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Blood parasites of some Anurans from southern Nigeria

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Abstract. Eighty nine wild anurans comprising of 14 species from nine genera i.e. *Afixalus dorsalis*, *Amietophrynus maculatus*, *A. regularis*, *Amnirana galamensis*, *Arthroleptis poecilonotus*, *Arthroleptis* sp., *Aubria subsigilata*, *Hoplobatrachus occipitalis*, *Hyperolus concolor*, *Hyperolus fusciventris*, *Hyperolus fusciventris burfoni*, *Hyperolus* sp., *Ptychadena mascareniensis* and *Silurana tropicalis* caught from three sampling sites: Okomu Oil Palm Plantation, Usen Cocoa Plantation and the banks of River Niger at Agenegbode, Edo State in southern Nigeria were examined for blood parasites. Nine anuran individuals (10.11%) were parasitaemic. Four species of blood parasites; microfilariae of *Folleyellides*, microfilariae of an unidentified filarid nematode, a *Trypanosoma* sp. and an intracellular blood parasite, were identified in the infected anurans. Of the four blood parasite species encountered, the microfilariae of *Folleyellides* was the most prevalent 7/89 (7.87%), occurring in 2 individuals of *Hoplobatrachus occipitalis* from Okomu and in 4 individuals of *Amietophrynus* spp.; in 3 individuals of *A. regularis* from Agenegbode and in 1 individual of *A. maculatus* from Usen and in 1 individual of *A. galamensis* from Agenegbode. It was followed by the *Trypanosoma* sp. with a prevalence rate of 3.37%, occurring in 1 individual of *Amietophrynus maculatus* from Usen and in 2 individuals of *Amietophrynus regularis* from Agenegbode. *Folleyellides* microfilariae seem to be a multi-host parasite occurring in four host species in contrast to the unidentified filarid detected only in *Aubria subsigillata*. The unidentified intracellular blood parasite bears close resemblance to *Hemoliva* sp. and was only detected in *A. regularis* from Agenegbode. With regards to sex, more females harboured blood parasites with a prevalence of 25% in contrast to males which had only 1.75%.

INTRODUCTION

Surveys of the blood parasites of anurans have been conducted in several geographical regions of the world including China (Werner, 1993), Costa Rica (Desser, 2001; McKenzie & Starks, 2008), Mexico (Bursey & Goldberg, 2001; Goldberg & Bursey, 2002), Thailand (Chutmongkonkul *et al.*, 2006), Uganda (Readel & Goldberg, 2010), Malaysia (Mohammad *et al.*, 2013) among others. Blood parasites found include viruses, rickettsiae, species of several genera of protozoa, and microfilariae (Desser, 2001; Sailasuta *et al.*, 2011). In spite of the numerous records and wide distribution of these parasites, the only existing record of such study in Nigeria was that of Omonona & Ekpenko (2011) in Oyo state.

In view of the dearth of information on this group of parasites infecting anurans in Nigeria, we undertook a preliminary investigation of the blood parasites occurring in the anurans from Edo State, Nigeria. In this paper, we report the presence of blood parasites in anurans collected from a number of locations within the State.

MATERIALS AND METHODS

Study area

This study was carried out in Edo State of Nigeria which is located in the south-western

region of the country (5°45' and 7°N and 6°52'E). In this study, anurans were collected from three different land use areas in Edo State; an Oil Palm Plantations at the Okomu Oil Palm Company (latitude 5°07' and 5°25' E and longitude 6°18' and 6°26' N), a Cocoa Plantation at Usen (6°45' 01.1N and 5°17' 28.5E), and at the banks of River Niger at Agenegbode (7°06' N and 6°45' E), located in the savannah-mosaic zone of northern Edo State.

Collection of anurans

Eighty nine anurans belonging to fourteen species from nine genera were collected between November 2012 and June 2013. The specimens were captured by hand in the field at night between 8:00 pm and 2:00 am.

Identification and processing of Anurans

The anurans were identified according to the descriptions by Schiøtz (1999) and Roedel (2000). The frogs were transferred to plastic containers with perforated lids while the toads were transferred to plastic baskets with covers and then transported to the laboratory. In the laboratory, the amphibians were euthanized in Benzocaine solution and the snout-vent lengths taken with a venier calliper. Blood specimens were either obtained from clipped toes or from the heart. Body fluid from the body cavity was also examined for the presence of parasites. The blood and body fluid specimens were first transferred to heparinized tubes. Thick and thin blood smears were prepared, air-dried and then fixed in absolute methanol for 2 minutes, redried in the air and later stained in Giemsa's stain (1 part Giemsa's stain: 5 parts phosphate buffer saline pH 7.0) in a staining trough for 15 minutes. The slides were removed and rinsed in distilled water, then erected to drain and dry. Each slide was examined microscopically at x10 and x40 magnifications to determine the presence of parasites and the micrographs taken using a Coolpix Digital Camera (3.34 Mega Pixels) attached to a Nikon Alpha Photo-2 Microscope. Slides which were positive with parasites were marked to indicate the infected anuran species. Parasites were identified according to Gardiner *et al.* (1988), Lainson *et al.* (2007) and Esslinger (1986).

RESULTS

All anurans appeared clinically normal at the time of sampling. From 89 anurans examined in this study, nine individuals (10.11%) were parasitaemic in the three sampling areas. Infections were found in only five species of the 14 species of the anurans examined: in two individuals of *Hoplobatrachus occipitalis* from Okomu, in one individual each from *Aubria subsigilata* and *Amietophrynus maculatus* from Usen, respectively, in four of *Amietophrynus regularis*, (three from Agenegbode and one from Usen) and in one of *Amnirana galamensis* from Agenegbode. Microfilariae of two filarial nematodes; namely *Folleyellides* sp. and an unidentified filarial nematode, a *Trypanosoma* sp. and an intracellular blood parasite were identified. The prevalence rates were as follows: 7.87% for the microfilariae *Folleyellides* sp., 1.12% for the unidentified microfilaria sp., 3.37% for *Trypanosoma* sp. and 2.25% for the intracellular blood parasites (Table 1). Mixed infections were also observed in two (2.25%) of the total anurans examined.

Microfilariae *Folleyellides* sp. (Plate 1) was detected in the blood of seven anuran specimens including two *Hoplobatrachus occipitalis*, four *Amietophrynus* specimens (one *A. maculatus* and three *A. regularis*) and one *Amnirana galamensis* with body length ranged from 62 µm to 87 µm (mean ± s.d. 71±7.27 µm). The microfilariae of the unidentified filarid nematode (Plate 2), which was detected only in *Aubria subsigilata* had a mean body length of 95±9.47µm ranged between 84 to 117µm. These microfilariae were sheathed with the sheath slightly extending beyond their tail end and in addition had a distinctive feature of bulbous posterior ends (Plate 2). The *Trypanosoma* sp. (Plate 3) was detected in the blood of one *A. maculatus* and two *A. regularis* specimens caught in Usen and Agenegbode, respectively. The mixed infections of three

Table 1. Parasitemic host species and the prevalence of the parasitic infections

Host species	Prevalence (%)			
	Folleyellides microfilariae	Unidentified microfilaria sp.	*Trypanosoma* sp.	Intracellular blood parasites
H. occipitalis	2/89(2.25)	–	–	–
A. galamensis	1/89(1.12)	–	–	–
A. regularis	3/89(3.37)	–	2/89(2.25)	2/89(2.25)
A. maculatus	1/89(1.12)	–	1/89(1.12)	–
A. subsigilata	–	1/89(1.12)	–	–
Total	7/89(7.87)	1/89(1.12)	3/89(3.37)	2/89(2.25)

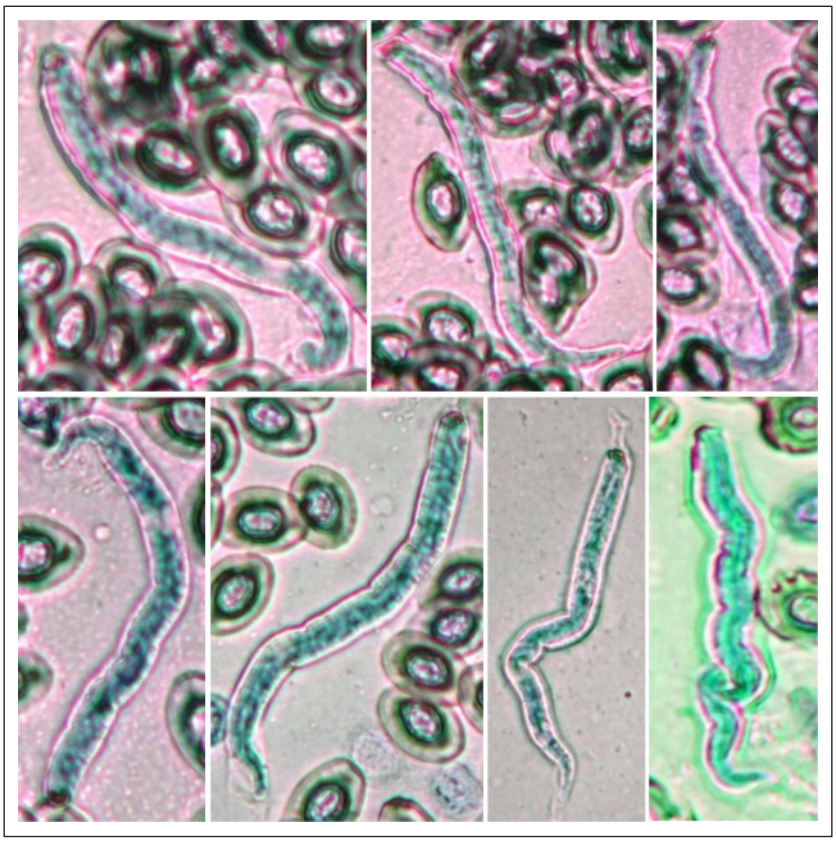

Plate 1A. Microfilariae of *Folleyellides* sp. from *H. occipitalis* from Okomu Oil Palm Plantation

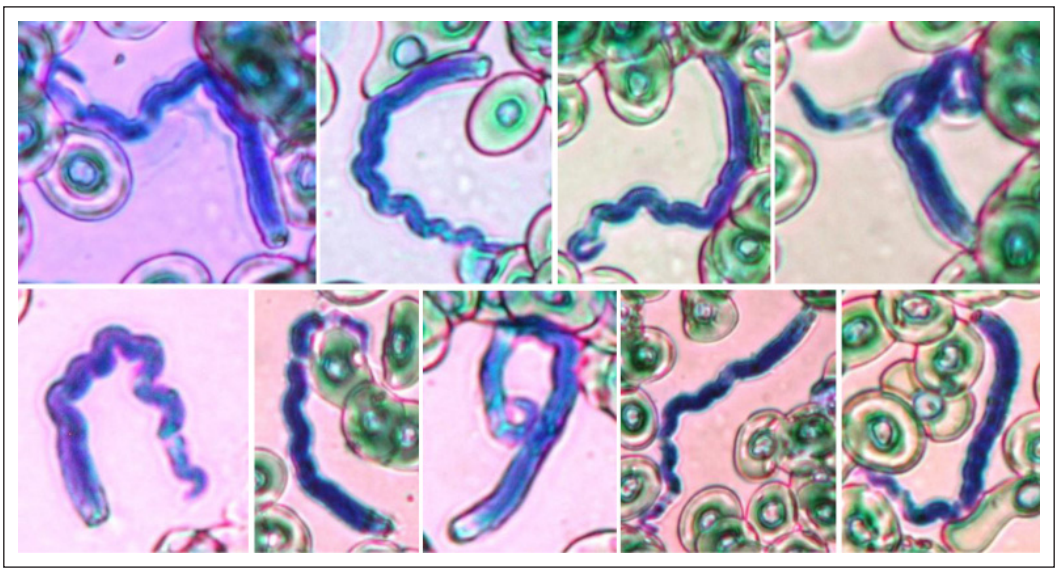

Plate 1B. Microfilariae of *Folleyellides* sp. from *A. regularis* from Agenegbode

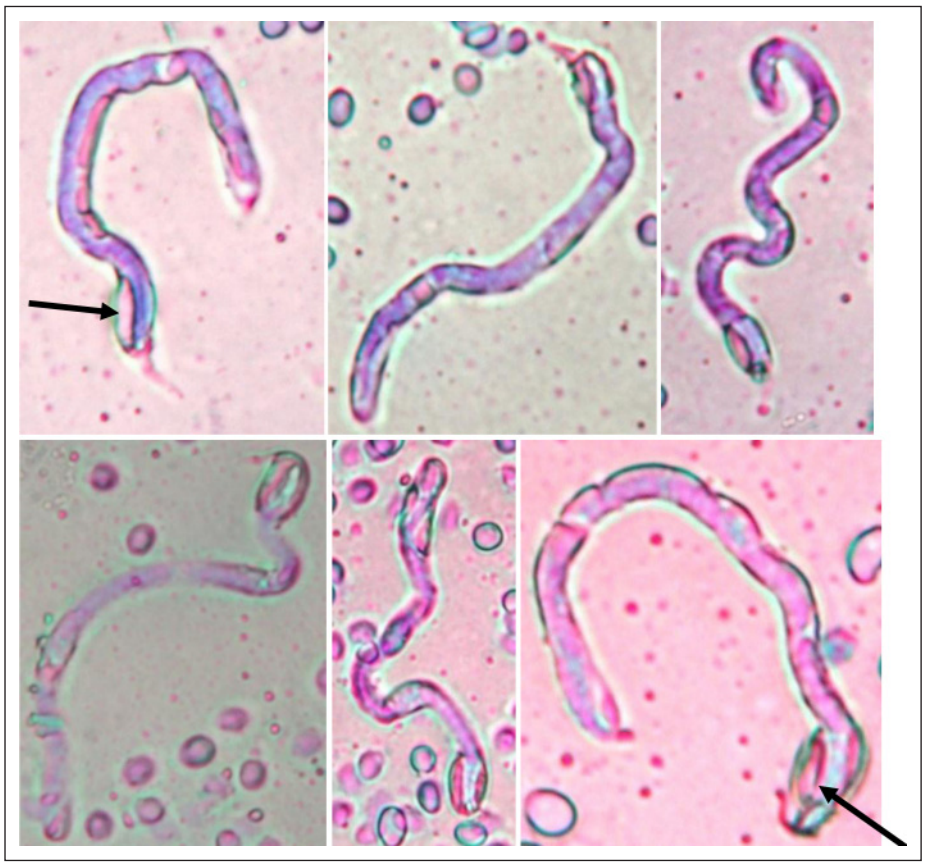

Plate 2. Microfilariae of an unidentified filarid from *Aubria subsigilata* from Usen (inflated posterior end indicated with arrows)

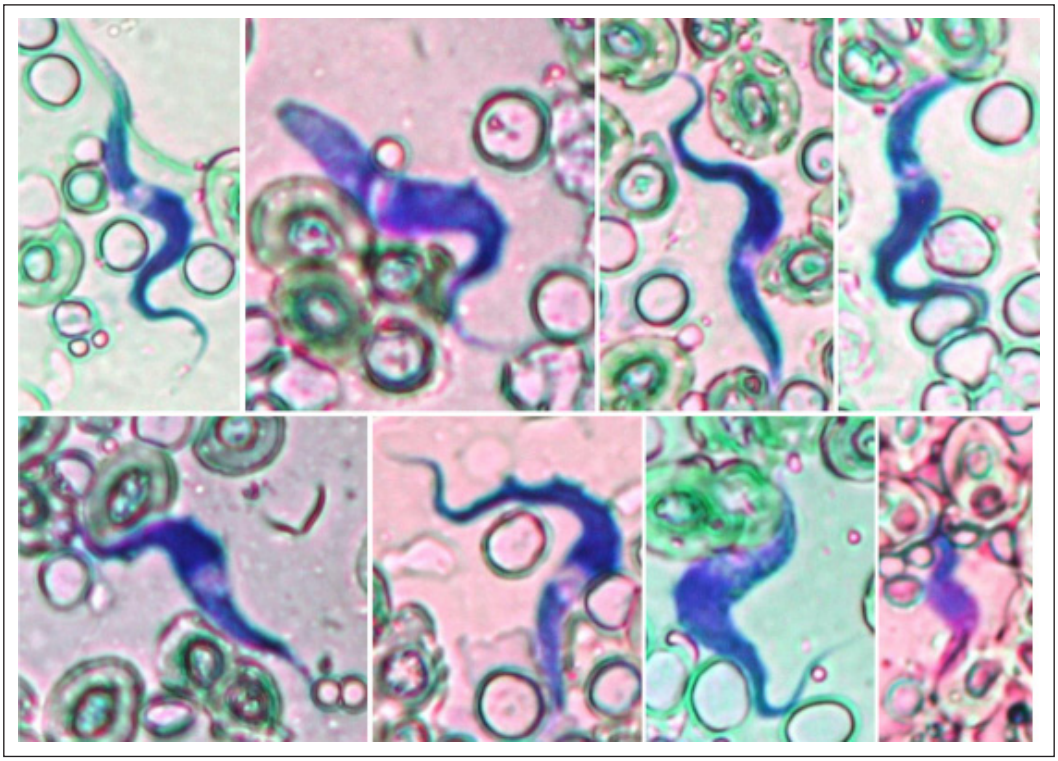

Plate 3A. *Trypanosoma* sp. from the blood of *Amietophrynus maculatus* from Usen

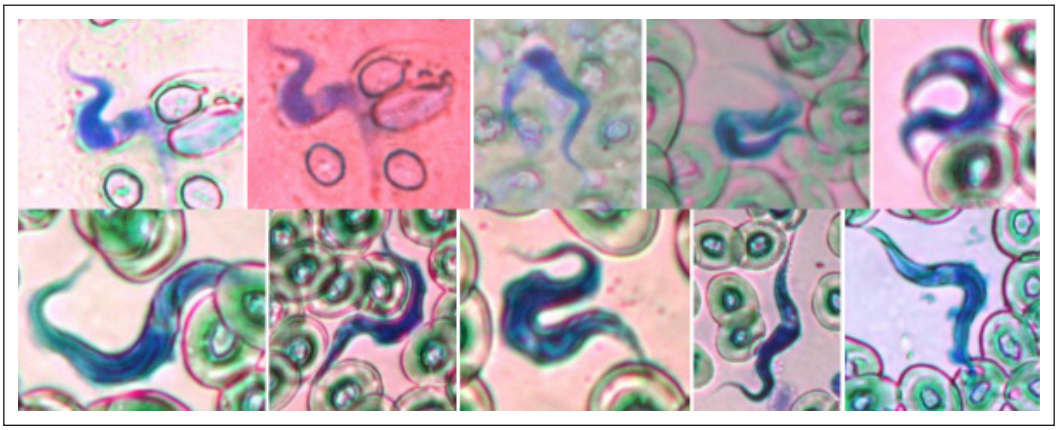

Plate 3B. *Trypanosoma* sp. from the blood of *A. regularis* from Agenegbode

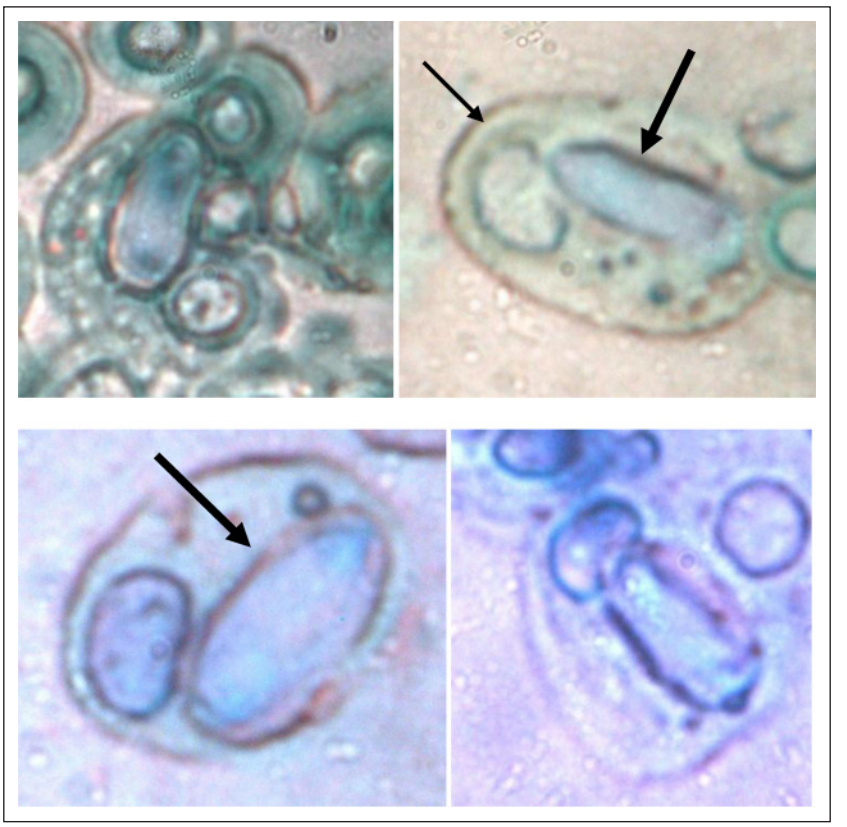

Plate 4. Intracellular blood parasites in *A. regularis* from Agenegbode (indicated with black arrows and r.b.c. indicated with thin black arrow)

blood parasites *Folleyellides* microfilariae, *Trypanosoma* sp. and the intracellular blood parasites (Plate 4) were recorded in two of the three *A. regularis* obtained from Agenegbode.

The results also showed that 57 (64.05%) anurans were males, while 32 others i.e. 35.96% were females. Of these figures, only one male from *Amietophrynus regularis* (1.75%) and 8 females (25%) were parasitaemic.

DISCUSSION

Results from this study have shown that anurans in Edo State irrespective of their locations (rainforest or savannah) are host to blood parasites. Until now, the only record available in the literature is the work of Omonona and Ekpenko (2011), who reported the presence of a *Trypanosoma* sp. in the blood of *Rana temporaria* in Ibadan, a south-western part of Nigeria. Other than the trypanosomes the anurans in southern Nigeria also habour other blood parasites including the microfilariae of filarial nematodes and intracellular parasites.

Microfilariae of *Folleyellides* were observed in the blood of *Hoplobatrachus occipitalis* caught at the Okomu Oil Palm Plantation and in *Amietophrynus regularis* specimens collected from the cocoa plantation at Usen and the banks of River Niger at Agenegbode, respectively. Aisien *et al.* (2003) had earlier reported *Folleyellides* sp. from the toads *A. regularis* collected at Agenegbode, Ogbonna and Auchi, all of which are located in the savannah-mosaic environment of Edo State. Therefore the present finding of the parasite in *A. regularis* from Agenegbode confirms the earlier report

of Aisien *et al.* (2003). With the finding of this parasite also present in *A. maculatus*, it seems that bufonids are generally susceptible to this parasite. This finding also confirms the report of Igetei (2012), who reported this parasite in *A. maculatus* collected from the Okomu Rubber Plantation. Result from the present study also confirms an earlier report that *A. galamensis* harbours *Folleyellides* sp. *Amnirana galamensis* collected from Agbede located in northern Edo State were also infected with the parasite (Aisien, M.S.O. personal communication). *Folleyellides* sp. infection has also been reported in *Hyperolius fusciventris burtoni*, a tree frog collected at the Okomu National Park (Imasuen *et al.*, 2012). *Folleyellides* sp. has also been recorded in anuran hosts outside Nigeria. For example, *Folleyellides striatus* was also detected in anurans from Costa Rica (McKenzie and Starks, 2008). According to Aisien *et al.* (2003), the vectors of the *Folleyellides* sp. are most likely mosquitoes, which were encountered in large numbers at the habitats of the infected anurans. Causey, as far back as 1939, suggested that vectors of *Folleyellides* spp. potentially included *Culex* and *Aedes* mosquitoes. Thus there is however a need for further investigation to establish which mosquito species is responsible for the transmission of this blood parasite among Nigerian anurans.

Another filarid nematode also detected in this study was found only in *Aubria subsigillata*. These microfilariae were longer than those of *Folleyellides* (95±9.47µm, with a range of 84µm to 117µm) sheathed and had bulbous posterior end (Plate. 2). The adults of this parasite which were recovered from the peritoneal cavity of *A. subsigilata* need to be identified.

The presence of *Trypanosoma* sp. in *Amietophrynus* sp. collected from Agenegbode and Usen represents new host and geographical records in Nigeria. Omonona and Ekpenko (2011) reported the occurrence of a *Trypanosoma* sp. in *Rana temporaria* from Ibadan in Oyo State, Nigeria. While the frog examined by these authors may have been a member of the Ranidae, the frog was certainly misidentified because *R. temporaria* is the European common grass frog, which has never been recorded in amphibian collections made in Nigeria by different investigators (Schiotz, 1963, 1964, 1966, 1967, 1969; Reid *et al.*, 1990; Oldham, 2000; Akani *et al.*, 2003; Onadeko and Roedel, 2009; Ogoanah, 2010; Imasuen, 2012). More anuran hosts need to be examined to determine which other ones habour trypanosomes in Nigeria. *Trypanosoma* spp. have also been detected in anuran species from other regions of the world (Werner, 1993; •ièkus, 2002; Leal *et al.*, 2008; McKenzie & Starks, 2008; Stenberg & Bowerman, 2010; Mohammad *et al.*, 2013).

In addition to trypanosome infection, toads from Agenegbode were also infected with intracellular blood parasites (Plate 4) which bear close resemblance to *Hemolivia* species. *Hemolivia* species (i.e *H. stellata*) are known to infect bufonid anurans (Petit *et al.*, 1990) and another species (*H. mariae*) infect lizards (Paperna & Smallridge, 2001; Lainson *et al.*, 2003, 2007) while *H. mauritanica* has been reported in the blood of an Algerian population of the spur-thighed tortoise, *Testudo graeca* by Tiar *et al.* (2010). However, this is the first record of intracellular blood parasites in the anurans of Nigeria. Other intracellular blood parasites such as *Haemogregarina*, *Lakesterella*, *Hepatozoon*, *Babesiasoma*, rickettsia, viruses and apicomplexans have been detected in anuran species elsewhere (Barta & Desser, 1984; Werner, 1993; Desser, 2001; Stenberg & Bowerman, 2008, 2010).

Of the two filarid nematodes found in this study, the unidentified species (Plate 2) seems to be confined to *Aubria subsigilata* since it was only recovered from this host. On the other hand *Folleyellides* is a multi-host parasite, infecting more than one hosts i.e. *H. occipitalis*, *A. regularis*, *A. maculatus* and *A. galamensis* as found in this study and from *Hyperolius fusciventris burtoni* (see Imasuen *et al.*, 2012). It will however be necessary to compare the morphometric characteristics of the adult worms from different host species before a final conclusion can be made on whether these worms are a single species or otherwise.

Microfilariae of different species of filarid worms have also been reported in anurans from other regions of the world, including Canada (Barta & Desser, 1984), Costa Rica (Desser, 2001), Thailand (Chutmongkonkul *et al.*, 2006), Malaysia (Rahman *et al.*, 2008) and Uganda (Readel & Goldberg, 2010).

Mixed infections of *Folleyellides* microfilariae, *Trypanosoma* sp. and an intracellular blood parasite, were only recorded in *Amietophrynus regularis* from Agenegbode. Studies by other investigators have also revealed the occurrence of mixed infections of blood parasites in anurans from elsewhere (Desser, 2001; Leal *et al.*, 2008; Stenberg & Bowerman, 2008, 2010; Readel & Goldberg, 2010).

In relation to sex, this study has shown that female anurans had higher prevalence rate of 25% among the 32 female specimens examined, as compared to only 1.75% (1 of 57) of male specimens examined. The reason for these differences in infection prevalence is not clear. However, more samples need to be examined to validate these differences.

In conclusion the present study has shown that anurans in Nigeria are indeed hosts to various blood parasites, including microfilariae of filarid nematodes, *Trypanosoma* sp. and intracellular parasites. Blood parasites were recorded in anurans from the three land use areas studied, with microfilariae of *Folleyellides* being the most prevalent and occurring as a multi-host parasite. The *Trypanosoma* sp was only recorded in members of the Bufonidae (*A. regularis* and *A. maculatus*). Mixed infections were however restricted to the toads collected from the savannah-mosaic. Overall, more female anurans haboured blood parasites (25%) than the males (1.75%).

Further investigations are needed to determine other blood parasites of amphibiabns in other locations and land use types in Nigeria. It will also be necessary ascertain which other amphibian hosts habour these parasites and which of these parasites have adverse effects on anuran health and population.

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