TECHNICAL MONOGRAPH NO. 17

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ZOOLOGICAL SURVEY
OF INDIA
1916

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Edited by the Director, Zoological Survey of India
1990

Published : December 1990

PRICE : Inland : Rs. 60.00

Foreign : £ 4·00 $ 6·00

PRODUCTION : PUBLICATION UNIT
Zoological Survey of India, Calcutta

Printed in India by Saakhhar Mudran, 4, Deshapran Sasmal Road, Calcutta-33
Produced by the Publication Division and Published by the Director,
Zoological Survey of India, Calcutta.

ZOOLOGICAL SURVEY OF INDIA

TECHNICAL MONOGRAPH

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INTRODUCTION

A) Historical development of soil protozoa :

The active existence of free-living protozoa in soil was not considered by earlier protozoologists, and their presence in culture of soil was attributed to wind-borne cysts that originally came from drying up of pools (Singh, 1975). Russell and Hutchinson (1909), the two agricultural chemists at Rothamsted Experimental station, England, first investigated the effect of partial sterilisation of soil on the production of plant food. They showed that when soils were heated and treated with certain volatile antiseptics and restored condition favourable to plant growth, there was an increase in soil fertility. A theory was thus advanced that the treatment by heat or volatile antiseptics removed some factor which in the untreated soils limited the growth of bacteria and hence the rate of ammonia production. It was the credit of Russell and Hutchinson who first drew attention to the fact that soil contained free-living protozoa usually preying on bacteria which aroused great interest because of its bearing on soil fertility. Thus according to this theory soil sickness is due to an excessive number of active (trophic) protozoa, which by their phagocytic action restrict the bacterial process going on in soil, while remedial effect of partial sterilisation is due to the killing of the protozoa. It may also be noted that a school of protozoologists maintained the view that protozoa were normally present in soil largely, if not exclusively, as cysts and were thus in inactive stage (vide Singh, 1975 for references).

Cutler (1920) devised a dilution culture method, using nutrient agar plates for counts of active and cystic protozoa of soil. By the use of this method Cutler *et al.* (1922) found large number of active amoebae and flagellates from a dunged plot at Rothamsted and demonstrated that rapid fluctuations in their numbers and in the bacterial numbers took place daily and seasonally. Interestingly however, no connection could be traced between temperature, moisture contents and rainfall and daily change in the numbers of any of the organisms. While an inverse correlation was found to exist between the numbers of active amoebae and the numbers of bacteria, a rise from one day to the next in the number of amoebae being correlated with a fall in the numbers of bacteria and vice versa. Cutler *et al.* (1922) thought that amoebae which feed on bacteria probably were responsible for the fluctuation in bacterial population (vide Singh, 1975 for further references). At a time when Sandon (1927) wrote his monograph on soil protozoa, there was neither a satisfactory method for the isolation and cultivation of soil amoebae, nor was there a system of classification of amoebae based on possible phylogenetic relationship.

B) Systems of classification of amoebae

As there is no constant body shape in amoebae without test, the classification of these organisms posed to be difficult and extremely controversial. They are important on the one hand as being components of soil and aquatic habitats and on the other as potential pathogen of man and animals.

Since it has been found that the locomotive form and pseudopodial characters of amoebae vary with the change in the chemical and physical conditions of the environment (Dobell, 1914, Minchin, 1922, Ray and Hayes, 1954, Kudo, 1959). Wenyon (1926), Calkins (1933), Kudo (1966) and others attempted to divide the order Amoebida as follows

(1) Family Amoebidae—amoebae that are not able to form temporary flagella.

(2) Family Paramoebidae—amoebae which in addition to nucleus, possess an accessory body (Nebenkörper) which during division divides with the nucleus.

(3) Family Dimastigamoebidae or Bistadiidae—amoebae which are able to form two or more flagella, by means of which they progress as flagellates.

(4) Family Endamoebidae—to include exclusively the parasitic forms (vide Singh, 1952 for references).

Singh (1952) pointed out that the erection of the family Dimastigamoebidae was not justified. He suggested that 'it is more logical to put species such as *Didascalus thorntoni* Singh, 1952 and *Schizopyrenus russelli* Singh, 1952 whose nuclear divisions are indistinguishable, in the same family rather than in two different families merely on the ground that the former produces temporary flagella with difficulty and the later can not. The character of flagella production then becomes of generic value only. Moreover, the physiological conditions under which amoebae produce flagella are not fully understood (Yuyama, 1971)' There are certain species of free-living aerobic soil amoebae belonging to the genera *Naegleria* and *Acanthamoeba* which have been found to cause primary fatal human amoebic meningo-encephalitis as a result of the trophozoites entering the nose during swimming or bathing in fresh water (vide Singh, 1975 for the literature on pathogenic free-living amoebae). *A. polyphaga* has been found to cause human eye infection leading to blindness (Singh and Hanumiah, 1979). Thus it has abolished the artificial barrier existing between parasitic and free-living amoebae. Moreover, *Entamoeba moshkovskii*, a free-living anaerobic amoeba discovered by Tshalaia (1941 1947) in Moscow, and also by other workers in different countries, resembling closely in morphology to *E. histolytica*, is not infective to any animals. So it is very difficult to tell whether amoebae are parasitic or not. So these findings clearly cast doubt on the validity for establishing a family for parasitic amoebae. Only the presence of an accessory body (Nebenkörper) does not throw any light on the probable evolutionary significance of amoebae. It is very clear that the system of Wenyon (1926), Calkins (1933) and Kudo (1966) is not based upon sound scientific basis and has not at all any possible phylogenetic value.

At the present time two major criteria on which higher taxa for amoebae have been based are (1) locomotive form and behaviour, and (2) mitotic pattern. Page (1976a) says (P. 63)—'To the present day the old controversy between nuclear and pseudopodial schools of taxonomy has been kept warm, with the current phase dating approximately from the earlier papers of Singh (1950, 1952) and Bovee (1953)' Schaeffer (1926) was the pioneer protozoologist to offer a comprehensive system of classification based on fundamental morphological characteristics and difference in locomotive forms of amoebae. He erected eleven new genera and described thirty-nine new species on the shape and character of pseudopodia and types of locomotion. It is to be mentioned that Schaeffer established three species, each from a single specimen and another species from two specimens. Regarding his system of classification, he presented his concept as follows The object of systematics in this group is of course to classify amoebas, not nuclei'

Bovee and Jahn (1965, 1966, 1973) and Jahn and Bovee (1965) tried to develop Schaeffer's system of classification further by adding new families and genera using pseudopodial types and trophic characters. The methodology attempted by Bovee and Jahn is as follows

For the study of amoebae from fresh water and marine sources, these workers usually kept the samples of water in clean glass jars and covered them loosely with a plastic or glass lined metal screw-cap cover. The samples were maintained in the laboratory at room temperature. They said under these conditions amoebae endured often for weeks to months in the samples, sometimes undergoing a bloom or a series of blooms. During observation, a drop of the water containing amoeba was examined microscopically on a slide after putting a coverslip at different intervals. Regarding the assessment of their work Singh and Hanumaiah (1979) concluded as follows

'How reliable this method is in studying healthy amoebae and in the creation of species in a genus is very difficult to assess. Using the above method Bovee (1970), established thirteen new species in the genus *Mayorella*'

Recently the system of classification given by Levine *et al.* (1980) is more or less the same as proposed by Page (1976a, b) who has recognized all the orders and suborders. Following is the detail of classification given by the Levine *et al.* (1980).

Sub class GYMNAMOEBIA Haeckel. 1962
Without test.

Order 1 AMOEBIDA Ehrenberg, 1830

Typically uninucleate mitochondria typically present no flagellate stage.

Sub order 1 TUBULINA Bovee and Jahn, 1966

Body branched or unbranched cylinder no bidirectional flow of cytoplasm , nuclear division mesomitotic.

Amoeba, Entamoeba, Saccamoeba

Sub order 2. THECINA Bovee and Jahn, 1966

Flattened, with more or less regular outline, often oblong, ovate or flabellate , often with discernible pellicle-like layer, which may be distinctly wrinkled , rolling movement of surface nuclear divisional pattern diverse.

Platyamoebae, Thecamoeba, Vannella.

Sub order 3. FLABELLINA Page, 1976

Flattened, broad, sometimes discoid with extensive hyaline zone but no obvious pellicle-like layer locomotion often accompanied by gentle eruption . nuclear division, where known, mesomitotic.

Flabellula, Rosculus.

Sub order 4. CONOPODINA Bovee and Jahn, 1966

Digitiform or mammilliform, usually blunt, normally unbranched hyaline sub-pseudopodia usually produced from a broad hyaline lobe , not discoid cysts seldom formed, nuclear division typically mesomitotic.

Mayorella, Paramoeba.

Sub order 5. ACANTHOPODINA Page, 1976

More or less finely tipped, sometimes filiform often furcate, hyaline sub-pseudopodia produced from a broad hyaline lobe not regularly discoid cysts usually formed nuclear division mesomitotic or metamitotic.

Acanthamoeba, Echinamoeba.

Order 2. SCHIZOPYRENIDA Singh, 1952

Body with shape of monopodial cylinder, usually moving with more or less eruptive hyaline, hemispherical bulges typically uninucleate, nuclear division promitotic, temporary flagellate stage in most species.

Naegleria, Tetramitus, Vahlkampfia.

Order 3. PELOBIONTIDA Page, 1976

Body with shape of thick cylinder monopodial, with true bidirectional fountain flow of cytoplasm common typically multinucleate, lacking mitochondria but with symbiotic bacteria in microaerobic habitats no flagellate stage known, but numerous non motile cils visible at fine-structural level, with variations of usual microtubular pattern.

Pelomyxa.

Since 1967 Page studied very diverse groups of amoebae growing them in clonal cultures, mostly on non-nutrient agar supplemented with a suitable species of bacteria as a food source, as advocated by Singh (1946, 1952, 1955, 1960). Although Page included in his studies locomotive form and behaviour, presence or absence of a flagellate stage, nuclear structure and nuclear division using Feulgen reaction, but he based his classification mainly on the locomotive morphology of amoebae. Page (1976a) concluded that 'Bovee and Jahn system has great value and further systems of Sarcodina as a whole will probably continue to be indebted to it'. Page (1967, 1971), using faulty techniques to study the nuclear division in amoebae, has described in some species completely abnormal stages of division claiming new types of mitosis (Singh and Hanumaiah, 1979).

Singh and Hanumaiah (1979) have rejected Page's system of classification for the following reasons. The salient points of their criticism are given below

Dobellina mesnili, a multinucleate amoeba without a flagellate stage and with no bidirectional flow of cytoplasm, can not be included in any one of the orders erected by Page (1976a). The presence of mitochondria in amoebida is not justified, because *Entamoeba histolytica* and *E. invadens* have no mitochondria. The formation of order Pelobiontida on the absence of mitochondria is not justified because *Pelomyxa palustris* has no mitochondria whereas other species of *Pelomyxa* have mitochondria (Daniels, 1973). Singh and Hanumaiah (1979) have concluded by saying (P-5)-'Suffice it to say that the characters used to create the orders are so ambiguous, indistinct and lacking sharp demarcation that they cannot be followed in practice. It is better to put all the amoebae without test in one order Amoebida, as done by nearly all the protozoologists' They liked to point out that the creation of the order Pelobiontida based on its anaerobic nature is not justified. *Entamoeba moshkovskii*, an uninucleate free-living amoeba also lives in semiaerobic habitat as the free-living *P. palustris*. Singh and Hanumaiah (1979) also conclude that a serious error in erecting sub orders, families and even genera on locomotive forms and behaviour has been made in grouping of amoebae showing different types of nuclear divisions. Thus such a classification of amoebae on morphological characters can not be of any possible phylogenetic value.

Earlier protozoologists established genera and species among small free-living and intestinal amoeba on the basis of their nuclear division. Several memoirs regarding nuclear division in free-living amoebae appeared after the work of Vahlkampf (1905). Different views have been postulated by different workers who have carried out cytological investigations. The confusion that appeared was mainly due to three principal causes (1) the study of amoebae grown in rich nutrient media, with uncontrolled bacterial food supply, which may lead to the production of abnormal forms, (2) lack of a suitable method for getting easily the normal stages of nuclear division, and (3) the use of cyto-logical techniques which are not specific for the location of chromosomal chromatin and non-chromatic materials during the different stages of nuclear division. Rich nutrient media, either in a solid or liquid form, promoting the growth of unsuitable organisms, which may lead to the production of abnormal forms (Singh 1945, 1946, 1955, 1960). The unsuitability of such methods can be judged by the following remark of Dobell (1914) who studied nuclear division in small, free-living amoebae. He says—'I have studied more than a dozen different species of free-living amoebae. Some of these have been obtained from the infusion of soil, hay and other organic substances others from fresh-water others again from sea. It is, of course, necessary, in the case of every form to discover at the outset the particular medium which is best suited to it and to discover what it eats and then take care that it is properly fed (P-142). I may add here that chance plays a large part in determining the success—or the reverse—which rewards the efforts of those who try to study the method of nuclear division in many species of amoebae. On several occasions I have worked extremely hard for several weeks on a flourishing culture of amoebae—making scores of preparations, systematically at all times of the day and examining thousands upon thousands of individuals. On at least two such occasion, I found almost complete series of stages in division in the first preparation I made from a culture of amoebae—and in large numbers of subsequent preparations of the same forms not a single stage' (P-146). Dobell was fortunate in getting the, details of nuclear division in only two species of amoebae during a period of seven years.

The use of haematoxylin and other non-specific aniline dyes and their combinations as the main criteria for differentiating the nuclear structure and behaviour during division, has created confusion in the past. The structure which has been called karyosomes, should be called nucleolus as it is Feulgen-negative, has been supported to contribute either partly or completely to chromosomal chromatin in some amoebae and not in others. It has also been observed that 'peripheral chromatin' has been found to be completely absent in some forms and present in others. Claim has also been made that 'peripheral chromatin' sometimes gives rise to chromosomes and sometimes disappears completely during division of the nucleus. It is to be mentioned that before Feulgen reaction came into use, it was impossible to locate chromosomal chromatin, during the nuclear divisional pattern. The difficulty of getting the various normal stages of nuclear division and the use of unsuitable cytological techniques resulted in the creation of a very large number of species of small free-living amoebae. Singh (1941, 1945, 1946, 1947a,b, 1948a,b, 1960) studied large number of very varied bacterial species on non-nutrient agar as food source for several species of small free-living amoebae and amoeboid organisms. He discovered *Aerobacter aerogenes*, a species of bacterium which was a non-toxic suitable food source for various species of free-living amoebae. This discovery led Singh (1946, 1955) to the use of non-nutrient agar and a suitable edible species of bacterium for the isolation and culture of amoebae from soil and other substrates. Non-nutrient agar discourages the growth of inedible bacteria and toxigenic micro-organisms coming from soil and other substrates.

For the study of nuclear division in small free-living amoebae, Singh (1950) devised agar film culture method. Singh (1952) combined agar-film method with Feulgen reaction to study the nuclear division of free-living amoebae obtained from clonal culture.

All the species of amoebae had a single more or less central spherical Feulgen-negative nucleolus in the resting nucleus stage. During mitosis Feulgen-positive chromatin gave rise to chromosomes. The nucleolus either persisted throughout division, giving rise to 'polar massess' as shown by Vahlkampf (1905) or it disappeared during division and a spindle with chromosomes arranged as an equatorial plate was formed. On the basis of these two distinct types of nuclear mitosis, Singh (1952) suggested the classification of the order Amoebida as follows

Family SCHIZOPYRENIDAE, Singh, 1952
emend. Singh and Das, 1970

Definition

The resting nucleus contains a more or less central Feulgen-negative nucleolus, which during mitosis divides to form 'polar masses' 'Inter-zonal bodies' may be present. Amoebae may have more than one nucleus, some genera may produce temporary flagella.

Type genus *Schizopyrenus* Singh, 1952

The other genera included in Schizopyrenidae by Singh and Das (1970) were

Sappinia (Dangeard, 1896), *Naegleria* (Alexeieff, 1912 emend. Singh

1952), *Didascalus* (Singh, 1952), *Tetramitus* (Perty, 1852 emend. Singh and Das, 1970), *Trimastigamoeba* (Whitmore, 1911 emend. Singh and Das, 1970), *Heteramoeba* (Droop, 1962 emend. Singh and Das, 1970).

Family HARTMANNELLIDAE Volkonsky, 1931
emend. Singh, 1952

Definition

The resting nucleus has either a single Feulgen-negative nucleolus or several nucleoli. During mitosis the nucleolus, or nucleoli, disappear and a spindle with chromosomes arranged as an equatorial plate resembling that found in higher animals and plants develops. Amoebae may be uni-or multinucleate no temporary flagella have been observed.

Type genus *Hartmannella* Alexeieff, 1912
emend. Singh, 1952

The other genera belonging to this family were *Amoeba* (Ehrenberg, 1830 emend. Singh and Das, 1970), *Pelomyxa* (Greff, 1874 emend, Singh and Das, 1970), *Dobellina* (Bishop and Tate, 1939 emend. Singh and Das, 1970).

Family ENDAMOEBIDAE Calkins, 1933
emend. Singh and Das, 1970

Definition

The resting nucleus contains a Feulgen-positive karyosome or Feulgen-positive chromatin granules. Feulgen-negative granules or Feulgen-negative nucleoli do not give rise to 'polar masses' and the nuclear membrane is always intact during mitosis. Amoebae may be uni—or multinucleate, and no temporary flagellate stage has been discovered.

Type genus *Entamoeba* Casagrandi and Barbagallo, 1895
emend. Singh and Das, 1970

The other genera included in Endamoebidae are *Endamoeba* (Leidy, 1879 emend. Singh and Das, 1970), *Hydramoeba* (Reynolds and Looper, 1928).

In the systems of classification of amoebae by Singh (1952) and Singh and Das (1970), the locomotive form and behaviour of amoebae were not considered. Recently Singh and Hanumaiah (1979) have attempted to unite Singh (1952) and Singh and Das (1970) systems of classification and the contributions made by pseudopodial school in a single scheme. They have shown, based on their own studies and of other workers, that amoebae are falling into three families based on nuclear divisional patterns, in the order Amoebida Kent, 1880.

Singh and Hanumaiah (1979) have fully admitted that the creation of the genus *Hartmannella* by Alexeieff (1912 a,b) and by Singh (1952) on nuclear division pattern has led to the inclusion in this genus of amoebae showing very diverse types of locomotive form and behaviour, as pointed out by Page (1976a). They (1979) have split the genus *Hartmannella* into many genera based on locomotive form and behaviour of amoebae and have defined the genus *Hartmannella* as follows

'The resting nucleus contains a single Feulgen-negative nucleolus. During mitosis the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is found. In active locomotion amoebae assume limax form. No temporary flagella are produced' (vide also Misra and Sharma, 1980).

In the present investigation the authors have followed the classification of Singh and Hanumaiah (1979) which in their opinion is an attempt to classify the order Amoebida Kent, 1880 on probable phylogenetic basis.

It should be mentioned that Chatton (1953) attempted to unite Schaeffer's (1926) system and contribution made by nuclear taxonomists in a single scheme. His first dichotomy was to divide the order Amoebida into two suborders on the presence or absence of flagellate stage. As pointed out before, such a division is neither justified nor is of any probable phylogenetic value.

Singh and Hanumaiah (1979) based on nuclear structure, nuclear division, locomotive form and behaviour, presence or absence of flagellate stage and other characters have added many more genera in the family Schizopyrenidae and Hartmannellidae. This system of classification, as suggested by Singh (1952), throws light on the evolution of amoebae from flagellate ancestors and not the flagellates from amoebae.

A detailed survey for the cystic, aerobic free-living soil amoebae was made covering most of the districts of West Bengal with all representative soil types (vide Mukherjee, 1974). Occasional reports (Halder, 1965, 1969 , Halder and Chakraborty, 1967 , Bhattacharya and Dey, 1978) are only available from this area with considerable amount of inadequacies. So, the necessity to have a comprehensive work on the systematics and distribution of naked soil amoebae from West Bengal was a long felt need.

While describing each species. generic criteria along with historical details have been presented followed by morphology including nuclear dividing patterns. Individual descriptions are followed by critical remarks which sometimes include the authors' comments.

A comprehensive note on 'exogenous amoebiasis' has also been added with a view that some of the members are pathogenic and are causative agents of a relatively new disease in humans, primary amoebic meningo-encephalitis (PAM).

A key for the identification of the described species has also been made. The authors thus hope that this monograph on cystic amoebae will stimulate more intensive and effective research in this field particularly in regard to their bioecological role as an important component of soil microbiota and their role as a human pathogen.

MATERIALS and METHODS

Characters of the soils of West Bengal

According to Roychaudhuri *et al.* (1963) soils of West Bengal can be broadly classified into (i) Laterite and Lateritic (Birbhum, Burdwan, Bankura, Midnapore districts), (ii) Red soil (Birbhum, Burdwan, Bankura, West Dinajpur, Midnapore), (iii) Ganga alluvial (Murshidabad, Nadia, 24 Parganas, Burdwan, Hooghly, Midnapore), (iv) Vindhya alluvial (Birbhum, Burdwan, Hooghly, Bankura, Midnapore), (v) Coastal soil (24 Parganas, Midnapore), (vi) Terrai soil (Jalpaiguri, Cooch Behar), (vii) Tea soil (Darjeeling).

Mukherjee (1974) has classifield the soils of West Bengal into eight distinct groups. Towards the southern part of the state, the maximum area is covered either by Ganga alluvium or Vindhya alluvium, while it is mainly Terrai and Teesta alluvium in the northern part of the state. In some districts of the state e.g., Purulia, Bankura, Birbhum and Midnapore, laterite, red and gravelly groups of soils are found. The soils of the state is either acidic or neutral. Acid soil prevails in the districts of Darjeeling, Jalpaiguri, Burdwan, Midnapore, Bankura, Purulia and Birbhum while the soil is mostly neutral in Murshidabad, Malda and Howrah districts.

The classification of the soils of West Bengal according to Mukherjee (1974) is given in Table 1

Soils examined

The soils used for the survey of aerobic free-living amoebae from different localities of West Bengal are given in Table 2.

Method of Soil sampling Soil samples were taken with the help of a sterile auger from a depth of 50-100 mm from different places in a field and thoroughly mixed to form a sample. It was passed through a 3 mm sieve to remove stones or other big objects. Soil temperature was noted by inserting a soil thermometer. The instruments for collection of soil were sterilised by autoclaving at under 15 lb pressure for 15 minutes. Soils were packed in polythene bags and kept at room temperature. Usually fresh soils were examined for the presence of amoeba.

Isolation and clonal culture of amoebae The method followed was the same as described by Singh (1955, 1975) and Singh and Hanumaiah (1979). About 10-20 ml of sterile non-nutrient Difco Bacto Agar (1.5% in distilled water , pH 6.6-6.8) was poured into a sterile Petri dish (diameter 9-10 cm). Young cultures of *Escherichia coli* (1-3 days old), growing on nutrient agar slopes, was sprayed. Pure line culture of *E. coli* was sprayed as a thick circular patch in the middle portion of the agar surface of about 2.0-2.5 cm in diameter (Bacterial circle). Small fragment of a soil sample was put in the centre of a bacterial circle and the plates were incubated at 25°C and at 30°C. The amoebae coming from soil were allowed to grow for a week or more till most of them form cysts.

For getting clonal cultures of amoebae from mixed populations of different types of amoebae growing on non-nutrient agar plate, the following method was used Cysts were put in sterile distilled water in cavity slides and diluted with water in cavity slides so that there were about 40-50 cysts in a cavity slide. Single cyst was picked up by micropipette under low power of a microscope and transferred to a bacterial circle on non-nutrient agar. The plates were incubated at 25°C and 30°C. The amoebae growing from single cyst were maintained with *E. coli* at 25°C for further study. Culbertson *et al.* (1968), Butt *et al.* (1968), Carter (1970), Singh and Das (1970) found that *Naegleria aerobia* (*N. fowleri*) from fatal human cases of primary amoebic meningo-encephalitis did not grow on non-nutrient agar containing 0.5% NaCl. Therefore, non-nutrient agar without antibiotics were used in this work for the isolation and culture of amoebae.

For monobacterial cultures of amoebae, cysts were treated with 2% w/v of HCl for 24 hours and thoroughly washed with sterile distilled water under low speed of centrifugation. They were then placed on *E. coli* bacterial circle. These amoebae excysted and multiplied vigorously.

Locomotive form of amoebae Locomotive form of amoebae were studied in hanging drop preparations at room temperature. Usually the method applied was same as suggested by Page (1967a, 1977) and Singh and Hanumaiah (1979). Active, young (12-36 hours old) cultures of amoebae on non-nutrient agar growing with *E. coli* were suspended in sterile distilled water in a centrifuge tube. The clumps were broken by drawing the water in a pipette and then gently blowing it back. This process was repeated several times and the amoebae were washed with sterile distilled water 3 to 5 times to remove as many bacteria as possible. Now the amoebae were suspended in 2-3 ml of water and hanging drop preparations were made. After an hour or more the locomotive form and behaviour of amoebae were observed under oil immersion lens and also under phase-contrast microscope.

Excystment Healthy mature cysts of 2-7 days old were centrifuged twice or thrice in sterile distilled water and were suspended in water containing *E. coli*. Hanging drop preparation was made and the slides were kept in moist chambers at 25°C. In presence of bacteria the cysts were excysted within 5-14 hours.

Flagellate transformation in amoebae In order to influence the flagellate transformation of amoebae most of the bacteria were eliminated by repeated washing, centrifugation of amoebae, as described earlier. Then hanging drop preparations were made in distilled water and the slides were kept in moist chamber in petri dishes. Flagellate transformation was observed during 24 hours at room temperature.

To study the morphological details of the flagellates they were put in a 10% formalin solution containing 0.1% neutral red (Singh and Hanumaiah, 1979). This method clearly demostrated the flagella and the basal body.

Measurement of the length of amoebae The length of amoebae in μm was measured when they were nicely stretched out (Table 3).

Measurement of cysts of amoebae The cysts were examined in the living condition under an oil immersion lens by putting a No. 1 coverslip on the culture of cyst on the agar and studied by using both light and phase contrast microscopy. The sizes were measured in μm (see Table 4).

Rate of locomotion of amoebae Rate of locomotion of different amoebae per minute was measured in μm.

Nuclear division in amoebae The method used for the study of nuclear division in amoebae was according to Singh and Hanumaiah (1979). Amoebae were subcultured 3-4 times every 24-48 hours. One or two drops of hot melted 1.5% agar, pH 6.6-6.8, was spread on a microscopic glass slide as a thin film by quickly covering with a No.1 17 mm coverslip. As soon as the agar had solidified, the coverslip was gently pushed from one side and removed from the slide with the help of a fine tipped sterile needle, leaving the film on the slide. The excess agar that had come out of the coverslip was cut off. About 5 mm square of the agar from the centre of film was removed, leaving a cavity in the agar. Large number of young and actively dividing amoebae from the agar plate was suspended in small quantity of distilled water containing freshly prepared *E. coli*. Then one or two drops of the suspension of amoebae were introduced under the film through the cavity by gently lifting the film. The slides were kept in moist chamber in petri dishes and the plates were incubated at 25°C. Every precaution was taken to avoid contamination of the films. Usually four to eight cultures were made from each strain of an amoeba and films were fixed within 15 hours at different intervals in Carnoy's fixative (glacial acetic acid one part, absolute alcohol six parts and chloroform three parts). The slides were placed in the fixative for a minute with the film surface downwards and then they were gently turned over

Amoebae were fixed in the fixative for 30-40 minutes and then put in 90% ethanol for 24 hours. The iron-alum haematoxylin was used for studying the nuclear division in amoebae. Slides, after the removal of film of agar, were placed in 4% iron-alum for 5-6 hours, washed quickly in distilled water and transferred to 0.4% haematoxylin in water. They were left in the stain for 16-18 hours, differentiated in 1.0% iron-alum, washed in distilled water and put in aqueous 0.5% lithium carbonate for 1 minute. The slides were then put under running tap water for 30 minutes, dehydrated in ethanol, cleared in xylol and mounted in DPX.

General Organization

A) LIFE CYCLE

For amoebae which do not experience a flagellate stage at any time, the life cycle is

Amoeba⇄Cyst

A simple alteration between a trophic or feeding amoeba and a dormant cyst.

An added dimension is found in the amoebo-flagellate life cycle

Flagellate⇌Amoeba⇌Cyst

With the flagellate stage capable of feeding and dividing as in *Tetramitus* (Rafalko, 1951), or only dividing as in *Tetramastigamoeba* (Singh and Hanumaiah, 1977a), or neither feeding or dividing as in *Naegleria* (Page, 1967a Rafalko, 1977).

Thus the presence of these life cycles with their alternative stages permits critical analysis of events involved in cellular differentiation.

B) MORPHOLOGY

I. THE TROPHOZOITE

The amoeboid stage is the sole reproductive and feeding phase of the life cycle. Examination reveals a disappointing sameness in regard to the various types of organelles present and the general organization of cyto—and nucleoplasm.

a. *Nuclear and cytoplasmic morphology*

The vesicular nucleus is the most readily visible structure present in the amoeba with large Feulgen-negative central nucleolus. A typical nuclear envelope is present. Cytoplasm tends to have a sparse appearance with minimal amounts of endoplasmic reticulum. A Golgi complex may or may not be present. Mitochondria represent another major type of organelle which show subtle differences in the various genera of amoebae.

Located at the uroid (posterior or tail region) is the contractile vacuole complex, usually characterized by several small vacuoles which, with continued fluid uptake, fuse to form a single large vacuole immediately prior to vacuolar emptying.

Among the small amoebae pseudopods are either of the lobose ectoplasmic variety or of the tapering hyaline type. Lobose pseudopods are typical of the limacine amoebae *(Hartmannella, Naegleria,* and *Tetramitus),* whereas tapering pseudopods are found in *Acanthamoeba* and are generally referred as acanthopodia (Page, 1967b).

b. *Phagocytosis and pinocytosis*

Food vacuoles are present in all amoebae, whether grown on agar with bacteria as a food source or axenically in a nonparticulate fluid medium (Ryter and Bowers, 1976). This is presumably a major pathway for uptake of nutrients into the organism and Weisman and Korn (1967) noted that *Acanthamoeba* exhibits remarkable selectivity in particle digestion. Although there are no specialized organelles, food intake in limacine amoebae appear to be restricted to the uroid region. Pinocytotic activity and associated turnover have been followed in *Acanthamoeba* using various tracer molecules (Chlapowski and Band, 1971 , Bowers and Olszewski, 1972 , Batzri and Korn, 1975).

c. *Contractile proteins*

In amoeboid organisms nonmuscle contractile systems have an obvious role in locomotion, as well as in food, pinocytotic, and contractile vacuole formation. *Acanthamoeba* actin has been isolated and characterized by Weihing and Korn (1971), representing about 0·2% of cell protein. A myosin ATPase has also been isolated from *Acanthamoeba*, accounting for about 0·3% of cell protein (Pollard and Korn, 1973a). Maruta and Korn (1977) isolated one myosin component of 400,000 MW from *Acanthamoeba*. Pollard *et. al.* (1970) studied cytoplasmic microfilaments *in situ* and purified F-actin from *Acanthamoeba*, subsequently Pollard and Korn (1973) observed this F-actin filaments attached to the inner face of the *Acanthamoeba* plasmamembrane. Their model has myosin crosslinking neighbouring F-actin membrane filaments which, in the presence of co-factor and ATP, produce a sliding motion. Superprecipitation of an actomyosin protein complex from *N. gruberi* has also been made possible (Lastovica and Dingle, 1971).

d. *Plasma membrane*

This membrane encloses the cytoplasm. Dykstra aed Aldrich (1978) have demonstrated cell coats in *Acanthamoeba* and *Naegleria*, which differ from the mucoid glycocalyx, typical of some of the large amoebae.

e. *Osmotic and ionic balance*

The contractile vacuole present in virtually all amoebae isolated from soil and freshwater, is generally assumed to have an osmoregulatory, nonexcretory function (Pal, 1972). Like most freshwater organisms, the small amobae are faced with two problems (1) uptake of water by osmosis from a dilute environment, and (2) loss of internal salts by diffusion. Isolated *Acanthamoeba* mitochondria are tolerant of low tonicity for prolonged periods of time (Klein and Neff, 1960). Volume changes in the mitochondria are due to the colloidal swelling or shrinkage and the former leads to increased respiratory activity making energy available for water or ion pumping. According to Klein (1961), *Acanthamoeba* maintains intracellular K and Na levels by cation binding. Osmotic and ionic balance have an important role in formation of the flagellate stage of amoeboflagellates.

f. *The amoebic genome*

The DNA composition of the amoebae has been of some value, in conjunction with morphological and physiological characteristics, in understanding phylogenetic relationships between genera and species. Fulton (1970) reported that strain NEG of *N. gruberi* is haploid and that another strain, NB-1, is diploid. The DNA content is 0·17 and 0·32 pg/amoeba, respectively. Ploidy characterizations are based on cell and nuclear volume, DNA content, patterns of ploidal inheritance, and ease in producing mutants. Evidence for sexual cycles in the non-amoebo-flagellate soil amoebae is generally lacking.

II. *THE CYST*

The environment of an organism living in the soil, is one of widely fluctuating growth conditions cycling from dry to wet, and back to dry, and an abundant bacterial food supply followed by food scarcity. Survival of soil amoebae requires some means by which they can pass from one period of favourable growth conditions to the next. This may simply be overcome by these organisms with the formation of a thick-walled cyst or dormant stage. This is one aspect of differentiation and is a necessary prerequisite for existence. Cyst also form in fluid media, so drying alone is not a trigger for encystment. Other factors influencing encystment are pH changes, waste accumulation, O_2 deficiency, and crowding of cells in culture Cysts exist in many shapes and sizes, with single or double walls, and with or without pores. The precystic amobae generally round up and exhibit a concomitant increase in cytoplasmic density, owing probably to loss of water from the cytoplasm. Wall material, produced in the cytoplasm, deposits uniformly about the plasma-membrane of the rounded amoeba. The build up of wall material increases with the age of the cyst, leading to thickening of the wall. In forms with a double wall, the outer component is the thinner of the two and often erodes as the cyst ages. The inner and outer walls are termed the endo-and ectocyst wall respectively. In forms with walls containing pores, the later become filled with an electron-lucent mucoid plug that effectively seals them. As encystment progresses, the cytoplasm of the amoeba increases in density. Food vacuoles are in most cases eliminated prior to formation of the wall.

Cyst wall composition

Cellulose component represent approximately one-third of the dry weight of the cyst wall while protein constituted another third. These two components are separated spatially and temporally from one another as the proteinaceous outer cyst wall (synthesized early in encystment) and the cellulose inner cyst wall (synthesized late in encystment). There is one lipoprotein component that may be bound to the cellulose inner wall thereby playing role in the control of cyst wall permeability.

g. *Encystment*

The organism of choice for studies in this area has been *Acanthamoeba*. Encystment media vary in specifics but are similar in the absence of a carbon or nitrogen source and the presence of divalent cations (Ca and/or Mg), otherwise they may or may not be buffered, their osmolarities vary and additional ions may be present. Three phases have been recognized leading to formation of the mature cyst (1) induction, characterized by rounding of amoebae, (2) synthesis of the protein and cellulose components of the cyst wall, and (3) dormancy, with reduced metabolic activity (Neff and Neff, 1969). Transmission electron microscopy has been used by Bowers and Korn (1969) to describe changes accompanying encystment of *Acanthamoeba*. Studies of Bauer (1967) have shown that wall formation starts at the time the amoeba begins to round up, with the laminar ectocyst depositing as a fibrous layer about 0·5 μm thick. The fibrogranular endocyst forms inside this, the two layers separated by a space. Ostioles are circular openings about 1 μm in diameter present in both walls, and are used as exits at excystment. The ostiole is sealed by an operculum or lid which resembles the two wall components. Within the cytoplasm, there is an increase in density, budding of vesicles from the Golgi complex, formation of acid phosphatase-positive autolysosomes, development of intracristal concretions within the mitochondria, localization of cytoplasmic microfilaments in the hyaline cortical region and reduction in both nuclear and nucleolar volume. Chemical analyses reveal an increase in neutral lipid and reduction in phospholipid and glycogen. At the time the cells are rounding, a decrease in intracellular actin is detected (Rubin and Maher, 1976). Neff and Neff (1969) detected phosphoprotein as diffused material at the surface of the rounded trophozoite of *Acanthamoeba*. Werth and Kahn (1967) identified cellulose in the *Naegleria* cyst wall and Schuster and Svilha (1968) demonstrated the presence of ribonucleo protein vesicles in the cytoplasm of encysting *Naegleria*. In another soil amoeba *Schizopyrenus russelli (Vahlkampfia russelli)* Maitra *et. al.* (1974) found a tripartite wall consisting of an inner electron-dense fibrillar layer, an outer electron-dense granular layer and between them an electron-lucent granular region or layer.

Concerning encystment of *Acanthamoeba*, Neff and Neff (1969) have generalized that factors controlling the phenomenon (1) is initiated by depletion (or absence) of some factor in the medium, (2) occurs in the absence of an exogenous energy supply and/or metabolites, and (3) is an example of single-cell differentiation. Mitomycin C (and trenimon) may induce encystment through an alteration in chromatin conformation, activating genes responsible for the initiation of dormancy.

Chagla and Griffiths (1974) noted an overall increase in DNA content in mature cysts and a rise in the proportion of binucleate amoebae. An increase in specific activity of DNA-dependent RNA polymerase was observed by Rudick and Weisman (1973), following induction of encystment in *Acanthamoeba*. This period of increase overlapped with a period of maximal uptake of ^{3}H uridine. The two events may be related to the synthesis of protein needed for cyst maturation. Rubin *et. al.* (1976) while detecting the increment of some of the acidic nucleolar proteins, and decrement or disappearance of others, postulated that these alterations may be related to gene regulatory functions, particularly coding for RNA.

Encysting *A. castellanii* exhibits elevated oxygen consumption correlated with an increased energy requirement. There is an increase in the acid phosphatase activity of encysting *Acanthamoeba* spp. (Lasman, 1967 Band and Mohrlok, 1969 Griffiths and Bowen, 1969). Activity of this lysosomal enzyme may have significance in regard to the degradative changes occurring at encystment, particularly the turnover of reserves in the synthesis of cyst wall materials. There is also an increase in the peroxisomal enzyme catalase in encysting population of amoebae (Band, 1959).

Encystment of *A. castellanii* was induced on a non-nutrient agar containing Mg and the biogenic amine taurine (Raizada and Krishna Murti, 1971). Magnesium ion and taurine are assumed to stimulate cAMP synthesis by activation of adenyl cyclase. Thus, encystment is regulated by the relative concentration of cAMP and phosphodiesterase, which is responsible for its conversion to noncyclic AMP. cAMP is believed to control the synthesis of cyst wall material from polysaccharide reserves. According to Steward and Weisman (1974) Golgi vesicles might contribute protein or oligosaccharide components to the developing wall.

Excystment

Excystment of amoebae can be triggered by a variety of factors. In some species, suspension of cysts in distilled water is sufficient to resumption of vegetative activity, and the stimulus is probably osmotic. Cysts of other species may respond to organic substances in the excystment medium, provided as bacterial food organisms, bacterial extracts, sterile complete growth medium, or component parts of the medium.

Chambers and Thompson (1972) noted that after transfer to growth medium, the process of excystment of *A. castellanii* began with the projection of a 'bud' through a pore or ostiole in the cyst wall. The operculum, which formed over the ostiole at excystment, was apparently digested prior to emergence, though other aspects of the cyst wall remain unchanged. Schuster (1963, 1975) found that *Naegleria* spp. apparently digest the mucoid plug sealing the cyst pore and then proceed to squeeze through the small hole. It may be noted that excysting *N. gruberi* leaves the cyst wall unaltered, though *N. fowleri* apparently digests some of the wall during the process. Induction of excystment in *Naegleria* has also been brought about by high hydrostatic pressure (Todd and Kitching, 1973). Averner and Fulton (1966) reported that CO_2 serves as a signal for activation of the dormant amoeba but the nature of the CO_2 sensitive trigger in the cyst is unknown. In activated cysts, a doubling of DNA occurred, but no increase in numbers of organisms RNA and protein also increased. Chambers and Thompson (1974) reported an obligatory period of aging of cysts of *A. castellanii* before amoebae excysted in significant numbers. Rastogi *et al.* (1973) reported that an amino acid mixture plus riboflavin was effective in promoting excystment of *S. russelli* and pH of the medium may also influence excystment Excystment in *A. culbertsoni* was initiated by peptone, tryptone and amino acids (Kaushal and Shukla, 1977).

III. *AMOEBOFLAGELLATE*

Amoeboflagellates have another patterns of differentiation, transformation from an amoeba to a flagellate. The flagellate is a dynamic, metabolically active state of the life cycle. The ease with which flagellation can be produced *in vitro* makes this an ideal system for the analysis of morphological and biochemical events leading to induction of the flagellate phenotype and synthesis of the flagellar apparatus.

KEY TO THE IDENTIFICATION OF FAMILIES, GENERA AND SPECIES OF SOIL AMOEBAE RECORDED FROM WEST BENGAL, INDIA.

KEY TO THE FAMILIES

Nuclear membrane persists throughout mitosis during which nucleolus/nucleoli form (s) 'polar masses' ; temporary flagellate stage may be found

Family Schizopyrenidae

Nuclear membrane along with nucleolus/nucleoli disappears during mitosis ; a spindle with chromosomes arranged as an equatorial plate ; no temporary flagellate stage has been found. Family Hartmannellidae

KEY TO THE GENERA AND SPECIES OF THE FAMILY SCHIZOPYRENIDAE

1. Feulgen-negative nucleolus dividing during mitosis to form 'polar masses' and interzonal bodies. Temporary flagella produced. 2
   Feulgen-negative nucleous dividing during mitosis to form only 'polar masses' 5
2. 'Polar masses' and 'interzonal bodies' present during division both in the amoeboid and flagellate stage ; four flagella present in the flagellate stage Genus *Tetramitus* 3
   'Polar masses' and 'interzonal bodies' present during division only in the amoeboid stage ; two flagella present in the flagellate stage ; the latter does not divide Genus *Naegleria* 4
3. Cysts single walled with an outer gelatinous layer ; fronto-lateral eruption of podia during active locomotion *Tetramitus rostratus.*
4. Cysts round or slightly oval having single refractile wall ; irregular podial bulging during active locomotion *Naegleria gruberi.*
5. No temporary flagellate stage produced Genus *Schizopyrenus* ... 6
   Temporary flagellate stage produced Genus *Didascalus* ... 7
6. Cysts spherical with two walls ; fronto-lateral podial bulging during active locomotion *Schizopyrenus russelli*
7. Cysts usually round with a single wall ; an outer thick transparent gelatinous layer ; fronto-lateral eruption of podia during active locomotion. *Didascalus thorntoni.*

KEY TO THE GENERA AND SPECIES OF THE FAMILY HARTMANNELLIDAE

1. Amoebae assume limax form during active locomotion Genus *Hartmannella* 2
   Amoebae have broad anterior hyaline lobopodium from which projection(s) (acanthopodium) appear(s) during active locomotion Genus *Acanthamoeba* 3
   Amoebae usually flattened ; anterior hyaline zone with finely pointed pseudopodia or microspines during active locomotion Genus *Echinamoeba* ... 4
   Amoebae fan-shaped or ovoid with flattened ectoplasmic hyaline veil during active locomotion ; breadth usually greater than the length Genus *Vannella* 5

2. Cysts double walled ; outer one being irregular *Hartmannella vermiformis.*
Cysts single walled with an outer gelatinous layer *Hartmannella crumpae.*
3. Cysts double walled ; both being generally irregular in outline giving a wrinkled appearance *Acanthamoeba rhysodes.*
Cysts double walled the ectocyst being uniformly irregular *Acanthamoeba culbertsoni.*
Cysts appearing single walled *Acanthamoeba glebae.*
Cysts double walled the endocyst being star shaped *Acanthamoeba astronyxis.*
Cysts double walled ; the ectocyst being fairly thick, very irregular and wrinkled *Acanthamoeba palestinensis.*
4. Cysts single walled circular or spherical with an outer gelatinous layer *Echinamoeba exundans.*
5. Cysts single walled ; spherical with an outer gelatinous layer *Vannella cutleri.*

OBSERVATIONS

Family SCHIZOPYRENIDAE Singh, 1952
emend. Singh and Das, 1970

Genus **Schizopyrenus** Singh, 1952

Definition .

Feulgen-negative nucleolus dividing during mitosis to form 'polar masses' Temporary flagella are not produced. The amoebae have limax locomotive form.

Schizopyrenus was selected as the type genus because amoebae included in it do not produce temporary flagella, and it is possible that the presence of temporary flagella may be considered by some authorities to exclude these forms from true amoebae.

Schizopyrenus russelli Singh

1. 1952. *Schizopyremes russelli* Singh, Phil. Trans. Roy. Soc. Lond., B **236** : 405.

The amoeba was first isolated by Singh, 1952 from Barnfield farmyard manured field soil and Broadbalk unmanured soil at Rothamsted Experimental Station, England. It was a very common amoeba in these soils. This amoeba has also been isolated by Singh and Hanumaiah (1979) from Central Drug Research Institute, Lucknow, garden soil, from Gomti river mud, from Chinhat lake mud, from sewage sludge and from garden soil of Baroda and Bombay. It was very common in these soils. In the present work *S. russelli* has been isolated from Midnapore district, West Bengal. The locality was Jhau forest' area, Digha. The soil was saline in nature. The collection time of soil was 1 8. 79 and the recorded temperature during collection was 30°C (Table 2). These observations show that *S. russelli* may be commonly present in different parts of the world.

Morphology

The length of amoebae in active locomotion has a range of 16.8 to 28.0μm, the mean length is 23.67 μm with standard error of mean ± 0.83 (Table 3). Type of locomotion is of limax type in a monopodial fashion. During change of direction which is usually followed through fronto-lateral bulging, the amoebae moved laterally .by lateral eruption. Occasionally they used reverse direction by 90% During reversal of direction a new hyaline pseudopodium is produced, which is limax in nature. The apex of the podium is generally hemispherical, sometimes circular with slight undulation on their terminal border. During active movement the amoeba moves 112 to 130 μm per minute, On careful observation no uroidal filaments are seen. The cytoplasm is granular with two types ol refractile granules, circular and cylindrical (Figs. 1 to 3). These granules are moving with the movements of amoebae. Well defined ectoplasm and endoplasm.

Vesicular nucleus with a central nucleolus distinctly present at the middle or posterior end of the amoebae (Figs. 1 to 3). The diameter of the nucleus has a range of 1·4 to 2.2 μm, mean diameter is 1.7μm with standard error of mean ± 0.66 (Table 3). Contractile vacuoles are 2 or 3 in number. occasionally one (Figs. 1 to 3). The diameter of the contractile vacuole has a range of 1.4 to 2.2 μm, mean 1.96μm, with standard error ± 0.12 (Table 3).

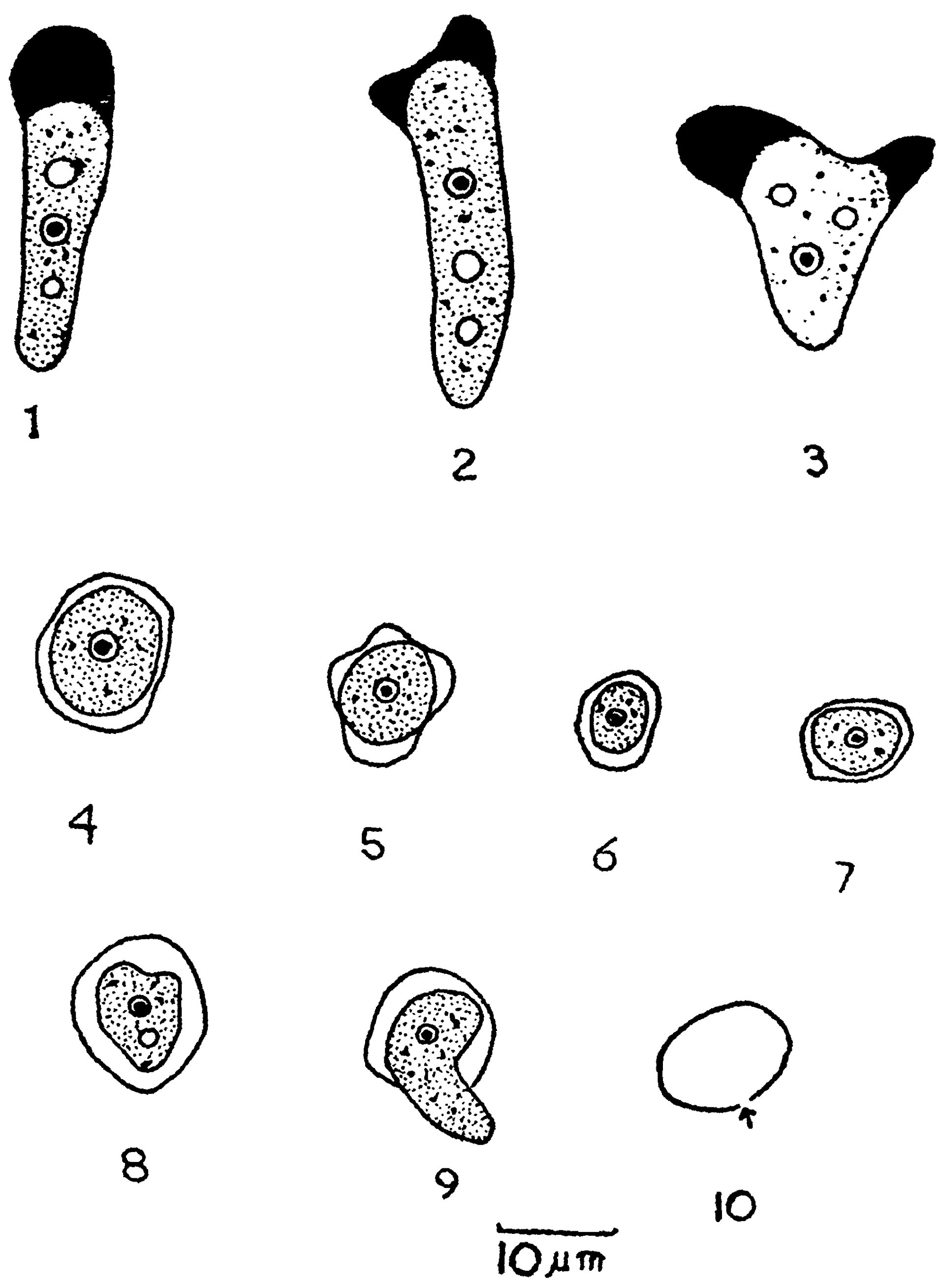
The living cysts are usually spherical in shape, and are variable in size (Figs. 4 to 7 and Pl. 1 Figs. 1 and 2). The diameter of the cyst has a range of 7.0 to 11.2 μm with a mean diameter 9.52μm and standard error of mean is ± 0.50 (Table 4). Each cyst consists of two walls (Pl. 1 Fig. 2). The endocyst is much thicker than ectocyst and looked dark coloured. Sometimes ectocyst has wavy irregular outline. There is a single nucleus having mean diameter, 0.7μm.

During excystment the endocystic wall is first dissolved and the amoeba begins to move within ectocystic wall. The amoeba comes out probably by digestion of cyst wall (Figs. 8 to 10).

Repeated efforts to produce a temporary flagellate stage have completely been failed.

The resting nucleus

In the living condition the nucleus is vesicular with a single nucleolus (Figs. 1 to 3). In Iron-alum haematoxylin preparations chromatic granules are seen occupying the inner periphery of the nuclear membrane (Fig. 11).



Camera lucida drawings of *Schizopyrenus russelli*.

Figs. 1 to 10 : Drawn in the living condition.
Figs. 1 to 3 : Trophic forms.
Figs. 4 to 7 : Cysts.
Figs. 8 to 10 : Excystment stages.

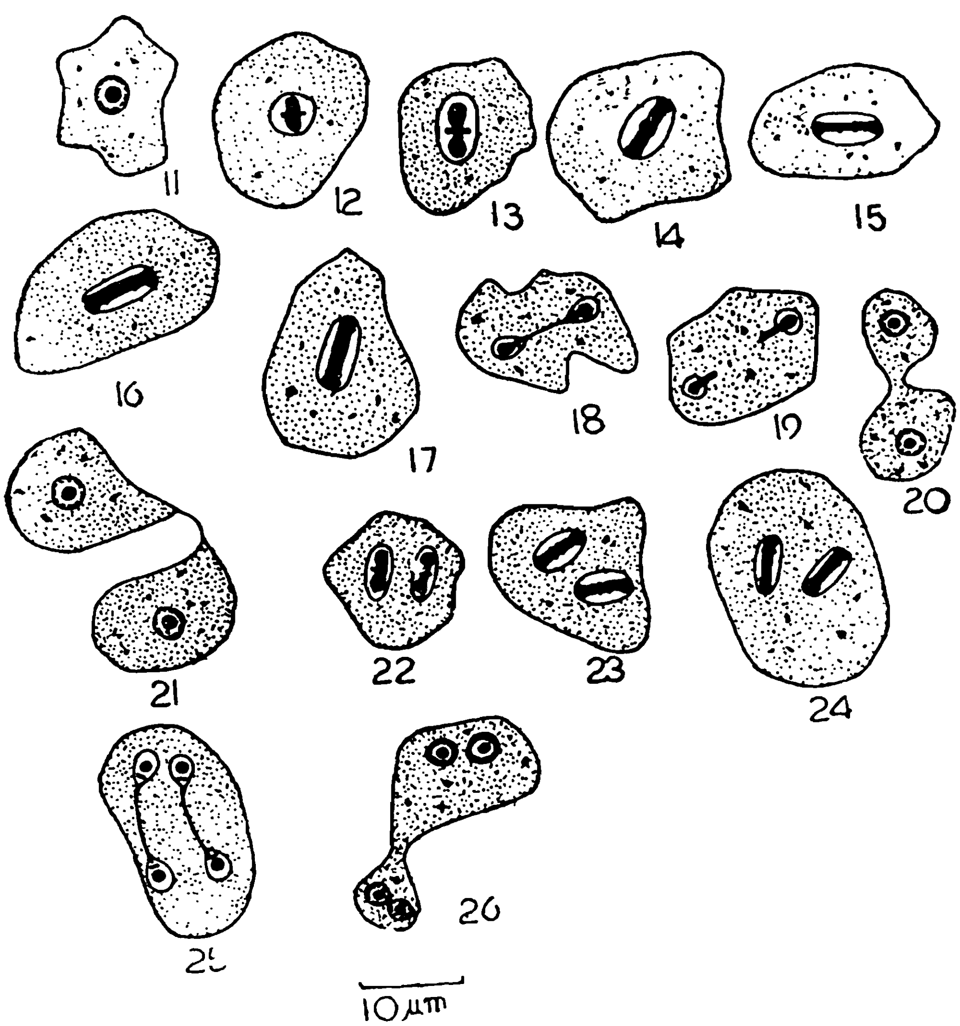
Mitotic division

Prophase

Amoebae do not become rounded during nuclear division. Nuclear division is initiated by the elongation of the nucleolus. Chromatic granules move from their position and lie on or near the nucleolus (Fig. 2). Gradually the nucleolus becomes dumble shaped and ultimately formed two distinct 'polar masses' (Fig. 13).

Metaphase

During this stage the chromatic granules are clearly distinguished at the equatorial region as a band like structure with the formation of connecting spindle from the two 'polar masses' the chromosome number and the spindle fibre could not be clearly distinguished (Fig. 14).



Figs. 11 to 26 : Fixed in Carnoy and stained with Iron-alum haematoxylin.
Fig. 11 : Ordinary individual and the structure of the resting nucleus.
Figs. 12 to 26 : Successive stages in division.

Anaphase

The band of chromatic materials divides into two equal halves. One half moves towards one pole and the other towards the other pole (Figs. 15 to 17). The nuclear membrane persists throughout division. It becomes elongated and constricts into two, giving rise to two daughter nuclei (Figs. 18 and 19).

Telophase

After the division of the nucleus, the two bands of chromatic material, after reaching the two 'polar masses', get mixed with the latter. Later on they break up into granules and occupy the position seen in the resting nucleus (Figs. 20 and 21). The 'polar masses' become nucleoli in the tho daughter amoebae. The amoebae later on elongates and constricts in the middle to give rise to two daughter individuals (Figs. 20 and 21).

Nuclear division of amoebae having two nuclei

The amoebae having two nuclei seem to be healthy and their nuclear division takes place normally. Both the nuclei divide at the same time (Figs. 22 to 26).

Critical comment

Singh (1952) created the genus *Schizopyrenus* in the family Schizopyrenidae for amoebae having 'polar masses' during nuclear division but having no temporary flagella. Page (1967a) has described *V. jugosa* n. sp, as having double-walled cysts, frequently with hilly or mound-like appearanc of the ectocyst. Singh and Das (1970) found that on morphological, cystic characters and mode of nuclear division and excystment, as described by Page (1967a), *V. jugosa* is exactly similar to *S. russelli*, though movement of the amoebae within the ectocyst is not clear as in that species. They have also stated that under similar cultural conditions and with the same food supply, *V jugosa* is smaller than *S. russelli*. The presence or absence of uroidal filaments, which is generally present in amoebae, has no diagnostic value as stated by Kudo (1959) and Singh and Das (1970). The authors fail to describe any uroidal filament in *S. russelli*. Moreover, Singh and Das (1970) suggested that in accordance with the system proposed by Singh (1952) *V. jugosa* should be transferred to the genus *Schizopyrenus*.

The amoeba originally described (1967a) as *Vahlkampfia jugosa*, has been found by Darbyshire *et al.* (1976) to produce flagellate stage.

and now it has been placed in a new genus *Paratetramitus* (*P. jugosa*). Thus it seems that the use of flagellum as a diagnostic feature in the classification of amoebae may not be always logistic and judicial.

Genus **Naegleria** Alexeieff, 1912, emend. Singh, 1952

Definition

'Polar masses' are found Feulgen-negative. 'Interzonal bodies' are present during late stages of nuclear division. Temporary flagella are produced. A flagellate stage has two flagella and no division takes place in this stage. The amoeba has limax locomotive form.

(2) **Naegleria gruberi** (Schardinger)

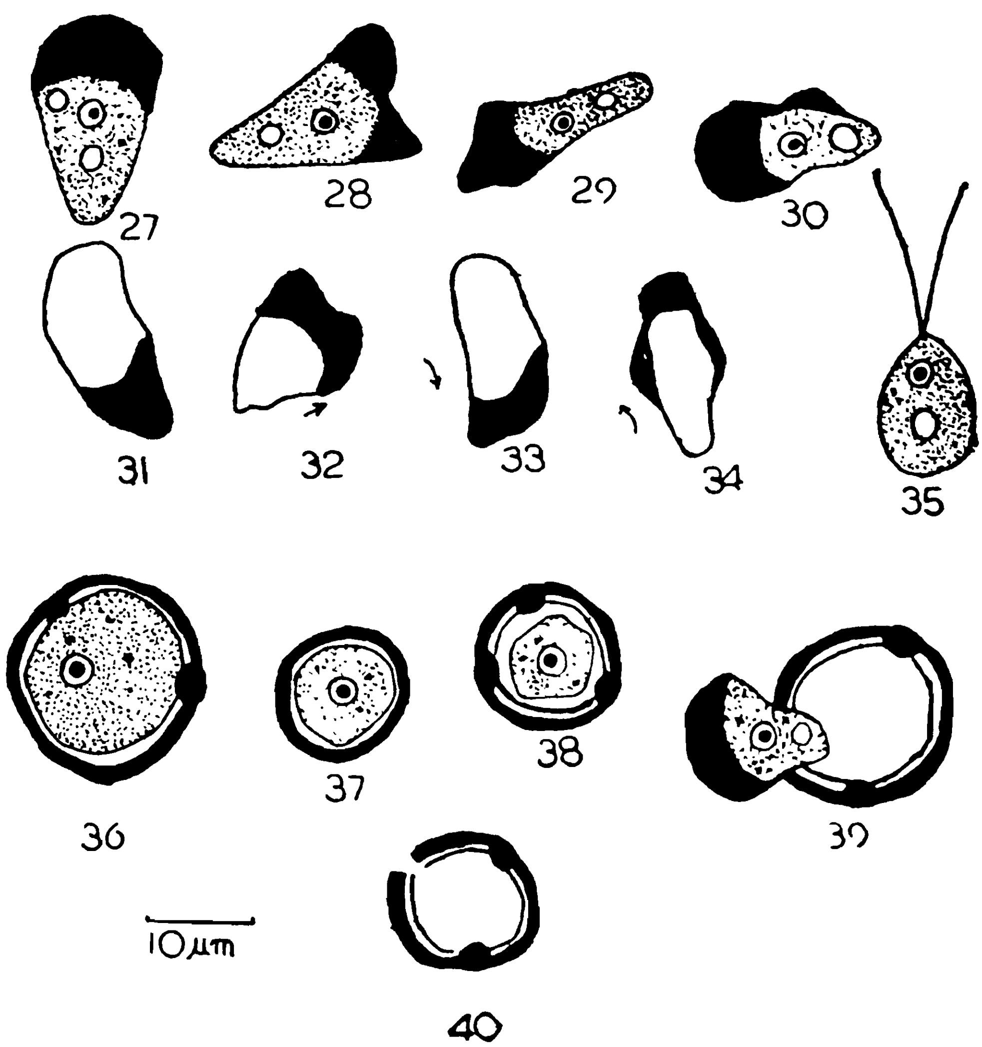
1. 1899. *Amoeba gruberi* Schardinger, S. K. Acad. Wiss. Wien., **108** : 713.

Schardinger (1899) first described this species in a diarrhoeic stool, as having amoeboid and flagellate stages. It has been recorded from many soils by Sandon (1927). Singh and Hanumaiah (1979) have isolated it from Central Drug Research Institute, Lucknow, garden soil, from Gomti river mud, from Chinhat Lake mud, from Baroda sewage sludge, from Poona and Bombay garden soils, from Mula river mud, Poona, and from Tap water in Lucknow. This is one of the most common amoebae found in different parts of the world as is evident from the reports of many workers. In the present work *N. gruberi* has been isolated from Howrah (Uluberia) Block seed farm soil, from Birbhum (Bolpur) upper Bangola paddy soil and from Raigunge (West Dinajpur) paddy soil. The collection times for the soils were 6.8.79, 11.9 80 and 12.1.81 respectively. The recorded soil temperatures varied from 28°C to 31°C (Table 2).

Morphology

Trophozoites have the range of length 9.8 to 15.4μm with mean length 13.16μm. The standard error of mean is ±0.55 (Table 3). The amoeba has at its anterior end a flat, hyaline semi circular or hemispherical pseudopodium. The posterior end of the amoebae is devoid of uroidal filament. The type of locomotion is of limax type. The change of direction is irregular and may be from any direction, usually from the antero-lateral position. During active locomotion the podial bulging is a typical diagnostic feature which usually occurs irregularly without maintaining any harmony. During locomotion in one direction generally number of pseudopodial bulgings are 1 to 4. During active movement the amoeba moves 62-122μm per minute. Cytoplasm is granular and the granules are found shifting during the movements of the amoebe (Figs. 26 to 34). Ectoplasm and endoplasm are distinct in an amoeba. It has a single vesicular nucleus and one or two contractile vacuoles. The diameter of the nucleus has a range of 1.4 to 2.2μm with a mean diameter 1.4μm. The mean diameter of the contractile vacuole is 5.6μm. Food vacuoles are very small sometime indistinguishable (Table 3).

The flagellate stage can be easily produced in *N. gruberi.* These stages have a more or less rigid oval shape and move actively in water. Each individual possesses two flagella, slightly longer than the body, arising from the anterior end. There is a single contractile vacuole and a nucleus (Fig. 35).



Camera lucida drawings of *Naegleria gruberi.*

Figs. 27 to 40 : Drawn in the living condition.

Figs. 27 to 34 : Trophic forms.

Fig. 35 : Flagellate stage.

Figs. 36 to 37 : Cysts.

Figs. 38 to 40 : Excystment stages.

The living cysts are round, very often slightly oval with variable sizes. It has a refractile wall. The cyst has clearly visible single nucleus, when observed in the living condition. The outer layer of the cyst is generally smooth but occasionally with irregular architecture. The thick inner layer is visible easily. The striking criterion of the cyst is the presence of pores plugged with some kind of a structureless substance (Figs. 36 and 38 and Pl. 1 Fig. 3). A few cysts have no pore (Fig. 37). The diameter of the cysts have the range 11.2 to 25.2μm with mean diameter 21.84μm and standard error of mean diameter is $\pm$ 1.37 (Table 4). During excystment the amoeba comes out through one of the plug after dissolving the structureless substance (Figs. 38 to 40).

The resting nucleus

The resting nucleus in the living condition consists of a distinct central spherical nucleolus. No chromatic granules could be seen (Figs. 27 to 30).

In iron-alum haematoxylin preparation the nucleolus stains deeply. Fine granular chromatic materials are observed encircling the border of the inner nuclear membrane (Fig. 41).

Mitotic division

Prophase

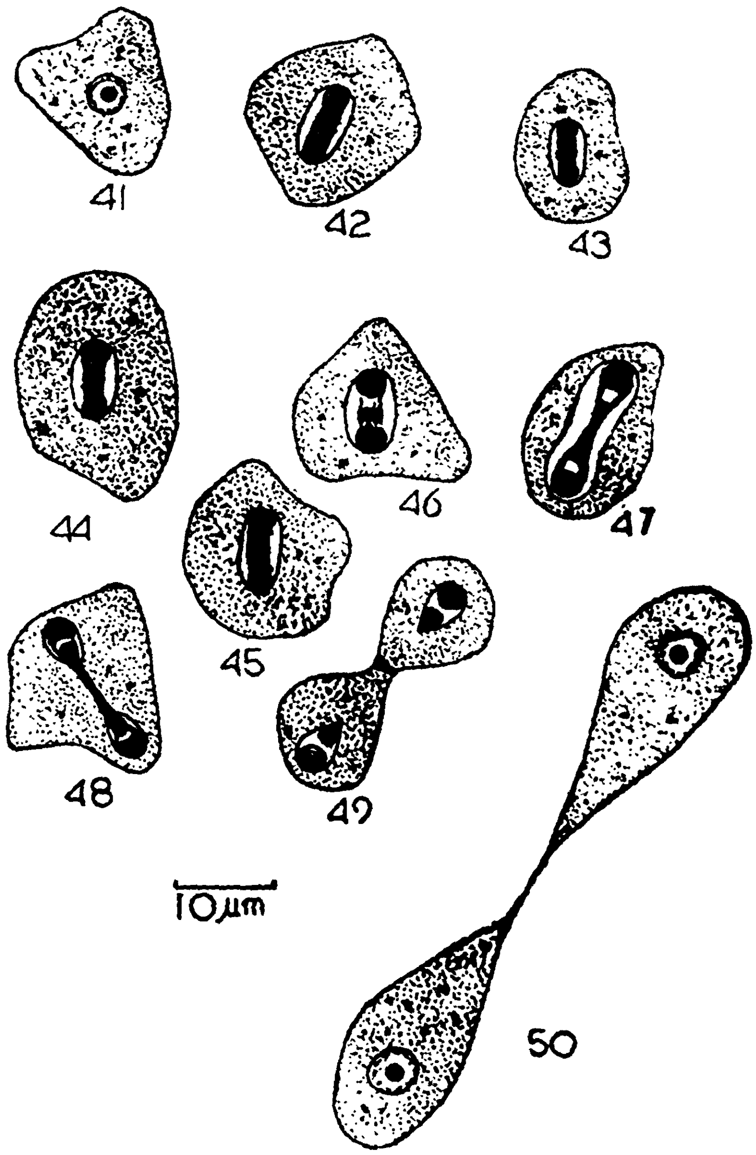
The amoebae do not become rounded or motionless during division. The beginning of the nuclear division is marked by the swelling of the nucleus and the elongation of the nucleolus. Chromatin granules lie besides the nucleolus. These granules begin to fuse and appear as a solid band surrounding the nucleolus. The nucleolus later on assumes a dum-bell-shaped appearance and divides into two equal halves to give rise to 'polar masses' (Fig. 42).

Metaphase

After the formation of the 'polar masses' a solid band of chromatin is seen occupying the position of the equatorial plate (Fig. 43). No individual chromosomes could be made out at this stage. The spindle connecting the 'polar masses' can be seen, though no distinct spindle fibres could be distinguished.

Anaphase

The band of chromatic material divides into two and each half moves towards the either pole (Figs. 44 and 45). At late anaphase stage certain granular non-chromatic substance, which Rafalko (1947) has called the 'interzonal body', can be distinctly seen lying half-way between the two polar masses (Fig. 46). The 'interzonal body' increases in size and divides into two. The nuclear membrane persists throughout division. It becomes elongated, constricts, giving rise to two daughter nuclei (Figs. 47 to 49).



Figs.	41 to 50 :	Fixed in Carnoy and stained with Iron-alum haematoxylin.
Fig.	40 :	Ordinary individual and the structure of the resting nucleus.
Figs.	42 to 50 :	Successive stages in division.

Telophase

After the nucleus has divided into two the 'interzonal bodies' are still connected for a time by a thread like structure (Fig. 48). The amoebae later on become elongated and constricts in the middle to give rise to two daughter individuals. The 'interzonal body' and the 'polar mass' of the daughter nuclei fuse together to give rise to the nucleolus. The chromatic material is broken up into granules, and these granules occupy the position as seen in the resting nuclei (Fig. 50).

Polar caps

During some of the stages of nuclear divisions, Ford (1914) described chromatic caps situated between the ends of the elongated nucleolus and the nuclear membrane in a free-living limax amoeba. This has also been reported by Rafalko (1947) and Singh (1952). Since it was very rarely found to be present in *N. gruberi* studied by the present authors, it has not been shown in the figures depicting in the nuclear division.

Critical remarks

The morphology, cystic character, excystment, nuclear structure and mode of nuclear division of *N. gruberi* has been studied by Rafalko (1947), Singh (1952), Chang (1958), Page (1967a), Sing and Das (1970) and others. The presence of 'interzonal body' during nuclear idvision in *N. gruberi*, has been clearly shown in the present work, emphasizing that the erection of the genus *Naegleria* based on the presence of 'interzonal bodies' is justified.

Studies on the nuclear division of *N. gruberi* invited lots of confusion in the past as a representative member of the free-living amoebae. Wenyon (1926) reported that *N. gruberi* (*Dimastigamoeba gruberi*) seemed to possess distinct 'interzonal bodies' although these structures, when stained with iron-alum haematoxylin, were interpreted as aggregated daughter chromosomes passing towards the two poles (vide his Fig. 5, page 105).

Genus : **Didascalus** Singh, 1952

Definition

'Polar masses' without 'interzonal bodies' are present during nuclear division. Temporary flagella are produced. The flagellate stage has two flagella and no division takes place in this stage. The amoebae have limax locomotive form.

(3) **Didascalus thorntoni** Singh

1. 1952. *Didascalus thorntoni* Singh, Phil. Trans. Roy. Soc. Lond., B **236** : 405.

Crump (1950) isolated an amoeba from Broadbalk field farmyard manured soil at Rothamsted, England and called species z. It was later studied by Singh (1952) and was named as *Didascalus thorntoni*.

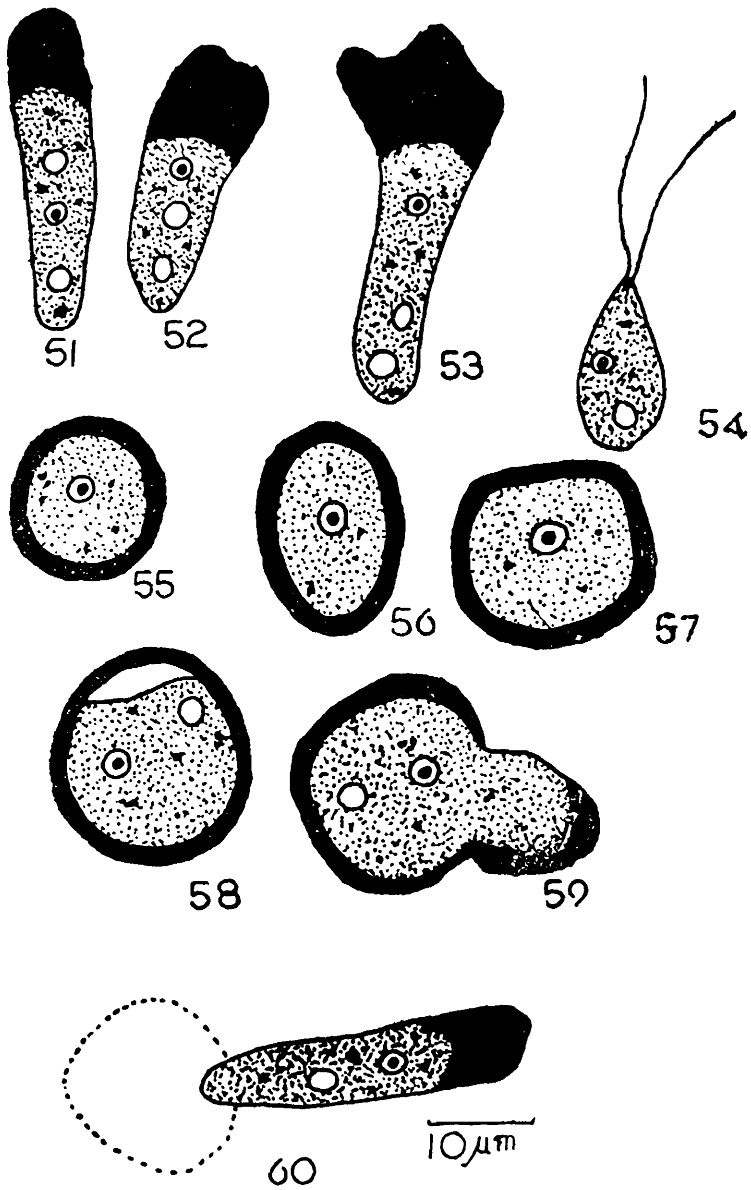
This amoeba has been isolated by Singh and Hanumaiah (1979) on two occasions, once from Central Drug research Institute garden soil and the other from Gomti river mud in Lucknow. Chang (1973) has mentioned that in an incidental examination of a small lake near the university of Cincinnati, U. S. A., he isolated *Naegleria* like amoeba that underwent sparingly flagellate transformation, even under most favourable conditions. It fails to form 'interzonal bodies' during mitosis. This was the first time that Chang (1973) found *Didascalus* during examination of waters for small free-living amoebae. In the present work *D. thorntoni* has been isolated from the soils of South 24 Parganas district. The locality was sagar Island. The collection time of the soil was 4.6.79 and the recorded soil temperature during collection was 32°C (Table 2). From the observation it appears that *D. thorntoni* is not so common amoebae as *S. russelli* and *N. gruberi*.

Morphology

D. thorntoni is a fast moving amoeba in hanging drop preparation. It's movement is typically limax-like. Anterior region has broad, hyaline, cylindrical pseudopodium, the boundary of which is smooth. The length of the amoebae in active locomotion has a range of 25.2 to 28.0μm the mean length is 26.32μm with standard error of mean $\pm$0.40 (Table 3). The change of directions if at all occurred, generally through the antero-lateral eruptions. No floating form is observed. The amoebae have a great fascination towards settling down on the substratum. Posterior end is slightly sticky, without uroidal filament. During active phase the amoebae move at a rate of 58.8 to 126μm per minute.

Endoplasm is smooth with a few refractile granules. Single spherical vesicular nucleus is present with a clear nucleolus. The diameter of the nucleus has a range of 1.4 to 2.1μm with a mean diameter 2.1μm. Sometimes the nucleus takes a cylindrical shape possibly due to the endoplasmic pressure during fast active movement. No food cup or food vacuoles could be observed. Contractile vacuoles are present, 2 to 3 in number. The diameter of the contractile vacuole has a range of 2.8 to 3.5μm with a mean diameter 3.5μm (Figs. 51 to 53).

The flagellate transformation is rare and usually occurring after 8 to 24 hours when the amoebae are suspended in distilled water. The flagellates have a more or less rigid, oval shape and move actively in water. Each individual possesses two flagella, slightly longer than the body, arising from the anterior end. There is a single contractile vacuole and nucleus (Fig.54).



Camera lucida drawings of *Didascalus thorntoni* :

Figs. 51 to 60 : Drawn in the living condition.
Figs. 51 to 53 : Trophic forms.
Fig. 54 Flagellate stage.
Figs. 55 to 57 : Cysts.
Figs. 58 to 60 : Excystment stages.

The living cysts are characteristic and variable in size. They are usually rounded having a single wall. The outside of the wall consists of a fairly thick transparent gelatinous layer (Figs. 55 to 57 and Pl. 1 Figs. 4 and 5). The diameter of the cyst has a range of 12.6 to 14.0μm with a mean 13.16μm and standard error of mean ±0.22. The mean diameter of cyst nucleus is 1.4μm (Table 4). The nucleolus inside the nucleus can be seen in some of the living cysts, although no chromatin granules can be distinguished.

During excystment the amoebae gradually detached themselves from the cyst wall and an active contractile vacuole appears. No preformed pore can be seen in the cyst, and some digestion of the cyst wall appears to occur (Fig. 59). The cysts eventually dissolve after emergence of amoebae (Fig. 60).

The resting nucleus

In the living condition the resting nucleus is round in shape with a prominent central nucleolus. During movement of the amoebae the nucleus is often seen as elongated due to the endoplasmic pressure. No chromatin granules could be observed (Figs. 51 to 53).

In iron-alum haematoxylin preparation, the nucleolus stains deeply having peripheral chromatin granules (Fig. 61)

Mitotic division

Prophase

The amoebae do not become rounded during division. The nuclear division is initiated by nucleolus becoming elongated. The chromatin granules move from their position and lie on or near the nucleolus (Fig. 62). The nucleolus becomes dum-bell-shaped, and chromatic granules begin to fuse together and lie as a band at the centre (Fig. 63). The dum-bell-shaped nucleolus divides into rwo halves known as 'polar masses'

Metaphase

After the formation of 'polar masses' the band of chromatin material can be seen occupying the position of the equatorial plate (Fig. 64). The spindle connecting the 'polar masses' can be clearly seen, although the spindle fibres could not be counted. It was impossible to count the number of chromosome.

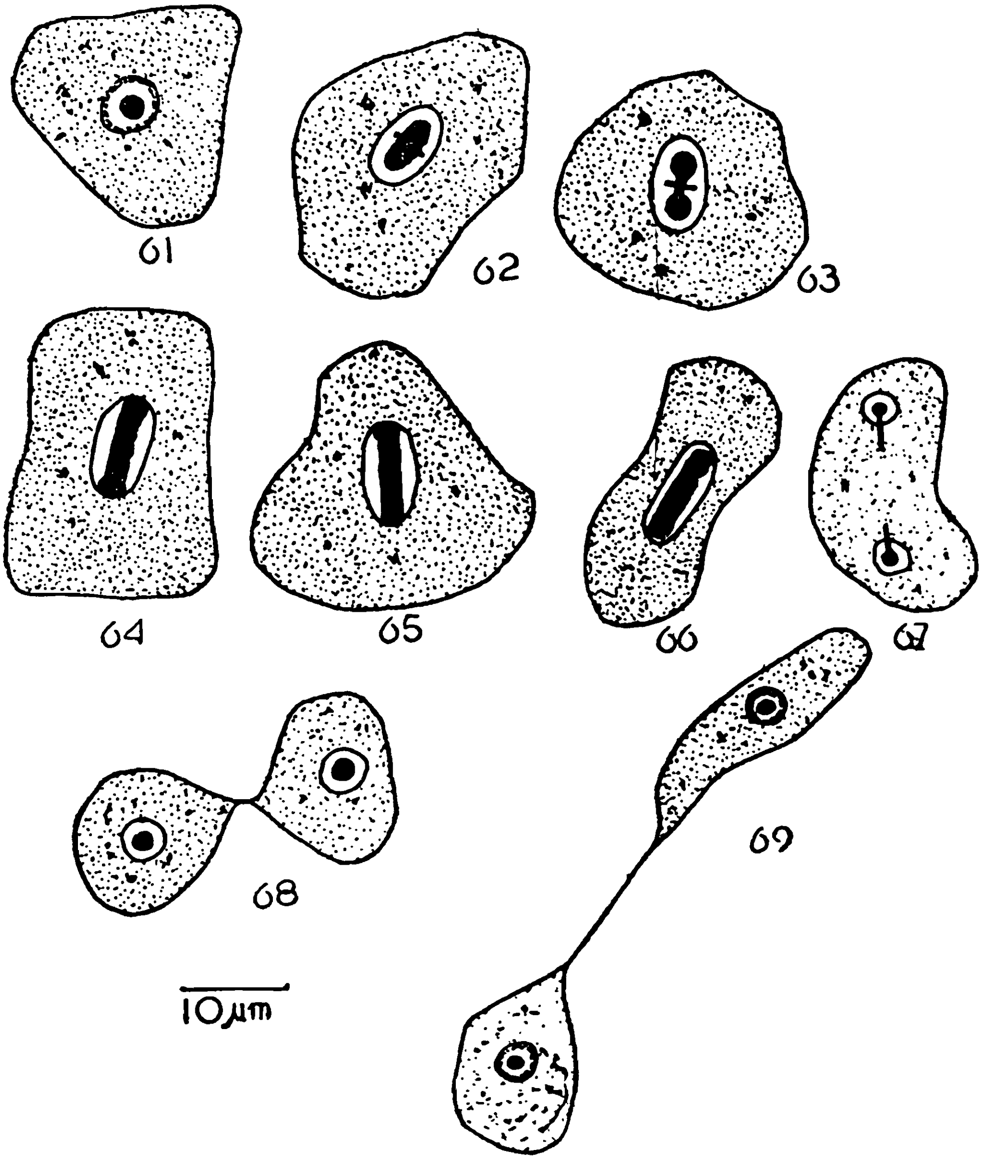
Anaphase

The band of chromatin divides into two equal halves, one half moves towards one 'polar mass' and the other half towards the other (Figs. 65 and 66).

As in *S. russelli* and *N. gruberi*, the nuclear membrane persists throughout division. It becomes elongated and constricts into two, giving rise to two daughter nuclei (Fig. 67).

Telophase

After the division of the nucleus, the two 'polar masses' remain connected by the spindle fibres which gradually become narrower and then disappear. The chromatic material breaks up into granules and occupy the position seen in the resting nuclei. The 'polar masses' become nuclei in the two daughter amoebae. The amoebae later on become elongated and constricts in the middle to give rise to two daughter individuals (Figs. 68 and 69).



Figs. 61 to 69 : Fixed in Carnoy and stained with Iron-alum haematoxylin.
Fig. 61 : Ordinary individual and structure of the resting nucleus.
Figs. 62 to 69 : Successive stages in division.

Remarks

The genus name is derived from the Greek word 'Didascalus' meaning school master—one who is prone to whip. The specific name is given in honour of Dr. H. D. Thornton. Singh (1952) contends that the only amoeba which can be placed in the genus *Didascalus* is *D. thorntoni*. Martin and Lewin (1914) described *Vahlkampfia soli* which has an outer gelatinous layer in the cyst and can produce temporary flagella. Since nuclear divisional stages of V *soli* had 'interzonal bodies' and 'polar masses', Singh (1952) concluded that Martin and Lewin's organism should be called *Naegleria soli*. The nuclear division of *S. russelli* is exactly similar to that of *D. thorntoni*. Due to the absence of 'interzonal bodies' and presence of outer gelatinous layer in the cyst, the species *Didascalus thorntoni* can be easily identified, from that of other described species of *Naegleria*.

Genus **Tetramitus** Perty, 1952, emend. Singh and Das, 1970

Definition

'Polar masses' and 'internal bodies' are present during division both in the amoeboid and flagellate stages. The flagellate stage is cone-shaped with broad anterior end having curved cytostomal groove walled in bilabial folds, which form the anterior end bent back to form a rostrum. The flagellate stage has four flagella. The amoebae have limax locomotive form.

Tetramitus rostratus Perty

1. 1852. *Tetramitus rostratus* Perty, Zur Kenntniss Kleinster Lebensformen nach Bau, Funktionen, Systematik mit speciqvarzeichniss der in der Schweiz. 1 vol. Bern. 228 pp.

This amoeba has been studied by several workers. Rafalko (1959) found it in faecal cultures from the faecal contents of a rat and the rectum of a cockroach. According to Sandon (1927) *T rostratus* is common in dirty waters, septic tanks of sewage works, etc., and found in soils from Kenya, Gough Island, Tristan da Cunha, and India (Pusa and Coimbatore). Singh and Hanumaiah (1979) isolated this amoeba from Central Drug Research Institute garden soil, from Gomti river mud, from Chinhat Lake mud and from sewage sludge sample in Lucknow. In the present work *T rostratus* have been found from 'tea plantation' soils of Darjeeling district, West Bengal. The locality was Chandman bazar. The collection time of soil was 5.1.80 and the recorded soil temperature during collection was 22°C (Table 2). These findings clearly show that *T rostratus* is very widely distributed throughout the world.

Morphology

The length of amoebae in active locomotion has a range of 16.8 to 23.8μm, the mean length is 21.56μm with standard error of mean $\pm$0.75 (Table 3). Amoeba has discrete monopodial pseudopodium which during locomotion behaves limax like. The eruption is a typical phenomenon and the point of eruptiveness is from the fronto-lateral direction. The boundary of the hyaline pseudopodia is smooth in outline. The anterior end of the amoebae is always broader than the posterior end. Posterior end is non-sticky in nature, without any filament. The endoplasm is granular with scanty refractile granules. The single vesicular nucleus usually observed at the anterior region of the amoebae and during the locomotion of amoebae, they are seen moving with the flow of the endoplasmic mass. During active movement the amoebae move at the rate of 63 to 112μm per minute. Diameter of the nucleaus has a range of 1.4 to 2.1μm and mean diameter is 2.1μm. There are 1 to 3 contractile vacuoles in an individual. The diameter of the contractile vacuole has a range of 2.8 to 3.5μm, with mean diameter 3.5μm (Figs. 70 to 74). The food vacuole and food-cup formation was not observed. The nature of adherence to the substratum is similar as described in *S. russelli* (Table 3).

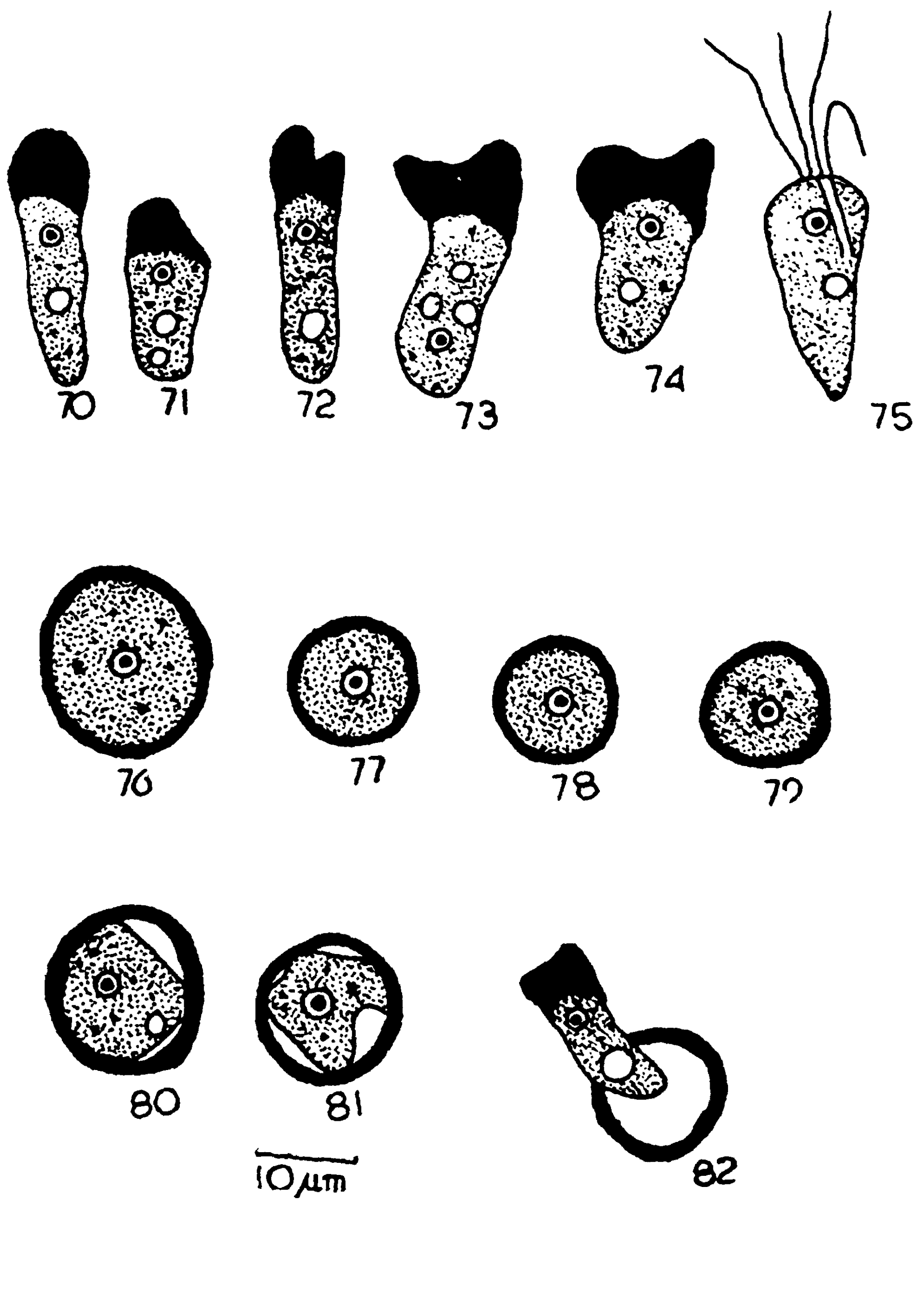
Living cysts are single layered with an outer gelatinous wall. Diameter of the cyst have a range of 9.8 to 14μm with a mean of 11.9μm and standard error of mean is$\pm$0.47 (Figs. 76 to 79 and Pl. 1 Fig. 6) (Table 4). The outer gelatinous wall is not so thick as described in the case of *D. thorntoni*. The size is very variable and shape is spherical. There is a single prominent nucleus in a single cyst. The mean diameter of the cyst nucleus is 1.4μm. During excystation the amoeba detaches itself from the cyst wall. As there is no preformed pore, so presumably some digestion of the wall occurs. The outline of the cyst wall and the place of emergence of amoeba can be seen in the empty cyst (Figs. 80 to 82).

Amoebae produce flagellate stage having 4 flagella. Flagellates have the conical shape in this state (Fig. 75). When the amoebae were maintained in culture on agar for a year or more they either did not produce the flagellate stage or only a few flagellates were produced.

The resting nucleus

The resting nucleus consists of a central spherical nucleolus. No chromatin granules could be seen (Figs. 70 to 74).

In iron-alum haematoxylin preparation nucleolus stains deeply with peripheral chromatin granules (Fig. 83).



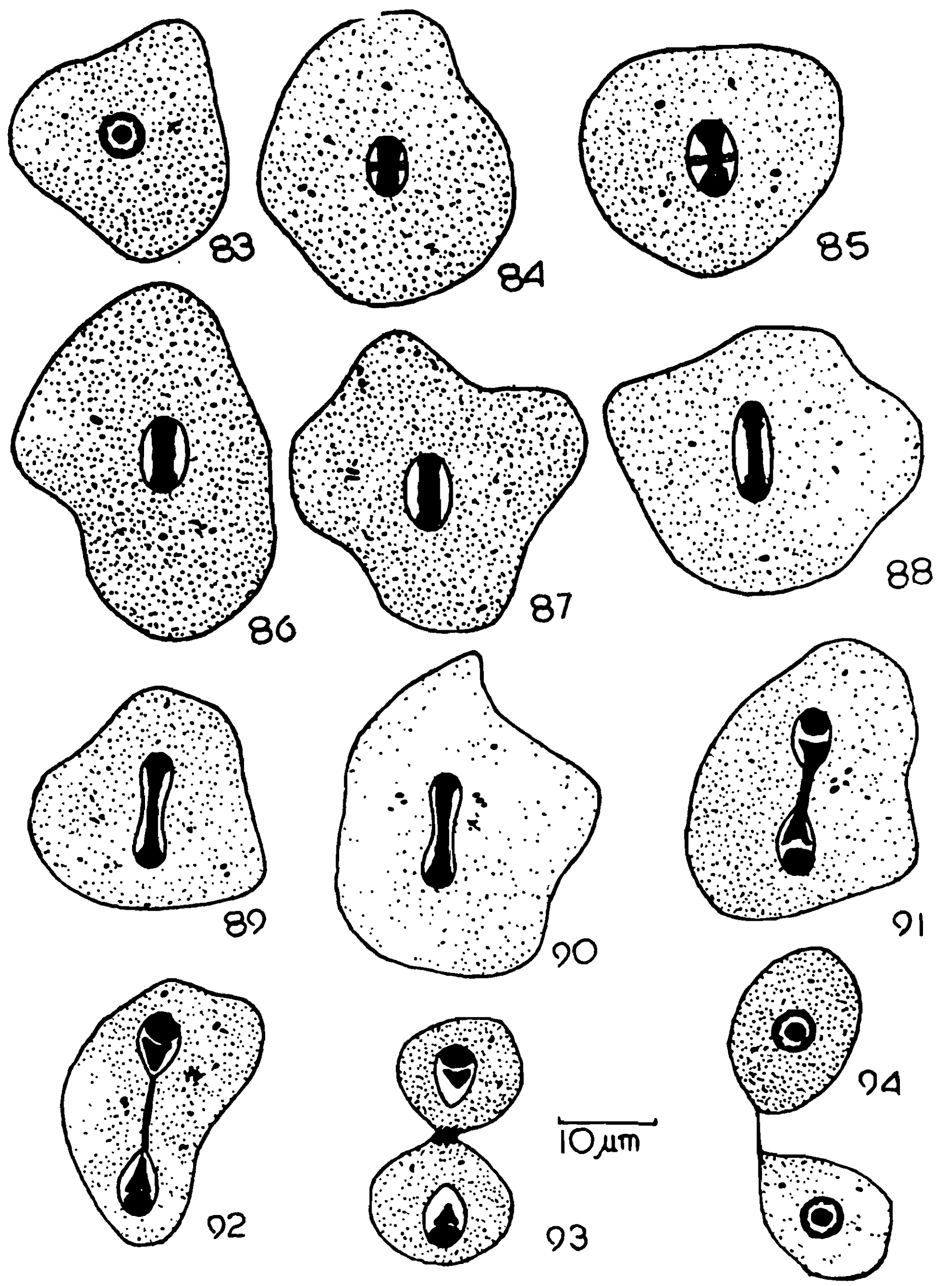
Camera lucida drawings of *Tetramitus rostratus*.

Figs. 70 to 82 : Drawn in the living condition.
Figs. 70 to 74 : Trophic forms.
Fig. 75 : Flagellate stage.
Figs. 76 to 79 : Cysts.
Figs. 80 to 82 : Excystment stages.

Mitotic division

Prophase

The amoebae do not rounded during nuclear division. The beginning of nuclear division is marked by the elongation of the nucleolus and the chromatin granules lie besides it (Fig. 84). Nucleolus later on assumes a dum-bell-shaped appearance and divides into two equal halves known as 'polar masses' (Fig. 85).



Figs. 83 to 94 Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.

Fig. 83 : Ordinary individual and structure of the resting nucleus.

Figs. 84 to 94 : Successive stages in division.

Metaphase

After the formation of 'polar masses' a solid band of chromatin is seen occupying the position of the equatorial plate. No individual chromosome could be made out at this stage. The spindle connecting the 'polar mass' could be seen, but no distinct spindle fibre could be distinguished (Fig. 86).

Anaphase

The band of chromatic material divides into two equal halves, and each half moves towards its pole (Fig. 87 and 88). After the chromatic material has moved to the two poles and get mixed with the nucleolus, certain granular substance, which Rafalko (1951) has called 'interzonal body' can be distinctly seen lying half way between the two 'polar masses' (Fig. 89). The 'interzonal body' increases in size and divides into two equal halves. The nuclear membrane persists throughout division. It becomes elongated and constrict to give rise to daughter nuclei (Figs. 90 to 92).

Telophase

After the nucleus has divided into two, the amoebae become elongated and constricts in the middle to give rise to two daughter individuals. The 'interzonal body' and 'polar mass' of the daughter nuclei fuse together to give rise to the nucleolus. Chromatic material is broken up into granules and these granules occupy the position as seen in the resting necleus (Figs. 93 and 94).

Critical remark

Rafalko (1951) described the mitotic division of *T rostratus* using Feulgen reaction. His divisional stages in *T rostratus* were confirmed by Singh and Hanumaiah (1979). The present observation is similar to that of Rafalko (1951) but the presence of intra-nuclear centrioles could not be detected as pointed out by Singh and Hanumaiah (1979). According to Singh and Hanumaiah (1979) and also as revealed in the present work the nuclear divisional stages given by Bunting and Wenrich (1929) and Hollande (1942) were either incomplete or faulty.

Singh (1952) described the nuclear divisional stage of *N. gruberi* which are more or less similar to *T rostratus* with the exception that 'polar caps' present at the ends of the 'polar masses' in *N. gruberi* is absent in *T rostratus*.

CRITICAL REMARKS ON THE INCLUSION OF THE GENERA IN THE FAMILY SCHIZOPYRENIDAE

Page (1974, p. 174) says 'For the benefit of non-specialist it should be emphasized that whichever nomenclature they wish to employ, Schizopyrenidae and Vahlkampfiidae (as used here) are exact equivalents, as are *Schizopyrenus* and *Vahlkampfia*. The difference is over the validity of *Vahlkampfia* and the familial name derived from it' It may be pointed out that Page (1976a) has included in Vahlkampfiidae only those genera of uninucleate amoebae which have limax, eruptive locomotive form and divide by promitosis. Singh and Hanumaiah (1979) have not only included limax amoebae but also those genera of amoebae which during locomotion may be oval, oblong, or ellipse and somewhat elongated or nearly fan-shaped and do not show eruptive movement in Schizopyrenidae based on promitotic nuclear division. Thus Vahlkampfiidae and Schizopyrenidae can not be equivalents. Singh (1952) pointed out that limax amoebae studied by Vahlkampf (1905) had 'interzonal bodies' during late stages of nuclear division and therefore it was *Naegleria gruberi* or a new species of *Naegleria*. The non-existence of *Vahlkampfia* and the justification for the erection of the genera *Naegleria* and *Didascalus*, on the presence and absence of 'interzonal bodies' have been discussed in great detail by Singh (1952), Singh and Das (1970) and Singh and Hanumaiah (1979). The claim of Page (1974, 1976b) that *Didascalus* is the same as *Naegleria*, although 'interzonal bodies' are found in most late stages of nuclear division in *Naegleria*, being absent only on rare occasions, is difficult to accept. Many protozoologists have found the constant presence of 'interzonal bodies' in *Naegleria* spp. (vide Singh and Hanumaiah' 1979 for the literature). Chang (1971) also did not recognize the existence of the genus *Vahlkampfia* and has agreed with Singh (1952) on the creation of the genera *Naegleria* and *Didascalus* on the presence and absence of 'interzonal bodies' and in placing the genera *Schizopyrenus*, *Naegleria* and *Didascalus* in the family Schizopyrenidae. In this work the criteria advocated by Singh (1952) and Singh and Hanumaiah (1979) to distinguish between the genera *Schizopyrenus*, *Didascalus*, *Naegleria* and *Tetramitus* have been employed.

Family HARTMANNELLIDAE Volkonsky, 1931 emend. Singh 1952
Genus **Hartmannella** Alexeieff, 1912 emend.
Singh and Hanumaiah, 1979

Definition

The resting nucleus contains a single Feulgen-negative nucleolus. During mitosis the nucleolus disappears and the spindle with chromosomes arranged as an equatorial plate is formed. In active locomotion amoebae assume limax form. No temporary flagella are produced.

Hartmannella vermiformis Page

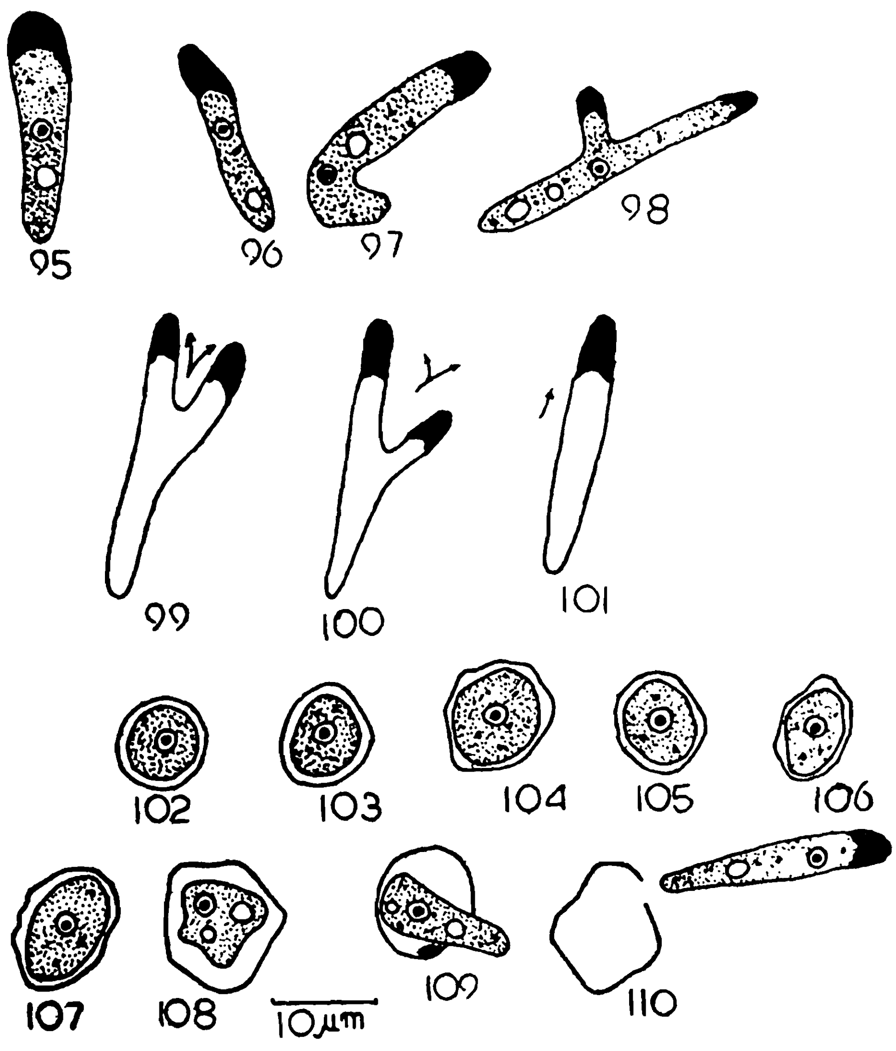
1. 1967. *Hartmannella vermiformis* Page, J. Protozool,, **14** : 499.

H. vermiformis was first isolated by Page (1967a) on two occasions, once from Pigeon lake, Wisconsin and on the other occasion from the Kankakee river at Schneider in Indianapollis. Singh and Hanumaiah (1979) isolated this amoeba from Central Drug Research Institute, Lucknow garden soil, from Chinhat lake mud, from sewage sludge sample in Lucknow and from Baroda garden soil. In the present work *H. vermiformis* has been isolated from a soil of Midnapore district, West Bengal. The locality was 'Jhau forest area' Digha. The soil was saline in nature. The collection time of soil was 3.8.79 and recorded soil temperature during collection was 30°C (Table 2).

Morphology

The length of amoebae in active locomotion has a range of 21 to 25.2 μm, the mean length is 22.96 μm with standard error of length ± 0.47 (Table 3). Locomotive amoebae are cylindrical-shape with tapering posterior end. The type of locomotion is typical limax-like with a symptom of monopodial homogenous steady flow from its anterior portion. During progression of locomotion, the bifurcation with assymetrical podium is observed which soon disappears to retain its original monopodial form. Change of direction is sometimes observed which always takes place from the anterior part of the body to its lateral sides. Anterior part of the podium is hyaline, transparent, without any temporary projections at its extremities. During active movement the amoebae can move at a range of 55 to 110 μm per minute. The vesicular nucleus is always one in number and usually occupies the middle of the cell. The mean diameter of the nucleus is 1.4μm. The contractile vacuole is single but sometimes two to three may be seen. The mean diameter of the contractile vacuoles are 2.1 μm. The endoplasm is filled with coarse granules which are scatteredly distributed (Figs. 95 to 101).

The cysts in living condition are variable in size with two walls (Figs. 102 to 107 and Pl. II Fig. 1). The outer wall is irregular. The diameter of cyst has a range of 5.6 to 9.8 μm, the mean diameter is 7.7 μm with a standard error ± 0.47 (Table 4). The cysts have single nucleus. The mean diameter of nucleus is 1.4μm.



Camera lucida drawings of *Hartmannella vermiformis*.
Figs. 95 to 110 : Drawn in the living condition.
Figs. 95 to 101 : Trophic forms.
Figs. 102 to 107 : Cysts.
Figs. 108 to 110 : Excystment stages.

During excystation the inner cyst wall is dissolved and the amoeba moves inside the outer wall. No pores were observed. Probably the digestion of cyst wall occurs to allow the passage of amoeba to come outside (Figs. 108 to 110).

Repeated efforts to produce a temporary flagellate stage have completely been failed.

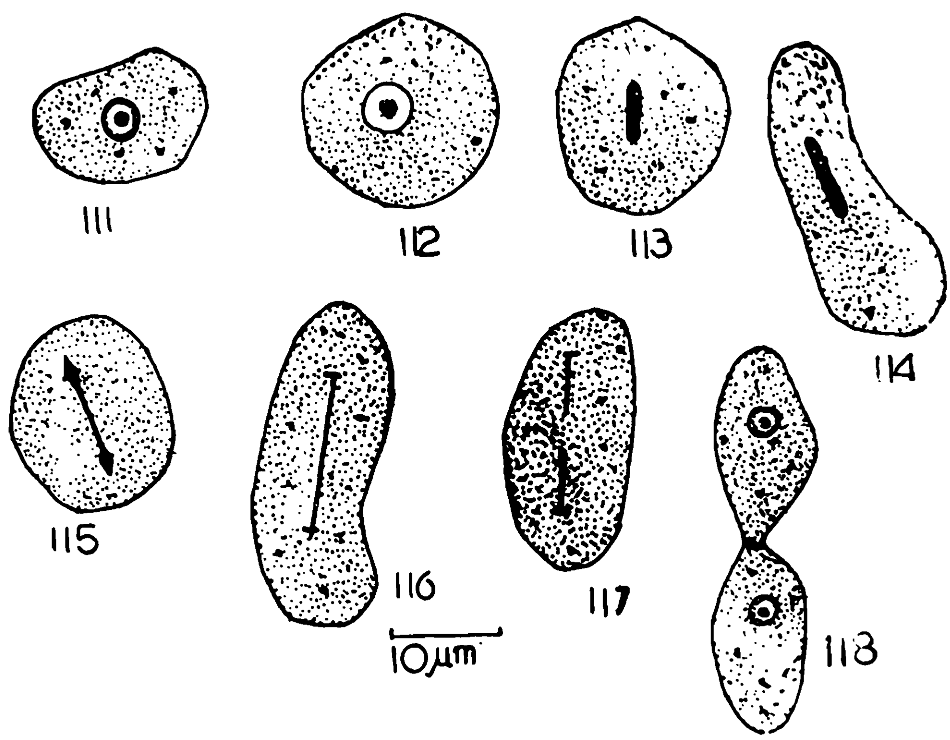
The resting nucleus

In living condition the nuclus consists of a central spherical nucleolus. No chromatin granules could be seen. In stained preparation there is a nucleolus and chromatin granules lies near the nuclear membrane (Fig. 111).

Mitotic division

Prophase

The amoebae become rounded and motionless during mitotic division. In this respect *H. vermiformis* differs from amoebae described earlier, that have 'polar masses' during nuclear division. In the early stages in the process of nuclear division the chromatic granules move to the centre and fuse (Fig. 112). The nucleolus gradually disappears, probably giving rise to the spindle.



Figs. 111 to 118 : Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 111 : Ordinary individual and the structure of the resting nucleus.
Figs. 112 to 118 : Successive stages in division

Metaphase

A spindle with a thick band of chromatic material occupying the equatorial-plane can be seen, and nuclear membrane disappears. No chromosomes or distinct spindle fibres could be seen (Fig. 113).

Anaphase

The band of chromatic material divides into two equal parts and moved to the two poles. The part of the spindle that lies between chromosomes and poles stains more deeply and appear like caps. They become smaller and smaller as the chromosome move to the two poles and finally disappear. When the chromosomes reach the two ends of the poles, they are connected by a thread-like structure, narrow in the middle, and the amoeba becomes elliptical (Figs. 114 to 117).

Telophase

A constriction appears in the middle of the elongated amoeba and two daughter individuals are produced. At the stage shown in Fig. 118, nuclear membranes appear and fragmented chromatic materials occupy the position seen in the resting nucleus.

Critical remarks

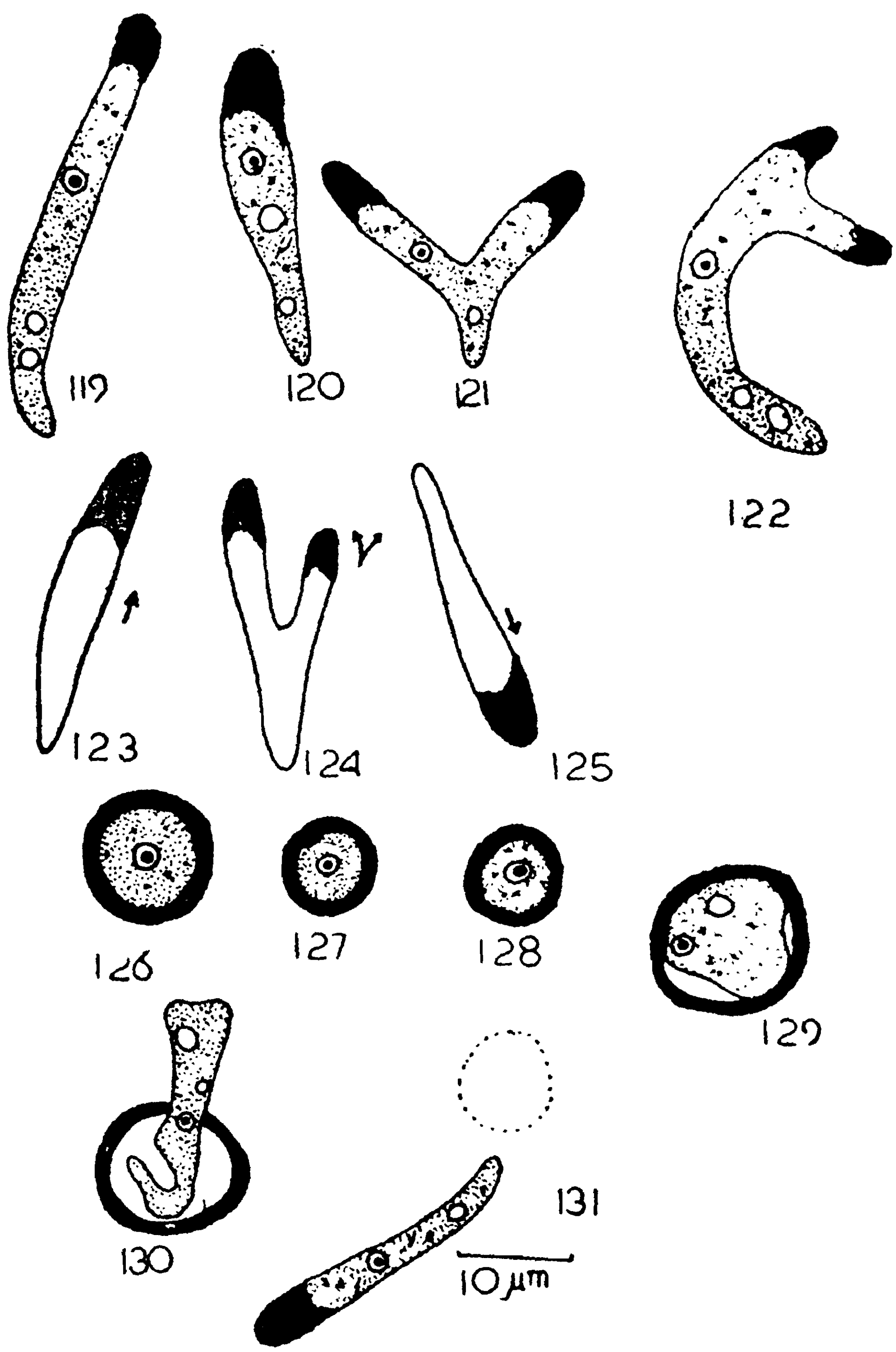
Page (1967a) found mass excystment of *H. vermiformis* on agar surface in three day. In some cysts the wall separated into distinct ectocyst and endocyst. The cysts in which this distinction was not visible, had separate layers closely apposed. The observation made by Page led him to the conclusion that the amoebae excyst by softening the cyst wall. Page also found few empty cyst walls after excystment of the amoebae. In the present work, *H. vermiformis* has been found to have an ectocyst and endocyst, and after excystment the ectocyst is left intact.

Page (1967a) observed that during nuclear division (his Figs. 86 to 90) amoebae become flattened and sometimes assumed rather peculiar projections. Singh and Hanumaiah (1979) showed that amoebae became rounded and motionless at the beginning of the nuclear division, and at no stage during division there were peculiar projections. We also could not detect any projections but other typical characters of amoebae in permanent preparations.

Hartmanella crumpae Singh and Hanumaiah

1. 1979. *Hartmanella crumpae* Singh and Hanumaiah, Monograph No. 1 of the Association of Microbiologists of India. Published by Indian J. Microbiol., 1-80.

Singh and Hanumaiah (1979) found *H. crumpae* to be very common soil amoeba. They have isolated it from Central Drug Research Institute, Lucknow garden soil, from Gomti river mud from Baroda sewage sludge, from tap water in Lucknow, from Bombay garden soil and from Mullah river mud, Poona. In the present work *H crumpae* has been isolated from a soil of Bankura district, West Bengal. The locality was Pratappur. The soil was of non-vegetative type. The collection time was 4.8.79, and the recorded soil temperature was 30°C (Table 2).



Camera lucida drawings of *Hartmannella crumpae*.

Figs. 119 to 131 : Drawn in living condition

Figs. 119 to 125 : Trophic forms.

Figs. 126 to 128 : Cysts.

Figs. 130 to 131 : Excystment stages.

Morphology

The length of the amoebae in active locomotion has a range of 35.0 to 42.0 μm, the mean length is 38.64 μm with standard error of length ± 0.78. The diameter of the contractile vacuoles have a range of 1.4 to 2.1 μm with a mean diameter 2.1 μm. The diameter of nucleus has a range of 0.7 to 1.4 μm with a mean diameter 1.4 μm (Table 3).

The observation on the living trophic form is same as described in *H. vermiformis* only with a noticeable change in the mode of direction during active locomotion (Figs. 119 to 125). During active movement the amoebae move at the rate of 80 to 125 μm per minute.

The living cysts are rounded or spherical and variable in size having a single wall with an outer gelatinous layer (Figs. 126 to 128 and pl. II Fig. 2). The nucleus having a nucleolus could be easily seen. The diameter of the cyst has a range of 6.3 to 9.8 μm with mean diameter 7.98 μm and standard error of mean is ± 0.40 (Table 4). The mean diameter of cyst nucleus is 1.4 μm (Table 4).

During excystment the amoeba gradually detaches itself from the cyst wall and move inside it. It comes out by digestion of cyst wall, which is eventually dissolved (Figs. 129 to 131). Repeated efforts to produce temporary flagellate stage have completely been failed.

The resting nucleus

The structure of the resting nucleus in living condition and stained preparation (Fig. 132) is similar to *H. vermiformis.*

Mitotic division

The different stages of nuclear division described for *H. vermiformis* also applies to *H. crumpae* (Figs. 133 to 140).

Remarks

Singh and Hanumaiah (1979) found that *H. crumpae* differ from *H. vermiformis* in the following characters

(1) *H. crumpae* cysts are rounded and spherical having single wall with an outer gelatinous layer, while the cysts of *H. vermiformis* have two walls, the outer being slightly irregular.

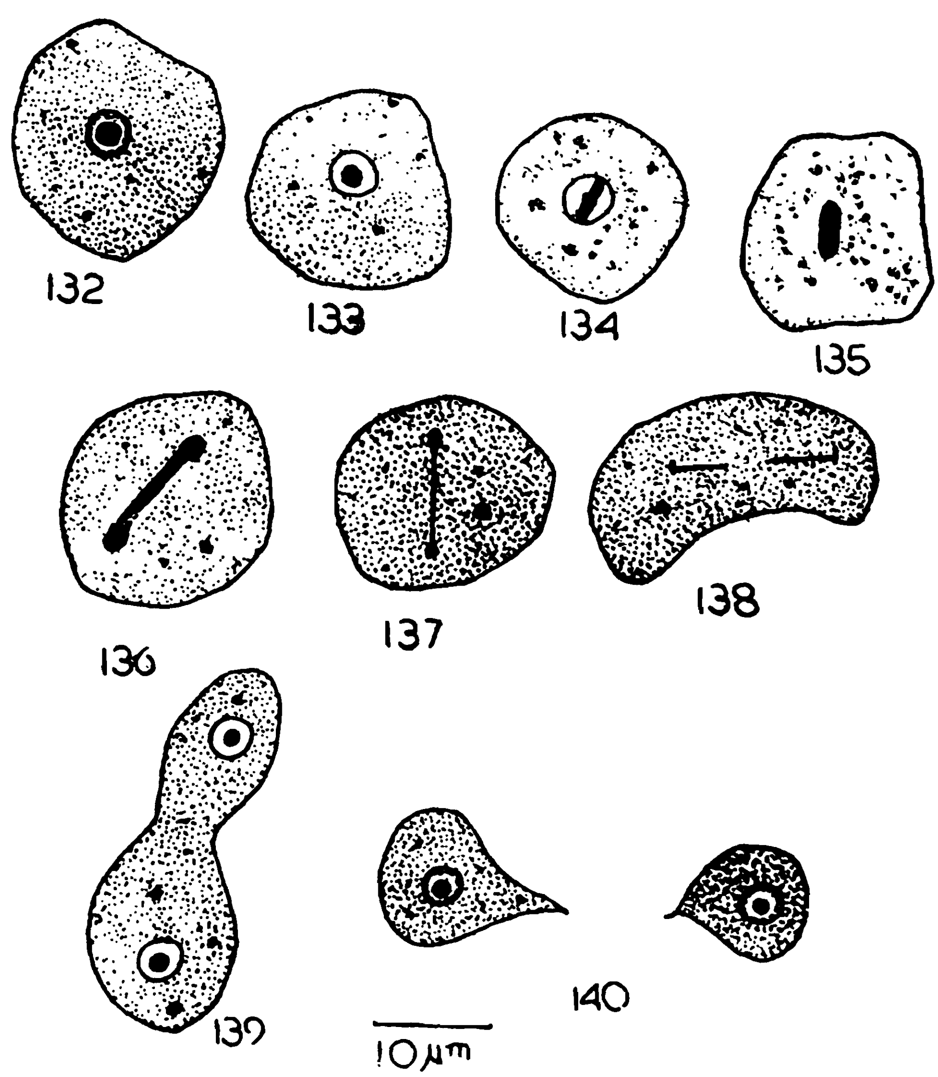
(2) During excystment the cyst wall of *H. crumpae* disappears, while the outer cyst wall of *H. vermiformis* remains intact after the emergence of amoebae from the cysts.

H. catabrigiensis Page, 1974 has a distinct ecto and endocyst and usually several bipyramidal crystals are present in the trophozoites. No such crystals could be found in *H. crumpae* (Singh and Hanumaiah 1979). The characters of *H. crumpae* as described in this work are exactly similar to the observations of Singh and Hanumaiah (1979).

Genus **Acanthamoeba** Volkonsky, 1931
emend. Singh and Hanumaiah, 1979

Definition

During mitosis the Feulgen-negative nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed. Amoebae in active locomotion with broad anterior hyaline lobopodium from which are produced singly or in twos or threes, several or many, hyaline projections (acanthopodia).



Figs. 132 to 140 : Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 132 : Ordinary individual and structure of the resting nucleus.
Figs. 133 to 140 : Successive stages in division.

Acanthamoeba rhysodes (Singh)

1. 1952. *Hartmannalla rhysodes* Singh, Phil. Trans. Roy. Soc. Lond., B **236** : 405
2. 1967. *Acanthamoeba rhysodes* Page, J. Protozool., **14** : 499.

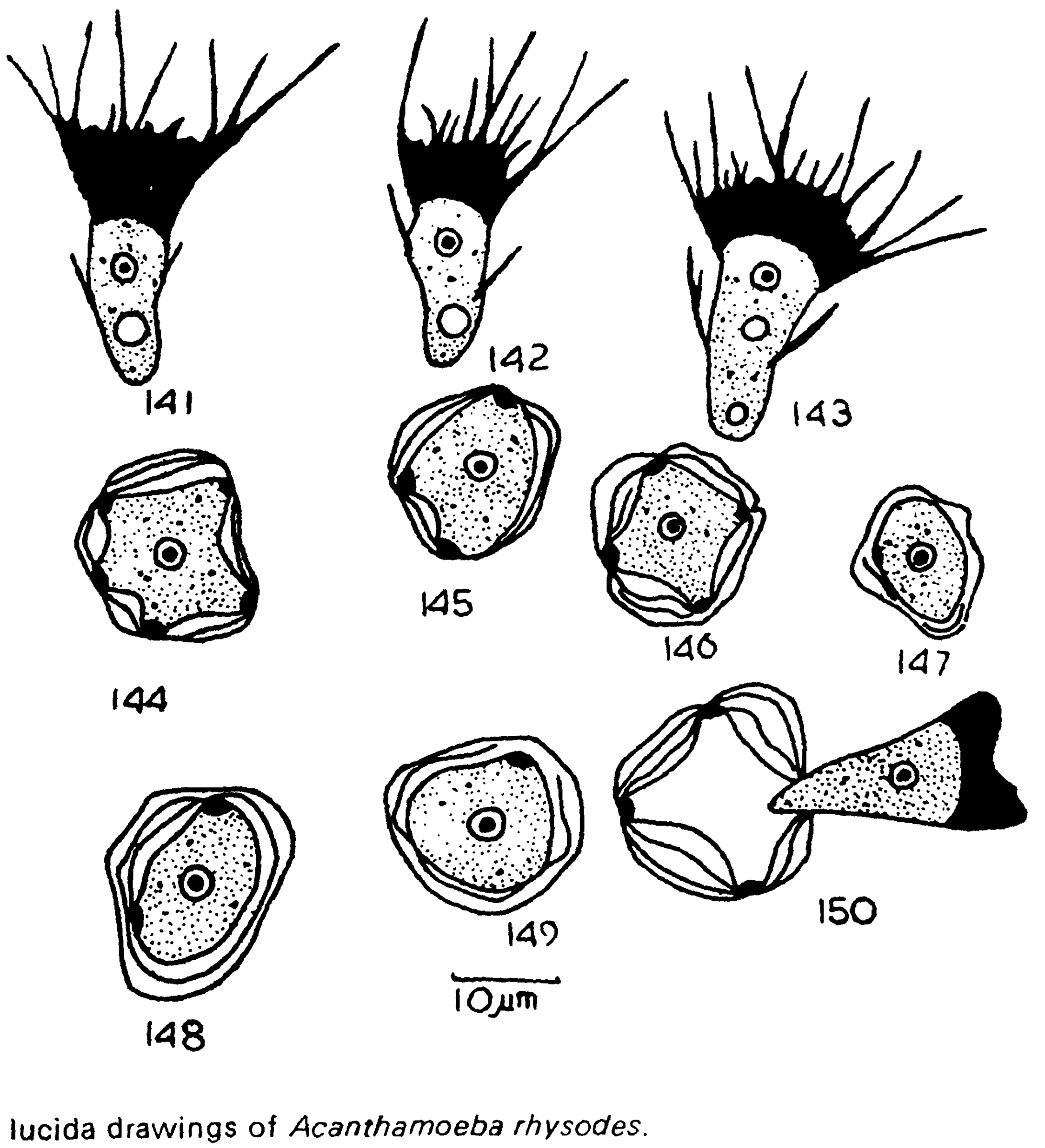
A. rhysodes (Hartmannella rhysodes) was found by Singh (1952) in soils receiving farmyard manure, complete minerals plus sulphate of ammonia and no manure in Barnfield and Broadbalk fields at Rothamsted Experimental Station, England. During quantitative studies of the amoebic population (Singh 1949) from 1945 to 1948, it was found to be present in large numbers in all the soils. It was the most common soil amoeba in Rothamsted soils. Singh and Hanumaiah (1979) isolated *A. rhysodes* from Central Drug Research Institute, Lucknow garden soil, from Gomti river mud, from Chinhat lake mud, from sewage samples from Lucknow and Baroda, from Bombay garden soil and from Mullah river mud, Poona. Chang (1971) in numerous examinations of surface water and sewage effluents found *A. rhysodes* most frequently. Several other workers have recorded the presence of *A. rhysodes* in soils and fresh water from different parts of the world. In the present work *A. rhysodes* has been isolated from the soil of West Dinajpur district, West Bengal. The locality was Asoke pally. The vegetation of the soil was mango. The collection time of the soil was 30.10.76 and the recorded soil temperature during collection was 28°C (Table 2).

Morphology

The length of amoeba in active locomotion has a range of 22.4 to 29.4 μm, the mean length is 26.04 μm with standard error of length ± 0.78 (Table 30). During active locomotion, the amoebae are moving slowly through their broader hyaline lobopodium at its anterior end. The boundary of the lobopodium is irregular with fine tipped acanthopodium. The tip of the acanthopodia usually single sometimes bifurcated from the base of their origin. The acanthopodia are very distinctly seen at the time of movement (Figs. 141 to 143). During active locomotion the amoeba can move at a rate of 14 to 32 μm per minute. Probably the formation of acanthopodium is associated with free active movement. During locomotion abrupt and prominent change of direction is not observed. There is a single nucleus and prominent contractile vacuoles in an amoeba. The mean diameter of the nucleus is 0.7 μm. The diameter of the contractile vacuoles has a range of 2.8 to 4.2 μm. Food vacuoles are clearly seen. Sometime food-cup formation could be observed.

The living cysts are very variable in size and shape (Figs. 144 to

149 and Pl. II Fig. 3). Each cyst consists of two walls which are generally irregular in outline and give a wrinkled appearance. Some of the cysts are pierced by one or more pores. These pores seemed to be plugged by some kind of structureless substance as is the case in the cysts of *N. gruberi* described earlier. Some cysts appear rounded with the contents of the inner cyst wall shrunken, presenting a polyhedral appearance. The diameter of cyst has a range of 7.0 to 14 μm with mean diameter 10.92 μm and standard error of mean is ± 0.90 (Table 4).



Camera lucida drawings of *Acanthamoeba rhysodes*.
Figs. 141 to 150 : Drawn in the living condition.
Figs. 141 to 143 : Trophic forms.
Figs. 144 to 149 : Cysts.
Fig. 150 : Excystment stages.

During the process of excystation the amoeba is coming outside from one of these pores through the removal of the structureless substance (Fig. 150).

Repeated efforts to produce temporary flagellate stage have completely been failed.

The resting nucleus

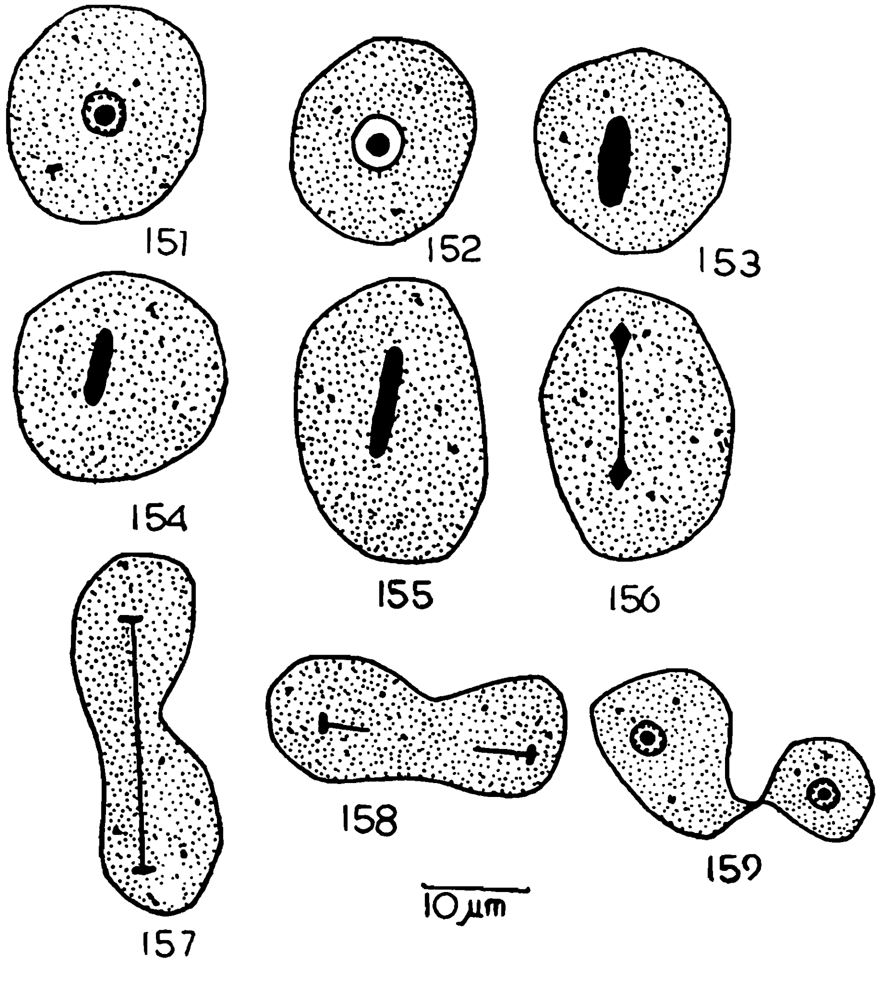
In the living condition single vesicular nucleus with a clear central nucleolus is seen. The chromatic granules could not be detected (Figs. 141 to 143).

In iron-alum stained preparation the nucleolus stains deeply. The peripheral chromatin granules is present (Fig. 151).

Mitotic division

Prophase

At the beginning of the nuclear division the amoebae become rounded



Figs. 151 to 159 : Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 151 : Ordinary individual and structure of the resting nucleus.
Figs. 152 to 159 : Successive stages in division.
Camera lucida drawings of *Acanthamoeba culbertsoni*.

and motionless. The chromatin granules move to the centre (Fig. 152). This gives the appearance of the fragmentation of the nucleolus. In iron-alum haematoxylin stain the nucleolus gradually disappears and probably give rise to spindle.

Metaphase

The chromatic material, assumes the shape of a solid band at the equatorial plate, and the nuclear membrane disappears (Fig. 153).

Anaphase

The band of chromatic material divides into two equal halves. Each half moves towards each pole. The parts of the spindle which lie between the chromosomes and the poles stain more deeply and appear like caps. They become smaller and smaller as the chromosomes move to the two poles and finally disappear. When the chromosomes reach the two ends of the poles they are connected by thread like structure. The shape of the amoeba becomes elliptical (Figs. 154 to 156).

Telophase

A constriction appears in the middle of the elongated amoeba and two daughter individuals are produced (Figs. 157 to 159). At this stage shown in Fig. 159, nuclear membrane appears and the nuclear structure is similar to that seen in the resting nucleus.

Critical comments

There is a great confusion regarding the validity of *A. castellanii* (Douglas 1930), *A. rhysodes* (Singh 1952) and *Acanthamoeba* sp. (Neff, 1957 strain). Neff (1957) isolated an amoeba in the U.S.A. and tentatively named it *Acanthamoeba* based mainly on cystic character as suggested by Volkonsky (1931). The diameter of the amoeba in the rounded condition was 12 to 40 μm (average approx. 20 μm). It appears that Neff was unaware of the existence of *A. rhysodes.* It must be admitted that it is not possible to distinguish between the three species either on their locomotive form or on cystic character. Adam (1964) considers *A. rhysodes* and *Acanthamoeba* Neff as synonym of *A. castellanii,* as judged by immobilization reaction. Page (1967b) holds similar view on locomotive form and cystic character of amoebae. Visvesvara and Balamuth (1975) found that *A. rhysodes* and *Acanthamoeba* Neff was serologically very similar and reached to the conclusion without studying *A. castellanii,* that *A. rhysodes* and *Acanthamoeba* Neff were synonym of *A. castellanii.* Serological studies carried out by Willaert *et al.* (1978) have revealed that *A. rhysodes* are much more closely related among themselves than they are with *A. castellanii*. Singh and Das (1970) found that under similar cultural condition and with the same bacterial food supply, *A. castellanii* was larger (diameter of amoeba in rounded condition 30 to 45 μm) than the strains of *A. rhysodes* (diameter of amoeba in rounded condition is 12 to 35 μm). *Acanthamoeba* Neff have been found to be non-pathogenic to mice (Culbertson *et al.*, 1965, Cursons and Brown, 1976 and others), while *A. rhysodes* is pathogenic (Culbertson *et al.*, 1965, 1966 Singh and Das, 1970 Singh and Hanumaiah, 1979 Datta and Hemlata, 1979 and Misra and Sharma, 1980). Judging from the size of *Acanthamoeba* Neff and *Acanthamoeba rhysodes* and their close serological relationship the former should be regarded as a non-pathogenic serotype of *A. rhysodes*. *A. rhysodes* was found most frequently in surface water and sewage effluents in U.S.A. by Chang (1971). It is also a very common amoeba in Indian soils as pointed out earlier in this work. At present *A. castellanii*, which is larger than *A. rhysodes* and non-pathogenic (Culbertson *et al.*, 1965 Singh and Das, 1970 and others) may be regarded as a distinct species. Cerva (1965) found that *Acanthamoeba* Neff was similar to but not identical with *A. castellanii*, as judged by complement-fixation test. It would be most interesting to carry out detail serological studies, nucleic acid pairing and isoenzyme patterns of *A. rhysodes* and *A. castellanii* to find out whether they are the same or different species.

Acanthamoeba culbertsoni (Singh and Das)

1 1959. *Hartmannella strain* A-1 Culbertson *et al.*, Amer. J. Path., **35** : 185.
2. 1967. *Acanthamoeba culbertsoni* Page, J. Protozool., **14** : 499.
3. 1970. *Hartmannella culbertsoni* Singh & Das, Phil. Trans. Roy. Soc. Lond., B **259** : 435.

Culbertson *et al.* (1959) found *Acanthamoeba* (*Hartmannella* A-1) as a contaminant of a mammalian cell culture. This amoeba was named by Singh and Das, *Acanthamoeba culbertsoni (H. culbertsoni)*. Singh and Hanumaiah (1979) found *A. culbertsoni* to be most common and have isolated it from Central Drug Research Institute, Lucknow garden soil, from Gomti river mud, from Chinhat lake mud, from a number of sewage sludge samples in Lucknow, from a sample of Baroda sewage sludge, from Baroda garden soil and from Mullah river mud. *A. culbertsoni* has also been isolated by many workers from soils and fresh water in different parts of the world. In the work *A. culbertsoni* has been isolated from 24 Parganas district, West Bengal. The locality was

Kakdwip, 8 No. Kalinagar area. The nature of crop production on soil was paddy. The collection time of the soil was 21.7.79 and the recorded soil temperature during collection was 28°C (Table 2).

Morphology

The length of the amoebae in active locomotion has a range of 21.0 to 26.6 μm, the mean length is 24.22 μm with standard error of mean ± 0.62. The mean diameter of the nucleus is 1.4 μm The diameter of the contractile vacuole has a range of 2.8 to 4.9 μm (Table 3).

The amoebae are very variable in size and during active locomotion have broad anterior lobopodium from which are produced singly or in twos or threes a number of slender hyaline projections (Figs. 160 to 162). This projections have been called by Page (1967b) as acanthopodia. The acanthopodia are eventually resorbed at the posterior end. outline of the amoebae is generally very irregular and they progress by flow into ectoplasmic lobopodium. There is a single nucleus and contractile vacuole in an individual (Figs. 160 to 162). When the amoebae are moving their rate of locomotion is 14.0 to 56.0 μm per minute.

Living cysts are rounded or oval and very variable in size (Figs. 163 to 166 and Pl. II Fig. 4), and have two walls. In some cysts the outer wall is nearly circular, in others it is irregular in outline (Pl. II Fig. 5). The great majority of the cysts are perforated by one or more pores or oppercula plugged by structureless substance as in *N. gruberi*. Few cysts have no pore (Fig. 166). The inner wall is in contact with the outer one at the point of the operculum. The diameter of the cyst has a range of 11.2 to 18.2 μm. The mean diameter is 15.26 μm with a standard error of mean ± 0.84. The mean diameter of the cyst nucleus is 1.4 μm (Table 4).

During excystment the amoeba escapes through a pore after dissolving the structureless substance. The cyst wall outline is still visible even after the amoeba has emerged (Figs. 167 to 169).

Repeated efforts to produce a temporary flagellate stage have completely been failed.

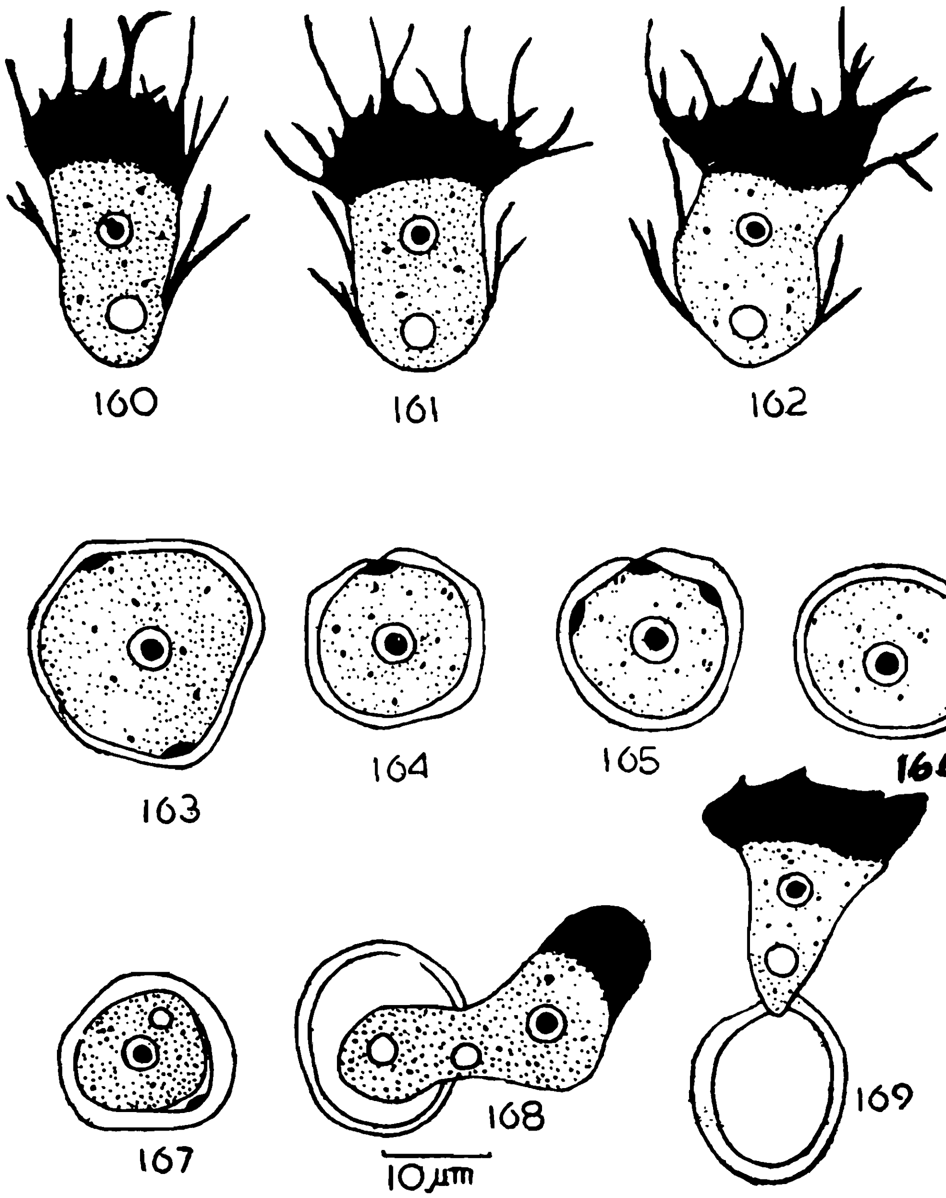
The resting nucleus

In the living amoebae the nucleus contains a farely large nucleolus and no chromatin granules could be seen (Figs. 160 to 162).

In iron-alum haematoxylin preparation the nucleolus and the chromatin granules, which are near the nuclear membrane, are distinctly seen. There is usually one nucleus in each amoeba two are rare.

Mitotic division

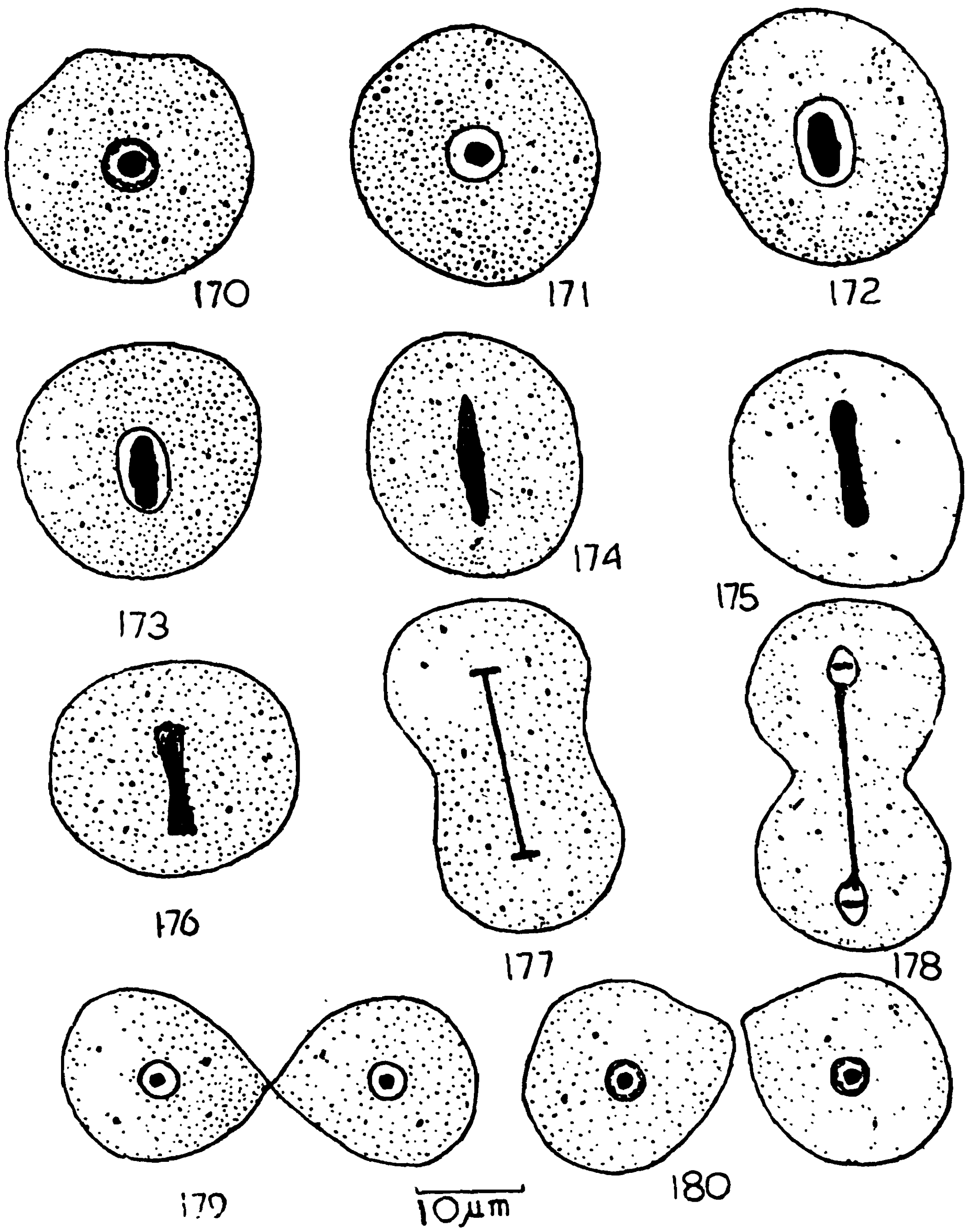
The amoebae become rounded and motionless at the beginning of the nuclear division. As the nuclear division resembles *A. rhysodes* in many respects, only a brief description of the stages of mitosis is given below.



Figs. 160 to 169 : Drawn in the living conditions.
Figs. 160 to 162 : Trophic forms.
Figs. 163 to 166 : Cysts.
Figs. 167 to 169 : Excystment stages.

Prophase

The chromatin granules move to the centre. This gives the appearance of the fragmentation of the nucleolus in iron-alum haematoxylin stain (Fig. 171).



Figs. 170 to 180 Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 170 : Ordinary individual and the structure of the resting nucleus.
Figs. 171 to 180 : Successive stages in division.

Metaphase

The small chromatic granules fuse and are seen as a band at the equatorial plate stage. Nucleolus gradually disappears and probably gives rise to the spindle. The chromosomes at equatorial plate stage could not be counted (Fig. 172).

Anaphase

The globular spindle gradually becomes elongated and the chromosomal plate divides into two equal halves which move to the two poles. The nuclear membrane disappears either at anaphase or in some cases earlier. The parts of the spindle which lies between the chromosome and the pole stain more deeply. They become smaller and smaller as the chromosomes move to the poles. The spindle connecting the solid lump of chromosomes at the two poles is narrow (Figs. 173 to 177).

Telophase

A constriction appears in the middle of the elongated amoeba, and the two daughter individuals are produced. A nuclear membrane appears surrounding each lump of chromatic material at the two pole. Later on the lump fragments give rise to chromatic granules. These granules occupy the position seen in the resting nucleus (Figs. 178 to 180).

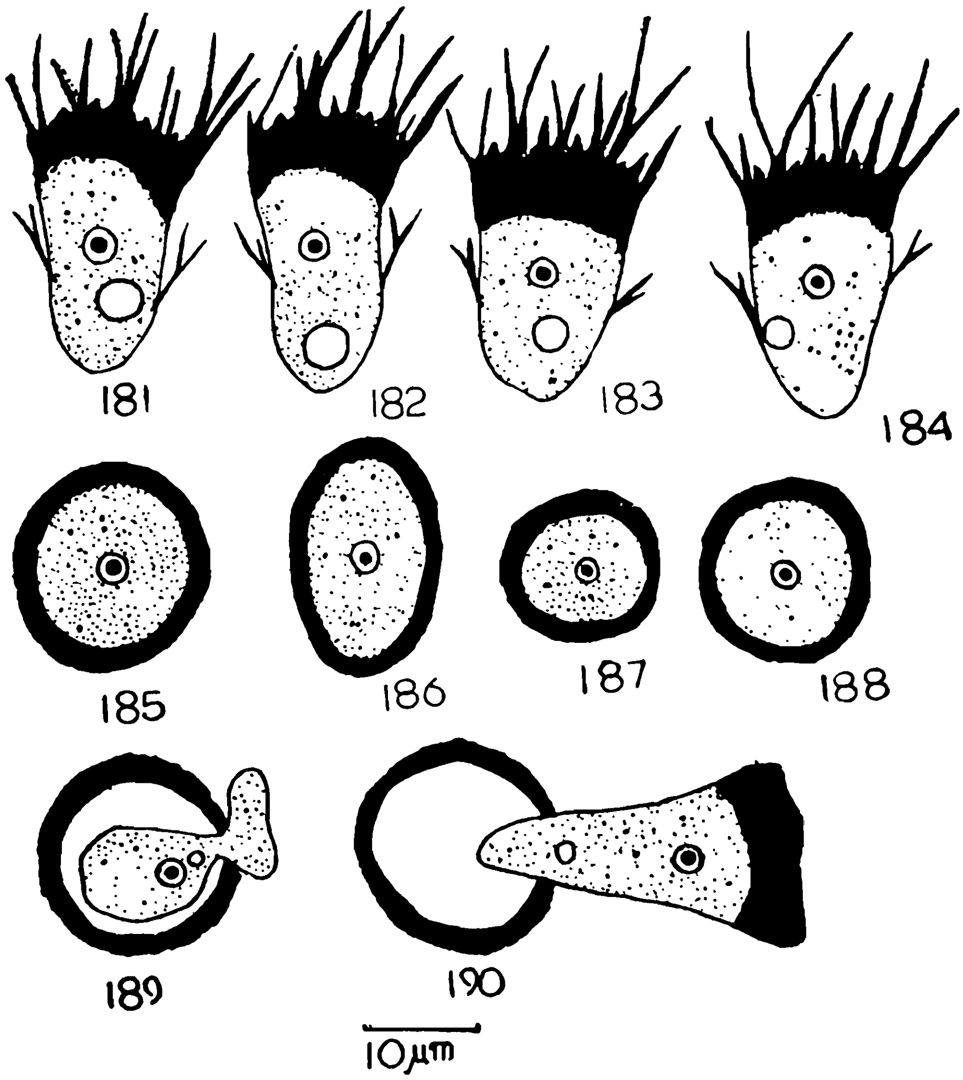
Critical remarks

Adam (1964) by the use of immobilisation reaction inferred that *A. culbertsoni* was serologically distinct from *A. rhysodes* and *A. castellanii* and suggested that it was a distinct species. Using complement fixation test, Culbertson *et al.* (1965) found that *A. culbertsoni* had very little antigenic relationship with other *Acanthamoeba* strains. Cerva (1965) carried out complement fixation tests and found that *A. castellanii* and *A. culbertsoni* were serologically related, though they showed significant differences between them. Cerva and Kramer (1973) found distinct differences in the titers in the homologous and heterologous anti-sera. Balamuth and Kawakami (1967) by the aid of agar-gel-diffusion techniques, have shown that *A. culbertsoni* is closely related to but not identical with *A. rhysodes.* Pant *et al.* (1968) by immobilisation and agglutination tests have confirmed the findings of Balamuth and Kawakami (1967). Visvesvara and Balamuth (1975) have concluded on the basis of cyst morphology and antigenic composition that *A. culbertsoni* differs from *A. rhysodes. Acanthamoeba culbertsoni* (Singh and Das, 1970) has been recognized as a distinct species in the genus *Acanthamoeba* by various protozoologists.

Acanthamoeba glebae (Dobell)

1. 1914. *Amoeba glebae* Dobell, Arch. Protistenk., **34** : 139.
2. 1952. *Acanthamoeba glebae* Singh, Phil. Trans. Roy. Soc. Lond., B **236** : 405.

Acanthamoeba glebae (*Amoeba glebae* Dobell, 1914) was first isolated from soil by Dobell (1914). Sandon (1927) recorded it from soils of Kenya, Sudan, Egypt, Palestine and Rothamsted. Singh (1952) reported *A. glebae (H. glebae)* from Barnfield (Plot 4A), complete minerals plus ammonium sulphate soil at Rothamsted. In the present work *A. glebae* has been isolated from 24 Parganas district, West Bengal. The locality was Bongaon, Shaktighat. The soil was manured and crop production was jute. The collection time of soil was 25.1.79 and recorded soil temperature was 28°C. Other strain of *A. glebae* had been isolated from the grassy soil of Nadia district, West Bengal. The locality was Krishnanagar and the recorded soil temperature was 28°C. These observations suggest that *A. glebae* is world wide in distribution (Table 2).



Camera lucida drawings of *Acanthamoeba glebae*.
Figs. 181 to 190 : Drawn in the living condition.
Figs. 181 to 184 : Trophic forms.
Figs. 185 to 188 : Cysts.
Figs. 189 & 190 : Excystment stages.

Morphology

The locomotive form and behaviour of *A. glebae* (Figs. 181 to 184), *A. culbertsoni* and *A. rhysodes* are similar. All the species of the amoebae have broad anterior hyaline lobopodium from which are produced singly or in twos or threes hyaline projections (acanthopodia). The length of the amoebae in active locomotion has a range of 22.4 to 28.0 μm, the mean length is 25.92 μm with standard error of mean ± 0.38. The diameter of nucleus has a range of 1.4 to 2.15 μm, with a mean 1.85 μm. The diameter of the contractile vacuole has a range of 4.2 to 7.0 μm with a mean 5.6 μm (Table 3). During active movement amoebae moves at the rate of 11.2 to 39.2 μm per minute.

Living cysts are usually rounded or spherical with a single wall (Figs. 185 to 188). The wall seems to consist of two layers in which no pores could be seen (Pl. II Fig. 6). The nucleolus is clearly seen in the nucleus of a living cyst, but no chromatin granules could be distinguished. The diameter of the cyst in living condition has a range of 11.2 to 15.4 μm with a mean 13.88 μm. The diameter of the nucleus has a range of 2.8 to 3.5 μm with a mean 3.01 μm (Table 4).

During excystment (Figs. 189 and 190) no pore can be seen, so presumably some digestion of the wall occurs. The outline of the cyst wall and the place of emergence of the amoebae can be seen in the empty cyst.

No flagellate stage could be produced by the methods described in connection with amoebae having flagellate stage.

The resting nucleus

The structure of the resting nucleus in the living condition (Figs. 181 to 184) and in iron-alum haematoxylin preparation (Fig. 191) is the same as reported in the case of *A. rhysodes* and *A. culbertsoni*.

Mitotic division

Prophase

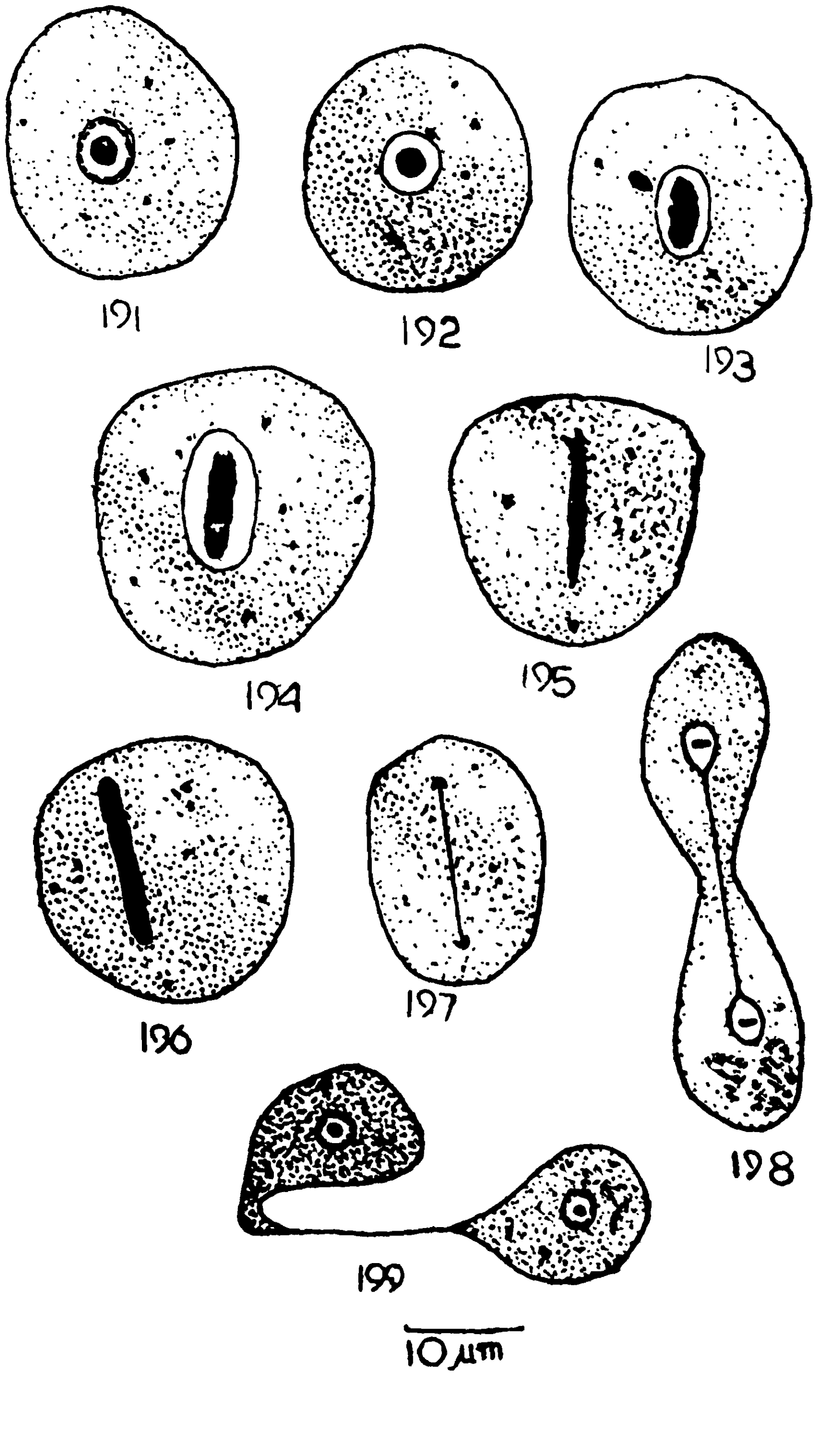
The amoebae become rounded and motionless during the beginning of the nuclear division as in the case of *A. rhysodes* and *A. culbertsoni*. The chromatic material moves to the centre and the nucleous disappears (Fig. 192).

Metaphase

A solid band of chromatic material can be seen at metaphase stage (Fig. 193).

Anaphase

The globular spindle gradually becomes very elongated and thinner. The fibre in the spindle are not very distinct. At late anaphase the nuclear membrane also disappears. The other stages of anaphase are the same as described for *A. rhysodes* and *A. culbertsoni* (Figs. 194 to 197).



Figs. 191 to 199 Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 191 : Ordinary individual showing the structure of resting nucleus.
Figs. 192 to 199 : Successive stages in division.

Telophase

A constriction appears at the middle of the elongated amoeba and the two daughter individuals are produced (Figs. 198 and 199). A new nucleus is formed as seen in the resting nucleus (Fig. 199).

Critical comments

All the three species of amoebae (*A. glebae*, *A. culbertsoni* and *A. rhysodes*) have exactly similar locomotive form and behaviour. Cysts of *A. glebae* are spherical or somewhat oval. The wall seems to be formed of two layers, with a thick smooth outer one. No pore could be seen in the cyst. *A. culbertsoni* cysts are rounded or oval and have two walls. The outer wall in some cyst is nearly circular, in others it is irregular in outline. Most of the cysts are perforated by one or more pores plugged by a structureless substance. The inner wall is in contact with the outer wall at the point of the pore. In the case of *A. rhysodes* cysts are very variable in size and shape each consists of two walls, irregular in outline and with a wrinkled appearance. The outer wall has folds and ripples and is irregularly stellate, with truncated rays or irregularly polyhedral in appearance. The two walls are in contact at places along the inner wall, where pores plugged with structureless substances are present.

It is clear from the cystic characters from *A. glebae* that it cannot be included in the genus *Acanthamoeba* as defined by Page (1967b). As suggested by Singh and Hanumaiah (1979) the genus *Acanthamoeba* should be recognized only on locomotive form and behaviour of amoebae and not on cystic character.

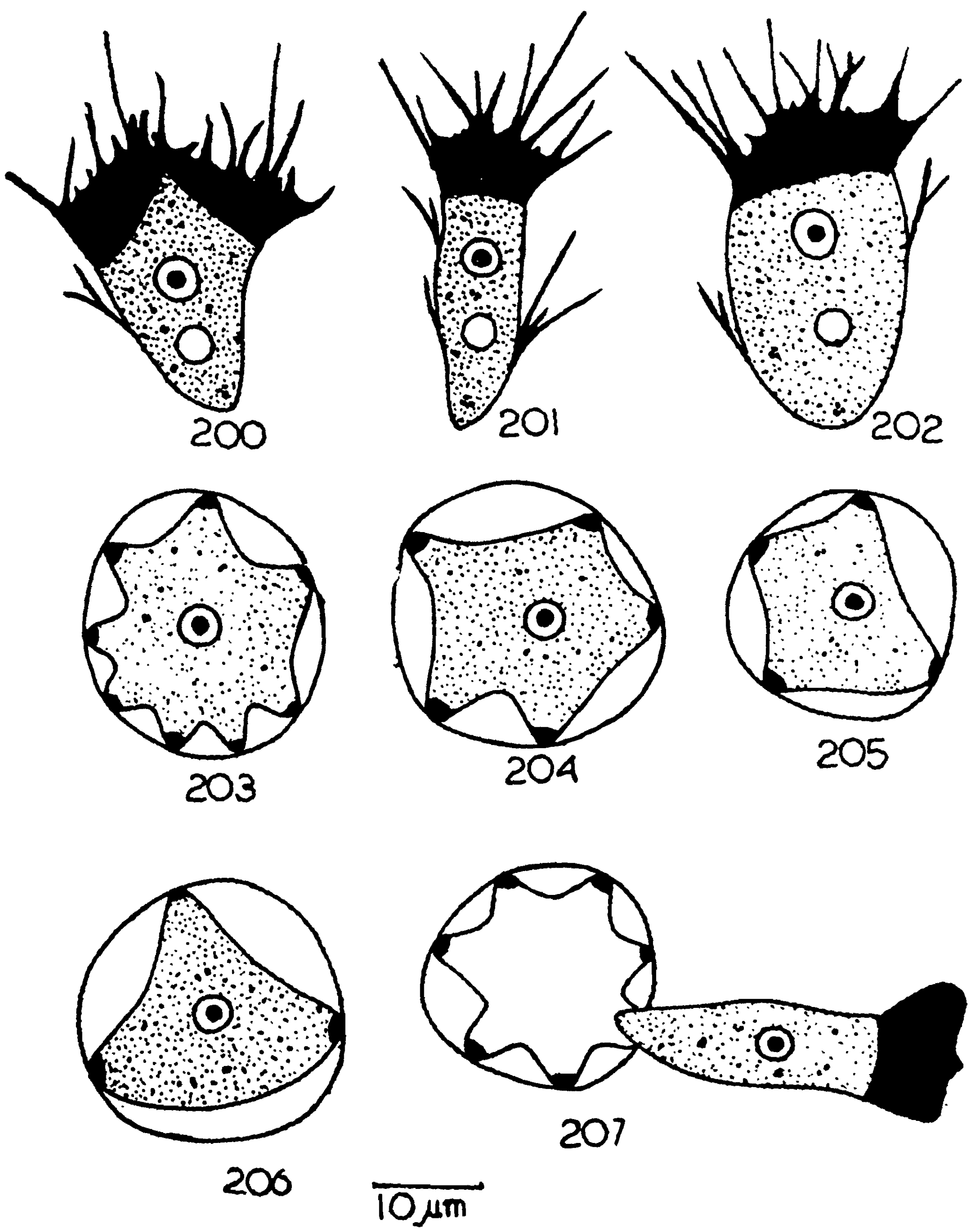
Acanthamoeba astronyxis (Ray & Hayes)

1. 1952. *Acanthamoeba astronyxis* Singh, Phil. Trans. Roy. Soc. Lond., B **236** : 405.
2. 1954. *Hartmannella astronyxis* Ray and Hayes, J. Morph., **95** : 159.

Ray and Hayes (1954) first described *A. astronyxis* (*H. astronyxis*). This amoeba has been isolated by Singh and Hanumaiah (1979) from Central Drug Research Institute, Lucknow, garden soil and from Baroda sewage sample. In the present work *A. astronyxis* has been isolated from the three different areas of West Bengal soils. The soils of Hooghly district, Murshidabad district and Malda district of West Bengal. The localities were respectively Mayapur, Khagra and English Bazar. The respective collection times were 5.6.79, 2.1.78 and 3.7.79 and the recorded soil temperatures were 28°C, 28°C and 30°C (Table 2).

Morphology

The locomotive form and behaviour of *B. astronyxis* (Figs. 200 to 202) are in no way different from the other species of *Acanthamoeba* described earlier. It has a single nucleus and contractile vacuole. The length of the amoebae in active locomotion has a range of 28.0 to 36.6 μm, mean length is 31.22 μm with standard error of mean is ± 0.59. The diameter of the contractile vacuole has a range of 7.5 to 7.0 μm (Table 3), amoebae move at a rate of 15.4 to 44.8 μm per minute.



Camera lucida drawings of *Acanthamoeba astronyxis*.
Figs. 200 to 207 : Drawn in the living condition.
Figs. 200 to 202 : Trophic forms.
Figs. 203 to 206 : Cysts.
Fig. 207 : Excystment stage.

Living cysts of *A. astronyxis* are very characteristic as described by Ray and Hayes (1954). They are uniform, consistent, distinctively biconvex, with the two walls in contact at a variable number of points sealed by parabolic opercula (Pl. III Fig. 1). The inner wall is star shaped (Figs. 203 to 206 and Pl. III Fig. 2). The range of diameter of the living cyst is 21.0 to 25.2 μm, the mean diameter is 22.76 μm with a standard error of mean $\pm$ 0.51 (Table 4).

During excystation the amoeba comes out through a pore (Fig. 207).

Repeated efforts to produce a temporary flagellate stage have completely been failed.

The resting nucleus

The resting nucleus in living condition and in stained preparation is similar to that described in other *Acanthamoeba* species earlier.

Mitotic division

As in the case of other *Acanthamoeba* species the amoebae become rounded at the beginning of the nuclear division. A detail description of nuclear division of *A. astronyxis* is given by Ray and Hayes (1954). This is similar to that found in *A. rhysodes* and other species of *Acanthamoeba*. Therefore the stages of nuclear division has not been presented in this work.

Critical remarks

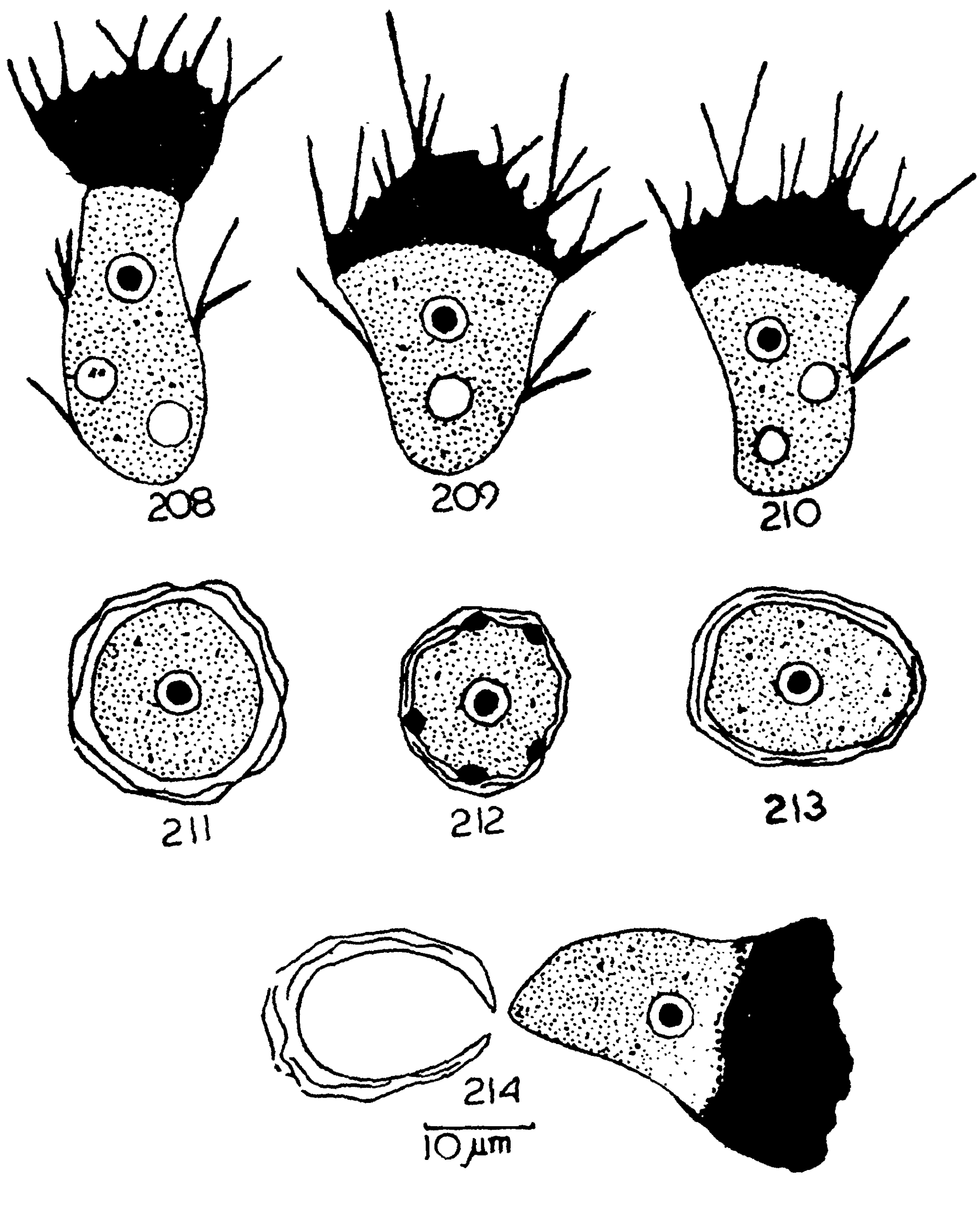
Ray and Hayes (1954) opine that with the change in physical and chemical condition of the environment the amoebae assume various shape. This is not true even when the locomotive form and the behaviour of *A. astronyxis* are studied under similar physical condition and physiological state. Page (1967b) found that *A. astronyxis* has similar locomotive morphology as other species of *Acanthamoeba*. This is in accord with the findings of Singh and Hanumaiah (1979) and the observations reported in this work.

A. astronyxis can be very easily distinguished from all the known species of *Acanthamoeba* on cystic character. *A. astronyxis* is serologically distinct from *A. rhysodes*, *A. culbertsoni* and *A. castellanii* as judged by immobilization reaction (Adam, 1964).

Acanthamoeba palestinensis (Reich)

1. 1933. *Mayorella palestinensis* Reich, Arch. Protistenk., **79** : 76.
2. 1952. *Acanthamoeba palestinensis* Singh, Phil. Trans. Roy. Soc. Lond., B, **236** : 405.

Reich (1933) had described *Acanthamoeba palestinensis (Mayorella palestinensis)* from soil in Palestine. In the present work this amoeba has been isolated from uncultivated field soil of 24 Parganas district, West Bengal. The locality was Ganga Sagar, Sagar Island. The collection time of soil was 18.1.79 and recorded soil temperature was 32°C (Table 2).



Figs. 208 to 214 : Drawn in the living condition.
Figs. 208 to 210 : Trophic forms.
Figs. 211 to 213 Cysts.
Fig. 214 : Excystment stage.

Morphology

The locomotive form and behaviour, structure of the resting nucleus in the living (Figs. 208 to 210) and in stained preparations (Fig. 215) are similar to those described earlier for other species of *Acanthamoeba*. The length of amoebae in active locomotion has a range of 16.8 to 22.4 μm, the mean length is 19.6 μm with standard error of mean ± 0.69. The diameter of the nucleus has a range of 0.7 to 1.4 μm. The mean diameter of contractile vacuole is 3.5 μm (Table 3). The amoeba moves during active locomotion at a rate of 12.6 to 42.0 μm per minute.

The living cysts are very variable in size (Figs. 211 to 213), consist of two walls. The ectocyst is fairly thick and very irregular and wrinkled (Pl. III Figs. 3 and 4). The great majority of the cysts, but not all of them appear to be pierced by one or more pores plugged with a structureless substance. The inner wall sometimes remains in contact with the ectocyst at the point of the pore. The nucleolus is distinct, but no chromatic granules could be seen. There is usually one nucleus, rarely two. The living cyst has a range of diameter from 11.2 to 15.4 μm. The mean diameter is 13.7 μm with the standard error of mean ± 0.57 (Table 4). The amoebae emerge during excystation through a pore. The outline of the cyst wall and the pore can be seen after the emergence of the amoebae (Fig. 214).

Efforts to produce temporary flagellate stage have completely been failed.

The resting nucleus

The structure of resting nucleus in living and stained preparation is same as described in *A. rhysodes*.

Mitotic pattern

The different stages of nuclear division of *A. palestinensis* has been given in detail by Singh and Das (1970). Similar pattern of nuclear division has been found in this work (Figs. 216 to 224). The nuclear mitosis is similar to that found in other species of *Acanthamoeba*.

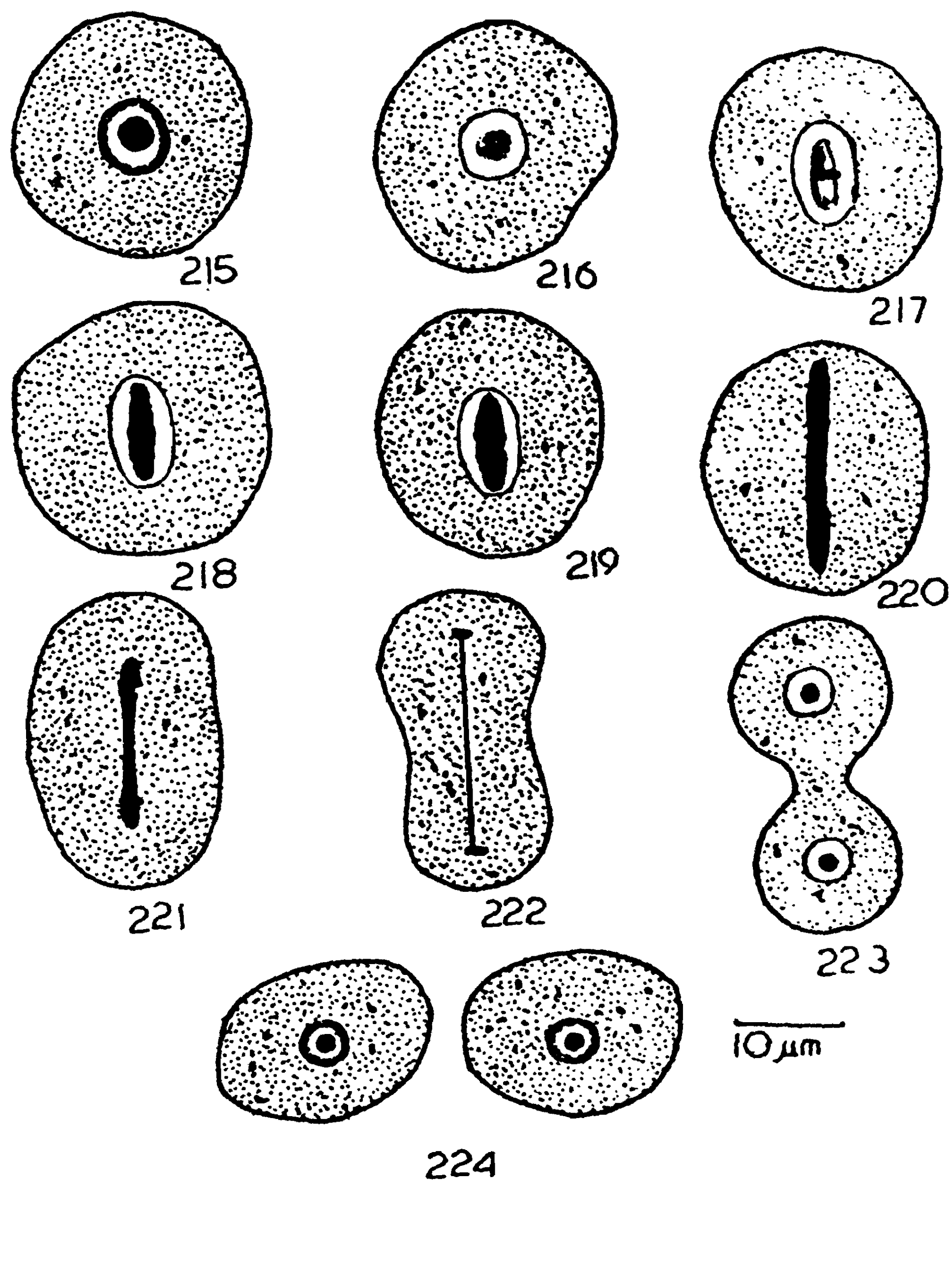
Remarks

Adam (1964) found that *A. palestinensis* was serologically unrelated to *A. rhysodes* and *A. culbertsoni* but was related to *A. castellanii* as judged by immobilization reaction. Siddiqui and Balamuth (1966) found some serological relationship between *A. rhysodes* and *A. palestinensis* by the use of diffusion precipitation and fluorescent-antibody technique. In our opinion the cyst characters are adequate to distinguish *A. palestinensis* as a distinct species.

Genus **Echinamoeba** Page, 1975, emend. Singh and Hanumaiah, 1979

Definition

The resting nucleus contains a central Feulgen-negative nucleolus. During mitosis the nucleolus disappears and spindle with chromosome arranged as an equitorial plate is formed. Amoebae in locomotion have usually flattened form and bear several to many finely pointed, non-anastomosing pseudopodia or micro-spines produced from anterior hyaline zone.



Figs. 215 to 224 Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 215 : Ordinary individual and the structure of the resting nucleus.
Figs. 216 to 224 : Successive stages in division.
Camera lucida drawings of *Echinamoeba exundans*.

Echinamoeba exundans (Page)

1. 1967. *Hartmannella vermiformis* Page, J. Protozool., **14**: 499.
2. 1976. *Echinamoeba exundans* Page, An illustrated key to freshwater and soil amoebae. Ambleside : Freshwater Biological Association.

A strain of *Hartmannella vermiformis* was isolated by Page (1967a) from the city lake, Tuskegee, Albama. This amoeba was later put in the genus *Echinamoeba*. *E. exundans* has been found to be a very common amoeba and has been isolated from Central Drug Research Institute, Lucknow garden soil, from Gomti river mud, from sewage sludge samples in Lucknow and from garden soil in Baroda by Singh and Hanumaiah (1979). In the present work this amoeba has been isolated from different sources. From the soils of Midnapur district and Cooch Behar district of West Bengal. The localities were Digha 'Jhau Forest' and Brahamanpara. The collection time were respectively 1.8.79 and 30.11.79. The type of vegetation of soils were paddy and bettle nut. The recorded soil temperatures were 30°C and 31°C (Table 2).

Morphology

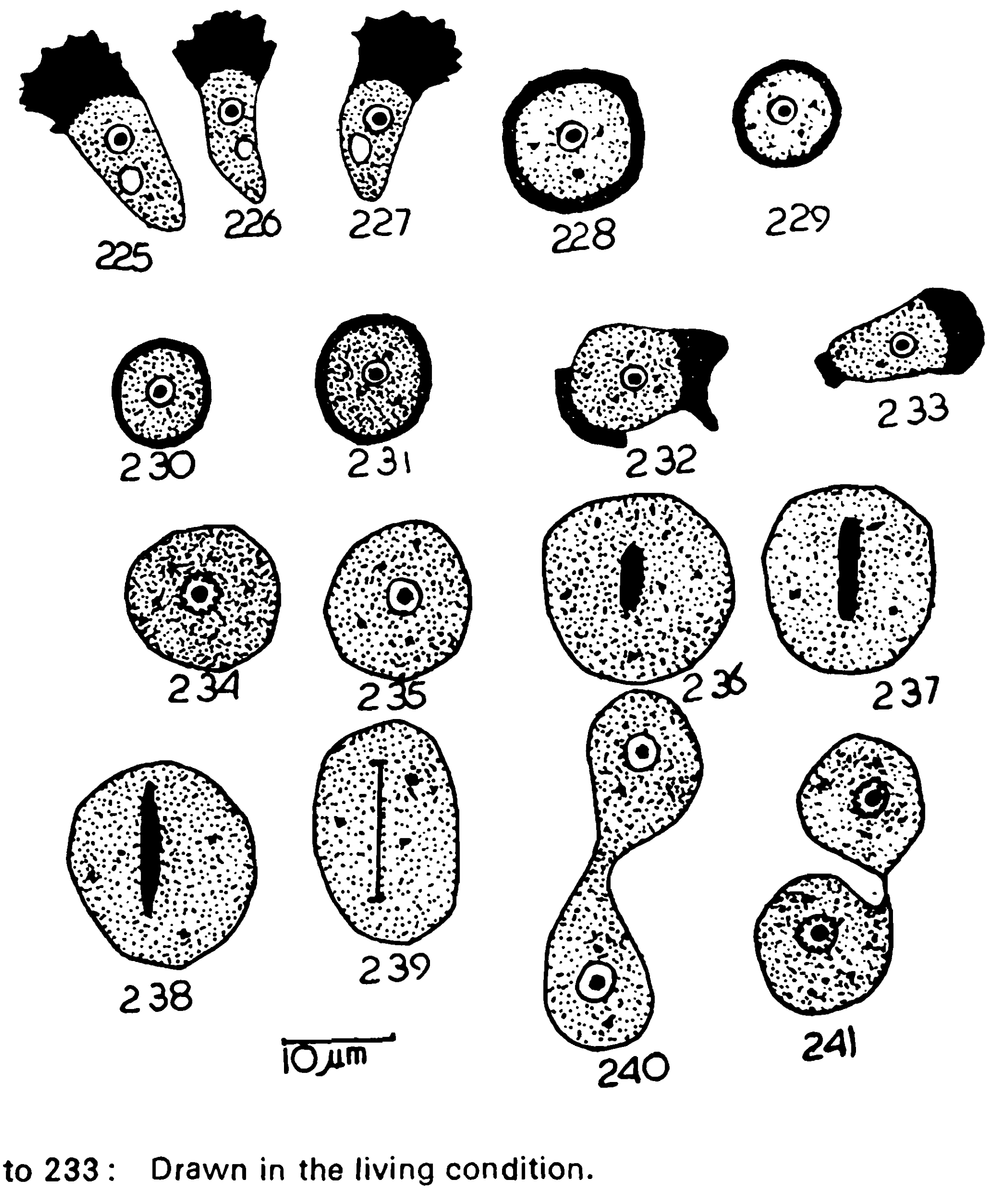
The amoebae are variable in size (Figs. 225 to 227). During locomotion they have usually flattened forms and ectoplasm has irregular border with short spine-like processes or micro-spines. Occasionally limax-like form is assumed and no micro-spine could be seen. Change of direction is usually by expansion of anterior end into new direction. There is a well defined ectoplasm and endoplasm. A single nucleus and a contractile vacuole is present in an individual. The length of the amoebae in active locomotion has a range of 15.4 to 22.4 μm. The mean length is 19.88 μm with a standard error of mean $\pm$ 0.71 (Table 3). Amoebae during locomotion move at a rate of 25 to 70 μm per minute. The cysts are circular or spherical with a single wall having an outer gelatinous layer (Figs. 228 to 231). A cyst contains a single nucleus with a nucleolus. The range of diameter of the cyst is 5.6 to 8.4 μm with a mean diameter 6.72 μm. The standard error of mean is $\pm$ 0.45. The mean diameter of the cyst nucleus is 1.05 μm.

During excystation the cyst is softened at a place and amoeba is attached to the remainder of the cyst wall (Fig. 232). Finally the entire cyst wall gets dissolved (Fig. 233).

The amoebae did not produce flagellate stage by the method described in this work.

The resting nucleus

The resting nucleus in the living condition consists of a central spherical nucleolus and no chromatic granules could be seen (Figs. 225 to 227). In stained preparation there is a nucleolus and chromatin granules lie beneath the nuclear membrane (Fig. 234).



Figs. 225 to 233 : Drawn in the living condition.
Figs. 225 to 227 : Trophic forms.
Figs. 228 to 231 : Cysts.
Figs. 232 & 233 : Excystment stages.
Figs. 234 to 241 : Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 234 : Ordinary individual and structure of the resting nucleus.
Figs. 235 to 241 : Successive stages in division.
Camera lucida drawings of *Vannella cutleri*.

Mitotic division

The amoebae become rounded and motionless at the beginning of mitotic division. The nuclear division resembles that found in *H. vermiformis* described earlier.

Prophase

The chromatin granules move to the centre and fuse (Fig. 235). The nucleolus gradually disappears probably giving rise to the spindle.

Metaphase :

The nuclear membrane disappears at metaphase stage. Chromosomes could not be distinguished (Fig. 236).

Anaphase

The spindle gradually becomes elongated and the band of chromatin divide into two equal halves (Fig. 237). The parts of spindle which lie between the chromosomes and poles stain more deeply. They become smaller and smaller as the chromosomes move to the two poles and finally disappear (Figs. 238 and 239).

Telophase

A constriction appears from the middle of the elongated amoebae and two daughter individuals are produced (Figs. 240 and 241).

Critical remarks

Singh and Hanumaiah (1979) pointed that the stages of nuclear division in *E. exundans* given by Page (1967a) are poor. The observations of the nuclear division given by Singh and Hanumaiah (1979) have been confirmed in the present study.

Genus **Vannella** Bovee, 1965, emend. Singh and Hanumaiah, 1979

Definition

Resting nucleus has a central Feulgen-negative nucleolus. During mitosis the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed. Amoeba in active locomotion fan-shaped or ovoid with flattened, hyaline ectoplasmic veil preceeding raised endoplasmic hump. Breadth of the amoebae usually greater than the length No longitudinal, lateral folds or wrinkles or dorsal ridges are present.

In *V. platypodia*, described by Singh and Hanumaiah (1979), radiate floating forms were present, while those were absent in *V. cutleri*. Thus this character used by Bovee, 1965 to define the genus *Vannella*, does seem to have any value either for generic or specific identification, as pointed out by Singh and Hanumaiah (1979).

Vannella cutleri Singh and Hanumaiah

1. 1979. *Vannella cutleri* Singh and Hanumaiah. Monograph No. 1 of the Association of Microbiologists of India. Published by Indian J. Microbiol., 1-80

This amoeba was first isolated from Chinhat lake mud, from Baroda garden soil and from Mullah river mud in Poona, India, by Singh and Hanumaiah (1979). In the present work this amoeba has been isolated from Purulia district, West Bengal. The locality was Purulia district town. The soil was unmanured and the vegetation was grassy. The collection time of the soil was 5.9.78 and the recorded soil temperature during collection was 31°C (Table 2).

Morphology

During active locomotion on glass-surface, the amoebae are often fan-shaped, spatulate or ovoid, with broad flattened, hyaline ectoplasmic veil preceeding raised endoplasmic hump. There is some tendency for the breadth to be greater and the amoebae frequently change their shape. No uroidal filaments are present (Figs. 242 to 246). Locomotion is by flow into ectoplasmic region. The change in direction is by lateral expansion of ectoplasmic zone. No radiate floating form is produced by this amoeba. There is a single contractile vacuole and a nucleus in an individual. Two nuclei in an amoebae are rare. During locomotion amoeba moves at a rate of 19.6 to 28.0 μm per minute. The length of the amoebae have a range of 8.4 to 19.6 μm with a mean length 14.14 μm and standard error of mean is $\pm$ 1.42. The mean diameter of nucleus and contractile vacuoles were respectively 2.1 μm and 1.4 μm (Table 3).

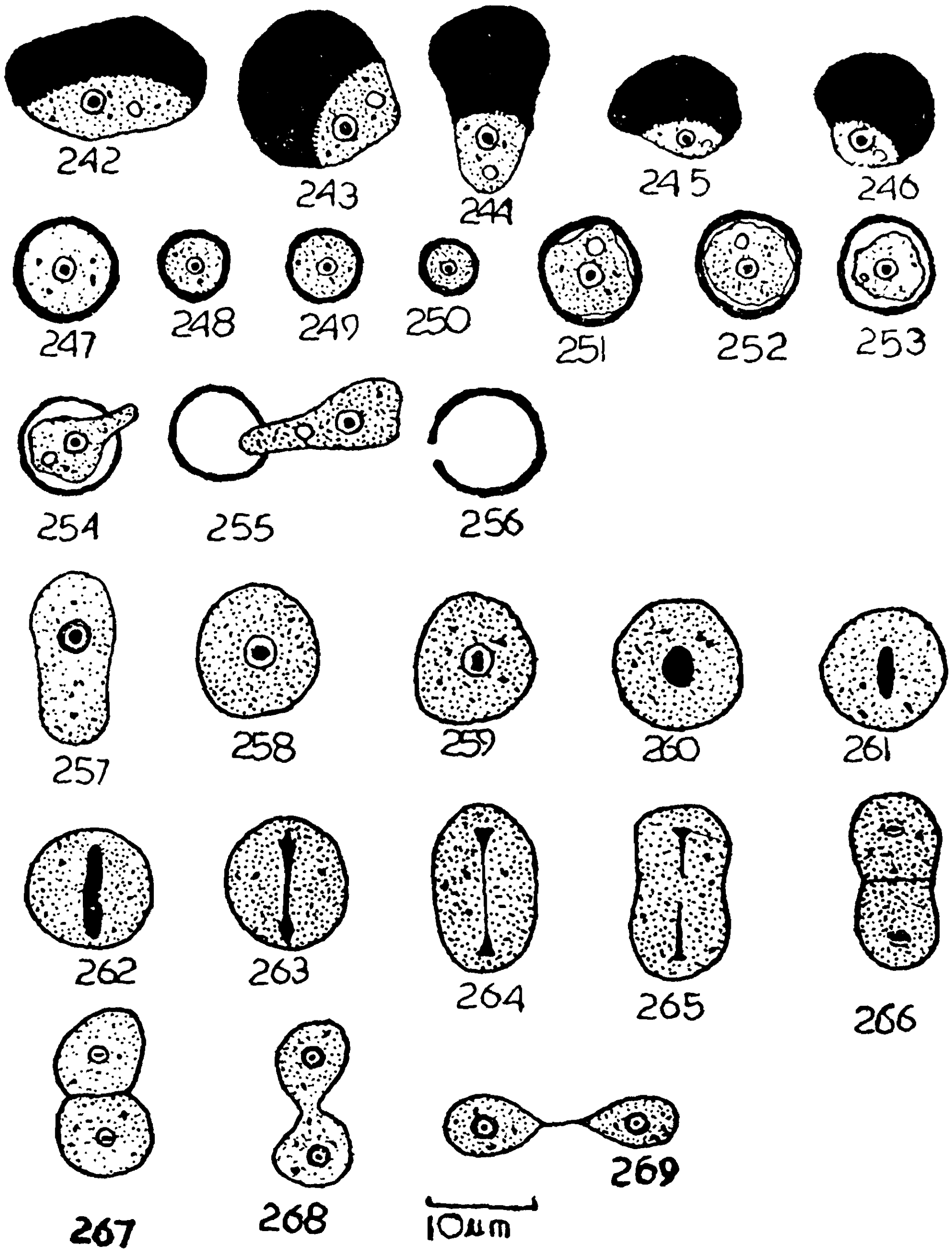
The cysts are variable in sizes and spherical having a single cyst wall with an outer gelatinous layer (Figs. 247 to 250 and Pl. III Fig. 6). The diameter of cysts has a range of 4.2 to 7.0 μm with a mean diameter 4.9 μm and standard error of mean is $\pm$ 0.31 The range of diameter of cyst nucleus is 0.7 to 1.4 μm. The mean diameter of cyst-nucleus is 1.05 μm.

During excystment, the amoeba detaches itself from the cyst wall and moves inside it. As there is no pre-formed pore, it seems that some digestion of the cyst wall occurs. The outline of the cyst wall and the place of emergence of the amoeba can be seen in the empty cyst (Figs. 251 to 256).

Repeated effort to produce a temporary flagellate stage have completely been failed.

Mitotic division

The resting nucleus in the living condition and in stained preparation is same as described for *E. exundans*. The amoebae become rounded off and motionless at the beginning of nuclear division. The mode of nuclear division in general is the same as described for *E. exundans* and for different species belonging to the genus *Acanthamoeba* (Figs. 257 to 269).



Camera lucida drawings of *Vannella cutleri*.

Figs. 242 to 255	:	Drawn in the living condition.
Figs. 242 to 246	:	Trophic forms.
Figs. 247 to 250	:	Cysts.
Figs. 251 to 256	:	Excystment stages.
Figs. 257 to 268	:	Fixed in Carnoy and Stained with Iron-Alum Haematoxylin.
Fig. 256	:	Ordinary individual and structure of the resting nucleus.
Figs. 257 to 269	:	Successive stages in division.

Remarks

V. cutleri differs from *V platypodia* and other species of *Vannella* in producing cysts in culture. The *Vannella* species have not been found to produce cyst under cultural condition.

CRITICAL REMARKS ON THE INCLUSION OF THE GENERA IN THE FAMILY HARTMANNELLIDAE

Alexeieff (1912a) created the genus *Hartmannella*, the members of which did not produce polar masses during nuclear mitosis He put *Amoeba hyalina* (Dangeard, 1900) as the type species of the genus *Hartmannella* based on nuclear division. Volkonsky (1931) erected a sub-family Hartmannellinae in the family Amoebidae to include only those amoebae whose resting nucleus contains a single nucleolus. He placed in this sub-family Hartmannellinae, *Hartmannella* (Type *H. glebae* Dobell 1914) in which the spindle is barrel-shaped or cylindrical and the cyst wall is smooth or slightly folded ; *Glaeseria* (Type *G. testadinis* Ivanic, 1926), with the same spindle shape, but with division occuring in the cyst, and an *Acanthamoeba* (Type *A. castellanii* Douglas, 1930) for amoeba with conical, pointed-ended spindle and rough cyst wall. Singh (1952) who emended the genus *Hartmannella* Alexeieff, 1912 also recognized it on mitotic division pattern. He included in this genus those amoebae whose resting nucleus contains a single Feulgen-negative nucleolus, and pass through a mitotic process in which the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate develops. Singh (1952) agreed with the erection of the genus *Glaeseria* on the cyst character. He pointed out that spindle shape and cyst character were inadequate for separating the genus *Hartmannella* from *Acanthamoeba*, and discarded the later genus. Singh (1952) also raised the sub-family Hartmannellinae to the status of a family Hartmannellidae, considering that the form of nuclear division justified this. Ray and Hayes (1954) and Culbertson (1971) have accepted Singh's terminology. Pussard (1965) while agreeing with Singh in considering spindle shape as unsatisfactory for generic differentiation, recognised the genus *Acanthamoeba* on the basis of its cyst structure, which he thought to be a decisive character. Page (1967a, b) also considered spindle shape to be a doubtful character for generic differentiation. He (1967a, b, 1968, 1974) has recognized the genus *Acanthamoeba* and has distinguished it from *Hartmannella*, both having similar nuclear divisional pattern, on locomotive form and behaviour and cystic character of amoebae. According to him, amoebae in *Acanthomoeba* during active locomotion on glass surface have broad anterior hyaline lobopodium from which are produced singly or twos or threes several or many, hyaline projections (Acanthapodia). Cysts are polyhedral or thickly bi-convex, wall consisting of more or less polygonal or stellate endocyst and more or less rippled ectocyst excystment takes place by removal of operculum at point of contact between endocyst and ectocyst. Amoebae having limax locomotive forms and moving usually by steady flow and with cyst wall smooth and rounded, where known, were included by Page (1967a) in the genus *Hartmannella*. Singh and Das (1970) and Singh (1975) pointed out that *A. glebae* Dobell, 1914 does produce acanthopodia, but the cysts are rounded or spherical with smooth wall and without pores or opercula. Page (1976a) in his revised classification of amoebae still insists to include both locomotive form and cyst character to define the genus *Acanthamoeba*. Page (1967a) has recognized *H. hyalina* as the type species of the genus *Hartmannella* created on locomotive form and behaviour of amoebae, although Alexeieff (1912a) recognized it on nuclear mitosis. Singh and Das (1970) and Singh (1975) pointed out that Dangeard's (1900) description, based entirely on fixed preparations doesn't refer to locomotion. Moreover, it is impossible to tell whether Dangeard was dealing with an amoeba that had limax locomotive form or had acanthopodia, or some other type of locomotive behaviour. Amoebae having several types of locomotive form have been found to have similar mitotic pattern, as found in *Hartmannella* and *Acanthamoeba* [see Singh and Hanumaiah (1979)].

Two principal systems of classification for amoebae without test have been advocated. One is based on locomotive form and behaviour and the other on patterns of nuclear divisions. Recently Singh and Hanumaiah (1979) have united both the systems in a single scheme. This system of classification throws light on the possible evolution of amoebae from flagellate ancestors. Singh and Hanumaiah (1979) have recognized both the genera, *Hartmannella* and *Acanthamoeba* only on locomotive form and behaviour of amoebae. They have described a new highly pathogenic amoeba, *A. invadens*, similar in locomotive behaviour and cystic character to non-pathogenic amoeba *A. glebae*. Singh and Hanumaiah (1979) have included the genera *Acanthamoeba* and *Hartmannella* in the family Hartmannellidae Volkonsky, 1931 emend. Singh, 1952. Amoebae belonging to the genera *Acanthamoeba* and *Hartmannella*, as judged by their locomotive behaviour, have also been found by Visvesvara and Balamuth (1975) to differ serologically and nutritionally. In the present work the characterisation of the genera *Acanthamoeba* and *Hartmannella* has been based as defined by Singh and Hanumaiah (1979). The genera *Echinamoeba* and *Vannella* have also been recognized in this work on the characters of the genera described by Singh and Hanumaiah (1979).

The inclusion in the family Thecamoebidae, of the genera *Sappinia, Thecamoeba, Vannella, Platyamoeba* and *Pessonella* based on locomotive form and behaviour as done by Page (1976a) does not perhaps reflect the possible phylogenetic system of classification of amoebae. Singh and Hanumaiah (1979) found that the nuclear division in *S. diploidea* was by the formation of 'polar masses' Therefore, they have placed the genus *Sappinia* under the family Schizopyrenidae. Singh and Hanumaiah (1979) found that the amoebae placed in the genus *Thecamoeba* Fromental, 1874 divide either by the formation of 'polar masses' or mesomitotic way. They have, therefore, put some species belonging to the genus *Thecamoeba* in the family Schizopyrenidae and the other species in the family Hartmannellidae and Singh *et. al.* (1981) have divided the genus *Thecamoeba* Fromental, 1874 into eight genera. Singh and Hanumaiah (1979) have recognized the genera *Vannella, Platyamoeba* and *Pessonella* on locomotive form and behaviour, but have included them in the family Hartmannellidae because the amoebae placed in them have mesomitotic pattern of nuclear division. This system of classification throws light on the probable evolution of these amoebae. Pussard (1973) also holds the view that genera with different mitotic pattern cannot belong to the same family.

BEARING OF NUCLEAR DIVISION ON THE POSSIBLE PHYLOGENETIC CLASSIFICATION OF AMOEBAE

Singh (1952) was the first to point out that the classification of amoebae on nuclear division patterns into families Schizopyrenidae and Hartmannellidae throws light on the possible evolution of amoebae. A form such as *N. gruberi* which during division has 'polar masses' and 'interzonal bodies' and can readily produce temporary flagellate stage, may be regarded as a primitive amoeba. During the course of evolution it seems that amoebae lose the power to produce 'interzonal bodies' although they are able to produce temporary flagella, as in the case in *D. thorntoni*. In a form like *S. russelli* no temporary flagellate stage is produced, although the nuclear division of this amoeba is indistinguishable from *D. thorntoni*, as are the active forms. Thus the primitive character of temporary flagella production in *S. russelli* is lost, but it retains the family character in having 'polar masses' In Hartmannellidae the Feulgen-negative nucleolus or nucleoli and the nuclear membrane disappear during the nuclear division, as happens in *A. glebae (H. glebae)*, *A. proteus* and *P. carolinensis.* A spindle with chromosomes arranged as an equatorial plate is formed, as in higher animals and plants. Judging from the advanced type of nuclear division in amoebae put in the family Hartmannellidae and the loss of the flagellate stage, it seems that amoebae are evolved from flagellate ancestors and not the flagellates from amoebae. Evidence, however is still too fragmented to allow a reconstructed phylogeny and it is safe to say that the assemblage is polyphyletic (Schuster, 1979 Bovee & Sawyer, 1979) with representative types having originated from different ancestral lineages. Presumably amoeboflagellates represent forms which have retained the ability to revert to this ancestral stage, giving rise to plastic flagellates (as in *Naegleria*) or flagellates with a highly organized cytoskeletal framework and a cytostome (as in *Tetramitus* or *Paratetramitus*). There is even less to say concerning the ancestry of *Acanthamoeba* and *Hartmannella*. Their mitotic patterns suggest an origin from a flagellate stock different from the one that gave rise to the amoeboflagellates (Schuster, 1979).

It may be pointed out that there is no justification to show an amoeba dividing by amitosis or in some such primitive way as was thought about hundred years ago. This is supported by the contributions of Band and Mohrlok (1973a, b) and Band *et al.* (1970). They have shown the methods for inducing cell division without mitotic nuclear division (amitosis). The induced amitotic cell division is similar to normal cytokinesis. However, the nucleus is partitioned during its interphase state so that the daughter products of amitosis are not viable. This is not a normal stage in the amoeba's life cycle.

At present three main types of nuclear divisions are known as pointed out by Singh (1952) and Singh and Das (1970). In the first group Feulgen-negative nucleolus or Feulgen-negative nucleoli give rise to 'polar masses' and the nuclear membrane nuclear division. The genera *Sappinia*, *Thecamoeba*. *Vannella*, *Platyamoeba* and *Pessonella* of the family Thecamoebidae, based on locomotive form and behaviour, as done by Page (1976a) perhaps does not have any phylogenetic significance. Singh and Hanumaiah (1979) found that the nuclear division in *S. diploidea* was by the formation of 'polar masses', they place the genus *Sappinia* in family Schizopyrenidae. Amoebae having thickened pellicle, and being placed in the genus *Thecamoeba* Fromental, 1874, are remarkable in having a great variety of nuclear structure. Although there is no sharp demarcation in their rugose or smooth character, Jahn *et al.*, (1974) have not only put the rugose and the smooth species into different genera but into different sub-orders. Page and Blakey (1979) studied the cell surface structure of eight species of *Thecamoeba* by electron microscope. There was no significant differences in the cell surface structures among seven species. Based on the presence of non-mucoid tegument in *Thecamoeba granifera* Page and Blakey (1979) have erected a new genus, *Dermamoeba*, for its reception. Singh *et al.* (1981) based on the stable characters of the resting nucleus and the mitotic pattern in thecamoebae, have sub-divided genus *Thecamoeba* Fromental, 1874 into eight genera, and have placed them either in Schizopyrenidae or in Hartmannellidae according to the pattern of their nuclear division. It is of interest to point out that since the amoebae having dermal thickening divide either promitotically or mesomitotically, it seems that they have been evolved from different ancestors. The possession of dermal thickening may be an acquired persists through out division. This type of division has been termed by some workers as promitosis. In the second group Feulgen-negative granules or nucleoli do not give rise to 'polar masses' and the nuclear membrane remain intact during division. In the third group Feulgen-negative nucleolus or Feulgen-negative nucleoli and the nuclear membrane disappears during mitosis. This type of division has been termed by Chatton (1953) as mesomitosis. Earlier workers reported centrioles during nuclear division in certain amoebae, but subsequent investigations including ultrastructural studies, did not reveal the presence of classical centrioles giving rise a mitotic figure like that in metazoans. Therefore, it is not proper to designate a nuclear division pattern in any amoebae as metamitosis. A few workers have more recently reported centrospheres in *Acanthamoeba* which has mesomitotic pattern of nuclear division (see Pussard, 1973). Singh and Hanumaiah (1979) have not used centrosphere as a character for identifying amoebae. They have included all the genera of amoebae showing mesomitotic pattern of nuclear division in the family Hartmannellidae. The same system has been adopted in the present work.

The nuclear division in amoebae having limax locomotive forms are of three distinct types, as found in Schizopyrenidae, Endamoebidae and Hartmannellidae, suggesting that they have been evolved from different ancestors. Singh and Hanumaiah (1979) have, therefore, included different genera of amoebae showing limax locomotive behaviour in different families according to their patterns of character for the survival of these amoebae under adverse conditions.

The nuclear structures and mitotic patterns in thecamoebae bear no relation to their cell surface structure. Therefore. the creation of *Dermamoeba* by Page and Blakey (1979) by cell surface structure may not perhaps be considered of any phylogenetic value.

Singh and Hanumaiah (1979) have recognized the genera *Vannella, Platyamoeba, Pessonella* on locomotive form and behaviour, but have included them in the family Hartmannellidae because the amoebae placed in them have mesomitotic pattern of nuclear division. Pussard (1973) also holds the view that genera with different mitotic patterns can not belong to the same families.

PATHOGENESIS

The study of Culbertson and co-workers (1958) reveals the potency of soil amoebae as pathogens. Hoare (1972) suggested the term 'exogenous amoebiasis' for the disease caused by aerobic free-living amoebae to distinguish it from 'endogenous amoebiasis' caused by anaerobic *Entamoeba histolytica*. Reports implicating these amoebae as the causal agents of a newly described disease of human, primary amoebic meningoencephalitis (PAM), appeared almost instantly (Fowler and Carter, 1965 Butt, 1966 Cerva *et al.*, 1968). Fowler and Carter (1965) were the first to report four fatal human cases of acute pyogenic meningitis caused by free-living amoebae belonging to the genus *Acanthamoeba Hartmannella*).

Human PAM infection have resulted from contact with amoebae in swimming pools (Cerva *et al.*, 1968 Jadin *et al* 1971) lakes (Butt *et al.*, 1968 Callicott *et al.*, 1968 Duma *et al.*, 1969 71) a mineral hot pool (Mandal *et al.*, 1970 Cursons and Brown, 1975) thermal streams (Van Den Driessche *et al.*, 1973 Cursons and Brown, 1976), mud puddles (Apley *et al.*, 1970) and a house hold water supply (Anderson and Jamieson, 1972 a, b). Carter (1970, 72) demonstrated clinical features and pathology of human cases. Human infection is routed through olfactory pathways as a result ofnasopharyngeal contamination with amoebae. Nagington *et, al.* (1974) reported that *Acanthamoeba polyphaga* caused human eye infection leading to blindness.

References on cases of PAM in India are not many. Only three such reports are available so far. The first report was that of Pan and Ghosh (1971) who recorded *Naegleria* from cerebro-spinal fluid of children in Calcutta. The second one was that of Bedi *et al.* (1972) who found one such case caused by unidentified amoebae from a 45 year old hindu villager from Gujarat. Mansurkar *et al.* (1974) studied PAM cases at Joslok Hospital, Bombay, from eight children where the pathogen was identified as *N. aerobia.*

Experimental animal pathogenicity

Culbertson *et al.* (1958) and Culbertson *et al.* (1959) first observed amoebae in brain and spinal cord sections from experimentally infected monkeys and mice which exhibited choriomeningitis and severe destruction lesions. Amoebae were found lying in the inflamatory exudate and also in the apparently normal nerve tissues. They also maintained these amoebae in cell culture fluid containing trypticase broth and inoculated intravenously to the mice and observed pulmonary granulomata containing huge amoebae which cause the death of mice. The work of Culbertson (1961) also revealed extensive haemorrhagic consolidation of lungs and abscess in the left temporal lobe of the brain of monkey by amoebae. Culbertson (1961) inoculated amoebae in two young rabbits which began to manifest the sign of fatality on sixth day. Culbertson *et al.* (1966) and Culbertson (1971) also found few strains of *Acanthamoeba (Hartmannella)* isolated from human nasal swab and soil, were of lower virulence, when given intranasally to mice.

Schuster and Dunnebacke (1977) reported the presence of intranuclear particles of an unknown nature in both amoebae and brain cells of mice dying from PAM. Adams *et al.* (1976) showed that mice which survived intravenous or intraperitoneal infection with *Naegleria,* were resistant to intravenous challenge with pathogens. Formalin-treated or freeze-thawed pathogenic or living non-pathogenic *Naegleria* administered to mice intravenously, protected animals from challenge with pathogens via intranasal or intravenous routs (John *et al.*, 1977). Live *N. fowleri* administered intraperitoneally protected mice from subsequent intranasal instillation of pathogens, with a 27% survival rate (Thong *et al.*, 1978). Amoeba dose and age, sex, and strain of mouse have been found to affect mouse mortality following intravenous inoculation with *N. fowleri* (Haggerty and John, 1978). Some deaths were also reported by Diffley *et al.* (1976) by *Naegleria* amoebae in subcutaneously inoculated guineapigs. Germ-free guineapigs were used by Phillips (1974) in a study in which pathogens were inoculated intranasally, intraorally, into skin lesions and into the conjunctival sac of the eye; the intranasal route caused death and *Naegleria* was found in the brain. Variability has also been encountered in producing mice mortality using pathogenic amoebae with long culture histories (Visvesvara and Callaway, 1974 , De Jonckneere *et al*, 1975 Wong *et al.*, 1977). This is most likely due to loss of virulence upon continued *in vitro* cultivation, which can be restored by repeated animal passage (cf Schuster, 1979).

Singh and Das (1972a, b) have produced PAM in mice using flagellate stage of pathogenic *Naegleria* which, in their opinion, is more likely to be encountered by humans in pools, lakes, etc. Singh and Hurtnumaiah, (1979) has described a species, *A. invadens* causing lethal pathogenicity in mice.

Natural animal infection

McConnell *et al.* (1968) reported *Acanthamoeba* infection in the lung of bovine by the cysts and trophozoites. Dwivedi and Singh (1965) also reported one case of pulmonary lesions in Indian buffalo caused by *Acanthamoeba* sp.

PAM ....Host-parasitse relationship

The early reports of PAM were not specific about the identity of the causal agent, except that it was an amoeba. *Acanthamoeba* was already known as a pathogen and not easily distinguished from *Naegleria*. With continued descriptions of new cases and the isolation of causal agents, it appeared that *Naegleria* was responsible for PAM (Culbertson, 1971). Now with careful analyses of cases, repeated studies of autopsied tissues, and application of immunochemical methods for identification, *Acanthamoeba* has also been identified as a causal agent of PAM (Martinez *et al.*, 1977 Willaert *et al.*, 1978).

a) *Aetiological difference between Naegleria and Acanthamoeba producing PAM*

Differences exist between PAM caused by these two amoebae. Cases of Naeglerial PAM are rapidly fatal, the victims almost invariably having a history of swimming or bathing in water that presumably contained the pathogen. Victims are generally young children, teenagers, or young adults—and in good health (Duma *et. al* 1969, 71). The portal entry of the amoebae is probably across the nasal mucosa, amoebae then migrating along the olfactory nerves to the olfactory lobes of the brain where within a matter of days, they proliferate and cause extensive damage to tissue. In contrast, *Acanthamoeba* infections are chronic and not necessarily the result of exposure to swimming or bathing. The portal entry is uncertain but need not be limited to the nasal passages and the olfactory mucosa. The invasion of brain tissue is probably secondary to establishment of amoebae at some other points in the body. Victims are of all ages and often reported to be in poor health. A feature useful in distinguishing the two different amoebae in brain tissue is that *Acanthamoeba* encysts, but *Naegleria* does not (cf. Schuster, 1979).

b) *The route of infection*

This experiment has been successfully done by laboratory studies in which mice have been infected by intranasal instillation of amoebae. Schematic pathways are produced by Martinez *et al.* (1973) as follows *Naegleria* infection occurs most likely by a nasal route.

i) Amoebae cross the nasal mucosa ii) invade (or are phagocytosed by) subtentacular cells that make up part of the olfactory epithelium iii) migrating along the olfactory nerves iv) cross the ciribriform plate v) enter the brain cavity.

The mode of entry in human infections is supported by the finding of damage of the olfactory lobes of human PAM victims. Amoebae when in the central nervous system, reproduce, causing extensive damage to host tissue (Martinez *et al.*, 1971 , Schuster and Dunnebacke, 1977 , Visvesvara and Callaway, 1974).

It is not clear till date whether the amoebae penetrate by direct pseudopodial activity, literally showing their way through the neutrophil, or whether their progress is aided by enzymes that act upon the cells, causing lysis. Food vacuoles seen within trophic amoebae in the brain, contain host tissue in various stages of digestion. Large numbers of amoebae come to occupy perivascular spaces and may use them as channels for deeper penetration into the brain. An inflamatory response is suggested by association between amoebae and host phagocytic cells. Germ-free guineapigs when experimentally innoculated with *Naegleria*, using various routes, like skin, mouth, conjunctival sac, nose , the nasal route only yielded positive infection (Phillips, 1974).

SUMMARY

1 A detailed survey on the aerobic small free-living amoebae from different soil types and different localities in West Bengal has been made.

2. Studies on the nuclear division, locomotive form and behaviour, cystic character, excystment and flagellate production have been carried out under standardised and reproducible cultural conditions by a method described by Singh and Hanumaiah (1979), which enables different specimens in all stages of mitosis to be obtained easily.

3. In the family Schizopyrenidae Singh, 1952, emend. Singh and Das, 1970 Feulgen-negative nucleolus or the nucleoli give rise to 'polar masses' and nuclear membrane remains intact during mitosis. *Schizopyrenus russelli, Naegleria gruberi, Didascalus thorntoni* and *Tetramitus rostratus* have been described.

(i) In *Schizopyrenus russelli*, nuclear division takes place by the formation of 'polar masses' no flagellate stage is found the amoebae progress in a limax-like manner. Cysts consist of two walls. During excystment the inner wall is dissolved first and the amoeba moves inside the outer wall. The amoeba comes out probably by digestion of cyst wall.

(ii) In *Naegleria gruberi*, the nuclear division takes place by the formation of 'polar masses' Feulgen-negative 'interzonal bodies' are present during the late stages of nuclear division. Temporary flagella are produced. The amoebae have limax locomotive form with noticeable eruptions. The cysts have pores plugged with some structureless substances. During excystment, amoeba comes out through one of these pores.

(iii) In *Didascalus thorntoni*, the nuclear division takes place by formation of 'polar masses' without 'interzonal bodies' Temporary flagella are produced and no division takes place in flagellate state. The amoebae have limax locomotive form. The cyst consists of single wall with an outer gelatinous layer. During excystment amoeba detaches itself from the cyst wall. As there is no pre-formed pore, some digestion of the cyst wall occurs and the cyst is eventually dissolved after the emergence of the amoebae.

(iv) In *Tetramitus rostratus* 'polar masses' and 'interzonal bodies' are present during nuclear division both in the amoeboid and flagellate stages. The flagellate is cone-shaped, having curved cytostomal groove. A cyst has a single wall with an outer gelatinous layer. During excystment the amoeba detaches itself from the cyst wall. As there is no pre-formed pore, so presumably some digestion of cyst wall occurs. The outline of the cyst wall and the place of emergence of amoeba can be seen in the empty cyst.

4. In the family Hartmannellidae, Volkonsky, 1931, emend. Singh, 1952 the Feulgen-negative nucleolus or nucleoli disappear during mitosis and a spindle with chromosomes arranged as an equatorial plate develops. In this family genera *Hartmannella, Acanthamoeba, Echinamoeba* and *Vannella* have been included based on the locomotive form and behaviour of amoebae.

(i) In the genus *Hartmannella* in which the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed during mitosis, and the amoebae show limax locomotive form. *H. vermiformis* and *H. crumpae* have been described.

H. crumpae differs from *H. vermiformis* in cystic character. Cysts of *H. crumpae* are found having a single wall with an outer gelatinous layer. During excystment the amoeba gradually detaches itself from the cyst wall and moves inside it. It comes out by digestion of cyst wall which is eventually dissolved. While cyst of *H. vermiformis* have two walls, the outer being slightly irregular. During excystment the inner wall is dissolved and the amoeba moves inside the outer wall. No pores were observed. Probably the digestion of cyst wall occurs to allow the passage of amoeba to come out.

(ii) In the genus *Acanthamoeba*, the nuclear division is similar to that found in the genus *Hartmannella*. The amoebae in active locomotion produce hyaline projections (acanthopodia). The described species are *A. rhysodes, A. culbertsoni, A. glebae, A. astronyxis* and *A. palestinensis*.

(iii) In the genus *Echinamoeba* the nuclear division is of the same type as described in the genus *Hartmannella*. The amoebae in active locomotion produce several to many finely pointed, non-anastomosing pseudopodia or microspines from anterior hyaline zone. The described species is *E. exundans.*

(iv) In the genus *Vannella*, nuclear division is of the same type as found in the genus Hartmannella. The amoebae in active locomotion are fan-shaped or ovoid with flattened, hyaline ectoplasm. The described species is *V. cutleri*.

5) Critical remarks on the inclusion of the genera in the family Schizopyrenidae and Hartmannellidae have been discussed in detail.

6) The role of nuclear division on the possible evolution of amoebae from flagellate ancestors and not the flagellates from amoebae has been discussed.

7) A brief note on 'exogenous amoebiasis' caused by some aerobic free-living amoebae has been added. Both *Acanthamoeba* and *Naegleria* have so far been identified as causal agents of this newly described disease of human, primary amoebic meningoencephalitis. Human case reports, host-parasite relationships, aetiological differences among *Naegleria* and *Acanthamoeba* regarding PAM, the route of infection, experimental animal pathogenicity and natural animal infection have been discussed.

Acknowledgements

The authors are thankful to the Director, Zoological Survey of India, for offering laboratory facilities to Dr. Prosad Basu and Dr. Mrinal Ghosh to continue the work.

They are deeply indebted to Dr B. N. Singh (deceased) of Central Drug Research Institute, Lucknow, India, for the identification of amoebae and for critical evaluation of the manuscript.

Best thanks are due to Dr. F. C. Page, The Institute of Terrestrial Ecology, The Culture Centre of Algae and Protozoa, Cambridge, England, Dr. S. S. Bamforth, Newcomb College, Tulane University, New Orleans, Louisiana, U. S. A., Dr. M. Pussard, Institut National de la Researche Agronomique, Dijon, France, Dr. E. C. Bovee, Department of Physiology and Cell Biology, U. S. Department of Commerce, Middle Atlantic Coastal Fisheries Center, Oxford, Maryland, U. S. A. for their wise suggestions, valuable comments and for helping with reprints from time to time throughout the study.

Thanks are due to Dr. R. P. Mukherjee, Scientist-in-Charge of Ecology Division, Zoological Survey of India, Dr. R. Roy, Department of Zoology, Darjeeling Government College. West Bengal, and Professor T N. Ghosh of School of Tropical Medicine, Calcutta for their active participation and constructive criticism all through the preparation of this monograph. Dr. A. Das of Protozoology Section, Zoological Survey of India kindly helped while preparing the key portion.

Thankful acknowledgement is offered to the fellow associates of Soil Zoology Laboratory, Zoological Survey of India, and Microbiology Division, Central Drug Research Institute, Lucknow for their sincere help and constant encouragement.

The cooperation of Dr. S. Bandopadhaya formerly of Protozoology Section and Sarbashri K. Dey and T N. Bhattacharjee of Zoological Survey of India for their useful technical assistance is thankfully acknowledged.

References

ADAM, K. M. G. 1964. A comparative study of hartmannellid amoebae. J. Protozool., **11** : 423-430.

ADAMS, A. C., JOHN, D. T and BRADLEY, S. G. 1976. Modification of resistance of mice to *Naegleria fowleri* infections. Infect. Immun., **13** 1387-1391.

ALEXEIEFF, A. 1912a. Sur les caracteres cytologiques et la systematéque des amibes du groupe *limax (Naegleria* nov. gen et *Hartmannia* nov. gen.*)* et des amibes parasites du vértebrés *(Proctamoeba* nov. gen.) Bull. Soc. Zool. Fr., **37** : 55-74.

ALEXEIEFF, A. 1912b. Quelques remarques complementaires sur la systématique des amibes du groupe *limax* Sur le genre *Sappinia* Dangeard. Bull. Soc. Zool. Fr., **37** : 149-168.

ANDERSON, K. and JAMIESON, A. 1972a. Primary amoebic meningoencephatitis. Lancet, **1** : 902-903.

ANDERSON, K. and JAMIESON, A. 1972b. Primary amoebic meningo-encephalitis. Lancet, **2** : 379.

APLEY, J., CLARKE, S. K. R., ROOME, A. P. C. H., SANDRY, S. A., SAYGI, G., SILK, B. and WARHURST, D. K. 1970. Primary amoebic meningoencephalitis in Britain. Br Med. J., **1** : 596-599.

AVERNER, M. and FULTON, C. 1966. Carbon dioxide signal for excystment of *Naegleria gruberi*. J. Gen. Microbiol., **42** : 245-255.

BALAMUTH, W. and KAWAKAMI, T 1967. Antigenic relationship among selected hartmannellid amoebae and amoebo-flagellates. J. Protozool., **14** (Suppl.) : 31.

BAND, R. N. 1959. Nutritional and related studies on the free-living soil amoeba, *Hartmannella rhysodes*. J. Gen. Microbiol., **21** : 80-95.

BAND, R. N. and MOHRLOK, S. 1969. The respiratory metabolism of *Acanthamoeba rhysodes* during encystation J. Gen. Microbiol., **59** : 351-358.

BAND, R. N. and MOHRLOK, S. 1973a. Observations on induced amitosis in *Acanthamoeba*. Exp. Cell Res., **79** : 327-337.

BAND, R. N. and MOHRLOK, S. 1973b. The cell cycle and induced amitosis in *Acanthamoeba*. J. Protozool., **20** 654-657.

BAND, R. N., MOHRLOK, S. and RUBIN, R. W. 1970. Separate induction of amitotic and mitotic division in *Acanthamoeba rhysodes*. Nature, **227** : 379-381.

BATZRI, S. and KORN, E. D. 1975. Interaction of phospholipid vesicles with cells. Endocystosis and fusion as alternate mechanisms for the uptake of lipid-soluble and water-soluble molecules. J. Cell Biol., **66** : 621-634.

BAUER, H. 1967. Ultrastruktur and Zellwandbildung von *Acanthamoeba* sp. Vierteljahresschr. Naturforsch. Ges Zürich, **112** : 173-197.

BEDI, H. K., DEVAPURA, J. C. and BOMB, B. S. 1972. Primary amoebic meningoencephalitis. J. Indian M. A., **58** : 13-14.

BHATTACHARYA, A. and DEY, G. C. 1978. Distribution of soil protozoa in association with nitrogen fixing bacteria. Bull. Zool. Sur. India, **1** (2) : 193-195.

BISHOP, A. and TATE, P. 1939. The morphology and systematic position of *Dobellina mesnili* nov. gen. (*Entamoeba mesnili* Klein, 1917). Parasitology, **31** : 501-511.

BOVEE, E. C. 1953. *Thecamoeba* (*Amoeba*) *corrugata* n. sp. as distinct from *Thecamoeba* (*Amoeba*) *striata*. Penard. Proc. Soc. Protozool., **4** : 14-15.

BOVEE, E. C. 1965. An emmendation of the amoeba genus *Flabellula* and a description of *Vennella* gen. nov. Trans. Am. Microsc. Soc., **84** : 217-227.

BOVEE, E. C. 1970. The lobose amoebas. I. A key to the suborder Conopodina Bovee and Jahn, 1966 and description of thirteen new or little known *Mayorella* species. Arch. Protistenk., **112** : 178-227.

BOVEE, E. C. and JAHN, T L. 1965. Mechanisms of movement in taxonomy of Sarcodina. II. The organization of subclasses and orders in relationship to the classes Autotractea and Hydraulea. Am. Midl. Nat., **73** : 293-298.

BOVEE, E. C. and JAHN, T L. 1966. Mechanisms of movement in taxonomy of Sarcodina. III. Orders, suborders, families and subfamilies in the superorder Lobida. Syst. Zool., **15** : 229-240.

BOVEE, E. C. and JAHN, T L. 1973. Taxonomy and Phylogeny, IN *The Biology of Amoeba* : K. W. Jeon (Ep.), Academic Press, New York and London.

BOVEE, E. C. and SAWYER, T K. 1979. Marine flora and fauna of the Northeastern United States Protozoa : Sarcodina Amoebae. NOAA Technical Report NMFS Circular 419, 1-56.

BOWERS, B. and KORN, E. D. 1969. The fine structure of *Acanthamoeba castellanii* (Neff Strain). I. Encystment. J. Cell Biol., **41** : 786-805.

BOWERS, B. and OLSZEWSKI, T. E. 1972. Pinocytosis in *Acanthamoeba castellanii.* Kinetics and Morphology. J. Cell Biol , **53** : 681-694.

BUNTING, M. and WENRICH, D. M. 1929. Binary fission in the amoeboid and flagellate phases of *Tetramitus rostratus* (Protozoa). J. Morph., **47** : 37-88.

BUTT, C. G. 1966. Primary amoebic meningo-encephalitis. New England. J. Med., **274** : 1473-1476.

BUTT, C. G., BARO, C. and KNORR, R. W. 1968. *Naegleria* (sp.) identified in amoebic encephalitis. Amer. J. Clin. Path., **50** : 568-574.

CALKINS, G. N. 1933. *The Biology of Protozoa.* 2nd ed., Baillière, Tindall and Cox, London.

CALLICOTT, J. H. Jr., NELSON, C. E., JONES, M. M., DOS SANTOS, J. G., UTZ, J. P., DUMA, R. J. and MORRISON, J. V Jr. 1968. Meningo-encephalitis due to free-living amoebae. J. Amer. Med. Ass., **206** : 579-582.

CARTER, R. F. 1970. Description of a *Naegleria* sp. isolated from two cases of primary amoebic meningo-encephalitis, and of the experimental pathological changes induced by it. J. Path., **100** : 217-244.

CARTER, R. F. 1972. Primary amoebic meningo-encephalitis. An appraisal of the present knowledge. Trans. Roy. Soc. Trop. Med. Hyg., **66** : 193-213.

CASAGRANDI, O. and BARBAGALLO, P. 1895. Ricerche biologiche and cliniche sull' *Amoeba coli* (Lösch), (Nota preliminare). Bull. Accad. Gioenia Sci. Nat. Catania, Fasc. XXXIX (N. S.) p. 4

CERVA, L. 1965. Immunological studies of hartmannellid amoebae. Progress in Protozoology, Int. Congr. Ser. no. 91225. Excerpta Medica Foundation, London.

CERVA, L. and KRAMAR, J. 1973. Antigenic relationships among several *limax*-amoebae isolates assessed with the indirect fluorescent antibody test (IFAT). Folia Parasit., **20** : 113-118.

CERVA, L., NOVAK, V and CULBERTSON, C. G. 1968. An outbreak of acute fatal amoebic meningo-encephalitis. Amer. J. Epidemiol., **88** : 436-444.

CHAGLA, A. H. and GRIFFITHS, A. J. 1974. Growth and encystation of *Acanthamoeba castellanii*. J. Gen. Microbiol., **85** : 139-145.

CHAMBERS, J. A. and THOMPSON, J. E. 1972. A scanning electron microscopic study of the excystment process of *Acanthamoeba castellanii.* Exp. Cell Res., **73** : 415-421.

CHAMBERS, J. A. and THOMPSON, J. E. 1974. Age-dependent excystment of the protozoan *Acanthamoeba castellanii.* J. Gen. Microbiol., **80** : 375-380.

CHANG, S. L. 1958. Cultural, cytological and ecological observations on the amoeba stage of *Naegleria gruberi*. J. Gen. Microbiol., **18** : 565-578.

CHANG, S. L. 1971. Small free-living amoebas : cultivation, quantitation, identification, classification, pathogenesis and resistance. IN : *Current Topics in Comparative Pathology* : T. C. Chang (Ed.). Vol. **1**, 201-254. Academic Press, New York and London.

CHANG, S. L. 1973. Etiological, pathological, epidemiological and diagnostical considerations of primary amoebic meningo-encephalitis. Presented before the 21st Annual Meeting of the Am. Soc. Trop. Med. Hyg. Nov. 6-10, 1972, and published in CRC Crit. Rev. Microbiol., **3** : 135-159.

CHATTON, E. 1953. Ordre des amoebiens nus ou Amoebaea. IN : *Traité de Zoologie* : P. P. Grasse (Ed.). **1** (2) Paris : Masson et Cie.

CHLAPOWSKI, F. J. and BAND, R. N. 1971. Assembly of lipids into membranes in *Acanthamoeba palestinensis*. II. The origin and fate of glycerol—3_H—labeled phospholipids of Cellular membranes. J. Cell Biol., **50** : 634-651.

CRUMP, L. M. 1950. Influence of the bacterial environment on the excystment of amoebae from soil. J. Gen. Microbiol., **4** : 16-21.

CULBERTSON, C. G. 1961. Pathogenic *Acanthamoeba (Hartmannella)*. Amer. J. Clin. Path., **35** : 195-202.

CULBERTSON, C. G. 1971. The pathogenicity of soil amoebas. Ann. Rev. Microbiol., **25** : 231-254.

CULBERTSON, C. G. ENSMINGER, P. W. and OVERTON, W M. 1965. The isolation of additional strains of pathogenic *Hartmannella* sp. *(Acanthamoeba)*. Proposed culture method for application to biological material. Amer. J. Clin. Path., **43** : 383-387.

CULBERTSON, C. G., ENSMINGER, P. W. and OVERTON, W M. 1966. *Hartmannella (Acanthamoeba)*. Experimental chronic granulomatous brain infections produced by new isolates of low virulence. Amer. J. Clin. Path., **46** : 305-314.

CULBERTSON, C. G., ENSMINGER, P. W and OVERTON, W M. 1968. Pathogenic *Naegleria* sp.—study of a strain isolated from human cerebrospinal fluid. J. Protozool., **15** 353-363.

CULBERTSON, C. G., SMITH, J. W., COHEN, H. K. and MINNER, J. R. 1959. Experimental infection of mice and monkeys by *Acanthamoeba*. Amer. J. Clin. Path., **35** : 185-197.

CULBERTSON, C. G., SMITH, J. W. and MINNER, J. R. 1958. *Acanthamoeba* : Observations on animal pathogenicity. Science, **127** : 1506.

CURSONS, R. T. M. and BROWN, T J. 1975. The 1968 New Zealand cases of primary amebic meningoencephalitis—myxomycete or *Naegleria* ? N. Z. Med. J., **82** : 123-125.

CURSONS, T. M. and BROWN, T J. 1976. Identification and classification of the aetiological agents of primary amoebic meningo-encephalitis. N. Z. J. Mar. Fresh. Res., **10** : 245-262.

CUTLER, D. W 1920. A method for estimating the number of active protozoa in the soil. J. Agric. Sci., **10** : 135-143.

CUTLER, D. W., CRUMP, L. M. and SANDON, H. 1922. A quantitative investigation of the bacterial and protozoan population of the soil. Phil. Trans. Roy. Soc. London, **B 211** 317-350.

DANGEARD, P. A. 1896. Contributions à l'étude des aerasiées. Botaniste, **5** : 1-20.

DANGEARD, P. A. 1900. Etude de la karyokinése chez l' *Amoeba hyalina* sp. nov. Botaniste, **7** : 49-83.

DANIELS, E. W. 1973. Ultrastructure. IN : *The Biology of Amoeba* : K. W. Jeon (Ed.). Academic Press, New York and London.

DARBYSHIRE, J. F., PAGE, F. C. and GOODFELLOW, L. P. 1976. *Paratetramitus jugosa*, an amoebo-flagellate of soils and freshwater, type species of *Paratetramitus* nov. gen. Protistologica, *T XII* : 375-387.

DATTA, T and HEMLATA. 1979. Temperature and salinity tolerance of free-living amoebae and their pathogenecity to mice. Indian J. Parasitol., **3** : 159-161.

DE JONCKHEERE, J., VAN DIJCK, P. and VAN DE VOORDE, H. 1975. The effect of thermal pollution on the distribution of *Naegleria fowleri.* J. Hyg., **75** : 7-13.

DIFFLEY, P., SKEELS, M. R. and SOGANDARES-BERNAL, F. 1976. Delayed type hypersensitivity in guinea pigs infected subcutaneously with *Naegleria fowleri*. Z. Parasitenkde., **49** : 133-137.

DOBELL, C. 1914. Cytological studies on three species of *Amoeba A. lacertae* Hartmann, *A. glebae* n. sp., *A. fluvialis* n. sp. Arch. Protistenk., **34** : 139-190.

DOUGLAS, M. 1930. Notes on the classification of the amoeba found by Castellani in cultures of a yeast-like fungus. J. Trop. Med. London, **33** : 258-259.

DROOP, M. R. 1962. *Heteramoeba clara* n. gen., n. sp., a sexual biphasic amoeba. Arch. Mikrobiol., **42** : 254-266.

DUMA, R. J., FERREL H. W., NELSON, C. W. and JONES, M. M. 1969. Primary amoebic meningo-encephalitis. New England J. Med., **281** : 1315-1323.

DUMA, R. J., ROSSENBLUM, W. I., MC. GEHEE, R. F., JONES, M. M. and NELSON, C. E. 1971. Primary amoebic meningo-encephalitis caused by *Naegleria*. Ann. Inter. Med., **74** : 923-931.

DWIVEDI, J. M., and SINGH, C. M. 1965. Pulmonary lesions in an Indian buffalo associated with *Acanthamoeba* sp. Indian J. Microbiol., **5** : 31-34.

DYKSTRA, M. J. and ALDRICH, H. C. 1978. Successful demonstration of an elusive cell coat in amoebae. J. Protozool., **25** : 38-41.

EHRENBBG, C. G. 1830. Organisation, Systematik and geographisches verhaltniss der Infusionsthierchan. K. Acad. Wiss., Berlin, 108 pp. + 8 pl.

FORD, E., 1914. On the nuclear division of a free-living *limax-amoeba. (Amoeba tachypodia* Glasser 1912). Arch. Protistenk., **34** : 190-197.

FOWLER, M. and CARTER, R. F. 1965. Acute pyogenic meningitis probably due to *Acanthamoeba* sp. : a preliminary report. Brit. Med. J., **2** : 740-742.

FROMENTAL, E. DE. 1874. Etudes sur les Microzoaires su infusoires proprement Dits. G. Masson, Paris.

FULTON, C. 1970. Amebo-flagellates as research partners the laboratory biology of *Naegleria* and *Tetramitus*. IN : *Methods in Cell Physiology*, D. M. Prescott (Ed.), **4** : 341-476.

GREEFF, R. 1874. *Pelomyxa palustris* (Pelobius) in amobenartiger organismus des sussen Wassers. Arch. Mikrosk, Anat. Entwicklungsmech, **10** : 51-73.

GRIFFITHS, A. J. and BOWEN, S. M. 1969. Lysosomal activity and its control in encysting *Hartmannella castellanii*. J. Gen. Microbiol., **59** : 239-245.

HAECKEL, E. H. 1862. "Betrachtungen uber die Grenzen und Verwandschaft der Radielarien und über die Systematik der Rhizopodien im Allgemeinen." Reimer, Berlin.

HAGGERTY, R. M. and JOHN, D. T 1978. Innate resistance of mice to experimental infections with *Naegleria fowleri*. Infect. Immun., **20** : 73-77.

HALDER, D. P. 1965. A simple medium for quick cultivation of soil protozoa. Proc. Zool. Soc., **18** (1) : 21-23.

HALDER, D. P. 1969. Studies on morphology, life history and bionomics of parasitic protozoa and free-living protozoa of the soil. Doctoral Thesis, University of Calcutta, India.

HALDER, D. P. and CHAKRABORTY, M. M. 1967. Some cytochemical observations on *Hartmannella* sp , isolated from a local soil samples. J. Protozool., **14** (Suppl.) : 34.

HOARE, C. A. 1972. (See Carter, R. F. 1972. Primary amoebic meningo-encephalitis. An appraisal of the present knowledge. Trans. Roy. Soc. Trop. Med. Hyg., **66** : P. 211.)

HOLLANDE, A. 1942. Etudes cytologique de quelques flagellés libre. Arch. Zool. Exp. Gén., **83** : 1-268.

IVANIC, M. 1926. Zur Kenntnis der Entwickelungsgeschichte einer neuen *Hartmannella*—Art (*H. testudinis* spec. nov.). Zool. Anz., **68** : 87-95.

JADIN, J. B., HERMANNE, J., ROBYN, G., WILLAERT, E., VAN MAERCHE, Y and STEVENS, W. 1971. Trois cas de meningo-encephalite amibienne primitive observes a Anvers (Belgique). [Three cases of primary amoebic encephalitis in Antwerp. (Belgium)] Ann. Soc. Belge. Med. Trop., **51** 255-266.

JAHN, T L. and BOVEE, E. C. 1965. Mechanism of movement in taxonom of Sarcodina. I. As a basis for a new major dichotomy into two classes, Autotractea and Hydraulea. Am. Midl. Nat., **73** : 30-40.

JAHN, T. L., BOVEE, E. C. and GRIFFITH, D. L. 1974. Taxonomy and evolution of the Sarcodina a reclassification. Taxon, **23** : 483-496.

JOHN, D. T., WEIK, R. R. and ADAMS, A. C. 1977. Immunization of mice against *Naegleria fowleri* infection. Infect. Immun., **16** : 817-820.

KAUSHAL, D. C. and SHUKLA, O. P. 1977. Excystment of axenically prepared cysts of *Hartmannella culbertsoni*. J. Gen. Microbiol., **98**: 117-123.

KENT, W. S. 1880-1881. A manual of the Infusoria, Vols. i-iii, London.

KENT, W. S. 1880-1882. A manual of the Infusoria. London: David Bogue.

KLEIN, R. L. 1961. Homeostatic mechanisms for cation regulation in *Acanthamoeba* sp. Exp. Cell Res., **25**: 571-584.

KLEIN, R. L. and NEFF, R. J. 1960. Osmotic properties of Mitochondria isolated from *Acanthamoeba* sp. Exp. Cell Res., **19**: 133-155.

KUDO, R. R. 1959. *Pelemyxa* and related organisms. Ann. N. Y Acad. Sci., **78**: 474-486.

KUDO, R. R. 1966. *Protozoology*. 5th ed. Charles C. Thomas, Springfield, Illinois, U.S.A.

LASMAN, M. 1967. Observations on acid phosphatase in *Mayerella palestinensis*. J. Cell Physiol., **69**: 151-154.

LASTOVICA, A. J. and DINGLE, A. D. 1971. Superprecipitation of an actomyosin-like complex isolated from *Naegleria gruberi* amoebae. Exp. Cell Res., **66**: 337-345.

LEIDY, J. 1879. Freshwater rhizopods of North America. Rept. U. S. Geol. Surv. Terr., 1-324.

LEVINE, N. D., CORLISS, J. O., COX, F. E. G., DEROUX, G., GRAIN, J., HONIGBERG, B. M., LEEDALE, G. F., LOEBLICH, A. R. III, LOM J., LYNN, D., MERINFELD, E. G., PAGE, F. C., POLJANSKY, G., SPRAGUE, V., VAVRA. J., and WALLACE, F. G. 1980. A new revised classification of the Protozoa. J. Protozool., **27**: 37-58.

MAITRA, S. C., SAGAR, P. and AGARWALA, S. C. 1974. Ultrastructural changes during encystment of *Schizopyrenus russelli*. J. Protozool., **21**: 507-511.

MANDAL, B. N., GUDEX, D. J., FITCHETT, M. R., PULLON, D. H. H., MALLOCH, J. A., DAVID, C. M. and APTHORP, J. 1970. Acute meninge-encephalitis due to amoeba of the order *Myxomycetale* (Slime mould). N. Z. Med. J., **71**: 16-23.

MANSURKAR, R. V., LAWANDE, R. R. V and NADKARNI, M. S. 1974.

"Pathogenic free-living amoebae and involvement of Central Nervous System." Indian Ass. Pathol. Microbiol., p. 187, Nov., New Delhi.

MARTIN, C. H. and LEWIN, K. 1914. Some notes on soil protozoa. Phil. Trans. Roy. Soc. London, B **205** : 77-95.

MARTINEZ, A. J., DOS SANTOS NETO, J. G., NELSON, E. C., STAMM, W. P. and WILLAERT, E. 1977. IN : *Pathology Annual*, S. C. Sommers and P. P. Rosen (Eds.) Vol. **12** : 225-250. Applenton, New York.

MARTINEZ, A. J., DUMA, R. J., NELSON, D. E. and MORETTA, F. C. 1973. Experimental *Naegleria* meninge-encephalitis in mice, penetration of the olfactory mucesal epithelium by *Naegleria* and pathogenic changes produced : a light and electron microscepe study. Lab. Invest., **29** : 121-133.

MARTINEZ, A. J., MARKOWITZ, S. M. and DUMA, R. J. 1975. Experimental pneumenitis and encephalitis caused by *Acanthamoeba* in mice. Pathogenesis and ultrastructural features. J. Infect. Dis., **131** : 692-699.

MARTINEZ, A. J., NELSON, E. C., JONES, M. M., DUMA, R. J. and ROSENBLUM, W I. 1971. Experimental *Naegleria* meninge-encephalitis in mice : An electron microscope study. Lab. Invest., **25** : 465-475.

MARUTA, H. and KORN, E. D. 1977. *Acanthamoeba* myosin II. J. Biol. Chem., **252** : 6501-6509.

McCONNELL, E. E., GARNER, F. M. and KIRK, J. H. 1968. Hartmannellosis innabull pathol. Vet (Basel), **5** : 1-6.

MINCHIN, E A. 1922. *An introduction to the study of protozoa.* London : Edward, Arnold.

MISRA, R. and SHARMA, A. K. 1980. Pathogenicity in mice of strains of *Acanthamoeba culbertsoni* (Singh and Das, 1970) and *A. rhysodes* (Singh, 1952) from Indian soils and the present status of the genera *Hartmannella and Acanthamoeba.* Indian J. Parasitol., **4** (2) 135-144.

MUKHERJEE, S. K. 1974. Soils of West Bengal. Symposium issue on 'Researches in Soil', Calcutta, University of Calcutta, 21-28.

NAGINGTON, J., WATSON, P. C., PLAYFAIR, J. J., McGILL, J., JONES, B. R. and STEELE, A. D. McG. 1974. Amoebic infection of the eye. Lancet, **2** : 1537-1540.

NEFF, R. J. 1957. Purification, axenic cultivation and description of a soil amoeba *Acanthamoeba* sp. J Protozool., **4** 176-182.

NEFF, R. J. and NEFF, R. H. 1969. The biochemistry of amoebic encystment. Symp. Soc. Exp. Biol., **23** : 51-81.

PAGE, F. C. 1967a. Taxonomic criteria for *limax*-amoebae, with description of three new species of *Hartmannella* and three of *Vahlkampfia*. J. Protozool., **14** : 499-520.

PAGE, F. C. 1967b. Redefinition of the genus *Acanthamoeba* with description of three species. J. Protozool., **14** : 709-724.

PAGE, F. C. 1968. Generic criteria for *Flabellula*, *Rugipes* and *Hyalodiscus* with description of species. J. Protozool., **15** 9-26.

PAGE, F. C. 1971. Two marine species of *Flabellula* (Amoebida, Mayerellidae). J. Protozool., **18** : 37-44.

PAGE, F. C. 1974. A further study of taxonomic critiria for limax amoebae, with descriptions of new species and a key to genera. Arch. Protistenk., **116** : 148-188.

PAGE, F. C. 1975. A new family of amoebae with fine pseudopodia. Zool. J. Linn. Soc., **56** : 73-89.

PAGE, F. C. 1976a. A revised classification of the Gymnamoebia (Protozoa : Sarcodina). Zool. J. Linn. Soc., **58** : 61-77.

PAGE, F. C. 1976b. *An illustrated key to Freshwater and Soil amoebae.* Freshwater Biological Association Scientific Publication No. 34, U.K.

PAGE, F. C. 1977. The genus *Thecamoeba* (Protozoa, Gymnamoebia): Species distinctions, locomotive morphology and protozoan prey. J. nat. Hist., **11** : 25-63.

PAGE, F. C. and BLAKEY, S. M. 1979. Cell surface structure as a taxonomic character in the Thecamoebidae (Protozoa Gymnamoebia). Zool. J. Linn. Soc., **66** : 113-135.

PAL, R. A. 1972. The osmoregulatory system of the amoeba, *Acanthamoeba castellanii*. J. Exp. Biol., **57** : 55-76.

PAN, N. R. and GHOSH, T N. 1971 Primary Amoebic Meningo-encephalitis in two children. J. Indian Med. Ass., **56** : 134.

PANT, K. D., PRASAD, B. N. K. and SINGH, L. M. 1968. Antigenic relationship among fifteen strains of pathogenic *Hartmannella*. Indian J. Exp. Biol., **6** : 227-229.

PERTY, M. 1852. Zur Kenntniss Kleinster Lebensformen nach Bau, Funktionen, Systematik mit Speciqverzeichniss der in der Schweiz, **1** vol. Bern. 228 pp.

PHILLIPS, B. P. 1974. *Naegleria* : Another Pathogenic ameba studied in germfree guineapigs. Am. J. Trop. Med. Hyg., **23** (5) 850-855.

POLLARD, T. D. and KORN, E. D. 1973a. *Acanthamoeba* myosin. I. isolation from *Acanthamoeba castellanii* of an enzyme similar to muscle myosin. J. Biol. Chem., **248** : 4682-4690.

POLLARD, T. D. and KORN, E. D. 1973b. The "Contractile" protenis of *Acanthamoeba castellanii*. Cold Spring Harbor Symp. Quant. Biol., **37** : 573-583.

POLLARD, T D., SHELTON, E., WEIHING, R. R. and KORN, E. D. 1970. Ultrastructural characterization of F-actin isolated from *Acanthamoeba castellanii* and identification of cytoplasmic filaments as F-action by reaction with rabbit heavy meromyosin. J. Mol. Biol., **50** : 91-97.

PUSSARD, M. 1966. Le genre *Acanthamoeba* Volkonsky, 1931 (Hartmannellidae-Amoebida). Protistologica, **2** : 71-93.

PUSSARD, M. 1973. Modalites de la division nucleaire et taxonomic chez les Amibes (Amoebida). Protistologica, **9** : 163-173.

RAFALKO, J. S. 1947. Cytological observation on the amoeboflagellate, *Naegleria gruberi* (Protozoa). J. Morph., **81** 1-44.

RAFALKO, J. S. 1951. Mitotic division in amoebo-flagellate, *Tetramitus rostratus*. J. Morph., **89** : 79-90.

RAIZADA, M. K. and KRISHNA MURTI, C. R. 1971. Changes in the activity of certain enzymes of *Hartmannella* (Culbertson strain A—1) during encystment. J. Protozool., **18** : 115-119.

RASTOGI, A. K., SAGAR, P. and AGARWALA, S. A. 1973. Role of riboflavine and certain amino acids in the excystment of *Schizopyrenus russelli*. J. Protozool., **20** : 453-455.

RAY, D. L. and HAYES, R. E. 1954. *Hartmannella astronyxis* : A new species of free-living amoeba. Cytology and life-cycle. J. Morph., **95** : 159-188.

REICH, K. 1933. Studien über die Bodenprotozoan Pülastinas. Arch. Protistenk., **79** 76-98.

REYNOLDS, B. D. and LOOPER, J. B. 1928. Infection experiments with *Hydramoeba hydroxena* nov. gen. J. Parasitol., **15** : 23-30.

ROYCHAUDHURY, S. P., AGARWAL, R., DATTA, R., BISWAS, N. R., GUPTA, S. P. and THOMAS, P. K. 1963. *Soil of India.* ICAR, New Delhi, India.

RUBIN, R. W., HILL, M. C., HEPWORTH, P. and BOEHMER, J. 1976. Isolation and electrophoretic analysis of nucleoli, phenol-soluble nuclear proteins, and outer cyst walls from *Acanthamoeba castellanii* during encystation initiation. J. Cell Biol., **68** 740-751.

RUBIN, R. W. and MAHER, M. 1976. Actin turnover during encystation in *Acanthamoeba.* Exp. Cell Res., **103** : 159-168.

RUDICK, V. L. and WEISMAN, R. A. 1973. DNA—dependent RNA polymerase from trophozoites and cyts of *Acanthamoeba castellanii* Biochim. Biophys. Acta, **299** : 91-102.

RUSSELL, E. J. and HUTCHINSON, H. B. 1909. The effect of partial sterilisation of soil on the production of plant food. J. Agric. Sci., **3** : 114-144.

RYTER, A. and BOWERS, B. 1976. Localization of acid phosphatase in *Acanthamoeba castellanii* with light and electron microscopy during growth and after phagocytosis. J. Ultrastruct. Res., **57** : 309-321.

SANDON, H. 1927. *The Composition and Distribution of the Protozoan Fauna of the Soil,* Oliver and Boyd, Edinburgh and London.

SCHAEFFER, A. A. 1926. Taxonomy of the Amoebas. Publ. no. 345, pp. 1-116. Carnegie Institute, Washington.

SCHARDINGER, F. 1899. Entwicklungskreis einer *Amoeba lobosa* (Gymnamoeba) : *Amoeba gruberi.* S. K. Acad. Wiss. Wien., **108** : 713-734.

SCHUSTER, F. L. 1963. An electron microscope study of the amoebo-flagellate, *Naegleria gruberi* (Schardinger). II. The cyst stage. J. Protozool., **10** : 313-320.

SCHUSTER, F. L. 1975. Ultrastructure of cysts of *Naegleria* spp. : a comparative study. J. Protozool., **22** : 352-359.

SCHUSTER, F. L. 1979. Small amoebae and amoebo-flagellates, IN : *Biochemistry and Physiology of Protozoa*, M. Levandowsky and S. H. Hutner (Eds.). Vol. **3** : 215-285. Academic Press, New York and London.

SCHUSTER, F. L. and DUNNEBACKE, T H. 1977. Ultrastructural observations on experimental *Naegleria* meningo-encephalitis in mice : intranuclear inclusions in amoebae and host cells. J. Protozool., **24** : 489-497.

SCHUSTER, F. L. and SVILHA, G. 1968. Ribonucleoprotein containing vesicles in cysts of *Naegleria gruberi*. J. Protozool., **15** : 752-758.

SIDDIQUI, W. A. and BALAMUTH, W. 1966. Serological Comparison of selected parasitic and free-living amoebae *in vitro*, using diffusion precipitation and fluorescent antibody techniques. J. Protozool., **13** 175-182.

SINGH, B. N. 1941. Selectivity in bacterial food by soil amoebae in pure mixed culture and in sterilised soil. Ann. App. Biol., **28** : 52-64.

SINGH, B. N. 1945. The selection of bacterial food by soil amoebae and the toxic effects of bacterial pigments and other products on soil protozoa. Brit. J. Exp. Path., **26** : 316-325.

SINGH, B. N. 1946. A method of estimating the numbers of soil protozoa especially amoebae, based on their differential feeding on bacteria. Ann. Appl. Biol., **33** : 112-119.

SINGH, B. N. 1947a. Studies on soil Acrasieae. 1. Distribution of species of Dictyostelium in soils of Great Britain and the effect of bacterial food on their development. J. Gen. Microbiol., **1** : 11-21.

SINGH, B. N. 1947b. Studies on soil Acrasieae. 2. The active life of *Dictyostelium* in soil and the influence there on of soil moisture and bacterial food. J. Gen. Microbiol., **1** : 361-367.

SINGH, B. N. 1948a. Studies on giant amoebiod organisms. 1. The distribution of *Leptomyxa reticulata* Goodey in soils of Great Britain and the effect of bacterial food on growth and cyst formation. J. Gen. Microbiol, **2** : 8-14.

SINGH, B. N. 1948b. Studies on giant amoeboid organisms. 2. Nuclear division and cyst formation in *Leptomyxa reticulata* Goodey with remarks on systematic position of the organism. J. Gen. Microbiol., **2** : 89-96.

SINGH, B. N. 1949. The effect of artificial fertilisers and dung on the numbers of amoebae in Rothamsted soil. J. Gen. Microbiol., **3** : 204-210.

SINGH, B. N. 1950. A culture method of growing small free-living amoebae for the study of their nuclear division. Nature, Lond., **165** : 65.

SINGH, B. N. 1952. Nuclear division in nine species of small free-living amoebae and its bearing on the classification of the order Amoebida. Phil. Trans. Roy. Soc. Lond., B **236** 405-461.

SINGH, B. N. 1955. Culturing soil protozoa and estimating their numbers in soil. IN : *Soil Zoology*. pp. 403-411. Butterworths Scientific Publications. London.

SINGH, B. N. 1960. Inter-relationship between micro-predators and bacteria in soil. Proc. 47th Indian Sci. Cong. Asso. Part II. 145-158.

SINGH, B. N. 1975. *Pathogenic and non-pathogenic amoebae.* The Macmillan Press Ltd., London and Basingstoke.

SINGH, B. N. and DAS, S. R. 1970. Studies on pathogenic and non-pathogenic small free-living amoebae and the bearing of nuclear division on the classification of the order Amoebida. Phil. Trans. Roy. Soc. Lond., B **259** : 435-476.

SINGH, B. N. and DAS, S. R. 1972a. Occurrence of pathogenic *Naegleria aerobia, Hartmannella culbertsoni* and *H. rhysodes* in sewage sludge samples of Lucknow. Current Sci., **41** 277-281.

SINGH, B. N. and DAS, S. R. 1972b. Intranasal infection of mice with flagellate stage of *Naegleria aerobia* and its bearing on the epidemiology of human meningo-encephalitis. Current Sci., **41** : 625-628.

SINGH, B. N. and HANUMAIAH, V 1977. A new amoebo-flagellate, *Tetramastigamoeba hoarei* n. g., n. sp. Protozoology, 3 : 183-191.

SINGH, B. N., MISRA, R. and SHARMA, A. K. 1981. Nuclear structure and nuclear division as the Lasis for the subdivision of the genus *Thecamoeba* Fromental, 1874. Protistologica, T XVII : 449-464.

SINGH, B N. and HANUMAIAH, V 1979. Studies on pathogenic and non-pathogenic amoebae and the bearing of mitotic division and locomotive form and behaviour on the classification of the order Amoebida. Monograph no. 1 of the Association of Microbiologists of India. Published by Indian J. Microbiol., 1-80.

STEWERT, J. R. and WEISMAN, R. A. 1974. A chemical and autoradiographic study of celulose synthesis during the encystment of *Acanthamoeba Castellanii.* Arch. Biochem. Biophys., **181** : 488-498.

THONG, Y H., SHEPHARD, C., FERRANTE, A and ROWAN-KELLY, B. 1978. Protective immunity to *Naegleria fowleri* in experimental amoebic meningo-encephalitis. Am. J. Trop. Med. Hyg., **27** : 238-240.

TODD, S. R. and KITCHING, J. A. 1973. Effects of high hydrostatic pressure on morphogenesis in *Naegleria gruberi* (Schardinger). J. Protozool., **29** : 421-424.

TSHALAIA, L. E. 1941. On a species of *Entamoeba* detected in sewage effluent. Med. Parsit., Moscow, 10 : 244 (in Russian). Trop. Dis. Bull. (1943), **40** : 311.

TSHALAIA, L. E. 1947. Contribution to the study of *Entamoeba moshkovskii.* Med. Parasit., Moscow, **16** 66 (Russian), Abstr. World. Med. (1948), 4 : 110.

VAHLKAMPF, E. 1905. Beitrage zur Biologie und Entwicklungsgeschichte von *Amoeba limax* U. S. W Arch. Protistenk., **5** : 167-220.

VAN DEN DRIESSCHE, E., VANDEPITTE, J., VAN DIOCK, P. J. DE JONCKHEERE, J. and VAN DE VOORDE, H. 1973. Primary amoebic meningo-encephalitis after swimming in stream water. Lancet, **11** : 971.

VISVESVARA, G. S. and BALAMUTH, W 1975. Comparative studies on related free-living and pathogenic amoebae with specieal reference to *Acanthamoeba.* J. Protozool., **22** : 245-256.

VISVESVARA, G. S. and CALLAWAY, C. S. 1974. Light and electron microscopic observations on the pathogenesis of *Naegleria fowleri* in mouse brain and tissue culture. J. Protozool., **21** : 239-250.

VOLKONSKY, M. 1931. *Hartmannella castellanii* Douglas et classification des *Hartmannelles.* Arch. Zool. Exp. Gen., **72** 317-339.

WEIHING, R. R. and KORN, E. D. 1971. *Acanthamoeba* actin : Isolation and properties. Biochemistry, **10** : 590-600.

WEISMAN, R. A. and KORN, E. D. 1967. Phagocytosis of latex beads by *Acanthamoeba.* I. Biochemical properties. Biochemistry, **6** : 485-497.

WENYON, C. M. 1926. *Protozoology*, **2** vols. Bailliere, Tindall and Cox, London.

WERTH, J. M. and KAHN, A. J. 1967. Isolation and preliminary chemical analysis of the cyst wall of the Amoeba-flagellate *Naegleria gruberi* J. Bacterial., **94** : 1272-1274.

WHITMORE, E. R. 1911. Studien über kulturamoben aus Manila. Arch. Protistenk., **23** : 81-95.

WILLAERT, E., STEVENS, A. R. and TYNDALL, R. L. 1978. *Acanthamoeba royreba* sp. n. from a human tumor cell culture. J. Protozool., **25** : 1-14.

WONG, M. M., KARR, S. L., JR. and CHOW, C. K. 1977. Changes in the virulence of *Naegleria fowleri* maintained *in vitro*. J. Parasitol., **63** : 872-878.

YUYAMA, S. 1971. The effect of selected chemical agents on the amoebo-flagellate transformation in *Naegleria gruberi*. J. Protozool., **18** : 337-343.

PLATES

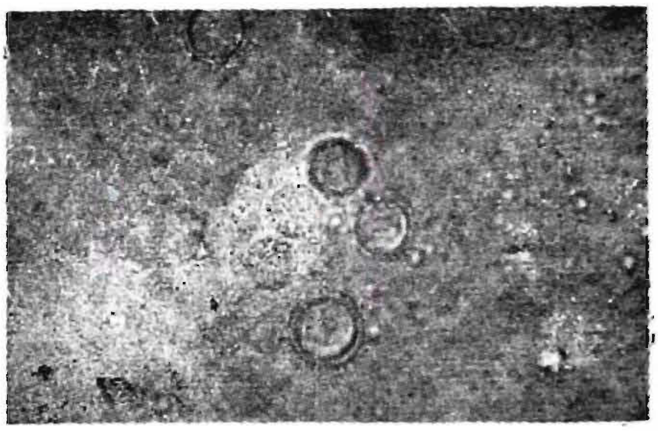
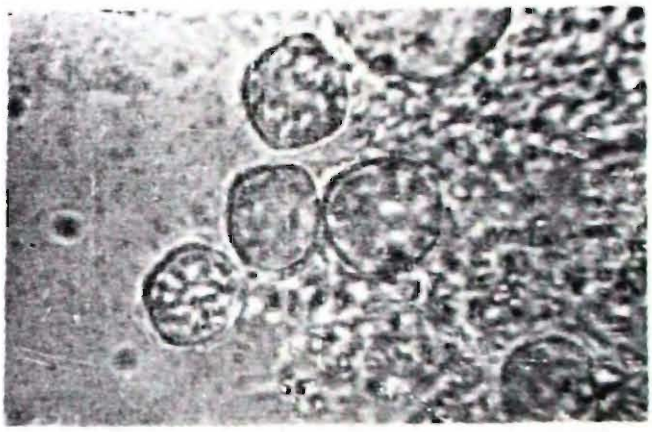


Fig. 1



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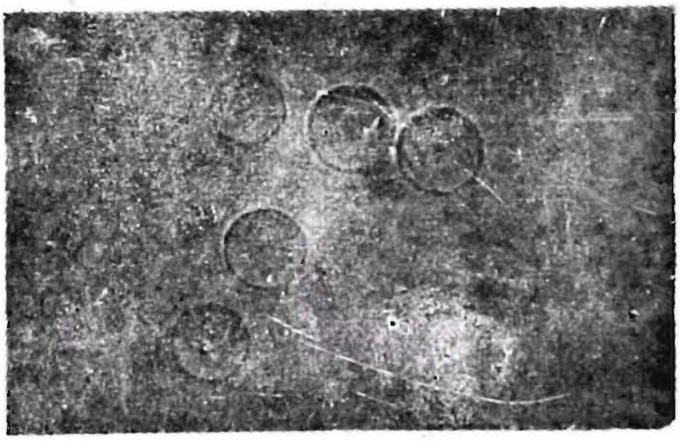

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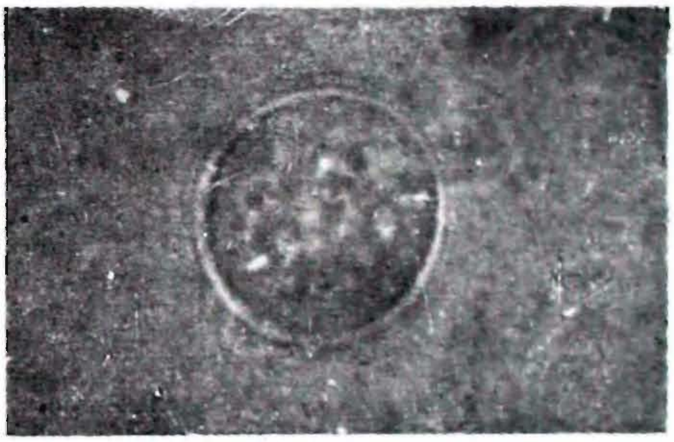


Fig. 4

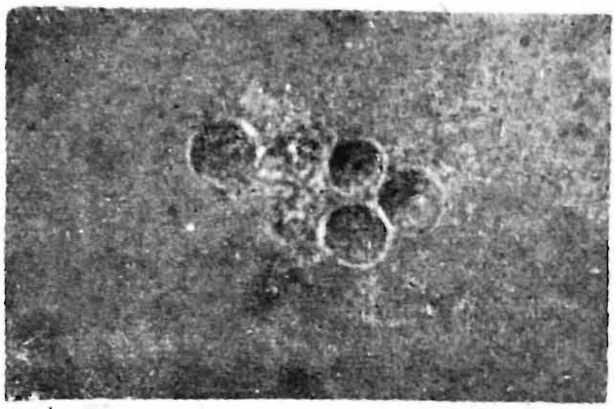
PLATE 'I'

Fig. 1 Photomicrographs of the living cysts of *Schizopyrenus russelli.* X 1050

Fig. 2 The cysts of *Naegleria gruberi* in culture media. X 680

Fig. 3 Living cysts of *Didascalus thorntoni.* X 910

Fig. 4 The cyst to *Tetramitus rostratus.* X 2690



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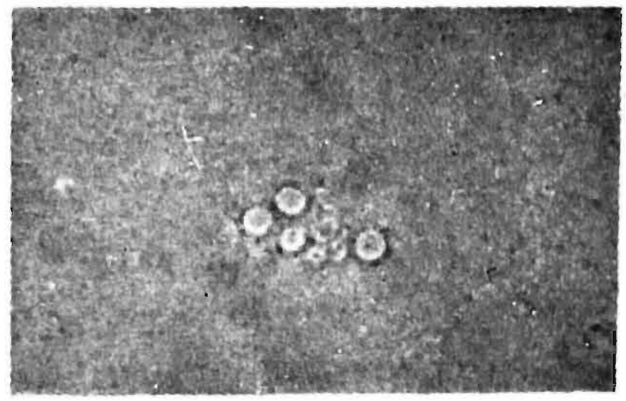

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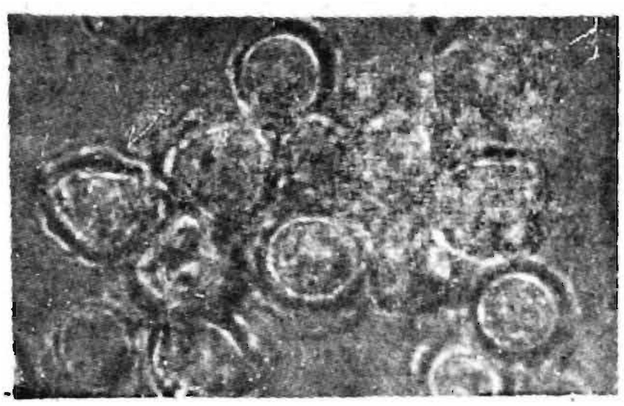


Fig. 7

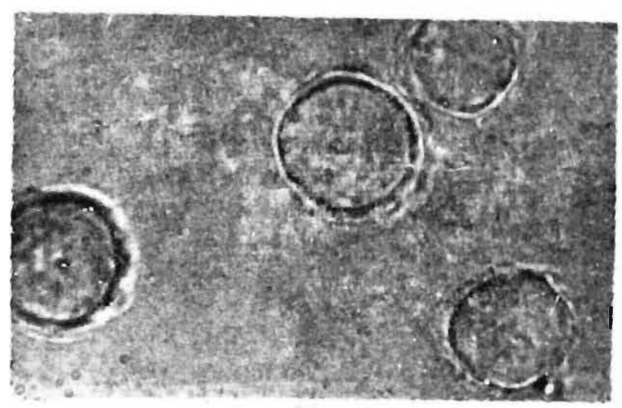


Fig. 8

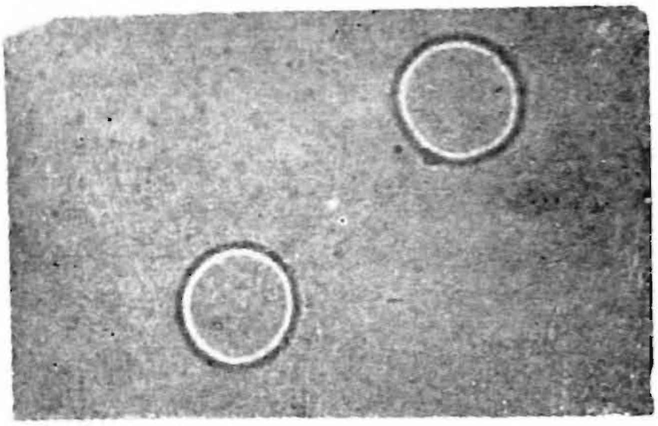
PLATE 'II'

Fig. 5 Photomicrographs of the living cysts of *Hartmannella vermiformis*. X 1300

Fig. 6 Living cyst of *Hartmannella crumpae* showing single wall with an outer gelatinous layer. X 625

Fig. 7 Living cyst of *Acanthamoeba rhysodes* showing wrinkled irregular outer wall. X 1830

Fig. 8 Cyst to *Acanthamoeba culbertsoni*. X 1440



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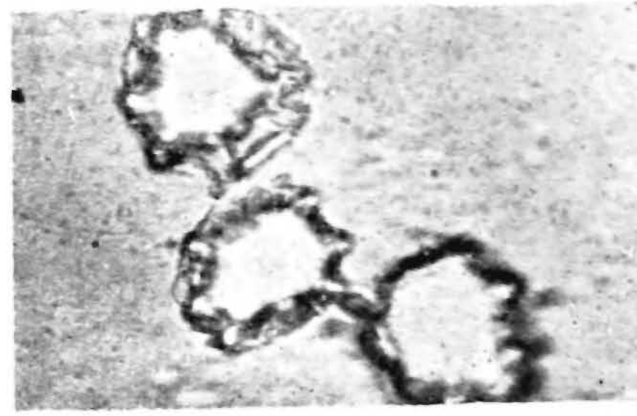

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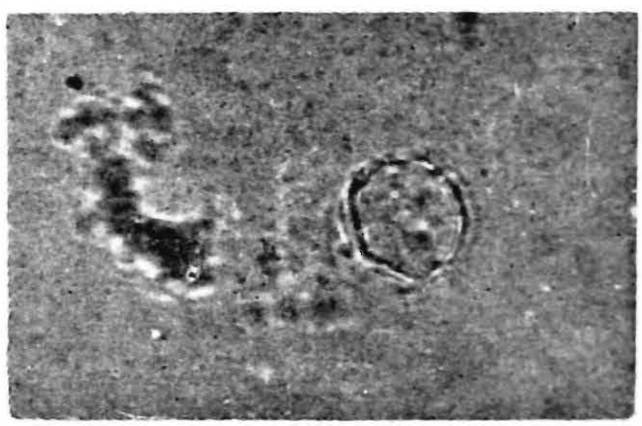

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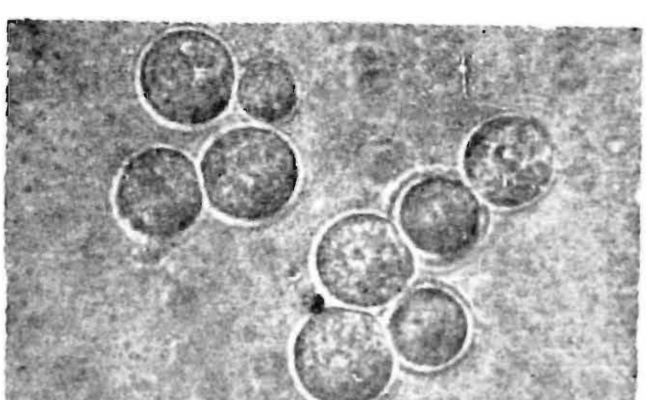

Fig. 12

Fig. 13

PLATE 'III'

Fig. 9 The cyst of *Acanthamoeba glebae* showing double layered outer wall. X 865

Fig. 10 Photomicrographs of *Acanthamoeba astronyxis*. X 1140

Fig. 11 The living cyst of *Acanthamoeba palestinensis* showing irregular wrinkled ectocyst. X 1240

Fig. 12 Cysts of *Echinamoeba exundans* having single wall with an outer gelatinous layer. X 2085

Fig. 13 The living cysts of *Vannella cutleri*. X 1840

TABLE—1

The types and nature of soils in West Bengal (district-wise)

District	Types of soil
Bankura	Vindhya alluvium, Laterite, Red.
Birbhum	Vindhya alluvium, Laterite, Red, Gravelly (Western part)
Burdwan	Gangetic alluvium, Vindhya alluvium, Laterite, Gravelly (Western part).
Calcutta	Gangetic alluvium, Coastal.
Cooch Behar	Terrai and Teesta alluvium.
Darjeeling	Terrai and Teesta alluvium, Tea soil.
Jalpaiguri	Terrai and Teesta alluvium.
Hooghly	Gangetic alluvium, Vindhya alluvium.
Howrah	Gangetic alluvium, Coastal.
Malda	Gangetic alluvium, Red.
Midnapore	Vindhya alluvium, Coastal, Laterite, Gravelly.
Murshidabad	Gangetic alluvium, Vindhya alluvium.
Nadia	Gangetic alluvium.
Purulia	Vindhya alluvium, Red, Gravelly.
West Dinajpur	Teerai and Teesta alluyium, Red.
24 Parganas	Gangetic alluvium, Coastal.

TABLE—2

Soils of West Bengal, Vegetation and the isolated species of amoeba

Districts	Localities	Vegetation	Soil Temperature	Species
24 Parganas	Ganga Sagar Island	Grassy	32°C	*Didascalus thorntoni, Acanthamoeba palestinensis.*
24 Parganas	Bongaon, Shaktighat	Jute	28°C	*Acanthamoeba glebae.*
24 Parganas	Kakdwip, 8 No. Kalinagar area	Paddy	28°C	*Acanthamoeba culbertsoni.*
Howrah	Uluberia, Block Seed Farm	Paddy	28°C	*Naegleria gruberi.*
Hooghly	Mayapur	Grassy	28°C	*Acanthamoeba astronyxis.*
Midnapore	Digha, Jhau forest area	Grassy	28°C	*Schizopyrnus russelli, Echinamoeba exundans, Hartmannella vermiformis.*
Nadia	Krishnanagar	Grassy	28°C	*Acanthamoeba glebae.*
Birbhum	Bolpur, Upper Bangola	Paddy	31°C	*Naegleria gruberi.*
Purulia	Purulia Town	Grassy	31°C	*Vannella cutleri*
Bankura	Pratappur	Grassy	30°C	*Hartmannella crumpae.*
Murshidabad	Behrampur, Khagra	Grassy	28°C	*Acanthamoeba astronyxis.*
Cooch Behar	Brahaman Para	Paddy, Beetle nut	31°C	*Echinamoeba exundans.*
West Dinajpur	Asoke Pally	Mango	28°C	*Acanthamoeba rhysodes.*
Malda	English Bazar	Grassy	30°C	*Acanthamoeba astronyxis.*
Darjeeling	Chandman Bazar	Tea Plant	22°C	*Tetramitus rostratus.*

TABLE—3

Measurement of 50 free-living aerobic soil amoebae of West Bengal in μm.

Sl. No.	Species	Length of Amoebae				Nucleus				Contractile vacuole			
		Range	Mean	S.D.	S.E. (—)	Range	Mean	S.D.	S.E. (—)	Range	Mean	S.D.	(S.E. —)
1.	*Schizopyrenus russelli*	16.8—28.0	23.67	4.0247	0.8392	1.4—2.2	1.70	0.1849	0.6653	1.4—2.2	1.96	9.3863	0.1221
2.	*Naegleria gruberi*	9.8—15.4	13.16	1.7708	0.5599	—	1 40	—	—	—	5.60	—	—
3.	*Didascalus thorntoni*	25.2—28.0	26.32	1.2865	0.4068	1.4—2.1	2.10	—	—	2.8—3.5	3.50	—	—
4.	*Tetramitus rostratus*	16.8—23.8	21.56	2.3977	0.7582	1.4—2.1	2.10	—	—	2.8—3.5	3.50	—	—
5.	*Hartmannella vermiformis*	21 0—25.2	22.96	1.5049	0.4759	—	1.40	—	—	—	2.10	—	—
6.	*Hartmannella crumpae*	35.0—42.0	38.65	2.4869	0.7864	0.7—1.4	1.40	—	—	1.4—2.1	2.10	—	—
7.	*Acanthamoeba rhysodes*	22 4—29.4	26.04	2.4569	0.7864	—	0.70	—	—	2.8—4.2	—	—	—
8.	*Acanthamoeba culbertsoni*	21.0—26.6	24.22	1.9853	0.6278	—	1.40	—	—	2.8—4.9	—	—	—
9.	*Acanthamoeba glebae*	22.4—28.0	25.92	1.9007	0.3801	1.4—2.1	1.85	0.3872	0.1224	4.2—7.0	5.60	1.1430	0.3614
10.	*Acanthamoeba astronyxis*	28.0—33.6	31.22	1.8724	0.5921	2.1—2.8	—	—	—	3.5—7.0	—	—	—
11.	*Acanthamoeba palestinensis*	16.8—22.4	19.60	2.1888	0.6921	0.7—1.4	—	—	—	—	3.50	—	—
12.	*Echinamoeba exundans*	15.4—22.4	19.88	2.2670	0.7169	—	—	—	—	—	0.70	—	—
13.	*Vannella cutleri*	8.4—19.6	14.44	4.4979	1.4223	—	—	—	—	—	1.40	—	—

TABLE—4

Measurament of 25 cysts of free-living aerobic soil amoebae of West Bengal in μm

Serial No.	Species	Diameter of the cysts				Nucleus			
		Range	Mean	S.D.	S.E. (±)	Range	Mean	S.D.	S.E. (±)
1.	*Schizopyrenus russelli*	7.0—11.2	9.52	1.5894	0.5026	—	0.7	—	—
2.	*Naegleria gruberi*	11.2—25.2	21.84	4.3377	1.3717	—	1.4	—	—
3.	*Didascalus thorntoni*	12.6—14.0	13.16	0.7229	0.2286	—	1.4	—	—
4.	*Tetramitus rostratus*	9.8—14.0	11.90	1.5121	0.4781	—	1.4	—	—
5.	*Hartmannella vermiformis*	5.6—9.8	7.70	1.5121	0.4781	—	1.4	—	—
6.	*Hartmannella crumpae*	6.3—9.8	7.98	1.2865	0.4068	—	1.4	—	—
7.	*Acanthamoeba rhysodes*	7.0—14.0	10.92	2.8615	0.9049	0.7—1.4	1.07	—	—
8.	*Acanthamoeba culbertsoni*	11.2—18.2	15.26	2.6767	0.8464	—	1.4	—	—
9.	*Acanthamoeba glebae*	11.2—15.4	13.88	1.3953	0.2790	2.8—3.5	3.01	0.3380	0.1068
10.	*Acanthamoeba astronyxis*	21.0—25.2	22.96	1.6432	0.5196	—	2.1	—	—
11.	*Acanthamoeba palestinensis*	11.2—14.4	13.70	1.8238	0,5767	2.1—2.1	—	—	—
12.	*Echinamoeba exundans*	5.6—8.40	6.72	1.4458	0.4572	0.7—1.4	1.05	0.3834	0.1565
13.	*Vannella cutleri*	4.2—7.00	4.90	0.9899	0.3130	0.7—1.4	1.05	—	—