SYNOPSIS. *Sarcocystis gracilis* n. sp. is described in the tortoise *Kinosternon scorpioides* (Reptilia, Chelonia) from the Island of Marajó, north Brazil. This appears to be the first record of a sarcosporidian from this group of reptiles.

The cysts are restricted to the skeletal musculature and may reach up to 8.0 mm in length. The cyst wall is extremely delicate, with no visible striations or spines: the trabeculae are well-developed and growth of the cyst appears to be largely from caps of proliferative cells at the poles.

The zoites are long and slender, averaging 18.4 × 1.4 μ in fresh preparations. They undergo sudden and rapid gliding movements. The parasite produces striking pathologic changes in the muscle.

*While Sarcocystis* has been described in a wide range of mammals and numerous species of birds, it appears to be uncommon in cold-blooded vertebrates (Table 1). Fantham and Porter (8) described what appear to be undoubted *Sarcocystis* infections in 2 fishes from Canada, *Salvelinus fontinalis* and *Salvelinus namaycush*. S. salvelini in the trout *Salvelinus fontinalis* and an unnamed species in the eel-pout *Zoarces anguillaries*. No *Sarcocystis* species have yet been reported in the Amphibia.

Among the reptiles, 4 species and 2 subspecies have been described in lizards: *S. platyacalys* from the gecko *Tarentola mauritania* (syn., *Platyacalys mauritania*) in Minorca (3), Algeria (16) and Tunisia (4); *S. gongyli* from the skink *Chalcides ocellatus* (syn., *Gongylus ocellatus*) in Sicily (16); *S. lacertae* in the lacertid *Lacerta muralis* from Lombardy, Italy (1); and *S. chameleo* from the chameleon *Chamaeleo fischeri* from Tanganyika (9). Ball (2) recorded the first sarcosporidia of reptiles from the New World, in the iguanid lizards *Uta stansburiana* and *Scoloporus occidentalis* from southern California, U.S.A. He wrote: “It has been a general custom to give specific status to a *Sarcocystis* described from a new host unless there was strong evidence based on morphology, or geographic distribution, or on close relationship of hosts that the new form was identical with one previously described. This seems justifiable in view of the general failure of cross-infection. On the basis of host distribution alone one would probably be justified in setting up new species . . . when the nearest reptilian hosts from which similar sarcosporidia have been described are on the Island of Minorca some 6,000 miles away.” In spite of this, Ball gave only sub-specific rank to the new parasites, which he named *S. lacertae utae* and *S. platyacalys scolopori*, respectively. We feel this to have been somewhat over-cautious and, for the reasons presented by Dr. Ball himself, have raised these names to specific level as *Sarcocystis utae* and *Sarcocystis scolopori* Ball 1944.

In snakes there are recorded *S. pythionis*, from the Australian carpet-snake *Morelia argus* (syn., *Python spilotes*) by Tiegs (17) and a dubious species, *S. atractaspispidis* in *Atractaspis leucomeles* from Kenya by Parenzan (14). The latter parasite would not seem to be a *Sarcocystis* species. The cysts occurred in the mesenteric membranes and lung and contained piriform “sporozoyta” 13-25 μ long. The more pointed end of the organism was sometimes drawn out into a flagellum-like prolongation. In the absence of illustrations it is impossible to say what this parasite may have been and we have thus omitted it from our accompanying table of recorded species.

As far as we are aware, there has been no report of *Sarcocystis* in the reptilian Order Chelonioidea (turtles and tortoises). It was with some interest, therefore, that we noted heavy infections in a number of tortoises, *Kinosternon scorpioides*, from the Island of Marajó, Pará State, northern Brazil.

MATERIALS AND METHODS

The muçuã, as it is called locally, is a small tortoise which rarely exceeds 25-30 cm in length. It has long been prized as a table delicacy in Pará, where it is served cooked in its own shell as “casquinho de muçuã.” In spite of governmental steps to protect this little tortoise from extinction by forbidding its sale, huge captures are still made annually in the swampy areas in which it lives.

In 1970 we had the opportunity to examine 206 specimens of *K. scorpioides*, which were being screened for *Salmonella* infection elsewhere in this Institute. Initially, all the animals were bled from the heart and thin blood-films were examined for parasites after staining with Giemsa’s stain. Fecal samples were examined for coccidia and other intestinal parasites.

No blood parasites were encountered, which was surprising in view of the frequency with which we had found haemogregarines in other reptiles of this region. The blood-films of 2 tortoises, however, contained scanty parasites which we interpreted as the zoites of *Sarcocystis*, from general structure and the complete absence of any intracellular forms. Subsequent examination of the muscles of both animals revealed very large numbers of sarcocysts and we feel that the blood forms were merely contaminants following the rupture of these cysts during the bleeding procedure.

In most instances the sarcocysts could be seen with the naked eye, usually as conspicuous white streaks in the flesh. In a few cases muscle-squash preparations were needed to verify suspected positives in which the cysts were small in size and number. These were presumably early infections.

Impression smears were made from skeletal muscle, heart, liver, spleen, lung, kidney and brain from a number of the more heavily infected animals. Individual cysts were dissected out of the muscle and crushed on slides for study of the zoites or “spores.” All preparations were air-dried and either fixed in methanol for the usual Giemsa staining, or in aqueous Boulin’s fluid for a modified Giemsa method (19, 20). Tissues were fixed in Carnoy’s fluid or neutral
Sarcocystis in Tortoises

Table 1. Sarcocystis species of cold-blooded vertebrates.

<table>
<thead>
<tr>
<th>Species of Sarcocystis</th>
<th>Host</th>
<th>Size of cyst</th>
<th>Size of zoites</th>
<th>Geographic areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. platydaactylus Bertram, 1892</td>
<td>Lizards: <em>Tarentola mauritanica</em> (Gekkonidae) <em>Platydaactylus mauritanica</em></td>
<td>2 mm × 400 μ</td>
<td>3-4 × 1 μ</td>
<td>Minorca; Algeria; Tunisia.</td>
</tr>
<tr>
<td>S. gongyli Trinci, 1911</td>
<td><em>Chalcides ocellatus</em> (Scincidae) <em>Gongylus ocellatus</em></td>
<td>200-800 × 30-60 μ</td>
<td>3-4 μ × 1 μ</td>
<td>Sicily</td>
</tr>
<tr>
<td>S. lacertae Babudieri, 1932</td>
<td><em>Lacerta muralis</em> (Lacertidae)</td>
<td>1.8-2 × 1 mm</td>
<td>6.5-7.3 × 1.5-2 μ</td>
<td>Lombardy, Italy.</td>
</tr>
<tr>
<td>S. utae Ball, 1944 (new status)</td>
<td><em>Uta stansburiana</em> (Iguanidae)</td>
<td>950 × 120 μ</td>
<td>5.5-7 × 1.5-2 μ</td>
<td>Southern California, U.S.A.</td>
</tr>
<tr>
<td>S. colocort Ball, 1944 (new status)</td>
<td><em>Sceloporus occidentalis</em> (Iguanidae)</td>
<td>600 × 180 μ</td>
<td>5.2-6 × 1.5-2 μ</td>
<td>“”</td>
</tr>
<tr>
<td>S. chamaeleonis Frank, 1966</td>
<td><em>Chamaeleo fischeri multituberculatus</em> (Chamaeleonidae)</td>
<td>15 mm × 500 μ</td>
<td>10-14 × 2-4 μ</td>
<td>Lushoto, Tanganyika</td>
</tr>
<tr>
<td>S. pyhonis Tiegs, 1931</td>
<td>Snakes: <em>Morelia arcos</em> (Pythonidae) <em>Python spilotes</em></td>
<td>1.1 mm long</td>
<td>4-7 μ long</td>
<td>Australia</td>
</tr>
<tr>
<td>S. gracilis sp. nov.</td>
<td>Turtles and Tortoises: <em>Kinosternon scorpioides</em></td>
<td>up to 8 mm × 170-230 μ</td>
<td>18.4 μ × 1.7 μ (fresh)</td>
<td>Island of Marajó, Belém, Pará State, Brazil.</td>
</tr>
<tr>
<td>S. salvelini Fantham &amp; Porter, 1943</td>
<td>Fish: <em>Salvelinus fontinalis</em> (Teleosti, Isospondyli)</td>
<td>500 μ long</td>
<td>5.2-8.8 × 1.5-2.5 μ</td>
<td>Canada</td>
</tr>
<tr>
<td>Unnamed Fantham &amp; Porter, 1943</td>
<td><em>Zoarces angularis</em> (&quot;eel pout&quot;)</td>
<td>1 mm long</td>
<td>6-15 μ × up to 3.5 μ</td>
<td>“”</td>
</tr>
</tbody>
</table>

* Previous scientific names of hosts are given in parentheses.

formol-saline; sections were cut at 4-5 μ and stained with Giemsa, hematoxyalin and eosin, or Gomori’s method (11).

Both cysts and zoites were examined, fresh, by phase contrast illumination. Photomicrographs were taken on a Zeiss W. L. research microscope, using Adox KB 14 and 17, 35 mm film and the zoites were measured by the method we have described for trypanosomes (16). Photomicrographs of the scale of a slide-micrometer are taken on each roll of film. This scale, printed at the same time as the photographs of the organims, is then used to set a pair of dividers so that the distance between their points is equivalent to 1.0 μ. The size of the zoite is easily calculated by “walking” the dividers down the mid-line.

RESULTS

Eighteen of the 206 tortoises examined were infected. The parasite described here is structurally different from those recorded in lizards and snakes and, geographically, the nearest reptilian hosts in which other Sarcocystis species have been described are iguanid lizards from southern California (2). All other reports are from the Old World.

In view of these differences we feel that a new specific name should be given to this sarcosporidian of the Brazilian tortoise *Kinosternon scorpioides*. Impressed by the graceful form and movement of the zoite stages, we therefore propose the name Sarcocystis gracilis n. sp.

The Cyst. As far as could be ascertained, the cysts are to be found only within the skeletal muscle fibers; a search of smears and sections revealed none in the heart muscle, involuntary muscle of the intestine, viscera or brain. They are of the cylindrical form so commonly seen in other species and may reach 8.0 mm in length and 170-230 μ in diameter. Generally their outline is smooth, occasionally with a few intuclings or convolutions (Fig. 6).

Possibly the electron microscope might reveal details in the cyst wall, but by the light-microscope it appears extremely delicate and structureless (Figs. 1, 5-9). Within the cyst there are prominent septae or trabeculae which “walking” the dividers down the mid-line.

The development of these trabeculae and the contained zoites can most clearly be seen in the large “Miescher's tubes” of *S. tenella* in the cap of “proliferative cells” at the tips of the cyst (Figs. 1, 9). Here the compartments may measure as little as 4 by 4 to 6 by 5 μ and contain single, rounded or oval parasites (Figs. 1, 9, 11). Division of these forms produces “packets” of zoites, the compartments sometimes being as large as 90 by 45 μ and containing many hundreds of parasites. Bertram (3) and Babudieri (1) both described degeneration of the central part of the larger cysts of *S. platydaactylus* and a similar destructive procedure can often be seen in the large “Miescher's tubes” of *S. tenella* in shee.

We saw no such process in the cysts of *S. gracilis* but, rather, a complete breakdown of cysts with liberation of the zoites and extensive host-cell response (Figs. 13-15). This will be discussed further in considering the pathology of the infection.
The Zoites. In comparison with other species of Sarcocystis the zoites of S. gracilis are surprisingly long and slender, averaging 18.4 by only 1.7 μ in fresh preparations (Figs. 2, 13). They are characteristically curved into a graceful crescent. At the more slender end there appears an optically more dense area which is probably the "conoid" or equivalent apparatus, described in ultrastructure studies on other species. Occasionally the zoite may be twisted on its longitudinal axis. The cytoplasm is generally dense and, instead of the large diffusely vacuolated area so often seen in the zoites of other species, there are a few small, widely scattered vacuoles that have a crisp "punched-out" appearance. At the blunter pole there may be one or 2 such vacuoles, with 1-3 more variously disposed towards the more slender end.

The nucleus, seen with difficulty in the living zoite, is a paler, oval area in the middle of the organism or slightly to the blunter end. There appeared to be a small diffuse karyosome, altho this could not be verified in stained material.

Table 2. Measurements in μ of zoites* of Sarcocystis gracilis sp. nov., from smears prepared in different ways.

<table>
<thead>
<tr>
<th>Type of preparation</th>
<th>Length</th>
<th>Width</th>
<th>Average Dimensions of Nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline suspension of fresh zoites</td>
<td>18.4 ± 0.60 (16-19)</td>
<td>1.7 ± 0.20 (1.5-2.0)</td>
<td>—**</td>
</tr>
<tr>
<td>Smear of zoites originally suspended in saline (Bouin fixation)</td>
<td>16.9 ± 0.92 (15-18.5)</td>
<td>1.9 ± 0.20 (1.5-2.3)</td>
<td>3.0 × 1.9</td>
</tr>
<tr>
<td>Smear of zoites made directly from a dissected cyst (Bouin fixation)</td>
<td>16.0 ± 0.85 (14-17.5)</td>
<td>1.9 ± 0.20 (1.5-2.3)</td>
<td>2.8 × 1.6</td>
</tr>
<tr>
<td>Smear of zoites made directly from a dissected cyst (Methanol fixation)</td>
<td>15.6 ± 0.60 (14-17)</td>
<td>1.9 ± 0.14 (1.5-2.1)</td>
<td>3.6 × 1.5</td>
</tr>
</tbody>
</table>

* 50 zoites measured.
** The nuclei were not clear enough to measure.

Fig. 1. Sarcocystis gracilis sp. nov. Tip of a cyst showing trabeculae and proliferative zone, with uni- and bi-nucleate parasites giving rise to zoites.

Fig. 2. Living zoites of S. gracilis as seen by phase contrast illumination.

Fig. 3. Zoites of S. gracilis. Crush smear of a cyst fixed in Bouin's fluid, staining by Giemsa's method.

TABLE 2. Measurements in μ of zoites* of Sarcocystis gracilis sp. nov., from smears prepared in different ways.

(Means ± standard deviations and range)
Fig. 4. *Sarcocystis gracilis* sp. nov. Leg muscle of a tortoise *Kinosternon scorpioides* showing large numbers of cysts. Approximately natural size.

Fig. 5. *S. gracilis*. Portion of fresh cyst as seen in a muscle-squash preparation. Note prominent septa or trabeculae. \( \times 300 \).

Fig. 6. Longitudinal sections of cysts of *S. gracilis*. Carnoy fixation, Giemsa staining. \( \times 140 \).

Fig. 7. Transverse section of cyst within muscle fiber, showing smooth cylindrical form and marked trabeculae. \( \times 560 \).

At first sight the zoites give the impression of extreme rigidity and immobility. A small number, however, periodically show a remarkably sudden and rapid gliding movement, executing sweeping curves that frequently remove them from the microscope field. This movement seems always directed with the more slender end forward.

We have concluded that the structure of air-dried, methanol-fixed zoites is often badly distorted, and many preparations were unsuitable for accurate measurement. Bouin fixation of dried smears (Figs. 10, 11) gave a picture closer to the living parasite but, even so, the smooth crescentic form tends to be lost and the nuclear membrane
Fig. 8. Sarcocystis gracilis sp. nov. Tangential section of a cyst. Fixation in Carnoy’s fluid, staining by Gomori’s method. × 350. The trabeculae are particularly well defined by this staining method.

Fig. 9. Longitudinal section of the tip of a cyst of S. gracilis showing the cap of proliferative cells and differentiation of the zoites and trabeculae. Note the very delicate nature of the cyst wall. Carnoy’s fixation, Giemsa stain. × 1,400.

Fig. 10 & 11. Smears of zoites of S. gracilis from a crushed cyst. Fixation in Bouin’s fluid and staining by Giemsa. Fig. 10 shows mature zoites, Fig. 8 shows a cluster of proliferative cells. × 1,980.

Pathology. Sarcocystis infections are generally associated with few or no apparent lesions, the cysts usually evoking no cellular response. Notable exceptions occur, as for example, the muscular degeneration in pigs infected with S. miescheriana (6) and the death of mice experimentally infected with Sarcocystis (vide Wenyon, 20, p. 766). Violent tissue reaction to the parasite has also been noted in trout...
Sarcocystis in Tortoises

Fig. 12. *Sarcocystis gracilis* sp. nov. Living zoites, photographed by phase contrast illumination. × 1,750.


Fig. 15. Longitudinal section of skeletal muscle of an infected tortoise showing the complete replacement of a cyst of *S. gracilis* by invading round-cells. Carnoy fixation, Giemsa staining. × 140.

Infected with *S. salvelini* (8) and in chameleons with *S. chamaeleonis* (9). It was with some interest that we noted marked lesions in some infected tortoises, usually associated with ruptured cysts that had released zoites into the surrounding muscle. Remnants of the cysts were visible in the center of intense round-cell infiltration (Figs. 13, 14). Eventually the entire sarcocyst may become completely replaced by a long, spindle-shaped mass of invading cells (Fig. 15) and the zoites appear to be destroyed.

The flesh of the uninfected muçü is firm and dark red. In heavily infected animals, however, it becomes pale, soft and pulpy, presumably due to the changes described. The fact that most of the tortoises that died in captivity proved to be very heavily infected suggests that infection may
scribed an eimeriid-type development of microgametes, and transmitted by way of teces and, as long ago as 1916, Crawley Toxoplasmea under the 3 separate families Sarcocystidae, Toxoplasmatidae and Besnoitiidae (10). Recently, however, Hutchison (12) and Frenkel et al. (7) have shown Toxoplasma to be an Isospora-like coccidium. The primary host appears to be the domestic cat.*

Clearly, interest must now be centered on the possibility that Sarcocystis, Besnoitia and Frenkelia may, too, be only part of the life-cycles of other coccidial parasites. There is, indeed, strong evidence that Sarcocystis is transmitted by way of feces and, as long ago as 1916, Crawley (5) was actually of the opinion that experimental mice, fed with zoites, developed male and female gametocytes of a coccidial nature in the intestinal epithelium. He described an eimeriid-type development of microgametocytes, and fertilization of macrogametes. Wenyon (20), however, felt that these sexual stages might really have belonged to Eimeria falciformis (a common coccidium of mice).

The type of life-cycle described for Toxoplasma fits well with that parasite's extremely wide distribution, and accounts for the fact that the asexual, proliferative and tissue-cyst stages are structurally indistinguishable in whatever host. With Sarcocystis, however, the situation would appear to be more complicated, for the tissue-cysts (Miescher's tubes) and contained zoites are usually structurally distinct in the different animals in which they have been described. The possibility that the introduction of a single parasite species into a variety of different hosts could result in such a degree of polymorphism seems most unlikely. It would be more plausible to argue that if Sarcocystis is a coccidium, then there are many different species involved, and this at once raises interesting problems regarding their hypothetical life-cycles. Can each be expected, for example, to have different primary and secondary hosts, like Toxoplasma, only on a more modest scale?

In the forest surrounding Belém we have a high rate of Sarcocystis infection in the 2 rodents Proechimys (Echimyidae) and Oryzomys (Cricetidae). We have also noted other infections in anteaters and opossums. These Sarcocystis species appear structurally distinct (15) and, till now, we have found no evidence of cross-infections. Both Proechimys and Oryzomys share the same terrestrial habitat and are frequently caught in the same traps. If we postulate the source of their Sarcocystis infections to be coccidia-like oocysts contaminating the forest floor, this absence of cross-infection strongly suggests that there is strict host-specificity. This brings us, inevitably, to consider that the entire life-cycle of each Sarcocystis species may take place within the one specific host. If they are coccidia, they might then be expected to produce oocysts in the intestinal (or bile duct) epithelium and asexual tissue-cysts (Miescher's tubes) in the muscle. Following the more typical coccidial pattern, the fecally passed oocysts would be infective only to the same animal species while possibly, like Toxoplasma, ingestion of the tissue-cysts might also lead to infection of the specific host.

Intestinal coccidia are certainly well known in most of those animals reported to harbor Sarcocystis. Eimeria species are common, for example, in many of the Proechimys we have examined, and we have already discussed the probable fecal transmission of Sarcocystis proechimyos from parents to offspring in a laboratory colony of these rodents. On the other hand, we found no evidence of coccidial infections in any of the 206 tortoises examined in the present work, including the 18 infected with Sarcocystis.

Clearly, very carefully controlled experiments will be needed to show if there is a connection between sarcosporidial and coccidial infection. They will, in most cases, be dogged by difficulties in securing completely clean animals. This has been achieved for Toxoplasma by the use of specific-pathogen-free cats and it is, we hope, only a matter of time before similar work can be undertaken with regards Sarcocystis.

SPECIFIC DIAGNOSIS OF SARCOCYSTIS GRACILIS N. SP.

Type Host: Kinosternon scorpioides (Reptilia, Chelonidae).

Geographic Area: Island of Marajó, Pará State, north Brasil.

Site of Infection: Thruout all skeletal muscle. Not seen in heart muscle, involuntary muscle.

Cysts: Cylindrical, with pointed ends: up to 8 mm long and approximately 230 μ diameter, as seen in fresh preparations. Relatively smooth outline, more rarely with invaginations or convolutions. Cyst wall a delicate and apparently structureless membrane; trabeculae well marked and dividing cyst into angular compartments containing up to many hundreds of zoites. Growth of cyst largely from terminal caps of proliferative cells.

Zoites: Long, slender, and curved into a graceful crescent. Up to 18.4 by 1.7 μ, when examined fresh. Nucleus central or slightly to the blunter end; a few small, widely scattered vacuoles giving the cytoplasm a "punched-out" appearance. Zoites show sudden and rapid gliding movements.

Pathology: Frequently produces marked pathologic changes in muscle.

Type Material: Slides deposited with the Dept. of Parasitology, London School of Hygiene and Tropical
We are grateful to Sr. Sebastião Fernandes de Oliveira for his painstaking technical assistance during this study, and to Dr. Mario Machado Sampaio who kindly drew our attention to the use of Gomori’s staining technique.

REFERENCES
