

ACTA PROTOZOLOGICA

Description of Two New Species of *Phleobum* Haldar and Chakraborty, 1974 along with a Major Taxonomic Revision of the Genus

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Summary. Two new species of *Phleobum* (Apicomplexa: Eugregarinida: Hirmocystidae) are described from adult grasshoppers in Kalyani, India. *Phleobum globuloepimeritum* n. sp. is described from *Oxya hyla hyla* (Insecta: Orthoptera: Acrididae) and *Phleobum elliptica* n. sp. from *Atractomorpha crenulata* (Insecta: Orthoptera: Pyrgomorphidae). In *P. globuloepimeritum*, the trophozoites are solitary, orange-coloured, have a maximum length of 352 µm with a hyaline globular epimerite and the protomerite in satellite is characteristically flanged. The gametocyst is orange, orbicular, averaging 300 × 250 µm in size, dehiscing through a pore after 50 h. The oocysts are uniformly smooth, ellipsoidal, measuring 9 × 5 µm. In *P. elliptica* trophozoites are also solitary, orange-coloured, having a maximum length of 157 µm with triangular epimerites. The protomerite flange is typically a bowel-shaped collar. The gametocyst is yellow-orange, ellipsoidal, averaging 240 × 165 µm and dehisces through a pore after 72 h, releasing smooth-walled, ellipsoidal, uniformly shaped oocysts measuring 5 × 3 µm. The two new species share traits, which define the genus such as: gamonts in association, satellites with characteristic flange and gametocysts dehiscence through a single pore. The epimerite of *P. globuloepimeritum* is hyaline and globular, which is unique among members of the genus. The gametocyst of *P. elliptica* is notably ellipsoidal. Basing on observation on all the described and present two species, generic characters of the genus *Phleobum* have been redefined. The genus is also transferred from the family Didymophyidae to the family Hirmocystidae as the gametocysts in all the species of *Phleobum* dehiscence through a pore.

Key words: Apicomplexa, Hirmocystidae, *Phleobum*, septate gregarines, grasshopper, *Oxya hyla hyla*, *Atractomorpha crenulata*.

Abbreviations: LD – length of deutomerite; LE – length of epimerite; LN – length of nucleus; LP – length of protomerite; TL – total length; WD – width of deutomerite; WE – width of epimerite; WN – width of nucleus; WP – width of protomerite. The ratios used are the ratio of the length of protomerite to total length (LP : TL) and the ratio of the width of protomerite to the width of deutomerite (WP : WD). PLUK – Protozoology Laboratory, University of Kalyani, West Bengal, India; HWML – Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska, USA.

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INTRODUCTION

The family designation for *Phleobum* has been historically unstable. In the year 1974, Haldar and Chakraborty established a new genus *Phleobum* for the cephaline gregarine, *Phleobum gigantinum* from the grasshopper, *Phlaeoba antennata* and placed it under the family Monoductidae Ray and Chakravarty, 1933. Later, Kundu and Haldar (1986) described the second species of *Phleobum*, *P. collarum* from *Phlaeoba infumata* and transferred the genus *Phleobum* to the family Didymophyidae as the gametocyst dehisces through a pore at one corner of the cyst wall. But this placement was incorrect as all members of the family Didymophyidae possess gametocytes that dehisce through a single large sporoduct. Currently, the taxonomic problems continue to exist at both the familial and generic status. In this study, we present a revision of the generic characters of *Phleobum*. The characteristic orange or yellowish-orange colour as well as the presence of “flange” in the protomerite of the satellite appear to be very unique features of all the gregarine species of *Phleobum*. In addition, we transfer the genus from the family Didymophyidae to the family Hirmocystidae in view of the dehiscence of the gametocyst through a pore instead of a duct. Prior to this communication, seven species of the genus were recorded, all from grasshopper in West Bengal, India. With the descriptions of two more new species in this paper the total number of the species now stands at nine. A thorough appraisal of the genus was necessary and, therefore, a comprehensive research was undertaken in this regards.

MATERIALS AND METHODS

The adult host insects, *Oxya hyla hyla* (Insecta: Orthoptera: Acrididae) and *Atractomorpha crenulata* (Insecta: Orthoptera: Pyrgomorphidae) were collected approximately once a week from different grassfields in Kalyani (N23°, E88.5°) from July, 1995 to October, 1997. Each time, the material was collected with help of an insect sweep net in the morning, between 6 to 8 a. m., and brought alive to the laboratory for investigation. A total of 315 adult *O. hyla hyla* and 417 adult *A. crenulata* were dissected and examined for parasites. 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 (Kudo 1966). Gametocysts were recovered from the hind gut and placed in moist chambers (> 80% relative humidity) for sporulation (Sprague 1941). The structure of the oocysts were studied by preparing oocyst suspensions: a drop of freshly prepared Lugol's iodine solution (Lugol's iodine was prepared by adding 1 g each of KI and iodine crystals in 100 ml of distilled water) was placed on the oocyst suspension and the slide was examined under the oil immersion lens of a phase contrast microscope.

Figures of stained specimens were drawn with the aid of a mirror type camera lucida. Measurements of both fresh and stained materials were taken using an ocular micrometer calibrated with a stage micrometer. All measurements, unless otherwise mentioned, are in micrometers. Thirty five specimens of each: trophozoites, mature gamonts and associations were randomly measured from the infected hosts. Similarly, forty gametocysts and fifty individual oocysts were measured. Measurement were taken from widest part of epimerite, protomerite, deutomerite, nucleus, gametocyst and oocyst and are presented in this paper as range values followed by means, standard errors and sample sizes in parentheses. Blue filters were used for measurements and daylight filters were used for observation of colour in living specimens. Nomenclature for shapes of planes and solids used in this manuscript conforms to Clopton (2004).

RESULTS

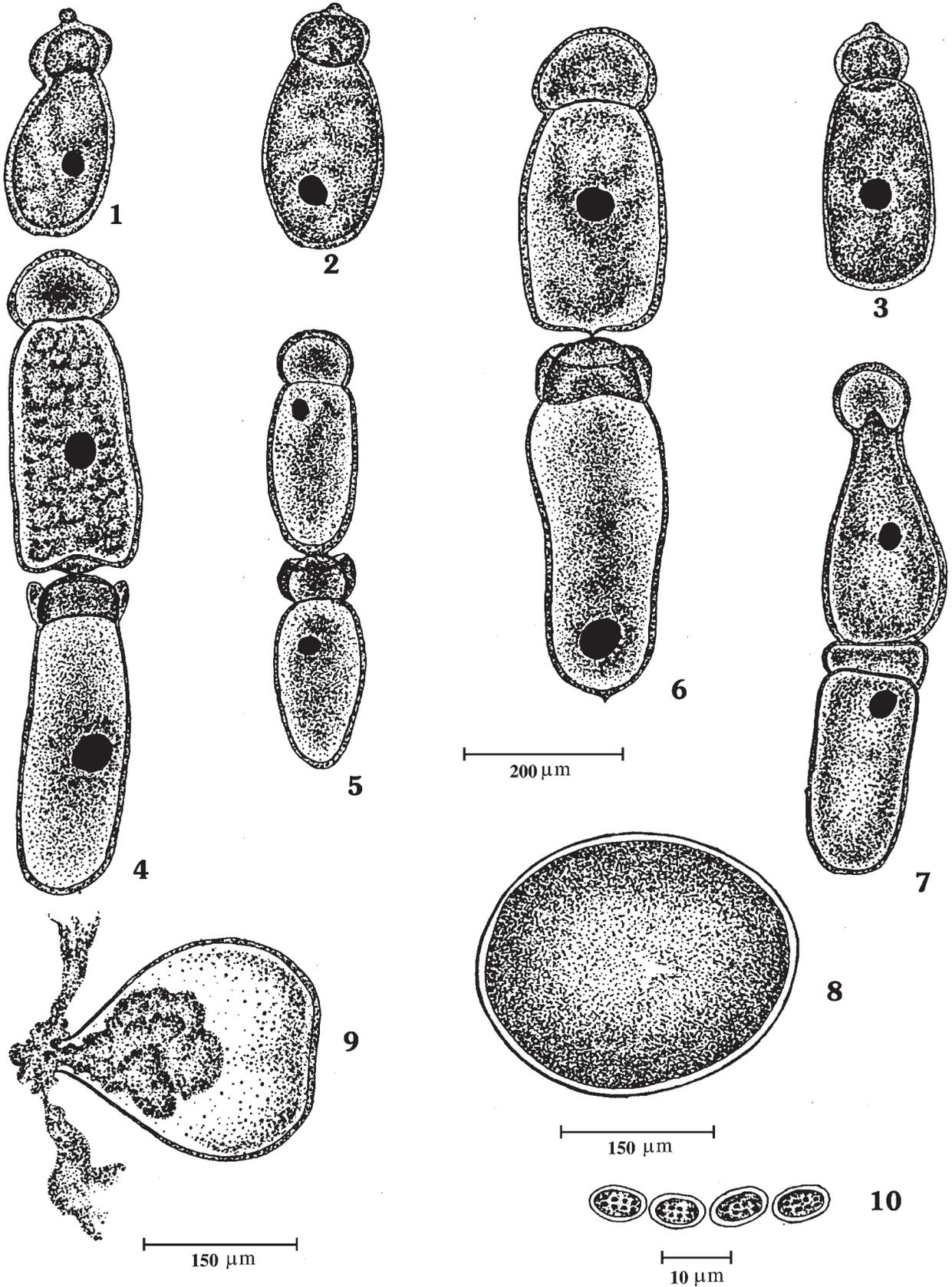
Phleobum globuloepimeritum n. sp.

Trophozoite (Figs 1, 11): These are solitary and orange-coloured. Epimerite is globular and hyaline. The protomerite is broadly or very broadly ovoid, covered by a thick pellicle (10–19, 14 ± 0.9 , 35). Cytoplasm of the protomerite is finely granulated in appearance. A sharp constriction separates the protomerite from the deutomerite. The deutomerite is somewhat ellipsoid with broadly rounded posterior extremity, covered by a well developed pellicle (7–11, 8.7 ± 0.4 , 35). Cytoplasm of the deutomerite appears densely granulated. The nucleus is orbicular to ellipsoidal and situated anywhere in the deutomerite.

Gamont (Figs 2–3, 12–13): These are solitary as well as in pairs and orange coloured. Solitary forms are oblong or cylindrical bodies. The protomerite is broadly or very broadly ovoid or dome-shaped. The pellicle is thick (14–23, 19 ± 1.1 , 35) and gives protomerite a very



Figs 1–10. Camera lucida drawings of different stages of life cycle of *Phleobum globuloepimeritum* n. sp. 1 – a mature trophozoite; 2–3 – young gamonts; 4–7 – associations of different shapes and sizes; 8 – a gametocyst; 9 – oocysts are liberated through a single pore; 10 – oocysts. Figs 1 to 7: Bar: 200 μ m.



broadly ovoid in outline. The cytoplasmic granules are concentrated in such a manner as to give a dome-like appearance within the protomerite. The deutomerite is cylindrical or oblong, densely granulated and covered by a thick pellicle ($6-11$, 9 ± 0.4 , 35). The nucleus is orbicular to ellipsoidal and situated anywhere in the deutomerite.

Association (Figs 4–7, 14–17): Associations are always caudo-frontal. The protomerite of the primite is broadly or very broadly ovoid. In the satellite anterior margin of the protomerite somewhat straight with two lateral protrusions that gives a characteristic flanged appearance, and in the middle there is an elevated portion that gives it a dome-shaped appearance. In a few instances two satellites are attached at the posterior extremity of the deutomerite of the primite (Fig. 17).

Gametocyst (Figs 8–9, 18–19) and oocyst (Figs 10, 20): Freshly collected gametocysts are orange-coloured, orbicular measuring $275-320 \times 240-265$ ($300 \times 250 \pm 5.5 \times \pm 4.8$, 40). The gametocyst dehisces through a pore after 50 h of development inside a moist chamber. Just before dehiscence, contracted central mass touches the periphery and a pore develops at that portion of the gametocyst wall. Ellipsoidal oocysts are linearly arranged while dehiscing but any point of contact between the two oocysts could not be observed. Oocysts are uniformly ellipsoidal and measure 9×5 ($9 \times 5 \pm 0 \times \pm 0$, 50). Sporozoites are arranged in linear fashion along the longitudinal axis of the oocysts.

Measurements: The summary of measurements (in micrometers) of fresh and preserved (fixed and stained) trophozoites and gamonts are given below:

	Fresh	Preserved
Trophozoite:		
TL	258–352 (317 ± 9.8 , 35)	252–343 (311 ± 11.5 , 35)
LE	13–18 (16 ± 0.3 , 35)	13–17 (15 ± 0.8 , 35)
WE	14–20 (17 ± 0.6 , 35)	14–19 (17 ± 0.8 , 35)
LP	35–78 (56 ± 2.6 , 35)	33–75 (54 ± 1.8 , 35)
WP	44–122 (81 ± 2.3 , 35)	42–120 (79 ± 2.9 , 35)
LD	209–256 (233 ± 7.9 , 35)	206–251 (228 ± 7.6 , 35)
WD	87–174 (132 ± 4.9 , 35)	84–171 (131 ± 4.5 , 35)
LN	22–34 (28 ± 1.8 , 35)	21–31 (26 ± 1.7 , 35)
WN	17–26 (22 ± 0.7 , 35)	17–25 (21 ± 0.5 , 35)
LP:TL	1:4.5–7.4 (5.4 ± 0.3 , 35)	1:4.6–7.6 (5.3 ± 0.4 , 35)
WP:WD	1:1.4–2.0 (1.8 ± 0.03 , 35)	1:1.4–2.0 (1.7 ± 0.03 , 35)
Gamont:		
TL	336–670 (472 ± 25.2 , 35)	332–661 (466 ± 28.1 , 35)
LP	57–134 (96 ± 5.1 , 35)	54–131 (93 ± 5.3 , 35)
WP	108–207 (148 ± 10.3 , 35)	104–203 (145 ± 7.1 , 35)
LD	288–536 (373 ± 23.1 , 35)	285–530 (368 ± 23.7 , 35)
WD	136–224 (174 ± 7.5 , 35)	134–219 (171 ± 6.1 , 35)
LN	28–80 (52 ± 2.9 , 35)	27–78 (50 ± 3.9 , 35)
WN	22–48 (37 ± 1.6 , 35)	21–46 (36 ± 2.1 , 35)
LP:TL	1:5.0–5.9 (5.4 ± 0.5 , 35)	1:5.0–6.1 (5.7 ± 0.4 , 35)
WP:WD	1:1.1–1.3 (1.2 ± 0.03 , 35)	1:1.1–1.3 (1.2 ± 0.02 , 35)
Primite in association:		
TL	342–665 (475 ± 25.1 , 35)	338–657 (472 ± 27.2 , 35)
LP	59–134 (96 ± 5.6 , 35)	58–130 (95 ± 6.4 , 35)
WP	108–207 (146 ± 9.2 , 35)	105–204 (144 ± 8.3 , 35)
LD	292–531 (375 ± 18.6 , 35)	288–527 (372 ± 17.2 , 35)
WD	140–226 (172 ± 9.4 , 35)	138–223 (170 ± 8.3 , 35)

Satellite in association:

TL = 345–670 (482 ± 29.5, 35)

LP = 61–131 (97 ± 6.1, 35)

WP = 110–205 (147 ± 9.3, 35)

LD = 295–539 (378 ± 25.2, 35)

WD = 140–220 (171 ± 9.6, 35)

342–655 (478 ± 26.1, 35)

60–127 (95 ± 6.2, 35)

108–201 (146 ± 8.1, 35)

291–528 (376 ± 23.1, 35)

136–217 (168 ± 8.7, 35)

Taxonomic summary:

Trophozoite: Globular, hyaline epimerite; cylindrical to oblong deutomerite, well developed pellicle, 317 in average total length; orange-coloured.

Gamont: In pairs, rarely two satellite attach with single primite 472 in average total length; orange-coloured.

Gametocyst: Orange, orbicular body, 300 × 250 in average dimensions.

Oocyst: Ellipsoidal, 9 × 5.

Type material: Catalog No. G₃A1–G₃A7. 7 slides containing syntypes have been deposited at the PLUK; 1 slide containing hapantotypes has been deposited at the HWML, Lincoln, Nebraska, USA; Catalog No. HWML 16637.

Type locality: India, West Bengal, Nadia district, Kalyani (N23°, E88.5°).

Host: *Oxya hyla hyla* (Insecta: Orthoptera: Acrididae) infesting grass field.

Location in host: Midgut.

Symbiotype: Two whole specimens deposited at the Zoological Survey of India, Government of India, Kolkata, West Bengal, India.

Prevalence of infection: 38.4% hosts (128 out of 315) were infected.

Etymology: The species has been named to emphasize the globular shape of the epimerite in the gregarine.

Affinities: The gregarine under report belongs to the genus *Phleobum* because it has the following specialized features: simple globular epimerite, gamonts in association, satellites with characteristic flange, gametocyst dehiscence through a single pore and ellipsoidal oocysts. The gregarine resembles *P. gigantinum* (Haldar and Chakraborty, 1974) in total length as well as in LP:TL and WP:WD ratios but differs from it in all other aspects. It has a close affinity with *P. collarum* Kundu and Haldar, 1986 in general shape of the trophozoites and gamonts, and structure of gametocysts and oocysts. But in *P. collarum*, the total length varies from 95.0 to 699.7, gametocysts and oocysts measure 436.8 × 436.8 and 7.7 × 4.4, respectively, while in the present case the total length varies from 258 to 670, and

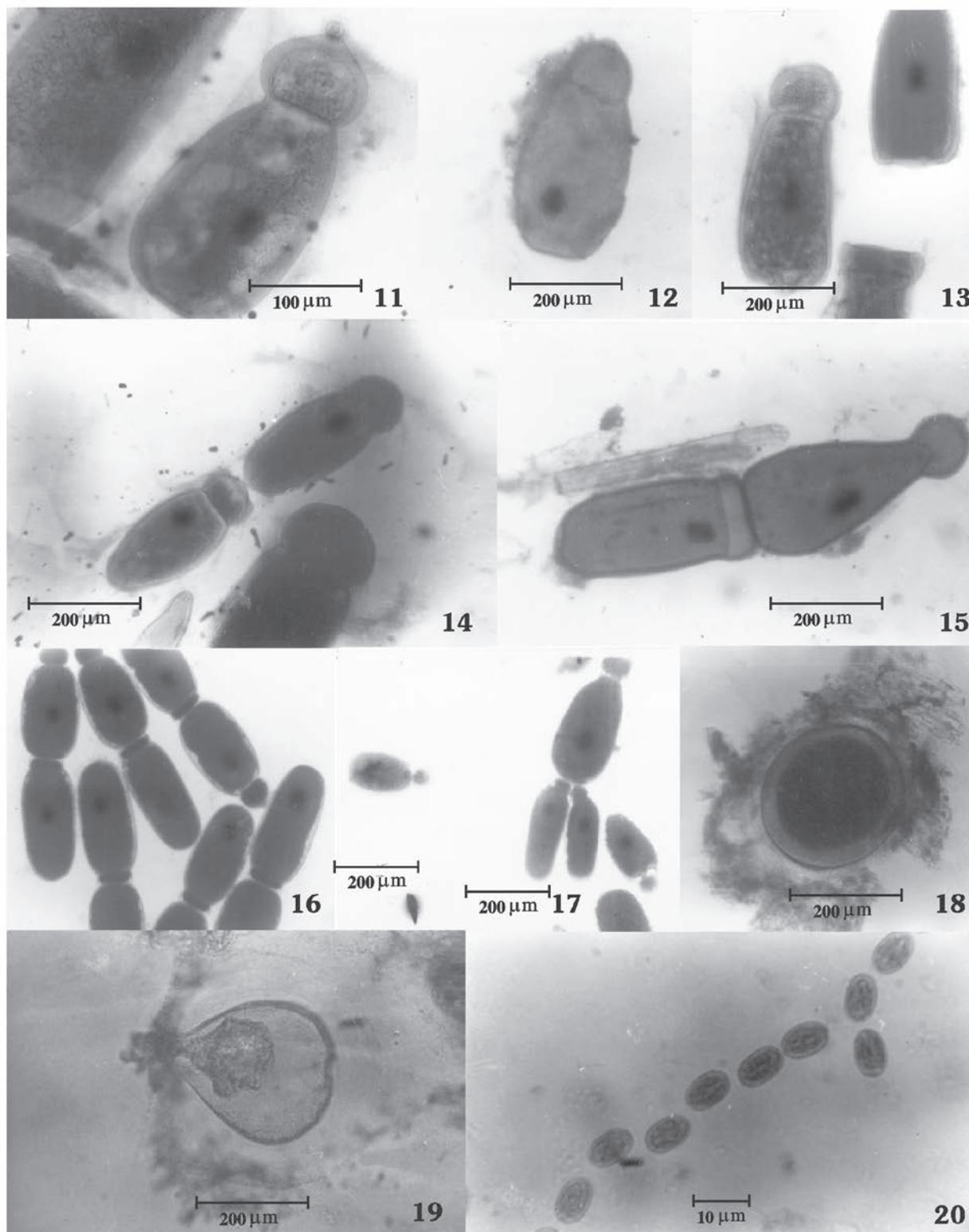
the gametocysts and oocysts measure 300 × 250 and 9 × 5, respectively. In addition to these, multiple lateral association is also encountered in the present species. Among other species, *P. subsphericum* shows resemblance with the present form in total length and in the structure of epimerite only, otherwise it is completely different from the present species. The flange, which is highly developed in the present form are also incipient in *P. subsphericum*. Another species *P. oxyum*, obtained from the host of the same genus (not belonging to the same species) but from different localities, shows no resemblance with the present form except in WP:WD ratios.

It, therefore, appears that the gregarine from *Oxya hyla hyla* is quite different from all the described species of *Phleobum*. The gregarine is designated as *Phleobum globuloepimeritum* n. sp. in this communication. The comparative characters of the related and the new species of *Phleobum* have been compiled in Table 1 to establish its distinctiveness.

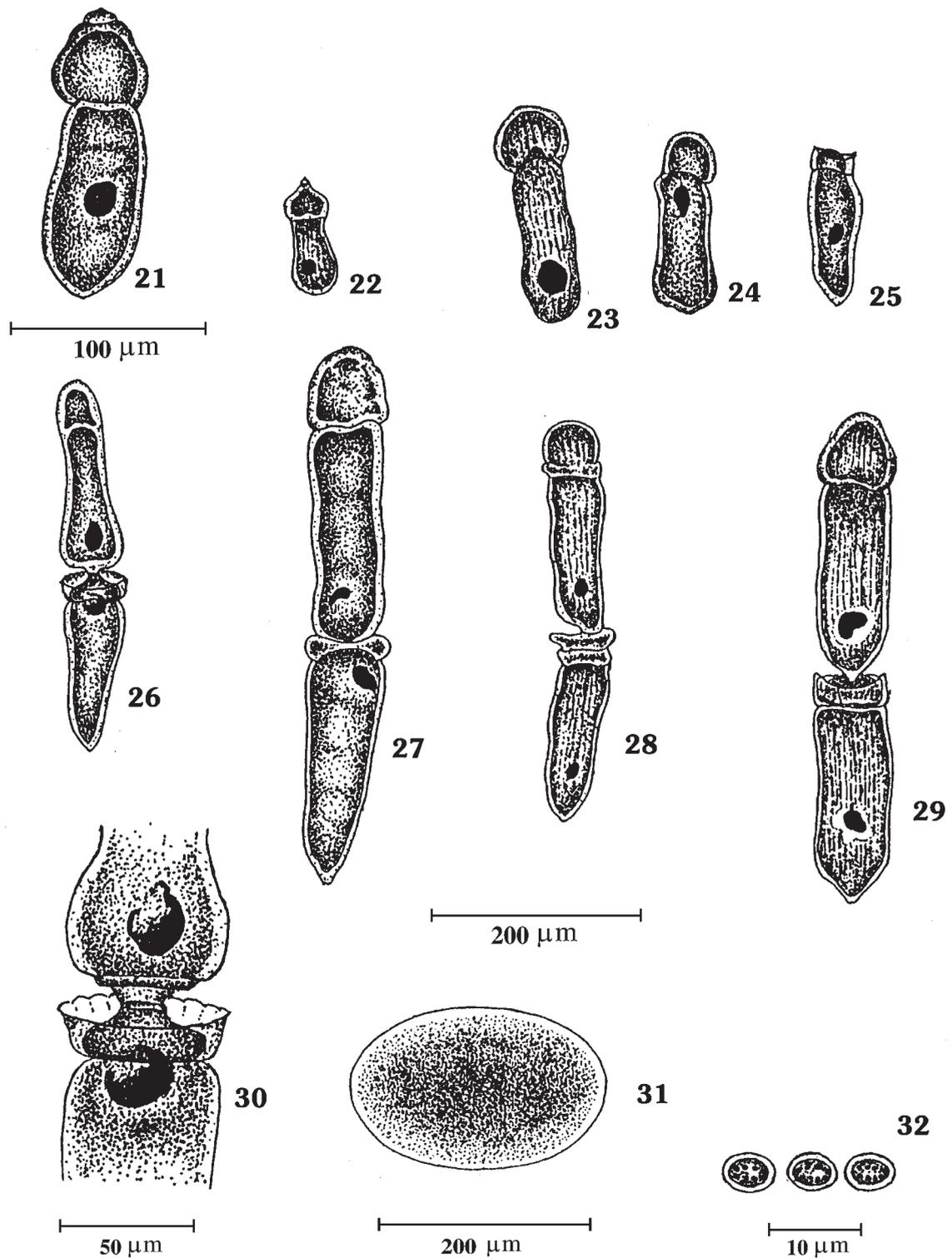
***Phleobum elliptica* n. sp.**

Trophozoite (Figs 21–22, 33): The epimerite is very small, somewhat triangular. The protomerite is more or less deltoid, broadly or very broadly ovoid or dome like. Its thick pellicle (4–7, 5 ± 0.2, 35) gives it a oblong or deltoid shape. The deutomerite is oblong, ends in a rounded or pointed proximity. Cytoplasmic granulation is very fine in nature. The nucleus is orbicular, situated anywhere in the deutomerite with a distinct nuclear membrane. Freshly collected specimens appear orange. Epicyteal striations are clearly discernable in some specimens.

Gamont (Figs 23–25, 34–36): Solitary as well as biassociative forms are observed and orange coloured. Gamonts are of two distinct types, one with broadly ovoid or dome-shaped protomerite and the other with shallowly oblong protomerite having slightly elevated margin, giving a slight depression at the middle. The deutomerite is oblong with pointed or rounded extremity. Both the protomerite and the deutomerite have thick pellicle (4–8, 6 ± 0.3, 35) and are filled up with fine as



Figs 11–20. Photomicrographs of *Phleobum globuloepimeritum* n. sp. **11** – mature trophozoite; **12–13** – gamonts; **14–15** – associations; **16** – cluster of associations (live); **17** – multiple lateral association (live); **18** – freshly collected gametocyst; **19** – oocysts are liberated through a single pore; **20** – oocysts.



Figs 21–32. Camera lucida drawings of different stages of life cycle of *Phleobum elliptica* n. sp. 21–22 – mature trophozoites; 23–25 – various types of gamont; 26–30 – associations of different shapes and sizes; 31 – a gametocyst; 32 – oocysts. Figs 22 to 29: Bar: 200 μm.

well as coarse granules. The nucleus is situated anywhere in the deutomerite. Closely set epicyteal striations are distinctly observed.

Association (Figs 26–30, 37–40): The organisms associate in pairs and association is always caudo-frontal. In most cases, the primites are larger than the satellites. Protomerite of primitive is broadly ovoidal in most cases, whereas in satellite it is almost shallowly oblong. Protomerite of the satellite bears characteristic flange on their anterior extremities. With further maturity, a clear cup-like depression replaces the flange. When highly enlarged, the free edge of the cup appears wavy with clear ridges on the inner side. This type of

structural modification helps in firm attachment during association.

Gametocyst (Figs 31, 41) and oocyst (Figs 32, 42): Freshly collected gametocysts are yellowish-orange, ellipsoidal, and measure $230\text{--}255 \times 155\text{--}175$ ($240 \times 165 \pm 3.2 \times \pm 2.8$, 40). Gametocyst dehisces through a pore after 72 h inside the moist chamber. Oocysts are uniformly ellipsoidal and measure 5×3 ($5 \times 3 \pm 0 \times \pm 0$, 50); sporozoites are arranged in linear fashion along the longitudinal axis of the oocysts.

Measurements: The summary of measurements (in micrometers) of fresh and preserved (fixed and stained) trophozoites and gamonts are given below:

	Fresh	Preserved
Trophozoite:		
TL =	103–157 (129 ± 3.9 , 35)	102–152 (127 ± 4.5 , 35)
LE =	8–11 (9 ± 0.5 , 35)	8–10 (9 ± 0.3 , 35)
WE =	14–18 (16 ± 0.6 , 35)	14–18 (16 ± 0.7 , 35)
LP =	20–40 (31 ± 0.4 , 35)	20–39 (31 ± 0.3 , 35)
WP =	22–44 (32 ± 0.8 , 35)	21–42 (31 ± 0.5 , 35)
LD =	79–106 (92 ± 2.9 , 35)	77–103 (90 ± 2.7 , 35)
WD =	29–50 (41 ± 1.7 , 35)	28–48 (40 ± 1.6 , 35)
LN =	18–22 (20 ± 0.8 , 35)	13–21 (19 ± 0.5 , 35)
WN =	12–15 (13 ± 0.4 , 35)	11–14 (12 ± 0.3 , 35)
LP:TL =	1:3.9–5.6 (4.1 ± 0.2 , 35)	1:3.8–5.5 (4.1 ± 0.2 , 35)
WP:WD =	1:1.1–1.3 (1.2 ± 0.03 , 35)	1:1–1.2 (1.1 ± 0.03 , 35)
Sporadin:		
TL =	146–311 (243 ± 11.1 , 35)	144–304 (241 ± 11.6 , 35)
LP =	37–89 (58 ± 4.1 , 35)	36–86 (56 ± 3.1 , 35)
WP =	36–112 (72 ± 4.5 , 35)	35–110 (71 ± 4.2 , 35)
LD =	88–222 (172 ± 10.2 , 35)	87–218 (171 ± 11.6 , 35)
WD =	36–122 (84 ± 5.2 , 35)	36–121 (83 ± 4.9 , 35)
LN =	18–47 (34 ± 2.1 , 35)	18–46 (34 ± 2.1 , 35)
WN =	13–44 (26 ± 1.9 , 35)	13–42 (25 ± 1.4 , 35)
LP:TL =	1:3.5–3.9 (3.6 ± 0.1 , 35)	1:3.4–4.1 (3.7 ± 0.2 , 35)
WP:WD =	1: 1–1.1 (1.0 ± 0.02 , 35)	1:1–1.1 (1.0 ± 0.03 , 35)
Primitive in association:		
TL =	158–311 (247 ± 13.2 , 35)	156–306 (244 ± 13.7 , 35)
LP =	39–89 (62 ± 3.4 , 35)	38–87 (61 ± 3.6 , 35)
WP =	38–112 (75 ± 4.5 , 35)	37–110 (74 ± 4.4 , 35)
LD =	96–222 (174 ± 12.2 , 35)	95–219 (172 ± 10.7 , 35)
WD =	39–120 (83 ± 4.6 , 35)	38–117 (82 ± 4.7 , 35)

Satellite in association:

TL = 151–290 (211 ± 14.1, 35)

LP = 37–81 (59 ± 3.6, 35)

WP = 36–111 (68 ± 3.7, 35)

LD = 92–209 (162 ± 10.3, 35)

WD = 36–122 (80 ± 4.8, 35)

150–286 (210 ± 12.7, 35)

37–80 (58 ± 3.2, 35)

35–109 (65 ± 4.1, 35)

90–206 (161 ± 9.4, 35)

35–120 (78 ± 4.6, 35)

Taxonomic summary:

Trophozoite: Triangular epimerite; deutomerite oblong; pellicle well developed, epicyteal striations present. 129 in average total length; orange coloured.

Gamont: In pairs, 243 in average total length; orange coloured.

Gametocyst: Yellowish-orange, ellipsoidal body, 240 × 165 in average dimensions.

Oocyst: Ellipsoidal, 5 × 3.

Type material: Catalog No. G₁A1–G₁A4. 4 slides containing syntypes have been deposited at the PLUK; 1 slide containing hapantotypes has been deposited at the HWML, Lincoln, Nebraska, USA; Catalog No. HWML 16638.

Type locality: India, West Bengal, Nadia district, Kalyani (23°N, 88.5°E).

Host: *Atractomorpha crenulata* (Insecta: Orthoptera: Pyrgomorphidae) infesting grass fields.

Location in host: Gastric caeca and Midgut.

Symbiotype: Two whole specimens deposited at the Zoological Survey of India, Government of India, Kolkata, West Bengal, India.

Prevalence of infection: 27.1% hosts (113 out of 417) were infected.

Etymology: The specific trivial name has been given to emphasize the ellipsoidal shape of the gametocyst of the gregarine.

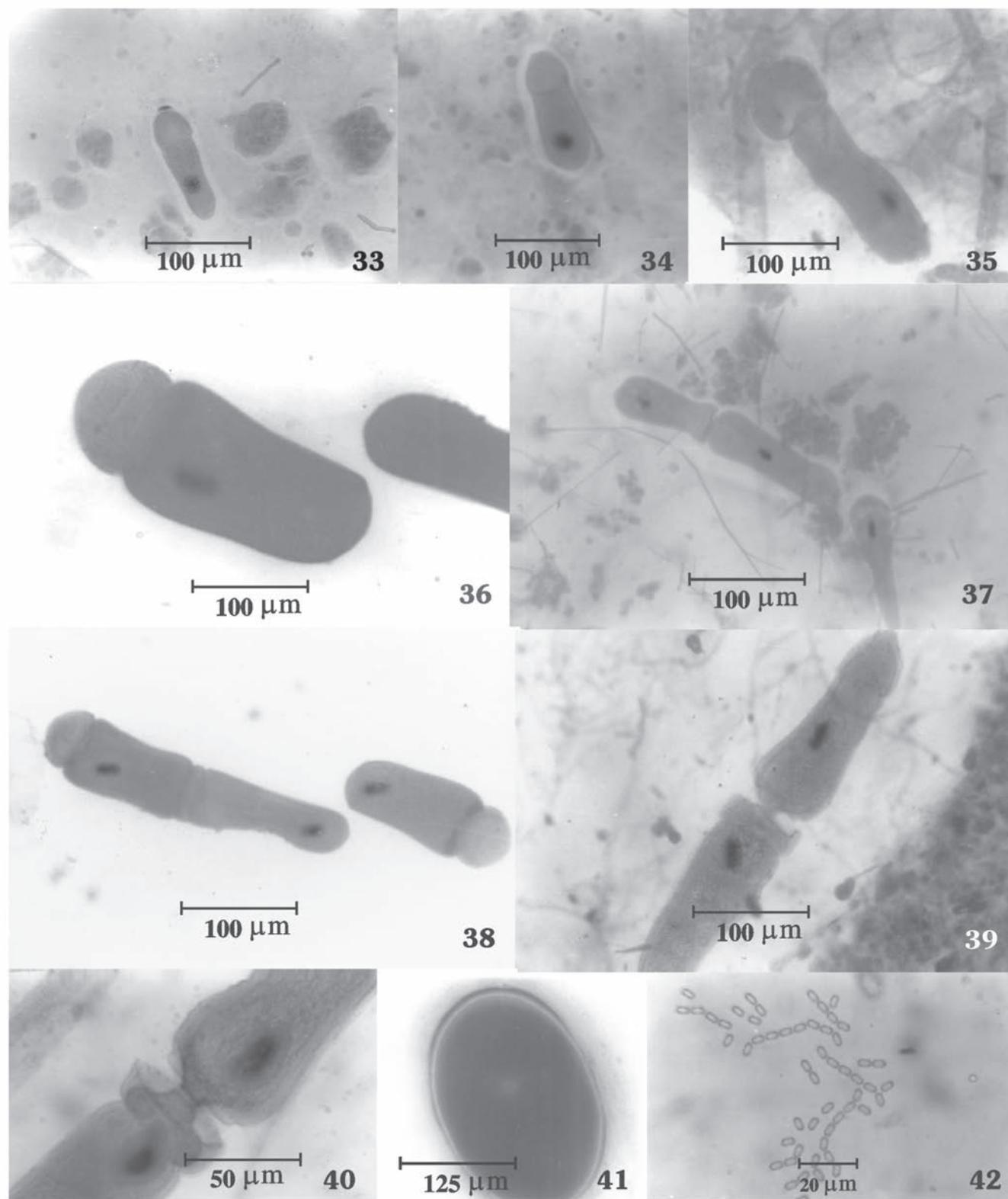
Affinities: The present form only shows resemblance with *P. longistriatum* in total length, and shape of the epimerite. In addition to other differences, structure of flange and shape of gametocyst are strikingly different. The other described species are entirely different from the present form (Table 1). The present form not only differs from the other species in metric data, but also in structural details. A very interesting bowl-shaped flange and ellipsoidal gametocyst have been observed, not reported in any of the previously described species. The unique morphology of the presently described gregarine justifies new species status.

DISCUSSION**A. Orange or yellowish-orange colour of the cytoplasm and its importance as a generic character of *Phleobum***

All previous workers dealing with the descriptions of species under *Phleobum* noted a characteristic orange or yellowish-orange colour of their cytoplasm. The same colouration has also been observed in the gregarines described in this communication. Initially, it was thought that the colour might have a direct relation with the diet consumed by the hosts of these gregarines. However, in several instances infections with species belonging to other genera of septate gregarines are commonly encountered in many grasshoppers, all of which consume a common diet in the grass fields, but in which the parasite cytoplasm was not orange but milky-white (Modak *et. al.* 2008). As such, the chance of the host diet as a reason for the origin of colour can perhaps be excluded. The colour might be carotenoid or some other pigments. The answer should be sought at the molecular level and a particular gene or a multiple gene complex might be playing a role in imparting the characteristic orange or yellowish-orange colouration in the cytoplasm of these gregarines. We are, therefore, tempted to include this specific colour as a generic character of the species belonging to *Phleobum*. It should be noted that similar pattern of carotenoid like colouration is also observed in several other genera like *Hyalospora*, *Ameobogregarina*, and *Tettigonospora* as well as in some species of *Gregarina* reported from gryllids.

B. The flange on the protomerite of satellite – its bearing on the generic identification

In all species of *Phleobum*, the protomerites in the satellite during association exhibit a characteristic rim like structure going around the protomerite that might be considered as ‘flange’ [According to the Encarta World English Dictionary, 1999 a flange is a projecting collar, rim, or rib on an object for fixing it to another object holding it in place or strengthening it].



Figs 33–42. Photomicrographs of *Phleobum elliptica* n. sp. **33** – young trophozoite; **34–36** – gamonts of various shapes and sizes; **37–40** – associations; **41** – freshly collected gametocysts; **42** – oocyst.

Table 1. Showing the comparative characters of two new species of *Phleobum* with closely related described species of the genus *Phleobum* Haldar and Chakraborty, 1974.

Characters	<i>Phleobum giganteum</i> Haldar, Chakraborty, 1974	<i>Phleobum (= Phlae- obum) collarum</i> Kundu, Haldar, 1986	<i>Phleobum (= Phlae- obum) subsphericum</i> Patra, Haldar, 2004	<i>Phleobum (= Phlae- obum) oxym</i> Chatterjee, Haldar, 2003	<i>Phleobum (= Phlae- obum) longistriatum</i> Patra, Haldar, 2004	<i>Phleobum globu- loepimeritum</i> n. sp.	<i>Phleobum elliptica</i> n. sp.
Total length	230.0–620.0	95.0–699.7	177.6–666.6	161.2–421.2	105.2–184.2	258–670	103–311
Epimerite	Absent	Spherical, hyaline body, 12.5–33.3	Subspherical, knob-like, hyaline body, 11.1–22.2	Triangular, 10.4 × 3.8	Conical; striped cytoplasm, 10.5–15.7	Globular, hyaline body, 16 × 17	Triangular, 9 × 16
Deutomerite	Cylindrical, pellicle well-developed; epi- cyteal striations present	Elongated, ellipsoidal to cylindrical; pellicle well- developed; epi-cyteal striations wanting	Elongated, cylindrical, thick pellicle	Cylindrical, pellicle is very thick	Elongated cylindrical, pellicle covering is very thick	Cylindrical to oblong in shape; well-developed pellicle; epi-cyteal stria- tions wanting	Oblong, pellicle well- developed; epi-cyteal striations present
Nucleus	Spherical; 50.0; several karyosomes	Oval; 25.0–99.9	Spherical or ellipsoidal; 33.3–77.7	Spherical or ellipsoidal 23.7 × 25.7	Oval or ellipsoidal, 10.5–36.8	Orbicular or ellipsoidal; 52 × 37	Orbicular; 34 × 26
Gamont	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	In pairs	In pairs; primate and satellite more or less in same size; in a few cases primites with an epimer- ite have been observed	In pairs; rarely two satel- lites attach with single primate	In pairs
Flange	Well-developed	Well-developed	Incipiently developed	Moderately developed	Incipiently developed	Well-developed	Bowl shaped
Gametocyst	Yellowish-white orbicu- lar body; 520.0–600.0; unequal gametocytes; dehiscence through a single enormous size sporoduct, 2.7 mm long	Orange coloured orbicu- lar body, with a transpar- ent gelatinous ectocyst; 436.8; gametocytes of equal or unequal size; dehiscence through a pore at one corner of the gametocyst wall liberating oocysts within a transparent mucoïd covering	Oval; bright lemon yel- lowish colour; 447.8 × 263.1–457.8 × 273.6 equal gametocytes dehis- cence by normal rupture through a pore on the cyst wall; liberating oocysts in singly	Orange coloured orbicu- lar body, 371.3 in average diameter	Orange coloured; orbicu- lar to ellipsoidal, 244.4 × 222.2–388.8 × 333.3, gametocytes of equal in size; dehiscence through a pore; oocysts within a transparent mucoïd covering	Orange coloured, orbicular; 300 × 250; gametocytes of equal or unequal size; dehiscence through a pore at one corner of the gametocyst wall liberat- ing oocysts in a linear fashion	Yellowish-orange, ellip- soidal body 240 × 165
Oocyst	Ellipsoidal; 6.0 × 4.0	Ellipsoidal; 7.7 × 4.4	Ellipsoidal; 8.1 × 7.1	Ellipsoidal; 5.6 × 3.5	Ellipsoidal; 6.1 × 5.1	Ellipsoidal; 9 × 5	Ellipsoidal; 5 × 3
*LP:TL	1:5.3	1:3.0–11.0	1:2.9–1:12:10	1:3.0–8.3 (4.7)	1:3.6–1: 9.4	1:5.0–6.1 (5.7)	1:3.4–4.1 (3.7)
*WP:WD	1:1.2	1:0.9–1.4	1:0.9–1:2.6	1:1.0–1.8 (1.3)	1:0.7–1: 1.1	1:1.1–1.3 (1.2)	1:1.0–1.1 (1.06)
Host	<i>Phleoba antennata</i>	<i>Phleoba infumata</i>	<i>Atractomorpha crenulata</i>	<i>Oxya</i> sp.	<i>Spathosternum</i> pr. <i>prasiniferum</i>	<i>Oxya hyla hyla</i>	<i>Atractomorpha crenulata</i>
Locality	Kalyani, India	Kalyani, India	Kalyani, India	Chandannagar, India	Kalyani, India	Kalyani, India	Kalyani, India

*Ratios of preserved gamonts.

In *P. gigantinum*, the type species, the flange is clearly observed (Haldar and Chakraborty 1974) and so in *P. collarum* (Kundu and Haldar 1986). Chakraborty, in his Ph. D. Thesis (University of Kalyani, 1975), described *P. gigantinum* in detail with illustrations. The flanges are smaller in size in *P. subsphericum*, *P. janovii*, *P. longistriatum* and *P. cloptoni* but medium in size in *P. oxyum* in comparison to the latter. These are very distinct in *P. globuloepimeritum* n. sp. and attain the maximum size in *P. elliptica* in the form of a bowl-shaped structure. As such, it is tempting to suggest that the presence of the flange on the protomerite in the satellite might be considered as an additional generic feature of *Phleobum*. However, like colouration, ‘flange’ like structures are also described from several species of other genera like *Protomaghalaensia*, *Torogregarina*, *Leidyna* and *Gregarina* and named variously as an “interlock,” “dovetail,” “lock-and-key,” or “cup-socket.”

C. The status of *Phleobum* as a valid genus

In 1974, Haldar and Chakraborty established a new genus of septate gregarines (Apicomplexa: Conoidasida), *Phleobum* from the grasshopper, *Phlaeoba antennata* in West Bengal. The genus was characterized as possessing: 1) trophozoite without an epimerite; 2) paired gamonts; 3) spherical nucleus with many chromatin granules; 4) gametocyst with ectocyst and endocyst dehiscing by a single elongated sporoduct; and, 5) oval oocysts not extruded in chains. *Phleobum gigantinum* was designated as the type species of the new genus. Later, in 1986, Kundu and Haldar described a second species, *P. collarum*, from the midgut and gastric caeca of another grasshopper, *P. infumata*. However, they observed some major variations in the generic character for which a new definition of the genus was proposed as: 1) development intracellular; 2) trophozoite with a simple, globular epimerite; 3) gamonts in association of two; 4) gametocyst with an ectocyst, dehiscing through a single pore at one corner of the cyst wall; and, 5) oocysts ellipsoidal, covered by a single hyaline covering at the time of their liberation. They amended the spelling of the genus *Phleobum* to *Phlaeobum* because the correct spelling of the host genus from which *P. gigantinum* was described is *Phlaeoba* and not *Phleoba*. However, as defined by ICZN Art 32.5.1, the original spelling is the nomenclaturally correct spelling, despite any agreement or disagreement with the correct spelling of the type host generic name. Levine (1985, 1988)

and Clopton (2002), both apparently reached to the same conclusion, which is accepted here also.

Five additional species were described subsequently from five different grasshopper species after 1986 (Patra and Haldar 2004, Chatterjee and Haldar 2003). These are *P. subsphericum* from *Atractomorpha crenulata*; *P. janovii* from *Aiolopus thalassinus tamulus*; *P. longistriatum* from *Spathosternum pr. prasiniferum*; *P. oxyum* from *Oxya* sp. and *P. cloptoni* from *Aiolopus thalassinus*. The new species were included into the genus based on the five main traits as outlined by Kundu and Haldar (1986). But in all the nine species of *Phleobum*, a unique set of characters are persistently observed. So the revised generic characters of the genus *Phleobum* are proposed in this communication. The characters are: 1) trophozoites with a simple knob like epimerites and very few in number; 2) cytoplasm of the freshly collected gregarines and gametocyst are orange or yellowish orange in colour; 3) the entire organism is covered by an excessively thick pellicle; 4) association is mostly in pairs but multiple lateral association (syzygy) with key-lock depression interface also found; 5) a flange is present around the protomerite in satellite during association leading to a firm key-lock depression interface; 6) gametocyst with an ectocyst in majority instances, dehiscing through a single pore at one corner of the cyst wall; 7) oocyst ellipsoidal, covered with single hyaline coat at the time of their liberation; and 8) the genus is specific for orthopteran insect host.

Type species: *Phleobum gigantinum*.

D. Placement of the Genus *Phleobum* under the Family Hirmocystidae Grassé, 1953

Haldar and Chakraborty (1974), while erecting the new genus *Phleobum* placed it under the family Monoductidae Ray and Chakravarty, 1933. Later, Kundu and Haldar (1986) incorrectly transferred the genus to the family Didymophyidae Legér, 1892 since any sporoduct has never been observed in any of the species of *Phleobum*.

As such, after thorough consideration of all the characters, especially: the 1) simple knob-like epimerite; 2) gametocysts dehiscing by a single pore, which can be considered as simple rupture; and 3) ellipsoidal oocyst; the placement of the genus *Phleobum* is proposed under the family Hirmocystidae Grassé, 1953.

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