

## Observations on the trematode parasite, *Microphallus* sp. using the prawn, *Macrobrachium vollenhovenii* as second intermediate host in the Niger Delta of Nigeria

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### Introduction

Adults of the trematode genus, *Microphallus* are intestinal parasites of birds and mammals while the metacercariae occur in decapod crustaceans (crabs, prawns and shrimps). In the microphallid life cycles described so far, prosobranch snails have been shown to be the first intermediate host, in which the developing sporocysts produce xiphidiocercariae, which encyst in decapod crustaceans. An exception to this rule is *Microphallus piriformes*, which has no decapod second intermediate host. The metacercariae are found only in the rough periwinkle, *Littorina saxatilis* (McCarthy *et al* 2000). Studies have shown that eating undercooked or raw sea food infected with *Microphallus* spp. can lead to gastrointestinal symptoms such as nausea, abdominal pain, and in severe cases, more significant infections in immunocompromised individuals (Africa *et al* 1940; Marcogilese 2004; EFSA 2024). *Microphallus* infection in humans is therefore of public health importance as a food borne infection (Chai and Jung 2019).

Only one *Microphallus* species, *M. bilobatus* has been reported from Africa by Deblock *et al* (2004) in *Charadrius marginatus* (Aves: Charadrii) in Namibia. In our studies of larval trematodes occurring in shell fishes collected in the Niger Delta Nigeria, the occurrence of various larval trematodes including xiphidiocercariae from the brackish water periwinkle, *Trypanotonus*

### Abstract

Crustaceans are known intermediate hosts of parasitic infections and also as a source of food borne zoonosis. In recognition of these roles, we undertook a study on the prawn, *Macrobrachium vollenhovenii* from water systems in the Niger Delta (Ovia River and Warri River) to determine its role as intermediate host of parasitic infections. The prawns were examined for infection from January to December, 2010 and the parasitic form isolated was the metacercaria of a *Microphallus* sp. Prevalence of the metacercaria in the specimens from Ovia River was 52.3% and 35.4% in those from Warri River. The infection intensity in both river systems was low, 1.2 in Ovia River and 3.0 in specimens from Warri River. The cysts measured  $443 \pm 50$  ( $378-510$ )  $\mu\text{m} \times 410 \pm 60.7$  ( $336-510$ )  $\mu\text{m}$ . The excysted parasite measured  $940 \pm 117.6 \times 471 \pm 44 \mu\text{m}$ , larger than *Microphallus bilobatus*, the only known species from Africa, which measures  $500 \times 210 \mu\text{m}$  in size. The parasite under study also differed from *M. bilobatus* by the possession of lateral folds. The uterus is post-acetabular and located in the intertesticular space bordered posteriorly by the excretory bladder. Infection experiments are needed to determine the identity and definitive host(s) of this parasite.

*fuscatus* had been reported (Awharitoma and Aisien 2011). The xiphidiocercariae were suspected to be the larval stages of *Microphallus* spp. In a follow-up study in which the prawn *Macrobrachium vollenhovenii* from the same region were examined, the metacercaria of a *Microphallus* sp. which uses this prawn as second intermediate hosts were recovered. In this paper, preliminary data on the parasite prevalence and aspects of the morphology of the metacercaria encountered are presented.

### Materials and methods

Freshly-caught prawns (*Macrobrachium vollenhovenii*) from Warri River (Delta State, Nigeria) were purchased at McIver Market, Warri, Delta State, and those from Ovia River (Edo State) were purchased from fishermen by the river side at Mile 18 Market along Lagos-Benin Road from January to December, 2010 and examined in the laboratory for parasites. The specimens were transported in a cooler containing ice packs to the laboratory. In the laboratory, prawns for examination were transferred to a kitchen sieve placed on top of a large bowl. Thereafter, the carapace was separated from the internal tissues of the prawns; the tissues were teased out and washed with normal saline (0.85% NaCl solution) into the bowl. By this process, the parasites filtered into the bowl while the host tissues were retained

on the sieve. The filtrate was transferred into conical wine glasses and more saline added till the glass was full. The glass was left to stand for about 45 minutes to allow for sedimentation of the parasites. The supernatant was discarded and fresh saline added to the sediment. The process was repeated 3-4 times until the supernatant became clear. Finally, the supernatant was discarded while the sediment was diluted in saline and transferred to a Petri dish. By gentle swirling, the parasites were concentrated at the middle of the Petri dishes followed by examination under a dissecting microscope. The encysted and excysted parasites were sorted and separated.

Intact and excysted metacercariae in the sediment were aspirated with a Pasteur pipette from the medium. The intact cysts were preserved in 3% formal-saline while the excysted metacercariae were flattened under mild cover slip pressure and fixed with 3% formal-saline. Alternatively, some excysted metacercariae were heat-killed on a microscope slide without pressure (Cribb and Bray 2010); these were first preserved in 70%

ethanol and after 24h transferred to 5% formal-saline. Before staining, the parasites were washed free of the fixative and stained in a dilute solution of acetocarmine, dehydrated in ethanol series and permanently mounted in Canada balsam. Description is based on 20 flattened specimens. Photomicrographs of the specimens from which line drawings were made were taken with a digital camera (Coolpix Digital Camera, 3.34 megapixels) attached to a Binocular microscope. Measurements were in micrometres ( $\mu\text{m}$ ).

## Results

A total of 243 *M. vollenhovenii* were examined from Warri River of which 86 (35.4%) were infected with metacercariae of a *Microphallus* sp. Mean intensity of infection was 3.0 (Table 1). The parasites recovered consisted of encysted and excysted juveniles with the latter predominant. From Ovia River, 262 prawns were examined and 137 (52.3%) were infected, with a mean intensity of 1.2 (Table 1).

**Table 1:** Prevalence of *Microphallus* sp. metacercariae in *Macrobrachium vollenhovenii* from Ovia and Warri Rivers, Nigeria

Month	Ovia River			Warri River		
	No Examined	Prevalence (%)	Mean intensity of infection	No Examined	Prevalence (%)	Mean intensity of infection
January	20	-	-	18	-	-
February	28	-	-	30	-	-
March	28	-	-	20	3(15.0)	1.0
April	24	8(33.3)	2.0	22	2(9.1)	5.5
May	18	16(88.9)	1.0	10	3(30.0)	2.7
June	20	20(100)	1.0	24	14(58.3)	1.1
July	15	13(86.7)	1.0	34	1(2.9)	14
August	26	18(69.2)	1.6	20	14(70.0)	1.0
September	18	12(66.7)	1.3	15	10(66.7)	1.1
October	20	13(65.0)	1.3	16	12(75.0)	1.3
November	20	14(70.0)	1.3	18	13(72.0)	1.1
December	25	23(92.0)	1.0	16	14(87.5)	1.0
Total	262	137(52.3)	1.3	243	86(35.4)	3.0

### (i). Metacercaria from *M. vollenhovenii*

Metacercariae recovered from the prawns (Figure 1) were ovoid, measuring  $443\pm 50.6\mu\text{m}$  ( $376\text{-}510\mu\text{m}$ )  $\times$   $410\pm 60.7\mu\text{m}$  ( $336\text{-}510\mu\text{m}$ ) enclosed in a single fragile cyst wall. Features clearly visible in the encysted parasite included the oral sucker, ventral sucker, intestinal caeca and the vitelline follicles.

Excysted parasites (Figure 2A-B). The parasites were released by the teasing process. Some metacercariae trapped in host tissues were observed digesting the tissues by proteolytic action. The excysted metacercariae had the characteristic features of the Microphallidae with full gonadal development. Body pyriform was  $940\pm 117.6\mu\text{m}$  ( $725\text{-}1168\mu\text{m}$ ) long and  $471\pm 44.0\mu\text{m}$  ( $416\text{-}577\mu\text{m}$ ) wide at testicular level. Tegumental spines extend from anterior extremity to level of ovary. Oral sucker terminal, wider than long, measuring  $84\pm 4.9\mu\text{m}$  ( $76\text{-}91\mu\text{m}$ )  $\times$   $94\pm 7.2\mu\text{m}$  ( $80\text{-}102\mu\text{m}$ ), prepharynx short,

$12\pm 2.5\mu\text{m}$  ( $7\text{-}15\mu\text{m}$ ) long, pharynx muscular  $40\pm 4.7\mu\text{m}$  ( $29\text{-}44\mu\text{m}$ ) long and  $29\pm 3.2\mu\text{m}$  ( $22\text{-}33\mu\text{m}$ ) wide.

Oesophagus  $320\pm 54.0\mu\text{m}$  ( $201\text{-}389\mu\text{m}$ ) long, bifurcating into two intestinal caeca, shorter than oesophagus. Acetabulum slightly larger than oral sucker,  $93\pm 9.1\mu\text{m}$  ( $80\text{-}113\mu\text{m}$ )  $\times$   $91\pm 6.9\mu\text{m}$  ( $80\text{-}102\mu\text{m}$ ).

Testes were symmetrical, measuring  $69\pm 6.6\mu\text{m}$  ( $55\text{-}87\mu\text{m}$ )  $\times$   $117\pm 8.2\mu\text{m}$  ( $91\text{-}127\mu\text{m}$ ), post-acetabulum, among vitelline follicles. Efferent ducts connecting testes to seminal vesicle not visible. Genital atrium postero-sinistral to sperm-filled seminal vesicle,  $167\pm 30.7\mu\text{m}$  ( $109\text{-}222\mu\text{m}$ )  $\times$   $105\pm 12.7\mu\text{m}$  ( $80\text{-}127\mu\text{m}$ ). Pars prostatica not well developed. Male copulatory papilla not extruded in most specimens, but those measured ranged  $40\text{-}66\mu\text{m}$  in length and  $55$  to  $80\mu\text{m}$  wide, ovary smooth, dextral to acetabulum, immediately anterior to right testes,  $134\pm 20.6\mu\text{m}$  ( $91\text{-}164\mu\text{m}$ )  $\times$   $99\pm 17.8\mu\text{m}$  ( $69\text{-}131\mu\text{m}$ ). Laurer's canal, ootype and

Mehlis's gland are in intertesticular region. Uterus is confined to hindbody, post-acetabulum in the intertesticular space (Figure 3; Figure 4 A-B). Eggs were undeveloped, 15 by 11µm. Vitelline follicles were arranged in two clusters, left with 7 follicles and right, 8-9.

Principal vitelline duct emerged from vitelline reservoir and united with oviduct, excretory vesicle V-shaped. In addition to the features observed in the flattened excysted metacercaria, the heat-killed specimens revealed the presence of lateral folds in this parasite (Figure 3). Mean body length of the heat-killed specimens was 781.16±38.3µm (724.68-845.46µm; n=14). A comparison of the morphometric parameters of the metacercariae from *Macrobrachium vollenhovenii* and the adult of *M. bilobatus* is presented in Table 2.

**Table 2.** Comparison of the morphometric parameters of the metacercariae from *M. vollenhovenii* and adult of *M. bilobatus* (units in µm)

Parameters	<i>M. bilobatus</i> (adult)	<i>M. vollenhovenii</i> metacercaria
Body length (L)	500	940±117.6
Width (W)	210	471±44.0
Oral sucker (L)	45-45	76-91
(W)	49-51	80-102
Prepharynx (L)	19-24	7-15
Pharynx (L)	23-26	29-44
(W)	26-32	22-33
Oesophagus (L)	160-160	201-389
Seminal vesicle (L)	46-65	109-222
(W)	47-40	80-127
Testis (L)	30-37	90-127
(W)	57-65	55-87
Ovary (L)	48-62	90-164
(W)	27-32	69-131
Ventral sucker (L)	36-49	80-113
(W)	--	80-102
Eggs (L)	16-19	15-15
(W)	--	11-11
Male papilla (L)	-	40-66
(W)	-	55-80
	Authority:	This study
	Deblock <i>et</i>	
	<i>al</i> 2004	

**Discussion**

On the African continent, *Microphallus bilobatus* remains the only species reported from *Charadrius marginatus*, an avian host in Namibia (Deblock *et al* 2004). Therefore, the finding of the metacercaria of a *Microphallus* sp. in *M. vollenhovenii* from the Niger Delta of Nigeria is of immense interest as it widens the geographical range of parasites from this genus. The question that needs to be answered is whether the metacercaria in question are those of *M. bilobatus* or that of a different species.

The first indication that the metacercaria from *M. vollenhovenii* might represent a different species is in the size range differences between it and *M. bilobatus*. While

the flattened specimens of the metacercaria from *M. vollenhovenii* on the average measured 940 × 471µm, adults of *M. bilobatus* measured 500 × 210µm. Even the heat-killed specimens, which were smaller than the flattened ones (781.16±38.3µm; 724.68-845.46µm) were still larger than adult *M. bilobatus*. Furthermore, *M. bilobatus* has an obviously longer prepharynx (19-24µm in length) when compared with the 7-15µm in the metacercaria from *M. vollenhovenii* (Table 2). While the reproductive organ in both parasites conform to the basic pattern described for microphallids, the genital atrium in *M. bilobatus* appeared more elaborate than that in the parasites from the prawn. We believe that greater details about the reproductive organs in the parasite under study will be revealed when adults are raised in experimental host(s) or recovered from natural definitive host(s).

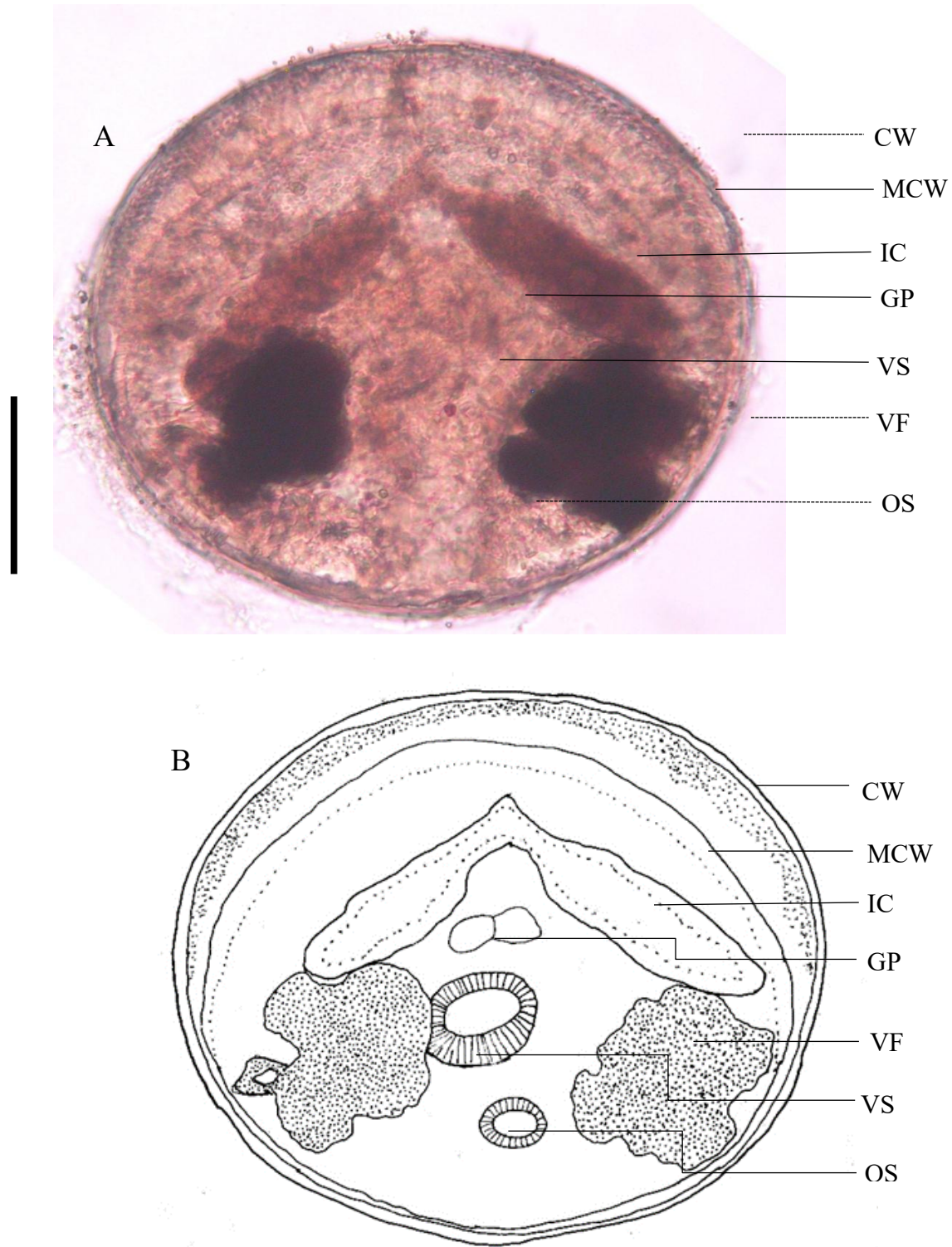
The presence of lateral folds in the Nigerian parasite further differentiates it from *M. bilobatus* and other *Microphallus* species. The uterus of the *Microphallus* sp. from the prawn is spherical to ovoid in shape (see Figs. 3. 4A and 4B) and is located postacetabular in the intertesticular space bounded by the V- shaped excretory vesicle posteriorly. In contrast, the uterus in *M. bilobatus* is more extensive; it is bilobed, extending beyond the testes laterally with projections extending almost to the posterior extremity of the parasite.

As have been reported in other microphallids (Sogandares-Berna 1965; Overstreet *et al* 1992), we observed *in vitro* egg production by the metacercaria from the Nigerian decapod. The excysted metacercaria from *M. vollenhovenii* produced eggs measuring 15 × 11µm (Figures 4 A and 4B). These eggs were slightly smaller than those reported for *M. bilobatus*, 16µm in length (Table 2). We cannot confirm if the few eggs observed in the uterus of the *Microphallus* sp. from *M. vollenhovenii* is attributable to its life stage (metacercaria) since we have not yet encountered adults of this parasite. However, only few eggs were also illustrated in the adult of *M. bilobatus* (Deblock *et al* 2004) and in adult of *M. sabaensis* collected from experimental definitive host (Diaz *et al* 2004). Adults of other *Microphallus* spp. have been shown to produce numerous eggs as in *M. limuli* with over 120 eggs *in utero* (Stunkard 1951), much more in *M. breviatus* (Deblock and Maillard 1975) and *M. koreana* (Guk *et al* 2008).

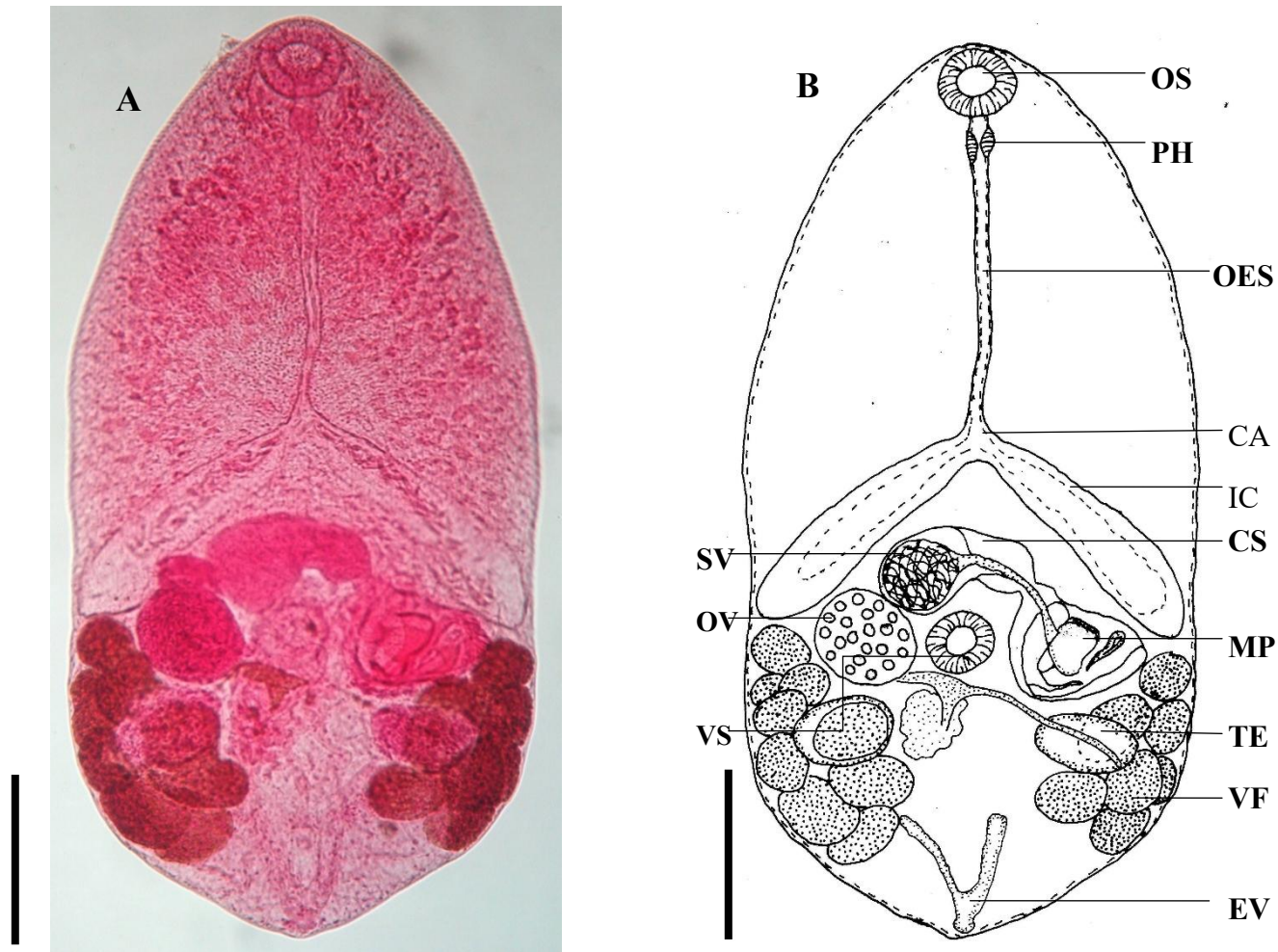
In a previous paper, we reported the occurrence of xiphidocercariae in *Tympanotonus fuscatus*, the brackish water periwinkle (Awharitoma and Aisien, 2011), which we presumed then to be the xiphidocercariae of *Microphallus* spp. Tentatively, the life cycle of this *Microphallus* sp. can be assumed to proceed as follows: it probably uses the periwinkle *T. fuscatus* as its first intermediate host; on emergence, the xiphidocercariae from this host encyst in *M. vollenhovenii*, which when consumed by the yet to be identified definitive host(s), transforms to the adult parasite in the small intestine of the host. Infection experiments are needed to establish the definitive host(s) of this parasite. It has however been established that most microphallids mature either in birds or mammals.

Lastly, *Microphallus* infection is considered a food-borne zoonosis, when humans, especially immunocompromised individuals consume improperly

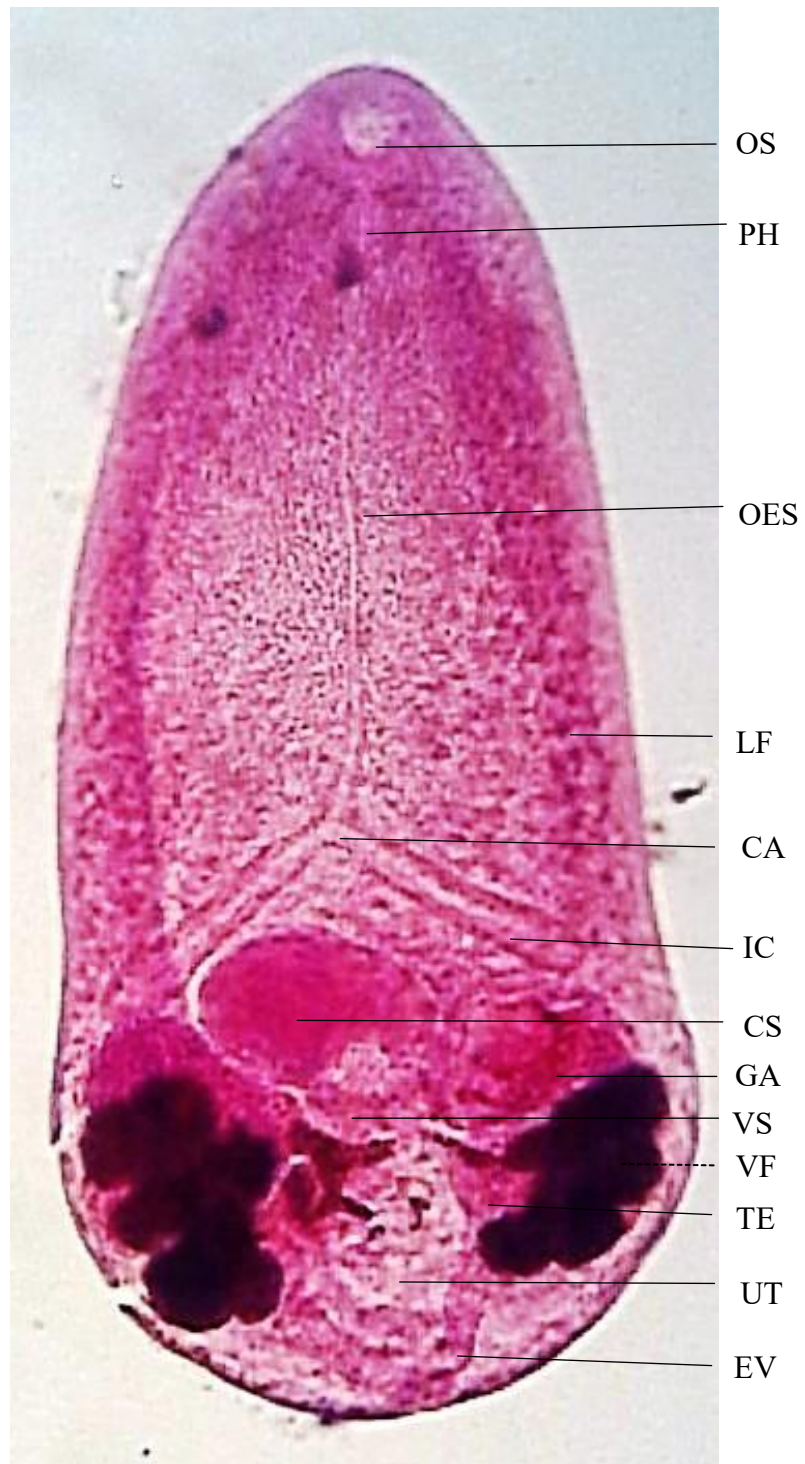
cooked sea food (Africa *et al* 1940; Marcogilese 2004; Chai and Jung 2019). It is therefore important to avoid the consumption of raw or improperly cooked sea food.



**Figure 1 (A-B).** Metacercaria of a *Microphallus* sp. recovered from *M. vollehovenii* from the Niger Delta of Nigeria. Abbreviations: CW, Cyst wall; GP, Genital primordium IC, Intestinal caecum; MCW, Metacercaria cyst wall; OS, Oral sucker; VF, Vitelline follicles; VS, Ventral sucker. Scale bar = 150 $\mu$ m.

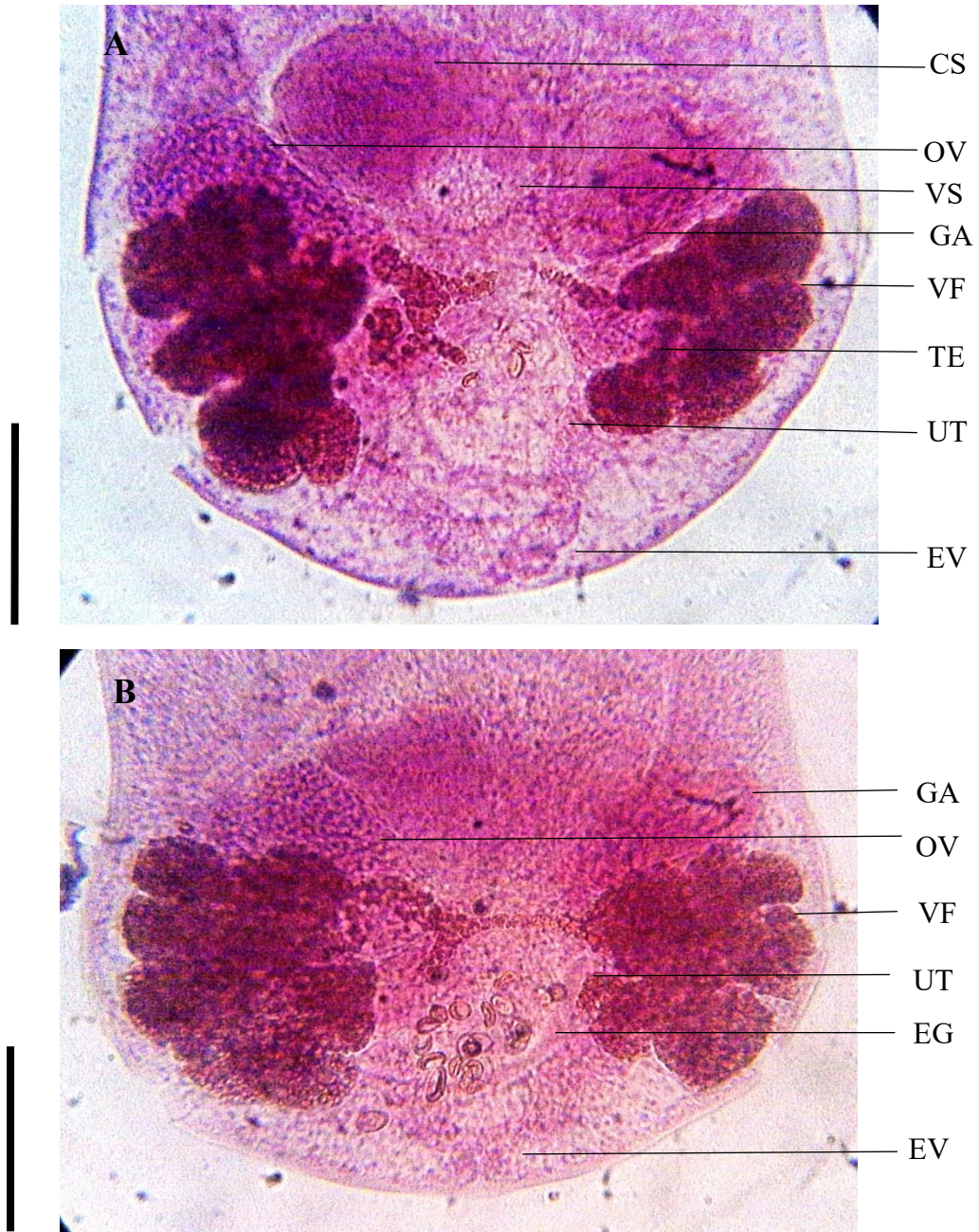


**Figure 2A and 2B.** Excysted metacercaria of *Microphallus* sp. recovered from *M. vollehovenii*. Abbreviations: CA, Caecal arch; CS, Cirrus sac; EV, Excretory vesicle; IC, Intestinal caeca; MP, Male papilla; OES, Oesophagus; OS, Oral sucker; OV, Ovary; PH, Pharynx; SV, Seminal vesicle; TE, Testis; F, Vitelline follicles; VS, Ventral sucker. Scale bar: 150µm



0.15

**Figure 3.** Heat-killed excysted metacercaria of *Microphallus* sp. recovered from *M. vollenhovenii*. **Abbreviations:** CA, Caecal arch; CS, Cirrus sac; EV, Excretory vesicle; IC, Intestinal caeca; MP, Male papilla; OES, Oesophagus; OS, Oral sucker; OV, Ovary; PH, Pharynx; SV, Seminal vesicle; TE, Testis; VF, Vitelline follicles; VS, Ventral ucker. Scale bar: 150µm



**Figure 4 A-B.** Posterior end of the *Microphallus* sp. recovered from *M. vollehovenii* from Warri River. A, posterior end showing uterus forming eggs; B, posterior end showing uterus with more eggs. Abbreviations: CS, Cirrus sac; EV, Excretory vesicle; EG, Egg; GA, Genital atrium; OV, Ovary; UT, Uterus; VF, Vitelline follicles. VS, Ventral sucker. Scale bars: 100 µm

### Conclusion

*Microphallus* sp. from *M. vollehovenii* which uses this prawn as a second intermediate host is reported. This parasite differs in several respect from *M. bilobatus*, the only species so far reported in Africa. Infection experiments are needed to obtain adults of the parasite and to establish the definitive host of this parasite using *M. vollehovenii* as second intermediate host in the Niger Delta of Nigeria.

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### Conflict of interest

Authors declare no conflict of interest.

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