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On Iron Flagellates

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Phil. Trans. R. Soc. Lond. B 1946 **232**, 311-342
doi: 10.1098/rstb.1946.0004

ON IRON FLAGELLATES

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(*Communicated by F. E. Fritsch, F.R.S.—Received* 8 *January* 1946—*Read* 14 *March* 1946)

[PLATES 8, 9]

CONTENTS

The physiology and morphology of iron- and manganese-depositing flagellates are investigated by means of cultural experiments, with special reference to *Anthophysa vegetans* Stein, *Siderodendron manganiferum* n.gen., n.sp., *Siphomonas Fritschii* n.gen., n.sp. and *Bikosoeca* (*Poteriodendron*) *petiolata* (Stein) n.comb.

Anthophysa multiplies in various liquid media containing small amounts of organic substances, hay decoction being specially favourable. Still better results are achieved with soil-water cultures, which afford the only successful cultures of *Siderodendron* and *Siphomonas*, while *Bikosoeca* also grows well in hay infusions. Addition of Fe$^{\cdot\cdot}$ and Mn$^{\cdot\cdot}$ is essential.

The brown colour of biological iron deposits is shown to be due to admixture of manganese compounds, while mere ferric precipitates are in microscopical amounts almost colourless. *Anthophysa* and *Siderodendron* deposit more manganese than iron, so that their stalks appear brown, while those of *Siphomonas* are generally light brown. The envelopes of *Bikosoeca* are almost entirely composed of ferric compounds and appear colourless or faintly yellowish.

All four organisms exhibit various modifications according to the habitat conditions. The formation of stalks and envelopes respectively depends on the availability of the relevant metals in

the form of lower oxides, but the organisms here described can also exist without producing these structures. The oxidation of ferrous and manganese compounds is catalysed by the cells of these flagellates, although the role of this process in the cellular metabolism is not known. Nutrition is holozoic, chiefly by ingestion of bacteria.

Like other holozoic flagellates these organisms cannot exist in the presence of an abundant bacterial vegetation owing to the resulting lack of oxygen. They thrive in quiet, well-aerated waters, with a small content of organic substances, above zones in which Fe and Mn compounds are reduced and from which ferrous and manganous compounds diffuse to the overlying oxidation zone, where these flagellates deposit $Fe^{\cdots}$ and $Mn^{\cdots}$ in a morphologically defined form. 'Iron' flagellates generally live in association and competition with iron bacteria of the *Leptothrix* group, the removal of which produces much better growth.

A description of the relevant flagellates and of their appearance under various conditions, as well as diagnoses of *Siderodendron* and *Siphomonas*, are given.

Introduction

Ehrenberg (1838, pp. 169–70) was the first to observe the deposition of iron oxide by the activity of micro-organisms. Cohn (1875) emphasized a relation between the occurrence of certain organisms like *Crenothrix polyspora* and *Cladothrix dichotoma* and a high content of iron compounds in the water. Winogradsky (1888, 1922), by his ingenious hypothesis relating to what he called 'Anorgoxydanten', did much to interest biologists and chemists in iron bacteria. These, according to his view, utilize the chemical energy furnished by the oxidation of ferrous into ferric compounds to reduce carbon dioxide so that they do not need to be supplied with organic substances.

A causal connexion was thus established between the property of depositing iron compounds at the surface of the organism and its energy relations. Winogradsky's theory aroused much controversy, but no one has inquired whether precipitation of iron compounds adjacent to the bodies of micro-organisms is always to be regarded as evidence of the utilization of oxidation energy, i.e. autotrophism, whether partial or complete (cf. Molisch 1910, pp. 32–3).

Apart from the iron bacteria, there are quite a number of Flagellata and Algae, which deposit iron and manganese compounds in a more or less definite manner. These have never been suspected of being chemo-autotrophic (cf. Cholodny 1926, p. 118), and there are, indeed, reasons opposed to such a suggestion. A list of such organisms is given in the following table.

These organisms belong to various nutritional groups. Some may possibly be chemo-autotrophic; some are photo-autotrophic, possessing chlorophyll and reducing CO_2; some are saprotrophic and some holozoic. These differences in nutritional behaviour do not favour any general explanation as to the role which oxidation and deposition of iron and manganese play in the life of these organisms. Such iron-depositing organisms have been spoken of as iron organisms in the wider sense (Gaidukow 1905; Dorff 1934). The term does not convey any definite meaning because nothing is known as to the adaptation of the organisms concerned to the occurrence of iron. *Anthophysa* is the first non-bacterial organism to be cultivated with a view to studying its relation to iron. The three other flagellates, dealt with in this paper, are likewise holozoic, so that one is concerned with a group of biologically co-ordinated forms.

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Iron-depositing organisms

I. Chlamydobacteriales
- *Gallionella ferruginea* Ehrbg.
- *Sphaerotilus natans* Ktzg.
- *S. discophorus* (Schwers) n.comb.
- *S. trichogenes* (Cholodny) n.comb.
- *Crenothrix polyspora* Cohn

II. Cyanophyceae

(*a*) Chroococcales
- *Aphanocapsa sideroderma* Naum.
- *A. siderosphaera* Naum.
- *Aphanothece ferriglobula* Dorff
- *Paracapsa siderophila* Naum.
- *Pseudoncobyrsa siderophila* (Naum.) Geitl.

(*b*) Chamaesiphonales
- *Chamaesiphon fuscus* (Rost.) Hansg.
- *C. siderophilus* Starm.
- *C. ferrugineus* Fritsch

(*c*) Hormogoneales
- *Lyngbya ferruginea* West
- *L. Martensiana* (Men.) Naum.
- *L. Molischi* Vouk
- *L. ochracea* (Ktzg.) Gomont
- *Hydrocoleus ferrugineus* Dorff (syn. *Microcoleus ferrugineus* Frémy?)

III. Chrysophyceae
- *Lepochromulina bursa* Scherffel
- *Chrysococcus rufescens* Klebs
- *Mallomonas* spp.
- *Synura uvella* Ehrbg.
- *Kephyriopsis ovum* Pascher & Ruttner
- *K. ellipsoidea* Pascher
- *Dinobryon marchicum* Lemm.
- *D. sertularia* Ehrbg.
- *D. utriculus* Stein
- *Anthophysa vegetans* (O.F.M.) Stein
- *Siderodendron manganiferum* n.gen., n.sp.
- *Siphomonas Fritschii* n.gen., n.sp.
- *Lagynion hemisphaericum* Naum.

Colourless Flagellata, possibly related to Chrysophyceae
- *Spongomonas intestinum* (Cienk.) Kent
- *Phalansterium digitatum* Stein
- *Rhipidodendron splendidum* Stein
- *Bikosoeca petiolata* (Stein) n.comb.
- *B. lacustris* James-Clark

IV. Volvocales
- *Chlamydomonas sidero gloea* Pascher & Jahoda
- *Chlamydoblepharis brunnea* Francé
- *Polytoma siderophilum* n.sp.
- *P. fusiforme* Korsch.
- *Thorakomonas sabulosa* Korsch.
- *T. irregularis* Korsch.
- *Granulochloris seriata* Pascher & Jahoda

Phacotaceae, e.g.
- *Phacotus lenticularis* Ehrbg.
- *Coccomonas orbicularis* Stein
- *Hemitoma maeandrocystis* Skuja
- *Pteromonas angulosa* (Cart.) Lemm.

V. Chlorococcales
- *Siderocelis ornata* (syn. *Oocystis ornata*) Fott
- *S. Kolkwitzii* Naum.

VI. Euglenineae
- *Trachelomonas* (entire genus!)
- *Ascoglena vaginicola* Stein
- *Colacium arbuscula* Stein
- *C. sideropus* Skuja
- *Euglena spirogyra* Ehrbg.

VII. Conjugales
- *Closterium juncidum* Ralfs
- *C. peracerosum* Gay
- *C. tumidum* Johnson
- *C. striolatum* Ehrbg.
- *C. dianae* Ehrbg.
- *C. angustatum* Ktzg.
- *C. didymotocum* Corda

and several others

VIII. Ulotrichales
- *Microspora rufescens* (Ktzg.) Lagerh.

A. *Anthophysa vegetans* Stein

I. *Provisional description*

Anthophysa occurs as spherical clusters of colourless cells situated at the ends of branched brown-coloured stalks. These clusters or colonies can also swim freely. Each cell possesses two flagella of unequal length, a contractile vacuole, a nucleus and often contains leucosin; food vacuoles are generally present.

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The common *Anthophysa* has so often been described that there should be no doubt about its identity. Actually its relation to certain other forms is in doubt: (1) *Cephalothamnion*, described by Stein (1878) as occurring attached to small Crustacea, only differs from *Anthophysa* in insignificant respects. The original figures of *Cephalothamnion* (Stein, plate V, figures 19, 20) show colonies composed of a smaller number of cells, but in *Anthophysa* the number varies with the prevailing conditions. The length of the main flagellum is given as $1\frac{1}{2}$–2 times that of the body in the latter (Lemmermann 1913, p. 96) and as equal to it in the former, but this dimension varies in many other flagellates and is difficult to measure accurately (Pringsheim 1942, p. 174). According to Lemmermann the stalks are soft and brown in *Anthophysa* and rigid and colourless in *Cephalothamnion*. Stein, however, in his description of the figure of the former refers to 'die die Starrheit der Aeste bewirkenden stabförmigen Skelettgebilde', and Gaidukow (1905, p. 252) and Molisch (1910, p. 58) separately refer to the same features. The stalks are therefore not always considered to be soft in *Anthophysa*. Lastly, there is scarcely any difference between Stein's figure 12, depicting the first stages of colony formation in the latter, and figure 21, showing an unbranched colony of *Cephalothamnion*. Even if there is an hereditary difference between the two types, they should be referred to the same genus. (2) There can hardly be any doubt as to the form which Stein and Bütschli (1883–7, plate XLI) had in mind when referring to *Anthophysa*, but there are no satisfactory figures. Those of Stein, though beautifully drawn, do not show correctly the shape of the cells, nor the way in which they are attached to the stalks. In particular, there is no knob at the end of the stalk, and the uppermost branches are tapering and not of equal thickness throughout. Cohn's (1853, plate 5, figures 1–3) and Fromentel's (1874, plate 26, figure 5) figures are more correct as regards the stalks, but inaccurate in other respects. (3) Senn (1900, p. 133) described under the name *A. Steinii* a form differing from the original *A. vegetans* in the possession of an eye-spot. The eye-spot is so small that it might easily be overlooked. All the strains observed in Prague had an eye-spot (Teichmann 1935). Stein (1878, 3, 1, plate V) figures cells with and without it, but he does not suggest that the difference is of taxonomic value. I was long in doubt as to the existence of a form devoid of an eye-spot, but eventually found it. The colonies did not react phototactically like those of the ordinary form. No other morphological or physiological differences could be detected. I propose to call both forms *A. vegetans* and to describe the variety without a stigma as var. *Sennii*. (4) *Anthophysa* is often found in nature as detached motile colonies. In my cultures it grew with or without stalks according to the prevailing conditions (cf. p. 320). Under certain circumstances single cells were observed moving actively or adhering to solid surfaces. Such stages, which would not have been recognized as belonging to the genus without culturing, may perhaps have been described as a separate species.

No colonial member of the colourless Chrysophyceae with an eye-spot is known. One such organism found in a ditch near Cambridge proved to consist of almost colourless colonies of *Uroglena* which became yellowish in subsequent subcultures. Klug (Vlk 1938, p. 463) observed a colourless modification of *Uroglena* which he believes to be identical with *Monas sociabilis* (Meyer 1897), but the former is possessed of an eye-spot, the latter is not. It shows, however, resemblance to *Anthophysa* and may possibly be the free-living state of *A. vegetans* var. *Sennii*. Meyer's drawing is good, but, like the still better illustration given by Liebmann (1938, p. 286), it does not help to decide the question whether *Monas sociabilis*

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is a distinct species. Another organism, which is at least very similar, has been described by Moroff (1904, p. 80) under the name of *Eucomonas sociabilis*. The clear and apparently reliable description might well be that of *Anthophysa* but for the single stiff flagellum equal in length to the body. The figures are, however, poor and probably drawn without the use of an immersion system, without which it is impossible to observe adequately essential features of these delicate organisms. Korshikov (1929) has described a *Synochromonas pallida*, but the generic difference between it and *Uroglena* is not clear. It is said to ingest bacteria and would in this respect resemble *Anthophysa*.

Single cells of *A. vegetans* would be regarded as a *Monas* with an eye-spot (= *Heterochromonas* Pascher), the only one so far described being *Monas vivipara* Ehrbg. which Dangeard (1910, p. 150) regards as representing single cells of *Anthophysa*. *Monas vivipara*, however, differs in the possession of a mouth-band and is insufficiently known to be distinguished from other undescribed species of *Monas* with a stigma, of which there are several. Two that have been grown in culture did not form colonies or stalks under circumstances which will lead to their formation in *Anthophysa*.

All the strains of *Anthophysa* I have seen and cultivated belonged definitely to *A. vegetans*, although there are slight physiological or morphological differences between them. Thus, one showed only a scanty formation of cysts, while others were always rich in them. Once a form was observed with cells which were shorter and broader than usual, but in thriving cultures this difference disappeared. This deviation from the type was therefore only due to poor nutrition, but the others were hereditary and therefore true varieties.

II. *Cultural technique and material*

According to Lemmermann (1910, p. 376) *Anthophysa* is an indicator of waters rich in iron. It is, in fact, generally associated with *Leptothrix*, the sheaths of which are impregnated with iron. If there is sufficient organic matter, *Cladothrix dichotoma*, *Zoogloea ramigera* and many other bacteria may be present. There is, however, often no evident precipitate of iron compounds in the waters frequented by *Anthophysa*. The latter was found in the municipal water supply of Prague by Molisch (1892), Adler (1904) and Ruttner (1906) at a time when insufficiently filtered water from the river Vltava (Moldau) was used for household purposes, but since the water is more carefully purified it has disappeared.

Adler (1904) first established that good development took place when ferrous ammonium citrate or manganous iron citrate were added to the Prague tap water. The form developed in cultures with the manganese salt had thicker, blackish brown stalks in place of the reddish brown ones in cultures without manganese. From other experiments Adler concluded that *Anthophysa* only needs the small traces of iron required for the growth of every organism. Teichmann (1935) confirmed Adler's results, using water from the river Vltava. His cultures were kept in the dark to avoid early oxidation and precipitation of iron compounds. *Anthophysa* has also been grown in enrichment cultures with hay and precipitated iron hydroxide (Winogradsky 1888, p. 263).

Teichmann employed the same inorganic medium as in his experiments with *Leptothrix discophora*, with the addition of various concentrations of ferrous ammonium citrate. Good results were obtained with strengths of 0·003–0·1 %; 0·5 % was too high. A neutral or slightly alkaline reaction was best. After a day the media were rendered turbid by bacteria,

later the colour changed from almost colourless to yellow. On the third or fourth day floccules of stalked *Anthophysa* colonies appeared. By repeated transference of single colonies into sterile water Teichmann attempted to obtain bacteria-free cultures, but always with negative results. The *Anthophysa* multiplied only in the presence of bacteria. Teichmann's cultures never grew particularly well, although serial subcultures could be made.

No readily recognizable growth of *Anthophysa* was to be found near Cambridge, but in a ditch with decaying *Spirogyra* there occurred, together with various colourless flagellates, especially *Chilomonas*, spherical colourless, many-celled colonies at first taken to be *Monas sociabilis* (figure 1). The presence of small eye-spots suggested that these colonies might belong to *Anthophysa*, and this was confirmed by cultures started with single colonies which developed brown stalks after two days.

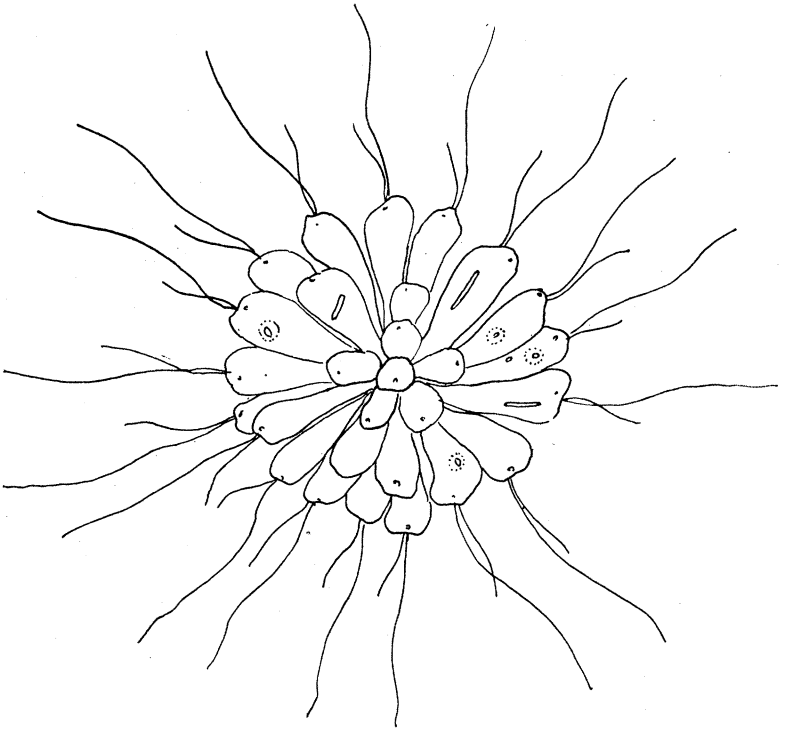

FIGURE 1. Free-swimming colony of *Anthophysa vegetans*, × 300.

By preparing enrichment cultures with boiled hay and ferric hydroxide the organism is obtained more readily. If, for example, black mud from a polysaprobic water is employed, iron organisms including *Anthophysa* begin to appear within a few weeks. Starting either from a natural growth or from such enrichment cultures single colonies are transferred to test-tubes containing small quantities of organic substances, soil and water (Pringsheim 1921, p. 93; 1936, p. 46; 1942, p. 172). After a few days colonies can be observed with a lens, swimming near the surface of the fluid; later stalked compound colonies appear as a brown ring adhering to the glass, a few millimetres below the surface of the water, while other free-swimming ones often appear as cloud-like aggregates.

The first strain (1) was taken from the ditch referred to above; a second one (2) from the grey mud of another ditch near Cambridge, containing *Beggiatoa*, *Sphaerotilus*, amoebae and other organisms. A third strain (3) was found in mud from a mountain tarn near

Wray Castle in the Lake District, a fourth (4) from a ditch with *Euglena* and other flagellates, beside the river Cam not far from Cambridge, and a last (5) from a ditch rich in decaying material in the garden of Trinity College, Cambridge.

Although the soil-water technique provides rich and long-living cultures, a liquid medium containing a dilute decoction of green hay with 0·01–0·025% ferrous ammonium citrate was also employed. This actually affords a better growth than Teichmann's solution. The concentrated hay decoction is neutralized with calcium carbonate before diluting. Cultures are kept in the dark at room temperature. At 25° C growth is quicker, but ceased sooner. By repeated washing of the colonies in sterile water bacteria were completely eliminated, but in their absence no multiplication of *Anthophysa* could be obtained in any medium. It cannot live without ingesting food particles, such as bacteria.

III. *Nutrition*

(*a*) *Liquid media*

Repeated attempts were made to grow *Anthophysa* in mineral solutions of various concentrations and pH, with nitrates or ammonium salts as the source of nitrogen, a ferrous and/or a manganous salt always being present. When ferrous ammonium citrate was used, there was slow and poor multiplication, while with ferrous sulphate no growth was observed, even if manganous sulphate was also present. These experiments do not prove conclusively that *Anthophysa* cannot be grown without organic food, but show it to be unlikely. Cultures containing organic substances and bacteria were therefore employed in an attempt to elucidate the mode of nutrition.

In these experiments ferrous ammonium citrate was usually employed, since this is obtainable as a pure ferrous salt, which can be sterilized as a 1% stock solution. The poor results obtained with this salt as the only organic compound are due to its low food value at concentrations tolerable to bacteria and to the lack of organic nitrogen. Heavy putrefaction was, however, also to be avoided. The following media were tested with the object of obtaining more abundant multiplication.

(1) *Sugar* as the main organic compound is not suitable. The concentration of dextrose has to be kept below 0·5%. With a complete mineral solution, 0·05–0·2% dextrose and ferrous ammonium citrate, the results are not much better than without sugar. Somewhat better growth is obtained if asparagin is substituted for inorganic sodium salts.

(2) Neutralized *beef extract* provides a fairly good nutritive solution in concentrations of 0·02–0·2%, with 0·02% ferrous ammonium citrate. Better results are obtained when dextrose is also present, but even so the growth is far inferior to that in hay cultures with iron. Peptones are even less suitable.

(3) *Hay extract* was tested in view of the good growth obtained with hay and ferric hydroxide. A decoction of bleached grass leaves left on the field during winter supplemented by ferrous ammonium citrate supplied the first satisfactory results. Extracts of green hay must be neutralized and sufficiently diluted, otherwise bacteria are too abundant. They provide a rich growth and were often used for experiments.

(4) *Extracts of soil*, leaf mould and peat rich in humus will also support growth after addition of iron, but they are not as effective as hay extract.

(*b*) *Soil-water media*

The duration and the health of the organism can be considerably improved by the addition of garden soil. Preliminary cultures, containing putrefiable organic material and soil and designed to produce a growth of colourless flagellates, often contain iron organisms (Pringsheim 1936, p. 53). Brownish or reddish rings composed of *Leptothrix* and other iron bacteria develop at a small distance from the surface of the fluid. This has been explained by the assumption that iron salts are reduced to ferrous compounds by anaerobic bacteria near the bottom of the tube, where penetration of oxygen is prevented by the soil; at a higher level the ferrous salts are oxidized by the iron bacteria (Pringsheim 1934, p. 298). *Anthophysa* is also able to utilize this source of ferrous compounds.

The garden soil employed contained appreciable amounts of iron. When extracted with hydrochloric acid, with evolution of carbon dioxide, the resulting yellow solution gave a strong Prussian blue reaction. Various *Trachelomonas* species grew well in cultures with this soil and formed brown, iron-containing envelopes. *Anthophysa*, however, was never abundant, whether further organic substances were added or not, and the stalks remained short and faintly coloured so long as additional iron and manganese were not provided. It thus requires a larger amount of heavy metals than *Trachelomonas*. Addition of 0·01–0·05% ferrous ammonium citrate favoured the growth in test-tubes with soil and distilled water. Addition of about 0·01% $MnSO_4.7H_2O$ further improved stalk formation, so that a thick brown ring developed near the surface of the fluid. In place of garden or other kinds of soil, sand with peat or acid leaf mould can be used. Leaf mould covered with soil, and supplemented with iron and manganese, provides the best medium so far prepared.

The favourable influence of humus substances is probably due to two properties. (1) They provide sufficient food only for a limited development of bacteria, an abundant growth of which is harmful, since they consume too much oxygen. Competition with bacteria is an important ecological factor for every micro-organism, even for holozoic ones. As a consequence, quantities of starch, wheat grains, etc., which further the growth of many heterotrophic flagellates, are not favourable to *Anthophysa* and other forms with similar nutritional needs. In order to grow them the equilibrium between feeding and food organisms has to be maintained. (2) Another effect of humus substances is to keep in solution iron compounds which would otherwise be precipitated (Pringsheim 1930, p. 35; 1934, p. 302 et seq.); in this respect their effect is similar to that of citrates. No substitutes have been found.

Apart from the presence of a suitable amount of bacteria, the growth of the stalked form of *Anthophysa*, like that of other iron organisms, involves the provision of dissolved ferrous compounds. Such organisms interfere with the oxidation of these compounds by atmospheric oxygen at a small distance from the surface of the liquid where their swarmers settle and form colonies. Observations on the influence of various concentrations of ferrous salts support this explanation. The ring of *Anthophysa* marks the boundary between zones of oxidation and reduction. Those stalks which project downwards into the region where oxygen is lacking and where the state of the heavy metals must be different, are faintly coloured and bear plentiful colonies, while the older ones above and nearer the point of settlement are brown.

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The height at which the ring first appears depends to some extent on the concentration of the heavy metal salts. *Anthophysa* was inoculated into tubes containing leaf mould and sand, to which 4, 2, 1, 0 drops respectively of a solution of 0·2% ferrous ammonium citrate and 0·2% manganous sulphate were added. Five days after inoculation growth was observed in all the tubes, being most abundant in those which had received 4 drops and very poor in those without any addition. The differences concerned especially stalk formation. Cultures with 4 drops showed long and thick stalks, those with 2 drops only short and thin ones, those with a single drop still shorter ones. The distances of settlement from the surface were: 4 drops, 1 cm.; 2 drops, 1·8 cm.; 1 drop, 3 cm.

These observations can be explained by the fact that *Anthophysa* lives in the zone where the lower oxide is transformed into the higher. Ferric salts are not poisonous because they are immediately precipitated. The boundary is therefore determined by the unsuitable conditions obtaining in the lower regions, which lack oxygen and exhibit a certain concentration of ferrous compounds, the two being interdependent. The oxygen is consumed by the cells of *Anthophysa* and by the chemical oxidation of the lower oxides, the concentration of which is responsible for the level of the boundary. This is also proved by the influence of the amount of lower oxides added, which shows that the level, at which the swarmers settle and develop into colonies, is determined by the concentration of lower oxides present at that level and the amount diffusing from the region of reduction to that of disappearance by oxidation.

(*c*) *Ingestion of bacteria*

Anthophysa fails to grow if the colonies used for starting subcultures in sterile media are thoroughly washed. This might be due to the necessity for bacteria to transform the media into a suitable condition. Food vacuoles are, however, regularly found in thriving cultures of *Anthophysa* so that the effect of the ingestion of bacteria on multiplication required investigation.

One or a few colonies were transferred to sterile liquid media, such as would either allow multiplication of *Anthophysa* and accompanying bacteria or only maintain living colonies for a restricted period without multiplication. For the former purpose media usually composed of hay or soil extract with ferrous and manganous salts, for the latter indifferent fluids (tap water, inorganic nutritive solutions, dilute soil extract) without addition of ferrous and manganous salts were used.

On inoculating media of the first type with washed colonies no multiplication of *Anthophysa* was obtained, while normal growth was observed with unwashed colonies. That the failure to multiply was not due to any harmful effect of washing was shown by the growth obtained when a loopful of fluid containing bacteria without *Anthophysa* cells was added. Similar, but more specific, results were obtained by using pure cultures of *Bacterium vulgare* and *B. coli*, instead of mixtures of unknown composition. The development of washed colonies in a dilute beef extract or hay medium with iron and manganese, to which one of the species mentioned had been added, was usually slightly inferior to that of colonies which had not been washed. This may have been due to unsuitability of the particular bacteria as a source of food, but also to the lack of dispersed bacteria after one or two days since they failed to multiply and quickly sedimented.

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The results obtained with fluids of the second type were somewhat different. Neither washed nor unwashed material would grow in such fluids without the addition of bacteria. When a loopful of bacteria was introduced into 10 c.c. of culture fluid containing one or two colonies of *Anthophysa* there was appreciable multiplication of the latter, but stalk formation was almost completely lacking. Subcultures of the same kind appear to remain indefinitely without stalk formation and the growth is never abundant. Nevertheless, there is no doubt that, under certain conditions, *Anthophysa* can multiply without ever excreting the iron or manganese oxides that build up the stalks. This condition was realized in the habitat, from which the first strain was derived, and it is common in nature.

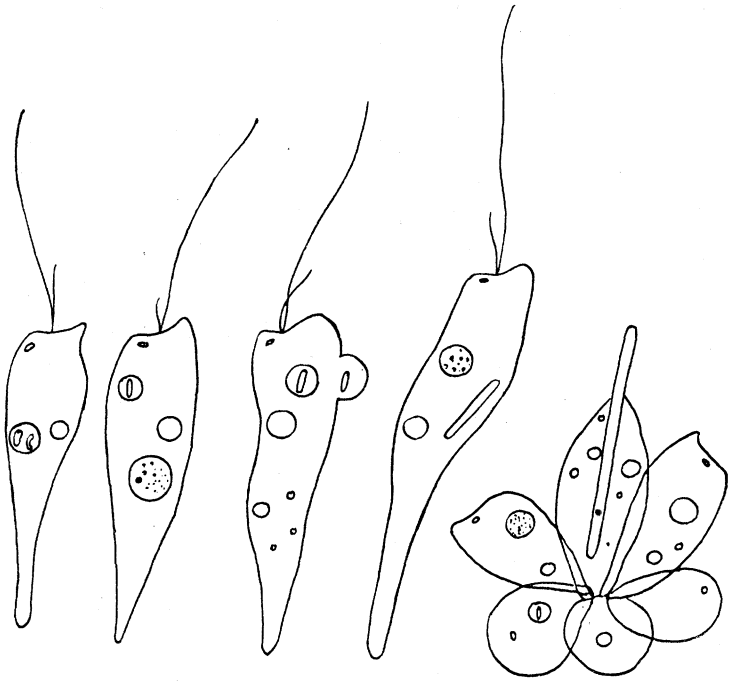

FIGURE 2. Ingestion of bacteria and food vacuoles in *Anthophysa*, × 1000. *a*, cells with food vacuoles; *b*, ingestion of a large bacterial rod.

The process of ingestion, which can best be observed in few-celled colonies or single cells imprisoned between debris, follows the same course as in other pigmented and colourless Chrysophyceae. Small particles, generally bacteria, are driven on to the cell surface by the flagella, most frequently near the base of the latter where the protoplasm appears to be most fluid and continually changes its contour forming short amoeboid pseudopodia or lip-like processes. Food particles can, however, also be taken in at other places. They are rapidly enveloped in a large projecting vesicle, filled with clear liquid (figures 2*a*, *b*). The bacterium almost immediately sinks into the cell, and as digestion proceeds becomes fainter and disintegrates into minute rounded particles. More accurate observations are rendered difficult by the small size of the cells which constantly alter their shape and situation.

IV. *Morphology*

Anthophysa colonies are very delicate, and proper fixation is scarcely possible. Our knowledge of its morphology therefore remained scanty, so long as reliance had to be placed on the material found in nature, which is disturbed when touched by any implement.

When grown *in vitro*, however, the development can be watched with a hand lens. Moreover, continuous microscopic observation was rendered possible by the use of micro-chambers mounted on slides. Three sides of the chamber consist of sealing wax or of glass strips fastened with solid paraffin, the fourth is left open. The chamber is closed by a cover-slip which allows of the use of immersion systems, an improvement on the device described by Schaudinn (1894; cf. also Bělař 1928, p. 772). If the chambers are filled with hay decoction or another medium containing iron and manganese they can serve as culture vessels, while a miniature soil-water culture can be prepared with mud and liquid. Although only objects adhering to the cover-slip can be observed under high powers, most of the features needed for an adequate description of organisms like *Anthophysa*, which settle near the surface of the fluid, can be detected. Such micro-chambers are especially suitable for long-term observations.

A typical growth of *Anthophysa* consists of numerous compound colonies, composed of unit colonies each derived from a single cell and individually attached to the delicate tips of branched stalks. The latter are excreted by the several unit colonies and there is no firm coherence between the two. The thicker stalks at the base of the compound colony are the oldest, and the forks are the result of fission of the individual unit colonies.

(*a*) *Structure of the cells*

The cells resemble those of other naked Monadaceae or Ochromonadaceae. As in most of these the shape of the cell changes, either rapidly owing to metaboly, or more slowly over longer periods, depending upon surrounding conditions, although the two kinds of changes overlap. Three forms of cells can be roughly distinguished. In a large colony the cells are clavate (1), the posterior end being prolonged into a nearly cylindrical tail, while the anterior end is rounded. Pressure of the cover-slip or other causes lead to the detachment of single cells which swim freely and assume an ovoid or pyriform shape (2). These resemble the single cells found in cultures under unfavourable conditions. The posterior end is either rounded or (3) drawn out into a fine thread, the tip of which is attached to solid particles, to the glass, etc.

The front margin is generally obliquely truncate, with one edge more protruded than the other, and in the type this bears a minute deeply coloured eye-spot. The flagella arise from the slight depression between the two unequal mounds. The anterior region continually changes, becoming broader and narrower, putting out processes which are subsequently withdrawn.

When cells escape from the colonies they exhibit rapid changes of shape, and it almost appears as if the form of the cells within the colony were directly conditioned by mechanical forces. The pressure of neighbouring cells certainly plays a part, but other forces must co-operate, since gradual changes such as renewed elongation after rounding off ensue after the cells have escaped. The effect of mutual pressure is seen also in the different appearance of the units in small and large colonies, which goes so far as almost to suggest different species (figure 3). In small colonies the cells are egg- or pear-shaped, much like the free-swimming ones, while in larger colonies they are much longer and club-shaped.

The two flagella are of different length, one being about four times as long as the other. According to Vlk (1938, p. 463) the longer one bears the ciliate appendages characteristic

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of Chrysophyceae, while the shorter one is a simple thread. Observation of stationary cells with dark-ground illumination showed no difference in the movements of the two flagella.

It is not yet clear whether there is any relation between the occurrence of free movement and the presence of food vacuoles. While in non-colonial Chrysophyceae ingestion of bacteria does not seem to take place in free-swimming cells, motile colonies of *Anthophysa* are probably able to ingest bacteria. Food vacuoles, though often abundant, are sometimes lacking and the cells can live for several days on their reserves, which consist of oily droplets and leucosin, the reserve characteristic of Chrysophyceae (Dangeard 1910, pp. 155–7).

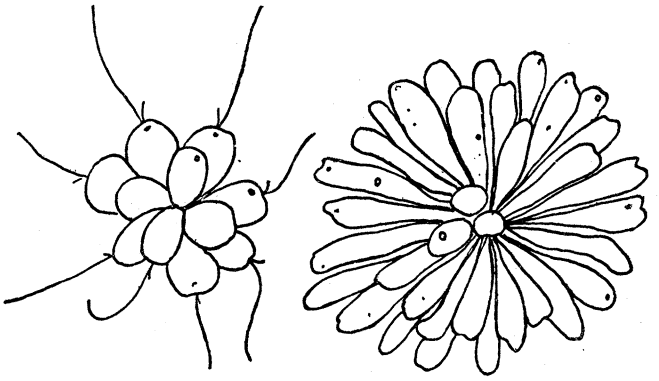

FIGURE 3. Different shapes of cells in small and large colonies, × 500.

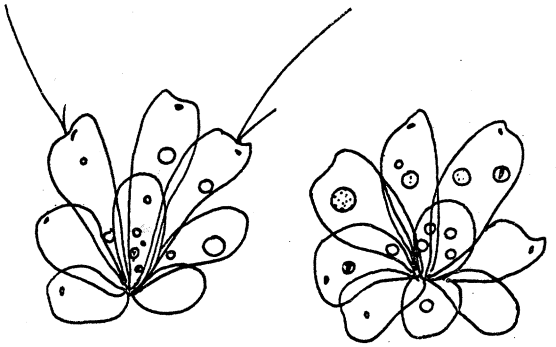

FIGURE 4. Small excentric colonies drawn during movement, × 1000.

(*b*) *Morphology of the colony*

The colonies are slightly elongate and the region, where the posterior ends of the cells are in close juxtaposition, is a little behind the centre. The tapering inner parts of the cells run for a short distance almost parallel, forming a strand from which the cells project in all directions. All but the middle cells are more or less bent so that the front ends are orientated nearly perpendicular to the surface of the colony. The excentric character is often more marked in attached than in free-swimming colonies (figure 4). The latter rotate around their longitudinal axis. In spite of the seemingly irregular twisting and rolling movements, the colonies obey a geotactic stimulus and aggregate near the surface, or a chemotactic one resulting in their accumulation just above the mud. The colonies with eye-spots are negatively phototactic.

(*c*) *The stalks*

The form of the stalks and their mode of connexion with the colonies is incorrectly shown in some of Stein's figures (1878, plate III, figure v), which have often been copied. Stein depicts rather solid stalks of almost uniform thickness, even at the top where the colonies are attached. Actually the upper parts of the stalks are frail and pale, and often difficult to detect. Between the older brown branches and the top of the stalks, the colour gradually fades, as mentioned already by Cohn (1853, p. 110) and Molisch (1910, p. 57). Owing to their delicate nature the tops of the stalks readily break and get carried away with the escaping colonies. The stalks are flexible (cf. p. 314) so that the attached colonies move gently to and fro under the action of the flagella (Saville Kent 1881–2, p. 267).

FIGURE 5. Compound colonies attached to floating particles and in process of formation, × 1000.

These facts were established with the help of the micro-chambers described on p. 321, which also made it possible to study the origin of the stalks. After a swarmer has settled on the cover-slip, it gives rise to an irregular cluster of a few cells, which multiply to form colonies (figures 5, 6). By mutual pressure of the cells the colonies become more regular. The flagellar movements go on continuously and help in the accumulation of food. The young stalk is exuded as a colourless, sticky, gelatinous mass, to which bacteria adhere. This mass pushes the colony over the surface of the glass until the lengthening stalk lifts it beyond the cover-slip. Older stalks generally hang from the place of attachment at some distance from the surface of the fluid, and a brown zone is produced by the numerous branched stalks which bear many unit colonies. Eventually a number of the cells form cysts, while the others disintegrate.

FIGURE 6. Portions of two stalks of different breadths, in cross-section on the right, × 1000.

It is still not clear how the unfinished stalks change into the thick brown ones, but the process must be a physico-chemical and not a biological one, since it takes place at some

distance from the living cells. It is not due to the penetration of iron compounds into a preformed organic mucilage (Molisch 1910, pp. 57–8), but to the mixture of brown manganic compounds with an almost colourless ferric hydroxide gel.

Experiments with various media show that iron salts alone do not suffice for the formation of the thick, dark brown stalks, but that manganese is necessary (cf. p. 315 and Adler 1904). It is actually possible to obtain stalks which give no Prussian blue reaction and consist only of manganese compounds. For this purpose soil-water cultures are not suitable, but hay decoction with 0·01% $MnSO_4.7H_2O$ can be used. The brown colour of the stalks thus obtained disappears on treatment with ferrous potassium cyanate and hydrochloric acid, being replaced by the white of the manganese compound corresponding to Prussian blue. Manganic hydroxide resembles the equivalent iron compound, except that its colour is brownish instead of reddish, although both compounds appear rather faintly coloured in thin layers. Brown oxides probably arise from manganese hydroxide by dehydration, followed by their oxidation to dark brown superoxides.

Molisch's view (1910, pp. 57–8) that an organic gelatinous mass is first exuded, which then attracts iron compounds, is apparently based on the observation that the stalks are at first faintly coloured and afterwards become deeper brown. Actually, however, the youngest tips already give a strong Prussian blue reaction whenever the older parts do so. There is no proof that any appreciable amount of organic matter is excreted. Molisch was misled by the general belief that the brown colour of the deposits in iron organisms is due to their rust-like nature, and that a structure not possessing such a colour could not be composed of iron compounds. This is the more remarkable as Molisch had a liking for experimenting with manganese compounds. In a medium containing both iron and manganese the older parts of the stalks show a dark inner well-defined thread and an outer, more translucent layer with an uneven surface (figure 6). Within the latter and on its surface are bacteria of various shapes, the presence of which renders it difficult to reach definite conclusions concerning the origin and fate of the layers of the stalk, since the bacterial growth increases on the older stalks. After several months stalks are no longer recognizable, only zoogloeae embedded in an amorphous mass of irregular 'iron' precipitates. In cultures containing *Bacterium coli* or *B. vulgare* only (cf. p. 315) there was no such settlement on the stalks, which retained their sharp outlines and long displayed clearly the darker brown inner thread.

Bifurcation is frequent, although in my cultures not as regular as shown in Stein's figures. The division of the colonies was not observed under the microscope, nor was it possible to follow up large compound colonies with many units to their very base.

As already mentioned the form and consistency of the stalks depend largely on the prevailing conditions, especially on the amount of food and on the manganese content of the medium. The stalks are short, faint and poorly developed when *Anthophysa* feeds mainly on bacteria; long, thick and brown when manganous compounds are available in appreciable concentrations, but little organic food is present. In the latter circumstances there appears the form with many branches, usually described as characteristic of *Anthophysa*, although rarely found in nature. No one modification of an organism can be regarded as the type unless it grows much more healthily than the others. All the various modifications of *Anthophysa* should be considered as equally characteristic of the species.

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(*d*) *Life cycle*

There is no inherent periodicity in the life cycle, the sequence of morphological development being determined by external influences. *Anthophysa* occurs as motile cells and few-celled aggregates, as many-celled colonies either free-swimming or borne on the ends of branched stalks, and as cysts.

When colonies are inoculated into suitable media they multiply considerably within 2 or 3 days before any stalks are formed. These appear after a further short period during which no free-swimming colonies are to be found. Examination of such cultures, preferably in micro-chambers, shows that the colonies first introduced separate into single cells which divide to form new colonies. These in turn again break up into single cells which usually settle on solid particles, or on the surface of the glass or of the water. As they divide, the cells deposit iron and manganese hydroxide at their posterior ends, the individual deposits uniting to form the young stalk.

The causes operating in producing the sequence of free-living and attached colonies are probably to be found in the momentary states of the iron and manganese compounds. Cultures with ferrous ammonium citrate are at first clear and almost colourless, but later turn turbid and yellow, indicating a change in composition (cf. p. 315), and this happens just when the formation of stalks commences. When suitable compounds of heavy metals are lacking and no stalks are formed, only a few generations of colonies are produced.

Entire colonies never undergo attachment. Nor is the distribution of colonies related to the direction of the light, as might be expected if the phototactic colonies were the starting-points for attached growth. In the origin of stalked colonies the swarmers fasten themselves by their drawn-out posterior ends and divide to form colonies as the stalk develops. When undisturbed, such clusters ultimately divide into two unit colonies, each of which excretes a stalk, the original one bifurcating. Any disturbance, however, may cause the branched colonies to dissolve with liberation of free-swimming stages, either entire unit colonies or fragments of them, down to single swarmers or amoeboid cells which can give rise to new colonies.

As conditions become less favourable for multiplication, some of the cells form cysts, which were first described by Dangeard (1910, p. 158). According to him they often contain two nuclei which subsequently fuse, and they possess the pore and stopper, characteristic of Chrysophycean cysts. Dangeard does not describe the endogenous origin typical of the class, although he mentions enveloping 'mucus' which may have been protoplasm. He failed to induce germination of the cysts, which I also have not seen.

In my cultures cysts were often formed in large numbers. They are 7–10 μ in diameter, with a thin but highly refractive wall, while pore and stopper are not very conspicuous. The wall is impregnated with a silicon compound and is often covered with a yellowish layer which, like the stopper of the cyst, gives the Prussian blue reaction.

Manifold cultures of a single strain over a period of several years afforded few cysts, although Teichmann (1935) observed many of them. No better success was achieved by varying the conditions of culture although a different strain produced many cysts in almost every older culture. Parallel series of cultures of both strains in six different media, inoculated with single colonies, previously cleaned by washing, confirmed the different behaviour of the two strains.

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B. *Siderodendron manganiferum* n.gen., n.sp.

I. *Discovery and culture*

Several other colourless flagellates depositing iron and manganese compounds (viz. *Phalansterium*, *Rhipidodendron* and *Spongomonas*) have been recorded from England (Jane 1938, p. 349; 1939, p. 121), but seem to be absent around Cambridge, probably because they prefer softer water. I have, however, found two new forms related to *Anthophysa* which I will describe here, the first under the name *Siderodendron manganiferum* n.gen., n.sp.

Siderodendron bears some resemblance to *Cladomonas fruticulosa* Stein (1878, plate VI, figures 6, 7), but the cells of the latter are embedded in the ends of the stalks. *Pycnobryon socialis* Fromentel (1874, plate IX, figure 10; plate XXVI, figure 9) is too inadequately figured and described to admit of its identification. It may possibly be either *Siderodendron* or *Anthophysa*.

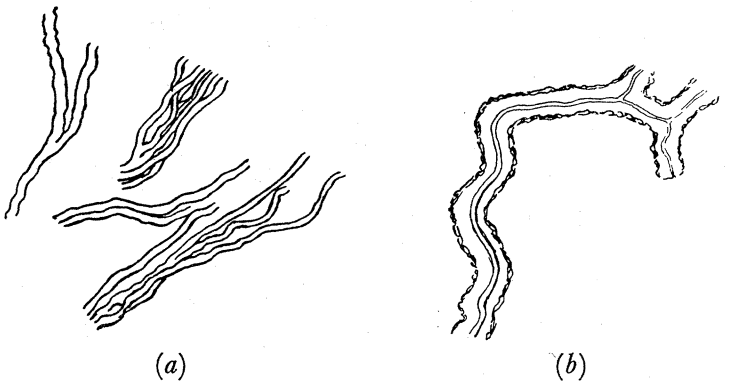
Siderodendron was discovered in the October plankton of the pond in the Botanic Garden, Cambridge, which consisted mainly of *Volvox* and *Cryptomonas*, and was found again in December, together with *Synura*, in a slow-flowing ditch with almost clear water in the garden of St John's College, Cambridge. The former sample contained small brown unidentifiable growths, from which cultures were prepared. After washing them three times in diluted soil extract they were transferred to three tubes with soil and water, two of which contained in addition a little starch and four grains of wheat respectively. After 4 days growth had commenced in these two cultures, wheat proving superior to starch, while soil and water alone afforded only a few small colonies. The organism appeared on the inner surface of the glass as rounded brown rosettes, which under low magnification showed a radiating structure with an excentric centre (plate 8, figure 23). Like those of *Anthophysa* the colonies were most frequent at a small distance from the surface of the liquid and diminished both in size and number downwards. At about 1–2 cm. below the surface, growth of *Siderodendron* was rarely detected. In older cultures the brown wreath is much more prominent, fluffy, feather-like colonies hanging down at a depth of about 2–3 mm. below the surface. Such cultures remain healthy for a long time and subcultures are easily prepared from them.

II. *Morphology*

The growth of *Siderodendron* at first sight bears some resemblance to that of *Anthophysa*, and the feather-like aggregates of the former might be mistaken for a poor growth of the latter. There is also some similarity under the microscope, since in both the brown growths consist of threads displaying an inner and an outer portion. Those of *Siderodendron*, however, are irregular tubes, the inner parts of which appear lighter and suggest a hollow structure. The composition of the whole dendroid aggregate is also different, since the larger branches are composed of a number of tube-like portions, which are almost of the same breadth throughout (plate 8, figure 24; figure 7 *a*, *b*).

No free-swimming colonies occurred, although small colourless *Monas*-like swarmers, which might have owed their presence to insufficient initial purification, were present. When material was carefully taken up with a wide pipette and dropped on to the slide with a little 1% osmic tetroxide, cells identical with the *Monas*-like organism were, however, observed in small numbers near the ends of the tubular stalks (figure 8). They constitute clusters of up to eight cells which are readily dislodged and do not form regular

colonies. The tops of the stalks immediately beneath these clusters are even more delicate than those of *Anthophysa* and are easily overlooked, nor is a definite connexion recognizable. The relation between the *Monas*-like cells and the brown stalks was more satisfactorily established in micro-chambers placed at a small angle to the horizontal when the growth which developed on the inner surface of the cover-slip could be observed without disturbance. Under these circumstances it could be seen that the brown parts of the stalks were continued as colourless, irregularly shaped gelatinous masses, to the tops of which a small number of cells were attached (figure 9 *a–c*; plate 8, figure 25). These are ovoid in shape, 6–7 μ long, and possess two very unequal flagella; there is a single contractile vacuole near the centre, and food vacuoles are also present. The cells show continual change of shape and to some extent also of position (figure 10).



(*a*) (*b*)

FIGURE 7. Stalks of *Siderodendron manganiferum*. *a*, interwoven tubular stalks, × 500; *b*, branching and tubular appearance, × 2000.

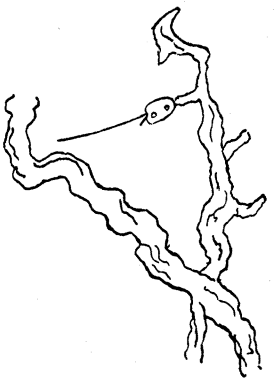


FIGURE 8. Cell attached to irregular stalk, × 750.

The stalks of *Siderodendron* thus originate in a manner similar to those of *Anthophysa*, although the former genus does not produce free-swimming colonies, the detached cells occurring singly or at the most in twos and threes. The stalks are not the combined products of a number of individuals, but each cell excretes its own tube, those formed by several cells cohering to produce an irregular rope-like structure. The colony derived from a single cell is therefore not really dendroid and only consists of the more or less complex aggregates resulting from the manifold mutual adherence of essentially independent tubes. It appears feathery or rosette-like according to the nutritive conditions.

III. *Nutrition*

The nutritive requirements are similar to those of *Anthophysa*. Without addition of manganese stalk formation is poor, even when ferrous compounds and suitable bacterial food are available. The relation to iron and manganese could not be investigated as thoroughly as for *Anthophysa*, because appreciable growth is only obtained when soil is present. The best medium for testing the effect of ferrous and manganous salts is leaf mould covered by sand, to which varying quantities of ferrous ammonium citrate and manganous sulphate are added. Owing to the non-homogeneous character of the soil, comparison is only possible between cultures of the same series. These show that growth increases up to a certain point with the concentration of iron and manganese. It is very poor and faintly coloured when heavy metals are deficient and fails altogether at higher concentrations.

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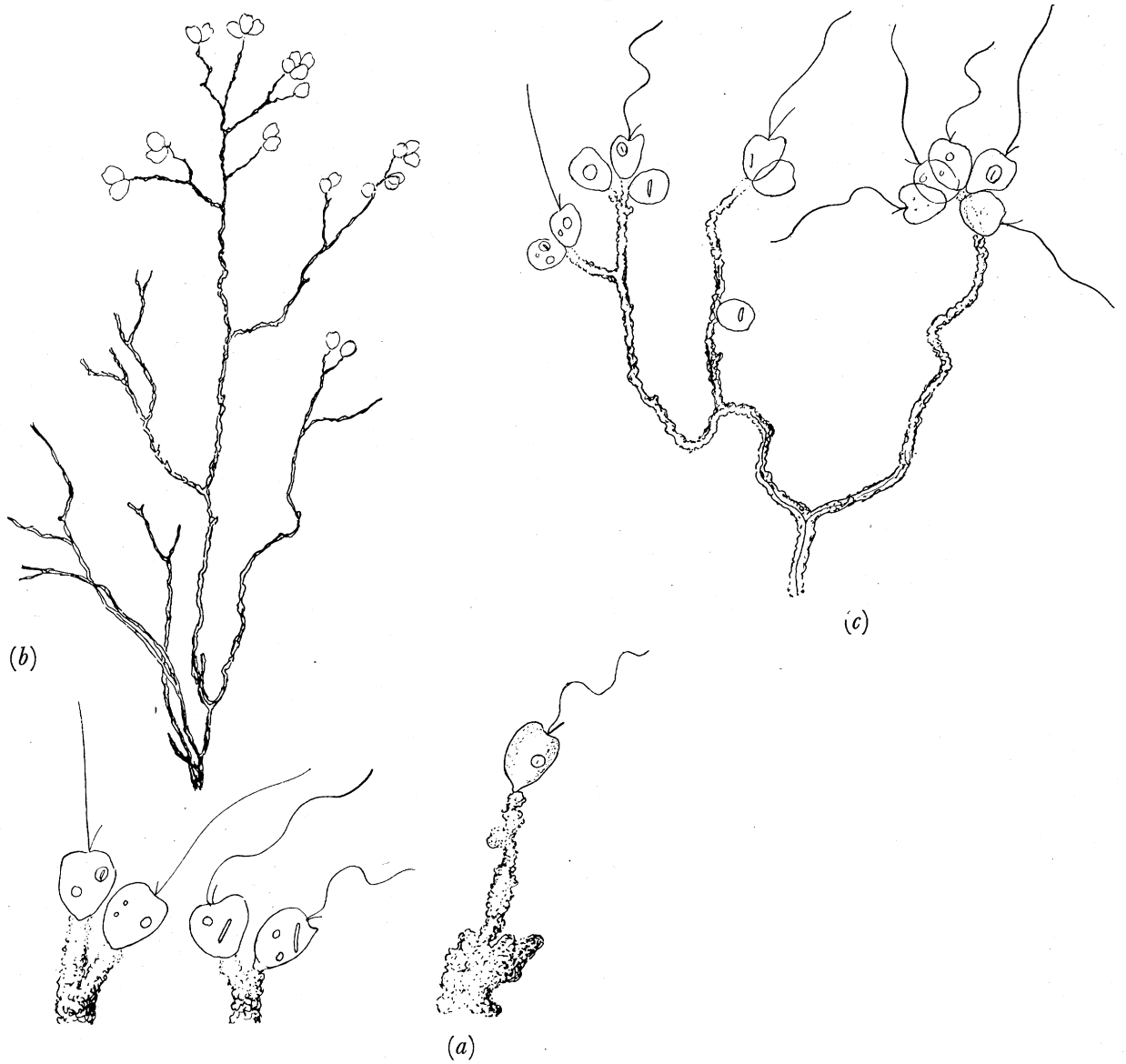

FIGURE 9. Stalk formation in *Siderodendron manganiferum*. *a*, stalk in process of formation, × 2000; *b*, branched colony, × 500; *c*, parts of a branched colony at a higher magnification, × 1000.

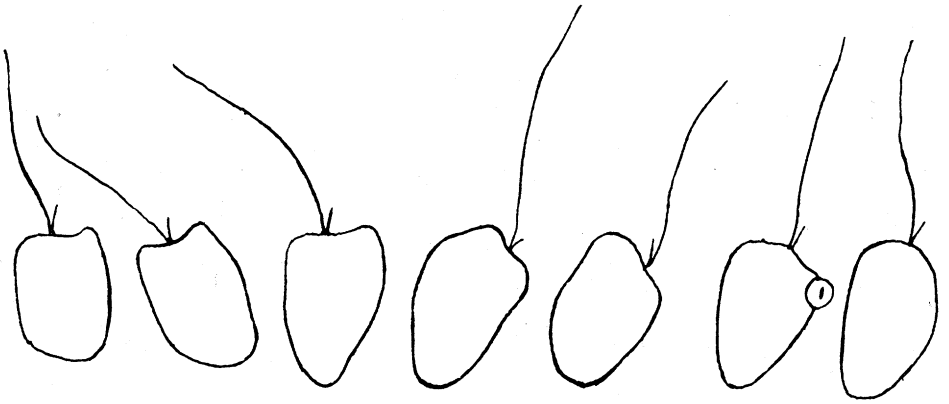

FIGURE 10. A single cell of *Siderodendron*, showing alteration in shape and position, × 400.

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If iron alone is supplied growth is not much increased, while manganese promotes it considerably; the strongest growth is obtained when both are added and results in the formation of dark brown stalks.

A very satisfactory development was obtained with leaf mould, soil and water, supplemented by 0·02% ferrous ammonium citrate and 0·01% manganous sulphate. Such cultures were clear and clean, but the growth was never abundant as when a small amount of organic material was added. A grain of pearl barley produced a considerable improvement. The feather-like colonies developing under such circumstances, are, however, observed only when the cultures remain undisturbed for several weeks.

The original material in both instances was free-floating, although all the growths observed in cultures were at least primarily attached. This may be a consequence of the small amount of fluid present.

C. *Siphomonas Fritschii* n.gen., n.sp.

I. *Description*

This flagellate, which is related to *Sideromonas*, was found in a polluted pool near Debden, Essex, containing various species of *Euglena* and *Trachelomonas* and a few *Anthophysa* colonies. Under low power one recognizes branched, slightly brownish growths, which turn blue with ferrous potassium cyanate and hydrochloric acid and which, when examined with a $\frac{1}{12}$th immersion lens, appear as hollow, cylindrical, rather regularly forked stalks, bearing minute flagellates. The posterior half of the body of each flagellate is enclosed in the end of the tube, like an egg seated in its egg-cup (figure 11). The same feature is found in

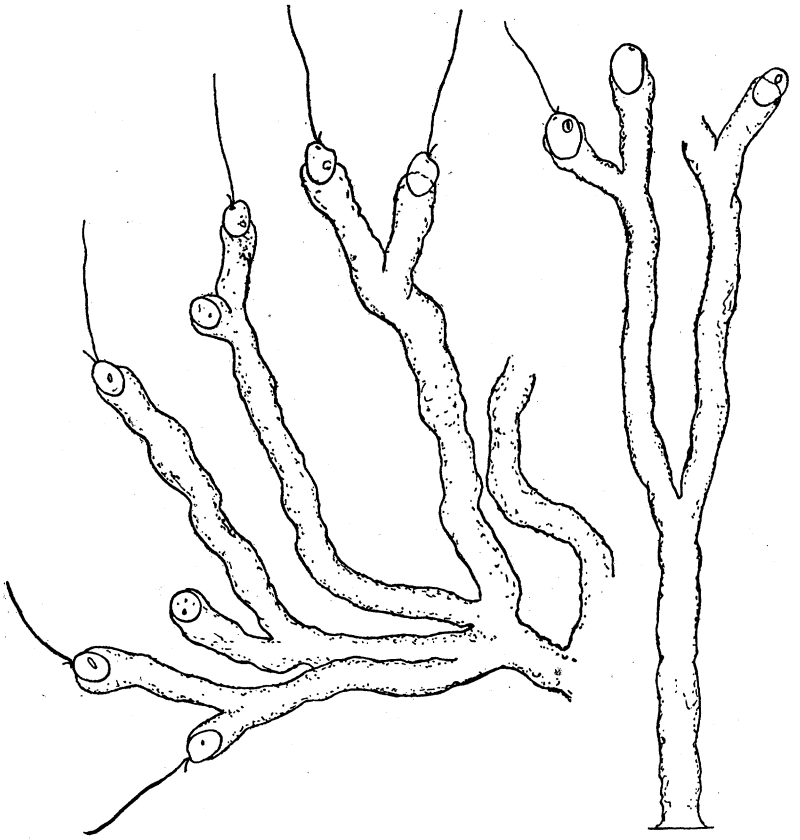

Figure 11. Branched tubular stalks of *Siphomonas Fritschii*, × 1000.

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Cladomonas fruticulosa Stein (1878, plate VI, figures 6, 7), with which the form under discussion has much in common, although it differs from it in two characters. *Cladomonas* is stated to have two equal flagella and is therefore grouped among Amphimonadaceae by Lemmermann (1913, p. 114), while in *Siphomonas* there are two unequal flagella; the longer is about three times the length of the body which is 6–7 μ long and 5 μ wide, the shorter only about 2–3 μ long (figure 12). The other difference, which also can hardly have been overlooked by Stein, is the presence of an eye-spot which is more conspicuous than that of *Anthophysa*. Stein and Lemmermann mention a contractile vacuole, which was not recognized in *Siphomonas* and in any case would be more difficult to observe than the eye-spot. *Siphomonas* differs from *Sideromonas* in (1) the branching of the stalk, (2) the mode of insertion of the cells which renders their aggregation in clusters impossible, and (3) the presence of the eye-spot.

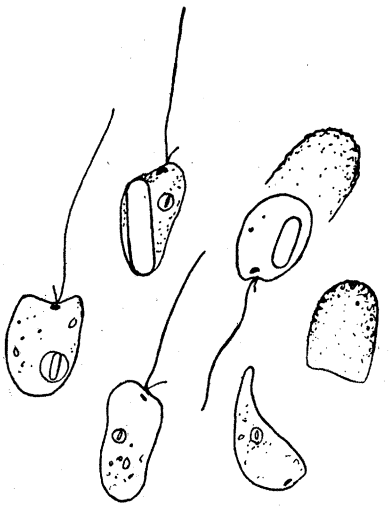


FIGURE 12. Cells of *Siphomonas*, partly with cup-shaped envelopes, × 2000.

II. *Culture*

In the sample from the habitat *Siphomonas* appeared after two weeks as a number of floating tufts. These were transferred to various soil-water media, with or without additional iron and manganese or organic substance. The only result was the formation of a slight brown precipitate on the glass when manganese was present; a few small flagellates, not necessarily belonging to the organism under discussion, were observed.

A further attempt was made with an arable soil which is slightly reddish in colour and contains clay and lime and has proved useful in the cultivation of various green flagellates. With this soil a more marked precipitate was obtained after a week and a few *Monas*-like flagellates with eye-spots were found. These were transferred singly to cultures made up with the same soil and supplied with a grain of pearl barley, as well as with one drop of a 1% solution of ferrous ammonium citrate and of a 1% solution of manganese sulphate. In these cultures *Siphomonas* grew as brownish tufts attached to the glass at about 1 cm. below the surface of the fluid. In the absence of additional organic matter only a few short, cup-shaped brown envelopes are formed. Although the flagellate stages multiply when iron and manganese are insufficient, tube formation does not take place.

Further experiments showed that *Siphomonas* prefers a slightly alkaline medium and that the conditions for the formation of long tubular stalks are difficult to create in a test-tube. An amount of manganous sulphate, sufficient to produce the characteristic growth of *Sideromonas*, results with *Siphomonas* only in the formation of short dark brown cups; one-quarter the concentration is necessary to produce the normal growth of *Siphomonas*. Abundant multiplication of the free-swimming stage takes place in cultures with increased organic food, for instance with three grains of wheat; such stages would be identified as a *Monas* with an eye-spot. When lime and iron are also present the characteristic growth form is produced. No cysts were observed.

The special conditions requisite for the culture of *Siphomonas* are therefore different from those demanded by the other forms here discussed. Abundant growth and the formation of forked tubes occur only with a rich supply of organic substances, a definitely alkaline reaction and a comparatively low concentration of manganous salts, while a ferrous salt is indispensable.

D. *Bikosoeca petiolata* (Stein) n.comb. (syn. *Poteriodendron petiolatum* Stein)

I. Poteriodendron (Bikosoeca), *an iron organism*

Unlike the three flagellates previously discussed, *Poteriodendron*, although described long ago (Stein 1878), has not been suspected of being an iron organism, since the goblet-shaped envelopes are not brown. I was, however, struck by the highly refractive and sometimes yellowish appearance of the envelopes and stalks (plate 9, figure 26), features already recorded by Reynolds (1927) for the closely related *Bicoeca Kepneri* (syn. *B. lacustris* James-Clark; cf. Picken 1941, p. 465). The generic name chosen by James-Clark (1868) was *Bicosoeca*, better spelt *Bikosoeca*. It was changed into *Bicoeca* by Stein for no apparent reason. The optical appearance of the envelopes recalls that of the sheaths of *Leptothrix ochracea*, and in correspondence with this they show a strong Prussian blue reaction. The organism has been found several times near Cambridge and has twice been isolated in culture. The first strain was obtained in September from a ditch between allotments at Cherry Hinton in a community comprising *Paramecium caudatum*, *Chilomonas paramecium*, *Cyathomonas truncata*, *Leptothrix discophora*, *Beggiatoa alba* and *Vitreoscilla beggiatoides* n.gen., n.sp., the second from an almost dry ditch at Barton, from which mud was transferred to a hay culture with iron and manganese in May. It grew associated with *Leptothrix* and *Chilomonas*.

A related form, *Bikosoeca lacustris* James-Clark, occurring in a ditch on Coldham Common near Cambridge in December, together with *Anthophysa*, *Thiothrix* and *Leptothrix* was cultured in the same way, but was lost before satisfactory growth had been obtained. It differed from the type in possessing a well-defined stalk which is stated to be 'absent or extremely short' (Picken 1941, p. 464). Zacharias (1894) has described a *Bikosoeca longipes*, but I am not sure of the identity of my strain with this form.

The two species *Poteriodendron petiolatum* and *Bikosoeca lacustris* should be included in the same genus, the former as *B. petiolata* (Stein) n.comb. The differences between *B. petiolata* (identical with *B. vacillans*, cf. below) and *B. lacustris* are enumerated by Picken (1941, p. 464), but are so slight that the reference of single individuals of *Poteriodendron* to *Bikosoeca* by Štolc (1887) and Picken is justified.

Both strains of *B. petiolata* were long maintained in diverse media containing for instance cereal grains with soil, but these cultures did not develop very satisfactorily, owing, as I learned subsequently, to putrefaction. It was first thought necessary to supply extra organic substance for the food bacteria, but experience with other holozoic flagellates, which thrive in mere soil-water cultures, led me to use such a medium for *Bikosoeca* with satisfactory results. The best cultures were, however, those with hay and water with the addition of 0·01–0·02% ferrous ammonium citrate; not more hay should be used than can, after sterilization, be completely covered with water. Very good results were obtained with old, seasoned grass leaves. The flagellate first develops at the margin and incompletely covers the surface.

The first cultures were started with free-swimming colonies, which under low power resemble *Dinobryon sertularia*. *Leptothrix discophora* (Schwers) Dorff appeared as a brown ring near the surface and served as a substratum for the flagellate. It did not seriously interfere with the development of the latter, although hampering its growth by competition.

The early cultures contained only single individuals within their stalked envelopes, a modification described by various authors as *Bicoeca vacillans* (Stolc 1887; Picken 1941, p. 462). Judging by Picken's and my observations this is identical with *Bikosoeca petiolata*. Colonies are formed only when there is a rich supply of ferrous compounds and an absence of mechanical disturbance. They are usually attached to floating particles by their stalks, but by the breaking away of such colonies free-swimming colonies result which carry with them small particles on which the primary cells had settled down.

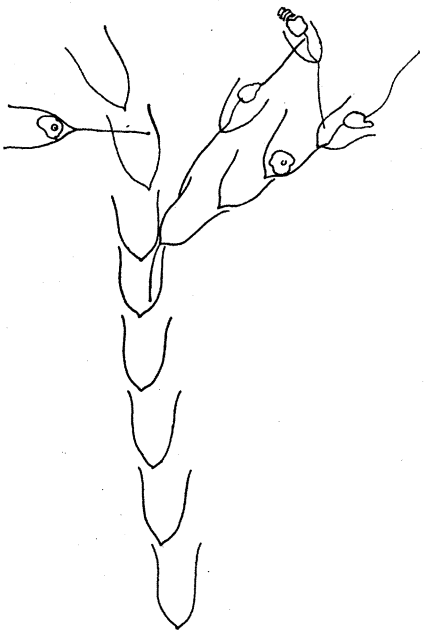


FIGURE 13. Chain formation in *Bikosoeca petiolata*, × 600.

When the cultures contain plentiful bacteria, no envelopes are formed. Their failure to develop is comparable to the absence of stalks in *Anthophysa* (cf. p. 324), but while such growth of the latter cannot be regarded as abnormal, *Bikosoeca petiolata* without envelopes makes a pathological impression.

Three problems still remained to be solved, viz. (1) the conditions determining the formation of the firm, glistening envelopes ascribed to *Poteriodendron*, (2) the conditions for the development of composite colonies, (3) the mode of formation of the branched colonies, figured by Stein. These problems could not be approached until, by washing small detached colonies with the help of a capillary pipette, admixtures of *Leptothrix* were removed and really satisfactory cultures obtained. When freed from competition for iron, *Bikosoeca* formed large colonies with firm envelopes, a further proof that this flagellate is an iron organism. There is apparently some connexion between the formation of firm envelopes and the tendency for the young cells to settle without stalk-formation inside the parent envelope with the production of chain-like colonies (figure 13).

II. *Morphology*

Despite their small size *Bikosoeca lacustris* and *B. petiolata* are well known, especially through Picken's researches (1941), although my strains differ in a few particulars. Stein's figures of the latter (1878, plate XI) show the envelopes of the individuals attached by stalks of considerable length to the insides of the parent envelope, often near the bottom.

Stokes's (1888) figure of *Stylobryon Abbottii* resembles Stein's figure of *Poteriodendron*, although the former differs in its two short and almost equal flagella and the very regular branching. I suspect that this is a misrepresentation of *Bikosoeca petiolata*. The dimensions given for the envelopes do not conform with the drawing. Deflandre (1928, p. 214, figure 1) portrays an irregularly branched form of *B. petiolata* and (figure 2) a form with well-developed envelopes, the latter under the name *B. petiolata* var. *Abbottii* (Stokes) Playfair (1921), in which all the envelopes are possessed of long stalks, while in Stokes's form only the primary cell is stalked.

Stein's figures of regularly branched *Poteriodendron* colonies are difficult to understand, since every envelope is occupied and there are even more than two daughter individuals within a single parent envelope. Division of a cell into more than two has never been observed. The same difficulty arises in interpreting some of Stein's figures of Craspedomonadaceae and *Dinobryon*.

In flourishing cultures containing iron there were often chains of twelve or more individuals, of which only the oldest were possessed of a stalk. Apparent bifurcations, similar to those of Stein, were also found, as well as chains of single stalked individuals. That these latter result from the settlement of swarmers on the outer surface of the envelopes of older individuals is shown by their long stalks. It remains unexplained why a stalk is formed only by those individuals which have previously led a free-swimming existence and which fail to settle on the inside of the envelope. It would be mere guess-work to suppose that the inner surface of the envelope differs in its properties from the outer. Moreover, stalked individuals are in rare instances even found attached inside the rim of older envelopes, where generally no stalks are produced (figure 14), and cells settling on older envelopes may or may not form a stalk. The swarmers can attach themselves to glass, to the water surface, to older envelopes, algae, hay particles, etc., so that there is no specialization.

In colonies showing bifurcation most of the forks are irregular, the angle of inclination and the place of attachment of the stalk to the older envelope varying considerably. Where such apparent bifurcation occurs, one fork only is derived by division from the parent, the sister cell remaining sessile within the rim of the parent envelope. The other fork is due to a swarmer having settled on the opposite side of the outer surface of the envelope and having secreted a stalk (figure 15). There is never more than one sessile daughter envelope within that of the parent and true branching does not occur.

A well-developed envelope of *Bikosoeca petiolata* is goblet-shaped, the lower part being conical with an apex of 60° and the rim being curved outwards. Picken, as well as Štolc, describe the envelope of *B. vacillans* as 'cylindrical, tapering posteriorly', although in one of Picken's drawings (1941, figure 1, p. 453) the typical shape is shown. It seems that he did not have the opportunity of examining well-developed envelopes (cf. p. 335) and since

he employed neither dark-ground illumination nor staining, the delicate rim would easily be overlooked. According to Lemmermann (1910, p. 343) the very delicate envelopes are often not clearly visible without staining. Klug (1936–7, p. 111) failed to stain or identify the substance composing the envelopes, although they readily take up gentian violet or methylene blue for example. When treated with ferrous potassium cyanate and hydrochloric acid, the Prussian blue reaction brings out the shape clearly (plate 9, figure 27).

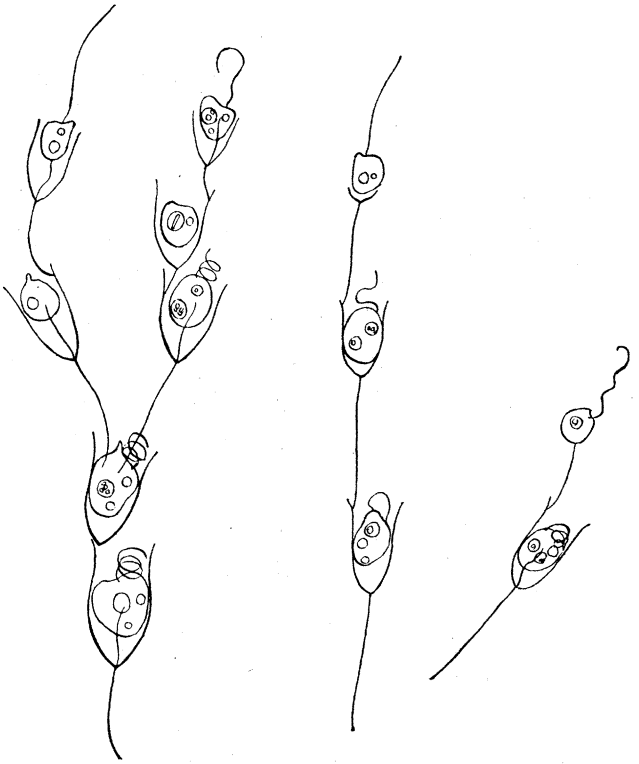


FIGURE 14. Colonies of *Bikosoeca*, showing various modes of connexion between the successive envelopes, × 1000.

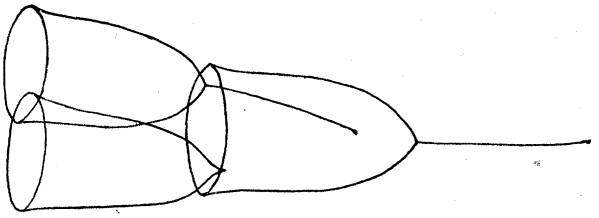
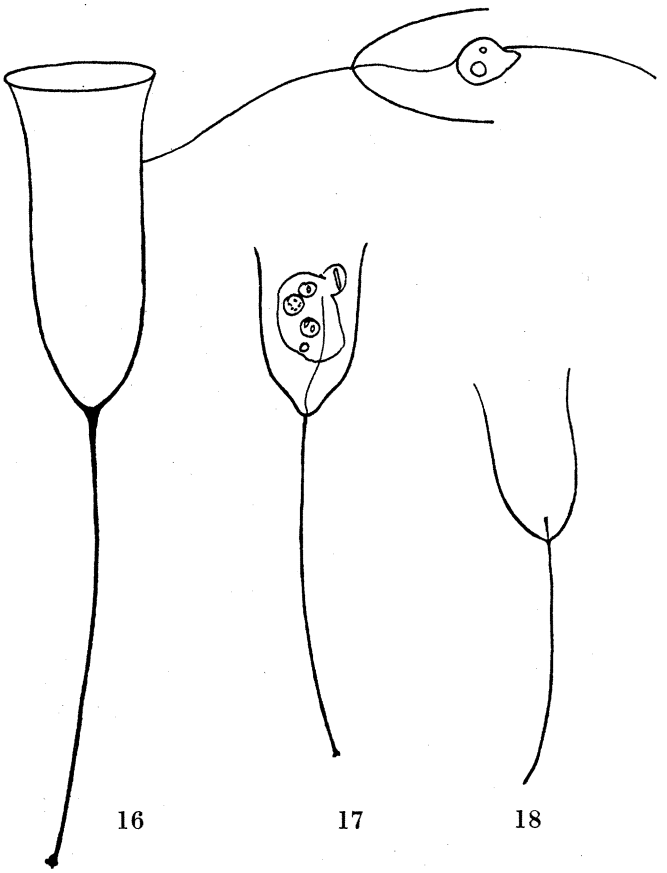


FIGURE 15. Formation of a forked chain in *Bikosoeca petiolata*, × 1500.

Envelopes which, except for the conical base, appear cylindrical are immature. Under favourable conditions they may become much longer than is shown in Picken's and the older figures (figure 16). Klug (1936–7, p. 111) states that the shape of the lower part is related to that of the young cell, which itself depends on the manner in which it settles down, and he does not consider that this feature is of much taxonomic value. There is no doubt, however, that different species can be distinguished by their envelopes which in my varied cultures presented much the same shape throughout, the only deviation being an occasional rounded instead of a pointed base (figure 17; cf. also figure 19). A ragged rim, such as is figured by Deflandre (1928, figure 1) was not observed.

Well-developed envelopes are only formed in cultures with a considerable supply of ferrous salts. When they are very thick, the usual slightly yellowish hue of the envelope becomes a very faint brown, but there is never a markedly brown tint, so that only little manganese can be present.

Even under conditions favouring the production of glistening envelopes there is a difference in optical appearance between the older and younger ones. The latter are delicate and may be difficult to detect, while the former are of some thickness, especially adjacent to the stalk, which has an appreciable width and broadens slightly below the base of the envelope; at this point the inner surface of the latter often bears a small knob (figure 18).



FIGURES 16–18. Special forms of envelopes in *Bikosoeca petiolata*, × 1500.

FIGURE 16. One of unusual length. FIGURE 17. Envelope with rounded base.

FIGURE 18. Envelope with basal knob.

In cultures with much organic food and little iron all the envelopes are delicate, but the individuals within them are more active than in cultures with well-developed envelopes. If the latter are examined at the right time, only the younger individuals are seen actively to ingest food, while the older envelopes are either empty or occupied by rounded protoplasts which do not take in food.

Cell division has been described and well figured by Klug (1936–7, p. 111) for *B. lacustris*, and by Picken (1941, p. 460) for *B. petiolata*. One daughter individual remains within the old envelope, possibly helping to complete it, the other either settles on the rim and at the

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best produces only a short stalk, or escapes to form a new colony elsewhere, when it develops a stalk about 20–25 μ long.

Division takes place rapidly and has several times been observed in hanging drops. Immediately after division both cells ingest food (figure 19), the one being attached by its backwardly directed flagellum to the bottom of the envelope, while the other adheres to the inner surface of the latter at a higher level. In the instance figured, this individual escaped and, after some to and fro movement, finally swam away. Its shape could not be clearly observed, but seemed to be bean-like. Swarmers are common in young cultures, but owing to their rapid movement and the difficulty of fixation, it has been impossible to decide whether the backwardly directed flagellum trails behind.

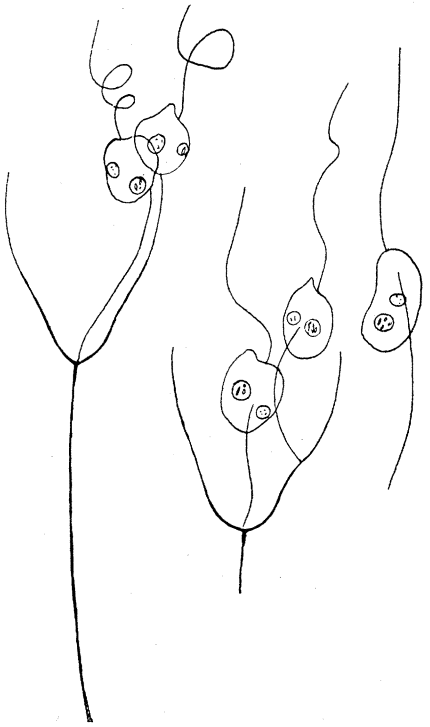

FIGURE 19. Daughter cells of *Bikosoeca* after division, × 1500.

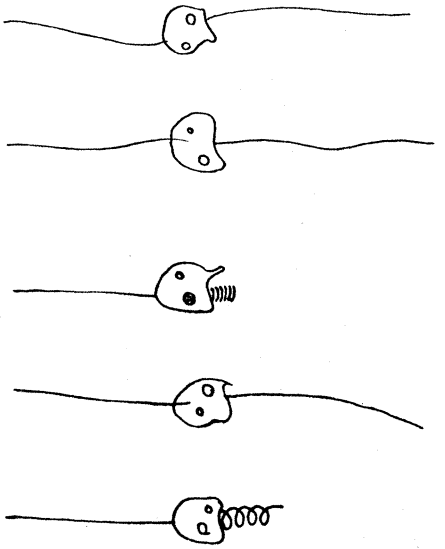

FIGURE 20. Successive drawings of a naked cell at short intervals, × 1000.

Naked cells, attached by their posterior flagellum and otherwise behaving just like those encased in an envelope, were found especially in cultures in which the capacity to form envelopes was diminished (cf. p. 332). Such cells exhibit continuous change in shape (figure 20).

In spite of rapid fission the actual rate of multiplication of both species is rather slow, even under favourable conditions, and certainly slower than in *Anthophysa*. The first mature envelopes were seen after a week.

III. *Taxonomic position*

The form here described as *Bikosoeca petiolata* was originally found as small colonies with delicate envelopes. In cultures it shows various modifications, ranging from the form known as '*Bicoeca vacillans*', with single individuals in very delicate envelopes, to chain-forming colonies with well-developed glistening envelopes, identified as Stein's *Poteriodendron petiolatum*. My form, however, differs in the following features: (1) only the primary envelopes

are stalked; (2) the flagellum in active cells generally shows a characteristic coil, a feature on which Picken lays emphasis in *B. vacillans*; (3) the collar-like process figured by Stein is absent. The protoplast in my form resembles that of Picken's *B. vacillans*, often showing a one-sided lip-like outgrowth which can be protruded into a minute process and might be regarded as a second anterior flagellum if not examined with an immersion lens (figure 21).

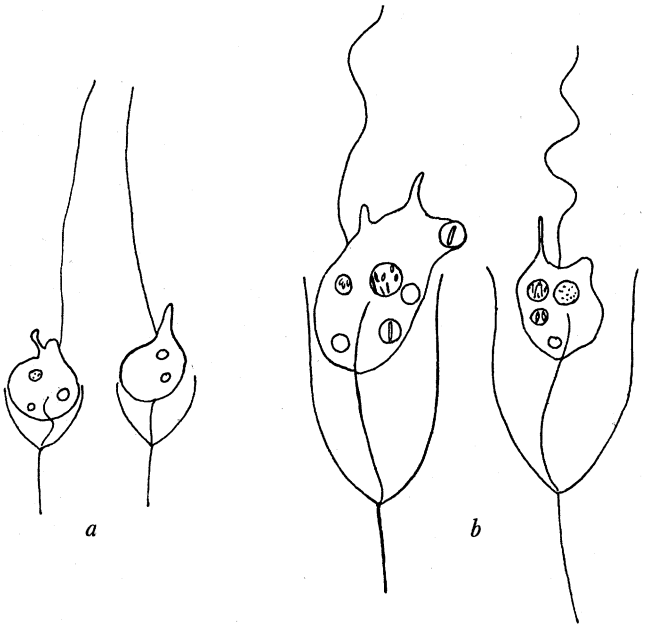

FIGURE 21. Cells of *Bikosoeca petiolata*, with protoplasmic processes, × 1500. *a*, with unfinished; *b*, with finished envelopes.

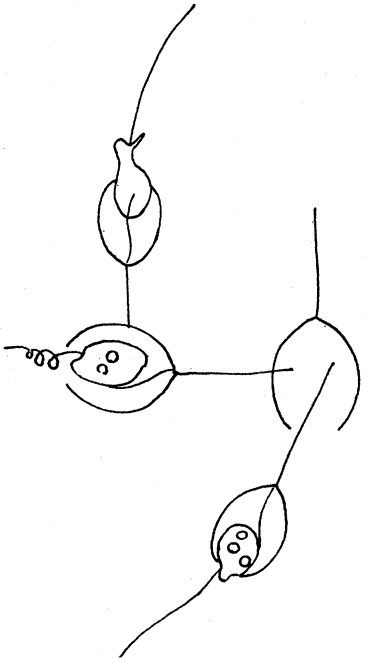

FIGURE 22. Colonies of *Bikosoecalacustris*, × 1000.

In spite of these differences Stein's species is believed to be identical with my and Picken's forms because easily observed features, such as the shape and proportions of the envelope, are common to all and only those characters, which are difficult to establish without modern optical equipment, are different. Nevertheless minute details, such as the varying breadth of the stalk, the enlargement at its apical end, the process sometimes found at the bottom of the envelope and the glass-like appearance of the whole structure, are well shown in Stein's figures.

The only morphological difference from *Bikosoeca* lies in the formation of colonies. These are, however, found in several species of this genus, viz. in *B. dinobryoides* (Lemmermann 1913, p. 71, figure 94, p. 67) and *B. socialis* (Lauterborn 1899, p. 372), as well as occasionally in *B. lacustris* (cf. Bütschli 1878, pl. XI). The last produces only few-celled colonies, which differ from those of *B. petiolata* in that the daughter cells never settle inside the envelopes so that the colonies are more irregular and there are no chains (figure 22).

E. Discussion

The micro-organisms listed on p. 313 are stated to deposit compounds of iron which have been identified by their reddish brown coloration. In the absence of manganese, however, ferric deposits appear colourless or only slightly yellowish, and the presence of iron would not be suspected. This is so in *Bikosoeca* and *Pteromonas*. In two strains of the latter genus,

probably belonging to *P. angulosa* (Cart.) Lemm., the Prussian blue reaction was shown by the wings as well as by the rest of the wall, which sometimes has a yellowish tint.

When manganese is lacking, the deposits under discussion have been interpreted as an organic matrix which subsequently may come to include iron recognizable by its distinctive hue. Actually this matrix consists of iron hydroxide which becomes coloured when a brown manganese compound is adsorbed. Variations in the quantities of the two metals present and consequent differences in colour are not solely due to the relative amounts available in the medium, but depend also on the properties of the organism concerned in their deposition. Thus, the envelopes of *Trachelomonas* are usually brown, while this is never true of the wings of *Pteromonas*; similarly the sheaths of *Leptothrix discophora* (*Sphaerotilus discophorus* mod. *manganiferus*) are brown, those of *L. ochracea* (*Sphaerotilus natans* mod. *ochraceus*) slightly yellowish.

The occurrence of iron organisms is determined not so much by the total quantity of iron and manganese present, as by their state. The amounts deposited are limited by the concentration of ferrous and manganous compounds which result from natural solution or more frequently from biological reduction. Since ferrous compounds are easily oxidized, the activities of iron organisms are restricted to regions between points of reduction and of oxidation. Manganese for the most part shows similar features, although the lower oxides are more stable. Deposition of manganese without iron has been found only in *Anthophysa*.

The rust-brown deposits seen in streams and marshes indicate the presence of iron-precipitating organisms. Certain forms found in the same habitats are classed by Dorff (1934, p. 52) and earlier writers as iron-loving, but of these only *Trachelomonas* and *Euglena spirogyra* give a distinct Prussian blue reaction.

The prominent role played by iron in determining the distribution of organisms in nature has been emphasized by Uspenski (1927). Though one of the commonest elements, it is readily precipitated and is then not available. This is also the chief reason why it is often difficult to prepare suitable media, especially when organic substances are excluded. It is, moreover, likely to be the principal cause of the success of soil-water cultures, which possess reducing power and contain complex iron-humus substances. Manganese, though indispensable to certain organisms and suspected of being indispensable to all, does not play so prominent a part in determining distribution, because it is needed in far smaller quantities and is not readily oxidized.

The iron organisms dealt with in this paper are holozoic flagellates, *Anthophysa*, *Siderodendron* and *Siphomonas* being members of the Monadaceae, a colourless family of Chrysophyceae, while *Bikosoeca* belongs to the Bikosoecaceae, the affinities of which are not clear. Their common characteristic lies in their capacity to deposit inorganic salts of heavy metals.

The weight of manganese and iron compounds precipitated by these flagellates is large compared with that of their bodies, most of which consists of water, so that the dry weight would certainly prove to be almost completely composed of inorganic substances. As none of them occur in large quantities, their geological importance as compared with *Leptothrix*, is negligible. *Anthophysa* and similar iron flagellates cannot even be taken as indicating a water rich in iron (Lemmermann 1910, p. 376), since they occur in places without a high content of dissolved iron. In such waters the heavy metals are concentrated around the organisms.

Whether deposition of iron and manganese is of any ecological value to the organisms concerned is doubtful. The production of stalks may give increased facility for holozoic nutrition, while the formation of envelopes and the building of colonies may constitute an obstacle to ingestion by other small organisms. Greater interest attaches to the nutrition of these holozoic flagellates, but little is known about their feeding habits. According to Cohn (1853, p. 10) *Anthophysa* does not occur in putrid waters and Lemmermann (1910, p. 344) includes the Bikosoecaceae in the *Dinobryon* association which avoids polluted habitats. This has been confirmed by observations in nature and by means of cultures.

All the organisms concerned grow best when there is little organic matter in the medium. Observation of other holozoic flagellates shows that most of them are likewise adapted to a scanty supply of organic matter, while saprotrophic flagellates, such as *Astasia*, *Polytoma* and *Chilomonas*, live and thrive in media containing large numbers of bacteria. The former need bacteria for food, but they are very susceptible to lack of oxygen and, under a cover-slip, cease to move in a few minutes, while *Astasia* for instance remains motile for a long time.

Anthophysa grows in diverse ecological habitats in which it varies morphologically. The stalked form (mod. *pedunculata*), regarded as typical, is a modification, which occurs where manganous salts exceed a certain minimum and little bacterial food is available. With a richer supply of organic food but little manganese and iron, the formation of stalks is greatly reduced or even completely suppressed, although multiplication and colony formation may be vigorous. These observations show that the excretion of stalk material is not essential to the well-being of the organism. The stalkless colonial form may be called mod. *animalis* (as opposed to *vegetans*). The occurrence of single cells and few-celled aggregates on the one hand, and of cysts on the other, indicate respectively too high a degree of putrefaction and exhaustion of the food supply.

In assessing the taxonomic position (cf. p. 314 seq.) of *Anthophysa* too much stress should not be placed on stalk and colony formation, which are not always manifested and are not essential morphological characters. Related forms are found among the colourless Monadaceae. The nearest may be *Monas sociabilis* H. Meyer, which forms similar colonies and might even be regarded as a free-living *Anthophysa* colony, since, under the conditions prevailing in Meyer's cultures, stalks could not possibly have been formed. Food vacuoles, which are conspicuous in *Anthophysa*, have, however, not been recorded. Among the pigmented Chrysomonadineae *Uroglena* is similar. If grown in the dark with organic substances its chromatophores shrink to very small leucoplasts, while the eye-spot persists. Confusion with *Anthophysa* might then easily occur. Like *Monas sociabilis*, *Uroglena* does not ingest solid food, but *Synochromonas pallida* Korshikov (1929) shows holozoic nutrition. The various genera mentioned are in any case closely related, and this is probably also true of *Cephalothamnion* and *Dendromonas*. Most of these genera are widely separated in most books, with the exception of that of Fritsch (1935, p. 555).

Siderodendron resembles *Anthophysa* in its brown stalks, but does not form colonies, so that it represents a simpler type similar to *Monas*, from which its swarmers cannot be distinguished. It also seemingly differs from *Anthophysa* in its planktonic habit. This is also so in *Siphomonas*, the different stalk-formation of which is related to the exudation of iron compounds by the entire posterior half of the cell and not only by the back end.

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Bikosoeca petiolata (*Poteriodendron*), like *Anthophysa*, occurs in two modifications, viz. mod. *vacillans* with single individuals inhabiting delicate, stalked envelopes, and mod. *Poteriodendron* with chains of well-developed glistening envelopes, of which only the primary one possesses a stalk, other individuals or chains being irregularly attached to it. The first modification appears when there is lack of iron and a good supply of bacteria, the second when iron is present and there are fewer bacteria. The slightly brownish tint, occasionally observed, is due to admixture of manganese.

Whether the envelopes in all species of *Bikosoeca* mainly consist of a ferric compound is not yet known. In *Dinobryon* envelopes with a content of iron are found. Many similar structures remain to be investigated in this respect.

F. Diagnoses

Siderodendron *n.gen.*

Monas-like cells, individually excreting long stalks which consist of manganic and ferric compounds and combine to form a rigid, brown, radiating structure. Stalks at first almost colourless and of irregular shape, later becoming brown except in the neighbourhood of the cells and cylindrical or somewhat tubular, several being intertwined to form thicker strands which in their entirety appear bushy or tree-like.

Siderodendron manganiferum *n.sp.*

Cells usually pyriform or ovoid, 6–7 μ long, 4–5 μ broad, with one contractile and several food vacuoles, the front end unequally protruded and continually altering in shape, the longer flagellum about $2\frac{1}{2}$–3 times as long as the body, the shorter half the length of the latter.

Habitat. Floating in slightly polluted waters, although perhaps originally attached to solid objects, at all times of the year.

Siphomonas *n.gen.*

Monas-like cells excreting tubular bifurcated stalks, in the tops of which they are inserted with the posterior ends of their bodies. The tubes contain iron and manganese and are light to dark brown.

Siphomonas Fritschii *n.sp.*

Cells usually ovoid, 6–7 μ long, 4–5 μ wide, with an eye-spot at the base of the unequal flagella which are 20 and 2–3 μ long respectively. Food vacuoles in which relatively large bacteria can be digested. Contractile vacuole not seen. Many cells without stalks.

Habitat. Floating in polluted alkaline water.

In conclusion I wish to thank Professor F. E. Fritsch for his kindly help in the preparation of this paper, especially in the correction of the manuscript, and Dr C. Robinow, who has devoted much time to the preparation of the photographs here reproduced.

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Explanation of Plates

Plate 8

Figures 23–25. *Siderodendron manganiferum*

Figure 23. Stalks under low power, × 45.

Figure 24. Branched colonies, × 65.

Figure 25. Young colony in micro-chamber, × 250.

Plate 9

Figures 26–27. *Bikosoeca petiolata*

Figure 26. Well-developed envelopes, × 580.

Figure 27. Envelopes showing the Prussian blue reaction, × 580.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 23

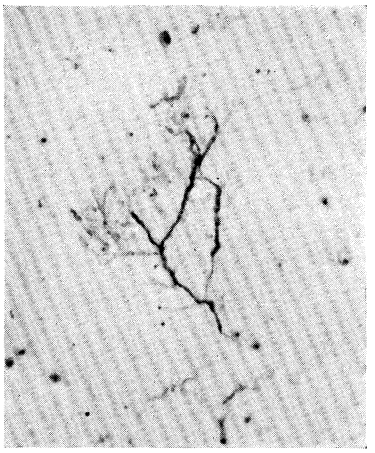

FIGURE 24

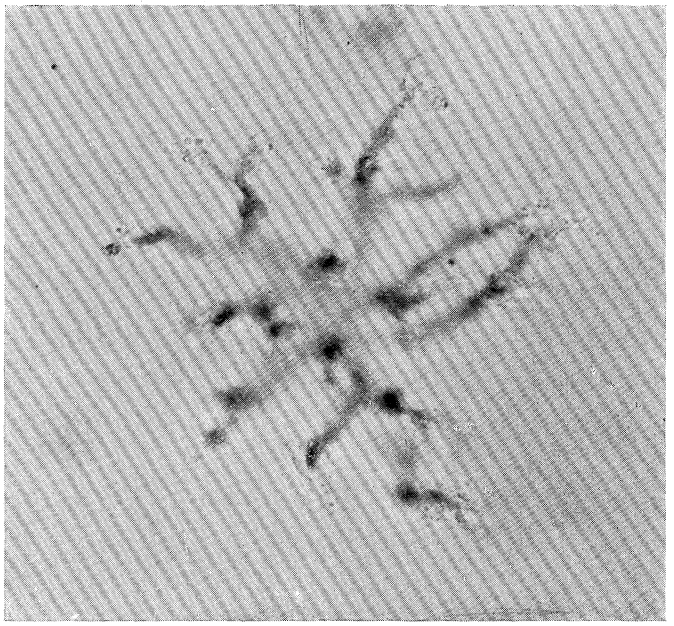

FIGURE 25

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

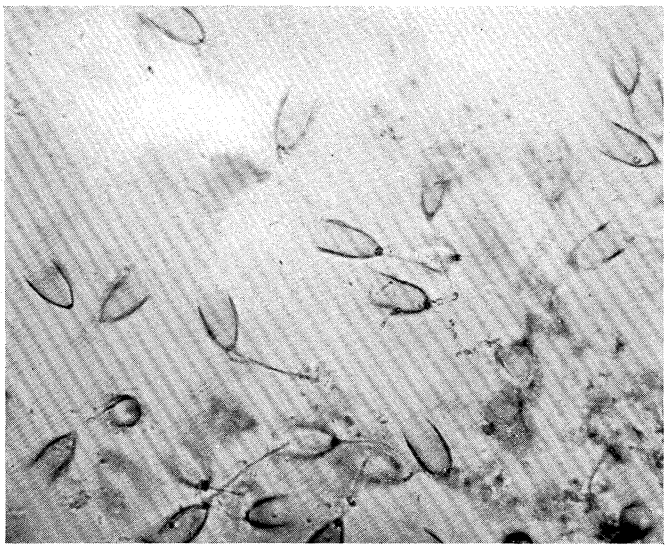

FIGURE 26

FIGURE 27

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 23

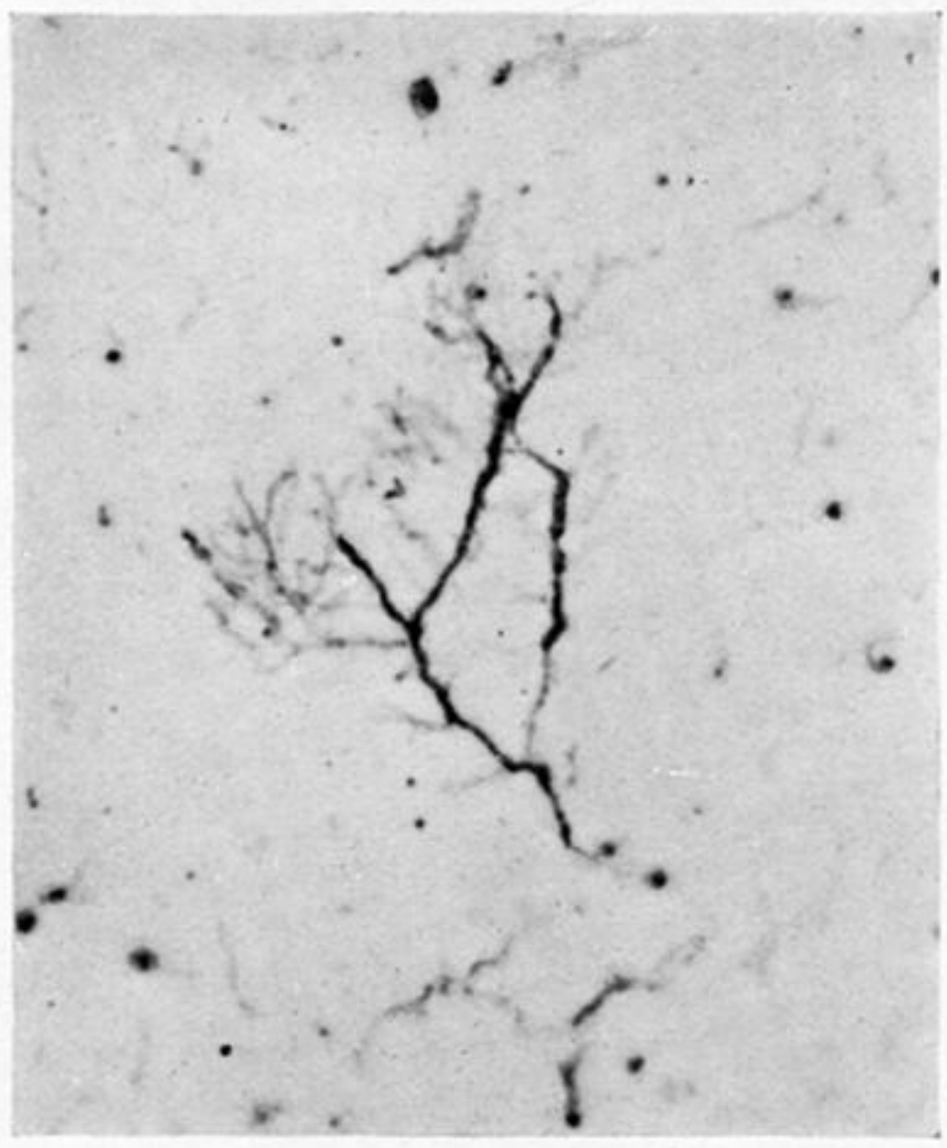

FIGURE 24

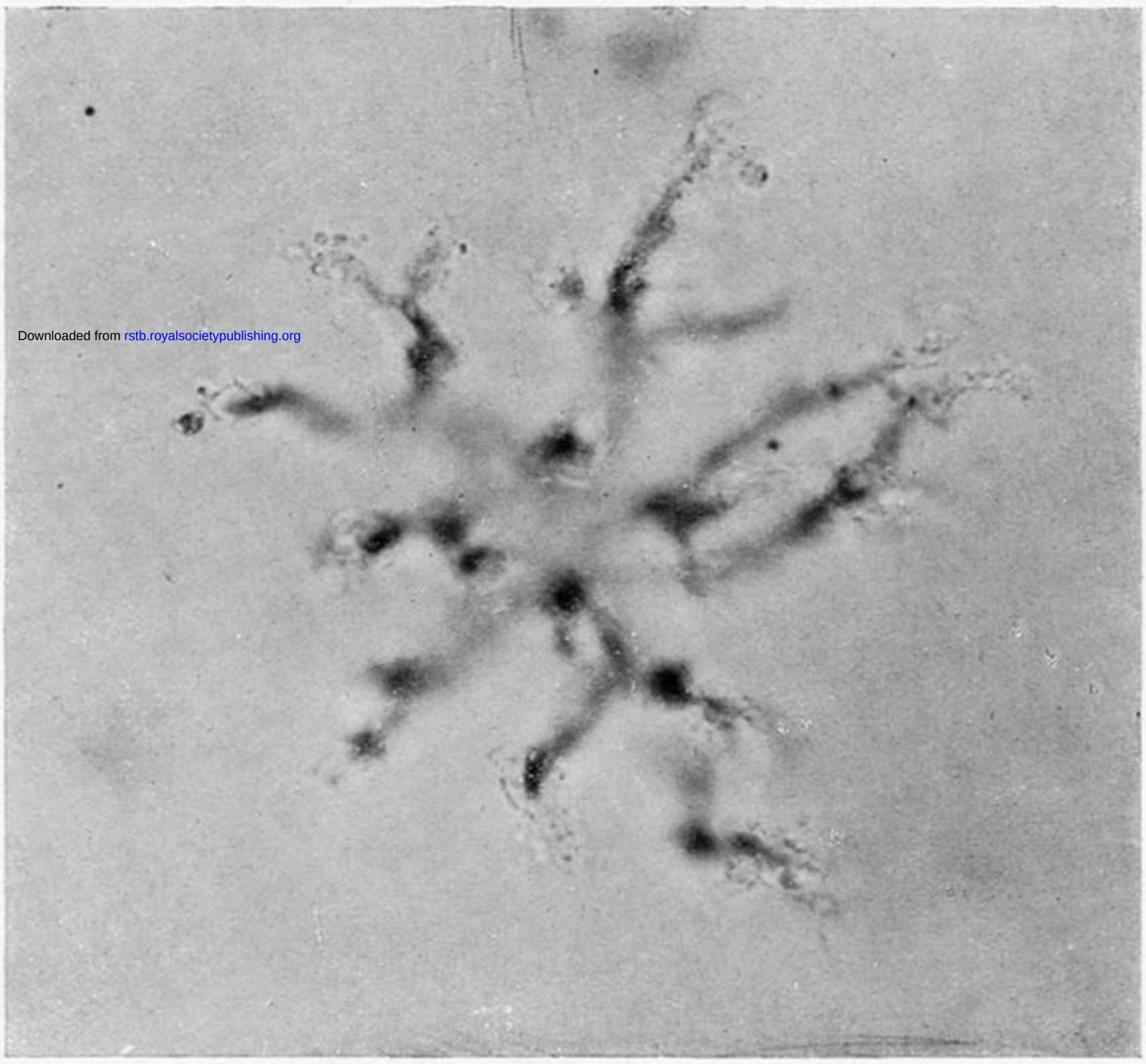

FIGURE 25

PLATE 8

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

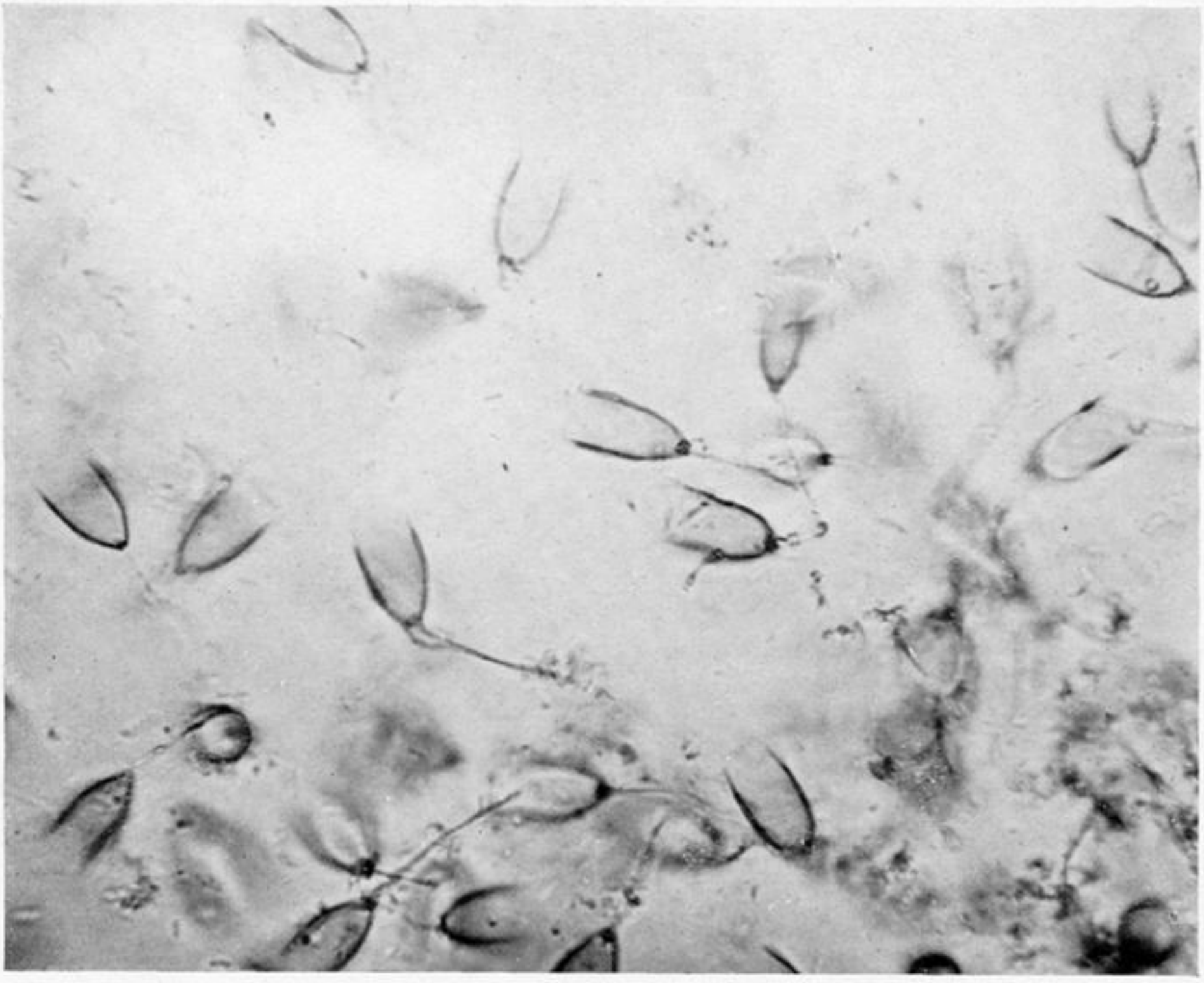

FIGURE 26

FIGURE 27

PLATE 9

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