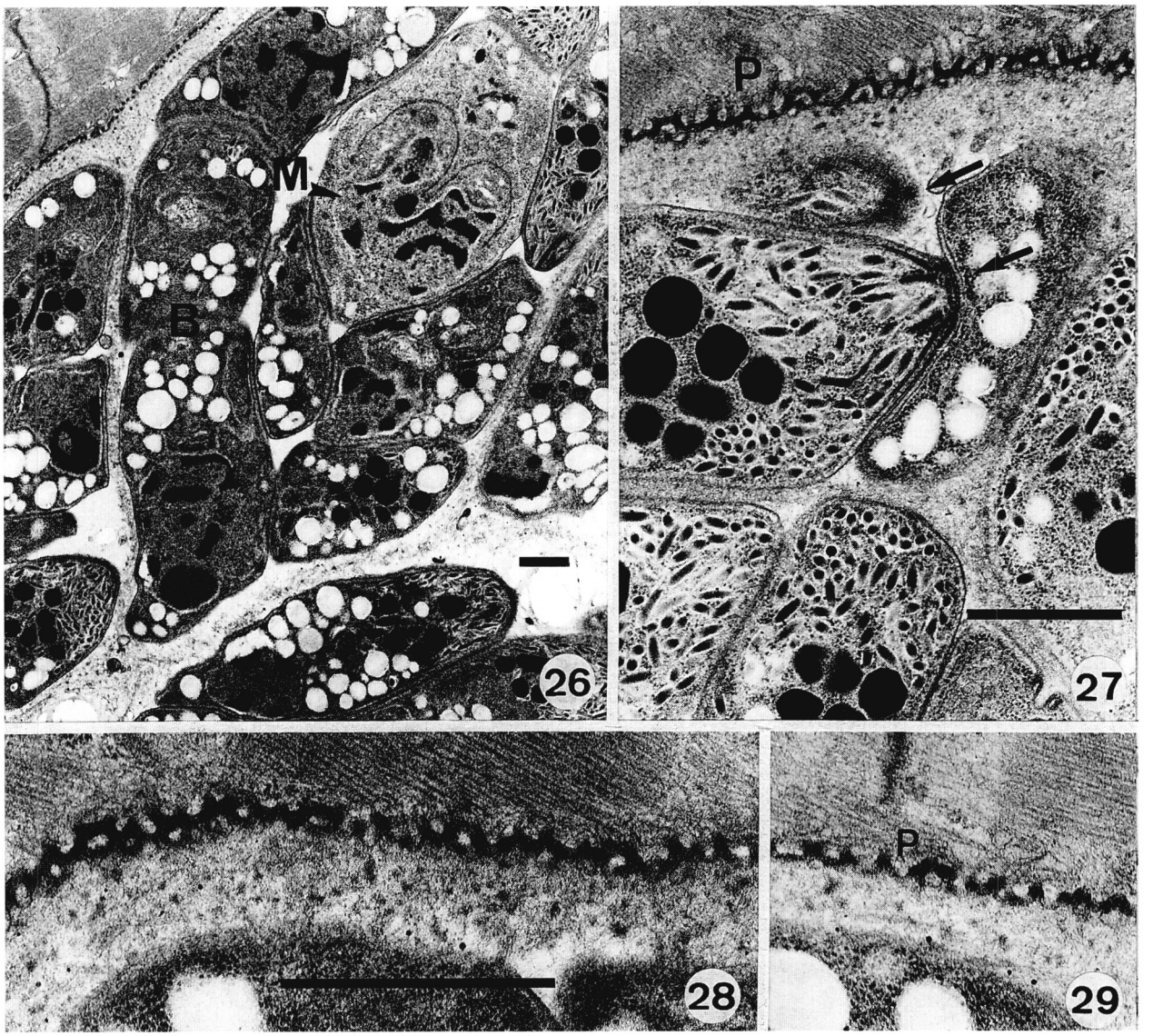
Type material. Histological sections of infected lizard and snake tissues (Accession number Caixa RL79) held in the authors' slide collections.

DISCUSSION

Among the *Sarcocystis* species of reptiles, *S. ameivamastigodryasi* n. sp. is similar to *S. kinosterni* in tortoises (Lainson & Shaw, 1971, 1972) in that their sarcocysts are relatively smooth cylinders, as seen by light microscopy. Although the definitive host of *S. kinosterni* is as yet unknown and the ultrastructure of its sarcocyst wall has not been studied, the possibility that the parasite is conspecific with *S. ameivamastigodryasi* is considered unlikely. Its sarcocysts are much larger (up to 8mm × 230 µm) and its bradyzoites very much longer and slimmer (18.4 × 1.7 µm).

The sarcocyst wall of *Sarcocystis* species is highly variable in its structure, as seen by both light microscopy and TEM (Dubey, Speer & Fayer, 1989), and we conclude that in reptilian hosts no specific pattern can be attributed to its morphology which might indicate a host/parasite coevolution. By the light microscope a thin, smooth sarcocyst wall is seen in a number of *Sarcocystis* species of mammalian hosts, while very conspicuous invaginations or protrusions, such as spines and villi, occur in the sarcocyst wall of other species in both mammals and some reptilian hosts. Finally, the presence of loculi formed by septa originating from the sarcocyst wall is commonly seen in both mammalian and reptilian parasites (Shaw & Lainson, 1969; Lainson & Shaw, 1971; Matuschka & Mehlhorn, 1984; Matuschka, 1987; Dubey, Speer & Fayer, 1989; Paperna & Finkelman, 1998). As far as we are aware, a TEM morphology of the sarcocyst wall similar to that seen in *S. ameivamastigodryasi* has not been previously recorded in reptile hosts, but it is reminiscent of the primary wall texture recorded for sarcocysts "type 1" and "type 2" of Dubey, Speer & Fayer (1989) in murine hosts.

Our necropsy of the experimentally infected snake (day 16 p/i) was too late, with the result that almost all the parasites found were developing or mature sporocysts. The finding of a single microgamont suggests an asynchronous development of the gamonts, a mechanism which would extend the period during which sporocysts are shed in the faeces. Studies on *S. bovicanis* in dogs showed that gametogony stages were



Figs 26-29. – *Sarcocystis ameivamastigodryasi* n. sp. Sarcocysts in the striated muscles of the lizard *Ameiva ameiva*. Fig. 26. Bradyzoites (B) and a dividing metrocyte (M): TEM. Bar = 1.0 µm.. Fig. 27. Details of the primary sarcocyst wall (P) and the apical ends of bradyzoites (arrowed): TEM. Bar = 1.0 µm. Figs 28, 29. High power magnification of the primary wall, showing the small osmiophilic protrusions alternating with tiny "windows" formed by a single unit membrane: TEM. Bar = 1.0 µm.

present as soon as 12 hours after the animals had been fed with sarcocyst infected meat (Sheffield & Fayer, 1980); the sporulation of *S. muris* is completed within 48 hours (Entzeroth Chobotar & Scholtyseck, 1985); and micro/macrogamonts of *S. fusiformis* could be found in cats necropsied 13-14 hours after they had been fed with infected meat (Scholtyseck & Hilali, 1978). In the case of both *S. cleithrionomyelaphis* and *S. muriviperae* gametogony is completed in 6-8 days and zygotes and oocysts are present by 9-10 days, after which almost all the oocysts complete sporulation and move into the *lamina propria* (Mehlhorn & Matuschka, 1986; Paperna & Finkelman, 1996a). In *S. singaporensis* of *Python reticulatus,* gamogony is completed by day 5 p.i. and sporogony by days 6-8 p.i. (Paperna & Martelli, in press) It would appear that the snake/lizard *Sarcocystis* species may have a similar developmental schedule, for Abdel-Ghaffar *et al.* (1990) showed that zygotes and young oocysts of *S. gongyli* could be found on day 8, and sporocysts by day 10, in snakes fed with infected lizards.

How the zygote moves from the intestinal epithelium into the *lamina propria* remains to be determined, but Entzeroth, Chobotar & Scholtyseck (1985) suggest three possible mechanisms. First, that the host cell and its contained parasite move as a unit into the *lamina propria*; second, that the zygote vacates its host-cell and migrates independantly; and third, that transfer of the

parasite is by another host cell of the leucocyte series. Our studies showed that the host cells of the presporulated oocysts become degenerate or destroyed, and this tends to support the second hypothesis of individual movement of the zygote to the subepithelial tissues. On the other hand, TEM suggests that the sporulated oocyst is, in fact, within a parasitophorous vacuole in a host cell: a contradition for which we at present have no explanation. The movement of the zygote from the epithelial cell into the *lamina propria* is also a feature of the heteroxenous eimeriid *Schellackia*, and Lainson *et al.*, (1976) described a similar degeneration or destruction of the host cell, prior to this migration, in *Sc. landauae* of the lizard *Polychrus marmoratus*. In this case it was noted that although the nucleus of the defunct host cell often accompanied the zygote, adhering to its surface, development of the oocysts seemed to be extracellular. A similar extracellular development appears to occur in *Sc.* cf. *agamae*, the sporulated oocysts of which are seen to occupy intercellular spaces in the subepithelial tissue (Paperna, 1992). In contrast, the oocysts of *Sc. ptyodactyli* were considered to be within a phagocytic cell (Paperna & Finkelman, 1996b). It seems likely, therefore that more than one mode of migration may be utilized by the zygotes of both *Schellackia* and *Sarcocystis*.

The periphery of what appears to be the parasitophorous vacuole enclosing the sporulating oocyst of *S. ameivamastigodryasi* n. sp., contains an extensive system of ER cisternae. Such apposed cisterae have been noted in the periphery of the PV housing other coccidians (Paperna & Lainson, 1999), including those containing the tachyzoites of *Toxoplasma* in macrophages (Jones & Hirsch, 1972), but their function remains uncertain. Similar electron-dense, flocculent contents have been recorded in the PV containing gamonts and sporulating oocysts of *S. muris* (Entzeroth, Chobotar & Scholtyseck,1985) and *S. muriviperae* (Paperna & Finkelman, 1996a).

With the differentiation of the sporocysts and consolidation of their hardened wall, the wall of the oocyst becomes thinner and reduced to an homogenous plasmatic substance. Such fragile oocysts occur in numerous *Sarcocystis* species (Dubey, Speer & Fayer, 1989), although the oocysts of *S. muriviperae* do remain sufficiently tough to induce processing faults and consequent interpretation of their ultrastructure (Paperna & Finkelman, 1996a). We experienced this problem to a small extent in the present studies, but were able to give what appears to be the first description of the changes occurring during the process of sporulation in *Sarcocystis*. The sporocyst wall formation differs in many respects from that of the oocyst, principally in that membranes differentiated from a special granular layer finally merge to form the final, thick wall with its overlying membranes. The electron-dense substance appearing at this time along the edge of the oocyst cytoplasm might be related to this process.

From the morphology of their sporocysts and their closely related hosts, we feel that the *Sarcocystis* encountered in the snake *M. boddaerti* is probably the same as that described here in *M. bifossatus*. On the other hand, our apparent failure to establish this parasite in a young boa-constrictor by feeding this snake with sarcocysts of *Ameiva* suggests that the *Sarcocystis* of snakes within the family Boidae may be another species. Further experiments are indicated to determine the relationship of *Sarcocystis* species in predatory snakes of different families, and to see if different lizards may serve as intermediate hosts of the same *Sarcocystis* species. We have noted the presence of sarcocysts in the skeletal muscles of a single specimen of another teiid lizard, *Kentropyx calcarata* Spix, 1825, which were macroscopically similar in size and form to those of *S. ameivamastigodryasi* n.sp., and which were shown to have a wall with the same ultrastructure (Paperna & Lainson, unpublished observations) Although *K. calcarata* is predominantly a forest species it prefers more open, sunny clearings, at times shared by *A. ameiva* and where the snakes *M. bifossatus* and *M. boddaerti* are likely to prey on both of these lizards.

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