Garnia karyolytica n. sp. (Apicomplexa: Haemosporina: Garniidae), a blood parasite of the Brazilian lizard Thecodactylus rapicaudus (Squamata: Gekkonidae)

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Summary:
Development of meronts and gametocytes of Garnia karyolytica nov.sp., is described in erythrocytes of the neotropical forest gecko Thecodactylus rapicaudus from Pará State, north Brazil. Meronts are round to subpherical and predominantly polar in position: forms reaching 12.0 x 10.0 µm contain from 20-28 nuclei. Macrogametocytes and microgametocytes are predominantly elongate, lateral in the erythrocyte and average 16.6 x 6.3µm and 15.25 x 6.24 µm respectively. Occasional spherical forms of both sexes occur in a polar or lateropolar position. All stages of development are devoid of malarial pigment. They have a progressively lytic effect on the host-cell nucleus, particularly the mature gamocytes, which enlarge and deform the erythrocyte. Possible vector(s) of garniid parasites are considered, and phlebotomine sandflies are high on the list of suspects.

KEY WORDS: Protozoa, Garnia karyolytica n. sp., haemosporine, Thecodactylus rapicaudus, gekkonid lizard, Brazil.

Résumé: Garnia karyolytica n. sp. (Apicomplexa : Haemosporina : Garniidae) parasite du sang du lézard brésilien Thecodactylus rapicaudus (Squamata : Gekkonidae)

Description du développement des mériotnes et des gamétoctyes de Garnia karyolytica n. sp., parasite des érythrocytes du gecko de forêts néotropicales Thecodactylus rapicaudus, capturé dans l’État de Pará (Nord Brésil). Les mériotnes, arrondies à subsphériques le plus souvent en position polaire, mesurent 12,0 x 10,0 µm et contiennent 20 à 28 noyaux. Les macrogamétoctyes et les microgamétoctyes sont le plus souvent allongées, en position latérale dans l’hématie et mesurent en moyenne respectivement 16,6 x 6,3 µm et 15,25 x 6,24 µm. Parfois des formes sphériques des deux sexes se trouvent en position polaire ou subpolaire. Tous les stades de développement sont dépourvus de pigment malarique. Les parasites ont une action lytique progressive sur le noyau de la cellule hôte et particulièrement les gamétoctyes mûrs qui hypertrophient et déforment l’érythroctye. Divers arguments sont supposés que les phlébotomes pourraient être les vecteurs des Garniidae.

MOTS CLÉS: Protozoa, Garnia karolytica n. sp., Haemosporina, Thecodactylus rapicaudus, Gekkonidae, Brésil.

INTRODUCTION
Among the parasitic protozoa, members of the suborder Haemosporina inhabit various cells in the peripheral blood of a wide range of reptiles, birds and mammals in both the Old and the New World, among which they are transmitted by a variety of haematophagous dipteran insects such as anopheline and culicine mosquitoes, midges (Culicoides), blackflies (Simulium) and tabanids (Chrysops). In the vertebrate host all haemosporines undergo a phase of asexual multiplication (merogony, or schizogony) and eventually produce separate, dimorphic male and female gametocytes (gamonts). Within the invertebrate host the male parasite produces a small number of flagellated microgametes, one of which fertilizes the female. The motile zygote (ookinete) forms an oocyst containing a large number of naked sporozoites: these are the infective stage of the parasite which, after migration to the salivary glands, are inoculated into a new host by the bite of the insect vector.

Until 1971 the suborder was divided into three families, by way of differences in development of the parasites in the vertebrate host and the role of different insect vectors in their transmission (Garnham, 1966): the family Plasmodiidae, which contains species of the genus Plasmodium in reptiles, birds and mammals, and has naturally received the bulk of the parasitologist’s attention due to the enormous medical importance of those species infecting man; the family Haemoproteidae, whose members infect similar hosts but are so far unknown in man; and the family Leucocytozoidae, genera of which are restricted to birds and reptiles. A fourth family, the Garniidae Lainson, Landau & Shaw, 1971, was later included in the Haemosporina (see table I) to contain other haemosporines commonly found in the blood of lizards. It at present contains three genera: Garnia, species of which undergo merogony and the production of gametocytes...
**Table I. Taxonomic characters separating families of the suborder Haemosporina*.**

<table>
<thead>
<tr>
<th>Character</th>
<th>Plasmodiidae</th>
<th>Leucocytozoidae</th>
<th>Haemoproteidae</th>
<th>Garniidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merogony in blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>With malarial pigment</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No (Garnia)</td>
</tr>
<tr>
<td>Gametocytes in mature erythrocytes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No (Fallisia)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No (Progarnia)</td>
</tr>
</tbody>
</table>


in the erythrocytes, but fail to produce the malarial pigment (haemozoin) that is characteristic of the genus *Plasmodium*, *Fallisia*, which has all these stages in the thrombocytes and leucocytes; and *Progarnia*, a blood parasite of crocodilians which inhabits the erythrocytes, thrombocytes and leucocytes (Lainson, 1995). Although some workers have preferred to modify the classical definition of the family Plasmodiidae and the genus *Plasmodium* in order to accommodate such saurian haemosporines (Telford, 1973, 1988; Ayala, 1978)), others (Garnham & Duggan, 1986; Boulard et al., 1987; Paperna & Landau, 1990; Euzeby, 1989-1990) have favoured their allocation to a separate family, Garniidae, and it is this classification that we follow in the present communication.

The lizard *Thecodactylus rapicaudus* has a wide geographical distribution in the New World, having been recorded in northern South America: in Venezuela, the Guyanas, Brazil, both sides of the Andes in Ecuador and Colombia and the eastern side of Peru and Bolivia; in Central America up to Mexico; and in the Lesser Antilles (Avila Pires, 1995). It is a relatively large, arboreal gecko found in both primary and secondary forest and sometimes in houses or animal shelters close to patches of trees. It is principally nocturnal in habits, and spends the daylight hours under the cover of loose bark, hollow trees and other secluded retreats.

The only named haemosporine parasite previously described in *T. rapicaudus* is *Plasmodium aurulentum* Telford, 1971, in specimens from Panama and the Canal Zone. In 1978, however, the same author recorded the presence of pigmentless gametocytes of an "undetermined haemosporian species" in the erythrocytes and proerythrocytes of this gecko from eastern Panama and Venezuela. In the absence of parasites that could confidently be regarded as stages of merogony, Telford concluded that additional material from *T. rapicaudus* needed to be examined "before a proper generic allocation" of the parasite could be made.

Our recent examination of blood films from a specimen of *T. rapicaudus* captured in Pará State, north Brazil has revealed abundant asexual and sexual stages of a non-pigmented, erythrocytic parasite which we believe to be that encountered and partially described by Telford (1978). A full description of the blood forms of this species of *Garnia* is the subject of the present paper.

The infected gecko was an adult male, captured by hand on the base of a large tree in primary forest near Novo Repartimento, about 60 km from Tucuruí, Pará, Brazil (3° 42'S: 49° 27'W), on 15th August, 1998. No infections were detected in three other adult specimens captured close to secondary or primary forest.

**DESCRIPTION**

When blood films were first examined on 15.08.98 we were able to locate only two mature female gametocytes after a long search. These aroused considerable interest, however, due to the extraordinary lytic effect the parasite had on the nucleus of the host erythrocyte (Figs 1 and 2). It was decided to maintain the infected lizard alive, in the hope that the parasitaemia would increase and provide additional stages of development of the blood forms. This in fact took place, possibly following exacerbation of the infection due to the stress of captivity, and when further blood films were made on 16 October 1998 they contained abundant parasites, including very young trophozoites, developing meronts and gametocytes. We propose the name of *Garnia karyolytica* n. sp. for the parasite, in view of its effect on the host-cell nucleus. All measurements are in μm.

**GARNIA KARYOLYTICA** N. SP. (Figs 1 and 2)

Asexual stages

The smallest parasites seen were compact, uninucleate bodies measuring only 3.0 × 2.0 (Fig. 2a) and presumably represent merozoites that have recently penetrated the erythrocyte. Subsequent merogonic stages (Figs 1b-f and 2b-f) show no amoeboid activity and
Fig. 1. *Garnia karyolytica* n. sp. in the gecko *Thecodactylus rapicaudus*. Developing meronts and gametocytes as seen in thin blood films fixed in absolute methyl alcohol and stained with Giemsa. 

- a: Normal, mature erythrocyte.
- b: Young, binucleate meront: the host-cell nucleus is already enlarged and with early signs of pycnosis.
- c-e: Developing meronts.
- f: Nearly mature meront with cytoplasm divided into clumps containing peripherally disposed nuclei.
- g, h: Young microgametocyte and macrogametocyte: the host-cell nucleus is already in an advanced state of pycnosis.
- i: Round form of macrogametocyte.
- j, k: Mature, elongated microgametocyte and macrogametocyte.
- l, m: Irregularly shaped macrogametocyte and microgametocyte, considered to be prematuration forms. Note the presence of azurophilic granules in the young and mature gametocytes and lysis of the erythrocyte nucleus. Bar = 10.0 μm.
Fig. 2. – Photomicrographs of *Gamia karyolytica* n. sp. in erythrocytes of the gecko *Thecodactylus rapicaudus*, as seen in thin blood films fixed in absolute methyl alcohol and stained with Giemsa. a: A merozoite that has recently entered an erythrocyte. B: Binucleate meront. c-e: Developing meronts: note early pycnosis of the erythrocyte nucleus (arrows). f: Almost mature meront: the cytoplasm has divided into clumps, with nuclei arranged at the periphery. g-k: Developing macrogametocytes. l: Mature macrogametocyte. m-o: Developing microgametocytes. p: Mature male (left) and female gametocytes. q, r: Rounded male (left) and female gametocytes. s: Ovoid male gametocyte. Non-parasitised erythrocytes are included for comparison in c. and j. Note extensive lysis of the erythrocyte nucleus (arrows), particularly in cells infected with gametocytes. Bar = 10.0 μm.
their finely vacuolated cytoplasm stains a delicate blue. During nuclear division the parasite usually maintains a smooth, rounded shape, finally producing from 20-28 nuclei and reaching up to 12.0 x 10.0 (Figs 1e and 2e). Prior to the formation of merozoites the cytoplasm is sometimes seen to divide into separate clumps, at the periphery of which the nuclei are arranged (Figs 1f and 2f). The exact number of merozoites produced is uncertain, as we encountered no fully segmented meronts. Most meronts occupied a polar position within the erythrocyte, but occasional lateral forms were seen.

Gametocytes

Dimorphism is apparent when the gametocytes are quite small, the males staining a delicate, pale pink due to the diffuse nuclear material and the females a clear blue, with a more clearly defined nucleus (Figs. 1g, b and 2g, h, m): a conspicuous karyosome can be seen in most of the parasites. Most gametocytes begin to assume an elongate shape at an early age (Figs. 2b, m), but a smaller number maintain a rounded or broadly ellipsoidal shape until they are quite large and apparently mature (Figs 1l and 2q, r, s). The cytoplasm of both sexes is finely vacuolated and usually contains a variable number of intensely staining azurophilic granules: these are larger and more abundant in the macrogametocyte. Single, large vacuoles (as commonly seen in the gametocytes of some saurian species of *Plasmodium* and *Haemoproteus*) were very rarely seen (Fig. 1g). The shape of the larger gametocytes is very variable. Elongated males and females may have pointed extremities (Fig. 2f), or a strange, wavy outline, particularly pronounced on the margin facing the host-cell nucleus (Figs. 1l, m and 2k, o). It is our impression that these are stages in the maturation of the gametocytes which, when fully developed, have a smooth outline with more rounded ends (Figs 1j, k and 2l). Similar irregularly shaped, prematuration gametocytes have been described for other *Garnia* species (Telford, 1970, 1978; Lainson, Landau & Shaw, 1971; Lainson, Shaw & Landau, 1975). Elongate macrogametocytes (50 measured) were 16.6 x 6.3 (13.3-21.4 x 4.4-8.1), shape index 2.6 (1.8-4.0): elongate microgametocytes (50 measured) were slightly smaller, 15.25 x 6.24 (12.6-18.5 x 4.4-8.1), shape-index 2.4 (1.6-3.3). Round to broadly ovoid macrogametocytes (13 measured) were 9.5 x 8.0 (7.4-11.1 x 6.6-9.6), shape index 1.2 (1.0-1.5): similarly shaped microgametocytes (16 measured) were 9.4 x 8.4 (7.4-11.8 x 7.4-9.6), shape-index 1.1 (1.0-1.4). The proportion of rounded/elongate macrogametocytes was 1:10, and that of the microgametocytes 1:7. The elongated gametocytes occupied a lateral position in the erythrocyte, sometimes curving slightly around the host-cell nucleus but not encircling it: rounded forms were predominantly polar or lateropolar. The sex ratio of all forms was one male parasite to 1.3 females.

Exoerythrocytic stages

No stages of the parasite were detected in cells other than the erythrocytes.

Effects on the host cell

Telford (1978) considered that the gametocytes of the Panamanian "strain" occupied mature erythrocytes, whereas three-fourths of the Venezuelan "strain" were in mature erythrocytes and the rest in immature red blood cells. We have found it difficult to say whether the parasitized erythrocytes are immature or mature, due to the profound effect this haemosporine has on its host-cell and nucleus. Even in the presence of a very small parasite the host-cell nucleus enlarges and shows early signs of pycnosis. The process is most pronounced in cells infected with growing gametocytes, both sexes of which are equally destructive (Figs 1g-m and 2g-s). The effect of growing meronts is notable, but less dramatic (Figs 1b-f and 2c-e). The host-cells are almost always enlarged, tend to become more rounded and are often distorted into unusual shapes, particularly by the larger gametocytes.

Type host


Locality

Primary forest, Novo Repartimento, near Tucuruí, Pará, north Brazil (3° 42'S: 49° 27'W).

Known geographical range

Eastern Panama to north Brazil.

Prevalence

Uncertain in the area of the present study, where one of four *T. rapicaudus* examined was infected. Telford (1978) recorded infections in one of 25 specimens in Panama and one of 22 in Venezuela: no "malaria parasites" were detected, however, in 36 from Panama by Kimsey (1985). By no means, then, can *G. karyolytica* be regarded as a common parasite of this lizard.

Pathology

The infected gecko appeared to be in good condition and survived well in captivity.

Etymology

The specific name refers to the lytic effect the parasite has on the nucleus of the host erythrocyte.
DISCUSSION

We are confident that the round and elongate gametocytes of the infected gecko belong to the same parasite because both forms have the same lytic effect on the host-cell nucleus and, other than their shape, they share the same morphological features. Among the species of Garnia described to date, the elongate gametocytes of G. karyolytica most closely resemble those of G. gonatodi, in the gekkonid lizard Gonatodes bumeralis, and G. multiformis of the iguanid Plica umbra (Telford, 1970; Lainson, Landau & Shaw, 1971; Lainson, Shaw & Landau, 1975). Meronts of G. gonatodi, however, are of highly variable shape, often amoeboid and may produce up to 50 merozoites. In mature erythrocytes, the meronts of G. multiformis are also variable in shape and produce an average of only eight merozoites. Neither of these haemosporines lyse the host-cell nucleus and, till now, this characteristic appears to be a unique feature of G. karyolytica within the Garniidae.

Telford (1978) referred to the parasites of T. rapicaudus in Panama and Venezuela as different “strains” and noted small but significant morphological differences in the gametocytes, principally in the length/width values of each sex. Mean measurements for female and male gametocytes of the Panamanian parasite were given as 19.3 × 12.8 (13 measured) and 18.4 × 11.3 (12 measured). Those of the Venezuelan parasite were 17.2 × 9.9 (17 measured) and 15.0 × 9.1 (8 measured). Morphologically and geographically, then, the latter is closer to the parasite described in the present paper. The significance of the recorded differences between the parasite from Panama and that from Venezuela and north Brazil will only become clear after the examination of more material. It is possible that there are two distinct species involved, rather than mere “strains”.

In his paper, Telford (1978) figured “cells of uncertain identity, some of which may be schizonts”. With the possible exception of his figure 43, however, these bear no resemblance to meronts of the parasite described in the present description. They have more the appearance of host cells (e.g. basophils) with cytoplasmic granules, which are far in excess of the number of nuclei produced in the meronts of G. karyolytica. The same author considered that some gametocytes of the Venezuelan parasite were “occasionally seen in white blood cells”, although in legends accompanying photomicrographs of these he cautiously refers to the host cells as “possibly a macrophage” and an “apparent monocyte”. An exhaustive search of blood films and smears of spleen, liver, lung, kidney and heart from the infected Brazilian gecko failed to reveal parasites in the white cells.

In drawing attention to the primitive nature of gekkonids, Telford discussed the fact that most haemosporines described in these lizards share some common features which also might be regarded as primitive; namely, lack of pigment in their stages in the erythrocytes, sexual difference in gametocyte size and the production of the irregularly shaped “prematurition gametocytes”. We certainly agree that members of the Garniidae are primitive parasites. Lainson (1995) described a new member of the family, Progamia archosauriae, in the South American crocodilian Caiman crocodilus crocodilus. The parasite undergoes merogony and gametogony principally in leucocytes and thrombocytes, but also produces pigmentless meronts in the erythrocytes: it thus shares characters of the genera Falisia and Garnia of modern-day lizards (see Table I). This, and the great antiquity of the crocodilians, which have remained relatively unchanged since their life with the dinosaurs some 160 million years ago, led to his suggestion that it was from a similar organism that the existing reptilian and avian haemosporines evolved. In conclusion: in spite of the steadily increasing list of garniid haemosporines (see Lainson, 1995 for a review) we are still woefully ignorant regarding their invertebrate vectors and, therefore, of their sporogonic stages. Phlebotomine sandflies and culicine mosquitoes have been incriminated as vectors of two reptilian Plasmodium species, however (Klein, Young & Telford, 1987; Klein et al., 1987, 1988), and it remains likely that these insects may also transmit members of the Garniidae. Indirect evidence that phlebotomine sandflies may be involved comes from the frequent presence of certain species of Lutzomyia on forest tree-trunks harbouring arboreal lizards which are hosts of different Garnia species. We have captured the sandflies Lutzomyia trinidadensis and Lutzomyia micropsyga actively feeding on Gonatodes bumeralis, the host of Garnia gonatodi (Lainson, unpublished observations); L. trinidadensis has been incriminated as the vector of Trypanosoma thecodactyli of Thecodactylus rapicaudus (Christensen & Telford, 1972) in Panama, and this sandfly’s fondness for the blood of T. rapicaudus has been confirmed by Kimsey (1985) in the same country; in north Brazil L. rotonensis has also been captured while feeding on T. rapicaudus (Lainson & Shaw, 1979). L. trinidadensis and L. rotonensis thus remain on the suspect list of vectors of G. karyolytica, although other arboreal sandflies, and culicines, must also be considered.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Wellcome Trust, London (to R.L.) and financed, in part, by the Instituto Nacional de Pesquisas
da Amazônia, Brazil (to R.D.N.). We thank Constância Maia Franco and Francisco Lima Santos for technical assistance, and Dr. Teresa de Avila Pires who identified the lizards.

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