I.—STUDIES ON SOIL PROTOZOA. PART I. PROTOZOAN FAUNA OF SOME MYSORE SOILS.

By H. S. Madhava Rao.

Although the occurrence of protozoa in soils has been well known since the work of Ehrenberg nearly a century ago, serious interest in them can only be regarded as having commenced with the publication by Russell and Hutchinson in 1909 of their theory of partial sterilisation. The earlier views, based on the belief that the protozoa were present in the soil merely as accidental stragglers from other more natural habitats, and existing for the greater part of the time only as inert cysts incapable of taking any active part in the life of the soil community, have now been entirely removed by the demonstration that, at any rate in the soils of temperate climates, they are present at all times of the year, leading an active life, often attaining very large numbers, and undoubtedly constituting an important component of the soil population. As to the role played by them in the soil economy, however, it must be confessed that the work of these seventeen years has yielded disappointingly little convincing information, and in view of the very wide diversity of forms occurring in the soil it seems very probable that this failure may be, in part at any rate, due to an over simplification of the problem by the assumption that they can all be considered together as leading very similar lives, or that at the most they need only be divided into the main classes of ciliates, flagellates, and amoebæ. Therefore the next step necessary was a study in detail of the life histories of these organisms in the soil under different natural conditions. Since little work in this connection with soil protozoa has been done in India there is no record of the type of the fauna met with in Indian soils. So, before taking up the study of the life history it was necessary to make a qualitative study of these fauna, the study of which has been confined for the present to the soils of Mysore.

EXPERIMENTAL.

Direct microscopic examination of soil gives but a faint idea of the wealth of life with which it teems and investigators who have employed this method for seeking protozoa have found little except shells of the testaceous rhizopods, and an occasional ciliate. The only practical method consists in inoculating the soil to be examined into a suitable culture medium and then examining the culture from time to time to find what protozoa develop. The objection may be

raised that some of the forms are so adapted to life in the soil that they are not capable of developing in the artificial conditions of such cultures, but the ingenious methods by which Martin and Lewin (*J. Agric. Sci.*, 1915, **7**, 109) obtained active protozoa direct from the soil itself yielded only forms which can also be found by the cultural method. One is therefore justified in assuming that the latter method is quite adequate. The testaceous rhizopods, however, multiply very slowly in cultures and as they can be detected by direct examination of the soil, this is the most satisfactory way of seeking them.

Although protozoa will develop in any of the media commonly used in soil bacteriology, either liquid or solid, all such media are not equally suitable. Cunningham and Lohnis (*Zentr. Bakt.*, 1914, ii, **39**, 596) have given some valuable data as to the development of protozoa in various bacteriological media inoculated with soils. Yakimoff and Zeren, (*Zentr. Bakt.*, 1924, ii, **63**, 37) have also described at length the different results obtained with soil cultures in various media such as vegetable infusions of several kinds, horse-dung infusion, soil extract, and dilute broth, with respect both to the species developing and to the rate at which they develop.

The medium used in the present work was 2 per cent. hay infusion containing 1·5 per cent. agar. About 10 gms. of the soil was placed in a petri-dish and 20 c.c. of the culture medium added and allowed to remain for a few days. The cultures were maintained throughout the year and samples taken at all seasons were inoculated into the medium.

In general, it is much more satisfactory when working with mixed cultures to identify living protozoa rather than dead forms. One great objection, however to using stained preparations for identification is that in the course of fixation and staining a large number of the organisms are lost, so that only those which are developing most strongly in the cultures are obtained in the final preparation.

To examine cultures a little of the liquid was taken with a loop, spread out on a slide and covered with a coverslip, and then examined under the microscope immediately. To kill the organisms without distortion 2 per cent. osmic acid has been used. Whenever possible, the protozoa have been examined in the living condition, by suspending a drop of the medium or a suspension in water in a hanging drop.

For the examination of finer structure, the protozoa were first fixed while moist, then stained and finally dehydrated. Several stains such as neutral red, methylene blue and Bismarck brown have been used. For rapid routine work eosin was found to be the best. Different fixatives

like Schaudinni's fixative, chromo-aceto-osmic acid, picroformal and picric-acetic acid also were tried. The best method is to use Schaudinni's fixative followed by Heidenhein's hæmatoxylin.

The following protozoa have been found:—

Pelomyxa palustris.—Large forms moving slowly by means of blunt pseudopodia. Endoplasm encloses sand particles and bacteria. Ectoplasm has a number of vacuoles. Nuclei are numerous. Length 200 microns. Reproduction by fission.

Amœba radiosa.—Body spherical with nearly rigid pseudopodia which are formed very slowly. The appearance resembles a starfish. The nucleus is spherical.

Amœba proteus.—Pseudopodia are numerous and very variable in form. There is a single large nucleus. A contractile vacuole is present. Size varies considerably. The maximum length is 300 microns.

Amœba limax.—This is the most commonly occurring soil protozoon. Very slow in movement. Contractile vacuole is single. The organism is largely responsible for devouring the bacteria. Length 100 microns.

Amœba polypodia.—Pseudopodia are numerous, and have a characteristic shape. There is a single contractile vacuole, and the nucleus is single. Reproduction by fission.

Amœba verrucosa.—Movement is very slow. Pseudopodia are broadly lobed and short. There is a delicate membrane enclosing the body proper. On the surface there are lines which cross each other, giving a wrinkled appearance.

Arcella discoides.—Shells smooth, regular, with a large circular aperture. Pseudopodia rarely visible. The diameter of the shell varies from 100 to 200 microns.

Dimastigamœba Gruberi.—There are two flagella. The cysts are spherical. Capable of amœboid and flagellate existence at different times.

Oicomonas termo.—There is a spherical nucleus. Body about 4 to 5 microns in diameter. There is a single flagellum. Spherical resistant cysts are formed.

Colpoda cucullus.—Length 50 to 100 microns. Macro-nucleus is spherical. Readily encysts under unfavourable conditions. Is bean-shaped and flat dorso-ventrally. The cytostome lies at the end of a groove in the middle of the body. Is provided with long cilia which form a tuft or brush at the opening. The side of the body in front of the cytostomal region is notched or lobed, each lobe corresponding with one of the longitudinal rows of cilia which cover the body. The single large contractile vacuole is at the posterior end. There are two nuclei. One of the most commonly occurring soil protozoa.

Colpoda steni.—Length 25 to 50 microns. Macro-nucleus is elongate. The opening of the pharynx is oblique, oval and turned completely to the right.

Colpidium striatum.—Body-length (50 microns) double the breadth, with longitudinal striations. Anterior extremity curved ventrally. The contractile vacuole is at the posterior end. Nucleus central.

Uronema marina.—Ventral side almost straight from the mouth to the front and drawn out, the dorsal side being curved. Ciliation short and thick. Nucleus round and central. The contractile vacuole is terminal posteriorly.

Laxophyllum rostratum.—Cilia of the anterior region longer. Nuclei nultiple. Contractile vacuole posterior. Trichocysts developed. Length 200 microns.

Euplotes patella.—Flattened dorso-ventrally. There is a special development of cilia on the central surface of the body, some being fused to form cirri. The marginal cilia are absent. Body is short in proportion to the breadth.

Carchesium polypinium.—Two spiral rows of cilia, one shorter than the other. Large horse-shoe shaped macro-nucleus. A contractile vacuole. Cytoplasm contains numerous food vacuoles.

Vorticella microstoma.—Body has a characteristic shape and appears to be segmented transversely. Single contractile vacuole. Cilia arranged spirally round the mouth.

Heteromita ovata.—Ovate body posteriorly wide. Two flagella. Length 25 to 40 microns.

Pleuromonas jaculans.—Kidney-shaped body, sometimes fixed. Contractile vacuole anterior. Nucleus posterior.

Condylostoma patens.—Body broadly ovate and wide posteriorly. Peristome broadly triangular. Contractile vacuole irregular. Two nuclei.

Uronema accuminata.—Allied to *U. marina.* Length 50 microns.

Cercomonas longicauda.—Oval or rounded. Two flagella, single contractile vacuole.

In this connection I describe some species which have not been recorded and are newly isolated from soils. Their description follows, with the names by which it is proposed to identify them.

Order.—*Holotricha*; Sub-order.—*Astomatea*; Family.—*Discophryidae*; Genus.—*Caudalinæ* (Gen. Nov. Madhava Rao).

Caudalina Bangalorensis (Sp. Nov. Madhava Rao).—The body length is 90 microns. At the broadest portion the body measures 18 microns, and tapers posteriorly. A little behind the anterior end it is slightly constricted giving the appearance of a neck. The anterior end is widened into a pentagonal disc. The head thus formed resembles a snake-head. The contractile vacuole is at the anterior end. Posteriorly there are two big processes which appear like arms and help in locomotion. They are broad at the base and taper towards the end. Between these two there are two more very small processes similar in appearance. Cilia are found throughout the body in longitudinal rows. At the anterior end round the sides of the disc they are elongated and closer. The head is 16 microns broad at the base and is reduced to half the size at the extremity. The macro-nucleus is nearly central and the micro-nucleus is found near this. Only a very few forms were obtained and the organism is still under observation.

Caudalina armata (Sp. Nov. Madhava Rao).—The body is elongated and tapers at both ends. It is roughly divided into two portions, the anterior portion being double the length of the posterior portion. It is broadest at about one-third the body length from the posterior end. At this point are given off two arms at right angles which in turn bend again at right angles. The organism swims with the help of these arms. Cilia are found throughout the margin and also on the outer margin of the food groove, there being no well defined gullet, however. The body-length is 80 microns and the maximum breadth is 20 microns. There is a single contractile vacuole and two nuclei.

Order.—*Oligotricha*; Genus.—*Octocirridæ* (Gen. Nov. Madhava Rao).

Octocirrus spheratus (Sp. Nov. Madhava Rao).—The organisms are slightly ovoid. The protoplasm can be differentiated into ectoplasm and endoplasm. At the anterior end there are eight cirri as long as the body and helping in locomotion. There is also one contractile vacuole. The organism is capable of forming cysts readily under unfavourable conditions. Immediately after encystation it will have short cilia all round the body but in the adult state it is characterised by the possession of eight cirri. Hence the name of the genus. Obtained from one particular soil. Diameter 30 microns.

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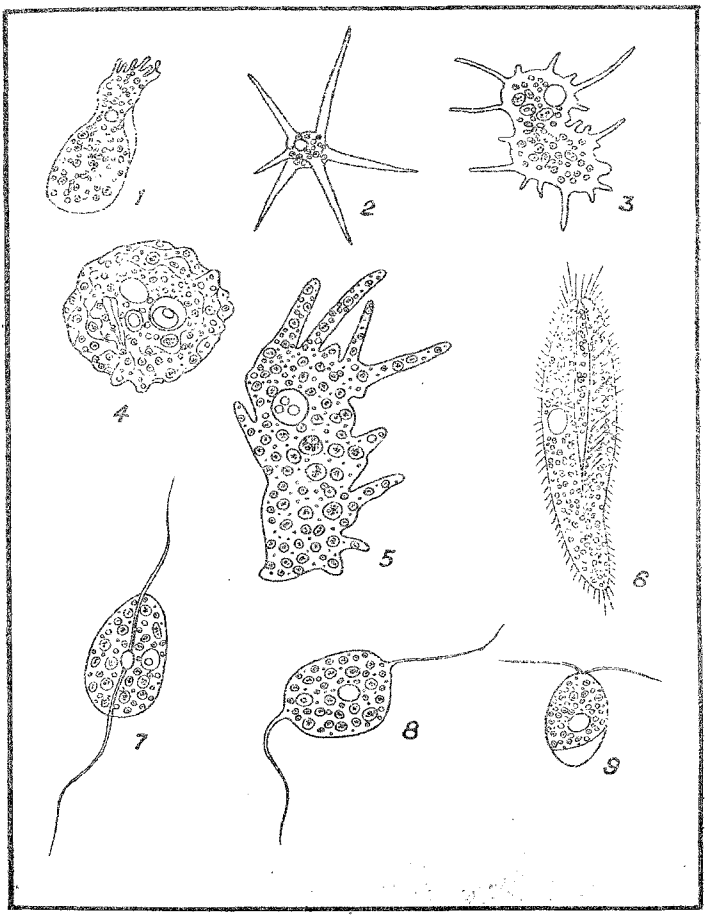
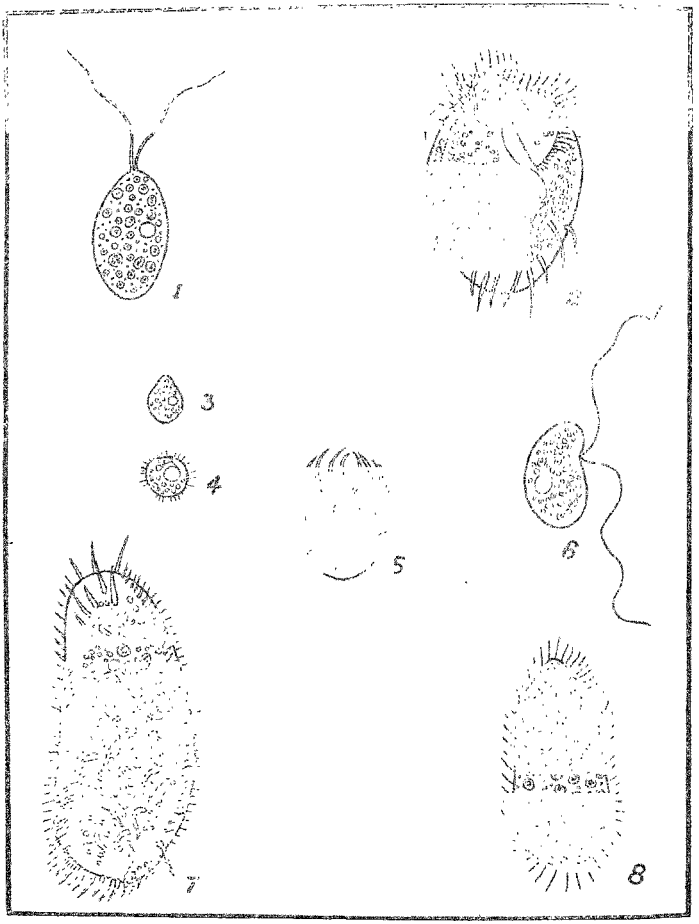


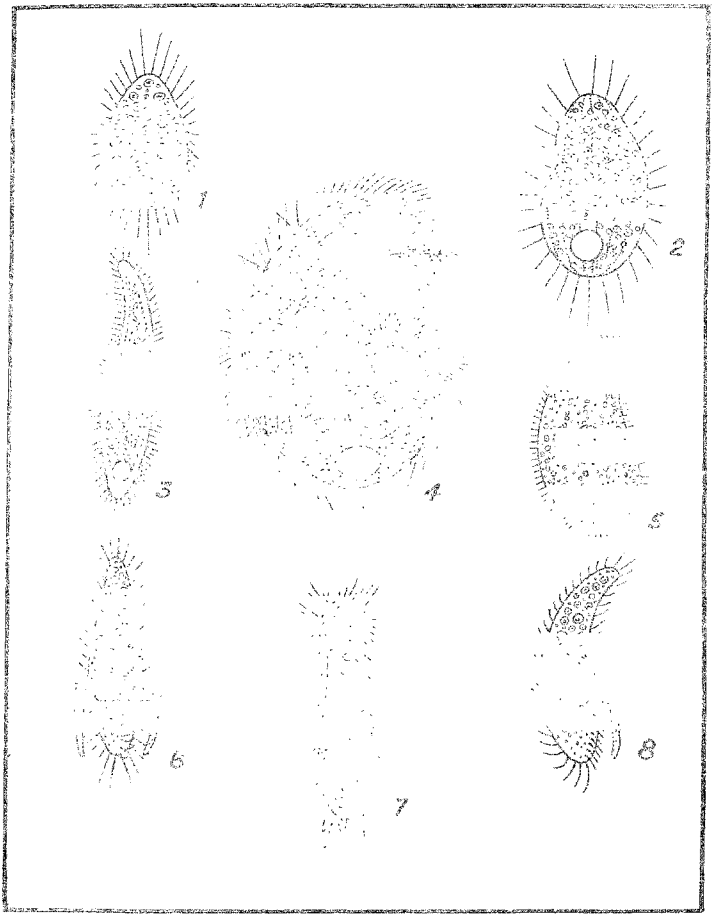
Magnification ..... 1 × 500.

1. *Amœba limax.*
2. *A. radiosa.*
3. *A. polypodia.*
4. *A. verrucosa.*
5. *A. proteus.*
6. *Balantiphorus sp?*
7. *Cercomonas sp?*
8. *Cercomonas longicauda.*
9. *Dimastigamœba Gruberi.*



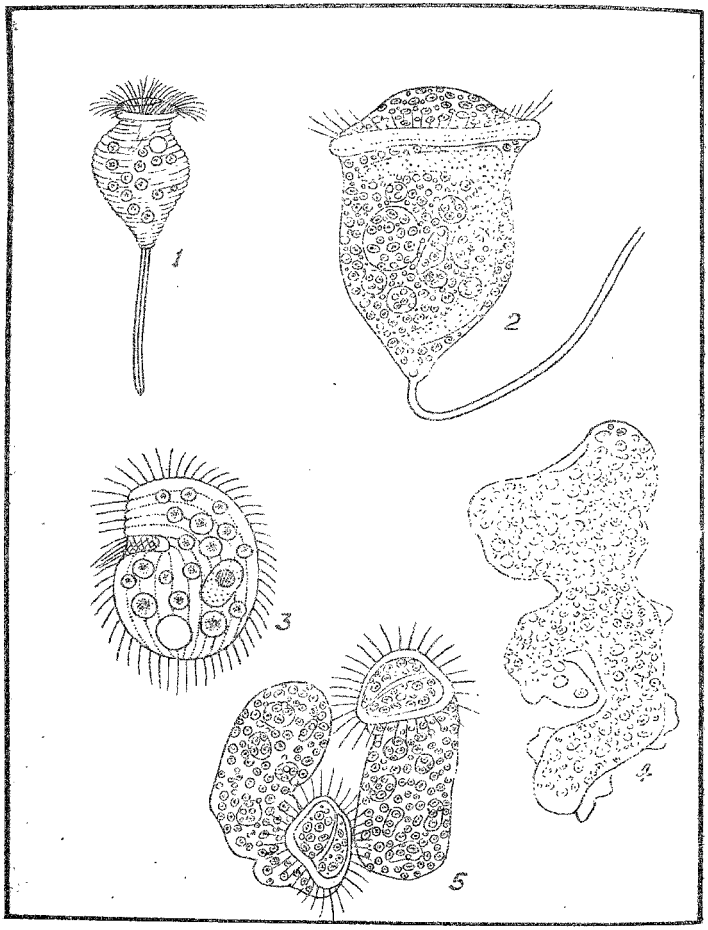
Magnification ..... 1 × 500.

1. *Oicomonas termo.*
2. *Euplotes patella.*
3. *Octocirrus spheratus* (Rao) ; cyst.
4. *Octocirrus spheratus* (Rao) ; developmental stage.
5. *Octocirrus spheratus* (Rao).
6. *Bodo saltans.*
7. *Pleurotricha lanceolata.*
8. *Pleurotricha grandis.*



Magnification ..... 1 × 500.

1. *Uronema accuminata.*
2. *Uronema marina.*
3. *Loxophyllum rostratum.*
4. *Colpoda cucullus.*
5. *Colpidium striatum.*
6. *Caudalina armata* (Rao) ; both arms visible.
7. *Caudalina Bangalorensis* (Rao).
8. *Caudalina armata* (Rao) ; side view.



Magnification ..... 1 × 500.

1. *Vorticella microstoma.*
2. *Epistylis umbellaria.*
3. *Colpoda steni.*
4. *Pelomyxa palustris.*
5. Developmental stage of *Vorticella.*