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An overview of the Dactylosomatidae (Apicomplexa: Adeleorina: Dactylosomatidae), with the description of *Dactylosoma kermiti* n. sp. parasitising *Ptychadena anchietae* and *Sclerophrys gutturalis* from South Africa

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ABSTRACT

Haemogregarine (Apicomplexa: Adeleorina) blood parasites are commonly reported from anuran hosts. Dactylosomatidae (Jakowska and Nigrelli, 1955) is a group of haemogregarines comprising *Dactylosoma* Labbé, 1894 and *Babesiosoma* Jakowska and Nigrelli, 1956. Currently *Dactylosoma* and *Babesiosoma* contain five recognised species each. In the current study, a total of 643 anurans, comprising 38 species, 20 genera, and 13 families were collected from South Africa (n = 618) and Belgium (n = 25), and their blood screened for the presence of dactylosomatid parasites. Three anuran species were found infected namely, *Ptychadena anchietae* (Bocage, 1868) and *Sclerophrys gutturalis* (Power, 1927) from South Africa, and *Pelophylax lessonae* (Camerano, 1882) from Belgium. Based on morphological characteristics, morphometrics and molecular results a new dactylosomatid, *Dactylosoma kermiti* n. sp. is described form *Pty. anchietae* and *Scl. gutturalis*. The species of *Dactylosoma* isolated from *Pel. lessonae* could not, based on morphological or molecular analysis, be identified to species level. Phylogenetic analysis shows species of *Dactylosoma* infecting anurans as a monophyletic group separate from the other haemogregarine groups. Additionally, the mosquitoes *Uranotaenia* (*Pseudoficalbia*) *mashonaensis* Theobald, 1901 and *U.* (*Pfc.*) *montana* Ingram and De Meillon, 1927 were observed feeding on *Scl. gutturalis in situ* and possible dividing stages of this new parasite were observed in the mosquitoes. This study is the first to describe a dactylosomatid parasite based on morphological and molecular data from Africa as well as observe potential stages in possible dipteran vectors.

1. Introduction

Intracellular haemogregarine blood parasites are a diverse group of adeleorinid coccidia (Apicomplexa: Adeleorina). These blood parasites are currently distributed among four families, namely Dactylosomatidae Jakowska and Nigrelli, 1955, Haemogregarinidae Léger, 1911, Hepatozoidae Miller, 1908, and Karyolysidae Labbé, 1894. The discovery of the first intraerythrocytic blood parasite was by Chaussat (1850) who reported on an unidentified parasite in the blood of frogs from Europe. Later Vulpian (1854) and Lankester (1871) provided information on similar frog blood parasites. These findings, together with the discovery of malarial parasites by Laveran (1881), are considered to have laid the foundation of our understanding of Apicomplexa today (see Jakowska and Nigrelli, 1955; Levine, 1971; Barta, 1991). The Dactylosomatidae is a small group of apicomplexan haemogregarines that currently comprises *Dactylosoma* Labbé 1894 and *Babesiosoma* Jakowska and Nigrelli, 1956. The family was initially named Dactylosomidae, but Becker (1970) later amended the name to

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Dactylosomatidae. According to Barta (1991), the taxonomic affinities of members of this family have been uncertain since their original discovery. They were often placed within genera now considered *incertae sedis* (Barta, 1991). This uncertain taxonomic placement may be the reason why, until recently, information on the biology of these parasites, beyond light microscopic observations in the vertebrate host, was non-existent (Boulard et al., 1982; Barta and Desser, 1986; Barta, 1989, 1991). To date, the life cycles and transmission mechanisms of only two species have been elucidated, namely, *Babesiosoma mariae* (Hoare, 1930) from a fish host and leech vector (Negm-Eldin, 1998), and *Babesiosoma stableri* Schmittner and McGhee, 1961 from a frog host and leech vector (Barta and Desser, 1989). Light microscopy constitutes the majority of observations on dactylosomatids and still plays an important role in documenting the development and parasitaemia of the parasite in its host. Members of this family have to date been recorded only in organisms with prolonged water contact, namely amphibian or fish hosts and leech vectors. This study aims to review our current knowledge of dactylosomatid parasites and provide a foundation for future taxonomic work. This will be achieved through the following objectives: (1) detailed literature review on current knowledge of this group; (2) determining the species diversity and phylogenetic relationships of the dactylosomatid parasites observed in anurans from South Africa and Belgium; and (3) describe a new species using morphological and molecular data.

1.1. Morphological and ultrastructural classification

Before ultrastructural studies (Boulard et al., 1982; Barta and Desser, 1986; Barta, 1991), dactylosomatids were considered closely related to Haemosporida and Piroplasmida (Levine, 1971, 1984). Similarly, the piroplasm *Anthemosoma garnhami* Landau, Boulard and Housin, 1969 was first considered a member of the Dactylosomatidae (see Landau et al., 1969; Vivier and Petitprez, 1969; Levine, 1971; Boulard et al., 1982; Chavatte et al., 2018). Levine (1971) also included *Haemohormidium* Henry, 1910 and *Sauroplasma* Du Toit, 1937 in this group. However, an ultrastructural study on *Dactylosoma ranarum* (Kruse, 1890) demonstrated typical apicomplexan structures, including a complete conoid, separating the genus *Dactylosoma* from the piroplasms and haemosporids (Boulard et al., 1982). Dactylosomatids instead fit within Coccidia and were subsequently moved to the order Eucoccidiorida (syn. Eucoccidiida) by Boulard et al. (1982), where they have remained (Barta, 1991). However, *Babesiosoma* was still considered as a junior synonym of *Haemohormidium* (see Levine, 1971), and was later transferred to Haemohormidiidae Levine, 1984, a family erected by Levine (1984) for the "piroplasm-like" parasites *Haemohormidium* and *Sauroplasma*. Later, Barta and Desser (1986) showed *B. stableri* to have ultrastructural features similar to *D. ranarum* as reported by Boulard et al. (1982), such as a parasitophorous vacuole, complete apical complex and a trilaminate pellicle. The shape of the merozoites with a large Golgi body associated with the nucleus and lipid inclusions with their conoid shape, size, distribution of the micronemes and rhoptries are also similar to *D. ranarum*, with the main difference being the number of merozoites produced (see Barta and Desser, 1986). Based on these observations *Babesiosoma* was resurrected and transferred back from Haemohormidiidae to Dactylosomatidae (see Barta and Desser, 1986; Barta, 1991).

1.2. Phylogenetic placement

Barta (1989) completed a phylogenetic analysis of the class Sporozoea using phenotypic characters to infer evolutionary relationships. That study showed the piroplasms and adeleorinid or adeleid coccidia to form a monophyletic group, separate from the eimeriid coccidia and haemosporidia. These findings showed that members of the Dactylosomatidae exhibit characteristics similar between some adeleid blood parasites and piroplasms, such as similar development and replication within intestinal epithelial cells of the definitive invertebrate vector instead of in the haemocoel (Barta, 1989). Furthermore, certain characters of dactylosomatids seem to be shared between various distantly and closely related blood inhabiting apicomplexan parasites. For example, intraerythrocytic development or asexual reproduction (exogenic merogony and binary fission) is a feature shared with various highly derived haemogregarines (*Haemogregarina* Danilewsky, 1885 (*sensu lato*) and *Cyrillia* Lainson, 1981), some piroplasms and all species of Haemosporidia (see Smith et al., 2000). These findings were supported by related studies focused on phenotypic characters of haemogregarines (Siddall, 1995; Smith and Desser, 1997; Smith et al., 2000). The analysis of these characters grouped species that shared definitive hosts, implying that the definitive hosts of apicomplexan parasites may be an informative character as initially suggested by Barta (1989). O'Donoghue (2017) proposed that the environment in which the hosts occur could also play a role in these patterns, e.g. the parasites of terrestrial versus aquatic hosts and vectors forming monophyletic clades.

Although most molecular phylogenetic studies tend to support relationships inferred from morphological or life history data, the evolutionary relationships of apicomplexan parasites are far from straightforward (O'Donoghue, 2017). A recent molecular phylogenetic study by Barta et al. (2012) on adeleorinid coccidia using 18S rDNA sequences, showed that monoxenous adeleorinid coccidia and heteroxenous haemogregarines (Hepatozoidae, Karyolysidae, Haemogregarinidae, and Dactylosomatidae) form a highly supported monophyletic group, separate from Haemosporidia and Piroplasmida. However, Piroplasmida appear to have closer affinities with the adeleorinid coccidia (and haemogregarines) than with the eimeriid coccidia, based on morphological or life history data, such as the shared developmental feature of syzygy (paring of gamonts) in the definitive host (O'Donoghue, 2017). On the other hand, species from Haemogregarinidae and Dactylosomatidae that undergo sporogonic development within their definitive host or vector without the formation of a resistant oocyst structure were shown to cluster together (Barta et al., 2012). These groups may have lost the need for such resistant oocysts as they are transmitted via the bite of their vectors (Hayes et al., 2006; Barta et al., 2012), whereas parasites in the other haemogregarine families are transmitted via ingestion of the definitive host. It is clear that additional taxon sampling, life cycle elucidation and molecular phylogenetic analysis, using multiple or faster-evolving markers may provide better support for these scenarios.

1.3. The genus Dactylosoma

Members of the genus *Dactylosoma* have undergone a fair number of taxonomic disputes, with *D. ranarum* being the first described species of the genus. Although Lankester (1871) is often given credit for the discovery of *D. ranarum* (see Saunders, 1960; Levine, 1971; Barta et al., 1987; Barta, 1991; Davies and Johnston, 2000), it is clear from the original descriptions (Lankester, 1871, 1882) that he was working with a species of *Lankesterella* Labbé 1899 (initially known as "*Drepanidium*" erected by Lankester (1871, 1882) however, this name had already been used by Ehrenberg (1861) for a ciliate). Nöller (1913) clarified that the first description of *D. ranarum* was by Kruse (1890) who believed he was describing additional developmental stages of the "*Drepanidium*" parasite that was *Lankesterella minima* (Chaussat, 1850). Kruse (1890) suggested that the parasite should be grouped with the haemogregarines, proposing the name *Haemogregarina ranarum* (Kruse, 1890) if the generic name "*Drepanidium*" was to fall away. Celli and San Felice (1891) also regarded it as *H. ranarum*, and Grassi and Feletti (1892) as a malarial parasite, due to its resemblance to human malaria. However, Labbé (1894) recognised differences between these genera, subsequently erecting *Dactylosoma* based on: (1) different elongated and amoeboid body forms; (2) areolar structure, vesicular nucleus and hyaline appearance of the protoplasm; (3) lack of pigment, but presence of retractile granules; (4) minimal effect on the host cell and nucleus;

Table 1
Recognised *Dactylosoma* species, host records, distribution, and morphometrics.

Species	Host(s)	Distribution	Morphometrics (given in μm). * Measurements obtained from figure scale bar.		References
			Primary Merogony:	Secondary Merogony:	
Dactylosoma kermiti n. sp.	*Ptychadena anchietae* (Plain grass frog) and *Sclerophrys gutturalis* (Guttural toad)	KwaZulu-Natal, South Africa	Trophozoites: 5.3–7.7 × 2.6–4.4. Meronts: 8.3–12.2 × 5.1–8.0. Merozoites: 5.0–6.6 × 1.8–3.2.	Meronts: 5.6–8.6 × 4.4–6.9. Merozoites: 4.2–5.5 × 1.8–3.5. Gamonts: 7.8–15.0 × 1.5–3.0.	Current study
Dactylosoma Iethrinorum Saunders, 1960	*Lethrinus nebulosus* (Spangled emperor), and *L. lentjan* (Pink ear emperor).	Waters of the Red Sea near Al Ghardaqa, Egypt	Meronts: 8.0 × 10.5. Merozoties: 1.9 × 2.4.		Saunders (1960)
Dactylosoma ranarum (Kruse, 1890) syn. *Dactylosoma splendes* Labbé 1894	*Pelophylax* kl. *esculentus*		Trophozoites: 3.0–4.0 × 1.5–2.0. Meronts: 10–15 × 2.0–3.0. Merozoites: 2.8 × 0.7. Meronts: 7.3 × 4.3. Merozoites: 4.3 × 1.3.	Meronts: 9.0 × 4.0. Merozoites: 2.0–3.0 × 1.0–1.5. Gamonts: 5.0–8.0 × 1.5–3.0. Meronts: 4.7 × 3.4. Merozoites: 3.4 × 0.9. Gamonts: 7.0 × 3.4.	Kruse (1890); Barta et al. (1987)
Dactylosoma salvelini Fantham, Porter and Richardson, 1942	*Salvelinus fontinalis* (Brook trout).	Province of Quebec, Canada		Meronts: 5.8–8.5 × 3.7–7.0. Gamonts: 4.4–7.8 × 1.5–3.0.	Fantham et al. (1942)
Dactylosoma sylvatica Fantham, Porter and Richardson, 1942	*Lithobates sylvatica* (Wood frog).	Province of Quebec, Canada	Trophozoites: 7.0–8.5 × 6.3–7.6. Meronts: 7.4–11.5 × 7.0–9.3.	Trophozoites: 4.4 × 3.0. Meronts: 5.2 × 4.0. Merozoites: 4.4–5.9 × 1.1–2.0. Gamonts: 7.0–12.6 × 1.5–3.0.	Fantham et al. (1942)
Dactylosoma taiwanensis Manwell, 1964	*Fejervarya limnocharis* (Alpine cricket frog)	Hualien, Hua Lien Hsien, Taiwan	*Trophozoites: 3.9 × 7.3.	* Meronts: 6.9–7.9 × 5.6–7.3 * Gamonts: 11.8–13.6 × 2.1–2.9.	Manwell (1964)

and (5) merogony ("sporulation") that gives rise to between 5 and 12 merozoites ("sporozoites") grouped in a rosette- or fan-like appearance. Labbé (1894) named the species he placed in this newly erected genus, *Dactylosoma splendens* Labbé 1894, however, according to Wenyon (1926) who supported the designation of Nöller (1913), Kruse (1890) had first used the species name "*ranarum*" for this parasite, thus *Dactylosoma splendens* was renamed *Dactylosoma ranarum* (Kruse, 1890).

Currently there are five recognised species of *Dactylosoma*, two of which infect fish hosts, namely *Dactylosoma iethrinorum* Saunders, 1960, and *Dactylosoma salvelini* Fantham, Porter and Richardson, 1942 (see Table 1). The remaining three species were described from anuran hosts. The first, *Dactylosoma ranarum*, is the most common and a cosmopolitan species reported from several anuran species. According to Barta (1991), this species has been recorded from *Pelophylax* kl. *esculentus* (Linnaeus, 1758) in France (Laveran, 1899), the Caucasus (Finkelstein, 1908), and Corsica (Boulard et al., 1982) and other European countries (França, 1908; Nöller, 1913). Furthermore, this species has also been recorded from Central and South America in *Rhinella marina* (Linnaeus, 1758) (see Walton, 1946) and in an unidentified toad from Pará, Brazil (Durham, 1902). Additionally, there have been reports in Africa from *Pel.* kl. *esculentus* collected in Constantine, Algeria (Billet, 1904), *Pelophylax saharicus* (Boulenger in Hartert, 1913) from Morocco (Seabra-Babo et al., 2015), and *Pelophylax ridibundus* (Pallas, 1771) (syn. *Rana ridibunda*) from North Africa (Walton, 1947, 1949), all included within the Palearctic realm. From the Ethiopian realm, hosts include *Hyperolius* sp. (syn. *Rappia marmorata*), *Amnirana galamensis* (Dumeril and Bibron, 1841) (syn. *Hylarana galamensis*, *Rana galamensis*), *Ptychadena oxyrhynchus* (Smith, 1849) (syn. *Rana oxyrhynchus*), *Ptychadena submascareniensis* (Guibé and Lamotte, 1953) (syn. *R. mascareniensis*) and *Sclerophrys regularis* (Reuss, 1833) (syn. *Amietophrynus regularis*, *Bufo regularis*) from the Gambia (Dutton et al., 1907). It is clear from the illustrations provided that the latter hosts were infected with several different blood parasites, all grouped as "*Drepanidia*" (Dutton et al., 1907; Walton, 1948). *Amnirana albolabris* (Hallowell, 1856) (syn. *Hylarana albolabris*, *Rana albolabris*) is reported as a host for *D. ranarum* from the Democratic Republic of the Congo (Schwetz, 1930; Walton, 1947) in Central Africa, and *Scl. gutturalis* (syn. *Scl. regularis*) in South Africa (Fantham et al., 1942). The giant bullfrog, *Pyxicephalus adspersus* Tschudi, 1838 is also reported to be infected with *D. ranarum* from an unspecified location possibly in sub-Saharan Africa (Walton, 1947). Lastly, there are also reports from *Sylvirana guentheri* (Boulenger, 1882) from Tonkin, northern Vietnam (Mathis and Léger, 1911) and Yung Foh Lee, Yang Ming Shan, Taiwan (Manwell, 1964). The type host and type locality for *D. ranarum* is *Pel.* kl. *esculentus and* probably Naples, Italy, respectively (Kruse, 1890). The second species, *Dactylosoma sylvatica* Fantham, Porter and Richardson, 1942 was described from the wood frog *Lithobates sylvaticus* (LeConte, 1825) (syn. *Rana sylvatica*) collected in the Province of Quebec, Canada. The third species is *Dactylosoma taiwanensis* Manwell, 1964, described from the Alpine cricket frog, *Fejervarya limnocharis* (Gravenhorst, 1829) (syn. *Rana Iimnocharis*) in Hualien, Hua Lien Hsien, Taiwan (see Table 1). In addition to the summary of reported species of *Dactylosoma* mentioned above, Netherlands et al. (2015) reported on an unidentified species of *Dactylosoma* in *Ptychadena anchietae* (Bocage, 1868) from northern KwaZulu-Natal, South Africa.

Species previously regarded as members of *Dactylosoma* are *Babesiosoma mariae* (Hoare, 1930) and *Babesiosoma jahni* (Nigrelli, 1929). Two enigmatic species previously assigned to *Dactylosoma*, *Dactylosoma tritonis* (Fantham, 1905) and *D. amaniae* (Awerinzew, 1914) are more likely inclusions of rickettsial organisms according to Levine (1988) and Barta (1991). *Dactylosoma clariae* (Haiba, 1962) (syn. *Cytauxzoon clariae* and *Haemohormidium clariae*) and *Dactylosoma tilapiae* Imam, Marzouk, Hassan, Derhall and Itman, 1985 were considered by Negm-Eldin (1998) to not contain sufficient proof to document the validity of these species (Smit et al., 2003). Species of *Dactylosoma* are characterised by similar merogonic development as for species of *Babesiosoma*, except for the morphologically distinct primary and secondary meronts and a varying number of merozoites produced in these cycles. In summary, *D. ranarum* undergoes primary merogony when a merozoite enters the host frogs' erythrocytes. Within erythrocytes, merozoites undergo simultaneous peripheral budding, transforming into a large multinucleate meront producing up to 16 merozoites arranged in the characteristic rosette- or hand-like nature for which the genus was named (see Labbé, 1894; Nöller, 1913; Barta, 1991; Lainson, 2007). Merozoites then separate and penetrate other erythrocytes, either repeating the cycle of primary merogony or

initiating secondary merogony. The cycle is repeated in secondary merogony with meronts producing up to eight merozoites that either repeat secondary merogony or mature into gamonts (see Nöller, 1913; Barta et al., 1987). To date, no complete cycles for any species of *Dactylosoma* have been elucidated. However, Barta (1991) used frogs captured on the island of Corsica, France, to experimentally infect the North American glossiphoniid leech *Desserobdella picta* (Verrill, 1872), the natural vector of *B. stableri* (see below) with *D. ranarum*. In his study, although no observations on the development of gametes or zygote formation were made, *D. ranarum* was found to undergo sporogonic development within the intestinal epithelium of this experimentally infected leech host. The oocysts observed appear to be polysporoblastic producing 30 or more sporozoites by a process of exogenous budding directly into the cytoplasm of the epithelial cell (Barta, 1991). Other haematophagous invertebrates have also been considered as potential vectors. Nöller (1913) was the first to experimentally attempt transmission of *D. ranarum* using the glossiphoniid leech *Hemiclepsis margmata* (Müller, 1774), however, this was unsuccessful despite repeated attempts. Boulard et al. (1982) also tested the mosquito *Culicoides nubeculosus* (Meigen, 1830) as the potential vector of *D. ranarum*, but the experiments yielded no results. Although phlebotomine sand flies are the known vectors for different blood parasites of anurans (Feng and Chung, 1940; Desportes, 1942), none thus far have been tested as potential vectors for any species of *Dactylosoma*.

1.4. The genus Babesiosoma

More than 60 years after the naming of the genus *Dacytylosoma*, the genus *Babesiosoma* was erected to incorporate the species that: (1) contain less granular but more vacuolated cytoplasm; (2) have a nucleus similar to species of *Babesia*, without a definite karyosome; (3) reproduce by schizogony or binary fission; and (4) do not produce more than four merozoites. Barta (1991) pointed out that certain of these characters, that, together, define *Babesiosoma*, are in general hard to unambiguously recognise. For example, (1) the less granular, more vacuolated cytoplasm indicates more amylopectin inclusions; (2) ultrastructurally a definite karyosome is present in the nuclei; and (3) the multiplication through binary fusion is dubious as this was probably confused with mature paired merozoites after merogonic duplication. However, the separation of the two genera is still considered justified as species of *Babesiosoma* produce only four merozoites during each merogonic cycle and double that number of sporozoites (eight) within oocysts in the definitive host (Barta, 1991) compared to species of *Dactylosoma* that produce up to 16 merozoites and double the number of sporozoites within their oocyst (Barta and Desser, 1986; Barta, 1991).

There are currently five recognised species of *Babesiosoma* (see Table 2), three described from fish hosts (Smit et al., 2003), namely *Babesiosoma bettencourti* (França, 1908), *Babesiosoma mariae* (syn. *Dactylosoma mariae*; *B. hannesi* Paperna, 1981) and *Babesiosoma tetragonis* Becker and Katz, 1965. The remaining two species are described from North American amphibian hosts. *Babesiosoma jahni* (syn. *Dactylosoma jahni*) is the type species of the genus, described from the Eastern newt *Notophthalmus viridescens* (Rafinesque, 1820). *Babesiosoma stableri* was described from the northern leopard frog *Rana pipiens* Schreber 1782 and was experimentally transmitted to *Anaxyrus americanus* Holbrook, 1836, *A. woodhousii* (Girard, 1854), *A. terrestris* (Bonnaterre, 1789), and *Rana catesbeianae* (Shaw, 1802). The latter ranid frog, *R. septentrionalis* (Baird, 1854), and *R. clamitans* (Latreille, 1801) have all been found infected naturally in Ontario, Canada (Barta and Desser, 1984); see Table 2.

Species previously regarded as belonging to *Babesiosoma*, but formally transferred to other genera are *Haemohormidium aulopi* (Mackerras and Mackerras, 1925), *Haemohormidium ophicephali* (Misra et al., 1969) (syn. *Babesiosoma hareni*, *Babesiosoma batrachi*, *Dactylosoma striata*, *Dactylosoma notopterae*), and *Haemohormidium rubrimarensis* (Saunders, 1960) (see Siddall et al., 1994; Cook et al., 2015). Enigmatic taxa, removed from the genus *Babesiosoma* are *incertae sedis* (*Babesiosoma*) *anseris* Haiba and El-Shabrawy, 1967, *incertae sedis* (*Babesiosoma*) *gallinanun* Fahmy, Arafa, Mandour, Kalifa and Abdel-Salem, 1979, and *incertae sedis* (*Babesiosoma*) *ptyodactyli* El-Naffer, Abdel-Rahman, and Khalifa 1979 (see Barta, 1991). Furthermore *Babesiosoma aegypta* Mohamed, 1978, *Babesiosoma tilapiae* Imam, Marzouk, Hassan, Derhall and Itman, 1985, and *Babesiosoma aegyptiacus* Eid, Negm-Eldin and Imam, 1991 were considered by Negm-Eldin (1998) to be supported by insufficient proof to document the validity of these species (Smit et al., 2003).

Babesiosoma species are characterised by merogonic replication within the vertebrate host leading to the formation of gamonts followed by sporogonic and merogonic replication in the invertebrate host and vector (see Jakowska and Nigrelli, 1956; Barta and Desser, 1986; Barta, 1991). In the vertebrate host, asexual development consists of primary and secondary merogony, except for *B. mariae*. The latter species was shown by Negm-Eldin (1998) to contain a third merogonic cycle (tertiary merogony). *Babesiosoma stableri* undergoes primary merogony when a merozoite enters a vertebrate (frog) host's erythrocyte. Within the erythrocytes, the merozoites undergo nuclear division, transforming into a binucleate meront. These binucleate meronts then undergo a second nuclear division, ultimately forming the characteristic and tetranucleate cruciform meront (four merozoites arranged in a cross shape) typical of members of *Babesiosoma* (see Barta and Desser, 1986; Barta, 1991). The cruciform meront's merozoites then separate and penetrate other erythrocytes, either repeating the cycle of primary merogony or initiating secondary merogony. The secondary merogonic cycle is completed in the same manner as primary merogony ultimately producing secondary merozoites that either repeat secondary merogony or mature into gamonts (Barta and Desser, 1986; Barta, 1991). For *B. mariae* in its fish host, the primary and secondary merogonic cycles are shorter compared to *B. stableri* and mature gamonts are produced from tertiary merozoites in the third merogonic cycle (Negm-Eldin, 1998). Mature gamonts are then ingested in the blood meal of the natural leech vector, *Desserobdella picta* and undergo syzygy, ultimately maturing into gametes that fuse to form an ookinete. The ookinete then penetrates intestinal epithelial cells to initiate sporogony. Mature sporozoites then make their way to the salivary glands, where they undergo similar merogonic replication as in the vertebrate host, producing merozoites that will repeat the merogonic cycle or enter the ductules of the salivary glands. When the leech takes its next blood meal, the merozoites are injected into its new vertebrate host (Jakowska and Nigrelli, 1956; Barta and Desser, 1986; Barta, 1991).

2. Materials & methods

2.1. Sample collection

A total of 643 anurans, belonging to 38 species of 20 genera from 13 families were collected at night using active sampling methods and their blood screened for the presence of dactylosomatid parasites as described previously (Netherlands et al., 2015). The majority of the samples were collected from South African anurans (n = 618) with the remaining samples collected from European anurans (n = 25). Anurans were collected from several sampling localities in South Africa and Belgium (Supplementary data S1). This study received the relevant ethical approval from North-West University's AnimCare ethics committee (ethics number: NWU-00372-16-A5). Ezemvelo KZN Wildlife issued research permits OP 526/2014, OP 839/2014, OP 4374/2015, OP 4092/2016, and OP 4085/2017. Agentschap Natuur & Bos issued the permit (ANB/BL/FF-V17-00091) for collection and sampling of amphibians in Belgium, with strict collection protocol followed as instructed by the permit office. Furthermore, authors (ECN, CAC, and LHdP) responsible for sample collection and processing of specimens

Table 2
Recognised *Babesiosoma* species, host records, distribution, and morphometrics.

Species	Synonym (s)	Host(s)	Distribution	Morphometrics (given in μm)	References
Babesiosoma bettencourti (França, 1908)	*Haemogregarina bettencourti* França 1908; *Desseria bettencourti* Siddall 1995	*Anguilla anguilla* (European eel)	In the Ancora, Este, Febros and Olivas river systems near Alcobaça, Portugal	Single meronts: 2.0–6.0 × 0.8–4.0. Meronts: 4.0–6.0 × 0.5–2.0 (Cruciform). Merozoits: 2.0–3.0 × 0.8–1.5. Gamonts: 3–10 × 1–4.	Cruz and Davies (1998)
Babesiosoma mariae (Hoare, 1930)	*Dactylosoma mariae* Hoare (1930); *Babesiosoma hannesi* Paperna 1981	*Haplochromis nubilus* (Blue Victoria mouthbrooder), *H. cinereus, H. serranus* and *H.* sp. (Pisces: Teleostei); *Oreochromis esculentus* (Singida tilapia).; *Serranochromis angusticeps* (Thinface largemouth tilapia). *Mugil cephalus* (*Flathead grey mullet), Chelon richardsonii* (South African mullet), and *C. dumerili* (Grooved mullet)	Victoria Nyanza, near Entebbe, Uganda. Swartkops estuary, Port Elizabeth, South Africa	Merozoites: 2.8 × 0.9. Trophozoites: 3.8–5.7 × 1.9–5.0. Gamonts: 6.6–8.5 × 1.9–3.5 (Crescent); 5.7–6.6 × 3.8–5.7 (Rounded).	Hoare (1930); Paperna (1981)
Babesiosoma tetragonis Becker and Katz, 1965 (Holotype US National Museum, No. 60066)	*Haemohormidium tetragonis* (Becker and Katz, 1965) Laird and Bullock, 1969	*Catostomus* sp.	Shasta River, Montague, California, USA.	Trophozoites: 2.2–3.7 × 4.4–6.6 (Elliptical); 3.3–4.8 × 3.8–5.5 (Oval). Meronts: 3.0–3.9 × 5.5–6.7 (Tetranueleate); 5.5–6.9 × 5.8–7.4 (Cruciform); Merozoites: 1.4–2.6 × 2.6–5.3. Gamonts: 3.0–4.8 × 6.1–8.2 (Macro); 1.6–2.8 × 4.9–7.2 (Micro). Extracellular merozoites: 1.5–3.1 × 2.3–3.4.	Becker and Katz (1965)
Babesiosoma jahni (Nigrelli, 1929)	*Haemohormidium jahni* (Nigrelli, 1929) Laird and Bullock, 1969; *D. Jahni* Nigrelli, 1929	*Notophthalmus viridescens* (Eastern newt)	Pennsylvania, USA		Nigrelli (1929); Jakowska and Nigrelli (1955)
Babesiosoma stableri Schmittner and McGhee, 1961	*Haemohormidium stableri* (Schmittner and McGhee, 1961) Laird and Bullock, 1969	*Lithobates pipiens* and experimental infection to *Lithobates catesbeianus, Lithobates septentrionalis Lithobates clamitans, Bufo americanus, Bufo woodhousei, and Bufo terrestis*	Purchased from Steinhilber and Co. Inc., Wisconsin, North America.	Trophozoites: 6.6 × 2.3. Immature binucleate stage: 7.0 × 2.3. Meronts: 7.6 × 2.4 (Binucleate). Meronts: 6.8 × 2.4–3.8 (Tetranueleate). Merozoites: 3.6 × 1.7. Gamonts: 6.1–10.3 × 2.0–2.7.	Schmittner and McGhee (1961)

have undergone specialised training in ethical handling of aquatic ectotherms (NWU Ectothermic Vertebrates Handling and Ethics).

2.2. Light microscopy screening

Blood was collected and processed following standard methods (Netherlands et al., 2015, 2018). Stained blood smears were screened at 1000× using a Nikon Eclipse E100 compound microscope and images of parasite stages captured and measured using the imaging software NIS Elements Ver. 4.60. Trophozoites, primary meronts and merozoites, and secondary meronts, merozoites and gamonts were measured for the dactylosomatid species. Measurements comprised the parasite's length and width. Measurements are in micrometres (μm), with the average, standard deviation and range given (in this order). Parasitaemia was calculated per 100 erythrocytes, with ~10^4 erythrocytes examined per blood smear.

2.3. DNA extraction, PCR amplification and phylogenetic analyses

Eleven (n = 11) ethanol-preserved blood samples from *Ptychadena anchietae* (n = 6), *Sclerophrys gutturalis* (n = 4), and *Pel. lessonae* (n = 1) parasitised with dactylosomatids were used for molecular work. Samples were selected based on a high intensity of dactylosomatid infection without other haemogregarine blood parasites present (i.e. *Hepatozoon* sp.) assessed using light microscopy screening. Genomic DNA extraction, polymerase chain reaction (PCR) amplification and sequencing of PCR products were all completed following the methods detailed in Netherlands et al. (2018). The software package Geneious R11 (http://www.geneious.com(Kearse et al., 2012)) was used to assemble and edit resultant sequence fragments. Species identity was verified against previously published sequences using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). Sequences obtained were deposited in the NCBI GenBank database under the following accession numbers [GenBank: MN879389 – MN879399].

Sequences for species of *Hemolivia* Petit, Landau, Baccam and Lainson, 1990, *Hepatozoon* Miller, 1908, *Karyolysus* Labbé, 1894, *Haemogregarina*, and *Dactylosoma* (parasitising amphibian, avian, reptilian and mammalian hosts) were downloaded from GenBank and aligned to the sequences generated in this study. As in previous studies on haemogregarines, *Adelina dimidiate* Schneider, 1875, *Adelina grylli* Butaeva, 1996 [GenBank: DQ096835–DQ096836] and *Klossia helicina* Schneider, 1875 [GenBank: HQ224955], from the suborder Adeleorina were selected as outgroup. Sequences were aligned using the Clustal W 2.1 alignment tool (Larkin et al., 2007) implemented within Geneious R11. GBlocks was used to remove any alignment gaps and ambiguities (Castresana, 2000; Talavera and Castresana, 2007). The final alignment contained 44 sequences at a length of 1637 nt. A model test was performed to determine the most suitable nucleotide substitution model, according to the Bayesian information criterion (BIC), using jModelTest 2.1.7 (Guindon and Gascuel, 2003; Darriba et al., 2012). The model with the best BIC score was the General Time Reversible (Tavaré, 1986) model, with a proportion of invariable sites (0.5100), and discrete gamma distribution (gamma shape 0.7840) (GTR + I + Γ). This optimal model of molecular evolution was used for all analyses. Phylogenetic relationships were inferred via Bayesian inference (BI) using MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001), and Maximum likelihood (ML) analysis using RAxML 7.2.8. (Stamatakis, 2014), implemented in Geneious R11. For the BI analysis, the Markov Chain Monte Carlo (MCMC) algorithm was run for 1 million generations, sampling every 100 generations. The first 25% of the trees were discarded as 'burn-in' with no 'burn-in' samples being retained. The Tracer tool (implemented from within Geneious R11) was used to assess convergence and the 'burn-in' period (Rambaut et al., 2018). For the ML analysis, nodal support was assessed using 1000 rapid bootstrap replicates. Furthermore, model-corrected (GTR + I + Γ) genetic distances for all the sequences used in the phylogenetic analysis were also calculated in PAUP* version 4.0a152 (Swofford, 2002).

2.4. Vector and life history evaluation

During anuran sampling, care was taken to search for potential haematophagous invertebrate vectors. When possible, following sampling, screening and releasing of collected individuals, sites that were identified to harbour larger numbers of infected individuals were revisited. The behaviour of anurans from these localities was documented *in situ*, via sedentary observations with infrared light (not to disturb the host or vectors). Macro-photography was also used to capture images of any possible haematophagous invertebrates feeding on the anurans. Haematophagous invertebrates observed feeding were collected using an aspirator. Specimens were either fixed in 70% alcohol to be identified later or dissected (to separate relevant organs and body parts) and smeared on a glass slide to screen for possible blood parasite development. When possible, the individual anurans the haematophagous invertebrates were feeding on were also collected. In November 2017 and January 2018, while collecting *Scl. gutturalis* (Supplementary data S1) for a separate study on microfilariid nematodes (Netherlands et al., 2020) in Sodwana Bay, site SB-1 (S27.488591°; E32.664259°), mosquitoes were observed feeding on calling *Scl. gutturalis* and collected using an aspirator. Collected mosquitoes housed in plastic jars (350 ml) with moist cotton wool were transported back to the NWU frog lab, to keep humidity levels up and provide mosquitoes with a source of water when necessary. Mosquitoes were euthanised between 1 and 7 days post-infection (dpi) with carbon dioxide (CO_2), identified using Jupp (1996) and dissected under a stereomicroscope using modified entomology pins. The mosquito's intestines and fat bodies were subsequently smeared on a glass slide using the same methods as for the blood smear preparation.

3. Results

Based on the reviewed literature it is clear that biological information on this group of haemogregarines is lacking. As a result 643 anurans were screened during the present study for dactylosomatids, 71 were found parasitised with haemogregarines conforming morphologically to species of *Dactylosoma*. The diagnosis was initially based on the hyaline appearance of the different elongated and amoeboid forms occurring in erythrocytes. Overall, three host species were found to be infected based on morphological and molecular data: *Pty. anchietae* (61/160) and *Scl. gutturalis* (9/73) from South Africa; and, *Pel. lessonae* (1/14) from Belgium. This study shows the efficacy of combining traditional morphological and modern molecular techniques in gathering data for biologically complex parasite groups hoping to promote an increase of future work on dactylosomatids.

3.1. Species descriptions

Phylum: Apicomplexa Levine, 1970
Class: Conoidasida Levine, 1988
Subclass: Coccidia Leuckart, 1879
Order: Eucoccidiorida Léger, 1911
Suborder: Adeleorina Léger, 1911
Family: Dactylosomatidae (Jakowska and Nigrelli, 1955)
Genus: *Dactylosoma* Labbé, 1894

3.1.1. *Dactylosoma kermiti Netherlands, Cook and Smit n. sp.*

Type-host: *Ptychadena anchietae* (Bocage, 1868) (Anura: Ptychadenidae).

Other host: *Sclerophrys gutturalis* (Power, 1927) (Anura: Bufonidae).

Site in host: Peripheral blood.

Definitive host and vector: Unconfirmed. Observed possible vectors (see below).

Type-locality (P. anchietae): Ndumo Game Reserve, KZN, South

Africa (S26.926179°, E32.332416°).

Other localities (*Scl. gutturalis*): Sodwana, KZN, South Africa (S27.488591°, E32.664259°).

Type-material: Hapantotype, 1 × blood smear with a parasitaemia of 5.7% from *Pty. anchietae* deposited in the protozoan collection of the National Museum, Bloemfontein, South Africa, under accession number [NMB P 534]. Parahapantotype, 1 × blood smear: *Pty. anchietae,* parasitaemia of 2.0%, deposited in the protozoan collection of the National Museum, Bloemfontein, South Africa, under accession number [NMB P 535].

Additional material: 2 × blood smears: *Scl. gutturalis* with a parasitaemia of 0.2%, respectivly, deposited in the Protozoan Collection of the National Museum, Bloemfontein, South Africa, under accession number [NMB P 536 - 537].

Representative DNA sequences: The sequence data specifically associated with *D. kermiti* (upon which the present biological description is based) have been submitted to GenBank and are as follows: Nuclear 18S rDNA (nu 18S) partial sequence: MN879389 – MN879398.

ZooBank registration: The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:F5CBE7E7-D57F-4854-A98B-A6D6ED6FD342. The LSID for *Dactylosoma kermiti* n. sp. is urn:lsid:zoobank.org:act:0D6D6A74-4136-4A9D-AE15-73FDFE12EBD3.

Etymology: The epithet is constructed in a positive form of a personal name. As the type host for this species is a frog, the species is named after the Muppet character, Kermit the Frog. Gender is male.

3.1.1.1. Description. Two distinct stages of merogony present in erythrocytes and occasionally in thrombocytes and monocytes of both infected type host *Pty. anchietae* (Fig. 1 A–L) and other host *Scl. gutturalis* (Fig. 2 A–L). The first stage characterised by large meronts producing up to approximately 12 merozoites. The second stage meront, seemingly smaller, produces up to six merozoites. The general appearance of the cytoplasm is hyaline, yet coarse. Parasitaemia of infected individuals (n = 70) given in percentage (%) was 0.4 ± 1.2 (0.1–5.7). The distribution range in the type host *Pty. anchietae* was larger with infected individuals collected from various localities and habitats, as compared to *Scl. gutturalis* that were only found infected at Sodwana Bay (Supplementary data S1).

Primary merogony: young trophozoites (Figs. 1 A, 2 A), rare, observed within erythrocytes, ovoid crescent shape, largely non-stained except peripherally. Nuclei not clearly defined, located at blunt end (broader end of the parasite). Condensed chromatin staining deep magenta (Fig. 1 A arrowhead). Measuring 4.2 ± 0.6 (3.6–4.9) long × 2.9 ± 0.8 (1.8–3.7) wide (n = 4).

Trophozoites (Fig. 1 B–D, 2 B) elongated to oval, usually tapering towards one end, distinct vacuoles frequently present (Fig. 1 B–D, 2 B arrow). Small round dense nuclei located at blunt end, chromatin staining deep magenta (Fig. 1 B–D). Trophozoites measure 6.7 ± 2.2 (5.3–7.7) long × 3.5 ± 1.2 (2.6–4.4) wide (n = 20).

Young primary meronts (Figs. 1E and 2C) large ovoid to round shape, causing slight displacement of the host cell nucleus, distinct vacuoles frequently present (Fig. 1 E arrow). Multinucleate, nuclei division located peripherally, chromatin staining bright deep magenta (Fig. 1 E arrowhead). Young meronts measure 7.8 ± 0.9 (6.3–9.5) long × 5.7 ± 1.3 (4.0–8.3) wide (n = 20).

Primary meronts (Fig. 1 F, G) varying in form, round to crescent shape, causing slight distortion or displacement of the host cell or nucleus, cytoplasm staining whitish-purple. Multinucleate, normally showing more than 10 nuclei located peripherally (Fig. 1 F, G arrowhead), chromatin staining deep magenta. Meronts measure 9.9 ± 1.2 (8.3–12.2) long × 6.9 ± 0.8 (5.1–8.0) wide (n = 10).

Primary merozoites (Fig. 1 H) elongate to ovoid, hyaline cytoplasm staining pinkish-purple. Small round dense nuclei located at blunt end, chromatin staining deep magenta (Fig. 1 H, arrowhead). Merozoites measure 5.6 ± 0.5 (5.0–6.6) long × 2.7 ± 0.4 (1.8–3.2) wide (n = 16).

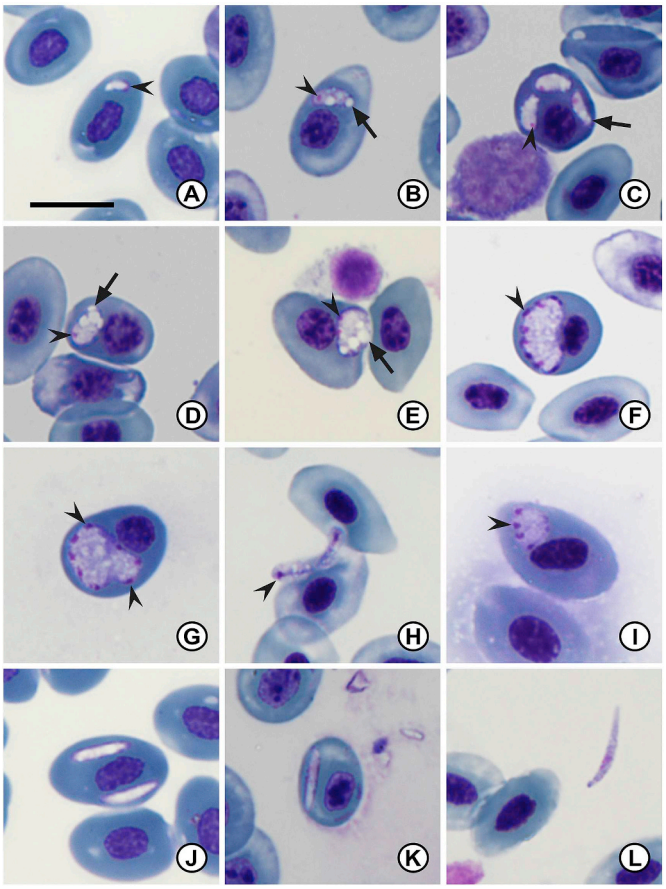

Fig. 1. (A–L). *Dactylosoma kermiti* n. sp. from the grass frog *Ptychadena anchietae*. (A–H) Primary merogony. (A) Young trophozoite. (B–D) Trophozoites. (E) Young meront. (F–G) Primary meronts. (H) Merozoites. (I–L) Secondary merogony. (I) Secondary meront. (J) Immature gamont. (K) Gamont. (L) Extracellular gamont. Arrowheads show condensed chromatin (A–I); arrows show vacuoles (B–E). All images captured from the deposited slides [NMB P 534 – 535]. Scale bar 10 μm.

Secondary merogony: young secondary meront (Fig. 2 D) irregular ovoid to round shape, cytoplasm staining whitish-purple, distorting the host cell and slight displacement of the host cell nucleus. Multinucleate secondary meront, nuclei located peripherally, chromatin staining bright pink to dark purple (Fig. 2 D, arrowhead). Young meronts measure 5.4 ± 0.5 (4.3–6.3) long × 4.6 ± 0.6 (3.4–5.8) wide (n = 19).

Secondary meronts (Figs. 1 I, 2 E–G) differ in form, ovoid to quadrilateral shape, mature forms have dactylate (hand-like) appearance (Fig. 2 F, G), cytoplasm staining dark purple. Multinucleate, normally between four and six nuclei located peripherally (Fig. 1 I, 2E–G arrowhead), chromatin staining deep magenta. Secondary meronts measure 7.2 ± 0.8 (5.6–8.6) long × 5.7 ± 0.9 (4.4–6.9) wide (n = 16).

Secondary merozoites (Fig. 2 F–G, L arrow) elongate to ovoid, hyaline cytoplasm staining purple. Small round dense nuclei located closer to the centre, chromatin staining deep magenta (Fig. 2 F–G, L arrowhead). Secondary merozoites measure 4.8 ± 0.5 (4.2–5.5) long × 2.3 ± 0.5 (1.8–3.5) wide (n = 11).

Gamonts (Fig. 1 J–L, 2 H–K) elongate and slender, often tapering to one end. In some cases slight displacement of the host cell nucleus is visible. Immature forms largely non-staining, except peripherally - staining dark purple (Fig. 1 J). Intracellular mature gamont forms have a hyaline appearance, although nuclei not clearly defined, nucleoplasm conglomeration visible off centre closer to blunt side (Fig. 1 K). Extracellular forms often elongate with a slight curvature, nuclei visible

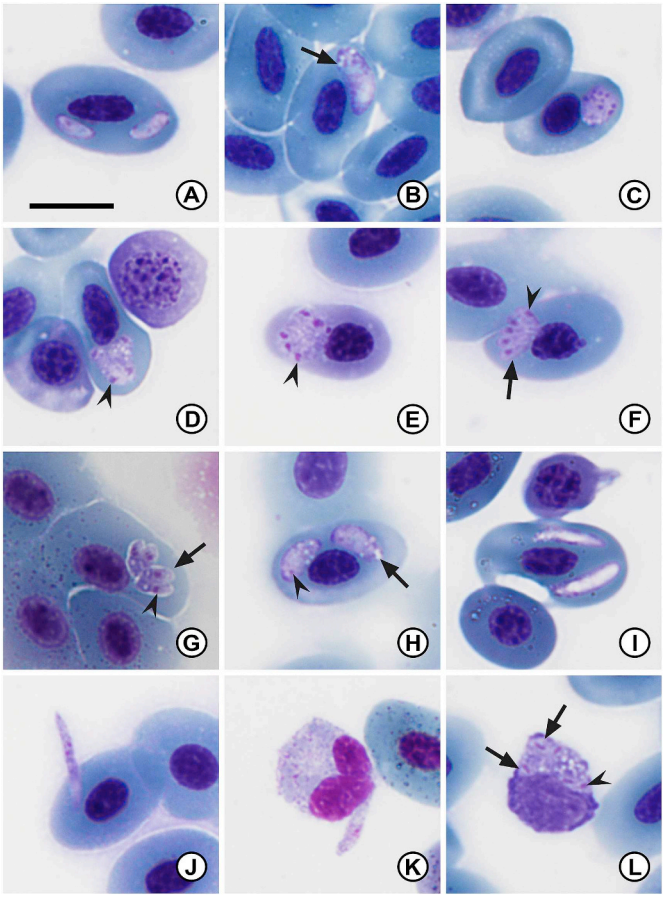

Fig. 2. (A–L). *Dactylosoma kermiti* n. sp. from the guttural toad *Sclerophrys gutturalis*. (A–D) Primary merogony. (A) Young trophozoite. (B) Trophozoites. (C) Young meront. (D–L) Secondary merogony. (D) Young secondary meront. (E) Secondary meront. (F–G) Secondary merozoites. (H–I) Gamont. (K) Extracellular gamont. (L) Secondary meront in leukocyte. Arrowheads show condensed chromatin (D–H, L); arrows show vacuoles (B) and merozoites (F–G). All images captured from the deposited slides [NMB P 536 – 537]. Scale bar 10 μm.

slightly off centre, staining dark purplish-pink. Gamonts measure 10.6 ± 2.5 (7.8–15.0) long × 2.2 ± 0.3 (1.5–3.0) wide (n = 40).

3.1.1.2. Remarks. *Dactylosoma kermiti* n. sp. can be characterised by its slender trophozoite and small merozoite stages; the number of primary (up to 14) and secondary (up to six) merozoites produced by meronts; and the vacuolated trophozoite stages. This species can be distinguished from all currently recognised species of *Dactylosoma* from anuran hosts, namely *D. ranarum*, *D. sylvatica*, and *D. taiwanensis*, based on several morphometric and developmental characteristics. Morphologically this parasite conforms closest to *D. sylvatica* with measurements overlapping across trophozoite, meront, merozoite and gamont stages. However, only a single, most probably the secondary merogonic cycle is reported, with meronts only producing up to eight merozoites. In comparison the number of merozoites produced by *D. kermiti* n. sp. differs in primary and secondary merogony with up to 14 or six merozoites observed in the primary and secondary meronts, respectively. Although measurements do overlap, *D. kermiti* n. sp. seems to possess generally more slender trophozoite stages and smaller merozoite stages. Also, *D. sylvatica* is reported from the wood frog *Lithobates sylvatica* from Canada. *Dactylosoma ranarum* is apparently a cosmopolitan species recorded from several hosts across Europe, Central and South America, Africa, and Asia. Based on morphometric data from the original description of *D. ranarum*, as well as from a more recent ultrastructural study of this species' morphology, several stages differ in size when compared to *D. kermiti* n. sp. (see Table 1). The most notable differences as compared to the relatively smooth to finely vacuolated cytoplasm of *D. sylvatica* and *D. ranarum* are the largely vacuolated trophozoite stages seen in *D. kermiti* n. sp. (Fig. 1 B, D–E; 2 B arrow). The third species, *D. taiwanensis*, described from the Alpine cricket frog *Fejervarya limnocharis* from Taiwan, is similar to *D. kermiti* n. sp. in that the trophozoite stages of both species contain vacuoles, the cytoplasm appears to be relatively coarse, and several measurements seem to overlap based on the scale provided. However, *D. taiwanensis*, similar to *D. sylvatica*, has meronts producing up to eight merozoites only. Likewise, the merozoites produced by *D. taiwanensis* are morphologically different from those of *D. kermiti* n. sp.: the nuclei seem to be larger and more prominent in comparison to the rest of the merozoites.

From the comparison of *D. kermiti* n. sp. to the currently recognised congeners infecting anurans, it is clear that these parasite species share close or overlapping morphology. Hence, since dactylosomatids possess several life stages in the vertebrate host, species can easily be misidentified. For example, gamont stages between the different species have largely comparable morphology and morphometrics. However, after careful review, screening a large number of infected hosts, comparing the stages observed in different individuals from different sites and seasons, we propose *D. kermiti* n. sp. as a new species.

3.1.2. Dactylosoma sp. from Belgium

Host: *Pelophylax lessonae* (Camerano, 1882) (Anura: Ranidae).

Site in host: Peripheral blood.

Vector: Unknown.

Localities: Haacht, Belgium (N50.979434°, E4.659686°).

Voucher material – 1 × blood smear with a parasitaemia of 0.3% from *Pel.* kl. *esculentus* deposited in the protozoan collection of the National Museum, Bloemfontein, South Africa, under accession number [NMB P 538].

Representative DNA sequences: The sequence data specifically associated with *Dactylosoma* sp. ex *Pel. lessonae* have been submitted to GenBank and are as follows: Nuclear 18S rDNA (nu 18S) partial sequence: MN879399.

3.1.2.1. Description. Two distinct stages of merogony present in erythrocytes of *Pel. lessonae* (Fig. 3 A–L). The first stage characterised by large dactylate meronts producing up to approximately 14 clear merozoites. The second stage meronts seemingly smaller, although no clear merozoites were observed. The cytoplasm has a hyaline appearance. Parasitaemia of infected individual (n = 1) was 0.3%.

Primary merogony: trophozoites (Fig. 3 A) elongated to oval, usually tapering towards one end, vacuoles in some cases present (Fig. 3 A arrow). Small round dense nuclei located at blunt end (Fig. 3 A arrowhead), chromatin staining deep magenta. Trophozoites measure 6.3 ± 0.9 (5.3–7.4) long × 3.8 ± 0.8 (2.6–4.9) wide (n = 10).

Young primary meronts (Fig. 3 B) round to ovoid causing slight displacement of the host cell nucleus. Multinucleate, nuclear division located peripherally, chromatin staining bright pink to dark purple (Fig. 3 B arrowhead). Young meronts measure 9.4 ± 1.1 (8.0–10.9) long × 8.0 ± 0.5 (7.0–8.6) wide (n = 7).

Primary meronts (Fig. 3 C–G) vary in form, round to pear shape, in some cases causing slight distortion or displacement of the host cell or nucleus, cytoplasm staining whitish-purple. Mature forms have dactylate appearance (Fig. 3 F–G). Multinucleate, normally showing more than 10 nuclei (Fig. 3 C-E arrowhead), staining deep magenta. Meronts measure 11.3 ± 2.8 (8.4–16.5) long × 9.1 ± 1.9 (7.1–12.6) wide (n = 10).

Primary merozoites (Fig. 3 F–H) elongate to ovoid, hyaline cytoplasm staining white to bluish-purple. Dense nuclei chromatin centrally located, staining deep magenta (Fig. 3 F–H, arrowhead). Merozoites (Fig. 3 F–H, arrow) measure 4.0 ± 0.6 (3.1–5.0) long × 1.9 ± 0.6

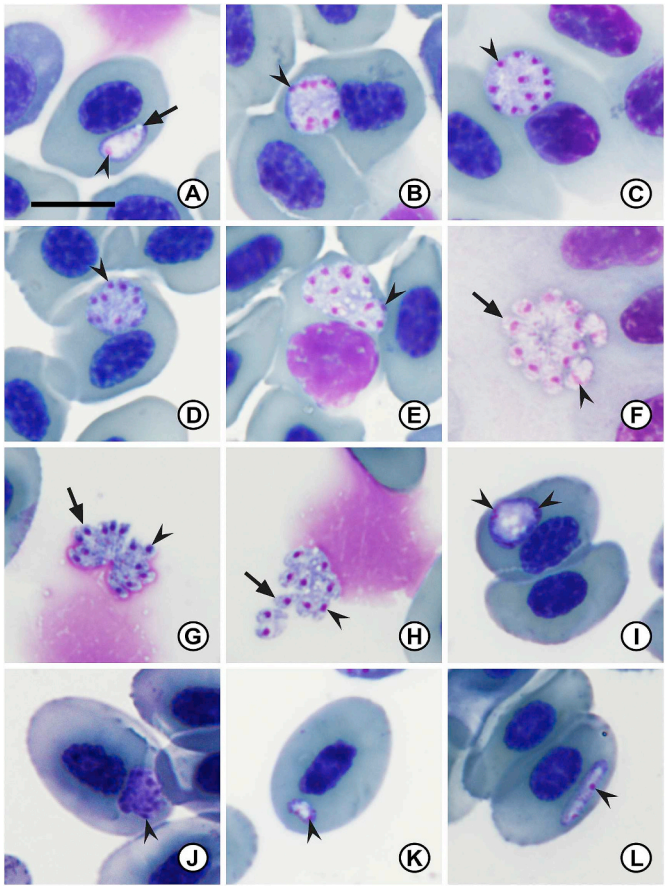

Fig. 3. (A–L). *Dactylosoma* sp. from *Pelophylax lessonae*. (A–D) Primary merogony. (A) Trophozoite. (B) Young meront. (C–G) Secondary meronts. (F–H) Merozoites, arrows. (I–L) Secondary merogony. (I) Young meront. (J) Meront. (K) Merozoite. (L) Gamont. Arrowheads show condensed chromatin; arrows show vacuoles (A) and merozoites (F–H). All images captured from the deposited slide [NMB P 538]. Scale bar 10 μm.

(1.1–3.1) wide (n = 20).

Secondary merogony: young secondary meront (Fig. 3 I) irregular ovoid shape, cytoplasm staining bluish-purple, occasionally causing displacement of the host cell nucleus. Multinucleate, nuclei located peripherally, staining deep magenta (Fig. 3 I, arrowhead). Young meronts measure 6.2 ± 1.1 (4.5–7.5) long × 4.5 ± 1.5 (2.7–7.3) wide (n = 7).

Secondary meronts (Fig. 3 J) ovoid shape, cytoplasm stains dark bluish-purple. Multinucleate, up to 6 nuclei located peripherally, chromatin staining dark purple (Fig. 3 J, arrowhead). Secondary meronts measure 7.7 ± 0.9 (5.9–8.9) long × 6.6 ± 1.1 (5.8–8.5) wide (n = 10).

Secondary merozoites (Fig. 3 K) rare, short crescent shape, hyaline cytoplasm staining purple. Small round dense nuclei located peripherally, staining deep magenta (Fig. 3 K arrowhead). Secondary merozoites measure 4.1 long × 2.3 wide (n = 1).

Gamonts (Fig. 3 L) elongate and slender shape, hyaline appearance, nuclei chromatin visible slightly off centre (Fig. 3 L arrowhead). Extracellular forms often elongate with slight curvature, nucleoplasm visible slightly off centre, staining dark purplish-pink. Gamonts measure 9.8 ± 1.8 (7.9–12.1) long × 2.3 ± 0.1 (2.2–2.5) wide (n = 5).

3.1.2.2. Remarks. The species of *Dactylosoma* under study possesses several phenotypic characteristics mentioned in the original description of *Dactylosoma splendens*, later synonymised with *D. ranarum*, such as the round shape of the meronts and the number of primary and secondary merozoites produced (ranging approximately between 12 and five). However, this synonymisation happened over 100 years ago when knowledge of the close resemblance between different species was unknown. Unfortunately, no precise measurement data are available from the original description of *D. splendens*, which was described from *Pel.* kl. *esculentus* in Paris, France (Labbé, 1894). However, based on the information of this species of *Dactylosoma* provided above, the parasite cannot, based on the data collected in the current study, be identified to species level.

Furthermore, this species of *Dactylosoma* can be characterised by its round young meront stages, the dactylate appearance of the mature meront stages, and the hyaline appearance of the cytoplasm. Although the trophozoite and gamont stages of *Dactylosoma kermiti* n. sp. are superficially similar in morphology and morphometrics, primary and secondary meront stages are wider (primary meront: 5.1–8.0 *vs* 7.1–12.6; secondary meront: 4.4–6.9 *vs* 5.8–8.5) and secondary merozoites stages slightly shorter (4.2–5.5 *vs* 4.1) in the species of *Dactylosoma* from *Pel. lessonae*. This species also conforms closely to *D. sylvatica*, however, the species from the current study produces more than eight merozoites in primary merogony and less than eight in secondary merogony as compared to *D. sylvatica*. Also the secondary meront stages are wider as compared to *D. sylvatica* (secondary meront: 5.9–8.9 × 5.8–8.5 *vs* 5.2–4.0).

3.2. Phylogenetic analysis

Sequence fragments between 1677 and 1744 nt long were isolated from the dactylosomatids collected from *Ptychadena anchietae* (n = 7), *Sclerophrys gutturalis* (n = 3) and *Pelophylax lessonae* (n = 1). Species of *Dactylosoma* formed a highly supported clade branching basally among the ingroup taxa that was sister to a large clade of species of *Haemogregarina, Hemolivia*, *Hepatozoon* and *Karyolysus*. *Dactylosoma* species isolates from European anurans (Palearctic realm) and identified as *D. ranarum* and *Dactylosoma* sp. ex *Pel. lessonae* formed a highly supported clade with *D. kermiti* n. sp. from African anurans (Ethiopian realm) as the sister taxon. The *Haemogregarina* clade branched next and formed a sister group to a large clade of *Hemolivia*, *Hepatozoon* and *Karyolysus* species. Intraleukocytic species of *Hepatozoon* (*Hepatozoon* clade A) and species of *Karyolysus* together formed a clade sister to a clade comprising species of *Hemolivia* and intraerythrocytic species of *Hepatozoon* (*Hepatozoon* clade B).

Based on 1637 nt sequence comparisons of the 18S rRNA gene (Supplementary data S2), the model-corrected genetic distance between the species of *Dactylosoma*, ranged between 0.2 and 0.5%. The interspecific divergence between *D. ranarum* and *D. kermiti* n. sp. was 0.5% (Supplementary data S2), and 0.2% between *D. ranarum* and *Dactylosoma* sp. ex *Pel. lessonae*. Several other haemogregarines also have an interspecific divergence below 1%, namely *Hepatozoon* cf. *clamatae* [GenBank: HQ224963] and *Hepatozoon* cf. *catesbianae* [GenBank: HQ224954] with 0.3%; *Hepatozoon chinensis* Han, Wu, Dong, Zhu, Li, Zhao, Wu, Pei, Wang, Huang, 2015 [GenBank: KF939620] and *Hepatozoon ayorgbor* Sloboda, Kamler, Bulantová, Votýpka et Modrý, 2007 [GenBank: EF157822] with 0.3%; *Hemolivia parvula* (Dias, 1953) [GenBank: KR069083] and *Hemolivia mauritanica* (Sergent and Sergent, 1904) [GenBank: KF992698] with 0.7% (Table S2).

3.3. Vector and life history evaluation

In the current study only phlebotomine sand flies, identified as species of *Sergentomyia* were observed feeding on *Pty. anchietae* and *Scl. gutturalis*. Mosquitoes identified as *U.* (*Pfc.*) *mashonaensis* and *U.* (*Pfc.*) *montana* were observed feeding on *Scl. gutturalis in situ* (Fig. 5 A–D). Unfortunately, although species of *Sergentomyia* were collected from localities with infected *Pty. anchietae*, observations were rare, and none were collected from infected hosts. In contrast several *U.* (*Pfc.*) *mashonaensis* and *U.* (*Pfc.*) *montana* were collected from *Scl. gutturalis*

parasitised with *D. kermiti* n. sp.

A total of 68 mosquitoes were collected, of which 15 fed on a parasitised *Scl. gutturalis in situ* (parasitaemia 0.2 ± 0.2 (0.1–0.5) %). Intraerythrocytic and extracellular stages of *D. kermiti* n. sp. were seen in smears made from *Uranotaenia* (*Pfc.*) *mashonaensis* and *U.* (*Pfc.*) *montana* between 1 and 7 dpi. After one dpi, several stages were observed in the gut contents of *U.* (*Pfc.*) *mashonaensis* and *U.* (*Pfc.*) *montana*. Probable intra- and extracellular meront stages of *D. kermiti* n. sp. were observed with undigested erythrocytes (Fig. 6 A–B). Stages were elongated to oval, and in most cases, vacuoles were present (Fig. 6 A-B arrow). Nuclei chromatin stained deep magenta (Fig. 6 B arrowhead).

Within one dpi, free gamonts coupled in syzygy in the gut contents of the mosquitoes (Fig. 6 C–D). Merging of gametes was visible, with nuclei fusing into a large nucleus staining light pink (Fig. 6 D arrow), with condensed chromatin, most likely from the different gametes, staining deep magenta (Fig. 6 D arrowhead). Cytoplasm vacuolated and staining dark purple. Immature ookinetes measure 10.4 ± 0.2 (10.2–10.5) long × 7.9 ± 1.3 (7.0–8.9) wide (n = 2). Subsequently, round ookinetes were formed, with a vacuolated cytoplasm staining dark purple (Fig. 6 E). Nucleus staining light pink (Fig. 6 E arrow) with condensed chromatin staining deep magenta (Fig. 6 E arrowhead). Ookinetes measured 11.6 ± 2.9 (8.5–15.9) long × 9.5 ± 4.0 (7.6–12.4) wide (n = 7), and developed into slightly larger immature oocysts (Fig. 6 F). Immature oocysts with a vacuolated cytoplasm staining dark purple, clearly visible nucleus staining light pink (Fig. 6 F arrow). Immature oocysts measure 16.2 long × 15.9 wide (n = 1).

Sporogony leading to the formation of at least six sporozoites was observed in the gut or haemocoel of the mosquito smears from six dpi (Fig. 6 G). Several free sporozoites were also observed at six dpi (Fig. 6 G). Free sporozoites elongate and broad; cytoplasm staining light purple, more or less with a centrally placed nucleus staining deep magenta (Fig. 6 H). One or two distinct vacuoles occurring either anterior or posterior to the nucleus, as well as deep-staining granules of various sizes. These sporozoites measure 12.7 ± 1.3 (10.5–14.2) long × 1.6 ± 0.2 (1.4–2.0) wide (n = 9). Also observed in the mosquitoes' gut or haemocoel was the remains of a probable meront producing roughly ten immature merozoites (Fig. 6 J), similar in shape and size to the secondary merogonic merozoites that occur in the peripheral blood of the vertebrate anuran host. Merozoites (Fig. 6 J arrow) elongate to ovoid, hyaline cytoplasm staining purple. Small round dense nuclei located closer to the centre, chromatin staining dark purple (Fig. 6 J arrowhead). Merozoites measure 5.9 ± 0.2 (5.6–6.2) long × 1.5 ± 0.1 (1.4–1.8) wide (n = 5).

In the gut or haemocoel of the mosquitoes, we observed a probable meront, producing approximately ten long and slender mature merozoites (Fig. 6 K). Mature merozoites similar in shape and size to extracellular gamont forms that occur in the vertebrate host. These forms are elongate often with a slight curvature, cytoplasm granulated staining dark purple. Nucleus visible, slightly off centre, staining light pink (Fig. 6 K arrow), in some cases condensed visible chromatin staining deep magenta (Fig. 6 K arrowhead). Mature merozoites measure 8.7 ± 3.0 (5.7–12.7) long × 3.0 ± 0.5 (2.2–3.8) wide (n = 9).

4. Discussion

In the present study, a total of 643 anurans were collected and screened for the presence of dactylosomatids. Out of 38 species from 20 genera of 13 families, only three species of three genera and three families were found infected. Morphological and molecular data indicate that the dactylosomatid found parasitising *Pty. anchietae* and *Scl. gutturalis* from South Africa represents a new species, *D. kermiti* n. sp.

The species of *Dactylosoma* found parasitising a single *Pel. lessonae* from Belgium, possesses several phenotypic characters of the synonymised *D. splendens*, originally described form *Pel.* kl. *esculentus* from Paris, France (Labbé, 1894). However, based on the data from the current study this species cannot be classified to species level.

4.1. Parasite prevalence and host association

Interestingly *D. kermiti* n. sp. had a prevalence of 38% and 12% in the frog and toad hosts, respectively *Pty. anchietae* and *Scl. gutturalis*. The distribution range of *D. kermiti* n. sp. in *Pty. anchietae* was also larger as compared to infected *Scl. gutturalis* that were found infected only from Sodwana Bay (Supplementary data S1). Frogs belonging to *Ptychadena*, are known to harbour a range of different parasites, which could be due to their ability to populate different habitat types successfully, and to their feeding habits and behaviour (Du Preez and Kok, 1992). A higher prevalence of different blood parasites is also reported from *Pty. anchietae* as compared to other species within the study area (Netherlands et al., 2015). In the present study, the prevalence of *Dactylosoma* was considerably higher as compared to the results of the anuran blood parasite survey of the same locality by Netherlands et al. (2015) in which 16.7% (13/78) of the *Pty. anchietae* screened were reported infected. These findings could be due to the severe droughts experienced in South Africa between 2015 and 2017 (Archer et al., 2017), affecting potential water-borne invertebrate vector numbers.

Parasitaemia of representatives of genera such as *Dactylosoma* may be maintained or even potentially increase within the host without re-infection (due to their development within the vertebrate host; that is the parasite's ability to multiply asexually in the peripheral blood), thus out-competing representatives of other blood parasite genera such as *Hepatozoon*, for which some species may need re-infection. This may be a possible explanation for the differences compared to the observations made by Netherlands et al. (2015), in which species of *Hepatozoon* were found to be the most prevalent blood parasite at the time, parasitising 39.7% (31/78) of the *Pty. anchietae* screened. These findings were in contrast to a more recent survey by Netherlands (2019) in the same area and on the same host, with species of *Dactylosoma* recorded to have a higher overall prevalence than species of *Hepatozoon*. Indeed, certain haemogregarines such as *Hepatozoon ixoxo* Netherlands et al. (2014), seem to be able to maintain a constant and high parasitaemia over long periods without re-infection (Netherlands et al., 2014). According to Conradie et al. (2017), this could be due to the dense capsule surrounding this species. Thus species of *Hepatozoon* which are not encapsulated, like the species parasitising *Pty. anchietae* and *Hepatozoon theileri* (Laveran, 1905), may not be as 'dormant' or 'maintain stable infection levels' as *H. ixoxo* (see Conradie et al., 2017). However, the longevity of representatives of an infrapopulation of blood parasites, without re-infection, needs to be tested experimentally before conclusions can be made with certainty.

4.2. Phylogenetic analysis

The 18S rRNA gene is a well-used marker for a variety of apicomplexan parasites with large datasets available for members of Adeleorina. This gene contains conserved regions for a wide range of organisms, with scattered variable regions providing sufficient evolutionary information to infer close and distant phylogenetic relationships (Medlin et al., 1988). Nonetheless, the 18S rRNA gene has been shown to possess limited resolution between closely related species within Adeleorina (Barta et al., 2012). Thus, while this gene provides sufficient information on the phylogenetic relationships between different genera, it is less informative for species differentiation. Although closely related haemogregarines such as *H. clamatae* and *H. catesbianae* that have an interspecific divergence below 0.5% based on the 18S rRNA gene were confirmed as separate species based on comparison of the internal transcribed spacer 1 (ITS-1) region (Boulianne et al., 2007). On the other hand, mitochondrial DNA has been shown to provide better resolution for species-level diagnostics and molecular phylogenetics of the intestinal eimeriid coccidia and would be a good alternative for future studies (Ogedengbe et al., 2011).

As expected the species of *Dactylosoma* from the present study and *D. ranarum* [GenBank: HQ224957] formed a well-supported clade,

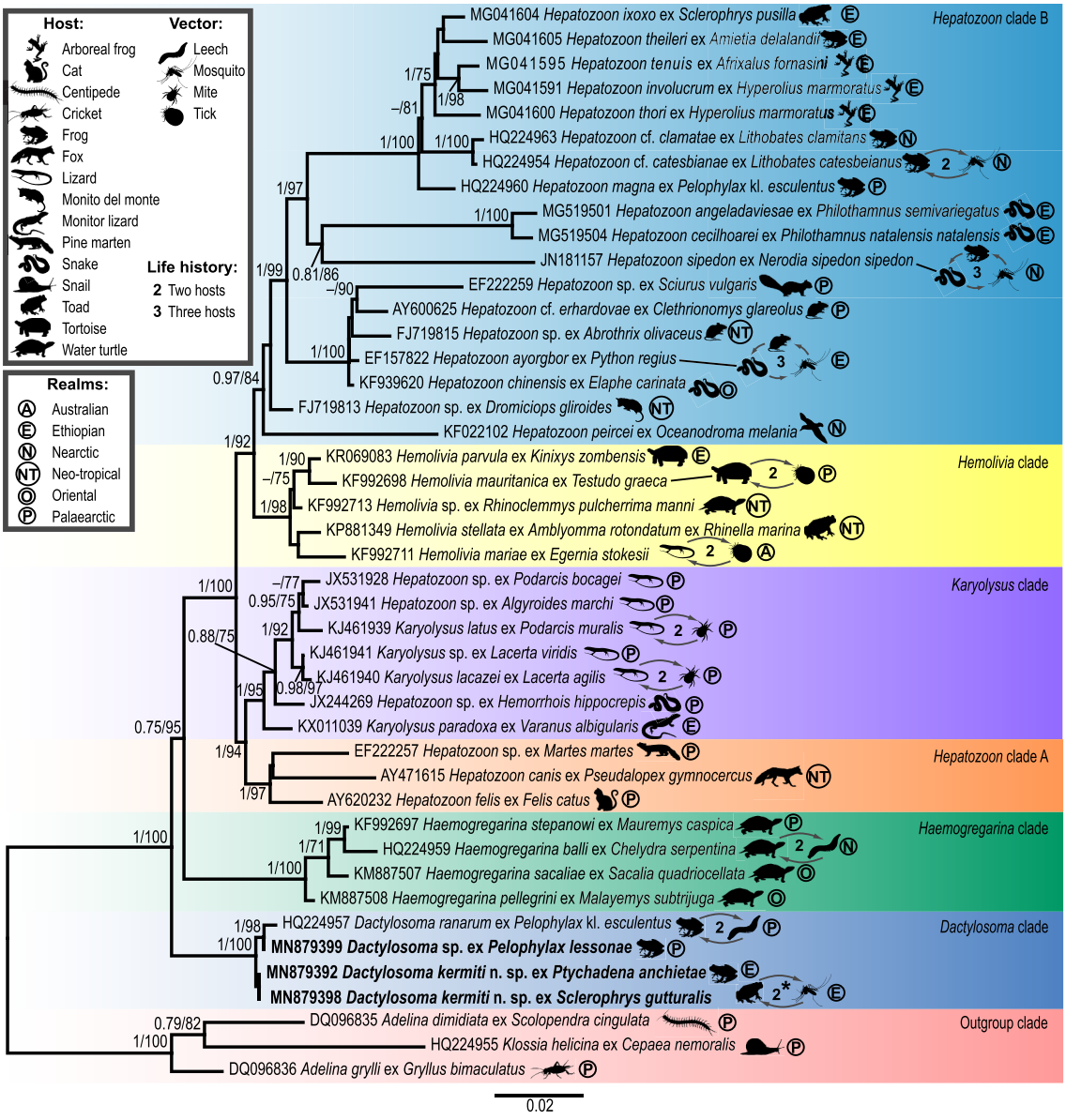

Fig. 4. Consensus phylogram of haemogregarines based on 18S rDNA sequences. Tree topologies for Bayesian inference (BI) and Maximum likelihood (ML) analyses were similar (represented on the ML tree), showing the phylogenetic relationships for *D. kermiti* n. sp. and *Dactylosoma* sp. ex *Pel. lessonae* (represented in bold), compared to other species of *Haemogregarina*, *Hepatozoon*, *Karyolysus*, *Hemolivia*, and *Adelina* and *Klossia* as outgroup. Clades that neither produced 0.80 posterior probability (BI) or 70 bootstrap (ML) nodal support values were omitted. The scale bar represents 0.02 nucleotide substitutions per site. The host, geographical distribution (according to the zoogeographical realms), and if known the vector and life history cycle are also provided for the different sequences using symbols and pictograms. Asterisks (*) indicate the proposed life history strategy of *D. kermiti* n. sp. based on data from the current study.

distinct from the other genera of which sequences were available (Fig. 4). These three species can be separated based on specific phenotypic characters and the conservative 18S rRNA marker. Model-corrected interspecific divergence ranges between 0.2 and 0.5% (Supplementary data S2). Although the interspecific divergence value within other haemogregarine genera (*Haemogregarina*, *Karyolysus*, *Hemolivia*, and *Hepatozoon*) is low (1% and below), based on the sequence comparisons of the conservative 18S rRNA gene, it seems as if interspecific divergence between anuran dactylosomatids is particularly low. Hence, increased sampling of species of *Dactylosoma*, especially from fish hosts, will be an interesting comparison, confirming if this trend of low interspecific divergence based on the 18S rRNA gene is consistent among all dactylosomatids or only those from anuran hosts. Also apparent from the phylogenetic analysis is the polyphyletic placement of different species of *Hepatozoon*. Apart from in *Hepatozoon* clade A and clade B, species of *Hepatozoon* were also observed in the *Karyolysus* clade. The latter species were most likely misidentified as belonging to *Hepatozoon*. A new genus *Bartazoon* Karadjian, Chavatte and Landau (2015), was proposed for species belonging to *Hepatozoon* clade B. The new genus was suggested for species transmitted solely by haematophagous insects and to resolve the polyphyletic placement of the genus *Hepatozoon* (Karadjian et al., 2015). However, as pointed out by Maia et al. (2016), the type species of the genus, *Hepatozoon perniciosum* Miller, 1908 may belong to the newly proposed genus *Bartazoon*, as other rodent haemogregarine species do. Thus until molecular data from the type species is available for comparison, it is suggested to rather continue to refer to species from *Hepatozoon* clade B as *Hepatozoon* as opposed to *Bartazoon* (see Maia et al., 2016).

4.3. Life history and vector identification

Prior to the availability of molecular data for haemogregarines,

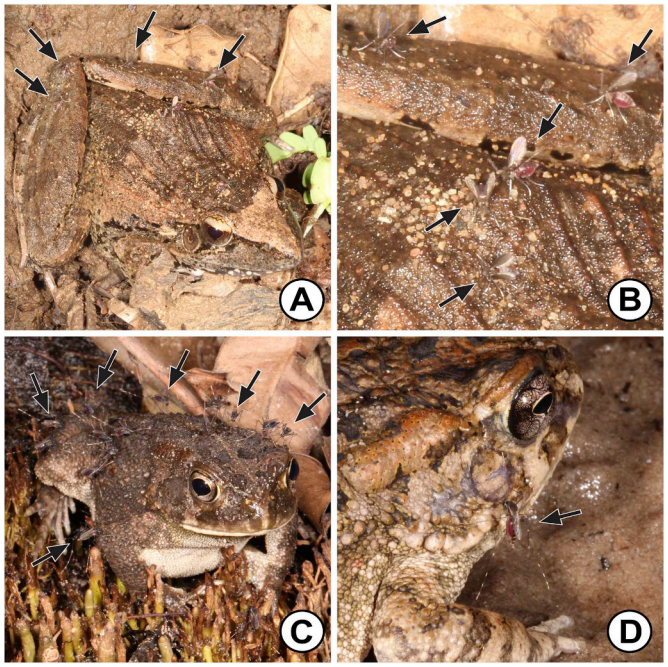

Fig. 5. (A-D). dipterans observed feeding on *Ptychadena anchietae* and *Sclerophrys gutturalis in situ.* (A–B). African phlebotomine sand flies (arrows) *Sergentomyia* sp. feeding on *Ptychadena anchietae in situ.* (C–D) Mosquitoes (arrows), *Uranotaenia* (*Pseudoficalbia*) *mashonaensis* and *U.* (*Pfc.*) *montana* feeding on *Sclerophrys gutturalis in situ.*

microscopic observations of peripheral blood stages, ultrastructural studies of different stages, and life cycle data were the primary methods to classify and distinguish between different haemogregarines (Boulard et al., 1982; Barta and Desser, 1986; Barta, 1989, 1991). Currently, life cycle elucidation via the natural vectors is limited to only two species, *B. mariae* and *B. stableri* (Barta and Desser, 1989; Negm-Eldin, 1998). The only attempt to elucidate the life cycle of a species of *Dactylosoma* was when Barta (1991) used the natural leech vector of *B. stableri, Des. picta*, to transmit *D. ranarum* from infected frogs from Corsica, France. Due to these observations and the life cycle data available for members of Dactylosomatidae, these parasites were considered to be transmitted to new hosts only through leech vectors (Barta, 1991; Negm-Eldin, 1998).

In southern Africa, three species of leeches are known to be haematophagous on amphibians, namely *Oosthuizobdella stuhlmanni* (Blanchard, 1897), *Marsupiobdella africana* Goddard & Malan, 1912 and *Hirudo michaelseni* Augener, 1936. Of these *leeches, M. africana* is the smallest (12 mm) and is reported to feed on species of *Xenopus* exclusively. However, this species also exhibits a phoretic interaction with freshwater crabs (Badets and Du Preez, 2014). *Oosthuizobdella stuhlmanni* is the second largest (33 mm) species, reported feeding on various anurans. The largest is *H. michaelseni* (65 mm), which is not host-specific and feeds on amphibians, but also on mammals and fish, with immature leeches feeding on freshwater snails. In the present study, anurans were examined for the presence of leeches, however no leeches were detected. Although it is likely that leech species haematophagous on amphibians are found in northern KZN, it would appear that they are not typical and do not act as the vector of anuran blood parasites from this area. Considering the proportion of infected anurans (from this area) parasitised with species of *Dactylosoma* and *Trypanosoma* Gruby, 1843 (Netherlands et al., 2015; Netherlands, 2019), which are commonly transmitted by leech vectors (Bardsley and Harmsen, 1973; Rhoden and Bolek, 2012), these findings support the role of other possible vectors, such as dipterans, in the transmission of these parasites.

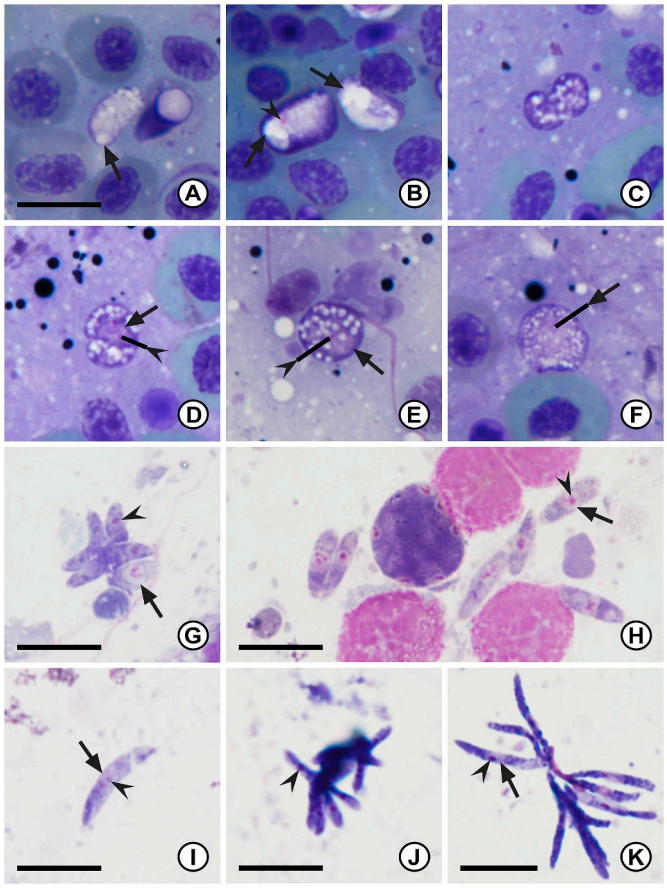

Fig. 6. (A–K). Possible development of *Dactylosoma kermiti* n. sp. in the gut or haemocoel from the mosquitoes *Uranotaenia* (*Pseudoficalbia*) *mashonaensis* and *U.* (*Pfc.*) *montana*, from infected *Sclerophrys gutturalis.* (A) Intracellular meront. (B) Intra- and extracellular meront. (C–D) Merging of gametes. (E) Ookinete. (F) Immature oocyst. (G–I) Free sporozoites. (J) Probable meront producing immature merozoites. (K) Probable meront, producing long and slender mature merozoites. Vacuoles – arrow (A–B); Nucleus – arrow (D–K); Condensed chromatin – arrowhead (B, D–K). Scale bars 10 μm.

Phlebotomine sand flies are other haematophagous invertebrates that are known to transmit blood parasites and have been reported as the vectors for species of *Trypanosoma*, *Leishmania* (Borovsky 1898) and even microfilarial worms (Feng and Chung, 1940; Desportes, 1942). In Africa, phlebotomines reported to feed on amphibian hosts are species of *Sergentomyia* (Franca and Parrot, 1920) and *Grassomyia* Theodor 1958 (see Krüger, 2015; Kvifte and Wagner, 2017). Species of *Sergentomyia* are the most widespread and diverse group of Phlebotominae in Africa. Although they are considered to primarily feed on reptiles (Kvifte and Wagner, 2017), in the present study, species of *Sergentomyia* were observed feeding on *Pty. anchietae* and *Scl. gutturalis*. Although none of the species of *Sergentomyia* collected took blood meals from infected hosts, the localities from where these sand flies were collected did have *Pty. anchietae* parasitised with *Dactylosoma kermiti* n. sp. Thus, even though no direct link was made between species of *Sergentomyia* collected in the current study and *Dactylosoma kermiti* n. sp., these dipterans remain a possible vector for this parasite.

Mosquitoes are known to be the vectors of amphibian haemogregarines, particularly for representatives of *Hepatozoon* Miller, 1908 (Desser et al., 1995; Smith, 1996). Even though no mosquitoes have been reported as potential vectors for representatives of *Dactylosoma*, the mosquito *C. nubeculosus* was experimentally tested by Boulard et al. (1982) as the vector for *D. ranarum* without success. *Uranotaenia* Lynch Arribalzaga 1891 is relatively species-rich with approximately 256 species occurring in nearly all zoogeographical regions (Coetzee, 2017).

Several members of the genus are known to feed selectively on amphibians (Cupp et al., 2004), with some species reported being attracted by the calls of their host species (Borkent and Belton, 2006; Camp et al., 2018). In the current study several *U.* (*Pfc.*) *mashonaensis* and *U.* (*Pfc.*) *montana* were collected from a calling *Scl. gutturalis* parasitised with *D. kermiti* n. sp. *in situ*. To determine if these mosquito species could serve as potential vectors of this parasite, collected blood-fed mosquitoes were kept alive to be screened for any form of development of *Dactylosoma kermiti* n. sp.

In only one dpi, several stages of development, from gamonts coupled in syzygy to possible immature oocysts, were observed in the gut contents of the screened mosquitoes. At six dpi several possible sporozoites and merozoites stages, and even a probable meront were observed in mosquito gut smears. Although this was only a preliminary observation with minimal data, the fact that development of a haemogregarine, most likely *Dactylosoma kermiti* n. sp., was reported in these hosts also suggests that dactylosomatids may not solely be transmitted by leech vectors.

Furthermore, it is interesting that stages of development were only found in the gut contents or haemocoel of infected mosquitoes. This possibly indicates that *Dactylosoma kermiti* n. sp. is transmitted through ingestion (new host consuming infected vector) rather than through bite by an infected vector. This is similar as for *Haemogregarina bigemina* Laveran and Mesnil, 1901, which is probably transmitted via the ingestion of infected gnathiids by fishes, rather than through feeding of the gnathiid on fish hosts (Davies and Smit, 2001). If this is the case, then the hypothesis that the closely related families Haemogregarinidae and Dactylosomatidae evolved to be transmitted via the bite of their definitive vectors is challenged (see Barta et al., 2012). However, resistant and thick-walled oocysts that are present in species of *Hepatozoon* that are known to be transmitted through ingestion, were not observed in the examined mosquitoes. Thus, although these mosquitoes are most likely the vectors of this dactylosomatid parasite, based on the development of different stages and the lack of other haematophagous invertebrates found feeding on *Scl. gutturalis* from this locality, more data on the process and site of development is required before any definite conclusions can be made on the life history of *Dactylosoma kermiti* n. sp.

Also if mosquitoes are the vectors of *Dactylosoma kermiti* n. sp., then its phylogenetic placement with *D. ranarum* (possibly leech-transmitted) based on the 18S rRNA gene contradicts the hypothesis that these parasites adapted within their invertebrate hosts to become vector transmitted rather than evolving with the vertebrate host (see Barta et al., 2012; O'Donoghue, 2017).

4.4. Concluding remarks

This study highlights the importance of screening and comparing anurans from different genera and even families, to not only increase the known biodiversity of these parasites, but also the range of hosts that they infect. The importance of using different techniques in the description of new species or identification of already existing species is also emphasised. Only *D. kermiti* n. sp. was found parasitising anurans from South Africa, whereas an unidentified species of *Dactylosoma* sp. isolated from *Pel. lessonae* (from the current study) and *D. ranarum* were found parasitising European anurans.

Future work should include the use of faster evolving markers to help increase phylogenetic resolution within *Dactylosoma*. Furthermore, more rigorous life-history experimental work, testing the role of *U.* (*Pfc.*) *mashonaensis* and *U.* (*Pfc.*) *montana* in the transmission development of *D. kermiti* n. sp. to *Scl. gutturalis*, should be done. These studies may even be extended to determining the possibility of phlebotomine sand flies, specifically belonging to *Sergentomyia*, as potential vectors.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.12.006.

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