

NEW SPECIES OF *EIMERIA* AND *ISOSPORA* (PROTOZOA: EIMERIIDAE) IN *GEOCHELONE* SPP. (CHELONIA: TESTUDINIDAE) FROM AMAZONIAN BRAZIL

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Summary:

Tetrasporocystic, dizoic oocysts of reptiles have been separated by some authors into the genera *Eimeria*, *Choleoeimeria* and *Acroeimeria* (Protozoa: Eimeriidae), based on the site and mode of development of their endogenous stages. The majority of *Eimeria* species have been, and still are, however, described on oocyst morphology alone. Four different oocysts with this basic morphology were encountered in the faeces of Brazilian tortoises, *Geochelone carbonaria* Spix, 1824 and are assigned to the genus *Eimeria*, with the view that they can readily be transferred to the genus *Choleoeimeria* or *Acroeimeria* if this is indicated by a future examination of their endogenous development. A morphological comparison distinguishes the oocysts from those of *Eimeria* spp., previously described in chelonids of the family Testudinidae, and the names *E. amazonensis*, *E. carbonaria*, *E. carajasensis* and *E. wellcomei* n. spp. are proposed. Coccidial infection appears to be common in *G. carbonaria*, with three of seven animals examined passing oocysts. Oocysts of *Isospora rodriguesae* n. sp. (Protozoa: Eimeriidae) are described in the faeces of *Geochelone denticulata* Linnaeus, 1766. They are morphologically very different from those of *Isospora testudae*, Davronov, 1985 in *Testudo horsfieldi*. *Eimeria motelo* Hürková et al., 2000, previously described in *Geochelone denticulata* from Peru, is here recorded in the same chelonid from Amazonian Brazil.

KEY WORDS : *Eimeria amazonensis* n. sp., *Eimeria carbonaria* n. sp., *Eimeria carajasensis* n. sp., *Eimeria wellcomei* n. sp., *Geochelone carbonaria*, *Isospora rodriguesae* n. sp., *Geochelone denticulata*, Chelonia, tortoises, Amazonian, Brazil.

Résumé : NOUVELLES ESPÈCES D'*EIMERIA* ET D'*ISOSPORA* (PROTOZOA: EIMERIIDAE) CHEZ *GEOCHELONE* SPP. (CHELONIA: TESTUDINIDAE) D'AMAZONIE AU BRÉSIL

Des oocystes de reptiles ont été classés par certains au sein des genres *Eimeria*, *Choleoeimeria* et *Acroeimeria* (Protozoa : Eimeriidae), en se basant sur le site et le mode de développement de leur stade endogène. La majorité des espèces d'*Eimeria* ont été et restent décrites à partir de la seule morphologie de leurs oocystes. Ainsi, quatre oocystes différents, selon cette étude morphologique basique, ont été observés dans les fèces de la tortue du Brésil *Geochelone carbonaria* Spix, 1824 et assignés au genre *Eimeria*, quitte à les transférer ultérieurement aux genres *Choleoeimeria* ou *Acroeimeria* suite à l'observation de leur stade endogène. Une comparaison morphologique permet de distinguer les oocystes d'*Eimeria* spp., précédemment décrits chez les chélonidés de la famille des Testudinidae, et de proposer les noms d'*E. amazonensis*, *E. carbonaria*, *E. carajasensis* et *E. wellcomei* n. spp. L'infection coccidienne semble assez commune chez *G. carbonaria*, avec trois animaux sur sept examinés. Les oocystes d'*Isospora rodriguesae* n. sp. (Protozoa : Eimeriidae) sont décrits dans les fèces de *Geochelone denticulata* Linnaeus, 1766. Ils sont morphologiquement très différents de ceux d'*Isospora testudae*, Davronov, 1985 chez *Testudo horsfieldi*. *Eimeria motelo* Hürková et al., 2000, précédemment décrit chez *Geochelone denticulata* du Pérou, est ici mentionné chez le même chélonidé de la région amazonienne du Brésil.

MOTS CLÉS : *Eimeria amazonensis* n. sp., *Eimeria carbonaria* n. sp., *Eimeria carajasensis* n. sp., *Eimeria wellcomei* n. sp., *Geochelone carbonaria*, *Isospora rodriguesae* n. sp., *Geochelone denticulata*, chélonidés, tortues, Amazonie, Brésil.

INTRODUCTION

Coccidia of the family Eimeriidae have been recorded in all classes of vertebrates and a number of invertebrates. Levine (1977), however, reckoned that species of *Eimeria*, the most frequently described genus of the family, had been described in as few as 1.2 % of the world's chordates, and in only 5.7 % of its mammals. He further calculated that if all the chordates were to be examined we could expect to have a list of about 34,000 species of this

coccidial and suggested the staggering total of some 45,000 in all their different hosts!

Paperna & Landsberg (1989) gave two new generic names to eimeriids of reptiles which underwent a strange development in the biliary or intestinal epithelium of the host. *Choleoeimeria* species at first occupy the distal part of the biliary epithelial cells, which subsequently become hypertrophied and, together with the host-cell nucleus, bulge out into the lumen of the gall bladder or its ducts, where schizogony and gametogony then take place. Species of *Acroeimeria* initiate their development immediately beneath the brush-border of the gut epithelial cells and undergo a similar mode of development on the surface of the epithelium.

Accepting this separation, the same basic structure of the oocysts of the three genera increases the danger

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of basing identification purely on the morphology of faecal oocysts, a common practice that has long been discussed (Parker & Duszynski, 1986; Frenkel *et al.*, 1987; Aquino-Shuster *et al.*, 1990; Finkelman & Paperna, 1994; Lainson & Paperna, 1999), and stresses the importance of information on the site and mode of development of the endogenous stages of coccidians in their hosts. This is particularly so when dealing with these parasites in reptiles.

A severe limitation in the number of animals that could be sacrificed for this purpose in the present investigation has, however, largely precluded this possibility and four different oocysts encountered in faeces of some specimens of the "red-footed tortoise" *Geochelone carbonaria* Spix, 1824 (Chelonia: Testudinidae) are here assigned to the genus *Eimeria* until such time as a study of their endogenous stages indicate whether or not some of them need to be transferred to the genus *Choleoimeria* or *Acroimeria*.

A new species of *Isospora* is recorded in the "yellow-footed tortoise", *Geochelone denticulata* Linnaeus, 1766, and *Eimeria motelo* Hürková *et al.*, 2000, previously described in this chelonid in Peru, is recorded in the same host in Brazil.

MATERIALS AND METHODS

This study was carried out, during the years 2006-2007, in areas of both primary and secondary rain-forest in the Salobo region of the Serra dos Carajás, Pará State, North Brazil (6° S, 50° 18' W). The tortoises were captured by hand, housed in separate cages to obtain faecal samples, and then released in compliance with current Brazilian wild-life protection laws. Description of the coccidia encountered is, therefore, limited to oocysts found in the faeces.

Faecal samples were suspended in 2 % aqueous potassium dichromate solution ($K_2Cr_2O_7$) in loosely covered Petri dishes maintained at approximately 24-26°C. When possible, the suspensions were examined at once for the presence of coccidial oocysts using normal light microscopy, $\times 16$ and $\times 40$ objectives and $\times 10$ eyepieces, and checked daily to determine the sporulation time of any oocysts detected. Sometimes, however, examination could only be made when faecal samples were dispatched to the laboratory in Belém, approximately one month after collection, and details of sporogony were then unobtainable. Measurements were made with an eyepiece micrometer and the oil-immersion lens: they are given in μm as means, followed by the range in parentheses, shape index (ratio of length/width) and number measured (n). Photomicrographs were prepared using a Zeiss "Photomicroscope III" and Kodak Plus-X 125 film.

In view of the absence of any satisfactory method of preserving coccidial oocysts with adequately maintained morphology (Duszynski *et al.*, 2008, and personal experience), type material is limited to photoholotypes.

RESULTS

EIMERIA AMAZONENSIS N. SP. (Figs 1-4, 18)

Description of the oocyst: mature forms ovoid, 11.7×9.1 ($10 \times 8-13 \times 10$), shape index 1.3 (1.2-1.4), n = 50. The fragile wall is of a single, colourless layer about 0.5 thick, with no micropyle but having two short, blunt protrusions at one end of the oocyst: there is no oocyst residuum or polar body. The ellipsoid sporocysts average 6.5×3.7 ($5 \times 3-7 \times 4$), shape index 1.7 (1.6-2), n = 40. There is a tiny dome-shaped Stieda body, but no subStieda structure could be detected. The sporocyst residuum of fine granules, mixed with larger globules, often obscures the slightly recurved sporozoites.

Sporulation: details not obtained.

Site of development: not ascertained.

Type host: the "red-footed jabuti", *Geochelone carbonaria* Spix, 1824 (Chelonia: Testudinidae).

Type locality: Salobo, Serra dos Carajás, Pará State, north Brazil (6° S, 50° 18' W), in secondary forest.

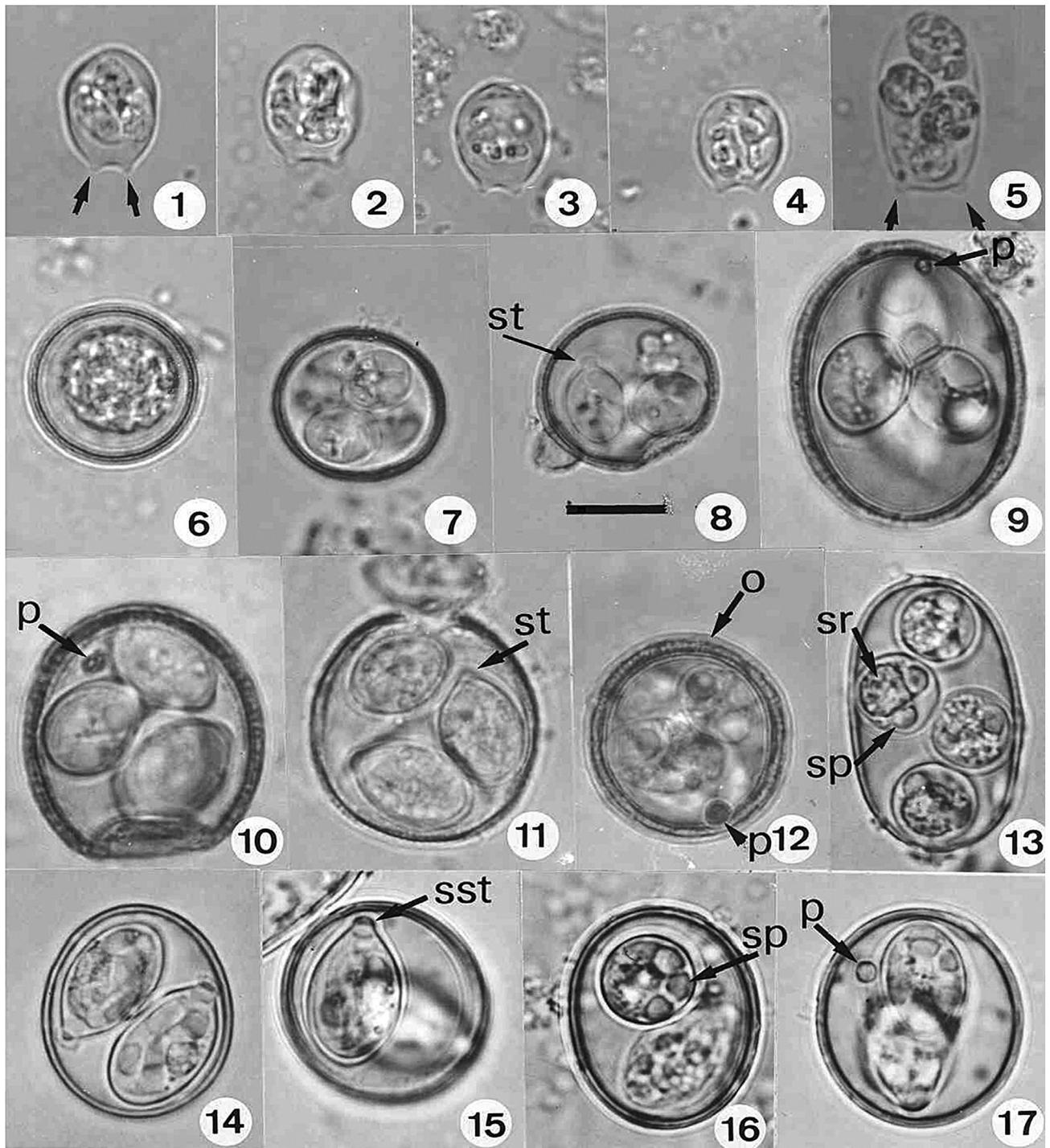
Prevalence: one of seven tortoises examined was infected (14.3 %).

Etymology: the specific name is derived from that part of Brazil in which the infected animal was captured.

Type material: photoholotype deposited in the Muséum National d'Histoire Naturelle, Paris, under the reference number P7378 (1-17).

EIMERIA CARBONARIA N. SP (Figs 6-8, 19)

Description of the oocyst: mature forms spherical to subspherical, 18.7×18.1 ($16.3 \times 16.3-21.5 \times 21.5$), shape index 1.0, n = 50. The wall is approximately 1.0 and of two striated layers, the outer one colourless and with only faintly discernible striations (pores), and the inner one brownish-yellow with conspicuous striations: it is fragile and the oocysts often become considerably deformed with age. There is no micropyle or oocyst residuum, but the frequent presence of a single small, spherical polar body (rarely two) of about 1.0 in diameter. The ovoid sporocysts average 11×7 ($10 \times 6.6-11.8 \times 7.4$), shape index 1.6, n = 40. They tend to almost fill the oocyst so that they are superimposed and their structure determined with difficulty. There is a nipple-shaped Stieda body (Fig. 8), but no sub-Stieda structure could be detected. Sporozoites occupy the full length



Figs 1-17. – Coccidial oocysts in faeces of the tortoises *Geochelone carbonaria* and *Geochelone denticulata* from Amazonian Brazil. Figs 1-4. *Eimeria amazonensis* n. sp. in *G. carbonaria*: note the consistent presence of two oocyst wall protrusions (arrows). Fig. 5. *Eimeria motelo* Hürková *et al.* in *G. denticulata*: note similar oocyst wall protrusions (arrows), but much larger and elongated oocyst compared with that of *E. amazonensis*. Figs 6-8. Unsporulated and sporulated oocysts of *E. carbonaria* n. sp. in *G. carbonaria*. Figs 9-12. Mature oocysts of *E. carajasensis* n. sp. from *G. carbonaria*: that in Figure 12 is an end-on view, showing the 2-layered, striated wall (o) to which a polar body (p) adheres. Fig 13. *Eimeria wellcomei* n. sp., also from *G. carbonaria*. Figs 14-17. *Isospora rodriguesae* n. sp. from *G. denticulata*. Bar in Figure 8 = 10 µm and applies to all other Figs. o = oocyst wall, p = polar body, st = Stieda body, sst = Stieda and sub-Stieda bodies, sp = sporozoite, sr = sporocystic residuum.

of the oocysts and are moderately recurved at their ends: refractile bodies were not visible. The sporocyst residuum is a conspicuous, loose mass of fine granules and larger globules.

Sporulation: exogenous, and completed after three days at approximately 24-26° C.

Site of development: not yet ascertained.

Type host: the "red-footed tortoise" *Geochelone carbonaria* Spix, 1824 (Chelonia: Testudinidae).

Type locality: Salobo, Serra dos Carajás, Pará State, north Brazil (6° S, 50° 18' W) in secondary forest.

Prevalence: two of seven (28.6 %) of the tortoises examined were infected.

Pathology: there were no outward signs of disease in either infected animal.

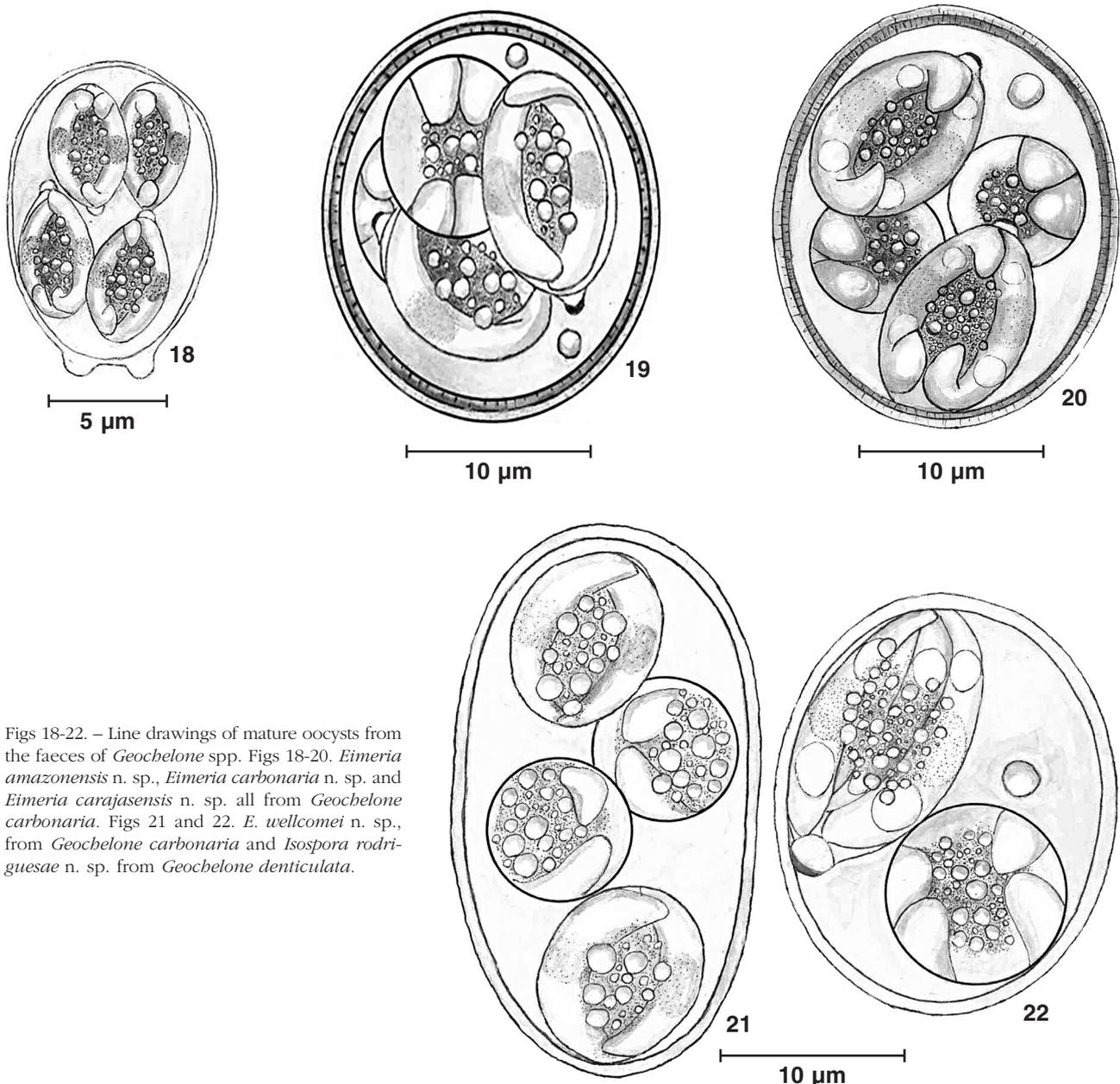
Etymology: the specific name is derived from that of the chelonid host.

Type material: photoholotype deposited in the Muséum National d'Histoire Naturelle, Paris, under the reference number P7378 (1-17).

Comments: one of the infected tortoises had a concomitant infection with *E. carajasensis* n. sp., described below, and the other with *E. wellcomei*, also described below.

EIMERIA CARAJASENSIS N. SP (Figs 9-12, 20)

Description of the oocyst: mature oocyst broadly ellipsoidal to sub-spherical, 22.4 × 19.3 (20 × 18-32 × 25), shape index 1.2 (1.1-1.3), n = 50. Oocyst wall brow-



Figs 18-22. – Line drawings of mature oocysts from the faeces of *Geochelone* spp. Figs 18-20. *Eimeria amazonensis* n. sp., *Eimeria carbonaria* n. sp. and *Eimeria carajasensis* n. sp. all from *Geochelone carbonaria*. Figs 21 and 22. *E. wellcomei* n. sp., from *Geochelone carbonaria* and *Isospora rodriguesae* n. sp. from *Geochelone denticulata*.

nish-yellow and approximately 1.0-1.5, composed of two striated layers, the inner one being darker and more markedly striated than the outer one. No micropyle or oocyst residuum, but frequently with a single spherical to irregularly-shaped polar body of 2.0-3.0 in diameter. Sporocysts ovoid, 12×8 ($11 \times 7-13 \times 8$), shape index 1.6, $n = 50$. There is a modest Stieda body but apparently no sub-Stieda structure. Sporozoites are strongly recurved at their extremities and have two refractile bodies. They surround a conspicuous sporocyst residuum of fine granules and larger globules, which is not enclosed in a membrane.

Sporulation: Some oocysts may be expelled already mature. Sporulation of others was exogenous, in less than 24 hours.

Site of development: not yet ascertained.

Type host: *Geochelone carbonaria* Spix, 1824 (Chelonia: Testudinidae).

Type locality: Salobo, Serra dos Carajás, Pará State, North Brazil (6° S, 50° $18'$ W) in secondary forest.

Prevalence: two of the seven *G. carbonaria* examined (28.6 %) were infected.

Pathology: no visible signs of disease.

Etymology: the specific name is derived from the hills of Carajás where the infected tortoises were found.

Type material: photoholotype deposited in the Muséum National d'Histoire Naturelle, Paris, under the reference number P7378 (1-17).

Comments: One of the tortoises had a concomitant infection with *E. carbonaria* n. sp., described above.

EIMERIA WELLCOMEI N. SP. (Figs 13, 21)

Description of the oocyst: mature oocyst ellipsoidal to cylindrical, 30.3×16.4 ($28 \times 15-32 \times 17$), shape index 1.8 (1.8-2.), $n = 50$. Oocyst wall approximately 1.0, colourless and of a single non-striated layer. No micropyle, oocyst residuum or polar bodies detected. Sporocysts sub-spherical to broadly ellipsoidal, 9.6×7.9 ($9 \times 7-10 \times 9$), shape index 1.2 (1.1-1.3), $n = 50$. No Stieda or sub-Stieda bodies. Sporozoites are strongly recurved. The sporocyst residuum is of fine granules and larger globules; refractile bodies were not visible in the sporozoites.

Sporulation: it was not possible to examine the faeces until one week after collection, when the oocysts were fully sporulated. It is, therefore, not known if sporulation is endogenous or exogenous or, if the latter, what time is required for its completion.

Site of development: not yet ascertained.

Type host: *Geochelone carbonaria* Spix, 1824 (Chelonia: Testudinidae)

Type locality: Salobo, Serra dos Carajás, Pará State, North Brazil (6° S, 50° $18'$ W) in secondary forest.

Prevalence: one of seven *G. carbonaria* examined was infected (14.3 %).

Pathology: the infected tortoise showed no outward signs of disease.

Etymology: the specific name is derived from that of Sir Henry Wellcome and in gratitude to the Wellcome Trust, London who have for so long financed the author's research in Brazil.

Type material: photoholotype deposited in the Muséum National d'Histoire Naturelle, Paris, under the reference number P7378 (1-17).

Comments: the single infected tortoise was also passing oocysts of *E. carbonaria*, described above.

ISOSPORA RODRIGUESAE N. SP. (Figs 14-17, 22)

Description of the oocyst: mature forms broadly ellipsoidal to subspherical, 24.5×21 ($23 \times 22-26 \times 22$), shape index 1.2 (1-1.2), $n = 50$. Oocyst wall a colourless, smooth, single layer, approximately 1.0 and with no striations or micropyle. No oocyst residuum but with a prominent, spherical polar body reaching up to 2.0×2.0 and frequently adhering to one of the sporocysts. Sporocysts are pear-shaped, 16×9.5 ($15 \times 9-17 \times 10$), shape index 1.7, $n = 40$. They possess a conspicuous cap-like Stieda body measuring about 2.0 wide and 0.5 deep, and a prominent sub-Stieda structure of approximately 2.5 wide and 1.0-2.0 deep. Sporozoites occupy the full length of the sporocyst, are only slightly recurved at their ends, and contain anteriorly and posteriorly located refractile bodies. The sporocyst residuum is a loose collection of fine granules and numerous larger globules.

Sporulation: exogenous, and completed by some oocysts within 24 hours at $24-26^{\circ}$ C.

Site of development: as yet not determined, but probably in the intestine.

Type host: the yellow-footed tortoise *Geochelone denticulata* Linnaeus, 1766 (Reptilia: Chelonia: Testudinidae).

Type locality: Salobo, Serra dos Carajás, Pará State, north Brazil (6° S, 50° $18'$ W). In a clearing in primary forest.

Prevalence: one infection recorded among 8 *G. denticulata* examined (12.5 %).

Pathology: the infected animal showed no signs of disease.

Etymology: the specific name of the parasite is given as a token of thanks to my colleague Dr. Izabel R. de C. Rodrigues who captured this juvenile, infected tortoise.

Type material: photoholotype deposited in the Muséum National d'Histoire Naturelle, Paris, under the reference number P7378 (1-17).

DISCUSSION

It was long the general view that there exists a strict host specificity among all members of the Eimeriidae and a differences of host species has fre-

Species & Reference	Host	Distrib ^a	Oocyst	Sporocyst
<i>E. amazonensis</i> n. sp. This paper	<i>Geochelone carbonaria</i>	Pará, North Brazil	Ovoid, 11.7 × 9.1 (10 × 8-13 × 10) W: 1 layer, smooth, colourless PB: - ve	Ellipsoid 6.5 × 3.7 (5 × 3-7 × 4) S: + ve
<i>E. broderi</i> Cerruti, 1930	<i>Testudo graeca</i>	Greece	Ovoid 30 × 19 (28-32 × 18-20) W: 2 layers, smooth, with micropyle	Ellipsoid 10 × 6-7 S: ?
<i>E. carbonaria</i> n. sp. This paper	<i>G. carbonaria</i>	Pará, North Brazil	Spherical-sub-spherical, 18.7 × 18.1 (16.3 × 16.3-21.5 × 21.5) W: 2 layers, brownish, striated PB: 1 or 2	Ovoid 11 × 7 (10 × 6.6-11.8 × 7.4) S: + ve
<i>E. carajasensis</i> n. sp. This paper	<i>G. carbonaria</i>	Pará, North Brazil	Ellipsoidal-sub-spherical 22.4 × 19.3 (20 × 18-32 × 25) W: 2 layers, brownish, striated PB: 1	Ovoid 12 × 8 (11 × 7-13 × 8) S: + ve
<i>E. geochelona</i> Couch <i>et al.</i> , 1996	<i>G. nigra</i>	Galápagos Islands	Ellipsoid-ovoid 21.6 × 18.1 (18 × 16-25 × 20) W: 2 layers, smooth, colourless PB: 1	Ellipsoid 10.7 × 7 (8 × 5-12 × 8) S: + ve
<i>E. jaboti</i> Carini, 1942	<i>G. denticulata</i>	São Paulo, Brazil	Spherical-sub-spherical 17 × 15-19 × 17 W: 3 layers, colourless PB: 1	Oval 10 × 6-11 × 6.6 S: - ve
<i>E. lainsoni</i> (Lainson <i>et al.</i> , 1990) Hůrková <i>et al.</i> , 2000	<i>G. denticulata</i>	Pará, North Brazil	Spherical-sub-spherical 19.2 × 18.6 (15 × 14-20 × 19) W: 1 layer, smooth, colourless PB: - ve	Ellipsoid 8.8 × 7.3 (8 × 7-9 × 7.5) S: - ve
<i>E. motelo</i> Hůrková <i>et al.</i> , 2000	<i>G. denticulata</i>	Iquitos, Peru	Ellipsoid 17 × 9.4 (15 × 8.5-19 × 11) W: 1 layer, smooth, colourless PB: - ve	Ellipsoid 8.9 × 4.4 (7.5 × 4.4-10 × 5) S: + ve
<i>E. paymei</i> Ernst <i>et al.</i> , 1971	<i>Gopherus polyphemus</i>	Georgia, USA	Ellipsoid 23.2 × 18.6 (19 × 16-26 × 20) W: 2 layers, brownish-yellow PB: 1-3	Ovoid 13.2 × 8.1 (12 × 7-14 × 9) S: + ve
<i>E. wellcomei</i> n. sp., This paper	<i>G. carbonaria</i>	Pará, North Brazil	Ellipsoid-cylindrical 30.3 × 16.4 (28 × 15-32 × 17) W: 1 layer, smooth, colourless PB: - ve	Subspherical-ellipsoidal 9.6 × 7.9 (9 × 7-10 × 9) S: - ve
<i>Isospora rodriguesae</i> n. sp. This paper	<i>G. denticulata</i>	Pará, North Brazil	Subspherical-ellipsoid 24.5 × 21 (23 × 22-26 × 22) W: 1 layer, smooth, colourless PB: 1	Pear-shaped 16 × 9.5 (15 × 9-17 × 10) S: + ve SS: + ve
<i>Isospora testudae</i> Davronov, 1985	<i>Testudo horsfieldi</i>	Uzbekistan	Spherical 25.6 (22.1-28.9) W: 2-layered, smooth PB: - ve	Ovoid 15.3-18.7 × 10.2-15.3 S: - ve

PB = polar body; S = Stieda body; SS = subStieda body; W = oocyst wall. Of all these species, only *E. broderi* has an oocyst micropyle. Oocysts of *E. amazonensis* n. sp. and *E. motelo* have two protrusions of the oocyst wall at one extremity.

Table I. – Morphological features, hosts and known geographical distribution of *Eimeria* and *Isospora* spp., in tortoises of the family Testudinidae.

quently been used as a taxonomic criterion. This began to be questioned, however, and it is now evident that there is considerable lack of host specificity at generic level among the eimeriids of chelonids, although it is considered unlikely to extend to different families (McAllister & Upton, 1988, 1989; McAllister *et al.*, 1991). For this reason it is proposed to largely limit morphological comparison of the parasites described in the present paper to species of the genus *Eimeria* and *Isoospora* that have previously been described in tortoises of the family Testudinidae Gray, 1825 (Table D).

The only species of *Eimeria* that needs to be compared with *E. amazonensis* n. sp. is *E. motelo* Hůrková *et al.*, 2000 described in *Geochelone denticulata* from Peru and re-encountered in same host in the present study. Our measurements of the oocyst agree closely with those of the original description (oocysts 17.1×10.8 vs 17×9.4 , and sporocysts 9×5 vs 8.9×4.4). The oocyst (Fig. 5) is larger and much more elongated than that of *E. amazonensis* n. sp., (17×9.4 vs only 11.7×9.1), and its sporocysts are larger (8.9×4.4 vs 6.5×3.7). Hůrková *et al.* suggested that the protrusions of the oocyst wall of *E. motelo* could be due to its “wrinkling”: from the present study, however, we have no doubt that they, and those of *E. amazonensis*, are constant morphological features (Figs 1-5). The following species of *Eimeria* were described from turtles in families other than the Testudinidae, but as the wall of their oocysts also have protrusions, we feel it appropriate to include them in a comparison with *E. amazonensis*. *E. mitraria* (Laveran & Mesnil, 1902) Doflein 1909, described in *Chrysemys picta marginata* (Emydidae), has oocysts which are considerably larger (15×10 vs 11.7×9.1): they are truncated at one end, which bears from 3-4 protrusions, while the other extremity is conical in shape. The oocysts of *E. stylosa* McAllister & Upton, 1989 from *Trachemys scripta elegans* (Emydidae) are, again, larger than those of *E. amazonensis* (16.5×13.1 vs 11.7×9.1): the wall has a variable number of much longer and pointed protrusions, usually two at one end and three at the other, but sometimes up to four at one end and seven at the other. Finally, *E. jirkamoraveci* Siroký *et al.*, 2006 in *Batrachemys heliostemma* (Chelidae) from Peru, has oocysts of similar size to those of *E. amazonensis* (10.6×8.9 vs 11.7×9.1) but they have three protrusions at one end. There is a distinct projection at the other extremity which contrasts with the smoothly rounded end in the oocyst of *E. amazonensis*. Among the *Eimeria* species with spherical to subspherical oocysts, *E. carbonaria* n. sp., can be differentiated from *E. jaboti* and *E. lainsoni* of *Geochelone denticulata*, by its 2-layered, brownish and striated oocyst wall. The ellipsoidal oocysts of *E. geochelona* in *Geochelone nigra* from the Galápagos Islands and *E. paynei* in *Gopherus polyphemus* from the USA, are of similar mean size to those of *E. carajasensis*, but the former

can readily be differentiated by their smooth, colourless and unstriated wall. *E. paynei* appears to be consistently ellipsoidal in shape and has the much smaller size range of $19-26 \times 16-20$ compared with that of *E. carajasensis* which is $20 \times 18-32 \times 25$: in addition, its oocysts may contain 1-3 polar bodies and a number of smaller granules, as opposed to a single polar body, and its sporocyst residuum was described as being enclosed in a membrane, unlike that of *E. carajasensis*. Oocysts of the latter parasite may undergo endogenous sporulation: there is no evidence that *E. paynei* does so.

Ellipsoidal to cylindrical oocysts, and sporocysts which lack a Stieda body, are features of the genus *Choleo-eimeria* in the biliary epithelium of reptiles (Paperna & Landsberg, 1989). *E. wellcomei* n. sp. is, therefore, a likely candidate for future transference to that genus. As far as we are aware, there has been no previous record of such a parasite in chelonids of the family Testudinidae.

Finally, the only previously recorded *Isoospora* species in chelonids of the Testudinidae is *I. testudae* Davronov 1985, described from *Testuda horsfieldi* in Uzbekistan. Its spherical oocyst with a bi-layered wall, absence of a polar body and sporocysts devoid of a Stieda body all clearly differentiate it from the oocyst of *I. rodriguesae* n. sp.

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REFERENCES

- AQUINO-SHUSTER A.L., DUSZYNSKI D.W. & SNELL H.L. Three new coccidia (Apicomplexa) from the Hood Island lizard *Tropidurus delanonis* from Galapagos archipelago. *Journal of Parasitology*, 1990, 76, 313-318.
- CARINI A. Sobre uma *Eimeria* da “*Testudo tabulata*”. *Arquivos de Biologia, São Paulo*, 1942, 26, 163-164.
- CERRUTI C. Su di um coccidio parassita de *Testudo graeca*, Linn. *Archivio Italiano di Scienze Mediche Coloniali e di Parassitologia*, 1930, 11, 328-331.
- COUCH L., STONE P.A., DUSZYNSKI D.W. & SNELL H.I. A survey of the coccidian parasites of reptiles from the islands of the Galapagos archipelago. *Journal of Parasitology*, 1996, 82, 432-437.

- DAVRONOV O. Coccidia of reptiles from southern Uzbekistan. *Parazitologiya (Leningrad)*, 1985, 19, 158-161.
- DUSZYNSKI D.W., UPTON S.J. & COUCH L. The Coccidia of the World. Available at: <http://biology.unm.edu/biology/coccidia/home.html> 2008.
- ERNST J.V., FINCHER G.T. & STEWART T.B. *Eimeria paynei* sp. n. (Protozoa: Eimeriidae) from the Gopher tortoise, *Gopherus polyphemus*. *Proceedings of the Helminthological Society of Washington*, 1971, 38, 223-224.
- FINKELMAN S. & PAPERNA I. The endogenous development of three new intranuclear species of *Isospora* (Apicomplexa: Eimeriidae) from agamid lizards. *Systematic Parasitology*, 1994, 27, 213-226.
- FRENKEL J.K., MEHLHORN H. & HEYDORN A.O. Beyond the oocyst: over the molehills and mountains of coccidia-land. *Parasitology Today*, 1987, 3, 250-252.
- HŮRKOVÁ L., MODRÝ D., KOUDELA B. & ŠLAPETA J. Description of *Eimeria motelo* sp. n. (Apicomplexa: Eimeriidae) from the yellow-footed tortoise, *Geochelone denticulata* (Chelonia: Testudinidae), and replacement of *Eimeria carinii* Lainson Costa & Shaw, 1990 by *Eimeria lainsoni* nom. nov. *Memórias do Instituto Oswaldo Cruz*, 2000, 95, 829-832.
- LAINSON R., COSTA A.M. & SHAW J.J. *Eimeria* species (Apicomplexa: Eimeriidae) of *Podocnemis expansa* (Schweigger) and *Geochelone denticulata* Linn.) from Amazonian Brazil (Reptilia: Chelonia). *Memórias do Instituto Oswaldo Cruz*, 1990, 85, 383-390.
- LAINSON R. & PAPERNA I. Some coccidia from the gall-bladder and intestine of the teiid lizard *Ameiva ameiva* and the gecko *Hemidactylus mabouia* in North Brazil. *Parasite*, 1999, 6, 151-162.
- LAVERAN A. & MESNIL F. Sur quelques protozoaires parasites d'une tortue d'Asie (*Damonia reevesii*). *Comptes Rendus des Séances de L'Académie des Sciences, Serie III, Sciences de la Vie*, 1902, 135, 609-614.
- LEVINE N.D. Introduction, History, and Taxonomy, in: The Coccidia, Hammond D.M. & Long P.L. (eds). University Press, Baltimore; Butterworths, London, 1977, 1-22.
- MCALLISTER C.T. & UPTON S.J. *Eimeria trachemydis* n. sp. (Apicomplexa: Eimeriidae) and other eimerians from the red-eared slider *Trachemys scripta elegans* (Reptilia: Testudines) in northcentral Texas. *Journal of Parasitology*, 1988, 74, 1014-1017.
- MCALLISTER C.T. & UPTON S.J. The Coccidia (Apicomplexa: Eimeriidae) of Testudines, with descriptions of three new species. *Canadian Journal of Zoology*, 1989, 67, 2459-2467.
- MCALLISTER C.T., UPTON S.J. & KILLEBREW F.C. Coccidian parasites (Apicomplexa: Eimeriidae) of *Graptemys caglei* and *Graptemys versa* (Testudines: Emydidae) from Texas. *Journal of Parasitology*, 1991, 77, 500-502.
- PAPERNA I. & LANDSBERG J.H. Description and taxonomic discussion of eimerian Coccidia from African and Levantine geckoes. *South African Journal of Zoology*, 1989, 24, 345-355.
- PARKER B.B. & DUSZYNSKI D.W. Polymorphism of eimerian oocysts: a dilemma posed by working with some naturally infected hosts. *Journal of Parasitology*, 1992, 72, 602-604.
- SIROKÝ P., KAMLER M. & MODRÝ D. A new *Eimeria* (Apicomplexa: Eimeriidae), possessing mitra-shaped oocysts, from the neotropical chelid turtle *Batrachemys bellostemma* (Testudines: Chelidae), and its comparison with *Eimeria mitraria* (Laveran & Mesnil 1902). *Memórias do Instituto Oswaldo Cruz*, 2006, 101, 555-558.

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