Nematode Parasites of Anurans from Three Cocoa farms in Ondo State, Nigeria.

*M. O Oniya and A. L Adeyekun
*Parasitology and Public Health Unit, Biology Department, Federal University of Technology, Akure, Nigeria.

*Corresponding author: onixous@gmail.com

Abstract
The research was designed to investigate the parasitic fauna of Anurans from cocoa farms in Ondo state. Amphibians are one of the most threatened groups of vertebrates. Many reasons are attributed to the decline of amphibian species such as global warming, habitat destruction and modification, others include: exploitation, pesticide use, introduced species, ultraviolet-B radiation (UV-B), pollution, parasites and diseases. A total of 31 frogs from 4 genera, *Hemisus, Ptychadena, Rana* and *Xenopus* and 7 toads from 1 genus, *Sclerophrys* were examined. 9 frogs were collected from Oluwateru farm at Iwoye Village; 7 frogs were collected from Folorunso farm at Ako-Igbatoro and 15 frogs from Obodulu farm in Idanre. 5 toads were collected from Oluwateru farm at Iwoye Village and 2 toads from Obodulu farm in Idanre. In all cases collection was done between 20:00hrs and 05:00hrs. Collected specimens were transported in sealed but ventilated containers to the laboratory where identification was done to species level. The frogs were anaesthetized until death in absolute chloroform soaked in cotton wool placed inside kill-jar for 3 minutes in the laboratory. The gastrointestinal tracts were cut open and the contents of the various sections were put into separate Petri dishes containing normal saline. The skin and the bladder were observed directly under a dissecting microscope for the presence of cysts and monogeneans. The parasites were fixed and preserved in 70% alcohol following standard procedure. Parasites recovered from the gastrointestinal tracts of the anurans include *Cosmocerca ornata,* Deising, 1861, *Cosmocerca cummutata,* Diesing, 1851 *Paracosmocerca mucronata,* Kung and Wu, 1945, *Ampliceacum africanum,* Taylor, 1924, *Gendria liberrei* Bain and Philipon, 1969 and *Chenospirura asturi* Hsu, 1957 Others were *Procamallus brevis* Kung, 1948 and *Camallanus dimitrovi* Durette- Desset and Batcharov, 1974. Some of the parasites are zoonotic while a few others are established parasites of African fishes and water Birds raising probable public health concerns from the findings. Further works aimed at unravelling the biodiversity of hosts and parasites in the lush ecosystem of Ondo state, as well as identification of organisms involved in the life cycle are noted.

Key words: Anurans, Parasitic fauna, Cocoa farms, Ondo State, Nigeria

Introduction
Amphibians are a class of tetrapods that evolved from lobe-finned fish and primitive tetrapods about 340 million years ago [1]. Anurans are usually less than 65cm in length and most species breed in aquatic environments. The most common of them in tropical Africa are the Frogs [2, 3]. They can be herbivorous or omnivorous and are consumed by both vertebrates and invertebrates. They are also used in pest control and play an important role as bio-indicators [4]. A parasitic organism lives on or in another organism (host) and obtains its food, protection, transportation and also performs its essential metabolism through the host. Monogeneans, common parasites of fishes, may externally infect aquatic life stages of amphibians. Some cestodes, acanthocephalans, and hirudineans may also reside on or in adult anurans, generally

as internal infections [5 and 6]. Many types of helminths may infect amphibians including *Echinostoma* spp, *Fibricola* sp, *Rhabdias bufonis* and *Ribeiroia ondatrae* [6] amongst others. Anurans have the capacity to carry extremely high parasite loads. As a resource for parasitological studies, there has been a number of significant papers and reviews of parasite groups of amphibians over the past century [7; 8; 9; 10; 11; 12; 13; and 14]. Some reports had been written about the parasites of Amphibians in Nigeria [15; 16; 17; 18; 19; 20; 21; 22; 23 and 24]. but not as extensive as in some parts of the world. Interestingly, tropical Nigeria has a limited number of publications in this field and none seem to have been done in Ondo State, which is a typical rainforest region of the country. In this region, many humans consume some of the amphibian species and the latter can also be found in some relative abundance despite the associated threats. This present research investigated the nematode parasites of anurans found in some Cocoa farms in Ondo State, Nigeria.

Materials and Methods

Ondo State is situated in the south western part of Nigeria with geographical coordinates of $5^0$45 ˈN, $4^0$20ˈE and $7^0$52ˈN, $6^0$05ˈE [25]. The state is bordered by Ekiti State in the north, Osun State by the west, Edo State at the eastern end, Ogun State and the Atlantic Ocean in the southern area. The study sites were cocoa farms with fresh flowing stream, making them good sites for amphibian habitat. The three cocoa farms where amphibians were collected include:

a. Oluwateru Family Farm at Iwoye Village ($7^0$25ˈN, $5^0$20ˈE), situated in Akure South Local Government Area (LGA), about 10 kilometres outside Akure township towards Ondo town
b. Folorunso Family Farm at Ako-Igbatoro Village ($7^0$09ˈN, $5^0$37ˈE), situated in Akure South LGA about 5 kilometres along Igbatoro road from Akure metropolis
c. Obodulu Cocoa farm ($7^0$24ˈN, $5^0$19ˈE), situated in Idanre LGA, around a rocky farmland in Idanre

The frogs and toads were collected between 20:00 and 05:00hrs in ponds, streams, underneath leaf litters and on trees. The specimens were handpicked and transported in sealed but ventilated containers to the Laboratory. Each container held specimens of averagely same size to prevent injury or death resulting from aggression. Safety precautions were put in place particularly against snake bites during collection by wearing thick boots and usage of hand gloves. Other measures included usage of whistles by all on site in case of emergency, keeping of bitter kola in the pockets (it is believed by the locals that it scares away snakes), usage of back packs instead of hand bags, sticks to remove leaf litters coupled with sharpened machetes and torch for proper illumination. A local who is very familiar with each site was engaged as a guide. An average of 12 specimens were collected per site. For proper identification, the specimens were first anaesthetized for 3 minutes in absolute chloroform soaked in cotton wool inside a kill-jar in the Laboratory. The smooth vein length (SVL) of each of the specimens were measured for taxonomic reasons and the sex of the animals were determined. The specimens were identified to the species level using identification keys by Rodel [3]. The specimens were examined for parasites 12 to 18 hours after collection. Dissections of the specimens were done 3-5minutes after anaesthetizing so as to recover life parasites. The various sections of the gastrointestinal tract were cut out systematically i.e. the Stomach, Oesophagus, and the intestine and put inside

Fig. 1: Map of Nigeria, showing Ondo State
Image Source: Rotowa et al. [28]

separate saline solutions in Petri dishes. The skin and the bladder were observed directly under a dissecting microscope to view the presence of monogeneans and cysts. The organs were teased using dissecting needle to facilitate the escape of the parasites into normal saline, then the Petri dishes were examined under a dissecting microscope. The parasites were lifted off the saline solution using Forceps/Pasteur pipette and placed inside another petri dish of saline solution before they were fixed for observation. The parasites were fixed by placing each of them inside small sterilized stainless steel vials, containing 70% alcohol and the container heated to make the parasite stretch out from the usual coiled position [20; 21 and 24]. The preservation of the parasites was done by removing them from the hot alcohol and placing them inside vials containing 70% alcohol. The recovered nematodes were cleared in lactophenol [21 and 23]. followed by examination under the dissecting microscope. The nematodes were identified using taxonomic keys provided by Yamaguti [26]. The prevalence rate was calculated as a percentage of the number of a particular host species infected with the specific helminth parasite divided by the total number of hosts examined, and mean intensity of infection was taken as the total number of parasites per host, and this was done for the whole animal population collected either infected or uninfected [27].

Results

A total of 31 frogs and 7 toads were collected from the sites. The species of frogs encountered during the study include: *Ptychadena longirostis* Peters, 1870 (n=2); *Ptychadena mascareniensis* Dumeril and Birron, 1841 (n=1); *Rana galamensis* Dumeril and Bibroni, 1841 (n=1); *Ptychadena retropunctata* Angel, 1949 (n=2); *Xenopus muelleri* Peters, 1844 (n=9); *Hemisus marmoratus* Peters, 1855 (n=4); *Ptychadena bibroni* Hallowell, 1845 (n=5) and *Ptychadena pumilio* Boulenger, 1920 (n=7). The toads encountered were *Sclerophrys maculata* Hallowell, 1854 (n=6) and *Sclerophrys pentoni* Anderson, 1893 (n=1).

The gut contents of *Rana galamensis* and *Ptychadena* spp were filled mostly with grasses and insects while *Xenopus muelleri* and *Hemisus memoratus* had Tadpoles in their gut. The contents were from the Oesophagus and the stomach while in the intestine, liquid to semi liquid matter was seen and could not be traced to any specific food substance. The gut contents of the *Sclerophrys* spp were insects e.g. grasshoppers, bugs and crickets. The contents were from the Oesophagus and the stomach while in the intestine, liquid or semi liquid matter were seen and could not be traced to any specific type of food substance. The gut contents were examined so as to help in knowing possible intermediate hosts of the parasites encountered. The parasites found in the alimentary canal of the frogs and toads were measured to help in taxonomic description and recorded (Table 1). They include: *Cosmocerca cummutata* Diesing, 1851, *Cosmocerca ornata*, *Procamallus brevis* Kung, 1948, *Camallanus dimitrovi* Durette- Desset and Batcharov, 1974, *Chenospirura asturi* Hsu, 1957, *Gendria liberrei, Paracosmocerca mucronata* Kung and Wu, 1945, *Ampliceacum africanum* Taylor, 1924 *and* Ascaridoid larvae Blanchard, 1849.The parasites were all nematodes belonging to 3 Orders (Oxyuridea, Spiruridea and Ascaridea) and 5 families (Oxyuridae, Camallanidae, Spiruridae, Quimperiidae and Heterochellidae) (Fig. 1). Frogs in Idanre were more infected than the other locations as highlighted in Table 2 and Iwoye had the highest number of infected toads (Table 3). The mean intensity was generally between 0.75-3.75 except for *Hemisus memoratus* that had 86 Ascaridoid larvae per host (Table 4). The overall prevalence for frogs recorded showed that *Sclerophrys maculata* had the highest infection rate in the study.

Table 1: Parasitic Species Recovered from Frogs and Toads from Different Sites

Parasites	Host		Site
	Frog	Toad	
Ampliceacum africanum	Nil	*Sclerophrys maculata; Sclerophrys pentoni*	Stomach, Oesophagus, Small intestine and Body Cavity
Chenospirura asturi	Nil	*Sclerophrys maculata*	Small intestine
Camallanus dimitrovi	Nil	*Sclerophrys maculata; Sclerophrys pentoni*	Stomach and Small intestine
Cosmocerca cummutata	*Ptychadena pumilio; Xenopus muelleri*	*Sclerophrys maculata*	Small intestine
Cosmocerca ornata	*Ptychadena pumilio; Hemisus memoratus*	Nil	Small intestine
Procamallus brevis	Nil	*Sclerophrys maculata*	Stomach and Small intestine
Gendria liberrei	Nil	*Sclerophrys maculata*	Small intestine
Chenospirura asturi		*Sclerophrys maculata*	Body Cavity
Paracosmocerca mucronata	Nil	*Sclerophrys pentoni*	Body cavity
Ascarididoid larvae	*Hemisus memoratus; Ptychadena bibroni*	Nil	Small intestine

Table 2: Prevalence and Mean Intensity of Infection in Frogs at the Different Sites

Parasites	Host	Location					
		Iwoye		Ako-Igbatoro		Idanre	
		Prevalence	Mean intensity	Prevalence	Mean intensity	Prevalence	Mean Intensity
Cosmocerca cummutata	*Xenopus muelleri*	100%	1.00	-	-	-	-
	Ptychadena pumilio		-	-	-	100%	2.00
Cosmocerca ornata	*Ptychadena pumilio*	-	-	-	-	100%	2.00
	Hemisus memoratus	-	-	-	-	50%	0.5
Ascarididoid larvae	*Hemisus memoratus*	-	-	-	-	100%	86
	Ptychadena bibroni	-	-	-	-	25%	0.25

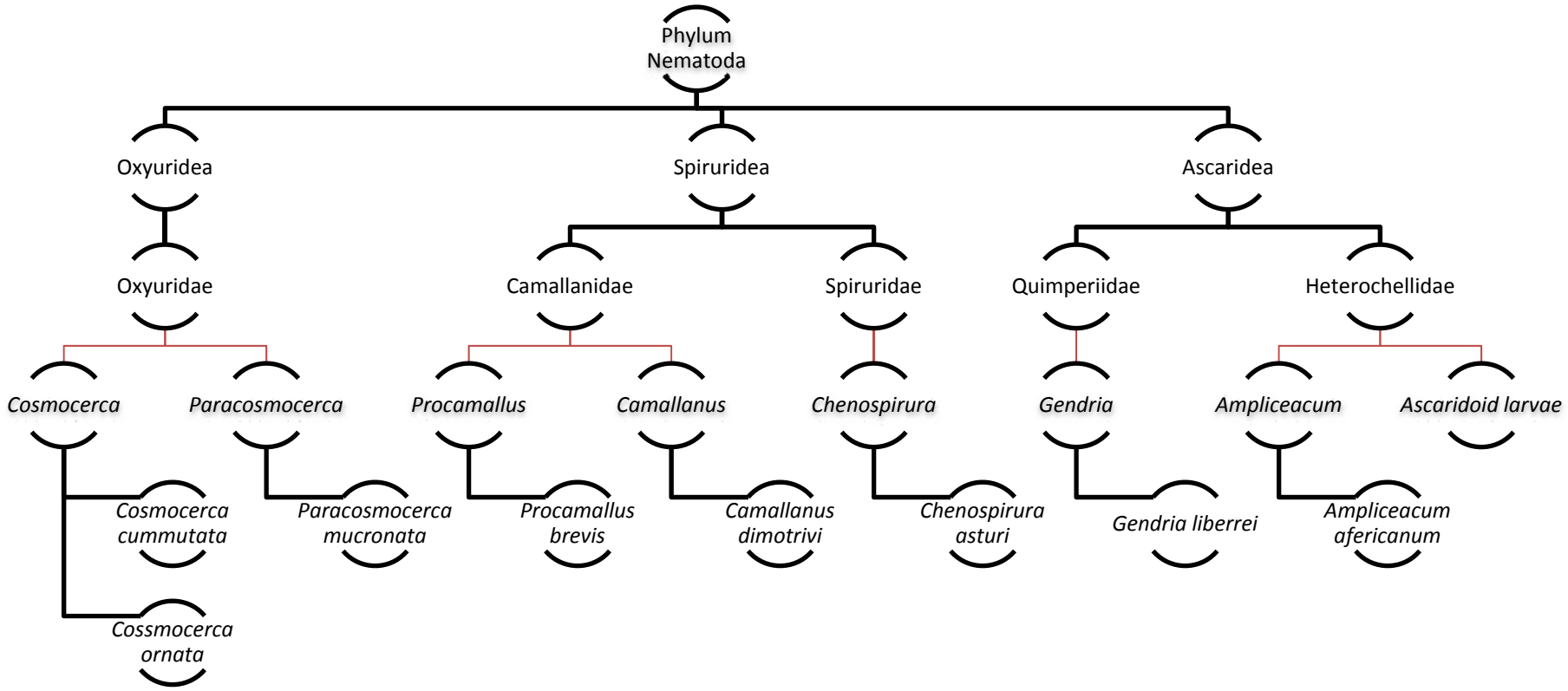

Fig 1: Pedigree of the Nematode Parasites encountered in the Anuran hosts

Adapted using taxonomic keys from Yamaguti [26]

Table 3: Prevalence and Mean Intensity of Infection in Toads at the Different Sites

Parasites	Host	Location			
		Iwoye		Idanre	
		Prevalence	Mean intensity	Prevalence	Mean Intensity
Ampliceacum africanum	*Sclerophrys maculata*	50%	0.75	50%	0.50
	Sclerophrys pentoni	-	-	100%	1.00
Camallanus dimitrovi	*Sclerophrys maculata*	50%	3.75	-	-
	Sclerophrys pentoni	-	-	100%	3.00
Cosmocerca cummutata	*Sclerophrys maculata*	25%	1.75	-	-
Procamallus brevis	*Sclerophrys maculata*	25%	0.75	-	-
Gendria liberrei	*Sclerophrys maculata*	25%	0.50	-	-
Chenospirura asturi	*Sclerophrys maculata*	25%	1.25	-	-
Paracosmocerca mucronata	*Sclerophrys pentoni*	.	-	100%	1.00

Table 4: Overall Prevalence of infection in the examined Animals

	Host Species									
	Sclerophrys maculata	*Sclerophrys pentoni*	*Xenopus muelleri*	*Hemisus memoratus*	*Rana galamensis*	*P. bibroni*	*P. pumilio*	*P. retropunctata*	*P. longirostris*	*P. mascarensis*
Cosmocerca cummutata	-	-	11.11%	-	-	-	28.57%	-	-	-
Cosmocerca ornata	-	-	-	33.33%	-	-	14.29%	-	-	-
Ascarididoid larvae	-	-	-	33.33%	-	20%	-	-	-	-
Ampliceacum africanum	50%	100%	-	-	-	-	-	-	-	-
Camallanus dimitrovi	33.33%	100%	-	-	-	-	-	-	-	-
Procamallus brevis	16.67%	-	-	-	-	-	-	-	-	-
Cosmocerca cummutata	16.67%	-	-	-	-	-	-	-	-	-
Cosmocerca ornata	-	-	-	-	-	-	-	-	-	-
Ascarididoid larvae	-	-	-	-	-	-	-	-	-	-
Gendria liberrei	16.67%	-	-	-	-	-	-	-	-	-
Chenospirura asturi	16.67%	-	-	-	-	-	-	-	-	-
Paracosmocerca mucronata	-	100%	-	-	-	-	-	-	-	-

Discussion

Seven toads from genus *Sclerophrys* and thirty-one frogs belonging to 4 genera, *Hemisus, Ptychadena, Rana* and *Xenopus* were examined out of the several seen for conservation reasons. The prevalence of infection in the observed species when compared to earlier reports in tropical Nigeria [20 and 23], showed that the frogs in this study had low worm burden. Previous studies from Nigeria also recorded the presence of cestodes and trematodes but only nematodes were encountered in this study.

Two of the parasites *Gendria liberrei* and *Chenospirura asturi* have been reported before in tropical Africa but are reported for first time in Nigeria in this study. The remaining parasites have been reported before in tropical Africa and in Nigeria. *Cosmocerca cummutata* recovered from the intestine of female *Ptychadena pumilio* in the Idanre farm had been reported in Brazil and Europe as a parasite of North American frogs [10], it was reported in Congo [29] and in Northern Nigeria as parasite of *Xenopus muelleri* [17]. Same parasite was also reported in Turkey as a parasite of the tree frog, *Hyla arborea* Linnaeus,1758 [30]. The report of this parasite in *Ptychadena pumilio* is new.

Cosmocerca ornata recovered from the gut of male *Hemisus memoratus* from the Idanre farm was also reported in Senegal from *Petropedetes natator* [31], in Nigeria from *Xenopus* spp [17], in South Africa from the native South African frogs [32], in Sudan from *Hoplobatrachus occipitalis* [33], and *Sclerophrys regularis* in Egypt, Zambia and Uganda [34; 35]. It was also reported in Nigeria from *Sclerophrys regularis, D. occipitalis,* and *Hemisus memoratus* [20,23].

Paracosmocerca mucronata was gotten from the intestine of the only *Sclerophrys pentoni* encountered in the study, it was reported as a parasite of *Xenopus muelleri* [20], this is the second time this parasite will be reported in another part of the Country. *Chenospirura asturi* was recovered from *Sclerophrys maculata* in Iwoye farm, it was reported as a parasite of water Birds [36]. *Camallanus dimitrovi* recovered from *Sclerophrys pentoni* has been reported extensively in the West African axis of the tropics. Its first report in Africa was in Togo in a general review of amphibian parasites [37], later in Nigeria from *Xenopus* spp [18] and from *Dicroglossus occipitalis* and *Hoplobatrachus occipitalis* [20 and 23]. *Procamallanus brevis* recovered from *Sclerophrys maculata* in this research was first reported in Tanzania [7], and reported Avery it in the northern part of Nigeria [17], and in both cases it was recovered from *Xenopus* spp.

Ampliceacum africanum, recovered from *Sclerophrys maculata* and *Sclerophrys pentoni,* was first reported from the mountainous region of the present day Tanzania from *Sclerophrys maculata* [7] and also reported in amphibians and reptiles, from some East and West African countries [40], Aisien *et al.* reported in Nigeria from *Sclerophrys maculata* and *D. occipitalis* [20]. *Gendria liberrei* was recovered from *Sclerophrys maculata,* a parasite already reported in Togo as a parasite of tilapia fish [12]. This is the first time the parasite will be reported in Nigeria.

The life cycle of *Cosmocerca cummutata, Cosmocerca ornata,* and *Paracosmocerca mucronata* of the family Oxyuridae starts by the female producing eggs in the large intestine of its host

making its host's rectum itch. The host scratches the area and transfers the eggs to the mouth where they travel to the intestine. Another way is by retrofection where eggs that are not transferred to extremities will hatch and crawl back into the intestines [39]. *Chenospirura asturi* a Spiruridae, undergoes indirect life cycle by using an arthropod intermediate host, most especially bugs or grasshoppers, while frogs and other vertebrates serve as definitive hosts [40].

The life cycle of *Camallanus dimitrovi* and *Procamallus brevis* of family Camallanidae involves a cyclopoid copepod crustacean as an intermediate host where development continues in the intestines of a vertebrate including freshwater fishes and turtles [39]. Females with fully developed first-stage larva burst from cheeks of a definitive host, releasing the larva, which are eaten by copepods that are then eaten by a definitive host [40]. Fertilization occurs when migrating from intestines of the definitive host to its head, after which all the males die [41]. *Ampliceacum africanum* and Ascaridoid larvae of the family Heterochelidae undergo viviparous direct life cycle in the stomach of the host. *Gendria liberrei* lays egg inside the host [40] and the eggs of this nematode can be effectively transferred from its natural host to a paratenic host i.e. amphibians and reptiles through the bite of Black flies which dwells in fast flowing streams with the natural hosts (fishes) [12].

The reported pathological effects of the parasites vary. *Cosmocerca cummutata* and *Paracosmocerca mucronata* changes the host's colour, causes ulceration and corrosion of alimentary canal [40]. *Chenospirura asturi* escalates the effects of other helminths and obstructs the intestinal passage, leading to serious mechanical damages [40]. *Camallanus dimitrovi* causes lesions, haemorrhage, mechanical damage, and associated diseases [39 and 40]. The recorded pathological effects of *Procamallanus brevis* are lesions, haemorrhage, mechanical damage, and associated diseases whereas, *Ampliceacum africanum,* causes major lesions and mechanical obstruction leading to a kwashiorkor like appearance of the host [40]. Ascaridoid larvae causes varying degree of mechanical damages depending on the numbers and stages of development while *Gendria liberrei* has no recorded pathological effect on the hosts.

Some of the observed parasites; *Cosmocerca cummutata*, *Paracosmocerca mucronata*, *Chenospirura asturi* and *Ampliceacum africanum* are zoonotic [40]. *Gendria liberrei* generally are parasites of African fishes. Dwellers and Farmers in the farm areas are prone to zoonotic infections since they are in contact with the anurans through various activities like farming, hunting (some of the anurans are edible) and fishing in the streams especially during rainy seasons.

CONCLUSION

The current study has described for the first time, the parasitic fauna of amphibians from the tropical rainforest of Ondo State, Nigeria and probable public health concerns from the findings. It further revealed the biodiversity of anurans in the study area confirming a relative abundance of the species since care was taken not to over exploit for investigation. Though of least concern, concerted efforts should be put in place to protect them from over hunting as they are

consumed by locals in the study area. Further works aimed at unravelling the biodiversity of hosts and parasites in the lush ecosystem, as well as identification of organisms involved in the life cycle continues.

AUTHORS' CONTRIBUTIONS

First Author designed the experiments, guided analyses and the writing of the Manuscript.
Second Author sourced literature, wrote the first draft of the manuscript and handled data analyses.
Both authors read and approved the final manuscript.

COMPETING INTERESTS

Authors declare no competing interests.

ETHICAL CONSIDERATIONS: Care were taken not to sacrifice more animals than necessary for the research following the standard procedures as established by the International Society of Applied Ethology [42,43]. In addition to this, the study conformed with the practice of reducing the number of amphibians used in research work to the smallest minimum possible as proposed by Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists [44].

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