

EIMERIA SPECIES (APICOMPLEXA: EIMERIIDAE) OF *PODOCNEMIS EXPANSA* (SCHWEIGGER) AND *GEOCHELONE DENTICULATA* (LINN.) FROM AMAZONIAN BRAZIL (REPTILIA: CHELONIA)

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Eimeria lagunculata, *Eimeria mammiformis* and *Eimeria podocnemis* n. spp., are described from the faeces of the fresh-water turtle *Podocnemis expansa*, in Pará State, north Brasil. Oocysts of *E. lagunculata* are ellipsoidal, 19.2 x 12.8 (17.0-20.7 x 11.8-14.1) μm , shape-index (= length/width) 1.5 (1.4-1.7). Oocyst wall about 0.5-0.7 μm thick, with a prominent stopper-like micropyle at one pole. No oocyst residuum and no polar body. Sporocysts elongate ellipsoidal, 11.0 x 5.4 (10.4-11.8 x 5.2-6.0) μm , shape-index 2.0 (1.8-2.1): no Stieda body. A compact, ellipsoidal sporocyst residuum lies between the two sporozoites, which possess a posterior and an anterior refractile body. Oocysts of *E. mammiformis* broadly ellipsoidal, 30.0 x 19.4 (23.0-37.0 x 16.3-21.5) μm , shape-index 1.5 (1.1-1.9). Oocyst wall about 0.7 μm thick, with a prominent micropyle: no oocyst residuum and rarely a single polar body. Sporocysts ellipsoidal, 15.3 x 7.9 (14.8-17.0 x 7.4-9.6) μm , shape-index 2.0 (1.8-2.2), with a tiny Stieda body. Sporocyst residuum bulky, ellipsoidal: sporozoites with two conspicuous refractile bodies. *E. podocnemis* has broadly ellipsoidal oocysts, 17.0 x 12.8 (14.8-19.2 x 11.8-14.1) μm , shape-index 1.3 (1.1-1.4). Oocyst wall about 0.5-0.7 μm thick, with no micropyle. No oocyst residuum, but always a single polar body. Sporocysts ellipsoidal, 9.7 x 5.2 (8.9-10.4 x 4.4-6.0) μm , shape-index 1.9 (1.6-2.0), with no Stieda body. Sporocyst residuum bulky, ellipsoidal: sporocysts with 2 refractile bodies. *Eimeria carinii* n. sp., is recorded from the tortoise *Geochelone denticulata*, also from Pará. Oocysts spherical to subspherical, 19.2 x 18.6 (15.0-20.0 x 14.0-19.0) μm , shape-index 1.0 (1.0-1.1). Oocyst wall about 1.2 μm thick. No micropyle. Oocyst residuum limited to a number (about 10-20) of scattered granules: no polar body. Sporocysts broadly ellipsoidal, and with no Stieda body: they measure 8.8 x 7.3 (8.0-9.0 x 7.0-7.5) μm , shape-index 1.2 (1.1-1.3). Sporocyst residuum bulky, spherical to ellipsoidal: sporozoites possess both posterior and anterior refractile bodies.

Key words: Apicomplexa – Eimeriidae – *Eimeria lagunculata* n. sp. – *Eimeria mammiformis* n. sp. – *Eimeria podocnemis* n. sp. – *Eimeria carinii* n. sp. – coccidia – oocysts – turtle – tortoise – *Podocnemis expansa* – *Geochelone denticulata* – Reptilia – Chelonia – Brazil

Faeces from 6 of 7 juvenile specimens of the fresh-water turtle *Podocnemis expansa*, housed in the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil, were found to contain coccidial oocysts considered to be those of 3 hitherto undescribed species of *Eimeria*.

Other oocysts were recorded in faecal specimens from 2 out of 5 tortoises, *Geochelone denticulata* (the "jaboti") captured in primary forest in the Serra dos Carajás, Pará,

and housed in the Parque Zoobotânico da Companhia Vale do Rio Doce, in the same locality. These, too, were regarded as representing a new species of *Eimeria*.

The 4 parasites are described below.

MATERIALS AND METHODS

Faecal samples from the young specimens of *P. expansa* were removed from the lower part of the intestine at autopsy and examined immediately. Those from *G. denticulata* were collected, freshly passes, from individually caged tortoises. Material from each animal was lightly triturated in 2.0% (w/v) aqueous $\text{K}_2\text{Cr}_2\text{O}_7$ and separately maintained in covered Petri-dishes at approximately 24-26 °C.

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Microscopic examination was made without concentrating the organisms by flotation methods, and 50 oocysts and 50 sporocysts of each parasite were measured, using an ocular micrometer, x 8 eyepieces and a x 100 neofluar objective; photomicrographs were prepared using a Zeiss Microflash II and Ilford Pan F film. Drawings are based partly on these photographs, but largely on direct observations on the oocysts while making measurements. All measurements are in micrometers (μm); they are given as means, with the range in parentheses, and are followed by the shape-index (= ratio of length/width).

RESULTS

Eimeria lagunculata n. sp. (Figs 1-3; 15)

Diagnosis: oocysts ellipsoidal, 19.2 x 12.8 (17.0-20.7 x 11.8-14.1), shape-index 1.5 (1.4-1.7). Oocyst wall very delicate, smooth, colourless, and of a single layer about 0.5-0.7 thick; structure of wall confirmed by rupturing the oocysts. There is a conspicuous "stopper-like" micropyle at one pole of the oocyst, measuring about 1.5-2.0 long x 1.0-1.5 wide. No oocyst residuum and no polar body. Sporocysts elongate ellipsoid, often with one side flattened, 11.0 x 5.4 (10.4-11.8 x 5.2-6.0), shape-index 2.0 (1.8-2.1), with a very delicate, colourless wall which has no Stieda body. Sporocyst residuum compact, usually ellipsoidal, and composed of numerous small granules lying between the two sporozoites. Sporozoites with posterior and anterior refractile bodies which are seen with difficulty.

Type host: *Podocnemis expansa* (Schweigger) (Reptilia: Chelonia: Pelomedusidae); a freshwater turtle.

Location in host: the ileum. No parasites were seen in the gall-bladder contents, liver and spleen. Oocysts described from the faeces.

Sporulation: endogenous; mature and developing oocysts were found in faecal material examined immediately after removal from the intestine.

Type material: oocysts preserved in 10.0% formol-saline and held in the Department of Parasitology, Instituto Evandro Chagas, Belém, Pará, Brazil.

Type locality: Belém, Pará, north Brazil. Turtles housed in the Museu Paraense Emílio Goeldi, Belém.

Prevalence: unknown in the wild; 5 out of 7 turtles examined in the Museum were infected. Of these, 1 was infected only with *E. lagunculata*, 1 with this parasite and *E. podocnemis*, and 3 with *E. lagunculata* and *E. mammiformis*.

Pathogenicity: infected turtles appeared healthy. Histopathology of the infection, however, remains to be studied.

Etymology: the specific name is from the Latin *laguncula* (a little flask), suggested by the flask-like appearance given to the oocyst by the micropyle.

REMARKS: the strange "stopper-like" micropyle of *E. lagunculata* differentiates the oocysts of this parasite from all the eimeriid species described from chelonids to date. Of the 28 we have encountered in the available literature, only 5 possess some form of micropyle; in no case, however, is this structure comparable with that of *E. lagunculata*, it being rather a mere thinning of the oocyst wall at one point or, at most, a flat cap-like body.

Apart from this difference in the micropyle structure, these species are readily differentiated by additional features, as follows.

Eimeria broderi Cerruti 1930, of the Greek tortoise *Testudo graeca*, has larger oocysts which are oval in shape and measure 28.0-32.0 x 18.0-20.0. The oocyst wall has two layers.

Eimeria chrysemydis Deeds & Jahn 1939, was first described from the north American turtle *Chrysemys picta marginata*. It has larger, pear-shaped oocysts, measuring 23.0 x 15.0; Deeds & Jahn (27.6 x 17.0; Wach & Christiansen, 1976) and a yellow, 2-layered oocyst wall.

Eimeria koormae Das Gupta 1938, of the Indian tortoise *Lissemys punctata* is readily distinguished by its spherical oocysts, averaging 14.0 in diameter, and its much more elongated sporocysts (shape-index 2.2).

Eimeria marginata Deeds & Jahn 1939 emend. Pellerdy, 1974, is from *Chrysemys picta marginata*. It has a larger, pear-shaped oocyst

measuring 20.0-28.0 x 15.0-21.0, and larger, elongate sporocysts of 10.0-14.0 x 15.0-21.0, shape-index 1.5.

Finally, *E. scriptae* Sampson & Ernst 1969, of *Pseudemys scripta elegans*, also from north America, is distinguished from *E. lagunculata* by its larger oocysts (24.2 x 13.7) and 2-3 layered oocyst wall.

Eimeria mammiformis n. sp.
(Figs 4; 5; 16)

Diagnosis: oocysts ellipsoidal, narrowing at the micropylar extremity, 30.0 x 19.4 (23.0-37.0 x 16.3-21.5), shape-index 1.5 (1.1-1.9). Oocyst wall a smooth, colourless, single layer about 0.7 thick: it bears a prominent, nipple-like micropyle at the narrow end of the oocyst, measuring about 2.5-3.0 high x 3.0 wide. No oocyst residuum and rarely a single polar body measuring 1.0-2.0 x 0.5-1.5. Sporocysts elongate ellipsoidal, narrowing at one and frequently with one side flattened. They measure 15.3 x 7.9 (14.8-17.0 x 7.4-9.6), shape-index 2.0 (1.8-2.2), and have a very delicate wall bearing a minute Stieda body. Sporocyst residuum bulky, usually in the form of an ellipsoidal mass of relatively large granules which often leave little of the sporozoites visible. Liberated sporozoites measure 14.8 x 3.7 in fresh preparations, and they have a large posterior refractile body and a smaller anterior one (Fig. 5).

Type host: *Podocnemis expansa* (Schweigger).

Location in host: the small intestine (ileum), as verified by intestinal scrapings. No parasites seen in gall-bladder contents or in the liver and spleen.

Sporulation: endogenous; mature and developing oocysts in faecal material examined immediately after its removal from the intestine.

Type material: oocysts preserved in 10.0% formol-saline and held in the Parasitology Department, Instituto Evandro Chagas, Belém.

Type locality: Belém, Pará, north Brazil; turtles housed in the Museu Paraense Emílio Goeldi, Belém Pará.

Prevalence: unknown in the wild; 4 of 7 captive turtles examined were infected, but all

were housed in the same aquarium. In 3 cases the animals had mixed infections with *E. mammiformis* and *E. lagunculata*.

Pathogenicity: infected turtles appeared healthy, but histopathology of the infection remains to be studied.

Etymology: the specific name is from the Latin *mamma*, breast; *forma*, shape (breast-shaped) in view of the nipple-like micropyle.

REMARKS: no *Eimeria* species possessing a micropyle comparable with that of *E. mammiformis* has been described previously from chelonid hosts (see remarks for *E. lagunculata*, above).

Eimeria podocnemis n. sp.
(Figs 6-8; 17)

Diagnosis: oocysts broadly ellipsoidal with rounded extremities, 17.0 x 12.8 (14.8-19.2 x 11.8-14.1), shape-index 1.3 (1.1-1.4). Oocyst wall smooth, colourless, single-layered and about 0.5-0.7 thick; there is no micropyle. No oocyst residuum: a single, pale polar body of about 2.0 x 1.5 is a constant feature and was always seen in the polar position of all oocysts examined. Sporocysts ellipsoidal, both extremities tending to be somewhat pointed, 9.7 x 5.2 (8.9-10.4 x 4.4-6.0), shape-index 1.9 (1.6-2.0); wall very delicate and with no Stieda body. Sporocyst residuum an ellipsoidal mass of 20-25 largish rounded granules, tending to obscure the sporozoites. Sporozoites with posterior and anterior refractile bodies, seen with difficulty.

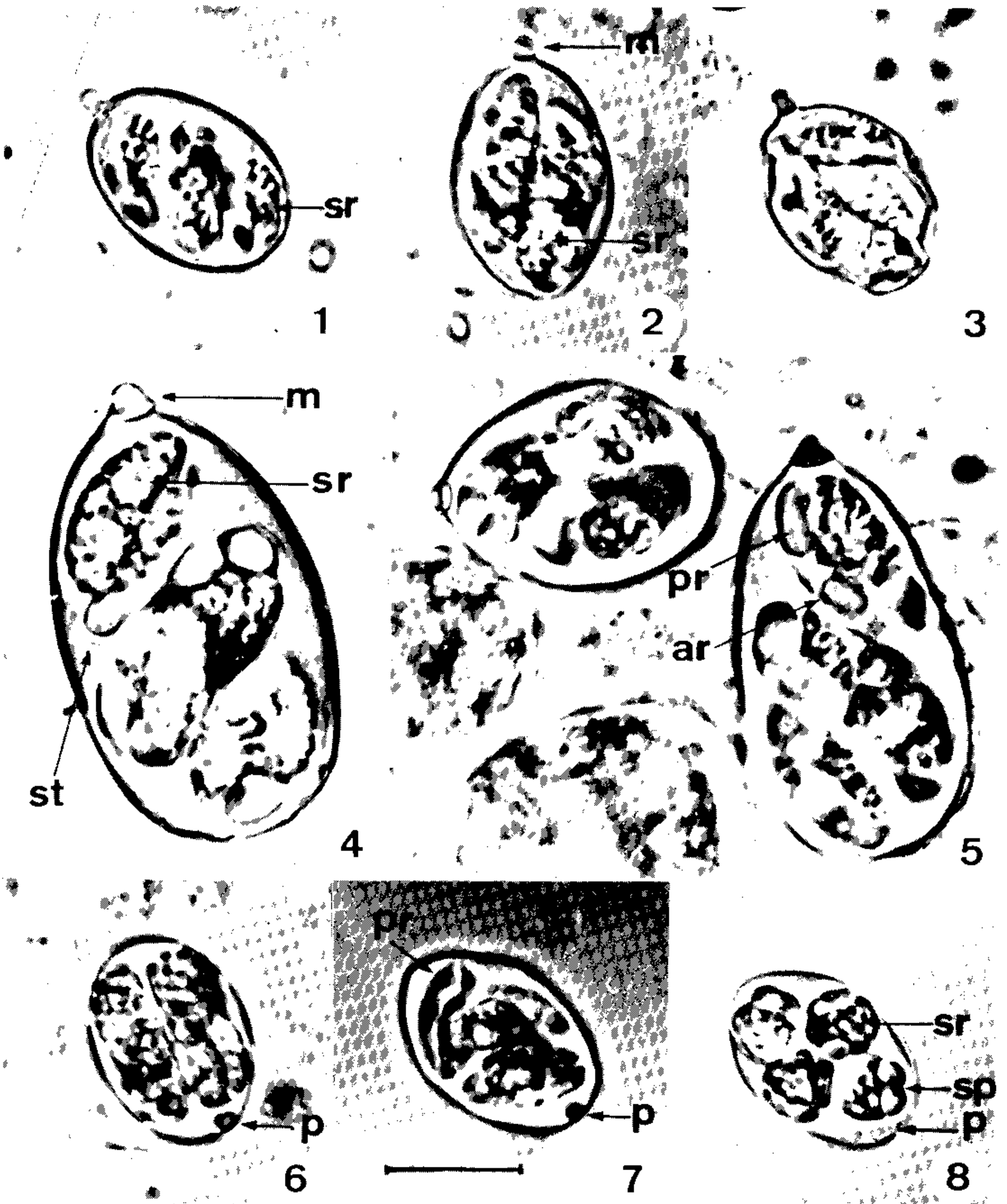
Type host: *Podocnemis expansa* (Schweigger).

Location in host: small intestine (ileum). No parasites seen in gall-bladder contents or in the liver and spleen.

Sporulation: endogenous; mature and developing oocysts seen in faecal material immediately after its removal from the intestine.

Type material: oocysts preserved in 10.0% formol-saline and held in the Department of Parasitology, Instituto Evandro Chagas, Belém.

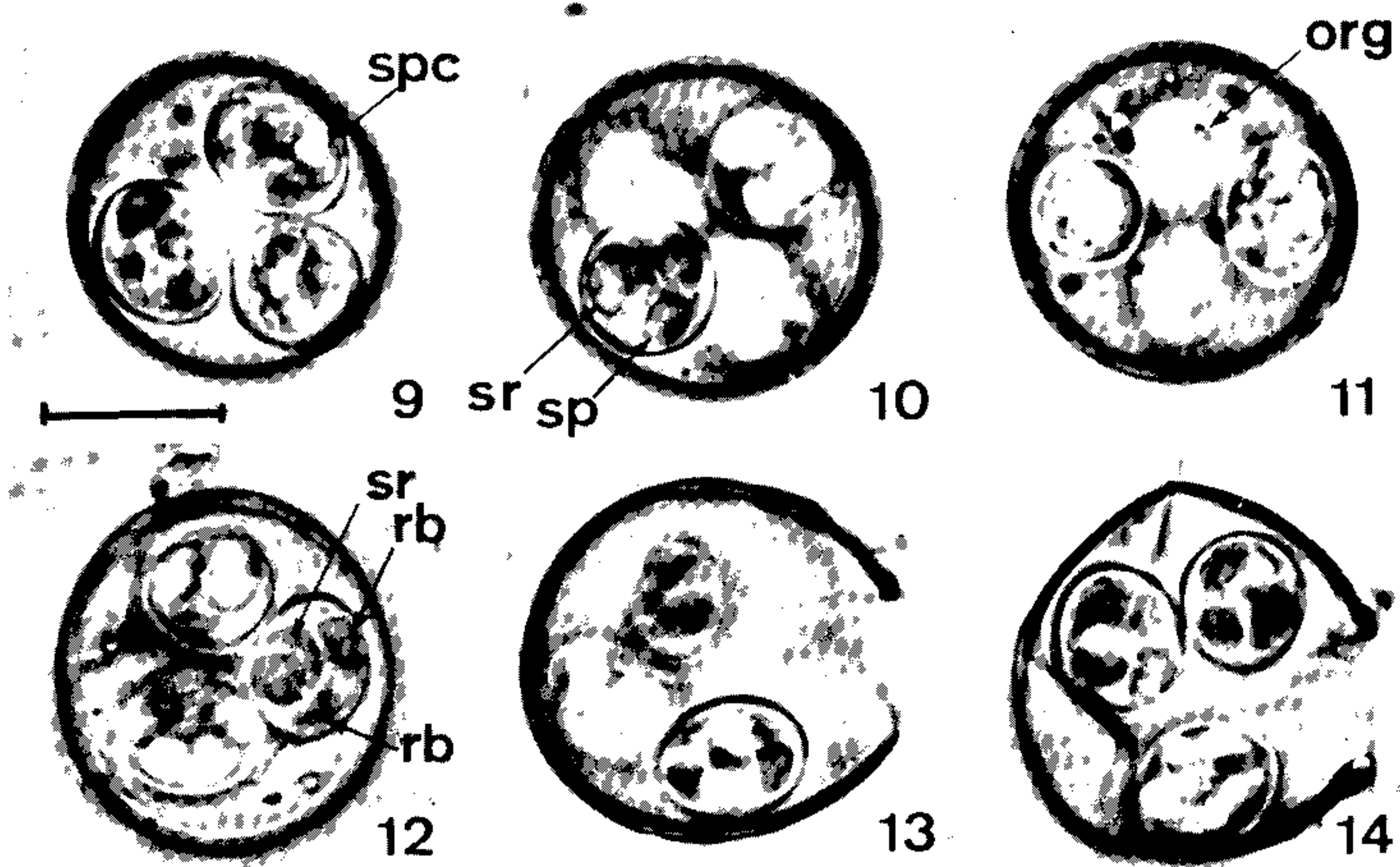
Type locality: Belém, Pará, north Brazil; turtle housed in the Museu Paraense Emílio Goeldi, Belém.



Photomicrographs of oocysts of *Eimeria* species from the Brazilian turtle *Podocnemis expansa*: bright-field microscopy. Figs 1-3: *Eimeria lagunculata* n. sp. Figs 4, 5: *Eimeria mammiformis* n. sp. Note wide size range of the oocysts. Figs 6-8: *Eimeria podocnemis* n. sp. In Fig. 8 all the sporocysts are seen end-on. Bar = 10.0 μ m. ar = anterior refractile body; m = micropyle; p = polar body; pr = posterior refractile body; sp = sporozoites; sr = sporocyst residuum; st = Stieda body.

Prevalence: not known in the wild. Only 1 of 7 captive turtles examined was infected, and this animal was also infected with *E. lagunculata*.

Pathogenicity: histopathology remains to be studied, but the infected turtle showed no signs of ill-health.



Figs 9-14: photomicrographs of oocysts of *Eimeria carinii* n. sp., from the Brazilian tortoise *Geochelone denticulata*. Bar = 10 μ m. org = scattered granules of oocyst residuum; rb = refractile bodies; sp = sporozoite; spc = sporocyst; sr = sporocyst residuum. Single-layered nature of the oocyst wall is shown in the ruptured oocysts in Figs 13 and 14.

Etymology: the specific name is derived from the generic name of the host, *Podocnemis expansa*, in which the parasite was first found.

REMARKS: there is no recorded species of *Eimeria* which combines the oocyst size-range and other morphologic features of *E. podocnemis*. The nearest is *E. jaboti* Carini 1942, which has no micropyle, no oocyst residuum, a single polar body and sporocysts having no Stieda body. The spherical-sub spherical oocysts measure 17.0 (round) to 17.0-19.0 x 15.0-17.0 (subspherical) and they have a relatively thick, 3-layered wall. The oval sporocysts measure only 10.0-11.0 x 6.0-6.6, and development is exogenous.

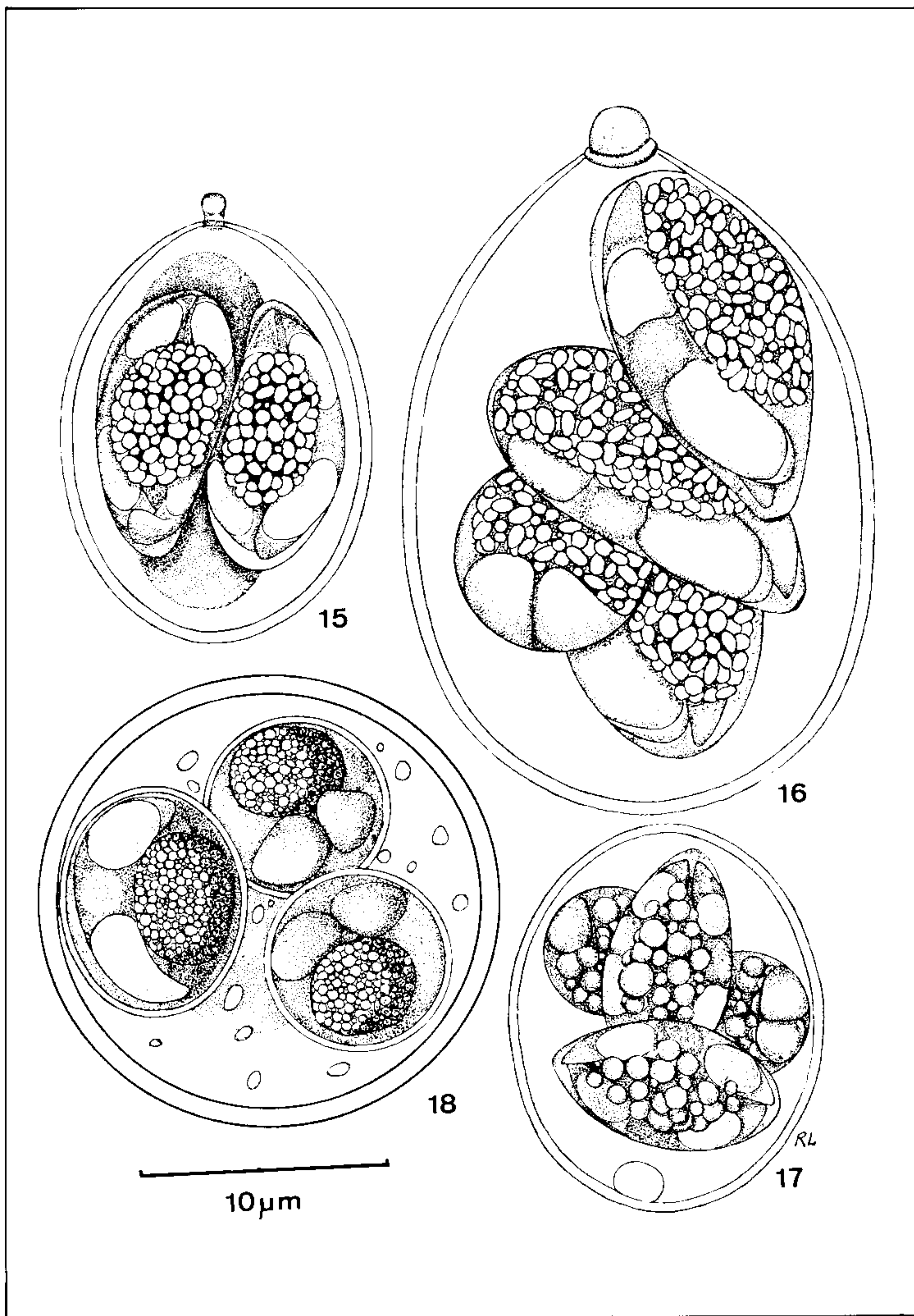
Eimeria carinii n. sp., described below in the present paper, differs from *E. podocnemis* in its thicker oocyst wall, the possession of an oocyst residuum of dispersed granules, and in having no polar body. Its sporocysts are much broader (shape-index 1.2) than those of *E. podocnemis*.

Eimeria carinii n. sp.
(Figs 9-14; 18)

Diagnosis: oocysts spherical to subspherical, 19.2 x 18.6 (15.0-20.0 x 14.0-19.0), shape-index 1.0 (1.0-1.1). Oocyst wall about 1.2 thick, and of a smooth, colourless, single layer which is relatively resistant to distortion; no micropyle. Oocyst residuum composed of a variable number (usually 10-20) of small, scattered granules measuring up to about 1.0 and in constant Brownian movement. There is no polar body. Sporocysts broadly ellipsoidal, 8.8 x 7.3 (8.0-9.0 x 7.0-7.5), shape-index, 1.2 (1.1-1.3), and with a relatively robust wall which has no Stieda body. Sporocyst residuum a bulky, spherical to ellipsoidal mass of granules lying between the two sporozoites. These are recurved at their ends and have an anterior and posterior refractile body each side of the centrally placed nucleus.

Type host: *Geochelone denticulata* (Linn.) (Reptilia: Chelonia: Testudinidae); the "jaboti" tortoise.

Location in host: uncertain, but as no oocysts were encountered in the gall-bladder contents, it is assumed that development of this coccidian is in the intestinal tract. Oocysts described in the faeces.



Line-drawings of mature oocysts of *Eimeria* spp., from the Brazilian turtle *Podocnemis expansa*. Fig. 15: *Eimeria lagunculata* n. sp. Fig. 16: *Eimeria mammiformis* n. sp. Fig. 17: *Eimeria podocnemis* n. sp. Fig. 18: mature oocyst of *Eimeria carinii* n. sp., from the Brazilian tortoise *Geochelone denticulata*.

Sporulation: exogenous, completed in 2 days.

Type material: oocysts preserved in 10.0% formol-saline and held in the Department of Parasitology, Instituto Evandro Chagas, Belém, Pará, Brazil.

Type locality: primary forest, Serra dos Carajás, Pará, north Brazil. Tortoises maintained in the Parque Zoobotânico da Companhia Vale do Rio Doce, Serra dos Carajás.

Prevalence: uncertain in the wild; 2 of 5 captive specimens of *G. denticulata* were infected, but all had been maintained together in the same compound for some time.

Pathogenicity: infected specimens of *G. denticulata* continued to pass oocysts for at least up to 2 months, without showing any signs of sickness.

Etymology: the specific name is given in honour of the late Prof. A. Carini.

REMARKS: recorded *Eimeria* species of chelonids having spherical to subspherical oocysts, an oocyst residuum, no polar body and no micropyle are differentiated from *E. carinii* by the following characters:

Eimeria dericksoni Roudabush 1937, of the north American chelonid *Amyda spinifera*, by its smaller oocysts (given as 10.8 x 10.0 in a redescription of the parasite by Wacha & Christiansen, 1977), thin oocyst wall of only 0.5, and sporocysts with a Stieda body.

Eimeria trionyxae Chakravarty & Kar 1943, in *Trionyx gangeticus* from India, differs from *E. carinii* in its smaller oocysts (14.4-18.5, mean 16.5) and its much more elongated, piriform sporocysts (12.4 x 6.2, shape-index 2.0).

Eimeria lutotestudinis Wacha & Christiansen 1976, from *Kinosternon flaviscens spooneri* in north America, has smaller oocysts (11.9 x 10.8), an oocyst residuum in the form of a compact, membrane-restricted mass of granules as opposed to the dispersed residuum of *E. carinii*, and an oocyst wall which is only 0.5 thick. The sporocysts have a Stieda body, which is absent in those of the latter parasite.

The only other *Eimeria* species previously described from *G. denticulata* (syn. *Testudo tabulata*) is *E. jaboti* Carini 1942, found in specimens of this animal in São Paulo State, south Brazil, and deserving, therefore, particular attention in our differential diagnosis. The colourless oocysts were described as "generally spherical", with a diameter of 17.0; although some measured 17.0-19.0 x 15.0-17.0, which is only just outside the size-range for *E. carinii* (15.0-20.0 x 14.0-19.0). The oocyst wall was described as having 3 layers, the outer one sometimes with a rough texture (although this is not clearly shown in the author's illustrations). The major features that distinguish *E. jaboti* from *E. carinii*, however, are the presence of a conspicuous polar body of from 2.0-4.0 in diameter, absence of the dispersed oocyst residuum seen in the latter coccidian, and the possession of oval (egg-shaped) sporocysts which measure 10.0-11.0 x 6.0-6.6, shape-index 1.5-1.7, and contrast sharply with the broadly ellipsoidal sporocysts of *E. carinii* (8.8 x 7.3, shape-index 1.2). Finally, the sporulation time for *E. jaboti* was given as 8-10 days: that of *E. carinii* is only 2 days.

These differences, we feel, warrant a new specific name for the parasite we have described above from the "jaboti", and it gives us particular pleasure to honour the name of the late Professor A. Carini, who contributed so much to Brazilian parasitology.

DISCUSSION

Why a few *Eimeria* species of chelonids should possess a micropyle and others not, remains a mystery, especially in view of the fact that the oocyst wall of both *E. lagunculata* and *E. mammiformis* is fragile and easily ruptured. Until the life-history of these and other chelonian coccidia is completely known, one can only speculate that some peculiarity in the transfer of parasites to new hosts requires the presence of a micropyle for a more ready liberation of the sporocysts and sporozoites in some species, and not in others.

In general the morphologic features of the oocyst of the different *Eimeria* species remain fairly constant, and the wide size-range of those of *E. mammiformis* is somewhat surprising (23.0 x 16.3-37.0 x 21.0). At first we suspected that we were dealing with two species of *Eimeria* with similar morphology but of different

size. Our measurements, however, revealed a continuous gradation from the smaller to the larger oocysts.

Further studies on the tissue stages of the above-described parasites are to be published separately.

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