

## *Stomatophora cloptoni* sp. n. (Apicomplexa: Eugregarinida) from the Seminal Vesicles of an Indian Earthworm

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**Summary.** In a survey of the endoparasitic acephaline gregarines in the district South 24 Parganas of West Bengal, India seminal vesicles of earthworm *Eutyphoeus orientalis* (Beddard) were found to be infested with a new species of the genus *Stomatophora* Drzhevetskii, 1907. *Stomatophora cloptoni* sp. n. is spheroidal and measures 95.0-135.0 ( $110.0 \pm 9.0$ )  $\mu\text{m}$  in diameter. A conical sucker 40.0-62.0 ( $55.0 \pm 1.5$ )  $\mu\text{m}$  is present marked by eight furrows dividing the lateral margin of the sucker into 8 lobes. Nucleus spherical, 18.0-24.0  $\mu\text{m}$  ( $20.0 \pm 2.0$ )  $\mu\text{m}$  in diameter and placed very close to the sucker. Gametocysts are ovoid and measure 59.0-86.0  $\times$  58.0-68.0  $\mu\text{m}$  and oocysts biconical with almost bluntly pointed ends and measure 13.0-19.0  $\times$  10.0-12.0  $\mu\text{m}$ .

**Key words:** Acephaline gregarines, earthworm, India, seminal vesicle, *Stomatophora cloptoni* sp. n.

### INTRODUCTION

Aseptate gregarine fauna have been reported from various parts of the world including India. But especially in India the search is far from complete. In an attempt to investigate the occurrence of acephaline gregarines in the oligochaete worms, seminal vesicles of earthworm *Eutyphoeus orientalis* (Beddard) collected from the South 24 Parganas district of West Bengal was infected with an undescribed species of *Stomatophora* Drzhevetskii, 1907, in their seminal vesicles. Little work has been done in India on the representatives of the

genus *Stomatophora* parasitizing the oligochaete worms. Only seven species of the genus *Stomatophora* (Hesse 1909; Pradhan and Dasgupta 1980; Roychoudhury and Haldar 1984; Bandyopadhyay *et al.* 2001, 2004) have so far been described from India. In this paper taxonomic description of one new species of acephaline gregarines of the genera *Stomatophora*, Drzhevetskii, 1907 as well as comparisons with previously described species, are provided.

### MATERIALS AND METHODS

Earthworms were collected and taken to the laboratory. They were dissected while alive and their seminal vesicles were carefully removed. These were placed on clean glass with a drop of 0.5% NaCl solution. A thin film of the seminal fluid was drawn out on a slide

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covered with a cover slip for examination of live protozoans under a phase contrast microscope. The content of seminal vesicles was semidried and fixed in Schaudin's fluid (20 min). The fixed smears were stored in 70% ethyl alcohol for removal of mercuric chloride. The slides were then passed through a descending series of alcohols (5 min each) and stored in distilled water. These were transferred to a 3% iron alum solution and stained with Heidenhain's haematoxylin solution (20 min). Differentiation (over night) was done with 1% iron alum solution. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol, cleared in xylene and mounted in Canada balsam. Photographs were taken with an Olympus camera. In each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation and number of sample size. All measurements are in  $\mu\text{m}$ . Method of describing shapes of planes and solids follows the guidelines of Clopton (2004).

## RESULTS AND DISCUSSIONS

### *Stomatophora cloptoni* sp. n. (Figs 1-11)

Phylum: Apicomplexa Levine, 1977; Order: Eugregarinida Leger, 1900; Family: Monocystidae Bütschli, 1882; Subfamily: Monocystinae Bhatia, 1930.

**Diagnosis:** Diameter of body (DB) = 95.0-135.0 (110.0  $\pm$  9.0); diameter of nucleus (DN) = 18.0-24.0 (20.0  $\pm$  1.7); diameter of sucker (DS) = 40.0-62.0 (55.0  $\pm$  1.5); length of gametocyst (LG) = 59.0-86.0 (65.0  $\pm$  1.9), width of gametocyst (WG) = 58.0-68.0 (61.0  $\pm$  1.7); length of oocyst (LO) = 13.0-19.0 (17.0  $\pm$  2.0); width of oocyst (WO) = 10.0-12.0 (10.8  $\pm$  1.9); DB: DN = 5.5:1; DB:DS (1.69:1); DN:DS = 1:0.36; LG:LO = 3.8:1; WG:WO = 5.6:1. Number of measurements made: 34

With the characters of genus *Stomatophora* Drzhevetskii, 1907, as given by Levine (1988); "gamonts spherical or ovoid; sucker petaloid, with radiating sides, oocysts biconical with a flattened button at each end, attached to each other end to end in long chains inside gametocyst". Gamont ellipsoid, flattened and compressed between its two poles. Sucker resembles a plate or disc marked by eight furrows and divided into eight lobes. In a few immature gamonts five or six petals are observed. Young gamonts slightly elliptical in shape. Ectoplasm about 1  $\mu\text{m}$  in thickness, without any external processes. Endoplasm coarsely granular. Granulation almost uniform. Sometimes a few patches of haematoxylin positive granules distributed at certain portions of the endoplasm. Sucker looks most prominent part of the body. Sucker appears a shallow cup-like depression, almost at the centre of the body. In a few young gamonts a very small mucron seen at centre of depression. Outer margin of

aperture of sucker almost rounded. Nucleus almost spherical and always seen closely associated with sucker. Karyosome diffused and sometimes one or two vacuoles seen inside nucleus. Gametocysts almost rounded. Oocysts biconical with a flattened button at each end.

### Taxonomic summary

**Type material:** *Stomatophora cloptoni* sp. n.

**Type host:** *Eutyphoeus orientalis* (Beddard)

**Symbiotype:** Host AH - 03/22/2002 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

**Site of infection:** Seminal vesicles.

**Type locality:** Dimond Harbour, South 24 Parganas, West Bengal, (22.11°N, 88.14°E).

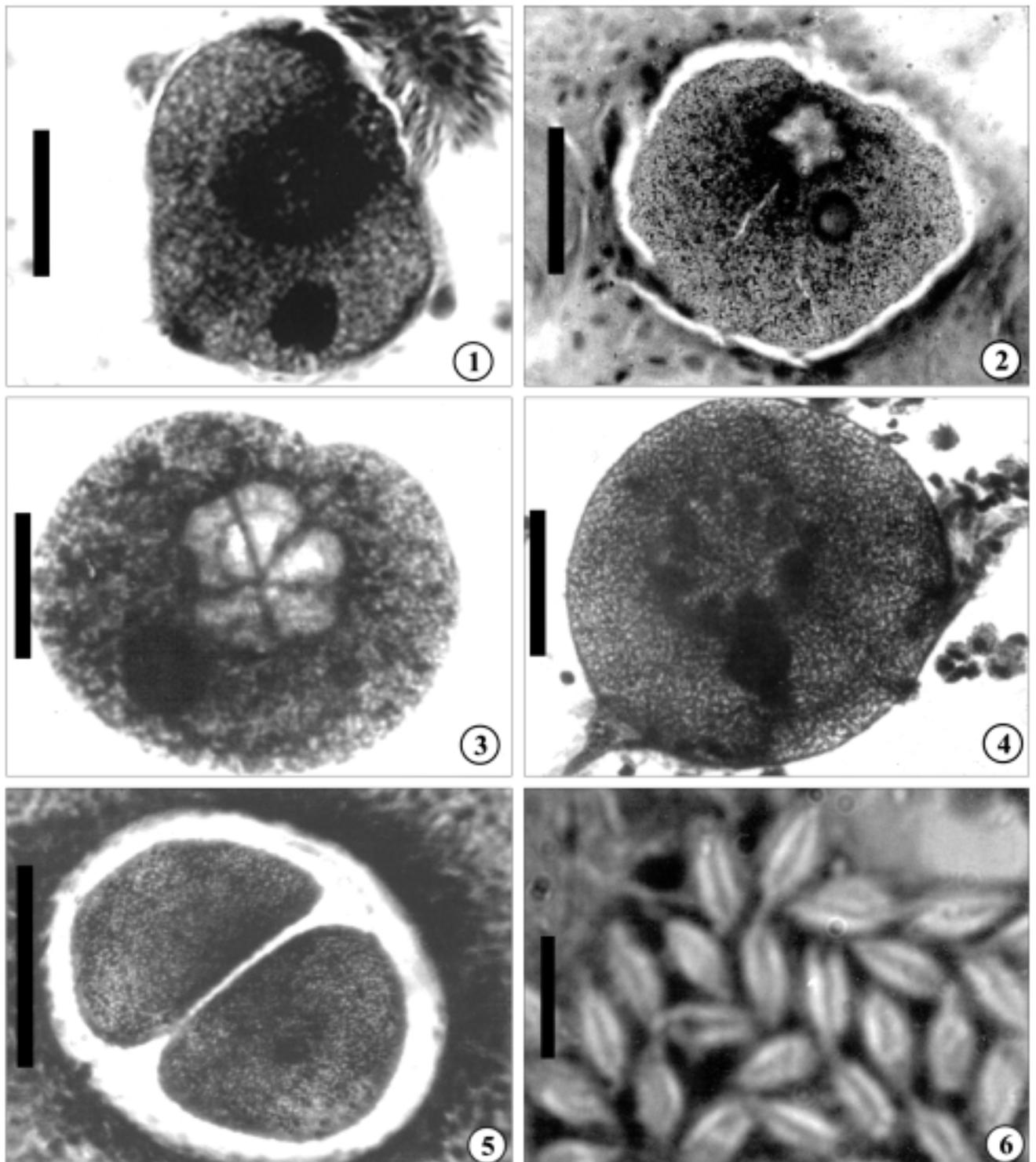
**Prevalance:** 7 out of 44 (15.9 %).

**Type material:** Holotype, slide EO/05/2004, and paratypes, slides EO/01/2004, EO/02/2004, EO/04/2004 EO/06/2004, EO/07/2004 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. Slide EO/03/2004 bearing some paratype materials are in the collection of the Museum of Zoological Survey of India (ZSI), Calcutta - 700016 (Catalogue No. 2506).

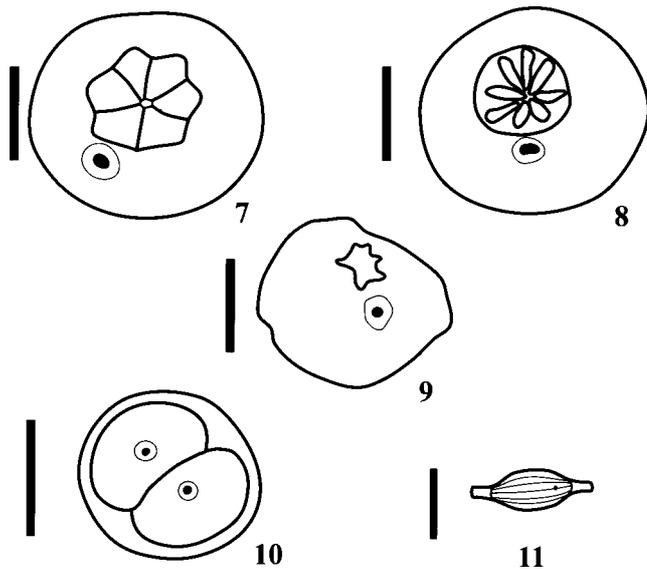
**Etymology:** The specific epithet "*cloptoni*" is given after the name of Dr Richard E. Clopton of Department of Natural Science, Peru State College, USA, for his outstanding contribution in the field of apicomplexan biology.

### Remarks

Spherical body with a cup-like sucker near the centre justifies the inclusion of the present form under the genus *Stomatophora* Drzhevetskii, 1907. At present there are ten described species of *Stomatophora*. No stomatophorid gregarines has been reported from the present host species, *Eutyphoeus orientalis* (Beddard). Considering the shape of gamonts, the present species can be compared with *S. globa* Pradhan et Dasgupta, 1980, *S. diadema* Hesse, 1909 and *S. coronata* (Bhatia, 1924). The size of the gamonts of *S. cloptoni* (95.0-135.0) is comparatively much greater than *S. globa* (30.6-97.2). The sucker of *S. globa* has a superficial similarity with the present species in having invisible mucron and long prolongation radiating outwards which are variable in number. But in *S. cloptoni* the prolongations radiating outwards are always eight in number and the sucker assumes a cup-like appearance. The nucleus of the gamonts of *S. globa* is rounded and karyosome is eccentric and well visible. In *S. cloptoni* although the



**Figs 1-6.** Photomicrographs of different stages of life cycle of *Stomatophora cloptoni* sp. n. **1, 3** - young gamonts; **2** - side view of gamont; **4** - mature gamont; **5** - gametocyst; **6** - oocysts. Scale bars 50  $\mu$ m (1-5); 10  $\mu$ m (6).



**Figs 7-11.** Camera lucida drawings of different stages of life cycle of *Stomatophora cloptoni* sp. n.; **7** - young gamont; **8** - mature gamont; **9** - side view of gamont; **10** - gametocyst; **11** - oocyst. Scale bars 50  $\mu$ m (7-10); 10  $\mu$ m (11).

nucleus is rounded the karyosome is situated near periphery. The size of the sucker of *S. globa* is comparatively smaller than *S. cloptoni* (12.6-39.6 vs. 40.0-62.0 in *S. cloptoni*). The sucker in *S. globa* is lacking any depression and any radiating sides. But the sucker in *S. cloptoni* is having a cup like depression with eight radiating sides. The maximum size of the gamonts of both *S. diadema* (105) and *S. cloptoni* (110.0) do not differ significantly. The whole body of the gamonts of *S. diadema* is marked by furrows and divided into a number of irregular lobes. But the body of gamonts of *S. cloptoni* does not have such type of furrows. In *S. diadema*, a prominent mucron is present whereas the new species under discussion does not have any prominent mucron in most of the mature gamonts. The size of the gametocysts of *S. diadema* is significantly larger (130-170) than that of *S. cloptoni* (59.0-86.0) but the size of the oocysts of *S. diadema* (8-12  $\times$  3-4) is significantly smaller than the oocysts of *S. cloptoni* (13.0-19.0  $\times$  10.0-12.0). The size of the gamonts of *S. coronata* (Bhatia 1924) is comparatively smaller than that of *S. cloptoni* (60.0-80.0 vs. 95.0-135.0 as in *S. cloptoni*). The shape of the body in *S. coronata* is also different, when it is compared with *S. cloptoni*. The main characteristic feature of *S. coronata* is the possession of the sucker which is an epimeritic structure slightly elevated off from the body. But in the new

species under discussion the sucker does not elevate off the body. The body of *S. coronata* is widely expanded at the anterior end, so as to make it resemble a top or a flower vase. But in *S. cloptoni* the shape of the body is never expanded anteriorly and as a result the flower vase like structure has not been noticed. Another significant difference is the change of structure during movement. In *S. coronata*, during movement, a sharp constriction appears round the middle of the body, causing it to assume an hour glass like shape, and later it may acquire a more regular outline again. But in case of the new species obtained from *Eutyphoeus orientalis* (Beddard) no sign of constriction appears during movements. In *S. coronata*, the sucker is surrounded by a well developed petaloid crown marked by epicytal striations. In case of *S. cloptoni*, sucker is not surrounded by any type of petaloid epicytal striations. The size of the oocysts also differs in both cases. The size of the oocysts in *S. coronata* is also smaller (9-11 by 5-6 and 7-7.5 by 3) than that the new species (13.0-19.0  $\times$  10.0-12.0).

Considering all these differences the parasite described has been given a separate species status under the genus *Stomatophora* Drzhevetskii, 1907 and is designated as *Stomatophora cloptoni* sp. n. in this paper.

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