The Bioscientist Vol. 4(1): 32-40, 2016 Available online at
http://www.bioscientistjournal.com

Full Length Research Paper

PARASITIC FAUNA OF AMPHIBIANS FROM RUMUJI-EMOHUA, RIVERS STATE, NIGERIA.

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ABSTRACT
A survey of the parasites of amphibians from Rumuji in Emohua Local Government Area of Rivers State, Nigeria, was conducted in the months of April and May, 2016. Anurans collected included *Amietophrynus maculatus, A. cameroonensis, Hoplobatrachus occipitalis, Ptychadena oxyrhynchus* and *Pty. pumilio*. Parasites recovered included a *Raillietiella* sp (Pentastomida): *Cylindrotaenia jaegerskioeldi* (Cestoda), *Mesocoelium monodi* and *Diplodiscus fischthalicus* (Digenea); *Rhabdias africanus, Rhabdias* sp., *Amplicaecum africanum, Oswaldocruzia hoepplii, Cosmocerca ornata*, and a larval ascaridoid (Nematoda). The co-occurrence of *Raillietiella* sp. *and R. africanus* in the lungs of *A. maculatus* is being reported for the first time in Nigeria. *Amietophrynus maculatus* is a new host record for *D. fischthalicus* in the country. The occurrence of *Oswaldocruzia hoepplli* in *A. cameroonensis* also increases the host range of the parasite in Nigerian anurans.
Key words: Amphibians, Parasites, Emohua, Rivers State, Nigeria.

INTRODUCTION
Amphibians are ectothermic, tetrapod vertebrates of the class Amphibia which exhibit a biphasic mode of life. They inhabit a wide variety of habitats, with most species living within terrestrial, fossorial, arboreal and freshwater aquatic ecosystems (Blackburn and Wake, 2011). They serve as paratenic or definitive hosts to a number of parasites (Imasuen *et al*., 2012), most of which are acquired from the consumption of intermediate hosts such as beetles and snails.

The Niger Delta is home to several amphibian species on which very limited studies have been undertaken (Akani *et al*., 2004; Ogoannah, 2010; Ogoannah *et al*., 2014). Urgent attention needs to be focused on this task in view of the numerous oil prospecting activities in this region and the attendant environmental pollution and degradation associated with them. These activities negatively impact on amphibian diversity and health (Akani *et al*., 2004). Unlike other bioclimatic zones of Nigeria where much information is available on the parasitic infections of amphibians hosts (Oladimeji *et al*., 1990; Aisien *et al*., 2001, 2003, 2004a, b, 2009; 2015a,b, Imasuen and Aisien, 2012; Imasuen *et al*., 2012; Nworah and Olorunfemi, 2013, Edo-Taiwo *et al*., 2014)

studies on this subject from the Niger Delta is still limited (Anyacho, 1997; Aisien *et al*., 2001; Oddo, 2008; Akani *et al*., 2011; Omereji, 2014; Sampson, 2014).

In view of the paucity of information on the amphibians of the Niger Delta and their endo-parasite fauna, there is a need to urgently fill this lacuna by undertaking relevant studies which will add to the pool of emerging information on this subject. The present study was therefore conducted to examine the amphibian diversity and parasitic infections of anurans from Rumuji in Emohua Local Government Area of Rivers State of Nigeria, results of which we present in this paper.

MATERIALS AND METHODS

The study area is located in Rumuji community (N4º56'32'', E6º 46'57'') in Emohua Local Government Area of Rivers State, Nigeria. It is located within the rainforest zone, with characteristic tropical climatic conditions. The vegetation in the community is composed of sparse secondary vegetation which included standing bushes and grass fields along with some nearby cassava farms. These were searched for the presence and collection of amphibians. Searches were also conducted along tracks on the road. The survey was carried out during the period spanning the last week of April, 2016 to the end of May, 2016. This was the early rainy season characterized by very few rainy days with much of sunshine and heat.

Adult frogs were hand captured, at night, between 7 pm and 11 pm, using the Visual Encounter and Acoustic Survey method (Crump and Scott, 1994). Individual frogs were transported live to the laboratory in moistened plastic bottles with perforated lids. Host specimens were identified (Roedel, 2000) and killed by exposure to chloroform vapour in an air-tight jar. The sexes of the anurans were determined by examining for the presence of vocal sacs, and examining the sex organs after they were dissected. The frogs were dissected and the oesophagus/stomach, small intestine, large intestine/rectum, lungs, urinary bladder were isolated and transferred to Petri dishes containing 0.72% NaCl solution and examined for parasites with a dissecting microscope. The body cavity was also examined for encysted parasites. Nematodes were fixed in hot 70% alcohol and preserved in fresh 70% alcohol. Cestodes and trematodes were flattened under cover slip pressure on a microscope slide and fixed in 5% formol-saline. The fixed specimens were preserved in the same fixative in labelled specimen bottles. Nematode parasites were cleared in lactophenol before examination. Cestodes and trematodes were washed free of fixative in three to four changes of tap water at 45mins intervals and then stained overnight in a dilute solution of acetocarmine. The specimens were dehydrated in alcohol series and permanent mounts made in Canada balsam. Parasites were identified with the protocols of Yamaguti (1971), Prudhoe and Bray (1982) and Khalil *et al*. (1994).

STATISTICAL ANALYSIS

The critical parasite quantification measures, namely prevalence and mean intensity, were computed according to Bush *et al*. (1997). The Data were log(x+1) transformed (Zar, 1984) and subjected to t-tests to determine significant differences in prevalence rates of the parasite species in infected hosts between locations. Standard deviation was computed for the mean intensity values. Statistical analyses were performed using Microsoft Excel. Kruskal-Wallis test was used to test for significant differences between parasite burdens in the predilection sites (Fowler and Cohen, 1994).

RESULTS

A total of sixty-one (61) anurans (15 females and 46 males) consisting predominantly of *Amietophrynus*

maculatus (38) and *Hoplobatrachus occipitalis* (15) were examined for parasites. Others included *A. cameroonensis* (1), *Ptychadena oxyrhynchus* (2) and *Ptychadena pumilio* (5). The host species abundance during the sampling period is shown in Table 1.

Table 1. Relative abundance of amphibian hosts in Rumuji, Rivers State

Host	Number examined	Abundance (%)	Males	Females
A. *cameroonensis*	1	1.64	0	01
A. maculatus	38	42.62	27	11
H. occipitalis	15	3.28	14	01
P. oxyrhynchus	2	1.64	02	00
P. pumilio	5	3.28	03	02
Total	**61**	**100**	**46**	**15**

The sites of infection in the anurans investigated are presented in Table 2; the small intestine was the most parasitized site. The parasites recovered from the anurans included a pentastomid (*Railletiella* sp.), one cestode (*Cylindrotaenia jaegerskioeldi*), two digenetic trematodes (*Mesocoelium monodi* and *Diplodiscus fischthalicus*). Nematodes were also recovered and they included *Rhabdias africanus*, an undetermined *Rhabdias* sp., *Amplicaecum africanum, Oswaldocruzia hoepplii, Cosmocerca ornata* and a larval ascaridoid encysted in the body cavity. A co-occurrence of the *Raillietiella* sp. and *R. africanus* was recorded in the lungs of two specimens of *A. maculatus* in a ratio of 2:9 in the first specimen and 26:2 in the other.

The prevalence of the parasites found ranged from 2.63% to 100.00%, while the mean intensity of infection ranged from 1.00 to 11.57 parasites/infected host (Table 3). The overall prevalence of infection in male and female amphibians is presented in Table 4. The overall prevalence of parasites in female frogs was higher (80%) than that of the males (57.8%) as shown in Tables 4, but the difference was statistically insignificant (t_{26} =0.256, p=0.800). *Amietophrynus cameroonensis* was represented by female hosts while *Pty. oxyrynchus* and *H. occipitalis* were represented by male hosts. In those anuran species represented by both sexes (*A, maculatus, P. pumilio*), parasite prevalence was higher in female hosts (91:81 for *A. maculatus*; 50:33.3 for *P. pumilio*). In *A. maculatus* which was the dominant species in the amphibian collection from Rumuji, *D. fischthalicus* and the larval ascaridoid infections were recorded only in male toads while the other parasites were encountered in both sexes (Table 5). While the prevalence values were higher in males for *R. africanus*, *A. africanum* and *C. ornata*, higher values were recorded for the *Raillietiella* sp., *M. monodi* and *O hoepplii* in female hosts. Of the 15 *H. occipitalis* examined, *C. ornata* occurred only on male frogs (Table 5). 99% confidence levels with a Kruskal-Wallis test showed a highly significant difference (K=71.32, p=0.1) between the predilection sites. The non-parametric Tukey-type multiple comparisons test revealed that significant differences existed between the helminth burden in the intestine and oesophagus/stomach, and between the large intestine/colon and the oesophagus stomach. However, significant differences did not exist between the other comparisons made.

Table 2. Endoparasites of anurans from Rumuji, Rivers State, Nigeria

Parasites	Hosts	Site of infection
Pentastomida		
Railietiella sp.	*A. cameroonensis*	Lungs
	A. maculatus	Lungs
Cestoda		
C. jaegerskioeldi	*P. pumilio*	Small intestine
Trematoda		
D. fischthalicus	*A. maculatus*	Rectum
M. monodi	*A. maculatus*	Small intestine
	P. oxyrynchus	Small intestine
Nematoda		
A. africanum	*A. maculatus*	Small intestine
	P. pumilio	Small intestine
R. africanus	*A. maculatus*	Lungs
Rhabdias sp	*P. pumilio*	Lungs
O. hoepplii.	*A. maculatus*	Small intestine
C. ornata	*A. maculatus*	Rectum
	H. occipitalis	Rectum
Larval ascaridoid	*A. cameroonensis*	Body cavity
	A. maculatus	Body cavity

Table 3: Prevalence of parasitic infections in the anuran host species from Rumuji, Rivers State

Parasite	Host	Prevalence (%)	*Mean intensity
Pentastomida			
Railietiella sp.	*A. cameroonensis*	100.0	15.0
	A. maculatus	18.42	11.6±4.43
Cestoda			
C. jaegerskioeldi	*P. pumilio*	20.0	1.0±0.00
Trematoda			
D. fischthalicus	*A. maculatus*	2.6	11.0±0.00
M. monodi	*P. oxyrhynchus*	50.0	9.0±3.25
	A. maculatus	18.4	6.6±5.41
Nematoda			
A. africanum	*A. maculatus*	29.0	1.8±1.61
	P. pumilio	20.0	2.0±0.00
R. africanus	*A. maculatus*	15.8	3.2±3.25
Rhabdias sp.	*P. pumilio*	20.0	1.0±0.00
O. hoepplii	*A. maculatus*	42.1	4.6±4.55
Larval ascaridoid	*A. cameroonensis*	100	2.0±0.00
	A. maculatus	5.3	1.5±1.41
C. ornata	*A. maculatus*	34.2	6.1±5.45
	H. occipitalis	13.3	2.0±0.00

Table 4: Overall prevalence of helminth parasites according to sex in the amphibians examined

Hosts	Male amphibians		Female amphibians	
	No. examined	**No. infected**	**No. examined**	**No. Infected**
A. maculatus	27	22	11	10
A. cameroonensis	0	0	1	1
H. occipitalis	14	2	0	0
P. oxyrhynchus	2	1	0	0
P. pumilio	3	1	2	1
Total	45	26	15	12

Table 5. Parasite prevalence and mean intensity in the amphibian hosts from Rumuji, Rivers State according to sex

		Males		Females	
Host	**Parasite**	**P**	**MI**	**P**	**MI**
A. cameroonensis	*Railietiella* sp.	-	-	100	15.0
A. maculatus	*Railietiella* sp.	14.8	1.8	27.3	24.7
	D. fischthalicus	3.7	11.0	-	-
	M. monodi	11.1	5.0	36.4	7.8
	R. africanus	22.2	3.0	9.1	2.0
	A. africanum	37.0	2.6	9.1	1.0
	O. hoepplii	37.0	3.3	54.6	6.8
	C. ornata	40.7	6.1	18.2	9.5
	Larval ascaridoid	7.4	1.5	-	-
P. oxyrhynchus	*M. monodi*	50.0	9.0	-	-
P. pumilio	C. jaegerskioeldi	33.3	1.0	-	-
	A. africanum	-	-	50.0	2.0
	Rhabdias sp.	-	-	50.0	1.0
H. occipitalis	*C. ornata*	14.3	2.0	-	-

DISCUSSION

The amphibian species encountered at Rumuji were few when compared to the 11 recorded at Egbeda (Emuohua) by Omereji (2014). Whereas Omereji (2014) recorded five tree frog species at Egbeda, these frogs were not encountered during the sampling period at Rumuji. This may be attributable to the short duration of the study, the nature of the sampling sites which consisted mainly of secondary forest and the season of collection which was the early part of the rainy season. Nevertheless, the number of parasite species (10) recovered from the anurans from Rumuji is quite comparable to the 12 reported from the Egbeda anurans.

Pentastomids (*Raillietiella* spp.) are known lung parasites of *Amietophrynus regularis* (Aisien *et al*., 2001; Ozemoka, 2012; Sampson, 2014) and a *Hyperolius* sp. (Ovwah, 2016). In this study, these parasites were recovered from *A. cameroonensis* and *A. maculatus*, which are new host records for these parasites. In contrast to the observation from previous studies, where these parasites were reported to occur separately in the lungs of infected hosts, we observed their co-occurrence with *R. africanus* (nematode) in the lungs of *A. maculatus*. This is the first report of such an occurrence in anurans of Nigeria.

The only cestode recovered in this study was *Cylindrotaenia jaegerskioeldi* from the small intestine of *Ptychadena pumilio*. This parasite is a multi-host parasite having been reported in *A.*

regularis and *A. maculatus* (Aisien *et al*., 2001, 2003, 2004), *Ptychadena* spp. (Aisien *et al*., 2003, 2009), *Aubria subsigilata* (Aisien *et al*., 2009) and tree frogs (Imasuen *et al*., 2012; Sampson, 2014). Sampson (2014) reported 52.2% prevalence of this parasite in the tree frog, *Afrixalus fulvovittatus*, which was attributed to the altered nature of the study environment in urban Port Harcourt. *Cephalochlamys compactus*, another cestode infecting *H. occipitalis* in locations in the Niger Delta (Aisien *et al*., 2001; Omereji, 2014) was not recorded in the *H. occipitalis* investigated at Rumuji.

It is surprising that only two trematodes (*D. fischthalicus* and *M. monodi*) were recorded in the anurans from Rumuji. In an earlier study by Omereji (2014) in two locations in Rivers State (Egbeda in Emohua and Odioku in Ahoada), five trematodes (*Mesocoelium monodi*, *Haematoloechus exoterorchis*, *Ganeo africana*, *Prosotochus exovitellosus* and *Progonimodiscus doyeri*) were recorded. Three of these parasites (*Haematoloechus exoterorchis*, *Ganeo africana*, *Prosotochus exovitellosus*) occurred in *H. occipitalis*, one (*P. doyeri*) in *Hymenochirus* sp. while *M. monodi* infected *Ptychadena* spp. (*P. bibroni* and *P. oxyrhynchus*). *Mesocoelium monodi* and *Diplodiscus fischthalicus* also infect anuran hosts elsewhere in Nigeria. *Mesocoelium monodi* has been recorded in several amphibian hosts including *Amietophrynus regularis, A. maculatus, Chiromantis rufescens, Hoplobatrachus occipitalis, Phrynobatrachus* sp., and in *Ptychadena* spp. (Aisien *et al*., 2001, 2003, 2004, 2009; Imasuen and Aisien, 2012; Imasuen *et al*., 2012). Whereas *D. fischthalicus* is already known to infect *H. occipitalis, R. galamensis, A. subsigillata* and *P. pumilio* (Aisien *et al*., 2001, 2003, 2004, 2009; Imasuen and Aisien, 2012; Imasuen *et al*., 2012), in *Phrynobatrachus francisci* (Omereji, 2014), this is the first record of *D. fischthalicus* in *A. maculatus*, thus extending the host range of the parasite.

All the nematodes recorded in this study have previous records in anurans investigated elsewhere in Nigeria (Aisien *et al*., 2001, 2003, 2004; Imasuen *et al*., 2012). *Rhabdias africanus* infects *Amietophrynus* spp. (Kuzmin, 2001) but there are reported occurrences of this lung worm in other anuran hosts that needs to be confirmed. This has become imperative with the recovery of *Rhabdias* spp. with features different from those of *R. africanus*. The *Rhabdias* sp. recovered from the lungs of a *Pty. pumilio* in this study and those from other anurans belong to this group. *Amplicaecum africanum* has been recovered from *A. regularis*, *A. maculatus*, and *H. occipitalis* (Aisien *et al*., 2001, 2003) while *O. hoepplli* commonly infects *Amietophrynus* and *Ptychadena* spp. *Amietophrynus cameroonensis* thus becomes a new host record for this nematode. *Cosmocerca ornata* has been reported from several hosts including *Amietophrynus* sp., *H. occipitalis, Ptychadena* spp., *Xenopus muelleri, Afrixalus dorsalis, Hyperolius fusciventris burtoni* and *H. hyloides* (Aisien *et al*., 2001; Imasuen *et al*., 2012). Encysted larval ascaridoids found in *Amietophrynus* spp. in this study have previously been reported in the body cavity of several anuran species (Aisien *et al*., 2001, 2003, 2004, 2009, 2015) and according to Imasuen *et al*. (2012) these nematodes use the amphibians as paratenic hosts to reach their definitive hosts.

It is not possible to comment on the parasite prevalence in those anuran hosts represented by only one of the sexes. However, in those species represented by both sexes (*A. maculatus*, *P. pumilio* and *H. occipitalis*), infection prevalence was consistently higher in the female hosts. Although this observation was not statistically significant, it may be related to their feeding habits. Gravid female frogs tend to forage more for food in order to satisfy their nutritional and those of the

developing egg mass. In this process, they consume more infected invertebrate intermediate hosts than female frogs.

CONCLUSION

In conclusion, this study has examined the parasitic infections of anurans from Rumuji-Emohua in Rivers State of Nigeria. Although all the parasites recorded in this work have been reported in anurans elsewhere in Nigeria, there are certain peculiarities that are noteworthy in this location. We reported for the first time in Nigerian anurans, the co-occurrence of pentastomids and *R. africanus* in the lungs of *A. maculatus*. This toad is also reported here as a new host record for *D. fischthalicus*. Pentastomids and *O. hoepplii* are also being reported new parasite records in *A. cameroonensis*. This study has added new information to the existing database on the amphibians of Rivers State and their parasitic infections.

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