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Parasite status of edible frogs *Hoplobatrachus occipitalis* from markets and lowlands in the commune of Daloa (Centre-West, Côte d'Ivoire)

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Abstract: The H. occipitalis frog is consumed by the largest ethnic groups in Côte d'Ivoire. As this animal protein is a common part of the diet of a large proportion of the Ivorian population, it is necessary to ensure that it is not harmful. With this in mind, a parasitological study was carried out on edible frogs Hoplobatracus occipitalis collected from two shallows and a market in the town of Daloa. This study made it possible to identify the different parasites of this species and to determine the epidemiological indices, i.e. the prevalence rate and the average intensity of infection for each species of frog, taking into account the sampling sites. The frogs were sampled by catching them in shallow areas using a dip net and by buying specimens of the species on the market. The samples were analyzed by dissecting the digestive system and associated organs of these amphibians, and then observing and identifying the helminth endoparasites. This analysis enabled two classes of helminths to be identified. These are: Nematodes and Trematodes. Analysis of the prevalence and average intensity of infestation indicated that nematode parasitosis was highest at the three sampling sites. In addition, the frogs collected at the Lobia market site were more parasitized by helminths than those collected in the two lowlands (Abattoir and UJLoG). Furthermore, no significant differences were observed between the three sampling sites. In short, these frogs appear to be parasitized but more in market, this risk can be minimized by food hygiene measures and better treatment of captured animals.

1. Introduction

Frogs are found naturally in all regions of the world, with the exception of the polar regions. Although they are generally absent from marine environments, some can adapt to brackish waters (Lecointre and Guyader, 2006). Their bare, moist skin facilitates gas exchange, making them sensitive to environmental variations (Guerry and Hunter, 2002). From an ecological point of view, frogs play an important role in food webs and are essential to the balance of aquatic and terrestrial ecosystems (Channing, 2001). Certain species of edible frog generate substantial income and are a significant source of animal protein for humans (Nzigidahera, 2006). Frogs, particularly the species Hoplobatrachus occipitalis, are prized for their appetising chicken-like flesh (Tokur *et al.*, 2008; Choukri *et al.*, 2023). This trend is a reality in Côte d'Ivoire, with high consumption of this species in various forms (smoked, braised and in soup) in the town of Daloa (Keita, 2022). However, agricultural crops in wetlands result in a high prevalence of parasites and a high rate of infection (Johnson *et al.*, 2007). In addition, the involvement of parasites and other pathogens in the decline of

amphibian populations has been demonstrated (Daszak *et al.*, 2003). The pathogenic effects of endoparasites on edible frogs are numerous: they can cause abnormalities such as slowed growth, skeletal deformities, impaired eyesight and reduced fecundity in hosts (Oungbe, 2020). According to Cissé, (2005), the presence of parasites in edible frogs could pose a public health problem. In West Africa, amphibian parasites have been the subject of numerous studies in natural environments. These include studies by Aisien *et al.* (2017) in Nigeria, Codjo *et al.*, (2022) in Benin and Assemian *et al.*, (2022) in Côte d'Ivoire.

The search of Frogs on Scopus gave more than 85,000 articles for all years from 1857 to now. This high finding is reduced to 1234 articles when associated Frogs to parasites. Then, a bibliometric analysis can be conducted using Scopus data and VOS viewer mapping to show profiler authors, collaborations and countries (Hassan *et al.*, 2020; Salim *et al.*, 2022; Gandasari *et al.*, 2024; Hammouti *et al.*, 2025; Kachbou *et al.*, 2025). The increase of publications is shown (a) and most of them are in agriculture, immunology and medicine (b), **Figure 1**. The most published authors are indicated via the overlay visualization (**Figure 2**), Bursey, C. R. Penn State Shenango, Sharon, United States is the top listed (orange node), followed by Johson (green node) and Rohr (light green node). **Figure 3** shows that the US is the most published country indicated by the great green node.

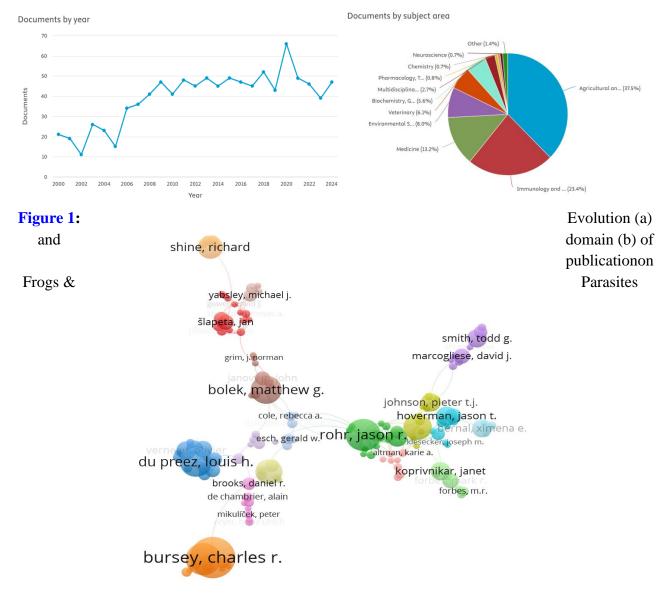


Figure 2: VOS viewer: Overlay visualization of Authors on Frogs & Parasites

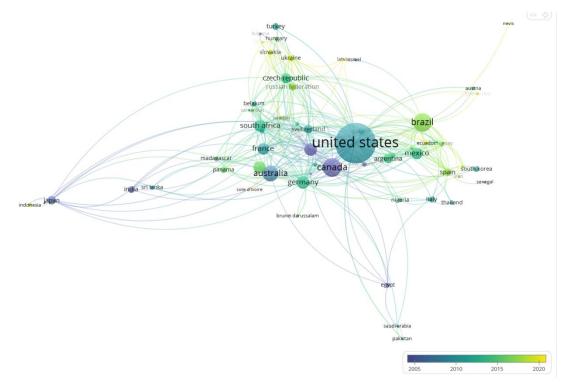


Figure 3: Countries of publication on Frogs & Parasites

The latter study focused on monitoring the health of the species studied in the farming environment. Data on the parasitology of *H. occipitalis* in highly anthropised habitats and sales outlets are therefore insufficient. It therefore proved necessary to assess the parasite status of *Hoplobatrachus occipitalis* frogs in the commune of Daloa. Ultimately, this study made it possible to determine the health risks associated with the consumption of *H. occipitalis* in Daloa and to make recommendations for the safe consumption of this animal protein.

2 Material and method

2.1 Study environment and sampling sites

Daloa (**Figure 4**), located in central-western Côte d'Ivoire, is the capital of the Haut Sassandra region. Its geographical coordinates lie between 06° 52'38' N and 06° 27'00' W (Konaté *et al.*, 2018). According to the 2021 general population and housing census, Daloa has 421,879 inhabitants (INS, 2021). The commune of Daloa has around forty neighbourhoods, the most emblematic of which are Tazibouo, Lobia, Abattoir 1 and 2, Millionnaire, Fatiga, Gbeuliville, Manioc, Savonnerie, Orly and Kennedy. Daloa also has a large community of non-natives, including ECOWAS populations (Burkinabe, Malians, Guineans, Senegalese and Nigeriens) and Lebanese and Mauritanian communities (PRICI, 2016). The choice of sampling sites was made according to the recommendations of Rödel and Ernst (2004), making it possible to select 3 sites. These were the Lobia market, the Abattoir lowlands and the Jean Lorougnon Guédé University (**Figure 5**).

2.2 Collection and identification of frogs

Specimens observed in the two shallows were captured in accordance with the standard technique of Rödel and Ernest (2004) between 6 and 10 am. Sampling consisted of capturing specimens in the water using a haze net. Frogs found out of the water were captured by hand or using a box. For market sampling (**Figure 6**), the frogs were purchased from retailers between 7 and 8 a.m. just as they arrived at the market.

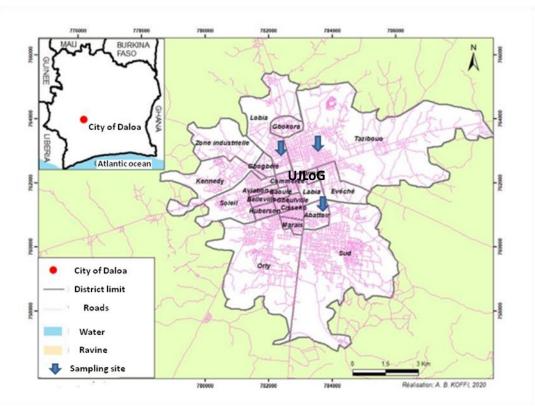


Figure 4: Geographical location of sampling sites (Source: BNETD, 2021)



Figure 5: Overview of sampling sites



Figure 6: Haze net (A) and *H. occipitalis* sold on the Lobia market (B)

2.3 Sex determination, morphometric and biometric measurements

Identification of the sex of the edible frog *Hoplobatrachus occipitalis* was based on morphological observations. The most obvious differences include the presence of vocal sacs in males and the larger size of females (Keita, 2023). Each frog was weighed using a precision balance. The frogs were then grouped into weight classes as follows [0; 50], [50; 100] and [100; 150].

2.4 Study of frog parasites

2.4.1 Collecting parasites from frogs

Using a pair of scissors, an incision was made along the mid-ventral line of the frog, from the anus to the snout. This incision was used to extract the digestive tract and its appendages. Each organ was placed in a Petri dish and opened longitudinally. The internal cavity was carefully rinsed with a pissette of water, and the water collected was examined under a binocular magnifying glass. After observation, the parasites were removed one by one using a fine brush and placed in Eppendorf tubes. Each Eppendorf tube containing the harvested parasites was labelled with information such as the host number, the host capture site and the location of the parasite within the host. The harvested parasites were fixed and stored in Eppendorf tubes containing 70% ethyl alcohol.

2.4.2 Identification of parasites

The preserved parasites were mounted between slides and coverslips, and images were taken for parasite identification. Trematodes and cestodes were identified with reference to the work of Assemian *et al.* (2016) and Oungbe (2020). Nematodes were identified based on the work of Oungbe (2020). The frog parasites were stored at the Tropical Biodiversity and Ecology Laboratory at the Jean Lorougnon Guédé University (Côte d'Ivoire). Using a pair of scissors, an incision was made along the mid-ventral line of the frog, from the anus to the snout. This incision was used to extract the digestive tract and its appendages. Each organ was placed in a Petri dish and opened longitudinally. The internal cavity was carefully rinsed with a pissette of water, and the water collected was examined under a binocular magnifying glass. After observation, the parasites were removed one by one using a fine brush and placed in Eppendorf tubes. Each Eppendorf tube containing the harvested parasites was labelled with information such as the host number, the host capture site and the location of the parasite within the host. The harvested parasites were fixed and stored in Eppendorf tubes containing 70% ethyl alcohol.

2.5 Parasite indices

Two parasite indices were considered in the present study: the prevalence rate (P) and the mean parasite intensity (MI) proposed by Blahoua*et al.* (2015).

2.5.1 Prevalence rate

The prevalence rate (P) of a parasite is defined as the percentage of infected individuals (ni) in relation to the total number of individuals examined (N).

$\mathbf{P} = \frac{ni}{N} \times \mathbf{100}$

Valtonen*et al.* (1997) have established the following classification system for hosts infested by parasite species:

- prevalence > 50%: dominant species;
- $10\% \le$ prevalence $\le 50\%$: satellite or intermediate species;
- prevalence < 10%: rare species.

2.5.2 Mean parasite intensity (MI)

The mean parasite intensity (MI) is the ratio between the number of individuals (Np) of a given parasite species in a sample and the number of infested host individuals (n) in the same sample. It therefore represents the average number of individuals of a parasite species per parasitized host in the sample. The formula is:

$$MI = \frac{Np}{n}$$

With regard to the average parasite intensity (PI), the current classification is that of Bilong and Njiné (1998):

- MI \leq 10: Species with very low average parasite intensity;

- $10 < MI \le 50$: Species with low average parasite intensity;

- $50 < MI \le 100$: Species with medium parasite intensity;

- MI > 100: Species with high average parasite intensity.

2.6 Statistical treatments

The Kruskal-Wallis rank test is a non-parametric alternative to the one-factor analysis of variance between classes (ANOVA 1). The Mann-Whitney U test is a non-parametric alternative to the Student t test for independent samples. Parametric tests have been applied when the normality of the distribution has been proven (Kinnear and Gray, 2005). Data summary tables and graphs were produced using Microsoft Excel (version 2016).

3.Results and Discussion

3.1 Specific diversity of Hoplobatracus occipitalis parasites

3.1.1 Parasites identified in frogs according to sampling site

Five parasite species were extracted from the specimens examined (**Table 1**). Four endoparasite species were identified in frogs from the two shallows (*Diplodiscus sp., Heamatoloechus johnson, Capillaria sp.* and *Oxysomatium brevicaudatum*). At the Lobia market, an additional species called *Cosmocerca ornata* was identified in addition to the 4 other parasite species in the 2 lowlands.

| Parasite species | Bottomland of Abattoir | Bottomland of UJLoG | Market of Lobia |
|----------------------------|---------------------------|------------------------|--------------------|
| Capillaria sp | Х | Х | X |
| Cosmocerca ornata | | | Х |
| Diplodiscus sp. | Х | Х | Х |
| Heamatoloechus johnson | Х | х | Х |
| Oxysomatium brevicaudatum | Х | Х | Х |
| Number of parasite species | 4 | 4 | 5 |

 Table 1: Parasite species identified according to sampling sites

These parasites are represented by five species (*Heamatoloechus johnson*, *Oxysomatium brevicaudatum*, *Diplodiscus sp.*, *Capillaria sp.* and *Cosmocerca ornata*). The same number of species of frog parasites were found in the Lobia market and four species in the lowlands. More or less the same frog parasite species were found in the shallows as in the markets, which could be due to the fact that most of the frogs sold in the markets were caught in the shallows (Keita *et al.*,

2022).Secondly, the greater parasitism of market frogs is thought to be due to the extra time spent by these frogs in the sun, in the heat and in the often-unhealthy environment of markets. The presence of the *Cosmocerca ornata* species only in markets could perhaps be explained by the ecology of this species, which lives in both aquatic (freshwater) and terrestrial environments, particularly in markets (Kirillov and Kirillova, 2016).In addition, this diversity of parasite species was confirmed by Assemian *et al.* (2016).

3.1.2 Parasites identified according to their location in the frog's body

The parasite species collected from frog specimens at the various sites are located in three different organs (**Table 2**). These are: the large intestine, the small intestine and the lungs. *Cosmocerca ornata* species are found in the small intestine and large intestine, *Diplodiscus sp.* is also found in two organs (large intestine and rectum) and *Oxysomatium brevicaudatium* (lung and large intestine). On the other hand, *Capillaria sp* and *Heamatoloechus johnson* were found in only one organ, the large intestine and the lung respectively.

| Table 2: Parasite s | species identified | according to their | location in the host |
|---------------------|--------------------|--------------------|----------------------|
| | peeres raemanea | according to mon | nooution in the noot |

| Parasite species | Location |
|----------------------------|-----------------------------------|
| Cosmocerca ornata | Small intestine / large intestine |
| Oxysomatium brevicaudatium | Large intestine / lung |
| <i>Capillaria</i> sp. | Large intestine |
| Heamatoloechus johnson | Lung |
| Diplodiscus sp. | Large intestine/ rectum |

3.1.3 Parasites identified in frogs according to host weight

The table shows that frogs from the Lobia market are the most parasitized by the 5 species and practically for the 3 weight classes]0,150] g (**Table 3**). In the 2 lowlands, the species *Oxysomatium brevicaudatum*, *Heamatoloechus johnson* and *Diplodiscus sp.* generally parasitise small and medium-sized frogs weighing]0,100] g. The species *Capillaria sp.* infests medium and large frogs]50,150] g.

Table 3: Parasites identified on frogs according to weight classes

| Doragita graciag | Bottomland | Bottomland | Market |
|---------------------------|---------------|--------------|--------------|
| Parasite species | of Abattoir | of UJLoG | of Lobia |
| Oxysomatium brevicaudatum |]0 ,100] g |]0,150] g |]0 ,150] g |
| Heamatoloechus johnson |]0 ,100] g |]0 ,100] g |]0 ,150] g |
| Diplodiscus sp. |]0 ,100] g |]0 ,100] g |]50 , 100] g |
| Capillaria sp. |]100 , 150] g |]50 , 100] g |]0 ,100] g |
| Cosmocerca ornata | | |]0 ,150] g |

3.1.4 Parasites identified in frogs by sex

Male frogs were infested by all parasite species except *Cosmocerca ornata* at all sites (**Table 4**). For female frogs, the parasites collected differed from one site to another.

| Daragita graciag | Bottomland | Bottomland | Market |
|---------------------------|-------------|------------|----------|
| Parasite species | of Abattoir | of UJLoG | of Lobia |
| Oxysomatium brevicaudatum | MF | MF | MF |
| Heamatoloechus johnson | MF | Μ | MF |
| Diplodiscus sp. | MF | MF | М |
| Capillaria sp. | Μ | Μ | MF |
| Cosmocerca ornata | | | F |
| M · male E · female | | | |

Table 4: Parasites identified on frogs according to the sex of the host

M: male, F: female

3.2 Parasitic indicators

3.2.1 Parasite prevalence by site, weight class and sex

Table 5 shows a high parasite prevalence for the 3 sampling sites, with higher values for frogs from the Lobia market (86.66%). Specifically, the species *Oxysomatium brevicaudatum* and *Capillaria sp.* had the highest and lowest parasite prevalence, respectively, in *Hoplobatrachus occipitalis* specimens at the sites sampled. *Oxysomatium brevicaudatum* is therefore the dominant species. Large frogs weighing [50, 150] g and females of *H. occipitalis* had the highest parasite prevalences (Figure 7).

 Table 5: Parasite prevalence of Hoplobatrachus occipitalis by site

| | Bottomland | Bottomland | Market | Total |
|--|-------------|------------|----------|-------|
| | of Abattoir | of Lobia | of Lobia | Total |
| Overall prevalence of parasites (%) | 66.66 | 53.33 | 86.66 | 68.88 |
| Prevalence of Cosmocerca ornata (%) | 0 | 0 | 20 | 6.66 |
| Prevalence of <i>Oxysomatium brevicaudatum</i> (%) | 46.66 | 40 | 66.66 | 51.11 |
| Prevalence of Capillaria sp. (%) | 6.66 | 6.66 | 20 | 11.11 |
| Prevalence of <i>Diplodiscus sp.</i> (%) | 20 | 13.33 | 6.66 | 13.33 |
| Prevalence of <i>Heamatoloechus johnson</i> (%) | 26.66 | 20 | 40 | 28.88 |

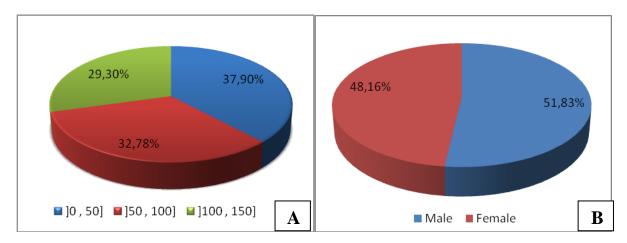


Figure 7: Over all parasite prevalence in *H. occipitalis* frogs as a function of weight class (A) and sex (B)

Statistical tests showed no significant difference between the parasite prevalence of the two sexes of frogs for the 3 sampling sites (Mann-Whitney (MN); p < 0.05).

Keita et al., J. Mater. Environ. Sci., 2025, 16(6), pp. 1049-1062

This high infestation rate (68.88%). in frogs could be justified by the sometimes poor quality of the water in which they live (Galli *et al.*, 2001). As the frog's diet is essentially insectivorous, the prey it consumes may also be parasite vectors, thus justifying the high infestation rate observed in this study. Our results are similar to those of Aisien *et al.* (2017), who obtained an overall infestation prevalence of 67.4% in anurans in Nigeria, where all of certain species were infested. Parasite prevalence varied according to the capture and sale sites. Frogs from the Lobia market had a prevalence of 86.66%, those from the Abattoir bottomland had a prevalence of 66.66%, and those from the UJLoG school field bottomland had a prevalence of 53.33%. These variations can be attributed to differences in environmental conditions and pollution levels. Urban lowlands are often more contaminated with organic waste, creating favourable conditions for parasites. These results are contrary to those of Blé *et al.* (2019) who found no significant difference between prevalence and sampling sites. The high parasite prevalence rate in frogs from the Lobia market could be linked to the multiple place of origin of this species. Studies carried out by Codjo *et al.* (2022) in Benin show the presence of this species in the catchment areas of the Mono, Ouémé and Niger rivers.

3.2.2 Mean parasite intensity according to sampling site

The average parasite intensity (**Table 6**) was very low (MI ≤ 10) for parasites collected from frogs from all three sites. *Oxysomatium brevicaudatum* had the highest values in frogs from the Abattoir shallows (MI= 5.54). Among the frogs from the Lobia market, the species Capillaria sp had the highest parasite intensity (MI= 7.33).In the UJLoG shallows, *Heamatoelochus johnson* had the highest mean parasite intensity (MI= 3). With regard to weight classes (**Figure 8A**), the mean parasite intensity is higher (MI= 5.43) for frogs of average size]50, 100]. Finally, this index, relative to sex (**Figure 8B**), is more or less the same for males and females, but slightly in favour of males.

| | Bottomland | Bottomland | Market |
|---------------------------------|-------------|------------|----------|
| | of Abattoir | of UJLoG | of Lobia |
| MI de Oxysomatium brevicaudatum | 5.42 | 2.25 | 5 |
| MI de Heamatoloechus johnson | 2.75 | 3 | 2.5 |
| MI de Diplodiscus sp. | 1.33 | 1 | 2 |
| MI de Capillaria sp. | 1 | 1 | 7.33 |
| MI de Cosmocerca ornata | 0 | 0 | 1.66 |

| Table 8: Average parasite intensity by sampling site | Table 8: | Average parasite | intensity by | sampling site |
|---|----------|------------------|--------------|---------------|
|---|----------|------------------|--------------|---------------|

MI: Mean parasite intensity

Out of 5 parasites, MI was higher in male frogs for 3 parasites. Statistical tests also showed no significant difference between the mean parasite intensities of males and females for the 3 sampling sites (Mann-Whitney (MN); p < 0.05). Photographs of the different species of parasites are shown in **figure 9**. Parasite prevalence and average parasite intensity varied according to the weight of the frog at the three sampling sites. The larger the frog, the greater the increase in these indices. This implies that larger specimens are more exposed to infection by endoparasites than smaller ones. In fact, the larger the frog, the more varied its diet, which exposes it to a higher risk of infestation. With regard to sex, although males had slightly higher parasite indices than females, statistical analyses did not show any significant differences between the indices of the two sexes.

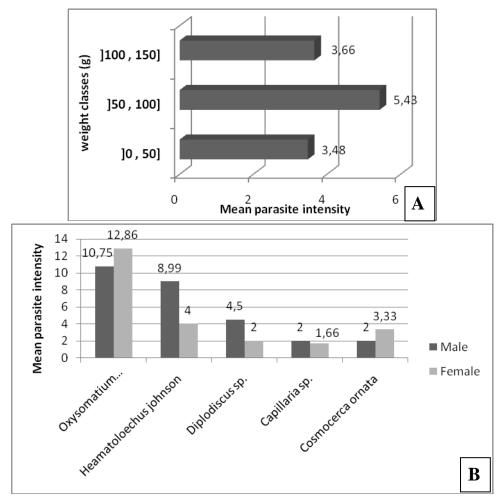


Figure 8: Average parasite intensity in *H. occipitalis* frogs as a function of weight class (A) and sex (B)



Figure 9: Photograph of parasites of *Hoplobatracus occipitalis* from the shallows and markets of the town of Daloa

(A): Oxysomatium brevicaudatum, (B): Diplodiscus sp., (C): Cosmocerca ornata, (D): Heamatoloechus johnson and (E): Capillaria sp.

Keita et al., J. Mater. Environ. Sci., 2025, 16(6), pp. 1049-1062

These results indicate that sex is not a major determining factor in the variation of parasite load in Hoplobatrachus occipitalis. This is consistent with the results of Blé *et al.* (2019) who found no significant difference between prevalence and mean infestation intensity by sex in *H. occipitalis*. The high prevalence and average intensity of helminths in frogs from the shallows and markets of Daloa pose significant health risks to consumers. Parasitic infections in humans can result from eating undercooked frogs, leading to illness. Work by Blé *et al.* (2016) shows that fresh frogs sold on markets are contaminated with *A. sobria* and *A. Hydrophila*, whereas dried frog meat is free of these bacteria.

Conclusion

This study revealed a significant diversity of parasites affecting the frog *Hoplobatrachus occipitalis* in the town of Daloa. Analysis of the 45 frogs collected revealed the presence of five parasite species in the frogs collected at the three sites. The frogs from the Lobia market were infested with five parasite species and those from the two shallows were infested with four parasite species. The prevalence of parasitic infestations and the average intensity varied according to collection site, with a higher parasite load in frogs from the market than in those from the two shallows. Large-weight frogs were also more infested with parasites than small-weight frogs at all sampling sites. In order to take this study further, it would be interesting to carry out additional studies to identify the sources and life cycles of the parasites, in order to better understand the transmission dynamics and develop more effective control measures. It would also be judicious to extend this study to the different seasons of the year and to other sites in the town of Daloa.

List of abbreviation

UJLoG: Jean Lorougnon Guédé University

Animal Ethics: A limited number of *Hoplobatrachus occipitalis* frogs were used for this study. Prior to dissection, all individuals were decerebrated and demedullated to limit their suffering.

Consent to publication: Not applicable

Availability of data and materials: The datasets used and analysed in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests. All authors confirm that they have read approved the content of the submitted article. They also declare that there are no conflicts of interest among the authors or with the publication ethics of the journal.

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Authors' contributions

KG: carried out the various manipulations in the field and in the laboratory, as well as the statistical analyses and drafting of the manuscript;

KM: was used to set up the method and identify parasites

KDAS: to help collect the frogs and carry out the dissections

ANE: was the scientific director of this study, so he supervised all the work.

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