Journal of Bioresources 9 (1): 54-59, January – June 2022

JOURNAL OF BIORESOURCES

journal webpage: http://jbr.rgu.ac.in

ISSN: 2394-4315 (Print)
ISSN: 2582-2276 (Online)

New Taxa

RESEARCH ARTICLE

Phleobum periplanae sp. nov. and *Neohirmorcystis canchipurae* sp. nov. (Aplicomplexa: Eugregarinida) from Manipur, India

Naorem Mohilal, Indira Yumnam, Loukrakpam Bina Chanu*

Department of Zoology, Manipur University, Canchipur- 795003, Manipur, India.

Corresponding author e-mail: bina.chanu@gmail.com
Article No.: NMJBR42; Received: 05.03.2022; Reviewed: 30.03.2022; Revised: 15.05.2022; Accepted and Published: 30.06.2022
Doi: https://doi.org/10.5281/zenodo.8369796

Abstract

During 2012 – 2018, a survey was conducted for the protozoan parasites in valley districts of Manipur. Sample insect, cockroaches were collected using Sweep-netting methods and dissected. Fresh and permanent slides of collected protozoa from the gut were prepared using Haedenhain's iron, haematoxylin, Giemsa and Eosin stains. Mean, standard deviation and percent of co-efficient of variations were calculated. Two new Eugregarine protozoans namely *Phleobum periplanae* sp. nov and *Neohirmorcystis canchipurae* sp. nov. were reported as new to science. *Phleobum periplanae* sp. nov. is characterized by spherical epimerite which measures 14.7 – 32.6 µm while the protomerite is broadly ovoid, covered by a thick pellicle which measure 26.9 – 56.9 µm. Fresh gametocytes are yellowish- orange and ellipsoidal. *Neohirmocystis canchipurae* sp. nov. is characterised by lack of epimerites with a trophozoite length of (159.6-192.6) µm. Gametocyst dehisces by simple rupture releasing spherical spores.

Keywords: *Periplanata americana,* Didymophyidae, and Neohirmocystidae.

1. Introduction

Medical entomology deals with arthropods which affect the health and well-being of human, vertebrate animals and is concerned with the vectors that affect human and animal health. The need for awareness of the diseases transmitted by insect bites is crucial as many of the diseases can be transmitted by insects acting as vectors as well as some can produce myiasis by fly larvae by entering the host through varying mechanisms ranging from oviposition of live, burrowing larvae on the host, on or near open wounds, to attachment to other bloodsucking insects.

Plenty of faunal types were listed from Manipur including micro-organism but work was completely lacking on the study of diversity of protozoan animals. So, a survey was conducted for this particular group of animals during 2012- 2018, where various insect hosts were collected for their gut content observation. Several Protozoan specimens were collected from their gut among which two protozoa of the genus *Phleobum* Haldar and Chakraborty, 1974 and *Neohormocystis* Gosh et al., 1986 were found new through study of their morphological variations and comparison to various literatures.

The genus *Phleobum* Haldar and Chakraborty, 1974 was first established with description of *Phleobum gigantinum* Haldar and Chakraborty, 1974 revealed from Grasshopper, *Phleobum antennata* Burn. The species was earlier placed under the family Monoductidae, citing the nature of the cyst's dehiscence through a single sporoduct (Ray and Chakraborty, 1933). Later the parasite was reported from the mid gut and hepatic caeca of *Phleobum infumata* Brunner and they were very similar to those obtained from *P. antennata*. On thorough investigation, major variations in genetic characteristics were noted after which a new definition of the genus *Phleobum* was proposed. Since the gametocyst dehisces through a pore at one corner of the cyst wall liberating oocysts within a transparent mucoid covering, the genus *Phleobum* was placed under family Didymophyidae Ray and Chakraborty 1933; Haldar and Chakraborty, 1974.

The genus *Neohirmocystis* Haldar and Chakraborty, 1933 belongs to the family Neohirmocystidae (Gosh et al., 1986). The genus is characterized by vestigial or apparently absent epimerite. The satellite have caudofrontal, protomerite and deutomerite and gametocysts dehiscence through simple rupture. Oocyst is spherical and double-walled.
The new *Phleobum* protozoa revealed from Manipur is named *Phleobum periplanae* sp. nov. after its host *Periplaneta americana* (Linnaeus) and that of *Neohirmocystis* is named *Neohirmocystis canchipurae* after its type locality. The morphological details of the two species are illustrated here in the present manuscript with diagrams and photographs.

2. Materials and methods

Sample specimen, cockroaches were collected using Sweep - netting method. Collected samples were brought alive to the laboratory and deactivated using 10% formalin solution. The gut of the insects were taken out, dissected in 0.5% saline solution 1X Phosphate Buffered Saline (1X PBS) solution and observed for the parasite contained.

2.1. Fresh Slides preparation

The guts were gently teased with the help of needles and forceps for emergence from lumen. Thin smears were prepared on clean glass slides by using the same solution. The slides were covered with cleaned cover slips and examined under phase contrast microscope to study the movement of the protozoan parasites.

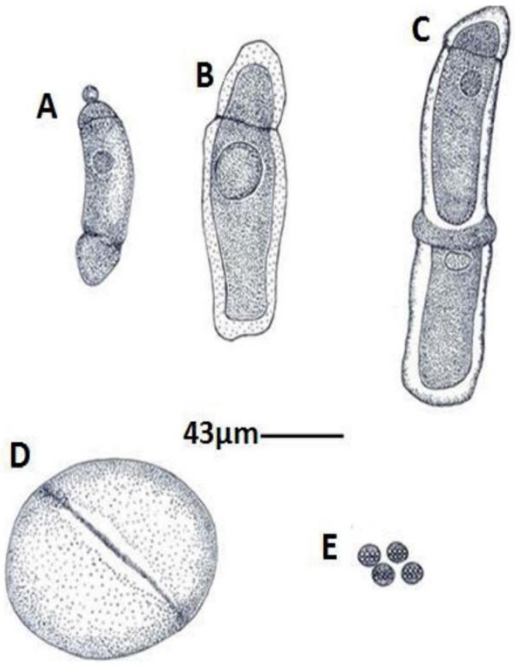

Figure 1: Camera lucida drawings of *Phleobum periplanae* sp. nov.; A – Mature trophozoite, B - Young trophozoite, C - Sporadin in sygyzy, D – Gametocyst and E - Spore with eight sporozoite.

Figure 2: Photomicrographs of *Phleobum periplanae* sp. nov.; A - Mature trophozoite; B - Young trophozoite, C - Group of syzygy; D – Gametocyst and E – Spore.

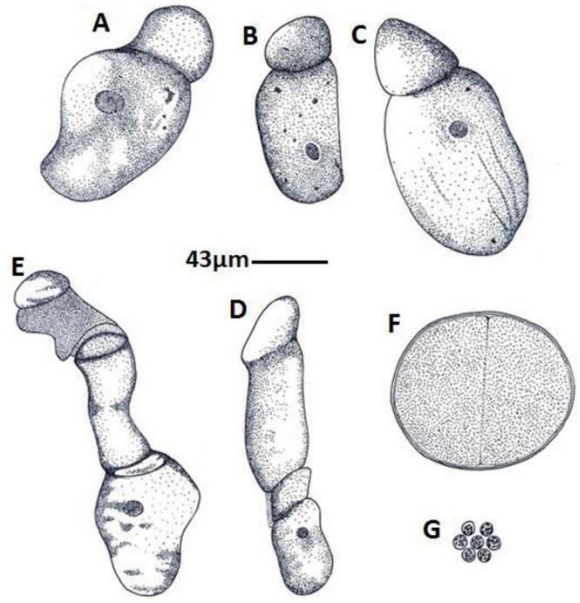

Figure 3: Camera lucida drawings of *Neohirmocystis canchipurae* sp. nov.; A - Mature trophozoite; B, C – Sporadin; D, E - Sporadin in syzygy; F – Gametocyst and G - Spore with eight sporozoite.

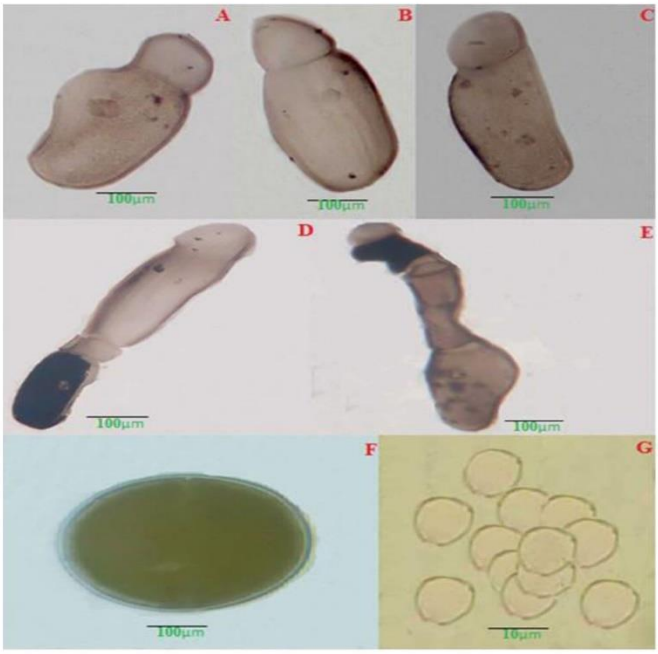

Figure 4: Photomicrographs of *Neohirmocystis canchipurae* sp. nov.; A - Mature trophozoite; B, C – Sporadin; D, E - Sporadin in sygyzy; F – Gametocyst and G – Spore.

Table-1: Statistical analysis of *Phleobum periplanae* sp. nov

Characters	R	X	SD	SE	CV%
TL	109.90 - 15.90	157.0	11.1	3.00	8.70
LE	14.70 - 32.60	24.0	6.7	1.30	25.48
LP	26.90 - 56.90	46.7	7.3	2.53	16.06
WP	37.40 - 69.50	58.8	7.4	2.64	13.69
LD	67.40 -103.20	93.04	6.0	1.36	7.42
WD	42.70 - 79.50	69.5	8.2	2.74	12.00
LN	22.60 - 49.50	40.1	7.6	2.41	17.24

2.2. Permanent Slides preparation

2.2.1. Heidenhain's Iron Alum Haematoxylin Stain

For permanent slides preparation, the guts of the hosts were smeared on a clean grease- free slide and dipped in Shaudinn's fluid (20-30 mins), Carnony fixative (20 mins) or aqueous Bouin fluid for 16-24 hour respectively. They were subsequently stained with Haemotoxylin for 30 mins and washed of over stained with 1% Iron alum solution. DPX is used as mounting agent and covered with cleaned glass covers.

Table 2: Comparative account of closely related species of *Phleobum*

Characters	*P. gigantinum* Gosh et al. 1986	*P. collarum* Kundu, Haldar 1986	*P. subsphericum* Modak et al. 2011	*P. loepimeritu* Larrain, Salas 2008	*P. periplanae* sp. nov.
Total length	230.0 – 620	95.0 – 699.7	177.6- 666.6	258 – 670	15.9 – 109.9
Epimerite	Absent	Spherical, hyaline body, 12.5 – 33.3	Sub spherical, knob - like hyaline body, 11.1 – 22.2	Globular, hyaline body, 16x17	Small spherical, 14.7 – 32.6
Deutomerite	Cylindrical, pellicle well – developed; epicyteal striations present	Elongated, ellipsoidal to cylindrical; pellicle well-developed; epicyteal striations wanting	Elongated, cylindrical, thick pellicle	Cylindrical to oblong in shape, well – developed pellicle; epicyteal striations wanting	Ellipsoidal with broadly rounded posterior and elevated margin; pellicle well developed; epicyteal striations present
Nucleus	Spherical; 50; several karyosomes	Oval; 25.0 – 99.9	Spherical or ellipsoidal, 33.3 – 77.7	Orbicular or ellipsoidal; 52x37	Orbicular; 35.7 – 63.2; fine and coarse karyosomes
Gamont	In pairs; satellite always larger than primate	In pairs, satellite smaller or larger than primite	In pairs; satellite and primate more or less same size but posterior tip of the primate fits firmly into the convexity of the protomerite of the satellite	Oval or ellipsoidal, 10.5 – 36.8	Orbicular or ellipsoidal; 52x37
Gametocyst	yellowish-white orbicular body; 520 – 600; unequal gametocytes; dehiscence through a single enormous size sporoduct, 2.7 mm long	Orange colored orbicular body, with a transparent gelatinous ectocyst; 436.8; gametocytes of equal or unequal size; dehiscence through a pore at one corner of gametocyst wall liberating oocysts within a transparent mucoid covering	Oval, bright lemon yellowish color; 447.8x 263.1- 457.7 x273.6 equal gametocytes dehiscence by normal rupture through a pore on the cyst wall; liberating oocysts in singly	Orange colored; orbicular to ellipsoidal, 244.4 x 222.2 – 388.8 x 333.3, gametocytes of equal or unequal size; dehiscence through a pore at one corner of the gametocytes wall liberating oocysts in a linear fashion	Yellowish – orange; ellipsoidal; 240 – 277.2 x 175.2 – 203.7; gametocyst dehisces through a pore after 72 hours inside moist chamber
Oocyst	Ellipsoidal; 6.0 x 4.0	Ellipsoidal; 7.7x4.4	Ellipsoidal, 6.1x5.1	Ellipsoidal; 9x5	Ellipsoidal; 12.2 x 9.5
LP: TL	1:5.3	1:3.0-11.0	1:3.6- 1:9.4	1:5-6.1	1: 3.5
WP: WD	1:1.2	1:0.9- 1.4	1:0.7- 1:1.1	1:1.1- 1.3(1.2)	1:1.0
Host	*Phlaeoba antennata*	*Phlaeoba infumata*	*Atractomorpha crenulata*	*Oxyla hyla hyla*	*Periplanata americana*
Locality	Kalyani, India	Kalyani, India	Kalyani, India	Kalyani, India	Canchipur, India

Table 3: Statistical analysis of *Neohirmocyctis canchipurae* sp. nov.

Characters	R	X	SD	SE	CV%
TL	160.5 – 193.5	172.8	10.0	2.25	5.81
LP	43.8 – 71.3	55.6	7.7	1.72	14.6
WP	60.2- 91.2	70.2	9.2	1.67	13.1
LD	111.8 – 150.5	118.3	9.2	2.07	7.71
WD	88.7 -101.8	101.8	8.7	1.96	8.62
LN	12.8– 42.3	21.2	7.4	1.65	34.9
WN	26.7 – 52.3	30.2	7.5	1.67	24.8

2.2.2. Giemsa Stain

The smear was prepared in 1X Phosphate Buffered Saline (1X PBS) soln. It was dried in air, fixed with acetone free absolute methyl alcohol for 5 -10 minutes and stained in Giemsa for 30 minutes. Slides were washed in buffered water, dried at air and mounted with DPX.

2.2.3. Haemotoxylin and Eosin (H&E)

Smeared slides were stained in Harris' haematoxylin (20 min), differentiated with 1% acid alcohol (10 sec) followed by washing and bluing by dipping the tissues in ammonia water for about 10 seconds. Using Bancroft and Gamble, 2002 method, the slides were counterstained with eosin for 2 minutes, dehydrated for 2 minutes, cleared in xylene for 2 minutes and finally mounted on DPX.

2.2.4. Temporary staining of oocysts

Drops of oocyst suspensions were taken on glass slides with drop of Lugol's iodine solution. The slides were covered with cleaned cover slips and edges were sealed with paraffin. Developmental stages of sporozoites were examined under oil immersion lens and photomicrographs taken.

Measurements were taken with the help of an ocular micrometer calibrated with a stage micrometer. Illustrations were drawn with the help of a camera lucida attached to microscope. The photomicrographs were taken using a digital camera fitted to an Olympus GE-52TRH microscope.

2.2.5. Abbreviations used

K= Karysome, LD = Length of deutomerite, LE= Length of epimerite, LMN=Length of macronucleus, LN = Length of nucleus, LP= Length of protomerite, N=Nucleus, WD =Width of deutomerite, WE= Width of epimerite, WMN=Width of macronucleus, WN=Width of nucleus and WP=Width of protomerite.

2.2.5. Ratios and Calculations

The ratios used in this article are LP: TL and WP:WD

P.C. of co-efficient of variation, CV% = $\frac{SD}{X}$x 100

3. Results

3.1. Phleobum periplanae sp. nov.

3.1.1. Description

Trophozoite: Solitary, the total length of the trophozoite measured 15.9-109.9 (143.1±11.17) µm (Table 1, Figures 1 and 2). The epimerite was very small, spherical and measured 14.7-32.6 (23.5±5.8) µm. The protomerite was broadly ovoid, covered by a thick pellicle 26.9-56.9 (45.8±7.3) µm × 37.4-69.4 (57.9±7.4) µm. Deutomerite was somewhat ellipsoidal with broadly rounded posterior and having slightly elevated margin, covered by a well-developed pellicle 67.4-103.2 (93.4±6.09) µm × 42.7-79.5 (68.6±8.23) µm. Fine cytoplasmic granulations were present. Nucleus is orbicular situated anywhere in the deutomerite with a distinct nuclear membrane which measured 22.6-49.5 (39.1±6.75) µm in length. Freshly collected specimens appeared orange. Epicyteal striations were clearly discernable in some specimens.

Sporadin: Solitary as well as bi - associative forms were observed. Solitary forms were oblong or cylindrical – shaped measuring 152.3-198.9 (186.9±10.06) µm in length. The protomerite was broadly ovoid or dome-shaped. The pellicle was 43.2-79.5 (66.9±8.8) µm × 42.5-72.5 (58.4±7.01) µm and protomerite was broad-shaped in outline. The deutomerite was oblong with rounded extremity. Both the protomerite and deutomerite had thick pellicle 109.1-156.2 (145.9±10.18) µm × 46.2-79.5 (68.8±7.67) µm and were filled with fine to coarse granules. The nucleus was situated anywhere in the deutomerite measuring 35.7-63.2 (41.4±6.49) µm in length. Closely set epicyteal striations were distinctly observed.

Association: Associations were always caudo-frontal. The protomerite of the primate was broadly ovoid, whereas in satellite it was almost shallow oblong. Protomerite of the satellite with characteristic flange on their anterior extremities. On maturation, a clear cup-like depression replaced the flange. When enlarged, the free edge of the cup appeared wavy with clear ridges on the inner side. This type of structural modification was associated with firm attachment during association.

Gametocyst: Freshly collected gametocyst were yellowish-orange in coloration, ellipsoidal and measured 240-277.2(261.1±30.4) µm × 175.2-203.7(188.3±7.11) µm. Gametocyst dehisced through a pore after 72 hours inside the moist chamber.

Spore: Spores were uniformly ellipsoidal and measured 12.2×9.5 µm in length and sporozoite were arranged in linear fashion along the longitudinal axis of the spores.

Specimen information

Type specimen : *Phleobum periplanae* sp.nov
Type host : *Periplaneta americana* (Order: Blattidae)
Type locality : Life Science Building, Manipur University with 24.7523° N and 93.9280°E Canchipur – 795003, Imphal - west
Site of infection : Mid gut

The statistical analysis for the species is given in Table 1.

Measurements

Holotype: Slide MU / 019/14_1 (*P. periplanae* sp. nov.)
Trophozoite:
LT = 100.3 LE =8.5
LP =10.7 WP =21.5
LD =91.8 WD = 32.25 and LN =11.2.

Sporadin:

LT =172.5 LP =45.6
WP =41.5 LD =126.4
WD =48.7 and LN = 36.7.

Paratypes (20): MU/0210/14_2–20 (*P. periplanae* sp. nov.)

Trophozoite:
TL = 15.9-109.9 (143.1±11.17) LE = 4.7-22.6 (13.5±5.8)
LP = 13.9-43.9 (35.8±7.3) WP = 20.4-59.4 (47.9± 7.4)
LD = 67.4-103.2 (93.4±6.09) WD = 22.7-62.5(51.6± 8.23)
LN = 9.6-39.5 (29.1±6.75) LP: LT= 1: 4.0 and WP: WD= 1:1.1

Sporadin:
TL = 152.3-198.9 (186.9±10.06) LP = 43.2-79.5 (66.9±8.8)
WP = 42.5-72.5 (58.4±7.01) LD = 109.1-156.2 (145.9±10.18);
WD = 46.2-79.5 (68.8±7.67) LN = 35.7-63.2 (41.4±6.49);
LP: LT = 1:3.5 WP: WD = 1:1.0.

3.1.2. Diagnosis and relationships

The specimen had a simple and spherical epimerite, paired sporadins, ellipsoidal nucleus with fine granules and ellipsoidal spore were not extruding in chains, so the gregarine justified its inclusion under the genus *Phleobum* Haldar and Chakraborty, 1933. The present species resembled *Phleobum gigantinum* Haldar and Chakraborty, 1933 in its P: WD ratios, but differs in many other aspects like the trophozoite, features of the protomerite in the sporadin and in the measurements of different parts of the body. Freshly collected gametocyst were yellowish- orange, ellipsoidal and 24 0- 277.2 µm in length. Trophozoites measured 15.9 – 109.9 µm in length. Epimerite was small spherical and measured 14.7 – 32.6 µm in length. Protomerite broadly ovoid and 26.9 – 56.9 µm X 37.4 – 69.4 µm in measurements. Deutomerite was ellipsoidal with broadly rounded posterior and having slightly elevated margin, well developed pellicle and fine cytoplasmic granulation. Nucleus was orbicular situated anywhere in the deutomerite (freshly collected gametocysts are yellowish- white, orbicular body with a length of 520 – 600.0 unequal gametocytes, dehiscence through a single enormous size sporoduct, 2.7 mm long; trophozoites with a length of 230.0- 620.0 µm, epimerite absent, deutomerite cylindrical, pellicle well developed and epicyteal striations present; nucleus spherical, 50.0 µm in length and with several karyosomes; gamonts in pairs, satellite always larger than primate, flange was well developed, and oocyst was ellipsoidal in case of *Phleobum gigantinum* (Haldar and Chakraborty, 1933). A comparative account of related species of *Phleobum* spp. was given in the Table 2.

3.2. Neohirmocystis canchipurae sp. nov.

3.2.1. Descriptions

Trophozoite: Elongated measuring 159.6-192.6 (171.9±10.0) µm in dimension (Table 3 and 4 and Figures 3 and 4). Epimerire absent. The protomerite was globular in shape and measured 43.9-71.3 (52.6±7.7) µm × 60.2-91.2 (70.2±9.2) µm in dimensions. The deutomerite was elongated, varied from ovoidal to elliptical and broadest near the posterior third with rounded posterior extremity which measured 110.9-149.6 (119.3±9.2) µm × 90.6-126.3 (100.9±8.7) µm in dimensions. Protomerite was separated from deutomerite by a thick septum. Protomerites were elongated with rounded posterior end. Granules were distributed uniformly in the cytoplasm. Nucleus was spherical in shape and situated in varied locations in the developing parasite measuring about 11.9-42.3 (21.1±7.4) µm × 20.7-52.3 (30.2±7.5) µm in dimensions.

Sporadin: Solitary as well as biassociative. They were obese – shaped measuring about 189.9-226.3 (199.4±8.3) µm in length. Protomerite was conical in shape and its length was greater than its breadth which measured 52.9-76.6 (62.1±7.2) µm × 42.9-76.6 (52.8±8.6) µm in dimensions. Deutomerite was elongated - obese shaped measuring 120.9-153.6 (130.2±7.7) µm × 82.9-115.3 (99.7± 7.7) µm in dimensions. Epicyteal striations were clear. The nucleus was like those that were present in trophozoite which measured 9.9-41.6 (29.1±7.2) µm in dimensions.

Association: It was caudo-frontal in nature. The structures of primite and satellite were different. Primite had a protomerite which was fan-shaped with high amount of cytoplasm while the satellite had protomerite which was dome-shaped and its cytoplasm was hyaline and clear in nature.

Gametocyst: Greyish white in colouration. Cyst were collected from the hind gut of the hosts and were spherical in shape which measured 109-156.2 (145.1±10.0) µm × 106-146.5 (131.1±13.0) µm in dimensions. The cysts dehiscenced within 48 hours by simple rupture.

Spores: Spherical shaped and double walled measuring 8.75×7.2 µm in diameter. Eight sporozoites were formed within 72 hours. Sporozoites were small, ovoid - shaped and arranged in circular fashion within the spore.

Abundance: 21 out of 50 (42%)

Paratypes: On slides MU/0210/14_2–20 (P. periplanae sp. nov.) and deposited in the Protozoan Collection of Parasitology Section, Department of Zoology, Manipur University, Canchipur-795003, India.
Holotype: On slides MU / 019/14_1 (P. periplanae sp. nov.), deposited in the Protozoan Collection of Parasitology Section, Department of Zoology, Manipur University, Canchipur-795003, India.
Etymology: The species is named after the host insect.

Species information

Type specimen : *Neohirmocystis canchipurae* sp. nov.
Type host : *Periplaneta americana* (Order: Blattodea)
Type locality : Thongju, Canchipur with latitude of 24.7528558 and longitude 93.9383985
Site of infection : Mid gut
Prevalence: 27 out of 50 (54%)

Paratype: On the slides MU/0213/14_2 – 20 (*N. canchipurae* sp. nov.) and deposited in the Protozoan Collection of Parasitology Section, Centre of Advanced Studies in Life Sciences, Manipur University, Canchipur -795003, India.

Holotype: On the slides No. MU / 022/14-1 (*N. canchipurae* sp. nov.) and deposited in the Protozoan Collection Laboratory of Parasitology Section, Department of Zoology, Manipur University, Canchipur – 795003, Manipur, India.

Etymology: The species was named after its type locality.

Dimensions: Fixed and stained Trophozoites as well as sporadins were measured in micrometers as below:

The statistical analysis for the species is given in the Table 3.

Table 4: Comparative account of existing species of *Neohirmocyctis*

Characters	***N. grassei* Gosh et al., 1986**	***N. dercentini* Gosh et al., 1986**	***N. canchipurae* sp. nov.**
Epimerite	Lacking	Lacking	lacking
Sporadin	Solitary as well as biassociative, young sporadins are elongated with globular protomerite while mature sporadins with hat-like, tongue-like or globular protomerite	Solitary as well as biassociative; solitary sporadins are obese in shape with conical protomerite	Solitary as well as biassociative, obese in shape with conical protomerite
Association	Caudo-frontal syzygy; primate with fan-shaped protomerite while it is dome-shaped in satellite	Caudo-frontal syzygy, associated partners are equal in size	Caudo-frontal, primate and satellite are different, primate has fan-shaped protomerite while satellite is dome-shaped
Gametocyst	Blackish white in color and rounded in shape with prominent ectocyst	White in color and spherical in shape	Greyish white in color and spherical in shape
Spore	Double-walled, spherical	Double-walled spherical in shape	Double- walled spherical
Development	Intracellular	Extracellular	Intracellular
LP: TL	1:3.07 – 12.3 (5.9)	1:4.2 – 10.2 (6.1)	1:3.7
WP: WD	1:08 – 2.7(1.4)	1:1.0 – 2.9(1.7)	1: 1.5
HOST	*Tribolim castaneum* (Herbst)	*Dercentina* sp.	*Periplaneta americana*

Holotype : Slide No-MU / 022/14_ 1 (*N. canchipurae* sp. nov.)

Trophozoite:
LT = 161.25 LP =43.0
WP = 65.4 LD =118.25
WD = 97.65 LN =16.65
WN=24.65 LP: LT= 1: 3.6 and WP: WD = 1: 1.6

Sporadin:
LT =192.4 LP =56.75
WP =44.65 LD =129.7
WD = 86.0 LN =10.75
LP: LT = 1: 3.5 WP: WD = 1: 1.9.

Paratypes (20): MU/0213/14_2 – 20 (*N. canchipurae* sp. nov.)

Trophozoites:
TL = 160.4-193.5 (171.9±10.0) LP = 44.8-71.3 (52.6±7.7)
WP = 60.2-91.2 (70.2±9.2) LD = 111.8-150.5 (119.3±9.2)
WD = 91.5-126.3 (100.9±8.7) LN = 12.8-42.3 (21.1±7.4)
WN = 21.6 -52.3 (30.2±7.5) LP: LT = 1: 3.7; WP: WD = 1: 1.5.

Sporadin:
TL = 190.8-226.3 (199.4±8.3); LP = 53.8-77.5 (62.1±7.2)
WP = 43.8-77.5 (52.8±8.6); LD = 121.8-154.5 (130.2±7.7)
WD = 8389-115.3(99.7± 7.7); LN = 10.0-42.5 (29.1±7.2)
LP: LT = 1: 3.5 WP: WD = 1: 1.9

3.2.2. Diagnosis and relationships

The specimen was characterised by lack of epimerites, Sporadins solitary and biassociative, satellite with septum during association, Gametocyst dehisces by simple rupture releasing spherical spores and Development intracellular or extracellula. In the present species, length of the trophozoite (159.6-192.6) µm was quite different to that of *Neohirmocystis grassei* and *Neohirmocystis dercetini* (Gosh et al., 1986). The present specimen differed in lack of epimerites; shape of spores, gametocyst and nucleus. Some similarities with *Neohirmocystis grassei* and *Neohirmocystis dercetini* were found. But there were differences in ratios of LP: TL and WP: WD (LP:TL=1:5.9/1:4.1 and WP: WD = 1:6.1/ 1:1.7 in *Neohirmocystis grassei* and *Neohirmocystis dercetini* respectively). Differences in the length of sporadins, diameters of gametocyst and other morphometric dimensions were found.
Based on the wide differences in the morphometric values and the geographical location of the present specimen, the present species was proposed as a new to science and it was named *Neohirmocystis canchipurae* sp. nov. after the type locality of host insect. A comparative account of closely related species of *Neohirmocystis* shown in the Table 4.

Acknowledgement

The authors are thankful to the Head, Department of Zoology, Manipur University, Canchipur, India for providing necessary laboratory facilities.

Authors contributions

All the authors equally contributed in field taxonomic work, manuscript preparation, language editing and finalization of the manuscript.

Conflict of interests

Authors have no conflict of interests

References

Ghosh S, Ray SK and Haldar DP.1986. Neohirmocystidae n. fam., a new family of septate gregarines (Apicomplexa: Sporozoae) from insects. Acta Protozoologica 25(4): 453-464.

Haldar DP and Chakraborty N.1974. A new cephaline gregarine, *Phleobum gigantinum* n. gen., n. sp. (Protozoa: sporozoa) from a grasshopper. Proceeding of Third International Congress of Parasitology 1: 8.

Kundu TK and Haldar DP.1986. *Phleobum collarum* sp. nov. a new septate gregarine (Apicomplexa: Sporozoa) from grasshopper *Phleobum infumata* Brunner with a new definition of the *Phleobum* (Haldar and Chakraborty, 1974). Archaelogy Protistenkd 132: 119 – 124.

Larraín P and Salas C. 2008. House fly (*Musca domestica* L.) (Diptera: Muscidae) development in different types of manure [Desarrollo de la Mosca Doméstica (*Musca domestica* L.) (Díptera: Muscidae) en DistintosTipos de Estiércol]. Chinese Journal of Agricultural Research 68 (2): 192 – 197.

Modak KM, Basu S and Haldar DP. 2011. Description of two new species of *Phleobum* Haldar and Chakraborty, 1974 along with a major taxonomic revision of the genus. Acta Protozoologica 50: 141 – 153. http://www.eko.uj.edu.pl/a.p

Ray HN and Chakraborty MM. 1933. Studies on Sporozoa from Indian millipes. II. The life history of cephaline gregarine, *Monoductus lunatus* n. gen., n. sp. Ibid 81(2): 352-360.