Paratenic and definitive hosts of *Diplopylidium triseriale* from a southern Nigeria rainforest biotope

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Abstract

The anuran paratenic and mammalian definitive hosts of *Diplopylidium triseriale*, a cestode parasite of mammalian carnivores were investigated in a rainforest biotope in Edo State, southern Nigeria. Of the 15 anuran species examined, three, including *Sclerophrys maculata* (formerly, *Amietophrynus maculatus)*, *Phrynobatrachus calcaratus* and *Ptychadena mascareniensis* harboured cysticercoids of the parasite. Cysts harbouring the cysticercoids were found on the liver in *P. calcaratus*, but were attached to the intestinal mesentery in *S. maculata* and *Pty. mascareniensis*. Prevalence in *P. calcaratus* was 27.3%, *S. maculata*, 8.3% and 10% in *Pty. mascareniensis*, but the highest infection intensity (69 cysticercoids/infected host) was recorded in *P. mascareniensis*, which is a new anuran paratenic host record for *D. triseriale*. Nigeria represents a new locality record for the parasite. *Civetticus civetta* was found to be the definitive host of *D. triseriale* and an undescribed *Joyeuxiella* sp. in the study area. It is presumed that *C. civetta* acquires infection with *D. triseriale* by consuming the infected anurans. Alternatively, infection may also occur following the consumption of reptilian hosts, which acquired infection from consuming infected anurans.

Keywords: *Diplopylidium triseriale*; *Joyeuxiella* sp.; anurans; *Civeticus civetta*; Nigeria.

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Introduction

The genus *Diplopylidium* (Cestoda) was established by Beddard (1913), who designated *D. genettae* from *Genetta donglolana* as the type species. Thereafter, several species have been described from viverrid mammals in various parts of the world. Members of the genus *Diplopylidium* are small to medium-sized intestinal cestode parasite of carnivores. The scolex in this group has an apically flattened rostellum, armed with crowns of hooks and four cup-like suckers. In this parasite group, there are two sets of genitalia per proglottid which in mature and gravid proglottids are pre-equatorial. The defining character of the genus is that the openings of the male reproductive system occur behind those of the female. According to Prudhoe and Bray (1982) and Jones (1994), cysticercoids of this genus occur in amphibians, reptiles and small mammals, while adult worms are parasitic in the small intestine of various mammalian carnivores.

Diplopylidium triseriale was described by Lühe (1898) from *Civeticttis civetta* and *Genetta afra* in Tunis. The cysticercoid (larval stage) was described by Lopez-Neyra (1927) from the snake, *Chlorophis irregularis*. Adults of *D. triseriale* have been reported several times in the Viverridae on the Iberian Peninsula (Jordano, 1951; Casanova *et al* 2000; Torres *et al* 2006; Millan and Casanova, 2007). The parasite was listed as one of the important animal parasites in Côte d'Ivoire, with the cysticercoid occurring in *Bufo maculatus* (now *Sclerophrys maculata*), *Phrynobatrachus accraensis* and *P. calcaratus* and the adult worms parasitic in the genet and other small carnivores in the Lamto region of the country (Baer, 1972). Another *Diplopylidium* species infecting the genet in Africa is *D. paurodex*, occurring in *Genetta rubiginosa* in Rhodesia (now Zimbabwe) (Mettrick and Beverley-Burton, 1961).

The aim of this study was three-fold: (i) determine the anurans serving as paratenic hosts of *D. triseriale* in the study location (ii) establish the mammalian definitive host of this parasite in the study area and (iii) describe the cysticercoids in the paratenic hosts and the adult worms recovered from the definitive host.

Materials and methods

Study area

The study was carried out between August and October, 2016 at the Okomu Oil Palm Plantation (Extension II), situated at Odigue-tue Village in Ovia North East Local Government Area of Edo State, Nigeria. The plantation covers an area of 11,416 hectares bounded by latitudes 06°38′-6°48′N and longitudes 5°48′-5°55′E. The area

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is located in a rainforest biotope which has been deforested for the establishment of an oil palm plantation on a commercial scale. The oil palm trees in this plantation were planted about two years ago in an undulating terrain, with mucuna grass forming the undergrowth. Rainy season in this area starts around the month of April and ends in October. The dry season lasts from November to March.

Amphibians

Amphibian specimens were collected from three locations within the plantation, namely, the area around the Management Quarters (06°41.142N, 005°49.964E), a seasonal lake close to the oil palm nursery (06° 40.095N, 005° 48.965E) and a swamp, which has been designated a biodiversity conservation area (06° 40.432N, 005° 49.915E), using the Visual Acoustic Encounter Survey method (Crump and Scott, 1994). The anurans were identified using the protocols of Schiøtz (1999) and Roedel (2000).

The amphibians were euthanized in Benzocaine solution and examined for their helminth parasitic infections. The sites examined included the gastro-intestinal tract (oesophagus/stomach, small intestine and the large intestine/rectum), lungs, liver/gall bladder, urinary bladder and the body cavity. The cysts found were transferred to Petri dishes containing 0.72% NaCl solution. The cysticercoids were freed from the cysts, counted and some flattened under cover slip pressure and fixed for 20 minutes with 5% formol-saline solution.

Definitive hosts

Two female specimens of Civet cats killed by a local hunter resident at Odigue-tue Village were supplied on 12th October and 23rd November, 2016, respectively. The first cat was an African Palm Civet (*Nandinia binotata*) while the second was the African Civet (*Civettictis civetta*). The animals were transported to the laboratory and dissected to recover parasites from the gastro-intestinal tract (GIT). For this purpose, the GIT was divided into portions of between 10 to12 cm and examined for parasites. Some of the cestodes recovered were flattened under cover slip pressure and fixed with 5% formol-saline.

Identification of larval and adult parasites

Prior to identification, the cestodes collected (cysticercoids and adult worms) were washed free of the 5% formol-saline preservative and then stained overnight in a dilute solution of acetocarmine. The specimens were thereafter dehydrated in alcohol series, cleared in xylene before permanent mounts were made in Canada balsam. Micrographs of encysted and freed cysticercoids and those of adult parasites were taken with the aid of a digital camera attached to a binocular microscope. Diagrams of the cysticercoid rostellar hooks were drawn from micrographs of the flattened but unstained larva. The parasites recovered were identified with protocols provided by Jones (1983, 1994).

Results

Prevalence of cysticercoids in anuran hosts

In total, 140 anuran specimens representing 15 species in seven families were examined for helminth parasitic infections (Table 1). The anurans included *Sclerophrys maculata* (formerly *Amietophrynus maculatus*) (Bufonidae), *Hemisus mamoratus* (Hemisotdae), *Hylarana galamensis* (Ranidae), *Afrixalus dorsalis*, *Leptopelis spritusnoctis*, *Hyperolius concolor*, *Kassina senegalensis* and *Phylictimantis boulengeri* (Hyperolidae), *Phrynobatrachus calcaratus* (Phrynobatrachidae), *Ptychadena bibroni*, *Pty. mascareniensis*, *Pty. oxyrynchus* and *Pty. pumilio* (Ptychadenidae), and *Hoplobatrachus occipitalis* (Hoplobatrachidae).

Table 1. Anuran hosts from Okomu Oil Plantation (Extension II), Odigue-tue, Edo State, Nigeria.

Anuran hosts	Number examined
Bufonidae	
Sclerophrys maculata	24
Hemisotidae	
Hemisus mamoratus	3
Ranidae	
Amnirana galamensis	2
Hyperolidae	
Afrixalus dorsalis	15
A. congicus nigeriensis	1
Leptopelis spritusnoctis	4
Hyperolius concolor	8
Kassina senegalensis	26
Phlyctimantis bouelengeri	1
Phrynobatrachidae	
Phrynobatrachus calcaratus	11
Ptychadenidae	
Ptychadena bibroni	4
Pty. mascareniensis	10
Pty. oxyrynchus	8
Pty. pumilio	14
Hoplobatrachidae	
H. occipitalis	9
Total	**140**

Cysts containing *Diplopylidium triseriale* cysticercoids were recovered from S. *maculata*, *P. calcaratus* and *Pty. mascareniensis*. Prevalence in *P. calcaratus* was 27.3%, *S. maculata* 8.3% and *Pty. mascareniensis*, 10%. The mean intensity of infection was highest in *P. mascareniensis* (69 cysticercoids/ infected host), followed by *P. calcaratus* (54 cysticercoids/infected host) and *S. maculata* (12 cysticercoids/infected host). The cysts (Figure 1) occurred in the liver parenchyma of *P. calcaratus* but were attached to the intestinal mesentery of *S. maculata* and *Pty. mascareniensis*. The cysts enclosed varying numbers of cysticercoids (Figures 2A and 2B), with

two to five larvae occurring in small cysts. In one infected specimen of *P. calcaratus*, as many as 30 cysticercoids were recovered from one large cyst.

Figure 1. Cysts habouring cysticercoids of *D. triseriale* attached to the intestinal mesentery of *S. maculata.*

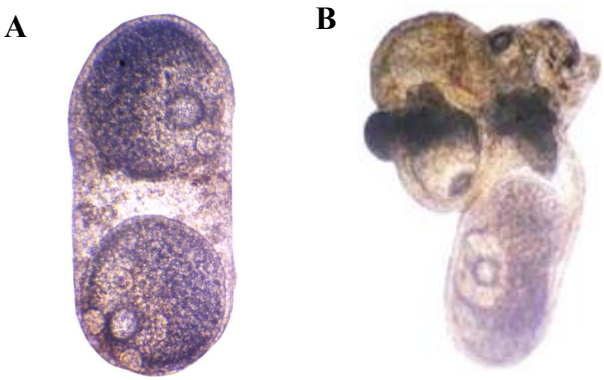


Figure 2A. Flattened cysts enclosing two cysticercoids.
Figure 2B. Cyst with three cysticercoids.

The morphometric parameters of *D. triseriale* cysticercoids are presented in Table 2. The cysticercoid larva (Figures 3A and 3B) which measured 536-938 µm in length is divided into two by a constriction at the anterior third of the body into the scolex and a pear-shaped hind body. The scolex bears four cup-shaped suckers (69.3-93.8 µm in length and 75.90-105.6 µm in diameter) and a spherical rostellum (Figure 4) bearing three alternating rows of hooks (18 hooks per row), with mean lengths of 65.8±1.6 µm, 52.2±1.3 µm and 41.1±2.0 µm, respectively (Table 2).

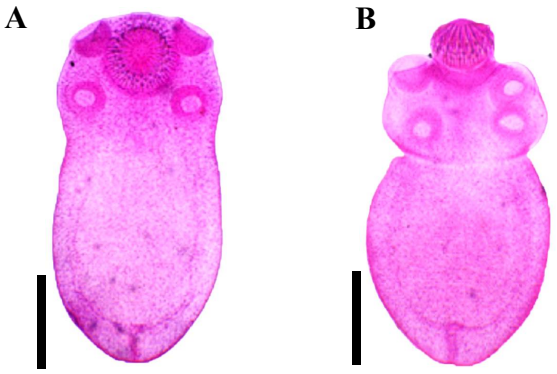
Of the two cats examined, the palm civet harboured no parasites. The GIT was filled with remnants of partially digested palm materials. The infected host (*C. civetta*) harboured a mixed infection of *Diplopylidium triseriale* (Figure 5A-E) and an undescribed *Joyeuxiella* sp. (Figure 6). No parasites were recovered from the stomach and in the first 30 cm of the small intestine. The two cestodes found occupied different segments of the small intestine, with *Diplopylidium triseriale* adults occupying the 31.5 cm to about 38cm segment, and the *Joyeuxiella* sp. the 41st to 58th cm segment. Thereafter, only detached gravid segments of both parasites were encountered. Reckoned by the number of scolesces counted, the infected cat harboured about 61 adults of *D. triseriale* and 54 of the *Joyeuxiella* sp.

The morphometric parameters of the adult worms are also presented in Table 2. Adult *D. triseriale* recovered had a mean length of 22.64±5.24 mm (range: 17.30-34.5 mm). The scolex (Figure 5A) ranged from 666.0-899.1 µm in diameter and the rostellum with a mean diameter of 472.3±38.9 µm was armed with three alternating rows of hooks characteristic of this species. The rostellar hook sizes were the same as those recorded

Table 2. Morphometric parameters of cysticercoids and adults of *Diplopylidium triseriale* from Nigeria.

Parameters	Cysticercoids		Parameters	Adult worms	
	Mean ± SD	Range		Mean ± SD	Range
Length	681.1±110.4	536-938	Length (mm)	22.64 ±5.24	17.30-34.50
Width	424.3±46.3	348-496	Scolex diameter (µm)	784.9±67.5	666.0-899.1
Scolex diameter	420.0±37.6	348.9-483.1	Rostellum diameter (µm)	472.3±38.9	399.6-532.8
Length of hindbody	444.4±62.6	362.3-550.2	Proglottids		
Sucker Length	78.3±8.1	69.3-93.8	Young proglottids (length)	251.3±25.5	201.3-281.8
Sucker width	92.5±8.3	75.90-105.6	Young proglottid (width)	745.4±69.6	603.9-818.6
Rostellum (length)	132.0±5.47	120.8-134.2	Mature proglottid (length)	531.4±87.7	442.9-684.4
Rostellum (width)	193.0±16.2	161.0-214	Mature proglottid (width)	951.9±33.8	899.1-993.1
Rostellar hook (length)			Late mature proglottid (length)	732.4±63.1	671.0-857.6
Anterior hook	65.8±1.6	62.04-67.98	Late mature proglottid (width)	1041.2±90.0	832.0-1140.7
Median hook	52.2±1.3	52.47-57.52	Gravid proglottid (length)	3887.2±1126.6	2497.5-4895
Posterior hook	41.1±2.0	36.30-42.90	Gravid proglottid (width)	1300.9±194.8	899.1-1598.4

in the cysticercoids. The young (Figure 5B), mature (Figure 5C) and the late mature segments (Figure 5D), (that is, proglottids preceding the gravid segments) were wider than long (Table 2). In contrast, the gravid segments (Figure 5E) were longer than wide (Table 2). The anlagen of the male genitalia first appeared in the 13th segment with about 34 visible testes (Figure 5B). In these young segments, the female genitalia were still very rudimentary. In the mature segments (Figure 5C), the size of the ovaries and the testicular follicles were either equal or the ovaries slightly larger than the testicular follicles; in the late mature proglottids (Figure 5D), the female genitalia became more prominent while the testes were observed to have degenerated. The gravid segments (Figure 5E) were filled with eggs and remnants of the male reproductive structures located at the anterior third of the proglottid. The scolex and mature proglottid of the *Joyeuxiella* sp. recovered from *C. civetta* are shown in Figures 6A and 6B.



Figures 3A-B. Cysticercoids of *D. triseriale*, A, showing flattened rostellar hooks; B, showing extruded rostellum and hooks. Scale Bar: A = 0.2 mm; B = 0.15 mm.

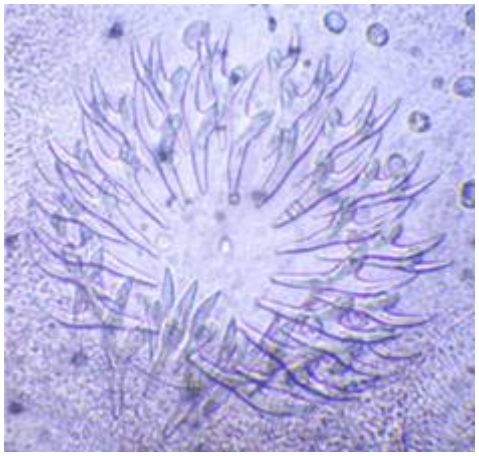


Figure 4a. Rostellar hooks of *D. triseriale* showing the three rows of hooks.

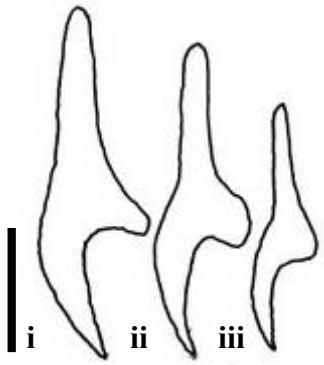
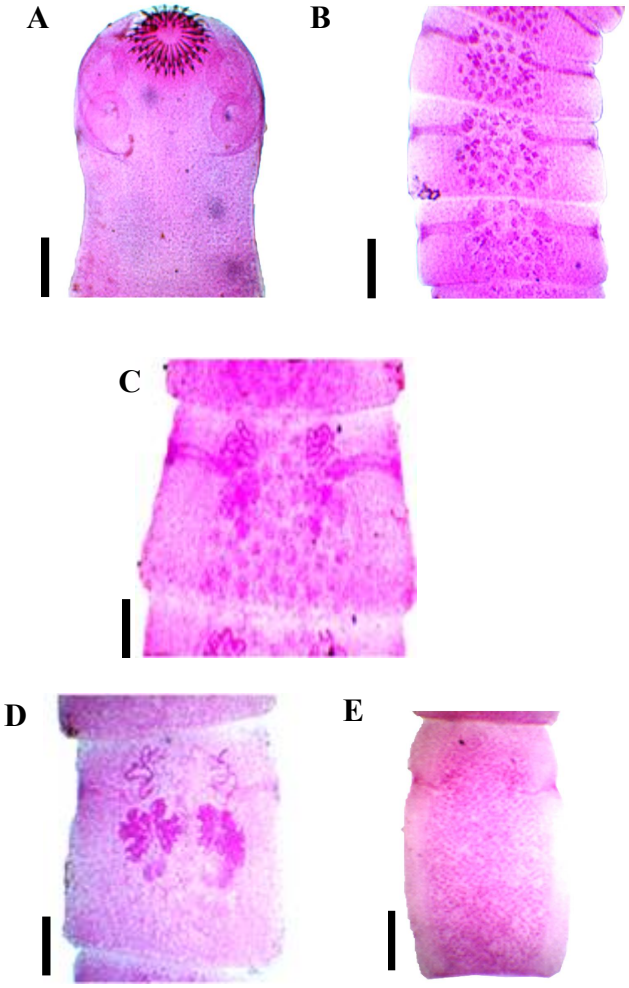
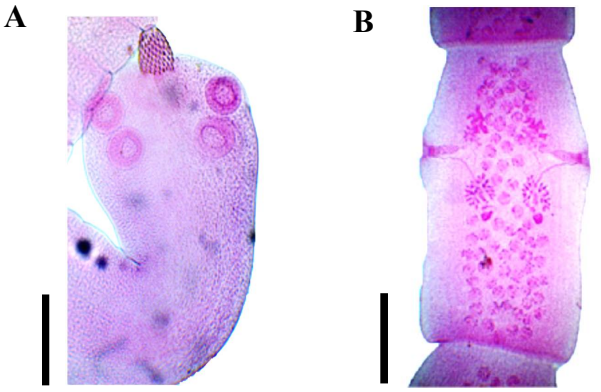


Figure 4b. The three rostellar hooks of *D. triseriale*: (i), anterior hook; (ii), median hook and (iii), posterior hook. Scale Bar: 20 µm.



Figures 5A-E. Adult of *Diplopylidium triseriale*, A, scolex of adult worm; B, early mature proglottid; C, mature proglottid; D, late mature proglottid and E gravid proglottid of adult worm. Scale Bar: 1 mm.



Figures 6A-B. *Joyeuxiella* sp. infecting *C. civetta.* A, scolex of adult worm; B, mature proglottid of adult worm. Scale Bar: A, 1 mm; B, 0.5 mm.

Discussion

This study is the first report of *D. triseriale* in anuran paratenic and mammalian definitive hosts in Nigeria, and has also provided some description of the cysticercoids of the parasites which was lacking in the account of Baer (1972). Previous examinations of domestic cats and the palm civet in the rainforest of southern Nigeria by Obiamiwe (1986) did not record any cestode infection in these carnivores but the author found *Dipylidium caninum* infection in domestic dogs.

Information about the life cycle of *Diplopylidium* spp. is fragmentary. According to Prudhoe and Bray (1982), coprophagus insects are presumed to serve as paratenic hosts for the cysticercoids of *Diplopylidium* spp. from which susceptible vertebrate hosts including amphibians, reptiles and small mammals acquire infection. As was observed in Côte d'Ivoire (Baer, 1972), anurans (*S. maculata*, *P. calcaratus* and *Pty. mascareniensis*) also serve as paratenic hosts for *D. triseiale* in Nigeria. Up until now, only proteocephalid cestodes have been reported to use anurans as paratenic hosts in Nigeria (Imasuen *et al* 2012) and we recovered them in this study from *H. occipitalis*. Two of the anuran hosts reported in Côte d'Ivoire (Baer, 1972), (*S. maculata* and *P. calcaratus*) also habour cysticercoids of *D. triseriale* in Nigeria. *Ptychadena mascareniensis* from which we additionally recovered cysticercoids in this study is a new anuran paratenic host record for the parasite.

There were differences in the prevalence of *D. triseriale* cysticercoids in the three anuran hosts harbouring the parasite. The prevalence in *P. calcaratus* was 27.3%, while prevalence in *S. maculata* and in *P. mascareniensis* were 8.3% and 10%, respectively. This prevalence pattern seems to mirror the values obtained by Baer (1972) in Côte d'Ivoire, where prevalence in *P. calcaratus* was 40%, *S. maculata*, 1.47% and *P. accraensis*, 2.3%. It seems therefore that in Côte d'Ivoire and Nigeria that *P. calcaratus* is the predominant anuran paratenic host with other infected anurans (*S. maculata* and *Pty. mascareniensis*) playing subordinate roles. However, an examination of the intensity of infection in the three hosts in Nigeria showed that *Pty. mascareniensis* had a higher infection intensity (69 parasites/infected host) than *P. calcaratus* (54 parasites/infected host) and *S. maculata* (12 parasites/infected host).

Adults of *D. triseriale* have been recovered from the genet in Côte d'Ivoire (Baer, 1972) and by a number of investigators on the Iberian Peninsula (Jordano, 1951; Casanova *et al* 2000; Torres *et al* 2006; Millan and Casanova, 2007). Our investigation has shown that the Civet cat (*Civeticttis civetta*) is the definitive host of *D. triseiale* in the study area (Odigue-tue). It can however not be ruled out that this parasite also infect other carnivores including the genet in the rainforest biotope of southern Nigeria. For now, it may be assumed that *C. civetta* acquires infection with *D. triseriale* by feeding on infected anuran hosts that habour the cysticercoids, giving a three host cycle.

It is however noteworthy that the cysticercoid of *D. triseriale* was originally described from the snake, *Chlorophis irregularis* (Lopez-Neyra, 1927). Since snakes are not insectivorous, the plausible route of infection for them is the consumption of infected anuran hosts, giving a four host cycle in this instance. The co-occurrence of a *Joyeuxiella* sp. with *D. triseriale* in the civet cat makes this latter route of infection very likely, as larval stages of *Joyeuxiella* spp. are known to occur in several reptilian hosts, including *Lacerta*, *Natrix*, *Coluber*, *Tropidonotus*, *Zamenis*, *Ailurophis*, *Hemidactylus*, *Varanus*, *Acanthodactylus*, *Trapellus*, *Stellio* and *Mabuya* (Joyeux and Baer, 1936; Fitzsimmons, 1961).

The *Joyeuxiella* sp. with previous record in mammalian carnivores in Nigeria is *J. pasqualei*. This cestode infects both domestic and wild cats (*Felis catus* and *F. serval*) in Nigeria (Jones, 1983). The *Joyeuxiella* sp. recovered from *C. civetta* differs in several respects from *J. pasqualei* and will therefore need a more detailed study. In conclusion, this study has reported the occurrence of the cysticercoids of *D. triseriale* in three anuran hosts in Nigeria. This is the first report of this parasite in Nigeria and the second in West Africa after Côte d'Ivorie. The anuran hosts and infection pattern in Nigeria are very similar to what was observed in Côte d'Ivoire, except that an additional anuran host, *Pty. mascareniensis* exist in Nigeria. Whereas the genet is the definitive host in Côte d'Ivoire and southern Europe, the civet cat plays this role in the rainforest biotope in southern Nigeria. A second cestode, a *Joyeuxiella* sp. which also infects the civet cat in this locality is different from *J. pasqualei*, which is parasitic in both domestic and serval cats.

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