ECOLOGY, TAXONOMY AND POSSIBLE LIFE CYCLES OF BLOOD PROTOZOANS INFECTING CRAG LIZARDS (*PSEUDOCORDYLUS* SPP.) FROM THE EASTERN FREE STATE HIGHLANDS

By

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Declaration

I, Johann van As, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree. I furthermore cede copyright of the dissertation in favor of the University of the Free State.

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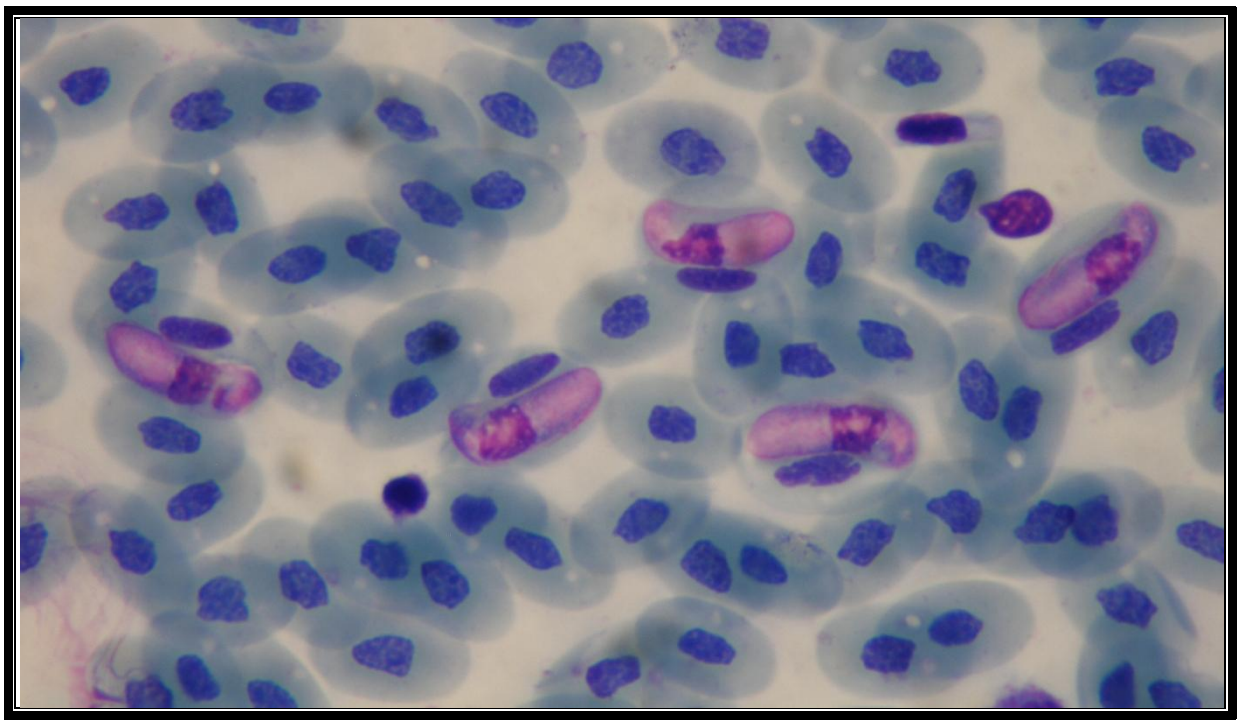

"Blut ist ein ganz besonderer Saft".

"Blood is a very special juice."

Johann Wolfgang von Goethe (1749 - 1832)

Contents

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE OVERVIEW

1.1 Overview and taxonomy of host lizard species

The Drakensberg mountain range forms the border between the mountain kingdom of Lesotho and three provinces of South Africa, namely the Eastern Cape, Kwa-Zulu Natal and the Free State. This mountain range has numerous ecological regions above 2500m and harbours many indigenous species of plants, insects, birds, reptiles and amphibians. Drakensberg basalts dominate the geology of the region, forming plateaus and steep cliffs, and creating habitats for this variety of plants and animals, and especially for rupicolous (rock inhabiting) cordylid lizards, of which one genus is the subject of this research.

The cordylid lizards (Reptilia: Sauria: Cordylidae) are endemic to sub-Saharan Africa, with their greatest diversity being seen in South Africa (Branch et al. 2005). Most members of this family are rupicolous, inhabiting rocky outcrops where they can be observed near to the entrance of (sometimes very narrow) crevices which they occupy. These lizards are sit-and-wait predators of mostly invertebrates, especially those associated with their habitat, and some are recorded as herbivorous.

Members of the Cordylidae are characterised by having strongly keeled scales, arranged in transverse rows and forming distinctive whorls. The family Cordylidae Fitzinger, 1826 has been considered a monophyletic group comprising four genera according to the traditional taxonomy of Loveridge (1944) and Lang (1991). The first of these four is the genus *Platysaurus* Smith, 1844, comprising the flat lizards, the only egg-laying representatives of this family found predominantly in southeast Africa; they are dorsoventrally flattened and are able to retreat into very narrow rock cracks. The second genus, *Chamaesaura* Fitzinger, 1843, or grass lizards, includes grassland specialists and snake-like lizards with greatly elongated tails and reduced limbs. Members of the third genus, *Pseudocordylus* Smith, 1838, or crag lizards, are rupicolous, occurring in crags in rocky outcrops in the Cape Fold, Drakensberg and Swaziland ranges (Fig. 1.1 A & B).

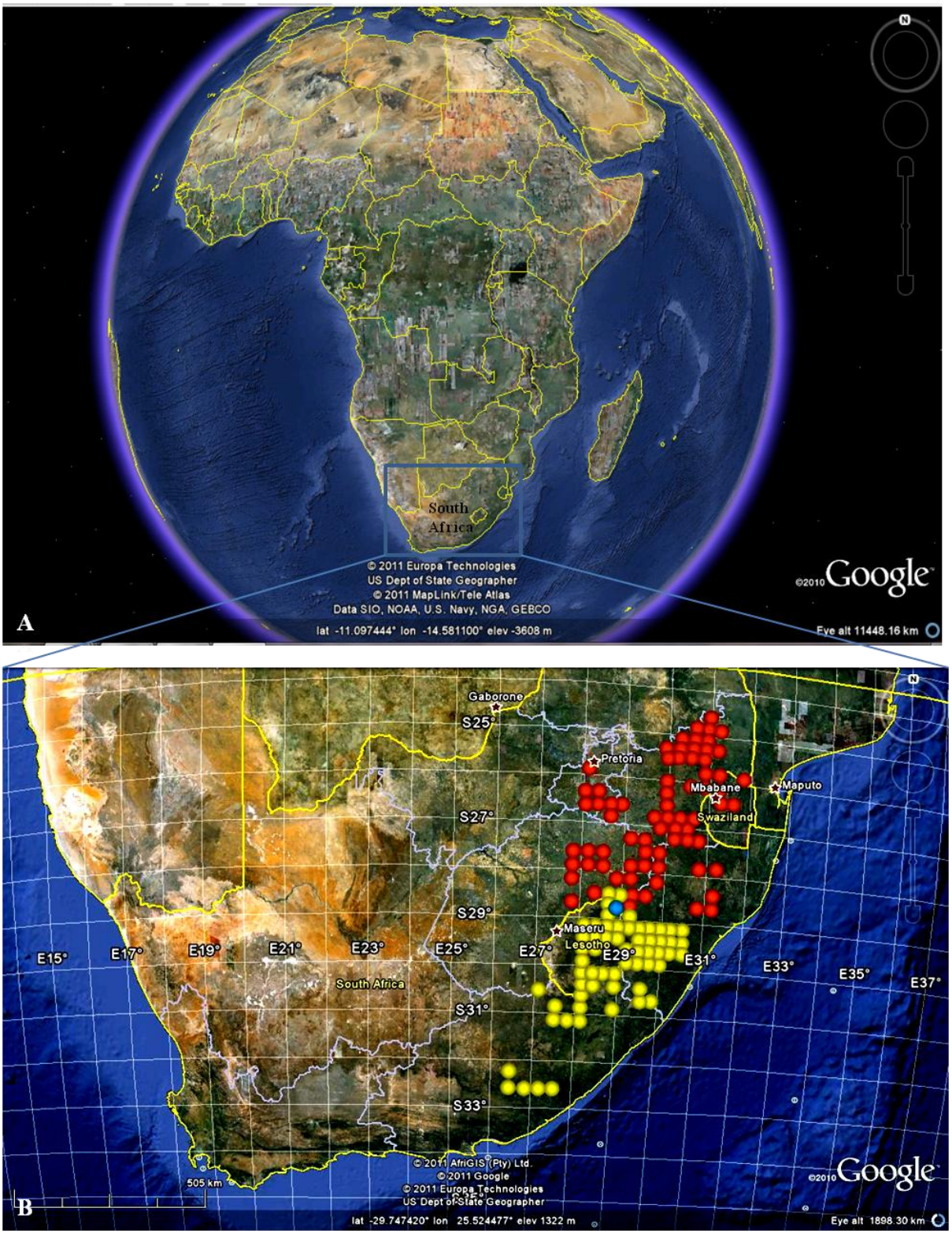

Figure 1.1 Google Earth images of the African continent **(A)** and South Africa **(B)**. Blue block in **A** indicates the map area shown in **(B)**. **(B)** Shows the geographical distribution records (from Bates, 2005) of *Pseudocordylus melanotus*, *P. subviridis* and *P. langi.* Red dots: *P. melanotus*; Yellow dots: *P. subviridis;* Blue dot: *P. langi*. Records were plotted by on a quarter degree grid. Scale bar: **(B)** = 505km.

Species within the final genus, *Cordylus* Laurenti, 1768, or girdled lizards, form an ecologically and morphologically diverse group, and range from South Africa to Angola and Ethiopia (Stanley et al. 2011). These four genera are represented by 80 named species and subspecies (Stanley et al. 2011), and three members of the genus *Pseudocordylus* form the focus in this thesis.

Numerous studies on the taxonomy of cordylid lizards have been conducted in recent decades (see Mouton, 1986; Mouton & van Wyk, 1989, 1990, 1994, 1995; Lang, 1991; Frost et al. 2001; Bates, 2005) resulting in dissimilar classifications and no firm conclusions on the status of members of this family. Of the four genera (see Loveridge, 1944; Lang, 1991), one remains still controversial, namely the genus *Pseudocordylus* Smith 1838 (see above). Bates (2005) summarised the taxonomic history of the Cordylidae and provided a critical review of the *Pseudocordylus melanotus* and *Pseudocordylus microlepidotus* Cuvier, 1829 species complexes. However, according to Bates (2005) the taxonomic status of *Pseudocordylus melanotus* (A. Smith, 1838), the subspecies *P. melanotus subviridis* (A. Smith, 1838) and *Pseudocordylus transvaalensis* FitzSimons, 1943 is still unresolved. Bates (2005) considers that the above mentioned taxa together with *Pseudocordylus langi* (Loveridge, 1944) and *Pseudocordylus spinosus* FitzSimons, 1947 form the *P. melanotus* complex. Most recently, molecular studies based on analysis of three mitochondrial (16s, 12s & ND2) and three nuclear genes (PRLR, KIF24 & MYH2) by Stanley et al. (2011) showed that the Cordylidae comprises 10 genera consisting of 10 well supported, monophyletic, lineages. The genus *Pseudocordylus* is retained by Stanley et al. (2011) and the two subspecies *Pseudocordylus melanotus melanotus* (A. Smith, 1838) and *Pseudocordylus melanotus subviridis* (A. Smith, 1838) are raised to full species level. For the purposes of this thesis the lizard names *Pseudocordylus melanotus*, *Pseudocordylus subviridis* and *Pseudocordylus langi*, as employed by Stanley et al. (2011) are used and, as the title suggests, the main focus of the thesis is to study the blood protozoans of these lizard species captured in the Eastern Free State Highlands, although microfilariae are also mentioned.

Jacobsen (1989) suggested that *Pseudocordylus melanotus* (Fig. 1.2 A) occurs in three allopatric populations (occurring in discreet geographic areas) in the North, South and West of the formerly Transvaal province.

Figure 1.2 Photographs of crag lizards investigated in this study. **(A)** *Pseudocordylus melanotus* from Platberg, Eastern Free State, **(B)** *P. subviridis* from the top of the escarpment of the Drakensberg in the Free State, **(C)** *P. langi* from Beacon Buttress at the top of the gully on the Sentinel trail in the Free State. **(A - C)** male lizards. Scale bar: **(A - C)** = 33mm.

According to Bates (2005) the main "southern population" is represented by sites in Mpumalanga, north western KwaZulu Natal and the Free State, and all are at altitudes of between 1400 to 2300m (see Fig.1.1 B red dots). Also, according to Bates (2005), *Pseudocordylus subviridis* (Fig. 1.2 B) occurs in two allopatric populations, one in the Drakensberg and surrounding areas (Lesotho, Free State, KwaZulu-Natal and Eastern Cape) and another population in the Amatole Mountains of the Eastern Cape at altitudes of 1400 - 3200m (see Fig 1.1 B yellow dots). *Pseudocordylus langi* (Fig. 1.2 C), reported to be a herbivorous lizard, occurs at higher altitudes (2805 - 3048m) and is known from a small area of the Drakensberg, the Mont-aux-Sources (Loveridge, 1944) and the Organ Pipe Pass (Broadley, 1964). Several new records of this lizard are now known from the Chain Ladders and Namahadi Pass close to where the type species was found (Bates, 2005) (see Fig.1.1 B blue dot). *Pseudocordylus langi* occurs microsympatrically with *P. subviridis* in these areas and thus their populations overlap.

1.2 Blood haematozoans of reptiles

The blood parasites of reptiles represent a rather unexplored field; however the diversity in documented infections is greater than the haemoparasite infections of mammals and birds (Telford, 2009). The often more restricted habitats and lower vagility (ability to move in a given environment) of terrestrial reptiles are, according to Telford (2009), major factors contributing to greater taxonomic diversity. This diversity is also probably considerably influenced by the greater phyletic age of the reptiles.

Reptilian blood parasites include, broadly, members of the protistan or protozoan phylum Apicomplexa Levine, 1970, the flagellated Kinetoplastida Honigberg, 1963, juvenile stages of the Nematoda (Potts, 1932), bacteria and viruses, as well as structures of uncertain status. Haemogregarines and haemococcidians, with the malarias and haemoproteids, are the most numerous apicomplexan haematozoans seen in reptiles and haemogregarines, in particular, form a major part of the study recorded in this thesis, although some of the malarias are also considered. Other lizard blood infections examined are 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. Also observed are juvenile nematode stages

(microfilariae), which are considered only briefly in this study. Thus far, no flagellates have been detected in cordylid lizards.

Haemogregarines are adeleorine apicomplexans (see below) that 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. Earlier, Labbé (1894) had proposed to divide the haemogregarines known at that stage into three distinct groups, on the grounds of the relative proportions of parasite to host blood cell. In *Drepanidium* Labbé, 1894, the parasite was no more than three fourths the host cell length. In *Karyolysus* Labbé, 1894, the parasite did not exceed host erythrocyte length and it exercised a destructive influence on the cell nucleus. For *Danilewskya* Labbé, 1894, the parasite exceeded host cell length 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. The term haemogregarine is now used to describe a variety of organisms representing the apicomplexan suborder Adeleorina (see section 1.7.1.1), and the blood dwelling forms comprise about 400 species representative of 6 genera: *Haemogregarina* Danilewsky, 1885, *Karyolysus* Labbé, 1894, *Hepatozoon* Miller, 1908, *Hemolivia* Petit, Landau, Baccam & Lainson, 1990, *Cyrilia* Lainson, 1981 and *Desseria* Siddall, 1995. The genus *Desseria* was erected to accommodate haemogregarines that lacked erythrocytic merogony, and are found in fish hosts (Siddall, 1995). The following year, Smith (1996), transferred the majority of reptilian haemogregarines to the genus *Hepatozoon* (see Table.1.1), but haemogregarines of chelonians were excluded and retained within the genus *Haemogregarina*. Some of the most complex life cycles of members of the genus *Hepatozoon* were elucidated particularly by Desser (1993) and his co-workers (see Desser & Bennett, 1993, Smith & Desser, 1997a, 1997b) and by Lainson et al. (2003). Several *Hepatozoon* species are examined in the current research.

Two other genera, *Dactylosoma* Labbé, 1894 and *Babesiosoma* Jakowska and Nigrelli, 1956 from another adeleorine family**,** the Dactylosomatidae, infect fish and reptiles (Barta & Desser, 1989). Leeches (Annelida: Hirudinea) are the only known vectors of dactylosomatids thus far, and according to Barta, (1991) all described species are aquatic. Haemococcidians,

which resemble haemogregarines, but differ from them in their general life cycles (see section 1.7.1.2.1); now include the genera *Lankesterella* and *Schellackia* Reichenow, 1919.

Reptilian malarias, like haemogregarines, have been known since at least the early 1900s (see Davies & Johnston, 2000) but it was between the 1970s to the 1990s that a remarkable amount of work on reptilian malarias and related species was undertaken. The fine, often descriptive, work of authors such as Ayala (1977), Telford (1972a, 1972b, 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 species) was undertaken and efforts were made to understand the effects of lizard malarias on host behavior (see Schall, 1996). New genera, such as *Garnia* (above) and *Billbraya* Paperna & Landau, 1990 were reported, as well new species of established genera such as *Plasmodium, Haemoproteus* and *Haemocystidium* Castellani & Willey, 1904 (see Paperna & Finkelman, 1996, Lainson & Paperna, 1996, Paperna & Landau, 1991 and Telford, 2005).

The first trypanosome life cycle from reptiles was studied by Robertson (1908, 1909), where epimastigotes of *Trypanosoma vittatae* Robertson, 1908 were found in leeches (*Glossiphonia* Johnson, 1817 and *Limnatis* Moquin-Tandon, 1827 species) that fed on a turtle *Emyda vittata* Boulenger, 1889. Since then, 81 species of reptilian trypanosomes have been described in chelonians (11 species), squamates (68 species) and crocodiles (2 species), but some of them may be synonyms (Telford, 2009). In Africa, 13 species have been described in lizards, comprising 6 species from the Gekkonidae, 2 species from the Cordylidae, one species from the Chamaeleonidae, 3 species from the Scincidae, and one from the Gerrosauridae. It is important to stress that the two species (*Trypanosoma zonuri* Telford, 1995 and *Trypanosoma cordyli* Telford, 1995) described in cordylids (*Cordylus cordylus* Linnaeus, 1758 and *Cordylus tropidosternum* Cope, 1896 respectively) were from Tanzania, a much more likely habitat for the vectors than in the cooler, raised, Drakensberg area examined in this thesis. So far, only one *Sauroleishmania* Ranque, 1973 species has been described from a gecko, *Pachydactylus turneri* (Gray, 1864), in the South African Gauteng Province (see below).

During the first half of the twentieth century some enigmatic new genera were reported from reptiles, 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). Stehbens & Johnston (1966) proved by transmission electron microscopy (TEM) that *Pirhemocyton* from Australian lizards is a viral infection, now known from the erythrocytes of lizards, turtles and snakes. Later, *Toddia*, like *Pirhemocyton*, was also shown to be a viral infection (De Sousa & Weigl, 1976). Classification of reptilian haematozoans is often based on morphological characters appearing in the vertebrate peripheral blood, as well as life cycles. However, more recently, authors such as Wozniak et al. (1994), Lang-Unnasch et al. (1998), Perkins & Martin (1999), Carreno et al. (1999), Matthew et al. (2000), Perkins & Keller (2001) and others have employed molecular techniques to differentiate particularly reptilian haemogregarines and malarias. The use of such molecular markers, which can be problematic (see Perkins & Keller, 2001) is not employed in this thesis, but is discussed in some detail in the final chapter (see Chapter 7).

1.3 Blood parasites of reptiles in Africa

Literature concerning the blood parasites of reptiles in Africa appears relatively scanty in comparison with that noted above on a world-wide scale. Sambon & Seligmann (1907), Bouet (1909), Catouillard (1909), Laveran & Pettit (1909), Fantham (1925), Hoare (1920, 1932), Garnham (1950), Pienaar (1962) and Ball et al. (1967) have probably made the most significant contributions to knowledge of blood parasites of reptiles from the African continent and this work was mostly focused in North, East and West Africa, and much less so in South Africa.

Sambon & Seligmann (1907) described a haemogregarine *Haemogregarina refrigens* Sambon & Seligmann, 1907 from a mole snake (*Pseudaspis cana* Linnaeus, 1758) (Serpentes: Colubridae) from South Africa and Bouet (1909) recorded *Haemoproteus mesnili* Bouet, 1909 in an African spitting cobra (*Naja nigricollis* Bogert, 1940) from the Ivory Coast. Fantham (1925) reported a haemogregarine from a puff adder (*Bitis arietans* Merrem, 1820) (Serpentes: Viperidae) in South Africa, while Fantham & Porter (1950) described two *Plasmodium* (*Ophidiella*) species, *Plasmodium pythonis* Fantham & Porter, 1950 from an

African rock python (*Python sebae* Gmelin, 1789) and *Plasmodium bitis* Fantham & Porter, 1950 from a puff adder (*B. arietans*) in South Africa. Several new species of parasites in South African reptiles were reported by Pienaar (1962), including a trypanosome (*Trypanosma mocambicum* Pienaar, 1962) in the blood of a Mozambican terrapin, *Peliosus sinuatus sinuatus* (Smith 1838), a species of lizard malaria (*Plasmodium zonuriae* Pienaar, 1962), a piroplasmid (*Sauroplasma zonurum* Pienaar, 1962) and infections of a viral nature (*Pirhemocyton zonuriae* Pienaar, 1962) in the girdled lizard, *Cordylus vittifer* Reichenow, 1887. A new haemogregarine (*Haemogregarina pelusiensi* Pienaar, 1962) from the terrapin, *Pelosios sinuatus sinuatus* was described from Mozambique (Pienaar, 1962), and Paperna (1989) found this infection in the Limpopo province close to the town of Pietersburg. Pienaar (1962) also recorded another suspected piroplasm, (*Serpentoplasma najae* Pienaar, 1962) from the blood of a black-necked cobra, *Naja nigricollis*, while Paperna & de Matos (1993a) reported new hosts and geographical locations of erythrocytic viral infections, of which some records were from South Africa. Later, Paperna et al. (2001) described *Sauroleishmania zuckermani* (Paperna, Boulard, Hering-Hagenbeck & Landau) 2001 from a gecko *Pachydactylus turneri* in Gauteng Province, close to where most of the laboratory work in this thesis was done. Most recently, a new haemoproteid namely, *Haemoproteus natalensis* Cook, Smit & Davies, 2010 was described from a hinged tortoise *Kinixys natalensis* Hewitt, 1935 in KwaZulu-Natal. However, in the Free State Province, work on the blood parasites of reptiles, or of any other vertebrate group, appears very limited.

About 30 species of haemogregarines, mostly *Hepatozoon* spp. have been described in lizards on the African continent (Table 1.1), and thus far, no *Hepatozoon* spp. have been recorded from lizards in the Free State Province, the focus of this thesis. In addition, a total of five species of *Haemoproteus* have been reported in tortoises from Africa (see Cook et al. 2010). The plasmodiid parasites of lizards are represented by 24 *Plasmodium* species and four *Haemocystidium* Castellani & Willey, 1904 species on the African continent (see Table 1.2). However, to date, only one species of filarial nematode has been described (*Befilaria pseudocordyli* Gibbons, 1989) from a Western Cape crag lizard (*Pseudocordylus microlepidotus*). Lastly, only two types of *Sauroplasma* infections have been described in South African lizards (Pienaar, 1962).

Table 1.1 Records of saurian haemogregarine and haemococcidian species from Africa.

Lizard host species by families	**Haemogregarine and haemococcidian species**	**Original host localities**	**Overall dimensions, intracellular gamonts in type description (µm), if recorded**	**References**
Agamidae				
Agama agama Linnaeus, 1758	*Haemogregarina agamae* Laveran et Pettit, 1909 [Probably *Schellackia agamae* (Laveran et Pettit, 1909)]	Senegal		Laveran & Pettit (1909), Levine (1988)
		Gambia		Todd & Wolbach (1912)
		Nigeria		Adler (1924) (cited in Bray, 1964)
				Theiler (1930) (cited in Bray, 1964)
		Central African Republic		Rogier (1974)
Agama colonorum Daudin, 1802 [possibly *Agama agama* or *Agama spinosa* Gray, 1831]	*Haemogregarina agamae* Laveran et Pettit, 1909 [Probably *Schellackia agamae* (Laveran et Pettit, 1909)]	Senegal	13 x 3	Laveran & Pettit (1909)
		Upper Senegal-Niger		Léger & Husnot (1912)
		Nigeria		MacFie (1914)
		French Sudan		Rousselot (1943)
	Schellackia agamae (Laveran et Pettit, 1909)	Central African Republic		Rogier (1977)
Agama mossambica Peters, 1854 (possible host)	*Hepatozoon argantis* Garnham, 1954	Kenya		Garnham (1954)

Table 1.1 Continued

Lizard host species by families	Haemogregarine and haemococcidian species	Original host localities	Overall dimensions, intracellular gamonts in type description (µm), if recorded	References
Chamaeleonidae				
Calumma brevicorne (Günther, 1879) [syn. *Chamaeleo brevicornis* Günther, 1879]	*Hepatozoon chabaudi* Brygoo, 1963	Madagascar	12 - 14.5 x 3.5 - 4.5	Brygoo (1963)
Chamaeleo chamaeleon Linnaeus, 1758 [syn. *Chamaeleo vulgaris* Duméril et Bibron, 1836]	*Haemogregarina chamaeleonis* Franchini, 1933	Libya	4-7 x 1 - 1.5	Franchini (1933)
	Haemogregarina chamoeleonis Rousselot, 1953	French Sudan		Rousselot (1953)
Furcifer oustaleti (Mocquard, 1984) [syn. *Chamaeleo oustaleti* Mocquard, 1894]	*Hepatozoon chabaudi* Brygoo, 1963	Madagascar		Brygoo (1963)
Furcifer pardalis (Cuvier, 1829) [syn. *Chamaeleo pardalis* Cuvier, 1829]	*Hepatozoon chabaudi* Brygoo, 1963	Madagascar		Brygoo (1963)
Cordylidae				
Pseudocordylus melanotus (Smith, 1838)	*Hepatozoon* sp. A	South Africa	18.7 x 5.6	Van As (2003)
	Hepatozoon sp. B		17.6 x 6.6	Current study
	Hepatozoon sp. C		24.2 x 6	Current study
Pseudocordylus subviridis (Smith, 1838)	*Hepatozoon* sp. A	South Africa	19.4 x 6.2	Current study
	Hepatozoon sp. C	Lesotho	34.2 x 6.6	Van As (2003)
Pseudocordylus langi (Loveridge, 1944)	*Hepatozoon* sp. D	South Africa	19.1 x 6.2	Current study
	Hepatozoon sp. E		16.5 x 5.9	Current study
Gekkonidae				
Tarentola mauritanica Linnaeus, 1758 [syn. *Platydactylus mauritanicus* Bőttger, 1873]	*Hepatozoon platydactyli* (Billet, 1900)	Algeria		Billet (1900)
	Hepatozoon annularis (El-Naffar, Mandour et Mohammed, 1991)	Egypt		El-Naffar et al. (1991)
	Hepatozoon burneti Lavier et Callot, 1938	Tunisia	35 x 6	Lavier & Callot (1938)
Tarentola sp. (possible host)	*Hepatozoon argantis* Garnham, 1954	Kenya		Garnham (1954)

Table 1.1 Continued

Lizard host species by families	Haemogregarine and haemococcidian species	Original host localities	Overall dimensions, intracellular gamonts in type description (µm), if recorded	References
Lacertidae				
Acanthodactylus boskianus Daudin, 1802	*Haemogregarina acanthodactylii* Ramadan, 1974	Egypt	10-11 x 5.5-5.6 (broad forms) 11.5 - 13.5 x 4 - 5 (slender forms)	Ramadan (1974)
	Hepatozoon boskiani (Catouillard, 1909)	Tunisia	8.5 - 16.5 x 4 - 16	Catouillard (1909)
	Haemogregarina damiettae Ramadan, Saoud, Mohammed et Fawzi, 1996	Egypt	16.5 - 22.5 x 5 - 7.5	Ramadan et al. (1996)
Acanthodactylus pardalis Lichtenstein, 1823	*Hepatozoon capsensis* (Conor, 1909)	Tunisia	8 x 3.4	Conor (1909)
		Senegal		Laveran & Pettit (1909)
		French Sudan		Rousselot (1953)
Acanthodactylus scutellarius [possibly *Acanthodactylus scutellatus* Audouin, 1827]	*Hepatozoon capsensis* (Conor, 1909)	Senegal		Laveran & Pettit (1909)
		French Sudan		Rousselot (1953)
Adolfus jacksoni Boulenger, 1899 [syn. *Lacerta jacksoni* Boulenger, 1899}	*Hepatozoon hamata* (Garnham, 1950)	Kenya	23 x 5	Garnham (1950)
Psammodromus algirus Linnaeus, 1758	*Hepatozoon psammodromi* (Soulié, 1904) (syns. *Haemogregarina lusitanica* França, 1912; *Haemogregarina pallida* França, 1908)	Algeria	16-22 x 6-8	Soulié (1904), Smith (1996)
		Algeria		Laveran & Pettit (1909)
Timon pater Lataste, 1880 [syn. *Lacerta ocellata* Daudin, 1802]	*Hepatozoon curvirostris* (Billet, 1904)	Algeria	13 x ?	Billet (1904)

Table 1.1 Continued

Lizard host species by families	Haemogregarine and haemococcidian species	Original host localities	Overall dimensions, intracellular gamonts in type description (µm), if recorded	References
Opluridae				
Oplurus cuvieri Gray, 1831 [syn. *Oplurus sebae* Duméril et Bibron, 1837]	*Schellackia brygooi* Landau, 1973	Madagascar		Landau (1973)
Oplurus cyclurus Merrem, 1820	*Schellackia brygooi* Landau, 1973	Madagascar		Landau (1973)
Scincidae				
Chalcides ocellatus (Forskal, 1775) [syn. *Gongylus ocellatus* Wagner, 1830]	*Hepatozoon sergentium* (Nicolle, 1904)	Tunisia		Nicolle (1904)
		Algeria		Laveran & Pettit (1909)
Trachylepis maculilabris (Gray, 1845) [syn. *Mabuia maculilabris* Schmidt, 1919]	*Hepatozoon hamata* (Garnham, 1950)	Kenya		Garnham (1950)
Trachylepis quinquetaeniata (Lichtenstein, 1823) [syn. *Mabuia quinquetaeniata* Boulenger, 1887]	*Hepatozoon gracilis* (Wenyon, 1909)	Sudan	16-17 x 1.5	Wenyon (1909)
		Egypt		Bashtar et al. (1987)
Trachylepis striata (Peters, 1844) [syn. *Mabuia striata* Boulenger, 1895]	*Karyolysus poleensis* Mutinga et Dipeolu, 1989	Kenya	6.9 x 5 - 5.6	Mutinga & Dipeolu (1989)
	Schellackia mabuyai Mutinga et Dipeolu, 1989	Kenya	7-9 x 2 - 3	Mutinga & Dipeolu (1989)
Trachylepis vittata (Oliver, 1804) [syn. *Mabuia vittata* , Boulenger, 1920]	*Hepatozoon mabuiae* (Nicolle et Comte, 1906)	Tunisia	14 - 17 x 5 - 6	Nicolle & Comte (1906)
Mabya quinquetaeniata (Lichtenstein)	*Hepatozoon gracilis* (Wenyon) 1909, Bashtar, Abdel-Ghaffar & Shazly 1987	Sudan	18 - 22.5 x 0.9 - 1.4	Wenyon (1909) Bashtar et al. (1987)
Varanidae				
Varanus albigularis albigularis Daudin, 1802	*Hepatozoon paradoxa* (Santos Dias, 1954)	Mozambique		Santos Dias (1954)
Varanus arenarius Duméril et Bibron, 1836 [possibly *Varanus griseus* Daudin, 1803]	*Hepatozoon varani* (Laveran, 1905)	French West Africa		Bouet (1909)
Varanus exanthematicus Bosc, 1792	*Hepatozoon varani* (Laveran, 1905)	French Sudan		Rousselot (1943)

Table 1.1 Continued

Lizard host species by families	Haemogregarine and haemococcidian species	Original host localities	Overall dimensions, intracellular gamonts in type description (µm), if recorded	References
Varanus griseus Daudin, 1803	*Hepatozoon borreli* (Nicolle et Comte, 1906)	Tunisia	7 - 8 x 2.3 - 2.5	Nicolle & Comte (1906)
	Hepatozoon varani (Laveran, 1905)	French Sudan		Rousselot (1953)
		Unknown		Laveran & Pettit (1909)
Varanus niloticus Linnaeus, 1758	*Hepatozoon borreli* (Nicolle et Comte, 1906)	Kenya		Ball (1967) (reporting a possible mixed infection)
	Hepatozoon varani (Laveran, 1905)	Transvaal	14 x 3	Laveran (1905)
		Senegal		Laveran & Pettit (1909)
		Portuguese Guinea		França (1911)
		Gambia		Todd & Wolbach (1912)
		Upper-Senegal-Niger		Léger & Léger (1914)
		French Sudan		Rousselot (1943)
		Kenya		Ball (1967) (reporting a possible mixed infection)
		Gambia		Wolbach (1914)
	Hepatozoon toddii (Wolbach, 1914)	Kenya	10.3 x 2.5	Ball (1967)

Table 1.2 Records of saurian plasmodiid species from Africa with morphometrical dimensions. Abbreviations: (LW) Length x Width (µm²).

Lizard host species by families	**Plasmodiid species**	**Original host Localities**	**Overall dimensions, intracellular meronts in type description (µm), if recorded (LW) (µm²)**	**Number of merozoites**	**Overall dimensions, intracellular gametocytes in type description (µm), if recorded (LW) (µm²)**	**Gametocyte size/ host cell nucleus ratio (µm)**	**General gametocyte morphology**	**Gametocyte size/ normal erythrocyte nuclei ratio (µm)**	**Effects of gametocytes on host cell**	**Reference**
					Agamidae					
Agama agama syn. *Agama colonorum* **Other hosts** *Agama cyanogaster* (Southgate, 1970) *Agama mossambica*	*Plasmodium* (*Sauramoeba*) *giganteum* Theiler, 1930	Gbanga, Liberia	9 - 18 x 4 - 11 (52 - 165)	28 - 74	9 - 22 x 4 - 10 (45 - 145)	1.2 - 5.3	Round to elongate or bulky	1.6 - 6.3		Theiler (1930) Bray (1959) Ball (1967) Garnham (1966)
Agama agama **Other hosts** *Acanthocercus atricollis* (Smith, 1849) *Agama hispida aculeata* (Kaup, 1827)	*Plasmodium* (*Lacertamoeba*) *agamae* (Wenyon,1909)	Bahr-El-Ghazal Province, Sudan	4 - 11 x 3 - 6 (12 - 55)	4 - 15	6 - 19 x 3 - 8 (33 - 105)	1.77 - 2.22		1.73 - 2.21	Hypotrophy Distortion of host cells Displacement and occasional distortion of nuclei	Wenyon (1909) Bray (1959) Garnham (1966) Petit et al. (1983) Schall (1990)
Agama mossambica	*Plasmodium* (*Lacertamoeba*) *mossambica* Telford, 2009	Morogoro Region, Tanzania	5 - 15 x 3 - 7 (20 - 75)	6 - 34	6 - 17 x 3 - 8 (36 - 84)	2.45	Elongate	2.35	Distortion of host cells Displacement and occasional distortion of nuclei Displacement of nuclei	Telford (2009)
Agama mossambica	*Plasmodium* (*Sauramoeba*) *giganteum* Theiler, 1930	Gbanga, Liberia	9 - 18 x 4 - 11 (52 - 165)	28 - 74	9 - 22 x 4 - 10 (45 - 145)	1.2 - 5.3	Dimorphic	1.6 - 6.3	Hypertrophy Distortion and occasional enlargement of host cell	Theiler (1930)

Table 1.2 Continued

Lizard host species by families	Plasmodiid species	Original host Localities	Overall dimensions, intracellular meronts in type description (µm), if recorded (LW) (µm²)	Number of merozoites	Overall dimensions, intracellular gametocytes in type description (µm), if recorded (LW) (µm²)	Gametocyte size/ host cell nucleus ratio (µm)	General gametocyte morphology	Gametocyte size/ normal erythrocyte nuclei ratio (µm)	Effects of gametocytes on host cell	Reference
Chamaeleonidae										
Chamaeleo brevicornis **Other hosts** *Calumma parsoni crucifer*(Cuvier, 1824)	*Plasmodium* (*Sauramoeba*) *robinsoni* (Brygoo, 1962) Telford and Landau, 1987	Moramanga Subprefecture, Madagascar	11 - 23 x 7 - 11 (90 - 184)	40-74	9 - 20 x 5 - 13 (72 - 221)	1.4 - 5.4	Oval to elongate or bulky	2.1 - 4.7	Hypertrophy Displacement and distortion of nuclei	Telford & Landau (1987)
Chamaeleo brevicornis	*Plasmodium* (*Lacertamoeba*) *brygooi* Telford and Landau, 1987	Périnet, Madagascar	6 - 9 x 5 - 8 (36 - 64)	10 - 16	9 - 15 x 5 - 10 (66 - 126)	0.5-1.2	Oval or elongate	0.5 - 1.1	Hypertrophy Distortion of cell Displacement and distortion of nuclei	Telford & Landau (1987)
Kinyongia fischeri (Reichenow, 1887)	*Plasmodium* (*Sauramoeba*) *acuminatum* Pringle, 1960	Tanga Region, Tanzania							Displacement of nuclei	Pringle (1960)
Kinyongia fischeri	*Plasmodium* (*Lacertamoeba*) *fischeri* Ball and Pringle, 1965	Tanga Region, Tanzania	9 x 6 (50)	21 - 25	8 - 11 x 5 - 8 (41 - 87)		Oblong to elongate		Distortion of host cell Displacement of nuclei	Ball & Pringle (1965)
Kinyongia oxyrhina (Klaver and Böhme, 1988)	*Plasmodium* (*Sauramoeba*) *michikoa* Telford, 1988	Kilombero district, Tanzania	6 - 15 x 4 - 8 (28 - 78)	12 - 32	6 - 14 x 4 - 8 (36 - 80)	1.81	Elongate	1.75	Hypotrophy	Telford (1988)
Kinyongia oxyrhina	*Plasmodium* (*Lacertamoeba*) *gologoloense* Telford, 1988	Morogoro Region, Tanzania	5 - 7 x 4 - 6 (20 - 42)	6 - 14	5 - 11 x 4 - 6 (20 - 54)	1.29	Ovoid or round	0.99	Displacement of nuclei	Telford (1988)
Trioceros werneri (Tornier, 1899)	*Plasmodium* (*Lacertamoeba*) *tanzaniae* Telford, 1988	Iringa Region, Tanzania	6 - 12 x 4 - 7 (28 - 70)	8 - 22	8 - 19 x 4 - 9 (48 - 112)	1.89	Elongate	2.07	Distortion of host cell Displacement and occasional distortion of nuclei	Telford (1988)

Table 1.2 Continued

Lizard host species by families	Plasmodiid species	Original host Localities	Overall dimensions, intracellular meronts in type description (µm), if recorded (LW) (µm²)	Number of merozoites	Overall dimensions, intracellular gametocytes in type description (µm), if recorded (LW) (µm²)	Gametocyte size/ host cell nucleus ratio (µm)	General gametocyte morphology	Gametocyte size/ normal erythrocyte nuclei ratio (µm)	Effects of gametocytes on host cell	Reference
Trioceros werneri	*Plasmodium* (*Lacertamoeba*) *arachniformis* Telford, 1988	Iringa Region, Tanzania	4 - 12 x 2 - 7 (12 - 49)	4 - 12	6 - 17 x 3 - 8 (30 - 75)	1.54	Elongate and thin	1.58	Hypertrophy Displacement and occasional distortion of nuclei	Telford (1988)
Triceros werneri	*Plasmodium* (*Lacertamoeba*) *uzungwiense* Telford, 1988	Iringa Region, Tanzania	4 - 8 x 3 - 6 (16 - 42)	4 - 12	5 - 13 x 3 - 7 (24 - 63)		Elongate	1.34	Distortion of host cell Displacement of nuclei Enlargement of proerythrocyte nucleus	Telford (1988)
				Cordylidae						
Cordylus t. tropidosternum (Cope, 1869)	*Plasmodium* (*Carinamoeba*) *cordyli* Telford, 1987	Tanga Region, Tanzania	4 - 7 x 3 - 6 (12 - 36)	4 - 11	5-8 x 4-7	0.74	Round or ovoid	0.89	Hypertrophy Distortion of host cell Distortion and displacement of nuclei	Telford (1987)
Cordylus vittifer (Reichenow, 1887)	*Plasmodium* (*Lacertamoeba*) *zonuriae* (Pienaar, 1962) Telford, 1987	Elandsfontein, South Africa	7 - 17 x 4 - 9 (36 - 120)	12 - 28	7 - 20 x 4 -10 (42 - 114)	1.69	Elongate	1.69	Hypertrophy Distortion and occasional enlargement of host cell Distortion and displacement of nuclei	Pienaar (1962) Telford (1987)
				Gekkonidae						
Cnemaspis barbouri Perret, 1986	*Plasmodium* (*Lacertamoeba*) *cnemaspi* Telford, 1984	Morogoro Region, Tanzania	6 - 13 x 3 - 7 (24 - 91)	8 - 24	7 - 14 x 3 - 9 (32 - 108)	2.04	Elongate (active) Ovoid or rounded (chronic)	1.77	Hypertrophy Distortion of host cell Displacement of nuclei	Telford (1984)
Hemidactylus platycephalus Peters, 1854	*Plasmodium* (*Lacertamoeba*) *uluguruense* Telford, 1984	Morogoro Region, Tanzania	4 - 10 x 2 - 6 (12 - 54)	4 - 12	5 - 10 x 4 - 7 (20 - 63)	0.97	Ovoid	1.07	Hypertrophy Distortion of host cell Enlargement and displacement of nuclei	Telford (1984)
Lygodactylus capensis grotei Sternfeld, 1911	*Haemocystidium lygodactyli* Telford, 2005	University campus, Morogoro, Tanzania			11 - 20 x 4 - 9.5 (62 - 140)				Distortion of host cell	Telford (2005)

Table 1.2 Continued

Lizard host species by families	Plasmodiid species	Original host Localities	Overall dimensions, intracellular meronts in type description (µm), if recorded (LW) (µm²)	Number of merozoites	Overall dimensions, intracellular gametocytes in type description (µm), if recorded (LW) (µm²)	Gametocyte size/ host cell nucleus ratio (µm)	General gametocyte morphology	Gametocyte size/ normal erythrocyte nuclei ratio (µm)	Effects of gametocytes on host cell	Reference
Lygodactylus capensis grotei	*Haemocystidium lygodactyli* Telford, 2005	Morogoro region, Tanzania	10.0 x 5.0 - 16.0 x 9.0		8 - 25 x 5 - 11		Elongate to oval		Distortion of host cell	Telford (2005)
Lygodactylus l. luteopicturatus Pasteur, 1964 **Other host** *Lygodactylus capensis grotei*	*Plasmodium* (*Lacertamoeba*) *loveridgei* Telford, 1984	Morogoro Region, Tanzania	5 - 15 x 3 - 7 (20 - 91)	6 - 26	8 - 23 x 3 - 11 (48 - 176)	3.19	Elongate, rarely rounded	2.89	Hypotrophy Distortion of host cell Displacement of nuclei	Telford (1984)
Tarentola mauritanica deserti (Linnaeus, 1758) **Other host** *Tarentola annularis* (Geoffroy De St-Hilaire, 1827)	*Haemocystidium tarentolae* (Parrot, 1927) Paperna & Landau, 1991	El Kantara, Algeria			8 - 18 x 4 - 12		Elongate		Slight hypertrophy and distortion of host cell Lateral displacement of nuclei.	Parrot (1927) Riding (1930) Paperna & Landau (1991)
Lacertidae										
Holaspis guentheri Gray, 1863	*Plasmodium* (*Lacertamoeba*) *holaspi* Telford, 1986	Morogoro Region, Tanzania	5 - 13 x 4 - 7 (25 - 66)	8-18	6 - 18 x 3 - 8 (28 - 98)	2.13	Elongate	2.25	Distortion of host cell Displacement and distortion of nuclei	Telford (1986)
Opluridae										
Oplurus cuvieri	*Haemocystidium opluri* Paperna & Landau, 1991	Baie de Loukaio, Madagascar			12 - 19 x 3 - 12		Oblong, oval		Lateral hypertrophy Displacement of nuclei	Paperna & Landau (1991)
Scincidae										
Trachylepis maculilabris	*Plasmodium* (*Lacertamoeba*) *maculilabre* Schwetz, 1931	Kisangani, Congo	10.0 x 6.9 (69)	15 - 20	7 - 13 x 5 - 8 (42 - 91)	2.62	Ovoid to elongate	3.61	Hypertrophy Distortion of host cell Displacement and occasional distortion of nuclei	Schwetz (1931)

Table 1.2 Continued

Lizard host species by families	**Plasmodiid species**	**Original host Localities**	**Overall dimensions, intracellular meronts in type description (µm), if recorded (LW) (µm²)**	**Number of merozoites**	**Overall dimensions, intracellular gametocytes in type description (µm), if recorded (LW) (µm²)**	**Gametocyte size/ host cell nucleus ratio (µm)**	**General gametocyte morphology**	**Gametocyte size/ normal erythrocyte nuclei ratio (µm)**	**Effects of gametocytes on host cell**	**Reference**
Trachylepis quinquetaeniata **Other hosts** *Trachylepis maculilabris* *Trachylepis striata*	*Plasmodium* (*Carinamoeba*) *mabuiae* (Wenyon, 1909), Telford, 1983	Bahr-El-Ghazal Province, Sudan	4 - 9 x 2 - 5 (10 - 30)	4-12	5 - 11 x 3 - 5 (18 - 44)	1.45	Elongate, rarely ovoid or round	1.38	Hypertrophy Occasional distortion of host cell Displacement and occasional distortion of nuclei	Wenyon (1909) Telford (1983)
Trachylepis striata	*Plasmodium* (*Sauramoeba*) *heischi* Garnham and Telford, 1984	Nairobi, Kenya	8 - 18 x 6 – 11 (48 - 144)	20-65	8 - 12 x 4 - 9 (60 - 120)	2.1 - 6.3	Large, spindle-shaped	3.1 - 6.9	Distortion of host cell Lateral displacement of nuclei	Garnham & Telford (1984)
Trachylepis striata **Other hosts** *Trachylepis maculilabris* *Trachylepis quinquetaeniata* *Trachylepis varia*	*Plasmodium* (*Lacertamoeba*) *pitmani* Hoare, 1932	Lake Victoria, Uganda	4 - 11 x 3 - 7 (12 - 66)	4-25	5 - 16 x 4 - 9 (25 - 91)	2.23	Ovoid	2.07	Distortion of nuclei	Hoare (1932) Garnham (1966)

1.4 Problems of taxonomy among reptilian haematozoans

For the purposes of the study, part of the classification system of Lee et al. (2000) is employed for the Protista or Protozoa, especially for the lower taxonomic ranks. There have been several attempts to re-define the classification of the Protozoa in recent years. Notable examples have been 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) noted in the Society of Protozoologists' (2000) publication that the Protozoa form an artificial group of eukaryotes, rather than a natural one. Patterson (2000) also concluded 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 believed, will probably come from a "molecular understanding of evolutionary relationships among taxa". As a result of the current uncertainties, Lee et al. (2000) divided the Protozoa into "Key Major Groups", many corresponding to phyla, others to orders. The phylum Apicomplexa, is one such group. Roberts & Janovy (2009) used a classification scheme of the parasitic protozoans based on work of Lee et al. (2000), Hausmann & Hülsmann (1996) and particularly Adl et al. (2005), where the latter authors established six, inclusive "super-groups" for the Eukaryota based on common structural characters. For the purpose of this thesis, the higher taxonomic ranks recorded in the text of Roberts & Janovy (2009) will be used.

1.5 Super-group Chromalveolata Adl et al. 2005

Adl et al. (2005) defined this group as organisms possessing a "plastid from a secondary endosymbiosis with an ancestral archaeplastid; plastid secondarily lost or reduced in some; with tertiary reacquisition of a plastid in others." This super-group comprises three kingdoms: Heterokontae, Alveolatae, and Eukaryomonadae of which the members are photosynthetic, heterotrophic, saprophytic or parasitic.

1.5.1 Super-phylum Alveolata Cavalier-Smith, 1991

This group includes three phyla: Dinoflagellata Bütschli 1885, Apicomplexa Levine, 1970 and Ciliophora Doflein, 1901.

1.6 Protozoan genera within the Phylum Apicomplexa Levine, 1970, with particular reference to those of lizards

The phylum Apicomplexa Levine, 1970 comprises unicellular endosymbionts, characterised by the presence of an apical complex, composed of one or more polar rings, a number of rhoptries and micronemes, a conoid and sub-pellicular microtubules. This phylum has the following two classes based on the taxonomic scheme reported in Roberts & Janovy (2009): Conoidasida Levine, 1988 and Aconoidasida Melhorn, Peters & Haberkorn, 1980.

In the Society of Protozoologists' classification system (Lee et al. 2000) and in the system reported in Roberts & Janovy (2009), 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 fall within the same class (Aconoidasida), but within the order Piroplasmorida Wenyon, 1926. Details of this classification are given below.

1.7 Class Conoidasida Levine, 1988

Organisms representing the class Conoidasida Levine, 1988 have organelles of the apical complex, and generally both sexual and asexual reproduction occurs, 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 be present for feeding. Homoxenous and heteroxenous species are known. This class is represented by seven orders of parasitic protozoans according to Roberts & Janovy (2009), of which the Eucoccidiorida Léger & Dubosq, 1910 are represented by two suborders.

1.7.1 Order Eucocciiorida Léger & Dubosq, 1910

Members representing the order Eucoccidiorida Léger & Dubosq, 1910 demonstrate merogony, gamogony and sporogony, and occur in vertebrates and/or invertebrates (Davies & Johnston, 2000).

1.7.1.1 Suborder Adeleorina Léger, 1911

Organisms within the suborder Adeleorina Léger, 1911 exhibit syzygy, with conjugation and subsequent sporogony usually in an invertebrate definitive host (Davies & Johnston, 2000). According to Davies & Johnston (2000) complex life-cycles exist, involving at least one cycle of merogony, followed by gametogony, syngamy and sporogony. Two types of meronts may occur. Roberts & Janovy (2009) record syzygy between micro and macro gamonts and infective sporozoites contained within an envelope. The Adeleorina comprises seven families, three of which contain genera of reptilian haemogregarines. About 400 species have been described mostly on the basis of presence in a new host and those species described before the late 1960s in lizards were known only from the erythrocytic stages.

1.7.1.1.1 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 "s*enso 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 (Table 1.1).

1.7.1.1.1.1 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. Telford et al. (2001) also placed *Haemogregarina floridiana* Telford, Wozniac & Butler, 2001 from an aquatic

snake in this genus on the basis of the erythrocytic meronts from peripheral blood being sequestered in the lung when they reach maturity.

1.7.1.1.1.2 *Haemogregarina* life cycles

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

Members of this genus occur in vertebrate hosts such as chelonians, fishes and possibly other ectotherms associated with an aquatic habitat. 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 or parasitic isopods, intracellular sporogony occurs in the intestinal epithelium and oocysts produce eight naked sporozoites. Four aflagellate microgametes form during microgametogenesis. After paring (syzygy) and fertilisation of the macrogamont by a microgamete, a monosporoblastic oocyst develops, lacking sporocysts. Eight sporozoites arise from a single germinal centre (Desser, 1993). Post-sporogonic merogony also occurs in the invertebrate host and sporozoites may infect leech tissue outside the intestine (Siddall & Desser, 1991).

Transmission is either by bite from the vector, when meronts release merozoites and concentrate near the end of the leech proboscis and transferred to the circulatory system of the vertebrate host, or perhaps when the invertebrate is ingested, where sporozoites are liberated from invertebrate tissue.

1.7.1.1.2 Family Hepatozoidae Wenyon, 1926 and genus *Hepatozoon* Miller, 1908

The Family Hepatozoidae Wenyon, 1926 contains the genus *Hepatozoon* Miller, 1908. Members of the genus demonstrate such diversity that the genus may be paraphyletic (see Barta, 2000).

1.7.1.1.2.1 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 (Davies & Johnston, 2000). Merogony does not usually occur within erythrocytes, but in vascular endothelial cells (Telford, 2009). 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 (Davies & Johnston, 2000).

More than 200 species of the genus *Hepatozoon* have been described in snakes worldwide (Levine, 1988) and according to Smith, (1996) they are considered to be the most frequent haemogregarines in these hosts. Since most descriptions in the past have been made on the basis of gamont morphology and not the life cycle characteristics, most of these species have been named *Haemogregarina.* However, Ball et al. (1967) revealed that *Haemogregarina rarefasciens* (Sambon & Seligmann, 1907) that infects the snake *Drymarchon corais* had a typical *Hepatozoon* life cycle. Later, following this work, several other authors (see Telford et al. 2002; Paperna & Lainson, 2004) transferred a few *Haemogregarina* species to *Hepatozoon* in accordance with Smith's (1996) recommendations. Today, the taxonomy of this genus is still tentative and according to Sloboda et al. (2007) descriptions of species without the life cycle are disputable. It is therefore essential to include sporogonic and gametogonic stages in new descriptions, if at all possible. This is a strategy adopted in this thesis.

The range of blood sucking invertebrates that parasitize reptiles includes ixodid and argasid ticks, mites, and assassin bugs; dipterans (sandflies, mosquitoes, tsetse flies), anopleurans (sucking lice), siphonapterans (fleas) and the hirudineans (leeches) (Smith, 1996). Most life cycle studies have been carried out using mosquitoes as possible definitive hosts and Smith (1996) considers *Culex*, *Aedes* and *Anopheles* as the main vectors of *Hepatozoon* species in ophidians. Low host specificity has been reported from members of this genus, for example Telford et al. (2004) reported *Hepatozoon sauritus* Telford, Wozniac & Butler, 2001 in four snake species, representing 3 genera. Ball (1967) observed in his experiments that *Hepatozoon rarefaciens* are transferred from a colubrid snake (*D. corais*) to a boa (*B. constrictor*) by means of a mosquito (*Culex tarsalis*). Other authors (see Landau et al. 1970; Paperna & Lainson, 2004) have shown that *Hepatozoon* host specificity is even less than the level of the first intermediate hosts, with various species of lizards representing the genera *Opluris*, *Podarcis* and *Tropidurus* being susceptible to *Hepatozoon terzii* and *Hepatozoon domerquei*. Dizoic, tetrazoic or hexazoic cysts were found in livers of above mentioned lizards under experimental conditions. Wozniak & Telford, (1991) successfully transmitted a *Hepatozoon* species from two species of colubrid snakes, (*Coluber constrictor* and *Nerodia fasciata*) to two species of *Anolis* lizards. Smith et al. (1994, 1996) showed that even amphibians can serve as first intermediate "vectors", at least under experimental conditions. Lowichik & Yaeger (1987) demonstrated that congenital transmission can represent yet another route of infection for *Hepatozoon*, in an ovoviviparous snake, *Nerodia fasciata*.

1.7.1.1.2.2 *Hepatozoon* life cycles

A general *Hepatozoon* life cycle in a lizard and mosquito is illustrated in Fig 1.3 and can be divided into three phases, involving a lizard as a vertebrate host and a mosquito as a definitive host and vector. The merogonic stages usually occur in the liver of a lizard (Fig. 1.3 C - H) while the gamogenetic and sporogonic stages occur in a mosquito (Fig.1.3 I - Q). Variations in this general life cycle pattern are documented (see below). A typical experimental life cycle with mosquitoes as vectors starts when gamonts are taken up with the vertebrate host blood and penetrate the intestinal wall of the mosquito; pairing (syzygy) of micro (male) and macrogamonts (female) occurs probably in the insect fat bodies (Telford, 2009). Microgamonts form two to four biflagellated microgametes, of which one microgamete fertilises the macrogamete to produce a zygote. The zygote grows and becomes an expanding, polysporocystic oocyst with multiple germinal centres.

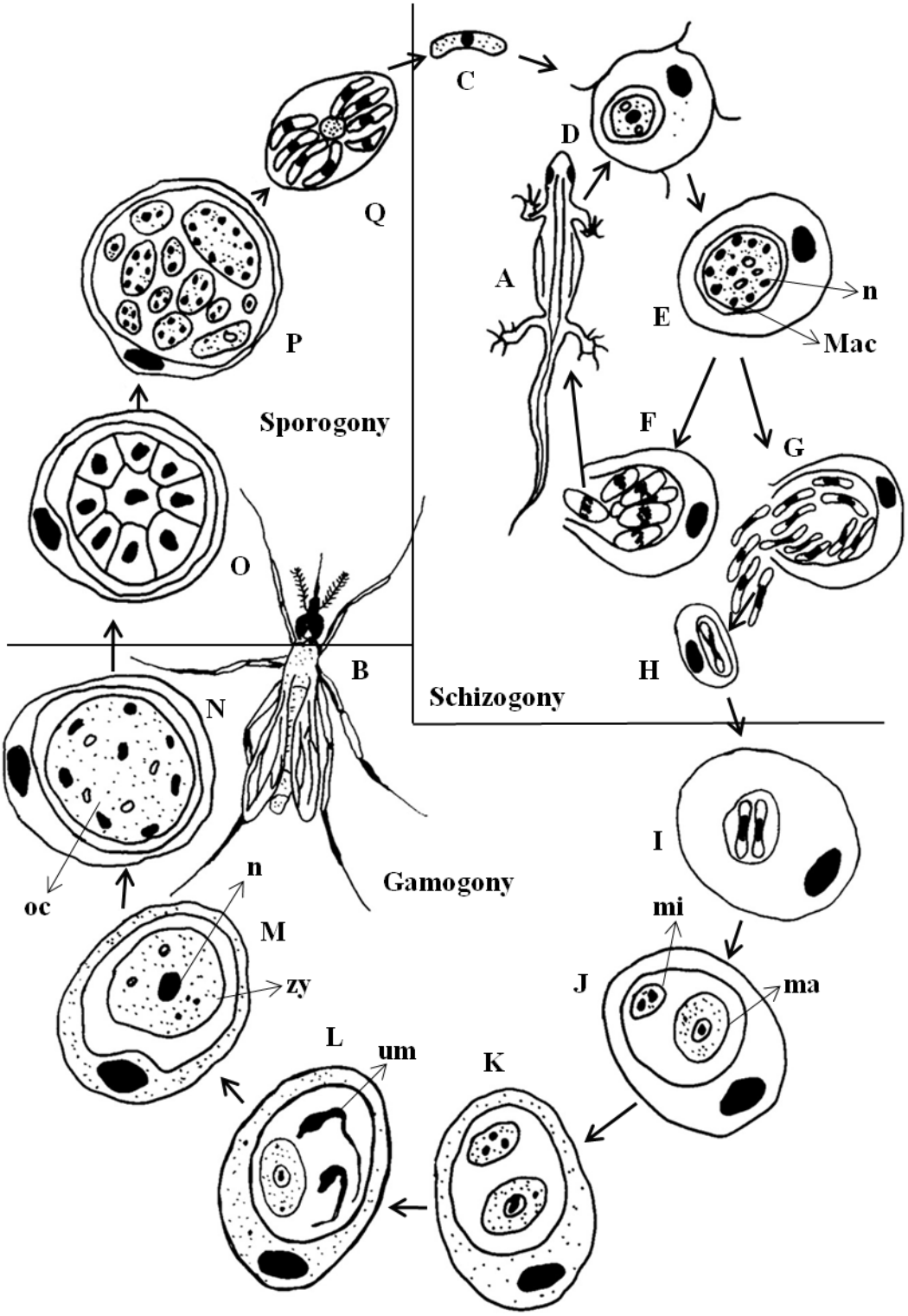

Figure 1.3 Diagrammatic representation of the life cycle of *Hepatozoon gracilis* (Wenyon, 1909) redrawn from Bashtar et al. (1987). **(A)** *Trachylepis quinquetaeniata.* **(B)** *Culex pipiens molestus.* **(C)** Free sporozoite invades liver cell of vertebrate host. **(D)** Trophozoite in liver parenchyma cell. **(E)** Macromeront (Mac) containing many nuclei (n). **(F)** Macromerozoites re-infecting liver cells. **(G)** Micromeront and micromerozoites invading host erythrocytes **(H)**. **(I)** Gamonts within parasitophorous vacuole within host cell in mosquito haemocoel. **(J)** Micro (mi) and macro (ma) gamonts. **(K)** Nuclei divide. **(L)** Uniflagellated microgametes (um). **(M)** Zygote (zy) with nucleus (n). **(N)** Oocyst (oc). **(O)** Sporoblast formation. **(P)** Sporoblast formation, repeated division of nuclei. **(Q)** Sporocyst containing sporozoites.

The location of the oocysts may vary, depending on the species and according to Telford (2009) may form in the haemocoel, thorax or in the head region of the mosquito. Some species have no preferred site while others are specific to an area. Oocysts develop, and contain, sporocysts which when mature produce 4 to 50 or more sporozoites (Telford, 2009). A typical *Hepatozoon* species characteristic is the number of sporozoites per sporocyst. When an infected mosquito with developed sporozoites is fed to a vertebrate host, the sporozoites leave the sporocysts and penetrate the intestinal wall (Telford, 2009) and enter the circulatory system of the vertebrate host. In most *Hepatozoon* species the sporozoites form meronts in liver or lung cells, specifically the hepatic parenchymal cells, Kupffer cells, endothelial cells of capillaries, or cells of the sinus walls, depending on the tissue involved. Macromeronts are the first generation and produce larger, fewer macromerozoites than micromeronts which are the product of the macromerozoites. Micromeronts form the second generation and produce more, longer micromerozoites which are liberated from the often bigger micromeronts (Telford, 2009) to invade erythrocytes in the peripheral blood.

Robin (1936a, 1936b) described a different mode of gametogony in mosquitoes with *Hepatozoon mesnili* Robin, 1936 in a blood meal taken from an infected gecko. In the mosquito gut lumen, the microgamont divided producing four nuclei. Flagellated microgametes did not form, but instead one nucleus entered the cytoplasm of the macrogamont, prior to the fusion of the two gamonts. The resulting zygote penetrated the gut wall into the haemocoel. Oocyst formation and sporogony ensued in the "normal" pattern. Miller (1908) described a similar mode of gametogony in mites infected with *Hepatozoon muris* from rodents. Ball & Oda (1971) argued that this different pattern of development may be enough justification for the taxonomic division of the genus *Hepatozoon.*

Another variation in the life cycle occurs in the sporogonic stages of *Hepatozoon lygosomarum* (Doré, 1919) in a mite (*Ophionyssus scincorum*) reported by Allison & Desser (1981) where uninucleate oocysts developed into polysporocystic oocysts in the intestinal caeca wall. Bashtar et al. (1984, 1987) presented yet another variation on the gametogonic process, where uniflagellated microgametes formed during microgametogenesis in the haemocoel of mosquitoes containing three species *Hepatozoon gracilis*, *Hepatozoon aegypti* and *Hepatozoon mehlhorni.* Smith & Desser (1997b) described production of uniflagellated

microgametes as "a retention of the ancestral condition". Molecular studies will probably give more insight in this matter in the future and Telford (2009) is of the opinion that it is premature to divide the genus based on variations in gametogony, sites of sporogony and zygote formation. More work needs to be done in this area of research to understand the complex life cycle patterns involved.

1.7.1.1.3 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.

1.7.1.1.3.1 The genus *Karyolysus* Labbé, 1894

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

Members of this genus have been reported from only lacertid lizards. Merogony occurs in the endothelial cells of lizards and gamonts primarily infect erythrocytes. Syzygy and sporogony exist 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).

Karyolysus species have been reported in only two genera of European lizards, (*Lacerta* Linnaeus, 1758 and *Podarcis* Wagler, 1830 spp.) (Sauria: Lacertidae). Members of this genus infecting lacertid lizards are transmitted by ingestion of a mite (*Sauronyssus saurarum*). Transovarian transmission occurs through female mite eggs to the larvae and represents the next generation of infection. Gamonts, after ingestion by the mite, leave their host cells (Svahn, 1975a) and pair 24h, post feeding. Microgametes pair with macrogametocytes, divide once, and produce two biflagellated microgametes, with one fertilising the macrogamete. Reichenow (1921) showed that the resulting zygote forms oocysts in the epithelial cells of the mite stomach in *Karyolysus lacetae* (Danilewsky) Reichenow, 1913 or in the podosomal region of the haemocoel in *Karyolysus lacazei* (Labbé)

Svahn, 1975b. Svahn (1975b) confirmed this and found oocysts of *Karyolysus latus* Svahn, 1975 in the stomach epithelium but not in the anterior portion of the haemocoel (Svahn, 1975b; Telford, 2009).

1.7.1.1.3.2 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 in the peripheral blood of a toad (*Bufo marinus*) from Brazil (Petit et al. 1990), a lizard from Australia and an African tortoise (see Davies & Johnston, 2000), with ticks as invertebrate hosts.

1.7.1.1.3.3 *Hemolivia* life cycles

In the vertebrate hosts merogony and cyst formation occur in endothelial cells and erythrocytes, while gamonts occur in erythrocytes (Davies & Johnston, 2000). 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.

The type species, *H. stellata* undergoes sporogony in the tick (*Amblyomma rotondatum* Koch, 1844), where the resulting zygote forms a star shaped oocyst producing sporokinetes in intestinal cells. Other intestinal cells are invaded and sporokinetes becomes sporocysts which gives rise to sporozoites. Petit et al. (1990) suggested that transmission can occur by any of the known modes for haemogregarines such as predation on a vertebrate host with cysts containing cystozoites, directly from a sporocyst-contaminated environment, or the ingestion of a sporocyst-infected tick.

Smallridge & Paperna (1997) extended the host range for this genus when they described *Hemolivia mariae* Smallridge & Paperna, 1997 from *Tiliqua rugosa* (Sauria: Scincidae) in

Australia. Transmission occurred by bite of a tick *Amblyomma limbatum*, known to infest these lizards. Stellate oocysts formed in the epithelial cells in the gut and possessed crystalline arms around which sporokinetes formed. Sporokinetes entered the intestinal cells, formed sporocysts and produced 8 - 20 sporozoites. Landau & Paperna (1997) reassigned *Hepatozoon mauritanicum* (Brumpt) Michel (1973) a haemogregarine of a tortoise (*Testudo graeca* Linnaeus, 1758) transmitted by a tick (*Hyalomma aegyptium* (Linnaeus, 1758)) to the genus *Hemolivia*. They found oocysts containing sporokinetes and sporocysts producing sporozoites in tick intestinal cells and gamonts, meronts and cysts containing cystozoites in erythrocytes, which confirmed the placement in the genus *Hemolivia*.

1.7.1.1.4 Family Dactylosmatidae Jakowska & Nigrelli, 1955

The dactylosomatids are heteroxenous blood parasites of ectothermic vertebrates that appear to use leeches as definitive hosts (Barta, 1991). This family was erected by Jakowska & Nigrelli in 1955 to place some enigmatic infections, and is one of the most poorly understood of all protozoan families (Lainson, 2007). This family comprises two genera, one of which may occur in reptiles.

1.7.1.1.4.1 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 (Levine, 1988), merogony and gamogony occur in the erythrocytes of the peripheral blood (Davies & Johnston, 2000). 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. Barta (1991) transferred some species infecting fish to the genus *Babesiosoma*.

1.7.1.2 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 (Roberts & Janovy

2009). 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.

1.7.1.2.1 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 has been 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*.

1.7.1.2.1.1 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 vertebrate 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.

1.7.1.2.1.2 The genus *Schellackia* Reichenow, 1919

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

Species in this genus has been described from lizards in Thailand (Finkelman & Paperna, 1998), tree frogs in Brazil (Paperna & Lainson 1995), geckos in Jordan (Paperna & Finkelman, 1996) and in an iguanid from Costa Rica (Desser, 1997). Levine (1988) listed 11 species infecting reptiles and amphibians, although, earlier Landau (1973) and Landau et al. (1974) identified two of these 11 species as *Lainsonia*.

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 (Davies & Johnston, 2000).

1.8 Class Aconoidasida Mehlhorn, Peters & Haberkorn, 1980

According to Davies & Johnston (2000), 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.

1.8.1 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 (Davies & Johnston, 2000).

1.8.1.1 Family Plasmodiidae Mesnil, 1903

This family includes *Plasmodium* Marchiafava & Celli, 1885, *Haemoproteus* Kruse, 1890, *Saurocytozoon* Lainson & Shaw, 1969, *Haemocystidium* Castellani & Willey, 1904 and seven other genera causing malaria or similar diseases in vertebrates. Only the first four (*Plasmodium*, *Haemoproteus*, *Haemocystidium* 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 (Davies & Johnston, 2000).

1.8.1.1.1 The genus *Plasmodium* Marchiafava & 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 an agamid lizard (*Agama agama*) and scincid lizard in

Africa. In the same year, Aragão & Neiva (1909) described two species, *Plasmodium diploglossi* Aragão & Neiva, 1909 and *Plasmodium tropiduri* Aragão & Neiva, 1909 from anguid and tropidurid lizards in Brazil. Until the 1960s species descriptions were slow, when Garnham (1966) listed 29 species. In the early 1990s Telford (1994) recognized 87 taxa. 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 (Table 1.2). 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. The distribution of only a few such malaria populations are known, and it is concluded that at least some parasite-host associations are ancient.

1.8.1.1.2 Reptile *Plasmodium* life cycles and vectors

Most published papers on reptile *Plasmodium* were of a taxonomic nature and until the early 1970s the complete life cycle, especially the sporogonic stage in a haematophagous invertebrate was unknown. The first evidence of sporogony (an oocyst) was found by Huff (1941) in the midgut of *Aedes aegypti*. Subsequently, several attempts were made to infect mosquitoes with saurian *Plasmodium*, but were all unsuccessful. Later, Baker (1961) was able to induce four *Aedes* species to feed on *Agama agama* infected with *Plasmodium giganteum* or *P. agamae*. The results were surprising, and only micro and macro gametocytes and ookinetes were seen in *A. aegypti* with no further evidence for sporogonic development for these parasites. Jordan (1964) showed that four species of mosquitoes could not be infected from feeding on *Plasmodium floridense* infected lizards (*Sceloporus undulatus* and *Anolis carolinensis*). Some positive results were found, however. Of 80 *Aedes aegypti* that fed on infected *S. undulatus*, one had an oocyst in the midgut, and one of 150 *C. quinquefasciatus* had 3 oocysts in the gut contents. One unidentified, small, dark, *Culex* sp. that fed on an infected lizard had 70 oocysts in the gut. In spite of these positive results, no sporozoites were found in the salivary glands by Jordan (1964). The exciting findings of Ayala (1970a) showed complete sporogonic development of *Plasmodium mexicanum* Thompson & Huff, 1944 in *Lutzomyia vexator* (Coquillett, 1907), and *L. stewardi* (Diptera: Phlebotominae). Evidence that these sandflies could transmit lizard malaria was confirmed when Ayala & Lee (1970) successfully transmitted the parasites to uninfected,

wild, juvenile lizards by means of inoculation. However, it was Klein et al. (1987) that first demonstrated that sandflies, after sporogony, could infect lizards by means of a bite. The vector list for saurian malaria grew when Petit et al. (1983) showed that the European fly *Culicoides nubeculosus* (Meigen, 1830) (Diptera: Ceratopogonidae) produced mature oocysts containing sporozoites derived from the African *Plasmodium agamae* Wenyon, 1909. Fialho & Shall (1995) confirmed that the sandfly *L. vexator* is the vector of *P. mexicanum* by feeding crushed sandfly gut contents containing oocysts to a lizard; this produced an infection in 14 days.

1.8.1.1.3 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, although Telford (2009) continues to recognize this genus (see Table 1.2). 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).

1.8.1.1.4 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. Landau et al. (1973) obtained mature oocysts containing sporozoites in *Culex pipiens*, but no sporozoite invasion of the salivary glands was observed. According to Telford (2009), *Culex pipiens* is probably not the natural host for *Saurocytozoon tupinambi*. Petit et al. (1983) and Klein (1985) reported completion of sporogony without oocyst rupture and salivary gland invasion in saurian plasmodiid species where unsuitable invertebrate hosts were involved.

1.8.2 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) named four families with the order, one of these, the Haemohormidiidae Levine, 1984, having a genus found in reptiles (*Sauroplasma* Du Toit, 1937).

1.8.2.1 Family Haemohormidiidae Levine, 1984

Members of this family undergo merogony, binary fission (Pienaar, 1962) and budding. The nucleus lacks an endosome or nucleolus and fish, reptiles and birds are hosts. Vectors are unknown (Davies & Johnston, 2000).

1.8.2.1.1 The genus *Sauroplasma* Du Toit, 1937

Type species: *Sauroplasma thomasi* Du Toit, 1937 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 (1937) 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. Telford (2009) noted that *Sauroplasma* infections have been documented in the blood of lizards from Africa, Europe, Asia and the Americas representing the families Cordylidae, Scincidae, Agamidae, Chamaeleonidae, Gekkonidae, Teiidae and Polychrotidae. These infections can certainly be overlooked as parasitic infections due to the resemblance to vacuoles in the cytoplasm of the host red blood cell. An example of this was when Uilenberg & Blanc (1966) reported a *Sauroplasma* species from a gecko (*Uroplatus fibriatus* (Schneider, 1797)) in Madagascar. When Frank (1974) re-examined the type material of the above mentioned species he was convinced that these were vacuoles and other artifacts that were mistaken for parasites. According to Telford (2009), any remaining doubt should have been resolved that *Sauroplasma* is parasitic in nature when Alberts et al. (1998) showed electron micrographs of *Sauroplasma* in erythrocytes of Cuban iguanas (*Cyclura nubila*). These infections were apparently not vacuoles or artifacts, but indeed parasitic infections that showed some similarity to piroplasms and Telford (2009) is of the opinion that their place in the piroplasmorids appears justified. Brygoo (1963) documented the presence of *Sauroplasma* in Madagascar where he illustrated a *Sauroplasma* infection in the blood of *Zonosaurus madagascariensis* and in the chameleon, *Chamaeleo verrucosus*. The taxonomic position of these infections became complicated when Svahn (1976) placed *Sauroplasma boreale* described from Swedish and Danish lizards in the Theileridae, because no reproduction was observed in the erythrocytes from these lizards. A further complication arose when Svahn (1976) considered the "budding" stages described by Du Toit (1937) and later Pienaar (1962) as double infections. Telford (2009) is of the opinion that *Sauroplasma* has little affinity with *Babesia* and *Theileria*, the mammal piroplasms. According to the sparse literature and in my own findings, *Sauroplasma* infections appear to have little visible effect on the host itself.

These parasites can possibly be transmitted by the bite of ticks and mites. According to Pienaar (1962) the prostigmatic mites (*Zonurobia circularis* Lawrence, 1935) that infest 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* Pienaar, 1962. 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 of *Cordylus polyzonus* A. Smith, 1838 from the Free State that I collected in my undergraduate years. TEM studies in my preceding MSc. study (Van As, 2003) confirmed the findings of Davies & Johnston (2000). More work needs to be done in order to understand the nature of these structures, their transmission and effects on the host), and further observations are made in this thesis. The issues of budding or double infections are also not resolved.

1.9 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 (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 (Davies & Johnston, 2000). Viral-like infections in reptiles probably include *Sauromella*, an infection of uncertain status (see Johnston, 1975), *Sauroplasma* (above), recently classified with the Protozoa as a piroplasm, and *Serpentoplasma* which is likely a similar infection found in the erythrocytes of snakes.

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 more 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 anaemia. 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 anaemia and excessive erythropoitic activity.

1.10 Reptilian filarial nematodes

1.10.1 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 juveniles 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). According to Bartlett (1986), the filaroid subfamily Dirofilariinae are known to be parasites of mammals, birds and reptiles. Anderson & Bain (1976) listed the genus *Foleyella* Seurat, 1917 as filarial parasites of reptiles representing the Dirofilariinae.

1.10.2 Family Oswaldofilariidae Chabaud & Choquet, 1953

This family is represented by three subfamilies (Oswaldofilariinae, Icosiellinae and Lemdaninae). Gibbons (1989) described *Befilaria pseudocordyli* Gibbons, 1989 as representing the Oswaldofilariinae from *Pseudocordylus microlepidotus* in the Western Cape. There is doubt about this species, since it was described from a *P. microlepidotus* specimen housed in the London Zoo.

1.10.2.1 Subfamily Splendidofilarinae Chabaud & Choquet, 1953 (Onchocercidae)

Three genera in this subfamily are known to be parasites of saurians (Hering-Hagenbeck et al. 2000). *Madathamugadia* Chabaud, Anderson & Brygoo, 1959, comprises two Madagascan species (Chabaud et al. 1959) and four species from the Ethiopian region (Bain et al. 1993), infecting three families of lizards, Cordylidae, Scincidae and Gekkonidae. *Madatahamugadia ineichi* Bain, Wanji, Petit, Paperna & Finkelman, 1993 was described from what Bain et al. (1993) identified as *Pseudocordylus microlepidotus melanotus* from the Golden Gate Highlands National park in the Eastern Free State. The host for this Splendidofilarine nematode is more likely *P. melanotus* or *P. subviridis* (see Chapter 5). A life cycle for *Madathamugadia hiepei* Hering-Hagenbeck, Boomker, Petit, Killick-Kendrick & Bain, 2000 was described in a gecko, *Pachydactylus turneri* (Gray, 1864) and a sandfly *Plebotomus dubosci* Neveu-Lemaire, 1906 from a colony coming from Senegal (Hering-Hagenbeck et al. 2000).

1.11 Mites as possible vectors for blood parasite infections

Mites are vectors of haemogregarines of the genus *Karyolysus* (see above) and these arthropods receive some attention in the current research. The first studies on mites of lizards were by Peters (1849) who erected the genus *Pterygosoma* Peters, 1849 for a mite species infesting *Agama mossambica*. Mégnin (1878) created the genus *Geckobia* Mégnin, 1878 for a mite from *Tarentola* (Sauria: Gekkonidae). Later, Trägardh (1905) contributed another genus from a geckonid lizard, *Platydactylus.* The same year saw the emergence of a new genus *Geckobiella* Banks, 1905 named by Banks (1905) from an iguanid lizard. The systematic paper of Hirst (1926) united the genera *Eupterygosoma* Trägardh (1905) with *Pterygosoma* and included the genus *Hirstiella* Berlise, 1920 from an unknown host. Vitzthum (1931) included the above mentioned five genera of mites in the family

Pterygosomidae. Later, Lawrence (1935, 1936) in his extensive work on mites of South African lizards regarded these genera as members of this same family and described three new genera *Zonurobia* Lawrence, 1935, *Scaphotrix* Lawrence, 1935 and *Ixodiderma* Lawrence, 1935 from lizards of the Cordylidae. According to Lawrence (1935), mites from the suborder Prostigmata parasitise the lizard families Gekkonidae, Agamidae, Cordylidae and Gerrhosauridae while representatives from the suborder Mesostigmata parasitise the lizard families Lacertidae and Scincidae. These two suborders are phylogenetically and morphologically divergent (Lawrence, 1935).

1.12 Mosquitoes as possible vectors for blood parasite infections

It is well known that mosquitoes are vectors for a spectrum of important diseases in vertebrates. Some haematophagous dipterans have been shown to be vectors for haemoparasites of reptiles, at least in experimental situations (see above sections). A diversity of mosquitoes is known from the Eastern Free State (see Jupp, 1996), and the present study aimed to investigate the role of four species of these as potential vectors of lizard haematozoans found in the region.

Culex (*Afroculex* Danilov) *lineata* Theobald, 1912 is the only representative of the subgenus *Afroculex* and according to Jupp (1996) has been recorded only in South Africa (Kwa-Zulu Natal, the Free State and Gauteng). Currently, nothing is known of the general biology of this species. Adult female mosquitoes of this species were observed feeding on *P. melanotus* in the current study and they eventually proved to have haemogregarine stages in their gut contents and elsewhere (see Chapter 3).

Three other mosquito species were investigated as possible viable vectors for haemogregarine infections in *P. melanotus* including *Culex andersoni bwambanus* Edwards, 1941, *Culex* (*Culex*) *pipiens* Linnaeus 1785 and *Culiseta* (*Allotheobaldia*) *longiareolata* Macquart 1838.

Culex andersoni bwambanus Edwards, 1941 occurs throughout South Africa and according to Hopkins (1952) is distinguishable from other *Culex* species by having a sclerotic tooth near the apex of the siphon in the larvae. Specimens are known to occur in the Eastern Free State

highlands (Muspratt, 1955). Jupp (1996) listed this species distribution as the Cape Province, Kwa-Zulu Natal, Gauteng (formerly Transvaal) and Lesotho.

Culex (*Culex*) *pipiens* Linnaeus 1785 has a worldwide distribution (Mattingly et al. 1951) and according to Muspratt (1955) occurs in Madagascar and the whole of Africa, except in West Africa. Larvae are common in standing water with high organic content.

Culiseta (*Allotheobaldia*) *longiareolata* Macquart 1838 has a cosmopolitan distribution and is widely distributed throughout South Africa (Muspratt, 1955). According to Lewis (1943) adult females seldom feed on humans. Van Pletzen & Van der Linde (1981) demonstrated that starved females feed readily on pigeons.

1.13 Study Hypotheses

Relatively little is known of haemogregarine and malaria infections in lizards from South Africa (see Van As, 2003). Owing to the limited knowledge of these infections, it is hypothesised that:

- these haemoparasites, of several species, are generally more widespread among South African cordylid lizards than previously thought, and particularly in the Eastern Free State Highlands
- haemogregarine and malarial infections may cross host species boundaries, especially in crag lizard species exhibiting sympatric distribution.
- species of the haemogregarine genus *Hepatozoon* are not host species specific, but may be host genus specific among these crag lizards
- malarial infections of the genus *Plasmodium* occur across a wide altitudinal gradient in lizards from the same cordylid family.

Currently, limited information exists on the role of vectors for haemoparasite infections in lizards. It is also hypothesised that:

- mosquitoes, but not mites, may serve as the final hosts for *Hepatozoon* and *Plasmodium* infections in cordylid lizards from the Free State Highlands.

- scale mites that infest crag lizards may serve as vectors for lizard *Sauroplasma* and filarial nematode infections.

1.14 Study Aims and Objectives

The aims and objectives of this study were to:

- use isolated populations of crag lizards from the Eastern Free State Highlands as a study group, to broaden the baseline biodiversity knowledge of haemoparasites in these lizards
- employ this same group of crag lizards to describe new species of blood parasites, especially haemogregarines, on the grounds of morphological and morphometrical criteria
- use transmission electron microscopy as an aid to gain a better understanding of light microscopy observations, thus contributing to knowledge of the fine structure of *Hepatozoon, Plasmodium* and *Sauroplasma* infections in crag lizards
- use impression, squash and histological techniques coupled with confocal microscopy to elucidate the development of *Hepatozoon s*pp. in the organs of *Pseudocordylus s*pp.
- capture wild mosquitoes, and undertake experimental methods to raise clean, laboratory mosquitoes in order to investigate their role as haemoparasite vectors
- use squash and histological methods to investigate the role of mites as vectors of crag lizard haematozoans
- employ this same sample of crag lizards to investigate and record briefly the presence of filarial nematodes in them.

The current research includes new records of blood parasites from crag lizards collected during surveys of sites in the Eastern Free State Highlands. Five species of haemogregarines are described from three species of lizards (*Pseudocordylus melanotus, Pseudocordylus subviridis* and *Pseudocordylus langi*), as well as the sporogonic stages of two of these haemogregarines in natural and laboratory-raised vectors. One lizard malaria, having some features of two previously described species, is recorded from two species of crag lizards (*P. melanotus* and *P. subviridis*). So-called *Sauroplasma* infections observed in almost all

specimens of *P. melanotus, P. subviridis* and *P. langi* are examined and presented as new locality records. Two (possibly three) species of previously undescribed microfilaria are also noted in the blood of crag lizards.

1.15 Layout of thesis

Chapter 1 is a general introduction to crag lizards and a taxonomic overview of haemoparasites in reptiles, especially in lizards. It records the saurian haemoparasites and the life cycles of some genera of parasitic blood protozoans, are highlighted. The role of mosquitoes and mites as possible vectors of haematozoans are also introduced. Finally, hypotheses, aims and objectives are set.

Chapter 2 emphasises the study areas where material was collected as well as specific methods used for this research.

Chapter 3 deals with five species of suspected *Hepatozoon,* from three crag lizard species *Pseudocordylus melanotus* from Platberg, *Pseudocordylus subviridis* from the North Eastern Drakensberg and *Pseudocordylus langi* from the Drakensberg escarpment. It reemphasises the taxonomy of *Hepatozoon* species, and focuses on the almost complete life cycles for *Hepatozoon* sp. A and *Hepatozoon* sp C in their lizard hosts and their mosquito definitive host, using a variety of methods. It also aims to demonstrate the possibility of 3 mosquito species serving as vectors for *Hepatozoon* spp. New infection and distribution records for the above infections are demonstrated.

Chapter 4 reports on morphological aspects of a species of saurian malaria from two species of crag lizards *Pseudocordylus melanotus* from Platberg, and *Pseudocordylus subviridis* from the North Eastern Drakensberg. Throughout this chapter new infection and distribution records for the above infections are illustrated.

Chapter 5 deals with the large numbers of so-called *Sauroplasma* and notes filarial nematode infections found in the blood of all three lizard hosts with new distribution records.

Chapter 6 is divided in two parts. The first involves a morphological overview of the different cell types found in lizard peripheral blood, focussing on leucocytes and thrombocytes, as well as differential leucocyte and thrombocyte counts. This chapter also attempts to correlate using statistical methods, leucocyte types and loads with parasite loads, hosts, and various environmental factors. The second section further examines mites and mosquitoes as possible definitive hosts and vectors for the blood infections of crag lizards and emphasises the general experimental approaches and findings.

Chapter 7 re-examines the hypotheses set in Chapter 1, arguing whether to accept or reject them. It draws general conclusions concerning the haematozoan infections, and their life cycles in the three species of crag lizards and invertebrates studied in the thesis. It also comments on the relatively modern history of molecular methods employed to identify apicomplexan parasites and solve problems of relatedness between them.

Chapter 8 lists the references used throughout this study

Some sections of this thesis were presented at conferences with published abstracts:

- VAN AS, J. 2006. Intraerythrocytic parasites from reptiles of the Free State and Lesotho Highlands. Paper presented at the 8th Herpetological Association of Africa Symposium, Potchefstroom, South Africa.

- VAN AS, J. SMIT, N. J. HEIDEMAN, N. & DAVIES, A. J. 2007. Intraerythrocytic parasites from crag lizards of the Free State Highlands. Zoological Society of Southern Africa, 8-11 July, Potchefstroom, South Africa.

- VAN AS, J. 2008. Haemogregarines (Apicomplexa: Adeleorina) of *Pseudocordylus* spp. from the Free State Highlands. Herpetological Association of Africa. Hosted by Dept. Zoology & Entomology, Qwaqwa Campus, University of the Free State, South Africa, at Sterkfontein Nature Reserve.

- VAN AS, J. SMIT, N. J. HEIDEMAN, N. J. L. & DAVIES, A. J. 2010. Haemogregarines (Apicomplexa: Adeleorina) of *Pseudocordylus* spp. and their possible definitive hosts from the Free State highlands. *Journal of the African Veterinary Association* **81:**187.

CHAPTER 2

STUDY SITES, MATERIAL AND METHODS

In a preceding study by the author (Van As, 2003) a survey of reptile blood parasites in the Free State province revealed a number of reptile species which could serve as host models for more detailed study of haemogregarine and malaria parasites. Members of the Cordylidae in the Free State, in particular, were found to have high parasitaemias and often mixed infections of blood protozoans and these were the lizards selected for the current research.

2.1 Study sites

Two study sites in the Eastern Free State were chosen on the basis of high cordylid diversity, especially among crag lizards. These were Platberg (Fig. 2.1, 2.2 A, 2.3 A & C), an inselberg, and part of the Drakensberg Massif about 55km northeast from the main Drakensberg range, and the Sentinel trail area (Fig. 2.1, 2.2 B & 2.3 B & D) of the Drakensberg, which is a favourite tourist attraction and one of the most breathtaking hiking trails in South Africa. These two study sites were chosen because they are disjunct, providing a clear boundary between the *Pseudocordylus melanotus* population sampled at Platberg and the microsympatric *Pseudocordylus subviridis* and *Pseudocordylus langi* populations from the Sentinel trail. These study sites were also chosen because both study sites are situated above the 2000m contour line (Fig. 2.2 A & B), and according to Mucina & Rutherford (2006), these unique conditions above 2000m lead to high levels of endemism in the fauna and flora. Lizards were collected opportunistically at these 2 study sites over a period of 5 years (2005 -2010); mostly during summer months (see Appendices 1 - 3 for specific dates).

2.1.1 Platberg

The Maluti-Drakensberg mountain range is part of the Karoo supergroup with basaltic peaks over 3000m above sea level. This mountain range and its associated inselbergs are represented by four species of the *Pseudocordylus melanotus* complex (Bates, 2005), which

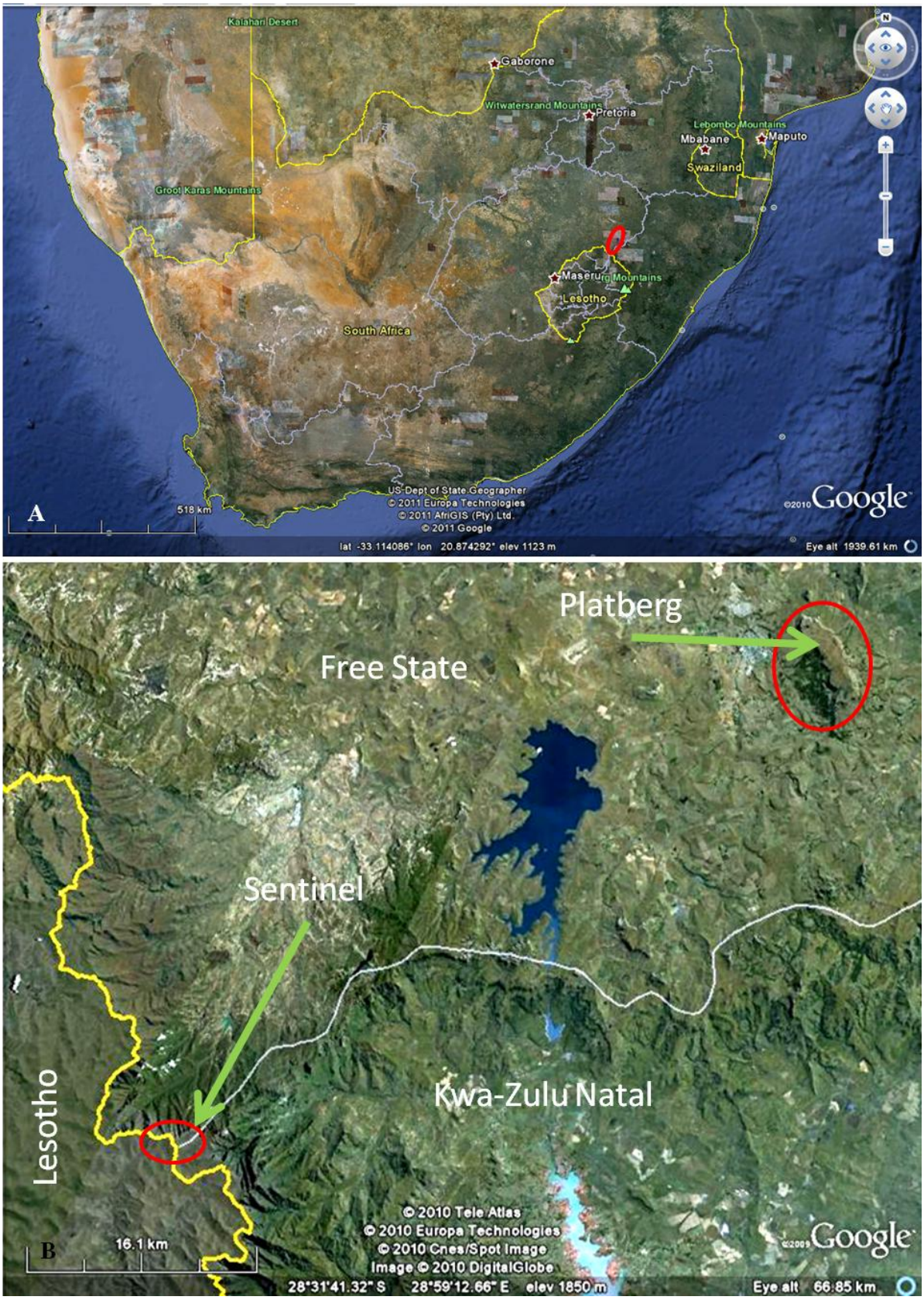

Figure 2.1 (A & B) Google Earth images of South Africa showing the provinces and study sites utilized in this research indicated in red circles. **(A)** Google Earth image of South Africa showing the provinces and the combined study sites in red circle, **(B)** Google Earth image of a portion of the Eastern Free State showing the two disjunct study sites in red circles. Scale bars: **(A)** = 518km **(B)** = 16.1km.

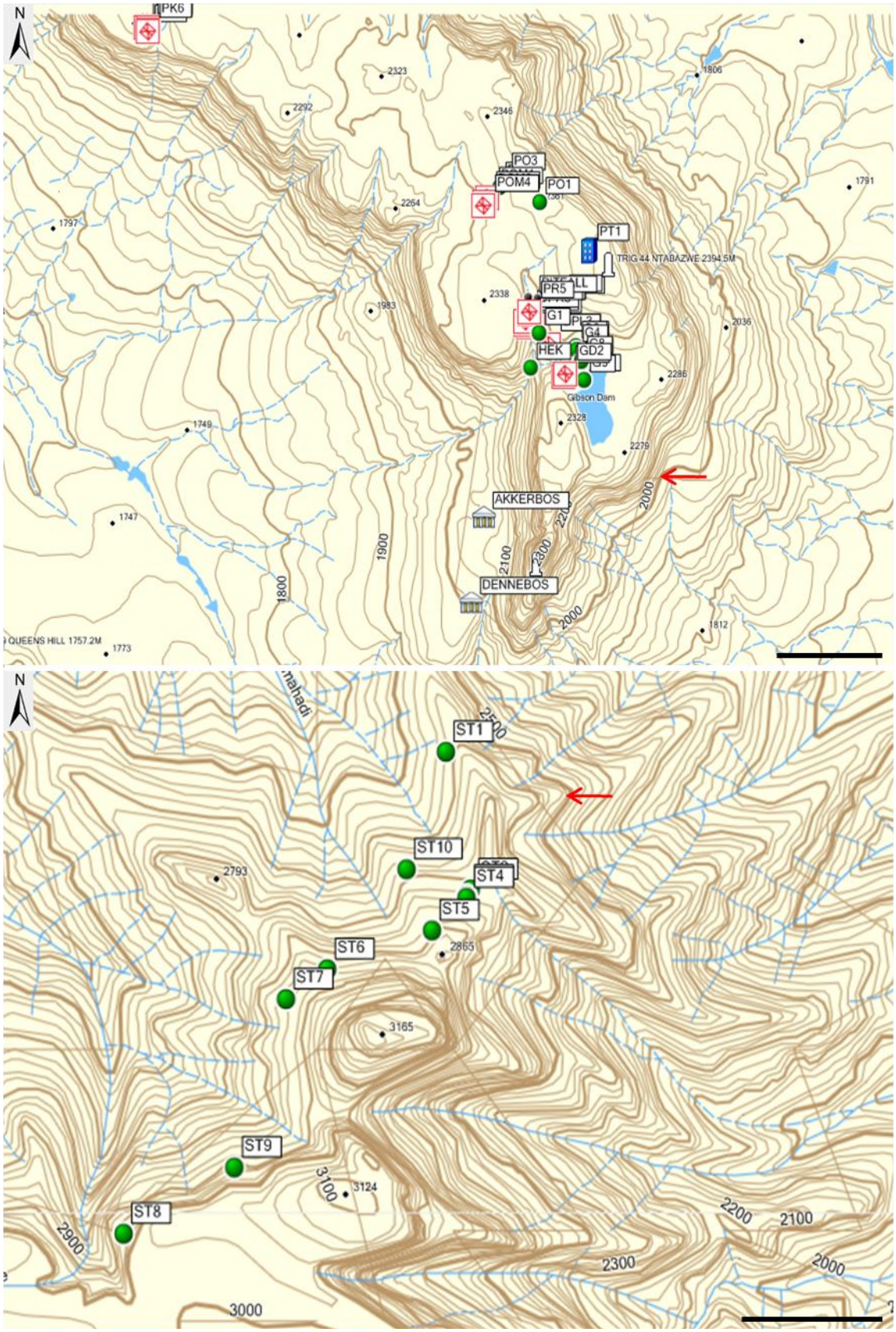

Figure 2.2 (A & B) Topographical maps (Mapsource®) of the two study areas showing GPS localities where lizards were collected for blood sampling. **(A)** South Eastern section of Platberg, with collection localities. Red arrow indicates 2000m contour line. **(B)** North Eastern region of the Sentinel area with collection localities. Red arrow indicates the 2500m contour line. Both study sites were above 2000m. Scale bars: **(A)** = 1000m **(B)** = 500m.

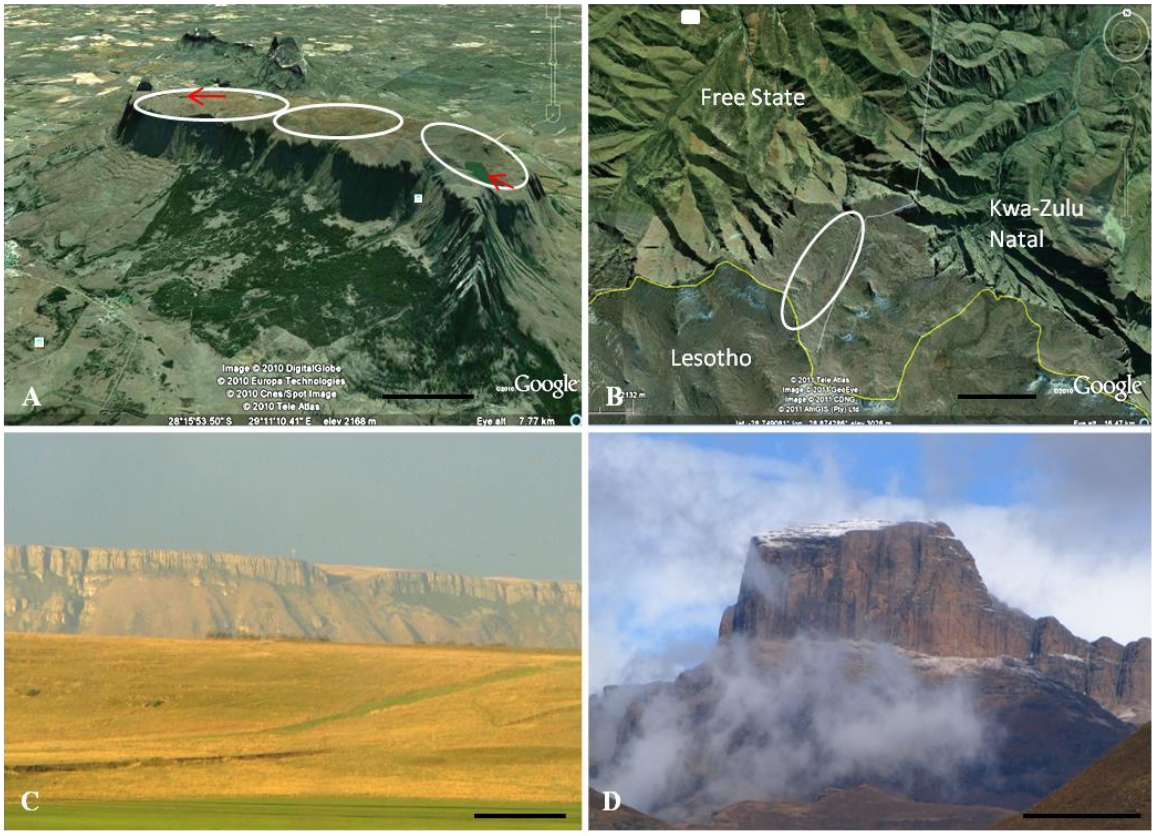

Figure 2.3 (A - D) Google Earth images and photographs of sampled areas. Google Earth images with study sites indicated by white circles, **(A)** Platberg, **(B)** Sentinel. **(C & D)** Photographs of Platberg taken in summer **(C)** and Sentinel taken in winter **(D)**. Red arrows in **(A)** indicate dams. Scale bars: **(A)** = 1000m **(B)** = 1500m **(C)** = 500m **(D)** = 250m.

includes *Pseudocordylus melanotus*, *Pseudocordylus subviridis*, *Pseudocordylus langi* and *Pseudocordylus spinosus.* Platberg is one of these inselbergs situated about half way between Johannesburg and Durban on the N3 Highway overlooking the town of Harrismith in the Eastern Free State (Brand et al. 2009). Platberg is situated approximately 55km north of the main Drakensberg/Maluti range and has altitudinal ranges from 1900m to the highest peak in the area of 2394.5m. The plateau is L- shaped with a surface area of about 3000ha and is predominantly grassland (Brand et al. 2009). This inselberg consists of capped igneous rock (Norman & Whitfield, 1998) with a definitive separation between the Stromberg volcanism on top and Clarens sandstone lower down. Two dams are situated at the top of Platberg (Fig. 2.3 A red arrows). Numerous outcrops are found throughout the plateau which constitutes perfect habitats for rock dwelling reptiles, especially *Pseudocordylus melanotus* (Fig. 2.4 A).

2.1.2 Sentinel trail

This study site consists of a well known hiking trail in the Free State province which leads up to the summit (~3200m) adjacent to the Royal Natal National Park (in Kwa-Zulu-Natal). The central feature of the Sentinel is a major free standing peak, accompanied by the Inner tower, Eastern Buttress and Devil's Tooth. The summit plateau on the Free State side is adjacent to the escarpment edge, the border of Lesotho to the south and the border with Kwa-Zulu-Natal on the East (Fig. 2.3 B). This summit area (2805 - 3048m) of the study site is the only locality known for *Pseudocordylus langi* (Fig. 2.4 C) where it, according to Bates (2005) forms a microsympatric population with *Pseudocordylus subviridis* (Fig. 2.4 B). *Pseudocordylus subviridis* has a wide altitudinal distribution, and it was collected along the trail area from the summit down towards the car park.

2.2 Lizard collection procedure

Pseudocordylus melanotus were located at Platberg (permit number: BBB002-00032-0035) near the town of Harrismith in the Free State (see above). Specimens were collected from various rock outcrops across Platberg (Fig. 2.5 A), from the western to eastern side. Collections of lizards were done mostly directly by hand, but sometimes a noose constructed

Figure 2.4 (A - C) Hosts collected by hand during fieldwork at Platberg and Sentinel. **(A)** *Pseudocordylus melanotus* from Platberg. **(B)** *Pseudocordylus subviridis* from the Sentinel trail area. **(C)** *Pseudocordylus langi* collected from the chain ladder area of the Sentinel trail in the North Eastern Drakensberg. Scale bars: **(A - C)** = 30mm.

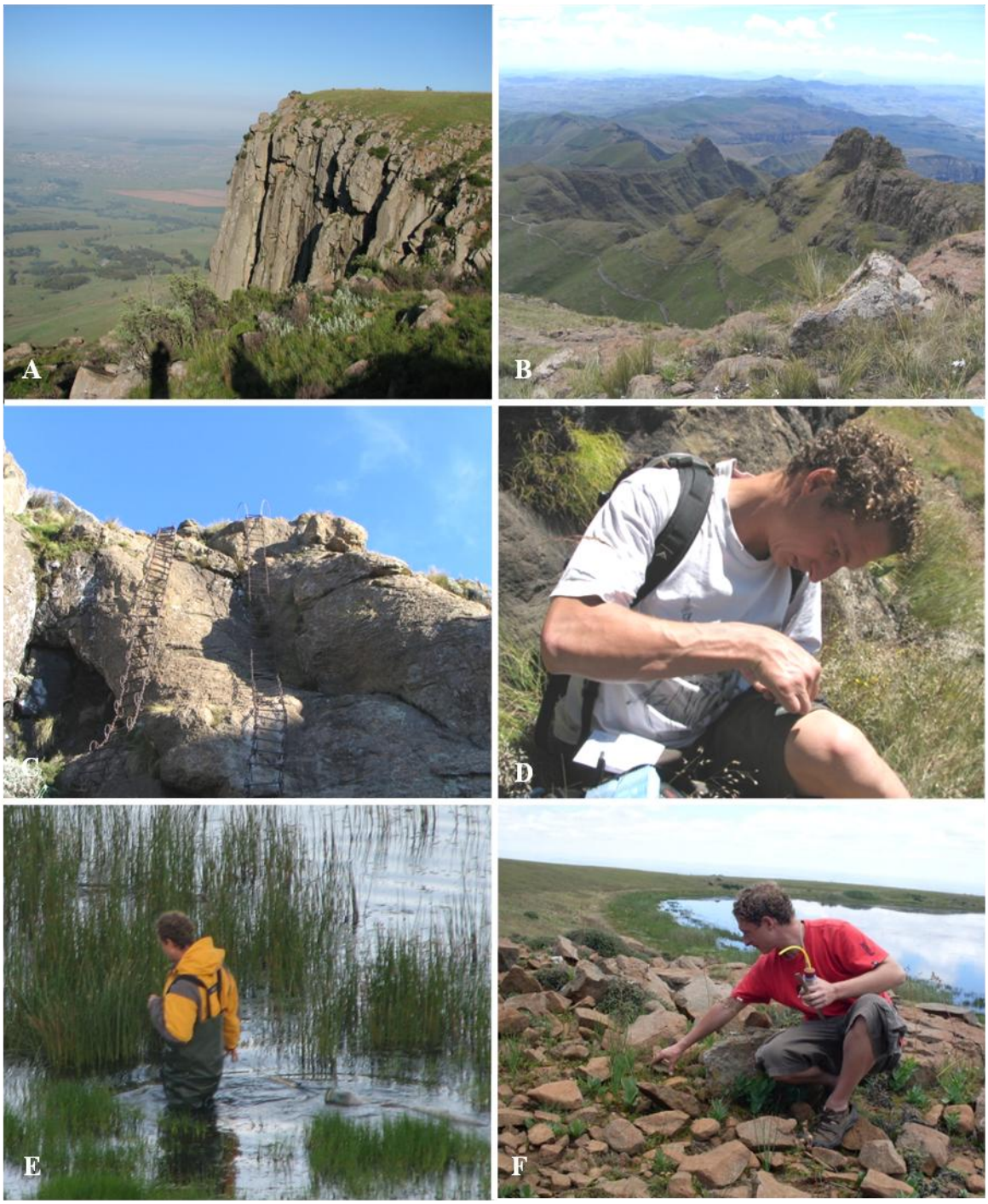

Figure 2.5 (A) Photographs of rocky outcrops at Platberg where *P. melanotus* was collected and studied, and **(B)** Sentinel trail area where *P. subviridis* was found. Further images of: **(C)** Chainladders (2805 - 3048m) on the Sentinel trail, the collection site for *P. langi*. **(D)** The author sampling blood and mites from lizards; **(E)** Collection of wild mosquito larvae at Platberg; and (**F)** Collection of female, wild *Culex* (*Afroculex*) *lineata* specimens on lizards in rock cracks.

from 1mm copper tubing with an attached looped nylon thread was used to collect specimens lodged deeply in rock cracks. *Pseudocordylus subviridis* and *P.langi* were collected while hiking along the Sentinel trail up to the summit plateau in the vicinity of the border of Free State, KwaZulu-Natal and Lesotho. Most collections of *P. subviridis* were carried out opportunistically on the trail (Fig. 2.5 B) at various altitudinal gradients (permit number: BBB002-00032-0035). At the chain ladders (from 2900 to 3100m), *P. langi* was collected near to its type locality (Fig. 2.5 C).

Collections of *P. langi* were carried out mostly by hand, but sometimes when individuals were out of reach, a thick rubber band was stretched and then released rapidly towards the lizard to dislodge it from the steep cliff face. Such lizards were then caught by hand in the air with a 100% success rate, and without harm. Fifteen representative host specimens of *P. subviridis*, 40 of *P. melanotus* from each population (male, female and juveniles) and seven *P. langi*, (male and female) were measured (Table 2.1.). Scale counts were performed on all captured specimens to identify the species using De Waal (1978) and Bates (2005). Blood samples and ectoparasitic mites (details below) were taken, where possible, from each specimen. A drop of Betadine (Adcock Ingram Ltd.) was placed on the site of blood collection to prevent secondary infections. Most lizards were released at their site of collection within 10 min of measuring, blood collection and sampling for mites. A few were used in laboratory experiments (see below). A Garmin E-trex legend (2005 model) G.P.S recording instrument was employed to record the sites where the lizards were captured.

2.3 Tissue collection procedure

2.3.1 Blood smear preparations

A drop of peripheral blood was taken from each lizard by toe clipping during daylight. This provided enough blood for 1 to 3 slides. Heart blood was taken from a lizard specimen by micropipette immediately post mortem. All blood smears were made (Fig. 2.5 D) on clean microscope slides, air dried, fixed in absolute methanol for 1 minute and stained in a 9:1 solution of buffered Giemsa (Gurr® Improved R66 solution) for 30 minutes in the laboratory. Blood smears were stored in dust free boxes until scanned individually for infections using a Nikon Eclipse photomicroscope. Measurements of blood parasites (Fig. 2.6 A - G) were taken using a 100 x oil immersion

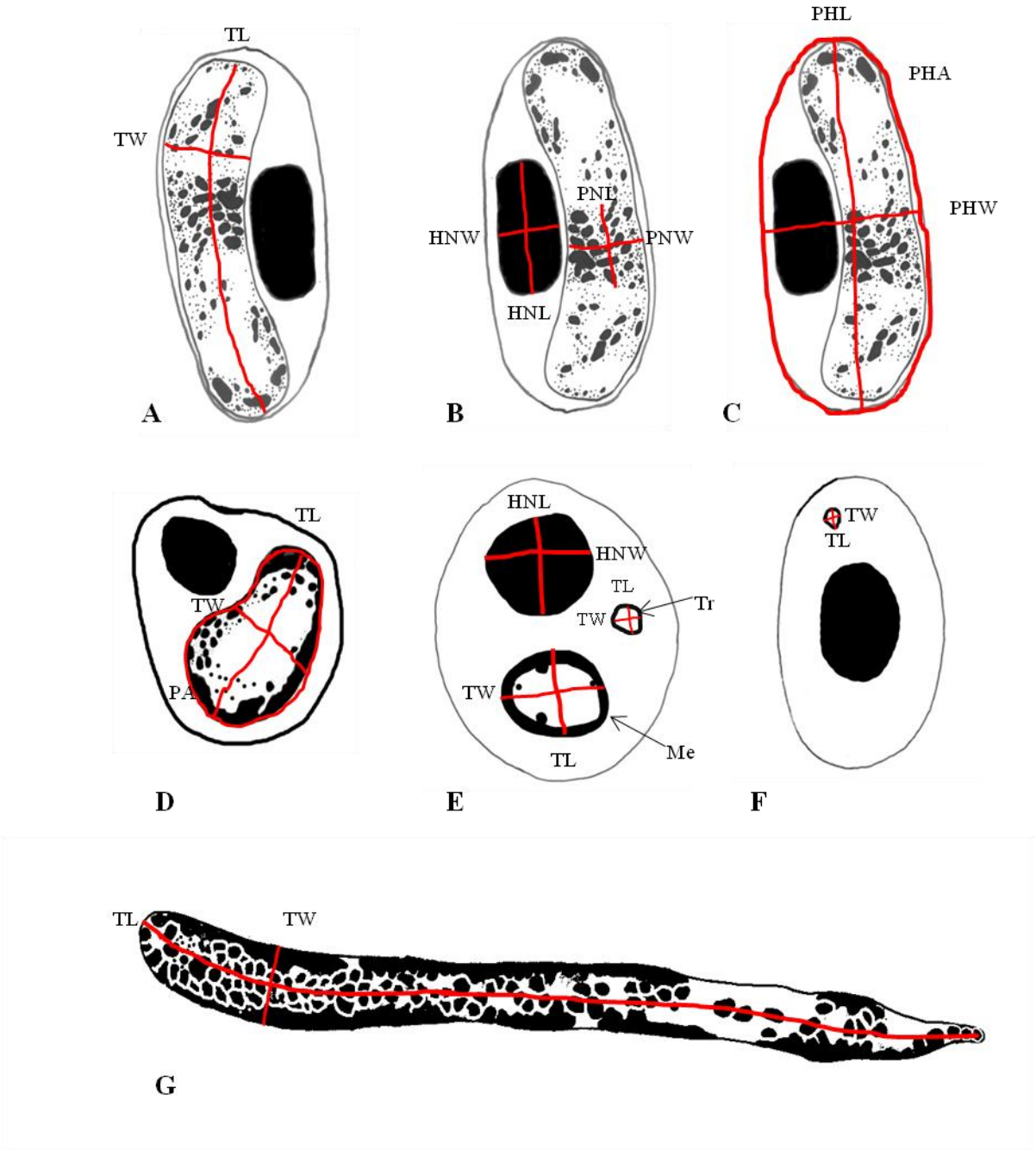

Figure 2.6 (A - G) Illustrations (not drawn to scale) of parasites and their measurements (red lines) taken for: **(A - C)** haemogregarines and parasitised host erythrocytes in **(B & C)**. **(D)** Malaria gametocytes, **(E)** malaria trophozoites, (Tr) and meronts (Me) in the cytoplasm of host cells; **(F)** so-called *Sauroplasma*; and **(G)** filarial nematodes, from stained blood smears. Abbreviations: **(A)** TL: Total length, TW: total width of haemogregarines; **(B)** PNL: Parasite nucleus length; PNW: parasite nucleus width; HNW: Host cell nucleus width; HNL: Host cell nucleus length**; (C)** PHL: Parasitised host cell length; PHW: Parasitised host cell width; PHA: Parasitised host cell area; (**D)** TL: Total length; TW: total width; PA: parasite area of malarial infection; (**E)** HNL: Host cell nucleus length; HNW: Host cell nucleus width; TL: Total length; TW: total width of meronts (Me) and trophozoite (Tr) respectively. **(F)** TL: Total length; TW: total width of so-called *Sauroplasma* infection. **(G)** TL: Total length; TW: total width of filarial nematode.

objective on a Zeiss Axioplan 2 photomicroscope using AxioVision Release 4.3 (11 - 2004) software, calibrated to a stage micrometer.

Table 2.1 Summary of snout vent lengths (AVG ± STDEV (MIN - MAX) mm) of three species of crag lizards used in the study.

Species	**Snout-vent length = AVG ± STDEV (MIN - MAX) mm**	**Number measured (N)**
Pseudocordylus melanotus	98.47 ± 17.13 (50 - 150)	40
Pseudocordylus subviridis	100.54 ± 6.41 (91.5 - 113)	15
Pseudocordylus langi	92.41 ± 4.15 (87 - 100)	7

Measurements were calculated ± Standard Deviation (SD), where appropriate. In addition to the standard photos taken, DIC (differential interference contrast) photos were taken using a Zeiss Axiophot photomicroscope.

2.3.2 Liver and lung smear preparations

An adult male *P. melanotus* specimen was euthanized with a mixture of 0.5ml Euthapent (Kyron Laboratories (Pty) Ltd.) and 1ml tapwater (see below). Small blocks of liver and other tissues were cut and smeared, or impressed, on clean microscope slides. Slides were fixed, stained and examined in the same way as the blood samples (see above).

2.4 Routine histology

Liver and lung tissue from the same specimen as above were removed and fixed in 10% buffered formalin and washed in tap water for 24 hours. Samples were dehydrated in 30%, and then a 50 - 90% series of ethanol concentrations for 60 minutes in each. Samples were transferred to 100% ethanol twice for 45 minutes each. Tissue samples were treated with two fresh changes of xylene for clearing. Tissue samples were embedded in paraffin wax (Paramat), and blocks were hardened at 0°C. Ribbons of sections were cut using a wax microtome (Reichert-Jung), placed on slides, and flattened on distilled water on a 30°C

hotplate. Slides were stained using standard Haematoxylin and Eosin (H&E) methods from Galigher & Kozloff (1964) and Humason (1979). Large cover slips were mounted over the stained sections with Entellan mounting medium. Sections were examined and photographed as above.

2.5 Ultramicroscopy

2.5.1 Transmission electron microscopy

2.5.1.1 Blood and tissue samples

One adult male lizard (*P. melanotus* from Platberg) was collected by hand and taken to the laboratory (permit number: BBB002-00032-0035). The lizard was euthanased and liver, heart, spleen and blood samples were taken in the same way as described above. Tissue samples were cut into 2mm² sections and four to five drops of fresh blood, and the tissue samples were dropped separately into 2.5% glutaraldehyde (10ml in 90ml 0.2M Sorensen's phosphate buffer at pH 7.2) and fixed for one hour. Glutaraldehyde-fixed blood was then centrifuged at 10 000rpm and the pellet and tissue samples were 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 and tissue pieces were rinsed in buffer and then dehydrated in a graded series (30 - 100%) of ethanol solutions. The final ethanol for dehydration (100%) was dried over a 4Å molecular sieve. Ethanol was removed by transfer of the samples (separate blood pellets and tissue pieces) through three changes of propylene oxide (1,2 epoxy propane). The pellets and tissue pieces were then left for 12 hours in a mixture of one volume propylene oxide mixed with three volumes of Agar 100 resin (Agar Scientific Ltd.). The epoxy resin mixture was made by mixing the following components: 23 ml Agar 100 resin, 12 ml methyl nadic anhydride and 15 ml dodecanyl succinic anhydride, with dropwise addition of 1.5 ml benzyl dimethyl amine during mixing (Agar Scientific Ltd.)

Blood pellets and tissue samples were then 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 according to Spurr (1969) was polymerised at 60 degrees Celsius for 48 hours. Sections, showing pale gold interference colours were cut from the blocks of embedded blood and tissue, using glass knives on a Reichert-Jung 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 Reynolds' lead citrate solution, washed with 0.02M sodium hydroxide solution followed by distilled water, before examination with a JEOL 1010 transmission electron microscope operated at 80 - 100 kV. Digital images were captured with a MegaView II side mounted digital Olympus camera with accompanying iTEM software.

2.5.2 Confocal microscopy

Giemsa stained slides were viewed on a Nikon Confocal C1 TE2000 - E inverted microscope with the aid of the following Lasers: 20mW 633nm RED He - Ne, 5mW 543nm GREEN He - Ne and 200mW 454 - 676nm BLUE Ar - ion, and the following lenses: 10X CFI Plan Fluor, 40X CFI Plan Fluor, 60X CFI Plan Apo and 100X CFI Plan Apo. Digital images were captured on the Nikon Confocal C1 TE2000 - E inverted microscope camera package.

2.6 Possible blood parasite vectors

2.6.1 Mites

Adult and nymph scale mite specimens were collected from the skin of *P. melanotus* and *P. subviridis* and identified using Lawrence (1935). *Ixodiderma inverta* Lawrence, 1935 and *Ixodiderma pilosa* Lawrence, 1935 nymphs and adults were carefully loosened from the skin of *P. melanotus* and *P. subviridis* with a fine pincette. Chigger mite nymphs, *Sauracarella whartoni* Lawrence (1949) identified from Lawrence (1949) were also collected in the same way, where possible, from the same host species. Squash preparations were made by pressing the collected mites between 2 microscope slides and then drawing the slides apart. Squash preparations were fixed and stained as for blood smears (see above). Stained squash preparations were scanned with a Zeiss stereo microscope using the 40x objective. Photographs were taken with a Nikon Coolpix 4500 digital camera custom fitted to a Nikon Eclipse microscope.

2.6.2 Mosquitoes

During sampling, especially at night, mosquitoes were observed feeding on *P. melanotus* at various rock outcrops on the plateau of Platberg. These mosquitoes were collected with great difficulty using an aspirator (Fig. 2.5 F). Feeding mosquitoes identified as *Culex* (*Afroculex*) *lineata* (Theobald, 1912) by Prof Maureen Coetzee (from the Malaria Entomology Research Unit, School of Pathology, WITS University, South Africa) were sucked off the lizard using the aspirator and kept alive in separate plastic vials for transportation back to the laboratory. Special care was taken to link each mosquito captured to the lizard it had fed on, and thus to its matching lizard blood smear/s. Searches for the larvae of *C. lineata* were conducted in the dams (Fig. 2.5 E) close to where the adult mosquitoes were collected.

Adult, female engorged *C. lineata* specimens were subsequently kept alive for as long as possible in 30cm^3 mosquito rearing cages. Cotton balls soaked in 10% sucrose solution were provided as a food source. Specimens that died were immediately dissected and the abdominal contents, thorax and head were squashed in three separate areas between 2 microscope slides and subsequently fixed and stained as for blood smears (above).

2.6.3 Laboratory experiments with possible vectors

Additional, easily obtainable, mosquito eggs and larvae of *Culex* (*Culex*) *pipiens*, *Culiseta* (*Allotheobaldia*) *longiareolata* and *Culex andersoni bwambanus*, identified from Jupp (1996) were collected from freshwater ponds in the town of Harrismith and were fed a 50:50 mixture of Nestum (Nestlé) and brewer's yeast (Robertsons) (Fig. 2.7 A). Emerged adult specimens (Fig 2.7 C - E) and other specimens collected were pinned on 0.15mm minutent insect pins for identification (Fig. 2.7 F). Alternatively, upon emerging as adults, female mosquitoes were placed in a mosquito rearing cage with a *Hepatozoon*-infected *P. melanotus* specimen (Fig. 2.7 B). This lizard was provided with natural sunlight and water, and fed on laboratory bred mealworms (Coleoptera: Tenebrionidae) and kept at 25 - 30ºC (50 - 60% Relative Humidity (RH)). This particular lizard was collected on 06/02/2010 and blood smears were made immediately to confirm the infection, and thereafter on a weekly basis for four months to reconfirm the infection until 02/06/2010, when the lizard was dissected for further examination. Mosquitoes were fed on this lizard a week after its initial collection.

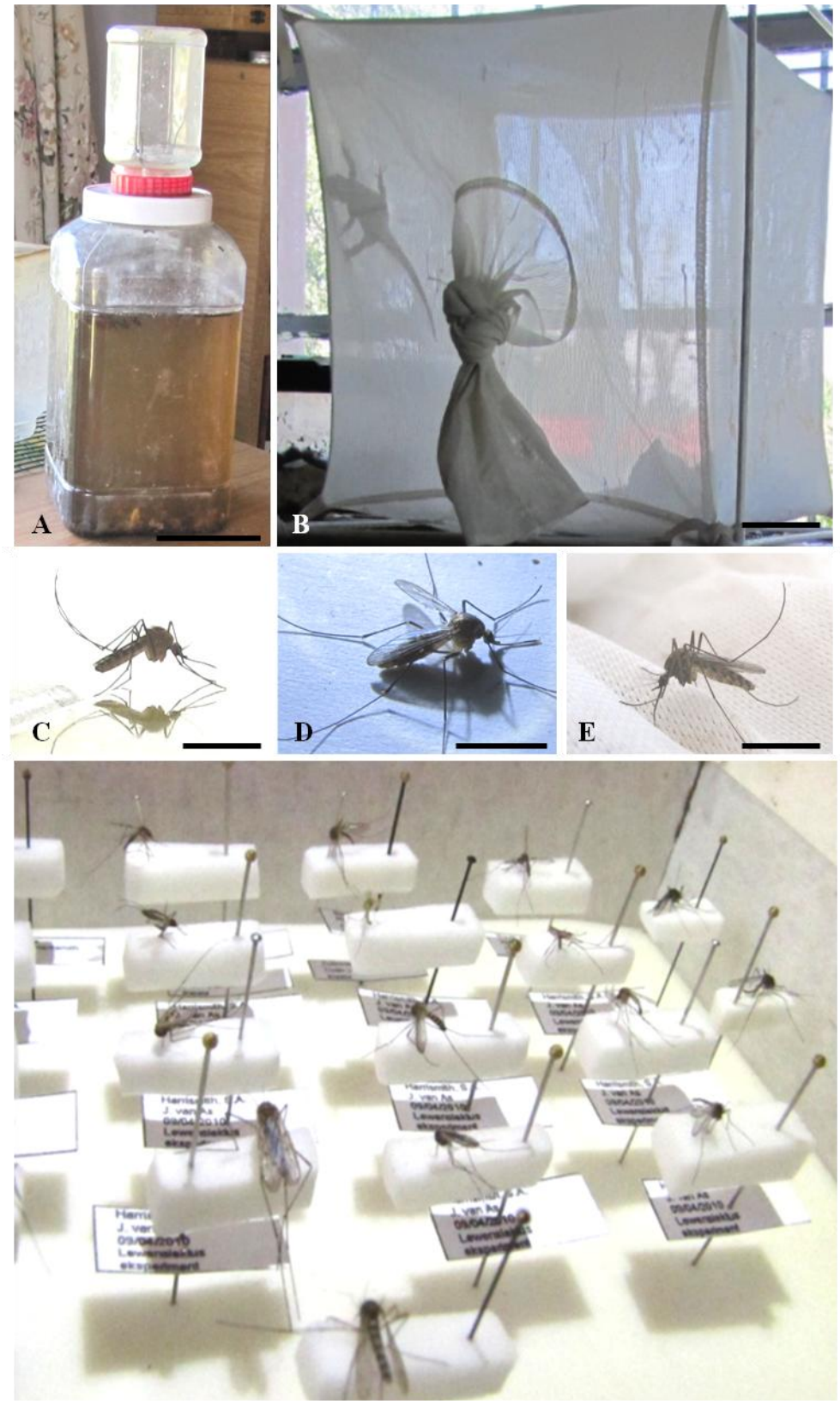

Figure 2.7 (A) Container used to breed and maintain mosquito larvae (from eggs) in pond water. The top plastic bottle is used to trap emerging adult mosquitoes. **(B)** Mosquito rearing cage with haemogregarine infected lizard on which starved mosquitoes will feed. **(C - E)** Adult *Culex* spp. of which the specimen in **(D)** are engorged with infected lizard blood; Specimen in **(E)** is in the feeding position. **(F)** Pinned mosquitoes used for identification. Scale bars: **(A)** = 75mm **(B)** = 100mm **(C - F)** = 10mm.

A total of 400 female mosquitoes was reared from eggs and kept alive with 2% sucrose solution soaked cotton balls. Mosquitoes were divided into 2 groups (200 per cage) with an infected lizard (*P. melanotus* in one cage, and *P. subviridis* in another cage). Mosquitoes were kept at 25 - 30ºC (50 - 60% RH) and were enticed to feed on the lizards by depriving them of the sucrose solution for 24 hours. Attempts were made to find sporogonic stages in other wild mosquitoes by means of routine dissections of the thorax, gut and salivary glands/ proboscis that were collected in the vicinity of the abovementioned lizards with no success. No larvae were found in any aquatic habitats at the top of Platberg. At the bottom of the mountain, mosquito eggs were found on the water surface of various dams and pans. These rafts, characteristic of the genus *Culex*, took 1 - 2 days to hatch. Subsequent larvae (Fig. 2.8 A) were kept in pond water maintained by methods described by Jupp (1996) (see above). Pupae stages (Fig. 2.8 B) were sucked off the surface with an aspirator and placed in a shallow petri dish filled with the same pond water inside the rearing cage to emerge as adults (Fig. 2.8 C) where the infected lizards were also housed (as explained above). During the course of the experiment, mosquitoes that were feeding on the captive lizards were collected and euthanased with ethyl acetate vapours (Fig. 2.8 D) for dissection and squash preparations (see below).

Mosquitoes were collected and euthanized in ethyl acetate vapours sequentially from 1 to 30 days post feeding on laboratory maintained lizards. Abdominal contents were dissected from of the abdomen of these mosquitoes. The thorax, head and abdominal contents were then squashed between 2 microscope slides, similar to the methods described in Davies & Smit, (2001) and Hayes et al. (2006) for blood-sucking crustaceans. Squashes were then fixed in absolute methanol for 1 minute and stained in a 9:1 solution of buffered Giemsa for 30 minutes. Stained squash preparations were scanned for possible *Hepatozoon* developmental stages on a Zeiss stereo microscope using the 40x objective. Photographs were taken with a Nikon Coolpix 4500 digital camera custom fitted to a Nikon Eclipse microscope.

2.7 Histology

2.7.1 Mites

Adult and nymph specimens of *I. inverta* were collected from *P. melanotus* from Platberg with a fine pincette. Half of the specimens (n=5) were fixed in 70 % ethanol and the other

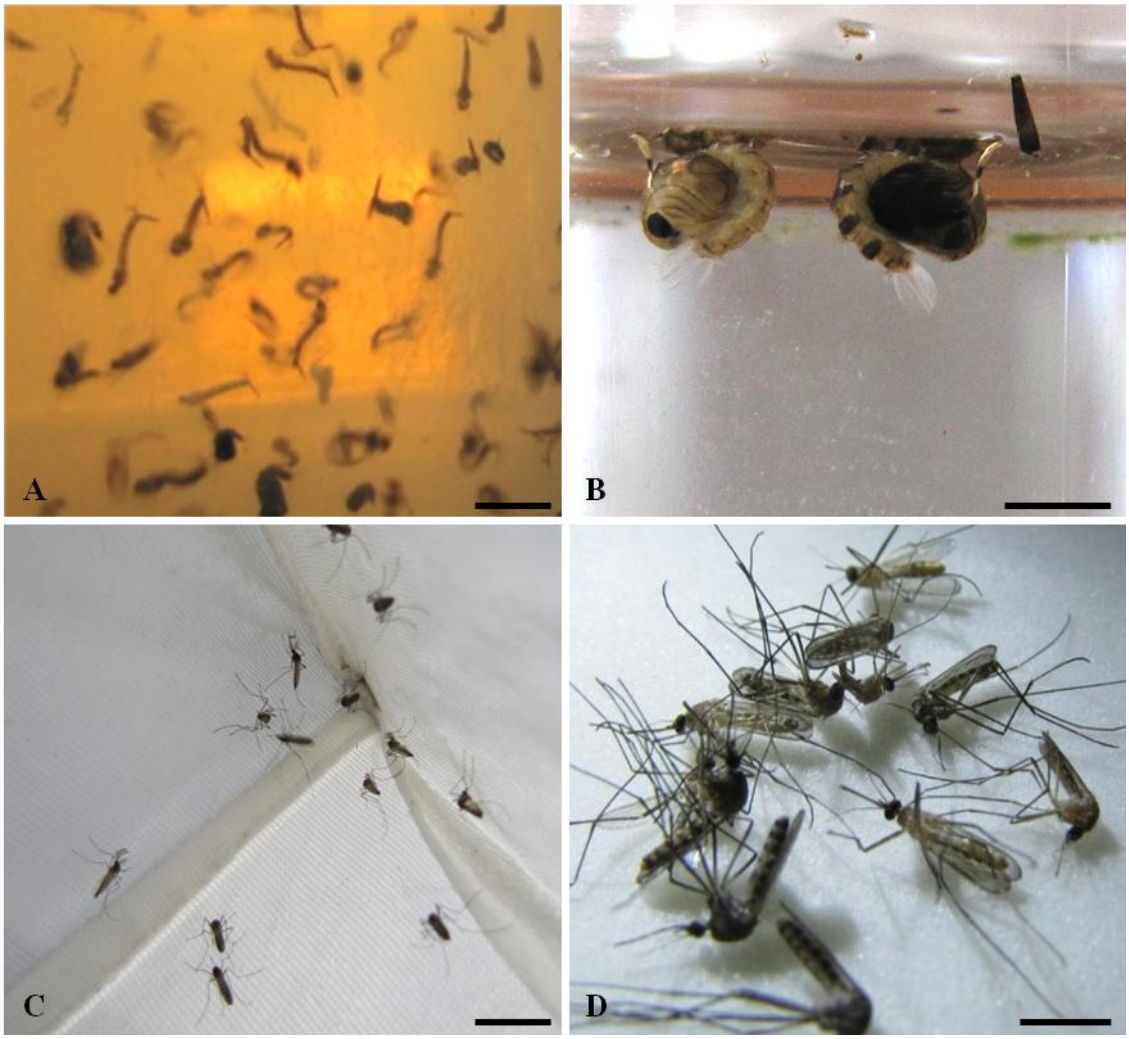

Figure 2.8 Larvae, pupae and adult mosquitoes used for obtaining sporogonic stages of haemogregarines. **(A)** Larvae maintained in pond water. **(B)** Pupae in Petri dish inside rearing cage. **(C)** Emerged adult mosquitoes in rearing cage. **(D)** Euthanased adult female mosquitoes prior to pinning and dissection. Scale bars: **(A - D)** = 10mm.

half (n=5) in 10% neutral buffered formalin. The specimens fixed in formalin were decalcified in 5.5% EDTA and tested for decalcification using a mixture of 5 % ammonium hydroxide and 5% ammonium oxalic acid solution. Specimens were washed in tap water and transferred to 30 % acetone for 30 minutes and then 50% acetone for 30 minutes. Samples were dehydrated in a graded ethanol series starting at 50% and left in a 70% solution overnight. Specimens were left in an oven overnight, and embedded in resin consisting of a mixture of Transmit LM resin, Transmit hardener and Transmit accelerator (Taab). Samples were polymerized in an oven at 60°C overnight. Thin (5µm) serial, sagittal and dorsoventral sections were cut using an Anglo Scientific microtome. Ribbons of sections were placed on a clean microscope slide and stretched on a 30°C plate using an albumin and glycerine solution as adhesive. All slides were marked with a diamond pen. Dried slides were stained using the standard Haematoxylin and Eosin (H&E) method (as above, see section 2.4). All sections were mounted with large cover slips with Entellan (Merck) mounting medium. Stained sections were examined and photographed with a Nikon Coolpix 4500 digital camera custom fitted to a Nikon Eclipse microscope.

2.8 Scanning electron microscopy

2.8.1 Mites

Pieces of lizard skin (5mm²) with engorged mites were fixed in 70% ethanol and dehydrated in a series of ethanol concentrations from 75 - 100% for 30 minutes at each stage. Samples were placed overnight in a small glass Petri dish containing hexamethyldisilazane (Sigma), which was allowed to evaporate, allowing the specimens to dry. Completely dried specimens were glued to stubs using Pratley putty (Pratley (Pty) Ltd.). Specimens were then sputter coated (BIO RAD) with gold, under vacuum, using standard methods and viewed using a JEOL WINSEM JSM 4600 scanning electron microscope (SEM).

2.8.2 Mosquitoes

Wild caught *C. lineata* specimens were euthanased with ethyl acetate vapours and air dried for 2 weeks. Samples were placed overnight in a small glass Petri dish containing 500µl hexamethyldisilazane, according to the method of Nation (1983). Completely dried specimens were glued to stubs using Pratley putty. Specimens were then sputter coated with

gold using standard methods and viewed with a Shimadzu SSX-550 Superscan scanning electron microscope (SEM).

2.9 Statistical analysis

In this study, Canonical Correspondence Ordination (CANOCO) techniques were used to analyse a variety of biological and environmental data sets and were applied using CANOCO v 4.5 statistical software (Ter Braak, 1994). Such techniques allow for the assessment of, for example, variability in parasitic infections, and then when combined with Monte Carlo permutation testing, statistical significance can be measured against complementary biological and physiological variables of the hosts (Ter Braak & Smilauer, 2002). Initially, however, the ordination approach allows for the expression of parasitic infections between hosts and surveys, without the need for correlating complementary biological, physiological, or explanatory data.

The biological data used in the present study comprised values for parasitaemias of *Hepatozoon* sp. A - E, *Plasmodium* sp. A, Filaria spp. and *Sauroplasma* sp. The values were entered as the number of parasites observed in a total field of ~1000 erythrocytes (ten fields of 100 erythrocytes). Additional data were leucocyte and thrombocyte counts, which were calculated in a similar manner to parasitaemias (as above) for each lizard species and the values were entered as a percentage (%) for each specific type of leucocyte, or thrombocyte observed in Giemsa stained slides. The above counts were done by examining Giemsa stained slides for each of the three species of lizards (*P. melanotus, P. subviridis* and *P. langi*). The gender of the lizard hosts (male, female and juveniles) were also entered as part of the biological data for the three species of crag lizards.

The environmental data entered for a redundancy analysis (RDA) constituted the following variables: Sites (Site 1 or Platberg, Site 2 or Sentinel, expressed as presence/absence in the data set), altitude expressed in meters above sea level (m.a.s.l.) for each host collection record, and proximity to dams at Site 1, (expressed as presence/absence of lizards collected further than 700 meters from the dams at the top of Platberg).

In this study, the largest portion of the total variance of the data sets were used to establish a first latent variable, and then a second was established that relied on the largest portion of the remaining variance in the data set (see Ter Braak & Smilauer, 2002). These two latent variables were then employed to construct an ordination diagram with two axes. The parasite loads and hosts were then initially presented in the diagram as points at the location of the values on the latent variables. Variables that were nearly identical or similar, were located close together, while samples located far apart represented those samples that comprised different compositions of parasitic loads. Thereafter, bi-plots were established, represented by arrows of explanatory data pointing in the direction of higher values, where correlations between the parasite load and host variables occurred (see Ter Braak and Smilauer, 2002). In this study, direct or constrained analyses were undertaken which involved overlaying captured variance of the explanatory variables onto parasite loads and host ordination diagrams. The linear response mode used to achieve this was a redundancy analyses (RDA), a derivative of principle component analyses (PCA), again using the CANOCO version 4.5 software package (Ter Braak, 1994).

CHAPTER 3

HEPATOZOON SPP. OF *PSEUDOCORDYLUS* SPP. (SAURIA: CORDYLIDAE) FROM SELECTED MONTANE LOCALITIES IN THE EASTERN FREE STATE

The previous chapters outlined the nature of the host lizards studied in the current chapter, namely, *Pseudocordylus melanotus*, *P. subviridis* and *P. langi* at the Platberg and Sentinel study sites in the Eastern Free State Highlands. They also detailed the methods used to examine the blood parasites of these lizards and their possible vectors from these locations. In the current chapter, haemogregarines of five distinct types, based especially on their general morphology, morphometric dimensions and staining properties in the blood films of male, female and juvenile lizards, are recorded. Blood films also contain other infections, including a *Plasmodium* sp., so-called *Sauroplasma* and filarial nematodes, and these will be considered in detail in Chapters 4 and 5.

For the purpose of this thesis, the five species of haemogregarines found in the *Pseudocordylus* species are described as *Hepatozoon* sp. A - E, mainly on the basis of the gamont stages in the peripheral blood, but also in some cases, their development patterns within lizard tissues and in potential vectors. The following 3 species, *Hepatozoon* sp. A - C, were found in 38/69 (prevalence, 55%) (18 female and 20 male) *Pseudocordylus melanotus* lizards from Platberg (Appendix 1). Two of the same species, *Hepatozoon* sp. A & C, were also found in 21/29 (prevalence, 72%) (4 female and 17 male) *P. subviridis* lizards from the Sentinel trail area in the North Eastern Drakensberg (Appendix 2). Lastly, *Hepatozoon* sp. D was found in 13/13 (prevalence, 100%) and *Hepatozoon* sp. E in 3/13 (prevalence, 23%) of the same *P. langi* individuals (8 females and 5 males) from the chainladders at the top of the Drakensberg escarpment (Appendix 3).

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

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

Type Locality: Platberg, Eastern Free State, 1900 - 2390m

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

Localities: Sentinel area, Northern Drakensberg, Eastern Free State, 2589 - 3050m

3.1.1 Systematics (Lee et al. 2000)

Phylum Apicomplexa Levine, 1970

Class Conoidasida Levine, 1988

Order Eucoccidiorida Léger & Dubosq, 1910

Suborder Adeleorina Léger, 1911

Family Hepatozoidae Wenyon, 1926

Genus *Hepatozoon* Miller, 1908

3.1.2 Prevalence

3.1.2.1 *Pseudocordylus melanotus*

This haemogregarine was found in the peripheral blood of 24/69 (prevalence, 34.8%) of *P. melanotus* sampled at Platberg. The infected lizards were 9 females and 15 males. Sometimes, this species formed mixed parasitaemias with other species of haemogregarines, saurian malaria and filarial nematodes. Mature gamonts were the most abundant stages in blood films, although younger gamonts and extracellular forms were also observed on rare occasions. In *P. melanotus*, *Hepatozoon* sp. A was found with other *Hepatozoon* infections described here with the following prevalences: co-infections of *Hepatozoon* sp. A & B in 4.3 % (3/69) of lizards; *Hepatozoon* sp. A & C in 7.2% (5/69) of individuals.

Hepatozoon sp. A was seen only in mature erythrocytes in the peripheral blood with parasitaemias ranging from 1/1000 (0.1%) to 170/1000 (17%). As well as forming mixed

infections of other *Hepatozoon* spp. (as mentioned above), this species occurred in the same blood films as *Plasmodium* sp. A, one filarial nematode species and in almost all the cases, so-called *Sauroplasma*. As noted above, these other infections will be described later in this thesis.

3.1.2.2 *Pseudocordylus subviridis*

The same haemogregarine (*Hepatozoon* sp. A) was found in the peripheral blood of 8/29 (prevalence, 27.5%) *P. subviridis* sampled at Sentinel. It was also found on occasion with other species of haematozoans. Mature gamonts of this haemogregarine were the most abundant stages in blood films, although younger gamonts and extracellular forms were also observed on rare occasions.

As in *P. melanotus*, *Hepatozoon* sp. A in *P. subviridis* was seen only in mature erythrocytes in peripheral blood smears. Parasitaemias overall ranged from 1/1000 (0.1%) to 18/1000 (1.8%) of erythrocytes infected. This species sometimes formed mixed infections with *Hepatozoon* sp. C (as mentioned above) but also occurred in the same blood films as *Plasmodium* sp. A, several filarial nematode species, and in almost all cases, so-called *Sauroplasma.*

3.1.3 Stages in the lizard peripheral blood

3.1.3.1 *Pseudocordylus melanotus*

In Giemsa-stained blood films from *P. melanotus*, immature gamonts (Fig. 3.1 A, black arrow) were rarely observed, forming parasitaemias of ~1/10000 (0.01%) of infected mature erythrocytes and measuring 11 x 3.5µm (n=1). Maturing or mature intraerythrocytic gamonts (Fig. 3.1 A - M) from the peripheral blood were broadly elongate, sausage-shaped organisms with a reflexed posterior pole (Fig. 3.1 C, D, F & H, yellow arrows) and a broadly rounded, or pointed, anterior extremity (Fig. 3.1 A, D - G, I, J & M, red arrows). Double infections (Fig. 3.1 D & E) of this species in erythrocytes were seen in blood films from one lizard. In some gamonts the anterior pole had a cap which stained a slightly deeper purple than the remaining parasite body (Fig. 3.1 D & E, red arrows). Mature gamonts measured 18.7 ± 1.4

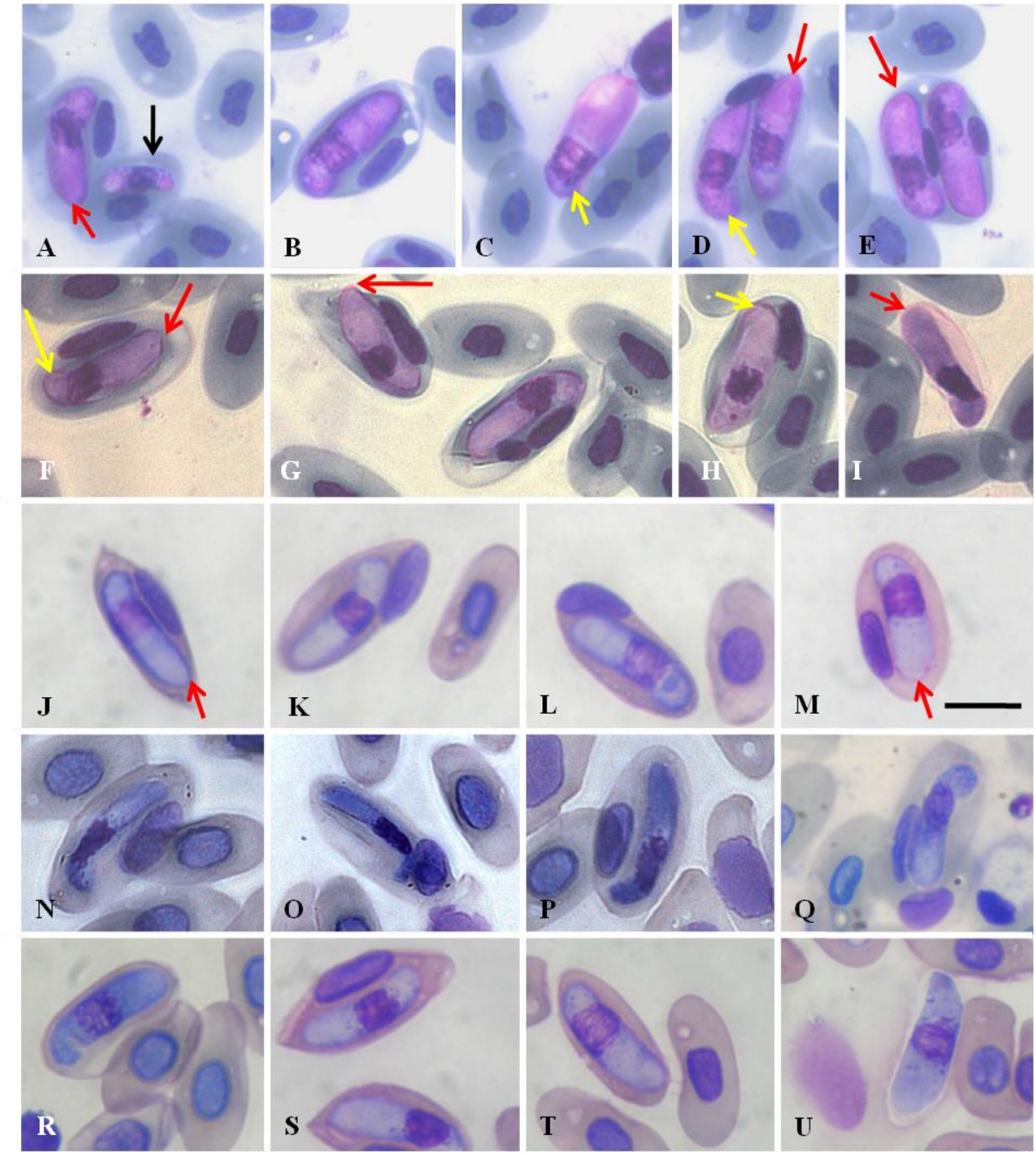

Figure 3.1 Micrographs of Giemsa stained blood films with *Hepatozoon* sp. A. from *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A)** Young (immature) gamont (black arrow). **(B - M)** Maturing and mature gamonts in erythrocytes from the peripheral blood of three different *P. melanotus* specimens **(B - E)**, **(F - I)** and **(J - M)**, showing different staining properties and morphological variation. **(N - U)** Gamont stages from heart blood; example in (**U)** is extracellular. Infected erythrocytes in **(F - I)** show a considerable degree of dehaemoglobinization. Yellow and red arrows indicate slightly recurved tails, and polar caps respectively. Scale bar: **(A - U)** = 10 µm.

(15.8 - 21.8) long by 5.7 ± 0.9 (3.2 - 7.3) µm wide at their broadest (n=75). Their cytoplasm stained mostly uniformly, sometimes with darker azurophilic granules anterior and posterior to the nucleus. The reflexed posterior extremity sometimes stained slightly deeper purple (Fig. 3.1 C, D, & F, yellow arrows). The gamont nucleus was dense and placed in the posterior third of the parasite body. The nucleus measured 5.8 ± 1.0 (3.9 - 7.9) long by 5.0 ± 1.0 (3.0 - 7.6) µm wide (n=75).

3.1.3.2 *Pseudocordylus subviridis*

Immature gamonts were rarely observed forming parasitaemias of ~2/1000 (0.2%) of mature erythrocytes (Fig. 3.2 A & B). These stages measured ~11µm x 3.5µm (n=2). The large nucleus, staining dark pink with banded chromatin, covered more than half of the parasite body and measured ~6µm x 3.5µm (n=2). Maturing and mature intraerythrocytic gamonts (Fig. 3.2 C - I) were elongate with a rounded anterior extremity (Fig. 3.2 C, E & F). The anterior pole was broader than the posterior pole, and bore an anterior cap in some individuals (Fig. 3.2 C & E red arrows). Gamonts measured 19.4 ± 1.0 (17.4 - 22.0) long by 6.2 ± 0.7 (5.4 - 7.6) µm wide (n=60) wide at the broadest. The cytoplasm stained uniformly pinkish with Giemsa, sometimes with scattered granules anterior and posterior to the nucleus. A slightly reflexed posterior was visible in some individuals (Fig. 3.2 C & D yellow arrows). The dense, rounded to oval nucleus stained deep pinkish-purple and contained finely stranded or slightly granular chromatin. It was centrally, or more posteriorly placed in the posterior third of the parasite body, and measured 6.5 ± 0.9 (5 - 10.1) long by 5.2 ± 0.5 (4 - 6.1) µm wide (n=60).

3.1.4 Effects on Host cell

3.1.4.1 *Pseudocordylus melanotus*

In general, infected *P. melanotus* erythrocytes stained lighter than non-infected erythrocytes, but were neither hypertrophied nor dehaemoglobulinized. However, in a blood film from one lizard specimen, infected red blood cells were dehaemoglobulinized and a degree of cellular hypertrophy was observed (Fig. 3.1 F - H).

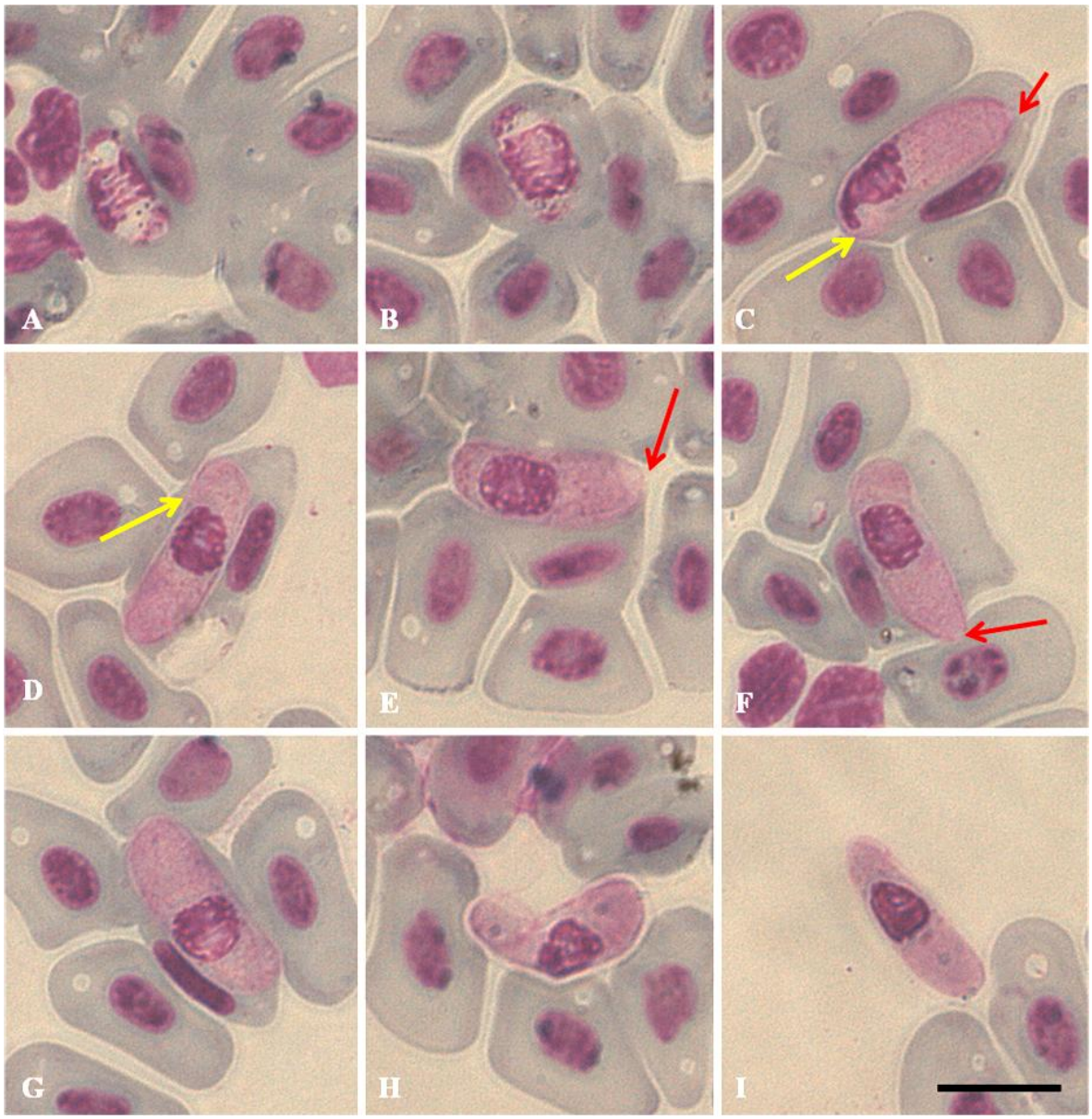

Figure 3.2 Micrographs of Giemsa stained peripheral blood films showing *Hepatozoon* sp. A. from *Pseudocordylus subviridis* from the North Eastern Drakensberg. **(A - B)** Immature gamonts in erythrocytes. **(C - G)** Maturing and mature gamonts in mature erythrocytes. **(H & I)** Extracellular gamonts. Yellow and red arrows indicate slightly recurved tails, and polar caps respectively. Scale bar: **(A - I)** = 10 µm.

The host cell nucleus was usually elongated, compacted, and displaced laterally (Fig. 3.1 A, B, F, G, J & M) sometimes almost terminally (Fig. 3.1 D, H, K & L), and occasionally fragmented (Fig. 3.1 E, G). Parasitised host cell nuclei measured 9.1 ± 1.2 (6.4 - 12.3) long by 2.9 ± 0.4 (1.9 - 3.8) µm wide (n=60) compared to normal, uninfected erythrocyte nuclei that measured 6.5± 0.6 (5.2 - 7.5) µm (n=60) in length and 4.0 ± 0.6 (3.3 - 4.9) µm in width (n=60). The total area of the parasitized erythrocyte nucleus was 24.5 ± 4.0 (15.5 - 32.1) µm² (n=60), compared to the non-infected erythrocyte nucleus that measured 23.7 ± 2.6 (16.4 - 26.7) µm² (n=60).

3.1.4.2 *Pseudocordylus subviridis*

Infected erythrocytes in this lizard species were generally not dehaemoglobulinized but were bigger, 21.6 ± 1.9 (17.6 - 26.1) long by 12.2 ± 1.8 (8.9 - 18.1) µm wide (n=60) with a total surface area of 210 ± 34 (141 - 294.7) µm² (n=60), than non-infected erythrocytes which measured 18.0 ± 1.0 (16.4 - 19.9) long by 9.8 ± 1.3 (7.0 - 11.5) µm wide (n=100), with a surface area of 148.3 ± 24 (121.8 - 191.6) µm² (n=100). The parasitized erythrocyte nucleus was compacted and displaced laterally (Fig. 3.2 A - G) and measured, 8.9 ± 0.9 (6.7 - 11.0) long by 2.9 ± 0.3 (2.1 - 3.7) µm wide (n=60) with a total surface area of 23 ± 3.4 (18.0 - 29.6) µm² (n=60). Uninfected erythrocytes had nuclear dimensions of 6.5 ± 0.6 (5.2 - 7.5) long by 4.0 ± 0.6 (3.3 - 4.9) µm wide (n=60) with a total surface area of 23.9 ± 2.9 (16 - 26) µm² (n=60).

3.1.5 Stages in heart blood of *Pseudocordylus melanotus*

Opportunity existed to examine heart blood from only *P. melanotus*. Initially, it was thought that some gamonts in heart blood, for example, Fig. 3.1 N & O, might belong to a different species from those in peripheral blood. However, gamonts of intermediate appearance between the two types (Fig. 3.1 P - S) were also discovered in heart blood, as well as those identical to those in peripheral blood (Fig. 3.1 T & U), suggesting all gamonts belonged to the same species. Heart blood gamont stages were elongated, with a rounded anterior extremity (Fig. 3.1 N, O & Q). The posterior pole was strongly reflexed, or sometimes curved (Fig. 3.1 N - Q) and occasionally stained darker than the remaining cytoplasm of the gamont. The host cell nucleus was mostly less compacted than in the peripheral blood stages, but still displaced laterally (Fig. 3.1 N, P, Q & S) and sometimes terminally (Fig. 3.1 O).

Occasionally, the host cell nucleus was absent from infected erythrocytes (Fig. 3.1 R & T), which was an unusual find. Overall, these heart blood gamonts measured 19.8 ± 0.9 (18.7 - 21.0) long by 3.6 ± 0.5 (3.1 - 4.2) µm wide (n=20), so that they tended to be longer and narrower than those in the peripheral blood. Except at the posterior pole, the main cytoplasm of heart blood gamonts stained paler than its periphery, sometimes with dark azurophilic granules just anterior and posterior to the nucleus (Fig. 3.1 S - U). The dense nucleus stained dark purple-blue and was situated centrally or in the posterior third of the parasite body, and measured 6.5 ± 0.8 (5.7 - 8.0) long by 2.6 ± 0.5 (2.2 - 3.8) µm wide (n=7) (Fig. 3.1 O - Q).

3.1.6 Effects on host cells in heart blood of *Pseudocordylus melanotus*

Infected erythrocytes in heart blood were larger in area, measuring 191.7 ± 21.3 (161 - 223) µm² (n=60) than non-infected cells which measured 138.8 ± 24.2 (120 - 192) µm² (n=100). In general, infected erythrocytes were paler stained than non-infected erythrocytes, but were not markedly dehaemoglobulinized.

3.1.7 Ultrastructure of *Hepatozoon* sp. A gamonts in host erythrocytes

Ultrathin sections of peripheral blood samples of *P. melanotus* examined by transmission electron microscopy (TEM) revealed intraerythrocytic gamonts in some erythrocytes (Fig. 3.3 A - C, Fig. 3.4 A - D). Gamonts, lying within a parasitophorous vacuole were also apparently surrounded by a capsule which was mostly tightly adherent to the pellicle and had not been evident in light micrographs, and compared closely with the ensheathed haemogregarine gamonts observed in the TEM studies of Stehbens & Johnston (1967). The capsule (Fig. 3.3 A & C, and especially Fig. 3.4 B - D) appeared to have protrusions into the erythrocyte cytoplasm. Each gamont demonstrated typically apicomplexan features that included the pellicle, a nucleus, rhoptries, numerous micronemes, Golgi, amylopectin and dense granules (Fig. 3.3 A - C & Fig. 3.4 A - D), some of which are described in more detail below. The host erythrocyte nucleus was displaced laterally by the parasite contained in its capsule and parasitophorous vacuole, and the periphery of this elongated erythrocyte nucleus (Fig. 3.3 A & Fig. 3.4 B) demonstrated electron dense heterochromatin.

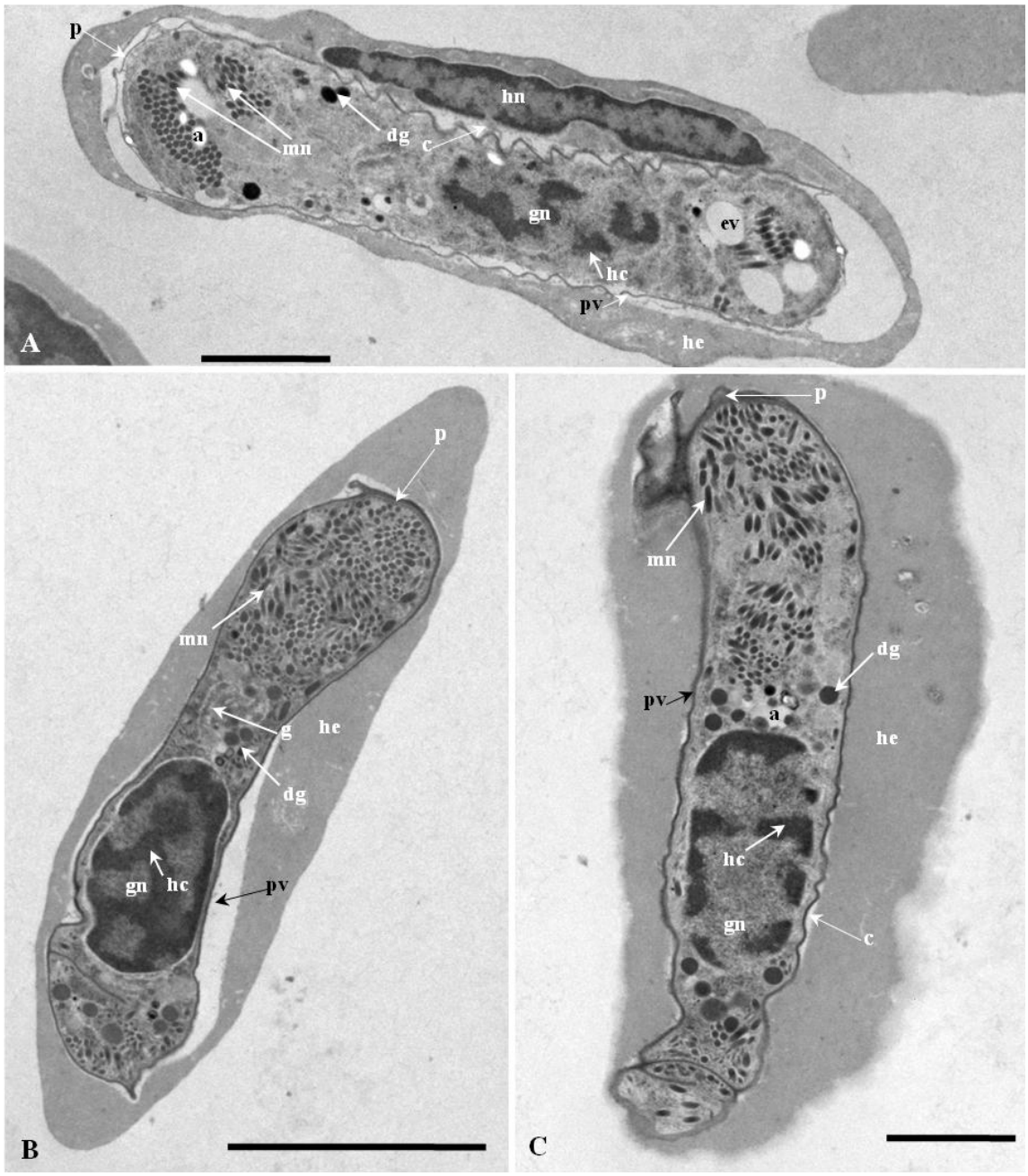

Figure 3.3 (A - C) Transmission electron micrographs of *Hepatozoon* sp. A. gamonts within parasitophorous vacuole (pv) and dense capsule (c) in *P. melanotus* erythrocytes. **(A)** Longitudinal section of *Hepatozoon* sp. A gamont in a host erythrocyte (he) showing the laterally displaced host cell nucleus (hn). The posterior part of the gamont shows electron-lucent vacuoles (ev). **(A, B & C)** Longitudinal sections of *Hepatozoon* sp. A. showing prominent apical complex organelles such as dense granules (dg) and micronemes (mn). Gamont nuclei (gn) with heterochromatin (hc) (electron dense areas) located in the second third of the parasite body. Note the slightly recurved parasite tail region in **(C)**. Scale bars: **(A & C) =** 2µm **(B) =** 5µm.

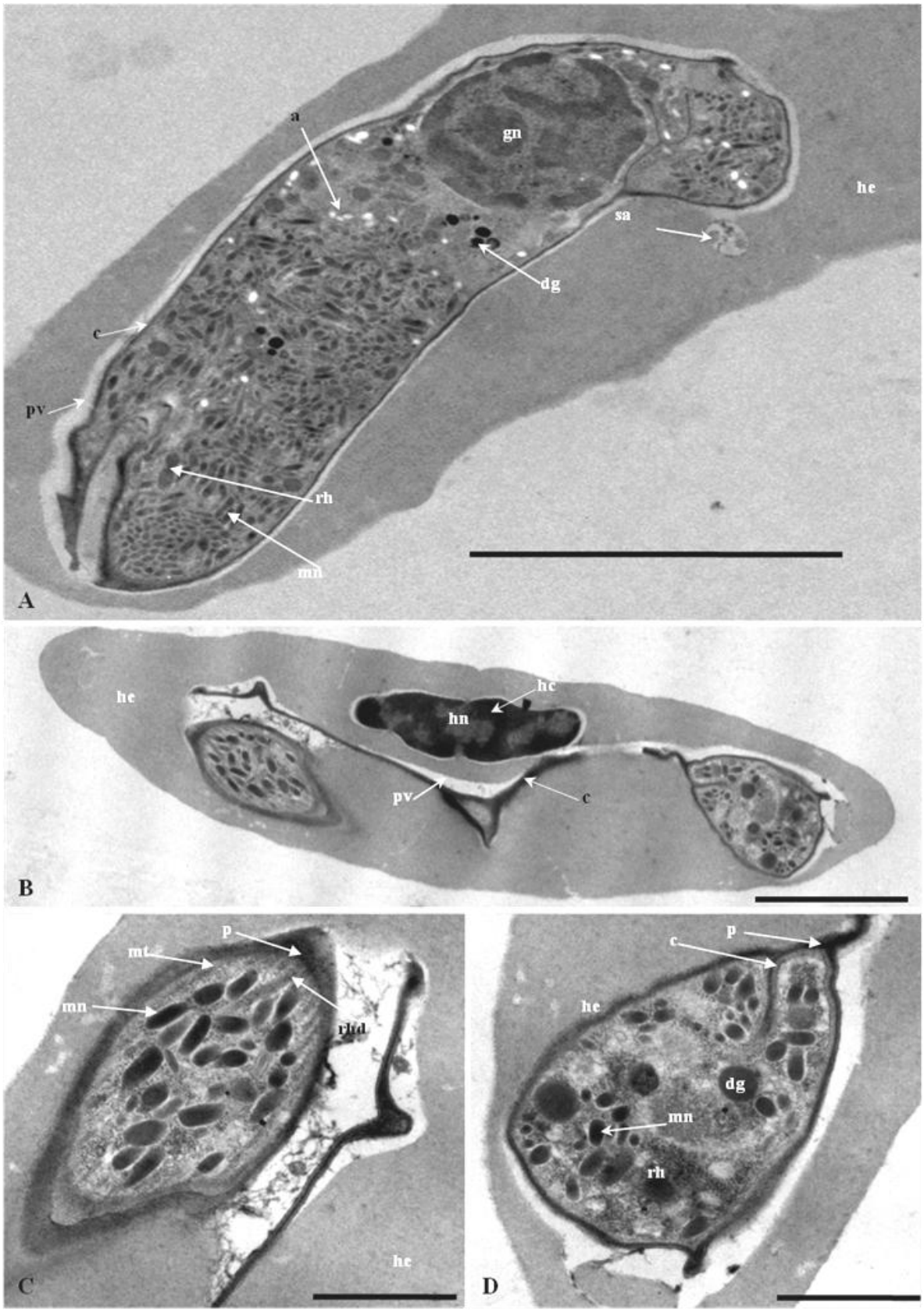

Figure 3.4 (A - D) Transmission electron micrographs of *Hepatozoon* sp. A. gamonts within parasitophorous vacuole (pv) and dense capsule (c) in *P. melanotus* erythrocytes. **(A)** Longitudinal section of gamont within parasitophorous vacuole showing the gamont nucleus (gn), rhoptries (rh), micronemes (mn), amylopectin granules (a) and dense granules (dg). Note the so-called *Sauroplasma* (sa) present in the cytoplasm of the host erythrocyte (he). **(B)** Cross sections of *Hepatozoon* sp. A gamont in a host erythrocyte (he) showing the laterally displaced host cell nucleus (hn) with electron dense heterochromatin (hc) areas, parasitophorous vacuole (pv) and dense capsule (c). **(C & D)** Enlarged micrographs of a cross section of the anterior **(C)** and posterior sections **(D)** showing characteristic apicomplexan structures like micronemes (mn), rhoptries (rh), dense granules (dg), rhoptry ducts (rhd) and microtubules (mt) beneath the pellicle (p) and capsule (c). Scale bars: **(A)** = 5µm **(B)** = 2µm **(C & D)** = 1µm.

Rhoptries (3 - 10 per gamont section) and micronemes (up to 180 per section) contained osmophilic, homogenous contents and were found anterior and posterior to the parasite nucleus (Fig. 3.3 A - C & Fig. 3.4 A - D), but the majority of these organelles were present anteriorly, perhaps corresponding to the cap region seen in some light micrographs. The gamont nucleus was prominent, often osmiophilic (with abundant heterochromatin), oval and situated in the posterior third (Fig. 3.3 A, B & C & Fig. 3.4 A) of the gamont, with the Golgi body lying immediately anterior to it, and close to dense granules (Fig. 3.3 B). Non parasitised erythrocytes (Fig. 3.3 D & E (white arrows)) in a vein from liver tissue showed rounded nuclei, compared to the displaced nuclei (eg. Fig. 3.3 A) of infected erythrocytes. A grazing section of the anterior region of the gamont (Fig. 3.4 B & C) revealed microneme ducts and microtubules towards the anterior extremity, closely associated with the pellicle. Micronemes, rhoptries and dense granules were also present in a cross section of the posterior region of the gamont (Fig. 3.4 D).

3.1.8 Merogonic stages in *Pseudocordylus melanotus*

Gamont stages, similar in appearance to those occurring in the peripheral blood were also observed in squash/smear preparations of *P. melanotus* liver tissue (Fig. 3.5 A - D) and had presumably escaped the general circulation during tissue preparation. These extracellular gamonts were also morphometrically identical to the blood stream forms in Figure 3.1 A - M. In liver squash/smear preparations additional haemogregarine stages were observed. Meronts were observed primarily in what were presumed to be hepatocytes or endothelial cells, and in the lizard specimen examined, no other organs harboured meronts. Young meronts (Fig. 3.5. E & F) presumably arose from sporozoites inoculated by a vector (see below) and these stages measured 18.3 ± 1.0 (17 - 19.4) in length by 17.1 ± 1.1 (16 - 18.4) µm in width (n=10) and apparently contained abundant amylopectin (Fig. 3.5 E). Dizoic meronts (Fig. 3.5 G & H) were rare in liver tissue and measured overall ~16.2 x 16.8 µm. Macromeronts (Fig. 3.5 I - L) were the most abundant in the liver smears and overall these measured 25.6 ± 2.3 (25.9 - 33.9) in length by 22.7 ± 2.3 (20.1 - 26.7) µm in width (n=10) with a surface area of ~681.2 µm² and contained 4 ± 1.8 (2 - 7) (n=10) macromerozoites.

Individual macromerozoites within macromeronts measured 20.8 ± 1.8 (15.8 - 22.4) in length by 5.2 ± 1.7 (2.9 - 8.5) µm in width (n=10), and were similar in general morphology to the

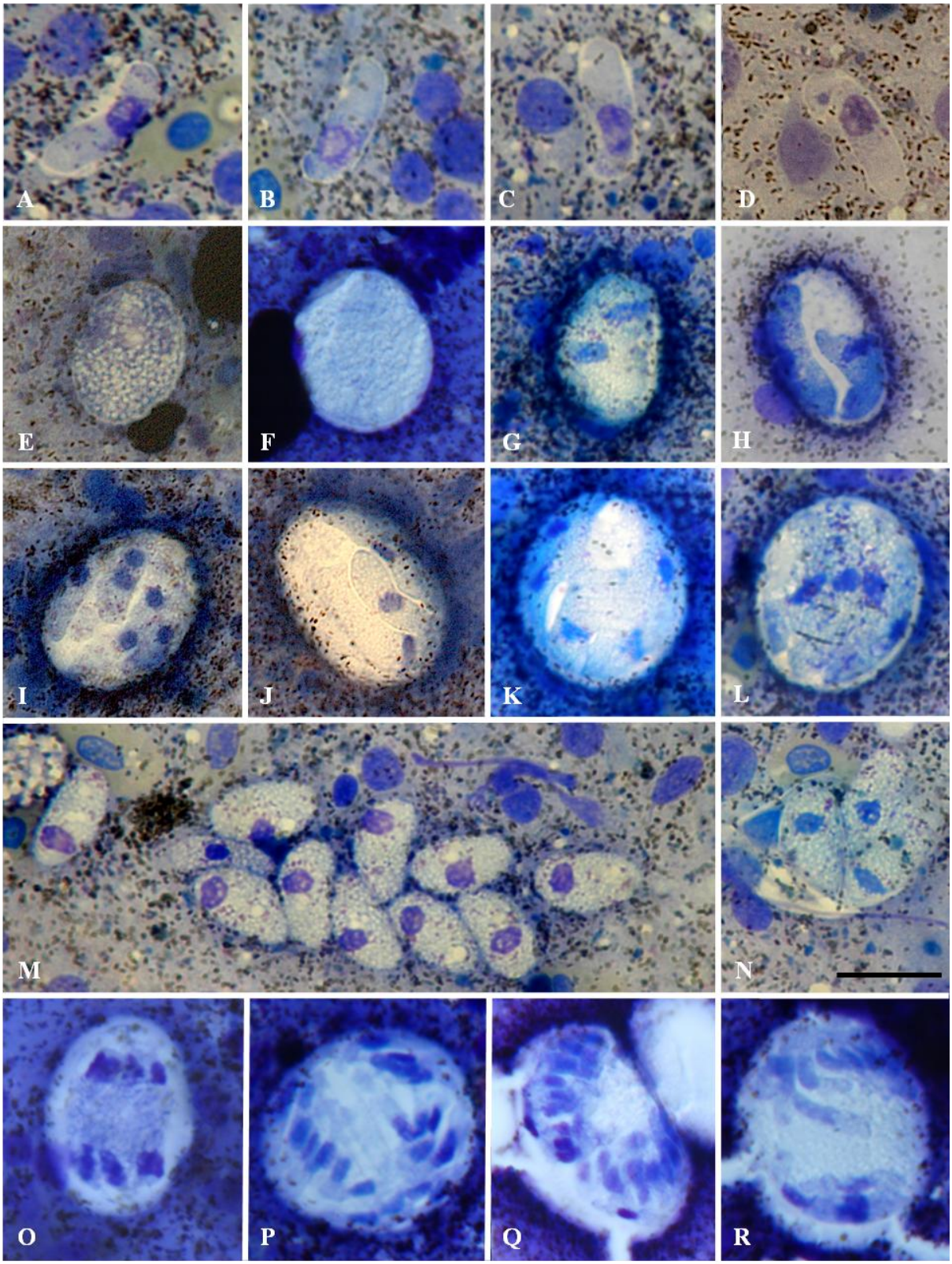

Figure 3.5 (A - R) Giemsa stained light micrographs of *Hepatozoon* sp. A mature gamonts and merogonic stages in *P. melanotus* liver tissue squashes. **(A - D)** Mature extracellular gamonts. **(E & F)** Young meronts with likely abundant amylopectin granules, especially in **(E)**. **(G & H)** Dizoic meronts. **(I - L)** Macromeronts with developing macromerozoites. **(M & N)** Ruptured macromeronts showing released macromerozoites. (**O - R)** Micromeronts with developing micromerozoites. **F, K & O - R** are DIC images. Scale bar: **(A - R)** = 20µm.

gamont stages in the peripheral blood, except that some were more pyriform in shape (Fig. 3.5 I - L). Macromerozoite cytoplasm stained whitish-blue with dark granules distributed randomly throughout. The nuclear periphery stained a dark blue with the centre staining dark purple. Macromerozoite nuclei measured 4.0 ± 1.0 (2.8 - 6.2) by 2.5 ± 0.4 (1.9 - 3.1) µm (n=10). Ruptured macromeronts (Fig. 3.5 M & N), released macromerozoites that were foamy in appearance and broader in morphometric dimensions than those within meronts. Extracellular macromerozoites measured 19.9 ± 1.1 (18.4 - 22.0) in length by 9.5 ± 1.3 (7.2 - 13.0) µm in width (n=13). Cytoplasm stained a light whitish-pink with distinct pink granules distributed around the nucleus. Elliptical nuclei (Fig. 3.5 M & N) were compact, stained a deep purple and measured 5.5 ± 0.8 (3.6 - 7.0) in length by 3.6 ± 1.1 (2.6 - 6.6) µm in width (n=13).

Micromeronts were also identified in lizard liver (Fig. 3.5 O - R) and were presumed to arise from macromerozoites. Each micromeront produced 9 - 24 micromerozoites. Micromeronts were generally slightly larger than mature macromeronts and measured 33 ± 5.4 (25 - 40) in length by 38.5 ± 5.3 (30 - 45) µm in width (n=7) with a surface area of 506.9 ± 111.9 (402.4 - 711.4) µm² (n=10). Micromerozoites were slender and more elongate than macromerozoites and were difficult to measure, since none was extracellular, but each was ~ 18 x 2.5µm.

3.1.9 Confocal microscopy and histology

Confocal micrographs of Giemsa-stained *P. melanotus* infected liver tissue showed likely abundant amylopectin within individual macromeronts (Fig. 3.6 A) with white light. With laser light, (red He - Ne, green He - Ne & blue Ar - ion) macromeronts were seen to contain four macromerozoites (Fig. 3.6 B & C) and had prominent nuclei shown by bright green fluorescence, while their cytoplasm fluoresced red. One macromeront contained a residuum of possible amylopectin (Fig. 3.6 C), while a micromeront with a thick capsule (yellow fluorescence) contained ~13 micromerozoites (Fig. 3.6 D) with nuclei fluorescing light green and cytoplasm pale red. Histological sections of infected *P. melanotus* liver tissue stained with H&E showed abundant melanomacrophage centres (Fig. 3.6 E & F white arrows), with some melanomacrophages surrounding meronts, and partially destroying them and their contained merozoites (Fig. 3.6 F).

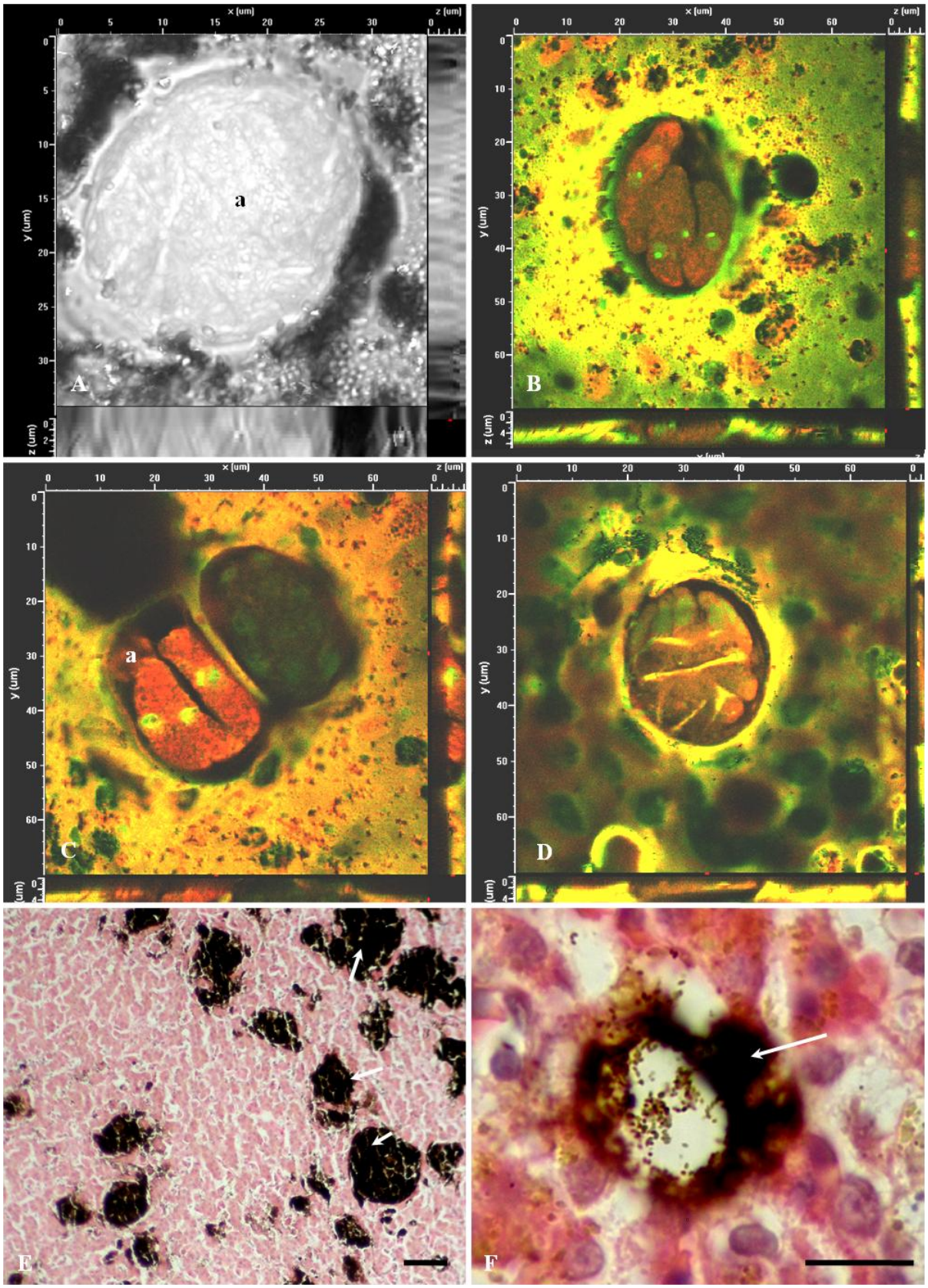

Figure 3.6 (A - F) Confocal and light micrographs of *Hepatozoon* sp. A meronts in the liver of *P. melanotus.* **(A - D)** Giemsa stained confocal micrographs of *Hepatozoon* sp. A meronts in lizard liver tissue. **(A)** Macromeront with white light. **(B)** Macromeront containing four macromeronts with prominent nuclei with laser light (red He-Ne, green He-Ne & blue Ar-ion). **(C)** Macromeronts with three or more macromerozoites and possibly amylopectin reserve (a) with laser light (red He-Ne, green He-Ne & blue Ar-ion). **(D)** Micromeront containing micromerozoites, surrounded by a seemingly thick capsule (yellow fluorescence) with laser light (red He-Ne, green He-Ne & blue Ar-ion). **(E & F)** Histological (H&E stained) sections of lizard liver showing melanomacrophage centres (white arrows). **(F)** Meront surrounded by, and partially destroyed by, melanomacrophages. Scale bars: **(E & F)** = 20µm.

3.1.10 Ultrastructure of meronts from *Pseudocordylus melanotus*

Ultrathin sections of infected *P. melanotus* liver examined by TEM revealed meronts in different stages of development (Fig. 3.7 A - F). Developing meronts as well as macromerozoites had abundant cytoplasmic amylopectin, as well as numerous dense bodies, possibly micronemes (Fig. 3.7 A - F). Binucleate meronts were observed (Fig. 3.7 B), and some with developing macromerozoites (Fig. 3.7 C - F) appeared to have spacious parasitophorous vacuoles within the host cells, probably mainly hepatocytes (as in Fig 3.7 B). One host cell, possibly an endothelial cell (Fig. 3.7 C), had a host nucleus that was displaced to one side. Meronts were frequently surrounded by melanin-containing granular melanomacrophages (as in Fig. 3.7 B - F), but were apparently protected from destruction at this stage, possibly by the parasitophorous vacuole and its limiting membrane, since no surrounding capsule could be detected by TEM.

3.1.11 Sporogonic stages in naturally feeding mosquitoes

Female *Culex* (*Afroculex*) *lineata* specimens were found feeding naturally on *P. melanotus* at night on the top of Platberg. These lizards were infected with *Hepatozoon* sp. A. In squash preparations made from *C. lineata* 1 - 7 days post feeding (d.p.f) on infected lizard blood, haemogregarines and red blood cells (in different stages of digestion) from the recent blood meal were observed in the gut contents (Fig 3.8 A - D). Morphometrically, these ingested gamonts had similar dimensions to gamonts in the erythrocytes of the host lizard and were broadly of two types. The apparently still encapsulated form (Fig. 3.8 A) measured 10 x 2.6µm (n=1). Free gamonts, which were long and slender (Fig. 3.8 B - D), measured 26.1 ± 2.1 (21.4 - 28.4) in length by 4.6 ± 0.6 (3.7 - 5.6) µm in width (n=10). Nuclei of these free gamonts measured 10 ± 1.8 (6.6 - 13.2) in length by 4.1 ± 0.8 (3.1 - 5.7) µm in width (n=10). Some free gamonts had a broad anterior (Fig. 3.8 C & D) and measured 24.3 ± 1.9 (22.9 - 26.8) in length by 5.4 ± 0.6 (4.7 - 6.4) µm in width at the broadest (n=10). Nuclear dimensions of these broader gamonts were 9.3 ± 2.1 (5.7 - 12.5) in length by 4.5 ± 0.6 (3.9 - 5.3) µm in width (n=10).

Gametogenesis in naturally infected *C. lineata*, and subsequent fertilization were not seen, but uninucleate and binucleate oocysts (Fig. 3.8 E & F) were present, 3d.p.f, in the gut contents of these mosquitoes and measured ~54 x 48 µm with an area of ~2093µm². The

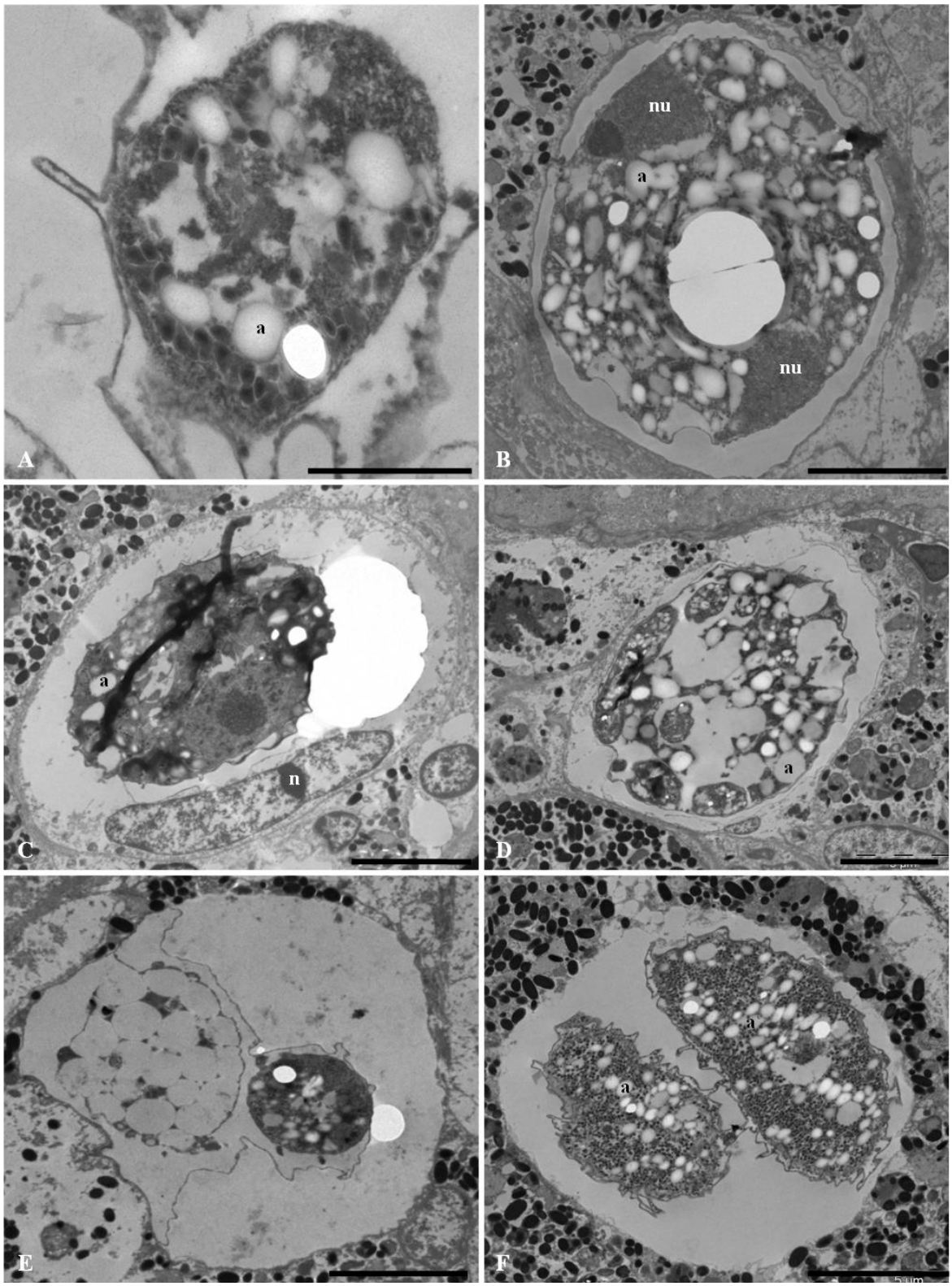

Figure 3.7 (A - F) Transmission electron micrographs of *Hepatozoon* sp. A meronts in infected *P. melanotus* liver tissue. **(A)** Cross section of a developing meront with dense granules, possibly micronemes and possibly amylopectin (a). **(B)** Young meront with two prominent nuclei (nu) and amylopectin, lying in a parasitophorous vacuole within hepatocyte. Portion of melanomacrophage in the top left of micrograph. Tear in section centrally. **(C & D)** Macromeronts beginning to form macromerozoites. Meront in **C** may lie in within the endothelial lining of a small blood vessel. Host cell nucleus (n). **(E)** Macromeront with cross section of a macromerozoite. **(F)** Dizoic macromeront with developing macromerozoites containing abundant amylopectin (a) and dense granules, possibly micronemes. Note the surrounding melanomacrophages in this electron micrograph. Scale bars: **(A)** = **1**µm **(B, D, E & F)** = 5µm **(C)** = 2µm.

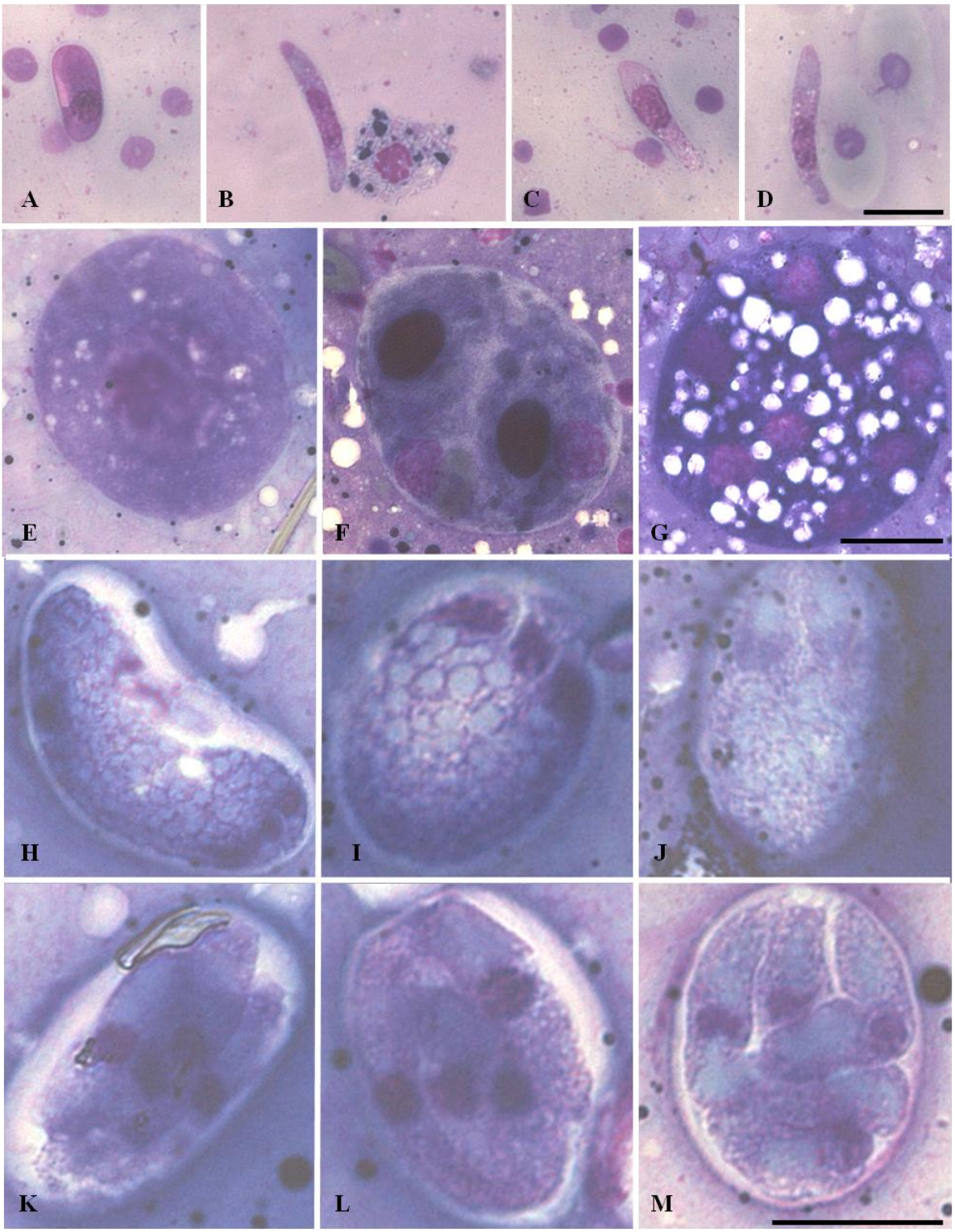

Figure 3.8 Micrographs of Giemsa stained squash preparations of gamonts and sporogonic stages of *Hepatozoon* sp. A. from the likely natural definitive host *Culex* (*Afroculex*) *lineata* fed on *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A - D)** Mature extracellular gamonts from the blood meal of the mosquito. Gamont in **A** is still encapsulated. **(E)** Uninucleate oocyst (exact source within the mosquito, unknown). **(F)** Binucleate oocyst. **(G)** Multinucleate oocyst signalling the onset of sporogony. **(H - J)** Sporocysts developing sporozoites. **(K - M)** Maturing and mature sporozoites within sporocysts. Scale bars: **(A - D)** = 10 µm (**E - G**) = 20µm (**H - M**) = 20µm.

nuclei of the oocysts measured ~26.9 x 25.8 µm with an area of ~693.5 µm². Six to seven days post feeding sporulating oocysts with 8 nuclei were present in the gut contents and measured ~25.5 x 26.1 µm with an area of 665.6 µm², and likely signalled the onset of sporogony (Fig. 3.8 G), although mature polysporocystic oocysts were not observed in these wild-feeding mosquitoes.

However, sporocysts with developing sporozoites were detected (Fig. 3.8 H - J) and overall these sporocysts measured 24.1 ± 1.1 (23 - 25.6) x 26.1 ± 1.5 (24.4 - 28.1) µm (n= 8) with an average area of 631.7 µm². Sporocysts containing maturing or mature sporozoites (Fig. 3.8 K - M) measured 31 ± 3.8 (26.7 - 39.2) µm in length by 21.8 ± 0.6 (17 - 25.6) µm in width and contained 4 ± 1.4 (2 - 8) large sporozoites (n=20). Sporozoite cytoplasm had a foamy appearance and sporozoites measured 22.4 ± 4.6 (16.8 - 32.4) µm in length by 5.1 ± 1.1 (4 - 9) µm in width (n=18). Each sporozoite nucleus was rounded and dense in appearance and measured 3.4 ± 1.0 (1.8 - 5.6) µm by 3.3 ± 0.7 (1.9 - 4.6) µm (n=18).

3.1.12 Observations in experimental mosquitoes (*Culex* and *Culiseta* spp.)

Mature gamonts (Fig. 3.9 A - C) of the same species of haemogregarine (*Hepatozoon* sp. A) were found in the gut of experimental mosquitoes (*Culex* and *Culiseta* spp.) one day post feeding on infected *P. melanotus*. Free gamonts (Fig. 3.9 B & C) were morphologically and morphometrically similar to those found in the gut contents of *C. lineata*, and measured 26.1 ± 2.1 (21.4 - 28.4) in length by 4.6 ± 0.6 (3.7 - 5.6) µm in width (n=10). Uninucleate and binucleate oocysts (Fig. 3.9 D & E) were found in the gut of *Culex pipiens* and *Culex andersoni* 3 days post feeding. Oocysts measured ~51 x 44 µm with an area of ~2243µm². Each oocyst nucleus measured ~7.7 x 8.2 µm with an area of ~64 µm². Oocyst-like structures were also seen in the gut contents of *Culiseta longiareolata*, but they could not be identified with enough certainty to be included in this description. No further sporogonic developmental stages were seen in these experimental mosquitoes.

3.1.13 Observations in the mite *Ixodiderma inverta*

Gamonts of the same species of haemogregarine were observed in squashes of the mite (*Ixodiderma inverta*) taken from infected *P. melanotus* hosts (Fig. 3.9 F & G).

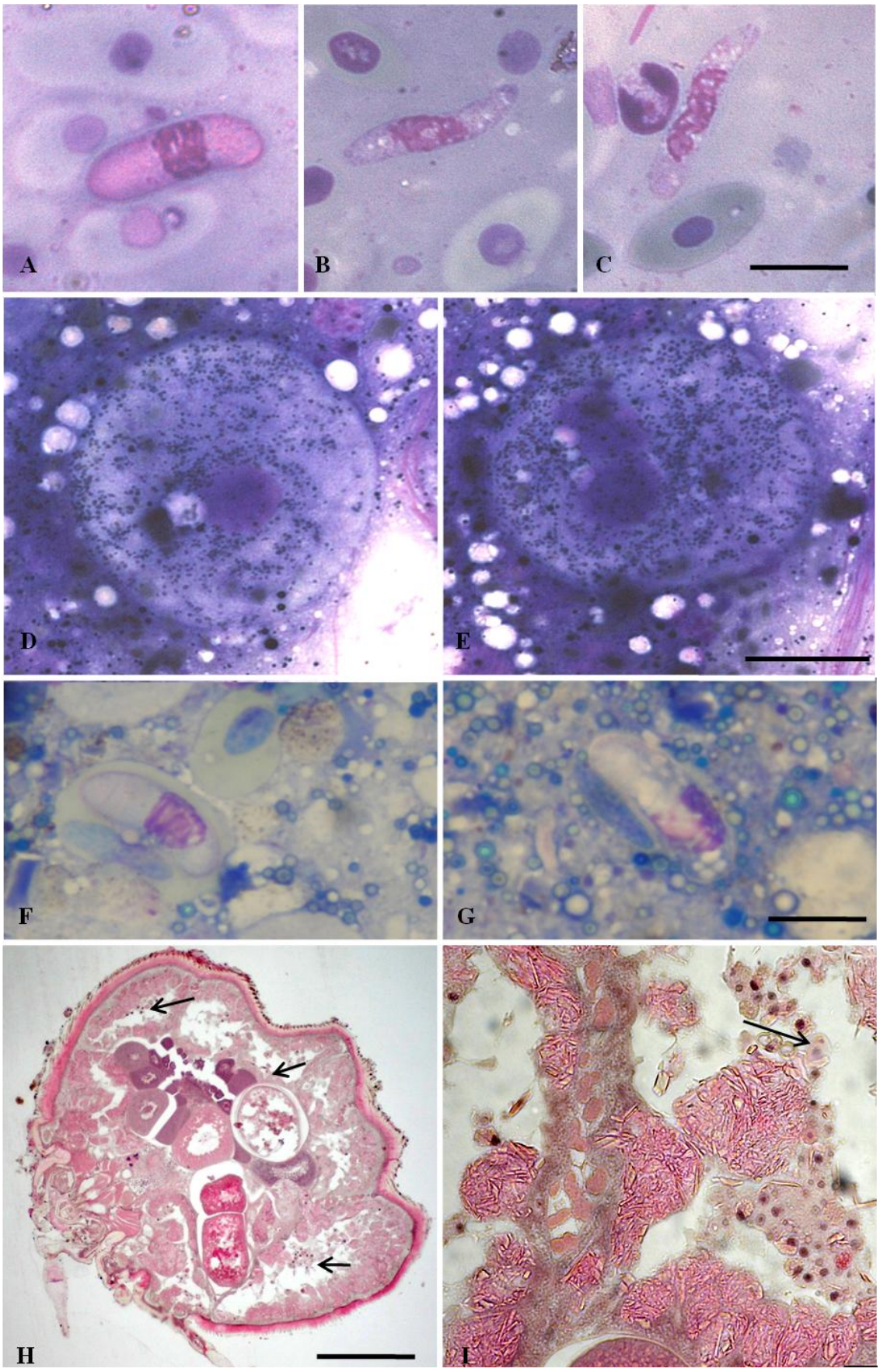

Figure 3.9 Micrographs of Giemsa stained squash preparations mature gamonts and oocysts of *Hepatozoon* sp. A. from experimental mosquitoes, *Culex pipiens* and *Culex andersoni* as well as gamonts in the mite, *Ixodiderma inverta* fed on *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A - C)** Mature extracellular gamonts in gut contents of the mosquito. **(D & E)** Uninucleate and binucleate oocysts in mosquitoes, though their exact source is unknown. **(F & G)** Free gamonts surrounded by erythrocytes in various stages of digestion in the gut contents of *Ixodiderma inverta*. **(H & I)** Histological sections of *I. inverta* showing ingested blood (arrows). Scale bars: **(A - C)** = 10 µm **(D & E)** = 20 µm **(F & G)** = 10 µm **(H)** = 100 µm **(I)** = 10 µm.

Morphologically and morphometrically these gamonts were identical to the intraerythrocytic gamonts described here from the blood of *P. melanotus*. No developmental stages were observed in subsequent H&E stained histological sections (Fig. 3.9 H & I) though mites contained digesting lizard blood cells.

3.1.14 Remarks

The haemogregarine reported here was likely found for the first time in five *P. melanotus* lizards from Clarens (28˚31'S, 28˚25'E) in the Eastern Free State (Van As, 2003), although it was not described in any great detail at that time. The haemogregarine described in the current study forms both immature and mature gamonts in the blood of *P. melanotus* and *P. subviridis*, but does not apparently divide in the erythrocytes, or cause their lysis. Light and TEM observations on blood stages suggest, therefore, that it is unlikely a member of the genus *Haemogregarina* or of *Karyolysus.* Squash/smear, histological, confocal and TEM studies also demonstrate that it forms macro- and micromeronts in lizard liver tissue, suggesting it belongs to the genus *Hepatozoon* (see Smith, 1996). This view is supported by its development in natural and experimental mosquito hosts, and the absence, apparently, of any development in mites. Although gametogenesis and fertilization were not observed in mosquitoes and mature, polysporocystic oocysts were not detected, young oocysts with one, two and multiple nuclei were seen, and numerous sporocysts containing sporozoites were located in naturally infected mosquitoes found feeding on lizards with this haemogregarine. For these reasons the haemogregarine is provisionally identified as *Hepatozoon* sp. A.

Hepatozoon sp. A infections in peripheral blood from *P. melanotus* and *P. subviridis* are morphologically alike in overall shape and staining properties, except for a few additional granules in the cytoplasm of the *P. subviridis* parasites (Fig. 3.2 C & E). Morphometrically the two infections differ only slightly in that the mature gamonts of the *Hepatozoon* from *P. subviridis* measure 17.4 - 22.0 x 5.4 - 7.6 µm, whereas those of the *Hepatozoon* from *P. melanotus* measure 15.8 - 21.8 x 3.2 - 7.3 µm, and thus they are almost identical in maximum length, and the latter are only marginally narrower. The current *Hepatozoon* also distorted infected erythrocytes in both lizard species to a similar extent. Based on all the above, it can be concluded that both P. *melanotus* and *P. subviridis* are infected with *Hepatozoon* sp. A.

When compared with other *Hepatozoon* species from the same hosts (*P. melanotus* and *P. subviridis*), mature gamonts of *Hepatozoon* sp. A (15.8 - 21.8 by 3.2 - 7.3 µm in *P. melanotus*; 17.4 - 22.0 by 5.4 - 7.6 µm in *P. subviridis*) are marginally longer (in maximum length) than those of *Hepatozoon* sp. B (15.8 - 18.8 by 5.5 - 7.8 µm, (see below), only in *P. melanotus*), but overall are shorter and narrower than those of *Hepatozoon* sp. C (15.5 - 32.6 by 3.9 - 8.6 µm in *P. melanotus*; 23.6 - 40.8 by 4.3 - 8.5 µm in *P. subviridis*, (see below). The parasite nucleus of *Hepatozoon* sp. A is more compact, deeper staining and more rounded, with little evidence of chromatin strands or blocks, compared with *Hepatozoon* spp. B & C in which nuclei predominantly have chromatin strands and chromatin blocks respectively. The cytoplasm of *Hepatozoon* sp. A also has fewer cytoplasmic granules compared with *Hepatozoon* spp. B & C (see below).

When compared to other lizard haemogregarines across Africa, *Hepatozoon* sp. A is unlike any described thus far, apart from the haemogregarine noted in the blood of *P. melanotus* at Clarens in the Free State (see above; Van As, 2003). However, it does overlap in size with *Hepatozoon mabuiae* (Nicolle et Comte, 1906) in the scincid lizard, *Trachylepis vittata* from Tunisia which measures 14 - 17 by 5 - 6µm (see Table 1.1). When *Hepatozoon* sp. A is compared with *Hepatozoon gracilis* Wenyon, 1909 (see Table 1.1), from another skink, *Trachylepis quinquetaeniata* in Sudan, *Hepatozoon* sp. A gamonts are much broader than those of *H. gracilis* which are long and slender (18 - 22.2 x 0.9 - 1.4µm), although both species caused a slight degree of hypertrophy of host erythrocytes, and lateral or sometimes terminal, displacement of host cell nuclei.

The merogonic stages of *Hepatozoon* sp. A follow the same overall development pattern as recorded by Wenyon (1909) and Bashtar et al. (1987) for *H. gracilis*. Wenyon (1909) reported macromeronts with 8 - 16 macromerozoites in the liver of the lizard and micromeronts with an "enormous number" of micromerozoites. Bashtar et al. (1987) reported "micromeronts" that produced 3 - 16 macromerozoites and "macromeronts" that produced up to 25 - 50 micromerozoites. *Hepatozoon* sp. A macromeronts in liver tissue measured 25.9 - 33.9 by 20.1 - 26.7 µm and produced 2 - 7 macromerozoites, fewer overall than the macromerozoites from *H. gracilis.*

The sporogonic stages of the current species follow the same general pattern as in the descriptions of Bashtar et al. (1987) for *H. gracilis.* Although the dimensions of the oocyst stages were not stated by Bashtar et al. (1987), they were present in the haemocoel of the experimental mosquito host *Culex pipiens molestus* on day 5 to 8 post infection. Large oocysts of the current species (~51 x 44 µm with an area of ~2243µm²) were seen in squashes of *Culex pipiens* and *C. andersoni* 3 days post feeding on *Hepatozoon* sp. A infected blood. Subsequent dissections of these mosquitoes revealed only these oocysts and possible sporocysts, but no sporozoites were seen in these experimental mosquitoes. In wild *Culex lineata* specimens (Fig. 3.10 A) collected while feeding on *Hepatozoon* sp. A infected lizards (Fig. 3.10 B), oocysts (Fig. 3.10 D & E) of similar size as in experimental mosquitoes (~54 x 48 µm with an area of ~2093µm²) were seen in the gut contents from 1 - 3 d.p.f. Elliptical sporulating sporocysts (Fig. 3.10 F) and subsequent stages with nuclear division (Fig. 3.10 G & H) were seen in *C. lineata.* Two *C. lineata* individuals also revealed sporocysts with developing sporozoites and these sporocysts (~24 x 26) µm were approximately similar to those of *H. gracilis* (~22 x 19) µm obtained from *C. pipiens molestus.* The current species apparently differed from *H gracilis* by having 2 - 8 large sporozoites per sporocyst, that ranged from 16.8 - 32.4 µm in length by 4 - 9 µm in width, whereas *H. gracilis* produced more sporozoites (8 - 24) per sporocyst, but these were smaller ~10 x 2 µm.

Although this haemogregarine (Fig. 3.10 C) was likely first found in *P. melanotus* in Clarens (Van As, 2003), this is the first report of a *Hepatozoon* species from such isolated populations of *P. melanotus* and *P. subviridis* at altitude and it is provisionally given the name *Hepatozoon* sp. A, as reported above. The elucidation of the merogonic stages in the lizard host liver tissue using a variety of methods, as well as careful study of the blood stages and the identification of sporogonic developmental stages in mosquitoes strongly support the parasite's designation to this haemogregarine genus (Fig. 3.10 D - H). It is proposed that *Culex* (*Afroculex*) *lineata* is the natural definitive host of *Hepatozoon* sp. A, although gametogenesis and fertilization in this host were not observed.

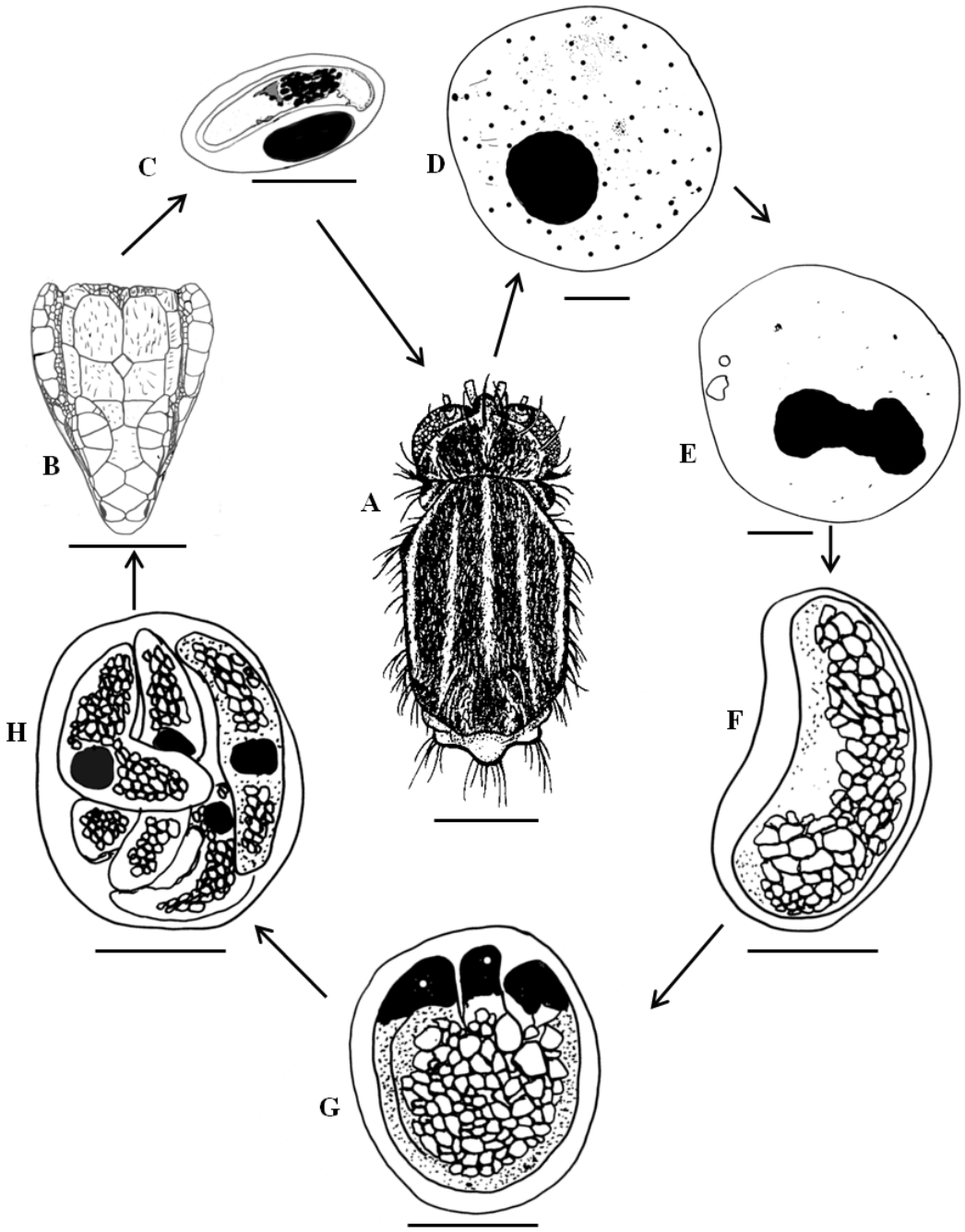

Figure 3.10 Diagrammatic representation of the presumed development of gamont and sporogonic stages of *Hepatozoon* sp. A in the natural vector *Culex* (*Afroculex*) *lineata* **(A)** (dorsal view, redrawn from Jupp, 1996), and of gamonts derived from liver meronts (not shown) in **(B)** *Pseudocordylus melanotus* (lizard head redrawn and adapted from Fitzsimons, 1943). **(B)** The lizard ingesting sporozoites from an infected mosquito. **(C)** After merogony occurs in lizard liver, micromerozites presumably become intraerythrocytic gamonts that are taken by *C. lineata* from peripheral blood stream of the lizard during a blood meal. **(D)** Following fertilization, an oocyst with a single nucleus presumably results in the mosquito. **(E)** Oocyst with dividing nucleus. **(F)** One of many sporocysts derived from multinucleated oocysts (not shown). **(G)** Developing sporocyst with dividing nuclei and cytoplasm. **(H)** Sporocyst with mature sporozoites in the mosquito, presumably a source of sporozoites for the lizard. Scale bars: **(A)** 500nm **(B)** 15mm **(C)** = 10µm **(D & E)** = 10µm **(F - H)** = 15µm.

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

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

Locality: Platberg, Eastern Free State, 1900 - 2390m

3.2.1 Systematics (Lee et al. 2000)

Phylum Apicomplexa Levine, 1970

Class Conoidasida Levine, 1988

Order Eucoccidiorida Léger & Dubosq, 1910

Suborder Adeleorina Léger, 1911

Family Hepatozoidae Wenyon, 1926

Genus *Hepatozoon* Miller, 1908

3.2.2 Prevalence

Hepatozoon sp. B was found in 8/69 (prevalence, 11.6%) *Pseudocordylus melanotus* specimens captured at various rocky outcrops on the summit of Platberg in the Eastern Free State. The infected lizards were six females and two males. These lizards also had mixed infections of other blood protozoans. This haemogregarine was found in 3/69 lizards with no other haemogregarine species. Additionally, this haemogregarine and *Hepatozoon* sp. C were found co-infecting 2/69 lizards (prevalence, 2.9%) and co-infecting 3/69 lizards (prevalence, 4.3%) with *Hepatozoon* sp. A. Gamonts of the current haemogregarine were intracytoplasmic and found in mature erythrocytes (Fig. 3.11 A - G). Extracellular stages were not seen in blood films.

3.2.3 Parasite description

In Giemsa-stained blood films parasites occurred singly in erythrocytes and some immature stages were noted for this species. Mature gamonts infected up to 22/1000 (2.2%) of mature red blood cells and were broadly elongated and sausage shaped, sometimes with a slightly broader anterior, and a detectable reflexed posterior region (Fig. 3.11 C & F red arrows). The main parasite body measured 17.6 ± 0.9 (15.8 - 18.8) in length by 6.6 ± 0.8 (5.5 - 7.8) µm in

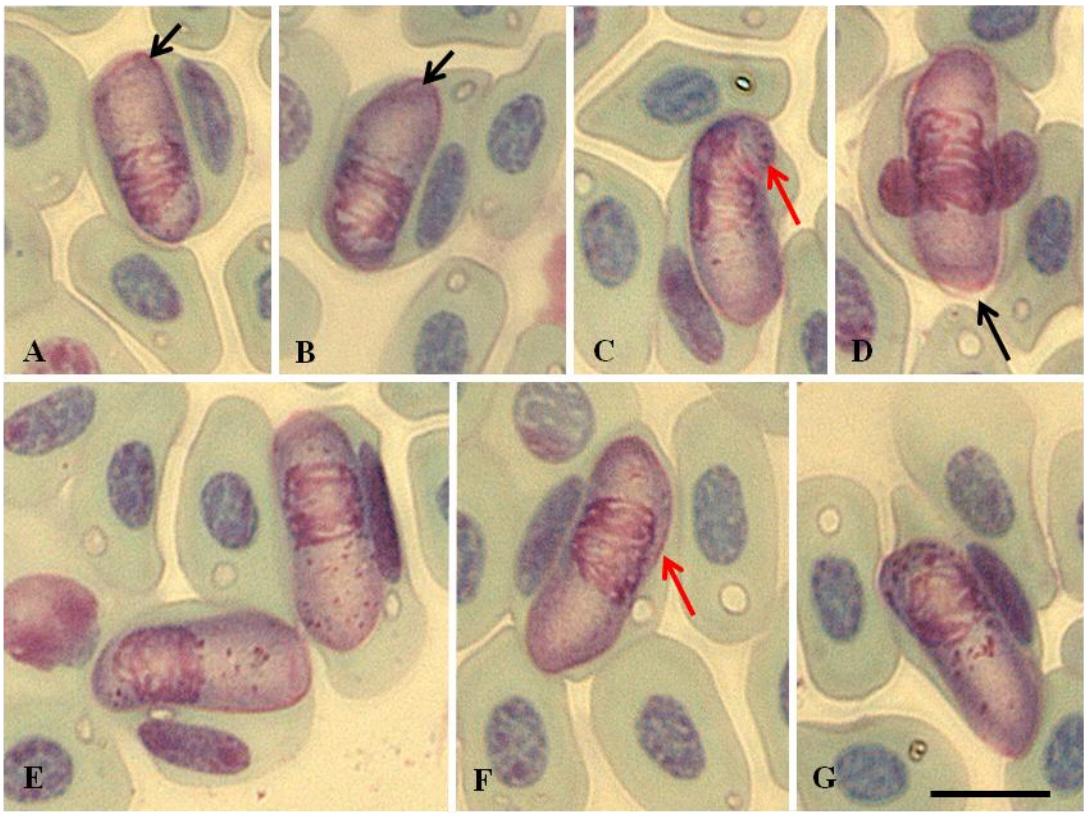

Figure 3.11 (A - G) Light micrographs of Giemsa stained peripheral blood films containing *Hepatozoon* sp. B from *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A - C)** Immature gamonts. **(D - G)** Mature gamonts, gamont in **(D)** appears to have split the host nucleus into two fragments. Red arrows indicate reflexed posterior region, black arrows indicate very faint pinkish/light purple cap. Note almost all erythrocytes have so-called *Sauroplasma* infections. Scale bar: **(A - G)** = 10 µm.

width (n=23), had a broadly pointed or sometimes flattened anterior pole (Fig. 3.11 C) and a rounded posterior pole associated with the reflexed region (Fig. 3.11 C, F).

Mature gamonts stained mostly pale purple with Giemsa and the cytoplasm had distinct, pinkish granules, especially in the vicinity of, and anterior to, the nucleus. Each nucleus stained pinkish purple and comprised stranded chromatin threads. These nuclei measured 6.3 ± 0.9 (5.0 - 7.8) µm (n=23) in length by 5.9 ± 0.7 (4.8 - 7.1) µm in width (n=23) and were located nearer to the posterior pole of the parasite body (Fig. 3. 11 A, B, D, E & G). The anterior end of the parasite bore a very faint pinkish/light purple cap (Fig. 3.11 A, B & D black arrows).

3.2.4 Effects on host cells

Effects on host cells were marked, the gamonts displacing the host nucleus to the side of the erythrocyte and reducing its nuclear width by ~50 % in some instances. Displaced nuclei of parasitised red blood cells measured 8.4 ± 1.0 (6.3 - 9.7) long by 2.9 ± 1.2 (2.3 - 3.7) µm wide (n=60), whereas non-parasitised erythrocyte nuclei measured 6.5 ± 0.6 (5.2 - 7.5) long by 4.0 ± 0.6 (3.3 - 4.9) µm in wide (n=60). The area of the non-parasitised erythrocyte nucleus measured 23.7 ± 2.6 (16.4 - 26.7) µm² (n=60) compared to the area of a compacted, parasitised red blood cell nucleus which measured 23.1 ± 3.8 (15.6 - 29.7) µm² (n=20). On rare occasions the infected host cell nucleus was apparently halved by the parasite (Fig. 3.11 D), but no erythrocytes without nuclei were observed.

3.2.5 Remarks

This parasite, from the peripheral blood of a *Pseudocordylus melanotus* population on a rock outcrop on Platberg, Eastern Free State, apparently lacks division stages in the blood of this lizard and thus it is provisionally allocated to the genus *Hepatozoon*, although other stages in its development are unknown. Although it resembles *Hepatozoon* sp. A (above) in its dimensions (see above) it is marginally shorter, and its apparently reflexed tail region, nuclear characteristics and cytoplasmic granules indicate that it may be a different species. It is also very different from *Hepatozoon* sp. C., which is much larger (see below). This is the first report of this haemogregarine in the peripheral blood of *Pseudocordylus melanotus.*

In general dimensions, two other African lizard haemogregarines, but from lacertids host, namely *Haemogregarina acanthodactylii* Ramadan, 1974 and *Hepatozoon boskani* Catouillard, 1909 from an Egyptian lizard *Acanthodactylus boskianus* (Lacertidae) (see Table 1.1), are similar to this second haemogregarine species from *P. melanotus* in size. For example, in width, this current, relatively broad, *Hepatozoon* species, measuring 15.8 - 18.8 by 5.5 - 7.8 µm, resembles *H. acanthodactylii* (which measures 10 - 11 x 5.5 - 5.6µm). On the other hand, *H. boskani* measures 4 - 8.5 by 16 - 16.5 µm and thus, the haemogregarine described just exceeds it in length. Gamonts of *H. boskani* are also apparently oval or slightly reniform, and sometimes broader at one end than the other and therefore differ from the current haemogregarine in overall appearance. The gamonts observed here also appeared to have reflexed tails, not characteristic of *H. acanthodactylii* or *H. boskani.*

Thus, it seems possible that the current haemogregarine is different from other cordylid haemogregarines described in this Chapter 3, and differs from other African lizard haemogregarines of broadly similar dimensions. Although merogony and sporogony are unknown for this species it is provisionally named as *Hepatozoon* sp. B.

3.3 *Hepatozoon* sp. C from *Pseudocordylus melanotus* (A. Smith, 1838) and *Pseudocordylus subviridis* (A. Smith, 1838)

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

Type Locality: Platberg, Eastern Free State, 1900 - 2390m

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

Localities: Sentinel area, Northern Drakensberg, Eastern Free State, 2589 - 3050m

3.2.1 Systematics (Lee et al. 2000)

Phylum Apicomplexa Levine, 1970

Class Conoidasida Levine, 1988

Order Eucoccidiorida Léger & Dubosq, 1910

Suborder Adeleorina Léger, 1911

Family Hepatozoidae Wenyon, 1926

Genus *Hepatozoon* Miller, 1908

3.3.2 Prevalence

3.3.2.1 *Pseudocordylus melanotus*

Hepatozoon sp. C was found in 16/69 (prevalence, 23.1%) of *Pseudocordylus melanotus* specimens captured at various rocky outcrops on the summit of Platberg in the Eastern Free State. The infected lizards were nine males and seven females. Of these 16 lizards, two (2.8%) were also infected with *Hepatozoon* sp. B, five (7.1%) with *Hepatozoon* sp. A, and nine (12.8%) were co-infected with *Plasmodium* sp. A. All lizards with an infection of *Hepatozoon* sp. C also had so-called *Sauroplasma.* Gamonts were mostly intracytoplasmic, and found in mature erythrocytes. Extracellular stages were also noted in blood films. Parasitaemias ranged from 1/1000 to 30/1000 red blood cells infected.

3.3.2.2 *Pseudocordylus subviridis*

The same haemogregarine was found in the peripheral blood of 16/29 (prevalence, 55.1%) of crag lizards (*Pseudocordylus subviridis*) sampled across an altitudinal gradient of the Sentinel trail. Sometimes, this species was found with other haematozoans. Mature gamonts were the most abundant in blood films, although immature, young and extracellular gamonts were also observed in blood preparations from this host. *Hepatozoon* sp. C was found together with *Hepatozoon* sp. A in 2/29 of these lizards (prevalence, 6.8%).

Hepatozoon sp. C infections were only seen in mature erythrocytes in the peripheral blood with a parasitaemia ranging from 1/1000 (0.1%) to 18/1000 (0.18%). This species was sometimes part of mixed infections of *Plasmodium* sp. A, several filarial nematode species and in almost all the cases, so-called *Sauroplasma*.

3.3.3 Parasite description

3.3.3.1 *Pseudocordylus melanotus*

In Giemsa-stained blood films erythrocytic parasites occurred singly. Immature gamonts (Fig. 3.12 A - D) were typically elongated, with rounded extremities and with a posteriorly placed nucleus consisting of dark, reddish-purple coarse, chromatin granules, especially peripherally. Reddish-pink pigment granules occurred in the cytoplasm of immature gamonts and appeared very characteristic of this species (Fig. 3.12 A - C). Apart from the granules, the cytoplasm of immature gamonts stained light purple. Young gamonts measured 22.7 ± 2.5 (19.4 - 32.1) in length by 4.7 ± 0.4 (3.9 - 5.4) µm in width (n=40). The posteriorly situated nucleus measured 7.2 ± 1.3 (3.9 - 9.9) in length by 4.3 ± 0.6 (3.3 - 5.4) µm in width (n=40).

Maturing gamonts (Fig. 3.12 E - H) infected up to 30/1000 (3%) of mature red blood cells and were elongated, sausage shaped, and sometimes had a broad anterior and had a reflexed posterior region, though this was not always easy to detect (Fig. 3. 12 E, F red arrows). The main body of these parasites measured 24.2 ± 4.8 (15.5 - 32.6) in length by 6 ± 1.3 (3.9 - 8.6) µm in width (n=40). Maturing gamonts (Fig. 3.12 E - I), stained mostly purple with Giemsa and the cytoplasm had the characteristic cytoplasmic granules seen in immature gamonts. The nucleus comprised two to five large chromatin blocks and a few chromatin strands, and stained a dark reddish-purple. The nucleus measured 4.8 ± 1.4 (3.2 - 8.2) in length by 4.7 ± 1.1 (2.6 - 6.8) µm in width (n=30) and was located in the second third of the parasite body, towards the posterior end. Extracellular gamonts were worm-like in appearance (Fig. 3.12 I) and measured ~30.1 by 8.5 µm (n=1). Nuclei of extracellular gamonts were of the same dimensions as those of maturing gamonts in erythrocytes.

3.3.3.2 Ultrastructure of the intracytoplasmic gamont

Ultrathin sections of infected *P. melanotus* blood examined by TEM revealed the recurved, posterior tail region of *Hepatozoon* sp. C (Fig. 3.12 J), which had not always been evident in light micrographs. Gamonts lay within a capsule, tightly adherent to the pellicle, and within a parasitophorous vacuole in the lizard host erythrocyte. The gamont nucleus contained osmiophilic, dense heterochromatin, as well as a large nucleolus (Fig. 3.12 J). The cytoplasm of the gamont contained dense granules and micronemes and these extended into the tail

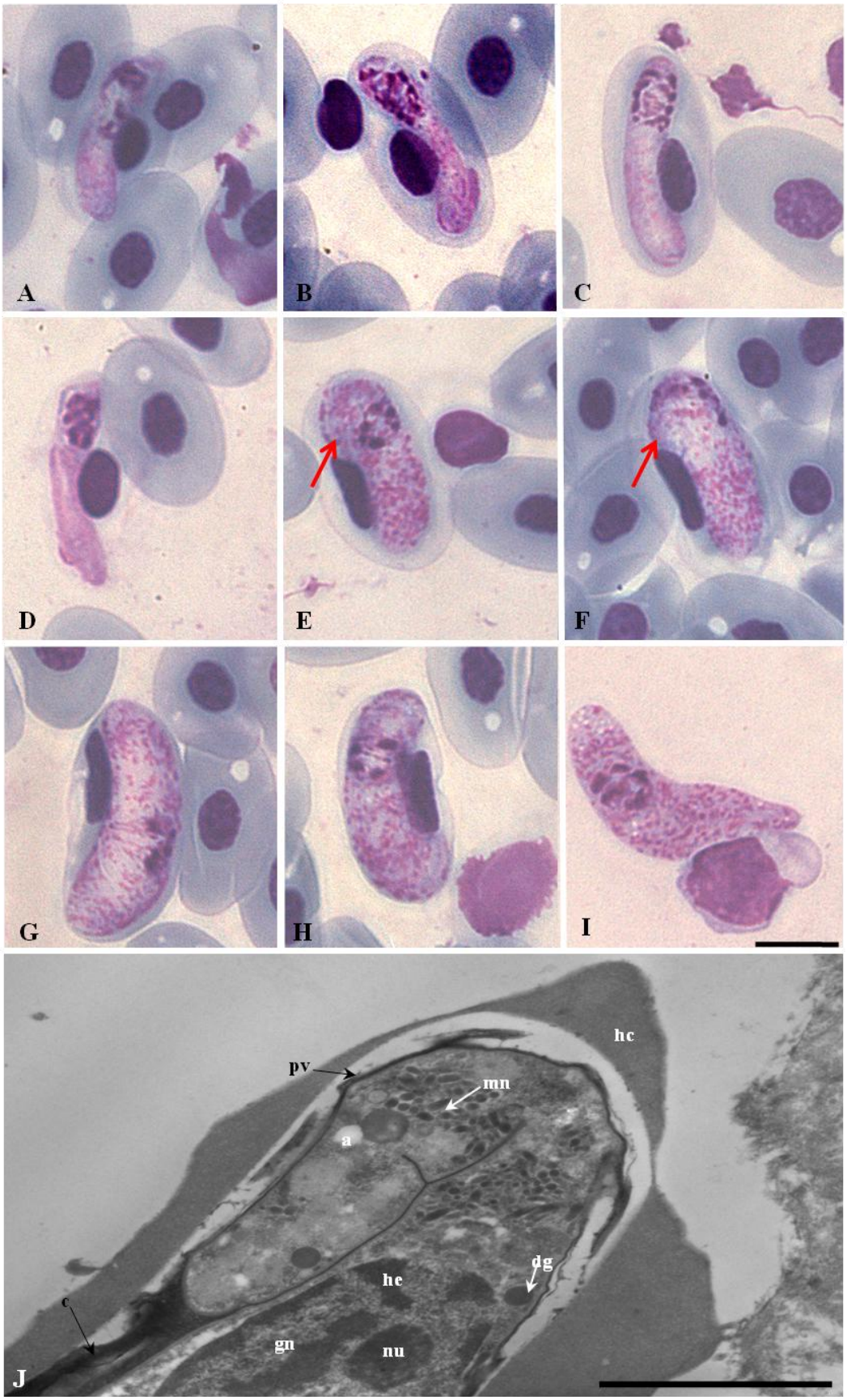

Figure 3.12 (A - I) Light micrographs of Giemsa stained peripheral blood films with *Hepatozoon* sp. C from *Pseudocordylus melanotus* from Platberg, Eastern Free State and transmission electron micrograph (TEM) of the same peripheral blood. **(A - D)** Immature gamont stages in erythrocytes. **(E - H)** Maturing and mature gamont stages. Note that some host erythrocytes appear dehaemoglobulized. **(I)** Extracellular gamont. Red arrows indicate the reflexed, posterior, tail region. **(J)** Transmission electron micrograph of a longitudinal section through the posterior, recurved tail of a gamont within a parasitophorous vacuole (pv) and capsule (c) in the host erythrocyte (hc), showing also a dense granule (dg) and micronemes (mn) which extend into the tail. Also present is a nucleolus (nu) within the gamont nucleus (gn) which contains heterochromatin (he). Scale bars: **(A - I)** = 10µm **(J)** = 2µm.

region. Electron-lucent vacuoles, possibly representing amylopectin, were also present in the cytoplasm of the gamont.

3.3.3.3 Stages in the peripheral blood of *P. subviridis*

Immature gamonts (Fig. 3.13 A - H) were present in blood films of 3/29 (prevalence, 10.3%) lizards with a parasitemias of 2/1000, 5/1000 and 10/1000 erythrocytes infected respectively. Immature stages were relatively rare compared to maturing or mature gamonts, and were rounded at each pole, narrow and elongated, and infected mature erythrocytes (Fig. 3.13 A - H). The cytoplasm of immature gamonts stained light purple having distinct, intensely pink-stained areas at each pole, extending towards the nucleus and round vacuoles were present anterior and posterior to the nucleus (Fig. 3.13 B, F, G & H red arrows). Overall young gamonts measured 13.2 ± 1.5 (11.1 - 17.3) µm long by 5.7 ± 1.6 (3.4 - 8.9) µm wide (n=30). Nuclei of immature gamonts measured 4.5 ± 1.0 (1.9 - 5.64) µm long by 4.9 ± 1.2 (2.6 - 7.1) µm wide (n=30). Erythrocytes infected with young gamonts measured 17.3 ± 3.2 (12.6 - 23.8) µm long by 13.4 ± 1.2 (11.2 - 16.2) µm wide (n=30) with a surface area of 193.4 ± 39 (124.6 - 264.2) µm² (n=30). Infected erythrocyte nuclei measured 7.1 ± 0.9 (5.3 - 8.7) µm long by 4.4 ± 0.6 (3.5 - 6.2) µm wide (n=30) with a surface area of 26.8 ± 4.3 (18.7 - 35.2) µm² (n=30).

Maturing gamonts (Fig. 3.13 I - N) were broader than immature gamonts, with the nucleus more defined. These maturing gamonts measured 23.1 ± 4.8 (15 - 31) (n=30) µm long by 6 ± 1.0 (4.0 - 7.9) µm wide (n=30). Their nuclei stained dark purple with coarse, compact chromatin strands and granules, and measured 6.1 ± 1.9 (2.9 - 9.6) µm long by 4.5 ± 1.4 (1.1 - 6.4) µm wide (n=30). Erythrocytes infected with maturing gamonts (Fig. 3.13 J - N) measured 20.9 ± 2.8 (15.2 - 26.1) µm long by 12.5 ± 1.9 (9.8 - 16.5) µm wide (n=30) with a surface area of 213.1 ± 39 (124 - 271.2) µm² (n=30). Infected erythrocyte nuclei measured 7.6 ± 1.1 (5.8 - 10) µm long by 3.8 ± 0.5 (3.2 - 5.3) µm wide (n=30) with a surface area of 25.4 ± 3.8 (20.1 - 33.0) µm² (n=30).

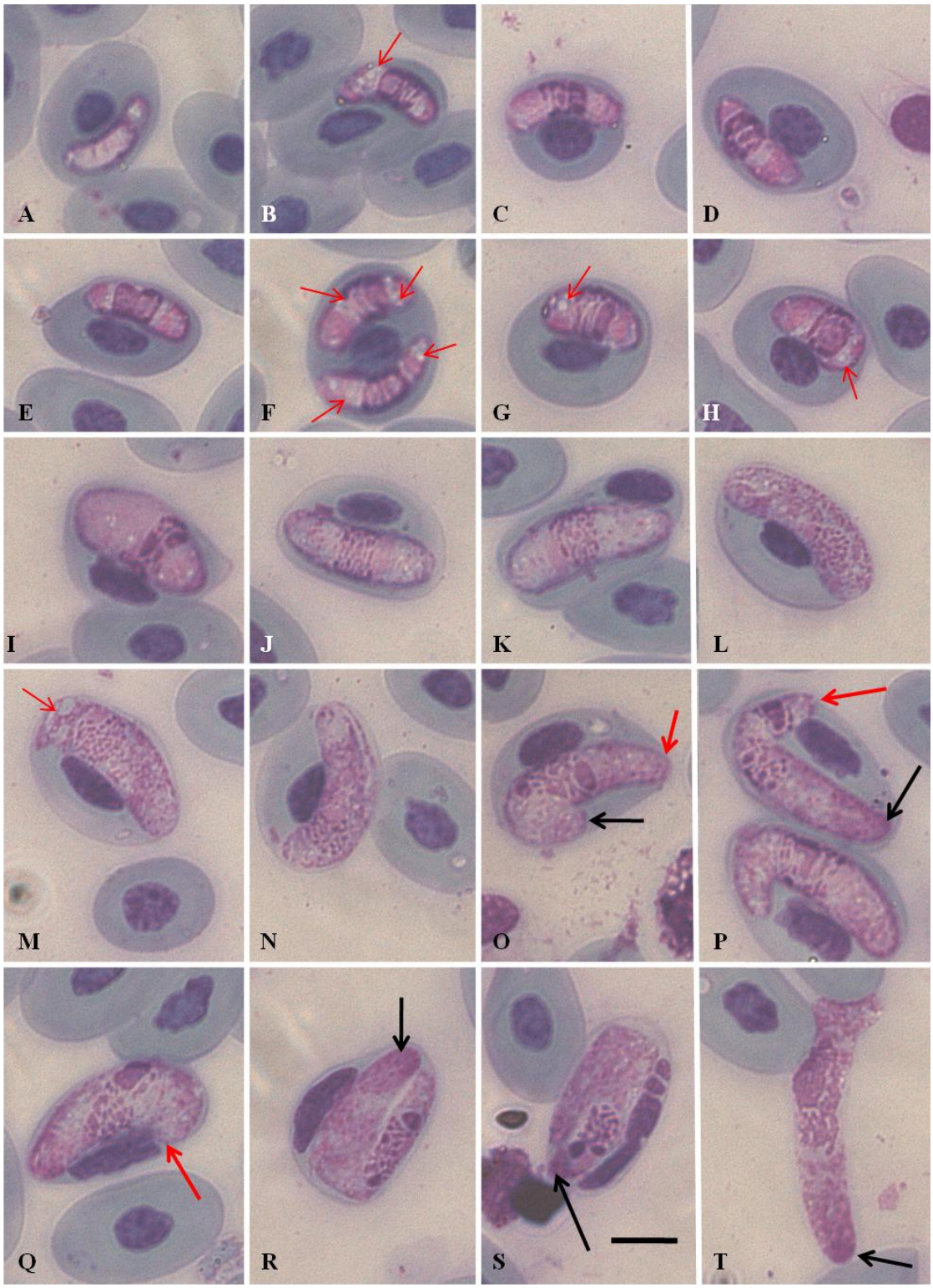

Figure 3.13 Micrographs of Giemsa stained blood films of *Hepatozoon* sp. C gamonts from *Pseudocordylus subviridis* from the North Eastern Drakensberg. **(A - N)** Immature and maturing gamonts. (**B, F, G & H & M)** red arrows indicate vacuolated cytoplasm. **(O - S)** Mature gamonts in erythrocytes displaying different effects on host erythrocytes. In the micrographs, black arrows indicate anterior caps. **(P & Q)** red arrows indicate reflexed posterior of the gamont. **(T)** Wormlike, extracellular gamont. Black arrow indicates its anterior cap. Scale bar: **(A - T)** = 10 µm.

Mature intraerythrocytic gamonts (Fig. 3.13 O - S) were elongated, with apparently curved edges and a broadly pointed anterior extremity (Fig. 3.13 O - Q red arrows). The anterior pole was slightly broader than the posterior pole with a cap which stained deep pinkish-purple (Fig. 3.13 O, P, R-T black arrows). Mature gamonts measured 34.2 ± 5.4 (23.6 - 40.8) µm long by 6.6 ± 1.0 (4.3 - 8.5) µm wide (n=75) at the broadest. The cytoplasm stained pale pinkish-purple, with pink granules in high concentrations anterior and posterior to the nucleus. The reflexed posterior extremity was clearly visible in most parasites, (Fig. 3.13 P & Q red arrows, R & S). Gamont nuclei stained deep purple, comprising two to five large, coarse, and many smaller, chromatin granules (Fig. 3.13 O - T). Nuclei measured 6.7 ± 2.4 (1.9 - 10.4) µm long by 5.0 ± 1.0 (2.5 - 76.9) µm wide (n=30) lying centrally within parasite body (Fig. 3.11 O), or closer to posterior (Fig. 3.13 R) or anterior (Fig. 3.13S) ends.

Extracellular mature gamonts were seen on six occasions, were worm-like, and generally narrower than their intracellular counterparts (Fig. 3.13 T); they measured 38.2 ± 2.4 (35 - 40) µm long by 7.4 ± 0.8 (6.6 - 8.5) µm wide (n=6). The cytoplasmic and nuclear staining was as in intracellular examples, and tail regions were also reflexed. In some individuals the nucleus was well forward in the parasite body, in others the nucleus lay centrally.

3.3.4 Effects on host cells

3.3.4.1 *Pseudocordylus melanotus*

Effects on host cells were marked, with some immature and mature gamonts displacing the host nucleus to the side of the erythrocyte and reducing its width considerably. Some maturing gamont stages also dehaemoglobulinized the red blood cells (Fig. 3.12 D - H). Infected red blood cells were larger than normal erythrocytes and measured 23.9 ± 0.9 (22.2 - 26.9) in length by 12.6 ± 1.0 (10.8 - 15.2) µm in width (n=30) with a surface area of 244.7 ± 18.4 (213 - 285) µm² (n=30), compared to non-infected erythrocytes with a surface area of 138.2 ± 24.2 (120 - 192) µm² (n=100).

3.3.4.2 *Pseudocordylus subviridis*

In general, infected erythrocytes stained lighter than non-infected erythrocytes, and as in *P. melanotus*, were sometimes dehaemoglobulinized (Fig. 3.13 Q - S). Infected cells were

bigger 191.7 ± 21.3 (161 - 223) µm² (n=60) than non infected cells which measured 144.5 ± 24 (121.8 - 191.6) µm² (n=100). Infections with mature gamonts resulted in the distortion of the host cell nucleus, including lateral compaction and displacement (Fig. 3.13 Q - S).

3.3.5 Stages in heart blood of *Pseudocordylus subviridis*

Stages of this type were only observed in blood smears taken directly from the heart, and the parasites generally did not appear as well preserved as in peripheral smears (Fig. 3.14 A - F). Gamont stages were elongated with rounded extremities. Their posterior poles were reflexed, sometimes curved (Fig. 3.14 C - E red arrows). The anterior extremity of the gamont bore a slightly darker staining cap (Fig. 3.14 C - F black arrows). Overall these gamonts measured 19.8 ± 0.88 (18.7 - 21.0) µm long by 3.5 ± 0.45 (3.1 - 4.2) µm wide (n=20). The cytoplasm of this species stained paler pinkish-purple, with pinkish granules anterior and posterior to the nucleus, though these granules were not as well defined as in parasites in peripheral blood smears. The dense nucleus stained dark purple, its position in the parasite body was variable, and it measured 6.5 ± 0.8 (5.7 - 8.0) µm long by 2.8 ± 0.5 (2.2 - 3.8) µm wide (n=7) (Fig. 3.14 C - E).

3.3.5.1 Effects on host cells from the heart blood of *Pseudocordylus subviridis*

Infected erythrocytes measured 182.1 ± 18.3 (174.0 - 198.6) µm² (n=45) and were larger than non-infected erythrocytes which measured 144.5 ± 24.4 (119.8 - 187.6) µm² (n=100). Infected erythrocytes stained lighter than non-infected erythrocytes and were markedly dehaemoglobulinized (Fig. 3.14 D & E). The host cell nucleus was often as compacted as in examples in the peripheral blood, and was also displaced laterally (Fig. 3.14 D & E**)** within the red blood cell.

3.3.6 Stages in the liver of *Pseudocordylus subviridis*

Gamont stages, similar in appearance to those in the peripheral and heart blood were observed in erythrocytes within liver impression smears (Fig. 3.14 G - J). These gamonts were also morphometrically identical to the gamonts in peripheral blood erythrocytes (described above) in Figure 3.13 O - S.

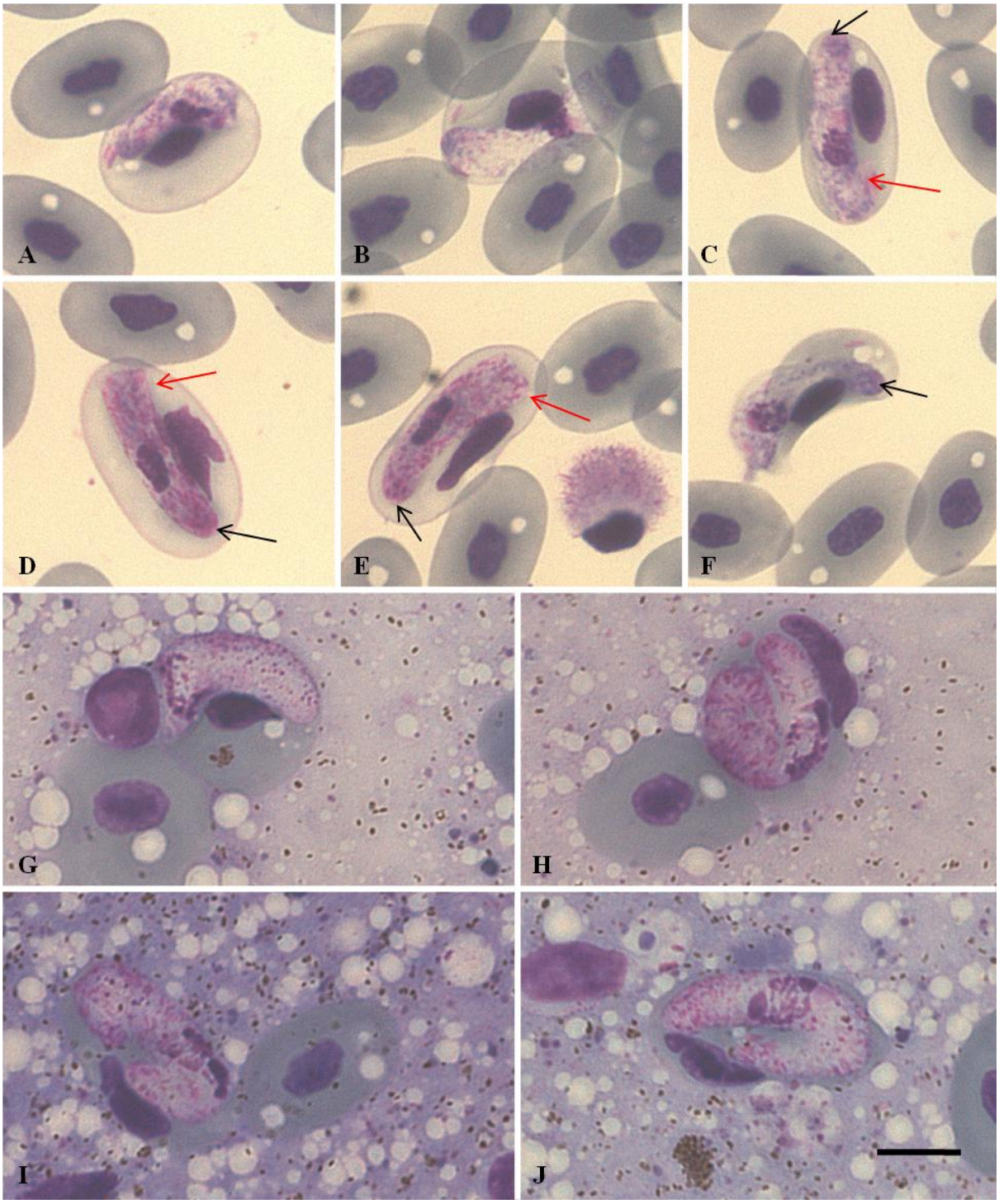

Figure 3.14 Micrographs of Giemsa stained heart blood films and liver tissue impressions of *Hepatozoon* sp. C gamonts from *Pseudocordylus subviridis* from the North Eastern Drakensberg. **(A)** Immature gamont and **(B - F)** maturing or mature gamonts in heart blood erythrocytes. **(C - E)** Red arrows indicate reflexed tail region. **(G - J)** Mature gamonts in erythrocytes from liver impression. Scale bar: **(A - J)** = 10 µm.

3.3.7 Merogonic Stages in the liver of *Pseudocordylus subviridis*

In liver smear preparations, meronts were observed primarily in hepatic cells, often surrounded by melanomacrophage pigment. In the specimen examined, only the liver harboured such developmental stages. Immature meronts (Fig. 3.15 A - E) measured 32 ± 5.5 (26 - 42) long by 18.4 ± 2.4 (15 - 21.8) µm wide (n=10) with a surface area of 511 ± 158 (332 - 790) µm² (n=10). Their contents were difficult to observe, but developing nuclei could be seen and granules, possibly of amylopectin, appeared abundant. Macromeronts (Fig. 3.15 F), containing ~8-9 macromerozoites each, measured 20.8 ± 1.8 (15.8 - 22.4) (n=10) µm long by 25.2 ± 2.1 (18.9 - 29.8) µm wide (n=10). Macromerozoite cytoplasm stained pale whitish blue, appeared foamy, and had pinkish granules distributed around the nucleus (Fig. 3.15 F).

Micromeronts (Fig. 3.15 G - J) were more abundant in liver impression smears and measured 29.2 ± 3.7 (24.9 - 32.2) long by 18.9 ± 4.05 (14.4 - 22.1) µm wide (n=10) with a surface area of 474.0 ± 147 (304 - 561) µm² (n=10). Micromeronts contained 8.7 ± 2.7 (2 - 11) (n=10) tightly-packed micromerozoites. Like the contents of immature meronts, those of the mature micromeronts were difficult to discern and only the micromerozoite nuclei and possibly, amylopectin reserves, could be observed in any detail. Micromerozoite nuclei stained a dark blue, and measured 4.0 ± 1.0 (2.8 - 6.2) by 2.5 ± 0.4 (1.9 - 3.1) µm (n=10).

3.3.8 Sporogonic stages

In squash preparations made 1 day post feeding from all three experimental mosquito species (*Culex andersoni bwambanus*, *Culex pipiens* and *Culiseta* (*Allotheobaldia*) *longiareolata*), haemogregarines and red blood cells (in different stages of digestion) from the recent blood meal were observed (Fig 3.16 A). Morphometrically these ingested gamonts had similar dimensions to gamonts in the erythrocytes of the host lizard**.** Gamonts in the gut contents of the mosquitoes thus had a broadened anterior (Fig. 3.16 A), were worm-like, and generally narrower than their intracellular counterparts (as in above description). However, they were of similar morphometric dimensions to the extracellular gamonts in peripheral blood of the

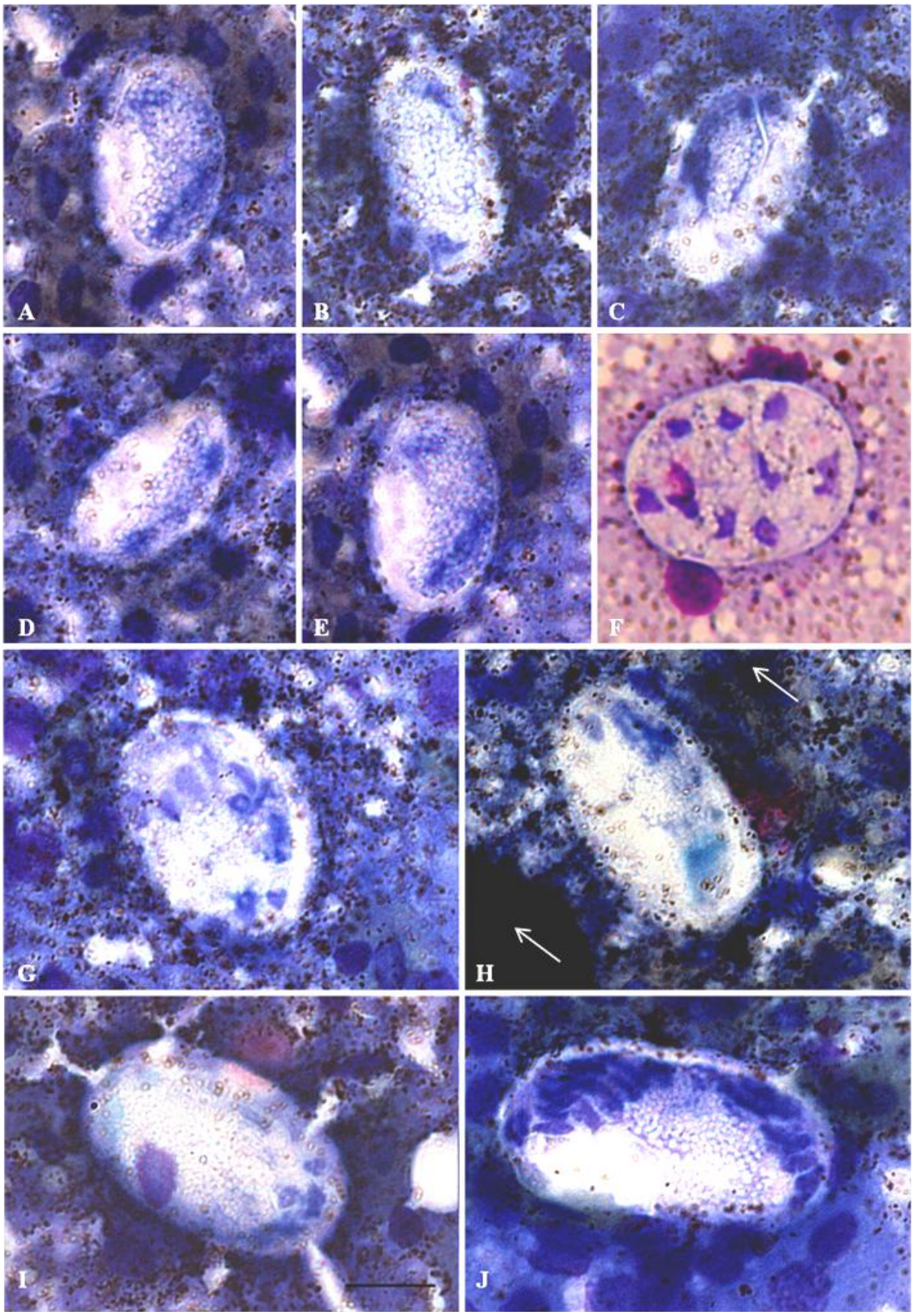

Figure 3.15 Micrographs of Giemsa stained liver tissue squashes of the merogonic stages of *Hepatozoon* sp. C. from *Pseudocordylus subviridis* from the North Eastern Drakensberg. **(A - E)** Developing meronts in liver. **(F)** Macromeront with fully developed macromerozoites. **(G - J)** Micromeronts showing different stages of micromerozoite development. White arrows indicate surrounding macromelanophage pigment in **(H)**. Scale bar: **(A - J)** = 10 µm.

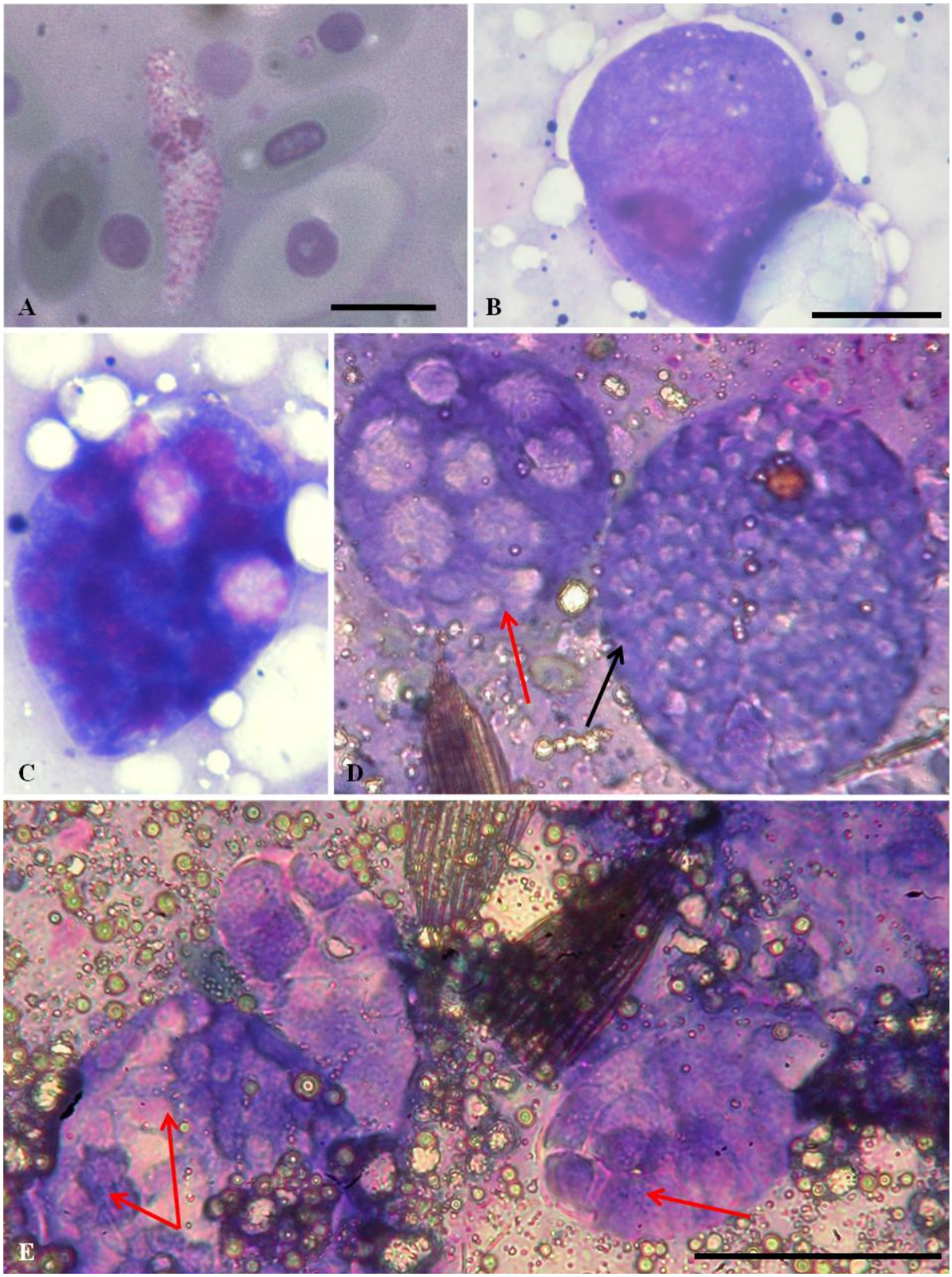

Figure 3.16 Micrographs of Giemsa stained squash preparation of *Culiseta* (*Allotheobaldia*) *longiareolata* and *Culex andersoni bwambanus.* **(A)** Mature, extracellular gamont of *Hepatozoon* sp. C from the blood meal of *C. longiareolata*. **(B & C)** Uninucleate and multinucleate oocysts from of *Culex andersoni bwambanus* and *C. longiareolata* respectively. Precise origin of oocysts is unknown. **(D)** Sporulating oocyst (red arrow) and maturing oocyst (black arrow) from the gut contents of *Culex andersoni bwambanus.* **(E)** Likely developing sporozoites (red arrows) within sporocysts from *Culex andersoni bwambanus*. Scale bar: **(A)** = 10 µm **(B & C)** = 20µm **(D & E)** = 30µm.

hosts. Cytoplasmic and nuclear staining was also similar. In most individuals the nucleus was well forward in the parasite body (Fig. 3.16 A).

Uninucleate and multinucleate oocysts (Fig. 3.16 B, C) were present in the gut contents of *C. longiareolata*. Uninucleate oocysts measured 52 ± 2 (50 - 54) µm in length by 46 ± 1.5 (45 - 48) µm in width (n=3) with an area of ~2392 µm². The nucleus of each uninucleate oocyst measured ~26.9 x 25.8 µm with an area of 603.48 µm². Multinucleate oocysts measured 55 ± 3.6 (51 - 60) µm in length by 46 ± 5 (40 - 52) µm in width (n=4) with an area of ~2530 µm².

Sporulating oocysts (Fig. 3.16 D) (red arrow) measured 40 ± 1.2 (39 - 42) µm in length by 45.3 ± 1.0 (44 - 47) µm in width (n=6). More mature, ruptured oocysts (Fig. 3.16 E) were also seen in mosquitoes. These contained numerous sporocysts with likely developing sporozoites (Fig. 3.16 E) and were frequently seen in squashes from all the experimental mosquitoes (*C. longiareolata*, *C. bwambanus* and *C. pipiens*) that had fed on *Hepatozoon* sp. C infected lizard blood.

3.3.9 Remarks

This species was probably first encountered in the blood of *P. subviridis* collected in Lesotho (29˚22’36’’S 28˚01’53E) (Van As, 2003), although, like the now named *Hepatozoon* sp A (above), it was not studied in great detail at the time. However, this is the first record of the Lesotho haemogregarine (designated *Hepatozoon* sp. C below) in *P. melanotus*, and from *P. subviridis* from the Free State and, as indicated earlier, it is much larger than the other four species (*Hepatozoon* sp. A, B, D, E) recorded from the three species of crag lizards at altitude in the Eastern Free State. Apart from its size, it is characterized by its cytoplasmic granules, the coarse nature of its nuclear chromatin and its recurved tail, seen in both blood films and TEM preparations. This species is most probably a *Hepatozoon* because of the meronts detected in lizard liver squashes, and the sporogonic stages in mosquitoes.

This species has been compared already with other species of *Hepatozoon* from *P. melanotus* and *P subviridis* (see sections 3.1.14 and 3.2.5). Several species of haemogregarines from other African saurians have also been considered earlier in this Chapter 3 (see sections 3.1.14

and 3.2.5) and *Hepatozoon* sp. C is different from these, especially in size, but also general morphology (see also below). The haemogregarine is also unlike most other gamont stages described from African saurians so far (Table 1.1), although its gamont dimensions in *P. melanotus* (15.5 - 32.6 by 3.9 - 8.6 µm) are reasonably close to those of *Hepatozoon burneti* Lavier et Callot, 1938 from a Tunisian gecko *Tarentola mauritanica* Linnaeus, 1758 which measure ~35 x 6µm. However, the intracellular gamont stages of *Hepatozoon* sp. C in *P. subviridis* are much longer (up to 40.8 µm long), and the extracellular stages of this species in *P. subviridis* also extended up to 40µm (see above). Mature gamont stages of the current species are similar in width, though not length, to those of *Hepatozoon boskiani* (mentioned in section 3.2.5), and those of *Haemogregarina damiettae* Ramadan, Saoud, Mohammed et Fawzi, 1996 from Egypt and *Hepatozoon psammodromi* (Soulié, 1904) from Algeria (see Table 1.1). Overall, however, the gamonts differ from known African saurian haemogregarines and haemococcidia, including the other four species reported in this chapter, in their extreme length and worm-like appearance when extracellular.

Beyond Africa, the closest match to the species described here is *Hepatozoon mesnili* Robin, 1936 from a Thailand gecko (*Gekko ventricillatus* Laurenti, 1768). *Hepatozoon mesnili* is characterized by falciform gamonts, 15 - 17 x 4 - 7 µm, with the anterior third of the gamont recurved on the median third. Extended gamonts of *H. mesnili* in an active infection measure 26 - 33 x 4.5 - 7.5µm and nuclei have prominent spaces between blocks of chromatin, giving them a vacuolated appearance. The dark staining reddish purple nuclei of the *Hepatozoon* species infecting *P. melanotus* also comprise several large chromatin granules, but extracellular gamonts are broader than in *H. mesnili*.

The merogonic stages of the current species follow the general life cycle pattern as reported by Robin (1936) for *Hepatozoon mesnili* in a Thailand gecko (*Gekko ventricillatus*). Meront stages (Fig. 3.17 B - G) of the current species compare morphometrically with those of *H mesnili* that measure 24 - 28 x 17 µm, but were found to occur in the lung of the gecko. Macromeronts of the current species were found only in the liver of *P. subviridis* (Fig. 3.17 A) and in the size range of 15.8 - 22.4 µm by 18.9 - 29.8 µm.

Sporogony of *H. mesnili* was obtained in *Culex fatigans* Wiedemann, 1828 and required about three weeks, while sporogony of the current species was obtained from experimental mosquitoes (*C. pipiens* and *C. andersoni*) and followed the same general pattern as for *H. mesnili* in *C. fatigans.* However, *H. mesnili* differed in having large oocysts (170 - 450 µm) in contrast to the oocysts of the current species in *C. pipiens* and *C. andersoni* that measured ~54 x 48 µm, although these were immature oocysts. Only developing sporocysts and immature sporozoites were seen in the current species, although mature sporocysts were observed in *H. mesnili*.

It has already been noted that this species is unlike the other two species (*Hepatozoon* sp. A found in *P. melanotus* from Platberg and in *P. subviridis* from the North Eastern Drakensberg and *Hepatozoon* sp. B from *P. melanotus* from Platberg). It is also unlike the other two species (*Hepatozoon* sp. D & E) from *P. langi* from the Drakensberg (see below). Interestingly, as well as the infection records found in *P. subviridis* from Lesotho (above; Van As, 2003), the current species (*Hepatozoon* sp. C) has been found in two disjunct populations, one *P. subviridis* population from the Drakensberg, and the other *P. melanotus* population 50km away in Platberg in South Africa.

As indicated above, this species in *P. melanotus* and *P. subviridis* is unique in that it has a distinctive large, granular body with an anterior cap, a reflexed tail, and a nucleus containing both large and small chromatin granules, and no other known species has a combination of these features. It is concluded, therefore, that *Hepatozoon* sp. C is new to science, and as well as its stages in the peripheral blood of the lizard, it undergoes merogonic development in the lizard liver and possible sporogonic development in three mosquito species (Fig. 3.17 A - K).

This next section is concerned with haemogregarines of two distinct types, based on general morphology, morphometric dimensions and staining properties, found in blood films from a third species of crag lizard, *Pseudocordylus langi*, collected at the top of the Drakensberg escarpment (~3050m) in the vicinity of the chain ladders, near the summit area of the Sentinel trail. Blood films also contained so-called *Sauroplasma*, like the other two *Pseudocordylus* spp. described in Chapter 5. One lizard also harboured a filarial nematode (described in Chapter 5).

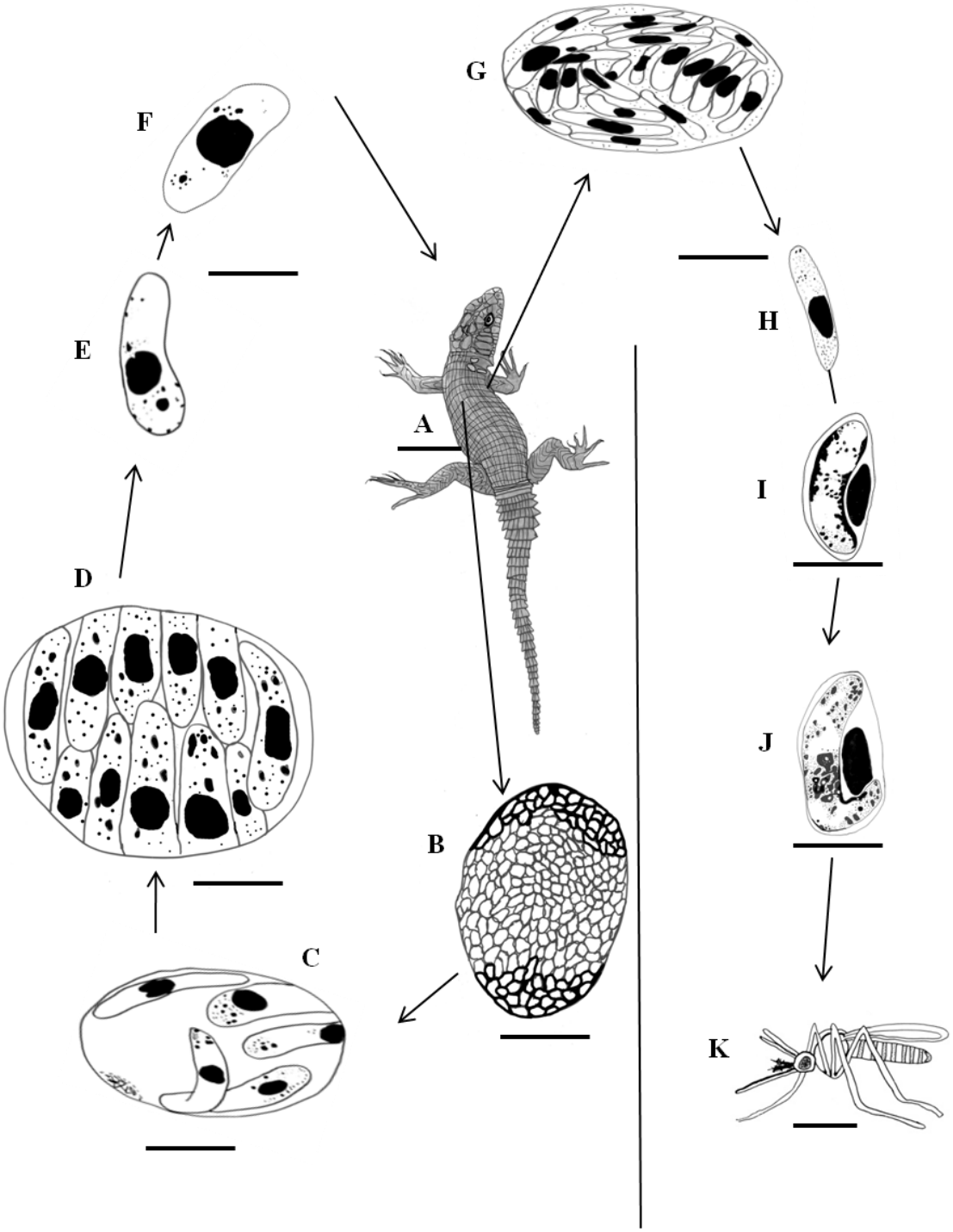

Figure 3.17 Diagrammatic representation of the merogonic stages of *Hepatozoon* sp. C in the liver of the crag lizard *Pseudocordylus subviridis*, the production of intraerythrocytic gamonts in the same host, and the transfer of gamonts to experimental vectors *Culex pipiens* and *Culex andersoni*. **(A)** *P. subviridis* with *Hepatozoon* sp. C infection. **(B)** Young meront in liver tissue. **(C)** Developing macromeronts. **(D)** Macromeront containing maturing macromerozoites. **(E & F)** Released macromerozoites in liver, presumably destined to form micromeronts in the liver of the same lizard. **(G)** Micromeront with micromerozoites in the liver of *P. subviridis*. **(H)** Micromerozoite presumably infecting host erythrocyte. **(I)** Immature gamont in lizard erythrocyte. **(J)** Mature *Hepatozoon* sp. C gamont in peripheral blood of *P. subviridis*. **(K)** Adult, female culicid mosquito (representing *C. pipiens* and *C. andersoni*) feeding on *Hepatozoon* sp. C infected lizard blood. Scale bars: (**A**) = 30mm (**B - J**) = 10µm (**K**) = 5mm.

In line with the other haemogregarines found in above section, the two described here will be assigned to the genus *Hepatozoon* (as *Hepatozoon* sp. D & E). However, these latter descriptions are based only on blood film stages and this placing is therefore tentative.

3.4 *Hepatozoon* sp. D. from *Pseudocordylus langi* (Loveridge, 1944)

Type Host: *Pseudocordylus langi* (Loveridge, 1944)

Type Locality: Escarpment area, Northern Drakensberg, Eastern Free State, 2950 - 3050m

3.4.1 Systematics (Lee et al. 2000)

Phylum Apicomplexa Levine, 1970

Class Conoidasida Levine, 1988

Order Eucoccidiorida Léger & Dubosq, 1910

Suborder Adeleorina Léger, 1911

Family Hepatozoidae Wenyon, 1926

Genus *Hepatozoon* Miller, 1908

3.4.2 Prevalence and general parasitaemia

This haemogregarine (Fig. 3.18) was found in the peripheral blood of 13/13 (prevalence, 100%) *Pseudocordylus langi* sampled from the chain ladders at Sentinel. It formed a mixed infection with *Hepatozoon* sp. E (described below) in 3/13 lizards (prevalence, 23%). *Sauroplasma* infections were also found in all 13 lizards.

Some gamont stages of this haemogregarine were seen in immature (erythroblasts), while others occurred in mature erythrocytes in peripheral blood smears, with parasitemias ranging from 1/1000 (0.1%) to 18/1000 (1.8%) across the host cell types.

3.4.3 Stages in the peripheral blood

Mature gamonts were the most abundant stages in blood films, although younger gamonts and extracellular forms were also observed. No division stages were found in blood smears.

Immature gamont stages were found in 1/13 lizards with a parasitaemia of 18/1000 (1.8%) and this stage infected mature erythrocytes (Fig. 3.18 A & B). Immature gamonts were elongate and slightly curved with a bluntly pointed, presumed anterior extremity. They measured 12.7 ± 1.4 (10.7 - 14.4) long by 3.8 ± 0.5 (3.2 - 4.6) µm wide (n=10). Their cytoplasm stained pinkish-purple and was vacuolated posteriorly, while their nuclei stained pale purple with stranded chromatin especially at the periphery of the nucleus. Young gamont nuclei measured 4.1 ± 3.7 (3.1 - 5.2) long by 4.5 ± 1.0 (3.3 - 6.4) µm wide (n=10).

Maturing and mature gamonts (Fig. 3.18 C - I) infected mature erythrocytes (Fig. 3.18 F), but were also seen in erythroblasts (Fig. 3.18 G). Intraerythrocytic gamonts (Fig. 3.18 C - H) were broadly elongated, with a rounded anterior extremity (Fig. 3.18 E - H red arrows) and some evidence of a capsule (Fig. 3.18 E, F). The anterior pole was also broader than the posterior pole, which often formed a characteristic, narrow, reflexed tail (Fig. 3.18 E, F). The anterior pole sometimes bore a characteristic deep purple staining cap (Fig. 3.18 C, E & F red arrows). Gamonts measured 19.1 ± 1.0 (15.4 - 28.1) long by 6.2 ± 1.1 (3.5 - 7.9) µm wide at the broadest (n=40). Cytoplasm stained pinkish-purple, sometimes with fine pink stippling granules (Fig. 3.18 C - I). The nucleus was often square, or oblong, in outline with finely stranded chromatin, and stretched across the width of the gamont. Each nucleus was usually centrally placed within the main parasite body. The gamont nucleus measured 5.1 ± 1.4 (2.5 - 8.7) long by 5.4 ± 1.5 (2 - 7.7) µm wide (n=30). Extracellular gamonts were seen only rarely (Fig. 3.18 I).

3.4.4 Effects on Host Cells

Some infected erythroblasts were dehaemoglobulinized (Fig. 3.18 G), staining lighter than their uninfected counterparts, but were not generally hypertrophied, except for some nuclei that were enlarged and pink-staining (Fig. 3.18 G). Infected red blood cells measured 18.4 ±

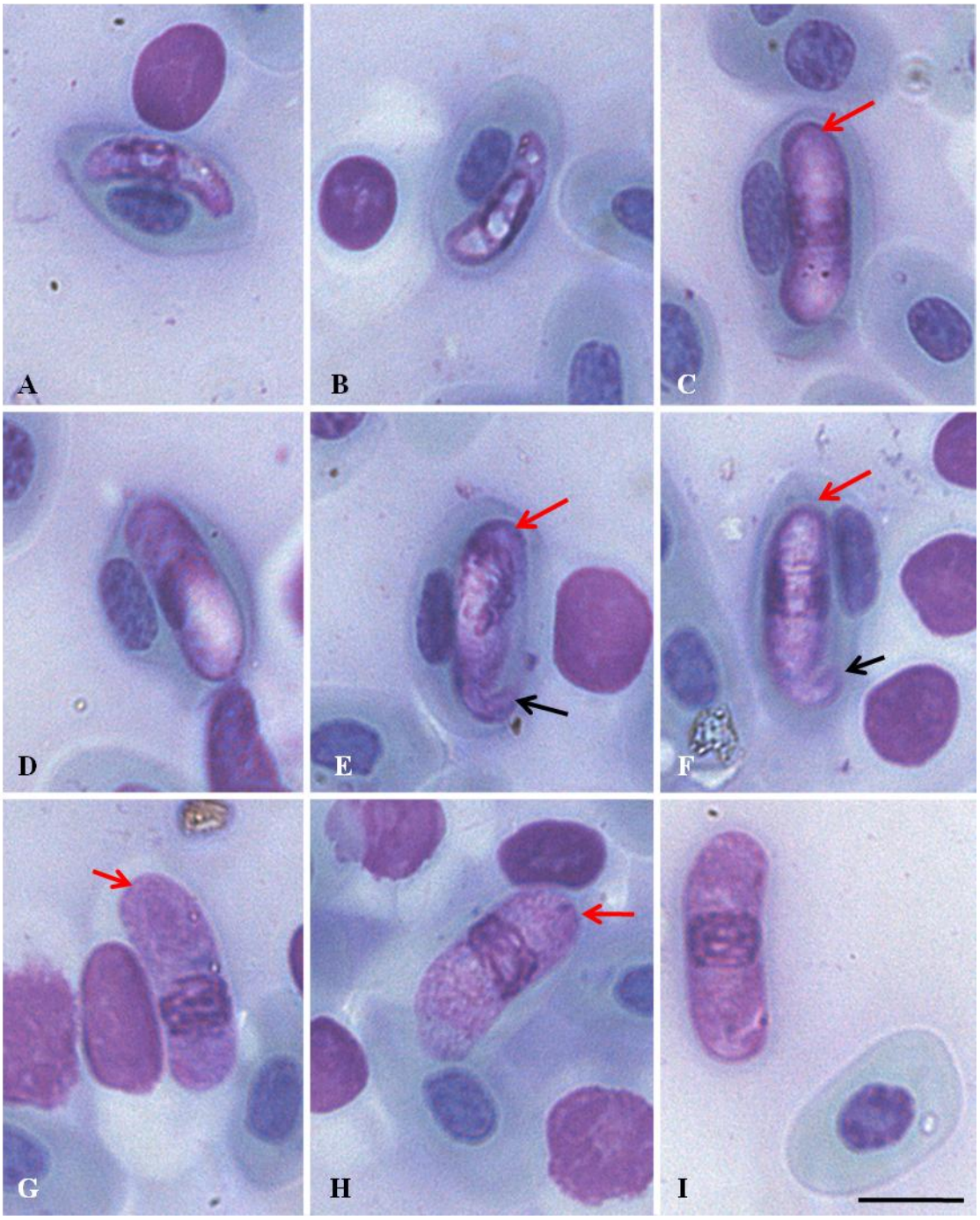

Figure 3.18 Micrographs of Giemsa stained blood films with *Hepatozoon* sp. D from *Pseudocordylus langi* from the Sentinel chain ladder in the North Eastern Drakensberg. **(A & B)** Immature gamonts. **(C - I)** Maturing and mature gamonts. **(E - H)** Gamonts in mature **(F)** and immature **(G)** erythrocytes. Host cell cytoplasm in **(G)** appears dehaemoglobulinized. Red arrows in **(C, E - H)** indicate rounded anterior end of parasite body, some with caps. Black arrows in **(E & F)** indicate reflexed, narrow posterior tails. **(I)** Extracellular gamont with an erythrocyte with so-called *Sauroplasma* sp. Scale bar: **(A - I)** = 10 µm.

1.6 (15.2 - 20.8) long by 12.9 ± 2.0 (9.6 - 18.5) µm wide (n=30), with a total surface area of 191 ± 25 (152.7 - 244.1) µm² (n=30). Non-infected erythrocytes measured 18.2 ± 1.2 (15.8 - 20.1) long by 9.9 ± 1.3 (6.9 - 11.2) µm wide (n=100), with a surface area of 151.1 ± 24.5 (120.3 – 194.4) µm² (n=100). The parasitized erythrocyte nucleus was slightly compacted and displaced laterally (Fig. 3.18 A, C, D, E & F) and measured 8.4 ± 0.6 (7.2 - 9.4) long by 4.1 ± 0.9 (2.7 - 6.1) µm wide (n=30) with a total surface area of 30.3 ± 7 (22.2 - 45.2) µm² (n=30). Uninfected erythrocytes had nuclear dimensions of 6.6 ± 0.9 (4.9 - 7.8) long by 4.5 ± 0.8 (3.9 - 4.9) µm wide (n=60) with a total surface area of 24.7 ± 3.1 (16.5 - 28) µm² (n=60).

3.4.5 Remarks

When compared with other *Hepatozoon* species from the blood of *Pseudocordylus* spp, mature gamonts of the haemogregarine described (19.1 x 6.2µm) here perhaps most closely resemble those of *Hepatozoon* sp. B in size, which measured 17.6 x 6.6µm. However, they differ in general morphology, mainly in the nucleus and the tail-like posterior ends seen in the current species. These features also distinguish it from *Hepatozoon* sp. A. The current haemogregarine differs by having a finely granular cytoplasm whereas *Hepatozoon* sp. B, and especially *Hepatozoon* C have more pronounced pinkish granules in their cytoplasm. Erythrocytes infected with the current haemogregarine were perhaps less distorted than in infections with *Hepatozoon* sp. A, B and C, with these species in some cases compacting and displacing laterally the host cell nucleus.

Morphometrically, *Hepatozoon* sp. D is in the same size range as *Hepatozoon mabuiae* (Nicolle et Comte, 1906) from the skink *Trachylepis vittata* (Table 1.1) and *H. damiettae* mentioned earlier (see section 3.3.9). However, it differs in staining properties and in the morphology of the nucleus from the above species. Features of *Heptozoon* sp. D are the likely encapsulated gamonts and its ability to infect erythroblasts; infections also resulted in hypertrophy of some host cell nuclei and occasional dehaemoglobulization. These features are not shared with *H. mabuiae* or *H. damiettae*.

In conclusion, *Hepatozoon* sp. D, the first haemogregarine to be described from an isolated population of *P. langi*, appears to be different from the other haemogregarines reported from cordylid lizards so far in this Chapter 3, and different from the other lizard haemogregarines

recorded from Africa. It appears to form only gamonts in erythrocytes, and no division stages have been detected. Thus, it is provisionally identified as a *Hepatozoon* species, and named *Hepatozoon* sp. D until more detailed studies of the merogonic and sporogonic stages can be undertaken.

3.5 *Hepatozoon* sp. E. from *Pseudocordylus langi* (Loveridge, 1944)

Type Host: *Pseudocordylus langi* (Loveridge, 1944)

Type Locality: Escarpment area, Northern Drakensberg, Eastern Free State

3.5.1 Systematics (Lee et al. 2000)

Phylum Apicomplexa Levine, 1970

Class Conoidasida Levine, 1988

Order Eucoccidiorida Léger & Dubosq, 1910

Suborder Adeleorina Léger, 1911

Family Hepatozoidae Wenyon, 1926

Genus *Hepatozoon* Miller, 1908

3.5.2 Prevalence and general parasitaemias

This second haemogregarine was found in the peripheral blood of 3/13 (prevalence, 23%) *Pseudocordylus langi* and occurred together with *Hepatozoon* sp. D (described above). Mature gamonts were the most abundant stage in blood films, although immature and extracellular mature gamonts were also observed. Infections with this second haemogregarine from *P. langi* were seen in erythroblasts and mature erythrocytes in the peripheral blood with parasitaemias ranging from 1/1000 (0.1%) to 20/1000 (0.2%). This species also formed mixed infections with the so-called *Sauroplasma* species.

3.5.3 Stages in the peripheral blood

Immature gamonts (Fig. 3.19 A - C) were present in blood films of one (1/13) lizard with a parasitaemia of 18/1000 erythrocytes infected. These younger stages were relatively rare compared with gamonts and they infected erythroblasts (Fig. 3.19 A & B) and mature erythrocytes (Fig. 3.19 C). Immature gamonts were slightly curved, relatively narrow and elongated, rounded both anteriorly and posteriorly, and measured ~11.1 x 3.8 µm (n=1). Their cytoplasm stained pinkish-purple with Giemsa and was vacuolated. The nucleus lay more or less centrally within the parasite body and the purple-stained chromatin was coarse.

Maturing and mature intraerythrocytic gamonts (Fig. 3.19 D - I) were elongate, had generally rounded anterior and posterior extremities, and well-defined nuclei. These stages measured 16.5 ± 1.0 (14.7 - 17.6) long by 5.9 ± 1.2 (4.0 - 7.7) µm wide at the broadest (n=20). The presumed anterior pole was slightly broader than the posterior pole, with cytoplasm posterior to the nucleus (possibly a posterior cap) staining deep pink, and sometimes appearing granular (Fig. 3.19 G & I red arrows), compared with the light purple region anterior to the nucleus. The cytoplasm of these stages was also characteristically vacuolated (Fig. 3.19 F - K).

Gamont nuclei stained deep purple. Chromatin was coarsely granular (Fig. 3.19 G) or comprised chromatin strands (Fig. 3.19 F, J & K). Nuclei were centrally placed or lay in the posterior third of the parasite body. The nucleus measured 5.4 ± 0.7 (4.1 - 6.1) long by 5.2 ± 0.9 (4.0 - 6.8) µm wide (n=15).

Extracellular mature gamonts (Fig. 3.19 J & K) were seen occasionally and were within the size range of the intracellular gamonts, measuring 14.7 x 6.8 µm and 17.3 x 7.0 µm (n=2). Cytoplasmic and nuclear staining was similar to that of the intracellular examples. The nucleus lay in the posterior half of the parasite body (Fig. 3.19 J & K). The cytoplasm in extracellular specimens was also characteristically vacuolated (Fig. 3.19 J & K).

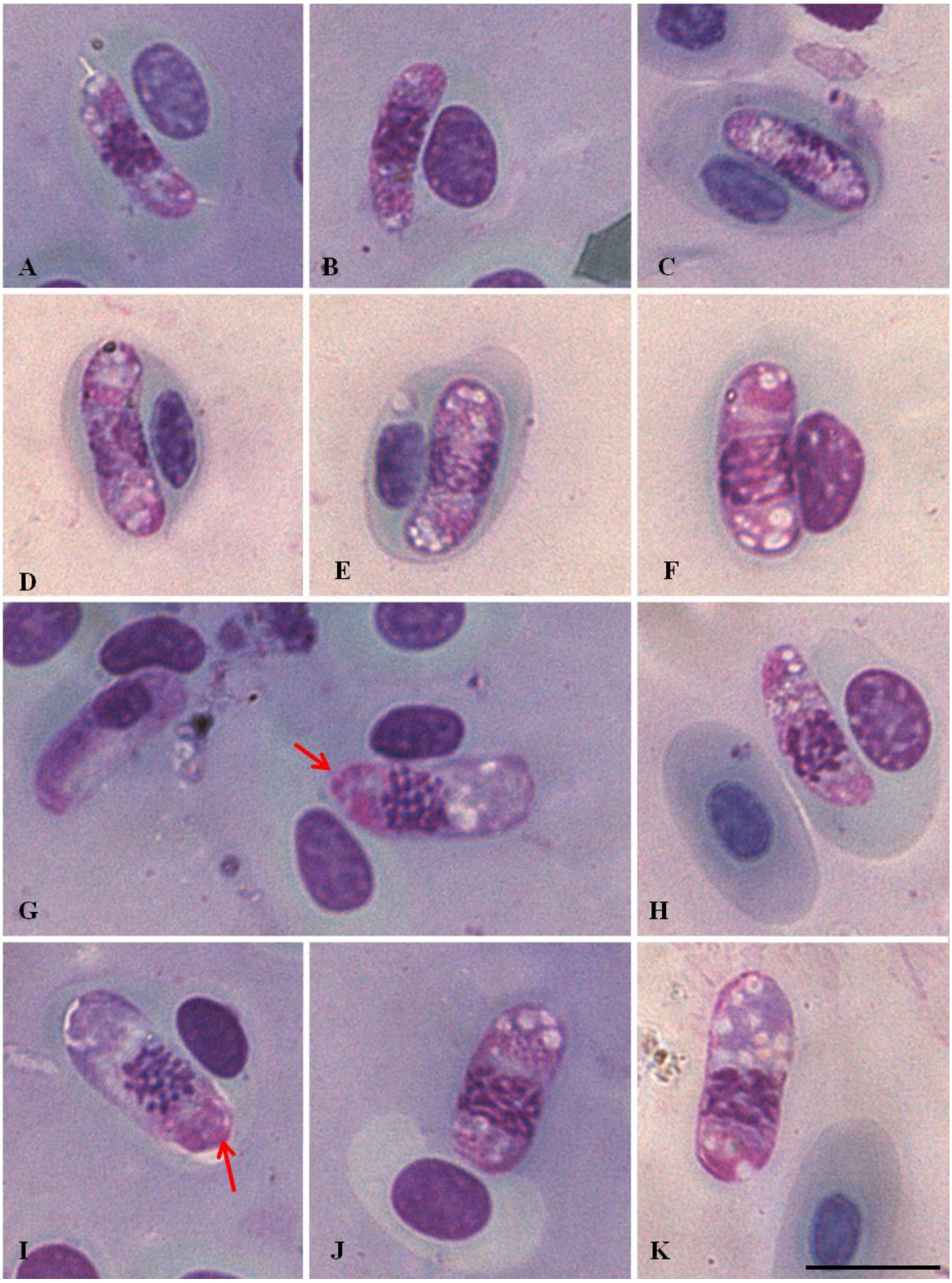

Figure 3.19 Micrographs of Giemsa stained blood films with *Hepatozoon* sp. E from *Pseudocordylus langi* from the Sentinel chain ladder in the North Eastern Drakensberg. **(A - C)** Immature gamonts. **(D - K)** Maturing and mature gamonts. **(F - I)** Gamonts in mature **(G)** and immature **(F, H)** erythrocytes. Host cell cytoplasm in **(H)** appears dehaemoglobulinized in comparison with that of the adjacent mature erythrocyte. Red arrows in **(G & I)** indicate pink-stained, rounded posterior ends. **(G, J & K)** Extracellular gamonts**. (G)** *Hepatozoon* sp. E gamont (red arrow) co-infection with *Hepatozoon* sp. D. gamont (no arrow). Scale bar: **(A - K)** = 10 µm.

3.5.4 Effects on host cell

In general, infected erythrocytes stained paler than non-infected erythrocytes, indicating some dehaemoglobulinisation, and were sometimes slightly hypertrophied; host nuclei were also sometimes slightly enlarged. Infected red blood cells measured 19.1 ± 1.1 (17.6 - 20.3) long by 12.1 ± 2.4 (9.1 - 14.6) µm wide (n=30) with a total surface area of 195.5 ± 39 (144.4 - 229.1) µm² (n=30). Non-infected erythrocytes measured 18.2 ± 1.2 (15.8 - 20.1) long by 9.9 ± 1.3 (6.9 - 11.2) µm wide (n=100), with a surface area of 151.1 ± 24.5 (120.3 - 194.4) µm². The parasitized erythrocyte nucleus was slightly compacted and displaced laterally (Fig. 3.19 C - F &I) and measured 8.0 ± 1.2 (6.6 - 10.7) µm long by 4.1 ± 1.3 (2.8 - 5.5) µm wide (n=30) with a total surface area of 27.1 ± 8.6 (18.2 - 38.5) µm² (n=30). Uninfected erythrocytes had nuclear dimensions of 6.6 ± 0.9 (4.9 - 7.8) long by 4.5 ± 0.8 (3.9 - 4.9) µm wide (n=60) with a total surface area of 24.7 ± 3.1 (16.5 - 28) (n=60) µm².

3.5.5 Remarks

Hepatozoon sp E. is morphologically unlike any of the other haemogregarine species from the three cordylid lizards described in this chapter. Morphometrically the current species is in the same size range as *Hepatozoon* sp. D, but does not possess a noticeable capsule. Furthermore, the current species has characteristic cytoplasm with large, rounded vacuoles. *Hepatozoon* sp E is morphometrically smaller than *Hepatozoon* sp. A - C from *P. melanotus* and *P. subviridis* (described above), and has a lesser impact on the host cell nucleus. Like *Hepatozoon* sp. D, the current species also had the ability to infect erythroblasts (Fig. 3.19 F & H). Erythroblast infections were never seen in *Hepatozoon* sp. A - C.

The current haemogregarine is within the same range (16.5 by 5.9 µm) as *Hepatozoon sauromali* (Lewis & Wagner, 1964) from the iguana *Sauromalus hispidus* Stejneger, 1891 from Mexico which measured overall 10 - 21 x 3.3 - 7.7 µm, although Telford (1966) reported gamonts of three slightly different sizes, 17.3 x 6.4µm, 15.2 x 6.1µm and 17 x 4µm. However, *H. sauromali* also has a narrow recurved tail and a capsule, which *Hepatozoon* sp. E does not have. Thus, the gamonts with their characteristic, highly vacuolated cytoplasm differ from most African saurian haemogregarines and haemococcidia, including all the other species reported here from the Cordylidae.

The parasite described here appears to develop only gamonts in the peripheral blood and no division stages have been detected. Given these features and the fact that it appears to be a species new to science, it is named *Hepatozoon* sp. E. Like the other cordylid *Hepatozoon* spp. B & D, the development in its vertebrate host and life cycle are unknown at this stage.

3.5.6 Discussion

The above five species of haemogregarines (*Hepatozoon* spp. A - E) were found in the blood of two isolated populations of crag lizards at an altitude above 2000m in the mountainous highlands of the Eastern Free State in South Africa. Although *Hepatozoon* sp. A & C were likely found in a previous study (Van As, 2003), these are the first detailed reports of these *Hepatozoon* species from such lizards, especially the merogonic stages of *Hepatozoon* sp. A from the liver of *P. melanotus* from Platberg and of *Hepatozoon* sp. C from the liver of *P. subviridis* from the North Eastern Drakensberg. Furthermore, sporogonic stages of *Hepatozoon* sp. A were found in mosquitoes (*Culex lineata*) feeding on *P. melanotus* lizards in their natural environment, while sporogony was observed in three species of experimental mosquitoes that fed on an *Hepatozoon* sp. C infected *P. subviridis* specimen.

Although mixed infections occurred in these crag lizards (Appendices 1 - 3), experimental lizards were sampled regularly in order to try and eliminate the possibility of mixed infections or sub-clinical infections. In addition, blood and the tissue stages of *Hepatozoon* sp. A & C in lizards, as well as their sporogonic stages in the mosquitoes could be separated on a morphological basis, including their size, shape, and staining characteristics.

The presence of extracellular gamonts shown in several micrographs throughout this chapter could have resulted from several factors. The host cells could have been weakened by the growth and/or feeding metabolism of the parasites, and could have ruptured as a result. In addition, physical pressure when making blood smears could also have resulted in the rupture of parasitized host cells.

Combinations of fieldwork, light, confocal, and transmission electron microscopy, as well as experimental studies with vectors were used in this Chapter 3. The section aimed to show the diversity of *Hepatozoon* infections in the peripheral blood of three species of high altitude lizards and the life cycle stages of two species, *Hepatozoon* sp. A & C infecting *P. melanotus* and *P. subviridis* from the two study sites. Likely vectors for *Hepatozoon* sp. B, D & E were not discovered in this study, and future work will include elucidating their life cycles.

CHAPTER 4

SAURIAN MALARIA (*PLASMODIUM* SP.) OF *PSEUDOCORDYLUS* SPP. (SAURIA: CORDYLIDAE) FROM SELECTED MONTANE LOCALITIES IN THE EASTERN FREE STATE

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 the vector and corresponding vector stages are unknown at present. Continuing searches through the literature suggest that these infections may eventually prove to be more than one species. However, for the present this infection is designated *Plasmodium* sp. A from the blood of *Pseudocordylus melanotus* and *Pseudocordylus subviridis*.

4.1. *Plasmodium* sp. A from *Pseudocordylus melanotus* (A. Smith, 1838) and *Pseudocordylus subviridis* (A. Smith, 1838)

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

Type Locality: Platberg, Eastern Free State, 1900 - 2390m

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

Other Localities: Sentinel area, Northern Drakensberg, Eastern Free State, 2589 - 3050m

4.1.1. Systematics (Lee et al. 2000)

Phylum Apicomplexa Levine, 1970

Class Aconoidasida Mehlhorn, Peters & Haberkorn, 1980

Order Haemospororida Danilewsky, 1885

Suborder Eimeriorina Léger, 1911

Family Plasmodiidae Mesnil, 1903

Genus *Plasmodium* Marchiafava and Celli, 1885

4.1.2 Prevalence

4.1.2.1 *Pseudocordylus melanotus*

Plasmodium infections (Fig. 4.1 A - J) were discovered in the peripheral blood of in 36/69 (prevalence 52.2%) specimens of *Pseudocordylus melanotus* captured on various rocky outcrops on the summit of Platberg in the Eastern Free State. Infected lizards were 21 males and 15 females. The peripheral blood of some lizards sometimes had mixed infections, comprising *Hepatozoon* spp. (A - C) (see Chapter 3), Filaria spp. and so-called *Sauroplasma* (see Chapter 5). Co-infections between *Plasmodium* and *Hepatozoon* were found in 23/69 lizards (prevalence of 33.3%). Mixed parasitemias of *Plasmodium* and *Hepatozoon* sp. A occurred in 10/69 (14.5%) (7 males and 3 females), *Plasmodium* and *Hepatozoon* sp. B in 2/69 (2.8%) (two females), and *Plasmodium* and *Hepatozoon* sp. C in 4/69 (5.8%) (two males and two females). One female lizard showed a co-infection of this *Plasmodium* sp. and *Hepatozoon* sp. A & B 1/69 (1.5%), four lizards 4/69 (5.8%) (three males and 1 female) co-infections between *Plasmodium* and *Hepatozoon* sp. A & C, and two lizards 2/69 (2.9%) (one male and one female) showed co-infections between *Plasmodium* and *Hepatozoon* sp. B & C.

4.1.2.2 *Pseudocordylus subviridis*

Plasmodium sp. A infections were also seen in the peripheral blood of 2/29 (prevalence, 7%) of *Pseudocordylus subviridis* captured on various rock outcrops along the Sentinel Trail in the North Eastern Drakensberg. *Plasmodium* sp. A infections were seen with so-called *Sauroplasma* infections but no mixed infections between *Plasmodium* sp. A and haemogregarines were observed.

4.1.3 Stages in the peripheral and heart blood of *Pseudocordylus melanotus*

4.1.3.1 Trophozoite and meront stages

The earliest forms of the parasite seen in blood smears were small, rounded to oval intra-erythrocytic trophozoites with a purple-stained periphery and a pinkish central vacuole (Fig. 4.1 A, B & E black arrows; Fig. 4.2 B red arrow). These stages infected mature red blood cells in the peripheral blood (Fig. 4.1 A, B & E) and mature or immature erythrocytes in heart blood (Fig. 4.2 B). The smallest, rounded forms (Fig. 4.1 A, B & E black arrows; Fig. 4.2 B

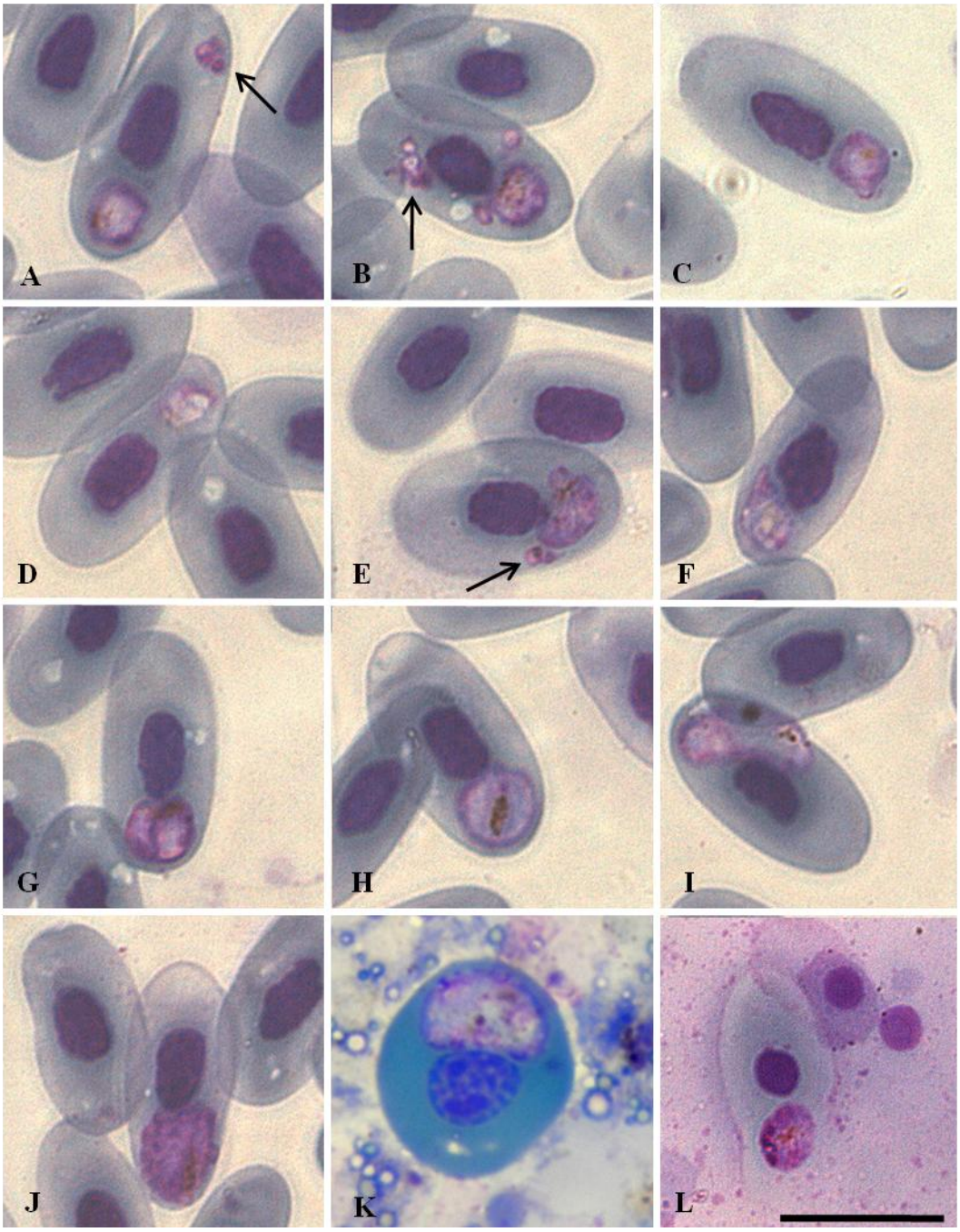

Figure 4.1 (A - J) Micrographs of Giemsa stained peripheral blood films with *Plasmodium* sp. A. from *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A - E)** Trophozoites (black arrows) and developing meronts (no arrows). **(C)** Amoeboid stage. **(E - J)** Probable gametocytes. (**E)** Likely young macrogametocyte above the arrowed trophozoite. **(F)** Possible young microgametocyte. **(G, H)** Possible gametocytes with a central pigmented strand. **(I)** Microgametocyte. **(J)** Macrogametocyte. **(K)** Intraerythrocytic microgametocyte in *Ixodiderma inverta* gut contents. **(L)** Intraerythrocytic meront in gut contents of mosquito (*Culex* (*Afroculex*) *lineata*). Scale bar: **(A - L)** = 10 µm.

red arrow) measured 1.9 ± 0.1 (1.6 - 2.2) by 1.6 ± 0.25 (1.4 - 2.3) µm (n=30), with a surface area of 4.0 ± 1.2 (1.9 - 6.4) µm² (n=30).

Later trophozoites, or perhaps early (immature) meronts (Fig. 4.1 A - D) & (Fig. 4.2 A) were similar to young trophozoites, except that were larger, their cytoplasm stained deeper pink generally, and some were broadly rounded or amoeboid in shape (Fig. 4.1 A - D) and measured 4.9 ± 0.3 (4.2 - 5.5) by 4.2 ± 0.7 (2.4 - 5.2) µm. These stages had a surface area of 28.8 ± 3.9 (11.9 - 26.9) µm² (n=30) and were mostly polar in position within host cells. Dark golden brown pigment granules were dispersed throughout these late trophozoites/early meronts, often forming aggregates (Fig. 4.1 B).

Meronts approaching maturity were best seen in heart blood where they contained large central vacuoles, and appeared to possess mostly a single granule of malarial pigment lying peripherally (Fig. 4.2 B - D). These meronts were also positioned in the polar regions of the host erythrocytes, and some host cells contained double infections of trophozoites and meronts (Fig. 4.2 B), or formed co-infections with so-called *Sauroplasma* (Fig. 4.2 C & E). Meronts measured 5.4 ± 0.7 (3.8 - 6.5) by 5.2 ± 0.9 (3.7 - 6.7) µm (n=18). The nuclei of developing merozoites (8 in number) were also observed in heart blood films, and these lay at the periphery of meronts (Fig. 4.2 E). No marked effects on the host cells were seen associated with trophozoite and early meront stages.

4.1.3.2 Gametocytes

Gametocytes were positioned in the polar regions of the host erythrocytes, and initially appeared tear-drop shaped (Fig 4.1 E, F) or rounded with a central strand of malarial pigment (Fig. 4.1 G, H). Later, they apparently became rather elongate or kidney-shaped, and pale pink-stained, with fine malarial pigment (Fig. 4.1 I), or rounded and deeply purple-stained and containing fine dark granules of malarial pigment (Fig. 4.1 J). Elongate or kidney-shaped pale-staining forms (Fig. 4.1 I) measured 6.9 ± 0.9 (6.5 - 7.7) by 3.4 ± 0.5 (2.6 - 4.1) µm (n=8) and may have represented microgametocytes. Deep staining forms (Fig. 4.1 J) measured 6.4 ± 0.9 (5.5 - 7.1) by 5.8 ± 0.5 (5.4 - 6.6) µm (n=8) and may have represented macrogametocytes. Some microgametocytes tended to curve around the host cell nucleus, and on rare occasions a slight displacement of the host cell nucleus was observed.

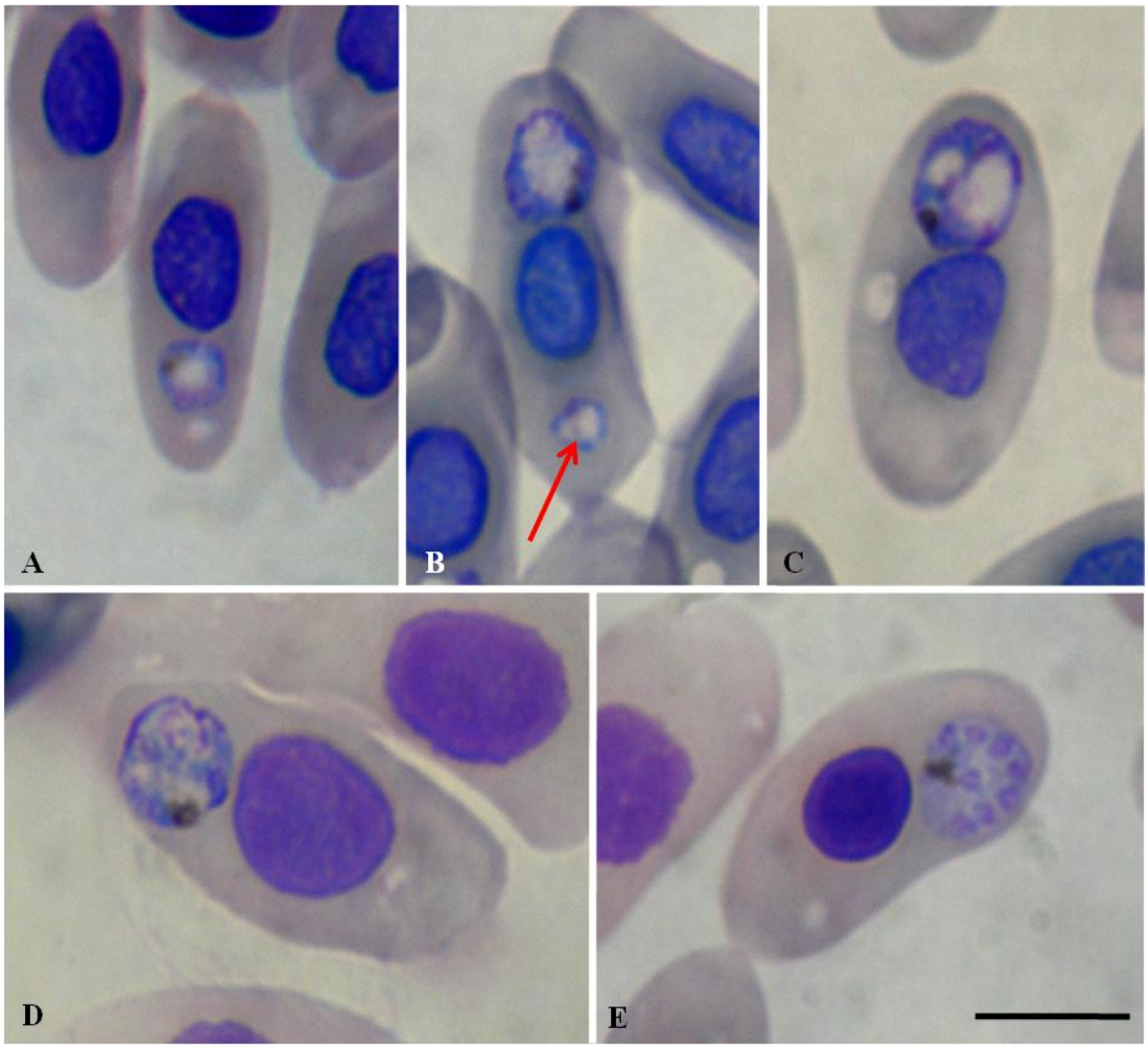

Figure 4.2 Micrographs of Giemsa stained heart blood films with *Plasmodium* sp. A. from *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A - D)** Young (red arrow) **(B)** and maturing **(A - D)** trophozoites and meronts. **(E)** Meront developing 8 merozoite nuclei. Many of these host erythrocytes appear immature in comparison with those seen in peripheral blood smears. Scale bars: **(A - L)** = 10 µm.

4.1.3.3 Observations in haematophagous invertebrates from *P. melanotus*

An intraerythrocytic gametocyte, likely a microgametocyte (Fig. 4.1 K), was seen in Giemsa stained smears of the gut contents of the scale mite (*Ixodiderma inverta*) infesting *P. melanotus* from Platberg; it had similar morphometrical dimensions to microgametocytes in the description above. Further searches of the gut/gut contents of scale mites did not reveal any sporogonic development. The gut contents of a mosquito (*Culex lineata*) seen taking a blood meal from *P. melanotus* contained possibly an intraerythrocytic meront (Fig. 4.1 L). However, subsequent examination of the gut, its contents, and the salivary glands of *C. lineata*, *C. pipiens*, *C. andersoni* and *Culiseta longiareolata* that fed on malaria infected lizard blood also failed to reveal any sporogonic or post-sporogonic stages for this species of *Plasmodium.*

4.1.4 Effects on host cells

Pseudocordylus melanotus erythrocytes infected with this species of *Plasmodium* occasionally stained lighter with Giemsa than non-infected cells, but were not markedly dehaemoglobulinized. Host cell enlargement was not detected either.

4.1.5 Ultrastructure of an intraerythrocytic meront from *P. melanotus* liver tissue

Transmission electron micrographs of liver tissue containing *Plasmodium* sp. A revealed a meront lying in a polar position in an erythrocyte from an infected lizard. Erythrocytes in the blood vessel of the hepatic tissue (Fig. 4.3 A) also contained so-called *Sauroplasma*. The meront was sectioned such that two nuclei were observed, and also present were a surface micropore, various intracytoplasmic vacuoles, and a structure resembling a dense body (Fig. 4.3 B). The meront showed no evidence of a parasitophorous vacuole, the parasite surface membrane apparently being in direct contact with the host erythrocyte cytoplasm.

4.1.6 Stages in the peripheral blood of *Pseudocordylus subviridis*

4.1.6.1 Trophozoites

The smallest trophozoites were round to oval in shape, or amoeboid (Fig. 4.4 A & B black arrows) and measured 1.9 ± 0.1 (1.8 - 2.2) µm long by 1.6 ± 0.2 (1.4 - 2.3) µm wide (n=32)

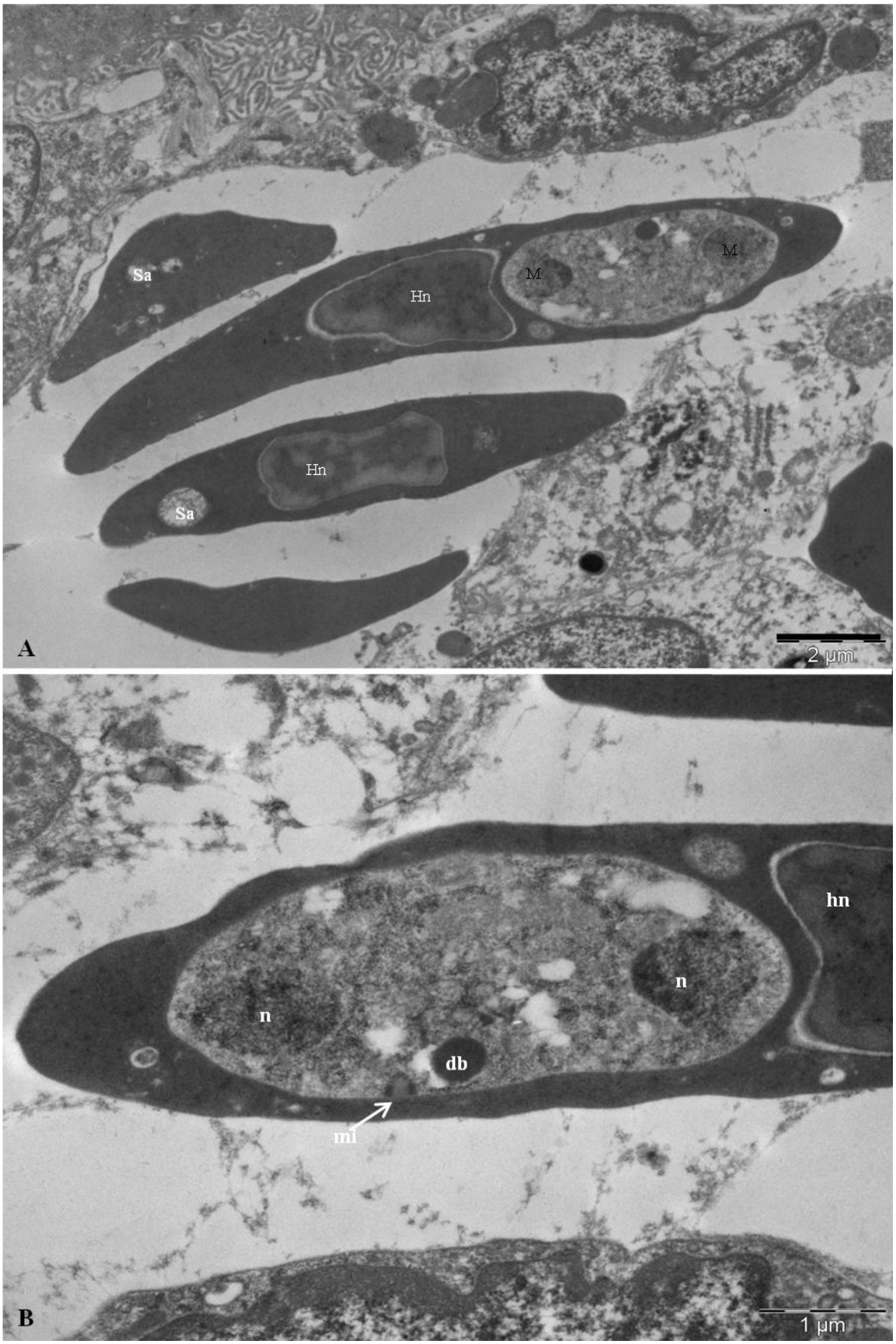

Figure 4.3 Transmission electron micrographs of a *Plasmodium* sp. A meront in an erythrocyte in the liver of *Pseudocordylus melanotus* from Platberg, Eastern Free State. **(A)** Low power transmission electron micrograph of a cross section of blood vessel containing an erythrocyte with host nucleus (Hn) infected with immature *Plasmodium* sp. A meront exhibiting two likely developing merozoite nuclei (n). Erythrocytes also contain so-called *Sauroplasma* (sa). **(B)** Detail of cross section of *Plasmodium* sp. A meront in the cytoplasm of an erythrocyte, showing the host nucleus (Hn), two parasite nuclei (n), a micropore (mi) and a possible dense body (db), and showing vacuolation, but no parasitophorous vacuole is visible. Scale bars: **(A)** = 2µm **(B) =** 1µm.

and closely resembled those seen in *P. melanotus* lizards. Trophozoites stained dark pinkish-purple with Giemsa peripherally, with some malarial pigment (Fig. 4.4 A) present, as well as a pale central vacuole.

4.1.6.2 Late trohozoites/early meronts

These stages (Figure 4.4 B - F) stained dark pinkish-purple at the periphery of the parasite, again with some malarial pigment visible, and their cytoplasm was largely vacuolated. No merozoite nuclei were detected. Meronts measured 4.6 ± 0.44 (4.0 - 5.6) µm in length by 4.2 ± 0.4 (3.5 - 5.0) µm in width (n=11). The surface area of meronts measured 20 ± 3.9 (16.9 - 25.7) µm². No marked effects on the host cells were seen.

4.1.6.3 Gametocytes

Macrogametocytes of this malaria were not observed, but microgametocytes lay mostly in polar positions within erythrocytes (Fig. 4.4 G). These stages contained scattered pigment granules and were broadly crescent-shaped or kidney-shaped. Such microgametocytes (Fig. 4.4 G) stained pale lilac with a pinkish periphery with Giemsa and measured 6.8 ± 0.6 (6 - 7.9) µm in length by 4.4 ± 0.9 (3.5 - 5.6) µm (n=10) in width. Apart from some gamonts that curved around the polar region of the erythrocyte nucleus, no effects on host cells were detected. So-called *Sauroplasma* was also observed in this lizard species (Figs. 4.4 A, E & G).

4.1.7 Discussion

Similar *Plasmodium* infections were encountered in a previous study (Van As, 2003) and in both species of lizards, but from different localities, namely in *P. melanotus* from Clarens in the Eastern Free State and in *P. subviridis* from Lesotho. Before Van As (2003), Pienaar (1962) had reported *Plasmodium zonuriae* Pienaar, 1962 from girdled lizards, *Cordylus vittifer*, captured in the South West Transvaal (now North West Province). The division stages in the peripheral blood of *P. melanotus* from Clarens (Van As, 2003) differed only from those of *P. zonuriae* in having a mainly eosinophilic cytoplasm and in carrying abundant fine pigment, but they were otherwise roughly the same dimensions as in the descriptions of Pienaar (1962).

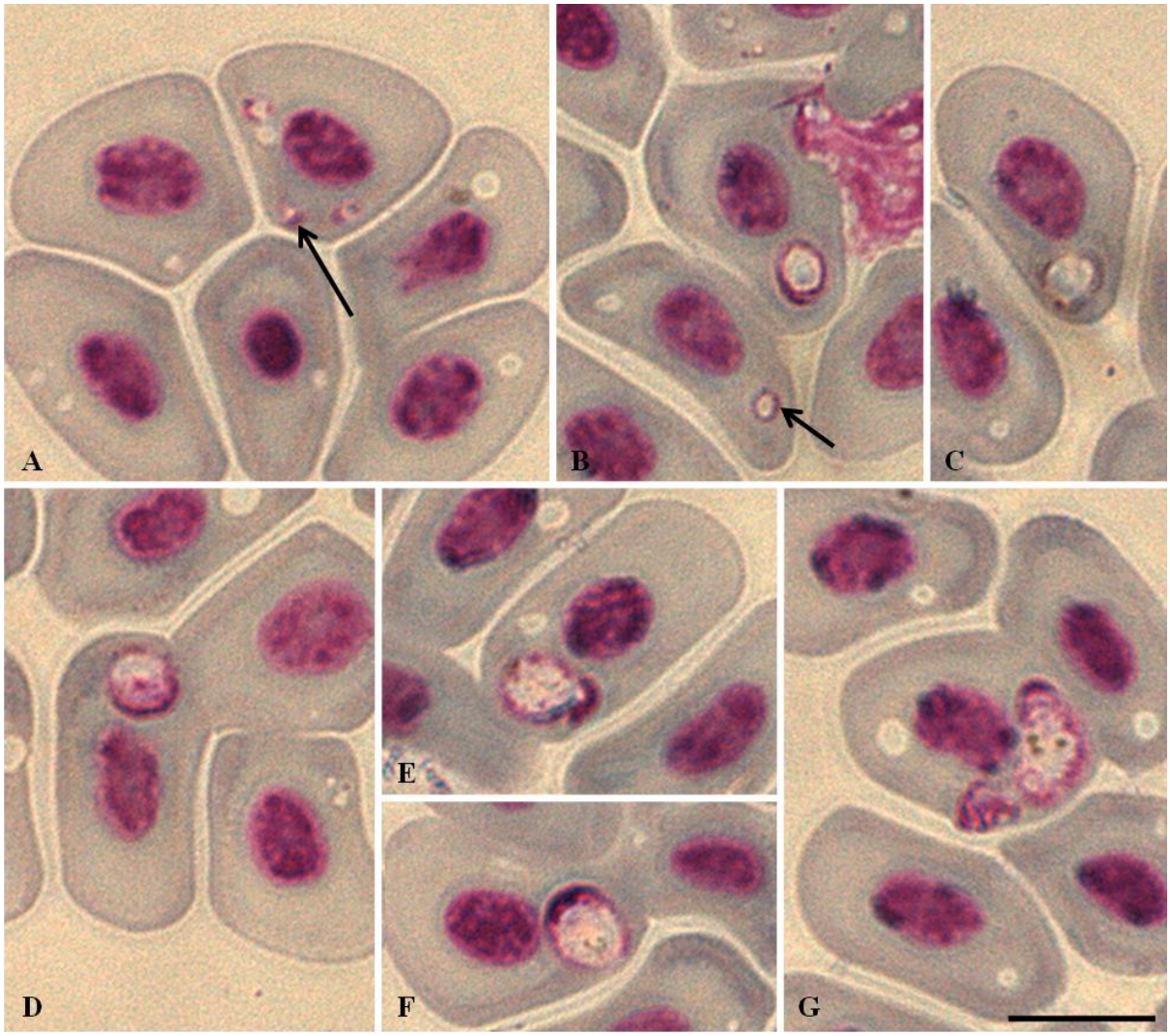

Figure 4.4 Micrographs of Giemsa stained peripheral blood films of *Plasmodium* sp. A from *Pseudocordylus subviridis* from the North Eastern Drakensberg. **(A & B** black arrows) Trophozoites. **(A - F)** Meronts in different stages of development. **(G)** Likely microgametocyte. So-called *Sauroplasma* is also present in many erythrocytes. Scale bar: **(A - G)** = 10µm.

Infections found in *P. subviridis* in Lesotho by Van As (2003) were also similar to Pienaar's (1962) parasites, but with different staining properties and greater morphometric variation in meronts and gametocyte dimensions (Van As, 2003).

Following Pienaar's (1962) description of malaria in a cordylid, Telford examined specimens in 1986 of the same lizard, *C. vittifer*, from roughly the same region as Pienaar (1962), as well as further north within South Africa, and confirmed that the specimens were infected with *P. zonuriae*. Telford also examined two specimens in 1972 (see Telford, 2009) of *Pseudocordylus microlepidotus* in the vicinity of Cape Town and found them to be infected with the same malaria. Later, Telford (1987) described *Plasmodium cordyli* Telford, 1987 from *Cordylus tropidosternum* in Tanzania and listed *C. vittifer* as a host for *P. cordyli*, from the same locations in South Africa (what is now North West Province) and in similar lizard numbers as when he recorded *P. zonuriae* (see Telford, 2009).

In the current study, trophozoites and early meronts of *Plasmodium* sp. A closely resemble those recorded by Van As (2003) and by Pienaar (1962), that is, they are similar in size, and in particular, are amoeboid like those of *P. zonuriae.* On the other hand, meronts of the *Plasmodium* infection in *P. melanotus* are similar in appearance to those of Telford's (1987) *P. cordyli*, and apparently produce 8 merozoites. Merozoite numbers recorded by Telford (1987) for *P. cordyli* were 8 - 14 (in *C. vittifer*), whereas Pienaar (1962) recorded 8 - 24 (average 18) in *P. zonuriae*. However, meront stages in the current study differ in their morphometrical dimensions, with those from *P. melanotus* being smaller overall (3.8 - 6.5 x 3.7 - 6.7 µm) than meronts of *P. zonuriae* described from *C. vittifer*, which measured 7 - 17 x 4 - 9 µm, according to Telford (2009), although these dimensions were not recorded by Pienaar (1962).

The gametocyte stages appear particularly problematic when comparisons are drawn. The macrogametocyte stages of *Plasmodium* sp. A found in the current study from *P. melanotus* match those of *P. zonuriae* (see Pienaar, 1962) in description, but also those of *P. cordyli* (see Telford, 1987), and seem similar in size to gametocytes (5 - 9 x 4 - 7 µm) in *C. vittifer* (see Telford, 1987), although those of Pienaar (1962) were slightly larger (8.0 - 8.4 x 4.6 µm).

However, the microgametocyte stages found in current *P. melanotus* closely resemble those of *P. zonuriae* in general appearance and size (Pienaar, 1962).

The *Plasmodium* species found in the erythrocytes of *P. subviridis* in the present study differed slightly from those found in the red blood cells of *P. melanotus* in that the trophozoites and meronts produced more pigment at the periphery. However, their microgametocyte stages appeared remarkably similar, and thus like those of *P. zonuriae.*

Comparing the two *Plasmodium* infections in the two host lizards in the current research, it appears they are the same, and thus both have features of Pienaar's (1962) *P. zonuriae* and Telford's (1987) *P. cordyli.* This suggests two possibilities, first that mixed infections of *P. zonuriae* and *P. cordyli* occur in these high altitude lizards, or, that both infections are of a polymorphic *P. zonuriae.* If the latter is true, then this calls into question Telford's (1987) observations in *C. vittifer* and his identification of *P zonuriae* and *P. cordyli* in this host. Overall, this appears such a complicated issue that only future molecular methods would likely solve the problem of the true identity of the malarias found in these Eastern Free State Highland lizards.

Outside South Africa, and other than *P. cordyli* in Tanzania (see above and Telford, 1987), *Plasmodium* sp. A is perhaps morphologically closest to *Plasmodium uluguruense* Telford, 1984 described from the gecko *Hemidactylus platycephalus* in the Uluguru Mountains, Tanzania (Telford, 1984). Meronts of *P. uluguruense*, however, were more variable in size and measured 4 - 10 x 2 - 6 µm, while meronts of *Plasmodium* sp. A infecting the erythrocytes of *P. subviridis* were more constrained in their dimensions measuring 4.0 - 5.6 µm in length by 3.5 - 5.0 µm in width. Gametocytes of the two species also differ. Gametocytes of *P. uluguruense* were larger and ovoid, and according to Telford (1984) the pigment was not dispersed within the gametocytes but formed an aggregate of dark greenish - yellow granules near the cell margin of both sexes of gametocytes; furthermore, these gametocyte stages measured 5 - 10µm x 4 - 7µm with a surface area of 20 - 63 µm². The smaller, microgametocytes of *Plasmodium* sp. A. of *P subviridis* had distinct pigment distributed around the periphery of the parasites, but sometimes centrally and they measured

6 - 7.9 µm in length by 3.5 - 5.6 µm in width. Microgametocytes of *P. uluguruense* were longer and larger than macrogametocytes.

Erythrocytes infected with the current species of *Plasmodium* were neither hypertrophied nor dehaemoglobulinized and rarely stained paler than non-infected cells. Pienaar (1962) concluded that infections of *P. zonuriae* in *C. vittifer* caused severe anaemic changes in the peripheral blood. Telford (1987) also reported that *P. zonuriae* meronts in erythrocytes of *C. vittifer* caused hypertrophy, and displaced and distorted nuclei. However, Telford (1987) also found that meronts in infected *P. microlepidotus* caused little distortion of either the host cell or its nucleus. In a similar manner, *Plasmodium* sp. A in the current study also showed little effect on host cells, but in some cases, meronts and gametocytes tended to curve around the host cell nucleus, though they never displaced or deformed it. Overall, stained slides of this species of *Plasmodium* (that is *Plasmodium* sp. A) seem to reveal that it is unique in terms of its staining properties, distribution of pigment and effects on the host cells, compared to most other Plasmodiid infections of African Reptiles, other than those highlighted above (see Table 1.2).

Although the gut contents of mosquitoes (*Culex lineata*) and prostigmatic mites (*I. inverta*) that fed on *Plasmodium* sp. A infected blood on *P. melanotus* lizards, revealed gametocyte and meront stages in host erythrocytes, no further development was seen in these haematophagous invertebrates. Pienaar (1962) thought that prostigmatic mites *Zonurobia circularis latior* Lawrence, 1935 associated with *C. vittifer* could host sporogony of *P. zonuriae*, but was skeptical about haematophagous dipterans serving as vectors. Although it was shown earlier that *C. lineata* hosted sporogonic stages for a haemogregarine of *P. melanotus*, as well as in other species of mosquitoes (*C. pipiens, C. andersoni* and *Culiseta longiareolata*) hosting sporogonic stages for a haemogregarine infecting *P. melanotus* and *P. subviridis* (see Chapter 3), no evidence was seen of sporogonic development of this *Plasmodium* species thus far.

Future work on the invertebrate hosts of *Plasmodium* sp. A of these cordylid lizards in the Eastern Free State will include setting up laboratory experiments, light traps and field observations to determine their definitive hosts and invertebrate vector(s), similar to

experiments carried out for *Hepatozoon* spp. found in these hosts. Although *P. langi* harboured 2 species of haemogregarines that formed mixed infections with so-called *Sauroplasma* and filarial nematodes, it is interesting to note that no evidence of malaria was seen in the blood of these highest altitude lizards. The fact that *P. langi* are known to be herbivorous, in conjunction with their specific high altitude habitats (and therefore the possible absence of specific malaria vectors), could be reasons that these malaria infections are not yet recorded from these lizards. However, potential absence of vectors goes against the obvious existence of haemogregarines and filariae in *P. langi* (Chapters 3 & 5).

CHAPTER 5

OTHER BLOOD INFECTIONS OF *PSEUDOCORDYLUS* SPP. (SAURIA: CORDYLIDAE) FROM SELECTED MONTANE LOCALITIES IN THE EASTERN FREE STATE

During routine examinations of peripheral blood smears and liver impressions from crag lizards as well as squashes of haematophagous ectoparasites that had fed on these lizards, two infections not described in Chapters 3 and 4 were observed. These were the abundant so-called *Sauroplasma* infections in the cytoplasm of the erythrocytes, and microfilaria occurring in the blood plasma. Each type of infection will be dealt with separately in this chapter.

5.1. So-called *Sauroplasma* infections of *Pseudocordylus* spp. from the Eastern Free State highlands

Sauroplasma Du Toit, 1937 are taxonomically controversial infections and are apparently haemosporidian in nature (Telford, 2009). These infections are currently considered as members of the Piroplasmorida Wenyon, 1926 and according to Keymer (1981) are rare blood parasites of chelonians, lizards and snakes. Frye (1991) described these haemoparasites as small (1 - 2µm) in diameter, and vacuole-like with punctual basophilic inclusions. According to Telford (2009), these infections, in the early stages are similar in appearance to anaplasmoid bodies and have been found in lizards from Africa, Europe, North and South America, distributed across several lizard families.

The following descriptions of so-called *Sauroplasma* infections all represent new distribution records and in the case of *P. langi*, also a new host record. Although these represent the first morphometrical descriptions from these localities, the 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, the data provided here are new and provide useful information on the distribution of these structures within erythrocytes, individual variations between them, and may aid future research in

resolving their taxonomic status. The following will involve three separate descriptions, based on the morphological and morphometrical characteristics seen in the three species of crag lizards examined in this study.

5.1.1. Systematics (Peirce, 2000)

Phylum Apicomplexa Levine, 1970

Class Aconoidasida Mehlhorn, Peters & Haberkorn, 1980

Order Piroplasmorida Wenyon, 1926

Family Haemohormidiidae Levine, 1984

Genus *Sauroplasma* Du Toit, 1937

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

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

Locality: Platberg, Eastern Free State, 1900 - 2390m

5.1.2.1 Prevalence

So-called *Sauroplasma* infections were found in all 69 lizards (100% prevalence) of this species collected at different sites, but mainly from rock outcrops on Platberg. The parasites were intracytoplasmic and found in mature erythrocytes and erythroblasts; they were always accompanied by one or more of *Plasmodium* infections (see Chapter 4), haemogregarines (see Chapter 3) and filarial nematode infections (see section 5.2 of this chapter). No intranuclear inclusions were observed in infected red cells. Mean parasitemia was 76.1 ± 24.0 (10 - 100) % of erythrocytes infected.

5.1.2.2 Parasite description

Rounded, ring-like and oval structures were seen in the cytoplasm of erythrocytes and erythroblasts, and each measured 1.2 ± 0.6 (0.1 - 2.6) µm by 1.7 ± 0.9 (0.2 - 3.0) µm (n=130). Mostly, these structures seemed to comprise an unstained vacuole-like centre (Fig. 5.1 A & B black arrows) and sometimes a pink staining region was seen along the periphery and in the

centre of the vacuole (Fig. 5.1 C & D black arrows). Apparently budding forms were observed in some blood smears (Fig. 5.1. E - G black arrows) and several smaller structures were present and scattered throughout the cytoplasm of the mature erythrocytes (Fig. 5.1. E red arrows). Intranuclear inclusions were not observed within erythrocytes and pink-staining areas, similar to those seen in *Pirhemocyton* infections, were not observed.

In a similar manner to those structures seen in *Sauroplasma boreale* Svahn (1976), the following forms were also observed, namely small anaplasmoid bodies without chromatin granules (Fig 5.1 A & B red arrows) and possibly anaplasmoid bodies with chromatin granules (Fig. 5.1 D red arrow), ring shaped bodies with pink staining regions (Fig. 5.1 C & D black arrows), signet ring like bodies with central vacuole and a darker staining periphery (Fig. 5.1 C & D yellow arrows), and irregular bodies of varying sizes with a large centrally vacuole (Fig. 5.1 A yellow arrow).

5.1.2.3 Ultrastructure of *Sauroplasma* infections from *P. melanotus*

Sauroplasma infections were seen in erythrocytes in a cross section of a minor blood vessel from lizard liver (Fig. 5.2 A).

Examination of erythrocytes by TEM revealed single or multiple daughter structures, or budding forms (Fig. 5.2 B, D & E). The apparent budding forms were surrounded by a boundary membrane (Fig. 5.2 D & E) and were filled with granular material dispersed in a largely electron-lucent matrix (Fig. 5.2 B, D & E black arrows). In one instance crystalline-like structures were present within the matrix (Fig. 5.2 B unlabelled white arrow). Small regions resembling dense heterochromatin were observed in some instances (Fig. 5.2 B - D white arrows). Budding appeared to occur as shown in Fig. 5.2 B, D & E and the daughter forms were filled with the same granular material as the mother structure. These daughter forms likely grow until they are the same size as the mother structure. Sometimes membranous whorls were seen at the periphery of the inclusion close to the limiting membrane (Fig. 5.2 C), with the centre of the structure remaining vacuole-like.

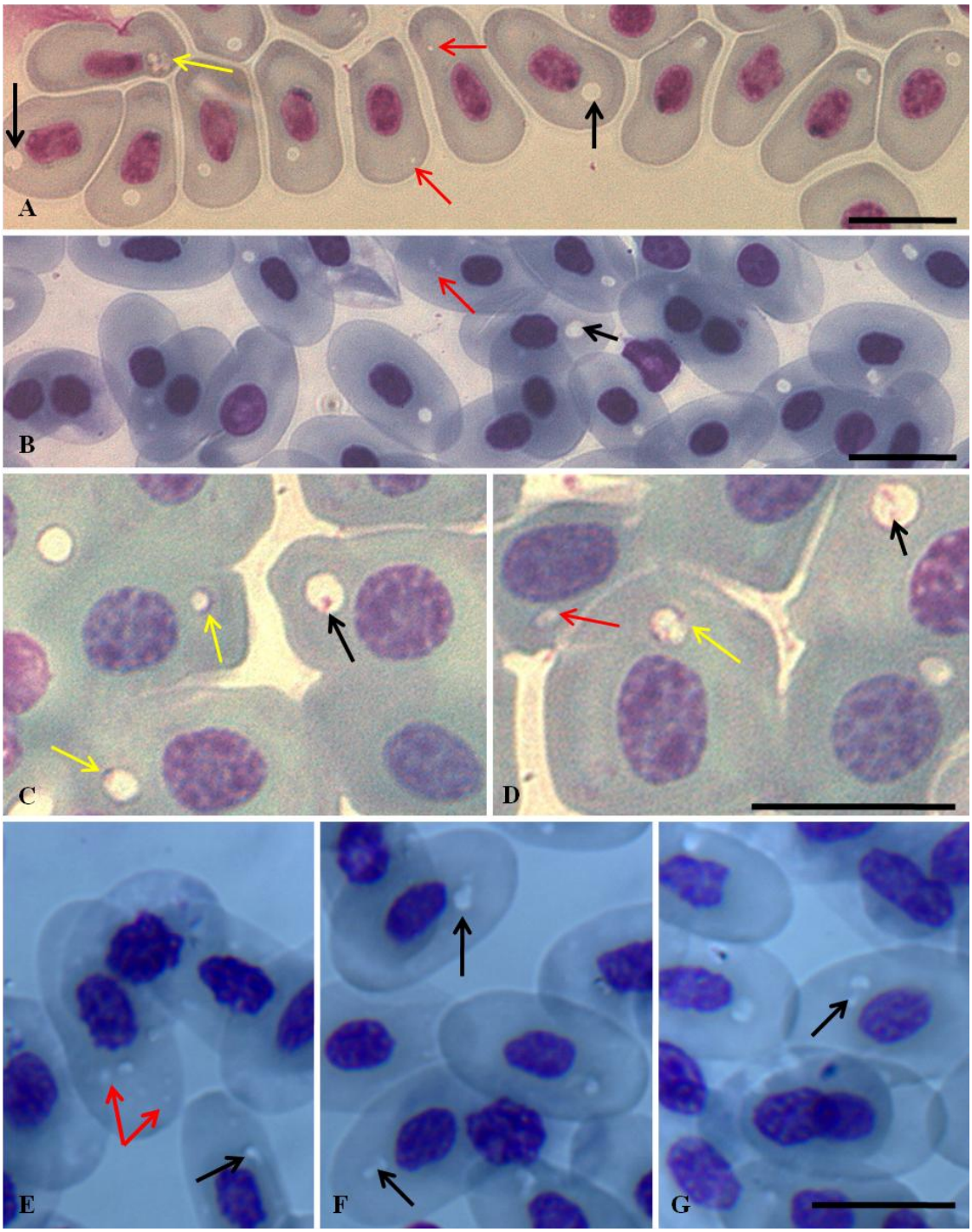

Figure 5.1 (**A - G**) Light micrographs of Giemsa stained blood films with so-called *Sauroplasma* infections in the red blood cells of *P. melanotus* from Platberg, Harrismith in the eastern Free State. Black arrows in **A & B** indicate relatively large unstained areas. Red arrows in **A & B** indicate small anaplasmoid bodies without chromatin granules. Yellow arrow in **A** indicates irregular bodies of varying sizes with a large centrally vacuole. Black arrows in **C & D** indicate pink staining regions. Yellow arrows in **C & D** indicate signet ring like bodies with central vacuole and a darker staining periphery. Black arrows in **E - G** show apparently budding forms. Red arrow in **D** indicates a small anaplasmoid body with chromatin granules, red arrows in **E** show possible daughter cells. Scale bars: (**A - G**) = 10µm

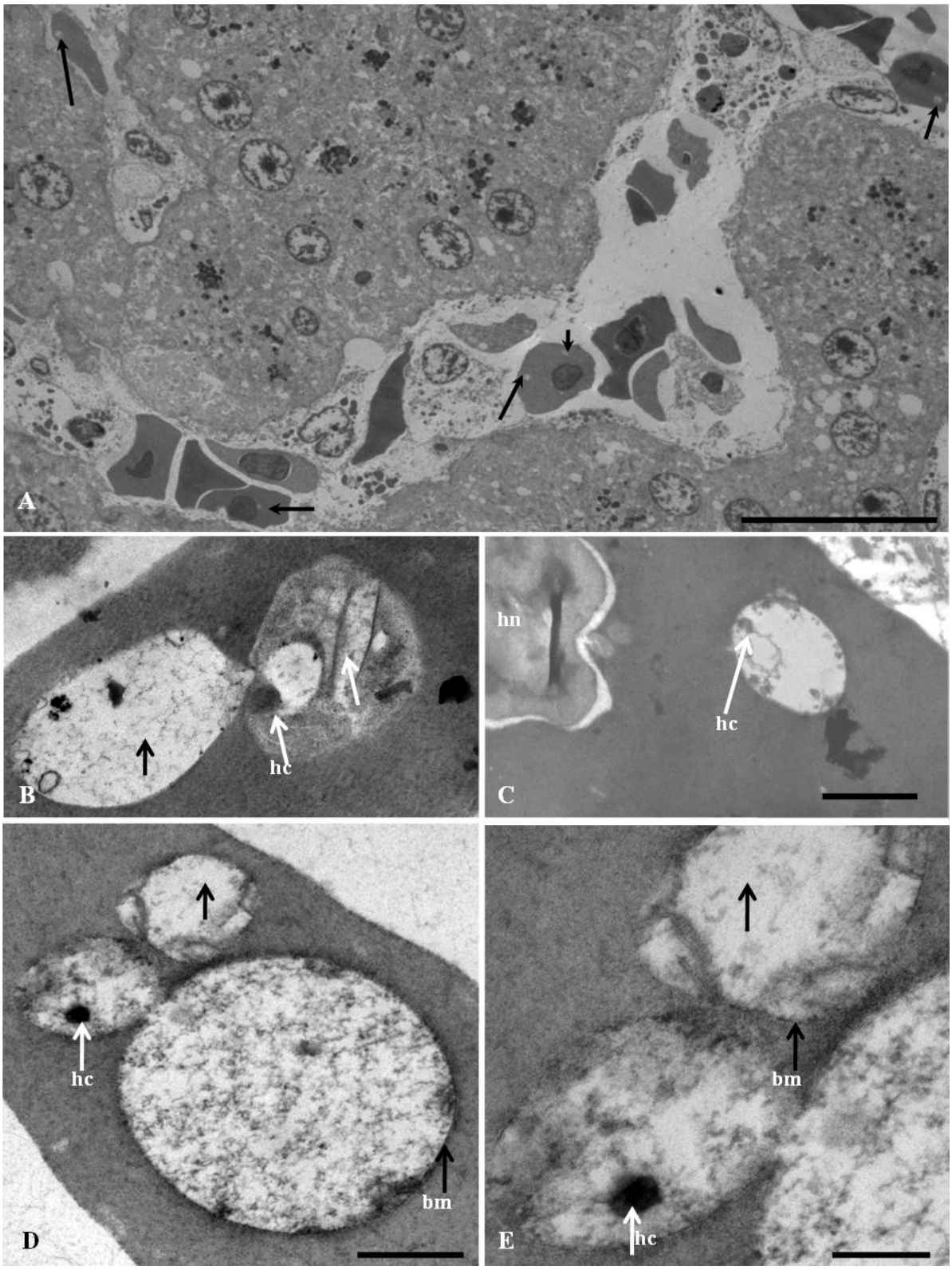

Figure 5.2 (A - D) Transmission electron micrographs of liver tissue showing erythrocytes from a so-called *Sauroplasma* infected *P. melanotus.* **(A)** Low power transmission electron micrograph of a cross section of blood vessel containing erythrocytes (black arrows) infected with so-called *Sauroplasma* sp. **(B)** Budding form with a small area of heterochromatin (hc). White arrow indicates crystalline-like structures within the matrix. **(C)** Membranous whorls at the periphery of the inclusion with a small area of heterochromatin (hc). **(D & E)** Higher magnification of apparently budding forms surrounded by a boundary membrane (bm) and electron dense regions resembling heterochromatin (hc). Unlabelled black arrows in **B, D & E** indicate the vacuole like centre with granular material dispersed in a largely electron-lucent matrix. Scale bars: **(A)** = 20µm **(B & C)** = 1µm **(D) =** 500nm **(E) =** 200nm.

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

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

Locality: Sentinel area and escarpment area, Northern Drakensberg, Eastern Free State, 2589 - 3050m

5.1.3.1 Prevalence

Sauroplasma infections were found in all 29 lizards (100% prevalence) collected in different localities, across an altitudinal gradient on various rock outcrops in the North Eastern Drakensberg. The parasites were intracytoplasmic, found in mature erythrocytes and erythroblasts, and accompanied *Plasmodium*, haemogregarine and filarial nematode infections (as noted above), but also occurred in the absence of these haematozoans. Mean parasitemia was 88.8 ± 8.7 (70 - 100%) of erythrocytes infected.

5.1.3.2 Parasite description

In Giemsa-stained blood films, one "bullet hole" structure was normally present in a single erythrocyte. On rare occasions two slightly smaller, but separate, structures were seen in an erythrocyte (Fig. 5.3 A red arrows). These structures were irregularly round and ring-like, and the main body of the structure measured 1.1 ± 0.6 (0.4 - 3.5) µm by 2.8 ± 1.0 (0.8 - 4.4) µm (n=100). Sometimes apparently budding forms were observed (Fig. 5.3 A, black arrows). The centre of the structure was largely unstained and vacuole-like, with a narrow, rim around its periphery (Fig 5.3 B, black arrow). No clear evidence of division was observed, apart from the presence of small "outgrowth" forms that suggested that multiplication or budding may have been in progress (Fig 5.3 A, black arrows) as well as the occurrence of two "bullet holes" in some erythrocytes. Pink-staining regions, similar to those seen in *Pirhemocyton* infections, were not encountered.

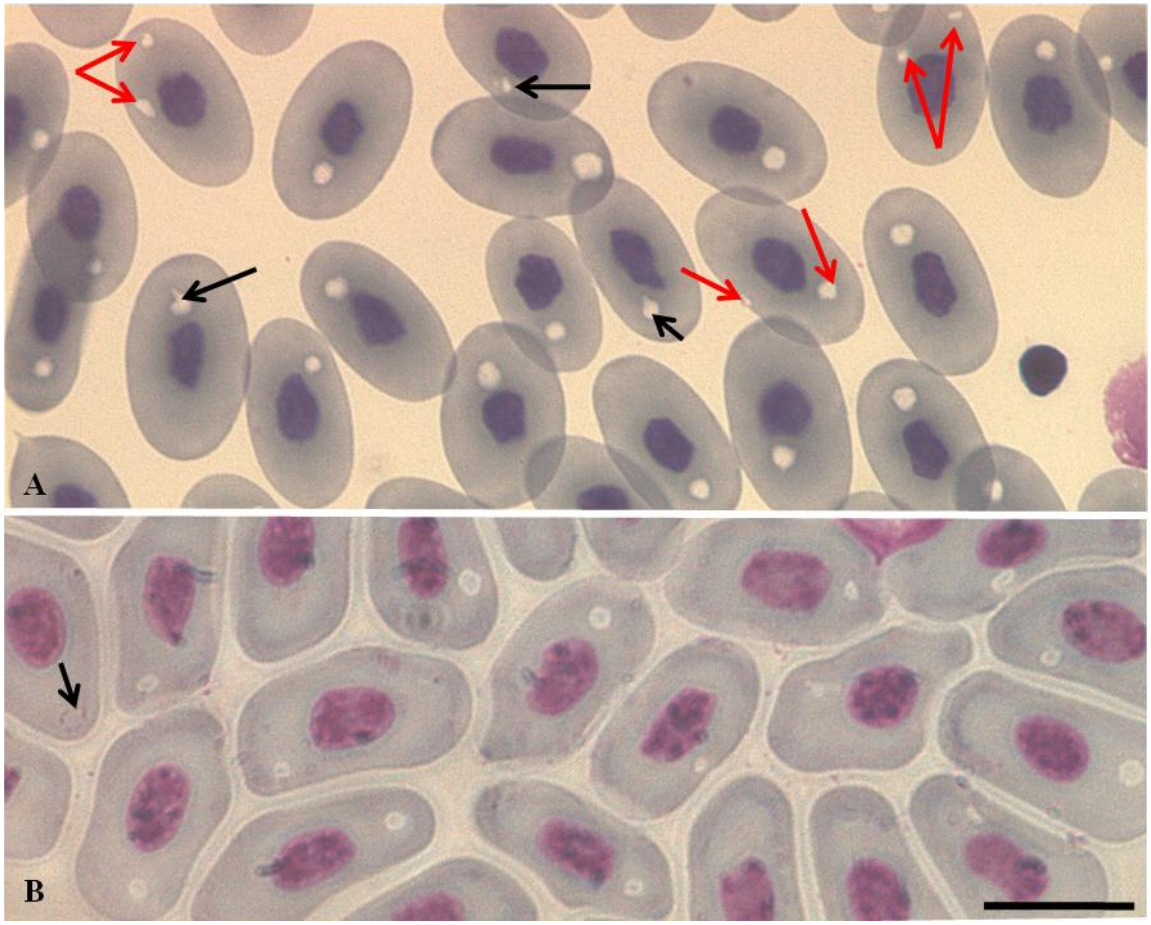

Figure 5.3 Micrographs of Giemsa stained blood films of so-called *Sauroplasma* infections in erythrocytes from *Pseudocordylus subviridis* from the North Eastern Drakensberg. **(A & B)** So-called *Sauroplasma* infections in erythrocytes from peripheral blood. Black arrows in **A** indicate apparent budding forms. Black arrow in **B** indicates signet ring like bodies with central vacuole and a darker staining periphery. Red arrows in **B** indicate separate, smaller structures within the cytoplasm of the host cell. Scale bar: (**A & B**) = 10µm.

5.1.4. *Sauroplasma* from *Pseudocordylus langi* (Loveridge, 1944)

Host: *Pseudocordylus langi* (Loveridge, 1944)

Localities: Escarpment area, Northern Drakensberg, Eastern Free State, 2589 - 3050m

5.1.4.1 Prevalence

So-called *Sauroplasma* infections were found in all 13 lizards (100% prevalence) collected from vertical cliffs at the chain ladders in the North Eastern Drakensberg. The parasites were intracytoplasmic and found in mature erythrocytes, accompanying haemogregarine and filarial nematode infections. Slightly lower intensities of infection than in the previous two species of lizards (see above) were recorded with mean parasitemia 44.0 ± 19.56 (20 - 90%) of erythrocytes infected.

5.1.4.2 Parasite description

In general, structures resembling "bullet holes" were present in erythrocytes and erythroblasts (Fig. 5.4 B, D & H). Each structure was irregularly round and ring-like, and the main body of the structure measured 1.2 ± 0.6 (0.5 - 3.9) by 3.2 ± 2.0 (1.2 - 4.3) µm (n=120). These bodies were vacuole-like, with a narrow, blue-stained rim at their periphery (Fig 5.4 A - I). No clear evidence of division was observed, but the presence of small forms suggested that multiplication or budding might have occurred (Fig 5.4 A, C & E, black arrows). Sometimes more than one intracytoplasmic inclusion was seen in a single host cell (Fig. 5.4 G & I black arrows), but pink-staining regions similar to *Pirhemocyton* infections were not encountered.

5.1.5. Effects on the host cells

Due to the small size of these intracytoplasmic inclusions (~1 - 4 µm across) relatively small areas within the erythrocyte cytoplasm appeared to be taken up by them. Thus, when compared to the effect of the larger haemoparasites described in Chapters 3 & 4 the effects on the host observed by microscopy seemed to be minimal.

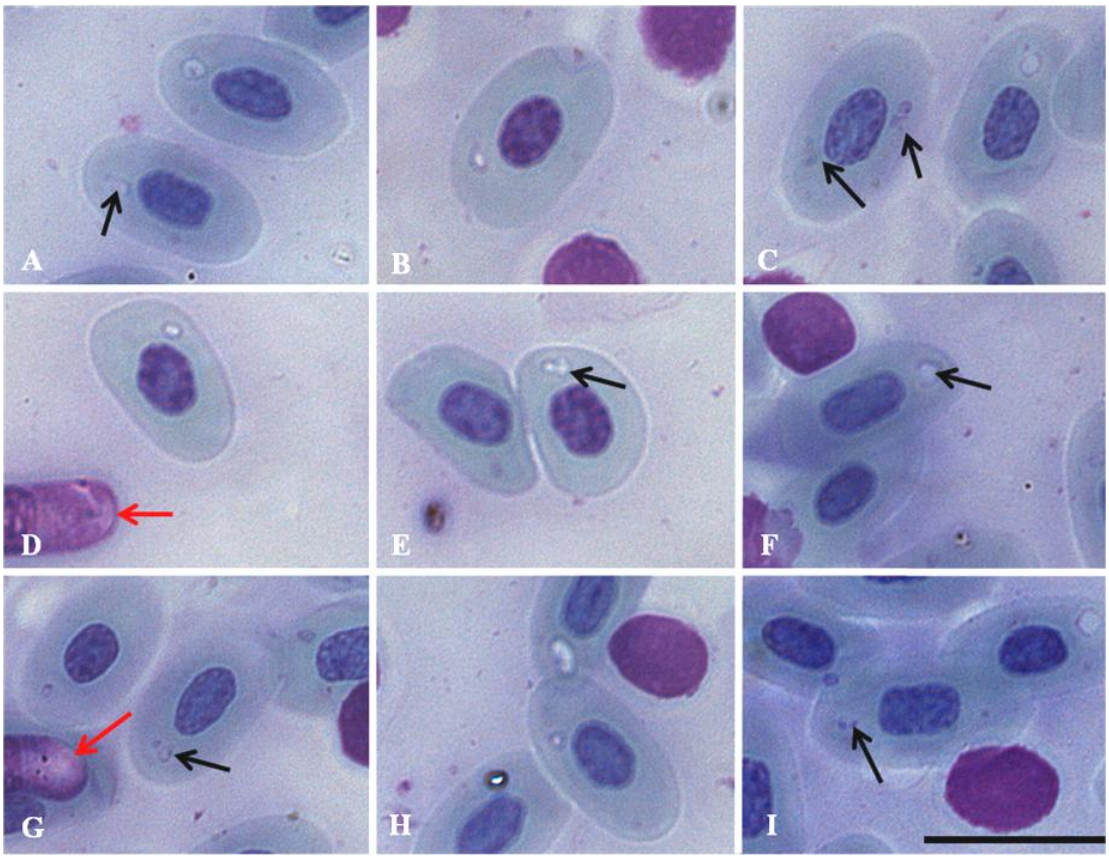

Figure 5.4 Micrographs of Giemsa stained blood films of so-called *Sauroplasma* sp. from *Pseudocordylus langi* from the Sentinel chain ladders in the North Eastern Drakensberg. **(A - I)** *Sauroplasma* infections of various sizes in cytoplasm of erythrocytes. **(D & G)** Show co-infections of *Hepatozoon* sp. D (red arrows). Black arrows indicate possible budding forms in **A, C & E**, black arrows in **G & I** indicates separate, smaller structures in the host cell. Scale bar: (**A - I)** =10µm.

5.1.6. So-called *Sauroplasma* infections in haematophagous invertebrates

In histological sections of *Ixodiderma inverta*, so-called *Sauroplasma* infections were seen in host erythrocytes present in the gut of the ectoparasitic mite (Fig. 5.5 A - C), which had fed on infected blood from *P. melanotus.* Furthermore, squash preparations from engorged *Ixodiderma pilosa* that fed on infected blood of *P. subviridis* also revealed so-called *Sauroplasma* infections in host erythrocytes (see Fig. 5.5 D - F). The gut contents of two species of mosquitoes (*Culex* (*Culex*) *pipiens* and *Culex andersoni bwambanus*) that fed on *P. subviridis* in the laboratory, also revealed host erythrocytes infected with so-called *Sauroplasma* (Fig. 5.5 G & H). Wild mosquitoes, (*Culex* (*Afroculex*) *lineata*) that fed on *P. melanotus* also had so-called *Sauroplasma* infections in host erythrocytes within their gut contents (Fig. 5.5 I). All these infections in the gut contents of the above mentioned haematophagous invertebrates were similar in morphology and morphometric dimensions than those in the host erythrocytes. No evidence of subsequent development was seen in histological sections of mites and squash preparations (*I. inverta* & *I. pilosa*) or squash preparations of the *Culex* mosquitoes.

5.1.7. Remarks

These so-called *Sauroplasma* infections in *P. melanotus* were first seen in a previous study (Van As, 2003) from the blood of *P. melanotus* from Clarens in the Eastern Free State, and in *P. subviridis* from different localities in Lesotho. Additionally they were also recorded in a wide spectrum of lizards in the same, previous study (see Van As, 2003). Morphologically, the infections observed previously in *P. melanotus* closely resembled those from the current *P. melanotus,* but were slightly narrower and broader (1.1 x 2.8µm) than the present infections which measure (1.2 x 1.7µm). They also resembled morphologically, infections in *P. subviridis* (see below), *Sauroplasma thomasi* of Du Toit (1937) and *Sauroplasma zonurum* of Pienaar (1962).

Telford (2009) concluded in his compilation of all known *Sauroplasma* infections worldwide that these infections are rare. Furthermore he stated that wherever these parasites occur, a prevalence of less than 0.1% to 6% is typical. In examining the literature cited here, the highest parasitaemia was found by Pienaar (1962) and this was 56% of the erythrocytes infected by *S. zonurum* in *Cordylus vittifer*. In the current study every single specimen,

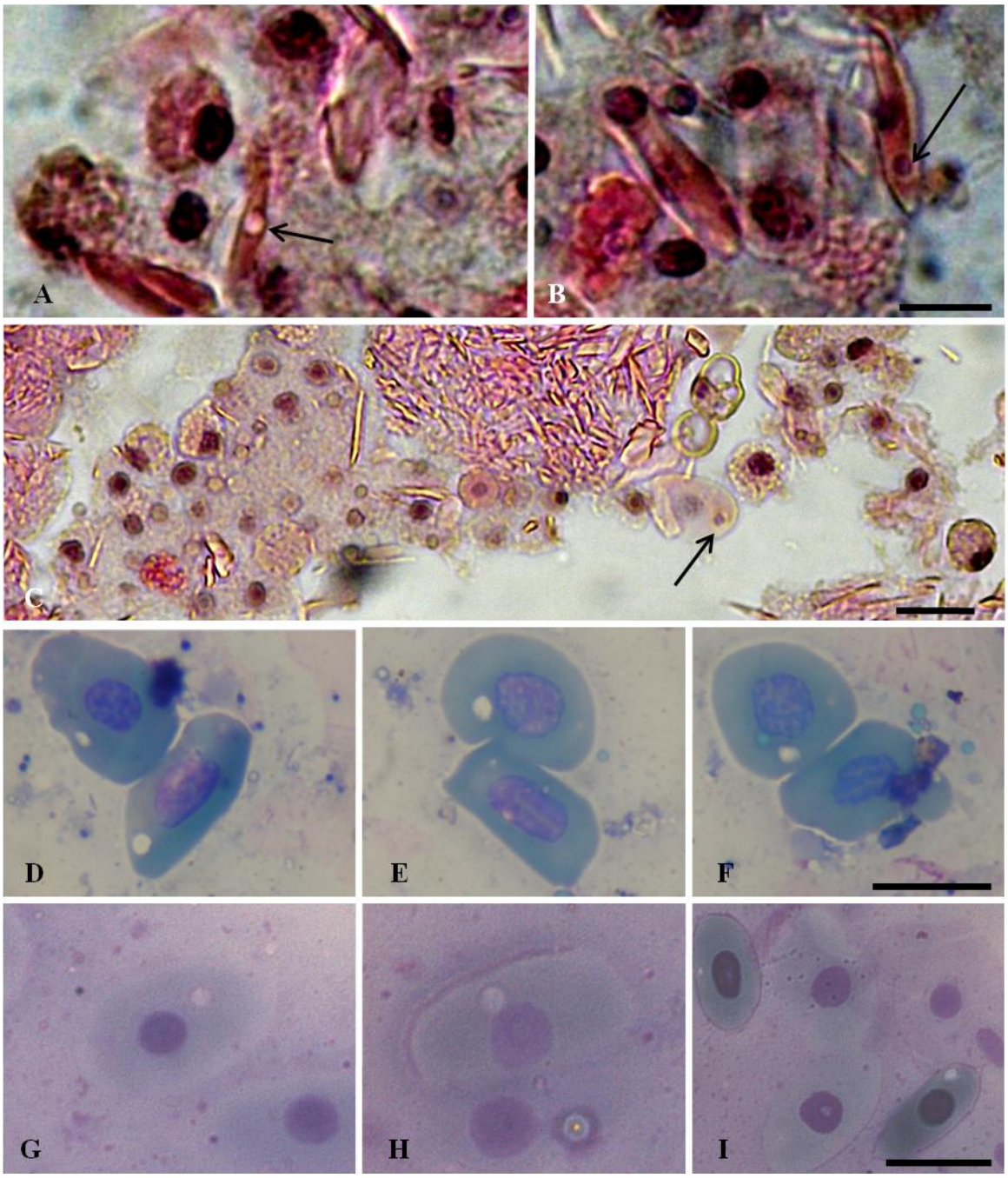

Figure 5.5 Light micrographs of so-called *Sauroplasma* infections in erythrocytes of *P. melanotus* and *P. subviridis* from Platberg, Eastern Free State and the North Eastern Drakensberg respectively. **(A - C)** Histological sections of *Sauroplasma* infections (black arrows) in erythrocytes in the gut of *Ixodiderma inverta* that fed on blood of *P. melanotus*. **(D - F)** So-called *Sauroplasma* infections in erythrocytes from the gut contents of the mite *Ixodiderma pilosa* that fed on infected blood from *P. subviridis* from the Drakensberg. **(G & H)** So-called *Sauroplasma* infections in erythrocytes from the gut contents of experimental mosquitoes *Culex (Culex) pipiens* **(G)** and *Culex andersoni bwambanus* **(H)** that fed on the blood of *P. subviridis*. **(I)** So-called *Sauroplasma* infections in erythrocytes of *P. melanotus* present in the gut contents of *Culex lineata*. Scale bars: **(A - I)** = 10µm.

comprising three species of crag lizards, was infected with so-called *Sauroplasma*. Furthermore, prevalence of infection tended to be high with mean parasitemia for the three lizard species ranging from ~44 - 88 % of red cells. Even the lowest prevalence of 10% in one of the *P. melanotus* specimens is considered a high infection rate by Du Toit (1937), Pienaar (1962), Svahn (1976) and Telford (2009).

These intracytoplasmic structures, because of their absence of pigment, mode of multiplication, size and shape (see Davies & Johnston, 2000; Peirce, 2000) have been described as parasites belonging to the Order Piroplasmorida Wenyon, 1926. Davies & Johnston (2000) also suspected them to be viral in nature because of their similarity with *Pirhemocyton*-like infections found in other members of the family Cordylidae. The TEM micrographs of this study do not reveal any apicomplexan structures, or provide any clear indication of the identity of *Sauroplasma*. No viral particles were found in the current electron micrographs, but *Sauroplasma* appears very similar to *Serpentoplasma* infections (see Section 3.2.4 on *Serpentoplasma* of snakes in the author's MSc thesis). TEM studies from the preceding MSc study [see *Sauroplasma* from *Smaug* (*Cordylus*) *giganteus* (Stanley et al. 2011) in Van As, 2003] 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 the Apicomplexa. However, when it is compared with *Serpentoplasma* from *Python sebae natalensis* and *Serpentoplasma* from *Bitis arietans* by TEM (Van As, 2003) it appears to be similar morphologically.

Although *Sauroplasma* was detected in mites and *Culex* mosquitoes in the current study, its mode of transmission also remains uncertain. Du Toit (1937) did not find any ticks on the two infected lizards that he studied, but speculated that *S. thomasi* infections are likely to be transmitted by ticks, like most other piroplasms. Pienaar (1962) also did not find any ticks on the lizards he examined and suggested that more likely vector could be the prostigmatic mites (*Zonurobia* spp.) that are usually associated with the cordylid lizards he investigated. Svahn (1976) stated that some facts pointed to the tick *Ixodes ricinus* Linnaeus, 1758 being the vector of her *Sauroplasma*, and that ticks were often seen on the lizards she collected.

Previously, *Sauroplasma* and *Sauroplasma*-like infections have been described from three species of cordylid lizards in South Africa (*C. giganteus*, *Cordylus jonesii* Boulenger, 1891 and *C. vittifer*), as well as from reptiles in Uzbekistan, lizards (*Lacerta agilis* Linnaeus, 1758) in Scandinavia, and geckoes (*Uroplatus fibriatus*) and chameleons (*Zonosaurus mascareniensis*) from Madagascar (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; Peirce, 2000 and Telford, 2009). These infections have also had their fair share of controversy. Svahn (1976) placed *Sauroplasma boreale* that she described (on the basis of new locality and different hosts) in the Theileridae since no reproduction in erythrocytes were observed and she regarded the reproductive stages from the descriptions of Du Toit (1937) and Pienaar (1962) as double infections. Apparent division and budding stages were seen in crag lizards in this study in light micrographs, as well as by TEM, thereby supporting the observations of Du Toit (1937) and Pienaar (1962). Similar parasites in lizards (*Gehyra variegata* (Duméril & Bibron, 1936)) from Australia were considered to be early trophozoites of the haemoproteid *Haemocystidium* Castellani & Willey, 1904 (see Johnston 1975), and certainly in malaria-infected blood of *P. melanotus* and *P. subviridis* these so-called *Sauroplasma* infections can be confused with early malarial trophozoites, although they do lack malarial pigment. Johnston (1975) also reported that some *Sauroplasma* infections can resemble the viral infection *Pirhemocyton*, since the albuminoid bodies of some of the latter infections are not unlike *Sauroplasma*, according to Davies & Johnston (2000). Therefore, although no *Pirhemocyton* was found in the current study, careful examination is needed when *Sauroplasma* infections are studied and classified, especially when only light microscopy methods are employed to examine them.

5.1.8 Discussion

Sauroplasma infections from the three crag lizard species examined in this study are clearly morphologically 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), and cannot yet be excluded from having a viral origin, rather than being a piroplasm. *Sauroplasma*-like infections are also more widespread than previously thought and perhaps cross specific, generic or family boundaries between

their hosts. However, the true identity of *Sauroplasma*, if it is an infection, the boundaries of its distribution and its mode of transmission between hosts are currently unknown. Future work should include conducting life cycle experiments using ectoparasites associated with these lizards.

5.2 Microfilariae infections of *Pseudocordylus* spp. from the Eastern Free State highlands

Fantham & Porter (1950) provided the first summary of reptile nematodes in South Africa and a little more than a decade ago, Hering-Hagenbeck & Boomker (2000) provided a checklist of nematodes parasitic in their reptile hosts. McAllister et al. (2010) updated the existing lists and added 10 new host records, especially from the Northern and Western Cape provinces in South Africa.

Nematode infections in cordylid lizards were first reported by Malan (1939), specifically *Physalopteroides impar* in *Cordylus niger* (Smith, 1844). McAllister et al. (2010) added a few new records for cordylid lizards in the Cape Province, of which one of these was *Abbreviata paradoxa* Linstow, 1908 in the same host, *Cordylus niger*. To the author's knowledge only two filarial nematodes have been described from cordylid lizards in South Africa. These were *Befilaria pseudocordyli* Gibbons (1989) described by Gibbons (1989) from the liver of *Pseudocordylus microlepidotus* Cuvier, 1829 and *Madatahamugadia ineichi* Bain, Wanji, Petit, Paperna & Finkelman, 1993 from what they identified as *Pseudocordylus microlepidotus melanotus*. The species description of Gibbons (1989) was based on material collected from a single specimen housed at the Zoological Gardens in Regent's Park in London. From the paper it is not clear if this lizard was captured in the South Western Cape in South Africa (where they naturally occur) or was zoo-bred in London. This therefore casts doubt on whether its nematode was an African endemic species, or a nematode infection acquired from the London Zoological Gardens. There is also uncertainty regarding the Bain et al. (1993) record. In McAllister et al.'s (2010) species list they spelt *Madathamugadia* (correct spelling) as "*Madatahamugadia"* and listed the host as *Pseudocordylus* (*Cordylus*) *microlepidotus* which only occurs in the Western Cape. In fact, in the original Bain et al. (1993) description the type locality was listed as Goldengate, Eastern Free State, thus ruling

Pseudocordylus (*Cordylus*) *microlepidotus* out as host as it does not occur in this locality. Since there is currently considerable confusion regarding the proper taxonomic status of cordylid lizards (see Chapter 1), and especially with those originally referred to as subspecies, it is likely that the host Bain et al. (1993) studied was *P. melanotus*, although the possibility that it might have been *P. subviridis* cannot be excluded.

The following section will be a brief overview of a number of morphologically and morphometrically different microfilarial nematodes that were observed in the blood of *P. melanotus, P. subviridis* and *P. langi* collected at different localities on Platberg and at the Sentinel trail in the Eastern Free State. These were of three different types, based on their different morphometrical dimensions and morphology in general. As they are not the main focus of this study, these filarial nematodes in the blood of the three crag lizards will be noted only briefly. Dissections of *P. melanotus* and *P. subviridis* also revealed several other unidentified nematodes in the small intestine, but these are not reported further here.

5.2.1 Systematics (Anderson & Bain, 1976)

Phylum Nematoda Potts, 1932

Class Chromadorea (Filipjev, 1917) Chitwood, 1933

Order Diplotriaenoidea Anderson, 1958

Family Oswaldofilariidae Chabaud & Choquet, 1953

Subfamily Splendidofilarinae Chabaud & Choquet, 1953 (Onchocercidae)

5.2.2 Microfilaria sp from *Pseudocordylus melanotus* from Platberg

Microfilariae were found in the blood plasma of 10/69 lizards (prevalence of 14.5%). Of those lizards infected, the numbers of filarial worms varied from 1 to 40 individuals present in a field of ~ 1000 mature erythrocytes.

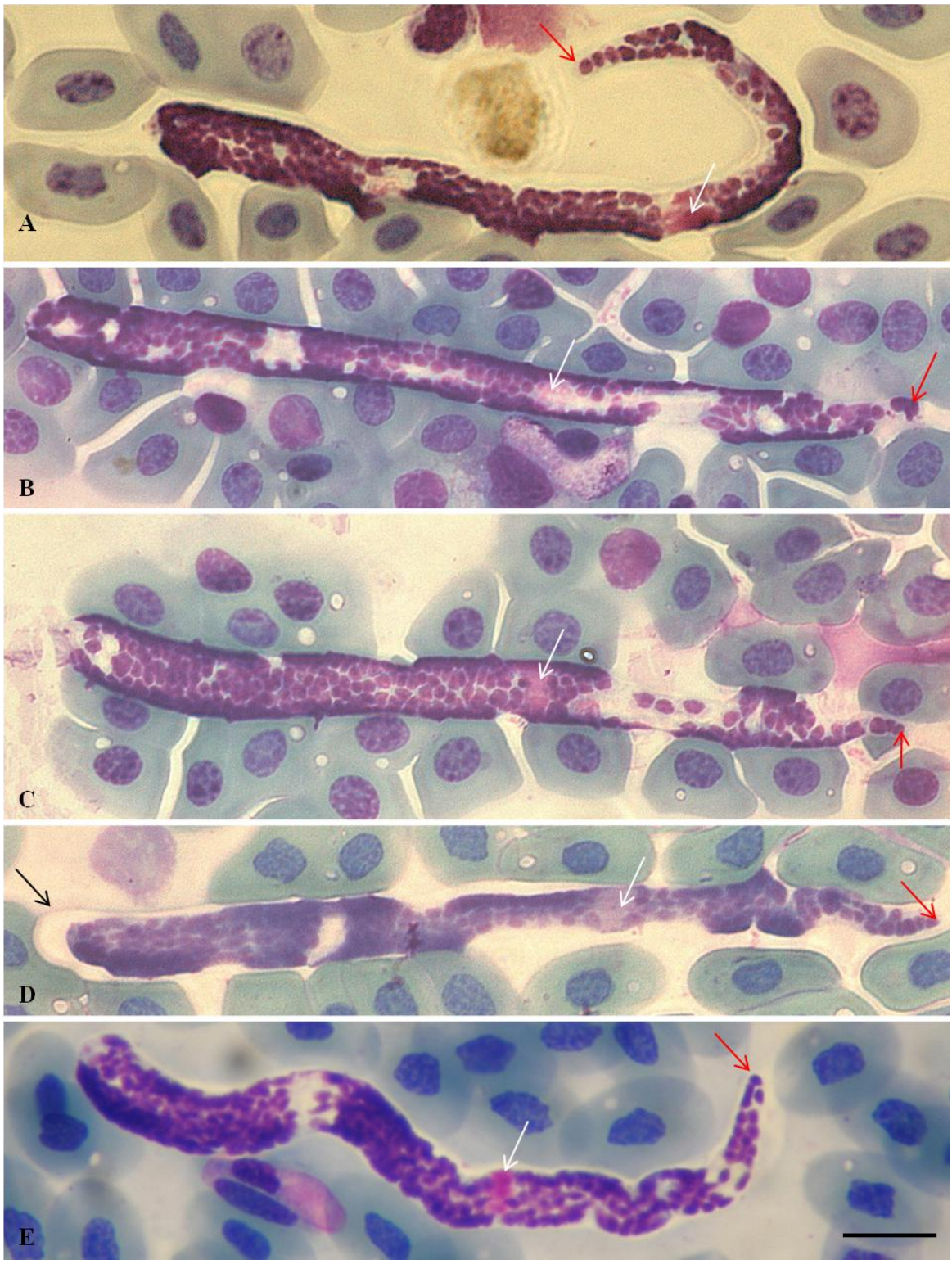

Figure 5.6 Light micrographs of Giemsa stained blood films of *P. melanotus* showing filarial nematodes (**A - E)**. Note the intraerythrocytic so-called *Sauroplasma* infections in **A - E,** and *Hepatozoon* sp. C & A in **B & E** respectively. Arrow in **(D)** indicates the inflated sheath. White arrows in **A - E** show organ primordia. Red arrows in **A - E** indicate a single nucleus at the tail tip. Scale bar: **(A - E)** = 10µm.

5.2.2.1 Parasite description

Microfilariae (Fig. 5.6 A - E) measured 100 ± 12.1 (79 - 118) µm in length by 6.3 ± 0.5 (5.2 - 7.3) µm in width (n=33) and some specimens possessed a pale pink or purple-stained, slightly inflated, sheath and a slightly rounded anterior (Fig. 5.6 D black arrow). The tail tip was relatively pointed but sometimes bluntly rounded, with one nucleus close to its terminus (Fig. 5.6 A - E, red arrows). Nuclei throughout the body region stained deep purple. Pink-staining organ primordia could be seen in the posterior third of the body (Fig. 5.6 A - E, white arrows).

5.2.2.2 Remarks

All of these microfilariae in the blood of *P. melanotus* were distinctly stained and had a characteristically pale pink staining sheath. Morphometrically, these filariae were longer 79 - 118µm when compared to the Giemsa stained filarial stages of *Madatahamugadia ineichi* which measured only 65 - 98µm long by 5 - 7µm wide, although unstained and heat stretched *M. ineichi* measured 112 - 125 µm long by 5.5 - 6 µm wide (Bain et al. 1993). Unfortunately Gibbons (1989) did not provide any description of the blood microfilaria stages of *Befilaria pseudocordyli* for comparison.

5.2.3. Microfilaria sp from *Pseudocordylus subviridis* from the North Eastern Drakensberg

Microfilariae were found in 15 of 29 lizards (prevalence of 51%) collected on the Sentinel Trail in the north-eastern Drakensberg.

5.2.3.1 Microfilaria in the peripheral blood

Microfilariae of this species measured 93 ± 7.5 (85 - 112) µm long by 4.9 ± 0.9 (3.8 - 6.7) µm wide (n=44), with an almost non-staining, loose-fitting, sheath and a broad, bluntly rounded anterior (Fig. 5.7 A & B). The tail tip was narrow, recurved and somewhat elongated and individual nuclei within it were difficult to count. Nuclei throughout the body stained deep red-purple. Organ primordia could be seen as colorless and red-stained areas towards the posterior third of the body (Fig. 5.7 B white arrow). The microfilaria formed mixed infections with *Hepatozoon* sp. A and so-called *Sauroplasma* (Fig. 5.7 B).

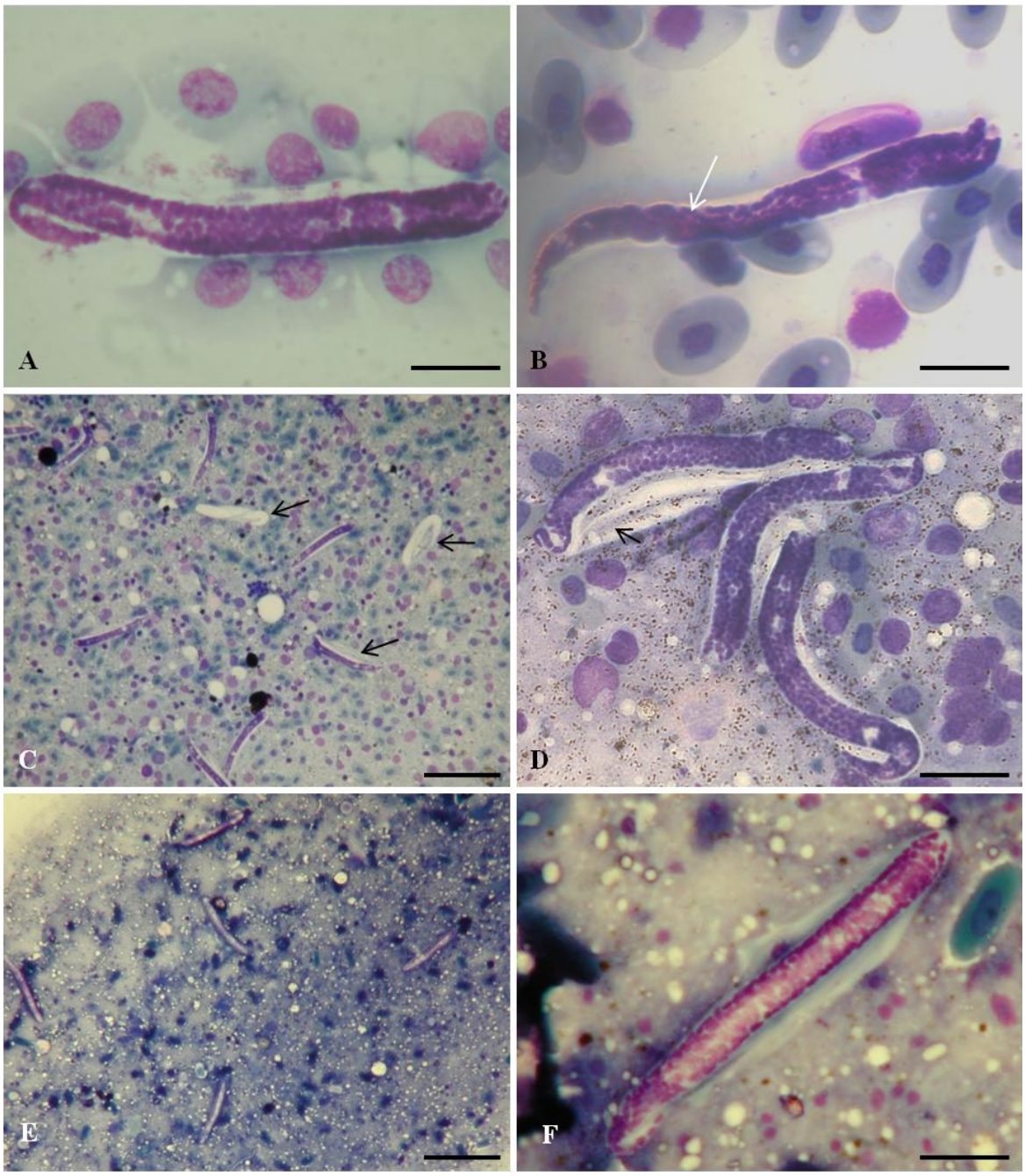

Figure 5.7 Light micrographs of Giemsa stained blood films of filarial nematodes in the blood and liver tissue of *P. subviridis* and its ectoparasitic mite, *Ixodiderma pilosa*, from the Drakensberg escarpment (3110m). (**A & B**) Filariae in the peripheral blood (note their narrow, recurved, tails); white arrow in **B** indicates red staining organ primordia, (**C & D**) microfilariae in lizard liver, black arrows indicates inflated sheaths. (**E & F**) Microfilaria in the gut contents of *Ixodiderma pilosa* from *P. subviridis*. Note the intraerythrocytic so-called *Sauroplasma* infections in erythrocytes in **A & B,** and *Hepatozoon* sp. A in **B**. Scale bars: (**A, B, D & F)** = 10µm **(C & F)** = 100µm.

5.2.3.2 Microfilaria in the liver

Microfilaria were also detected in the liver (Fig. 5.7 C & D) of the same lizard species. Interestingly, the liver stages were slightly smaller and measured 90 ± 6.5 (77 - 99) µm long by 4.2 ± 0.4 (3.8 - 5.0) µm wide (n=30). Morphologically, however, these filarial were the same as the blood forms, but had a more inflated, light purple sheath (Fig. 5.7 C & D black arrows). A non-staining organ primordium was seen in the posterior third of each nematode, in the same position as microfilariae from the peripheral blood, but without any red stained area.

5.2.3.3 Microfilaria in the mite, *Ixodiderma pilosa* Lawrence, 1935

Ectoparasitic, prostigmatic mites (*Ixodiderma pilosa* Lawrence, 1935) (see Fig. 6.8 E) were collected from a lizard infected with microfilaria and morphologically similar microfilariae were observed in the mite gut contents (Fig. 5.7 E & F). These microfilariae stained reddish purple and interestingly, in terms of the morphometrical dimensions, these were of about the same length (93.9 ± 7.3 (79 - 116) µm) and width (4.4 ± 0.6 (3.8 - 6.3) µm) as those found in the lizard peripheral blood.

5.2.3.4 Remarks

These *P. subviridis* lizards were collected in various localities along the Sentinel Trail. Compared to the microfilariae in *P. melanotus*, their microfilariae were different morphologically and morphometrically, suggesting that the nematodes may be specific to each lizard species. Morphometrically these *P. subviridis* microfilariae were in the same length range (77 - 99µm) as *M. ineichi* which measured 65 - 98µm long, but were thinner (3.8 - 5.0µm) compared to filarial stages of *M. ineichi* which measured 5 - 7µm wide. Adult nematode species were found in the stomach and in the rectum of one *P. subviridis*, but were not identified with confidence.

5.2.4. Microfilaria sp from *Pseudocordylus langi* (Loveridge, 1944)

Microfilariae were seen in the blood of one, gravid, female lizard of 13 lizards (prevalence 7.6%) examined. In this lizard the microfilariae were seen with *Hepatozoon* sp. D & E (not shown in micrographs) and numerous so-called *Sauroplasma* sp. infections (Fig. 5.8 A - C).

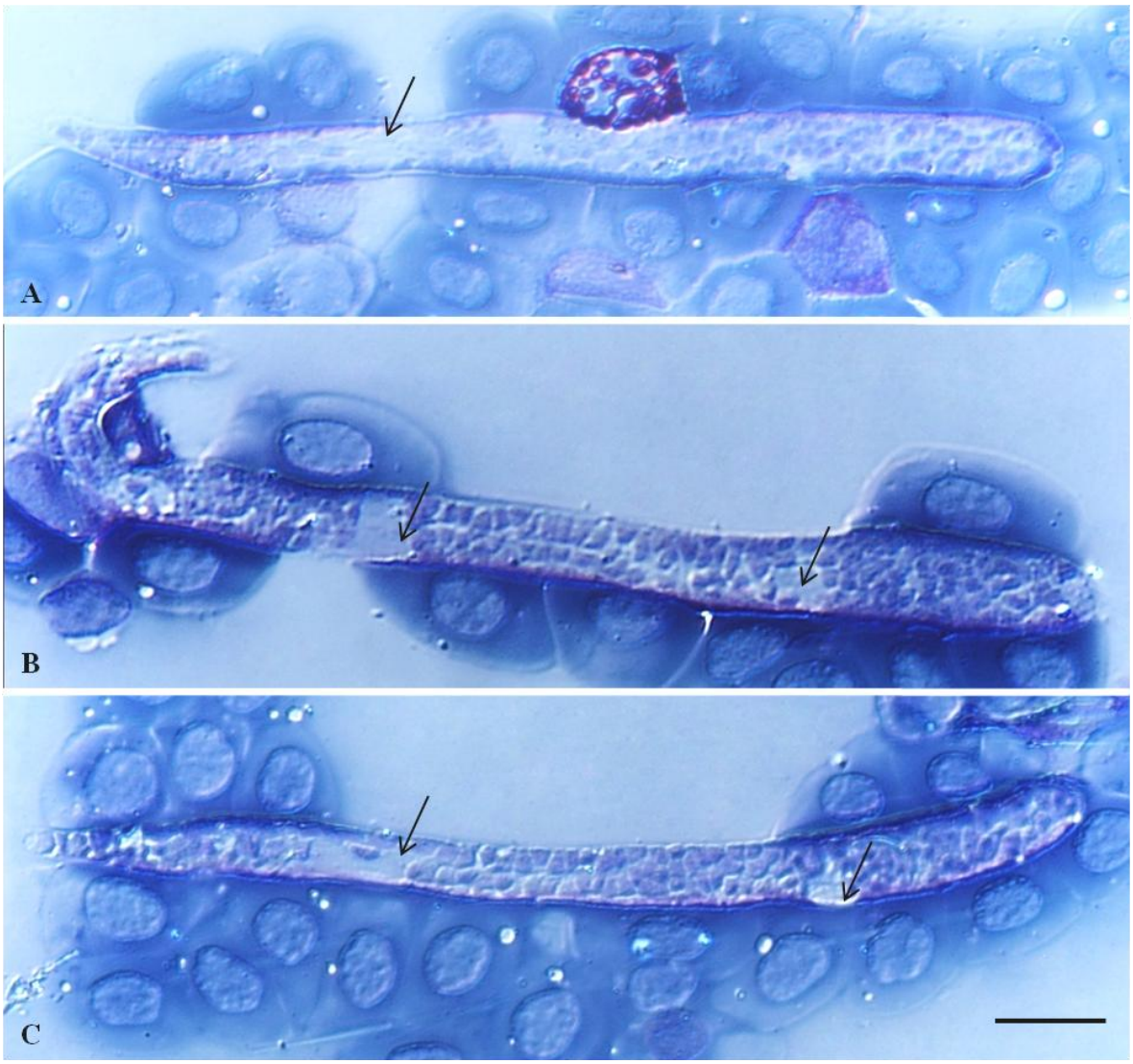

Figure 5.8 (A - C) DIC micrographs of Giemsa stained blood films of microfilariae and so-called *Sauroplasma* from the peripheral blood of *Pseudocordylus langi* from the Sentinel chain ladders in the North Eastern Drakensberg. Black arrows indicate non-staining organ primordia. Scale bar: **(A - C)** = 10µm.

5.2.4.1 Microfilaria in the peripheral blood

Microfilariae from these lizards (Fig. 5.8 A - C) measured 107 ± 4.5 (90 - 125) µm long by 4 ± 0.9 (3.0 - 4.4) µm wide (n=30) with a blue-stained, tight fitting, sheath and a rounded anterior. The tail was slender and sharply pointed (Fig. 5.8 A & C), with a single nucleus at its tip. Nuclei in the body region stained deep blue. As above, non-staining organ primordia could be seen in the anterior and posterior thirds of each nematode (Fig. 5.8 A - C black arrows).

5.2.4.2 Remarks

These microfilariae seem distinct from the other forms described in this study. Morphometrically they were the longest of all the microfilariae observed. The characteristically blue-stained body had a sharp pointed tail. These microfilaria were also longer and thinner (90 - 125 µm long by 3.0 - 4.4µm wide) than *M. ineichi* which measured only 65 - 98µm long by 5 - 7µm wide (Bain et al. 1993).

5.2.5 Discussion

Further studies on the microfilarial nematodes noted in this section in the blood of the three *Pseudocordylus* species from the Free State need to be conducted, to identify the species, since the nematodes described are all juvenile stages in nematode life cycles. Adults in the organs of hosts need to be collected and, importantly, the hosts themselves must be identified correctly. Based on the information in this Chapter 5, it seems likely that the description of *M. ineichi* in Bain et al. (1993) is from *P. subviridis* rather than from *P. melanotus.* This is because morphometrically the filarial nematodes in Giemsa stained smears from *P. subviridis* are most similar to *M. ineichi* (see above). Future work should include obtaining *P. subviridis* and *P. melanotus* specimens from the Golden Gate Highlands National park (the *M. ineichi* type locality) to establish the correct host of this species.

CHAPTER 6

GENERAL MORPHOLOGY OF CIRCULATING BLOOD CELLS, STATISTICAL ANALYSIS OF BIOLOGICAL AND ENVIRONMENTAL DATA, AND ADDITIONAL INFORMATION CONCERNING LIKELY VECTORS OF HAEMOPARASITES ASSOCIATED WITH *PSEUDOCORDYLUS* SPP.

In the previous chapters, intracytoplasmic parasites were described that occurred primarily within erythrocytes in the circulating blood of lizards. In contrast, and because of their association with the immune system, the first half of this chapter (Section 1) focuses on the morphological properties of the general leucocyte series of haemoparasite-infected crag lizards, as well as their relative numbers in blood smears, although erythrocyte characteristics are also considered. Attempts at correlating haemoparasite load with numbers of leucocytes and erythrocyte dimensions in infected individuals, and some environmental factors are also demonstrated.

The leucocyte counts presented in this Chapter 6 are compared with those of Pienaar (1962) for lizards in a broad, general sense. These comparisons are made with lizards of different species, but representing the same family (the Cordylidae). The information given in this chapter is not a detailed haematological study and is intended only as a general guide. For example, Pienaar (1962) used comprehensive haematological methods for his differential leucocyte counts, including a series of cytochemical staining techniques. For leucocyte counts, he first expressed the number of erythrocytes per cm^3 of blood, using a Levy-Hauser counting chamber and modified red blood cell diluting fluid. His leucocyte and thrombocyte counts were determined by the "differential count technique" with a Netz micrometer eyepiece. By counting 1000 to 2000 cells and knowing the erythrocytes per cm^3, he calculated the leucocyte and thrombocytes for the same volume of blood. In the current study, only Giemsa stained blood films are examined. Leucocyte counts given for the three species of crag lizards are calculated differently from Pienaar's (1962) methods, and are outlined in Chapter 2. In general, counting and categorising the different cell types of the

leucocytic series (excluding Type 1 eosinophils, which were not seen) are done in a field of ~100 erythrocytes, repeating this 10 times (that is in fields of ~ 1000 erythrocytes) in different portions of each slide for each preparation. The total number of cell types and haemoparasite infected cells are expressed as in percentages (%).

In contrast to Section 1 of this Chapter 6, the second section (Section 2) provides additional information concerning the likely, or possible, invertebrate haematophagous vectors that may aid in transmitting some parasitic infections recorded in Chapters 3 - 5. It demonstrates the external morphology of these arthropods and their adaptations to a parasitic existence, as well as showing some further examples of apicomplexan parasites and their developmental stages observed within these invertebrates,

6.1 SECTION 1

As noted above, the purposes of this section of this chapter are to give a general overview of the morphology and composition of the leucocyte types and erythrocytes present in Giemsa stained blood films of these crag lizards, as well as highlighting the pertinent literature relating to them. The final part of this Section 1 demonstrates attempts to correlate leucocyte and thrombocyte types and numbers with parasite load, lizard hosts, and some environmental factors with multivariate statistical methods. Use of Canonical Correspondence Ordination (CANOCO) plots is employed to see if there are correlations between the above mentioned variables.

6.1.1 History of reptilian haematological work

The earliest published works on the blood of reptiles were concerned with descriptions of the individual cellular components, often comparing them with those of other vertebrates (see Saint Girons, 1970). Circulating blood cells of the different reptile species have been described by Ryerson (1949), Taylor & Kaplan (1961), Pienaar (1962), Heady & Rogers (1963), Hartman & Lessler (1964), Szarski & Czopek (1966), Duguy (1970), Saint Girons (1970), Mateo et al. (1984), Canfield & Shea (1988), Cannon et al. (1996), Alleman et al. (1992), Sevinç et al. (2000), and Uğurtaş et al. (2003). According to Saint Girons (1970), one of the principle difficulties in comparative studies of reptilian blood is to determine the

different cellular lineages. Because of the different views on how haemopoiesis is achieved, there is also confusion in the terminology of the individual cell types and, to a lesser degree, the cells themselves. Pienaar (1962) compiled a comprehensive list of synonyms used in the nomenclature by major previous authors. For the purpose of this chapter, a slightly simplified version of the nomenclature of Pienaar (1962) will be used and this was outlined by Saint Girons (1970).

The overall cellular composition of blood films in the current study corresponded in general with the blood picture given by Pienaar (1962) for lizards representing the Cordylidae. The major types of mature circulating cells in the blood of reptiles are erythrocytes, granulocytes (granular leucocytes), lymphocytes, monocytes, plasma cells and thrombocytes. All of the above cells are nucleated and, according to Saint Girons (1970), do not correspond exactly between species in their staining properties or chemical composition. The great majority of workers agree that most cells in the circulating blood are derived from a multipotent haemoblast-type cell, which is capable of differentiating in the spleen and bone marrow into erythrocytes, granulocytes (basophils, eosinophils, neutrophils and azurophils), lymphocytes and monocytes.

6.1.2 Erythrocytes in circulating blood films of *Pseudocordylus* spp.

The parasitic protozoans described in Chapters 3 & 4 were found in the cytoplasm of mostly mature erythrocytes and these host cells are the most abundant of all circulating cells. Although this Chapter 6 focuses mainly on cells of the leucocytic series, some brief comments on erythrocytes will be made here. Erythrocytes are nucleated, slightly oval cells, and rounded at each end (Fig. 6.1 A black arrow) and their dimensions in crag lizards in the current study are recorded in Table 6.1. Their nuclei are chromophilic, slightly oval and centrally placed (Fig. 6.1 B, F, G, M & N). Heterochromatin masses are visible in the nuclei of erythrocytes in Giemsa stained slides (Fig. 6.1 A black arrow) and are also easily discernable in transmission electron micrographs (Fig 6.2 A & Fig. 6.3 A). Such chromatin masses are apparently dependent on the age of these cells (Pienaar, 1962; Saint Girons, 1970). In Giemsa stained blood smears, the light-blue staining cytoplasm appears homogenous and translucent. In TEM micrographs, the homogenous nature of the erythrocyte cytoplasm can also be seen (Fig. 6.2 A white arrow & Fig. 6.3 A).

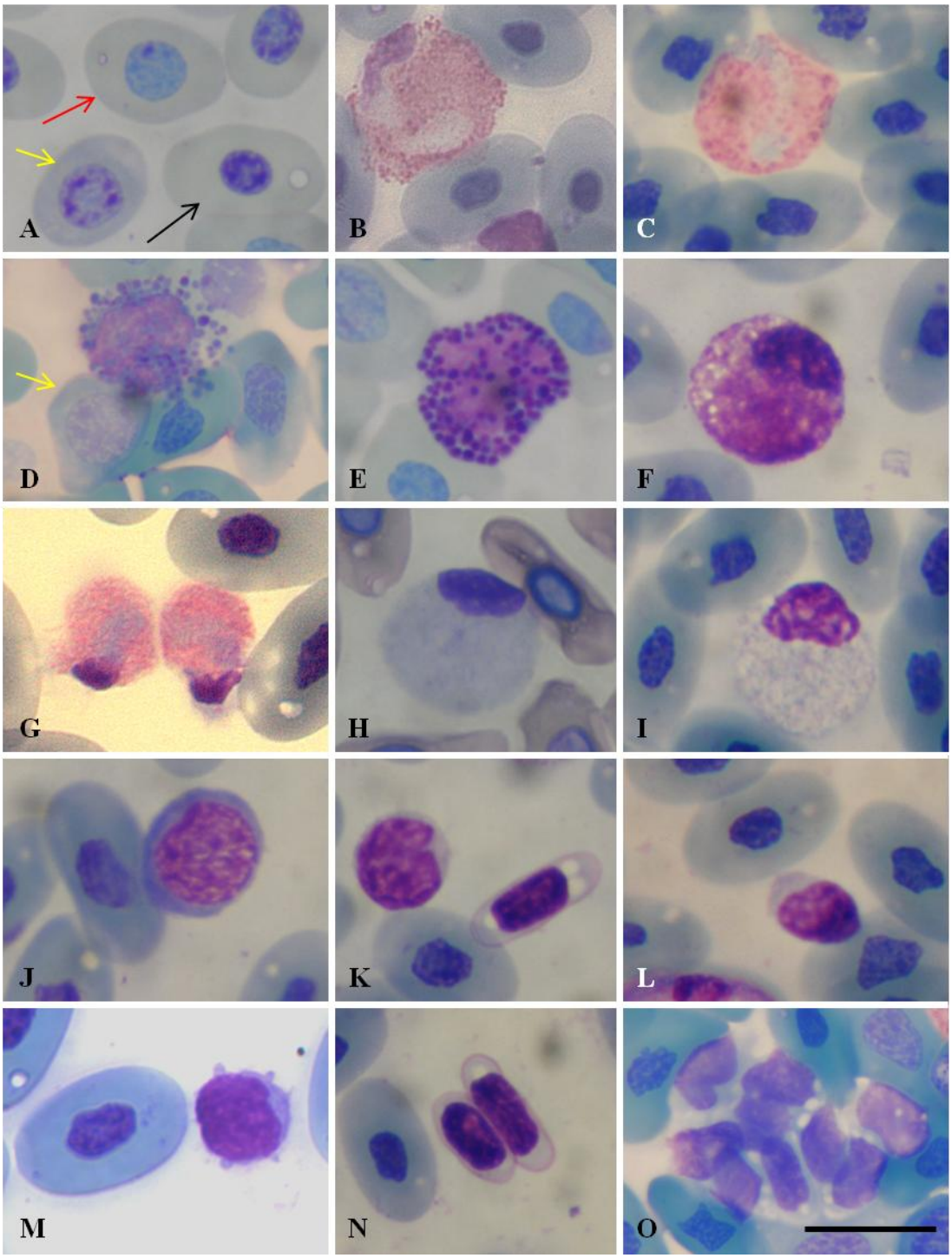

Figure 6.1 Micrographs of Giemsa stained blood films with cells of the erythrocytic and leucocytic series from *Pseudocordylus melanotus* from Platberg. **(A)** Immature erythrocyte (red arrow) with so-called *Sauroplasma*, mature erythrocyte with so-called *Sauroplasma* (black arrow), and degenerate erythrocyte (yellow arrow). **(B & C)** Possible eosinophills **(D & E)** Basophils with degenerate form in **(D)**. **(F)** Azurophil. **(G)** Heterophills. **(H)** Monocyte. **(I)** Macrophage. **(J)** Large lymphocyte. **(K)** Small lymphocyte with thrombocyte. **(L)** Small lymphocyte**. (M)** Small lymphocyte with cytoplasmic projections. **(N)** Two thrombocytes. **(O)** Clump of thrombocytes. Scale bar: (**A - O)** = 10µm.

Slightly different shades of cytoplasmic staining were observed among the three lizards in this study, but this may have been due to variations in the different batches of Giemsa stain applied. According to Pienaar (1962), the erythrocyte cytoplasm is characteristically acidophilic due to the presence of haemoglobin, although the cytoplasm is slightly less intensely staining in the perinuclear area. The cytoplasm in parasitised erythrocytes sometimes appears to stain a little lighter blue with Giemsa, or lose colouration (see Chapters 3 - 5), indicating de-haemoglobulinization. In addition, Reyerson (1949) demonstrated granules in the erythrocyte cytoplasm using critical staining methods, Bhattacharya & Brambell (1925) apparently indicated the Golgi apparatus, and Pienaar (1962) showed a variety of cellular properties using a range of haematological methods and procedures.

6.1.3 Erythroblasts

Immature erythrocytes are more rounded than mature erythrocytes, with a large nucleus which is less chromophilic than in mature red cells (Fig. 6.1 A red arrow). According to Saint Girons (1961), immature erythrocytes are common in young or moulting animals, and Pienaar (1962) observed high numbers of immature erythrocytes in reptiles heavily infected with haemoparasites, perhaps because of mature host erythrocyte destruction in this instance. Various other developmental stages of the erythrocyte series and mitotic forms are present in the circulating blood of reptiles and these reflect the processes of erythropoiesis in association with thyroid activity (Saint Girons, 1970).

6.1.4 Senile erythrocytes

Senile or degenerate stages of red blood cells were sometimes abundant in blood smears of *Pseudocordylus* spp. and these were generally larger than normal erythrocytes. These stages were seen in individuals with high haemoparasite infections. Their cytoplasm and nuclei often stained lighter with Giemsa (Fig. 6.1 A & D yellow arrows). Apparently, their nuclei are sometimes barely visible according to Pienaar (1962), who studied these cell types in *Cordylus vittifer*.

6.1.5 Remarks

Saint Girons & Saint Girons (1969) compiled a list, comparing the morphometrical dimensions of erythrocytes in four orders of reptiles representing 29 families and 76 species. From this table it was clear that the Cordylidae (represented by two species, *Cordylus cordylus* and *Cordylus vittifer*) displayed some unique differences in terms of size, compared with other lizards. The main difference was the larger, more rounded erythrocyte nucleus of these species in relation to that of most other lizards in Saint Girons & Saint Girons (1969) study.

Morphometrically the erythrocytes in cordylid lizards appear to be moderately variable between species and to a lesser degree, some variability also occurs in different specimens within a species. For example, erythrocyte dimensions given by Girons & Saint Girons (1969) for *Cordylus vittifer* were 17.2 x 9.5 µm with a total surface area of 128.0 µm² and the erythrocyte nuclei measured 7.1 x 4.6 µm with a surface area of 25.6 µm². Pienaar's measurements of the same lizard species were in the same range (12 - 18 x 7 - 10) µm with a mean of 16.5 x 8.5 µm for mature erythrocytes. Interestingly, erythrocyte measurements for *Cordylus cordylus* reported by Girons & Saint Girons (1969) were 17.3 x 9.8 µm with a surface area of 133.4 µm² and nuclei 6.3 x 4.5µm with a surface area of 22.2 µm². Pienaar (1962) listed the erythrocyte dimensions for *Cordylus gigantus* (16.8 x 10) µm which were slightly larger and more rounded than erythrocytes in *C. vittifer*.

Erythrocyte measurements of the three species of cordylid lizards in the present study are summarised in Table 6.1 and seem to be unique for each species. The smallest in surface area were those of *P. melanotus* (138.2 µm²), then *P. subviridis* with slightly larger cells (144.5 µm²) and *P. langi* with the largest erythrocytes (151.1 µm²). These measurements, when loosely correlated with host altitudinal distribution show interesting results. *Pseudocordylus langi*, occurring at the highest altitudes (above 3000m), has the largest erythrocytes in surface area. *Pseudocordylus subviridis* on the other hand, occurs at lower altitudes (from above ~3000 to below 2000m), overlapping the altitudinal ranges of the other 2 species since it occurs sympatrically with *P. langi* and *P. melanotus*; it has erythrocytes in the medium range surface area (144.5 µm²). *Pseudocordylus melanotus* occur at the lowest altitudes (~2000m and below) of all three lizards, and has erythrocytes with the smallest

Table 6.1 Morphometrical dimensions of mature erythrocytes and erythrocyte nuclei in *P. melanotus*, *P. subviridis* and *P. langi* from the two study sites of Platberg and Sentinel in the Eastern Free State.

Pseudocordylus melanotus	**(Mean ± Stdev (Min - Max) µm (n)**
Erythrocyte length	18.0 ± 1.0 (16.4 - 19.9) (100)
Erythrocyte width	9.8 ± 1.2 (7.0 - 11.8) (100)
Erythrocyte area (µm²)	138.2 ± 24.2 (120 - 192) (100)
Erythrocyte nucleus length	6.5 ± 0.6 (5.2 - 7.5) (60)
Erythrocyte nucleus width	4.0 ± 0.6 (3.3 - 4.9) (60)
Erythrocyte nucleus surface area (µm²)	23.7 ± 2.6 (16.4 - 26.7) (60)
Pseudocordylus subviridis	
Erythrocyte length	18.1 ± 1.1 (16.9 - 20) (100)
Erythrocyte width	9.4 ± 1.4 (7.1 - 11.5) (100)
Erythrocyte area (µm²)	144.5 ± 24 (121.8 - 191.6) (100)
Erythrocyte nucleus length	6.5 ± 0.6 (5.2 - 7.5) (60)
Erythrocyte nucleus width	4.0 ± 0.6 (3.3 - 4.9) (60)
Erythrocyte nucleus surface area (µm²)	23.9 ± 2.9 (16 - 26) (60)
Pseudocordylus langi	
Erythrocyte length	18.2 ± 1.2 (15.8 - 20.1) (100)
Erythrocyte width	9.9 ± 1.3 (6.9 - 11.2) (100)
Erythrocyte area (µm²)	151.1 ± 24.5 (120.3 - 194.4) (100)
Erythrocyte nucleus length	6.6 ± 0.9 (4.9 - 7.8) (60)
Erythrocyte nucleus width	4.5 ± 0.8 (3.9 - 4.9) (60)
Erythrocyte nucleus surface area (µm²)	24.7 ± 3.1 (16.5 - 28) (60)

surface area of the three lizards (138.2 µm²). A possible explanation for these observations could be that the high altitudes at which the lizards occur, with more demand for oxygen transport, and therefore more haemoglobin carried in the erythrocyte cytoplasm to meet these demands. *Pseudocordylus langi* erythrocyte cytoplasm also stains slightly darker with Giemsa (see Chapters 3 & 5) and this could indicate more haemoglobin in this location. It may be that the different *Pseudocordylus* species have different sized erythrocytes naturally, but is very interesting to note that in the present case, there seems to be a set of geographical boundaries determining variations in the morphometrical dimensions of erythrocytes for each species used in the study.

Without measuring the precise haemoglobin content of these high altitude lizards, it can only be assumed that they may have more haemoglobin for oxygen transport than cordylids at lower altitudes, like *C. cordylus* with smaller erythrocytes with a surface area of 133.4 µm² (see Girons & Saint Girons, 1969). However, there could be other explanations for differences in erythrocyte dimensions. Wintrobe (1933) postulated that the size of erythrocytes reflects the systematic position of a species in the evolutionary scale, while in a similar manner Saint Girons (1970) suggested that the size of erythrocytes is related to the systematic position of the families from which they are derived.

6.2 Granulocytes

6.2.1 Eosinophils (Eosinophilic granulocytes)

Eosinophils are large, rounded cells with a granulated cytoplasm that normally stains brilliant pinkish, yellow-orange with Giemsa (Fig. 6.1 B & C). These granulated types of leucocytes were rarely encountered during routine examinations for haemoparasites in the current study. Pienaar (1962) distinguished between two types of eosinophils in *Cordylus vittifer.* The Type 1 eosinophils contained spindle shaped or crystalloid granules and Type 2 eosinophils contained course spheroidal granules in the cytoplasm. Pienaar (1962) stated that a sharp rise in Type 1 eosinophils was associated with heavy 'worm' and haemoprotozoal infections. Saint Girons (1970) reported that these cells were very variable in morphometrical dimensions compared with the same types of cells in other animal groups. Only Type 2 eosinophils were observed in the blood of *Hepatozoon* sp. A - E infected individuals and they made up a small fraction of the total leucocyte counts (Table 6.2). The number of this type of

eosinophil in all three *Pseudocordylus* spp. was slightly higher compared to the Type 2 eosinophils in *C. vittifer* from Pienaar (1962) which he calculated as 0.12% for young males, 0.07 % for adult females and 0.18 % for adult males. Ultrastructurally, an eosinophil was seen in sections of blood from *P. melanotus*. Characteristically the cytoplasm contained large, pleomorphic, oval to elongate granules (Fig. 6.3 B red arrow). The nucleus was eccentrically located and showed peripherally and centrally located heterochromatin areas (Fig. 6.3 B white arrows). Interestingly, eosinophil cytoplasmic processes (Fig. 6.3 B black arrows) were clearly seen in electron micrographs.

6.2.2 Basophils (Basophilic granulocytes)

This type of leucocyte is rounded, sometimes slightly oval, with its cytoplasm filled with extremely chromatophilic granules (Fig. 6.1 D & E). In some cases the cytoplasmic granulations obscure the nucleus. Pienaar (1962) described the normal, intact forms appearing as "microscopic blackberries" and found a striking variation in the morphology of these cells according to his counts in *C. vittifer*. These cells constituted 6.3% of the differential counts of the leucocytes in his study. Basophil cells in *Pseudocordylus* spp. were seen only occasionally and therefore were low in number, as in Pienaar's (1962) counts for *C. vittifer*. Pienaar found that these cells were fragile, and with stains such as Giemsa, the basophilic material was sometimes partially or totally dissolved from the cytoplasm. This could be a major reason for the low basophil counts in the current study of infected lizards because most of the cells could only be identified as basophil "ghosts" with scattered bluish granules in a largely smudged cytoplasm (Fig. 6.1 D).

6.2.3 Azurophils (Azurophilic granulocytes) and Heterophils

Pienaar (1962) stated the following regarding the first of these cell types (azurophils): "No single leucocyte type or group of leucocytes occurring in the blood of the lower vertebrates has induced such complete misapprehension, ambiguity of description and speculative or controversial discussion as this group of leucocytes". A wide range of morphological type variations was found by Pienaar (1962) and he was of the opinion that there exists absolute confusion in the haematological literature about the identity of these cells. The same cells were referred to as monocytes or large mononuclears by some authors (Ryerson, 1949; Jordan, 1938 and Wood, 1935). Michels (1937) referred to them as heterophils and Bernstein

(1938), Wood, (1935) and Rabelais (1938) referred to them as neutrophils. Pienaar (1962) found these cell types in *Cordylus* and in other reptiles where they exhibited the morphological features of a granulocyte and also characteristics of mammalian monocytes. Saint Girons (1970) described azurophils as relatively small, with irregular eccentric nuclei that varied greatly in size. Azurophils located in *Pseudocordylus* spp. in the current study appeared to have the eccentric nuclei characteristic of heterophils, but with a much deeper staining cytoplasm (Fig. 6.1 F). These cells are also reported to contain intracytoplasmic lipid bodies, but Pienaar (1962) found these bodies absent in those from *Cordylus*. Senile cells of this type may apparently contain many vacuoles (Saint Girons, 1970). Heterophils (Fig. 6.1 G) were identified on some occasions in blood films of *Pseudocordylus* spp. and constituted a small fraction of the total leucocyte counts in infected crag lizards (Table 6.2). Ultrastructurally, a likely heterophil (Fig. 6.2 A blue arrow) had characteristic electron dense granules (Fig. 6.2 A yellow arrows) in the cytoplasm, and an eccentric nucleus with electron dense heterochromatin areas (Fig. 6.2 A white arrows).

6.2.4 Neutrophils (Neutrophilic granulocytes)

Pienaar (1962) characterised these cells as circular to oval, frequently forming groups of two or three cells beside each other in blood smears. The early stage had a basophilic cytoplasm and as they matured the nucleus took an eccentric position and the intragranular cytoplasm took on an acidophilic tinge (Pienaar, 1962). Saint Girons (1970) was of the opinion that these types of leucocytes were probably not homologous to the neutrophils of mammals, but could be regarded as morphological variants of azurophilic granulocytes (see above), and they were also described as heterophils (mononuclear neutrophils). According to Ryerson (1949), the name neutrophils has also been applied to eosinophilic granulocytes with cylindrical granules, which are the Type 1 eosinophils of Pienaar (1962). These cells are also known as polymorphonuclear amphophilic leucocytes (Pienaar, 1962; Telford, 1975). This adds to the nomenclatural confusion as describe above. Such cells were not identified with confidence in any *Pseudocordylus* spp. blood smears in the current study.

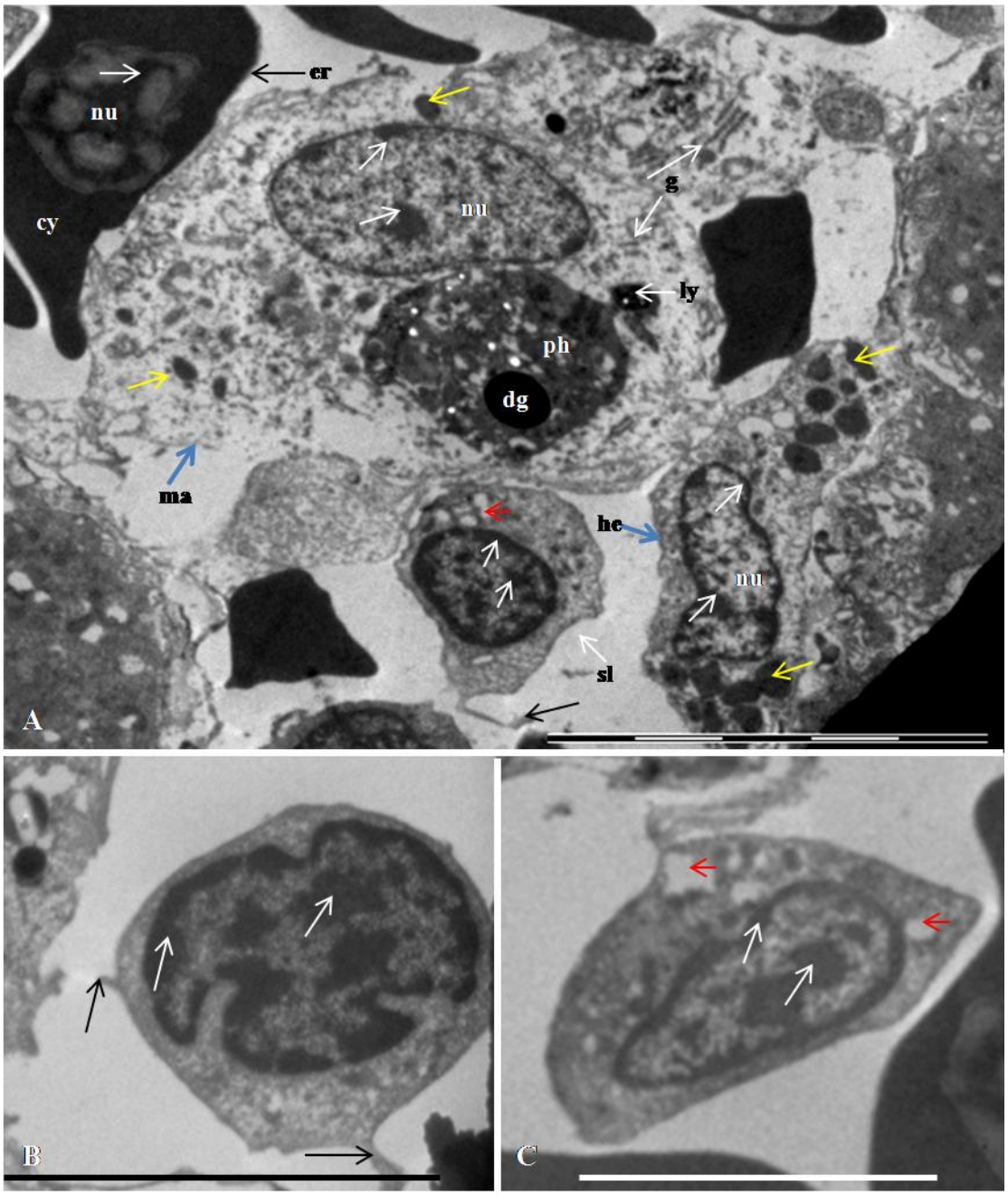

Figure 6.2 Transmission electron micrographs of the crag lizard blood showing some cells of the white cell series, including a macrophage, suspected heterophil, and lymphocytes, as well as possibly a thrombocyte, and an erythrocyte. **(A)** Transmission electron micrograph showing large macrophage [(ma) blue arrow)] containing an intracytoplasmic phagosome (ph) with a dense granule (dg), possibly fusing with a lysosome (ly). The macrophage cytoplasm also contains parallel cisternae, possibly those of a Golgi apparatus (g). Also present in this micrograph are a likely heterophil [(he) blue arrow)] with granules (yellow arrows) and an eccentric nucleus (nu), a small lymphocyte [(sl) white arrow)] showing clear vacuoles in the cytoplasm (red arrow) and a irregularly rounded nucleus that contains peripherally and centrally located heterochromatin (white arrows), as well as an erythrocyte [(er) black arrow)] showing the cytoplasm (cy) and nucleus (nu) with electron dense heterochromatin areas (white arrow). **(B)** Another **s**mall lymphocyte with scant cytoplasm and two cytoplasmic processes (black arrows). Irregularly rounded nucleus contains peripherally and centrally located heterochromatin (white arrows). **(C)** Possible thrombocyte with intracytoplasmic vacuoles (red arrows) and a slightly elongate nucleus with heterochromatin and possible nucleoli (white arrows). Scale bars: **(A)** = 10µm, **(B & C)** = 5µm.

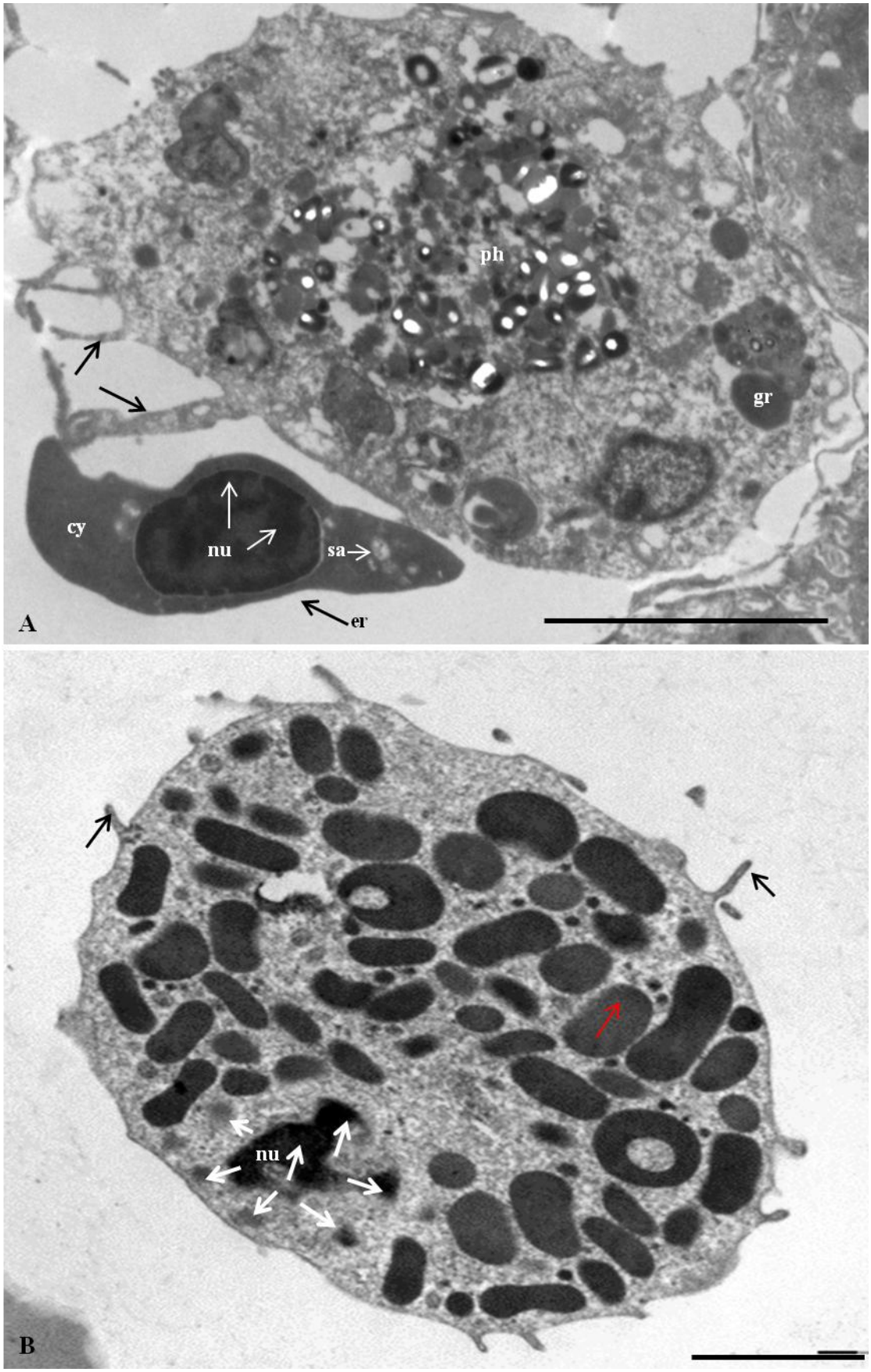

Figure 6.3 Transmission electron micrographs of further examples of lizard blood leucocytes and an erythrocyte. **(A)** Macrophage with cytoplasmic granules of variable density (gr) and large, central phagosome (ph). Note the macrophage cytoplasmic processes (black arrows). Erythrocyte lies beneath the macrophage [(er) black arrow)] showing the cytoplasm (cy) and nucleus (nu) with electron dense heterochromatin areas (white arrow). Note the so-called *Sauroplasma* (sa) infections in the cytoplasm. **(B)** Likely eosinophil with large, pleomorphic oval to elongate cytoplasmic granules (red arrow). Grazing section through eccentrically located nucleus (nu) shows peripherally and centrally located heterochromatin areas (white arrows). Note also the cytoplasmic processes in **B** (black arrows). Scale bars: **(A)** = 5µm **(B)** = 2µm.

Table 6.2 Thrombocyte, differential leucocyte and *Hepatozoon* sp. A - E infected erythrocyte counts in *Pseudocordylus melanotus*, *Pseudocordylus subviridis* and *Pseudocordylus langi* (expressed as % of erythrocytes)

Pseudocordylus melanotus	**Females (n=17)**	**Males (n=27)**	**Juvenile (n=1)**
Thrombocytes, leucocyte types and *Hepatozoon* sp. A - C	Mean %	Mean %	Mean %
Thrombocytes	3.07	4.63	3.45
Type 2 Eosinophils	0.77	1.46	1.31
Basophills	1.23	1.48	0.93
Small Lymphocytes	5.09	5.60	9.61
Large Lymphocytes	2.23	2.34	2.61
Monocytes / Macrophages	0.29	0.24	0.19
Azurophil	0.17	0.16	0.09
Heterophil	0.14	0.12	0.09
Hepatozoon sp. A	0.53	16.48	0.00
Hepatozoon sp. B	2.18	0.19	0.00
Hepatozoon sp. C	0.82	0.56	0.00
Pseudocordylus subviridis	**Females (n=6)**	**Males (n=10)**	**Juveniles (n=2)**
Thrombocytes, leucocyte types and *Hepatozoon* sp. A & C	Mean %	Mean %	Mean %
Thrombocytes	1.69	1.16	1.53
Type 2 Eosinophils	0.36	0.78	0.62
Basophil	0.76	1.04	0.37
Small Lymphocyte	7.49	2.83	17.86
Large Lymphocyte	1.69	3.03	2.47
Monocytes / Macrophages	0.55	0.70	0.44
Azurophil	0.13	0.14	0.13
Heterophil	0.15	0.12	0.54
Hepatozoon sp. A	1.33	6.60	0.00
Hepatozoon sp. C	2.33	9.50	0.00
Pseudocordylus langi	**Females (n=7)**	**Males (n=3)**	
Thrombocytes, leucocyte types and *Hepatozoon* sp. D & E	Mean %	Mean %	
Thrombocytes	3.8	1.8	
Type 2 Eosinophils	0.9	0.8	
Basophil	0.7	2.0	
Small Lymphocyte	8.3	6.2	
Large Lymphocyte	7.2	4.1	
Monocytes / Macrophages	0.1	0.4	
Azurophil	0.1	0.2	
Heterophil	0.1	0.2	
Hepatozoon sp. D	9.1	8.2	
Hepatozoon sp. E	0.1	2.0	

6.2.5 Non- granulocyte leucocytes

6.2.5.1 Monocytes and Macrophages

Monocytes were seen only rarely in blood films (Fig. 6.1 H) and are believed to differentiate into macrophages (Fig. 6.1 I). Macrophages are named according to their function and a phagocytic element is implied (Pienaar, 1962; Jurd, 1994); these cells were seen in the circulating blood of *Pseudocordylus* spp. (as above) as well as in TEM sections.

Interestingly, the lizard from which TEM sections were prepared was heavily infected with two *Hepatozoon* species, a *Plasmodium* sp and so-called *Sauroplasma* infections. Ultrastructurally, the two macrophages shown in Fig. 6.2 A & Fig. 6.3 A contained phagosomes in their cytoplasm, emphasizing their important role in ingesting foreign matter. The intracytoplasmic phagosome shown in Fig. 6.2 A possessed a dense granule of uncertain identity, and possibly the fusing of the phagosome with a lysosome was in progress. The same electron micrograph shows the macrophage cytoplasm with parallel cisternae, possibly those of a Golgi apparatus.

6.2.5.2 Lymphocytes

In general, lymphocytes are considered modified haemocytoblasts or stem cells (Saint Girons, 1970) that can differentiate into all other types of corpuscles. According to Zimmerman et al. (2009), reptiles possess both innate and adaptive immune systems and the innate system includes components such as non-specific leucocytes that respond quickly as a non-specific first line of defence against a broad range of pathogens. There is confusion regarding the exact origins of these cells in reptiles. According to Zimmerman et al. (2010), T cells are a class of lymphocytes that are known to only regulate antibody production and not produce antibodies themselves. T cells have been identified in various reptiles representing the squamates, chelonians and tuataras (Burnham et al. 2005), and T helper cells are known to regulate other immune cells. Zimmerman et al. (2010) explain further that activated T cells can differentiate into cytotoxic T cells or T helper cells. The cytotoxic T cells attack altered or damaged cells, such as cancerous cells and can also kill viral or bacterial infected cells by rapidly triggering apoptosis. Another class of lymphocytes, B cells, produces antibodies when stimulated by an antigen (Zimmerman et al. 2010) and does not require the antigen to be processed by an intermediate cell (Coico et al. 2003). These B

cells can therefore recognize antigens in their natural state (Zimmerman et al. 2010). According to Zimmerman et al. (2009), B cells release antibodies in response to immunization after a latent period of a week, and these cells have been demonstrated to have phagocytic abilities in amphibians and teleost fish (Li et al. 2006), as well is in turtles (Zimmerman et al. 2009).

Other than erythrocytes, lymphocytes are the most numerous cells in blood films, as was the case in Pienaar's (1962) work, and in the counts for crag lizards in the current study (Table 6.2). Lymphocytes are variable in size with a weakly basophilic cytoplasm with Giemsa stain (Fig. 6.1 J - M). Azurophilic granules and chromophobic vacuoles apparently occur in the cytoplasm (Pienaar, 1962). Three types of lymphocytes were described by Pienaar (1962). These were large lymphocytes (as in Fig. 6.1 J), (14.5µm or larger in diameter), small lymphocytes (as in Fig. 6.1 L), (5.5 - 10µm in diameter) and all intergrades were regarded by Pienaar (1962) as medium lymphocytes (as in Fig. 6.1 K & M) (with a diameter of 10 - 14.5µm). Cytoplasmic projections can clearly be seen in Fig. 6.1 M. Lymphocyte counts in blood films of *Pseudocordylus* spp. were given only as large and small lymphocytes (see Table 6.2). In line with Pienaar's (1962) findings, the largest composition in Giemsa-stained blood films of the three species of *Pseudocordylus* was small lymphocytes, followed by the larger lymphocytes. Ultrastructural observations of small lymphocytes in the current research showed clear vacuoles in the cytoplasm (Fig. 6.2 A red arrows) and irregularly rounded nuclei (Fig. 6.2 A & Fig. 6.2 B) that contained peripherally and centrally located heterochromatin (Fig. 6.2 A & B white arrows).

6.2.6 Thrombocytes

Normal, mature thrombocytes are oblong in shape (as in Fig 6.1. K & N) and induce blood clotting, like the platelets of higher vertebrates. Pienaar (1962) stated that the cytoplasm of thrombocytes is of a delicate, viscous in nature, and is rarely seen in their natural state. Apparently these cells can be seen only in their natural shape when thin smears are meticulously made, rapidly dried and efficiently stained. According to Pienaar (1962) even under ideal conditions, these cells show considerable cytoplasmic and nuclear distortions, with irregular outlines and long cytoplasmic attenuations that give them a stellate appearance. Owing to the viscous nature of these cells, they tend to stick together in groups of 2 or more,

and this was especially evident in the current case when smears that were not prepared immediately after a blood sample was collected (Fig. 6.1 O). Another diagnostic feature of these cells is a longitudinally grooved nucleus with a deep furrow or depression along its long axis (Pienaar, 1962). Ultrastructural observations of these cells confirm a slightly elongate nucleus with peripherally and centrally distributed heterochromatin (Fig. 6.2 C white arrows) and intracytoplasmic vacuoles (Fig. 6.2 C red arrows).

6.2.7 Possible relationships between leucocyte and thrombocyte counts, parasite load, lizard hosts, and environmental data.

Leucocyte, thrombocyte and infected red blood cell counts from stained blood smears give an overall representation of the blood picture of some of the three crag lizard species examined in this study. The information provided in Table 6.2 was pooled with other biological data (leucocytes and thrombocytes, haemoparasite parasitaemia, hosts) and environmental data (sites, altitude and proximity to dams at site 1, Platberg) to see whether any correlations between these variables could be detected. Although more data are needed for clear assessment of the associations between parameters, the following observations were made.

Findings of the multivariate statistical assessment of variability of parasitic infections are graphically presented in the redundancy analyses (RDA) in Fig. 6.4 and Fig. 6.5. Findings in Fig. 6.4 show that a large variability in the parasitic infections of the host reptiles was obtained. These variations result in the formation of three groups of host lizards whose infections are similar. The first grouping includes PLAN-M (*P. langi* males) and PLAN-F (*P. langi* females) and the closely associated HEP-D (*Hepatozoon* sp. D) and HEP-E (*Hepatozoon* sp. E) infections. This grouping also appears to be closely associated with the L-Lymphocyte (large lymphocyte) and to lesser extent S-Lymphocyte (small lymphocyte) explanatory variables. Another grouping includes PSUB-F (*Pseudocordylus subviridis* females) and PSUB-M (*Pseudocordylus subviridis* males) which correlate with HEP-C (*Hepatozoon* sp. C) and HEP-A (*Hepatozoon* sp. A). This grouping appears to be driven by the Monocyte explanatory variable. Both of these groups are together largely driven by altitude (m.a.s.l) (meters above sea level) and Site 2 (Sentinel).

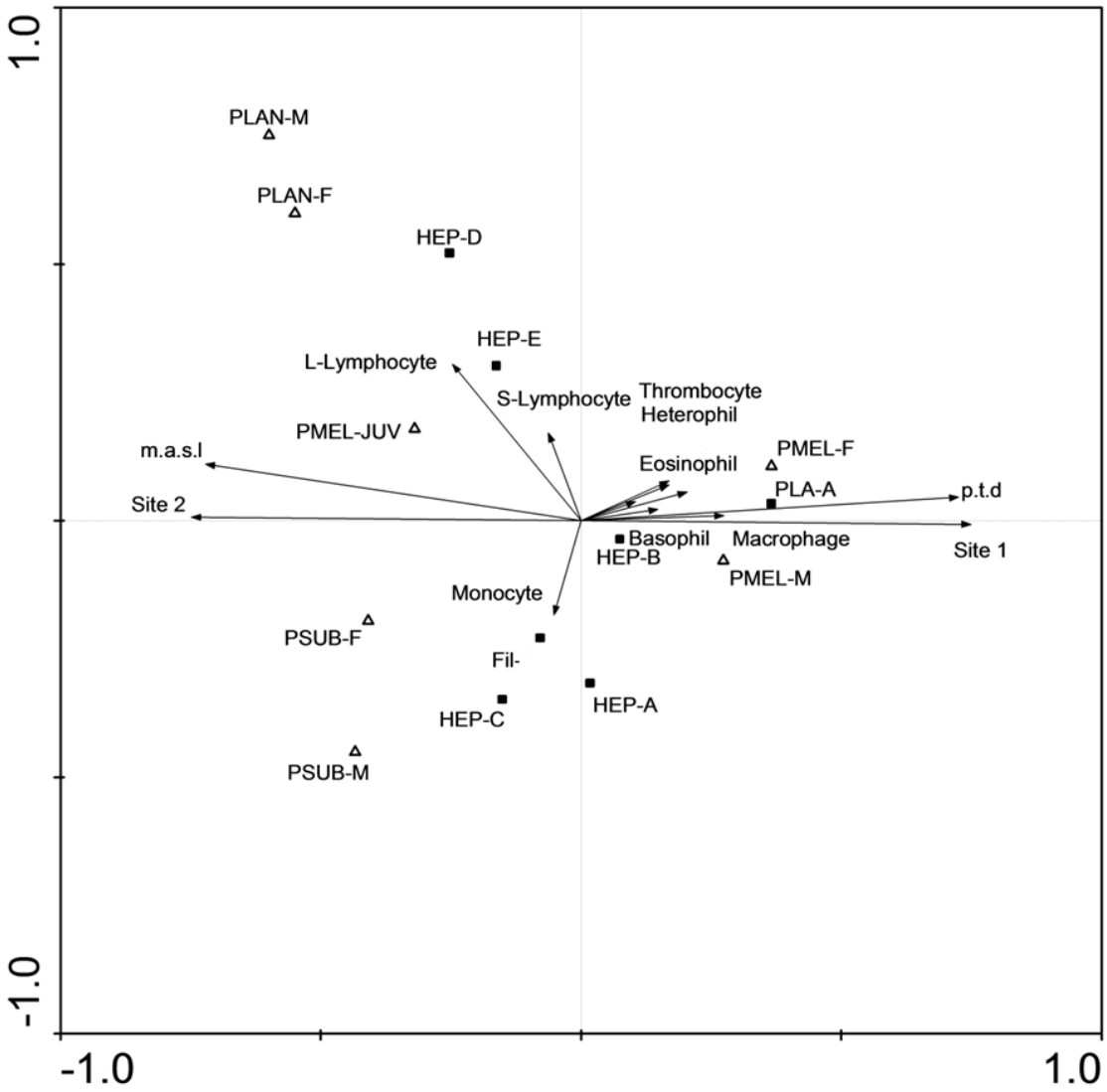

Figure 6.4 Canonical correspondence ordination (CANOCO) plot of 3 lizard species males, females and juveniles, from 2 sites (Platberg and Sentinel), to examine the strength of association between haemoparasite load (*Hepatozoon sp.* A – E, *Plasmodium* sp. A, Filaria spp. (that is the biological data) and the number of different types of leucocytes (explanatory data) per individual. The eigenvalues of Axis 1 (horizontally) and Axis 2 (vertically) are 52.3 and 27.8, respectively, with 80.1% of variation of data explained. Significant differences were noted in biological data set between haemoparasite load and lizard species from the 2 sites was (P = 0.001). Lizard hosts from which leucocyte counts and parasitaemias were calculated are indicated by triangles, and are abbreviated as follows: (PLAN-M) = *P. langi* male; (PLAN-F) = *P. langi* female; (PMEL-JUV) = *P. melanotus* juvenile; (PMEL-M) = *P. melanotus* male; (PMEL-F) = *P. melanotus* female; (PSUB-F) = *P. subviridis* female; (PSUB-M) = *P. subviridis* male. Parasite species are indicated by square blocks and are abbreviated as follows: (HEP-A) = *Hepatozoon* sp. A; (HEP-B) = *Hepatozoon* sp. B; (HEP-C) = *Hepatozoon* sp. C; (HEP-D) = *Hepatozoon* sp. D; (HEP-E) = *Hepatozoon* sp. E; (PLA-A) = *Plasmodium* sp. A; (FIL) = Filaria spp. Explanatory data are shown by arrows, and are abbreviated as follows: (m.a.s.l) = meters above sea level; (p.t.d) = proximity to dam; (site 1) = Platberg; (site 2) = Sentinel; (S-lymphocyte) = small lymphocyte; the other types of white blood cells (leucocytes) are written out in full, as are thrombocytes.

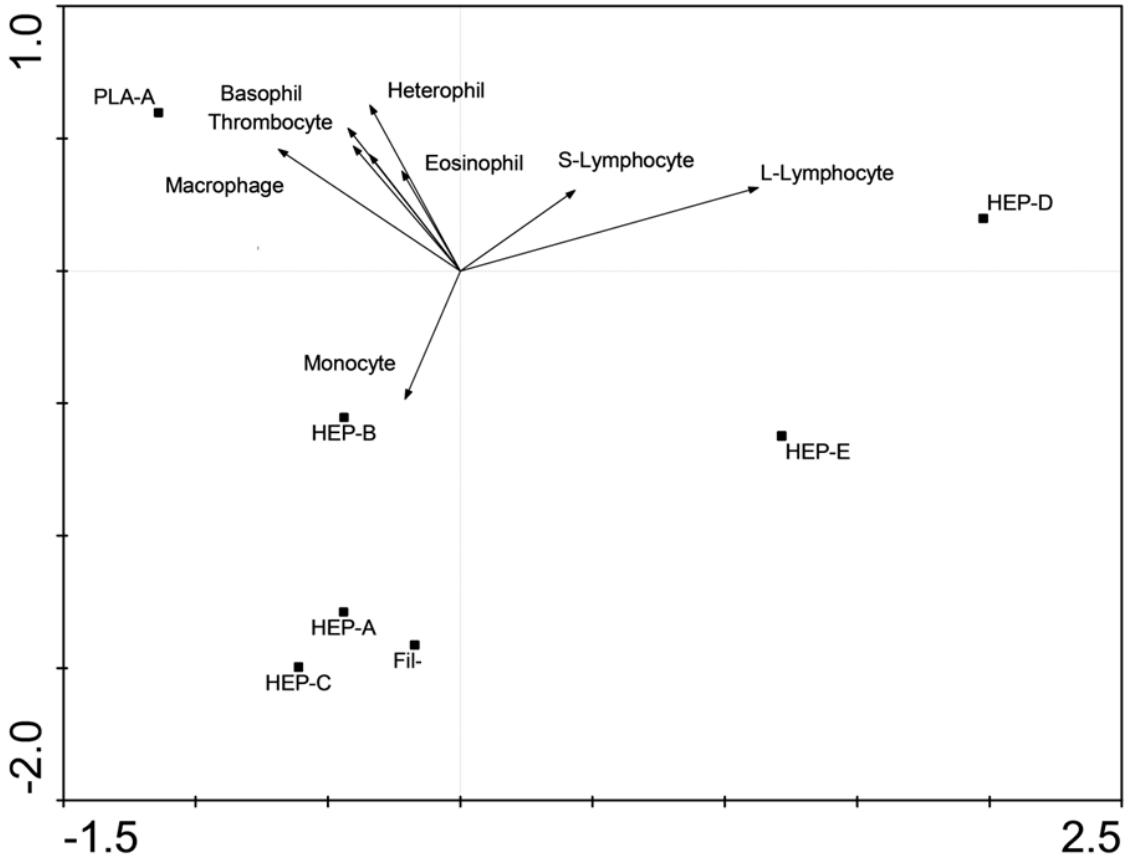

Figure 6.5 Canonical correspondence ordination (CANOCO) plot of haemoparasite load (*Hepatozoon* sp. A - E, *Plasmodium* sp. A, Filaria spp. (that is the biological data) and the number of different types of leucocytes (explanatory data) per individual. The eigenvalues of Axis 1 (horizontally) and Axis 2 (vertically) are 48.9 and 25.2, respectively, with 74.1% of variation of data explained. No significant differences in biological data set between haemoparasite load and types of leucocytes were noted (P = 0.466). Parasite species are indicated by square blocks and are abbreviated as follows: (HEP-A) = *Hepatozoon* sp. A; (HEP-B) = *Hepatozoon* sp. B; (HEP-C) = *Hepatozoon* sp. C; (HEP-D) = *Hepatozoon* sp. D; (HEP-E) = *Hepatozoon* sp. E; (PLA-A) = *Plasmodium* sp. A; (FIL) = Filaria spp. Explanatory data are shown in arrows, and are abbreviated as follows: (S-lymphocyte) = small lymphocyte; (L-lymphocyte) = large lymphocyte; the other types of white blood cells (leucocytes) are written out in full, as are thrombocytes.

The third, and remaining group includes PMEL-M (*Pseudocordylus melanotus* males), PMEL-F (*Pseudocordylus melanotus* females), and PLA-A (*Plasmodium* sp. A) and these are weakly associated with Thrombocyte, Heterophil and Eosinophil explanatory variables. This last group is largely driven by p.t.d (proximity to dam) and Site 1 (Platberg). Significant differences (P = 0.001) were seen between the occurrence of *Hepatozoon* sp. A - E, *Plasmodium* sp. A, Filaria spp. and so-called *Sauroplasma* infections in the blood of the three species of *Pseudocordylus* lizards. Altitude, expressed in meters above sea level (m.a.s.l) may have a slight impact on the number of large lymphocytes, as it constitutes part of the top 20 driving variables, but is more closely related to Site 2 (Sentinel). However, the effects between altitude and parasite loads are unclear, and although manual assessment of the correlation between altitude and parasite load is significant (P=0.001), this is likely due to the driving influence from Site 2 (Sentinel). Proximity to dam (p.t.d) was found to be a significant variable in this data set, and is associated with Site 1 (Platberg); thus proximity to dam and Site 1 (Platberg) are shown to be important variables driving variation in the data set, and indicate a significant relationship (P = 0.001).

A second assessment comparing parasite loads and leucocyte and thrombocyte counts is shown in Figure 6.5. In this assessment, although no significant relationships were found between the parameters (P=0.466), weak correlations between small and large lymphocytes and the different parasites were obtained. The number of monocytes was the most dissimilar parameter between the leucocytes, perhaps not surprising since these cells were observed only rarely in blood films (see above). This assessment similarly showed that variability in the parasite loads resulted in the formation of three groups, where similar loads were obtained. These groups included PLA-A (*Plasmodium* sp. A), alone in one group (Group 1), Fil (Filaria spp.) and HEP-A, B & C (*Hepatozoon* sp. A - C) in a second (Group 2) and HEP-E (*Hepatozoon* sp. E) and HEP-D (*Hepatozoon* sp. D) in the third (Group 3). These groups were weakly associated with Monocytes (group 2), S-Lymphocytes and L-Lymphocytes (Group 3) and the remaining blood physiology parameters with Group 1.

6.3 SECTION 2

6.3.1 Likely vectors of hemoparasites associated with *Pseudocordylus* spp.

This section deals with some haematophagous invertebrates that have been noted already in earlier chapters, but exploring further their role as possible vectors in transmission of haemoparasites to crag lizards of the eastern Free State highlands. Here, a morphological overview of mites and mosquitoes is provided as well as some additional results obtained from the study of transmission of *Hepatozoon* spp. from lizard to mosquito hosts.

The often, very high, infections of blood haematozoans found in these three species of crag lizard (see Chapters 3 - 5) suggest that the haematophagous invertebrate vectors must be abundant and perhaps be able to transfer between lizard populations relatively easily. For example, the same species of haemogregarines (based on morphometrical and morphological characteristics) were found in two different species of crag lizards from completely disjunct localities and at different altitudes. Other blood infections, such as *Plasmodium* sp. A and so-called *Sauroplasma* sp., may also represent the same species in both lizard hosts from these separate localities.

Although discussed in greater depth in the Introduction (Chapter 1), a few recognised examples of haematozoan transmission are as follows. Brumpt and Lavier (1935) showed transfer of the piroplasm, *Pirhemocyton lacerate* between two lizards, *Lacerta viridis* by means of direct inoculation. Transmission of a haemogregarine was demonstrated by Lewis and Wagner (1964) by feeding lizards infected, crushed mites (*Hirstiella* sp.), containing sporocyst stages of *Hepatozoon sauromali* Lewis and Wagner, 1964. Ayala & Lee (1970) showed that a sandfly (*Lutzomyia vexator*) could transmit a malaria parasite (*Plasmodium mexicanum*) to lizards. The above examples suggest that natural transmission of at least some of the blood parasites in this study may take place with the aid of haematophagous vectors under natural conditions.

The three lizard species captured and sampled during fieldwork were found to be infested with at least four genera of prostigmatic mites. Crag lizards often occur in groups of two or more, sometimes even existing with different species of lizards, representing different

families, and it is not inconceivable that these ectoparasitic mites play an important role in transmitting blood parasites. However, in terms of vectors for *Hepatozoon* infections, thus far, only eight species of haematophagous invertebrates are known to be naturally infected with *Hepatozoon* oocysts or sporocysts of reptilian origin (Telford, 2009). These are: some mites *Ophionyssus* sp. (see Ramanandan Shanavas & Ramachandran, 1990), *Ophionyssus scincorum* (see Allison & Desser, 1981) and *Hirstiella* sp. (see Lewis & Wagner, 1964); two species of ticks *Amblyomma dissimile* (see Ball et al. 1969) and *Hyalomma cf. aegyptium* (see Paperna et al. 2002); and two species of reduvids, *Triatoma arthurneivae* (see Rocha e Silva, 1975) and *Triatoma rubrovaria* (see Osimani, 1942; Talice, 1929). Dipterans include *Glossina palpalis* (see Chatton & Roubaud, 1913; Macfie, 1916) and *Lutzomyia vexator occidentis* (see Ayala, 1970b). Adding to this list, the mosquito *Culex* (*Afroculex*) *lineata* is proposed to serve as the likely vector of *Hepatozoon* sp. A (see Chapter 3) under natural conditions, while *Culex andersoni* and *Culex pipiens* were found to probably aid in the transmission of *Hepatozoon* sp. A & C under experimental conditions (see Chapter 3 and below). Although no sporogonic developmental stages have been found in the gut contents of scale mites associated with *Pseudocordylus* spp used in this study, *Hepatozoon* spp. gamonts have been found in the gut contents of *Ixodiderma inverta* Lawrence, 1935 and *Ixodiderma pilosa* Lawrence, 1935 that fed on infected lizard blood (see below).

6.3.2 Parasitic mites infesting *Pseudocordylus* spp.

Lawrence (1935) discovered and described three mite genera (*Zonurobia, Scaphotrix* and *Ixodiderma*) from cordylid lizards in South Africa. Adult and nymph *Ixodiderma inverta* were seen on the skin of *P.melanotus* and *P. subviridis* specimens in the present case, sometimes at relatively high infestations (Fig. 6.7 A). The starting point for the search for the possible definitive hosts transmitting *Hepatozoon* species were the adult and nymph stages of *I. inverta* occurring on *P. melanotus.* Squash preparations from engorged mites revealed *Hepatozoon* (see Fig 3.9 F & G), *Plasmodium* (see Fig 4.1 K), and *Sauroplasma* species (see Fig 5.5 A - C) as well as leucocytes and erythrocytes in different stages of digestion in the mite gut contents. These infections in the lizard host erythrocytes and various other types of leucocytes and erythrocytes in the gut contents of the mite corresponded to the overall blood picture of individual lizard specimens. The host blood cells and parasites in the gut were partially digested in most mite squashes which sometimes made it difficult to make a positive

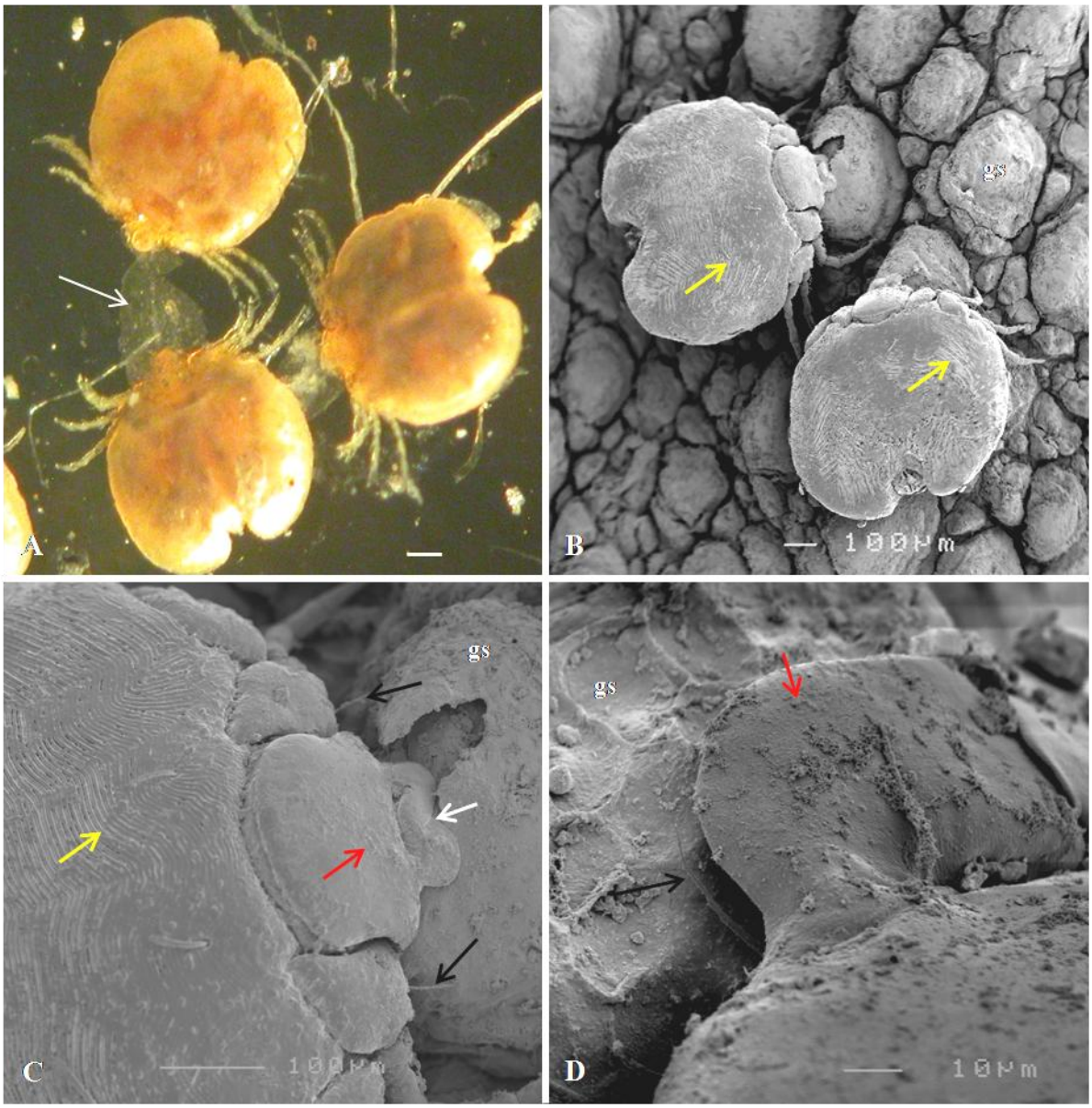

Figure 6.6 Light and scanning electron micrographs of *Ixodiderma inverta* nymphs from or on *P. melanotus* from Platberg, Eastern Free State. **(A)** Engorged mites, white arrow indicates small piece of host skin. **(B)** Scanning electron micrographs of nymphs in feeding position, **(C & D)** Scanning electron micrographs of nymphs in feeding position on a granular scale (gs) showing the tips of the spines on the palpi (black arrows) and the large chitinous scute (red arrow). Yellow arrows in **(B & C)** indicate cross striations. Scale bars: **(A - C)** =100µm **(D)** = 10µm.

Table 6.3 Summary of blood protozoans (*Hepatozoon*, *Plasmodium* and so-called *Sauroplasma* infections) present in the gut contents of nymphal and adult *Ixodiderma inverta* Lawrence, 1935 from the skin of *P. melanotus* male, female and juvenile specimens from the summit of Platberg 1900 - 2390m. Abbreviations: Nymph (N), Adult (A).

Stage of mite: Nymph (N), Adult (A)	**Lizard Host gender**	**Blood protozoans present in mite gut contents**		
		***Hepatozoon* spp**	***Plasmodium* sp. in host erythrocytes**	**So-called *Sauroplasma* sp. in host erythrocytes**
N & A	♂	C	-	*
A	♀	-	-	*
N & A	♂	-	*	*
N & A	♂	A	*	*
N & A	juv	-	-	*
N & A	♀	B	-	*
N & A	♂	-	-	*
N & A	♂	-	*	*
A	♀	-	-	*
N & A	♂	C	-	*
N & A	juv	--	-	*
N	♀	B	*	*
N & A	♂	A & C	-	*
N & A	♀	-	-	*
N & A	♂	-	-	*
N & A	♀	-	-	*
N	♀	A & C	*	*
N & A	♂	-	-	*
N	♂	A	*	*
N	♂	A	*	*

identification. Furthermore, although blood infections of the lizard host were seen in the gut contents of some *I. inverta* specimens, no definite evidence of sporogonic stages, or any other developmental stage were seen (Table 6.3). More detailed studies are needed in order to exclude these mites as vectors of blood protozoans of lizards.

Other species representing the Pterogostomatidae were also collected and scanned for possible sporogonic stages, which included *Ixodiderma pilosa* and *Zonurobia semilunaris* infesting *P. melanotus, P. subviridis* and *P. langi*. Chigger mites (Trombiculinae: *Sauracarella* sp.) were seen on occasion on the skin of all three lizard species. *Ixodiderma inverta* Lawrence, 1935 (Fig. 6.6 A) were the most abundant ectoparasites infesting both *P. melanotus* and *P. subviridis.* These mites are adapted to feed and live on the skin of crag lizards and have a similar method of feeding to Ixodid ticks (Lawrence, 1935).

According to Lawrence (1935), their hypostome is inserted through one of the granular scales, as seen in electron micrographs (Fig. 6.6 B - D) where they feed on blood from capillaries directly beneath the skin. When mites were removed from the skin of their hosts, a small piece of skin came away with it, in all cases (Fig. 6.6 A white arrow). When these were examined microscopically it was evident that the mandibles and hypostome had penetrated the granular scales completely, and the swollen fleshy apex of the mouthparts was effectively preventing retraction of the mouthparts. Presumably the hypostome enlarges in the skin of the host, after the mandibles have punctured the skin.

Adults were usually found together on the legs (Fig. 6.7 A black arrow) and tail of the lizard where they resembled the armoured osteoderms of the lizard. Nymphs of this species were usually seen attached to the lateral side on the granular scales of the lizard (Fig. 6.7 A white arrows). In the generic description of this mite, Lawrence (1935) noted that its dorsal surface had become the ventral surface and *vice versa*, therefore the dorsal side is in contact with the host while feeding (Fig. 6.6 B); this means that this mite lies on its back when feeding. Another extraordinary observation from Lawrence was the peculiar placement of the mouthparts, which were vertically placed with the epistome above and the mandibles underneath forming the hypostome, including a large scute in the nymphs (see Fig. 6.6 B - D and Fig. 6.8 D). This scute is a large chitinous plate that lies ventrally and is a unique

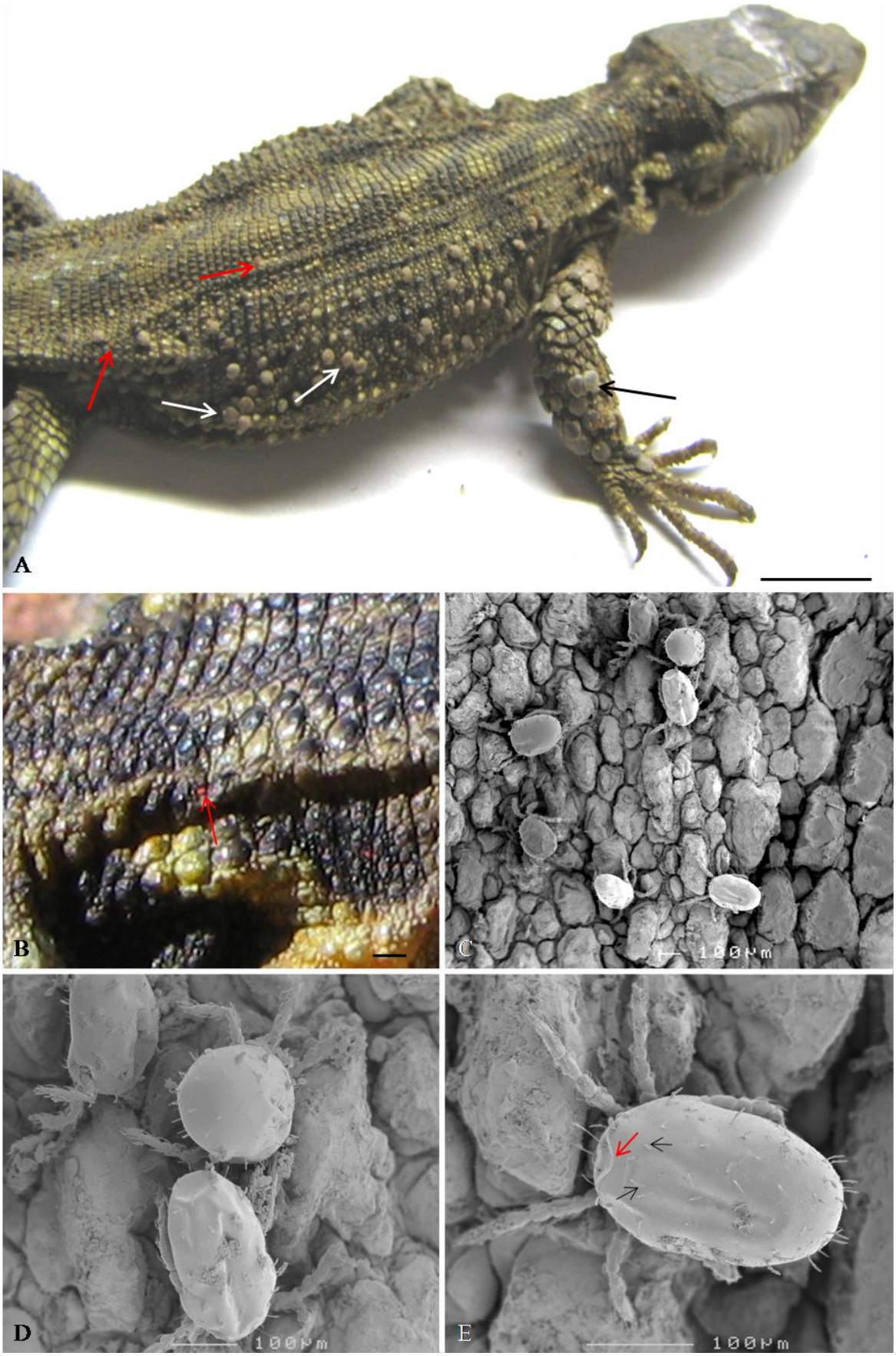

Figure 6.7 Feeding ectoparasitic mites infesting *Pseudocordylus subviridis* **(A)** and *Pseudocordylus melanotus* **(B)** from the Sentinel trail area in the Northern Drakensberg and Platberg respectively. **(A & B)** Red arrows indicate chigger mite nymphs (*Sauracarella whartoni*), White arrows indicate nymphs and black arrows indicate and adult scale mites (*Ixodiderma inverta*). **(C - E)** Scanning electron micrographs of *Sauracarella whartoni* nymphs in the feeding position on the skin of *Pseudocordylus malanotus.* Red arrow in **(E)** indicates the bluntly triangular median projection, black arrows indicates submedian setae. Scale bars: **(A)** = 20mm **(B)** = 1mm **(C - E)** = 100µm.

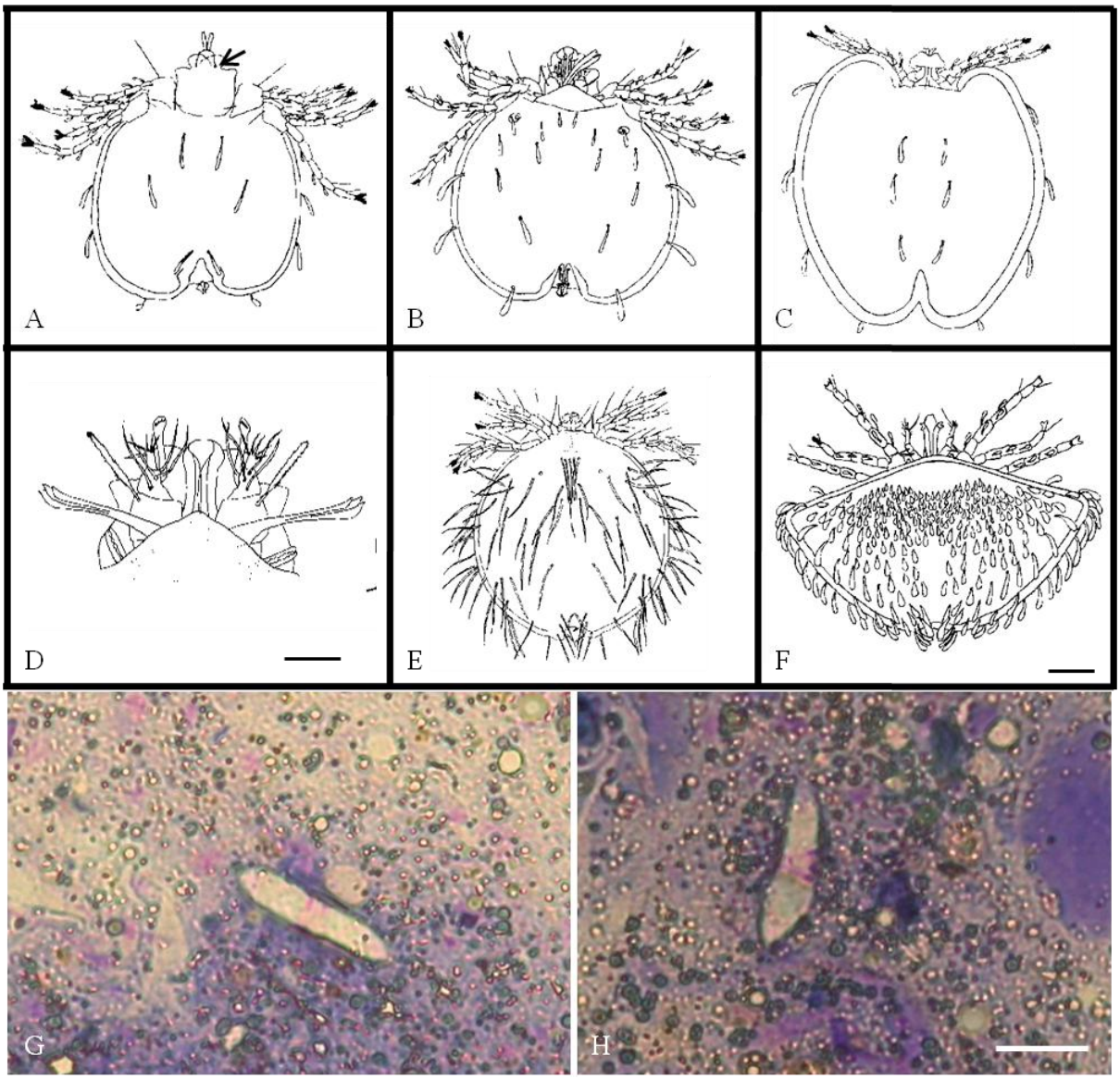

Figure 6.8 Illustrations of representatives of the Pterogostomatidae infesting crag lizards and Giemsa stained light micrographs of presumably *Hepatozoon* sp. A in the gut contents of *Ixodiderma pilosa*. **(A - D)** *Ixodiderma inverta,* **(A & B)** Nymph dorsal and ventral view, respectively, **(C)** adult *I. inverta,* dorsal view, **(D)** Mouthparts of *I. inverta*. **(E)** Adult *Ixodiderma pilosa*. **(F)** Adult *Zonurobia semilunaris.* **(G & H)**, *Hepatozoon* sp. A. gamonts in the gut contents of *Ixodiderma pilosa* from *P. subviridis.* **(A - F)** redrawn from Lawrence, 1935. Scale bars: **(A - C, E & F)** = 100µm **(D)** = 50µm **(G & H)** = 10µm.

morphological adaptation absent in all other genera in the suborder Prostigmata. This plate completely overlies the large hypostome, the other mouthparts, and especially the coxae of the palps and legs.

According to Lawrence (1935), the small palpi are completely hidden by this scute so that only the tips of the spines on the palpi are visible when these nymphal mites are attached (Fig. 6.6 C & D black arrows). This scute is shovel like (Fig. 6.6 D red arrow) and transparent when viewed closely on the skin of the host. The mouthparts can be seen by microscopy and with a hand lens. The anterior part of this scute is obliquely incised so that the one half partly overlaps the other half (Figs. 6.6 C white arrow & 6.8 A black arrow). According to Lawrence (1935), representatives of *Zonurobia* spp. (Fig. 6.8 F) also have a thin transparent, chitinous layer overlying the coxae of the palps, but the large chitinous plate evident in nymphs of *Ixodiderma* sp. could be an adaptation to protect the incompletely developed mouthparts of the nymphal stages when the host moves through rock cracks and over uneven, rocky areas. Furthermore, the apparent dorsal surface is cross-striated (Fig. 6.6 B & C yellow arrows) which could also be an adaptation for protection against abrasion from hard surfaces in the host's habitat.

Only a few specimens of *Ixodiderma pilosa* Lawrence, 1935 (Fig. 6.8 E) were obtained from the skin of *P. subviridis*. All individuals but one showed completely digested blood in the gut contents, but one mite had gamonts of *Hepatozoon* sp. A in these contents (Fig. 6.8 G & H). No other stages or any evidence of development was seen in subsequent squash preparations or histological sections of these mites.

Sauracarella whartoni Lawrence, 1949 were seen on the skin of *P. subviridis* (Fig. 6.7 A red arrows and on *P. melanotus* (Fig. 6.7 B red arrow). Scanning electron micrographs, revealed that these mites appeared in clusters on the skin of *P. melanotus* (Fig. 6.7 C & D), with its characteristic bluntly triangular median projection (Fig. 6.7 E red arrow) and associated submedian setae (Fig. 6.7 E black arrows) as was also seen in the description of Lawrence (1949). Squash preparations of these chigger mites did not yield evidence of any possible development for blood protozoans. Tentatively, these chigger mites were eliminated as possible final hosts transmitting *Hepatozoon* spp, due to the absence of any sporogonic

developmental stages in their gut contents. These chigger mite nymphs cannot, however, be completely ruled out as possible vectors for other haematozoans, since host red blood cells, sometimes with a *Sauroplasma* or probable trophozoites from *Plasmodium* infections were seen in the gut contents, often severely degraded. Further studies are needed on the involvement of these nymphs in the life cycles of *Plasmodium*, so-called *Sauroplasma* and even filarial nematode infections of these lizards.

6.3.3 Mosquitoes as possible vectors for haemoparasites in *Pseudocordylus* spp.

Most life cycles of *Hepatozoon* spp. have been studied using mosquitoes, mostly *Culex* and *Aedes* species (Telford, 2009). Salivary transmission of haemogregarines has been shown to be possible at least in experimental situations. Mosquitoes, namely *Culex pipiens* (see Ebraheem et al. 2006) and *Culex neavei* (see Rashdan & El-Sebaii, 2006) have been shown to transmit *Hepatozoon matruhensis* to a snake *Psammophis schokari* by bite. Telford et al. (2001, 2004, 2005) found haemogregarine oocysts and sporocysts in the proboscis of mosquitoes. Just recently, Telford (2009) found an oocyst with mature sporocysts of *Hepatozoon horridus* that fed on a snake host, in the salivary glands of the mosquito. Therefore, these findings do suggest that salivary transmission is possible in nature and according to Telford (2009), sporogony in the proboscis or in the proximity of the proboscis does not have to occur in a high percentage to maintain such high prevalences, due to the high abundance of these mosquito vectors.

In the last stages of this study (November 2010), at a field site on Platberg, mosquitoes were seen taking blood meals from *P. melanotus* at night. These mosquitoes, (Fig. 6.9 A & B) were identified as *Culex* (*Afroculex*) *lineata* Theobald, 1912. The subgenus *Afroculex* Danilov, 1989 is represented solely by the above species. Morphologically, adult females bear five white longitudinal lines comprising creamy white scales (Jupp, 1996) and these run the whole length of the scutum (Fig. 6.9 C & D black arrows). The scutum surface also comprises black setae and scales (Fig. 6.9 C & D red arrows). Scales shown by SEM in Fig. 6.9 G have been seen in some squash preparations of *C. lineata* (as seen in Fig. 3.16 D & E).

Searches for developmental stages of apicomplexans in the proboscis (Fig 6.9 F), salivary glands, gut, fat bodies and the haemocoel of wild engorged mosquitoes revealed *Hepatozoon*

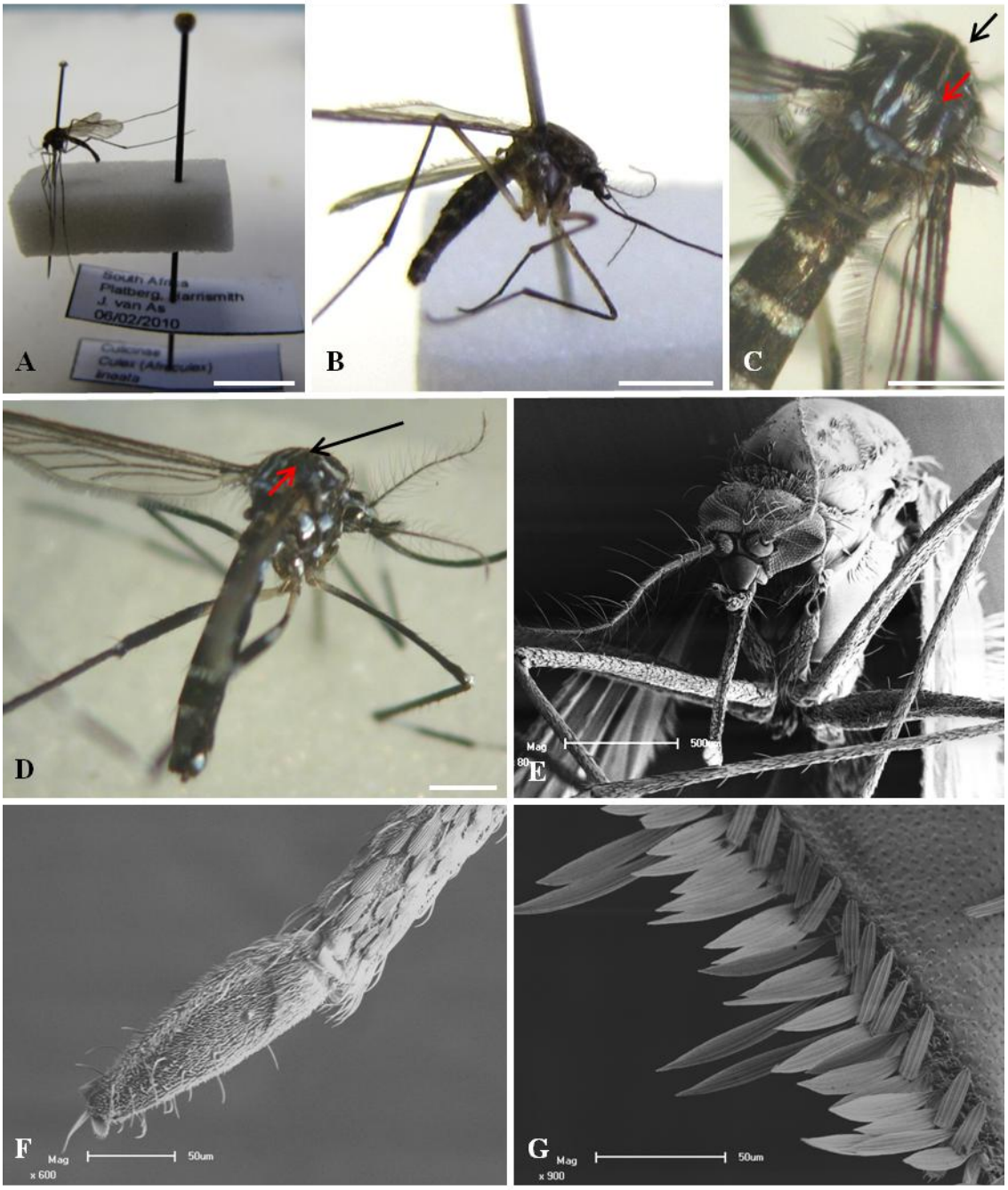

Figure 6.9 Light and scanning electron micrographs of *Culex* (*Afroculex*) *lineata*. **(A & B)** Female *C. lineata* pinned for collection and identification. **(C & D)** Micrographs of *C. lineata* showing 5 prominent lines of creamy-white scales (black arrows) against the black setae and scales (red arrows) on the scutum. **(E - G)** Scanning electron micrographs of a female *C. lineata* showing the head and thorax region in **(E)**, distal end of the proboscis in **(F)** and characteristic scales in **(G)**, often seen in micrographs of gut contents. Scale bars: **(A)** = 5mm **(B)** = 3mm **(C - E)** = 500µm **(F & G)** = 50µm.

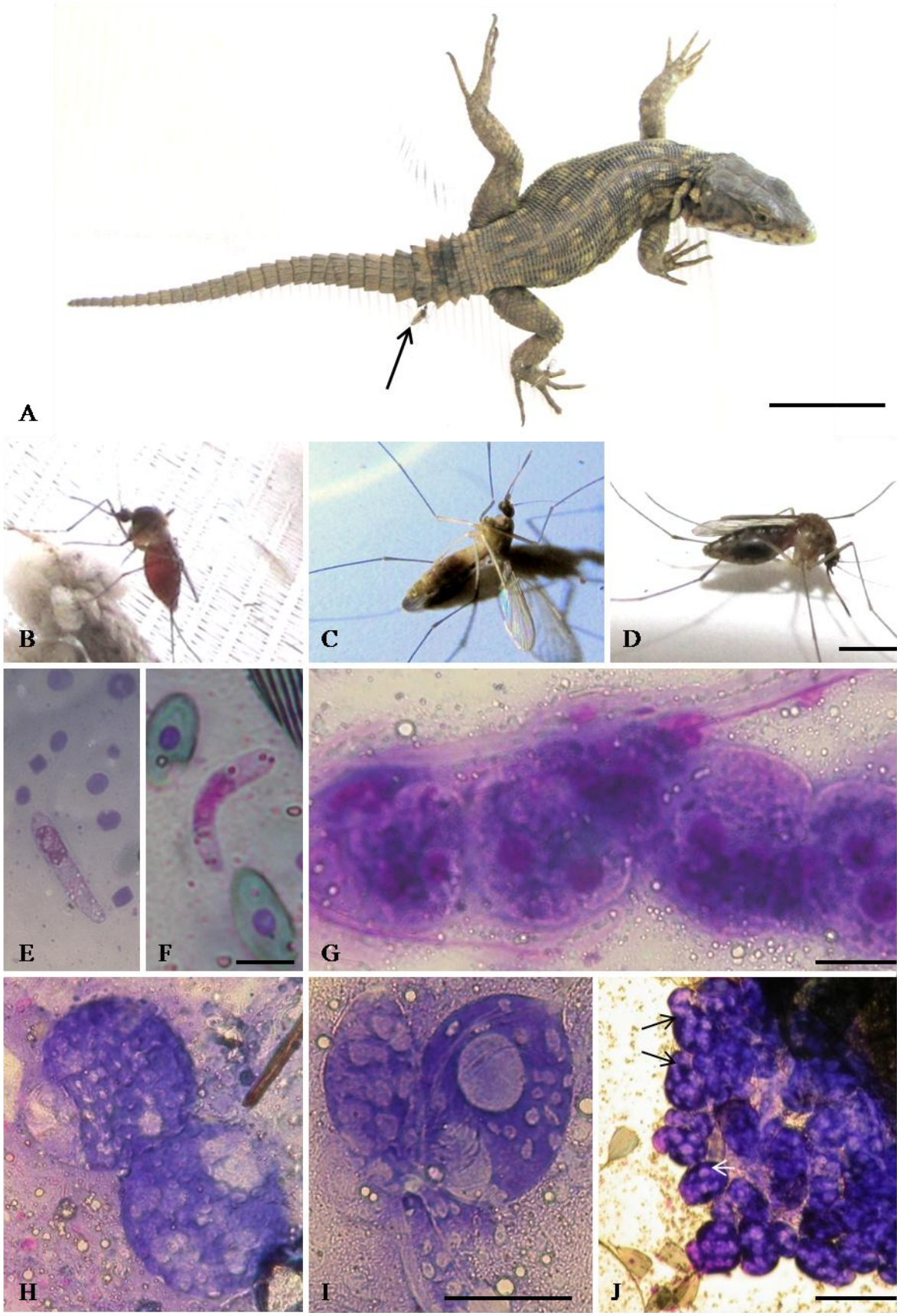

Figure 6.10 A *Hepatozoon* and so-called *Sauroplasma* infected, captive *Pseudocordylus melanotus* specimen with a female *Culex pipiens* (black arrow) taking a bloodmeal. (**B**) Fully engorged *Culex* sp. that survived only 1 day post feeding. **(C & D)** laboratory reared *Culex* sp. containing infected blood meals 6 & 9 days post feeding respectively. **(E - F)** *Hepatozoon* sp. A gamonts in the gut contents of *Culex andersoni* and *Culex pipiens* respectively. Note the so-called *Sauroplasma* infections of host erythrocytes in (**F**). **(G - J)** likely sporogonic stages of *Hepatozoon* sp. A in the gut contents of the same mosquitoes, 3 d.p.f. **(G)** Oocysts with dividing nuclei at 9 d.p.f. (**H, I)** Sporulating oocysts. **(J)** Ruptured oocyst revealing numerous sporocysts (black arrows) with sporozoites (white arrows). Scale bars: **(A) =** 30mm **(B - D)** = 1mm **(E & F)** = 10µm **(G)** = 20µm **(H & I)** = 25µm **(J)** = 50µm.

Table 6.4 Summary of *Hepatozoon* sp A, B & C gamonts and sporogonic stages apparently in the gut contents of wild caught, engorged, *Culex* (*Afroculex*) *lineata* mosquitoes directly after feeding on infected blood from *Pseudocordylus melanotus* specimens in rock crevices from Platberg.

Days post feeding (d.p.f)	***Hepatozoon* spp. gamonts from lizard blood present in mosquito gut contents**	**Sporogonic stages:**
0.5	C	-
0.5	B	oocyst
0.5	A	oocyst
0.7	A	-
1	A	oocyst
1	A	young oocyst oocyst
1	A	sporulating oocyst sporozoites
1	A	oocyst sporulating oocyst
1	A	oocyst sporulating oocyst
1	A	oocyst oocyst
2	C	-
3	B	oocyst
7	A	oocyst
9	B & C	-
10	B & C	-
11	A	oocyst sporulating oocyst sporozoites

Table 6.5 Summary of *Hepatozoon* sp A, B & C gamonts and sporogonic stages apparently in gut contents of mosquito species (of the genera *Culiseta* and *Culex*) that fed on infected, captive *P. melanotus* and *P.subviridis* male specimens.

Mosquito species	**Days post feeding (d.p.f)**	***Hepatozoon* species present in gut contents**	**Sporogonic stages of *Hepatozoon* spp.**
Pseudocordylus melanotus			
Culiseta (*Allotheobaldia*) *longiareolata*	1	A	oocyst
Culiseta (*Allotheobaldia*) *longiareolata*	2	A & B	oocyst
Culex andersoni bwambanus	3	B	oocyst
Culex andersoni bwambanus	23	A	oocyst sporulating oocyst
Culiseta (*Allotheobaldia*) *longiareolata*	24	A & B	oocyst sporulating oocyst
Pseudocordylus subviridis			
Culex (*Culex*) *pipiens*	1	A	oocyst
Culex andersoni bwambanus	4	A & C	oocyst
Culex andersoni bwambanus	5	A	oocyst
Culiseta (*Allotheobaldia*) *longiareolata*	6	C	oocyst
Culex (*Culex*) *pipiens*	7	A	oocyst
Culex (*Culex*) *pipiens*	8	A	oocyst
Culex (*Culex*) *pipiens*	9	A & B	oocyst
Culiseta (*Allotheobaldia*) *longiareolata*	10	A	oocyst
Culiseta (*Allotheobaldia*) *longiareolata*	11	A & B	possible oocysts
Culiseta (*Allotheobaldia*) *longiareolata*	12	A	possible oocysts
Culex andersoni bwambanus	13	A	possible oocysts
Culex (*Culex*) *pipiens*	14	C	possible oocysts
Culiseta (*Allotheobaldia*) *longiareolata*	15	A & B	possible oocysts
Culex (*Culex*) *pipiens*	16	C	possible oocysts
Culiseta (*Allotheobaldia*) *longiareolata*	17	A	possible oocysts
Culex (*Culex*) *pipiens*	18	A	possible oocysts
Culex andersoni bwambanus	19	A	possible oocysts
Culex andersoni bwambanus	20	A	-
Culex andersoni bwambanus	21	A	possible oocysts
Culex andersoni bwambanus	22	C	oocyst sporulating oocyst

sp. A, B and C gamonts only in the gut, in mosquitoes that fed on lizard blood (infected with *Hepatozoon* spp.) 4/16 (25%) had late phases of oocyst development, including sporulating oocysts, apparently in their gut contents (Table 6.4). In a further two mosquito specimens (12.5%), all the sporogonic stages of *Hepatozoon* sp. A were found including sporozoites (see Fig. 3.4 A - M) again, apparently in the gut contents.

After depriving captive bred, adult female mosquitoes of sucrose solution for 24 hours, some of the mosquitoes fed on *Hepatozoon* and so-called *Sauroplasma* infected blood from a captive *Pseudocordylus melanotus* specimen (Fig. 6.10 A). Only very few *Culex pipiens, Culex andersoni* and *Culiseta longiareolata* specimens took a bloodmeal. In general, these mosquitoes were reluctant to feed on the lizards, only 2.5% of mosquitoes fed on *P. melanotus* and 10% of mosquitoes fed on *P. subviridis* (Table 6.5). Interestingly, mosquitoes that were fully engorged died quickly, surviving only for 1 day post-feeding (as in the example in Fig. 6.10 B).

Other mosquitoes that took only a little blood and then fed on sucrose solution could be kept alive for a maximum of 24 days post-feeding. However, most mosquitoes died in under a week (such as those in Fig. 6.10 C & D), when they were immediately dissected and examined for any possible sporogonic stages of haemogregarines. Examples of these were *Culex andersoni* and *Culex pipiens*, and they had gamonts (Fig. 6.10 E & F) in their gut contents, and those that were kept alive longer had different phases of oocyst development (Fig. 6.10 G), seemingly in their gut contents. Only three (0.75%) mosquitoes survived past 20 days post-feeding and had fully sporulated oocysts, with sporocysts forming sporozoites (Fig. 6.10 H - J), apparently in the gut contents. From numerous dissections of most of the 400 mosquitoes examined, no evidence of sporozoites occurring in the salivary glands was seen, or in the proboscis, suggesting that transmission of at least some of the *Hepatozoon* species observed is by ingestion of the infected mosquitoes.

In this specific case, these mosquitoes seem to be the preferred definitive hosts for *Hepatozoon* infections. Although not all the apicomplexan life cycle stages were observed (such as gametogenesis) within the experimental mosquitoes, it seems more likely that development takes place within a mosquito definitive host rather than in the prostigmatic

mites known to infest *Pseudocordylus* species, especially in respect of *Hepatozoon* sp. A & C described in Chapter 3.

Future work regarding the life cycles of haemoparasites will include more detailed studies on the mites and their association with the lizards. Continued searches for other haematophagous dipterans will be undertaken to find the vectors of *Sauroplasma*, *Plasmodium* and microfilarial blood infections.

6.3.4 Discussion

The identification of the different types of cells in blood smears from these lizards is at best challenging, since various stages and intermediate phases of each are present at any given time. Furthermore meticulous staining regimes using various techniques are necessary to characterize each leucocyte type precisely. It is also virtually impossible to characterize every type of cell, applying it to all species, since different lizard groups have different morphological cell types. Identification of thrombocytes, lymphocytes and basophils in the blood of these lizards was not very difficult and these cell types seem to be relatively homogenous across the Squamates. Other cell types and lineages proved to be more difficult to characterize, especially azurophils and heterophils, and neutrophils were not identified with confidence. Transitional forms also presented a problem, since these were difficult to identify without knowledge of the haematopoietic organs and processes involved in blood cell production and maturation in these lizards. This chapter provided a comparison and characterization of the general, circulating cell types with the blood picture of different species within the same family of lizards. A relationship between erythrocyte surface area and altitude was suggested, perhaps indicating increased haemoglobin with height above sea level in these lizards. Attempts were also shown to relate locality and host collection data with leucocyte and haemoparasite numbers, and these statistical analyses also proved to be a difficult undertaking, indicating that more data are needed for highlighting these relationships, if any.

Discovering diverse groups of blood parasites, often in high numbers, in *Pseudocordylus* lizards at isolated, high altitude habitats was surprising. Finding two species of *Hepatozoon* in the blood of two different species of lizards in disjunct localities emphasized that the

vectors are likely haematophagous, gregarious and abundant. Mosquitoes served as a good model to study the potential transmission of haemogregarines in particular. They were bloodthirsty, easily maintained, available in high numbers, and with their relatively quick life cycles, successful sporogonic development in their body contents was shown. Future studies on acarine ectoparasites should focus on their life history in close association with their hosts.

CHAPTER 7 CONCLUDING REMARKS

Although the first meticulous, morphological, descriptions of African, reptilian haemoparasites were done over a century ago, for instance, by Sambon & Seligmann (1907), in depth research into the taxonomy of these organisms remains scanty. It was only relatively recently that the life cycles of lizard haemogregarines, for example, began to be resolved (Ball & Oda, 1971; Allison & Desser, 1981; Bashtar et al. 1984, 1987 and Smith & Desser, 1997b) and even then, such studies on lizards were sporadic, usually concentrated in small localities, and most work was done on continents other than Africa. Life cycle and taxonomic studies of haemogregarines and other haematozoans of lizards are thus still relatively young and unexplored fields, and were essentially non-existent in South Africa until the current research was undertaken

As outlined in Chapter 1, the main aims of this study were to contribute to knowledge of baseline biodiversity and taxonomy of the haemoparasites of crag lizards, using morphological and morphometrical characteristics, and to elucidate and characterize life cycle stages, especially of haemogregarines in both their lizard and invertebrate, haematophagous hosts. The aim of broadening knowledge of the baseline biodiversity of blood parasites in lizards was considered essential, but even more so was the aim to contribute further to their taxonomy by studying their life histories, if possible. In the case of haemogregarines, the taxonomy of members of this broad group is usually tentative, especially if only morphological and morphometrical characteristics are examined. In the past, haemogregarines were described often only on the basis of the morphology of the gamont stages in blood films but, with Smith's (1996) reorganization of the genus and his recommendations, understanding of the taxonomy of the genus *Hepatozoon* became a little more coherent. Futhermore, Sloboda et al. (2007) suggested that descriptions of such new species from then on should be based on life cycle stages in both the vertebrate and invertebrate hosts. Therefore, the current type of study which benefits not only baseline biodiversity, but also contributes to the life history of these

parasites in both vertebrate and invertebrate hosts, greatly improves understanding of this group of blood parasites as a whole.

7.1 Haemoparasites in their crag lizard (vertebrate) hosts

When reviewing the broader literature for this study (Chapter 1), as well as the findings from a previous, related, study (Van As, 2003) it was apparent that very little was known about haemogregarine and malaria infections in lizards from South Africa. On the basis of the limited knowledge of these infections, four hypotheses were formulated (see Chapter 1):

1) **Haemoparasites, of several species, are generally more widespread among South African cordylid lizards than previously thought, and particularly in the Eastern Free State Highlands**

The previous study of this type (Van As, 2003) was essentially a basic survey of blood parasites of reptiles in the Free State and Lesotho. From that work, it was apparent that haemoparasites were widespread and abundant in the South African reptiles sampled, and therefore for the current research *Pseudocordylus* lizards were selected as good host models to study haemoparasites. Infections in crag lizards in the present case often formed high parasitaemias and occurred frequently with high prevalence among populations (see Chapters 3 - 5). In the case of some haemogregarines, two types of merogony in lizard hepatic tissue were also recorded, supporting their likely classification as *Hepatozoon* spp. Every lizard sampled in the current study seemingly had some form of haemoparasite infection in the peripheral blood and these parasites were especially prevalent in lizard populations on Platberg, which is essentially a terrestrial island, as well as in populations of the Drakensberg escarpment. It is therefore possible to accept the first hypothesis.

2) **Haemogregarine and malarial infections may cross host species boundaries, especially in crag lizard species exhibiting sympatric distribution**.

It is also possible to accept this second hypothesis, based on the findings in Chapters 3 & 4. It was interesting to observe that an isolated population of *P. melanotus* lizards on Platberg had the same *Hepatozoon* sp. A & C, and the same species of *Plasmodium*, as *P. subviridis* lizards over 50 kilometers away and, at a higher altitude, in the North Eastern Drakensberg. As noted earlier, these two species of lizards do have a patchy sympatric distribution in the eastern Free State, where their distribution overlaps at roughly above and below ~2000m.

3) **Species of the genus *Hepatozoon* are not host species specific, but may be host genus specific among these crag lizards.**

Hepatozoon infections suspected to be of five different species were found in the peripheral blood of three species of crag lizards occurring in the Eastern Free State highlands. This third hypothesis can be accepted partially on the basis that *Hepatozoon* sp. A & C were found in *P. melanotus* and *P. subviridis* occurring in disjunct localities (see above). However, *Hepatozoon* sp. B in *P. melanotus* and *Hepatozoon* sp. D & E found in the blood of *P. langi* appeared unique to these hosts based on morphological and morphometrical grounds. The third hypothesis can also be accepted on the grounds that all five species of haemogregarines found in the crag lizards differed markedly from haemogregarines of other lizards of different genera, but within the same family, the Cordylidae. These haemogregarines were also distinct from those of other species of lizards occurring sympatrically with crag lizards, but representing different lizard families.

4) **Malarial infections of the genus *Plasmodium* occur across a wide altitudinal gradient in lizards from the same cordylid family.**

This hypothesis can be accepted on the basis of the findings in Chapter 4, where the same *Plasmodium* species (with some features of both *Plasmodium zonuriae* and *Plasmodium cordyli*) was found in *P. melanotus* and *P. subviridis* from disjunct localities across a wide altitudinal gradient of about 1000 meters ~ (2000 to 3000m). Acceptance of this hypothesis is supported by the literature. Ten years after Pienaar (1962) described *P. zonuriae* in cordylid lizards from what is now the North West province, Telford found in 1972 (see Telford, 2009) the same species of malaria in *Pseudocordylus microlepidotus* near Cape Town. Telford also located in 1986 (see Telford, 2009) the same malaria species in *Cordylus vittifer* from the southwest Transvaal (now the North West Province). Later, Telford (1987) described *P. cordyli* from *Cordylus tropidosternum* in Tanzania and listed *Cordylus vittifer* as a host for *P. cordyli*, from the southwest Transvaal (see Chapter 4).

7.2 Haemoparasites of their crag lizard in their invertebrate hosts

Although in the last few decades some work has been done regarding definitive hosts and vectors of haemoparasites of lizards, it has been undertaken mostly on continents other than Africa and this type of work is still in its infancy (see above). Thus, in Africa, and especially in South Africa, very little is known about definitive hosts and vectors of the haemoparasites of lizards.

Another aim of this study was, therefore, to find likely vectors responsible for transmitting haemogregarines between crag lizards, as well as documenting the possible sporogonic stages occurring in these hosts using various techniques. In this context, the role of mites and mosquitoes was explored, to determine whether they could serve as possible definitive hosts for haemogregarines and likely transmit these and other blood infections such as *Plasmodium*, so-called *Sauroplasma* and filarial nematode infections.

Owing to the limited information that exists on the role of definitive hosts and vectors for haemoparasite infections in lizards in South Africa, two hypotheses were formulated:

1) **Mosquitoes, but not mites, may serve as the final hosts for *Hepatozoon* and *Plasmodium* infections in cordylid lizards from the Free State Highlands.**

This hypothesis can likely be accepted in the case of some *Hepatozoon* infections of crag lizards, where development was demonstrated to occur in mosquitoes, though not in mites. Chapter 3 outlines the findings and the second section of Chapter 6 gives an overview of mites and mosquitoes as possible vectors. Scale mites (see Chapter 6) were abundant on the skin of most lizards sampled in this study, but their gut contents revealed only ingested blood, that contained *Hepatozoon* gamonts, as well as *Plasmodium* meronts. Subsequent sporogonic stages of *Hepatozoon* and *Plasmodium* were not observed in these mites. Mosquitoes that fed on infected lizard blood contained sporogonic stages of *Hepatozoon* spp. associated with the insect gut, although their exact location was not determined. These sporogonic stages were confirmed for the first time in experimental situations as well as in dissected wild mosquitoes that fed on lizards at night (see Chapter 3). In the first stages of this part of the study, it was assumed that mosquitoes or sandflies could be possible vectors in transmitting *Plasmodium* infections, in line of the findings of Huff (1941), Baker (1961), and Jordan (1964) on mosquitoes (Diptera: Culicidae) and Ayala (1970) on sandflies (Diptera: Phlebotominae). However, only mosquitoes and mites were observed feeding on lizards, and mosquitoes collected from *Plasmodium* infected lizards in the field did not yield any subsequent developmental stages associated with their gut, or in their salivary glands. Mosquitoes used in experiments produced similar results. Future work in this area of research will include looking for other haematophagous dipterans associated with the general habitat of these particular lizards.

2) **Scale mites that infest crag lizards may serve as vectors for lizard *Sauroplasma* and filarial nematode infections.**

Currently, this hypothesis can neither be accepted nor rejected and further studies need to be conducted. Although it was exciting to discover both so-called *Sauroplasma* infections within host erythrocytes and filarial nematodes in squashes of scale mites, no conclusions can be drawn at this stage. In general, the piroplasms are known to be transmitted via acarine hosts, but knowledge of their involvement in transmission of the so-called *Sauroplasma* infections of lizards is tentative at best. For example Du Toit (1937) speculated that *Sauroplasma thomasi* from a cordylid lizard is likely to be transmitted by ticks. Later, Pienaar (1962) suggested that the so-called *Sauroplasma* infections in other species of cordylids could be transmitted by prostigmatic mites (*Zonurobia* spp.). In Chapter 5, there was speculation about the possibility of prostigmatic mites (*Ixodiderma* sp.) transmitting these so-called *Sauroplasma* infections to crag lizards (*Pseudocordylus* spp.). However, this is a difficult area of study, since the exact nature, biology and the taxonomic placement of these unusual infections are all still uncertain. Thus far, it is not known whether scale mites might aid in transmission of filarial nematodes, although the possibility cannot be eliminated, as yet. Hering-Hagenbeck et al. (2000) described the life cycle of a nematode, *Madathamugadia* sp. in a gecko, but the vector was a sandfly (see Chapter 1). Future work will include molecular characterization of these infections in lizards to establish their taxonomic status, as well as detailed life cycle work on the mites inhabiting infected hosts. Histology and TEM may also be useful tools in determining the life history of these infections.

7.3 New contributions

This research project led to the discovery of new host and locality records for blood parasites from crag lizards collected during surveys of sites in the Eastern Free State Highlands. Five species of haemogregarines were found in the blood of three species of lizards (*Pseudocordylus melanotus*, *Pseudocordylus subviridis* and *Pseudocordylus langi*), as well as the sporogonic stages of two of these haemogregarines in natural and

laboratory-raised vectors. Two, almost certainly new, haemogregarine species were studied histologically and ultrastructurally, and new morphological and morphometrical information regarding the merogonic stages in the liver were presented for the first time, confirming their likely status as members of the genus *Hepatozoon.* A curious lizard malaria with some features of two known *Plasmodium* species was also presented from two species of crag lizards (*P. melanotus* and *P. subviridis*) as well as some ultrastructural observations. So-called *Sauroplasma* infections observed in all specimens of *P. melanotus, P. subviridis* and *P. langi* were examined and presented as new locality records; some attempts were made to define these infections by ultrastructural means. Two (possibly three) species of previously undescribed microfilaria were also noted in the blood of crag lizards. A blood picture of these infected lizards was given in line with work of previous authors, and several leucocyte types were studied ultrastructurally.

7.4 Recommendations

7.4.1 Future taxonomic work based on molecular methods

The bulk of literature regarding the taxonomic placement of parasites of the types examined in this thesis is often described as "controversial "or "uncertain". In future, it is important therefore to characterize these infections molecularly, but in conjunction with morphological descriptions. Molecular taxonomy becomes especially important in elucidating the life cycles of undescribed or partially described parasites.

The taxonomy of the phylum Apicomplexa has had a stormy and volatile past and, to some extent, this controversy continues. Recent molecular studies have shed some light on the associations between groups within the Apicomplexa, but for the few genera that have been studied, it is not long ago that internal transcribed spacers (ITS) were first employed to determine the degree of relatedness between apparently closely related groups within the phylum (see below). For example, nucleotide sequences of ITS regions have been determined for species of *Plasmodium* (see Shippen-Lentz et al. 1987), *Toxoplasma* (see Payne et al. 1996), *Eimeria* (see Barta et al. 1997) *Cryptosporidium* (see

Cai et al. 1992), *Perkinsus* (see Goggin, 1994), *Theileria* (see Kibe et al. 1994) and *Neospora* (see Holmdahl, 1996). Interestingly, molecular studies have resulted in some cases in formerly completely different species being designated morphs of the same species (see Cai et al. 1992; Goggin, 1994; Holmdahl, 1996). Molecular characterization has also proved important in identifying pathogenic protozoans. The work of Cevallos et al. (1993) showed that some pathogenic protozoans are morphologically identical, but immunologically distinct and therefore, also molecularly separate entities.

In order to separate host and parasite DNA, which can be a problem, Wozniak et al. (1994) designed conserved primers and produced PCR (polymerase chain reaction) products for lizard and parasite in equal proportions. Such PCR-based ribotyping assays were then used to differentiate, and detect interspecific transmission of blood parasites in reptile populations (Wozniak et al. (1994). Wozniak et al. (1994) also demonstrated that the haemogregarines examined formed a monophyletic group, distant from the malarias. Feldman et al. (1995) discovered that many *Plasmodium* species have an insertion of ~200 nucleotides in a portion of SSU rRNA (small subunit ribosomal RNA) gene in theory, making it theoretically possible to distinguish the host and parasite genes, based on size differences. However, haemogregarine and host SSU rRNA genes are more or less the same size and cannot be separated by such means. Lang-Unnasch et al. (1998) conducted a phylogenetic study of the rRNA genes of the plastid genome of several apicomplexan taxa, and found a *Plasmodium-Babesia-Hepatozoon* grouping. Carreno et al. (1999) also used SSU rRNA genes in their exploration of the phylogenetic placement of haemogregarines and concluded that one species of *Hepatozoon* clustered with *Plasmodium knowlesi* and *Plasmodium gallinaceum.* Later, SSU rRNA studies of Matthew et al. (2000) showed a well supported grouping of *Hepatozoon-Plasmodium-Cryptosporidium.* Thus, some molecular studies appear to support a distant relationship between the malarias and haemogregarines, while others imply the exact opposite.

Although Perkins and Martin (1999) considered Wozniak et al's (1994) primers were not specific for haemogregarines because they also amplified host SSU rRNA genes, Matthew et al. (2000) used these conserved primers, but extracted the parasite DNA from oocysts from the intermediate hosts (ticks). Perkins & Keller (2001) used primers HEMO1 and HEMO2 to extract haemogregarine genomic DNA from whole blood samples, and showed that this method could be used as a rapid diagnostic tool for detecting haemogregarine infections in peripheral blood. Perkins & Keller (2001) also used a novel screening procedure to differentiate between host and parasite genes.

After 2001, universal primers for amplifying part of the 18S rRNA gene of *Hepatozoon* species became available, and various studies showed that blood parasites could be detected using optimized PCR protocols and sequencing of the DNA. Studies from Ujvari et al. (2004), Criado-Fornelio et al. (2007), Merino et al. (2009), Gabrielli et al. (2010) and Harris et al. (2011) demonstrated that molecular surveys can be as effective as blood smear examinations and may detect very low parasitaemias, often missed in microscopical blood smear examinations. According to Maia et al. (2011), such molecular methods, once optimised, should become essential tools in assessing blood parasite prevalence in a large number of hosts. Such molecular methods have already been employed in estimating prevalences of blood parasites in their host populations, detecting very low parasitaemias, and confirming host specificity of parasites in host populations (see Maia et al. 2011; Harris et al. 2011).

Today, these molecular techniques are more refined than previously and more accepted, and have become important in solving taxonomic and phylogenetic problems for parasite groups like those discussed in this thesis. It is proposed that future work on the crag lizard hosts, their blood parasites and their likely vectors must aim to employ molecular techniques as outlined above, in conjunction with the morphological descriptions and life cycle observations on which the foundations of this thesis were laid.

CHAPTER 8

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ABSTRACT

The study of blood parasites of reptiles is a relatively new and unexplored field in South Africa. Therefore, the general aims of this research were to explore the haemoparasite fauna of cordylid lizards, *Pseudocordylus melanotus, Pseudocordylus subviridis* and *Pseudocordylus langi*, and especially to search for the definitive hosts and likely vectors of their haemogregarines. Surveys of lizard blood were conducted at various altitudinal gradients on the Sentinel Trail in the escarpment area of the Drakensberg, and at the top of Platberg, near Harrismith, both in the Free State. Five species of haemogregarines were identified, all suspected to belong to the genus *Hepatozoon*, and none was known from previously published records. These occurred in the blood of *P. melanotus, P. langi* and *P. subviridis* from the two disjunct study sites, and mostly were accompanied by other haemoparasites including a saurian malaria, so-called *Sauroplasma*, and filarial nematodes. Developmental stages of two of the *Hepatozoon* spp. were documented in the internal organs of *P. melanotus* and *P. subviridis* by means of light and confocal microscopy, histology, and transmission electron microscopy. Life stages were also observed in ectoparasitic lizard mites, by means of stained histological sections, and in stained squashes of mites and mosquitoes. Three species of experimentally reared mosquitoes were found to act as likely definitive hosts for *Hepatozoon* spp. of *P. melanotus* and *P. subviridis*, while wild caught *Culex* (*Afroculex*) *lineata* appeared to serve as a definitive host, and therefore possible vector for an *Hepatozoon* species of *P. melanotus* at the top of Platberg. A saurian malaria that appeared to have features of two previously described species was recorded in *P. melanotus* and *P. subviridis.* New locality records for so-called *Sauroplasma* and filarial nematodes were also documented for the three species of crag lizards. Some aspects of the fine structure of two haemogregarines, the *Plasmodium* sp. and so-called *Sauroplasma* infections were recorded for the first time in the erythrocytes of the *Pseudocordylus* spp. Differential leucocyte and thrombocyte counts were performed on the three crag lizard species and, with erythrocyte characteristics, compared with those of cordylid lizards in previous studies. Several types of leucocytes were characterized ultrastructurally, as well as by light microscopy, and attempts were made to correlate statistically leucocyte counts, and host and environmental data, with parasite loads. Finally, mites and mosquitoes

associated with crag lizards were explored further as possible vectors of lizard haemoparasites, and studied using light and scanning electron microscopy.

Key words: South Africa, haemoparasites, crag lizards, mosquitoes, mites, life cycle, morphology, taxonomy, final hosts, vectors.

OPSOMMING

Die bestudering van bloed parasiete van reptiele is ‘n relatiewe nuut en onbekende veld in Suid-Afrika. Dus was die algemene doelwit van hierdie navorsing om die bloed parasiet fauna van akkedisse in die Cordylidae, *Pseudocordylus melanotus, Pseudocordylus subviridis* en *Pseudocordylus langi* te ondersoek en veral die finale gashere en moontlike vektore op te spoor. Akkedis bloed is in die Vrystaat teen verskillende hoogtes versamel by die Sentinel voetslaanpad in die Drakensberg en bo-op Platberg naby Harrismith, wat afsonderlike geïsoleerde areas is. Vyf spesies haemogregarines is geïdentifiseer, waarvan almal klaarblyklik aan die genus *Hepatozoon* behoort en wat voorheen nog nie beskryf is nie. Hierdie haemogregarines het voorgekom in die bloed van *P. melanotus, P. langi* en *P. subviridis* en is gevind tesame met ander bloed parasiete, insluited akkedis malaria, sogenaamde *Sauroplasma* infeksies en filariale nematode. Die ontwikkelings-stadiums van twee *Hepatozoon* spp. in die interne organe van *P. melanotus* en *P. subviridis* is waargeneem deur middel van van lig, transmissie- en konfokale mikroskopie asook met behulp van histologiese tegnieke. Lewenssiklus-stadia in ektoparasitiese myte en muskiete is waargeneem deur middel van histologiese snitte asook in gekleurde preparate van myte en muskiete. Drie spesies eksperimenteel geteelde muskiete was heel moontlik die finale gashere van *Hepatozoon* spp. in *P. melanotus* en *P. subviridis.* Dit blyk of wilde muskiete *Culex* (*Afroculex*) *lineata* die finale gasheer en dus die vektor vir ‘n *Hepatozoon* sp. van *P. melanotus* op Platberg kon wees. ‘n Akkedis malaria met eienskappe van twee voorheen beskryfde spesies, is gevind in die bloed van *P. melanotus* en *P. subviridis.* Nuwe lokaliteits-rekords is opgeteken vir sogenaamde *Sauroplasma* en filariale nematood infeksies in hierdie gasheer spesies. Sommige aspekte van die fyn-struktuur van twee haemogregarines, die *Plasmodium* sp. asook die sogenaamde *Sauroplasma* infeksies in the eretrosiete van *Pseudocordylus* spp., is vir die eerste keer opgeteken. Differensiële leukosiet- en trombosiet-tellings is uitgevoer op die bloed van die drie gasheer spesies en, tesame met hul eretrosiet eienskappe, met dié van voorheen bestudeerde akkedisse vergelyk. Ultrastrukturiële eienskappe van verskeie soorte leukosiete is deur middel van lig mikroskopie vasgestel en daar is gepoog on leukosiet-tellings, gasheer en omgewings data met parasiet ladings te korreleer. Uiteindelik is myte

en muskiete, wat geassosieërd met die akkedisse voorkom, verder met behulp van lig en skanderings-elektron mikroskopie ondersoek as moonglike vektore van akkedis bloed parasiete.

Sleutel woorde: Suid Afrika, bloed parasiete, krans akkedisse, muskiete, myte, lewenssieklus, morfologie, taksonomie, finale gashere, vektore.

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Appendix 1 Summary of three *Hepatozoon* species (*Hepatozoon* sp. A - C), present in the peripheral blood of male and female *Pseudocordylus melanotus* individuals with their corresponding slide numbers and dates, examined from Platberg, Eastern Free State. Haematozoans are estimated per 1000 erythrocytes. Abbreviations: (EC) extracellular gamonts, (I) immature gamonts, (M) mature gamonts.

				***Hepatozoon* spp. parasitaemia**		
Collection date	**Slide no**	**Host Gender**	***Hepatozoon* species**	***Hepatozoon* sp. A**	***Hepatozoon* sp. B**	***Hepatozoon* sp. C**
31/10/07	dpr4	♀	*Hepatozoon* sp. A	(M)1/1000	0	0
11/03/08	g13	♀	*Hepatozoon* sp. A	(M)3/1000	0	0
15/03/07	g4	♀	*Hepatozoon* sp. A	(M)2/1000	0	0
05/10/05	mrc1	♀	*Hepatozoon* sp. A	(M)7/1000	0	0
21/04/07	pp11	♀	*Hepatozoon* sp. A	(M)135/1000	0	0
25/01/08	pp17	♀	*Hepatozoon* sp. A	(M)4/1000	0	0
31/10/07	dpr5	♂	*Hepatozoon* sp. A	(M)2/1000	0	0
15/03/07	g8	♂	*Hepatozoon* sp. A	(M)1/1000	0	0
21/04/07	pp10	♂	*Hepatozoon* sp. A	(M)170/1000	0	0
21/04/07	pp12	♂	*Hepatozoon* sp. A	(M)1/1000	0	0
21/04/07	pp14	♂	*Hepatozoon* sp. A	(M)21/1000	0	0
21/04/07	pp16	♂	*Hepatozoon* sp. A	(M)20/1000	0	0
25/01/08	pp23	♂	*Hepatozoon* sp. A	(M)18/1000	0	0
22/03/07	pp5	♂	*Hepatozoon* sp. A	(M)167/1223	0	0
21/04/07	pp7	♂	*Hepatozoon* sp. A	(M)15/1000	0	0
06/02/10	ppe	♂	*Hepatozoon* sp. A	(M)10/1000	0	0
02/10/07	gd10	♀	*Hepatozoon* sp. A & B	(M)3/1000	(M)12/1000	0
17/10/07	pk3	♀	*Hepatozoon* sp. A & B	(M)1/1000 (EC)15/1000 (M)12/1000	(I)4/1000	0
22/03/07	po1	♂	*Hepatozoon* sp. A & B	(I)15/1000	(M)4/1000	0
17/10/07	pom2	♀	*Hepatozoon* sp. A & C	(M)2/1000	0	(M)1/10 000
07/03/09	k3	♂	*Hepatozoon* sp. A & C	(M)43/1000	0	(M)5/1000
17/10/07	pk4	♂	*Hepatozoon* sp. A & C	(M)2/1000	0	(I)4/1000
02/02/09	pp27	♂	*Hepatozoon* sp. A & C	(M)25/1000		(M)5/1000
22/04/07	pt1	♂	*Hepatozoon* sp. A & C	(M)3/1000	0	(I)1/1000
02/10/07	gd9	♀	*Hepatozoon* sp. B	0	(I)2/1000	0
21/03/07	po3	♀	*Hepatozoon* sp. B	0	(M)22/1000 (I)1/1000	0
04/01/10	ppb	♀	*Hepatozoon* sp. B	0	(M)1/10 000	0
02/02/09	pp24	♀	*Hepatozoon* sp. B & C	0	(M)15/1000	(M)2/1000
15/03/07	g2	♂	*Hepatozoon* sp. B & C	0	(M)1/1000	(M)4/1000
15/03/07	g10	♀	*Hepatozoon* sp. C	0	0	(M)1/1000
22/03/07	pk1	♀	*Hepatozoon* sp. C	0	0	(M)12/1000 (I)1/1000
17/10/07	pk5	♀	*Hepatozoon* sp. C	0	0	(M)30/1000
25/01/08	pp28	♀	*Hepatozoon* sp. C			(M)3/1000
07/03/09	ppm3	♀	*Hepatozoon* sp. C	0	0	(M)20/1000 (I)1/1000 (EC)1/1000
17/10/07	dpb1	♂	*Hepatozoon* sp. C	0	0	(M)3/1000
15/03/07	g1	♂	*Hepatozoon* sp. C	0	0	(M)3/1000
02/10/07	gd3	♂	*Hepatozoon* sp. C	0	0	(M)1/10 000
02/10/07	gd7	♂	*Hepatozoon* sp. C	0	0	(M)3/1000

Appendix 2 Summary of two *Hepatozoon* species (*Hepatozoon* sp. A & C), present in the peripheral blood of male, female and juvenile *Pseudocordylus subviridis* individuals with their corresponding slide numbers and collection dates, examined from the Sentinel in the North Eastern Drakensberg. Haematozoans are estimated per 1000 erythrocytes. Abbreviations: (EC) extracellular gamonts, (M) mature gamonts, (I) immature gamonts, juv = juvenile.

				***Hepatozoon* spp. Parasitaemia**	
Collection date	**Slide number**	**Gender**	***Hepatozoon* species**	***Hepatozoon* sp. A**	***Hepatozoon* sp. C**
08/03/05	PMS6	♂	*Hepatozoon* sp. A	(M)18/1000 (I)3/1000	0
21/03/07	ST10	♀	*Hepatozoon* sp. A	(M)1/1000	0
21/03/07	ST2	♂	*Hepatozoon* sp. A	(M)3/1000	0
21/03/07	STA	♂	*Hepatozoon* sp. A	0	(M)3/1000
22/11/08	TF1	♀	*Hepatozoon* sp. A	(M)2/1000	0
21/03/07	ST4	♂	*Hepatozoon* sp. A & C	(M)4/1000	(M)4/1000
22/11/08	STX	♂	*Hepatozoon* sp. A & C	(M)10/1000	(M)4/1000
15/04/06	PMS1	♂	*Hepatozoon* sp. A & C	(M)8/1000	(M)3/1000
11/10/06	MHO 0595	♀	*Hepatozoon* sp. C	0	(M)9/1000
11/10/06	MHO 0804	♂	*Hepatozoon* sp. C	0	(M)12/1000 (EC)3/1000 (I)10/1000
17/02/10	PMS4	juv	*Hepatozoon* sp. C	0	(M)3/1000
11/10/06	QQ0008	♂	*Hepatozoon* sp. C	0	(M)16/1000, (I)1/1000, (EC)5/1000
11/10/06	QQ0006	♂	*Hepatozoon* sp. C	0	(M)18/1000, (I)5/1000, (EC)3/1000
21/03/07	ST1	♂	*Hepatozoon* sp. C	0	(M)20/1000
21/03/07	ST3	♂	*Hepatozoon* sp. C	0	(M)10/1000, (EC)8/1000
21/03/07	ST5	♂	*Hepatozoon* sp. C	0	(M)12/1000
21/03/07	ST6	♂	*Hepatozoon* sp. C	0	(M)3/1000
21/03/07	ST7	♂	*Hepatozoon* sp. C	0	(M)8/1000
21/03/07	ST9	♂	*Hepatozoon* sp. C	0	(M)12/1000, (EC)3/1000
28/06/05	TT0	♀	*Hepatozoon* sp. C	0	(M)3/1000
18/02/10	PMS3	♂	*Hepatozoon* sp. C	0	(M)2/1000, (I)2/1000, (EC)1/1000

Appendix 3 Summary mature gamont stages of two *Hepatozoon* species (*Hepatozoon* sp. D & E), present in the peripheral blood of male, female and juvenile *Pseudocordylus langi* individuals with their corresponding slide numbers and collection dates, examined from the Sentinel escarpment area in the North Eastern Drakensberg. Haematozoans are estimated per 1000 erythrocytes. Abbreviation: juv = juvenile

Collection date	Slide no	Gender	Haemogregarine species	*Hepatozoon* spp. parasitaemia	
				***Hepatozoon* sp. D**	***Hepatozoon* sp. E**
18/01/06	bc1	♀	*Hepatozoon* sp. D	2/1000	0
18/01/06	bc2	♀	*Hepatozoon* sp. D	4/1000	0
11/10/06	Mho 0501	♂	*Hepatozoon* sp. D	8/1000	0
09/04/09	pl1	♂	*Hepatozoon* sp. D	2/1000	0
11/10/06	qq0004	♀	*Hepatozoon* sp. D	1/1000	0
11/10/06	qq0015	♀	*Hepatozoon* sp. D	38/1000	0
17/01/07	tc2	♀	*Hepatozoon* sp. D	5/1000	0
18/01/07	tc3	♀	*Hepatozoon* sp. D	10/1000	0
19/01/08	Tg1	♂	*Hepatozoon* sp. D	1/1000	0
22/01/08	tg11	♀	*Hepatozoon* sp. D	3/1000	0
15/01/08	qq0010	♂	*Hepatozoon* sp. D & E	20/1000	4/1000
21/03/07	st8	♀	*Hepatozoon* sp. D & E	4/1000	1/1000
17/01/07	tc1	♂ juv	*Hepatozoon* sp. D & E	20/1000	2/1000