The Genus Cyclospora (Apicomplexa: Eimeriidae), with a description of Cyclospora schneideri n.sp. in the snake Anilius scytale scytale (Aniliidae) from Amazonian Brazil - A Review

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A review is made of the recorded species of the coccidian genus Cyclospora and major events leading up to the discovery of C. cayetanensis, which is responsible for serious outbreaks of diarrhoea in man and is one of the aetiological agents of “traveller’s diarrhoea”. Humans appear to be the specific hosts, with the entire life-cycle in the intestine: to date there is no convincing evidence that the disease is a zoonosis. A description is given of oocysts and endogenous stages of C. schneideri n.sp., in the snake Anilius scytale scytale. Sporulation is exogenous and completed after about one week at 24-26°C. Mature oocysts 19.8 × 16.6 (15.1 × 13.8-25.7 × 20.1), shape-index 1.2 (1.0-1.3): no oocyst residua or polar bodies. Oocyst wall a single colourless, smooth layer with no micropyle: it is rapidly deformed or broken. Sporocysts 13.6 × 9.4 (11.3 × 8.3-15.1 × 9.9), shape-index 1.4 (1.2-1.5) with an inconspicuous Stieda body. Sporozoites 11-13 × 2.5-3. Endogenous stages are intracytoplasmic in the epithelial cells of the small intestine and with the characters of the Eimeriorina.

Key words: Cyclospora - cyclosporiasis - Cyclospora cayetanensis - Homo sapiens - Cyclospora schneideri n.sp. - oocysts - endogenous stages - Anilius scytale - snakes - Brazil

Background history

Description of the coccidian Cyclospora cayetanensis of humans in 1992 naturally resulted in an explosion of papers on this parasite, largely clinical and epidemiological. It is not the object of the present paper to attempt a revision of the 200 or more publications that ensued, admirably compiled by Steve Upton (2001), but rather to discuss the history of the genus, the species recorded in non-human hosts and the major events that led up to the discovery of Cyclospora in man. A description is given of a new species encountered in the Brazilian snake Anilius scytale scytale.

The type species of the genus Cyclospora, C. glomericoloca, was described in the millipede Glomeris (Diplopoda) by Aimé Schneider in 1881 and, to date, appears to be the only species encountered in an invertebrate host. Previously, Eimer (1870) had noted the presence of a parasite with cyclosporan morphology in the intestine of the mole Talpa europaea, but gave it no name, and it remained for Schaudinn (1902) to give a full description of the life cycle of this parasite, which he named C. caryolytica. Tanabe (1938) later described the development of what he considered to be the same species in another mole referred to as Moger a wogura coreana from Japan.

Schaudinn described the asexual and sexual stages of C. caryolytica in both the small and large intestine of the mole, where they develop within the nucleus of the epithelial cells. With the growth of these stages the nucleus disintegrates and the host cell becomes a mere sac containing the parasite: heavy infection may result in a fatal enteritis. Schaudinn noted two types of meronts and suggested that the one producing larger merozoites gave rise to the macrogamonts, while the other gave smaller merozoites which were destined to become microgamonts. Tanabe (1938) was unable to demonstrate more than a single type of meront for what he considered to be C. caryolytica in M. wogura coreana and suggested that Schaudinn was dealing with a mixed infection of that parasite and the merogonic stages of a concomitant Eimeria infection. The consensus of opinion now is that such morphologically different asexual stages merely represent different generations of meronts rather than a sexual dimorphism: Lainson (1965), for example, noted three different types of merozoites produced during the asexual division of C. niniae in the snake Ninia sebae sebae. In typical eimerid fashion, the microgamont of C. caryolytica produces a large number of flagellated microgametes and, following fertilization of the microgamonts and development of a resistant membrane around the zygote, the resulting oocysts are expelled, unsporulated, in the faeces. Sporulation is completed in 4-5 days, with the formation of two sporocysts, each containing two sporozoites and a sporocystic residuum.

There followed a succession of descriptions of other Cyclospora species in reptiles, principally snakes (Table), all with intracytoplasmic development in epithelial cells of the intestine. Subsequently, however, Pellerdy and Tanyi (1968) described the oocysts of a second species in the European mole and named it C. talpae. They found microgamonts and macrogamonts in the liver and, in a more recent study, Mohamed and Molyneux (1990) have shown that these sexual stages develop within the nucleus of the bile-duct epithelial cells. Merogony appears to be limited to mononuclear cells in the capillary sinusoids of
the liver. Oocysts entering the intestine with the bile are voided in the faeces and exogenous sporulation is completed in about two weeks.

Duszynski and Wattam (1988b) re-described the oocysts of *C. talpae* in *T. europaea* from England and, in addition, noted that some oocysts were present which differed from those of *C. talpae* in minor details (principally in size). Whether or not they belonged to yet another species of *Cyclospora* was not decided.

Ford and Duszynski (1988, 1989) turned their attention to faecal samples from other members of the Insectivorida and encountered three further species of *Cyclospora*. *C. megacephali* was described in the “eastern mole” *Scalopus aquaticus*, and both *C. ashtabulensis* and *C. parascalopi* in the “hairy-tailed mole” *Parascalops breweri*. The site of development in these animals was not ascertained.

Finally, Ford et al. (1990) gave the name of *C. angimurinensis* to oocysts they found in the faeces of the heteromyid rodent *Chaetodipus hispidus* from the US, and Northern Mexico. Once again, the site of endogenous development was not determined.

**Human cyclosporiasis**

In 1979 Ashford published a paper recording the presence of what were most probably oocysts of *Cyclospora* in the faeces of three patients in Papua New Guinea, two of whom were suffering from diarrhoea. The oocysts contained two sporocysts, but Ashford was unable to identify the parasite to generic level due to difficulties in determining the number of sporozoites in the sporocysts. This important finding, strangely overlooked in much of the literature, was followed by a series of publications on similar findings in patients, most of whom were suffering from acute “traveller’s diarrhoea” acquired in areas with poor standards of hygiene, or in immunocompromised (Aids) patients (Soave et al. 1986, Long et al. 1990, Hart et al. 1990, Shlim et al. 1991). Long et al. (1991) proposed the term “cyanobacterium-like bodies” (CLB) for the cysts, because of a superficial ultrastructural resemblance to unicellular members of the blue-green algae. Bendall et al. indicated the coccidial nature of the cyanobacterium-like bodies in the 1993 edition of the *Lancet*, illustrated by photomicrographs of the sporulated oocysts containing two sporocysts. It was at the 41st annual meeting of the American Society of Tropical Medicine and Hygiene in 1992, however, that Ortega and colleagues finally published unpublished evidence showing that the sporocysts each contained two sporozoites, and that the parasite was, therefore, a member of the genus *Cyclospora*. The name *C. cayetanensis* was later proposed by these authors in an abstract of this presentation (Ortega et al. 1992), although Ashford et al. (1993) questioned the validity of the name in view of what they considered to be an inadequate written description of the parasite, and the absence of illustrations. Subsequently, Ortega et al. (1994) described the light and electron microscope morphology of the oocysts in detail and the specific name *C. cayetanensis* now remains in firm usage.

Growing epidemiological evidence pointed to the transmission of *C. cayetanensis* by way of contaminated food or water and to the fact that the parasite enjoyed a world-wide distribution in various countries of Central and South America, Asia, Africa and Europe, and in Australia. The remarkable efficiency of transmission and the medical importance of human cyclosporiasis first became apparent, however, following a series of explosive outbreaks of acute diarrhoea among large numbers of guests at a number of social events in the US and Canada, during the years 1996-1998 (Chambers et al. 1996, Herwaldt & Ackers 1997, Herwaldt & Beach 1999). Careful investigations finally traced the source of infection to unwashed raspberries imported from Guatemala and, during studies in that country (Bern et al. 1999), an infection-rate of 2.3% was found in the stools of 5552 persons examined in governmental health centres and hospitals. Positive faeces were most commonly found in children and persons suffering from gastroenteritis, and the authors noted a seasonality of cyclosporiasis. Thus, an infection-rate of 3.8% in May rose to a peak of 6.7% in June, and subsided to zero during the period August to November of the same year. A study of raspberry farm workers and family members showed 6 of 182 persons (3.29%) to be passing oocysts. Finally, in a case-control analysis of 68 infected individuals, 62 (91%) admitted drinking untreated water about two weeks before the onset of illness (Bern et al. 1999).

Further outbreaks of human cyclosporiasis among participants at social events in the US were attributed to the consumption of fresh basil or lettuce in salads and side-dishes (Anon. 1997a, b, Lopez et al. 2001). In Germany, 34 persons developed acute diarrhoea diagnosed as cyclosporiasis, and the source of infection was again considered to be salad side-dishes of lettuce, imported from southern Europe, spiced with fresh leafy herbs (Döller et al. 2002). Epidemiological investigations in endemic areas of other countries revealed oocysts of *C. cayetanensis* on green leafy vegetables, in sewage water, and even in tap-water (Ortega et al. 1997a, Sturbaum et al. 1998, Sherchand et al. 1999, El-Naga 1999, Cam et al. 2001).

**The source of *C. cayetanensis* in water and food**

Attempts have been made to find non-human hosts of *C. cayetanensis* and show that human cyclosporiasis is in fact a zoonosis: there is as yet, however, no conclusive evidence that this is so and the few claims of success have been thrown into doubt by the conflicting findings of other workers. Garcia López et al. (1996) reported the finding of oocysts considered to be those of this parasite in the pooled faeces of some 600 young chicken from a poultry farm and in pooled caecal contents from another 50 from a market near Monterey, Mexico, and there is a report of similar oocysts in the faeces of a duck in Peru (Zerpa et al. 1995). In an endemic area of Nepal, Sherchand et al. (1999) found oocysts suspected to be those of *C. cayetanensis* in two chickens during a survey of 196 domestic animals: all the other animals were negative. In a survey of Peruvian children in Peru, Bern et al. (2002), suggested that human cyclosporiasis was most frequently associated with the ownership of domestic animals. In contrast to all these findings, however, Eberhard et al. (1999a) found no morphologically compatible oocysts in
chickens, ducks, turkeys, pigeons, pigs, cattle, horses, goats, dogs, cats, and guinea-pigs in Haiti, despite their living in or near houses with human infection: they concluded that domestic animals were not a reservoir of *C. cayetanensis* and that man appears to be the only host. Yai et al. (1997) reported oocysts that appeared to be those of *C. cayetanensis* in two dogs from São Paulo, Brazil, but Carollo et al. (2001) found no signs of infection in 140 stray dogs they examined in the same area.

To date, then, available evidence suggests that humans are the specific hosts of *C. cayetanensis* and the sole source of oocysts, following their faecal contamination of food and water. Supporting this is histological evidence showing that the entire life-cycle of the parasite takes place in the human intestine (Sun et al. 1996, Ortega et al. 1997b), and the striking host specificity shown by other primate species of *Cyclospora*, namely *C. cer- copithecii* and *C. colobii* of monkeys and *C. papionis* of baboons, even when there is a geographical overlap of the hosts and parasites (Eberhard et al. 1999a, 2001). To what extent such specificity extends to *Cyclospora* species recorded in other hosts is uncertain. The genus would appear to be particularly common in snakes (Table), and this review extends the list of ophidian hosts with the description of a new species in the snake *Anilius scytale scytale* (Aniliidae).

*A. scytale scytale*, is a relatively small, burrowing “pipe-snake” found in the Guianas, North Brazil, Venezuela and Amazonian Colombia, Ecuador and Peru. It is harmless, but because of its vivid red colour with black rings it is often mistaken for the venomous coral snake *Micrurus*. Seldom seen above ground, it is most commonly found when flushed out during heavy rain.

Only two of these snakes were available for study, in December, 1990 and January 2004, both from the state of Pará, North Brazil. They were passing coccidial oocysts in their faeces and sporulation was in both cases complete in approximately one week in 2% aqueous potassium dichromate solution (K₂Cr₂O₇) at 24-26°C. Oocysts were measured using normal light microscopy, an eye-piece micrometer and the oil immersion lens. Dimensions are given in µm as means, followed by the range in parentheses, the shape index (ratio of length/width) and the number measured (n). Tissues for histology were fixed in 10% buffered neutral formalin and embedded in paraffin wax: sections were cut at 4 µm. Photo-micrographs were made with a Zeiss “Photomicroscope III” and Kodak TMX100 film.

*Cyclospora schneideri* n.sp.

(Figs 1-18)

**Description of the oocyst** - Mature forms (Figs 14-18) ovoid to subspherical or, more rarely, spherical: 19.8 × 16.6 (15.1 × 13.8-25.7 × 20.1), shape index 1.2 (1-1.3), n = 100. No oocyst residuum or polar bodies. Oocyst wall approximately 0.5-1 thick, colourless, smooth, and apparently of a single layer: no micropyle or striations. It is fragile and soon becomes highly deformed. The two dzoic sporocysts average 13.6 × 9.4 (11.3 × 8.3-15.1 × 9.9), shape index 1.4 (1.2-1.5), n = 77; there is an inconspicuous nipple-like Stieda body. The sporozoites measure 11-13.0 × 2.5-3, n = 13, and curve slightly around a sporocystic residuum of fine granules and larger globules. Refractile bodies were not detected.

**Endogenous stages** - These develop, in inconspicuous parasitophorous vacuoles, in the cytoplasm of the epithelial cells of the small intestine. In histological sections of the infected gut, most of the developing parasites were clearly located above the host cell nucleus, but it is possible that others commenced development below and, with growth, eventually positioned themselves between or above the nuclei. Mature meronts (Figs 1, 2) were scanty but appeared to be of a single, small type of approximately 16 ×14 (n = 4): they produce 6-12 merozoites, measuring an average of 10 × 2.5 (n = 4) and segmentation leaves no residuum. Growing microgamonts (Figs 8, 9) reach up to 33 × 26 and contain many bulky, heavily stained nuclei distributed predominantly at the periphery of the parasite. Mature forms (Figs 10, 11) have a residuum containing a few big vacuoles, and shed a large number of micromegames measuring approximately 5.0 × 1. Young macrogamonts (Figs 3-5) have a poorly staining nucleus containing a densely staining karyosome (Fig. 4). Mature forms (Fig. 7) may reach up to 24 × 20 (15 × 10 - 24 × 20), n = 10, and contain very prominent small and large wall-forming bodies (Figs 6, 7). The wide size range of the mature macrogamonts results in an equally wide range in the size of the zygotes and the oocysts. This at first gave the impression that both snakes were infected with two different species of *Cyclospora*. Demonstration of a smooth gradation between the dimensions of the smallest and the largest oocysts, however, militates against this possibility.

**Sporulation** - Exogenous, in approximately one week. **Host** - The snake *Anilius scytale scytale* (Linnaeus), (Aniliidae). **Type locality** - Capanema, state of Pará, North Brazil. **Type material** - Oocysts in 10% buffered formalin, histological sections of the endogenous stages and phototypes in the Department of Parasitology, Instituto Evandro Chagas and the Muséum National d’Histoire Naturelle, Paris; Accession No. 2257. **Prevalence** - Uncertain. Only two *A. s. scytale* were examined, and both were infected. **Pathology** - No apparent pathology. **Etymology** - The specific name is in honour of Aimé Schneider, who founded the genus *Cyclospora*.

As far as I am aware, this is the first coccidian to be described in *Anilius s. scytale*. Among the *Cyclospora* species previously described in snakes, *C. niniae* Lainson, 1965 resembles *C. schneideri* n.sp., in the fragility of the oocyst wall, the size of the sporocysts and their possession of a modest Stieda body. The oocysts of *C. niniae*, however, contain a conspicuous polar body, and measure only 14.6 × 13.3. Phisalix (1923, 1924a, 1933) made a number of revisions to her measurements of the oocysts of *C. vipersiae*, but on the basis of her final measurements of 16.8 × 10.5 they are substantially smaller than those of *C. schneideri* n.sp., as are those of *C. babaulti*, *C. tropidonoti*, and *C. zamensis* (17 × 10) (Table). Without cross-infection experiments the host specificity of ophid-
ian Cyclospora species remains uncertain, but the wide zoological difference and wide geographical separation of A. s. scytale and the European colubrids and vipers makes it most unlikely that C. schneideri n.sp., is conspecific with any one of the four species described by Phisalix.

I have commented on the similarity of the oocysts of the four species of Cyclospora named by Phisalix (Lainson 1965) and suggested that C. babaulti, C. tropidonoti and C. zamenis might be synonyms of C. viperae. Duszynski et al. (1999) appear to have in part agreed with this suggestion and have listed C. babaulti as a synonym of C. viperae and C. tropidonoti as a synonym of C. zamenis. They went further in suggesting that the oocysts of C. viperae “… appear to be misidentifications of Sarcocystis spp.” and that “… perhaps all species from reptiles are misidentifications of Isospora or Sarcocystis spp.” I feel it best, however, to include the five reptilian species of Cyclospora described by Phisalix in the accompanying

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### Table

<table>
<thead>
<tr>
<th>Species</th>
<th>Hosts</th>
<th>Oocysts (µm)</th>
<th>Endogenous stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. glomericolata</td>
<td>Glomeris sp. (Diplopoda)</td>
<td>25-36 × 9-10</td>
<td>Not described: oocysts in lumen of gut</td>
</tr>
<tr>
<td>C. caryolytica</td>
<td>Talpa europaea, Mogera vogura coriana</td>
<td>18 × 12.5</td>
<td>Intranuclear in epithelial cells of small and large intestine</td>
</tr>
<tr>
<td>C. viperae</td>
<td>Coluber scalaris, Coronella austriaca,</td>
<td>16.8 × 10.5</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td></td>
<td>Natrix vipherinus, Vipera aspis (Reptilia: Ophidia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. babaulti</td>
<td>Vipera berus (Reptilia: Ophidia)</td>
<td>17 × 10</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td>C. scincii</td>
<td>Scincus officinalis (Reptilia: Squamata: Scincidae)</td>
<td>10 × 7.0</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td>C. tropidonoti</td>
<td>Natrix natrix, Natrix stolata (Reptilia: Ophidia)</td>
<td>17 × 10</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td>C. zamenis</td>
<td>Coluber viridiflavus viridiflavus (Reptilia: Ophidia)</td>
<td>17 × 10</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td>Cyclospora sp.</td>
<td>Hemidactylus frenatus (Reptilia: Squamata: Gekkonidae)</td>
<td>16.9-30.3 × 16.7-26</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Ninia sebae sebae (Reptilia: Ophidia)</td>
<td>14.6 × 13.3</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td>C. cercopithecii</td>
<td>Cercopithecus aethiops (Primates)</td>
<td>8-10 × 8-10</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. talpae</td>
<td>Talpa europaea (Insectivora: Talpidae)</td>
<td>15-18 × 10-12</td>
<td>Intranuclear in epithelial cells of bile ducts and capillary sinusoids of the liver</td>
</tr>
<tr>
<td>C. megacephali</td>
<td>Scalopus aquaticus (Mammalia: Insectivora)</td>
<td>18.5 × 15.7</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. ashtabulensis</td>
<td>Parascalops breviri (Mammalia: Insectivora)</td>
<td>18.0 × 14.3</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. parascalopi</td>
<td>Parascalops breviri (Mammalia: Insectivora)</td>
<td>16.5 × 13.6</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. angimurinensis</td>
<td>Chaetodipus hispidus (Mammalia: Rodentia)</td>
<td>19-24 × 16-22</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>Homo sapiens (Primates)</td>
<td>8.6 × 8.6</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
<tr>
<td>C. colobi</td>
<td>Colobus guereza (Primates)</td>
<td>8-9 × 8-9</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. papionis</td>
<td>Papio anubis (Primates)</td>
<td>8-10 × 8-10</td>
<td>Not ascertained</td>
</tr>
<tr>
<td>C. schneideri n.sp.</td>
<td>Anilius scytale scytale (Reptilia: Ophidia)</td>
<td>19.8 × 16.6</td>
<td>Intracytoplasmic in epithelial cells of the intestine</td>
</tr>
</tbody>
</table>

*a: opinions are divided regarding the taxonomy of Japanese moles, with some authors considering that Mogera vogura coreana is a synonym of Talpa micrura coreana and vice versa (Nowak & Paradiso 1983, Duszynski & Wattam 1988a).
Table until such time as conclusive evidence is provided with which to sink them. Their re-examination by DNA analysis might confirm or refute their present taxonomic status, and in this respect it is of interest that Eberhard et al. (1999a) considered that the three species *C. cercopithecus*, *C. colobi*, and *C. papionis* of non-human primates could not be separated by morphology of their oocysts but only at molecular level.

Endogenous stages of *Cyclospora schneideri* n.sp., in the epithelial cells of the small intestine of the snake *Anilius scytale scytale*, as seen in histological sections. Figs 1, 2: mature, segmented meronts. Figs 3-6: developing macrogamonts. Fig. 6: nearly mature macrogamont with formation of the oocyst wall-forming bodies. Fig. 7: mature macrogamont with small wall-forming bodies (arrow) and large wall-forming bodies (arrow head). Figs 8, 9: young microgamonts, with large nuclei located at the periphery of the parasite. Figs 10, 11: mature microgamonts shedding microgametes. Note the large vacuoles in the residuum. Fig. 12: intracellular zygote or young oocyst, with developing oocyst wall already apparent (OW). Fig. 13: extracellular oocyst in contents of the intestinal lumen (L). Bar in Fig. 10 = 10 μm, and applies to all Figs, staining by haematoxylin and eosin.
It is now becoming apparent that species of *Cyclospora* infect a wider range of hosts than previously supposed and it should come as no surprise that reptiles are hosts of *Cyclospora* when one considers the wide host range of other members of the Eimeriidae. Thus, species of *Eimeria* are recorded in millipedes, centipedes, coleopterans, fish, amphibians, reptiles, birds and mammals (Levine 1988); the type species of *Isospora*, *I. rara*, was described in the gastropod *Limax* sp., and other species are common parasites of amphibia, reptiles, birds and mammals (including man); *Caryospora* species are found in reptiles, birds and mammals. By light microscopy, it is clearly difficult to determine the number of sporozoites in a sporocyst of an oocyst as small as that of *C. cayetanensis* (diameter 8.6 µm), and other *Cyclospora* species of similar size may well have been erroneously assigned to the genus *Isospora* or *Sarcocystis*. However, due to the much larger size of the oocysts of both *C. niniae* and *C. schneideri* n.sp. (14.6 × 13.3 and 19.8 × 16.6, respectively), it has not been difficult to determine the dzoic nature of their sporocysts, particularly when they are seen in an end-on position (Figs 15, 18).

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