Description of *Rhynchocystis silvae* sp. nov (Apicomplexa: Eugregarinida) From *Metaphire peguana* Rosa (1890) of Dhaka, Bangladesh

Dakka, Bangladeş'den Bir Solucan Türü *Metaphire peguana* Rosa (1890)'da Yeni Bir Gregarin Parazit Türü *Rhynchocystis silvae* sp. nov. (Apicomplexa: Eugregarinida)

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ABSTRACT

Objective: Biodiversity studies in search of endoparasitic acephaline gregarines of earthworms revealed a new species under the genus *Rhynchocystis* Hesse, 1909.
Methods: The species has been obtained from the seminal vesicles of the earthworm, *Metaphire peguana*. The parasite was identified using standard methodology.
Results: Gamonts are solitary, elongated with a conical, enlarged head and blunt posterior end. The mature gamont measures 419.90-430.95 (425.05±4.70) µm in length and the width at the widest portion is 44.2-50.83 (47.88±2.85) µm and 15.47-17.68 (16.50±1.14) µm in the posterior end. The nucleus is elongated and positioned at the posterior end. The nucleus measures 17.68-22.01 (19.74±1.95) µm×11.05-15.47 (13.40±1.95) µm. The measurement of the mucron is 13.26-17.68 (15.47±1.86) µm×17.68-22.1 (19.44±1.71) µm. Gametocysts are ovoid with two unequal sized gametocytes. The gametocyst measures 95.03-97.24 (96.20±1.14) µm×77.35-81.77 (79.56±1.86) µm. Large and small gametocytes measure 57.46-61.88 (59.81±1.76) µm×70.72-75.14 (72.48±1.71) µm and 30.94-37.57 (34.47±2.74) µm×61.88-66.3 (64.38±1.84) µm respectively. Oocysts are biconical, measuring 11.05-15.47 (12.81±1.71) µm×6.63-8.84 (7.70±1.20) µm.
Conclusion: A new gregarine parasite species is described. *(Turkiye Parazitol Derg 2012; 36: 178-81)*

Key Words: *Rhynchocystis silvae* sp. nov, gregarine, earthworm, seminal vesicles, Bangladesh

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ÖZET

Amaç: Toprak solucanlarının endoparazitik asefalin gregarinlerinin araştırılmasına yönelik biyoçeşitlilik çalışmaları *Rhynchocystis*, Hesse, 1909 cinsine dahil yeni bir türü ortaya koymuştur.
Yöntemler: Yeni saptanan tür, *Metaphire peguana*'nın seminal vesiküllerinden elde edilmiştir. Parazitin teşhisinde standart yöntemler izlenmiştir.
Bulgular: Gamontlar soliter, konik şekilde uzamış bir baş ve küt bir posterior uca sahiptir. Ergin gamont uzunluğu 419.90-430.95 (425.05±4.70) µm ve genişliği posterior uçta 15.47-17.68 (16.50±1.14) µm iken en geniş kısımda 44.2-50.83 (47.88±2.85) µm olarak ölçülmüştür. Nukleus uzamış olup, posteriorda yerleşir. Nukleus ölçüleri 17.68-22.01 (19.74±1.95) µm×11.05-15.47 (13.40±1.95) µm'dir. Mukron ölçüleri 13.26-17.68 (15.47±1.86) µm×17.68-22.1 (19.44±1.71) µm'dir. Gametosistler oval ve farklı büyüklükte iki gametosit içerir. Gametositler 95.03-97.24 (96.20±1.14) µm×77.35-81.77 (79.56±1.86) µm'dir. Büyük ve küçük gametosit ölçüleri sırasıyla, 57.46-61.88 (59.81±1.76) µm×70.72-75.14 (72.48±1.71) µm ve 30.94-37.57 (34.47±2.74) µm×61.88-66.3 (64.38±1.84) µm'dir. Ookistler bikonik ve 11.05-15.47 (12.81±1.71) µm×6.63-8.84 (7.70±1.20) µm boyutlarındadır.
Sonuç: Yeni bir gregarin parazit türü tanımlanmıştır. *(Turkiye Parazitol Derg 2012; 36: 178-81)*

Anahtar Sözcükler: *Rhynchocystis silvae* sp. nov., gregarin, toprak solucanı, seminal vesikül, Bangladeş

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INTRODUCTION

"Gregarines" are a diverse and successful group of protozoan parasites belonging to the phylum Apicomplexa. Gregarines are specific parasites of a wide range of invertebrates from Phyla Arthropoda (including crustaceans, insects, myriapods) and Annelida (including polychaetes, oligochaetes and leeches). Of the two major groups of gregarines, aseptate and septate, earthworms harbour the aseptate one. The aseptate or acephaline forms are characterized by a non-septate body and they are the common endoparasites of the seminal vesicles of earthworms**.** There are not many important studies on gregarine protozoan parasites infesting invertebrates throughout the world. The researches on aseptate gregarines have gained momentum since 1980. Ruston (1) described *D. minuta* from *Lumbricus terrestris*. Bandyopadhyay and Mitra (2) described one species, *D. indica* from *Lampito mauritii* collected from India. Later, a study of aseptate gregarines from earthworms revealed a new species of the genus *Rhynchocystis* Hesse 1909, which is described here. Levine (3) listed some species under the genus *Rhynchocystis*. These were *Rhynchocystis awatii*, *R. cognetti, R. cuneiformis, R. pilosa, R. hessei, R. mamillata, R. oculata, R. ovata, R. pessoai, R. piriformes, R. porrecta*. All of these species of *Rhynchocystis* were described from seminal vesicles of oligochaete hosts. During the present biodiversity studies of the aseptate gregarines from the earthworms of Bangladesh, a new species belonging to the genus *Rhynchocystis* previously defined by Hesse in 1909 was identified. This paper deals with a unique form of a *Rhynchocystis* species obtained from the seminal vesicles of the earthworm, *Metaphire peguana,* collected from North Badda of the Dhaka district, Bangladesh. This new species of aseptate gregarine is described for the first time in the present study.

METHODS

For the present study 30 host specimens were collected from the drainage soil of North Badda, Dhaka. The specimen collections were conducted between January 2011-June 2011. The collected earthworms were kept in soil in an earthen tub and brought to the laboratory alive. Some of the earthworms were dissected while alive and their seminal vesicles were carefully removed. The fluids of the seminal vesicle were placed on clean glass with a drop of 0.6% NaCl solution. A thin film of seminal fluid was drawn out on a slide and covered with a cover slip for examination of living protozoans under a light microscope. After initial study of living protozoans the content of the seminal vesicles were semidried and fixed in Schaudin's solution for 20 minutes. After fixation, the smears were stored in 70% ethanol for removal of mercuric chloride. The slides were then passed through a descending series of alcohol (5 minutes each) and placed in distilled water. These were transferred to a 3% iron alum solution (Over night) and stained with Heidenhain's hematoxylin solution for 20 minutes. Differentiation was done with 1% iron alum solution under the low power objective lens of the light microscope. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol, cleared in xylin and mounted in D.P.X. All measurements are presented in micrometers (µm) as mean (±) SD followed in parentheses by the range. Photographs were taken with an Olympus Phase Contrast Microscope fitted with a digital camera. The methodology used to describe shapes of planes and solids of the gregarines is in accordance with Clopton (4). The following abbreviations are used for the gregarine parasite in this paper. GL=Gamont length, GWwa=Gamont width at the widest part (anterior end), GWp=Gamont width (posterior part), LN=Length of nucleus, WN=Width of nucleus, LG=Length of gametocyst, WG=Width of gametocyst, LGL=Large Gametocyte Length, LGW=Large Gametocyte Width, SGL=Small Gametocyte Length, SGW=Small Gametocyte Width, LO=Length of oocyst, WO=Width of oocyst.

RESULTS

Phylum-Apicomplexa Levine, 1988
Order - Eugregarinida Leger, 1900
Family- *Monocystidae* Biitschli, 1882
Subfamily - *Rhynchocystinae* Bhatia 1930
Genus- *Rhynchocystis*, Hesse, 1909
***Rhynchocystis silvae* sp. nov (Figure 1 a-c, Table 1, 2)**

Mature gamont measures 419.90-430.95 (425.05±4.70) µm×15.47-17.68 (16.50±1.14) µm. The widest part of it is 44.2-50.83 (47.88±2.85) µm width. The nucleus measures 7.68-22.01 (19.74±1.95) µm×11.05-15.47 (13.40±1.95) µm. Mucorn measures 13.26-17.68 (15.47±1.86) µm×17.68-22.1 (19.44±1.71) µm. The gametocyst is 95.03-97.24 (96.20±1.14) µm in length and 77.35-81.77 (79.56±1.86) µm in width. Large and small gametocytes measure 57.46-61.88 (59.81±1.76) µm×70.72-75.14 (72.48±1.71) µm and 30.94-37.57 (34.47±2.74) µm×61.88-66.3 (64.38±1.84) µm respectively. Length of the oocyst is 11.05-15.47 (12.81±1.71) µm and width is 6.63-8.84 (7.70±1.20) µm.

The genus *Rhynchocystis* was detected by Hesse in 1909. Bhatia, placed it in a new sub family Rhynchocystinae in 1930 and the subfamily was defined as, "Gamont ovoid, spherical or elongated with a conical or cylindroconical trunk at the anterior end, solitary: oocyst biconical with similar non appendiculate ends, with eight sporozoites" by Levine (1). The morphology of the present form obtained from the seminal vesicles of the earthworm, *Metaphire peguana* collected from Dhaka, the capital of Bangladesh is studied thoroughly. The trophozoite of the present form is usually long

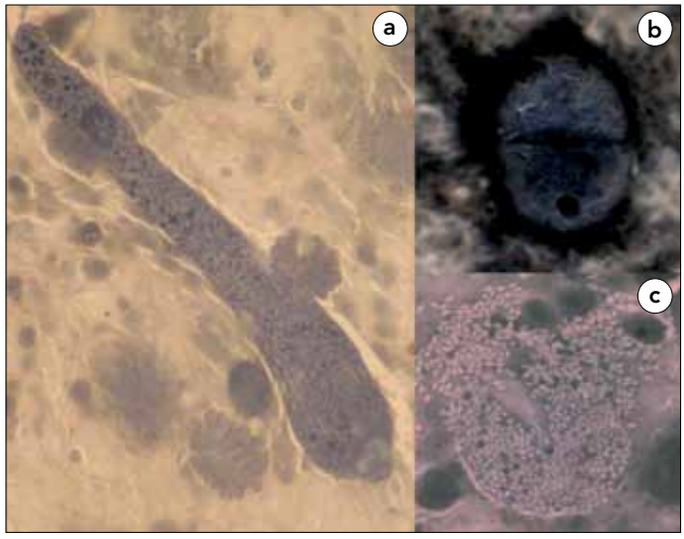

Figure 1. a-c) Photomicrographs of different stages of the life cycle of *Rhynchocystis silvae* sp. nov. obtained from the seminal vesicles of the earthworm, *Metaphire peguana*. a) A mature gamont; b) Gametocyst with two unequal sized gametocytes; c) Oocysts

Table 1. Summarized statistics of different body parts of the gamonts, gametocysts and oocysts. All measurements are in µm

Characters	Mean	Range	Standard Deviation
Gamont length (GL)	425.05	419.90-430.95	4.70
Gamont width at the widest part (anterior part) (GWwa)	47.88	44.2-50.83	2.85
Gamont width (Posterior part) (GWp)	16.50	15.47-17.68	1.14
Length of nucleus (LN)	19.74	17.68-2.01	1.95
Width of nucleus (WN)	13.40	11.05-15.47	1.95
Length of gametocyst (LG)	96.20	95.03-97.24	1.14
Width of gametocyst (WG)	.925	88.4-99.45	5.823
Large gametocyte length (LGL)	59.81	57.46-61.88	1.76
Large gametocyte width (LGW)	72.48	70.72-75.14	1.71
Small gametocyte length (SGL)	34.47	30.94-37.57	2.74
Small gametocyte width (SGW)	64.38	61.88-66.3	1.84
Length of oocyst (LO)	12.81	11.05-15.47	1.71
Width of Oocyst (WO)	7.70	6.63-8.84	1.25

and slender. Its anterior end is broad and rounded but the rest of the body tapers towards the posterior extremity. It is cylindrical and blunt. The anterior end of the parasite is provided with a small conical mucorn which emerges from the base of a concavity formed by the infolding of the ectosarc. Endoplasm is dense with paraglycogen grains. Epicyte is thin, transparent and quite delicate. Ectoplasm is relatively thick. The nucleus is small, elongated and near the posterior end, typically contains a central karyosome surrounded by a layer of chromatic granules which lies half way between it and the thick nuclear membrane. Gametocysts have two unequal sized gametocytes. Oocysts are biconical. Detailed measurements are presented in Table 1.

Taxonomic Summary

Type Material: *Rhynchocystis silvae* sp. nov

Type Host: *Metaphire peguana*.

Type Locality: North Badda, (23.7667° N and 90.4333° E.) Dhaka district, Bangladesh

Site of infestation: Seminal Vesicles.

Prevalence: 21/30=(70%)

Holotype: R1/301/2011, deposited at the Parasitology Laboratory of the Department of Zoology, University of Kalyani, Kalyani -741235, West Bengal, India.

Paratypes: R2/302/2011, deposited at the Parasitology Laboratory of the Department of Zoology, University of Kalyani. Kalyani-741235, West Bengal, India.

Symbiotype: MP/11/09, deposited at the Parasitology Laboratory of the Department of Zoology, University of Kalyani.

Etymology: The species is named in honour of Dr. Joseph D'Silva, former Professor of the Department of Zoology from the University of Dhaka, Bangladesh for his contribution in the field of Parasitology.

DISCUSSION

Being a parasite of seminal vesicles of oligochaetes and having a cylindrical anterior end, with little differentiation, the parasite justifies its inclusions under the taxa: family Monocystidae, Subfamily Rhynchocystinae and the genus *Rhynchocystis* Hesse, 1909. The genus *Rhynchocystis* is characterized by the presence of some sort of anterior epimerite like apparatus. Only ten *Rhynchocystis* species have been described so far from all over the world by Levine (1), and only few of them are described from India. The species presented here has been described from the earthworm of Bangladesh for the first time. Among the described species, *R. pilosa* and *R. porrecta* are more closely related with the new gregarine species presented in this study by Troisi (5). In *R. pilosa* the epimerite like apparatus is highly specialized for attaching the parasite with host cells, whereas the other species of the same genus rarely or never uses its anterior apparatus for the purpose of attachment and it may be reduced to a small, conical, protoplasmic structure called the mucorn by Troisi, (5). In *R. pilosa* the body is usually long and slender, anterior end broad and rounded, while the rest of the body tapers toward the posterior extremity, which is pointed. The average size of *R. pilosa* is 217.3µ×25.5µ. The present form differs from *R. pilosa* in having a more elongated and cylindrical body with a blunt posterior end and it measures 419.90-430.95 (425.05±4.70) µm×44.2-50.83 (47.88±2.85) µm. The anterior pole of *R. porrecta* and the present form possesses a very well developed mucorn which is made up of smooth hyaline protoplasm. But in *R.* pilosa this type of attaching apparatus surrounding the mucorn is lacking. The ectosarc of *R. pilosa* is thick, the cuticle thin with close longitudinal ridges. The cuticular hairs are also present in *R. pilosa*, but in *R. porrecta* the epicyte is thin, transparent and marked by 36 to 48 longitudinal ridges. Longitudinal myonems are also present in the myocyte of both *R. pilosa* and *R. porrecta*. Myonem is absent in the present form. The endoplasm of *R. pilosa* is viscous, packed with oval paraglycogen grains whereas the endoplasm of *R. porrecta* is composed of dense granular protoplasm within many ovoid paraglycogen grains which resemble the present species. Nucleus in the present form is small, elongated and found in the posterior portion of the gamont, measuring 17.68-22.01 (19.74±1.95) µm×11.05-15.47 (13.40±1.95) µm. However, in *R. pilosa* the nucleus is rounded and large while it is of a vesicular type in *R. porrecta*. The gametocysts of *R. porrecta* contain two approximately equal sized gametocytes. Spores are biconical, symmetrical and measures 27.7 µm - 28.0 µm×11.8 to 12.1 µm in *R. porrecta*. The cyst of *R. pilosa* is ovoid and measures 94.9 µm×4.2 µm. They are comparatively small and measure 13.3 µm to 5.0 µm. The gametocysts of the present form are also ovoid with two unequal sized gametocytes and the gametocyst measures 95.03-97.24 (96.20±1.14) µm×77.35-81.77 (79.56±1.86) µm. Tye oocyst of the present form is biconical. It is 11.05-15.47 (12.81±1.71) µm in length and 6.63-8.84 (7.70±1.20) µm in width.

Besides these, the adult trophozoites of *R. pilosa* and *R. porrecta* undergo a process of asexual reproduction, plasmotomy. *R. porrecta* also undergo a process of degeneration in their life cycle. However it is not found in the present species. Moreover, the hosts are also different. *R. pilosa* was described from the seminal vesicles of *Lumbricus castanues*, *L. terrestris*, *Helodrilus foetidus* while the *R. porrecta* was obtained from the seminal vesicles of *L.*

Table 2. A comparison between different characters of R. silvae sp. nov. with closely related species is presented. All measurements are in µm

Species Characters	*R. pilosa* Hesse (1909)	*R. porrecta* Hesse (1909)	*R. silvae* sp. nov
Host(s)	Lumbricus castaneus, Lumbricus terrestris and Helodrilus foetidus	Lumbricus rubellus and Helodrilus foetidus	Metaphire peguana
Locality	Europe (Philadelphia)	Europe (Philadelphia)	North Badda, Dhaka District, (Bangladesh)
Gamonts	Long and slender. Anterior end broad, rounded and posterior extremity is pointed. It measures 217.3µ in length by 25.5µ in width	Snake like with enlarged head. Total length of the gamont is 21/2 mm. Anterior end swollen average 32µ to 36µ in diameter and rest of the body tapers gradually to a point average 28µ in diameter	Elongated with a conical, enlarged head and blunt posterior ends. Mature gamont length 419.90-430.95 (425.05±4.70) µm and width at the widest part is 44.2-50.83 (47.88±2.85) µm. Width of the posterior end is 15.47-17.68 (16.50±1.14) µm
Mucorn	Present. "Cuticle hair" is also present for attacking to the host	Present. But "Cuticle hair" is absent	Present at the anterior part. It measures 13.26-17.68 (15.47±1.86) µm×17.68-22.1 (19.44±1.71) µm
Longitudinal Myonems	Present	Present	Absent
Nucleus	Rounded, large with central karyosome and at the anterior end	Vesicular type	Small, elongated and at the posterior end. It measures 17.68-22.01 (19.74±1.95) µm×11.05-15.47 (13.40±1.95) µm
Endoplasm	Viscous, packed with oval paraglycogen grains	Composed of dense granular protoplasm within many ovoid paraglycogen grains	Composed of dense granular protoplasm with ovoid paraglycogen grains
Gametocysts	Ovoid, and measures 94.9µ×84.2µ	Speherical, contains two equal sized gametocytes. Each gametocyte produces isogametes	Ovoid, with two unequal sized gametocytes. Gametocysts measure 95.03-97.24 (96.20±1.14) µm×77.35-81.77 (79.56±1.86) µm
Spores	Small and measures 13.3µ to 5.0µ	Biconical, symmetrical and measures 27.7µ to 28.0µ×11.8µ to 12.1µ	Biconical and measures 11.05-15.47 (12.81±1.71) µm×6.63-8.84 (7.70±1.20) µm
Plasmotomy	Present	Present	Absent
Degeneration	Absent	Present	Absent
References	Troisi, 1933	Troisi, 1933	Present study

rubellus and *Helodrilus foetidus*. On the contrary the present form has been described from the *Metaphire peguana*. A comparative table: Table 2 contains the comparison of the present species with other closely related species. It is seen from the table that the present form differs in morphology, morphometrics, host preference and distribution pattern from the other two previously described *Rhynchocystis* species. Moreover, it is evident that the present species obtained from *Metaphire peguana* is also totally different from the two closely related species *R. porecta* and *R. polisa*. After careful consideration, it can be concluded that the present form should not be accommodated into any known species under the genus *Rhynchocystis*. Therefore, a separate status has been proposed for the present form and it is also described for the first time from *Metaphire peguana* of Bangladesh. Hence the name *Rhynchocystis silvae* sp. nov. is being proposed here.

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Conflict of Interest
No conflict of interest was declared by the authors.

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