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Introductory Chapter: Nematodes - A Lesser Known Group of Organisms

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<http://dx.doi.org/10.5772/intechopen.68589>

1. Introduction

Nematodes are a group of lesser-known but the most abundant group of multicellular organisms on earth. They can be defined as a group of thread/worm-like, transparent, bilaterally symmetrical, pseudocoelomate and multicellular organisms that are free-living or parasitic to plants or animals. Numerically, they form the most abundant phylum within the meio- and mesofauna. However, for many of us, nematodes are something unseen and unheard. It is assumed to be due to their small size as well as their habit of remaining hidden in soil, water, plant and animal tissues. Nematodes, being ubiquitous, are associated with plants, insects, other invertebrate and vertebrate animals including domestic animals and even human beings. They exhibit different modes of life—parasitic (plant and animal), free-living, predatory, insect associates, entomopathogenic, terrestrial, aquatic (marine and freshwater) etc. The plant parasites may be migratory ectoparasites (feeding at different places but the body remaining outside of plant tissue) or migratory endoparasites (feeding at different places at the same time migrates inside the plant tissue) and some of them may be sedentary (in the forms with obese females like *Meloidogyne* sp.). Some are semiendoparasites (half of the body embedded in plant tissues while half remains outside), for example, *Tylenchulus semipenetrans*.

2. Brief history

Our knowledge of animal parasitic nematodes is much more ancient than that of plant-parasitic and free-living forms. Animal parasitic forms were known to us as early as 1500 BC. Large round worm like *Ascaris lumbricoides* and the dreaded Guinea worm, *Dracunculus medinensis*, etc., were known at that time [1]. On the other hand, soil nematodes remained unknown to us for a long period of time. It is assumed that this is due to the hidden mode of life these organisms lead as well as due to their minute size. Borellus [2] was the first to observe a free-living

nematode, *Turbatrix aceti* (vinegar eel). Needham [3] reported the first plant-parasitic nematode. Systematics of nematodes was first published by Rudolphi [4]. Leidy [5] was the first one to describe a freshwater nematode, *Tobrilus longus*. Dujardin [6] for the first time described a dorylaim nematode, *Dorylaimus stagnalis*.

It is almost impossible to make a list of all nematologists the world has had so far. However, an effort is being made to highlight some of the important contributions made by the past and present nematologists. In nematode taxonomy, Bastian [7] made a historic contribution through his descriptions of 100 new species under 23 new and 7 known genera. Schneider [8] and Bütschli [9, 10] gave detailed accounts of free-living nematodes. Örley [11] provided the first comprehensive survey on the taxonomy of free-living nematodes which included 202 species belonging to 27 genera. Modern generic and specific descriptions are based mainly on the de Man's works [12]. His monograph [13] is regarded the "Bible of Nematologists" and his indices for expressing nematode morphometric values are still used with some modifications and additions. Cobb is considered as the "Father of Nematology in the United States." He published a series of very valuable papers.

There are several other nematologists whose contributions deserve to be mentioned. Filipjev [14–16] made significant changes in the classification of nematodes. Micoletzky [17] reported 142 genera and 931 species. The present classification of Nematoda is mainly based on the hypothesis of Paramonov & Filipjev. Chitwood's book [18] "An Introduction to Nematology" is a golden piece of work in the history of Nematology. Valuable contributions made by Thorne in the form of his monographs on Dorylaids [19], Cephalobidae [20] and Tylenchida [21], and in the form of his book [22] "Principles of Nematology" need special mention. Goodey [23] gave much information related to soil and freshwater nematodes. Contributions made by Meyl [24], Grasse [25] and Gerlach & Riemann [26, 27] still prove to be milestones in terms of changes in nomenclature, synonymisations and reviews. Andrásy's contributions in the field of nematode taxonomy [28–32] will always remain a great asset of Nematology forever. Blaxter et al. [33] and De Ley & Blaxter [34] revised the classification of phylum Nematoda based on molecular and morphological characters. Eyal et al. [35], Steiner [35, 36–43], Füchs [44–48], Rahm [49–52], Allgen [53–56], Altherr [57–60], Pearse [61], Hirschmann [11, 62, 63], Kirjanova [64–66], Wieser [67–69], Timm [70], Golden [71], Loof [72–74], Coomans et al. [75], Inglis [76], etc., also contributed significantly to the field of Nematology. Contributions made by Siddiqi [77], Jairajpuri and Ahmad [78] are highly valued.

3. Smart lifestyle of smart organisms

It is impossible to think of a habitat, macro or micro, without nematodes like hot springs, low oxygen conditions, acid environments, rocky mountains, deep sea trenches, polar regions, aerial region, subterranean region, decaying organic debris, plant roots, stems, flowers and seeds. Thus, in habitat diversity, nematodes are the masters. This vast distribution may be attributed to their surprisingly versatile life. Nematodes may be bacterial and fungal feeders, parasites of plant, predators and parasites of animals (insects to humans and livestock). Many species cause deaths to insects (entomopathogenic). Such nematodes that kill economically important

pests are popularly called as “Farmers best friend” [79]. Some nematodes may simply develop phoretic relationship (meant for only transport from one place to other) with the insects.

Nematode body is described by many as “tube within a tube.” Nematodes have a very simple body plan. However, they can successfully survive a wide range of geo-physico-chemical conditions. In unfavorable conditions, they can switch their food preference, a condition known as omnivory. They can survive without any detectable metabolic activity (*cryptobiosis*) or simply they can lower their rate of metabolism (*dormancy*). The young ones (juveniles) can also survive unfavorable conditions through a kind of survival stage in which metabolic activities are suppressed (*dauer stages*). Some species can survive complete dryness.

So far, Arthropoda is the largest phylum in the kingdom Animalia. However, nematodes are the most abundant organisms. Four of every five multicellular animals on our planet are nematodes [79]. Nearly 90% of the multicellular animals on earth are nematodes [12, 80, 81]. An average of 15,000–20,000 juveniles of *Anguina tritici* is present in a single wheat gall. Many million individuals per m² in soil and bottom sediments of aquatic habitats may be present and it is not uncommon to find more than 50 species in a handful of soil. Nathan Augustus Cobb, referred to as the Father of Nematology in the United States [13] very rightly said, “*If all the matter in the universe except nematodes were swept away, our world would still be recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes.*”

4. Role of nematodes in our life

In agriculture: Most of the soil nematodes are microscopic. However, their direct and indirect roles in a country's economy are massive. Annual crop losses due to nematodes have been estimated to more or less \$80 billion. In many developing countries, the population increases at a very fast pace while the size of fertile land decreases due to industrialization, expansion of urban area, transport system, etc.

In ecosystem functioning: In the food chain of subterranean ecosystem, nematodes play a very important role. Many of them are bacterial and fungal feeders which contribute to decomposition of organic materials and thus increase fertility, while many others are parasites of plants attacking a wide range of plants. Many others attack human beings and livestock. A good number of them are predators and thus feed on soil microarthropods, nematodes, etc.

In experimental biology: A good number of them have successfully been used as experimental models, for example, *Caenorhabditis elegans* and *Pristionchus pacificus*. Nematodes, specially the bacterial and fungal feeders, are easy to culture in the laboratory. They can complete the whole life cycle in a few days. Many trials can be done on several generations in a short period of time. As their body is transparent, their internal structures can be observed without going through the process of tedious dissections.

In ecological studies: All of the species are equally good for ecological studies. There are several other species which are considered to be reliable bioindicators too. Nematode community structure can be used as a bioindicator in environmental monitoring [52].

In pest management programs: The entomopathogenic nematodes like the species of *Steinernema*, *Heterorhabditis*, *Neosteinernema*, etc., have been used in successful management of many economically important insect pests [82].

5. External and internal morphology of nematodes

With a few exceptions, all the nematodes are vermiform (worm-like). They show a great range of species-specific variability in their body morphologies.

Body shape or body posture: Generally, nematodes have elongated, spindle-shaped body. However, pear-shaped, lemon-shaped or saccate body also occurs. Nematode body usually tapers toward anterior (head) and posterior ends. Nematodes' body posture on head is interestingly very specific. The body may remain straight or slightly/strongly curved ventrally; or spiraled or exceptionally dorsally curved.

Body size: Nematodes show a great range of variability in their body size. It ranges from less than 82 μm (*Grieffella minutum*—marine) to more than 8 meter (*Placentonema gigantissima*—placenta of whale). Most of the free-living and plant-parasitic nematodes are small in size, while the predatory nematodes are large.

Body wall: The outer body wall (exoskeleton) of nematode is known as cuticle. Externally, it bears longitudinal or transverse striations or both. Besides the longitudinal and transverse striations, the cuticle may possess differently modified structures called cuticular ornamentations—dots, warts, depressions, elevations, projections or spines from the posterior margins of the annules. Below the cuticle, there lies the hypodermis and the musculature. The cuticle is made of mainly protein with small amounts of lipids and carbohydrates. It is semipermeable. Cuticle varies from species to species in terms of thickness and structure. It is mainly composed of three layers—cortical layer, median layer and basal layer. The number of layers in the cuticle is more in animal parasitic forms (e.g., 7–9 layers in *Ascaris*).

Hypodermis: As has been mentioned above, the hypodermis lies below the cuticle. It is a thin layer and is characterized by the presence of four longitudinal invaginations also called chords (dorsal—1, ventral—1 and laterals—2) in the coelomic cavity.

Somatic musculature: It is a layer of spindle-shaped muscle cells attached to the hypodermis. Each of these muscle cells has sarcoplasmic and fibrillar parts.

Lip or cephalic region: Lip region is the anteriormost part of the body and it differs in different groups of nematodes. It may be continuous or set off from the body.

Lips and labial papillae: There are six lips arranged circularly around the oral opening. Two of them are in the lateral sectors, two are in subventral sectors and two are in subdorsal sectors. Each lip carries three papillae except the laterals which carry two papillae. The labial papillae are arranged in inner and outer circlets. There is only one papilla on each lip in the inner circlet, while two papillae each are there on each lip in the submedian sectors. The lateral lips carry one papilla each.

Cephalic framework: It is a ring or basket-like cuticularized structure present around the stoma. It may be weakly or strongly cuticularized and it varies from species to species.

Amphid: It is a paired structure considered to be chemoreceptor organs. These are present in the lateral sectors of the body in the anterior esophageal region. The amphids open to exterior and the openings of amphids may be circular, oval, slit-like or pore-like and may be located on the lateral lips or close to or far posterior to them.

Deirids: Like amphids, deirids are also paired structures. They are circular, thickened and are present on cuticle in the mid-lateral sectors in the pharyngeal region around the level of excretory pore.

Phasmids: Phasmids are also circular and paired and are present in the mid-lateral regions. Generally, these are present posterior to anus (females) or cloaca (males). However, their positions may be adanal, pre-anal or even further anterior. Either the phasmids may be just opposite to each other or one of these may be shifted anterior or posterior.

Stoma: The anteriormost part of the digestive tract is the stoma. It varies in shape and size in different nematode groups having different food and feeding habits. Bacterial and fungal feeders have tubular or funnel-shaped or barrel-shaped stoma (**Figure 1(A), (B)**), whereas plant-parasitic tylenchids (**Figure 1(C)**) and aphelenchids have a protractible, hypodermic needle-like stylet/spear. The predators, on the other hand, have wide and spacious stoma which may or may not be provided with tooth, teeth or denticles (**Figure 1(E) and (F)**). The terminology used for the feeding apparatus is different in different nematode groups. In the dorylaim nematodes, it is called odontostyle, while in nygolaims, it is named onchiostyle. In case of mononchs, it is simply called buccal/stomal cavity. The buccal cavity in mononchs is generally provided with dorsal tooth, a pair of subventral teeth, denticles, etc.

Esophagus: It is also called pharynx. It is a roughly tubular structure. It connects the stoma with intestine. It varies in shape and size in different groups. In Tylenchida and Rhabditida, it is tripartite (having three different parts) (**Figures 2(A), (B) and 3**).

Esophageal glands: These are also called as pharyngeal or salivary glands. Esophageal glands are nothing but unicellular, uninucleate cells found embedded in pharyngeal tissue. There is variation in the number of these glands in different groups. Tylenchids usually have three glands, while the dorylaims have five glands. In tylenchs, the glands may extend over the intestine forming a kind of lobe.

Esophago-intestinal junction: It is also called cardia. It is a disc or tongue-like structure. It connects the pharynx with intestine. It prevents the food in intestine from coming back to pharynx.

Intestine: It is a tubular structure made up of a single layer of comparatively large cells. It is the longest part in the digestive system connecting the cardia anteriorly and the rectum (in all groups except dorylaims) or prerectum posteriorly (dorylaims).

Prerectum: In Dorylaimida, the intestine posteriorly connects with prerectum. It is different from the intestine proper in color, thickness, texture of the food containing in it. The length of prerectum is variable and is different from species to species.

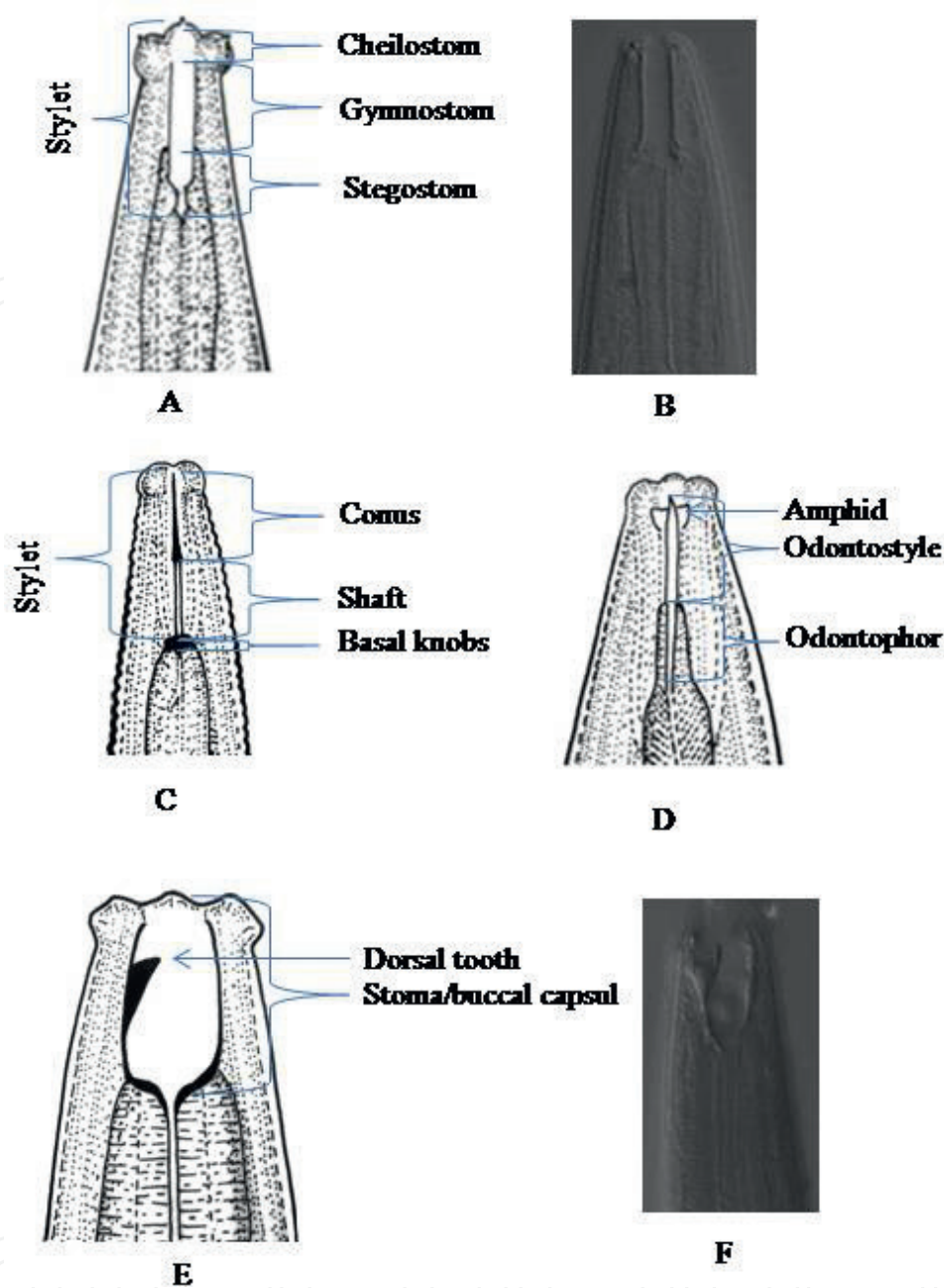


Figure 1. (A) Rhabditid (bacterial & fungal feeder) stoma, (B) photograph of rhabditid stoma, (C) Tylenchida (plant-parasitic) stoma, (D) Dorylaim (predatory, some are plant parasites) stoma, (E) Mononchid (predator) stoma, (F) photograph of Mononchid stoma.

Rectum: It connects anteriorly with intestine or prerectum and posteriorly with anus. The junction with intestine is provided with sphincter (circular-contractile ring made of muscles) muscles. In many species, the anterior end of rectum may carry three unicellular glands.

Anus/cloaca: Females have separate openings for both digestive and reproductive systems—anus and vulva. Anus is the end point of the rectum. It opens to the exterior. Males, on the other hand, have a common opening for both digestive and reproductive systems to the exterior and is called cloaca.

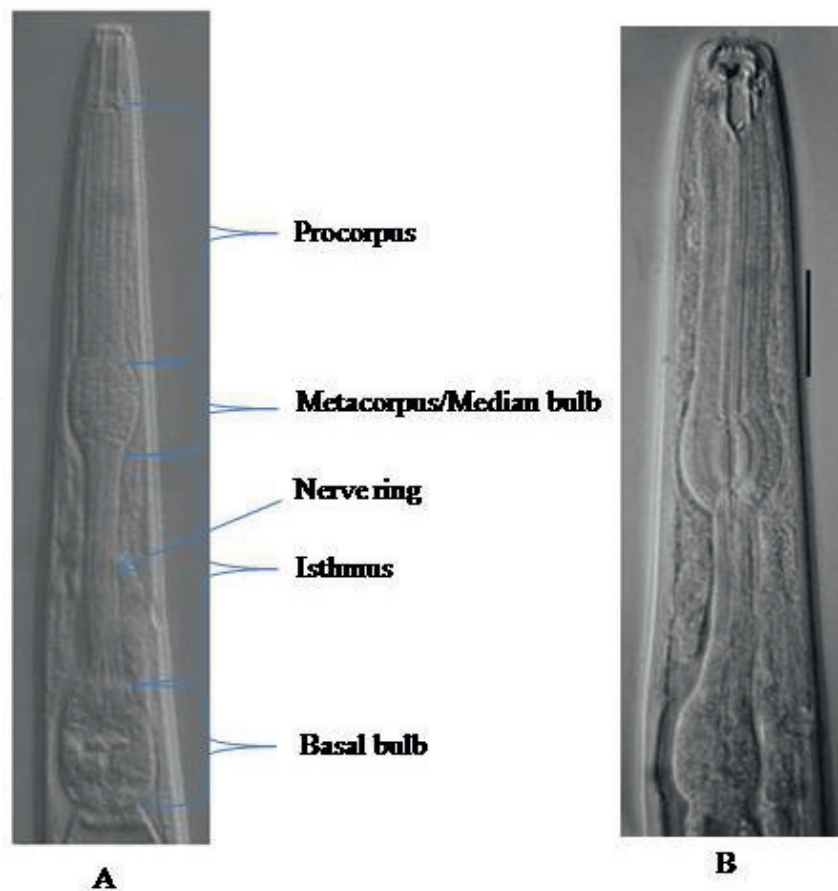


Figure 2. (A) Rhabditid (bacterivorous) pharynx, (B) Diplogastrid (omnivore-predator) pharynx.

Female reproductive system: Reproductive system in females is composed of ovary, oviduct, spermatheca, uterus, vagina and vulva. The reproductive system may be single (*monodelphic*) or paired (*didelphic*). The gonad(s) may be positioned anterior to vulva (*prodelphic*) or posterior to the vulva (*opisthodelphic*), or on both the sides (*amphidelphic*). Both the reproductive systems may be positioned on the same side (*didelphic-prodelphic*, e.g., *Meloidogyne*). A nonfunctional gonad which is also reduced in size may be present in addition to the functional one. This condition is known *pseudo-prodelphic* (anterior) or *pseudo-opisthodelphic* (posterior) gonad. The reduced, nonfunctional branch is called as *prevulval uterine sac* (anterior) or *postvulval uterine sac* (posterior).

Male reproductive system: The components of male reproductive system are very important for proper identification. In many instances, studying only the female characteristics is not enough for species level identification. The male sexual characters comprise of testis, seminal vesicle, ejaculatory duct, cloacal chamber and its associated glands, spicules, gubernaculum, lateral guiding pieces, copulatory muscles, genital papillae and bursa. Testis may be single (*monorchic*) or paired (*diorchic*). The testis is outstretched with the tip directed anteriorly in monorchic condition. However, in dio-orchic condition, one testis is placed in reversed condition with the whole of it directing the opposite side.

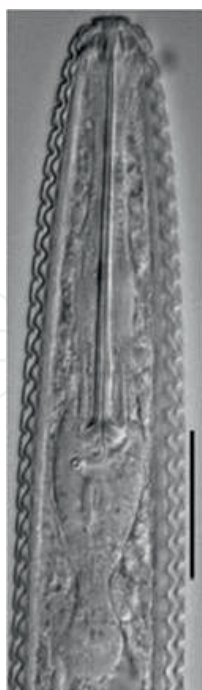


Figure 3. Criconematid (plant-parasitic) pharynx (procorpus and metacorporus fused, isthmus very short, basal bulb recorded).

Tail: Tail in nematodes may be of different shapes and lengths. It may be short, long, long conoid, whip-like, filamentous, conoid, digitate, clavate, hemispheroid, etc. It may be with phasmids, scutella (singular—scutellum), caudal glands, caudal pores, caudal setae, spinneret, mucro, etc. Tail may differ in shape and length in different sexes.

6. Collection and processing of samples

Soil samples: The soil samples should be taken from a depth of 10–25 cm after removing the topmost dry layer of soil and should be kept in airtight polythene bags. Each sample should be tied so that the soil particles are not disturbed. Loosely tied soil samples may not give a good collection of nematodes as they may die due to desiccation before processing the samples. All relevant information such as host, locality, and date of collection should also be noted. Till further processing the samples should be kept undisturbed, away from sunlight at 20–25°C.

Plant materials: For studying nematodes which are ectoparasites of roots, the samples should be collected from around the roots of the host plant. Effort should be made to collect the fine roots too. For endoparasitic nematodes, direct observation of the affected parts after staining is suitable.

Staining the roots with acid fuchsin solution: First, prepare stock solution of acid fuchsin by dissolving 3.5 g of acid fuchsin in 250 ml acetic acid and then increase the volume up to 1000 ml by adding distilled water. Secondly, dip the roots thoroughly in 5.25% NaOCl and keep for about 4 min. Thirdly, wash the roots by using tap water for about 45 s and then keep the roots

immersed in water for 15 min to avoid any residue of NaOCl. It may otherwise affect staining. Now, the roots should be transferred to a glass beaker containing 30–50 ml of tap water. Take 1 ml of stock solution and pour into the glass beaker containing roots and tap water. Boil the same for about 30 s. Let it cool down to room temperature and drain the stained solution. Rinse the roots again in running tap water. Now, the roots can be teased with the help of needles under a stereoscopic microscope to examine the presence of any endo- or semiendo-parasitic nematodes such as *Meloidogyne incognita*, *Tylenchulus semipenetrans*, etc.

Isolation of nematodes from soil samples: There are many techniques employed to isolate nematodes from soil samples. Some of them are Oostenbrink's elutriator, Seinhorst's elutriator, Cobb's decanting and sieving technique, Baermann's funnel technique, Maceration-filtration technique, Mistifier extraction technique, Sugar floatation technique, etc. However, a combination of Cobb's [83] decanting and sieving technique and Baermann's funnel technique is commonly used in a slightly modified way. It is very good to isolate vermiform, active nematodes. The drawback of this technique is that it cannot isolate the immobile, inactive individuals and also the eggs.

Modified Cobb's decanting and sieving technique: In this, around 500 cc of sample is taken in a bucket and mixed with water thoroughly. The debris and pebbles, if present, are removed, and soil crumbs (in case of soil samples) are broken manually. The bucket is then filled with water and the suspension is stirred thoroughly to make it homogeneous. It is then left undisturbed for about half a minute so as to allow the heavy soil particles to settle down to the bottom of the bucket. The suspension is then passed into another bucket through a coarse sieve (2 mm pore size), which retains debris, roots and leaves. The suspension in the second bucket is again stirred thoroughly and left for another half a minute and then poured through a BSS 300mesh sieve (pore size 53 μ m). The catch on the sieve containing nematodes and very fine soil particles is collected in a beaker. The process is repeated twice for good recovery of nematodes.

Modified Baermann's funnel technique: The residue collected in the beaker is poured on a small coarse sieve which is already lined with tissue paper. The small coarse sieve is then placed in a Baermann's funnel fitted with stoppered rubber tubing. Tap water is slowly poured into the funnel until it touched the bottom of the sieve. Care should be taken to avoid trapping of air bubbles at the bottom of the sieve as the nematodes containing in the coarse sieve will not migrate down the funnel in the area where there are bubbles. The nematodes will migrate from the sieve into the clear water of the funnel and settle at bottom. After 24 h, a small amount of water containing the nematodes can be drained from the funnel into a glass cavity block.

Killing and fixation: The nematodes collected in cavity blocks should be left undisturbed for some time so as to allow them to settle to the bottom of the cavity blocks. Excess water should then be removed with a fine dropper. Disposable syringe with very fine hypodermic needle can be easier to handle for removing excess water from the cavity blocks. Use of a hot fixative will simultaneously kill and fix the nematodes. There are several fixatives like TAF (8 ml formalin + 2 ml triethanolamine + 90 ml distilled water), FG (8 ml formalin + 2 ml glycerin + 90 ml distilled water).

Dehydration: After 24 h of fixation, the nematodes should be transferred into a mixture of glycerin-alcohol (5 parts glycerin + 95 parts 30% alcohol) in a small cavity block. Picking individually and transferring several nematodes is not easy, and it is not good for health too as the fixative is formaldehyde-based. It can be avoided by simply drawing the fixative out of the cavity block by using a fine-tipped dropper or a disposable syringe. Then, remove the fixative as much as possible and add glycerin-alcohol and keep the same in desiccator containing anhydrous fused calcium chloride. In 3–4 weeks' time, the nematodes will be dehydrated completely.

Mounting and sealing: Take a clean glass slide and place a small drop of anhydrous glycerin and transfer the nematodes from the cavity block to this drop and make them settle on the surface of the slide. Take 3 cubes of wax (approximately 2 mm²) and place around the glycerin drop at around 120° to each other. It is preferable to place three pieces of glass wool of same thickness as of the nematodes around the nematodes to prevent flattening. Take a circular glass cover slip (18 mm diameter) and gently place on it and keep the slide on a hot plate to allow the wax to melt and seal the slide.

Measurements and drawing: For taxonomic studies or for any pest-management program, proper identification is the key to success. For proper identification, measurements of different body parts are inevitable. All measurements can be made on specimens mounted in dehydrated glycerine with an ocular micrometer. The ocular micrometer should be calibrated first by using a stage micrometer. For denoting dimensions of nematode, De Man [84] introduced a system. It was further modified in 1880. There have been many changes made by some famous nematologists like Cobb [26], Thorne [20], Caveness [85], etc. Besides those changes, these morphometric parameters are still known as the De Man's indices/formula and are given below.

n = Number of specimens measured.

L = Body length.

V = Distance from anterior end to vulva/total body length $\times 100$.

a = Body length/greatest body diameter.

b = Body length/length of pharynx.

b' = Body length/distance of base esophageal glands from anterior end.

c = Body length/tail length.

c' = Tail length/diameter of tail at anus or cloaca.

s = Stylet length/diameter of body at base of stylet.

T = % Total length of testis relative to total body length.

G^1 = % Total length of anterior female gonad in relation to total body length.

G_2 = % Total length of posterior female gonad in relation to total body length.

7. Nematode trophic groups

Ecological studies using nematodes as models use the feeding habit as the basis of categorization. Nematodes show all possible modes of feeding. Such type of classification is far away from the systematics of the nematode species concerned. All the species sharing a common mode of feeding are considered in a single category. Many nematode ecologists have proposed several trophic groups. The trophic groups of nematodes are herein proposed as follows -

1. *Plant-feeding*: This group includes those nematodes feeding on plant tissues. Such nematodes possess a spear (Tylenchida) or an odontostyle (Dorylaimida). This group may be further divided into
 - i) Migratory ectoparasites—This group is represented by those species which feed at different places but never enter into the plant tissue. They can penetrate the stylet deep into the cortex, for example, members of the family Dolichodoridae, Criconematidae, etc. The feeding may also be restricted only to the epidermal cells and root hairs as in case of the members of the families Tylenchidae, Psilenchidae, etc., in which the stylet is not so strong.
 - ii) Migratory endoparasites—It is represented by those nematodes which migrate inside plant tissues, for example, *Radopholus*.
 - iii) Sedentary endoparasites—It includes the groups in which the females become obese, for example, *Meloidogyne*.
 - iv) Semiendoparasites—This group includes those nematodes in which half of the body is embedded inside plant tissues, while the rest of the body is exposed to the external environment, for example *Tylenchulus*.
2. *Bacterial-feeding*: This group is represented by those nematodes having cylindrical or barrel-shaped or slightly wide feeding apparatus such as rhabditid and diplogastrid species.
3. *Omnivore-predators*: This type of feeding habit is found in some diplogastrid species in which the stoma is provided with armatures such as tooth, teeth, denticles, etc.
4. *Predatory*: Many nematode species of Mononchida, Dorylaimida (Naigolaimina), Rhabditida (Diplogastrina) live on the soil microarthropods, nematodes, etc.

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