SOIL NEMATODE COMMUNITY STRUCTURE ALONG ELEVATION GRADIENT IN BHAGIRATHI BASIN, UTTARAKHAND: A MORPHOLOGICAL AND MOLECULAR APPROACH

A Thesis Submitted by

PRIYANKA KASHYAP

For award of the Degree of

DOCTOR OF PHILOSOPHY
IN
WILDLIFE SCIENCES

Under the Guidance of

DR.V.P. UNIYAL

WILDLIFE INSTITUTE OF INDIA
Dehradun, Uttarakhand, India

SAURASHTRA UNIVERSITY
Rajkot, Gujarat, India

JANUARY, 2021

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Dated: /01/2021

Certificate

Certified that the work incorporated in the thesis "**Soil Nematode Community Structure Along Elevation Gradient in Bhagirathi Basin, Uttarakhand: A Morphological And Molecular Approach**" submitted by **Ms. PRIYANKA KASHYAP** was carried out by the candidate under my supervision and guidance.

To the best of my knowledge: (i) the candidate has not submitted the same research work to any other institution for any degree/diploma, associateship, Fellowship or other similar title (ii) the thesis submitted is a record of original research work done by the student during the period of study under my supervision, and (iii) the thesis represents independent work on the part of the student.

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CERTIFICATE FOR PRE Ph.D. PRESENTATION

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I certify that the Research work was appreciated by all who remain present and there was no comments made for this research work.

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Date:

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Summary

Gangotri National Park (GNP) is a protected area and has fewer human disturbances, allowing soil nematodes to diversify. However, it has harsh climatic conditions at high altitudes, which may hinder the nematode community structure. Systematic study of the soil inhabiting nematodes associated with forest vegetation of Gangotri National Park is discussed in this thesis. Since sizeable number of soil inhabiting nematodes species were collected from two valleys of GNP, it is not possible to explain all the recorded species. Therefore, a comprehensive taxonomic study of nematodes belonging to two families of order Rhabditida and plectida each namely, the Cephalobidae and Plectidae has been done for this thesis. A compiled inventory of the soil nematode genera recorded from GNP during this study has also been provided in this thesis. A total of 62 nematode genera and fifteen species were identified from high altitude region of GNP. Of which, three species are new records from India viz. ***viz.*** **Anaplectus granulosus** *(Bastian, 1865) de Coninck and Schuurmans Stekhoven, 1933***,** **Stegelletina devimucronata** *(Sumenkova, 1964) Bostrom and De Ley, 1996,* **Ceratoplectus armatus** *(Butschli, 1873) Andrassy, 1984, three new records from Uttarakhand* ***(Plectus parvus*** *Bastian, 1865,* **Plectus parietinus** Bastian, 1865 ***and Plectus minimus*** *Cobb, 1893) and all 15 species are new records from Gangotri National Parks. Estimation of overall evolutionary divergence over sequence Pairs in COI gene and 18S r RNA gene for the barcode region were calculated for individuals collected and cultured. In cases of discrepancy between morphological taxonomy and molecular based sequence, the specimens were morphologically examined and identified to ascertain their identity. Fifty-five genera were recorded from subalpine Deodar (SAD), 52 genera from sub-alpine blue pine (SAP) vegetation, and 44, 42, and 31 genera were recorded from* *subalpine Himalayan Birch (SAB), alpine scrub Artemisia (ASA), and dry alpine scrub Caragana (ASC) respectively. Among vegetations, 36 genera significantly differed among various vegetation types. The mean abundance of soil nematodes varied among vegetation types. Nematode diversity and nematode generic richness were relatively higher at a low elevation region of subalpine Deodar forest and lowest at high elevational alpine dry scrub Caragana. Trophic grouping of nematodes included 26 genera of bacterivores, 6 fungivores, 13 plants parasitic, 10 omnivores, and 7 were predators. The abundance of bacterivores- Acrobeloides, Cephalobus, Cervidellus, Chiloplacus, Stegelletina, Cylindrolaimus, Wilsonema, Panagrolaimus were significantly different among subalpine and alpine scrub vegetation types. The maturity indices of soil nematode communities in ecosystem formed by different vegetation were estimated along the elevational gradient. The present study showed that highest stability among the vegetations was found in lower elevation Deodar dominated forest and lowest in high elevation Caragana dominated scrubland. The El values significantly different among vegetation types. The structural index is an excellent means to understand whether the system is mature or structured where high value represents structured and low value as a disturbed ecosystem. In the present study, the values of SI were statistically significant among the vegetation type. Pairwise multilevel comparisons of nematode community composition between various vegetation types showed a significant difference between the subalpine and alpine regions. The result showed that nematode composition in Caragana scrubland is close to ASA, showing similar nematode composition. It may be concluded that the vegetation type is a significant factor for soil nematode diversity as every vegetation type has a different local environment and physicochemical properties that affect the soil biota, specifically*

soil nematodes being present at various trophic levels in the soil food web. Among the various vegetation types in Gangotri National Park's high-altitude areas, there are substantial differences in the taxonomic composition, the energy flow channels, and nematode community structures, which provide actual examples for understanding the role of soil nematodes in key soil ecological processes in the region. We found that the soils from subalpine and alpine vegetations differed significantly in physicochemical characteristics and their soil inhabiting nematode communities. Nematode communities also changed with elevation, distinctly in the nutrient-deficient alpine dry scrubs. The study also showed that soil abiotic parameters correlated with the nematode composition.

Experimental study using Open Top Chamber were also conducted to understand the effect of temperature on soil nematodes where soil and the air temperature was ~1 °C higher inside OTC in comparison with the control plot outside for almost throughout the year in 2017 except in August- September in Bhojwasa. Bacterial feeder abundance is higher in OTC but was not significant. Acrobeloides and Rhabdolaimus are the only bacterial feeder whose density significantly increased by the elevated condition of OTC. Predatory nematode abundance, Nematode channel ratio and plant parasitic nematode Index was slightly lower in OTC compared to control plot. Maturity Index was lower in Open Top chamber and significantly different. Dissimilarity tests based on the Bray-Curtis distance showed that nematode communities from the OTC were significantly different from the control plots when assessed at the generic level. But has no inter-annular difference on community composition. The temperature difference was nearly 1.9 °C higher inside OTC from May 2018 - May 2019, and the soil moisture was different among the treatment and control plot, suggesting that warming has indirect effect on nematode diversity by *affecting soil moisture in treatment plot. As a result, it can be hypothesized that warming-induced soil moisture could be the the major reason for differences in nematode responses between OTC and Control. As our study is a pioneer study in the region evaluating the effect of vegetation type on soil nematode diversity therefore, more similar studies are required to comprehend the relationship between nematode diversity and soil ecosystems of high altitude vegetations.*

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Chapter 1

Introduction

1.1 Soil Nematode: Diversity and Importance

Nematodes constitute almost 90% of all multicellular animals in number (Hugot et al., 2001) with a biomass of 2.0 ton per ha in soil (Killham, 1994). Soil nematodes inhabit sites ranging from pristine to extremely polluted site. So far approx 25,000 species have been known, and there could be 100 million nematode species still to be discovered (Blaxter, 2003; Lambshead, 1993).

Soil nematodes are responsible for supporting ecosystem services as they play an important role in three crucial ecological processes i.e., soil food web, maintenance of microbial biomass, and mineralization and help disperse both the organic matter and the decomposers in the soil. Bacterial and fungal-feeding nematode liberates substantial amount of nitrogen when feeds on their prey groups, thus responsible for much of the plant available nitrogen in the majority of soils (Ingham et al. 1985). Nematodes are recognized as a major consumer group in soils, generally grouped into various trophic groups based on their feeding habits i.e bacterivores, fungivores, predators, omnivores and plant parasites (Yeates et al. 1993).

Nematodes were categorized among colonizer-persister (c-p) values of 1-5. Nematodes with c-p value 1 are short lived, have high fecundity, graze on enriched medium where as nematodes with c-p value 5 are predominantly predators and, omnivores having longer life span, low fecundity, vulnerable to disturbance (Bongers, 1990). The c-p value categorization forms the basis for the maturity index which provides the status of soil health condition (Bongers, 1990).

Nematodes have been considered as bioindicators due to various traits. They are considered as the most numerous and diverse integrant of the soil micro fauna and provides high intrinsic information regarding soil health status (Yeates, 2003; Bongers and Ferris, 1999). Despite being contributing less to a soil biomass, their inhabitance across various trophic levels in the soil environment is highly important (Barker and Koenning, 1998). They contribute directly to the bioreactions in soils by regulating important processes i.e nutrient cycling and decomposition. Indirectly, they also manages nutrients availability by regulating the microbial biomass, disseminating microbes and and excreting reserve nitrogen as ammonium in soil. Nematode abundance, their genetic diversity and being present at various sites led to the nematode community assessment suitable in making predictions about soil environment conditions (past and present status) and act as bio-indicators for soil health as well as soil disturbance.

Since the beginning of nematode taxonomic studies, focus was more prominent on plant parasites owing to their commercial importance whereas the free-living nematodes were ignored and received comparatively less attention as they were not directly related with economic loss. Recent studies showed their importance as bioindicators in soil ecology where they constitute an integral part of food chain. Different nematode species respond differently to degradation of environmental quality degradation and have various features to indicate alteration in the systems (Freckman, 1988; Gupta and Yeates, 1997).

1.2 Monitoring Soil Nematode Diversity and Distribution

Life strategy of nematodes reflects the quality of soil which ultimately indicates the soil condition. Nematodes vary in sensitivity to pollutants and environmental disturbance and provide a strong basis for analysis of faunal diversity in soil for environmental assessment systems (Bongers and Ferris, 1999) and will be very useful in impact assessment of climate change in the IHR.

Soil nematodes represent an important component of soil faunal community in the cold ecosystem (Ruess et al., 1999; Hoschitz and Kaufmann, 2004; Tong et al., 2010). Soil inhabiting nematode communities and its structural changes were considered as a potential instrument for assessing aboveground plant conditions, soil health status and biomonitoring system (Bongers, 1990; Wang et al., 2009; Pen-Mouratov et al., 2010) as they respond rapidly to disturbance and enrichment (Korthals et al. 1996; Tenuta and Ferris, 2004). In soil, nematodes have direct proximity with their microenvironment and occupy prime positions in soil food web (Neher, 2001). Numerous studies have suggested that nematode community structure and diversity act as ecological indicators, to understand the effects of disturbances on soil microbial community in forest ecosystem (Butenko et al., 2017; Kitagami et al., 2017; Salamun et al., 2017). Various indices can depict the environmental disturbance using nematode genera, life strategy and trophic structure (Bongers, 1990; Freckman and Ettema, 1993). Combined effect of soil nematode population and various ecological factors offers benefits for the development of soil quality index (Doran and Parkin, 1994).

Soil inhabiting nematodes plays a major role in some crucial soil ecological processes (Yeates, 1987) and have a substantial impact on ecosystem functioning (Yeates, 2003; Bakonyi et al., 2007). Therefore nematode community structure can be used to reflect differences in soil food web dynamics in different vegetation types. The abundance of various nematode trophic groups in soil system transfigured into ecological indices and parameters can be used to assess soil health status, disturbance level and decomposition pathway (Gomes et al., 2003) and to compare different vegetation types (Winslow, 1960; Yeates, 1970). Subsequent studies were conducted in various ecological systems, ranging from natural forests to agriculture systems on the changes of nematode community structure (Hyvonen and Persson, 1990; Coleman et al., 1991; Freckman and Ettema, 1993; Neher and Campbell, 1994; Yeates, 2007; Zhang et al., 2012; Kitagami et al., 2017).

Rise in soil temperature due to global warming may increase nutrient availability and cause indirect changes by increasing nutrient mineralization of soil organic matter and altering soil microbial biomass consequently changes the vegetation cover and plant species composition (Graglia et al., 1997), which will alter litter quality and the net primary productivity ultimately affecting the soil microflora and fauna. High altitude soil fauna composition is dominated by soil-inhabiting nematodes and microarthropods (Sohlenius et al., 1997; Heal, 1997). Interestingly nematode community has been suggested as a potential instrument for assessing soil conditions and biomonitoring system (Bongers et al., 1989) as they respond rapidly to disturbance and enrichment (Korthals et al., 1996; Tenuta and Ferris, 2004) increasing microbial activity leads to change in microbial feeders in a community. In soil, nematodes have direct contact with their microenvironment indicating the community structure and occupy key positions in soil food webs (Neher, 2001). Various indices can depict the environmental disturbance using nematode genera, life strategy, or trophic structure (Bongers, 1990; Freckman and Ettema, 1993). In the cold ecosystem like in our study area, there is a lack of earthworms which are known as ‘ecosystem engineers’, and nematode forms the dominating underground taxa and acts as a valuable tool for assessment of climate change impact in the soil ecosystem.

Studying soil biodiversity and distribution patterns of species along elevation gradients in the high altitudes and understanding diversity, behind these patterns is central to ecology and conservation biology in the Indian Himalayan Regions (IHR).

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Chapter 2

Review of Literature

Nematodes are one of the most ancient and diverse group of animals on this planet (Wang et al., 1999). Our knowledge of animal nematodes is very old, but work on plants and soil nematode started very late. The first free-living soil nematodes (*Turbatrix aceti*) was recorded by Borellus (1656). Early work done mostly on plant-parasites (Berkeley, 1855; Scopoli, 1977) due to their economical importance. Most of the studies concentrated on plant-parasitic nematodes associated with cereals and other crops (Steinbuch, 1799; Berkeley, 1855; Schacht, 1859; Schmidt, 1871; Scopoli, 1977). Wheat gall nematode *Anguina tritici* was the first nematode discovered by Needham (1743). The relationship of plant parasitic nematodes and free living nematodes was first recognized by Dujardin (1845). Monograph by Bastian (1865) was considered to be as beginning of nematology (Thorne, 1961). He made outstanding contributions by mentioning 100 species of nematodes in a single paper. Schneider (1866) elucidated histological and anatomical account of soil inhabiting nematodes. The first detailed description of free-living nematode genera and species by differentiating parameters were given by Butschli (1873, 1876). Orley (1880, 1885) worked on the genus *Rhabditis* and published a monograph on free-living nematodes. Encyclopedia of nematodes was given by Maupas (1899) and later he also described new species under genus *Rhabditis* (Maupas 1915, 1916). Main turning point in the history of nematodes came when Cobb (1906) published a series of excellent papers on nematode taxonomy comprising detailed morphological studies and new terminologies. Filipjev (1918, 1921) categorized five families on the basis of structure of the amphids, cuticle, female gonads, pharynx, and tail. Major work on morphology of nematode has been recorded in the form of book by Chitwood (1950); Goodey (1951, further revised in 1963) De Coninck (1965) and monographs by Thorne (1936-1975) in 90s.

During this time, various studies were carried out on nematodes around the world. Some important mentions are Steiner (1914) in Switzerland, Rahm (1937) worked on nematodes in North China, Allgen (1935, 1952) documented the freshwater and free-living nematodes from Mt. Kenya and Niederkongo, Ordens whereas De Coninck and Stekhoven (1933) documented nematodes from Belgian coast. Altherr (1950, 1952, 1953, 1963 and 1976) recorded the soil inhabiting nematodes from Swiss National Park, French Jura, Vaudois, Lorraine and Austrian Alps. Kirjanova (1951) reported the soil inhabiting nematodes from Hungry Steppe of Uzbekistan.

A breakthrough in the ecology of free-living soil nematodes was the seven-year study carried out in Denmark by Nielsen (1949); he worked on nematode faunae of different soils, their physiological ecology and reckoning to ecosystem services. Brzeski (1962) studied on the nematodes of mosses in Bialowieza forest (located on the border between Belarus and Poland). Inglis (1983) provided an outline classification of the Nematoda phylum and Coomans (1962, 1971, and 2002) revised the systematic and morphology of nematodes and its status under current scenario and in its near future. A phylogenetic system for nematodes was drafted by Lorenzen (1981). Later, Jacob (1984) drafted a classification scheme consisting of free-living, animal and plant parasites of continental and marine origin. Centres of ecological study on nematodes were developed in Sweden, Poland, Italy, Germany and Russia, New Zealand (Prejs, 1970; Wasilewska, 1970; Sohlenius, 1973; Tsalolikhin, 1976; Yeates, 1978; Zullini, 1976; Sudhaus, 1981). In the US, surge on soil nematology occurred in 80's (Norton, 1978; Yeates and Coleman, 1982; Stinner and Crossley, 1982). The studies of Ingham et al. (1985) encouraged in the positive aspect of free-living soil nematodes in nutrient cycling and agricultural productivity.

Istvan Andrássy (1952-2012) significantly contributed to taxonomy of nematodes and published and compiled innumerable extremely useful data on nematode systematic and classification. He also wrote book entitled "Free-living nematodes of Hungary" with three volumes (2005, 2007 and 2009). Holovachov (2014, 2015) and coworkers (2012, 2014i, 2014ii) contributed a lot in systematic and morphology of order plectida and published number of articles on nematodes from Sweden. Heyns (2002) documented a checklist of free-living nematodes of water habitats from South Africa. Gagarin (1993, 2001) studied free living freshwater nematodes of Russia and its other adjacent countries under various order namely Areaolaimida, Chromadorida, Enoplida Mononchida, and Monhysterida. Loof (1999, 2001) also worked on the freshwater nematodes of Central Europe and published two taxonomic based books in the series "Freshwater fauna from Central Europe" (Susswasserfauna von Mitteleuropa). Holovachov (2001, 2004, 2005, 2006, 2014) provided information on the systematic and development of order Plectida. Holovachov and Bostrom (2006) proposed some new genera (Deleyia and Panagrolobus) under subfamily Cephalobinae.

Some other major significant contributions in nematology field with their useful publications are Fuchs (1915-38), Rahm (1928-1937), Allgén (1933-52), Hirschmann (1951-1956), Kirjanova (1951-1958), Dougherty (1953, 1955), Wieser (1953, 1954), Timm (1956-1971), Golden (1956-1986), Brzeski (1960-1989), Clark (1961-1963), Inglis (1961-1983), Sher (1961-1970), Coomans and co-workers (1962-1988), Heyns

(1962-1980), Taylor (1964-1966), Yeates (1967-1970), Lamberti (1975), Eroshenko (2002), Abolafia and Pena-Santiago (2001, 2007), Holovachov et al. (2003), Tabassum and Shahina (2002, 2008) and Weimin et al. (2010).etc.

In India history of nematode research is still too young and data deficient in many aspects and it is not possible to compare the present data with the past when it comes to Indian Himalayan Region. In 1855, the first vertebrate nematode was recorded and the first plant nematode by Barber (1901). A species of Criconema was reported around the roots of mango tree in Bangalore by Cobb (1913). Goodey (1951) documented two new species from India. Other major taxonomic work conducted in past by Krishnan (1933), Ayyar (1934), Dastur (1936), Luthra and Vasudeva (1939), Khera (1951), Sanwal (1951, 1957, 1960, 1971) is mainly on control, pathogenicity and disease complexes.

The first centre in India to start research on taxonomy of soil and plant nematodes was the Aligarh Muslim University where M.R. Siddiqi initiated his work on nematodes under the guidance of Professor M.A. Basir in 1955. Siddiqi (1959) published his first paper on nematodes. His contribution made a landmark in the field of nematology in India. During the same period, Das (1960) published interesting articles on the nematode of Andhra Pradesh.

The young team of nematologists (Siddiqi, Jairajpuri, S.H. Khan, E. Khan, and others) resulted in around 100 publications on the taxonomy of soil and plant nematodes comprising of >150 new species and their description from 1961 to 1965 in international repute journals. Siddiqi (1959-1990) and Jairajpuri (1961-1990) documented more than 300 new taxa and always named among a few foremost taxonomists of nematodes in the world.

At present more than 200 nematologists are involved in nematode research at various institutes in India, namely department of Zoology and Botany at Aligarh Muslim University Aligarh; Indian Agriculture research Institute (IARI), New Delhi; regional station of IARI at Hissar, Ludhiana, Solan; Central Potato Research Institute (CPRI), Shimla mainly contributing on potato cyst nematodes; Central Plantation Crops Research Institute, Krishnapuram and Kasargod; National Bureau of Plant Genetic Resources, New Delhi; Zoological Survey of India, Calcutta. Aligarh Muslim University (AMU), Zoological Survey of India (ZSI), Indian Agricultural Research Institute (IARI), Punjab Agriculture University (PAU), Ludhiana; and Haryana Agricuture University (HAU), Hissar are some major contributors in field of nematology in our country. Khera (1968-1974) has made a significant contribution on freshwater nematode taxonomy.

Since 1990s, many workers have contributed in field of nematology. Jairajpuri with coworkers (1992-2002); Ahmad, W and coworkers (1992-2013); Ahmad, I and coworkers (1992-2013); Tahseen and coworkers (1992-2013), Khan and coworkers (2002-2012) from Agriculture Muslim University are among some renowned nematologist whose contribution has enhanced the knowledge of nematodes and formed the base for present studies.

We have extracted 209 entries of total publication on work done on soil nematodes in Indian Himalayan Region (Kashyap et al., 2016). Out of which 12.56% work is conducted in Uttarakhand. Fauna of Uttarakhand documented 196 species under 93 genera and 37 families of which 17 species of nematode for the first time from the state and two from India (Rizvi, 2010). Major work has been done in Doon valley and Garhwal region in crop fields, sal and teak forest (Khera and Chaturvedi, 1967;

Jauhari and Lal, 2001; Jauhari and Lal, 2001; Rizvi, 2010; Sharma, 2011). Sharma (2014-15) studied the nematode fauna of Rajaji National Park and Govind Wildlife Sanctuary. Plant and Soil Nematodes have been published in Fauna of Uttarakhand, State Fauna Series by Zoological Survey of India (Rizvi, 2010). Records of Rhabditida in Doon valley was documented by Rizvi (2008-2010, 2013). Effect of soil moisture on population of nematode was studied by Jauhari and Lal (2001) on tea plantation in Doon valley. Soil nematodes in high altitude regions of IHR studied by various scientist namely Baqri and Bohra, 2005 who studied nematodes of western himalayas; Number of new species under Genus *Enchodelus* was recorded by Baqri and Jairajpuri, 1974, 1975; Description of two new species of Myctolaimus Cobb, 1920 and two new species of the Genus *Criconemoides* Taylor, 1936 was given by Hussain et al., 2004; Jairajpuri, 1963 respectively.

For years, morphological identification was used as only tool for identification of nematodes. As our information on nematodes of economical importance increased, it became evident that morphological identification alone cannot uncover the complete scenario of pathological aspects and differences among nematode populations morphologically delimited species. With the advancement of technology, Molecular approch used with the ability of identifying nematodes at the species level. The utility of molecular diagnostics for nematodes first studied by Curran et al., 1985. Techiques became useful for classification, identification, diagnostics, and phylogenetic relationships among taxa (Subbotin and Moens, 2006). Various techniques comprises of polymerase chain reaction, multiplex PCR, real-time PCR (Madani et al., 2005., McCuiston et al., 2007, Skantar et al., 2007), protein electrophoresis, denaturing gradient gel electrophoresis (Esbenshade and

Triantaphhllou, 1985, Ercolini, 2004, Bhadury et al., 2006; Okada and Oba, 2008), Restriction fragment length polymorphism (Curran et al., 1986), Random amplified polymorphic DNA (Caswell-Chen et al., 1992), Amplified fragment length polymorphism (Folkertsma et al., 1996), nematode DNA sequencing, and barcoding (Bac et al., 2008, Floyd et al.. 2002). Molecular methods provide an efficient alternative to classical morphological identification for nematode species assessment. High throughput sequencing technology used to generate large amounts of sequence data at low cost and very short time span. One of most important advantage of these molecular teechniques is the capability to identify huge numbers of species among complex communities (Opik et al., 2008). Vanderknaap et al. (1993) used an arbitrarily primed PCR technique used to distinguish closely related bacterivors species that could not be differentiated morphologically and showed that the molecular method can be used in ecological aspects. The first phylum Nematoda molecular phylogenetic framework was produced by Blaxter et al. (1998). Initially they recognized 3 major nematode clades. De Ley and Blaxter (2002, 2004) further used molecular data to update the Nematoda phylum classification. A MOTU method developed using a molecular barcode, PCR and sequencing of the small subunit ribosomal RNA gene for nematodes (Floyd et al., 2002). A simple PCR protocol for detection of the agricultural importance root-knot nematode species, Meloidogyne javanica and Meloidogyne arenaria isolated from soil was developed by Qiu et al. (2006).

Hamilton et al. (2009) used PCR with species specific primers and sequencing of isolated nematodesdirectly from soil for their characterization which provided enough taxonomic data to explain the community structure of the region though the nematodes were only separated into Enoplea and Chromadorea. Other major contributors on molecular studies of nematodes are Powers et al. (2009), Foucher and Wilson (2002), Waite et al. (2003), Donn et al. (2008), Griffiths et al. (2006), Bhadury et al. (2008), Hamilton et al. (2009). Shah et al. (2013) studied the molecular comparison of some nematode species using RAPD. The detection of strawberry latent ring spot virus and Arabis mosaic virus in vector nematodes by Kulshrestha et al. (2005). Genetic studies is lacking behind as very few has been documented from India Himalayan Region. Recently Abebe et al. (2011) gave a review and compared the various techniques used in the nematodes taxonomy in the context of trends and recent developments including their use in nematode taxonomy, and biodiversity. Still these molecular techniques have some limitations and their consistency and reliability is suspected. Therefore, morphological characterization is still remaining crucial for soil nematodes identification.

In recent years, taxonomic studies have become very important because correct identification is required for all applied and experimental work. As mentioned in past, most of the work was focused on plant parasite leaving behind the other taxa of nematode such as bacterivores, fungivores which play an important role in some major ecological processes and reacts to abiotic and biotic factor of environment. Soil inhabiting nematode community structure responds to changes in soil temperature and moisture within few weeks. Rhabditida and Tylenchida are sensitive to cold (Sohlenius and Bostrom, 1999). During the small scale experiment, Papatheodorou et al. (2004) reported that the *Cephalobus* and *Acrobeles* density increased in warm plots and density of *Chiloplacus* increased in cold plots.

There is important point to consider when soil temperature and moisture effects on nematode communities are examined, that microclimatic conditions are important in evaluating nematode community structure (Hoschitz and Kaufmann, 2004) in natural ecosystems. Therefore, inspecting different microhabitats is necessary to understand temperature and moisture effects on the entire ecosystem and the water films thickness on soil mass surface determines different aspects of nematode biology, such as nematode activity, their feeding and population growth (Yeates et al., 2003; Strong, 2003). It is widely known that nematode community structure is sensitive to environmental disturbances (Bongers and Bongers, 1998). Ecological research concerned with the study of few nematode species in relation to functional groups of other organisms such as viruses, arbuscular mycorrhizal fungi, plant pathogensand to other groups of nematodes were studied by various scientists. (Eisenback and Griffin, 1987; Lamberti and Roca, 1987; Smith, 1987; Sikora and Carter, 1987). In recent years, studies on soil nematode in correlation with soil parameters and plant diversity were studied by Hanel (2000), Sohlenius and Bostrom (2001), Matlack (2001), Renco et al. (2012), Kostenko et al. (2015) and Cortois et al. (2017). Major studies were performed on nematode community structure in high altitude forest in Beskydy Mountains spruce forests, deciduous forests of Vihorlat, Slovakia and Changbai Mountain, China (Yuen, 1996; Wasilewska, 1997; Yeates et al., 2000; Mladenov et al., 2004; Hanel and Cerevkova, 2010). Some studies are available on soil-inhabiting nematode diversity and abundance in forest ecosystems (Yeates, 2007; Baniyamuddin et al., 2007; Hanel, 2008; Rizvi, 2008; Tomar and Ahmad, 2009; Tong et al., 2010; Butenko et al., 2017; Kitagami et al., 2017). Few studies on community structure in alpine regions were done by Kergunteuil et al. (2016), Ya Shen et al. (2019) and along elevational zonation by Dong et al. (2017). Recently, several studies have considered altitudinal gradients as propitious aspect to evaluate evolutionary hypotheses for species niche breadth to changing environment (Korner, 2007; Rasmann and Pellissier, 2015; Alexander et al., 2015).The molecular technique has been applied recently for evaluating nematode community structures. Major examples are Derycke et al. (2010), Dong et al. (2017), Kitagami et al. (2017), Treonis et al. (2018). Soil inhabiting nematode community analyses conducted for biological assessments for soil environments in north India by various nematologists (Baniyamuddin et al., 2007; Rizvi, 2008; Kumar and Ahmad, 2017). However, research in the study area across different vegetation along elevation gradient is still lacking.

2.1 Taxonomic Literature on Rhabditida and Plectida

Order Rhabditida (emphasis on family Cephalobidae)

Individuals belonging to order Rhabditida inhabits various habitats i.e. terrestrial, freshwater and even in marine habitats. They are largely bacterivores and very few are predatory in nature (Diplogasterids) while a minor section represents parasitic associations (*Heterorhabditis* and *Steinernema*).Order Rhabditida was formed by Chitwood (1933) for bacterial feeder rhabditids. Dujardin in 1845 formed the genus *Rhabditis* (*R. terricola* as type species). Later in 1865, four new species of the genus *Rhabditis* was explained by Bastain and morphology of the genus was described by Butschli in "'Beitrage Zur Kenntniss der freilebenden Nematoden" (1873). Systematics and Phylogeny of *rhabditis* was studied by Osche (1952). De Man (1876, 80, 84) recognized 37 species of *rhabditis* was recognized by De Man (1876,1880,1884) and later under family Rhabditidae, 7 new species were described by Micoletzky (1922). Sudhaus made a remarkable contribution in biology, ecology, phylogeny and systematic of Rhabditidae and published a series of articles along the years (1974-2011). Other scientists who also made contribution to the taxonomy of *rhabditida* are Anderson (1979, 1983), Fuchs (1931), Timm (1957-1961i, 1961ii), Farkas (1973), Belogurov (1977), Zeiden and Geraert (1989), J Gerber and Giblin-Davis (1990) Smart and Nguyen (1994), Heyns (1995), Gagarin (2000), Eroshenko (2002), Abolafia and Pena-Santiago (2001, 2007), Holovachov et al. (2003), Tabassum and Shahina (2002, 2008) and Ye,W et al. (2010).

Meagre information on Rhabditida is available from India because of the infrequent studies done on this category. Some new genera and new species of Rhabditida were reported by Khera (1968, 1969, and 1971) who is also considered among Indian pioneers in nematology. Tahseen and Jairajpuri (1988) reported a new species *Teratorhabditis andrassyi*. Later in 2004, Tahseen et al. proposed and described another new genus *Metarhabditis*. New species were added to genera *Oscheius* (Tahseen and Nisa, 2006) and *Poikilolaimus* (Tahseen et al., 2009). In 2009, they also explained the detailed systematics and biogeography of the *Distrolabrellus* (a monotype genus) and described a new species of *Cruznema*. New genus *Sclerorhabditis* was proposed by Ahmad et al. (2007).

Cephalobids constitute mainly the bacteriovors; they are capable of surviving extreme environment. It consists of single superfamily Cephaloboidea Filipjev, 1934. Back in 1656, when Borellus noticed vinegar eel worms; first record of cephalobid was observed; later these worms were named as *Vibrio aceti* by Muller (1783). The genus *Turbatrix* were proposed by Peters (1927) and Turbatrix aceti were acceptedas its type species. Bastian described the first cephalobid way back in 1865. Cobb (1924) suggested *Acrobeles* as a genus and proposed the *Acrobeles* and *Acrobeloides* subgenera and laterboth the generawere redescribed by Thorne. Systematic, morphology and taxonomy of the *Acrobeles* was elucidated by Thorne (1925). He (1937) also revised the Cephalobidae which has a great value and importance till date. He also proposed and placed genera *Placodira, Chiloplacus, Cervidellus* and *Zeldia* under subfamily Acrobelinae. Thorne suggested Panagrolaimoidea (superfamily) with Panagrolaimidae and Panagrolaiminae with addition of genera *Panagrellus* and *Panagrobelus* as genera. Steiner suggested the genus *Procephalobus* under Panagrolaimidae, and formed the genera *Eucephalobus, Tricephalobus* and *Pseudacrobeles* (Steiner 1934, 1936, and 1938).

Heyns formed few genera and added various species in the group cephalobids. Numbers of papers on cephalobids was published by Heyns (1962, 1968 and 1969) and Andrassy (1967). Suborder Cephalobina along with three superfamilies Elaphonematoidea, Cephaloboidea and Panagrolaimoidea was proposed by Andrassy (1974). Later in 1984, seven families and eleven subfamilies were introduced in Cephalobina suborder by Andrassy. He also put up new genera *Stegelletina*, *Panagrocephalus*, *Acrobelophis*, and *Panagrobelium*. Genus *Acromoldavicus* was proposed by Nesterov (1970). Cephalobids were considered as most abundant nematode group in desert ecosystem (Sohlenius, 1979). In 1979 and 1984, Details on cephalobids morphology using Scanning electron microscopy (SEM) was performed by Sauer and coworkers.

Extensive work on the taxonomy of cephalobids taxonomy was performed by Bostrom (1984-2000). Initially in 1984, He elucidated the *Chiloplacus minimus* morphological variability and differentiated the details of species of *Eucephalobus* viz., *E. oxyuroides*, *E. mucronatus* and *E. striatus* using SEM. Later In (1988a, 1988b) he worked on systematic and morphological aspect to understand the structure and importance of labial probolae of Cephalobidae. Similar studies on the morphology, morphometric and taxonomy of the cephalobids was performed by Rashid et al. (1984, 1989). From Antarctica, *Panagrolaimus magnivulvatus* was described by Bostrom (1995).

Using ultrastructural studies, De Ley and coworkers (1990-97) revised the term stomal components in Cephalobidae, Rhabditidae and Panagrolaimidae and also added fair number of species besides revision of genera under cephalobidae. Various new species and five new genera under Cephalobina were proposed by Siddiqi (1993, 2002). Embryogenesis, Morphology and oviposition of *Acrobeloides nanus* were studied by Bird et al. (1994).

Genus *Penjatinema* with new species *P. natalense* was proposed by Heyns and Swart (1998). Later, Second species of this rare genus *P. novaezeelandiae* from New Zealand under Cephalobidae were described by Holovachov et al. (2009). Karegar et al. (1998) explained three species of *Cervidellus* and one new and one known and one new species of *Stegelletina*. In 2001, a new genus *Acroukrainicus* and its type species *A. sagittiferus* weredescribed by Holovachov et al. Revision of some genera under cephalobids viz. *Acrobeloides, Cephalobus, Chiloplacus, Pseudacrobeles, Panagrolaimus, and Nothacrobeles*were carried by Santiago (2002, 2003, 2005, 2006, and 2009) and they also provided the identification key of these species. In *Acrobeles complexus*, sperm protein was studied by Yushin et al. (2016)

Various other scientists who made crucial and valuable contributions to the taxonomy and morphology of cephalobids, are Anderson (1965), Anderson and Hooper (1970, 1971), Abolafia and Pena-Santiago (2002, 2003i, 2003ii, 2003iii, 2003iv), Baldwin et al. (2001), Rashid and Heyns (1990i, 1990ii), Mundo-Ocampo et al. (2003), Nadler (2006), Poiras et al. (2002), Shahina and De Ley (1997), Steiner (1934), Stock et al. (2005), Sanwal (1959, 1960), Stock and Nadler (2006), Shokoohi et al. (2007), Smythe and Nadler (2006), Taylor et al. (2004), Van De Velde et al. (1994), Waceke et al. (2005), and Yeates (1969).

Five new genera and eight new species of Cephalobidae were proposed by Siddiqi (1993). A new genus *Acroukrainicus,* a new genus was proposed by Holovachov et al. (2001). Further, Holovachov and Bostrom (2006) proposed some new genera (*Deleyia and Panagrolobus*) under subfamily Cephalobinae. Abolafia and Peña-Santiago (2002; 2003ii; 2006; 2009) besides adding new species, revised the genera, *Acrobeloides, Chiloplacus, Pseudacrobeles, Nothacrobeles, Panagrolaimus, Cephalobus* and also provided keys to species identification. Extensive studies were carried out on Cephalobids by Holovachov and co workers (2011, 2014). Thorough descriptions of some new species of cephalobidae were given by Abolafia and co workers (2014 and 2016). Redescription of Cephalobidae was given by Nguyen et al. (2016). In India, So far no such substantial work has been done on the taxonomy of this group of nematodes. Some important contributions on taxonomy of cephalobids are Khera (1968), Rathore et al. (1992), Tahseen et al. (1999), Rizvi and Bhutia (2009), and Rizvi (2010).

Order Plectida

Malakhov (1982) proposed Plectida for the families which were initially assigned in order Chromadorida (Lorenzen, 1981) or Araeolaimida (Andrassy, 1976) by various authors. Bastian (1865) described the type genus *Plectus*. The family Plectidae was elevated to the order level based on molecular phylogeny by Blaxter et al. (1998).

Five superfamilies were suggested for the order Plectida by Holovachov and Boström (2004) i.e., Plectoidea, Ceramonematoidea, Camacolaimoidea, Haliplectoidea and Leptolaimoidea. The other authors who contributed to this field include Kirjanova (1958), Allen and Noffsinger (1968) who studied the genus *Plectus* and revised the genus *Anaplectus* respectively. Heyns and Coomans (1980, 1983) described new and known species of *Chronogaster* from fresh water nematodes of South Africa. One known and two new species of *Plectus* from Canada were described by Ebsary (1985). Kito et al. (1991), reported species of Plectus i.e., *P.frigophil*us and *P. antarcticus* de Man, 1904, while working on plectida species of Soya coast in East Antarctica. From east Antarctica, Bostrom (1995) described *Plectus acuminatus* Bastian, 1865. A new species of *Chronogaster* described by Gagarin and Thanh (2004) from Vietnam. Order Plectida got lot of attention in recent years. Significantcontribution was made by Holovachov in field of evolution, phylogeny, and morphology of the superfamily Plectoidea Orley, 1880. From Ukraine, Holovachov and Susulovsky (2000) described nematodes belonging to family Plectidae. Holovachov (2001, 2004, 2005, 2006, 2014) provided information on the systematic and development of order Plectida.

Juvenile stages of species of subfamily Wilsonematinae under SEM was studied by De Ley et al. (2002). A new species of *Wilsonema* was decribed by Holovachov and Hanel (2004) from Poland. Holovachov and De Ley (2006) provided characteristic information of order Plectida in the book entitled “Freshwater nematodes: Ecology and Taxonomy”. Systematics and morphology of Camacolaimoidea and Leptolaimoidea superfamilies of order Plectida studied by Holovachov and Bostrom (2004). They also published a manual on identification of order Plectida in 2010. A comparative information of the systematic and morphology of *Ereptonema, Wilsonema, and Neotylocephalus* was explained by Holovachov et al. (2003) and also separately published the revision of the genera *Tylocephalus* and *Anaplectus* in 2004. Further, Holovachov et al. (2009) explained the systematic position and morphology of the genus *Hemiplectus* under Plectida. Holovachov (2014, 2015) and coworkers (2012, 2014i, 2014ii) contributed a lot in systematic and morphology of order plectida and published number of articles on nematodes from Sweden. A new genus *Neotylocephalus* was proposed by Ali et al. (1969) from Marathwada. In the same year *Wilsotylus* from Bangalore was also proposed by Chawla et al. (1969). Description of a new species under family Wilsonematidae was given by Ganguly and Khan (1986) and the developmental biology and description of a new species *Plectus zelli* was given by Tahseen et al. (1992). Later six known species of Plectus were also recorded from India (Tahseen and Mustaqim, 2011).

Table 1 Soil nematode (Species belonging to orders Rhabditida and Plectida) reported from Indian Himalayan States

Species	J and K	Ladakh	H.P	Uttarakhand	Sikkim
Acrobeloides nanus	✓			✓	
Cephalobus pseudoparvus				✓	
Acrobeloides buetschlii				✓	
Acrobeles ciliatus				✓	
Acrobeles timmi				✓	✓
Acrobeloides buetschlii				✓	✓
Acrobeloides nanus	✓	✓		✓	
Bursilla vernalis				✓	
Caenorhabditis elegans				✓	
Caenorhabditis kashmirensis	✓				
Cephalobus persegnis				✓	✓

Species	**J and K**	**Ladakh**	**H.P**	**Uttarakhand**	**Sikkim**
Cephalobus pseudoparvus			✓	✓	
Cervidellus neftasiensis				✓	
Cervidellus vexilliger	✓	✓			
Chiloplacus demani	✓	✓			
Chiloplacus magnus				✓	
Chiloplacus minimus				✓	
Chiloplacus subtenuis				✓	
Chiloplacus symmetricus				✓	
Chiloplectus indicus				✓	
Diploscapter coronatus	✓				
Eucephalobus mucronatus				✓	
Eucephalobus oxyuroides				✓	✓
Macrolaimellus longicauda				✓	
Mesorhabditis cranganorensis				✓	✓
Mesorhabditis spiculigera	✓			✓	
Pelodera strongyloides	✓				
Plectus cirratus				✓	✓
Plectus grandulatus				✓	
Plectus thornei				✓	
Protorhabditis elaphri				✓	
Protorhabditis neoxylocola	✓				
Protorhabditis oxyuroides				✓	
Pseudacrobeles eurystoma				✓	
Pseudacrobeles multicinctus				✓	
Pseudacrobeles pauciannulatus				✓	
Pseudacrobeles tabacum				✓	
Pseudacrobeles variabilis				✓	
Pseudacrobelus eurystoma				✓	
Stegorhabditis abursata	✓				
Steinernema feltiae				✓	

Species	J and K	Ladakh	H.P	Uttarakhand	Sikkim
Teratocephalus costatus				✓	
Teratocephalus terrestris				✓	
Teratorhabditis mangiferae	✓				
Wilsonema auriculatum				✓	
Wilsonema otophorum				✓	
Zeldia punctata				✓	

2.2 Indian Himalaya Region and Backdrop of Current Study

The earth's climate has been changing and evolving. Some of the changes have been due to natural causes others are influenced by anthropogenic causes such as deforestation, atmospheric emissions, led to aerosols and gases being accumulating in the atmosphere enhancing already existing risks for natural and human systems which can be seen by comparing the status of the Indian Himalayan Region (IHR), glacier melt runoff, snowmelt runoff, and total stream flow and their distribution. These changes have not only altered the above ground biota but the soil structure and vegetation affecting below-ground soil biota. Above ground biodiversity status has been documented well since long time however the status of below-ground biodiversity is less understood, despite known links between below-ground soil biodiversity and above-ground systems, and regulating ecosystem functions (Leon et al., 2003; Todd et al., 2006; Heneghan et al., 2008; Cuesta et al., 2012). Soil nematodes constitute the major part of soil biota as they are present at different trophic level in soil and any alteration of the nematode community induced by climate change will have a considerable influence on ecosystem functioning.

Himalayan forests provide a variety of products and services which are essential for sustainable livelihood of people in mountain. Characterization of nematode community of a study area provides a glimpse of current environmental conditions and provides condition of decomposition pathway. Alterations of the nematode community structure induced by global change may have a considerable influence on ecosystem functioning. However, whether minor changes in soil temperature and moisture have any significant effect on nematode community structure is not studied well. In India, most of work has been focused on agricultural fields because of its economical importance and very less work has been done in high altitude forest region in Uttarakhand, one of the states of IHR. In Indian Himalayan Region (IHR) ecosystem, only study in Ladakh and Jammu and Kashmir by Devetter et al. (2017), they have worked on the nematode linkage with habitats specifically along the elevation gradient rather than vegetation. Information with respect to soil nematodes in forest ecosystem is very less (Deysarkar, 2008; Rizvi, 2009, 2010, 2013; Kumar and Ahmad, 2017). Latitude, climate and vegetation type are key factors in the distribution of soil nematodes (Dagang, 2017) and no such work is done in Uttarakhand along elevational gradient.

It is observed that most of the studies are result of random surveys in most of the states of IHR. Moreover, these studies are attempts to identify the important nematode pests of agricultural crops, which show that considerable work has been done on nematodes residing in agricultural fields and studies on nematodes from forest ecosystems are very meagre. There is an increase in number of studies on nematodes during last decades which may be attributed to the fact that there is advancement in technology during this period. It is also observed that most of the work has been conducted on plant-parasitic nematodes while there are very few studies on bacteriovors, fungivores and omnivores nematodes. There are a large number of studies on ecology and behavior of agricultural field nematode followed by taxonomy, pathogenicity and their control. Studies on themes like genetics, forest ecosystem in IHR region, climate change are needed. Soil nematode community structure and their changes were found to be one of the best biological tools for assessing soil processes and plant conditions in terrestrial ecosystems (Wang et al., 2009; Pen-Mouratov et al., 2010).

2.3 Objectives

The present study aimed to create baseline data of soil nematode community structure including diversity for long term monitoring and to determine the sensitivity of soil nematodes in two sites in the subalpine and alpine region (*Deodar*, *Pinus*, *Betula* dominated subalpine and scrub dominated alpine sites) to environmental change by altering temperature by the installation of Open top experiment setup. High altitude terrestrial ecosystems are stressed by extreme climatic conditions and strongly nutrient-limited due to direct and indirect effects of snow cover and low temperature in the alpine and subalpine region. Hence, minor change may lead to change in community structure of various free-living nematodes which is sensitive to change which will be useful for evaluating the impact of climate change in Indian Himalayan system which may alter predator-prey interactions and have significant effects on the soil food web, nutrient cycling, and microbial biomass in the soil. Therefore, Nematode community structures were assessed and the use of nematode biodiversity to indicate climate change in IHR is discussed using OTC experimental setup.

Specifically, in the subsequent six chapters, the study explores soil nematode community structure and relationship with its immediate environment. Further it also indulges experimental analysis of soil nematode as bioindicators for climate change using Open Top Chamber in subalpine and alpine region of GNP scrutinizing the following objectives:

1. To analyze soil nematode diversity across different vegetation types.

2. To determine the relation between soil nematodes and soil physiochemical parameters.

3. To study the effect of change in temperature and humidity on soil nematode through experimental setup of Open Top Chamber (OTC) in sub-alpine and alpine region of Gangotri National Park.

4. Morphological and molecular characterization of some bacteriovorous nematodes.

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Chapter 3

Material And Methods

3.1 Study Area

The IHR is one of the richest biodiversity regions on the earth. The largest glacial cover after the polar region is in the Indian Himalaya and the surrounding high mountains of Asia (Dyurgerov, 2001) which are termed as the 'third pole'. There are 9575 glaciers spread across the Indian part of the Himalayas (Sangewar and Shukla, 2009). Indian states namely Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh and northern region of West Bengal constitute IHR. Bhagirathi basin covers an area of 6,921 km². Bhagirathi sub-basin I and II constitute upper Bhagirathi basin.

Bhagirathi originates from Gaumukh at the foot of Gangotri and Khatling glaciers in Garhwal Himalaya. Later joined by other tributaries namely Kedar Ganga at Gangotri, Jadh Ganga at Bhairongathi, Kakora gad and Jalandhari gad near Harsil. Siyan Gad near Jhala, Asi Ganga near Uttarkashi, Bhilangna river near old Tehri. The Bhilangna itself rises at the foot of the Khatling Glacier approximately 50 km (31 mi) south of Gaumukh.The river flows from its source for 205 km (127 mi) before confluence with the Alaknanda River at an elevation of 475 m (1,558 ft) in Devprayag. The river is known as Ganges River downstream of its confluence. The Tehri dam lies at the confluence of the Bhagirathi and the Bhilangna, at 30°22′32″N 78°28′48″E, near Tehri. Gangotri national park covers a total area of 2,390 km^{2}in the Uttarakashi district of Uttarakhand. Gangotri National Park (30°50'N, 78°45' E - 31°12' N, 79°02' E) covers a wide altitudinal range from 1200 to 6000 m and lies at upper catchment of the River Bhagirathi. The vertical forest distribution of high altitude forest of GNP typically changes with elevational variations. GNP represents the high altitude areas of western IHR and comes under biographical zone 2B of Western Himalaya (Rodgers and Panwar, 1988). High altitude of GNP comprises of two valleys - Gangotri and Nelang. The study area comprises different vegetation types from elevation ranging above 3000 to 5000 m in Gangotri and Nelang valley of GNP. Nelang valley gives rise to Jadhganga which is one of the largest tributaries of the Bhagirathi River, and confluence at bhairongathi in Uttarkashi district. Nelang valley of Gangotri National Park is the highest valley of the basin. Climate and vegetation vary greatly with elevation from lower forest to higher glacier regions. The highest elevation covered by ice and bare rocks, alpine steppes at a higher altitude (>3800m) and upper region (>3700m) of Gangotri valley is relatively drier and forms a transition zone between moist and arid region, stunted tree line exists between 3700 - 3800 m in Gangotri valley Vegetation comprised of Himalayan Birch (*Betula utilis*), subalpine mixed conifer forests with West Himalayan Fir(*Abies pindrow*), Deodar (*Cedrus deodara*) and Blue Pine (*Pinus wallichiana*) which is the dominant species in the region (Champion and Seth, 1968). The natural vegetation is primarily dominated by the Deodar at a lower elevation, Blue Pine and Himalayan Birch from middle elevation gradient to the tree-line and alpine scrub at transition zone between moist and dry region (*Artemisia* sp, *Juniperus* sp). Alpine dry scrubs (*Caragana sp.*) in cold desert region of Nelang (4000-5000 m) mainly characterized by very low temperature, low rainfall with scanty and specialized vegetation. Study area comprise of different vegetation type along the elevation gradient. Snow leopard (*Panthera uncia*), Tibetan wolf (*Canis lupus chanko*), Himalayan brown bear (*Ursus arctos isabellinus*), and blue sheep (*Pseudois nayaur*) are known from alpine and Trans-Himalayan region.

Intensive study was conducted in Bhagirathi sub basin I comprising of two zones i.e. subalpine; and alpine region of Gangotri and Nelang valley in GNP (Table 2). No prior study exists on soil nematode community in the study area.

Table 2 Forest type in upper Bhagirathi basin (Champion and Seth, 1968)

Zone	Forest type	Elevation
Sub-alpine	Coniferous forest, Scrub / krummholtz	3000-treeline
Alpine	Moist herbaceous forest, Grasslands, Scrub steppe, Peat land / Marshland	Above tree line

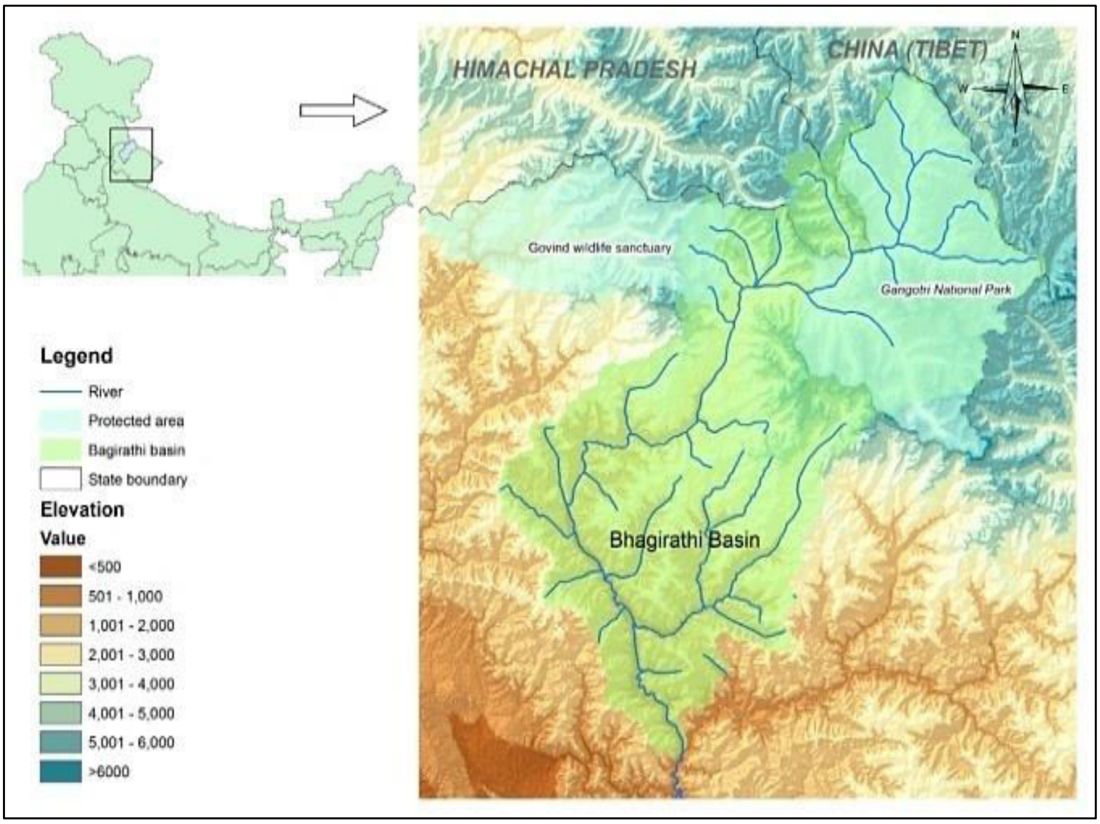

Figure 1 Digital Elevation Map of Bhagirathi Basin, Uttarakhand showing Gangotri National Park

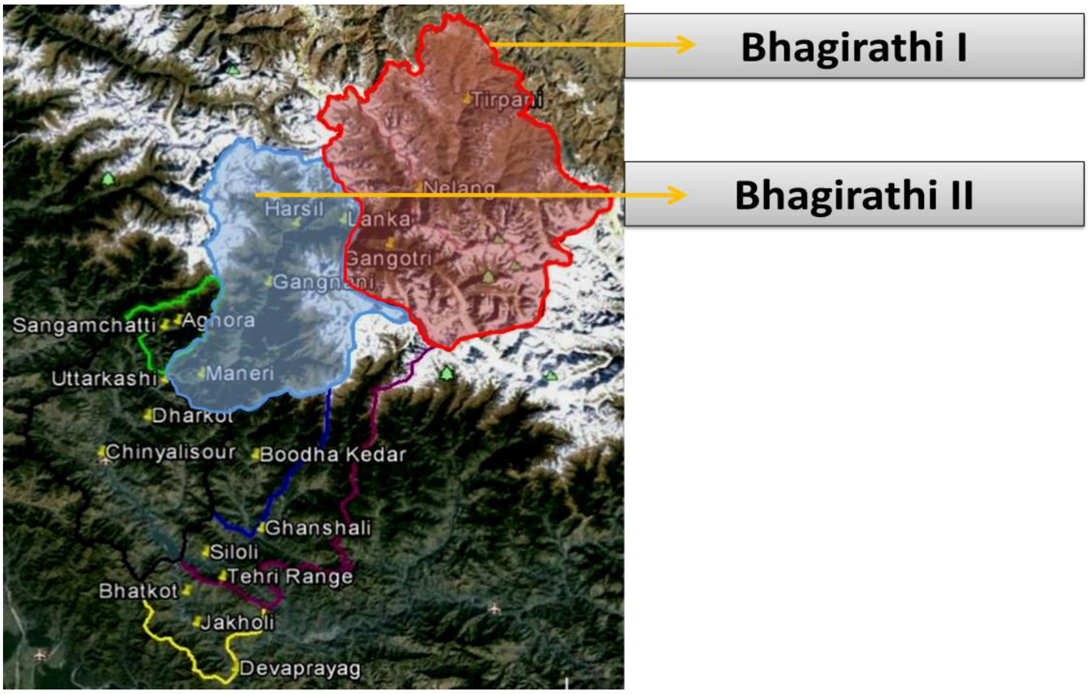

Figure 2 Map showing sub-basin I and II of Bhagirathi basin

Gangotri Valley (3000 – 4000 m)

Nelang Valley (4000 – 5000 m)

Figure 3 Sampling sites in Gangotri National Park.

PHOTOGRAPHS OF SURVEY SITES
GANGOTRI VALLEY

Gangotri

Kanku

Kanku-Chirwasa

Chirwasa

Bhojwasa

Gaumukh

PHOTOGRAPHS OF SURVEY SITES
NELANG VALLEY

Sumla Top

Sonam

Janaktaal

Jadhung

Nelang Base camp

Rangmanch Ghad

3.2 *Methods and Analysis*

3.2.1 *Sampling Design*

The entire landscape of Bhagirathi sub-basin I was sampled along the elevation gradient. Samples were collected from various vegetation types along elevational range of 3000-5000 m. Four random sites were selected in each vegetation type categorized on the basis of dominant plant community in the region Subalpine-Deodar (SAD), Blue Pine (SAP), Himalayan Birch (SAB), alpine scrub *Artemisia* (ASA), alpine dry scrub *Caragana* (ASC). Three samples per site and total of 12 samples from each vegetation types were collected (3 samples x 4 sites x 5 vegetation types). Five soil cores were taken to get maximum diversity which constituted one composite sample. Stratified random sampling was followed. Within the stratums, sites were selected randomly.At each sampling site, beneath the host plant, leaf litter (if present) as well as the upper layer of soil was removed. Soil samples were collected at 10-15 cm depth using soil auger of diameter 3 cm (Rizvi, 2008).

3.2.2 *Sampling from Experimental Plots*

In May 2017 and 2018, 24 soil cores were collected from experimental plots (6 Treatment Plots + 6 Control Plots x 3 Sites (with replicates) x 2 years = 24 total samples). Cores were taken 10 cm deep using soil auger of diameter 3 cm and shovel in case of rocky soil from the center of the plot to minimize any edge effects. Samples were transferred to the lab in airtight bags. Soil nematodes were extracted from 100 g samples by using cobs sieving and decantation technique Nematodes were identified up to the genus level by BX53 DIC /BF Olympus research microscope with an attached DP27 digital camera. Nematode genera were allotted to trophic groups and colonizer- persister groups and ecological indices were calculated. Nematodes were identified according to Yeates et al. (1993). All nematodes extracted from soil samples were identified and counted.

3.2.3 Soil Processing and Extraction of Soil Nematode.

Samples were stored in fridge to avoid drying of soil after collection.

Nematodes were extracted from a sample taken from each composite soil sample using decantation and sieving (Cobb, 1918) method (Fig. 4) Stepwise processes were carried as follows:

- 200-250 gm of soil placed in a bucket mixed with 4 litres of water and stirred thoroughly using glass rod. More water were added to fill the bucket just below rim and kept still for 20-30 sec.
- Next suspension waspoured through 2mm sieve in another bucket. Remains of pebbles and other residue on sieve were discarded and suspension in the bucket were stirred and left undisturbed for another 20-30 seconds and poured over fine 300 mesh sieve i.e. 53µm pore size.
- Soil nematodes remain on the sieve along with finesand and mud particles were collectedin abeaker by adding some water. Next aliquot containing nematodes were poured over the tissue paper layered on the coarse gauge and placed over a Petri dish filled with water making sure water touches a centre of the gauge so that nematodes from aliquot swim down the water in Petri-dish through the gauge.

Fixation of Nematodes

The suspension containing the nematodes in the Petri plate is transferred in the 30ml tubeand kept undisturbed for 2-3 hours so that nematodes could settle down at the bottom. Using adropper of appropriate length, excess water is removed carefully. The nematodes isolated as above are ready for fixation. Remove water and leave the nematodes in as little water aspossible. Formalin- Acetic Acid fixative (FAA) gave satisfactory results for fixation of soil nematodes (Fixative used - 8 ml of 40% Formalin + 2 ml Glycerol + 1-2 drops of Glacial Acetic Acid + 90 ml water).

Next, formalin-acetic acid fixative were boiled in the microwave or heater and poured over nematode suspension in the tubes after bubbles subside which were formed due to boiling.Tubes were capped and left undisturbed for 1 hourand then labeled with locality, host, site and date.

Picking and dehydration

Nematodes were picked manually based on their size shape and structure and transferred in ethanol-glycerol solution and placed in a desiccators for dehydration at least for 15 days.

Next step is preparing slides of each kind for its identification under high power microscope. Nematodes were picked under microscope from cavity blocks and placed over a slide and mounted using wax and heating it on slide warmer table. Specimens were identified to the genus level using an inverted microscope at 100 X magnification according to Cobb, 1918; Southey, 1986; Thorne, 1974; Jairajpuri and Ahmad, 1992; Bongers 1994; Siddiqi, 2000; De Ley et al., 2003. Nematode genera will be allotted to trophic groups Yeates et al., (1993) and colonizer-persister groups based on Bongers (1990).

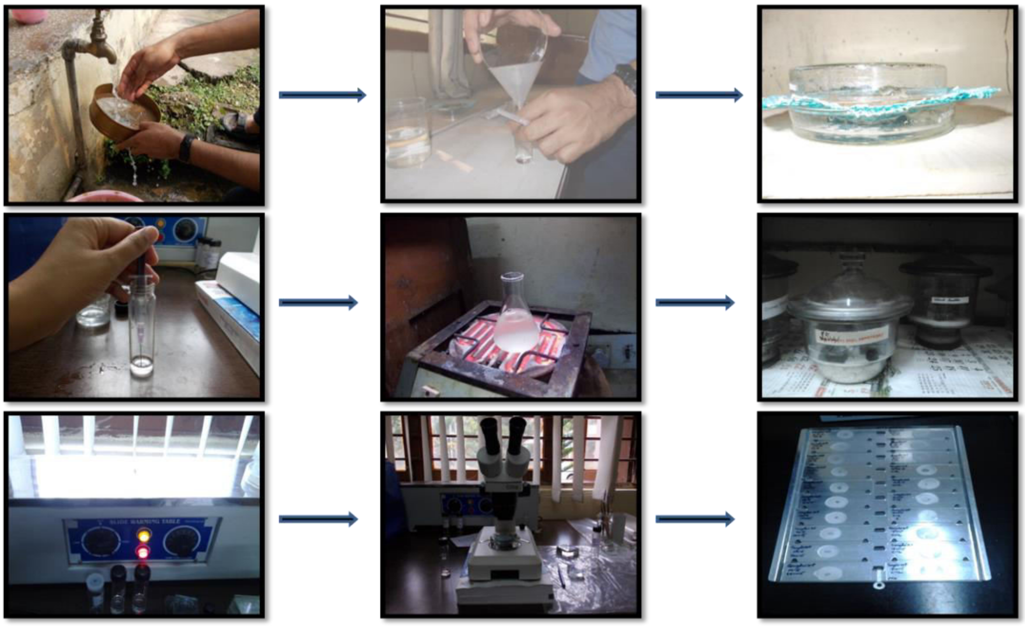

Figure 4 Showing processing of samples (Cobb's sieving and decantation technique; collection, fixation; Picking and dehydration, slide preparation.

Soil analysis

Different parameters such as soil reaction (pH), soil moisture, organic carbon, total nitrogen, phosphorus and soil texture were analyzed in the laboratory by using standard protocols. Significant variation in soil properties at different altitudinal range was observed. Soil has also been sampled along the elevation among different vegetation ranging from 3000-5 000 masl and from experimental apparatus i.e Open Top Chambers and control plots.

The processed soil samples were used to analyse different soil parameters such as the electrical conductivity ($\mu Sc\ m^{-1}$) determined by the method of Jackson (1967); soil pH determined in 1: 2.5 soil: water ratio using the pH meter with glass electrodes (Jackson, 1973). The organic carbon in the soil was estimated by methods suggested by Walkley and Black (1934); total Nitrogen content by alkaline potassium permanganate (Kjeldahl, 1883); $NaHCO_3$ extractable P (Olsen et al., 1954) by spectrophotometer, ammonium acetate extractable K (Hanway and Heidel, 1952) by flame photometer.

Installation of Open Top Chamber to study effect of temperature on soil nematodes in subalpine and alpine region of Gangotri National Park

OTC features and design

Open top chambers (OTCs) are cost effective and innovative device to understand the effects of altered climatic factors such as temperature, humidity on growth dynamics and response of soil microbial community activity. Chambers are hexagonal in shape and opened on the top (Fig. 5 and 6). The opened design ensures that the system has airflow and receive same amount of precipitation during rainfall. They are installed on relatively flat surface with same vegetation cover. The minimum distance between two adjacent chambers is about 5 to 6 m. A plot is left untreated adjacent to each OTC within 2-3 m with e same area and vegetation type serves as a natural control plot (Fig. 5). Due to the thick snow cover, low temperatures, and strong winds in winter season of Himalayan alpine region, Samples were not collected during winter season, which lasts on average from Mid November till March.

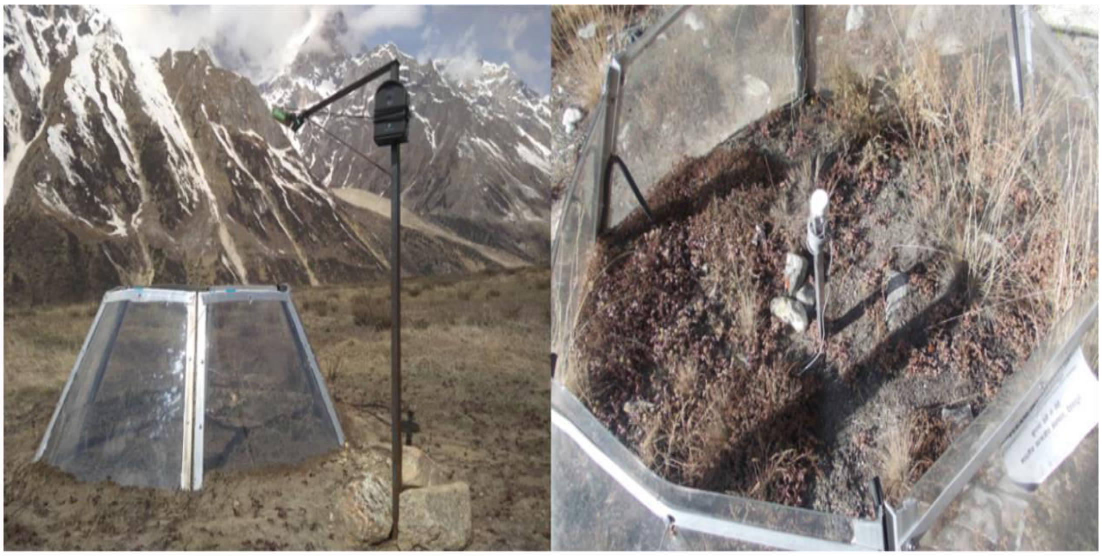

Figure 5 Installed Open Top Chamber with data logger

Materials required and construction

The chambers are built with high quality multilayered polycarbonate sheets (4 mm thickness) with high solar transmittance (transmits ~ 92% of visible light, reflect 4% of incoming radiation and pass on ~ 85% of incoming energy). Each site is provided with two replicates.

Equipments used with OTC setup

Weather stations (HOBO U23 Pro v2 Temp/RH and HOBO U23 Pro v2 2X external temperature data logger) are installed in the OTC and the adjacent outside control, at a height of 14-15 cm above the surface for recording air relative humidity and air temperature every hour. Soil temperature and moisture is also measured below ground suface at 5 cm depth.

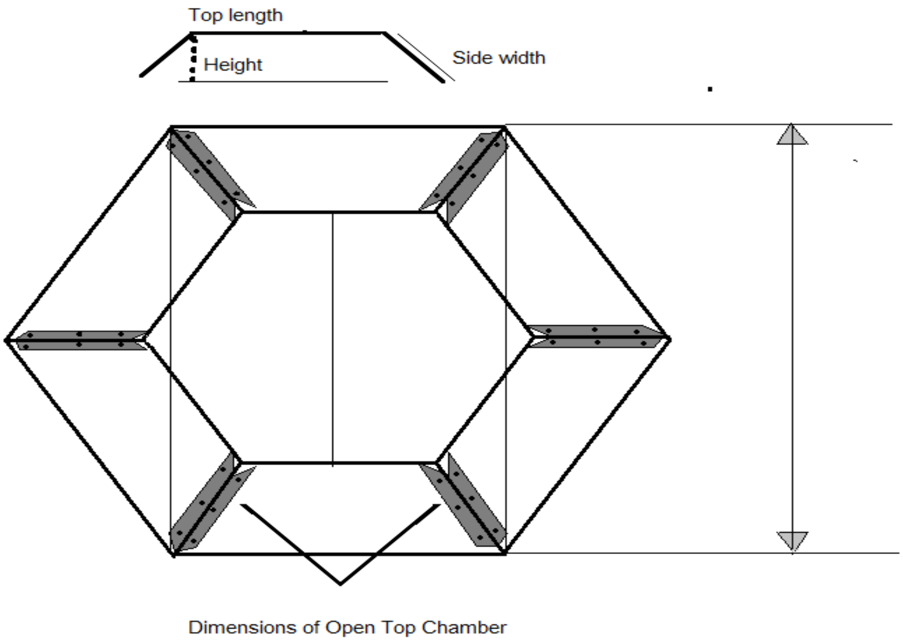

Figure 6 Dimensional sketch of OTC

3.2.4 Molecular Analysis

Nematodes (bacterivores) were cultured and maintained in agar medium till their final use. Nematode extraction buffer (NEB) method (Troccoli, et al., 2008) with modifications wherever required was followed for genomic DNA isolation. After purification DNA samples were subjected to PCR (polymerase chain reaction) using nematode specific primers. Next step, DNA samples were sequenced, and then compared (using BLAST search) against gene bank data to identify related taxa based on sequence similarity and Performing analyses of the database to examine patterns and processes in soil nematode diversity.

The Neighbor-Joining method used to infer the evolutionary history. The phylogeny of the group was constructed using the evolutionary distance data (Saitou et al. 1987). Estimation of Overall Evolutionary Divergence over Sequence Pairs in COI gene and 18S rRNA gene for the barcode region were calculated for individuals collected, using the analytical tools the Kimura 2 Parameter model (Kimura, 1980). MEGA7 is used for evolutionary analyses.

3.2.5 Community analysis and Diversity Indices

A detailed description of the formulae used are given below

Frequency N: The number of samples containing nematodes.

Absolute frequency (AF): Genus frequency /total number of samples x 100:

Mean density (D): Number of nematode specimens of the genus in samples / total number of the samples collected;

Relative density (RD) %: Genus mean density / Sum of the mean density of all nematode genera x 100.

The total number of genera in each sample will be recorded (richness). Nematode communities will be investigated in terms of the total abundance, abundance of trophic groups, number of taxa, Maturity Index, Maturity Index 25, Channel Index, Nematode Channel Ratio (NCR), Channel index (CI), Shannon-diversity Index and similarity. Indices values are collectively used to elucidate the indicative role of nematode communities.

Maturity indices were calculated based on c-p scale assigned to different genera of nematode communities, ranging from r-strategy colonizers cp-1(enrichment colonizers) and cp-2 (disturbance colonizers) to K strategy persisters' cp-5 (persisters). Details for calculation of maturity index (MI) and plant parasite index (PPI) can be found in Bongers (1990).

- MI -free-living nematodes with cp1-5, (Bongers, 1990, Bongers and Bongers, 1998);

- MI25 free-living nematodes with cp2-5 (Bongers et al., 1995; Neher and Campbell, 1994);
- Channel ratio (CI) = (0.8Fu2/{3.2Ba1 + 0.8Fu2})
- Enrichment index (EI) = (e/e+b) X 100, and
- Structure index (SI) = (s/s+b) X 100 to understand the dynamics of soil food web in Microsoft excel.

The b component is calculated as $\Sigma k_b n_b$, where k_b are the weightings assigned to guilds which indicates basal characteristics of the soil food web (0.8[Ba2+Fu2]) and n_b are the nematode abundance in the guilds. Similarly, the e and s components are calculated using the guilds indicating enrichment ([3.2Ba1] + [0.8Fu2]) and structure (Ba3-Ba5, Fu3-Fu5, Ca2-Ca5, Om3-Om5,), respectively (Ferris et al., 2001).

Shannon-weaver diversity index (H'); Simpson’s index (λ); evenness (J'); richness (GR) diversity indices were calculated in R software.

3.2.6 Statistical Analysis

Kruskall-Wallis analysis of variance (ANOVA) performed to detect significant differences in nematode abundance, nematode diversity, nematode specific indices, and soil properties among different vegetation types. Multiple comparisons were performed by using Dunn test among vegetation types. Spearman's rank correlation coefficient and their significance at $P<0.05$ level among various soil physicochemical parameters and nematode trophic groups were calculated. Nematode generic data were assessed for normality and transformed by ln (n+1) wherever required. To understand the effect of temperature factor using OTC chambers, to determine the amount of variance in different nematode generic taxa data in between elevated and control plots, planned comparison ANOVA is used. Centered principal component analysis (PCA) was performed to compare the nematode taxon compositions with environmental variables. Non-metric multidimensional scaling (NMDS) was performed to investigate soil inhabiting nematode community in ordination space among vegetation types. NMDS is a robust unconstrained ordination method which depends on dissimilarity rank order in a community and often used in community ecology (Kruskal, 1964; Minchin, 1987). To test if nematode composition in nematode communities differs among vegetation types, Permutational multivariate analysis of variance (PERMANOVA) was performed to investigate the community structure in the experimental and control plot and the ‘adonis’ and ‘betadisper’ functions (vegan package) for the PERMANOVA(999 permutations) is utilized to test if communities significantly grouped by treatment and year. We also conducted pairwise multilevel comparisons with dissimilarity matrix using ‘pairwise.adonis’ function to assess differences between vegetation types. These analyses were performed in R statistical platform.

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CHAPTER 4

Soil Nematode Diversity Among Different Vegetation Types

4.1 Background

Vegetation type is an important factor for determining nematode community composition (Song et al., 2017). Different vegetation types have different nematode communities (Yeates, 2007) and compositions of aboveground floral composition were propounded to affect soil-inhabiting nematode community structure by altering abiotic factors (Keith et al., 2009). Some studies are available on soil-inhabiting nematode diversity and abundance in forest ecosystems (Yeates, 2007; Baniyamuddin et al., 2007; Hanel, 2008; Rizvi, 2008, Tomar and Ahmad, 2009; Tong et al., 2010; Butenko et al., 2017; Kitagami et al., 2017). In Indian Himalayan Region (IHR) ecosystem, only study in Ladakh and Jammu and Kashmir by Devetter et al. (2017), they have worked on the nematode linkage with habitats specifically along the elevation gradient rather than vegetation.

Fragmented studies (mainly taxonomic) were done in lower altitude region of Gangotri landscape. To the best of our knowledge, no study has been conducted to assess the soil nematode community structure across different vegetation types above elevational range>3000 m in high-altitude forests of GNP. Characterization of soil nematode community of such a high-altitude wilderness area can provide a glimpse of current micro-environmental conditions. It may also elucidate the details of the decomposition pathway therein, for long-term monitoring and to understand the soil health of the region. The objectives of the study were to investigate and compare the nematode assemblage patterns in different vegetation types of high altitude region of GNP.

4.2 Methodology

Samples were collected in October 2018. In total, 60 samples from various vegetation types within the elevational range of 3000-5000 m. Four random sites selected in each vegetation type categorized based on the dominant plant community in the region, Subalpine-Deodar (SAD), Blue Pine (SAP), Himalayan Birch (SAB), alpine scrub *Artemisia* (ASA), dry alpine scrub *Caragana* (ASC). Three samples per site and a total of 12 samples from each vegetation type were collected (3 samples x 4 sites x 5 vegetation types). Five soil cores were taken to get maximum diversity, which constituted one composite sample. At each sampling site, beneath the host plant, leaf litter (if present) and the upper layer of soil were removed. Soil samples were collected at 10-15 cm depth using a soil auger of diameter 3 cm (Rizvi, 2008). These were packed in an airtight polythene bag and transferred to the laboratory for analysis.

Kruskall-Wallis analysis of variance (ANOVA) was used to detect significant differences in nematode abundance, nematode diversity, nematode specific indices, and soil properties among different vegetation types. Multiple comparisons were performed by using Dunn test among vegetation types. Spearman's rank correlation coefficient and their significance at $P<0.05$ level among various soil physicochemical parameters and nematode trophic groups were calculated. Permutational multivariate analysis of variance (PERMANOVA) was used to test if nematode composition in nematode communities differs among vegetation types using the ‘adonis’ and ‘betadisper’ functions (vegan package) for the PERMANOVA (999 permutations). Pairwise multilevel comparisons with dissimilarity matrix using ‘pairwise.adonis’ function were conducted to assess differences among vegetation types. These analyses were performed in R statistical platform.

4.3 Results

Nematode faunal composition

A total of 62 nematode genera were identified, of which 36 genera significantly differed ($p<0.05$) among various vegetation types. (Table 3). Fifty-five genera were recorded from SAD, 52 genera from sub-alpine blue pine (SAP) vegetation, and 44, 42, and 31 genera were recorded from subalpine Himalayan Birch (SAB), alpine scrub *Artemisia* (ASA), and dry alpine scrub *Caragana* (ASC) respectively. The mean abundance of soil nematodes varied from 896.5 to 373 (Table 4). Trophic grouping of nematodes included twenty six genera of bacterivores, six fungivores, thirteen plant parasitic, ten omnivores, and seven were predators. The abundance of bacterivores- *Acrobeloides, Cephalobus, Cervidellus, Chiloplacus, Stegelletina, Cylindrolaimus, Wilsonema, Panagrolaimus* were significantly different among subalpine and alpine scrub vegetation types, whereas the abundance of *Cephalobus*($p=0.01$) and *Chiloplacus* ($p<0.01$) were significantly higher in SAB than SAD. *Axonchium* was significantly higher in subalpine -Deodar than the other subalpine vegetation type ($p<0.01$). Some bacterivores (*Anaplectus, Rogerus*), plant-parasitic (*Dorylaimellus, Axonchium*), and predatory nematodes (*Tripyla*) were absent in the alpine scrub region, whereas, *Protorhabditis, Campydora, Mylonchulus*, and *Oriverutus* were only present in SAD.

Table 3 Relative abundance of nematode genera in different vegetation types (Mean ± standard error). Means (%) followed by different superscript letters are significantly different by the Post hoc-test at the 5% level of significance ($p<0.05$). Means followed by different superscript letters are significant. (Where ASA-Alpine scrubsArtemisia; ASC-Alpine scrub Caragana; SAB-Subalpine Birch; SAD-Subalpine Deodar; SAP-Subalpine Pine).

Genera	**c-p value**	**ASA**	**ASC**	**SAB**	**SAD**	**SAP**
Bacteriovores						
Acrobeles	2	6.95±1.76	5.38±0.94	5.26±1.45	6.89±2.13	6.07±2.18
Acrobeloides	2	10.02±2.36^{a}	6.05±1.46^{a}	6.57±2.21^{ab}	1.955±0.87^{b}	1.65±0.67^{b}
Alirhabditis	1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.13±0.08
Amphidelus	4	0.67±0.49	0.35±0.24	0.00±0.00	0.00±0.00	0.00±0.00
Anaplectus	2	0.00±0.00	0.00±0.00	0.12±0.12	0.65±0.32	0.4±0.27
Cephalobus	1	0.16±0.16^{a}	0.44±0.21^{a}	2.66±0.66^{b}	0.08±0.05^{a}	1.92±0.81^{ab}
Cervidellus	2	6.25±2.06^{ab}	12.96±3.39^{a}	1.75±0.85^{bc}	1.87±1.24^{c}	0.00±0.00^{c}
Ceratoplectus	2	1.78±0.65	1.41±0.58	0.59±0.23	2.40±1.08	3.5±1.65
Chiloplacus	2	3.79±1.18^{ab}	6.8±1.27^{a}	4.91±0.84^{a}	0.58±0.14^{b}	3.39±1.09^{ab}
Cryptonchus	4	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.06	0.00±0.00
Cylindrolaimus	3	0.12±0.12^{ab}	0.00±0.00^{a}	5.43±2.55^{bc}	2.60±1.01^{c}	6.22±2.58^{c}
Eucephalobus	2	1.23±0.55	1.66±0.55	0.44±0.29	0.14±0.09	0.95±0.62
Mesorhabditis	1	0.44±0.32	0.00±0.00	0.12±0.12	0.60±0.27	0.4±0.33
Monhystera	1	0.24±0.24	0.00±0.00	0.4±0.27	0.41±0.21	0.19±0.12
Nothacrobeles	1	0.76±0.6	0.78±0.77	0.61±0.34	0.79±0.26	0.23±0.23

Panagrolaimus	1	2.78±0.75^{a}	1.46±0.61^{ab}	0.00±0.00^{c}	0.41±0.24^{bc}	0.27±0.13^{bc}
Protorhabditis	1	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.06	0.00±0.00
Plectus	2	4.04±0.79	4.17±0.85	4.54±2.21	5.71±2.4	9.18±2.92
Prismatolaimus	3	6.48±3.87	1.87±0.68	2.33±0.94	9.75±3.24	13.61±4.69
Pseudacrobeles	2	1.66±0.75	1.37±0.84	0.2±0.2	1.22±0.34	0.62±0.21
Rogerus	2	0.00±0.00	0.00±0.00	0.28±0.19	0.96±0.76	0.08±0.08
Rhabdolaimus	2	2.78±1.48	3.22±1.1	1.17±0.76	0.51±0.21	0.56±0.22
Stegelletina	2	3.55±1.14^{ab}	4.8±0.77^{a}	2.24±0.56^{bc}	1.22±0.7^{bc}	0.67±0.51^{c}
Teratocephalus	3	1.54±0.77	2.98±0.53	0.91±0.72	2.56±1.01	1.74±0.82
Tylocephalus	2	0.00±0.00	0.00±0.00	0.00±0.00	0.12±0.11	0.05±0.05
Wilsonema	2	1.54±0.77^{a}	1.38±0.63^{a}	4.75±1.14^{b}	5.60±1.79^{ab}	11.4±3.49^{b}
Fungivores						
Aphelenchus	2	4.03±1.66	0.77±0.48	4.16±1.7	0.77±0.42	0.62±0.4
Aphelenchoides	2	6.14±1.69	3.53±0.7	3.65±1.14	3.23±1.14	2.11±0.91
Dorylaimoides	4	0.24±0.24^{a}	0.00±0.00^{a}	5.55±0.95^{b}	1.77±0.5^{ab}	1.86±0.99^{ab}
Filenchus	3	1.14±0.68^{ab}	3.31±0.8^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Paraphelenchus	2	2.51±1.28^{ab}	3.44±0.83^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Tylencholaimus	4	0.36±0.36^{ab}	0.00±0.00^{a}	0.71±0.21^{ab}	0.80±0.23^{b}	0.42±0.21^{ab}
Plant feeders						
Axonchium	5	0.00±0.00^{a}	0.00±0.00^{a}	0.2±0.2^{a}	1.06±0.13^{b}	0.22±0.17^{a}
Basiria	2	0.00±0.00	0.00±0.00	1.11±0.45	1.31±0.55	0.25±0.25

Coslenchus	2	0.00±0.00^{a}	2.72±1.08^{b}	0.00±0.00^{a}	2.72±1.16^{b}	0.00±0.00^{a}
Ditylenchus	2	1.89±0.95^{ab}	2.52±0.77^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Dorylaimellus	5	0.00±0.00^{a}	0.00±0.00^{a}	1.5±0.62^{b}	1.28±0.32^{b}	1.53±0.58^{b}
Helicotylenchus	3	0.08±0.08	3.11±2.12	2.09±0.49	0.96±0.85	2.11±0.81
Hemicycliophora	3	0.69±0.48^{a}	1.15±1.15^{a}	3.73±0.63^{b}	1.92±1.19^{ab}	0.43±0.43^{a}
Hoplolaimus	3	0.48±0.48^{ab}	0.00±0.00^{a}	3.47±0.51^{c}	1.94±0.54^{bc}	0.48±0.33^{ab}
Merlinius	3	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.41±0.76^{b}	0.17±0.17^{ab}
Paratylenchus	2	0.00±0.00^{a}	0.00±0.00^{a}	0.4±0.4^{a}	1.49±0.68^{b}	0.17±0.17^{a}
Pratylenchus	3	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.13±0.5^{b}	0.25±0.25^{ab}
Tylenchorhynchus	3	1.81±0.68^{ab}	2.71±0.89^{ab}	2.93±0.62^{a}	1.5±0.44^{ab}	0.42±0.42^{b}
Tylenchus	2	0.00±0.00^{a}	0.00±0.00^{a}	2.21±0.73^{b}	3.14±1.59^{ab}	1.64±0.58^{ab}
Omnivores						
Actinolaimus	5	0.72±0.5^{ab}	0.00±0.00^{a}	1.19±0.48^{abc}	2.07±0.24^{c}	1.57±0.38^{bc}
Aporcelaimellus	5	1±0.69^{ab}	0.00±0.00^{a}	1.89±1.01^{abc}	3.45±0.94^{c}	1.85±0.57^{bc}
Aporcelaimus	5	2.56±0.81	1.3±0.74	2.19±0.74	2.55±0.76	2.24±0.27
Campydora	4	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.52±0.2^{b}	0.00±0.00^{a}
Dorylaimus	4	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.26±0.32^{b}	1.59±0.47^{b}
Eudorylaimus	4	4.94±1.10	5.78±1.12	6.65±1.55	4.05±1.09	7.66±1.14
Mesodorylaimus	5	3.12±1.70^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Moshajia	4	0.56±0.37^{ab}	0.00±0.00^{a}	3.65±0.60^{c}	2.22±0.76^{bc}	2.61±0.5^{c}
Oriverutus	4	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.72±0.26^{b}	0.00±0.00^{a}

Thornenema	4	0.56±0.37^{ab}	0.00±0.00^{a}	1.27±0.39^{b}	1.44±0.29^{b}	0.9±0.31^{ab}
Predators						
Clarkus	4	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.38±0.23^{ab}	1.03±0.42^{b}
Coomansus	4	1.46±0.47	0.66±0.5	0.72±0.72	0.11±0.11	0.25±0.12
Discolaimus	5	1.12±1.12^{ab}	0.00±0.00^{a}	2.85±1.52^{bc}	4±0.86^{c}	2.07±0.56^{c}
Discolaimoides	5	5.81±1.2^{a}	5.23±1.01^{a}	1.49±0.54^{b}	1.68±0.53^{ab}	0.97±0.51^{b}
Mylonchulus	4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.32±0.21
Prionchulus	4	1.88±0.82^{ab}	6.71±1.36^{a}	0.29±0.19^{b}	0.3±0.20^{b}	0.08±0.08^{b}
Tripyla	3	0.00±0.00^{a}	0.00±0.00^{a}	0.72±0.21^{b}	0.41±0.22^{ab}	0.81±0.46^{ab}

According to the proportions in soil nematode communities' trophic structure, the trophic groups differed in abundance among various vegetation types (Table 3). The bacterivores were the most numerous trophic group among all the vegetation types. The abundance of plant parasites, omnivores were significantly higher in the SAD than in other vegetation types. Absolute abundance of total nematodes was significantly greatest in the SAD followed by SAP, SAB, ASA, and least in ASC. The total number of nematodes, plant parasites, bacterivores, and omnivores showed significant differences among different vegetation types. Bacterivores were the most diverse and abundant trophic group in all vegetation types, and fungivores were least abundant in subalpine vegetation types. Plant parasites were least abundant in Caragana and Artemisia dominated scrubland and were observed in larger numbers in subalpine vegetation types (BB>PP>O>PR>FF in SAD; BB>O>PP>PR>FF in SAP; BB>PP>O>FF>PR in SAB; BB>FF>O>PR>PP in ASA; BB>FF>PP>O>PR in ASC).

Table 4 Abundance of various feeding groups (Mean ± SE). Mean followed followed by different superscript letters are significantly different by the Post hoc-test at the 5% level of significance (p<0.05). (Where BB-Bacterivores; FF-Fungivores; PP-Plant parasite; O-Omnivores; PR-Predators and Nem-Total nematode abundance)

Trophic Group	**ASA**	**ASC**	**SAB**	**SAD**	**SAP**
BB	216±25.2^{ab}	221.5±44.6^{a}	212.125±33.4^{ab}	366±43.8^{ab}	373.75±53.1^{b}
FF	74±11.6	56.5±7.1	58.25±20.2	55±14.0	26.875±10.6
PP	18.5±4.4^{b}	43±13ab	85.5±16.05^{a}	213±77.1^{a}	43.75±16.9^{ab}
O	60.5±19.4^{ab}	25.5±4.7b	83.75±16.2^{a}	193±51.5^{a}	102.875±12.7^{a}
PR	21±7.9	26.5±3.9	49.5±9.1	69.5±16.2	35.5±7.9
Nem	390±47.8^{ab}	373±41.98^{a}	498.3±49.46^{abc}	896.5±144.55^{c}	582.7±50.44^{bc}

The cp1, cp2, cp3, cp4, cp5 groups of soil nematodes accounted for 4.29%, 47.05%, 21.05%, 17.05, and 10.55% of the total number of soil inhabiting nematodes, respectively in GNP comprising all vegetations. The variance analysis result showed that the values of cp3, cp4, and cp5 significantly differed ($p < 0.05$) among the vegetation types. The proportions of soil nematodes in the cp1 and cp2 groups in the SAD were higher than those in other vegetation types and least in ASC (Table 5).

Table 5 Mean value of the different c-p group taxa (Mean±SE). Mean followed by different superscript letters are significantly different by the Post hoc-test at the 5% level of significance (p<0.05)

c-p value	ASA	ASC	SAB	SAD	SAP
cp1	15.5±4.1	13±8.5	19±4.4	21±8.6	18.1±3.4
cp2	209±20.3	216±33.5	221.2±39.4	321±38.8	260.8±50
cp3	59.5±18.27^{a}	73±15^{a}	101.6±5.18^{ab}	252.5±63.5^{b}	150.5±17.7^{b}
cp4	50±13.8^{a}	49.5±6.3^{ab}	102.75±18.2^{ab}	164±36^{b}	102.1±14.8^{ab}
cp5	56±14.4^{ab}	21.5±3.7^{a}	44.5±17.4^{a}	131.2±36^{b}	51.1±10.3^{ab}

Soil Nematode Community Indices

The nematode diversity indices (MI, MI15, MI25, H') of soil nematodes were different but not statistically significant among vegetation types. The highest MI and H' values were in the vegetation SAD, whereas the lowest MI, MI5, and MI25 values were in ASC. The plant parasitic index in ASC and PPI/MI values of the vegetation ASA were significantly lower than those of other vegetation types (Table 6). Evenness (J') significantly differed, whereas the Simpson index (λ) was almost similar among vegetation types. The enrichment index (EI) and channel index (CI) exhibited significant differences among the vegetation types, and the structure index (SI) in the ASC was significantly ($p < 0.05$) lower than the subalpine vegetation types. (Table 6 and Fig 7). Nematode generic richness (GR) of the overall nematode community was higher in SAD and statistically significant (p<0.05).

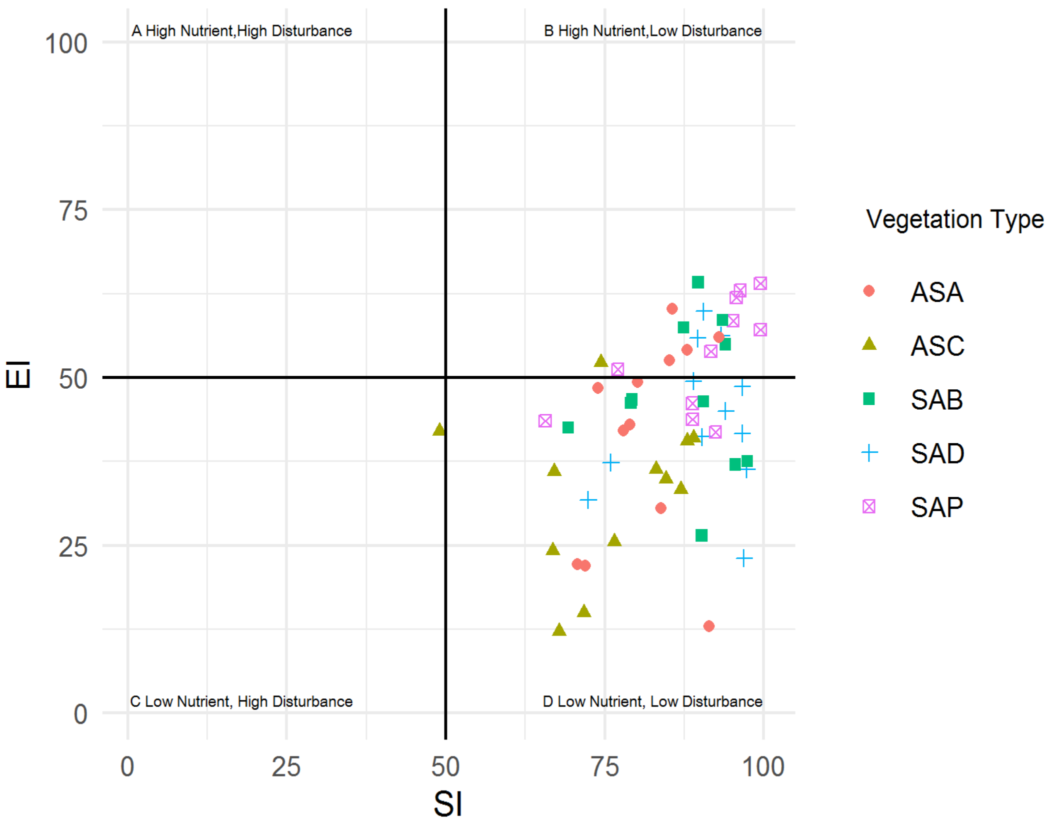

Figure 7 Enrichment index (EI) and structure index (SI) values for the five vegetation type. SAD, Subalpine Deodar; SAB, Subalpine Birch; SAP, Subalpine Pine; ASA, Alpine scrub *Artemisia*; ASC, Alpine dry scrub *Caragana*. Food web diagnostic comes within quadrant B and D (Majority falls within low nutrient low disturbance) for all, and the means are from Ferris et al. (2001).

Dissimilarity test based on the Bray-Curtis distance showed that soil inhabiting nematode community structure and the community composition among high altitude vegetation types were significantly different when evaluated at the genera level (PERMANOVA p-value =0.001, Fig. 8).Only those genera are plotted that contributed the most to the Bray-Curtis measures between vegetation types. They are not necessarily significantly different. There are number of genera that differ between one or more vegetation types compared.

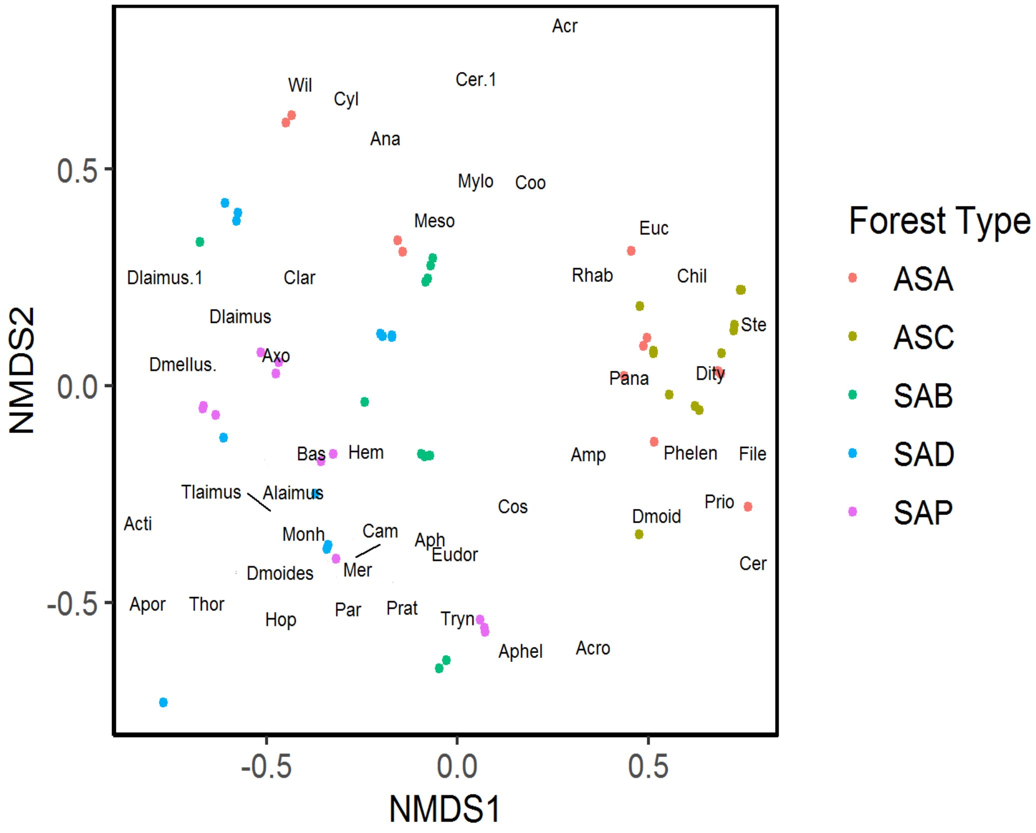

Figure 8 Bray-Curtis dissimilarity (NMDS ordination) of nematode communities based on abundances of nematode genera. Each point display the community found in an individual sample (n=12 samples per vegetation type x 5 types= 60). Points that are close proximity have more similar communities than points that are distant from each other. Colors show vegetation type, names of nematode genera are overlaid.

Abbr:Acro:Acrobeloides;Ana:Anaplectus;Aphel:Aphelencoides;Cer:Cervidellus;Cer.1:Ceratoplectus;Chil:Chiloplacus;Coo:Coomansus;Cyl:Cylindrolaimus;Dity:Ditylenchus;Dmoid: Discolaimoides;Euc:Eucephalobus;File:Filenchus;Mylo:Mylonchulus;Pana:Panagrolaimus ;Phelen:Paraphelenchus;Prio:Prionchulus;Rhab:Rhabdolaimus;Ste:Stegelletina;Tryn:Tylenchorynchus;Wil:Wilsonema.

To test significance, we compared the relative abundance of genera across vegetation types with Kruskal-Wallis test as relative abundance was not normally distributed (Table 3). There were differences in soil nematode composition among various vegetation typeswith a significant difference (p<0.05) in the relative abundance of bacterial feeders (*Ceratoplectus, Chiloplacus, Stegellitina, Cylindrolaimus, Wilsonema and Panagrolaimus)*, omnivores-predators (*Mesodorylaimus, Prionculus, Discolaimus, Tripyla, Aporcelaimus, Moshajia*) and some plant parasite and fungal feeders (Table 3). Major difference was seen between subalpine *Deodar* and alpine scrub *Caragana*and *Artemisia.* Pairwise multilevel comparisons of nematode community composition between various vegetation types showed significant difference between subalpine and alpine scrub region particularly SAD, ASA, and ASC (Table 6).

Table 6 Pairwise multilevel comparisons of nematode composition between different vegetation types. Subalpine deodar (SAD), Pinus (SAP), Birch (SAB), Alpine Scrub-*Artemisia* (ASA), and *Caragana* (ASC). Bray-Curtis dissimilarity metrics were used to assess differences in community composition. Asterisks indicate significant differences in community composition (p< 0.05).

Vegetation type	**ASA**	**ASC**	**SAB**	**SAD**
ASC	0.071	-	-	-
SAB	0.0067	0.0025**	-	-
SAD	0.004**	0.0025**	0.01*	-
SAP	0.0025**	0.0025**	0.0533	0.0533

Table 7 Soil nematode community indices of the five vegetation types. Means followed by different superscript letters are significantly different by the Post hoc-test at the 5% level of significance ($p<0.05$)

Index	**ASA**	**ASC**	**SAP**	**SAD**	**SAB**
MI	2.72±0.09	2.58±0.072	2.87±0.13	3.09±0.18	2.91±0.18
MI15	2.76±0.09	2.66±0.07	2.91±0.13	3.08±0.14	2.94±0.14
PPI	2.33±0.36^{b}	2.59±0.14^{b}	3.3±0.36^{a}	3.29±0.31^{a}	3.19±0.26^{a}
PPI/MI	0.84±0.13	1.01±0.07	1.16±0.14	1.1±0.13	1.12±0.12
EI	40.41±(5.94)^{ab}	31.27±(4.69)^{b}	54.52±(3.22)^{a}	38.48±(6.85)^{ab}	45.38±(3.5)^{ab}
CI	48.96±(9.91)^{ab}	58.6±(11.81)^{b}	13.7±(5.81)^{a}	30.85±(10.98)^{ab}	38±(12.1)^{ab}
SI	82.08±(2.52)^{b}	79.13±(2.66)^{b}	94.52±(1.48)^{a}	93.5±(1.16)^{a}	87.8±(2.67)^{ab}
H'	2.73±0.09	2.76±0.05	2.66±0.1	3.05±0.14	2.9±0.07
λ	0.91±0.01	0.92±0.006	0.89±0.01	0.93±0.01	0.93±0.00
J’	0.9±0.02^{ab}	0.92±0.01^{a}	0.83±0.02^{a}	0.88±0.01^{ab}	0.91±0.01^{b}
GR	20.62±1.26^{a}	20.12±1.09^{a}	25±2^{ab}	33.75±4.02^{b}	25.25±1.11^{ab}

4.4 Discussion

The study focused on soil nematode diversity among different vegetation types at the high altitude region of Gangotri National Park. GNP is a protected area and has fewer human disturbances, allowing soil nematodes to diversify but have harsh climatic conditions at high altitudes. Nematode diversity and nematode generic richness were relatively higher at a low elevation region of subalpine Deodar forest and lowest at high elevational alpine dry scrub *Caragana*. A similar study in the forests ecosystem by Yeates (2007) showed higher diversity and generic richness at lower elevation forest vegetation soils linked with a resilient and sustainable ecosystem. It can be inferred that the soil ecosystem in the subalpine vegetation types is more suitable for sustaining ecosystem balance and soil biota. The results agree with similar studies in the Beskydy Mountains spruce forests, deciduous forests of

Vihorlat, Slovakia, and Changbai Mountain, China (Hanel, 1996; Hanel and Cerevkova, 2010; Zhang et al., 2012). The values of cp3, cp4, and cp5 significantly differs ($p < 0.05$) among the vegetation types. Omnivorous nematodes have a long life cycle and are highly sensitive to disturbance (Ferris et al., 2001), and any change in the system will affect the high trophic level nematodes. Mostly, more predatory nematodes are present in less disturbed soil. Therefore, high trophic soil inhabiting nematodes may be considered an important component in long-term monitoring and climate change studies to understand the soil ecosystem pattern/change in the Indian Himalayan Region.

Bacterivores were recorded as a dominant group in all vegetation types and showed significant differences among various vegetation types. The results support findings of earlier studies that state dominance of bacterivores specifically in the forested regions (Yuen, 1996, Wasilewska, 1997, Yeates et al., 2000; Mladenov et al., 2004; Hánel and Cerevkova, 2010). Keith et al. (2009) reported that *Pinus* roots stimulate Bacterivores in contrast to *Betula* roots, which showed the importance of different plant species root inputs in governing nematode trophic structure. The current study by channel index has also been confirmed, which showed higher bacterial decomposition in SAP than SAB. The current study also found a higher abundance of fungivores in higher pH soil of alpine scrub (pH 8.4) than subalpine vegetation (pH 5.0).

Moreover, soil nematode taxa and populations also vary among forest types (Yeates, 2007). Abundance of Plant parasites and omnivores were comparatively higher in subalpine vegetation with acidic soil than in alpine vegetations. The relative abundance of predatory nematodes was high in vegetation where the soil is acidic, similar results achieved by Keith et al. (2006). Total nematode abundance and bacterivores significantly correlated with nitrogen, soil organic carbon, and soil moisture. Similar results were recorded in previous studies where bacterivores were higher in nitrogen-rich soil (Forge and Simard, 2001; Savin et al., 2001; Postma-Blaauw et al., 2005).

Regarding nematode generic richness, it decreased from low altitude subalpine vegetation to high altitude alpine scrubland and was significantly different among various vegetation types. Among the nematode genera found, *Acrobeloides* were predominant in ASA and SAB, whereas *Prismatolaimus* were the predominant genera in SAD, and SAP and *Cervidellus* in ASC and was significantly affected by the vegetation types. Earlier studies in the natural forest ecosystem reported that *the Acrobeloides* genus predominates (Hanel, 1996 and Cerevkova and Renco, 2009), which may be due to the accumulation of litter (Hanel, 2001).

The abundance of the genera *Aphelenchoides, Acrobeles, Plectus, Cephalobus, and Helicotylenchus, Eudorylaimus, Acrobeloides* represents the soil inhabiting nematode community in SAB. Similar diversity reported from Birch forest of south Bohemia, a territory of Slovak and Czech Republic, coniferous forest of southern Mississippi and Pine forest of Sweden where genera *Aphelenchoides, Paratylenchus, Plectus, Cephalobus, Filenchus, Helicotylenchus* along with *Acrobeloides* documented as the dominant genera (Hanel, 2000; Sohlenius and Bostrom, 2001; Matlack, 2001). Whereas, *Filenchus* and *Paratylenchus* were absent in the SAB and SAP due to the different elevation range of Birch and Pine-dominated regions in the study area. The most abundant nematode genera in the alpine region were *Acrobeles, Acrobeloides, Cervedillus, Plectus, Paratylenchus, Eudorylaimus, Discolaimoides* *and Aphelenchoides.* The abundance of *Teratocephalus* was highest in high altitude *Caragana dominated* region may be due to their ability to withstand extremely low temperature (Ruess et al., 1999; Hoschitz and Kaufmann, 2004).

The maturity indices of soil nematode communities in ecosystem formed by different vegetation were estimated along the elevational gradient. Generally, MI value varies from low to high according to disturbance level where the low value of MI indicates enriched disturbed soil ecosystem and high value as in pristine region indicates undisturbed ecosystem (Bongers and Ferris, 1999). The present study showed that highest stability among the vegetations was found in lower elevation Deodar dominated forest (3.09±0.18) and lowest in high elevation *Caragana* dominated scrubland (2.58±0.07), although the difference was not statistically significant. The low value of MI in high altitude scrub land showing lower stability may be due to harsh weather and low soil moisture in the region. The PPI/MI ratio is used to assess natural soils of forests or grasslands (Cerevkova, 2006; Cerevkova and Renco, 2009). The lower value of PPI/MI ratio indicates comparatively poor nutrient conditions than the nutrient rich soil (Bongers and Korthals, 1995; Bongers et al., 1997). In the current study, the mean ratio varies among vegetation types. Results showed that alpine scrub has a significantly lower PPI/MI ratio than in the subalpine forests. El and SI provide information on the ecosystems soil food web dynamics and stability (Ferris et al., 2001; Ferris et al., 2004). The present study revealed that vegetation types in the subalpine region are comparatively nutrient richer than alpine vegetation. The El values were significantly different among vegetation types. The structural index is an excellent means to understand whether the system is mature or structured where high value represents structured and low value as a disturbed ecosystem.

Previous studies reported that forest soil has a high SI value due to high trophic level linkage and a high abundance of predatory and omnivores in soil biota (Ferris and Matute, 2003; Tomar and Ahmad, 2009). In the present study, the values of SI were statistically significant among the vegetation type. Similar results on EI-SI were reported from alpine meadows of the Tatra National Park, Slovak Republic (Hanel, 2017). Results showed significant differences in the structural index among vegetation types, and EI-SI for alpine vegetation falls under quadrant D i.e., low nutrient and low disturbance (Ferris et al., 2001).Various nematode species composition is responsible for maintaining a structured soil food web (Hanel and Cerevkova, 2006). Channel index provides the data on the contribution of bacterial and fungal feeders in the decomposition pathway where higher values (>50%) indicate a fungal dominated decomposition pathway while lower value (<50%) signifies the bacteria dominated decomposition pathway in the soil. The mean values of CI in the present study vary (13.7-58.6) among vegetation types.

Pairwise multilevel comparisons of nematode community composition between various vegetation types showed a significant difference between the subalpine and alpine regions. The result showed that nematode composition in *Caragana* scrubland is close to ASA, showing similar nematode composition. It may be concluded that the vegetation type is a significant factor for soil nematode diversity as every vegetation type has a different local environment and physicochemical properties which affect the soil biota, specifically soil nematodes being present at various trophic levels in the soil food web. Among the various vegetation types in Gangotri National Park's high-altitude areas, there are substantial differences in the taxonomic composition, the energy flow channels, and nematode community structures, which provide actual examples for understanding the role of soil nematodes in key soil ecological processes in the region. We found that the soils from subalpine and alpine vegetations differed significantly in physicochemical characteristics and their soil inhabiting nematode communities. Nematode communities also changed with elevation, distinctly in the nutrient-deficient alpine dry scrubs. The study also showed that soil abiotic parameters correlated with the nematode composition. Our study is a pioneer study in the region showing the effect of vegetation type on soil nematode diversity; therefore, more similar studies are required to comprehend the relationship between nematode diversity and soil ecosystems of high altitude vegetations.

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CHAPTER 5

Assessment of Micro-Fauna Habitats and Correlation with Soil Nematode Diversity in High Altitudes of Gangotri National Park

5.1 Methodology

Soil analysis

Different parameters such as soil reaction (pH), soil moisture, organic carbon, available nitrogen, potassium, and phosphorus and soil texture were analyzed in the laboratory by using standard protocols. Significant variation in soil parameters along altitudinal gradient was observed. Soil has also been sampled along the elevation among different vegetation ranging from 3000-5000 masl.

The processed soil samples were used to different soil parameters such as electrical conductivity (µSc m^{-1}) determined by the method of Jackson (1967); soil pH determined in 1: 2.5 soil: water ratio using the pH meter with glass electrodes (Jackson, 1973). The organic carbon in the soil was estimated by methods suggested by Walkley and Black (1934); total N content by alkaline potassium permanganate (Kjeldahl, 1883); $NaHCO_3$ extractable P (Olsen et al., 1954) by spectrophotometer.

Statistical analysis

Statistical analysis of the data was done by using non-parametric Kruskal Wallis test (Zar, 1984) with a probability, the mean rank was compared at $P< 0.05$ by using SPSS 21.0 software. All parameters (i.e. soil reaction, electrical conductivity, soil moisture andavailable NPK) used for Spearman's correlation coefficient was calculated to find the relationships. Principal Component Analysis (PCA) was also performed to establish the relation between soil physicochemical properties, trophic groups, and nematode genera.

5.2 Result

Fifty-seven genera belong to 30 families and eight orders from Gangotri Valley and Thirty-four genera belongs to 20 families and eight orders from Nelang valley were recorded. *Aporcelaimus* and *Discolaimus* were the most dominant genera and also among the predators with the highest frequency of occurrence 88.24% and RD 4.71; 6.41% respectively, followed by *Plectus* and *Wilsonema* (frequency of occurrence 82.35%). *Cryptonchus*, *Tylocephalus*, and *Alirhabditis* found to be rare with a frequency of occurrence 5.88% and RD 0.03%. Whereas, among the predators, Mylonchulus recorded as a rare genus (N=1 and AF=5.88%). Plectus and *Wilsonema* were the most dominant genera among the bacterivorous with the highest frequency of occurrence 82.35 % and RD 6.07; 6.87% respectively, while *Cryptonchus* was the rarest with a frequency of occurrence 5.88% and RD 0.03%. Among plant parasite, *Dorylaimellus* was the most dominant genus (N=11, AF= 64.7%) while *Pratylenchus*, *Merlinius,* and *Coslenchus* were the least common (N=3, AF=17%). The most frequent genus among fungivores was *Aphelencoides* (N=12, AF=70%) and least was *Tylenchus* (N=8). *Moshajia* was the most dominant genus in omnivores (N=14, AF= 82%) whereas *Campydora* was least frequent N=3. In Nelang valley, *Stegelletina* was the most dominant genera and also among the predators with the highest frequency of occurrence 100% and RD 4.89. Various genera dominate among the bacterivorous with the high frequency of occurrence, while *Amphidelus* and *Cephalobus* were the least with the frequency of occurrence 20% and RD 0.5% and 0.28% respectively (Table 8).

Table 8 Population structure of soil-inhabiting nematodes in Gangotri Valley and Nelang Valley

Genera	**Gangotri Valley**		**Nelang Valley**	
	AF%	RD%	AF%	RD%
Bacteriovors				
Acrobeles	76.47	5.55	95	6.79
Acrobeloides	70.59	3.64	95	6.79
Alirhabditis	5.88	0.03	x	x
Cephalobus	35.29	0.83	20	0.28
Cervidellus	17.65	1.04	95	10.85
Chiloplacus	64.71	1.91	95	4.95
Cryptonchus	5.88	0.03	x	x
Eucephalobus	17.65	0.31	65	1.78
Pseudacrobeles	52.94	0.83	55	2.11
Stegelletina	41.18	1.08	100	4.89
Plectus	82.35	6.07	95	4.06
Anaplectus	29.41	0.45	x	x
Ceratoplectus	52.94	1.94	75	1.95
Cylindrolaimus	70.59	3.61	x	x
Prismatolaimus	64.71	10.86	60	1.5
Rhabdolaimus	35.29	0.59	75	2.95
Wilsonema	82.35	6.87	50	1.33
Teratocephalus	47.06	1.77	75	2.89
Mesorhabditis	29.41	0.45	25	0.56
Monhystera	29.41	0.38	x	x
Nothacrobeles	35.29	0.56	75	0.83
Panagrolaimus	17.65	0.21	75	2.39
Protorhabditis	5.88	0.03	x	x
Rogerus	23.53	0.49	x	x
Tylocephalus	5.88	0.03	x	x
Amphidelus	x	x	20	0.5
Fungivore				
Aphelencus	52.94	1.67	45	1.45

Aphelenchoides	70.59	3.3	90	3.73
Tylencholaimus	64.71	0.62	x	x
Paraphelenchus	x	x	70	3.73
Filenchus	x	x	55	2.34
Dorylaimoides	58.82	2.29	x	x
Plant Parasite				
Basiria	35.29	0.97	x	x
Axonchium	52.94	0.66	x	x
Coslenchus	17.65	1.25	25	1.22
Ditylenchus	x	x	70	2.67
Helicotylenchus	52.94	1.39	20	2.73
Hemicycliophora	52.94	1.77	10	0.56
Hoplolaimus	58.82	1.8	x	x
Merlinius	17.65	0.73	x	x
Paratylenchus	23.53	1.01	x	x
Pratylenchus	17.65	0.69	x	x
Tylenchus	47.06	2.43	x	x
Tylenchorynchus	58.82	1.67	70	2.17
Dorylaimellus	64.71	1.42	x	x
Omnivore				
Campydora	17.65	0.28	x	x
Eudorylaimus	76.47	5.66	90	5.34
Moshajia	82.35	2.71	x	x
Oriverutus	17.65	0.42	x	x
Thornenema	76.47	1.42	x	x
Dorylaimus	52.94	1.53	x	x
Mesodorylaimus	x	x	40	5.06
Actinolaimus	82.35	2.01	55	1.78
Aporcelaimellus	82.35	3.05	x	x
Aporcelaimus	88.24	2.78	x	x
Predator				
Clarkus	23.53	0.38	x	x
Coomansus	17.65	0.28	55	1.33
Discolaimus	88.24	3.78	x	x

Discolaimoides	64.71	1.7	90	4.56
Mylonchulus	5.88	0.07	x	X
Prionchulus	23.53	0.21	75	3.95
Tripyla	35.29	0.49	x	x

Bacterial feeders dominate in the study area, followed by predators, omnivores, and plant-parasites in abundance. Bacterivores (44%) represented the highest generic diversity, followed by predators (19%), plant parasites (18%), omnivores (12%), and fungivores (7%). Based on individual abundance, bacterivores represented the highest abundance (49%), followed by predators 15%, omnivores 14 %, plant parasite 13%, and fungivores 9% in Gangotri valley while in high elevational one Nelang valley, Bacterivores (55%) represented the highest genetic diversity, followed by fungivores (15%), predators (12%), plant parasites (12%), and omnivores (6%) (Fig 9).

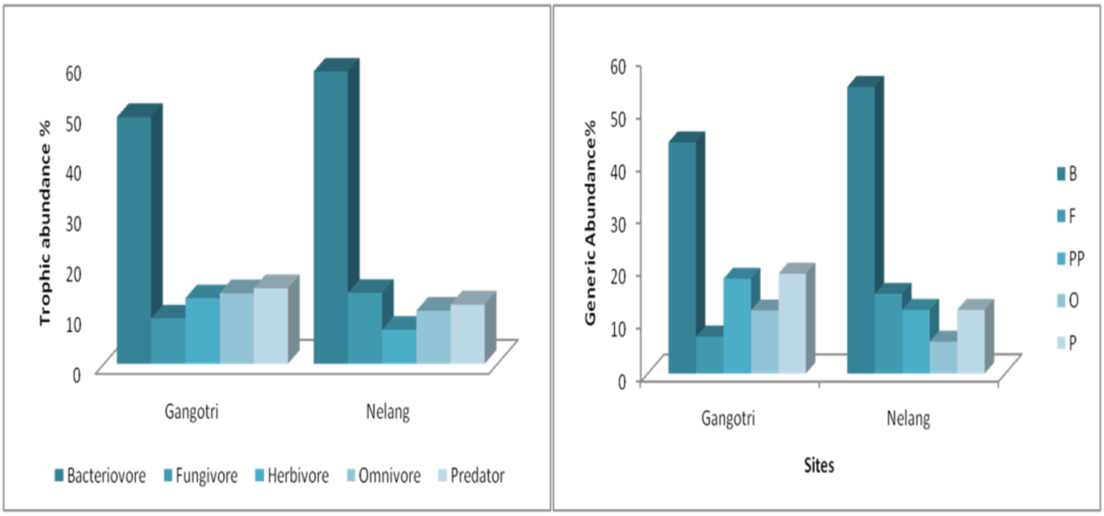

Figure 9 Trophic Abundance and Generic abundance in Gangotri and Nelang Valley

Among 10 orders, Dorylaimida (24%) represented the most abundant order (Fig. 10), followed by Rhabditida (17.2%), Plectida (17.1%), Tylenchida (13.9%), Triplonchida (10.4%) Monhysterida (1%), Aphelenchida (8.1%), Araeolaimida (3.5%), Mononchida (4.6%) Enoplida (1%), in Nelang valley while in Gangotri valley, Dorylaimida (29.3%) represents the most abundant order followed by Rhabditida (17.4%) and Monhysterida (1%) as least abundant order. Dorylaimida found to be the most abundant group indicating the fewer disturbances in the Gangotri and Nelang valley. The result is in agreement with the findings of other studies on soil nematode community structure in forest areas (Johnson et al., 1972; Thomas, 1978; Sohlenius and Wesilewska, 1984; Neher et al., 2005).

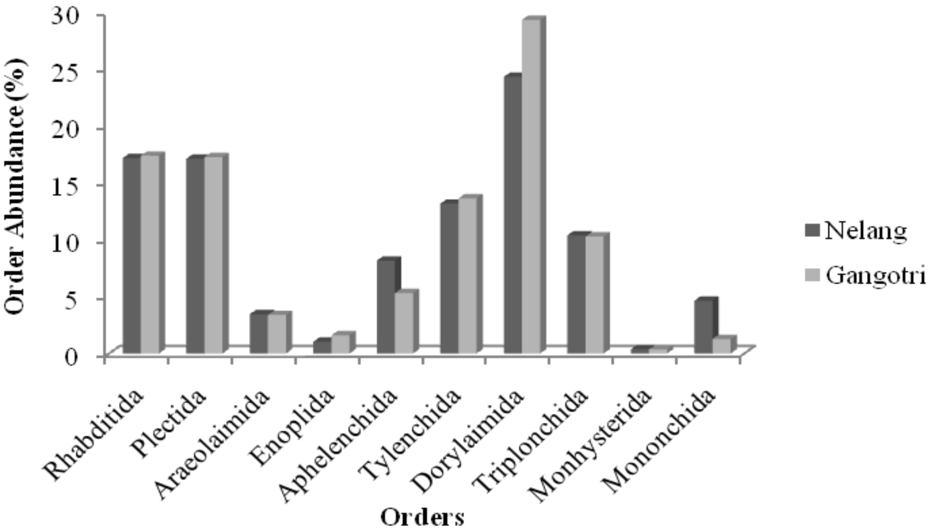

Figure 10 Ordinal diversity (Abundance) in Gangotri and Nelang Valley.

Relationships between Trophic group and soil properties

Bacterial feeders were the most diverse and abundant functional group in all vegetation types and fungivores were least abundant in sub alpine vegetation types. Plant parasites were least abundant in *Caragana* and *Artemisia* dominated scrub land (B>PP>PR>O>FF in SAD; B>O>PR>PP>FF in SAP; B>O>PP>FF>PR in SAB; B>PR>FF>O>PP in ASA; B>FF~PR>PP>O in ASC). The communities were determined, mainly by pH, nitrogen, soil moisture and soil organic carbon contents, which are mostly related to vegetation. There was no significant difference in phosphorus content, while soil moisture content was higher in subalpine vegetation types except in SAB where soil moisture is comparatively low (Table 9).

Table 9 Soil physicochemical properties in different vegetation types. Different lower case letters denote significant differences across forest types $p<0.05$.

Parameters	SAD	SAP	SAB	ASA	ASC
PH	5.7±0.15^{a}	5.4±0.06^{a}	5.5±0.11^{a}	8.3±0.17^{b}	8.5±0.14^{b}
EC	137.5±14.28^{ab}	134.5±8.7^{a}	171.6±5.1^{b}	67±14.9b^{c}	68.25±6^{c}
N	0.47±0.06^{a}	0.49±0.08^{a}	0.04±0.01^{b}	0.25±0.05^{c}	0.23±0.06^{c}
P	0.52±0.17^{a}	0.36±0.1^{a}	0.44±0.09^{a}	0.37±0.05^{a}	0.28±0.03^{a}
SM	23.8±4.7^{b}	14.7±3.5^{ab}	8.61±1.23^{ab}	7.03±0.86^{a}	7.1±1.1^{ab}
OC	8.71±1.3^{a}	8.1±1.6^{a}	5.5±1.2a^{b}	3.36±0.6^{b}	4.3±0.9^{ab}

Trophic groups differed significantly between the subalpine and alpine vegetation types ($p < 0.05$) except fungivores and predators. Organic carbon and soil moisture were lower in high altitude alpine vegetation as compared to sub alpine vegetation type (Fig 11 and Table 9). The pH varies from acidic to alkaline from subalpine to alpine dry scrubs (5.4-8.8). Soil properties were significantly correlated with the nematode trophic groups (Table 9).Total nematode abundance and bacterial feeders were positively correlated with soil moisture, soil organic carbon and nitrogen content, whereas, total nematode abundance was negatively correlated with soil pH. Soil of subalpine vegetation type was acidic, while, soil of alpine scrub was found to be alkaline in nature. Phosphorus showed no significant correlation with trophic groups. The predatory nematodes showed neutral responses to the various soil properties.

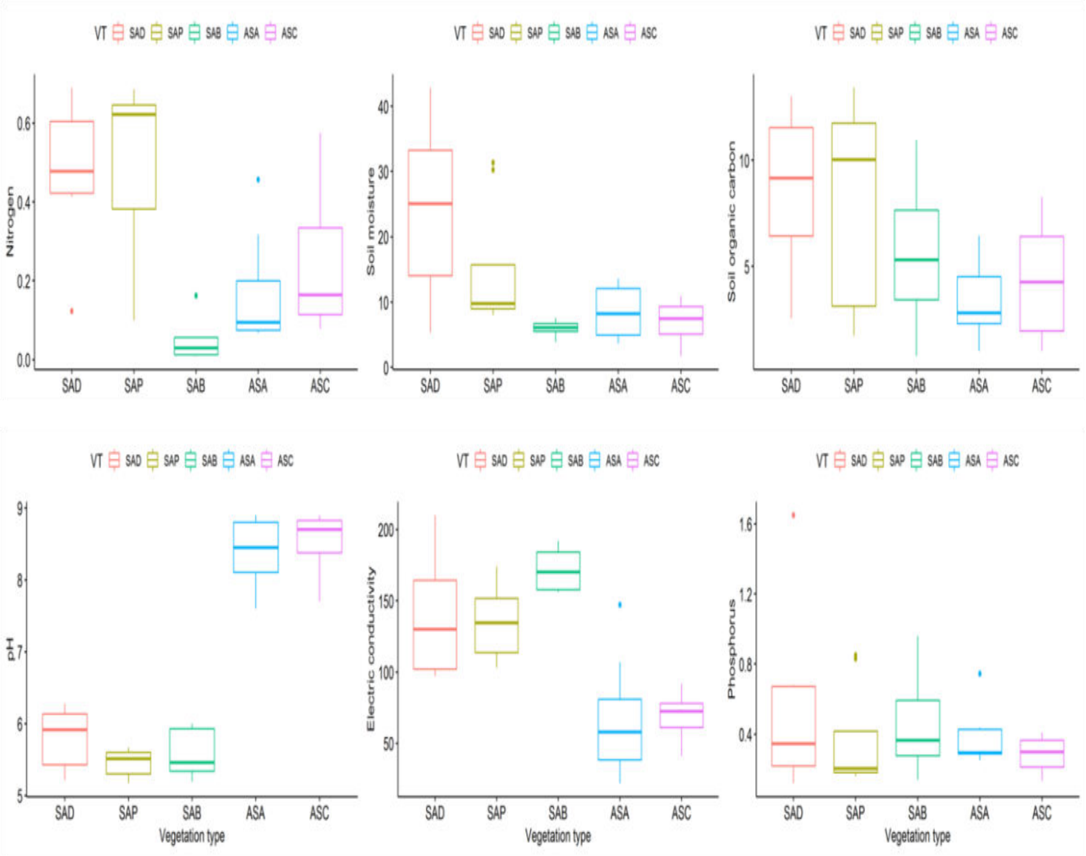

Figure 11 Soil parameter across different vegetation types-Nitrogen content; Soil moisture; soil organic carbon; soil pH; Electric conductivity and phosphorus content among vegetation types.

The study showed that the plots with higher nitrogen, organic carbon, and soil moisture are associated with higher faunal diversity of bacterial and fungal feeders and also with the occurrence of genera, Nothacrobeles, Axonchium, Dorylaimus, and Pseudacrobeles. Omnivores and predators were highly contributing feeders. The analysis also highlighted that the majority of genera showed a negative relation with elevation, while phosphorus and potassium were the least contributing factors in the study area. The correlation analysis among the different soil parameters showed that SOC has significant positive correlations with nitrogen (r=0.964), phosphorous (r=0.794), and soil moisture (r=0.661).

Table 10 Spearman correlation coefficients between the variables in Gangotri National Park. Asterisks indicate significant correlation at probability levels of 0.05(*), 0.01(**) and 0.0001 (***).

	PH	EC	N	P	SM	OC	BB	FF	PP	O	PR	Nem
PH		0	ns	ns	ns	0.01	0.01	ns	ns	0.01	ns	0.003
EC	-0.59		ns	ns	ns	ns	ns	ns	0.004	0.003	ns	0.005
N	-0.14	-0.13		ns	0.007	0	0.005	ns	ns	ns	ns	0.002
P	-0.07	-0.08	-0.25		ns	ns	ns	ns	ns	ns	ns	ns
SM	-0.23	0.19	0.51	-0.12		0.01	0.04	ns	ns	ns	ns	0.003
OC	-0.39	0.18	0.59	-0.1	0.37		0	ns	ns	ns	ns	0.003
BB	-0.4	0.17	0.53	-0.06	0.32	0.67		ns	ns	ns	ns	0
FF	0.07	0.07	-0.09	0.12	-0.06	0	0		0.025	ns	ns	0.03
PP	-0.25	0.44	-0.02	-0.08	-0.07	0.19	-0.05	0.35		0	0.03	0.001
O	-0.38	0.55	-0.04	-0.15	0.1	0.19	0.28	0.28	0.58		0.003	0
PR	-0.11	0.06	0.12	-0.24	-0.09	0.06	0.09	0.21	0.34	0.46		
Nem	-0.46	0.43	0.31	-0.12	0.16	0.54	0.68	0.34	0.49	0.78	0.45	

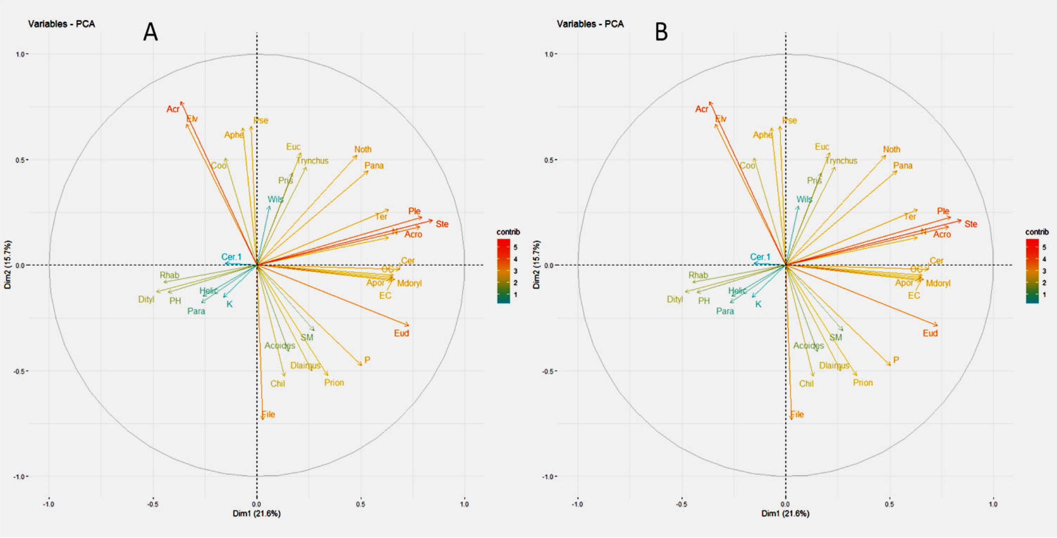

Figure 12 Principal Component Analysis on soil parameters and nematode data of Gangotri (A) and Nelang Valley (B). Environmental variables and nematode genera are marked by arrows.

The principal component analysis (Fig. 12A) explains 43.8% of the variation. The first axis explains 27.8 % and 16% defined by the second axis for Gangotri valley.

In the case of Nelang valley, the principal component analysis explains 37.3 % of the variation. The first axis explains 21.6 % and 15.7% defined by the second axis for Nelang valley. Omnivores and predators were highly contributing feeders. The analysis also highlighted that the majority of genera showed a negative relation with elevation except for the Acrobeles at an elevation range of 4000-5000m. Bacterivores showed a positive relation with Organic carbon and nitrogen. *Helicotylenhus, Ditylenchus, and Paratylenchus* were associated with pH and Potassium levels in Nelang valley (Fig. 12B). Soils samples collected from high altitudes (>3500m) has significantly less SOC and soil nutrients except from 3700 to 3800m in gangotri valley. This may be explained as the impact of long term human influence in the forms of camping and altering the natural habitat. As an example, famous trekking destination and pilgrimage site of Bhojbasa is situated in this range. Soil moisture and soil organic carbon decreases along the elevation, it may be due to harsh weather conditions at higher altitude where no vegetation can survive. The high affinity between soil physicochemical characteristics and abundance of nematode in different ecosystem has been used as criteria to assess soil health and to understand the dynamics of food webs. This study may further help in climate change study in current scenario of global warming.

Trends along climatic gradients

Both abiotic factors (soil properties) and biotic factors (trophic links, relationships with plant diversity) were studied to understand the underground ecological parameters driving the pattern in nematode abundance and diversity in high altitude region of GNP.

Spearman correlation analysis showed the different trophic group reacts with the climatic gradient. mainly Soil moisture, Soil total organic carbon, and nitrogen, where organic carbon and nitrogen showed significant correlation with bacterial feeders and total nematode composition whereas elevation showed a significant negative correlation with plant parasite and total nematode composition and soil moisture were positively related with the various trophic group what was not significant except with omnivores and the total nematode composition (Table 10).

5.3 Discussion

The soil in the park was sandy loam throughout whereas pH varies from acidic to basic i.e 5 ~8 from low to high elevation but has no correlation with nematode community structure of Gangotri valley. Bacterial decomposition pathway recorded in the study area attributes to less acidic to slightly neutral pH ranges from 5.22-to-6.02 indicating good soil fertility which might relate to soil organic matter turnover in the Gangotri region (Porazinska et al., 1999; Popovinci and Ciobanu, 2000). The plots with higher nitrogen, organic carbon and soil moisture are associated with higher faunal diversity of bacterial and fungal feeders and also with the occurrence of genera, *Nothacrobeles, Axonchium, Dorylaimus*, and *Pseudacrobeles.*

Omnivores and predators were highly contributing feeders. The analysis also highlighted that the majority of genera showed a negative relation with elevation except the *Acrobeles at* elevation range of 4000-5000m while phosphorus and potassium were the least contributing factors in the study area.The correlation analysis among the different soil parameters showed that SOC has significant positive correlations with nitrogen (p=0.007), soil moisture (p=0.038) and nitrogen with phosphorous (r=0.029) in Gangotri valley.

The current study also found higher abundance of fungivores in lower pH soil of subalpine vegetation (pH 5.06) compared to alpine scrub (pH 8.4). This may be hypothesized due to higher dietary base (fungus) of fungivores in lower pH zones as suggestive from works by Alexander (1977) and Yeates and coworker (1996). These studies conferred soils with lower pH levels to be favorable for fungal growth due to higher acid tolerance and lower competition with other soil micro biota. Moreover soil nematode taxa and population also varies among forest types (Yeates, 2007). The abundances of plant parasites and omnivores were higher in Deodar forest with acidic soil than in other vegetations. The relative abundance of predatory nematodes was high in vegetation where soil is acidic in nature. Similar results were achieved by Keith et al. (2006). Total nematode abundance and bacteriovores were significantly correlated with nitrogen, soil organic carbon, and soil moisture. Similar results were recorded in previous studies where bacterivores were higher in nitrogen rich soil (Forge and Simard, 2001; Savin et al., 2001; Postma-Blaauw et al. 2005). Bacterivors showed a positive relation with organic carbon and nitrogen. *Helicotylenhus, Ditylenchus* and *Paratylenchus* were associated with pH and Potassium level in Nelang valley. Bacterivorous nematodes were positively related to Nitrogen which conforms to the findings of Ou W et al. (2005); Moreno et al. (2006) and Devetter et al. (2017). SOC and soil moisture was higher in lower altitudes as compared to the higher elevations, where cold and dry climate prevails, and the soil remains devoid of any vegetation cover most of the time. Soils samples collected from high altitudes >3500m were significantly low in SOC and soil moisture except from 3500-3600 and 3700-3800 m elevation. This pattern is may be due to human activities like camping in the area which is a major stopover in the Gaumukh and Tapovan pilgrim route in this elevation.Among various elevation range, there is a comparatively good amount of phosphorus content in birch dominated area at mid elevation range (3500-3800 m) than the other vegetation occupied region along the elevation. This result conforms to the study done in Nanda Devi biosphere reserve forest. The correlation analysis indicated that the soil of this area was also rich in nutrients such as nitrogen and phosphorous at lower elevation where vegetation prevails below tree line up to 3800 m elevation range. The nutrient content of the soil changes along with the changes in temperature as it is dependent on the decomposition of organic matter. Thus, any future increment in the temperature of this region may increase the soil's organic carbon and nutrients such as nitrogen and phosphorous. On the other hand, soil samples collected from high altitudes (>3500 m) significantly lacks SOC and soil nutrients except from at 3400-3500 and 3700 - 3800 m. This may be explained as the impact of long term human influence in the forms of camping and altering the natural habitat. As an example, the famous trekking destination and pilgrimage site of Chirwasa and Bhojbasa are situated in this range. Soil moisture and soil organic carbon decreases along the elevation, it may be due to lesser or no tree crown cover, harsh weather conditions at higher altitude of alpine region above tree-line i.e >3800 m where dry alpine scrub *Artemisia* is prevalent. This provides the baseline trend of soil parameter in the study area.

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CHAPTER 6

Experimental Setup of Open Top Chamber in Sub-Alpine and Alpine Region of Gangotri National Park To Study The Effect of Change in Temperature and Humidity on Soil Nematode

6.1 Methodology

Experimental warming set up: Open Top Chambers (OTCs)

Six hexagonal OTCs, each chamber was built with high-quality multilayered polycarbonate sheets (4 mm thickness) with high solar transmittance (transmits ~ 92% of visible light, reflect 4% of incoming radiation and pass on ~ 85% of incoming energy). Each site provided with a minimum of two replicates. Chambers installed in the spring of the year 2016. Open top chambers (OTCs) are innovative and cost-effective devices for investigating the effects of altered climatic factors such as temperature, humidity on growth dynamics, and yield response of plants and soil microbial community activity. These chambers are hexagonal in shape and opened on the top. The opened design ensures that the system has airflow and receives the same amount of precipitation during rainfall. A plot is left untreated adjacent to each OTC within two meters having the same area and similar vegetation and serves as a natural control. Due to the thick snow cover, strong winds, and low temperatures during winter in the Himalayan alpine region, experiments can only be conducted during the growing season, which lasts on average from May till mid of November.

Weather stations (HOBO U23 Pro v2 Temp and HOBO U23 Pro v2 2X external temperature data logger) are installed in the OTC and the adjacent outside control, at a height of 15 cm above the ground surface for recording air temperature every hour. Soil temperature and moisture are also measured at 5 cm depth. Samples were collected every year from chambers and adjacent control plots at each site. Following the same procedure as mentioned above after sample collection.

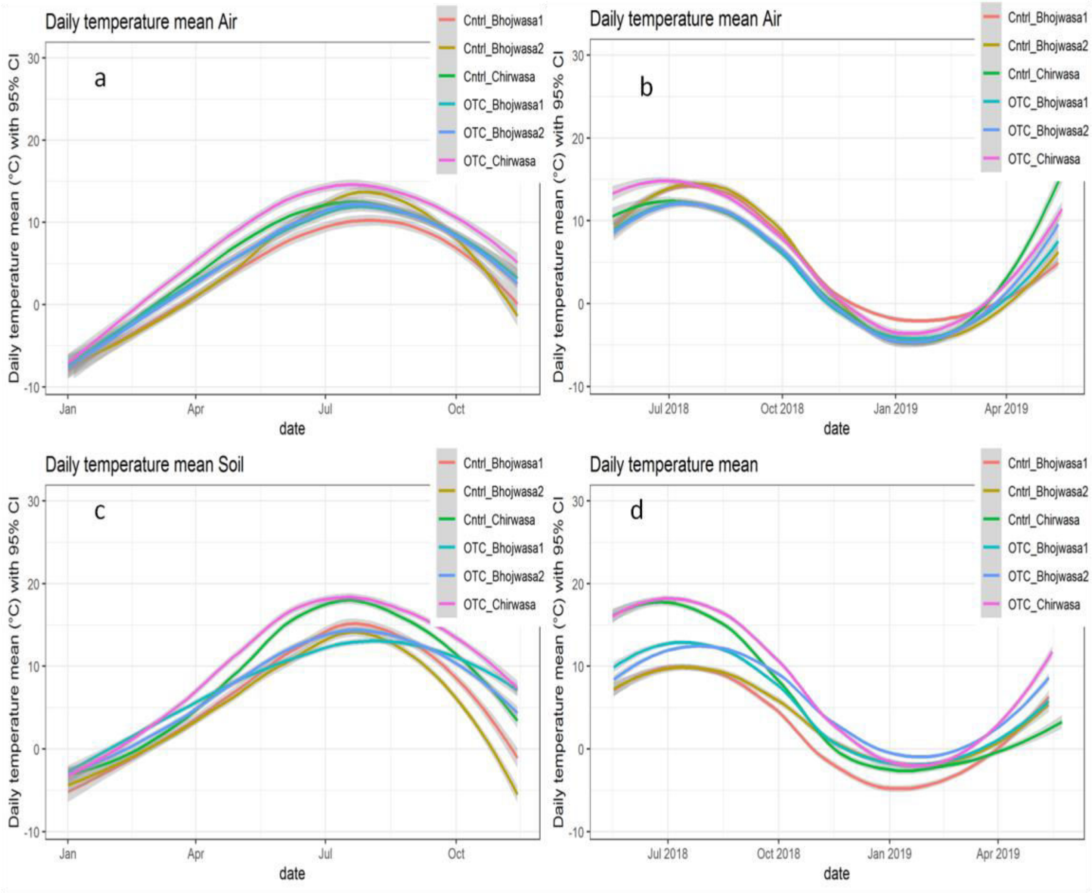

Figure 13 a-c Daily mean temperature of air and soil (Jan-Nov, 2017); b-d Daily temperature mean of air and soil (May 2018-April 2019).

6.2 Results

There is difference in temperature between experimental and control plots but has no significant effect of on means of daily temperature variability. Comparison of means of soil and air temperature inside OTC and control plot depicted significant difference in case of maximum air and soil temperature in both Chirbasa and Bhojbasa (p-value <0.01). However, though the minimum and average soil and air temperature were slightly (~1 degree C) higher inside OTC than control plot for both places, the differences were not statistically significant except for minimum air temperature (p-value<0.5) for both years (Fig. 8). Nematode community structure was studied in treatment and control plots to understand the effect of change in temperature on soil mesofaunal group. High altitude terrestrial ecosystems are stressed by extreme climatic conditions and strongly nutrient-limited due to direct and indirect effects of snow cover and low temperature in the alpine and subalpine region. Hence minor change may lead to change in community structure of various free-living nematodes which is sensitive to change which will be useful for evaluating the impact of climate change in Indian Himalayan system. Dissimilarity tests based on the Bray-Curtis distance showed that nematode communities from the treatment plot were significantly different from the control plots when assessed at the generic level (PERMANOVA p=0.011) (Fig. 14).But no inter-annual difference in the community (PERMANOVA p=0.179) with a significant decrease in the relative abundance of predaceous genera *Tripyla, Discolaimus* and an increase in *Rhabdolaimus* and *Pseudacrobeles and Acrobeloides* for the treatment plot (Table 10)

Table 11 Average density of nematode taxa in control and OTC plots (values marked with asterisk are significantly different (p<0.05).

Genera	Control	OTC	Genera	Control	OTC
Acrobeles	3.58±0.62	2.35±1.11	*Axonchium*	3.66±0.53	2.3±0.54
Acrobeloides	4±0.71	5.55±1.11*	*Tylenchus*	3.62±0.94	3.2±1.19
Cervidellus	2.66±0.18	2.7±0.57	*Hoplolaimus*	5.91±1.97	2.9±0.47
Chiloplacus	6.37±2.89	3.05±0.59	*Tylenchorynchus*	6.54±2.10	3.95±0.69
Eucephalobus	4.87±1.32	4.9±0.63	*Pratylenchus*	4.41±1.86	1.45±0.40
Pseudacrobeles	2.29±0.20	3.2±0.48**	*Helicotylenchus*	7.29±3.32	2.15±1.06
Stegelletina	1.41±0.55	2.3±0.60	*Aporcelaimellus*	3.08±1.14	1.55±0.83
Plectus	3.45±0.51	1.5±0.67	*Eudorylaimus*	8.16±1.05	4.9±1.87
Anaplectus	0.83±0.39	1±0.54	*Oriverutus*	3.33±1.51	2.25±0.83

Ceratoplectus	1.41±0.46	1.7±0.64	*Discolaimus*	9.79±3.21	1.55±0.55**
Cylindrolaimus	2.41±0.18	1.15±0.55	*Discolaimoides*	3.12±0.90	1.75±1.04
Prismatolaimus	1.41±0.41	1.7±0.61	*Dorylaimellus*	5.66±1.03	4.7±0.72
Rhabdolaimus	4.62±0.95	8.5±1.54*	*Tripyla*	3.5±1.14	1.75±0.85*
Wilsonema	1.79±0.68	2.45±0.95	*Mononchus*	1.08±0.27	1.25±0.37
Teratocephalus	0.12±0.13	0.5±0.32	*Clarkus*	1.95±0.41	1.6±0.81
Aphelencus	1.87±0.40	1.6±0.28	*Prionchulus*	2.08±0.61	1.55±0.42
Aphelencoides	1.70±0.33	1.95±0.49	*Mylonchulus*	1.5±0.66	1.45±0.60

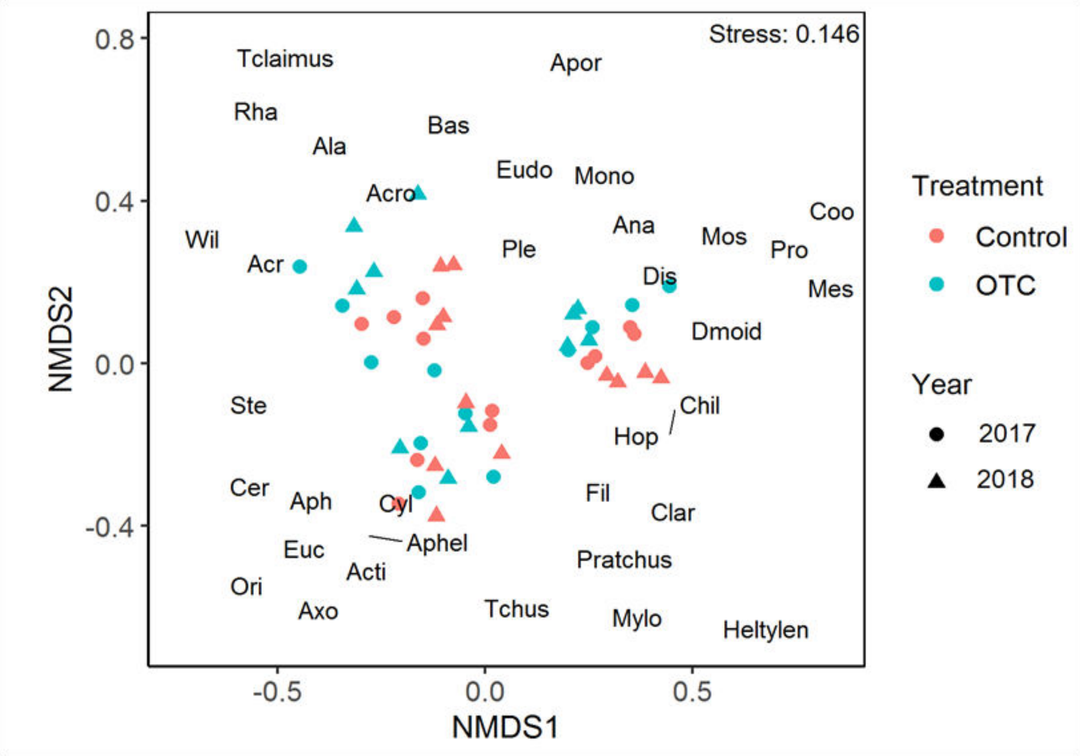

Figure 14 NMDS ordination (Bray-Curtis dissimilarity) of nematode communities based on relative abundances of nematode genera. Each point reflects the community found in an individual sample (n=12 per treatment x 2 years). Points that are close together have more similar communities than points that are far apart.

To identify nematode food web properties the enrichment index (EI) and structural index (SI) were calculated. These are the extension to the maturity index (Ferris et al.

2001). Enrichment and structural index guild plot showed a control plot was more enriched but less structured than the control plot based on their response to disturbance and enrichment (Fig. 15). It can be concluded that an increase in temperature increased the disturbance level inside the OTC. The OTC plots have a comparatively greater abundance of nematodes but the nematode community was less diverse and mature (lower MI in OTC) compared to the control plots but was not statistically significant.

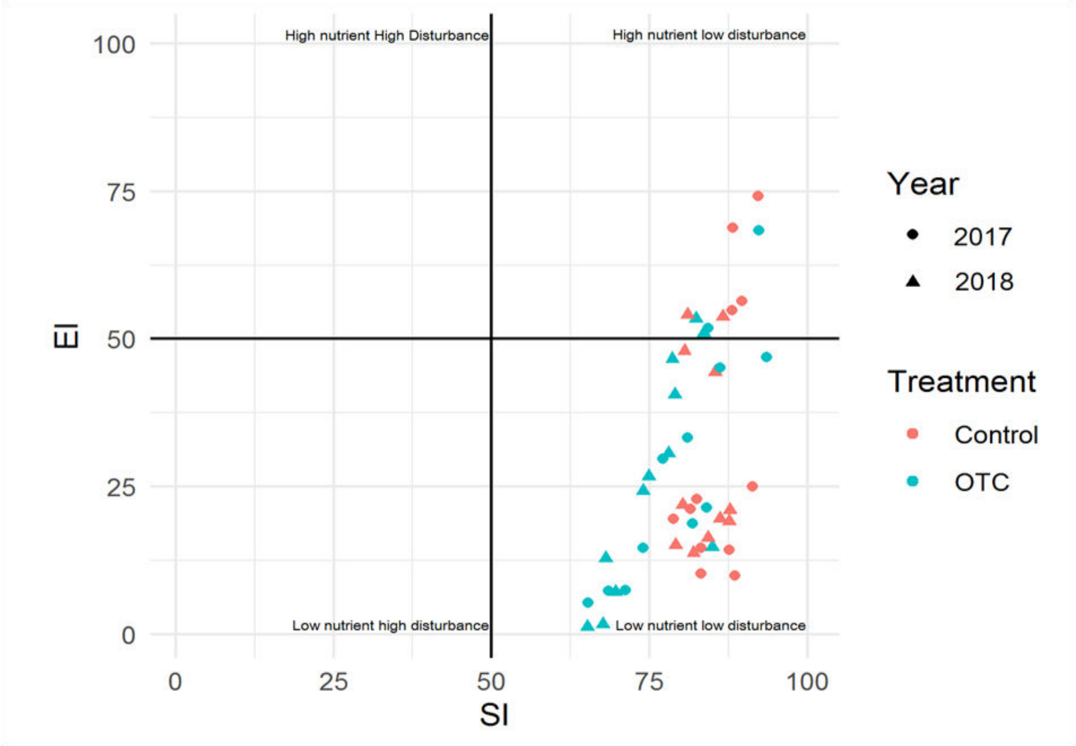

Figure 15 Structure - Enrichment plot by treatment and year. Quadrants are labeled after Ferris et al. (2001). Colors show treatment: Control plots are red and OTC plots are blue. Shapes show year: circles are 2017 and triangles are 2018.

There was a significant effect of treatment on total nematode abundance, MI by year. Overall, Genera richness, Shannon Index was greater in Control plots but was not statistically significant while MI and total nematode abundance were significantly greater in control plots (p-value=0.005, 0.030 respectively) (Table 12). Structural

Index was significantly greater in OTC plots (p-value=0.002) SI indicates an increase in trophic linkages which corresponds to disturbance level (physical or chemical). Plant-parasitic nematode abundance was greater in the OTC plots than the control plots. In terms of the trophic group, Bacteriovores increased in OTC plots which may be due to the increase in microbial activities in treatment plot, and Predators and omnivores significantly decreased in Open top chambers (p-value=0.001, 0.04).

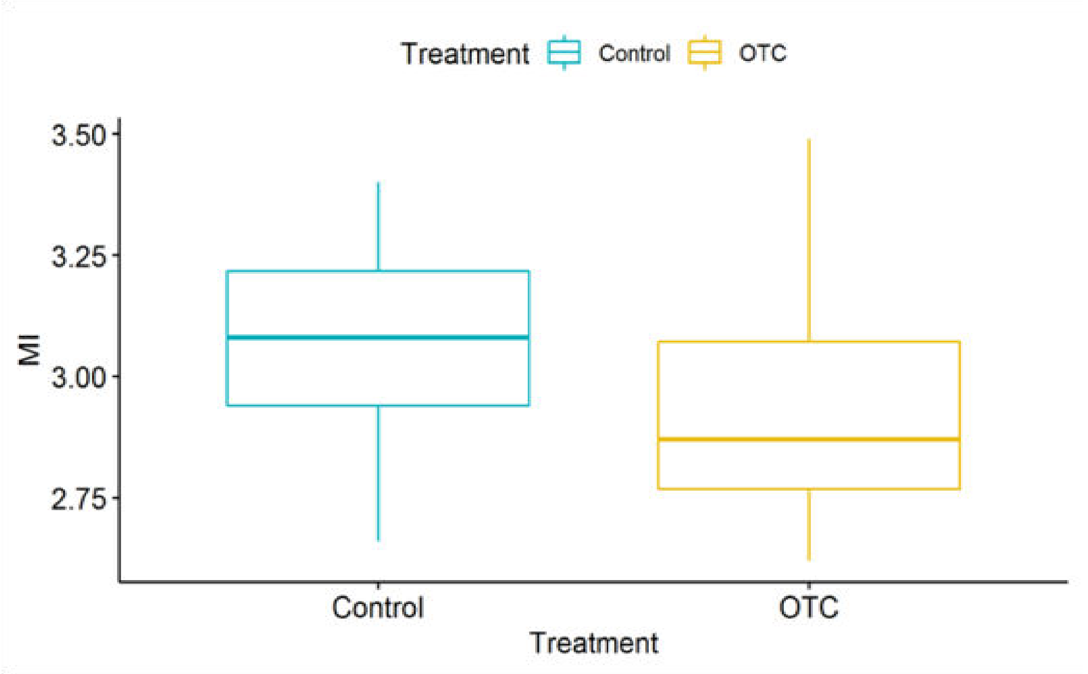

Figure 16 Maturity index across Open top chamber and Control plot.

Table 12Mean Value ±SD of various nematode community Parameter for control plot and OTC Plots

Indices	Control	OTC
Maturity Index(MI)	3.06±2.91	2.91±0.21
Nematode Channel Ratio(NCR)	0.76±0.11	0.78±0.10
Plant parasite Index(PPI)	0.38±0.28	0.28±0.11
Shannon diversity(H')	3.16±0.11	3.1±0.15
Enrichment Index(EI)	30.71±20.88	27.58±19.1
Structural Index(SI)	84.19±5.68	77.67±8.04

Table 13 Mean Value ±SD of soil nematode trophic groups in control plot and OTC Plots

Trophic group	Control	OTC
BF	47.3±17.0	48±19.3
FF	14.9±6.90	11.7±5.88
PP	12.6±8.15	25.9±19.5
O	21±8.28	16.7±12.0
PR	30.7±12.8	19.3±11.4

6.3 Discussion

Soil and the air temperature was nearly ~1 °C higher inside OTC in comparison with the control plot outside for almost throughout the year in 2017 except in August-September in Bhojwasa. Bacterial feeder abundance is higher in OTC but was not significant. Acrobeloides and Rhabdolaimus are the only bacterial feeder whose density is significantly increased by the elevated condition of OTC.

Nematode channel ratio and plant parasitic Index was comparatively slightly lower in OTC .Maturity Index was lower in Open top chamber and significantly different (P-value 0.0091).Predatory nematode abundance was also found lower than the control plot but was not significant. Dissimilarity tests based on the Bray-Curtis distance showed that nematode communities from the OTC were significantly different from the control plots when assessed at the generic level (p=0.001).but has no inter-annular difference on community composition (p=0.179).Results showed that an increase in plant parasite nematodes may be due to the decrease in the number of predaceous nematodes as they consume the plant parasite nematodes. Thick vegetation cover inside OTC may be one of the explanations of this. The temperature difference was nearly ~1.9 °C higher inside OTC from may 2018-may 2019, but the soil moisture was different across the treatment and control plot, suggesting that warming has indirect effect on nematode diversity by affecting soil moisture in treatment plot. As a result, warming-induced soil moisture could be the the major reason for differences in nematode responses between OTC and Control and for the decrease in soil nematodes trophic groups except for bacterial feeders and Plant parasites in OTC plots in the present study.

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CHAPTER 7

Morphological and Molecular Characterization of Bacteriovorous Nematodes

7.1 Methodology

7.1.1 Culturing of Nematode

Single bacterial feeder nematodes (adult female) were selected and individuals were picked onto previously prepared agar plates (plates were incubated at 27-30 °C for bacterial growth to prepare feed for nematodes). Plates were monitored daily for up to three to four weeks to identify growth and multiplication of nematodes on cultured plates.

Nematodes cultured as above are hand picked from culture plates and placed in distilled water for cleaning and fixed using Formalin- Acetic Acid fixative (FAA) (composition of fixative - 8 ml of 40% Formalin + 2 ml Glycerol + 1-2 drops of Glacial Acetic Acid + 90 ml water). Next, formalin-acetic acid fixative were boiled in the microwave or heater and poured over nematode suspension in the tubes after bubbles subside which were formed due to boiling. Tubes were capped and left undisturbed for 1 hour and then labeled with locality, host, site and date.

Nematodes were picked manually transferred in ethanol-glycerol solution and placed in a desiccators for dehydration at least for 15 days. Nematodes were picked under microscope from cavity blocks and placed over a slide and mounted using wax and heating it on slide warmer table. Specimens were identified to the genus level using an inverted microscope at 100 X magnification.

7.1.2 DNA extraction

Freezing and thawing method were used to isolate nematode DNA. Individual nematodes were handpicked from culture plates and rinsed using sterile water to clean prior to DNA extraction. Each nematode was placed to a PCR tube (measuring 0.2 ml) having solution of 50 µl of 0.2 M Tris-HCl maintained at pH 8.0 and 5 µl proteinase K (20 mg/ml). Next, samples sonicated at 60°C for 10 min in a GT Sonic ultrasonic water bath (GTsonics) and incubated at 60°C for 30 min. Samples were mixed on a vortex mixer and frozen for 5 min at -80°C in freezer, next incubated for 10 min at 90°C. Samples were mixed on a vortex again. The cycle of freeze/thaw was repeated, after vortexing again for 30 sec at high speed samples were centrifuged at 2000 rpm for 2 min, stored at -20°C until ready for use or used directly for amplification.

7.1.3 PCR amplification and sequencing

PCR amplification was performed on a fragment of the 18S small subunit (SSU) rRNA gene and mitochondrial COI (cytochrome c oxidase I) region. A 20 ul reaction was carried out using 1X PCR Buffer, 0.5 µM of each primer, 0.15 mM of each dNTP, 1.5 mM MgCl2, 1 U of Taq DNA polymerase (Invitrogen), ultrapure distilled water, and 2 µl of DNA template of nematode sample lysate.

PCR conditions applied were: Initial denaturation for 4 min at 94°C. PCR used 34 cycles in a thermocycler. The DNA chain denaturation into two separated strands occured at 94°C for 30 s, annealing of primers at 56°C for 30 s; and extension of new strand at 72°C for 1 min, with a final extension at 72°C for 10 min in a DNA thermal cycler (Applied Biosystem by Thermo fisher scientific).

The successful PCR products were prepared for sequencing with ExoSAP protocol using two enzymes Shrimp Alkaline Phosphatase and Exonuclease I to strip the DNA of unwanted dNTPs and primers 0.1µL of SAP, 0.2uL of Exo, 2.7µL of milliQwater and 10uL product are mixed together. Next the mixture is incubated for 20min at 37°C, Next at 80°C for 15min. Once they had been prepared for sequencing, the samples were sent to outsource sequencing facility to be sequenced using the same primers as the PCR.

DNA sequence analyses

Sequences were visualized and edited using Chromas 1.6 (Technelysium Pty Ltd., South Brisbane, Australia). To cross check, we compared our querry sequences using GenBank BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). CLUSTAL W was used to compare DNA sequence data implemented in BioEdit v 7.0.9.0 software (Hall 1999). The BLAST result showed the obtained sequences from mitochondrial genes 99% to 100% matched with their respective species. The evolutionary history was inferred using the Neighbor-Joining method using the Kimura 2 Parameter model (Kimura, 1980) from the sequences acquired. The phylogeny of the group was constructed using the evolutionary distance data (Saitou et al. 1987). Estimation of Overall Evolutionary Divergence over sequence pairs in COI gene and 18S r RNA gene for the barcode region were calculated for individuals collected and cultured, using the analytical tools on using the Kimura 2 Parameter model (Kimura, 1980). Evolutionary analyses were conducted in MEGA7. In cases of discrepancy between morphological taxonomy and molecular based sequence, the specimens were morphologically examined and identified to ascertain their identity.

7.2 Results

7.2.1 Taxonomy of bacteriovorous nematodes of Gangotri national park

Order: **Rhabditida** Chitwood, 1933

Rhabditida consist of parasitic and free-living forms. Body size ranging from microscopic forms to the size visible to bare eyes. Cuticle ranges from smooth to finely annulated; longitudinal striae may present. Continuous or offset lip region with 3 or 6 lips; In some species, labial probolae may also be present. Shape of amphid varies circular, pore-like, or slit-like. Ccheilostom, gymnostom and stegostom of different sizes and shapes constitute Stoma which may vary according to feeding habits (narrow, collapsed, or tubular in free-living bacteriovores; fine needle-like stylet in plant parasitic type and more or less spacious stoma with armature in predators. Lateral fields with 2-7 incisures. Pharynx consists of corpus, isthmus and basal bulb. Median swelling may or may not be present in corpus. Well-developed basal bulb or overlaps over intestine. Excretory system well-developed; excretory pore may distinct or indistinct. Intestine having narrowed to wide lumen. Didelphic, amphidelphic or monodelphic, prodelphic female reproductive system. Post uterine sac present or absent. Males with or without genital papillae. Varied shaped spicules, Tail of variable shapes, similar or different in both the sexes.

Type suborder:
Rhabditina Chitwood, 1933

Family **Cephalobidae** Filipjev, 1934

Transverse annulated cuticle. Sharp border on lateral fields, irregularly separated by longitudinal lines. Anteriorly six cephalic probolae and three labial probolae in lip region. Amphids present at lateral lips. Tubular stoma, generally narrow;cheilostom wider than gymnostom and stegostom. Dorsal wall of metastegostom have tiny tooth-like projection. Corpus cylindrical, isthmus strong and narrow and Pharyngeal basal bulb with grinder. Monodelphic, prodelphic female reproductive system, reflexed ovary posterior to vulva. Post- uterine sac short. Strong spicules. Genital papillae present. Distinct phasmids. Tail short, conoid to elongated conoid. Mostly similar in both sexes.

Type genus: ***Cephalobus*** Bastian, 1865.

Order **Plectida Malakhov, 1982**

Superfamily **Plectoidea:**

Cuticle with annulation. No sub-cuticular ornamentation. Lateral fields having two lateral alae. Hypodermal glands and pores may present. Somatic setae present. Six equal lips are present in lip region. Four prominent cephalic sensilla present. Amphids present as transverse slit or unispiral. Funnel-shaped stoma constituting cheilostom, gymnostom may be cylindrical or arched with sclerotized lining and a stegostom tapers posteriorly. Transverse or longitudinal valves in terminal or subterminal basal bulb with denticles. Didelphic, amphidelphic female reproductive system; reflexed ovaries, present on alternate sides of intestine. Caudal glands present.Sometimes, small papillae surrounds the spinneret.

Type subfamily:
Plectinae Örley, 1880
Other subfamilies
Anaplectinae Zell, 1993
Wilsonematinae Chitwood, 1951.

Order- **PLECTIDA**
Superfamily- **PLECTOIDEA**Orley, 1880
Family- **PLECTIDAE**Orley, 1880
Subfamily-**PLECTINAE** Orley, 1880
Genus- ***Ceratoplectus*** Andrassy, 1984
Species- ***Ceratoplectus armatus*** (Butschli, 1873) Andrassy, 1984

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National Park, Bhojwasa, 18.x.2016, 4♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 1 ♀♀, coll. P. Kashyap.

Dimensions: Female- L= 0.43 (0.37-0.47) mm, a=23.42(21.9-24.9) µm, b=3.46 (3.13-3.70) µm, c= 10.76 (9.5-12.3) µm, c′=3.47 (2.7-4.0) µm, V= 52.90(51.0-57.3) µm, T=41.0(30-46) µm.

Description: Small body (0.37- 0.47 mm long), ventrally curved on heat fixation, thin cuticles 0.8-1 thick, finely annulated 0.6-0.8 µm wide. Thin and scattered somatic setae. Labial region 2.5-3 µm and 8-9.5 µm wide and not offset. Cephalic setae extending on the anterior margin 3.0-4 µm long directed forward and outward. Amphids about oval 2.5-3.0 µm wide round to oval. Stoma cylindrical 15-19 µm long. Oesophagus 84-97 µm long, 22-27% of body length. nerve ring at the level of Isthmus at anterior end. Oval shaped basal pharyngeal bulb, with valves. Excretory pore at 69-76 µm from the anterior. Amphidelphic female reproductive system. Vulva having transverse slit at midbody, usually flat or slightly protruding. Vagina slightly curved or straight occupies 1/3 of vulval body diameter. Rectum 1.0-1.5 times longer than anal body diameter. Vulva-anus distance e varies between 173-184 µm. Tail conical, ventrally curved, gradually narrowing, 40-46 µm long, anal body width vary between 11-13 µm. Three caudal glands and a terminal spinneret present at tail end.

Habitat and Locality: Soil near Betula and Deodar patches in subalpine and alpine region of Gangotri National Park.

Distribution: Galapagos archipelago, Kenya, St. Kitts (West Indies), Brazil, Bolivia, Peru, Australian Lizard Island, Slovakia.

Remarks: ***First record from India***. All the measurements and descriptions are in agreement with earlier description

Order- **PLECTIDA**

Superfamily- **PLECTOIDEA**Orley, 1880

Family- **PLECTIDAE**Orley, 1880

Subfamily-**PLECTINAE** Orley, 1880

Genus- ***Anaplectus*** De Coninck and Schuurmans Stekhoven, 1933

Species- ***Anaplectus granulosus*** (Bastian, 1865) de Coninck and Schuurmans Stekhoven, 1933

Material Examined: India, Uttarakhand, District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 3 ♀♀, coll. P. Kashyap

Dimensions: Female- L= 0.90 (0.86-0.92) µm, a=26.42 (25.7-27.1) µm, b=3.44 (3.12-3.75) µm, c= 16.58 (13.0-20.0) µm, c′=2.63 (2.14-3.13) µm, V= 56.28(55.1-57.4) µm, T= 57(49-69) µm.

Description: Body Cylindrical. Annulated cuticle. At anterior region, gradually narrows in pharyngeal region and posteriorly at tail; ventrally arcuate in posterior region. Somatic setae absent, caudal setae present. Labial region truncate, slightly offset from body. Indistinct Labial sensilla with visible nerve endings. Setiform cephalic sensilla, begins on 3rd or 4th annule. Lip region consists of six separate lips. Inner labial sensilla's nerve endings open in cheilostom and nerve ending of outer labial sensilla open in anterior of lips. A transverse slit- amphid, visible at level with anterior region of stegostom. Nerve ring at the level of anterior part of isthmus. Posterior to nerve ring, papilliform deirid present inside lateral field. Excretory cells at the level of posterior part of isthmus. Cuticularized excretory duct, refractive ring appears as semicircular sclerotized structure surrounding the excretory pore. Narrow, undifferentiated cheilostom present and marked by lips. Shorter gymnostom having sclerotized gymnorhabdia (arcute). Stegostom comprises of two parts: broad cylindrical tube anteriorly and posterior narrow posterior part, encased by pharyngeal muscular tissue. Pharynx noticeably divided into anterior corpus, middle isthmus and bulbus below isthmus. Corpus moderately widens posteriorly. Isthmus narrower than corpus and separated by a break in cuticular lumen and muscular tissue from corpus. At corpus-isthmus junction, orifices of subventral gland open in lumen of pharynx. Oval basal bulb, having grinder. Conoid or cylindrical glandular cardia surrounded by intestinal tissue. Three gland-like cells surrounds intestine-rectum junction. Didelphic, amphidelphic female reproductive system, ovary branched, antidromously reflexed. Anterior genital structure present on right side and posterior genital part on left side in intestine. Narrow and short oviduct. Broad and long uterus which is divided into proximal spermatheca, crustaformeria in centre region and tubular (thick walled) section in distal part. Vagina straight, cylindrical and short without sclerotizations. Slightly protruding pore-like vulva, lips often or sometimes sunken. Short rectum consist of strong cuticularized lumen. Conoid, ventrally arcuated tail, with blunt rounded terminus. Caudal glands and spinneret is noticeably present.

Habitat and Locality: Soil around Pinus wallichiana trees in Chirwasa, Gangotri National Park, Uttarakhand.

Distribution: Known distribution from Germany, Sweden, Costa Rica, Romania, Indonesia, Bulgaria, Netherlands, United Kingdom, United States of America, Belgium, Denmark, Austria, France, New Zealand.

Remarks: ***New record from India***. All the measurements and descriptions are in agreement with earlier description.

Order- **PLECTIDA**

Superfamily- **PLECTOIDEA**Orley, 1880

Family- **PLECTIDAE**Orley, 1880

Subfamily-**PLECTINAE** Orley, 1880

Genus- ***Plectus*** Bastian, 1865

Species- ***Plectus parvus*** Bastian, 1865

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Gaumukh, 18.x.2016, 8♀♀, coll. P. Kashyap.

Dimensions: Female- L= 0.35 (0.35-0.36) µm, a=21.05(20.7-21.3) µm, b=2.67 (2.61-2.72) µm, c= 8.68 (8.4-9.0) µm, c′=3.42 (3.23-3.62) µm, V= 43.7(42.6-44.4) µm, T = 41.25 (40-42) µm.

Description: Female- Body slightly arcuate upon heat fixation, tapering towards ends, more at posterior end. Cuticle 0.9-1.0 µm thick; finely striated outer and inner cuticles. Striae comparatively more prominent at tail region. Two cuticular alae on lateral fields. Somatic setae scattered. Cervical region have 2-4 pair of somatic setae. Lip region continuous with body. Triangular Lips, radially ridges appears to be amalgamated. Anteriorly directed cephalic sensilla 1.6-2.0 µm long and setose. Small, circular amphidial fovea located at middle to posterior level of stoma and have prominent ductus amphidialis. Cylindrical Stoma; stegostom gradually tapers at base. Pharynx comprising of cylindrical corpus, narrow isthmus and an oval shaped basal bulb of 13-15 µm long. Nerve ring encircles pharynx at 58-60 µm. Excretory pore slightly below the nerve ring with cuticularised duct, distance from anterior at 65-77 µm. Intestine with wide lumen and granular. Amphidelphic didelphic reproductive system; dorsally reflexed antidromously ovaries. On right side of intestine, anterior ovary is present and and posterior ovary on left side; infrequently crosses each other.Vagina measures 22-26% of body diameter; usually with a pair of sphincter muscles. Ovoid vulva nearly equatorial transverse slit; slightly protruded vulva lips. Cylindrical tail, slightly arcuate, tapers to a bluntly rounded terminus with spinneret.

Habitat and Locality: From soil of moss covered area of alpine region at 4000 m elevation in Gangotri National Park.

Distribution: In India- Uttar Pradesh; elsewhere- Turkey, Antarctica, United Kingdom, Germany, Romania, Sweden, Austria, Italy, Netherlands, Bulgaria, Denmark, United States of America, and Costa Rica.

Remarks: ***New record from Uttarakhand***. All the measurements and descriptions are in agreement with earlier description.

Order- **PLECTIDA**
Superfamily- **PLECTOIDEA**Orley, 1880
Family- **PLECTIDAE**Orley, 1880
Subfamily-**PLECTINAE** Orley, 1880
Genus- ***Plectus*** Bastian, 1865
Species- ***Plectus minimus*** Cobb, 1893

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Gaumukh, 18.x.2016, 4♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Bhojwasa 18.x.2016, 1 ♀♀, coll. P. Kashyap

Dimensions: Female- L= 0.39 (0.38-0.39) mm, a=21.05(20.7-21.3) µm, b=2.67 (2.61-2.72) µm, c= 8.68 (8.4-9.0) µm, c′=3.42 (3.23-3.62) µm, V= 43.7(42.6-44.4) µm, T =29.4 (29-30) µm.

Description: Female adult: Small to medium-sized (0.38-0.39 µm) slender body, slightly curved ventrally upon fixation, tapering at both ends. Outer cuticle with transverse striations and inner cuticle smooth. Fine and sparse somatic setae (3 pairs) in the cervical region. Lip region fused with body contour. Papilloid labial sensilla (both outer and inner) Setose cephalic sensilla (2.0-2.5 µm long) and anteriorly directed. Oval shaped amphids, mostly at mid level of stoma. Plectoid type-thin-walled, tubular, weakly cuticularised stoma. Slightly cuticularised, rod-shaped Cheilostom; cuticularised and parallel-walled gymnostom; Wide stegostom with abruptly narrow down at base. Pharynx consists of a cylindrical corpus with indistinct isthmus and ovoid basal bulb with simple grinder. Nerve ring positioned at about 50-52% of pharynx length. Excretory pore situated after the nerve ring with the duct forms a loop first and joins the renette cell. Cardia measures 4-7 µm in length and have narrow lumen. Intestine with wide lumen. Thin-walled rectum. Crescent-shaped slit as anus. Didelphic amphidelphic female reproductive system, Dorsally reflexed ovaries. Anterior and posterior ovaries are on opposite side of intestine. Vulva equatorial having transverse slit. Ventrally arcuate tail with a crooked spinneret at terminal. Tail gradually tapers towards tip. Pairs of two subdorsal, one subventral and one lateral together as caudal setae pointing downwards linearly arranged caudal glands present with prominent nuclei. Terminal seta positioned at anterior to tail terminus (at about 8.5-10 µm).

Habitat and Locality: Soil from alpine region near Gaumukh at elevation 3900 m above tree line in Gangotri National Park, Uttarakhand, India

Distribution: In India- Uttar Pradesh; Elsewhere- Sweden, Spain, Congo, Democratic Republic of the, Ecuador.

Remarks: ***New record from Uttarakhand***. All the measurements and descriptions are in agreement with earlier description.

Order- **PLECTIDA**

Superfamily- **PLECTOIDEA**Orley, 1880

Family- **PLECTIDAE**Orley, 1880

Subfamily-**PLECTINAE** Orley, 1880

Genus- ***Plectus*** Bastian, 1865

Species- ***Plectus parietinus*** Bastian, 1865

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Gaumukh, 18.x.2016, 4♀♀, coll. P. Kashyap.

Dimensions: Female- L= 1.53 (1.47 -1.68) mm, a=17.20 (16.68 -17.67) µm, b= 4.9 (4.69 -5.08) µm, c= 14.34 (13.65 -15.12) µm, c′= 2.84 (2.77 -2.89) µm, V= 52.57 (50.62 -53.85) µm, T= 108 (103 -111) µm.

Description: Female- Body large sized (1.3-1.6 mm) ventrally curved on fixation, wide (92-95 µm) at mid body region. Distinctly annulated thick cuticle, 3.5- 4 µm on mid region. Lip region strongly set off by a constriction, lip diameter measures 17-19 µm wide. Circular amphid, 3- 4 µm wide, located at anterior to mid of stoma. Stoma measures 40 µm long. Pharynx differentiated into anteriorly corpus, middle narrow isthmus and basal pharyngeal bulb pyriform shaped 42-43 µm wide. Nerve ring is located at 156- 165 µm from anterior region. Excretory pore present slightly below nerve ring, distance from anterior end to excretory pore is 173-183 µm i.e. ~ 67 % of pharyngeal length. Didelphic amphidelphic female reproductive system well developed reflexed ovaries, measuring 260-279 µm and 243-258 µm respectively. Vulva has transverse slit. Distance between vulva and anus measures 618 µm. ventrally arcuate tail, 103-111 µm long with a spinneret at terminal. Tail gradually tapers towards tip.

Habitat and Locality: Collected from soil of alpine region in between bhojwasa and Gaumukh at elevation of 3800 m in Gangotri National Park, Uttarakhand.

Distribution: Sweden, Germany, Romania, Antarctica, Netherlands, United Kingdom of Great Britain and Northern Ireland, United States of America, Denmark, South Africa, Faroe Islands.

Remarks: Length of the body is slightly longer i.e 0.16 mm than the earlier reported species of *P. parietinus.* Common species in aquatic and terrestrial habitat.

Order- **PLECTIDA**

Superfamily- **PLECTOIDEA**Orley, 1880

Family- **PLECTIDAE**Orley, 1880

Subfamily-**PLECTINAE** Orley, 1880

Genus- ***Plectus*** Bastian, 1865

Species- ***Plectus assimilis*** Butschli, 1873

Material Examined: India, Uttarakhand, District Uttarakashi, Gangotri National park, Chirwasa, 19.x.2016, 4♀♀, coll. P. Kashyap.

Dimensions: Females- L = 0.76 (0.75-0.78) mm, a = 16.66 (16.0-17.2) µm, b = 3.73 (3.60-3.81) µm, c = 11.35 (9.32-13.39) µm, V = 50.19 (49.30-51.89) µm, T = 88.33 (84-92) µm.

Description: Medium sized (0.75-0.78 mm), stout body, slightly tapering at anterior region and posteriorly where body tapers into short tail. Transverse striae marked on cuticle, prominently in cephalic part of the body. Two longitudinal alae interrupts striae on each side of body. Four cephalic setae taper at end with broad base. Cephalic setae located over two annules after lips. Rounded truncate- lip region, very low, 10-11 µm wide. Very low six connate lips not set off from body contour. Lips delineated or outlined by starting of transverse striae. Stoma wide and 20-24 µm long. Amphids 10-12 µm from anterior located at level of anterior region of stoma, diameter (2-2.5 µm). Esophageal region~one-fourth of body length. Excretory pore located at 113-115 µm from anterior region i.e approx 54-61% of esophagus length, present slightly posteriorly to the nerve ring. Basal pharyngeal bulbus 30-31 µm long.Vulva equatorial present at 393-397 µm from anterior region i.e 50-51% of whole body length, vulva lips flat. Rectum 23-29 µm long. Tail short (c = 9.32-13.39) tapering rapidly to very narrow tip. Anal body diameter 20 µm and tail 80-82 µm long. Total of five caudal setae are present - three subventral and two subdorsal. Tail with spinneret 2-2.5 µm long.

Habitat and Locality: Collected from mixed forest (Birch, Blue pine and Deodar) of Chirwasa in Gangotri National park, Uttarakhand.

Distribution: Romania, Sweden, United States of America, Austria, Bulgaria, Costa Rica, United Kingdom of Great Britain and Northern Ireland

Remarks: All the measurements and descriptions are in agreement with earlier description.

Order- **PLECTIDA**

Superfamily- **PLECTOIDEA** Orley, 1880

Family- **TERATOCEPHALIDAE**Andrássy, 1958

Genus- ***Teratocephalus*** De Man, 1876

Species- ***Teratocephalus costatus*** Andrássy, 1958

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Bhojwasa, 18.x.2016, 7♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 4 ♀♀, coll. P. Kashyap

Dimensions: Female- L= 444 (408-463) µm, a = 32.55 (29.1-35.4) µm, b=3.87 (3.84-3.89) µm, c=4.54 (4.3-4.7) µm, c'=13.07 (12.6-13.3) µm, V=51.87 (50.9-52.6) µm, T= 98 (86-107) µm.

Description: Body arcuate ventrad upon fixation. Cuticle thick and coarsely annulated, with longitudinal ridges (n=8) extending throughout the body. Six lips (leaf like appearance) 3-4 µm long, deep incisures separates those most of the lip length and reinforced by borders which are strongly sclerotized, and rounded apically. The lips (subdorsal and subventral) have a single setiform sensillae. Raised cuticle incised surrounds excretory pore anteriorly (aperture -0.5 µm). Cylindroid oesophagus, 104-105 µm long, constricted immediately below or at level of nerve ring. Oval basal bulb, 13.5-14 µm long, large and smooth bulb flaps. Nerve ring at 65% of oesophagus length, 68 µm from anterior end. Vulva has flat transverse slit. Monodelphic, prodelphic female reproductive system, short and reflexed ovary, short postuterine sac, thick walled vagina. Anal aperture has transverse slit and the posterior lip slightly bulged. Tail 86-107 µm long with a bifid terminus and anal body diameter (6.5-8 µm).

Habitat and Locality: In soil of betula forest near bhojwasa and Chirwasa, Gangotri national park, Uttarakhand, India

Distribution: In India- Uttarakhand; Elsewhere- Sweden, Germany, Romania, Netherlands, Svalbard and Jan Mayen.

Remarks: All the measurements and descriptions are in agreement with earlier description.

Order- **PLECTIDA**

Superfamily- **PLECTOIDEA** Orley, 1880

Family- **PLECTIDAE** Orley, 1880

Subfamily- **WILSONEMATINAE**Chitwood, 1951

Genus- ***Wilsonema*** Cobb, 1913

Species- ***Wilsonema otophorum*** (de Man, 1880) Cobb, 1913.

Material Examined: India, Uttarakhand, District Uttarkashi, Gangotri National park, Chirwasa, 19.x.2016, 8♀♀, coll. P. Kashyap.

Dimensions: Female- L= 0.26 (0.25-0.27) mm, a = 16.12 (15.4-16.8) µm, b=2.95 (2.78-3.12) µm, c=9.92 (9.5-10.3) µm, c'=2.92 (2.6-3.2) µm, V=52.25 (50.6-53.9) µm, T=26 µm.

Description: Female- Small and fusiform body ventrally curved on fixation. Annulated, thin cuticle. Deirid setiform close to excretory pore. Amphid aperture circular, (2.0-3.5 µm) wide located at the mid region of stoma. Plectoid stoma, cylindrical corpus. Nerve ring present at anterior region of Isthmus and excretory gland cells are present posterior part of Isthmus. Oval pharyngeal basal bulb having valves. Didelphic, amphidelphic female reproductive system.Vulva equatorial and transverse. Short vagina. Sphincter muscle encircles vagina. No Epiptygmata. Stout and short rectum, 1-1.5 times ABD long. Tail gradually tapers, ventrally arcuate with five caudal setae. Caudal glands present.

Habitat and Locality: From soil of deodar forest at elevation 3000-3400 m in Gangotri National Park, Uttarakhand, India.

Distribution: In India- Uttarakhand; Elsewhere- Germany, Sweden, Romania, Netherlands, Bulgaria, Congo, Democratic Republic of the, Costa Rica.

Remarks: All the measurements and descriptions are in agreement with earlier description.

Order- **RHABDITIDA**
Suborder- **CEPHALOBINA**
Super family**- CEPHALOBOIDEA** Filipjev, 1934
Family- **CEPHALOBIDAE**Filpijev, 1934
Subfamily -**ACROBELINAE** Thorne, 1937
Genus- ***Acrobeles*** von Linstow, 1877
Species- ***Acrobeles ciliatus*** von Linstow, 1887

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Bhojwasa, 18.x.2016, 7♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 10 ♀♀, coll. P. Kashyap

Dimensions: Female L=0.41 (0.39-0.43) mm, a= 15.18 (14.7-15.7) µm, b= 3.29 (3.06-3.47) µm, c= 8.54 (7.6-9.4) µm, c′= 3.0 (2.6-3.1) µm, V= 60.15 (58.8-61.9) µm, T= 47.66 (42-53) µm.

Description: Body nearly cylindrical and stout, tapering towards ends, single thick cuticle. Lateral fields consisted of two incisures. Head region is off set from the anterior neck part, three deeply furcated labial probolae, having usually 5 tines on inner sides and five to eight at outer margins, and two apical tines which are elongated and appears to be V-shaped under microscope. Cephaloboid pharynx-cylindrical corpus, with longer procorpus than metacorpus; comparatively narrow isthmus than metacorpus; basal bulb ovoid with well developed valve in middle. Excretory pore located at 49-60 µm from anterior region. Monodelphic-prodelphic usually reflexed reproductive system. Well developed spermatheca. Vulva flat without protrusion located mid to posterior part. Phasmids at 47-49% of tail length. Tail conoid.

Habitat and Locality: Soil around roots of Himalayan Blue Pine and Deodar in GNP, Uttarkashi, Uttarakhand.

Distribution: In India- Uttarakhand, Punjab, Haryana, Bihar; Elsewhere- Germany, Sweden, Romania, Netherlands, Greece, Belgium, Denmark, New Zealand, Ukraine

Remarks: All the measurements and descriptions are in agreement with earlier description. This species is being recorded from the first time from Gangotri National Park, Uttarakhand.

Order- **RHABDITIDA**
Suborder- **CEPHALOBINA**
Super family**- CEPHALOBOIDEA** Filipjev, 1934
Family- **CEPHALOBIDAE**Filpijev, 1934
Subfamily -**ACROBELINAE** Thorne, 1937
Genus- ***Acrobeles*** von Linstow, 1877
Species- ***Acrobeles timmi*** Chaturvedi and Khera 1979

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Bhojwasa, 18.x.2016, 3♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 4 ♀♀, coll. P. Kashyap

Dimensions: Female- L= 0.43 (0.40-0.48) mm, a=16.3 (15.28-17.04) µm, b=3.28 (3.10-3.46 µm, c= 9.22 (8.49-9.72) µm, c′=2.76 (2.61-2.93) µm, V= 61.2 (59.7-62.4) µm, T= 48.25(44.1-57.2) µm.

Description: Cuticle thick, double and coarsely striated. Lateral fields with four incisures. Body tapering at both extremities. Lip diameter 10-12 µm. Labial probolae 10-14 µm long deeply bifurcated with number of paired tines along with it. Head offset 11-13 µm wide deep. Pharynx consists of sub-cylindrical corpus, short isthmus and terminal bulb (valvated). Excretory pore opens at level of isthmus (57-65 µm). Deirid present. Body diameter varies from 26-30 µm. Cephaloboid female reproductive system. Presence of postvulval uterine sac and flat vulva without protusion. Vulval -anus distance varies 114-139 µm. Anal body diameter varies 17-21 µm. Tail conoid.

Habitat and Locality: From mixed forest (*Deodar*, Pine and Betula) in subalpine region of Gangotri National Park, Uttarkashi, Uttarakhand.

Distribution: In India- Uttarakhand, Punjab, Haryana, Bihar, Rajasthan, Sikkim, Goa.

Remarks: Measurements and features are in conformity with original description. Species is being recorded first time from GNP, Uttarakhand.

Order- **RHABDITIDA**
Suborder- **CEPHALOBINA**
Super family**- CEPHALOBOIDEA** Filipjev, 1934
Family- **CEPHALOBIDAE**Filpijev, 1934
Subfamily -**ACROBELINAE** Thorne, 1937
Genus- Chiloplacus Thorne, 1937
Species- *Chiloplacus demani* Thorne, 1925 (Schneider, 1939).

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Chirwasa 19.x.2016, 4 ♀♀, coll. P. Kashyap

Dimensions: Female- L= 0.68 (0.61-0.70) µm, a=23.13 (22.50-23.68) µm, b=3.65 (3.51-3.73 µm, c= 16.11 (14.54-16.75) µm, c′=2.1 (2.0-2.1) µm, V= 64.53 (63.17-65.42) µm, T=42.5 (41.2-44.0) µm.

Description: Female body ventrally curved. Cuticle markedly annulated. Lateral field having three distinct incisures. Lip region have six lips fused in pairs. U-shaped deep primary axils and V-shaped shallow secondary axils. Labial probolae 4-6 µm long. Stoma cephaloboid. Pharyngeal corpus slightly tapering at the end giving spindle shape structure, corpus- isthmus junction is indistinct. Oval shape basal bulb with valve. Monodelphic prodelphic female reproductive system. Bluntly rounded tail terminus

Habitat and Locality: Deodar patch in Kanku, subalpine region in Gangotri National Park, Uttarkashi, Uttarakhand

Distribution: In India- Ladakh, Punjab, Haryana; elsewhere- Belgium, Hungary, Slovakia, Poland, Spanish Mainland, Netherlands.

Remarks: First record from Uttarakahnd. All measurements and features are in accordance with original description.

Order- **RHABDITIDA**
Suborder- **CEPHALOBINA**
Super family**- CEPHALOBOIDEA** Filipjev, 1934
Family- **CEPHALOBIDAE** Filpijev, 1934
Subfamily -**ACROBELINAE** Thorne, 1937
Genus- ***Acrobeloides*** (Cobb, 1924) Steiner and Buhrer, 1933
Species- ***Acrobeloides nanus*** De Man, 1880.

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Bhojwasa, 18.x.2016, 10♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 6 ♀♀, coll. P. Kashyap.

Dimensions: Female- L= 0.39 (0.36-0.42) µm, a=21.03(20.6-21.6) µm, b=3.51 (3.3-3.75 µm, c= 18.10 (17.47-18.75) µm, c′=1.88 (1.83-1.99) µm, V= 65.0 (63.76-66.33 µm, T= 28.28 (26.5-30) µm.

Description: Body cylindrical, ventrally curved upon fixation. Cuticle annulated, head region continuing with neck. Lip region 6 -7 µm wide. Cephalic probolae not absent. Labial probolae present, conical-rounded probalae. Pharynx cephaloboid .Pharyngeal corpus spindle shaped with swollen metacorpus. Corpus broader, narrower isthmus. Oval-shaped basal bulb with well-developed valve. Monodelpic-prodelpic female reproductive system. Vulva flat, vaginal 1-3 of body width, Rudimentary post-vulval sac. Conoid tail with rounded terminus.

Habitat and Locality: Soil of Deodar patch in between Kanku and Chirwasa in GNP, Uttarakhand.

Distribution: In India- Ladakh, Punjab, Haryana, Uttarakhand; elsewhere-Worldwide Asia, Korea, Iran, America, Europe.

Remarks: All measurements and features are in accordance with original description.

Order- **RHABDITIDA**

Suborder- **CEPHALOBINA**

Super family**- CEPHALOBOIDEA** Filipjev, 1934

Family- **CEPHALOBIDAE** Filpijev, 1934

Genus- ***Stegelletina*** Andrassy, 1984

Species- **S*tegelletina devimucronata*** (Sumenkova, 1964) Bostrom and De Ley, 1996

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Bhojwasa, 18.x.2016, 6♀♀, coll. P. Kashyap.

Dimensions: Female- L= 0.29 (0.29-0.31) µm, a=18.35(17.9-18.6) µm, b=3.26 (3.25-3.27) µm, c= 14.5 (14.2-14.7) µm, c′=2.0 (1.9-2.1) µm, V= 65.51(64.74-66.22) µm, T=20.66(20-21) µm.

Description: Female- Moderately arcuate ventrad body. Annulated cuticle; Lateral field having three lines. Lip region 7.8-8 µm wide. Slender-conical labial probolae, around 3 µm long, single bifurcation at their tip forming two prongs. Cheilorhabdia small and rounded; rest of the stoma region is slightly sclerotized and usually unnoticeable. Oval shaped basal bulb with well developed valves. Excretory pore at the level of isthmus. Corpus is 1.5-2.5 times long as isthmus. Vulva flat or slightly protruding. Vulva located at about 2-3rd of body length. Phasmids located around anterior half of tail. Straight and short vagina. Tail finely rounded with ragged mucro.

Habitat and Locality: From soil near Juniper species in alpine region of bhojwasa at elevation 4000 masl in Gangotri National Park, Uttarakhand.

Distribution: Turkey, Portugal, Greece.

Remarks: **This is a new record from India.** This population is similar to S. devimucronata (Sumenkova, 1964; Akbar Karegar et al., 1998) Bostrom and De Ley, 1996 with only difference in number of lateral lines with type population which could be due to fixation or slide preparation method.

Order- **RHABDITIDA**
Suborder- **CEPHALOBINA**
Super family**- CEPHALOBOIDEA** Filipjev, 1934
Family- **CEPHALOBIDAE** Filpijev, 1934
Subfamily- **CEPHALOBINAE** Filipjev, 1934
Genus- ***Pseudacrobeles***Steiner, 1938
Species- ***Pseudacrobeles pauciannulatus*** (Marinari Palmisano, 1967) De Ley, Siddiqi & Boström, 1993

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Chirwasa 19.x.2016, 8♀♀, coll. P. Kashyap

Dimensions: Female- L= 0.39 (0.35-0.45) mm, a = 22.67 (20.8-23.8) µm, b=3.15 (3.06-3.28) µm, c=8.44 (7.8-8.88) µm, c'=4.35 (3.72 - 5.52) µm, V=62.2 (61.1-62.9) µm, T=47(41-58) µm.

Description: Female- Ventrally arcuate body on fixation. Cuticle 1.0-1.3 µm thick, annulated 1.3-1.5 µm wide. Head region continuous with body contour. Three low lips, conical or rounded, lip diameter 6-7 µm; stoma 10-12 µm long, sparingly sclerotized. Small and oval cheilorhabdions with the presence of dorsal metarhabdial tooth. Oesophagus having moderately expanded corpus, narrow and short isthmus and oval shaped pharyngeal basal bulb. Nerve ring located at anterior region of isthmus, 60-71% of oesophagal length. About the level of nerve ring, excretory pore present at 80 to 89 µm from anterior region. Monodelphic, prodelphic female reproductive system. Ovary with double flexure behind vulva; anteriorly, vagina oblique approx one-third of vulva body wide long and posterior uterine sac 8 -15 µm long. 12-15.5 µm long rectum. Three incisures on lateral field ending at phasmid. Phasmid present at 27-31% of tail length. Straight and conical tail with pointed terminus, without annulations on tail tip.

Habitat and Locality: Collected from soil of *Pinus wallichiana* dominated region on the way to Chirwasa in Gangotri National park, Uttarakhand.

Distribution: In India- Punjab, Uttarakhand

Remarks: All measurements and features are in accordance with original description.

Order- **RHABDITIDA**
Suborder- **CEPHALOBINA**
Super family**- CEPHALOBOIDEA** Filipjev, 1934
Family- **CEPHALOBIDAE** Filpijev, 1934
Genus- ***Pseudacrobeles***Steiner, 1938
Species- ***Pseudacrobeles eurystoma*** (Andrássy, 1967) Abolafia & Peña-Santiago, 2001

Material Examined: India, Uttarakhand, District Uttarakahi, Gangotri National park, Bhojwasa, 18.x.2016, 7♀♀, coll. P. Kashyap.; District Uttarakashi, Gangotri National Park, Chirwasa 19.x.2016, 5 ♀♀, coll. P. Kashyap

Dimensions: Female- L= 0.59 (0.55-0.66) mm, a = 28.47 (25.8-33.0) µm, b=3.67 (3.61-3.67) µm, c=12.34 (11.1-12.9) µm, c'=3.84 (3.3 - 3.9) µm, V=63.80 (63.1-64.6) µm, T=48.33 (44-51) µm.

Description: Female- Body ventrallv arcuate on fixation. About 1-1.2 µm thick cuticle. Lateral field with 3 lines further extending to the phasmid. Low lip region with three conical pointed lips. Labial papillae variously developed, ranging from not pro-truding to distinctlv protruding. Small and oval cheilorhabdions. Stoma 10-14 µm long, walls are weakly sclerotized. Metarhabdion with indistinct dorsal tooth. Cylindrical oesophagus, short isthmus 17-28 µm long. Excretory pore located at posterior part of the corpus, Hemispherical cardia. Numerous nuclei are present in Intestine. Cephalobid female reproductive svstem. Post-vulval uterine sac present, 0.5-1.5 times vulval body diameter. 15-21 µm long rectum, 1.3-1.8 times ABD. Tail conoid with pointed tip, with or without mucro. Phasmid present posteriorly to anus and 9-21 µm in size.

Habitat and Locality: Soil of deodar forest in Kanku, Gangotri National Park, Uttarakhand, India.

Distribution: In India- Punjab, Uttarakhand, Haryana.

Remarks: All measurements and features are in accordance with original description.

7.2.2 Molecular Analysis Results

We have performed morphological characterization for cultured bacterivores using the molecular markers COI and 18S rRNA genes. Availability of 18S rRNA sequencesof nematode in public domain reflects its utility in molecular phylogenetic studies (Bhadury et al., 2006b; Meldal et al., 2007). For marine nematodes barcoding, Cytochrome c oxidase subunit 1 (COI) gene has been efficiently used to understand taxonomic relationships among closely related species (Blouin et al., 1998; Derycke et al., 2005, 2010). In this study the 18S rRNA gene region was amplified using primers 18S NemF TGTCTCAAAGATTAAGCCATGC and 18S NemR

GGGCGGTGTGTACAAAGG (Avo et al. 2017) and the COI region was amplified using the primer pair JB3F TTTTTTGGGCATCCTGAGGTTTAT (Bowles et al., 1992) and JB5R AGCACCTAAACTTAAAACATAATGAAAATG (Derycke et al., 2005).

Purification and Sequencing of the COI and 18S rRNA Genes

1% agarose gel were used to analyse the pcr product and expected size product were excised and purified (MinElute PCR Purification Kit by Qiagen) following the instruction manual of the manufacturer. Next, the PCR products were sequenced to characterize the gene for *Plectus parietinus, Plectus parvus, Caenorhabditis elegans, Wilsonema otophorum* and compared with available data of Genbank. For the purpose of DNA-based identification, BLAST used as a first step to retrieve published sequences that match a query sequence in the GenBank database. The BLAST searches gave matches with query sequences from the NCBI database.

The phylogenetic tree constructed based on the partial sequences for the bacterivores species generated in this study is shown in Fig 17 and 18. neighbor-joining method used to infer the evolutionary history. 1000 replicates was extracted to represent the evolutionary history and bootstrap consensus of the taxa for analysis. Branches corresponding to partitions reproduced in bootstrap replicates less than10% are collapsed. The replicate trees percentage in which the associated taxa clustered together in the test is shown along with the branches. Kimura 2-parameter used to compute the evolutionary distances and are in the units of the number of base substitutions per site. Codon positions includes 1st+2nd+3rd+noncoding. In pair-wise sequence comparisons, all sites containing missing data and alignment gaps were eliminated (Pair-wise deletion option).

Species-specific variable sites in COI

In relation to the genome of studied bacterivores species, Out of 389 positions, there were 252 conserved sites, 137 variable sites, 66 parsimony informative sites and 71 singleton variable sites were observed in Plectus parietinus, Plectus parvus, Caenorhabditis elegans respectively. These sites can be used to differentiate studied nematode species. The nucleotide compositions of the entire sequenced region of COI are as follow: T =44.6%, C=13.6%, A =23.5%, G =18.3%. For COI gene-based NJ tree with 1000 bootstrap replicates showed Plectus parietinus are close to Plectus parvus. The analysis involved 9 nucleotide sequences. All positions with missing data and gaps were removed. There were a total of 389 positions in the final dataset (Fig. 17). Among each species, there were 374, 333, and 0 conserved sites, 15, 56, and 0 variable sites, 0,12, and 0 parsimony informative sites and 15,44, and 0 singleton variable sites were observed in *Plectus parietinus, Plectus parvus, Caenorhabditis elegans* respectively. The nucleotide compositions of the sequenced region of COI are as follow: T=44.1%, 45.4%,43.4%; C=15.8%, 12.2%,10%; A =23.1%, 23.2%, 26.5%; G =17%, 19.2%, 20.1% for Plectus parietinus, Plectus parvus, Caenorhabditis elegans respectively.

To estimates evolutionary divergence over sequence pairs between groups and within groups in COI gene were shown with the number of base substitutions per site from averaging over all sequence pairs between groups shown in Table 15 and 16 respectively and overall mean distance 0.156 (Table 14).

Table 14 Estimates of Overall Evolutionary Divergence over Sequence Pairs in COI gene

	d (mean distance)	*SE*
Overall mean distance	0.156	0.013

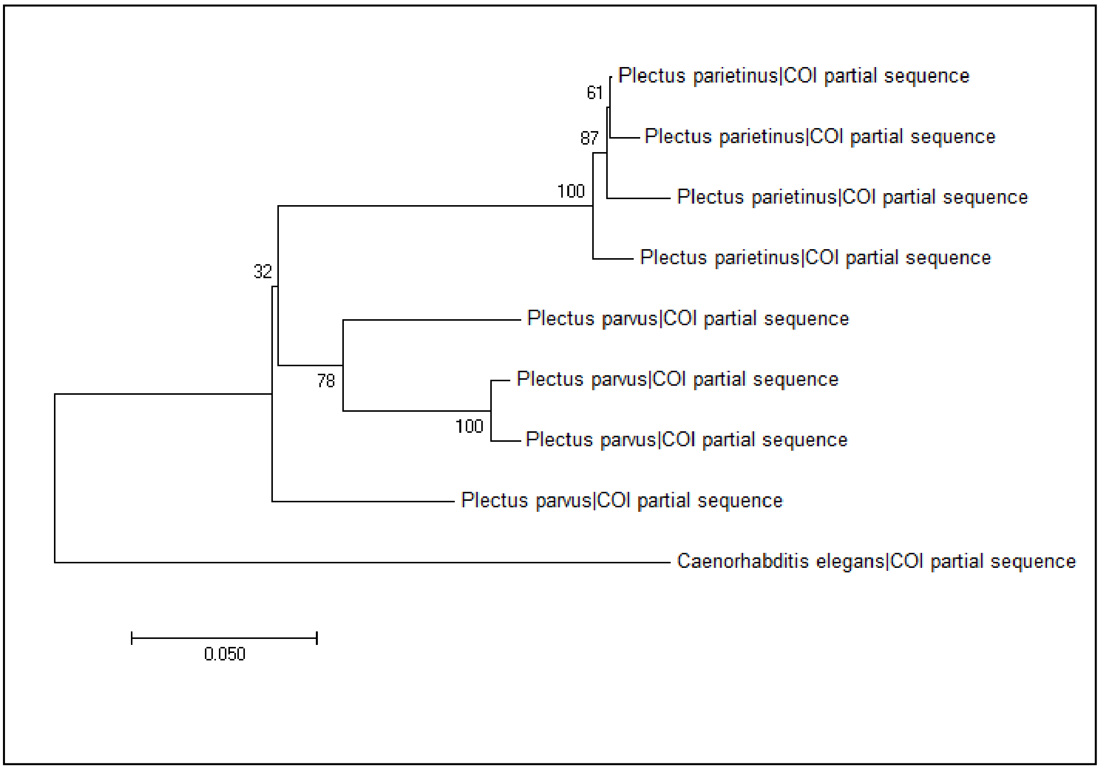

Figure 17 NJ tree analysis of K2P distances in *P. parientinus, P.parvus*, and *C. elegans* in COI gene.

The Neighbor-Joining method was used to infer evolutionary history. The optimal tree with the sum of branch length = 0.51794272 is shown. The replicate trees percentage in which the associated taxa clustered together in the test is shown along with the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Table 15 Estimates of Evolutionary Divergence over Sequence Pairs between Groups in COI gene (The number of base substitutions per site from averaging over all sequence pairs between groups are shown).

Species	***Plectus parietinus***	***Plectus parvus***	***Caenorhabditis elegans***
Plectus parietinus		0.018	0.033
Plectus parvus	0.157		0.028
Caenorhabditis elegans	0.322	0.290	

Kimura 2-parameter model (Kimura, 1980) was used for analyses. Nine nucleotide sequences were used for analysis. Codon positions include 1st+2nd+3rd+noncoding. All positions with missing data and gaps were removed. Total of 389 positions were present in the final dataset. MEGA7 were used for evolutionary history analysis (Kumar et al, 2016).

Table 16 Estimates of Evolutionary Divergence over Sequence Pairs within groups in COI gene (The number of base substitutions per site from averaging over all sequence pairs within each group are shown).

Species	***d***	***SE***
Plectus parietinus	0.020	0.005
Plectus parvus	0.089	0.012
Caenorhabditis elegans	n/c	n/c

18S r RNA Data Analysis:

In relation to the overall genome of studied bacterivores species, Out of 735 positions, there were 194 conserved sites; 541 variable sites; 538 parsimony informative sites and 3 singleton variable sites for all were observed in Plectus parietinus, Plectus parvus, Caenorhabditis elegans, Wilsonema otophorum respectively.These sites can be used to differentiate nematode species. The nucleotide compositions of the entire sequenced region of 18S r RNA are as follow:

T =25.2%, C=20.2%, A =26.2%, G =19%. For 18S r RNA gene-based NJ tree with 1000 bootstrap replicates showed Plectus parietinus are close to Plectus parvus. The analysis involved 9 nucleotide sequences. All sites with missing data and gaps were removed. There were total of 735 positions in the final dataset.

Among species-specific variable sites, Out of 735 positions, there were 677, 635, 726, conserved sites; 2,0 variable sites; 0 parsimony informative sites and 0 singleton variable sites for all were observed in *Plectus parietinus, Plectus parvus, Caenorhabditis elegans, Wilsonema otophorum* respectively. These sites can be used to differentiate four nematode species. The nucleotide compositions of the entire sequenced region of 18S r RNA are as follow in percentage: T =25.2, 24.6, 41.3, 25.6; C=21.8, 23.0, 12.5,21.2; A =25.2, 24.9, 27.8, 25.7; G =27.9, 27.6, 18.3, 27.5 in *Plectus parietinus, Plectus parvus, Caenorhabditis elegans, Wilsonema otophorum* respectively.

Table 17 Estimates of Overall Evolutionary Divergence over Sequence Pairs in 18S r RNA gene (The number of base substitutions per site from averaging over all sequence pairs between groups are shown.

	d (mean distance)	***SE***
Overall mean distance	1.015	0.091

Table 18 Estimates of Evolutionary Divergence over Sequence Pairs between Groups in 18S r RNA gene (The number of base substitutions per site from averaging over all sequence pairs between groups are shown.

Species	***Plectus parietinus***	***Wilsonema otophorum***	***Plectus parvus***	***Caenorhabditis elegans***
Plectus parietinus		0.006	0.087	0.362
Wilsonema otophorum	0.027		0.089	0.310
Plectus parvus	1.019	1.026		0.212

Caenorhabditis elegans	1.624	1.55	1.379	

To estimate evolutionary divergence over sequence pairs between groups and within groups in 18S r RNA gene were shown with the number of base substitutions per site from averaging over all sequence pairs between groups shown in table 19 and 20 respectively and overall mean distance = 2.01 (Table 18).

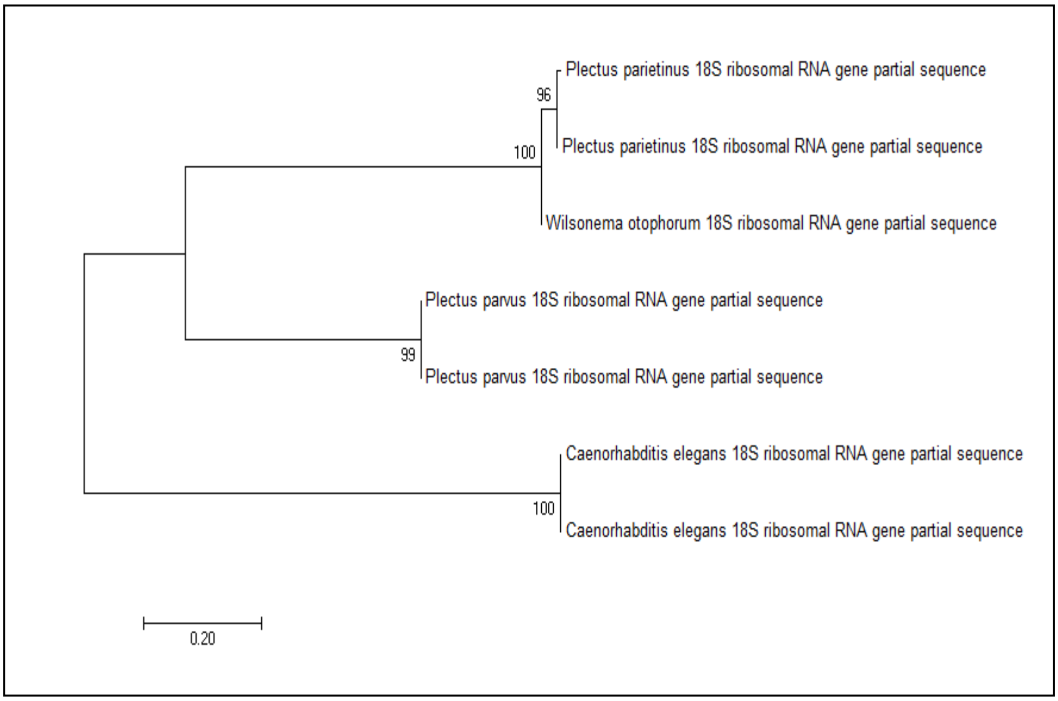

Figure 18NJ tree analysis of K2P distances in *P. parientinus, P. parvus,* and *C. elegans* in 18S ribosomal RNA gene.

The optimal tree with the sum of branch length = 2.01524852 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site.

Table 19 Estimates of Evolutionary Divergence over Sequence Pairs over Sequence Pairs within groups in 18S r RNA gene (The number of base substitutions per site from averaging over all sequence pairs within each group are shown).

Species	***d***	***SE***
Plectus parietinus	0.003	0.002
Wilsonema otophorum	n/c	n/c
Plectus parvus	0.00	0
Caenorhabditis elegans	0	0

Plates of Bacteriovorous Nematodes

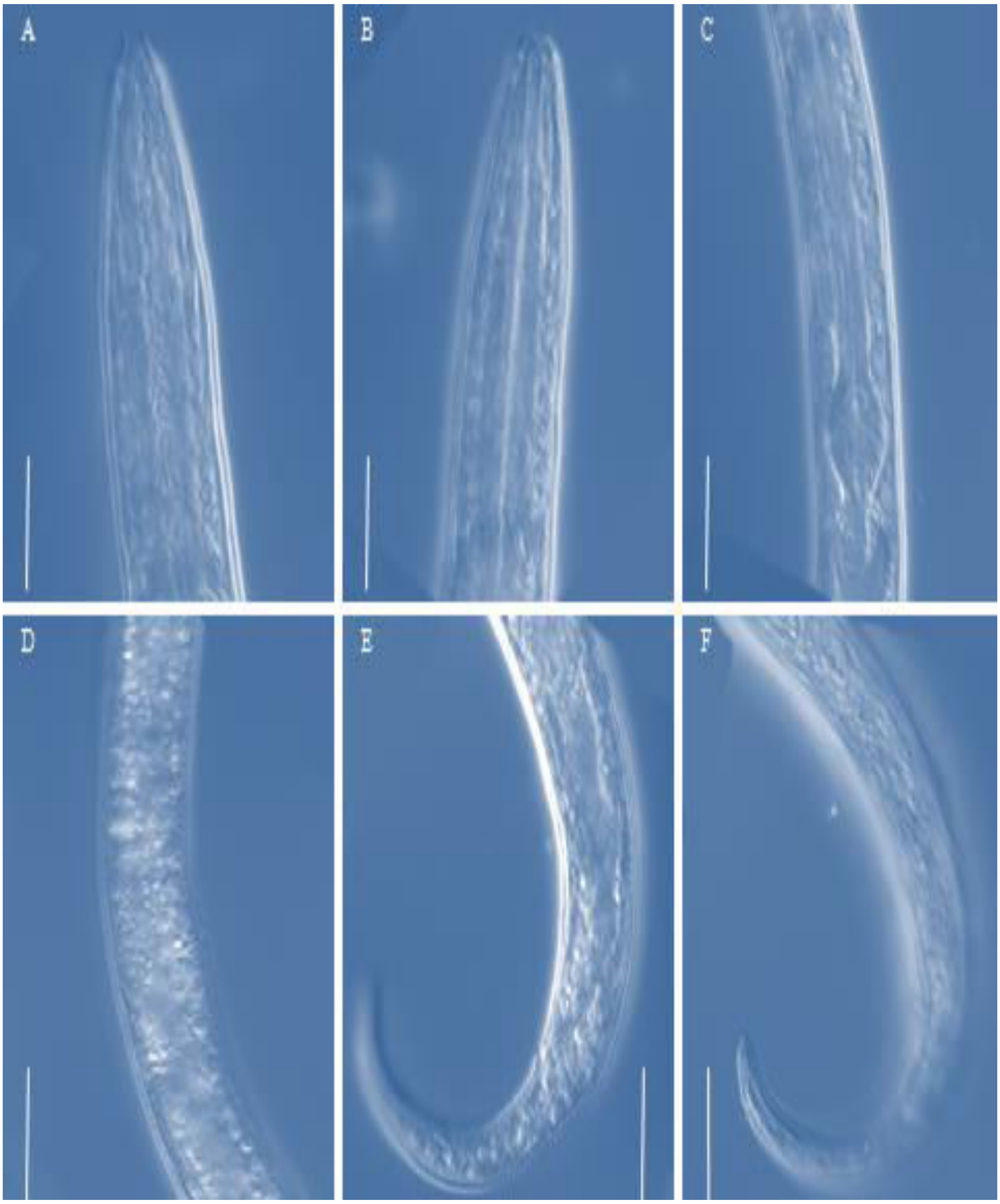

Plate 1 ***Ceratoplectus armatus*** (Butschli, 1873) Andrassy, 1984 sp.: A-B- Anterior region, C-Pharyngeal bulb region, D-Vulval region, E-F- Tail region [scale=10µm].

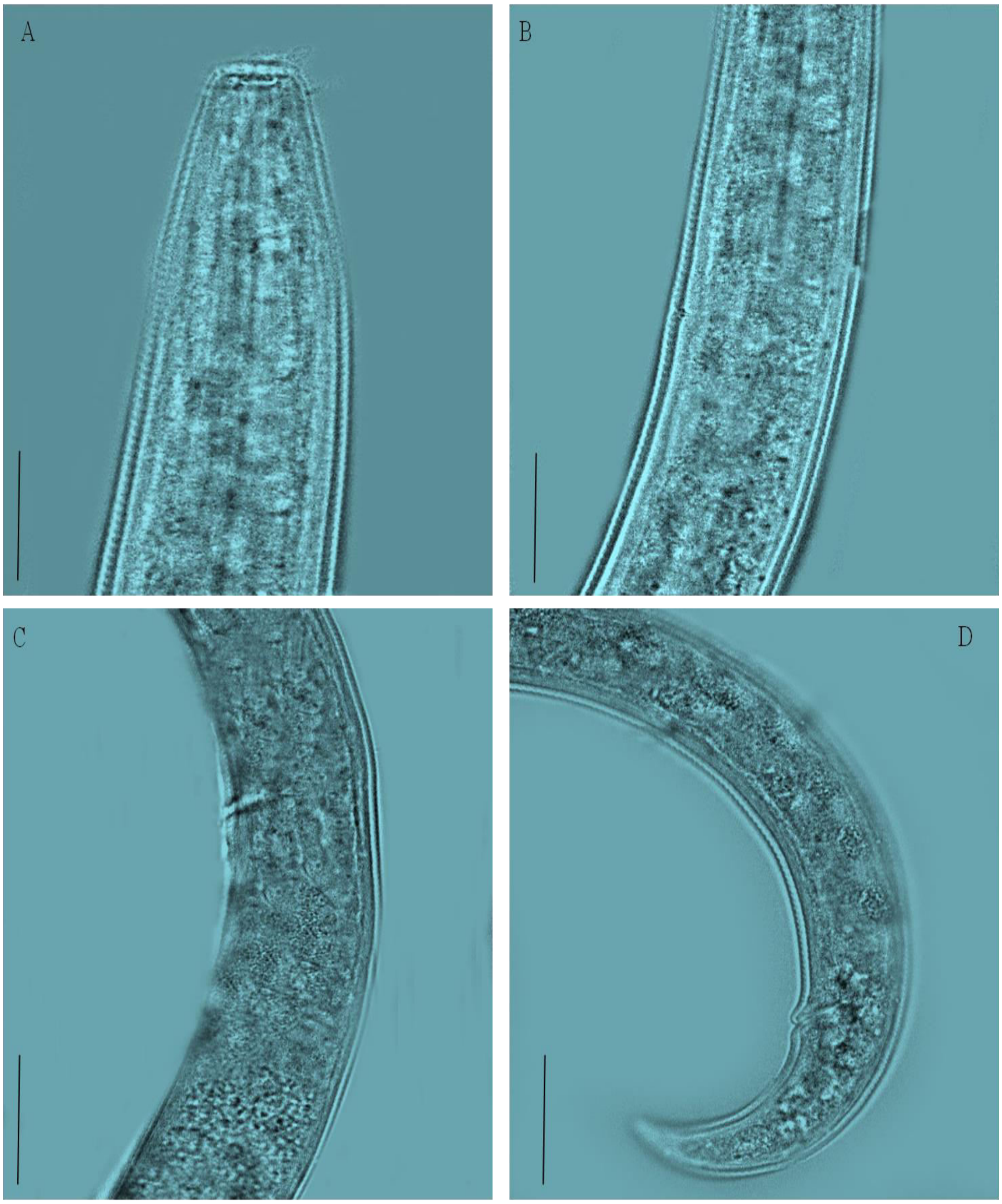

Plate 2 ***Anaplectus granulosus*** (Bastian, 1865) de Coninck and Schuurmans Stekhoven, 1933 sp.: A- Anterior region,B- Excretory pore, C- Vulval region, D- Tail region [scale=10µm].

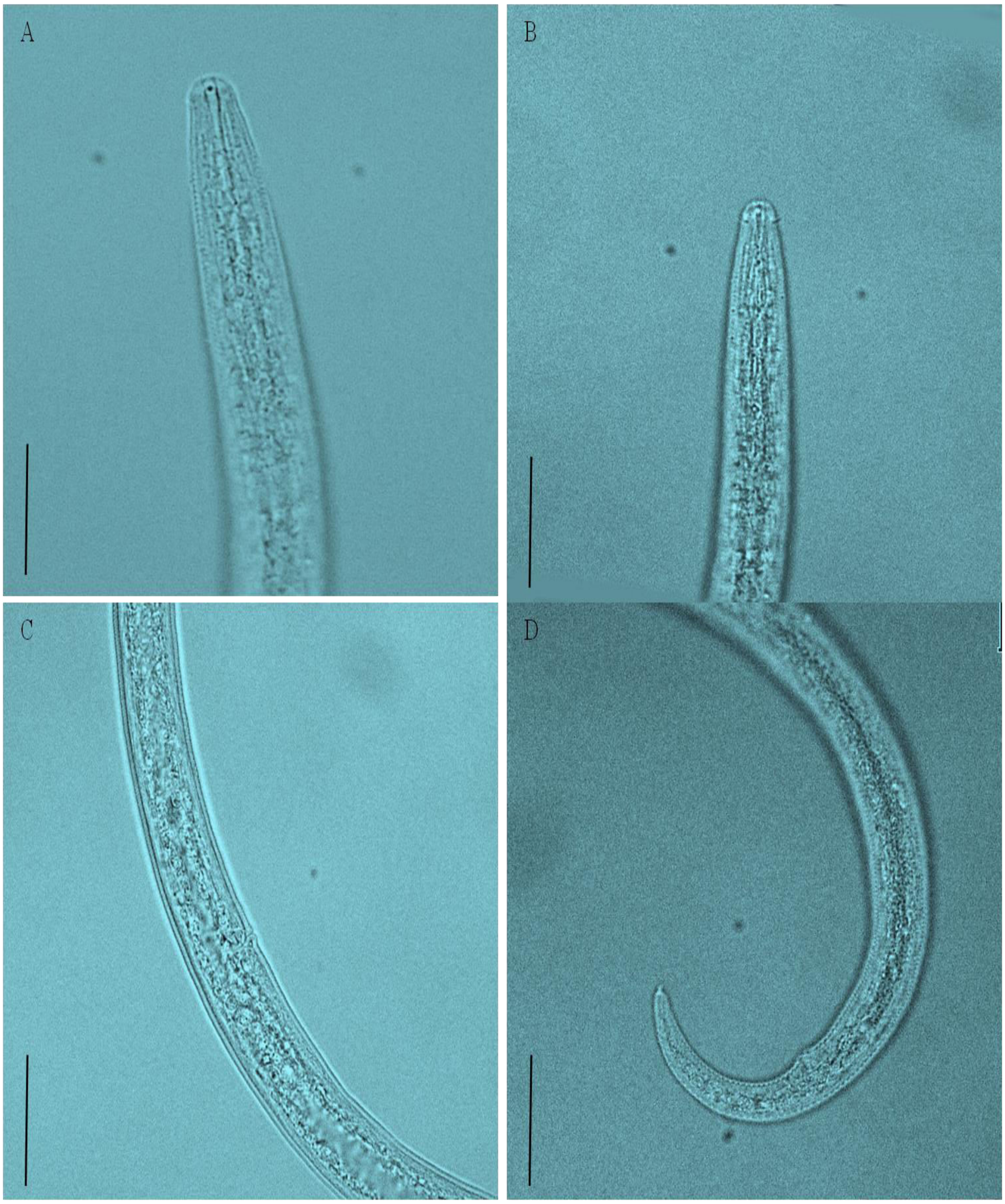

Plate 3 ***Plectus parvus*** Bastian, 1865 sp.: A, B-Anterior region, C- Vulval region, D-Tail region [scale=10μm].

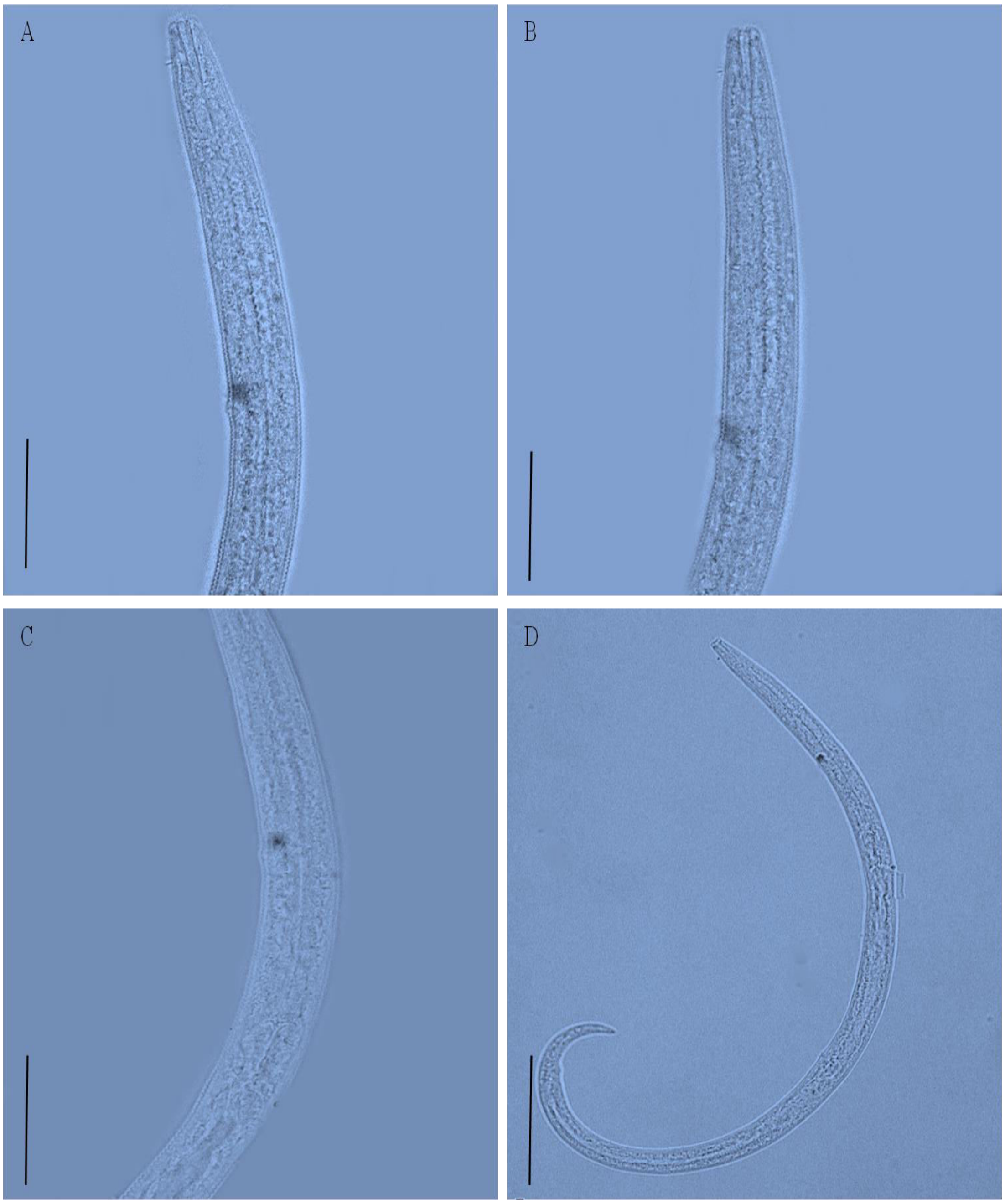

Plate 4 ***Plectus minimus*** Cobb, 1893 sp.: A, B - Anterior region, C- Pharyngeal region, D- Entire body [scale=10 µm, D= 40 µm].

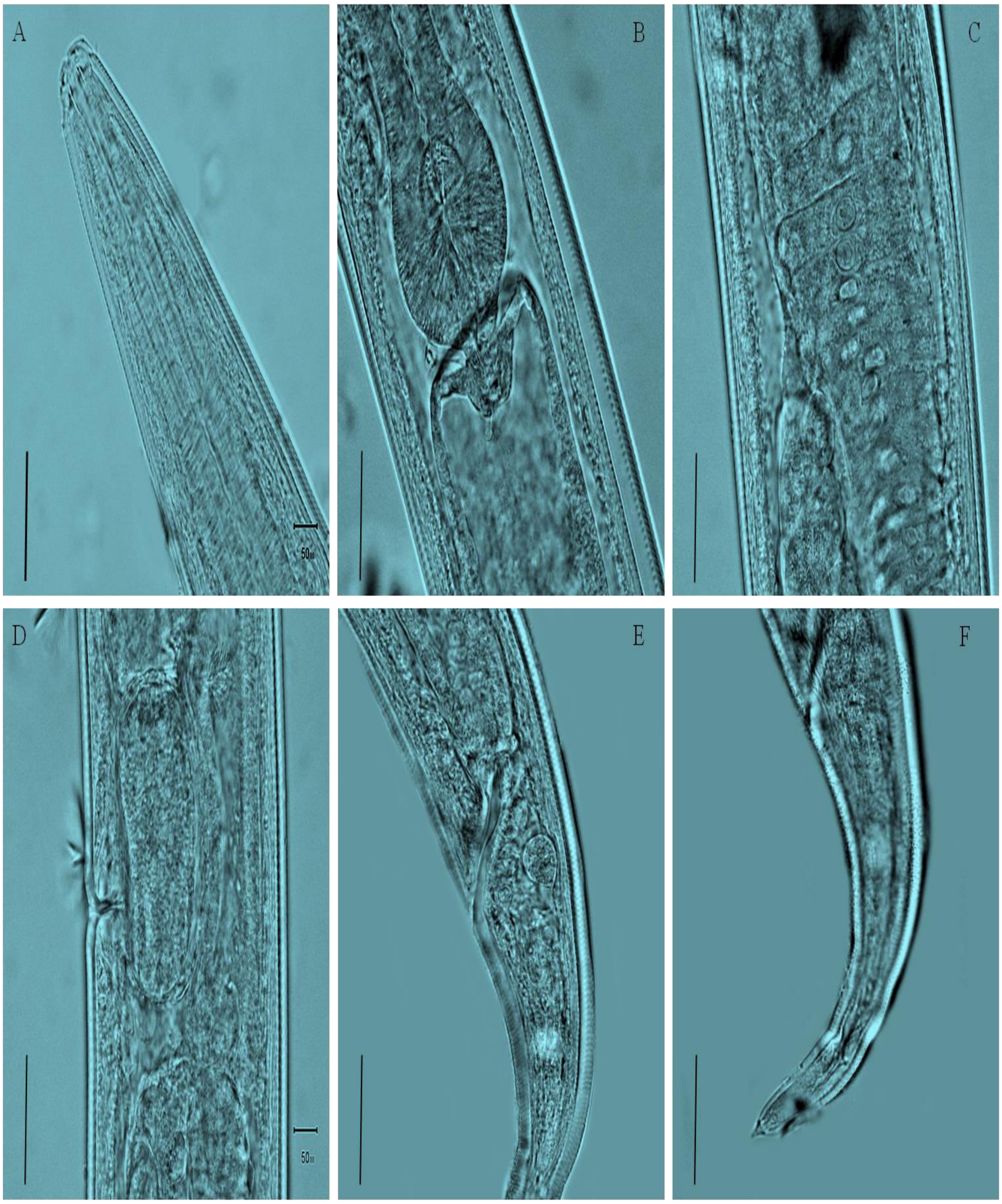

Plate 5 ***Plectus parietinus*** Bastian, 1865 sp.: A- Anterior region, B- Pharyngeal bulb, C- Ovary, D- Vulva, E= Anus region, F- Tail [scale=10 µm, D= 40 µm].

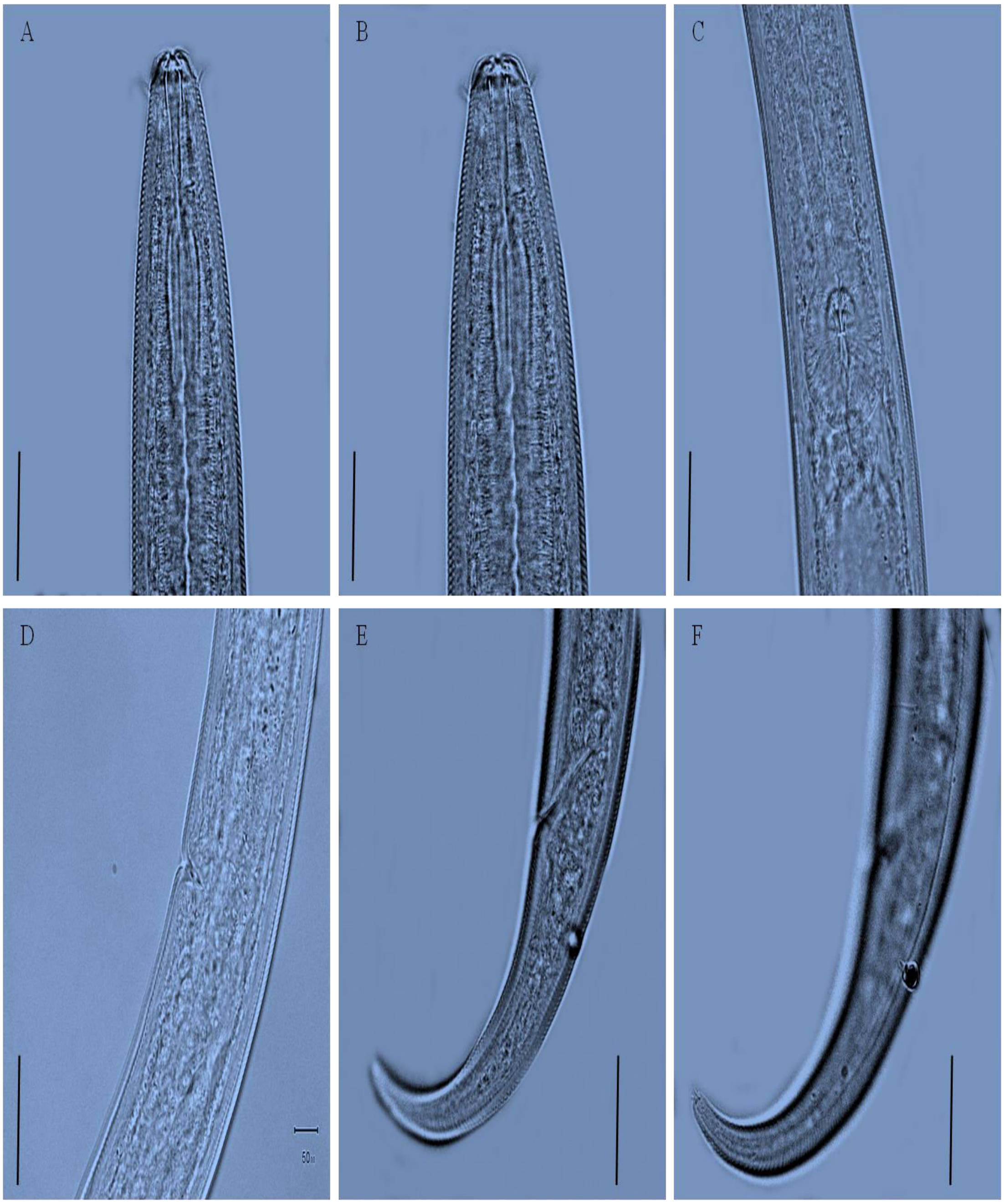

Plate 6 ***Plectus assimilis*** Butschli, 1873 sp. : A,B- Anterior region, C- Pharyngeal bulb, C- Vulva, E- Anus region, F- Tail [scale=10 μm].

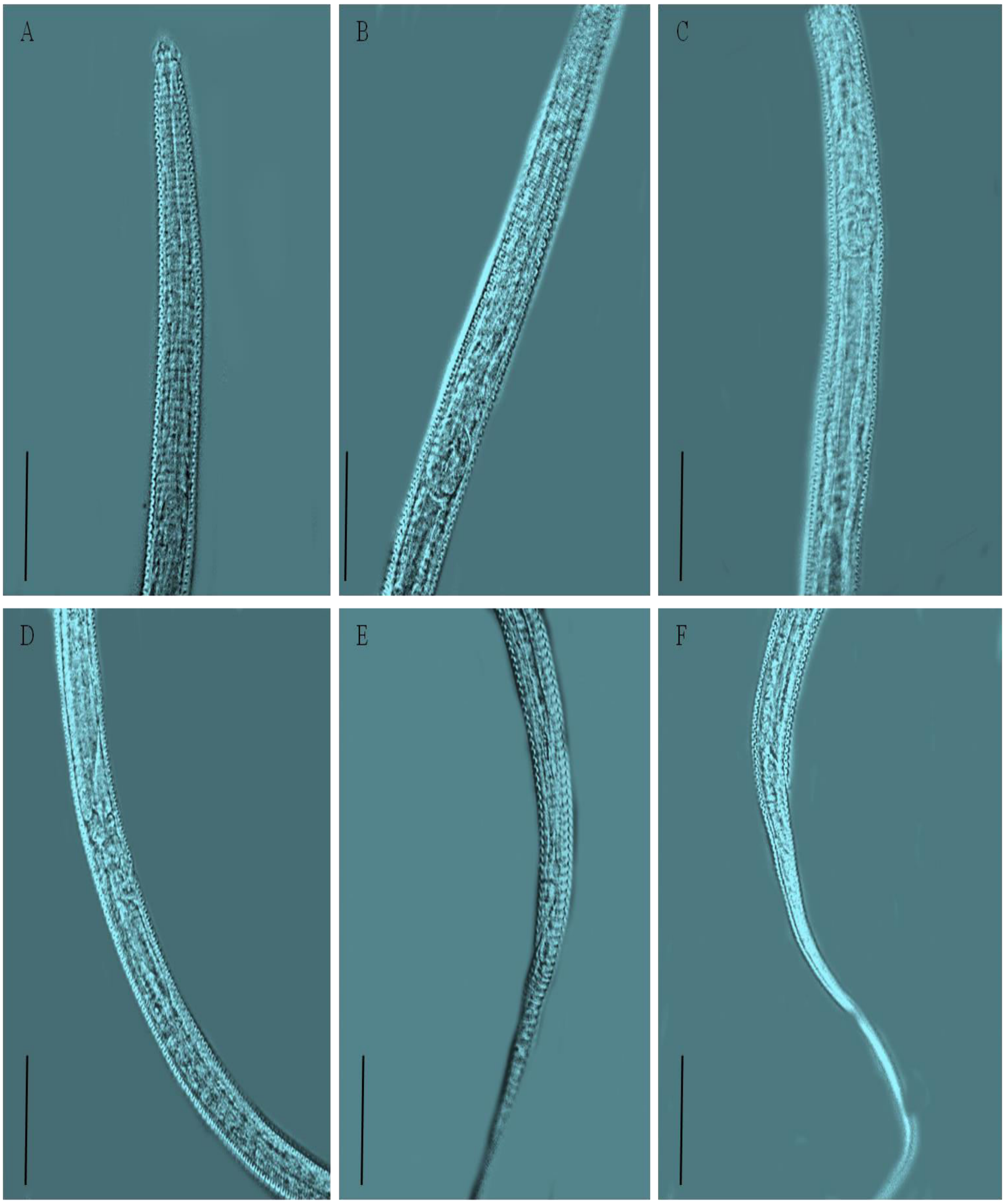

Plate 7 ***Teratocephalus costatus*** Andrássy, 1958 sp.: A- Anterior region, B, C- Pharyngeal bulb, D- Vulva, E,F- Tail [scale=10 µm].

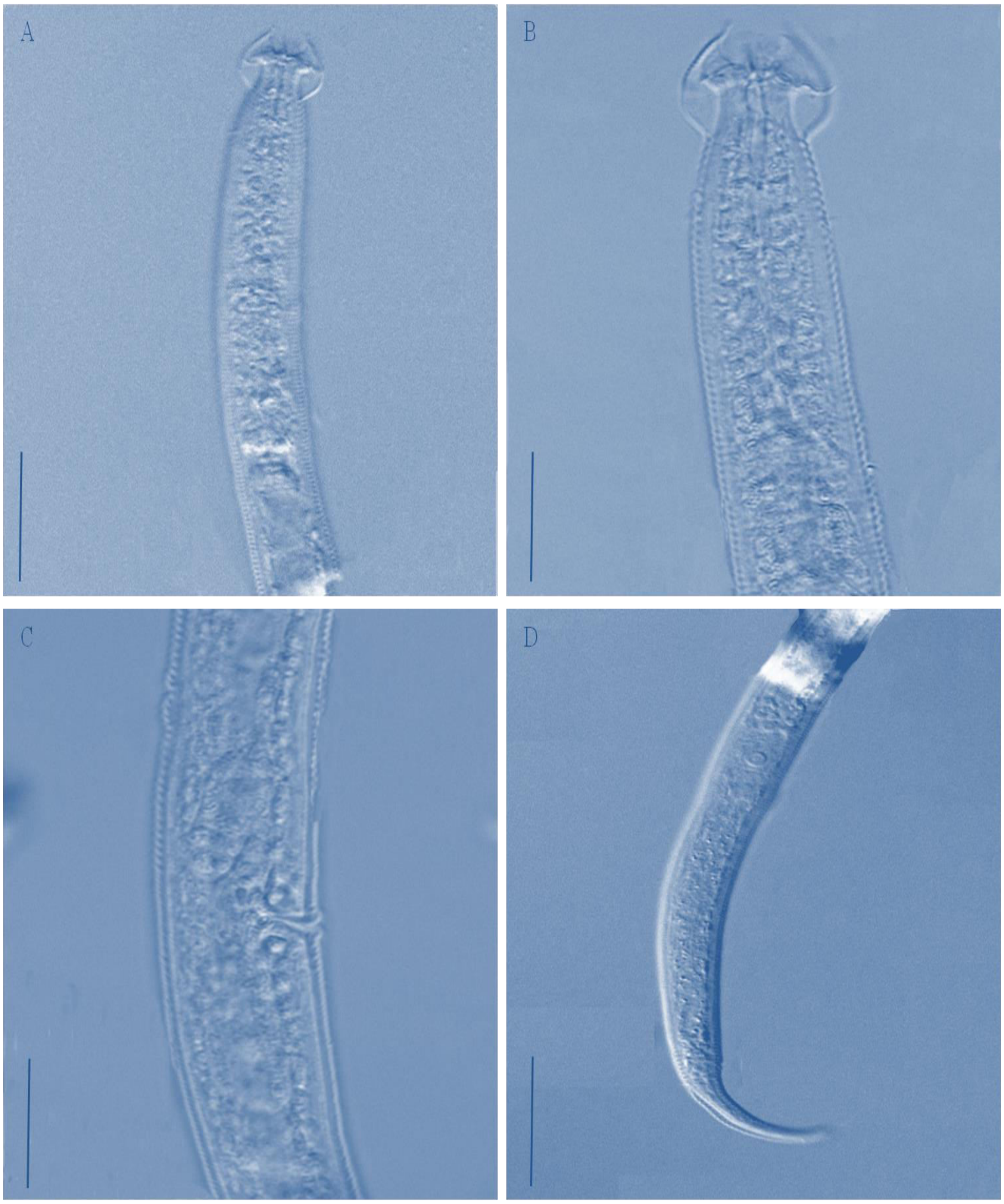

Plate 8 ***Wilsonema otophorum*** (de Man, 1880) Cobb, 1913. sp.: A, B- Anterior region, C- Vulva, D- Tail [scale=10 µm].

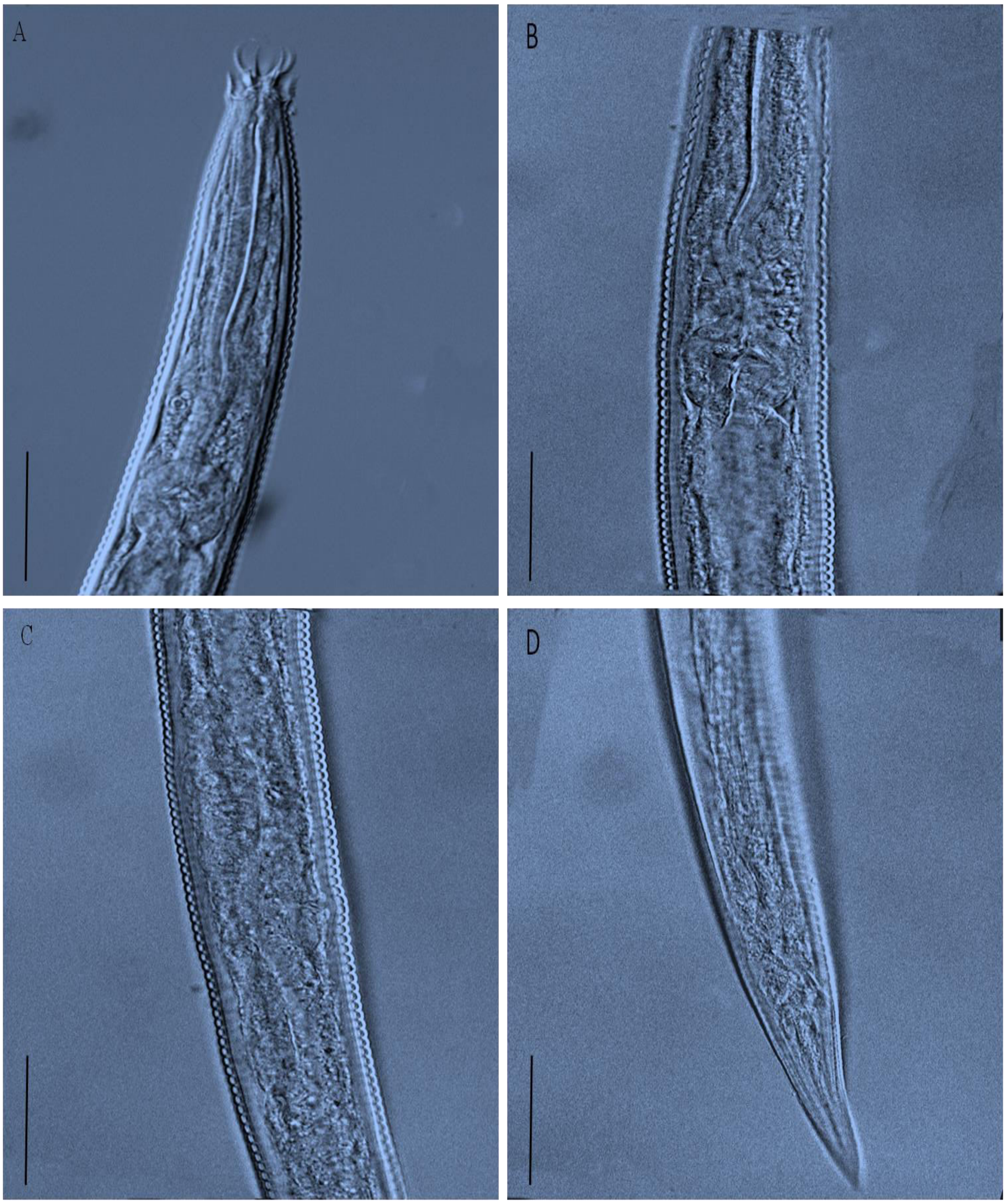

Plate 9 ***Acrobeles ciliatus*** von Linstow, 1887 sp.: A- Anterior region, B- Pharyngeal bulb, C- Vulva, D- Tail [scale=10 µm].

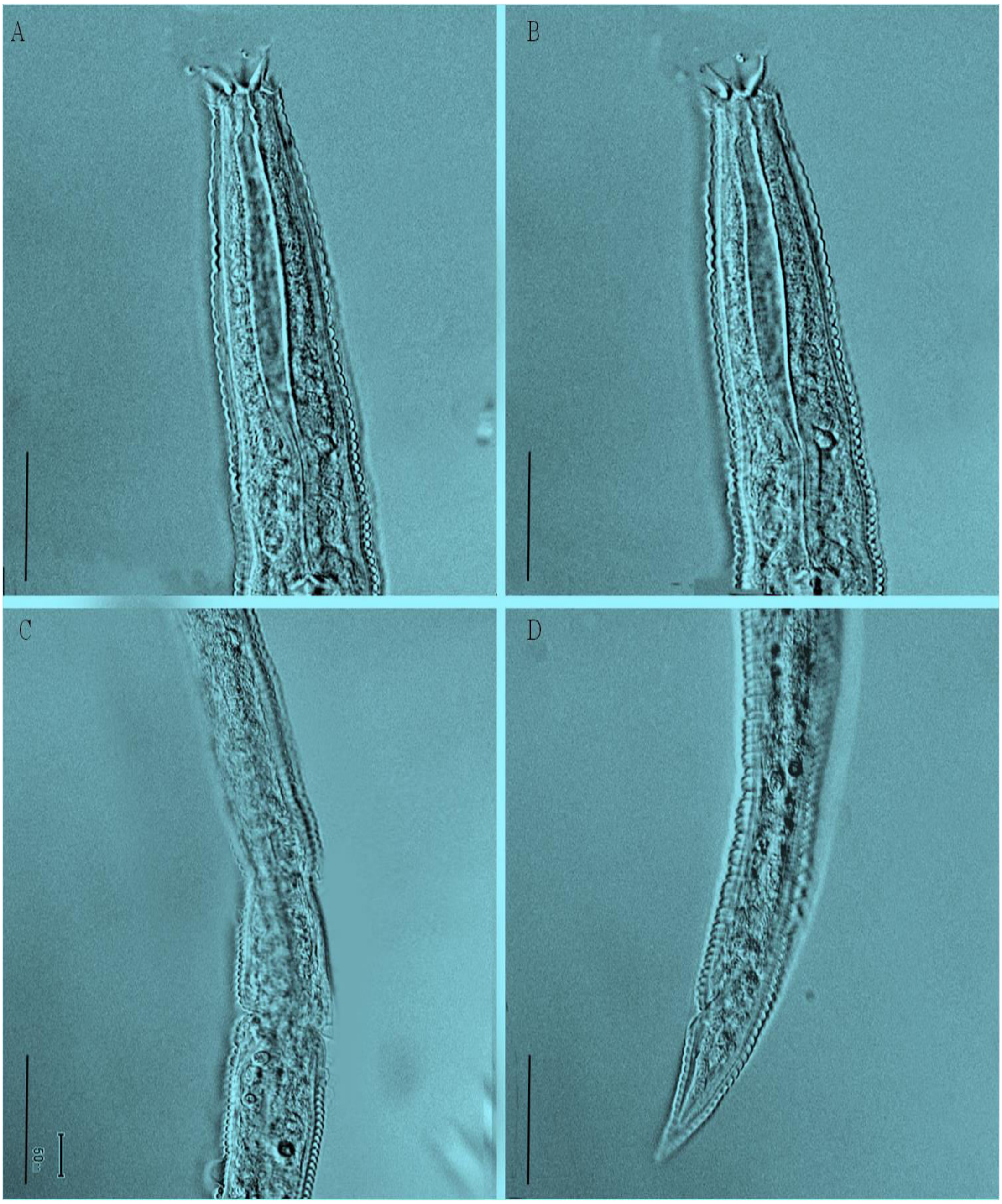

Plate 10 ***Acrobeles timmi*** Chaturvedi and Khera 1979 sp.: A, B- Anterior region, C- Vulva, D- Tail [scale=10 µm].

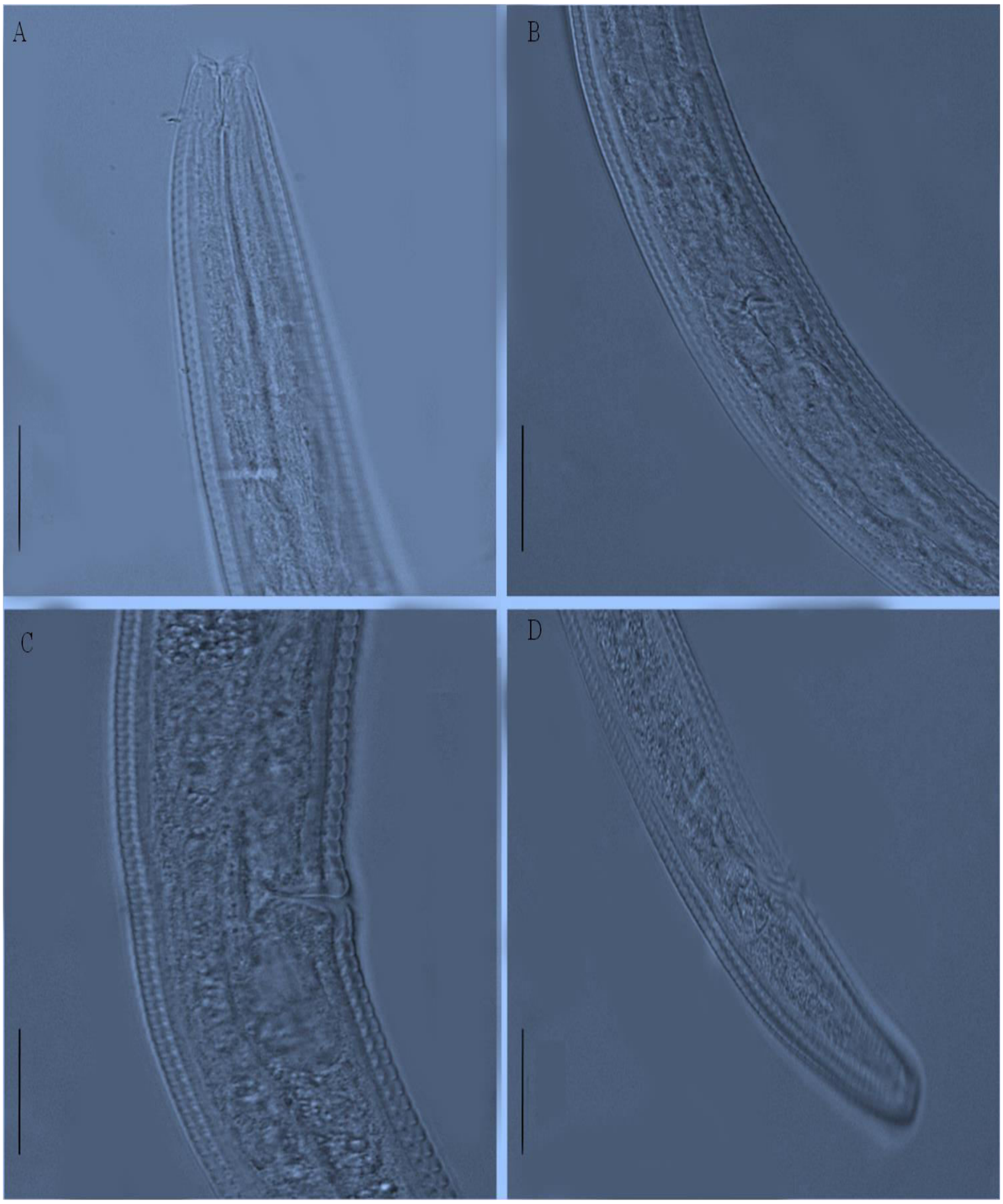

Plate 11 ***Chiloplacus demani*** Thorne, 1925 (Schneider, 1939) sp.: A- Anterior region, B- Pharyngeal bulb, C- Vulva, D- Tail [scale=10 µm].

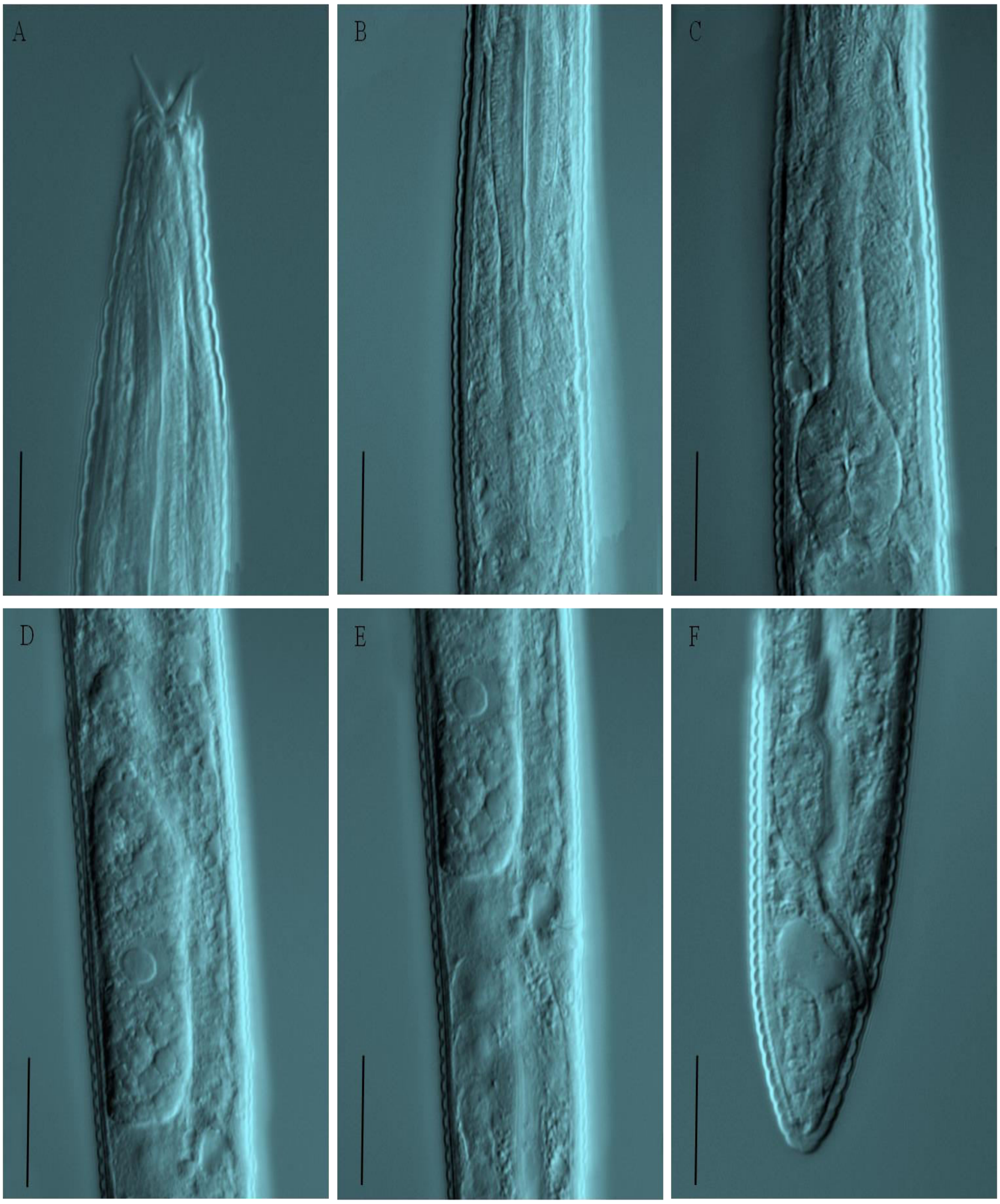

Plate 12 ***Acrobeloides nanus*** De Man, 1880 sp.: A- Anterior region, B- Pharyngeal bulb, C- Vulva, D- Tail [scale=10 μm].

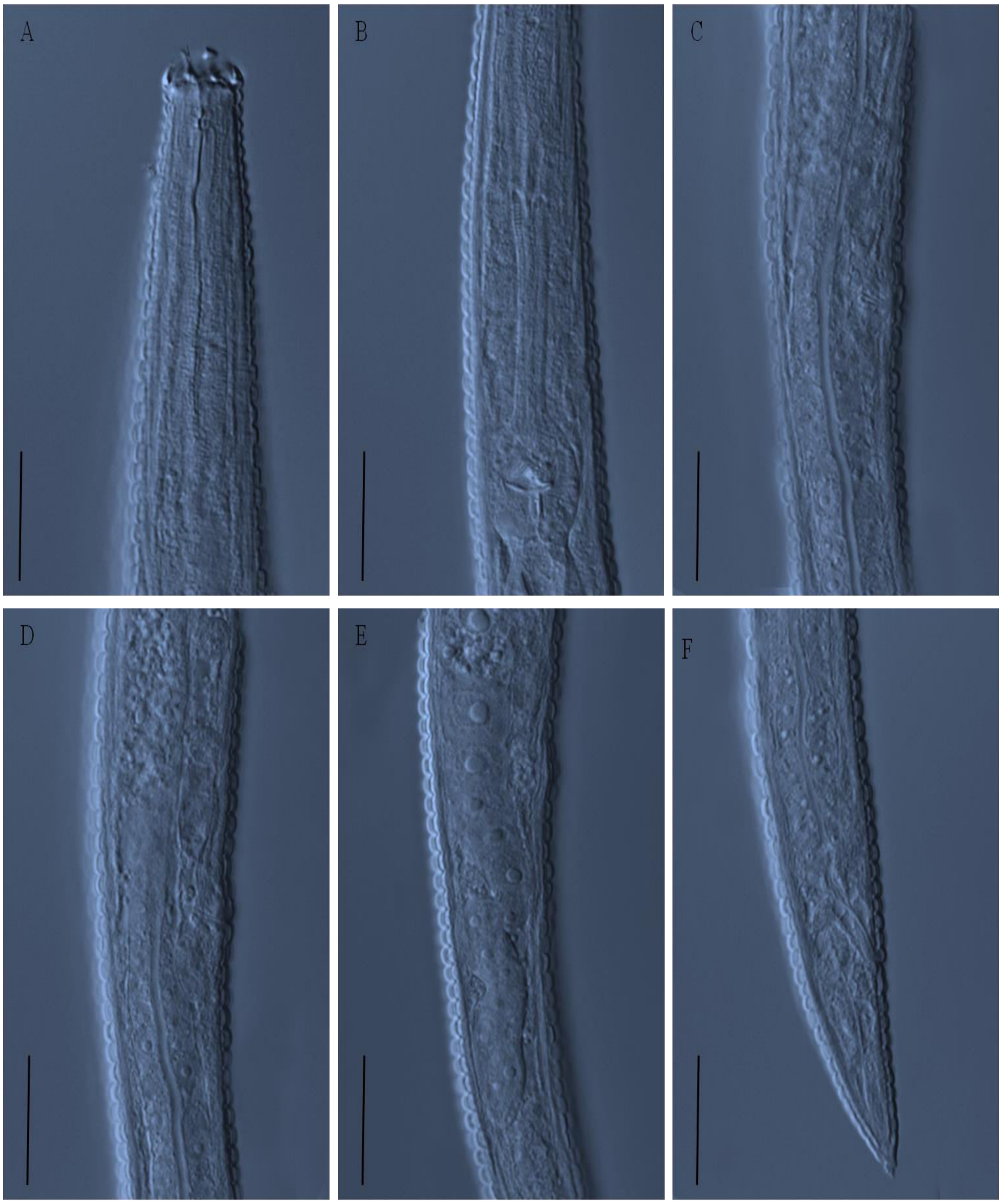

Plate 13 ***Stegillitina devimucronata*** (Sumenkova, 1964) Bostrom and De Ley, 1996 sp.: A- Anterior region, B- Pharyngeal region, C, D, E- Vulva Region, F- Tail [scale=10 μm].

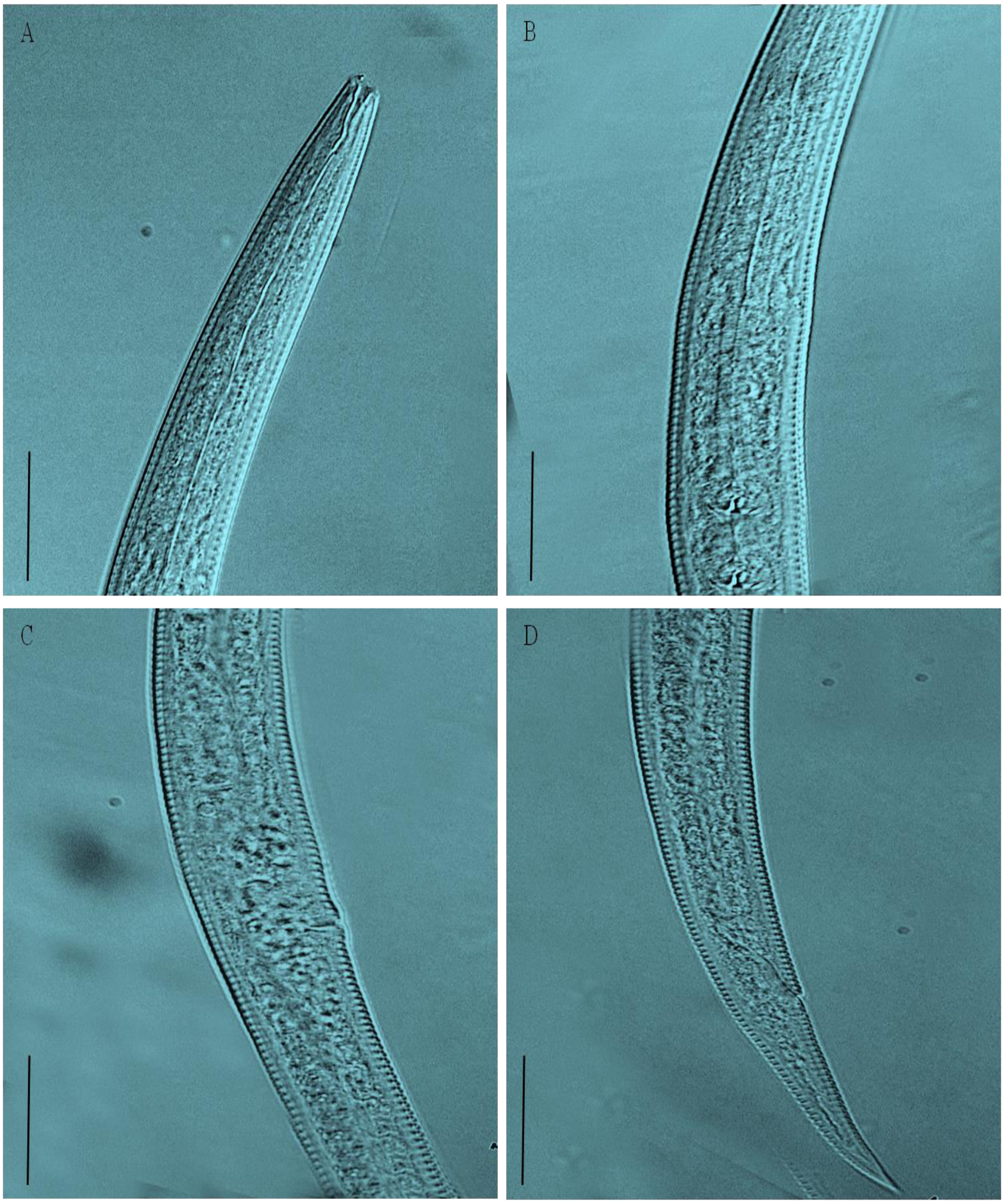

Plate 14 ***Pseudacrobeles pauciannulatus*** Marinari Palmisano, 1967 sp.: A- Anterior region, B- Pharyngeal region, C- Vulva Region, D- Tail [scale=10 µm].

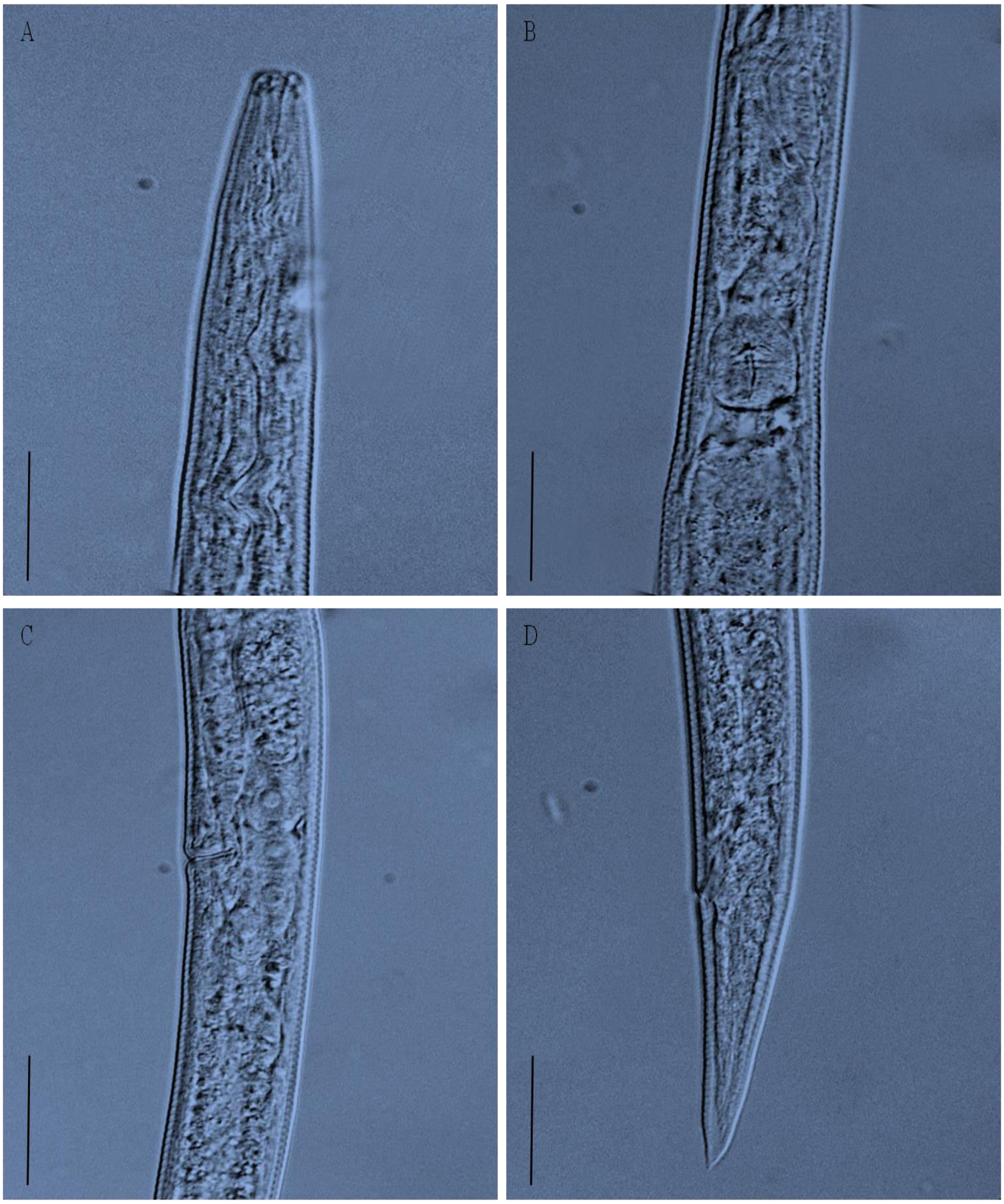

Plate 15 ***Pseudacrobeles eurystoma*** (Andrássy, 1967) Abolafia & Peña-Santiago, 2001 sp.: A- Anterior region, B- Pharyngeal region, C- Vulva Region, D- Tail [scale=10 µm].

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Conclusion

The current study on sil nematode community structure tried to achieve some basic insights: firstly, New records of bacterivores nematode are also recored from India and Uttarakhand itself namely ***Ceratoplectus armatus*** (Butschli, 1873) Andrassy, 1984; ***Anaplectus granulosus*** (Bastian, 1865) de Coninck and Schuurmans Stekhoven, 1933; ***Stegelletina devimucronata (***Sumenkova, 1964) Bostrom and De Ley, 1996 (from India); ***Plectus parvus*** Bastian, 1865***, Plectus parietinus*** Bastian, 1865 and ***Plectus minimus*** Cobb, 1893 from Uttarakhand. All the species described in the studies are first recorded from high altitude region of GNP. Secondly, documentation of genera richness of major nematode families as inventories in Indian Himalayan sub alpine and alpine vegetational zone along altitudinal gradient of Gangotri National Park, gathered as basic crucial information as well as to appraise variation along climatic gradient using OTC experiment; Thirdly, correlating the basic data of soilnematode abundance with different environmental parameters to know what drives their assemblage pattern. Molecular characterization of four cultured bacterivores were performed.The knowledge regarding variety of genera sampled across different habitats demonstrate their importance in practical conservation and also will help to publicize its importance in understanding soil health status as well as in developing strategies of mitigation and conservation management based on biological information. The universal problem in analyzing diversity and distribution of soil nematodes to monitor environmental changesor its bioindicator property is to establish the baseline data on nematode assemblages and environmental parameters. So output of this research work provided the baseline information on nematode assemblage in high altitude region of Indian Himalayan where a minor change may affect the nematode community structure. And can be used for the future long term monitoring studies on soil microfauna. Indicator properties of soil nematods using various nematode specific indices explored in the current research strongly supports that nematode assemblages can be useful as as tools for monitoring changes to natural assemblages in correlation with changes in habitat parameters. The study will certainly be helpful to promote soil nematode as model microfaunal group for long term monitoring and climate change studies.

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List of Publications

Kashyap, P., Rizvi, A.N. and Uniyal, V.P. (2020). Assessment of Soil Physicochemical Properties along an Altitude Gradient in High Altitude Region of Gangotri National Park, Uttarakhand, India. Journal of Ecophysiology and Occupational Health. 20(3and4), 134-139,

Bhardwaj, M, Kashyap, P., Uniyal, V.P. (2017) Common Blue Apollo, *Parnassius hardwickei*. pp 37 In: Sathyakumar, S., Mathur, V.B. (eds.) Wildlife Watch-Series III. A user guide for monitoring wildlife species in the Indian Himalayan Region. Wildlife Institute of India, Dehradun, pp 62.

Kashyap, P., Bhardwaj, M. and Uniyal, V.P. (2016). Bibliography of soil nematodes of Indian Himalayan Region. Chapter published WII Envis, Wildlife Institute of India, Dehradun, Uttarakhand. pp 239-256.

List of Conferences/ Seminar attended

Participated in the knowledge sharing and networking workshop on “Biodiversity, Forest Conservation and Management” organized by G.B. Pant National Institute of Himalayan Environment and Sustainable Development Kosi-Katarmal, Almora during May 24- 25,2018.

Presented a paper in National mission on Himalayan studies (NMHS)-Himalayan Researchers Consortium (HRC-2018) held during April 26-27, 2018 in Dehradun and got the second best presentation award on theme “Himalayan faunal and microbial diversity assessment”.

Participated in workshop “Connecting Science with Practice and Policy for Resilience towards Climate change risks in Uttarakhand” organized by central Himalayan association (CHEA), Nainital on 27th June, 2017.

Presented a paper on soil nematode of Bhagirathi basin in 1st Himalayan Research Seminar held at Wildlife Institute of India, Dehradun on 23rd September, 2016.