Prevalence and Mean Intensity of Nematode Parasites in Anurans of the Genus *Sclerophrys* in Burkina Faso (West Africa)

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Abstract

A study aimed at better understanding parasitic infections was conducted on nematode parasites infecting anurans of the genus *Sclerophrys* between June 2022 and November 2023 in the provinces of Ganzourgou, Kadiogo, and Houet in order to preserve them. To do this, anurans were collected at night using the VAES method (Visual and Acoustic Encounter Survey), then dissected in order to search for nematodes. The results indicate that 743 anurans were collected. These anurans belong to four species: *Sclerophrys maculata*, *S. regularis*, *S. pentoni* and *Sclerophrys xeros*. Of the 743 anaintensities examined, 669 individuals were infected with nematodes, representing an overall prevalence of 90.04%. Nine species of nematodes were identified: *Amplicaecum pesteri*, *Cosmocerca ornata*, *Oswaldocruzia* sp., *Physaloptera* sp., *Rhabdias africanus*, *Rhabdias* sp., *Cosmocercella* sp., *Cosmocercoides* sp. and *Aplectana* sp. The most widespread species was *Cosmocercoides* sp. (58.95%), while *Cosmocerca ornata* was the least prevalent (0.26%). No significant variation was observed in prevalence in relation to the infected organ ($p = 0.7128 > 0.05$), sex (p-value = $0.06031 > 0.05$) and season (p-value = $0.2365 > 0.05$). This study provides significant information on the population of *Sclerophrys* anurans and their nematode parasites. The high overall prevalence in these toads of the genus *Sclerophrys* reveals that these populations could be threatened by mortality induced by this high parasite load. In addition, the parasite load was notably higher in environments with increased pollution due to the use of chemical fertilizers. Therefore, the preservation of these organisms would require the adoption of sustainable agricultural practices.

Keywords: Anurans of the genus *Sclerophrys*, Nematodes, infection, Conservation, Burkina Faso

Introduction

Amphibians, also known as batrachians, comprise 8579 species (Frost, 2016) and represent a class of vertebrates naturally distributed worldwide, except in the Arctic and Antarctic regions. They are generally absent from marine environments; however, some species have adapted to brackish waters (Benito-Espinal, 1997; Lecointre & Le Guyader, 2006). Anurans are the most diverse order of amphibians in the world (Lescure, 1991). To date, 36 anuran species belonging to eleven families have been identified in Burkina Faso, including five species of the genus Sclerophrys (Ayoro et *al*., 2020). These animals have ecological interests. Indeed, they are excellent bioindicators of the health and conservation status of aquatic environments (Rödel, 2000; Channing, 2001; Guerry & Hunter, 2002) as well as biological tools to identify the ecological stress of their habitat (Welsh et Ollivier, 1998; Adams, 1999).

In addition, these organisms play a crucial role in food webs and are essential for maintaining the balance of wetland ecosystems (Channing, 2001). Moreover, they are biological control agents contributing to the control of pest populations, insect pests and vectors of diseases (Channing, 2001). Anurans are also an important food source for humans and animals (Angulo, 2008; Mohneke et *al*., 2009; Mohneke et *al*., 2010). Moreover, these vertebrates are used in ethnozoology in traditional medicine (Simmaco et *al*., 1998; Zhou et *al*., 2006; Mohneke et *al*., 2011). On the paratological level, toads host a diverse range of parasites, particularly nematodes (Goldberg et *al*., 2001). These parasites can have adverse effects on their populations in terms of growth retardation, skeletal deformities, vision impairment, reduced host fecundity, absorption inhibition, feeding behavior and health (Knudsen et *al*., 2001; De Montaudouin et *al*., 2003; Lafferty et *al*., 2008; Mohammad et *al*., 2015), which could affect the viability of amphibian populations. Despite their pathogenic potential, studies on nematode fauna infecting amphibians remain limited in Burkina Faso. Indeed, rare studies have focused on anuran parasites and associated platyhelminths (Soubeiga et *al*., 2020; 2025). It is to fill this information gap that this study was conducted. The objective of this study is to contribute to a better knowledge of the bio-ecology of parasites associated with anurans in order to contribute to their preservation. More specifically, it is (i) to inventory the host Anurans of the parasites; (ii) to determine the prevalences and intensities of infestation and (iii) to establish the distribution of prevalences according to sex, organs, season and site.

Materials and Methods
Study Sites

The toads examined in this study were captured in urban and peri-urban water bodies of Kadiogo, Ganzourgou and Houet. The study was conducted in the two largest cities of Burkina Faso, Kadiogo and Houet, which together represent nearly two-thirds (65.8%) of the urban population, as well as the corresponding pollution levels (INSD - FRISTAT, 2019). The province of Ganzourgou is particularly noteworthy because Sclerophrys toads are consumed there and provide a source of income to local communities. These toads are also among the widely distributed anuran species (Ayoro et *al*., 2020).
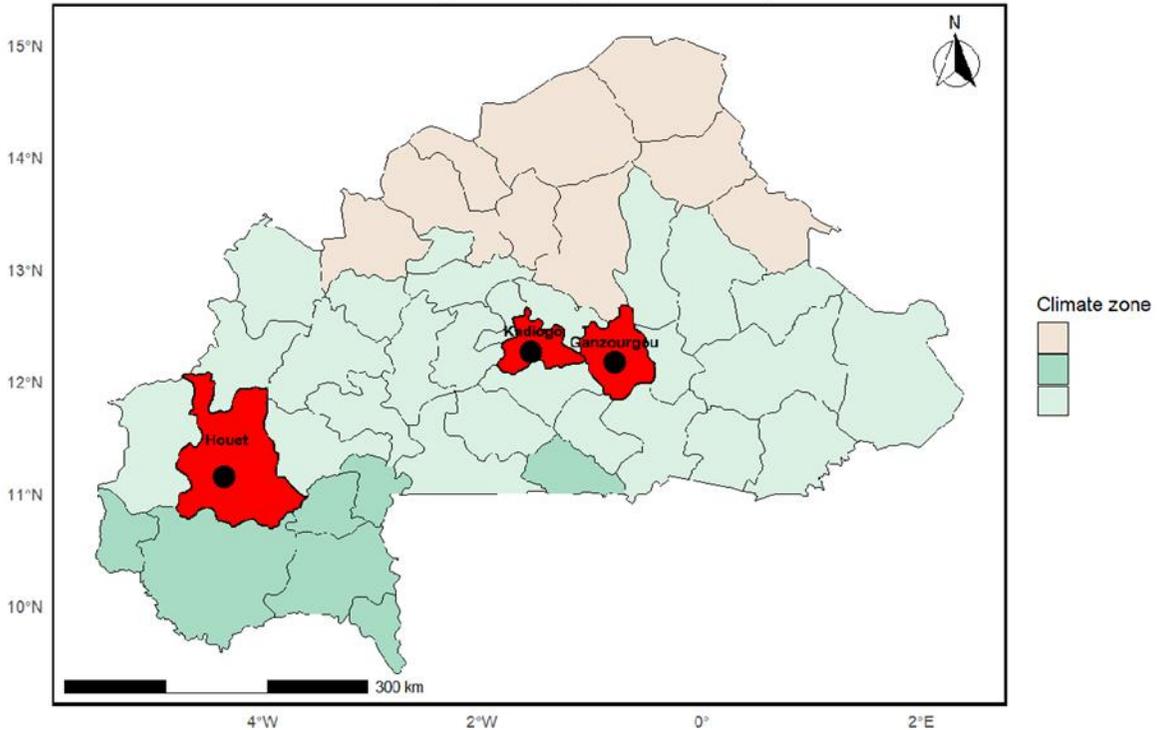
Figure 1: Capture sites for toad specimens

Toad Sampling and Collection of Parasitic Nematodes

The toads were collected from June 2022 to November 2023. The sampling was manually carried out near the water reservoirs (canals, ponds and dams) between 6:00 PM and 9:00 PM, then from 4:00 AM to 6:00 AM. Toad specimens were captured using the visual and acoustic inventory method described by Crump and Scott (1994), which involved searching for toads by lifting rocks, scanning water bodies with headlamps and locating individuals through their calls. Each caught toad was individually placed in a cotton bag and transported to the Laboratory of Animal Biology and Ecology (LBEA) at Joseph KI-ZERBO University for identification.

In the laboratory, toads were identified using the identification keys of Rödel (2000), Rödel & Branch (2003) and Rödel et al. (2005). They were then euthanized by immersion in a solution of 1,1,1-trichloro-2-methyl-2-propanol hemihydrate (MS222). A mid-ventral incision (from mouth to cloaca) was made to remove the internal organs (digestive tract, liver, heart, gallbladder and gonads) in order to search for parasitic nematodes.

Nematodes were examined in temporary mounts after removing one drop of lactophenol for 3 to 5 minutes. This clarifying agent allows detailed observation of the characteristics of nematodes. Morphometric measurements of nematodes, including body and organ dimensions, were taken using a

standard ZEISS ICS 25 optical microscope equipped with an optical micrometer.

The nematodes collected were observed under a light microscope to determine the morpho-anatomical characteristics likely to allow their identification using taxonomic keys (Yamaguti, 1961; Baker, 1987; Khalil et *al*., 1994). After identification, the nematodes were immediately fixed and stored in 70% ethanol.

Statistical analyses

The prevalence (P) and average intensity (MI) of parasitic infections were calculated according to the definitions provided by Anderson (1993). Prevalence indicates the percentage of hosts infected with a specific nematode species and was calculated as the ratio of infected hosts (Ni) to the total number of examined hosts (Ht), multiplied by 100:

$$P\,(\%) = (Ni/Ht)\,100$$

The mean intensity (MI) indicates the average number of parasites per infected host and was calculated by dividing the total number of parasites (p) by the number of infected hosts (n):

$$IM = n/p$$

A Chi-square (χ^2) test was used to compare parasite prevalence by gender and prevalence by season at the three sampling sites. Principal component analysis (PCA) was used to compare prevalence rates according to location. Differences were considered significant at the 5% threshold. These tests were performed using R.4 2.1 software.

Results
Parasite Hosts

The inventories of anuran hosts (N=743 individuals) made it possible to identify four species belonging to the genus *Sclerophrys* and the family of Bufonidae. *Sclerophrys maculata* (Hallowell, 1854), *S. pentoni* (Andersson, 1893), *S. regularis* (Reuss, 1833) and *S. xeros* (Tandy et *al*., 1976). Nine species of nematodes are identified on the hosts anurans and classified into five families (Table 1).

Table 1: Summary table taking into account the inventoried anuran species, the parasites by anuran species

Family of parasitic nematodes	Species of parasitic nematodes	Host of parasitic nematodes
Physalopteridea (Leiper, 1908)	*Physaloptera* sp.	*S. r*
Ascarididea Blanchard, 1849	*Amplicaecum pesteri:Orneoascaris chrysanthemoides* Skrjabin, 1916	*S.r; S.m; S.p*
Oxyuridae Cobbold, 1864	*Aplectana* sp.	*S.r; S.m; S.p;S.x*
	Cosmocercoides sp.	*S.r; S.m; S.p;S.x*
	Cosmocercella sp.	*S.r*
	Cosmocerca ornata	*S.r*
Trichostrongylidae : Molineoidea (Leiper, 1912)	*Oswaldocruzia* sp.	*S.r; S.m; S.p; S.x*
Rhabdiasidae Railliet, 1916	*Rhabdias africanus*	*S.r; S.m; S.p; S.x*
	Rhabdias sp.	*S.r; S.m*

Legender : *H.o*: *Hoplobatrachus occipitalis*; *S.r* : *Sclerophrys regularis*; *S.m* : *Sclerophrys maculata*; *S.p* : *Sclerophrys pentoni*; *S.x* : *Sclerophrys xeros*

Prevalence and mean intensity of parasitic nematodes in Anurans according to infested organs

Table 2 presents the prevalence of nematode infections in anurans based on the infested organs. ANOVA tests indicated p-values above the significance threshold (p = 0.7128 > 0.05 for prevalence by organ; p = 0.8890 > 0.05 for mean intensity by organ). No significant variation was observed in prevalence or mean intensity in relation to the type of organ or the species of parasite. Thus, there is no relationship between the type of organ, the species, and the level of infestation in the host.

Table 2: Prevalence and mean intensity of parasitic nematodes in anurans from Burkina Faso according to infested organs

Organs of predilection	Parasites	Abundance	Prevalence P (%)	Mean intensity (IM)
Stomach	*Physaloptera* sp.	61	0.40	20.30
Small intestine	*Amplicaecum pesteri*	129	3.36	5.16
Large intestine	*Aplectana* sp. *Cosmocerca ornata* *Cosmocercella* sp. *Cosmocercoides* sp. *Oswaldocruzia* sp.	2300	84.65	3.43
Lungs	*R. africanus* *Rhabdias* sp.	1140	20.86	7.35

In contrast, the distribution of parasite abundance by organ is highly significant ($p < 0.001$), indicating that certain organs are preferentially targeted by parasites (for example, the small intestine and lungs host significantly more parasites than the stomach). Of the 9 species of parasites, five (5) are found in the Large intestine (84.65%), two (2) in the Lungs (20.86%) and one species in the other organs, that are Small intestine (3.36%) and the Stomach (0.40 %).

Overall prevalence and mean intensity of parasitic nematodes in anurans according to host sex

Qualitatively, a greater number of parasite species is inventoried in female sex anurans (9 nematode species) than in male anurans (6 nematode species), which would indicate a greater sensitivity of females to nematodes. Table 3 presents the prevalence of nematode infections in anurans based on the sex of the host species. A Chi-square (χ^2) test indicated p-values above the significance threshold for differences in prevalence between sexes (X-squared = 3.5289, df = 1, p-value = $0.06031 > 0.05$). Consequently, the observed differences in prevalence and mean intensity of infections are not statistically significant between males and females.

Table 3: Overall prevalence and mean intensity of parasitic nematodes in anurans from Burkina Faso according to host sex

Sex	Nematodes	Prevalence	Mean intensity	
Female	*A.pesteri*	2.8	7.92	
	Aplectana sp.	36.36	18.72	
	C.ornata	1.17	6.8	**51.27%**
	Cosmocercella sp.	0.47	49	
	Cosmocercoides sp.	61.07	34.44	
	Oswaldocruzia sp.	22.38	11.23	
	Physaloptera sp.	0.7	20.33	
	R.africanus	21.21	10.49	
	Rhabdias sp.	2.33	2.8	
Male	*A.pesteri*	4.14	2.46	
	Aplectana sp.	39.81	16.25	
	Cosmocercoides sp.	59.55	29.45	
	Oswaldocruzia sp.	33.44	7.18	**38.76%**
	R.africanus	16.56	5.06	
	Rhabdias sp.	0.64	1	

In contrast, females demonstrate greater species richness, hosting 9 nematode species compared to 6 in males. This difference may suggest that females have a higher ecological exposure or susceptibility to infections.

Overall prevalence and mean intensity of parasitic nematodes in anurans according to seasons

The overall prevalence of nematodes in anurans collected during the rainy season is 46.16% and 43.87% during the dry season. When comparing infection prevalence between the two seasons, as shown in Table 4, no significant difference was found based on a chi-square test (X-squared = 1.4013, df = 1, p-value = 0.2365 > 0.05). Therefore, the levels of parasitism in the anurans collected do not appear to be influenced by the season. However, a statistically significant difference in mean infection intensity was observed between the seasons (p = 0.0367 < 0.05), with certain species, such as *Cosmocercoides* sp., showing higher intensity during the dry season. In contrast, overall prevalence and total abundance did not vary significantly between the seasons. Species richness remained consistent across both seasons (9 species), indicating that while nematode diversity is stable, the intensity of infestation varies.

Table 4: Overall prevalence and mean intensity of parasitic nematodes in anurans from Burkina Faso according to seasons

Season	Nematodes	Abundance	Mean intensity	Prevalence globale (%)
Rainy	*Aplectana* sp.	2300	21.67	
	Oswaldocruzia sp.	1257	7.57	
	R. africanus	518	6.64	
	Rhabdias sp.	4	1.33	
	A. pesteri	52	10.40	**46.16%**
	Cosmocercoides sp.	7164	16.70	
	Physaloptera sp.	0	0.00	
	C.ornata	0	0.00	
	Cosmocercella sp.	0	0.00	
Dry	*Aplectana* sp.	2332	17.66	
	Oswaldocruzia sp.	1667	30.30	
	R.africanus	928	14.27	
	Rhabdias sp.	24	2.67	
	A.pesteri	117	7.80	**43.87%**
	Cosmocercoides sp.	6828	31.03	
	Physaloptera sp.	61	20.34	
	C.ornata	98	49.00	
	Cosmocercella sp.	6	6.00	

Overall prevalence and mean intensity of parasitic nematodes in anurans according to collection sites of host species

Figure 1 presents the abundance of nematodes according to the collection sites of the anurans, which are located in the provinces of Kadiogo (urban and peri-urban areas), Ganzourgou (urban and peri-urban areas), and Houet (urban and peri-urban areas).
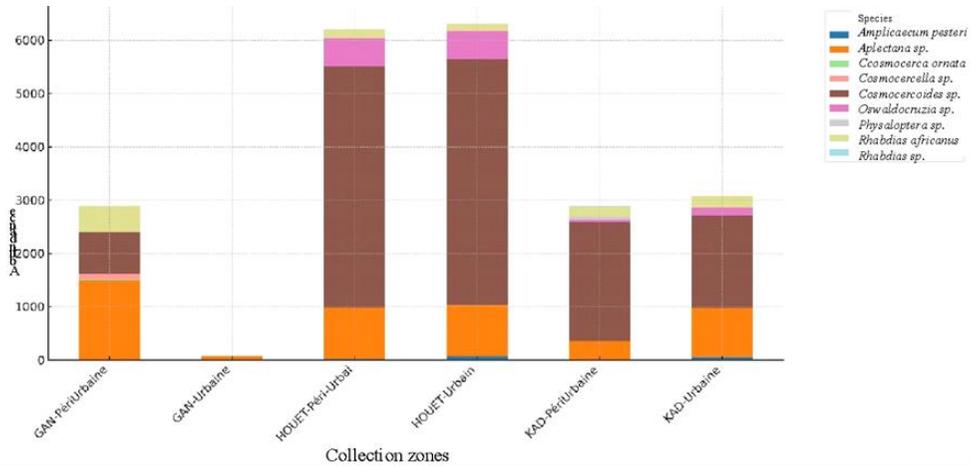
Figure 2: Histogram showing the abundance of parasitic nematodes in anurans from Burkina Faso according to the collection sites of host species. KAD: province of Kadiogo; GAN: province of Ganzourgou; Houet: province of Houet

Nematode abundance is greatest in the Houet province, followed by Kadiogo and Ganzourgou. This indicates that nematodes are more abundant in Houet, or that anurans from Houet have greater exposure to nematodes compared to those from Kadiogo and Ganzourgou. This trend may be attributed to Houet's higher levels of industrialization and a greater number of agricultural zones, which typically involve increased chemical usage compared to Kadiogo and Ganzourgou.

Distribution of nematode species according to anuran species and provinces

The Principal Component Analysis (PCA) depicts the relationship between nematode species and amphibian collection sites. Notably, four species of nematodes *Cosmocercoides* sp., *Aplectana* sp., *Rhabdias* sp., and *R. africanus* were found at all sites excepted Ganzourgou urban areas. The Ganzourgou urban areas are home to unique species, such as *Cosmocerca ornata* and *Cosmocercella* sp., which were not present at the other two sites (Figure 2). Furthermore, six (6) species of nematodes were simultaneously observed at both the Houet and Kadiogo sites: *Rhabdias* sp., *R. africanus*, *Aplectana* sp., *Cosmocercoides* sp., *Oswaldocruzia* sp., and *Amplicaecum pesteri*. No nematodes common to the Kadiogo and Ganzourgou sites were identified in this study.
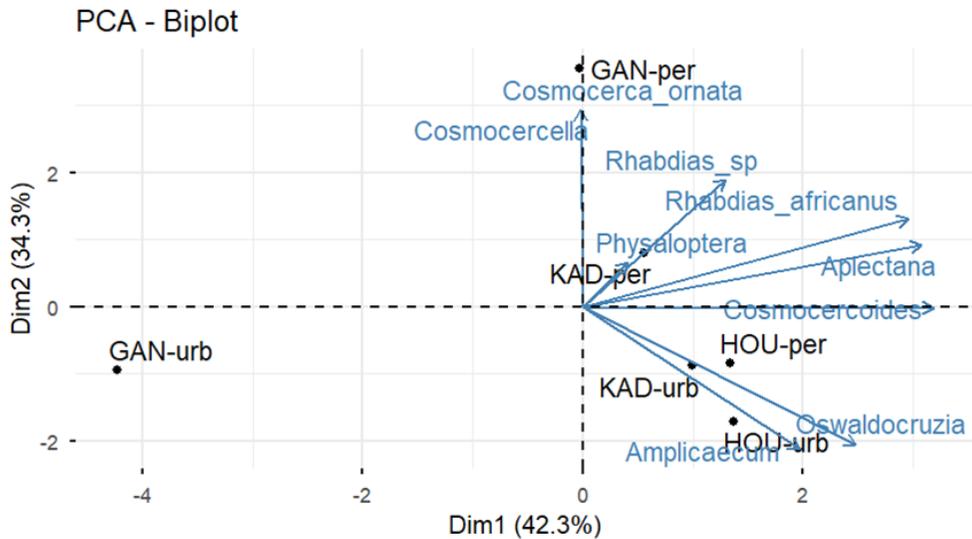
Figure 3: illustrates the Principal Component Analysis (PCA) with the species and abundance matrix of nematodes species according to anuran species and provinces. KAD-urb: province of Kadiogo urban area; KAD-per: Kadiogo peri-urban area; GAN-urb: province of Ganzourgou urban area; GAN-per: peri-urban area; HOU-urb: province of Houet urban area; HOU-per: Houet peri-urban area

Discussion

This study focused on four anuran species from the family Bufonidae, all of which are consumed in the Ganzourgou province of Burkina Faso (Mohneke et *al*., 2010). The anuran species identified in this research have been previously reported by Ayoro et *al*. (2020) in Burkina Faso and in various countries within the sub-region (Akani et *al*., 2011; Omereji, 2014; Reuben, 2014; Ukawu, 2014). The parasitic nematodes infecting anurans of the genus *Sclerophrys* in urban and peri-urban areas across three sites in Burkina Faso include nine species from four families: Oxyuridae, Rhabdiasidae, Trichostrongylidae, and Physalopteridae. All these nematodes, recorded for the first time in Burkina Faso, have also been identified in other African countries, including Benin (Aisien et *al*., 2011), Nigeria (Aisien et *al*., 2001, 2003, 2009, 2015, 2017b; Imasuen et *al*., 2012), Togo (Durette-Desset & Batcharov, 1974), and Cameroon (McAllister et *al*., 2010).

The Oxyuridae, the most numerous family of nematodes, are known to infect amphibians (Moravec et *al*., 1987; Patterson-Kane et *al*., 2001). Similarly, the genus *Oswaldocruzia* (family Molineidae) serves as a cosmopolitan parasite of both amphibians and reptiles (Ben Slimane & Durette-Desset, 1996). The genus *Rhabdias* (family Rhabdiasidae) specifically targets the lungs of amphibians and reptiles (Baker, 1987). It has a cosmopolitan distribution and is found in any environment where its hosts, such as frogs and toads, reside (Goater, 1992).

The overall prevalence of 90.04% indicates a significant nematode parasitic infestation in *Sclerophrys* toads collected in Burkina Faso. This high level of infestation can be attributed to the parasitic specificity of nematodes for these toads. On land, anurans are particularly susceptible to nematodes with direct life cycles (monoxenous), such as *Oswaldocruzia* sp., *Rhabdias* sp., and the Cosmocercinae, as most nematodes infect these amphibians through skin penetration or egg ingestion. Additionally, previous studies have shown that bufonid anurans tend to harbor a greater number of nematode species, as observed in this research (Bolek & Coggins, 2003). This is likely due to the fact that terrestrial toads primarily feed on ants, beetles, and other terrestrial invertebrates (Hirai & Matsui, 2002).

Among the 743 toads analysed, 667 were found to be infected with nematodes. *Cosmocercoides* sp. was present in nearly all large intestines of the four *Sclerophrys* species, highlighting their strict (oioxenous) infestation. The distribution of nematode abundance across different organs is highly significant, suggesting that certain organs are preferentially parasitized. For example, the small intestine and lungs host more parasites than the stomach. Previous studies (González & Hamann, 2008; González & Hamann, 2015) have shown that the preferred site of infestation for the genus *Physaloptera* is the stomach, while nematodes of the genus *Rhabdias* tend to favor the lungs. Our findings also indicate that nematodes are localized in the small intestine, large intestine, and stomach of the toads studied. Similar results have been reported by other authors (Aisien *et al.*, 2011; Imasuen *et al.*, 2012).

Our analysis of the influence of host sex on parasitic prevalence in these anurans from various environments showed that, regardless of the nematode species or the host's origin, sex does not significantly impact infection rates. Thus, the variability in nematode contamination is not influenced by the sex of the anurans. Previous studies have similarly found no correlation between host sex and parasite community structure (Santos & Amato, 2010; Santos *et al.*, 2013). While prevalence did not differ between sexes, females exhibited greater species richness, with 9 nematode species compared to 6 in males, suggesting that they may have higher ecological exposure or susceptibility.

When comparing infection prevalence between the rainy and dry seasons, no significant difference was observed. Therefore, parasitism in the collected anurans does not depend on season. This result is in line with work carried out by Oungbe (2021) in the south-eastern part of Côte d'Ivoire. Indeed, this author reported that there was no significant difference between parasite prevalence from one site to another depending on the season.

The urban and peri-urban zones of Kadiogo share several nematode species, including *Aplectana* sp., *Cosmocercoides* sp., *Amplicaecum* sp., *Physaloptera* sp., and *Oswaldocruzia* sp. In contrast, the peri-urban zone of

Ganzourgou hosts unique species, such as *C. ornata* and *Cosmocercella* sp., which are absent in the other areas. Some species, notably *Aplectana* sp., *Cosmocercoides* sp.*, Rhabdias* sp., and *R. africanus*, are common to all three areas.

This investigation reveals that nematode prevalence is highest in Houet province (97.94%), followed by Kadiogo (84.51%) and Ganzourgou (83.12%). This pattern can be attributed to the fact that the Ganzourgou province is less industrialized and less exposed to chemical products compared to the Kadiogo and Houet sites. The differences in infestation levels among the sites may be linked to environmental pollution from chemical fertilizers. Authors such as Hulme (2017), Goly et *al*. (2022), and Čeirāns et *al*. (2023) have noted that the development of directly transmitted parasites is influenced by environmental characteristics and pollution, which can affect the host's immune status. Immunosuppression may contribute to imbalances in parasite populations (Rollins-Smith, 2017). Similar findings have been reported by Aisien et *al*. (2017a) and Imasuen et *al*. (2012).

Conclusion

Examination of four *Sclerophrys anuran* species found in Burkina Faso revealed nine genera of parasitic nematodes. The overall high prevalence, as well as site-specific prevalence, indicated significant parasitic infestations in *Sclerophrys xeros* and *S. regularis*. The majority of nematodes were identified in the large intestine, small intestine, and lungs of the collected toads.

In this study, the analysis of the influence of season and sex on the prevalence and abundance of nematodes in these anurans from different environments indicated that, regardless of the parasite species or the host's origin, neither sex nor season significantly affected these parameters.

Finally, parasite abundance and diversity varied among the collection sites in this study. These findings will be essential for the management and conservation of anuran species, whose ecological significance is well established.

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