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Examination of Prevalence and Sex-Related Variation in Gill and Gastrointestinal Tract Parasite Infestation of *Clarias* Species in 'Tella' Area of River Taraba, Nigeria

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Abstract

The presence of certain parasites in *Clarias* species consumed in Nigeria has continued to pose some challenges to fish production in many parts of Nigeria. The present study was aimed at assessing the sex-related distribution of parasites in gills and gastrointestinal tracks of *Clarias* species consumed in Nigeria. Two hundred and sixty (260) *Clarias* species obtained from River Taraba, Taraba state was examined for the presence of gill and digestive tract parasites. One hundred and forty (140) of them were *Clarias gariepinus* and one hundred and twenty (120) were *Clarias anguillaris*. Analysis was carried out using standard parasitological techniques. The gills of the fish were removed and observed under a stereo microscope for parasites. The intestines were cut opened and viewed under the stereo microscope section-by-section. Parasites seen were recovered, fixed, and preserved in 5% formalin before they were later processed. Two gill parasites, *Macrogyrodactylus* sp. (a monogenean) and *Ergasilus sarsi* (a copepod) were recovered. The relationship between sex and gill parasite infestation was significant ($p<0.05$). Three parasite species namely *Tetracampos ciliotheca* and *Monobothroides woodland* (cestodes) and *Procamallanus laeviconchus* (nematode) were recovered from the digestive tract of both *Clarias* species in the river. Relationships between the sex of fish and digestive tract infestation were also significant ($p<0.05$).

Keywords: Gill parasites, *Macrogyrodactylus, Ergasilus, Tetracampos ciliotheca, Procamallanus laeviconchus*.

1. Introduction

Clarias gariepinus is generally considered to be one of the most important tropical fish species for aquaculture in West Africa [1, 2]. Since the last three decades, *C. gariepinus* has been considered to hold great promise for

aquaculture in Africa; it has a wide geographical spread, high growth rate, resistance to handling and stress, and it is well appreciated in a wide number of African countries both for food and aquaculture [3].

Most fish in the wild are infested by parasites. These are sometimes obvious but more often are difficult to detect other than by special techniques and usually appear to have little effect on the host fish [4]. The emerging need to culture fish for protein consumption for the teeming rapidly growing populations in developing countries has made it necessary to intensify studies on the parasite fauna of African freshwater fishes [2]. Knowledge of parasites that infect/infest different species of fish is very crucial for the management, prevention and control of the parasites. With the current interest in aquaculture, information on the types of parasites that infect *Clarias* species will be desirable since parasites that infect wild species can also infect the cultured ones; also, parasites of fish form an integral part of their biology and reflect the life habits of the fish [4]. Development of aquaculture during the last few decades has resulted in much greater attention being paid to problems posed by parasites and their importance for the fishery, leading to constraints in the productivity of aquaculture [5]. Aside from direct losses caused by mortality, parasites may have a considerable impact on the growth and behaviour of fish, their resistance to other stressing factors and their susceptibility to predation; their presence may also reduce the marketability of fish [6, 7]. Knowledge of parasites of fish is highly needed because such parasites may rank among the most sensitive of bioindicators [8].

2. Materials and Methods

2.1. Study Area

River Taraba is located on latitude 8° 34'N and longitude 10°15'E and Tella (fish collection area) on latitude 8° 24'N and10°32'E. A 100 square kilometre around River Taraba has an approximate population of 14,326 people and an average elevation of 178 meters above sea level. The river has its source in the Mambila Plateau of Sardauna Local Government Area of Taraba state. The river traverses the three districts of Gashaka, Bukundi and Gassol, in the Gashaka, Bali, and Gassol Local Government Areas. The Northern border of Gassol is the River Benue and River Taraba; the latter flows north through the area to its confluence with the river Benue (Fig. 1).

Like most parts of northern Nigeria, Taraba State has wet and dry seasons. The wet season lasts, on average, from April to October. Mean annual rainfall varies between 1058 mm in the north around Jalingo and Zing, to over 1300 mm in the South around Serti and Takum. The wettest months are August and September. (www.getamap.net/map/nigeria). The dry season lasts from November to March. The driest months are December and January with relative humidity dropping to about 15%. The mean annual temperature around Jalingo is about 28 °C with maximum temperatures varying between 30 °C and 39.4 °C. The minimum temperatures range between 15 °C to 23 °C. The Mambila Plateau has climatic characteristics typical of a temperate climate. (www.getamap.net/map/nigeria).

Rainfall distribution and topography are the most important factors influencing the pattern of vegetation in Taraba State. The vegetation may be classified into three broad types: the Northern Guinea, the Southern Guinea and the Mountain Grassland and forest vegetation (www.getamap.net/map/nigeria). The fish collection point falls within the Northern Guinea savannah.

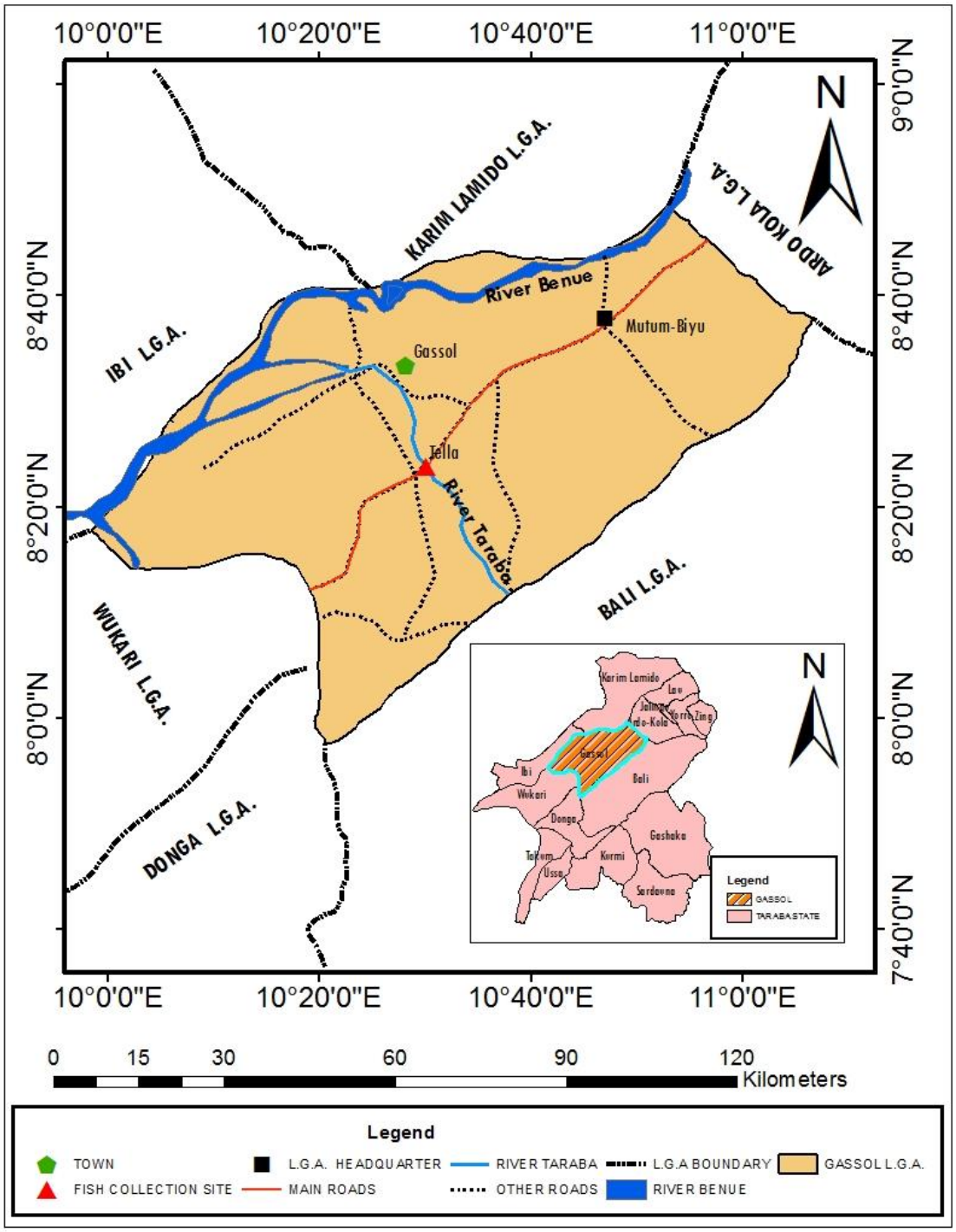

Fig-1. Map of Gassol Local Government Area showing site of fish collection (▲)
Source: Modified from the administrative map of Taraba State.

2.2. Collection of Fish

Clarias species were purchased alive from the artisanal fisher folk at a fish-landing site along River Taraba at Tella between January 2013 and October 2013. The fish were then placed in a water-filled plastic container and taken to the biology laboratory of the Taraba State University for parasitic examination. A total of 260 fish were collected and examined.

2.3. Identification of Clarias Species

Fish identification started in the field. Black spots/patches on the caudal fins and sometimes on the body were used to identify *C. anguillaris* and their absence, *C. gariepinus*. To confirm the identification made in the field, the gill rakers of the two species were examined; *C. anguillaris* has fewer and thicker gill rakers than *C. gariepinus.*

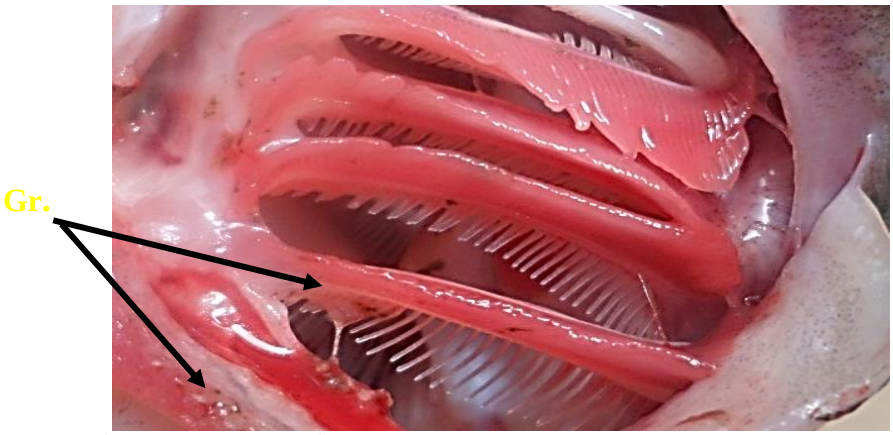

Plate-I. Gills of *C. anguillaris* showing fewer, shorter and thicker gill rakers (Gr; shown with arrows) than in *C. gariepinus.*

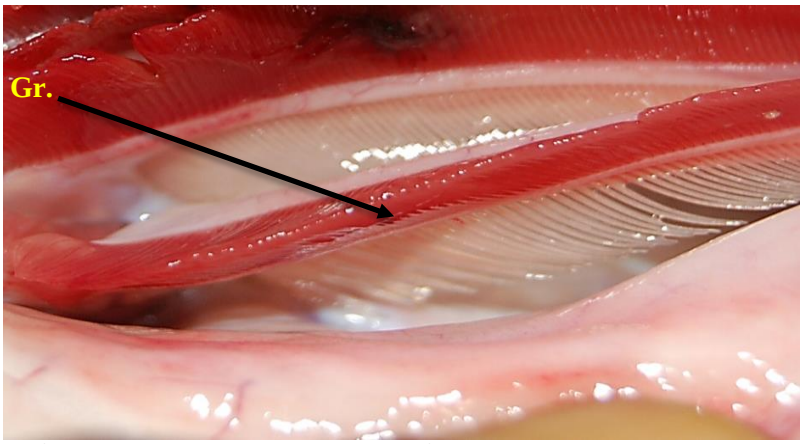

Plate II. Gills of *Clarias gariepinus* show more, longer, and finer gill rakers (Gr.; arrow) than in *Clarias anguillaris.*

2.4. Sex Determination

The sex of fish was determined by external examination of their genital apparatus; the males have a genital papilla while the females have none. The sexes were confirmed by internal examination after dissection to expose the paired testes in the males and paired ovaries in the females.

2.5. Collection and Preservation of Gill-Parasites

Gills were obtained by cutting them out at their upper and lower extremities. They were placed in a Petri dish containing distilled water and separated into individual gill arches using a pair of dissecting scissors and forceps. The gill filaments on the arches were examined in succession for parasites under a dissecting microscope. Infested gills, along with attached parasites were placed in a specimen bottle. The parasites were fixed by pouring hot water on them and then left for 1 to 2 min. Water from the bottle was then discarded, making sure no solids were discarded. Ten per cent (10%) of formalin was then added to the top of the bottle. The specimen bottle was then labelled with the fish's autopsy number, number of parasites and date of collection written in pencil on a piece of paper and inserted into the specimen bottle. The bottle was finally capped and kept in a safe cupboard in the laboratory.

2.6. Collection and Preservation of Digestive-Tract Parasites

The abdominal cavity of the fish was opened from the anus to just anterior to the area below the pectoral fins. The digestive system was cut around the anus and the oesophagus. All organs were extracted and placed in a Petri dish; the liver and spleen were discarded. The intestine was untangled using the fingers, the slit open longitudinally from the anus to the oesophagus, including the stomach with a pair of scissors. The gall bladder was opened as well. Large undigested food materials in the stomach were noted and discarded. The entire digestive system was examined section by section under a dissecting microscope. Parasites found were carefully removed and placed in a cavity block containing distilled water. The parasites that were firmly attached to the intestinal wall were cut out along with a small portion of the host tissue at the attachment site. The live parasites were allowed for about two minutes in distilled water and fixed by popping them into hot water (70-80 °C). Contents of the cavity block were then transferred using a pipette or a pair of forceps into a specimen bottle containing 5% formalin. A piece of paper labelled in pencil with the fish autopsy number, sex, and date examined was inserted into the specimen bottle containing the parasites.

2.7. Processing of Preserved Parasites

The preserved parasites recovered from the fish were taken to the Parasitology and Entomology Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where permanent slides were prepared.

2.7.1. Copepods

The parasites were extracted from the gills and transferred to a cavity block containing distilled water to wash off the preservative. The copepods were then placed on a slide and dabbled with filter paper to remove excess distilled water. A drop of Berlese's fluid was placed on the copepod and covered with a cover slip. Berlese's fluid acts both as a clearing agent and mounting.

2.7.2. Nematodes

Preserved nematodes were washed with distilled water to remove the preservative. The distilled water was then drained and replaced with glycerine to clear them. They were then mounted on a slide using glycerine-gelatine.

2.7.3. Monogeneans and Cestodes

The monogeneans found on the gills of *Clarias* species were preserved along with the gills as proposed. The infested gills along with the formalin were poured into a Petri dish and the formalin was drained using a plastic pipette. The gills were washed with distilled water to remove the preservative, and then examined under the dissecting microscope; the parasites in the debris were sucked up with a pipette and placed in a cavity block containing distilled water. The distilled water was drained using a pipette under a dissecting microscope to avoid missing the parasite. The cestodes on the other hand were picked from the specimen bottle using a disposable pipette and placed in a cavity block. They were washed with distilled water to remove the preservative. Distilled water was then added along with two drops of Mayer's acid haematoxylin to the parasite and left to stain overnight. The diluted stain was drained carefully using a pipette the next day and worms were rinsed with distilled water. A drop of 0.3% hydrochloric acid was added to distilled water in the cavity block to de-stain the parasite. Worms were then rinsed with distilled water; fresh distilled water was added to the worm in the cavity block then a drop of ammonia (0.03%) was added to stop the de-staining. Worms were rinsed with distilled water and dehydrated in a graded series of ethanol (30%, 50%, 70%, 90% and absolute) in succession for forty minutes each. The cavity block was kept covered during this process to prevent rehydration from the atmosphere. After about forty minutes, the absolute ethanol was replaced gradually with methyl salicylate. The dehydration time for the monogeneans in the graded series of alcohol was reduced to fifteen minutes each because of their small size. Individual parasites were transferred onto a glass slide and mounted in thinned Canada balsam.

2.8. Measurement and Identification of Parasites

A permanent slide was prepared for each of the processed parasites and was snapped under the microscope using a digital camera. The parasites were thereafter measured using a calibrated ocular micrometre and the measurements were recorded in micrometres. Parasites were identified following previous publications.

2.9. Data Analysis

The IBM SPSS version 20 statistical package was used for statistical analysis. Chi-square was used to test the significance of the relationships between sex and gill and digestive tract parasites of *Clarias* species at $p < 0.05$ confidence interval.

3. Results and Discussion

3.1. Overall Prevalence of Gill and Digestive Tract Parasites of Clarias Species

The overall prevalence of gill and digestive tract parasites of *Clarias gariepinus* and *Clarias anguillaris* are shown in Table 1. Out of the 140 *C. gariepinus* and 120 *C. anguillaris* examined; 23 (16.4%) and 23 (19.2%) respectively, were infested by gill parasites, and 52 (37.1%) *C. gariepinus* and 46 (38.3%) *C. anguillaris*, by intestinal parasites.

3.2. Gill Infestation about the Sex of Fish

Sex-related gill and digestive tract parasite infestations of *C. gariepinus* and *C. anguillaris* are shown in Table 1. Out of 71 male and 69 female *C. gariepinus* examined for gill parasites, 8(11.3%) and 15 (21.7%) respectively were infested. Chi-square showed that the relationship between sex and infestation of the gills of *C. gariepinus* was statistically significant ($p<0.05$). On the other hand, gill infestation about sex in *C. anguillaris* showed that 11(16.4%) of the 67 male fish and 12 (22.6%) of the 53 female fish examined were infested. Chi-square showed that the relationship between sex and gill infestation in *C. anguillaris* was significant ($p< 0.05$)

Table-1. Gill infestation in species and sex of *Clarias*

Clarias spp.	Sex	NE	NI	NP	MI ±SE	Prevalence (%)
C. gariepinus:	**M**	71	8	24	3.00 ± 0.55*	11.3
	F	69	15	40	2.67 ± 0.67*	21.7
C. anguillaris:	**M**	67	11	25	22.27 ± 0.45*	16.4
	F	53	12	34	2.83 ± 0.46	22.6

Results are means ±SE (n = 3). *Statistically significant ($p< 0.05$; one-way ANOVA followed by Duncan multiple range post hoc test) compared between sexes. M: male, F: female, NE: number examined, DT: digestive tract, NI: number infested, MI: mean intensity, NP: number of parasites, and SE: standard error of means. $\chi 2$ values are 0.11 and 1.04 for *C. gariepinus* and *C. anguillaris* respectively.

3.3. Digestive Tract Infestation According to Species and Sex of Clarias

Table 2 shows digestive tract infestation about sex of *C. gariepinus* and *C. anguillaris*. 30 (42.3%) of the 71 males examined and 22 (31.9%) of the 69 female fish examined were infested. The relationship between the sex of fish and digestive tract infestation in *C. gariepinus* was not significant ($P \leq 0.05$). In C. *anguillaris*, 26 (38.8%) of 67 males and 20 (37.7%) of 53 females were infested. The relationship between digestive tract infestation and sex of fish was not significant ($p<0.05$).

Table-2. Digestive tract infestation in species and sex of *Clarias* species

Clarias spp.	Sex	NE	NI	NP	MI ±SE	Prevalence (%)
C. gariepinus:	**M**	71	30	141	4.70 ± 0.45*	42.3
	F	69	22	86	3.91 ± 0.34*	31.9
C. anguillaris:	**M**	67	26	103	3.96 ± 0.56*	38.8
	F	53	20	73	3.65 ± 0.48*	37.7

Results are means ±SE (n = 3). *Statistically significant ($p< 0.05$; one-way ANOVA followed by Duncan multiple range post hoc test) compared between sexes. M: male, F: female, NE: number examined, DT: digestive tract, NI: number infested, MI: mean intensity, NP: number of parasites, and SE: standard error of means. $\chi 2$ values are 0.14 and 1.64 for *C. gariepinus* and *C. anguillaris* respectively.

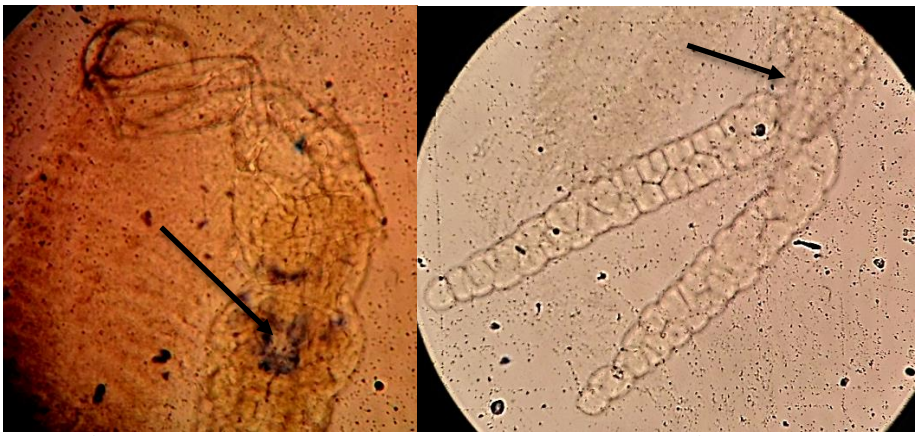

Plate-III. The anterior part of *Ergasilus sarsi* shows attachment organs (arrows) encircling a gill filament and the posterior part shows eggs; 400x.

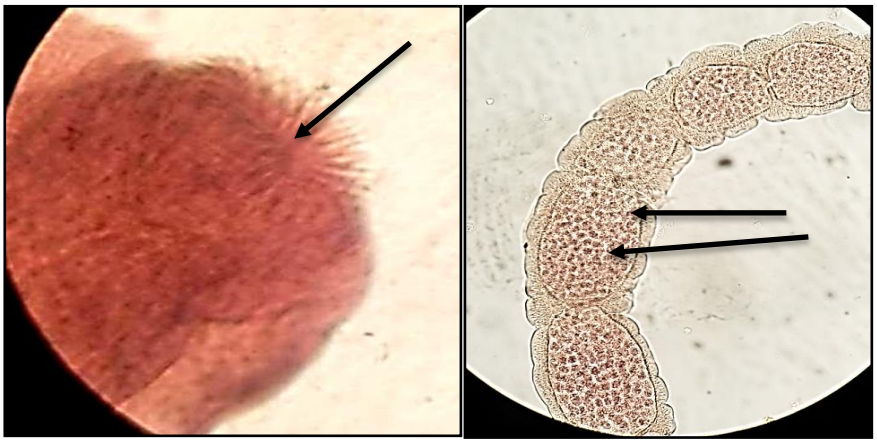

Plate IV. The scolex of *Tetracampos ciliotheca* shows attachment hooks (single arrow) and gravid proglottids (double arrows); 400x.

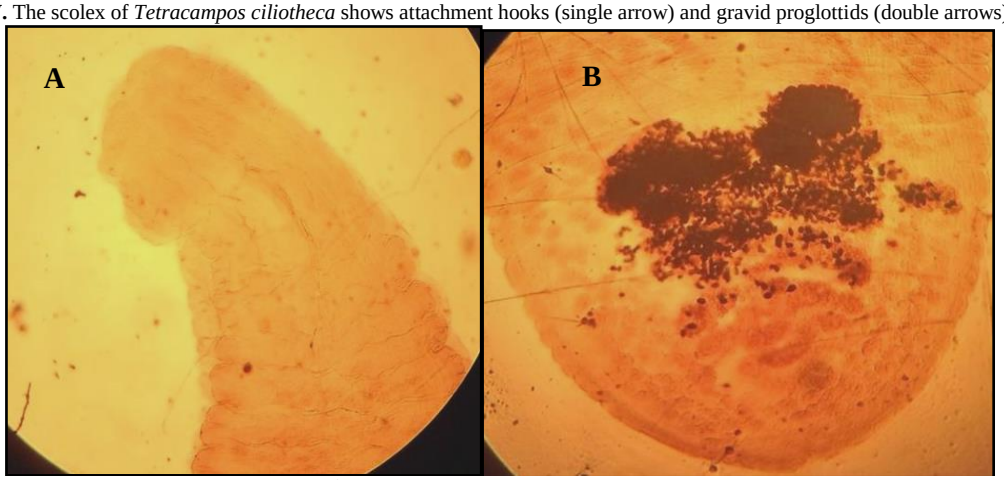

Plate V. The anterior (A) and posterior (B) portions of *Monobothrioides woodland;* 400x.

4. Discussion

The recovery of gill and digestive tract parasites from two *Clarias* species in River Taraba at Tella has established that these fish species are infested by at least 2-gill parasites and at least three intestinal parasites. It is not unlikely that more parasites than those recovered in this study infested *Clarias* species and other fish species in the river. The same groups of parasites recovered from the two species of Clarias have also been recovered from other parts of northern Nigeria by Shotter [9], Shotter [10]; Aken'Ova [11], Aken'Ova [12] in Zaria; Yakubu, *et al.* [13] in Jos; Oniye, *et al.* [14] in Zaria and Ayanda [15] in Ilorin. This implies that host identity plays a very important role in the establishment of the parasitic fauna characteristic of fish host species.

Macrogyrodactylus sp. and *Ergasilus sarsi* recovered from the gills of both *Clarias* species in River Taraba have also been recovered from Clarias species by several workers Shotter [9] and Aken'Ova [16] in Zaria, northern Nigeria. The descriptions of the parasites agree with those given by these workers and with the original descriptions by Prudhoe [17]; Paperna [18] and Thurston [19].

Two of the three helminth species recovered from the digestive tract of the two species of Clarias in River Taraba, i.e the cestode *Tetracampos ciliotheca* (syn. *Polyonchobothrium clariae*) and the nematode *Procamallanus laevionchus* have been found in *Clarias* species in Zaria by Shotter [10] and Oniye, *et al.* [14]. *Monobothrioides woodlandi*, the second of two cestodes from Clarias in River Taraba, does not appear to have been recovered from any species of *Clarias* in Zaria or any part of northern Nigeria for that matter, by any of the workers mentioned earlier. The morphology of this worm conforms to that described by Mackiewicz and Beverley-Burton [20], which they recovered and described from a related species, *Clarias mellandi* in Lake Chali, Bangweulu, in northern Zambia. The identification of this worm to species is not quite conclusive until more material and descriptions of other species of *Monobothrioide*s are available for comparison. A species of a monozoic cestode was recovered from the digestive tract of *Clarias gariepinus* by Oniye, *et al.* [14] and identified as *Monobothrium* but it was not described so it is not possible to say whether their species and *Monobothrioides* are the same.

The finding in this study that more of both species of *Clarias* were infested by intestinal parasites than by gill parasites is surprising because the gills are continuously exposed to any potential infective stages of parasites in water, which infest the gills directly. On the other hand, many parasites of the digestive tract such as some helminths generally use an intermediate host that is consumed by the definitive host to acquire infection. The chances of acquiring digestive tract parasites and gill parasites should therefore be similar since they may both result from feeding and respiration respectively, which are vital for the survival of the fish and are continuous. One reason that may explain the disparity in the prevalence of the two categories of parasites is the availability of infective stages of the gill parasites and infected intermediate hosts of the intestinal helminths recovered [11, 12, 21].

More female than male *C. gariepinus* and *C. anguillaris* being infested with gill parasites as observed in this study can be explained by the fact that female fish actively look for breeding places during the rainy season, increasing their chances of meeting infective stages of the parasites. The monogenean life cycle is direct, so it would have a greater chance of infesting the fish, unlike parasites that need an intermediate host. Paperna [21], stated that the majority of Ergasilus species infest different species of fish, except those of cichlids which are host specific. Though the infestation of the gills according to the sex of fish was not significant, more female fish than males were infested. Goselle, *et al.* [22], in contrast, obtained higher infestation in male than female fish. The reason they gave was that male fish were more active than females during the breeding period, and so are more prone to infestation by the parasite or its infective stage. The probable reason for the findings of this study may also concur with that of Goselle, *et al.* [22], since the difference between male and female fish was not significant.

Unlike infestation of the digestive tract, infestation of the gills showed that male fish had a higher prevalence. This might likely be because the fish collection period had entered the host's breeding period during which the male fish are more actively searching for mates. Anosike, *et al.* [23], in Plateau state and Oniye, *et al.* [14] in Zaria also observed higher infestation in the male fish. Ayanda [15], however, obtained equal infestation in males and females when he worked on the intestinal helminths of wild *Clarias gariepinus* in a reservoir at Ilorin. The reason Ayanda gave for equal prevalence in male and female fish was that all the fish used in the investigation were collected during the breeding period when both were actively swimming about and so had similar chances of being infested by intestinal parasites.

5. Conclusion

The study showed that parasitic distribution in the gills and digestive tracts of *Clarias* species investigated in this study was heavily influenced by sex and seasons of collection. More attention should therefore be directed at preventing the infestation of *Clarias* species which form the most preferred fish in Nigerian delicacies to stop any disease transmitted by these endo parasites.

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