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Diversity of anurans and their gastrointestinal helminth parasites within the University of Ibadan and environs, Oyo State, Nigeria

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Abstract

Anurans play a crucial role as bio-indicators of environmental changes in their ecosystem and their parasite populations can act as markers of environmental health since changes in their population may be related to changes in the quality of their environment. Therefore, this study was carried out to explore the anurans' diversity and their gastrointestinal helminths parasites within the University of Ibadan campus in order to ascertain the richness of the in the environment. A total of 225 anurans were captured using a combination of live capture sampling techniques and auditory-visual methods from four sampling locations within University of Ibadan, namely- Awba Dam, Heritage Park, Awba-Stream, and Ona River. The collected anurans were identified and examined for gastrointestinal parasites using standard protocols. Five anuran species; *Sclerophrys maculate*, *S. pusilla*, *S. regularis, S. steindachneri* and *Bufo bufo* were encountered and all were infected with parasites. *Cephaloclamys compactus, Aplectana chamaeleonis* and *Ophidascaris* (Trematoda), *Pharyngodon* and *Kalicephalus* (Nematoda) and *Acanthocephalus bufonis* (Acanthocephalan) were identified parasites. Ona River had the highest anuran diversity having all the five encountered amphibians. This study has provided valuable insight to anurans diversity including prevalence and intensity of their parasite.

Introduction

Anurans (frogs and toads) are mostly a carnivorous group of short-bodied, tailless amphibians as described by Frost (2021). Members of the Family Bufonidae are regarded as "true toads" while true frogs belong to the Family Ranidae (Cannatella 1997). The term "frog" refers to aquatic or semi-aquatic species with smooth, wet skins, whereas "toad" refers to terrestrial species with dry, warty skins (Badger and Netherton 1995). According to Frost (2023), the Order Anura comprises of about 7,600 species in 55 families. With over 1,000 described species globally, the African continent harbours a diverse assemblage of anurans are adapted to various habitats, ranging from rainforest to savanna and some can be found in the deserts (Rahman *et al* 2020). Key generas of anurans in tropical Africa include: *Hyperolius*, *Amietophrynus*, and *Phrynobatrachus* (Aisien *et al* 2017).

These Anurans inhabit various habitats with unique characteristics that influence their diversity and the parasite they harbour. Importantly, they play a crucial role in the functioning and maintenance of tropical African ecosystems; their ecological contributions span nutrient cycling, food webs maintenance, and biological control of pests.

Due to their permeable skin and aquatic life stages, anurans are susceptible to a wide range of parasitic infections. Common parasites associated with these anurans are; protozoans, helminths, and ectoparasites. Protozoans, such as *Trypanosoma* and *Leptotheca*, often infect the blood or tissues of anurans, causing various diseases (Barta and Desser 1989). Helminths, including trematodes and nematodes, typically inhabit the digestive tract, though some species can invade other organs (Goldberg *et al* 2021). Ectoparasites, like leeches and mites, attach to the skin or the external orifices of anurans (Poynton and Whitaker 2001). The impact of these parasites on host populations and communities can be significant, thereby causing morbidity and mortality resulting to anurans population declines (Daszak *et al* 2003). They can also influence their hosts' behaviour, reproduction, and survival, causing a change in the community dynamics (Koprivnikar *et al* 2012).

Due to habitat loss, pollution, disease and other environmental problems, many anuran species are in decline or may soon go extinct (Hayes *et al* 2010). Certain parasites that infect anurans have also been found to cause disease in humans; for example, the fungal pathogen, *Batrachochytrium dendrobatidis* is responsible for chytridiomycosis, a disease that has wiped out numerous anuran populations globally (Van Rooij *et al* 2015). Anuran parasite populations can act as markers of environmental health since changes in their population may be related to changes in the quality of the water, habitat deterioration, or other environmental stressors (Oliveira *et al* 2019). Furthermore, majority of studies on anurans and their parasites carried out recently were done in the South-South and North Central Nigeria (Aisien *et al* 2004, 2017; Onadeko and Ogoanah 2017); it is therefore pertinent to research on anuran parasites diversity in the South West Nigeria, since such studies have not been carried out in the last decade. Hence, this study was carried out to evaluate the current anurans' diversity and their gastrointestinal helminths parasites within the University of Ibadan campus.

Materials and methods

Study area

The University of Ibadan (UI), is located in Ibadan, the largest city in West Africa. The University features separate botanical and zoological gardens, various parks, and a dam. The campus is situated at a topographic elevation ranging from 185 to 230 meters, resulting in a gently undulating relief. It is in a region in southwest Nigeria characterized by a humid environment. The rainy season commences in April and lasts until November, with a brief dry spell in August. Typically, the dry season extends from November through February. The mean annual rainfall varies between 788 and 1884mm. Although Ibadan's environment is typical of a tropical rainforest, urbanization and the consequent population increase have led to severe forest degradation and sparse vegetation (Oyetunji *et al* 2020).

This study was conducted in four distinct locations within and around the University of Ibadan: Awba Dam, Heritage Park, Awba Stream, and Ona River (Ajibode).

Ethical approval

The ethical approval was obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of University of Ibadan.

Sample size determination

The sample size was estimated using the formula reported by Aisien *et al* (2017):

$$N = [Z2(P)(1 - P)]/d2$$

N=Sample size, Z=1.96, d=absolute error or precision = 0.18, P=Previous prevalence studies obtained, P=30%, which was the value obtained earlier by Aisien *et al* (2004). N=24.99 ≈ 25

Based on the formula, the minimum sample size for the study on the diversity of anurans' parasites at the University of Ibadan was calculated to be 25.

Sample collection

The anurans (n=225) were collected using the Acoustical Encounter Survey (AES) sampling technique according to Rodel and Ernst (2004) and Onadeko and Ogoanah (2017). Sampling was between 6-10pm weekly from June 2023 to February 2024. This timing was chosen based on the nocturnal nature of most anuran species. Attempts to capture them in the early morning proved abortive, further validating the chosen time frame. Safety boots were worn during the collection as a precaution against potential snake encounters. A flashlight was also used to ensure proper visibility during the night.

Upon capture, the anurans were carefully placed in a small square plastic pack, and then transferred to an improvised sample container with holes drilled on the cover to aid ventilation. This ensured the well-being of the specimens during transportation. A little water was added to the container to create and maintain a humid micro-environment, which is crucial for the survival of these terrestrial anurans. They were thus transported live to the Parasitology Laboratory, University of Ibadan and maintained prior to identification and parasitological examination.

Examination and identification of samples

Before dissection, each anuran was checked for ectoparasites. This step ensured that any parasite present on the external body of the anurans were accounted for in the study. Each of their Snout to Vent Length (SVL) was measured to determine age. Sex was determined by checking for the presence or absence of vocal sacs as described by Aisien *et al* (2017). The anurans captured were predominantly females with an average SVL of 10.5, which indicates that they were mostly adults. They were identified to species using their skin type, tarsals, presence or absence of web between feet and colouring as described by Channing and Rödel (2019).

The anurans were euthanized by immersion in benzocaine solution followed by postmortem examination (Aisien *et al* 2017), after which each sample was placed on a dissecting slab. Two slits were made on the side of each anuran, allowing access to the Gastrointestinal Tract (GIT). The GIT was then cut open using dissecting scissors, and the various parts were separated into petri-dishes filled with distilled water, as these facilitated a thorough search for the presence of parasites. Then the stomach, small and large intestine, lungs, gall bladder, urinary bladder, and other body cavities were examined for parasites following Aisien *et al* (2017) outlined procedure. The isolated parasites from the GIT were viewed using a compound microscope at ×4 and ×10 magnifications. The parasites were identified based on the guide provided by Imkongwapang *et al* (2012) and Aisien *et al* (2017).

Data analysis

Data were analyzed using descriptive statistics. Chi-square test was used to assess the association between parasites and hosts, while one way Analysis of Variance (ANOVA) was used to determine variations in parasite distribution patterns in relation to different study locations.

Results

Parasites found and their respective hosts

A total of 225 anurans made up of five species namely: *Sclerophrys maculate* (Plate 1), *Sclerophrys pusilla* (Plate 2), *Sclerophrys regularis* (Plate 3), *Bufo bufo,* and *Sclerophrys steindachneri* (Plate 4) were identified during the study (Table 1).

Table 1: Number of anurans in relation to sampling locations

Anuran species	Awba Stream	Awba Dam	Heritage Park	Ona River
Sclerophrys regularis	20	16	19	22
Sclerophrys pusilla	17	13	18	10
Sclerophrys maculata	9	16	16	7
Bufo bufo	10	0	0	8
Sclerophrys steindachneri	0	0	0	10
Total	**56**	**45**	**53**	**57**

Helminths parasites found include: trematodes, nematodes and acanthocephalans (Table 2). Some of the identified species were *Cephalochlamys compactus* (Plate 5), *Ophidascaris* species (Plate 6), and *Capillaria* species (Plate 7), encysted larval *Ascarididia* (Plate 8). The Pearson Chi-Square test and the Likelihood Ratio test was p< 0.001, this suggests that the distribution of parasites among different species of anurans is unlikely to occur by chance alone, emphasizing a relationship worthy of deeper investigation.

Prevalence and intensity of anuran parasites

From the data shown in Table 3, the parasite with the highest prevalence was *Pharyngodon* species, with a prevalence of 20.83%, with a mean intensity of 7±4.5. This indicates that approximately one in every five anurans in the studied population was infected with this parasite. *Cephalochlamys compactus* and *Ophidascaris* species had the second-highest prevalence at 16.67% each with intensities of 4.5±3.5 and 4.3±3.2, respectively. *Acanthocephalus bufonis* had a prevalence of 12.5% with a mean intensity of 4.7±2.1. There were six other parasites: *Amplicaecum africanum, Aplectana chamaeleonis, Gorgoderina tarascea, Kalicephalus,* and encysted larvae of Ascaridida had equal prevalence (4.2%); out of these six, *Aplectana hylambatis* had the highest mean intensity of 14.0±7.0 while *Gorgoderina tarascea* was least (1.0±8.1). The parasites prevalence and mean intensity were not statistically significant across the sampled locations (p>0.05, f-value 0.424).

Discussion

The study provided a valuable insight into the diversity, prevalence and intensity of parasitic infections in these amphibian hosts within and around University of Ibadan. The findings underscore the importance of considering both host species and geographic location when assessing parasite diversity and prevalence within the anuran populations. Since many anurans are carnivorous in nature (Fabrezi and Cruz 2020), the different assemblage of parasites found depict the arrays of lower animals distributed within and around the study area.

An intriguing aspect of the study was the observation that although the captured anurans were predominantly *S. pusilla*, they harboured a diverse array of parasites in their gastrointestinal tracts. This suggests that factors beyond host species alone, such as environmental conditions and ecological interactions, may influence parasite diversity and transmission dynamics. Looking at the association between the type of anuran host and their parasitic infections, this association indicates the specificity of certain parasites to particular anuran hosts. Parasites often evolve to exploit specific hosts, leading to a strong correlation between host and parasite types. This specificity can be influenced by various factors, including the host’s physiology, behaviour, and habitat, which can create conducive environments for certain parasites abundance as described by Kołodziej-Sobocińska (2019).

Furthermore, from the measures of association, with a Phi value of 1.303 and a Cramer's V value of 0.652, both indicated a strong association between parasite type and anuran host type, these results underscore the profound influence of host species on parasite diversity and distribution (p<0.001). Also, the strength of the association, as indicated by these results, suggests that the anuran host plays a significant role in determining the diversity and distribution of parasites. This could be due to the host’s immune response, which can act as a selective pressure on the parasite community, favouring those that can evade or withstand the host’s defense. Moreover, the observed association might reflect underlying ecological dynamics. For example, certain anuran species might inhabit areas with higher exposure to parasites, leading to a higher incidence of parasitism. Alternatively, some anuran species might have behaviours or life history traits that make them more susceptible to certain parasites.

In addition, the observed prevalence and mean intensity of different parasite species in the anuran population provide valuable insights into the complex dynamics of host-parasite interactions. The high prevalence of *Pharyngodon* species could be indicative of its broad host range, or it could suggest that the environmental conditions at the study site are particularly conducive for this parasite. It could also be a reflection of the life cycle of this parasite, which might involve multiple hosts or stages, thereby increasing its chances of infection. The fact that *C. compactus* and *Ophidascaris* species share similar prevalence but differ in intensity could be due to differences in their life cycles, transmission modes, or host specificity. For instance, *C. compactus* might have a higher reproductive rate or a shorter generation time, leading to a higher parasite load per infected individual (Hagmayer *et al* 2020).

The significant presence of *A. bufonis,* despite its lower prevalence compared to other parasites, might suggest that this parasite has a high impact on its host, possibly due to its virulence or the physiological stress it imposes on the host. The lower prevalence of *Gorgoderina tarascea* and *Kalicephalus* species could be due to their narrow host range, lower transmission rates, or the absence of suitable environmental conditions for their propagation.

Considering the parasites mean intensity, high mean intensity could indicate a high level of parasitism going in the study area among anuran population, and this could have significant implications for the health and survival of the anuran population (Lettoof *et al* 2013). These findings underscore the importance of considering both the prevalence and intensity of different parasite species when studying host-parasite interactions. They highlighted the need for further research to understand the factors driving these patterns and their implications for anuran health and conservation. Moreover, they emphasize the role of parasites as integral components of biodiversity, contributing to the complexity and resilience of ecosystems.

The lack of a statistically significant difference in the mean number of parasites across the four locations, provides an intriguing perspective on the role of location in shaping host-parasite dynamics.

Plate 1: *Sclerophrys maculata*

Plate 2: *Sclerophrys pusilla*

Plate 3: *Sclerophrys regularis*

Plate 4: *Sclerophrys steindachneri*

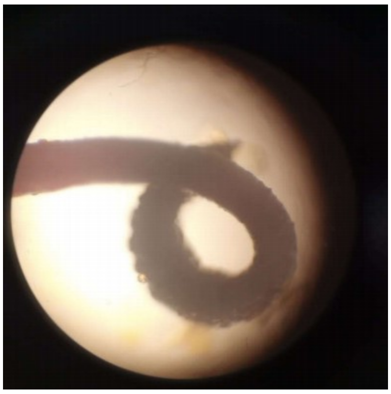

Plate 5: *Cephaloclamys compactus* (x10)

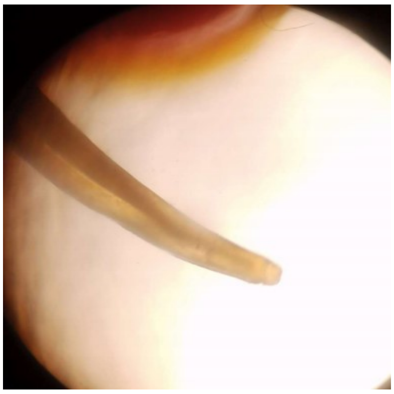

Plate 6: *Ophidascaris* (x10)

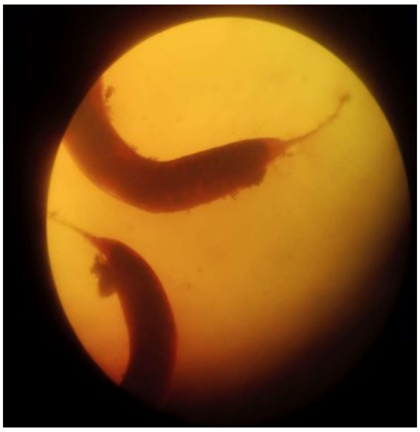

Plate 7: *Capillaria* (x10)

Plate 8: Encysted Larval *Ascaridida* (x10)

Table 2: Parasites, anuran hosts, and location found in the hosts

Parasites	Hosts	Location in hosts
Trematoda		
Cephalochlamys compactus	*Sclerophrys regularis*	Stomach x 5
		Intestine x 6
	Sclerophrys maculata	Intestine x 3
	Sclerophrys steindachneri	Stomach x 1
		Intestine x 4
Cephalochlamys species	*Sclerophrys regularis*	Intestine x 3
Gorgoderina tarascae	*Sclerophrys regularis*	Stomach x 1
Pleurogenoides gastroporus	*Sclerophrys regularis*	Stomach x2
		Intestine x 2
Aplectana chamaeleonis	*Sclerophrys pusilla*	Stomach x 2
		Intestine x 4
Aplectana hylambatis	*Sclerophrys steindachneri*	Stomach x 7
		Intestine x 13
	Sclerophrys regularis	Stomach x 5
		Intestine x 3
Nematodes		
Kalicephalus species	*Sclerophrys regularis*	Stomach x 2
Oswaldocruzia duboisi	*Sclerophrys steindachneri*	Stomach x 3
		Intestine x 3
Amplicaecum africanum	*Sclerophrys regularis*	Stomach x 3
		Intestine x 7
Ophidascaris species	*Bufo bufo*	Stomach x 4
	Sclerophrys pusilla	Intestine x 5
	Sclerophrys regularis	Stomach x 3
		Intestine x 5
Capillaria species	*Sclerophrys regularis*	Stomach x 6
		Intestine x 9
Encysted Larval Ascaridida	*Sclerophrys steindachneri*	Stomach x 5
Cosmocercella species	*Sclerophrys pusilla*	Stomach x 7
		Intestine x 8
Pharyngodon species	*Sclerophrys regularis*	Intestine x 9
	Bufo bufo	Stomach x 7
	Sclerophrys pusilla	Stomach x 6
		Intestine x 12
Acanthocephala		
Acanthocephalus bufonis	*Sclerophrys steindachneri*	Intestine x 6
	Sclerophrys pusilla	Stomach x 6
		Intestine x 2
	Bufo bufo	Intestine x 2
Oxysomatium macintoshii	*Sclerophrys maculata*	Stomach x3

x- number found in each location within each of the anuran

One possible interpretation of this finding is that the sampling locations share similar environmental conditions, such as temperature, humidity, and vegetation, which could influence the life cycles of the parasites and their ability to infect hosts. This could suggest that the parasites are relatively generalist in nature, able to thrive across a range of conditions. Alternatively, the anuran hosts across the sampling locations might have similar behaviours or physiological traits that make them equally susceptible to parasitic infection, regardless of location. This could indicate that host characteristics play a more significant role than location in determining parasite prevalence and intensity.

The diversity of parasites in this study suggests that the location may not play a significant role in the diversity or intensity of parasitic infection in anurans, this could imply that other factors, such as host characteristics or broader environmental conditions, may be more influential in shaping these host-parasite dynamics.

Conclusion

This study of anuran species and helminths parasites in the anurans within the University of Ibadan has provided valuable insight to their diversity as well as prevalence and intensity of the parasites. The high prevalence underscores the potential impact of these parasites on anuran health and survival, and the need for conservation efforts that will prevent their extinction.

Finally, while there is still much to learn about the intricate relationships between anurans and their parasites, this study represents a significant step forward in our understanding of these relationships. It is hoped that these findings will inform future research and contribute to the development of effective strategies for managing parasitic infections in anuran populations.

Table 3: Parasite prevalence and mean intensity

Parasites	Prevalence (%)	Mean Intensity
Acanthocephala		
Acanthocephalus bufonis	12.5	4.7±2.1
Oxysomatium macintoshii	8.3	2.5±6.1
Nematodes		
Amplicaecum africanum	4.2	10.0±6.0
Capillaria	8.3	7.5±2.5
Oswaldocruzia duboisi	8.3	3.0±1.9
Pharyngodon	20.8	7.0±4.5
Kalicephalus	4.2	2.0±5.4
Cosmocercella	8.3	7.5±1.2
Encysted larval Ascaridida	4.2	5.0±2.3
Ophidascaris	16.7	4.3±3.2
Trematoda		
Aplectana chamaeleonis	4.2	6.0±2.5
Aplectana hylambatis	8.3	14.0±7.0
Cephalochlamys compactus	16.7	4.5±3.5
Gorgoderina tarascea	4.2	1.0±8.1
Phleurogenoides gastroporus	4.2	4.0±3.7

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Conflict of interest

The authors declare that, there is no conflict of interest.

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