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An encapsulated haemogregarine from the evileye pufferfish in South Africa

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Developmental stages of a haemogregarine were found within polychromatocytes and erythrocytes in Giemsa-stained blood smears from six evileye pufferfish (*Amblyrhynchotes honckenii*) caught at Koppie Alleen in the De Hoop Nature Reserve, South Africa. This unusual haemogregarine, *Haemogregarina* (*sensu lato*) *koppiensis* sp. nov., was characterized by encapsulated gamonts with recurved tails, features more common in haemogregarines infecting amphibian and reptilian erythrocytes than in those from fish. *Haemogregarina koppiensis* is only the third species of fish haemogregarine to have been described from South Africa.

INTRODUCTION

Haemogregarines are widespread among marine fishes (Davies, 1995) but only two species, *Desseria* (*Haemogregarina*) *fragilis* (Fantham, 1930) Siddall, 1995 and *Haemogregarina* (*sensu lato*) *bigemina* Laveran & Mesnil, 1901, are described from southern Africa (see Fantham, 1930; Smit & Davies, 1999; Davies & Smit, 2001). This paper reports a third species from this region found in the heart blood of *Amblyrhynchotes honckenii* (Bloch, 1795) (Teleostei: Tetraodontidae), the evileye pufferfish, caught in the De Hoop Nature Reserve. The mature stages of this haemogregarine are characterized by their unusual encapsulation within the host cells and their recurved tails.

MATERIALS AND METHODS

Pufferfish were captured at Koppie Alleen, De Hoop Nature Reserve in October 1999 and November 2000. They were lured with dead fish as bait and caught with hand nets in deep pools during evening low tide. Fish were identified and measured as described elsewhere (see Smit & Davies, 1999; Davies & Smit, 2001). Heart blood smears from each fish were fixed in absolute methanol, stained with phosphate-buffered Giemsa (pH 6.8) and subsequently examined for blood parasites. The methods reported by Davies & Merret (2000) for screening, measuring and determination of prevalence of blood infections were applied.

RESULTS

The heart blood of six out of eight (overall prevalence 75%) evileye pufferfish examined (total length range: 138–200 mm) was found to contain a haemogregarine which was unlike either of those previously reported from South Africa, or any known species. This unusual infection is described below.

Phylum APICOMPLEXA Levine, 1970
Class COCCIDEA Leuckart, 1879
Order EIMERIIDA Léger, 1911
Suborder ADELEINA Léger, 1911
Family HAEMOGREGARINIDAE Léger, 1911
Genus *Haemogregarina* Danilewsky, 1885

Haemogregarina (*sensu lato*) *koppiensis* sp. nov.

The stages of this haemogregarine observed in blood films were trophozoites, meronts and merozoites, which all occurred in low numbers (<0.001% polychromatocytes/erythrocytes infected), and intraerythrocytic and extracellular gamonts which were relatively common (0.3% of erythrocytes). No intraleucocytic stages were seen, except in one smear where a degenerate trophozoite was found inside a small lymphocyte.

Trophozoites were small, elongate, with a rounded, presumed anterior end and a pointed posterior (Figure 1A). They measured 5.8 ±0.6 (5.0–6.4) μm long by 2.6 ±0.3 (2.3–3.0) μm wide (N=6) and were found individually in polychromatocytes and erythrocytes of all the infected fish. The cytoplasm of these stages stained light pinkish-blue with Giemsa. The centrally placed nucleus was diffuse and deeply stained. Trophozoites in polychromatocytes were situated close to the nucleus of the host cell, sometimes apparently in direct contact with it. Where they were found infecting erythrocytes (Figure 1A), trophozoites lay closer to the limiting membrane of the host cell cytoplasm than the nucleus.

Large meronts, presumably arising from trophozoites, were found singly within host cells, which were often enlarged compared with similar uninfected cells. They lay adjacent to the nucleus of primarily polychromatocytes, but also erythrocytes, in four of the six infected fish (Figure 1B). These oval-shaped parasites measured 6.8 ±0.6 (6.1–8.0) μm long by 2.7 ±0.2 (2.3–3.0) μm wide (N=6). Cytoplasm stained pale blue, except at the periphery of the meront, which was darker blue. The

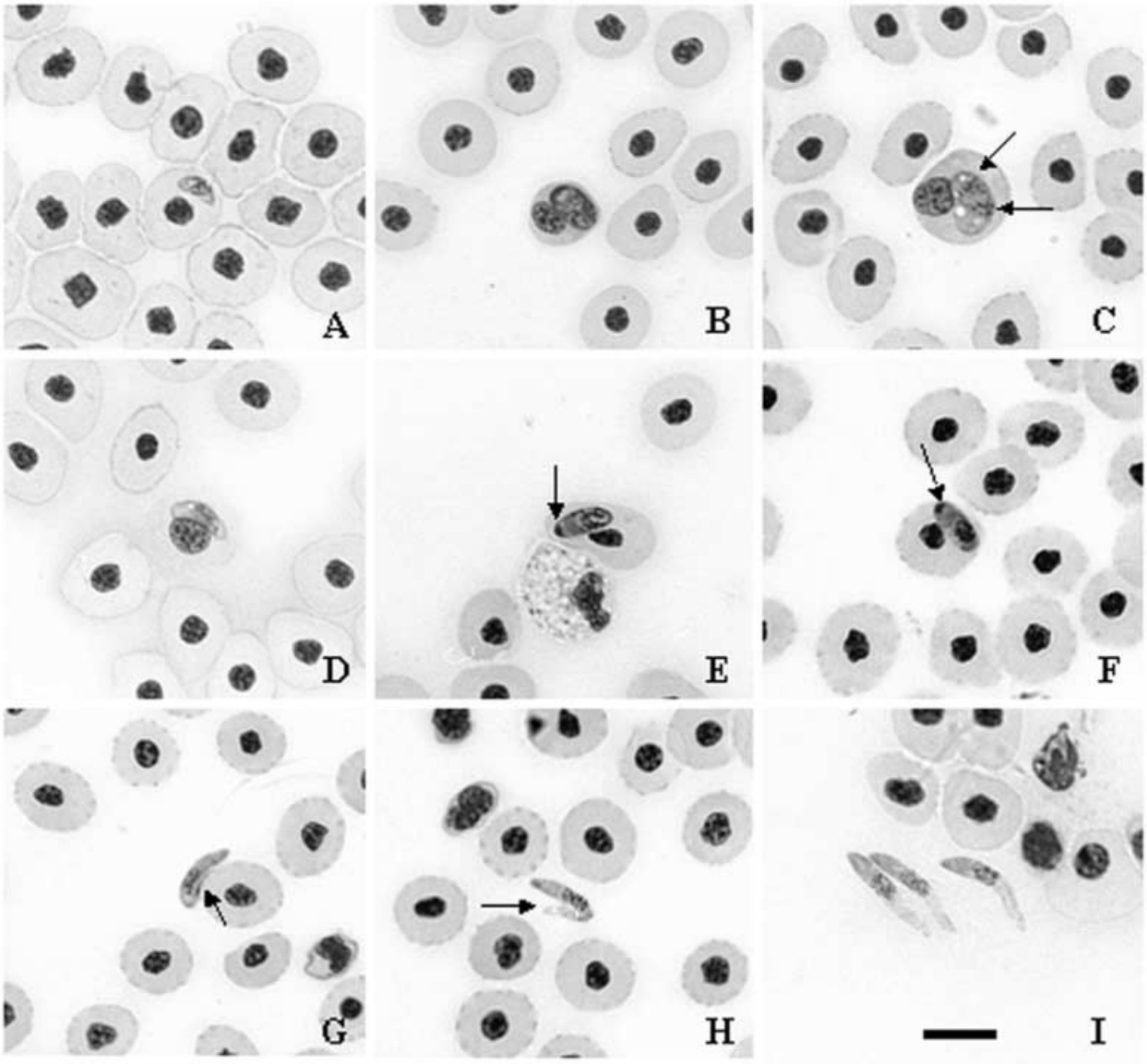

Figure 1. Light micrographs of the developmental stages of *Haemogregarina koppiensis* in Giemsa-stained blood films of *Amblyrhynchotes honckenii*. (A) Intra-erythrocytic trophozoite; (B) deep staining meront in polychromatocyte; (C) dividing meront with two daughter nuclei (arrows) within enlarged polychromatocyte, and note also vacuoles within the parasite cytoplasm; (D) merozoite or young gamont with nucleus in posterior two-thirds; (E & F) intra-erythrocytic encapsulated gamonts with dark staining anterior cap (arrows); (G) extra-erythrocytic gamont with recurved tail closely adherent to the body (arrow); (H) extra-erythrocytic gamont with recurved tail (arrow) clearly visible; (I) extra-erythrocytic gamonts with straight tails. Scale bar: 10 m.

diffuse nucleus was granular, filled almost two-thirds of the meront and stained deep purple. No vacuoles were present in the cytoplasm. The nuclei of the largest meronts (longer than 7 μm) were in division, with the two daughter nuclei appearing at opposite ends of the body of the parasite (Figure 1C). Four to seven vacuoles were distributed randomly in the cytoplasm of the dividing meronts.

Merozoites or immature gamonts, presumably derived from the division of the meronts, were larger than the trophozoites, but more slender than the meronts, with one end broad (anterior) and the other pointed (posterior) (Figure 1D). They occurred individually in host cells and measured 6.7 $\pm$0.9 (5.5–8.4) μm long by 2.4 $\pm$0.3 (2.0–2.7) μm wide (N=8). Their cytoplasm stained light pinkish-blue and a single vacuole was present between the nucleus and the broad anterior end. The nucleus, situated in the posterior two-thirds of the parasite, was not as diffuse as in the previous stages and stained darkish pink.

The largest forms seen were identified as gamonts, they always occurred singly in mature erythrocytes and were the dominant form in the six infected fish. The gamonts were characteristically elongate and encapsulated (Figure 1E,F). In this condition they measured 7.3 $\pm$0.9 (6.1–9.8) μm long by 2.8 $\pm$0.4 (2.0–3.6) μm wide (N=20), but were later shown to have a tightly adherent recurved tail which added to their overall length (see below). The capsule stained dark blue, the parasite cytoplasm was light blue, except for the deep purple staining anterior cap present in almost all cases and a few randomly distributed vacuoles were present in the cytoplasm of some of the specimens. In the encapsulated state, the

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nucleus was situated in the posterior half of the body, ~4.5 μm from the anterior and 1 μm from the posterior end, and each nucleus measured 2.5 ±0.3 (2.3–3.2) μm long by 2.1 ±0.2 (1.8–2.5) μm wide (N=20). Nuclei were diffuse and consisted of deep purple staining chromatin granules. Gamonts were situated in close proximity to the nucleus of erythrocytes, but only in a few cases did they seem to distort the host cell. Five gamonts were also seen in the process of escaping from erythrocytes. In these cases no capsules were detected. A number of extracellular gamonts conforming generally to the description of the intraerythrocytic stages were also found, but without the deep-staining capsule and cap (Figure 1G). These stages were slightly larger than the intraerythrocytic forms and measured 9.7 ±0.4 (8.9–10.2) μm long by 2.3 ±0.4 (1.8–3.0) μm wide (N=10).

In two of the infected fish a second type of extracellular gamont was found. This was characterized by an elongated body with a pointed anterior end and a long recurved tail more than half the length of the body of the haemogregarine (Figure 1H). The bodies of these stages were 9.7 ±0.4 (8.9–10.7) μm and the tails were 5.5 ±0.9 (4.1–6.6) μm long, making the total length 15.2 ±1.0 (13.6–16.6) μm (N=15). At their widest point, these specimens were 2.1 ±0.2 (1.8–2.5) μm. The diffuse nucleus of this stage consisted of deep staining granules and was situated in the anterior half of the parasite ~5.5 μm from the anterior and 7.5 μm from the posterior ends respectively. The cytoplasm stained bluish purple, with purple staining granules lying anterior to the nucleus in some gamonts. A single vacuole was present near the posterior end of the tail in some specimens. In one blood film, three extracellular gamonts with straight, rather than recurved, tails were also observed (Figure 1I). These specimens were more deeply stained anterior to the nucleus than posteriorly (Figure 1I).

The vector of this parasite is unknown, but one of the infected fish was parasitized by 14 haematophagous larvae of a *Gnathia* sp. Three of the infected fish also harboured a few specimens of an unidentified *Caligus* sp.

Remarks

It is difficult to compare the overall body sizes of encapsulated gamonts with extracellular types. In encapsulated haemogregarines from other ectothermic vertebrates, intracellular forms, which are probably constrained within the capsule, can gain considerable size upon escaping from the host cell (Ball, 1958; Davies & Johnston, 2000). Morphometric data in the current study suggested at first that there were two main kinds of gamonts (encapsulated intracellular stages and similar extracellular forms, and rather different extracellular types with a recurved tails). However, closer examination of the morphological characteristics of this apicomplexan revealed that the gamonts are likely all the same form, with the tail closely adherent to the body in the encapsulated intracellular stages so as to make it scarcely visible, but with it released in most extracellular forms. It is concluded therefore that the gamonts of this species are monomorphic.

The new haemogregarine is named *Haemogregarina* (*sensu lato*) *koppiensis* sp. nov. The species name is derived from the collection site, Koppie Alleen, in the De Hoop Nature Reserve.

Taxonomic summary

Haemogregarina (*sensu lato*) *koppiensis* sp. nov.

Type host

Amblyrhynchotes honckenii (Bloch, 1795).

Type specimen

Holotype: deposited at the National Museum, Bloemfontein, South Africa, Slide no. NMBP 228. Paratypes: deposited at the National Museum, Bloemfontein, South Africa, Slides no. NMBP 229, NMBP 230, NMBP 231.

Type locality

Koppie Alleen, De Hoop Nature Reserve (34°28′S 20°30′E).

Diagnosis

All stages intrapolychromatocytic, intraerythrocytic or extracellular. Trophozoites 5.8 ±0.6 (5.0–6.4) μm long by 2.6 ±0.3 (2.3–3.0) μm wide (N=6). Meronts 6.8 ±0.6 (6.1–8.0) μm long by 2.7 ±0.2 (2.3–3.0) μm wide (N=6). Merozoites 6.7 ±0.9 (5.5–8.4) μm long by 2.4 ±0.3 (2.0–2.7) μm wide (N=8). Encapsulated intraerythrocytic gamonts 7.3 ±0.9 (6.1–9.8) μm long by 2.8 ±0.4 (2.0–3.6) μm wide (N=20). Nuclei of encapsulated intraerythrocytic gamonts 2.5 ±0.3 (2.3–3.2) μm long by 2.1 ±0.2 (1.8–2.5) μm wide (N=20). Extracellular gamonts with recurved tail, 15.2 ±1.0 (13.6–16.6) μm long by 2.1 ±0.2 (1.8–2.5) μm wide (N=15). Nuclei of extracellular gamonts with recurved tail 3.3 ±0.3 (2.7–4.1) μm long by 1.8 ±0.1 (1.6–2.0) μm wide (N=15). Extracellular gamonts with straightened tail 14.2 ±0.1 (14.1–14.3) μm long by 2.3 ±0.1 (2.3–2.5) μm wide (N=3). Nuclei of extracellular gamonts with straight tail 3.63 ±0.5 (3.2–4.1) μm long by 2.0 ±0.2 (1.8–2.3) μm wide (N=3).

Vector

Unknown.

DISCUSSION

Haemogregarina koppiensis has features that are unusual for fish haemogregarines, but more in common with those infecting amphibian and reptilian erythrocytes. According to Davies & Johnston (2000), encapsulated gamonts are recorded from only a small number of fish haemogregarines, such as *Desseria (Haemogregarina) londoni* (Yakimoff & Kohl-Yakimoff, 1912) Siddall, 1995 and *Desseria (Haemogregarina) lepidosirensis* (Jepps, 1927) Siddall, 1995. In comparison, many encapsulated haemogregarines infect toads, lizards and tortoises (see Davies & Johnston, 2000), more than 20 particularly fine examples having been described by Sambon & Seligmann (1907) from snake erythrocytes. Where the formation of such capsules has been examined by transmission electron microscopy, their creation seems closely connected with that of the inner membrane of the parasitophorous vacuole (see Davies & Johnston, 2000).

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Although several fish haemogregarines have recurved tails, to the authors' knowledge the only encapsulated type similar to that described here is *D. lepidosirensis* from the primitive freshwater South American lungfish, *Lepidosiren paradoxa*. Originally, two species of such haemogregarines were described from *L. paradoxa*, namely *Haemogregarina lepidosirensis* Jepps, 1927 and *Haemogregarina bertonii* Schouten, 1941. Siddall (1995) concluded that they are the same species and, because no merogony was reported, he synonymized them in the genus *Desseria*. In *D. lepidosirensis*, unlike *H. koppiensis*, the recurving tail is clearly visible in the intraerythrocytic stages and as many as four parasites infect a single host red cell (see Jepps, 1927). Furthermore, the gamont in Jepps' (1927) drawing has a body length of 13 μm and a tail of 6.21 μm (total length=19.21 μm), and is thus much larger than *H. koppiensis*. The two species also differ in the position of the gamont nucleus.

The presence of a deeply stained anterior cap in the present species places it in the 'rovignensis group' proposed by Laird (1952) for fish haemogregarines. This group includes species listed by Davies (1995) as *Cyrilia (Haemogregarina) uncinata* (Khan, 1978) Lainson, 1981, *Haemogregarina aeglefini* Henry, 1913, *Haemogregarina anarhichadis* Henry, 1912, *Haemogregarina coelorhynchi* Laird, 1952, *Haemogregarina rovignensis* Minchin & Woodcock, 1910, as well as *Haemogregarina johnstoni* Davies & Merrett, 2000 (see Davies & Merrett, 2000). The observation of mature meront division in some erythrocytes justifies the placement of *H. koppiensis* in the genus *Haemogregarina* (*sensu lato*) (see Siddall, 1995).

Recent observations in South Africa support the transmission of *H. bigemina* between its intertidal fish hosts by the blood-sucking stages of the isopod *Gnathia africana* Barnard, 1914 (see Smit & Davies, 1999; Davies & Smit, 2001). Although gnathiid larvae were found on evileye pufferfish, we have no evidence currently that they transmit *H. koppiensis*.

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