Introduction to Protozoa:

Protozoa are mostly single-celled, animal-like organisms. Although some are colonial or form loose aggregations, most live and function as separate cellular individuals. Most protozoa are chemoheterotrophs, i.e., organisms using preformed organic compounds for both energy and carbon. Most do not contain green pigments and are not capable of using light as an energy source (however there are exceptions). They live in a variety of habitats including fresh and salt water and inside multicellular organisms including humans and other animals. Most protozoa are free-living organisms that obtain nutrients from decaying organic materials or feed on bacteria and smaller eukaryotic cells. Some are parasites and some are pathogens capable of causing disease in humans and other animals.

Since protozoa live in diverse habitats and function as individuals subject to a multitude of environmental challenges, it is not surprising that they (as a group) have evolved a variety of specializations. Some of these are described below.

1. **Locomotor structures –** Locomotor structures are specializations allowing cells to travel through their environment as a means of dispersing, locating food sources or escaping potential predators.

    a) **Cilia** – cilia (singular = cilium) are short, hair-like structures found on the surfaces of protozoa called **ciliates** (phylum Ciliophora). As described earlier (Eukaryotic cell structure and function), each cilium is surrounded by the cell membrane and is supported by a cytoskeleton of microtubules arranged in a characteristic 9 plus 2 pattern. They are capable of whip-like motion involving MAPs such as dynein and are coordinated by microtubules arranged just inside the cell membrane. Cilia can be distributed more or less uniformly all over the cell surface, can occur in rows or patches, or be grouped together in tufts. Most cilia are used for swimming and allow ciliates to move smoothly through their watery habitats, some provide a jumping motility, and cilia arranged in tufts called **cirri** allow cells to walk or jump along solid surfaces.

    b) **Flagella** – flagella (singular = flagellum) are long, whip-like structures found on the surfaces of many eukaryotic cells. Like cilia they are surrounded by the cell membrane and contain microtubules arranged in a characteristic 9 plus 2 pattern. Some cells such as those in the genus *Trypanosoma* have a single flagellum enclosed in a double layer of membrane running the length of the organism in a fin-like manner. Flagella are usually ness numerous than cilia and are often used to pull cells through their environments.

    c) **Pseudopodia** – Pseudopodia or false feet (singular = pseudopodium) are extensions of the protoplasm associated with amoeba-like organisms. These vary considerably in size and shape but are like cilia and flagella are surrounded by the cell membrane and often supported by microtubules (not arranged in a 9 plus 2 pattern). Pseudopodia typically form in a flowing fashion, and allow amoeba-like organisms

to creep slowly along solid surfaces. Some extend through holes in glass skeletons like a multitude of spokes radiating from spherical wheels.

2. **Food gathering structures –** Structures involved in food gathering activities are often those used for locomotion, including cilia, flagella and pseudopodia. In addition to these are structures involved in ingestion and digestion of foods. Many protozoa are **holozoic**, i.e., organisms that take in whole organisms for food. They have no means of biting off small portions of their prey as would large, multicellular animals.

    a) **Cytostome** – The cytostome or cell mouth (cyto = cell, stoma = mouth) is a region on the surface of a cell where endocytosis can occur. Ciliated protozoa such as *Paramecium* can take in food only through their cytostome because the rest of the cell is covered by a tough pellicle.
    b) **Lysosomes** – Lysosomes contain the digestive enzymes needed to break down food materials taken in through endocytosis.
    c) **Cilia** and **flagella** can sweep food along the cell surface toward the cytostome and sometimes line a region of the cell called an oral funnel or oral groove.
    d) **Pseudopodia** – Amoeba-like protozoa use their pseudopodia to capture food by extending them out and around the food and fusing them to form food vacuoles.

3. **Osmoregulatory structures –** The contractile vacuoles present in many types of freshwater protozoa are used primarily to pump excess water out of cells. They are connected to the endoplasmic reticulum, so also have circulatory function and may be used to eliminate liquid wastes, i.e., also have excretory function.

4. **Protective Structures –** Protozoa live in potentially dangerous environments, and have evolved a variety of protective structures that help them survive.

    a) **Pellicle** – The pellicle is a tough, flexible layer found outside the cell membrane on all types of ciliated protozoa. It helps provide the cell with a characteristic shape and protects it against physical damage.
    b) **Skeletons** – The skeletons of protozoa are usually made of glass (silica dioxide) or calcium carbonate. Radiolaria have glass skeletons perforated with numerous holes allowing pseudopodia to extend out through them. Foraminifera have skeletons of calcium carbonate that resemble the shells of mollusks (e.g., the chambered nautilus). Skeletons provide protection against predation and also support the protoplasm.
    c) **Trichocysts** – Trichocysts are dart-like structures that can be shot out from certain cells. They are made of protein, are often barbed and attached to the cell surface by microscopic threads. Trichocysts are released in response to chemical and/or physical stimuli and may be used for defense or attachment.

5. **Life cycle stages –** Protozoa often live in habitats subject to change due to climate and seasons, while some live parts of their lives inside different types of hosts. To survive variations in living conditions, protozoa can switch between two different stages.

a) **Trophozoites –** Trophozoites (troph = activity), are active protozoa sometimes called vegetative cells. While in their trophozoite form, protozoa are engaged in feeding, reproducing and moving about actively. Vernal pools, i.e., those filled with water during the spring, contain many trophozoites. As the weather warms up and pools dry out in the summer sun, the protozoa go into a resting state.

b) **Cysts** – Cysts are dormant structures produced by many types of protozoa under certain circumstances. They are metabolically inactive and much more resistant to heat, drying, radiation and chemicals than are **trophozoites** (active vegetative cells). Cysts allow protozoa to survive when their watery habitats dry out during summer months or freeze solid during the winter. They also allow gastrointestinal parasites to survive passage through the stomach without being damaged by stomach acids.

Protozoan Reproduction – Protozoa like fungi and algae can reproduce themselves both asexually and sexually. There are many variations on these basic themes, but some of the most commonly encountered forms of reproduction are introduced below.

1. **Asexual reproduction –** Asexual reproductive processes allow individuals to reproduce without interacting with other cells. In eukaryotic organisms, asexual reproduction requires **mitosis** (the separation of the chromosome) and **cytokinesis** (the separation of the cytoplasm forming new daughter cells). Some specific examples include:

   a) **Binary fission** – Binary fission is a process involving the separation of the cytoplasm across the long axis of the cell. Most protozoa reproduce by binary fission.
   b) **Schizogony** – Schizogony or multiple-fission is a process involving the splitting of one cell into many daughter cells. Sporozoans in the genus *Plasmodium* reproduce by means of schizogony while inside human RBCs.
   c) **Budding** – Budding involves an unequal division of the protoplasm and results in the formation of a bud at the margin of a cell. If conditions are favorable, the bud will grow and eventually separate from the cell, but if conditions are poor, the bud may die with little consequence to the cell.

2. **Sexual reproduction** – In order to reproduce sexually, protozoa must interact with other, genetically different cells. Sometimes this involves plasmogamy, karyogamy and meiosis, but not always, and these terms are rarely used in zoology texts. Two examples of sexual reproduction include:

   a) **Syngamy** – Syngamy involves the fusion of two haploid cells to form a diploid zygote. Protozoa in the genus *Plasmodium* undergo syngamy while inside mosquitoes.
   b) **Conjugation** – Conjugation requires that two cells with different genetic content meet and position themselves side-by-side. Portions of the cell membranes fuse allowing the formation of a cytoplasmic bridge, and then segments of genetic material (DNA) are exchanged between the two cells. Following conjugation, the cells separate again, but each one is now carrying a new combination of genetic material.

Medical Protozoology – Introducing Some Medically Significant Protozoa

Though most protozoa are free-living organisms, feeding on decaying organic materials, bacteria and other cells, some protozoa are parasites and some are pathogens. Protozoa of medical significance are responsible for killing millions of humans, and remain a threat to human health throughout the world. Some examples of medically significant protozoa include:

1. ***Giardia lamblia*** – Protozoa identified as *Giardia lamblia* or *G. intestinalis* are flagellated organisms that infect the small intestine and occasionally the bile ducts of humans and other animals causing giardiasis. They enter the body in cyst form along with contaminated food or water, and each cyst contains two trophozoites. The trophozoites released within the intestine attach to the mucosa where they apparently feed on mucous and other epithelial secretions. If they are numerous (covering much of the intestinal mucosa) they interfere with digestion and absorption of nutrients, especially fats resulting in diarrhea often accompanied by large amounts of yellowish fatty mucous. *Giardia* are more commonly pathogenic in children than in adults.

2. ***Trichomonas vaginalis*** – Various species of *Trichomonas* may live within the human host, but only *T. vaginalis* is pathogenic and causes trichomoniasis. These organisms live primarily in the vagina, but may travel to the cervix or vulva, and can infect the urethra and prostate in males. Infection causes inflammation accompanied by a creamy-white discharge, with severe itching and chaffing. Transmission may be direct via sexual intercourse or may involve transfer from mother to infant during childbirth. Some infections have been acquired in poorly maintained pools and hot tubs. According to some texts, around 20-40% of women in some areas have *Trichomonas* infections.

3. ***Entamoeba histolytica*** - Although most amoeba-like protozoa are free-living in fresh and salt water, many are parasites, and some are pathogenic. *Entamoeba histolytica* are amoebae that live in the large intestines of humans and other animals. They enter their human hosts in the **cyst** form usually with water or contaminated vegetable material. The cyst walls are digested away in the stomach and duodenum, allowing the **trophozoites** (four per cyst) to be released. The trophozoites live in the caecum and reproduce by binary fission. In most cases they cause no or little damage, living on food material that is passing through the host. However, the name histolytica (which means tissue lysing or splitting) indicates that these organisms can invade the tissues causing damage.

   In about 10% of infections, the amoebae invade the intestinal mucosa causing tissue lysis and ulceration resulting in dysentery. In severe infections they may penetrate the submucosa, muscularis and serous membrane to enter the peritoneal cavity (often resulting in secondary bacterial infection). Symptoms vary depending upon severity and location of the infection, but typically include nausea, cramps and diarrhea. More severe infections result in abdominal tenderness, dysentery, dehydration and general incapacitation. Symptoms may develop within days of exposure or as much as a year later depending on host condition. Rarely amoebae travel via the portal system to the liver causing amoebic hepatitis.

4. ***Acanthamoeba*** and other genera – There are a number of other intestinal amoebae, and there are also cases of amoebae infecting other areas of the body. Amoebae in the genera *Acanthamoeba*, *Naegleria* and *Balamuthia* are normally free-living in soil and water but have been found to cause eye infections (keratitis), skin infections and primary amoebic meningoencephalitis, a rapidly fatal infection of the brain and meninges. These amoebae can enter the nasal cavities and travel via the ethmoid bone into the brain where they cause extensive hemorrhage and tissue damage. Death can occur in less than one week. Contaminated outdoor pools and hot baths have been found to harbor amoebae.

5. ***Plasmodium vivax*, *malariae*, *ovale*,** and ***falciparum* –** Protozoa in genus *Plasmodium* are sporozoans recognized as the causative agents of **malaria**. The vector involved in the transmission of malaria is a mosquito in the genus ***Anopheles*.** The parasites enter their mammalian host along with saliva from the mosquito bite, and in this stage are called **sporozoites**. These enter tissue cells (exo-erythrocytic stage) such as the liver, where they may persist indefinitely. Eventually some parasites enter the bloodstream (erythrocytic stage) where they infest RBCs and reproduce asexually via **schizogony**. The red blood cells lyse, and the parasites, now called **merozoites**, are released in great numbers. The maturation of parasites within the RBCs occurs at intervals of 48 hours in *Plasmodium vivax*, *ovale*, and *falciparum*, and of 72 hours in *malariae*, which accounts for the cyclic symptoms of the disease. The rupture of infected RBCs releases hemoglobin and causes fever. Symptoms begin with severe chills (shivering, teeth chattering, etc.) followed by fever, headache and nausea - fever may reach as high as 106 degrees F. Fever is followed by sweating and a drop in temperature (sometimes to below normal) that lasts until the next cycle begins. Within the bloodstream, some of the merozoites undergo meiosis to form **gametocytes**. A mosquito can pick up gametocytes from the blood of a mammalian host, and the parasites undergo sexual reproduction (**syngamy**) within the mosquito gut. The name Malaria means "bad air", and was applied due to the association of disease with the air of swamps (before the mosquito connection was recognized). It was also called black water fever - due to the production of dark urine from hemoglobin breakdown.

6. ***Toxoplasma gondii*** – Sporozoans identified as *Toxoplasma gondii* are recognized as the causative agents of **toxoplasmosis**, and are known to infect all kinds of mammals and birds. In the 1970's it was estimated that between 17 and 35% of all Americans carred the organisms. Dogs and cats also serve as reservoirs and recent findings suggest the protozoa change the behavior of rodents to increase their susceptibility to predation by cats. Transmission can occur from contact with feces or flesh of infected animals (eating raw meat) and can also occur prenatally. Symptoms in adults include fever, rash, enlarged lymph nodes and eye disturbances. The parasites may be found free in the blood or within various tissue cells. In fetuses or infants born infected, nervous system damage is common, and fatal encephalitis is not unusual. Pregnant women and infants can be tested for the presence of antibodies against *Toxoplasma*.

7. ***Cryptosporidium parvum*** – Protozoa identified as *Cryptosporidium parvum* are the most common cause of **cryptosporidiosis** in immunocompromised patients. The organisms develop within the microvilli of the intestinal mucosa and can be transmitted through animal feces and contaminated water. Some noscomial (hospital acquired) cases have

also occurred. These parasites can also infect the lungs and the gal bladder causing potentially deadly disease symptoms.

8. ***Balantidium coli*** – Although most ciliated protozoa are free-living, some are parasites, and some are pathogens. *Balantidium coli* are ciliates that are parasitic in the gut of man and other animals. They enter their human hosts in cyst form, along with contaminated food. Digestive enzymes in the gut dissolve the cyst walls, releasing the trophozoites within the colon. In most cases, these feed on bacteria and fecal debris without causing disease symptoms, but rarely they invade the mucosa and submucosa of the gut causing abscesses and ulcerations. Symptoms typically include chronic diarrhea alternating with constipation, but can result in severe dysentery. Fatal cases occur occasionally.

9. **Hemflagellates** – Flagellated protozoa inhabiting the bloodstream are known as hemoflagellates and include organisms in two genera, *Trypanosoma* and *Leishmania*. Species of *Trypanosoma* cause African sleeping sickness and Chaga's disease, while *Leishmania* organisms cause a variety of diseases including Kala-azar and oriental sore. All hemoflagellates live within the circulatory system of their host and are transmitted by insect vectors.

    a) ***Trypanosoma gambiense*** and ***T. rhodesiensis*** – Hemoflagellates identified as ***Trypanosoma gambiense*** and ***T. rhodesiensis*** are recognized as the causative agents of **African sleeping sickness** and are transmitted by insect vectors (Tse tse flies) in the genus **Glossina**. These trypanosomes may be passed to many alternate hosts (antelope, pigs, monkeys, dogs, etc.) that form a huge reservoir in addition to humans. The Trypanosomes live in the salivary glands of the flies and so enter their host when the flies bite to feed. The bites itch, and form lesions, and the following infection is accompanied by fever and headache at irregular intervals. In this form, it may last for weeks or months making the victim susceptible to other diseases. Eventually (if not treated) the disease-causing organisms may enter the cerebrospinal fluid (CSF) and the symptoms of true "sleeping sickness" occur. During this stage, the victim "sleeps" a lot and is unable to engage in physical activity. Muscular convulsions and trembling are common. If not treated, coma and death are inevitable. If the disease-causing organisms do not enter the CSF, the symptoms disappear spontaneously and the hosts recover. Prevention requires control of the Tse-tse flies or elimination of the alternate hosts (which is highly unlikely).

    b) ***Trypanosoma cruzi*** – Hemoflagellates identified as ***Trypanosoma cruzi*** are recognized as the causative agents of **Chaga's disease**, and are transmitted by insect **vectors** ("true bugs" in the genus *Triatoma*) called "kissing bugs" that feed on human blood. The trypanosomes enter the body when infected excrement, deposited by feeding bugs, is scratched into the wound. They then enter the bloodstream or lymphatic system, and migrate to tissue cells. The trypanosomes reproduce within the tissue cells and are later released when the cells lyse. Symptoms include edema at the site of infection (at bite or conjunctiva) with later development of acute headache, fever and sometimes severe skin lesions (especially in children). Chronic infections may involve enlargement of the liver, spleen, and lymph glands, with anemia and nervous system disorders. Treatment is not overly successful, and

chronic infections may result in death. Prevention involves elimination of the vectors.

Currently, the protozoa responsible for causing malaria, sleeping sickness, Chaga's disease, dysentery and other diseases are more commonly encountered in tropical regions than here in the United States. Microbiologists are concerned that changes in world climate will result in more widespread disease as vectors expand their ranges in response to global warming.