MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Additional morphological characters of the female reproductive system of camallanid *Procamallanus* (*Procamallanus*) *pseudolaeviconchus* Moravec and Van As, 2015 from the catfish *Clarias gariepinus*

Azza F. EL-Tantawy, Mohamed I. Mashaly and Mohamed M. EL-Naggar

Zoology Department, Faculty of Sciences, Mansoura University, Egypt

***Corresponding author** e-mail: dr.moh_mashaly@mans.edu.eg– Tel: +201004063132*

Received:6/6/2023
Accepted: 18/6/2023

Abstract: This is the first time to identify the nematode *Procamallanus* (*Procamallanus*) *pseudolaeviconchus* in Egypt from the stomach and intestine of the Nile catfish *Clarias gariepinus.* In this study, the female reproductive system has been studied in detail for the first-time using phase contrast microscopy. It consists of a single ovary, an oviduct, a seminal receptacle, a uterus, a vagina, and a vulva. The ovary appears telogonic and differentiated into 3 main regions namely germinal, growth and maturation zones. In the proximal widened (sac-like) region of the ovary, the oocytes reach their maximum maturation and size and assume cuboidal form. Four oocyte forms could be detected in the sac-like region of the ovary. In most forms, the nuclear membrane is surrounded by a thin layer of dense material and the cytoplasm contains different types of secretory bodies and vesicles. In between, the different forms of mature oocytes, lie non-nucleated cytoplasmic regions, some of them have no cytoplasmic inclusions while others contain dense granular bodies. Moreover, relatively large, spherical membrane-bound vacuoles, containing secretory bodies and vesicles, are seen in between the mature oocytes. There are constrictions between the ovary and oviduct, the oviduct and seminal receptacle and between the seminal receptacle and uterus.

keywords: Nematoda, Procamallanus, Reproductive system, Clarias gariepinus

1.Introduction

The African catfish, *Clarias gariepinus* Burchell, 1822, is considered one of the most essential commercially farmed fish in Egypt and other African countries. Three camallanid nematode species namely *Procamallanus laeviconchus* Wedl, 1861, *Procamallanus pseudolaeviconchus* Moravec and Van As, 2015 (in the stomach) and *Paracamallanus cyathopharynx* (Baylis, 1923) Yorke and Maplestone, 1926 (in the posterior end of the intestine), were found to infect the African catfish, *C. gariepinus*.

Moravec and Van As [1] were the first to describe the nematode, *P.* (*P.*) *pseudolaeviconchus* from *Clarias stappersi* Boulenger, 1915, *C. theodorae*, Weber, 1897 and *C. gariepinus* Burchell, 1822. Previous studies of *Procamallanus* species in marine fishes in Egypt recorded only *Procamallanus* (*Procamallanus*) *elatensis* Fusco and Overstreet, 1979 from siganid fishes. Hussein et al. [2] recorded two species of *Procamallanus* from Red Sea fishes at Hurghada and Safaga, Egypt namely *Procamallanus* (*Procamallanus*) *annulatus* and *Procamallanus* (*Procamallanus*) *elatensis*. Noor El-Din et al. [3] reported the pathological effects associated with the nematode *P. laeviconchus* infecting the stomach and intestine of the Nile catfish *C. gariepinus*. Novel information on the morphological and molecular characteristics of *P. pseudolaeviconchus* was made by Svitin et al. [4].

The female reproductive system of nematodes is widely used as an important diagnostic character in the systematics of nematodes. Many authors focused on the study of the female reproductive system of different nematode species. For example, using light microscopy, Bert et al. [5] examined the cellular gonducts architecture of several species and genera within the family Pratylenchidae. Moreover, Zograf et al. [6] studied the morphology of the female reproductive system in several representatives of the genus *Steinernema* using light and electron microscopy.

This is the first time to identify *P. pseudolaeviconchus* in Egypt from the stomach and intestine of the Nile catfish *C. gariepinus.* Previous descriptions of *P. pseudolaeviconchus* were concentrated on the general morphological characters using light and SEM. However, few of these studies considered detailed morphological and anatomical characteristics of the internal organs, particularly the reproductive system [5, 6]. Therefore, it was found necessary to make extensive and detailed observations on the female reproductive system of *P. pseudolaeviconchus* to precisely illustrate the structures of its organs. The study was extended to follow, in detail, the embryonic development and maturation of the oogonia until they reach the mature oocytes.

2. Materials and methods

A total of 154 specimens of *C. gariepinus* were collected by trammel nets from the Damietta branch of the River Nile at Mansoura City, Dakahlia Governorate, Egypt from April 2020 to March 2021. Fishes were dissected as soon as possible after their capture. To collect nematode parasites, individual fishes were transferred into a dissecting dish, the abdominal cavity of each fish was opened via a medio-sagittal incision using a sharp scissor beginning from just behind the operculum to the end of the abdominal cavity. The alimentary canal was removed, and the separated intestine was longitudinally opened by means of a fine needle and left in a Petri dish containing 0.9 % normal saline solution. The parasites were dislodged from the tissues by scratching the intestinal wall using a fine dissecting needle under a stereomicroscope to allow the freeing of the entangled parasites from the intestinal wall. The parasites were allowed to settle out in a cubic glass dish.

For studying the morphological and anatomical features of living specimens, each parasite was transferred with a drop of saline to a clean glass slide, flattened by the withdrawal of saline by a filter paper and then covered with a coverslip. Flattened parasites were examined and photographed using Leitz Laborlux 20 EB phase-contrast research microscope provided with Omax digital camera. Identification and measurement methods of *P. pseudolaeviconchus* were done according to Moravec and Van As [1].

For whole-mount preparations, specimens were flattened between a slide and a coverslip, preserved either in 10% formaldehyde or in alcohol-Formalin-Acetic Acid (AFA) fixative for about 24 hrs. Then washed in distilled water. The parasites were then stained in either Semichon's Acetocarmine or light green stain. After staining, specimens were washed in distilled water, dehydrated in an ascending series of ethyl alcohol, cleared in xylene, and mounted in Canada balsam. Stained preparations were then examined and photographed using Leitz Laborlux 20 EB phase-contrast research microscope provided with OMAX digital camera.

3. Results (Figs.1,2,3)

During the examination of the young female specimen, the cuticle was ruptured and internal organs including part of the intestine and reproductive organs were released outside the worm. The female reproductive system of *P.* (*P*) *psudolaeviconchus* is monodelphic and consists mainly of a single ovary, an oviduct, a seminal receptacle, a uterus, a vagina, and a vulva (Fig. 1a, d, e). In specimens where the reproductive organs are released outside the body, the ovary appears telogonic (i. e. the new germ cells originate only at the distal end of the gonad) and bounded by a thin epithelial layer along its length (Fig. 1b). The ovary can be differentiated into 3 main regions namely germinal, growth and maturation zones (Fig. 1b, c, d). The germinal zone of the ovary begins at the anterior region of the glandular oesophagus as a narrow small, cap-like cell (diameter; 5.5 x 10 µm) followed by a cylindrical, tubular, structure packed with oogonia that are attached to each other by rachis (a cytoplasmic extension of the cap cell). Oogonial cells are apparently small (diameter; 5.5 x 5.5 µm), attain spherical shape, and contain a conspicuous nucleus (diameter2-2.5µm) (Fig. 1b). The diameter and size of the oocytes increase as the ovary proceeds posteriorly where it forms its largest and widened region (sac-like structure), near the anterior third of the intestine (Fig. 1d).

In the growth zone, the young oocytes are packed together and attain pentagonal and hexagonal shapes (Fig. 1c). Their diameter ranges between 15 X 25 to 24.8 X 37.2 µm. The nucleus is relatively large (11-18 µm in diameter) and possesses a conspicuous, dense nucleolus measuring 3.5-6.2 µm in diameter. In most oocytes examined, the nucleoli are centrally located while in a few cells, they are peripherally located. It was evident that each nucleolus is surrounded by a thin, transparent layer of the nucleoplasm while the cytoplasm surrounding the nuclear membrane is denser (Fig. 1c). In the widened posterior (sac-like) region of the ovary, the oocytes reach their maximum maturation and size (47- 48 X 28-35 µm) and assume cuboidal form with larger nuclei (12-17.5 µm diameter) and nucleoli (4-5 µm diameter) (Fig. 1d). In well-flattened mature oocytes released from a leak in the posterior widened region of the ovary (maturation zone), the cells acquire different shapes and sizes according to their developmental stage. Some oocytes (oc1) are comparatively smaller in size where the nucleus has no nucleolus and fills three-quarters of the cell, and the cytoplasm forms a thin layer around the nucleus (Fig. 2a). The second form of the mature oocyte (oc2) has a moderate size and lacks a nucleus, but the cytoplasm is apparently filled with a dense granular matrix (Fig. 2a, b). The third form of the mature oocyte (oc3) has a conspicuous nucleus, like the first type lacking nucleolus, but the cell size is comparatively large and contains dense granules. There is evidence of the chromosomes inside the nucleus (Fig. 2b). The fourth type of the mature oocyte is large and possesses a conspicuous nucleus provided with a centrally or peripherally located nucleolus surrounded by a thin translucent area of the nucleoplasm (Fig. 2c). In most forms of the oocytes, the nuclear membrane is surrounded by a thin layer of dense material and the cytoplasm contains different types of secretory bodies and vesicles including clusters of dense granules, small vesicles, each with centrally located dense granule, and vacuoles with multivesicular vesicles containing large dense granules (Fig. 2c). In between the different forms of mature oocytes, lie non-nucleated cytoplasmic regions which may represent part of the rachis. Some of these regions have no cytoplasmic inclusions while others contain dense granular bodies (Fig. 2c). Moreover, relatively large, spherical membrane-bound vacuoles, containing secretory bodies and vesicles, are seen in between the mature oocytes (Fig. 2a, c).

The proximal widened region of the ovary (the sac-like region) leads to a narrow oviduct possessing a lumen lined with an epithelial layer and measures (356.13± 24.56) µm in length and (21.27± 5.93) µm in width (Fig.1e). Evidence indicates the presence of passing oocytes in the oviduct lumen. There is a constriction between the proximal region of the ovary and the oviduct. The latter dilates into an oval-shaped chamber known as a seminal receptacle measuring (142± 4) µm in length and (68.40± 2.65) µm in breadth and filled with mature spermatozoa (Fig.3a). It is believed that, in nematodes, fertilization occurs in the seminal receptacle and fertilized ovum (zygote) passes into the uterus where it undergoes meiotic division and forms masses of blastomeres. There is a constriction between the oviduct and the seminal receptacle and between the seminal receptacle and the uterus (Fig. 3a). The posterior region of the seminal receptacle dilates to form a uterine chamber that extends posteriorly to reach the level of the posterior region of the body, a short distance from the rectum. In some specimens, the anterior region of the uterus was found to contain mature oocytes like those found in the posterior sac-like region of the ovary. In most specimens examined, the uterus begins as a wide chamber containing 3-4 rows of developing embryonated larvae (Fig. 3b), then decreases in size as it proceeds posteriorly where it contains 1-2 rows of developing embryonated larvae (Fig.3c). The vaginal tube originates from nearly the middle region of the uterus and extends anteriorly where it opens into the vulva at the ventral surface of the worm (Fig.3d, e)). The released intact vaginal tube appears to be covered externally by an epithelial layer and supported by transverse muscle fibres. The opening of the vulva lies nearly at the middle region of the body and appears to be guarded by 2 elevated conspicuous lips (Fig.3e). In old female worms, the uterus is filled with first-stage rhabditiform larvae (Fig.3f) which are seen, in some well-flattened specimens, expelled from the worm and moving around (Fig. 3e, g). Each larva consists of a muscular oesophagus and intestine (Fig. 3f, g) and measures (202.87± 43.45) µm in length and (20.08± 2.37) µm in width.

4. Discussion

It should be emphasized that this is the first time for the reproductive system of a *Procamallanus* species to be described in such detail using phase-contrast microscopy. The ovary was found to be telogonic and differentiated into germinal, growth and maturation zones. The germinal zone begins as a small, cap-like cell which was suggested by Wharton and Chappell [7] to release a substance that prevents the germ cells from entering meiosis and thus ensures the mitotic proliferation of germ cells at the distal tip of the gonad. The proximal region of the ovary of *P. pseudolaeviconchus* swells to form a sac-like structure filled with oocytes. In this respect, the ovary of *P. pseudolaeviconchus* resembles that of other nematodes characterized by one or two swellings (sacs) at its proximal region [6, 8, 9]. These sacs are separated from each other by a simple narrowing (constriction) as in the case of the nematode *Steinernema carpocapsae.* Similar constrictions were observed in the present study between the ovary and oviduct, oviduct, and seminal receptacle, and between the seminal receptacle and uterus. The exact function of these constrictions is not known but it seems probable that they serve as a "valve" that can control the passage of mature oocytes from the ovary to the oviduct and from the oviduct to the seminal receptacle where they are fertilized. Also, this constriction controls the passage of fertilized ovum from the seminal receptacle to the uterus where they develop into embryonic developing larvae. Zograf et al. [6] detected similar valves between the ovary sacs in the nematodes *Steinernema arenarium* and *S. feltiae* and suggested that these valves may prevent premature movement of ripening oocytes to that part of the genital system where they may meet spermatozoa. Another important anatomical character of *P. pseudolaeviconchus* is the presence of at least 4 differentiated forms of the oocytes in the proximal sac-like region of the ovary as well as in the anterior portion of the uterus which indicates the potential reproductive division of the oocytes. Most of these forms of mature oocytes contain secretory bodies of different sizes and forms including single and aggregated dense granules, small vesicles with central dense granules and large vacuoles with multivesicular bodies. The precise function of these secretory inclusions is not known but it seems likely that they serve as a source of nutritive material that provides the cell with the necessary energy required for completing the next stages of embryonic development. The large vacuoles with dense secretory bodies observed in close contact with the mature oocytes in the posterior sac-like region of the ovary could represent remnants of the vitelline (yolk) globules which may function like the nurse cells recorded in other nematodes. The non-nucleated cytoplasmic regions observed between the oocytes might represent remnants of the rachis which are still present in the sac-like region of the ovary and have an anchoring and supporting function for the oocytes.

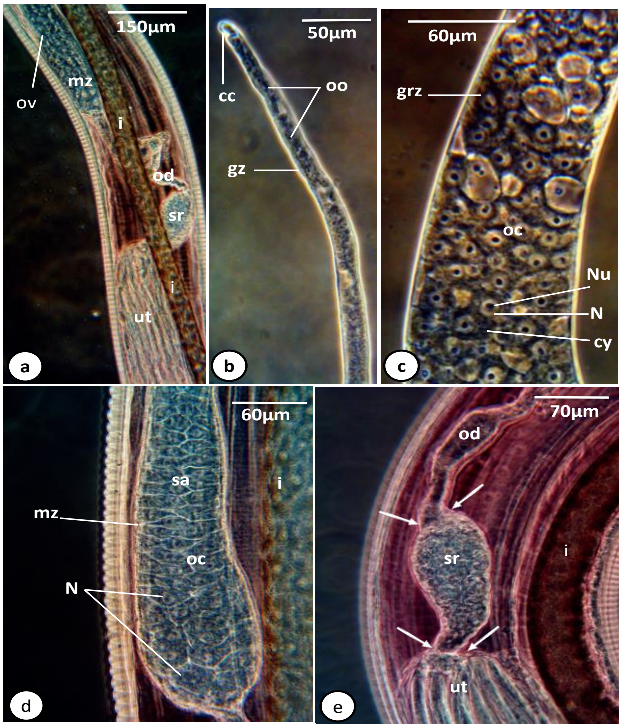

Fig.1. Phase-contrast microscope photographs showing structural details of the female reproductive system of *Procamallanus(P) pseudolaeviconchus.* a. The middle region of

the female reproductive system consists of the mature zone (mz) of the ovary (ov), oviduct (od), seminal receptacle (sr) and anterior part of the uterus (ut). i, Intestine. Scale bar= 150 µm. b. The germinal zone (gz) terminates with a cap-like cell (cc) and contains small oogonia (oo). Scale bar= 50 µm. c. Growth zone (grz) of the ovary containing young oocytes (oc) at the region immediately following the germinal zone. cy, Cytoplasm surrounding the nuclear membrane; N, nucleus; Nu, nucleolus. Scale bar= 60 µm. d. Enlarged view of the posterior region of the maturation zone (mz) forming sac-like structure (sa) packed with hexagonal and pentagonal-shaped oocytes (oc) containing large nuclei (N). i, Intestine. Scale bar= 60 µm. e. The seminal receptacle (sr) is a widened region between the oviduct (od) and the uterus (ut). Note the presence of a constriction between the oviduct and seminal receptacle and between the seminal receptacle and uterus (arrows). i, Intestine. Scale bar= 70µm.

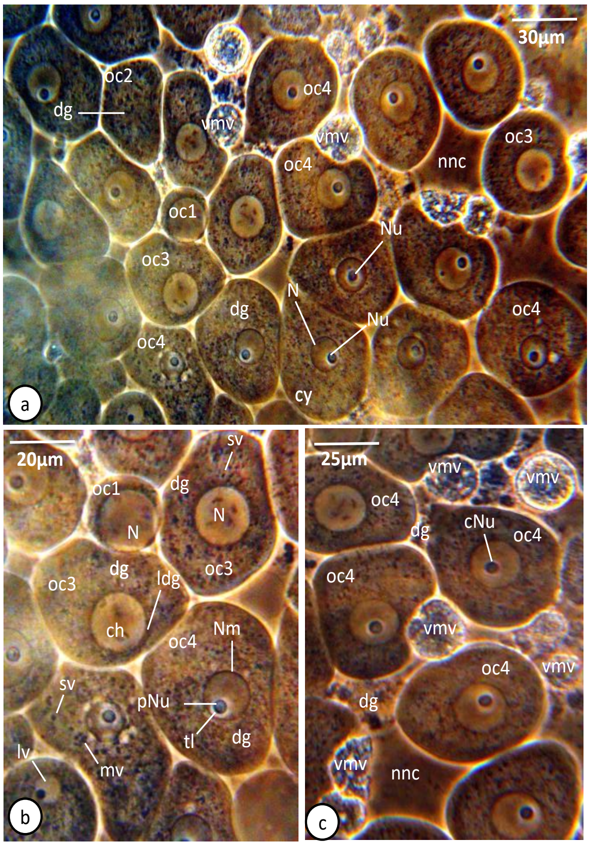

Fig.2. Phase-contrast microscope photographs showing released, well-flattened oocytes and associated structures. a. Four forms of maturing oocytes, small cell (oc1) possessing large nucleus and narrow cytoplasmic layer but without nucleolus, medium-sized oocyte (oc2) without nucleus but the cytoplasm has dense granules (dg), large oocytes (oc3) possessing large nucleus without nucleolus, but the cytoplasm is wide and contains dense granules, and large oocytes (oc4) possessing nucleus (N) and nucleolus (Nu) and greater cytoplasm (cy) with dense granules (dg). nnc, None-nucleated cytoplasm; vmv, vacuole with multivesicular bodies. Scale bar= 30 µm. b. Enlarged portion of (a) showing the detailed structure of oc1, oc3 and oc4. ch, Chromosomes; dg, dense granules; ldg, a narrow layer of dense granules; lv, large vesicle with dense granules; Nm, nuclear membrane; pNu, peripheral nucleolus; sv, small vesicle with dense granules; tl, translucent layer of the nucleoplasm. Scale bar= 20 µm. c. Enlarged portion of (a) showing the detailed structure of oc4 surrounded by vacuoles with multivesicular bodies (vmv) and non-nucleated cytoplasmic regions (nnc). cNu, Central nucleolus; dg, dense granules. Scale bar= 25 µm.

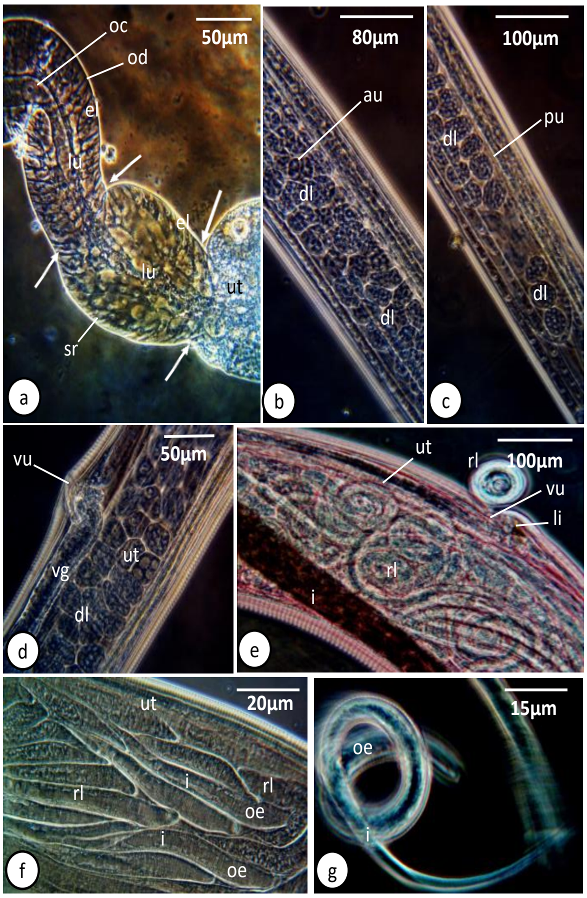

Fig. 3. Phase-contrast microscope photographs showing structural details of the posterior region of the female reproductive system of *Procamallanus* (*P*) *pseudolaeviconchus.* a. Both the oviduct (od) and seminal receptacle (sr) possess a lumen (lu) lined with an epithelial layer (el). arrows, Constrictions; oc, oocyte; ut,

uterus. Scale bar= 50 µm. b. The anterior region of the uterus (au) with 3-4 rows of developing larva (dl). Scale bar= 80 µm. c. The posterior region of the uterus (pu) with 1-2 rows of developing larva (dl). Scale bar= 100 µm. d. The middle region of the body showing the vulva (vu), vaginal tube (vg) and the uterus (ut) with developing larvae (dl). Scale bar= 50 µm. e. The uterus (ut) in an old female containing the first stage of rhabditiform larva (rl). Note the presence of these larvae coming out of the vulva (vu). i, Intestine; li, lip of the vulva. Scale bar= 100 µm. f. Uterus (ut) packed with the first stage rhabditiform larva (rl) and each consists of a long muscular oesophagus (oe) and intestine (i). Scale bar= 20 µm. g. Released, free swimming, first stage rhabditiform larva. i, Intestine; oe oesophagus. Scale bar= 15 µm.

5. References

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