sciendo

DOI 10.2478/helm-2023-0006

HELMINTHOLOGIA, 60, 1: 84 – 93, 2023

Observations on the biology of *Postharmostomum ntowi* Hodasi, 1967 (Trematoda: Brachylaimidae) based on intermediate and definitive hosts found in Nigeria

E. O. ALARI[1], A. P. UGBOMEH[2], A. O. AWHARITOMA[1], E. E. ENABULELE[1], M.S.O. AISIEN[1,*]

[1]Laboratory of Parasitology Research, Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, *E-mail: *aisien@uniben.edu*; [2]Department of Applied and Environmental Biology, Rivers State University, Port Harcourt, Nigeria

Article info

Received July 13, 2022
Accepted February 14, 2023

Summary

Following the recovery of the metacercariae of a brachylaimid trematode from the rectum of the frog *Amnirana galamensis* from Ase in Delta State, Nigeria, we investigated the land snails in the locality to establish their roles in the life cycle of the parasite. Of the four land snails investigated from Ase (*Limicolaria aurora*, *Archachatina marginata*, *A. papyracea*, and *Thapsia oscitans*), and a *Limicolaria* sp. from Tombia (Bayelsa State), four harboured larval stages of the bracylaimid. Only *L. aurora* and the *Limicolaria* sp. harboured cercariogenous sporocysts and are therefore presumed to serve as the first intermediate hosts of the parasite. Metacercariae were recovered from the *Limicolaria* spp. and the *Archachatina* spp. and so serve as the second intermediate hosts. No larval brachylaimids were recovered from *T. oscitans*. Metacercariae from *L. aurora* and *A. papyracea* were cultured *in vivo* in 14 days old chicks of *Gallus gallus domesticus*. Parasites recovered from the experimental hosts 7, 14, 21 and 28 days post-infection, showed progressive development of the parasite with the full maturity attained by the 28th day post-infection. Adult parasites recovered from the experimental birds and from free range chicken purchased from Ase and Tombia showed that the brachylaimid infecting these birds was *Postharmostomum ntowi*, a parasite previously reported in domestic chicken in Ghana. There is need to investigate the host range of the parasite in Nigeria as this trematode is also known to infect the Guinea fowl in Ghana.

Keywords: *Postharmostomum ntowi*; snail host; larval stages; domestic chicken, Nigeria

Introduction

In a survey of the helminth parasites of anurans from Ase, a location in the freshwater creeks of the Niger Delta of Nigeria, Aisien *et al*. (2017) recorded the metacercariae of a brachylaimid trematode in the rectum of *Amnirana galamensis*. Since the stage recovered was the metacercaria, it was doubtful if the frog was the definitive host of the trematode. Previous reports of brachylaimids in the West African sub-region have been in avian hosts. *Postharmostomum ntowi*, a parasite of the gastrointestinal tract of domestic chickens and guinea fowl was described by Hodasi (1967, 1976) in Ghana. Okon and Enyenihi (1980) reported an unnamed *Postharmostomum* sp. in the caecum of domestic chicken in Oron, Cross River State of Nigeria. Awharitoma *et al*. (2003) found metacercariae of a bachylaimid in the land snail *Limicolaria aurora* collected from locations in the Niger Delta of Nigeria. *In vivo* cultivation of the metacercariae in domestic chicks yielded adults of *Brachylaima fuscatum*.

In order to determine the snail intermediate host(s) of the metacercariae we recovered from *A. galamensis*, we examined various

* – corresponding author

land snails found in Ase for the presence of larval brachylaimids, with a view to establishing the roles these snails play in the life cycle of the parasite. We also infected 14 days old chicks of *Gallus gallus domesticus* with metacercariae recovered from *Limicolaria aurora* and *Archachatina papyracea*, to determine whether it was a susceptible host to this trematode. In this paper, we report on the larval stages recovered from the snail intermediate hosts and the adult recovered from experimental hosts and natural infections in free range *Gallus gallus domesticus* from the study areas.

Materials and Methods

Snail hosts

The snails examined (*Archachatina marginata*, *A. papyracea*, *Limicolaria aurora*, *Limicolaria* sp. and the semi-slug *Thapsia oscitans* were collected from their natural habitats at two locations: Ase (05°20´N; 006°19´E) in Delta State and Tombia (05°00´N; 006°15´E) in Bayelsa State, both in the Niger Delta of Nigeria. While *A. marginata*, *A. papyracea*, *L. aurora* and *T. oscitans* were collected from Ase, the unidentified *Limicolaria* sp. was collected from Tombia.

Laboratory maintenance and examination of snails

In the laboratory, snails from the two sites were transferred into plastic containers with perforated lids. The perforations were screened with fine nettings to protect the snails from flies. The snails were fed on cabbage leaves *ad libitum* until examined. The containers were cleaned out and fresh cabbage provided every other day.

Snails were cracked and dissected to isolate the digestive gland (hepato-pancreas), kidney, heart and surrounding pericardium. Each part was teased open in a Petri dish containing normal saline (0.85 % NaCl) and examined under a dissecting microscope. Parasite stages recovered were identified, counted and preserved. Sporocysts and cercariae were fixed and preserved in 70 % alcohol; metacercariae were flattened under cover slip pressure, fixed and preserved in 5 % formol-saline.

Infection experiments

Day-old chicks of *Gallus gallus domesticus* were ordered from a local hatchery, raised for 14 days in experimental cages, under hygienic conditions in the laboratory. Prior to experimental infection, the birds were weighed, starved of food and water for 6 hours and then anaesthetized by injection of Euthapent (Sodium pentabarbitone) to the thigh (30 µg/g body weight), to decrease the urge to defecate. Each bird was thereafter infected with 50 live metacercariae by cloacal drop infection method (Herman & Bacha, 1978). If a chick defecated immediately after infection, it was assumed that the metacercariae were discharged with the stool and was re-infected with another dose of 50 metacercariae. Following full recovery from the effects of the anaesthesia, the birds were first provided with water containing glucose (0.9 g/l) and subsequently fed chick mash *ad libitum*. Control birds were administered 0.2 ml of 0.85 % NaCl solution without metacercariae. Eight chicks were each infected with 50 metacercariae recovered from *Limicolaria aurora*. Two additional chicks were infected each with 50 metacercariae obtained from *Archachatina papyracea* collected from Ase.

Recovery of parasites from infected birds

In order to recover developing parasites, a pair of the infected birds was sacrificed on the 7th, 14th, 21st and 28th day post-infection. The gastro-intestinal tract (small intestine, large intestine, the intestinal caecae and rectum) was examined under a dissecting microscope for sub-adult and adult worms. The site of recovery was recorded and the number of worms recovered from each bird was counted and the percentage recovery calculated for each pair.

Natural infection in free-range domestic chicken

Following the successful recovery of sub-adult and adult parasites from the experimental birds, two hens and one cock were purchased from Ase while two other hens were purchased from Tombia. The gastro-intestinal tracts of these birds were examined for natural infection with brachylaimids.

Preservation of parasites

Larval brachylaimids (sporocysts and cercariae) recovered from first snail intermediate hosts were fixed and preserved in 70 % ethanol; metacercariae from the snail intermediate hosts, sub-adults and adults from experimental hosts were flattened under cover slip pressure and fixed with 5 % formol-saline. Adult worms from naturally infected free range birds were also fixed and preserved following the procedure used for worms from the experimental birds.

Table 1. Snail hosts examined for larval brachylaimid infections from Ase and Tombia (Metacercariae in *Archachatina* spp.; cercariae and metacercariae combined in *Limicolaria* sp.; details given under results)

Host	Ase	Tombia	Total No examined	No. infected	Prevalence (%)	Mean Intensity
A. marginata	04	-	04	3	75.0	4.33
A. papyracea	04	-	04	2	50.0	120.8
L. aurora	30	-	30	25	83.3	98.6±47.3
Limicolaria sp.	-	22	22	22	100	39.9±9.1
T. oscitans	02	-	02	-	-	-

Examination of parasites

Larval stages (sporocysts, cercariae and metacercariae) were examined as temporary mounts in lactophenol. Sub-adult and adult parasites were washed free of preservative (formol-saline) in four changes of tap water, over four hours, stained overnight in a dilute solution of acetocarmine and thereafter dehydrated in alcohol series, cleared in xylene before they were permanently mounted in Canada balsam.

Photography

Photomicrographs of the different parasite stages were taken with a Digital Camera, (DFK MKU 130-10 x 22, Imaging Source, Germany) attached to a binocular microscope.

Ethical Approval and/or Informed Consent

No ethical approval was required nevertheless we followed the guidelines for Ethical Conduct in the Care and Use of Non-human Animals in Research by the American Psychological Association.

Results

As shown in Table 1, a total of 62 snail specimens were examined for infection with larval brachylaimids, 40 from Ase and 22 from Tombia. The snails examined consisted of *A. marginata* (n=4), *A. papyracea* (n=4), *L. aurora* (n=30), *Limicolaria* sp. (22) and *T. oscitans* (2). The prevalence and mean intensity of infection are also shown on Table 1. Of the five land snails examined, all except the semi slug *T. oscitans* harboured larval brachylaimids. Larval stages encountered included branched sporocysts with cercaria (Figs. 1A and 1B) brevicaudate cercariae (Fig. 1C) and metacercariae (Fig. 1D). Sporocysts were recovered in only three specimens (6.38 %) of *Limicolaria* spp. (one specimen of *L. aurora* from Ase and two specimens of *Limicolaria* sp. from Tombia). While all three larval stages occurred in the *Limicolaria* spp. only metacercariae were recorded in *A. marginata* and *A. papyracea*.

Mean measurement ±SD of the larval stages is given in micrometer (µm), followed by the range in parenthesis.

Cercariae

The brevicaudate cercariae recovered from *L. aurora* measured 366 ± 30 (310 – 416) x 118 ± 10 (102 – 135) with the tail lengths ranging from 40 – 86. The oral sucker is longer than wide, 83 ± 5 (76 – 96) x 76 ± 4 (66 – 83), while the acetabulum is slightly wider than long, 72 ± 6 (63 – 83) x 74 ± 5 (63 – 84).

Metacercariae

The morphometric measurements of metacercariae (n=20) recovered from *L. aurora* collected from Ase are presented as follows: Body length 1388 ± 271 (938 – 2157); Greatest width, 613 ± 86 (429 – 737); Forebody length (FBL) 564 ± 103 (375 – 831); FBL as percentage of body length 41 ± 3 % (35 % – 47 %); Oral sucker length 253 ± 44 (147 – 295; Oral sucker width (OSW) 260 ± 44 (161 – 335); Ventral sucker length 167 ± 25 (107 – 208); Ventral sucker width (VSW) 171 ± 21 (121 – 214); OSW/VSW ratio 1.5 ± 0.1 (1.2 – 1.8); Distance between suckers 299 ± 90 (174 – 523); Pharynx length 130 ± 22 (80 – 161); Pharynx width 126 ± 21 (80 – 161).

Metacercariae recovered from the *Limicolaria* sp. from Tombia measured as follows: Body length (BL) 1352 ± 232 (1045 – 1929); Greatest width 660 ± 57 (536 – 737); Forebody length (FBL) 579 ± 98 (442 – 831); FBL as percentage of BL 43 ± 4 (31 – 48); Oral sucker length (OSL) 290 ± 26 (241 – 335; Oral sucker width (OSW) 304 ± 24 (268 – 348); Ventral sucker length (VSL) 182 ± 18 (147 – 214); Ventral sucker width (VSW) 188 ± 14 (161 – 214); OSW/VSW ratio 1.6 ± 0.1 (1.5 – 1.9); Distance between suckers

Table 2. Measurement of metacercariae of *Postharmostomum ntowi* from *Limicolaria aurora* collected from Ase, Delta State, Nigeria.

Characters	Ase	
	Mean±SD	Range
Body Length (BL)	1387.6±271	938–2157
Greatest width	613.1 ± 85.8	938–2157
Forebody length (FBL)	563.5 ± 102.9	375–830.8
FBL as %tage of BL	40.8 ± 3.1	34.6–47.1
Oral sucker length (OSL)	253.3 ± 43.7	147.4–294.8
Oral sucker width (OSW)	260.0 ± 43.6	160.8–335.0
Ventral sucker length	167.2 ± 24.9	107.2–207.7
Ventral sucker width	170.5 ± 20.8	120.6–214.4
OSW/VSW ratio	1.5 ± 0.1	1.2–1.8
DBS	298.8 ± 90.2	174.2–522.6
Pharynx length	130 ± 22.2	80.4–160.8
Pharynx width	126.3 ± 20.8	80.4–160.8

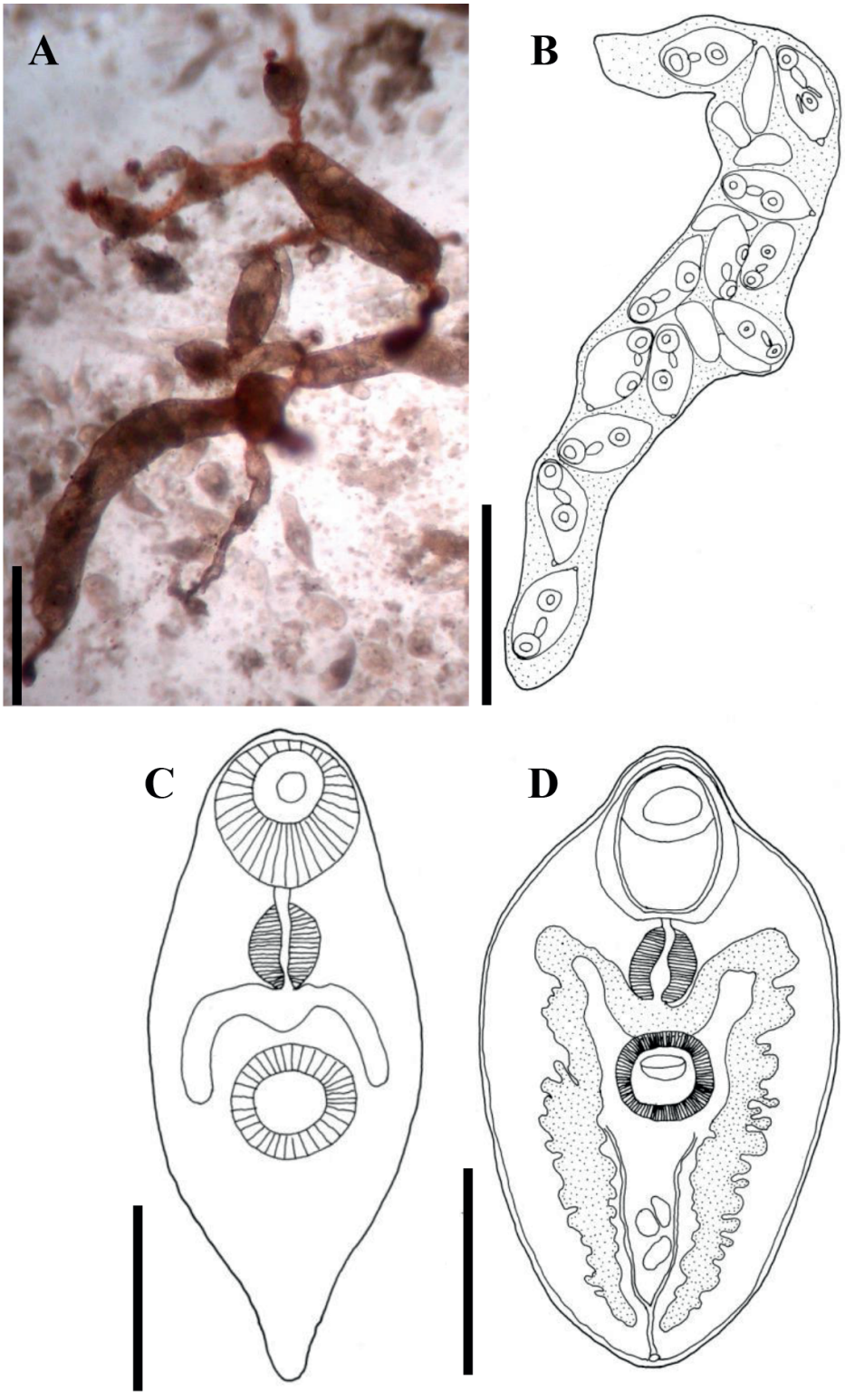

Fig. 1. A - Branched cercariogenous sporocysts of *P. ntowi* from *L. aurora*; B - Cercariae within a sporocyst; C.Cercaria; D. Metacercaria. Scale Bar: A, B = 0.5mm; C= 0.1 mm; D = 0.05 mm

DBS 263 ± 81(161 – 442); Pharynx length 149 ± 20 (121 – 188); Pharynx width 146 ± 17 (121 – 174).

Parasites recovery 7, 14, 21 and 28 days post-infection

A pair of chicks infected with metacercariae recovered from *L. aurora* was sacrificed on the 7th, 14th, 21st and 28th day post-infection, respectively, and the developing, mature or gravid parasites recovered. The respective numbers of parasites recovered from the infected chicks are shown in Table 3. All parasites whether juveniles, mature or gravid adults were recovered from the caecum. The recovery rate (RR) ranged from 8 to 19 %.

The pair of chicks infected with metacercariae from *A. papyracea* was also sacrificed on the 28th day post-infection. Five gravid parasites were recovered from the first chick and one from the second (RR, 6 %). No parasites were recovered from the control birds. The measurements of the parasites recovered are shown in Table 4.

The prominent features in the parasites recovered 7 days post-infection were the oral sucker, the pharynx, intestinal caecae and the acetabulum. The primordia of the genital organs were present but not measureable at this stage (Fig. 2A). The parasites recovered 14 days post-infection were morphologically similar to the preceding stage (7 days), except that they were larger and the primordia of the reproductive organs were clearly visible and measureable (Fig. 2B). At 21 days post-infection, the testes and the ovary were clearly differentiated; the uterus (without eggs) and the vitellaria were also visible (Fig. 2C). The parasites recovered 28 days post-infection were gravid and the vitellaria which were restricted to the lateral fields in the parasites from 21 days post-infection now filled both the extra-caecal and intra-caecal spaces (Fig. 2D).

Brachylaimid infections in free range Gallus gallus domesticus from Ase and Tombia

Adult birds purchased from Ase and Tombia were killed and their intestinal tracts examined for brachylaimid infection. The three birds purchased from Ase were all infected (prevalence, 100 %) with 8, 19 and 6 adult (gravid) parasites (Fig. 3), respectively. Of the 19 worms recovered from chicken number 2, 17 were from the caecum while two were from the rectum. Parasites from hen 1 and the cock were all recovered from the rectum. Measurements for the gravid parasites from Ase are also presented in Table 4.

Of the two hens purchased from Tombia, only one was infected with two (2) gravid worms.

Discussion

The long and complex history of the genus *Postharmostomum* was summarized by Valadão *et al*. (2018). The account showed that *Postharmostomum* was first reported from a young chicken from Italy by Wagener (1852), who misidentified it as *Distoma dimorphum* Diesing, 1850. The error was corrected six years later by Diesing (1858) who described the parasite found by Wagener (1852) as a new species and named it *Distoma commutatum*. Thereafter, several reports of the occurrence of the parasite were published in Europe and Africa. Witenberg (1923) described a caecal fluke which was assigned to a new genus and species with the name *Postharmostomum gallinum* from parasites found in chicken from Turkestan in Central Asia. The parasite was differentiated from *D. commutatum* on account of the shape of the oral sucker, position of the genital pore and most especially, the anterior extent of the vitellaria, which reach the pharynx in *P. commutatum* but did not exceed the ventral sucker in *P. gallinum*. Shortly after its description, *P. gallinum* was synonymised with *P. commutatum* as it was believed that Wagener (1852) mischaracterized the vitellaria of *P. commutatum*, especially as no parasites with vitellaria extending anteriorly to caecal bifurcation were found in the caeca of chickens world-wide, even in areas where *P. commutatum* was originally described (Pereira & Cuocolo,1939; Deiana & Arru, 1963). The morphological characteristics of the trematode recovered in this study shows it clearly belongs to the genus *Postharmostomum* (Brachylaimidae).

From the results obtained in this study, it has been established that *Amnirana galamensis* was an accidental host of the metacercariae found in its gut, since no further development occurred in this host. Similar accidental infections with brachylaimid metacercariae have been reported in *Osteolaemus tetrapsis*, the African dwarf crocodile (Enabulele *et al*., 2013) and the fresh water turtle *Pelusios castaneus* (Adebayo, 2021). The infection in *A. galamensis* may have arisen from the consumption of an infected snail intermediate host. Of the five land snails investigated, it was only the semi-slug *T. oscitans* that did not harbour larval brachylaimids. Even this observation needs further investigation since other slugs have been reported to serve as intermediate hosts of brachylaimid parasites in Africa (Canaris, 1963; Sirgel *et al*., 2012) and elsewhere (Thiengo & Amato, 1995; Valente *et al*., 2016). Therefore, despite the negative result recorded in this study, the role of slugs in the transmission of brachylaimids cannot be overlooked. It is either that *T. oscitans* is not susceptible to the brachylaimid under study or that the number of specimens of this slug examined was too few.

Brachylaimids use two land snails as first and second intermediate hosts, respectively (Lewis, 1969; Mas-Coma & Montoliu, 1986; Lewin, 1992; Cribb & Barton, 1991; Gibson & Bray, 1994). The first snail host according to Mas-Coma and Montoliu (1986, 1987, 1995) is very specific, as brachylaimid species use only one or a very small number of closely related mollusc species inhabiting the local area. Since the cercariogenous sporocysts occurred only in *Limicolaria* spp. (*L. aurora* and *Limicolaria* sp.), these snails must be the first intermediate hosts of the brachylaimid occurring in them. *Limicolaria* spp. are also known to play this role for other brachylaimids in Africa. For example, Canaris (1963) reported that *L. martensiana* served as first intermediate host for an undetermined *Postharmostomum* sp. in Uganda while Awharitoma *et al*. (2003) reported the occurrence of the sporocysts of *Brachylaima fuscatum* in *L. aurora* from Nigeria. The recovery of sporocysts from the digestive glands (hepatopancreas) of *Limicolaria* is in

Table 3. Parasite recovery from infected chicks 7, 14, 21 and 28 days post-infection.

Chicks	Parasite recovery days post-infection								Total	Recovery rate (%)
	7th day		14th		21st		28th			
Set 1	1	15	-	-	-	-	-	-	16	16
Set 2	-	-	1	7	-	-	-	-	8	8
Set 3	-	-	-	-	2	15	-	-	17	17
Set 4	-	-	-	-	-	-	7	12	19	19

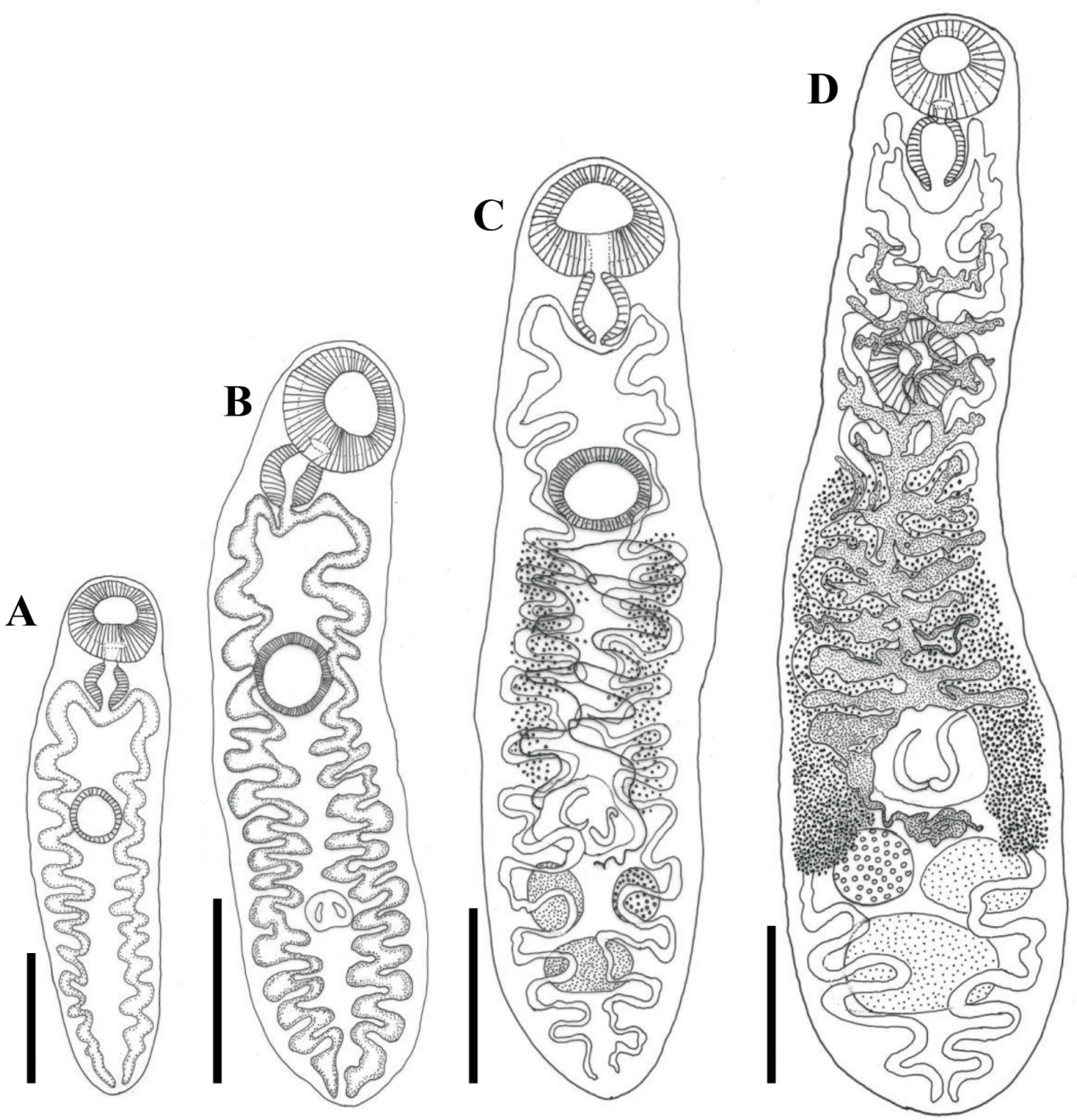

Fig. 2. Developing stages of *Postharmostomum ntowi* recovered from the experimental host (*Gallus gallus domesticus*) after: A - 7days; B - 14 days; C - 21 days and D - 28 days post-infection. Scale bars: A= 0.5 mm; B-D = 1mm

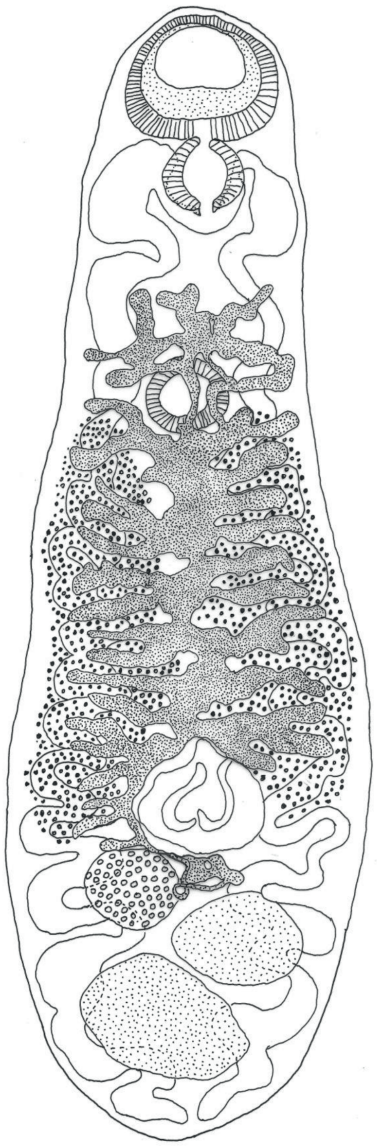

Fig. 3. Adult *Postharmostomum ntowi* recovered from naturally infected *Gallus gallus domesticus* purchased from Ase, Delta State, Nigeria. Scale bar: 1 mm

agreement with observations in other studies (Robinson, 1949; Mas-Coma & Montoliu, 1987; Butcher & Grove, 2001; Gracenea & Gonzalez-Moreno, 2002; Awharitoma *et al.,* 2003; Gonzalez-Moreno & Gracenea, 2006; Segade *et al*., 2011).

The fact that only three specimens of the 62 snails examined harboured sporocysts confirms the earlier observations by Mas-Coma and Montoliu (1986, 1987, 1995) that in brachylaimid species, the prevalence of cercariogenous sporocysts in the first intermediate host is remarkably low. In brachylaimid life cycles, the first intermediate host can also serve as second intermediate host. We believe that *L. aurora* from Ase and the *Limicolaria* sp. from Tombia also serve as second intermediate hosts judging by the high prevalence of metacercaria in these snails (see Table 1). *Archachatina* spp. (*A. marginata* and *A. papyracea*) most probably serve only as second intermediate hosts. The similarity in the size range and morphology of the metacercariae from *L. aurora* and the unidentified *Limicolaria* sp. is an indication that they belong to the same species.

It needs to be mentioned here that the larval stages of *P. commutatum* and *P. ntowi* share some similarities but also have some differences. Both parasites have branched cercariogenous sporocysts harbouring brevicaudate cercariae. In both species the oesophagus is absent, the caeca is saccular and lateral. However, the cercariae of *P. ntowi* have longer body size (310 – 415) as against 186 – 193 µm for *P. commutatum* (Alicata 1940); the cercariae of *P. ntowi* lack the body spines that occur in *P. commutatum*; the genital primordia which are visible in the cercaria of *P. commutatum* (Alicata, 1940) are not detectable at this stage of *P. ntowi*. Again, except for the differences in body size, metacercariae of both parasites are quite similar: have sinuous intestinal caeca which extend to the posterior of the body; both genital structures are present but not well defined. As remarked by Valadão *et al*. (2018), who conducted a molecular phylogenetic study of *P. commutatum* (= *P. gallinum*), DNA sequences from other isolates of *Postharmostomum*, (including the one studied here), may yet confirm the cosmopolitan distribution of *P. commutatum* or reveal a hidden diversity related to this genus of avian trematodes.

The progressive development of the metacercariae of the studied brachylaimid from juveniles to mature adults and finally to gravid worms in *Gallus gallus domesticus* (Figs. 2 A-D) is an indication that the domestic chick is a susceptible experimental host. However, the recovery of gravid adults from free range *Gallus gallus domesticus* from Ase and Tombia confirms that these birds are the definitive host of this brachylaimid. Moreover, the infection prevalence in both experimental and naturally infected hosts is very similar.

Results obtained in this study have shown that the parasite recovered from *Limicolaria* spp. and *Archachatina* spp. is not *B. fuscatum* previously recorded by Awharitoma *et al*. (2003). In contrast, the brachylaimid parasites recovered from experimental and naturally infected domestic chicken from Ase and Tombia is *Postharmostomum ntowi* described by Hodasi (1967) from domestic chicken and the Guinea fowl in Ghana (Hodasi, 1976). Okon and Enyenihi (1980) provided neither measurements nor illustrations for the *Postharmostomum* sp. they reported from chickens in Oron, Nigeria but judging from the infection site reported (the caeca), it is likely that this parasite was also *P. ntowi*. The similarities shared by the two parasite populations from Ghana and Nigeria, respectively, include: elongated body lengths that are in the same size range (see Table 5), intestinal caeca that is continuously sinuous down to the posterior extremity as well as the testes and ovary arrangement (the testes are obliquely tandem while the ovary is opposite the anterior testis). Another shared feature is the anterior reach of the uterus which terminates close to the posterior end of the pharynx. As in the type specimens of *P.*

Table 4. Morphometric measurement of *Postharmostomum ntowi* from experimental hosts and hosts with natural infections.

Parameters	7 days (n=5		14 days (n=7)		21 days (n=7)		28 days (n=8)		Natural infections (n=11)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Body Length (BL)	2458±202	2164–273	3510±389	2930–4029	5865±1010	4562–7192	6639±865	5561–7726	8639.8±927	7060–10089
Body width	729±45	683–799	899±94.2	766–1032	1517±270	1199–1864	1640±229	1432–2098	2183±363	1765–2731
Forebody length (FBL)	1026±119	866–1199	1246±88	1132–-1365	1755±286	1399–2231	1944±123	1764–2098	2546±219.	2098–2930
FBL as %tage of BL	41.6±1.44	40.0–43.9	35.7±2.59	32.2–39.0	30.1±1.79	26.7–32.1	29.6±2.9	25.9–34.5	29.7±3.0	25.7–34.5
Oral sucker length	446.2±44.7	399.6–499.5	528.0±30.0	466.2–566.1	675.5±65.8	599.4–765.9	720.1±90.7	599.4–832.5	1044.4±60.1	965.7–1165.5
Oral sucker width	456.2±49.4	383.0–499.5	551.8±24.4	532.8–599.4	761.1±107.8	632.7–932.4	786.7±115.3	566.1–932.4	1135.2±113.9	990.0–1365.3
Ventral sucker length	273.4±15.3	254.6–294.8	423.2±210.1	335.0–899.1	480.5±68.9	399.6–566.1	553.6±35.3	499.5–599.4	678.1±84.6	566.1–832.5
Ventra sucker width	273.4±26.1	227.8–294.8	358.0±27.6	321.6–388.6	518.5±76.6	399.6–599.4	545.3±81.4	466.2–732.6	687.2±96.8	532.8–832.5
OSW/VSW ratio	1.7±0.2	1.3–1.9	1.6±0.1	1.4–1.7	1.5±0.1	1.3–1.6	1.5±0.2	1.1–1.7	1.7±0.3	1.4–2.6
Pharynx length	203.7±27.5	174.2–227.8	260.3±24.3	214.4–281.4	399.6±19.2	366.3–432.9	407.9±42.7	366.3–466.2	560.0±66.3	466.2–666.0
Pharynx width	243.9±25.8	214.4–281.4	279.5±27.3	254.6–321.6	383.0±31.9	333.0–432.9	403.8±41.5	333.0–466.6	469.2±54.6	366.6–566.1
Distance between suckers	576.1±87.8	466.2–699.3	718.3±74.1	632.7–832.5	980.0±186.3	765.9–1265.4	1111.4±188.3	799.2–1332.0	1416.±186.2	1065.6–1665.0
Oesophagus length	48.2±20.3	26.8–80.4	38.0±15.7	26.8–67.0	85.6±26.2	66.6–133.2	86.6±38.0	33.3–133.2	92.3±25.1	66.6–133.2
Cirrus length	-	-	-	-	507.8±106.6	399.6–599.4	627.±38.9	566.1–666.0	812.5±137.0	566.1–1032.3
Cirrus width	-	-	-	-	591.1±147.4	432.9–732.6	638.3±53.3	532.8–666.0	865.8±194.2	566.1–1298.7
Anterior testis length	-	-	-	-	541.1±116.6	399.6–666.0	521.7±80.7	432.9–632.7	716.0±160.3	499.5–999.0
Anterior testis width	-	-	-	-	582.8±79.3	499.5–666.0	510.6±71.9	399.6–599.4	899.1±201.6	699.3–1398.6
Posterior testis length	-	-	-	-	557.8±187.1	399.6–765.9	471.8±74.2	399.6–599.4	722.6±115.4	532.8–832.5
Posterior testis width	-	-	-	-	757±298.3	499.5–1032.3	782.6±151.5	499.5–899.1	1115.6±235..6	732.6–1565.1
Ovary length	-	-	-	-	416.3±69.3	333.0–499.5	399.6±75.9	333.0–532.8	559.4±96.5	432.9–732.6
Ovary width	-	-	-	-	474.5±50.0	432.9–532.8	394.1±32.7	366.3–432.9	462.9±90.8	299.7–566.1
Egg length	-	-	-	-	-	-	-	-	30.5±2.5	26.8–34.8
Egg width	-	-	-	-	-	-	-	-	17.2±2.3	13.4–20.1

Table 5. Comparison of the morphometrics of *P. ntowi* from experimental and natural infections in Nigeria with the Ghanian specimens.

Parameters	Natural infections (Ghana)	28th day post-infection in chicks (Nigeria)		Natural infection (Nigeria) (this study)	
	Range	Range	Mean±SD	Range	Mean±SD
Body length (BL)	6300–11100	5600–700	6600±800	7100–10000	8600±900
Maximum width	1700–3100	1400–2100	1600±230	1800–2700	2200±400
Oral sucker length	840–1130	600–830	720±90	1000–1200	1000±60
Oral sucker width	780–1070	570–930	790-±120	1000–1400	1100±100
Ventral sucker length	560–880	500–600	550±40	600–800	700±80
Ventral sucker width	533–780	470–730	550±80	530–830	700±100
OSW/VSV ratio	-	1100–1700	1500±200	1400–2600	1700±300
Distance between suckers	-	800–1330	1110±190	1100–1700	1400±200
Forebody length (FBL)	-	1770–2100	1940±120	2100–2900	2500±200
FBL as percentage of BL	-	25.9–34.5	29.6±2.9	25.7–34.5	29.7±3.0
Pharynx length	400–630	370–470	410±40	500–700	600±70
Pharyx width	400–590	330–470	400±41	400–600	500±50
Oesophagus length	-	30–130	90±40	70–130	90±3
Anterior testis length	470–1180	430–630	520±80	500–1000	700±200
Anterior testis width	440–1000	400–600	510±70	330–730	900±200
Posterior testis length	810–1380	400–600	470±70	500–800	700±120
Posterior testis width	560–1030	500–900	780±15	700–1600	1100±240
Cirrus pouch length	350–740	570–670	630±38	600–1030	800±140
Cirrus pouch width	290–590	530–670	640±53	600–1300	900±200
Ovary length	400–650	330–530	400±76	400–700	600±100
Ovary width	300–520	370–430	390±32	300–600	500±100
Egg length	23–33	-	-	30–35	30±3
Egg width	15–19	-	-	13–20	17±2

ntowi, the vitelline follicles are both extra-caecal and intra-caecal, extending anteriorly to a point behind the posterior edge of the ventral sucker and posteriorly to the anterior regions of the ovary and the anterior testis (Fig. 2D and Fig. 3). This finding has shown that *P. ntowi* is not restricted to Ghana but may be widespread in the West African sub-region. Okon and Enyenihi (1980) remarked that humid climate (as in coastal area of Ghana and Oron in Cross River State of Nigeria), may be a factor in the development and transmission of the parasite. This may be so as Ase, where we recorded this parasite is located in a freshwater creek environment where it rains most of the year. It should therefore be expected that this brachylaimid may be a common parasite of domestic chicken in the humid coastal communities of southern Nigeria.

In conclusion, this study has unravelled the life cycle of *P. ntowi* and has shown that *Limicolaria aurora* and the unidentified *Limicolaria* sp. are the first intermediate host of *P. ntowi* in Ase and Tombia. The two *Limicolaria* spp. along with *A. marginata* and *A. payracea* serve as second intermediate host for this parasite. *Thapsia oscitans* is apparently the exception as no infection with larval stages of *P. ntowi* was recorded in this snail. It has also been shown that the domestic chicken (*Gallus gallus domesticus*) is not only a susceptible experimental host for *P. ntowi*, but the definitive host for this parasite with the record of natural infections in free-range birds. Full maturity is attained in about 28 days under experimental conditions with recovery rates ranging from 8 to 19 %. The confirmation of *P. ntowi* infection in *Gallus gallus domesticus* in Nigeria extends the geographical range of the parasite. Since according to Hodasi (1976) *P. ntowi* infection is not restricted to domestic chicken in Ghana, it will be necessary to investigate the host range of this parasite in Nigeria.

Conflict of Interest

Authors state no conflict of interest

References

Adebayo, D.I. (2021): *Helminth parasites of water turtles from Ondo*

State, Nigeria. B.Sc. Dissertation, Nigeria, Benin City: University of Benin

Aisien, M.S.O., Ugbomeh, A. P., Awharitoma, A.O. (2017): Parasitic infections of anurans from a freshwater creek community in Delta State, Niger Delta of Nigeria. *Helminthologia*, 54 (2): 132 – 144. DOI: 10.1515/helm-2017-0017

Alicata, J.E. (1940): The life cycle of *Postharmostomum gallinum*, the caecal fluke of poultry. *J Parasitol*, 26: 135 – 143

Awharitoma, A.O., Okaka, C.E., Obaze, S.E. (2003): Larval stages of *Brachylaima fuscatum* in the terrestrial snail *Limicolaria aurora* from southern Nigeria. *J Helminthol*, 77: 1 – 5. DOI: 10.1079/JOH2002155

Butcher, A.R., Grove, D.I. (2001): Description of the life-cycle stages of *Brachylaima cribbi* n. sp. (Digenea: Brachylaimidae) derived from eggs recovered from human faeces in Australia. *Syst Parasitol*, 49: 211 – 221. DOI: 10.1023/a:1010616920412

Canaris, A.G. (1963): *Postharmostomum* sp. Witenberg, 1923 (Brachylaimidae: Trematoda) in land snails from Uganda, East Africa. *J Parasitol*, 49: (5, Section 2, 38) 26

Cribb, T.H., Barton, D.P. (1991): *Zeylanurotrema spearei* sp. n. (Digenea: Brachylaimidae) from the cane toad, *Bufo marinus* in Australia. *Zool Scr*, 20: 207 – 213. DOI: 10.1111/j.1463-6409.1991.tb00286.x

Deiana, S., Arru, E. (1963): Il ciclo biologic di *Postharmostomum commutatum* (Dies, 1858) ricostruito sperimentalmentein Sardegna. *Riv. Parassitol*., 24: 163 – 177

Deising, K.M. (1858): Revision der Myhelminthen. Abteilung: Trematoden. *S B Akad. Wiss. Wien,* 32: 307 – 390

Enabulele, E.E., Ozemoka, H.J., Awhariroma, A.O., Aisien M.S.O. (2013): Parasitic infections of the African dwarf crocodile (*Osteolaemus tetraspis*) and the ornate Nile monitor (*Varanus ornatus*) from Nigeria. *Acta Parasitol*, 58(2): 191 – 197. DOI: 10.2478/s11686-013-0131-0

Gibson, D.I., Bray, R.A. (1994): The evolutionary expression and host-parasite relationship of the Digenea. *Int J Parasitol*, 24(8): 1213 – 1226. DOI: 10.1016/0020-7519(94)90192-9

Gonzalez-Moreno, O., Gracenea, M. (2006): Life cycle and description of a new species of brachylaimid (Tremaoda: Digenea) in Spain. *J Parasitol*, 92(6): 1305 – 1312. DOI: 10.1645/GE-821R.1

Gracenea, M., Gonzalez-Moreno, O. (2002): Life cycle of *Brachylaima mascomai* n. sp. (Trematoda: Brachylaimidae), a parasite of rats in the Llobregat Delta (Spain). *J Parasitol*, 88(1): 124 – 133. DOI: 10.1645/0022-3395(2002)088[0124:LCOBMN]2.0.CO;2

Herman, S.M., Bacha, W.J. (1978): Growth, development and site location of the trematode *Himasthla quissetensis* in the chick following cloacal drop infections with cercariae. *J Parasitol*, 64(5): 827 – 830

Hodasi, J.K.M. (1967): Digenetic trematodes from domestic fowl in Ghana. *J Helminthol*, 41(4): 329 – 336. DOI: 10.1017/S0022149X0002188X

Hodasi, J.K.M. (1976): The helminth parasites of the helmet guinea fowl (*Numia meleagris galeata* Pallas) in Ghana. *Bull Anim Health Prod Afr,* 24: 81 – 87

Lewin, J. (1992): Parasites of the sand lizard (*Lacerta agilis* L.) in Poland. *Acta Parasitol*, 37: 19 – 24

Lewis, J.W. (1969): Studies on the life history of *Brachylaimus oesophagei* Shaldybin, 1953 (Digenea: Brachylaemidae). *J. Helminthol*., 43: 79 – 98. DOI: 10.1017/s0022149x00003941

Mas-Coma, S., Montoliu, I. (1986): The life cycle of *Brachylaima ruminae* n. sp. (Trematoda: Brachylaimidae), a parasite of rodents. *Z Parasitenkd*, 72: 739 – 753. DOI: 10.1007/BF00925095

Mas-Coma, S., Montoliu, I. (1987): The life cycle of *Dollfusinus frontalis,* a brachylaimid trematode of small mammals (insectivora and rodentia). *Int J Parasitol*, 17(6): 1063 – 1079. DOI: 10.1016/0020-7519(87)90159-7

Mas-Coma, S., Montoliu, I. (1995): Life cycle of *Pseudoleucochloridium pericardicum* n. sp. (Trematoda: Brachylaimidae), a parasite of shrews (Insectivora: Soricidae) in the Oriental Pyrenees. *Res. Rev. Parasitol*., 55: 155 – 171

Okon, E.D., Enyenihi, N.U. (1980): A study of parasites of local fowls in Oron, Cross River State, Nigeria. *Niger. J. Parasitol*., 1: 82 – 86

Pereira, C., Cuocolo, R. (1939): A propósito de *Postharmostomum commutatum*. *Arq. Inst. Biol*. (*Sao Polo*), 10: 73 – 88

Robinson, E.J. (1949): The life history of *Postharmostomum helicis* (Leidy, 1847) n. comb. (Trematoda: Brachylaemidae). *J Parasitol*, 35: 531 – 533

Segade, P., Crespo, C., Garcia, N., Garcia-Estevez, J.M., Arias, C., Iglesias, R. (2011): *Brachylaima aspersae* n. sp. (Digenea: Brachylaimidae) infecting farmed snails in NW Spain: morphology, life cycle, pathology and implications for heliciculture. *Vet Parasitol*, 175: 273 – 286. DOI: 10.1016/j.vetpar.2010.10.026

Sirgel, W.F., Artigas, P., Bargues, M.D., Mas-Coma, S. (2012): Life cycle of *Renylaima capensis*, a brachylaimid trematode of shrews and slugs in South Africa: two-host and three-host transmission modalities suggested by epizootiology and DNA sequencing. *Parasit Vectors*, 5: 169. DOI: 10.1186/1756-3305-5-169

Thiengo, S., Amato, S.B. (1995): *Phyllocaulis variegatus* (Mollusca: Veronicellidae), a new intermediate host for Brachylaima sp. (Digenea: Brachylaimatidae). *Mem. Inst. Oswaldo Cruz*, 90(5): 621 – 622. DOI: 10.1590/S0074-02761995000500015

Valadão, M.C., Silva, B.C.M., Lopez-Hernández, D., Araújo, J.V., Locke, S.A., Pinto, H.A. (2018): A molecular study of the caecal fluke of poultry *Postharmostomum commutatum* (=*P. gallinum*) (Trematoda: Brachylaimidae). *Parasitol Res*, 117: 3927 – 3934. DOI: 10.1007/s00436-018-6102-5

Valente, R., Inés Diaz, J., Salomóna, O.D., Navone, G.T. (2016): The role of *Phyllocaulis variegatus* (Mollusca: Veronicellidae) in the transmission of digenean parasites. *Rev Mex Biodivers*, 87: 255 – 257. DOI: 10.1016/j.rmb.2016.01.017

Wagener, G.R. (1852): Enthelminthiea, n. III. *Archiv. Anat. Physiol*., 555 – 567

Witenberg, G. (1923): Harmostominae in Skryabin K.I. [Trematodes of domestic birds]. *Arb. Inst. Exp. Vet. Moscow*, 2: 193 – 256