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Pathological changes and description of *Procamallanus* (*Spirocamallanus*) *spiralis* Baylis from the freshwater fish, *Parachanna obscura* Gunther

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## ARTICLE INFO

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## ABSTRACT

**Objective:** To report the morphological details of the two species of *Procamallanus* with scanning electron microscopy.

**Methods:** To gain insights into the phenology of *Procamallanus* spp., scanning electron microscopy descriptions of male and female parasites along with attendant histopathological consequences to the hosts species were undertaken. The two species were obtained from the intestines of the freshwater fish, *Parachanna obscura*. Parasites were fixed in 2.5% glutaldehyde and postfixed in osmium tetraoxide, sputter-coated and examined with the electron microscope.

**Results:** Nominal parasitic species of *Procamallanus* could be distinguished with spiral thickenings in front of the buccal capsule. The male specimens of the parasite (n = 213) were recorded in a prevalence of 12.7% while the female specimens (n = 68) were recorded in a prevalence of 20.5%. An overall prevalence of 14.6% was obtained in the fish species. The two *Procamallanus* species differed from the described species of the genus *Procamallanus* (*Spiracamallanus*) with having the posterior end not ventrally bent, tail not short but elongate and straight bending slightly with no spikes, inner depression at the posterior region, excretory pore near the posterior end, wider cephalic region with an inner sieve-like compartment, longitudinal cephalic papillae, papillae of external circles distinctly larger and lateral cuticular extensions. These male and female species of *Procamallanus* exhibited novel morphometrics descriptions in the number of preanal and postanal papillae and different body and shape relationship. The histopathological changes seen in the host included hyperplasia of intestinal villi, mucosal hypercellularity of lamina propria and calcification of *Procamallanus* spp. in Nigeria describing the morphological details of the two species of *Procamallanus*.

1. Introduction

During a survey of parasites of the freshwater fish, *Parachanna* obscura (P. obscura), in tropical Africa, two species of the nematode genus *Procamallanus* were recovered from the intestines comprising genus *Procamallanus* Baylis 1923 and the subgenus *Spirocamallanus*. Chabaud[1] and Petter[2] considered *Spirocamallanus* as a separate genus while Rodrigues *et al.*[3] also supported the subgeneric status of *Spirocamallanus* within the genus *Procamallanus* to accommodate species with spiral thickenings in

the buccal capsule. Apparently, there are some confusions regarding the taxonomic status and validity of the group. Hoffman[4] reported that parasites of the genus *Procamallanus* (order Spirurida, family camallanidae) are intestinal parasites of freshwater fishes and occasionally of Amphibians. Yeh[5] and Bashirullah[6] argued that *Spirocamallanus* Olsen 1952 should be retained as the generic epithet, while Rigby and Rigby[7] elevated all subgenera into full generic rank.

Moravec, Thatcher[8] and Gibbons[9] recognized *Procamallanus* Baylis, 1923, *Spirocamallanus* Olsen, 1952, *Spirocamallanoides*[10], *Punctocamallanus*[11] and *Denticamallanus* as subgenera of *Procamallanus*. Among these subgenera, only representatives of *Procamallanus* and *Spirocamallanus* have been reported from freshwater fishes of Africa[12]. It is reported that the type specimen of *Procamallanus laeviconchus* (Wedl, 1862) is the only African species in which the life cycle has been studied and the first stage

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larvae identified[13].

Bilal and Abdullah[14] reported that approximately 34 species of *Procamallanus* have been reported from different fishes in the world and that 24 species have been recorded from South and Central America[10] with nine species recorded from Bangladesh[15].

In Nigeria, parasitic fauna of freshwater fishes has been studied by numerous researchers<sup>[16-21]</sup>. Thatcher<sup>[22]</sup> reported that parasitic nematodes cause considerable economic loss in commercial fisheries because their presence makes fish unmarketable. The aim of this paper is to report the morphological details of the two species of *Procamallanus* with scanning electron microscopy (SEM), which is the first description of the parasites from Nigeria freshwater bodies. The morphological details of freshwater parasites are therefore very important for comparison with the original description.

## 2. Materials and methods

## 2.1. Description of the study area

The Lekki lagoon is a part of an intricate system of waterways made up of lagoons and creeks that are found along the coast of South-western Nigeria from the Dahomey boarder to the Niger Delta stretching over a distance of about 200 km. It is fed by the rivers Oshun and Saga discharging into north-western parts of the lagoon. Lekki lagoon supports a major fishery in Nigeria (Figure 1)<sup>[23]</sup>.



Figure 1. The map of Lekki Lagoon, Lagos, Nigeria.

The lagoon is located in Lagos State, Nigeria and lies between longitudes  $4^{\circ}0'0''$  and  $4^{\circ}15'0''$  E and between latitudes  $6^{\circ}25'0''$  and  $6^{\circ}35'0''$  N. It has a surface area of about 247 km<sup>2</sup> with a maximum depth of 6.4 m. A greater part of the lagoon is shallow and less than 3.0 m deep.

Lekki lagoon experiences both dry and rainy seasons typically of the southern part of Nigeria. The vegetation around the lagoon is characterized by shrubs and *Raphia sudanica* and oil palms, *Elais guinensis*. Floating grasses occur on the periphery of the lagoon while coconut palms, *Cocos nucifera*, are widespread in the surrounding villages. The rich fish fauna of the lagoon includes *Heterotis niloticus*, *Gymnarchus niloticus*, *Clarias gariepinus*, *Malapterurus electricus*, *Synodontis clarias*, *Chrysichthys nigrodigitatus*, *P. obscura*, *Mormyrus rume*, *Calabaricus calamoichthys*, *Tilapia zillii*, *Tilapia galilaeus*, *Hemichromis fasciatus* and *Sarotherodon melanotheron*.

#### 2.2. Collection and examination of specimens

A total of 281 specimens (males, n = 213) and (females, n = 68) were collected from the guts of *P. obscura* and subjected to parasitologic investigation. The nematode parasites were observed by their wriggling movement in the intestines of the fish hosts. The parasitic organisms were carefully removed, counted and fixed in 2.5% glutaldehyde solution in 0.1 mol/L phosphate. Some of the recovered helminthes were also fixed in 70.0% alcohol and sent to Natural History Museum of London, United Kingdom, for their identification to species level.

# 2.3. SEM

For SEM investigation, after fixing in 2.5% glutaldehyde, the nematode were later rinsed in the same buffer solution 3 times at 15 min interval to remove debris and to hold pH (7.4%) steady during the fixation process. Post fixation was done in 1.0% osmium tetra oxide for 2 h. The samples were later dehydrated through a graded ethanol series. After dehydration, the parasites were dried to the critical point in liquid  $CO_2$  and then were brought above critical point (31.1 °C and 1073 Psi) to become a dense vapour phase. The samples were later sputter-coated and examined with SEM at the Department of Biological Sciences, Central Michigan University, USA.

## 2.4. Histopathological analysis

For histopathological analysis, infected tissues of the fish host were fixed in Bouins fluids for 7 h. They were later preserved in 10% phosphate buffered formalin and embedded in paraffin wax, blocked, sectioned and stained with haematoxylin and eosin. The stained tissues were washed in tap water and the over-stained ones were destained in 1% acid alcohol. The tissues were mounted using distyrene plasticizer xylene mountant, which replaced xylene balsam, dried and examined under the microscope.

## 3. Results

The SEM photomicrographs of the female species of *Procamallanus* were shown in Figures 2 and 3.



A: Cephalic end showing the spiral ridges of buccal capsule (lateral view); B: Apical view of the cephalic region showing amphid; C: Sublateral view of the cephalic region showing cephalic alae; D: Caudal region showing the vulva.



**Figure 3.** SEM micrograph of *Procamallanus* sp. E: Cuticular body showing papillae; F: Caudal region showing the tail; G: Body showing the presence of alae; H: Smooth cuticle showing the presence of compressed alae.

The description is based on a female species recovered from the intestine of *P. obscura*. The nematode is medium-sized with very thick transversely striated cuticle. Mouth apertures were completely oval with four very conspicuous external cephalic papillae with a cephalic region width of 94.5  $\mu$ m and a length of 90.7  $\mu$ m (Figure 2A). A longitudinal, erect papillae (Figure 2B) located on the top of the cuticle close to the spiral ridges of buccal capsule was also evident. The cephalic region had a length of 3.4  $\mu$ m and width of 1.7  $\mu$ m. The buccal cavity was measured 27.7  $\mu$ m by 26.3  $\mu$ m (Figure 2A). Several body ornamentations run throughout the cuticle of the nematode. Spiral ridges of the buccal capsule were 7 in number, a pair of amphid (Figure 2B) were present, and the cephalic region was more wider than some of the already described species with spiral thickenings internal to the cephalic region and close to the external cephalic papillae.

A circular ornamentation was also observed close to one of the

external cephalic papillae with an irregular outer surface. There were 6 cephalic alae, 2 on top of the cuticle and 4 on the lateral side (Figures 2A and 2B).

The posterior end of the Procamallanus sp. had a short tail (Figure 3F) with 13 lateral alae and papillae (Figure 3E). There were 4 compressed cuticular alae before the conical tail. The tail length was 47.6 µm with a diameter of 10.5 µm. The vulva was postequitorial with a length of 13.6 µm and width of 6.7 µm (Figure 2D). The width of the body was 309.3 µm, with papillae of the exhibiting dimensions of 28.1 µm by 21.5 µm. Cuticle smoothed with streaks of demarcated lines, with lateral papillae of different and shapes sizes, some oval while others were irregular. Deirid was very small, 2.0 µm length and 1.6 µm width. Some of the papillae were large with 10 lengths from 21-28 µm and width of 11-25 µm. The papillae had an average length of 7 µm and width of 102 µm. The spiral ridges of buccal capsule did not reach anterior to the end of the capsule. The length of phasmid was 7.3 µm and its breadth was 12.0 µm. There was no cuticular body extensions as found in the male species (Figure 4F).

#### 3.1. Description of the male species

The second putative Procamallanus species was a male and was also recovered from the intestines of P. obscura. The SEM revealed new morphological data different from the first Procamallanus sp. examined (Figures 4A-D). The cephalic region was spindle shape with the length of 83.2 µm and width of 122.1 µm. There were four external cephalic papillae and compressed alae. Mouth circular was measured 27.9 µm by 8.2 µm. Amphid presented and was close to the external cephalic papillae and spiral thickening surrounding the mouth. The posterior region was rounded with a short pointed tail, 61.3 µm in length and 31.2 µm in width while the posterior region diameter was 191.3 µm. There was a depression on both sides of the lateral region of the posterior end (Figure 4B). Three longitudinal caudal alae were present near the lateral inward depression with length of 4.9 to 10.7 µm and width from 4.9 to 7.4 µm. These three longitudinal caudal alae were adjacent to one another. Phasmid was found on the right lateral side at the caudal region. The middle cuticular body was measured 252.9 µm. The male organ located at the lateral region had dimensions of 60.6 µm by 29.0 µm. Another circular compressed ala was located very close to the male reproductive organ. There was lateral cuticular extension on the body which terminated very close to the caudal region. This lateral cuticular extension of the body was absent in the female species. All the details of this male species were shown in Figure 4.

## 3.2. Histopathological changes in the fish host

The histopathological changes observed as a result of the parasitic infections of *Procamallanus* spp. revealed different pathological lesions (Figure 5). The tissue sections revealed thickening of the tunica muscularis with a localized area containing section of an intraluminal nematode with minimal inflammation. There was also thickening of the mucosa with sloughing of epithelial cells into the lumen with a moderate goblet cell hyperplasia. There was hypercellularity of the mucosa, severe mucosal necrosis and lymphocytic infiltrations of the lamina propria and mucosa thickened





Figure 4. SEM of Procamallanus sp.

A: Caudal region showing the tail; B: Apical view of the caudal region showing the caudal alae and papillae; C: Sublateral view of the cephalic region showing external papillae; D: Apical view of the cephalic region; E: Male reproductive organ; F: Cuticular body extension.



Figure 5. Section through the intestine of Parachanna argus showing different pathological conditions.

A: Normal section; B: Intramural nematode with surrounding inflammatory cells; C: Necrosis of surface epithelium with accumulation of necrotic debris in lumen; D: Severe mucosal necrosis with accumulation of necrotic debris in lumen.

#### 4. Discussion

The SEM study of the two species of Procamallanus (Spirocamallanus) was undertaken. Both male and female Procamallanus species were recovered from the intestine of the fish hosts. The two species in this report were based on the descriptions provided by Ali[24] and nominally belonged to the subgenus Aspiculus due to the lack of spicules. The subgenera Aspiculus, Monospiculus, Isospiculus and Procamallanus were created by Ali[24] who synonymized the group based on morphological characters. Due to the advances in SEM technology, there are previously unreported taxonomically important morphological features in these two species of the genus Procamallanus. In the first female described for the species, a prominent longitudinal erect papillae at the cephalic region on top of the cuticle has never been reported in any species of Procamallanus.

In an effort to reconcile the group, Moravec et al.[25] redescribed Procamallanus (Spirocamallanus) fulvidraconis and reported two spicules and a gubernaculum with unequal arms in the species. There were no spicules in these species examined and the longitudinal erect papillae at the cephalic region was never reported[8,25,26]. Moreover, Moravec et al.[26] who reported only female species and suggested extremely rare occurrence of male species or absence of males due to parthenogenesis in the genus Procamallanus together with Bashirullah and Williams[27]. In this study, however, male and female species of the genus were recovered. Moravec et al.[26] noted that camallanid nematodes from New Caledonian had 22 spiral ridges in the buccal capsule which differed from our study. This reported Nigerian species of the genus Procamallanus is, however, with only 7 inner spiral ridges of buccal capsule. Higher taxonomical grouping in the family Camallanidae is entirely based on gross differences in buccal capsule morphology. The cephalic regions of these species differ from the already described species with ridges in between the four papillae of the external circle which are distinctly larger than the already described species on the morphology of Procamallanus annulatus and P. monotaxis reported lack of spiral ridges in the inner surface of capsules[25,27-31]. This is in agreement with Procamallanus species in this report without spiral ridges in the buccal capsule but 7 spiral ridges observed in the first female species. Moravec et al.[25] also reported 10-12 spiral ridges in the male species of Procamallanus (Spirocamallanus) anguillae in Thailand. The buccal cavity of these Procamallanus is also wider and broader. The preanal papillae observed in these species are 4 which is in contrast to the studies of several authors[26-28,30]. The tail length is also longer (47.6 µm) together with the length and width of the excretory pore. The two species of Procamallanus described in this paper conform to Procamallanus aspiculus for lack of spicules described by Agarwal[32] from an Indian siluroid fish which was again described by Khera[33]. The species was first described by Baylis[34] with the creation of Procamallanus laeviconchus while he also described Procamallanus spiralis from a single specimen from the siluroid fish, Heterobranchus anguillaris. This paper present the first SEM description of the genus Procamallanus in Nigeria. The histopathological effect of the nematode in the intestine of the fish host revealed some pathological changes such as goblet cell hyperplasia, hypercellularity of the mucosa and lymphocytic infiltration of the lamina propria. This is also in agreement with the study of Noor El-Din et al.[35] which also reported goblet cell hyperplasia cellular infiltration and penetration of the parasite to the submucosa layer of the fish host, Clarias gariepinus by Procamallanus laevicondius. Costa and Camargo[36] reported that the infection by Procamallanus limits the potential capacity of nutrients absorption by the fish host, Bivibranchia velox. Bamidele[16] also reported some histopathological changes on some fish hosts of Lekki Lagoon, Lagos, Nigeria such as necrosis, lymphocytic infiltration,

sloughing of cells into the lumen and goblet cell hyperplasia. In Nigeria, this is the first report of *Procamallanus aspiculus* from *P. obscura*. This report on pathological alterations of the nematode, *Procamallanus* and the occurrence of male and female species of the genus in Lekki Lagoon is an indication that there should be control measures in the water body so as to increase the marketability values of the fishes.

#### **Conflict of interest statement**

We declare that we have no conflict of interest.

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