Punjab Univ. J. Zool. Vol. 6, pp. 7-18, 1991.

STUDIES ON THE MORPHOLOGY OF HEMOCYTES OF *PHYSA ACUTA* AND *BELLAMAYA BENGALENSIS**

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Abstract: A study was made on the hemocyte morphology of freshwater snails *Physa acuta* and *Bellamaya bengalensis*. The hemolymph directly obtained from the heart was allowed to settle for 30 minutes before fixation and staining. Both snail species showed two basic types of hemocytes known as spreading hemocytes and round hemocytes. Light microscopy of these hemocytes revealed behavioural distinctions. In both the snails studied, the spreading hemocytes predominates. Mean percentage of these cells constitutes 90.51±3.71 and 93.38±2.03 for *P. acuta* and *B. bengalensis* respectively. Similar values for round hemocytes were 6.62±1.22 and 9.48±1.01 percent. Majority of the spreading hemocytes were mononucleated. However, less than 2% of the total blood cells were binucleated or multinucleated, with unequal nuclei termed as mother nucleus and daughter nucleus. Round hemocytes were always mononucleated. The mean size of the mononucleated spreading hemocytes was 1378.42±578.08 and 1745.26±990.39 $(\mu m)^2$, while that of the binucleated cells was 1843±1117 and 3202.0± 1646.39 $(\mu m)^2$ for *P. acuta* and *B. bengalensis*.

The nucleus of spreading hemocyte was rounded to oval, kidney shaped or lobulated. Only spreading hemocytes produced filopodia and pseudopodia in almost all directions and were capable of exhibiting rapid changes in shape, but no net motion. The number of pseudopodia produced by spreading hemocytes lie between 9-27 with an average value of 17.47±5.77 and 13.70±4.89 for *P. acuta* and *B. bengalensis* respectively. Maximum average length of pseudopodia of a single spread hemocyte was 327.37±143.67μm and 226.84±95.68μm for *P. acuta* and *B. bengalensia*.

The round hemocytes were characterized by the presence of more of less circular or weavy boundry, and no pseudopodia. They also posses comparatively hyaline (clear) cytoplasm alongwith few granules and vacoules of various sizes. The mean area occupied by round hemocytes was 416.0±140.22 and 950.0±928.10 $(\mu m)^2$ for *P. acuta* and *B. bengalensis*, with a typical rounded to oval nucleus.

Only spreading hemocytes fuse with one another to form synctia but round hemocytes did not fuse either with each other or with spreading hemocytes. However, fusion among spreading hemocytes was reversible. The present findings are discussed in the light of available studies on the molluscan blood in general and gastropod hemolymph in particular.

Key words: *Physa acuta, Bellamaya bengalensis*, hemocytes, snails.

INTRODUCTION

The work done on various aspects of molluscan hemocytes have been reviewed by Cheng (1981), Cowden and Curtis (1981) and Sminia (1981). Cheney (1971) has reviewed literature describing leukocyte forms and functions within the invertebrate phyla. The most valueable data on blood cell structure and function have been obtained from the studies on pulmonate snails, e.g., *Helix aspersa* and *H. pomatia* (Wagge, 1955, 1955; Prowse and Tait, 1969, Bayne, 1974), *Biomphalaria glabrata* (Pan, 1958; Tripp, 1961; Faulk *et al.,* 1973; Cheng and Auld, 1977) and *Lymnaea stagnalis* (Muller, 1956: Stang-Voss, 1970; Sminia, 1972, 1977 a, b, Sminia and Barendsen, 1980).

*Part of the Ph.D. work of the author

There is a good deal of disagreement on the types of blood cells present in molluscs (Geogre and Ferguson, 1950; Muller, 1956; Kostal, 1969; Cheney, 1971; Sminia, 1972; Cheng and Auld 1977). Many workers think that gastropod blood cells must be classified on the basis of morphological difference (Muller, 1956; Kress, 1968; Cheng *et al.,* 1969) but some have highlighted the importance of functional differences (Brown and Brown, 1965; Prowse and Tait, 1969 Davis and Partridge, 1972).

The presence or absence of cytoplasmic granules in the molluscan leukocytes have also been studied and discussed (Cuenot, 1891; de Bruyne, 1895; Kollmann, 1908; Drew, 1910; Takatsuki, 1934; George and Ferguson, 1950; Dundee, 1953; Cheng and Rifkin, 1970; Feng *et al*., 1971; Foley and Cheng, 1972). One type of blood cell was distinguished in the gastropod mollusc by Cuenot (1892), Baecker (1932), Haughton (1934), Pan (1958), Stang-Voss (1970), Meuleman (1972), Sminia (1972), Faulk *et al.* (1973), Renwrantz *et al.* (1979), Sminia and Barendsen (1980), Dikkeboom *et al.* (1984).

The concept of two type of blood cells have been documented by Kollmann (1908), Wagge (1955), Glatsoff (1964), Brown and Brown (1965), Feng *et al.* (1971), Davis and Partridge (1972), Foley and Cheng (1972), Cheng (1975), Harris (1975), Yoshino (1976), Cheng and Auld (1977), Cheng and Guida (1980 a,b), Schoenberg and Cheng (1980, 1981), Ottaviani (1983), Tanveer (1989, 1990). There are other workers who investigated three type of blood cells in gastropod molluscs i.e George and Ferguson (1950); Kress (1968); Cheng *et al.,* (1969). While Muller (1956) was the only who described four blood cell types.

The blood cells of gastropods are often described by various names *e.g.*, leucocytes, lymphocytes, hemocytes, amoebocytes, granulocytes and macrophages (Wagge 1955, Kress 1968, Cheng *et al.* 1969, Davis and Partridge 1972, Cheng and Auld 1977).

In the present study we have used the terms spreading hemocytes and round hemocytes which explain the description more approperiately. Unfortunately no significant work has been done on blood cells of gastropod snails in Pakistan. It was, therefore, considered desireable to undertake a study on hemocytes of local gastropod snails with a view to provide basic information regarding their structure, which contribute to the understanding of phagocytosis.

MATERIALS AND METHODS

The gastropod snails used in the present studies were *Physa acuta* (Physidae) and *Bellamaya bengalensis* (Viviparidae). The information regarding snail collection sites have been provided by Tanveer (1989) while details comprising maintenance of snails have been given by Tanveer *et al.,* (1989). In all, laboratory bred snails of age 32 weeks for *P. acuta* and 60 weeks for *B. bengalensis* were used. Their respective weight and shell lengths were 68.0$\pm$1.3 mg, 12.01$\pm$0.32mm and 1190.0$\pm$0.33 mg, 22.6$\pm$0.37 mm. For the details of getting hemolymph samples, staining and measuring techniques see Tanveer (1990).

RESULTS AND DISCUSSION

Among the hemocytes studies for *P. acuta* and *B. bengalensis,* two distinct cell types i.e., spreading hemocytes and round hemocytes were observed. Spreading hemocytes constitute 90.51±3.71 and 93.38±2.03 precent of the total blood cells. Round hemocytes were 6.62±1.22 and 9.48±1.01 *for P. acuta* and *B. bengalensis*. The detailed morphological characteristics of these cells are given below.

Spreading hemocytes

These are amoeboid cells with hgihly variable shape. Their cytoplasm which contain certain granular structures was not differentiated into ecto and endoplasm. On the base of these granules they have been given the name of granulocytes (Cheng and Guida, 1980 a, b; Tanveer, 1989). These cells have a tendency to spread on contact with a solid substratum and the degree of this spreading depends somewhat on the time for which they are left undisturbed and on the temperature and the medium in which the cells are kept. Whenever they come in contact with solid substratum, they extend pseudopodia of different shape and size in almost all directions. Another remarkable ability of these cells is to merge with each other. This mergence is such that they only loose their cytoplasmic identity while their nuclei always remain distinct. However, this mergence was found to be reversible. By this mergence hemocytes of all the snails studied are capable of forming aggregates, some of which appear to be syncytia. Pair of these cells connected by cytoplasmic bridges are also commonly observed (Plate 1-2). Such bridges range from the width of a single pseudopodia to that of an entire cell. Some times several cells are found surrounding unidentified non cellular debris. This merging may be an extension of their tendency to phagocytose foreign particles. This further seems to suggest that in their reaction to solid objects, the cells are not able to identify self from nonself and they react in the same manner to another cells of their own type (Plate 1-2) as they would to a foreign solid object.

This property of molluscan hemocytes to fuse and form multinucleated cells *in vitro* was first observed and reported by Geddes (1880). The original interpretation that they represent clots, has since been modified by Michel (1888), Drew (1910), Dundee (1953), Narain (1972) and Foley and Cheng (1972). In addition, Spark and Pauley (1964), Cheng and Galloway (1970), Stang-Voss (1970), Cheng and Guida (1980a), have also reported the presence of multinucleated giant cells in sections of molluscs both gastropods and plecypods under pathological condition. Cheng (1981) designated these cells as multinucleated macrocytes and assumed that they represent the fused granulocytes *in vitro*.

The area of spreading hemocyte of *P. acuta* and *B. bengalensis* was 1378.42±578.08 and 1745.26±990.39 $(\mu m)^2$ respectively. For further details see Table 1. It was also noted that spreading hemocytes showed great diversity of size even within the same snail species. This great diversity in size have also been quoted by various workers (Yoshino, 1976, Cheng and Auld, 1977, Lo Verde, 1979, Cheng and Gudia, 1980b, Khan, 1986). Their reproted dimensions were between 7-70 μm. However, Tanveer (1990) reported that mean area occupied by the mononucleated spreading hemocyte was 1301±578.83 and 1021.67±344.81 $(\mu m)^2$ for *Lymnaea*

acuminata and *Indoplanorbis exustus*, which showed much more variation within the same species.

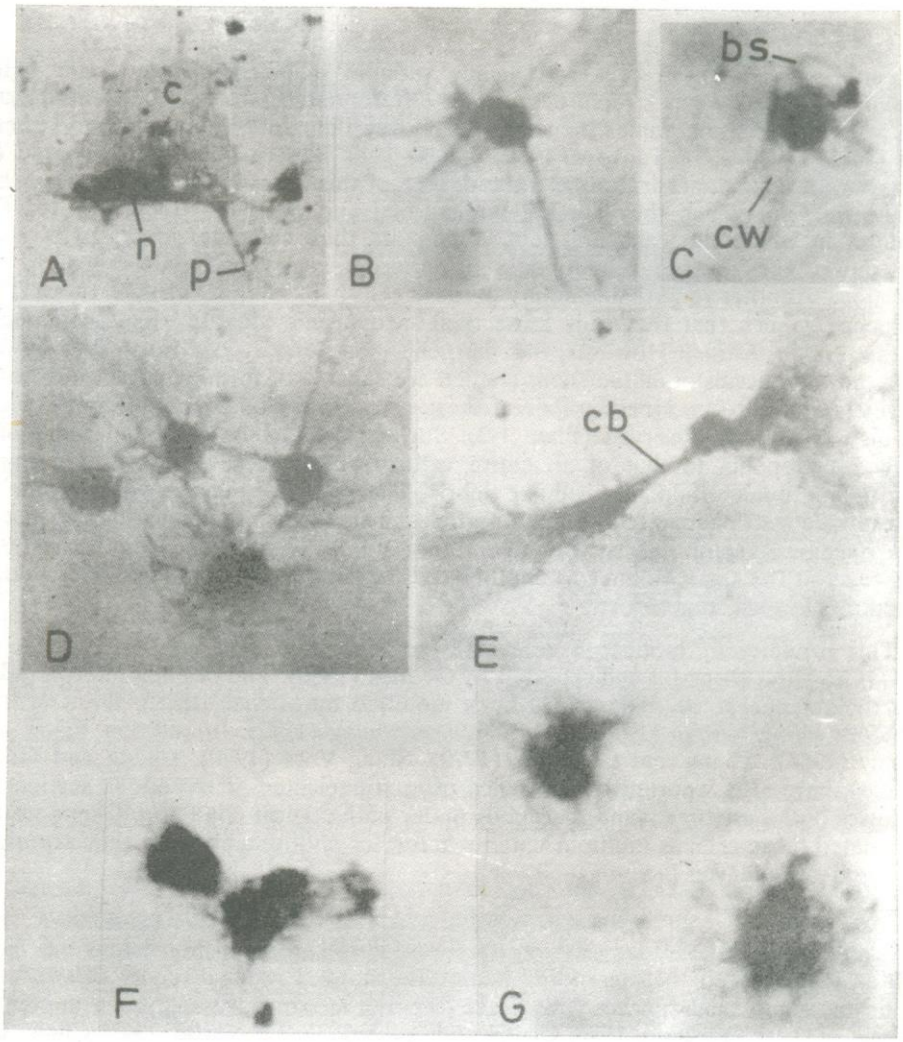

Fig. 1. Hemocytes of *Physa acuta* (1000X)
c = cytoplasm; cb = ctoplasmic bridge; cw = cytoplasmic web; n = nucleus; p = pseudopodia

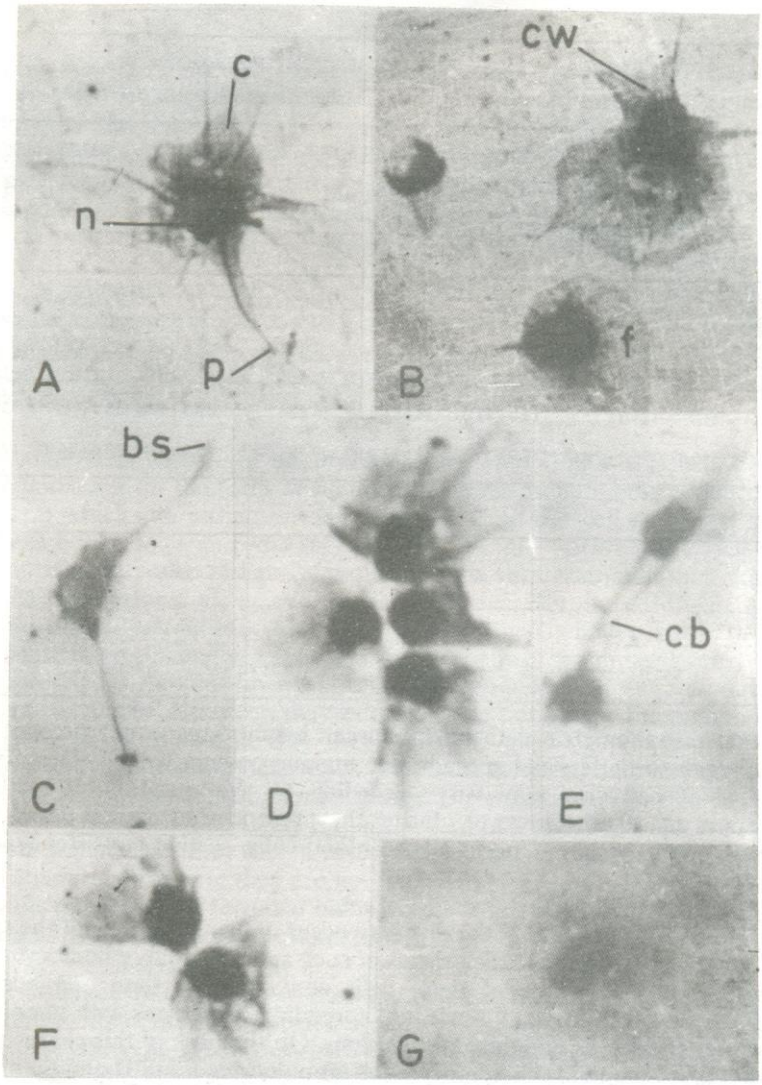

Fig. 2. Hemocytes of *Bellamaya bengalensis* (1000X)

Table 1: Some morphological characteristics and dimentions (mean± S.D.) of stained hemocytes of Physa acuta **(a) and** Bellamaya bengalensis **(b).**

Mononucleated Spreading Hemocytes

Sample (n)	cell's area (μm)^2	nucleus area (μm)^2	cell/nucleus area	Pseudopodia total number	minimum length(μm)	maximum length(μm)
(a) 30	1378.42±578.08	110.05±61.63	14.54±6.54	17.47±5.77	27.37±14.08	327.37±143.67
(b) 30	1745.26±990.39	197.37±64.69	8.67±4.15	13.79±4.89	32.37±16.89	226.84±95.68

Binucleated Spreading Hemocytes

Sample size (n)	cell area (μm)^2	mother nucleus area (μm)^2	daughter nucleus area (μm)^2	cell/mother nucleus ratio	cell/daughter nucleus ratio	total number	Pseudopodia minimum length (μm)	maximum length (μm)
(a) 30	1843.4±111.7	138.0±7.58	113.0±12.04	13.84±0.86	16.42±1.59	18.2±5.54	13.4±5.27	334.0±62.69
(b) 30	3202.0±1646.39	238.0±70.14	174.0±85.90	12.91±4.09	20.79±8.87	15.8±4.44	28.0±14.03	190.0±119.79

Round Hemocytes

Sample size (n)	cell area (μm)^2	nucleus Area (μm)^2	cell/nucleus ratio
(a)10	416.2±140.22	62.6±18.53	6.82±1.73
(b)10	950.0±928.18	130.0±98.38	7.91±4.34

Consistant morphometric data on molluscan haemocytes is not present because these cells began to flatten and spread after making contact with surface. Therefore measurement of cell size may vary according to the duration of setting time (Renwarantz *et al.,* 1979). However, during the present investigation equal time (30 minutes) was given for spreading to all the blood cells, so that consistency in results could achieved.

It was interesting to note that the diversity occur only in area/size of the spreading hemocyte while the staining characteristics of such small and large hemocytes showed no difference. Unusally some of these cells contain more than one nucleus and accordingly termed as bi or multinucleated spreading hemocytes with unequal nuclei termed as mother nucleus and daughter nucleus. On the base of this character Cheng and Guida (1980b) named such hemocytes as granulocytes I and II and their reported dimensions (minimum and maximum) were 3.94±0.61, 5.17±0.86 and 3.70±0.90, 8.54±3.13μ respectively for *Bulinus truncatus rohlfsi*.

During the present investigation, among the binucleated spreading hemocytes the maximum area was occupied by *B. bengalensis* (3202±1646.39μm^2) and minimum

area by hemocytes of *P. acuta* (1843.4±1117.7μm^2). It may be mentioned here that the double nucleated spreading hemocytes were quite rare in the two snail species studied. The percentage of these cells was 1.95 and 2.0 of the total hemocytes of *P. acuta* and *B. bengalensis*.

In the two snail species studied, another important characteristic of spreading hemocytes is to extend pseudopodia in almost all directions (Plate 1-2). The pseudopodia may differ in number, shape and length. The proximal protion of the the pseudopodia usually include some cytoplasm, but due to the tapering construction of each pseudopodia, at the tip there is little visible cytoplasm. Diameter of the typical pseudopodia at its tip was 0.2 to 0.6 um. Similar values (0.3 - 0.5 um) have been reported for pseudopodia of *B. truncatus rohlfsi* by Cheng and Guida (1980b).

The pseudopodia whereever and whenever are in contact with solid substratum retained the property of spreading. The formation of pseudopodia is not restricted to a foreign object only, they will be formed as a reaction to the presence of another haemocyte also (Plate 1-2). This property leads to the formation of large aggregates of cells (Plate 1-2).

In some cells there is a basal ectoplasmic web (Plate 1-2), while in others the pseudopodia are in the form of filopods (the first pseudopodia produced by the cell, Plate 1-2) which can radiate in any direction from the cell and may have a narrow core extending to some distance. Cheng *et al.* (1979) have stated that fine pseudopodia/filopodia radiate along the glass substrate and that the cytoplasmic granules may extend along certain pseudopodia. But it is difficult to see why a pseudopodia will not be formed towards a solid object which is not in the plane of the glass substratum. Cheng *et al.* (1979) by the help of Scanning Electron microscope also reproted for the first time that the free terminals of all the filopods/pseudopods terminate as a bulb. However, they were not able to find any functional significance of such terminal bulbs except that they were the growing points of the filopods or pseudopods for *B. glabrata* hemocytes. The same terminal bulb has also been reported by Cheng and Guida (1980b) for *B. truncatus rohlfsi*. According to another view put forth by Rajaraman *et al.* (1974) is that the terminal bulbs or webs are the specialized attached organells as they are in mammalian cells. However, their exact function is still controverical because they are not necessarily found in all the cases as studied so far. In the present study terminal bulbs were not observed, as they can only be seen by the help of Scanning Electron microscope.

Variations in the number of pseudopodia produced by the spreading hemocytes were observed in the two species studies. This variability probably points to the species specific characteristics and/or may also depend on the mechanism of phagocytosis of that particular species. For the number of pseudopodia, their maximum and minimum length, see Tables 1. It is clear from the Table that *P. acuta* and *B. bengalensis* produced an average of 17.47±5.77 and 13.79±4.89 pseudopodia. The only work of significance in this regard have been documented by Cheng and Guida (1980b), their reported number of pseudopodia produced by the hemocytes of *B. truncatus rohlfsi* was 1-20. They did not reprot the average number or standard

deviations.

As far as the length of the pseudopodia is concerned, there also existed great variations. Among the species studied the maximum average length of pseudopodia of single spread hemocytes was observed for *P. acuta,* i.e., 327.37±143.67μm followed by *B. bengalensis* (226.84±95.68μm). The other reported maximum lenghth for pseudopodia was upto 35μ beyond the margin of the cell's body in *B. truncatus rohlfsi* (Cheng and Guida, 1980b). It is clear that this value is less than the values observed during the present investigation. Another important thing to note is the length of pseudopodia that may vary according to duration of setting time.

The other morphological and structural characteristics of the two species are similar with the exception of variability in the number and length of pseudopodia, which probably may not be of much significant importance except showing species specificity.

The spreading nucleus of hemocytes was found to be rounded to oval, kidney shape or lobulated and rarely with a constriction near the middle. Similar findings has also been reported by Sminia (1972) and Cheng and Guida (1980b). The nucleus area $(m\mu)^2$ of single nucleated spreading hemocyte was 110.05±61.63 and 197.37±64.79 for *P. acuta* and *B. bengalensis* (Table 1). The nucleus area of double nucleated spreading hemocyte was 138.0±7.58 and 113.0±12.04, for mother nucleus and daughter nucleus of *P. acuta.* Similar findings for *B. bengalensis* were 238.0±70.14 and 174.0±85.90 for mother nucleus and daughter nucleus respectively.

In both the snail species studied the cell to nucleus ratio of the speading hemocytes was greater for *P. acuta* (14.54±6.54) as compared to the similar ratio for the spreading hemocytes of *B. bengalensis* (Table 1).

In addition to the presence of neuclei the cytoplasm of spreading hemocytes, contain granules of various sizes ranging from 0.4 to 1.6 μm in diameter. Presence of such granules have also been reported by Cheng and Guida (1980b).

Round hemocytes

These cells constitute less than 10 percent of the total hemocyte counts of the two snail species studied. These cells differ from the speading hemocytes in the presence of very few granules in the cytoplasm, having more or less circular or somewhat weavy boundry in the spread from. They also possess comparatively hyaline (clear) cytoplasm and vacoules of various sizes. These cells differ from the spreading hemocytes in the absence of filopodia or pseudopodia. In this regard our observations are in line with those of Cheng and Auld (1977, *B. glabrata*); Guida and Cheng (1980, *B. truncatus rohlfsi*); Sminia (1981, *L. stagnalis)* Tanveer (1990, *L. acuminata* and *I. exustus*).

The area $(\mu m)^2$ occupied by the round hemocytes of the two snail species studied was 416.0 ± 15.22 and 950.0 ± 928.10 for *P. acuta* and *B. bengalensis* respectively.

It is also clear that round hemocyte (even the biggest) are smaller than the average spreading hemocyte of the two snail species studied in the present investigation, although difference exist in the area of round hemocyte within the species nevertheless, morphological characteristics of cytoplasm and nucleus are similar in the two snail species studied.

A typical round to oval nucleus in the round hemocyte was observed in the present investigation (Plate 1-2) which has previously been reported by many workers also (Cheng and Auld, 1977; Sminia and Barendson, 1980; Sminia, 1981 and Tanveer, 1990).

The nucleus area $(\mu m)^2$ of round hemocyte was 62.6 ± 35.85 and 130.0 ± 98.38 for *P. acuta* and *B. bengalensis* respectively. The respective nucleus to cytoplasm ratio for these cells was 6.82 ± 1.73 and 7.91 ± 4.31 for the two snail species studied (Table 1).

Comparatively higher nucleus to cytoplasm ratio in hyalinocytes (round hemocytes) has been noted by Sminia (1981) in *L. stagnalis* and Khan (1986) in *Angiospira alternata alternata*. However, their reproted nucleus to cytoplasm ratio was less as compared to the similar ratio for spreading hemocytes in the present study.

Acknowledgement: Financial aid provided by PSF, Project No. P-PU/Bio (117) is gratefully acknowledged

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