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Introduction

It has been known for a considerable time that protozoa may be important in soil fertility. It was felt that it would be interesting and valuable to determine the protozoans present in a virgin prairie. Such a prairie is located near Crocker, Iowa.

The present investigation on a virgin prairie is centered upon (1) what organisms would be present, (2) the organisms at each station and depth, and their description by drawing, and (3) correlations of the organisms at each station with other stations and depth with depth.

Method and Materials

A prairie belonging to Mrs. Lydia Moeckley, was used in this study. It is an inland prairie that has never been turned by the plow, and has been in the family since 1855. The prairie is located in Lincoln Township, Polk County, Iowa, or more specifically, Range 24 West, Township 81 North, Section 32, and approximately in the center of the section. The prairie is approximately twelve hundred feet square with a total of thirty-three acres. The general topography is low and rolling, and at several points throughout the prairie there are small, temporary pools.

At the prairie, nine stations were selected for the collection of soil samples. Station A was on a low rolling area where the soil was moist but not wet. Soils at Station B and H contained the most moisture. Station B was located at the southwest corner of the prairie about twenty feet from a temporary pond. At a depth of thirty inches water was standing in the augered hole so that contamination at this depth was possible. At station H water was also standing at the thirty-inch level although a temporary pond was some distance away. The driest stations were station C and I. Station C represented the highest and driest point of any station. The other stations, D, E. F, and G, were located to give special representation to the prairie.

Previous to obtaining the soil samples, eighty-one six-inch test tubes were prepared by the Schoenbourn (1949) method. These

were air dried and cotton stoppers applied. The test tubes were then autoclaved at fifteen pounds pressure for twenty minutes. They were then placed in a special rack and taken to the prairie.

In obtaining the samples at each station an eight-inch post hole auger was used to remove soil to a depth of thirty-two inches, and care was taken to keep foreign soil from falling into the newly formed hole. At each station soil samples were taken at the surface, two, four, six, eight, twelve, eighteen, twenty-four, and thirty-inch levels. A knife that had been sterilized in the field with the aid of a flame from canned heat was used to scrape away foreign material that might have fallen from the surface. After use at each depth, the knife was again sterilized by the above method. Surface samples were obtained before each hole was augered. The procedure was to remove a cotton stopper from a sterilized test tube, flame the tube, and take a quantity of soil from the surface into the test tube. Flaming of the tube and cotton stopper followed obtaining the samples. After samples were taken, they were returned to the rack with depth and station indicated. The procedure for the taking of depth samples was modified only by the use of a sterile knife. When obtaining depth samples, leather gloves were worn to avoid cutting the hands if a test tube should break. When taking the samples no rainfall was noted and the temperature was warm and humid.

Through preliminary soil experimentation the Noyes' (1916) Starch Peptone agar medium was selected for all subsequent work. Constituents of the Noyes' Starch Peptone agar medium are water 1000. c.c., agar 15. grams, starch 2. grams, and peptone .05 grams.

To check if air contamination occurs during the collecting of the soil samples, a control was prepared for each station. Nine one-pint jars containing sterile Noyes' media and thirty cc. of distilled water were prepared. When soil samples were collected at a given station, one of the above media jars was opened about five feet from the augered station. When soil samples were obtained at each station the lid of the control jar was applied, and the nine control jars from each station were returned to the laboratory. Daily observation of the controls was practiced for six weeks.

One-pint Mason jars with zinc glass-lined lids were used for laboratory cultivation of the soil protozoa. From the Noyes' Starch Peptone medium the following were used: Difco Standard Bacto agar, Argo corn starch, Difco Proteose-peptone, and distilled water. These constituents were heated and one hundred cc. of medium was placed in each of the one-pint Mason jars; the lids were applied

without tightening. Autoclaving of the media jars was carried out at fifteen pounds pressure for twenty minutes. The media jars were then allowed to cool. A sterile room was used for transferring soil samples from the test tubes to media jars, and all materials were placed in the sterile room at least thirty minutes before transfers were made.

Actual transfer was made by removing the cotton stopper from a test tube containing the soil sample and flaming. By the aid of a sterile knife blade, three grams of soil sample were weighed upon sterile filter paper. The three grams of soil were then placed in a one-pint Mason jar that contained Noyes' Starch Peptone agar, and added were twenty-five to thirty cc. of the singly distilled water. Distilled water in the amount of twenty-five or thirty cc. was added to each jar depending upon temperature and humidity of the laboratory. In the summer months the additional five cc. were added to compensate for evaporation. As each soil sample was transferred, a new filter paper was used and the old one discarded. When all twenty-seven soil transfers were completed, lids were tightened slightly, and the culture jars were placed under a sixty-watt lamp.

Culture examinations were made daily for a period of six weeks. Twenty-seven culture jars, or samples from three stations, were observed during an examination period. Samples from the culture jars were obtained with the aid of fine tipped micropipettes. The lid of the culture jar was raised slightly and the sample was then placed directly upon a glass slide and a cover slip was applied. Micropipettes once put to use were treated as follows: micropipettes were rinsed in tap water, and then emersed for twenty minutes in a boiling 250 cc. beaker which was filled with distilled water. The boiling distilled water was heated on a six hundred-watt heater, and was used continuously during culture examination.

For each soil sample a chart was prepared plotting the organisms observed against the date of excystment, and to facilitate the study of morphological characteristics the following stains were used: methyl green, neutral red, Nolands' solution, Lugols' solution and Janus green.

Observation and Data

From the charts that have been prepared plotting the different individual species observed at a given station against the different depths at which samples were obtained from the station, station B and C are representative. Charts 1 and 2 shows the organisms observed at station B, with this station containing the most organisms

Chart I

Protozoans observed at Station B at varying depths

	Surface	2″	4″	6″	8″	12″	18″	24″	30″
Colpoda steini	X		?						
Colpoda maupasii	X		X						
Polytoma uvella	X	X							
Monas vivipara	X								
Amoeba present*	X	X	X	X	X	X	X	X	X
Monas socialis	X	X							
Drepanomonas sp.	X	X							
Amoeba sp. No. 1.	X	X							
Distigma proteus	X								
Halteria grandinella	X								
Actinophrys sol.	X								
Trachelocera sp.	X								
Amoeba sp. (albida)	X	X	X						
Amoeba radiosa	X								
Cyrtolophosis sp. (mucicola)	X								
Trinema enchelys		X							
Allas sp. (diplophysa)	X								
Cercomonas longicauda	X	X							
Euglypha filifera	X								
Menodium incurvum		X	X						
Amoeba striata		X							
Cyrtolophosis sp.		X							
Cyrtolophosis elongata		X	X						
Cercomonas sp.		X			X				
Cercobodo sp.		X							
Amoeba sp. No. 3.		X							
Tetramitus sp.			X						
Cercomonas crassicauda				X					
Petalomonas sp.		X							
Metopus sp.			X						
Amoeba less than 10u					X	X			

*Includes limax or Valkampfia type organisms, or both size 10-30u.

Chart 2

Protozoans observed at Station B at varying depths.

	Surface	2″	4″	6″	8″	12″	18″	24″	30″
Euglypha sp.	X	X							
Bodo sp. (globosa)	X	X	X	X					
Amoeba sp. No. 2.	X								
Raphidiophrys sp.	X	X							
Centrophyxis sp.	?								
Flag. sp. No. 4.	X								
Flag. sp. No. 5.		X							
Flag. sp. No. 6.		X							
Flag. sp. No. 7.		X							
Flag. sp. No. 9.	X								
Flag. sp. No. 10.	X								
Testaceae sp. No. 1.	X								
Testaceae sp. No. 2.		X							
Ciliate sp. No. 3.			X						
Cyclidium glaucoma	X								
Heliozoa sp.	X								
Uroleptus sp.	X								
Bodo sp.	X								

Chart 3

Protozoans observed at Station C at varying depths

	Surface	2″	4″	6″	8″	12″	18″	24″	30″
Colpoda steini	X		X	X	X				
Colpoda maupasii	X	X	X						
Colpoda inflata		X	X						
Amoeba present*	X	X	X	X	X	X	X	X	X
Cohnilembus sp. (vexillarius)	X								
Cercomonas crassicauda		X	X						
Cercomonas sp.	X		X	X	X				
Trinema enchelys	X	X							
Monas socialis	X								
Cyrtolophosis elongata	X	X							
Bodo sp. (globosus)	X								
Amoeba striata	X								
Amoeba sp. (albida)	X	X	X	X					
Amoeba less than 10u		X	X	X	X		X		
Euglypha sp.	X								
Enchelys sp.		X							
Microthorax sp.	X								
Flag. sp. No. 2.	X								
Flag. sp. No. 3.	X								
Flag. sp. No. 10.					X				

*Includes limax or Valkampfia type organisms, or both size 10-30u.

Chart 4

The Mastigophora present at the different stations

	A	B	C	D	E	F	G	H	I
Allas sp. (diplophysa)	X	X		X	X	X		X	X
Distigma proteus	X	X		X		X	X	X	
Astasia curvata						X			
Petalomonas angusta var.	X							X	X
Petalomonas sp.		X							
Bodo sp. (globosus)	X	X	X	X			X		
Cercomonas crassicauda	X	X	X	X			X	X	X
Cercomonas longicauda	X	X				X		X	X
Monas socialis	X	X	X					X	X
Menoidium incurvum		X							
Polytoma uvella	X	X						X	
Cercomonas sp.	X	X	X		X	X	X	X	
Flag. sp. No. 1							X		
Oikomonas sp.						X			X
Flag. sp. No. 3.			X	X					
Cercobodo sp.	X	X				X	X		
Oikomonas termo	X			X		X			X
Monas vivipara		X					X	X	
Flag. sp. No. 2.			X						
Flag. sp. No. 6.		X							
Cercobodo sp. (vibrans)							X		
Flag. sp. No. 10	X	X	X	X					X
Bodo sp.		X							
Flag. sp. No. 9.		X		X				X	
Allantion sp. (tachyploon)	X			X					
Flag. sp. No. 4		X							
Tetramitus sp.		X						X	
Flag. sp. No. 7.		X							
Flag. sp. No. 8.		X							
Spiromonas sp.	X								
Flag. sp. No. 5.		X							

Chart 5

The Sarcodina present at the different stations

	A	B	C	D	E	F	G	H	I
Amoeba present*	X	X	X	X	X	X	X	X	X
Amoeba less than 10u	X	X	X	X	X	X	X	X	X
Amoeba sp. No. 1.		X							X
Euglypha sp.	X	X	X	X		X	X		X
Amoeba sp. No. 2.		X							
Amoeba sp. No. 3.	X	X				X	X	X	X
Actinophrys sol.		X						X	
Testaceae sp. No. 1.		X							
Trinema enchelys		X	X	X		X		X	
Trinema (lineare)							X		X
Centropyxis sp. (arcelloides)								X	
Testaceae sp. No. 2.		X							
Euglypha filifera		X							
Amoeba radiosa	X	X		X	X	X	X		X
Amoeba sp. (albida)	X	X	X		X				X
Heliozoa ap.		X						X	
Amoeba striata		X	X						
Raphidiophrys sp.		X							
Euglypha (denticulata)		X							
Testaceae sp. No. 3.									X
Amoeba sp. No. 4.						Air control culture			

*Includes linax or Valkampfia type organisms, or both size 10-30u.

Chart 6

The Ciliophora present at the different stations.

	A	B	C	D	E	F	G	H	I
Oxytricha sp.				X					
Trachelocera sp.		X							
Euplotes carinatus								X	
Halteria grandinella	X	X		X				X	
Drepanomonas sp.	X	X				X	X	X	
Microthorax sp.			X	X		X			
Tillina sp.						X			
Cyrtolophosis elongata	X	X	X	X	X	X	X	X	X
Cyrtolophosis sp. (mucicola)	X	X		X		X	X	X	X
Cyrtolophosis sp.	X	X					X		
Cohnilembus sp. (vexillarius)			X						
Blepharisma sp.	X			X		X		X	X
Colpoda inflata	X		X		X	X	X	X	
Colpoda maupasii	X	X	X	X	X	X	X	X	X
Enchelys sp.			X		X				X
Colpoda steini	X	X	X	X	X	X	X	X	X
Nassula sp.								X	
Metopus sp.	X			X					
Oxytricha minor							X		
Ciliate sp. No. 3.		X							
Cyclidium glaucoma		X						X	
Uroleptus halseyi								X	
Uroleptus mobilis var. americanus				X					X
Uroleptus sp.	X			X			X	X	X
Ciliate sp. No. 1.							X		
Ciliate sp. No. 2.				X					
Metopus es	X					X			
Ciliate sp. No. 4.						X			
Gonostomum sp.	X				X	X			X
Ciliate sp. No. 5.									X
Ciliate sp. No. 6.	X								
Ciliate sp. No. 7.	X								
Vorticella sp.	X							X	

recorded from the prairie of all the different stations. Chart 3 shows the organisms observed at station C.

However, in the original thesis work by Mote, (1953) charts have been prepared for all the different stations, as well as drawings and a description of all protozoa observed from the prairie. To conserve space the additional charts plotting the individual species present at a given station against the depth of that station, and the description of each of the protozoans, are not included in this paper. But illustrations of all protozoa observed from the prairie may be seen on Plate I through VIII.

Charts 4, 5, and 6 represents each individual species observed, plotted against its presence at the different stations. Here, one may see the distribution of a species in relation to its range over the entire prairie.

Discussion

Examination of each control jar indicated only two instances of airbourn activity. One jar contained the organism *Amoeba albida,* as described by Sandon (1927), and this organism was also common to the prairie soil. One other amoeba was observed in a control jar but no final identificiation could be made.

From the eighty-one samples, eighty-five species of protozoa were recorded and described from the prairie, and during the early examination of cultures one or more species not listed may have been present. The ciliates predominated with thirty-two species recorded from the soil, and thirty-one species of flagellates were observed. Twenty-two species of amoebas were observed, but it is possible that more than one species was listed under the classification "Amoeba present." A large number of small limax and small Valkampfia type organisms, or both, appeared in the majority of cultures. Many of these forms probably belonged to the same organisms, but no species identification was undertaken for any of the small amoeba forms. One cyst had all the appearances of Naegleria gruberi, but other cysts were present, and some resembled the genus Hartmanella. The trophic stage of Naegleria and Hartmanella are said to be very similar except for size. In other cases the trophic amoeba were not like the above genera.

Of the eighty-one soil samples examined by the cultural method only six were found to be entirely free from protozoa, and when these six samples were recultured they again gave a negative result. It was further observed that all the protozoan free cultures were from soil samples taken from the lower depths, with station A giving rise to three of the six. Providing unforseen physical and chemi-

cal irregularities within the culture it would indicate the absence of cysts from these six soil samples.

Charts 1, 2, and 3 show two of the nine station charts plotting the different individual species of a given station against the different depths at which samples were obtained from the station. A comparison of these charts indicates that a number of different species occur in the upper limits of the soil, and as depth increases the number of different species become less. These findings are in accord with Sandon's statement that the majority of organisms are found in the upper ten to twenty cms. of the soil providing maximum numbers represent the number of different species. By comparing the results of the different stations one can obtain the distribution of any given species. For example, *Colpoda steini* was found in cultures from the surface to eight inches in depth. However, *Halteria grandinella* was never observed below the surface in any cultures. Other organisms can be compared on a similar basis; however, pinpoint stratifying of any organism is impossible except for protozoa observed at the surface.

A comparison of the individual station charts also verifies Waksman's (1916) statement that few protozoan species are found below twelve inches in depth. However, in 1916 this same author made the statement that at times protozoa were found below the twelve-inch depth even to a depth of twenty or thirty inches below the surface, but even though these organisms were observed the "cases are exceptional and should not be taken into account." The results of this study would conflict with the above statement indicating an extensive amoeba population occurs in the subsoil of the prairie, either in the active or encysted condition. In this study small amoebas made their appearance in seventy-five of the eighty-one samples cultured, but in some cultures the numbers were more numerous than others.

Charts 4, 5, and 6 list the protozoa recorded from the prairie, plotting each species against the station or stations where they were observed. From these charts one can observe that some species were found at all stations, organisms like *Colpoda steini*, *Colpoda maupasii*, and many small limax amoebas listed under "Amoeba present." Other protozoa were recorded from only one station. For example, *Menoidium incurvum*, *Astasia curvata*, *Lembus vexillaris*, and a number of flagellated species. Several other protozoa were present at the majority of stations but not all, such as *Monas vivipara*, *Actinophrys sol.*, *Distigma proteus*, and a number of others listed in the charts. From this information it would appear

that not all the protozoa had the same distribution range over the prairie, but some cysts had a general distribution range while others were less general.

During this study no individual attention was given to chemical and physical conditions of the soil, but at station B considerable moisture was present with water standing at the thirty-inch depth. Yet less than four hundred feet from this station was station C which was the driest of the stations. A comparison of the results from these two stations show station B with the largest number of individual species recorded from the prairie, and only a few species were recorded from station C. These results would indicate a considerable amount of moisture may be essential for the development of certain protozoa, and when that moisture is lacking certain protozoa trophozoites or cysts will not be present.

Some investigators have spoken of certain protozoa as being common to almost every soil, or dominant soil forms. Sandon lists several of these species some of which were also found quite common in this study. Common to Sandon's list and the present study are *Oilomonas termo, Cercomonoas sp. Colpoda, Allantion, Cyrtolophosis sp.* and several limax amoebas. The family Trichopelmidae to the writer's knowledge has not been listed by other investigators, although three species were found on the virgin prairie studies.

Experimentation with this soil problem suggest the need for future studies in the field of soil protozoology.

Summary

1. From an Iowa virgin prairie eighty-one soil samples were cultured with Noyes' Starch Peptone medium, observed daily for at least six weeks, and all the protozoa that excysted were described and illustrated.

2. From the eighty-one soil samples cultured only six were found to be free from protozoa.

3. A total of eighty-five protozoa were recorded and described from the prairie. Thirty-two species of ciliates were observed, and thirty-one species of flagellates. Twenty-two species of amoebas were recorded, but other species not listed may have been present under the classification "Amoeba present."

4. From the cultured soil samples it appears the distribution of soil protozoa over the Iowa virgin prairie varies for a given species.

a. Some protozoa were found only once indicating a restricted range.

b. Some protozoa were found to have a limited range appearing at several stations.

c. Some protozoa made their appearances at each station indicating a general distribution.

5. Depth distribution for a given species at all the stations show close relationship.

6. This study confirms the fact that the majority of individual species are located in the upper ten to twelve cms. of the soil, and few species of protozoa occur in the subsoil.

7. Many protozoa described as common by other investigators were also common to this Iowa virgin prairie with the exception that many armoured Trichopelmidae are recorded for the first time.

8. Need for future studies in soil protozoology.

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Explanation of Plate I

Figure

1. Allas sp. (diplophysa).
2. Distigma proteus.
3. Astasia curvata.
4. Petalomonas angusta var.

4a. Same organism but a cross section.

5. Petalomonas sp.
6. Bodo sp. (globosus).
7. Cercomonas crassicauda.

7a. Same organism with a different body form.

7b. Same organism with a different body form.

8. Cercomonas longicauda.
9. Monas socialis.
10. Menoidium incurvum.
11. Polytoma uvella.
12. Cercomonas sp.

12a. Same organism with a different body form.

12b. Same organism with a different body form.

12c. Same organism with a different body form.

12d. Same organism with a different body form.

13. Flagellate sp. No. 1.
14. Oikomonas sp.
15. Flagellate sp. No. 3.

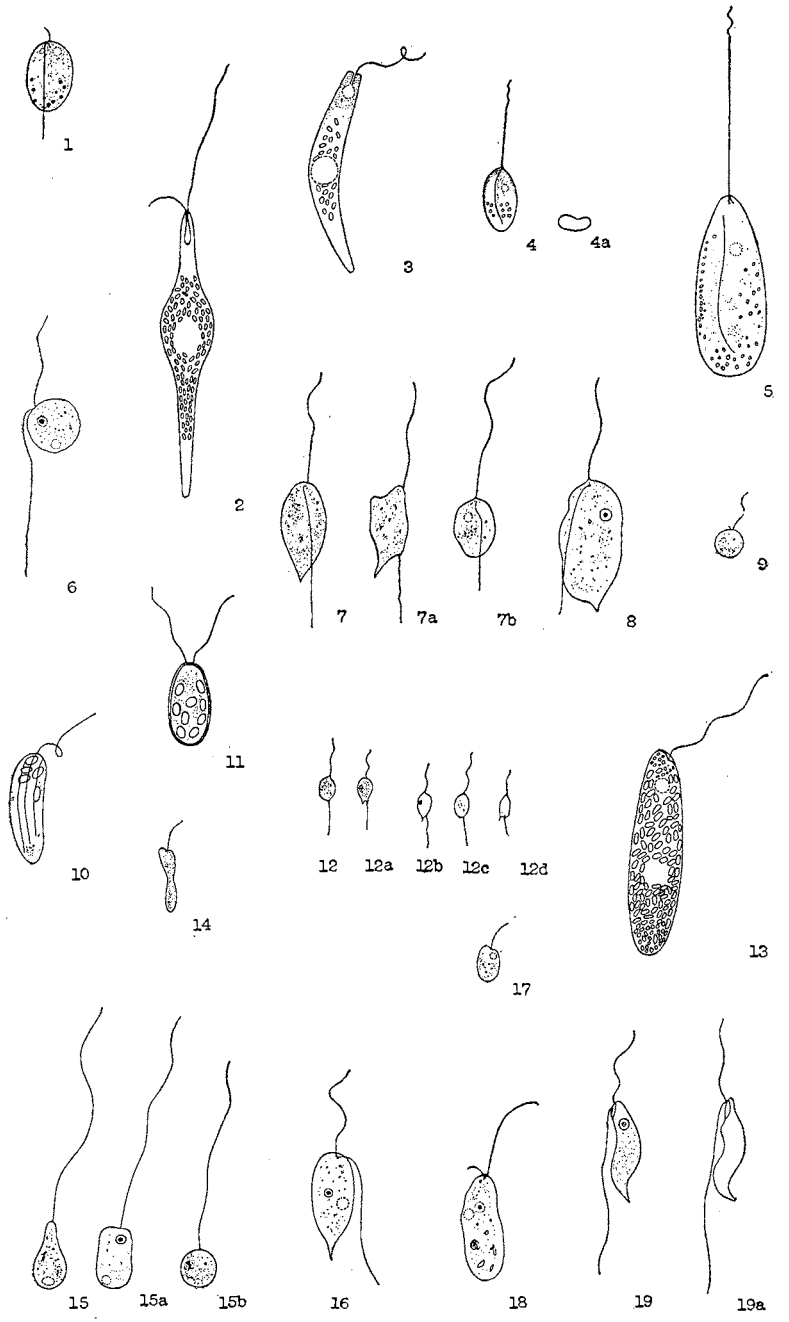
15a. Same organism with a different body form.

15b. Same organism with a different body form.

16. Cercobodo sp.
17. Oikomonas termo.
18. Monas vivipara.
19. Flagellate sp. No. 2.

19a. Same organism with a different body form.

PLATE I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19a

Explanation of Plate II

Figure
20. Flagellate sp. No. 6.
21. Cercobodo sp. (vibrans).
22. Flagellate sp. No. 10.
22a. Same organism with a different body form.
23. Bodo sp.
24. Flagellate sp. No. 5.
25. Flagellate sp. No. 9.
25a. Same organism with a different body form.
25b. Same organism with a different body form.
26. Allantion sp. (tachyploon).
27. Flagellate sp. No. 4.
28. Tetramitus sp.
29. Flagellate sp. No. 7.
30. Flagellate sp. No. 8.
31. Spiromonas sp.
31a. Same organism with a different body form.
1. Flagellated amoeba.
2. Amoeba present.
3. Flagellated amoeba.
4. Cyst of an amoeba (Naegleria gruberi).
4a. Cyst of an amoeba.
4b. Cyst of an amoeba.
5. Flagellated amoeba.
6. Amoeba present.
7. Amoeba present
7a. Amoeba present.
8. Amoeba less than 10u.
9. Amoeba albida.

Explanation of Plate III

Figure
10. Amoeba sp. No. 1.
11. Euglypha sp.
11a. Aperture scales of above species.
11b. Body scales of the above species.
12. Amoeba sp. No. 2.
13. Amoeba sp. No. 3.
14. Actinophrys sol.
15. Testaceae sp. No. 1.
16. Trinema enchelys.
17. Centropyxis sp. (arcelloides).
18. Testaceae sp. No. 2.
19. Testaceae sp. No. 3.

Explanation of Plate IV

Figure
20. Euglypha filifera.
21. Amoeba radiosa.
22. Amoeba sp. (albida) cyst.
22a. Amoeba sp. (albida).
22b. Same organism with a different body form.
22c. Same organism with a different body form.
22d. Same organism with a different body form.
23. Euglypha sp.
23a. A single aperture scale of the above species.
24. Amoeba sp. No. 4.
24a. Same organism with a different body form.
24b. Same organism with a different body form.
24c. Same organism with a different body form.
25. Heliozoa sp.
26. Amoeba striata.
27. Amoeba sp. No. 3.

PLATE II

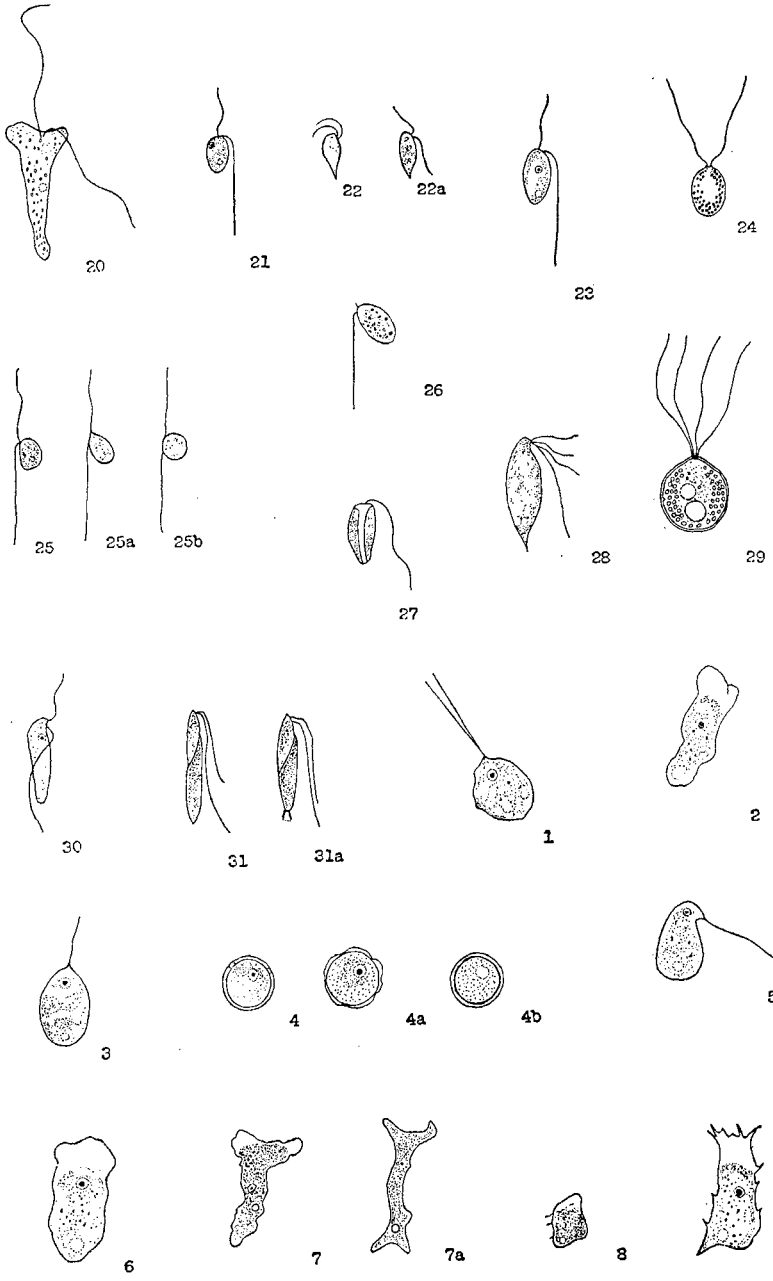
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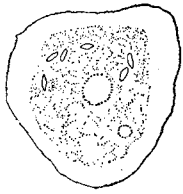
PLATE III



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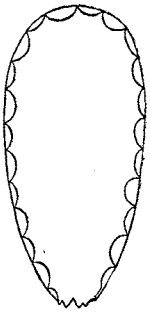

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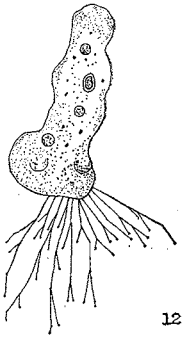
11b

11a



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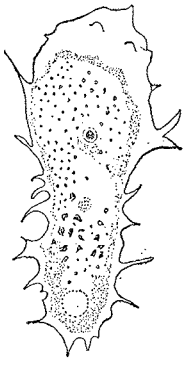

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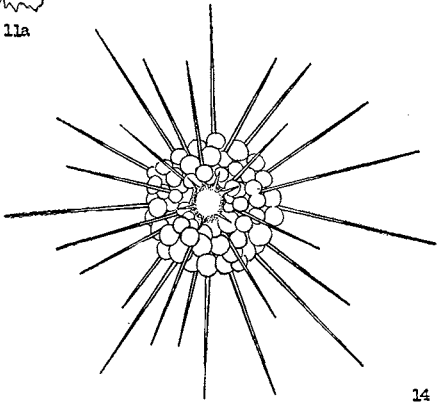

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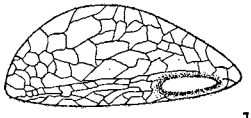

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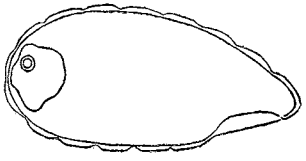

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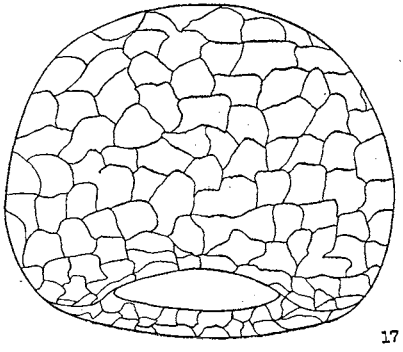

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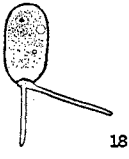

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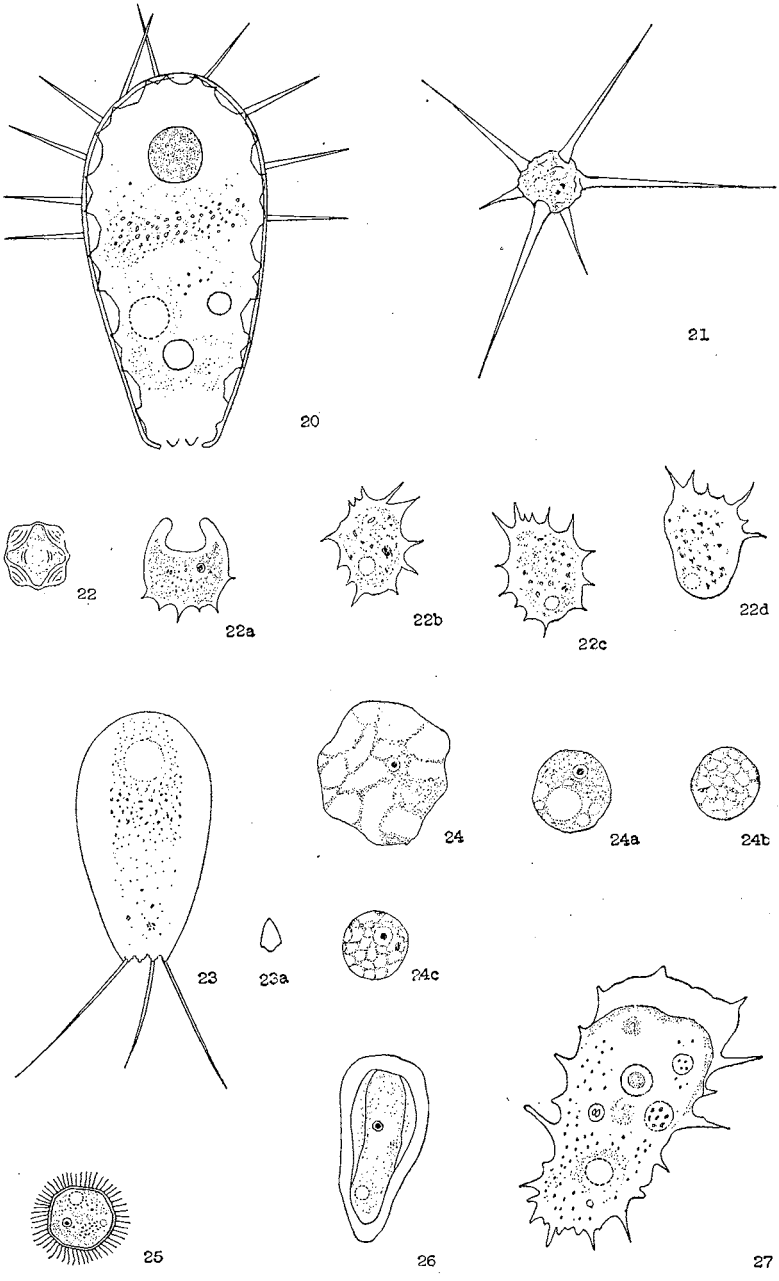
PLATE IV



Explanation of Plate V

Figure
1. Oxytricha sp.
2. Trachelocera sp.
3. Euplotes carinatus.
4. Halteria grandinella.
5. Drepanomonas sp.
6. Microthorax sp.
7. Tillina sp.
8. Cyrtolophosis elongata.
9. Cyrtolophosis sp. (mucicola).
10. Cyrtolophosis sp.

Explanation of Plate VI

Figure
11. Cohnilembus sp. (vexillarius).
12. Blepharisma sp.
13. Colpoda inflata.
14. Colpoda maupasii.
15. Enchelys sp.
16. Colpoda steini, the schlanke junge form.
17. Colpoda steini, the junge rundliche form.
18. Nassula sp.
19. Metopus sp.
20. Oxytricha minor.

Explanation of Plate VII

Figure
21. Metopus es.
22. Ciliate sp. No. 3.
23. Cyclidium glaucoma.
24. Uroleptus halseyi.
25. Uroleptus mobilis var. americanus.
26. Ciliate sp. No. 1
27. Uroleptus sp.

Explanation of Plate VIII

Figure
28. Ciliate sp. No. 2.
29. Ciliate sp. No. 4.
30. Gonostumum sp.
30a. Ciliate sp.
31. Ciliate sp. No. 5.
32. Ciliate sp. No. 6.
33. Ciliate sp. No. 7.

3420 South Union
Des Moines, Iowa

PLATE V

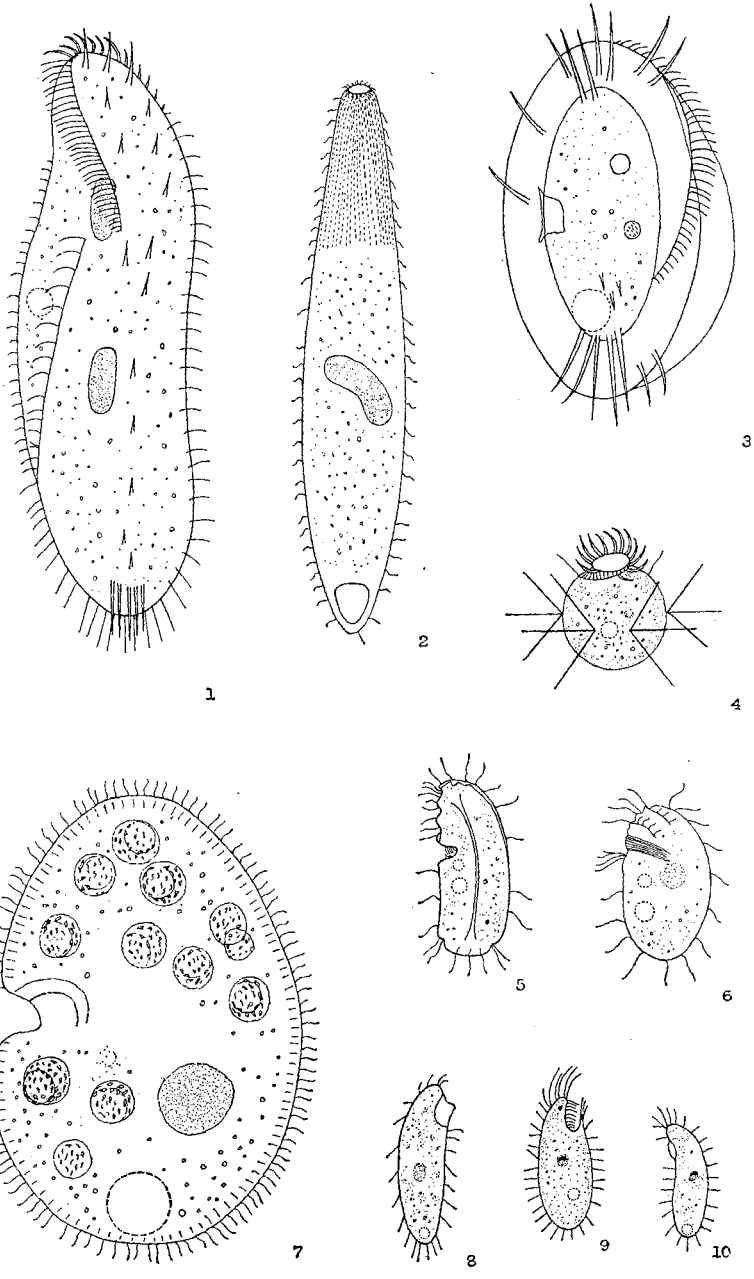
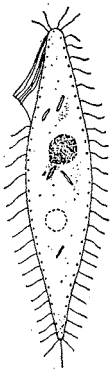


PLATE VI



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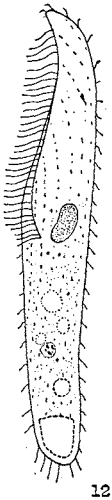

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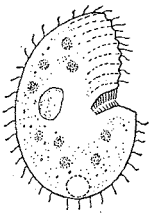

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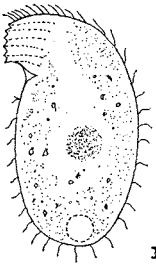

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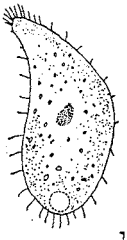

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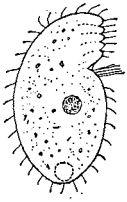

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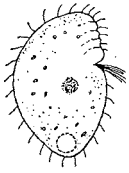

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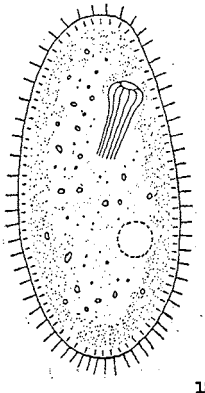

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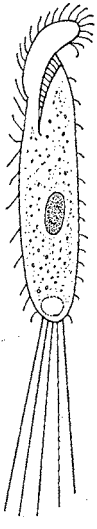

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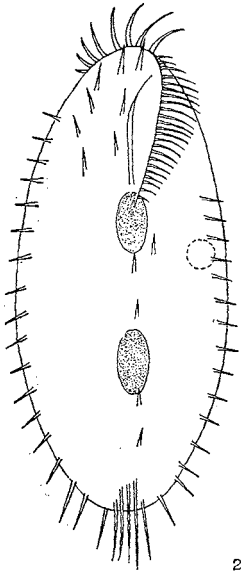

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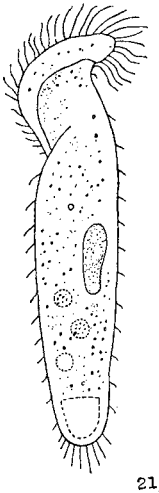
PLATE VII



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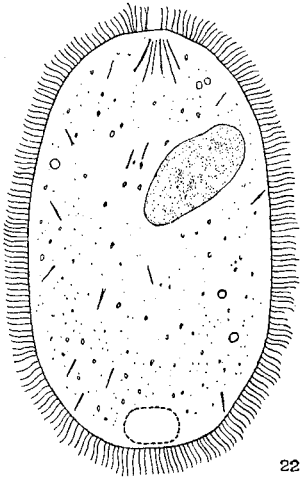

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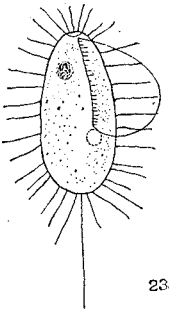

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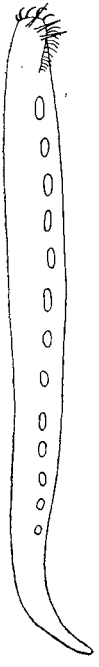

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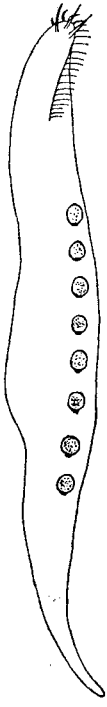

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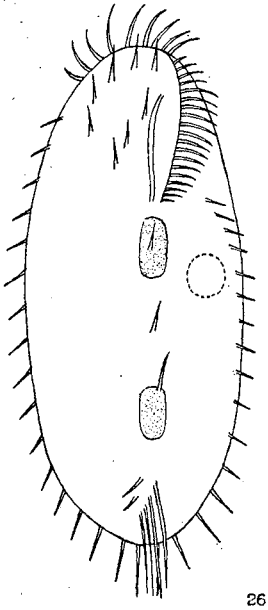

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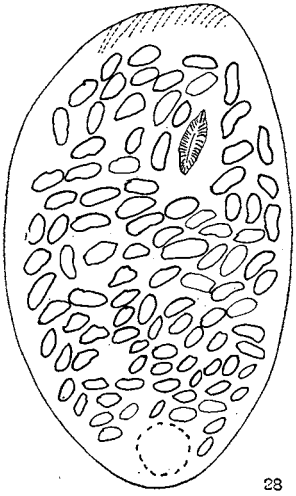
PLATE VIII



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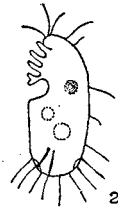

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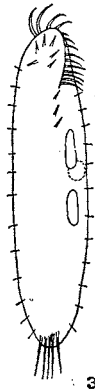

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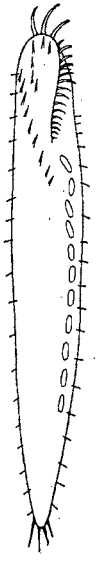

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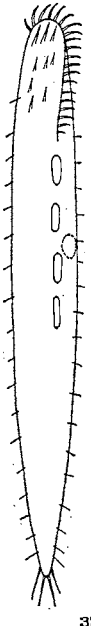

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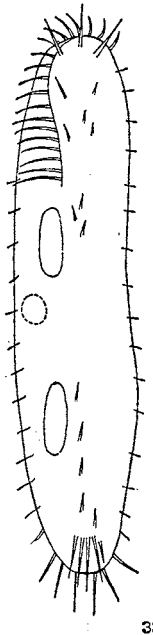

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