

**METARADIOPHYRYA EGYPTIACA N.SP. AN ASTOMATOUS CILIATE  
FROM THE ALIMENTARY CANAL OF ALLOLOBOPHORA  
CALIGINOSA (ANNELIDA: OLIGOCHAETA).**

By

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**Abstract**

In this study, 639 of *Allolobophora caliginosa* were collected from different regions of Nile Delta of Egypt. *Metaradiophrya egyptiaca* n. sp. was recorded in intestinal worms from Bajour (Monoufia Governorate) only. Ciliate species ranged in size from 100-137 $\mu$ m (125.7 $\pm$ 9.9 $\mu$ m) in length & 57-88 $\mu$ m (72.5 $\pm$ 10.7) in width. Macronucleus is long, band-shape, varied between 71-106 $\mu$ m (92.8 $\pm$ 10.2 $\mu$ m) in length x 5.6-10.3 $\mu$ m (7.3 $\pm$ 1.5 $\mu$ m) in width. Ciliate has 2 rows of contractile vacuoles on both side of macronucleus, each row of 4-6 vacuoles. There were 75-84 kineties on body dorsal side and 56-60 kineties on ventral one. The 58 ectoplasmic fibers restricted in anterior ventral depressed region, from which 12-13 ones end on ectoplasm left clear area and 9 fibers from distal end of the right shaft.

**Keywords:** *Metaradiophrya egyptiaca*, *Allolobophora caliginosa*, Astomatous ciliate, Egypt.

**Introduction**

Earthworms (Annelida, Oligochaeta) are recognized to harbor symbiotic astomatous ciliates (order Astomatida) in intestines (Pitelka, 2013). These ciliates lack mouth and covered by uniform cilia; and some astomes have suckers, but others use various hooks or barbs. Genus *Metaradiophrya* belongs to class Oligohymenophorea, subclass Hymenostomatia, order Astomatida & family Radiophryidae (Lynn, 2008). Heidenrich (1935) established genus *Metaradiophrya* with 2 *Hoplitophrya* species Stein, 1860, *H. lumbrici*, and *H. falcifera* Stain, 1861. Genus *Metaradiophrya* is characterized by its anterior ventrally placed attaching apparatus consisted of a curved ventrally directed central spine (hook), continuous with a larger shaft lying on the right side of the cell body. Also, some species have a smaller shaft which is attached to the central spine and laid on the left side of the cell body. Many ectoplasmic fibers are connected to attaching apparatus for supporting (collectively referred to as cytoskeleton). The number and distribution of ectoplasmic fibers were constant and species character (De Puytorac, 1954). Several *Metaradiophrya* species described as *M. asymmetrica* (Beers, 1938); *M. chlorotica*, *M. wenrichi* (Williams, 1942);

*M. lituiformis* (Georgevitch, 1941); *M. hova-ssei*, *M. varians*, *M. gigas*, *M. heidenreichi* and *M. bifulta* (De Puytorac, 1954); *M. gardneri* (Rees, 1961); *M. pheretimi* (Bhandari, 2012) & *M. simplex* (Fokam, 2012). De Puytorac (1954) gave a monograph on the astomatous ciliates, with wide morphological and taxonomic data of *Metaradiophrya* and diagnostic descriptions for known species. Molecular techniques were used in exploring genetic diversity and phylogenetic position of subclass Astomatia (Fokam *et al*, 2011), but these techniques for specific astomatous ciliates identification not yet used.

In Egypt, insufficient studies were done on the endosymbiotic ciliates of annelids, and the present study aimed to explore biodiversity of the ciliates infecting the Egyptian Annelids, especially Oligochaetes.

**Materials and Methods**

*Allolobophora caliginosa* worms were collected from Bajour (Menoufia Governorate), Marg (Qalyobia Governorate), Embaba (Giza Governorate) and Magaga (Menia Governorate). Worms were trapped by a labeled plastic container with some local damp soil and immediately transport to laboratory. They were put in a sieve under running tap water to remove attached debris and soil grains. Each one was cut transversely into

small parts in clean dish; each part was dissected on clean glass slide with a drop of normal saline solution (0.8%), covered and microscopic examination. After examination, slides were semidried and fixed for 20 min. in Schaudin's solution (saturated aqueous solution of mercuric chloride & few drops of glacial acetic acid). A group of slides were washed in ethanol to remove excess mercuric chloride, transferred to 3% iron alum solution for 3hrs and stained with Heidenhain's haematoxylin stain (20min.), differentiated in 1% iron alum solution, washed in distilled water, dehydrated, cleared in xylene and mounted in Canada balsam. Another group of slides were stained in haematoxylin stain for 30min., transferred to a tap water for one minute, and then dipped in eosin stain. Silver line system was revealed by the wet silver nitrate method (Foissner, 2014). Photomicrographs were taken by a Kodak digital camera (model 1450Z) attached to a compound microscope.

### Results

From 639 totally collected worms, 44 ones were from Bajour (Menoufia G.), of which 12 ones were infected with *Metaradiophrya egyptiaca* n. sp. This species was very active and usually found in the anterior two thirds of the host's intestine.

General morphology: *Metaradiophrya egyptiaca* n. sp. has a broad short and dorso-ventrally flattened body, which was slightly widest at the middle portion (Fig.1 A). The anterior extremity was more or less pointed while the posterior one round or truncated in a plane that was oblique on body longitudinal axis. The dorsal side was convex and the ventral one was more or less flattened (Fig. 1A). Laterally, the body thickness gradually increased from the posterior extremity to anterior one. Ventrally, an anterior concavity formed a depression, immediately below the attaching apparatus, where the cilia were beat independently creating circular waves. This region was often closely applied to the host's tissue to fix ciliate into the intestinal wall. Body length varied between

100 $\mu$ m & 137 $\mu$ m (125.7 $\pm$ 9.9 $\mu$ m), but width varied between 57 $\mu$ m & 88 $\mu$ m (72.5 $\pm$ 10.7 $\mu$ m) on the body modally. Some cases of cellular division were observed.

The cilia were slightly longated at the cell posterior part (11 $\mu$ m) than those in the anterior one, generally, did not exceed 7 $\mu$ m; besides, the cilia were shorter on the ventral surface than those on the dorsal one. By observing the active ciliate, the cilia synchronous waves of strokes originated in the body lower right side as an oblique wave (wave direction form an angle with body longitudinal axis) that propagated upward on the upper left side (Fig.1B).

The ciliate moved in saline media either by rotating forward its longitudinal axis as counterclockwise (Fig.1Fa) or progressing on its ventral side; of body anterior part up forming an angle with movement direction of (Fig. 1Fb). Macronucleus was long, band-shape, narrow and somewhat irregular in outline (Fig. 2A), varied between 71 $\mu$ m & 106 $\mu$ m (92.8 $\pm$ 10.2 $\mu$ m) x 5.6-10.3 $\mu$ m (7.3 $\pm$ 1.5 $\mu$ m) in width. Sometimes, macronucleus formed a kind of spiral path along the animal longitudinal axis (Fig. 2B). The micronucleus was hardly seen, being spherical, 2.6 $\mu$ m in diameter for an individual ciliate 130x88 $\mu$ m, situated on the ciliate lower right side adjacent to macronucleus (Fig. 1 A & Fig. 2 A).

The ciliate has two rows of contractile vacuoles arranged on either side of macronucleus. Each row composed of 4-6 vacuoles more or less straightened in course (Fig. 1A & Fig. 2A). Average vacuoles number in a single ciliate was 9 (12-7vacuoles), smaller ones were about 2.7 $\mu$ m in diameter and the larger ones reached 8 $\mu$ m.

Attaching apparatus (cytoskeleton) was typical in general form for generic characterization. It formed an inverted asymmetrical V shape, where the shaft, lied on body right side was much longer than central ventrally curved spine prominently on ciliate anterior medial line (Fig. 1A). This system was easily stained by H & E (Fig. 2D). The shaft

size varied between 17µm & 28µm (22±3) in length. Central spine varied between 8µm & 12µm (10.2±1.2) in length with an irregular diameter; reached 2.5µm in its width. No evidence of an articulation between the spine and shaft. On left side of central spine was an area of ectosarc with neither any ectoplasmic fibers nor kineties (Fig. 1C, D & Fig.2 E). This area was up to 9µm long with sometimes appearance of second short shaft connected to the attaching apparatus.

There were 75-84 kineties on the dorsal side and 56-60 kineties on body ventral one, with a total number of 131-144 kineties around the body. Intervals between kineties on ventral side was asymmetrical, these intervals between the kineties on the ciliate left side were greater than those on right side, 1µm & 0.7µm respectively, (Fig. 1E). On dorsal side, the intervals between kineties were alike and more closely spaced than intervals of kineties on ventral one, 0.6µm (Fig. 2C). Moreover, the dorsal kineties on anterior region were generally directed toward the right where they converge slightly. Ventral kineties on left side turned more anteriorly to the right direction in course mid line to end around attachment apparatus (Fig.1C).

Starting from the distal end of right shaft to the hook base about 30-41 kineties originated ventrally, but dorsally were 55-60 ones. In the left clear area, 9-14 kineties originated ventrally and 20-24 ones originated dorsally (Fig.1C). Posteriorly, dorsal and ventral rows of kineties showed little or no convergence toward the midline but end independently along truncated posterior border (Fig. 2E).

Ectoplasmic fibers, supported the attaching apparatus, were mainly restricted on anterior ventral depression region (Fig. 1D & Fig. 2F). The longest ones were located on the depression left side, and gradually shorten towards right side. Ectoplasmic fibers were beside to the ciliary rows and nearly parallel with them. On the ciliate right side, the fibers were closely packed than those on the left side. The number of fibers was 58, which course forward to attachment apparatus, from which 12-13 ones end on ectoplasm left clear area. Dorsally, 9 fibers originated from the right shaft distal end that course posteriorly for a certain distance (Fig. 1D & Fig. 2F). Generally, ectoplasmic fibers supported right shaft of distal end were the thickest.

Table 1: Comparison between *Metaradiophrya egyptiaca* n. sp. and other related species.

Species	Body Dimen. (µm)	Contract. vacuoles	Attaching apparatus		Ectoplas. fibers (ventral)	Kineties of body
			Shaft (µm)	Spine (µm)		
<i>Metaradiophrya egyptiaca</i> Present work	125.7 x 72.5	4 - 6 each side	22 ± 3.1	10.2 ± 1.2	57-58	(131-144) 75-84 dorsal 56-60 ventral
<i>M. gigas</i> De Puytorac, 1954	550 x 165	10-12 each side	40 x 3.5	17 x 4	65	(137) 66 dorsalex71 ventral
<i>M. lumbrici</i> De Puytorac, 1954	150 x 75	5-9 each side	25-30 x 2.5	10- 11	62 longer in med-line	(155) 84 dorsalex71 ventral
<i>M. heidenreichi</i> De Puytorac, 1954	195 x 75	----	35 x 2.6	12-13	58 asymmetric arrangement	(138) 75 dorsalex63 ventral
<i>M. asymmetrica</i> Beers, 1938	115-150 x 55-70	3-6 each side	30 x 2	10 fused with shaft	25-30	(100-120) with interval 1 µm
<i>M. simplex</i> Fokam, 2012	115-223 x 52-150	8- 23 in two rows	44-70	No spine	74	57-102 dorsal 46-70 ventral
<i>M. wenrichi</i> Williams, 1942	76-152 x 29-58	-----	similar to <i>M.</i> <i>lumbrici</i>	similar to <i>M.</i> <i>lumbrici</i>	----	----

### Taxonomic summary

*Type host:* *Allolobophora caliginosa* (Annelida: Oligochaeta).

*Type locality:* Hosts were collected from Bajour (Menoufia Governorate). 30°25'54.5" N 31°02'08.5"E

*Prevalence:* 12/639 (1.8%)

*Site of infection:* Intestine.

*Etymology:* Species name *egyptiaca* derived from the country name, Egypt.

*Type specimens:* Permanent prepared slides deposited in Parasitological Collection, Zoology Department, Faculty of Science, Ain Shams University, Cairo 11566, Egypt.

## Discussion

In this study, *Metaradioprhya egyptiaca* n. sp. was found in a population of *Allolobophora caliginosa*. The morphological characteristics determined its generic name, coincided with those characterizing this genus (Heidenreich, 1935; Hovasse, 1945; De Puytorac, 1954), ciliate with the prominent anterior inverted V-shaped attachment apparatus.

The attaching apparatus composed of a curved ventrally directed central spine or hook, which was a part of a larger shaft lying on the cell body the right side. In some species, apparatus included a smaller second shaft attached to the central spine and lying on cell body left side.

The present species was similar to *M. gigas*, *M. lumbrici*, *M. heidenreichi*, *M. asymmetrica*, *M. pheretimi*, *M. gardneri*, *M. simplex*, *M. wenrichi* and *M. lituiformis* without the second shaft, which was well developed on the left side of other species, e.g. *M. varians*, *M. bifulta* and *M. hovassei*. Within the range of size of the present parasite were *M. lumbrici* Heidenreich (150x75µm), *M. heidenreichi* De Puytorac (195x75µm), *M. asymmetrica* Beers (115-150x55-70µm) and *M. wenrichi* Williams (76-152x29-58µm).

Heidenreich (1935) described *M. lumbrici* from *Lumbricus rubellus* and *Eisenia foetida* and reported that hook articulated with the slightly curved right half of the attaching apparatus. But, in the present study, no evidence was observed for any kind of movement to hook with the right shaft of attaching apparatus. De Puytorac (1954) characterized *M. lumbrici* by 5-9 contractile vacuoles on each side; 84 dorsal and 71 ventral kineties, which were irregularly spaced (totaling 155 kineties). Paisán *et al.* (2009) described *M. lumbrici* from the intestine of *Eisenia foetida*, they reported that contractile vacuoles were 12 on each side, besides, 83 dorsal and 54 ventral kineties (totaling 137 ones).

Generally, the present ciliate possessed 4-6 as maximum contractile vacuoles on ciliate each side, 56-60 ventral and 75-84 dorsal kineties that totaled 131-144 kineties around

the body. Heidenreich (1935) reported that the ectoplasmic fibers on the left half of *M. lumbrici* body originated ventrally along the left antero-lateral margin of the cell quite as if the missing left half of the inverted V-shaped apparatus was present. But, other fibers originated ventrally along right shaft of V-shaped apparatus, which was well-known marked in the present species. On the other hand, Williams (1942) reported that the longest ventral ectoplasmic fibers of *M. lumbrici* were present on the central portion of the concavity and were shorten gradually on both sides. Also, he noted that fibers on the ciliate right side were finer than those on the left side. In present species, the longest ectoplasmic fibers were present on the ciliate left side decreased gradually in lengths toward extremity, besides, fibers on the ciliate right side, especially around the right shaft end were thicker than those on the left side.

*M. heidenreichi* differed from the present species in that right shaft of attachment apparatus exceeded 35µm in length (compared to 28µm in the present one). Besides, proximal end of spine was abruptly curved at right angle, enlarged with a rounded base for articulation with the right shaft of attaching apparatus. The last feature was not seen in the present ciliate where the spine was completely fused with the right shaft without evidence of any articulation or movement to spine detection during continuous studying of live specimens. De Puytorac (1954) described 58 ventral supporting ectoplasmic fibers in *M. heidenreichi*, with an asymmetrical arranged with the shortest fibers was present in latero-ventral side while longest ones were located in mid-ventral side and extended over almost the body length. In the present study, the fibers in mid-ventral side did not extended to body posterior region.

*M. asymmetrica* was described by Beers (1938) from *Eisenia lonnbergii* intestine. This ciliate was characterized by having only ventral ectoplasmic fibers supporting the attachment apparatus, restricted to the dep-

ressed antero-ventral surface. He reported that all of supporting fibers (25-30 ones) originated from the right shaft and those on the left half of ciliate arise in the midline near the hook base and diverged slightly over the body posterior course. Also, Beers stated that a small area on the right side was devoid of fibers. In the present ciliate, 12-13 fibers originated ventrally from the ectoplasm left clear area; and the ventral fibers were directly developed along the extremity of right shaft without free-fiber area. The total number of kineties of *M. asymmetrica* was 100-120 ones with an interval 1  $\mu\text{m}$ , but the present one possessed 131-144 kineties. Williams (1942) described new species of *Metaradiophrya*, *M. wenrichi* from unidentified earthworm species. He reported that his species was very similar to *M. lumbrici* in general dimensions with the attachment apparatus morphometric data. On the other hand, he added that macronucleus was anteriorly curved strongly to right, sometimes; swell anteriorly so that the entire macronucleus resembled a spear. But, he neither gave details about kineties number, contractile vacuoles nor attachment apparatus-supporting fibers.

Away from the present ciliate size, many species of *Metaradiophrya* were described from *Allolobophora* sp. intestines; *M. falcifera* from *A. caliginosa* by Cépède (1910), De Puytorac (1954) and Rees (1961), *M. hovassei* from *A. chlorotica* and *A. caliginosa* by De Puytorac (1954) and Rees (1961) respectively, and *M. gigas* from *A. savignyi* by De Puytorac (1954). *M. falcifera* was characterized by a conspicuous torsion in the body anterior part to the right side, which resulted on the displacement of skeletal right shaft behind the anterior end of cell. Besides, ciliate possessed a large left skeletal branch; a homologue of skeletal left stem in *M. varians*, *M. biflta* and *M. hovassei*. By contrast, the present ciliate has an area of ectosarc on the spine left side, which have neither kineties nor skeletal elements. *M. gigas* was differentiated than the present ciliate by its huge

size, reached 550 $\mu\text{m}$ x165 $\mu\text{m}$ , 10-12 contractile vacuoles in each row and 137 kineties around the body.

### Conclusion

In the present study, it is justified to consider *Metaradiophrya* a new species; *Metaradiophrya egyptiaca* characterized by moderate size, ventral ectoplasmic fibers restricted to antero-ventral depression with characteristic arrangement, without left skeletal element and low number of contractile vacuoles, 4-6 in each row.

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#### Explanation of Figures

Fig. 1: A- General morphology of the body. Rs: right shaft, Cs: Central spine, Ma: Macronucleus, Mi: Micronucleus, Cv: Contractile vacuoles. B- Synchronous waves of cilia strokes originated in lower right side of body as oblique waves. C- Arrangement of kineties around attaching apparatus. Ca: Clear area. D- Arrangement of ectoplasmic fibers around attaching apparatus (ventral side). E- Silver line system of ciliate. Ki: Kineties. F- Modes of movement of ciliate.

Fig. 2: A- General morphology. Black arrowhead: micronucleus, white arrowhead: contractile vacuole. Heidenhain's haematoxylin. B- Macronucleus exhibits a kind of spiral path along the longitudinal axis of ciliate. Heidenhain's haematoxylin. C- Kineties on dorsal side, narrowly spaced. D- Attaching apparatus of ciliate and its ectoplasmic fibers. Haematoxylin & Eosin stain. E- Kineties on the ventral side of ciliate, note left kineties converge toward attaching apparatus. F- Enlarged view for antero-ventral area showing ectoplasmic fibers. Left black arrowhead: fibers on distal end of left shaft, right black arrowhead: longest fibers, white arrowhead: short fibers originate from extremity of left shaft



