A redescription of *Haemogregarina fitzsimonsi* Dias, 1953 and some comments on *Haemogregarina parvula* Dias, 1953 (Adeleorina: Haemogregarinidae) from southern African tortoises (Cryptodira: Testudinidae), with new host data and distribution records

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Abstract: Blood films were examined from 154 wild and captive tortoises from four provinces of South Africa, including Gauteng, Kwazulu-Natal, North West and Western Cape. The five species of chelonians studied were *Chersina angulata* (Schweigger), *Kinixys belliana belliana* (Gray), *K. lobatsiana* Power, *K. natalensis* Hewitt, and *Stigmochelys pardalis* (Bell). Two species of haemogregarines, previously reported from Mozambique, were identified in blood films, namely *Haemogregarina fitzsimonsi* Dias, 1953 and *Haemogregarina parvula* Dias, 1953. Additional stages of development (trophozoites and probable meronts, merozoites and immature gamonts) in blood preparations from South Africa warranted the redescription of *H. fitzsimonsi*. A variety of hosts and broad host distribution range were observed for this haemogregarine, with all five species of tortoises parasitized, wild and captive, from all four provinces, in all seasons. In contrast, only two individuals of *K. b. belliana* and one *S. pardalis*, all three captive in Kwazulu-Natal, contained *H. parvula* with encapsulated stages resembling those of *Hemolivia mauritanica* (Sergeant et Sergeant, 1904). For *H. fitzsimonsi*, parasite prevalences, but not parasitaemias, were significantly higher in captive than wild *S. pardalis*; captive female *S. pardalis* also showed a significantly greater prevalence of infection than males, but younger, lighter hosts were not significantly more heavily parasitized than older, heavier individuals. The ticks, *Amblyomma marmoreum* Koch, 1844 and *A. sylvaticum* (De Geer, 1778), found attached to some tortoises, may prove to be definitive hosts for the two species of haemogregarines observed.

Key words: blood parasites, haemogregarines, *Haemogregarina fitzsimonsi*, *Haemogregarina parvula*, tortoises, *Chersina angulata*, *Kinixys* spp., *Stigmochelys pardalis*, Africa

Few records exist of haematozoans infecting African land tortoises (Chelonina, Cryptodira, Testudinidae) and these were published mainly during the early part of the last century (see Lainson and Naiff 1998). Examples of early descriptions were of: *Haemogregarina mauritanica* Sergeant et Sergeant, 1904 in *Testudo graeca* (L.) (syn. *Testudo mauritanica*) from Algeria (Sergeant and Sergeant 1904); *Haemamoeba testudinis* Laveran, 1905 in *Stigmochelys pardalis* (Bell) (syns. *Testudo pardalis*, *Geochelone pardalis*) from southern Africa (Laveran 1905); *Plasmodium roumei* Bouet, 1909 in *Kinixys belliana belliana* (Gray) (syns. *Testudo belliana*, *Cinixys belliana*) from the Ivory Coast (Bouet 1909); a *Haemocystidium* sp. from west African tortoises *K. b. belliana*, *Kinixys erosa* (Schweigger) (syns. *Testudo erosa*, *C. erosa*) and *Kinixys homeana* (Bell) (syn. *Cinixys homeana*), all housed at the Zoological Gardens in London (Plimmer 1912); and *Haemogregarina bruneti* Commes, 1919 and a trypanosome in *K. homeana* from Mali (Commes 1919). Some

of these protozoans have since been ascribed to different genera with *Haemamoeba* and *Haemocystidium* spp. becoming *Haemoproteus* spp., and *H. mauritanica* being transferred to the genus *Hemolivia* Petit, Landau, Baccam et Lainson, 1990 (see Lainson and Naiff 1998, Široký et al. 2007). The most recent research on blood protozoans from land tortoises in Africa (Dias 1953), reported two species of haemogregarines (Apicomplexa, Adeleorina, Haemogregarinidae) and one haemoproteid (Apicomplexa, Haemospororida, Haemoproteidae) from two individuals of *K. b. belliana* (syn. *Kinixys belliana zuluensis*) from the coastal town of Maputo, Mozambique. For the haemogregarines, Dias (1953) recorded intraerythrocytic gamont-like stages of *Haemogregarina fitzsimonsi* Dias, 1953 and immature and mature forms of *Haemogregarina parvula* Dias, 1953 within intraerythrocytic capsules.

In the current study, five species of tortoises were examined, both wild and captive, from four provinces within South Africa, across all seasons. The two haemogregarine

species of Dias (1953) were observed, together with previously unrecorded stages of *H. fitzsimonsi*, necessitating a redescription of this species. Distribution and host records were expanded for both *H. fitzsimonsi* and *H. parvula*. Prevalences and/or parasitaemias with *H. fitzsimonsi* were compared for captive and wild *S. pardalis*, female and male tortoises, and for younger and older animals. Tortoise ectoparasites that might serve as definitive hosts for the two haemogregarine species were identified and finally, doubts were cast on the current classification of the two apicomplexans as members of the genus *Haemogregarina* (sensu stricto) Danilewsky, 1885.

MATERIALS AND METHODS

Sample collection. Tortoises ($n = 154$) of five species, the angulate tortoise *Chersina angulata* (Schweigger), Bell's hinged tortoise *Kinixys belliana belliana*, the Lobatse hinged tortoise *K. lobatsiana* Power, the Natal hinged tortoise *K. natalensis* Hewitt, and the leopard tortoise *Stigmochelys pardalis* were examined from the following provinces of the Republic of South Africa: Gauteng (G); Kwazulu-Natal (KZN); North West Province (NWP); and Western Cape (WC) (Table 1). These provinces represented a diverse range of habitats, from the savannah of G, tropical/subtropical coastal habitats of KZN, semi-arid conditions of NWP, to the arid-fynbos of WC. Vetter (2002) was used to name authorities for tortoises examined.

Wild tortoises ($n = 84$) were examined from Rustenburg ($25^{\circ}42'S$, $27^{\circ}5'E$) and from rural markets, both in NWP, and at Hondeklip Bay ($30^{\circ}19'S$, $17^{\circ}16'E$) and Velddrif ($32^{\circ}54'S$, $18^{\circ}46'E$) in WC (Fig. 1). Chelonians (except from rural markets) were captured on foot from October to February (the South African spring and summer) of 2006 and 2007, using a hooked stick to displace dense vegetation, and were examined once, in the field. Since wild tortoises tended to hibernate deep in the holes of larger animals from April to September (autumn and winter), field sampling was suspended during this period. Additional wild tortoises held in rural markets, were each examined once during spring, summer, autumn, and winter between March 2006 and December 2007. Long-term captive tortoises ($n = 70$) were sampled at the National Zoological Gardens (NZG), Pretoria ($25^{\circ}44'S$, $28^{\circ}11'E$), in G, and at Crocodile Creek, Crocodile Valley, and Flag Animal Farm, all three at Ballito ($29^{\circ}32'S$, $30^{\circ}33'E$), in KZN (Fig. 1). Captive tortoises were housed in similar small huts with outdoor pens in which they could be caught easily. These individuals, except for those from the NZG (sampled once during spring, November 2007), were each examined twice between June 2006 and November 2007. The exact origins of rural market (classified as wild, above) and captive tortoises were unknown; however, these animals were understood to have been taken locally (for rural markets), or have been surrendered or confiscated by Nature Conservation and placed in regional zoological facilities (captive animals).

Blood (~ 0.1 – 0.5 ml) was taken by needle and syringe from veins and arteries of the fore and hind limbs, jugular veins, or the coccygeal vein of the tail of wild and captive tortoises (see McArthur et al. 2004). Effects of seasonality on prevalence of infection, and parasitaemia, were recorded for wild specimens, with these animals being examined only once (see above). Ex-

Fig. 1. Locations in South Africa at which tortoises were examined for haematozoans. (a) Rustenburg, including rural markets in NWP, where wild *Kinixys lobatsiana* and *Stigmochelys pardalis* were examined. (b) Hondeklip Bay and (c) Velddrif in WC, where wild *Chersina angulata* were examined. (d) NZG in G, where captive *K. lobatsiana* and *S. pardalis* were examined and (e) Ballito, including Crocodile Creek, Crocodile Valley and Flag Animal Farm, in KZN, where captive *C. angulata*, *Kinixys belliana belliana*, *K. natalensis* and *S. pardalis* were examined.

cept for those at the NZG, captive tortoises of all five species were marked with nail varnish on their shells following removal of the first blood sample to allow future identification, so that the effects of seasonality and levels of parasitaemia could be assessed over time. Each *S. pardalis* in the NZG's collection was sexed and then weighed (kg) using a set of industrial scales because of the substantial size of some tortoises; these approaches allowed assessment of prevalence and parasitaemia in relation to host gender and size, following blood sampling.

Blood films. Thin blood films from all tortoises were fixed in absolute methanol for ~ 10 minutes, Giemsa-stained (FLUKA, Sigma-Aldrich) for 20 minutes, and then screened initially using a $\times 100$ oil immersion objective on an Olympus BX41 light microscope. Subsequently, images of blood parasites were captured using a $\times 100$ oil immersion objective on a Carl Zeiss Axiocam digital camera attached to a Zeiss Axioplan 2 photomicroscope, measurements (μm) were taken using AxioVision Release 4.3 (11-2004) software, calibrated to a stage micrometer, and the parasite species infecting the wild and captive host tortoises were recorded (Table 1). Parasitaemias were calculated as infection levels per 100 red blood cells, with $\sim 10^4$ erythrocytes examined per blood film.

Ectoparasites. Prior to tortoise release, either to the wild at the site of capture, or back into captivity, ectoparasites that might be definitive hosts for tortoise blood parasites were collected from the chelonians and fixed in 10% neutral buffered formalin before identification in the laboratory.

When appropriate, the validity of results was tested statistically using Student's *t*-test, and the parasitaemia/weight correlations tested using Conoco for Windows 4.5.

RESULTS

General observations on haemogregarines within the blood of tortoises

Of the South African tortoises ($n = 154$: 84 wild; 70 captive) screened for blood parasites, 25% (38/154) overall were parasitized by the two haemogregarine species, *H. fitzsimonsi* (Table 1, Figs. 2–14) and *H. parvula* (Table 1, Figs. 15–17). Haemogregarine prevalence varied with host status (wild or captive) and distribution (Table 1). Only captive *C. angulata* lacked haemogregarines and thus, among wild or captive tortoises, *H. fitzsimonsi* was found in the five species of hosts (*C. angulata*, *K. b. belliana*, *K. lobatsiana*, *K. natalensis* and *S. pardalis*) from four provinces (G, KZN, NWP, WC), at prevalences from 5% to 66%; *H. parvula* was detected only in captive hosts (*K. b. belliana* and *S. pardalis*) from KZN and at prevalences up to 14%. In addition, *H. fitzsimonsi* occurred in wild *C. angulata*, *K. lobatsiana* and *S. pardalis* at parasitaemias between ~1.8% and ~2.5%, and in captive *K. b. belliana*, *K. lobatsiana*, *K. natalensis* and *S. pardalis* at levels between ~2.3% and ~5.1%. Parasitaemias of *H. parvula* in captive *K. b. belliana* and *S. pardalis* were between ~2.5% and ~2.6%.

When prevalence of *H. fitzsimonsi* in wild and captive tortoises was examined over the seasons (Table 1), no effect of seasonality was indicated. Younger stages of *H. fitzsimonsi*, that is, trophozoites, probable meronts and merozoites (Figs. 2–5) were found during spring and summer in three captive *S. pardalis* from G, and in two wild *C. angulata* from WC. The tortoises with *H. parvula* were located in winter and spring in KZN, and only one individual, an *S. pardalis*, had both *H. fitzsimonsi* and *H. parvula*.

A significantly higher prevalence of 35% (13/37) of infection with *H. fitzsimonsi* occurred in captive *S. par-*

dalisis at the NZG, compared with that of 5% (2/41) in the wild-caught specimens of this tortoise ($p = 0.0002$). However, no significant difference ($p = 0.04$) in parasitaemia occurred between captive (~5.1%) and wild-caught specimens of this tortoise (~2.5%). Female *S. pardalis* from the NZG showed a significantly greater prevalence of infection (43.5%, 10/23) than males (21.4%, 3/14) ($p = 0.002$), and a negative correlation ($r^2 = <1.00$) between increasing weight (kg) of *S. pardalis* and parasitaemia with *H. fitzsimonsi*, detected at the NZG, was not significant statistically ($p = 0.095$).

Difficulty was experienced in determining numbers of tortoises, wild and captive, infested with ticks, and numbers of ticks per tortoise, as these arthropods tended to reside in deep host skin folds and were not readily detected. However, a few specimens collected from *K. b. belliana*, *K. lobatsiana* and *S. pardalis*, were identified as the common African tortoise tick, *Amblyomma marmoreum* Koch, 1844 while the tick species parasitizing *C. angulata* was *Amblyomma sylvaticum* (De Geer, 1778) (see Fielden and Rechav 1994). Thus, 7 specimens of *A. marmoreum* were removed from a wild *S. pardalis* and a wild *K. lobatsiana*, both from NWP, and from a captive *K. b. belliana* from KZN, while 7 specimens of *A. sylvaticum* were taken from a wild *C. angulata* from WC.

Haemogregarina (sensu lato) *fitzsimonsi* Dias, 1953 Developmental stages within the blood of tortoises

Trophozoites: occurring singly within immature erythrocytes (Fig. 2), $3.7 \pm 0.4 \mu\text{m}$ (3–4.3 μm) long by $1.0 \pm 0.1 \mu\text{m}$ (0.8–1.1 μm) wide ($n = 10$), curved with rounded or bluntly pointed ends, finely vacuolated cytoplasm stained whitish-blue, nucleus $1.7 \pm 0.1 \mu\text{m}$ (1.5–1.8) by $0.9 \pm 0.2 \mu\text{m}$ (0.6–1.2 μm) ($n = 10$) centrally placed, or closer to one pole than the other, loosely arranged chromatin, stained pink or purple.

Table 1. Host species, host status (W = wild, C = captive), and seasons (Sp = spring, Su = summer, A = autumn, W = winter) in which tortoises were examined. Location of captured individuals, including Gauteng (G), Kwazulu-Natal (KZN), North West Province (NWP) and Western Cape (WC), parasite prevalence (%) with *H. fitzsimonsi* (*H. f*) and *H. parvula* (*H. p*) and seasons in which parasitized tortoises were found. Total parasite prevalence for individual and all provinces.

Host tortoises	Status (n)/Seasons tortoises were examined	Hosts parasitized/ Province (Prevalence, %)/ Seasons in which infections were recorded								Hosts parasitized All provinces (Prevalence, %)	
		G		KZN		NWP		WC			
		<i>H. f</i>	<i>H. p</i>	<i>H. f</i>	<i>H. p</i>	<i>H. f</i>	<i>H. p</i>	<i>H. f</i>	<i>H. p</i>		
<i>Chersina angulata</i>	W (16)/Su							6/16 (38)	Su	0/16 (0)	6/16 (38)
	C (4)/Sp, Su	0/1 (0)	0/1 (0)	0/3 (0)	0/3 (0)						0/4 (0)
<i>Kinixys belliana belliana</i>	C (14)/Sp, Su, W			6/14 (48)	Sp	2/14 (14)	Sp				8/14 (57)
<i>Kinixys lobatsiana</i>	W (27)/A, W					2/27 (7)	A	0/27 (0)			2/27 (7)
	C (2)/Su	1/2 (50)	Su	0/2 (0)							1/2 (50)
<i>Kinixys natalensis</i>	C (3)/Sp, W			2/3 (66)	Sp	0/3 (0)					2/3 (66)
<i>Stigmochelys pardalis</i>	W (41)/Sp, Su, W					2/41(5)	Su	0/41(0)			2/41(5)
	C (47)/Sp, Su, W	13/37 (35)	Sp	0/37 (0)	3/10 (30)	W	1/10 (10)	W			17/47 (36)
Total/Province (%)	C & W (154)	14/40 (35)	0/40 (0)	11/30 (37)	3/30 (10)	4/68 (6)	0/68 (0)	6/16 (38)	0/16 (0)		
Total (%)											38/154 (25)

Binucleate meronts, or closely apposed paired individuals: curved with rounded or bluntly pointed ends (Figs. 3, 4), $4.0 \pm 0.2 \mu\text{m}$ ($3.6\text{--}4.3 \mu\text{m}$) long by $1.9 \pm 0.4 \mu\text{m}$ ($1.3\text{--}2.6 \mu\text{m}$) wide ($n = 10$), cytoplasm vacuolated, stained whitish-blue, paired nuclei with loose chromatin, $1.2 \pm 0.1 \mu\text{m}$ ($1.1\text{--}1.3 \mu\text{m}$) long by $0.5 \pm 0.1 \mu\text{m}$ ($0.5\text{--}0.6 \mu\text{m}$) wide ($n = 5$), stained pinkish-purple, often lying centrally within parasite body; these stages occasionally associated with single individuals (trophozoites or merozoites) within a single immature erythrocyte (Fig. 4).

Merozoites: likely the result of meront division, lying in pairs within immature erythrocytes, each $3.8 \pm 0.3 \mu\text{m}$ ($3.4\text{--}4.3 \mu\text{m}$) long by $1.3 \pm 0.1 \mu\text{m}$ ($1.1\text{--}1.5 \mu\text{m}$) wide ($n = 10$); cytoplasm much less vacuolated than in trophozoites and meronts, stained whitish-blue (Fig. 5); nucleus similar to that of trophozoite, centrally placed, or closer to one pole than the other, stained pink, $1.6 \pm 0.2 \mu\text{m}$ ($1.3\text{--}1.9 \mu\text{m}$) long by $1.0 \pm 0.1 \mu\text{m}$ ($0.9\text{--}1.1 \mu\text{m}$) ($n = 10$). No parasitophorous vacuole evident surrounding merozoites, trophozoites or meronts.

Immature gamonts: lying singly (Fig. 6), or frequently in adjacent (Figs. 7, 8) or opposing (Fig. 9) pairs in parasitophorous vacuoles, within mature erythrocyte cytoplasm, or extracellular (Fig. 10); slender and elongate, or curled (Figs. 8, 9), $17.8 \pm 1.2 \mu\text{m}$ ($14.3\text{--}19.6 \mu\text{m}$) long by $2.3 \pm 0.4 \mu\text{m}$ ($1.6\text{--}3.0 \mu\text{m}$) wide ($n = 12$); whitish-blue staining cytoplasm without vacuolation; rectangular or oval nucleus, chromatin more condensed than in previous stages, stained purple, usually nearer one pole of individual than the other, $2.0 \pm 0.4 \mu\text{m}$ ($1.4\text{--}2.8 \mu\text{m}$) long by $0.9 \pm 0.1 \mu\text{m}$ ($0.7\text{--}1.0 \mu\text{m}$) wide ($n = 10$).

Mature gamonts: lying singly (Figs. 11, 12), or occasionally in pairs (Fig. 13) within mature erythrocytes (Figs. 11–13), $17.5 \pm 0.3 \mu\text{m}$ ($17.1\text{--}17.7 \mu\text{m}$) long by $3.9 \pm 0.5 \mu\text{m}$ ($3.3\text{--}4.3 \mu\text{m}$) ($n = 36$), also extracellular (Fig. 14); elongate, curved, sometimes with one broad pole and opposite, a small recurved tail; whitish-blue stained cytoplasm without vacuoles or granules; pink or purple stained nucleus, square or oval, $4.8 \pm 0.3 \mu\text{m}$ ($4.5\text{--}5 \mu\text{m}$) long by $2.9 \pm 0.4 \mu\text{m}$ ($2.4\text{--}3.2 \mu\text{m}$) ($n = 36$), lying nearer one pole (anterior ?) than the other; parasitophorous vacuole (or thin capsule ?) of intracellular gamont narrower than in immature stages, appearing to persist in extracellular form (Fig. 14). Host cell nuclei compressed and displaced by meront and gamont stages.

Vertebrate type host: *Kinixys belliana belliana* (Gray).

Vertebrate hosts from this study: *Chersina angulata* (Schweigger), *Kinixys belliana belliana* (Gray), *K. lobatsiana* Power, *K. natalensis* Hewitt and *Stigmochelys pardalis* (Bell) (Testudinidae, Cryptodira).

Type locality: Maputo, Mozambique.

Localities in this study: Gauteng (National Zoological Gardens); Kwazulu-Natal (Crocodile Creek, Crocodile Valley, Flag Animal Farm), Ballito; North West (Rustenburg, rural markets); Western Cape (Hondeklip Bay, Velddrif).

Site of infection: Peripheral blood.

Vector: Unknown.

Deposition of voucher specimens: Protozoan collection of the South African Museum, Cape Town, South Africa (blood film of *C. angulata* with mature gamonts, SAM A25092; blood film of *K. b. belliana* with mature gamonts, SAM A25093; blood film of *K. lobatsiana* with mature gamonts, SAM A25094; blood film of *K. natalensis* with mature gamonts, SAM A25095; blood film of *S. pardalis* with trophozoites, probable meronts, merozoites, immature gamonts and mature gamonts, SAM A25096).

Remarks. The gamont stages of *H. fitzsimonsi* described by Dias (1953) were long, slender and curved, with one end broader than the other, and they measured $15.84\text{--}18.81 \mu\text{m}$ long by $3.63\text{--}5.61 \mu\text{m}$ wide. Their cytoplasm was homogeneous, while nuclei were square in outline, often placed excentrically within the parasite body and sometimes condensed; each nucleus was $7.5 \mu\text{m}$ long by $4.62 \mu\text{m}$ wide. The surface membrane of the gamont formed a halo, slightly brighter than the parasite cytoplasm. In the current study, the mature gamont stages of the haemogregarine parasitizing five species of tortoises, from four provinces sampled in South Africa, appeared identical to those of *H. fitzsimonsi* in general appearance, comparable in size, and similar in having a prominent parasitophorous vacuole. The mature gamont form from South African tortoises is thus identified as a development stage of *H. fitzsimonsi*.

Haemogregarina bruneti, which Combes (1919) reported from *K. homeana* captured near Bamako, Mali, has gamonts of greater width ($7 \mu\text{m}$), but similar length ($16.8 \mu\text{m}$), to those of *H. fitzsimonsi*, with these gamonts possibly contained within a thin capsule. The gamont nucleus of *H. bruneti* is irregular, gamont cytoplasm is reticulated with ~20 characteristic granules, and the shape and volume of the host erythrocyte is altered markedly by the parasite presence. Dias (1953) concluded that this was a different species from *H. fitzsimonsi* and we concur with his opinion.

Dias (1953) also described briefly, a dumb-bell like form of *H. fitzsimonsi*, absent from the present material. However, we detected haemogregarine stages which Dias (1953) did not report, namely trophozoites, and probable meronts, merozoites and immature gamonts. These forms occurred alongside what are interpreted here as the mature gamonts of Dias' haemogregarine in two wild-caught *C. angulata* from Velddrif and three *S. pardalis* from the NZG, and therefore they are provisionally identified as developmental stages of *H. fitzsimonsi*.

Dias (1953) collected his two parasitized tortoises in Maputo, Mozambique, but in the current study tortoises from four South African provinces were infected, indicating that *H. fitzsimonsi* is not host-specific among land tortoises from southern Africa, and has a broad geographical range.

Figs. 2–17. Giemsa-stained blood films of *Stigmochelys pardalis* with *Haemogregarina fitzsimonsi* (Figs. 2–14) and *Haemogregarina parvula* (Figs. 15–17) infecting immature (Figs. 2–5, 15) and mature erythrocytes (Figs. 6–14, 16, 17). **Fig. 2.** Trophozoite. **Figs. 3, 4.** Meronts in division, or closely opposed individuals. **Fig. 5.** Paired merozoites. **Figs. 6–10.** Immature gamonts singly, or in pairs, within erythrocytes, or extracellularly. **Figs. 11–13.** Mature gamonts singly or in pairs within erythrocytes. **Fig. 14.** Extracellular mature gamont. **Fig. 15.** Immature encapsulated gamont. **Figs. 16, 17.** Mature encapsulated gamonts. Scale bar (Figs. 2–17) = 10 μ m.

Haemogregarina (sensu lato) *parvula* Dias, 1953

Developmental stages within the blood of tortoises

Two stages, probably immature and mature intraerythrocytic gamonts (Figs. 15–17) of *H. parvula* were observed in two captive specimens of *K. b. belliana*, and one captive *S. pardalis*, both in KZN. The *S. pardalis* was also parasitized by *H. fitzsimonsi*, but this haemogregarine was not observed in the blood of the two infected *K. b. belliana*.

Immature and mature gamonts of *H. parvula* were broadly oval, with ill-defined perimeters, and enclosed by a non-staining capsule. Only two immature gamonts were observed, but their overall outline was similar in size to that of mature gamonts, measuring 10.9 and 12.1 μ m long, by 5 and 6.4 μ m wide ($n = 2$). The nucleus of this younger stage was not visible and the parasite cytoplasm, which was difficult to discern, appeared to stain pale blue,

and lack granules or vacuoles (Fig. 15). This stage was observed in immature erythrocytes, where host nuclei were displaced.

Mature gamonts of *H. parvula* occurred in mature erythrocytes, also inducing host nucleus displacement. Overall measurements of mature gamonts in *K. b. belliana*, were 11.3 ± 0.4 μ m (11–12.1 μ m) long by 6.2 ± 0.6 μ m (5.4–7.2 μ m) wide ($n = 6$), with nuclei measuring 3.2 ± 0.9 μ m (2.4–4.9 μ m) long by 4.4 ± 0.4 μ m (4–5.1 μ m) wide ($n = 6$). Mature gamonts (Figs. 16, 17) in *S. pardalis* measured 11.5 ± 0.4 μ m (11–12 μ m) long by 6 ± 0.3 μ m (5.4–6.1 μ m) wide ($n = 5$), with nuclei measuring 3.2 ± 0.9 μ m (2.2–4 μ m) long by 4.4 ± 0.3 μ m (4–4.6 μ m) wide ($n = 5$). The nucleus of mature gamonts (Figs. 16, 17), stained deep purple and lay terminally, suggesting that the gamont body may be bent double within the capsule. Cytoplasm stained pale blue and lacked granules, or vacuolation. Extracellular gamonts were not seen.

Vertebrate type host: *Kinixys belliana belliana* (Gray).
Vertebrate hosts from this study: *Kinixys belliana belliana* (Gray), *Stigmochelys pardalis* (Bell) (Testudinidae, Cryptodira).

Type locality: Maputo, Mozambique.

Localities in this study: Kwazulu-Natal (Crocodile Creek, Flag Animal Farm), Ballito.

Site of infection: Peripheral blood.

Vector: Unknown.

Deposition of voucher specimens: Protozoan collection of the South African Museum, Cape Town, South Africa (blood film of *K. b. belliana* with immature and mature intraerythrocytic gamonts, SAM A25097; blood film of *S. pardalis* with mature intraerythrocytic gamonts, SAM A25098).

Remarks. The gamont stages observed in *K. b. belliana* and *S. pardalis* resemble Dias' (1953) description of encapsulated *H. parvula* very closely, and as in the current study, he reported low parasitaemias. Dias' gamonts were 9.2–13.2 µm long by 5.67–5.94 µm wide, and were thus slightly longer, but of similar width to those recorded in this study. The parasite nucleus also occurred at one pole, in both the mature gamont of this and Dias' parasite, while the immature gamont exhibited no stained nucleus. Furthermore, host erythrocyte nuclei were greatly displaced in both the current and Dias' material. The similarities between Dias' *H. parvula* and the haemogregarine reported here draw us to the conclusion that they are identical. Thus, the host and geographical ranges of *H. parvula* have been extended within KZN.

Northern KZN (the source of *H. parvula* in this study) shares a subtropical climate and general habitat type with Maputo, Mozambique from which Dias (1953) obtained his parasitized tortoises. This suggests that, unlike *H. fitzsimonsi*, *H. parvula* may be a habitat-specific haemogregarine. However, the encapsulated blood gamonts of *H. parvula* are also close in size and appearance to those of *Hemolivia mauritanica* from the spur-thighed tortoise, *Testudo graeca*, from Algeria (12–15 by 6 µm) (see Sergeant and Sergeant 1904), and from the same tortoise host from Bulgaria and Turkey, and the marginated tortoise *Testudo marginata* Schoepff from Greece (both, 10–14 by 4–7 µm) (Široký et al. 2005). The possibility that *H. parvula* is a member of the genus *Hemolivia*, or may be *H. mauritanica* itself, is discussed further below.

DISCUSSION

Among them, five South African tortoise species appear to contain the haemogregarine species, *Haemogregarina fitzsimonsi* and *Haemogregarina parvula*, recorded by Dias (1953) from Maputo, Mozambique. Siddall (1995) regarded *H. parvula* as a synonym of *H. fitzsimonsi*, with the latter taking priority. However, in the current study, both parasites were detected in only one tortoise, a captive *Stigmochelys pardalis* in Kwazulu-Natal (KZN), and

thus we prefer, at present, to retain their status as separate species of haemogregarines.

Haemogregarina parvula was observed in a new host (*S. pardalis*) and in a new locality (Ballito) within KZN, but no stages of development, other than those noted by Dias were observed. On the other hand, the distribution and host range of *H. fitzsimonsi* were much greater than for *H. parvula*, and additional blood stages were located, with probable meronts, paired merozoites and paired immature gamonts pointing to the existence of intraerythrocytic division. Superficially, this would support the classification of *H. fitzsimonsi* as a member of the genus *Haemogregarina* sensu stricto (see Siddall 1995). However, it is difficult to accept that South African savannah and arid-land tortoises (from Gauteng, North West Province, and the Western Cape) are infested with leeches, supposedly the vectors of chelonian *Haemogregarina* spp. (see Siddall 1995), even if tortoises in KZN encounter moist conditions. These observations, coupled with the existence of ticks of two species, *Amblyomma marmoreum* and *A. sylvaticum*, found parasitizing some host animals, suggest that *H. fitzsimonsi* may not be a *Haemogregarina* sp., but a species of *Hemolivia*, or of *Hepatozoon* Miller, 1908, both of which use ticks as definitive hosts (see Davies and Johnston 2000).

Haemogregarina parvula may also be a haemogregarine of this type, since it clearly has encapsulated stages in host erythrocytes, a feature of *Hemolivia* species (see Široký et al. 2007). The similarity in morphology and size between the encapsulated forms of *H. parvula* and *Hemolivia mauritanica* from *Testudo graeca* and *T. marginata* is striking (see above). However, whether *H. mauritanica* can exist outside the vertebrate host genus *Testudo* is unclear at present, and the parasite may not be conspecific with *H. parvula* from *Kinixys belliana belliana* and *S. pardalis*. Furthermore, proving that *H. fitzsimonsi* and *H. parvula* are members of a genus such as *Hemolivia*, rather than *Haemogregarina*, will require detailed cycle studies like those of Michel (1973), Landau and Paperna (1997) and recently, Široký et al. (2004, 2005, 2007). To date, *H. mauritanica* is the only species of the genus *Hemolivia* recorded from land tortoises and a tick vector, *Hyalomma aegyptium* L. (see Široký et al. 2007). In his study, Dias (1953) reported the tick *Amblyomma nuttalli* Dönitz, 1909 to parasitize *K. b. belliana*. However, this tick species was not found in the present study.

Haemogregarina fitzsimonsi can be detected in all seasons in South African tortoises. As judged by host weight, it also exists in younger and older *S. pardalis* housed at the National Zoological Gardens (NZG), Pretoria. Comparison of data from wild and captive specimens showed that prevalence of *H. fitzsimonsi* infections in this tortoise is significantly greater in captive hosts, but not parasitaemia. Furthermore, female *S. pardalis* at the NZG showed a significantly higher prevalence of infection with *H. fitzsimonsi* than males. In the wild, female tortoises roam

widely through male territories (Branch 1998, 2008), possibly increasing their vector contact as they move from one territory to another. However, although greater tick intensities in captive situations may increase the likelihood of haemogregarine transmission, we have no evidence of this at the NZG, and a gender difference in prevalence is difficult to explain at present. In contrast to our observations, Široký et al. (2005) detected no substantial difference in prevalence of *H. mauritanica* among female, male and juvenile *T. marginata* from Greece. However,

potential host gender influences on prevalence have been observed recently with haematozoan infections in wild-caught fish from Brazil (Davies et al. 2008).

Acknowledgements. We are most grateful to Prof. Jorge Eiras, University of Oporto, Portugal, for obtaining the work of Dias (1953) for us and translating it from Portuguese. We would also like to thank the National Zoological Gardens, Crocodile Creek, Crocodile Valley and Flag Animal Farm for allowing us to examine their tortoises. This work has been funded by a University of Johannesburg Sasol Fund Research Grant.

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Received 9 February 2009

Accepted 23 March 2009