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Diversity and parasitic infections of tree frogs in the high conservation value area of Okomu Oil Palm Plantation, Edo State, Nigeria

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Abstract

Agricultural land use is one of the drivers of amphibian decline and it also impacts on the severity of amphibian parasitism. As part of our investigations of the effect of land use on biodiversity and parasitism, we studied the helminth parasitic infections of tree frogs from the High Conservation Value Area (HCVA) at the Okomu Oil Palm Plantations located in Oke Community, Edo State, Nigeria. The frogs were identified, euthanized and then dissected. The gastrointestinal tract and other organs of the viscera were examined for parasites. Ten species of tree frogs were encountered at the HCVA from which seven platyhelminth and three nematode species were recovered. Notable among the parasites was a tetracotyle strigeoid larva, which is a new parasite record in Nigeria. The number of tree frog species and the number of helminth parasites recovered at the HCVA was low when compared with the records at the Okomu National Park. Habitat fragmentation and loss of adequate canopy cover have negatively impacted on tree frog diversity in this sanctuary. Although the HVCA did not provide adequate cover for tree frogs, the idea of a conservation area in the midst of oil palm plantations is desirable and should be encouraged.

Introduction

Agricultural land use is one of the drivers of habitat change in southern Nigeria, where vast acres of rainforest habitats are being replaced by oil palm plantations, with attendant negative impact on flora and fauna. As part of the broad study to understand land use type and the effect on amphibian diversity and parasitism, Imasuen (2012) investigated the amphibians at the Okomu National Park (a protected rainforest biotope) and their parasitic infections. The study revealed over 40 amphibian species in the five compartments accessed. The study also reported on the helminth parasite fauna and chytridiomycosis in the amphibians collected from this rainforest reserve (Imasuen *et al* 2009, 2011, 2012a, b; Imasuen and Aisien 2015). Subsequent studies by Igetei (2013) and Ovwah (2019) investigated the effects of monoculture plantations on amphibian diversity and parasitism at the Okomu Rubber and Oil Palm plantations, respectively. Igetei (2013) recorded 16 species of amphibians and 22 helminth parasite spp. The study at the Okomu Oil Palm Plantations (Okomu-Udo) recorded 18 anuran species and 40 species of helminth parasites (Ovwah 2019).

The Oil Palm industry being one of the drivers of habitat change, plantation developers are required to create buffer zones designated as High Conservation Value Areas (HCVAs) within the plantations. These protected sanctuaries serve as safe havens for various animals including amphibians. We investigated the tree frogs in the HCVA at the Okomu Oil Palm Plantation (Extension II) located at Oke Community in Ovia North East Local Government Area (LGA) of Edo State, Nigeria and report on the diversity and parasitic infections of tree frogs in this enclave.

Materials and methods

Study area

The study was undertaken at Okomu Oil Palm Plantation (Extension II) situated at Oke Community in Ovia North East LGA of Edo State, Nigeria, for 12 months (between August 2016 and July 2017). The plantation covers an area of 11,416 hectares bounded by latitudes 06°38′N to 06°48′N and longitudes 005°48′E to 005°55′E) in the rainforest biotope. The area has an undulating topography with the mucuna grass forming the undergrowth within the plantations. The wet season starts in April and ends in October while the dry season lasts from November to March.

Collection of anurans and recovery of helminth parasites

The anurans were collected by hand at night between 8pm and 12 midnight near the Management Quarters (06°41′N, 005°49′E), a seasonal lake close to the oil palm nursery (06°40′N, 006°48′E) and a swamp designated as a biodiversity conservation area (06°40′N, 005°49′E), using the Visual Acoustic Encounter Survey (VAES) method. The anurans were identified using the protocols of Schiøtz (1999) and Roedel (2000) and thereafter euthanized with Benzocaine solution (Goater *et al* 1987). The snout-vent length (SVL) was measured and the sexes determined. The anurans were dissected and the sites

examined for parasites included the gastrointestinal tract, lungs, urinary bladder, liver/gall bladder and the body cavity. The different sections were excised and placed in separate Petri dishes containing 0.72% saline solution, cut open and examined for parasites with a dissecting microscope. The recovered parasites were preserved as follows: the cestodes, monogeneans and digeneans were flattened under cover slip pressure, fixed and preserved in 5% formol-saline. Nematodes were fixed with hot 70% ethanol and preserved in fresh 70% ethanol in specimen bottles.

Identification of helminth parasites

The flatworms (cestodes, monogeneans and digeneans) were washed in 4 changes of tap water, thereafter stained in a dilute solution of acetocarmine, dehydrated in alcohol series, cleared in xylene and permanent mounts made in Canada balsam. The nematodes were cleared with lactophenol and examined as temporary mounts using a binocular microscope. The parasites were identified with appropriate keys (Yamaguti 1971; Andersen *et al* 1974; Prudhoe and Bray 1982; Khalil *et al* 1994). Micrographs of the parasites were taken with a digital camera (DFK MKU 130-10 x 22, Image Source, Germany) attached to a binocular microscope. Prevalence and mean intensities were calculated for each parasite taxon recovered (Bush *et al* 1997).

Results

Two families of tree frogs (Arthroleptidae and Hyperolidae), represented by five genera and ten species were encountered in the HCVA as shown in Table 1.

Prevalence and mean intensity of helminth parasites in tree frogs

A total of 215 tree frogs were examined, from which 11 species of helminth parasites were recovered (Table 2). Parasites were recovered from nine of the ten tree frogs encountered in the HCVA. *Afrixalus nigeriensis* did not habour any parasite while *L. viridis* was infected with only one parasite. The helminth parasites recorded include Cestoda: *Cylindrotaenia* sp., *Nematotaenoides* sp.; Monogenea: *Polystoma dorsalis*; Digenea: *Mesocoelium monodi*, *Ostioloides rappiae* and a strigeoid trematode larva (Tetracotyle-type larva); Nematoda: *Cosmocerca ornata*, *Aplectana mackintoshi*, *Camallanus* sp., *Physaloptera* sp. and an unidentified oxyurid nematode. The prevalence and mean intensity of infection of the parasites recorded are presented in Table 2.

Of the 11 helminth parasites recovered, four (*Cylindrotaenia* sp., *Nematotaenoides* sp., *P. dorsalis* and the Tetracotyle strigeoid larva) occurred only in *A. dorsalis* at low prevalence and infection intensity (Table 2). *Mesocoelium monodi* occurred in *H. concolor* (phase C) (P/MI = 2.1/7.0) while *O. rappiae* was recovered in *H. concolor* Phase C and *L. spiritusnoctis* (P/MI = 2.1/1.0 and 7.2/1.0), respectively. Infection with *C. ornata* occurred in all the tree frogs except *P. boulengeri*; *A. mackintoschi* infected only two frogs (*L. spiritusnoctis* and *K. senegalensis*) while *Camallanus* sp. was recorded only in *K. senegalensis*. On the other hand, *Physaloptera* sp. was recorded in *H. fusciventris burtoni*, *H. concolor* (phase C) and *L. spiritusnoctis*. An unidentified oxyurid nematode infected *P. boulengeri* albeit at very low infection intensity.

With regards to seasonal prevalence (Table 3), more hosts seven (07) were infected during the wet season than the four (4) recorded in the dry season. Parasite prevalence in the wet season ranged from 2.2% to 100%; in the dry season it ranged from 3.7% to 50%. Infection with *C. ornata* was recorded in *Afrixalus dorsalis* in both seasons but with a higher prevalence in the dry season (29.6% in the dry season *vs* 9.3% in the wet season).

In relation to host sex, more parasites were encountered in male tree frogs (10) than in the females (4) (Table 4). Parasites with high prevalence among the male frogs included *C. ornata* in *K. senegalensis* (64.5%) and *L. viridis* (57.1%), and *Physaloptera* sp. in *L spiritusnoctis* (33.3%). Except in a few cases (*C. ornata* in *H. fusciventris* -75%; *Physaloptera* sp. in *L. spiritusnoctis*-80%), prevalence values in female frogs were mostly below 25%. Parasites that occurred in both sexes included *Cylindrotaenia* sp. (in *A. dorsalis*), *C. ornata* (in *H. concolor*, phase C and *A. dorsalis*) and *Physaloptera* sp. (in *L. spiritusnoctis*).

Table 1: Tree frogs encountered in the HCVAs at the Okomu Oil Palm Plantations (Extension II) at Oke Community, Edo State

Family	Genus	Species
Arthroleptidae	*Leptopelis*	*L. spritunoctis*
		L. virids
Hyperolidae	*Hyperolius*	*H. fusciventris burtoni*
		H. concolor (phase A)
		H. concolor (phase B)
		H. concolor (phase C)
	Kassina	*K. senegalensis*
	Afrixalus	*A. dorsalis*
		A. nigeriensis
	Phlyctimantis	*P. boulengeri*

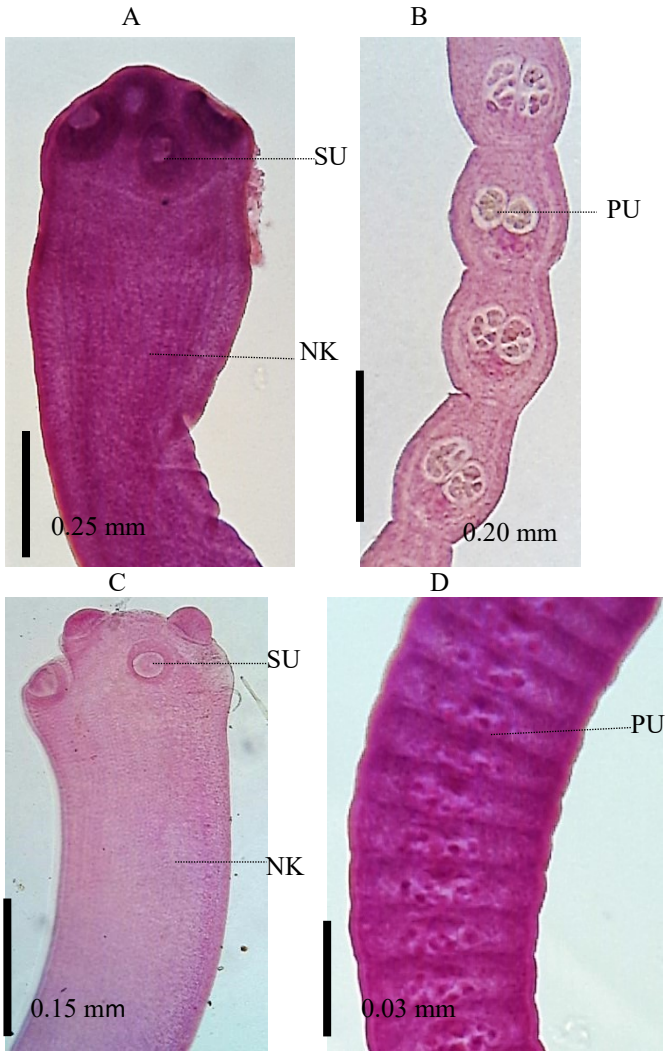

Figure. 1A-D. Cestode species infecting tree frogs from the HCVA at Okomu Oil Plantation (Extension II), Edo State, Nigeria. A. Scolex, B. Gravid proglottids of *Cylindrotaenia* sp.; C. Scolex, D. Gravid proglottid of *Nematotaenoides* sp. *Abbrevitions*: NK, Neck; PU, Paruterine organs; SU, Sucker.

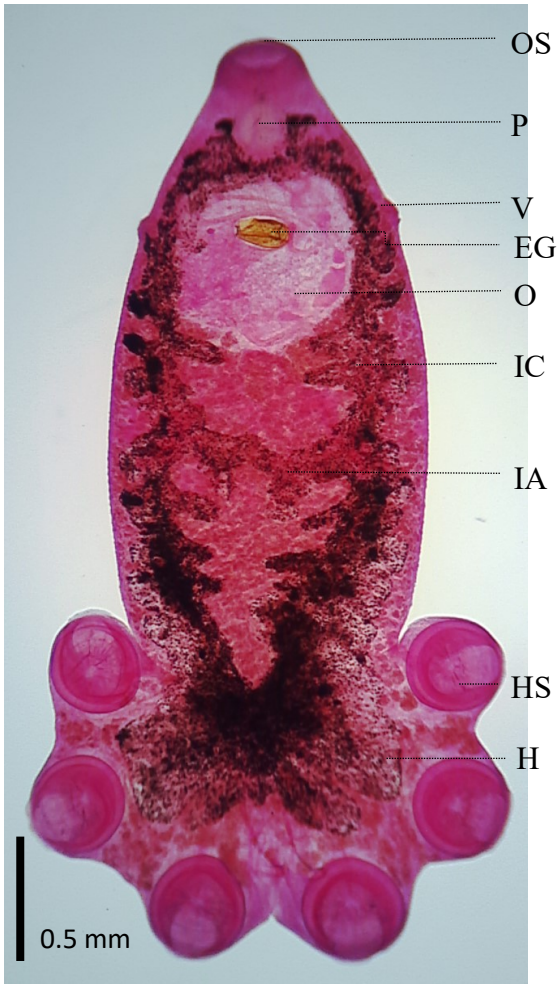

Figure 2. *Polystoma dorsalis* infecting *Afrixalus dorsalis* from the HCVA, Okomu Oil Plantation (Extension II), Edo State, Nigeria. *Abbreviations*: EG, Egg; HP, Haptor; HS, Haptoral sucker; IA, Intercaecal anastomosis; IC, Intestinal caecum; OV, Ovary; OS, Oral sucker; PH, Pharynx; VG, Vagina.

Discussion

From this study, there is no evidence that the enclave designated as HCVA has profited from this status in terms of the amphibian diversity recorded therein. Only ten species of tree frogs were encountered in comparison to the 23 species occurring at the ONP (Imasuen *et al* 2012). Habitat fragmentation caused by the surrounding Oil Palm plantations must have adversely impacted on the faunal diversity in this sanctuary. Moreover, the absence of adequate canopy cover due to the absence of large trees in the HCVA had a limiting effect on the number of tree frog species thriving at the HCVA.

Only two classes of helminth parasites (platyhelminths and nematodes) infected the tree frogs from the HCVA. This pattern was also observed in the frogs investigated at the ONP, which is a protected sanctuary (Imasuen *et al* 2012) and from two other non-protected rainforest biotopes where acanthocephalan cysthacanths were additionally recorded (Imasuen *et al* 2019). A major difference between the ONP and HVCA is that ONP is a protected sanctuary with a primary forest devoid of anthropogenic activities. On the other hand, the HCVA is a secondary forest that is yet to recover from human activities at the onset of the palm plantations. Consequently, the frogs from the ONP harboured more helminth parasites species (13) than the nine (9) in frogs from the HCVA because of the more conducive environment at the national park. Furthermore, more polystome species (5) were recorded from the tree frogs at ONP than those from the HCVA.

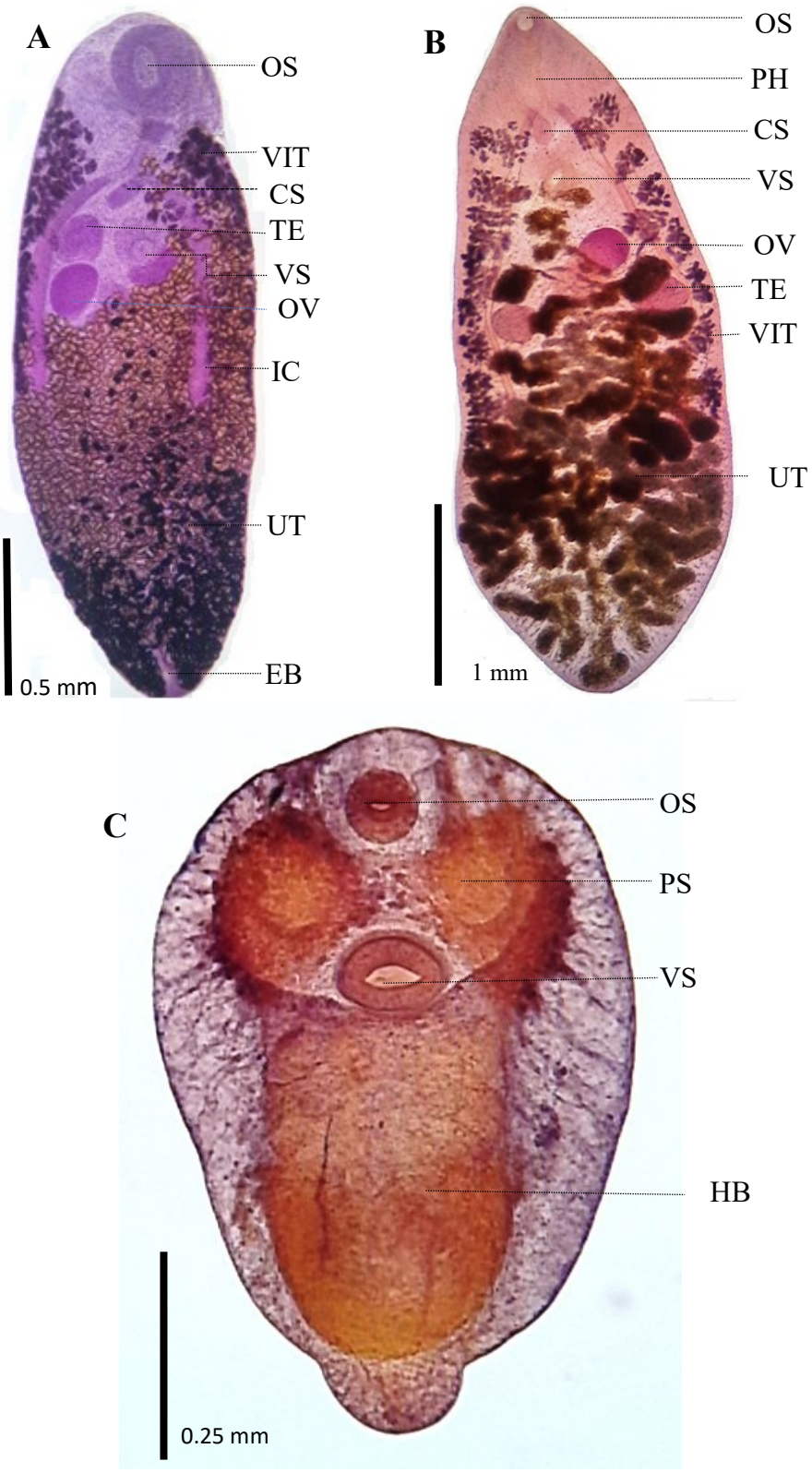

Figure 3A-C. Digeneans infecting tree frogs in the HCVA at Okomu Oil Plantation (Extension II), Edo State, Nigeria. A. *M. monodi*; B. *O. rappiae*; C. Tetracotyle strigeoid larva. *Abbreviations* CS Cirrus sac; EB, Excretory bladder; HB, Hindbody; OS, Oral sucker; OV, Ovary; PH, Pharynx; PS, Pseudo sucker; TE, Testis; UT, Uterus; VIT, Vitellaria; VS, Ventral sucker.

Table 2: Prevalence and mean intensity of infection in tree frogs from the HCVAs in Okomu Oil Palm Plantation (Extension II) at Oke Community, Edo State, Nigeria

Parasite	*Afrixalus dorsalis* (n=114); P(MI)	*Afrixalus nigeriensis* (n=01); P(MI)	*H. concolor* (phase A) (n=17) P(MI)	*H. concolor* (phase B) (n=02) P(MI)	*H. concolor* (phase C) (n=48) P(MI)	*H. fusc. burtoni* (07) P(MI)	*K. senegalensis* (33) P(MI)	*L. spirtusnoctis* (14) P(MI)	*P. boulengeri* (01) P(MI)	*L. viridis* (07) P (MI)
Cestoda										
Cylindrotaenia sp.	6.2 (2.2)	-	-	-	-	-	-	-	-	-
Nemataenoides sp.	2.0 (1.0)	-	-	-	-	-	-	-	-	-
Monogenea							-			
P. dorsalis	1.0 (1.0)	-	-	-	-	-	-	-	-	-
Digenea										
M. monodi	-	-	-	-	2.1 (7.0)	-	-	-	-	-
O. rappiae	-	-	-	-	2.1(1.0)	-	-	7.2 (1.0)	-	-
Strigeoid larva	1.8 (3.5)	-	-	-	-	-	-		-	-
Nematoda								-		
C. ornata	14.0(5.3)	-	5.9 (1.0)	50 (1.0)	14.6 (15.8)	43.0 (8.7)	57.6 (3.6)	14.2 (15.8)	-	57.1 (2.5)
A. mackintoshi	-	-	-	-	-	-	13.0(3.0)	7.1 (10)		-
Camallanus sp.	-	-	-	-	-	-	6.1(1.0)	-	-	-
Physaloptera sp.	-	-	-	1.4 (1.8)	10.4 (1.8)	14.3 (2.0)	-	50.0(2.0)	-	-
Unidentified oxyurid nematode	-	-	-	-		-	-	-	100.0 (1.0)	-

The polystomes recorded in the tree frogs from the ONP included *Polystoma dorsalis* from *Afrixalus dorsalis*, *P. chiromantis* from *Chiromantis rufescens*, *P. gracei* from *Leptopelis hyloides*, *P. okomuense* from *Phlyctimantis boulengeri* and a *Polystoma* sp. recorded in *Hyperolius sylvaticus*. Only *P. dorsalis* was recorded at the HCVA. We presume that this low polystome presence to be a consequence of the altered nature of the HCVA, which was largely devoid of canopy cover as occurs at the ONP. This exposure not only excluded some of the hosts (e.g. *Chiromantis rufescens* and *Hyperolius sylvaticus*) but also created unfavourable ecological conditions, which may have hindered the ability of the monogeneans to successfully complete their life cycles. Interestingly, tree frogs from the pesticide-contaminated cocoa plantations at Ugboke, Edo State harboured more parasites (13) than those from the HCVA. Studies have shown that parasite prevalence tend to be higher in pesticide-contaminated environments because of the immunosuppressive effect of pesticides on the frogs, thereby rendering them more susceptible to infection (Pietrock and Marcogliese 2003; Rohr *et al* 2008; Aisien *et al* 2011). While more cestode and nematode species were recorded in frogs from the cocoa plantations, monogeneans were conspicuously absent. Their absence this time around was not occasioned by lack of canopy cover (which the cocoa plantations provided), but presumably due to the pesticide contamination of the cocoa plantation environment. Thus, it is evident that the prevalence of polystomes in these frogs can be influenced by the loss of canopy cover or can be due to environmental contamination, factors which adversely affect the development and transmission of their free-living oncomiradial stage (Pietrock and Margoliese 2003).

One noteworthy parasite encountered at the HCVA was the tetracotyle-type larva of a strigeoid trematode (Figure 3C), recovered from *A. dorsalis*. In an earlier publication, Edo-Taiwo *et al* (2014), reported some larval strigeoids occurring in some anurans from southern Nigeria. They included two cyst types; the type I (rounded in shape) occurred in the body cavity of several tree frogs including *Afrixalus dorsalis*, *A. nigeriensis*, *Chiromantis rufescens*, *Hyperolius sylvaticus*, *Hyperolius* sp. and *Leptopelis hyloides* from the ONP and *Hyperolius fusciventris* from Okomu Oil Palm Plantation (OOPP). The type II cysts were ovoid in shape and occurred in the body cavity of *A. dorsalis* and *Ptychadena bibroni* from the OOPP. While Cyst type I was susceptible to trypsin digestion, Cyst type II was not. Also, at the OOPP, the mesocercaria of *Alaria* sp. was recovered from the lungs of *Ptychadena pumilio*. At a third location (Usen) sited in a forest-savanna biotope, *A. dorsalis* harboured the type I cyst and the unencysted metacercaria of another strigeoid larva in the body cavity, giving a total of four strigeoid larvae types. In the present study, none of these species were recorded in the anurans at the HCVA. In contrast, the strigeoid trematode larva recorded in this location was a

Table 3: Seasonal prevalence of helminth parasites in the tree frogs of the HCVAs at Okomu Oil Palm Plantation (Extension II) at Oke Community, Edo State, Nigeria

Parasite	Host	Wet Season			Dry Season		
		No of host examined	No of host infected	Prev. (%)	No of host examined	No of host infected	Prev. (%)
Cestoda							
Cylindrotania sp.	*A. dorsalis*	87	7	8.0	27	-	-
Nematotaenoides sp.	*A. dorsalis*	87	2	2.3	27	-	-
Monogenea *P. dorsalis*	*A. dorsalis*	87	-	-	27	1	3.7
Digenea	*L. spirtusnoctis*	13	1	7.7	1	-	-
O. rappiae	*H. concolor* (phase C)	44	1	2.2	4	-	-
	A. dorsalis	87	2.	2.3	27	-	-
Strigeoid larva							
Nematoda	*K. senegalensis*	33	19	57.6	-	-	-
C. ornata	*L. spiritusnoctis*	13	3	23.0	1	-	-
	H. concolor (phase C)	44	5	11.1	4	-	-
	H. concolor (phase B)	1	1	100	1	-	-
	H. concolor (phase C)	12	-	-	5	1	20
	A. dorsalis	87	8	9.3	27	8	29.6
	H. fusciventris	1	-	-	6	3	50
	A. galamensis	-	-	-	10	2	20.0
	L. viridis	7	4	57.1	-	-	-
	P. boulengeri	1	1	100	-	-	-
A. mackintoshi	*L. spritusnoctis*	13	1	7.7	1	-	-
	K. senegalensis	33	1	3.0	-	-	-
	K. senegalensis	3	2	6.1	-	-	-
Camallanus sp.	*L. spiritusnoctis*	13	7	53.8	1	-	-
	H. concolor (phase C)	44	-	-	4	-	-
	H. concolor (phase B)	1	-	-	6	1	16.7
	P. boulengeri	1	1	100	-	0	-
Unidentified oxyurid nematode							

tetracotyle-type larva in *A. dorsalis*. Tetracotyle larvae are metacercariae of *Strigea* spp., parasitic in birds of prey (vultures and owls). They use freshwater fishes and amphibians (tadpoles or adult frogs) as intermediate hosts (Hoffman 1960; Hamann *et al* 2023). Adults of four species (*Strigea. neotidis*, from *Neotis cafra denhami*; *S. lilensis* from *Pseudogyps africanus*; *S. cuncumae* from *Cuncuma vocifer vocifer* and *S. rhodesiensis* from *Pseudogyps africanus*) have been reported in the gastrointestinal tract of birds of prey in Central Africa (Biseru 1956). It will be of interest to know the definitive hosts of this species in the HVCA ecosystem. Unlike the observation that sex had no significant effect on parasite prevalence ($p>0.05$), overall prevalence according to season was the opposite; prevalence was significantly higher in the wet season ($p<0.05$) than in the dry season.

Conclusion

Although the HCVA is designated as a protected area, its tree frog diversity did not reflect this status considering the few species (10) recorded in this study. This figure sharply contrasts with the species number (23) recorded at the ONP. While there were similarities in the classes of helminth parasites that infect tree frogs at the HCVA and at the Okomu National Park, the tree frogs at the ONP harboured more parasites than those from the HCVA. Although the HCVA is free of anthropogenic activities, the absence of adequate canopy cover may have hindered the development and transmission of some monogeneans (polystomes) even though the appropriate hosts were present in the environment. Investigation of the tree frogs in this enclosure revealed the presence of a tetracotyle-type larva (strigeoid larava), which is a new record in the amphibians of Nigeria.

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Table 4: Prevalence of helminth parasites in tree frogs from the HCVAs at Okomu Oil Palm Plantations (Extension II) at Oke Community, Edo State, Nigeria according to sex

Parasite	Host	Male			Female		
		No of host examined	No of host infected	Prev. (%)	No of host examined	No of host infected	Prev (%)
Cestoda							
Cylindrotaenia sp.	*A. dorsalis*	69	7	10.1	45	1	2.2
Nematotaenoides sp.	*A. dorsalis*	69	2	2.9	45	-	-
Monogenea							
P. dorsalis	*A. dorsalis*	69	-	-	45	1	2.2
Digenea							
M. monodi	*P. calcaratus*	16	-	-	4	1	25
	H. concolor (phase C)	32	-	-	16	1	6.3
O. rappiae	*L. spiritusnoctis*	9	-	11.1	5	-	-
Strigeoid larva	*A. dorsalis*	69	2	2.9	45	-	-
Nematoda							
C. ornata	*K. senegalensis*	31	20	64.5	2	-	-
	L. spiritusnoctis	9	2	22.2	5	-	-
	H. concolor (phase A)	7	-	-	-	-	-
	H. concolor (phase B)	1	1	100	-	-	-
	H. concolor (phase C)	32	3	9.4	16	1	6.3
	A. dorsalis	69	7	10.1	45	8	17.8
	H. fusciventris	3	-	-	4	3	75.0
	L. viridis	7	4	57.1	-	-	-
	P. boulengeri	1	1	100	-	-	-
A. mackintoshi	*L. spiritusnoctis*	9	1	11.1	2	-	-
	K. senegalensis	31	1	3.2			
	K. senegalensis	319	3	9.7	2	-	-
Camallanus sp.	*L. spiritusnoctis*	9	3	33.3	5	4	80.0
Physaloptera sp.	*H. concolor* (phase C)	32	2	6.3	16	-	-
	H. concolor (phase B)	3	-	-	-	-	-
Unidentified oxyurid nematode	*P. boulengeri*	1	1	100	-	-	-

Conflict of interest

The authors declare that there is no conflict of interest.

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