



Prevalence of Intestine dwelling ciliates and Morphological details of *Anoplophrya infundibulii* (n. sp.) from Earthworm, *Pheretima posthuma* from Nashik district of Maharashtra, India

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ABSTRACT

Many ciliates species are the common parasites found in Indian earthworm *Pheretima posthuma*. During the period of two years (Jan. 2007 to Dec. 2008) total 2609 number of earthworm animals were examined. In the first year (Jan. 2007 to Dec. 2007) 1146 *P. posthuma* were examined, 693 of these were positive for ciliate infection. The percentage of prevalence being (60.47%), in second year (Jan. 2008 to Dec. 2008) total 963 animals were examined, 560 of these were positive with ciliates. The percentage of prevalence was 58.15%. A month wise analysis of the percentage of prevalence of ciliates during the first year (Jan. 2007 to Dec. 2007) was maximum in June to August (83.33%, 81.88%, 84.32%), minimum in April and May (36.19%, 36.67%) and moderate in remaining months. While In second year (Jan. 2008 to Dec. 2008) the maximum percentage of prevalence showed during August and September (84.72%, 81.33%), minimum in February to May (49.43%, 45.45%, 50.67%, 50.98%) and moderate in remaining months. While the observation of gut of the host *P. posthuma* it is seen that the four species of genus *Anoplophrya* were investigated. Out of that the two species are new to the sciences which are *A. chakravartii*, *A. krishnamurthii*, *A. nikamai* n. sp. and *A. infundibulii* n. sp. The parasite ciliates were first observed in 0.6% saline solution fixed in Schaudin's fixative and then stained with phaspho-tungastic acid hematoxyline stain.

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Introduction

Anoplophrya was reported by Stein (1860). Genus *Anoplophrya* belong to order Astomatida of Class Oligohymenoporea which is representative of the primitive and most controversial Ciliophoran. *Anoplophrya* belongs to Subclass Astomatia (Schewiakoff 1896), or Hymenostomatia (Levin 1980). Ciliates belongs to this subclass are 'mouth less' symbionts (sometimes Parasites) living in guts of annelids especially oligochaetes Somatic monokinetids like those of other Oligohymenoporea; with a divergent post ciliary ribbon (absent in some genera) distinct anteriorly directed kinetodesmal fibril originating near triplets 5-7; a radial transverse ribbon near triplets 3,4 (reduced to distinct) and extending laterally towards adjacent kinety; cortical cytoskeleton in thigmotactic region may be conspicuously developed as anterior attachment structure.

The order Astomatida, composed of entirely endosymbiotic form, has long puzzled protozoan phylogeneticists, although progress has certainly been made in recognizing that the loss of a mouth here is a secondary and thus regressive character, not a primitive one and that features of the infraciliature *senso lato* may be used to advantage in trying to determine the most likely origin of the group. From an overall approach the Astomes may continue to be characterized as relatively large uniformly ciliated mouth less ciliates. Unique characters include an almost universal infraciliary endoskeleton is of varying complexity and, in many species, the areas thigmotactic ciliature or even elaborately developed 'hold-fast' organelles in the form of

hooks, spines, spicules or suckers. Fission is straightward, particularly in that it is uncomplicated by Stomatogenesis, but separation of the product is often incomplete, result in formation of catenoid colony (Corliss 1979). The typical body form is ovoid to elongate, with a flattening apparently related to the habit of lying up against the intestinal epithelium of the host's digestive system.

Rows of somatic cilia converge at the poles in characteristic patterns. The suture area or 'air secante' at anterior end pole is of particular diagnostic importance. Contractile vacuoles are sometime straight out in long line, and mucocysts are present. Feeding is by osmotrophy. The parasitic mode of life tends to simplification of the structural differentiation of any persisting organ system by reduction in numbers of repeated element and restriction structural modifications among parts of series of organs. The astomatous ciliate such as *Anoplophrya*, lives in the fluids of the digestive tract of earthworms and absorb food from the medium in which they are immersed, exhibit the loss of pharyngeal or organelles consisting in a other ciliates, of especially differentiated cilia and even membranelles the function of which in the ancestor of the astomatous ciliates was to pick up organisms such as bacteria and pass them in to and down the pharynx into the endoplasm often shaping them into food ball at the inner end of this circumscribed route. Ciliates serving this function, there are structural differentiation and their specialized groupings have been described by Lund (1933) in *Paramecium* and Rosenberg (1937) in *Nyctotherus*.

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Since *Anoplophrya* has neither mouth or pharynx, it is a matter of interest to find out how far its parasitic mode of life has reduced the pharyngeal apparatus and the motorium, found near the pharynx and from which fibrils are sent out to the pharyngeal apparatus and to other parts of ciliary mechanism. Because of the relatively simple structure of the astomatous ciliates their systematic has been in a state of confusion. Heidenreich (1935) described few species of *Anoplophrya*.

Several later described species of *Anoplophrya* are *A. allure* Ceped, *A. complanata* and a species by Exsemplerskaja. He considered the commonly accepted name. *A. striata*, Dujardin is as a synonym of the earlier *A. lumbrici* (Shrank 1803).

Genus *Anoplophrya* was also redscribed by Claparede (1860), Leidy (1877); Kent (1881), Balbiani (1885) and Butschli (1888). The general body form is elongate, cylindrical or slightly flattened, with rounded ends. The posterior end of the body is tapering in some species. The body is striated with clearly defined often depressed line which runs longitudinally and sometimes spirally. The contractile vacuoles are usually placed in rows upon the edges. The macronucleus is almost always long and band form, and generally extending through the entire length of the body. Micronucleus may be distinct in some species where as absent in other. Reproduction is affected by simple cross division or by budding at the posterior end, and is frequently combined with chain formation. The main characteristics are the entire absence of mouth. A number of workers consider this mouth- less forms representing the most primitive order of holotrichs (Class Oligohymenophorea, de Puytorac et al., 1974). It is also not unlikely that are secondarily degenerate forms showing some specialization which may be associated with their entirely symbiotic mode of life (Corliss 1956).

There is a single compact macronucleus with a single lenticular micronucleus. Macronucleus is of elongated or ribbon shaped where as in some cases it is spherical. Taxonomically, the Astomatida is dividing in three major groups. Du Puytorac (1972) has recognized super families. Genus *Anoplophrya* has super family Anoplophryodae.

Family Anoplophryidae erected by Cepede (1910). He also recognized in his monograph several families like Discophryidae, Kofoidellidae, Haptophryidae and Maupasellidae for ciliates from different invertebrate groups. Subsequently several contributions have been made by Ghosh (1918). Heidenreich (1935) Georgevitch (1941), de Puytorac (1972, 1974) has contributed a series of papers on astomates. Ghosh (1918) described a new species *A. lloydi* from the seminal vesicle of Indian earthworm from *Pheretima posthuma* and *A. pheretimi*. Raychaudhuri, Haldar and Chakravarty (1969) from alimentary canal of same host.

Genus *Anoplophrya* has following species.

- A. lumbrici* (Shrank, 1803)
- A. lloydi* (Ghosh, 1918)
- A. marylandensis* (Conklin, 1935)
- A. orchestii* (Summer, 1936)
- A. chakravartii* (Lalpotu, 1976)
- A. perionychis* (Lalpotu, 1976) and (T.T. Shaikh, 2006)
- A. foldii* (T.T. Shaikh, 2006)
- A. bifoldii* (Bhandari, 2010)
- A. pheretimi* (Bhandari, 2010)
- Anoplophrya infundibulii* (n. sp.)

Material and methods

The hosts were collected from different parts of hilly regions of Nashik dist (Deola, Nandgaon; Surgana, Kalwan Satana) of Maharashtra state. Due care was taken and the hosts were collected in moist soil with decaying leaves were present and the temperature was maintained by using ice bags around them. Mostly the hosts were collected during morning and evening.

During a period of two years (Jan. 2007 to Dec. 2008) the earthworm species *Pheretima posthuma*, were examined for ciliate infection. In which four species of ciliates were investigated from the gut of earthworms, all the four species of ciliates are belongs to Genus *Anoplophrya*, they are,

1. *Anoplophrya chakravartii*
2. *Anoplophrya krishnamurthii*
3. *Anoplophrya nikamai* n. sp.
4. *Anoplophrya infundibulii* (n. sp.)

Out of four the two species are new to the science. The present morphological study is concern with *Anoplophrya infundibulii* (n. sp.). The hosts were collected from Nashik district of Maharashtra. Earthworms were examined for the ciliates. Entire alimentary canal of host animal was examined. For the observation or detection of the faecal matter was mixed with 0.6% saline solution and observed under microscope. When sample found positive it was treated with permanent preparation, for that tungsten phosphoric haematoxyline method was used along with Lugol's solution and hyposolution. For fixation of ciliates and gregarines Schaudinn's fixative was used. Dry silver impregnation method was also used for ciliates.

Topography-



Plate- 1 showing Map of Nashik District of Maharashtra, India

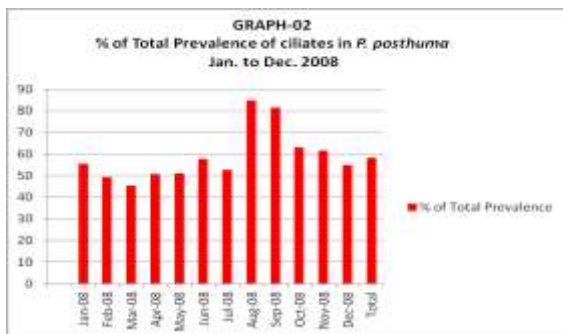
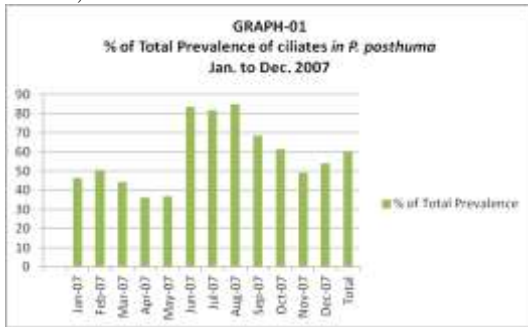
Result and Discussion

The Percentage Prevalence ciliates in *Pheretima Posthuma* are counted for the period of two years, January 2007 to December 2008 which is shown year wise in table No. 1 and 2, with the graphs.

During the period of two years (Jan. 2007 to Dec. 2008) total 2169 earthworms were examined. In the first year (Jan. 2007 to Dec. 2007) 1146 individuals were examined, 693 of these were positive for ciliate infection. The percentage of prevalence was found 60.47%. In second year (Jan. 2008 to Dec. 2008) total 963 *P. posthuma* species were examined 560 of these were positive. The percentage of prevalence was 58.15%.

A month wise analysis of the prevalence in first the year (Jan. 2007 to Dec. 2007) showed the maximum percentage of prevalence during June to August (83.33%, 81.88%, 84.32%), minimum in April and May (36.19%, 36.67%) and moderate in remaining months. In second year (Jan. 2008 to Dec. 2008) the maximum percentage of prevalence showed during August and

September (84.72%, 81.33%), minimum in February to May (49.43%, 45.45%, 50.67%, 50.98%) and moderate in remaining months. The details of the number of earthworms examined and the month wise prevalence are shown in Table No. 1, and 2.



Morphological details:

Description of the species:

This species of *Anoplophrya* was found in the intestine of earthworm *Pheretima posthuma*. Hosts were collected besides the Girna River and Deola (Nashik) college campus. The parasites were usually numerous in the infected worms. They were found only in the posterior part of the intestine.

In living condition the ciliate appears as transparent, elongated and bell shaped with distinct granular, elongated macronucleus with spherical and large contractile vacuoles. It shows very fast movements, it moves anterior narrow end forwards, and due to rounded bell shaped body it rotates itself in a circular manner and pushes forward. These ciliates also perform variety of movements.

The body is elongated; anterior end is narrow and elongated, while posterior end is broad and rounded. This gives bell shape appearance to the body of the organism. Body is slightly concave only at antero-ventral side where as convex at the dorsal side. Posterior side is rounded. It is seen that both the lateral sides of the body show very short folds. The body measures 50 μ to 87.5 μ in length and 22.5 μ to 32.5 μ in width. Body is covered with uniform coat of cilia. Cytoplasm appears uniformly granulated; both ectoplasm and are distinct.

Macronucleus is elongated, centrally placed, both the ends are rounded, anterior end is slightly narrow than the posterior. It covers total 1/3 of body length approximately. The length of the macronucleus is 15 μ to 27.5 μ , where as width is 5 μ to 7.5 μ . Its outline is smooth. The micronucleus is small and spherical and lies close to the middle of right side of the macronucleus. The number of contractile vacuoles is varying from 6 to 10. They are rounded and present at the posterior end, in the circular manner in the cytoplasm and looks like a group of pearls. The number of kineties is 60 to 120 depending up on the body dimensions.

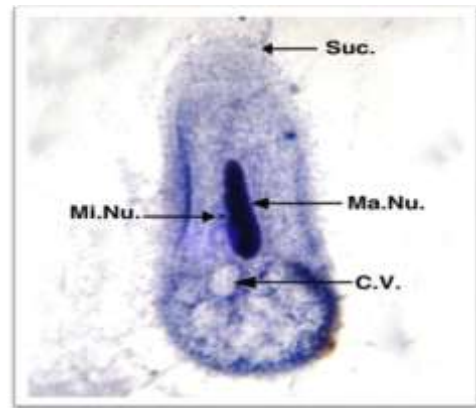


Fig. A- *Anoplophrya infundibulii* (n.sp.) W.M



Fig. B- *A. infundibulii* (n.sp.) Binary Fission

Comments

The genus *Anoplophrya* is almost very common in alimentary canal of several oligochaetes since nineteenth century many early research workers have been published and reported several species of this genus including Shrank (1803), S. Raychaudhari et al (1969) and Puytorac (1954, 1959, 1960, 1961 and 1972).

As already stated that de Puytorac, (1972) had erected a super family Anoplophryodae for this ciliate and also listed number of host from oligochaetes and astomatous ciliates found in them. He also gave an up to list of 15 species of genus *Anoplophrya* found in different host from different countries of the world. He described only one species of *Anoplophrya* from Indian earthworm (*Peretima posthuma*) Gosh (1918) have describe the same species from the seminal vesicles of these worms from Bengal. *A. lumbrici* which was reported by Schrank (1803), Heidenrich (1935) rediscrined species of *Anoplophrya* including *A. allure* (Ceped), *A. complanata*, (Rossolimo) *A. merylandnesis* (Conklin).

M. K. Biwas (1974) has rediscrined the same species i.e. *A. lumbrici* from a new host *Pheretima peguyana* from Calcutta. *A. chakravartii* and *A. perionychis* (n.sp.) described by Lalpotu from the host *Perionychis* (1976), *A. foldi* from *P. posthuma* (n.sp.) by T. T. Sheikh (2006) from Aurangabad district and *A. bifoldii* (n. sp.) and *A. chakravartii* (redescribed) from *P. posthuma* is described Bhandari (2010).

The present species shows different shape and body dimensions than that of the previous species. The shape of body is cylindrical to pyriform in *A. branchiarm* (Stein, 1852), oval in *A. pheretimi* (Raychaudhari, 1969), oval and elongated in *A. lumbrici* (Sharnk, 1803), elongated oval in *A. perionychis* and *A. chakravartii* (Lalpotu, 1979), elongated, oval, anterior end is narrow, posterior end is broad in *A. merylandnesis* (Konlkin, 1935), elongated, oval small, both ends are rounded in *A. krishnamurthii* (Bhandari, 2010), elongated with left lateral fold

in *A. foldii* (Shaikh, 2006), elongated with both lateral folds in *A. Bifoldii* (Bhandari, 2010), leaf like in *A. nikamai* (n. sp. by present author), while in present species body shape is elongated and bell shaped.

The number contractile vacuoles is single in *A. branchiarm*, 3 to 6 in two rows in *A. pheretimi*, 3 in posterior end in *A. lumbrici*, 5 very small in two rows in *A. perionychis*, 3 to 7 in *A. chakravartii*, 4 in *A. merylandnesis*, 4 arranged in two rows in *A. krishnamurthii*, 7 to 18 in *A. foldii*, 19 to 24 scattered irregularly in *A. bifoldii*, single large and oval in *A. nikamai* while 6 to 10 arranged in circular disc at the posterior end in present described species.

Macronucleus is long and showing banding pattern in *A. branchiarm*, long and ribbon like in *A. pheretimi*, ribbon shaped with irregular outline in *A. perionychis* & *A. krishnamurthii*, elongated in *A. chakravartii*, ribbon like with several fine projection in *A. merylandnesis*, ribbon like in *A. foldii*, elongated and ribbon like in *A. bifoldii*, long and S-shaped in *A. nikamai* (n. sp.) while in the present species it is shorter than all the above species with narrow anterior end and broad & rounded posterior end. Comparative analysis of various species of *Anoplophrya* is shown in Table No. 3.

The species described by present author is compared with previously described species. It is seen that some distinct features are found in this species which are as follows.

1. Body is bell shaped, narrow anterior end and broad rounded posterior end.
2. Number of contractile vacuoles is 6 to 10 and are rounded arranged in circular manner at the rounded posterior end in the cytoplasm.
3. Macronucleus is ribbon like in *A. pheretimi*, *A. lumbrici*, *A. perionychis*, *A. merylandnesis*, *A. krishnamurthii*, *A. foldii* and in *A. bifoldii*. It is long with bands in *A. branchiarm*, elongated in *A. chakravartii* while it is elongated with narrow anterior end and broad rounded posterior end in the present species.

In view of its distinct features this species is considered new to the science, it looks very beautiful because its bell shape. It is designated as *Anoplophrya infundibulii* (n.sp.).

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TABLE 1.				
SHOWING THE MONTHWISE PREVALENCE OF CILIATES IN EARTHWORMS (<i>Pheretima Posthuma</i>) during the period from Jan.2007 To Dec.2007				
Sr No	Months	Total No. of	No. of	% of Total
		Hosts Examined	.+ve samples	Prevalence
1	Jan-07	140	65	46.43
2	Feb-07	75	38	50.67
3	Mar-07	102	45	44.12
4	Apr-07	105	38	36.19
5	May-07	90	33	36.67
6	Jun-07	150	125	83.33
7	Jul-07	138	113	81.88
8	Aug-07	112	95	84.82
9	Sep-07	95	65	68.42
10	Oct-07	44	27	61.36
11	Nov-07	47	23	48.94
12	Dec-07	48	26	54.17
	Total	1146	693	60.47

TABLE 2.				
SHOWING THE MONTHWISE PREVALENCE OF CILIATES IN EARTHWORMS (<i>Pheretima posthuma</i>) during the period Jan.2008 To Dec.2008				
Sr No	Month	Total No. of	No. of	% of Total
		Hosts Examined	.+ve samples	Prevalence
1	Jan-08	45	25	55.56
2	Feb-08	87	43	49.43
3	Mar-08	77	35	45.45
4	Apr-08	75	38	50.67
5	May-08	102	52	50.98
6	Jun-08	104	60	57.69
7	Jul-08	110	58	52.73
8	Aug-08	72	61	84.72
9	Sep-08	75	61	81.33
10	Oct-08	65	41	63.08
11	Nov-08	47	29	61.70
12	Dec-08	104	57	54.81
	Total	963	560	58.15

Table 3- Comparison of the present species with the species of *Anoplophrya*

Sr. No.	1	2	3	4	5	6	7	8	9	10
particulars	<i>A. branchiarms</i> Stein (1852)	<i>A. pheretimi</i> S. Raychaudhari (1969)	<i>A. lumbrici</i> sharnk (1803)	<i>A. perionychis</i> Lalpotu (1979)	<i>A. chakravertii</i> Lalpotu (1979)	<i>A. merylandnesis</i> Konlkin (1935)	<i>A. kirshnamurthii</i> (n. sp.) Bhandari (2010)	<i>A. foldii</i> T T Shaikh (2006)	<i>A. bifoldii</i> (n. sp.) Bhandari (2010)	<i>A. infundibulii</i> (n. sp.) present author (2010)
Body shape and dimensions	Cylindrical to pyriform L – 104 to 136 μ	Oval shape L – 49.5 to 188.8 μ W – 23.1 to 46.5 μ	Oval elongated L-180 to 250 μ W-50 to 70 μ	Elongated oval L-67 to 120 μ W-28 to 43 μ	Elongated oval ant. End. Narrow post. End broad L-63 to 179.6 μ W-28.4 to 94.6 μ	Elongated oval ant. End. Narrow post. End broad L-36.72 μ W-16.42 μ	Elongated oval small both ends rounded L-25.63 to 39.6 μ W-16.2 to 20.97 μ	Elongated with left lateral fold L-58 to 112 μ W-30 to 48 μ	Elongated with both left lateral folds L-132.81 to 186.4 μ W- 32.62 to 55.92 μ	Elongated bell shaped ant. End narrow post. End broad L-50-87.5 μ W-22.5-32.5 μ
Contractile vacuoles	Single	Two, rows each side of macronucleus 3 to 6 in each	3, post. End	5, two very small	3-7	4, arranged in longitudinal rows	Four, arranged in two rows, 2 in each row	Two rows each side of macronucleus 7-18 in each rows	19-24 scattered irregularly	6 to 10 circularly arranged at the post. end
macronucleus	Long band formed 30-89 μ	Long ribbon shaped 33-92 μ	Ribbon shaped 100-120 μ	Ribbon shaped irregular outline	Elongated ant. end pointed post. end broad	Ribbon shaped several fine projections	Ribbon shaped with irregular outline 23.3 to 69.9 μ	Ribbon shaped	Elongated ribbon shaped L-93.21 to 51.45 μ W-4.6 to 6.99 μ	Somewhat elongated ant. end narrow rounded post end broad rounded L-15 to 27.5 μ W-5 to 7.5 μ
micronucleus	Spherical	Spherical	Elongated somewhat banana like	Spherical	Spherical	Spherical
Kineties	60 to 120
Host	<i>P. posthuma</i>	<i>Octoplasium cyancum</i>	<i>Perionychis</i>	<i>Perionychis</i>	<i>Lumbricus terrestris</i>	<i>P. posthuma</i>	<i>P. posthuma</i>	<i>P. posthuma</i>	<i>P. posthuma</i>
Locality	West Bengal	California	Aurangabad Dist.	Parbhani Dist.	Baltimore	Partur Jalna	Aurangabad Dist.	Aurangabad Dist.	Nashik Dist.