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Biodiversity of frog haemoparasites from sub-tropical northern KwaZulu-Natal, South Africa

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ABSTRACT

Since South Africa boasts a high biodiversity of frog species, a multispecies haemoparasite survey was conducted by screening the blood from 29 species and 436 individual frogs. Frogs were collected at three localities in sub-tropical KwaZulu-Natal, a hotspot for frog diversity. Twenty per cent of the frogs were infected with at least one of five groups of parasites recorded. Intraerythrocytic parasites comprising *Hepatozoon*, *Dactylosoma*, and viral or bacterial organisms, as well as extracellular parasites including trypanosomes and microfilarid nematodes were found. A significant difference ($P < 0.01$) in the prevalence of parasitaemia was found across species, those semi-aquatic species demonstrating the highest, followed by semi-terrestrial frog species. None of those species described as purely terrestrial and aquatic were infected. *Hepatozoon* and *Trypanosoma* species accounted for most of the infections, the former demonstrating significant differences in intensity of infection across species, families and habitat types ($P = 0.028$; $P = 0.006$; $P = 0.007$ respectively). Per locality, the first, the formally protected Ndumo Game Reserve, had the highest biodiversity of haemoparasite infections, with all five groups of parasites recorded. The other two sites, that is the area bordering the reserve and the Kwa Nyamazane Conservancy, had a lower diversity with no parasite infections recorded and only *Hepatozoon* species recorded respectively. Such findings could be ascribed to the anthropogenic impact on the latter two sites, the first by the rural village activities, and the second by the bordering commercial sugar cane agriculture. Future studies should include both morphological and molecular descriptions of the above parasites, as well as the identification of potential vectors, possibly clarifying the effects human activities may have on frog haemoparasite life cycles and as such their biodiversity.

1. Introduction

Amphibians are the most threatened vertebrate group, suffering large-scale declines in species diversity since at least, according to historical data, the 1970s (Stuart et al., 2004). A decade ago the IUCN's Global Amphibian Assessment indicated that a third of the estimated amphibian species had declined or become extinct (Stuart et al., 2004; Beebee and Griffiths, 2005). Such declines may be attributed to a number of factors ranging from habitat destruction, pollution and exploitation, to climate change and disease (Beebee and Griffiths, 2005). The disease known as chytridiomycosis (amphibian chytrid), caused by the fungal pathogen *Batrachochytrium dendrobatidis*, has been responsible for major global amphibian declines (Readel and Goldberg, 2010). Along with chytrid, amphibians are host to a wide variety of parasites (du Preez and Carruthers, 2009; Netherlands et al., 2014a), including intraerythrocytic and extracellular haemoparasites ranging from protozoans, comprising both intracellular apicomplexans (Davies and Johnston, 2000) and extracellular flagellates (Acosta et al., 2013), to extracellular nematode microfilariae (Baker, 2008) as well as those intracellular parasites of uncertain identity such as the viral and bacterial infections (Davies and Johnston, 2000; Davis et al., 2009). The most attention, however, has been given to those parasites of the first three groups mentioned, most likely due to the frequent findings and thus greater basis of knowledge of these organisms in anuran hosts. Furthermore, of these three groups, those of the Protozoa, particularly the apicomplexans, would appear to be the most studied of all (see Davies and Johnston, 2000; Netherlands et al., 2014a; Netherlands et al., 2014b).

However, since few parasite surveys on frogs have been carried out in sub-Saharan Africa, the degree of this haemoparasite diversity remains unknown (Readel and Goldberg, 2010; Netherlands

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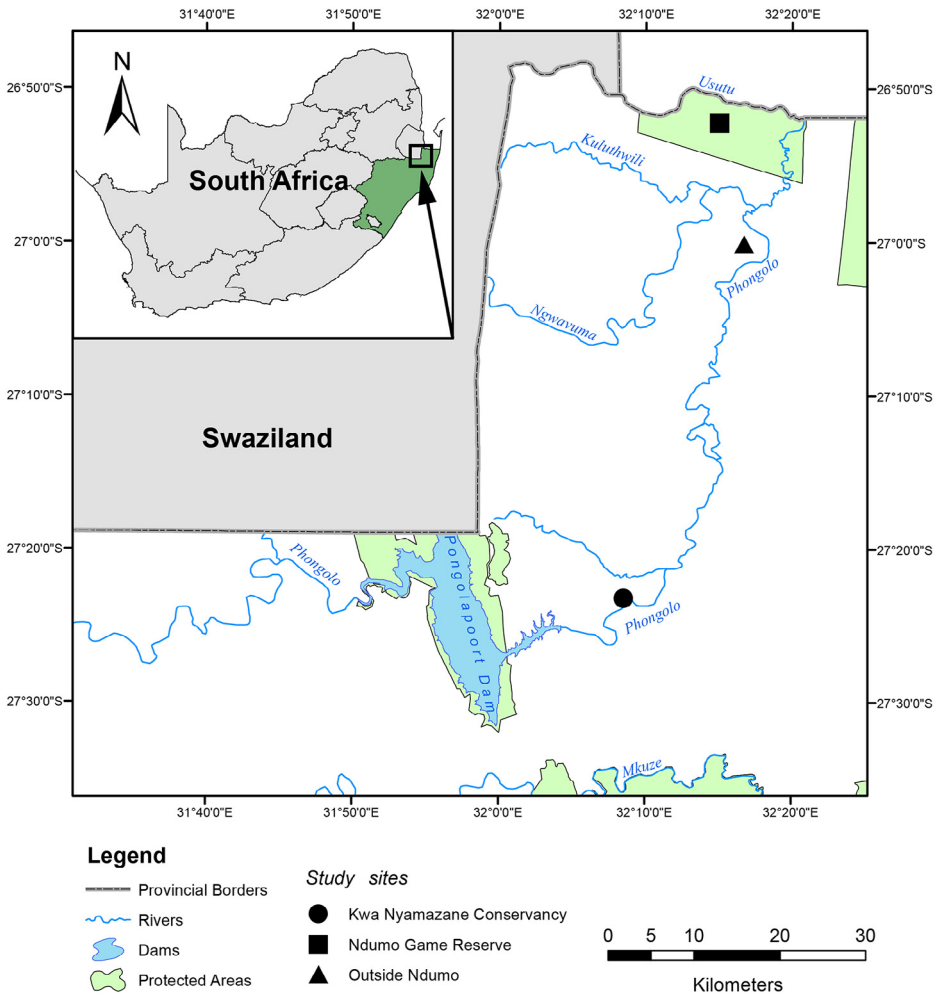

Fig. 1. Map displaying the three sampling localities in northern KwaZulu-Natal, South Africa. Map displaying the three sampling localities at which frogs were surveyed for haemoparasite biodiversity, top to bottom: Ndumo Game Reserve (NGR), outside NGR and Kwa Nyamazane Conservancy, in northern KwaZulu-Natal, South Africa. All sampling sites were directly or indirectly linked to the Phongolo River.

et al., 2014a, 2014b). Yet, such diversity of knowledge regarding these parasites is necessary before further studies can be done on elucidating the effects that these parasites may have on their natural hosts, and the role these parasites may have in amphibian conservation.

Southern Africa currently boasts 159 known species of frogs in 33 genera and 13 families (du Preez and Carruthers, 2009; Channing and Baptista, 2013; Channing et al., 2013a, 2013b; Conradie, 2014). This study presents the results of a haemoparasite survey of frogs from three localities in KwaZulu-Natal, South Africa (see Fig. 1). Localities include the formally protected Ndumo Game Reserve (NGR) and the reserve's anthropogenically impacted surrounds, as well as Kwa Nyamazane Conservancy (KNC). All three sampling areas fall within a sub-tropical region known for its biological richness and as such the province, KZN, boasts the highest diversity of frog species in South Africa (see du Preez and Carruthers, 2009). The following study thus aimed to determine and record, through a multispecies haemoparasite survey on frogs, if this parasite diversity paralleled that of its rich frog diversity.

2. Materials and methods

2.1. Study area and frog collection

Ndumo Game Reserve (NGR) (26°52′00.0″S 32°15′00.0″E) is situated in the West of the Maputaland bioregion, close to the borders of South Africa, Swaziland and Mozambique. The Maputaland bioregion, located in northern KwaZulu-Natal (KZN) and crossing into southern Mozambique, is one of the most biologically rich areas in southern Africa (Haddad, 2003) and has a sub-tropical climate. Ndumo is a large reserve, holding 10,117 ha of diverse habitats, including floodplains, sub-tropical bush, savannah and woodland, to riparian forest (Wesołowska and Haddad, 2009). The area directly surrounding the NGR (27°00′13.8″S 32°16′49.9″E) is not formally protected and thus is covered in rural tribal villages, causing the vegetation to be heavily impacted by the villagers' livestock and subsistence farming practices. Approximately 80 km to the south lies the Kwa Nyamazane Conservancy (KNC) (27°23′34.9″S 32°08′40.8″E), a small conservation area running along the Phongola River and surrounded by large sects of agricultural land, most of it utilised for sugar cane farming. These localities were specifically chosen as all three are located on, and are thus supplied by a permanent water source, the Phongola River (see Fig. 1).

Frogs were collected via active sampling at night in all three localities as mentioned above. All these sites were visited during the warmer and rainy months of February and November 2012, April and November 2013 and February 2014. During collection possible invertebrate vectors feeding on frogs were searched for; however none were observed. Captured frogs were held in disposable plastic bags and transported back to a field laboratory either at the NGR

or KNC, where they were identified to species level using du Preez and Carruthers (2009).

2.2. Preparation and screening of frog blood

A drop of blood was collected from each frog via cardiac or femoral venipuncture using a sterile heparinised insulin syringe. A portion of this blood was used to prepare a thin blood smear, which once air-dried in a dust-proof container was fixed immediately using absolute methanol and stained thereafter using a modified solution of Giemsa stain (FLUKA, Sigma-Aldrich, Steinheim, Germany); the other portion was dropped into a microcentrifuge tube with an equal volume of 70% ethanol for future molecular analysis. All frogs were processed the morning after collection and were released within 24 h of capture.

Smears were screened using a 100 × immersion oil objective on a Nikon Eclipse E800 compound microscope (Nikon, Amsterdam, Netherlands), and images were captured with an attached Nikon digital camera. The average parasitaemia was calculated per 100 erythrocytes, with ~10^4 erythrocytes examined per blood smear following Cook et al. (2009). The estimated average parasitaemia for extracellular parasites were calculated as number of parasites/per-slide (ps) with an approximate field of 20,000 blood cells examined. This study received the relevant ethical approval (North-West University ethics approval no.: NWU-00005–14-S3), as well as approval to do research from the appropriate conservation authorities (Ezemvelo KZN Wildlife, permits: OP 674/2012, OP 5139/2012, OP 526/2014, and OP 839/2014.).

2.3. Statistical analysis

The Monte Carlo variant of the Fisher's exact test, set to 10,000 replicates with a confidence interval of 99%, was employed to investigate significance in variation of prevalence between species, families, habitat types and sampling periods. The habitat types were established based on those described by du Preez and Carruthers (2009). Frog species were classified as terrestrial (those species thriving and breeding away from a permanent water source for most of their lives), semi-terrestrial (species thriving away from a permanent water source, but needing such a source to breed), semi-aquatic (species requiring a position near a permanent water source for most of their lives in order to survive and breed) and aquatic (species permanently living and breeding in a water source, rarely leaving that source). The Kruskal–Wallis test was used since it is suitable for non-parametric data and does not assume normal data distribution and equal sample size. It was applied to determine significance levels ($P < 0.05$) of variation between infection intensity across species, families, habitat types, and sampling periods. It was further employed to determine significance of variation of the overall intensity of *Hepatozoon* and *Trypanosoma* across frog species, families, habitats and sampling periods. A non-parametric Levenes's test was used to verify the equality of variances in the samples (homogeneity of variance, $P > 0.05$) (Nordstokke and Zumbo, 2007, 2010). All statistical analyses were performed using IBM SPSS Statistics ver. 22 (SPSS, 2013).

3. Results

Blood smears were collected from 436 frogs of 29 species, 6 genera and 11 families (Table 1). Of these 15/29 (52%) of the frog species were infected with haemoparasites, making up 87/436 (20%) of the total number of frogs (Table 2). Five groups of haemoparasites were recorded including intraerythrocytic haemogregarine and haemogregarine-like species of the genus *Hepatozoon* and *Dactylosoma* respectively, and intraerythrocytic organisms of a viral or bacterial nature, the species of which could not be identified; furthermore, extracellular flagellate parasite species of the genus *Trypanosoma* and microfilarid nematode species were observed.

Hepatozoon species accounted for most of the infections at 59/436 (14%), followed by *Trypanosoma* species at 46/436 (11%); viral or bacterial infections, microfilarid infections and *Dactylosoma* species, accounted for 6/436 (1%), 2/436 (0.5%) and 13/436 (3%) of the overall prevalence respectively (Table 2). As for the intensity of the groups, *Hepatozoon* showed an overall (all infected frogs pooled) intensity of 5%, the *Dactylosoma* an overall intensity of 1%, the viral or bacterial infections an overall intensity of 87%, the *Trypanosoma* an overall intensity of 11 per blood slide, and the microfilarid nematode infections an overall intensity of 15 per blood slide (Table 2). The overall prevalence of haemoparasites (all parasite groups pooled) varied significantly by frog species ($\chi^2 = 163.475$, $P < 0.01$). *Ptychadena anchietae* demonstrated the highest prevalence at 47/78 (60%) and *Chiromantis xerampelina* the lowest at 1/44 (2%) (Table 2). Upon division of the frog species into groups including aquatic (two species), semi-aquatic (17 species), terrestrial (one species) and semi-terrestrial (nine species) (see Table 1), it was observed that only the semi-aquatic and semi-terrestrial groups contained infected species (Table 2). These two groups varied significantly in prevalence of infection ($\chi^2 = 87.000$, $P < 0.01$), with 79% of the infected individuals from the semi-aquatic group and only 21% from the semi-terrestrial group. Of the semi-aquatic group, the genus *Ptychadena* had the highest diversity of haemoparasites, infected with all types as recorded in Table 2. Furthermore, *P. anchietae*, of all the infected frog species, revealed the highest prevalence of parasites, making up 47/87 (54%) of the total with 47/78 (60%) of the *P. anchietae* themselves found to be infected.

Hepatozoon species accounted for most of the infections followed by *Trypanosoma* species, significance of intensity calculated via the use of the Kruskal–Wallis test. *Hepatozoon* intensity across frog species ($\chi^2 = 17.683$, $P = 0.028$), across families ($\chi^2 = 11.717$, $P = 0.006$), and across the different habitat types ($\chi^2 = 7.227$, $P = 0.007$) showed a significant difference. *Hyperolius marmoratus*, in the semi-aquatic group, and *Amietophrynus maculatus*, in the semi-terrestrial group, accounted for the highest intensities (Table 2). *Hepatozoon* intensity across the different sampling periods, however, showed no significant variance ($\chi^2 = 4.177$, $P = 0.552$). *Trypanosoma* intensity across frog species ($\chi^2 = 11.919$, $P = 0.028$) showed a significant difference; however, across families ($\chi^2 = 3.802$, $P = 0.664$), habitat types ($\chi^2 = 0.330$, $P = 0.585$) and sampling periods ($\chi^2 = 6.675$, $P = 0.147$), no significant difference was observed. In this case *Hyperolius tuberilinguis*, in the semi-aquatic group, and *Chiromantis xerampelina*, in the semi-terrestrial group, accounted for the highest intensities (Table 2).

Per locality, it was observed that the NGR, with 26 species examined, showed a prevalence of 77/360 (21%) as compared with outside the NGR, with eight species examined and a prevalence of 0/54 (0%), and the KNC, with seven species examined and a prevalence of 10/22 (45%). Furthermore, the NGR had a higher diversity of haemoparasites, including 50/360 (14%) infected with *Hepatozoon* species, 11/360 (3%) with *Dactylosoma* species, 5/360 (1%) with viral or bacterial organisms, 46/360 (13%) with *Trypanosoma* species and 2/360 (0.6%) with microfilaria as compared with the KNC frogs that were only infected with *Hepatozoon*.

4. Discussion

On the whole, 20% (87/436) of the frogs in this study were infected with at least one haemoparasite group, some infected up to five. This was similar to previous comparable studies such as that of Readel and Goldberg (2010) in western Uganda documenting a 17% (30/180) prevalence, even though this was found to be approximately half that of other studies in Africa (see Mohammed and Mansour, 1959; Ball, 1967; Readel and Goldberg, 2010). In this study,

Table 1
Frog species divided into associated habitat types and listed alphabetically with families, as well as numbers collected at Ndumo Game Reserve (NGR), the locality bordering NGR (BNGR) and the Kwa Nyamazane Conservancy (KNC).

Habitat type	Frog species	Family	Locality	No. collected
Terrestrial (1)	*Breviceps adspersus*	Breviciptidae	NGR	4
Semi-terrestrial (9)	*Amietophrynus garmani*	Bufonidae	NGR	23
			BNGR	2
			KNC	5
	Amietophrynus gutturalis	Bufonidae	NGR	1
			KNC	3
	Amietophrynus maculatus	Bufonidae	NGR	9
	Chiromantis xerampelina	Rhacophoridae	NGR	43
			BNGR	1
	Leptopelis mossambicus	Arthroleptidae	NGR	2
	Schismaderma carens	Bufonidae	NGR	7
	Tomopterna cryptotis	Pyxicephalidae	NGR	6
	Tomopterna krugerensis	Pyxicephalidae	NGR	1
	Tomopterna natalensis	Pyxicephalidae	NGR	1
Semi-aquatic (17)	*Afrixalus aureus*	Hyperoliidae	NGR	14
	Afrixalus delicatus	Hyperoliidae	NGR	7
	Afrixalus fornasinii	Hyperoliidae	NGR	2
	Cacosternum boettgeri	Pyxicephalidae	BNGR	11
			KNC	1
	Hemisus marmoratus	Hemisotidae	NGR	22
	Hildebrandtia ornata	Ptychadenidae	NGR	6
	Hyperolius argus	Hyperoliidae	BNGR	24
	Hyperolius marmoratus	Hyperoliidae	NGR	20
			BNGR	10
			KNC	6
	Hyperolius pusillus	Hyperoliidae	NGR	10
			BNGR	1
			KNC	3
	Hyperolius tuberilinguis	Hyperoliidae	NGR	12
	Kassina maculata	Hyperoliidae	NGR	8
	Kassina senegalensis	Hyperoliidae	KNC	3
	Phrynobatrachus mababiensis	Phrynobatrachidae	NGR	13
	Phrynomantis bifasciatus	Microhylidae	NGR	1
	Ptychadena anchietae	Ptychadenidae	NGR	77
			KNC	1
	Ptychadena mascareniensis	Ptychadenidae	NGR	5
			BNGR	2
	Ptychadena mossambica	Ptychadenidae	NGR	19
Aquatic (2)	*Xenopus laevis*	Pipidae	NGR	1
	Xenopus muelleri	Pipidae	NGR	46
			BNGR	3
Total	29	11	3	436

Hepatozoon species accounted for most of the infections at 14%, which was equally true for the survey done in Uganda by Readel and Goldberg (2010). On the contrary, Ball (1967), during a survey completed in Tanzania and Kenya, found a considerably higher prevalence of 29%. Readel and Goldberg (2010) suggested this may be attributable to availability of insect vectors. Trypanosome species were the second most common parasite infecting frogs in this study at 11%, just slightly higher than the 6% reported by Readel and Goldberg (2010). Both the results of the present and the Readel and Goldberg (2010) studies' conducted in Africa are in contrast to what has been recorded in other similar studies but on different continents (see Barta and Desser, 1984; Barta et al., 1989), in which the *Trypanosoma* demonstrate a higher prevalence to that of *Hepatozoon* or any other haemoparasite groups (see Werner, 1993). Microfilarid infections from the South African frogs studied here, were also seen to occur in low numbers, similar to Readel and Goldberg (2010). Infections not reported by Readel and Goldberg (2010) and Ball (1967), but reported in this study, were a *Dactylosoma* species and viral or bacterial infections. The only other study in Africa to report on parasite intensities was that by Readel and Goldberg (2010), in which *Hepatozoon* species contained an average intensity of 2.3%, *Trypanosoma* species had an average intensity of 7.2 parasites, and Microfilariae had an average intensity of 11.2 parasites. In comparison the total parasite intensity for the current study was 5.3% for *Hepatozoon* species, 10.6 for *Trypanosoma* and 14.5 for Microfilariae.

Similar to Readel and Goldberg (2010) significant differences ($P < 0.01$) in the prevalence of parasites among frog species were recorded during the current study, with *P. anchietae* showing the highest prevalence and *C. xerampelina* the lowest. Both frog species prefer habitats close to water (classified in this study as being semi-terrestrial) (du Preez and Carruthers, 2009). *Ptychadena anchietae* is a grass frog and is often found around the water's edge whilst *C. xerampelina* is an arboreal frog species. Since the abundance of possible vectors associated with water, such as mosquitoes and leeches, would be high in such habitats, it may explain the high prevalence of haemoparasites recorded from *P. anchietae*. The reason for such a low prevalence in *C. xerampelina*, particularly for *Hepatozoon* species, which may be mosquito transmitted in such an environment (Desser et al., 1995; Davies and Johnston, 2000), cannot be explained. This result is particularly peculiar since both frog species were infected with trypanosomes (only a single individual of *C. xerampelina*), which are mosquito and leech transmitted (Barta and Desser, 1984). One of the only possibilities could be, since *Hepatozoon* is transmitted via the ingestion of the infected invertebrate or vertebrate (Davies and Johnston, 2000), that *C. xerampelina* prefers a diet not inclusive of mosquitoes and other frogs. Future diet studies may help to clarify this finding.

Division of the frog species into groups showed that only the semi-aquatic and semi-terrestrial groups contained infected species, these two groups varying significantly in prevalence of infection

Table 2
Frog species listed alphabetically and categorised according to their habitat type. Shown are the number of frogs examined and infected, prevalence of the five haemoparasite groups (P) and the intensity of the infections (I), along with the reference to the figures of these parasites (Fig.) in parentheses.

Habitat type	Frog species	Examined	Infected	*Hepatozoon* spp., P; I (Fig.)	*Dactylosoma* spp., P; I (Fig.)	Viral or bacterial organisms, P; I (Fig.)	*Trypanosoma* spp., P; p/s (Fig.)	Microfilariae, P; p/s(Fig.)
Semi-terrestrial	*Amietophrynus garmani*	30	7	5/7; 8.4% (Fig. 2A–B)			2/7; 21 p/s (Fig. 2L–M)	
	Amietophrynus gutturalis	4	3	3/8; 2.4% (Fig. 2A–B)				
	Amietophrynus maculatus	9	7	7/7; 19.5% (Fig. 2A–B)			3/7; 3 p/s (Fig. 2O–P)	
	Chiromantis xerampelina	44	1				1/1;26 p/s (Fig. 2Q)	
Semi-aquatic	*Hemisus marmoratus*	22	2	2/2; 0.8% (Fig. 2B)				
	Hildebrandtia ornata	6	1	1/1; 0.3% (Fig. 2E)				
	Hyperolius marmoratus	36	4	3/4; 20.5% (Fig. 2D)			1/4; 1 p/s (Fig. 2N)	
	Hyperolius tuberilinguis	12	1				1/1; 20 p/s (Fig. 2R)	
	Kassina maculata	8	1				1/1; 4 p/s (Fig. 2M–S)	
	Phrynobatrachus mababiensis	13	1	1/1; 0.3% (Fig. 2B)			1/1; 8 p/s (Fig. 2O)	
	Phrynomantis bifasciatus	1	1				1/1; 6 p/s (Fig. 2O–P)	
	Ptychadena anchietae	78	47	31/47; 2.4% (Fig. 2C)	13/47; 1% (Fig. 2F–G)	4/47; 75% (Fig. 2I)	29/47; 13.3 p/s (Fig. 2L–P,R,T)	1/47; 1 p/s
	Ptychadena mascareniensis	5	2	1/2; 0.2% (Fig. 2B)			2/2; 8.8 p/s (Fig. 2O)	
	Ptychadena mossambica	19	7	5/7; 3% (Fig. 2B)		2/7; 99% (Fig. 2J)	4/7; 6 p/s (Fig. 2O,N,R)	
	Schismaderma carens	7	2	1/2; 0.8% (Fig. 2A–B)				1/2; 28 p/s (Fig. 2K)
Total	15	294	87	59; 5.3%	13; 1%	6; 87%	46; 10.6 p/s	2; 14.5 p/s

Prevalence = P; intensity = I; per slide = p/s (Figure reference = Fig.).

($P < 0.01$). The semi-aquatic group had the highest prevalence, likely attributable to the *Ptychadena* species, one of them *P. anchietae*. The *Ptychadena* species also showed the highest diversity of haemoparasites, infected with all five recorded groups. Furthermore, of all the frog species, *P. anchietae* was the only species to be parasitised with a species of *Dactylosoma*. These parasites are closely associated with water and thus are suggested to be transmitted by a leech vector (Barta, 1991). Reports of *Dactylosoma* parasitising frogs in Africa are numerous, accounts of these organisms from at least five countries and approximately eight species of frog (see Barta, 1991). One such report was from South Africa from the bufonid *A. regularis* (most likely *Amietophrynus gutturalis*) by Fantham et al. (1942). In all these reports the *Dactylosoma* species are referred to as a single species *Dactylosoma ranarum* (see Barta, 1991); however, only future molecular work will be able to clarify if the species here is one of the same. Furthermore, the Ptychadenidae were the only frogs found infected with viral or bacterial organisms. Viral or bacterial infections have been recorded from a cosmopolitan distribution of amphibians (see Desser, 1987). Unfortunately, very little is known about the identity, classification and effect of these organisms (see Desser, 1987; Davies and Johnston, 2000; Davis et al., 2009). Alves de Matos and Paperna (1993) presented the most recent study of uncertain erythrocyte virus infections from *P. anchietae* in South Africa. These virus or bacterial infections were found to be similar to several different viruses of the Frog Erythrocytic Virus (FEV) group such as *Toddia*, *Pirhemocyton* and other Rickettsiales. In contrast to the above findings in both the semi-aquatic and semi-terrestrial groups, the frog species from the terrestrial as well as aquatic groups were not observably parasitaemic. Since *B. adspersus* (terrestrial) spends most of its life underground (du Preez and Carruthers, 2009), contact with vectors would be rare. However, since the semi-aquatic group had the highest prevalence of parasites, most likely due to the frequent contact with vectors, it was expected that those of the aquatic group would be equally parasitised. Yet, as in Readel and Goldberg (2010), the species of *Xenopus* (aquatic) here were found to be uninfected. Such a finding is surprising as it would be expected that *Xenopus* should contain a rather high prevalence of parasites, particularly as it is well known that leeches feed on these frogs (see Badets and Du Preez, 2014; Kruger and Du Preez, 2015). Possible explanations for this could be that haemoparasites infecting *Xenopus* are extremely host specific or were simply not present in the area sampled.

Intensity of *Hepatozoon* species across frog species and families, as well as across habitat types were found to be significant ($P = 0.007$). *Hyperolius marmoratus* (Hyperolidae) of the semi-aquatic group, and *A. maculatus* (Bufonidae) from the semi-terrestrial group, had the highest intensity. *Hyperolius marmoratus* may be found permanently on the edges of water, where vector abundance and contact rates are likely to be high, thus accounting for this high intensity. *Amietophrynus maculatus* appears to favour more static, shallow water bodies, which are also favoured by mosquito species, the high contact rates with possibly *Hepatozoon* infected mosquitoes would thus be high. *Hepatozoon* species have been reported and described from a few *Hyperolius* species in Africa, though the majority have been reported from bufonid species such as *A. maculatus* (see Netherlands et al., 2014b). *Trypanosoma* species intensities varied significantly only across species ($P = 0.028$), being highest in the semi-aquatic *H. tuberilinguis* and the semi-terrestrial *C. xerampelina*. These two species are permanently associated with water, and thus always in close association with an abundance of possible vectors. A plethora of trypanosome species have been described and reported from numerous African frog species, unfortunately many reports contain inadequate taxonomic descriptions and in numerous cases they are simply referred to as a *Trypanosoma* sp. without any morphological data provided on the specific parasite (see Bardsley and Harmsen, 1973; Telford, 2009).

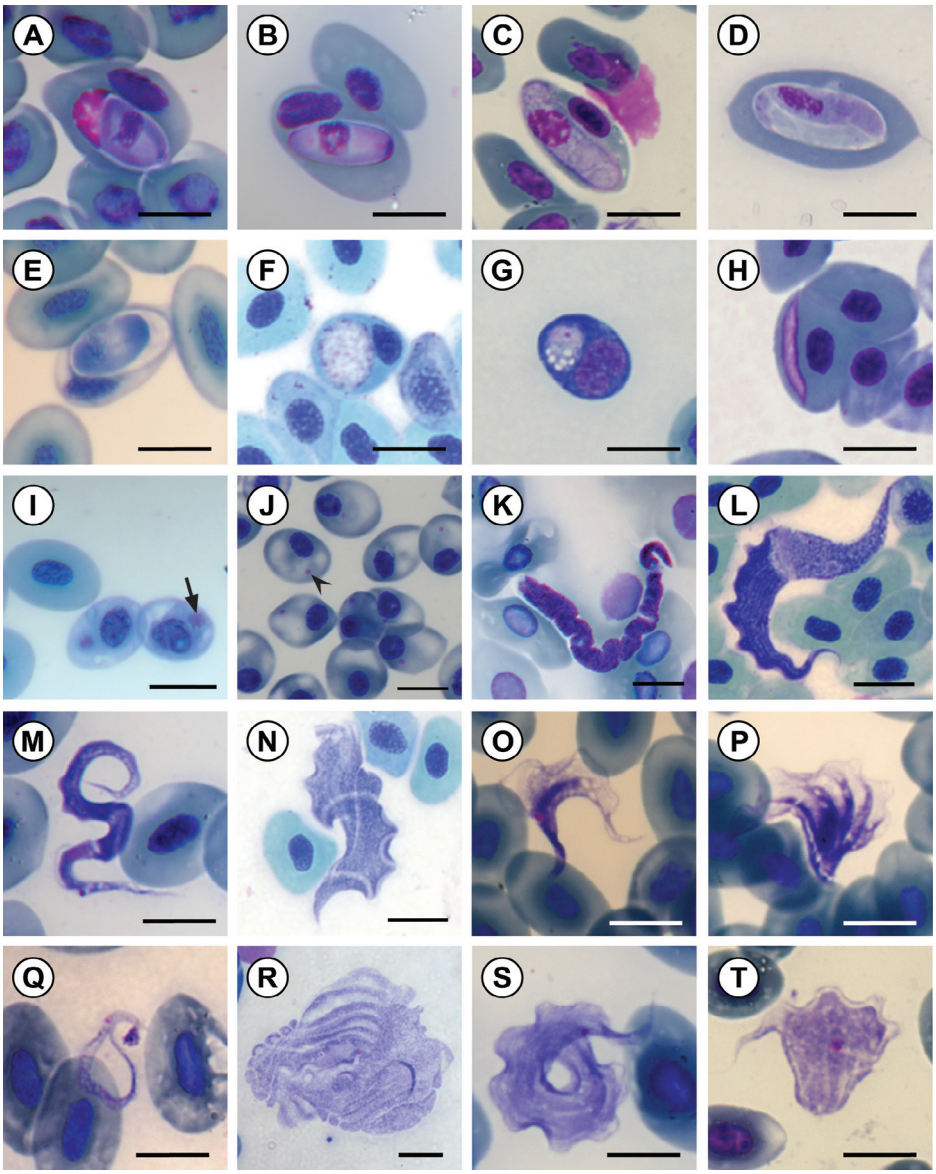

Fig. 2. Micrographs of various frog haemoparasites encountered in the current study. Haemoparasites from the peripheral blood of 15 frog species collected from three localities in northern KwaZulu-Natal, stained with Giemsa stain (A–E) gamonts of Hepatozoon species; (F–G) primary and secondary stage gamonts of Dactylosoma species; (H–J) viral or bacterial inclusions; (K) microfilarid nematode species; (L–T) Trypanosoma species. Scale bar: 10 µm.

Furthermore, since trypanosomes are known to be pleomorphic, the true diversity seen in this study cannot be realised, and thus future molecular work along with morphological description is intended in order to differentiate between species and life stages of these organisms. Intensity across sampling periods for both *Hepatozoon* and *Trypanosoma* species was insignificant ($P = 0.552$ and $P = 0.147$ respectively), likely due to sampling occurring only during the wet seasons.

Parasites have always been seen in a negative light, especially with regards to human and livestock health. However, within the natural environment parasites may be seen as a crucial part of a functional and healthy ecosystem. Parasites make a profound impact on the biodiversity of an ecosystem by influencing aspects such as host competition, migration, speciation and stability (Combes, 1996). Furthermore, parasites reflect their host species' environmental interactions, revealing feeding behaviour, geographical ranges and social systems (Dobson et al., 2008). In a stable and healthy natural ecosystem parasites and their hosts have had the opportunity to co-evolve, the parasite causing few pathogenic effects in a healthy host animal. If however, this well established co-existence is disturbed by, for example, habitat destruction or the indiscriminate movement of host animals between habitats; pathogenic effects may become apparent resulting in the destabilisation of the host population (see Combes, 1996). Additionally, in light of the above, the loss of parasite diversity would very likely have unforeseen, but grave consequences, especially with respect to regulation of host populations and their abundance within the communities (Dobson et al., 2008). As aptly put by Dobson et al. (2008), if the main aim of conservation biologists is to conserve fully functional food webs, it is imperative that parasites are thus also included within biodiversity conservation. Ndumo Game Reserve was found to harbour the highest diversity of both frog species and haemoparasites as compared with the other two sites (see Table 1), which are impacted by rural village settlements and peripheral commercial sugar cane agriculture respectively. Anthropogenic impacts, as found in Readel and Goldberg (2010), may account for the lack of diversity in these

two sites, affecting vector distributions and contact rates between frog hosts and vectors.

This study represents the first multispecies haemoparasite survey done on frogs in South Africa. It is anticipated that through future work, including both morphological and molecular descriptions of the parasites reported in this study, that the biodiversity of this region will be elucidated. Furthermore, it is hoped that with this biodiversity knowledge and the identification of potential vectors, the effects human activities may have on frog haemoparasite life cycles and as such their biodiversity will be clarified.

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Conflict of interest

The authors declared that there is no conflict of interest.

References

Acosta, I.C.L., Costa, A.P., Nunes, P.H., Gondim, M.F.N., Gatti, A., Rossi, J.L., et al., 2013. Morphological and molecular characterization and phylogenetic relationships of a new species of trypanosome in *Tapirus terrestris* (lowland tapir), *Trypanosoma terrestris* sp. nov., from Atlantic Rainforest of southeastern Brazil. Parasit. Vectors. 6, 349.

Alves de Matos, A.P., Paperna, I., 1993. Ultrastructure of erythrocytic virus of the South African anuran *Ptychadena anchietae*. Dis. Aquat. Org. 16, 105–109.

Badets, M., Du Preez, L., 2014. Phoretic interaction between the kangaroo leech *Marsupiobdella africana* (Hirudinea: Glossiphoniidae) and the cape river crab *Potamonautes perlatus* (Decapoda: Potamonautidae). Int. J. Parasitol Parasites Wildl. 3, 6–11.

Baker, D.G., 2008. Flynn's Parasites of Laboratory Animals. John Wiley & Sons, pp. 149–150.

Ball, G.H., 1967. Blood sporozoans from East African Amphibia. J. Eukaryot. Microbiol. 14, 521–527.

Bardsley, J.E., Harmsen, R., 1973. The trypanosomes of anura. Adv. Parasitol. 11, 1–73.

Barta, J.R., 1991. The dactylosomatidae. Adv. Parasitol. 30, 1–37.

Barta, J.R., Desser, S.S., 1984. Blood parasites of amphibians from Algonquin park, Ontario. J. Wildl. Dis. 20, 180–189.

Barta, J.R., Boulard, Y., Desser, S.S., 1989. Blood parasites of *Rana esculenta* from Corsica: comparison of its parasites with those of eastern North American ranids in the context of host phylogeny. Trans. Am. Microsc. Soc. 108, 6–20.

Beebee, T.J.C., Griffiths, R.A., 2005. The amphibian decline crisis. A watershed for conservation biology? Biol. Conserv. 125, 271–285.

Channing, A., Baptista, N., 2013. *Amietia angolensis* and *A. fuscigula* (Anura: Pyxicephalidae) in southern Africa: a cold case reheated. Zootaxa 3640, 501–520.

Channing, A., Hillers, A., Lötters, S., Rödel, M.O., Schick, S., Conradie, W., et al., 2013a. Taxonomy of the super-cryptic *Hyperolius* nasutus group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species. Zootaxa 3620, 301–350.

Channing, A., Schmitz, A., Burger, M., Kielgast, J., 2013b. A molecular phylogeny of African Dainty Frogs, with the description of four new species (Anura: Pyxicephalidae: Cacosternum). Zootaxa 3701, 518–550.

Combes, C., 1996. Parasites, biodiversity and ecosystem stability. Biodivers. Conserv. 5, 953–962.

Conradie, W., 2014. The King of the Dwarves: a new cryptic species of Dainty Frog (Anura: Pyxicephalidae: Cacosternum) from the eastern Great Escarpment of South Africa. Zootaxa 3785, 438–452.

Cook, C.A., Smit, N.J., Davies, A.J., 2009. A redescription of *Haemogregarina fitzsimonsi* Dias, 1953 and some comments on *Haemogregarina parvula* Dias, 1953 (Adeleorina: Haemogregarinidae) from southern African tortoises (Cryptodira: Testudinidae), with new host data and distribution records. Folia Parasitol. 56, 173–179.

du Preez, L., Carruthers, V., 2009. A Complete Guide to the Frogs of Southern Africa. Struik Nature, Cape Town.

Davies, A.J., Johnston, M.R.L., 2000. The biology of some intraerythrocytic parasites of fishes, amphibians and reptiles. Adv. Parasitol. 45, 1–107.

Davis, A.K., Devore, J.L., Milanovich, J.R., Cecala, K., Maerz, J.C., Yabsley, M.J., 2009. New findings from an old pathogen: intraerythrocytic bacteria (family Anaplasmatacea) in red-backed salamanders *Plethodon cinereus*. EcoHealth 6, 219–228.

Desser, S.S., 1987. *Aegyptianella ranarum* sp. n. (Rickettsiales, Anaplasmataceae): ultrastructure and prevalence in frogs from Ontario. J. Wildl. Dis. 23, 52–59.

Desser, S.S., Hong, H., Martin, D.S., 1995. The life history, ultrastructure, and experimental transmission of *Hepatozoon catesbianae* n. comb., an apicomplexan parasite of the bullfrog, *Rana catesbeiana* and the mosquito, *Culex territans* in Algonquin Park, Ontario. J. Parasitol. 81, 212–222.

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to Linnaeus: how many parasites? How many hosts? Proc. Natl. Acad. Sci. U. S. A. 105, 11482–11489.

Fantham, H.B., Porter, A., Richardson, L.R., 1942. Some haematozoa observed in vertebrates in eastern Canada. Parasitology 34, 199–226.

Haddad, C.R., 2003. Fruit chafers (Coleoptera: Scarabaeidae: Cetoniini) of the Ndumo Game Reserve and Tembe Elephant Park, KwaZulu-Natal. Afr. Entomol. 11, 130–133.

Kruger, N., Du Preez, L.H., 2015. Reproductive strategies of the Kangaroo Leech, *Marsupiobdella africana* (Glossiphoniidae). Int. J. Parasitol Parasites Wildl. doi:10.1016/j.ijppaw.2015.01.005.

Mohammed, A.H.H., Mansour, N.S., 1959. A general survey of blood protozoa in some Egyptian amphibians and reptiles. Bull. Zool. Soc. Egypt. 14, 21–26.

Netherlands, E.C., Cook, C.A., Smit, N.J., du Preez, L.H., 2014a. Redescription and molecular diagnosis of *Hepatozoon theileri* (Laveran, 1905) (Apicomplexa: Adeleorina: Hepatozoidae), infecting *Amietia quecketti* (Anura: Pyxicephalidae). Folia Parasitol. 61, 293–300.

Netherlands, E.C., Cook, C.A., Smit, N.J., 2014b. *Hepatozoon* species (Adeleorina: Hepatozoidae) of African bufonids, with morphological description and molecular diagnosis of *Hepatozoon ixoxo* sp. nov. parasitising three *Amietophrynus* species (Anura: Bufonidae). Parasit. Vectors 7, 552.

Nordstokke, D.W., Zumbo, B.D., 2007. A cautionary tale about Levene's tests for equality of variances. J. Edu. Res. Policy Stud. 7, 1–14.

Nordstokke, D.W., Zumbo, B.D., 2010. A new nonparametric Levene test for equal variances. Psicologica 31, 401–430.

Readel, A.M., Goldberg, T.L., 2010. Blood parasites of frogs from an equatorial African montane forest in western Uganda. J. Parasitol. 96, 448–450.

SPSS, 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp, Armonk, NY. IBM Corp. Released.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S., Fischman, D.L., et al., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783–1786.

Telford, S.R., 2009. Hemoparasites of the Reptilia: Color Atlas and Text. CRC Press, New York.

Werner, J.K., 1993. Blood parasites of amphibians from Sichuan Province, People's Republic of China. J. Parasitol. 79, 356–363.

Wesołowska, W., Haddad, C.R., 2009. Jumping spiders (Araneae: Salticidae) of the Ndumo Game Reserve, Maputaland, South Africa. Afr. Invert. 50, 13–103.