Protozoa

Introduction

Protozoa (meaning "first animals") are heterotrophic, single-celled or colonial eukaryotes. Individuals are microscopic and range in size from a few to hundreds of micrometers, depending on the species. Most protozoa are animal-like (heterotrophic) because their carbon and energy must be obtained by eating or absorbing organic compounds originating from other living organisms. As eukaryotes they have several **organelles** , including at least one **nucleus** that contains most of the cell's deoxyribonucleic acid (DNA).

Beyond this broad description, it is difficult to define protozoa because they are so diverse and only distantly related to each other. While the term "protozoa" is commonly used, it has little basis in evolutionary history, or phylogeny, of these organisms. Taxonomic systems try to assign organisms to a **monophyletic** group, that is, one that includes an ancestor and all of its descendants. Plants, animals, and fungi are monophyletic groups; protozoans are not. (The understanding of evolutionary relationships of uni-cellular eukaryotes is in a state of flux.) Further complicating a precise definition of protozoa is the close relationship between some protozoa and unicellular algae. Modern taxonomic treatments recognize these similarities and group protozoa, photosynthetic unicellular algae, and slime molds together as **protists** or protoctists. Whichever term one prefers, the classification is not monophyletic. Despite the fact that protozoa is not a proper taxonomic name, it is a useful, functional term. Ecologists differentiate between autotrophic and heterotrophic components of an ecosystem, and it is natural to separate the animal-like protozoa from the photosynthetic algae based on their nutritional mode. (However, *Euglena,* which can be induced to lose their chloroplast, illustrate why unicellular algae are included with protozoa.) .

General characteristics

1- Organisms in which the individual is a single cell, i.e. consists of a single undivided mass of protoplasm which is capable of independent existence in a suitable environment; if many such individuals be combined together to form a **colony**, as frequently occurs, there is no differentiation of the individuals except for reproductive purposes, and never for tissue-formation as in the Metazoa.

2- The body always contains chromatin or nuclear substance, which may be disposed in various ways, but usually forms one or more concentrated masses termed nuclei, which can be distinguished sharply from the general body protoplasm or cytoplasm. The protoplasmic body may be naked at the surface, or may be limited and enclosed by a distinct envelope or cell-membrane, which is not usually of the nature of cellulose, except in holophytic forms. the differentiation of the body-substance into nucleus and cytoplasm separates them at once from the Bacteria, in which the chromatin is distributed evenly through the body protoplasm.

3- Organs serving for locomotion and for the capture and assimilation of solid food are usually present, but may be wanting altogether when the mode of nutrition is other than holozoic; chlorophyll, on the other hand, is only found as a constituent of the body-substance in the holophytic Flagellata.'

4- most protozoa are motile (able to move). The way they move is one of the important characteristics historically used to divide them into major groups: **amoebae, flagellates**, and **ciliates**. **Apicomplexa**, formerly called **Sporazoa**, is a fourth group of generally obligate parasitic protozoa.

-Amoebae crawl along surfaces by extending a cytoplasm -filled **pseudopod** (false-foot) that bulges outward from any edge of the cell.

- **Flagellates and ciliates** use specialized organelles, flagella and cilia, that differ primarily in length and number, to propel the cells through water.

Flagella are whiplike structures that usually occur one to a few per cell and have an undulating motion.

Cilia are shorter and move in concert, like oars, with alternating power and recovery strokes. Sporozoa are either nonmotile or very slow.

5- Most protozoa reproduce most of the time by **equal binary fission**, in which a cell divides into two daughter cells after the chromosomes have been duplicated and distributed between them. This asexual mode of reproduction leads to rapid population growth of a clone of genetically identical cells. To these characters it may be added that reproduction is effected by some form of fission (**multiple fission**), or division of the body into smaller portions.

6- However, sex is widespread in protozoa and complicated life histories do exist. Sexuality is associated with environmental change and interrupts asexual reproduction; sex in protozoa usually marks the end of the existence of a genetically unique individual, when it becomes the gamete (reproductive cell). In the vast majority of Protozoa, if not in all, a process of **conjugation** or **syngamy** occurs at some period in the life-cycle, the essential feature of the process being fusion of nuclear matter from distinct individuals.

7- Other organelles that are widely distributed among protozoa include

a- **food vacuoles**, in which ingested particles are digested, and **lysosomes** that fuse with food vacuoles and supply digestive enzymes.

b- **Contractile vacuoles**, common in freshwater protozoa, eliminate water that moves into the cells by **osmosis** .

c- **Extrusomes** are associated with the membrane of many protozoa and contain material that can be ejected from the cell. Some extrusomes secrete an amorphous material that is involved in formation of a capsule or cyst, and others discharge a pointed projectile that may serve for protection or predation.

d- The thousands of "**trichocysts**" distributed over the surface of the ciliate *Paramecium* are extrusomes that discharge rapidly in response to physical stimulation and are probably effective deterrents to some predators.

e- Ciliates are unique among protozoa in having two kinds of nuclei: the **micronucleus,** which is involved only in sexual reproduction; and the **macronucleus,** which is involved only in the production of messenger ribonucleic acid (mRNA) for cell function.

8- Protozoa are ubiquitous (found everywhere); they are present in all aquatic or moist environments, and their cysts can be found in even the most inhospitable parts of the biosphere. Most are free-living and eat bacteria, algae, or other protozoa. Protozoa are important components of aquatic and soil ecosystems, where they eat bacteria that are too small to be efficiently captured by most animals and are in turn eaten by other organisms. **Bacterivorous** protozoa also are abundant in activated sludge sewage treatment plants and, in fact, are necessary for their proper functioning. There are several protozoa of medical and economic importance. Examples include the flagellate ***Trypanosoma***, which causes African sleeping sickness; the amoeba ***Entamoeba histolytica***, which can attack the intestinal wall and cause amoebic dysentery, and the sporozoans of the ***Plasmodium*** species, which cause malaria.

Body wall

The body-form may be constant or inconstant in the Protozoa, according as the **body-substance** is or is not limited at the surface by a firm envelope or cuticle. When the surface of the protoplasm is :

1- **naked**, as in the common amoeba and allied organisms, the movements of the animal bring about continual changes of form. The protoplasm flows out at any point into processes termed ***pseudopodia***, which are being continually retracted and formed anew. Such movements are known as **amoeboid**, and may be seen in the cells of Metazoa as well as in Protozoa. The pseudopodia serve both for locomotion and for the capture of food. If equally developed on all sides of the body, the animal as a whole remains stationary, but if formed more on one side than the other, the mass of the body shifts its position in that direction, but the movement of translation is generally slow. If the animal remains perfectly quiescent and inactive, the laws of surface-tension acting upon the semifluid protoplasmic body cause it to assume a simple spherical form, which is also the type of body-form generally characteristic of Protozoa of floating habit (Radiolaria, Heliozoa).

2- In the majority of Protozoa, however, the protoplasm is limited at the surface by a firm membrane or **cuticle**, and in consequence the body has a definite form, which varies greatly in different species, according to the habit of life. As a general rule those forms that are fixed and sedentary in habit tend towards a radially symmetrical structure; those that are **free swimming** approach to an ovoid form, with the longest axis of the body placed in the direction of movement; and those that **creep** upon a firm substratum have the lower side of the body flattened, so that dorsal and ventral surfaces can be distinguished; it is very rare, however, to find a **bilaterally symmetrical type** of body-structure amongst these organisms. In some cases the cuticle may be too thin to check completely the changes of form due to the movements of the underlying protoplasm; instances of this are seen amongst the so-called" metabolic "Flagellata, in which the body exhibits continually changes of form, termed euglenoid "movements, due to the activity of the superficial contractile layer of the body manifesting itself in ring-like contractions passing down the body in a manner similar to the peristaltic movements of the intestine.

Body-substance

The body-substance of the Protozoa is **protoplasm**, or, as it was originally termed ,**sarcode**, which is finely alveolar in structure, the diameter of the alveoli varying generally between. At the surface of the body the alveoli may take on a definite honeycomb-like arrangement, forming a special" alveolar layer "which in optical section appears radially striated. Besides. the minute protoplasmic alveoli, the protoplasm often shows a **coarse vacuolation** throughout the whole or a part of its substance, giving the body a frothy structure. When such vacuoles are present they must be carefully distinguished from the contractile vacuoles and food-vacuoles ,from the former they differ by their non-contractile nature, and from the latter by not containing food-substances.

In many Protozoa and especially in those forms in which there is no cuticle, the body may be supported by a **skeleton.** The material of the skeleton differs greatly in different cases, and may be wholly of an organic nature, or may be impregnated with, or almost entirely composed of, inorganic mineral salts, in which case the skeletal substance is usually either silica or carbonate of lime. From the morphological point of view the skeletons of Protozoa may be divided into two principal classes, according as they are formed internal to, or external to, the body in each case.

1- **Internal skeletons** are best seen in the spherical floating forms comprised in the orders Radiolaria and Heliozoa; such skeletons usually take the form of spicules, radiating from the centre to the circumference; and often further strengthened by the formation of tangential bars, producing by their union a lattice-work, which in species of relatively large size may be formed periodically at the surface as the animal grows so that the entire skeleton takes the form of concentric hollow spheres held together by radiating beams. The architectural types of these skeletons show, however, an almost infinite diversity, and cannot be summarized briefly.

2- **External skeletons** have usually the form of a shell or house, into which the body can be retracted for protection, and from which the protoplasm can issue forth during the animal's phases of activity. Shells of this kind, which must be carefully distinguished from cuticles or other membranes that invest the body closely, are well seen in the order Foraminifera; in the simplest cases they are monaxon in architecture, that is to say, with one principal axis round which the shell is radially symmetrical, and at one pole is a large aperture through which the protoplasm can creep out. In addition to the principal aperture, the shell may or may not be pierced all, over by numerous fine pores, through which also the protoplasm can pass out.

Protoplasmic body

The **protoplasmic body** of the Protozoa is frequently differentiated into two zones or regions: a more external, termed the **ectoplasm** or ectosarc, and a more internal, termed the **endoplasm** or endosarc. The ectosarc is distinguished by being more clear and hyaline in appearance, and more tough and viscid in consistence; the endoplasm, on the other hand, is more granular and opaque, and of a more fluid nature. The ectoplasm is the protective layer of the body, and is also the portion most concerned in movement, in excretion, and perhaps also in sensation and in functions similar to those performed by the nervous systems of higher animals. The endoplasm, on the other hand, is the chief seat of digestive and reproductive functions.

As the protective layer of the body, the ectoplasm forms the envelopes or membranes which invest the surface of the body, and which are differentiations of the outermost layer of the ectoplasm. Thus in most Flagellata the ectoplasm is represented only by the more or less firm outer covering or **periplast.** Even when such envelopes are absent, however, the ectoplasm can still be seen to exert a protective function; as, for instance, in those Myxosporidia which are parasitic in the gall-bladders or urinary bladders of their hosts, and which can resist the action of the juices in which they live so long as the ectoplasm is intact, but succumb to the action of the medium if the ectoplasm be injured. In many Infusoria the ectoplasm contains special organs of offence termed **trichocysts**, each a minute ovoid body from which, on stimulation, a thread is shot out, in a manner similar to the nematocysts of Cnidaria. Similar organs are seen also in the spores of Myxosporidia, as the so-called **polar capsules**; but in this case the organs are not specially ectoplasmic, and appear to serve for adhesion and attachment, rather than for offence.

The connexion of the ectoplasm with movement is seen in the simplest forms, such as *Amoeba*, by the fact that all pseudopodia arise from it in the first instance. In forms with a definite cuticle, on the other hand, the ectoplasm usually contains contractile fibres or myonemes, forming, as it were, the muscular system of the organism. The dependence of the motility of the animal upon the development of the ectoplasm is well seen in Gregarines, in which other organs of locomotion are absent; in forms endowed with active powers of locomotion a distinct ectoplasmic layer is present below the cuticle; in those Gregarines incapable of active movement, on the other hand, the ectoplasm is absent or scarcely recognizable. From the ectoplasm arise the special organs of locomotion, which, when present, take the form of pseudopodia, flagella or cilia.

Pseudopodia, as already explained, are temporary protoplasmic organs which can be extruded or retracted at any point; they fall naturally into two principal types, between which, however, transitions are to be found:

1-first, slender, filamentous or ***filose*** pseudopodia, composed of ectoplasm alone, which may rem ain separate from one another, or may anastomose to form networks, and are then termed ***reticulose***. Filose pseudopodia are more adapted for the function of capturing food.

2-secondly, thick, blunt, so-called ***lobose*** pseudopodia, which are composed of ectoplasm with a core of endoplasm, and never form networks. In forms showing active locomotor powers the pseudopodia are usually more lobose in type.

Flagella

are long, slender, vibratile filaments, generally few in number when present, and usually placed at the pole of the body which is anterior in progression. Each flagellum performs peculiar lashing movements which cause the body, if free, to be dragged along after the flagellum in jerks or leaps; if, however, the body be fixed, the action of the flagellum or flagella causes a current towards it, by which means the animal obtains its food-supply. A flagellum which is anterior in movement has been distinguished by the convenient term ***tractellum****;* sometimes, however, the flagellum is posterior in movement and acts as a propeller, like the tail of a fish; this type is the termed ***pulsellum***. The flagellum appears to arise in all cases from a distinct **basal granule**, and in some cases, as in the genus

Trypanosoma, there is a portion of the nuclear apparatus set apart as a distinct kinetic nucleus, with the function, apparently, of governing the activities of the flagellum.

Cilia

are minute, hair-like extensions of the ectoplasm, which pierce the cuticle and form typically a furry covering to the body. Though perhaps primitively derived from flagella, cilia, in their usual form, are distinguished from flagella by being of smaller size, by being present, as a rule, in much greater numbers, and above all by the character of their movements. In the place of the complicated lashing movements of the flagella, each cilium performs a simple stroke in one direction, becoming first bowed on one side, by an act of contraction, and then straightened out again when relaxed. The movements of the cilia are coordinated and they act in concert, though not absolutely in unison, each one contracting just before or after its neighbour, so that waves of movement pass over a ciliated surface in a given direction, similar to what may be seen in a cornfield when the wind is blowing over it. Primitively coating the whole surface of the body evenly, the cilia may become modified and specialized in various ways.

Undulating membranes

Besides the organs of locomotion already mentioned, there may be present so-called undulating membranes, in the form of thin sheets of ectoplasm which are capable of performing: sinuous, undulating movements by their inherent contractility. In some cases distinct contractile threads or myonemes have been described in these membranes. Undulating membranes appear to be formed either by the fusion together of a row of cilia, side by side, or by the attachment of a flagellum to the body by means of an ectoplasmic web, in which case the flagellum forms the free edge of the membrane, as in the genus. *Trypanosome.*

Ectoplasm

Returning to the ectoplasm, the excretory function exerted by this layer is seen by the formation in it of the peculiar **contractile vacuoles** found in most free-living Protozoa. A contractile vacuole is a spherical drop of watery fluid which makes - its appearance periodically at some particular spot near the surface of the animal's body, or, if more than one such vacuole is present, at several definite and constant places. Each vacuole grows to a certain size, and when it has reached the limit of its growth it discharges its contents to the exterior by a sudden and rapid contraction. There is, apparently, in most. if not in all cases, a definite pore through which the contractile vacuole empties itself to the exterior. On account of the relatively large size which the contractile vacuole attains it bulges inwards beyond the limits of the ectoplasm and comes, to lie chiefly in the endoplasm, to which it is sometimes, but erroneously, ascribed. In the most highly differentiated Protozoa, for instance, the Ciliata, the ectoplasm contains an apparatus of excretory channels, situated in its deeper layers, and forming as it were a drainage-system, from which the contractile vacuoles are fed. The fluid discharged by the contractile vacuoles appears to be chiefly water which has been absorbed at the surface of the protoplasmic body, and which has filtered through the protoplasm, taking up the soluble waste nitrogenous products - of the metabolism and the gaseous products of respiration; hence the contractile vacuoles may be compared in a general way to the urinary and respiratory organs of the Metazoa.

One of the first consequences of the parasitic habit of life is - the disappearance of the contractile vacuoles, which are hardly ever found in truly parasitic Protozoa, that is to say, in forms which live in the interior of other animals and nourish themselves at their expense. They are also very frequently absent in marine forms.

Mechanisms of a nervous nature are very seldom found in Protozoa, but in some Ciliata special tactile bristles are found, and it is possible that flagella, and perhaps even pseudopodia, may be sometimes tactile rather than locomotor in function. Pigment-spots, apparently sensitive to light, may also occur in some Flagellata.

Endoplasm

The endoplasm, as already stated, is the chief seat of nutritive and reproductive processes. In many Flagellata the ectoplasm is represented only by the thin envelope or periplast, so that the tvhole body is practically endoplasm. When the two layers are well differentiated the endoplasm is more fluid and coarsely granular, and contains various organs, chief amongst them in importance being the nucleus, which must be considered specially and may be put aside for the present.

In considering the functions of ingestion and assimilation of food a distinction must be drawn between those Protozoa which absorb solid food-particles, that is to say, which are holozoic in habit, and those which, being holophytic, saprophytic or parasitic in habit, absorb their nourishment in a state of solution. Only in holozoic forms is a special apparatus found for ingestion or digestion of food; in all other forms nutriment is absorbed by osmosis through the body-wall, presumably at any point of the surface. In holozoic forms we must distinguish further those in which the protoplasm is naked at the surface from those in which the body is clothed by a firm cuticle or cell membrane. In naked forms food-particles are taken in at any point of the body-surface, either by means of the pseudopodia, 'or by the action of flagella causing them to impinge upon the surface of the body. In either case the food is absorbed by the protoplasm simply flowing round it and engulfing it, and the food passes into the interior of the body in a tiny droplet of water forming what is termed a **food-vacuole**. Into the food-vacuole the surrounding protoplasm secretes digestive enzymes, so that each such vacuole represents a minute digestive cavity, in which the food is slowly digested, rendered soluble, and absorbed by the surrounding protoplasm. The insoluble residue of the food is finally rejected by expelling the food-vacuole and its contents from the surface of the body at any convenient point.

The simple process of food-absorption described above for the more primitive naked forms is necessarily modified in detail, though not in principle, in corticate Protozoa, that is to say, in forms provided with a cuticle. In the first place, it becomes necessary to have a special aperture for the ingestion of food, a cell-mouth or ***cytostonae.*** Primitively the **cytostome** is a simple pore or interruption of the cuticle, but in forms more highly evolved the aperture is prolonged inwards in the form of a tube lined by ectosarc and cuticle, forming a gullet or oesophagus which ends in the endoplasm. Food-particles are forced by the action of cilia or flagella down the oesophagus and collect at the bottom of it in a droplet of water which, after reaching a certain size, passes into the endoplasm as a **food vacuole** in which the food is digested. For rejection of the insoluble residue of the food-vacuoles, a special pore or cell-anus (***cytopyge*)** may be present. In the Ciliata there is often a distinct anal tube visible at all times, but as a rule the anus is only visible at the moment that faecal matter is being ejected from it, though fine sections show that the pore is a constant one. In the higher Flagellata, on the other hand, the oesophageal ingrowth forms commonly a sort of cloacal cavity, into which the contractile vacuole or vacuoles discharge themselves, and into which also the food-vacuoles evacuate their residues.

Besides the food-vacuoles already described, and the nuclear apparatus presently to be dealt with, the endoplasm may contain various **metaplastic products**, that is to say, bodies to be regarded as stages in the upward or downward metabolism of the protoplasmic substance. Such substances may take the form of coarse granules of various kinds, crystals, vacuoles or droplets of fatty or oily nature, pigment-grains, and other bodies. In the holophytic Flagellata the endoplasm contains also various organs proper to the vegetable cell, such as chlorophyll-bodies (chromatophores), pyrenoids, grains of a starchy nature (paramylum), and so forth, which need not be described here in detail.

The Nucleus

The nucleus in Protozoa is usually a compact, fairly conspicuous structure, composed of chromatin combined in various ways with an achromatic substance or substances. Sometimes the chromatin is distributed in smaller masses through the nucleus, producing a granular type of nucleus; more often the chromatin is more or less concentrated in a central mass forming a so-called **karyosome**, consisting of an achromatic plastinoid substance impregnated with chromatin. If the karyosome is large and there is very little chromatin between it and the nuclear membrane, the nucleus is of the type termed **vesicular**. A nuclear membrane is not, however, always present, and true **nucleoli**, of the type found in the nuclei of metazoan cells, are not found in Protozoa.

A given individual may have more than one nucleus, and the number present may amount to many thousands, as ' in the plasmodia of **Mycetozoa**. In such cases the nuclei may be all of one kind, that is to say, not markedly different in size, structure or function, so far as can be seen; or there may be a pronounced morphological differentiation of the nuclei correlated with a difference of function. Thus in the class **Infusoria** two nuclei are found in each individual; a **macronucleus** which is somatic in function, that is to say, which regulates the metabolism and vital processes of the body generally, and the **micronucleus**, which is generative in function, that is to say, which remains in reserve during the ordinary, " vegetative "life of the organism and becomes active during the act of syngamy, after which the macronucleus is absorbed or cast out and a new somatic nucleus is formed from portions of the micronuclei which have undergone fusion in the sexual act. Thus the micronucleus of the **Infusoria** can be compared in a general way with the germ-plasm of the Metazoa, like which it remains inactive until the sexual union. On the other hand, in some Flagellata a differentiation of the nucleus of quite a different type is seen, a smaller, kinetic nucleus being separated off from the larger, trophic or principal nucleus. The kinetic nucleus has the function, apparently, of controlling the locomotor apparatus, so that the specialization of these two nuclei is of a kind quite different from that seen in the **Infusoria.**

Besides the nuclear substance which is concentrated to form the principal nucleus or nuclei, there may be present also **extranuclear granules** of chromatin, so-called **chromidia,** scattered throughout the whole or some part of the protoplasmic body. Chromidia may be normally present in addition to the principal nucleus, or may be formed from the principal nucleus during certain phases of the life-cycle. In some cases the entire nucleus may become resolved temporarily into chromidia, from which a new nucleus may be formed again later by condensation and concentration of the scattered granules. When the chromidia are numerous and closely packed they may form a so-called **chromidial network** . Recent observations on the reproduction of some Sarcodina have shown that the chromidia may possess great importance in the life-cycle as representing generative chromatin which, like the micronucleus of the Infusoria mentioned above, remains in reserve until, by the process of syngamy, the nuclear apparatus is renewed; while the principal nuclei represent, like the macronuclei, somatic or vegetative chromatin which becomes effete and is cast off or absorbed when syngamy takes place.

The nuclear apparatus may be supplemented by other bodies of which the nature is not always clear. Such is the socalled" Nebenkern "of *Paramoeba eilhardi,* apparently of the nature of a centrosome. Sometimes the karyosome acts like a centrosome during the division of the nucleus, and sometimes true centrosomes are present. Flagella also commonly arise from basal granules of a centrosoinic nature, blepharoplasts in the correct sense of the term; these blepharoplasts are always in connexion with the nucleus, or with the kinetic nucleus if there is one distinct from the trophic nucleus, as in the genus *Trypanosoma* and allied forms.

Reproduction of the Protozoa

The mode of reproduction in these organisms is the same as that of the cell generally, and takes always the form of fission of some kind; that is to say, of division of the body into smaller portions, each of which represents a young individual. The division of the body is preceded by that of the nucleus, if single, or of each nucleus in the cases where there are two different nuclei; if, however, more than one nucleus of the same kind be present, the nuclei may be simply shared amongst the daughter-individuals, this mode of division being known as **plasmotomy**. Other organs of the body may either, like the nucleus, undergo fission, or may be formed afresh in the daughter-individuals.

The division of the nucleus in Protozoa may take place by the direct method or by means of mitosis.

1- **Direct division**, without mitosis, is of very common occurrence; the division may be simple or multiple, that is to say, into only two parts, or into a number of fragments formed simultaneously. An extreme case of multiple fission is seen in the formation of the **microgametes** of *Coccidium schubergi,* where the nucleus breaks up into a great number of chromidia, which become concentrated in patches to form the several daughter-nuclei. In some cases, on the other hand, multiple daughter-nuclei are formed by **rapidly repeated** simple division of the parent nucleus. The mode of division may be different in different nuclei of the same individual; thus in the Infusoria the macronucleus divides by direct division, the micronucleus by mitosis.

2- **The mitosis** of the Protozoa is far from being of the uniform stereotyped pattern seen in the Metazoa, but, as might have been expected, often shows a much simpler and more primitive condition. **Centrosomes** are often absent, and their place may be taken, as stated above, by other bodies. The nuclear membrane may be retained throughout the mitosis. Definite chromosomes can, as a rule, be made out, but the chromosomes are often very numerous and minute, without definite form, and divide irregularly.

Binary fission

The simplest method of fission in Protozoa is that termed **binary**, where the body divides into two halves, which may be equal and similar, so that the result is two sister-individuals impossible to distinguish as parent and offspring.

Gemmation or Budding

In many cases of binary fission, however, the resulting daughter-individuals may be markedly unequal in size, so that one may be distinguished as the parent, the other as the offspring. If the daughter individual be relatively very small, and formed in a more or less imperfect condition at first, the process is termed gemmation or budding. The buds formed in this way may be either external, formed on the surface of the body, or internal, that is, formed in special internal cavities, from which the offspring are later set free, as in many Acinetaria.

Multiple fission

Gemmation may be correlated with multiple nuclear fission in such a way that buds are formed over the whole body surface of the organism, which thereby undergoes a process of simultaneous multiple fission into numerous daughter-individuals. Rapid multiple fission of this kind is termed **sporulation**, and is a form of reproduction which is of common occurrence, especially in parasitic forms. Usually the central portion of the parent body remains over as a residual body (*Restharper*), but sometimes the parent organism is entirely resolved into the daughter-individuals, which are termed **spores**.

Life-cycles of the Protozoa

It is probable that in all Protozoa, as in the Metazoa, the life-history takes its course in a series of recurrent cycles of greater or less extent, a fixed point, as it were, in the cycle being marked by the act of **syngamy** or **conjugation**.

The life-cycle of a given species may be very simple or it may be extremely complex, the organism occurring under many different forms at different phases or periods of its development. The **polymorphism** of the Protozoa is best considered under three categories, according to the three main causes to which it is due, namely,

1- polymorphism due to adaptation to different conditions of existence
2- polymorphism due to differences of size and structure during growth
3- olymorphism due to the differentiation of individuals in connexion with the process of syngamy or sexual conjugation.

1- **Polymorphism in Relation to Life-conditions**.--*As* a protection against unfavourable conditions, or for other reasons, most Protozoa have the power of passing into a resting condition, during which the vital functions may be wholly or in part suspended. In the resting phase the animal usually becomes enveloped in a resistant membrance or **cyst** secreted by it, and is then said to be encysted. The formation of a cyst may be a response to conditions of various kinds. Very commonly it is formed to protect the organism against a change of medium, as in the case of freshwater forms liable to desiccation, or of parasites about to pass out of the bodies of their hosts. In other cases the organism passes into the resting state in order to absorb ingested nutriment or in order to enter upon reproductive phases.

As a preparation for encystment,

1- organs of locomotion, if present, are retracted or cast off;

2-contractile vacuoles cease to be formed; and
3-the food-vacuoles disappear, usually by digestion of their contents and rejection of the waste residue.
4-The body becomes rounded off and more or less spherical in form, and the protoplasm becomes denser, that is, less fluid and more opaque, but at the same time of diminished specific gravity, by loss of water.

5-The cyst is then secreted at the surface as a layer of varying thickness and toughness.

In the encysted condition many Protozoa are capable of being transported by the wind, a fact which explains their appearance in infusions and liquids exposed to the air. In favourable conditions the cysts germinate, that is to say, the envelope is dissolved and the contained organism or organisms are set free to enter upon the strenuous life once more.

In the Mycetozoa, organisms adapted to a semi-terrestrial life in moist surroundings, the protoplasm is capable, when desiccated, of passing into a tough condition resembling sealing wax, which, when moistened, assumes again its normal appearance and active condition.

Resting phases, analogous to encystment, are seen in the spores of various forms, especially those of parasitic habit, which are commonly enclosed in tough, resistant envelopes or sporocysts, and enveloped as a protection against change of medium or of host. Within the sporocyst multiplication of the sporoplasm may take place to form more or fewer sporozoites. The sporocysts usually show definite symmetry and structure; infinitely variable in different species. In a suitable medium the spores germinate by rupture of the sporocysts and escape of the contents.

2- **Polymorphism in Relation to Growth and Development**.--In many species of Protozoa there is hardly any difference to be observed between different individuals during their active phases except in size. Those individuals about to multiply by fission are slightly above the normal in dimensions; on the other hand, those resulting from recent fission will be smaller than the average; and such differences are, it need hardly be said, more pronounced when the fission is of the unequal binary type, or in cases of gemmation or multiple fission. In cases also where a given strain of a species is becoming senile, it is sometimes observed that the individuals are markedly undersized on the average.

On the other hand, it is often the case that the young individuals resulting from a recent act of multiplication may differ from adult individuals of the species, not merely in size, but in structural characters, to such an extent that their relationship to the adult forms could not be determined by simple inspection without other evidence. This is especially true of those species in which multiplication by sporulation occurs, giving rise to numerous small spores which may at first be in a resting condition, enveloped in protective sporocysts, but which sooner or later become free, motile individuals known technically as swarmspores. Thus in many Sarcodina the adult is a large amoeboid organism which produces by sporulation a great number of relatively minute swarm-spores. These may be either, as in the common *Amoeba proteus,* amoeboid organisms, so-called amoebulae or pseudopodiospores, or, as in the Foraminifera and Radiolaria, flagellated organisms, so-called flagellulae or flagellispores. Sometimes, as in many Mycetozoa, amoeboid and flagellated phases may succeed each ether rapidly in the development of the swarm-spores. The familiar *Noctiluca miliaris* is another instance of a species which produces by sporulation numerous tiny swarm-spores quite different from the parent form in their characters. Such instances could be multiplied indefinitely amongst the Protozoa.

When the young individuals differ greatly from the adults in structure and appearance they may be regarded as larval forms, and it is interesting to note that such forms appear to be just as much recapitulative, in the phylogenetic sense, as are the larvae of many Metazoa. A striking instance is that of the Acinetaria, in which the swarm-spores produced by gemmation are ciliated, and thus betray affinities with the Ciliata which could hardly be suspected from a study of the adult forms alone.

Similarly, in the genus *Trypanosoma,* the young forms often show a *Herpetomonas-like* structure which is probably of phyletic significance. The swarm-spores of Sarcodina and of *Noctiluca* mentioned above can, perhaps, be regarded in the same light. On the other hand, many larval forms cannot be considered as exhibiting recapitulative characters, but merely as adaptations to environment or other special life-conditions. This is especially true, as in Metazoa, of parasitic forms, subject as they are to great vicissitudes, to cope with which the most finely adjusted adaptations are necessary on the part of the organism.

3- **Polymorphism in Relation to Sex**. - In all Protozoa of which the life-cycle has been made known in its entire course, a process of **syngamy** or sexual union has been found to occur. There are still many forms in which syngamy remains to be discovered. It is quite possible, therefore, that Protozoa exist in which syngamy does not occur. In view, however, of the widespread occurrence of sexual processes amongst unicellular organisms, both of animal and vegetable nature, and the fact that extended observation continually brings to light new instances of this kind, it is safer, in cases amongst the Protozoa in which syngamy is not known to occur, to explain its apparent absence by the imperfections of the present state of our knowledge, than to suppose that in such forms sexual phenomena are entirely lacking in the life-cycle. The process of syngamy, though greatly diversified in different forms, consists essentially of one and the same process in all cases; namely, the fusion of nuclear matter from two distinct individuals. Hence true syngamy may be distinguished as **karyogamy** from the process of **plastogamy,** or fusion of the protoplasmic bodies.

. The individuals whose nuclei undergo fusion are termed ***gametes***. They may be in no way different from each other or from ordinary individuals of the species, or, on the other hand, they may be highly differentiated in size, form and structure. The two gametes may undergo complete fusion into one body, thus giving rise to an individual termed generally a **zygote** or **copula**, but which may bear special names in special cases (e.g. **vermicule** or **ookinete** of the malarial parasites.); such a process is termed sometimes copulation.

On the other hand, the bodies of the two gametes may remain distinct, and portions of the nucleus of each be exchanged between them; to this condition the term **conjugation** is sometimes specially applied. The act of syngamy may be performed in the free condition, or in the resting state, within a cyst.

The significance of syngamy has been much discussed, and it is very difficult to make positive statements upon this point. By comparing the life-cycles of different forms it is found that syngamy sometimes precedes, sometimes follows, a period of great reproductive activity on the part of the organism. Thus in such a form as *Noctiluca,* syngamy between two full-grown individuals is followed by rapid sporulation and the production of a swarm of young individuals; on the other hand, in Foraminifera and Radiolaria, rapid sporulation of adult individuals produces a numerous progeny of young forms which may go through the process of syngamy and produce zygotes that simply grow into the adult form. Comparing these two types of development, instances of which might be greatly multiplied, it is seen that in one case syngamy follows a period of growth and precedes a period of proliferation in the life-cycle, and that in the other case exactly the reverse is true. Hence it follows that syngamy must not be regarded as in any way specially connected with reproduction, but must be considered in its relation to the lifecycle as a whole, and in those instances in which syngamy is followed by increased reproductive activity the explanation must be sought in the general physiological effects of the sexual process upon the vital powers of the organism.

In the Metazoa the sexual process is always related to the production of a new individual, that is to say, of a multicellular organism for which there is no analogy amongst the Protozoa, although an approach to the Metazoan condition is seen in colony-forming Flagellata, such as *Volvox* and its allies. The reproduction of Protozoa is analogous to the ordinary process of cell-division and multiplication which is going on at all times in the bodies of the Metazoa, and which can be observed in the production of the gametes; that is to say, in the period of the lifecycle immediately preceding the sexual process in the Metazoa, just as much as in the developmental phases which follow syngamy and result in the building up of a new Metazoan individual. Hence, so far as the Protozoa are concerned, the phrase" sexual reproduction "is an incongruous combination of words; reproduction and sex are two distinct things, not necessarily related or in any direct causal connexion; and in order to arrive at any theory of sex it is necessary first of all to clear away all misconceptions or preconceived notions arising from analogies with the multicellular Metazoan individual.

Many observations indicate that the vital powers of the Protozoa become gradually weakened, and the individual tends to become senile and effete, unless the process of syngamy intervenes. The immediate result of the sexual union is a renewal of the vitality, a rejuvenescence, which manifests itself in enhanced powers of metabolism, growth and reproduction. These facts have been most studied in the Ciliata. It is observed that if these organisms be prevented from conjugating with others of their kind they become senile and finally die off. It has been found by G. N. Calkins, however, that if the senile individuals be given a change of medium and nourishment, their vigour may be renewed and their life prolonged for a time, though not indefinitely; there comes a period when artificial methods fail and only the natural process of syngamy can enable them to prolong their existence. The results obtained by Calkins are of great interest, as indicating that under special conditions of the environment the necessity for the sexual process may be diminished and the event may be deferred for a long time, if not indefinitely. Hence it is quite possible that in many Protozoa the process of syngamy may be in abeyance, just as there are plants which can be propagated indefinitely by suckers or cuttings without ever setting seed; and it is possible that the inoculative or artificial transmission of parasitic Protozoa from one host to another, as in the case of pathogenic trypanosomes, without any apparent diminution in their vital powers, is an instance of this kind.

As a general rule, in order that syngamy may be attended by beneficial results to the organism, it is necessary that the two conjugating individuals should be from different strains, that is to say, they should not be nearly related by descent and parentage. Thus F. Schaudinn found that in order to observe the sexual union of the gametes of Foraminifera it was necessary to bring together gametes of distinct parentage. On the other hand it has been observed that in many Protozoa, especially in parasitic forms, syngamy takes place between individuals of common parentage. Thus in *Amoeba coli*, a single individual becomes encysted and its nucleus divides into two; after each nucleus has undergone certain maturative changes they give rise to pronuclei which conjugate and initiate a new developmental cycle. Syngamy between sister individuals, or **autogamy,** as it has been termed, is not, however, confined to parasitic Protozoa; it has been observed in *Actinosphaerium* by R. Hertwig. The benefit to the organism, if any, arising from autogamy can only be supposed to result from the rearrangement and reconstitution of the nuclear apparatus. The frequent occurrence of autogamy suggests that in many Protozoa the nature of the environment diminishes the importance of the sexual process, at least so far as the mixture of nuclear material from distinct sources is concerned; and, since autogamy is most common in parasitic forms, this result may, in the light of G. N. Calkins's experiments, be ascribed in great part to the frequent changes of environment and nutrition to which parasitic forms, above all, are subject.

True syngamy consists, as has been said, of nuclear fusion or karyogamy. It rarely, if ever, happens, however, that such fusion takes place without the conjugating nuclei having undergone some process of reduction by elimination of a portion of the nuclear substance, in a manner analogous to the maturation of the germ-cells in the Metazoa. The chromatin thus eliminated may be cast out from the body of the organism as one or more so-called polar bodies; or may be absorbed in the cytoplasm; or may remain in the cytoplasm and be left over in the residual protoplasm in cases where syngamy is followed by a process of rapid multiplication by sporulation; but in all cases the chromatin removed from the nucleus is rejected in some way or other and plays no part in the subsequent development of the organism. The nuclei of the gametes which have completed this process are then ripe for syngamic fusion and are termed **pronuclei**; the union of two pronuclei produces a single nucleus termed a **synkaryon**.

It is certain that in many, if not in all, cases the nuclear substance that is rejected as a preliminary to syngamy consists of somatic or vegetative chromatin; that is to say, of chromatin that has been functional in regulating the ordinary vital functions, metabolism, growth, reproduction, during previous generations, and has become effete; while on the other hand the chromatin that persists to form the pronuclei is generative chromatin which has remained in reserve for the sexual act and has retained its peculiar powers and properties unimpaired. The truth of this explanation is extremely obvious in such forms as the Infusoria, where somatic and generative chromatin are concentrated into two distinct and entirely separate nuclei. In some Rhizopoda also the body contains one or more principal nuclei and a mass of chromidia, and it has been observed that as a preparation for syngamy the principal nuclei are eliminated and the pronuclei are formed from the chromidia; in such cases, therefore, it is reasonable to regard the principal nuclei as representing somatic chromatin, the chromidia as generative chromatin. In other cases, however, for example *Actinosphaerium*, the chromidia must be interpreted, from their behaviour, as somatic chromatin, and the principal nuclei as generative chromatin; hence R. Goldschmidt has proposed the special term *sporetia* for those chromidia which represent reserve generative chromatin. In the majority of Protozoa, however, the nuclear substance is not differentiated in such a way that it can be distinguished by any visible peculiarities into somatic and generative chromatin.

The process of reduction is not limited, apparently, to the elimination of somatic chromatin, but a portion of the generative chromatin is also cast off. Thus in the Infusoria not only the somatic macronucleus, but also a considerable portion of the generative micronucleus, is absorbed at each act of conjugation. The elimination of generative chromatin is perhaps of importance as a factor in heredity and the production of variations, or possibly for sex determination, as will be discussed below; it is difficult to suggest any other explanations for it, unless it be supposed that during the exercise of ordinary vital functions a portion of the generative chromatin be rendered effete as well as the somatic chromatin.

From the considerations set forth in the foregoing paragraphs it must be supposed that the synkaryon, the fusion-product of the two pronuclei in syngamy, consists at first purely of generative chromatin, which must speedily become differentiated into the regulative somatic chromatin of the ensuing generations and the generative chromatin held in reserve for the next act of syngamy. Such a differentiation can be actually observed in the Infusoria, where immediately after conjugation the synkaryon divides into one or more pairs of nuclei, each pair becoming the two unequally sized nuclei of an ordinary individual, sometimes with, even at this stage, an apparently wanton elimination of nuclear substance. Thus the somatic and generative chromatin of the Protozoa offer a certain analogy with the soma and germplasm of Metazoa; but in making such comparisons the distinction between a physiological analogy and a morphological homology should be borne clearly in mind.

It has been stated above that the two gametes of a given species of Protozoa may be perfectly similar and indistinguishable, or may be very different one from the other. The condition with similar gametes is termed isogamy, that with differentiated gametes anisogamy. Every transition can be found from complete isogamy and pronounced anisogamy in the Protozoa; in tracing, however, the evolution of specialized gametes it must be remembered that we are dealing only with visible morphological differences mainly of an adaptive nature, without prejudice to the question of the possible existence of a fundamental sexual antithesis in all gametes, present even when not perceptible. The sex philosopher O. Weininger has urged that sex is a fundamental attribute of living things, and that the living substance, protoplasm, consists of arrhenoplasm and thelyplasm united in varying proportions. Certain observations of F. Schaudinn tend to support this view; in *Trypanosoma noctuae*, for example, Schaudinn found that the process of reduction in one gamete took an opposite course to that which it took in the other gamete. In one gamete certain portions of the nucleus were retained and certain other portions rejected; in the maturation of the other gamete the portions rejected and the portions retained were the reverse. Hence Schaudinn was led to regard the indifferent individuals as essentially hermaphrodite in nature, and therefore capable of giving rise to gametes of either order by elimination of one or the other set of sexual elements; a theory which throws further light on the elimination of generative chromatin mentioned above. It is possible, therefore, that the gametes of Protozoa may possess sexual characters intrinsically different even when perfectly similar so far as can be perceived. It is very probable, for instance, that the isogamy in Gregarines is a state of things derived secondarily from a primitive condition of anisogamy (see Gregarines) .

The simplest possible condition of the gametes is seen in the free-swimming Ciliata, forms which in other respects are the most highly organized of Protozoa; here the individuals which conjugate are only distinguished from ordinary individuals of the species by the fact that their nuclei have undergone very complicated processes of reduction and nuclear elimination. In these forms there is also no difference between young and adult individuals, beyond scarcely perceptible differences of size between individuals about to divide and those that are the products of recent division, so that these species are practically monomorphic in the active condition. In forms, however, which, like *Vorticella*, are of sessile habit, small free-swimming individuals are liberated which seek out and conjugate with the ordinary sessile individuals. Here we have an instance of a morphological differentiation of the gametes which is clearly adaptive to the life-conditions of the species. In other Protozoa there may be, as already stated, differences, more or less pronounced, between young and adult individuals, and syngamy may take place either between young individuals (microgamy) or between adults (macrogamy); the gametes may be in either case ordinary individuals of the species, not specially differentiated in any way, or on the other hand they may be differentiated from ordinary individuals, while still similar and isogamic amongst themselves; or, finally, they may be anisogamic; that is to say, differentiated into two distinct types. Thus in the Radiolaria, for example, an 3 individual breaks up by a process of sporulation into numerous minute flagellated swarmspores; these may be all of one kind, termed isospores, which develop directly without undergoing syngamy; or they may be of two kinds, termed anisospores, both different in their character from the isospores, and incapable of development without syngamy.

When the gametes are differentiated the divergence between them almost always follows parallel paths. One gamete is distinguished by its smaller size, its greater activity, and its comparative poverty in granules of reserve food-material; hence it is termed the microgamete. The other gamete is distinguished by its greater bulk, its pronounced sluggishness and inertness, and its tendency to form and store up in the cytoplasm reserve nutriment of one kind or another; hence it is termed the macrogamete, or, as some prefer to write it, the megagamete (better megadogamete). When these differences are very pronounced, as, for instance, in the Coccidia and other Sporozoa, a condition is reached which is practically indistinguishable from that seen in the sperm and ova of the Metazoa. Hence the microgamete is generally regarded as male, the macrogamete as female; and these terms may be conveniently used, although they do not in themselves imply more than would the words positive and negative, or any other pair of terms expressive of a fundamental contrast. The microgamete may become reduced to a mere thread of chromatin, which may possess one or two flagella for purposes of locomotion, as in Coccidia, &c., or may move by serpentine movements of the whole body, which resembles in its entirety a flagellum, and is often wrongly so termed. In contrast with the microgamete, its correlative, the macrogamete, tends to become a bulky, inert body, often with great resemblance to an ovum, its cytoplasm dense and granular, packed with reserve food-materials as an egg contains yolk, and without organs of locomotion or capacity for movement of any kind. Hence the macrogamete is the passive element in syngamy, which requires to be sought out and" fertilized "by the active microgamete, a division of labour perfectly analogous to that seen in the male and female gametes of Metazoa. In those cases where syngamy takes place by interchange of nuclear substance between two gametes which remain separate from one another, as in the Infusoria, each gamete forms two pronuclei, which are distinguished by their behaviour as the active and passive pronuclei respectively. The active pronucleus of each gamete passes over into the body of the other and fuses with its passive pronucleus to form a synkaryon. A similar method of procedure occurs also in *Amoeba coli,* according to F. Schaudinn.

When gametes are not very highly specialized they may still retain the power of multiplication by division possessed by ordinary individuals, so long as they have not undergone the process of nuclear reduction preliminary to syngamy. If, however, the gametes are highly specialized they may forfeit the power of multiplication. In this respect the microgametes are worse off than the other sex; on account of the great reduction of the body-protoplasm, and the entire absence of any reserve materials, they must either fulfil their destiny as gametes or die off. The macrogametes, on the other hand, with their great reserves of cytoplasm and nutriment, are more hardy than any other forms of the species, and are able to maintain their existence in periods of famine and starvation when all other forms are killed off. Moreover they may regain the power of multiplication by a process of parthenogenesis, a term originally applied in the Metazoa to cases where a germ-cell of definitely female character, that is to say an ovum, acquires the power of reproduction without fertilization by syngamy. A macrogamete multiplying by parthenogenesis first goes through certain nuclear changes whereby it is set back, as it were, from the female to the indifferent condition, and it is then able to multiply by fission like any ordinary, non-sexual individual of the species. Parthenogenesis has been described by F. Schaudinn in the malarial parasites and in *Trypanosome noctuae.* In both cases the female forms are able to persist under adverse conditions after all other forms have perished, and then by parthenogenesis they may multiply when conditions are more favourable, overrun the host again, and cause a relapse of the disease of which they are the cause. S. v. Prowazek has described in *Herpetomonas muscae-domesticae* an analogous process of multiplication on the part of male individuals, and has coined the term etheogenesis for this process, but the statement needs confirmation, and as a general rule the microgamete is quite incapable of independent reproduction under any circumstances.

It is often found that not only are the gametes differentiated, but that their immediate progenitors may also exhibit characters which mark them off from the ordinary or indifferent individuals of the species. In such cases the parent-forms of the gametes are termed gametocytes, and they may differ amongst themselves in characters which render it possible to distinguish those destined to produce microgametes from those which will produce the other sex. The parent-individuals of the microgametes, or microgametocytes, are distinguished as a general rule by clearer protoplasm, free from coarse granulations, and a larger nucleus, more rich in chromatin. The macrogametocytes, on the other hand, usually have coarsely granular cytoplasm, rich in reserve food-stuffs, and a relatively small nucleus. The gametocytes produce the gametes by methods that vary according to the degree of specialization of the gametes. In isogamous forms, of which good examples are furnished by many Gregarines (q.v.), the gametes are produced by a process of sporulation on the part of the gametocytes, a certain amount of residual protoplasm being left over. In forms with pronounced anisogamy, for instance, Coccidia or Haemosporidia, the microgametes are produced by sporulation in which almost the whole mass of the body of the gametocyte may be left over as residual protoplasm, together with some portion of the nucleus; in the other sex, however, the process of sporulation may be altogether in abeyance, and the macrogametocyte becomes simply converted into the macrogamete after going through a process of nuclear reduction.

The gametocytes may, however, possess the power of multiplication without change of character for many generations; or, to put the matter in other words, the sexual differentiation may be apparent not merely in the generation immediately preceding the gametes, but in many generations prior to this. Thus a given species may consist of three different types of adult individuals, male, female and indifferent, each multiplying in its own line. Complicated alternations of generations are the result, and if at the same time there is a well-marked difference between young and adult forms of the species the height of polymorphism is reached. Very commonly a double series of generations occurs, the non-sexual or indifferent forms multiplying apart from the sexually differentiated individuals and the generations immediately descended from them in such cases the series of non-sexual generations is termed schizogony, the series of sexual generations gametogony or sporogony. Schizogony and sporogony usually occur as adaptations to, or at least in relation with, distinct conditions of life. Thus in parasitic forms, as well illustrated by the Coccidia, the organisms multiply by schizogony when overrunning the host, that is to say, when nutriment is abundant; sporogony begins as a preparation for passing into the outer world, in order to infect new hosts. In' the

Haemosporidia, in which transmission from one vertebrate host to another is effected by means of blood-sucking ectoparasites (Diptera, ticks, leeches, &c.), the schizogony goes on in the vertebrate host, the sporogony in the invertebrate host. In free-living, non-parasitic forms, schizogony may go on under ordinary conditions, while sporogony supervenes as a preparation for a marked change in the life-conditions; for instance, a change of medium, or at the approach of winter. It is interesting to note that, as a general rule, the differentiation of sexual forms seems to be a preliminary to the production of more resistant forms capable of braving adverse conditions or violent changes in the conditions of life; a phenomenon which is in support of the hypothesis that syngamy has a strengthening effect on the vitality of the species.

Classification of the Protozoa.

Various attempts have been made to separate the Protozoa into two primary subdivisions. E. Ray Lankester divided them into two main groups, the Gymnomyxa, with naked protoplasm and indefinite form, and the Corticata, with the protoplasm limited by a firm membrane, and consequently with a definite body-form. In many of the corticate groups, however, there must be placed amoeboid, non-corticate forms, such as *Mastigamoeba* amongst the Flagellata, or the malarial parasites amongst the Sporozoa. Hence if Lankester's classification be used, it must be without a hard and fast verbal definition. F. Doflein, on the other hand, has divided the Protozoa into Plasmodroma, with organs of locomotion derived from protoplasmic processes, *i.e.* pseudopodia or flagella, and Ciliophora, with locomotion by cilia. It may be doubted, however, if the distinction between flagella and cilia is so fundamental and sharply defined as this mode of classification would imply. W. H. Jackson has proposed to unite the forms bearing flagella and cilia into one section, Plegepoda, and distinguishes two other sections, Rhizopoda (= Sarcodina) and Endoparasita (= Sporozoa).

Four main groups of Protozoa, of the rank of classes, are universally recognized, however they may be combined into larger categories; these are the Sarcodina, Mastigophora, Sporozoa and Infusoria.

The Sarcodina are characterized by the body being composed of naked protoplasm, not covered by any limiting cuticle, although in many cases a house or shell is secreted into which the protoplasm can be partly or entirely withdrawn. No special organs of locomotion, either flagella or cilia, are ever present in the adult, and locomotion and capture of food are effected in the manner named *amoeboid*, by more or less temporary extrusions or outflow of the protoplasm, which are termed pseudopodia, as in *Amoeba.* The Mastigophora are so named because organs of locomotion are always present in the adult in the form of one or more flagella, each flagellum (Gr. μ tOT, whip) a delicate, thread-like extension of the protoplasm, endowed with a special contractility which enables it to perform lashing, whip-like movements. The body protoplasm is sometimes naked, in which case it may be amoeboid, but is more usually limited by a cuticle, varying in thickness in different types.

The Sporozoa, with the exception of a few forms of dubious position, are exclusively internal parasites of Metazoa, absorbing their food from the internal juices and secretions of their hosts, and never exhibiting in their trophic phases any organs of locomotion or for the ingestion and digestion of solid food. The body-protoplasm may be naked and amoeboid or limited by a cuticle. The reproduction is specialized in correlation with the parasitic habit, and results typically in the formation of a number of minute germs or spores, by which the infection of fresh hosts is affected. It must not be supposed, however, that spore-formation is confined to this class of Protozoa.

The Infusoria., a name originally of much wider application, is now restricted to denote those Protozoa in which locomotion or capture of food is effected by means of special organs termed *cilia*, minute hair-like contractile extensions of the protoplasm differing from flagella not only in their usually smaller size and greater number, but also in the mode of contraction and movement. The cilia may be present throughout life or only in an early stage of the individual. The body is always limited by a cuticle and the nucleus seems to be invariably double, being divided into two parts specialized in function and differing in size, termed respectively macronucleus and micronucleus.

Comparing these four subdivisions with one another, it may be said at once that the Sporozoa and Infusoria are highly specialized classes, each well marked off from the other subdivisions. The Sarcodina and Mastigophora, on the other hand, include the most primitive types of Protozoa and are delimited from one another by a somewhat arbitrary character, the presence or absence of a flagellum in the adult. Thus Mastigamoeba is a form which unites the characters of the Sarcodina and Mastigophora, having an amoeboid body which bears a flagellum, and it is classed among the Mastigophora merely because the flagellum is retained throughout life; if the flagellum were absent in the adult condition it would be placed among the Sarcodina, many of which have flagella in their young stages but lack them when adult. Hence Biitschli considered the Rhizomastigina (i.e. *Mastigamoeba* and its allies) as the most primitive group of Protozoa, representing the common ancestral form of all the classes; and on this view the flagellated young stages of many Sarcodina would represent recapitulative larval stages.

Bi.itschli's theory of Protozoan phylogeny implies that a flagellum is an organ of most primitive nature, possessed perhaps by the earliest forms of life; and it must be remembered that flagella are borne by many Bacteria. On the other hand, one would imagine, from general considerations, that living beings possessing a flagellum would have been preceded in evolution by others that did not bear so definite an organ. The flagellum itself is generally regarded as a vibratile process or extension of the protoplasm, comparable in its nature to a slender pseudopodium endowed with peculiar powers of movement. More knowledge with regard to the nature and formation of the flagellum is needed in order to decide this point, and particularly with regard to the question whether the flagella of Bacteria are of the same nature as those of Protozoa.

It has been much debated whether the earliest forms of life were of the nature of plants or animals. Many authors consider the question settled beyond all debate by a process of trenchant deductive reasoning. It is argued that animals require other organisms for their nutriment, and that plants, that is to say green plants, do not; therefore plants must have preceded animals. On the other hand, the morphologist will urge that green plants derive. their peculiar powers of metabolism from t he possession of very definite cell-organs, namely chromatophores containing chlorophyll; and will argue that living things without such organs must have preceded in evolution those possessing them. The whole dispute is based on the assumption that plant and animal represent the two fundamental modes of metabolism; whereas the study of the Bacteria shows the possibility of many other modes of life. Many Bacteria exhibit processes of metabolism totally different from those generally laid down in textbooks as characteristic of living matter; some are killed by free oxygen; others can absorb free nitrogen, and various other" abnormal "properties are manifested by them. Hence the primitive organisms may have been neither plant nor animal in their nature, but may have possessed, like the Bacteria at present, many different methods of metabolism from which plant and animal are two divergent paths of evolution.

Classification

Protozoa were previously often grouped in the kingdom of Protista, together with the plant-like algae and fungus-like slime molds. As a result of 21st-century systematics, protozoa, along with ciliates, mastigophorans, and apicomplexans, are arranged as animal-like protists. With the possible exception of Myxozoa, protozoa are not categorized as Metazoa.[5] Protozoa are unicellular organisms and are often called the animal-like protists because they subsist entirely on other organisms for food. Most protozoa can move about on their own. Amoebas, paramecia, and trypanosomes are all examples of animal-like protists.

Sub-groups

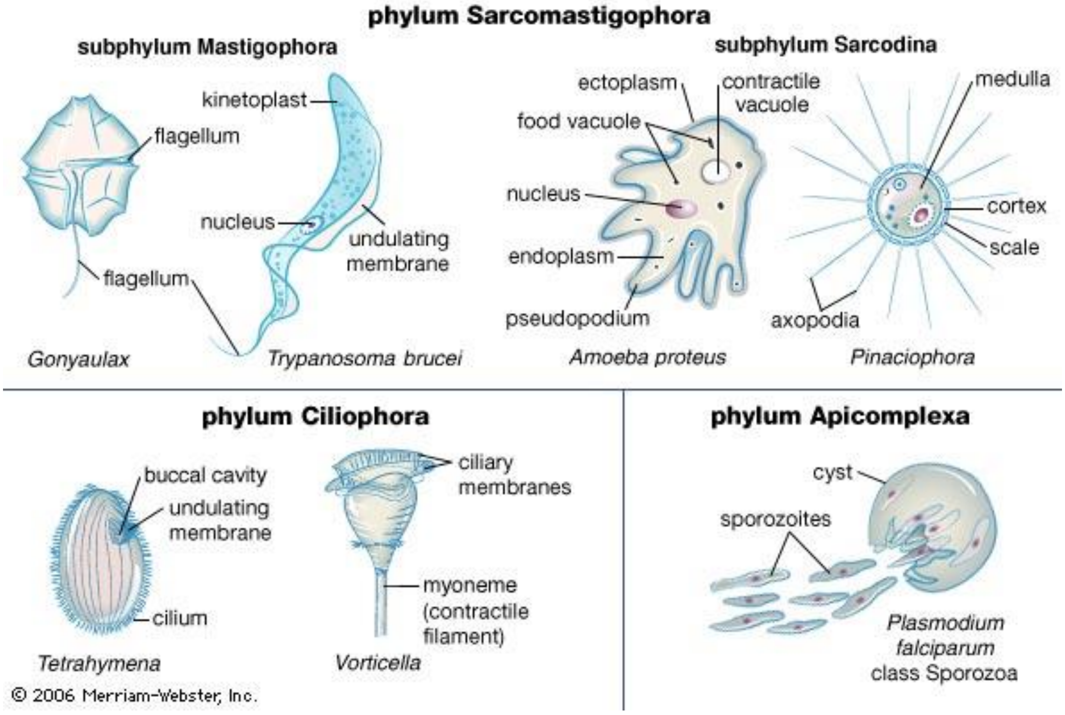
The classification of protozoa has been and remains a problematic area of taxonomy. Where they are available, DNA sequences are used as the basis for classification but for the majority of described protozoa such material is not available. They have been and still are mostly on the basis of their morphology and for the parasitic species their hosts. Protozoa have been divided traditionally on the basis of their means of locomotion.

- Flagellates (e.g., *Giardia lamblia*)
- Amoeboids (e.g., *Entamoeba histolytica*)
- Sporozoans (e.g., *Plasmodium knowlesi*)
  - Apicomplexa
  - Myxozoa
  - Microsporidia
- Ciliates (e.g., *Balantidium coli*)

Protozoa had been divided into four phyla reflecting the means of locomotion:

- phylum Sarcomastigophora
  - Subphylum Mastigophora (includes flagellates)
  - Subphylum Sarcodina
  - Subphylum Opalinata
- phylum Sporozoa (includes apicomplexans)
- phylum Cnidospora
  - Subphylum Myxosporidea
  - Subphylum Microsporidea
- phylum Ciliophora (includes ciliates)

Phylum Sarcomastigophora



A- Subphylum Mastigophora

phylum of unicellular heterotrophic protozoans of the kingdom Protista. Most of the approximately 1,500 species of Mastigophora are propelled by one or more flagella, and members of the group are sometimes referred to as **flagellates**. Some have pseudopodia, temporary arm like protrusions of cytoplasm that help the cell move about and enwrap particles of food. Asexual reproduction by equal longitudinal binary fission or repeated fission , but some species reproduce sexually by a process called syngamy, the fusion of two gametes produced by meiosis (i.e., fertilization). Some parasitic members of the Mastigophora are the causative organisms of disease in humans and other animals. ***Trypanosomes***, for example, are the cause of African sleeping sickness and Chagas' disease, and giardiasis is caused by the mastigophoran ***Giardia lamblia***.

Diagnosis

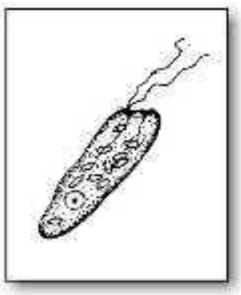
1- Principle phase possess one or more flagella
2- Often parasitic but rarely intracellular.
3- Have only one type of nucleas, do not have meganucleas.
4- Do not form large number of spores after syngamy.

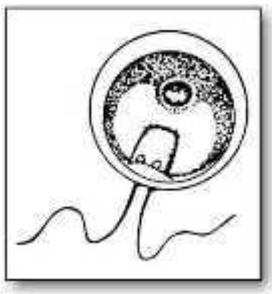
The flagellates are divided taxonomically into two classes, those resembling plants, Phytomastigophorea (phytoflagellate), and those resembling animals, Zoomastigophorea (zooflagellate).

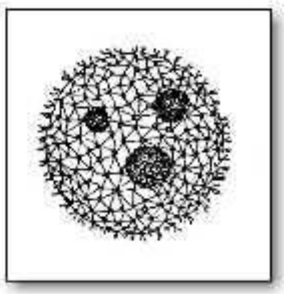
Class phytomastigina

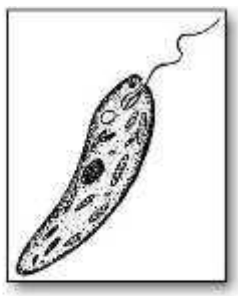
Mastigophora which possess chromatophores and being holophytic. They includes:

1- **Order Chryssomanadina**: yellow, brown or colourlessphytomastigina without starch reserves , but usually have leucosin and oil, without gullet or transverse groove, often amoeboid, e.g. ***Chrysamoeba*** .
2- **Order Cryptomonadina** : Green, yellow, brown or colourless phytomastigina with starch reserves, with gullet or with longitudinal groove , but without transverse groove, very rarely amoeboid. e.g. ***Cryptomonas* and *Chilomonas.***
3- **Order Euglenoidina** : Phytomastigina which have numerous green chromatophores or are colourless , with reserves of paramylum and sometimes also oil. With gullet ; contractile vacuole opening by reservoir usually into the gullet. Without transverse groove, with stout pellicle. e.g. ***Euglena***
4- **Order Dinoflagellata:** The Dinoflagellata (syn. Dinophyceae) contain either numerous yellow-brownish plastids or none. 2 flagellae, one of which longitudinally and the other transversally undulating. Frequently, the cells are subdivided by a sulcus and the annulus, the latter separating the upper epi- from the lower hypoconus (see e.g. ***Ceratium*** images). Complex shell with most diverse shapes and often trichocyst-like "extrusomes". Dioptric stigms as light sensors. Dinoflagellates exhibits all kinds of food behaviour: photoautotrophy, heterotrophy and there free-living or parasitic, partially osmotroph. Some species can occur in blooms and may then account for severe intoxication (e.g. *Gymnodinium* and *Gonyaulax*blooms). *Noctiluca* species are bioluminescent . Of eminent importance are dinoflagellates as zooxanthellae in corals, radiolaria and foraminifers.
5- **Order Phytomonadina** (syn. Volvocina) possess one single, cup-shaped green plastid and a stigma. Ratio of pigments is similar to that of higher plants suggesting the phytomonadina to be the group in which their common ancestor may be found. 2, 4 or 8 smooth flagellae. There are solitary and colonial phytomonads with the colonies being plate-, sphere- or egg-shaped. In the case of colonies, differentiation into somatic and generative cells is observed. After generation of daughter colonies the somatic cells die. Besides asexual also sexual propagation with equally (isogamy) or unequally sized (anisogamy) gametes. Phytomonadina are haplonts with zygotic meiosis. Almost exclusively limnic distribution. e.g. ***Chlamydomonas & Volvox***.

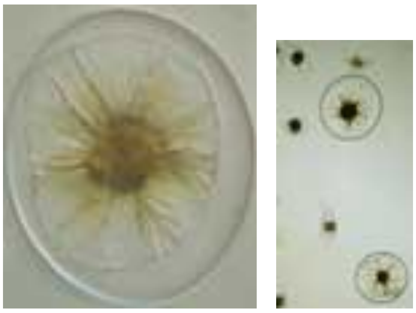

Chilomonas

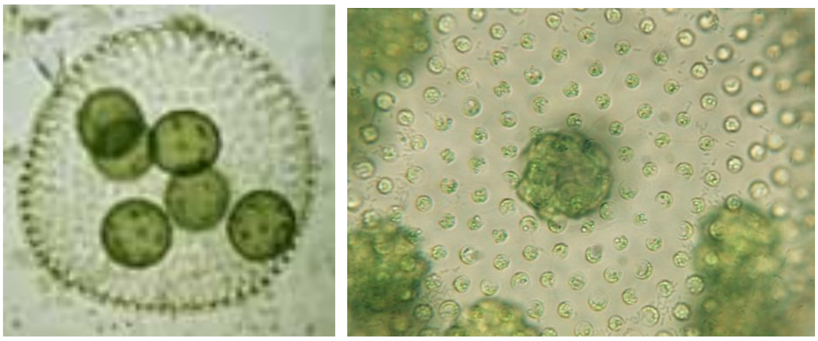

Chlamydomonas


Volvox


Euglena


Ceratium spp.


Noctiluca scintillans


Volvox aureus

Class Zoomastigina

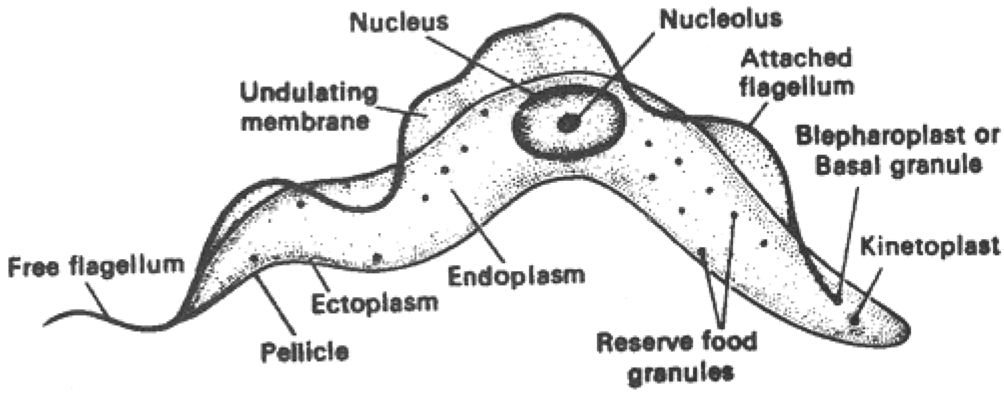
1- **Order Protomonadina** comprise a heterogenous group of flagellates with one or two, rarely with up to four flagellae. Two groups are of special interest.

a- **Choanoflagellates** possess one single, long flagellum which is along the half length ensheathed by a collar. It therefore reminds of the poriferan choanocytes This suggests choanoflagellates to be the common ancestor of multicellular animals. Eg. *Codosiga*

b- **Kinetoplastids** exhibit a large, specialized mitochondrion, the **kinetoplast,** which lies at the basis of the flagellum. This mitochondrion contains the highest amount of DNA amongst so far known mitochondria and is therefore thought to represent the closest relative to the bacterial ancestors of mitochondria which may be found in the alpha subdivision of Proteobacteria **.eg** ***Leishmania & Trypanosoma***

Trypanosoma

Trypanosoma is a zooflagellate protozoan parasite it is usually founding the blood of vertebrates, finally invading cerebrospinal fluid. It is an endoparasite, blood parasite, extra cellular parasite. It has a nucleus, a flagellum, undulating membrane, blepharoplast (basal granule) and Kinotoplst. Trypanosoma is polymorphic and has four forms; **Leis mania**, **Leptomonad**, **Crithidial** and **Trypanosomal** (= Metacyclic) stages. It reproduces asexually, no sexually reproduction.



Trypanosoma gambiense

It is **digenetic**; it completes its life cycle in two hosts. The primary or **principal** or **definite** host is **man** and the intermediate or secondary host vector is the insect, tsetse fly or bug. Three important species of *Tryanosoma* for which man is host are: *Trypanosoma gambiense, T.rhodesiensi and T. cruzi.*

Trypanosoma gambiense was discovered by **Forde** in 1901. *T. gambiense* is the causative agent of **African sleeping** sickness or **Gambian trypanosomiasis**. *Trypanosoma gambiense* is confined to west and central parts of Africa, particularly Nigeria and Congo. **The chief vector hosts of** *T. gambiense* transmitting the disease from one to another is the **tsetse fly**, Glossina palpalis. Occasionaly, *Glossina tachinoides* also act as a vector.

T. rhodesiensi causes **Rhodesian trypanosomiasis**, it is confined to east and central parts of Africa, particularly Rhodesia. The insect vectors for *T. rhodesiense* are tsetse flies mainly *Glossina morsitans* and *G. pallidipes*.

T. cruzi **is the causative agent of South American trypanosomiasis** or **Chaga's disease**. T. ceuzi is transmitted by bugs like Triatoma and Panstrongylus. **Symptoms of Chaga's disease are fever, diarrhoea, anaemia and enlargement of lymphoid glands, etc**.

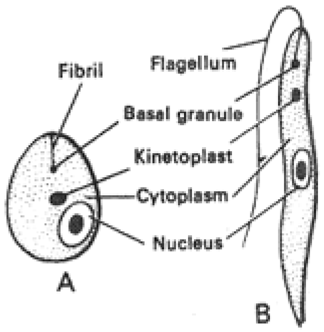
T. brucei causes **Nagana disease**, Vector is *Glossina morsitans*.

T. evansi causes **surra disease**, Vector is tabanid fly.

2. *Leishmania*

Leishmania is a pathogenic blood flagellate. It is digenetic intermediate host, (vector) is **sandfly** belonging to the genus Phlebotomus. *Leishmania donovani* causes **Kala-azar** or **visceral leishmaniasis**. Kala-azar is also called **Dum fever**, infaction occurs ciefly in spleen and liver, secondarily in bone marrow and intestinal villi. *L. tropica* causes '**Oriental sore**' or **cutaneous leishmaniasis** in men or Delhi sores. Direct transmission of Oriental sore by contact with wound is also possible.

L. brasiliensis causes '**Espundia**' or **naso-oral leishmaniasis** in South America. Espundia is characterized by lesion upon skin and mucous membrane of nose, mouth, pharynx and rarely of vagina.



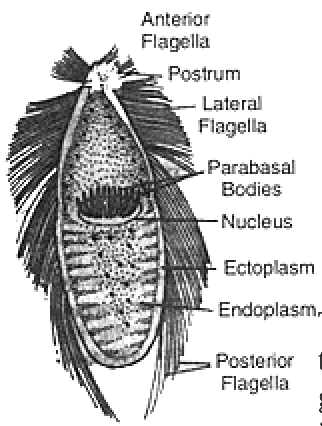
Leishmania donovani
A. ***Leishmaina form,***
B. ***Leptomonad form***

3. *Giardia*

Giardia is commonly nick nameed as the "Grand Old Man of Intestine". It occurs in the upper part of the human intestine. Transmission occurs through food and water contamined with faeces with faeces of aninfected person. There are two nuclei and four pairs (one anterior and three posterior) of backwardly directedflagella. Two supporting needle-like **auxostyles** are also present. It causes epigastric pain, abdominal discomfort, diarrhoea, headache and sometimes fever. The disease caused by Giardia is popularly known as giardiasis. All times the parasites form cysts which pass out of the stool and become the source of infection to others.

4. *Trichomonas vaginalis*
It inhabits vagina of women and causes the disease known as **leucorrhoea**. The disease is characterised bt burning sensation, itching and forthy discharge. Transmission is through sex. In males the parasite produces irritation in urethra. An axostyle is present. Nutrition is both holozoic and saprobic.

Trichomonas tenax (= T. buccalis) resides in the buccal cavity while T. homonis is found in large intestine. Both are nearly harmless.



Trichonympha

5. Trichonympha campanula
This zooflagellate occurs as a symbiont in the intestine of the termites. *Trichonympha* secretes cellulose digesting enzymes β-glucosidases which convert cellulose into glucose. The digested food is shared by the termite. Without Trichonympha the termites starve and die.

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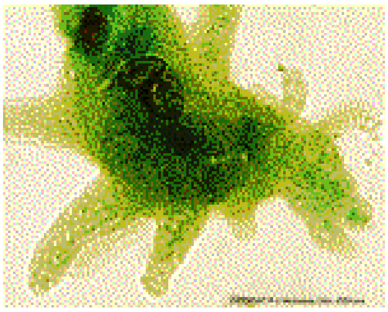
Subphyum Rhizopoda(= Sarcodina)

Rhizopods are heterotropic protozoans lacking permanent locomotive organelles like cilia or flagellae. To move and to catch prey, they use transient cellular extensions, the so called pseudopods. According to their shape and organization they are called lobopods (lobular), filopods (fathom-like), rhizo- or reticulopods (net-like) and axopods (more or less stable and fixed). The different pseudopodial extensions are also used for classification.

"Amoeboid" and "**amœba**" are often used interchangeably even by biologists, and especially refer to a creature moving by using pseudopodia. Most references to "amoebas" or "amoebae" are to amoeboids in general rather than to the specific genus *Amoeba*. The genus *Amoeba* and amoeboids in general both derive their names from the ancient Greek word for change.

1- Class Amoebina

In contrast to all other rhizpods, amoebina are devoid of any shell-like structure and are therefore extremely flexible. Nonetheless, in most cases a cell polarity can be observed. Pseudopods are mostly lobopods, rarely filpods. During migration, a clear ecto- and a granular endoplasm can be distinguished in many cases. Amoebina living in habitats which dry out seasonally are able to survive in cysts. Most amoebina, though, live in fresh waters or the sea, feeding on bacteria, protozoa or algae. Some species in the gut of bilaterians. Eg. ***Amoeba, pelomyxa, Entamoeba***

2- Class Testacea

Testacea live in a single-chambered shell consisting of organic material in which inorganic stuff like diatom shells or grains of sand are incorporated. Pseudopods are mostly lobular or fathom-like, exceptionally also reticular. The cytoplasm is diveded into the extruded pseudopods, the "nutritional zone" in which food is digested and finally the most inward and sheltered region containing the nuclei. Mostly asexual propagation by simple cell division which may, in the case of soft shells, may also include the shell itself. Sexual propagation so far unknown. Testacea a typical inhabitants of fresh water and are frequently found in mosses and the leave-detritus-containing top soil of fagan woods.eg. ***Arcella***


Arcella


Difflugia

3- Class Foraminifera

Like testacea, foraminifera possess a shell. This can be monothalamous (single-chambered) or polythalamous (multi-chambered). The shell is made of organic material which incorporates chalk, grains of sand or other things. The chambers are built successively and are connected to both, the following chamber as well as to the extracellular space. Reticulopods are predominant and can extend from the terminal aperture or from the pores in each chamber. "Granular streaming", the intracllular movement of vesicles, mitochondria or other cellular components along the pseudopods, which contain bundles of microtubules, can be frequently observed. Propagation can be sexual or asexual, in some species also heterogony has been observed. Foraminifera are facultatively marine and live mostly in coral reefs, on rocky shores or in sandy ground as part of the mesopsammon. Many species contain zooxanthellae. Foraminifera have an important ecological function as main source of marine sediments.

Elphidium (Foraminifera)

Elphidium is a genus of foraminiferan protozoa, one of the more common genera found near coasts. Like other forams, fossils from different species are used to date rocks.

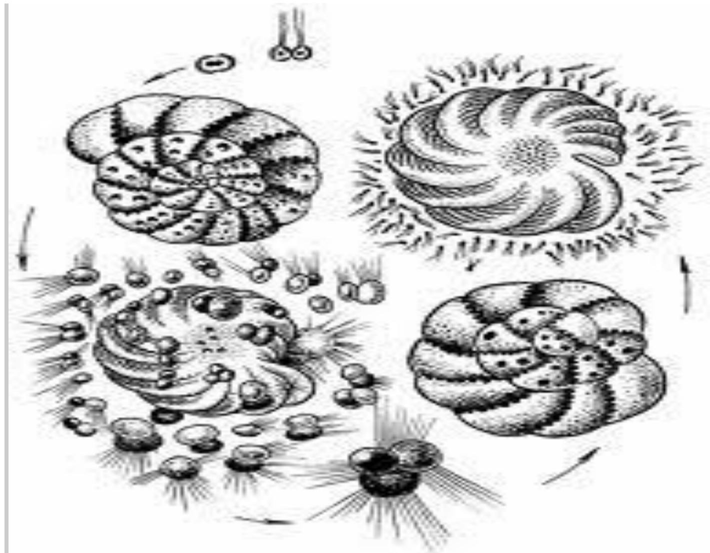
Description

Elphidium is one of the more beautiful foraminiferans and once seen easy to recognize. The test (shell) is lenticular, composed of finely perforate, bilamellar, optically radial, or less commonly granular, calcite, planispirally enrolled. Coiling is involute or partially evolute, with seven to twenty chambers in the final whorl and may have umbilical plug on each side. In some species the rim is sharp, keel-like, in others more rounded. The most diagnostic feature, perhaps, are the retral processes (small backward extensions of the chamber walls) that cross the sutures; giving some the appearance of tiny rolled up glass baskets .

Life Cycle

Elphidium shows dimorphism with alternating generations. The complete cycle for *Elphidium crispum* takes two years in the shallower marine regions, although it may be delayed at deeper stations. Asexual reproduction reaches a peak in spring of first year. Sexual reproduction begins early in the second spring as temperatures begin to rise.

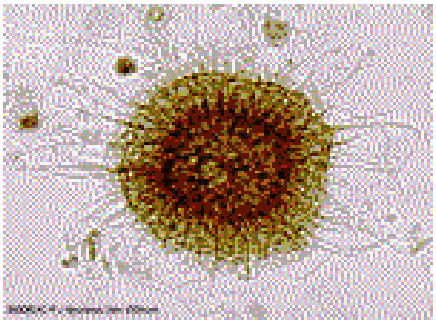
There are two forms , small called **microspheric** form and large form called **megalospheric** form. These forms correspond to the alternation of generationin the life cycle . The microspheric form usually becomes multinucleated at an early stage , reproducing a sexually by multiple fission to form small individuals called **amoebuli** which grow to megalospheric form, while the megaspheric form which remain uninucleate till to about to reproduce by multiple fission to produce gametes . The gametes conjugate outside in open sea to produce zygotes and the microspheric form then develops and matures during the second summer. It has been shown in some species that the microspheric form is diploid and the megalospheric forms is haploid.



4- Class Heliozoa

Heliozoa exhibit a sphaerical cell-body containing one or more nuclei. Along the axopods, mitochondria, vesicles and other particles may be transported underneath the plasma membrane in a process called "granular streaming". Often, an apparently looser ectoplasm with pulsatile vacuoles can be distinguished from a denser endoplasm with nuclei. Mostly asexual propagation, sexual propagation known only from *Actinophrys* and *Actinosphaerium.*

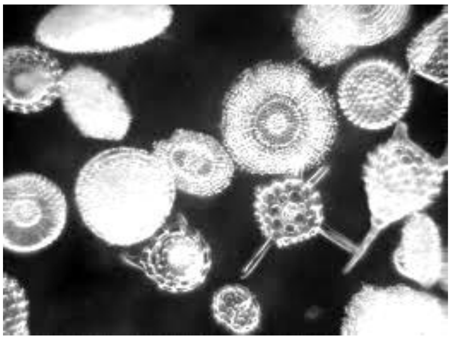
Actinosphaerium



Actinophrys

5- Class Radiolarian

In contrast to heliozoa, radiolaria exhibit a "central capsule" which separates the intra- from the extracapsular cytoplasm. Nucleus or nuclei are always located in the intracypsular cytoplasm. Most radiolaria possess a species' specific skeleton , mostly with star-like appearance, which is made out of silicic acid or strontium sulfate. Most radiolaria are found in the deeper planktonic strata of warm-water seas. Acantharia are found mainly in the upper plankton.



Radiolaria different types of Radiolarian capsules

Multicellular Amoebas: Slime molds

Amoebas seem to have connections with two phyla composed of multicellular organisms of the lineage of fungus-like protists, the so-called **slime molds**. These two defunct phyla were the **Myxomycota** (i.e., plasmodial slime molds, now classified in the taxon **Myxogastria**), and **Acrasiomycota** (i.e. cellular slime molds, now divided into the taxa **Acrasida** and **Dictyosteliida)**. These two phyla use amoeboid movement in their feeding stage. The former is basically a giant multinucleate amoeba, while the latter lives solitary until food runs out; in which a colony of these functions as a unit. Myxomycete slime molds use amoeboid gametes, as well.

giant marine amoeboids, the **xenophyophores,** that did not fall into any of these categories.

Subphylum Opalinata

The **opalines** are a small group of peculiar protists, currently assigned to the family Opalinidae, in the order Slopalinida. Their name is derived from the opalescent appearance of these microscopic organisms when illuminated with full sunlight . Most opalines live as endocommensals in the large intestine and cloaca of anurans (frogs and toads), though they are sometimes found in fish, reptiles, molluscs and insects. The unusual features of the opalines, first observed by Antoine van Leeuwenhoek in 1683, has led to much debate regarding their phylogenetic position among the protists.

Taxonomy and phylogeny

The relationships opalines and other protists has been a subject of great controversy since the late 19th century, and is not completely resolved at present. Initially, microscopists believed that the thousands of rhythmically beating hair-like structures which cover their surface were cilia, and they placed the opalines in Ciliophora. In the early 20th century other aspects of opaline biology clearly differentiated them from the ciliates, and they were placed in Sarcomastigophora, with the amoebae and flagellates. In the 1980s, detailed ultrastructural studies of *Opalina ranarum* revealed that they share many features with the heterokonts of the family Proteromonadidae. A new order—Slopalinida Patterson 1985—was proposed to include the members of the families Proteromonadidae Grassé 1952 and Opalinidae Claus 1874. In 2004, the first reliable opaline genetic sequence data supported the monophyletic nature of the order Slopalinida. The authors of that study considered the opalines to be a family (Opalinidae) within the order Slopalinida.

There are currently about 200 recognized species of opalines in 5 genera:

1- *Opalina* Purkinje and Valentin 1835,
2- *Protoopalina* Metcalf 1918,
3- *Cepedea* Metcalf 1920,
4- *Zelleriella* Metcalf 1920, and
5- *Protozelleriella* Delvinquier et al. 1991.

Two additional genera, *Hegneriella* Earl 1971 and *Bezzenbergeria* Earl 1973, have not been considered as valid by subsequent authors . The 5 recognized genera differ in terms of the number of nuclei, the appearance and location of the falx (two short, sickle-shaped rows of flagella), and whether the long rows of flagella (called "kineties") cover the body evenly or if there is a "bald spot". Due to the differences in body shape among the different life cycle stages within a species, the use of overall body shape - whether flat or cylindrical - to differentiate the genera has been de-emphasized.

Life cycle

Like many parasites, the life cycle of opalines is rather complex. The most comprehensive study published so far concluded that the life cycles of 10 *Opalina* species, 1 *Zelleriella* species and 1 *Protoopalina* species are all "remarkably similar" . A more recent study found that *Cepedea couillardi* fits the standard opaline life cycle model described below, while that of *Opalina proteus* is completed entirely in the tadpole stage of the host. Very little is known about the life cycles of opalines in fish, reptile or arthropod hosts.

1- Asexual phase in adult anuran host. The basic opaline life cycle begins with the large, multinucleate **trophonts** in the adult anuran cloaca. Through much of the year, the trophonts grow and divide continually to yield more trophonts. Nuclear divisions maintain the appropriate number of nuclei during this phase. As the host's breeding season approaches, the trophonts enter a phase known as **palintomy** -- a series of cell divisions with little or no overall growth or nuclear divisions. The resulting opalines, which become gradually smaller with fewer nuclei per individual, are called **tomonts**. At some point the small tomonts undergo encystment, and the **cysts** are released into the environment (i.e. the breeding pool of the anuran host) along with the feces.

2- **Sexual and asexual phases in larval anuran host.** Once cysts are eaten by foraging tadpoles, they excyst (hatch) to yield **gamonts**. The gamonts divide further, including a meiotic division, to yield haploid **gametes**. Each gamete has only one nucleus and may be either a **microgamete** or a **macrogamete**. Syngamy occurs between one microgamete and one macrogamate, to yield a diploid **zygocyst** with one nucleus. The zygocyst has two possible fates. It may be shed along with the feces of the tadpole host; and if eaten by another tadpole, it will excyst (hatch) to yield more gamonts in the new host. Alternatively, the zygocyst may excyst in its original host and grow into a multinucleate **protrophont**. In this case, the protrophont grows into a trophont and the whole cycle starts over again. The cycle from protrophont to cyst may occur in either the tadpole or adult hosts. Some evidence suggests that the life cycle transitions of opalines may be governed by the hormonal cycles of the host.

Hosts and commensal lifestyle

Lacking a mouth, opalines feed by taking in nutrients from their surroundings by pinocytosis. While the opalines are often referred to as "parasites", two lines of evidence suggest that they are actually commensals which do no harm to their anuran hosts.

1. They are found almost exclusively in the large intestine and cloaca. Since the anuran absorbs the nutrients from its food in the small intestine, the opalines are probably not depriving their hosts of nutrients. It is believed that the opalines are simply living off the "left-over" nutrients in the feces, possibly supplemented by the biochemical contributions of the rich bacterial flora which also reside there.
2. Anuran hosts containing many thousands of opalines appear to be completely healthy, with no obvious irritation or other pathological signs on their intestinal or cloacal walls.

Only about a dozen reports of opalines in fishes have been published, and even fewer on opalines from reptile or salamander hosts. Their scarcity outside of anuran hosts had led many to speculate that the others are just incidental infestations—maybe the infested snake had just eaten an infested frog, for example. However, opalines have been found in saltwater fish which have no access to anurans. Also, the populations of opalines in fish hosts are often very high, suggesting that they are probably reproducing in the fish host.

The pathogenicity (if any) of opalines in fish hosts is not yet known. One study found no irritation or other pathological signs on the rectal epithelium of *Symphysodon aequifasciata* infested with *Protoopalina symphysodonis*, but stated that "most infected animals died".

In vitro culture of opalines

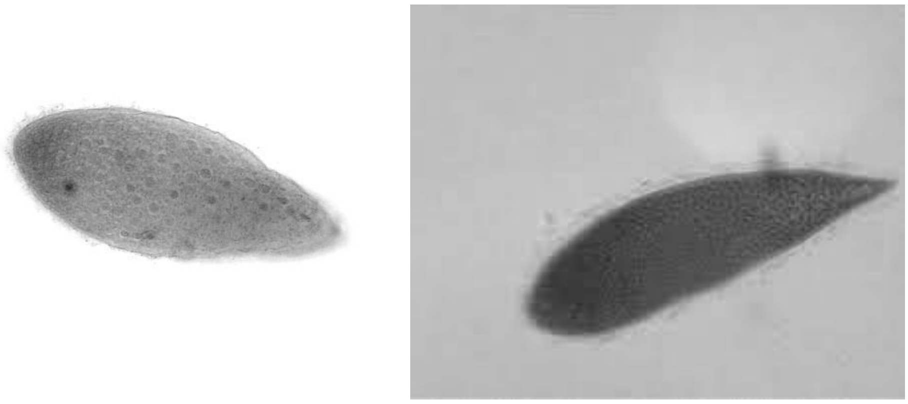
Successful culturing of opalines in artificial media for periods of 1 month or more has been reported. This technique will aid tremendously in future studies of all aspects of opaline biology.

Opalina

The body, which reaches 1 mm in length, is not completely symmetrical, is leaflike, and covered with numerous flagella. The ectoplasm and endoplasm are well demarcated and sometimes have dozens, even hundreds, of nuclei. There is no mouth opening; feeding is by osmosis. Reproduction is asexual (binary division) and sexual (copulation).

Several dozen species of *Opalina* have been described. They usually live in the intestines of amphibians; they are found less frequently in the intestines of fish and reptiles. The sexual process occurs in the spring in the gut of a tadpole. Cysts that are evacuated from the intestine of an animal host, such as a frog, are deposited on the bottom of a body of water, where they are swallowed by tadpoles that have just hatched. In the tadpoles' intestines, small opalinids hatch from the cysts, which divide to form uninucleate sexual individuals, or gametes. The gametes fuse in pairs, and multinucleate opalinids develop from the zygotes.

Until recently, the *Opalina* were considered to be a subclass of Infusoria. However, opalinids lack the two principal characteristics of infusorians: **two types of nuclei** ,the **sexual process of conjugation** and **horizontal binary fission .**



Opalina

Phylum Apicomplexa (Sporozoa)

The name of the taxon *Apicomplexa* is derived from two Latin words - *apex* (top) and *complexus* (infolds) - and refers to a set of organelles in the sporozoite. The older taxon **Sporozoa** grouped the *Apicomplexa* together with the Microsporidia and Myxosporida.

Sporozoa is a phylum of mainly parasitic spore-forming protozoans that have a complex life cycle with sexual and asexual generations. They include the organisms that cause malaria, babesiosis, coccidiosis, and toxoplasmosis. Also called Apicomplexa gathers several species of obligate intracellular protozoan **parasites** classified as Sporozoa or Sporozoans, because they form reproductive cells known as spores. Many sporozoans are parasitic and pathogenic species, such as *Plasmodium (P. falciparum, P. malariae, P. vivax), Toxoplasma gondii, Pneumocysts carinii, Coccidian, Babesia, Cryptosporidum (C. parvum, C. muris),* and *Gregarian.*

Sporozoans have no flagellated extensions for locomotion, with most species presenting only gliding motility, except for male gametes in the sexual phase, which have a flagellated stage of motility.

General morphological features

All members of this phylum have an infectious stage - the sporozoite - which possess three distinct structures in an apical complex. The apical complex consists of a set of spirally arranged microtubules (the conoid), a secretory body (the rhoptry) and one or more polar rings. Additional slender electron dense secretory bodies (micronemes) surrounded by one or two polar rings may also be present. It is this structure that gives the phylum its name.

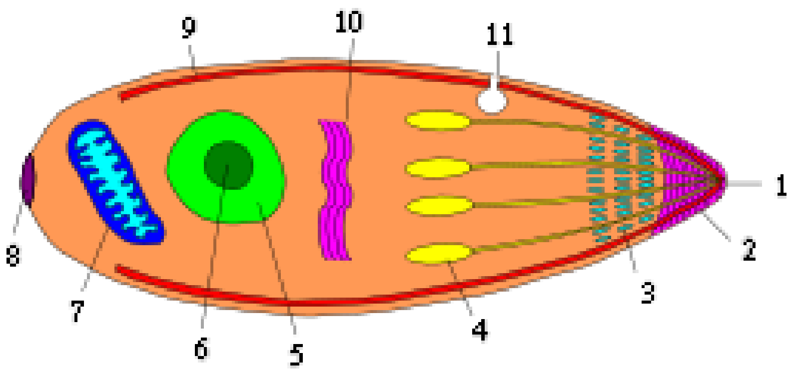
A further group of spherical organelles are distributed throughout the cell rather than being localized at the apical complex and are known as the dense granules. These typically have a mean diameter of about 0.7 micrometers. Secretion of the dense-granule content takes place after parasite invasion and localization within the parasitophorous vacuole and persists for several minutes

The apical complex includes vesicles called rhoptries and micronemes, which open at the anterior of the cell. These secrete enzymes that allow the parasite to enter other cells. The tip is surrounded by a band of microtubules, called the *polar ring*, and among the Conoidasida there is also a funnel of tubulin proteins called the *conoid*. Over the rest of the cell, except for a diminished mouth called the micropore, the membrane is supported by vesicles called alveoli, forming a semi-rigid pellicle.

The presence of alveoli and other traits place the Apicomplexa among a group called the alveolates. Several related flagellates, such as *Perkinsus* and *Colpodella* have structures similar to the polar ring and were formerly included here, but most appear to be closer relatives of the dinoflagellates. They are probably similar to the common ancestor of the two groups. Another similarity is that many apicomplexan cells contain a single plastid, called the apicoplast, surrounded by either 3 or four membranes. Its functions are thought to include tasks such as lipid and heme biosynthesis, and it appears to be necessary for survival. Plastids are generally considered to share a common origin with the chloroplasts of dinoflagellates, and evidence generally points to an origin from red algae rather than green.
Other morphological findings that are common to all members of this phylum include://

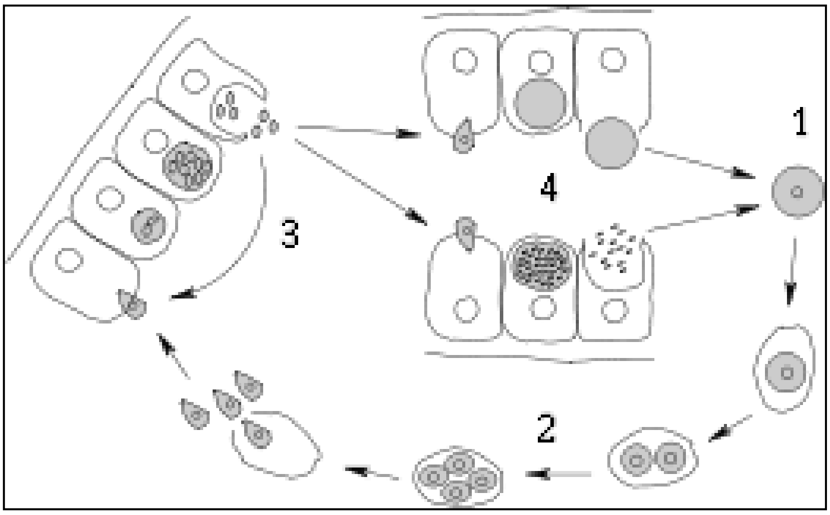
- The nucleus is haploid.
- Flagellae are found only in the motile gamete. These are posteriorly directed and vary in number (usually one to three).
- Basal bodies are present. Although hemosporidians and piroplasmids have normal triplets of microtubules in their basal bodies and coccidians and gregarines have 9 singlets.
- The mitochondria have tubular cristae.
- A Golgi apparatus is present.
- Centrioles, chloroplasts, ejectile organelles and inclusions are absent.
- Colourless plastids are present in some species.

The cell is surrounded by a pellicle of three membrane layers (the alveolar structure) penetrated by micropores



Apicomplexan structure: 1-polar ring, 2-conoid, 3-micronemes, 4-rhoptries, 5-nucleus, 6-nucleolus, 7-mitochondria, 8-posterior ring, 9-alveoli, 10-golgi apparatus, 11-micropore.

Reproduction and Life cycle



Generic life cycle of an apicomplexa: 1-zygote (cyst), 2-sporozoites, 3-merozoites, 4-gametocytes.

The Sporozoa reproduction cycle has both asexual and sexual phases. The asexual phase is termed **schizogony** (from the Greek, meaning generation through division), in which **merozoites** (daughter cells) are produced through multiple nuclear fissions. The sexual phase is known as **sporogony** (i.e., generation of spores) and is followed by **gametogony** or the production of sexually reproductive cells termed **gamonts**. Each pair of gamonts form a gamontocyst where the division of both gamonts, preceded by repeated nuclear divisions, originates numerous gametes. Gametes fuse in pairs, forming zygotes that undergo meiosis (cell division), thus forming new **sporozoites**. When sporozoites invade new host cells, the life cycle starts again. This general description of Sporozoan life cycle has some variation among different species and groups.

Most members have a complex life-cycle, involving both asexual and sexual reproduction. Typically, a host is infected via an active invasion by the parasites (similar to entosis), which divide to produce *sporozoites* that enter its cells. Eventually, the cells burst, releasing *merozoites* which infect new cells. This may occur several times, until *gamonts* are produced, forming gametes that fuse to create new cysts. There are many variations on this basic pattern, however, and many Apicomplexa have more than one host.

Taxonomy

These form the following five taxonomic groups:

1. **The Gregarines** are generally one-host parasites of invertebrates.
2. **The Adeleorins** are one-host parasites of invertebrates or vertebrates, or two-host parasites that alternately infect haematophagous (blood-feeding) invertebrates and the blood of vertebrates.
3. **The Eimeriorins** are a diverse group that includes one host species of invertebrates, two-host species of invertebrates, one-host species of vertebrates and two-host species of vertebrates. The eimeriorins are frequently called the coccidia. Somewhat confusingly this term is often used to include the adeleorins.
4. **Haemospororins** often known as the malaria parasites, are two-host Apicomplexa that parasitize blood-feeding dipteran flies and the blood of various tetrapod vertebrates.
5. **Piroplasms** where all the species included are two-host parasites infecting ticks and vertebrates.

General features

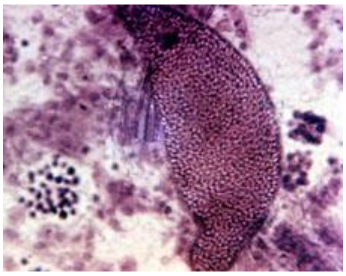
Within this phylum there are three groups - coccidians, gregarines and haemosporidians. The coccidians and gregarines appear to be relatively closely related.

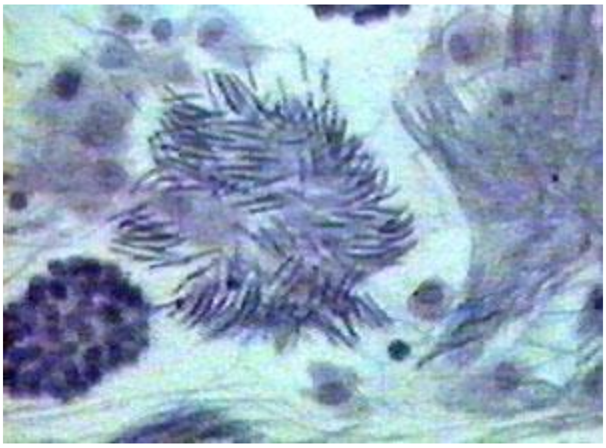
Gregarines

The gregarines are generally parasites of annelids, arthropods and mollusks. They are often found in the guts of their hosts but may invade the other tissues. In the typical gregarine life cycle a trophozoite develops within a host cell into a plasmodium. This then divides into a number of merozoites by schizogony. The merozoites are released by lysing the host cell which in turn invade other cells. At some point in the life cycle gamonts are formed. These are released by lysis of the host cells and group together by syzygy. Each gamont forms multiple gametes. The gametes fuse with another to form oocysts. The oocysts leave the host to be taken up by a new host.

Monocystis

Several species of *Monocystis* are parasitic in the seminal vesicles of the earthworm. They belong to the class Sporozoa and are placed in the order Gregarinida.
The adults, or mature trophozoites, are commonly to be found within the seminal vesicles of the worm. There is a thick pellicle beneath which, in the plasmagel, are longitudinal myonemes. In the granular plasmasol there are paramylum granules and an ovoid nucleus with a prominent nucleolus. Locomotion of the parasite is characterised by contraction of the myonemes during wriggling; it is known as gregarine motion.





The parasite feeds on the cytoplasm of a sperm morula by extruding enzymes and absorbing the digested products through the pellicle. It will frequently move to another morula and consume the cytoplasm, before it is fully-grown. Often numerous sperm tails adhere to the pellicle, giving the *Monocystis* a ciliated appearance.

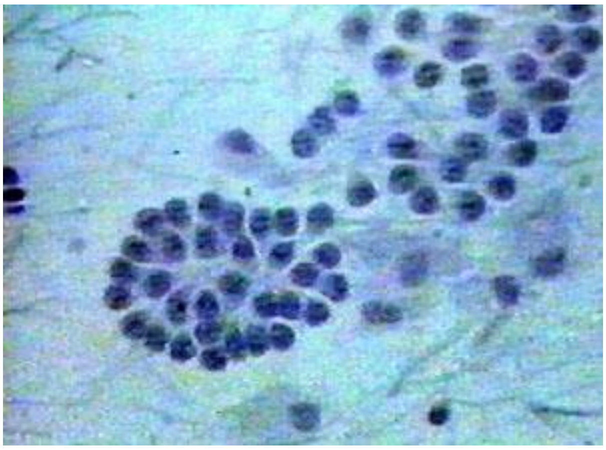
Excretion and respiratory activities are carried out by diffusion.

Reproduction

When two mature adults come together they secrete a common wall and form a conjugative cyst. Within the cyst mitotic and meiotic divisions occur. There is no further development until another earthworm, which first entails the liberation of the cysts into the soil, swallows the cysts. This latter is accomplished by birds, or other animals, eating the earthworm. The cysts are not digestible and are voided with the faeces. Another worm now swallows the cyst, the cyst coat is digested and the freed sporozoites migrate to the seminal vesicles by boring through the gut wall into the coelom of the earthworm. Cysts may live in the soil for a considerable period.

The majority of earthworms are infected by the parasite. In fact, in many years, I have not found the absence of the parasite in the seminal vesicles of any worm examined. Quite often there is a heavy infestation. Despite this, the presence of the parasite seems to cause little inconvenience to the worm. Sperm are produced in such quantity that the destruction of quite large amounts has little effect on the reproductive capacity of the worm.

Smear Preparation of the Contents of the Seminal Vesicles.



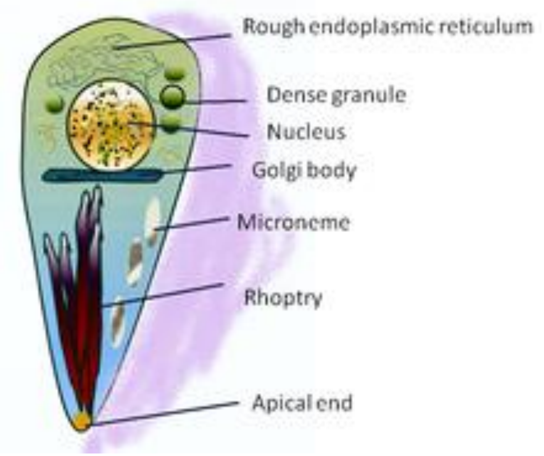
For this preparation the worm should be killed with chloroform and opened dry. A mid-dorsal incision is made, about segments 9 - 15. This will clearly show the white seminal vesicles. These are cut off and placed in a watch-glass. The material is covered, with about five times its bulk, with 0.75% saline, and is then teased thoroughly with needles, to release the contents of the seminal vesicles. A drop of the milky fluid obtained, is placed on a cover-glass, dried by warming and fixed in alcohol. This is then stained with Ehrlich's haematoxylin and again dried by warming. The cover-glass is placed on a microscope slide, in the centre of which a drop of Canada Balsam has been placed, and is then examined microscopically. Apart from the presence of the parasite, all the developing stages of the spermatozoa (the morula, resembling a blackberry) will be seen. .

Coccidians

Coccidians are generally parasites of vertebrates. Like gregarines they are commonly parasites of the epithelial cells of the gut but may infect other tissues. The typical coccidial life cycle while similar to that of the gregarines differs in zygote formation. Some trophozoites enlage and become macrogamete while others divide repeatedly to form microgametes. The microgametes are motile and must reach the macrogamete to fertilize it. The fertilized macrogamete forms a zygote which in its turn forms an oocyst which is normally released from the body.

Toxoplasma gondii

The parasite consists of only a single eukaryotic cell and re
the host. Virtually all birds and mammals can serve as intermedia
parasite replicates asexually, but the cat is the final host wherein
takes place. In humans, infection can originate from contact with
or through consumption of undercooked, infected meat. Upon ing
penetrate the gut-wall and infect cells of the host wherein they st
several division rounds parasites emerge from the infected cell, d
and quickly invade neighboring cells to repeat

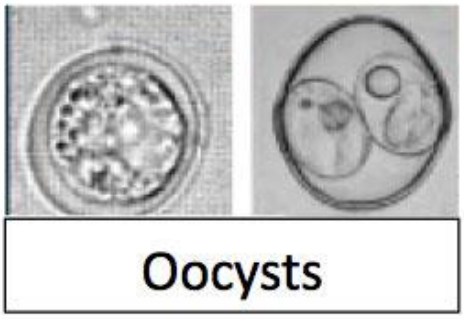


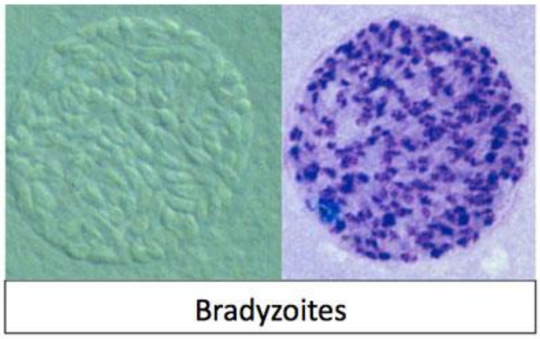
T. gondii tachyzoites

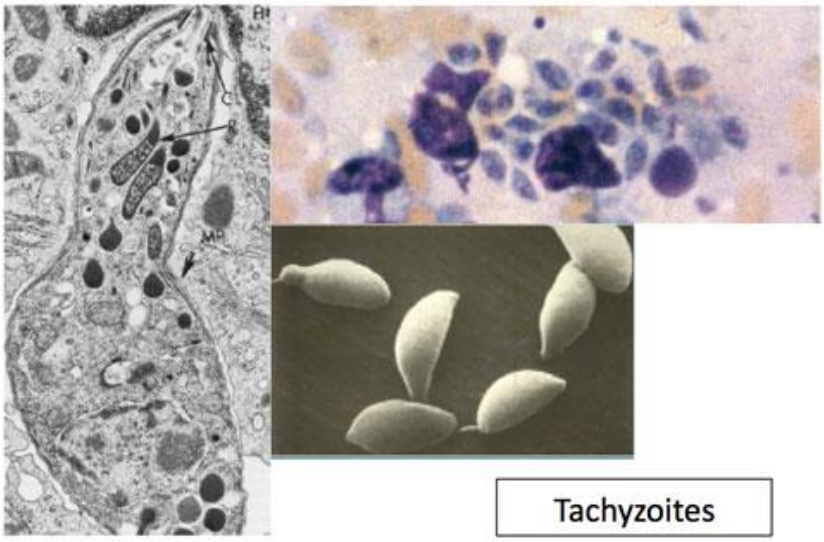
Diagram of *Toxoplasma gondii* structure

Life cycle

- **Stages**
  - Oocysts (resistant stages for environmental transmission; only develop in and shed by cats)
  - Tachyzoites (rapidly dividing tissue stages)
  - Bradyzoites (slow dividing, encysted tissue stages)

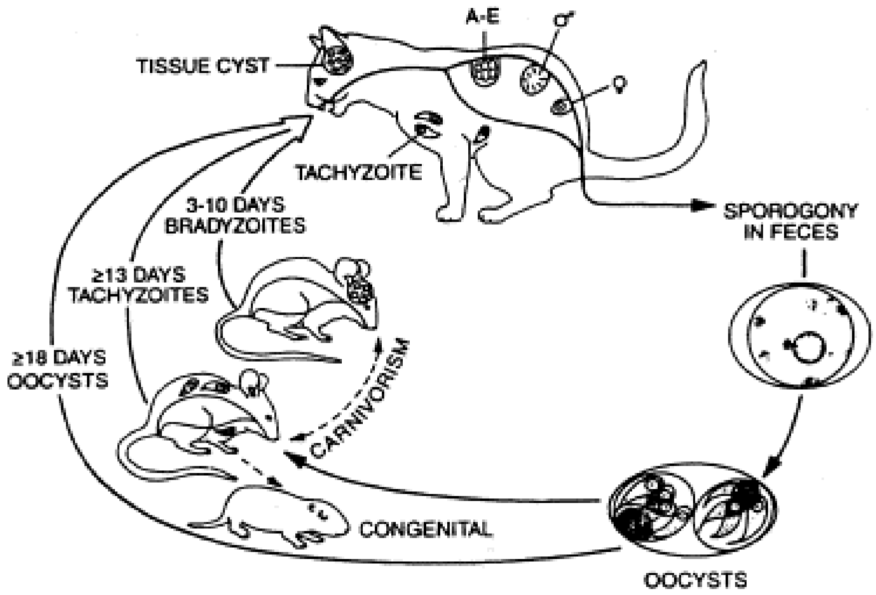






The life cycle of *T. gondii* has two phases. The sexual part of the life cycle (coccidia like) takes place only in cats, both domestic and wild (family Felidae),[which makes cats the parasite's primary host. The second phase, the asexual part of the life cycle, can take place in other warm-blooded animals, including cats, mice, humans, and birds. The hosts in which asexual reproduction takes place is called the intermediate host. Rodents are the typical intermediate host.

In both kinds of hosts, the Toxoplasma parasite invades cells and forms a space called a vacuole. Inside this specialized vacuole, called a parasitophorous vacuole, the parasite forms bradyzoites, which are the slowly replicating versions of the parasite. The vacuoles containing the reproductive bradyzoites form cysts mainly in the tissues of the muscles and brain.



Toxoplasma's resistance to anti-toxoplasmosis medication varies, but the cysts are very difficult to eradicate entirely. Inside the vacuoles, *T. gondii* replicates itself (by endodyogeny) until the infected cell fills with parasites and bursts, releasing tachyzoites, the motile, asexually reproducing form of the parasite. Unlike the bradyzoites, the free tachyzoites are usually efficiently cleared by the host's immune system, although some of them manage to infect cells and form bradyzoites, thus maintaining the infection.

Tissue cysts are ingested by a cat (e.g., by feeding on an infected mouse). The cysts survive passage through the stomach of the cat and the parasites infect epithelium of the small intestine where they undergo sexual reproduction and oocyst formation. Oocysts are shed with the feces. Animals and humans that ingest oocysts (e.g., by eating unwashed vegetables) or tissue cysts in improperly cooked meat become infected. The parasite enters macrophages in the intestinal lining and is distributed via the blood stream throughout the body.

Similar to the mechanism used in many viruses, Toxoplasma is able to dysregulate its host’s cell cycle by halting cell division before mitosis (the G2/M border). This dysregulation of the host’s cell cycle is caused by a heat-sensitive secretion (with a molecular mass larger than 10 kDa). Infected cells secrete the factor which inhibits the cell cycle of neighboring cells. The reason for Toxoplasma’s dysregulation is unknown, but studies have shown that infection is preferential to host cells in the S-phase and host cell structures with which Toxoplasma interacts may not be accessible during other stages of the cell cycle.

Acute stage *Toxoplasma* infections can be asymptomatic, but often give flu-like symptoms in the early acute stages, and like flu can become, in very rare cases, fatal. The acute stage fades in a few days to months, leading to the latent stage. Latent infection is normally asymptomatic; however, in the case of immunocompromised patients (such as those infected with HIV or transplant recipients on immunosuppressive therapy), toxoplasmosis can develop. The most notable manifestation of toxoplasmosis in immunocompromised patients is toxoplasmic encephalitis, which can be deadly. If infection with *T. gondii* occurs for the first time during pregnancy, during an activity such as changing cat litter of a cat infected with *T. gondii* (uptake of cyst by inhalation, followed by ingestion as the mucus is cleared), the parasite can cross the placenta, possibly leading to hydrocephalus or microcephaly, intracranial calcification, and chorioretinitis, with the possibility of spontaneous abortion (miscarriage) or intrauterine death.

- Toxoplasmosis is a multisystemic infection characterized by granulomatous inflammation associated with tachyzoite proliferation in the tissues.
- Organs and tissues commonly affected include lymph nodes, liver, lung, brain/spinal cord, and eye.
- Toxoplasmosis may cause focal or generalized lymphadenitis, encephalitis, pneumonitis, myocarditis, and retinochoroiditis.
- There may be associated fever, weight loss, and lethargy.
- Congenital infection with Toxoplasma is associated with neurologic disease, birth defects, stillbirth, and ocular disease in humans and some other animals.

- **Transmission**
  - Ingestion of tachyzoites or bradyzoites in mammalian or avian tissues
  - Ingestion of sporulated oocysts from areas or articles contaminated by feline feces (e.g., soil, water, vegetation)
  - Transplacental or transmammary transfer of tachyzoites
- **Prepatent Period and Environmental Factors**
  - Cats shed oocysts:
    - in 3 to 10 days following ingestion of bradyzoites in raw meat
    - in 19 to 48 days following ingestion of oocysts
  - Oocysts shed by cats sporulate (become infective) in 1 to 5 days and survive for months to years in the environment.

Haemosporidia

The Haemosporidians have more complex life cycles that alternate between an arthropod and a vertebrate host. The trophozoite parasitizes erythrocytes or other tissues in the vertebrate host. Microgametes and macrogametes are always found in the blood. The gametes are taken up by the insect vector during a blood meal. The microgametes migrate within the gut of the insect vector and fuse with the macrogametes. The fertilized macrogamete now becomes an ookinete which penetrates the body of the vector. The ookinete then transforms into an oocyst and divides initially by meiosis and then by mitosis (haplontic life cycle) to give rise to the sporozoites. The sporozoites escape from the oocyst and migrate within the body of the vector to the salivary glands where they are injected into the new vertebrate host when the insect vector feeds again.

Haemosporidia is divided into 2 orders:

- Order Chromatorida (with pigmented intraerythrocytic parasites)
  - Suborder Laveraniina
    - Family Plasmodiidae
    - Family Haemoproteidae

- Order Achromatorida (with non-pigmented intraerythrocytic para
  - Suborder Babesiina includes agents of piroplasmosis eg.
    - Genus *Babesia*
  - Suborder Theileriina includes parasites of erythrocytes and diverse white blood cells with sexual reproduction by exoerythrocytic or by exo- and endoerythrocytic schizogony. Genus *Leucocytozoon*

Plasmodiidae

The **Plasmodiidae** are a family of apicomplexan parasites, including the type genus *Plasmodium*, which is responsible for malaria. This genus was created in 1903 by Mesnil. They are one of the four families in the order Haemospororida.

Diagnostic criteria
The diagnostic criteria of the *Plasmodiidae* are:

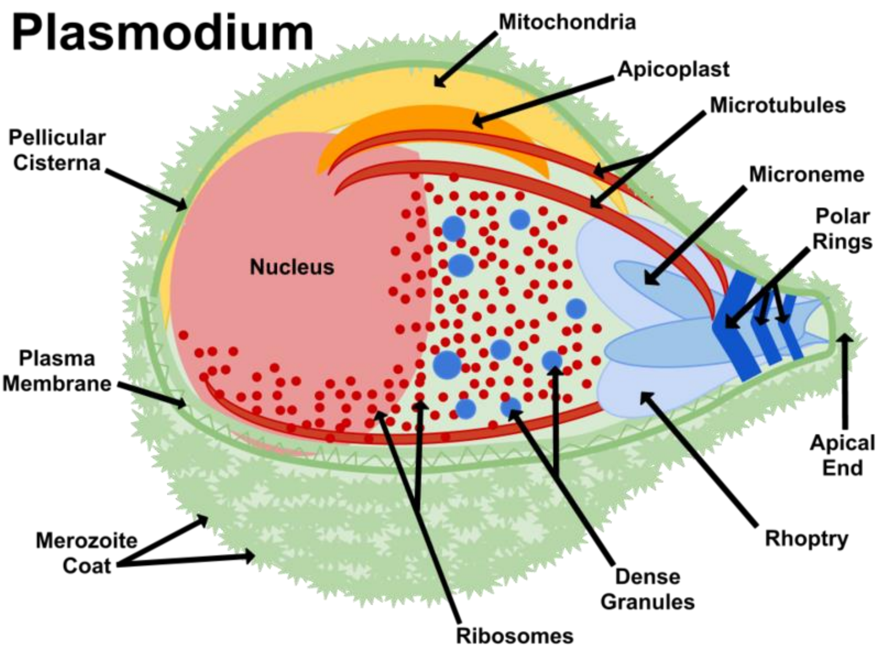
- macrogametes and microgamonts develop independently
- syzygy absent
- microgametocyte produces 8 flagellated microgametes
- zygote is motile (known as an ookinete)
- conoid present in ookinete stage only
- sporozoites naked in oocyst (that is without a sporocyst)
- sporozoites have three walls.
- heteroxenous: merogony and gamogony occur in vertebrate host and fertilization and sporogony in definitive host (a blood sucking insect)
- hemozoin pigment is produced

Plasmodium

Plasmodium is a genus of Apicomplexan parasites. Infection by these organisms is known as malaria. The genus *Plasmodium* was described in 1885 by Ettore Marchiafava and Angelo Celli. Currently over 200 species of this genus are recognized .Of the over 200 known species of *Plasmodium*, at least 11 species infect humans. Other species infect other animals, including monkeys, rodents, birds, and reptiles. The parasite always has two hosts in its life cycle: a vector—usually a mosquito—and a vertebrate host.

<u>History of malaria</u>

The organism itself was first seen by Laveran on November 6, 1880 at a military hospital in Constantine, Algeria, when he discovered a microgametocyte exflagellating. In 1885, similar organisms were discovered within the blood of birds in Russia. There was brief speculation that birds might be involved in the transmission of malaria; in 1894 Patrick Manson hypothesized that mosquitoes could transmit malaria. This hypothesis was independently confirmed by the Italian physician Giovanni Battista Grassi working in Italy and the British physician Ronald Ross working in India, both in 1898. Ross demonstrated the existence of *Plasmodium* in the wall of the midgut and salivary glands of a *Culex* mosquito using bird species as the vertebrate host. For this discovery he won the Nobel Prize in 1902. Grassi showed that human malaria could only be transmitted by *Anopheles* mosquitoes. It is worth noting, however, that for some species the vector may not be a mosquito.



As a protist, the plasmodium is a eukaryote of the phylum Apicomplexa. Unusual characteristics of this organism in comparison to general eukaryotes include the rhoptry, micronemes, and polar rings near the apical end. The plasmodium is known best for the infection it causes, malaria.

The genome of several *Plasmodium* species—*Plasmodium falciparum*, *Plasmodium knowlesi*, *Plasmodium vivax*, *Plasmodium berghei* and *Plasmodium yoelii*—have been sequenced. All these species have genomes of about 25 megabases organised into 14 chromosomes consistent with earlier estimates. The chromosomes vary in length from 500 kilobases to 3.5 megabases and it is presumed that this is the pattern throughout the genus.
The plasmodium contains a degenerated chloroplast called an apicoplast.

Diagnostic characteristics of the genus *Plasmodium*

- Merogony occurs both in erythrocytes and other tissues
- Merozoites, schizonts or gametocytes can be seen within erythrocytes and may displace the host nucleus
- Merozoites have a "signet-ring" appearance due to a large vacuole that forces the parasite's nucleus to one pole
- Schizonts are round to oval inclusions that contain the deeply staining merozoites
- Forms gamonts in erythrocytes
- Gametocytes are 'halter-shaped' similar to *Haemoproteus* but the pigment granules are more confined
- Hemozoin is present
- Vectors are either mosquitos or sandflies
- Vertebrate hosts include mammals, birds and reptiles

Life cycle
The life cycle of *Plasmodium*, while complex, is similar to that of several other species in the Haemosporidia.
All the *Plasmodium* species causing malaria in humans are transmitted by mosquito species of the genus *Anopheles*. Species of the mosquito genera *Aedes*, *Culex*, *Culiseta*, *Mansonia* and

Theobaldia can also transmit malaria but not to humans. Bird malaria is commonly carried by species belonging to the genus *Culex*. The life cycle of *Plasmodium* was discovered by Ross who worked with species from the genus *Culex*.
Both sexes of mosquitos live on nectar. Because nectar's protein content alone is insufficient for oogenesis (egg production) one or more blood meals is needed by the female. Only female mosquitoes bite.
1- **Sporozoites** from the saliva of a biting female mosquito are transmitted to either the blood or the lymphatic system of the recipient. It has been known for some time now that the parasites block the salivary ducts of the mosquito and as a consequence the insect normally requires multiple attempts to obtain blood. The reason for this has not been clear. It is now known that the multiple attempts by the mosquito may contribute to immunological tolerance of the parasite. The majority of sporozoites appear to be injected into the subcutaneous tissue from which they migrate into the capillaries. A proportion are ingested by macrophages and still others are taken up by the lymphatic system where they are presumably destroyed. ~10% of the parasites inoculated by the mosquitoes may remain in the skin where they may develop into infective merozoites.[

2-Dendritic cells

It is known that the murine parasites can infect, survive and replicate within plasmacytoid dendritic cells of the spleen and that these infections may be productive. The importance of this site of replication in mice has yet to be established and it is currently unknown if these cells support parasite replication in other species.

3-Hepatic stages

The majority of sporozoites migrate to the liver and invade hepatocytes. For reasons that are currently unclear each sporozoite typically penetrates several hepatocytes before choosing one to reside within. Once the sporozoite has ceased migration it undergoes an initial remodelling of the pellicle, with disassembly of the inner membrane complex and the appearance of a bulb that progressively enlarges until the initially elongated sporozoite has transformed into a rounded form. This rounded form then matures within the hepatocyte to a schizont containing many merozoites. In some *Plasmodium* species, such as *Plasmodium vivax* and *Plasmodium ovale*, the parasite in the hepatocyte may not achieve maturation to a schizont immediately but remain as a latent or dormant form and called a **hypnozoite**. Although *Plasmodium falciparum* is not considered to have a hypnozoite form, this may not be entirely correct (. This stage may be as short as 48 hours in the rodent parasites and as long as 15 days in *P. malariae* in humans.

A proportion of the hepatic stages may remain within the liver for considerable time - a form known as hypnozoites. Reactivation of the hypnozoites has been reported for up to 30 years after the initial infection in humans. The factors precipating this reactivation are not known. In the species *Plasmodium ovale* and *Plasmodium vivax*, but not in *Plasmodium malariae*, hypnozoites have been shown to occur. It is not yet known if hypnozoite reactivaction occurs with any of the remaining species that infect humans but this is presumed to be the case.

The development from the hepatic stages to the erythrocytic stages has, until very recently, been obscure. Within the hepatocyte the parasites develop into huge multinucleated schizonts within a parasitophorous vacuole. Thousands of merozoites are formed and released into the host cell cytoplasm by complete disintegration of this parasitophorous vacuole membrane. This is associated with degeneration of the host cell's mitochondria and cessation of protein synthesis which is probably due to the lack of mitochondially produced ATP. This process results in death and detachment of the infected hepatocyte and is followed by the formation of merosomes which may contain hundreds or thousands of merozoites. The membrane of the merosome is then formed from that of the hepatocyte membrane but the hepatocyte proteins within the membrane are lost. In contrast the membrane of the merozoites is formed by repeated invagination of the parasite's own membrane. This host derived membrane presumably provides protection from the immune system while the merozoites are transported to the lung. These merosomes lodge in the pulmonary capillaries and slowly disintegrate there over 48–72 hours releasing merozoites. Erythrocyte invasion is enhanced when blood flow is slow and the cells are tightly packed: both of these conditions are found in the alveolar capillaries.

Infection of the liver may be influenced by the iron regulatory hormone **hepcidin** and this may play a role in preventing superinfection despite repeated inoculation.

4- Erythrocyte stages

After entering the erythrocyte, the merozoite lose one of their membranes, the apical rings, conoid and the rhopteries. Phagotropy commences and both smooth and granular endoplasmic reticulum becomes prominent. The nucleus may become lobulated.

Within the erythrocytes the merozoite grow first to a **ring-shaped form** and then to a larger **trophozoite** form. In the **schizont** stage, the parasite divides several times to produce new **merozoites**, which leave the red blood cells and travel within the bloodstream to invade new red blood cells. The parasite feeds by ingesting haemoglobin and other materials from red blood cells and serum. The feeding process damages the erythrocytes.

Erythrocytes infected by *Plasmodium falciparum* tend to form clumps - rosettes - and these have been linked to pathology caused by vascular occlusion. This rosette formation may be inhibited by heparin. This agent has been used in the past as part of the treatment of malaria but was abandoned because of an increased risk of haemorrhage. Low molecular weight heparin also disrupts rosette formation and may have a lower risk of bleeding in malaria. Rosetting has been shown to be due to the binding of the erythrocyte major protein (the *var* gene product) to the ABO blood group protein. Blood group A is preferred over group B which in turn is preferred over group O. This has been shown to be due to different fits of blood group protein to the erythrocyte major protein. The binding side on the erythrocyte major protein is opposite to the heparin binding site on the same protein.

5- Merozoites

The budding of the merozoites from interconnected cytoplasmic masses (pseudocytomeres) is a complex process. At the tip of each bud a thickened region of pellicle gives rise to the apical rings and conoid. As development proceeds an aggregation of smooth membranes and the nucleus enter the base of the bud. The cytoplasm contains numerous large ribosomes. Synchronous multiple cytoplasmic cleavage of the mature schizont results in the formation of numerous uninucleate merozoites.

Escape of the merozoites from the erythrocyte has also been studied. The erythrocyte swells under osmotic pressure. A pore opens in the erythrocte membrane and 1-2 meorozites escape. This is followed by an eversion the entire erythrocyte membrane, an action that propels the merozoites into the blood stream.

6-Gametocytes

Most merozoites continue this replicative cycle but some merozoites differentiate into male or female sexual forms (gametocytes) (also in the blood), which are taken up by the female mosquito. This process of differentiation into gametocytes appears to occur in the bone marrow. Five distinct morphological stages have recognised (stages I - V). Female gametocytes are produced about four times as commonly as male. In chronic infections in humans the gametocytes are often the only forms found in the blood. Incidentally the characteristic form of the female gametocytes in *Plasmodium falciparum* gave rise to this species's name.

Gameteocytes appear in the blood after a number of days post infection. In *P. falciparum* infections they appear after 7 to 15 days while in others they appear after 1 to 3 days. The ratio of asexual to sexual forms is between 10:1 and 156:1 The half life of the gametocytes has been estimated to be between 2 and 3 days but some are known to persist for up to four weeks.

7-Gametocyte morphology

The five recognised morphological stages were first described by Field and Shute in 1956. One constant feature of the gametocytes in all stages that distinguishes them from the asexual forms is the presence of a **pellicular complex**. This originates in small membranous vesicle observed beneath the gametocyte plasmalemma in late stage I. Its function is not known. The structure itself consists of a subpellicular membrane vacuole. Deep to this is an array of longitudinally oriented microtubules. This structure is likely to be relatively inflexible and may help to explain the lack of amoeboid forms observed in asexual parasites.
Gametocyte elongation is driven by the assembly of a system of flattened cisternal membrane compartments underneath the parasite plasma membrane and has a supporting network of microtubules. The sub-pellicular membrane complex is analogous to the inner membrane complex, an organelle with structural and motor functions that is well conserved across the apicomplexa.
Early **stage one** gametoctyes are very difficult to distinguish from small round trophozoites. Later stages can be distinguished by the distribution of pigment granulues. Under the electrom microscope the formation of the subpellicular membrane and a smooth plasma membrane are recognisable. The nuclei are recognisably dimorphic into male and female. These forms may be found between day 0 and day 2 in *P falciparum* infections.

In stage **two** the gametocyte enlarges and becomes D shaped. The nucleus may occupy a terminal end of the cell or lie along its length. Early spindle formation may be visible. These forms are found between day 1 to day 4 in *P falciparum* infections.

In stage **three** the erythrocyte becomes distorted. A staining difference between the male and female gametoctyes is apparent (male stain pink while female stain faint blue with the usual stains). The male nucleus is noticeably larger than the female and more lobulated. The female cytoplasm has more ribosomes, endoplasmic reticulum and mitochondria.

Electron dense organelles (osmophilic bodies) are found in both sexes but are more numerous in the female. The osmophilic bodies are thought to be involved in egress of the gametocyte from the erythrocyte. These organelles are found between day 4 and day 10 in *P. falciparum* infections. They are connected to the gametocyte surface by ducts and are almost absent after transformation into the female gamete.

In stage **four** the erythrocyte is clearly deformed and the gametocyte is elongated. The male gametocytes stain red while the female stain violet blue. In the male pigment granules are scattered while in the female they are more dense. In the male the kinetochores of each chromosome are located over a nuclear pore.

In stage **five** the gametocytes are clearly recognisable on light microscopy with the typical banana shaped female gametocytes. The subpellicular microtubules depolymerise but the membrane itself remains. In the male gametocyte exhibit the is a dramatic reduction in ribosomal density. Very few mitochondria are retained and the nucleus enlarges with a kinetochore complex attached to the nuclear envelope. In the female gametocytes there are numerous mitochondria, ribosomes and osmophillic bodies. The nucleus is small with a transcription factory.

Stages other than **stage five** are not normally found in the peripheral blood. For reasons not yet understood , stages **I to IV** are sequestered preferentially in the bone marrow and spleen. Stage V gametocytes only become infectious to mosquitoes after a further two or three days of circulation.

Within the gametocytes are poorly studied **Garnham bodies (G bodies**). These are membranous whorls within the cytoplasm and are highly diverse in morphology. They occur in both immature and mature gametocytes. Hemozoin is present within them. Their function is currently unknown.

Infection of mosquito

In the mosquito's midgut, the gametocytes develop into gametes: the process of activation and gametogenesis occur within 15 minutes of ingestion. and fertilize each other resulting in formation of a diploid zygote: this usually occurs within one hour of ingestion. Zygotes immediately undergo meiosis and differentiate within 24 hours of ingestion into motile, invasive ookinetes. It has been shown that up to 50% of the ookinetes may undergo apoptosis within the midgut. The reason for this behavior is unknown. While in the mosquito gut the parasites form thin cytoplasmic extensions to communicate with each other. These structures persist from the time of gametocyte activation until the zygote transforms into an ookinete. The function of these tubular structures remains to be discovered.

The ookinetes penetrate the midgut epithelium and escape the midgut, then attach themselves onto the exterior of the gut membrane beneath the basal lamina where they differentiate into oocysts. As in the liver the parasite tends to invade a number of cells before choosing one to reside in. The reason for this behavior is not known. Here they divide many times (usually ~11) to produce large numbers (~8,000) of tiny elongated sporozoites. These sporozoites migrate to the salivary glands of the mosquito where they are injected into the blood and subcutaneous tissue of the next host the mosquito bites.

The pellicle of the ookinete is composed of three membranes: the plasma membrane, and the two linked intermediate and inner membranes which form one flattened vacuole located beneath the plasma membrane. Beneath this vacuole is found an array of microtubules that are connected to the inner membrane by intramembranous particles. The pellicle differs from all other apicomplexan motile stages by the presence of large pores whose function is currently unknown.

The invasion process appears to be dependent on a serine protease produced by the mosquito in the midgut epithelial cells and in the basal side of the salivary glands.

The escape of the gametocytes from the erythrocytes has been until recently obscure. The parasitophorous vacuole membrane ruptures at multiple sites within less than a minute following ingestion. This process may be inhibited by cysteine protease inhibitors. After this rupture of the vacuole the subpellicular membrane begins to disintegrate. This process also can be inhibited by aspartic and the cysteine/serine protease inhibitors. Approximately 15 minutes post-activation, the erythrocyte membrane ruptures at a single breaking point a third process that can be interrupted by protease inhibitors.

Effects on the mosquito

Infection of the mosquito has noticeable effects on the host. The presence of the parasite induces apoptosis of the egg follicles. The development of the parasite in the mosquito is temperature dependent with higher temperatures being associated with more rapid development. Higher temperatures appear to enhance the mosquito's immune system leading to a lower average infection rate.

Survival of infected mosquitoes is enhanced in starvation conditions compared to uninfected controls. Development within the mosquito involves several insulin like peptides. Blocking this pathway results in reduced parasite development. It appears that the parasite is capable of altering the physiology of the mosquito host and this alternation under starvation conditions is favourable to the host. Infection appears to reduce fecundity (ability to reproduce) and to increase survival of the mosquito.

Ingested peptides within the blood meal may influence the infection rate and survival of the mosquito. Insulin-like growth factor 1 and insulin can persist within the blood meal in the midgut for up to 30 hours. Both peptides can cross the epithelial surface of the midgut and affect the mosquito's physiology. Insulin like growth factor 1 can extended the mosquito lifespan, reduce the rate of infection and the parasite load. Insulin, in contrast, tends to have the opposite effects.

Phylum Cnidospora

Cnidospora is a subphylum of microscopic spore-forming parasites in the phylum Protozoa. The subphylum was divided into two classes, the Myxosporidea (now classified as higher-animals, i.e. Metazoa) and the Microsporidea (now classified as fungi)

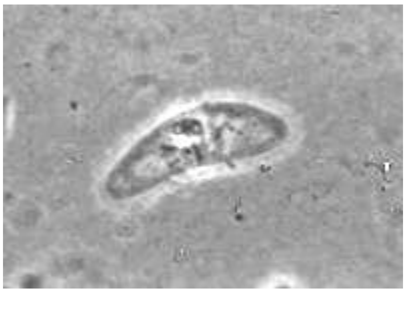
Myxosporea

The **Myxosporea** are a class of microscopic parasites, belonging to the Myxozoa. They have a complex life cycle which comprises vegetative forms in two hosts, an aquatic invertebrate (generally an annelid) and an ectothermic vertebrate, usually a fish. Each host releases a different type of spore. The two forms of spore are so different that until recently they were treated as belonging to different classes within the Myxozoa.

Life cycle

a- Myxosporean stage

In the vertebrate host, organisms belonging to the Myxosporea are characterised by spores composed of several cells, contained within between 1 and 7 shell "valves". These cells include 1 or two amoeboid infective germ cells, and 2 to 7 nematocyst-like polar capsules. During some stages of the life cycle, the germ cells are completely contained within cells of the host.



Alataspora solomoni, a myxosporean parasite found in the gall bladder of Atlantic Horse Mackerel. This species has two "valves" arranged in a banana-shape, with two polar capsules, one on either side of the suture between the valves

The myxosporean species are typically defined by the size and shape of the spores released by vertebrate hosts. For instance, the genus Ceratomyxa is a common parasite of the gallbladder of many fish species; they have "boomerang-like" spores with two polar capsules resembling eyes in the middle of the spore. Most species within the myxosporea are sized between 10 µm and 20 µm, however *Myxidium giganticum* is up to 98 µm long.

The spore shell consists of shell "valves", which are joined together along "suture lines". Some species contain polysaccharide reserves in the form of β-glycogen particles, which are concentrated in a central "iodinophillous vacuole".

The shell valves may have smooth or ridged surfaces, may be drawn out into lateral "alate" projections, and may or may not have a mucous coating. These adaption probably serve to increase the buoyancy of the spore in the water column, aiding dispersal. The valves are formed of resistant, non-keratinaceous protein.

b- Actinosporean stage

The alternate stage of the life cycle is generally released by an annelid or polychaete worm, typically resembles three or four hooks united by the base, and can also be used for identification. These organisms were classified in the class Actinosporea, until careful experimentation supported by analysis of the 16S Ribosomal Subunit RNA sequence in the early 1990s allowed matching of several actinosporeans with their myxosporean equivalent.

Transmission

Until the 1980s, direct transmission of myxosporeans was presumed. In 1984, it was shown experimentally that spores of *Myxobolus cerebralis* failed to produce infections when fed to salmonids .To reproduce successfully, this species requires a tubificid worm as an intermediate host, in which the spores develop into a "species" of the "genus" *Triactinomyxon*. These spores develop inside the oligochaete into forms which are infective to salmonids. Such a life cycle, with two different sexual stages, resulting in two kinds of resistant spores, is unique amongst the parasitic organisms. This mode of life cycle has been confirmed in several other *Myxobolus* species.

This mode of infection has also been proved in other families. *Ceratomyxa shasta*, an economically important parasite of salmonids, has been shown to use a polychaete worm as an alternate host. Surprisingly, however, direct transmission between fish has also been demonstrated, so far in three species of *Enteromyxum*.

Examples of Myxosporean genera are *Kudoa*, which attacks fish muscle, and *Myxobolus*, which attacks the hearts of freshwater fishes.

Microsporidia

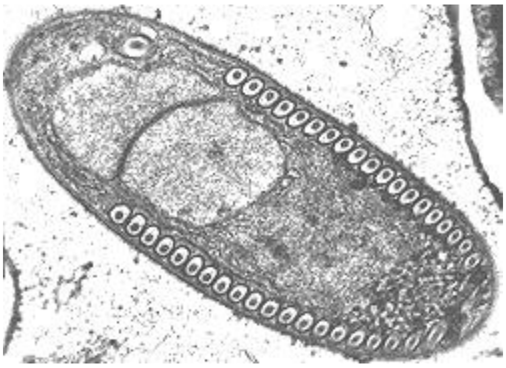
The **microsporidia** constitute a phylum (**Microspora**) of spore-forming unicellular parasites. They were once thought to be protists but are now known to be fungi. Loosely 1500 of the probably more than one million[species are named now. Microsporidia are restricted to animal hosts, and all major groups of animals host microsporidia. Most infect insects, but they are also responsible for common diseases of crustaceans and fish. The distinguished species of microsporidia usually infect one specific host or a related group of hosts. Several species, most of which are opportunistic, also infect humans.

Approximately 10 percent of the species are parasites of vertebrates, including humans.

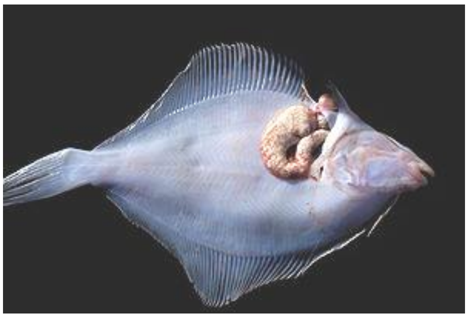
After infection they influence their hosts in various ways and all organs and tissues are invaded, though generally by different species of microsporidia. Some species are lethal, and a few are used in biological control of insect pests. Parasitic castration, gigantism, or change of host sex are all potential effects of microsporidian parasitism (in insects). In the most advanced cases of parasitism the microsporidium rules the host cell completely and controls its metabolism and reproduction, forming a xenoma. .

[Replication takes place within the host's cells, which are infected by means of unicellular spores. These vary from 1-40 µm, making them some of the smallest eukaryotes.[*citation needed*] Microsporidia that infect mammals are 1.0-4.0 µm. They also have the smallest eukaryotic genomes.

Microsporidium was once the vernacular name for a member of the class **Microsporidea** in the protozoan subphylum Cnidospora.



Sporoblast of *Fibrillanosema crangonycis*



Xenoma on flatfish caused by *Glugea stephani*

Morphology

Microsporidia lack mitochondria and possess, instead, mitosomes. They also lack motile structures such as flagella.
Microsporidia produce highly resistant spores to survive outside the host for up to several years. Spore morphology is useful in distinguishing between different species. Spores of most species are oval or pyriform, but rod-shaped or spherical spores are not unusual. A few genera produce spores of unique shape for the genus.

The spore is protected by a wall, consisting of three layers:

- an outer electron-dense *exospore*
- a median, wide and seemingly structureless *endospore*, containing chitin
- a thin internal *plasma membrane*

In most cases there are two closely associated nuclei, forming a *diplokaryon*, but sometimes there is only one.
The anterior half of the spore contains a harpoon-like apparatus with a long thread-like *polar filament*, which is coiled up in the posterior half of the spore. The anterior part of the polar filament is surrounded by a *polaroplast*, a lamella of membranes. Behind the polar filament there is a posterior *vacuole*.

Infection

In the gut of the host the spore germinates, it builds up osmotic pressure until its rigid wall ruptures at its thinnest point at the apex. The posterior vacuole swells, forcing the polar filament to rapidly eject the infectious content into the cytoplasm of the potential host. Simultaneously the material of the filament is rearranged to form a tube which functions as a hypodermic needle and penetrates the gut epithelium.

Once inside the host cell, a sporoplasm grows, dividing or forming a multinucleate plasmodium, before producing new spores. The life cycle varies considerably. Some have a simple asexual life cycle, while others have a complex life cycle involving multiple hosts and both asexual and sexual reproduction. Different types of spores may be produced at different stages, probably with different functions including autoinfection (transmission within a single host).

Medical implications

The microsporidia often cause chronic, debilitating diseases rather than lethal infections. Effects on the host include reduced longevity, fertility, weight, and general vigor. Vertical transmission of microsporidia is frequently reported. In the case of insect hosts, vertical transmission often occurs as transovarial transmission, where the microsporidian parasites pass from the ovaries of the female host into eggs and eventually multiply in the infected larvae. *Amblyospora salinaria* n. sp. which infects the mosquito *Culex salinarius* Coquillett, and *Amblyospora californica* which infects the mosquito *Culex tarsalis* Coquillett, provide typical examples of transovarial transmission of microsporidia.

Microsporidia, specifically the mosquito-infecting *Vavraia culicis*, are being explored as a possible 'evolution-proof' malaria-control method. Microsporidian infection of *Anopheles gambiae* (the principal vector of *Plasmodium falciparum* malaria) reduces malarial infection within the mosquito, and shortens the mosquito lifespan. As the majority of malaria-infected mosquitoes naturally die before the malaria parasite is mature enough to transmit, any increase in mosquito mortality through microsporidian-infection may reduce malaria transmission to humans.

Clinical

A number of species may infect humans: these include *Trachipleistophora hominis*.

Classification

For some time microsporidia were considered as very primitive eukaryotes, especially because of the lack of mitochondria, and placed along with the other Protozoa such as *diplomonads*, *parabasalia* and *archamoebae* in the protist-group Archezoa. More recent research has falsified this theory of early origin (for all of these). Yet microsporidia are proposed to be highly developed and specialized organisms, which just dispensed functions that are needed no longer, because they are supplied by the host. Furthermore, spore-forming organisms in general do have a complex system of reproduction, both sexual and asexual, which look far from primitive.

Nowadays microsporidia are placed within the Fungi or as a sister-group of the Fungi with a common ancestor.

Forming of clades is largely based on habitat and host. Three classes of Microsporidia are proposed by Vossbrinck and Debrunner-Vossbrinck, based on the habitat: Aquasporidia, Marinosporidia and Terresporidia.

Phylum Ciliophora

What Is Infusoria?

The term infusoria is an old one that has changed in meaning over the years. At one time it referred to just about any microscopic, or nearly microscopic, organism that lived in fresh water. That use of that word long ago became outdated in the scientific community. However, the term infusoria is still used by many within the aquarium community, even by younger fish enthusiasts.

Ciliophora is protozoa which at least as young possess cilia. They are never amoeboid Free-living ciliates can be found in almost any habitat that has water – in soils, hot springs and Antarctic sea ice. Symbiotic species live as commensals in sea urchins or as parasites of lobsters and fish. Ciliate life histories can have specialised forms for dispersal and for resisting desiccation.

General characters

- They are characterised by three main features: they exhibit nuclear dimorphism; undergo conjugation as a sexual process; and typically have cilia at some stage in their life cycle.
- They are the 'top' predators in microbial food webs, and were likely the major predatory group before the evolution of animals.
- Parasitic ciliates can cause morbidity and death of animals, and are becoming particularly important in aquaculture operations.
- They can have complex life cycles, including **macrostome** or cannibalistic stages, **swarmers** or dispersal stages and **cyst** or desiccation-resistant stages.
- The **kinetid**, an organellar complex in the cell cortex, is composed of at least one kinetosome and its cilium associated with two microtubular ribbons and a striated kinetodesmal fibril, whose patterned arrangement identifies a ciliate to a particular major clade or class.
- Their macronuclei divide in two ways, which suggest that macronuclear division evolved independently twice in the phylum**:**
  - **a-** **heterotrich ciliates** divide their macronucleus principally using extramacronuclear microtubules
  - **b-** **intramacronucleate** ciliates divide their macronucleus with intramacronuclear microtubules.

Classification

There are an estimated 7,500 species of ciliate known. Ciliates are classified on the basis of cilia arrangement, position, and ultrastructure. This work now involves electron microscopy and comparative molecular biology to estimate relationships. In the most recent classification of ciliates, the group is divided into eight classes. We list them here, along with common or well-known genera within each class:

- **Class Prostomatea**
  Benthic, mostly marine forms.
- **Class Litostomatea**
  Includes *Balantidium* and *Didinium*
- **Class Karyorelictida**
  Benthic, mostly marine forms.
- **Class Spirotrichea**
  Includes *Stentor*, *Stylonychia*, and tintinnids.
- **Class Phyllopharyngea**
  Includes suctorians.
- **Class Nassophorea**
  Includes *Paramecium* and *Euplotes*.
- **Class Oligohymenophorea**
  Includes *Tetrahymena*, *Vorticella*, and *Colpidium*.
- **Class Colpodea**
  Includes *Colpoda*.

The Ciliate subdivisions divided into:

- **Holotrich Ciliates.**
  Are those whose bodies are more or less uniformly covered with cilia. The cilia usually run in rows called kineties which often form curved or spiral patterns characteristic of the particular ciliate. This category has largely been abandoned in recent reclassifications of the ciliates.

- **Heterotrich Ciliates.**
  Are those which have, in addition to normal ciliature, specialized structures such as cirri or membranelles. These usually take the form of long cilia or a formation of membranelles around the mouth, or cirri which function as legs.

- **Peritrich Ciliates.**
  In these, ciliature is restricted to the (usually circular) zone around the mouth, the rest of the body having no cilia.

- **Colonial Ciliates.**
  Not used as a taxonomic group, but included here as colonial organisms are fairly common, and their coordinated behaviour can be spectacular when encountered. Most of the colonial ciliates are peritrichs of one kind or another.

- **Suctorians.**
  These organisms do not at first sight resemble the usual ciliates, but they are classified amongst them as they have ciliated larvae, and also have the nuclear dualism characteristic of the other ciliates. The adult forms have no cilia, but possess long hollow contractile tentacles through which they suck the contents of the prey organism.

Ciliata : Morphology

We often think of unicellular organisms as having simple, primitive structure. This is definitely an erroneous view when applied to the ciliates; they are probably the most complex of all unicellular organisms. Unlike multicellular organisms, which have cells specialized for performing the various body functions, single-celled organisms must perform all these functions with a single cell, and so their structure may be much more complex than the cells of larger organisms. Movement, sensitivity to the environment, water balance, and food capture must all be accomplished with the machinery in a single cell.

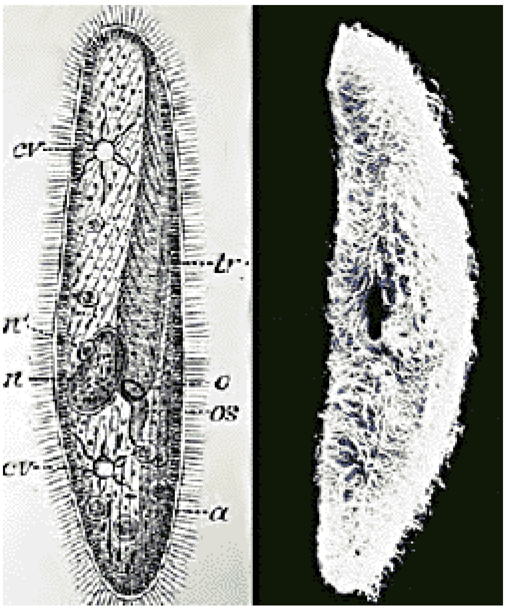
Ciliates include some of the largest free-living unicellular organisms (the ciliate *Stentor* can reach 2 millimeters in length), and include a wide variety of forms. We will use *Paramecium*, depicted at left, as a more or less typical ciliate for demonstrating features of ciliate anatomy.

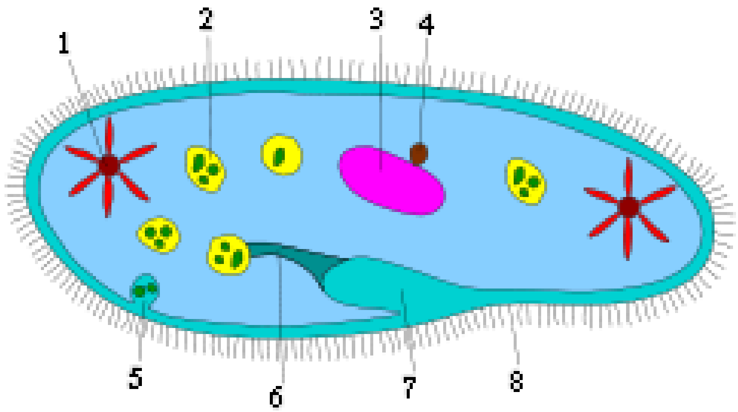
Unlike other eukaryotes, ciliates have two kinds of nuclei. The **micronucleus** (labeled n' on this diagram) contains chromosomes, with two copies of each chromosome; hence this nucleus is **diploid**, as is common in eukaryotes. A cillate may have one or several micronuclei. In the much larger **macronucleus** (n), the genetic material is in the form of short pieces of DNA, each of which may exist in tens of thousands of copies. At cell division, the micronuclei divide through **mitosis**, while in most ciliates the macronucleus simply pinches apart into two.

Aside from the nuclei, a ciliate contains several **vacuoles**, or round membranous structures that enclose food, waste, or various structures. **Digestive vacuoles** form at the end of the **gullet** (os) as food particles are ingested, and then circulate through the cell. Waste remaining in these vacuoles is discharged through a particular point in the cell membrane known as the **cytoproct**. The star-shaped **contractile vacuole** (cv) gathers excess water through microtubule-lined channels (the "rays" of the star) and periodically pumps it out through another special pore.

The outer layer, or **cortex**, is a complicated structure, separated from the interior of the cell by a layer of microfilaments. Each hair-like **cilium** is associated with a set of tubules and structural protein molecules that make up a **kinetosome**. In turn, kinetosomes are arranged in rows known as **kineties**. The cilia beat in waves to propel the organism forward and

also move food into the **cytostome**, the ciliate's "mouth," labelled (o). The mouth is sometimes set back in an **oral groove** (tr). The kineties around the cytostome are often arranged in a specialized way to generate water currents that funnel food particles into the cell. Also part of the cortex are the **extrusomes**, organelles that can rapidly eject short threadlike structures. These extrusomes function in predation, defense, and in forming cysts in various ciliates.

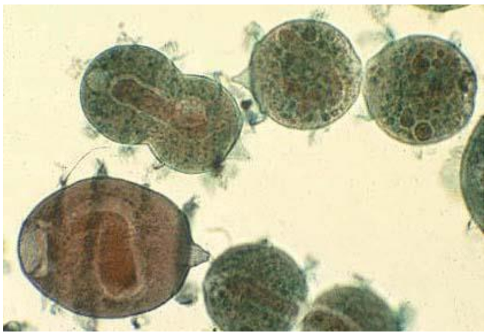




Cell structure of a ciliophoran: 1-contractile vacuole, 2-digestive vacuole, 3-macronucleus, 4-micronucleus, 5-cytoproct, 6-cytopharynx, 7-cytostome, 8-cilium.

Ciliata: Ecology

Most ciliates are free-living forms. Relatively few are parasitic, and only one species, *Balantidium coli*, is known to cause human disease. Some other ciliates cause diseases in fish and may present a problem for aquaculturists; others are parasites or commensals on various invertebrates. Still others live in great numbers in the digestive tracts of many hoofed mammals, where they serve to stabilize the large populations of symbiotic bacteria that break down cellulose in the animals' food.



Free-living ciliates may feed on bacteria, algae, or even other ciliates; *Didinium*, shown above, is a rapacious hunter and consumer of other ciliates. Some ciliates harbor symbiotic bacteria or algae. Free-living ciliates may be found almost anywhere there is liquid water, but different forms predominate in different habitats. Ciliates in soils tend to be small forms that can form resistant **cysts** in order to survive long periods of drying. Tintinnids abound in the marine plankton, where they and other ciliates may consume up to 90% of the production of planktonic bacteria and algae. Large ciliates are common in freshwater environments, in particular those that have been organically enriched (such as by sewage). By listing and counting the ciliate species in a sample of water, it is possible to estimate quickly how much organic material -- which could include pollution -- is present.

Reproduction and sexual phenomena

Ciliate undergoing the last processes of binary fission, a form of asexual reproduction

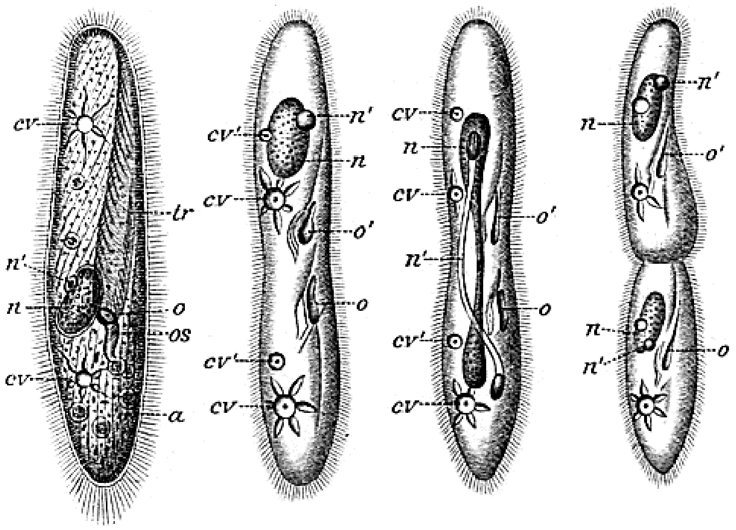
Asexual reproduction

Ciliates reproduce asexually, by various kinds of fission. During fission, the micronucleus undergoes mitosis and the macronucleus elongates and splits in half . The cell then divides in two, and each new cell obtains a copy of the micronucleus and the macronucleus.

Typically, the cell is **divided transversally**, with the anterior half of the ciliate (the *proter*) forming one new organism, and the posterior half (the *opisthe*) forming another. However, other types of fission occur in some ciliate groups. These include ***budding*** (the emergence of small ciliated offspring, or "swarmers," from the body of a mature parent); ***strobilation*** (multiple divisions along the cell body, producing a chain of new organisms); and ***palintomy*** (multiple fissions, usually within a cyst).

Sexual reproduction

Fission may occur spontaneously, as part of the vegetative cell cycle. Alternatively, it may be proceed as a result of self-fertilization (**autogamy**), or it may follow **conjugation**, a sexual phenomenon in which ciliates of compatible mating types exchange genetic material. During conjugation, two cells form a bridge between their cytoplasms, the micronuclei undergo meiosis, the macronuclei disappear, and the haploid micronuclei are exchanged over the bridge. In some ciliates (such as Vorticella), conjugating cells become permanently fused, and one conjugant is absorbed by the other. In most ciliate groups, however, the cells separate after conjugation, and both form new macronuclei from their micronuclei. Conjugation and autogamy are always followed by fission.



Conjugation is often induced by lack of food. Two ciliates of opposite mating types come close together and form a cytoplasmic bridge between the two cells; the micromuclei divide by **meiosis**, the macronuclei disintegrate, and the conjugating cells exchange haploid micronuclei over the cytoplasmic connection. They then separate, reform new macronuclei from their micronuclei, and divide. This may not sound very sexy, but remember that the essence of sexual reproduction is forming a new organism from the combined genetic material of parents. After conjugation, each ciliate partner has acquired new genetic material, and divides to give rise to progeny with a new combination of genes. This is essential to the survival of ciliate lineages; most ciliates cannot reproduce indefinitely by asexual fission, and eventually die out if prohibited from conjugating.

Unlike most other eukaryotes, ciliates have two different sorts of nuclei: a small, diploid micronucleus (reproduction), and a large, polyploid macronucleus (general cell regulation). The latter is generated from the micronucleus by amplification of the genome and heavy editing. Division of the macronucleus occurs by amitosis, the segregation of the chromosomes is by a process whose mechanism is unknown. This process is by no means perfect, and after about 200 generations the cell shows signs of aging. Periodically the macronuclei must be regenerated from the micronuclei. In most, this occurs during *conjugation*. Here two cells line up, the micronuclei undergo meiosis, some of the haploid daughters are exchanged and then fuse to form new micronuclei and macronuclei.

Feeding

Most ciliates are heterotrophs, feeding on smaller organisms, such as bacteria and algae, and detritus swept into the oral groove (mouth) by modified oral cilia. This usually includes a series of membranelles to the left of the mouth and a paroral membrane to its right, both of which arise from *polykinetids*, groups of many cilia together with associated structures. The food is moved by the cilia through the mouth pore into the gullet, which forms food vacuoles. This varies considerably, however. Some ciliates are mouthless and feed by absorption (osmotrophy), while others are predatory and feed on other protozoa and in particular on other ciliates. Some ciliates parasitize animals, although only one species, *Balantidium coli*, is known to cause disease in humans.

Food vacuoles are formed through phagocytosis and typically follow a particular path through the cell as their contents are digested and broken down via lysosomes so the substances the vacuole contains are then small enough to diffuse through the membrane of the food vacuole into the cell. Anything left in the food vacuole by the time it reaches the cytoproct (anus) is discharged via exocytosis. Most ciliates also have one or more prominent contractile vacuoles, which collect water and expel it from the cell to maintain osmotic pressure, or in some function to maintain ionic balance. In some genera, such as Paramecium, these have a distinctive star-shape, with each point being a collecting tube.

Specialized structures in ciliates

1- In some forms there are also body **polykinetids,** for instance, among the spirotrichs where they generally form bristles called *cirri*. More often body cilia are arranged in *mono-* and *dikinetids*, which respectively include one and two kinetosomes (basal bodies), each of which may support a cilium. These are arranged into rows called *kineties*, which run from the anterior to posterior of the cell. The body and oral kinetids make up the ***infraciliature,*** an organization unique to the ciliates and important in their classification, and include various fibrils and microtubules involved in coordinating the cilia.**The infraciliature** is one of the main component of the cell cortex.

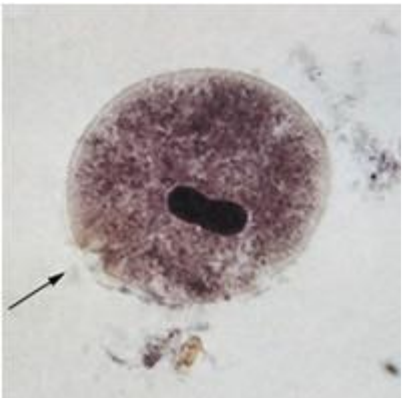
2- the ***alveoli***, small vesicles under the cell membrane that are packed against it to form a pellicle maintaining the cell's shape, which varies from flexible and contractile to rigid.

1- Numerous **mitochondria** and **extrusomes** are also generally present.

The presence of alveoli, the structure of the cilia, the form of mitosis and various other details indicate a close relationship between the ciliates, Apicomplexa, and dinoflagellates. These superficially dissimilar groups make up **the alveolates**.

Ciliates contain two types of nuclei: the somatic "macronucleus" and the germline "micronucleus". Only the DNA in the micronucleus is passed on during sexual reproduction (conjugation). On the other hand, only the DNA in the macronucleus is actively expressed and results in the phenotype of the organism. Macronuclear DNA is derived from micronuclear DNA by amazingly extensive DNA rearrangement and amplification.

The macronucleus begins as a copy of the micronucleus. The micronuclear chromosomes are fragmented into many smaller pieces and amplified to give many copies. The resulting macronuclear chromosomes often contain only a single gene. In *Tetrahymena*, the micronucleus has 10 chromosomes (5 per haploid genome), while the macronucleus has over 20,000 chromosomes.



A trophozoite of *Balantidium coli*