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Plasmodium carmelinoi n. sp. (Haemosporida: Plasmodiidae) of the lizard *Ameiva ameiva* (Squamata: Teiidae) in Amazonian Brazil

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Summary:

Plasmodium carmelinoi n. sp. is described in the teiid lizard *Ameiva ameiva* from north Brazil. Following entry of the merozoites into the erythrocyte, the young, uninucleated trophozoites are at first tear-shaped and already possess a large vacuole: with growth, they may assume an irregular shape, but eventually become spherical or broadly ovoid. The vacuole reduces the cytoplasm of the parasite to a narrow peripheral band in which nuclear division produces a schizont with 8-12 nuclei. At first the dark, brownish-black pigment granules are restricted to this rim of cytoplasm but latterly become conspicuously concentrated within the vacuole. The mature schizonts are spherical to ovoid and predominantly polar in their position in the erythrocyte. They average 5.4 x 4,9 µm (4.4 x 4.4 - 6.6 x 5,9 µm), shape index 1.1, n = 50: 8-12 merozoites are produced and measure approximately 2.0 x 1,0 µm. Mature gametocytes are also polar in position, and spherical to subspherical. The macrogametocytes measure 5.7 x 5,2 µm (4.4 x 4.0 - 5.9 x 5,1 µm), shape index 1.1, n = 50 and, following staining by Giemsa's method, possess a compact, pink-staining nucleus and a clear blue, faintly stained cytoplasm. Microgametocytes are slightly larger, 6.0 x 5,0 µm (5.2 x 4.4 – 6.2 x 5,2 µm), shape index 1.2, n = 45. They stain an over-all pink colour due to the dispersed nuclear chromatin. The vacuoles in both the macro- and microgametocytes are considerably smaller than those of the schizonts and of ovoid or spindle shape: they contain most of the pigment granules. The sex ratio, as seen in an inicial intense infection, was 1 male to 2.2 females. Prevalence of infection was low (5 %) but, due to the very low parasitaemia which may result in a failure to detect parasites, it is probably higher than this.

KEY WORDS: *Plasmodium carmelinoi* n. sp., Ameiva ameiva, Teiidae, lizard, Amazonian Brazil.

Résumé : *Plasmodium carmelinoi* n. sp. (Haemosporida : Plasmodiidae) chez le lézard *Ameiva ameiva* (Squamata : Teiidae) de la région amasonienne du Brésil

Plasmodium carmelinoi n. sp. est décrit chez le lézard Ameiva ameiva *au nord du Brésil. À la suite de l'entrée des mérozoïtes dans l'érythrocyte, les jeunes trophozoïtes uninucléaires sont initialement en forme de larme et possèdent déjà une grande vacuole ; au cours de leur croissance, ils peuvent présenter une forme irrégulière, mais ils deviennent finalement sphériques ou ovoïdes. Les vacuoles réduisent le cytoplasme du parasite à une étroite bande périphérique dans laquelle la division nucléaire produit un schizonte à 8-12 noyaux. Au début, des granules de pigment noir-brunâtre sont limités à cette bande de cytoplasme, mais ils peuvent rapidement se concentrer dans la vacuole. Les schizontes matures sont sphériques ou ovoïdes et prédominent en position polaire dans l'érythrocyte. Ils mesurent 5.4 x 4,9 µm (4.4 x 4.4 - 6.6 x 5,9 µm), index de forme 1.1, n = 50 : 8-12 mérozoïtes sont produits et mesurent environ 2.0 x 1,0 µm. Les gamétocytes matures sont également en position polaire, sphériques ou ovoïdes. Les macrogamétocytes mesurent 5.7 x 5,2 µm (4.4 x 4.0 - 5.9 x 5,1 µm), index de forme 1.1, n = 50. Après coloration par la méthode de Giemsa, ils montrent un noyau compact rosé et un cytoplasme faiblement coloré en bleu clair. Les microgamétocytes sont légèrement plus grands, 6.0 x 5,0 µm (5.2 x 4.4 – 6.2 x 5,2 µm), index de forme 1.2, n = 45. Ils prennent une couleur entièrement rose en raison d'une chromatine nucléaire dispersée. Les vacuoles, tant dans les macro que les microgamétocytes, sont bien plus petites que celles des schizontes et sont ovoïdes ou en forme de fuseau ; elles contiennent la plupart des granules de pigment. Le sex-ratio, observé dans une infection intense initiale, était de un mâle pour 2,2 femelles. La prévalence de l'infection était basses (5 %), mais, en raison d'une très faible parasitémie qui peut mettre en échec la détection du parasite, elle est probablement plus élevée.*

MOTS CLÉS : Plasmodium carmelinoi *n. sp., Ameiva ameiva, Teiidae, lizard, Amazonian Brazil.*

INTRODUCTION

Ameiva ameiva (Linnaeus, 1758) can rightly be considered as the most well known and beautiful of all lizards in South and Central America, where its vivid colouration makes it a welcome sight in gardens, and most open, sunny areas. It is essentially ground inhabiting, and with a wide geographic distribution in Panama, Brazil, French Guiana, Suriname, Guyana, Venezuela, Colombia, Ecuador, Peru, Bolivia and Argentina (Avila-Pires, 1995).

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A. ameiva is host to a surprising number of haematozoa, including *Plasmodium cnemidophori* Carini, 1941 (Lainson & Shaw, 1969); *P. pifanoi* Scorza & Dagert, 1956; *P. diminutivum* Telford, 1973; *Garnia telfordi* Lainson, Landau & Shaw, 1971; *Hemolivia stellata* Petit, Landau, Baccam & Lainson, 1990 (Lainson, de Souza & Franco, 2007); and two as yet unidentified haemogregarines, one in the erythrocytes and the other in the monocytes of the peripheral blood (Lainson, de Souza & Franco, 2003).

It was during studies on the latter two parasites that scanty infections with a *Plasmodium* specie were encountered in the blood of four specimens of this lizard examined. Although morphology of the rare schizonts seen suggested to the authors that they were probably dealing with a previously undescribed parasite, the chronic nature of these infections and the paucity of parasites made them unsuitable for its full description. In addition to further scanty infections, however, continuing examination of this series of blood films eventually revealed an extremely heavy infection, in one lizard, presumably recently acquired, and it is this that has been used for the following description of *Plasmodium carmelinoi* n. sp.

MATERIAL AND METHODS

Specimens of *A. ameiva* were captured by hand in an area of savannah and degraded forest near Capanema, Pará State, north Brazil (1º 12´ S - 47º 11´ W) in June, 2003. They were bled by puncture of the orbital sinus using glass pipettes drawn out to a very fine point, and thin blood films were air dried, fixed in absolute methyl alcohol and stained by Giemsa's method. All measurements of the parasites are in µm: they are given as means, followed by the range in parentheses, the shape-index (ratio of length/width) and the number measured (n). A random count of 100 mature gametocytes was used to establish the sex ratio. Photographs were prepared using a Zeiss "Photomicroscope III" and Kodak Plus-X 125 film.

DESCRIPTION

PLASMODIUM CARMELINOI N. SP. (Figs A-H; 1-11)

Development is in the mature erythrocytes and parasitaemia may sometimes be intense (usually considered to indicate a recent infection), with multiple infection of a single cell extremely common (Figs A, B, C, F and 1, 7, 9, 11). The infected erythrocyte is only occasionally enlarged, when it principally shows an increase in length. Apart from displacement or slight indentation, the host cell nucleus is of normal appearance.

Asexual stages

Following entry of merozoites into the erythrocyte, the developing trophozoites are frequently tear-shaped and may be variably positioned in the cell. There is the rapid formation of a large vacuole, resulting in the cytoplasm of the tiny parasite being reduced to a peripheral ring containing the undivided nucleus (Fig. A). With growth, the parasites now become polar in their position within the erythrocyte and may assume an irregular outline (Fig. 2): a few granules of dark brownish-black pigment now appear (Figs A, B). At first these are confined to the cytoplasm, but with nuclear division they become concentrated in the growing vacuole which may now occupy over half of the parasite (Figs C, D, 4, 5). The dividing nuclei form a partial or complete ring in the peripheral rim of cytoplasm (Figs C, D, F & 4-7). Rarely, however, some schizonts may show the presence of a smaller vacuole containing only a few pigment granules and with the merozoites being budded off from an evenly distributed cytoplasm (Fig. E): the mature, spherical or broadly ovoid schizonts produce 8-12 nuclei and average 5.4 × 4.9 (4.4 × 4.4 - 6.6 × 5.9), shape-index = 1.1, n = 50. From 8-12 merozoites are produced, measuring approximately 2.0 × 1.0. No exoerythrocytic schizonts were detected.

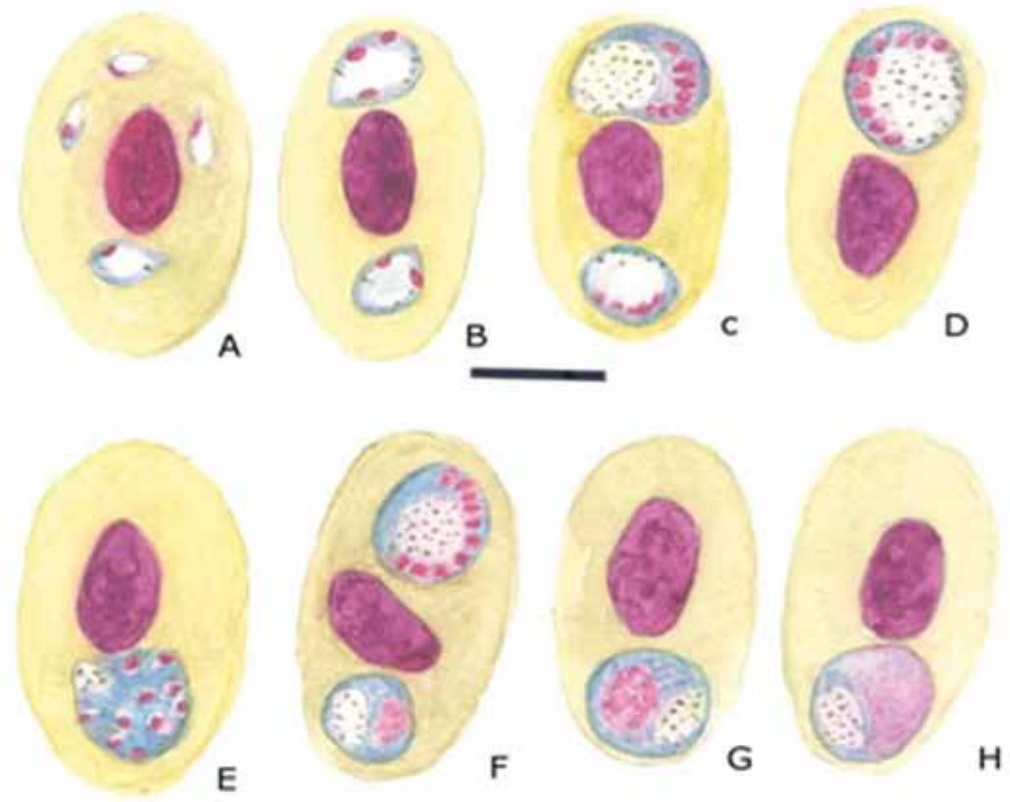
Gametocytes

These are also polar in their development in the infected cell and, like the gametocytes of other members of the Haemosporida stained by Romanovsky stains, the sexes are differentiated by the blue cytoplasm and compact, pink nucleus of the macrogametocyte and the overall pink colour of the microgametocyte due to its widely dispersed nuclear chromatin (Figs G, H). Although a vacuole containing the bulk of the pigment is present in the gametocyte, it is much smaller that that of the asexual stages and commonly flattened into an oval or spindle shape. The spherical or subspherical male and female gametocytes are of similar size: macrogametocytes 5.7 × 5.2 (4.4 × 4.0 – 5.9 × 5.1), shape-index 1.1, n = 50. Microgametocytes average 6.0 × 5.0 (5.2 × 4.4 – 6.2 × 5.2) shape-index 1.2, n = 45. The sex ratio was calculated as 1 male: 2.2 females

Type host: the lizard *Ameiva ameiva* Linnaeus, 1758 (Squamata: Teiidae).

Type locality: Capanema, Pará State, North Brazil (1º 12´ S - 47º 11´W).

Prevalence: of 206 *A. ameiva* examined, infections were detected in only 12 (5.83 %). In view of the difficulty in detecting infections with extremely low parasitaemia, however, it is likely that the true prevalence is much higher.



Figs A-H. – *Plasmodium carmelinoi* n. sp. in the teiid lizard *Ameiva ameiva*. A. Multiple infection of an erythrocyte with four young trophozoites, already showing a well developed cytoplasmic vacuole; B-D. Progressive nuclear division and increasing concentration of pigment granules within the vacuole; E. Unusually compact, mature schizont of *P. carmelinoi* with ten merozoites and a small vacuole containing the pigment; F. Double infection with a mature schizont, with ten nuclei (above), and a young macrogametocyte (below); G. Mature macrogametocyte: note the smaller, flattened vacuole containing the bulk of the pigment; H. Mature microgametocyte, again showing concentration of the pigment in a small, flattened vacuole. Bar = 5 µm.

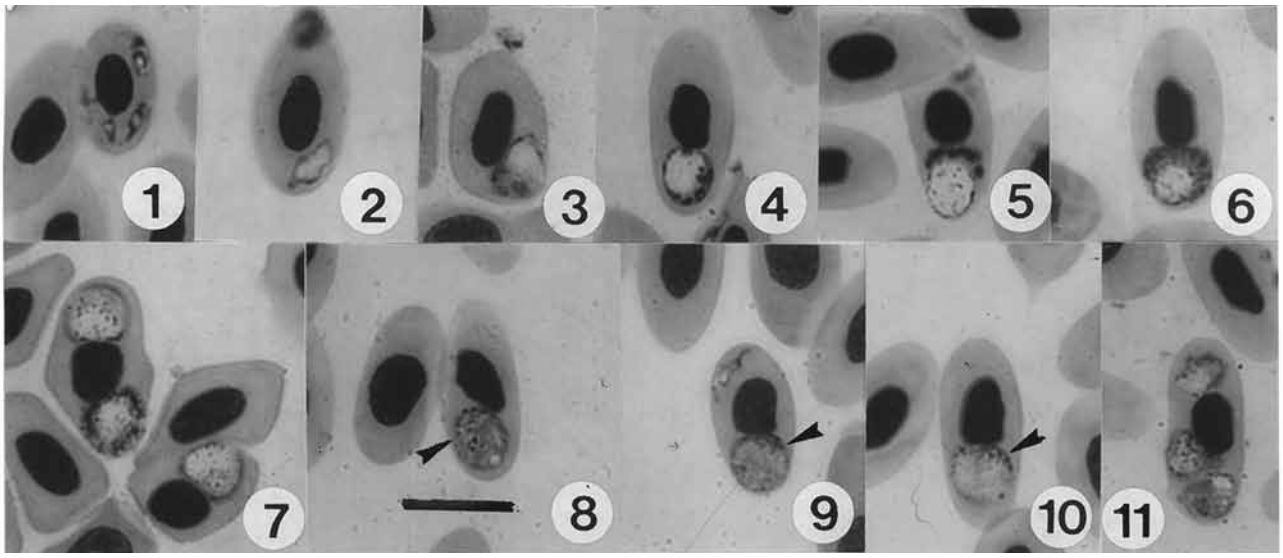
Pathology: on no occasion did infected lizards exhibit signs of disease due to the parasite.

Etymology: the specific name is given as a token of thanks to Manoel Carmelino Mendes de Souza, who first drew the senior author's attention to the presence of the parasite in *A. ameiva*.

Type material: stained blood film (2160) is to be deposited in the Muséum National d'Histoire Naturelle, Paris.

DISCUSSION

The morphology of *P. carmelinoi* n. sp., particularly that of the schizonts, sets it apart from other species of saurian plasmodia, in particular the following parasites previously recorded in the lizard *A. ameiva*. *P. cnemidophori* has elongated gametocytes measuring up to 16 × 7 µm and large schizonts producing up to 60 or more merozoites; although *P. pifanoi* is similar to *P. carmelinoi* n. sp. in having schizonts producing only 6-12 merozoites, its gametocytes were described as elongated and measuring 15-17 × 6-8 µm; *P. diminutivum* has rounded gametocytes of very similar size (5.6 × 4.8 µm) to those of *P. carmelinoi* n. sp., but the parasite has tiny schizonts with only 4-6 nuclei, often in fan-shaped arrangement, and lacks the large pigment-filled vacuole of *P. carmelinoi*. *Garnia telford* is a non-pigmented parasite of the erythrocytes of *A. ameiva* and thus easely distinguished from *P. carmelinoi* which produces a large quantity of conspicuous black pigment; *Hemolivia stellata*, another pigmentless parasite, is remarkable in its ability to infect its type amphibian host, *Bufo marinus*, and the lizard *A. ameiva* (Lainson, de Souza & Franco, 2007). In the latter host it is readily differentiated from *P. carmelinoi* n. sp., by its production of cystic forms in the



Figs 1-11. – *Plasmodium carmelinoi* n. sp. in the teiid lizard *Ameiva ameiva*. 1. Four young irregularly shaped trophozoites in the same erythrocyte; 2. Growing uninucleate trophozoite, of irregular shape and assuming the polar position of development in the host cell: note the large vacuole and peripheral band of cytoplasm; 3-6. Progressive nuclear division of the growing schizont to form from 8-12 merozoites: the large vacuole now contains most of the pigment granules; 7. One erythrocyte containing a mature microgametocyte (above) and a mature schizont (below), and another with a single microgametocyte; 8. A nearly mature macrogametocyte; 9. Mature macrogametocyte and a growing trophozoite in the same erythrocyte; 10. Mature microgametocyte: note the smaller vacuole of the gametocytes (arrow heads) containing most of the pigment granules; 11. Erythrocyte containing two developing gametocytes (below) and a growing schizont (above). Bar = 10 µm.

erythrocytes and its elongated, encapsulated and stain-resistant gametocytes.

As is regrettably the case with the majority of saurian *Plasmodium* species, the vector of *P. carmelinoi* is as yet unknown.

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