Phylum Protozoa: General characters and Classification

Introduction

The animals included in phylum Protozoa can be defined as microscopic and a cellular animalcules without tissues and organs. They have one or more nuclei. Protozoa exist either singly or in colonies. Almost about 50,000 species are known till date.

Anton Van Leeuwenhoek was the first to observe protozoa (Vorticella convellaria) under a microscope. He called them animalcules. Gold fuss coined the term Protozoa which in Greek means first animals (Proto= first; zoans=animals). Hyman and other zoologists preferred to call them as acellular animals.

The body of protozoans is unicellular. They are generally referred to as acellular rather than unicellular as the so called single cell performs all the life activities. Though it is structurally equivalent to a single cell of the metazoan body, it is functionally equivalent to the whole metazoan animals.

General Characters of Phylum Protozoa

- Protozoan animals exhibit protoplasmic grade of organization. There is division of labor among various organelles of the cell.
- These are solitary (*Euglena*), or colonial (*Proteospongia*)
- They may be free living (*Amoeba*) or symbiotic (Parasitic, mutualistic or commensalistic)
- Body symmetry is symmetrical (Actinopodeans) or radial (sessile forms) or bilateral (*Giardia*) or absent (*Amoeba*)
- Locomotion is brought about by pseudopodia or flagella or cilia or myonemes.

- Nutrition is holozoic or holophytic or osmotrophic (Saprophytic or parasitic). Digestion is intracellular. Some forms like Euglena are mixotrophic (perform more than one type of nutrition)
- Exchange of respiratory gases takes place by diffusion through the general body surface. Respiration is anaerobic in some parasitic forms.
- Excretion occurs by diffusion across general body surface or by contractile vacuoles. Contractile vacuoles serve mainly for Osmoregulation and are common in freshwater forms.
- Asexual reproduction takes place by binary fission or multiple fission or plasmotomy or budding.
- Sexual reproduction takes place by syngamy or conjugation
- Many forms undergo encyctment to tide over unfavorable conditions
- Somotoplasm and germplasm are not differentiated. Hence they are immortal (exempt from natural death).

Classification of Phylum Protozoa

Phylum Protozoa is a large and varied group. This phylum has a number of problems in its classification. As per one of the classification given out by Hyman, Hickman and Storer, this phylum is divided into two subphyla on the basis of organs of locomotion. These two subphyla are further divided into 5 classes.

Most accepted classification of protozoa is given by BM Honigberg and others based on the scheme given by the committee on Taxonomy and Taxonomic problems of the society of Protozoologists divides this phyla into 4 subphyla.

SUBPHYLUM I: SARCOMASTIGOPHORA (Gr. Sarcodes=fleshy; mastix=whip; phoros=bearing)

The locomotion in this subphylum is brought about by flagella or pseudopodia or both. Other important feature of this subphylum is the presence of monomorphic nuclei. This subphylum is further divided in to 3 super classes:

Superclass 1: Mastigophora (Gr. Mastix=whip; phoros=bearing)

The body of the animals belonging to this super class is covered by pellicle. The locomotory organelles are flagella. In this super class the asexual reproduction occurs by longitudinal binary fission. This super class includes 2 classes:

Class 1: Phytomastigophora (Gr. Phyton=plant; Mastix=whip; phoros=bearing)

They have chromatophores with chlorophyll. The nutrition in these organisms is mainly holophytic which takes place by phototrophy. These are free living organisms. The reserve food in these organisms is starch or paramylon. These organisms may have 1 or 2 flagella.

Ex: *Euglena, Ceratium, Noctiluca*

Class 2: Zoomastigophora (Gr. Zoon=animal; Mastix=whip; phoros=bearing)

These organisms do not have chlorophyll bearing chromatophores. These are mostly parasitic. The nutrition in these organisms is holozoic or saprozoic. The reserved food is glycogen. They may have one to many flagella.

Ex: *Leishmania, Trypanosoma, Trichomonas, Trichonympha*

Superclass 2: Opalinata

The organisms belonging to this super class live as commensals or parasites in the gut of anurans. Their body is covered by oblique rows of cilia-like flagella. These organisms may have 2 or many nuclei also the nuclei are monomorphic. They undergo asexual reproduction by binary fission or by syngamy. Sexual reproduction takes place by anisogamy.

Ex: *Opalina, Zelleriella*

Superclass 3: Sarcodina (Gr. Sarcode=fleshy)

The locomotion in the organism belonging to this superclass is brought about by pseudopodia. Their body is amoeboid without definite pellicle. The nutrition is holozoic or saprozoic. This super class is further divided into 3 classes:

Class 1: Rhizopodea (Gr. Zoon=animal; Mastix=whip; phoros=bearing)
The pseudopodia of the animals in this class are in the form of lobopodia, filopodia or reticulopodia without axial filaments. This class includes amoebas, foraminiferans and mycetozoans. These animals are mostly free living and a few are also parasitic. In amoebas, the body is naked; in foraminiferans the body is covered by porous calcareous shell.
Ex: *Amoeba, Entamoeba, Elphidium*
Class 2: Piroplasmea
The animals belonging to this class are parasitic. Locomotory structures are absent in this class. Spores are also absent. These are the small parasites in the red blood cells of vertebrates.
Ex: *Babesia*

Class 3: Actinopodea (Gr. Actis=ray; podos=foot)
The pseudopodia of the animals belonging to this class are in the form of axopodia with axial filaments, radiating from the spherical body. These are planktonic. This class includes Heliozoans, Radiolarians and acanthareans. Radiolarians and acanthareans are marine forms whereas heliozoans are both marine and fresh water forms. Skeletons of radiolarians have siliceous shells. The shells of dead radiolarians accumulate on the ocean floor to form radiolarian ooze.
Ex: *Collozoum, Actinophrys, Acanthometra*

SUBPHYLUM II: SPOROZOA (Gr. Actis=ray; podos=foot)
The animals belonging to this subphylum are exclusively endoparasites. Special locomotory organelles are absent in these animals. Sometimes pseudopodia are present which are useful only for ingestion of food. Sporozoites are merozoites bear anterior apical complex that helps penetrate host cells. This subphylum includes 3 classes:
Class 1: Telosporea

The Sporozoites are long in these animals. Reproduction is both asexual and sexual. They are blood and gut parasites of vertebrates. Sexual reproduction is by isogamy or anisogamy.

Ex: *Monocyctis, Eimera, Plasmodium*

Class 2: Toxoplasmea

In this class reproduction is only asexual type which takes place by internal budding where two daughter cells are produced within the mother cell and the mother cell is finally destroyed in the process of reproduction. Spores are absent.

Ex: *Toxoplasma*

Class 3: Haplosporea

The spores in this class are amoeboid. Also reproduction is only asexual type taking place through multiple fissions.

Ex: *Haplosporidium, Ichthyosporidium*

SUBPHYLUM III: CNIDOSPORA (Gr. Knide=nettle; spora=seed)

The animals belonging to this subphylum are parasitic. Special kind of locomotory organelles are absent in these animals. Spores are present with one or more polar filaments. Polar filaments are special and unique features of these animals. When these spores infect a host, the polar filament is discharged and it gets attached to the host tissue. This subphylum includes 2 classes:

Class 1: Myxosporidea

The spores of the animals of this class are large and develop from several nuclei. These are generally extracellular parasites. The spores of this class have two polar filaments and have two to three valves

Ex: *Myxobolus*

Class 2: Microsporidea

The spores of the animals of this class are small and are developed from only one nucleus. These spores have single valve. These are generally intracellular parasites. Many of the animals of this class have a single polar filament.

Ex: *Nosema bombycis*

SUBPHYLUM IV: CILIOPHORA (La. Cilium=eye lid with lashes; phoros=bearing)

Ciliophorans are complex of all the protozoans. The locomotory organelle of all the animals of this subphylum is cilia. These cilia also help in feeding at some stage of the life cycle of the animals. The nuclei of these organisms are dimorphic. Macronucleus is vegetative and polyploid. Micronucleus is reproductive and diploid. Asexual reproduction takes place by binary fission. Sexual reproduction takes place by conjugation. Only one class is included in this subphylum:

Class 1: Ciliatea

The locomotory organelles of these animals are numerous hair-like cilia. One or more contractile vacuoles are present in these forms. The nucleus is dimorphic including both macro nucleus and micronucleus.

Ex: *Paramoecium, Vorticella, Balatidium*

PARAMECIUM

Paramecium or **Paramoecium** is a genus of unicellular ciliated protozoa. They are characterised by the presence of thousands of cilia covering their body. They are found in freshwater, marine and brackish water. They are also found attached to the surface. Reproduction is primarily through asexual means (binary fission). They are slipper-shaped and also exhibit conjugation. They are easy to cultivate and widely used to study biological processes.

Paramecium Classification

Paramecium is unicellular and eukaryotic, so they are kept in the kingdom Protista. They are ciliated protozoan and come under phylum Ciliophora.

The common species of *Paramecium* include:

Paramecium aurelia

Paramecium caudatum

Paramecium woodruffi

Paramecium trichium

Domain	Eukaryota
Kingdom	Protista
Phylum	Ciliophora
Class	Oligohymenophorea
Order	Peniculida
Family	Parameciidae
Genus	*Paramecium*

- The cell size varies from 50 μ to 300 μ. The cell is ovoid, slipper or cigar-shaped
- The cellular cytoplasm is enclosed in a pellicle. Pellicle consists of an outer plasma membrane, inner epiplasm and a layer of alveoli, present in between both the layers. The pellicle is elastic and gives the cell its definite but changeable shape
- Cilia project from the depressions in the pellicle and cover the entire body surface. They are used for locomotion and taking nutrient-rich water inside the gullet

- Protoplasm is divided into outer ectoplasm and inner endoplasm, which is granular
- Trichocysts are present and embedded in the ectoplasm. They are a defensive organ
- The endoplasmic granules reserve food. Some of the granules are secretory or excretory
- Paramecia contain at least **two nuclei**, micronuclei (one or more) and one macronucleus. Micronuclei have diploid chromosomes and take part in the reproduction. Macronuclei regulate all vital metabolic activities and growth. The macronucleus has multiple copies of the genome, i.e. polyploid
- Contractile vacuoles are present and their number varies from species to species. They are required for osmoregulation and expel the additional absorbed water
- At the midpoint, there is an oral groove on the ventral side known as the vestibule. Food is drawn inside the cell due to coordinated movement of cilia
- The oral groove opens in the mouth known as **cytostome** and to the gullet or pharynx
- There are numerous food vacuoles present for digesting food
- There is an anal pore present on the ventral surface in the posterior half of the cell known as **cytoproct or cytopyge**, which helps in egesting undigested food

Paramecium Locomotion

The coordinated movement of thousands of cilia propels paramecium. Paramecium can rotate around its axis and move in the reverse direction on encountering an obstacle.

Paramecium Nutrition

They are mostly heterotrophic. They feed on bacteria, algae, yeast and other microorganisms. They are holozoic. The food-laden water is drawn inside by the movement of cilia and it goes to the cytostome and to the gullet (cytopharynx).

The food gets loaded at the posterior end of cytopharynx. It gets surrounded by vacuoles, pinches off and circulates in the endoplasm. The food is acted upon by digestive enzymes present in the food vacuoles.

The undigested residue is egested through the temporary anal pore (cytopyge).

Some of the *Paramecium* species, e.g. *Paramecium bursaria, etc.* form a symbiotic relationship with green algae. Algae are present as an endosymbiont and provide food to paramecium by photosynthesis, in turn, the algae get a safe and protective habitat.

Paramecium may have intracellular bacteria known as **kappa particles.** Paramecium with kappa particles has the ability to kill other strains of paramecium.

Paramecium Reproduction

Asexual Reproduction in paramecium is by binary fission. The mature cell divides into two cells and each grows rapidly and develops into a new organism. Under favourable conditions, Paramecium multiplies rapidly up to three times a day. Binary fission divides a cell transversely and followed by mitotic division in the micronucleus. Macronucleus divides amitotically. The gullet also divides into two halves.

Although the favoured mode of reproduction in Paramecium is mostly asexual, they reproduce sexually too, when there is a scarcity of food.

Sexual reproduction in Paramecium is by various methods.

In **conjugation,** two complementary paramecia (syngen) come together and there is a transfer of genetic material. An individual has to multiply asexually 50 times before reproducing by conjugation.

In the process of conjugation, the conjugation bridge is formed and united paramecia are known as conjugants. Macronuclei of both the cells disappear. The micronucleus of each conjugant forms 4 haploid nuclei by meiosis. Three of the nuclei degenerate. The haploid nuclei of each conjugant then fuse together to form diploid micronuclei and cross-fertilization takes place. The conjugants separate to form exconjugants. They are identical, but different from the earlier cells. Each exconjugate undergoes further division and forms 4 daughter Paramecia. Micronuclei form a new macronucleus.

Paramecium also shows **autogamy** i.e. self-fertilization. A new macronucleus is produced, which increases their vitality and rejuvenates them.

Cytogamy is less frequent. In cytogamy, two paramecia come in contact but there is no nuclear exchange. Paramecium rejuvenates and a new macronucleus is formed.

A Paramecia undergoes ageing and dies after 100-200 cycles of fission if they do not undergo conjugation. The macronucleus is responsible for clonal ageing. It is due to the DNA damage.

Protozoan Parasites

The name 'proto-zoa' literally means 'first animals' and early classification systems grouped the protozoa as basal members of the animal kingdom. However, they were recognized as a discrete assemblage on the basis of their unicellularlity and were assigned to the taxon Protozoa (but still invariably figured as the trunk of the animal tree of life). Members of the subkingdom Protozoa are quite disparate; indeed the taxon has never been considered a natural assemblage of organisms but rather one of convenience. More recently, the protozoa have been classified together with several algal and fungal groups in the kingdom Protista (protozoa representing the motile protists). Irrespective of contemporary classification systems, most parasitological texts continue to use the name protozoa for historical reasons.

Protozoa are eukaryotic organisms (with a membrane-bound nucleus) which exist as structurally and functionally independent individual cells (including those species which are gregarious or form colonies). None have adopted multicellular somatic organisation characteristic of metazoan organisms. Instead, protozoa have developed relatively complex subcellular features (membranes & organelles) which enable them to survive the rigours of their environments. Most protozoa are microscopic organisms, only a few grow to a size large enough to be visible to the naked eye. As unicellular eukaryotes, protozoa display all the same essential life activities as higher metazoan eukaryotes: they move about to survive, feed and breed.

Plasmodium Life cycle

Plasmodium species that infect humans

Until recently, there were four *plasmodium* species that were considered responsible for malaria disease in humans: ***P. vivax***, ***P. falciparum***, ***P. ovale*** and ***P. malariae***. In 2008, ***P. knowlesi***, a species that used to infect exclusively apes of the genous *Macaque*, was recognised by WHO as the fifth *plasmodium* species that infect humans.

Transmission routes

The main mode of transmission of the disease is by bites from infected Anopheles mosquitoes that have previously had a blood meal from an individual with parasitemia. Less common routes of transmission are via infected blood transfusion, transplantation, infected needles, and from a mother to her fetus during pregnancy.

Plasmodium life cycle

The life cycle (Figure 1) is almost the same for all the five species that infect humans and follows three stages:

(I) ifection of a human with sporozoites
(II) asexual reproduction
(III) sexual reproduction

The two first stages take place exclusively into the human body, while the third one starts in the human body and is completed into the mosquito organism.

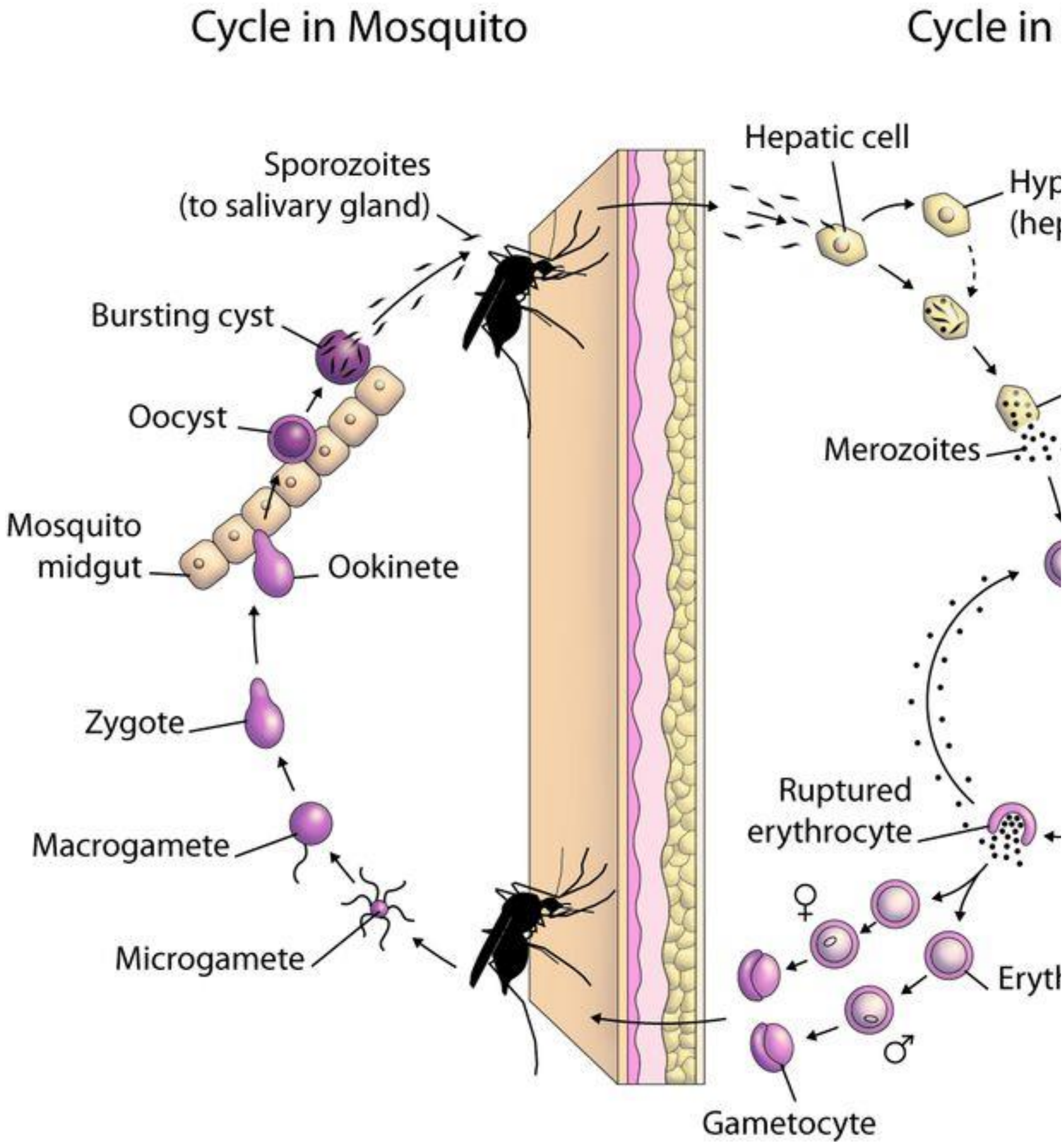

Figure 1. *Plasmodium* life cycle
(Source: Open Course Ware)

The human infection begins when an infected female anopheles mosquito bites a person and injects infected with **sporozoites** saliva into the **blood circulation**. That is the first life stage of plasmodium (**stage of infection**).

The next stage in malaria life cycle is the one of asexual reproduction that is divided into different phases: the pre- erythrocytic (or better, **exoerythrocytic**) and the erythrocytic **phase**. Within only 30- 60 minutes after the parasites inoculation, sporozoites find their way through blood circulation to their first target, the **liver**. The sporozoites enter the liver cells and start dividing leading to **schizonts** creation in 6-7 days. Each schizont gives birth to thousands of **merozoites** (**exoerythrocytic schizogony**) that are then released into the blood stream marking the end of the exoerythrocytic phase of the asexual reproductive stage.

It is worth mentioning that, concerning ***P. vivax*** and ***P. ovale,*** sporozoites may not follow the reproduction step and stay dormant (**hypnozoites**) in the liver; they may be activated after a long time leading to relapses entering the blood stream (as merozoites) after weeks, months or even years. The exoerythrocytic phase is not pathogenic and does not produce symptoms or signs of the disease. Its duration is not the same for all parasite species.

Merozoites released into the blood stream, are directed towards their second target, the **red blood cells** (RBCs). As they invade into the cells, they mark the beginning of the erythrocytic phase. The first stage after invasion is a ring stage that evolves into a **trophozoite**. The trophozoites are not able to digest the haem so they convert it in haemozoine and digest the globin that is used as a source of aminoacids for their reproduction. The next cellular stage is the **erythrocytic schizont** (initially immature and then mature schizont). Each mature schizont gives birth to **new generation merozoites** (**erythrocytic schizogony**) that, after RBCs rupture, are released in the blood stream in order to invade other RBCs. This is when parasitaemia occurs and cinical manifestations appear. The liver phase occurs only once while the erythrocytic phase undergoes multiple cycles; the merozoites release after each cycle creates the febrile waves.

A second scenario into the RBCs is the parasite differentiation into male and female **gametocytes** that is a non pathogenic form of parasite. When a female anopheles mosquito bites an infected person, it takes up these gametocytes with the blood meal (mosquitoes can be infected only if they have a meal during the period that gametocytes circulate in the human's blood). The gametocytes, then, mature and become **microgametes** (male) and **macrogametes** (female) during a process known as gametogenesis. The time needed for the gametocytes to mature differs for each plasmodium species: 3- 4 days for *P. vivax* and *P. ovale*, 6- 8 days for *P. malariae* and 8- 10 days for *P. falciparum*.

In the mosquito gut, the microgamete nucleus divides three times producing eight nuclei; each nucleus fertilizes a macrogamete forming a **zygote**. The zygote, after the fusion of nuclei and the fertilization, becomes the so- called **ookinete**. The ookinete, then, penetrates the midgut wall of the mosquito, where it encysts into a formation called oocyst. Inside the oocyst, the ookinete nucleus divides to produce thousands of **sporozoites** (**sporogony**). That is the end of the third stage (stage of sexual reproduction/ sporogony). Sporogony lasts 8- 15 days**.**

The oocyst ruptures and the sporozoites are released inside the mosquito cavity and find their way to its salivary glands but only few hundreds of sporozoites manage to enter. Thus, when the above mentioned infected mosquito takes a blood meal, it injects its infected saliva into the next victim marking the beginning of a new cycle.

TREATMENT

Treatment varies greatly depending on the species, drug resistance and severity of the infection.
Drugs include: Chloroquine, Sulfadoxine-pyrimethamine, Quinine, Primaquine

Treatment should be continued until the elimination of the parasites is confirmed.

PREVENTION and CONTROL

Because infection is transmitted by *Anopheles* mosquitoes, prevention includes reducing the possibility of being bitten. This may be accomplished by the use of topical repellents, protective clothing and bed netting while sleeping. When traveling in endemic areas, prophylactic use of the appropriate drugs (e.g. Chloroquine) can prevent infection.

Long-term control involves reducing the mosquito populations by eliminating standing water breeding habitats, treating breeding areas with insecticides and spraying sleeping quarters with insecticides.

Anopheles mosquitoes are able to evolve resistance to insecticides rapidly and the parasites can attain resistance to drugs, making the control of malaria difficult.