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# Parasitic helminth fauna of tree frogs from cocoa plantations at Ugboke, Edo State, Nigeria

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## Abstract

Cocoa pods are vulnerable to pest and diseases which often cause huge financial losses to farmers. To address this challenge and improve yield, cocoa farmers often resort to pesticide application on the cocoa trees. This leads to deposition of pesticides residues on the cocoa trees, the undergrowth and plantations floor with devastating consequences for amphibians including tree frogs. Previous studies have reported the immune suppressive nature of pesticides on anurans thereby rendering them more susceptible to infection. This study is part of an on-going investigation of the effect of pesticide application on the pattern of helminth parasitic infections of anurans in pesticide-treated cocoa plantations at Ugboke, Edo State. A total of 354 tree frogs belonging to three genera (*Leptopelis, Hyperolius* and *Afrixalus*), consisting of 14 species were examined. A high species richness (d=1.971) and diversity (H'=2.215) was recorded. Ten species (71.43%) of these frogs were infected while four (28.57%) were uninfected. The overall prevalence of parasitic infections was 30.23%. Thirteen helminth parasites including three cestodes, two digeneans and eight nematode species were recovered. A significantly higher ($P<0.05$) prevalence of infection was recorded during the wet season (31.64%) than in the dry (10.53%). There was high parasite diversity with low prevalence and infection intensity of infection as previously reported for other anurans collected from the same cocoa plantations.

**Keywords**: Tree frogs, helminth parasites, cocoa plantations, pesticides, Edo State

## Introduction

Plantations are large expanses of land with trees cultivated for commercial purposes. The cultivation process involves clearing a vast portion of the natural vegetation, and replacement with the desired crop, resulting in deforestation and habitat fragmentation. Often times, some of the plantations are exposed to chemical contamination from herbicides, fungicides and pesticides. For example, cocoa plantations are subjected to periodic spraying with pesticides, especially during the wet season, to prevent or combat pest and disease infestations. Cocoa pods are vulnerable to pests (e.g. myriads and stem borer) and diseases (e.g. black pod and cocoa swollen shoot virus). These pests and diseases cause huge financial losses of as much as 30% or even more in severe cases to farmers (David, 2005). Residues of the pesticides applied are deposited on tree tops (branches and leaves), the undergrowth and leaf litter on the plantation floor, which are respectively micro-habitats for different species of frogs.

Apart from their direct toxicity on anuran hosts, they are also known to affect the immune system of exposed tadpoles, resulting in higher prevalence of helminth infections in adult frogs (Kiesecker, 2002; Rohr *et al*., 2008). Pesticide-contaminated environments are also known to reduce the survival/infectivity of free-living stages of parasitic helminths, resulting in reduced infection intensity (Lafferty and Kuris, 1999; Morley *et al.,* 2003; Peitrock and Marcogliese, 2003).

Tree frogs are anurans that have their abode/dwellings on tree tops, shrubs and herbs in the undergrowth of the forest. They live and reproduce in the arboreal microhabitats, though they sometimes visit the ground in search of food and mates. They can be heard often making con-specific mating calls from these plants, especially those over hanging or close to water bodies.

This report is part of a study undertaken to determine the helminth parasites of amphibians from cocoa plantations at Ugboke, Edo State (Edo-Taiwo, 2018). Two previous publications from this study have dealt with the parasitic infections of leaf litter frogs (Edo-Taiwo and Aisien, 2020a) and ground dwelling anurans (Edo-Taiwo and Aisien, 2020b) from these plantations. The present paper is focused on the helminth parasites of tree frogs collected from these cocoa plantations.

The only report available on the helminth parasites of tree frogs in Nigeria is the work of Imasuen *et al.* (2012a), at Okomu National Park (a protected sanctuary for flora and fauna). The aim of this paper is to report on the helminths parasitic infections in the tree frogs from the pesticide/herbicide-contaminated environment in the cocoa plantations, comparing them with those from non-contaminated habitats.

## Materials and Methods

Tree frog specimens were sampled from a number of contiguous cocoa plantations (6°32'20'' and 6°45'35''N and 5°15'20'' and 5°17'46''E) at Ojo Camp-Ugboke of Usen, in Ovia South-West Local Government Area of Edo State, Nigeria. The cocoa plantations are traversed by streams and rivulets. Pesticides used for pest controls include Gammalin, Avesthrin (Cypermethrin 10% EC), Scorpion, Best, Instakill and Ridonul Gold 66WP while Weed crusher was employed as herbicide. According to the farmers, the cocoa trees were sprayed weekly during the wet season; there was no pesticide application during the dry season.

The frogs were sampled at night (between 19.00 hrs and 01.00 hrs) by active searches using the Visual Acoustic Encounter Surveys method (Crump and Scott, 1994) for 15 months (August 2012 – October 2013) covering both the wet and dry seasons. Collected specimens were identified following the protocols of Roedel (2000, 2007) and Schiotz (1963, 1999). The frogs were thereafter euthanized with Benzocaine solution (Goater *et al*., 1987; Muzzall, 1991) and the snout-vent length (SVL) taken before dissection. Sex determination was either by visual inspection (for vocal sacs in males) or after dissection (for gonads). The gastro-intestinal tract (oesophagus/stomach, small intestine, large intestine/rectum), body cavity, liver/gall bladder, lungs, heart and the urinary bladder were examined for parasites. The parasites recovered were processed according to Aisien *et al.* (2001). Strigeoid metacercariae were incubated in 0.85% normal saline containing 0.5% trypsin at 37°C in order to free the juvenile parasite. Parasites were examined with a binocular microscope and identified with the aid of appropriate keys (Yamaguti, 1961, 1971; Prudhoe and Bray, 1982; Khali *et al.*, 1994) to the lowest possible taxon. Prevalence and mean intensity of parasitic infection among the tree frogs were calculated for each parasite taxon recovered (Bush *et al.*, 1997). Alpha diversity indices were used to estimate species richness and diversity. Chi-square test was used to compare prevalence of infection between males and females; and between wet and dry seasons.

## Results

Fourteen species of tree frogs belonging to two families (Arthroleptidae and Hyperoliidae) and three genera (*Leptopelis, Hyperolius* and *Afrixalus*) were examined in this study for helminth infections. Among these were *Leptopelis occidentalis* (08), *L. spiritusnoctis* (10), *Leptopelis* sp. (03), *Hyperolius concolor* (47), *H. fusciventris* (01), *H. fusciventris burtoni* (91), *H. picturatus* (22), *H. sylvaticus* (01), *Hyperolius* spp. 1–4 (66, 01, 52 and 01, respectively), *Afrixalus dorsalis* (50) and *A. nigeriensis* (01). The species richness (d) and diversity (H') were 1.971 and 2.215, respectively.

Infection was recorded in 10 species (71.43%) of the frogs while four (28.57%) were uninfected. The uninfected tree frog species included *H. fusciventris*, *H. sylvaticus*, *Hyperolius* sp. 2 and *A. nigeriensis.* The overall prevalence of infection among the tree frogs was 30.23% (Table 1). The Hyperoliids were more infected (30.63%) than the Arthroleptids (28.57%), but this difference was not significant (p>0.05)

Thirteen helminth parasites were recovered from various predilection sites in the tree frogs (Table 2). The parasites included three species of cestodes (*Cylindrotaenia jaegerskioeldi,* larval *Proteocephalus* spp. 1 and 2), two digeneans (*Ostioloides rappiae* and a strigeoid trematode larva) and eight nematode species (*Amplicaecum* sp., *Aplectana* sp., *Camallanus* sp., *Cosmocerca ornata*, *Cosmocerca* sp., *Foleyellides* sp., *Physaloptera* sp. and an unidentified oxyurid nematode).

Table 1: Overall prevalence and mean intensity of parasitic infection in tree frogs from cocoa plantations at Ugboke

| Species | Number examined | Number infected | Prevalence (%) | Number of parasites | Mean intensity |
|---|---|---|---|---|---|
| Arthroleptidae | | | | | |
| *L. occidentalis* | 08 | 01 | 12.5 | 05 | 5.0 |
| *L. spiritusnoctis* | 10 | 02 | 20.0 | 08 | 4.0 |
| *Leptopelis* sp. | 03 | 02 | 66.7 | 10 | 5.0 |
| Hyperoliidae | | | | | |
| *H. concolor* | 47 | 10 | 21.3 | 20 | 2.0 |
| *H. fusciventris* | 01 | - | - | - | - |
| *H. fusc. burtoni* | 91 | 25 | 27.5 | 147 | 5.88 |
| *H. picturatus* | 22 | 02 | 9.1 | 10 | 5.0 |
| *H. sylvaticus* | 01 | - | - | - | - |
| *Hyperolius* sp. 1 | 66 | 33 | 50.0 | 277 | 8.39 |
| *Hyperolius* sp. 2 | 01 | - | - | - | - |
| *Hyperolius* sp. 3 | 52 | 19 | 36.5 | 140 | 7.37 |
| *Hyperolius* sp. 4 | 01 | 01 | 100.0 | 01 | 1.0 |
| *A. dorsalis* | 50 | 12 | 24.0 | 75 | 6.25 |
| *A. nigeriensis* | 01 | - | - | - | - |
| Total | 354 | 107 | 30.23 | 693 | 6.48 |

The most infected micro-habitat was the small intestine (in 16 hosts with 9 parasite species) with prevalence ranging from 1.2 to 66.7% (mean intensity, 1.0 to 36.0). The least preferred site was the oesophagus (Table 2). *Cylindrotaenia jaegerskioeldi, Proteocephalus* sp. 2, *O. rappiae*, the strigeoid trematode larva, *Aplectana* sp., *Foleyellides* sp. and *Physaloptera* sp. were generalists, infecting 3 to 7 hosts. *Proteocephalus* sp. 1, *Amplicaecum* sp., *Camallanus* sp., *C. ornata, Cosmocerca* sp. and the unidentified oxyurid nematode infected a single host each. *Aplectana* sp. was the most recurrent parasite encountered, occurring in seven host species with prevalence ranging from 1.1 to 12.5% (MI, 1.0 to 6.0), followed by *Proteocephalus* sp. 2 (in 6 host species; prevalence, 2.1 to 100%; M.I, 1.0 to 11.5±17.88). A total of 322 male and 34 female tree frogs were examined in this study. Prevalence of infection was higher in male tree frogs than in the female, but the difference was not significant (P>0.05). Parasites were recovered from 99 (30.75%) male and 08 (23.53%) female frogs.

Prevalence and intensity of infection of the different helminth parasites in relation to sex of the tree frogs is presented in Table 3. Twelve (92.3%) helminth species were recorded in the males and six (46.2%) in the females. Three cestodes (*C. jaegerskioeldi,* larval *Proteocephalus* spp. 1 and 2) occurred in male tree frogs; only one (*C. jaegerskioeldi*) was recorded in a female of *H. fusciventris burtoni* (Table 3). The two digeneans encountered in this study were recorded in both sexes. *Ostioloides rappiae* infected different *Hyperolius* spp. with the highest prevalence and intensity of infection occurring in the female of *Hyperolius fusciventris burtoni.* Although the strigeiod trematode larva had its highest prevalence (31.8%) in male *Hyperolius* sp. 1, the highest intensity (12.5±3.54) occurred in *A. dorsalis*. With respect to nematode parasites, *Amplicaecum* sp, *Camallanus* sp., *Cosmocerca* sp., *Foleyellides* sp. and the unidentified oxyurid nematode infected only male frogs while *C. ornata* was recorded only in the females of *A. dorsalis* (Table 3). Both sexes of tree frogs were infected with *Aplectana* sp. and *Physaloptera* sp. Prevalence of infection was significantly higher (P<0.05) during the wet season (31.64%) than in the dry (10.53%). Almost all (91.7%) the parasites species were recorded during the wet season with the exception of *Amplicaecum* sp. which incidentally was recorded only in the dry season (Table 4). Infection was recorded in all frogs during the wet season except in *Hyperolius* sp. 4. Only a few frogs harboured infections in the dry season (Table 4)

Table 2: Prevalence and mean intensity of endohelminth parasites of tree frogs from cocoa plantations at Ugboke

| Parasite | Host | Predilection Site | Prevalence (%) | Mean intensity ± S.D |
|---|---|---|---|---|
| Cestoda | | | | |
| *C. jaegerskioeldi* | *A. dorsalis* | Small intestine | 2.0 | 3.0 |
| | *H. fusc. burtoni* | Small intestine | 4.4 | 2.0±1.16 |
| | *H. picturatus* | Small intestine | 4.6 | 7.0 |
| | *Hyperolius* sp. 1 | Small intestine | 13.6 | 3.2±1.72 |
| | *Hyperolius* sp. 3 | Small intestine | 1.9 | 1.0 |
| | *Leptopelis* sp. | Small intestine | 66.7 | 4.0±2.83 |
| *Proteocephalus* sp. 1 | *Hyperolius* sp. 1 | Attached to S.I & Liver | 1.5 | 1.0 |
| *Proteocephalus* sp. 2 | *A. dorsalis* | Attached to liver | 6.0 | 4.7±3.97 |
| | *H. concolor* | Attached to liver | 2.1 | 1.0 |
| | *H. fusc. burtoni* | Attached to liver | 4.4 | 11.5±17.88 |
| | *H. picturatus* | Attached to liver | 4.6 | 3.0 |
| | *Hyperolius* sp. 3 | Attached to liver | 19.2 | 1.2±0.81 |
| | *Hyperolius* sp. 4 | Attached to liver & stomach | 100.0 | 1.0 |
| Digenea | | | | |
| *Ostioloides rappiae* | *Hyperolius* sp. 1 | Small intestine | 1.5 | 1.0 |
| | *Hyperolius* sp. 3 | Small intestine | 3.9 | 2.0±1.41 |
| | *H. fusc. burtoni* | Small intestine | 1.2 | 6.0 |
| | *H. concolor* | Small intestine | 4.3 | 1.5±0.71 |
| Strigeiod trematode | *A. dorsalis* | Large intestine | 4.0 | 12.5 |
| | *H. fusc. burtoni* | Body cavity | 15.4 | 6.0 |
| | *Hyperolius* sp. 3 | Body cavity | 17.3 | 5.3 |
| | *Hyperolius* sp. I | Body cavity | 31.8 | 10.5 |
| Nematode | | | | |
| *Amplicaecum* sp. | *Leptopelis* sp. | Small intestine | 33.3 | 2.0 |
| *Aplectana* sp. | *A. dorsalis* | Small intestine | 10.0 | 5.6±3.21 |
| | *H. concolor* | Small & Large intestine | 2.1 | 6.0 |
| | *Hyperolius* sp. 3 | Large intestine/rectum | 3.8 | 6.0±7.07 |
| | *L. occidentalis* | Small & Large intestine | 12.5 | 5.0 |
| | *L. spiritusnoctis* | Large intestine/rectum | 10.0 | 2.0 |
| | *Hyperolius* sp. 1 | Large intestine/rectum | 4.5 | 1.0 |
| | *H. fusc. burtoni* | Large intestine/rectum | 1.1 | 2.0 |
| *Camallanus* sp. | *H. concolor* | Large intestine/rectum | 2.1 | 1.0 |
| *Cosmocerca* sp. | *Hyperolius* sp. 3 | Small intestine | 1.9 | 5.0 |
| *C. ornata* | *A. dorsalis* | Large intestine/rectum | 2.0 | 4.0 |
| *Foleyellides* sp. | *A. dorsalis* | Body cavity | 2.0 | 1.0 |
| | *H. concolor* | Small intestine | 8.5 | 1.5±0.58 |
| | *H. fusc. burtoni* | Body cavity | 1.1 | 1.0 |
| | *Hyperolius* sp. 1 | Body cavity | 4.5 | 6.0±8.66 |
| | *Hyperolius* sp. 3 | Body cavity | 1.9 | 22.0 |
| *Physaloptera* sp. | *H. concolor* | Small intestine | 4.3 | 1.5±0.71 |
| | *Hyperolius* sp. 1 | Oesophagus/stomach | 3.0 | 2.5±2.12 |
| | *L. spiritusnoctis* | Oesophagus/stomach | 11.1 | 6.0 |
| Oxyurid nematode | *Hyperolius* sp. 3 | Small intestine | 1.9 | 36.0 |

Table 3: Prevalence and mean intensity of parasitic helminths based on sex of tree frogs from Ugboke cocoa plantations

| Parasite | Hosts | Male | | Female | |
|---|---|---|---|---|---|
| | | Prevalence (%) | MI ± S.D | Prevalence (%) | MI ± S.D |
| Cestoda | | | | | |
| *C. jaegerskioeldi* | *A. dorsalis* | 2.3 | 3.0 | - | - |
| | *H. fusc. burtoni* | 3.9 | 1.7±1.16 | 7.1 | 3.0 |
| | *H. picturatus* | 5.6 | 7.0 | - | - |
| | *Hyperolius* sp. 1 | 13.6 | 3.2±1.72 | - | - |
| | *Hyperolius* sp. 3 | 2.0 | 1.0 | - | - |
| | *Leptopelis* sp. | 66.7 | 4.0±2.83 | - | - |
| *Proteocephalus* sp. 2 | *Hyperolius* sp. 1 | 1.5 | 1.0 | - | - |
| *Proteocephalus* sp. 3 | *A. dorsalis* | 7.0 | 4.7±3.97 | - | - |
| | *H. concolor* | 2.2 | 1.0 | - | - |
| | *H. fusc. burtoni* | 5.2 | 11.0±17.38 | - | - |
| | *H. picturatus* | 5.6 | 3.0 | - | - |
| | *Hyperolius* sp. 3 | 20.0 | 1.2±0.45 | - | - |
| | *Hyperolius* sp. 4 | 100 | 1.0 | - | - |
| Digenea | | | | | |
| *O. rappiae* | *Hyperolius* sp. 1 | 1.5 | 1.0 | - | - |
| | *Hyperolius* sp. 3 | 4.0 | 2.0±1.41 | - | - |
| | *H. fusc. burtoni* | - | - | 7.1 | 6.0 |
| | *H. concolor* | 4.4 | 1.5±0.71 | - | - |
| Strigeiod trematode larva | *A. dorsalis* | 4.7 | 12.5±3.54 | - | - |
| | *H. fusc burtoni* | 14.3 | 7.2±11.37 | 21.4 | 2.0±1.00 |
| | *Hyperolius* sp. 3 | 20.0 | 7.0±6.27 | - | - |
| | *Hyperolius* sp. I | 31.8 | 10.5±35.48 | - | - |
| Nematode | | | | | - |
| *Amplicaecum* sp. | *Leptopelis* sp. | 33.3 | 2.0 | - | - |
| *Aplectana* sp. | A. *dorsalis* | 11.6 | 5.6±3.21 | - | - |
| | *Hyperolius* sp. 3 | 4.0 | 6.0±7.07 | - | - |
| | *H. concolor* | - | - | 50.0 | 6.0 |
| | *Hyperolius* sp. 1 | 4.5 | 1.0 | - | - |
| | *H. fusc. burtoni* | 1.3 | 2.0 | - | - |
| | *L. occidentalis* | 16.7 | 5.0 | - | - |
| | *L. spiritusnoctis* | 14.3 | 2.0 | - | - |
| *Camallanus* sp. | *H. concolor* | 2.2 | 1.0 | - | - |
| *Cosmocerca* sp. | *Hyperolius* sp. 3 | 2.0 | 5.0 | - | - |
| *C. ornata* | *A. dorsalis* | - | - | 14.3 | 4.0 |
| *Foleyellides* sp. | *A. dorsalis* | 2.3 | 1.0 | - | - |
| | *H. concolor* | 8.9 | 1.5±0.58 | - | - |
| | *H. fusc. burtoni* | 1.3 | 1.0 | - | - |
| | *Hyperolius* sp. 1 | 4.5 | 6.0±8.66 | - | - |
| | *Hyperolius* sp. 3 | 2.0 | 22.0 | - | - |
| *Physaloptera* sp. | *H. concolor* | 4.4 | 1.5±0.71 | - | - |
| | *Hyperolius* sp. 1 | 3.0 | 2.5±2.12 | - | - |
| | *L. spiritusnoctis* | - | - | 333 | 6.0 |
| Oxyurid nematode | *Hyperolius* sp. 3 | 2.0 | 36.0 | - | - |

Table 4: Seasonal prevalence and intensity of infection of parasitic endohelminths in tree frogs from Ugboke cocoa plantations

| Parasite | Hosts | Wet | | Dry | |
|---|---|---|---|---|---|
| | | ᴾPrevalence (%) | Mean intensity ± S.D | Prevalence (%) | Mean intensity ± S.D |
| Cestoda | | | | | |
| *C. jaegerskioeldi* | *A. dorsalis* | 2.3 | 3.0 | - | - |
| | *H. fusc. Burtoni* | 4.7 | 2.0±1.16 | - | - |
| | *H. picturatus* | 2.3 | 7.0 | - | - |
| | *Hyperolius* sp. 1 | 13.6 | 3.2±1.72 | - | - |
| | *Hyperolius* sp. 3 | 2.0 | 1.0 | - | - |
| | *Leptopelis* sp. | 50.0 | 6.0 | 100 | 2.0 |
| *Proteocephalus* sp. 2 | *Hyperolius* sp. 1 | 1.5 | 1.0 | - | - |
| *Proteocephalus* sp. 3 | *A. dorsalis* | 6.8 | 4.7±3.97 | - | - |
| | *H. concolor* | 2.2 | 1.0 | - | - |
| | *H. fusc. burtoni* | 4.7 | 11.5±18.06 | - | - |
| | *H. picturatus* | 4.6 | 3.0 | - | - |
| | *Hyperolius* sp. 3 | 19.2 | 1.2±0.45 | - | - |
| | *Hyperolius* sp. 4 | - | - | 100 | 1.0 |
| Digenea | | | | | |
| *O. rappiae* | *Hyperolius* sp. 1 | 1.5 | 1.0 | - | - |
| | *Hyperolius* sp. 3 | 3.9 | 2.0±1.41 | - | - |
| | *H. fusc. burtoni* | 1.2 | 6.0 | - | - |
| | *H. concolor* | 4.4 | 1.5±0.71 | - | - |
| Strigeiod trematode larva | *A. dorsalis* | 4.5 | 12.5±3.54 | - | - |
| | *H. fusc. burtoni* | 16.5 | 6.1±10.22 | - | - |
| | *Hyperolius* sp. 3 | 19.2 | 7.0±6.27 | - | - |
| | *Hyperolius* sp. 1 | 31.8 | 10.5±35.48 | - | - |
| Nematode | | | | - | - |
| *Amplicaecum* sp. | *Leptopelis* sp. | - | - | 100 | 2.0 |
| *Aplectana* sp. | *A. dorsalis* | 11.4 | 5.6±3.21 | - | - |
| | *Hyperolius* sp. 3 | 3.8 | 6.0±7.07 | - | - |
| | *H. concolor* | 2.2 | 6.0 | - | - |
| | *Hyperolius* sp. 1 | 4.5 | 1.0 | - | - |
| | *H. fusc. burtoni* | 1.2 | 2.0 | - | - |
| | *L. occidentalis* | 12.5 | 5.0 | - | - |
| | *L. spiritusnoctis* | 12.5 | 2.0 | - | - |
| *Camallanus* sp. | *H. concolor* | 2.2 | 1.0 | - | - |
| *Cosmocerca* sp. | *Hyperolius* sp. 3 | 1.9 | 5.0 | - | - |
| *C.ornata* | *A. dorsalis* | 2.3 | 4.0 | - | - |
| *Foleyellides* sp. | *A. dorsalis* | 2.3 | 1.0 | - | - |
| | *H. concolor* | 8.9 | 1.5±0.58 | - | - |
| | *H. fusc. burtoni* | 1.2 | 1.0 | - | - |
| | *Hyperolius* sp. 1 | 4.5 | 6.0±8.66 | - | - |
| | *Hyperolius* sp. 3 | 1.9 | 22.0 | - | - |
| *Physaloptera* sp. | *H. concolor* | 4.4 | 1.5±0.71 | - | - |
| | *Hyperolius* sp. 1 | 3.0 | 2.5±2.12 | - | - |
| | *L. spiritusnoctis* | 14.3 | 6.0 | - | - |
| Oxyurid nematode | *Hyperolius* sp. 3 | 1.9 | 36.0 | - | - |

## Discussion

High species diversity (H'= 2.215) and richness (d=1.971) were recorded among the tree frogs of the cocoa plantations under study, although the species were not evenly distributed. Some tree frog species, including *A. nigeriensis, H. fusciventris, H. sylvaticus, Hyperolius* spp. 2 and 4 were sparsely represented as only a single individual of each was encountered. The species number recorded (14) was however lower than the 23 recorded in the protected environment at the Okomu National Park by Imasuen *et al.* (2012). Furthermore, forest specialist such as *Chiromantis rufescens*, reported at the Gelegele Forest Reserve and the Okomu National Park was not encountered in this study, possibly due to the contaminated and altered nature of the plantation environment.

An overview of the pattern of parasitic infections in this study showed generally low prevalence and intensity of infection of the individual helminth parasites recorded. This observation is similar to earlier reports on the pattern of parasitic infections observed in leaf litter frogs (Edo-Taiwo and Aisien, 2020a) and ground dwelling amphibians (Edo-Taiwo and Aisien, 2020b) collected from the same cocoa plantations. This means that irrespective of the microhabitat occupied by anurans in the cocoa plantations, the limiting effect of residual pesticide on the transmission and establishment of parasites is the same. It manifests in high parasite diversity characterized by low prevalence and infection intensity (Edo-Taiwo and Aisien., 2020a, b). An additional factor possibly responsible for the low parasite prevalence intensity is the arboreal location (habitat) of these frogs, which makes them less exposed to infection in comparison to their ground-dwelling and leaf litter counterparts. For example, at the Okomu National Park which is devoid of anthropogenic activities and pesticide contamination, prevalence/infection intensity values were equally low (Imasuen *et al.*, 2012).

The parasite species number (13) and prevalence of infection (30.23%) recorded among the tree frogs are marginally lower than the values (15 parasites/ 37.4% prevalence) recorded among the leaf-litter frogs (Edo-Taiwo and Aisien, 2020a), but was much more lower than the 33 parasites/ 60.36% prevalence recorded among the ground dwelling amphibians (Edo-Taiwo and Aisien, 2020b). While the low infection values recorded among the leaf-litter frogs may be connected to the pesticide-contaminated environment (which impeded the development of the free-living stages of their parasites), the prevalence/ intensity of infection recorded among the tree frogs must have other causes. It can be assumed that they are isolated from parasitic stages in water and soil by their arboreal abode. Furthermore, it is possible that pesticide application may also have eliminated some arthropod vectors that could have transmitted parasites to them in this microhabitat. The immuno-suppressive effects of pesticides on the pre-metamorphic stages of the ground-dwelling anurans may have rendered them more susceptible to infection, hence the higher prevalence recorded in this group. Nevertheless, the infection intensity among these frogs was generally low, arising from the inhibitory effects of the pesticides on the free-living stages of parasites (Pietrock and Marcogliese, 2003) and possibly the elimination of arthropod hosts transmitting infection to these anurans.

The absence of *Mesocoelium* infection among the tree frogs in the cocoa plantations is noteworthy. Among the leaf-litter frogs, two unidentified *Mesocoelium* spp. were recorded (Edo-Taiwo and Aisien, 2020a), while among the ground dwelling anurans six tentative spp. were reported (Edo-Taiwo and Aisien, 2020b). Similarly, the tree frogs from the Okomu National Park were infected by *Mesocoelium monodi* and *M. monas* (Imasuen *et al.*, 2012). In contrast to the frogs from these other habitats, no *Mesocoelium* infection was recorded in the tree frogs from the cocoa plantations. *Mesocoelium* spp. are transmitted by molluscan intermediate hosts (Thomas, 1965). It is presumable that pesticide action may have reduced the population of the snail intermediate hosts, thus reducing the contact between the tree frog and the snail hosts when the frogs occasionally forage for food on the plantation floor. The transmission of *Ostioloides rappiae* among these frogs was unaffected as infection with this digenean was recorded among four *Hyperolius* spp. encountered in the plantations. The intermediate host of this parasite is unknown but it can be assumed that some survived pesticide application to effect the transmission of these infections.

Parasites recovered from the tree frogs were mainly adult stages which have these frogs as definitive hosts. There were however, some larval stages which use anurans as paratenic hosts. Edo-Taiwo and Aisien (2020a) recorded *Proteocephalus* spp. 1 and 2 from *Artholeptis* spp. among the leaf-litter frogs and from some ground dwelling anurans (*Ptychadena* spp. *Sclerophrys regularis* and *Aubria subsigillata*). The same *Proteocephalus* spp. were also recovered from the tree frogs (*Proteocephalus* sp.1 in *Hyperolius* sp.1 and *Proteocephalus* sp. 2 larva from *A. dorsalis* and five *Hyperolius* spp.) (see Table 2). Suspected final host for these cestodes include arboreal snakes and birds. Prevalence of infection was higher in male frogs (30.75%) than in the females (23.53%) just as more males (322) than the females (34) were collected during the study. This is understandably so since the males make the conspecific calls which were used in locating the frogs, especially during the breeding season.

A significantly ($P<0.05$) higher prevalence of infection was recorded during the wet season (31.64%) than in the dry (10.53%). The wet season is the spawning season for these frogs and this activity brings them in contact with the larval stages of parasites (especially nematodes) that occur in the aquatic milieu or others that occur in other microhabitats to take advantage of the high environmental humidity to reach their hosts. Moreover, this is also the season when the arthropod intermediate host of some parasites occur in their abundance to effect their trophic transmission to their definitive hosts. This pattern of infection has also been observed by other authors (Aisien *et al.*, 2001, 2011, 2017).

Three species of cestodes occurred in the tree frogs investigated in this study. *Cylindrotaenia jaegerskioeldi* (*Baerietta*) had a wide hosts range occurring in *Leptopelis* sp., *Afrixalus dorsalis*, *H. fusciventris burtoni*, *H. picturatus*, *Hyperolius* spp. 1 and 3. The two *Proteocephalus* spp. larvae recovered possibly use the tree frogs as paratenic hosts. Other researchers have also reported the occurrence of larval *Proteocephalus* spp. in amphibian hosts (Ulmer and James, 1976; Mckenzie, 2007; Aisien *et al.*, 2011; Imasuen *et al.*, 2012). According to Khalil *et al.* (1994), Proteocephalid cestodes have cosmopolitan distribution and species of *Proteocephalus* have also been recorded in freshwater fishes and reptiles (Scholz and de Chambrier, 2003).

The digenean *Ostioloides rappiae* was recovered only from the small intestine of Hyperolid tree frogs (*H. concolor, H. fusciventris burtoni*, *Hyperolius* spp. 1 and 3). This host specificity agrees with earlier reports by other workers. This trematode was reported from the duodenum of *H. fusciventris burtoni* collected from Cote d' Ivoire (Maeder *et al.*, 1970a). Gassmann (1975) reported it from *Hyperolius nasutus, H. tuberculatus, H. viridistriatus* and *Scotobleps gabonicus* in Cameroon. In addition to hyperolids (*H. fusciventris* and *Hyperolius* sp.), *Ostioloides rappiae* was reported in *Afrixalus dorsalis* collected from Okomu National Park, in Edo State, Nigeria (Imasuen and Aisien, 2012).

The other digenean recorded in this study was the metacercaria of a strigeiod trematode which occurred in high abundance. This observation contrasts with the report of King *et al.* (2008), which observed a decrease in the abundance of strigeoid metacercariae as a result of pesticide pollution. In the same study, King *et al.* (2008) also reported the presence of four non-strigeoid metacercariae (*Clinostomum* sp., *Fibricola* sp., *Alaria* sp. and echinostomes) from pesticide-polluted agricultural land in Canada. The occurrence of strigeiod trematodes had earlier been reported from several tree frogs (*A. dorsalis*, *A. nigeriensis*, *H. fusciventris*, *H. picturatus*, *H. sylvaticus*, *Hyperolius* sp., and *Leptopelis hyloides*) and *P. bibroni* from the Okomu National Park and the Okomu Oil Palm Plantation in Edo State, Nigeria (Edo-Taiwo *et al.*, 2014). Other hosts of strigeiod trematode metacercariae in Africa include *D. occipitalis* and *B. regularis* reported by Pike (1970) in the Sudan. Infected tree frogs serve as intermediate hosts of these trematodes for their trophic transfer to their definitive hosts (Imasuen *et al.*, 2012; Edo-Taiwo *et al.*, 2014), which include birds and mammals (King *et al.*, 2010).

Nematodes were the predominant parasites among the parasites infecting the tree frogs in the cocoa plantations. *Aplectana* sp., *Foleyellides* sp. and *Physaloptera* sp. were generalists, infecting between three to seven anuran hosts. The microfilariae of filariid nematodes including *Folleyellides* are transmitted by *Culex* and *Aedes* mosquitoes to new amphibian hosts (Causey, 1939). The same observation was made by Aisien *et al.* (2017), who reported two *Folleyellides* species in the anurans investigated at Ase, a location in the Niger Delta of Nigeria. The authors remarked that the high prevalence/intensity infection of these nematodes (47.7%/10.2 worms per infected host) bore direct relationship with the high population of mosquitoes observed in the locality. The presence of various water bodies inside the cocoa plantations must have provided good breeding ground for these mosquitoes. Adults (egg-laying females) *Physaloptera* sp. were recovered from *L. spiritusnoctis, H. concolor* and *Hyperolius* sp. 1 in this study. This is contrary to reports of Baker (1987) and Anderson (2000), who noted that adult *Physaloptera* were usually parasites of reptiles, birds and mammals. Other authors (Aisien *et al.,* 2009; Igetei, 2013; Edeigbe, 2015; Ovwah, 2016; Oseki, 2016) have similarly reported adult *Physaloptera* spp. in amphibians studied in southern Nigeria. Nevertheless, most records of *Physaloptera* in amphibians are the larval forms (Goldberg and Bursey, 2001, 2008; González and Hamann, 2007; Goldberg *et al.,* 2009; Imasuen, 2012; Ovwah, 2016) which are thought to use amphibians as transport hosts. These parasites need further studies and characterization as they may represent new species.

*Camallanus* species are commonly encountered in pipid anurans (Southwell and Kirshner, 1937; Yeh, 1960; Thurston, 1970; Avery, 1971; Tinsley *et al.*, 1979; Jackson and Tinsley, 1995, 1998) and *H. occipitalis* (Durette-Desset and Batcharov, 1974; Aisien *et al.*, 2001, 2003, 2004, 2009, 2011, Igetei, 2013; Ovwah, 2016) but its occurrence in hosts like *H. concolor* is uncommon. Aisien *et al.* (2017) recorded an immature female specimen in this tree frog collected from Egbeda in Rivers State. The authors concluded that this parasite may be a new addition to the parasites that use anurans as paratenic hosts.

## Conclusion

Despite pesticide use, high species diversity and richness of tree frogs were recorded in the cocoa plantations investigated in this study. However, generally low prevalence and infection intensity of helminth parasites as previously reported with leaf litter frogs and ground dwelling anurans was also observed. The low parasite prevalence/intensity of infection is indicative of the inhibitory influence of pesticides on the development and transmission of parasites in this environment.

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