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Exploration and biodiversity of nematode in Nilgiri forest ecosystem

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Abstract
The survey was conducted by collecting the , soil and root samples randomly in the forests and from tree species such as *Tectonagrandis, Spathodea campanulata, Jacaranda mimosifolia, Grevillea robusta, Pongamia pinnata* and *Kijelia pinnata* with an aim of understanding the distribution and occurrence of the nematodes and their role in soil health in the forest soils.

Keywords: Biodiversity, nematode, Nilgiri, forest ecosystem

Introduction

Biodiversity of micro-organisms like bacteria, fungi, nem atodes play an indispensible role in the ecosystem. Interestingly the abundance and the interaction of these micro organisms are more in the undisturbed soils of forest ecosystem. Nematodes are the most common groups occurring in the soil as free living forms like bacterial-feeders, fungal-feeders, plant parasites, predators and omnivores. All these types of nematodes coexist in the soil together with the plant parasitic nematodes. Nematodes of certain trophic groups play an important role in organic matter decomposition, mineral and nutrient cycling and as bio indicators of pollution. The nematodes genus identified from the samples included bacterial feeders like *Rhabditis* sp., *Acrobeles* sp., *Diplogaster* sp., and. plant parasitic or herbivorous nematodes such as *Helicotylenchus* sp., *Tylenchorynchus* sp., *Hemicroconemoides* sp., *Psilenchus* sp., *Xiphinema* sp., *Helicotylenchus* sp., fungal feeding nematodes such as *Aphelenchus* sp., *Filenchus* and *Dorylaimoides* sp., predatory nematode *Mononchus* sp., and *Dorylaimus* sp., and entomopathogenic nematodes such as *Steinernema* sp. The free living nematodes identified play an important role in maintaining the soil health of the forest ecosystem. The bacterial feeding nematodes were found to be predominant in the forests in lower elevations *viz*., Kallar and Burliyar. The population of fungal feeder and also bacterial feeders were observed more in the forests of higher altitudes Hence, an exploration for nematodes in the Nilgiri forests would give an insight on the occurrence of the nematode biodiversity and their functional roles in the soil food web their community structure can provide important insights regarding many aspects of ecosystem function.(Ekschmitt, and Korthals 2006) [4] Nematodes (free-living and plant-parasitic) may be the most useful group for community indicator analysis because more information exists on their taxonomy and feeding roles (Hunt, 1993) [6] than does for other mesofauna. Soil nematodes can be placed into at least five functional or trophic groups and they occupy a central position in the detritus food web Hooper and Cowland. 1986 [5] Most kinds of soil nematodes do not parasitize plants, but are beneficial in the decomposition of organic matter (Ingham and Coleman. 1983) [7]. These nematodes are often referred to as free-living nematodes. Although the plant-parasitic nematodes are relatively well-known, most of the free-living nematodes have not been studied very much. Identification of these groups is extremely difficult, and there are only a few nematode taxonomists in the world who can formally describe new species of free-living nematodes to science. 1. Survey or nematodes in selected forests of Nilgiris 2. Collection and categorization of trophic groups of nematodes.

Materials and Methods

Collection of Soil Samples

Nematodes are usually unevenly distributed in the field especially in the forest soils. Plant parasitic nematodes are abundant within the root zone and free living nematodes are distributed in all regions. Therefore, sampling was done near the root zone and in a random manner with an aim of collecting both plant parasites and free living respectively. Hand shovel and auger were used for digging and collecting soil. Samples of 250 g were collected at a depth 20 to 40 cm as the deep inside the drip line removing the top layer soil filled with dried leaves and other debris. About 20 random soil samples were collected from each forest area during the preliminary survey. A total of 100 samples were collected during the second survey at the rate of 20 samples in each forest area. Samples were collected in polythene bags, sealed, labelled and stored in a cool place in order to avoid desiccation of nematodes until processing.

Extraction of nematodes

a. Processing soil samples by Cobb's (1998) Sieving and Decanting methods

This method takes advantage of the difference in size and specific gravity between nematodes and other soil components. The equipments used for processing were plastic basins of two to three litres capacity, a stirring stick about 45 cm long and 2.5 cm wide and test sieves of 20,100 and 350 for soil analysis. The sieves were about 20 cm in diameter. The coarse sieve had 20 mesh to remove large stones and plant debris and a fine sieve of 100 to 350 mesh sieves were used for the recovery of nematodes. Plastic beakers of capacity of 500 ml were processed through modified Baermann's funnel method.

b. Recovery of nematodes by Modified Baermann's Funnel Method (Schindler, 1961) [3]

A moulded sieve of wire gauge over which one to two layers of paper tissue was trimmed down to the edges of gauge. The soil suspension collected from the fine sieve was filtered through the above wire gauge placed in a Petri dish with sufficient water to wet the soil samples. The setup was allowed to remain for 48 hours without any disturbance. After 48 hours, the nematodes collected in the Petri plates were poured into a 50 ml beaker then collected in vials. These samples were subjected to fixation for identification and characterization of nematodes.

c. Killing and Fixing of Nematodes

Nematode suspension @ 250 ml was concentrated to a small quantity in vials. The vials were plunged in hot water at 60°C in a beaker for 2 minutes. The concentrated nematode suspension was poured back into a beaker and equal quantity of hot fixative was added to it. 4% formalin (formalin 4ml and distilled water 96ml) was used as hot fixative in killing the nematodes. The fixed samples called as wet collection are used for further studies.

d. Slide Preparation

Glass slides were washed with ethanol in order to make it free of micro-organisms. Few drops of fixatives used in the fixation of nematodes were put over the glass slide. The nematode meant for identification was taken out from the nematode suspension with the help of a pick and placed exactly over the drops of fixative. A cover slip was placed over it. Wax or nail polish coating was given over the cover slip to maintain it as a permanent slide for further characterization of nematodes.

Categorization of nematodes in to different trophic groups

The soil samples collected during the survey from the forests of Kallar, Burliyar, Katteri, Coonoor, Aravangadu, Kunjapanai and Kotagiriand, Ooty were processed and analyzed. The wet collections belonging to different samples were observed under compound microscopes and the species identified. The samples were found to be of a mixed population of tardigrades, mites and different groups of nematodes.

Different trophic groups of nematodes observed were

- Bacterial feeding nematodes
- Fungal feeding nematodes
- Predatory nematodes
- Herbivorous nematodes/ Plant parasitic nematodes
- Ento-pathogenic nematodes (EPN)

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Results and Discussion

Characterization of Bacterial Feeding Nematodes

***Rhabditis* sp.**

In these nematodes, the body tapers at extremities *viz.*, "mouth", or stoma, is a hollow tube for ingestion of bacteria. The lips are closed, not forming an open cup. The oesophageal collar is always present. The metarhabdions are with 5 or 3 tubercules in place of teeth, often anisoglottid or anisomorphic. Pharyngeal cavity is long cylindrical. Intestine is sparsely covered with fat-particles, the large containing cells of which can sometimes be recognized. Vulva median, ovaries paired, oppposed reflexed. The tail of females is of various shapes whereas for males tail is usually leptoderan. Spicules are separate. Movements active.

***Acrobeles* sp.**

Body length is between 0.3 and 1.1 mm. Cuticle is single, often ornamented with minute dots, annules simple or, rarely, divided into blocks by longitudinal striae. . Head is with two kinds of appendages. Cephalic prolobae is more or less triangular, also with fringes. Amphids are circular, well visible. Stoma consists of the usual five elements. Oesophageal procorpus is generally cylindrical, terminal bulb strong. Location of excretory pore varies between 1/4 and more than 3/4 of oesophagus length. Ovary is anterior with two flexures: postvulval uterine sac present. Males are generally with eight pairs of genital papillae. Tail in both sexes are conoid, acute.

***Diplogaster* sp.**

The labia are seldom well developed; however, a hexaradiate symmetry is distinct. The external circle of labial sensilla may appear setose but they are always short, never long and hairlike. The stoma may be slender and elongate or spacious or any gradation between these two. The stoma may be armed or unarmed; the armature may be movable teeth, fossores, or a pseudostylet. The corpus is always muscled and distinct from the postcorpus that is divisible into an isthmus and glandular posterior bulb. The metacorpus is almost always valved. The female reproductive system may have one or two ovaries and males may or may not have caudal alae; however, a gubernaculum is always present. The male tail most commonly has nine pairs of caudal papillae; three are preanal and six are caudal.

Characterization of Fungal Feeding Nematodes

***Aphelenchus* sp.**

Body tapers anteriorly. Cuticle is transversely striated. Head and lips are slightly offset. Spear shaft with slight thickenings at the base. Stylet is without basal knobs. Oesophageal glands overlap intestine dorsally. Vulva is posterior, ovary outstretched, prodelphic; a post vulval sac is also present.Female tail is short, bluntly rounded. Spicules paired slender, not fused, with minute rostrum. Gubernaculum is Vshaped. Postuterine sac is long. Male tail is with well-developed caudal alae supported by prominent ribs.

***Dorylaimoides* sp.**
It is classified as an omnivore of medium size body, Cuticle dorylaimoid, outer layer thin and with fine transverse striations; inner layer thicker. Odontostyle asymmetrical, ventral side shorter than the dorsal one and dorsally bent anteriorly. Odontophore usually arcuate or angular.Esophagus slender and weakly muscular in anterior region, expanding abruptly into a cylindrical basal bulb which occupies 1/4 to 1/3 of total esophagus length. Female genital system didelphic or mono opisthodelphic. Vulva a transverse slit. Tail similar in both sexes: long and filiform to short and rounded.

Characterization of Predatory Nematodes
***Mononchus* sp.**
Body tapers posteriorly. Head truncate; no papillae. Integument with longitudinal striae, Dorsal tooth in the anterior part of stoma, points forward. Pharyngeal cavity is oval, with a single hook projecting from upper surface. Oesophagus is about 1/4th of the total body length. Intestinal cells have distinctly tessellated arrangement. Oesophago-intestinal junction is non-tuberculate. Vulva is slightly posterior to middle of body. Lateral canals are broad, very indistinct, recognizable behind intestine. Gonads are paired, rarely single and prodelphic.

***Dorylaimus* sp.**
Longitudinal body ridges 38-42, very prominent, uniformly spaced by 4-5 um. Ventromedianvulval papillae well developed, generally two anterior and two posterior to level of vulva. Spermatheca long, 350x80 um, oval. Sperms spindle shaped, 10-15 um in length.

Characterization of Plant Parasitic Nematodes
***Helicotylenchus* sp.**
Length, female: 0.47-0.53 mm Body arcuate to c-shaped when relaxed; annules distinct. Lateral fields not areolated, with 4 incisures, about one-fourth of body width. Lip region hemispherical, slightly offset, with 3-5 (usually 4) annules and a prominent oral depression terminally; framework heavily sclerotized, with conspicuous outer margins extending posteriorly through 3 to 4 body annules, which are much narrower at that region than at others. Ovaries paired, symmetrical, but sometimes the posterior one is reduced. Vulva prominent, a depressed transverse slit. Intestine not overlapping rectum. Tail slightly tapering, with a hemispherical annulated terminus, usually with greater curvature dorsally than ventrally, devoid of any ventral projection or mucro, with 6-13 annules.Inner incisures of lateral fields usually not fusing for some distance on tail.

***Hemicroconemoides* sp.**
Body plump, straight or slightly ventrally curved, tapering on short distance at both ends. Number of annules 51-164. Cuticle with two detached layers, closely adpressed; annulation strong, not retrorse. Lateral field not marked. Vulva posterior. Vulval lips plain; vulval flaps occasionally present. Tail short, conoid to rounded. Labial framework heavily sclerotized. First anterior annules only weakly differentiated. No submedian lobes. Amphid apertures slit-like. Stylet strong; basal knobs directed forward, generally with a jointed anterior process; rarely rounded; never sloping backwards.

Tylenchorhynchus sp.
Body is vermiform, spiral to straight. Labial region is rounded or anteriorly flattened. Anterior lip annulus is generally not divided into sectors. Rarely faint or marked lip sectors are present. Phasmidsare small, near anus.Cephalids and caudalidare present Tail is more curved dorsally, sometimes rounded. Stylet and labial framework is average-sized. Median bulb rounded with average-sized valve. Glands overlap intestine dorsally and ventrally. Epiptygmais present, inconspicuous.

***Psilenchus* sp.**
The mouthpart is a needlelike stylet which is used to puncture cells during feeding. Caudal striae extend completely to the terminus. Deirids prominent, opposite nerve ring. Spear slender, devoid of basal knobs. Tails are elongate, varies from cylindrical to clavate. Ovaries paired, outstretched. An elongated spermatheca present in each uterus. Spicula curved, tapering, cephalated, resting on a thin, troughlike gubernaculum. Bursa crenate, rising near a point about opposite proximal ends of spicula and extending past anus a distance equal to about twice anal body diameter. Male phasmids located near posterior ends of bursa.

***Xipninema* sp.**
Adult stages of this nematode range in length from 1.3 to 2.2 mm. However, the *X. americanum* sensustricto ranges from 1.6 to 1.8 mm in length with an odontostyle and odontophore length slightly greater then 100 Âµm The tail of *X. index* adults has a distinct, finger-like, protuberance. Under the dissection microscope, the protruberance provides a convenient method for separating *X. index* from co-occurring *Xiphinema* species. Length of adults of this species range from 2.9 to 3.3 mm. Males are extremely rare.

***Longidorus* sp.**
Body is slender, frequently a slight protrusion at vulva. Lips are flattened, set off by a knob-like expansion. Wide bilobedamphidial apertures are obscure, just behind lateral lips. Oesophagus with anterior part is often convoluted. Rectum is shorter than the anal body diameter. Tail is conoid, but sometimes with short broad peg in adults. Ovaries are didelphic, reflexed. Vagina is reaching about half-way across body. Vulva slit is slightly oblique and posterior.

Characterization of Entomopathogenic Nematodes
***Steinernema* sp.**
Cuticle smooth, head slightly rounded. Six distinct lips united, each 1 papilla. Four cephalic papillae. Amphids crescent-shaped, narrow. Stoma partially collapsed; cheilorhabdions represented by a thick ring of sclerotized material just beneath the lips. Below this there isanother sclerotized ring that represents the prorhabdions. Other part of stoma forming anasymmetrical funnel with thick anterior end. Esophagus muscular with a cylindrical procorpus followed by a slightly swollen non-valvatedmetacorpus, isthmus, and basal bulb with a valve. The nerve ring is surrounding the isthmus just anterior to tile basal bulb. Excretory pore opening usually anterior to nerve ring. Lateral fields and phasmids inconspicuous. Gonads amphidelphic, reflexed. Vulva a transverse slit from slightly to very protruding from the body surface, with or without a thick flap. The vagina is short leading into paired uteri. Eggs deposited initially, but they later hatch inside the females and the juveniles bore their way out. First generation females larger than those of the second

generation. Tail with a prominent postanal swelling, terminating with a rounded projection in the first generation females and sometimes a fine mucro in second generation females of certain isolates.

Tardigrades

Tardigrades are commonly called as water bears about 0.5 mm (0.02 in) longmicro organism which are short and plump, with four pairs of legs, each ending in claw. They are prevalent in mosses and lichens and feed on plant cells, algae and small invertebrates. They live in all environments feed on species such as nematodes, tardigrades, bacteria, algae and mites and play important role in the soil ecosystem.

Mites

Soil mites commonly found in forest areas often assist in the breakdown of organic matter. They feed on fungi and nematodes and are extremely important in maintaining soil health and fertility. Mites are among the most important predators in the soil ecosystem. Several Oribatids have been reported to feed on parasitic nematodes and play a role in managing their population

Table 1: Different trophic groups of nematodes observed in the forest soil ecosystem

S. No.	Trophic Level of Nematode	Genus of Nematodes Identified
1.	Bacterial feeding nematodes	*Rhabditis* sp. *Acrobeles* sp. *Diplogaster* sp.
2.	Plant parasitic nematodes/ Herbivorous nematodes	*Helicotylenchus* sp. *Tylenchorynchus* sp. *Hemicroconemoides* sp. *Psilenchus* sp. *Xiphinema* sp.
3.	Fungal feeding nematodes	*Aphelenchus* sp. *Dorylaimoides* sp.
4.	Predatory nematodes	*Mononchus* sp. *Dorylaimus* sp.
5.	Entomopathogenic nematodes	*Steinernema* sp.

Table 2: Nematodes observed in the forest soil samples collected under various tree species

S. No.	Location	Tree Species	Nematodes Identified
1.	Kallaru	*Garcinia mangostana* *Myristica fragrans*	*Acrobelus* sp. *Helicotylenchus* sp. *Psilenchus* sp. *Rhabditis* sp. *Acrobeloides* sp. *Steinernema* sp. *Diplogaster* sp.
2.	Kotagiri	*Jacaranda mimosifolia* *Eucalyptus* sp. *Delonix regia* *Tectonagrandis*	*Rhabditis* sp. *Mononchus* sp. *Aphelenchus* sp. *Acrobelus* sp. *Diplogaster* sp. *Helicotylenchus* sp. *Hemicriconemoides* sp.
3.	Ooty	*Grevillearobusta* *Jacaranda mimosifolia* *Eucalyptus spp.*	*Chromadoria* sp. *Aphelenchus* sp. *Longidorus* sp. *Filenchus* sp.
4.	Burliyar	*Pongamia pinnata* *Jacaranda mimosifolia* *Myristica fragrans* *Garcinia mangostana*	*Rhabditis* sp. *Psilenchus* sp. *Acrobeloides* sp. *Mononchus* sp.
5	Coonoor	*Pongamia pinnata* *Jacaranda mimosifolia*	*Mononchus* sp. *Aphelenchus* sp. *Dorylaimoides* sp. *Helicotylenchus* sp. *Tylenchorhynchus* sp.

Rhabditis sp.

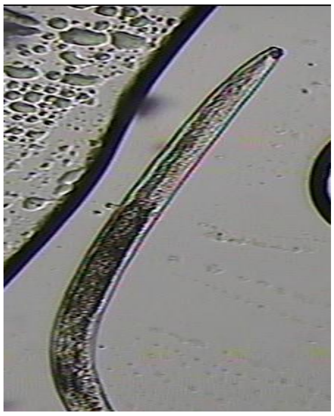
Fig 1: Bacterial Feeding Nematodes

Aphelenchus sp.

Fig 2: Fungal Feeding Nematodes

Helicotylenchus sp.

Pratylenchus sp.



Tylenchorynchus sp.

Hemicroconemoides sp.

Fig 3: Plant Parasitic Nematodes

Entomopathogenic Nematode

Steinernema sp.

Predatory Nematode

Mononchus sp.

Tardigrade Mite

Fig 4: Other micro arthropods identified

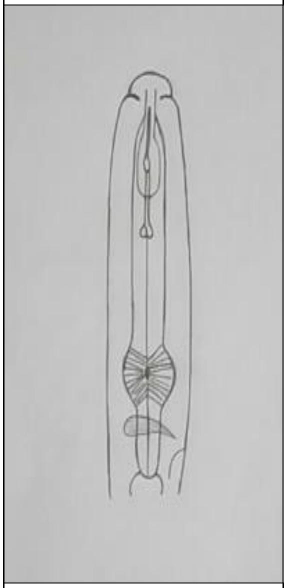
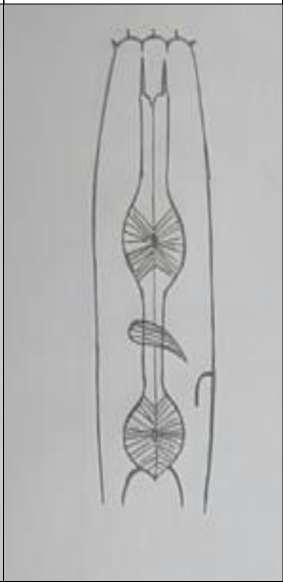
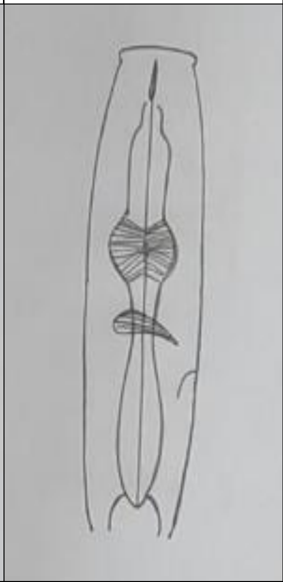
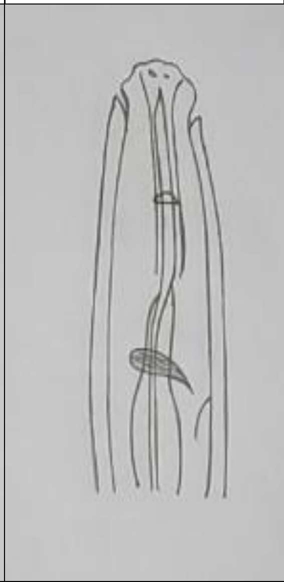
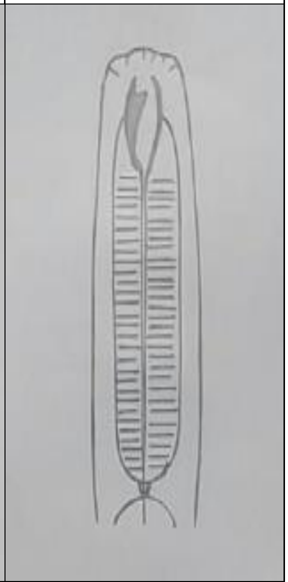
Plant Parasitic Nematode	Bacterial Feeding Nematode	Fungal Feeding Nematode	Predatory Nematode	
				
Tylenchorynchus sp.	*Rhabditis* sp.	*Aphelenchus* sp.	*Dorylaimus* sp.	*Mononchus* sp.

Fig 5: Categorization of Nematodes based on their feeding habit

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