Observations on Hypotrichous Ciliates:
The Genera *Stichotricha* and *Chaetospira*

BY

JOAN FROUD, M.Sc.

(From the Department of Biology, Wye College, University of London)

CONTENTS

INTRODUCTION

WHILE examining the empty cells of certain pondweeds, I became interested in two hypotrichous ciliates that occurred there with some regularity and often in considerable numbers. The one that drew my attention first was a loricate organism that I identified as a species of *Chaetospira* Lachmann (1856). The other was a non-loricate form which was much less modified for a restricted habitat, and migrated from cell to cell even when adult. This second organism answered to the description of *Stichotricha* Perty (1852), although its morphology did not precisely accord with that of any of the species recognized by Kahl (1932).

Whereas the free-swimming scavenging Hypotricha, such as *Euplotes* and *Stylonychia*, have been exhaustively studied, little has been recorded of the behaviour and reproduction of the cell-inhabiting species, although the restriction on their movements makes them comparatively easy to observe for long periods at a time. The following notes, though fragmentary, may serve to fill some gaps in our knowledge; and they may also help to clear up some taxonomic confusion. After a brief historical survey, I propose to deal first with *Stichotricha*, since it is the more comparable with 'normal' hypotrichs: more precise information as to its structure, life-history, and behaviour undoubtedly helps in the interpretation of the highly modified *Chaetospira*.

HISTORY

The confusion in the nomenclature of these genera is almost impossible to resolve. *Stichotricha* was first described in 1852 by Perty, 'lancelet-shaped with the extensible anterior end narrow, flat, drawn out and carrying the peristome. On one side of this is a row of large obliquely-standing cilia.'

Although his description was so incomplete that the ciliate 'could have been a *Loxodes* or *Amphileptus*' (Lachmann, 1856), Perty's name *Stichotricha secunda* for the type species has been retained. He placed it in the Oxytrichina. Four years later Lachmann (1856) described two species of a new ciliate genus *Chaetospira*, *C. mülleri* and *C. mucicola*, both of them freshwater and loricate. Of this genus he wrote, 'when extended the proboscis (Rüssel) forms more than one turn of a spiral and the first membranelle is longer and stronger than the rest'. He placed *Chaetospira* near *Stentor* but realized that it was related to *Stichotricha*. In 1862 Strethill Wright described, but did not figure, another species of *Chaetospira*, which he named *C. maritima* on account of its marine habitat. A third freshwater species, *C. remex*, was described by Hudson (1875); but this, together with Bolton's *C. cylindrica*, recorded in 1878, was relegated by Savile Kent (1880) to the genus *Stichotricha*. Gruber (1879) had already said that *Stichotricha* and *Chaetospira* were identical, and his *S. urnula*, described in 1883, certainly is very closely related indeed to *Chaetospira* and possibly forms a bridging species between the two genera. Entz (1884) again identified *Chaetospira* and *Stichotricha* and said that his newly described *S. inquilinus* was none other than *C. mülleri*. Möbius (1888) recorded *C. maritima* from the Kiel Canal and figured it. Meanwhile eight other species of *Stichotricha* had been described. Some of these are possibly species of the genus *Chaetospira*.

Another complication was introduced by Sterki (1897), who, while giving the most complete description as yet of *Chaetospira mülleri*, seems not to have known of this genus. He called his ciliate *Spirotricha paradoxa*, and he appreciated the differences between it and *Stichotricha*. Kahl (1932) returned Sterki's *Spirotricha*, together with a variety *S. paradoxa univacuolata* Illowaisky (1913), to the genus *Chaetospira*, species *mülleri*. In the same work Kahl recorded *C. entzi* as another bridging species between *Chaetospira* and *Stichotricha*, the thirteen described species of which he reduced to eight.

MATERIAL AND METHODS

Chaetospira was first found by me on algal encrustations at water level on the sides of a shallow glass jar of pond water in which were growing *Lemna*, *Cladophora*, and *Riccia*. The sources of the material were not recorded. Later collections were made from ponds in or near London. These never yielded cysts as did the first batch, and the *Chaetospira* were always found in the dead cells of *Lemna trisulca* or *Riccia fluitans*. Once they occurred in the root of *Lemna minor* and once in the dead cells of some unrecognizable plant debris.

Stichotricha was found inhabiting the empty cells of leaves of *Lemna trisulca* and roots of *L. minor*. These two species of *Lemna*, collected over a period of 18 months, were kept in tongue jars in the laboratory.

Most of the observations were made on living material and consisted in watching behaviour, division, and, in *Chaetospira*, lorica formation. Certain individuals of this genus could be watched for several weeks; but *Stichotricha*

rarely stayed in the same cell for more than a day. However, this was long enough for all the stages of asexual reproduction to be followed.

In feeding experiments the animals were placed in pond water enriched with bacteria and small undetermined flagellates, or in a carmine suspension.

For cytological purposes *Lemna* leaves or roots having a fair ciliate population were fixed in alcoholic Bouin or Champy, Zenker or Schaudinn and stained with Heidenhain's iron haematoxylin, haematein (after Dobell),
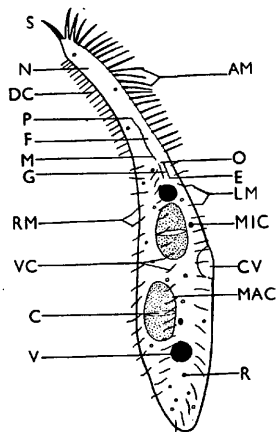
TEXT-FIG. I

Delafield's haematoxylin, or haemalum by the Vicelle method. This method I had direct from Professor Hovasse, who recommended it for demonstrating micronuclei: after fixation wash overnight in tap-water, rinse in distilled water, and stain in a very weak aqueous solution of haemalum for 24 hours. Feulgen following Champy was frequently used.

STICHOTRICHA INTERMEDIA N. SP.

Trophic Phase (Text-fig. 1)

This species, like others of the genus, has a contractile, flexible, spindle-shaped body, drawn out anteriorly into a proboscis (N), usually bent but never spiral, bearing along its left border a row of powerful adoral membranelles (AM). The whole animal is slightly spirally twisted especially when extended in feeding (Text-fig. 2, fig. 1). The terminal membranelle is larger and set apart (S). Ventrally there run three longitudinal rows of cilia curving posteriorly to the right (RM, LM, VC). At the base of the proboscis lies the mouth (O) and to the right of it a hyaline ectoplasmic membrane (M). There are two

macronuclei (MAC), and the contractile vacuole (CV) is dorsal and on the left, roughly half-way along the length of the body.

In the adult of this species, length varies from 40 to 170 μ: when extended about two-fifths is occupied by the proboscis. It is characterized by two rows of dorsal hair-like cilia (DC), all the other species, according to Kahl (1932), having three rows. There are in this species, in *S. secunda* (for a full account of ciliation in *S. secunda* see Stein, 1859), and probably in all others, a row of fine, stiff, seta-like cilia supporting the ectoplasmic membrane on the right of the peristome. These form a parabolic curve (P), and, following Sterki's nomenclature for similar structures in *Spirotricha* (=*Chaetospira*, 1897), they may be called the paroral cilia. The membrane, together with its cilia, runs back to the mouth from the point where the proboscis is bent. Endoral cilia (E) line the gullet.

The pellicle is very thin. There is no differentiation into ectoplasm and endoplasm, but the cytoplasm of the proboscis is hyaline and contains only a few greenish, refringent granules (R). These are more numerous in the main mass of the body, where also lie the nuclei, contractile and food vacuoles (V). The contractile vacuole, which at diastole projects on the left dorsal surface, contracts every 15–20 seconds—at more frequent intervals in young than in older individuals. The anus is mid-dorsal and the creature emerges from the sheltering cell just enough to allow the faecal material to be deposited outside (Text-fig. 2, fig. 1 A).

Perty (1852) did not mention the nucleus. Kahl (1932), in his monograph on Ciliates, says of the nucleus of *Stichotricha* only that it is in two pieces. Obviously this refers to the macronucleus. The two lie in tandem, each an ovoid structure with its long axis parallel with that of the ciliate. In the living organism the macronucleus appears as a refringent greenish body. About half-way along its length is a transverse cleft. In the preserved ciliate the macronucleus is made up of a chromatin network surrounding a number of vacuole-like spheres (cf. Willis, 1942, and Wenrich, 1929b). Embedded in the side of, or somewhere near, each macronucleus is a micronucleus. This stains densely and uniformly in interphase, but is not visible in the living animal.

Behaviour

The species of *Stichotricha* under consideration is intermediate in its habits between the freely swimming *S. secunda* and the loricate *S. socialis*. Unlike the former it may remain in one particular cell for several days, but never inhabits the same cell permanently as does *S. socialis*.

When feeding, the animal is extended (Text-fig. 2, fig. 1), the proboscis projecting beyond the aperture of the sheltering cell and bending to one side, the posterior end of the body being braced to one or more sides of the cell wall by the posterior cilia.

When disturbed the animal withdraws rapidly to the inmost corner of the cell, moulding its shape to fit against the wall. At the same time the proboscis

is contracted and thickened but never retracted into the body (cf. *Chaetospira*). Withdrawal is brought about by the adoral membranelles. The ciliate usually emerges again quickly and continues feeding. It may, however, remain withdrawn for a longer time, especially if environmental conditions are unfavourable, e.g. increased temperature or gradual desiccation. These conditions, amongst others not yet determined, also cause the migration of the animal to a new site. The behaviour of the ciliate during migration is the same as that of the swarmer after asexual reproduction, and is dealt with in the following section.

Stichotricha usually feeds on bacteria and other small organisms. Occasionally it will swallow larger organisms such as coloured flagellates. *Chlamydomonas* spp. measuring 7 μ in diameter were fed to these *Stichotricha*, and even though the flagellates were as broad as the proboscis (i.e. three times the diameter of the gullet), they were ingested (Text-fig. 2, fig. 3). As there are no records of feeding methods in *Stichotricha*, a brief account based on my observations may be of interest.

A feeding current is promoted by the adoral membranelles and is sufficiently powerful to bring in particles from some considerable distance.

Small particles, about 1 μ in diameter, are ingested indiscriminately if they get carried to the food groove. Larger particles, 7–10 μ in diameter (width is the important dimension), are undoubtedly selected. Particles of carmine and faecal debris of this size are always rejected. Flagellates are always accepted.

Playing an important role in selection is the membrane and its paroral cilia. These are so placed in relation to the row of adoral membranelles that a V-shaped food groove lies between them, its floor formed by a strip of proboscis cytoplasm running the length of the membrane and back to the mouth. Normally the membrane is held stiffly, and curves outward gently from the base of the food groove directing the current. In doing this it plays a passive role (Text-fig. 2, fig. 4).

When a large edible particle is brought into the food groove, the membrane, moved by the parorals, closes over it and appears to push it down into the gullet. With very large edible particles, such as the *Chlamydomonas* already mentioned, strenuous gulping movements of the cytoplasm take place as in *Stokesia* (Wenrich, 1929a). While the monad is trapped by the membrane and is revolving in the food groove at the base of the peristome, the cytoplasm within the body surges forward and surrounds it. The combined efforts of the displaced cytoplasm and the membrane finally bring about ingestion (Text-fig. 2, fig. 5). After this the cytoplasm that had piled up at the base of the proboscis returns to its normal position.

Rejection of large particles is accomplished by spasmodic jerks of the membrane.

Asexual Reproduction

Preparatory stages. Animals about to divide by binary fission have not necessarily reached a maximum size. Whereas the ciliate in Text-fig. 2, fig. 1,
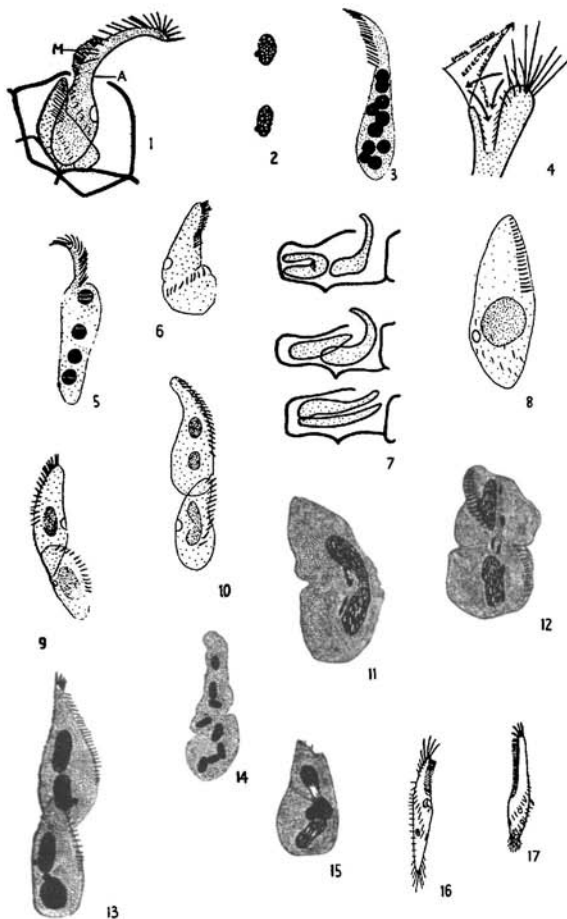
TEXT-FIG. 2. *Stichotricha intermedia* n.sp.

(Except where otherwise stated, drawings, made with aid of camera lucida, are of living specimens × 360.)

1. Left side view of adult feeding. Contracted position also shown. M, ectoplasmic membrane. A, position of anus. 2. Nuclear apparatus of adult. × 800. Feulgen. 3. Specimen that has fed well on algae. 4. Diagram of proboscis seen end on at the bend, showing feeding currents. 5. Displacement of cytoplasm in swallowing a large food particle. 6. Onset of cytoplasmic fission. 7. The products of fission after separation but before migration. Freehand. 8. The two macronuclei have fused prior to fission. 9. After fission but before separation, each daughter ciliate with a single macronucleus. 10. The single macronucleus of each daughter dividing. 11. The fusion macronucleus and two micronuclei dividing during binary fission. Haemalum. × 800. 12. The macronucleus and two micronuclei of each daughter dividing. Haemalum. × 800. 13. The two macronuclei of each daughter ciliate dividing. Haemalum. × 800. 14. Each daughter ciliate with four macronuclei. Haemalum. 15. The two macronuclei of a daughter ciliate dividing after separation. Haemalum. × 800. 16. *S. simplex*, after Kahl. 17. *S. gracilis*, after Möbius.

has grown to 96 μ before dividing, that in fig. 6 has divided when only 52 μ in length. Other workers have found that the rate of multiplication in ciliates may be independent of size (Harding, 1937, and Chatton and Beauchamp, 1923). Adolph (1931) came to the conclusion that 'when the processes which have to do with age come to a certain point, fission occurs regardless of how much body substance is present'. In the case of the organism described here it may be that the size of the containing cell in some way determines division. The onset of fission is marked by the withdrawal of the ciliate and consequent cessation of feeding, whereas other Hypotricha are active during division. Beating of the posterior adoral membranelles continues.

Cytoplasmic fission. Fission begins on the oral side just behind the peristome and extends by an oblique line across to the dorsal side behind the contractile vacuole (Text-fig. 2, fig. 6). The posterior half acquires new organs, whereas the anterior half retains those of the parent, as in *Euplotes harpa* (Wallengren, 1901). The new membranelles appear before cleavage is complete, but in what order I am unable to say. In addition a contractile vacuole is working in the posterior half by this time. While the line of cleavage deepens, the dividing ciliate rotates from time to time and may slowly expand and contract. When fission is complete, the two resulting ciliates remain one behind the other for some time, their membranelles beating actively. About 10 minutes later the posterior individual or swarmer moves up towards the opening of the cell and lies alongside (Text-fig. 2, fig. 7) or in front of the anterior individual, and it is difficult to distinguish one from the other. The whole process occupies about 2 hours.

Fate of the swarmer. The two individuals produced as a result of binary fission may remain together within the same cell, providing there is sufficient room for both to protrude and feed. If this happens, then the anterior one extends its neck and begins to feed shortly after fission, whereas the swarmer spends a preliminary period within the enclosing cell, extending and retracting, rotating and 'exploring'. After this it partially emerges and commences feeding. This remaining together of the products of fission sometimes results in groups of four individuals; but these are independent and not alined as in *S. socialis* (Gruber, 1879).

Migration and settlement. It is more usual for the swarmer to migrate after division. Before doing so it makes excursions to the aperture of the cell, pushing its sister ciliate back. These exchange movements continue for some time, the swarmer seeming to become bolder and emerging tentatively through the cell opening. Finally, it squeezes the broader posterior end of its body through and escapes. Its behaviour now depends upon local conditions.

If there is plenty of *Lemna* and it comes into contact with this, it will crawl over the plant in a persistent and exploratory manner, searching for a possible settling place. It does this by means of the membranelles and the few body cilia. The crawling is a jerky progression in straight lines, alternating with sudden backing movements brought about by a reversed beat of the membranelles. In backing the front part of the body is bent on the rest and

then straightened suddenly so that the ciliate points in a new direction in which it now proceeds. The front end is continually poking into crevices and empty cells. Sometimes these will be entered. Feeling all round the inside of the cell, using the larger anterior membranelles, projecting the anterior end through the aperture of the cell as if testing the nature of the surrounding water, and then backing and twisting round inside, the swarmer seems to test thoroughly the new abode. It may remain here or leave and begin the process of trial again. No feeding is done during this migratory period. As with Hypotricha in general and the swarmers of the Peritricha, the ciliate at this stage is markedly thigmotactic.

If, however, there is not much *Lemna*, and the swarmer swims straight out into open water after binary fission, then its behaviour is different. The swimming is continued. This is a rapid forward spiralling movement brought about by the membranelles and by the fact that the body is lop-sided owing to the disposition of the curved proboscis. As a result of the spiralling a much larger volume of water is explored. Although the movement is usually forward, reversals are not infrequent, and the bending of the front end, with subsequent change in direction, results sooner or later in some solid surface being contacted. Then the crawling and exploring movements begin.

The duration of this free-swimming phase varies from one to many hours (cf. *Chaetospira*).

Cytology. The behaviour of the macronuclei can be followed in the living ciliate. Fusion of the two macronuclei takes place before the cytoplasm cleaves (Text-fig. 2, fig. 8). Shortly after fission has started, the fusion nucleus divides into two, one of which passes forwards into what will be the anterior individual, while the other takes up its position in the swarmer (Text-fig. 2, fig. 9). Before fission is completed each macronucleus divides again. The products of this division, however, do not become the definitive nuclei of the new individuals, for they are seen to undergo the initial stages of a further division before the daughter ciliates separate. This division may be completed before or after separation (Text-fig. 2, figs. 14 and 15). Presumably the four macronuclei now in each product of division fuse two by two to form the two adult macronuclei, the line of fusion being represented by the cleft characteristic of hypotrichous nuclei.

The behaviour of the micronuclei can be followed only in stained material. While the fusion macronucleus is dividing, the two micronuclei divide to form four and then eight. The fate of these is obscure for only two persist in each daughter ciliate. Perhaps as in *Kahlia simplex* (Horvath, 1936) some of them disintegrate and do not participate in the final stages of fission (Text-fig. 2, figs. 11, 12, and 13).

While dividing, the chromatin of the macronucleus is in the form of beaded threads (Text-fig. 2, fig. 14), short and thick in the early and late stages, long and attenuated in the middle stages. These threads are so numerous and crowded together that it is impossible to count them. The same is the case with the micronuclei; and here extremely small size adds to the difficulty.

TABLE I. *Showing Chief Distinctive Structural Features of Nine Stichotrich Species*

(.. indicates no record)

SPECIES	secunda	marina	aculeata	socialis	saginata	gracilis	opisthoto-noides	simplex	intermedia n.sp.
FOUNDER	Perty	Stein	Wrzesni-owski	Gruber	Möbius	Möbius	Smith	Kahl	Froud
DATE	1852	1867	1870	1880	1888	1888	1897	1930	1944
HABIT	Solitary	Solitary	Solitary	Social	Solitary	Solitary	Solitary	Solitary	Solitary
HABITAT	Chara & Myriophyllum	Ulva	Sphagnum	Flooded meadows	Ulva	..	Old infusions	..	Lemna
LORICA	Absent	Absent	May or may not be present	Gelatinous	Absent	Absent	Absent	Absent	Absent
LENGTH IN μ	130–200	180–260	100	200	200	100	55	85–120	40–170
PROBOSCIS	Bent	⅓ body length	Straight ⅓ body length	Bent ⅓ body length	Thick	..	Bent dorsally	⅓ of body length	Bent ⅓ body length
FRONTAL CIRRI	..	..	..	3–4	..	5	..	5	None
ROWS OF BODY CILIA	4	4 ridged	4	4	4	2	..	2	3
LENGTH OF DORSAL CILIA IN μ	20	7	..	..	..	..	..	7	5

Systematic Position

Of the eight species of *Stichotricha* described by Kahl (1932), *S. simplex* (Text-fig. 2, fig. 16) agrees most closely with the one described here (see Table I). Both have three rows of cilia on the narrow, spindle-shaped body and the proboscis occupies a third of the body length. Both are non-loricate and, though widespread, are not profuse. The size range of *S. simplex*, 85–120 μ in length, is comparable with that for my species, but it has five frontal cirri whereas mine has what might be called a frontal cirrus, but this is really the first membranelle, which sticks out horn-like as does that in *S. secunda*. The dorsal cilia of *S. simplex* measure 7 μ as compared with 5 μ in the new species. These are only small differences, and it might be considered that my species is identical with *S. simplex*; but it does not seem wise to give it the same name until more is known about them both.

Again it is possible that the species described here is identical with *S. gracilis* Möbius, sketched by him in 1888. This species also shows reduction of body ciliation (Text-fig. 2, fig. 17) although Kahl (1932) attributes this to oversight in observation. No account of *S. gracilis* was given. If it does have only two rows of body cilia (see Table I), then it has gone farther in the process of cilium-reduction than my species, which is intermediate between it and the more typical species. Here again, until more is known about *S. gracilis*, it seems best not to identify mine with it.

S. opisthotonoides (see Table I) is also so incompletely described that it is impossible to say whether I have been dealing with it rather than with a new species. Like mine it is freshwater, solitary, naked, and has a bent proboscis. Its small size (55 μ) might well be accounted for by the fact that the specimens measured by Smith (1897) were all young ciliates, in which case they would agree with the species described here.

On these grounds a new species is proposed for this *Stichotricha*, and because several of its characters are intermediate between those of other species of the genus it is called *S. intermedia*.

The following is the diagnosis of *Stichotricha intermedia*:

Systematic position. Stichotricha intermedia n.sp. (Spirotricha, Hypotricha, Oxytrichidae).

Description. Solitary; non-loricate; length of adult 40–170 μ, two-fifths of which is occupied by a bent proboscis; three rows of body cilia; dorsal cilia 5 μ in length, two rows.

Habitat. Lemna.

Locality. London, England.

<div align="center">CHAETOSPIRA MÜLLERI LACHMANN</div>

Trophic Phase (Text-fig. 3).

Chaetospira is a flask-shaped organism varying in length from 60 to 200 μ. When feeding, the neck of the flask is drawn out into a spiral (PM), which may form as much as a third of the length of the body, and runs in an

anti-clockwise direction. On this proboscis are borne the powerful adoral membranelles (AM). Of these the first few are stouter and more widely separated than the rest. The first (s) in particular is very conspicuous, differing from the others in size, appearance, and behaviour. In addition to the cilia of the membranelles there are accessory peristomial cilia (VC), stiff, sensory dorsal cilia (DC), and a small undetermined number of body cilia. Opposite the adoral membranelles on the left-hand margin of the peristome and running from the first twist of the spiral back to the mouth (o) is a thin
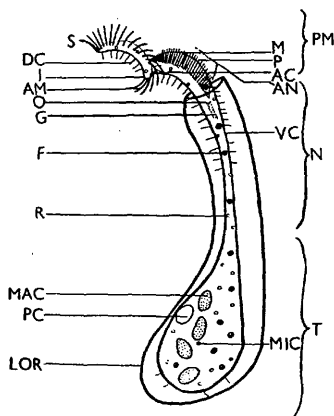
TEXT-FIG. 3

ectoplasmic membrane (M) supported by a series of stiff paroral cilia (P). The mouth lies at the base of the proboscis and leads into a gullet (G) which penetrates the neck. This and the proboscis are composed of hyaline cytoplasm containing a few of the refringent granules which in a well-nourished specimen fill the base of the flask-shaped body (T). This contains the contractile vacuole (PC), food vacuoles (F), and the nuclei (MAC, MIC). There may be another contractile vacuole (AC) on the proboscis, left and dorsal to the adoral membranelles, on the first turn of the spiral. As in *Stichotricha* the pellicle is very thin and the body is plastic, without any real distinction between ectoplasm and endoplasm. The anus is far forward on the proboscis, dorsal, to the left and a little way behind the first turn of the spiral (Text-fig. 4, fig. 18).

Hitherto the nucleus of *Chaetospira* has not been adequately described. In Sterki's words, 'Two almost globular endoplasts were seen not very distinctly.' Kahl, in 1932, said that the nucleus of *C. mülleri* is in two separate pieces. Both these remarks refer to the macronucleus. Now my species of *Chaetospira* has its macronucleus in rounded pieces, usually four, as in *Gastrostyla*

(Weyer, 1930). But as Lachmann, the discoverer of *C. mülleri* (with which species I think I am dealing), did not mention the nucleus, there is no justification for setting up a new species until the cytology of the genus has been studied.

In the living animal nuclei cannot be seen. Feulgen-stained specimens (Text-fig. 4, fig. 20) show the macronucleus to be made up of a chromatin network surrounding a number of vacuole-like spheres as in *Stichotricha*. The number of macronuclei in trophic individuals varies from two to eight

TABLE II. *Showing that Macronuclear Number is independent of Body Length in* C. mülleri (*fixed material*)

| No. of macronuclei	8	6	5	4	3	2	1
Body length in μ of individuals	31 54	32 42 57	30 32 42 54 69 128	32 41 54 72 91 111 132	40 69	35 72	35 65

(cf. *Urostrongylum contortum* Kahl (1932)). There is no correlation between the number of macronuclei and the size of the individual (see Table II). This may be accounted for by one or more of the following assumptions:

1. Endomixis might be occurring in these apparently ordinary individuals. Against this is absence of disintegrating macronuclei.
2. Mitosis might be completed before cytoplasmic fission starts. Against this is the rareness of division figures; but supporting it are the individuals of all sizes with five to eight nuclei.
3. In many ciliates, e.g. *Stentor*, the adult macronucleus is the product of several 'Placenten' (Bělař, 1926). This might account for the large number of macronuclei in some of the small ciliates, and for what looks like the fusion of macronuclei in pairs in a few cases.

The micronucleus is usually single, but at times two or more have been seen. It lies near or in a notch in the side of one of the macronuclei (Text-fig. 4, fig. 21). In interphase it is densely and uniformly stained. Consequently when it lies above or below a macronucleus it cannot be seen. This may account for the variation in number recorded above. On the other hand this might be due, as in the case of the macronucleus, to precocious mitosis.

Feeding

Chaetospira feeds on the same kinds of food and in the same way as *Stichotricha*. I have seen it ingest *Bodo*, diatoms, and considerable lengths of algal filaments. Sterki (1897) observed this and noted also that the posterior adoral membranelles are responsible for rejection. This I have not seen. The
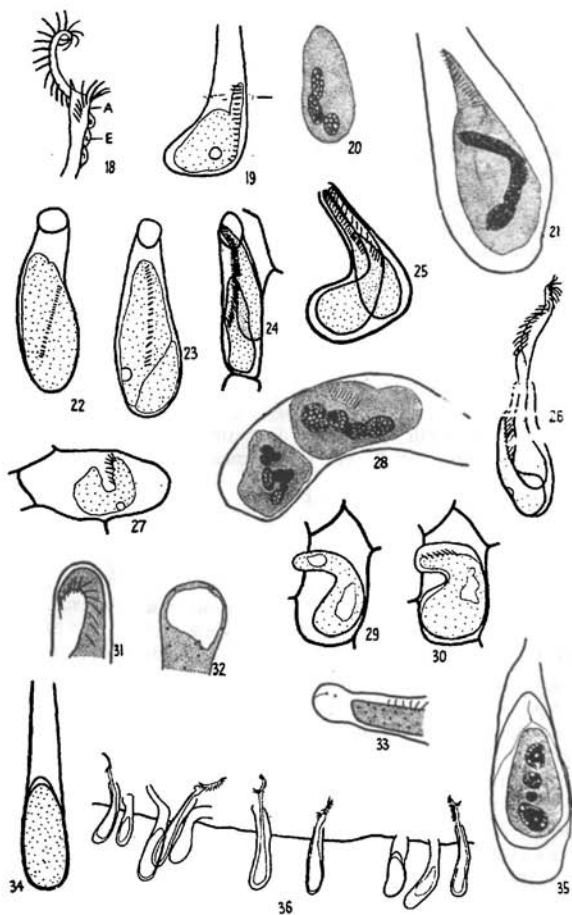
TEXT-FIG. 4. *Chaetospira müilleri*

(*Except where otherwise stated, drawings, made with aid of camera lucida, are of living specimens* × 360.)

18. Diagrammatic sketch of proboscis, ventral view, showing three successive positions of a faecal pellet about to pass out through the anus A. E, ectoplasmic membrane. 19. Retraction of proboscis in a contracted animal. 20. Nuclear apparatus of adult. × 580. Feulgen. 21. Fusion macronucleus prior to fission. × 580. Haemalum. 22. Dormant phase prior to fission. 23. Cytoplasmic fission. 24, 25, and 26. Different appearances of the products of fission after separation but before migration. Freehand. 27. Swarmer, exploring a new site. 28. The nuclear picture immediately after fission. Haemalum. × 800. 29. Formation of lorica: vacuolation. 30. Same: withdrawal. 31. Neck of new lorica before it is open. Freehand. 32. Adding new length of neck to lorica: vacuolation. 33. Same: withdrawal. 34. Cyst. 35. Same, stained Heidenhain's iron haematoxylin. × 580. 36. Group of feeding, resting, and encysted individuals. × 72.

membranelles can be seen moving only during extension or retraction of the proboscis and at certain times during asexual reproduction. At other times their rate of beating is so rapid that they appear motionless. I could see no differential beating of the posterior ones such as one would expect if they were the rejecting agents.

In a large, fully extended, feeding *Chaetospira*, food vacuoles are formed every 20 seconds. If cysts are ingested, the contents are utilized and the empty cases egested. The colour of a diatom disappears 10 minutes after ingestion, and *Bodo* is immobilized in less than 1 minute.

Asexual Reproduction

Preparatory stages. Individuals about to divide by binary fission are large and have their cytoplasm packed with greenish granules. The first sign of division is the withdrawal of the proboscis and rounding of the anterior end of the now pear-shaped body. Because the adoral zone is contracted, no feeding is done and the creature is sluggish, but the posterior membranelles continue to move though slowly and discontinuously. When not moving they are closely pressed to the body. This dormant period lasts for several hours (Text-fig. 4, fig. 22).

Cytoplasmic fission. Fission takes place as in *Stichotricha* (Text-fig. 4, fig. 23). All the dividing ciliates that I watched were in loricas and not easy to observe on account of the disposition of the sheltering cells, so that I was not able to determine how or when the ciliation of the posterior individual is laid down. It is this posterior half that acquires new organs, whereas the anterior half retains those of the parent. While the two new ciliates are still attached by a strand of cytoplasm dorsally, the membranelles of both begin to beat, but no attempts are made at this stage to emerge and feed. After some time separation is effected and the two inhabitants of the lorica now roll round one another, frequently changing places (Text-fig. 4, figs. 24 and 25).

Escape of the swarmer. The behaviour of the two individuals is different. The membranelles of the posterior individual or swarmer are less well developed than those of the anterior. They appear ragged and beat haphazardly so that the swarmer makes jerky movements. It will come up to the base of the neck of the lorica, stick out its now pointed anterior end, and then withdraw rapidly to the back end of the lorica. When it does this the anterior individual is first displaced from the neck end and retires, then moves forward again when the potential swarmer withdraws. The movements of the anterior individual with its clear-cut membranelles are more regular; and after a time it extends its proboscis at intervals through the lorica neck and feeds (Text-fig. 4, fig. 26). Feeding is interrupted when the swarmer makes its forward exploratory movements. Sometimes both are forward together (Text-fig. 4, fig. 25).

Not for long do the two remain in one lorica. They are too cramped and it is impossible for them both to feed. The swarmer comes more and more frequently to the lorica neck, pushing the other back: it makes more and more

frequent essays at extending its proboscis through the lorica mouth, and finally squeezes out through the neck and swims away. The anterior individual is now left in possession of the lorica. It immediately proceeds to feed and carries on as a typical trophic *Chaetospira*.

Migration and settlement. The swarmer acts as a distributive phase. It is still unlike the adult in that the proboscis is relatively undeveloped, with few membranelles and not spirally twisted but bent over to one side as in *Stichotricha*. The size of the swarmer varies with that of the parent, but an average length of 46 μ was obtained from fifteen specimens. On leaving the parent lorica the behaviour of the swarmer is very similar to that of *Stichotricha* (Text-fig. 4, fig. 27).

The duration of this free-swimming phase varies. In one case, escape from the parent lorica was followed by immediate contact with a leaf of *Lemna*, discovery of an empty cell 5 minutes after, and its exploration and acceptance 10 minutes after this. In another case, the free-swimming ciliate was watched for half an hour before it finally settled.

I must remark here upon the striking resemblance between this distributive organism and the corresponding stage in *Stichotricha*. Morphologically they are identical except for small details in ciliation and in the nature of the nucleus. The *Chaetospira* swarmer is never free for such long periods as that of *Stichotricha*. As regards adaptation to a loricate existence, *Chaetospira* has gone much farther than the other ciliate, and the adult is considerably modified accordingly. But here is an example of a 'young' stage, retaining during its development the facies of a less-specialized genus of the family. Possibly this resemblance has been responsible for some of the confusion of the two genera, observers having mistaken swarmers of *Chaetospira* for free-swimming individuals of *Stichotricha*. In view of this some revision of stichotrich species seems necessary.

Cytology. Owing to the innumerable greenish granules present in the cytoplasm during division, it is impossible to follow nuclear behaviour in the living organism. Consequently my observations have had to be made on preserved material. This is unsatisfactory for the nuclear pictures are so variable that I am as yet at a loss to explain many of them. All I can say at present is that I believe the following to happen during binary fission:

The macronuclei fuse (Text-fig. 4, fig. 21). The fusion nucleus divides into two, four, eight, or more, usually. Cytoplasmic fission separates an anterior individual with four macronuclei from a posterior one with eight (Text-fig. 4, fig. 28). These eight fuse in pairs forming the four adult macronuclei. The fusions are not necessarily simultaneous. Moreover, they can take place before or after the daughter cells have separated. The micronuclear cycle has not been followed.

Lorica Formation.

The adult lorica (Text-fig. 4, fig. 36). This is flask-shaped, with its base embedded in an empty plant cell and its neck protruding from the opening.

It is thin and perfectly smooth. I can find no evidence for its being sticky, except perhaps when it is first laid down. The accumulation of debris around the neck of the flask, described by Möbius (1888) and Sterki (1897), is due to the method of feeding: for in *Stichotricha*, where there is no lorica but the same mode of feeding, the debris collects round the opening of the cell sheltering the animal. In both these ciliates rejected solid matter tends to be deposited where the quickly travelling water-current meets the static pond water. This is outside the ambit of the membranelles at the base of the proboscis and, in *Chaetospira*, on a level with the mouth of the lorica.

Möbius (1888) called the lorica chitinous, though he makes no mention of chemical tests to support his statement. Tests for chitin are difficult to make on such a small object. With picro-nigrosin and Mann's methyl blue eosin, I got positive results: the lorica stained blue in each case. Yet with iodine in potassium iodide followed by zinc chloride the result was negative. A negative result was also obtained with Millon's reagent, suggesting that the lorica is not protein.

Origin of the lorica. The lorica is laid down in the first place by the swarmer. This, as soon as it has found a suitable settling place, becomes inactive, appears to withdraw its membranelles and cilia, rounds off the pointed anterior end, and undergoes a peculiar process of vacuolation. Just before this there are streaming movements in the cytoplasm. Then the greenish granules with which it is well stocked pass to the periphery and large vacuoles appear throughout the body (Text-fig. 4, fig. 29). This leads to inflation and the ciliate becomes half as large again. By this means the limits of the new lorica are determined, for it must be larger than the animal it shelters and allow for growth. Complete immobility follows for about 5 minutes. Then there is a slight oscillating movement of the greenish granules. Meanwhile the vacuolation has increased, the lorica becomes very definite on the surface of the cytoplasm, and, 10 minutes or so after, the animal seems to be composed almost entirely of one enormous vacuole and appears dead. Only 5 minutes after this, vacuolation is reduced, movement of the cytoplasm recommences, and withdrawal from the lorica begins (Text-fig. 4, fig. 30). This starts in the region of the membranelles, which now reappear and, apparently better developed, become active. The contractile vacuole can now be seen again. Roughly an hour after the swarmer first becomes free it is established. That there is some close connexion between the granules and lorica-formation is obvious, but I am unable to say how the material in the granules reaches the surface and gets deposited there. In each case, however, some of the granules are left behind adhering to the newly formed lorica neck when the ciliate withdraws. Moreover, although normally rare in the peristome region, at this time they are numerous there.

Opening of the mouth of the lorica takes a variable time and I am unable to say whether it is accomplished by mechanical or chemical means. The ciliate moves backwards and forwards in the lorica, using its adoral membranelles. As it moves forwards, the proboscis creeps along the neck of the

lorica to which its dorsal surface is closely applied. When it gets to the closed end it bends over, still keeping in close contact. At this stage the ciliate seems to be pushing against the closed end with the back of its neck (Text-fig. 4, fig. 31). This lasts only a short time, and is followed by return to the base of the flask. Then the cycle of movements is repeated. Finally a break through is made and the ciliate can extend its proboscis and feed.

Additions to the lorica. Unlike the ciliates *Lagenophrys tatersalli* Willis (1942) and *Folliculina ampulla* Fauré-Fremiet (1932), where no additions are made to the adult lorica, *Chaetospira* adds to its from time to time. More frequent additions are made in the young than in the old ciliate. In all its stages the process of addition is similar to that of lorica formation by the swarmer, except that when inflation takes place the front end of the vacuolating *Chaetospira* emerges as a sphere from the mouth of the lorica (Text-fig. 4, fig. 32). The surface of this sphere is continuous with the neck of the lorica. After some time, during which presumably the surface of the sphere is converted into lorica material, the cytoplasm within the sphere reorganizes itself into the peristome of the ciliate. This now withdraws from the sphere which shrinks a little (Text-fig. 4, fig. 33). An opening is made at the distal pole of the sphere and it contributes a new length of neck to the lorica. Old specimens have very long necks. The rest of the surface of the ciliate contributes a new layer within the old lorica wall.

Encystment

Cysts occurred only in one batch of material, where they were discovered in encrustations at water-level in the winter of 1944–5. The first stages of encystment were not seen, nor was excystment.

Living cysts are pear-shaped and measure from 35 to 55 μ in length and 15 to 20 μ in breadth. Each lies in the lorica of the individual which forms it, and has a perfectly smooth wall 1 μ thick, except at the pointed end lying next the neck of the lorica. Here it thickens to 2 μ or more (Text-fig. 4, fig. 34). The contents are in close contact with the cyst all the way round and are finely granular and homogeneous, very different from the cytoplasm of the adult. There are no greenish granules, no cilia, no contractile vacuole or other organs.

The most common number of macronuclei in a cyst is three (Text-fig. 4, fig. 35), but cysts with two to six have been seen. This suggests that nuclear division might take place within the cyst as in *Gastrostyla* (Weyer, 1930) and *Kahlia simplex* (Horvath, 1936). One or two micronuclei are present. These I have not seen dividing. The nuclei have the same appearance as those of the unencysted adult.

No work has yet been done on the factors causing encystment. Since the pH of the medium has been maintained constantly at 7, this factor alone is not responsible. Since cysts were obtained only in winter months, drought and/or lowered temperature might be responsible. Cysts were not obtained the following winter (1945–6), so temperature alone is not the cause.

SUMMARY

1. The structure, behaviour, and asexual reproduction of a plant-cell-inhabiting hypotrichous ciliate are described.

2. The systematic position of the ciliate is discussed and it is placed in the genus *Stichotricha* Perty as *S. intermedia* n.sp.

3. New information concerning behaviour, nuclear apparatus, and asexual reproduction is given for the loricate *Chaetospira mülleri* Lachmann. Cysts are described for the first time.

REFERENCES

ADOLPH, E., 1931. *The Regulation of Size as Illustrated in Unicellular Organisms.* London (Baillière, Tindall & Cox).
BĚLAŘ, K., 1926. *Der Formwechsel der Protistenkerne.* Jena (Gustav Fischer).
BOLTON, T., 1878. Midland Naturalist, **1**, 76.
CHATTON, E., and BEAUCHAMP, P., 1923. Arch. Zool. exp. gén., **61**, 123.
DOBELL, C., 1914. Arch. Protistenk., **34**, 139.
ENTZ, G., 1884. Mitt. zool. Sta. Neapel, **5**, 289.
FAURÉ-FREMIET, E., 1932. Bull. biol., **66**, 77.
GRUBER, A., 1879. Z. wiss. Zool., 33. Reported in Quart. J. micr. Sci., **3**, 282.
—— 1883. Z. wiss. Zool., 38. Reported in Ann. Mag. nat. Hist., **11**, 316.
HARDING, J. P., 1937. J. exp. Biol., **14**, 422.
HORVATH, J. v., 1936. Arch. Protistenk., **86**, 482.
HUDSON, C. T., 1875. Monthly Microscopical Journal, **14**, 165.
ILLOWAISKY, S., 1913. Trd. gidrobiol. st. Glubokoe Moskva, **5**, 132. With German résumé.
KAHL, A., 1932. *Tierwelt Deutschlands*, Teil 25, 1 Ciliata, 3 Spirotricha, p. 399. Jena (Gustav Fischer).
KENT, W. S., 1880. *A Manual of the Infusoria.* London (David Bogue).
LACHMANN, C. F. J., 1856. Arch. Anat. Physiol., p. 364.
MÖBIUS, K., 1888. Arch. Naturgesch., **1**, 81.
PERTY, M., 1852. *Zur Kentniss kleinster Lebensformen.* Bern (Jent & Reinert).
SMITH, J. C., 1897. Amer. mon. micr. J., **18**, 109.
STEIN, F., 1859. *Der Organismus der Infusionstiere*, I. Leipzig (Wilhelm Engelmann).
STERKI, V., 1897. Amer. Nat., **31**, 535.
STRETHILL WRIGHT, T., 1862. J. micr. Sci., **2**, 220.
WALLENGREN, H., 1901. Zool. Jb. (Anat.), **15**, 1.
WENRICH, D. H., 1929a. Trans. Amer. micr. Soc., **48**, 221.
—— 1929b. Ibid., **48**, 352.
WEYER, G., 1930. Arch. Protistenk., **71**, 139.
WILLIS, A. G., 1942. Quart. J. micr. Sci., **83**, 171.
WRZESNIOWSKI, A., 1870. Z. wiss. Zool., **20**, 467.