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CHILOMASTIX MESNILI
(WENYON, 1910)

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I. INTRODUCTORY

In the course of enquiries into the diarrhoeas and dysenteries of the Anglo-Egyptian Sudan we have found that *Chilomastix mesnili* (Wenyon, 1910) is very frequently present in the motions of Europeans and Natives alike. We have never found it to be present entirely by itself, as it is, in our experience, always associated with one or more of the following organisms:—*Löschia coli*, *Löschia histolytica*, *Entamoeba nana* Wenyon and O'Connor 1917,

Blastocystis hominis, *Giardia intestinalis*, *Octomitus hominis*, or *Trichomonas intestinalis* synonym *T. confusa* Stiles, but we did not observe it in the one case of infection with *Enteromonas hominis* da Fonseca 1915, which we have found here.

Although it seems quite harmless when present in small numbers, still we believe that when present in large numbers it may be the causal agent of diarrhoea, and, therefore, because it is such a *very common* intestinal flagellate it merits consideration.

Moreover, if only casually inspected in the living and moving condition in the faeces it is apt to be mistaken for a *Trichomonas*, and in its preserved and stained condition it has so frequently confused observers as to its morphology, even down to the present year, that we feel justified in attempting to clear up a few points. The following remarks are illustrated by photomicrographs, and though it must be admitted that it is impossible to obtain entirely satisfactory photographs of these minute animals, still photographs exclude the personal equation which must always be present in drawings.

II. HISTORY

The history of *Chilomastix mesnili* may be divided into three periods as follows:—

1. The Unrecognised Parasite.
2. Wenyon's Discovery.
3. Present Day.

1. *The Unrecognised Parasite.* The first record of this parasite, with which we are acquainted, is to be found in Davaine's '*Traité des Entozoaires*' published in 1860. On page 6 of the Synopsis with which this work begins he describes two varieties of *Cercomonas hominis*, viz., a large and a small. The large variety he obtained from the motions of cholera patients during the epidemic of 1853-1854 in Paris. So abundant were they that, at times, every drop examined contained many specimens. In this variety of *C. hominis* he saw with difficulty a flagellum arising from the broad end of the pear-shaped body. Also towards the anterior extremity he noticed a longitudinal feature which gave the appearance of a buccal orifice. He also gives a good illustration of this form, and, in our minds, there is no doubt that he saw and recognised a *Chilomastix*.

His smaller parasite, forming variety or species B, is by consensus of opinion considered to be *Cercomonas hominis*.

We therefore have two distinct parasites called by one name, but with regard to Davaine's species A it certainly does not belong to Dujardin's genus *Cercomonas* which was founded in 1841 and was placed in his Order III, i.e., among organisms without a mouth.

Therefore *Cercomonas hominis* Davaine 1860 pro parte becomes a synonym of *Chilomastix mesnili*, but it is the genus *Cercomonas* Davaine 1860 nec Dujardin 1841 which is a synonym of the genus *Chilomastix* Alexeieff 1911.

Thirty-three years pass before we meet with another known observation upon *Chilomastix mesnili*, and this time it is described by Roos (1893) in his paper 'Ueber Infusoriendiarrhöea.' We have been unable to consult this paper, but according to Gäbel (1914) the author states that he found in one case parasites with the following characters:—

'In der Nähe des vorderen Endes befindet sich eine Mundöffnung, die sich oft bis zur Körpermitte erstreckt. Vorn an demselben sitzen drei nicht ganz körperlange Geisseln. Sonst ist die Oberfläche glatt, und nur das hintere Ende trägt einen kurzen, unbeweglichen, stachelartigen Fortsatz.'

Gäbel gives a copy of two of Roos' drawings and they are typical of *Chilomastix*, and he considers that Roos was first to describe accurately a species of this genus. In another case Roos met with a typical *Trichomonas* with an undulating membrane. Roos, however, appears to have called both forms *Trichomonas intestinalis*; therefore *Trichomonas* Roos 1893 pro parte nec Donné 1837 becomes a synonym of *Chilomastix*, and *T. intestinalis* Roos 1893 pro parte nec Leuckart 1879 is a synonym of *C. mesnili*.

The next description is by Epstein (1893), who uses the term *Monocercomonas hominis* Grassi 1879 in his papers in the *Prager Medizinische Wochenschrift*, Nos. 38, 39, and 40, but only No. 39 contains the illustrations, of which figs. A 1, 2, and 3 were drawn from living forms by Dr. Czerny, and 4 and 5 by Professor Hatschek, after fixation by sublimate. Drawing 1 shows a pear-shaped parasite with a large deep cytostome and with two flagella; drawing 5 illustrates a similar object, and figs. A 1, 4 and 5 and fig. B 7, the illustration with the mouth and three flagella, leave the reader in no doubt that Epstein saw a *Chilomastix*,

as no axostyle and no undulating membrane are illustrated in these drawings, although other illustrations depict an obvious *Trichomonas*.

But Grassi (1888) very distinctly stated that his species *M. hominis* was a *Trichomonas*; therefore Epstein's *Monocercomonas* is not Grassi's, and therefore the synonym of the genus must run *Monocercomonas* Epstein 1893 nec Grassi 1879, while *M. hominis* Epstein 1898 nec Grassi 1879 becomes a synonym of *Chilomastix mesnili*.

2. *Wenyon's Discovery*. Seventeen years after the last entry in the preceding period the parasite was again observed by Wenyon (1910), who saw it in the motions of a native of the Bahamas. He remarked that in its movements there was little to distinguish it from *Trichomonas* except that the undulating membrane so characteristic of this genus was absent. He described the body as pear-shaped, with three long flagella directed forwards from the blunt end of the body and with a very large cytostome, the edges of which were produced into two lips, within which a flagellum or membrane could be made out displaying a constant undulatory movement. The length of the parasite varied, but the largest forms were about 15 microns long and 7 microns broad. Encysted forms, 7 by 5·5 microns, were seen, containing the characteristic nucleus, the large cytostome and some other details, but it was impossible to decide whether these cysts were reproductive or merely protective.

The flagellate was described as showing undoubted affinities with *Trichomonas* and *Trichomastix*, and was placed in the new genus *Macrostoma* Alexeieff 1909 because it agreed with *M. caulleryi* Alexeieff in all particulars, except the undulating membrane in the cytostome, which, it was just possible, had been overlooked. It was therefore called *Macrostoma mesnili* Wenyon 1910. It was further noted that the flagellate produced no ill-effects on its host, and that its presence was only discovered in the routine examination of the faeces.

It is now necessary to digress from the actual history of the parasite and to enquire what Alexeieff meant by his genus *Macrostoma*.

Alexeieff (1909) describes, but does not figure, a new genus

and a new species of flagellate which he calls *Macrostoma caulleryi*, and which he found in tadpoles and in an axolotl. The length of the parasite was 20 to 25 microns and the breadth 8 microns. It was pear-shaped, with three anterior flagella, and was characterised by possessing a very large cytostome with one border developed into a sort of lip. The nucleus was situate anteriorly.

Unfortunately the name *Macrostoma* had been used by Latreille in 1825 for a mollusc, and in subsequent years by other authors for various kinds of animals, but Latreille's genus has priority, and the other names, including Alexeieff's, lapse.

For the benefit of the Medical reader we may here point out that a generic name already in use can be discovered if it is given in Agassiz's *Nomenclatoris Zoologici* published in 1846, Scudder's *Nomenclator Zoologicus* published in 1882 or in the Zoological Society's *Index Zoologicus* No. I, 1880-1900 (its supplement by Waterhouse published in 1904) or in No. II, 1901-1910. These works are so well arranged that in a few minutes it is possible to know whether the proposed generic word is contained therein. If it is not to be found in these works then it is much more difficult to be sure that it has never been used before.

Returning to Alexeieff's *Macrostoma*, this must lapse and must become a synonym of whatever generic name is finally fixed for the parasite we are considering, and the synonym must read *Macrostoma* Alexeieff 1909 nec Latreille 1825.

In 1910, Alexeieff reported that he had obtained a flagellate from *Box salpa* which was identical with that described by Wenyon, whose parasite he had also seen in a Russian sailor suffering from cholera. At the same time he stated that he had found that the name *Macrostoma* was not available for these parasites, and suggested that they should be classified in Perty's genus *Tetramitus* which we must now, very briefly, consider.

Perty (1852) established this genus for two free living flagellates which he named *T. descissus* and *T. rostratus*, and which he illustrated as possessing *four equal anterior flagella*. Fresenius (1858) gave a brief description of *T. rostratus*, but did not advance matters, while Dallinger and Drysdale (1871) described and figured the life history of a 'Calycine Monad' which by general agreement is identified with *T. rostratus*, but they did not improve upon Perty's descriptions. Stein (1878) carefully figured the two species mentioned above, but he depicts *T. descissus* with *a long trailing flagellum*. He also described a third species which we believe to be identical with *Callodictyon triciliatum* Carter 1865.

Bütschli (1878) investigated *T. descissus*, and recorded that the flagella were of unequal length.

Klebs (1892) redescribed Perty's two original species, and added a third: *T. pyriformis*. He insisted upon the broad size of the mouth and upon the *inequality of the flagella*, of which *the fourth is always the longest*, and in two at all events of the three known species *is trailing*. Klebs' descriptions have been accepted by Blochmann, by Senn, and by Hartmann and Chagas, and are, as far as we know, the latest original writings upon species of Perty's genus *Tetramitus*. It will thus be seen that these species possess four anterior flagella, while Alexeieff's and Wenyon's parasites have only three. Again in *Tetramitus* one flagellum is trailing, while no such flagellum is described by Alexeieff or by Wenyon. Therefore we are of the opinion that it was incorrect to classify Alexeieff's and Wenyon's parasites in the genus *Tetramitus* Perty 1852. Nor had this difficulty escaped Dobell (1909), who had suggested that the name *Tetramitus* should be reserved for free living quadriflagellate organisms of the type described above, while Alexeieff (1910) proposed, that if the free living species belonging to the genus *Tetramitus* were found to be different from the parasitic, then this name should be retained for these free living forms, while that of *Chilomastix* should be given to the parasitic organisms; and, as we have shown above, there are considerable morphological differences between the parasites described by Alexeieff and by Wenyon and the generally accepted definition of a *Tetramitus*, and therefore the two parasites should be called *Chilomastix caulleryi* (Alexeieff 1909) and *Chilomastix mesnili* (Wenyon 1910), respectively, even though Alexeieff started the existence of the new genus without any description, and indeed without carefully differentiating it from *Tetramitus*.

Though Alexeieff did not give sufficient distinguishing features for the genus *Chilomastix* in 1910, he did so in 1911, and therefore the genus stands as *Chilomastix* Alexeieff 1911, and not 1910.

3. *Present Day*. The period which we are about to describe begins with much confusion, new genera and new species being formed for Wenyon's parasite because mistakes were made with regard to the morphology of the forms which various observers described.

Prowazek (1911) instituted the genus *Fanaepepea* for a flagellate *Fanaepepea intestinalis* which he discovered first in 1903 in the alimentary tract of a baboon, and later in persons suffering from Ankylostomiasis in Samoa. The organism was described and figured as a pear-shaped or rounded flagellate, with an anteriorly situate nucleus containing a small karyosome. In front of the nucleus lay two small blepharoplasts from which sprang two *anterior flagella*. Alongside the nucleus there was a large cytostome, the lips of which were strengthened by a chromatic band containing a third flagellum which arose from a third blepharoplast. This flagellum gave rise to a short cytostomic undulating membrane. No axostyle was described or depicted. In fig. 16 a twisted and grooved parasite is illustrated, which is a point of some little interest, as will be seen below. He also gives a figure of binary fission.

It seems to us that the characters of *Fanaepepea intestinalis* are the same as those of *Chilomastix mesnili*, because:—

1. In fig. 8 of his paper he depicts a typical *C. mesnili* but calls it *Trichomonas nov.*, but he shows no axostyle.
2. In fig. 10 of the same paper he shows another typical *C. mesnili*, but calls it *Fanaepepea intestinalis*.
3. In 1914 he, together with Werner, clearly states that *Fanaepepea* is nearly identical with Wenyon's *Macrostoma*.

Therefore the name *Fanaepepea intestinalis* becomes merely a synonym of *Chilomastix mesnili*.

Nattan-Larrier (1912) reported *C. mesnili*, under the term *Tetramitus mesnili*, as being present on the Ivory Coast of West Africa. He thinks that in the case which he studied the parasite may have had some pathological importance. In the discussion upon this paper Chatton said that he had seen a similar parasite in a leech *Haemopsis sanguisuga* in Belfort in the year 1910.

Brumpt (1912) published an account of a case of Colitis due to *Tetramitus mesnili* (Wenyon 1910). In this paper he gave illustrations of the parasites, free and encysted. He also took exception to Alexeieff's views that the flagellate, which that observer had found in the intestines of *Box salpa*, was identical with *C. mesnili*, and called it *Tetramitus bocis*, because he said that the illustrations of the two parasites were totally different. Now Alexeieff seems to have clearly recognised the three anterior flagella and the fourth

cytostomic flagellum, and as we have been unable to see his original drawings we prefer to consider that, until further evidence is forthcoming, the parasites in man and in *Box salpa* are the same.

Alexeieff (1912) published another paper of which we have been able to obtain only some notes and some copies of his drawings of *C. caulleryi*, including those showing binary fission and cyst formation. According to these notes and drawings he considered *C. caulleryi* and *C. mesnili* as good species.

Gäbel (1914) described the same organism which he found in a German who had travelled in Tunisia. He created a new genus and species, based upon some misconceptions, using the terms *Difämus tunensis*. He considered that the parasite was pathogenic.

Prowazek and Werner (1914) contributed some remarks upon *C. mesnili*, pointing out that it was closely related to *Fanapepea intestinalis* which Prowazek had found in Sawai, Samoa, Saipa and India. They considered that it was probable that the nuclear changes in binary fission would be found to be promitotic in character, and they gave an excellent illustration of a cyst.

Chatterjee (1915), under the name *Macrostoma*, reported the parasite's presence in the dejections of six persons suffering from intestinal troubles in Bengal.

Wenyon (1915), under the terms *Tetramitus mesnili* (*Macrostoma mesnili*), gave a further account of the parasite in question, giving the length as from 3 to 15 microns. He states that he has never seen an undoubted dividing form, but considers that the presence of specimens with two tail-like prolongations in fresh preparations may indicate simple longitudinal division. He also describes cyst formation and figures two cysts, one with a single and the other with four nuclei. When examining these drawings one is struck with the fact that the nuclei do not appear to be typical of *Chilomastix*, and, as we shall see later, Wenyon finally decided that they were not *C. mesnili* cysts, but those of an amoeba.

Mackinnon (1915), in a paper mainly dealing with the genus *Embadomonas* (which appears to us to be the same as Wenyon and O'Connor's new genus *Waskia*), drew attention to its close affinity to *Chilomastix* Alexeieff 1911.

Da Fonseca (1915 and 1916) reported that *C. mesnili* existed in Brazil, and also added to the genus several new species derived from mammals.

Fantham and Porter (1915) reported its presence in the motions of soldiers from the Mediterranean War Zone.

Archibald, Hadfield, Logan and Campbell (1916) found it to be present four times in the large number of motions of soldiers which they examined during the Gallipoli campaign.

Chatterjee (1917) stated that he had found it eighteen times in stools of chronic dysentery in Bengal.

Wenyon (1917) found it in films of faeces from the Philippine Islands and from Panama; and Wenyon and F. W. O'Connor (1917), in their excellent paper upon '*Problems affecting Intestinal Protozoal Infections*,' noted that the parasite is often flattened and leaf-like and that its long-drawn-out flattened posterior extremity may be twisted and folded in a variety of ways, thus producing grooves and spiral turns. This is an important observation to which we shall return later. They note that binary fission probably takes place but has not yet been observed. They lay great stress on the cysts, which they state can be found in the formed stools, and they point out that the four-nucleated cyst illustrated in Wenyon's (1915) paper does not belong to *C. mesnili* but to *Entamoeba nana*, a mistake which everyone who has worked at this small organism knows is very liable to occur even when one is well acquainted with some of the stages of the cyst—and it must be remembered that *C. mesnili* is generally accompanied by a number of other parasites (*vide* the list given in the Introductory Section of this paper). They also state that *intermission* of the presence of the parasite in the stools is not so marked in the case of *C. mesnili* as it is with other intestinal parasites.

From this history it will be evident that a considerable number of observations have been made with regard to the parasite in question, but unfortunately a great deal of confusion has arisen owing to mistakes as to the number of anterior flagella, the presence or absence of the cytostomic flagellum and its action, while much more confusion has arisen as to the cystic form of the parasite and its pathogenicity. Moreover, the names used for the same parasite are numerous and confusing, and yet it is essentially a *common human intestinal flagellate*, and therefore we consider that any contribution, however small, which attempts to clear up some of these points may be of use to workers in the Tropics.

III. GEOGRAPHICAL DISTRIBUTION

Chilomastix mesnili appears to be widely distributed all over the world.

So far as we know, it has been recorded in man from the following places:—

Europe:—France, Germany, Russia, Salonika War Area and Turkey.

Asia:—India and the Philippine Islands.

Oceania:—Samoa, Sawai and Saipa.

America:—Brazil, Panama and the West Indies.

Africa:—The Ivory Coast, Tunisia, Egypt and the Anglo-Egyptian Sudan.

IV. ZOOLOGICAL DISTRIBUTION

So far its principal host is man, in whom it would appear that all races will be found to be infected, or susceptible, as it is recorded in Europeans, Natives of India, Natives of Samoa, Negroes and Arabs.

It has also been found in a baboon, and in a fish *Box salpa*, but, with regard to this last host, Brumpt has raised the doubt as to whether it is not a separate species, for which he suggests the name *Chilomastix bocis*.

V. THE LIVING PARASITE

Chilomastix mesnili in the fresh condition in the faeces is usually pear-shaped (figs. 1-4, 6-7 and 9-11), but, at times, it may assume other forms, being fusiform (fig. 5) or roundish (fig. 14). It measures on an average, and excluding the very small developmental forms, some 11 to 18 microns in length, if the caudal appendix is included, and some 1·6 to 4·0 microns less if this is excluded. In breadth it attains some 6 to 8 microns in greatest measurement.

With regard to its orientation, the anterior end is the broad portion of the pear-shaped animal, and is characterised by the presence of the nucleus and by the origin of the three flagella. The ventral surface is that on which the cytostome opens.

When fresh faecal matter is examined the parasite may be

readily seen dashing quickly about among the débris, but it is not until this rapid motion has abated that it is possible to see that it moves with its broad extremity placed anteriorly.

When moving still more slowly it is possible to see that this anterior end is provided with three free equal anterior flagella. These flagella as a rule are not longer than the body, and work by lashing from side to side.

Rotatory movements can also be observed, as can a peculiar motion of the anterior portion of the body, which appears to consist of a dorsal twisting of the antero-ventral part, while the posterior part of the body is not affected. It is a quick movement, and gives one the impression that the antero-ventral portion is quickly pulled dorsally for a short distance and then quickly returned to its original position. If the parasite is fixed while performing this dorsal twist it appears as represented in fig. 15.

This twist naturally gives rise to the appearance of lines and grooves running more or less diagonally backwards across the body.

There is a very evident and large cytostome (figs. 3 and 4) situate anteriorly, and extending posteriorly a considerable distance into the body of the flagellate. This cytostome possesses very distinct lips, which open (figs. 4 and 5) and close (figs. 8 and 13). There is also a movement of the nuclear or inner lip which resembles that of a short undulating membrane. It would appear to be due to the action of a fourth or posterior flagellum (figs. 6 and 11) lying in the inner lip and pulling outwards the inner wall of the cytostome into the form of a bow-shaped membrane (figs. 7, 9, 10 and 11), which returns to its original position when the flagellum has ceased to contract. There can be little doubt that the action of this membrane helps the flagellate to convey motionless food particles to the cytostome. It seems to us that these thickened mobile margins (figs. 4 and 5) of the lips of the cytostome are of great service in enabling the flagellate to secure a firm hold on its food. To exemplify this we may quote an incident which we have noticed. We have seen a parasite swim up to a chain of bacilli evidently much too long to be completely engulfed by the cytostome. This chain was promptly seized by the lips of that opening and then the parasite set to work to try to swallow its prey, but while so engaged a passing flagellate, doubtless attracted by the commotion, stopped

and seized hold of the other end of the chain with its cytostome, whereupon a struggle ensued which demonstrated in an admirable manner the movements of the flagella, the lips of the cytostome and the caudal appendix, as it lasted for some ten minutes. The second comer appeared never to get a firm hold of the chain, which was being jerked about at the time that it arrived, because it was repeatedly thrown off, but the first one had a firm grip, and it appeared to us, watching the struggle, that the thickened lips helped that hold considerably, as they appeared to be very strong; but, be that as it may, the second flagellate gave up the game and went away, leaving the first claimant alone with its gargantuan feast.

The food of the parasite appears to be the micro-organisms to be found in the intestinal contents. We have never observed red blood corpuscles in its interior.

It seems to be readily killed in the adult condition by exposure to cold, dryness and light.

We have been unable to cultivate the flagellate on ordinary laboratory media aëroically or anaëroically.

In sand we have merely obtained some organism not unlike that described by Ross and Thompson (1916), by the Thomsons (1916), and by Chatterjee (1914).

We have met with an allied species in white rats, originally brought from England, but bred for several years in the Sudan, and also in the Sudan Gerbil (*Gerbillus pygargus*).

VI. MORPHOLOGY

We have studied the structure of the parasite entirely by films made from faecal matter, as we have been unable to obtain a post-mortem in a case known to harbour the organism. The flagellates were killed by osmic acid, fixed in Schaudinn's fluid and stained by iron haematoxylin.

In specimens so prepared the most striking object (fig. 1) is the large roundish, oval, or slightly irregular nucleus, which measures some 2.8 microns in its greatest diameter and lies in close proximity to the anterior extremity.

As a rule the nucleus stains homogeneously, but in well decolourised specimens (fig. 1 *a*) the rim is more darkly tinted and

there is a lighter centre. There are often two or three darker and thicker spots of variable size and appearance situate on this rim, but these may be absent and there may be one large, more or less rounded, central karyosome, or two medium-sized karyosomes lying in the central portion and not attached to the rim. In other instances three small karyosomes may be seen.

Situate anteriorly or antero-laterally to the nucleus two chromatic particles can usually be readily seen (fig. 5), but, when careful decolourisation has been conducted, it will be observed that there are really three (fig. 2) chromatic particles or blepharoplasts. One of these is situate anteriorly, and gives rise to *two of the anterior flagella*; the second lies externally and is the origin of the *third anterior flagellum* and of the chromatic outer rim of the cytostome which, as we shall see later, is probably homologous to a 'Parabasal.' The third and small blepharoplast lies posterior to the first and internal to the second, and gives rise to a fourth or *cytostomic flagellum* (fig. 2).

We have failed to observe any trace of a rhizoplast.

Situate externally to the nucleus, and extending considerably posterior thereto, lies the large cytostome (figs. 3 to 13). It measures some 2.8 microns in length by some 1.2 microns in breadth when widely open, but, of course, its dimensions vary considerably, and its general characters can be best judged by an examination of the figures just quoted.

If figures 3, 4, 5 and 13 are examined it will be seen that the margins of the mouth show well defined siderophilous thickenings, which in fig. 5 appear to run continuously from one blepharoplast, all around the cytostomic opening to the other blepharoplast. This condition has been noted by many observers, and has produced much misconception as to the nature of these lips.

In this quiescent condition it will be noted that the outer lip is, as a rule, distinctly wavy, while the inner or nuclear lip is straight (figs. 3, 4, and 5).

When more carefully studied, even in this quiescent condition, it can be seen that the thicker and more important siderophilous rim runs down the whole length of the outer lip, and curving around the posterior end of the cytostome ascends the inner lip for some distance, as is shown in figs. 3 and 6. It now stops, but its outer

aspect is overlapped by the nuclear siderophilous rim, as can be easily seen when the two are separated as in figs. 3 and 6, but cannot be observed in figs. 5 and 13, in which it is impossible to say where one structure begins and the other ends.

The inner or nuclear siderophilous rim appears to us to take the black stain less deeply (fig. 6) than the outer rim. It starts from the third blepharoplast (fig. 2), or from a fusion of the first and third when this latter is absent, and runs posteriorly down the inner or nuclear lip of the cytostome (fig. 3), being placed externally to the anteriorly running prolongation of the external rim (fig. 6). It is capable of contraction and of being displaced outwards (figs. 6, 9, 10 and 11), and when this takes place it raises a bow-shaped membranous expansion (fig. 7) from the inner aspect of the cytostome. This movement may be carried across the mouth so that the membrane touches, or almost touches, the outer lip and so temporarily divides the mouth into an anterior and a posterior opening (fig. 8). When this takes place it is seen (figs. 6 and 11) that not merely is this rim attached to a blepharoplast, but it is also attached near to the postero-internal angle of the cytostomic opening.

When its contraction is over it returns to its original position in the inner rim of the mouth, and by this alternate movement produces a flickering and undulating movement in the cytostome very reminiscent of the undulating membrane of a *Trichomonas*.

If the specimen is subject to somewhat rough treatment this internal siderophilous rim can be seen hanging in the cytostome (fig. 12) and then produces the appearance of a free cytostomic flagellum, but, should the treatment be even rougher, it may be torn from the cytostome and appear as a short free fourth anterior flagellum as figured by Gäbel.

These artefacts are extremely interesting, showing that this internal rim is really a fourth posteriorly directed flagellum which penetrates into the cytoplasm, and is capable, on contraction, of raising a membrane, homologous to an undulating membrane in such an organism as a typical *Trichomonas*, such as is depicted in fig. 16.

If this is admitted we have still to explain the outer siderophilous rim, and this appears to us to be homologous to the '*Parabasal*' or chromatic thickening at the base of the undulating membrane of the *Trichomonas*, as can be seen by an examination of fig. 16. It will, however, be observed that there are two great differences between the

undulating membrane or fourth flagellum of the *Trichomonas* and its parabasal and the siderophilous rims of the cytostome of the *Chilomastix*, and these are:—

1. Both organellae are dorsal in *Trichomonas* and ventral in *Chilomastix* (assuming that the cytostome marks the ventral surface in both flagellates).
2. In *Trichomonas* the flagellum is situate external to the parabasal, while in *Chilomastix* this position is reversed.

These two differences appear to us to be co-related. We believe that in development and evolution the posterior flagellum and the parabasal growing backwards from the blepharoplasts of the *Trichomonas* for some unknown reason took the dorsal side of the nucleus, and meeting with no obstruction were able to grow backwards through the whole length of the flagellate (fig. 16). In *Chilomastix* for some unknown reason they grew down the ventral side, and in so doing their relationship to one another would be exactly the reverse to that found in *Trichomonas*, viz., the parabasal would be external and the flagellum internal. Moreover, they would meet with the cytostome round which the parabasal curves, thus checking the growth of the flagellum. The curving of the parabasal round the cytostome reminds us of the way in which the same organella winds round the axostyle in *Devescovina*. We therefore see a homology between the flagellum in the inner lip of the cytostome of *Chilomastix* and that in the undulating membrane of a *Trichomonas*, and between the siderophilous thickening in the outer lip of the cytostome of *Chilomastix* and the parabasal of a *Trichomonas*.

We now turn to ask ourselves whether this parabasal has any other function besides that of being a thickening to the outer lip of the cytostome. We have already referred to the curious twisting to and fro movement observed in the anterior portion of the body of the parasite at times, and we believe that it is this movement which produces the twisted appearance depicted in fig. 15.

We suspect that this movement is brought about by the action of the parabasal, and our reasons for this suggestion, for it is nothing more than a suggestion, are:—

1. Because we can find no other structure which can produce this movement.
2. Because the parabasal is often found to be wavy (figs. 3, 4, 5 and 6), as though caught in the act of movement.

3. Because of its persistence in cysts indicating some important function.

The twisted condition of the parasite when caught performing this movement gives rise to the appearance of a groove sloping diagonally and posteriorly along or across the back portion of the body (fig. 15).

The cytoplasm of the flagellate is colourless, finely granular and contains, as a rule, a number of food vacuoles (fig. 1). No contractile vesicle can be seen.

The periplast is thin, apparently elastic, and without any marked thickenings. It is prolonged posteriorly into the so-called 'caudal appendix or tail,' which may (fig. 1) or may not (fig. 6) contain a slight amount of cytoplasm at its base. It is this appendix which gives the pear-shape to the flagellate, but it is not always present, and then the shape of the parasite is rounded (fig. 14). It may possibly function as an organ of temporary attachment of the flagellate to some neighbouring structure.

VII. DEVELOPMENT

We have met with two forms of development, viz., *binary fission* and *cyst formation*. We have never seen any sign of multiple fission, but we have come across a number of small forms.

Binary Fission. Judging by the rarity of these forms in the faeces, as experienced by ourselves and other observers, we are inclined to the view that this form of reproduction is intended for the propagation of the parasite in its host, and we think that until the bowel in a case of infection can be studied it will be unlikely that any complete series of the changes undergone by the parasite will be obtainable. At all events, we have been unable to find any such series, and the few dividing forms which we have met with have produced exceedingly poor photographs. We therefore wish such views as are set forth here to be considered as merely tentative.

Starting with the non-dividing nucleus, we have the condition of affairs represented in fig. 2, viz., the nucleus with its chromatic apparatus and three blepharoplasts with their attached three anterior flagella, one parabasal, and one posterior flagellum. The earliest stage of mitosis which we have observed is that set forth in fig. 17,

in which the nucleus seems to be gathering its chromatin into two masses, and there are only two distinct blepharoplasts with four flagella. The mouth is exceedingly indistinct, although the parabasal is just visible.

In the next stage (metaphase) which we have met with, the chromatin particles are arranged in an equatorial plate consisting of four chromosomes. Starting from the left there is a blepharoplast with two flagella attached; then comes a second blepharoplast with one flagellum and also with a chromatic line running over the length of the nucleus and passing on a level between the anterior and the second chromosome to reach the right end of the nucleus, where it joins with another blepharoplast also carrying a flagellum. This chromatic line appears to indicate a *paradesmose*. Just posterior to this right-handed blepharoplast there lies a fourth blepharoplast giving rise to two flagella, while posterior again some remains of the mouth margins can be distinguished.

A further stage (anaphase) seemed to be indicated by a form which showed two chromatin particles (chromosomes) at each end of the nucleus. Just outside each pole (right and left) of the nucleus there lay a chromatic particle connected by fine lines to the intranuclear chromosomes, while these in their turn were also connected by fine lines, thus making a spindle.

The difficulty which we have with this stage is that whereas the equatorial plate had four chromosomes, each end of this nucleus had two (and no more) chromosomes, as the specimen was well decolourised and the four chromosomes were seen very clearly. The blepharoplasts were now situate some little distance from the nucleus and showed the same general arrangement as in the preceding specimen, except that there was no *paradesmose*, and that the first blepharoplast gave off only one, and not two flagella as mentioned above. In the penultimate stage (late anaphase) which we have seen, the chromatin particles were gathered into two fair-sized lumps more or less fused together at either end of the nucleus, which was considerably elongated, while the blepharoplasts and flagella were a replica of the second division stage described above.

The last stage (telophase) which we have observed is that depicted in figs. 18 and 19, in which it will be observed that the parasite is much elongated and that the two nuclei lie at opposite

poles, and that two or three blepharoplasts give rise to three flagella. A mouth is visible near each nucleus in these specimens, but is only partially in focus in the illustrations.

These are the only stages which we have observed. They are obviously incomplete, and they are peculiar: for example, we can offer no explanation of the apparent reduction in the number of the chromosomes, and we must leave the clearing up of this and many other points in this mitosis to future investigations.

Cyst formation. We have seldom found the cysts in the faeces, and then only in cases of fairly severe diarrhoea. The typical cyst (figs. 22 and 23) usually measures some 7 by 5·6 microns, and is now extremely easy to identify, being somewhat egg-shaped (figs. 22 and 23), that is to say, having its anterior end narrower than its posterior. The cyst wall (figs. 21, 22 and 24) is separated from the parasite by a clear space. In its youngest stages (figs. 20 and 21) it is possible to discern the nucleus, and a blepharoplastic mass (situate anteriorly) from which run two chromatic lines which appear to be the mouth margins, viz., the parabasal and the posterior flagellum, and sometimes other rather vague siderophilous lines running from front to back which represent remains of the anterior flagella. Sometimes the nucleus looks as though it was composed of four portions closely joined together.

The only development which we have seen to take place in the cyst has been the disappearance of all the siderophilous lines, leaving the cyst with a very distinct nucleus containing a single large karyosome (fig. 24; it is also well shown in fig. 21 which represents an earlier stage), and surrounded by a clear space but without any evident membrane. Such a nucleus appears to us to be of the nature of a '*Protokaryon*.'

Just outside the nucleus, and often lying in close contact with it, is a chromatic mass (figs. 22 and 24), and, as a rule, one or two other small siderophilous particles can be seen lying in the cytoplasm.

We have seen signs of division twice; first it was merely in the nucleus (fig. 25), which consisted of two chromatic poles connected by means of a thick chromatic rod. This appeared to us to be an early stage of a promitotic division. Outside this dividing nucleus lay the usual chromatic or siderophilous mass, which was elongated in the same direction as the dividing nucleus.

The second showed two nuclei embedded in granular protoplasm (fig. 26).

These cysts are extremely difficult to photograph, but we have preferred to give untouched photographs, however imperfect, rather than drawings. We look upon the cysts as the means by which the parasite changes its host.

Young forms. We have met with numerous small forms, of which the smallest we have seen (fig. 27) measured some 4·2 by 2·2 microns. It possessed a well marked nucleus and three flagella, but we were unable to make out any mouth, and it contained no micro-organisms. In the photograph some organisms are lying over but not inside the parasite.

The next larger type of flagellate is depicted in fig. 28, in which a well marked mouth can be seen, and the development can proceed through such a type as that depicted in fig. 29 till by growth it reaches the adult form.

We have no evidence as to where these young forms come from, though judging by the size and the absence of the mouth it is possible that they come from the cysts.

Such are the few, fragmentary and unsatisfactory details which we are able to give of the development of *Chilomastix mesnili*, and therefore we will now pass on to consider its classification.

VIII. CLASSIFICATION

As the protozoal organism which we have described above. possesses permanent flagella which serve as organs of locomotion and also assist in the capture of food, it belongs to the Class *Mastigophora* Diesing 1866, which is divided into sub-classes as follows :—

DIAGNOSTIC TABLE OF THE *Mastigophora*

A. <i>Body inflated with gelatinous substance...</i>	...	Sub-class 1
<i>Cystoflagellata</i>
Haeckel 1873
B. <i>Body not so inflated :—</i>		
I. Periplast markedly thickened with two
flagella arising in the middle of the body ;
one trailing and one lying in the trans-
verse groove		Sub-class 2
<i>Dinoflagellata</i>
Bütschli 1885

II. Periplast thin with a variable number and arrangement of flagella... ..	Sub-class 3
<i>Euflagellata</i>
Cohn 1887 |
|---|---|

The parasite in question obviously belongs to Cohn's *Euflagellata*, which may be classified into the following orders :—

DIAGNOSTIC TABLE OF THE *Euflagellata*

A. <i>Chromatophores often present</i> :—	Order 1
I. With cellulose envelope	<i>Phytomonadina</i>
Blochmann 1895
II. Without cellulose envelope :—	Order 2
a. Small forms without oesophagus or vacuole system	<i>Chromomonadina</i>
Klebs 1892
b. Large forms with oesophagus and vacuole system	Order 3
<i>Euglenoidina</i>
Bütschli 1884
B. <i>Chromatophores absent</i> :—	Order 4
I. Amoeboid forms in which the food is captured by pseudopodia	<i>Rhizomastigina</i>
Bütschli 1884
II. Non-amoeboid forms in which the food is usually captured by flagella	Order 5
<i>Protomonadina</i>
Blochmann 1895

Our parasite certainly belongs to Blochmann's *Protomonadina*, which, with the above definition, includes the orders Binucleata and Polymastigina, and can be sub-divided as follows :—

DIAGNOSTIC TABLE OF THE *Protomonadina*

A. <i>No tendency to bilateral symmetry in undividing forms</i> . Anterior flagella vary from one to many, in addition to which a trailing flagellum or undulating membrane may be present ...	Sub-order 1
<i>Monozoa</i>
Hartmann and Chagas
1911
B. <i>More or less tendency to bilateral symmetry in undividing forms</i> as shown by the arrangement of the flagella, the duplication of the axostyles, sometimes of the nucleus, and more rarely of the cytostome. Undulating membranes absent	Sub-order 2
<i>Diplozoa</i>
Hartmann and Chagas
1911

As the parasite in question does not show any signs of bilateral symmetry it belongs to the Sub-order 1 *Monozoa*, which is classifiable into the following families :—

DIAGNOSTIC TABLE OF THE *Monozoa*

A. <i>One flagellum present</i> :—				Family 1
I. With a collar	...	...	...	<i>Craspedomonadidae</i> Stein 1878
II. Without a collar :—				Family 2
a. Blepharoplast not separate from the nucleus	...	...	...	<i>Oicomonadidae</i> Senn 1900
b. Blepharoplast separate from the nucleus				Family 3 <i>Trypanosomidae</i> Doflein 1901
B. <i>Two flagella present</i> :—				
I. Both anterior :—				Family 4
a. Unequal	...	...	...	<i>Monadidae</i> Stein 1878 emendavit Senn 1900
b. Equal	...	...	...	Family 5 <i>Amphimonadidae</i> Kent 1880 emendavit Bütschli 1884
II. One anterior and one trailing flagellum :—				
a. Trailing flagellum free :—				Family 6
1. In horny sheath and with lip or proboscis-like process	...	...	...	<i>Bikocidae</i> Stein 1878
2. Without sheath or process	...	...	...	Family 7 <i>Bodonidae</i> Bütschli 1884
b. Trailing flagellum in part attached to the body (except <i>Tricercomonas</i> with three anterior flagella)	...	...	...	Family 8 <i>Cercomonadidae</i> Kent 1880 emendavit Bütschli 1884
C. <i>Three to six anterior flagella</i> with or without one trailing flagellum (except <i>Embadomonas</i> with one anterior and one cytostomic flagellum)				Family 9 <i>Tetramitidae</i> Kent 1880
D. <i>Numerous anterior flagella</i>				Family 10 <i>Callimastigidae</i> Da Fonseca 1915

IX. TETRAMITIDAE

It is sufficiently evident that the organism we are considering comes under the heading Tetramitidae, which may be defined as follows :—

‘*Protomonadina* with three to six anterior flagella (with the exception of *Embadomonas* which has only two flagella, one

anterior and one lying in a large cytostome with siderophilous lips), with or without a rhizoplast, with or without one trailing flagellum which may or may not form an undulating membrane, and with or without an axostyle and a cytostome.

Habitat : Free living and parasitic.

Type genus : *Tetramitus* Perty 1852.'

It is not possible in Khartoum to obtain a full literature, and the following list of genera which come under this family merely comprise *those with which we are acquainted*, and therefore does not pretend to be a complete list.

With this proviso and because so many human parasites are included in this family which, with the above definition, represents not merely Saville Kent's *Tetramitidae* but also his *Trimastigidae*, we consider that it is necessary briefly to consider every genus which at any time has been classified in these families.

Our reason for this is because we wish clearly to define each genus which is properly classifiable in the *Tetramitidae*, in order to do away with the existing confusion and in order to prepare a diagnostic table with a view to defining clearly the position of the genus *Chilomastix*.

We shall not consider the new genus *Tricercomonas* Wenyon and O'Connor 1917 as it appears to have very definite affinities with the genus *Cercomonas*, notwithstanding its three anterior flagella, and therefore has no place in the *Tetramitidae*; but with regard to some thirty-four genera which from time to time have been placed in one or other of Saville Kent's families we have the following remarks to make :—

GENUS 1. *Chloraster* Ehrenberg 1836:—Ehrenberg (1836) instituted this genus for *C. gyrans*, which having its cytoplasm coloured green was removed from the *Tetramitidae* by Bütschli and was subsequently classified by Blochmann in the family *Polyblepharididae* Dangeard 1888 which belongs to his order *Phytomonadina* and therefore does not further concern us.

GENUS 2. *Trichomonas* Donné 1837. In his work '*Recherches microscopiques sur la nature du mucus*,' Donné (1837) described and figured an organism which he found in innumerable quantities in vaginal mucus.

He considered that it possessed a single flagellum which at times was bifurcated distally, a series of three to five cilia with very rapid

rotatory movement, and that at times it was elongated posteriorly into a tail. The name was first spelt *Tricomonas*, but afterwards altered to *Trichomonas*.

Dujardin (1841) described *T. limacis* from *Limax agrestis* in much the same terms, and so did Perty (1852) with regard to *T. batrachorum*, though he depicted the axostyle, but Stein's figures of Perty's organism show clearly the three anterior flagella, the undulating membrane, the posterior free flagellum, the axostyle, the nucleus and the cytostome, and in this way was laid the foundations upon which the main features of the genus were placed.

Returning now to the type *T. vaginalis*, this was re-studied in 1884 by Blochmann, who illustrated the three anterior flagella, the undulating membrane, the axostyle and the nucleus, but in the same year Künstler produced a much better illustration showing the three anterior flagella taking their origin from a blepharoplast from which the undulating membrane also arose, while this shows a trace of a parabasal. The nucleus is also represented, while the axostyle shows exceedingly clearly. He also saw the cytostome. Bensen (1910) figured two blepharoplasts, one of which is connected with the nucleus by means of a rhizoplast, and he also gave an illustration of a cyst. Thus the type species *T. vaginalis* was brought into line with the results of researches upon the species found in animals, of which a number have been carefully described and drawn by Dobell, Alexeieff, Martin and Robertson, Kuczynski, and by Kofoid and Swezy.*

With regard to the Sudan, there have been strikingly few observations upon species of this genus. Stevenson (1911) figured *T. batrachorum* from *Bufo regularis*. Since then we have observed several species in animals which we hope to describe in a future paper.

We have only very rarely seen *T. intestinalis* (Leuckart 1879) in man, and this is very curious, considering the very large number of

* Since writing the above, we have had the good fortune to procure a copy of Künstler's paper on *Trichomonas vaginalis* in which he states: 'Le *Trichomonas vaginalis* présente quatre flagellums antérieurs.' In this country it is almost impossible to obtain specimens of this organism from the vagina, but by good fortune we obtained a few specimens from the urine of one person, and certain of these had undoubtedly four flagella; which facts point to the type of the genus *Trichomonas* as being in reality a *Tetratrichomonas*, unless there are two different parasites in the vagina, viz., a *Trichomonas* and a *Tetratrichomonas*.

examinations which we have made during the last four years. We have so far only once met with *T. vaginalis* in the Sudan.

GENUS 3. *Pyramimonas* Schmarda 1849:—This genus was founded by Schmarda for *P. tetrarhynchus* of which drawings are given by Stein who called it *Pyramidomonas*.

It was placed in an appendix to the Chlamydomonadidae by Bütschli, while Doflein classifies it in Dangeard's Polyblepharididae, which belongs to Blochmann's order *Phytomonadina*. It can therefore be excluded from the *Tetramitidae*.

GENUS 4. *Tetramitus* Perty 1852. This genus is of importance because it is the type of the family which we are studying, and because *considerable confusion* has arisen not merely in medical but also in zoological literature as to its exact definition.

The history of the genus has, however, already been described in the section dealing with the history of *Chilomastix mesnili*, while its definition will be given later on in this paper, and therefore we merely draw attention to what is known with regard to the life history.

Perty described binary fission, and Dallinger and Drysdale confirmed the binary division mentioned by Perty, and also reported sexual conjugation followed by encystment and minute spore formation from which the young animalcules arose.

GENUS 5. *Callodictyon* Carter 1865. There is a great deal of confusion about this genus. In the first place, German authors spell it *Collodictyon*, which appears to be wrong. In the second place, it was established for *C. triciliatum*, which according to Bütschli is the same organism as that described by Stein as *Tetramitus sulcatus*. Klebs disagrees with this, but only on the ground that *T. sulcatus* has four and not three flagella, while Carter might easily have missed one flagellum.

Blochmann considers that they are one and the same species, and it must be admitted that the representations of the two species admit of this explanation.

Blochmann defines the genus as follows:—

‘Colourless, anteriorly broad, posteriorly broad or drawn out finely, with a deep ventral longitudinal furrow, and four anteriorly springing flagella.’

To which may be added that two of the flagella are longer than the other two.

It obviously belongs to the *Tetramitidae*.

GENUS 6. *Tetraselmis* Stein 1878:—The only species belonging to this genus was described by Carter in 1858 as *Cryptoglana cordiformis*, but Stein considered that it should form a separate genus, hence the origin of *Tetraselmis*; but Diesing had already created the genus *Carteria* in 1865 for this species; therefore *Tetraselmis* becomes merely a synonym of *Carteria*. The animalcule possesses green endoplasm and is classified by Blochmann in the family *Chlamydomonadina*, which belongs to his Order *Phytomonadina*, and is therefore excluded from the Tetramitidae.

GENUS 7. *Monocercomonas* Grassi 1879. This genus was subdivided by its author into four sub-genera, which, thanks to Stiles' valuable researches, are easy to understand. They are:—

(a) *Monocercomonas*. Stiles has shown that the type of this sub-genus must be *M. coronellae*, found parasitic in *Coronella austriaca*, and it is the only species left, as Grassi himself eliminated five out of his seven species, leaving only *M. hominis*, which is recognised to-day as a *Trichomonas*, and *M. coronellae*. Doflein, however, considers that this latter species is the same as Dobell's *Trichomastix serpentis*.

(b) *Trichomonas*. We have already considered this genus.

(c) *Retortamonas*. According to Stiles, this is the same as Grassi's genus *Plagiomonas*. The type and only species is *R. gryllotalpae* Grassi 1879. Doflein considers this sub-genus to be a *Bodo*, and certainly, judging by Grassi's figures, it does not appear to belong to the Tetramitidae.

(d) *Schedocercomonas*. This sub-genus originally included four species, of which *S. gryllotalpae* and *S. melolonthae* were transferred by their author in 1881 to *Monocercomonas* under the single name *M. insectorum* Grassi 1881. The third species *S. caviae*, found in the guinea-pig, is unrecognisable, as its diagnosis rests upon the words 'quasi senza corda'; moreover Stiles was unable to trace a reference to it later than its origin in 1879, and therefore it may be eliminated. The fourth species is the well-known *Herpetomonas muscaedomesticae*.

Thus all the species of Grassi's sub-genera disappear, and the genus *Monocercomonas* Grassi 1879 is left with only two species, viz., *M. gryllotalpae* and *M. melolonthae*, which are considered by their author to be identical, and, of the two, priority is given to the former, which is believed to be the same organism as *M. cetoniae* Jollos 1911 but different from *M. bufonis* Dobell 1909. It has been carefully studied by Mackinnon, and will be defined later.

A flagellate has been found by Chatterjee in man in choleraic and diarrhoeal motions which he refers to this genus, but there is no axostyle depicted in his drawings, and it is uncertain to what genus it belongs.

GENUS 8. *Trimastix* Saville Kent 1880. This genus was created for *T. marina* Saville Kent 1880 found in salt water, which is the only and hence the type species.

It possesses three flagella, one of which is directed anteriorly and two posteriorly, of which one is free while the other is retained in the angle or depression formed between the body and a membranous non-vibratile expansion of the right lateral border.

No cytostome was observed, but the angle or depression mentioned above is in all probability this organella, though this cannot be stated with any certainty as the organism has never been investigated by modern methods.

It is peculiarly interesting from our present point of view, as it is a free living organism with a flagellum retained in part of its course in what is apparently a cytostomic depression.

GENUS 9. *Dallengeria* Saville Kent 1880. It was created by Saville Kent in 1880 for the free living species *D. drysdali* Saville Kent 1880, and is so remarkably like genus (13) *Elvirea* created by Parona in 1887 for a parasitic species found in the intestine of *Ciona intestinalis* Linnaeus, that it seems to us unnecessary to differentiate these genera, although the species may be different, until they are examined by modern methods, especially as the former was found in animal macerations.

GENUS 10. *Cimaenomonas* Grassi 1881 was instituted mainly for *C. batrachorum* (Perty 1852) which was considered by Perty and others to be a *Trichomonas*, to which genus Grassi's drawings, according to Stiles, clearly show that it belongs. *Cimaenomonas* therefore becomes a synonym of Donn's *Trichomonas*.

GENUS 11. *Polymastix* Btschli 1884. This genus was created by Btschli for the organism named *Trichomonas melolonthae* by Grassi in 1882. It has been carefully re-examined by Hamburger (1911) and by Mackinnon (1913), and is now known to possess four flagella arranged in two groups, each belonging to a separate blepharoplast between which lies a cytostome. A vesicular nucleus lies behind the blepharoplast, from which an axostyle arises. The

characteristic feature of the genus is the peculiar condition of the periplast, which is raised into numerous longitudinal ribs or folds.

Unfortunately Künstler stated that *P. melolonthae* possessed six flagella. This naturally led to much confusion until Alexeieff formed the genus *Hexamastix* as will be mentioned below.

GENUS 12. *Trichomastix* Blochmann 1884. This genus was created by Blochmann for the parasite known as *Trichomonas lacertae* Bütschli, because its fourth flagellum, instead of forming an undulating membrane as in the case of a typical *Trichomonas*, remains free as a trailing flagellum.

Unfortunately it is necessary to alter this generic name, because it was originally introduced by Svellen van Vollenhoven in 1878 for a genus of the Hymenoptera with a type species *Trichomastix polita* v. Vollenhoven 1878, and as such was published on page 160 of Volume XXI of the *Tijdschrift voor Entomologie*, together with an illustration of the insect on Plate 9, fig. 4.

Raillet (1893) spelt the name *Trichomastyx*, but this slight change in spelling is not sufficient alteration to hold good.

We had written in this manuscript what we considered to be a suitable name when we received Kofoid and Swezy's paper, in which they suggest the name *Eutrichomastix*. This is a sufficient alteration and must stand, though we would have preferred some such term as *Axomastix*, thus making a greater difference in the nomenclature between the Hymenopteron and the Protozoon.

Besides the type, quite a number of species are known to belong to this genus, thanks to the labours of Dobell, Mackinnon, Martin and Robertson, Alexeieff and Parisi. We have met with several species, some probably new, in various animals in the Anglo-Egyptian Sudan.

GENUS 13. *Elvirea* Parona 1887 :—As already stated above we believe that this genus should be absorbed into genus (9) *Dallengeria*.

GENUS 14. *Costia* Leclerq 1890 :—Leclerq formed this genus for the organism known as *Bodo necator* Henneguy 1884. Leclerq's name cannot stand, as the same word was used by Kirchner (1867) for a Hymenopteron. In this instance a change of name is most useful, because Senn (1900) instituted the genus *Costiopsis* for the parasite called by Weltner (1894) *Tetramitus nitschei*, which Moroff's (1903) researches proved to be the same as *Costia necatrix* synonym *Bodo necator*. Therefore the name *Costia* is excluded.

GENUS 15. *Costiopsis* Senn 1900. As just explained above, this name takes the place of *Costia* Leclerq. Unfortunately, situate in Khartoum in time of war, we have been unable to refer to the original papers of Hennequy, Leclerq, Nitsche and Weltner and of Moroff, and have obtained our knowledge of these organisms from Neresheimer's work and the writings of Senn.

The principal characters of the genus are that the body is dorso-ventrally flattened and asymmetrical, and that the true morphological anterior end is about the middle of the left lateral border. On the ventral surface there is a depression which serves as a suckorial disc which becomes funnel-shaped in front, and in this the cytostome lies, near the morphological anterior end, where also arise the four flagella, of which two are thick, long and trailing, while the other two are fine and short and remain in the mouth cavity. In the middle of the body there is a vesicular nucleus with a contractile vacuole on either side. There is no undulating membrane and an axostyle is not mentioned, and is therefore assumed to be absent.

The organisms can swim about in water, but are essentially ecto-parasites of the skin of young gold fish, carp, &c.

GENUS 16. *Devescovina* Foà 1905. The Monozoa by means of Da Fonseca's family *Callimastigidae* appear to be very closely related to Leidy's family *Trichonymphidae*, but, be this as it may, we quite agree with Kofoid and Swezy that the genus *Devescovina* should be removed from this family and should be included within the *Tetramitidae* near *Eutrichomastix*, and we note that Janicki (1915) appears to have done this in a paper which we have been unable to see.

The genus *Devescovina* was created by Foà (1905) for a flagellate organism which she obtained from the Neuropteran *Calotermes castaneus* Burmeister found in Honolulu.

The parasite, which is more or less oval in form, possesses three equal anterior flagella which spring from an anteriorly situate blepharoplast. There is also a long trailing flagellum, an anteriorly situate nucleus, and a well marked axostyle, around the anterior portion of which the thick parabasal is wound.

It would appear from Kofoid and Swezy's paper that Janicki (1915) probably created a new genus *Foaina* which is classified in the Polymastigina, i.e., in our

definition of the *Protomonadina*, but we have been unable to obtain any references to this genus and therefore cannot say whether it is or is not classifiable in the Tetramitidae.

GENUS 17. *Macrostoma* Alexeieff 1909. The subject of this genus has already been discussed in the section dealing with the history of *C. mesnili*, and it will suffice to remind the reader that the original use of the word was *Macrostoma* Latreille 1825 for a mollusc, the second *Macrostoma* Risso 1826 for a fish, and the third *Macrostoma* Agassiz 1839 for a fish, so that Alexeieff's name was at least the fourth use of the same word for an animal.

GENUS 18. *Trimitus* Alexeieff 1910 :—'This genus was created by Alexeieff for a flagellate organism which he called *T. motellae*. It is described as having two anterior flagella and one posterior which traverses the body. We have been unable to refer to Alexeieff's original paper with regard to this parasite, but da Fonseca seems to regard it as of doubtful value, and if the above are its only characters we fail to see any essential difference between it and genus 8, *Trimastix* Saville Kent 1880 and therefore temporarily, until it is further studied, exclude it from our list.

GENUS 19. *Tetratrichomonas* Parisi 1910. Alexeieff (1909) found a trichomonas-like parasite in the terminal intestine of *Salamandra maculosa*, *Triton cristatus* and *Alytes obstetricans*, and subsequently in *Haemopsis sanguisuga*. This parasite, measuring 10 to 14 by 4 to 7 microns, possessed four free unequal anterior flagella and an undulating membrane thrown into long loose folds, as well as a thinnish axostyle and a nucleus rich in chromatin and bounded by a very definite membrane. He named this organism *Trichomonas prowazeki*, but as it possesses four and not three anterior unequal flagella, Parisi created a new genus with it as the type.

GENUS 20. *Chilomastix* Alexeieff 1910. Though Alexeieff started the existence of this genus in 1910 without any description, and indeed without carefully differentiating it from Tetramitus, still he rectified this in 1911, from which date the history of the genus really begins. It seems to us that the organisms to be comprised in it vary in shape but are often more or less pyriform, and that they possess three anterior flagella which are more or less equal and which spring from one, two or three anteriorly situate blepharoplasts, behind which lies the anteriorly situate nucleus. The cytostome is large and has lips possessing marginal chromatic thickenings which spring from the blepharoplasts, of which the one in the nuclear lip

is of the nature of a flagellum and can pull that side of the mouth outwards, thus forming the homologue of an undulating membrane. The other lip thickening, which is larger than the nuclear, appears to us to be morphologically a parabasal. There is no axostyle. The type species is *Chilomastix caulleryi* (Alexeieff, 1909).

GENUS 21. *Fanapepea* Prowazek 1911 :—In the historical section of this paper with regard to *Chilomastix mesnili* we have a sufficient account of this genus, of which the type *Fanapepea intestinalis* Prowazek 1911 is, in our opinion, identical with *C. mesnili*.

GENUS 22. *Embadomonas* Mackinnon 1911. This genus was formed by Mackinnon for a parasite which she found in the intestine of a trichopterous larva. Her latest (1916) definition of the genus, but slightly modified, is as follows :

'Small, twisted, slipper-shaped flagellates characterised by a very large cytostome with prominent more or less siderophilous lips, two flagella one anterior and one cytostomic arising from two blēpharoplasts situate at the anterior border of the cytostome, an anteriorly placed spherical nucleus and a definite periplast.'

As will be noted below, this definition covers the characters described by Wenyon and O'Connor in 1917 for the genus *Waskia*, which therefore becomes a synonym of *Embadomonas*.

The type species is *E. agilis* Mackinnon 1911, found in the intestine of trichopterous larvae and larvae of *Tipula*. The other known species are *E. alexeieffi* Mackinnon 1912, with prominent markedly siderophilous cytostomic lips, found in the intestine of larvae of *Tipula*, and *E. intestinalis* (Wenyon and O'Connor, 1917) synonym *Waskia intestinalis* Wenyon and O'Connor 1917 found in man at Alexandria.

We agree with Mackinnon that this genus is closely related to *Chilomastix*.

GENUS 23. *Protrichomonas* Alexeieff 1911. This genus was created in 1911 by Alexeieff for a parasite which he found in the oesophagus of *Box salpa*, which he called *P. legeri*, and which possessed an axostyle and three anterior flagella but no undulating membrane.

GENUS 24. *Hexamastix* Alexeieff 1912. *Hexamastix* was instituted by Alexeieff for a species of flagellate which he described in 1909 as being present in the terminal intestine of species of *Triton taeniatus* from the forest of Senart. This parasite resembled a *Trichomastix* in not possessing an undulating membrane, but differed therefrom in having six anterior flagella which were very often united in a large portion of their length. The nucleus, which contained but little chromatin, was placed anteriorly.

No name was given to this parasite, nor is there any illustration in the *Comptes Rendus de la Société de Biologie* of the Meeting of the 17th July, 1909.

Alexeieff's description resembles that of a *Trichomonas* found by Künstler in 1882 in the intestine of larvae of *Melolontha vulgaris*. It also had six anterior flagella and a mouth opening just behind the flagella. Bütschli placed Künstler's parasite in his new genus *Polymastix*, but he noted the difference between Grassi's and Künstler's description of the periplast.

Alexeieff (1911) again referred to this parasite, giving illustrations and pointing out that it possessed an axostyle like that of a *Trichomonas* and calling it *Polymastix batrachorum*.

Mackinnon (1912), in writing on *Polymastix melolonthae*, refers to this parasite in the following terms:—

'The flagellate with six flagella and *without striated periplast* placed provisionally by Alexeieff (1911) in this genus as *P. bufonis* seems to me a very doubtful species.'

We give this quotation in full, as it is the only reference which we possess to the name *Polymastix bufonis*.

In the same year Alexeieff instituted the new genus *Hexamastix* for the parasite known as *Polymastix batrachorum* Alexeieff 1911, of which *P. bufonis* Mackinnon 1912 must be a synonym.

The name of the parasite therefore becomes *Hexamastix batrachorum* (Alexeieff, 1911), and is at present the only species of the genus. It has an axostyle, a cytostome, and six anterior flagella, but no undulating membrane or trailing flagellum and no thickenings in its periplast.

GENUS 25. *Difämus* Gäbel, 1914:—This genus was formed for *Difämus tunensis* Gäbel 1914, a flagellate found in man and possessing all the characteristics of a species of the genus *Chilomastix* and not distinguishable from *C. mesnili*, of which it becomes a synonym.

GENUS 26. *Cyathomastix* Prowazek and Werner 1914. *Cyathomastix* owes its existence to the fact that Rodenwaldt (1911) described a human parasite which, though in general appearance like a *Trichomonas*, yet differed therefrom in that the undulating membrane was merely represented by an undulating peristome. This undulating margin, taking its origin from one blepharoplast, runs around the cytostomic opening and ends in the second blepharoplast. The three anterior flagella all arise from one blepharoplast along with one of the ends of the thickened cytostomic margin.

To this parasite Prowazek and Werner gave the name *Cyathomastix hominis*, and, at present, it forms the sole species of this genus. It only differs from *Chilomastix* in possessing an axostyle, and perhaps a mistake has been made owing to the line produced by the peculiar twist at times found in *C. mesnili*. At all events confirmation of its existence is required. At present we consider it to be the same as *C. mesnili*.

GENUS 27. *Hexamastix* Derrieu and Raynaud 1914:—*Hexamastix* was formed by these authors for a flagellate which they found in a case of chronic dysentery occurring in a man living at Oued-el-Alleng in Algeria. It closely resembled a *Trichomonas* in structure except that it possessed five anterior flagella. This parasite they named *H. ardindelteili*, but we have just noted above that the generic name *Hexamastix* has been used by Alexeieff for quite a different organism, and therefore Derrieu and Raynaud's parasite is left without a generic name, but this was altered by Chatterjee in the succeeding year in his new genus to which we will refer below.

GENUS 28. *Tetratrichomastix* Mackinnon 1914. This genus was formed by Mackinnon for *Eutrichomastix*-like parasites found along with *Eutrichomastix trichopterae* (Mackinnon 1910) in the alimentary canal of larvae belonging to the genus *Tipula*. The only difference between *Eutrichomastix* and *Tetratrichomastix* is that the former has three and the latter four anterior flagella.

The type species is *Tetratrichomastix parisii* Mackinnon 1914, apparently named after Parisi because he is of the opinion that the number and method of attachment of flagella are of real importance in classification.

GENUS 29. *Pentatrichomonas* Chatterjee 1915. Chatterjee instituted this genus for a flagellate which he found in the intestine of man in Bengal, and which at the time he called *P. bengalensis*. This organism agrees in most particulars except measurement with

that mentioned above under the heading *Hexamastix* Derrieu and Raynaud, and therefore Chatterjee's generic name takes the place of this *Hexamastix*, but Derrieu and Raynaud's specific name has priority, and the parasite becomes known as *Pentatrichomonas ardindeiteili* (Derrieu and Raynaud, 1914) until some definite difference between the two is established.

More recently Chatterjee states that he has found this organism to be present in thirty-two cases of chronic dysentery.

GENUS 30. *Enteromonas* da Fonseca 1915. This genus contains one parasite, *E. hominis* da Fonseca 1915, found in the fresh faeces of a dysenteric patient in Brazil.

According to Fantham's review, *E. hominis* is a spherical or more rarely pear-shaped organism (i.e. with a tail) with a diameter varying from 5 to 6 microns, and possessing a rigid periplast which encloses an alveolar endoplasm, often with inclusions such as bacteria. In the endoplasm and lying close to the anterior end of the body is the nucleus, measuring 1 micron in diameter, and being of the nature of a protokaryon with a karyosome. Anterior to the nucleus and united thereto by a rhizoplast lies the single blepharoplast, from which three flagella arise, two of which are small and anterior while one is large and trailing.

There is no axostyle and no cytostome, while division by longitudinal fission is recorded. Da Fonseca's paper is not illustrated.

More recently da Fonseca has defined the genus and given illustrations of *E. hominis*, but this definition will be given below.

GENUS 31. *Chilomitrus* da Fonseca 1915. Da Fonseca (1915) found a flagellate in the caecum in *Cavia aperea* Linnaeus 1766 (the wild ancestor of the domesticated *Cavia cobaya* Marcgrave 1648), and in *C. porcella* Linnaeus 1791 (Gmelin) which was characterised by possessing four equal anterior flagella and an anteriorly situate cytostome in close proximity to which lay the blepharoplast from which the flagella arose, and which appeared to pass out through the mouth. The blepharoplast is said to be joined to the anteriorly situate nucleus by means of a rhizoplast. There is no axostyle and no undulating membrane. The type species is *C. caviae* da Fonseca 1915.

We believe that there is no real difference between this genus and the next to be described below, viz., *Tetrachilomastix* da Fonseca 1915, because:—

1. Martin and Robertson (1912) figured the four flagella of their *Chilomastix* as escaping through the cytostomic opening in fig. 3 of their Plate 10, and also, but less markedly, in fig. 4, and partially in some other figures.
2. Da Fonseca says nothing about the siderophilous thickening of the cytostomic lips, but such a thickening is depicted in his fig. 11.
3. The general descriptions of both genera agree.

GENUS 32. *Tetrachilomastix* da Fonseca 1915. This is the name given by da Fonseca to the parasite described by Martin and Robertson (1911) as *Chilomastix gallinarum*, because it has four anterior flagella and not three.

By the kindness of Miss Robertson we have been able to examine some of the original slides of this type from the fowl, and agree with da Fonseca that it is worthy of being placed in a separate genus from *Chilomastix*, but we, *unfortunately*, believe that it is the same genus as that observer's *Chilomitus*, and we are not certain which name has priority, because his 1915 papers, viz., his thesis, his paper in the *Brazil Medico* of April 2nd and of September 22nd, are not available for us. In his 1916 paper he gives *Tetrachilomastix* as a sub-genus of *Chilomastix* on page 10, and *Chilomitus* as a genus on page 11.

It is therefore evident that he created *Chilomitus* as a proper genus, and *Tetrachilomastix* only as a sub-genus, but, as far as rules go, this makes no difference, and after considering matters we prefer at present to retain his name *Tetrachilomastix*, of which *Chilomitus* becomes a synonym.

GENUS 33. *Eutrichomastix* Kofoid and Swezy 1915. This is the new name proposed by Kofoid and Swezy for the genus *Trichomastix*, which name was already in use, as explained above, when suggested by Blochmann in 1884 for Bütschli's *Trichomonas lacertae*.

GENUS 34. *Waskia* Wenyon and O'Connor 1917. This genus was created in March 1917 by Wenyon and O'Connor for a small flagellate which they found in man in Alexandria, and which they named *W. intestinalis* Wenyon and O'Connor 1917.

This flagellate is oval in form, being 4 to 9 by 3 to 4 or more microns, with the anterior end rounded and the posterior end pointed, and with a cytostome at the side of the anterior end. A long thin flagellum springs from the anterior end of the body, while a second stouter and shorter flagellum arises from the inner part of the anterior wall of the cytostome, and passing backwards and outwards projects from this organella for a considerable distance. The nucleus with a central karyosome and a spherical nuclear membrane occupies the anterior end of the body. On the nuclear membrane and towards the cytostome lie two granules from which the flagella arise.

Stages of binary fission have been observed and cyst formation. The cysts are pear-shaped bodies, 4.5 to 6 microns in length.

The name *Waskia* was derived from the Orwa-el-Waska section of the 19th General Hospital in Alexandria.

This description must be compared with Mackinnon's definition of the genus *Embadomonas* given above.

After reading this, and comparing the illustrations, we are of the opinion that the genera are the same but the species may be different. *Waskia intestinalis* therefore becomes *Embadomonas intestinalis* (Wenyon and O'Connor 1917).

X. GENERA

After considering the various points raised above it will be observed that the following genera can be excluded from the family *Tetramitidae*, either because they belong to other families or because their names become synonyms of other genera. The genera which may be so excluded are:—

1. Chloraster; 3. Pyramimonas; 7. all the sub-genera;
10. Cimaenomonas; 13. Elvirea; 14. Costia; 17. Macrostoma;
18. Trimitus; 21. Fanapepea; 25. Difamus; 26. Cyatho-

mastix; 27. Hexamastix; 31. Chilomitus; 34. Waskia; while 12. Trichomastix disappears and becomes 33. Eutrichomastix.

After excluding the above, the family is left with nineteen genera, which may be defined as follows:—

GENUS 1. *Trichomonas* Donné 1837

Parasitic Tetramitidae possessing an axostyle, a small cytostome without thickened lips, three anterior flagella, and one posterior flagellum, which being attached to the body of the organism for a portion of its length gives rise to a well defined undulating membrane, supported by a chromatic rod or parabasal, and then ends freely. There is no contractile vacuole, and the nucleus is situate anteriorly. *Type species*: *T. vaginalis* Donné 1837, found in *Homo sapiens*.

GENUS 2. *Tetramitus* Perty 1852

Free living Tetramitidae without an axostyle (as far as is known) and with a large cytostome, with three anterior and one posterior free trailing flagellum, but without an undulating membrane. Contractile vacuole present. *Type species*: *T. descissus* Perty 1852, found in water from swamps.

GENUS 3. *Callodictyon* Carter 1865

Free living Tetramitidae with four unequal anterior flagella, and a deep ventral longitudinal furrow. *Type species*: *C. triciliatum* Carter 1865 synonym *Tetramitus sulcatus* Stein 1878.

GENUS 4. *Monocercomonas* Grassi 1879

Parasitic Tetramitidae with an axostyle but without a cytostome, and with four anterior flagella. *Type species*: *M. gryllotalpae* (Grassi 1879), from the larvae of insects.

GENUS 5. *Trimastix* Saville Kent 1880

Tetramitidae, perhaps parasitic, perhaps free living, without an axostyle (as far as is known), with one anterior, one free trailing and one cytostomic, becoming a free, posterior flagellum. A contractile vacuole is present. *Type species*: *T. marina* Saville Kent 1880, found in a vessel of seawater containing Fuci in an advanced state of decomposition.

GENUS 6. *Dallengeria* Saville Kent 1880

Probably parasitic Tetramitidae without known axostyle, and with one anterior and two trailing flagella, the latter arising laterally. No known contractile vacuole. *Type species*: *D. drysdali* Saville Kent 1880, found in animal macerations.

GENUS 7. *Polymastix* Bütschli 1884

Parasitic Tetramitidae with an axostyle, a small cytostome, four anterior flagella, and with the periplast thickened in places by ribs or folds. *Type species*: *P. melolonthae* Grassi 1882, found in the intestine of *Melolontha*, *Cetonia* and *Tipula*.

GENUS 8. *Costiopsis* Senn 1900

Ecto-parasitic Tetramitidae with dorso-ventrally flattened bodies and with a sucker-like depression on the ventral surface and an oval body, of which the true anterior end is towards the middle of the left lateral border, being marked by the origin of the flagella. Without an axostyle but with a cytostome and four flagella, two of which are long, thick and trailing, while the other two are thin and short and lie in the mouth depression or sucker. Contractile vacuoles are present. *Type species*: *C. necatrix* (Henneguy 1884) with numerous synonyms.

GENUS 9. *Devescovina* Foà 1905

Parasitic Tetramitidae with an axostyle, three anterior flagella and one trailing flagellum, while the thick parabasal is wound round the anterior part of the axostyle. *Type species*: *D. striata* Foà 1905, found in *Calotermes castaneus* Burmeister in Honolulu.

GENUS 10. *Tetratrichomonas* Parisi 1910

Parasitic Tetramitidae resembling *Trichomonas* but possessing four anterior flagella, two long and two short. *Type species*: *T. prowazeki* (Alexeieff 1909), found in Salamanders and Tritons.

GENUS 11. *Chilomastix* Alexeieff 1911

Parasitic Tetramitidae without an axostyle but with a large cytostome, the lips of which are thickened on the side of the nucleus by a flagellum which can give rise to a short membrane.

There are three anterior flagella and no contractile vacuole. *Type species*: *C. caulleryi* (Alexeieff 1909), found in tadpoles and in an axolotl.

GENUS 12. *Embadomonas* Mackinnon 1911

Parasitic Tetramitidae without an axostyle, but with a large cytostome with deeply staining border often thrown into folds, and one anterior and one posterior flagellum which generally lies in the cytostome. Periplast definite. *Type species*: *E. agilis* Mackinnon 1911, found in the intestine of larvae of Trichoptera and Tipula.

GENUS 13. *Protrichomonas* Alexeieff 1911

Parasitic Tetramitidae with an axostyle and three anterior flagella, but without a cytostome and without an undulating membrane. *Type species*: *P. legeri* (Alexeieff 1910), from the oesophagus of *Box salpa*.

GENUS 14. *Hexamastix* Alexeieff 1912

Parasitic Tetramitidae with an axostyle and six anterior flagella, but without an undulating membrane or trailing flagellum or thickened periplast. *Type species*: *T. batrachorum* (Alexeieff 1911), found in the intestine of *Triton taeniatatus*.

GENUS 15. *Tetratrichomastix* Mackinnon 1914

Parasitic Tetramitidae resembling *Eutrichomastix*, but with four anterior flagella. *Type species*: *T. parisii* Mackinnon 1914, found in the intestine of the larvae of Tipula.

GENUS 16. *Pentatrichomonas* Chatterjee 1915

Parasitic Tetramitidae resembling *Trichomonas*, but possessing five anterior flagella. *Type species*: *P. ardindefteili* (Derrieu and Raynaud 1914), found in the intestine of man in Africa and India.

GENUS 17. *Enteromonas* da Fonseca 1915

Parasitic Tetramitidae without axostyle or cytostome, but with two anterior and one free trailing flagellum. *Type species*: *E. hominis* da Fonseca 1915, found in a case of dysentery in man in Brazil.

GENUS 18. *Tetrachilomastix* da Fonseca 1915

Parasitic Tetramitidae resembling *Chilomastix*, but with four anterior flagella. *Type species*: *T. gallinarum* (Martin and Robertson 1912), found in the caeca of fowls.

GENUS 19. *Eutrichomastix* Kofoid and Swezy 1915

Parasitic Tetramitidae with an axostyle and a cytostome, without thickened lips and with three anterior and one free trailing flagellum, but without an undulating membrane or contractile vacuole. *Type species*: *Eutrichomastix lacertae* (Blochmann 1884), found in the intestine of *Lacerta agilis*.

XI. AFFINITIES

Our views of the possible phylogenetic relationships of these genera can be gathered from the diagram given below. It will be observed that we take as the ancestor of the family a flagellate with three anterior flagella, no axostyle, no cytostome and no posterior flagellum, and we believe that the genera varied first according to the development or non-development of an axostyle and then of a mouth and later of a free trailing flagellum, while finally both sides developed an undulating membrane; but in the case of those without an axostyle and with a large cytostome this membrane became cytostomic, while in the other it was non-cytostomic. In other words, if the cytostome is considered as indicating the ventral portion of the body, then in the one case the membrane is ventral and in the other it is dorsal.

XII. SUB-FAMILIES

It is quite evident that, if these views as to the possible relationship of the genera are accepted, they indicate that the family *Tetramitidae* can be sub-divided into two sub-families as follows:—

A. Tetramitidae without an axostyle	...	Sub-family I
		<i>Tetramitidinae</i>
		Chalmers and Pekkola 1917
B. Tetramitidae with an axostyle		Sub-family II
		<i>Trichomonadinae</i>
		Chalmers and Pekkola 1917

POSSIBLE AFFINITIES OF THE GENERA OF THE TETRAMITIDAE

XIII. TETRAMITIDINAE

Further it is obvious that the parasite we are considering belongs to the Tetramitidinae, and that the genera of this sub-family are:—

1. *Tetramitus* Perty 1852.
2. *Callodictyon* Carter 1865.
3. *Trimastix* Saville Kent 1880.
4. *Dallengeria* Saville Kent 1880.
5. *Costiopsis* Senn 1900.
6. *Chilomastix* Alexeieff 1911.
7. *Embadomonas* Mackinnon 1911.
8. *Enteromonas* da Fonseca 1915.
9. *Tetrachilomastix* da Fonseca 1915.

These nine genera can be differentiated as follows:—

- A. *Cytostome absent or unknown* :—
 - I. Two anterior and one trailing flagellum ... 1. *Enteromonas*
 - II. One anterior and two trailing flagella 2. *Dallengeria*
- B. *Cytostome present* :—
 - I. *Trailing flagellum is free* :—
 - a. Body dorso-ventrally compressed and asymmetrical, ventral surface with deep depression which serves as a sucker and contains the cytostome and two short fine flagella. The two thick long trailing flagella issue from this depression ... 3. *Costiopsis*
 - b. Body more or less symmetrical and not compressed and arranged as above :—
 1. One anterior flagellum, one said to be trailing and free, and one posteriorly directed through the body from which it escapes as a free flagellum 4. *Trimastix*
 2. Flagella, three anterior and one trailing and free ... 5. *Tetramitus*
 - II. *Free trailing flagellum absent* :—
 - a. Chromatic thickenings in the lips of cytostome. One of these thickenings is either known or suspected to be formed by a flagellum
 1. One anterior and one free posterior flagellum which generally lies in the cytostome ... 6. *Embadomonas*
 2. Three anterior flagella ... 7. *Chilomastix*
 3. Four anterior flagella ... 8. *Tetrachilomastix*
 - b. Chromatic thickenings not known to be present in lips of cytostome
 - Four anterior flagella ... 9. *Callodictyon*

XIV. GENUS CHILOMASTIX

As the parasite we are considering has a large cytostome, the lips of which show chromatic thickenings, one of which is a flagellum, and as it has three anterior and no free trailing flagellum, it is obvious that it belongs to the genus *Chilomastix* Alexeieff 1911.

The synonyms of *Chilomastix* are :—

1. *Cercomonas* Davaine 1880 pro parte nec Dujardin 1841.
2. *Trichomonas* Roos 1893 nec Donné 1837.
3. *Monocercomonas* Epstein 1893 nec Grassi 1879.
4. *Macrostoma* Alexeieff 1909 nec Latreille 1825.
5. *Fanaepea* Prowazek 1911.
6. *Tetramitus* Brumpt 1912 nec Perty 1852.
7. *Difämus* Gäbel 1914.
8. *Cyathomastix* Prowazek and Werner 1914.

XV. CHILOMASTIX SPECIES

The species of *Chilomastix* known to us are :—

1. *C. caulleryi* (Alexeieff 1909) synonym *Macrostoma caulleryi* Alexeieff 1909, found in the intestine of tadpoles.
2. *C. mesnili* (Wenyon 1910), synonyms :—
 - (a) *Cercomonas hominis* Davaine 1869 pro parte.
 - (b) *Monocercomonas hominis* Epstein 1893 nec Grassi 1879.
 - (c) *Trichomonas intestinalis* Roos 1893 pro parte nec Leuckart 1879.
 - (d) *Macrostoma mesnili* Wenyon 1910.
 - (e) *Fanaepea intestinalis* Prowazek 1911.
 - (f) *Tetramitus mesnili* Brumpt 1912.
 - (g) *Difämus tunensis* Gäbel 1914.
 - (h) *Cyathomastix hominis* Prowazek and Werner 1914.
 - (i) *Tetramitus bocis* Brumpt 1912.
 - (k) *Chilomastix intestinalis* Kuczynski 1914.
3. *C. motellae* Alexeieff 1912. The only reference which we possess as to this parasite is its name, which is contained in *Bulletin de l'Institut Pasteur* for 1912, page 847, and in da Fonseca's paper in 1916. It is not included in the list of species given by

Mackinnon (1916), and as we have been unable to obtain Alexeieff's original paper, we are unable to give a single *specific* character.

It seems to have been discovered in the intestine of a species of the genus *Motella* Cuvier, possibly *M. tricirrata*, but we are not certain about this.

4. *C. bocis* (Brumpt 1912) synonym *Tetramitus bocis* Brumpt 1912, found by Alexeieff in the intestine of *Box salpa* and considered to be identical with *Chilomastix mesnili*. Brumpt, however, says that Alexeieff's illustration is quite different from the human parasite, but he does not say wherein the difference lies, and therefore we retain Alexeieff's original view, that it is the same as *C. bocis*, pending further research.

5. *C. intestinalis* Kuczynski 1914. This parasite was found by Kuczynski in the caecum of *Cavia cobaya* Marcgrave 1648 in Europe, and subsequently by da Fonseca in Brazil. Its length is 13 to 16 microns and its breadth 7 to 9 microns, but as regards its structure as described or illustrated by the authors quoted, we confess to be unable to find any point of sufficient importance to indicate a specific difference between it and *C. mesnili*. The hosts of course are different, but this is not a specific character, and therefore we consider *C. intestinalis* to be a synonym of *C. mesnili*.

6. *C. bittencourti* da Fonseca 1915. This flagellate was found by da Fonseca in the caecum of *Epimys norvegicus* (Erxleben 1777) in Brazil, and we have found a similar parasite in white rats and in the gerbil (*Gerbillus pygargus* F. Cuvier 1838) in the Anglo-Egyptian Sudan.

7. *C. caprae* da Fonseca 1915. This *Chilomastix* was found by da Fonseca in the rumen of *Capra hircus* Linnaeus 1758 in Brazil.

8. *C. cuniculi* da Fonseca 1915. This parasite was found by da Fonseca in Brazil in the caecum of *Oryctolagus cuniculus* (Linnaeus 1758).

As a result of the above considerations we reduce the known species of *Chilomastix* to the following:—

1. *C. caulleryi* (Alexeieff 1909).
2. *C. mesnili* (Wenyon 1910).

3. *C. motellae* Alexeieff 1912.
4. *C. bittencourti* da Fonseca 1915.
5. *C. caprae* da Fonseca 1915.
6. *C. cuniculi* da Fonseca 1915.

We give the following as a provisional diagnostic table :—

A. *Characters known to us :—*

- I. *Size large ; 20-25 microns in length :—*
Flagella easily seen in cysts which resemble
C. mesnili cysts in size and appearance ... 1. *Caulleryi*
- II. *Size medium ; 11-18 microns in length :—*
 - a. Cytostome long ; cysts about 7×5.6 microns in which the anterior flagella are difficult to see ... 2. *Mesnili*
 - b. Cytostome very short ; cysts large, about 8×6 microns, in which the anterior flagella are very distinct ... 3. *Bittencourti*
- III. *Size small ; 7-12 microns in length :—*
 - a. Nucleus with a central karyosome connected to the blepharoplast by a rhizoplast ; size 9-12 microns in length ... 4. *Caprae*
 - b. Nucleus without a central karyosome and without a rhizoplast ; size 7-9 microns in length ... 5. *Cuniculi*

B. *Characters unknown to us :—*

- Found in species of *Motella* ... 6. *Motellae*

XVI. CHILOMASTIX MESNILI

The parasite which we are considering is found in man, measures 11 to 18 microns in length, possesses a large cytostome and has cysts measuring 7 by 5.6 microns in which the anterior flagella, in contradistinction to the posterior or cytostomic flagellum and the parabasal (or siderophilous mouth parts) are difficult to see.

We therefore classify it as *Chilomastix mesnili* (Wenyon 1910), which we define as follows :—

Chilomastix of medium size, with a long cytostome and possessing cysts, measuring 7 by 5.6 microns, in which the traces of the anterior flagella are difficult to discern.

Habitat : *Homo sapiens*. *Baboon*. *Cavia cobaya*. *Box salpa* (?).

This completes our study of the classification of *this common human intestinal flagellate*, and we now turn to the consideration of its possible pathogenicity.

XVII. PATHOGENICITY

Wenyon considers that *C. mesnili* is a harmless parasite of man, and states that it can be recognised in a formed motion by its characteristic cysts. He states that it is present intermittently in the stools, but that his intermittency is not so marked as in the case of other protozoal parasites, and with this we are in agreement. We also believe that an infection can persist for years, but we believe that when the parasite increases in numbers it becomes pathogenic and causes diarrhoea.

We think that Wenyon's views and our own can be brought into agreement in that perhaps he has dealt mostly with *carriers* of the flagellate, while we have met both *carriers* and *patients*, and we use the term carrier in the same sense as when used for the *Bacillus typhosus*.

We further think that any lowered condition of the general health will enable *C. mesnili* to flourish and to cause diarrhoea, but we have never seen it cause dysentery, though, in our opinion, there is no reason why it should not do so.

It is, however, most desirable to distinguish between the pathogenicity of *C. mesnili* and that of any associated parasite, e.g. we certainly accused *C. mesnili* as being the cause of the diarrhoea which was really due to *Octomitus hominis*, which, once recognised and treated, cured the patient. On the other hand, when after prolonged search only a very few specimens of *Löschia coli* can be found and the faeces are swarming with *C. mesnili*, and when treatment for the latter parasite, which resists Emetine, is successful in stopping the diarrhoea, we think that it is the causal agent.

XVIII. TREATMENT

The treatment which we favour is to place the patient on a very restricted diet and to administer purgatives night and morning for several days consecutively, and then to give some intestinal

disinfectant, e.g. Salol. Our idea is to clear away as much of the adherent mucus of the bowel as possible and then to give whatever lethal drug is chosen by the practitioner, and we prefer Salol mixed with bicarbonate of soda. We think that we have been successful with this method of treatment.

XIX. SUMMARY

In this paper we have brought forward such facts as we have been able to observe with regard to the structure and development of *Chilomastix mesnili* (Wenyon 1910), and have endeavoured to illustrate our observations by photomicrographs.

We have also endeavoured to make clear the position of the genus *Chilomastix* as regards the other genera of the *Tetramitidae*, and in so doing have been compelled to review such information as we were able to obtain in Khartoum with regard to these various genera.

We have also attempted to differentiate from one another the known species of *Chilomastix* with the view of finding whether any animal is a carrier of the parasite. Personally, we think that man is the important carrier of *C. mesnili*, and that the infection spreads from man to man by means of the cysts, otherwise it is not possible to explain the world-wide distribution.

Finally we have discussed the pathogenicity of *C. mesnili* infections.

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ADDENDUM

For the purpose of completing our remarks upon the genera of the Tetramitidae we add a diagnostic table of the sub-family *Trichomonadinae*.

DIAGNOSTIC TABLE OF THE *Trichomonadinae*A. *Cytostome absent* :—

I. Three anterior flagella	...	...	...	1. <i>Protrichomonas</i>
II. Four anterior flagella...	...	...	...	2. <i>Monocercomonas</i>

B. *Cytostome present* :—

I. Without undulating membrane :—

a. *Without trailing flagellum* :—

1. Periplast thickened in places; four anterior flagella	...	...	...	3. <i>Polymastix</i>
2. Periplast not thickened; six anterior flagella	...	...	...	4. <i>Hexamastix</i>

b. *With trailing flagellum* :—

1. Three anterior flagella :—

x. Without parabasal	...	...	...	5. <i>Eutrichomastix</i>
y. With parabasal wound around the axostyle	...	...	...	6. <i>Devescovina</i>

2. Four anterior flagella...	...	...	...	7. <i>Tetratrichomastix</i>

II. With undulating membrane :—

1. Three anterior flagella	...	...	...	8. <i>Trichomonas</i>
2. Four anterior flagella	...	...	...	9. <i>Tetratrichomonas</i>
3. Five anterior flagella	...	...	...	10. <i>Pentatrichomonas</i>

KHARTOUM,

June 10th, 1917.

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EXPLANATION OF PLATES

PLATE VI

- FIG. 1. *Chilomastix mesnili* (large form) showing the general appearance and the three anterior flagella. Note the food vacuoles and especially the large vacuole lying in the cytoplasm just internal to the mouth opening. $\times 3,300$ diameters. Photomicrograph.
- FIG. 1a. A well-differentiated nucleus showing the nuclear membrane and the karyosomes. $\times 2,000$ diameters. Photomicrograph.
- FIG. 2. The same photograph as Fig. 1, but printed lightly to show the three blepharoplasts from which the anterior flagella, the parabasal, and the posterior flagellum can be seen arising. $\times 3,300$ diameters. Photomicrograph.
- FIG. 3. *C. mesnili* showing the mouth in an open position and the posterior flagellum quiescent. $\times 3,300$ diameters. Photomicrograph.
- FIG. 4. *C. mesnili* showing the peculiar twist found at times and a widely open mouth. $\times 2,300$ diameters. Photomicrograph.
- FIG. 5. *C. mesnili* showing a widely open mouth with apparently a circumoral siderophilous band running from one of the blepharoplasts to another. $\times 2,000$ diameters. Photomicrograph.
- FIG. 6. *The Cytostome* showing the end of the parabasal on the inner lip, the posterior attachment and the outer movement of the posterior flagellum. $\times 3,300$ diameters. Photomicrograph.
- FIG. 7. *The Cytostome* to show the outward movement of the posterior flagellum and the membrane which it carries with it. $\times 3,300$ diameters. Photomicrograph.
- FIG. 8. *The Cytostome* with membrane and posterior flagellum extending so far outwards that they touch the parabasal and divide the mouth opening into anterior and posterior portions. $\times 3,300$ diameters. Photomicrograph.

Fig. 1

Fig. 1a

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Chilomastix mesnili (WENYON 1910)

PLATE VII

- FIG. 9. *Pear-shaped Form* showing general shape, mouth opening, with posterior flagellum and attached membrane and long caudal appendix. $\times 1,600$ diameters. Photomicrograph.
- FIG. 10. Cytostome and flagella. $\times 1,300$ diameters. Photomicrograph.
- FIG. 11. Shows slight outward movement of the posterior flagellum and its attached membrane and the general arrangement of the parabasal. $\times 1,300$ diameters. Photomicrograph.
- FIG. 12. Posterior flagellum apparently lying loose in the cytostome, probably an artefact. $\times 1,500$ diameters. Photomicrograph.
- FIG. 13. *Rounded Small Form* showing side view of mouth with apparently continuous siderophilous margins. $\times 1,500$ diameters. Photomicrograph.
- FIG. 14. *Rounded Form* showing the three anterior flagella. $\times 3,000$ diameters. Photomicrograph.
- FIG. 15. *Twisted Form*. $\times 1,600$ diameters. Photomicrograph.
- FIG. 16. *Tetratrichomonas gallinarum* (Martin and Robertson 1912) from a stained film kindly lent by Miss Robertson. $\times 2,000$ diameters. Photomicrograph.
- FIG. 17. *Binary Fission*. Early stage. $\times 2,000$ diameters. Photomicrograph.
- FIG. 18. *Binary Fission*. Late stage. $\times 1,000$ diameters. Photomicrograph.
- FIG. 19. *Binary Fission*. Late stage. $\times 1,000$ diameters. Photomicrograph.
- FIG. 20. *Cyst* showing nucleus, blepharoplast, posterior flagellum and parabasal. Note egg-shape and cyst wall. $\times 1,500$ diameters. Photomicrograph.
- FIG. 21. *Cyst* with clearly shown nucleus and parts of the cytostome rim. $\times 1,500$ diameters. Photomicrograph.
- FIG. 22. *Cyst* showing nucleus with large siderophilous mass lying in the cytoplasm. $\times 2,000$ diameters. Photomicrograph.
- FIG. 23. *Cyst* to show typical shape and breaking up, prior to disappearance, of the mouth parts. $\times 1,500$ diameters. Photomicrograph.
- FIG. 24. *Cyst* to show the cyst wall. $\times 1,500$ diameters. Photomicrograph.
- FIG. 25. *Cyst* showing division of nucleus. $\times 1,500$ diameters. Photomicrograph.
- FIG. 26. *Cyst* showing two nuclei somewhat obscured by overlying debris. $\times 2,000$ diameters. Photomicrograph.
- FIG. 27. *Young Form* without mouth. $\times 1,500$ diameters. Photomicrograph.
- FIG. 28. *Older Form* with mouth. $\times 2,000$ diameters. Photomicrograph.
- FIG. 29. *Larger Rounded Form*. $\times 1,000$ diameters. Photomicrograph.

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 19

Fig. 20

Fig. 22

Fig. 24

Fig. 27

Fig. 28

Fig. 21

Fig. 23

Fig. 25

Fig. 26

Fig. 29

Chilomastix mesnili (WENYON 1910)