

## Original Research Article

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

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**Abstract:** During 2012–2018, a survey was conducted for the protozoan parasites in valley districts of Manipur. Cockroaches were collected using Sweep-netting methods and dissected. Fresh and permanent slides of collected protozoa from the gut; the sample insect were prepared using Haedenhain's iron, haematoxylin, Giemsa and Eosin stains. Mean, standard deviation and percent of co-efficient of variations are calculated. Two new Eugregarine protozoans namely, *Phleobum periplanae* sp. nov. and *Neohirmocystis canchipurae* sp. nov. were revealed new to science. Only 9 species of *Phleobum* protozoa has been identified so far. The present study added a new species to this group of protozoa. *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. A new species of *Neohirmocystis* is described here. Only a few species of *Neohirmocystis* protozoa have been identified. *Neohirmocystis canchipurae* sp. nov. is characterised by lack of epimerites with atrophozoite length of (159.6-192.6) µm. Gametocyst dehisces by simple rupture releasing spherical spores.

**Key words:** Didymophyidae, Neohirmocystidae and *Periplanata americana*.

## Introduction

Medical entomology deals with insects affecting the health and well-being of man and vertebrate animals and it concerns with the vectors that affect human and animal health. The need to be aware of 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.

A number of faunal types including microorganisms were listed from Manipur e.g. fungal diversity (Premila, 2013) and antibacterial diversity (Nimaichand *et al.*, 2015); but work is

lagging on the study of diversity of protozoan parasites. So, a survey was conducted for this particular group of animals during 2012- 2018 to fill the lacunae, where various insect hosts were collected for their gut content observation. Numerous 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 to scientific knowledge 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* Burnn. The species was earlier 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 that 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 dehiscence 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). There are a total of nine species of *Phleobum* identified so far. *Phleobum* is an important septate gregarine protozoa observed in the gut of grass hoppers. Prominent work on the ultrastructural details on one species of *Phleobum* i.e. *Phleobum elliptica* Modak et al., 2011 revealed that, there is apical protuberance on protomerite and the structure may be helpful in delineation of new species (Sil et al., 2017).

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 satellites 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 genus *Periplaneta americana* (Linnaeus) and that of *Neohirmocystis* is named *Neohirmocystis canchipurae* after its type locality. The morphological details of the two new species are illustrated here in the present manuscript with morphological details, diagrams and photographs.

## 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 the insects were taken out, dissected in 0.5% saline solution 1X Phosphate Buffered Saline (1X PBS) soln. and observed for the parasite contained.

## 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 soln. The slides were covered with cleaned cover slips and examined under phase contrast microscope to study the movement of the protozoan parasites.

## Permanent slides preparation

### 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 Shadinn's fluid (20-30 mins), Carnoy fixative (20 mins) or aqueous Bouin fluid for 16-24 hour respectively. They were subsequently stained with Haematoxylin for 30 mins and wash if over stained with 1% Iron alum solution (Kudo, 1960). DPX is used as mounting agent and covered with cleaned glass covers. 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.

## Abbreviations used

K= Karyosome, 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.

## Calculations

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

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

## Results

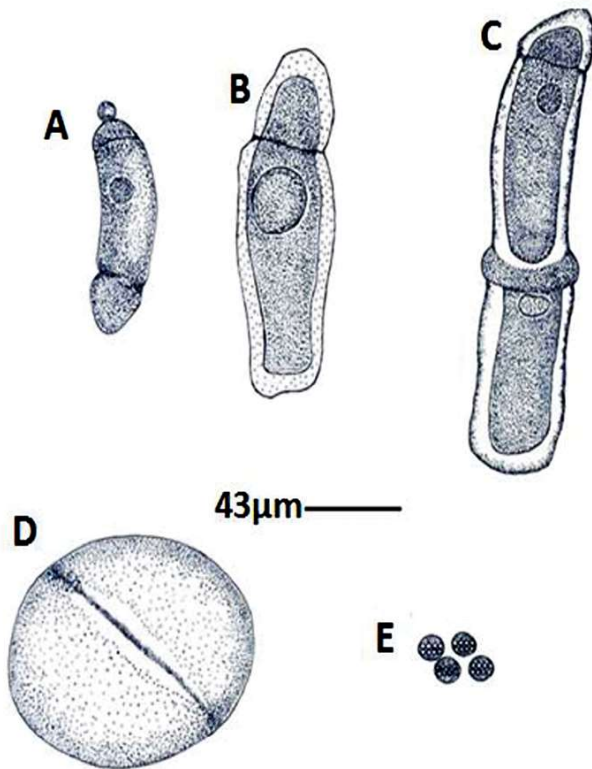
*Phleobum periplanae* sp.nov.

## Description

**Trophozoite:** Solitary, the total length of the trophozoite measured 15.9-109.9 (143.1±11.17) µm. The epimerite was very small, spherical and measured 14.7-32.6 (23.5±5.8) µm. The

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

Characters	R	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

**Fig. 1.** Camera lucida drawings of *Phleobum periplanae* sp. nov.;

A - Mature trophozoite, B - Young trophozoite, C - Sporadin in syzyzy, D - Gametocyst and E - Spore with eight sporozoite.

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 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. Epicyte striations were clearly discernable in some specimens.

**Fig. 2.** Photomicrographs of *Phleobum periplanae* sp. nov.;

A - Mature trophozoite, B - Young trophozoite, C - Group of syzyzy, D - Gametocyst and E - Spore.

**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 epicyte 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 gametocysts were yellowish-orange in coloration, ellipsoidal and measured 240-277.2

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

Characters	<i>P. gigantinum</i> Gosh <i>et al.</i> 1986	<i>P. collarum</i> Kundu, Haldar 1986	<i>P. subsphericum</i> Modak <i>et al.</i> 2011	<i>P. loepimeritu</i> Larrain, Salas 2008	<i>P. periplanae</i> sp. nov.
<b>Total length</b>	230.0 – 620	95.0 – 699.7	177.6- 666.6	258 – 670	15.9 – 109.9
<b>Epimerite</b>	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
<b>Deutomerite</b>	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
<b>Nucleus</b>	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
<b>Gamont</b>	In pairs; satellite always larger than primate	In pairs, satellite smaller or larger than primate	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
<b>Gametocyst</b>	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
<b>Oocyst</b>	Ellipsoidal; 6.0 x 4.0	Ellipsoidal; 7.7x4.4	Ellipsoidal, 6.1x5.1	Ellipsoidal; 9x5	Ellipsoidal; 12.2 x 9.5
<b>LP: TL</b>	1:5.3	1:3.0-11.0	1:3.6- 1:9.4	1:5-6.1	1: 3.5
<b>WP:WD</b>	1:1.2	1:0.9- 1.4	1:0.7- 1:1.1	1:1.1- 1.3(1.2)	1:1.0
<b>Host</b>	<i>Phlaeoba antennata</i>	<i>Phlaeoba infumata</i>	<i>Atractomorphacrenulata</i>	<i>Oxyla hyla hyla</i>	<i>Periplanata americana</i>
<b>Locality</b>	Kalyani, India	Kalyani, India	Kalyani, India	Kalyani, India	Canchipur, India

**Table 3.** Statistical analysis of *Neohirmocytis 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

(261.1±30.4)µm ×175.2-203.7(188.3±7.11)µm. Gametocyst dehisced through a pore after 72 hrs inside the moist chamber.

**Spore:** Spores were uniformly ellipsoidal and measured 12.2×9.5µm 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

**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.

#### 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:

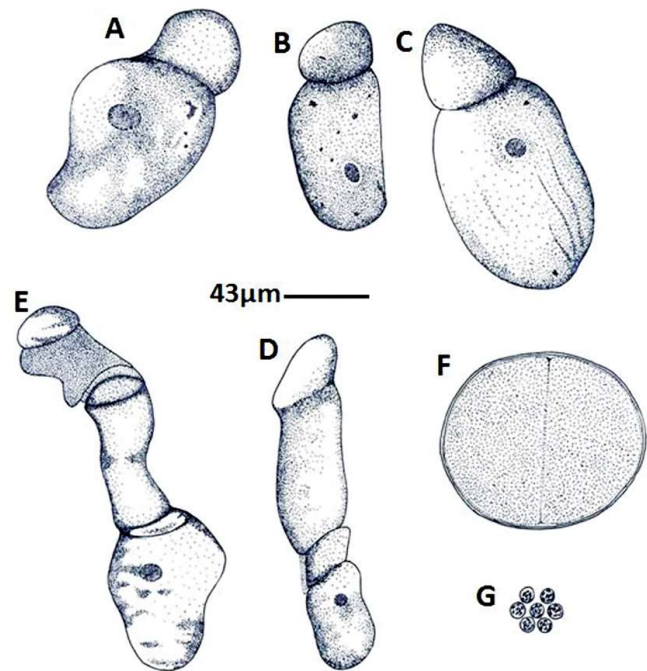
TL = 15.9-109.9 (143.1±11.17)      LE = 4.7-22.6 (13.5±5.8)  
LP = 13.9-43.9 (35.8±7.3)      WP = 20.4-59.4 (47.9± 7.4)  
LD = 67.4-103.2 (93.4±6.09)      WD = 22.7-62.5(51.6± 8.23)  
LN = 9.6-39.5 (29.1±6.75)      LP: LT= 1: 4.0 and WP: WD= 1:1.1

#### 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.

The statistical analysis for the species is given in the table no.1.

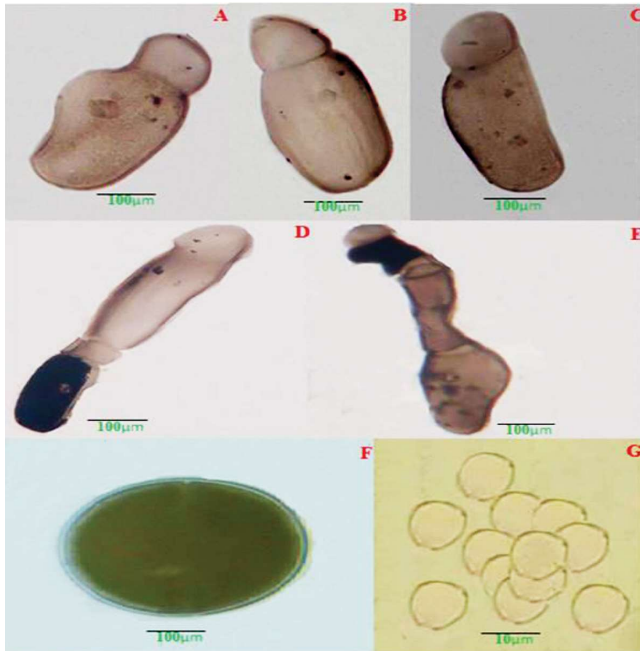
#### *Neohirmocystis canchipurae* sp.nov



**Fig. 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.

#### Descriptions:

**Trophozoite:** Elongated measuring 159.6-192.6 (171.9±10.0)µm in dimension. 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



**Fig. 4.** Photomicrographs of *Neohirmocystis canchipuræ* sp. nov.;

A - Mature trophozoite; B, C - Sporadin; D, E - Sporadin in syzygy; F - Gametocyst and G - Spore.

deutomerite was elongated, varied from ovoidal to elliptical and broadest near the posterior third with rounded posterior extremity which measured  $110.9-149.6$  ( $119.3 \pm 9.2$ )  $\mu\text{m} \times 90.6-126.3$  ( $100.9 \pm 8.7$ )  $\mu\text{m}$  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 lies in varied locations in the developing parasite measuring about  $11.9-42.3$  ( $21.1 \pm 7.4$ )  $\mu\text{m} \times 20.7-52.3$  ( $30.2 \pm 7.5$ )  $\mu\text{m}$  in dimensions.

**Sporadin:** Solitary as well as biassociative. They were obese - shaped measuring about  $189.9-226.3$  ( $199.4 \pm 8.3$ )  $\mu\text{m}$  in length. Protomerite was conical in shape and its length was greater than its breadth which measured  $52.9-76.6$  ( $62.1 \pm 7.2$ )  $\mu\text{m} \times 42.9-76.6$  ( $52.8 \pm 8.6$ )  $\mu\text{m}$  in dimensions. Deutomerite was elongated - obese shaped measuring  $120.9-153.6$  ( $130.2 \pm 7.7$ )  $\mu\text{m} \times 82.9-115.3$  ( $99.7 \pm 7.7$ )  $\mu\text{m}$  in dimensions. Epicyteal striations were clear. The nucleus was just like that were present in trophozoite which measured  $9.9-41.6$  ( $29.1 \pm 7.2$ )  $\mu\text{m}$  in dimensions.

**Association:** It was caudo-frontal in nature. The structures of primitive and satellite were different. Primitive 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  $109-156.2$  ( $145.1 \pm 10.0$ )  $\mu\text{m} \times 106-146.5$  ( $131.1 \pm 13.0$ )  $\mu\text{m}$  in dimensions. The cysts dehiscenced within 48 hours by simple rupture.

**Spores:** Spherical shaped and double walled measuring  $8.75 \times 7.2$   $\mu\text{m}$  in diameter. Eight sporozoites were formed within 72 hours. Sporozoites were small, ovoid-shaped and arranged in circular fashion within the spore.

#### Species information

**Type specimen :** *Neohirmocystis canchipuræ* 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. canchipuræ* 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. canchipuræ* 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

**Holotype :** Slide No-MU / 022/14\_1 (*N. canchipuræ* 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 = 83.89-115.3(99.7± 7.7); LN = 10.0-42.5 (29.1±7.2)

LP: LT = 1: 3.5 WP: WD = 1: 1.9

The statistical analysis for the species is given in the table no.3.

**Discussions:**

*Phleobum periplanae* sp. nov. 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). WP: 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- 277.2µm 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 is 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

**Table 4.** Comparative account of existing species of *Neohirmocystis*.

Characters	<i>N. grassei</i> Gosh et al. 1986	<i>N. dercentini</i> Gosh et al. 1986	<i>N. canchipurae</i> 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	1:0.8 – 2.7(1.4)	1:1.0 – 2.9(1.7)	1: 1.5
HOST	<i>Tribolium castaneum</i> (Herbst)	<i>Dercentina</i> sp.	<i>Periplaneta americana</i>

and Chakraborty, 1933). A comparative account of related species of *Phleobum* spp. was given in Table no. 2.

*Neohirmocystis canchipuræ* sp. nov. 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 extracellular.

In the present species, length of the trophozoite (159.6-192.6)  $\mu\text{m}$  which 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 was 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 new to science and it was named *Neohirmocystis canchipuræ* sp. nov after the type locality of host insect. A comparative account of closely related species of *Neohirmocystis* spp. is shown in table 4.

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