ON SOME MORE PROTOZOAN PARASITES FROM TRICHOPTERA.

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(Plate III and 8 Text-Figures.)

Introduction.

In a recent paper (*Journal of Parasitology*, 1910, III. pp. 245—54) I described a new trichomonad (*Trichomastix trichopterorum*)[1] and a spirochaete from the hind-gut of certain trichopterous larvae. I also mentioned the occurrence along with these parasites of another peculiar flagellate, which, at the time, I was disposed to consider as closely related to *Macrostoma caulleryi*, Alexeieff.

The present paper deals chiefly with this flagellate, which seems to be an entirely new form, and for which, after more careful study, I am reluctantly obliged to erect a new genus, *Embadomonas*.

It is extremely interesting to find that the caddis-fly larvae also sometimes harbour the flagellate, *Crithidia campanulata*, Léger, and a microsporidian which I regard as *Gurleya légeri*, Hesse. The first of these has previously been recorded from the larvae of *Chironomus* (Léger, 1902) and *Ptychoptera* (Léger and Duboscq, 1909), and the second from *Ephemerella* (Hesse, 1903), all of which are insect larvae living alongside caddis-worms, and probably feeding very similarly. The likelihood of mutual infection with common parasites is, in such cases, very great. I thus feel strengthened in the view, expressed in my last paper, that the spirochaete of trichopterids is identical with those found in *Chironomus* (Léger, 1902) and in *Ptychoptera contaminata*

[1] Through an oversight I gave the specific name of this trichomonad as "*trichopterae.*" I take this opportunity of correcting it to "*trichopterorum.*"

(Léger and Duboscq, 1909); and I believe that the same spirochaete will be found in other aquatic insect larvae in the future.

Observations such as these lead one to protest against the present tendency to regard each new parasite as peculiar to one host—a tendency which leads to the frequent formation of new species on insufficient grounds, and is surely opposed to the principles of common-sense.

On two or three occasions I saw some small amoebae in the hind-gut of the caddis-worm. The amoebae were very few in number, and I never obtained any satisfactory stained preparations. I shall content myself here with some brief observations made on the living organisms.

MATERIAL AND METHODS.

The caddis-flies and their larvae and pupae were collected in the neighbourhood of Aberdeen at various times from April 1909 till November 1910. Larvae from ponds near Dundee were found to be similarly infected. The infected individuals belonged mainly to the genera *Limnophilus* and *Anabolia*.

The microsporidian occurred in the fat-body of the larva: with this exception, the parasites were all found in the hind-gut along with the trichomonad. I saw no trace of them in the upper regions of the alimentary tract.

I attach prime importance to the study of the living parasites. This can be done either by observing them as they move about within the transparent gut of the young larva, or in teased-out preparations of the gut contents under a waxed-down coverslip.

Examination in soda solution of material that had been fixed in osmic vapour (Schewiakoff's method) was found useful for determining the number of flagella, and their point of origin.

For permanent preparations, fixation with sublimate alcohol (Schaudinn's formula) and staining with Heidenhain's iron-haematoxylin gave the best results in the case of the flagellates. Attempts to stain *Embadomonas* by the modified haematoxylin method used by Rosenbusch for trypanosomes (*Arch. f. Protistenk.* XV. 1909, p. 263) proved unsuccessful.

The microsporidian was first smeared, and then fixed and stained variously; but better results were got by fixing the infected larva whole, and cutting sections of it: portions of these sections were quite thin enough to display the individual spores. Fixation with sublimate

alcohol and staining with Delafield's haematoxylin was found the most satisfactory method. Iron-haematoxylin was also successful; and prolonged staining with Giemsa's stain, followed by rapid dehydration and clearing, was useful.

Description of the Parasites.

(*a*) *Embadomonas agilis*, nov. gen. et nov. sp.

This interesting new flagellate was found in about 60% of the trichopterid larvae examined by me. I never came across it in any of the pupae, but on one occasion I found two individuals in the intestine of an adult fly.

The body of the parasite varies from 8 μ to 14 μ in length, with an average maximum breadth of 4·5 μ. It is of definite slipper shape, with the broadest portion about half-way between the middle and the posterior end; thence it tapers back abruptly into a well-defined point (Pl. III, figs. 1 and 2). The anterior end is rounded, very flexible, and usually bent back at a considerable angle to the long axis of the body, forming, as it were, a heel to the slipper. (See Pl. III, fig. 3.) Very striking is the large cytostome, which extends from about 2 μ from the anterior end to a point almost half-way along the body: its posterior border is often produced into a sort of lip. To carry out the slipper simile, we should compare the cytostome to the opening into which the foot would be thrust.

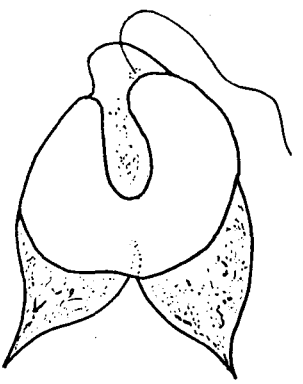
There is only one slender *flagellum*. It arises from a highly refractive point at the anterior margin of the cytostome, and is about three-quarters the length of the body. It describes a wide spiral movement, by means of which the flagellate progresses, rotating with great rapidity round its long axis. The backward inclination of the anterior end of the body leaves the cytostome facing almost directly forward as the animal swims, and bacteria of relatively large size are swept into it in the current produced by the flagellum. It is not unusual to see an *Embadomonas* swimming about with a long bacillus sticking out of its cytostome, which gives it rather a rakish appearance.

In preparations stained with iron-haematoxylin the *nucleus* appears as a diffuse, dark-staining area occupying all the anterior region above the cytostome (Pl. III, figs. 1—3). Well differentiated specimens show a number of dark chromatin masses in a somewhat lighter background. The lateral limits of the nucleus are not definite. Often it is

possible to make out 1—3 dark granules lying on the anterior border of the cytostome. It is very difficult to be certain of the exact point of origin of the flagellum, which is exceedingly slender, and apt to become invisible on differentiation; but it seems to arise from the most central of these granules.

The *cytoplasm* is often highly vacuolated, and is crowded with masses of bacteria that have been ingested.

Curiously enough, though the flagellate is sometimes pretty abundant, I have only once seen a living individual in process of longitudinal *division*, and I have searched my stained material for division stages in vain. Text-fig. 1 illustrates the dividing flagellate. The cytostome has become widely extended in a lateral direction, while



Text-fig. 1. Drawing of living *Embadomonas agilis*, apparently in process of longitudinal division.

the longitudinal split has already advanced about one-third along the body, starting from the posterior end. I could detect only one flagellum. While I watched, the split advanced a little, and the cytostome showed a tendency to divide in half. At this point, the organism's movements, which had been getting slower and slower, ceased altogether, and it died without completing division. *Embadomonas* is even more sensitive than *Trichomastix*, and seldom survives removal from the host for more than an hour.

I have never seen anything suggesting encystment in this organism.

Systematic position. The name *Embadomonas*—ἐμβάς, a slipper—was suggested by the characteristic shape of the organism. *Embadomonas* does not agree closely with any parasitic flagellate hitherto described, and, in the present unsatisfactory state of the classification,

I cannot define the position that this new genus should occupy. On reading Alexeieff's brief, unillustrated description of *Macrostoma caulleryi* from the tadpole of the frog (*Compt. Rend. Soc. Biol.* (1909), LXVI. p. 199), I was inclined to think that my parasite might be related thereto, even though *Macrostoma* was described as having three flagella and a "vesicular" nucleus. Lately, Wenyon has published a paper (*Parasitology* (1910), III. 2, p. 210) on another species of *Macrostoma*—*M. mesnili*—from human faeces. From this account with its figures, it is abundantly clear to me that *Embadomonas* has very little in common with *Macrostoma*, beyond the possession of a large cytostome.

The new genus may be defined thus: *Slipper-shaped mono-flagellate, with pointed posterior end and blunt, rounded, anterior end, which is bent back at an angle with the long axis of the body. A large cytostome occupies almost all the anterior half of the body. The slender flagellum arises from a basal granule on the anterior margin of the cytostome. The nucleus is a diffuse mass containing several chromatin clumps: it lies in the extreme anterior end, in front of the cytostome.*

(*b*) *Crithidia campanulata*, Léger.

In five of the two hundred larvae examined, the hind-gut contained the adult and encysting stages of a flagellate, which agrees, in all essentials, with *Crithidia campanulata*, Léger. For reasons given above, I do not intend to create a new species, although both previous records of the parasite were drawn from dipterous larvae.

The flagellate was always found in the upper regions of the hind-gut along with the *Trichomastix*. I never saw them in the mid-gut, and my infected material was too limited to allow of complete working out of the life-cycle.

The parasites were mostly in the rounding-off and semi-encysted condition, but a few were still typical *crithidian flagellates* (Pl. III, figs. 4 and 5). Léger and Duboscq (1909) have given a clear and concise description of *Crithidia campanulata* as found in *Ptychoptera*. My parasite does not differ from theirs in any important feature. The larger flagellates are about 12 μ long by 2·5 μ broad: smaller individuals measure only 5 $\mu \times$ ·75 μ. The flagellum projects for some 6—10 μ, and is continued on to the body along the undulating membrane. It takes its origin in a minute basal granule, lying in front of the rod-shaped kineto-nucleus. The kineto-nucleus is pressed up against the anterior

border of the trophonucleus. I saw no division stages among the flagellate individuals, nor any sign of so-called "sexual" forms.

My recent experience of the extraordinary variability of the herpetomonad flagellates and their allies has made me very reluctant to assume from a few samples that my specimen is a true *Crithidia*, and not just a stage in the encystment of a *Herpetomonas*. Particularly is this the case with the flagellates found in the hind-gut, where, in the process of rounding off, a more or less crithidian-like stage is liable to be gone through by *Herpetomonas* itself. I recognize, however, that the present flagellate possesses a slight undulating membrane: according to Patton's definition of the genera this character serves to distinguish *Crithidia* from *Herpetomonas*, even when the other test—*i.e.* the position of the kineto-nucleus relative to the trophonucleus—fails.

The characteristic feature of *C. campanulata* consists in the bell-shaped encysting stages. Pl. III, figs. 9—12 illustrate these, as I found them in the caddis-worms. Léger and Duboscq describe them as attached in masses to the epithelial lining of the gut, with the mouth of each bell directed downwards. I never found the parasite so plentiful as Léger and Duboscq figure it, and the "bells" were more often seen clinging to the bacterial tangles that almost block the intestine of the larva. The "mouth" of the bell seems to arise through the gradual broadening of the anterior end of the flagellate (Pl. III, figs. 6—8) and the withdrawal and dissolution of the attaching flagellum, rather than by the encysting stages coming to lie on their sides, as Léger and Duboscq describe.

Very small, rounded-off individuals measured only 3—4 μ in length, but the final stage of the cyst was not seen.

(*c*) *Entamoeba*, sp.

In three larvae (*Limnophilus rhombicus*) I noticed a few small amoebae moving about among the bacteria in the hind-gut. Text-figures 2—5 were drawn from the living organisms. I did not succeed in staining them. The average dimensions of the amoebae were about $12\,\mu \times 10\,\mu$. As will be seen from the figures, the form varies from oval to round, one end showing an even outline, while from the other break out several blunt pseudopodia. The endoplasm is very granular, and passes insensibly into the clearer ectoplasm. I saw no contractile vacuole. The nucleus appears as a small, clear, oval or circular area in the posterior portion of the body.

The amoebae moved about rather slowly. On one occasion they showed after some time a tendency to round off and become motionless: I was then able to detect in one or two cases a delicate cyst-wall (Text-fig. 5). Though watched for a long time after that, the amoebae showed no further signs of development. I saw no division stages.

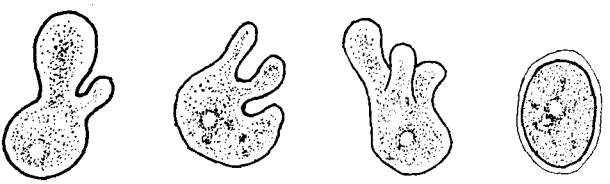


Fig. 2. Fig. 3. Fig. 4. Fig. 5.

Text-figs. 2, 3 and 4. Drawings of living *Entamoeba* from hind-gut of caddis-worm, showing the lobose pseudopodia.
Text-fig. 5. The same, encysted.

Systematic position. The scanty nature of my observations on this form makes it impossible to define a new species. From the rare occurrence of the parasites, and the very slight degree of the infection, I am inclined to suppose that the true host is some other animal, and that their presence in the trichopterid larvae was due to the swallowing of a few chance cysts, which in rare instances have found a suitable field for development. At first I suspected that I might be dealing with *Amoeba chironomi*, Porter, the different form of the pseudopodia being called forth by a slightly different medium. *Amoeba chironomi*, however, is characterized by the possession of a contractile vacuole, an unusual feature in a parasitic amoeba, and one not exhibited by the amoeba in question.

The development of a cyst-wall precludes the possibility of confusion with leucocytes.

(*d*) *Gurleya légeri*, Hesse.

This microsporidian infests the fat-body of the larval caddis-fly. I found it only three times, though I dissected fully two hundred larvae. This is in accordance with the observations of Hesse (1902), who describes it as occurring in the fat-body of only 4 % of the larvae of *Ephemerella ignita*. I think it possible, however, that, as I was primarily interested in the intestinal parasites of my caddis-worms, I may often have overlooked the early stages of the microsporidian in the fat-body. The infected larvae were all found in the late summer of

1910, towards the close of my investigation; the spores were ripe, and the infection so intense that I could not fail to notice it.

The parasitised larva moves about sluggishly, and has a swollen, congested appearance. On opening the body I was at once struck by the curious, chalk-white colour of the fat-body. Examination showed that the whole tissue was replaced by the masses of spores of some microsporidian. These were all at an advanced stage of development: the majority showed four spores within each pansporoblast, but in many cases the outlines of the pansporoblast were lost, and the spores set free.

Figs. 13—14 (Pl. III) illustrate the parasite as I found it. The pansporoblasts are oval to round in form, and measure from $8\,\mu \times 5\,\mu$ to $11\,\mu \times 6\,\mu$. The pear-shaped spores ($4—5\,\mu \times 2{\cdot}5—3\,\mu$) are arranged therein in two superimposed rows: sometimes the narrow ends all point the same way (Text-fig. 6), more often the narrow ends of the upper row lie above the broad ends of the row beneath (Text-figs. 7 and 8). The large pansporoblasts occasionally hold only three spores, and

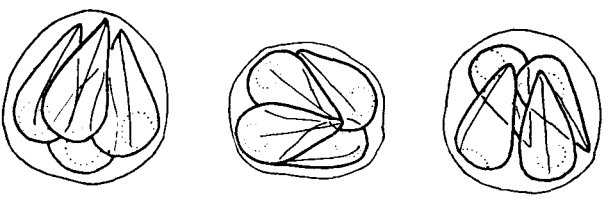


Fig. 6. Fig. 7. Fig. 8.

Text-figs. 6, 7 and 8. Drawings of the living pansporoblasts of *Gurleya légeri*, to show the arrangements of the spores.

these seem bigger than where there are the usual number. These spores would correspond to Hesse's macrospores, but I do not find the difference in size between the macrospores and microspores so great as he describes it in the parasite from *Ephemerella*. If sulphuric acid be brought in contact with the spores, the filament is shot out to a length of nearly $25\,\mu$.

Systematic position. The genus *Gurleya*, Doflein, is distinguished from other microsporidia of the *Oligosporea* by the possession of tetrasporous pansporoblasts. At present the genus contains three species:

Gurleya tetraspora, Doflein (1898),
G. légeri, Hesse (1903),
and *G. francottei*, Léger and Duboscq (1909).

Of these, the first occurs in the hypodermis of *Daphnia maxima*, and is described as having "oval" spores. Unfortunately, measurements were not taken[1]. *G. francottei*, found in the cells of the intestinal epithelium of *Ptychoptera contaminata*, is a good species with very characteristic crescent-shaped spores.

I see at present no reason for separating the *Gurleya* of trichoptera from *Gurleya légeri*, found in a similar position in *Ephemerella contaminata*, and agreeing with it in all essential points. I regard this as still another instance of the infection by common parasites, to which similar animals on the same feeding-grounds are subject.

From what I have already shown, I think it is clear that trichopterous larvae offer an unusually rich field to the seeker after protist parasites. The following is a list of the parasites hitherto mentioned or described by various authors: with the probable exception of *Gurleya légeri*, they do not seem to be harmful to the host[2].

Class BACTERIA.

Bacillus sp.*
Streptothrix sp.*

Class PROFLAGELLATA, Doflein.

Spirochaeta sp.*

Class FLAGELLATA.

Crithidia campanulata, Léger*.
Embadomonas agilis, nov. gen. et nov. sp.*
Trichomastix trichopterorum, Mackinnon*.

Class RHIZOPODA.

Entamoeba sp.*

Class SPOROZOA.

Sub-Class TELOSPORIDIA.

Ancyrophora uncinata, Léger[3].
Asterophora elegans, Léger.

[1] It would appear to be difficult to distinguish *G. tetraspora* from *G. légeri* on morphological grounds.

[2] Those marked with an asterisk have been recorded by me.

[3] It is interesting to notice that Léger also found this gregarine in the intestine of the aquatic larvae of *Dytiscus* sp. and *Colymbetes* sp.

Discorhynchus truncatus, Léger.
Gregarina mystacidarum, Frantz.
Pileocephalus chinensis, A. Schneider.
P. heerii, Kölliker.

Sub-Class NEOSPORIDIA.

Gurleya légeri, Hesse*.

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EXPLANATION OF PLATE III.

All figures drawn to scale, using Zeiss' achromatic objective 1/12" or apochromatic 2 mm. homog. immersion, with compensating oculars 8 and 12.

Figures 1—12 stained with iron-haematoxylin, Figs. 13 and 14 with Delafield's haematoxylin.

I. *Embadomonas agilis*, nov. gen. et nov. sp.

Fig. 1. Individual viewed from front, showing cytostome.

Fig. 2. Large individual from same aspect, but with sides of cytostome projecting in lip-like manner.

Fig. 3. *Embadomonas* in side-view, to show slipper-like form.

II. *Crithidia campanulata*, Léger.

Fig. 4. Slender flagellate individual showing narrow undulating membrane, long flagellum, basal granule, kineto-nucleus, and trophonucleus with central karyosome.

Fig. 5. Plump flagellate with well-marked undulating membrane.

Fig. 6. Shortening of flagellum and broadening out of anterior end to form attaching surface of encysting stages.

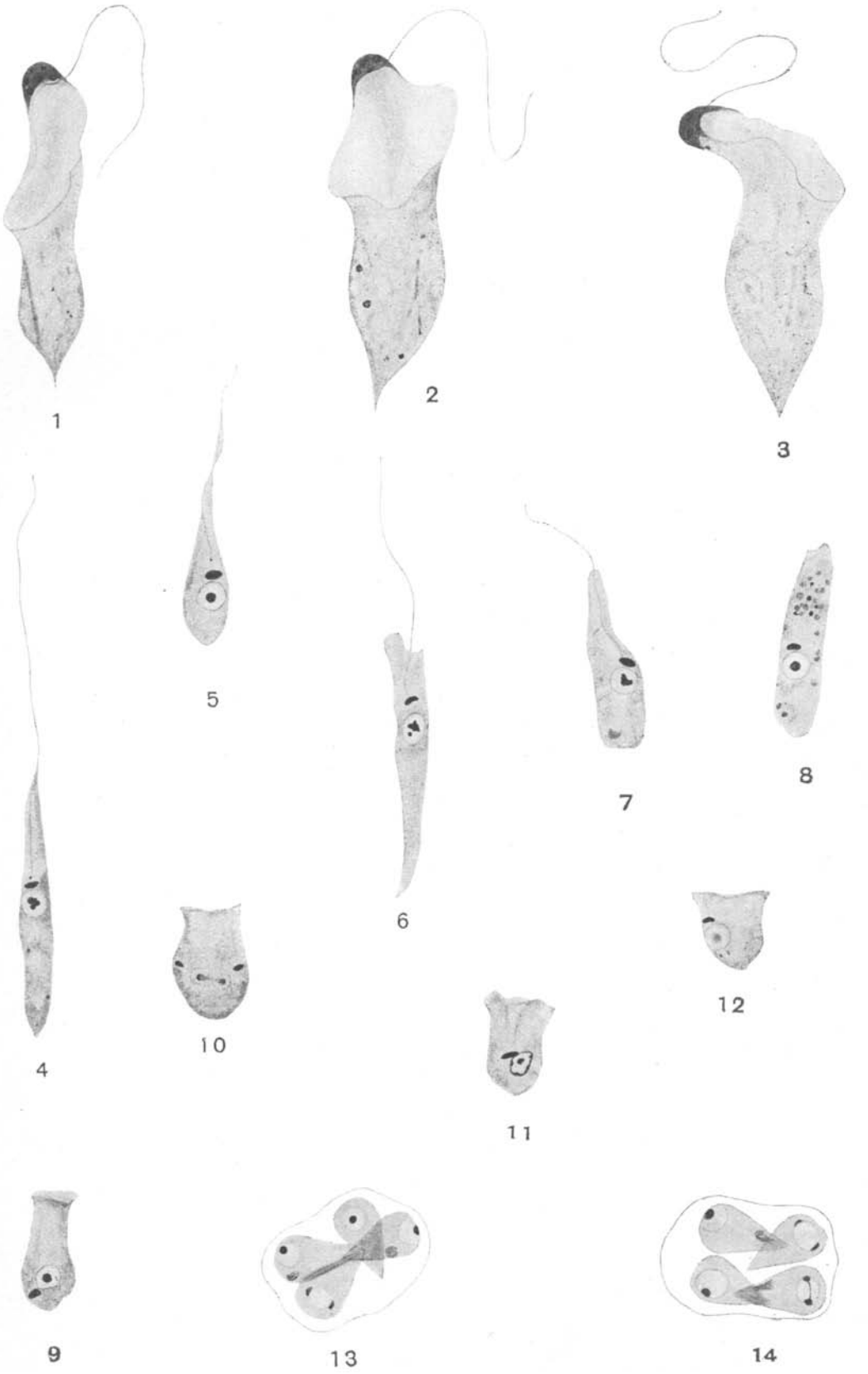
Fig. 7. Early stage of rounding up.

Fig. 8. Encysting form in which the cytoplasm is full of darkly-staining granules.

Figs. 9—12. Campanulate encysting stages. In fig. 10 the nuclei are dividing.

III. *Gurleya légeri*, Hesse.

Figs. 13 and 14. Typical tetrasporous pansporoblasts. In fig. 14 some of the nuclei are seen in division.



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