British and Other Freshwater Ciliated Protozoa

Part I
Ciliophora: Kinetofragminophora

Colin R. Curds

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Keys and notes for the identification of the free-living genera

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Foreword

The two *Synopses* devoted to *British and other freshwater ciliated Protozoa* (Nos. 22 & 23) have been published exactly a century after the appearance between 1880–2 of W. Saville Kent's classic *Manual of the Infusoria*. Dr Curds is a member of the scientific staff of the British Museum (Natural History) as was Saville Kent when he wrote his manual, emphasising the important role of this great national museum in the field of taxonomy and the importance that it has placed upon the collection of living material as well as upon the maintenance of its vast collections of dead specimens.

The remarkable resistance of ciliated protozoa to desiccation by cyst formation has led to their easy transport in the air and on the outsides of other animals, hence their lack of respect for national boundaries. Thus this *Synopsis* is not restricted to the British kinetofragminophorans but includes those from elsewhere. It is however restricted to their genera: to run down these protozoa to species often involves elaborate techniques with expensive apparatus outside the scope of this *Synopses* series, which is designed to be a set of handbooks for use in the field and laboratory by amateur and professional biologists from VIth-form level upwards. Extending the coverage to specific level would also increase their already generous size so they could no longer be described as 'handbooks'. The reference list at the back of each *Synopsis* should be of assistance if a particular specimen needs to be 'tracked down' to species level.

Although there are two *Synopses* devoted to freshwater ciliates, each is complete in itself; the introductory chapters and the associated figures (pp. 1–59) will be repeated with minor changes in each part.

The editors acknowledge, as has the author, their gratitude to the Natural Environment Research Council for a grant to cover the artist's fees. They also thank both author and artist for the skill and care with which they have used their respective skills to produce a work which is both scientifically accurate as well as aesthetically pleasant to read and use.

R. S. K. Barnes

Estuarine and Brackish-water
Sciences Association

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A Synopsis of the British Freshwater Ciliates Part I. The Kinetofragminophora

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Contents

Introduction

Ciliated protozoa are found in all moist habitats. They are generally cosmopolitan in their distribution and may be free-living or symbionts. They are extremely common, frequently numerous and indeed it is rare to find a sample of natural water without some ciliates being present. In spite of their abundance they are recognised to be a difficult group to identify and this is partly due to the lack of a suitable key both for specialist and non-specialist alike. The now classical keys of Kahl (1930–35, 1934) are still used by most specialists for identification purposes; while these have the merit of being keys to species they are long outdated. Patterson (1978) has recently published an English translation of the keys to the genera in one of Kahl's (1930–35) works. The non-specialist has tended to use Kudo (1966) which while containing many generic descriptions does not include them all nor does it contain keys and is also rather out of date.

Until recently ciliates were classified as a subphylum within the phylum Protozoa. It is now generally recognised that ciliates are so distinctive and diverse as to merit the rank of phylum in their own right. There are about 1125 described genera comprising some 7200 species. With so many taxa now available it is not easy for a second 'Kahl' to be written, indeed it would need a team of specialists writing within their own small fields to accomplish the task within a reasonable length of time. Here to keep the number of taxa down to a manageable figure it was necessary to include only freshwater free-living genera as listed in Corliss (1979). Thus all freshwater ciliates living freely or growing as symphoriants on the outside of aquatic animals and plants have been dealt with but no exclusively entozooic genera have been included. Similarly no ciliate genera reported from marine or brackish waters have been described herein unless at least one species has been identified in freshwater. We realise that these restrictions are partly arbitrary but even so ≏400 genera have been included in the two *Synopses* devoted to freshwater ciliates.

Although these form part of the series of *Synopses of the British Fauna*, a British ciliate is not easy to define since ciliates are so cosmopolitan and this is complicated by so few protozoologists having identified ciliates within the British Isles. We have therefore included *all* free-living freshwater genera as all are at least potentially to be found in British waters. The only likely exceptions to this are certain ciliates which grow as symphoriants on specific species of aquatic animals. For example, there are several chonotrichs and suctoria which have been found growing on the exoskeleton of specific crustaceans endemic to Lake Baikal in Russia. Since the numbers of these

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examples are so low it seemed worthwhile retaining them since the key may then be used on a world-wide basis.

We have aimed the key at both the specialist and non-specialist. Ciliate taxonomy has moved so rapidly in the last decade that it is difficult for the ecologist to keep abreast of these changes. It has been our intention to provide an up-to-date résumé. We have purposely not included the specific names of ciliates in diagrams and descriptions unless necessary to do so for some nomenclatorial reason, this will prevent the novice from recording species from any examples given, which evidently was the case in the past with many non-specialists using works such as Kudo (1966). However, we do wherever possible cite references to descriptions of species and keys to species where they exist. Similarly because size varies considerably from species to species within a genus we have not included scales on diagrams but have indicated in the text when the size of the species within a particular genus is particularly large or small.

Since much of the skill in identification lies in knowing precisely what features to look for, we have included an extensive introductory review on the morphology and biology of these animals. Similarly a review on staining and handling techniques is included since at some stage during an identification it is often necessary to display a particular structure by staining.

The original drawings of Figs. 41–243 are deposited in the Protozoa Section, Department of Zoology, British Museum (Natural History).

General structure and biology

All ciliates are single-celled microscopic animals, although their overall range is about 15–2000 µm long most species lie within the range of 40–200 µm long. The shape of the **body** is generally simple, often ovoid or spherical but there are many variations and the terms most commonly used in the descriptions given in this book are defined in Fig. 1. It should be pointed out that it is rare for a ciliate to be completely and regularly spherical or ovoid, they may be slightly elongated or compressed and thus the terms used in the descriptions are the nearest equivalents. Many genera are rounded in cross-section but others may be flattened either dorso-ventrally or laterally and the position of the **oral opening** is usually used to define the ventral surface. Thus where the flattening is lateral the oral aperture will lie on an edge rather than on a surface and in this case there are ventral and dorsal edges and lateral surfaces. In certain flattened ciliates the opening may lie so closely to an edge that it is difficult to know if it is on a surface or along an edge; in cases like these the position of the **stomatogenic kinety** is often considered to be more important than the position of the oral aperture for defining the ventral aspect of the cell. The longitudinal axis is defined by the direction of the kineties which generally run along the longitudinal body axis although there is frequently some spiralling present. The **kinetodesmata** always lie on the animal's right of the line of kinetosomes so that the anterior and posterior can be defined (Fig. 2A). In practice the anterior end is usually held forwards whilst swimming. Perhaps it should be noted here that right and left in descriptions throughout the text refer to those sides of the animal not of the observer.

The outside of all ciliates is covered by a cell wall or **pellicle**, many remain highly plastic but in other cases the pellicle is thicker and/or made more rigid by the addition of **ribs** or **ridges** and this is often referred to as body armour or the **cuirass**. Similarly, several genera with armour possess spines or spikes which frequently project from the body. Other ciliates retain their plasticity, but produce a shell-like structure known as a **lorica** inside which the animal is protected. Some ciliates live permanently trapped inside the lorica, others leave sporadically to return later. Several ciliate genera attach themselves to the base of the lorica and these may then extend out of the lorica to feed and contract back inside for protection. Loricas may be membranous, gelatinous or pseudochitinous and they commonly include debris from the local environment in their construction. Many ciliate genera produce **cysts** for protective purposes, usually to withstand desiccation or some other environmental hazard. Protective cysts have thick walls but there are also certain

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Round/spherical   Barrel   Oval/ovoid

Reniform   Pyriform   Cylindrical

Clavate   Flask   Cigar

Transversely truncate   Obliquely truncate   Heart

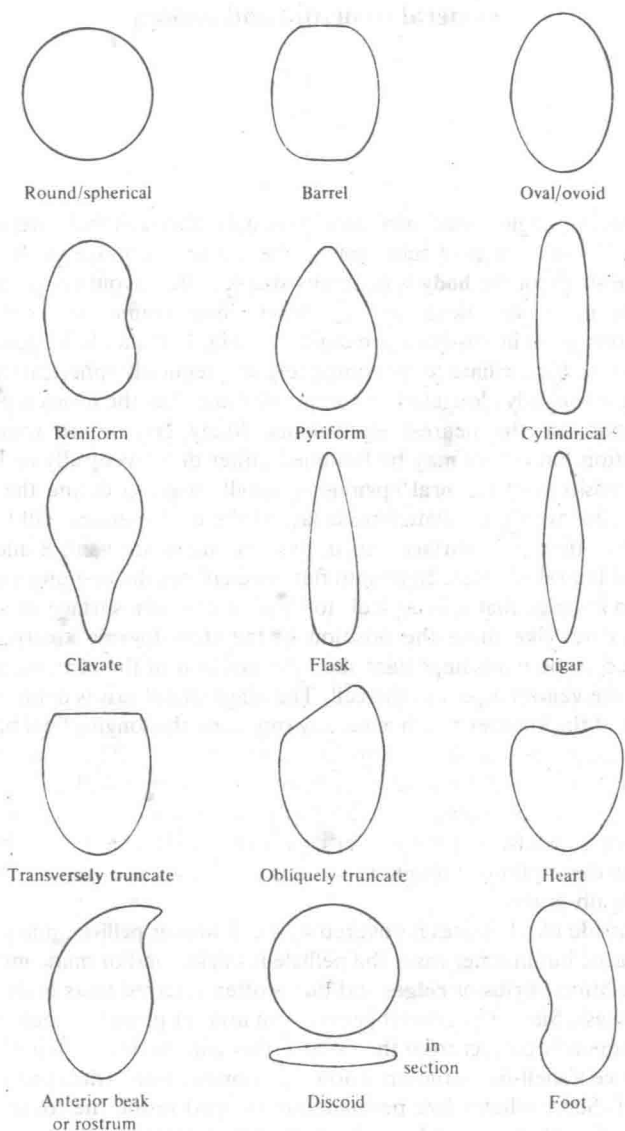Anterior beak   Discoid   Foot
or rostrum

in
section

Fig. 1. Shapes of ciliates. Organisms will rarely be exact replicas, the nearest approximation will be given in the text. When two descriptive names are used in the above diagram they represent the two- and three-dimensional equivalents respectively.

genera that can produce thin-walled reproductive cysts. The best known examples of these include the genus *Colpoda* and its relatives which undergo several divisions within the cyst to produce up to eight daughter cells which then emerge.

Some ciliates are able to protect themselves by means other than the possession of a solid external covering; certain genera secrete a mucilage from subpellicular vesicles called **mucocysts** and others defend themselves by the expulsion of fibrous harpoon-like **trichocysts**. These two organelles are not easily distinguished by light microscopy and for this reason we have used the all-embracing term trichocyst throughout the key. True fibrous explosive trichocysts are restricted to the peniculine hymenostomes and the hypostome nassulid suborder Microthoracina.

The majority of ciliates swim freely in the water but there are many others which are attached to surfaces of some kind, these surfaces may be inanimate objects or other living organisms such as plants and animals. Attachment to the surface may be direct or via a stalk or the lorica. **Stalks** may be contractile or non-contractile, and they may be branched giving rise to colonial forms or they may be unbranched.

Tentacles and tentacle-like structures appear in several subclasses. In the suctoria they represent the sites for ingestion, there being no other oral aperture and no cilia present. However, in the hypostomes, gymnostomes and vestibulifera there are always cilia present, the tentacle-like structures do not represent the ingestion site and there is always a single oral aperture situated elsewhere on the body.

All ciliates, with very few exceptions, possess **cilia** at some stage in their life history. Cilia are fine hair-like organelles (about 0.25 µm diameter, length variable but often 7–10 µm long) that project out from the body from a subpellicular basal granule known as a **kinetosome** (Fig. 2A). Cilia beat rhythmically for locomotion and to create feeding currents. In the rare cases where cilia are absent either completely or, more commonly, during a particular stage in the life cycle their kinetosomes always persist. Kinetosomes are usually arranged in rows along the body axis, and a single row of them is known as a **kinety** (Fig. 2A). There are usually several kineties arranged on the body surface and associated with these structures are longitudinally orientated subpellicular cytoplasmic fibrils known as **kinetodesmata** (Fig. 2A) which arise close to the base of a kinetosome and extend anteriorly on the right of the kinety concerned. Individual kinetodesmal fibrils are relatively short, thus the longitudinal fibre travelling along the right-hand side of a kinety is not a continuous fibre as is apparent from the light microscope, but a series of over-lapping fibrillar units. The complete collection of kineties plus any oral ciliary organelles that may be present are referred to collectively as the **infraciliature** which may be displayed by the application of various silver-impregnation techniques (see *Practical methods*) and is therefore frequently referred to as the 'silver-line system'. In certain ciliates, particularly the spirotrichs, cilia may be reduced and largely replaced

by **cirri** (Fig. 3C). These latter organelles are complexes of numerous long cilia loosely grouped together to form stout, tapering organelles that are rounded in cross-section. Although coordinated, cirri do not beat uniformly but are often used for 'walking' over solid surfaces.

With the exception of the suctoria* and the astomes (only one free-living representative) all ciliates possess a single **oral aperture** (Fig. 2A,B,C). We have purposely used the term oral aperture to denote the actual hole, slit or indentation in the body without any regard to any oral ciliary structures which may be present since the term loosely includes all of the precise protozoological terms of **cytostome, buccal overture** and **vestibular aperture**. While the technical differences between these structures can be precisely defined (see below) we would point out that it is not usually easily possible for the microscopist to be able to differentiate between a buccal cavity and a vestibulum without considerable effort. For example, it would be simple to key out the Vestibulifera by the presence of a vestibulum, however, in practice this would be valueless to the user since it is not easy to recognise a vestibulum. However, the position and nature of the oral aperture can also be of great importance for identification. In most cases the oral aperture lies somewhere in the anterior body half but there are several examples of subequatorial oral apertures. When the position of an oral aperture is of importance in the key, several illustrative examples are usually given.

The oral region of many ciliates contains compound ciliary organelles that are specialised for feeding although some in the course of time have regained their locomotory function. These compound structures are of two types and are called **undulating membranes** and **membranelles** according to their structure. An undulating membrane is simply a line or arc of cilia set close together, in a single row, so that they more or less permanently coalesce into a membrane. They are set on the right of the oral area. Conversely, membranelles are composed of two or three rows of cilia forming a block, the free ends of which adhere together to form triangular or trapezoidal flaps. They are typically arranged on the left of the oral area (Fig. 2A,C).

In the subclass Gymnostomata, most species are without oral ciliature and the **cytostome** is at, or near to, the surface of the body and located apically, subapically or laterally (Fig. 2D–F). The cytostome is the two-dimensional hole which marks the cell mouth proper denoting the end of any ciliation. Any passage beyond the cytostome is known as a **cytopharynx** and is always unciliated. The cytopharynx is frequently strengthened by a series of rods or **trichites** and the complex formed is known as the **cytopharyngeal apparatus** (Fig. 2D,F). The **trichites** or rods encircle the cytopharynx to form a basket-like structure which may be straight (**rhabdos** type, Corliss, 1979) or

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* This branch of zoology has yet to develop a colloquial language and such words as 'suctoria' can be used in two contexts: firstly formally Suctoria, referring to the subclass of that name and secondly informally, when the lower-case or small 's' is used, suctoria. Protozoa and protozoa is another example.
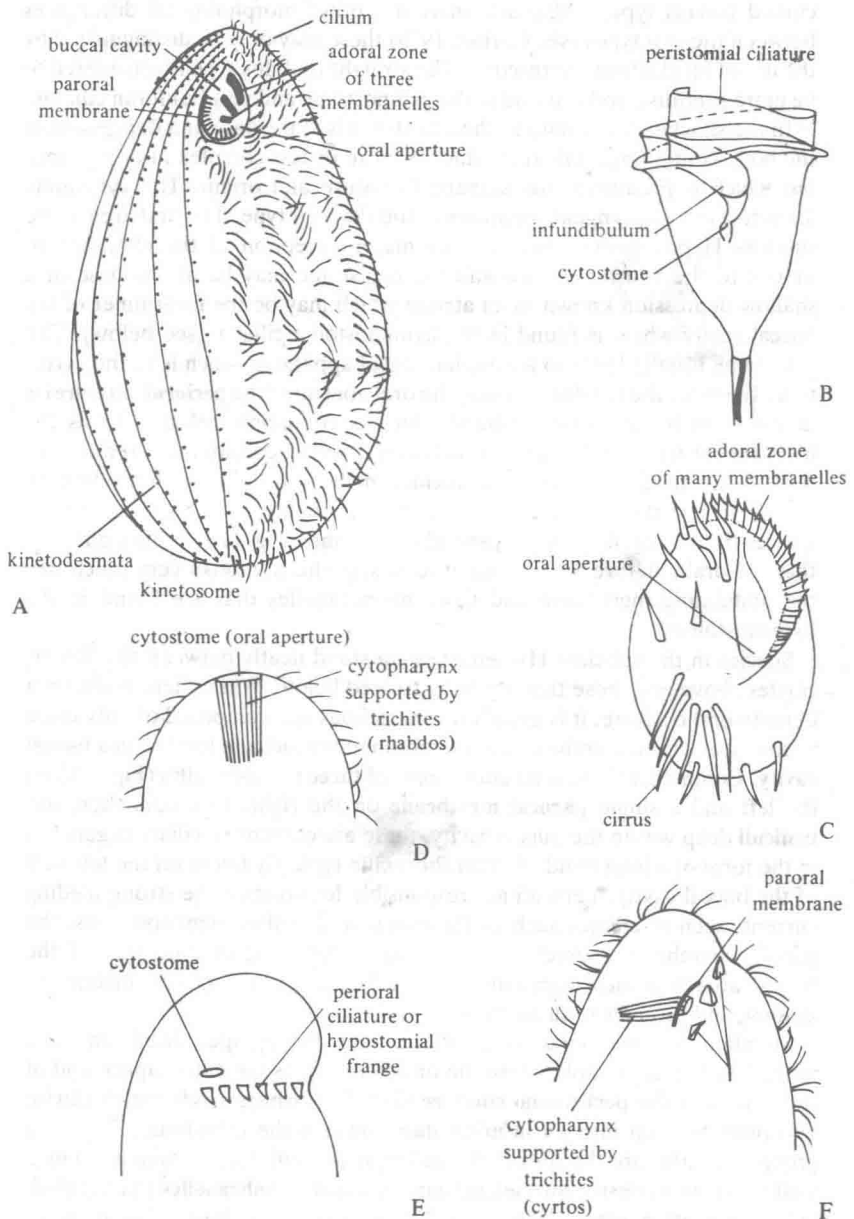
Fig. 2. Ciliary structures in ciliated protozoa. A, *Tetrahymena*, impregnated with silver on left, as seen in living specimen on right. B, *Vorticella*, a peritrich. C, *Euplotes*, a hypotrich. D, *Holophrya*, a simple gymnostome. E, *Nassula*, a hypostome. F, *Furgasonia*, a hypostome.

curved (**cyrtos** type). Although there are other morphological differences between the two types (see Corliss, 1979) these may only be distinguished by the use of an electron microscope. The straight rhabdos type is considered to be more primitive and is found in the gymnostome and vestibuliferan ciliates.

In the subclass Vestibulifera the cytostome is at the base of a depression in the body containing a ciliature which is more or less complex in appearance but which is predominantly somatic in nature and origin. The cytostome leads to a cytopharyngeal apparatus of the rhabdos type. The oral area in the subclass Hypostomata has, with the major exception of the chonotrichs, moved to the ventral surface and the cytostome may be at the base of a shallow depression known as an **atrium** which may be the forerunner of the buccal cavity which is found in the hymenostome ciliates (see below). The cytostome usually leads to a cytopharyngeal apparatus which is of the cyrtos type. In some, the ciliature around the oral aperture (the **perioral ciliature**) is in the form of an extensive band which is commonly referred to as the **hypostomial frange** (Fig. 2E). This frange may be considerably reduced in some forms to three short **pseudomembranelles** (Fig. 2F) which are located on the left of the oral aperture and may lie within an atrium. In certain species there is even a fourth 'paroral membrane' (Fig. 2F) on the right such that the oral ciliature as a whole at least superficially looks very much like the undulating membrane and three membranelles that are found in the hymenostomes.

Species in the subclass Hymenostomata stand neatly between the 'lower' ciliates above and those that are to be treated below. While there is always a definite oral ciliature, it is usually inconspicuous and composed of only three or four specialised membranes or membranelles which are located in a **buccal cavity**. Commonly there is an **adoral zone of three membranelles** (Fig. 2A) on the left and a single **paroral membrane** on the right. In others there are **peniculi** deep within the buccal cavity, these are compound ciliary organelles in the form of a long band of often short cilia typically found on the left wall of the buccal cavity. Peniculi are responsible for creating the strong feeding currents seen in ciliates such as *Paramecium*. In other hymenostomes, the paroral membrane (sometimes in multiple segments) on the right of the buccal area becomes dominant and the buccal cavity may be distinct or shallow and difficult to distinguish.

Members of the subclass Peritricha are highly specialised and are immediately recognisable. Here the oral field covers the entire apical end of the body and the **peristomial ciliature** (Fig. 2B), which winds anticlockwise and plunges deep into an **infundibulum** towards the cytostome. The final group of ciliates are the spirotrichs and their oral ciliature is dominated by a well developed conspicuous **adoral zone of many membranelles** (AZM) (Fig. 2C) which often extend out onto the surface of the body. These membranelles are used both for feeding and locomotion.

Ciliates feed on a variety of food sources although it is generally in the form of other microorganisms such as bacteria, algae and other protozoa. In
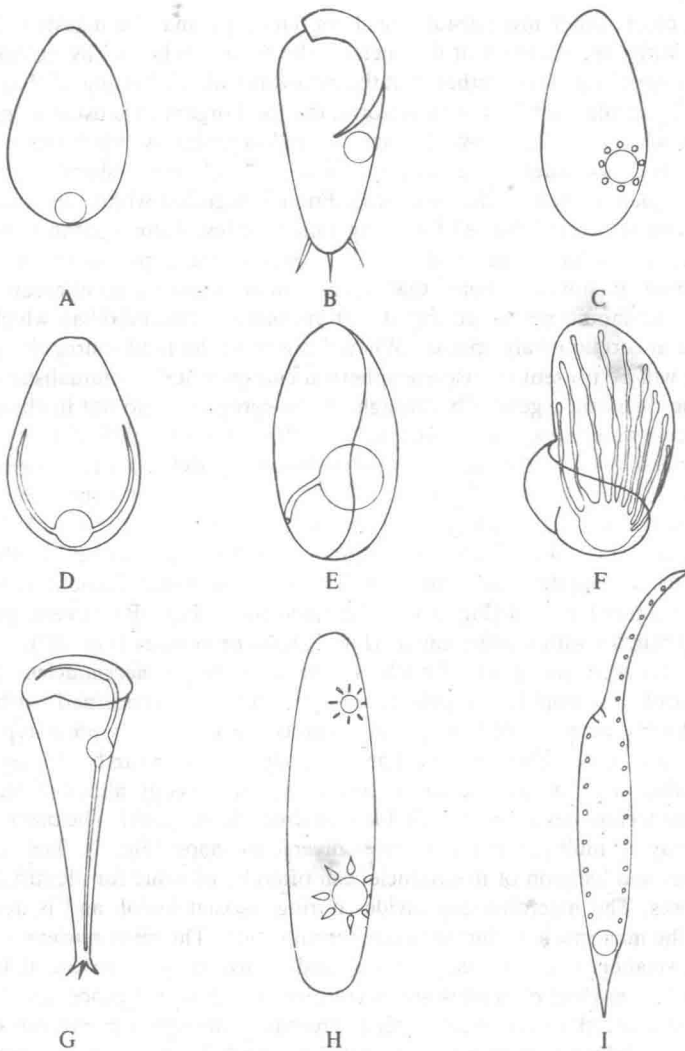
Fig. 3. Contractile vacuoles in ciliates, their location and their accessory structures. A, *Tetrahymena*, single terminal vacuole. B, *Stylonychia*, single lateral vacuole. C, *Bursostoma*, vacuole with satellite vesicles. D, *Climacostomum*, vacuole with two serving canals. E, *Lembadion*, vacuole with expulsion canal. F, *Tillina*, vacuole with several serving canals. G, *Stentor*, vacuole with two long serving canals. H, *Paramecium*, two vacuoles with radiating serving canals. I, *Dileptus*, large terminal and many lateral vacuoles.

rarer cases, other invertebrates such as flatworms may be ingested. In all cases with the exception of the suctoria the food is collected by means of a ciliary current and it is gathered at the cytostome where it is engulfed to form a food vacuole. In the case of suctoria, the food organism is usually another ciliate which is sucked down through the hollow tentacles into the predator's body. **Food vacuoles** are usually spherical although lemon-shaped vacuoles are frequently seen in the peritrichs. Food is digested within the vacuoles which move around the cell following a more or less distinct pathway before undigested remains are voided to the exterior via a permanent pore or **cytoproct**. It should be noted that when a ciliate is feeding upon green algae the latter should not be confused with mutualistic zoochlorellae which are found in certain ciliate species. When algae form the food source the green areas will be present in discrete spherical clumps whereas mutualistic algae will be distributed generally throughout the cytoplasm and not in clumps.

Excess water is expelled from ciliates by the pulsating action of transparent vacuoles known as **contractile vacuoles**. Water is discharged to the exterior via a pore, or pores, which may in some species open into the contractile vacuole via a canal. Contractile vacuoles may be solitary (Fig. 3A–G) or numerous (Fig. 3H–I), and the position, structure and number is often of great use for identification purposes. The most commonly encountered type is the solitary terminal (Fig. 3A) or lateral vacuole (Fig. 3B). Several genera have vacuoles with serving canals (Fig. 3D–G) or vesicles (Fig. 3C).

Ciliates have two kinds of nucleus. The much larger **macronucleus** is the vegetative or trophic nucleus of ciliates and it is concerned with the 'day-to-day running' of the cell. It controls the organism's phenotype. In many cases it is a single spherical or oval body that often can be displayed in the living cells by the use of phase-contrast microscopy although various staining techniques are more reliable (see *Practical methods*). The macronucleus may be multiple and sometimes diverse in shape (Fig. 4). The shape, number and location of macronuclei can often be of value for identification purposes. The macronucleus divides during asexual fission and is derived from the micronucleus during sexual reproduction. The **micronucleus** is very much smaller than the macronucleus and is invariably spherical; it is the generative nucleus of ciliates and is concerned with sexual processes. There may be more than one micronucleus present. Although a ciliate can often survive indefinitely without a micronucleus, and there are several amicronucleate strains of *Tetrahymena* that have been growing for several decades, the presence of a macronucleus is vital to the ciliate's survival.

Most ciliates are able to reproduce both asexually and sexually. Asexual reproduction is the normal method of increasing the numbers of a species within a population and sexual processes serve to ensure the transference of genetic material from one organism to another.

Asexual reproduction in ciliates is usually by **binary fission**; this takes place transversely across the somatic kineties. However, other specialised methods of asexual reproduction, such as **budding**, are known to occur in certain
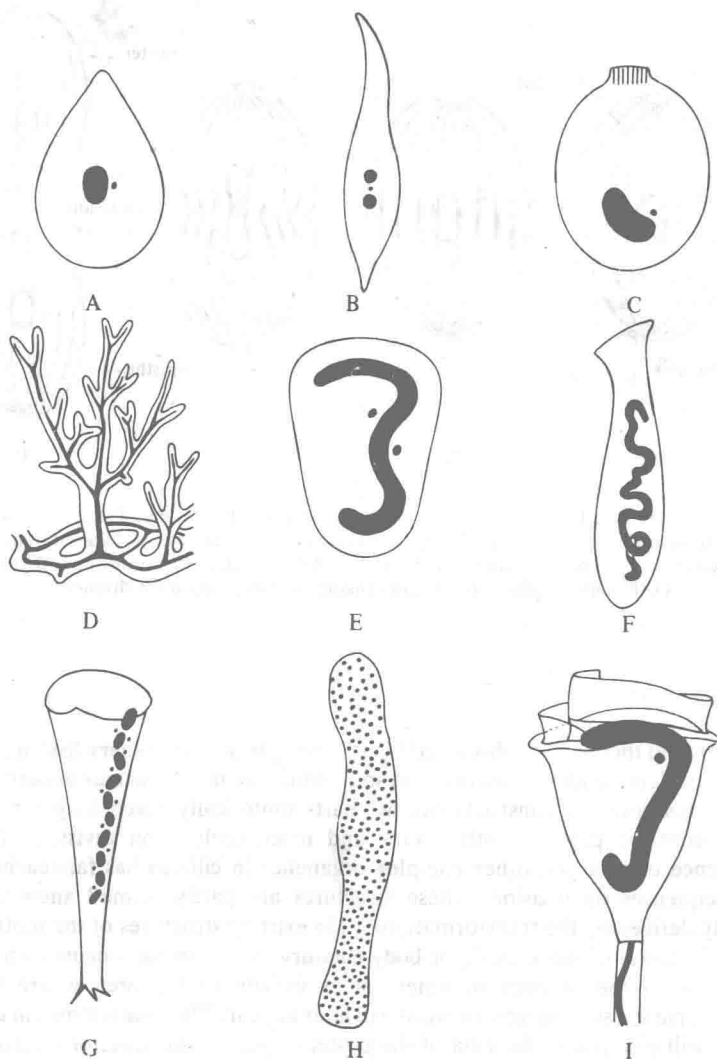
Fig. 4. Macro- and micronuclear diversity in ciliates. A, *Tetrahymena*. B, *Litonotus*. C, *Belonophrya*. D, *Dendrosoma* (only macronucleus shown). E, *Euplotes*. F, *Spathidium* (only macronucleus shown). G, *Stentor*. H, *Myriokaryon* (only macronuclei shown, up to 3000 per cell). I, *Vorticella*.
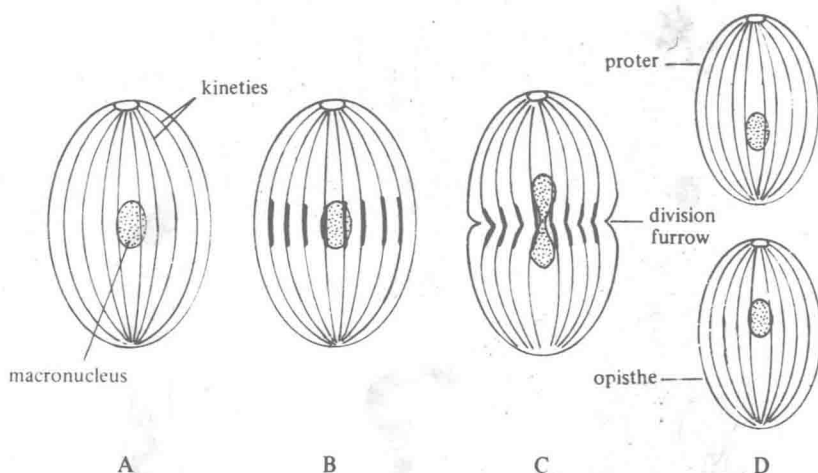
Fig. 5. Transverse binary fission in a primitive ciliate with telokinetal stomatogenesis. A, Ciliate prior to division. B, Ciliate at onset of division showing increased numbers of kinetosomes in equatorial region. C, Ciliate and macronucleus dividing. D, Daughter cells (proter and opisthe) immediately after division.

groups and these will be discussed later. During **transverse binary fission**, the micronucleus divides by mitosis and splits while the macronucleus undergoes DNA synthesis and constricts into two parts amitotically. Each daughter cell thus contains part of both micro- and macronucleus on division. The presence of cilia and other complex organelles in ciliates has far-reaching consequences on division. These structures are partly formed anew and partly derived by the transformation of the existing structures of the mother cell. Renewal of the **somatic** or **body ciliature**, for example, begins with an increase in the number of kinetosomes usually in the area where the transverse division, furrow or waist will later appear. The kinetosomes in this zone will give rise to the cilia of the posterior half of the anterior daughter cell (**proter**) as well as to the anterior ciliature of the posterior daughter (**opisthe**). The formation of the oral ciliature (**stomatogenesis**) varies considerably from group to group but four major methods are commonly recognised.

In the more primitive ciliates (Fig. 5) where there is little or no specialised oral ciliature, either all or some of the kinetosomes of the somatic kineties at the apex and equator are involved. Those in the equator which are transected at fission simply turn in at the anterior end of the forming opisthe to produce the new oral area. This type of stomatogenesis is called **telokinetal**. In the more advanced groups a single somatic kinety or several somatic kineties in