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Steinina blattidaensis sp. nov.: A New Species of Septate Gregarines of the Genus Léger and Dubscq (1904) from *Periplaneta americana* (Order: Blattidae) of Manipur, India

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ABSTRACT

The present paper study with the life history and the morphology of the new cephaline gregarines from *Periplaneta americana*. The parasite has been assigned a new species *Steinina blattidaensis*. sp. nov. has been described as the type of the genus Léger and Dubscq (1904). The parasite has the following ratios: LP: TL=1:7.5; WP: WD=1:1.4. The present percentages of infection in the mid-gut of the parasite were 21 out of 50 (42%). A new specie *Steinina blattidaensis*. sp. nov has been created according to the (Order: Blattidae). The morphological details of the parasite of different stages supported with photomicrographs are also provided.

Keywords: *Steinina blattidaensis*. sp. nov., *Periplaneta americana*, Morphology, Cephaline gregarines

Abbreviations: TL: Total Length; LE: Length of Epimerite; LP: Length of Protomerite; WP: Width of Protomerite; LD: Length of Deutomerite; WD: Width of Deutomerite; LN: Length of Nucleus; WN: Width of Nucleus; LP:LT: Length of Protomerite:Total Length; WP:WD: Width of Protomerite:Width of Deutomerite

INTRODUCTION

The genus Steinina was established by Léger and Dubscq [1] to include a gregarine *Steinina ovalis* (Stein). The characters of the genus Steinina by Léger and Dubscq [1] are: Sporont Solitary, Epimerite a short retractile digitiform process in the early stage and becoming flattened transparent button-like. Cyst spherical, dehiscence by simple rupture and spores biconical. Later many workers like Ashworth and Rettie [2], Ishii [3], Watson [4], Foerster [5], Hoshide [6], Obata [7], Théodoriès and Jolivet [8], Théodoriès and Desportes [9] and Théodoriès et al. [10] described many new species under the genus from different parts of the world. However, the literature extent reveals that only four cephaline gregarines under the genus Steinina have so far been reported from India [11-13]. Later on Gupta and Haldar [14], while studying the cephaline gregarines from the stored-grain pests of West Bengal, found gregarines from the mid gut of the larva of the *Tenebrionid bettle*, *Palorus* sp. and described *Steinina palorousi*.

In course of the present investigation, one species of Steinina is described which is discovered from the valley districts of Manipur. The description of the species is made in details with illustrative diagrams and photomicrographs. The gregarine has been described as new species which has distinctive features of its own.

MATERIALS AND METHODS

The samples were collected from various grass fields of Manipur with the help of net were kept in glass tubes and brought alive to the laboratory for examination. These were decapitated; their guts carefully dissected out under a dissecting microscope and gently pressed to expel the parasites from the gut lumen. Thin smear preparations were fixed in Schaudinn's fixative and subsequently stained with Heidenhain's haematoxylin [15]. Gametocysts were recovered from the hind gut and placed in moist chambers

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(80% relative humidity) for sporulation [16]. The structures of the oocysts were studied by using Lugol's iodine solution. Figures of stained specimens were drawn with the aid of a camera lucida **(Figure 1)**. Measurements of fresh materials were taken using an ocular micrometer calibrated with a stage micrometer. All measurements, unless other-wise mentioned were in micrometers. Measurements were taken from widest part of protomerite, deutomerite, nucleus, gametocyst and oocyst and presented in this paper as range values, followed by means, standard errors and sample sizes in parentheses. Nomenclature for shapes used in this paper conforms to those of Clopton [17].

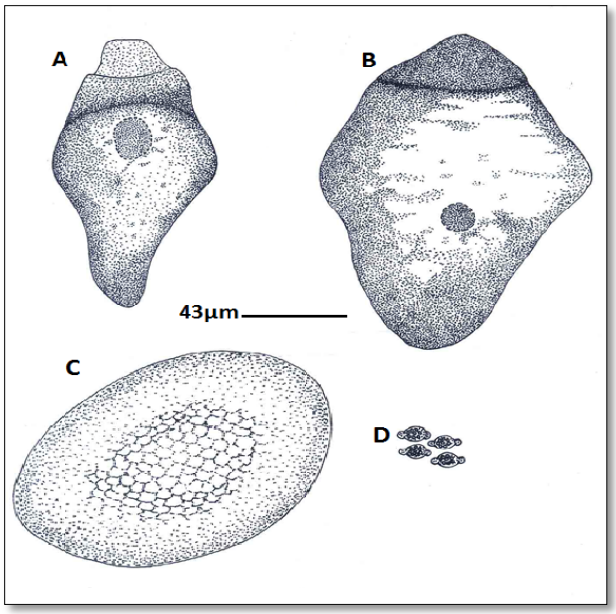

Figure 1. *Camera lucida* drawings of *Steinina blattidaensis* sp. nov. A) Mature trophozoite; B) Sporadin; C) Gametocyst; and D) Spore with knobs.

RESULTS AND DISCUSSION

Description

Trophozoite: Trophozoite are found freely in the lumen in the host's gut. Trophozoite are so far encountered in the smear preparations. These are solitary, elongated obese in shape and are of various sizes and measures 109.9-159 (143.1 ± 11.17) µm in total length. The epimerite of the mature trophozoite is short retractile digitiform process, later become flattened button-like. The epimerite measures 14.7-32.6 (23.5 ± 5.8) × 17.5-39.6 (24.2 ± 6.06 µm in average. The protomerite is hemispherical or dome-shaped. The protomerite is broader at its posterior extremity and measures 14.4-40.2 (23.9 ± 6.72) × 37.4-69.4 (57.9 ± 7.4) µm in average. The septum between the protomerite and the deutomerite is thin and there is also a slight constriction between the protomerite and the deutomerite. The deutomerite is elongated and is broadest near the septum. And it become narrower towards the posterior end, terminating in a pointed to rounded extremity. The deutomerite measures 99.6-134.4 (109.3 ± 8.01) × 55.7-89.5 (65.3 ± 7.91) µm in average. The nucleus is spherical with a single deeply stained endosome and always situated at the anterior half of the deutomerite. It measures 13.7-29.4 (21.7 ± 6.40) × 15.7-32.4 (22.9 ± 5.17) µm in average **(Figure 2)**.

Sporadin: Sporadins are solitary, with a short protomerite and a long deutomerite. The young sporadin are very small and are of various sizes. Some are triangular in shape, the anterior end of which is broader, gradually tapering towards the posterior end. The sporadin measure 152.3-198.9 (186.9 ± 10.06) µmin total length. The protomerite is dome-shaped and measures 28.9-64.3 (38.9 ± 8.16) × 39.3-75.2 (49.3 ± 8.16) µm in average. The septum between the protomerite and the deutomerite is convex in shape. The nucleus is spherical and is situated at the centre of the deutomerite and measures 109.1-156.2 (119.9 ± 10.14) × 99.7-139.5 (109.6 ± 8.88) µm in average. The deutomerite is the largest segment of the body. It is broadest at the anterior one-third of its length. It unevenly tapers towards the posterior extremity and end in a cone. The cytoplasm of both protomerite and the deutomerite is highly granular. The nucleus is subspherical or spherical in shape and measures 10.5-26.3 (18.2 ± 4.81) × 9.5-29.3 (19.3 ± 5.79) µm in average **(Figure 2)**.

Gametocyst: Gametocysts collected from the hind gut of the infected hosts are oval in shaped and measures 101.5-155.2 (137.0 ± 12.6) × 63.5-102.1 (83.1 ± 9.6) µm in dimensions. The cyst wall is smooth and the cenreal part is more condensed than its periphery. After 48 hrs of development inside the moist chamber the spores are formed within the cyst **(Figure 2)**.

Spore: Spores are biconical. The button-like hyaline, subconical knobs form the two poles of the spores. The spore measure 13.5 × 10.5 µm in dimensions. After 40 h, the sporozoites are clearly discernable. These are eight in number and are arranged in a circular fashion inside the spore **(Figure 2)**.

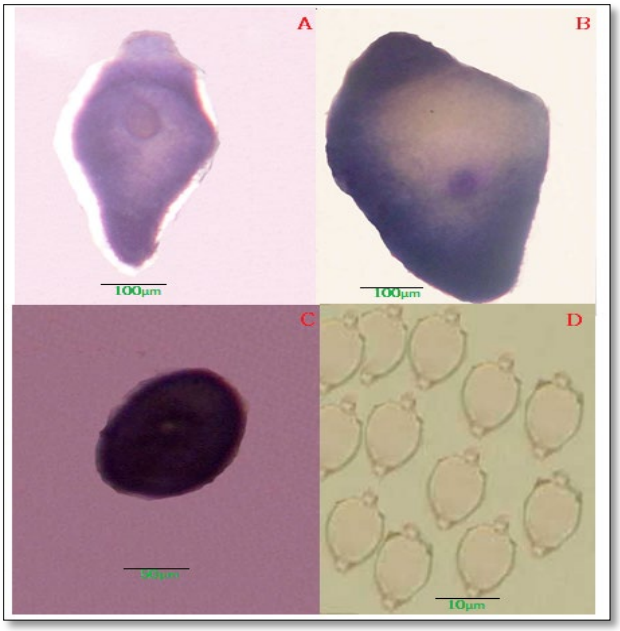

Figure 2. Photomicrographs of *Steinina blattidaensis* sp. nov. A) Mature trophozoite; B) Sporadin; C) Gametocyst; and D) Spore with knobs.

Taxonomic summary

Type material: *Steinina blattidaensis*. sp. nov.

Type host: *Periplaneta americana* (Order: Blattidae)

Type locality: Canchipur, Imphal (west)

Site of infection: Mid gut

Prevalance: 21 out of 50 (42%)

Paratype: MU/0213/14, deposited in the Protozoan Collection of Parasitology Section, Centre of Advanced Studies in Life Sciences, Manipur University, Canchipur-795003, India

Holotype: MU/022/14, deposited in the Protozoan Collection of Parasitology Section, Centre of Advanced Studies in Life Sciences, Manipur University, Canchipur-795003, India

Measurements: Summary of measurements in micrometers of preserved (fixed and stained) Trophozoites and Sporadins are provided **(Table 1)**.

Table 1. Showing R, $\bar{X}$, SD, SE and CV% of measurement of *Steinina blattidaensis*. sp. nov.

Different parts	R	$\bar{X}$	SD	SE	CV%
TL	109.9-15.9	156.9	11.1	2.99	7.80
LE	14.7-32.6	23.5	5.8	1.30	24.68
WE	17.5-39.6	24.2	6.06	1.35	25.04
LP	14.4-40.2	23.9	6.72	1.68	28.11
WP	37.4-69.5	57.9	7.4	1.65	12.79
LD	99.6-134.4	109.3	8.01	1.79	7.32
WD	55.7-80.5	65.3	7.91	1.76	12.11
LN	13.7-29.4	21.7	6.40	1.43	29.41
WN	15.7-32.4	22.9	5.17	1.15	22.57

Paratype (20): Slide No. MU/0213/14

Trophozoite

TL=109.9-159 (143.1 ± 11.17)

LE=14.7-32.6 (23.5 ± 5.8)

WE=17.5- 39.6 (24.2 ± 6.06)

LP=14.4-40.2 (23.9 ± 6.72)

WP=37.4-69.4 (57.9 ± 7.4)

LD=99.6-134.4 (109.3 ± 8.01)

WD=55.7-89.5 (65.3 ± 7.91)

LN=13.7-29.4 (21.7 ± 6.40)

WN=15.7-32.4 (22.9 ± 5.17)

LP:LT=1:7.5

WP:WD=1:1.4

Sporadin

TL=152.3-198.9 (186.9 ± 10.06)

LP=28.9-64.3 (38.9 ± 8.16)

WP=39.3-75.2 (49.3 ± 8.16)

LD=109.1-156.2 (119.9 ± 10.14)

WD=99.7-139.5 (109.6 ± 8.88)

LN=10.5-26.3 (18.2 ± 4.81)

WN=9.5-29.3 (19.3 ± 5.79)

LP:LT=1:5.3

WP:WD=1:2.5

Holotype: Slide No. MU/022/14

Trophozoite

LT=148

LE=21.5

WE=26.87

LP=19.5

WP=48.75

LD=105

WD=69.87

LN=21.5

WN=23.2

LP:LT=1:7.5

WP:WD=1:1.4

Sporadin

LT=172

LP=32.2

WP=48.37

LD=139.7

WD=125.7

LN=17.7

WN=12.5

LP:LT=1:5.3

WP:WD=1:2.5

DISCUSSION AND CONCLUSION

The present gregarine possesses solitary sporadins, bears short retractile digitiform process in its epimerite, gametocyst and dehisces by simple rupture releasing biconical spores, thereby justifying its inclusion under the genus Steinina Léger and Duboscq [1] **(Table 2)**.

The present species quite resembles *S. ovalis* Léger and Dubsoscq [1] in the ratio of WP:WD, cyst and spore. But it differ from the species in the feature of the trophozoite, the protomerite, deutomerite of the sporadin and in the measurement of different parts of the body (Trophozoite 109-159 µm in size are solitary, dome shaped protomerite of sporadin 28.9-64.3 × 39.3-75.2 µm, deutomerite of sporadin is convex in sharp 109.1-156.2 × 99.7-139.5 µm in average in the present species). It also come close to the measurement range given for *S. minor* Obata, 1953 specially in the ratio of LP:TL but there is no agreement in other features like the shape of the triphozoite, epimerite, nucleus and cyst (Trophozoite elongated 109.9-159 µm in total length, Epimerite of the mature trophozoite is a short retractile digitiform process, 14.7-32.6 × 17.5-39.6 µm , Nucleus is spherical 13.7-29.4 × 15.7-32.4 µm and cyst is oval, 101.5-155.2 × 63.5-102.1 µm in dimensions in the present species). The gregarine, therefore is given a separate specific status for which the name *Steinina blattidaensis* .sp. nov. is proposed.

Table 2. Showing the comperative characters of different species of Steinina.

Characters	*S. ovalis* Léger and Duboscq [1]	*S. minor* Obata [2]	*Steinina blattidaensis* sp. nov.
Total Length	100 µm in length	72 µm in length	109.9-15.9 µm in length
Epimerite	Short, retractile digitiform process, later become fattened button-like	Little cone	Short, retractile digitiform process, later become flattened button-like
Protomerite	Cylindrical	Hemispherical	Hemispherical or done-shaped
Deutomerite	Ovoidal, terminating in the obtuse angled cone	Widest at 1/3rd from the anterior end	Deutomerite is elongated and is broadest near the septum
Nucleus	Spherical with one large karyosome	Spherical with one large	Spherical with a single deeply stained endosome
Sporadin	Solitary and ovoidal	Solitary, small obese	Solitary and triangular in shape
Gametocyst	Spherical and ovoidal	Spherical	Oval
Spore	Biconical broad through middle, 9.0 × 7.5 µm in size	-	Biconical broad through middle, 13.5 × 10.5 µm in size
LP:TL	1:2.5	1:3.3-7.2	1:7.5
WP:WD	1:1.4	1:1.0-1.3	1:1.4
Host	*Tenebrio molitor* L. Larva	Tenebrionidae larva	*Periplaneta americana*
Locality	France	Hiroshima, Japan	Chanchipur, Manipur

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