

**Research Article** 

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# Gregarine Parasite Belonging to the Family Diplocystidae Bhatia, 1938 Reported from the Cockroaches *Blatta orientalis* (Dictyopetra: Blattidae) from Kashmir

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## Abstract

During the present study *Diplocystis kasmirensis* n. sp. (Sporozoa: Eugregarinida) were collected from Haemocoel including gut surface of *Blatta orientalis* Linnaeus (Dictyoptera; Blattidae) with description to their morphology, life cycle stages and taxonomy.

Keywords: Protozoan; Cockroach; Camera lucida; Srinagar; India

## Introduction

Protozoan diseases of insects are ubiquitous and comprise an important regulatory role in insect populations [1,2]. The main route of infection by protozoans in insects is through mouth, however in some cases transmission may be through egg of host (trans ovum). One important and common consequence of protozoan infection is a reduction in the number of offspring produced by infected insects.

Although protozoan pathogens play a significant role in the natural limitation of insect populations. Involved protozoan species have successfully been utilized for suppression of insect pests of agricultural, horticultural, forestry, medical and veterinary importance in different parts of the world [3].

Gregarines were first found in cockroaches by Siebold. Most gregarines have been found in the midgut but *Protomagalhaensia serpentula* and *Diplocystis* sp. [3] also have been reported from the body cavity [4].

## Materials and Methods

The cockroaches for the present study were collected at night with the help of forceps from toilet, main holes' bathroom from different localities of Srinagar, and were then dissected with the help of scissors, forceps and needles and the alimentary tract was drawn out intact by grasping the head and thorax and gently pulling apart.

The field collected Insects were anesthetized with chloroform or by putting them in a refrigerator for 5-10 minutes [5]. The anterior and posterior extremities of the host were clipped off as close to the ends of the insect as possible with scissors, forceps and needles and the alimentary tract was drawn out intact with the help of forceps, by grasping the head and thorax and gently pulling apart. The intestine came out attached to the head. It was then slit lengthwise with fine needles, placed flat on a slide, and the masses of food were then teased out carefully to form a layer as thin, nearly transparent as possible and scanned for protozoan parasites. At the same time the Malpighian tubules, fat bodies, other tissues and body cavity was also scanned for the protozoans. The protozoan parasites were first observed unfixed in live condition [6].

The number of hosts and protozoan parasites collected from them were recorded and placed in sampling bottles till further processing. After examining protozoans in live condition, they were processed for permanent slide preparations for detailed microscopic examination and identification [7]. Thin smear of host intestine, Malpighian tubules and body fluid was prepared on a clean slide or cover slip and was dropped face down into a petri-dish of fixative. The slide or cover slip was allowed to float on the surface with no air bubbles. This held the specimens against the glass until they were affixed through fixation of parasites. In each case, material on cover slips was allowed to fix for at least 5 minutes. After fixing in AFA solution the parasites were stained with Semichon's Acid and finally dehydrated and mounted in DPX to get the correct picture of protozoan parasites [8-10].

## Key to the Families of Order Eugregarinida

- 1. Mature trophozoites with primary segmentation only (distinguishing the protomerite and deutomerite); no secondary segmentation is present.
- 2. Mature trophozoites with secondary segmentation of the protomerite and deutomerite **Metameridae.**
- 3. Epimerite simple, not dilated into a lobate sucker.
- 4. Epimerite dilated into a lobate sucker.
- 5. Gametocysts dehisce by simple rupture Cephaloidophoridae.
- 6. Gametocysts dehisce through one or more sporoducts.
- 7. Epimerite apparently, an extension of protomerite, without distinct neck or stalk; parasitic in crustacean -**Cephalobidae**.
- 8. Epimerite without four distinct cup-like suckers and a distinct neck; parasitic in insects **Hermocystidae**.
- 9. Gametocysts with single sporoduct Didymophyidae.
- 10. Gametocysts with more than one sporoduct Gregarinidae.

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Received October 07, 2016; Accepted November 11, 2016; Published November 15, 2016

**Citation:** Rehana R, Bhagat RC, Rukhsana AF, Sofi TA (2016) Gregarine Parasite Belonging to the Family Diplocystidae Bhatia, 1938 Reported from the Cockroaches *Blatta orientalis* (Dictyopetra: Blattidae) from Kashmir. J Vet Sci Technol 7: 399. doi: 10.4172/2157-7579.1000399

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Page 2 of 3

- 11. Epimerite apparent in mature trophozoites, complex or at least strongly differentiated.
- 12. Epimerite in mature trophozoites absent, vestigial, invaginated or reduced to a small cap or spherule.
- 13. Epimerite in mature trophozoites absent or reduced and invaginated; oocysts ovoidal or ellipsoidal with or without equatorial suture **Stenophoridae**.
- 14. Epimerite in mature trophozoites reduced to a small cap or spherule; oocyst ellipsoidal **Cnemidosporidae.**
- 15. Epimerite with bristle or epicytic processes Brustiophoridae.
- 16. Epimerite varied, if present epicytic processes strong, digitate or hooked; not bristle-like.
- 17. Oocysts biconical, cylindric conical, crescentic or irregular, liberated singly or in clumps, but not in chains; gametocyst with or without secondary cyst wall when present usually lose and gelatinous **Actinocephalidae**.
- 18. Oocysts pruse-shaped or hat-shaped, emitted in chains; gametocyst with loose, rugose, secondary cyst wall – Stylocephalidae.
- 19. Trophozoites solitary or associated in pairs and spores are ovoid to spherical **Diplocystidae**.
- 20. Trophozoites range from spheroid to much elongated type and spores are typically spindle shaped **Monocystidae**.

#### Diagnostic characters of the family Diplocystidae

Coelomic or gut parasites of insects; trophozoites solitary or associated in pairs; spores round or oval, with eight sporozoites. Syzygy is precocious with fusion of the gamonts. The oocysts are ellipsoidal.

**Diagnostic characters of genus** *Diplocystis*: Trophozoites spherical to oval. Association of two individuals begin early in spherical form, spores round or oval with eight sporozoites.

#### Diplocystis kasmirensis n. sp.

Host: Blatta orientalis Linnaeus (Dictyoptera; Blattidae)

Site of infection: Throughout Haemocoel including gut surface

#### Locality: Srinagar

Out of 12 host specimens, four host specimens of *Blatta orientalis*, were found to be infected throughout the haemocoel including gut surface.

## Description

The sporadins (Figures 1a-1c) are rounded to ovoidal, nucleus spherical with one karyosome measuring 15.375  $\mu$ . The cytoplasm was finely granulated. Sporadin measuring 51.25  $\mu$  in diameter. They were biassociative (Figures 1d and 1e) and in apposition head to head; obese with no protomerite. The association measures 112.75  $\mu$  in length and 61.65  $\mu$  in width. The associated gamonts vary in size [11].

The cysts (Figure 1f) are spherical, measuring 97.75  $\mu$  in diameter with the sporulation being partial. There are certain spaces on the wall which are meant for the emergence of the sporoducts. Only one sporoduct is visible. Spores (Figures 1g-1i) oval measuring 10.25  $\mu$  in diameter.

# Discussion

The critical examination of the present species revealed that certain important features such as the spherical trophozoites without the protomerite, the associated sporadins, the oval spores led to its placement in the genus *Diplocystis* Kunstler [12].

While comparing the present specimens with *Diplocystis schneideri* Kunstler, [4] collected from the posterior half of mid gut surface of *Blattella germanica* and *Periplanata americana*, the present specimen differed from it in host locality, as it was found throughout haemocoel of *Blatta orientalis*. The shape and size of spores also varied among both the species as round vs oval, 2.0 vs 10.25  $\mu$  (Table 1).

Diplocystis major Cuenot was observed through out the haemocoel including gut surface and young stages were found in the gut epithelium of *Gryllus* spp. when present specimen was compared with *D. major* it differed in having different sizes and shapes of the spores, oval vs. rounded and measuring  $10.25 \ \mu$  vs  $8.0 \ \mu$  in diameter (Table 1) [13,14].

When compared with *D. minor* (Table 1) found throughout haemocoel of *Gryllus* sp., there was difference in the shape and size of spore of both the species as oval vs round measuring 8.0 vs  $10.25 \mu$ .

On comparison with *Diplocystis chandigarhensis* Bala [5] from *Periplanata americana* observed in fat bodies, the present specimens of parasites were found throughout haemocoel including gut surface. There was variation in the size of cysts and spores as  $4.5 \times 2.5$  vs  $10.25 \mu$  and 50 vs 97.75  $\mu$  [15,16].

Hence, all the above morphological characters and measurements discussed with the already known species of the genus *Diplocystis* made differences clear with the presently considered specimens [17]. Taking into account the above-mentioned differences, it became necessary to create a new species for which the name *Diplocystis kasmirensis* n. sp. is proposed after the name of the study state i.e., Kashmir.



Figures 1a-1c: Sporadins of Diplocystis kashmirensis n. sp. 100X.



Figures 1d and 1e: Schizogonic stages of Diplocystis kashmirensis n. sp. 100X.

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Page 3 of 3

		Species				
Particulars		Diplocystis schneideri Kunstler, 1887	Diplocystis major Cuenot, 1901	Diplocystis minor Cuenot, 1901	Diplocystis <i>chandigarhensis</i> Bala, 1988	Diplocystis <i>kasmirensis</i> n. sp. (Present specimen)
Spore	Shape	Round-Oval	Oval	Oval	Oval	Round-Oval
	Size	6.0 µ	8.0 µ	5.0 µ	4.5 × 2.7 μ	10.25 µ
Cyst	Shape	-	-	-	Spherical	Spherical
	Size	-	-	-	50 µ	97.75 µ
Host		Blattella germanica and Periplanata americana	<i>Gryllus</i> sp.	<i>Gryllus</i> sp.	Periplanata americana	Blatta orientalis
Site		Mid gut surface (Post. half)	Haemocoel including gut surface and young stages found in the gut epithelium	Haemocoel	Fat bodies	Haemocoel including gut surface

Table 1: Comparison of present specimen with four species of Diplocystis.



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Figures 1f and 1g: Life cycle stages of Diplocystis kashmirensis n. sp.



Figures 1h and 1i: Schizogonic and Sporadin stages of *Diplocystis* kashmirensis n. sp.

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