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BLOOD PARASITES OF FREE STATE AND LESOTHO REPTILES

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July 2003

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INTRODUCTION

1.1 Distribution and evolution of reptiles

Evolutionary history and fossil evidence suggest that reptiles have existed for more than 300 million years. According to this evidence this was, and is, a very successful group of vertebrates. In this time-scale reptiles have successfully radiated onto most of the continents, with the exception of Antarctica. They are thought to have arisen from amphibians during the Carboniferous period and the earliest reptile fossils are about 315 million years old. According to Branch (1998), living reptiles are either remnants of this period, or a recent flowering has taken place since the extinction of the dinosaurs, 65 million years ago. They are so successful in terms of diversity and radiation that there are more species of reptiles in South Africa than mammals. In the western deserts they exceed the birds in number, if not diversity (Branch 1998). Furthermore, there are more endemic reptile species in South Africa than any other vertebrate group and according to Branch (1998) in the period from 1988-1998, 83 new species were described, that is, one in every 44 days.

The key to the reptiles' success was the development of the amniotic egg, which is resistant to desiccation and without the free-living tadpole stage. The absence of this typical aquatic larval stage was instrumental in freeing reptiles from the aquatic world. Some have evolved cleiodic eggs, with thick shells and yolk stores. Reptiles also have particular features that they share with amphibians: scaly skin, the presence of lungs and of four legs, at least in the primitive forms. In some cases evolution has brought the loss of two and even four external appendages. According to Low (1978) there are four orders of reptiles, namely the Crocodylia (crocodiles, caimans and alligators), Chelonia (tortoises, terrapins and turtles), Squamata (lizards, amphisbaenians and snakes) and Sphenodonta (the tuataras). The lizard-like tuataras are restricted to a few islands on the north coast of New Zealand. The other three orders are well represented in South Africa, comprising 480 species.

1.2 Squamata of the Free State

The first contribution to the knowledge of the Free State reptiles was that of Boettger (1883), who recorded a few lizard and snake species from Smithfield. FitzSimons added much more to this subject in his books on the lizards (FitzSimons 1943) and snakes (FitzSimons 1962) of Southern Africa. Later, De Waal (1978) compiled a list of the Free State squamates, collecting in 16 habitats, in each degree unit, making this the most intensive survey to date in Africa. He also sampled in every quarter degree in the Free State. His results are summarised in Table 1.

A more recent survey by Bates (1996) shed new light on the diversity of reptiles in the Free State. He added 11 lizard and two snake species to the list, bringing the total of known representatives of Squamata of the Free State to 53 lizard, one amphisbaenian and 38 snake species. The following table (Table 1) is a complete list of known squamates in the Free State, as well as Bates' newer records indicated with asterisks.

Table 1 A summary of reptiles of the Free State from De Waal (1978) and Bates (1996). Newer records from Bates (1996) are indicated by asterisks.

Host
Class: **Reptilia**
Order: **Squamata** Oppel, 1811
Suborder: **Sauria** MacCartney, 1802
Family: **Gekkonidae** Cuvier, 1817
Afroedura anivaria (Boulenger, 1894)
Afroedura karroika halli (Hewitt, 1935)
*Hemidactylus maboeia (Moreau de Jonnes, 1818)
Lygodactylus capensis capensis (A. Smith, 1849)
Pachydactylus bibronii (A. Smith, 1845)
Pachydactylus capensis capensis (A. Smith, 1845)
*Pachydactylus laevigatus laevigatus Fisher, 1888
Pachydactylus maculatus ocelatus (Hewitt, 1927)
Pachydactylus mariquensis mariquensis (A. Smith, 1849)

Table 1 (Continued).   A summary of reptiles of the Free State from De Waal (1978) and Bates (1996). Newer records from Bates (1996) are indicated by asterisks.

*Pachydactylus vansoni FitzSimons, 1933
Ptenopus garrulus garrulus (A. Smith, 1849)
Family: **Agamidae** Gray, 1827
Agama atra Daudin, 1802
Agama hispida (Linnaeus, 1758)
Agama makarikarica FitzSimons, 1932
Family: **Chameleonidae** (Methuen & Hewitt, 1915)
*Bradypodion sp (Qua Kwa & Zastron varieties)
*Bradypodion dracomontanum Raw, 1976
*Bradypodion cf. *karooicum* (Methuen & Hewitt, 1915)
Chameleo dilepis dilepis Leach, 1819
Family: **Scincidae**
Acontias gracilicauda gracilicauda Essex, 1925
Afroablepharus wahlbergii (A. Smith, 1849)
Mabuya occidentalis (Peters, 1867)
Mabuya striata punctatissima (A. Smith, 1849)
Mabuya sulcata sulcata (Peters, 1867)
Mabuya varia (Peters, 1876)
Mabuya variegata punctulata (Bocage, 1872)
Mabuya variegata variegata (Peters, 1869)
Tetradactylus africanus africanus (Gray, 1838)
*Tetradactylus seps (Linnaeus, 1758)
*Tetradactylus tetradactylus (Lacèpéde, 1803)
Family: **Cordylidae** Gray, 1837
*Chamaesaura aenea (Wiegmann, 1843)
Cordylus cordylus cordylus (Linnaeus, 1758)
Cordylus giganteus A. Smith, 1844
Cordylus polyzonus polyzonus A. Smith 1838
Cordylus vittifer vittifer (Reichenow, 1887)

Table 1 (Continued). A summary of reptiles of the Free State from De Waal (1978) and Bates (1996). Newer records from Bates (1996) are indicated by asterisks.

Pseudocordylus melanotus melanotus (A. Smith, 1838)
Pseudocordylus melanotus subviridis (A. Smith, 1838)
Pseudocordylus spinosus FitzSimons, 1947
*Tropidosaura essexi Hewitt, 1927
Family: **Lacertidae** Bonaparte, 1831
Eremias burchelli Dumeril & Bibron, 1839
Eremias lineoocellata lineoocellata Dumeril & Bibron, 1839
Eremias namaquensis Dumeril & Bibron, 1839
Ichnotropis squamulosa Peters, 1854
Nucras intertexta (A. Smith, 1838)
Nucras lalandii (Milne-Edwards, 1829)
Nucras taeniolata ornata (Gray, 1864)
Varanus exanthematicus albigarus (Daudin, 1802)
Varanus niloticus niloticus (Linnaeus, 1766)
Suborder: **AMPHISBAENIA**
Family: **Amphisbaenidae** Gray, 1825
Monopeltis capensis capensis A. Smith, 1848
Suborder: **SERPENTES** Linnaeus, 1758
Family: **Typhlopidae** Gray, 1825
Rhinotyphlops lalandei (Schlegel, 1844)
Typhlops bibronii (A. Smith, 1846)
Family: **Leptotyphlopidae** Stjneger, 1891
*Leptotyphlops conjunctus conjunctus (Jan, 1861)
Leptotyphlops scutifrons scutifrons (Peters, 1854)
Family: **Colubridae** Gray, 1825
Aparallactus capensis A. Smith, 1849
Crotahopeltis hotamboeia (Laurenti, 1768)
Dasypeltis scabra (Linnaeus, 1785)
Dispholidus typus typus (A. Smith, 1829)

Table 1 (Continued). A summary of reptiles of the Free State from De Waal (1978) and Bates (1996). Newer records from Bates (1996) are indicated by asterisks.

Duberria lutrix lutrix (Linnaeus, 1785)
Lamprophis aurora (Linnaeus, 1754)
Lamprophis fuliginosus fuliginosus (Boie, 1827)
Lamprophis fuscus Boulenger, 1893
Lamprophis guttatus (A. Smith, 1843)
Lamprophis inoratus Dumeril & Bibron, 1854
*Lycodonomorphus laevissimus (Günter, 1862)
Lycodonomorphus rufulus (Lichtenstein, 1823)
Lycophidion capense capense (A. Smith, 1831)
Philotamnus natalensis occidentalis Broadley, 1966
Prosymna bivittata Werner, 1903
Prosymna sundevalli sundevalli (A. Smith, 1849)
Psammophis crucifer (Daudin, 1803)
Psammophis leightoni trinasalis Werner, 1902
Psammophis notostictus Peters, 1867
Psammophylax rhombeatus rhombeatus (Linnaeus, 1754)
Psammophylax tritaeniatus (Gunter, 1868)
Pseudaspis cana (Linnaeus, 1785)
Xenocalamus bicolor bicolor Gunther, 1868
Family: **Elapidae** Boie, 1827
Aspidelaps lubricus lubricus (Laurenti, 1786)
Elaps dorsalis A. Smith, 1849
Elaps lacteus (Linnaeus, 1754)
Hemachatus haemachatus (Lacépède, 1788)
Naja nivea A.Smith, 1849
Family: **Viperidae** Gray, 1825
Atractaspis bibronii A. Smith, 1849
Bitis arietans arietans (Merrem, 1820)
Bitis artropos (Linnaeus, 1754)
Causus rhombeatus (Lichtenstein, 1823)

1.3 Parasites of Squamata and aims of the project

Several groups of parasites are known from most types of reptiles, including the Squamata (see Mader 1996). Work on parasites of reptiles in general is mostly confined to Europe and the Americas, although Mackerras (1961) compiled a comprehensive list of blood parasites of Australian reptiles.

It is impossible in a study like this one to focus on all parasites found in the Squamata. The aims of this project are therefore to concentrate on the blood parasites, since these are readily accessible and once sampled, allow reptiles to be returned to the wild shortly after capture, without harm. It focuses particularly on blood parasites of Squamata of the Free State, but also includes some work in Lesotho. The project also aims to provide baseline studies, so that in future more detailed research will be possible. In doing a survey of reptiles, many of which are protected species, an initial assessment of the identity and taxonomy of their blood parasites is necessary. This approach can identify taxonomic problems that merit future, more detailed work. For example, the study makes preliminary, yet determined attempts to identify the nature of infections in the blood of CITES listed red data species *Cordylus giganteus* A. Smith, 1844 in the northeastern parts of the Free State. This particularly involves the use of transmission electron microscopy (TEM) techniques.

By surveying the blood parasites of these animals, a deeper understanding of the population structures and general biology of their parasites can be achieved. This also provides a greater knowledge of the distribution of these infections and whether they might be of a pathological nature. Focusing on the biology of blood parasites in reptiles might just lead to a greater insight into parasites infecting man, like the malarias, which are common in lizards. A well-known example of such an event using non-human parasite models was that involving Ronald Ross, who famously completed his work on human malaria in 1898 by observing the transmission of bird malaria parasites (see Roberts & Janovy, 2000).

1.4 Blood parasites of reptiles

The blood parasites of reptiles represent a rather unexplored field. These parasites were first recorded in the late 1800s, and the work of Robertson (1906) and Sambon & Seligmann (1907) are classical examples of early research on turtle and snake haemogregarines, blood parasites broadly related to the malarias. Labbé (1894) proposed to divide the haemogregarines known at that stage into three distinct groups, on the grounds of the relative proportions of the parasite to the host blood cell. In *Drepanidium* Labbé, 1894, the parasite was no more than three fourths the host cell in length. In *Karyolysus* Labbé, 1894, the parasite did not exceed the host erythrocyte in length and exercised a destructive influence on the cell nucleus. For *Danilewskya* Labbé, 1894, the parasite exceeded the host cell and doubled up in it. Sambon & Seligmann (1907) later noted that except for the substitution of the name *Lankesterella* Labbé, 1899 for *Drepanidium* and *Haemogregarina* Danilewsky, 1885 for *Danilewskya*, Labbé's classification was followed by the great majority of authors at that time.

During the first half of the last century, work on blood parasites of reptiles tended to be sporadic. Some enigmatic new genera were reported, including *Toddia* França, 1911, *Pirhemocyton* Chatton & Blanc, 1914, *Cingula* Awerinzew, 1914, *Tunetella* Brumpt & Lavier, 1935, *Sauroplasma* Du Toit, 1937, *Serpentoplasma* Pienaar, 1954 and *Sauromella* Pienaar, 1954 (see Davies & Johnston, 2000) as well as coccidian genera such as *Schellackia* Reichenow, 1919. In the 1960s, Mackerras (1961) reported on the haematozoa of Australian reptiles and Stehbens & Johnston (1966) proved by TEM that *Pirhemocyton* from the same region is a viral infection, now known from the erythrocytes of lizards, turtles and snakes. The 1970s revealed evidence that *Toddia*, like *Pirhemocyton*, is a viral infection in the blood of snakes (De Sousa & Weigl 1976). Furthermore, from the 1970s to the 1990s the work of authors such as Ayala (1977), Telford (1972, 1973, 1988, 1989, 1993), Lainson & Paperna (1996) and Schall (1990, 1996) on lizard malarias (mainly *Garnia* Lainson, Landau & Shaw, 1971, *Haemoproteus* Kruse, 1890 and *Plasmodium* Marchiafava & Celli, 1885) was undertaken (see Ayala 1977; Schall, 1996). This period also saw efforts to understand the effects of reptilian blood parasites on host behavior (see Schall, 1996). The 1990s onwards have also

produced descriptions of reptilian blood parasites, including new genera such as *Hemolivia* Petit, Landau, Baccam & Lainson, 1990 and *Billbraya* Paperna & Landau, 1990 and new species of established genera such as *Schellackia, Plasmodium* and *Haemoproteus* (see Paperna & Finkelman, 1996, Lainson & Paperna, 1996 and Paperna & Landau, 1991). Also during this period, except for those in chelonians, the majority of reptilian haemogregarines has been transferred to the genus *Hepatozoon* Miller, 1908 by Smith (1996). Finally, some of the most complex life cycles of members of this genus have been elucidated, particularly by Desser (1993) and his co-workers (see Desser & Bennett, 1993, Smith, Desser & Martin, 1994, Smith & Desser, 1997a, 1997b) and by Lainson, Paperna & Naiff (2003).

Literature concerning the blood parasites of reptiles in Africa appears scanty in comparison with that noted above. Sambon & Seligmann (1907), Fantham (1925) and Pienaar (1962) have probably made the most significant contributions to knowledge of these blood parasites in South Africa, although in the last decade there has been some research done on the blood parasites of reptiles in this region (see Paperna & De Matos, 1993a). Pienaar (1962) described several new species of parasites in South African reptiles, including a trypanosome (*Trypanosoma mocambicum* Pienaar, 1962) in the blood of a Mozambican terrapin, *Pelosios sinuatus sinuatus*, a species of lizard malaria (*Plasmodium zonurae* Pienaar, 1962), a piroplasmid (*Sauroplasma zonurum* Pienaar, 1962) and infections of a viral nature (*Pirhemocyton zonurae* Pienaar, 1962) in the girdled lizard, *Cordylus vittifer* Reichenow, 1887. A new haemogregarine (*Haemogregarina pelusiensi* Pienaar, 1962) from the terrapin, *Pelosios sinuatus sinuatus* and another suspected piroplasm (*Serpentoplasma najae* Pienaar, 1962) were also recorded from the blood of a black-necked cobra, *Naja nigricollis* Bogert, 1940 by Pienaar (1962). Paperna & de Matos (1993a) reported new hosts and geographical locations of erythrocytic viral infections, of which some records were from South Africa. However, in the Free State Province, work on blood parasites of reptiles or any other vertebrate group appears very limited.

1.5 Problems of Taxonomy

Several of the reptilian blood infections in this study are known or suspected to be of viral origin, although some of these are currently classified with the so-called piroplasms of the protozoan Phylum Apicomplexa Levine, 1970. Other infections in the study result from the presence of haemogregarines and the malarias, both groups, like the piroplasms, belonging to the Apicomplexa. Also observed are nematode stages (microfilariae), which will be considered last in this section and which are considered only briefly in this study.

1.6 Reptilian viral and viral- like infections

Classification of viral and viral-like infections in the blood of reptiles is probably unwise, given the current uncertainties concerning their identity, nomenclature and classification (see Davies & Johnston 2000). Some infections are thought to result from icosahedral viruses (e.g. *Pirhemocyton*) related to the iridoviruses, others may be herpesviruses, and yet more may be oncornaviruses (see Davies & Johnston 2000). Viral-like infections in reptiles probably include *Sauromella*, an infection of uncertain status (see Johnston 1975), and *Sauroplasma*, recently classified with the Protozoa as a piroplasm (see Section 1.7 below). *Serpentoplasma* may be a similar infection.

Chatton & Blanc (1914) noted an organism resembling a piroplasm within the red blood cells of the North African gecko (*Tarentola mauritanica*), which they named *Pirhemocyton tarentolae* Chatton & Blanc, 1914. Later, Brumpt (1936) defined the genus *Pirhemocyton* Chatton & Blanc, 1914 as "nonpigmented endoglobular parasites of saurian red cells with diffuse chromatin or central chromatin dot, giving rise, in infected blood, to albuminoid inclusions in the red corpuscles. Multiplication and replication unknown". It was Stehbens & Johnston (1966) who discovered the viral nature of *Pirhemocyton* by examining its ultrastructure and a recent study of this infection also confirmed its viral nature (Paperna & de Matos 1993b). Johnston (1975) listed 35 hosts for *Pirhemocyton*. All these viruses comprise icosahedral, intracytoplasmic, iridovirus-like particles (see Paperna & de Matos 1993b).

According to Pienaar's (1962) post-mortem observations there is a chance that severe infections such as these in reptiles may run a fatal course. Heavy invasions of the erythrocytes with *Pirhemocyton* invariably lead to extensive aniso- and poikilocystosis, gross cellular distortion and cytolysis leading to severe anemia. Cellular deformation and disruption is effected primarily through the association of the parasite with the "curious" albuminoid bodies that appear in the cytoplasm of the host cells. In this condition, according to Pienaar (1962), there is nuclear displacement.

Sauromella haemolysus Pienaar, 1954 is the only parasite of its type reported from the blood of lizards. The parasite was originally noted in a South African lizard (*Pachydactylus capensis* (A. Smith, 1845)), which is also found in the Free State. According to Pienaar (1962) this endoglobular parasite was of a doubtful nature and could possibly be of the *Anaplasma* type. He described forms as minute, dark, spherical or rod-like bodies. These bodies could occur singly or in groups, and could be associated with a *Pirhemocyton*-type infection since the red cell stroma de-haemoglobinized. According to Pienaar (1962) multiplication was apparently effected through binary or multiple fission, and the infection appeared to be of an acutely pathological nature, as it not only destroyed the haemoglobin pigment of the host cell, but may have also caused heavy anemia and hyperactive erythropoitic activity.

1.7 Reptilian Protozoa or so-called Protozoa

For the purposes of the study, the classification system of Lee *et al.* (2000) is employed for the Protozoa. There have been several attempts to re-define the classification of the Protozoa in recent years. Notable examples have been firstly by Levine *et al.* (1980), then Corliss (1994), who designed a "user friendly", six-kingdom classification of life and then Cavalier-Smith (1998), who elevated the Protozoa to kingdom status. However, Patterson (2000) notes in the Society of Protozoologists' publication that Protozoa form an artificial group of eukaryotes, rather than a natural one. Patterson (2000) also concludes that although the "bricks (groups with distinctive ultrastructural identities)" and the "cement (phylogenetic systematics)" for a "systematic edifice" exist, "the plans" are lacking. Such plans, he believes, will probably come from a "molecular

understanding of evolutionary relationships among taxa". As a result of the current uncertainties, Lee *et al.* (2000) divides the Protozoa into "Key Major Groups", many corresponding to phyla, others to orders. The phylum Apicomplexa, is one such group.

PHYLUM APICOMPLEXA LEVINE, 1970

The phylum Apicomplexa comprises unicellular endosymbionts, characterised by having an apical complex, composed of one or more polar rings, a number of rhoptries and micronemes, a conoid and sub-pellicular microtubules. The phylum has the following three classes: Perkinsasida Levine, 1987, Conoidasida Levine, 1988 and Aconoidasida Melhorn, Peters & Haberkorn, 1980.

In the Society of Protozoologists' system (Lee *et al.* 2000), the haemogregarines found in the current study may fall within the class Conoidasida, order Eucoccidiorida Léger & Duboscq, 1910, suborder Adeleorina Léger, 1911, or in the suborder Eimeriorina, Léger 1911 of the same order (Eucoccidiorida). The malarias are all classified within the class Aconoidasida, order Haemospororida Danilewsky, 1885 and the so-called piroplasms within the same class (Aconoidasida), but the order Piroplasmorida Wenyon, 1926. Details of this classification are given below.

CLASS CONOIDASIDA LEVINE, 1988

Such organisms have organelles of the apical complex, and generally both sexual and asexual reproduction occur, followed by sporogony. Sporogony results in oocysts with infective sporozoites. Cellular motility exists, but flagella are found only on the microgametes of some taxa. Pseudopods may exist for feeding. Homoxenous and heteroxenous species are known.

Order Eucoccidiorida Léger & Dubosq, 1910

Members of this order demonstrate merogony, gamogony and sporogony and these occur in vertebrates and/or invertebrates.

Suborder Adeleorina Léger, 1911

Organisms within this suborder exhibit syzygy, with conjugation and subsequent sporogony usually in an invertebrate definitive host. Complex life-cycles exist, involving at least one cycle of merogony, followed by gametogony, syngamy and sporogony. Two types of meronts may occur. The Adeleorina comprises seven families, four of which contain genera of reptilian haemogregarines.

Family Hepatozoidae Wenyon, 1926

This family contains only the genus *Hepatozoon* Miller, 1908. Members of the genus demonstrate such diversity that the genus may be paraphyletic (see Barta, 2000).

The genus *Hepatozoon* Miller, 1908
Type species *Hepatozoon muris* (Balfour, 1905) Wenyon, 1926 in *Rattus norvegicus*

The majority of species within the genus have been reported on the appearance of their gamonts in the erythrocytes and/or leucocytes of vertebrate hosts, including reptiles. Merogony does not usually occur within erythrocytes, but in vascular endothelial cells. Latent monozoic and dizoic cysts can also exist in vertebrate tissues. In invertebrate hosts such as mites, ticks, insects and possibly leeches, microgametes may be flagellated, but no sporokinetes are formed. Normally in the haemocoel of these same invertebrates, large polycystic oocysts are produced with sporocysts containing four to 16 or more sporozoites. Transmission occurs when the vertebrate host ingests the infected invertebrate, or through predation on another vertebrate containing tissue cysts.

More than 121 species of the genus *Hepatozoon* have been described worldwide (Levine 1988, Smith 1996). The range of blood sucking invertebrates that these parasite utilise include ixodid and argasid ticks, mites, assassin bugs (Hemiptera: Reduviidae); Diptera (sandflies, mosquitoes, tsetse flies), Anopleura (sucking lice), Siphonaptera (Fleas) and the Hirudinea (leeches) (Smith, 1996).

Family Haemogregarinidae (Neveu-Lemaire) Léger, 1911

The numerous species comprising this family, particularly those of the genus *Haemogregarina* Danilewsky, 1885 have been described as a "taxonomic mess" and the genus itself, a "taxonomic repository of poorly described forms" (Barta, 2000). In fact, Mohammed and Mansour (1959) recommended the qualifier *"senso lato"* to include species whose life cycles have not yet been described or studied and *"senso stricto"* for those with a known life history. Representatives of the family Haemogregarinidae comprise three genera, but only one of these, *Haemogregarina*, is known from reptiles.

The genus *Haemogregarina* Danilewsky, 1885
Type species: *Haemogregarina stepanowi* Reichenow, 1885 in *Emys orbicularis*

More than 300 *Haemogregarina* species have been described in many groups of vertebrates (Desser, 1993). Siddall (1995) listed 19 chelonian species infected with representatives of the genus *Haemogregarina (senso stricto)*. A further 10 chelonian species were added to this list by Smith (1996). Siddall (1995) also recommended that all the remaining species that parasitise fish, turtles, snakes, crocodilians, lizards, and birds that he could not place in the genera *Haemogregarina (sensu lato)*, *Cyrilia* Lainson, 1981 *and Desseria* Siddall, 1995 be transferred to the genus *Hepatozoon*. Smith (1996) completed this task. *Haemogregarina* are adeleid coccidia with heteroxenous life cycles. Generally, the gamont stages that are a product of merogony occur in the erythrocytes of the vertebrate host and according to Davies & Johnston (2000), the sporozoites, a product of sporogony, occur in haematophagous invertebrates. Desser (1993) noted that the

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characteristics of *Haemogregarina* species are that they have small oocysts with eight sporozoites, formed from a single germinal centre.

Members of this genus (*Haemogregarina*) occur therefore in vertebrate hosts such as chelonians, fishes and possibly other ectotherms. In their vertebrate hosts, vermicular meronts exist in blood cells and fixed tissue cells, with gamonts mainly in erythrocytes. In their invertebrate hosts, such as leeches, sporogony occurs in the intestinal epithelium and oocysts produce eight naked sporozoites. Post-sporogonic merogony also occurs in the invertebrate host and transmission is by bite, when merozoites are transferred to the vertebrate host, or perhaps when the invertebrate is ingested.

Family Karyolysidae Wenyon (1926)

Karyolysids may represent a sister taxon to piroplasms of veterinary importance (see Barta, 2000). They have definitive hosts in common (arachnids) and both initiate merogony in these invertebrate hosts, or in their progeny. Their vertebrate hosts are amphibians and reptiles. The family contains two genera, both of which parasitise reptiles.

The genus *Karyolysus* Labbé, 1894

Type species: *Karyolysus lacertae* (Danilewsky, 1886) Reichenow, 1913 in *Lacerta muralis*

Members of this genus probably infect only lacertid lizards. Merogony occurs in the endothelial cells of lizards and gamonts primarily infect erythrocytes. Syzygy and sporogony occur in the gut of female mites, forming motile sporokinetes (sporozoites, according to Barta, 2000). These enter mite eggs to form sporocysts with 20-30 sporozoites each (merozoites, according to Barta, 2000). Transmission occurs when the vertebrate host eats an infected mite of the next generation (mite nymph).

The genus *Hemolivia* **Petit, Landau, Baccam & Lainson, 1990**

Type species: *Hemolivia stellata* **Petit, Landau, Baccam & Lainson, 1990 in** *Bufo marinus* and *Amblyomma rotondatum*

This genus has been reported from a toad from Brazil, a lizard from Australia and an African tortoise (see Davies & Johnston, 2000), with ticks as invertebrate hosts. In the vertebrate hosts merogony and cyst formation occur in endothelial cells and erythrocytes, while gamonts occur in erythrocytes. In ticks, sporogony exists in cells of the intestine and a typically star-shaped oocyst is produced. Numerous sporokinetes (possibly sporozoites) from the oocyst invade the intestinal cells, form sporocysts and then sporozoites (possibly merozoites). Transmission occurs when the vertebrate host ingests an invertebrate containing sporocysts/sporozoites and by predation of another vertebrate with tissue cysts.

Family Dactylosmatidae Jakowska & Nigrelli, 1955

This family comprises two genera, one of which may occur in reptiles. Dactylosomatids are heteroxenous blood parasites of ectothermic vertebrates that appear to use leeches as definitive hosts (Barta 1991).

The genus *Dactylosoma* **Labbé, 1894**

Type species: *Dactylosoma ranarum* (Lankester, 1882) Wenyon, 1926 in *Rana esculenta*

In vertebrate hosts that include fishes, newts, anurans and possibly lizards, merogony and gamogony occur in the erythrocytes of the peripheral blood. Primary merogony yields six to 16 merozoites by budding to form a "hand-like" structure. Secondary merogony may produce six individuals that form the gamonts. In the leech, budding produces 30 or more sporozoites within cells of the intestinal lining, but transmission has not been demonstrated.

Suborder Eimeriorina Léger, 1911

This suborder contains numerous genera and species, many of uncertain taxonomic status. Species develop in both vertebrates and invertebrates, some alternating between them. Syzygy does not occur and microgamonts produce many microgametes. Sporozoites develop within oocysts (or membranes corresponding to the oocyst wall) and sporocysts may be present. Zygotes are often motile. Upton (2000) divided the Eimeriorina into nine families, one of which (Lankesterellidae Nöller, 1920) has members occurring in the blood of reptiles.

Family Lankesterellidae Nöller, 1920

This family shares some characteristics with the representatives of the family Haemogregarinidae (Neveu-Lemaire) Léger, 1911, having stages in blood cells, and members with heteroxenous life cycles. The representatives of the family Lankesterellidae can be distinguished from those of the family Haemogregarinidae in having sexual and replicative stages in the tissues (gut, connective tissue and/or viscera) of the vertebrate host. In this family therefore, merogony, gamogony and sporogony occur in the gut, connective tissue and viscera of the vertebrate host. According to Desser (1993), the oocysts are asporoblastic and thus a variable number (eight commonly) of sporozoites are produced, and these enter the red and white blood cells of the host. The dispersive agents of these parasites are haematophagous invertebrates that ingest the sporozoites, but development of these is minimal (probably maturation only) in the intermediate hosts. Few life cycles have been described, and more confusion arises because the descriptions of lankesterellid sporozoites and haemogregarinid gamonts resemble each other. Twenty species have been described within the family Lankesterellidae, many of which that could just as well be members of the Haemogregarinidae, because relatively little attention was given to the multinucleate stages.

Representatives of the Lankesterellidae consist of two genera (*Lankesterella* Labbé, 1899 and *Schellackia* Reichenow, 1919), according to Upton (2000). *Lankesterella* spp. are suspected to parasitise reptiles, whereas *Schellackia* spp. are known to do so. The genus

Lainsonia Landau, 1973 has also been recognised as a member of the same family (Lankesterellidae) (see Desser, 1993; Davies & Johnston, 2000), but this genus is not recorded by Upton (2000), who presumably, like Levine (1988), regarded it as synonymous with the genus *Schellackia*.

The genus *Lankesterella* Labbé, 1899

Type species: *Lankesterella minima* (Chaussat, 1850) Nöller, 1920 in *Rana esculenta*

Members of this genus undergo merogony, gamogany and sporogony in cells of the reticuloendothelial system and have oocysts with 32 or more sporozoites. Sporozoites exist in blood cells, but dormant sporozoites may also occur in the vertebrate tissues. In invertebrates such as mites, mosquitoes or leeches, sporozoites undergo little or no development. Transmission occurs when the invertebrate is ingested, or by predation between vertebrates.

The genus *Schellackia* Reichenow, 1919

Type species: *Schellackia bolivari* Reichenow, 1919 in *Acanthodactylus vulgaris*

Species within this genus undergo merogony, gamogony and sporogony in the intestinal epithelium or lamina propria, with possible development in the spleen and liver. The oocyst yields eight sporozoites that enter erythrocytes and lymphocytes, but dormant sporozoites can occur in tissues. In invertebrates, such as mites and some Diptera, sporozoites exist without development. Transmission occurs on ingestion of an infected invertebrate or by predation between vertebrates.

CLASS ACONOIDASIDA MEHLHORN, PETERS & HABERKORN, 1980

Members of this class are without a conoid, except for the ookinete of some species of Haemospororida Danilewsky, 1885. There are two orders found in reptiles, the Haemospororida and the Piroplasmorida Wenyon, 1926.

Order Haemospororida Danilewsky, 1885

This order contains apicomplexans that do not demonstrate syzygy. About eight flagellated microgametes are produced and the zygote is motile (ookinete). Sporozoites are naked and the life cycle is heteroxenous. Blood–sucking insects usually transmit these parasites. Merogony occurs in the vertebrate host and sporogony in the invertebrate. Pigment (haemozoin) may be formed from host cell haemoglobin, with the macro- and microgametes that develop independently.

Family Plasmodiidae Mesnil, 1903

This family includes *Plasmodium* Marchiafava and Celli, 1885, *Haemoproteus* Kruse, 1890, *Saurocytozoon* Lainson and Shaw, 1969 and seven other genera causing malaria or similar diseases in vertebrates. Only the first three (*Plasmodium, Haemoproteus* and *Saurocytozoon*) occur in reptiles. The genera (and subgenera) are differentiated by: the morphology of the erythrocytic stages; development in the tissues of the vertebrate host and the vector.

The genus *Plasmodium* Marchiafava and Celli, 1885

Type species: *Plasmodium malariae* (Feletti & Grassi, 1889) Int. Com. Zool. Nomen., 1954 in *Homo sapiens* and other primates

Numerous species of this genus have been described in the blood of reptiles, birds and mammals. The parasites exist as meronts in erythrocytes and other tissues, and gametocytes in erythrocytes, which characteristically produce pigment. Invertebrate hosts are mostly anopheline mosquitoes, midges and possibly mites. The oocyst stages of *Plasmodium* exist in the stomach wall of the invertebrate, and sporozoites occur in the salivary glands. The parasites are transmitted and distributed through the bite of the invertebrate.

Peirce (2000) regards *Plasmodium* in reptiles as comprising about 90 species or subspecies divided into subgenera including: *Asiamoeba, Carinamoeba, Fallisia, Garnia, Lacertamoeba, Ophidiella, Parasplasmodium, Sauramoeba* and possibly *Billbraya*. Presumably, *Progarnia* would be another.

The first known saurian *Plasmodium* species was described by Weynon (1909) from the rainbow lizard *Agama agama* in Africa. According to Schall (1990), half of the known malaria parasites are described from lizards, these comprising seventy-six of the 196 *Plasmodium* species. Of Schall's (1990) listed *Plasmodium* species that infect lizards, six species are present in Africa, and one in South Africa. Lizard malaria has been found on all the warm continents, except Europe. Schall (1990) stated that most of the well-known families of lizards are infected with malaria, in temperate woodlands, tropical rain forests and cool upland tropical habitats. Only a few distributions of such malaria populations are known, and it is concluded that at least some parasite-host associations are ancient.

The genus *Haemoproteus* Kruse, 1890

Type species: *Haemoproteus columbae* Kruse, 1890 in *Columba livia*

According to Peirce (2000), *Haemocystidium* Castellani & Willey, 1904 is synonymous with this genus. Within *Haemoproteus,* merogony occurs in the endothelial cells of blood vessels and gamonts exist in erythrocytes. Pigment is formed and vectors are hippoboscid flies, *Culicoides* spp. (Ceratopogonidae) or *Chrysops* spp. (Tabanidae).

The genus *Saurocytozoon* Lainson & Shaw, 1969

Type species: *Saurocytozoon tupinambi* Lainson & Shaw, 1969 in *Tupinambus nigopunctatus*

Meronts occur in lymphocytes, gamonts in leucocytes and pigment is not formed. Oocysts are large and slow to develop, forming hundreds of slender sporozoites. Vectors are presumed to be culicine mosquitoes.

Order Piroplasmorida Wenyon, 1926

This order contains generally pyriform, round, rod-shaped or amoeboid organisms found in the erythrocytes of a variety of vertebrates. Oocysts, spores and pseudocysts are absent, as are flagella. Subpellicular microtubules may be present, and polar rings and

rhoptries occur. Asexual reproduction is present and sexual reproduction may well exist. Merogony occurs in vertebrates and sporogony in invertebrates such as ticks. They are therefore heteroxenous parasites. Peirce (2000) names four families with the order, one of these, the Haemohormidiidae Levine, 1984, having a genus found in reptiles (*Sauroplasma* Du Toit, 1937).

Family Haemohormidiidae Levine, 1984

Members of this family undergo merogony and binary fission. The nucleus lacks an endosome or nucleolus and fish, reptiles and birds are hosts. Vectors are unknown.

The genus *Sauroplasma* Du Toit, 1938

Type species: *Sauroplasma thomasi* Du Toit, 1938 in *Cordylus giganteus* A. Smith, 1844.

Binary fission or budding into daughter cells exists in reptiles. Vectors are unknown. Peirce (2000) makes no mention of the very similar genus *Serpentoplasma* Pienaar, 1962 and Davies & Johnston (2000) were not convinced that *Sauroplasma* is of protistan origin.

These so-called piroplasms were discovered by Du Toit (1937) in girdled lizards *Cordylus giganteus* in Africa. According to Du Toit the degree of infection varied and the parasites were small in comparison with the host erythrocytes. The smaller forms were anaplasmoid forms consisting of granules or small ring-shaped bodies. According to Du Toit (1937), these bodies arose from an anaplasmoid body with the gradual enlargement of the central vacuole. Multiplication subsequently took place by binary fission or by a process of budding. In the first, the spherical bodies elongated, and the "nuclear" material concentrated at the two opposite extremities. A constriction appeared in the middle of the elongated parasite, this constriction tightening until two separate and approximately equal daughter cells were formed. The second procedure, a budding process, achieved the same result. According to Du Toit, (1937), this process was very similar to that seen in many mammalian piroplasms.

Possibly, these parasites can be transmitted by the bite of ticks and mites. According to Pienaar (1962) the prostigmatic mites (*Zonurobia circularis*) that infect these giant girdled lizards may transmit *Sauroplasma* infections. Pienaar (1962) described a new piroplasm from a cordylid lizard *Cordylus vittifer* and named it *Sauroplasma zonurum*. He also recorded a piroplasm from a black-necked cobra (*Naja nigricollis)* and named it *Serpentoplasma najae*. He described it in a similar way to Du Toit, referring to these parasites as sporozoans. Davies & Johnston (2000) suggested that these structures are unlikely to be of a protistan origin, after examining a specimen *Cordylus polyzonus* A. Smith, 1838 from the Free State I collected in my undergraduate years.

1.8 Reptilian filarial nematodes

These are classified below broadly according to Roberts and Janovy (2000).

Phylum Nematoda Potts, 1932

Nematodes are typically multicellular, bilaterally symmetrical, elongated cylindrical animals and tapered at both ends. They possess a pseudocoele and a complete digestive system with an anterior mouth and a posterior anus. The body is covered with non-cellular cuticle and body wall muscles are all longitudinal. Most species are dioecious, but some are hermaphroditic, others parthenogenetic. Most are oviparous, some ovoviviparous.

Family Onchocercidae Leiper, 1911

Members of this family live in amphibian, reptilian, avian and mammalian tissues. All species of filaroids have arthropods as intermediate hosts, most of which deposit third stage larvae on the vertebrate host when they bite. These juveniles often migrate to areas such as subcutaneous tissues, intermuscular connective tissue, the body cavity and lymph nodes, where they develop into adult male and female worms. Adults mate and the female releases microfilariae that migrate to the blood stream. These are ingested when the vector bites. Members of about 14 genera of filarial nematodes can be found in reptiles (Mader, 1996).

1.9 The current study

The current work includes new records of blood parasites from reptiles collected during surveys of sites in the Free State and Lesotho. Two hundred and four lizards, one amphisbaenian and 59 snakes were screened for blood parasites. *Pirhemocyton* infections are described from numerous specimens of *Agama atra atra* Daudin, 1802. *Sauroplasma thomasi* Du Toit, 1937 and *Sauromella haemolysus* are redescribed, and *Sauroplasma thomasi* infections from *Cordylus giganteus* were observed and analysed by transmission electron microscopy (TEM). Eight new distribution records for *Sauroplasma* are reported, involving five families of lizards. In addition, nine new distribution records for *Serpentoplasma* are described across three families of snakes, including preliminary ultrastructural studies by TEM of blood infections of a captive African rock python (*Python sebae natalensis* (Gmelin, 1789)) and *Serpentoplasma* infections of a puff adder (*Bitis arietans* (Merrem, 1820)). Previously unreported haemogregarines are recorded from girdled lizards (*Pseudocordylus melanotus melanotus* (A. Smith, 1838)) and *Pseudocordylus melanotus subviridis* (A. Smith, 1838)), Agamid lizards (*Agama atra atra* Daudin, 1802) and a striped skaapsteker (*Psammophylax tritaeniatus* (Günther, 1868)). Five possible new infections of lizard malarias are described from three families of lizards, and five species of unidentified microfilaria are noted in the blood of Agamid, Gekkonid and Cordylid lizards.

MATERIALS AND METHODS

All reptiles were collected in the Free State with permission from the Free State Province Department of Tourism, Environment and Economic Affairs (Permit nr. HK/P1/03894/002) (see appendix A). Reptiles collected in Lesotho were collected with permission from the Ministry of Environment and Tourism, Lesotho. Reptiles were captured at various localities in the Free State and Lesotho (Fig. 2.1 & 2.2), and then released back into their habitats at the same collection sites. Table 2.1 provides details of the reptiles collected in nine main areas.

2.1 Collections

Representatives of the Gekkonidae Cuvier, 1817, Agamidae Gray, 1827, Scincidae, Cordylidae Gray, 1837, Lacertidae Bonaparte, 1831 and Varanidae Hardwicke & Gray, 1828 were collected by hand where possible. A noose was used to catch specimens lodged inside rock cracks and a crowbar was employed to lift large rocks. *Cordylus giganteus* were collected by inserting a 10cm nail into earth above burrows and a nylon noose was attached to the nail. Specimens were trapped in the nooses as they left the burrows. After blood samples had been taken, specimens were released back into the same burrows.

All snakes were collected by hand and a 1.2m long (9mm diameter) aluminium rod was used to pin venomous snakes to the ground. These snakes were then put head-first into Perspex pipes ranging from 10mm to 60mm in diameter for further investigation.

2.2 Blood sampling, light microscopy and image capture

Reptile blood was sampled mainly in the field, but also in the laboratory. Lizard blood was collected from toe clips or a tail end, using sharp scissors. Snakes were put in Perspex pipes (as above) to make tail clips. In some cases a micropipette was used to draw blood from the clip site. Blood samples were smeared on clean glass slides marked with each reptile's identity, age and sex (if possible), as well as date and site of capture.

Smears were then air-dried and stored in dust-free slide boxes for transport back to the laboratory.

In the laboratory, these thin blood films were fixed in absolute methanol for at least 60 seconds. Fixed blood films were then stained with 10% Gurr's Giemsa Improved R66 stain solution (10ml in 90ml tap water) for up to one hour. Blood films were then examined with a Zeiss Axiophot photo microscope using 63X and 100X oil immersion objectives. Slides were also examined at Kingston University (UK) with a Zeiss Axiophot 20 microscope and a 100X oil immersion objective lens. Images from the Axiophot 20 were captured by Nikon digital camera (DN100), and stored on computer discs as JPEG files. These images were then measured using an Eclipse Net (Nikon) image analysis package calibrated to a stage micrometer.

2.3 Transmission electron microscopy

Two giant girdled lizards (*Cordylus giganteus*), one puff adder (*Bitis arietans*) and one captive African rock python (*Python sebae natalensis*) (permit nr: HK/P19C/03894/002) were sampled for electron microscopy. Four to five drops of fresh blood from each reptile were dropped into 2.5% glutaraldehyde (10ml in 90ml 0.2M Sorensen's phosphate buffer at pH 7.2). Glutaraldehyde-fixed material was then centrifuged at 10000rpm and the pellet washed in 0.2M Sorensen's phosphate buffer and post-fixed with a 2% solution of osmium tetroxide in 0.2M phosphate buffer. Post-osmication, the pellet was rinsed in buffer and then dehydrated in a graded series (30 – 100%) of ethanol solutions. The final dehydration in ethanol (100%) was dried over a 4Å molecular sieve. Ethanol was removed by transfer of the sample through three changes of propylene oxide (1,2 epoxy propane). The pellet was then left for 12 hours in a mixture of one volume propylene oxide mixed with three volumes of Agar 100 resin. The epoxy resin mixture was made by mixing the following components: 23 ml Agar 100 resin, 12 ml methyl nadic anhydride and 15 ml dodecaenyl succinic anhydride, with dropwise addition of 1.5 ml benzyl dimethyl amine during mixing.

Blood pellets were transferred from the propylene oxide/Agar 100 mixture into freshly made Agar 100 resin mixture (2 x 24 hour changes), then transferred to fresh Agar 100 resin mixture in silicone rubber embedding moulds. The resin mixture was polymerised at 60 degrees Celsius for 48 hours. Sections, showing pale gold interference colours were cut from the blocks of embedded tissue, using glass knives on a Huxley Mark II ultramicrotome and collected on copper, 300 hexagonal-mesh grids. The sections on the grids were stained for 20 minutes with a solution of 10% uranyl acetate in Analar grade methanol, washed with Analar methanol and allowed to dry. They were then stained for 20 minutes with Reynold's lead citrate solution, washed with 0.02M sodium hydroxide solution followed by distilled water, before examination with a JEOL JEM-1010 transmission electron microscope operated at 80-100 kV. Digital images were captured using a Soft Imaging Systems' Mega View III camera mounted in the microscope column.

All chemicals used were obtained from Agar Scientific Ltd, 66A Cambridge Road, Stansted, Essex, England with three exceptions. The Analar methanol and the 4Å molecular sieve were supplied by BDH Laboratory Supplies, Poole, Dorset, England, and the Analar ethanol was obtained from Hayman Ltd, East Ways Park, Witham, Essex, England.

2.4 Cell infection data

Levels of infection (intensity) among blood cells (erythrocytes) were calculated by taking 10 random fields on each slide with the 63X objective and 2 x converter and with a Zeiss Axiophot 20 microscope and a 100X oil immersion objective lens. In each sample field the number of cells ranged from four to 150 but on average ±50 blood cells were counted of which some were infected. This was repeated 10 times in different areas of the slide. Filarial nematodes were counted in a field using the 10x objective, with an average of 500 host cells counted in the same field.

2.5 Digital imaging

Pictures of hosts were taken from field guides of Branch (1998) and Patterson (1987) by aid of a Nikon Coolpix 990 digital camera. Images were digitally reduced of noise, resampled, cropped, sharpened and flipped in Corel draw™ 10. These selected images only serve as illustrations for the thesis and are not intended for publication purposes.

2.6 Miscellaneous

Names of host species collected from South Africa are provided with author names. Those host localities from other than South African are without authors. The author name of Scincidae was not found.
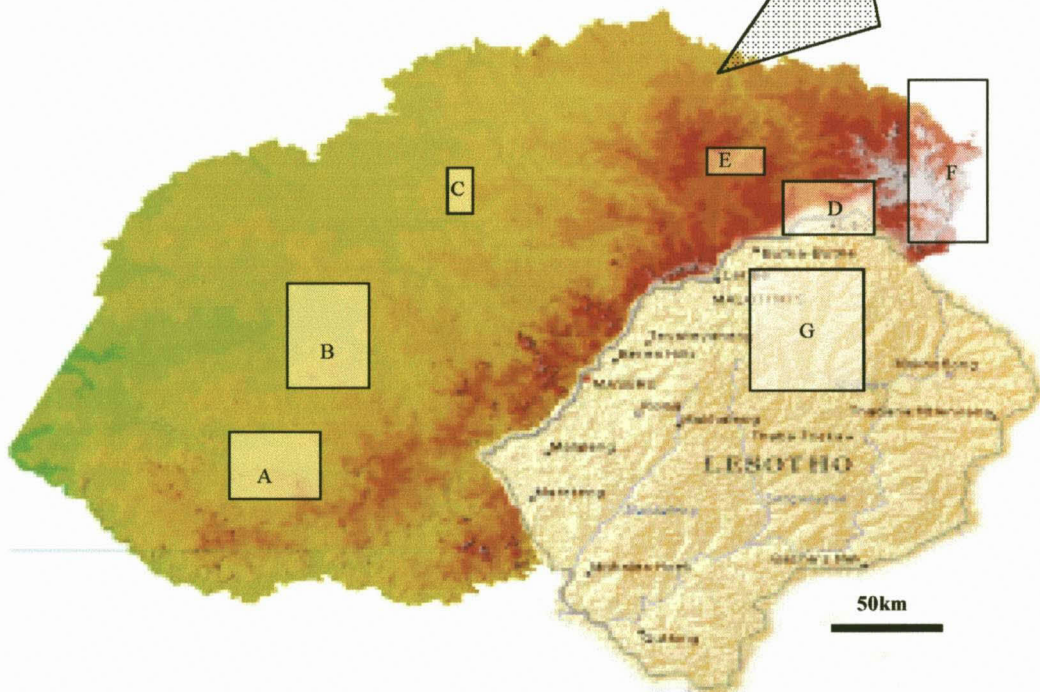
Fig.2.1 Map of South Africa, Free State and Lesotho showing collection localities. Collections on farms and other sites are shown in marked areas.   A: Jagersfontein district (The farm Zuurfontein), B: Bloemfontein and Brandfort districts (The farms Deelhoek and Hopefield), C: Koppies district (The farm Koffielaagte), D: Clarens district, E: Reitz district, F: Harrismith district G: Lesotho. Map taken and digitally adapted from Microsoft Encarta™ Scale: 50 Km.

Fig.2.2 Map of Lesotho where collections were made showing the three Catchment areas (highlighted areas) with the nearby villages indicated in red. Scale 50 km and 5 km respectively. Map taken and digitally adapted from Microsoft Encarta™

Table 2.1 Main collection sites where specimens were collected. The total number of specimens collected is indicated in column Number Captured. In South Africa eight different districts are presented where collec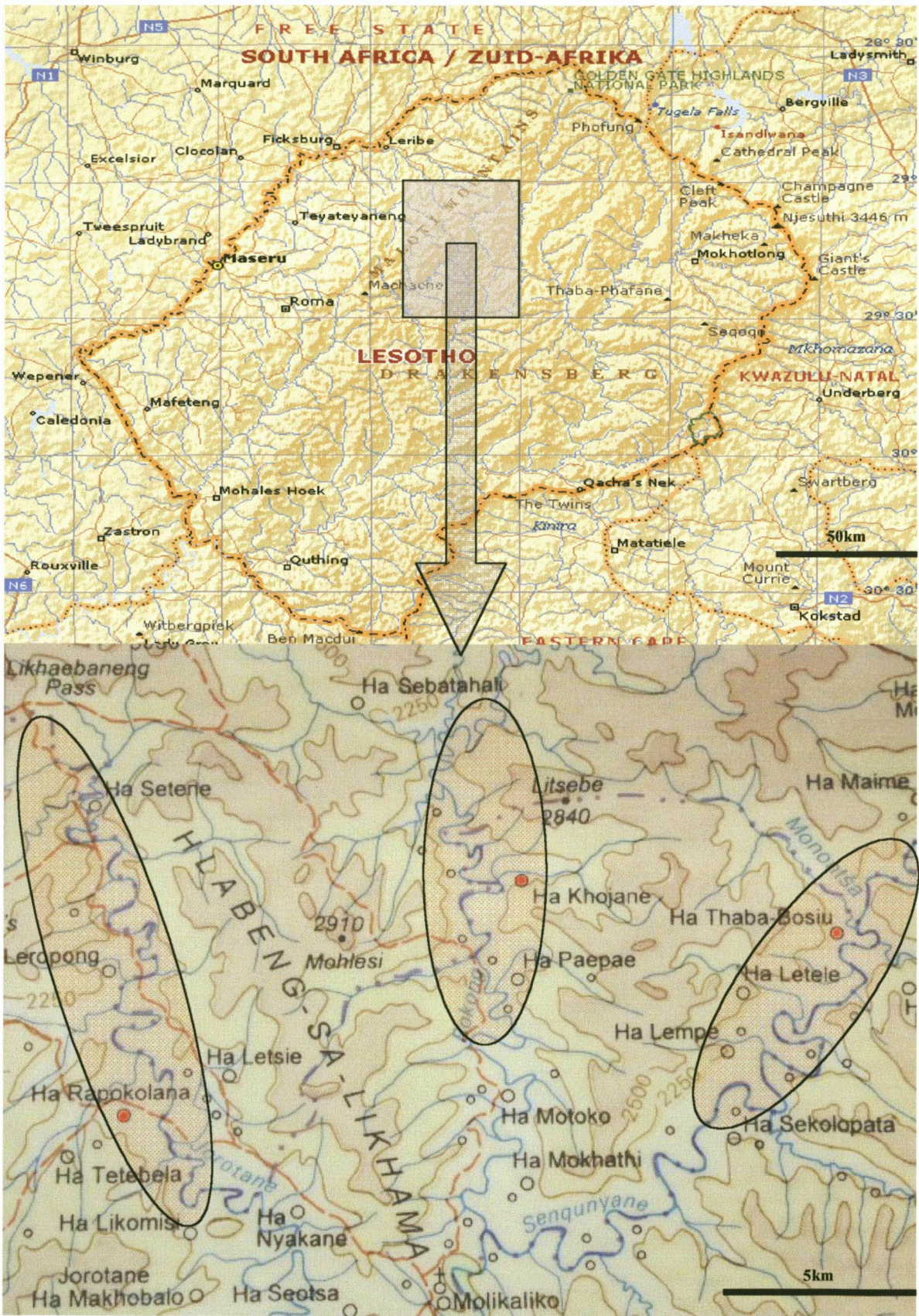tions were made. Collected specimens from various parts in Lesotho and endemic species to these regions are given. The presence of collected specimens in a particular district site is indicated with X

Host	NUMBER CAPTURED	BLOEMFONTEIN	CLARENS	ZUURFONTEIN	DEELHOEK	REITZ	HARRISMITH	BRANDFORT	KOFFIELAAGTE	LESOTHO	ENDEMIC
Sauria											
Gekkonidae Cuvier, 1817											
L .capensis	1	X									
P. bibronii	2			X							E
P. capensis	4	X		X	X						E
Agamidae Gray, 1827											
A. atra	65	X	X	X			X	X		X	E
A. hispida	1			X							E
Scincidae											
A. gracilicauda	2	X								X	E
M. capensis	3			X							
M. striata punctatissima	1	X									
M. sulcata	6			X							
Cordylidae Gray, 1837											
C. giganteus	31								X		E
C. polyzonus	46	X		X				X			E
P. melanotus melanotus	12		X				X				E
P. melanotus subviridis	35									X	E
Lacertidae Bonaparte, 1831											
N. intertexta	1						X				E
Varanidae Hardwicke & Gray, 1828											
V. albigularis	1			X							

Table 2.1 (continued). Main collection sites where specimens were collected. The total number of specimens collected is indicated in column Number Captured. In South Africa eight different districts are presented where collections were made. Collected specimens from various parts in Lesotho and endemic species to these regions are given. The presence of collected specimens in a particular district site is indicated with X

Host	NUMBER CAPTURED	BLOEMFONTEIN	CLARENS	ZUURFONTEIN	DEELHOEK	REITZ	HARRYSMITH	BRANDFORD	KOFFIELAAGTE	LESOTHO	ENDEMIC
SERPENTES Linnaeus, 1758											
Colubridae Gray, 1825											
A. capensis	5				X						
C. hotamboeia	6	X									
D. scabra	18				X						
L. aurora	2	X			X						
L. capense	2	X			X						
L. fuliginosus	12	X			X						
L. guttatus	5									X	
P. cana	1	X									
P. notostictus	1				X						
P. tritaeniatus	6	X			X						
Elapidae Boie, 1827											
E. sundevallii media	3				X						
H. haemachatus	2					X				X	E
Viperidae Gray, 1825											
B. arietans	2	X									
C. rhombeatus	1		X								

RESULTS

A list of the reptiles collected in the Free State and Lesotho for this study is provided below. The terminologies of De Waal (1978) and Branch (1998) are used in the descriptions of these reptiles. For each species the following information is provided: synonyms, range and distribution patterns, characteristics, general biology and breeding, and a short note on the haematological findings for the present study. Most parasitic infections are new host records from the Free State, except for the viral infection known as *Pirhemocyton,* found earlier in the blood of the skink *Mabuya capensis* (Gray, 1830) and the agama *Agama atra* by Paperna & De Matos (1993a). *Sauroplasma thomasii* from *Cordylus giganteus* and *Sauromella haemolysus* from *Pachydactylus capensis* have also been reported previously from the Free State.

3.1 HOSTS COLLECTED

SAURIA: GECKONIDAE

Lygodactylus capensis capensis (A. Smith, 1849) Cape dwarf gecko (Fig. 3.1A)

Synonyms: *Hemidactylus capensis* (A. Smith, 1849)

Lygodactylus strigatus Gray, 1864

Range: Mpumalanga, KwaZulu-Natal, Northern Cape, northwestern Free State, Zaire, Angola and Botswana (De Waal, 1978).

Characteristics: Nostril bordered by two nasals, anterior nasals separated by one granule; mental with deep lateral clefts; post-mentals two to three; supra-labials seven to nine, infra-labials six to seven, males pre-anal pores five; original tail with six or seven scales above.

Biology: These geckos have been translocated to various regions such as Bloemfontein, Port Elizabeth and Grahamstown (Branch 1998). They prefer to forage in low shrubs, but can also tolerate urban areas. They live for 15-18 months and sexual maturity is reached in eight months. Hard-shelled eggs are laid in cracks or under loose bark.

Haematological observations: A single specimen was caught in the urban area of Bloemfontein (Fig. 2.1) and was infested with mites. The peripheral blood showed a high infection of a hitherto undescribed haemogregarine. It also had a suspected viral infection (possibly *Pirhemocyton*).

Pachydactylus capensis capensis (A. Smith, 1845) Cape thick-toed gecko (Fig. 3.1B)

Synonyms: *Tarentola capensis* A. Smith, 1845

Pachydactylus elegans Gray, 1845

Pachydactylus leopardinus Sternfeld, 1911

Range: Throughout Plateau areas of Southern Africa (FitzSimons 1943; Loveridge 1947).

Characteristics: Nostrils bordered by two or three nasals, anterior nasals in contact or separated by one granule; supra-labials six to eight, seldom five or nine; infra-labials five to seven; five transversely enlarged adhesive lamellae under fourth toe; two or three underdeveloped tubercles on either side of base of tail; original tail verticillate, four scale rows per vertical above, with second, third or last row enlarged, into keeled tubercles.

Biology: Commonly found under stones, logs, old termitaria and occasionally in cracks of houses. Nocturnal and prey on insects. Clutches of two eggs are laid in old termitaria; incubation time is 90-110 days.

Haematological observations: Four specimens were collected and examined from Bloemfontein and Jagersfontein districts; all four were infested with mites. The blood smears showed a heavy infection of so-called *Sauromella haemolysus*.

Pachydactylus bibronii (A. Smith, 1845) Bibrons thick-toed gecko (Fig. 3.1C)

Synonyms: *Tarentola bibronii* A. Smith, 1845

Homodactylus tuneri Gray, 1864

Pachydactylus bibronii var. *stellatus* Werner, 1910

Pachydactylus bibronii pulitzerae Schimdt, 1933

Range: Restricted populations in the Cape Provinces, just extending into adjacent Free State and Namibia (Branch 1998). Mpumalanga and KwaZulu-Natal, Botswana, Malawi, Zambia, Angola to Tanzania (Fitzsimons 1943; Loveridge 1947).

Characteristics: Large stout gecko with strongly keeled tubercles separated by granular scales on back. Middle row of scales below toes and above scansors not enlarged (Branch 1998). Ten to 13 transversely enlarged adhesive lamellae under fourth toe. Original tail verticillate, four to six rows per scale above, one row consisting of large keeled stellate tubercles; sub-caudals in periods of two, sometimes divided anteriorly.

Biology: These geckos are found in rock outcrops. Many of them are translocated to urban areas and can sometimes be found in and around houses. They are gregarious and can often be found in dense colonies. They feed on a variety of insects, but smaller geckos can also be taken. Two eggs are laid under bark or in a rock crack.

Haematological observations: Two of these geckos were collected on the farm Zuurfontein (Fig. 2.1) and both specimens had a malaria infection. Both of these geckos also had a suspected viral infection.

SAURIA: AGAMIDAE

Agama atra atra Daudin, 1802 South African rock agama (Fig. 3.1D)

Synonyms: *Agama micropholis* Matschie, 1890

Agama micropterolepis Boulenger, 1896

Agama holubi Bocage, 1896

Agama atra var. *rudis* Boulenger & Power, 1921

Range: Throughout the Cape Province, absent from sandy areas, South Namibia and east to the escarpment KwaZulu-Natal and Maputuland (Branch 1998). Southern Namibia and southeastern corner of Botswana (De Waal 1978). No specimens recorded in Kruger National Park (Pienaar 1966).

Characteristics: Mid body scale rows 120-150, seldom as low as 109 and high as 170. Supra-labials 10-15, mostly 13-14; pre-anal pores in males10-16; fourth toe longer than third. Lamellae under fourth toe 16-20, seldom 15 or 22. White vertebral streak running from nape to base of tail; throat, chest and ventral parts of upper arm greenish blue; lateral side of body rust-red; tail often yellow with dark cross bands.

Biology: Agamas live in rocky outcrops and in mountain ranges. They are colonial and can form dense colonies, according to Branch (1998) up to 165 specimens per hectare can be found. Male territories are approximately 90m. Their diet is mostly insectivorous, but plant matter can also be taken. Females dig a shallow hole in damp soil and lay eggs which take 2-3 months to hatch.

Haematological observations: Sixty-five of these lizards were collected from various parts of the Free State and Lesotho, but the greatest number of these was from a farm Zuurfontein near Jagersfontein (Fig 2.1). Most lizards (precise numbers not recorded) were infested with mites and some had engorged ticks behind their legs or in the neck folds. The blood infections of these lizards showed a great diversity of parasites.

Infections ranged from viral-like infections (*Pirhemocyton*), haemogregarines, suspected malaria and filarial nematodes.

Agama hispida (Linnaeus, 1758) Southern spiny agama (Fig. 3.1E)

Synonyms: *Lacerta hispida* Linnaeus, 1758

       Agama aculeata Merrem, 1820

       Agama armata Peters, 1845

       Agama infralineata Peters, 1877

       Agama brachyura Boulenger, 1885

       Agama distanti Boulenger, 1902

Range: Two varieties, Southeastern (Kalahari form) and the Eastern variety. The Eastern variety occurs in South central and an isolated population in the Northwestern Free State, (Branch 1998).

Characteristics: Medium-sized agama with broad head and rounded snout. Ear holes small and tympanums cannot easily be seen. Scales overlap from head towards tail (Branch 1998). According to de Waal (1978), mid body scale rows 84-112 supra-labials 10-14, pre-anal pores in males 10-14; fourth toe shorter than third; fifth toe shorter than equal to first; tail shorter than body and head in females; males dorso-ventrally dark; females with large dark spots on either side of vertebral band.

Biology: These lizards dig a short tunnel at the base of a bush in sandy areas; their main diet is ants and beetles. They are solitary and females lay seven to 11 eggs in spring.

Haematological observations: A suspected viral infection (*Pirhemocyton*) was present in the peripheral blood of one specimen caught on the farm Zuurfontein.

SAURIA: SCINCIDAE

Mabuya capensis (Gray, 1830) Cape three-striped skink (Fig. 3.1F)

Synonyms: *Scincus trivittatus* Cuvier, 1829

Tiliqua capensis Gray, 1838

Tiliqua ascensionis Gray, 1830

Euprepes merremi Dumeril & Bibron, 1839

Range: Zambia, Botswana, Zimbabwe (Broadley 1966), Namibia (Mertens 1955), rest of South Africa except for arid areas of the Western Cape (FitzSimons 1943). According to Branch (1998) there are relict populations in the Inyanga Mountains in Zimbabwe and Luiwa Plain in Zambia.

Characteristics: In De Waal's (1978) terminology, centre of nostril always posterior to structure of rostal. Supra-nasals always in contact; pre-frontals usually in contact; supra-labials four; 32-38 scale rows around middle of body; lamellae under fourth toe 15-20; light brown or grey-brown; three pale stripes on back extending to tail.

Biology: Found in numerous habitats, including around houses, termitaria and in open fields. They dig in loose sand, favour fallen logs and rocky outcrops. The female gives birth to five to 18 young, but in Pretoria and Port Elizabeth females have been known to lay clutches of eggs.

Haematological observations: Three specimens were collected on the farm Zuurfontein. Two of these had mite infestations and all three had *Sauroplasma* -like infections.

Mabuya sulcata sulcata (Peters, 1867) Koppie skink (Fig. 3.1G)

Synonym: *Euprepes sulcata* Peters, 1867

Range: From Southern Angola, south through Namibia, Northern half of the Cape and the south Free State (FitzSimons 1943).

Characteristics: Centre of nostril posterior to suture of rostral/ first supra-labials; supra-nasals always in contact (De Waal 1978). Coloration varies between sexes; males completely jet-black on dorsal and ventral sides. Females and juveniles pale olive to olive brown with six golden stripes on dorsal surface.

Biology: These are active skinks that live in rocky outcrops and feed on insects. They shelter in rock cracks where they have three to five young. According to Branch (1998) there are informal reports that these skinks also lay eggs.

Haematological observations: Six of these skinks were collected on the farm Zuurfontein near Jagersfontein. The peripheral blood showed an infection of *Sauroplasma*-like inclusions in all six specimens studied.

Mabuya striata punctatissima (A. Smith, 1849) Common striped skink (Fig. 3.1H)

Synonyms: *Euprepes punctatissimus* A. Smith, 1849

Euprepes sunderallii A. Smith, 1849

Euprepes grützneri Peters, 1869

Range: Eastern temperate highveld regions of South Africa to southeastern Botswana. Relict populations exist on the Eastern Highlands of Zimbabwe (Broadley 1966).

Characteristics: Centre of nostril posterior to suture of rostral or super-labial; supra-nasals in contact; pre-frontals usually separated; lamellae under fourth toe 16-21; yellow dorso-lateral stripe; scales between these stripes are pale spots.

Biology: They feed on invertebrates and are active climbers of rocks and the habitats are varied. According to Branch (1998) the southern populations give birth to three to nine young.

Haematological observations: Infections resembling *Sauroplasma*, were present in the red blood cells of one specimen collected in Bloemfontein suburbs.

Acontias gracilicauda gracilicauda Essex, 1925 Legless skink (Fig. 3.11)

Range: North East Cape, Free State, Southern Mpumalanga and Northern Cape Province (Broadley 1966). Two isolated populations in Little Namaqualand, Eastern Cape and Free State (Branch 1998)

Characteristics: According to De Waal (1978), three sub-oculars; second supra-labials usually entering eye, five supra-labials, scale rows 18; sub-caudals 30 to 40. According to Branch (1998), lower eyelids opaque, coloration pale golden olive to olive brown.

Biology: These skinks show a preference for compact, moist soils. Females give birth to two young in February (Branch 1998).

Hematological observations: Infections resembling *Sauroplasma*, were present in the peripheral blood erythrocytes of one specimen captured in Bloemfontein and one captured in Lesotho.

SAURIA: CORDYLIDAE

Cordylus giganteus A. Smith, 1844 Giant girdled lizard (Fig. 3.1J)

Synonym: *Zonurus derbianus* Gray, 1845

Range: Northeastern half of the Free State and adjacent areas of southern Gauteng (Loveridge 1944). Scattered populations in southeast Mpumalanga and extreme western KwaZulu- Natal (Branch 1998).

Characteristics: Nasals separated by rostral; four large occipital spines; supra-labials five to seven; males with nine to 13 pores and a patch of differentiated femoral scales; females with 10-13 femoral pores (De Waal 1978). Tails have very large spines. Lateral sides are golden yellow.

Biology: These vulnerable lizards live in colonies in burrows they dig in soil. These are usually about 17 meters apart, 50 centimeters under ground and 2 meters long. They are long lived, up to 20 years and females give birth to one or two young every two-three years.

Haematological observations: Thirty-one of these lizards were collected on the farm Koffielaagte near Kroonstad, in the Koppies district (Fig. 2.1). The locality consists of two disjunct populations, separated by cultivated lands. Light microscopy showed *Sauroplasma thomasi* infections in all captured specimens, but TEM studies failed to reveal the true nature of these infections and further examination is needed to establish what these enigmatic structures represent.

Cordylus polyzonus polyzonus A. Smith 1838 Karoo girdled lizard (Fig. 3.1K)

Range: Southern half of Free State, Karoo and Western Cape, into Namibia (FitzSimons 1943).

Characteristics: Two nasals; supra-nasals in contact; occipitals four to eight, supra-labials anterior to sub-ocular four, dorsal scales 39-45 transverse and 32-41 longitudinal rows; femoral pores in males 10-15, lamellae under fourth toe 13-16, mostly 15 to 16, dorsal color brown to black, ventral lighter brown below.

Biology: Found on low lying koppies in rocky outcrops. Insectivorous and two or three large young are born in late summer.

Haematological observations: Forty six of these shy lizards were caught in Bloemfontein, Jagersfontein and Brandfort districts (Fig. 2.1). Most of these were collected on a farm at Zuurfontein and most of these lizards were infected with malaria. This type of infection is not mentioned in existing literature for *C. polyzonus polyzonus*, and therefore this represents a new host record for this blood infection. All of the specimens examined also harbored a *Sauroplasma*-like infection. The specimens collected in the Brandfort district had viral infections, haemogregarines, malarias and microfilarial nematodes. These are also new host records.

Pseudocordylus melanotus melanotus (A. Smith, 1838) Highveld girdled lizard (Fig. 3.1L)

Synonym: *Cordylus (Pseudocordylus) melanotus* (A. Smith, 1838)

Range: Eastern Free State (FitzSimons 1943), Gauteng to Mpumalanga escarpment to northern KwaZulu Natal and North to Northeastern Free State (Branch 1998).

Characteristics: *Pseudocordylus m. melanotus* has a divided fronto-nasal and the femoral pits in females are only shallow pits. Breeding males has one to 17 glandular femoral scales, a broad brown band around neck and a diffuse grey patch on throat.

Biology: Found in large colonies with a single dominant male. They are ambush predators and feed on small insects, as well as vegetable matter. One to six young are born in late summer.

Haematological observations: A total of 12 lizards were collected, four from the Clarens district and five in the Harrysmith district in Collins Pass (Fig. 2.1). All of these lizards had heavy infections of *Sauroplasma* and some of them had malaria and haemogregarines. None of these parasitic haematozoan infections have been recorded from this host in previous surveys.

Pseudocordylus melanotus subviridis (A. Smith, 1838) Drakensberg girdled lizard (Fig. 3.2A)

Range: Mount-aux-Sources through Lesotho and Transkei, with an isolated population in the Amatola Mountains in Eastern Cape (Branch 1998).

Characteristics: According to Branch (1998) this smaller race does not exceed 118cm in snout-vent length and has undivided fronto-nasal, with large lateral scales.

Biology: Found in large colonies with a single dominant male. They are ambush predators and feed on small insects, as well as vegetable matter. One to six young are born in late summer.

Haematological observations: Thirty-five lizards were collected during a survey done in Lesotho. The infections ranged from viral-like (*Pirhemocyton*), *Sauroplasma*-like to three types of haemogregarines, as well as malaria and filarial nematodes.

SAURIA: LACERTIDAE

Nucras intertexta (A. Smith, 1838) Spotted sandveld lizard (Fig. 3.2B)

Synonyms: *Lacerta intertexta* A. Smith, 1838

Nucras tesselata var. *ocellata* Boulenger, 1910

Range: Western Free State, Northern Cape, Botswana, Namibia, Northern Mpumalanga, southern Mozambique and south eastern Zimbabwe (Broadley 1972).

Characteristics: Parietal foramen present, three nasals, three to six granules between supra-ciliaries and sub-oculars (De Waal 1978). Eleven to fifteen pre-femoral pores on each thigh. Belly cream white and lateral scales have distinct cream spots.

Biology: These lizards forage widely in open savanna and search for slow moving food and even other lizards.

Haematological observations: One of these lizards was collected on Collins Pass in the Harrismith district (Fig. 2.1), and had a *Sauroplasma*-like infection.

SAURIA: VARANIDAE

Varanus exanthematicus albigularus (Daudin, 1802) Rock or Tree Leguaan (Fig. 3.2C)

Synonyms: *Tupinambis albigularus* Daudin, 1802

Varanus gillii A. Smith, 1831

Monitor exanthematicus var. *capensis* Schlegel, 1844

Varanus exanthematicus ionidesi, Laurent, 1964

Range: Savannas of southern and eastern Africa (Broadley 1966). Absent from West Cape (Branch, 1998)

Characteristics: Large stout lizard with sharp claws and stocky limbs. Head with a bulbous snout. Tail longer than body, cylindrical at base and compressed laterally. Dorsal dark brown, with five to six pale yellow markings on back (Branch 1998).

Biology: These lizards live in burrows or under fallen logs. They are scavengers, insectivorous and will kill and eat any animal small enough to swallow (Branch 1998).

Haematological observations: One juvenile was sampled. It was found in a rocky outcrop on the farm at Zuurfontein (Fig. 2.1). Two mosquitoes were observed taking a blood meal on one of its eyelids. The blood smears revealed a *Sauroplasma*-like infection in the erythrocytes, as well as haemogregarines.

SERPENTES: BOIDAE

Python sebae natalensis (Gmelin, 1789) African rock python (Fig. 3.3F)

Synonyms: *Coluber speciosus* Bonnaterre, 1789

Boa hieroglyphica Schneider, 1801

Python bivittatus Kuhl, 1820

Python natalensis A. Smith. 1833

Hortulia sebae Gray, 1840

Range: Restricted to north and north-eastern parts of Southern Africa, Eastern Cape, Pondoland and Transkei (FitzSimons 1962).

Characteristics: Rostral as broad as deep; two elongate pits on either side; internasals one and a half times as long as broad; supraocular broken into two shields; a pair of frontals broken into smaller shields; eight to 12 scales around eye; 12 to 15 upper labials;

first two are deeply pitted; smooth scales 71 to 93 at midbody; ventrals 265 to 286; anal plate divided or undivided; vestiges of hind legs are visible as spurs either side of vent (FitzSimons 1962).

Biology: Common in well flooded valleys, plantations and bushveld. Seldom far from permanent water where they may lie submerged for long periods on end. Non-venomous with needle-sharp solid recurved teeth. Diurnal and feeds on mammals, can swallow large prey. Female lays 30-50 (exceptionally up to 100 eggs) and incubates and protect the eggs.

Haematological observations: One captive specimen in Bloemfontein had a haemogregarine infection (*Hepatozoon sebae* (Laveran & Pettit, 1909) Smith, 1996) in its red cells. It also had a *Serpentoplasma*-like infection.

SEPENTES: COLUBRIDAE

Lamprophis aurora (Linnaeus, 1754) Aurora-or Night snake (Fig. 3.2D)

Synonym: *Coluber aurora* Linnaeus, 1754

Range: Eastern Cape, Free State, Lesotho, Southern KwaZulu Natal and Mpumalanga (FitzSimons 1962).

Characteristics: Pre-ocular one; post-oculars two; supra-labials eight, fourth and fifth entering orbit; infra-labials eight (De Waal 1978). Scales smooth with 21-23 rows; sub-caudals 28-34 (Branch 1998).

Biology: This snake is not common and feeds on nestling rodents. It can sometimes be found in old termitaria.

Haematological observations: Two specimens were collected. One on the farm Deelhoek (Fig. 2.1) 30 kilometers north of Bloemfontein and one in Bloemfontein suburbs itself. Both of these snakes had what appeared to be a *Serpentoplasma* infection in the red blood cells.

Lamprophis guttatus (A. Smith, 1843) Spotted house snake (Fig. 3.2E)

Synonyms: *Lycodon guttatus* A. Smith, 1843

  Alopecion annulifer Dumeril & Bibron, 1854

Range: Southern Cape, Great Namaqualand, KwaZulu-Natal, Eastern Mpumalanga, Eastern Free State. Inland Mountains of the Cape and Cape Fold Mountains (FitzSimons 1962)

Characteristics: A small slender snake, with 21-25 scale rows and large eyes. Blotches on dorsal side, varying according to habitat.

Biology: It shelters in rocky areas where it preys on geckos and other lizards. The female lays three to six elongated eggs in midsummer.

Haematological observations: All five specimens captured in Lesotho had an infection resembling *Serpentoplasma* in the erythrocytes of this snake species.

Lamprophis fuliginosus (Boie, 1827) Common house snake (Fig. 3.2F)

Synonyms: *Lycodon fuliginosus* Boie, 1827

  Lycodon unicolor Schlegel, 1837

  Boaedon quadrivittatum Hallowel, 1857

  Boaedon quadrilineatum Dumeril, 1859

  Alopecion variegatum Bocage, 1867

Boodon bipraeocularis Günhter, 1888

Boodon lineatus var. *plutonis* Werner, 1902

Range: All over Sub Saharan Africa (Branch 1998).

Characteristics: Posterior sub-linguals usually in contact, separated by prolongation of anterior sub-linguals; pre-oculars one or two (De Waal 1978). Large head with two pale yellow streaks along head, small body scales with 27-29 rows (Branch 1998).

Biology: These nocturnal snakes forage for rodents and sometimes bats, they seldom take lizards. They usually occupy old termitaria and can be found in almost any habitat, as they usually occur nearby houses.

Haematological observations: Twelve specimens were collected in Bloemfontein suburbs and on the farm Deelhoek. All the specimens studied had *Serpentoplasma*-like blood infections.

Lycophidion capense capense (A. Smith, 1831) Cape wolf-snake (Fig. 3.2G)

Synonyms: *Lycodon capensis* A. Smith, 1831

Lycodon horstokii Schlegel, 1837

Range: Throughout most of Southern Africa, except the western parts of the Cape and Namibia (FitzSimons 1962).

Characteristics: Flattened head; post-nasal touches - first upper labial. Ventral scales are 150-205 (Branch 1998). Sub-caudals 32-41 in males; 24-31 in females (De Waal 1978).

Biology: This species prefers well-vegetated areas and the Cape skink (*Mabuia capensis*) is an important part in their diet.

Haematological observations: A *Serpentoplasma*-like infection was observed in the erythrocytes of two specimens captured in Bloemfontein and the farm Deelhoek.

Pseudaspis cana (Linnaeus, 1758) Mole-snake (Fig. 3.2H)

Synonyms: *Coluber canus* Linnaeus, 1758

Coluber elegantissimus Laurenti, 1768

Coluber ocellatus Gmelin, 1789

Cadmus cuneiformis Theobald, 1868

Coronella phocarum Günter, 1872

Ophirhina anchietae Bocage, 1882

Range: Throughout Southern Africa and on Robben Island, elsewhere Angola and Kenya (Branch 1998).

Characteristics: Ventral scales 186-197 in males, 206-218 in females (De Waal 1978). A large thick snake with hooked nose. Body scales smooth, but sometimes keeled in black specimens from Western Cape (Branch 1998) and counts 25-31 rows. Eyes with round pupils. Young blotched, older specimens darker and more uniform in color.

Biology: They feed on moles, rodents, other snakes, eggs and lizards. They are constrictors that live underground.

Haematological observations: One specimen was collected from Bloemfontein; the presence of a *Serpentoplasma*-like infection was found in the erythrocytes of this snake.

Dasypeltis scabra (Linnaeus, 1758) Rhombic egg-eater (Fig. 3.2I)

Synonyms: *Anodon typus* A. Smith, 1929

Rachiodon abyssinus Dumeril & Bibron, 1854

Dasypeltis scaber var. *capensis* Peters, 1864

Dasypeltis scaber var. *mosambicus* Peters, 1864

Dasypeltis scaber var. *breviceps* Peters, 1864

Dasypeltis lineolatus Peters, 1878

Dasypeltis scabra loveridgei Mertens, 1954

Range: Africa, except high mountain peaks and dense lowland forest, Southern Arabia.

Characteristics: Post-ocular scales two; pre-ocular scales one; dorsal scales 21-28 and 17-22 anterior to vent (De Waal 1978). Lateral scales small and have serrated scales. Tail short, head rounded.

Biology: This snake is very common and can be found in old termitaria. It is adapted for a special diet of only eggs.

Haematological observations: A *Serpentoplasma*-like infection was observed in the red blood cells of 18 specimens captured from the farm Deelhoek.

Crotaphopeltis hotamboeia (Laurenti, 1768) Herald or Red-lipped snake (Fig. 3.2J)

Synonyms: *Coronella hotamboeia* Laurenti, 1768

Coronella virginia Laurenti, 1768

Coluber bicolor Leach, 1819

Ophis heterurus Duvernoy, 1833

Dipsas hippocrepis Reinardt, 1843

Dipsas inorhatus A. Smith, 1849

Oxyropus melanocrotaphos Cope, 1860

Range: Tropical Africa south of the Sahara, southwards over eastern Africa to Cape Town; absent from arid western deserts (FitzSimons 1966).

Characteristics: Ventral scales 146-160 in males, 146-161 in females; anal plate entire. Dorsal color dark brown with uniform white spots. Distinct black patch from eye to neck; upper lip orange red.

Biology: These snakes live in marshy areas and feed at night on amphibians. They can also be found in old termitaria.

Haematological observations: A *Serpentoplasma*-like infection was observed in the erythrocytes of six specimens from Bloemfontein.

Psammophylax tritaeniatus (Günther, 1868) Striped skaapsteker (Fig. 3.2K)

Synonym: *Rhagerrhis tritaeniatus* Günther, 1868

Range: Tanzania to Southern Zaire, Namibia, KwaZulu-Natal, Free State, Eastern Cape (Broadley 1966).

Characteristics: Ventral scales 151-160 in males, 153-169 in females; anal plate usually divided. Three black edged dark stripes on back, vertebral line is yellow.

Biology: These snakes forage in grass for small mammals; female lays five to18 eggs in a hole.

Haematological observations: Six specimens were collected in the Free State. Two were collected in Bloemfontein district and four on the farm Deelhoek, 30 Km north of Bloemfontein. All six of these snakes had a haemogregarine infection as well as *Serpentoplasma*-like infections in the blood. These also represent new records.

Psammophis notostictus Peters, 1867 Karoo sand snake or Whip snake (Fig.3.2L)

Synonyms: *Psamophis moniliger* var. *notostictus*

   Psammophis sibilans var *stenocephalus* Bocage, 1887

Range: Cape Province to Southwestern Angola (Broadley 1975). Southern Free State to Gauteng, Northern Province, Botswana and Tanzania (Branch 1998).

Characteristics: Upper labials four and five enter the eye; 17 scale rows; one pre-ocular; the tail is long: 80-107 sub-caudals; striped or blotched coloration.

Biology: These snakes are fast predators of skinks and lacertids. They live in old termitaria, under rocks and in rock cracks.

Haematological observations: One specimen was collected on the farm at Deelhoek (Fig. 2.1). Its erythrocytes contained *Serpentoplasma*-like inclusions, as well as an undescribed haemogregarine.

Aparallactus capensis A. Smith, 1849 Cape centipede eater (Fig. 3.3A)

Synonyms: *Cercocalamus collaris* Günther, 1863

          Aparallactus punctatolineatus Boulenger, 1895

          Aparallactus bocagii Boulenger, 1895

          Aparallactus lubberti Sternfeld, 1910

Range: Free State, KwaZulu-Natal, Eastern Cape (Broadley 1966).

Characteristics: Supra-labials six; ventral scales 141-157 in males, 159-173 in females; dorsum dark brown (De Waal 1978). Lower labials separated by mental black collar and brown head (Branch 1998).

Biology: These snakes are abundant in old termitaria, where they shelter and feed.

Haematological observations: A total of five specimens were collected at the farm Deelhoek near the Soetdoring Nature Reserve in the Bloemfontein district (Fig. 2.1). These snakes were easily obtained from old termitaria. Their blood contained a *Serpentoplasma*-like infection.

SEPENTES: ELAPIDAE

Elapsoidea sundevallii media Broadley, 1971 Sundevall's garter snake (Fig. 3.3B)

Range: Highveld areas of Free State and Mpumalanga, North Cape in the Kimberly region (Broadley 1971).

Characteristics: Ventral scales 161-164 in males; 143-152 in females; sub-caudals 21-22 in males, 14-17 in females, dorsum with alternating black and white cross bands.

Biology: These are slow moving snakes and reluctant to bite. Rarely seen, they live in old termitaria and under rocks. The venom has not yet been studied.

Haematological observations: Three specimens were collected on the farm Deelhoek 30 km north of Bloemfontein. All of these specimens had a *Serpentoplasma*-like infection in the blood.

Hemachatus haemachatus (Lacèpéde, 1788) Rinkhals (Fig. 3.3C)

Synonyms: *Vipere haemachate* Lacèpéde, 1788

Naja capensis A. Smith, 1826

Range: Eastern parts of the country, south of the 25° South latitutude line (FitzSimons 1966).

Characteristics: Sub-caudals 40-47 in males, 38-45 in females; ventrum black with white cross bands on ventral part of neck (De Waal 1978). Keeled scales, 17-19 rows, and broad head, interscaly areas of spread hood sometimes yellow-orange tinged (De Waal 1978).

Biology: Nocturnal, eats small vertebrates, spread a hood when cornered and can spit venom up to three meters.

Haematological observations: One specimen was collected near Excelsior in the Reitz district, Eastern Free State (Fig. 2.1). Its blood contained a *Serpentoplasma*-like infection similar to that seen in all the other snakes. The inclusions were, however, much bigger

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than those *Sauroplasma* infections found in lizards. Another specimen from Lesotho (Fig. 2.2) also had the same kind of *Serpentoplasma*-like infection.

SEPENTES: VIPERIDAE

Causus rhombeatus   (Lichtenstein, 1823) Night adder (Fig. 3.3D)

Synonyms: *Naja (Coluber)* var. *nigrum* Cuvier (sic) Boie, 1827

> *Causus rhombeatus* var. *taeniata* Sternfeld, 1912

Range: Sudan and Somalia, Cape, Zaire and Angola (Broadley 1966). Eastern regions and in Eastern Free State (FitzSimons 1966).

Characteristics: Ventral scales 136, anal plate entire, sub-caudals 23, dorsal scales in 19 rows on nape and mid-body, 13 anterior to vent.

Biology: It rests during the day in undergrowth, termitaria and under logs. At night it forages for amphibians, the venom glands are large, extending into neck.

Haematological observations: One specimen was collected in the Eastern Free State. Its blood contained *Serpentoplasma* –like inclusions.

Bitis arietans (Merrem, 1820) Puff adder (Fig. 3.3E)

Synonyms: *Vipera (Echidna) arietans* Merrem, 1820

> *Vipera inflata* Burchell, 1822

> *Vipera brachyura* Cuvier, 1829

> *Clotho lateristriga* Gray, 1842

> *Vipera inflata* Burchell, 1822

> *Vipera brachyura* Cuvier, 1829

> *Clotho lateristriga* Gray, 1842

Range: Throughout Africa, absent from rain forests and extreme deserts (FitzSimons 1962).

Characteristics: Ventral scales 135-142 in males, 134-146 in females, anal plate entire, sub-caudals 26-32 in males, 16-20 in females, yellow or white inter-ocular band (De Waal 1978). Heavily keeled scales, large triangular head with nostrils pointed upward (Branch 1998).

Biology: It emerges at dusk and waits and ambushes its prey. This snake relies on its camouflaging and poison action.

Haematological observations: Two specimens were collected in the Bloemfontein district. Both of these specimens also had *Serpentoplasma*-like infections in the peripheral red blood cells.

The following table (Table 3.1) provides a summary of infections in reptiles collected. It includes parasite species, hosts, localities and levels of infection.

Table 3.1 Summary of parasitic infections examined and presented from some of the reptiles collected with their localities. N indicates the number of specimens examined and (%) gives parasite prevalence. Intensity was calculated by number of infected erythrocytes in 10000 erythrocytes. In each case the highest intensity found is given.

Viral and viral-like infections (including so-called piroplasms)	Host	Locality	N (%)	Intensity
Pirhemocyton	Agama atra atra	Bloemfontein (26°04'S, 29°13'E), Zuurfontein (29°54'S, 25°22'E), Lesotho, Clarens (28°31'S, 28°25'E)	65/65(100)	2800/10000
Pirhemocyton	Cordylus polyzonus polysonus	Ha Rapokolana (29°22'36''S 28°01'53E)	1/1 (100)	8900/10000
Sauroplasma thomasi	Cordylus giganteus	Koffielaagte (27°29'S, 27°28'E)	31/31 (100)	7400/10 000
Sauroplasma	Cordylus polyzonus polyzonus	Zuurfontein (29°54'S 25°22'E)	38/46 (82.6)	6900/10 000
Sauroplasma	Mabuya sulcata sulcata	Zuurfontein (29°54'S 25°22'E)	6/6 (100)	1100/10 000
Sauroplasma	Mabuya striata punctatissima	Bloemfontein (26°04'S 29°13'E),	3/3 (100)	600/10 000
Sauroplasma	Nucras intertexta	Bloemfontein (26°04'S 29°13'E),	1/1 (100)	3700/10 000
Sauroplasma	Acontias gracilicauda gracilicauda	Bloemfontein (26°04'S 29°13'E),	1/1 (100)	600/10 000
Sauroplasma	Varanus exanthematicus albigularus	Zuurfontein (29°54'S 25°22'E),	1/1 (100)	2500/10 000
Sauromella haemolysus	Pachydactylus capensis	Deelhoek (28°54'S, 26°09'E)	4/4 (100)	900\10000

Table 3.1 (continued). Summary of parasitic infections examined and presented from some of the reptiles collected with their localities. N indicates the number of specimens examined and (%) gives parasite prevalence. Intensity was calculated by number of infected erythrocytes in 10000 erythrocytes. In each case the highest intensity found is given.

Viral and viral-like infections (include-ing so-called piroplasms)	Host	Locality	N (%)	Intensity
Sauroplasma	*Pseudocordylus melanotus subviridis*	Ha Rapokolana (29°22'36''S 28°01'53E), Ha Khojane (28°22'S, 28°01'E) and Ha Thaba Bosiu (29°20'15''S 28°11'58''E), Lesotho.	39/39 (100)	6780/10000
Sauroplasma	*Pseudocordylus melanotus melanotus*	Clarens (28°31'S, 28°25'E)	5/5 (100)	5620/10000
Serpentoplasma	*Bitis arietans arietans*	Bloemfontein (26°04'S 29°13'E)	2/2 (100)	800/10000
Serpentoplasma	*Crotaphopeltis hotamboeia*	Bloemfontein (26°04'S 29°13'E)	6/6 (100)	700/10000
Serpentoplasma	*Lycophidion capense capense*	Deelhoek (28°54'S, 26°09'E	2/2 (100)	2700/10 000
Serpentoplasma	*Causus rhombeatus*	Clarens (28°31'S, 28°25'E),	1/1 (100)	100/10000
Serpentoplasma	*Elapsoidea sundevallii media*	Deelhoek (28°54'S, 26°09'E),	3/3 (100)	85/10 000
Serpentoplasma	*Hemachatus haemachatus*	Excelsior	1/1 (100)	2500/10000
Serpentoplasma	*Hemachatus haemachatus*	Ha Rapokolana (29°22'36''S 28°01'53E)	1/1 (100)	3540/10000
Serpentoplasma	*Lamprophis aurora*	Deelhoek (28°54'S, 26°09'E)	2/2 (100)	750/10000
Serpentoplasma	*Lamprophis fuliginosus*	Deelhoek (28°54'S,26°)	12/12 (100)	900/10 000

Table 3.1 (continued). Summary of parasitic infections examined and presented from some of the reptiles collected with their localities. N indicates the number of specimens examined and (%) gives parasite prevalence. Intensity was calculated by number of infected erythrocytes in 10000 erythrocytes. In each case the highest intensity found is given.

Viral and viral-like infections (include-ing so-called piro-plasms)	Host	Locality	N (%)	Intensity
Serpentoplasma	*Psammophylax tritaeniatus*	Bloemfontein suburbs	5/5	300/10 000
Haemogregarines	**Host**	**Locality**	**N (%)**	**Intensity**
Hepatozoon sp.A	*Agama atra atra*	Ha Khojane (28°22'S, 28°01'E)	3/3 (100)	1263/10000
		Zuurfontein (29°54'S 25°22'E)	7/62 (3.5)	2200/10000
Hepatozoon sp. B	*Pseudocordylus melanotus melanotus*	Clarens (28°31'S, 28°25'E)	5/5(100)	6850/10000
Hepatozoon sp. C	*Pseudocordylus melanotus subviridis*	Ha Thaba Bosiu (29°20'15''S 28°11'58''E	7/8 (87.5)	1000/10000
Hepatozoon sp. D.	*Pseudocordylus melanotus subviridis*	Ha Rapokolana (29°22'36''S 28°01'53E),	4/4 (100)	1850/10000
Hepatozoon sp. E.	*Pseudocordylus melanotus subviridis*	Ha Rapokolana (29°22'36''S 28°01'53E),	3/3 (100)	550/10000
Hepatozoon sp. F	*Psammophylax tritaeniatus*	Bloemfontein (26°04'S 29°13'E)	5/5 (100)	150/10000
Hepatozoon (Haemogregarina) sebae	*Python sebae natalensis*	Bloemfontein (26°04'S 29°13'E)	1/1 (100)	450/10000

Table 3.1 (continued). Summary of parasitic infections examined and presented from some of the reptiles collected with their localities. N indicates the number of specimens examined and (%) gives parasite prevalence. Intensity was calculated by number of infected erythrocytes in 10000 erythrocytes. In each case the highest intensity found is given.

| Malarias	Host	Locality	N (%)	Intensity
Plasmodium sp. A	*Cordylus polyzonus polyzonus*	Zuurfontein (29°54'S 25°22'E)	17/38 (44.7)	3800/10000
Plasmodium sp. B	*Pseudocordylus melanotus melanotus*	Clarens (28°31'S, 28°25'E)	1/5 (20)	1200/10000
Plasmodium sp .C	*Pseudocordylus melanotus subviridis*	Ha Rapokolana (29°22'36''S 28°01'53''E)	3/4 (75)	800/10000
Plasmodium sp. D	*Pachydactylus bibronii*	Zuurfontein (29°54'S 25°22'E)	2/2 (100)	4100/10000
Microfilariae	**Host**	**Locality**	**N (%)**	**Intensity**
Microfilaria sp. A	*Agama atra atra*	Ha Rapokolana (29°22'36''S 28°01'53''E) Ha Khojane (28°22'S, 28°01'E) Zuurfontein (29°54'S 25°22'E)	Exact numbers not recorded	Exact numbers not recorded
Microfilaria sp. B	*Cordylus polyzonus polyzonus*	Zuurfontein (29°54'S 25°22'E)	Exact numbers not recorded	Exact numbers not recorded
Microfilaria sp. C	*Pseudocordylus melanotus subviridis*	Ha Rapokolana (29°22'36''S 28°01'53''E) Ha Khojane (28°22'S, 28°01'E) Ha Thaba Bosiu (29°20'15''S 28°11'58''E	Exact numbers not recorded	Exact numbers not recorded
Microfilaria sp. D	*Pseudocordylus melanotus melanotus*	Clarens (28°31'S, 28°25'E)	Exact numbers not recorded	Exact numbers not recorded

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Figure 3.1. Photographs of reptiles collected in the Free State and Lesotho. **A:** *Lygodactylus capensis capensis* (A. Smith, 1849), **B:** *Pachydactylus capensis capensis* (A. Smith, 1845), **C:** *Pachydactylus bibronii* (A. Smith, 1845) **D:** *Agama atra atra* Daudin, 1802 **E:** *Agama hispida* (Linnaeus, 1758) **F:** *Mabuya capensis* (Gray, 1830), **G:** *Mabuya sulcata sulcata* (Male & Female) (Peters, 1867) **H:** *Mabuya striata punctatissima* (A. Smith, 1849), **I:** *Acontias gracilicauda gracilicauda* Essex, 1925 **J:** *Cordylus giganteus* A. Smith, 1844, **K:** *Cordylus polyzonus polyzonus* A. Smith 1838, **L:** *Pseudocordylus melanotus melanotus* (A. Smith, 1838). **A-C, E-I, K&L** are taken from Branch (1998) and digitally adapted and reproduced. This is only for illustration purposes for this thesis and not for any publication purposes.
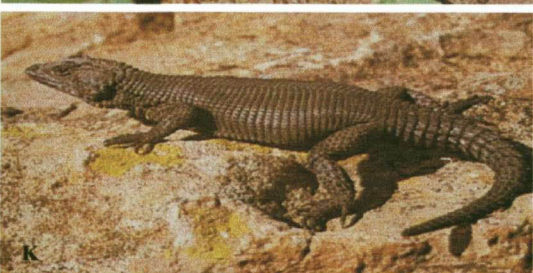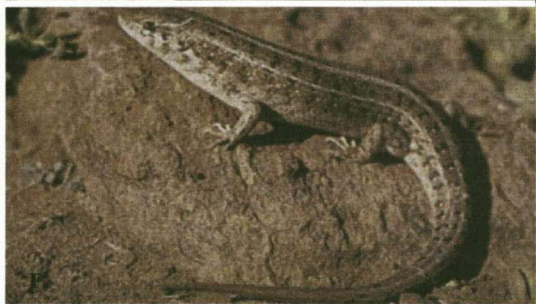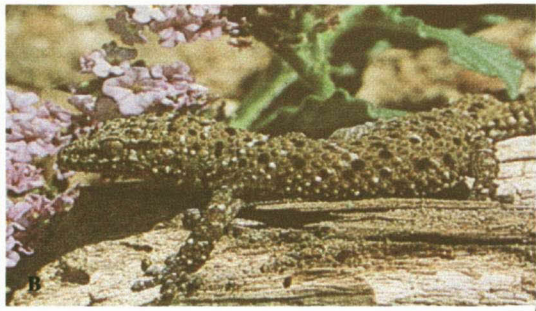
Figure 3.2. Photographs of reptiles collected in the Free State and Lesotho. **A:** *Pseudocordylus melanotus subviridis* (A. Smith, 1838), **B:** *Nucras intertexta* (A. Smith, 1838), **C:** *Varanus exanthematicus albigularus* (Daudin, 1802), **D:** *Lamprophis aurora* (Linnaeus, 1754), **E:** *Lamprophis guttatus* (A. Smith, 1843), **F:** *Lamprophis fuliginosus fuliginosus* (Boie, 1827), **G:** *Lycophidion capense capense* (A. Smith, 1831), **H:** *Pseudaspis cana* (Linnaeus, 1785), **I:** *Dasypeltis scabra* (Linnaeus, 1785), **J:** *Crotaphopeltis hotamboeia* (Laurenti, 1768), **K:** *Psammophylax tritaeniatus* (Günter, 1868), **L:** *Psammophis notostictus* Peters, 1867. **B & C, E, G & H, J-L** were taken from Branch (1998), **D & F:** were taken from Patterson (1987) and digitally adapted and reproduced. This is only for illustration purposes for this thesis and not for any publication purposes. **A & I:** Taken by author.
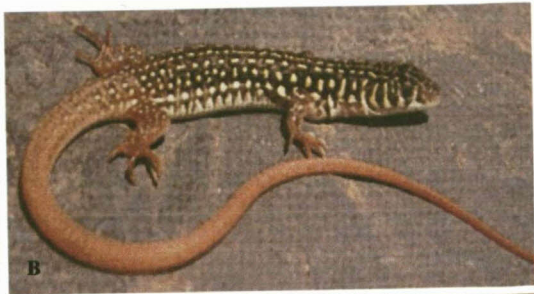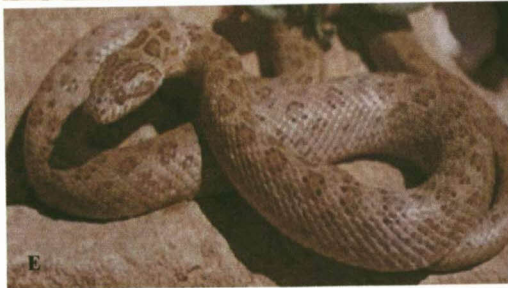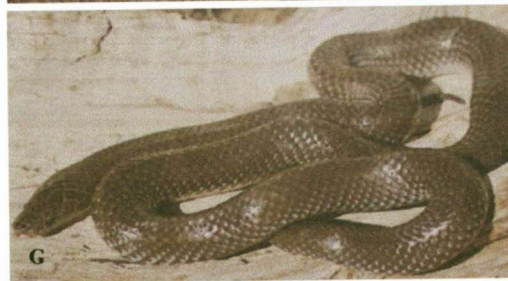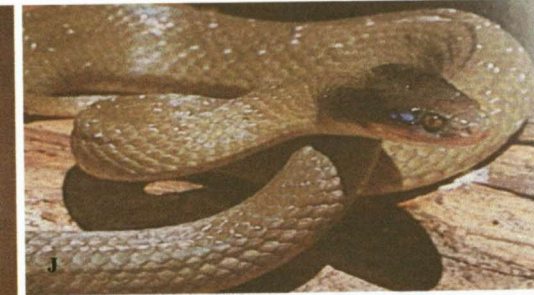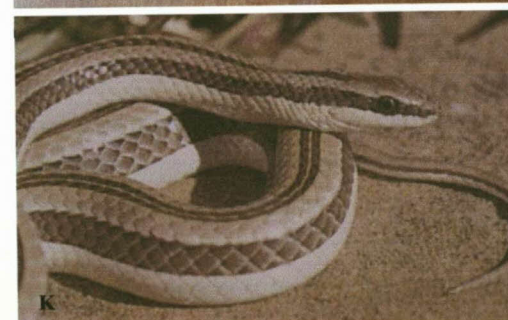
Figure 3.3. Photographs of reptiles collected in the Free State and Lesotho. **A:** *Aparallactus capensis* A. Smith, 1849, **B:** *Elapsoidea sundevallii media* Broadley, 1971, **C:** *Hemachatus haemachatus* (Lacèpéde, 1788), **D:** *Causus rhombeatus* (Lichtenstein, 1823), **E:** *Bitis arietans arietans* (Merrem, 1820), **F:** *Python sebae natalensis* (Gmelin, 1789). **A & B:** were taken from Branch (1998), **E:** was taken from Buys & Buys (1983) and digitally adapted and reproduced. This is only for illustration purposes for this thesis and not for any publication purposes. **C & D:** Taken with permission from Prof. L.H. du Preez, **F:** was taken by author.

BLOOD PARASITES

As reported above, a variety of blood parasites were seen in the reptiles captured in the Free State and the border regions of Lesotho, including viral infections (*Pirhemocyton*) and suspected viral infections (*Sauroplasma, Sauromella, Serpentoplasma*), protozoans (haemogregarines of the genus *Hepatozoon*, malaria parasites of the genus *Plasmodium* and possibly *Haemoproteus*) and filarial nematodes (microfilariae). Many infections probably provide the basis for new parasite species and most yield new host records.

Of those infections noted in the previous section 3.1 (Hosts collected), only host blood samples that were adequate enough to provide good descriptions are recorded below. For the remainder, further collections will be required before they can be examined in sufficient detail to allow description.

3.2 VIRUSES AND SUSPECTED VIRAL INFECTIONS

3.2.1. *PIRHEMOCYTON* CHATTON & BLANC 1914

Pirhemocyton from *Agama atra atra* Daudin, 1802

Type host: *Tarentola mauriticana*

Type localities: Morocco, Spain

Present study:

Localities: Bloemfontein; Zuurfontein (29°54'S, 25°22'E); Clarens (28°31'S, 28°25'E); Lesotho

Host: *Agama atra atra* Daudin, 1802

Pirhemocyton infections were observed in the lizard *Agama atra atra* collected in the Bloemfontein, Clarence and Jagersfontein districts (Fig. 2.1) and in Lesotho (Fig. 2.2). Such infections have been described from lizards in Australia by Stehbens & Johnston
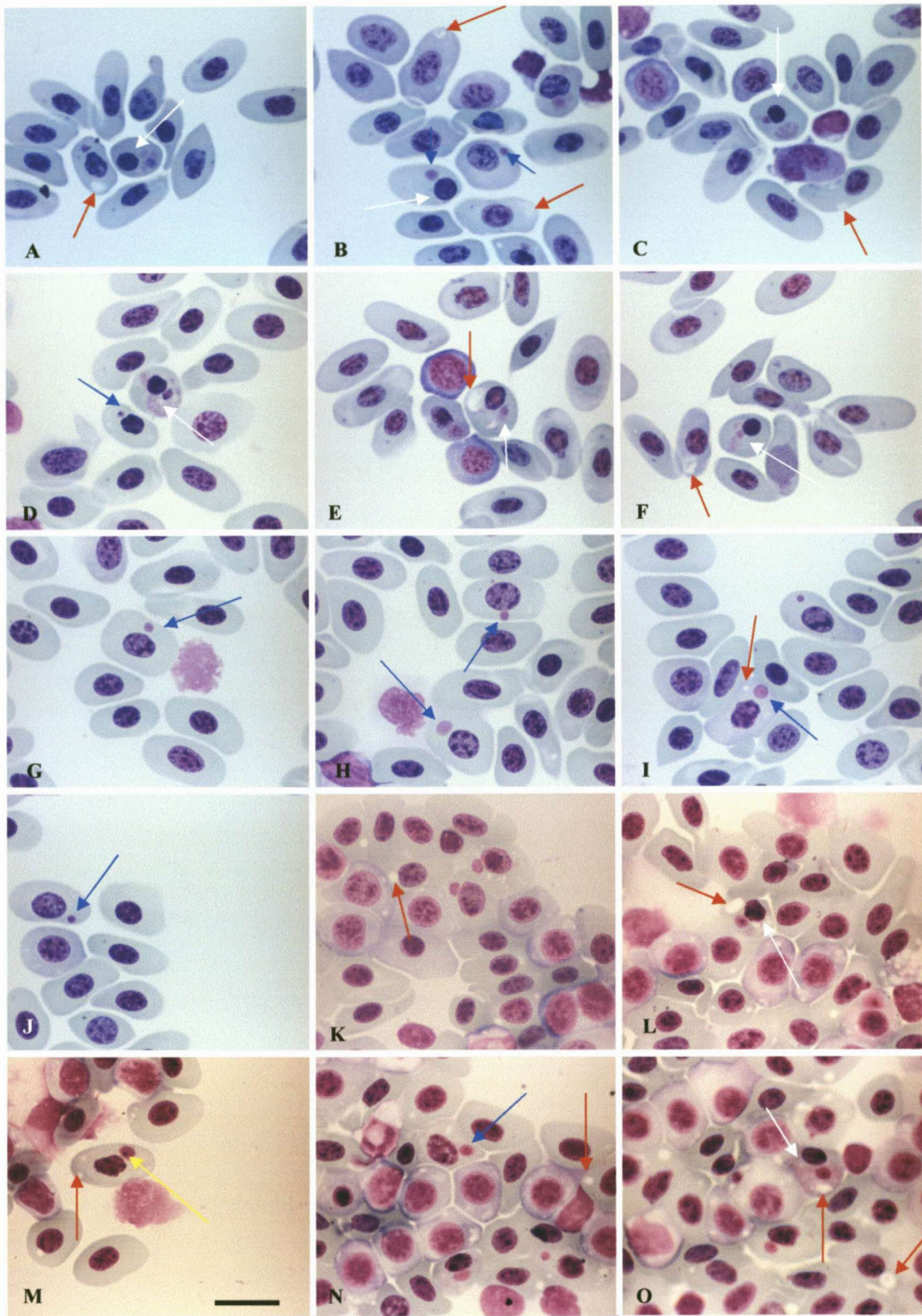
Figure 3.4. Light micrographs of Giemsa stained blood films of *Pirhemocyton* infections of 15 different specimens of *Agama atra atra* collected at different localities. **A-C:** *Pirhemocyton* infections from two ♂ and one ♀ specimen from the farm at Zuurfontein, **D-F:** *Pirhemocyton* infections from three ♂ specimens collected in the Bloemfontein district, with **D** (white arrow) showing areas of pinkish granules, **E & F** showing comet tail effects, **G-I:** *Pirhemocyton* infections from two ♀ and one ♂ specimen collected in Bloemfontein, **J:** *Pirhemocyton* infections from one ♂ specimen collected at Ha Thaba-Bosiu, **K-M:** *Pirhemocyton* infections from two ♀ specimens collected at Ha Khojane, **N-O:** *Pirhemocyton* infections from one ♂, one ♀ and one juvenile collected at Ha Rapokolana. **A-C, E&F, I, K-O** (red arrows): albuminoid bodies. **B, D, G-J, N** (blue arrows): rounded purple staining bodies, **M** (yellow arrow): deep red pigment inside purple staining areas, **A-F, L, O** (white arrows): effects on host cell: rounded nucleus is distinctively displaced to the lateral side in the cell. Scale: **A-O:** 10 μm.

(1966) and Johnston (1975), while Paperna & De Matos (1993b) studied the ultrastructure of the same type of infection from the same species of lizards from the Free State.

In the present study, these infections were abundant in all of the captured lizards and infections varied from low levels among erythrocytes to almost 100% red cells infected. Infections could be seen as purple bodies (Fig.3.4A-O), sometimes accompanied by an albuminoid body (vacuole) (Fig.3.4A-C, E&F, I, K-O (red arrows)). Albuminoid bodies measured 2.88 ± 0.55 (0.26-5.5) μm (n=30). The centre of infection itself was largely stained deep purple with Giemsa, measuring 2.2 ± 0.57 (0.9-3.5) μm (n=60) in diameter. Although in general, infection centres were seen as purple staining bodies which were mostly round (Fig.3.4B, D, G-J, N (blue arrows)), sometimes a deep red pigment was visible within the round body (Fig.3.4M (yellow arrow)). In addition, patches of fine pink granules were sometimes visible scattered within the host erythrocyte cytoplasm (Fig.3.4D (white arrow)), or forming a comet-tail leading from the round body (Fig.3.4E, F), and associated effects on host cells were marked, these cells appearing rounded with a distinctive round nucleus, sometimes displaced to one side of the cell. (Fig.3.4A-F, L, O (white arrows)). Some infected host erythrocytes were pale-stained with Giemsa (Fig. 3.4I (red and blue arrows)). *Pirhemocyton* infections were also found in *Agama atra atra* in Lesotho, (Fig.3.10B, E-I (blue arrows)) where they were accompanied by another parasite, *Hepatozoon* sp. A, illustrated in Fig.3.10A-I.

Pirhemocyton from *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

Host: *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

Locality: From the village of Ha Rapokolana (29°22'36''S 28°01'53E), Lesotho (Fig. 2.2)

A similar infection, but apparently producing smaller intraerythrocytic bodies was seen in this lizard and was accompanied by *Hepatozoon* sp. E. (Fig.3.14A-F). This possible *Pirhemocyton*, was found in almost all erythrocytes (Fig.3.14A-F). Some cells appeared

to have multiple infection centres (Fig.3.14C&D (red arrows)) and small granular areas were observed. As previously described for *Pirhemocyton*, the infection itself had a rounded, purple-stained centre (Fig.3.14A-F (blue arrows)), measuring 1.29 ± 0.9 (0.25-2.33) μm (n=60) in diameter. However, no albuminoid bodies like those in infections of *Agama atra atra* were observed. Host cells did not seem to be unduly affected by these infections, in contrast to the infection described above.

Comments

Pirhemocyton zonurae was described from a cordylid lizard (*Cordylus vittifer*) in Southern Africa (Pienaar 1962). The current study demonstrates the first record of a possible *Pirhemocyton* infection of *Pseudocordylus melanotus subviridis* (A. Smith, 1838) in South Africa, thus extending the range of distribution of these viral infections among the Cordylidae. However, this differs from most other known *Pirhemocyton* infections, including that seen in *Agama atra atra* (above), in that it is not accompanied by an albuminoid body. Morphometrically, the infected areas are also smaller (1.29 ± 0.9 (0.25-2.33) μm (n=60) in diameter) than those (2.2 ± 0.57 (0.9-3.5) μm (n=60) in diameter) for *Pirhemocyton* from *Agama atra atra* and the characteristic red pigment is also absent from the current infection.

3.2.2. *SAUROPLASMA* DU TOIT, 1937

***Sauroplasma* from *Cordylus giganteus* (A. Smith, 1844)**

Type species: *Sauroplasma thomasi* Du Toit, 1937

Type Host: *Cordylus giganteus* (A. Smith, 1844)

Type locality: Wesselsbron (27°52'S, 26°21'E) and Odendaalsrust (27°51'S, 26°41'E), Free State

Present study:

Host: *Cordylus giganteus* (A. Smith, 1844)

Locality: On the farm Koffielaagte (27°29'S, 27°28'E), near Kroonstad

Sauroplasma infections, indistinguishable from those of Du Toit (1937) were found in all of 31 *C. giganteus* captured on a farm near Kroonstad. The infected lizards were 10 males and 21 females.

Light microscopy

Parasites were intracytoplasmic and found in mature erythrocytes of which 7400/10 000 (74%) cells were infected. The effects on the host cells were seemingly minimal (Fig. 3.5A-F), as they were of normal size and shape and only a small area of the host cytoplasm was normally affected. In some heavy infections, larger areas within the cell cytoplasm appeared to be vacuolated, constituting a significant lesion in the infected cells.

In Giemsa-stained blood films parasites usually occurred singly, but occasionally in pairs, in a single erythrocyte. They were rounded, ring-like structures, sometimes with several small buds or protrusions (not shown in light micrographs). The main body of the structure measured 1.51 ± 0.7 (1.35-1.79) µm by 1.58 ± 0.74 (1.46-1.62) µm (n= 50), with buds 0.61 ± 0.54 (0.45-0.78) µm by 0.67 ± 0.48 (0.47-0.81) µm (n=25). The centre of the parasite was largely unstained and vacuole-like, but a narrow rim around most of its periphery was deeply stained with Giemsa, sometimes bearing purple-stained granules. Except for budding forms, no evidence of division was observed and no pink-stained regions similar to those seen in *Pirhemocyton* infections were observed in host erythrocytes.

Electron microscopy

Examination of blood samples by transmission electron microscopy (TEM) revealed something of the curious nature of these structures. Images showed multiple daughter structures or budding forms (Fig.3.5G & H). These were filled with lightly granular material embedded in an otherwise largely electron-lucent matrix, surrounded by a boundary membrane (Fig.3.5G & H (black arrows)). In just one image the faint impression of a structure resembling a small region of heterochromatin was observed (Fig. 3.5H (white arrow)), but this was the only image of its type.
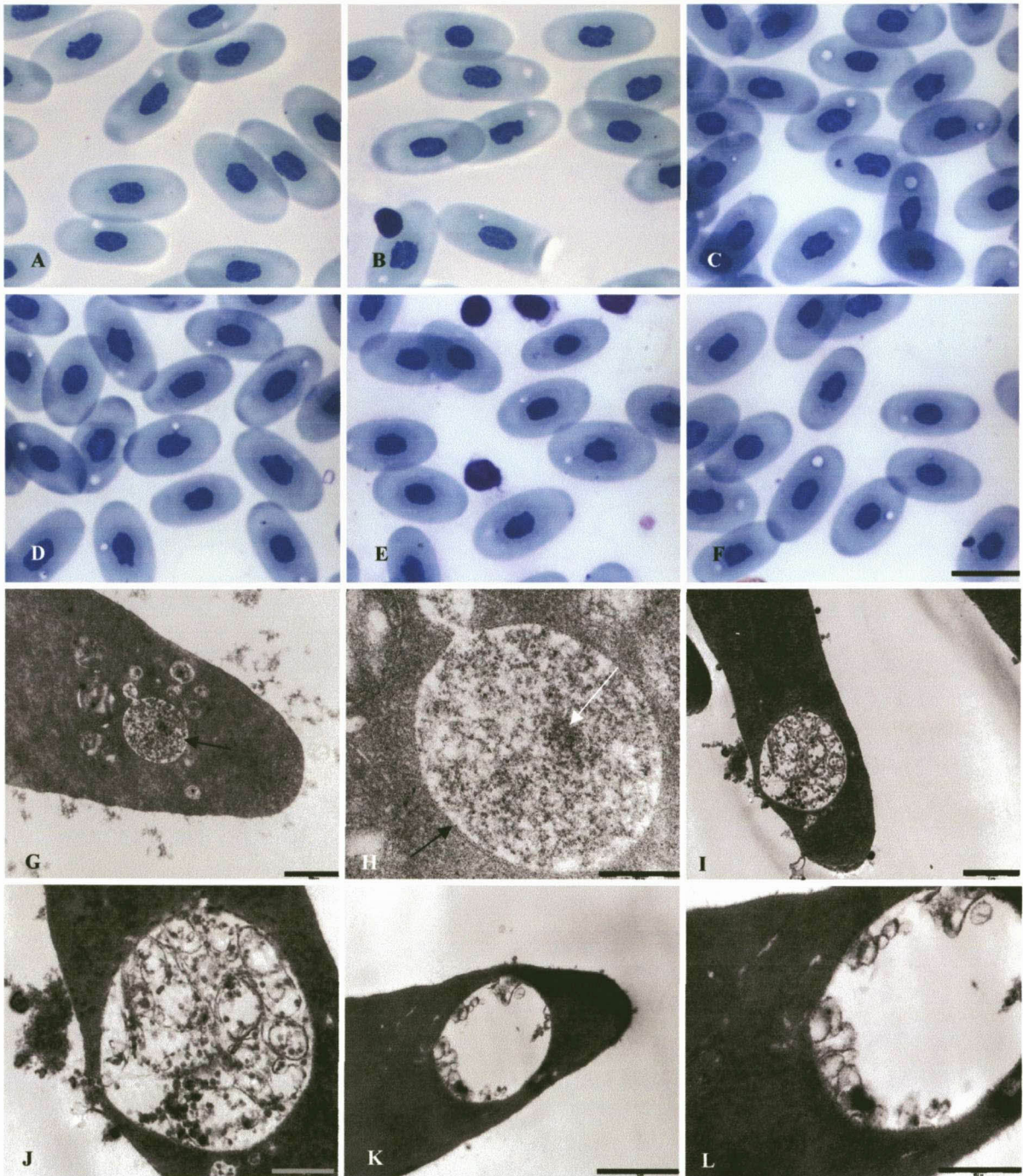
Figure 3.5. Light micrographs of *Sauroplasma thomasi* Du Toit, 1937. **A-F:** *Sauroplasma thomasi* from Giemsa stained blood films of six different specimens of *Cordylus giganteus* A. Smith, 1844. **G-L:** Transmission electron micrographs of *S. thomasi* in mature erythrocytes of *C. giganteus*. **G & H:** Multiple daughter parasites or budding forms, with boundary membrane (black arrows). **H:** (white arrow) A small area of heterochromatin. **G & H:** Budding: surface of mother structure expands until a bud is produced. **I & J:** Membranous whorls and granules within the structure. **K & L:** Peripheral membranous whorls with a prominent vacuolated center. Scale: **A-F:** 10 μm, **G, H, J & L:** 500 nm, **I & K:** 1μm.

Budding appeared to occur when the surface of the mother structure expanded to produce a bud that was also filled with granular material (Fig. 3.5G & H.). Presumably, by a process of constriction, the bud eventually separates from the mother structure and grows until roughly the same size of the mother. Some structures also seemed to be filled with membranous whorls and particulate material (Fig. 3.5I & J), while in others, the membranous material was confined to the perimeter of the structure and the centre was vacuole-like (Fig. 3.5K & L).

These structures have been described as parasites belonging to the Order Piroplasmorida Wenyon, 1926 because of their absence of pigment, mode of multiplication, size and shape (see Davies & Johnston (2000); Peirce (2000)). They were also suspected to be viral in nature because of their similarity with *Pirhemocyton*-like infections found in other members of the family Cordylidae (see Davies & Johnston, 2000). The current TEM study does not reveal any apicomplexan structures, or provide any clear indication of the identity of *Sauroplasma*, including whether or not it might be of host origin. No viral particles were found in the current electron micrographs, but *Sauroplasma* appears very similar to *Serpentoplasma* and the latter infection might be of viral origin (see Section 3.2.4 on *Serpentoplasma* of snakes).

Comments

By light microscopy, the intra-erythrocytic structures found in this study are identical to those described by Du Toit (1937) and later Pienaar (1962) from *C. giganteus* and therefore they are identified as *Sauroplasma thomasi*. Although leading to no firm conclusions about its identity, this is the first description of the ultrastructure of *S. thomasi*. The infection found in *C. giganteus* also closely resembles those found in all other species of lizards collected in this study. This leads to the suspicion that *Sauroplasma*-like infections are more widespread than previously thought and that they may cross specific, generic and even family boundaries. The true identity of *Sauroplasma*, the boundaries of its distribution and its mode of transmission between hosts are currently unknown.

Additional records of *Sauroplasma*

The following descriptions of *Sauroplasma* all represent new host records, as far as is known. Although they are the first morphometrical descriptions in new host erythrocytes, these descriptions are not considered sufficient to distinguish between species, especially since the identity of *Sauroplasma* is currently unclear and all forms are round, ring-like, budding structures. However, these data provide useful new information on the dispersion of these structures and individual variations between them. Furthermore, since host specimens were collected from areas separated by extensive mountain ranges, rivers and grasslands, these descriptions may well prove eventually to be those of new species, if protozoan, or strains, if viral.

Sauroplasma from *Cordylus polyzonus polyzonus* A. Smith, 1838

Host: *Cordylus polyzonus polyzonus* A. Smith, 1838.

Locality: On the farm Zuurfontein (29°54'S 25°22'E), Jagersfontein district, Free State.

Sauroplasma infections were found in 38 of 46 *C. polysonus* captured on a farm near Jagersfontein (Fig. 2.1). The infected lizards were 21 males and 25 females. The parasites were intracytoplasmic and found in mature erythrocytes of which an average 6900/10 000 (69%) cells were infected. The effects on the host cells were seemingly minimal (Fig. 3.6A-E). In some heavy infections large areas within the cell cytoplasm appeared to be vacuolated.

In Giemsa-stained blood films the structures occurred singly, or in pairs in a single erythrocyte (Fig.3.6A-C), although some were multiple (Fig.3.6D & E). They were irregularly round, ring-like structures. The main body of the structure measured 2.37 ± 0.64 (0.37-2.20) μm by 1.36 ± 0.7 (0.26-2.46) μm (n= 60). The centre was largely unstained and vacuole like, but a narrow rim around most of its periphery was deep stained with Giemsa. No evidence of division was observed.

Sauroplasma from *Mabuya sulcata sulcata* (Peters, 1867)

Host: *Mabuya sulcata sulcata* (Peters, 1867)

Locality: on the farm Zuurfontein (29°54'S 25°22'E), Jagersfontein district, Free State.

Sauroplasma infections were found in all six *M. sulcata* (Peters, 1867) captured on a farm near Jagersfontein (Fig. 2.1) and infected lizards were four males and two females. The parasites were intracytoplasmic and found in mature erythrocytes of which 1100/10 000 (11%) cells were infected. The effects on the host cells were seemingly minimal (Fig. 3.6F & G) (arrows)). Only small areas within the cell cytoplasm appeared to be vacuolated.

In Giemsa-stained blood films the structures occurred singly within erythrocytes (Fig.3.6F & G). They were round and ring-like with no buds or protrusions. The main body of the structure measured 0.66 ± 0.67 (0.37-0.87) μm by 0.655 ± 0.77 (0.21-1.10) μm (n= 24) and its centre was largely unstained and vacuole like. A narrow rim around most of its periphery, deep stained with Giemsa (Fig3.6F & G). No evidence of division was observed, but faintly pink-staining regions similar to those seen in *Pirhemocyton* infections were seen in some cases.

Sauroplasma from *Nucras intertexta* (A. Smith, 1838)

Host: *Nucras intertexta* (A. Smith, 1838)

Locality: Bloemfontein district, Free State.

Sauroplasma infections were found in one specimen of *Nucras intertexta* (A. Smith, 1838) captured in the Bloemfontein area (Fig. 2.1). The infected lizard was a male. The structures were intracytoplasmic and found in mature erythrocytes of which 3700/10 000 (37%) cells were infected. The structures themselves were quite large and bullet hole shaped.

In Giemsa-stained blood films *Sauroplasma* occurred singly in erythrocytes (Fig.3.6H & I). Each was a round, ring-like structure with no buds or protrusions. The main body of the structure measured 1.35 ± 0.4 (1.20-1.45) μm by 1.33 ± 0.46 (1.17-1.49) μm (n= 30). The centre was unstained and vacuole-like (Fig. 3.6H & I). No evidence of division was observed and pink-staining regions like those seen in *Pirhemocyton* infections were absent.

Sauroplasma from *Mabuya striata punctatissima* (A. Smith, 1849)

Host: *Mabuya striata punctatissima* (A. Smith, 1849).
Locality: Bloemfontein district, Free State.

Sauroplasma infections were found in all three *Mabuya striata punctatissima* (A. Smith, 1849) specimens captured in the Bloemfontein suburbs. The infected lizards were all males. The structures were intracytoplasmic and found in mature erythrocytes of which 600/10 000 (6%) cells were infected. The effects on the host cells were seemingly minimal (Fig. 3.6J & K), although in some heavy infections large areas of host cytoplasm appeared to be vacuolated (Fig. 3. 6J & K).

In Giemsa-stained blood films *Sauroplasma* occurred singly (Fig. 3.6J), or in pairs (Fig. 3.6K) in a single erythrocyte. They were irregularly round, ring-like structures with the main body of the structure measuring 1.44 ± 0.57 (0.61-2.24) μm by 1.54 ± 0.59 (0.91-1.97) μm (n= 40). No buds were observed. The centre of the parasite was largely unstained and vacuole-like, without a staining rim at its periphery. No evidence of division was observed and no pink-staining regions similar to those seen in *Pirhemocyton* infections were noted.

Figure 3.6. *Sauroplasma* Du Toit, 1937 infections of various lizards. **A-E:** *Sauroplasma* from *Cordylus polyzonus polyzonus* A. Smith, 1838, **A-C:** Single infections, **D & E:** multiple infections, **F & G:** *Sauroplasma* from *Mabuya sulcata sulcata* (Peters, 1867), **H-I:** *Sauroplasma* from *Nucras intertexta* (A. Smith, 1838). **J & K:** *Sauroplasma* from *Mabuya striata punctatissima* (A. Smith, 1849) with vacuolated cytoplasm in infected areas. **L & M:** *Sauroplasma* from *Acontias gracilicauda gracilicauda* Essex, 1925 with large vacuoles in infected cells. **N & O:** *Sauroplasma* from *Varanus exanthematicus albigilarus* (Daudin, 1802) with vacuolated areas in infected cells.
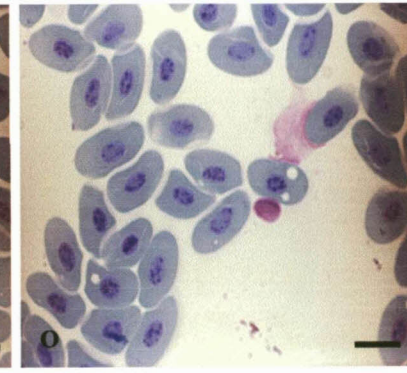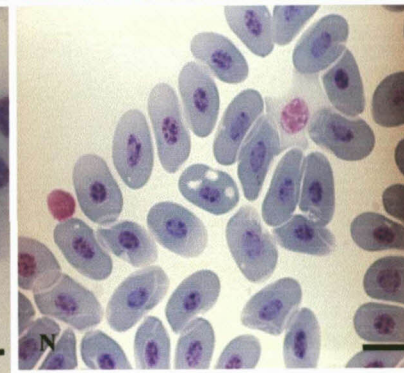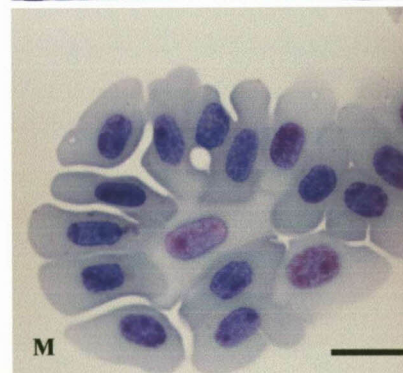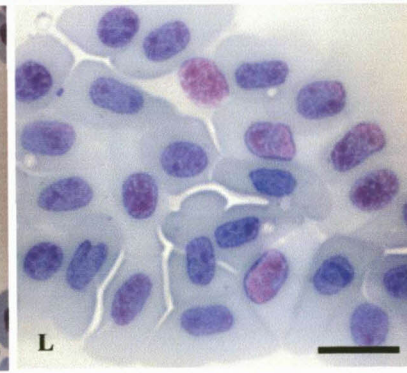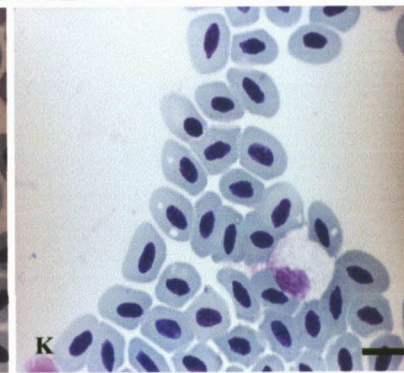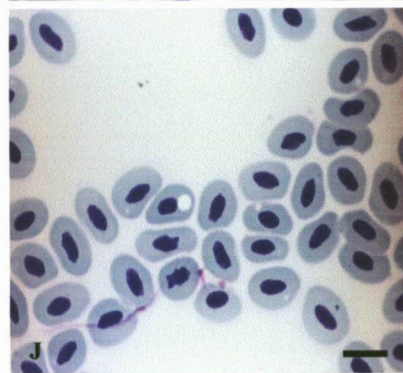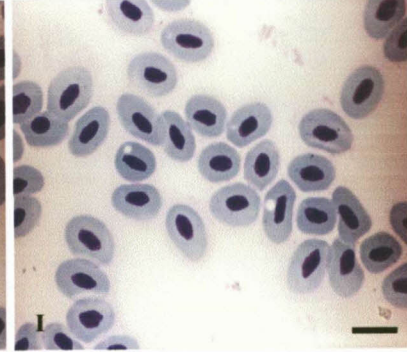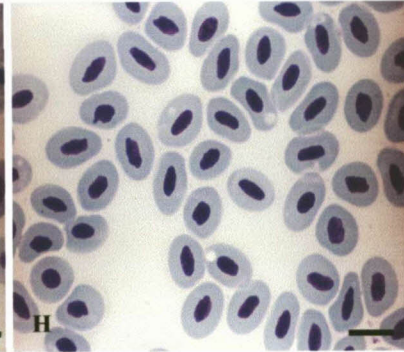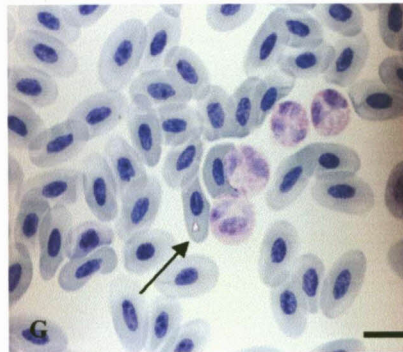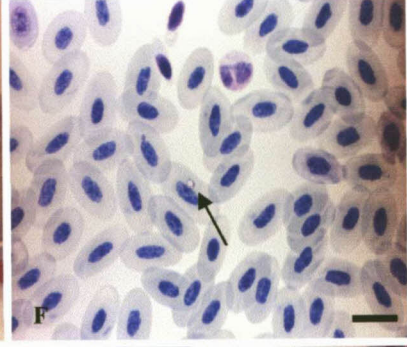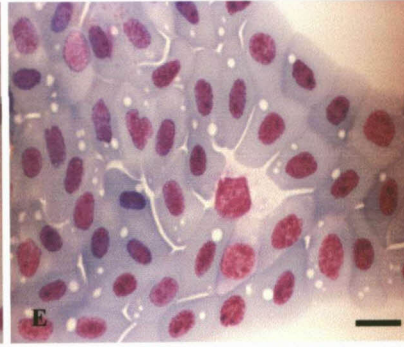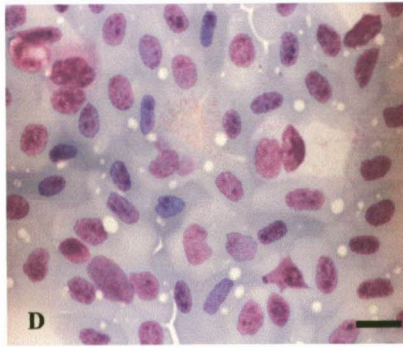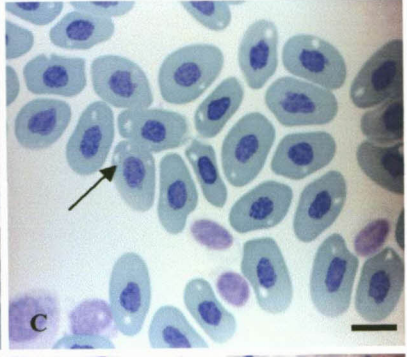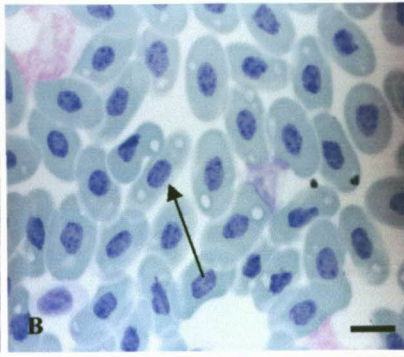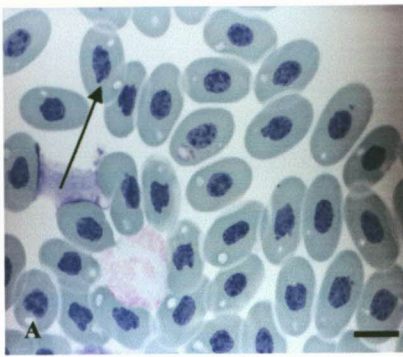
Sauroplasma from ***Acontias gracilicauda gracilicauda*** **Essex, 1925.**

Host: *Acontias gracilicauda gracilicauda* Essex, 1925.

Locality: Bloemfontein district, Free State.

Sauroplasma infections were found in one specimen of *Acontias gracilicauda gracilicauda* Essex, 1925. The sex of this specimen was undetermined. The parasites were intracytoplasmic and found in mature erythrocytes of which 600/10 000 (6%) cells were infected. These infections involved large areas of host cytoplasm which appeared vacuolated (Fig. 3.6L & M).

In Giemsa-stained blood films the structures occurred singly, in pairs, or up to three were present in a single erythrocyte and were irregularly round, ring-like structures. The main body of the structure measured 2.5 ± 0.9 (1.1-3.8) μm by 1.76 ± 0.86 (0.98-2.97) μm (n= 20). No buds were observed. The centre of the parasite was largely unstained and vacuole-like, with no narrow rim around its periphery. No evidence of division was observed and no pink-staining regions similar to those seen in *Pirhemocyton* infections were noted.

Sauroplasma from ***Varanus exanthematicus albigularus*** **(Daudin, 1802)**

Host: *Varanus exanthematicus albigularus* (Daudin, 1802).

Locality: On the farm Zuurfontein (29°54'S 25°22'E), Jagersfontein district, Free State.

Sauroplasma infections were found in one specimen of *Varanus exanthematicus albigularus* (Daudin, 1802). The sex of this specimen was undetermined. The structures were intracytoplasmic and found in mature erythrocytes of which 2500/10 000 (25%) cells were infected. The effects on the host cells were marked (Fig. 3.6N & O), sometimes involving large areas of host cytoplasm that appeared to be vacuolated (Fig. 3.6N & O).

In Giemsa-stained blood films the structures occurred singly, in pairs, and up to three were present in a single erythrocyte. As in other infections, they were irregularly round, ring-like, structures. The main body of the structure measured 1.4 ± 0.81 (1.0-3.8) µm by 1.8 ± 0.97 (1.12-3.2) µm (n= 25) and no buds were observed. The centre of the parasite was largely unstained and vacuole-like, without a narrow rim at its periphery. No evidence of division was observed and no pink-staining regions similar to those seen in *Pirhemocyton* infections were noted.

Sauroplasma from **Pseudocordylus melanotus subviridis** (A. Smith, 1838) and **Pseudocordylus melanotus melanotus** (A. Smith, 1838).

Hosts: *Pseudocordylus melanotus subviridis* (A. Smith, 1838) and *Pseudocordylus melanotus melanotus* (A. Smith, 1838).

Localities: Clarens district (28°31'S, 28°25'E), Eastern Free State and Jorotane river valley near village of Ha Rapokolana (29°22'36''S 28°01'53E), Lesotho.

Sauroplasma infections were found in all 44 lizards collected in the different localities noted above. Infections varied from only a few cells affected to nearly all erythrocytes infected. The parasites were intracytoplasmic and found in mature erythrocytes, often accompanying *Plasmodium*, haemogregarine and filarial nematode infections. Effects on the host cells were apparently minimal (Fig. 3.18A-O (blue arrows)); although in some cases multiple infections occurred (Fig. 3.18K (blue arrow)). In heavy infections, relatively large areas within the cell cytoplasm appeared to be vacuolated (Fig. 3.18A-D, F).

In Giemsa-stained blood films, one to five structures were present in a single erythrocyte. They were irregularly round and ring-like, and the main body of the structure measured 1.6 ± 0.45 (0.30-2.47) µm by 2.45 ± 0.51 (0.91-3.1) µm (n= 50). No buds were observed. The centre of the parasite was largely unstained and vacuole-like, with no narrow rim around its periphery. No clear evidence of division was observed, but the presence of

small forms suggested that multiplication or budding may have occurred. Pink-staining regions similar to those seen in *Pirhemocyton* infections were not encountered.

Comments

Sauroplasma and *Sauroplasma*-like infections, have been previously described from three species of lizards in South Africa (*C. giganteus, Cordylus jonesii* and *Cordylus vittifer*), as well as from chameleons (*Zonosaurus mascareniensis*) and geckoes (*Uroplatus fibriatus*) in Madagascar, unidentified reptiles in Uzbekistan, and lizards (*Lacerta agilis*) in Scandinavia (Du Toit (1937); Pienaar (1962); Brygoo (1963); Uilenberg & Blanc (1966); Zakharyan (1970) and Svahn (1976)). Most of these infections have been grouped with the piroplasms (see Du Toit (1937); Pienaar (1962); Svahn (1976); Levine (1971, 1988); Johnston (1975); Frye (1991); Barnard & Upton (1994) and Peirce (2000)), however, similar parasites in lizards (*Gehyra variegata*) in Australia were considered to be early trophozoites of the haemoproteid *Haemocystidium* Castellani & Willey, 1904 (see Johnston 1975).

Johnston (1975) also reported that some *Sauroplasma* infections can resemble the viral infection *Pirhemocyton*. Davies & Johnston (2000) examined blood samples of *Lacerta agilis* from Scandinavia containing both *Sauroplasma* and a *Pirhemocyton*-like infection. Viral particles were found by TEM, but the authors were unable to state with certainty that *Sauroplasma* is viral in nature.

By light microscopy, *Sauroplasma thomasi* could easily be interpreted as a piroplasm infection, and is similar to the enigmatic infections of *Serpentoplasma* (seen in snakes), *Chelonoplasma* Frye, 1981 (in tortoises) and *Haemohormidium* Henry, 1910 and *Haematractidium* Henry, 1910 (from fishes). It could also be a viral infection, since the albuminoid bodies of some *Pirhemocyton* infections are not unlike *Sauroplasma* (see Davies & Johnston (2000)).

Current TEM studies (see *Sauroplasma* from *Cordylus giganteus*) have revealed that *Sauroplasma* is probably not a typical apicomplexan because of the apparent absence of an apical complex including rhoptries, micronemes and other characteristic structures of

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the Apicomplexa. However, when it is compared with *Serpentoplasma* from *Python sebae natalensis* and *Serpentoplasma* from *Bitis arietans* by TEM they appear to be of very similar infections, possibly of viral origin (see electron microscopy section of *Serpentoplasma* infections of *Bitis arietans*).

3.2.3. *SAUROMELLA* PIENAAR, 1954

Sauromella from Pachydactylus capensis (A. Smith, 1845)

Type species: *Sauromella haemolysus* Pienaar, 1954.

Type Host: *Pachydactylus capensis* (A. Smith, 1845).

Type Locality: On the farm Elandsfontein, Potchefstroom district.

Present study:

Host: *Pachydactylus capensis* (A. Smith, 1845).

Locality: On a farm Deelhoek, Bloemfontein district.

Sauromella haemolysus was found in the peripheral blood of *Pachydactylus capensis* captured in the Bloemfontein district. Two males, one female and one juvenile were infected. The parasites were intracytoplasmic and found in mature erythrocytes of which 900\10000 (9%) cells were infected. The effects on host cells were mostly minimal (Fig. 3.7A-F), but in some heavy infections large areas within the cell cytoplasm appeared to be vacuolated (Fig. 3.7B, D-F (arrows)).

Sauromella comprised a small, round or streaked, purple or dark blue-stained area, sometimes associated with a large vacuole (Fig.3.7A, C, D, & E). The purple structure appeared to become larger as it matured and exhibit peripheral granules (Fig.3.7D & E (red arrow)), and measured 1.79 to 2.57 µm by 0.94 to 1.48 µm (n= 21). Vacuoles were of similar size, but sometimes slightly larger (Fig. 3.7B, E, & F). No evidence of division was observed, but the pink-staining regions and vacuoles were similar to those seen in viral *Pirhemocyton* infections. These infections were indistinguishable from those found by Pienaar (1954) in the same host.
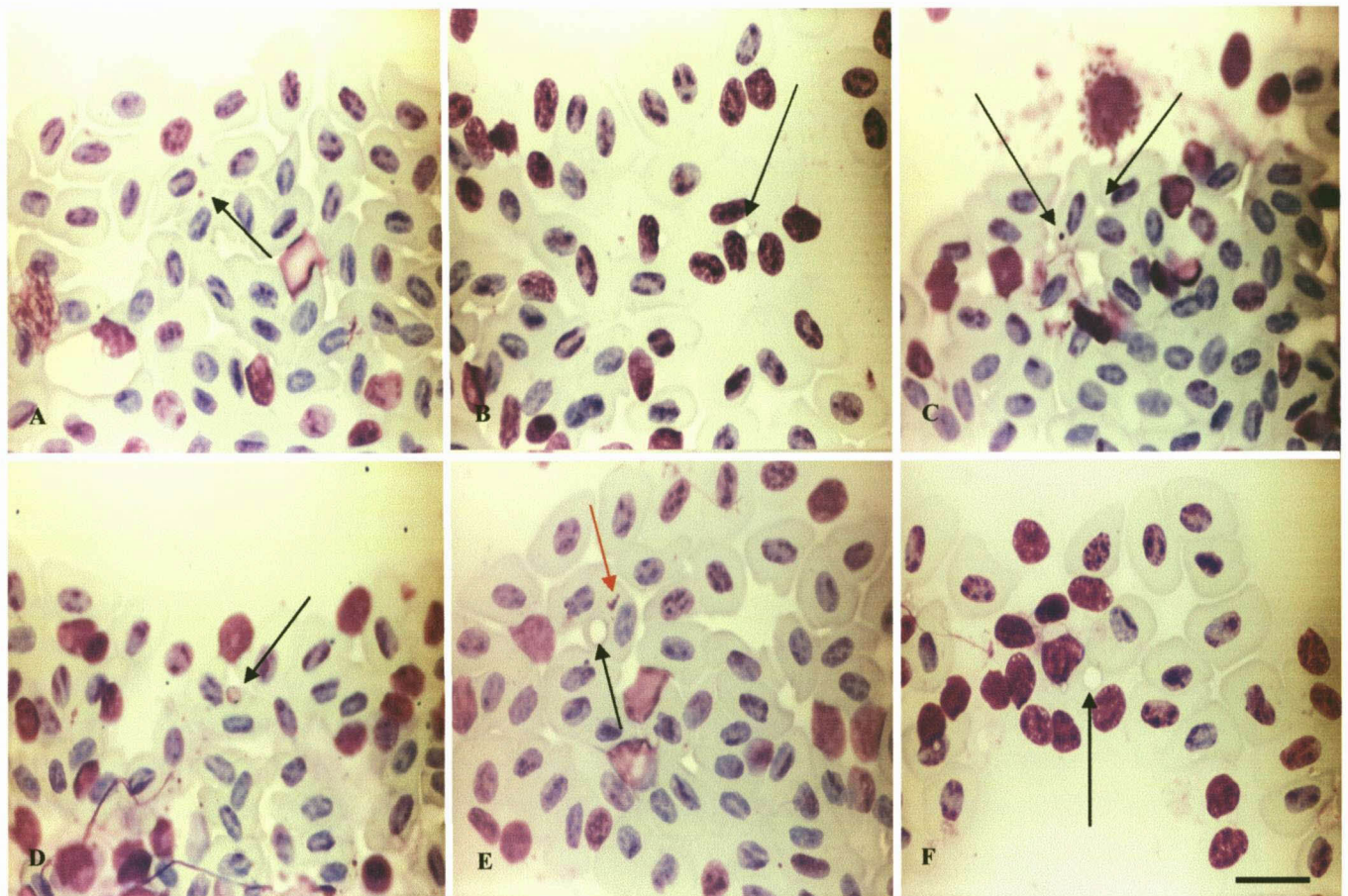
Figure 3.7. *Sauromella haemolysus* Pienaar, 1954 in Giemsa stained blood films from two ♂, one ♀ and one juvenile (**A**-♂, **B**-♂, **C**-juvenile **D-F**, ♀) *Pachydactylus capensis* (A. Smith, 1845) specimens. **A, C, D:** Pigmented structures, **B, E, & F:** unstained and vacuolated apparent structures. Red arrow in **E** points to stained and streaked structure. Scale: **A-F:** 10 µm.

3.2.4. *SERPENTOPLASMA* PIENAAR, 1954

The following species are new host records of *Serpentoplasma* found in snakes. Their morphology appears to be similar to *Sauroplasma* infections found in lizards, although some resemble *Pirhemocyton.*

Serpentoplasma from *Crotaphopeltis hotamboeia* (Laurenti, 1768)

Host: *Crotaphopeltis hotamboeia* (Laurenti, 1768)

Locality: On the farm Deelhoek (28°54'S, 26°09'E), Bloemfontein district, Free State.

These infections were similar to those described by Pienaar (1962). They were found in six (two males and four females) *Crotaphopeltis hotamboeia* (Laurenti, 1768)specimens, collected in the Bloemfontein district. The structures were intracytoplasmic and occurred as prominent round and oval bodies (Fig. 3.8A-C). They were found in mature erythrocytes of which 700/10 000 (7%) cells were infected.

In Giemsa-stained blood films the structures occurred singly, or up to three in mature erythrocytes. They were irregularly round, vacuole-like structures (Fig. 3.8A-C). The main body of the structure measured 1.8 ± 0.93 (1.4-2.3) μm by 1.94 ± 0.7 (1.2-2.10) μm (n=36). The centre of the parasite was largely unstained and vacuole-like, with no narrow rim around its periphery. No pink-staining bodies similar to those seen in *Pirhemocyton* infections from lizards were noted.

Serpentoplasma from *Lycophidion capense capense* (A. Smith, 1831)

Host: *Lycophidion capense capense* (A. Smith, 1831).

Locality: On the farm Deelhoek (28°54'S, 26°09'E), Bloemfontein district, Free State.

These infections were also similar to those described by Pienaar (1962). They were found in two male specimens, collected in Bloemfontein and on a farm at Deelhoek in the Bloemfontein district. The structures were largely vacuole-like intracytoplasmic and

occurred as small round and oval bodies (Fig. 3.8D-F). They were found in mature erythrocytes of which 2700/10 000 (27%) cells were infected.

In Giemsa-stained blood films parasites occurred singly in mature erythrocytes. The main body of the structure measured 1.12 ± 0.84 (0.4-1.5) μm by 0.97 ± 0.71 (0.5-1.64) μm (n=20). The centre of the parasite was largely unstained and vacuole-like, with a narrow rim around its periphery. Small pink-staining bodies similar to those seen in *Pirhemocyton* infections from lizards were also noted (Fig. 3.8F).

Serpentoplasma from *Elapsoidea sundevallii media* Broadley, 1971

Host: *Elapsoidea sundevallii media* Broadley, 1971.

Locality: On the farm Deelhoek (28°54'S, 26°09'E), Bloemfontein district, Free State.

These *Serpentoplasma* infections also showed some similarities with *Pirhemocyton*. They were found in three specimens collected on a farm Deelhoek in the Bloemfontein district. The structures were intracytoplasmic and occurred as small oval bodies (Fig. 3.8G). They were found in mature erythrocytes of which 85/10 000 (0.85%) of the cells were infected.

In Giemsa-stained blood films the structures occurred singly in mature erythrocytes. They were irregularly round, dark-purple structures with some dark pigment around the periphery of the organism. The main body of the structure measured 0.98 ± 0.99 (0.68-1.2) μm by 0.65 ± 1.2 (0.55-1.1) μm (n=11) and its centre appeared solid.

Serpentoplasma from *Hemachatus haemachatus* (Lacèpéde, 1788)

Host: *Hemachatus haemachatus* (Lacèpéde, 1788).

Locality: Near the village of Excelsior and in the village of Ha Rapokolana(29°22'36''S 28°01'53E), Lesotho.

Intra-erythrocytic infections were found in two male specimens, collected near the village of Excelsior in eastern Free State and in the village of Ha Rapokolanathe Lesotho highlands. The structures were intracytoplasmic, measured 0.67 ± 0.57 (0.4-1.2) μm by 1.16 ± 0.6 (0.6-1.67) μm (n=30) and occurred as rounded vacuole-like structures, one in each infected erythrocyte (Fig. 3.8H). They were found in mature erythrocytes of which 2700/10 000 (27%) cells were infected. Bodies with a purple-stained periphery (Fig. 3.8H (red arrow)), and vacuole-like bodies (Fig. 3.8H (black arrows)), were observed.

Serpentoplasma from *Causus rhombeatus* (Lichtenstein, 1823)

Host: *Causus rhombeatus* (Lichtenstein, 1823).

Locality: Clarens district (28°31'S, 28°25'E), Eastern Free State.

These *Serpentoplasma* infections also showed great similarities with those of lizard *Pirhemocyton*. They were found in one female specimen, collected on a road in the Clarens district. The structures were intracytoplasmic, occurring as small or prominent rounded, oval and elongate purple-stained bodies, with or without a vacuole or pale-stained region of host cytoplasm (Fig. 3.8I-L (arrows)). They were found in mature erythrocytes of which 100/10 000 (1%) of the cells were infected.

In Giemsa-stained blood films the structures occurred singly in mature erythrocytes. The effects on the host cells (Fig. 3.8I-L) were most evident in cells containing deep-stained bodies and pale-stained cytoplasm (Fig. 3.8I). The main body of the purple-stained structure measured 2.68 ± 1.4 (1.89-2.90) μm by 3.89 ± 1.1 (2.24-4.2) μm (n=12).
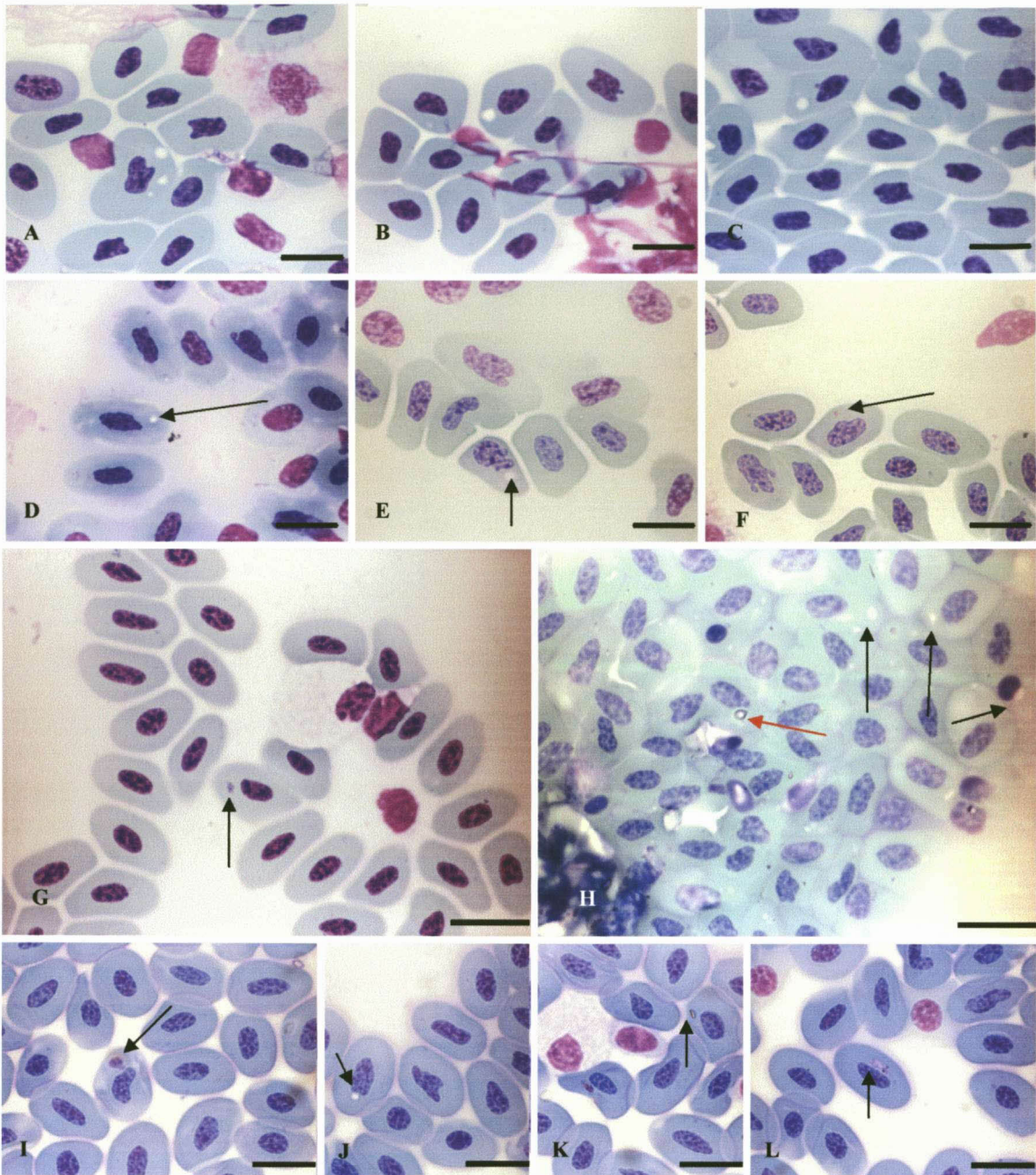
Figure 3.8. *Serpentoplasma* Pienaar, 1954 infections in Giemsa stained blood films from different snake species: **A-C**: from *Crotaphopeltis hotamboeia* (Laurenti, 1768) with prominent vacuole-like bodies. **D-F**: similar vacuole-like structures from *Lycophidion capense capense* (A. Smith, 1831). **G**: from *Elapsoidea sundevalli media* Broadley, 1971 with pink-stained bodies similar to *Pirhemocyton* Chatton & Blanc, 1914 infections. **H**: from *Hemacatus haemachatus* (Lacèpéde, 1788), with purple staining periphery (red arrow). **I-L**: from *Causus rhombeatus* (Lichtenstein, 1823) (see arrow in K) but also similar to *Pirhemocyton* consisting of purple stained bodies (**I** (arrow)). Scale: **A-L**: 10 μm

Serpentoplasma **from *Bitis arietans* (Merrem, 1820).**

Host: *Bitis arietans arietans* (Merrem, 1820).

Locality: Bloemfontein district, Free State.

Serpentoplasma infections were found in two specimens of *Bitis arietans arietans*. One male and one female were sampled.

Light microscopy

The structures were intracytoplasmic and occurred as tiny round or oval bodies (Fig. 3.9A-C). They were found in mature erythrocytes of which 800/10 000 (8%) cells were infected.

In Giemsa-stained blood films parasites occurred singly in mature erythrocytes. They were ring-like structures, without any small buds or protrusions. The main body of the structure measured 1.1 ± 0.99 (0.9-1.4) µm by 1.4 ± 0.81 (0.91-1.35) µm (n=30), and was largely unstained and vacuole-like, with no narrow rim around its periphery. No evidence of division was seen.

Electron microscopy

Blood samples from the same specimen of *Bitis arietans* showed by TEM (Fig.3.9D-I) that these *Serpentoplasma* infections were largely similar to *Sauroplasma* infections from lizards (Fig.3.5G-L). Stages with rather fibrous and whorled contents, and particulate matter were very close in appearance to those seen in Fig. 3.5G-J of *Sauroplasma* infections. Furthermore, stages of *Serpentoplasma* were present with a vacuole-like centre and marginal whorled material (Fig. 3.9G & H), also similar to those seen in *Sauroplasma* (Fig. 3.5G, H). However, in *Serpentoplasma*, the forms with marginated contents also showed small particles both within the body itself, and on the surface of the host erythrocyte (Fig. 3.9H & I). These particles are reminiscent in position and appearance to particles observed by Daly, Mayhue, Menna & Calhoun (1980) in the erythrocytes of the yellowbelly water snake, *Nerodia erythrogaster flavigaster*. Daly et al. (1980) interpreted their structures as resembling oncornaviruses.
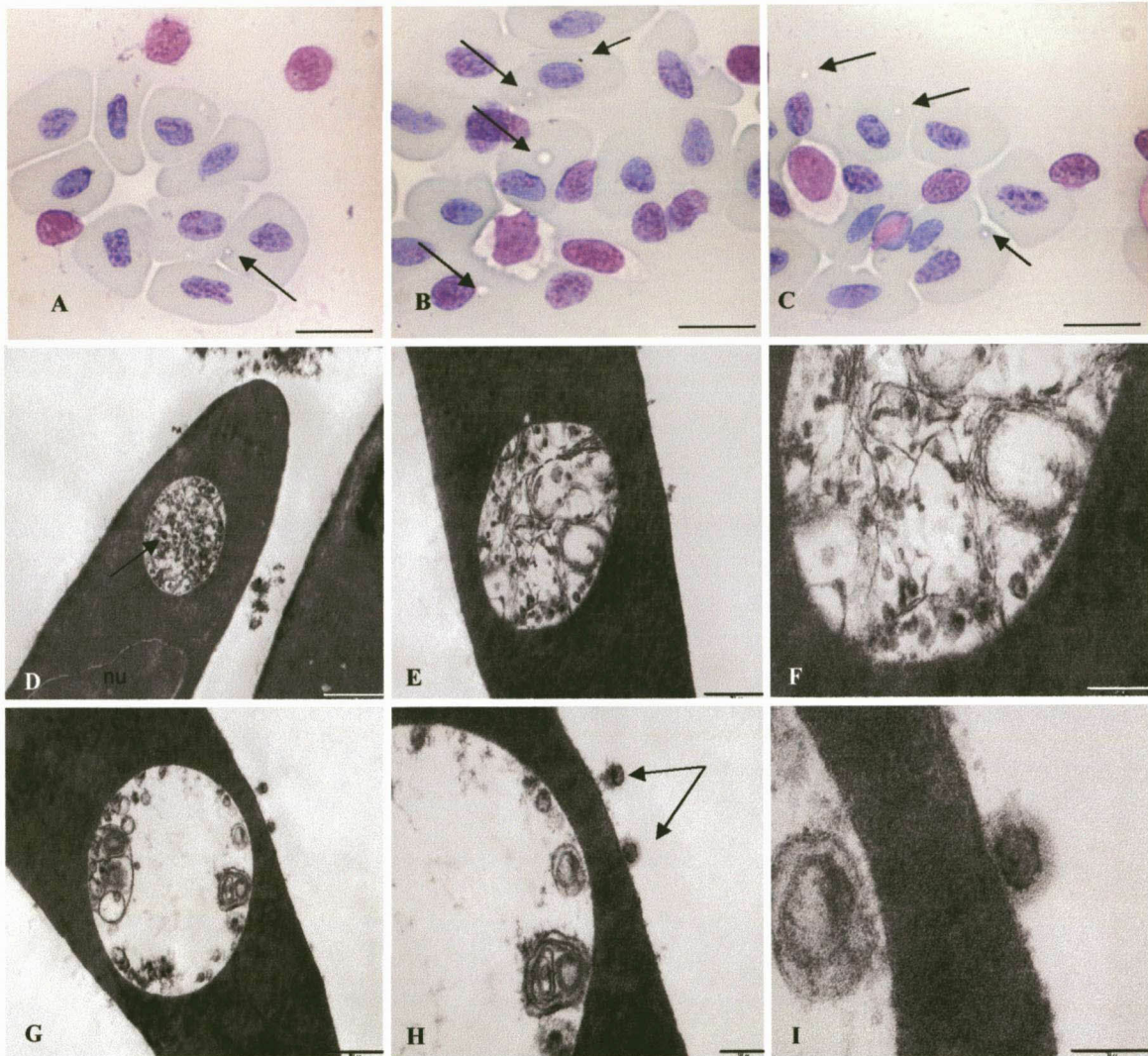
Figure 3.9. **A-C:** Light micrographs of *Serpentoplasma* Pienaar, 1945 infections in Giemsa stained blood films of the Puff adder (*Bitis arietans arietans* (Merrem, 1820). **D-I:** transmission electron micrographs of *Serpentoplasma* infections in peripheral blood cells of the puff adder (*Bitis arietans*) collected in the Bloemfontein district. **D-F:** Structures filled with granular and fibrous material, and membranous whorls, **G & H:** structures with marginated whorled contents. **I:** possible viral particles attached inside vacuole and on surface of host erythrocyte. Scale: **A-C:** 10 μm, **D:** 1 μm, **E & G:** 500 nm, **F & H:** 200 nm, **I:** 100 nm.

3.3. PROTOZOA

3.3.1. HAEMOGREGARINES

All haemogregarines noted in these studies have been allocated the genus name *Hepatozoon,* in line with the recommendations of Smith (1996). When their life cycles are elucidated, however, they may prove to be *Hemolivia, Schellackia* or other genera of haemogregarines reported from reptiles.
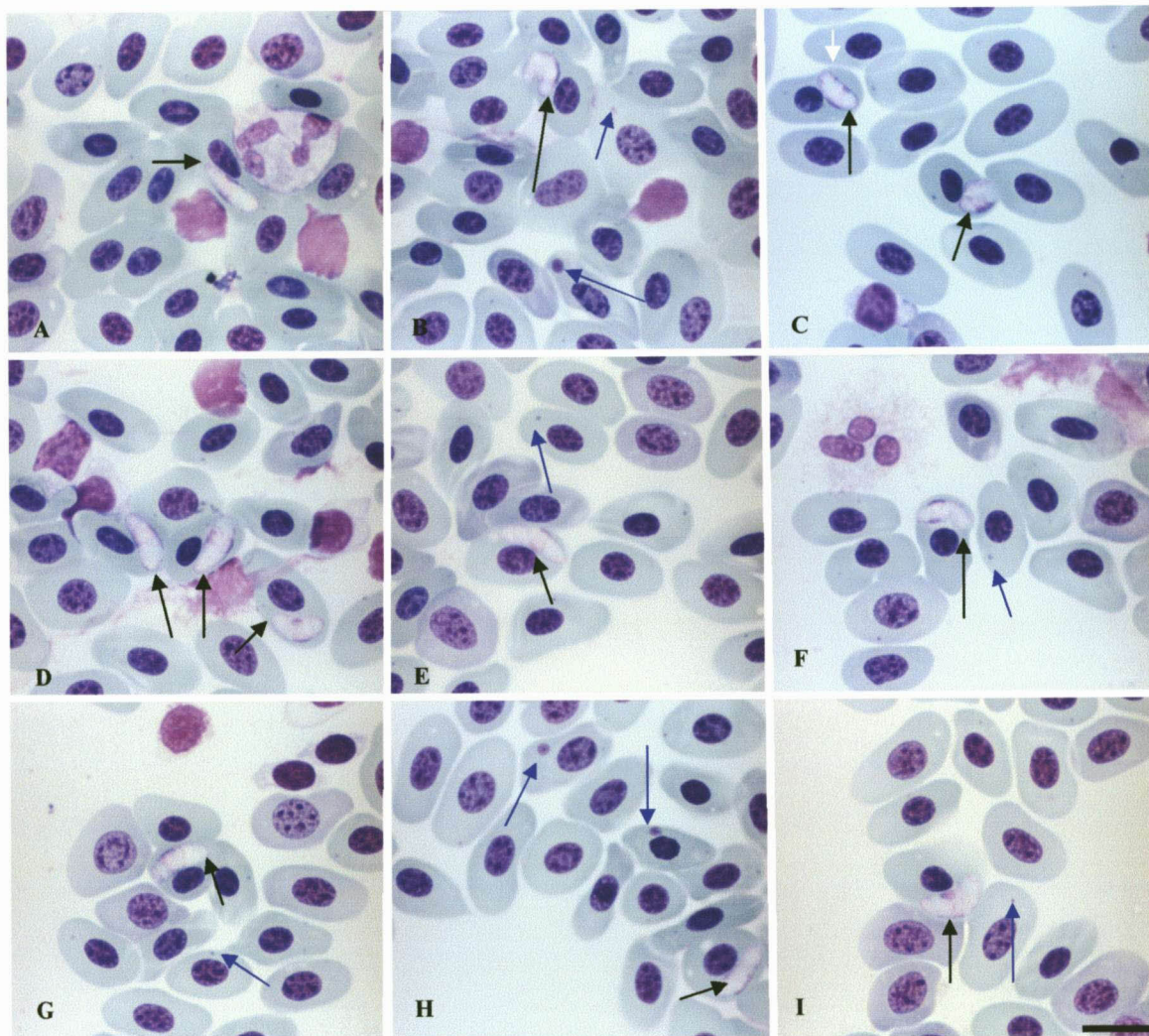
Hepatozoon sp. A from _Agama atra atra_ Daudin, 1802

Host: *Agama atra atra* Daudin, 1802.

Localities: Bokong River near Ha Khojane (28°22'S, 28°01'E), Lesotho. On the farm Zuurfontein (29°54'S 25°22'E) Jagersfontein district, Southern Free State.

Hepatozoon sp. A was found in three of three *Agama atra atra* captured in three areas in the Bokong catchment. The first site was a river bank near the village of Ha Khojane (28°22'S, 28°01'E), where a male specimen was collected (Fig. 3.10A-I). Another male was caught roughly halfway up the adjacent mountain on its southwest facing slopes (29°18'S, 28°19'E) with the same infection. A gravid female was collected above the snowline on top of the mountain (29°18'S, 28°07'E), and had a similar blood picture. This parasite was also found in seven other specimens of the same host from the southern parts of the Free State on the farm Zuurfontein (29°54'S 25°22'E). All these lizard specimens had morphologically the same infections of *Hepatozoon* and *Pirhemocyton.* The haemogregarines were intracytoplasmic and found in mature erythrocytes. Mature and immature stages of these parasites were noted.

In Giemsa-stained blood films parasites occurred singly in erythrocytes. As noted above, these infections were accompanied by *Pirhemocyton* infections (Fig. 3.10B, E, F, H & 1 (blue arrows)), either in the same cells as the haemogregarine or in erythrocytes lacking

Figure 3.10 A-I: Light micrographs of Giemsa stained blood films showing *Hepatozoon* Miller, 1908 sp. A infecting red blood cells of *Agama atra atra* Daudin, 1802 collected near the village of Ha Khojane in Lesotho. **B, E, F, H & I** (Blue arrows)): *Pirhemocyton* Chatton & Blanc, 1914 infections accompanying the *Hepatozoon* sp. A infection. **B & C** (black arrows)): slightly elongated immature stages, **A, D & G** (black arrows): pale whitish cap at the anterior end of the more mature parasites. Scale: **A-I**: 10 μm.

the apicomplexan. Only gamont stages of the haemogregarine were recognised in blood films and these were typically only slightly elongate when immature (Fig. 3.10 B-C (black arrows)). The cytoplasm of these immature gamonts stained pale blue with Giemsa, and the nucleus, which lay slightly nearer the posterior end, consisted of stranded, pale-stained chromatin. Cytoplasm contained dark peripheral granules in the vicinity of the nucleus. Immature stages measured 4.2 ± 0.7 (3.1-5.3) μm by 3.8 ± 0.85 (2.7-4.9) μm (n= 12).

Mature gamonts were found in 220\10000 (0.2%) of mature erythrocytes and were elongate. These measured 8.8 ± 2.06 (7.31-10.36) μm long by 3.7 ± 0.93 (3.54-3.9) μm wide (n = 62). They were generally pale-stained with Giemsa, while the nucleus stained pale pink and the anterior end of the parasites bore a pale, almost whitish, cap (Fig. 3.10A, D & G (black arrows)). The nucleus measured 3.1 ± 0.9 (2.7-3.5) μm by 3.6 ± 0.8 (3.4-3.9) μm (n = 26). The nucleus was located in the posterior half of the parasite body.

Comments

These infections are the first haemogregarines reported from *Agama atra atra* in South Africa. The parasite differs from all other similar species in its overall morphological dimensions: mature gamonts are much smaller than cordylid haemogregarines and have a pale stained nucleus. This is the first description of a *Hepatozoon* species from *Agama atra atra*. In comparison with an unnamed haemogregarine described from the agamid *Agama ruppelli* by Ball (1967) from East Africa, the gamont stages in *A. ruppelli* measured 9.8 by 4.3μm with a lightly stained nucleus and prominent granules in the cytoplasm, while those of *Hepatozoon* sp. A gamonts were smaller (8.8 ± 2.06 (7.31-10.36) μm long by 3.7 ± 0.93 (3.54-3.9) μm wide (n=62)). *Hepatozoon* sp. A were also generally pale-stained with Giemsa, while the nucleus stained pale pink and the anterior end of the parasites bore a pale, almost whitish, cap and did not have the granules characteristic of the parasite from *A. ruppelli*. It is interesting that *Hepatozoon* sp. A was found in *Agama atra atra* located in disjunct populations.

***Hepatozoon* sp. B from *Pseudocordylus melanotus melanotus* (A. Smith, 1838)**
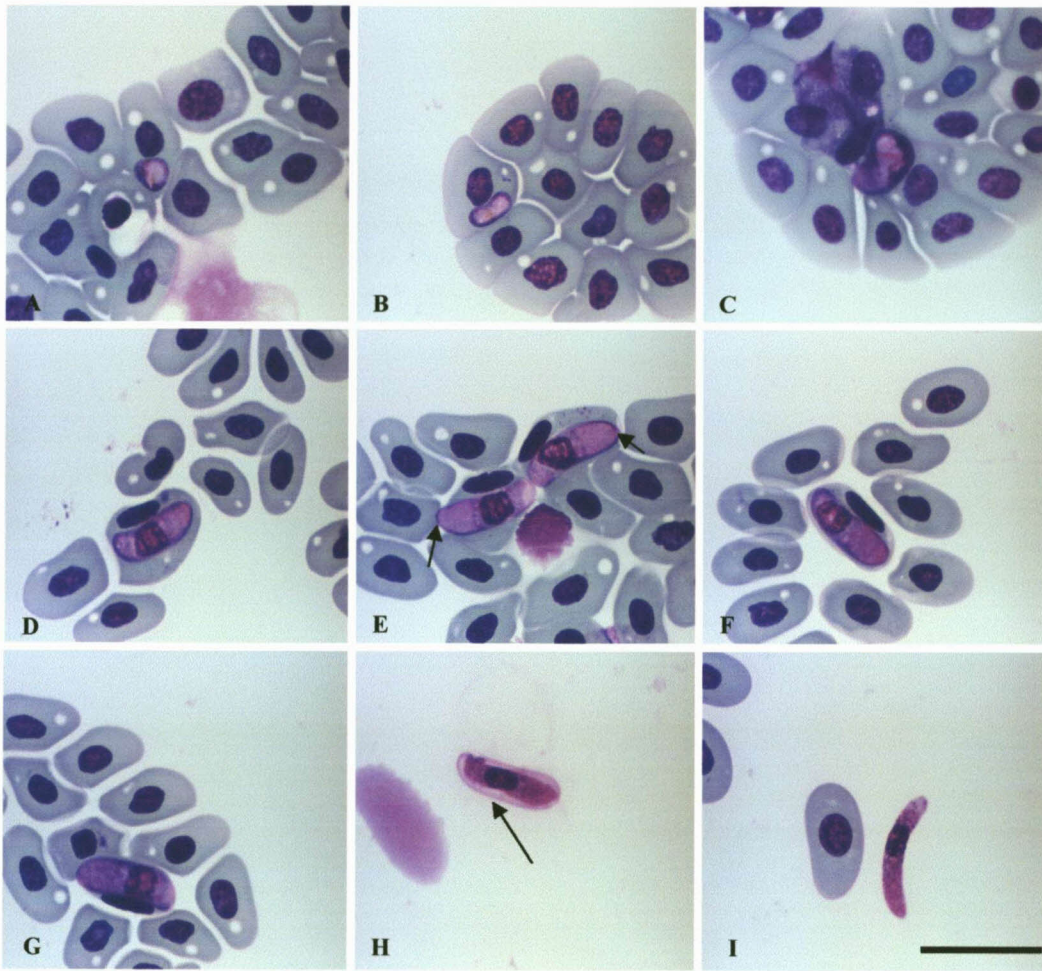
Host: *Pseudocordylus melanotus melanotus* (A. Smith, 1838).

Locality: Clarens (28°31'S, 28°25'E), Eastern Free State.

Hepatozoon sp. B was found in all five *Pseudocordylus melanotus melanotus* (A. Smith, 1838) captured at an isolated rocky outcrop (28°31'S, 28°25'E) near Clarens in the Eastern Free State. The infected lizards were two males and three females (of which one also had a *Plasmodium* infection (Fig. 3.11A-C). The haemogregarines were usually intracytoplasmic, found in mature erythrocytes. Extracellular and immature stages were noted. Effects on host cells were marked, the mature stages displacing the host nucleus to the side of the erythrocyte and apparently reducing its width by up to 50 %.

In Giemsa-stained blood films parasites occurred singly in erythrocytes and extracellular parasites were also present (Fig. 3.11H-I). Only gamont stages were recognised in blood films and these were typically rather elongate when immature (possibly those in Fig. 3.11A-C, but these could also be malarial parasites). These immature gamonts stained purple with Giemsa and their nuclei were difficult to discern. Immature stages measured 2.5 ± 0.95 (2.25-4.4) μm by 4.2 ± 0.87 (3.1-7.21) μm (n=10).

Mature gamonts infected 6850/10 000 (69%) of cells and were elongate structures sometimes with a slightly reflexed posterior region. The main body of the parasite measured 19.2 ± 0.83 (18.4-20.7) μm by 6.2 ± 0.7 (5.9-6.6) μm (n=23). The mature gamont stained mostly deep purple to purple with Giemsa and its cytoplasm was relatively free of granules, while its nucleus stained reddish purple and comprised dense strands of chromatin. The anterior end of the parasite bore a characteristic pinkish cap (Fig. 3.11E (arrows)). The nucleus measured 5.8 ± 0.9 (5.5-6.4) μm (n=23) and was located nearer to the posterior end of the parasite body.

Figure 3.11. Light micrographs of *Hepatozoon* Miller, 1908 sp. B. from peripheral blood of *Pseudocordylus melanotus melanotus* (A. Smith, 1838). collected in Clarens in the eastern Free State. **A-C:** possible immature stages of *Hepatozoon* sp. B or perhaps a species of *Plasmodium* Marchiafava and Celli, 1885. **D-G:** mature gamonts in mature erythrocytes. **H-I:** Extracellular gamont in capsule (arrow). **I:** Free gamont. Scale: **A-I:** 20 μm.

The intraerythrocytic mature gamont was surrounded by a purple-stained capsule (see Fig. 3.11H which shows an extracellular example). This was only slightly bigger than the gamont. Extracellular mature gamonts appeared longer and narrower than their intracellular counterparts when free of the capsule and measured 21.4 μm by 3.2 μm (n=1). The tail regions of these extracellular forms were slightly curved.

Comments

This investigation led to the first description of this parasite from *Pseudocordylus melanotus melanotus* in Clarens (28°31'S, 28°25'E), Eastern Free State. It compared with the general morphology of *Haemogregarina boskani* Catouillard, 1909 from a Tunisian lizard *Acanthodactylus boskianus*. *Haemogregarina boskani* gamonts were oval or slightly reniform with sometimes a broader end than the other, while *Hepatozoon* sp. B differed in having rounded nuclei and cytoplasm relatively free of granules. It was also wider than all of those haemogregarines described in the Cordylidae in the current survey. *Hepatozoon* sp. B has nuclear dimensions of 5.8 ± 0.9 (5.5-6.4) μm (n=23) and the nucleus is located nearer to the posterior end of the parasite body. The overall dimensions of *Hepatozoon* sp. B were 19.2 ± 0.83 (18.4-20.7) μm by 6.2 ± 0.7 (5.9-6.6) μm (n=23). *Haemogregarina boskani* measured 8.5-16.5 μm by 4-16 μm with prominent chromatophillic granules. Furthermore, *Hepatozoon* sp. B has characteristically purple to deep purple-stained capsules with a light purple cytoplasm and a rounded compact slightly posteriorly situated nucleus that are not common in other haemogregarines found in lizards.

***Hepatozoon* sp. C from *Pseudocordylus melanotus subviridis* (A. Smith, 1838)**
Host: *Pseudocordylus melanotus subviridis* (A. Smith, 1838).
Locality: Ha Thaba Bosiu (29°20'15''S 28°11'58"E), Lesotho.

Hepatozoon sp. C was found in seven of eight *Pseudocordylus melanotus subviridis* captured at Ha- Thaba Bosiu in Lesotho. The infected lizards were five males and three
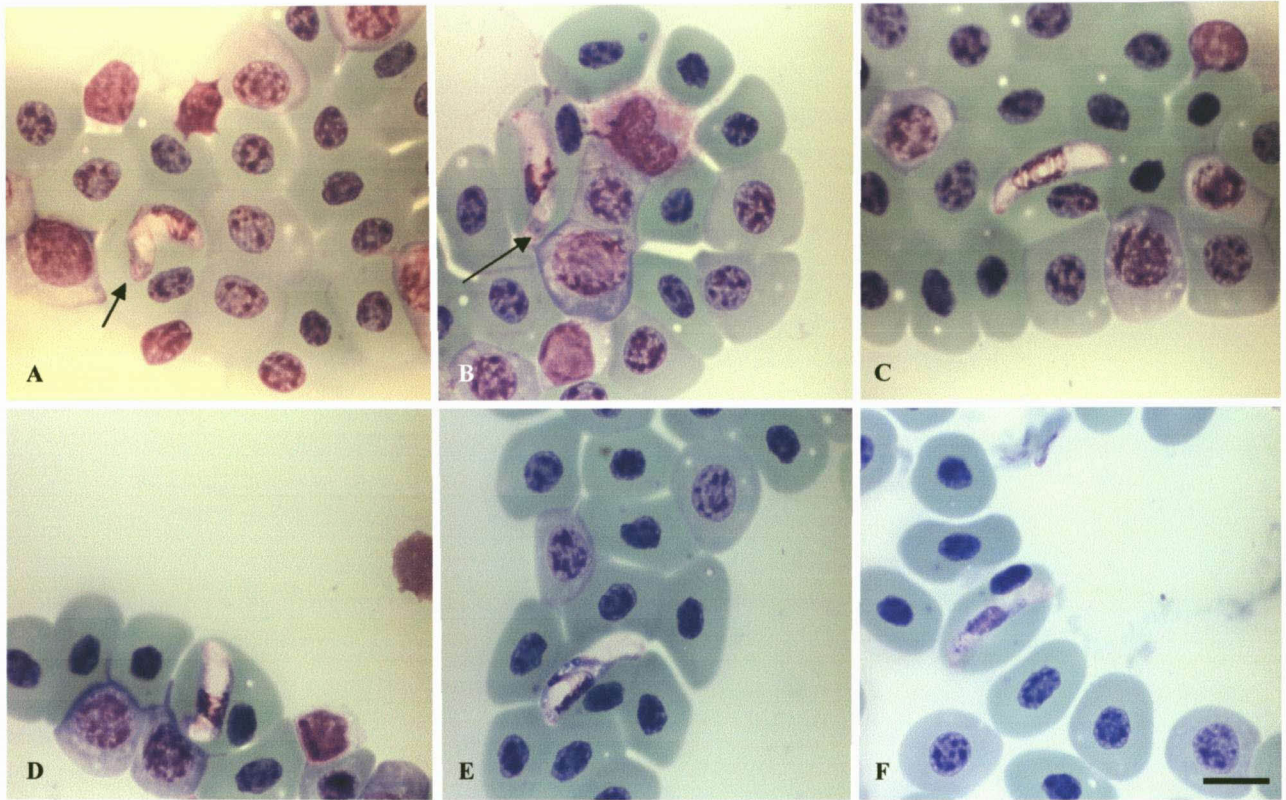
Figure 3.12. **A-F:** Light micrographs of *Hepatozoon* Miller, 1908 sp. C collected from the peripheral blood of *Pseudocordylus melanotus subviridis* (A. Smith, 1838) in the Syncunjane river catchment near the village Ha Thaba Bosiu. **A-F:** Various gamont stages parasitizing mature erythrocytes. **A:** immature gamont with dense nucleus near the posterior end, note the polar cap at the anterior end (arrow). **B:** slender gamont with dense pigmentation along one extremity (arrow), note the lateral displacement of the host cell's nucleus. **C, D, E:** different gamonts with dense nuclear pigmentation. **F:** mature gamont in mature erythrocyte with a compact dense nucleus. The effect on the host cell is marked with a displacement of the host cell nucleus. Scale: **A-F:** 10 μm.

females. The parasites were intracytoplasmic and found in mature erythrocytes, of which 1000/10 000 (10%) of cells were infected.

Effects on host cells were marked, the mature stages enlarging the host cell and displacing the host nucleus to one side or the end of the erythrocyte (Fig 3.12A-F). Sometimes the host nucleus was reduced in size (Fig 3.12D, F & G).

In Giemsa-stained blood films parasites occurred singly in a single erythrocyte. Extracellular parasites were not detected and only mature gamont stages were recognised. Mature gamonts (Fig. 3.12A-F) were elongate structures with a slightly reflexed posterior region (Fig 3.12B (arrow)).   The main body of the parasite measured 19.5 ± 0.89 (17-25) μm by 2.24 ± 0.99 (2.30-3.52) μm (n=30).

In mature gamonts the cytoplasm stained pale pink or purple with Giemsa, and the centrally or posteriorly placed nucleus (measuring 3.45 ± 0.5 (3-4) μm by 1.61 ± 0.44 (2-4) μm (n=30) consisted of banded chromatin that stained purple. Parasite cytoplasm also contained purple-stained granules posterior to the nucleus (Fig. 3.12F). The anterior end of the mature gamont sometimes bore a characteristic cap (Fig. 3.12A (arrow)).   A capsule was not visible

Comments

This is the first description of a haemogregarine in the Cordylidae from Lesotho. This new parasite seems to be very narrow in comparison to the rest of *Hepatozoon* descriptions in this thesis, but broader than *Hepatozoon gracilis* from an Egyptian skink (Bashtar, Abdel-Ghaffar & Shazly 1987). *Haemogregarina acanthodactylii* Ramadan, 1974 described from an Egyptian lizard *Acanthodactylus boskianus* by Ramadan (1974) had broad forms with rounded ends, a rounded, centrally placed, nucleus and red staining granules. These measured 10-11 by 5.5-5.6μm for the broad forms and 11.5-13.5 by 4-5 μm for the slender ones. However, *Hepatozoon* sp. C was longer and its morphometrical dimensions were (19.5 ± 0.89 (17-25) μm by 2.24 ± 0.99 (2.30-3.52) μm (n=30) in contrast with *Haemogregarina acanthodactylii*. These slender gamonts of *Hepatozoon*

sp. C have a marked effect on host cells when mature, which can mean displacement of the host cells nucleus to one extremity of the cell, where the nucleus itself is laterally compacted.

Hepatozoon sp. D from *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

Host: *Pseudocordylus melanotus subviridis* (A. Smith, 1838).

Locality: Jorotane river near village of Ha Rapokolana (29°22'36''S 28°01'53E), Lesotho.

Hepatozoon sp. D was found in all of *Pseudocordylus melanotus subviridis* captured at the Jorotane Catchment in Lesotho. The infected lizards were two males and two females. The parasites were intracytoplasmic and found only in mature erythrocytes and 1850/10 000 (19%) of cells were infected. Effects on host cells were marked, the mature stages displacing the host nucleus to one side and enlarging the host cells (Fig. 3.13A, D, E, F, G, H, K & L). Enlarged host cells were also sometimes de-haemoglobinised (Fig. 3.13A, D, F, H, K, L & M).

In Giemsa-stained blood films parasites occurred singly in mostly mature erythrocytes and extracellular parasites were also present. Only gamont stages were recognised in blood films and these were typically rather elongated even when very immature (Fig. 3.13A (red arrow)). These very young gamonts stained lightly purple with Giemsa, and a centrally placed nucleus consisting of granular chromatin stained dark purple with Giemsa. Cytoplasm contained two distinct pink-stained areas on either side of the host nucleus. Immature gamonts measured 8.5 ± 1.5 (5.2-10.1) µm long and 2.2 ± 5.7 (2.0-2.9) µm (n=11) wide.

Maturing and mature gamonts were elongate structures often with a reflexed posterior region when intracellular. The mature gamont stained mostly pale purple with Giemsa and its cytoplasm contained abundant characteristic pink-stained granules (Fig. 3.13E). Its nucleus stained pink and comprised large granules of chromatin (Fig. 3.13E (arrow)).

The anterior end of the parasite bore a characteristic pink-stained cap (Fig. 3.13A, F, G, L, M (arrows)). The nucleus measured 5.2 ± 0.7 (4.8-6.61) μm by 3.64 ± 1.12 (3.1-3.92)

Figure 3.13. Light micrographs of *Hepatozoon* Miller, 1908 sp. D. from peripheral blood of *Pseudocordylus melanotus subviridis* (A. Smith, 1838) collected in the Jorotane River catchment in Lesotho. A-C (red arrows): Immature gamonts. A-M: mature gamont stages in mature erythrocytes. Black arrows of A, F, G, L & M: indicate the polar caps, arrows of E: indicate nuclear granules. A, D, E, F, G, H, K, & L: mature stages displacing the host nucleus to one side and causing severe hypertrophy. A: immature and mature gamonts, B: maturing and mature gamonts. C: possible early stages of host cell occupation. D: damaging effects of a mature gamont on the host cell. E: different nuclear positions, note the folding of the tail. F: Dehaemoglobinisised effect on the host cell, arrow shows polar cap. G: folding of the haemogregarine with lateral displacement of host cell nucleus. H, K, L & M: dehaemoglobinisised host cells B, I, & J: characteristic folding of mature gamonts around host cell nucleus with maturing gamont in M: (black arrow). N: extracellular gamont. Scale: A-N: 10µm
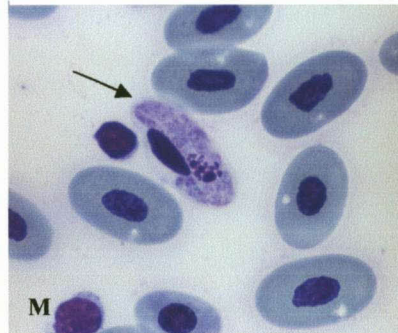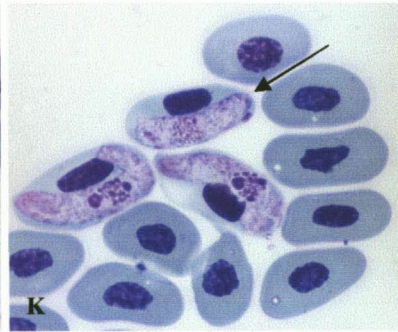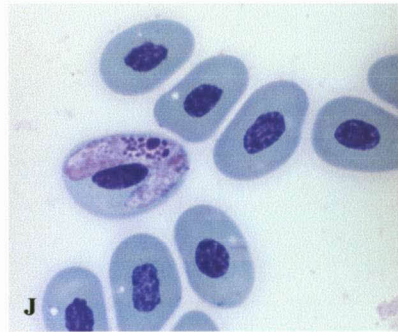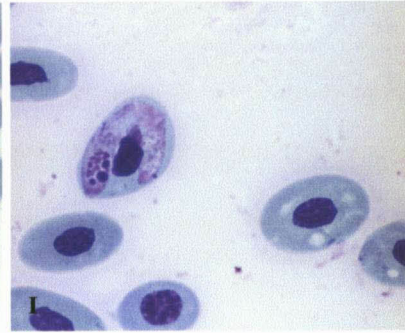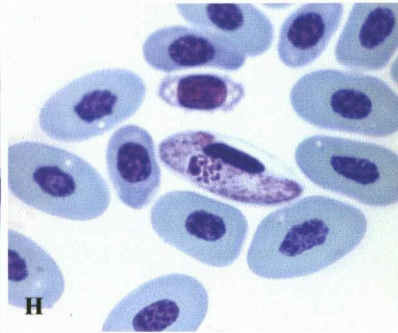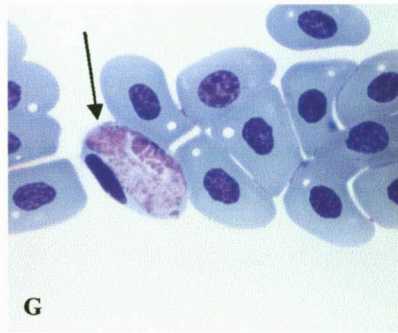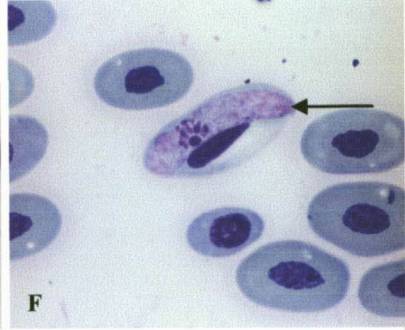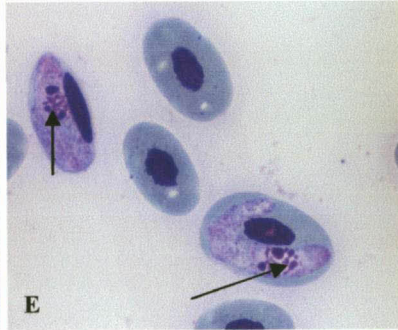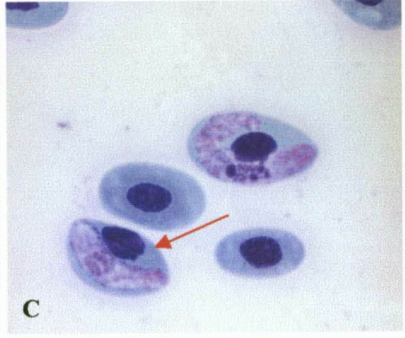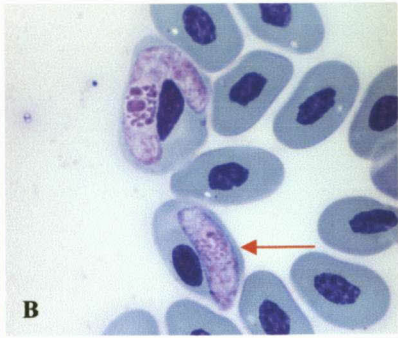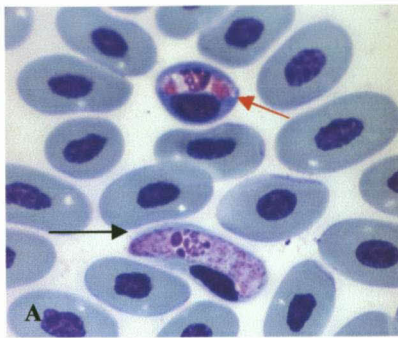
µm (n=12) and was located centrally (Fig. 3.11J) or commonly nearer to the anterior end of the parasite body (Fig. 3.11B). The main body of the parasite measured 25.9 ± 0.8 (25.1-27.05) µm by 5.25 ± 0.98 (4.98-5.56) µm (n=20), with a reflexed tail measuring 5.87 ± 0.94 (5.25-7.84) µm (n=12), that was therefore roughly a third of the length of the main body of the haemogregarine. Extracellular mature gamonts were rather worm-like in appearance, appearing much longer and thinner than their intracellular counterparts (Fig. 3.13N). They measured 35µm by 3.2 µm (n=2). The tail regions of these extra cellular forms were often, though not invariably, reflexed.

Comments

These are most likely new host and species records. The gamonts differ from those of other species of *Hepatozoon* found in such lizards by having a characteristically large, curved granulated body and a heavily granulated nucleus consisting of both large and small granules. It is a little similar morphologically to described species like those of *Haemogregarina damiettae* Ramadan, Saoud, Mohammed & Fawzi, 1996 in an Egyptian lizard *Acanthodactylus boskianus* (see Ramadan, Saoud, Mohammed & Fawzi, 1996). One end of this haemogregarine was broader with the nucleus closer to one end than the other, but it measured 15 µm-16.5 µm by 2.5 µm-7.5 µm whereas *Hepatozoon* sp. D measures 25.9 ± 0.8 (25.1-27.05) µm by 5.25 ± 0.98 (4.98-5.56) µm (n=20) and differs from *Haemogregarina damiettae* in the absence of a thick sheath and in its nuclear arrangements. The current species has a distinct nuclear arrangement with heavily pink staining chromatin with one or two darker and larger granules. Although the nuclei of *Haemogregarina damiettae* are in the same way diffused they consist of discreet strands whereas *Hepatozoon* sp. D has a granulated nucleus which measures 5.2 ± 0.7 (4.8-6.61) µm by 3.64 ± 1.12 (3.1-3.92) µm (n=12) and is located centrally. Infections of *Hepatozoon* sp. D had a severe effect on the host cells. Infections of mature gamonts caused dehaemoglobinised areas in some host cells. The gamonts can also occupy all or most of the host cell cytoplasm and displace the nucleus to one side of the cell (Fig. 3.13A, E, G, H, K, L, & M).

Hepatozoon sp. E from *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

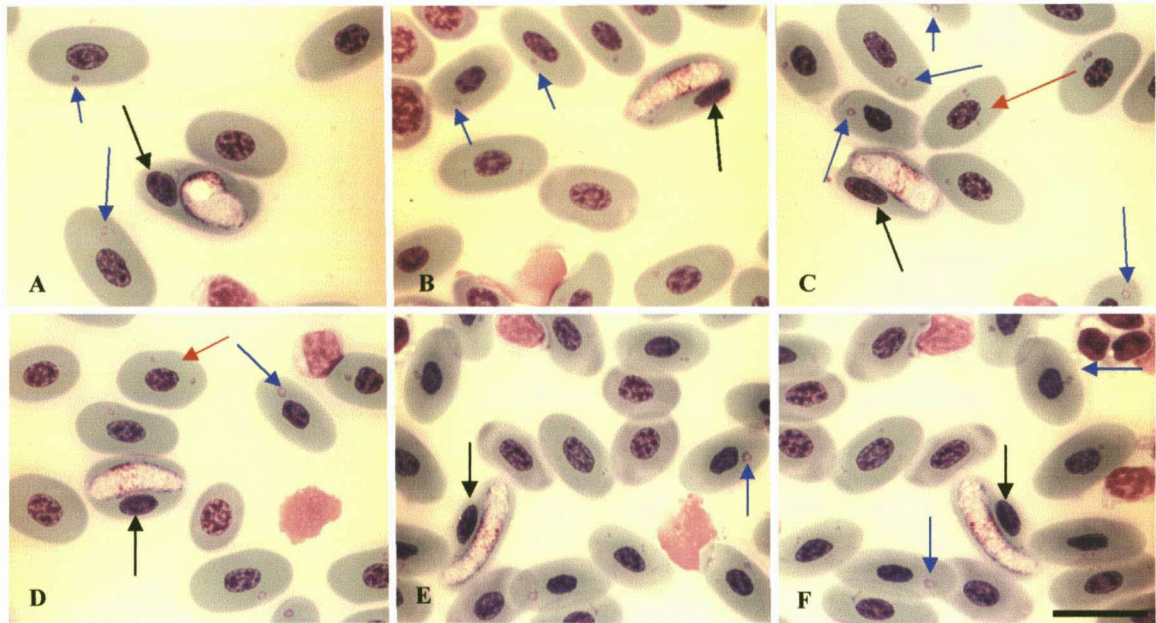Host: *Pseudocordylus melanotus subviridis* (A. Smith, 1838).

Localities: In the village of Ha Rapokolana (29°22'36''S 28°01'53E), Lesotho.

Hepatozoon sp. E was found in three of three *Pseudocordylus melanotus subviridis* captured in the village of Ha Rapokolana in the Jorotane catchment. Three male specimens were collected and sampled.

The parasites were intracytoplasmic and found in mature erythrocytes of all three male specimens and 550/10 000 (5.5%) of cells were infected in one of them. Effects on host cells were marked, both the immature and mature stages displacing the host nucleus to the side of the erythrocyte (Fig. 3.14A-F). In most cases the host nucleus was laterally compacted (Fig. 3.14A-F (black arrows)).

In Giemsa-stained blood films parasites occurred singly in an erythrocyte. These infections were accompanied by *Pirhemocyton* or (*Sauroplasma*-like) infections (Fig. 3.14A-F (blue and red arrows), but not in the same cells. Only gamont stages were recognised in blood films and these were typically rather oval when immature (Fig. 3.14A). Immature gamonts stained purple with Giemsa, the possible posteriorly placed nucleus consisting of granular chromatin stained dark purple with Giemsa. Their cytoplasm contained dark granules peripherally and a large vacuole. Immature stages measured 3.6 ± 0.5 (3-4.4) μm by 4.8 ± 0.71 (4.5-6.2) μm (n=11).

Mature gamonts were thin, elongate structures with rounded extremities, measuring 11.46 ± 0.41 (9.24-13.8) μm by 2.4 ± 0.4 (2.2-3.54) μm (n=30). The mature gamont stained mostly deep blue purple to purple along its sides with Giemsa and its cytoplasm was finely granulated. Nuclei stained pale reddish purple and comprised finely stranded chromatin (Fig.3.14A-F (black arrows)). The nucleus measured 2.4 ± 0.47 (2.1-3.2) μm by 3.15 ± 0.66 (2.4-3.7) μm (n=20) and was located towards the anterior half of the parasite body.

Figure 3.14. A-F: Light micrographs of *Hepatozoon* Miller, 1908 sp. E. from the peripheral blood of *Pseudocordylus melanotus subviridis* (A. Smith, 1838) captured in the village of Ha Rapokolana in the Jorotane catchment. **A-F** (black arrows): show lateral compacted host nuclei. **A-F** (blue arrows): *Pirhemocyton* Chatton and Blanc 1914 -like infections. **C & D** (red arrows): multiple infection centres of a possible *Pirhemocyton* infection. **A:** Immature stage of the haemogregarine. Scale: **A-F:**10μm.

Comments

These infections most likely represent new records for *Pseudocordylus melanotus subviridis* in its locality and new species compared with all others described. *Hepatozoon* sp. E differs from all other species in the overall morphological dimensions: the mature gamont is much slimmer than others described from cordylid lizards with characteristically pigmented granules in the cytoplasm and nucleus. It shows some resemblance to the general morphology of *Haemogregarina capensis* Conor, 1909 from an Egyptian lizard *Acanthodactylus pardalis*, but this has a nucleus in the form of a rectangular band. *Hepatozoon* sp. E has a characteristically granulated nucleus measuring 2.4 ± 0.47 (2.1-3.2) μm by 3.15 ± 0.66 (2.4-3.7) μm (n=20). *Haemogregarina capensis* measured 8μm by 3.4 μm and slightly affected the host cell with a displaced host cell nucleus. *Hepatozoon* sp. E gamonts measured 11.46 ± 0.41 (9.24-13.8) μm by 2.4 ± 0.4 (2.2-3.54) μm (n=30) and displaced the host nucleus and in some infections caused dehaemoglobinised areas in the host cell.

Hepatozoon (*Haemogregarina*) *sebae* (**Laveran and Pettit, 1909**) **Smith, 1996 from** *Python sebae natalensis* (**Gmelin, 1789**)

Type Host: *Python sebae* (Gmelin, 1789)

Type Locality: Senegal.

Present study

Host: *Python sebae natalensis* (Gmelin, 1789).

Locality: Bloemfontein, Free State (snake bred in captivity by a licenced breeder).

Hepatozoon (*Haemogregarina*) *sebae* (Laveran and Pettit, 1909) Smith, 1996 was found in an African rock python, *Python sebae* (Gmelin, 1789) in Senegal, North Africa. The same species was found in the present study, but in a different variety (Southern race) of African rock python (*Python sebae natalensis*). This subspecies has larger shields and a different colour pattern than the typical race (Branch 1998).

The current investigation led to the discovery of *Hepatozoon sebae* in a captive African rock python (*Python sebae natalensis*) (permit nr: HK/P19C/03894/002 (appendix A)) in the Bloemfontein district. The infected snake was a female. The parasites were intracytoplasmic and found in mature erythrocytes of which 450/10 000 cells were infected. Effects on host cells were marked, the mature stages displacing the host nucleus to one side of the erythrocyte.

Light microscopy

In Giemsa-stained blood films parasites occurred singly in erythrocytes. Extracellular parasites were absent. Only gamont stages were recognised in blood films and these appeared to be mature (Fig. 3.15A-C). These stages stained bluish purple, with a nucleus that stained deep purple with Giemsa, positioned near the posterior end of the parasite. Cytoplasm contained coarse dark granules, especially in the anterior portion of the parasite (Fig. 3.15A-C).

Mature gamonts were overall elongated and slightly curved structures with rounded ends (Fig. 3.15A-C). The main body of the parasite measured 11.36 ± 1.1 (10.4-12.33) µm long and 3.8 ± 1.2 (3.22-4.51) µm wide (n=10). A small pinkish-red cap occurred on the anterior end of the parasite, and the entire haemogregarine appeared to be surrounded by a capsule. The nucleus measured 2.3 ± 2.5 (2.1-2.5) by 4.42 ± 2.7 (4.24-4.46) µm (n=10) and was located nearer to the posterior end of the parasite body. The parasite closely resembled the type description of Laveran & Pettit (1909).

Electron microscopy

Initial observations on *Hepatozoon (Haemogregarina) sebae* by TEM showed that the gamont had a typical apicomplexan structure. The extracellular haemogregarine shown in Fig. 13D-F is surrounded by a typical plasma-membrane or membranous sheath (pellicle) (Fig. 3.15F) and it contains abundant micronemes (Fig. 3.15E & F), a few rhoptries (Fig. 3.15E & F) and a distinct nucleus (Fig. 3.15D & E). A capsule is not visible surrounding this extracellular form. *Serpentoplasma*–like structures similar to those of lizards (See Fig. 3.5G-L) and of snakes (Fig. See Fig. 3.9D-I) were also seen
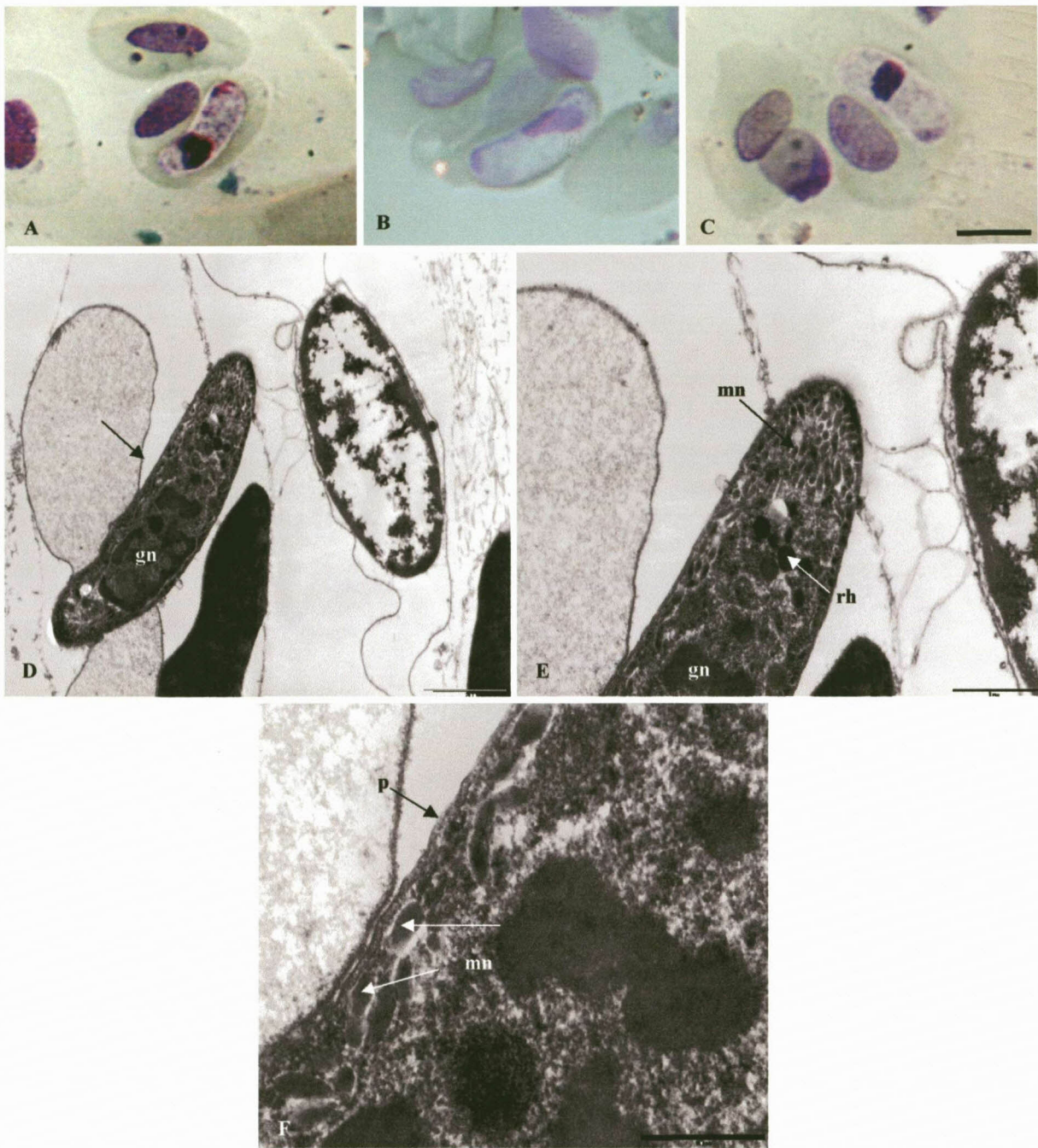
Figure 3.15. A-C: light micrographs of *Hepatozoon* (*Haemogregarina*) *sebae* (Laveran and Pettit, 1909) Smith, 1996 in Giemsa stained films of peripheral red blood cells of a captive African rock python (*Python sebae natalensis* (Gmelin, 1789)). **D-F:** Transmission electron micrographs of infections from peripheral erythrocytes of the same python (*Python sebae natalensis*), **D-F:** extracellular haemogregarine showing the gamont nucleus (gn), rhoptries (rh) micronemes (mn) and pellicle (p) in **F. E & F:** abundant micronemes (mn) and rhoptries (rh). **D & E:** (gn): gamont nucleus. Scale: **A-C:** 10 μm, **D:** 2 μm, **E:** 1 μm, **F :** 500 nm.

(not shown).   Further TEM studies are needed to elucidate the fine structure of these enigmatic infections and those of the apicomplexan in any detail.

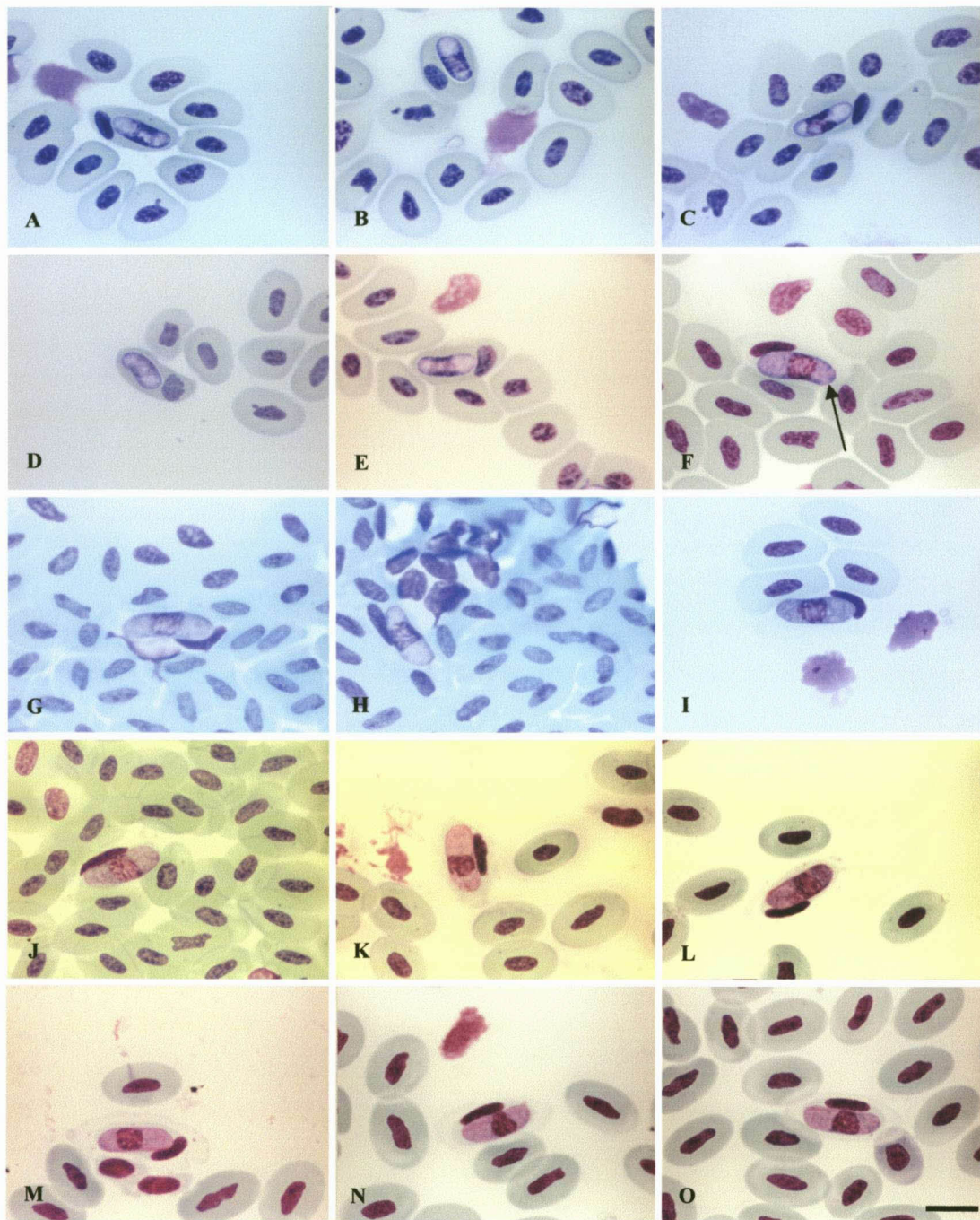### *Hepatozoon* sp. F from *Psammophylax tritaeniatus* (Günther, 1868)

Host: *Psammophylax tritaeniatus* (Günther, 1868).

Locality: Bloemfontein, Free State.

Hepatozoon sp. F was found in all five *Psammophylax tritaeniatus* (Günther, 1868) specimens captured in the Bloemfontein district.   The infected snakes were two males, two females and a juvenile.   The parasites were intracytoplasmic and found in mature erythrocytes of which an average of 150/10 000 (1.5%) of cells were infected.   Effects on host cells were marked, the mature stages displacing the host nucleus to one side of the erythrocyte and causing severe dehaemoglobinisation of the host cells.   The juvenile male snake had immature gamonts (Fig. 3.16A-C), with the host erythrocyte nuclei showing lateral displacement and compaction.   The young female snake had a series of developmental stages in its cells (Fig. 3.16D-F).   A gravid female (Fig. 3.16G-I) and two adult males (Fig. 3.16J-L) and (Fig. 3.16M-O) respectively, had mature gamonts in their blood and a severe effect on the morphology of the host cells was noted.   The host nucleus was compacted to about half of its original width.   The cytoplasm was severely dehaemoglobinised and the cell perimeter stretched to about twice its original size.

In Giemsa-stained blood films parasites occurred singly in erythrocytes.   Extracellular parasites were absent.   Only gamont stages were recognised in blood films and these were typically elongate when immature (Fig. 3.16A-E).   These immature gamonts stained pale blue with Giemsa, and the nucleus, which was located centrally, or slightly posteriorly, consisted of banded chromatin and stained a deep purple with Giemsa. Immature stages were small and measured 7.8 ± 0.96 (7.1-8.5) μm long and 4.1 ± 0.7 (3.5-4.7) μm wide (n=15), with nuclei 3.32 ± 0.7 (2.88-3.76) by 4.56 ± 0.79 (4.30- 4.94) μm (n=15).

Figure 3.16. **A-O:** light micrographs of Giemsa-stained blood films showing *Hepatozoon* Miller, 1908 sp. F from the peripheral blood of five different specimens of striped skaapstekers (*Psammophylax tritaeniatus* (Günther, 1868)) collected in the Bloemfontein district. **A-E:** immature gamonts. **A-C:** immature stages from a young ♂ 20cm SVL. **D-F:** immature stages and mature stage **F:** of a young ♀ 31cm SVL. **G-I:** different maturity stages of gamonts from a gravid ♀, 61cm SVL. **J-L:** mature gamonts from a mature ♂ (62cm SVL.) showing cell degradation. **M-O:** mature gamonts from a mature ♂ showing severe hypertrophy of the cells and hemoglobin loss. **F:** mature gamont showing rounded anterior and posterior regions, (arrow). Scale: **A-O:** 10 μm.

Mature gamonts were also elongate structures with rounded anterior and posterior regions (Fig. 3.16F (arrow)). The main body of the parasites measured 12.65 ± 0.83 (10.2-15.1) µm long by 6.02 ± 0.88 (5.4-6.65) µm (n=35). The mature gamonts stained mostly pale purple with Giemsa and their cytoplasm largely lacked granules, while their nuclei stained dark purple and comprised strands or granules of dense chromatin. A cap was detected at the anterior end of some mature gamonts, but was not visible in most parasites. The nucleus measured 4.5 ± 0.6 (4.14-4.97) by 5.01 ± 0.79 (4.57-5.46) µm (n=20). In mature gamonts, and as in immature forms was mostly located in the posterior half of the parasite body.

Comments

This is the first record and description of a haemogregarine from *Psammophylax tritaeniatus* in South Africa. One distinguishable characteristic of this species is the damage it causes to the host cell. The host cell cytoplasm is often robbed of haemoglobin, and may increase in volume (hypertrophy) and its nucleus is distorted. Infected cells are therefore changed in form and their function may be altered. This species shows some resemblance to the dimensions of *Hepatozoon bitis* (Fantham, 1925) Smith, 1996 found in the puff adder, but now considered a synonym (according to Peirce & Bengis (1998) of *Hepatozoon dogieli* Hoare, 1920). The dimensions of *H. bitis* were 12.5-15.3 µm and for *H. dogieli* 14 by 6µm. *Hepatozoon* sp. F had dimensions of 12.65 ± 0.83 (10.2-15.1) µm long by 6.02 ± 0.88 (5.4-6.65) µm (n=35). This species was also compared with the descriptions given by Sambon & Seligmann (1907) of *Hepatozoon stattocki* Sambon & Seligmann, 1907 in the diamond snake *Python (Morelia) spilotes*. It had nearly the same morphometrical dimensions, but effects on the red cells were different. Whereas the current parasites compressed and elongated the nucleus, *H. stattocki* had little or no influence on the cell, with the nucleus only slightly displaced and parasite occupying nearly half the cell.

SAURIAN MALARIA

3.3.2. PLASMODIDAE

A number of endoglobular pigmented parasites were found in representatives of the Cordylidae, Agamidae and Gekkonidae. Apart from previous descriptions by Pienaar (1962) and Garnham (1966), Ayala (1977) listed nine species of *Plasmodium* from African lizards and Mutinga & Dipeolu (1990), noted 10 more that they had found in Kenya (see Dipeolu & Mutinga 1989). Mutinga & Dipeolu (1990), focusing particularly on *Mabuya striata* and *Agama agama*, also reported that these lizards could carry up to four concurrent infections with different malaria species.

Clearly, *Plasmodium* species in lizards can be difficult to identify, particularly if they form mixed infections. For this reason, precise identification of the species (or mixed species) found in the current work awaits further study, especially as some infections were found in only one or two lizards. Preliminary searches through the literature, do suggest that at least some of these infections will eventually prove to be new species. However, they are designated *Plasmodium* species A, B, C & D at present.

Plasmodium **sp. A from** *Cordylus polyzonus polyzonus* **A. Smith, 1838**
Host: *Cordylus polyzonus polyzonus* A. Smith, 1838
Locality: On the farm Zuurfontein (29°54'S 25°22'E) Jagersfontein district, Southern Free State.

Plasmodium sp. A infections were discovered in the blood of *Cordylus polyzonus polyzonus* A. Smith, 1838 in 17 of 38 specimens captured on a farm Zuurfontein in the Jagersfontein district, Southern Free State. These infections appeared different from all descriptions given for malarias occurring in the family Cordylidae in South Africa.
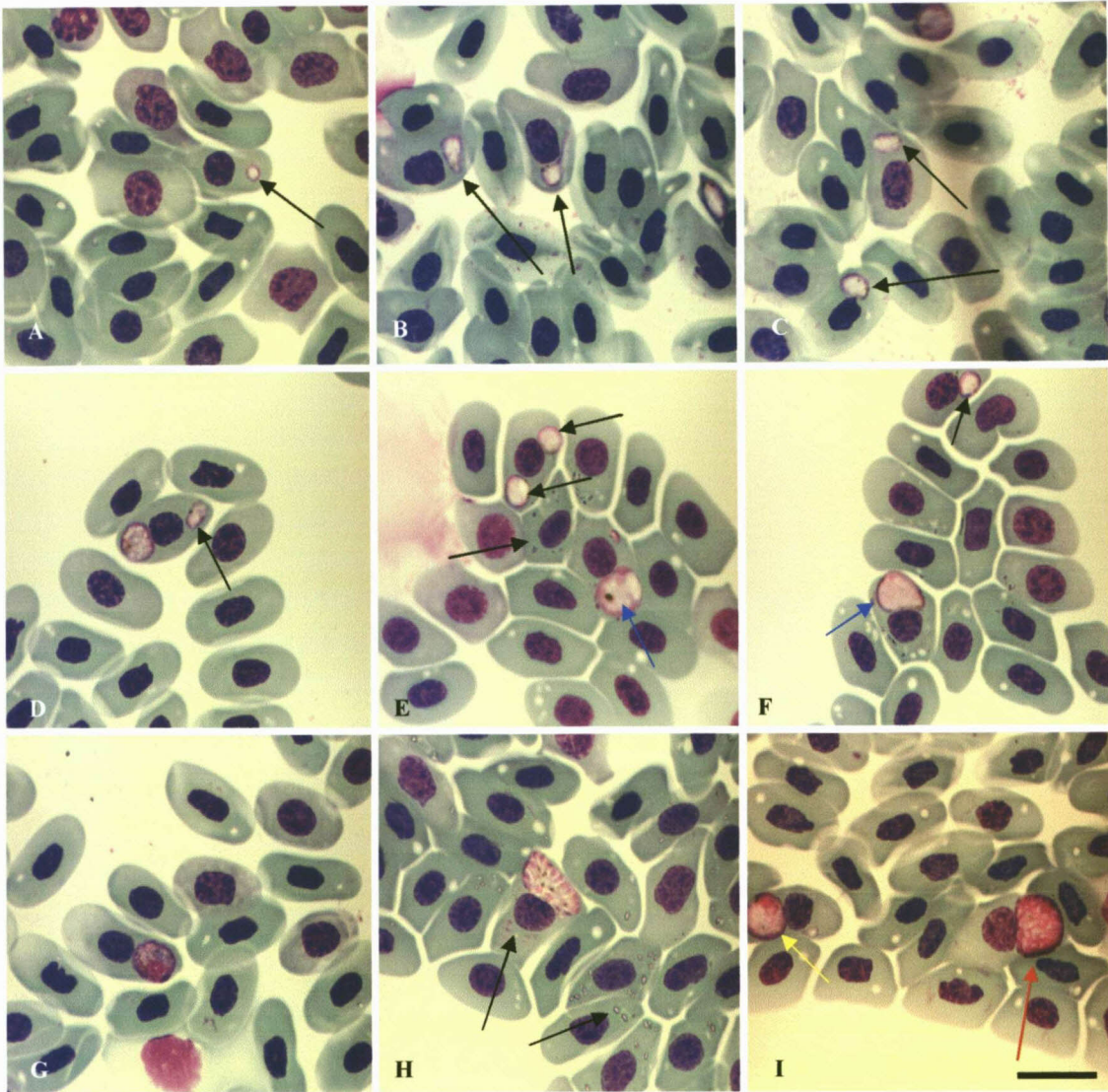
Figure 3.17. Light micrographs of Giemsa stained blood films showing *Plasmodium* Marchiafava and Celli, 1885 sp. A from *Cordylus polyzonus polyzonus* A. Smith, 1838 captured on the farm Zuurfontein in the Southern Free State. **A-F:** (black arrows): trophozoites with fine malaria pigment, **E:** two trophozoites located at opposite poles of the host cell **D-F:** different maturing meronts (**E & F**) (blue arrows). **E, F, & H:** coarse pigment granules within erythrocyte cytoplasm (arrows). **G-I:** gametocytes and meronts, **G:** microgametocyte, **H:** meront probably yielding 16 merozoites, **I:** macrogametocyte (yellow arrow) with meront (red arrow). Scale: **A-I:** 10 µm.

Trophozoites

The earliest asexual forms of the parasites were situated mostly singly in a polar position within mature erythrocytes. Some erythrocytes contained two trophozoites located at opposite poles of the host cell (Fig. 3.17E). These trophozoites were mostly round to oval in shape, but sometimes they were rather triangular, or elongate with extensions (Fig. 3.17A-C). They measured 2.21 ± 0.98 (1.12-4.89) μm by 3.1 ± 1.45 (2.15-5.22) μm (n=10) in diameter. Trophozoites stained dark purple with Giemsa around their periphery, while the vacuole-like centre was relatively unstained. Fine malaria pigment granules were found within some trophozoites, and these, and much coarser pigment granules were found scattered throughout the blood films (Fig 3.17B, E, F & H). Host cells containing trophozoites and other stages of this parasite (below) were largely of normal appearance.

Meronts

These stages varied from round to rather triangular in shape (Fig.3.17F (blue arrow), I (red arrow) & H) and were also polar within erythrocytes. Parasite cytoplasm became increasingly eosinophilic as development of the meront progressed (compare Fig. 3.17F (black arrow) with Fig 3.17I (red arrow)), and merozoite nuclei were seen forming a deep purple band around the perimeter of the maturing meront (Fig 3.17I (red arrow)) these measured 4.48 ± 3.4 (3.4-5.56) μm across (n=11). As they approached maturity, triangular or elongate meronts were seen to contain at least 16 merozoites (Fig. 3.17H) and measured 6.25 ± 4.1 (5.1-7.4) μm (n=13) in diameter.

Gametocytes

Two distinct types of gametocytes were observed and because of their small size, both were suspected to represent immature stages (Fig. 3.17G & I (yellow arrow)). Both types lay in a polar position within the host erythrocyte, and often a trophozoite shared the same host cell (Fig. 3.17D, I). The presumed male gametocyte (microgametocyte) (Fig.3.17G) was round and stained deep purple with an eccentrically placed red-stained nucleus. Presumed macrogametocytes were also round in shape (Fig. 3.17I (yellow

arrow)) and measured 8.8 ± 2 (8.2-9.4) by 6.17 ± 1.4 (5.8-6.54) μm (n=12) and stained light purple, with a peripherally placed nucleus stained deep purple or red.

Comments

When compared with *Plasmodium zonurae* Pienaar, 1962 the trophozoites of this species were smaller (2-2.8) μm than *Plasmodium* sp. A (2.21 ± 0.98 (1.12-4.89) μm (n=10) in diameter). *Plasmodium zonurae* trophozoites also had a light blue cytoplasm with reddish blue chromatin, while *Plasmodium* sp. A stained a dark purple with Giemsa along the periphery of the trophozoite with fine pigment in the centre of the parasite. The occurrence of fine pigment in *Plasmodium* sp. A was one of the main differences between these parasites.

Young meronts of *Plasmodium* sp. A measured 4.48 ± 3.4 (3.4-5.56) μm across (n=11) and more mature ones measured 6.25 ± 4.1 (5.1-7.4) μm (n=13) in diameter. Young segmenters were seen to produce at least 16 merozoites, whereas *P. zonurae* produced an average of 18 merozoites. Microgametocytes measured 12.5 ± 4.0 (12.3-12.8) μm by 6.4 ± 3.1 (6.2-6.6) μm (n=12) in contrast with 8-8.4 by 4.2 μm for *P. zonurae*. *Plasmodium* sp. A was overall bigger than the *Plasmodium* described from *Cordylus vittifer* by Pienaar (1962) and carried considerably more pigment.

Plasmodium sp. B from *Pseudocordylus melanotus melanotus* (A. Smith, 1838)

Host: *Pseudocordylus melanotus melanotus* (A. Smith, 1838).
Locality: Clarens (28°31'S, 28°25'E), Eastern Free State.

Plasmodium infections were discovered in the blood of in one specimen of *Pseudocordylus melanotus melanotus* captured on a rocky outcrop in the Clarens district, Southern Free State and in the village of Ha Rapokolana(29°22'36''S 28°01'53E), Lesotho. The lizard also contained a *Sauroplasma*-like infection (Fig.3.18 (blue arrows)).
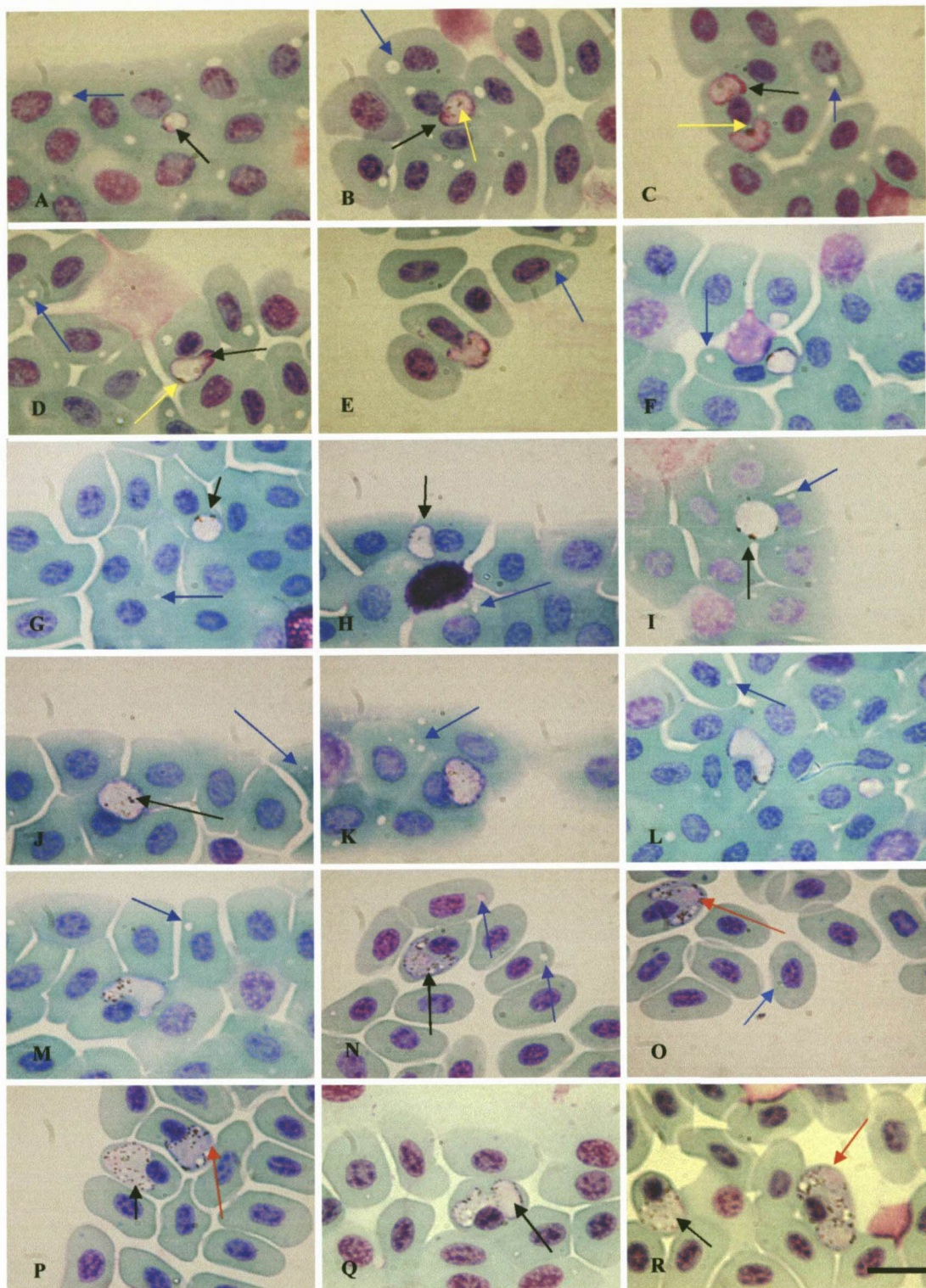
Figure 3.18. A-R: light micrographs of lizard malaria and *Sauroplasma* Pienaar, 1937 infections of three lizards. **A-E:** light micrographs of *Plasmodium* Marchiafava and Celli, 1885 sp. B from *Pseudocordylus melanotus melanotus* (A. Smith, 1838) captured on a rocky outcrop in the Clarens district. Blue arrows of **A-E** indicate a *Sauroplasma*-like infection. **A:** (black arrow): oval trophozoite with a purple stained periphery and a pinkish central vacuole. **B & D:** trophozoites/ possible early meronts **D:** (Yellow arrow): amoeboid meront. **C & E:** gametocytes. **F-M:** *Plasmodium or Haemoproteus* Kruse, 1890 sp. C from *Pseudocordylus melanotus subviridus* (A. Smith, 1838) captured near the village Ha Rapokolana. **F-M** (blue arrows): *Sauroplasma*-like infection. **F-I:** shows fine malaria pigment. **K-M:** suspected juvenile gametocytes. **N-R:** *Plasmodium or Haemoproteus* sp. D from *Pachydactylus bibronii* (A. Smith, 1845) collected on the farm Zuurfontein, Free State. **N:** (blue arrows) *Sauroplasma*-like infection. **N-R:** gametocytes. Scale: **A-R:** 10 µm.

Trophozoites and possible early meronts

The earliest infecting forms of the parasite seen in blood smears were small, oval trophozoites with a purple-stained periphery and a pinkish central vacuole (Fig. 3.18A (black arrow)) 0.98 ± 0.97 (1.14-2.12) µm to 2.30 ± 0.88 (2.47-3.35) µm (n=15) in diameter. Larger trophozoites/ possible early meronts (Fig. 3.18B, D) were similar in staining properties to young trophozoites, but some were triangular or amoeboid in shape (Fig. 3.18D (yellow arrow) and measured 3.3 ± 1.1 (3.1-5.8) µm in width by 3.8 ± 1.4 (3.4-6.1) µm in length. Mature meronts were not observed. No marked effects on the host cell were seen associated with trophozoite and early meront stages, or with presumed gametocytes (below).

Gametocytes

Stages presumed to be juvenile gametocytes were positioned in the polar regions of the host erythrocytes, and some host cells contained two such parasites (Fig. 3.18C). They were kidney-shaped or somewhat amoeboid (Fig 3.18C & E), stained deep pink with Giemsa and contained fine granules of malaria pigment. The biggest of these forms measured 4.0 ± 0.9 (3.84-5.55) µm in width by 5.8 ± 1.9 (4.47-7.10) µm (n=12) in length.

Comments

The parasites were smaller than *Plasmodium* sp. A and about the same dimensions (4.0 ± 0.9 (3.84-5.55) µm in width by 5.8 ± 1.9 (4.47-7.10) µm (n=12)) as *P. zonurae* but had different staining properties and carried lots of fine pigment in the mainly eosinophilic cytoplasm.   Macrogametocytes also had more pigment than those described from Cordylidae in South Africa by Pienaar (1962).

Plasmodium or *Haemoproteus* **sp. C from** *Pseudocordylus melanotus subviridis* **(A. Smith, 1838)**

Host *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

Locality: Village of Ha Rapokolana (29°22'36''S 28°01'53E), Lesotho.

Plasmodium sp. C infections were discovered in the blood of three of four *Pseudocordylus melanotus subviridis* captured near the village Ha Rapokolana in Lesotho. These infections were similar to *Plasmodium zonurae* in the girdled lizard *Cordylus vittifer*, and were accompanied by a *Sauroplasma*-like infection (Fig. 3.18F-M (blue arrows)).

Trophozoites

Trophozoites were round to oval in shape and measured 2.25 ± 0.84 (1.12-5.16) μm (n=20) in diameter. They stained dark purple with Giemsa peripherally, with some malaria pigment (Fig.3.18F-I) with a pale central vacuole. No marked effects on the host cell were seen and no meronts were identified.

Gametocytes

Suspected juvenile gametocytes (Fig. 3.18K-M) of this malaria were also observed. They were mostly polar in position within erythrocytes, rounded, rather amoeboid or broadly crescent-shaped and contained pigment granules. They stained either pinkish or pale lilac with Giemsa and measured 3.4 ± 0.8 (3.2-4.5) μm in width and 5.54 ± 1.0 (5.18-6.97) μm (n=14) in length. No effects on host cells were detected.

Comments

These specimens were found only in one developmental stage, therefore it will be considered as *Plasmodium* or *Haemoproteus* sp. C. When compared with *Plasmodium zonurae* this *Plasmodium* or *Haemoproteus* species appears similar in the general morphology. *Plasmodium* or *Haemoproteus* sp. C has round or oval-shaped trophozoites with dimensions of 2.25 ± 0.84 (1.12-5.16) μm (n=20) in comparison with 2-2.8 μm of *P. zonurae*. The gametocytes varied slightly in size, being 3.4 ± 0.8 (3.2-4.5) μm in width

and 5.54 ± 1.0 (5.18-6.97) µm (n=14) in length in *Plasmodium* or *Haemoproteus* sp. C, whilst *P. zonurae* had gametocytes that measured 8-8.4 by 4.2 µm.

Plasmodium or Haemoproteus sp. D from *Pachydactylus bibronii* (A. Smith, 1845)

Type Host: *Pachydactylus bibronii* (A. Smith, 1845).

Type Locality: On the farm Zuurfontein (29°54'S 25°22'E) Jagersfontein district, Southern Free State.

Plasmodium infections were observed in the blood of two specimens (male and female) of *Pachydactylus bibronii* (A. Smith, 1845) captured on the farm Zuurfontein. These infections were accompanied by a *Sauroplasma*-like infection (Fig.3. 18N (blue arrows)).

Gametocytes

Only gametocytes were observed in the blood of these geckos (Fig. 3.18N-R) and two distinct types of gametocytes were observed. Presumed macrogametocytes were broadly crescentic in form (Fig.3.18R (black arrow)), stained faintly lilac or purple, had fine pigment granules and measured 8.4 ± 0.7 (8.2-8.7) µm by 9.3 ± 0.44 (8.8-9.4) µm (n=20). Microgametocytes stained deeper purple, and scattered, slightly coarser, pigment granules were present (Fig. 3.18P (black arrow), Q & R (red arrow)), and they measured 9.4 ± 0.51 (8.87-10.2) by 5.6 ± 0.45 (5.5-5.9) µm (n=20). Both types of gametocytes usually lay at one extremity of the host cell. Large forms (Fig. 3.18R (red arrow)) filled the cell cytoplasm, causing displacement of the host cell nucleus and general cell enlargement.

Comments

These infections, like those of the previously described malaria (from Lesotho) could be *Haemoproteus* sp. if it can be proved that there is lack of merogony in the red cells. Certainly some stages of the parasite almost fill the host cell cytoplasm (Fig. 3.18R) as in *Haemoproteus*. Only gametocytes were present in the blood cells and if these prove to be a *Plasmodium* infection, then it is possible that it is an old infection. These infections

correspond closely to the gametocytes described as *Plasmodium mexicanum* Thompson & Huff, 1944 from *Sceloporis undulates*, but the current species differs as it seems to produce more pigment.

3.4. MICROFILARIAE

3.4.1. NEMATODA

A number of different microfilarial nematodes were observed in the blood of *Agama atra atra*, *Cordylus polyzonus* and *Pseudocordylus melanotus subviridis* collected in different localities in Lesotho and the Free State.

Microfilaria sp A from *Agama atra atra* Daudin, 1802

Specimens were collected (exact numbers not recorded) in the village of Ha Rapokolane, Ha Khojane in Lesotho and on the farm Zuurfontein (29°54'S 25°22'E) in the southern Free State.

These microfilariae (Fig. 3.19A-F) measured 54 ± 0.8 (40-60) μm (n=10) long with a pale pink or purple-stained, highly inflated, sheath and a slightly pointed anterior. The tail tip was relatively slender and bluntly rounded, with two single nuclei close to its terminus. Nuclei in the tail region stained deep purple, the remainder, almost black. Pink-staining organ primordia could be seen towards the centre of the nematode.

Comments

All of these microfilariae were distinctly stained and had a characteristically inflated sheath. It seems that these unidentified species are specific to *A. atra atra* hosts even over disjunct localities.

Microfilaria sp B from *Cordylus polyzonus polyzonus* A. Smith, 1838

Specimens were collected from various sites on the farm Zuurfontein (29°54'S 25°22'E) in the southern Free State.

Microfilariae from these lizards (Fig. 3.19G-K) measured 82.5 ± 1.8 (80-95) µm (n=20) long with a pale blue-stained, loose fitting, sheath and a rounded anterior, with eight pairs of nuclei clearly visible. The tail tip was slender and sharply pointed (Fig. 3.19G-J), with 5-6 elongate, widely spaced single nuclei. Nuclei in the tail region stained deep purple, the remainder, almost black. As above, pink-staining organ primordia could be seen towards the centre of the nematode.

Comments

These microfilariae seem distinct if compared to the other described forms in this study. It has a robust thick body with a characteristically blue-stained body with a sharp pointed tail.

Microfilaria sp C from *Pseudocordylus melanotus subviridis* (A. Smith, 1838)

These microfilariae were found in numerous lizards (exact numbers not recorded) collected in the Bokong, Jorotane and Syncunjane river catchments in Lesotho.

Microfilariae of this species measured 95 ± 0.955 (40-110) µm (n=20) long with an almost colourless, loose-fitting, sheath and a broad, bluntly rounded anterior (Fig. 3.19L-O). The tail tip was rather broad and stumpy and individual nuclei within it were difficult to count. Nuclei throughout the body stained deep red-purple. Organ primordia could be seen as colourless and red-stained areas towards the centre and two-thirds down the body of the nematode.

Comments

These lizards were collected in various localities separated by high mountain ranges. It seems from their general morphology that these microfilariae are specific to these lizards

(*Pseudocordylus melanotus subviridis*) in Lesotho. These are morphometrically the biggest forms of nematode observed in the blood plasma of all lizards infected examined in this study.

Microfilaria sp D from *Pseudocordylus melanotus melanotus* (A. Smith, 1838)

Lizards with this microfilarial nematode were collected in the Clarens district in the eastern Free State.

Microfilariae in Fig. 3.19P-R measured 55 ± 1.4 (40-77) μm (n=20) long with deep purple-staining nuclei. The anterior tip of each was pale-stained. The parasites were irregularly coiled with a rough outline and the sheath that seemed to be tight fit.

Comments

These lizards were collected deep in the Maluti Mountains. They were collected from a few sites relatively close to each other, and it seems that these species of microfilariae are specific to these subspecies of lizards. More surveys will clarify this.

Figure 3.19. Light micrographs Giemsa-stained blood films of filarial nematodes found in the blood plasma of *Agama atra atra* Daudin, 1802, *Pseudocordylus melanotus melanotus* (A. Smith, 1838), *Pseudocordylus melanotus subviridis* (A. Smith, 1838) and *Cordylus polysonus* A. Smith, 1838. A-F: microfilaria sp A in six different specimens of *Agama atra atra* collected on the farm Zuurfontein, G-K: microfilaria sp B in blood plasma from *Cordylus polysonus* collected in same locality (Zuurfontein). L-O: microfilaria sp. C from *Pseudocordylus melanotus melanotus* collected in Clarens, P-R: microfilaria sp. D from *Pseudocordylus melanotus subviridis* collected in the village of Ha Khojane, Lesotho. Scale: A-R: 20 μm.
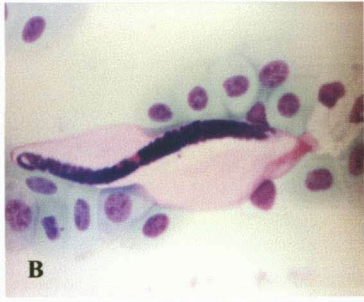
DISCUSSION

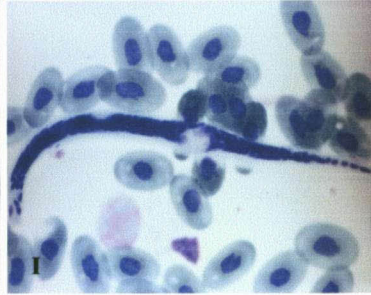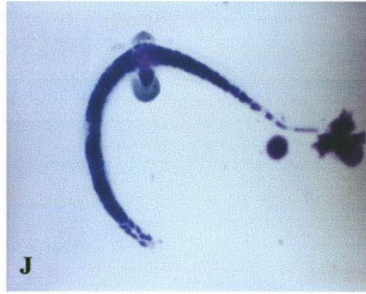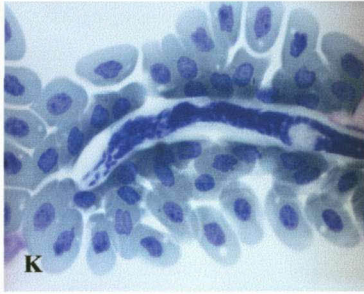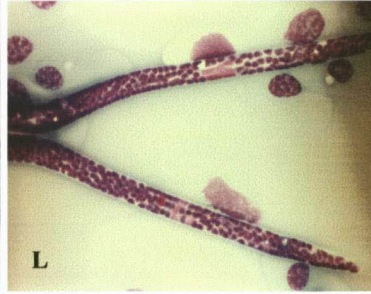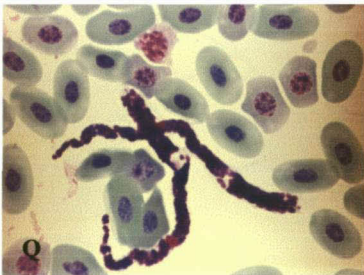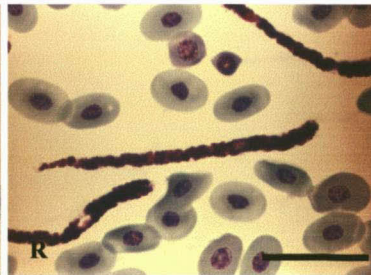The Free State is an area of high altitude grasslands and deep soils where the summers are hot with frequent thunderstorms and the winters are dry and cold. It was noted by Lynch (1983) that 75% of the Free State consists of grassland with 20% karoo in the South West and the remaining 5% is thornveld in the North West. The Eastern parts are mountainous with montane grasslands and scattered patches of Afromontane forests. Temperature increases from east to west while rainfall, elevation and reptile diversity increase from west to east. The overall climatic conditions have an influence on reptile diversity, i.e. it increases to the east. Therefore, most of the sample areas in the present study were selected in the eastern parts of the Free State and in the heavily mountainous and rocky areas in Lesotho.

Of the 59 ophidian specimens representing 13 snake species, and 204 lizards consisting of 15 species in six families, examined during this study all apparently revealed at least some kind of blood infection. Some specimens with wide distribution ranges like the puff adder (*Bitis arietans*), and others with specific and endemic distribution patterns like giant girdled lizards (*Cordylus giganteus*), showed over dispersed parasite infections. Others like the Karoo girdled lizards (*Cordylus polyzonus*), which are distributed throughout the South western parts and consist of scattered and isolated populations living almost exclusively on dolerites, were found to have high and sometimes mixed infections. These reptiles could serve as manageable models for future studies to investigate parasite populations, since each isolated outcrop serves as an island for the host population.

A broad spectrum of blood parasites (summarised in Table 3.1) ranging from viral infections and apicomplexans to nematodes were found in all locations. No clear patterns were found in terms of the distribution arrangements of parasites. Apart from parasites that are over dispersed, all of the reptiles sampled apparently had some sort of an infection in their peripheral erythrocytes. It is well-known that reptiles are an ancient group and some of them have remained virtually unchanged, perhaps for many millions

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of years. It could thus be assumed that they possibly had time to adapt to their infections, but the question arises why most reptiles have an infection and why it is that the infections are so severe in some members of populations. Furthermore, infections like *Sauroplasma, Hepatozoon* and *Plasmodium* undoubtedly have a destructive effect on the host cells, but how do they affect the hosts overall? Surely if a cell is robbed of its haemoglobin content (by several infections) the normal metabolic functions would be changed, most likely to the detriment of the host. Ultimately what effect does this have on the population dynamics?

One of the reasons that the giant girdled lizards (*Cordylus giganteus*) are regarded as (SA RDB) vulnerable is because they have become isolated due to roads and agricultural events. Apart from their stagnating gene pools, should one be concerned when almost every blood cell appears infected? Although the nature of this study was not to determine the physiological state of reptiles, no overall apparent effects on the hosts itself could be observed. Could these infections be due to human disturbance? Human disturbance has created island populations instead of the once free areas for the exchange of genes regime amongst animals. These induced host isolations may have carried on for the last few hundred years and on a comparative morphological level perhaps no differences could be seen in their parasites. It would be interesting to see the genetic or molecular changes in these parasites trapped in these human induced refuges.

Very few habitats these days are in pristine condition and some human influence could perhaps have an effect on the parasite infections of reptiles. However, lizards from pristine habitats with almost no human impact, like those from the highlands of Lesotho, are seemingly healthy, but have a blood picture that is abnormal, with extremely high levels of (often mixed) infections. From an objective point of view something is wrong. Is it normal for these undisturbed reptiles to have such high infection levels? Clearly, it would seem to be so.

Haemogregarines discovered in the blood of some reptiles in this study undoubtedly constituted new host records and very likely new species, with the exception of

Haemogregarina (*Hepatozoon*) *sebae* (Laveran & Pettit, 1909) Smith, 1996 from the African python. The current investigation led to the discovery of this latter parasite from a different race (southern race) of python, *Python sebae natalensis*. This was also the first description of the ultrastructure of *H. sebae natalensis* and, although an initial and rather brief study, it clearly demonstrated the apicomplexan features of this haemogregarine. Distribution ranges and probably numbers of species for reptilian haemogregarines are extended during this study to include six likely new species of haemogregarines present in the blood cells of a snake, cordylid and agamid lizards. They have been named *Hepatozoon* sp. A to F for the purpose of this dissertation, because their life cycles are not known at this stage. When these have been determined it may still be appropriate that they are named *Hepatozoon*, or it may be necessary to transfer these new haemogregarines to different genera, such as *Hemolivia, Karyolysus* or *Schellackia*. Some *Hepatozoon* spp. found in this study were particularly interesting. Two in particular deserve further study, namely, the distinctive huge *Hepatozoon* sp. D from Lesotho in the lizard *Pseudocordylus melanotus subviridus*, and *Hepatozoon* sp. F. from Bloemfontein which has severe effects on host erythrocytes.

It seems that these *Hepatozoon* spp. have adapted to infect isolated populations of species of hosts. The Lesotho highlands provide a number of isolated localities where a spectrum of new parasites and infections were discovered. The catchment area of Mohale Dam in Lesotho consists of the Syncunjane, Bokong and Jorotane rivers separated by huge mountains with peaks sometimes exceeding 2900m in height. These barriers probably isolate host populations where distinct morphological differences between *Hepatozoon* spp. are evident. Future studies will involve in-depth sampling in these and other locations to map the different host and haemogregarine species distribution. Furthermore, agamid lizards, which are likely an older group than cordylid lizards in evolutionary terms, seem more adapted to their parasites, and their parasites to them, to such an extent that their haemogregarines, filarial nematodes and *Pirhemocyton* infections are host (species) specific. Although host specific parasites have probably evolved in most of the major reptile groups, it seems from the current survey that these parasites have evolved particularly to *Agama atra atra* hosts. Although these lizards

have a wide distribution range and were collected in various disjunct localities, infections in the blood of these lizards seemed identical, hence the tentative conclusion regarding the host specificity.

Lizard malaria has been found on five continents and in all the major families of lizards as well as in some snakes. Infections of representatives of the Plasmodidae are so far not recorded from any representatives of the Testudes and Rhynchocephalia. Ayala (1977) was of the opinion that lizard malaria is most common in wet tropical forests, is distributed as far north as Japan and as far south as New Zealand. These distributions probably reflect, in part, that more surveys were conducted in those areas. The subject of reptile malaria had been studied, (according to Ayala, 1977), by about 80 authors who had contributed significantly in their 153 papers in their knowledge of infections in Africa, Australasia and the Americas. In the African region a total of 16 species have been described thus far, with one, *Haemoproteus mungutti* Mutinga and Dipeolu, 1989 from *Agama agama* and another *Plasmodium pitmani* Hoare, 1932 from a skink *Mabuya striata*, while in South Africa three species have been recorded by Pienaar (1962) and Garnham (1966) in three families of lizards (Agamidae, Cordylidae and Gerrhosauridae Fitzinger, 1843). Investigations in the present study of the blood of *Pseudocordylus melanotus subviridis*, *Pseudocordylus melanotus melanotus*, *Cordylus polyzonus polyzonus* (Cordylidae) and *Pachydactylus bibronii* (Gekkonidae) revealed four likely new species of lizard malaria, namely *Plasmodium* sp. A-D, although some may prove to be *Haemoproteus* sp. These are new host and distribution records for these parasites. In general, lizards live their entire lifetime in a small habitat range with no migratory movements as in birds. Therefore, *Plasmodium* infections in lizards are well suited for evolutionary and epidemiological studies in natural infections. According to Ayala (1977), typical saurian infections follow the same general cycles as their bird and mammal counterparts: a period of initial rise, then a peak followed by a gradual or abrupt decline. A chronic period is usually interrupted by a relapse. Future studies in South Africa should involve complete life cycle studies of this prehistoric, but successful group.

Transmission of representatives of the Plasmodidae between South African lizards remains unresolved. Pienaar (1962) was of the opinion that although *Tabanus* and *Haematopota* spp. (Diptera: Tabanidae) are prevalent it seems inconceivable that these flies could act as vectors transmitting *Plasmodium* infections. However, it was noted that all lizards that harbored a *Plasmodium* infection, had infestations of prostigmatic mites. These acarines could be considered potential vectors, but Ayala (1977) considered that these would not support sporogonic development, although parasites might be retained in the stomach for several days. Although much of the skin of many reptiles seems resistant to insect bites, it actually consists of an extensive vascular system where haematophagous arthropods can easily take a blood meal. Numerous "soft" areas can serve as possible and accessible sites for attachment of acarines or as a feeding spot for capillary feeders such as Diptera (Culicidae), or Hemiptera (Reduviidae) which are undoubtedly present in the Free State. I observed a few culicine mosquitoes feeding on the soft skin beneath the eye of a monitor lizard lodged in a rock crack. Ayala (1971 & 1973) proved that lizards can be infected following inoculation with sporozoites from heavily infected sandflies (Diptera: Phlebotidae). It thus seems that transmission from invertebrate vectors to reptile hosts may take place by inoculation of salivary fluids, or due to regurgitation when taking a blood meal, or if the invertebrate is ingested. For specimens collected in South Africa and Lesotho, it seems unlikely that these lizards prey on minute mites that are usually engorged. I doubt that lizards can even see these highly camouflaged acarines safely wedged between their scales. The four likely new *Plasmodium* species are described from disjunct localities, and each will have their own characteristic haematophagous invertebrates. Jupp (1996) reported numerous culicine and toxorhynchitine mosquitoes from South Africa some of which are found in Free State (*Aedes aegipi, A caballus, A circumluteolus, A dentatus, A durbanensis, A hirsutus, A luridus, A luteolateralus, A mcintoshi, A mixtus, A natalensis, A natronius, A sudanensis, A unidentatus, A vittatus, Culex annulioris, C lineate, C pipiens, C poicilipes, C quinquefasciatus, C salisburiensis, C theileri, C tigripes, C univittatus, Culiseta longiaeriolata* and *Mansonia uniformes*) and in Lesotho (*Aedes hirsutus, A juppi, A unidentatus, Culex andersoni, C. theileri, C salisburiensis, C. univittatus* and *Culiseta longiaeriolata*). *Plasmodium* sp. A, B & D are described from the Free State and

Plasmodium sp. C. was described from Lesotho. Apart from the two subfamilies of mosquitoes that could likely be responsible for transmission, the only common link between infections is the presence of prostigmatic mites. Future studies will involve seeking vectors that are likely to be responsible for *Plasmodium* infections. These invertebrates will be prepared for investigation by light microscopy (for example, by histology), TEM and confocal laser scanning microscopy.

The enigmatic and so-called piroplasms of reptiles, *Sauroplasma* and *Serpentoplasma*, were found in most of the reptiles collected. Morphologically some of these apparent infections appeared similar to *Pirhemocyton* infections, which are viral in nature and now often designated LEV (lizard erythrocytic virus) or SEV (snake erythrocytic virus) (see Telford & Jacobson 1993; Davies & Johnston 2000). Strangely perhaps, *Toddia* was not seen in snakes from South Africa, but *Sauromella* (from the lizard *Pachydactylus capensis*) was found to be particularly close to *Pirhemocyton* in appearance, suggesting that this is also a viral infection.

Attempts were made to study the nature of *Sauroplasma* and *Serpentoplasma* by means of transmission electron microscopy (TEM). The threatened and CITES listed giant girdled lizards (*Cordylus giganteus*) are endemic and found only in small isolated areas. They are considered vulnerable South Africa Red Data Species due to massive habitat destruction by farmers producing monocultures (mostly maize and corn) and the recently booming (illegal) pet trade. These lizards serve as an excellent model to try and understand the biology of *Sauroplasma* infections, because they are colonial, viviparous, long-lived and reasonably easily obtained. Ultrastructural studies failed to give a firm indication of the nature of these infections. Their boundary membrane, heterochromatin and their budding suggested a possible eukaryotic identity, but no structures consistent with those found in the Apicomplexa were observed. However, when they were compared with *Serpentoplasma*, *Sauroplasma* infections were found to be very similar, and *Serpentoplasma* might just be viral in nature (see below). Future studies will involve attempts to finally resolve the identity of these strange infections, to find their mode of transmission, and an assessment of their pathogenicity to their host lizards.

A puff adder (*Bitis arietans*) and a captive African rock python (*Python sebae natalensis*) were used to study *Serpentoplasma* infections by means of TEM. The infections were similar in morphometrical dimensions and morphological appearance to those (*Sauroplasma*) found in lizards. By TEM they were also reminiscent of the viral infections seen by Daly *et al.* (1980) in water snake erythrocytes, but again further studies are needed to confirm this. Future studies will also focus on the mode of transmission of *Serpentoplasma*.

Mixed infections were common amongst host species. In general, *Sauroplasma* was observed together with haemogregarines and with *Plasmodium* species. *Pirhemocyton* infections and *Hepatozoon* sp. A. were found to coexist in the erythrocytes of *Agama atra atra*. All six likely new species of haemogregarines were accompanied by *Sauroplasma* infections. It was not uncommon to find malaria, haemogregarines, nematodes (microfilariae) and so-called piroplasms in the same specimens of some lizards. The identity of the microfilariae, the location of their adult stages and their mode of transmission are further problems that require resolution.

Transmission of *Pirhemocyton, Sauroplasma* and *Serpentoplasma* also remains unresolved. It is possible that prostigmatic mites transmit these infections via their salivary glands while having a blood meal. On most wild reptilian specimens these engorged ectoparasites were found and I also observed on several occasions mosquitoes feeding on geckos and on the eyelids of a monitor lizard (*Varanus exanthematicus albigularus*). This latter lizard's blood revealed a *Sauroplasma*-like infection, as well as haemogregarines.

Pathological effects on host cells were apparent. Several infections revealed infected blood cells showing nuclear displacement hypertrophy and dehaemoglobinisation. These infections must have an effect on the host itself. Infections like those of *Sauroplasma* that infect nearly every cell, possibly reduce the haemoglobin content extensively. The *Hepatozoon* sp. F from the striped skaapsteker (*Psammophylax tritaeniatus*) was another amazing example of a parasite that dehaemoglobised the host erythrocyte, displaced the

host cell nucleus and reduced it in size. As a result of these infections perhaps less oxygen is available at a cellular level which might reduce the mobility of the reptile. These lower energy levels could have an impact on the reproduction abilities. Schall (1996) did some ecological work on malaria parasites affecting lizard behaviour, effects on showy male traits being a good example.

Even though short in geological time, reptiles have had at least a few hundred million years to successfully adapt to their parasite infections. The present survey, although relatively scanty in reptile diversity, showed an awesome diversity of parasites in their erythrocytes. Some of the infections may prove to be detrimental to their host's health, and, like any system under pressure, the host may then experience further increase in parasite loads. The outcomes of this study opened up one main question: what are the normal levels of parasite loads in reptiles? Is it only in the present study area that such levels of infection are seen? Are the host reptiles adapted to these seemingly heavy levels of infection or is there something that we do not see?

Huchzermeyer, Williamson and Swanepoel (1986) briefly examined (reported in a conference presentation) the blood of a few lizards from the Eastern Cape, where they found infections ranging from viral to haemogregarines and malarias, but the present investigation was the first study of its kind in the Free State and in Lesotho. These remarkable findings of infections of such high frequencies and the presence of mixed infections, proves that these varied and relatively unknown parasites are indeed widespread and possibly threatening to their hosts. Are we witnessing gradual extinction of these successful reptiles, due to parasitic infections? It is regrettable that so few studies like this one have been conducted. Further studies like these might indicate whether these infections are increasing, and, if so, are they a cause for concern? Should future nature conservation efforts for red data species like the endemic giant girdled lizards be focused on monitoring intraerythrocytic infections in view of the demonstrable effects of these on the host cells? Certainly, further studies on these fascinating infections are urgently needed.

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ABSTRACT

The study of blood parasites of reptiles is a relatively new and unexplored field of research in South Africa. The Free State province and the Lesotho highlands provide a range of reptiles in which their intraerythrocytic parasite fauna were explored. Objectives of this study were to set a baseline of blood parasite diversity and to identify the enigmatic *Sauroplasma* Du Toit, 1937 and *Serpentoplasma* Pienaar, 1954 infections in lizards and snakes, respectively. Surveys were conducted in various localities in the Free State and Lesotho. Although low in diversity, 204 specimens representing 14 species of lizards, and 59 specimens representing 13 species of snakes were investigated for the presence of blood parasites. Three known infections were found: *Sauroplasma thomasi* Du Toit, 1937, *Sauromella haemolysus* Pienaar, 1954 and *Hepatozoon* (*Haemogregarina*) *sebae* (Laveran and Pettit, 1909) Smith, 1996. These were redescribed and *S. thomasii* and *H. sebae* were examined by aid of transmission electron microscopy. The investigation led to the discovery of six new records and possibly new species of haemogregarines named *Hepatozoon* sp. A-F, four new records and possibly new species of lizard malaria named *Plasmodium* sp. A-D, and a viral infection possibly of the *Pirhemocyton* type. Furthermore nine new host and distribution records for *Sauroplasma* in lizards and nine for *Serpentoplasma* in snakes are described. Ultrastructural investigations of *S. thomasi* in *Cordylus giganteus* A. Smith, 1844, *Serpentoplasma* in *Bitis arietans arietans* (Merrem, 1820) and *H. sebae* in *Python sebae natalensis* (Gmelin, 1789) were the first to examine the nature of infections in this manner. This is the first comprehensive survey of the biodiversity of blood parasites in reptiles in the Free State and Lesotho highlands.

Key words: Reptiles, blood parasites, haemogregarines, lizard malaria, *Sauroplasma*, *Serpentoplasma*, *Pirhemocyton*, Free State, Lesotho.

OPSOMMING

Die studie van bloedparasiete van reptiele is 'n relatiewe nuwe en onbekende veld van navorsing in Suid Afrika. Die Vrystaat Provinsie en Lesothohooglande het 'n verskeidenheid van reptiele waarvan hul intraeretrositiese parasietfauna bestudeer was. Die doel van die studie was om 'n basislyn van bloedparasietdiversiteit op te stel, en om die enigmatiese *Sauroplasma* Du Toit, 1937 en *Serpentoplasma* Pienaar, 1954 infeksies in akkedisse en slange onderskeidelik te identifiseer. Opnames was in verskeie lokaliteite in die Vrystaat en in Lesotho gedoen. Alhoewel laag in biodiversiteit, was 204 eksemplare, verteenwoordigend van 14 spesies akkedisse en 59 slange, verteenwoordigend van 13 spesies, ondersoek vir die teenwoordigheid van bloedparasiete. Drie bekende infeksies was gevind: *Sauroplasma thomasi* Du Toit, 1937, *Sauromella haemolysus* Pienaar, 1954 en *Hepatozoon* (*Haemogregarina*) *sebae* (Laveran and Pettit, 1909) Smith, 1996. Die material is herbeskryf en *S. thomasii* en *H. sebae* was deur middel van transmissie-elektron mikroskopie ondersoek. Die ondersoek het tot die ontdekking van ses nuwe rekords en moontlik nuwe haemogregariene spesies, naamlik *Hepatozoon* sp. A-F, vier nuwe rekords en moontlike nuwe spesies van akkedis-malaria, naamlik *Plasmodium* sp. A-D, en 'n virus-infeksie wat moontlik van die *Pirhemocyton* tiepe is, gelei. Verder is nege nuwe gasheerverspreidingsrekords vir *Sauroplasma* spp. in akkedisse en nege vir *Serpentoplasma* spp. in slange beskryf. Ultrastrukturele ondersoeke van *S. thomasi* in *Cordylus giganteus* A. Smith, 1844, *Serpentoplasma* sp. in *Bitis arietans arietans* (Merrem, 1820) en *H. sebae* in *Python sebae natalensis* (Gmelin, 1789) was die eerste keer gedoen op hierdie manier om die aard van infeksies vas te stel. Hierdie is die eerste samevattende opname van die biodiversiteit van bloedparasiete van reptiele in die Vrystaat en Lesothohooglande.

Sleutelwoorde: Reptiele, bloedparasiete, haemogregarienes, akkedis-malaria, *Sauroplasma, Serpentoplasma, Pirhemocyton*, Vrystaat, Lesotho.

ACKNOWLEDGEMENTS

APPENDIX A

Permit om lewende Wilde en/of Uitheemse Reptiele in gevangenskap aan te hou

HIERDIE PERMIT IS NIE OORDRAAGBAAR NIE

Kragtens die Ordonnansie op Natuurbewaring Nr 8 van 1969, word magtiging hiermee verleen aan:

PERMITHOUER BESONDERHEDE

Mnr. van As
J.

ID Nommer   7707225055089

Nauhaus 12
Brandwag
Bloemfontein.

Suid-Afrika

Tel No (H)   051-4300691
Tel No (W)   051-4012370

Faks No: ~

om die volgende **Reptiele**   genoem in die Ordonnansie op Natuurbewaring Nr 8 van 1969,   **in gevangenskap aan te hou** by bogenoemde adres, onderhewig aan die voorwaardes op die keersy gemeld.

Spesie	Wetenskaplike naam	Lengte	Geslag	Identifikasie	Nommer	
Afrika Rotsluislang	Python sebae natalensis	-	-	-		
Totale Spesies	1				Totaal	

Handtekening van Permithouer

Goedgekeur: namens die LUR: Omgewingsake en Toerisme

CHIEF DIRECTOR
ENVIRONMENTAL AFFAIRS
AND TOURISM

Vervaldatum	Permit Nommer	Uitreikdatum
31/12/2003		2003 -01-
Stuur Permit terug na vervaldatum	HK/P19C/03894/002	PO BOX 264

FREE STATE
PROVINCIAL ADMINISTRATION

1

P.O. Box 517
BLOEMFONTEIN
9300 South Africa

Tel : 051 - 4470407
Fax: 051 - 4475240

PERMITTEE DETAILS

Mnr. van As
J.
Nauhaus 12
Brandwag
Bloemfontein.

Suid-Afrika

ID Number   7707225055089

Tel No (H)   051-4300691
Tel No (W)   051-4012370

Fax No

In terms of Nature Conservation Ordinance no 8 of 1969, permission is hereby granted to the holder of this permit to:

Collect reptiles in the Free State for a M.Sc. Project.

Subject to the following conditions:

1. This permit is invalid unless all requirements of any other legislation in respect of the act mentioned are complied wi

2. This permit is invalid if it is not signed by the permittee and is not transferable.
3. All specimens are to be released at the point of collection within 24 hour of capture.
4. All wounds from capture and drawing blood are to be treated appropiately before release.
5. No more than one voucher specimen per site is allowed to be collected for identification. Such specimens must be fixated in the field and deposited as soon as possible with the National Museum, following each field trip. The neceessary collection data as required by the National Museum must accompany each specimen.
6. Live specimens can be transported backto, or kept in captivity at the laboratory and or University premises for not longer than two months.
7. A register must be kept of all animals caught and released, with dates and localities.

Permittee's Signature

Approved   on behalf of the MEC: Department of Tourism, Environment and Ec

W.J. BOING
ASSISTANT DIRECTOR