

Morphological and Histological Studies on the Vermicomposting Indian Earthworm *Eudrilus eugeniae*

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Abstract: *Earthworm* is a potential contributor in organic waste disposal or vermicomposting. *Eudrilus eugeniae* collected from moist subsurface soil and under stones in the University of Agriculture Sciences, GKVK, Bangalore, India, were studied. *E. eugeniae* can also be utilized for protein source in animal feed. The external features, growth, reproductive morphology and histology were investigated. The Indian *E. eugeniae* has higher bodyweight as compared to the African counterparts, in spite of comparable lengths. Morphology of the reproductive parts and histology of the ovary and oviduct of these worms are elucidated by histological staining methods. There have been no previous reports about these clitellar earthworms from the Indian subcontinent. The histological details of the ovary reveals the presence of larger follicles towards the periphery which shows degenerative changes, while the smaller primary follicles and oocyte are concentrated in the center. Further, the posterior part of the oviduct shows the presence of dense mass of sperms in its large lumen which confirms the process of internal fertilization in *Eudrilus eugeniae*. Hence, this first report is to help towards increasing breeding practices of these earthworms in these regions having good temperature and climatic conditions for ideal growth of this organism.

Key words: *Eudrilus Eugeniae* • Reproductive Morphology And Histology • Ovary • Oviduct

INTRODUCTION

Earthworms are soil dwelling organisms involved in the process of soil formation and organic matter decomposition. *Eudrilus eugeniae* (Kinberg) is the commonly type of earthworms used for vermicomposting in tropical and sub-tropical countries [1]. Vermicomposting, a mesophilic process carried out by earthworms, involves ingestion, digestion and absorption of organic waste followed by excretion of castings

through the worm's metabolic system, enhancing the levels of plant-nutrients of organic waste during their biological activities [2].

The earthworm species, *E. eugeniae* is indigenous to Africa but has also been bred in the USA, Canada, Europe and Asia, where it is commonly called the African nightcrawler, to be used as fish bait [3]. It grows well at a temperature of more than 25°C but best at 30°C [4], attaining maximum weight, length and number of segments in about 15 to 20 weeks [5]. Size of the worms

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may depend on habitat and ranges in from about 10cm in length to huge specimens of over 12cm [6]. The total number of segments in *E. eugeniae* varies from about 80 to over 100 with the location of a thick cylindrical collar - the clitellum between segments 13 - 20 [7]. It has a purple sheen and the posterior segments evenly taper to a point [8].

Among the tropical earthworms, there is a great taxonomic diversity. The dominant ones are belong to the families Almidae, Glossoscolecidae, Megascolecidae, Ocnodrilidae and Eudrilidae. *E. eugeniae* belongs to Eudrilidae⁶. Variability in terms of morphological measurements has been reported among different species of earthworms [9-11]. Variation in total body length as measured from the first to last segment was reported by Vitturi *et al.* [12].

The aim of this research work is to report morphological characteristics of the vermicomposting earthworm *E. eugeniae* and study the external reproductive morphology of the Indian *E. eugeniae*. Structure and functions of the ovary and the oviduct are elucidated through histological studies.

MATERIALS AND METHODS

Earthworms: The earthworm *E. eugeniae* (Annelida; Oligochaeta; Eudrilidae) was collected from moist subsurface soil and under stones in the University of Agriculture Sciences, GKVK, Bangalore, India. The earthworms were collected from the same site in order to reduce variability in biotype. In the laboratory, the earthworms *E. eugeniae* were kept in an earthen pot (volume 5 Litres), which was half filled with a mixture of loamy and humus soil supplemented with cow dung, dry leaves and vegetable waste and moistened with deoxygenated tap water (treated soil) [13]. The earthworms were kept in earthen pot for a minimum of ten days in order to allow them to adapt to experimental conditions (28 + 2°C, 79 + 2R.H). During the period of acclimatization, the worms were fed with air dried cow manure and poultry droppings (5 g dry wt) every 15 days.

Earthworms of similar sizes were carefully selected from the earthen pots for further studies.

External Reproductive Morphology: *E. eugeniae* worms were taken in a small beaker and anaesthetized using chloroform, After 5 minutes, the earthworms were taken in a wax tray for the study of external morphology. The earthworms were pinned dorsally and observations were made on the external morphological characters of the

mouth and anterior segments, the position of the spermathecal openings; the clitellar segments and the female and male genital openings or apertures. Observations were also made on inter segmental grooves, the dorsal pores and the arrangement of setae.

Morphology of the Ovary and Oviduct: The adult female worms were used for investigations. *E. eugeniae*, were anaesthetized using chloroform and pinned at anterior and posterior regions on a wax tray. After careful positioning of the worms, a median longitudinal i.e. mid dorsal opening was made using a fine scissors on the skin above the clitellum thus exposing the body cavity. The body cavity was exposed completely in the anterior region and the skin exposed was accordingly pinned on the wax tray using needle and forceps. The alimentary canal or the digestive system was carefully excised to expose the reproductive structures. The wax tray was later shifted to the dissection microscope "Leica WILD M 420" (Germany) with micro zoom built in illumination. Detailed observations were made on the position and morphology of the female reproductive structures consisting of the ovaries and the oviduct.

Histological Studies: A pair of ovaries located on the 13th segment which are palmate were removed from the clitellar worms onto a clean watch glass and immediately fixed in Bouin's and formalin fixatives for further histological studies.

Briefly, the ovaries and the oviducts fixed in Bouin's fluid for 24 h, were washed in running tap water for a few hours and stored in 80% alcohol for further tissue wax preparations using routine standard laboratory procedures. The ovaries and the oviducts were then processed using step by step dehydration with different grades of alcohols, then cleared using xylene.

Finally, after cleaning, the tissues were infiltrated with molten paraffin wax at 55-58°C and appropriate sizes of the tissue blocks were prepared. The serial sections of 5-7 microns of the ovary and the oviducts were cut in a Rotary microtome using fine blade. After deparaffination and down grading in alcohols, the sections of the ovary and the oviduct were stained in Haematoxylin followed by Eosin. The permanent slides were prepared using DPX mountant. The detailed microscopic observations were done in a stereo research microscope (Leica, Germany). The size variations and the cell measurements were done using stage micrometer. Selective microphotographs of the ovary and the oviducts were taken in a stereo research microscope with an attached camera (Leica, Germany).

RESULTS

The External Morphology: The body of the clitellar earthworm, *Eudrilus eugeniae* is reddish brown in colour, round or cylindrical in shape and metamerically segmented. The mean individual length and live weight, is 12.5cms and 3-4g. (Fig. 1) The arrangement of setae is lumbricine. The anterior region shows the prostomium which is epilobus. On the ventrolateral surface, the body segments 8, 9, 10, 11 and 12 shows the presence of spermathecal openings. Exactly on the 12th segment, a pair of female genital openings is present. Segments 13, 14 and 15 forms the unsegmented collar like structure called the clitellum. On the 16th segment, pair of male genital openings is present, bellow the clitellum (Fig. 2).

Morphology of the Ovary: The ovaries are the female gonads in the Earthworm, *E. eugeniae*. A pair of ovaries is located in the 13th segment below the alimentary canal in the coelomic cavity (Fig. 3). Each ovary is a granular, creamish white, cauliflower like measuring 1-2 mm in size and approximately weighing 0.1 milligram with rich blood supply.

The Morphology of the Oviduct: The oviducts are small, minute coiled structures found behind the ovaries. The oviduct is about 2-3mm in length. The anterior part of the oviduct is larger, the oviducal funnel, continues as a coiled tubular structure and opens on the ventral side between 12th and 13th segments as female opening or aperture externally.

Histology of the Ovary: The ovary is covered by a membranous covering of the germinal epithelium made up of cuboidal cells. Within the ovary there are many ovarian follicles in different stages of development (Fig. 4). In between the follicles, there is a connective tissue and rich supply of blood vessels. The larger follicles are seen at the periphery and their number ranges from 20-30 in the section. Each follicle has many layers of follicular cells. The follicular cells are small, oval and the nucleus in them is large and measures 2-3 microns. The smaller primary follicles and oocytes are seen at the centre or below the larger follicles and their size varies from 1-1.5 microns. Further the larger follicles show degenerative changes (Fig. 5). The follicular cells as well as the oocyte show the changes. The oocyte membrane shows shrinkage and undulated with dense substances evenly distributed in the cytoplasm (Fig. 6). The nucleus in them also shows shrinkage and folding in the membrane.



Fig. 1: General morphology of the clitellar earthworms *Eudrilus eugeniae*



Fig. 2: Magnified view of the anterior end of *Eudrilus eugeniae* showing the female and male genital openings. CL-clitellum, FA-female genital aperture and MA- male genital aperture

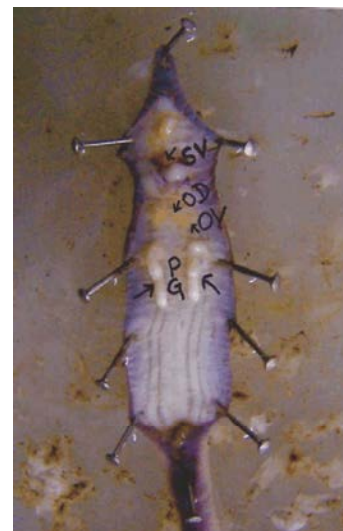


Fig. 3: Location and morphology of the ovary of the worms *E. eugeniae*.SV-seminal vesicle, OV-ovaries, OD-oviduct, PG-prostate gland

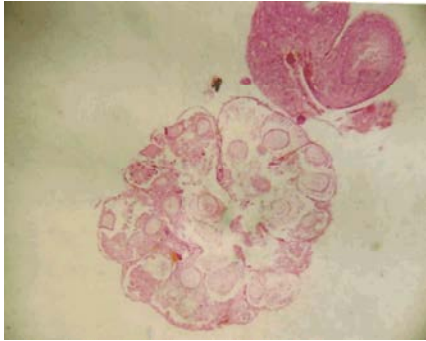


Fig. 4: Histology of the ovary in the clitellar *E. eugeniae* showing the follicles in different stages of development. (20X, HE)

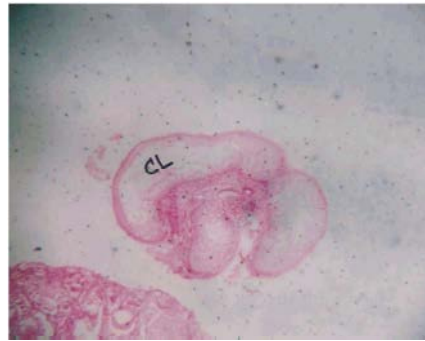


Fig. 7: Histology of the anterior part oviduct in the worms *E. eugeniae* showing central lumen-CL. (20X)

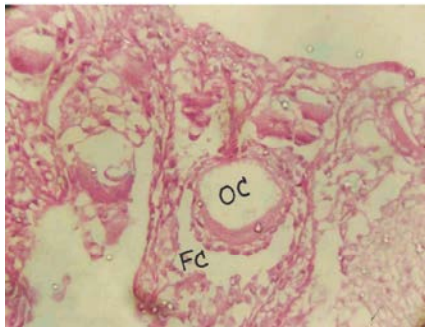


Fig. 5: HE staining of the section of the ovary in Fig. 4 showing the older follicles with degeneration. (100X). OC-oocyte, FC-follicle cells

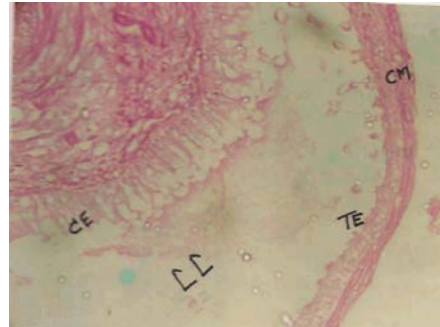


Fig. 8: HE staining of the cross section of the oviduct showed in Fig. 7 in the worms *E. eugeniae* showing the outer thinner epithelium-TE, circular muscles-CM, columnar epithelial cells-CE and large lumen-LL. (100X)

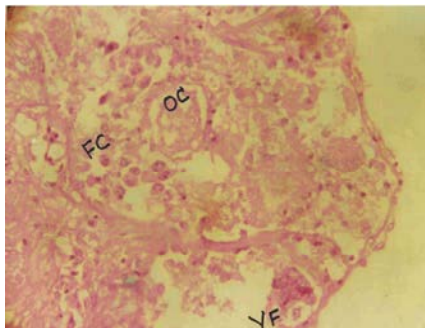


Fig. 6: HE staining of the cross section of the ovary in the clitellar *E. eugeniae* showing YF-young follicles, OC-oocytes with degeneration and FC-follicle cells. (100X)

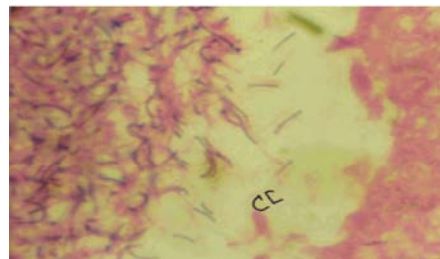


Fig. 9: HE staining of the magnified section of the oviduct in the worms *E. eugeniae* from the posterior part of the oviduct showing the presence of sperms in the central lumen-CL

Histology of the Oviduct: The anterior part of the oviduct in *E. eugeniae* is the oviducal funnel. It is larger and shows large lumen or cavity (Fig. 7), its diameter ranges from 10-12 microns. The outer covering is made up of cuboidal epithelium and a thin layer of circular and longitudinal muscles. A single layer of columnar epithelium surrounds or lines the large central cavity (10-12microns) (Fig. 8). Each columnar cell is tall and

ciliated. The posterior part of the oviduct is somewhat round and circular in outline and has a diameter of 8-10 microns. Histologically this part of the oviduct is similar to that of the anterior part except with regard to the large central cavity and the columnar epithelial cells are little smaller and do not show ciliated condition, but what is more evident is the presence of dense mass of sperms in their lumen (Fig. 9).

DISCUSSION

Even though studies of the *Eudrilus eugeniae* from Africa (Lagos in Nigeria) and other places like Spain, UK, USA and Canada have been reported, there are hardly any reports on their India counterpart. This study was undertaken to fill this lacunae in existing information about these earth worms. Clitellar worms *E. eugeniae* were collected from the University of Agriculture Sciences, GKVK, Bangalore, India for this purpose. We have recorded the average size and adult weight of these Indian *E. eugeniae* [14]. External reproductive morphology of the earthworms was also noted. Structure and functions of the ovary and the oviduct are elucidated through histological studies.

Previous information about *Eudrilus* populations from 14 different locations within Lagos State in Nigeria [3] report them to be purplish grey with head dark and tail light in colour. Length of the worms ranges from 11.5 to 15°C m and the clitellar region was observed to be between 13-20 segments and the species found in India were observed to be reddish brown in colour, with an average length of about 12.5 cm and clitellum between 13-15 segments. Weight ranges from 0.45 g to 1.26 g, whereas it is between 3-4g for their Indian counterparts. Even though the lengths of the worms are average, the live body weights are higher when compared to that of earthworms of different locations of Lagos and Nigeria (Fig. 1). On the ventrolateral surface, the body segments 8-12 shows the presence of spermathecal openings. A pair of female genital openings is present on the 12th segment. Segments 13-15 form the unsegmented collar like clitellum. Below the clitellum, on the 16th segment, a pair of male genital openings is present.

A detailed study of morphology and histology of the ovary (Figs. 3-6) and oviduct has been done as shown in Figs. 7-9. Histology of the ovary in the clitellar *E. eugenia* showed the follicles in different stages of development including the older follicles with degeneration. Histology of the anterior part oviduct in the worms of *E. eugeniae* showed a central large lumen, outer thinner epithelium, circular muscles, columnar epithelial cells. At a higher magnification, the posterior part of the oviduct shows the presence of sperms in the central lumen. This agrees with other reports of internal fertilization in *Eudrilus* earthworms from Ghana [15].

E. eugeniae would be a good candidate species for earthworm protein production for animal feed. Hence, the high body weight of the Indian earthworm would

contribute towards higher biomass and protein production. Vermicomposting, the microbial composting of organic wastes through earthworm activity, helps in the disposal of organic wastes from domestic, agricultural and industrial sources which are increasingly causing environmental and economic problems. *Eudrilus* earthworms have potential role in this process. Hence, study of their size, weight, lifespan, time to reproductive maturity etc. would be very helpful in breeding practices in the Indian subcontinent.

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