

Parasitic endohelminths of tree frogs from two rainforest habitats in Edo State, Nigeria

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Abstract

Tree frogs are generally regarded as arboreal frogs and those associated with water during their breeding season as aquatic/arboreal frogs. Obazuwa Wetlands and Ikpako Riparian Habitat are both located in Ovia North East and Ovia South West Local Government Areas of Edo State, Nigeria. Frogs were captured from both habitats based on Acoustic Encounter Survey (AES) and the Visual Encounter Survey (VES) techniques. A total of 169 tree frogs were examined; 103 from Obazuwa Wetlands and 66 from Ikpako Riparian Habitat. The specimens from both sites comprised 08 species. Helminth parasites belonging to four taxa were recovered, which included: Cestoda, Trematoda, Nematoda and Acanthocephala. A prevalence of 39.8% and 30.5% were recorded for Obazuwa Wetlands and Ikpako Habitat, respectively. The helminth parasites were restricted to one of both habitats except for *Foleyellides* sp., which was common to both sites. Some of the helminth parasites were reported for the first time in some of the hosts such as *Baerietta jaegerskioeldi* (in *Afrivalus nigeriensis* and *Leptopelis spiritusnoctis*), *Amplificaecum africanum* (in *Hyperolius guttulatus*, *Aplectana macintoshii* (in *L. spiritusnoctis*), *Cosmocerca commutata* (in *A. dorsalis* and *L. spiritusnoctis*) and *Physaloptera* sp. (in *Hyperolius concolor* Phase B and *H. concolor* Phase C). The occurrence of *Foleyellides* sp. in 6 species of tree frogs with a prevalence range of 23.1-75.1% whereas only two species have been previously reported is exceptional and of pathological concern.

Keywords: Tree frogs; helminthes; wetlands; riparian habitats; Edo State; Nigeria.

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Introduction

Tree frogs are generally regarded as arboreal frogs although their different reproductive behaviour has classified them further as arboreal/aquatic or arboreal/non-aquatic. The former associate with water during breeding season in which case the eggs or tadpoles develop in water, while the latter have their young developing from eggs in the absence of water (Crump, 2009). The tree frogs investigated in this research belong to the first category. Information on the helminth parasites of Nigerian amphibians from the different bioclimatic zones in the country is building up. Of the four zones investigated: rainforest (Aisien *et al* 2001; 2009, Imasuen *et al* 2012a, b), Savannah mosaic and transitional forest (Aisien *et al* 2003; Ozemoka, 2013), Guinea Savannah (Avery, 1971, Aisien *et al* 2004) and mangrove (Aisien *et al* 2015), only the investigations by Aisien *et al* (2003) and Aisien *et al* (2009) sparingly reported on helminth parasites of tree frogs. The first detailed report on helminth parasites of tree frogs was by Imasuen *et al* (2012a) from Okomu National Park wherein 25 species of tree frogs were encountered and 5 classes of helminth parasites were recovered from the hosts. This study would be the second

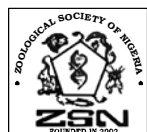
known detailed report exclusively on tree frogs in Nigeria, from two different rainforest habitats, Obazuwa Wetlands (subsequently referred to as OBW) and a riparian habitat at Ikpako (hereafter referred to IKR habitat).

Materials and methods

Study area

Obazuwa Wetland is situated in a rainforest environment of Obazuwa Village, Edo State, Nigeria. Geographically, Obazuwa is located in Ovia North East Local Government Area of the state, between Latitudes 6°56'N and Longitudes 5°63'E. Activities around the wetland are strictly bounded by rules restricting human activities such as farming, laundry and bathing since it serves as a major source of water supply for the community. The water level ranged from 4.56 to 5.20 m.

Ikpako is geographically located in Ovia South West Local Government Area of Edo State, Nigeria, situated approximately between 6°45'N and 5°52'E in a rainforest environment. The sampled area is characterized with anthropogenic activities such as farming, washing and fetching of water for domestic use.



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Collection and examination of specimens

Specimens of tree frogs were captured at night by Acoustic and Encounter Survey (AES) and the Visual Encounter Survey (VES) techniques (Crump and Scott, 1994). The captured frogs were transferred into plastic bottles with perforated tops and then transported to the laboratory. Identification of the frogs was carried out based on standard protocols (Schiotz, 1963, 1999; Rödel, 2000; Rödel *et al* 2005).

The specimens were anaesthetized for a few minutes in benzocaine solution, followed by post-mortem examination. The sections examined for parasites included gastro-intestinal tract (oesophagus, stomach, small intestine and large intestine/rectum), lungs, urinary bladder, liver/gall bladder and body cavity. The sections were placed in petri dishes containing 0.72% NaCl (physiological saline) and teased for examination under a dissecting microscope. Cestodes, monogeneans and trematodes were flattened under cover slip pressure on microscope slide and fixed in 5% formol-saline. Fixed specimens were preserved in specimen bottles containing fresh 5% formol-saline. Nematodes recovered were fixed in hot 70% ethanol and preserved in specimen bottles containing fresh 70% ethanol. Flat worms were washed free of the preservative in several changes of water, stained overnight in diluted acetocarmine solution, dehydrated and permanent mount made in Canada balsam. Each nematode was cleared in lactophenol prior to examination (Aisien *et al* 2001, 2003, 2004). Cysts were crushed under cover slip pressure to identify the parasite group. Appropriate keys were used in the identification of parasites (Yamaguti, 1961; Smyth and Smyth, 1980; Prudhoe and Bray, 1982; Khali *et al* 1994). Prevalence and mean intensity of infection for each parasite were calculated. Photomicrographs of the parasites were taken using a Sonny digital camera (20.1 mega-pixels) attached to a microscope.

Results

In this study, a total of 169 tree frogs belonging to 3 families, 4 genera and 8 species were examined. One hundred and three frogs were collected from OBW while 66 were collected from IKR habitat. Six species of the frogs were encountered at each of the sites (Table 1). The helminth parasites recovered from these tree frogs are presented in Table 2. The parasites belong to four taxa: Cestoda, Trematoda, Nematoda and Acanthocephala. Six helminth parasites were recorded at each of OBW and IKP habitat with the prevalence of 39.8% and 30.5%, respectively, but the difference was not statistically significant ($p>0.05$). *Foleyellides* sp. was the only parasite species common to both sites. Other parasites were restricted to one of both habitats, although a wider range of infected host (6 species of tree frogs) was recorded at OBW while only 2 species of tree frogs were infected at IKR habitat.

The helminth parasites recovered at OBW include *Baerietta* sp. (Figure. 1), metacercariae of strigeoid

trematode cyst (Type 1) (Figure. 2), *Aplectana* sp., *Cosmocerca ornata*, *Foleyellides* sp. and acanthocephala larvae (Table 2). The range of prevalence was from 3.4%-75% and the mean intensity was between 1 and 90.5 parasites/infected frog. The highest prevalence of 75.0% was recorded for *Foleyellides* species infecting *A. nigeriensis* followed by 66.7% for *A. paradorsalis* infected by the same parasite. The lowest prevalence of 3.4% was recorded for *L. spiritusnoctis* infected by two parasites, *Baerietta* sp. and *Foleyellides* sp. Larval stages recorded were metacercariae of strigeoid trematodes and Acanthocephala with a prevalence of 7.7% each and mean intensity of 90.5 and 1 parasite/infected host for *A. dorsalis* and *C. rufescens*, respectively. *Baerietta* sp. and *Foleyellides* were found to be multi-host parasites while *C. ornata* infected two hosts. *Aplectana* sp. metacercariae cyst (Type 1) of strigeoid trematode and Acanthocephala larva infected one host each. The metacercarial cyst Type 1 has rounded bodies divided into an outer hyline layer with projections at the dark fringes and a dark inner core enclosing the developing larvae.

Table 1: Tree frogs species examined from Obazuwa wetlands and Ikpako riparian habitat in Edo State, Nigeria.

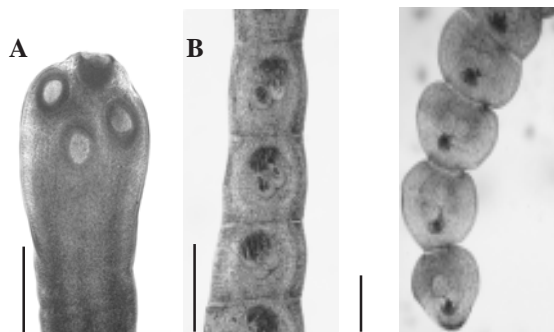
Family	Locations			Total
	Tree frog species	Obazuwa Wetlands	Ikpako	
Arthroleptidae	<i>Leptopelis spiritusnoctis</i>	29	3	32
Hyperoliidae	<i>Hyperolius concolor</i> Phase A	2	2	4
	<i>Hyperolius concolor</i> Phase B	-	1	1
	<i>Hyperolius concolor</i> Phase C	15	11	26
	<i>Hyperolius fusciventris</i>	-	1	1
	<i>Hyperolius guttulatus</i>	-	1	1
	<i>Afrixalus dorsalis</i>	26	42	68
	<i>Afrixalus nigeriensis</i>	12	5	17
	<i>Afrixalus paradorsalis</i>	6	-	6
	<i>Chiromantis rufescens</i>	13	-	13
	Total	103	66	169
Rhacophoridae				

At IKR habitat, only nematodes were recovered from the tree frogs examined which were *Amplichaecum africanum*, *Physaloptera* sp., *Aplectana macintoshii*, *Cosmocerca commutata* (Figure 3), *Camallanus* sp. and *Foleyellides* sp. (Table 2). The prevalence range was between 3.3% and 100%, while the range of intensity of infection was between 1 and 6 parasites/infected host.

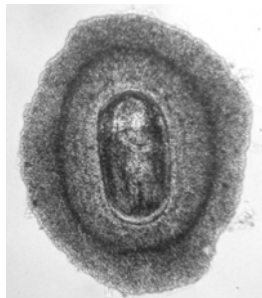
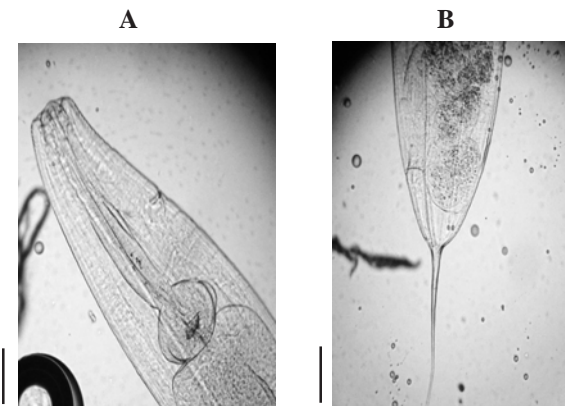
Table 2: Prevalence and mean intensity of parasitic endohelminths of tree frogs from Obazuwa Wetlands and Ikpako riparian habitat in Edo State, Nigeria.

Parasite	Host	Obazuwa			Ikpako	
		Site	%	MI	%	MI
Cestode						
<i>Baerietta</i> sp.	<i>A. dorsalis</i>	A	7.7	3	-	-
	<i>A. nigeriensis</i>	A	16.7	3	-	-
	<i>L. spiritusnoctis</i>	A	3.4	1	-	-
Trematode						
Strigeoid metacercaria cyst	<i>A. dorsalis</i>	B	7.7	90.5	-	-
Nematode						
<i>Amplificaecum africanum</i>	<i>H. guttulatus</i>		-	-	100	1
<i>Aplectana mackintoshii</i>	<i>L. spiritusnoctis</i>		-	-	33.3	2
<i>Aplectana</i> sp.	<i>A. dorsalis</i>	B	3.8	5	-	-
<i>Camallanus</i> sp.	<i>H. concolor</i> Phase C		-	-	9.1	1
<i>Cosmocerca commutate</i>	<i>A. dorsalis</i>		-	-	2.4	6
	<i>L. spiritusnoctis</i>		-	-	3.3	5
<i>Cosmocerca ornate</i>	<i>A.nigeriensis</i>	B	8.3	5	-	-
	<i>L. spiritusnoctis</i>	B	6.9	5	-	-
<i>Foleyellides</i> sp.	<i>A. dorsalis</i>	C	21.7	3.3	3.5	4
	<i>A. nigeriensis</i>	C	75.0	3.3	-	-
	<i>A. paradorsalis</i>	C	66.7	10	-	-
	<i>C. rufescens</i>	C	23.1	4.5	-	-
	<i>H. concolor</i> Phase A	C	50.0	1	-	-
	<i>H. concolor</i> Phase C	C	53.3	14.4	27.3	6
	<i>L. spiritusnoctis</i>	C	3.4	1	-	-
	<i>Physaloptera</i> sp.	<i>H. concolor</i> Phase B		-	-	100
	<i>H. concolor</i> Phase C		-	-	100	3
Acanthocephala						
Acanthocephala cysthacanth	<i>C. rufescens</i>	D	7.7	1	-	-

A represents small intestine; B, large intestine/rectum; C, body cavity; D, urinary bladder and MI means mean intensity.

**Figure 1.** *Baerietta* sp. infecting some tree frogs at Obazuwa Wetland.

A, scolex; B, mature proglottid and C, late mature proglottid
Scale bar: A and B = 0.5 mm, C = 0.3 mm

**Figure 2.** Strigeoid metacercaria cyst (type 1) recovered from *A. dorsalis* at Obazuwa wetland. Scale bar: 0.3 mm.**Figure 3:** *Cosmocerca commutate* recovered from *A. dorsalis* and *L. spiritusnoctis* at Ikpako Riparian Habitat. A: anterior end, B: posterior end. Scale bar: A and B = 0.4 mm.

Discussion

One hundred and three specimens which comprise of 6 species of tree frogs were encountered at Obazuwa Wetlands while 66 specimens made up of 6 species were recorded at Ikpako Riparian Habitat. Although no numerical difference is revealed among the amphibian species, not more than four species were observed to cut across both habitats. This variation and the broad difference in the

number of specimens captured may be due to the pristine characteristics of the Obazuwa Wetlands forest vegetation as compared to the riparian habitat. Moreover while anthropogenic activities in the former habitat are under strict control (Olomukoro and Oviojie, 2015), the activities at the latter such as farming, laundry and collection of water for domestic use are not regulated. This finding is consistent with studies such as IUCN (2009) and Aisien *et al.* (2017b) which confirmed that habitat alteration and land use impact on amphibian diversity.

Most of the helminth parasites recorded in this study have been recorded in Nigeria or elsewhere. *Baerietta* sp. was the only cestode recovered in the study from the small intestine of *A. dorsalis*, *A. nigeriensis* and *A. paradorsalis*. Although some amphibian species have been reported to harbour this parasite such as *Bufo* (*Sclerophrys*) *regularis* (Aisien *et al* 2001), *S. regularis*, *S. maculatus* (*maculata*) and *Ptychadena mascareniensis* (Aisien *et al* 2003), *A. dorsalis* is the only tree frog from which it has been previously recovered (Imasuen *et al* 2012a). The genus *Baerietta* a synonym of *Cylindrotaenia* and *C. jaegskoeldi* has been reported to occur in only two tree frogs *A. dorsalis* (Aisien *et al* 2015) and *A. fulvovittatus* (Aisien *et al* 2017b), *Aubria subsigulata* and *Ptychadena longirostris* (Aisien *et al* 2009), *S. maculata* and *Phrynobatrachus latifrons* (Aisien *et al* 2011), *Pty. bibroni* (Aisien *et al* 2015), *Pty. bibroni* and *Pty. mascareniensis* (Aisien *et al* 2017c) are other records of *C. jaegskoeldi* occurrence in non- tree frogs in Nigeria. The occurrence of *Baerietta* sp. in the tree frogs *A. nigeriensis* and *L. spiritusnoctis* at Obazuwa Wetlands creates new hosts record for the parasite in Nigeria. Although the prevalence of this parasite is generally low, an appreciable prevalence of 16.7% was recorded for *A. nigeriensis*. The prevalence values recorded for *Baerietta* sp. in this study are consistent with reports from previous investigations. Imasuen *et al* (2012a), reported 7.4% for *A. dorsalis* at the Okomu National Park while Aisien *et al* (2003) recorded 14.3% for *P. mascareniensis*, a non tree frog from a savanna mosaic habitat, similar to the 7.7% and 16.7% recorded for *A. dorsalis* and *A. nigeriensis*, respectively, in this study. *Baerietta* sp. was not recorded at IKR habitat and this could have resulted from the unavailability of infected prey items of the frogs which serve as first intermediate hosts at this site. The absence of *Baerietta* sp. at the riparian habitat is an indication that the prevailing conditions of the external environment were not conducive enough for the parasite to thrive (Aisien *et al* 2015, 2017a). Several studies such as McKenzie (2007) have shown that parasite diversity also declines with habitat alteration.

The metacercarial cyst (Type I) recorded in the present study was first reported by Imasuen *et al* (2012a) and also reported by Edo-Taiwo *et al* (2014). Edo-Taiwo *et al* also reported another strigeoid metacercarial cyst (Type II) which differs from the Type I in its ovoid shape. The finding in this study buttresses the use of tree frogs as intermediate host to this particular strigeoid trematode irrespective of

whether the environment is altered or protected, rainforest or derived savannah biotope. The definitive hosts of strigeoid trematodes are aquatic reptiles, piscivorous birds and carnivorous mammals (Stunkard, 1973). Although the prevalence (7.7%) of metacercarial cyst (Type I) in the current study was low compared to 13.3% at Okomu National Park and 32% at Okomu Oil Plantation, the mean intensity of 90 parasite/infected host was much higher than the 3.3 parasite/infected host recorded at Okomu National Park and 25.1 parasite/infected host recorded at Okomu Oil Plantation (Edo-Taiwo *et al* 2014). When the infected number of species with the larval trematode at OBW and ONP is compared, the observable difference of 1 species of tree frogs, *A. dorsalis* at OBW as against 6 species recorded at ONP and 4 at OOP could have been due to the availability of water all year round at OBW which may have reduced the chances of these tree frogs feeding on the first intermediate host of this parasite which are usually snails. On the contrary, the lakes at ONP are seasonal thus making it possible for the frogs in this environment to have access to the infected snails. Moreover, Olomukoro and Oviojie (2015) reported low abundance of molluscs at OBW during their study period. The absence of strigeoid larval trematode at the IKR habitat could be attributed to the inability of the first intermediate host snail to thrive in that habitat due to the adverse effects of environmental alteration which affects the ability of parasites to complete their life cycles and maintain infection in their normal host (Pietroock and Marcogliese, 2003; Lafferty and Kuris, 2005). The finding buttresses the use of amphibians as transport host of this parasite to its definitive host where maturity is attained.

Amplificum africanum was not documented in the first report of helminth parasites of tree frogs in Nigeria (Imasuen *et al* 2012a), therefore its occurrence in *H. guttulatus* in a riparian habitat is an expansion of its host range in Nigeria. However, reports of its occurrence in several amphibian species such as *Sclerophrys maculata*, *Sclerophrys regularis*, *Dicroglossus* (*Hoplobatrachus*) *occipitalis*, *Pty. mascareniensis*, *Amietophrynus* (*Sclerophrys*) *cameroonensis* and *Amietophrynus* (*Sclerophrys*) *maculatus* (*maculata*) have been documented (Aisien *et al* 2001, 2003, 2009; 2017a; Amuzie *et al.*, 2016; Amuzie and Ekerette, 2017). The 100% prevalence recorded in this study might have been due to low sample size.

Members of the Cosmocercidae family recovered were *Aplectana macintoshii*, *Cosmocerca ornata*, *C. commutata* and unidentified species of *Aplectana*. These parasites are a wide range of nematodes known to infect amphibians of varied biogeographical radius (Aisien *et al* 2003, 2004, 2009; McAllister *et al* 2010; Krillova and Krillov, 2015; Goldberg *et al.*, 2016). In Nigeria, *A. macintoshii* was first reported from some non-tree frogs: *Amietophrynus* sp, *Ptychadena bibroni*, *Pty. pumilio* and *Pty. mascareniensis* (Aisien *et al* 2017b). It was first reported from a tree frog, *Afraxalus fulvovittatus*, by Aisien *et al.* (2017a). It's occurrence in *L. spiritusnoctis* in this

investigation does not only expand the host range but also confirm its occurrence in the country. Although *C. commutata* and *Aplectana* sp. have been previously reported in the country from two tree frogs *H. fusciventris* and *L. hyloides* (Aisien *et al* 2009), this study has expanded their host range by their occurrence in *A. dorsalis*.

Cosmocerca ornata has been reported from a wide range of amphibians in Nigeria (Aisien *et al* 2001, 2003, 2004, 2009, 2015, 2017a, b; Imasuen, 2012). However, its occurrence in tree frogs in Nigeria were first recorded by Imasuen *et al* (2012a) from *A. dorsalis*, *L. hyloides* and *L. spiritusnoctis*. Aisien *et al* (2015) also reported it from *Hyperolius concolor*. It is of interest to know that while *A. macintoshii* and *C. commutata* were recovered from IKR habitat, *Aplectana* sp. and *C. ornata* were recorded from OBW. The reason for this is not certain. Elsewhere, *C. commutata* was reported from a tree frog, *Hyla arborea* in Southwest Turkey with a low prevalence of 5.5% (Düsen and Öz, 2004) not so different from 2.4% and 3.3% recorded in this study for *A. dorsalis* and *L. spiritusnoctis*, respectively.

Camallanus sp. and *Physaloptera* sp. were found to parasitize hyperolid species: *Hyperolius concolor* Phase C and *Hyperolius concolor* Phase B and C, respectively. There is no known literature reporting the occurrence of these nematodes in Nigerian tree frogs. Therefore, *H. concolor* Phase C and *H. concolor* Phase B and C are new host record in Nigeria as far as tree frogs are concerned. *Camallanus dimitrovi* and *C. siluranae* have been reported to parasitize semi-aquatic and aquatic frogs, *Hoplobatrachus occipitalis* (Aisien *et al* 2003, 2004) and *Silurana tropicalis* (Imasuen and Aisien, 2015), respectively. It is an indication that these tree frogs such as *H. concolor* Phase B and *H. concolor* Phase C though arboreal, also forage on common or related invertebrate intermediate hosts as other frogs when they alight to feed. *Sclerophrys maculata* (Aisien *et al* 2011), *Pty. oxyrhynchus*, *Pty. bibroni* and *Pty. pumilio* and *H. occipitalis* (Aisien *et al* 2009; Aisien *et al* 2015), *Pty. oxyrhynchus* and *Pty. bibroni* are amphibians which have been reported to harbor both larval and adult forms of *Physaloptera* sp. In this study, the adult stage was recovered from *H. concolor* phase B, which is a new host record in Nigeria. The initial occurrence of its larval form in Nigeria gave the impression that the parasite was using the frogs to transport itself to the definitive host usually reptiles which feed on amphibians (Imasuen *et al*, 2012b; Edo-Taiwo *et al*, 2014; Goldberg *et al*, 2016). However, finding the adult form in amphibians has changed that view partially. Further studies are required to ascertain whether they are accidental hosts. The larvae of *Physaloptera* in cysts have been reported to occur in a number of tree frogs in Brazil: *Hyla boans*, *H. fasciata*, *H. granosa*, *H. leali*, *H. leucophyllata*, *H. marmorata* (Bursey *et al*, 2001) and *H. microcephala* (Goldberg *et al*, 2002). These authors have also recorded unencysted larvae in the hosts listed above which has raised a question whether the anurans in question represent paratenic host or the presence of the larvae is a

byproduct of diet (Goldberg *et al*, 2009).

An exceptional finding in the present study is the occurrence of *Foleyellides* sp. in 6 species of tree frogs (Table 2) compared to only 2 species recorded in the country previously, *H. fusc. burtoni* by Imasuen *et al*. (2012a) and *H. guttulatus* by Aisien *et al* (2017b). Likewise, the moderate to high prevalence (23.1%- 75.0%) of infection is worthy of note. Six hosts were found to be infected at OBW, while only 2 of the tree frogs were parasitized at IKR habitat. This could be attributed to the favourable breeding ground provided by the wetlands for *Anopheles* mosquitoes, the vectors of the filarid parasitic nematode. All the tree frogs infected in this study, represent new host records for *Foleyellides* sp. in Nigeria. A non-tree frog host previously reported to harbour the parasite was *Sclerophrys maculata* (Aisien *et al*, 2004, 2017a).

Although acanthocephalan larvae (*Centrorhynchus* cystacanth) have been recovered from a number of amphibians previously (Düsen and Öz, 2004; Smales, 2005; Bursey *et al*, 2001 Imasuen *et al*, 2012b), its record in *C. rufescens* in the present study provides a new host record for the parasite. *Acanthocephalus ranae* was reported to infect *Hyla arborea* in Turkey. Prevalence and mean intensity were both low, 5.5% and 1.5 parasites/infected host, respectively (Düsen and Öz, 2004), similar to the 7.7% prevalence and 1.0 parasite/infected host recorded in this study.

The present study has revealed that the tree frogs and the parasites infecting them at OBW and IKR habitat differed which would have been influenced by the habitat alteration particularly at Ikpako. The high prevalence of *Foleyellides* sp. among the tree frogs at the OBW resulting from the constant availability of water to its vector, *Anopheles* mosquito, is of conservation significance as wetlands are a refugia for wild life especially amphibians which possess a biphasic mode of life.

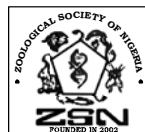
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