NEMATODES

Definition: "Nematodes are triploblastic (body is composed of three layers-cuticle, hypodermis and muscle layer), bilaterally symmetrical, unsegmented, pseudocoelomate and vermiform animals".

Nematodes are thread-like roundworm that lives in wide range of environments including soil and fresh and salt water. There are species of nematodes that feed on fungi, bacteria, protozoans, other nematodes and plants. They can also parasitize insects, animals and humans. Nematodes that feed on plant parts are called plant parasitic nematodes (PPN) and are ubiquitous in agricultural soils. The life cycle of nematode includes egg, larva (juveniles) and adults, and they can overwinter at any of these stages.

General Characteristies: Generally nematodes are popularly known as eelworm, nemas, threadworms etc.

1. They are fusiform, cylindrical and essentially tapering towards both ends.
2. In transverse section, they are vermiform and circular.
3. They are bilaterally symmetrical animals.
4. The nematode body is more or less transparent.
5. They lack the circulatory and respiratory systems.
6. They have four larval stages between the eggs and the adults.
7. These are dioecious (unisexual) animals, but some are monoecious (bisexual).
8. They are aquatic, terrestrial and parasites in their habitat.
9. Cilia are absent.
10. Plant parasitic nematodes have no appendages.
11. The mouth of nematodes is at the anterior end and the terminus is at the posterior end. The excretory pore, vulva and anus are on the ventral side and the opposite side is called the dorsal side. The right and left sides are called lateral sides.
12. Most of the nematodes are oviparous.
13. Reproductive organs: females have one or two ovaries and the female sexual opening is known as vulva. Males have one or two testes. Their sexual opening is called **cloaca** and copulatory organs are known as **spicules**.

Size: Plant parasitic nematodes are small, about 300-1000 microns (0.3-1.0 mm) with some up to 4.0 mm long by 15-35 microns wide.

Shape: The typical nematode shape is a long and slender worm like animal.

Males: Cylindrical, filiform and tapering at both ends.

Females: Most of the females are cylindrical or filliform but in some species females are pear/lemon shaped (pyriform), oval, round and elliptical. The examples of such nematodes are Meloidogyne and Heterodera spp.

Anatomy: The whole nematode body is covered with an elastic, colourless layer known as cuticle. Cuticle is supported by the next layer called hypodermis. The body cavity contains a fluid through which circulation and respiration taks place. The digestive system is hollow tube extending from the mouth to oesophagus, intestine, rectum and anus. All plant parasitic nematodes have a hollow stylet or spear which is used to puncture plant cells. The reproductive system is well developed. Females have one or two ovaries followed by an oviduct and uterus terminating in vulva The male reproductive structure is similar to the female but there is a testes replacing ovaries and the genito- anal opening is common called

cloaca. Reproduction in nematodes is through eggs and may be sexual, hermaphroditic or parthenogenetic. Many species lack males.

Egg (I^{st} stage larva)
I----------Hatching
II^{nd} stage larva
I----------First moulting
III^{rd} stage larva
I----------Second moulting
IV^{th} stage larva (Adult)

LIFE CYCLE OF A TYPICAL NEMATODE

Classification of Plant Parasitic Nematodes: Plant parasitic nematodes have been observed from the two orders of Class-Nematoda of Phylum-Nemathelminthes in Kingdom-Animalia. The two subclasses of Class-Nematoda are (1) Adenophorea or Aphasmidia and (2) Secementia or Phasmidia. The Plant parasitic nematodes are found in the order Dorylamida in the Subclass-Aphasmidia (Adenophorea) and in the order Tylenchida of Subclass Phasmidia (Secementia).

Table: Classification of Plant Parasitic Nematodes

Sub Class		**Order**	**Familiy**	**Plant Pathogenic Genera**
1.	Aphasmidia (Adenophorea)	Dorylamida	Dorylamidae Longidoridae Trichodoridae	*Xiphinema* *Longidorus* *Trichodorus*
2.	Phasmidia (Secernentia)	Tylenchida	Tylenchidae	*Anguina* *Tylenchus* *Ditylenchus*
			Tylenchorhynchidae	*Tylenchorhynchus*
			Pratylenchidae	*Pratylenchus* *Radopholus*
			Criconematidae	*Criconemella* *Hemicycliophora*
			Hoplolamidae	*Hoplolaimus*
			Heteroderoidae	*Heterodera* *Globodera* *Meloidogyne*
			Aphalanchoididae	*Aphalachoides*
			Belanolaimidae	*Belanolaimus*

MORPHOLOGY OF NEMATODES

Body size: There is great variability in the size of nematodes. Free-living nematodes in soil and freshwater vary in length from about 150 um (microns) to 10 mm. Marine species vary from 83 um to about 50 mm in length. The animal parasites range from about 1 mm to 6 or 7 meters in length, the largest being a nematode parasitic in whales. Plant parasites range from 0.25 mm to approximately 12 mm in length.

Body shape: Nematodes are elongate, cylindrical, unsegmented worms. They are bilaterally symmetrical. In some plant and animal parasites the body of the adult female (and juveniles stages) may be greatly modified. The body may be spindle-shaped (**fusiform**), pear-shaped, lemon-shaped or variations of saccate. In elongate-cylindrical species the anterior end is usually bluntly rounded with the oral aperture (mouth opening) terminal. The posterior end may be bluntly rounded or pointed; sometimes it is tapered to a point or it may be long and **filiform**. The opening of the excretory system (**excretory pore**) is located in the anterior portion of the body in a ventro-median position. The opening of the female reproductive system (the **vulva**) is usually midway in the body in a ventro-median position. The opening of the digestive system (**anus**) is ventro-median near the posterior end. In males the digestive and the reproductive systems join posteriorly to form a **cloaca** which opens on the ventral side near the terminal end. The following terms are used to refer to areas of the nematode: **adanal** (located near the anus), **caudal** (located near the tail), **cervical** (in the neck region).

Body wall: The body wall is composed of the **cuticle** which may be considered to be an **exoskeleton**; underneath the cuticle is the **hypodermis** and the muscular layer composed of a single layer of elongate spindle-shaped muscles.

Cuticle: The cuticle is predominately protein with traces of lipids and carbohydrates. About 17 to 19 amino acids have been identified. The thickness of the cuticle varies among species but on the average it is about 1/34 of the diameter of the body. The cuticle is basically composed of 3 layers, the **cortical**, **median** (matrix) and **basal**. In some large nematodes (*Ascaris*) the basic 3 layers can be subdivided so that a total of 7-9 distinct layers have been observed. In other nematodes such as root-knot nematode only 2 layers (cortical and basal) are present. The primary function of the cuticle is to provide a certain rigidity and to protect the nematode from harmful materials in the environment. The cuticle is permeable to water and to some ions. It is known that the permeability of the cuticle is in some way controlled by the living nematode; certain dyes and stains do not penetrate the cuticle readily when the nematode is alive but the cuticle becomes permeable when the nematode is dead. The external surface of the cuticle is nearly always marked by transverse **annules** (not to be confused with segmentation). Longitudinal annules are sometimes present. In some forms the cuticle may have punctations, bars, spines, and overlapping of the annules. Many marine forms have numerous **somatic setae** which vary in length, some species have setae whose length exceeds the width of the body. Somatic setae are believed to function as tactile sense organs.

Hypodermis: The hypodermis is a thin layer of tissue immediately underneath the cuticle. The cells and cell nuclei of this layer are located in the lateral hypodermal **chords** which extrude into the **pseudocoelom**. There are also extensions of the hypodermis dorsally and ventrally; these are the dorsal and ventral chords. The chord area contains the longitudinal nerves, e.g. the ventral, lateral and dorsal nerve chords, and in some groups the lateral chords contain excretory canals. The cuticle is secreted by the hypodermis, as is the lining of the esophagus and the rectum. That the hypodermis is an active layer of the body wall is evidenced by the fact that a new cuticle must be formed between each juvenile stage, usually a total of 4 times, since there are usually 5 stages in the life cycle.

Somatic Muscles: Immediately beneath the hypodermis is a single layer of muscles. These muscles are intimately associated with the hypodermis and are attached to it throughout their length. The muscles occur in 4 quadrants, lying between the lateral, dorsal and the ventral chords. There are two subdorsal quadrants and two subventral quadrants. Each muscle is elongate spindle-shaped and each has a connection to the nervous system. The muscles are connected to the dorsal nerve cord, lateral nerves and ventral nerve cord. In movement the muscles of the subdorsal and subventral quadrants are alternately contracted and relaxed and this accounts for the dorso-ventral movement of vermiform nematodes. The connection of the muscle with the nerve cord is unique because the extension from the muscle to the nerve cord is by an extension of the muscle that does not contain nerve tissue. In other animals the nerves extend to the muscles.

Specialized Muscles: There are muscles associated with the **buccal cavity** and with the piercing organ called the **stylet** or **spear**. The **esophagus** contains muscles that are used to open and close the **lumen** during feeding. Some muscles extend from the esophagus and intestine to the body wall to maintain the position of these structures in the body cavity. Muscles are also associated with the female and male reproductive system and with the rectum and anus.

Pseudocoelom: The pseudocoelom is the body cavity (the area between the body wall and the digestive tract). It is filled with fluid which bathes the muscles, the digestive system, the reproductive system, and some or all of the excretory system. The fluid in the pseudocoelom serves as a transport system for oxygen, food materials and products of metabolism.

Digestive System: The digestive system consists of the buccal cavity (**stoma**, mouth cavity), the esophagus, **esophago-intestinal valve**, the intestine and the **rectum** (cloaca in males). The head region of nematodes typically shows specialized morphology adapted to its lifestyle, as demonstrated by the plant-parasitic nematode at left below.

Buccal Cavity: The buccal cavity is at the anterior end of the esophagus; it is lined by cuticle and is variable in shape and size. It may be elongate-cylindrical to globular; sometimes it is armed with large teeth and various kinds of smaller teeth or **denticles**.

In the case of the plant-parasitic nematodes the buccal apparatus is in the form of the stylet (spear) that is used to penetrate plant cells and tissues (as shown at left). The stylet is hollow and serves as a piercing-sucking organ. To some extent the form of the buccal cavity is correlated with the foods that are utilized by various kinds of nematodes, e.g. bacteria, protozoa, algae, and predaceous forms that feed upon small worms and other nematodes. Some nematode parasites of higher animals use the buccal cavity to attach themselves to their feeding sites e.g. hookworms. The spear of a **dorylaim**, called an **odontostylet**, forms in a cell far back in the left submedial wall of the esophagus and moves forward to its place in the pharynx as each molt occurs. The spear of a **tylench** is called a **stomatostylet** because it develops in the pharynx along with other portions of the oral cavity.

Esophagus: The esophagus is that portion of the digestive system between the buccal cavity and the intestine. The lining of the esophagus is cuticular and is shed with the cuticle during molting. The lumen is **triradiate**; one ray is ventral and two subdorsal. All or part of the esophagus is equipped with radial muscles which open and close the lumen during feeding. In some groups portions of the lumen may be modified into more valves which serve as special pumping devices during feeding. The esophagus may be cylindrical, or 2 part, or 3 part and its shape is used as a basis for classification in some groups. The esophagus is separated from the intestine by an esophago-intestinal valve which prevents the intestinal contents from moving forward into the lumen of the esophagus.

Intestine: The intestine, extending from the esophago-intestinal valve to the rectum, is a tube composed of a single layer of epithelial cells. The number of cells comprising the intestine varies from few (8-10) in small nematodes to many (several hundred thousand) in forms like *Ascaris*. The inner surface of each intestinal cell consists of **micro-villi**. The intestinal cells frequently contain stored food material such as glycogen, fatty acids and proteins. Sometimes inorganic crystals and granules are present.

Rectum and Cloaca: In females, the rectum connects the intestine with the anal opening. It is usually relatively short and is lined with cuticle. In some groups **anal glands** are associated with the rectum. In males the reproductive and digestive systems are joined posteriorly to form a cloaca.

Reproductive System: The female reproductive system usually consists of two opposed tubular **gonads** (**amphidelphic**, **didelphic**), one extending anteriorly and one posteriorly from the vulva (external opening). However, in some groups the posterior gonad or the anterior gonad may be **vestigial** and non-functional so that forms with a single gonad (**monodelphic**) are not uncommon. Possessing one ovary located anterior to the vulva is termed **prodelphic**. The female gonad consists of a distal **cap cell** followed by a **germinal zone** where **oocytes** develop, a **growth zone**, an **oviduct** and the **uterus** and **vagina**. There is frequently a **spermetheca** (for storage of **spermatozoa**) located at the anterior end of the oviduct.

The male reproductive system may consist of one (**monorchic)** or two (**diorchic**) gonads. If there are two the testes are opposed. The male gonad consists of the **testis** (germinal zone and growth zone), the **seminal vesicle** (for storage of sperm) and the **vas deferens** which functions as an **ejaculatory duct** and opens into the cloaca. The male reproductive system has various copulatory structures associated with it. The most conspicuous of these are the **spicules**, the **gubernaculum**, and in some forms the **caudal alae** (also called the **bursa**).

Nervous System: The **circumesophageal commissure** is the central portion of the nematode nervous system to which are connected anteriorly and posteriorly directed nerves and nerve chords. This structure encircles the esophagus and is called the **nerve ring**. Nematodes possess a number of structures thought to be chemosensory and the presence or absence, location, shape and size are frequently utilized in classification. These include the **amphids** (a pair of lateral, innervated organs usually opening on or near the lip region), and the **phasmids** (lateral caudal papillae, with glandular connections in the lateral field and believed to be chemoreceptive). A series of **cephalic papillae** is arranged around the mouth of nematodes. **Caudal papillae** are found on the tails of adult males and are usually arranged around the cloaca. **Deirids** are exceptionally large cervical papillae situated near the nerve ring.

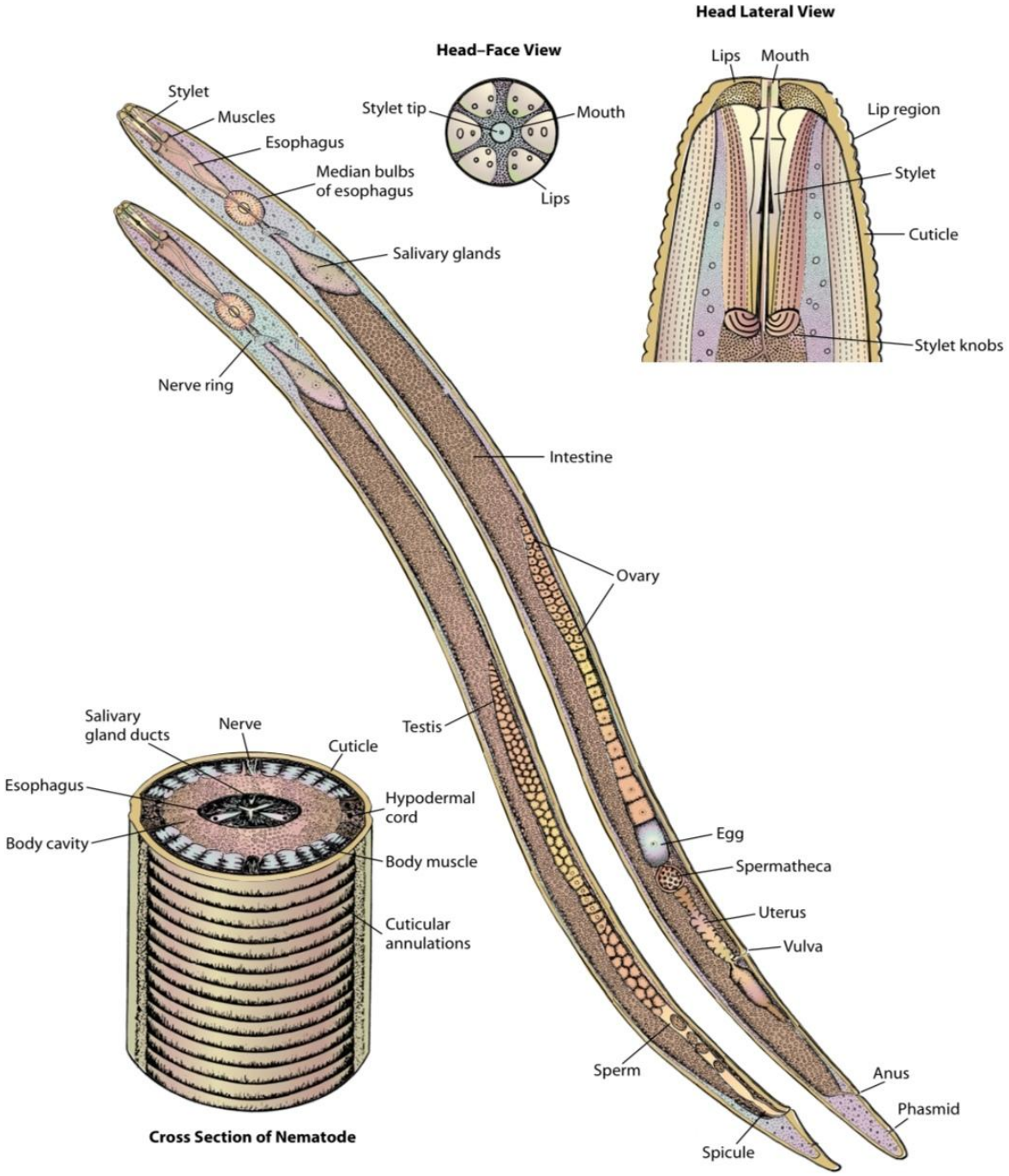

FIGURE 15-2 Morphology and main characteristics of typical male and female plant parasitic nematodes.

Genus- *Meloidogyne* spp.
(Root – knot nematodes)
Classification: Kingdom- Animalia, Phylum- Nematoda, Class- Secernentea, Order Tylenchida, Family- Heteroderoidae

The root – knot nematodes are sedentary endoparasites of underground plant parts. The eggs are retained in a gelantinous matrix, which normally protrudes out of the host tissues. About 200 to 300 oval eggs are found in a single egg mass which makes its size larger than the female body.

Morphology:

The female is saccate to globose, 0.4-1.3 mm. long, and usually embedded in root tissues which are often swollen or galled. Its body is soft, pearl white in colour and does not form a cyst. The neck protrudes anteriorly and the excretory pore is anterior to the median bulb and often near the stylet base. Its vulva and anus is terminal. The female stylet is shorter and more delicate with small basal knobs. The paired gonads have extensive convoluted ovaries that fill most of the swollen body cavity. There are six large unicellular rectal glands in the posterior body which produce a gelatinous matrix, which is excreted via the rectum to form an egg sac in which many eggs are deposited.

The male has long, thin, cylindrical shape of a worm but the lip region has a distinct head cap, which includes a labial disc surrounded by lateral and medial lips. The head skeleton is usually weaker and the stylet less robust and shorter. Infective second stage juveniles, often free in the soil, are usually 0.3-0.5 mm long; they are less robust, the stylet is delicate with small basal knobs.

Life cycle: The life cycle starts from the egg usually in the one-celled stage deposited by the female. Development of the embryo starts within hour of deposition, resulting in two, four, eight cells, etc., The embryo and the first stage larva move within the egg but not very active.

After the first moult, the second stage infective juvenile is formed within the egg. Larval hatch occurs under suitable physical condition but not depending on host root exudates or hatching factor. The emerging second stage larvae are found free in the soil. They attack new host root tissue in the region behind the root tip (meristamatic zone). The larvae which develop into females establishes feeding site in the pericycle region and become sedentary. Subsequently three moults occur and the larvae develop into females with spherical body embedded in the host tissue. The neck region is unaltered.

During feeding the larvae pierce the cell wall with secretions cause enlargement of cells in the vascular cylinder and increased cell division in the pericycle. The nematode feeding stimulates the development of a typical nurse cell system called ‘Syncytium’ or ‘ Giant cell’. These cells are multinucleate which contain dense cytoplasm and enlarged nuclei with several mitochindrial and golgi bodies and are metabolically active.

The larvae that develop into adult males are initially parasitic. After moulting three times they leave the host as a worm like from and come closer to the females for copulation. Parthonogenesis is reported to be common in Meloidogyne. For development of a mature female it takes around 30 days which may vary depending upon the species of the host and parasite and environmental factors like temperature and soil type.

Genus - *Heterodera* spp.
(Cyst nematodes)

Second stages larvae usually penetrate the root just behind the growing point. These larvae grow rapidly and thee moults occur in the host. In about 5 –6 weeks after penetration , the white cysts are clearly visible which protrude from the root surface. These young cysts are packed with eggs and upon death the body wall hardens due to quinone tanning into a tough resistant brown covering known as cysts. The cysts get separated from the root and fall into soil.

Larval emergence from cysts is often in response to rot exudates from a host plant. The best emergence of juveniles occurs as a result of a rise in temperature after a period of low temperature. Maximum emergence of larvae from cysts under Indian condition takes place at a temperature of 20 -22°C. The cysts continue to release eggs over a period of 3 –4 years at the rate of 50 per cent viable eggs per year. There is only one generation of the nematode in a year.

Multiplication of nematode is favored by soil texture. Migration of second stage juveniles is favoured by light textured soils. The host cells close to the head region of the sedentary female being to modify and finally enlarge to form multinucleate syncytium with a thick outer boundary. The female feeds from this nurse cell system and grows. The swollen adult female protrudes out of the root tissues and later changes into brown cysts.

Although cyst nematodes induce giant cell formation, gall formation is not distinct. Each syncytium is associated with only one nematode in the case of cyst nematodes unlike the root – knot nematodes where one or more nematodes are associated with a syncytium. Nuclei are enlarged in the syncytium caused by the root – knot nematode but in cyst neamtode nuclei is relatively small. The syncytia are bound by the vascular elements especially xylem which develops specific wall in growths. There are enlarged nucleoli and irregular nuclei. Abundant mitochondria, Golgi bodies, protoplasts and dense endoplasmic reticula are also found in the syncytia.