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Parasitic infections of anurans from a freshwater creek community in Delta State, Niger Delta of Nigeria

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Summary

The parasitic infections of 125 anurans belonging to 13 taxa from Ase community in Delta State in the Niger Delta of Nigeria was investigated. An overall prevalence of 77.6% was recorded and the 32 parasites recovered included one pentastomid, four cestode, four monogenean, ten digenean and thirteen nematode taxa. Among the anurans examined, *Ptychadena bibroni* harboured the highest number of parasites. Most of the parasites encountered were adults but some occurred as larvae, which either used the amphibians as intermediate or transport hosts. *Hylarana galamensis* is considered an accidental host for the larval brachylaimid trematode and, a new host record for the larval strigeiod while *Polystoma galamensis* infection of this frog is made for the first time outside the savannah biotope. *Hymenochirus* sp. is a new host record for *Progonimodiscus colubrifer*. Two *Foleyellides* spp., one occurring in *Hyperolius concolor*, *Afrixalus fulvovitattus* and *Sclerophys regularis* and the other in *H. galamensis* occur in the study area.
Keywords: Parasitic infections; anurans; Ase creek; Niger Delta; Nigeria

Introduction

The vegetation zones of Nigeria include the mangrove and fresh water swamps of the Niger Delta, followed by the rainforest, derived savannah, guinea savannah, montane forest and the sahel savannah of the extreme north. Investigations of the parasites of amphibians in Nigeria have mostly been undertaken in the forest and savannah biotopes of the country (Thurston, 1967, 1970; Avery, 1971; Aisien and Du Preez, 2009; Aisien *et al*., 2001, 2003, 2004a, 2009, 2011, 2015; Imasuen and Aisien, 2012, 2015; Imasuen *et al*., 2012a). Investigations have also been carried out on pipid anurans (*Hymenochirus curtipes* and *Xenopus tropicalis*) obtained from the Lagos (coastal) area of the country (Jackson and Tinsley, 1995, 1998a, b).

Some information is available on the parasites of anurans from the freshwater swamp of the Niger Delta, Nigeria (Oddo, 2008) but none from the freshwater creeks of this region. Recent visits to Ase, a community located on the shores of Ase Creek in Delta State of Nigeria, afforded us the opportunity to collect and examine anurans from the locality for their parasitic infections. In this paper we report on the diverse parasitic infections of these frogs, some of which are being reported for the first time in Nigeria while others represent new geographical records for the country.

Materials and Methods

Study area

The amphibians investigated were collected from Ase town (05.17°N; 06.18°E), Delta State in the Niger Delta of Nigeria between July and September, 2014 and then in the months of June and August in 2015. The town lies on the shores of the Ase Creek on the course of Ase River, which is a tributary of the Forcados

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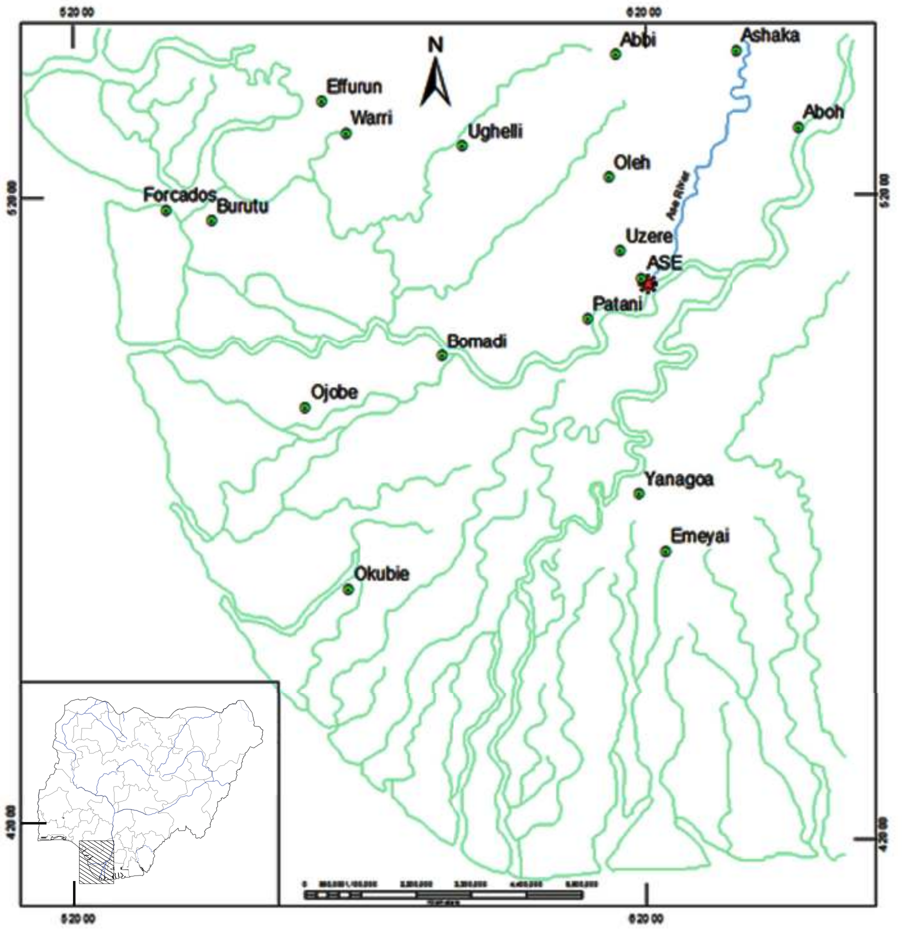

Fig. 1. Map of the Niger Delta, Nigeria showing the location of Ase

River, the western branch of River Niger in the Delta of southern Nigeria (Fig 1). The climate of the area is tropical and it is divided into the rainy (February to October) and dry (November to January) seasons, with intermittent rains in the month of December. During the later part of the rainy season (September and October), the area gets inundated and aquatic species of both plants and animals are carried ashore with some getting stranded when the water recedes.

Amphibians

All 13 amphibian species (except *Hymenochirus* sp.) were collected by hand at night using the Visual Acoustic Encounter Survey (VAES) method (Crump and Scott, 1994). Specimens of *Hymenochirus* sp. were caught after the pond harbouring them was drained. The anurans were transported to the laboratory and specimens collected were identified using the protocols of Schiøtz (1963, 1967, 1999) and Roedel (2000). The anurans were euthanized by immersion in Benzocaine solution followed by post-mortem examination. The gastro-intestinal tract (oesophagus/stomach, small intestine, large intestine/rectum), lungs, urinary bladder, liver/gall bladder and the body cavity were examined for parasites. Flatworms (cestodes, monogeneans and digeneans) were flattened under cover slip pressure on microscope slides and fixed with 5 % formol-saline. Prior to staining, the specimens were washed free of the fixative and then stained overnight in a dilute solution of acetocarmine. The parasites were dehydrated in alcohol series, cleared in xylene and permanent mounts made in Canada balsam. Nematodes were fixed and preserved in 70 % alcohol and were cleared for examination in lactophenol. Cysts of helminth parasites were isolated, crushed under cover slip and the enclosed parasitic group identified. Parasites were identified using appropriate keys (Yamaguti, 1971; Prudhoe and Bray, 1982; Khalil *et al.*, 1994) and either drawn using a drawing tube attached to a Nikon Alpa-Phot-2-microscope or photomicrographs taken with a Coolpix Digital Camera (3.34 Megapixels) attached to the same microscope.

Frog specimens investigated in the study have been deposited in the Amphibian Collection of Prof. A.B.M. Egborge Museum, University of Benin, Benin City, Nigeria. Parasite specimens deposited at the Natural History Museum, London and their NHMUK

Table 1. Helminth parasites of amphibians from Ase creek, Delta State of Nigeria.

Parasite	Habitat
Pentastomida	
Raillietiella sp.	Lungs
Cestoda	
Cylindrotaenia jaegerskioeldi	Small intestine
Cephalochlamys compactus	Small intestine
Larval Proteocephalid	Body cavity
Proteocephalus sp.	Small intestine
Monogenea	
Polystoma aeschlimanni	Urinary bladder
Polystoma baeri	Urinary bladder
Polystoma galamensis	Urinary bladder
Polystoma pricei	Urinary bladder
Digenea	
Diplodiscus fischthalicus	Large intestine/rectum
Ganeo africana	Small intestine
Metahaematoloechus exoterorchis	Lungs
Metahaematoloechus micrurus	Lungs
Mesocoelium monodi	Small intestine
Mesocoelium sp.	Small intestine
Ophidiscus sp.	Large intestine/rectum
Brachylaimid (metacercariae)	Large intestine/rectum
Progonimodiscus colubrifer	Large intestine/rectum
Strigeoid larva	Small intestine
Nematoda	
Amplicaecum sp.	Small intestine
Ascaridoid larva I	Body cavity
Ascaridoid larva II	Stomach mucosa
Batrachocamallanus siluranae	Oesophagus/stomach
Capillaria sp.	Small intestine
Cosmocerca ornata	Large intestine/rectum
Folleyelides sp. I	Body cavity
Folleyelides sp. II	Body cavity
Rhabdias africanus	Lungs
Rhabdias sp. I	Lungs
Rhabdias sp. II	Lungs
Rhabdias sp. III	Lungs
Oswaldocruzia hoepplii	Small intestine

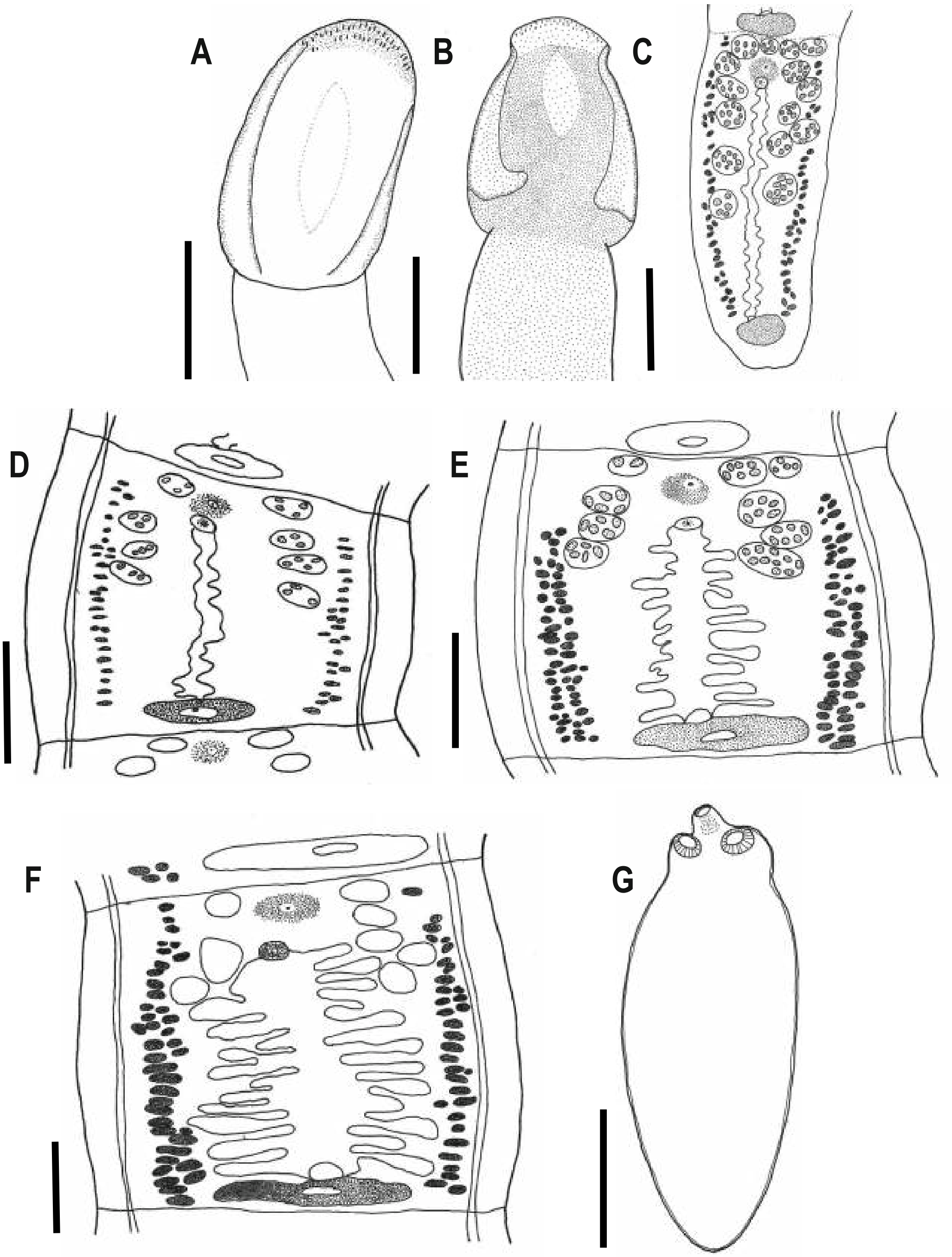

Fig. 2. Some cestodes infecting anurans at Ase creek. Figs. A-F – *Cephalochlamys compactus* infecting *H. occipitalis*. A,B, scoleces (different specimens); C, D – mature proglottid (different specimens); E,F – gravid proglottids; G – Larva of *Proteocephalus* sp. infecting *H. galamensis*. Scale bar: A,C-F = 0.30 mm; B,G = 0.2 mm.

accession numbers are as follows: *Cephalochlamys compactus* (2016.12.8.1-3); *Proteocephalus* sp. larva (2016.12.8.4); Proteocephalid larvae (2016.12.8.5); *Polystoma aeschlimanni* (2016.12.8.8-9); *Polystoma baeri* (2016.12.8.10-11); *Polystoma pricei* (2016.12.8.12-13); *Diplodiscus fischthalicus* (2016.12.8.14-15); *Ganeo africana* (2016.12.8.16-17); *Metahaematoloechus exoterorchis* (2016.12.8.18); *Metahaematoloechus micrurus* (2016.12.8.19); *Mesocoelium monodi* (2016.12.8.20-21); strigeoid trematode larva (2016.12.8.22-23); *Progonimodiscus colubrifer* (2016.12.8.24-25); *Batrachocamalanus siluranae* (2016.12.8.28-35); *Oswaldocruzia hoepplii* (2016.12.8.36-50) brachylaimid (*Postharmostomum*) metacercariae (2016.12.8.51-60); *Amplicaecum* sp. (2016.12.8.61-66; *Foleyellides* sp. I (2016.12.8.67-77 and *Foleyellides* sp. II (2016.12.8.78-97).

Results

In this study, 125 anurans belonging to 13 taxa were examined: *Afrixalus fulvovittatus* (01), *Sclerophys maculata* (02), *S. regularis* (01), *Hoplobatrachus occipitalis* (05), *Hylarana galamensis* (44), *Hymenochirus* sp. (06), *Hyperolius fusciventris* (01), *Hyperolius fusciventris burtoni* (07), *Hyperolius concolor*, phase B (16), *Ptychadena bibroni* (23), *P. mascareniensis* (11), *P. oxyrynchus* (10) and *P. pumilio* (05), with an overall parasite prevalence of 77.6 %.

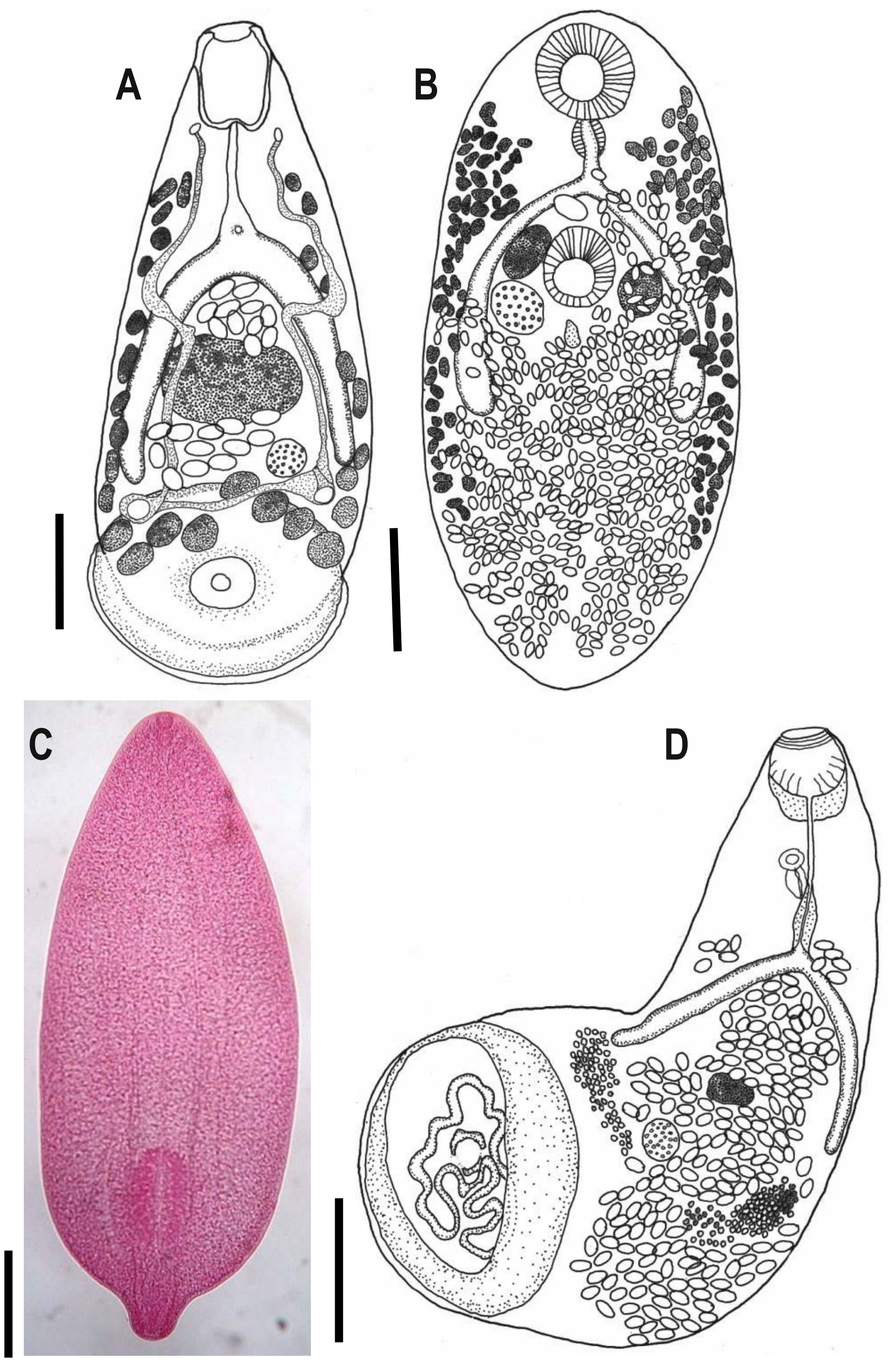

Fig. 3. Some digenetic trematodes infecting anurans from Ase creek.
A – *D. fischthalicus*; B – *Mesocoelium* sp.; C – Larval strigeoid; D – *P. colubrifer*. Scale bar: A, D = 0.5 mm; B,C =0.25 mm.

Table 2. Prevalence of parasites in the amphibians from Ase, Delta State, Nigeria

Parasites	Amphibian species								
	S. maculata n= 02	*S. regularis* n= 01	*Hymenochirus sp.* n= 06	*H. galamensis* n= 44	*H. occipitalis* n= 05	*P. bibroni* n= 23	*P. mascareniensis* n= 11	*P. oxyrynchus* n= 10	*P. pumilio* n= 05
Pentastomida									
Raillietiella sp.	100 (3.0)	--	--	--	--	--	--	--	--
Cestoda									
C. jaegerskioeldi	--	--	--	--	--	8.7 (1.0)	9.1 (1.0)	--	--
C. compactus	--	--	--	--	40 (1.5)	--	--	--	--
Proteocephalid cestode (larva)	--	--	16.7 (1.0)	--	20 (9.0)	18.8 (3.3)	18.2 (4.0)	--	--
Proteocephalus sp. (larva)	--	--	--	4.6 (2.0)	--	--	--	--	--
Monogenea									
P. aeschlimanni	--	--	--	--	--	--	--	--	40 (2.0)
P. baeri	--	--	--	--	--	12.5 (1.0)	--	--	--
P. galamensis	--	--	--	2.3 (1.0)	--	--	--	--	--
P. pricei	--	--	--	--	--	--	9.1 (1.0)	--	--
Digenea									
D. fischthalicus	--	--	--	--	40 (2.5)	4.3 (2.0)	--	10 (2.0)	40 (2.5)
G. africana	--	--	--	--	20 (3.0)	--	--	--	--
M. exoterorchis	--	--	--	--	20 (5.0)	--	--	--	--
M. micrurus	--	--	--	--	20 (1.0)	--	--	--	--

M. monodi	--	100 (1.0)	--	--	--	65.2 (15.6)	81.8 (10.1)	50 (26.0)	100 (18.8)
Mesocoelium sp.	--	--	--	29.5 (6.2)	--	--	--	--	--
Ophidiscus sp.	--	--	--	--	--	4.4 (1.0)	--	--	--
Brachylaimid (metacercariae)	--	--	--	2.3 (3.0)	--	--	--	--	--
P. colubrifer	--	--	100 (2.8)	--	--	--	--	--	--
Strigeoid larva	--	--	--	11.4 (4.6)	--	--		--	--
Nematoda							--		
Amplicaecum sp.	--	--	--	2.9 (6.0)	--	8.7 (4.0)	--	--	--
Ascaridida larva I	--	--	1.7 (1.0)	--	--	--	9.1 (1.0)	--	--
Ascaridida larva II	--	--	--	--	20 (1.0)	12.5 (2.5)	--	10 (12.0)	40 (5.0)
B. siluranae	--	--	18.2 (1.0)	--	--	--	--	--	--
Capillaria sp.	--	--	66.7 (2.5)	--	--	--	72.7 (7.9)	--	40 (2.0)
C. ornata	100 (1.5)	--	--	63.6 (13.5)	--	65.2 (4.6)	--	--	20 (1.0)
Foleyellides sp I.	--	100 (2.0)	--	--	--	--	--	--	--
Folleyelides sp. II	--	--	--	47.7 (10.2)	--	--	--	--	--
Rhabdias africanus	--	--	--	18.8 (15.9)	--	--	--	--	--
Rhabdias sp. I	--	--	--	--	--	--	18.2 (3.0)	--	--
Rhabdias sp II	--	--	--	--	--	17.4 (2.8)	--	--	--
Rhabdias sp III	--	--	--	--	--	--	--	20 (2.0)	--
O. hoepplii	50 (1.0)	--	--	--	--	13.0 (2.0)		--	--

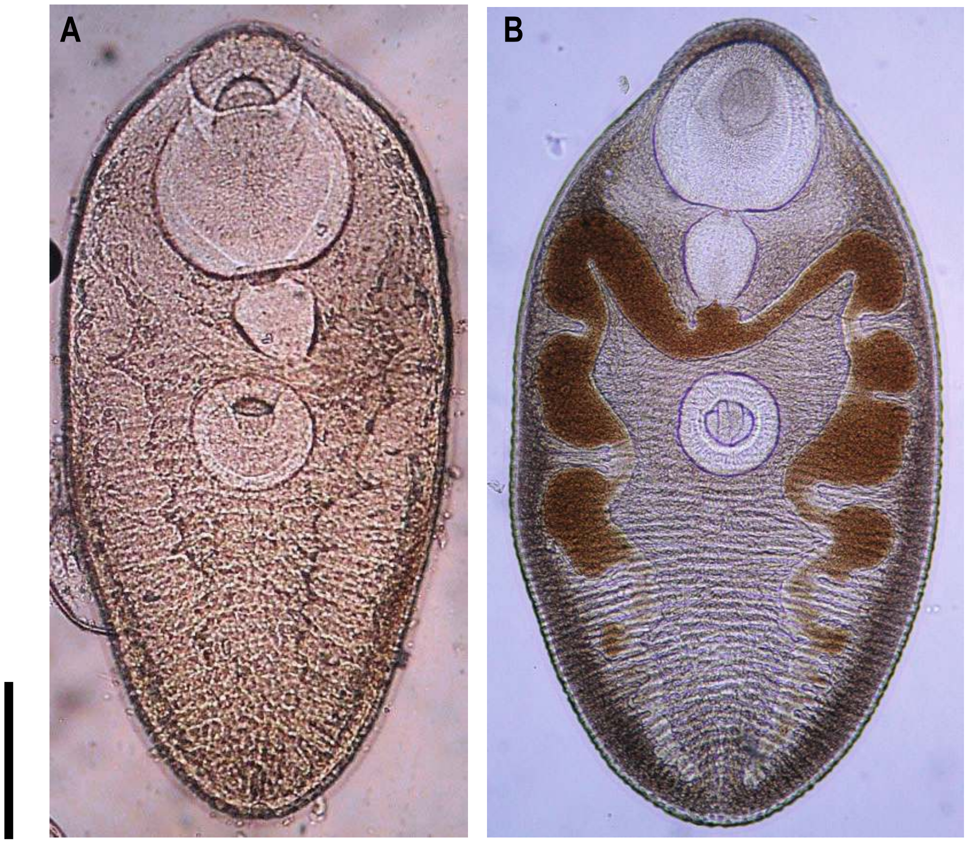

Fig. 4. Metacercariae of the brachylaimid trematode recovered from A – *H. galamensi*; B – *L. aurora*. Scale bar: 0.2mm

A total of 32 parasites were recovered and they consisted of one pentastomid; four cestode, four monogenean, 10 digenean and 13 nematode species. The sites of infection in the hosts are presented in Table 1. The prevalence and mean intensities (in parentheses) of the parasites recovered and the hosts from which they were recovered are presented in Table 2. No parasites were recorded in *H. fusciventris burtoni*; *P. bibroni* harboured 11 parasite species, followed by *H. galamensis* (9) and *P. mascareniensis* (8). The remaining frogs harboured between two and six parasite species (Table 2). A majority of the parasites parasitized only a single host, but some others including *C. jaegerskioeldi*, the proteocephalid cestode larva, *D. fischthalicus*, *M. monodi*, *Amplicaecum* sp., ascaridida larvae I and II, *Capillaria* sp., *C. ornata*, and *O. hoeppli* were generalist parasites, infecting between two and five different amphibian hosts (Table 2). Most of the parasites (26) were represented by adult stages while only six occurred as larvae and these included cestoda: (larval proteocephalid and *Proteocephalus* sp.); digenea (Brachylaimid metacercariae and the strigeoid larva); nematoda (ascaridida larvae I and II). The most parasitized habitat was the small intestine, followed by the lungs and the large intestine/rectum.

The pentastomid recovered was a *Raillietiella* sp., while the cestodes included *C. compactus* (Fig. 2A – F) *Proteocephalus* sp. (Fig. 2G), *C. jaegerskioeldi* and cyst of a proteocephalid, which was a generalist parasite. Four *Polystoma* spp. infecting four different anuran hosts, all with low prevalence and intensity were recorded (Table 2). Ten digenetic trematodes, some of which include *D. fischthalicus* (Fig. 3A), an undetermined *Mesocoelium* sp. (Fig. 3B), a larval strigeoid (Fig. 3C), *P. colubrifer* (Fig. 3D) and a brachylaimid trematode metacercaria (Figs. 4A – B) were recovered. Nematode parasites formed the largest group encountered, notably two larval ascaridida, one of which was encysted in the body cavity of *Hymenochirus* sp. and the second (Fig. 5A – D) embedded in the stomach mucosa of three anuran hosts (Table 2). Two species of *Foleyellides* were also observed to infect anurans in the study area; one occurring in the body cavity of *H. concolor* (prevalence 25 %; mean intensity, 5.3), in the single specimen of *A. fulvovittatus* examined and *S. regularis* (Fig. 6A – B) and the other in *H. galamensis* (Fig. 6C – D).

Discussion

The anuran species encountered during this study were rather few in view of the fact that Ase community is a humid freshwater envi-

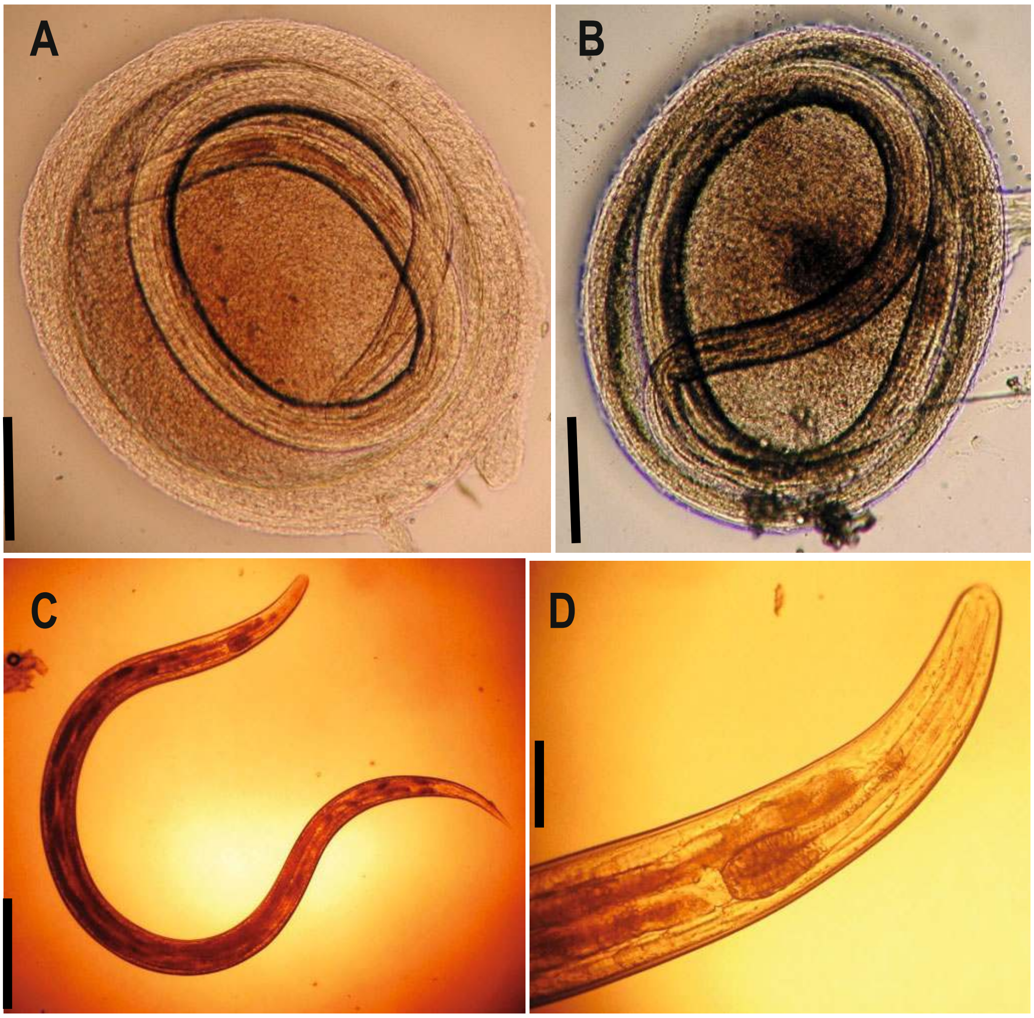

Fig. 5. Encysted larval ascaridida infecting anurans from the Ase creek. A,B – encysted larval (whole worm); C – excysted larva; D – anterior end of excysted larva. Scale bar: A,B = 0.3mm; C = 0.5 mm; D = 1 mm

ronment, which should support a rich amphibian species diversity. The dominant species in this location was *H. galamensis* which was frequently encountered especially close to the water front. *Ptychadena* spp. were encountered inland near water puddles while the few *H. occipitalis* were caught near dug out fish ponds not far from human dwellings. This frog was hard to find because it is hunted and consumed by the locals. Among the tree frogs, *Hyperolius concolor* (phase B) was the most common, occurring among shrubs and fence hedges in the community.

Although as many as 32 parasites species were recorded in the anurans investigated, the following have previous records in Nigerian amphibians; pentastomida: *Raillietiella* sp. in bufonids; cestoda: *C. jaegerskioeldi*, cysts of a proteocephalid and *C. compactus*; monogenea: *P. aeschlimanni*, *P. baeri*, *P. galamensis* and *P. pricei*; digenea: *D. fischthalicus*, *G. africana*, *M. exoterorchis*, *M. micrurus*, *M. monodi* and *Ophidiscus* sp.; nematoda: *Amplicaecum* sp., *B. siluranae*, *C. ornata*; *Foleyellides* spp., *O. hoepplli* and the larval ascaridida I (Aisien *et al*., 2001, 2003, 2004a, 2009; Jackson and Tinsley, 2001; Oddo, 2008; Imasuen, 2010; Imasuen *et al*., 2012a). Nevertheless, *C. compactus* is of interest, being a generalist parasite, infecting pipid anurans in the rainforest and savannah-mosaic (Aisien *et al*., 2003; Imasuen and Aisien, 2015) and *H. occipitalis* in the Niger Delta region as earlier reported by Aisien *et al*. (2001) and recorded in the present study. While the juveniles of *Proteocephalus* sp. recovered from *H. galamensis* represent a new geographical record for Nigeria, this cestode has been reported elsewhere by other investigators (Goldberg *et al*., 2001; McKenzie, 2007). Until now, *Polystoma galamensis* was thought to be restricted to frogs from the savannah biotope (Aisien *et al*., 2004b) but its finding in Ase, albeit in very low prevalence changes the ecological restriction earlier ascribed to this parasite. Up to now we have not recorded the co-occurrence of *M. exoteror-*

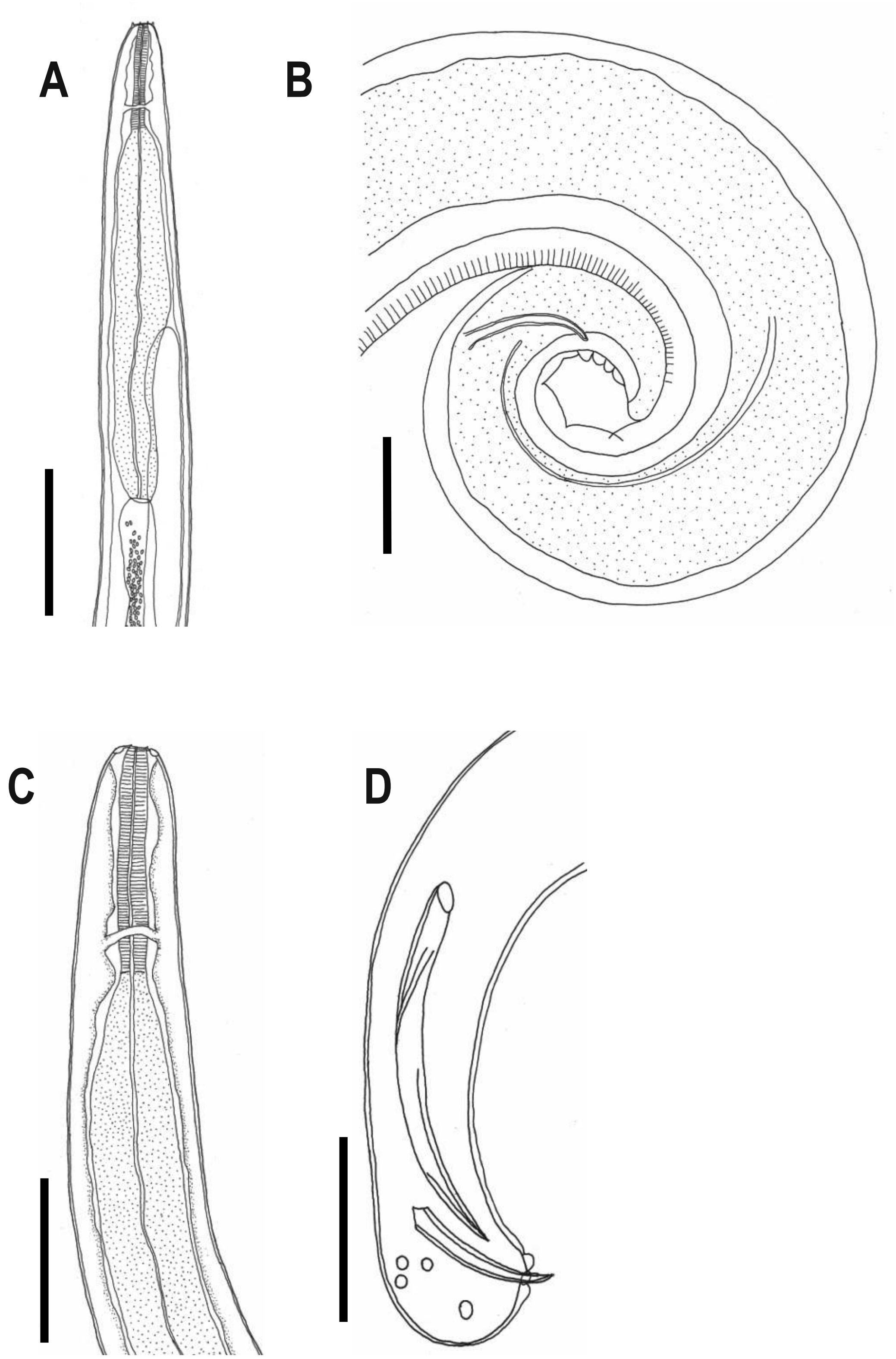

Fig. 6. *Foleyellides* spp. infecting anurans at Ase Creek. A – Anterior; B – posterior end of the species occurring in *S. regularis* and *Hyperolius concolor*; C – anterior end; D – posterior end of the species occurring in *H. galamensis*. Scale bar: A,B = 0.1 mm; C,D = 0.3 mm.

chis and *M. micrurus* in the same host in Nigeria. The two species have always occurred separately in the infected hosts (Aisien *et al*., 2001, 2003, 2004a). Fischthal and Thomas (1968) had reported their co-occurrence in frogs examined in Ghana, which we also now observe in Nigeria. *Mesocoelium* sp. (Fig. 3B), which is different from *M. monodi* both in size and morphological characteristics was found in *H. galamensis*. The parasite had a mean length of 1.01mm (0.67 – 1.40mm) and was 0.56 mm (0.44 – 0.78mm) in diameter. Although the parasite has some resemblance of *M. cameroonensis* by the short length of the intestinal caeca, it however differs from this parasite in some respect: the testes are slightly smaller than the ovary, contrary to what is observed in *M. cameroonensis*; the vitellaria extend to the posterior third of the parasite beyond what is described for *M. cameroonensis* (see Saoud, 1964; Dronen *et al*., 2012).

One interesting finding in this study was the recovery of the metacercariae of a brachylaimid trematode from the rectum of *H. galamensis*. Members of the Brachylaimidae are parasitic mostly in domestic and wild birds as well as in some small mammals (Alicata, 1940; Ulmer, 1951a, b; Sirgel *et al*., 2012) and use terrestrial gastropods as first and second intermediate hosts. The only brachylaimid trematode known so far to have an amphibian definitive host is *Zeylanurotrema spearei*, which is parasitic in the urinary bladder of *Bufo marinus* (see Cribb and Barton, 1991). We have recovered metacercariae identical to the ones we recovered from *H. galamensis* in the snail, *Limicolaria aurora*. Since no further development of this parasite occurred in *H. galamensis*, this frog is most likely an accidental host, which probably acquired the parasite from the consumption of an infected snail or vegetation contaminated with shed metacercariae. Awharitoma *et al*. (2003) found the metacercariae of *Brachylaima fuscatum* in the snail *L. aurora*. Adults of this parasite were recovered from domestic chicks infected by cloacal drop (Herman and Bacha, 1978), 29 days post-infection. In view of the close similarity among the metacercariae of brachylaimids, it needs to be experimentally determined if the metacercariae recovered from *L. aurora* and *H. galamensis* are those *B. fuscatum* or some other member of the Bracylaimidae. This clarification is necessary, because, another brachylaimid trematode (*Postharmostomum ntowi*) is known to infected domestic chicken in Ghana (Hodasi, 1969).

Progonimodiscus colubrifer appears to be a common parasite of pipid anurans as this parasite also infects *Xenopus tropicalis* collected from the Lagos area of Nigeria (Jackson and Tinsley, 1998b). *Hymenochirus* sp. is therefore a new host record for the parasite. *Hylarana galamensis* is also a new host record for the strigeoid metacercaria previously reported in *A. dorsalis* by Edo-Taiwo *et al*. (2014). According to Niewiadomska (2002), trematodes with this metacercarial type include *Diplostomum*, *Neodiplostomum* and *Alaria* spp., and they presumably use these frogs as intermediate hosts.

Among the nematodes, two *Foleyellides* species infect anurans in Ase community. One species is a generalist parasite, infecting two tree frogs and *S. regularis*. Infection with this species occurs in *S. regularis* in the savannah-mosaic (Aisien *et al*., 2003) and in *H. fusciventris burtoni* from the Okomu National Park (Imasuen *et al*., 2012a), while the second which is restricted to *H. galamensis* infects the same host in the savannah-mosaic (Aisien *et al*., 2003). The high prevalence and intensity of infection recorded for this parasite (47.7 %/10.2 worms per infect host) bears direct relationship with the high population density of mosquitoes in the locality. This observation is in agreement with Anderson (2000), who had incriminated haematophagus insects in the transmission of these parasites. In addition to *R. africanus* (Kuzmin, 2001), which infects bufonids there are other undetermined *Rhabdias* spp. infecting *Ptychadena* spp. in Ase community and other locations in southern Nigeria (M.S.O. Aisien, unpublished observations). There is a need to establish the identities of these other members of the Rhabdiasidae infecting Nigerian amphibians. The finding of a *Capillaria* sp. in *P. mascareniensis* and *P. pumilio* increases the host range for the parasite in Nigerian amphibians. A previous record of this parasite was made by Thurston (1970), who reported the presence of this parasite in the intestine *Xenopus muelleri*. Another vertebrate host harbouring *Capillaria* sp. in Nigeria is the lizard *Agama agama* reported by Adeoye and Ogunbanwo (2007). As already reported by Imasuen *et al*. (2012b), the larval ascaridida found in this study, whether in the body cavity or in the stomach mucosa, most probably use the infected anurans as transport hosts.

In conclusion, our study of the parasitic infections of anurans in Ase community reveals an interesting biotope with a low amphibian diversity whose members habour a rich community of parasites. From the 125 specimens (spread across 13 species) examined, 32 parasite species representing three helminth Phyla were recorded. The digenea and nematoda were the predominant groups represented by 10 and 13 species, respectively. Parasites recovered exhibited different life cycle patterns. While parasites like the monogeneans have a direct life cycle, others including the filariids are transmitted by arthropod (mosquitoes) vectors, others use the anurans either as intermediate or paratenic hosts.

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