

# Abundance, Diversity, and Distribution of Platyhelminthes of the African Tiger Frog (*Hoplobatrachus occipitalis* Günther 1858) from Two Climatic Zones in Burkina Faso (West Africa)

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## Abstract

From June 2022 to November 2023, frogs (*Hoplobatrachus occipitalis*) from the urban and peri-urban areas of Ouagadougou, Ganzourgou, and Bobo-Dioulasso were examined for Platyhelminthes. The aim was to inventory frog Platyhelminthes in two climatic zones in Burkina Faso. The collection method used was a visual and acoustic approach. After each collection, the frogs were euthanized and dissected to examine the lungs, bladder, digestive tract, and general cavity for Platyhelminthes. 1203 Platyhelminthes in 14 species were collected from 238 frog specimens. The Platyhelminthes collected belonged to five (05) genera: *Diplodiscus*, *Ganeo*, *Halipegus*, *Haematolechus*, and *Cephalochlamys*. The overall prevalence of Platyhelminthes was high (69.75%) in the 10 sites surveyed. Of the 238 specimens examined, 166 were infested with Platyhelminthes. The frog's small intestine was the preferred zone for most of these Platyhelminthes. These results could be used for large-scale studies to assess the impact of these Platyhelminthes on frog morbidity and mortality.

## Keywords

Amphibians, Anurans, Conservation, Helminthes, Parasites, Burkina Faso

## 1. Introduction

Anurans are the world's most diverse amphibians or batrachians [1]. They comprise 55 families divided into 451 genera and 6968 species [2]. These animals are vital to man in terms of food, agriculture, and the economy [3]. In terms of food, certain Anuran species, such as the African tiger frog (*Hoplobatrachus occipitalis* Günther), are an important source of animal protein in Nigeria, Benin, and Burkina Faso [4] [5]. This frog (*Hoplobatrachus occipitalis*) is an important source of animal protein in the diet of the local population of Ganzourgou, Gourma, and southeastern Burkina Faso [6] [7]. [8] noted that these Anurans were used to treat hypertension, tuberculosis, conjunctivitis, abortion, and Kwashiorkor. However, amphibian populations are declining worldwide, and many factors, such as habitat loss, toxins, and pathogens, are implicated in this decline [9] [10]. In addition, anthropogenic stressors, such as livestock grazing wetlands, cause amphibians to become more susceptible to infection due to a lowered immune system [11]. The causes of these declines are multiple, ranging from habitat destruction and degradation, pollution, and the synergistic action of climate change to the removal of natural resources for human consumption and disease [12]. According to [13], pathogens such as Platyhelminthes and new emerging diseases are major causes of the decline of the frog population. These pathogens generally cause significant mortalities, mainly in the ranks of wild amphibian populations in Africa. In addition, high levels of Platyhelminthes infestation cause significant mortalities in African anurans [14]. These parasites can cause numerous pathologies and even death in their hosts [15]. Both [16] and [17] provide experimental evidence that a Trematode parasite, *Ribeiroia* sp., is responsible for the severe limb deformities observed in certain amphibian populations in several regions of North America. However, minimal research on amphibian Platyhelminthes has been conducted thus far in Burkina Faso. This study aims to further the understanding of the Platyhelminthes associated with the African tiger frog (*Hoplobatrachus occipitalis*) in Burkina Faso, focusing on their diversity and distribution.

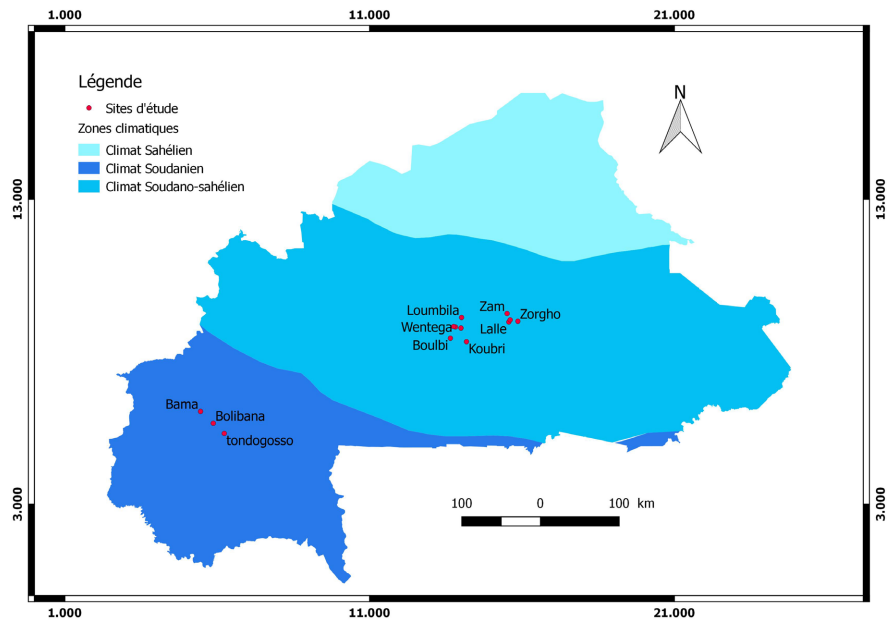
## 2. Materials and Methods

### 2.1. Study Area

The frogs examined in our study were captured in bodies of water in the urban and peri-urban areas of Ouagadougou, Ganzourgou, and Bobo-Dioulasso (Figure 1). The study was conducted in the two largest cities of Burkina Faso (Ouagadougou and Bobo Dioulasso) as they concentrate practically two-thirds (65.80%) of the urban population with its corollaries of pollution [18]. The Ganzourgou province was chosen as the site because of the high diversity of the frog (*Hoplobatrachus occipitalis*). Also, the local community consumes this frog a lot.

This species was chosen because amphibians are edible and have a high breeding potential. *H. occipitalis* is the most widely sold species, as it is easy to harvest and has a large thigh with a high economic value. They are also widely distributed

amphibian species in Burkina Faso [19].



**Figure 1.** Map showing collection sites for African tiger frogs (*Hoplobatrachus occipitalis*).

## 2.2. Frog Sampling

The frog collection period ran from June 2022 to November 2023. Sampling was carried out by capture near the ponds between 6 pm and 9 pm and between 4 am and 6 am. Frog species were collected by hand using mist nets and the visual and acoustic approach of [20] after examining the frogs' hiding places on rocks, looking around the ponds with headlamps, and locating them by their croaks. Systematic visual searches consisted of scraping through leaf litter, lifting rocks and logs and looking around or within burrows and termite mounds. Additional supports of acoustic signals were applied [21]. Specimens were collected by hand and photographed. Each frog captured alive was placed in a cotton bag and transported to the laboratory for identification and parasite testing. In the laboratory, the frogs were identified using the keys of [22]-[24].

## 2.3. Collection and Identification of Platyhelminthes

Frogs were euthanized by immersion in a benzocaine solution (14% - 20%) for dissection [25]. The lungs, bladder, digestive tract, and appendages were dissected and placed separately in a Petri dish containing 0.72% saline solution, then examined to harvest Platyhelminthes. The contents of each organ were excavated in saline solution and examined under a dissecting loupe. Platyhelminthes specimens were fixed with 5% formalin saline, removed after 24 hours, and placed in 70% alcohol. Specimens were stained with acetic carmine and permanently mounted between slides and coverslips in a drop of Canada balsam. After drying in an oven, the preparation was observed under a ZEISS optical microscope (ICS 25 standard) equipped with an optical micrometer. Cestodes and Digenea were

identified using the identification keys of [26] [27].

## 2.4. Data Analysis

Abundance ( $A$ ), prevalence ( $P$ ), and average intensity ( $IM$ ) were calculated using the method of [28]. The parasite abundance ( $A$ ) is represented by the ratio of the total number of individuals of a parasite species ( $N$ ) in a sample of hosts to the total number of hosts ( $H$ ) in a sample. It is, therefore, the average number of individuals of a parasite species ( $N$ ) per host examined. It is expressed as follows:

$$A = N/H$$

The prevalence rate (in %) is calculated as the ratio between the number of host species infected ( $N_i$ ) by a Platyhelminth species and the total number of hosts examined ( $H_t$ ). The following mathematical formula expresses it:

$$P(\%) = N_i/H_t \times 100$$

Average infection intensity refers to the number of parasites ( $P$ ) per host ( $N$ ) ( $N$  is the total number of hosts infected by a Platyhelminth species). The subsequent formula expresses it:

$$IM = P/N$$

The parasite abundance as a function of sites was presented using bar plots. The Venn diagram was used to present the relationship between Platyhelminth species and attachment sites using the “draw.quintuple function” from the “VennDiagram” package. A Spearman correlation test was used to assess the strength of the correlation between parasite abundance and parameters such as weight and height using the ggscatter function from the “ggpubr” package. Parasite abundance as a function of season and sex was presented in boxplots using the ggviolin function in the “ggplot2” package. All statistical tests were performed with R software version 4.2.0 at a significance level of 5%.

## 3. Results

### 3.1. Platyhelminthes Hosts

Two hundred thirty-eight (238) specimens of *Hoplobatrachus occipitalis* belonging to the family Dicroglossidae were collected for examination and testing for Platyhelminthes. The 238 collected specimens included 180 female and 58 male frogs, for a total prevalence of 69.75%. By sex, the prevalence of females and males collected was 65.00% and 84.48% respectively. According to the season, the prevalence of infection was 78.30% in the rainy season and 46.11% in the dry season.

### 3.2. Platyhelminthes

Of the 238 specimens of *Hoplobatrachus occipitalis* analyzed, a total of 14 species of Platyhelminthes were encountered (Table 1). These species belonged to five (05) genera, divided into four (04) genera of Digenea (*Diplodiscus*, *Ganeo*, *Halipegus*, *Haematoloechus*) and one (01) genus of Cestodes (*Cephalochlamys*): *Dip-*

*Iodiscus fischthalichus*, *Diplodiscus lali*, *Diplodiscus subclavatus*, *Diplodiscus amphichrus*, *Ganeo africana*, *Ganeo glottoides*, *Halipegus* sp., *Haematoloechus variegatus*, *Haematoloechus nanchangensis*, *Haematoloechus parviplexus*, *Haematoloechus micrurus*, *Haematoloechus exoterorchis*, *Haematoloechus* sp., *Cephalochlamys namaquensis*.

**Table 1** summarizes the different species inventoried during the study.

**Table 1.** List of Platyhelminthes, their hosts, and attachment organs.

Classes	Parasite Species	Hosts	Infected Organs			
			Lungs	Small Intestine	Large Intestine	Stomach
Trematodes	<i>Haematoloechus</i> sp.	<i>Ho. occipitalis</i>	+	-	-	-
	<i>Haematoloechus variegatus</i>	<i>Ho. occipitalis</i>	+	-	-	-
	<i>Haematoloechus nanchangensis</i>	<i>Ho. occipitalis</i>	+	-	-	-
	<i>Haematoloechus exoterorchis</i>	<i>Ho. occipitalis</i>	+	-	-	-
	<i>Haematoloechus micrurus</i>	<i>Ho. occipitalis</i>	+	-	-	-
	<i>Haematoloechus parviplexus</i>	<i>Ho. occipitalis</i>	+	-	-	-
	<i>Diplodiscus fischthalichus</i>	<i>Ho. occipitalis</i>	-	-	+	-
	<i>Diplodiscus lali</i>	<i>Ho. occipitalis</i>	-	-	+	-
	<i>Diplodiscus subclavatus</i>	<i>Ho. occipitalis</i>	-	-	+	-
	<i>Diplodiscus amphichrus</i>	<i>Ho. occipitalis</i>	-	-	+	-
	<i>Ganeo africana</i>	<i>Ho. occipitalis</i>	-	+	-	-
	<i>Ganeo glottoides</i>	<i>Ho. occipitalis</i>	-	+	-	-
	<i>Halipegus</i> sp.	<i>Ho. occipitalis</i>	-	-	-	+
Cestodes	<i>Cephalochlamys namaquensis</i>	<i>Ho. occipitalis</i>	-	+	-	-

Legend: + = present and - = absent; *Ho. Hoplobatrachus*.

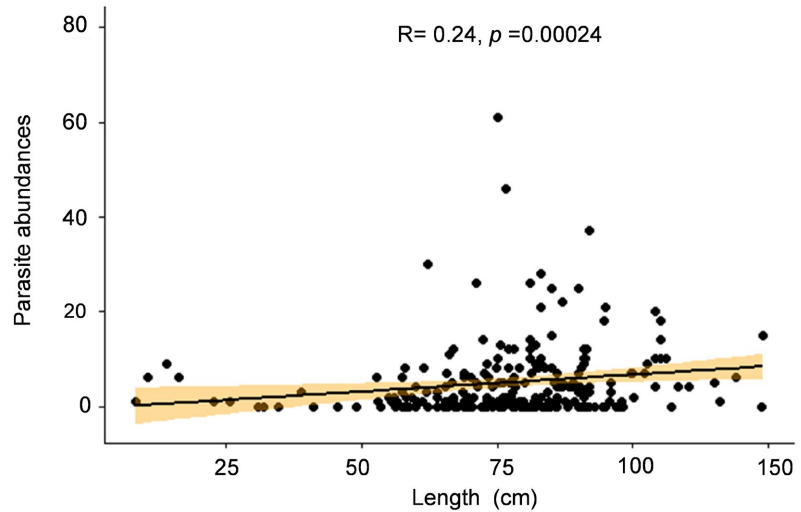
### 3.2.1. Platyhelminthes Abundance as a Function of Host Frog Size

The specimens vary in size from 5 to 150 cm. The correlation between Platyhelminthes abundance and host frog size is shown in **Figure 2**. Analysis of this figure shows that, as frog size increases, so does Platyhelminthes abundance ( $r = 0.24$  and  $p = 0.00024$ ). The Correlation and Linear Regression Test applied to the data indicates that Platyhelminthes abundance positively correlates with host frog size ( $p < 0.05$ ). However, frogs between 50 and 60 cm in size hosted the highest number of Platyhelminthes. As a result, this study shows that size significantly affects the abundance of Platyhelminthes in these frogs.

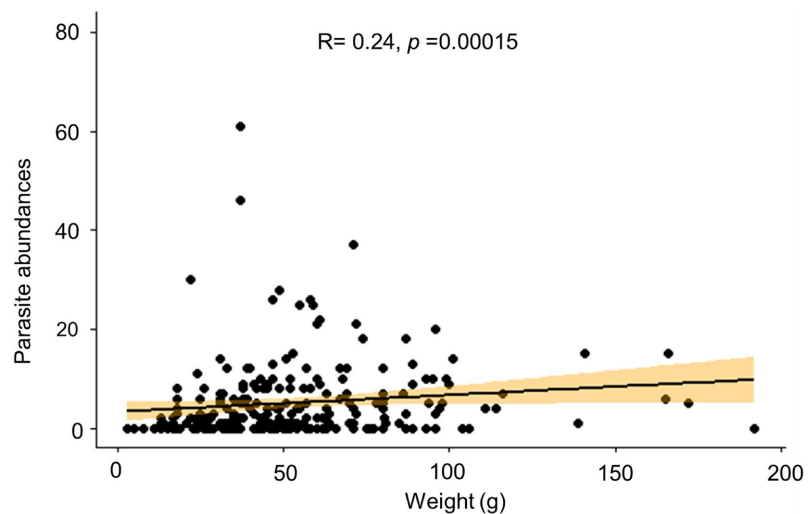
### 3.2.2. Platyhelminthes Abundance as a Function of Weight

The frog specimens collected weighed between 20 and 195 g. Frogs weighing between 5 and 100g exhibited a significant prevalence of Platyhelminthes (**Figure 3**). The analysis of this image indicates that frog weight correlates positively with

the abundance of Platyhelminthes ( $r = 0.24$  and  $p = 0.00015$ ). The Correlation and Linear Regression Test applied to the data indicates that Platyhelminthes abundance varied with frog weight ( $p < 0.05$ ). Accordingly, their weight significantly influences the number of Platyhelminthes infesting these frogs.



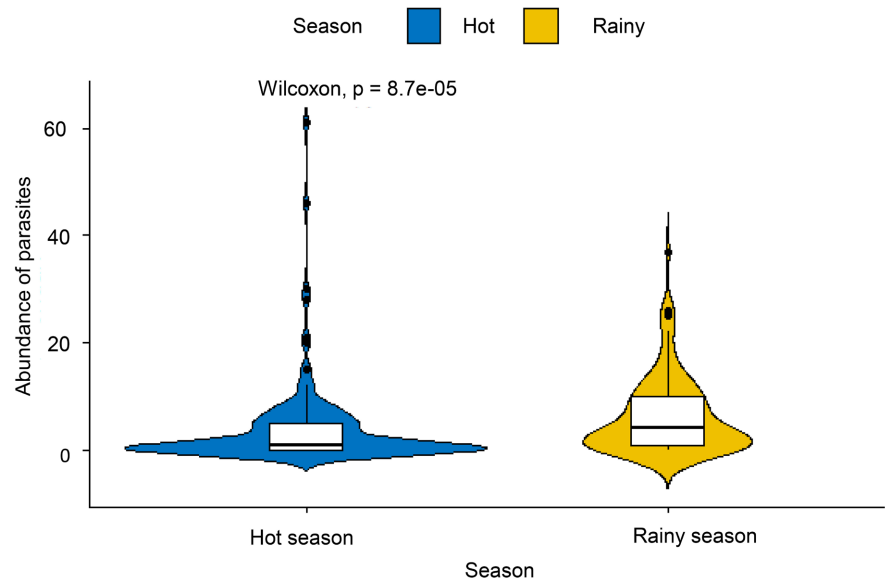
**Figure 2.** Correlation line between Platyhelminthes abundance and host frog size.



**Figure 3.** Correlation line between Platyhelminthes abundance and host frog weight.

### 3.2.3. Seasonal Abundance and Prevalence of Platyhelminthes

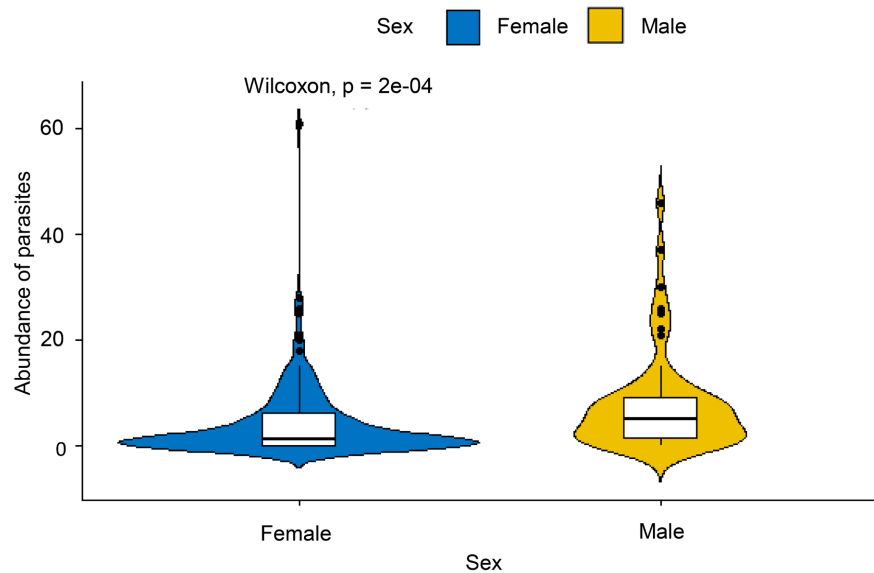
The analysis of Platyhelminthes abundance in *Hoplobatrachus occipitalis* as a function of season showed that there was no significant difference in abundance according to season with the Wilcoxon test ( $p = 0.000087 < 0.05$ ) (Figure 4). The abundance of amphibians collected depended on the season. The prevalence of Platyhelminthes in frogs collected in both rainy and dry seasons ranged from 0 to 100%. Comparing this prevalence between the two seasons, as shown in Figure 4, we found no significant difference between the rainy and dry seasons (chi-square test,  $p = 0.16 > 0.05$ ).



**Figure 4.** Platyhelminthes abundance by season.

### 3.2.4. Abundance and Prevalence of Platyhelminthes by Sex

The prevalence is higher in males (84.48%) than in females (65.00%). **Figure 5** shows Platyhelminthes abundance as a function of host frog sex. The Wilcoxon test yields a p-value below the significance threshold ( $p \leq 0.05$ ). As a result, parasite abundance varies by host sex, although there is no significant difference (**Figure 5**). However, prevalence was higher in males (84.48%) than in females (65.00%).

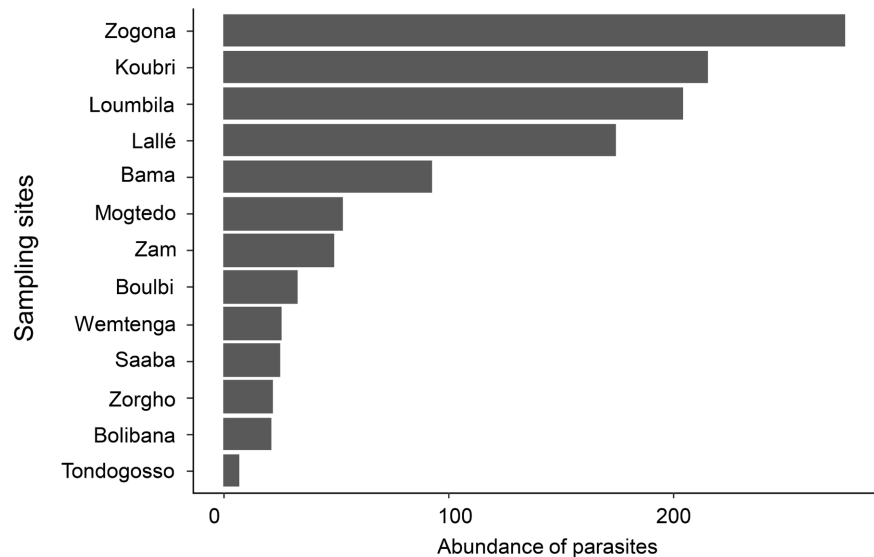


**Figure 5.** Platyhelminthes abundance by sex.

### 3.2.5. Platyhelminthes Prevalence According to Site

**Figure 6** illustrates the prevalence of Platyhelminthes as a function of African tiger frog sampling sites, *i.e.*, the urban and peri-urban areas of Ganzourgou, Ouaga-

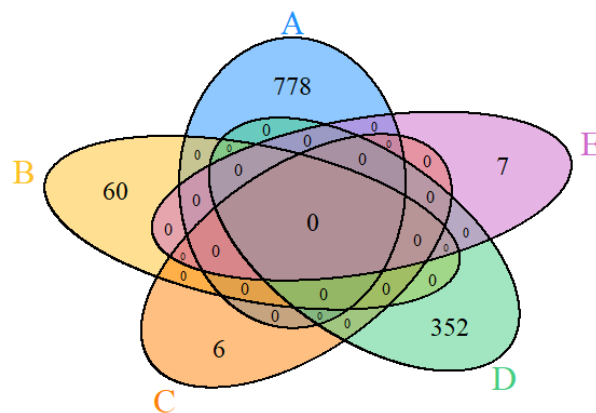
dougou, and Bobo-Dioulasso. Platyhelminthes prevalence was highest in the commune of Tondogosso (100%), followed by Bama (90.91%) and Koubri (88.00%). Moreover, the lowest prevalence was observed in the commune of Zorgho (34.78%). This outcome indicates that frogs in Tondogosso exhibit a higher prevalence of infection or contact with Platyhelminthes than those in Zorgho.



**Figure 6.** Prevalence of Platyhelminthes species by site.

### 3.2.6. Relationship between Platyhelminthes Species According to Attachment Sites

**Figure 7** shows no relationship between Platyhelminthes species according to their attachment sites within amphibians. Indeed, each genus of Platyhelminthes had its own preferred attachment site. All Cestodes of genus *Cephalochlamys* were collected from the small intestine, Trematodes of genus *Ganeo* and genus *Haematoloechus* from the lungs, those of genus *Diplodiscus* from the large intestine, and those of genus *Halipegus* from the stomach.



**Figure 7.** Relationship between Platyhelminthes species according to attachment sites. A: Lung trematodes; B: Small intestine trematodes; C: Small intestine cestodes; D: Large intestine trematodes; E: Stomach trematodes.

## 4. Discussion

The study concerned the only frog species belonging to the Dicroglossidae family in Burkina Faso (*Hoplobatrachus occipitalis*). Specimens of this species are not only consumed in Burkina Faso [6] but also in other African countries such as Nigeria [4], Côte d'Ivoire [29], and Benin [6]. This study revealed an overall prevalence of 69.75% of Platyhelminthes infection in African tiger frogs. This study enabled us to identify one species of Cestode, *Cephalochlamys namaquensis*, and 13 species of Digenea, six of which were recorded for the first time in Burkina Faso. The recorded prevalence indicates a significant parasite infection among the sampled frogs. In fact, out of 238 frog specimens examined, 166 species were infested with Platyhelminthes. Fourteen species of Platyhelminthes were identified in the examined frogs; however, *Cephalochlamys namaquensis* has previously been documented in *Hoplobatrachus occipitalis* in Burkina [30]. These Platyhelminthes recovered from these amphibians were similar to those reported from other African countries [31]-[34], and the prevalence was of the order of that observed in amphibians from the Nigerian savannah [33] [34]. *Ganeo africana* has been reported in *Hoplobatrachus occipitalis* from Porto Novo in southern Benin [32] and recorded in this host in the Pendjari Biosphere Reserve.

Among the *Haematoloechus* species described by [35], two (02) were subsequently reported by [31] on *D. occipitalis* in Ghana. *Diplodiscus fischthalicus* was also found in *D. occipitalis* in the Guinean savannah at New Bussa, Nigeria. *Cephalochlamys compactus* is an intestinal parasite of *Hoplobatrachus occipitalis*. It has so far been recovered from *H. occipitalis* collected in the Niger Delta region of Nigeria, namely from Warri and Ase in Delta State, as well as from Egbeda and Degema in River State [33] [36] [37]. Among the *Diplodiscidae* encountered in this study, *Diplodiscus fischthalicus* appears to be a multi-host or heteroxenous parasite. [34] collected *D. fischthalicus* from *Hoplobatrachus occipitalis*, while [38] originally described the parasite from *Rana angolensis* in Ethiopia. Additionally, [39] collected and identified *Diplodiscus* specimens in 1916 as *D. subclavatus* from *H. occipitalis* in Ghana. [40] also identified *Diplodiscus* specimens collected from *H. occipitalis* and *Ptychadena mascareniensis* from Cameroon as *D. subclavatus*.

Our study examined the impact of sex on parasite abundance in amphibians from different environments, demonstrating that sex significantly affects parasitism. Indeed, males exhibit a higher prevalence of infestation than females. This disparity may be partially attributed to hormonal variations and divergences in the evolution of immune defense mechanisms corresponding to specific reproductive roles [41].

It is worth noting that [42] showed that parasitism levels in Bangladesh were frequently elevated in male toads compared to females. They believed that sex hormones impede parasite establishment in female amphibians. The effect of biological parameters such as size and mass on the diversity and abundance of Platyhelminthes in amphibians in the course of this work showed the existence of sig-

nificant correlations between the abundance and diversity of Platyhelminthes species collected and the size, as well as the mass, of the host body. Amphibian size and mass are key determinants of parasite abundance and prevalence. This suggests that larger amphibians have a more varied diet, ingest more significant quantities of food, and provide more surface area for parasite colonization [43]. Larger size is also linked to a longer life span and more infection time, which favors parasitism [44]. Indeed, previous studies demonstrated that large amphibians harbored more Platyhelminthes species than small amphibians [45]-[47].

While capturing frogs, we noted the use of pesticides on these sites, which could explain the difference in infestation between sites. Indeed, [48] demonstrated that pesticides are stressful for aquatic organisms, including amphibians. As anurans become compromised, they become more vulnerable to predation and colonization by new parasitic species. [49] have shown that pesticides have an immunodeficient effect on frogs, which can make them more susceptible to infestation by Platyhelminthes.

## 5. Conclusion

The present study revealed the presence of five genera of Platyhelminthes in the African tiger frog (*Hoplobatrachus occipitalis*): One (1) species of Cestodes (*Cephalochlamys namaquensis*) and Thirteen (13) species of Digenea. These Platyhelminthes' overall prevalence or infestation rate was low (69.75%) across the three sites surveyed. The preferred organs for infestation of these Platyhelminthes are the lungs, stomach, small intestine, and large intestine of frogs. These findings may serve as a foundation for extensive research to determine how these Platyhelminthes affect amphibian morbidity and mortality.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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