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

Sunil N. Bhamare¹, Susheel V. Nikam² and Bhimrao N, Jadhav³
¹Department of Zoology, K.R.A. College Deola, Nashik (M.S.) India.
²Department of Zoology, Dr. B.A.M. University, Aurangabad, Maharashtra, India.
³Department of Zoology, Shri. Muktanand College Gangapur, Aurangabad (M.S.), India.

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 Oligohymenophorea; with a divergenic 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 be used to advantage in trying to determine the most likely origin of the group. From an overall approach the Astomes may be used to advantage in trying to determine the most likely origin of the group.

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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 Schauddin’s fixative and then stained with phaspho-tungastic acid hematoxyline stain.

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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 characteristics are the entire absence of mouth. A number of 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, Kofoideellidae, 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. lloydii* from the seminal vesicle of Indian earthworm from *Pheretima posthuma* and *A. pheretimi*. Raychaudhuri, Haldar and Chakravarty (1969) from alimentary canal of same host.


**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 nikaum n. sp.* 4. *Anoplophrya infundibuli* (n. sp.)

Out of four the two species are new to the science. The present morphological study is concern with *Anoplophrya infundibuli* (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 the faecal material 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](image)

**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.

![Graph 01](image1.png)

![Graph 02](image2.png)

**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 whereas 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 50μ to 87.5μ in length and 22.5μ to 32.5μ 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 15μ to 27.5μ, where as width is 5μ to 7.5μ. 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 kinetics is 60 to 120 depending up on the body dimensions.

**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. Raychaudhuri 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 (*Pheretima 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) rediscribed species of *Anoplophrya* including *A. allure* (Ceped), *A. complanata*, (Rossolimo) *A. merylandensis* (Conkin).

M. K. Biwas (1974) has rediscribed 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. bifoldi* (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. phe reti m* (Raychaudhuri, 1969), oval and elongated in *A. lumbrici* (Shrank, 1803), elongated oval in *A. perionychis* and *A. chakravartii* (Lalpotu, 1979), elongated, oval, anterior end is narrow, posterior end is broad in *A. merylandensis* (Konkin, 1935), elongated, oval small, both ends are rounded in *A. krishnamurthii* (Bhandari, 2010), elongated with left lateral fold...
The number of contractile vacuoles is single in *A. branchiarm*, 3 to 6 in two rows in *A. pereretimi*, 3 in posterior end in *A. lumbrici*, 5 very small in two rows in *A. perionychis*, 3 to 7 in *A. chakravertii*, 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. nikamii* 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. pereretimi*, ribbon shaped with irregular outline in *A. perionychis & A. krishnamurthii*, elongated in *A. chakravertii*, 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. nikamii* (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 in circular manner at the rounded posterior end in the cytoplasm.
3. Macronucleus is ribbon like in *A. pereretimi, 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. chakravertii* 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 infundibilii* (n.sp.).

**Reference:**


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Kijenskij, G. (1925): 


### TABLE 1.
**SHOWING THE MONTHWISE PREVALENCE OF CILIATES IN EARTHWORMS (Pheretima Posthuma) during the period from Jan. 2007 To Dec. 2007**

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Months</th>
<th>Total No. of Hosts Examined</th>
<th>No. of +ve samples</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Jan-07</td>
<td>140</td>
<td>65</td>
<td>46.43</td>
</tr>
<tr>
<td>2</td>
<td>Feb-07</td>
<td>75</td>
<td>38</td>
<td>50.67</td>
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<tr>
<td>3</td>
<td>Mar-07</td>
<td>102</td>
<td>45</td>
<td>44.12</td>
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<tr>
<td>4</td>
<td>Apr-07</td>
<td>105</td>
<td>38</td>
<td>36.19</td>
</tr>
<tr>
<td>5</td>
<td>May-07</td>
<td>90</td>
<td>33</td>
<td>36.67</td>
</tr>
<tr>
<td>6</td>
<td>Jun-07</td>
<td>150</td>
<td>125</td>
<td>83.33</td>
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<tr>
<td>7</td>
<td>Jul-07</td>
<td>138</td>
<td>113</td>
<td>81.88</td>
</tr>
<tr>
<td>8</td>
<td>Aug-07</td>
<td>112</td>
<td>95</td>
<td>84.82</td>
</tr>
<tr>
<td>9</td>
<td>Sep-07</td>
<td>95</td>
<td>65</td>
<td>68.42</td>
</tr>
<tr>
<td>10</td>
<td>Oct-07</td>
<td>44</td>
<td>27</td>
<td>61.36</td>
</tr>
<tr>
<td>11</td>
<td>Nov-07</td>
<td>47</td>
<td>23</td>
<td>48.94</td>
</tr>
<tr>
<td>12</td>
<td>Dec-07</td>
<td>48</td>
<td>26</td>
<td>54.17</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1146</td>
<td>693</td>
<td>60.47</td>
</tr>
</tbody>
</table>

### TABLE 2.
**SHOWING THE MONTHWISE PREVALENCE OF CILIATES IN EARTHWORMS (Pheretima posthuma) during the period Jan. 2008 To Dec. 2008**

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Month</th>
<th>Total No. of Hosts Examined</th>
<th>No. of +ve samples</th>
<th>% of Total</th>
</tr>
</thead>
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<td>1</td>
<td>Jan-08</td>
<td>45</td>
<td>25</td>
<td>55.36</td>
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<tr>
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<td>Feb-08</td>
<td>87</td>
<td>43</td>
<td>49.43</td>
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<tr>
<td>3</td>
<td>Mar-08</td>
<td>77</td>
<td>35</td>
<td>45.45</td>
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<tr>
<td>4</td>
<td>Apr-08</td>
<td>75</td>
<td>38</td>
<td>50.67</td>
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<td>5</td>
<td>May-08</td>
<td>102</td>
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<td>50.98</td>
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<td>6</td>
<td>Jun-08</td>
<td>104</td>
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<td>57.69</td>
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<td>7</td>
<td>Jul-08</td>
<td>110</td>
<td>58</td>
<td>52.73</td>
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<tr>
<td>8</td>
<td>Aug-08</td>
<td>72</td>
<td>61</td>
<td>84.72</td>
</tr>
<tr>
<td>9</td>
<td>Sep-08</td>
<td>75</td>
<td>61</td>
<td>81.33</td>
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<td>10</td>
<td>Oct-08</td>
<td>65</td>
<td>41</td>
<td>63.08</td>
</tr>
<tr>
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<td>Nov-08</td>
<td>47</td>
<td>29</td>
<td>61.70</td>
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<tr>
<td>12</td>
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<td>Total</td>
<td>963</td>
<td>560</td>
<td>58.15</td>
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<tr>
<td>Sr. No.</td>
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<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>----------------------------------</td>
<td>----------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td></td>
<td>Body shape and dimensions</td>
<td>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-36.7 to 170.6 µ W-28.4 to 49.6 µ Elongated oval small both ends rounded L- 25.63 to 39.6 µ W-16.2 to 20.97 µ Elongated oval 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</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contractile vacuoles</td>
<td>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 Two rows each side of macronucleus 7-18 in each rows 19-24 scattered irregularly arranged at the post. end 6 to 10 circularly arranged at the post. end</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>macronucleus</td>
<td>Long band formed 30-89 µ Long ribbon shaped 33-92 µ Q 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 Elongated ribbon shaped Somewhat elongated ant. narrow rounded post end broad rounded L-15 to 27.5 µ W-5 to 7.5 µ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>micronucleus</td>
<td>…… …… …… …… …… …… Spherical Spherical Elongated somewhat banana like Spherical Spherical Spherical</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kineties</td>
<td>…… …… …… …… …… …… …… …… …… …… …… …… …… …… …… …… 60 to 120</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Host</td>
<td>…… P. posthuma Octoplasium cyanicum Perionychis P. posthuma P. posthuma P. posthuma P. posthuma</td>
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</tr>
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