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Haemogregarine blood parasites in the lizards *Podarcis bocagei* (Seoane) and *P. carbonelli* (Pérez-Mellado) (Sauria: Lacertidae) from NW Portugal

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Abstract In Iberian and Canarian lizards, haemogregarines have been recorded infecting erythrocytes, but most of the records correspond to mature gametocytes. We analysed blood smears from 75 specimens of *Podarcis bocagei* (Seoane) and 33 specimens of *P. carbonelli* (Pérez-Mellado) from localities of north-western Portugal. We found haemogregarines in 74.7% of *P. bocagei* and 69.7% of *P. carbonelli*. Our observations show characteristics of the haemogregarines other than the morphology of the mature gametocytes. In histological sections of the liver of four hosts latent cysts with sporozoites and meronts with merozoites were detected. Both traits have been described as typical of the genera *Hepatozoon* Miller, 1908 and *Hemolivia* Petit, Landau, Baccam & Lainson, 1990. We suggest that not only *P. bocagei* and *P. carbonelli* from Portugal but other species of Iberian and Canarian lacertids might also be infected by species belonging to one or both genera.

Introduction

The haemogregarines (*sensu lato*) (Apicomplexa: Adeleorina) are protozoan parasites infecting a variety of hosts, among which are many reptile species. They have an indirect life-cycle involving merogony and the formation of gametocytes in the circulatory system and/or visceral tissues of a vertebrate host, and gamogony proper and sporogony within the gut of an invertebrate vector (Telford, 2008). The best known and, in many cases, the only reported stages are those in erythrocytes (and often less in leukocytes). This has prevented an accurate identification of these infecting forms. Thus, despite the large number of nominal species of haemoprotozoa in the literature, the life-cycle and/or the intermediate host have been described for only a few species (Telford, 2008).

Most records of blood parasites in species of *Lacerta* Linnaeus, 1758 (*sensu lato*) concern southern Europe and North Africa. In Iberian and Canarian lacertid lizards, haemogregarines have been recorded from erythrocytes in lizards of the genera *Podarcis* Wagler (Alvarez-Calvo, 1975; Amo et al., 2005b), *Timon* Tschudi (Alvarez-Calvo, 1975; Amo et al., 2005a), *Iberolacerta* Arribas (Amo et al., 2004) and *Gallotia* Boulenger (Bannert et al., 1995; Martínez-Silvestre et al., 2001, 2005). The specific identity of these parasites remains to be elucidated. In the case of the Canarian lizards of the genus *Gallotia*, a mite of the genus *Ophionyssus* Megnin was suspected to be involved as a vector in the life-cycle of these haemogregarines (Bannert et al., 1995).

In this paper, we describe gametocytes parasitising erythrocytes of *Podarcis bocagei* (Seoane) and

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P. carbonelli (Pérez-Mellado) and also record, for the first time in Iberian lizards, other stages from the host liver.

Materials and methods

Sampling was carried out during spring (April–May) in two coastal localities from the Douro littoral region and one inland locality in the Tras-Os-Montes e Alto Douro Province. The coastal localities were: Madalena, UTM squares 29T NF25 (n = 37 *Podarcis bocagei* collected); and Torreira, near Aveiro, 29T NF21 (n = 33 *P. carbonelli* collected). The inland locality, Vila Pouca de Aguiar, UTM 29T PF19 (n = 38 *P. bocagei* collected), is located near Sierra de Alvao at an altitude of 650 m.

For each lizard, the snout-vent length (SVL) was measured with a caliper (±0.01 mm). Thin blood smears were made from blood obtained by cutting off the tail of the lizards (Sevinç et al., 2000) (tails were also used for genetic analysis). Blood smears for each individual were prepared immediately, air-dried and stained using the standard MayGrünwal/Giemsa staining method; then they were randomly examined using a ×100 oil immersion lens following standard routines (Sacchi et al., 2007). Four lizards were humanely killed and the liver was dissected out and cut into 2–3 mm slices. Tissue slices were dehydrated and embedded in paraffin, and 7 µm thick sections were cut and mounted on gelatin-coated slides (Goldman, 1983); then the sections were rehydrated and stained with H & E.

Results

Most gametocytes were found infecting red blood cells, although some of them were observed outside the cells (Fig. 1I). Different developmental stages were observed in the gametocytes (Fig. 1): (i) immature gametocytes, oval and with a large central nucleus (Fig. 1A); (ii) pre-mature gametocytes with a nucleus containing spiral chromatin (Fig. 1E, F, G); (iii) pre-mature gametocytes with a dense, homogeneous, central nucleus (Fig. 1H); (iv) mature gametocytes encapsulated in a parasitophorus vacuole (Fig. 1K, L, LL). Measurements of mature gametocytes were 12 ± 1 (10–13) µm in length and 4 ± 1 (3–6) µm in width.

Fig. 1 Haemogregarine from *Podarcis* spp.: A. merozoite; B. immature gametocyte; C–G. gametocytes at different stages of maturity (note the spiral chromatin in images E, F and G); H. premature gametocyte with compact central nucleus; I. free gametocyte; J. double infection and lysis of the infected erythrocyte; K–LL. nuclear lysis of the infected cell (note the parasitophire vessel in images K–LL); M. liver cyst containing an sporozoite; N. liver meront containing merozoites ▶

Variation in the shape of the infected erythrocytes was detected. Thus, parasitised erythrocytes were perceptibly wider than non-infected ones.

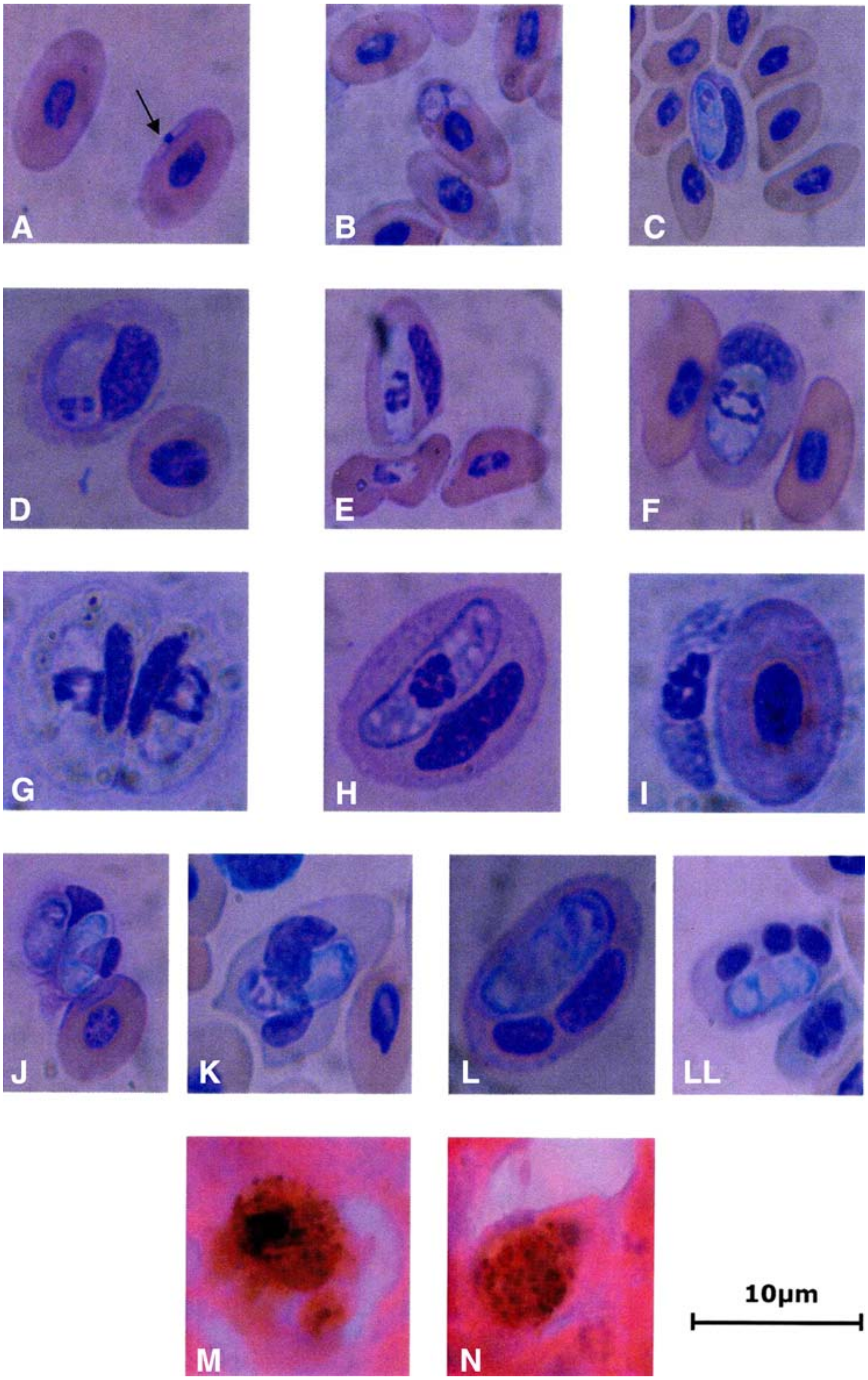
The prevalence of infection was 74.7% for *Podarcis bocagei* and 69.7% for *P. carbonelli*.

In the liver, the presence of latent cysts and meronts was observed. Cysts, 10 ± 1 (9–11) µm long and 9 ± 1 (8–10) µm wide, contained 1–2 cystozoites (Fig. 1M). Meronts, 13 ± 1 (11–16) µm long and 12 ± 1 (10–16) µm wide, contained a variable number (always > 30) of merozoites (Fig. 1N) at different stages of development.

Discussion

The different developmental stages found in this study agree with those described by Paperna et al. (2002). The observed immature gametocytes (Fig. 1A) are considered as merozoites by several authors (Smith et al., 2000).

Morphological characteristics of gametocytes have been rejected by many authors (Smith & Desser, 1997; Jakes et al., 2003) as valid criteria for differentiating genera of haemogregarines because: (i) gametocytes of two or more species may be present in the same vertebrate host (Smith, 1996); (ii) mature and immature gametocytes of the same species might be considered as different species (Jakes et al., 2003); and (iii) there may be confusion between macro- and microgametocytes, which could also be considered as different species (Smith, 1996; Paperna & Lainson, 2004). Thus, other traits, such as developmental stages found in vertebrate hosts, invertebrate hosts involved in the life-cycle, and the biogeography and ecology of the vertebrate hosts, have been used to differentiate genera of these blood parasites. Using some characteristics of the developmental stages, Siddall (1995) transferred 41 species of *Haemogregarina* Danilewskyi, 1885 (those using fish as vertebrate hosts) to *Desseria* Sidall, 1995 and *Cyrilia*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Lainson, 1981, including those species parasitising chelonians, to *Haemogregarina* (*sensu stricto*); and suggested that the species belonging to *Haemogregarina* (*sensu lato*), parasitising amphibians, saurians, ophidians, crocodiles, birds and mammals, should be transferred to *Hepatozoon* Miller, 1908. On the other hand, although species of *Hemolivia* Petit, Landau, Baccam & Lainson, 1990 had been considered as amphibian parasites, the host range of the species of this genus was extended into reptiles by Smallridge & Paperna (1997). Moreover, Landau & Paperna (1997) reassigned *Hepatozoon mauritanicum* (Brumpt, 1938), parasite of the tortoise *Testudo graeca* (Linnaeus), to *Hemolivia*. As occurs in the hosts examined in this study, some species of *Hepatozoon* and *Hemolivia* may produce cysts in the internal organs (especially liver and lungs) of their vertebrate hosts (Landau et al., 1972; Smith, 1996; Telford, 2008). The above-mentioned latent cysts have been recorded for those species of *Hepatozoon* that need two vertebrate hosts in their life-cycle (Landau et al., 1972) but also in species of *Hepatozoon* with only one vertebrate host in their life-cycle (Smith & Desser, 1998). The sporozoites (contained in the latent cysts) are infective forms in some species of *Hepatozoon*, but not in the species of *Haemogregarina*, *Cyrillia*, *Desseria* and *Karyolysus* Labbé, 1894 in which the only infective form are merozoites (Smith et al., 2000).

Cysts containing single cystozoites within erythrocytes have been found in *Hemolivia* spp. but not in *Hepatozoon* spp. (see Telford, 2008). As we have not found these forms in the hosts examined in the present study, we might consider that the species described here parasitising *P. bocagei* and *P. carbonelli* belonged to *Hepatozoon*, but the absence of the cysts makes it difficult to evaluate this possibility. In view of this, and considering the likelihood that not all species of *Hemolivia* develops such cysts, we consider it pragmatic to make no definitive allocation of these forms to one of both of *Hepatozoon* and *Hemolivia* at the present time.

Although more than 70 species of *Hepatozoon* have been recorded from saurians (Smith, 1996; Paperna et al., 2002) only five have been found parasitising lizards of the genus *Podarcis*: three in *P. muralis* (Laurenti) from France, two in *P. hispanica* (Steindachner) from Spain and one in *P. bocagei* from Spain (Alvarez-Calvo, 1975). On the other hand, no species of *Hemolivia* have been recorded from lizards in Europe. Taking into account the similarity of the parasitic forms found in both host species examined, *P. bocagei* and *P. carbonelli*, which themselves are very closely related in terms of the phylogeny of lacertids, being separated by only a short evolutionary period (Harris & Sa Sousa, 2001), and which are ecologically similar in relation to their feeding habits, distribution and habitats (strict syntopy) (Carretero et al., 2002), we suggest that the haemoparasites found may belong to one and the same species. We also suggest that these blood parasites in saurians from the Iberian Peninsula and Canary Islands could represent a species of *Hepatozoon* or *Hemolivia*.

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References

Alvarez-Calvo, J. A. (1975). Nuevas especies de hemococcidios en lacértidos españoles. *Cuadernos de Ciencias Biológicas, 4*, 207–222.

Amo, L., Fargallo, J. A., Martínez-Padilla, J., Millán, J., López, P., & Martín, J. (2005a). Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*. *Parasitology Research, 96*, 413–417.

Amo, L., López, P., & Martín, J. (2004). Prevalence and intensity of haemogregarinid blood parasites in a population of the Iberian rock lizard, *Lacerta monticola*. *Parasitology Research, 94*, 290–293.

Amo, L., López, P., & Martín, J. (2005b). Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. *Parasitology Research, 96*, 378–381.

Bannert, B., Lux, E., & Sedlaczek, J. (1995). Studies on endo and ectoparasites of Canarian Lizards. In G. A. Llorente, A. Montori, X. Santos, & M. A. Carretero (Eds.), *Scientia Herpetologica: Papers submitted from 7th O.G.M. of Societas Europaea Herpetologica*, Barcelona, Sept 15–19, 1993. Barcelona: Asociacion Herpetologica Espanola, pp. 293–296.

Carretero, M. A., Sa Sousa, P., Barbosa, D., Harris, D. J., & Pinho, C. (2002). Sintopís estricta entre *Podarcis bocagei* y *Podarcis carbonelli*. *Boletín de la Asociación Herpetológica Española, 13*, 20–24.

Goldman, J. E. (1983). Immunocytochemical studies of actin localization in the central nervous system. *Journal of Neuroscience, 3*, 1952–1962.

Harris, D. J., & Sa Sousa, P. (2001). Species distinction and relationships of the western Iberian *Podarcis* lizards (Reptilia, Lacertidae) based on morphology and mitochondrial DNA sequences. *Herpetological Journal, 11*, 129–136.

Jakes, K. A., O'Donoghue, P. J., & Cameron, S. L. (2003). Phylogenetic relationships of *Hepatozoon* (*Haemogregarina*) *boigae*, *Hepatozoon sp.*, *Haemogregarina clelandi* and *Haemoproteus chelodina* from Australian reptiles to other Apicomplexa based on cladistic analyses of ultrastructural and life-cycle characters. *Parasitology, 126*, 555–559.

Landau, I., Michel, J. C., Chabaud, A. G., & Brygoo, E. R. (1972). Cycle biologique d'*Hepatozoon domerguei*; discussion sûr les caractères fondamentaux d'un cycle de coccidie. *Zeitschrift für Parasitenkunde, 38*, 250–270.

Landau, I., & Paperna, I. (1997). The assignment of *Hepatozoon mauritanicum*, a tick-transmitted parasite of tortoises, to the genus *Hemolivia*. *Parasite, 4*, 365–367.

Lane, T. J., & Mader, D. R. (1996). Parasitology. In D. R. Mader (Ed.), *Reptile medicine and surgery*. Philadelphia: WB Saunders Company, pp. 185–202.

Martínez-Silvestre, A., Marco, I., Rodríguez-Dominguez, M. A., Lavín, S., & Cuenca, R. (2005). Morphology, cytochemical staining, and ultrastructural characteristics of the blood cells of the giant lizard of El Hierro (*Gallotia simonyi*). *Researches in Veterinary Sciences, 78*, 127–134.

Martínez-Silvestre, A., Mateo, J. A., & Silveira, L. S. (2001). Presencia de protozoos intraeritrocitarios en el lagarto gigante de La Gomera (*Gallotia simonyi gomerana*). *Boletín de la Asociación Herpetológica Española, 12*, 90–92.

O'Dwyer, L. H., Moço, T. C., & Da Silva, R. J. (2004). Description of the gamonts of a small species of *Hepatozoon* sp. (Apicomplexa, Hepatozoidae) found in *Crotalus durissus terrifucus* (Serpentes, Viperidae). *Parasitology Research, 92*, 110–112.

Oppliger, A., Célérier, M. L., & Clobert, J. (1996). Physiological and behavior changes in common lizards parasitized by haemogregarines. *Parasitology, 113*, 433–438.

Oppliger, A., Clobert, J., Lecomte, J., Lorenzon, P., Boudjemadi, K., & John-Alder, H. B. (1998). Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. *Ecological Letters, 1*, 129–138.

Paperna, I., Kremer-Mecabell, T., & Finkelman, S. (2002). *Hepatozoon kisrae* n. sp. infecting the lizard *Agama stellio* is transmitted by the tick *Hyalomma* cf. *aegyptium*. *Parasite, 9*, 17–27.

Paperna, I., & Lainson, R. (1907). *Hepatozoon* cf. *terzii* (Sambon & Seligman, 1907) infection in the snakes *Boa constrictor constrictor* from North Brazil: Transmission to the mosquito *Culex quinquefasciatus* and the lizards *Tropidurus torquatus*. *Parasite, 11*, 175–181.

Sacchi, R., Pupin, F., Zuffi, A. L., Scali, S., Bonconpagni, E., Binda, A., et al. (2007). Blood cell morphology of the Moorish gecko, *Tarentola mauritanica*. *Amphibia-Reptilia, 28*, 503–508.

Sevinç, M., Ugurtas, I. H., & Yildirimhan, H. S. (2000). Erythrocyte measurements in *Lacerta rudis* (Reptilia, Lacertidae). *Turkish Journal of Zoology, 24*, 207–209.

Siddall, M. E. (1995). Phylogeny of adeleid blood parasites with a partial systematic revision of the haemogregarine complex. *Journal of Eukaryote Microbiology, 42*, 116–125.

Smallridge, C., & Paperna, I. (1997). The tick-transmitted haemogregarinid of the Australian sleepy lizard *Tiliqua rugosa* belongs to the genus *Hemolivia*. *Parasite, 4*, 359–363.

Smith, T. G. (1996). The genus *Hepatozoon* (Apicomplexa: Adeleina). *Journal of Parasitology, 82*, 565–585.

Smith, T. G., & Desser, S. S. (1997). Phylogenetic analysis of the genus *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina). *Systematic Parasitology, 36*, 213–221.

Smith, T. G., & Desser, S. S. (1998). Ultrastructural features of cystic and merogonic stages of *Hepatozoon sipedon* (Apicomplexa: Adeleorina) in northern leopard frogs (*Rana pipiens*) and northern water snakes (*Nerodia sipedon*) from Ontario, Canada. *Journal of Eukaryote Microbiology, 45*, 419–425.

Smith, T. G., Kim, B., Hong, H., & Desser, S. S. (2000). Intraerythrocytic development of species of *Hepatozoon* infecting ranid frogs: Evidence for convergence of life cycle characteristics among Apicomplexans. *Journal of Parasitology, 86*, 451–458.

Svahn, K. (1975). Blood parasites of the genus *Karyolysus* (Coccidia, Adeleidae) in Scandinavian lizards. Description and life cycle. *Norwegian Journal of Zoology, 23*, 277–295.

Telford, S. R. (2008). *Hemoparasites of the reptilia. Color atlas and text*. Boca Raton: CRC Press, 376 pp.

Wozniak, E. J., Kazacos, K. R., Telford, S. R., & Maclaughlin, G. L. (1996). Characterization of the clinical and anatomical pathological changes associated with *Hepatozoon mocassini* infections in unnatural reptilian hosts. *International Journal for Parasitology, 26*, 141–146.