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Unusual haemogregarines parasitizing intertidal teleosts from the subtropical east coast of South Africa, with the description of *Haemogregarina kunegemina* sp. nov.

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Of three intertidal fish species collected on the east coast of South Africa, 67% (127/190) had haemogregarine infections. Horned rockskippers, Antennablennius bifilum *Günther, 1861, demonstrated 77% parasite prevalence, maned blennies,* Scartella emarginata *Günther, 1861, 53% prevalence, and a single specimen of the hotlips triplefin,* Helcogramma obtusirostre Klunzinger, *1871, was also parasitized. Less than 1% of* A. bifilum *and* S. emarginata *erythrocytes were infected, but ~2% of those of* H. obtusirostre. *Haemogregarines in* A. bifilum *and* S. emarginata *were morphologically similar to* H. bigemina *Laveran & Mesnil, 1901, but uncharacteristic clusters of four merozoites were observed in* S. emarginata *and paired gamonts were smaller overall than those of the type species, although close in size to* H. bigemina *reported elsewhere. Intraerythrocytic gamonts in* H. obtusirostre, *occurred mainly in fours, a characteristic of the European species originally named* H. quadrigemina *Brumpt & Lebailly, 1904. Additionally however, this South African species infrequently demonstrated eight intraerythrocytic gamonts and host cells commonly had spiny perimeters and were de-haemoglobulinized. Owing to the differences observed, this species is described as new to science and named* Haemogregarina kunegemina *sp. nov. Possible haemogregarine developmental stages were found in first and second stage pranizae of the gnathiid isopod,* Gnathia pilosus *Hadfield, Smit & Avenant-Oldewage, 2008, that had fed on the three fish hosts. These are the first reports of haemogregarines from teleosts of the subtropical east coast of South Africa.*

Keywords: haemogregarines, intertidal fish, South Africa, fish blood protozoans, gnathiid isopods, *Haemogregarina bigemina*, *Haemogregarina kunegemina*, *Gnathia pilosus*, Blenniidae, Triptrygiidae

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INTRODUCTION

Haemogregarines are apicomplexan protozoans and were first reported from the blood of fish by Laveran & Mesnil (1901) in northern France. Siddall (1995) classified fish haemogregarines in three genera, including *Cyrilia* Lainson, 1981 from freshwater fish, and *Desseria* Siddall, 1995 and *Haemogregarina* (*sensu lato*) Danilewsky, 1885 from freshwater and marine fish. This classification relied on haemogregarine development in fish and vector (leech) hosts although such complete development was known for only a few species (see Davies & Johnston, 2000). Members of the genera *Cyrilia* and *Haemogregarina* are partially characterized by intraerythrocytic division, whereas those of the genus *Desseria* lack this property (see Siddall, 1995).

Haemogregarina (*sensu lato*) *bigemina* Laveran & Mesnil, 1901 is a fish haemogregarine that appears in peripheral blood films as intraerythrocytic trophozoites, meronts undergoing binary fission, and paired gamonts, characteristic of the species (Davies *et al.*, 2004). Remarkably, it is an apparently cosmopolitan haemogregarine, recorded from the blood of at least 96 species of teleost fish, across 70 genera and 40 families (Davies *et al.*, 2004). On the south and west coasts of South Africa, *H. bigemina* is found in intertidal fish of the families, Blenniidae, Clinidae and Gobiesocidae (see Davies *et al.*, 2004; Hayes *et al.*, 2006). In addition, developmental stages of *H. bigemina* occur in haematophagous juveniles (pranizae) of the gnathiid isopod, *Gnathia africana* Barnard, 1914 from South Africa, indicating that the haemogregarine is likely transmitted by arthropods rather than leeches (Davies & Smit, 2001).

A further four fish haemogregarines are currently known from South Africa: *Haemogregarina* (*sensu lato*) *koppiensis* Smit & Davies, 2001 from the evil eye pufferfish, *Amblyrhynchotes honckenii* Bloch, 1795 (see Smit & Davies, 2001, 2005); *Desseria zei* Smit & Davies, 2006 from *Zeus capensis* Valenciennes, 1835 (see Smit & Davies, 2006); a *Desseria* sp. from flathead mullet, *Mugil cephalus* Linnaeus, 1758 (see Smit *et al.*, 2002); and *Haemogregarina curvata* Hayes, Smit, Seddon,

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Wertheim & Davies, 2006 from a variety of intertidal fish and the marine leech *Zeylanicobdella arugamensis* De Silva, 1963 (see Hayes *et al.*, 2006).

These latter four haemogregarines and *H. bigemina* are known only from the cold temperate west coast or the warm temperate south coast of South Africa. In these locations and, likely influencing the life cycles of marine animals, the cold Benguela Current (southern Atlantic Ocean) flowing up the west coast, mixes with the warm Agulhas Current (Indian Ocean) moving down the east coast, providing the relatively warm temperate conditions seen on the south coast (Branch *et al.*, 2002). However, the current paper reports for the first time haemogregarines in intertidal teleost fish from the subtropical east coast of South Africa, where the Agulhas Current provides warm water from the tropics and likely accompanying changes in the marine life (Branch *et al.*, 2002). Haemogregarines from this locality are compared with *H. bigemina* and the European fish haemogregarine originally named *Haemogregarina quadrigemina* Brumpt & Lebailly, 1904. Unusual developmental stages are observed in the fish, as well as remarkable changes to some host erythrocytes, and a new species of haemogregarine is named. Possible haemogregarine development is observed in pranizae of the gnathiid, *Gnathia pilosus* Hadfield, Smit & Avenant-Oldewage, 2008.

MATERIALS AND METHODS

Intertidal fish of three species in two teleost families were collected from rock pools in March, June and September 2006, February 2007 and October 2008 at Tinley Manor (29°27′S 31°17′E) and Sheffield Beach (29°29′S 31°15′E) on the east coast of South Africa, north of Durban (see Table 1). Fish were captured using hand-held nets or a baited hook-and-line, identified using Smith & Heemstra (1995), measured (total length (TL)), examined for haematophagous ectoparasites (see below), and kept in small 20 l aerated aquaria. Prior to further examination, fish were anaesthetized with clove oil (Griffiths, 2000; Chanseau *et al.*, 2002), and slides with thin blood films (one per fish) were prepared from caudal vein blood, taken using a 25 gauge needle. Films were air dried, fixed with methanol, stained with phosphate-buffered Giemsa solution, and screened with a 100× oil immersion objective on a Zeiss Axioplan 2 photomicroscope (Smit & Davies, 1999; Davies & Smit, 2001). Microscopic images of blood protozoans were captured and measured with Zeiss Axiovision 4.7; measurements were calculated as means ± standard deviation (range). Fish caught on the east coast were also hosts to the ectoparasitic blood-sucking juvenile (praniza) stages of *Gnathia pilosus* (see Hadfield *et al.*, 2008). Gnathiids were collected from fish with brushes or broad mouthed pipettes, drained of residual seawater on absorbent paper and each was crushed, smeared, fixed and stained for screening either immediately or at 1–30 days post-feeding (d.p.f.) on fish blood. Methods for preparing and screening gnathiids d.p.f. followed Davies & Smit (2001) and Hayes *et al.* (2006). Following examination, fish were released at the site of capture.

RESULTS

Infections with haemogregarines

Haemogregarines were found in two members of Blenniidae, the horned rockskipper *Antennablennius bifilum* Günther, 1861 and the maned blenny *Scartella emarginata* Günther, 1861, and a single member of the Triptrygiidae, the hotlips triplefin *Helcogramma obtusirostre* Klunzinger, 1871. Parasites were restricted to erythrocytes, with trophozoites, meronts, and gamonts present, but no intraleucocytic stages (see Laird, 1953). Prevalence of haemogregarines in host fish was 67% overall; *A. bifilum* showed 77% prevalence, *S. emarginata*, 53%, and the single *H. obtusirostre* was also parasitized (Table 1). Parasitaemias involved <1% of erythrocytes in *A. bifilum* and *S. emarginata*, and ~2% of erythrocytes in *H. obtusirostre* (Table 2). Overall, trophozoites and meronts occurred in <0.1% of erythrocytes, while gamonts occupied ~1% of erythrocytes (Table 2). Gnathiids (*Gnathia pilosus*) were the only haematophagous ectoparasites found on the fish, and their abundance varied among the three fish hosts (Table 3). Prevalence of gnathiids on fish was 31% overall; *A bifilum* showed 44% prevalence, *S. emarginata* 12% and the single *H. obtusirostre* was also parasitized (Table 3). Of the three stages of pranizae found on fish (P1, P2 and P3), P1 juveniles were the most prevalent (80%), with P2 and P3 stages contributing 18% and 2% respectively (Table 3).

Table 1. Fish identity, mean length ± SD and range (in mm), number infected with haemogregarina (prevalence %) and date of capture.

Fish			Fish infected by year/month (prevalence) with haemogregarines				
Families and species of teleosts	Size of fishes, TL ± SD (range) in mm	Number of fish infected (prevalence)	2006			2007	2008
			March	June	September	February	October
Blenniidae							
Antennablennius bifilum Günther, 1861	59 ± 9.3 (43–81.3)	82/106 (77%)	3/4 (75%)	6/6 (100%)	11/13 (85%)	20/35 (57%)	42/48 (88%)
Scartella emarginata Günther, 1861	63.9 ± 10.9 (44–110.4)	44/83 (53%)	3/12 (25%)	10/15 (67%)	9/19 (47%)	12/26 (46%)	10/11 (91%)
Triptrygiidae							
Helcogramma obtusirostre Klunzinger, 1871	39	1/1 (100%)	0/0	0/0	0/0	0/0	1/1 (100%)
Total		127/190 (67%)	6/16 (38%)	16/21 (76%)	20/32 (63%)	32/61 (53%)	53/60 (88%)

TL, total length; SD, standard deviation.

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Table 2. Fish species, parasitaemias with haemogregarines and percentages of haemogregarine life stages found in erythrocytes.

Fish	Parasitaemia (intensity of infection) expressed overall or by stages of haemogregarine in erythrocytes			
Families and species of teleosts	**Overall parasitaemia**	**Trophozoites**	**Meronts**	**Mature/immature gamonts**
Blenniidae				
Antennablennius bifilum Günther, 1861	<1%	<0.1%	<0.1%	>1% (mature/immature gamonts)
Scartella emarginata Günther, 1861	<1%	<0.1%	<0.1%	>0.1% (mature/immature gamonts)
Triptrygiidae				
Helcogramma obtusirostre Klunzinger, 1871	2%	<0.01 %	<0.01 %	>1% (mature gamonts)

SYSTEMATICS

Phylum APICOMPLEXA Levine, 1970
Class CONOIDASIDA Levine, 1988
Order EUCOCCIDIORIDA Léger & Duboscq, 1910
Suborder ADELEORINA Léger, 1911
Family HAEMOGREGARINIDAE Léger, 1911
Genus *Haemogregarina* Danilewsky, 1885
Infection cf *Haemogregarina bigemina* Laveran & Mesnil, 1901
(Figure 1A–E)

MATERIAL EXAMINED

Voucher material: in the collection of the South African Museum, Cape Town (*Antennablennius bifilum* blood film SAM A25102 with trophozoites, meronts, mature gamonts from Tinley Manor (29°27′S 31°17′E), collected by M.L. Ferreira, 22 March 2006. *Scartella emarginata* blood film SAM A25103 with trophozoites, meronts, mature gamonts from Tinley Manor, collected by M.L. Ferreira, 24 March 2006. *Antennablennius bifilum* blood film SAM A A25104 with trophozoites, meronts, mature gamonts from Sheffield Beach (29°29′S 31°15′E), collected by M.L. Ferreira, 14 February 2007. *Scartella emarginata* blood film SAM A25105 with trophozoites, meronts, mature gamonts from Sheffield Beach, collected by M.L. Ferreira, 15 February 2007). Other material: in the collection of N.J. Smit (80 *A. bifilum* blood films; 42 *S. emarginata* blood films).

DESCRIPTION

Trophozoites. In both fish species (*Antennablennius bifilum* and *Scartella emarginata*) intraerythrocytic trophozoites occurring singly in erythrocytes; elongate, with broad anterior and narrower, roundly pointed, posterior pole (Figure 1A). From *A. bifilum* 5.5 ± 0.6 (4.5–6.3) by 2.1 ± 0.4 (1.3–2.8) μm (N = 20), from *S. emarginata* 5.0 ± 0.9 (3.2–6.8) by 1.9 ± 0.5 (1.1–3.0) μm (N = 20). Cytoplasm stained light blue. Nucleus situated predominantly centrally within the trophozoite body, stained purple, with chromatin loosely arranged. Nuclei 2.7 ± 0.6 (1.7–4.1) by 1.6 ± 0.4 (1.1–2.6) μm (N = 20) in *A. bifilum*, 2.4 ± 0.4 (1.8–3.3) by 1.4 ± 0.4 (0.7–2.4) μm (N = 20) in *S. emarginata.*

Meronts. Meronts mostly singly in erythrocytes in both fish species, occasionally in pairs, elongate, one pole slightly broader than the other (Figure 1B). From *A. bifilum* 6.1 ± 0.8 (5.2–8.0) by 2.5 ± 0.4 (1.8–3.6) μm (N = 20), from *S. emarginata* 5.7 ± 0.9 (3.3–7.1) by 2.6 ± 0.6 (1.3–4.0) μm (N = 20). Cytoplasm stained pale blue, without granules. Nuclei stained purple, often with stranded chromatin, 2.9 ± 0.4 (1.9–3.6) by 1.7 ± 0.4 (1.0–2.5) μm (N = 20) from *A. bifilum*, 2.5 ± 0.6 (1.5–3.8) by 1.8 ± 0.6 (0.7–2.7) μm (N = 20) from *S. emarginata.* Meronts apparently producing two merozoites in *A. bifilum*, but two to four in *S. emarginata* (Figure 1B). Individual merozoites 3.6 ± 0.8 (2.1–4.6) by 2.3 ± 0.5 (1.9–3.0) μm (N = 10), with nuclei, each 2.4 ± 0.6 (2.0–3.8) by 1.0 ± 0.4 (0.8–2.1) μm (N = 10).

Gamonts. Gamonts in pairs in erythrocytes (Figure 1C, D) in both fish species, with free gamonts resembling those in erythrocytes (Figure 1E); slightly broader anterior than posterior poles, anterior generally rounded, occasionally pointed; posterior straight or re-curved and always narrow; in *A. bifilum* 8.0 ± 0.5 (6.7–8.9) by 1.3 ± 0.2 (0.8–1.8) μm (N = 50) in *S. emarginata* 9.0 ± 1.0 (5.4–10.2) by 1.4 ± 0.3 (0.7–2.3) μm (N = 50). Cytoplasm stained pale blue or pinkish, granule at posterior extremity for some gamonts from *S. emarginata.* Nuclei with loosely arranged chromatin, situated approximately halfway or more posterior in parasite body, 2.7 ± 0.4 (1.9-3.6) by 1.0 ± 0.2 (0.6–1.3) μm (N = 50) from *A. bifilum*, and 2.6 ± 0.6 (1.6–5.0) by 1.0 ± 0.2 (0.6–1.4) μm (N = 50) from *S. emarginata.* Tight-fitting parasitophorous vacuole visible in some instances (Figure 1A).

REMARKS

In *Antennablenius bifilum* and *Scartella emarginata* blood films, mature gamonts appeared paired within erythrocytes,

Table 3. Classification of *Gnathia pilosus* pranizae from fish by stage collected from March 2006 to October 2008. P1, Praniza 1; P2, Praniza 2; P3, Praniza 3.

Families and species of teleosts	Number of fish infected (prevalence)	P1	P2	P3	Total
Blenniidae					
Antennablennius bifilum Günther, 1861	47/106 (44%)	92	14	0	106
Scartella emarginata Günther, 1861	10/83 (12%)	30	16	3	49
Triptrygiidae					
Helcogramma obtusirostre Klunzinger, 1871	1/1 (100%)	8	0	0	8
Total	58/190 (31%)	130/163 (80%)	30/163 (18%)	3/163 (2%)	163

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resembling those of *H. bigemina* (see Laveran & Mesnil, 1901; Davies *et al.*, 2004). Trophozoites from *A. bifilum* and *S. emarginata* were close to each other in size, while meronts from *A. bifilum* were slightly larger than in *S. emarginata*. Importantly, meronts from *S. marginata* produced four as well as two merozoites, whereas those from *A. bifilum* apparently gave rise to only two. This former characteristic is not typical of *H. bigemina* from its type hosts (see Davies *et al.*, 2004) and is not reported from other South African infections of this haemogregarine (Smit & Davies, 1999; Davies & Smit, 2001; Hayes *et al.*, 2006). Furthermore, there was no evidence of mixed *H. bigemina*-like and *H. kunegemina* sp. nov. infections in the current study to account for the extra merozoites seen in *S. emarginata* (see below). However, division of an *H. bigemina*-like organism in Australian fish also produces occasionally up to 4 individuals in a single erythrocyte (Smit *et al.*, 2006), highlighting apparent variations in the development of this haemogregarine across continents.

Gamonts in *A. bifilum* (6.7–8.9 by 0.8–1.8 μm) were overall smaller than in *S. emarginata*, (5.4–10.2 by 0.7–2.3 μm). The latter may have represented more mature stages, because of their increased length and width, a granule at the posterior pole, and a visible parasitophorous vacuole in some instances; thus their appearance was similar to that of mature *H. bigemina* gamonts in a variety of New Zealand intertidal fish (Laird, 1953), although gamonts from New Zealand material were somewhat longer (9.1–14.9 by 1.0–2.2 μm) than those from *S. emarginata*. The type material for *H. bigemina* described by Laveran & Mesnil (1901) in intertidal blennies *Lipophrys pholis* (Linnaeus, 1758) and *Coryphoblennius galerita* (Linnaeus, 1758) from St Martin's cove, near Cape de la Hague, France also demonstrated

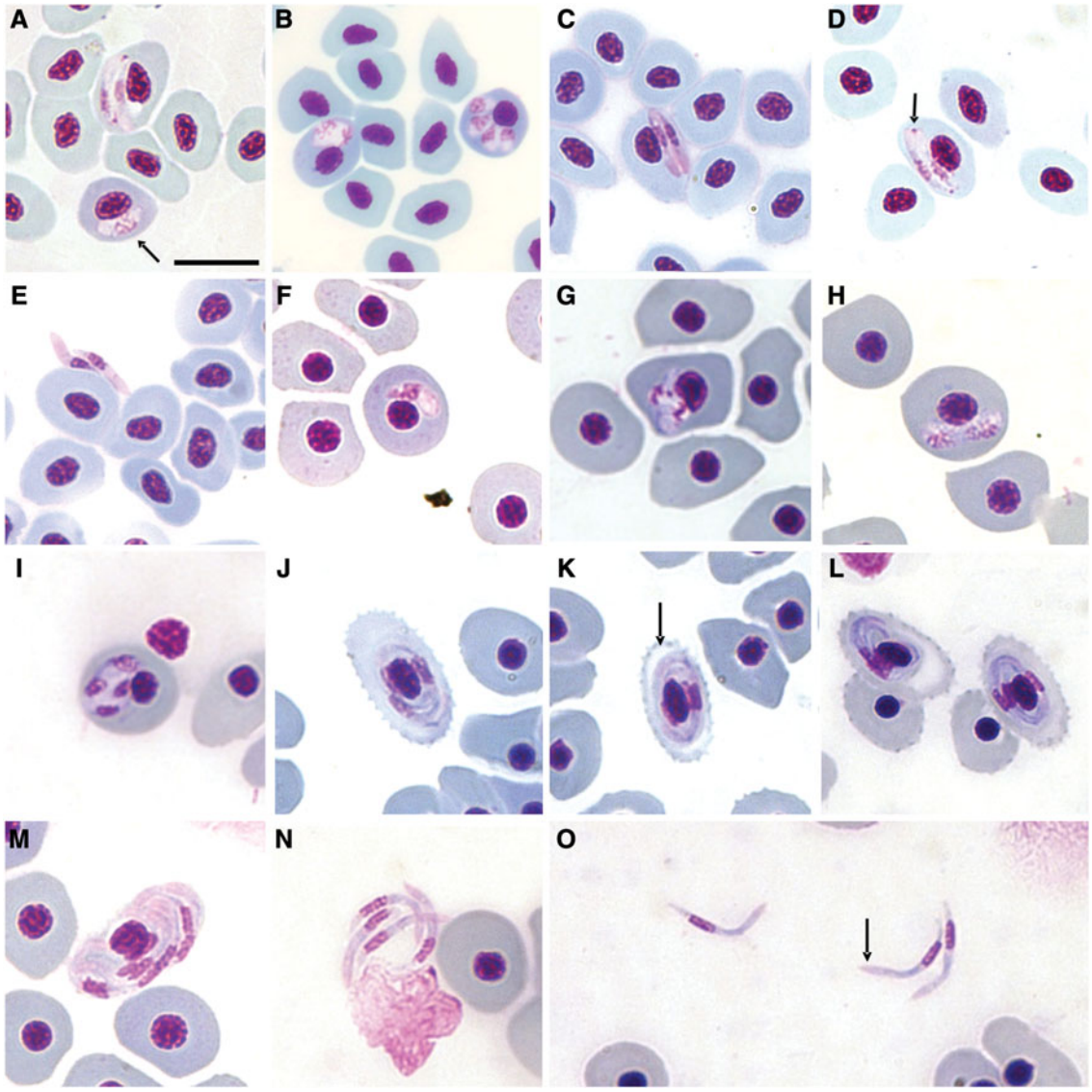

Fig. 1. Micrographs of Giemsa stained blood films from *Antennablennius bifilum* Günther, 1861 and *Scartella emarginata* Günther, 1861 showing the *Haemogregarina bigemina*-like organism (A–E), and of *Helcogramma obtusirostre* Klunzinger, 1871 illustrating the *Haemogregarina kunegemina* sp. nov. (F–O). (A.) Trophozoite (arrowed) and paired, mature gamonts in host erythrocyte above within parasitophorous vacuole (visible just below host nucleus); (B) meronts producing two or four individuals (the latter seen only in *S. emarginata*); (C, D) paired matured intracellular gamonts (arrow indicating granule at posterior extremity); (E) Extracellular gamonts; (F) trophozoite; (G–I) meronts apparently producing two or four merozoites; (J, K, L) four mature gamonts within host erythrocytes demonstrating spiked periphery and de-haemoglobulinization (arrow in K); (M) eight gamonts in erythrocyte; (N, O) extracellular gamonts (arrow indicating pinkish cap). Scale bar: A–O = 10 μm.

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longer gamonts (12 by 1.5–2 μm) than those in *S. emarginata* and division produced only two individuals.

Smit & Davies (1999) reported *H. bigemina* from *Clinus superciliosus* (Linnaeus, 1758) and *Clinus cottoides* Valenciennes, 1836 from Jeffreys Bay on the south coast of South Africa, and gamonts from *A. bifilum* and *S. emarginata* from the east coast are close in size to those from both *C. superciliosus* (9.0–13.2 by 1.2–2.2 μm) and *C. cottoides* (9.8–12.3 by 1.6–2.5 μm). Similar *H. bigemina* infections were later documented from De Hoop Nature Reserve, also on the south coast, infecting *Blennioclinus brachycephalus* (Valenciennes, 1836), *Chorisochismus dentex* (Pallas, 1769), *C. superciliosus*, *C. cottoides*, and *Parablennius cornutus* Linnaeus 1758 (see Davies & Smit, 2001; Hayes *et al.*, 2006) and from *Clinus agilis* Smith, 1931 at Mouille Point on the west coast of South Africa (Hayes *et al.*, 2006). In the present study, it is concluded that the haemogregarine found in *A. bifilum* and *S. emarginata*, despite its unusual development in the latter fish species, is also likely *H. bigemina*, making this the first record of this haemogregarine in these fish hosts and the first report of it from the east coast of South Africa.

Haemogregarina kunegemina sp. nov.
(Figure 1F–O)

TYPE MATERIAL

Hapantotype: in the collection of the South African Museum, Cape Town (*Helcogramma obtusirostre* blood film SAM25106 with trophozoites, meronts, mature gamonts from Sheffield Beach (29°29′S 31°15″E), collected by M.L. Ferreira, 27 October 2008).

DIAGNOSIS

Gamonts intraerythrocytic, encircling host nucleus. These stages predominantly in fours, with vacuoles near pinkish cap and occasionally, granules posteriorly. Haemogregarine-infected erythrocytes elongated when gamonts present compared with uninfected cells. Host erythrocytes usually with a spiny perimeter, and others de-haemoglobulinized.

DESCRIPTION

Trophozoites. Intraerythrocytic trophozoite stage observed only once (Figure 1F); elongate, with broad anterior, and narrower pointed posterior, 6.9 × 1.8 μm (N = 1). Cytoplasm stained light blue. Nucleus situated towards the posterior pole, stained purple, 2.3 × 1.6 μm (N = 1).

Meronts. Meronts of irregular shape (Figure 1G–I) occurring singly within erythrocytes, 8.5 ± 1.5 (8.4–10.1) by 8.4 ± 1.9 (7.0–17.2) μm (N = 3). Cytoplasm stained pale blue and chromatin of developing merozoite nuclei, deep purple. Meronts apparently producing two and four merozoite nuclei (Figure 1H, I). Individual merozoites 3.5 ± 0.8 (2.6–4.6) by 2.0 ± 0.4 (1.3–2.4) μm (N = 10), with nuclei, each 2.6 ± 0.6 (2.0–3.3) by 1.6 ± 0.3 (1.3–1.9) μm (N = 10).

Intracellular gamonts. Four gamonts normally in erythrocytes (Figure 1J–L), exceptionally eight (Figure 1M). These stages difficult to measure, owing to overlapping and encircling of host nucleus, but ~12.4 × 0.7 μm. Cytoplasm stained blue. Gamont nucleus elongate, narrow, purple stained, 3.7 ± 0.3 (3.3–4.1) by 1.0 ± 0.2 (0.8–1.3) μm (N = 7) (Figure 1M). Little or no parasitophorus vacuole evident.

Extracellular gamonts. Slender forms, slightly broader anteriorly, curved or bent at ~120°, occurring singly, in pairs, or fours; individuals (Figure 1N, O) 12.9 ± 0.7 (11.5–14.1) by 1.2 ± 0.2 (0.9–1.5) μm (N = 20). Cytoplasm stained bluish, sometimes with vacuoles near pinkish cap (Figure 1O, arrowed), occasionally a few granules posteriorly. Nucleus in probable posterior half of parasite body; nucleus elongate, narrow, stained purple, 3.2 ± 0.3 (2.5–3.9) μm by 1.1 ± 0.1 (0.9–1.4) μm (N = 20) (Figure 1N, O).

Haemogregarine infected erythrocytes were elongated when gamonts were present compared with uninfected cells. Many of these erythrocytes had spiny perimeters (Figure 1J–L), while others were de-haemoglobulinized (Figure 1K (arrowed), L). Infected cells measured 15.4 ± 2.5 (13.2–26.0) μm in length and 8.4 ± 1.9 (7.0–17.2) μm (N = 26) in width, whereas uninfected cells were 11.7 ± 0.9 (10.2–13.3) μm by 8.2 ± 1.2 (6.0–11.1) μm (N = 25).

ETYMOLOGY

This parasite broadly resembles *Haemogregarina quadrigemina* Brumpt & Lebailly, 1904 (see below), thus the new species epithet is derived from the Zulu word *kune* which means 'four', referring to the four gamonts present in the erythrocytes, replacing the *quadri* of *quadrigemina*.

REMARKS

Haemogregarina kunegemina sp. nov. observed in *Helcogramma obtusirostre* broadly resembles *Haemogregarina quadrigemina*, as it was described originally from its type host *Callionymus lyra* Linnaeus, 1758 at Luc-sur-Mer, France (Brumpt & Lebailly, 1904). The organism from *C. lyra*, like *H. kunegemina*, normally produces four comma-shaped, individuals in each parasitized erythrocyte. In *H. quadrigemina*, however, these stages are larger (17 by 1.8 μm, compared with ~12.9 by 1.2 μm) and arranged in a barrel-like formation. On the other hand, and unlike *H. quadrigemina*, *H. kunegemina* gamonts have vacuoles near an anterior cap, and parasitized erythrocytes commonly have spiny perimeters, and some show de-haemoglobinization. Siddall (1995) regarded *H. quadrigemina* as a junior synonym of *Haemogregarina callionymi* Brumpt & Lebailly, 1904, a shorter, stouter haemogregarine (12 by 2.5 μm), believing the former to be merogonic stages of the latter, since both were reported from *C. lyra*. However, not only would it be unusual for haemogregarine merozoites (*H. quadrigemina*) to exceed gamonts (*H. callionymi*) in length, but also, if the life cycle of *H. quadrigemina* (currently unknown) proves similar to that of *H. bigemina*, then the slender forms resulting from division in erythrocytes would be gamonts, not merozoites (Davies & Smit, 2001). The relationship between the two haemogregarines from *C. lyra* (if any) is obviously complex and requires further study but, for the purposes of this paper, the name *H. quadrigemina* is retained.

Another haemogregarine, *Haemogregarina clavata* Neuman, 1909, found originally in *Buglossidium luteum* (Risso, 1810), at Naples, Italy (Neumann, 1909) is also characterized by having four mature gamonts in host erythrocytes. However, these measure 32 μm long by 2.5 μm (Neumann, 1909), greatly exceeding the size of *H. kunegemina* gamonts. Furthermore, this species was synonymized by Siddall (1995)

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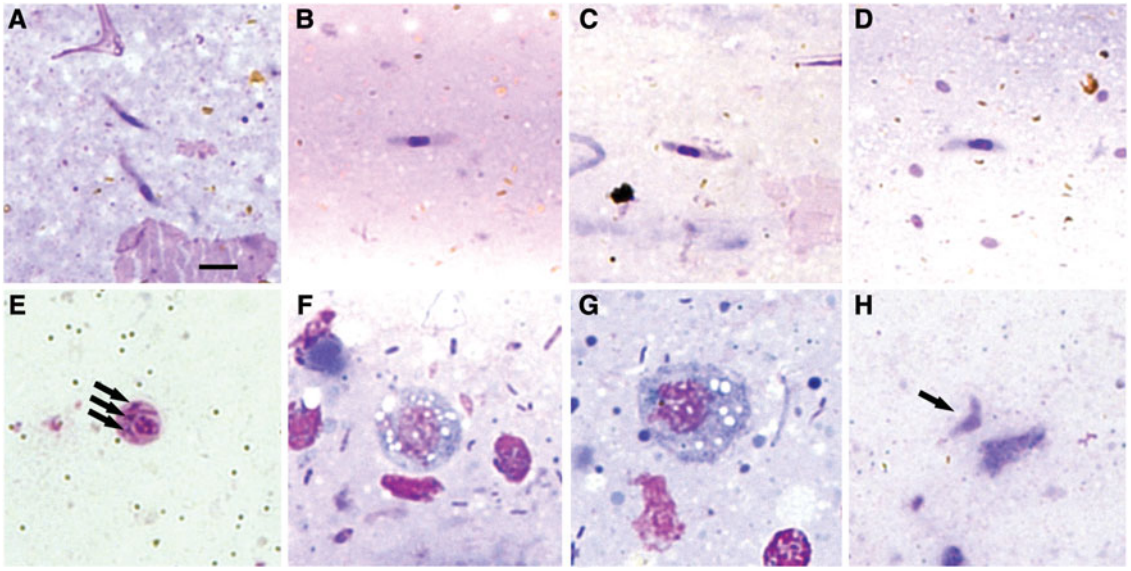

Fig. 2. Micrographs of Giemsa stained smears from *Gnathia pilosus* that fed on *Antennablennius bifilum*, *Scartella emarginata* and *Helcogramma obtusirostre*. (A) Gamonts freed from erythrocytes; (B–D) free gamonts; (E) syzygy, showing macrogamont (right) encircled by microgamont with microgamont nuclei (arrows); (F, G) oocysts; (H) merozoite-like stage (arrow). Scale bar = 5 μm.

with *Haemogregarina simondi* Laveran & Mesnil, 1901, which produces eight gamonts in each parasitized erythrocyte (Laveran & Mesnil, 1901).

Haemogregarina kunegemina was found in only one *H. obtusirostre* on the east coast and the significance of the eight gamonts observed in one erythrocyte is not understood currently, although it may represent atypical development. Clearly, *H. kunegemina* most closely resembles *H. quadrigemina* in appearance, but we consider the differences in size and remarkable effects on the host cells, to be sufficient to regard the former as a species new to science.

Haemogregarines in *Gnathia pilosus* pranizae (Figure 2A–H)

MATERIAL EXAMINED

Voucher material: in the collection of the South African Museum, Cape Town (squash preparation of *Gnathia pilosus* praniza SAM A25107, with free gamonts, oocysts, merozoite-like stages from Tinley Manor (29°27′S 31°17′E), collected by M.L. Ferreira, 10 October 2008). Other material: in the collection of N.J. Smit (12 squash preparations of *Gnathia pilosus* pranizae).

DESCRIPTION

Free haemogregarine gamonts (Figure 2A–D) were observed up to 20 days post-feeding (d.p.f.) in squashes of P1 and P2 stages that had fed on *A. bifilum* and *H. obtusirostre*, but none was found in pranizae from *S. emarginata*. These likely immature gamonts measured 6.2 ± 1.5 (0.7– 8.5) by 1.0 ± 0.2 (0.7–1.6) μm (N = 22). Suspected pairing of gamonts (syzygy) (Figure 2E) was recorded up to 15 d.p.f. At this stage, the microgamont wrapped itself around the macrogamont, with the microgamont nucleus replaced by individual microgamete nuclei (see Davies & Smit, 2001). Oocysts (Figure 2F, G) were observed in pranizae from *S. emarginata* from 3 d.p.f., and were rounded, pale blue stained, with a vacuolated cytoplasm, and had a centrally placed nucleus staining deep magenta. These stages measured 9.7 ± 0.6 (9.1–10.7) by 9.6 ± 0.9 (8.3–11.3) μm in diameter (N = 7). No sporozoites or meronts were observed. However, merozoite-like stages were found in pranizae that fed on all three hosts (*A. bifilum*, *S. emarginata* and *H. obtusirostre*), 26 d.p.f. Two merozoites (Figure 2H) were 4.6 by 1.5 μm, and 5.0 by 1.7 μm; the remainder were too poorly preserved to measure. Many smears contained a bacterial flora, presumed to comprise the symbionts needed to help digest fish blood in these pranizae (Davies, 1995).

REMARKS

The association of haemogregarines, intertidal fish and gnathiid isopods was examined by Davies & Smit (2001) and *Gnathia africana* was considered the likely vector of *H. bigemina* in South Africa. *Antennablennius bifilum*, *S. marginata* and *H. obtusirostre* caught during the current survey were hosts to praniza stages of *G. pilosus*. It was also evident that haemogregarine stages, particularly gamonts, were present in some of these pranizae and immature oocysts found in in *G. pilosus* were similar to those in *G. africana*, which measured 8.3–10.8 μm across (Davies & Smit, 2001). Suspected merozoites were however smaller than first, second and third generation merozoites found in *G. africana* (see Davies & Smit, 2001), but these results suggest, yet again, that gnathiids may act as vectors for fish haemogregarines. However, the current data were insufficient to firstly, identify the species of haemogregarine(s) accurately in the gnathiid, and secondly, to propose a life cycle for the apicomplexans.

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