

**A new endoparasitic gregarine genus, *Stomatocystis indica*
gen. nov., sp. nov. (Apicomplexa: Sporozoea: Stomatophorinae)
from the seminal vesicles of an Indian earthworm
(Annelida: Oligochaeta) *Amyntas diffringens* Baird**

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Abstract

During the exploration of acephaline gregarines from oligochaete hosts in India, unique forms were obtained from the seminal vesicles of the earthworm, *Amyntas diffringens* Baird in Midnapore of West Bengal and are being proposed as a new genus *Stomatocystis* gen. nov. under the subfamily Stomatophorinae Bhatia, 1930. This gregarine possess oblong body, a mucron transformed into a sucker, complex myocyte and navicular oocysts with truncated ends, thereby justifying its inclusion under the subfamily Stomatophorinae of the family Monocystidae. By possessing, at the anterior end of the body, a crater-like sucker, rimmed by a collar (4.8–7.1 µm), inside of which are parallel protoplasmic ridge-like processes (8.3–10.5 µm in length) which are separated by furrows (1.1–1.6 µm spaced apart), the gregarine can not be assigned to any of the 10 genera under the subfamily Stomatophorinae. Gamonts are oblong. Length and width of gamonts range from 44.0–96.0 (70.5 ± 1.1) µm and 29.0–59.0 (44.0 ± 1.2) µm respectively. Length and width of nucleus range from 11.5–20.0 (17.0 ± 1.1) µm and 9.5–15.0 (13.0 ± 1.2) µm respectively. Gametocysts are ovoidal and their diameter (GD) ranges from 72.0–102.0 (90.0 ± 1.9) µm. Oocysts navicular. Length and width of oocysts range from 7.9–11.0 (9.0 ± 1.1) µm and 3.0–4.0 (3.7 ± 1.1) µm respectively. This species is described as *Stomatocystis indica* gen. nov., sp. nov. in this paper.

Key words: Acephaline gregarine, new genus, *Stomatocystis indica* gen. nov., sp. nov., earthworm, seminal vesicle, India

Introduction

Exploration of acephaline gregarine fauna inhabiting oligochaete hosts in India have discovered representatives of the genera *Apolocystis* Cognetti de Martiis 1923; *Monocystis* Stein, 1848; *Nematocystis* Hesse, 1909; *Stomatophora* Drzewiecki, 1907 and *Zygocystis* Bhatia, 1930 [Hesse 1909; Ghosh 1923 (cited by Levine 1988); Bhatia & Chatterjee 1925; Bhatia & Setna 1926; Kar 1946; Kalavati 1979; Subbarao *et al.* 1979; Pradhan & Dasgupta 1980a, b; 1982; 1983a, b; Roychoudhury & Haldar 1984; Bandyopadhyay *et al.* 2001; 2004; 2006a, b, c; Bandyopadhyay & Mitra 2004; 2005a, b, c, d, e; 2006a, b].

In the present study an unique form of acephaline gregarine was obtained in the seminal vesicle of the earthworm, *Amyntas diffringens* Baird in East Midnapore district of West Bengal, India. By the presence of oblong body, a mucron transformed into a sucker, navicular oocysts with truncated ends the species has been included under the subfamily Stomatophorinae Bhatia, 1930 of the family Monocystidae Bütschli, 1882. At present there are 10 genera under the subfamily Stomatophorinae. The present species has no similarity with any one of the existing genera except the genera *Stomatophora* Drzewiecki, 1907 and *Craterocystis* Cognetti de Martiis, 1918. The genus *Stomatophora* is characterized by the presence of ovoid gamont and petaloid sucker with radiating sides, while the genus *Craterocystis* possesses globular gamont covered laterally with "hairs". However, the presence of a crater-like sucker, protoplasmic ridge-like processes separated by furrows set the new genus apart from the genera *Stomatophora* and *Craterocystis*. So, a new genus has been created to accommodate this species which is described below.

Material and methods

Twenty three earthworms (*Amyntas diffringens*) 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 covered with a cover slip for examination of live protozoa under a phase contrast microscope. The content of seminal vesicles was semidried and fixed in Schaudin's fluid for 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 alcohol (5 min each) and stored in distilled water. These were transferred to a 3% iron-alum solution and stained with Heidenhain's haematoxylin solution for 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 micrometers. Method of describing shapes of planes and solids follows the guidelines of Clopton (2004).

Results and discussions***Stomatocystis* gen. nov.****Phylum: Apicomplexa Levine, 1977****Order: Eugregarinida Leger, 1900****Family: Monocystidae Bütschli, 1882****Subfamily: Stomatophorinae Bhatia, 1930***Diagnosis*

Gamonts cylindroconical. Solitary and also in the syzygy. Lacks papillae and filaments. Sucker anterior, conical with truncated round protoplasmic collar that rims the stomatal opening. Protoplasmic ridges line the lateral surface. Sucker lodged on the anterior end. Gametocyst ovoidal. Oocysts navicular with drawn out truncated ends.

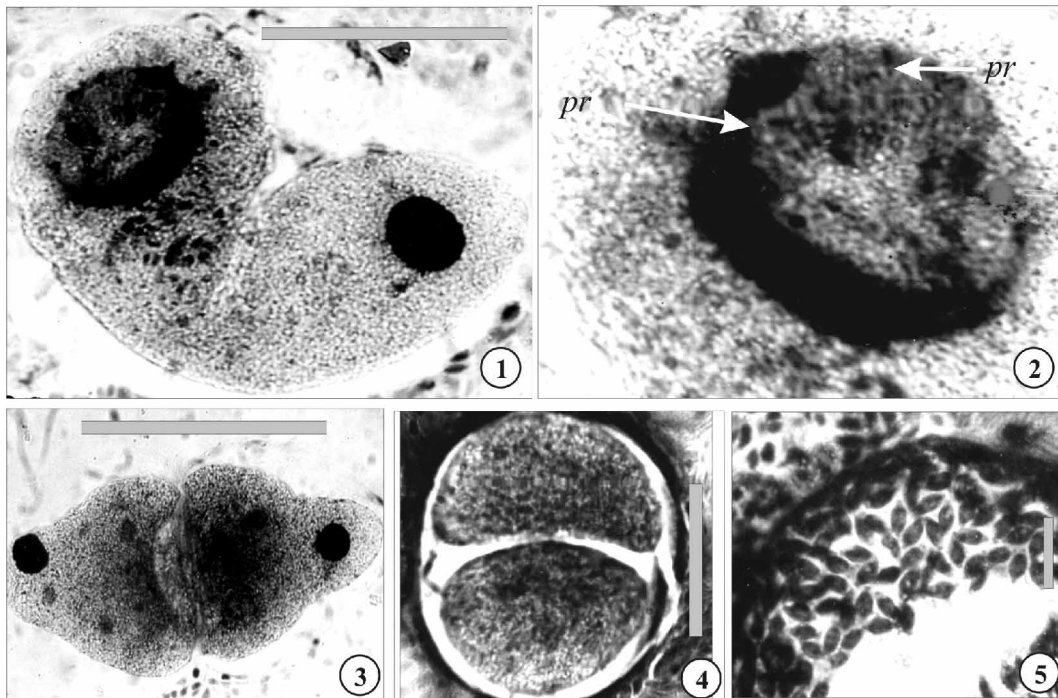
***Stomatocystis indica* gen. nov., sp. nov.**

(Figs. 1–9, Table 1)

Thirty two specimens were examined. Body length (BL) 44.0–96.0 μm ($70.5 \pm 1.1 \mu\text{m}$); Body width (BW) 29.0–59.0 μm ($44.0 \pm 1.2 \mu\text{m}$); Nucleus length (NL) 11.5–20.0 μm ($17.0 \pm 1.1 \mu\text{m}$); Nucleus width (NW) 9.5–15.0 μm ($13.0 \pm 1.2 \mu\text{m}$); Stomata diameter (StD) 22.0–41.0 μm ($31.0 \pm 1.2 \mu\text{m}$); Gametocyst diameter (GD) 72.0–102.0 μm ($90.0 \pm 1.9 \mu\text{m}$); Oocyst length (OL) 7.9–11.0 μm ($9.0 \pm 1.1 \mu\text{m}$); Oocyst width (OW) 3.0–4.0 μm ($3.7 \pm 1.1 \mu\text{m}$); BL/BW 1.6:1; BW/StD 1.4:1; NL/NW 1.3:1; OL/OW 2.4:1

Mature gamonts oblong in shape having a round epimerite at the anterior end, narrowing gradually to a round posterior end. Anterior epimeritic structure contains in its centre a crater-like sucker having a rim projected slightly outside. The epimerite-like structure is almost round to elliptical in shape having six to eight shallow indentations on the periphery progressing slightly towards the centre and very prominent in the young individuals. In mature gamonts the epimerite-like structure is discernible only in certain angle of distortion.

A sucker is present in the centre of the epimeritic structure. The sucker is broadly funnel-shaped with a truncated broad base having a roundish stomata at the external margin lodged on the surface of the epimeritic structure on a band-like protoplasmic collar. Inside the collar arise longitudinal parallel protoplasmic ridge-like processes, 8.3–10.5 μm in length, spaced 1.1–1.6 μm apart, and adhered to the inner margin of the collar. Ectoplasm is thin, without any external processes. Sarcoplasm and myoplasm layers are not very distinct.



FIGURES 1–5. Photomicrographs of different stages in the life cycle of *Stomatocystis indica* **gen. nov., sp. nov.** from seminal vesicles of earthworm *Amyntas diffringens* Baird. 1. a slightly curved trophozoite showing the sucker and epimerite-like structure; 2. enlarged view of the sucker showing protoplasmic ridge-like processes in its lateral margin; 3. syzygy; 4. gametocytes within a gametocyst; 5. navicular oocysts. *Abbreviation:* pr-protoplasmic ridges. Scale bars: Figs. 1–4 (50 μ m), Fig. 5 (10 μ m)

Endoplasm is granular, without any inclusions. Only a few haematoxylin positive granules are seen accumulated in the endoplasm. The vesicular nucleus is spheroidal to ellipsoidal in shape and placed generally in the posterior part of the gamont. But in young specimens, it may be placed anteriorly. Karyosome is located near the periphery of the nucleus resulting in the formation of a vacuole at the centre.

Taxonomic summary

Type material: *Stomatocystis indica* **gen. nov., sp. nov.**

Type host: *Amyntas diffringens* Baird

Symbiotype: Host AD/ NG/03/2001 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Type locality: East Midnapore, West. Bengal, India

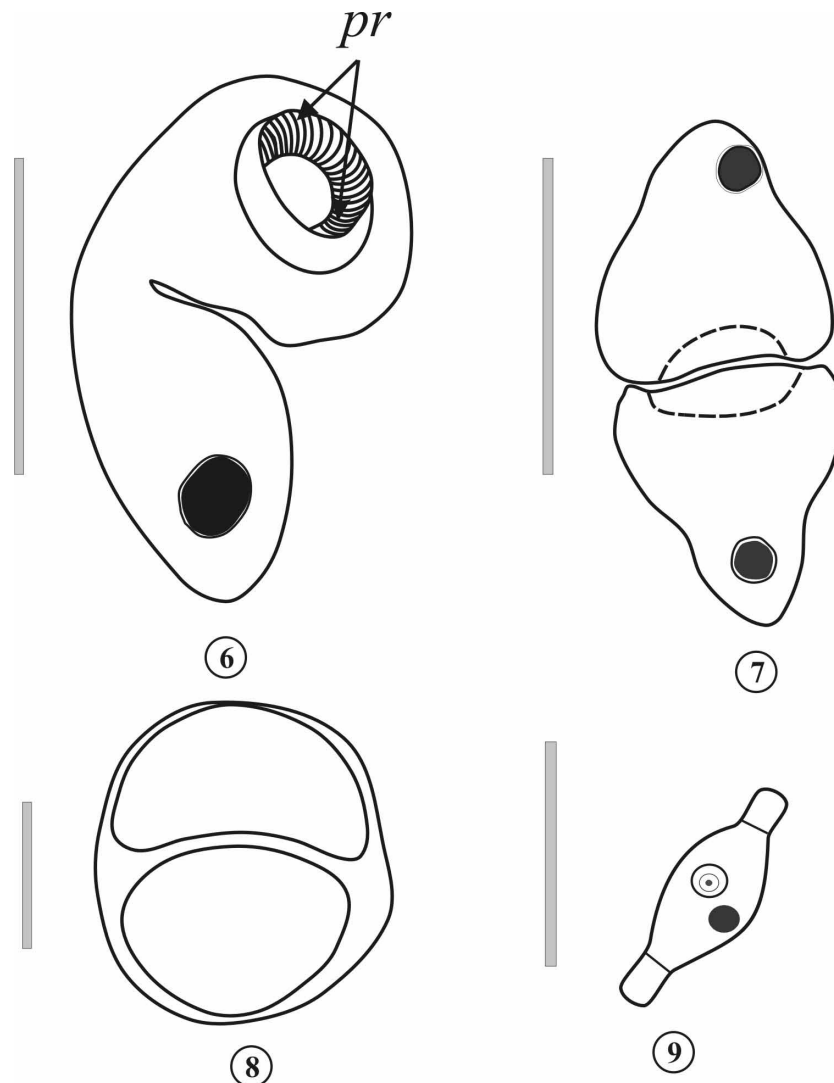
Prevalance: 9 out of 23 (39%).

Number of specimens measured: 32

Type material: Holotype, slide NG/01/2001, and Paratypes, slides NG/02/2001, NG/05/2001, NG/07/2001 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Etymology: This species was named after the motherland, India.

Site of infection: Seminal vesicles.



FIGURES 6–9. Camera lucida drawings of different stages in the life cycle of *Stomatocystis indica* **gen. nov., sp. nov.** from seminal vesicles of earthworm *Amyntas diffringens* Baird. 6. a mature gamont showing central mucron within the sucker; 7. syzygy; 8. gametocytes within a gametocyst; 9. oocyst. *Abbreviation:* pr-protoplasmic ridges. Scale bars: Figs. 7–8 (50 μ m), Fig. 9 (10 μ m)

TABLE 1. Morphological comparisons of 10 genera of the family Stomatophorinae with *Stomatocystis* **gen. nov.**

| Genera | Gamont characters | Sucker |
|---|--|--|
| <i>Stomatophora</i> Drzewiecki, 1907 | Solitary. Spherical or ovoid | Petaloid, with radiating sucker |
| <i>Craterocystis</i> Cognetti de Martiis, 1918 | Solitary. Globular, covered laterally with “hairs”, deep myocyte wall well developed, with strong myonemes going from sucker to lateral face | Anterior |
| <i>Astroccystella</i> Cognetti de Martiis, 1918 | Solitary. Body flattened, stellate, cut into 5–9 deep lobes arising from a central region which contains the nucleus and forms a sucker | Central, round |
| <i>Albertisella</i> Cognetti de Martiis, 1918 | Solitary. Ovoid, myocyte deep, comprising myonemes running from the base of the sucker to the opposite face | Central with smooth wall |
| <i>Beccaricystis</i> Cognetti de Martiis, 1918 | Solitary. Body cylindrical, lobed laterally | Anterior, axial cluster of myonemes present |
| <i>Parachoanocystoides</i> De Saedeleer, 1930 | Solitary. Ovoid body. A retractile, pseudopodia like tentacle bearing cytoplasmic “hairs” arises from the base of the sucker | Anterior |
| <i>Zeylanocystis</i> Dissanaike, 1953 | Solitary, also in syzygy. Rounded when in syzygy, bordered by papillae and filaments that may be absorbed to form a rim | |
| <i>Arborocystis</i> Rees and Howell, 1966 | Solitary. Large, pear shaped, broadly rounded anteriorly and sharply pointed posteriorly | Anterior, with a circle of contractile processes forming the mucron |
| <i>Chakravartiella</i> Mishra and Roychoudhury, 1973 | Solitary. Elongate and cylindrical. Myonemes present | Anterior, with a circle of contractile processes forming the mucron |
| <i>Stomatocystis indica</i> gen. nov., sp. nov. | Solitary, also in syzygy | Anterior, conical with truncated round bottomed protoplasmic collar rim the stomatal opening, protoplasmic ridges line the lateral surface. Sucker lodged on epimerite |

Discussion

A parasite was obtained in the coelom of an oligochaete annelid, *Amyntas diffringens* Baird. The body of the gregarine is nearly cylindrical and has a differentiated anterior end, justifying its inclusion under the family Monocystidae Bütschli, 1882. Of the five subfamilies under the Monocystidae, it shares distinctive characteristics of two different subfamilies. First, by forming early syzygy it shows affinity with the subfamily Zygozystinae Bhatia, 1930, and second, in having a stomata and sucker, it shows an affinity with the subfamily Stomatophorinae Bhatia, 1930. However, the presence of stomata and sucker should be given priority. It is probable that due to its free coelom-dwelling habit the formation of syzygy early in the life history stage has been acquired by the parasite. Formation of early syzygy is reflected in almost all the 'free' coelom-dwelling forms. Considering all these characters, the parasite under report has been assigned to the subfamily Stomatophorinae. At present there are 10 genera under the subfamily Stomatophorinae. A comparative chart of these 10 genera along with the present one has been presented in Table 1. From the above comparison it is evident that the species under discussion has no close similarity with any one of the existing genera except the genera *Stomatophora* Drzewiecki, 1907 and *Craterocystis* Cognetti de Martiis, 1918. The genus *Stomatophora* is characterized by the presence of ovoid gamont and petaloid sucker with radiating sides, while the genus *Craterocystis* possesses globular gamont covered laterally with "hairs". However, the presence of crater-like sucker rimmed by a collar, inside of which is provided with longitudinal, parallel, protoplasmic ridge-like processes separated by furrows set it apart from the genera *Stomatophora* and *Craterocystis*. With the exception of that character, the species contains ovoid to ellipsoidal gametocyst and navicular oocyst extended terminal ends. Considering all these characters, the present species can not be accommodated under any known genus within subfamily Stomatophorinae. Therefore, a new genus, *Stomatocystis* gen. nov. is proposed for the type specimen *Stomatocystis indica* gen. nov., sp. nov.

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