ESI Preprints **Not Peer-reviewed**

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

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Doi: 10.19044/esipreprint.12.2025.p290

Approved: 16 December 2025
Posted: 18 December 2025

Cite As:
Soubeiga, P., Boungou, M., Thiombiano, G.N., Chabi, A.M.B., Zougouri, N.E., Ouédraogo, N.A., Bagayan, M. & Sinare, Y. (2025). *Prevalence and Mean Intensity of Nematode Parasites in Anurans of the Genus Sclerophrys in Burkina Faso (West Africa)*. ESI Preprints. https://doi.org/10.19044/esipreprint.12.2025.p290

Abstract

As part of a study aimed at improving understanding of parasitic infections in anurans for their conservation in Burkina Faso, a survey 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. Anurans were collected at night using the Visual and Acoustic Encounter Survey (VAES) method, then dissected and examined for nematode infections. A total of 743 individuals of the genus *Sclerophrys* (family Bufonidae) were examined, with 669 found to be infected, resulting in an overall prevalence of 90.04%. Seasonal prevalence was 87.94% during the rainy season and 92.35% during the dry season, with the difference being statistically significant ($p < 0.05$). Nine nematode species were identified: *Amplicaecum pesteri*, *Cosmocerca ornata*, *Oswaldocruzia* sp., *Physaloptera* sp., *Rhabdias africanus*, *Rhabdias* sp., *Cosmocercella* sp., *Cosmocercoides* sp., and *Aplectana* sp. The most prevalent species was *Cosmocercoides* sp. (58.95%), while *Cosmocerca ornata* was the least prevalent (0.26%). This study provides significant insights into the *Sclerophrys* anurans population and their nematode parasites. Four Bufonidae toad species— *Sclerophrys maculata*, *S. regularis*, *S. pentoni*, and *Sclerophrys xeros*—were found to be infested by these nine nematode species. The high overall prevalence indicates a significant parasitic burden in these *Sclerophrys* toads. Furthermore, the parasitic load was notably higher in environments with increased pollution from chemical fertilizer use.

Keywords: Parasitic nematodes, anurans, *Sclerophrys*, Burkina Faso

Introduction

Amphibians, also known as Batrachians, comprise 8,579 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 constitute the most diverse order of amphibians globally (Lescure, 1991). To date, 36 anuran species belonging to eleven families have been recorded in Burkina Faso, including five species of the genus *Sclerophrys* (Ayoro et *al*., 2020). These animals serve as excellent bioindicators of aquatic environments (Rödel, 2000; Channing, 2001; Guerry & Hunter, 2002). As poikilothermic organisms (Morin, 2008), their moist, permeable skin and associated gas exchanges render them highly sensitive to environmental fluctuations. Consequently, anurans are valuable indicators of ecological stress within their habitats (Welsh & Ollivier, 1998; Adams, 1999). Ecologically, these organisms play a crucial role in trophic networks and are essential for maintaining the balance of wetland ecosystems (Channing, 2001). They consume pest insects, thereby reducing harmful

insect populations, which benefits agricultural productivity and contributes to malaria vector control (Channing, 2001).

Anurans also represent a significant food source for both humans and animals. Locally, they have long been considered as an essential source of protein (Angulo, 2008; Mohneke et *al*., 2009). In Burkina Faso, studies of Mohneke et *al*. (2010) indicate that nearly all anurans collected in their survey are consumed in the Ganzourgou province, where they are highly valued and used in traditional medicine. For instance, toads are used in soup preparations to treat respiratory infections, measles, and boils (Mohneke et *al*., 2011). Furthermore, direct contact between toad skin and scorpion sting sites or infected wounds is believed to benefit patients due to the antibacterial properties of compounds secreted by toad skin (Simmaco et *al*., 1998; Zhou et *al*., 2006).

However, toads serve as hosts for a diverse range of parasites, particularly nematodes (Goldberg et *al*., 2001). According to De Montaudouin et *al*. (2003), Lafferty et *al*. (2008), and Mohammad et *al*. (2015), parasitic nematodes can have various pathogenic effects, including growth retardation, skeletal deformities, impaired vision, and reduced fecundity in the host. Additionally, several nematode species are known to inhibit nutrient absorption in amphibian hosts, thereby altering their physiology, feeding behavior, and overall health (Knudsen et *al*., 2001). Despite their pathogenic potential, studies on the nematode fauna infecting amphibians remain limited. In Africa, research efforts include contributions from Aisien et *al*. (2001, 2003, 2009, 2011, 2015, 2017), Imasuen et *al*. (2012), and Ozemoka & Aisien (2021) in Nigeria; Maeder et *al*. (1969a), Murith (1981a, 1981b), Kouamé et *al*. (2015), Assemian (2016), Konan et *al*. (2016), and Oungbe et *al*. (2023) in Ivory Coast; Bourgat et *al*. (1976) in Benin; Skrjabin (1916), Rees (1964), and Fischthal & Thomas (1968) in Ghana ; Rödel (2000) and Joger & Lambert (2002) in Senegal ; Rödel (2000) and Rödel & Bangoura (2004) in Sierra Leone; Durette-Desset et *al*. (1974) in Togo ; Saoud (1964) and Gassmann (1975) in Cameroon; Meskal (1970) in Ethiopia; and Kuzmin (2001) and Halajian (2013) in South African. In Burkina Faso, although a few studies on anuran parasites (Soubeiga et *al*., 2020; 2025) have focused on associated platyhelminths, research on the nematode parasites of anurans remains scarce. Therefore, this study aims to inventory the nematode parasites of anurans in Burkina Faso, determine their prevalence and mean intensity,and ultimately contribute to enhanced conservation efforts for anuran populations.

Materials and Methods

Study Sites

The toads examined in this study were captured from water bodies in urban and peri-urban areas of Ouagadougou, Ganzourgou, and Bobo-Dioulasso (Table1). The study was conducted in Burkina Faso's two largest cities, Ouagadougou and Bobo-Dioulasso, which together account for nearly two-thirds (65.8%) of the urban population, along with 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 for local communities. These toads are also among the most widely distributed anuran species in Burkina Faso (Ayoro et *al*., 2020).

Table 1: Capture sites for toad specimens

Sites	Latitudes	Longitudes
Urban areas of Houet	N11°17'49.0"	W4°20'28.7"
Peri-urban areas of Houet	N11°22'28.8"	W°24'46.3"
Peri-urban areas of Kadiogo	N12°12'20.27124''	W1°21'1065''
Urban areas of Kadiogo	N12°22'46.37568''	W1°30'5.0868''
Peri-urban areas of Ganzourgou	N12°19'772''	W000°50'180''
Urban areas of Ganzourgou	N12°14.751'	W000°37.510'

Toad Sampling and Collection of Parasitic Nematodes

Toad were collected from June 2022 to November 2023. Sampling was conducted manually near water reservoirs (canals, ponds, and dams) between 18:00 and 21:00, and again from 04:00 to 06:00. Toad specimens were captured using the visual and acoustic survey method outlined by Crump and Scott (1994), which involved searching for toads by lifting rocks, scanning around water bodies with headlamps, and locating individuals by their calls. Each captured toad was placed individually in a cotton bag and transported to the Laboratory of Animal Biology and Ecology (LBEA) at Joseph KI-ZERBO University.

Once in the laboratory, toads were identified using identification keys provided by 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 the cloaca) was made to remove the internal organs (digestive tract, liver, heart, gallbladder, and gonads) for the examination of parasitic nematodes. Nematode specimens did not require staining due to their rigid cuticle, which prevents dye penetration. The procedures followed the method outlined by Aisien et *al*. (2009).

Nematodes were examined in temporary mounts after clearing in a drop of lactophenol for 3 to 5 minutes. This clearing agent allows for

detailed observation of nematode characteristics. Morphometric measurements of nematodes, including body and organ dimensions, were taken using a ZEISS ICS standard 25 light microscope equipped with an optical micrometer.

Nematode identification was conducted using relevant taxonomic keys (Yamaguti, 1961; Baker, 1987; Khalil et *al*., 1994). Specimens were examined under a Carl ZEISS light microscope, with identification based on their anatomical and morphological characteristics. After identification, the nematodes were immediately fixed and preserved in 70% ethanol.

Data analyses

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

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

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):

$$IM = n/p$$

Descriptive statistics were used to characterize the sample, including the total number of anurans examined and infected, overall and species-specific prevalence, and mean abundance and intensity across different categories.

To assess the influence of biological and environmental factors on parasitic parameters, analyses of variance (ANOVA) were conducted. The significance threshold was set at $p < 0.05$.

Results

Parasite Hosts

A total of 743 specimens representing four anuran species belonging to a single genus, *Sclerophrys*, were collected for examination and screening of parasitic nematodes . These species include *Sclerophrys maculata* (Hallowell, 1854), *S. pentoni* (Andersson, 1893), *S. regularis* (Reuss, 1833), and *S. xeros* (Tandy *et al.*, 1976), all members of the family Bufonidae. The identified nematodes were classified into five families: Oxyuridae, Trichostrongylidae, Physalopteridae, Rhabdiasidae, and Ascarididae, comprising nine species: *Cosmocerca ornata* Diesing, 1861, nec Duj.,1845 ; *Cosmocercoides* sp. Wilkie, 1930; *Cosmocercella* sp. Steiner, 1924;

Aplectana sp. Railliet & Henry, 1916; *Amplicaecum pesteri* (or *Orneoascaris chrysanthemoides* Skryjabin, 1916); *Physaloptera* sp. Rud.,1819; *Oswaldocruzia* sp. Travassos, 1917; *Rhabdias* sp. Stiles & Hassall, 1905; and *Rhabdias africanus* Kuzmin, 2001. Among the 743 anurans collected, the overall prevalence of parasitism was 90.04%. Within this study, *Sclerophrys regularis* was the most heavily parasitized species, with a prevalence of 67.70%, followed by *Sclerophrys maculata* at 11.44%, *Sclerophrys xeros* at 5.51% and *Sclerophrys pentoni* at 2.96%.

Prevalence and mean intensity of parasitic nematodes in Anurans from Burkina Faso 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

Parasites	Organs of predilection	Abundance	Prevalence P (%)	Mean intensity (IM)
Amplicaecum pesteri	Small intestine	129	3.36	5.16
Aplectana sp.	Large intestine	864	14.80	7.85
Cosmocerca ornata	Large intestine	6	0.26	3.00
Physaloptera sp.	Stomach	61	0.40	20.30
Cosmocercella sp.	Large intestine	96	0.13	96.00
Cosmocercoides sp.	Large intestine	104	1.61	8.67
Oswaldocruzia sp.	Large intestine	1230	3.50	28.77
R. africanus	Lungs	1110	19.24	7.76
Rhabdias sp.	Lungs	30	1.61	2.50

In contrast, the distribution of parasite abundance by organ is highly significant ($p < 0.001$), indicating that certain organs are preferentially target by parasites (for example, the small intestine and lungs host significantly more parasites than the stomach).

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

Table 3 presents the prevalence of nematode infections in anurans based on the sex of the host species. ANOVA tests indicated p-values above the significance threshold for differences in prevalence between sexes ($p =$

0.9202 > 0.05). Similarly, the ANOVA test for mean nematode intensity between sexes also yielded a p-value above the threshold (p = 0.5395 > 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
	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
	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 from Burkina Faso according to seasons

The prevalence of nematodes in anurans collected during both the rainy and dry seasons ranges from 0 to 100%. When comparing infection prevalence between the two seasons, as shown in Table 4, no significant difference was found based on a chi-square test (p = 0.16 > 0.05). Therefore, the levels of parasitism in the anurans collected do not appear to be influenced by the season.

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

Season	Nematodes	Abundance	Prevalence (%)	Mean intensity
Rainy	*Aplectana* sp.	2300	27.17	21.67
	Oswaldocruzia sp.	1257	42.56	7.57
	R. africanus	518	20.00	6.64
	Rhabdias sp.	4	0.77	1.33

Season	Nematodes	Abundance	Prevalence (%)	Mean intensity
	A. pesteri	52	1.28	10.40
	Cosmocercoides sp.	7164	57.94	16.70
	Physaloptera sp.	0	0.00	0.00
	C.ornata	0	0.00	0.00
	Cosmocercella sp.	0	0.00	0.00
Dry	*Aplectana* sp.	2332	37.40	17.66
	Oswaldocruzia sp.	1667	15.58	30.30
	R.africanus	928	18.41	14.27
	Rhabdias sp.	24	2.55	2.67
	A.pesteri	117	4.25	7.80
	Cosmocercoides sp.	6828	62.32	31.03
	Physaloptera sp.	61	0.85	20.34
	C.ornata	98	0.57	49.00
	Cosmocercella sp.	6	0.28	6.00

The prevalence of nematodes in anurans collected during both the rainy and dry seasons ranged from 0 to 62.32%. When comparing infection prevalence and abundance between the two seasons, as shown in Table 3, no significant differences were found using ANOVA tests ($p = 0.9357 > 0.05$ for prevalence and $p = 0.9381 > 0.05$ for abundance). 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.

Overall prevalence and mean intensity of parasitic nematodes in anurans from Burkina Faso 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).

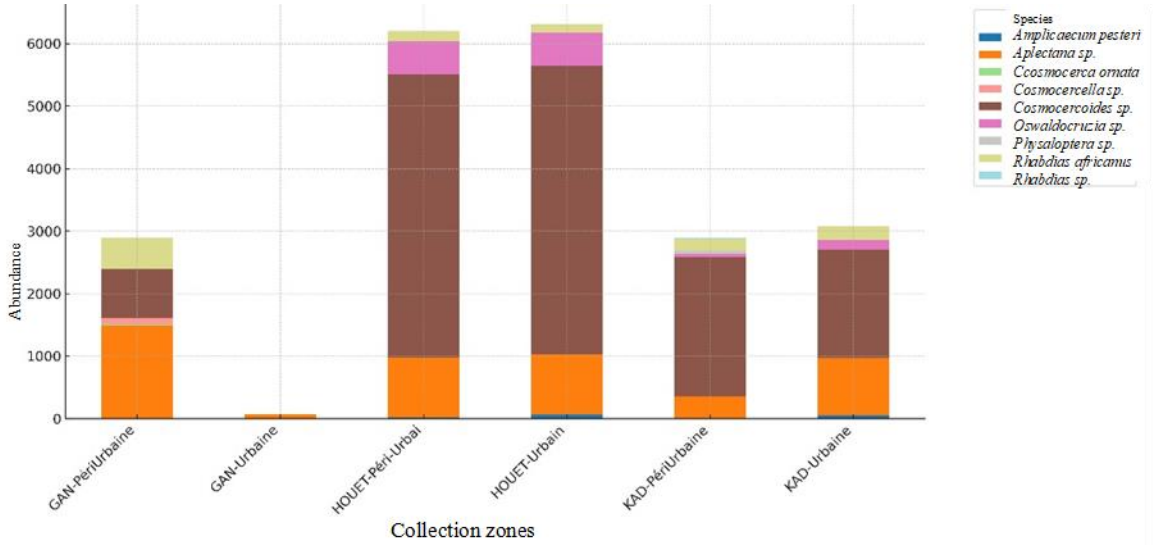

Figure 1: Histogram showing the abundance of parasitic nematodes in anurans from Burkina Faso according to the collection sites of host species. KAD: province of Kadiogo; GAN: province of Ganzourgou ; Houet: province of Houet.

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.

Relationship between different nematode species according to collection sites

Figure 2 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 three sites. The Ganzourgou site is home to unique species, such as *Cosmocerca ornata* and *Cosmocercella* sp., which were not present at the other two sites (Figure 2). Furthrmore, 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.

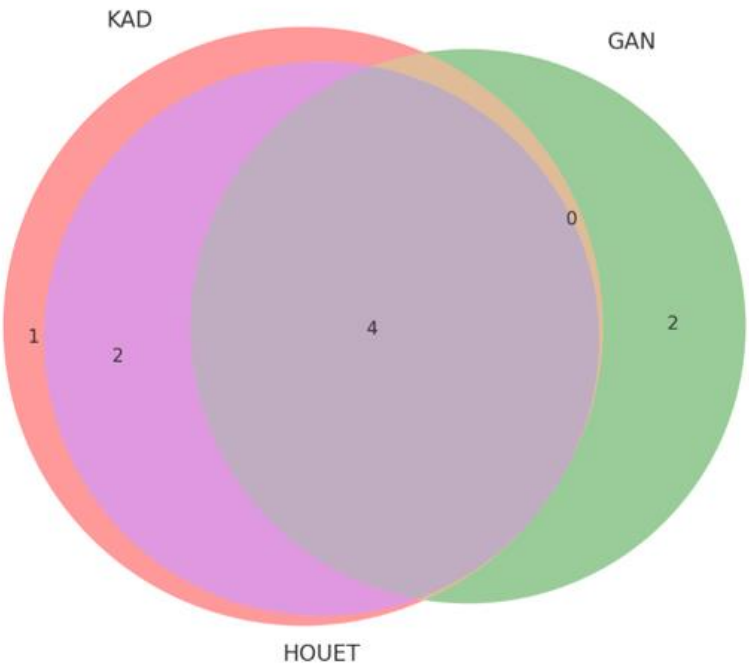

Figure 2 illustrates the relationship between nematode genera according to the anuran collection sites. KAD: province of Kadiogo; GAN: province of Ganzourgou ; Houet: province of Houet

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 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 particulary 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 organ 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 south-eastern 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., and *Oswaldocruzia* sp. In contrast, the peri-urban zone of Ganzourgou hosts unique specie, 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 chemicals 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.

Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All data are included in the content of the paper.

Funding Statement: The authors did not obtain any funding for this research.

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