# Pattern of parasitic infections in anurans from a mangrove community of the Niger Delta, Nigeria

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## Abstract

Helminth parasites of anurans from Ijala Ikeren, a mangrove-contiguous community, in the Niger Delta of Nigeria were investigated. A total of 76 anurans belonging to 4 families, 5 genera and 9 species were examined and they include *Afrixalus dorsalis*, *A. fulvovittatus*, *Amietophrynus maculatus*, *Hyperolius concolor* phase B, *H. concolor*, phase C, *H. fusciventris burtoni*, *H. guttulatus*, *Hoplobatrachus occipitalis*, *Ptychadena bibroni* and *P. oxyrynchus*. A total of 13 helminth parasites were recovered, with an overall prevalence of 56.6%. Prevalence of cestode parasites was 9.21%, trematodes, 18.42% and nematodes, 28.96%. The cestodes recovered were *Baerietta jaegerskioeldi* and a larval proteocephalid; trematodes included *Haematoloechus exoterorchis*, *Mesocoelium cameroonensis* and *M. monodi*, while the nematodes included *Chabaudus leberrei*, *Cosmocerca ornata*, larval *Physaloptera*, *Rhabdias africanus*, *Rhabdias* sp., two larval ascaridoids (one encysted in the body cavity and the other in the mucosa of stomach) and an unidentified intestinal nematode. The mean intensity of infection was generally low except for *Mesocoelium* spp. (*M. cameroonensis* and *M. monodi*) where an over-dispersion was observed. The encysted ascaridoid larvae recovered from the stomach of *Ptychadena oxyrynchus* represents a new species and is an addition to the group of nematodes that use anurans as transport hosts. In conclusion, the mangrove environment at Ijala-Ikeren sustains a low diversity of amphibians which habour a low number of parasite species, whose low intensity of infection may be attributable to the parasite-hostile nature of the habitat.

**Keywords:** anurans, mangrove, helminth parasites, Niger Delta, Nigeria.

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## Introduction

The amphibian diversity in mangrove communities of Nigeria is poorly known and so also is the pattern of parasitic infections in anurans from these areas. Aisien *et al* (2001) reported on the endoparasites of amphibians from south-western Nigeria. In that paper, we reported on the parasites of anurans from three locations and we described Benin City as being in the rainforest while Warri and Sapele were assigned to the mangrove forest. Aisien *et al* (2009) observed that Warri and Sapele were more appropriately located in the fresh water swamps of the Niger Delta. Therefore, our publication (Aisien *et al*, 2001) actually described the parasites of amphibians from the rainforest and fresh water swamps. At present, there is no report on the composition of amphibians from the mangroves of Nigeria. This is so, because of the brackish nature of the environment and only very few amphibian species are known to be tolerant of such conditions. For example, *Fejervarya cancrivora* (the crab-eating frog), native to south-east Asia (Kuang-Yang, 2011) can tolerate brief excursions into sea water. Another important factor that may be excluding amphibians from the mangroves of the Niger Delta is the oil prospecting activities and the associated environmental pollution and degradation (Akani *et al*, 2004).

Amphibians are definitve hosts to helminth parasites including cestodes, monogeneans, digeneans, nematodes and acanthocephalans. In addition, they also serve as intermediate hosts (Sessions and Ruth, 1990; Bolt *et al*, 1993; Moravec and Kaiser, 1994; Torres and Puga, 1996; Krone and Streich, 2000; Thiemann and Wassersug, 2000; Imasuen *et al*, 2012a) and transport hosts (Moravec and Kaiser, 1994; Nickol, 1985; Eberhard and Brandt, 1995; Jackson and Tinsley, 1998; Moravec and Škoríková, 1998; Gonzalez and Hamann, 2007; Santos and Amato, 2010) in the trophical transmission of helminth infections to a number of vertebrate hosts. Imasuen *et al* (2012a) showed anurans as intermediate and paratenic hosts of helminth infections in the rainforest and derived savannah biotopes of Nigeria. It will therefore be of interest to know the role(s) they play in the life cycle of parasites in the mangrove community.

Whereas information abound on the helminth parasite fauna of amphibians from other bioclimatic zones of

Nigeria (Aisien *et al*, 2001, 2003, 2004, 2009; Imasuen and Aisien, 2012, 2015; Imasuen *et al*, 2012b), corresponding information on the amphibians of the mangrove biotope is lacking. There is a need to know which parasites are adapted to the anurans domicile in this brackish water environment, because, the ability of parasites to complete their life cycles and maintain infection in their normal hosts is dependent on the nature of the external environment to which their free-living stages are exposed (Pietrock and Marcogliese, 2003). In order to fill this information gap, we undertook a survey of the amphibians in a mangrove swamp (Falcorp Mangrove Park) and the mangrove contiguous community of Ijala Ikenren in Delta State of Nigeria. We also examined the parasitic infections of the amphibians collected from this location, which we report in this paper.

## Materials and methods

### *Study area*

Amphibians were collected from different locations in Ijala Ikenren community located at the upper reach of Warri River estuary of the western Niger Delta in the months of May and June, 2014. The community lies between Latitudes 05.5368833°N and 05.5590333°N; Longitudes 005.6926167°E and 005.7013500°E. The Ijala Ikenren wetland can be described as an intertidal creek mangrove swamp forest nourished by Ijala Creek which links to the Warri River. The mangrove fringes are made up of sand-dredged built-up areas that are being converted to residential areas.

Vegetation in the area is predominantly mangrove with the dominant type being the red mangrove, *Rhizophora* species with *R. racemosa* being the dominant species. There is also some presence of *R. harrisoni* and *R. mangle* which prefer drier habitats towards the built-up areas. The built-up area is composed of various forest trees with *Elaeis guineensis* (oil palm) as one of its major tree forms. The uncultivated lands are dominated by shrubs and grasses, including *Andropogon gyanus* (gamba grass), *Sida acuta* and herblike *Chromolena odorata*.

The topography of the area consists of a flat inundated land that easily drains into the creeklets that traverses the entire area. Rainfall pattern is characterized by a long rainy season from March/April through October. Mean annual rainfall averages around 4,000 mm, making it one of the wettest areas in Africa. The dry season months are January and February, during which there may be occasional rainfall. Relative humidity rarely dips below 60% and fluctuates between 90 and 100% for most of the year.

### *Sampling and identification of amphibians*

The amphibians were sampled at night using the Vocal Acoustic Encounter Survey (VAES) method. The locations where amphibian activities were recorded and catches made are listed in Table 1. The amphibians were transported to the laboratory and identified using the protocols of Schiøtz (1963, 1967, 1999) and Roedel (2000).

**Table 1.** Locations of amphibian activity and catches in Ijala Ikeren Community.

| Locality | Coordinates |
|---|---|
| Along the trunk road, opposite side of the refinery pipelines | (a) 05.55878′N; 05.70000′E<br>(b) 05.55742′N; 05.69713′E<br>(c) 05.55771′N; 05.69484′E |
| Earth road leading to Ikala Ikeren Community | 05.55479′N; 05.69856′E |
| Bush side and puddles along Ijala Ikeren Community road | 05.55586′N; 0569744′E |
| Sand dredging site partly overgrown with grasses | 05.55654′N; 05.69713′E |

### *Parasite collection, preservation and identification*

The amphibians were euthanized in a solution of Benzocaine and post-mortem examination carried out on them. The gastro-intestinal tract (oesophagus/ stomach, small intestine and large intestine/rectum) and other sites, including the lungs, urinary bladder, liver/ gall bladder and the body cavity were examined for parasites. The parasites were isolated using a stereo microscope and then viewed for identification with a Nikon Alpha-Phot-2 binocular microscope at x4 or x10 magnifications. The parasites were identified with the aid of appropriate keys (Yamaguti, 1971; Prudhoe and Bray, 1982; Baker, 1987; Khalil *et al*, 1994). The cestodes and trematodes recovered were flattened under cover slip pressure and fixed with 5% formol-saline. Nematodes were preserved in 70% ethanol. The cestodes and trematodes were washed free of preservative in several changes of tap water and then stained overnight in a dilute solution of acetocarmine. The stained specimens were dehydrated in alcohol series (50%, 70%, 90% and 100%), cleared with xylene and permanent mounts made in Canada balsam (Aisien *et al*, 2001, 2003). The nematodes were cleared with lactophenol before examination. Photo-micrographs of parasites were taken with the aid of a digital camera attached to the Nikon Alpha-Phot-2 microscope.

### *Prevalence and mean intensity of infection*

Prevalence of parasites was calculated as a percentage of the number of a particular host species infected with a specific helminth parasite divided by total number of hosts examined. The mean intensity of infection refers to the average number of parasites per host (calculated only for the infected hosts examined).

## Results

No amphibians were encountered in the mangrove

swamp, but in the bushes, water puddles and ponds in the built-up areas, a number of anurans were caught (Figures 1A-J). In total, 76 anurans belonging to 4 families, 5 genera and 9 species were examined and the number of specimens examined for each species is in parenthesis. The anurans include *A. dorsalis* (23), *A. fulvovittatus* (02), *A. maculatus* (03), *H. concolor* phase B (13), *H. concolor* phase C (13), *H. fusciventris burtoni* (02), *H. guttulatus* (01), *Hoplobatrachus occipitalis* (06), *Ptychadena bibroni* (04) and *P. oxyrynchus* (09).

The helminth parasites recovered from the infected hosts are as follows: Cestoda: *B. jaegerskioeldi* and cysts of a proteocephalid cestode; Trematoda: *H. exoterorchis*, *M. cameroonensis* and *M. monodi* while members of the Nematoda recovered included *C. leberrei*, *C. ornata*, larva of a *Physaloptera* sp., *R. africanus*, *Rhabdias* sp., larvae of two ascaridoid nematodes; one encysted in the body cavity and the other in stomach mucosa (Figure 2) and an unidentified intestinal nematode. No parasites were recovered from the following frogs: *A. fulvovitattus*, *H. concolor* (phase B), *H. fusc. burtoni* and *H. guttulatus*.

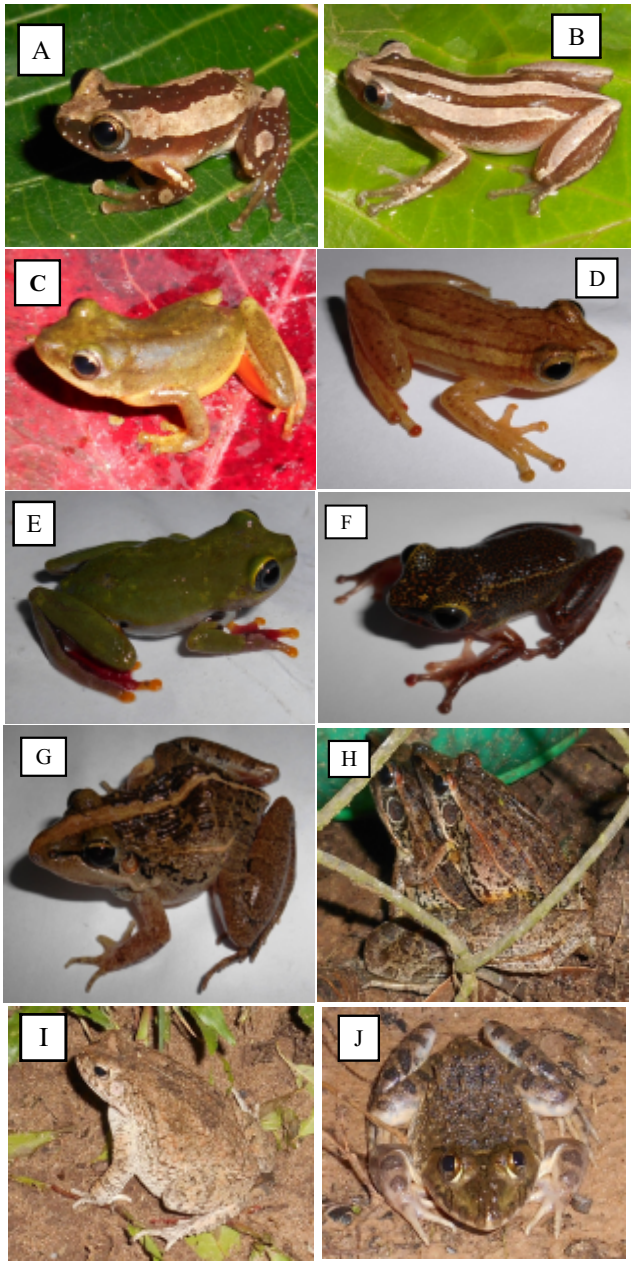


**Figure 1A-J.** Anurans species encountered at Ijala Ikeren community. **A.** *Afrixalus dorsalis*; **B.** *Afrixalus fulvovittatus*; **C.** *Hyperolius concolor* (phase C); **D.** *H. concolor* (phase B); **E.** *Hyperolius fusciventris burtoni*; **F.** *Hyperolius guttulatus*; **G.** *Ptychadena bibroni*; **H.** *Ptychadena oxyrynchus*; **I.** *Amietophrynus maculatus*; **J.** *Hoplobatrachus occipitalis*.

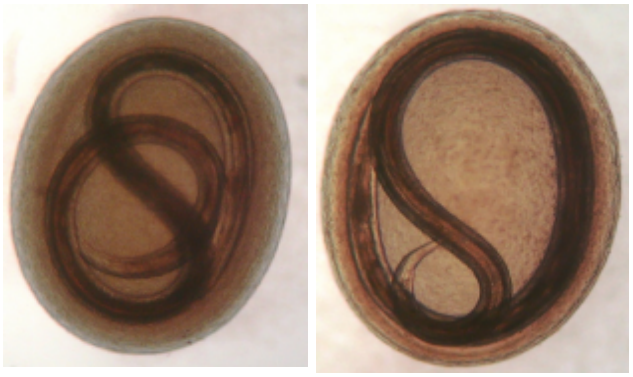

**Figure 2.** Encysted larvae of unidentified nematode infecting *Ptychadena oxyrynchus*. Scale bar = 0.3 mm. Bar length = 1.09 cm.

The sites within the hosts from which the parasites were recovered are presented in Table 2, while the prevalence and mean intensity of parasites in the infected anurans are summarized in Table 3. *Baerietta* was recorded in *A. dorsalis* and *P. bibroni*, both at low prevalence rates but with a higher intensity of infection in *A. dorsalis*. Cysts of the proteocephalid cestode were encountered in only one of the nine *P. oxyrynchus* examined. Although the prevalence values for trematode parasites were generally high in hosts in which they were recovered (33 to 100%), it was only for the *Mesocoelium* spp. (*M. monodi* and *M. cameroonensis*) that an over-dispersion was observed. For example, of the seven specimens of *P. oxyrynchus* infected with *M. cameroonensis*, three specimens harboured one, six and seven specimens, respectively; two frogs harboured 14 and 16 parasites, respectively; one specimen harboured 58 parasites while the last one harboured 179 parasites. In the three specimens of *A. maculatus* infected with *M. monodi*, one was infected with 4 parasites, the second with 11 parasites and the third with 42 parasites while three of the four *P. bibroni* infected with *M. monodi* harboured 1, 2 and 13 parasites, respectively. Prevalence of the nematodes parasites ranged from 7.7% to 75% but the mean intensity values for these parasites were generally low, with the highest value at 5.5 parasites per infected host (Table 3). Among these round worms, *Cosmocerca ornata*, larvae of *Physaloptera* sp. and the ascaridoid larva occurred as multi-host parasites. While *Rhabdias africanus* was recovered from *A. maculatus*,

an unidentified *Rhabdias* sp. was recovered from *P. bibroni*.

**Table 2.** Heminth parasites of amphibians from the mangrove of the Niger Delta.

| Parasite | Site |
|---|---|
| **Cestoda** | |
| *Baerietta jaegerskioeldi* | small intestine |
| Proteocephalid cesode (cyst) | body cavity |
| **Trematoda** | |
| *Haematoloechus exoterorchis* | lungs |
| *Mesocoelium camerunensis* | small intestine |
| *Mesocoelium monodi* | small intestine |
| **Nematoda** | |
| *Chabaudus leberrei* | small intestine |
| *Cosmocerca ornata* | large intestine, rectum |
| *Physaloptera* sp. larva | oesophagus, stomach |
| *Rhabdias africanus* | lungs |
| *Rhabdias* sp. | lungs |
| Ascaridoid larva I | body cavity |
| Encysted ascaridoid larva II | stomach mucosa |
| Unidentified nematode | intestine |

**Table 3.** Helminth parasites of anurans from Ijala Ikeren community.

| Parasite | Host | Prevalence (%) | Mean intensity |
|---|---|---|---|
| **Cestoda** | | | |
| *B. jaegerskioeldi* | *A. dorsalis* | 21.7 | 4.0 |
| | *P. bibroni* | 25.0 | 2.0 |
| Proteocephalid cestode (cyst) | *P. oxyrynchus* | 11.1 | 3.0 |
| **Trematoda** | | | |
| *H. exoterorchis* | *H. occipitalis* | 33.3 | 8.0 |
| *M. cameroonensis* | *P. oxyrynchus* | 66.6 | 48.3 |
| *M. monodi* | *A. maculatus* | 100.0 | 19.0 |
| | *P. bibroni* | 75.0 | 5.3 |
| **Nematoda** | | | |
| *C. leberrei* | *H. occipitalis* | 16.7 | 1.0 |
| C. *ornata* | *H. concolor* | 7.7 | 1.0 |
| | *H. occipitalis* | 19.7 | 2.0 |
| | *P. bibroni* | 75.0 | 4.0 |
| Unidentified | *Hyperolius* sp. 1 | 16.7 | 3.5 |
| intestinal nematode | *H. concolor* | 7.7 | 1.0 |
| *Physaloptera* sp. | *H. occipitalis* | 16.7 | 1.0 |
| (larva) | *P. oxyrynchus* | 44.4 | 4.5 |
| | *P. bibroni* | 25.0 | 2.0 |
| *Rhabdias africanus* | *A. maculatus* | 33.3 | 1.0 |
| *Rhabdias* sp. | *P. bibroni* | 50.0 | 3.0 |
| Ascaridoid larva I | *H. occipitalis* | | |
| | *P. oxyrynchus* | 16.7 | 2.0 |
| Encysted ascaridoid | *P. oxyrynchus* | 22.2 | 5.5 |
| larva II | | 11.1 | 4.0 |

## Discussion

In this study, nine amphibian species were recorded in the mangrove at Ijala Ikeren. This species number is low, especially when compared with number of species recovered from other humid forest biotopes investigated in southern Nigeria (Ogoannah, 2010; Imasuen, 2010). This low species diversity is however not peculiar to the mangrove in Nigeria. For example, in the Bhitarkanika mangroves in the east coast of India Jena *et al* (2013) recorded only 14 species. It thus seems that mangroves generally, because of their brackish nature are not a conducive environment for amphibians.

In all, 13 helminth parasite species, predominated by nematodes were recovered from the anurans collected from Ijala Ikeren. This number is rather low when compared to those of other humid habitats previously investigated in southern Nigeria (Aisien *et al*, 2001, 2009; Imasuen *et al*, 2012b). At the Okomu National Park, tree frogs alone were infected with 19 species of helminth parasites (Imasuen *et al*, 2012b). Even more significant is the fact that more parasites were recovered from anurans investigated in the savannah-mosaic, which receives less precipitation. For example, a total of 25 helminth parasites were recorded by Aisien *et al* (2003) from anurans investigated from this biotope. The explanation for this low parasite species number must lie in the nature of the environment in Ijala Ikeren.

Although the anurans investigated were not located directly in the mangrove swamp, the environment outside the swamp may not also be too tolerable for the parasites as shown by the acidic pH values (5.3 to 6.1) obtained for some of the ponds outside the swamp. According to Pietrock and Marcogliese (2003), the more the hydrogen ion concentration (pH) deviates from the species-specific optimum, the more it will affect the physiological properties of the free-living parasite stages, leading to impaired survival and/or reduced infectivity. This observation supports the recorded low mean intensity of infection obtained for almost all the parasites found except the two *Mesocoelium* spp. It may be that the hosts for the *Mesocoelium* spp. (*P. oxyrynchus*, *A. maculatus* and *P. bibroni*) live and breed in microhabitats that are favourable to these trematodes and permit such levels of infection intensity.

In contrast, *H. occipitalis* which we often encountered in some of the acidic ponds were infected with only five helminth parasites species (Table 3) all with low mean intensity of infection, which points to the fact these habitats were not conducive for the development of the parasites which usually infect this frog elsewhere. For example, in rain forest and freshwater swamps (Aisien *et al*, 2001) and in the derived savannah (Aisien *et al*, 2003) as many as 12 to 13 parasites were recorded in *H. occipitalis* examined from these locations. Although we recovered parasites from two of the tree frogs (*A. dorsalis* and *H. concolor* phase C), the majority of them (*A. fulvovittatus*, *Afrixalus* sp., *H. fusc. burtoni*, *H. guttulatus*, *Hyperolius concolor* phase B) did not habour any parasite. Even those that were infected had low intensity of infection. This may not be unconnected with their arboreal location, which to an extent shields them from parasitisation. Nevertheless, it is noteworthy that tree frogs investigated at the Okomu National Park (Imasuen *et al*, 2012b) haboured diverse parasitic infections (cestodes, monogeneans, digeneans and nematodes) albeit at low intensity. Therefore, the near absence of parasitic infections in the tree frogs of Ijala Ikeren may

not be unconnected with the prevailing unfavourable environmental conditions.

The prevalence of the helminth parasites encountered in the frogs of Ijala Ikeren is in agreement with the pattern observed elsewhere, where nematodes were observed to predominate among the parasites infecting amphibians (Aisien *et al*, 2001, 2003, 2004). While some of the parasites encountered have direct life cycles (*B. jaegerskioeldi*, *C. ornata*, *Rhabdias* spp.), which exposes them to the external environment during their free-living stages, others including the proteocephalid cestode, *Physaloptera* sp., *H. exoterorchis* and the larval ascaridoids have strategies which protect their vulnerable larval stages from direct contact with the environment. They either use insect vectors (as with *H. exoterorchis* and *Physaloptera* sp.) to transmit their larval stages to the amphibians or develop encysted larval forms within the amphibians hosts (proteocephalid cestode, the larval ascaridoids).

In this study, we observed that most of the parasites encountered have the infected amphibians as definitive hosts indicated by the presence of adult and egg-laying or ovoviviparous individuals. Larval parasite stages, including the encysted proteocephalid cestode larva, *Physaloptera* sp. larva and ascaridoid larva I and II use them as transport or paratenic hosts in their trophical transmission to their definitive hosts as previously observed by several investigators (Moravec and Kaiser, 1994; Nickol, 1985; Eberhard and Brandt, 1995; Jackson and Tinsley, 1998; Moravec and Škoríková, 1998; Gonzalez and Hamann, 2007; Santos and Amato, 2010; Imasuen *et al*, 2012a).

Except for the larval ascaridoid II recovered from *P. oxyrynchus*, all the other parasites encountered in this study have been reported in anurans previously investigated elsewhere in Nigeria (Aisien *et al*, 2001, 2003, 2004; Imasuen *et al*, 2012b). Ascaridoid larva II is different from other encysted nematode larvae reported by Imasuen *et al* (2012a). Unlike other encysted nematode larvae which are attached to the walls of organs of the viscera, this parasite occurred buried in the mucosa of the stomach wall of *P. oxyrynchus*. Furthermore, the texture of the cyst was also different from those we have encountered in previous studies. It was difficult to crush under the cover slip even when pressure was applied. It seems excystment of this nematode may only be achieved by specific enzyme action within the definitive host. This parasite may be common and also a multi-host parasite of anurans in the Niger Delta, as it has been recovered in another location in the region from *Hopolobatrachus occipitalis*, *Ptychadena bibroni*, *Ptychadena oxyrynchus* and *P. pumilio* (M.S.O. Aisien, personal communication).

In conclusion, this study has revealed the low amphibian diversity in the mangrove at Ijala Ikeren. It has also given an insight into the helminth parasites infecting anurans in this environment, characterized by low species number and intensity of infection. These results show that the mangrove environment is not conducive for many amphibian species and does not sustain the development of the free-living stages of the parasites that infect them. While some of the parasites found have amphibians as their definitive hosts, others such as the encysted proteocephalid cestode, the *Physaloptera* sp. larva and the two ascaridoid larvae use them as transport hosts to reach their definitive hosts.

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