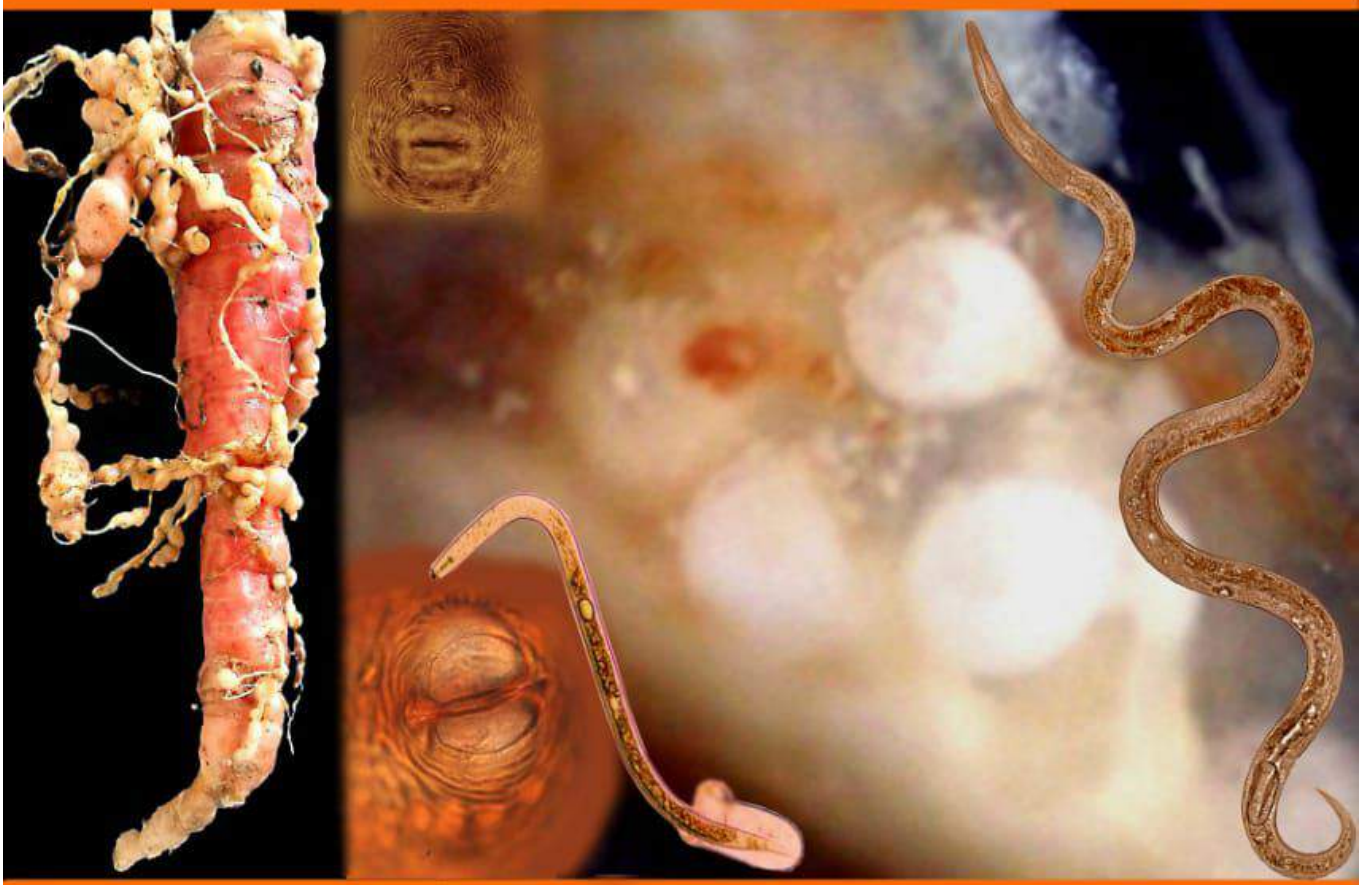
Textbook of Plant Nematology



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1st Edition

PSN Publication

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PREFACE

"Take a handful of soil, from the top of mountains and the depths of the seas. Extract the living organisms in some water, and among the other forms of life you will find elongate, threadlike, active animals. These are nematodes"; wrote Victor H. Dropkin in his erstwhile 1980 book; "Introduction to Plant Nematology". Thus, the nematodes of different kinds are found across the environments and habitats. Plant parasitic nematodes are of considerable importance worldwide and are costly impediments to crop production & productivity; their devastating effects on crops have major economic and social impacts. Phytoparasitic nematodes are one of the most abundant, highly diversified and ecologically significant groups with wide host range and geographical distribution. Th feeding action of plant parasitic nematodes, not only results in mechanical and physical injury to the tissues causing disturbance and distortion in plant growth process, leading to production losses but also affects the quality and marketability of the produce especially of root and tuber crops. This ultimately leads to food insecurity worldwide. In developing countries including Pakistan, nematode problems coupled with secondary infections, are serious and complex as climatic conditions and crop culture practices favour the activities and reproduction of these destructive parasites. However, there are some nematode species that play a beneficial role in the ecosystem, though; more research is required to learn about them.

Considering the importance of diversity and roles of nematodes in the ecosystem, and the fact that plant nematology is taught in degree programmes at various universities, not a single textbook of the subject has been produced in Pakistan. Thus, a dire need of such a textbook has been felt for quite some time for the benefit of students specializing in the subject at graduate and postgraduate levels. Therefore, this book has been designed to cover the course outline of "Plant Nematology", approved by the National Regulator- the Higher Education Commission (HEC) of Pakistan for B.S. and M.S. (Plant Pathology) degree programmes.

The book contains 16 chapters covering the latest information on the major fundamental and advanced aspects of plant parasitic nematodes. The book includes; basic introduction, global and local historical development of nematology, as well as its importance in agriculture. It explains the techniques for the study of nematodes including their morphology, anatomy, structure and systematics; and the outline classification of the nematode fauna of Pakistan. The textbook also provides for taxonomy of important parasitic nematode genera along with their parasitism and habitat. It has a chapter on the most important nematodes; root-knot, cyst, and some other important species; their life cycles, diseases caused by them, as well as disease symptoms, etiology and their management. Nematode-microbe interaction, nematode population dynamics and crop/yield loss assessment, also make part of the book. Exclusive chapters on nematodes as vectors of plant viruses, entomopathogenic nematodes and molecular nematology are also included in the book.

The authors hope that this "Textbook of Plant Nematology" with a touch of Pakistan perspective, would serve as a major guide book for students of Plant Nematology, Plant Pathology, Plant Protection, Microbiology, Environment, and Agriculture as a whole. Though the intended audiences of the book are students and researchers; the extension personnel, growers and those working in crop production and crop protection, would also benefit.

We have tried our best to put in the book, all the needed and available authentic scientific information of nematology, but to err is human, and we take the responsibility of any error or omission. However, the book can be improved in future edition(s). Thus, we would appreciate receiving any views, comments and constructive criticism as well as suggestions for improvement of the contents and quality of the book in future editions.

We dedicate this book to the initiators of the science of Nematology in Pakistan but especially to the two dedicated Pakistani Nematologists, who got this science recognized in Pakistan, established Pakistan Society of

Nematologists (PSN) and the National Nematological Research Centre (NNRC), i.e., Dr. M.A. Maqbool (1941-2016) and Prof. Dr. Shahina Fayyaz (1959-2020), who not only assisted Dr. Maqbool in all his endeavours, but further strengthened the NNRC, the PSN, and the Pakistan Journal of Nematology (PJN).

We are very grateful to the two outstanding champions of agricultural research for writing the Forewords to the book; a living legend and developer of Pakistan's National Agricultural Research System and the Founder Chairman of Pakistan Agricultural Research Council (PARC)- Dr. Amir Muhammed, and Dr. Zafar A. Handoo, a very senior Nematologist at the US Department of Agriculture, ARS, Beltsville, MD, USA. Dr. Handoo also kindly reviewed the book and advised on many technical aspects of the manuscript.

Finally, the financial support of Pakistan Science Foundation to Pakistan Society of Nematologists for publication of the book, is acknowledged.

Manzoor Hussain Soomro
Erum Iqbal
Firoza Kazi

FOREWORD

Plant parasitic nematodes are recognized as one of the most important limiting factors in crop production worldwide and Pakistan is no exception. They cause billions of dollars' worth of damage to global crop production annually. Plant Nematology was initiated in Pakistan during 1952 with the first ever report of ear-cockle disease on wheat from wheat growing region of Punjab province. However, systematic research in Plant Nematology began with a PL-480 project funded by the Pakistan Agricultural Research Council (PARC) in late1970s and this science further developed with establishment of the National Nematological Research Centre (NNRC) at the University of Karachi under auspices of PARC. It is satisfying for me as the then Founder Chairman of PARC that a small initiative in the fag end of 1970s, the NNRC became a truly National Centre and Pakistan Society of Nematologists (PSN) was established in early 1980s. PSN has continued to publish internationally recognized Pakistan Journal of Nematology (PJN) since 1983 and established the science of Plant Nematology in Pakistan so well.

I understand that Nematology forms an important part of the academic course of the undergraduate and postgraduate degree programs in agriculture and plant sciences in Pakistan. Higher Education Commission (HEC) of Pakistan has prescribed a common syllabus for Nematology course throughout Pakistan; however, there has not been even a single textbook published in Pakistan that covers the syllabus prescribed by HEC. Thus, I hope this book would serve as an important resource that fulfils the needs of the students in the universities of Pakistan and beyond. This book would also be very useful for the teaching faculty, research and extension personnel and others concerned with plant protection.

I sincerely appreciate and congratulate the authors; Prof. Dr. Manzoor Hussain Soomro, President of Economic Cooperation Organization Science Foundation (ECOSF) based in Islamabad, Patron-in-Chief of the Pakistan Society of Nematologists (PSN) and the Editor-in-Chief of PJN;

Dr. Erum Iqbal, Associate Editor of Pakistan Journal of Nematology (PJN); and Dr. Firoza Kazi, a senior scientist and nematode taxonomist associated with NNRC and PSN and an Associate Editor of PJN since long, for bringing out this comprehensive "Textbook of Plant Nematology". Authors have extensive experience of working in Nematology. Prof. Soomro of course having served as IPM Expert in FAO-UN, Professor of Plant Protection, Chairman of Pakistan Science Foundation and lately as President of ECO Science Foundation, has made important contributions in Nematology as well as in the fields of Agriculture, Plant Protection and in promotion of science and technology in Pakistan and beyond.

I take this opportunity to encourage all experienced scientists to write books based on their local, national and international experiences, for better learning of the younger generations.

Dr. Amir Muhammed
Founder Chairman of PARC
Former President of Pakistan Academy of Sciences

FOREWORD

Plant parasitic nematodes constitute a major constrain for agricultural crops worldwide. Significant contributions and achievements have been made in recent years globally; however, the emerging nematode problems limiting the production of major food, feed and industrial crops and their management strategies currently are big challenges across the world.

The aim of this textbook is to provide basic and advanced knowledge on plant parasitic nematodes with a global perspective. It targets practitioners, professionals, scientists, researchers, students, and other personnel working on crop protection and biosecurity about plant-parasitic nematodes.

The book entitled, Textbook of Plant Nematology, is an excellent book for students at the undergraduate and postgraduate levels, teachers of nematology/entomology/plant pathology and a perfect guide to applied plant protection practitioners, and a reference for researchers in and outside the country.

This book compiles and updates information on plant-parasitic nematodes and their alarming threat worldwide. This book is conveniently divided into 16 concise chapters with information on their identification; geographical distribution; systematics; symptoms; biology and ecology; and a detailed account of diagnostic procedures, such as sampling, isolation/detection, and identification with morphological and molecular characterization.

The stupendous effort of Prof. Dr. Manzoor Hussain Soomro, Dr. Erum Iqbal and Dr. Firoza Kazi deserve much admiration in bringing out this comprehensive and voluminous book. I am sure that its readers particularly students will find it very useful and informative.

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Chapter 1

INTRODUCTION TO AND HISTORICAL DEVELOPMENT OF NEMATOLOGY

Introduction

Nematology is an important discipline of agricultural sciences and deals with the study of nematodes. The name nematode was derived from Greek words nema (thread) and oides (resembling). Nematodes, also called roundworms, threadworms, eelworms or nemas, are a large group of organisms belonging to the phylum Nematoda.

Nematology is onere of the most dynamic, exciting and relatively a new discipline in the agricultural sciences. It was first recognized as an independent discipline during the middle of previous century. The development of Nematology is largely attributed to the discovery of the importance of nematodes in agricultural ecosystems and their impact on society. During the early years of the 20^{th} century, studies of nematodes made rapid progress. Cobb, the "Father of Nematology" did the pioneering work in Nematology at the United States Department of Agriculture in 1907 and continued till his death in 1932. He made major discoveries in the areas of nematode taxonomy, morphology and methodology with significant contributions. He coined the word "Nematology" for the science dealing with nematodes and for developing many techniques for the study of nematodes which are still used by Nematologists. Intensive efforts of other Nematologists helped in establishing various roles of nematodes in reducing the yield and quality of crops and also in their management.

During the past few decades the scope of Nematology expanded rapidly with the emergence of several new areas such as application of DNA based techniques in; nematode taxonomy, free-living nematodes in environmental monitoring and exploiting entomopathogenic nematodes in insect pest control.

Nematodes are the most abundant multicellular animals on earth and a diversified group in the animal kingdom that occurs worldwide in virtually every environment. Four out of every five metazoans on earth are nematodes, but are often overlooked because most of them are microscopic in size. Nematodes as parasites of plants, animals and humans are of considerable concern to agricultural, veterinary and medical scientists. Nematodes are second only to insects in the number of species in the animal kingdom. However, only 3 percent of all nematode species have been studied and identified so far. One cubic meter of soil may contain millions of individual nematodes belonging to several different taxonomic groups.

Nematodes have adapted to live in many different habitats and occur in wide variety of shapes, sizes and structures. Nematodes are typically bilaterally symmetrical, triploblastic, unsegmented, pseudocoelomate, vermiform animals with colorless or transparent body (Jairajpuri, 2002). Out of the total nematode population on the earth, 50% are marine nematodes, 25% are free living soil nematodes, 15% are animal parasites while 10% are plant parasites; but these plant parasitic nematode impact almost every plant on this earth and even every part of the plant including roots, stems, leaves, fruits, buds and seeds are causing heavy economic losses.

All phytoparasitic nematodes are characterized by the presence of a stylet, which is located in the stoma or mouth and have a channel through which the secretions and food passed. This structure allows phytoparasitic nematodes to pierce the wall of the host cells and inject enzymes that partially digest the cell contents before the nematodes suck it into their digestive system. In general, the plant parasitic nematodes are lower invertebrates having slender, elongate, spindle shaped or fusiform body, tapering at both ends and circular in cross section. The length of nematodes may vary from 0.2 mm (*Paratylenchus*) to over 12 mm (*Paralongidorus maximus*). Their body width varies from 0.01 to 0.05 mm. In few genera, the females on maturity assume pear shape (*Meloidogyne*), globular shape (*Globodera*), reniform (*Rotylenchulus reniformis*) or saccate shape (*Tylenchulus semipenetrans*). Despite their tiny size, they have highly specialized systems i.e., nervous system,

muscular system, excretory system and reproductive system; however, they lack circulatory and respiratory systems. The lifecycle of a typical nematode is divided into six stages; i.e., an egg, four stages of juveniles and adult. The duration of lifecycle of different species is different depending upon some ecological factors such as temperature, soil moisture and host plant.

Historical Development of Nematology

N. A. Cobb, an American Scientist, the founding Father of Nematology, first proposed the word Nematology in 1914 and recognized Nematology as an independent discipline; since then this term has been used in the discipline of agricultural science. Nematology as an independent discipline has its recognizable beginnings in the mid to late 19th century. Earlier, nematodes were frequently recorded by various writers in Medicine and Zoology (Hoeppli, 1959). However, some of the important milestones in the history of nematology viz., free-living, animal and human parasites are as follows:

- **6000-4000 BC:** Vedas are the earliest religious scriptures in the human history written 6000-4000 BC that contain occasional references to nematode parasites of human beings by the name Krmin or Krmi in Sanskrit (meaning worms). Other words; Sarpah (meaning snake) and Asparah (move retrogressively) were also used in Vedas.
- **3000 BC:** Charak recognized 20 different organisms as Krimis in his Samhita, which included nematodes besides arthropods and leeches.
- The first fossil of plant parasitic nematodes "*Oligaphelenchoides atrebora*" is 26 million years old.
- **2700 BC:** The oldest record of human parasitic nematode is *Ascaris lumbricoides*, the intestinal worm of humans appeared in the "Yellow Emperor Classic of Internal Medicine" from China.
- **1553-1550 BC:** Roundworm *Ascaris lumbricoides* and guinea worm *Dracunchulus medinensis*as parasites of man were known to Egyptians in "Papyrus Ebera" a manuscript of ancient civilizations of the Mediterranean and Middle East.

- **1250 BC:** The earliest written account of a nematode may be found in the Old Testament of the Bible "And the Lord sent fiery serpents among the people, and they bit the people; and much people of Israel died". Some historians believe that the fiery serpents that attacked the people of Israel and killed them were guinea worms as recorded in Bible (Chapter 21: Verse 6-8), as this nematode is known to inhabit the region near the Red Sea.
- **430 BC:** Hippocrates reported pin worm *Enterobium vermicularis* from women and horse.
- **384-322 BC:** Aristotle, often called the "Father of Zoology" mentioned animal parasitic nematodes viz., roundworms, flatworms and ascarids on several occasions in his "Historia Animalium".
- **181-146 BC:** Agatharchides of Cuidus described guinea worm.
- **53 BC-7AD:** A. C. Celsus distinguished roundworms (nematodes) from flatworms (cestodes).
- **200 AD:** Columella reported *Neoascaris vitulorum* from a calf.
- **180 AD:** C. Galen was the first who reported nematodes *Trigla* spp., from fish, the European red mullet.
- **1200-1280 AD:** Albertus Magnus provided the first record of nematodes from birds (Falcons).
- **1519-1630:** Caesalpinus discovered the giant kidney worm *Dioctophyma renale* from a dog (approximately 100 cm x 1.5 cm long).
- **1547:** Venergia reported Filarid (*Spiruria*) from falcon.
- **1656:** P. Borellus reported first free-living nematode, vinegar eel *Turbatrix aceti* in Europe.
- **1667:** Robret reported *Ponegrellus redvivus* eel in paste.
- **1683:** E. Tyson was the first who used a crude microscope to study nematodes, and described the nematode anatomy of the human intestinal roundworm, *Ascaris lumbricoides* and a nematode egg.
- **1951:** D. Gubanov reported *Placentonema gigantisma* (8 meters) as the largest nematode discovered in the placenta of a sperm whale.

Plant parasitic nematodes did not receive as much or as early attention as did animal parasites because they were less conspicuous to ancient scientists. The earliest information of a plant parasitic nematode is

however preserved in a famous line "Sowed cockle, reaped no corn," by William Shakespeare penned in 1594 in "Love's Labour's Lost', Act IV, Scene 3, most certainly has reference to blighted wheat caused by the plant parasitic nematode, *Anguina tritici.*

- **1743:** T. Needham discovered the first plant parasitic nematode *Anguina tritici*, the wheat gall nematode from England that caused ear cockle disease of wheat. It was named as "*Vibrio tritici*".

From 1750 to the early 1900's, Nematology research continued to be descriptive and taxonomic, focusing primarily on free-living nematodes, and plant and animal parasites. It was during the middle of 19^{th} century when Nematology commenced as a science with some important landmarks and the root parasitic nematodes were beginning to receive attention with their discovery in different regions of the world.

A series of some excellent monographs were published; which advanced the field of Nematology, including the detailed description with fine illustrations of several species of plant parasitic nematodes as follows:

- **1786:** O. F. Muller reported free-living nematodes from fresh water.
- **1855:** M. J. Berkeley observed nematode galls caused by *Meloidogyne* spp. on cucumber plants in green house in England.
- **1857:** J. Kuhn described a stem and bulb nematode, *Ditylenchus dispsaci* from malformed floral heads of teasel, *Dipsacus fullonum*.
- **1859:** H. Schacht reported the decline in sugar beet due to nematode in Germany later named as *Heterodera schachtii*.
- **1865:** H. C. Bastian gave the comprehensive monograph of free-living nematode (Anguillolidae) which marked the beginning of Nematology as a science.
- **1871:** A. Schmidt described the cyst nematode as *Heterodera schachtii*, the sugar beet nematode from Germany.
- **1871:** J. Kuhn was the first to use soil fumigation to control *Heterodera schachtii*, applying carbon disulfide (CS_2) treatments in sugar beet fields in Germany.

- **1873 & 1876:** O. Butschli made the first detailed description, illustration and morphology of free-living nematodes including a number of characters that are still in use in differentiating genera and species of nematodes.
- **1880:** L. Orley gave introduced the system of classification in his "Monograph of the Anguillulidae" for 202 nematode species representing 27 genera of free-living and plant parasitic nematodes with keys to both, genera and species.
- **1884:** J. G. deMan provided the taxonomic monograph of soil and fresh water nematodes of The Netherlands. He also gave the measurement of nematodes called de Man formula, still used in taxonomy today. Dichotomic keys were first published by de Man.

During early 20th century, Nematology experienced rapid growth and many advances were made. A number of scientists in many countries became active Nematologists which yielded a considerable account of important fundamental and applied knowledge about nematode biology. During the second half of the 20th century, Nematology developed more swiftly and became full-fledged discipline of science.

- **1907:** N. A. Cobb, Nematologist of USA described detailed morphology, taxonomy and life habits of plant parasitic nematodes**;** developed techniques for nematode extraction from soil and the methods for sampling for the study of nematodes, which are still used by Nematologists, such as Cobb's sieving extraction procedure.
- **1914:** N. A. Cobb was the first who proposed the word "Nematology and is thus called the "Father of Nematology". He wrote a valuable book, *Contribution to the Science of Nematology* containing articles on marine, soil, fresh water and insect nematodes with excellent illustrations.
- **1918:** N. A. Cobb published a lab manual *Estimating the Nema Population of Soil* for the benefit of new workers in this field.
- **1922:** Micoletzky wrote the most voluminous (650 pages) monograph on the free-living non-marine Nematoda comprised of 144 genera and 931 species.

- **1933:** Tom Goodey wrote the books *Plant parasitic nematodes & the diseases they cause* and *Soil & Fresh water Nematodes.*
- **1934:** I. N. Filipjev wrote a book in Russia on nematodes of agricultural importance. It was translated into English by Schuurman-Stekhoven in 1941 as *A Manual of Agricultural Helminthology*.
- **1936 & 1939:** G. Thorne produced monograph on Dorylaimida.
- **1941 & 1949:** G. Thorne published monograph on Tylenchida
- **1950:** B. G. Chitwood wrote the book, *An Introduction to Nematology*, was an outstanding compilation and served as an important guide book for students of Nematology.
- **1959: J**. R. Christie wrote a book, *Plant Nematodes: Their Bionomics and Control*, that was the first compilation of plant parasitic nematodes.
- **1960:** G. Steiner, wrote an article "Nematology-An Outlook", in *Nematology: Fundamentals and Recent Advances* Edited by J. N. Sasser and W. R. Jenkins.
- **1961:** G. Thorne wrote an excellent textbook, *Principles of Nematology* which still serves as a guide for many beginner students of Nematology.

In the following years, several publications have covered a wide range of aspects of Nematology viz., taxonomy, research and knowledge, documenting the significance of nematodes to agriculture. Some of the most significant publications are as follows:

- **1986 & 2000:** M. R. Siddiqi published a classical monograph on Nematology entitled, "*Tylenchida: Parasites of Plants and Insects*" Edition I & II. It was given the names of "Milestone of Nematology" by I. Andrassy and "Taxonomist Bible for the Tylenchida" by E. Geraert.
- **Blaxter *et al*., (1998) and De Ley and Blaxter (2002)** revised the classification of phylum Nematoda based on molecular and morphological characters.
- **2005:** Andrássy's contributions in the field of nematode taxonomy (2005 a-c) will always remain a great asset of Nematology forever.

Molecular Advancement in Nematology

During the last few decades the scope of Nematology expanded rapidly with the emergence of several new areas such as application of molecular or DNA based techniques in nematode taxonomy; molecular characterization of nematodes as bio-pesticides (Entomopathogenic nematodes as insect pests), bio-indicators (free-living marine nematodes in sea pollution monitoring) and bio-fertilizers (free-living soil nematodes in environmental monitoring).

Hereunder is the chronological development in molecular aspects of nematology.

- **1974:** Epstein H. F. first time the protein of *C. elegans* was readily analyzed by SDS-PAGE.
- **1981:** Files and Hirsh, the first genetically identified *C. elegans* gene "*unc-54* (myoin gene)" was cloned.
- **1984:** E. C. Lawson took a step towards nematode identification with the use of biochemical and molecular diagnostic tools such as; the enzyme- linked immunosorbent assay (ELISA) and isoelectric focusing (IEF).
- **1990:** T. S. Harris and his colleagues did the identification of single *Meloidogyne* juveniles by polymerase chain reaction (PCR) amplification of mitochondrial DNA.
- **1992:** Sulston J. and his team initiated the genome sequencing project of *C. elegans.*
- **1997:** T. O. Powers proposed the ITS regions of mDNA as targeted sequence for identification purposes.
- **2008:** Hillier L.W. and his team completed whole genome sequencing of.*C. elegans*.

The use of PCR technology enables nematologists to diagnose the disease causing nematodes rapidly and accurately. Furthermore, the use of PCR is adopted by the European and Mediterranean Plant Protection Organization (EPPO) and used in standardized phytosanitary protocols.

Some Early Records of Nematodes of Indo-Pakistan Subcontinent

- **1901:** C. A. Barber reported the root-knot nematode, *Heterodera radicicola* (*Meloidogyne sp.*) on roots of tea from India.
- **1913:** E. J. Butler reported "Ufra disease of rice" caused by *Ditylenchus angustus* in Bengal.
- **1917:** C. M. Hutchinson reported the occurrence of wheat gall nematode, *Anguina tritici* in the province of Punjab of the undivided India.
- **1935:** H. Chowdhury also reported the occurrence of *Anguina tritici* in the provice of Punjab, causing ear-cockle disease of wheat.

Development of Nematology in Pakistan

During early 1950s, the nematological research was initiated by individual scientists at different institutions of the country. These pioneer scientists of Nematology contributed a great deal to the knowledge of plant, soil and marine nematodes from time to time. Some of the important findings made in Nematology are highlighted below.

- **1952:** A. Sattar and A. Hafeez reported wheat gall nematodes on wheat from D.G. Khan, Muzaffarabad and Jhang Districts.
- **1961-1962:** S. A. Akhtar gave the first report on the occurrence of a large number of plant and soil nematodes from Lahore.
- **1962-1963:** R. W. Timm, described number of new and known species of marine nematodes from Karachi.
- **1963:** A. Kafi reported a number of plant parasitic nematodes along with their hosts and habitats from Pakistan in a technical document of FAO.
- **1965-1970:** Inamullah Khan and his students (M. Ashraf, Riaz Ahmad Chohan and Safdar A. Anwar) did research on plant diseases caused by nematodes and their control at the University of Agriculture, Faisalabad.
- **1968:** S. H. Ashrafi and his associates initiated research on plant parasitic nematodes at PCSIR, Karachi.

- **1970-1992:** Manzoor Saeed, Hanif A. Khan and their co-workers at PCSIR, Karachi reported number of plant parasitic and soil nematodes and their association with different hosts from Karachi and other parts of Sindh. They also undertook research on management of plant parasitic nematodes including development of nematicides. S. A. Anwar and co-workers recorded several plant parasitic nematodes from various fruits and vegetables of Punjab. Riaz Ahmad Chohan and his associates focused their research on a number of nematode species of agricultural importance especially root-knot nematodes (*Meloidogyne* spp.) and citrus nematode (*Tylenchulus semipenetrans*) and their management including the use of bacterium *Pasteuria penetrans* at the University of Agriculture Faisalabad. Research work on basic and applied aspects of Nematology was started at Crop Diseases Research Institute (CDRI) at National Agricultural Research Centre (NARC), Islamabad by Anjum Munir and at CDRI Karachi Campus, Karachi by Aly Khan. It was also in the 1970s that G.R. Solangi began research in plant nematology at Sindh Agriculture University Tandojam and trained students like Manzoor Hussain Soomro.
- **1974:** National Nematological Research Centre (NNRC) was establishment at the University of Karachi by M. A. Maqbool with the guidance and support of Zain-ul-Abedin (Dean, Faculty of Science, University of Karachi).

  Subsequently, under the leadership of M. A. Maqbool and A. Ghaffar, a Professor of Plant Pathology, Department of Botany, University of Karachi, the nematological research particularly the identification of major nematode species was initiated systematically in the country.
- **1980:** Pakistan Society of Nematologists was founded by M. A. Maqbool.
- **1983:** Publication of Pakistan Journal of Nematology (PJN) was initiated by the Pakistan Society of Nematology (PSN) in 1983 with A. Ghaffar as Editor-in-Chief till his death in 2015. Afterwards, Manzoor Hussain Soomro, then the President of PSN took over as the Editor-in-Chief of PJN, the responsibilities he continues to date (January 2022).

- **1986:** A. Hafiz published a book on "Plant Diseases" of Pakistan including the diseases causes by nematodes. It was the first agricultural text book of its kind in Pakistan.
- **1996:** M. A. Maqbool and F. Shahina initiated research on entomopathogenic nematodes as bio-control agents.
- **1998:** M. A. Maqbool and K. Nasira initiated the research on marine nematodes as pollution indicators.
- **2005-todate:** Significant progress has been made by NNRC in the morphological, biochemical, molecular biology, genomics, of plant parasitic nematodes as well as beneficial nematodes.

NNRC, a Centre of Excellence in nematode taxonomy in the Near East Region (as declared by the FAO in 1992), since its inception has been rendering valuable services in providing diagnostic and advisory services, conducting agricultural research and educational programs, with professionalism and ethical practices. The Centre has also been technically supporting various institutions all over the country. NNRC during it's over 47 years of existence, has attained a globally accepted research and education institution in Nematology. In these endeavors, several pioneer scientists and educationists have selflessly rendered valuable services. The journey of NNRC of almost five decades will remain incomplete without mentioning the endeavors and leadership of M. A. Maqbool, A. Ghaffar and Shahina Fayyaz, consistently supported by Manzoor Hussain Soomro. They all contributed immensely in the development of Plant Nematology in Pakistan.

NNRC is the permanent office of PSN and PJN; and with the efforts of Prof. Manzoor Hussain Soomro, PSN is an accredited member of the International Federation of Nematology Societies (IFNS-http://www.ifns.org/).

Importance of Nematology in Agriculture

Plant parasitic nematodes are one of the major limiting factors for production of major crops worldwide. Overall, they cause an estimated annual crop loss of $78 billion worldwide and an average of 10–15%

crop yield losses. Pakistan alone loses more than 2 billion rupees annually due to plant parasitic nematode infestation in different crops.

The wide distribution, extensive host range and involvement with other microorganisms in disease complexes put nematodes on top of the list of plant pests affecting agricultural production globally. Often the plant damage caused by nematodes is overlooked because the resulting nonspecific symptoms, such as slow growth, stunting, and yellowing, can also be attributed to nutritional and water-associated disorders.

However, many nematode species are beneficial to agriculture and the environment. For example, some have proven to be important allies in the biological control of insects and other pests, and some contribute to soil fertility by helping cycle nutrients through the soil.

Plant Nematology has been growing more rapidly and gaining more importance in comparison with other traditional plant protection disciplines. At present, education and research in plant nematology occupies a significant and important place among those of other plant protection disciplines. Effective methods have been developed for preventing the harmful effects of parasitic nematodes without a heavy reliance on pesticides, besides understanding and effective promotion of the beneficial aspects of agriculturally advantageous nematodes.

Importance of Nematode Identification in Agriculture

Research on nematodes directly benefits humanity and greatly enhances our understanding of the earth's biodiversity. The importance of nematodes especially in agricultural productivity was realized only in the middle of the last century during the Second World War. The discovery of soil fumigants established the economic importance of plant parasitic nematodes. Inspite of the vast knowledge about the harms and control aspects of parasitic nematodes, there are lacunae in the emerging areas of nematode problems.

The identification of new or potentially harmful species of nematodes is important in crop production and aids in the development and evaluation

of quarantine or regulatory procedures to minimize their spread. As world travel and the transportation of plant material increases, the need to monitor the movement of destructive nematodes increases. Correct identification of nematode species is basic requirement for efficient nematode control and successful plant quarantine operations.

Preventive regulatory programs have minimized or prohibited the introduction of parasitic nematodes to new agricultural areas efficiently and have proved cost-effective to minimise future crop losses. In 1941, for example when the golden cyst nematode of potatoes was discovered in Long Island, New York, the immediate implementation of a federal quarantine on this serious pest of potatoes helped prevent the spread of this species in the United States, thereby saving annually millions of dollars in crop loss due to this exotic pest.

Quick and accurate nematode identifications are very important in the release of shipments of various domestic and foreign plant material, food stuffs and wood products detained at various ports by Animal and Plant Health Inspection Service (APHIS) inspectors. The results of identifications are used by APHIS personnel for taking appropriate regulatory actions beneficial to the public, and are significant because they save importers and exporters from losing millions of dollars in product deterioration and other losses, if and when the shipment of crops products would not be allowed to be unloaded. For example, in 1997, a devastating seed gall nematode (*Afrina wevelli*) was identified at several occasions intercepted by APHIS USA from *Eragrostis* sp. seed galls from South Africa.

Most nematodes feed on bacteria, fungi, or other microscopic creatures; thus as such, they are a major component of soil and sediment ecosystems. One species that feeds on soil bacteria, *Caenorhabditis elegans* is better characterized than that of any other multicellular organism. *C. elegans* is studied as a model system in molecular and developmental biology, and is providing insights into many other areas of biology and medicine. Three specialists on the biology of this worm were the 2002 recipients of the Nobel Prize in Medicine.

Chapter 2

BASIC TECHNIQUES FOR THE STUDY OF NEMATODES

To study plant parasitic and free-living nematodes, various techniques and methods are used. The basic techniques and methods used in collection, extraction and processing are explained below.

Sampling and Sample Collection

In collecting plant and soil samples for nematode examination, it should be kept in mind that many nematodes feed on plants, and can be most easily found where food for them is abundant. Therefore, a good way to proceed is to sample soil around the roots of growing plants.
Soil samples are collected from the root zone of plants at a depth of 15-30 cm along with some root system (fine feeder roots). In case of trees and other deep rooted perennials, 60cm depth might be more appropriate for collection of soil samples. Sampling can be carried out at random or systematically in different patterns. Samples are always taken when the soil is moist in the vicinity of plant roots; very wet or very dry surface soil should not be sampled. Collected soil and root samples are placed in polythene bags to avoid drying. Nematodes are rarely distributed evenly in a field; thus it is important to collect samples from several areas within a field. To obtain a reliable estimate of the nematode species, random sampling of a large area / field has to be done with several sub samples (10-100) and mixed to draw a composite sample for assay. The greater the number of sub-samples / cores for each sample, the more accurate the assessment will be. This is a basic procedure outlined by Cobb (1918).

For diagnostic purposes, samples should be collected in the middle of the season and/or at final harvest. For predictive population estimation, the samples are often taken early in the season, at or before planting when plant-parasitic nematode numbers are typically very low; better predictive assessments can even be made from the end of season samples from the previous crop.

Collected samples should be kept cool and moist by placing them in an ice chest/box during transit to avoid getting them hot or dry; and be processed as soon as possible. The plastic bags are tied with rubber bands, tagged and labeled clearly with permanent markers, containing necessary information about host (crop/cultivar), sampling date, location (possibly with Global Positioning System- GPS), disease symptoms, previous crop history, size of the sampled area, name and address of the farmer.

Storage

Normally the samples are processed as soon as they are collected and brought to laboratory. However, when samples need to be stored for longer than 1-2 days, it is advisable to keep them in a refrigerator at 4-5°C temperature.

Methods of Nematodes Extraction from Soil and Roots

A number of methods are available for extracting the nematodes from soil or plant parts. The most popular methods are Cobb's sieving and decanting method (Cobb, 1918), Baermann funnel method (Baermann, 1917) and its modifications; and the centrifugal flotation method (Southey, 1986). Practically, one or more methods are often combined to extract nematodes.

Cobb's Sieving and Decanting Method (Cobb, 1918): This is a basic, quick and easy technique for nematode extraction from soil, using a set of 3 to 4 sieves of different mesh pore sizes in a downward order of; bigger, medium and smaller pore sized sieves. The sieving technique is also known as the "bucket sieving" method. It is widely used as it enables the extraction of a large number of both active and inactive nematodes in a relatively short time.

This technique is explained in sequential manner in **Fig. 2.1 (a-j)**. In this technique, 250-500 g of soil sample is placed in a plastic tub/bucket containing water **(Fig. 2.1. a,b)**. The mixture is vigorously stirred to get a homogenous suspension and is soaked for 15-20 seconds. The lumps or

clods are gently broken with fingers and the stones and debris removed **(Fig. 2.1 c,d)**.

It is then left for 1-2 minutes to settle the heavy particles to the bottom of the bucket. The soil suspension is stirred again and the supernatant water decanted through the coarse sieve (36 mesh size) into the 2nd bucket and the residue left in the first bucket is discarded **(Fig. 2.1 e)**. Any roots present on 36 mesh sieve are collected and the debris discarded **(Fig. 2.1 f-h)**.

The contents of 2nd bucket are then stirred, allowed to settle for 30 seconds and then poured gently through a 100 mesh sieve into a 3rd bucket. The deposits on the sieve (100 mesh) are transferred into a 250 ml beaker by rinsing the sieve with a gentle stream of water **(Fig. 2.1 i,j)**.

It is then examined to check the presence of cysts (*Heterodera* and *Globodera*) and large sized nematodes (*Longidorus* and *Xiphinema* etc.) under a stereomicroscope.

The above process is repeated by adding about one liter of water to get optimum number of nematodes. Most of the nematodes in the soil sample will now be in the water of the 3rd bucket. The residue left in the second bucket is then discarded. The soil suspension of 3rd bucket is stirred and allowed to settle and then the supernatant water poured from the 3rd bucket gently through the fine sieves (270 and 325 mesh); the small size nematodes would be collected on these fine sieves; most of the nematodes will remain on the fine sieve (325 mesh). The 3rd bucket contents are then allowed to run down the drain with a trap whereby any remaining nematodes can be trapped and then treated to avoid any spread through drainage. The deposits on 270 and 325 mesh sieves are washed by a gentle stream of water from a dropping bottle into separate glass beakers to collect smaller size nematodes **(Fig. 2.1 i-j)**. The quantity of water is minimized by decanting after allowing the nematodes to settle at the bottom of the beakers.

The samples so collected in the beakers often become dirty due to the presence of fine soil particles, thus it may be processed further through

Baermann funnel technique. The combination procedures allows extraction of nematodes from large soil samples and is easier to examine under binocular microscope. During soil processing fine sieves are commonly blocked with soil particles and can be removed by gently tapping the frame with hand on the underside of the bottom sieve.

Fig. 2.1. Cobb's Sieving and Decanting Method (from NNRC).

Baermann Funnel Method (Baermann, 1917)

A Baermann funnel is made by attaching a short piece of rubber tubing to a funnel stem and placing a pinchcock or clamp at the bottom of the rubber tubing. The funnel is then placed in an upright position with a suitable support on a ring stand or a rack specially designed to hold multiple funnels and partly filled with water. This method is used to recover active nematodes only (**Fig.2.2 a-f**).

A tissue paper is placed over the plastic basket that just fits the inside of coarse mesh sieve on the funnel (**Fig.2.2 a,b**). The beaker containing nematode sample which is already processed by Cobb's sieving method, is now inverted in the funnel through plastic basket/ sieve, covered by tissue paper and the water containing nematodes slowly pours through it (**Fig.2.2 c,d**). The water level should be adjusted until the water just covers the sample. The funnel is then left for 48 hrs.

Nematodes migrate from the sample through the tissue and sink to the bottom of the funnel stem. Water sample containing nematodes is drawn after 24-48h from the funnel stem by opening the clamp or pinchcock into a beaker. The sample should not be more than 100 ml (**Fig.2.2 e**).

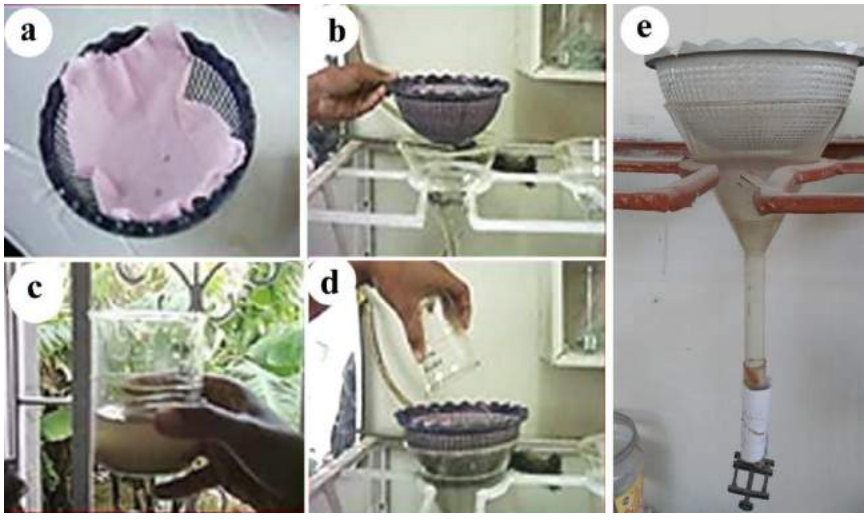


Fig.2.2. Baermann Funnel Method (from NNRC).

Modified Baermann Funnel Method: There are many modifications of Baermann funnel method. Lack of oxygen and the problem of nematodes lodging on the slopping funnel sides are overcomed by replacing the funnel with a Baermann pan or shallow extracting tray dish that can be used to extract nematodes from large quantities of soil. It is also called pie-pan method or Whitehead tray method. It is a simple technique and can be used to extract both ectoparasitic and endoparasitic nematodes from soil and plant tissues, respectively.

A coarse plastic or metal sieve is placed in a suitable size container e.g., basin, pan, bowl or tray. Small supports are used to provide space between the base of the sieve and the collecting tray. A tissue paper is placed over the sieve and a known weight of soil is spread on it. Water is then added carefully under the sieve inside the edge of the tray until the soil / root are just immersed. The tray(s) are left for 24-48 h.

After 48 h the inner sieve with tissue paper and soil is gently removed, leaving a clear water suspension containing nematodes that have migrated through the tissue into the water. The nematodes are concentrated by pouring the water suspension from the tray into a beaker and allowing the nematodes to settle down. After 30 minutes, the supernatant water can be drawnout/siphoned off. The sieve is reimmersed in fresh water for further extraction of nematodes. Nematodes can also be extracted from roots and other plant tissue by this method.

Centrifugal Flotation Technique (Southey, 1986): This technique is very good for extracting sluggish nematodes e.g., *Criconematoides* and also dead nematodes. Centrifugal flotation is generally more efficient nematode extraction method than sieving, Baermann or other techniques. This method is often used to clean extract obtained by sieving. In this method nematodes are extracted from soil and organic debris by floating them out in a solution with a greater specific gravity than their own average density.

Sugar (sucrose) is the most used solute in this method because it is easily available and relatively cheap. A solution with a specific gravity of about

1.18 (484 g of cane sugar dissolved in water and made up to one liter) is suitable. This suspension is so clean that the nematodes can be collected directly on very fine sieve.

The suspension is collected by sieving method in two 50ml centrifuge tubes which are balanced before spinning at 1750rpm for 4-5 minutes. The supernatant is poursed off and replaced with sucrose solution (sp.gr. 1.18). The tube is balanced, shaken and spinned for 1-2 minutes. The supernatant is poured through two or three fine sieves (325 mesh). The sieves are washed in a beaker and nematodes collected for examination.

Extraction of Cyst Nematodes

The suspension of 100 mesh sieve is examined for the presence of cyst nematodes by pouring the soil suspension into a Petri dish and examining under a binocular microscope. The cysts are collected with glass dropper and transferred into another glass cavity block.

Extraction of Root-Knot Nematodes

Infested roots are collected and cut into 1-2 cm segments for observation. Small pieces of the cut plant roots are placed in a Petri dish and water is poured over; then observed under the dissecting microscope. Using dissecting needles or syringe needles, the egg-mass are pulled and the root-knot females are gently removed out of the gall. These females are picked with the help of a glass dropper and transferred to a glass cavity block for identification.

Extraction of Vermiform Nematodes from Plant Parts / Roots

Extraction of vermiform nematodes from the plant parts including roots is done using different methods; however, the following two techniques are considered more efficient and convenient.

Waring Blender Technique or Maceration Technique (Stemerding, 1964)

This method is quicker and more efficient than other methods such as; root incubation, for extraction of migratory and endoparasitic nematodes from various parts of plant viz., roots, leaves and stem. This technique is also used for extracting *Meloidogyne* eggs from roots.

The plant parts/ roots are gently washed to remove soil particles, copped/cut into 1 cm pieces, and 2-3 g pieces and placed in the electric blender with about 100 ml of water. The blender contents are macerated for 10-20 seconds and poured through a 36 and 325 pores mesh sieves. The 325 mesh sieve water can be placed in a Baermann funnel to get clear suspension. Nematodes separate themselves from the inert debris by wriggling through the filter. That way the live vermiform nematodes are collected in a beaker or dish and observed under light microscope.

Root Incubation Method (West, 1957)

This method is used for examining the nematodes from large number of root samples. Endoparasites, immature stages and males of sedentary parasites are extracted by this method. The roots are washed gently with tap water to remove the soil particles. Washed roots are cut into small pieces of 5-10 cm length. Large diameter or flesh roots can be split longitudinally to help the nematodes emerge. The roots are kept in containers with closable lids, e.g., screw cap jars, closed Petri dishes or sealed polyethylene bags with enough water to submerge the roots at 20-25°C. After 24 h of settling, the excess water is siphoned/poured off for nematode examination. Extraction can be continued by adding more water and reclosing the container. Within 4-7 days, most of the nematodes are recovered. Soil or root pieces (about 100 mg) can be spread on a tissue paper placed over the net that is further placed onto a rectangular plastic tray, just bigger in size. Water is then poured from outside of the net till it touches the soil or roots layer which become moistened. This arrangement is left overnight for nematodes to naturally move down in the water on the principle of gravity. On second day, the net with tissue is removed/lifted from one side and water allowed to rinse down in the tray. Water from the tray is collected into a beaker and left

for 0.5-1 hour. Then the excess water is poured off and the contents with small amount of water are transferred into a Petri dish for examination of nematodes under the microscope.

Microscopic Observations

Direct Examination of Plant Material

Nematodes can usually be seen by examining small amount of plant tissue with a stereoscopic microscope at magnifications from 15x to 50x using transmitted / or indicent light.

In this method, the roots are washed to remove as much soil as possible. Small pieces of infected plant tissue- roots, stem, leaves, buds or seeds are examined in clear water in an open Petri dish by teasing the tissue apart with dissecting needles under a microscope. Endoparasitic nematodes if present will float out and can be collected with a handling needle or fine pipette, fixed in 3% formaldehyde and examined for species identification at higher magnifications.

The sample should be re-examined after 2-3 h as nematodes tend to migrate out from the damaged tissues. This is the best method for root-knot and cyst nematodes as swollen female can be observed easily. Careful observation of plant material reveals the presence of nematodes in galls or swellings on roots/tubers/rhizomes and the white, yellow or brown pinhead sized bodies adhering to the roots, swollen or malformed leaf stem or other tissue and roots lesions.

Staining Nematodes in Plant Tissue

Staining is used to detect nematodes in infected plant tissue. A wide range of staining techniques has been reported. It includes the most common staining technique- the acid fuchsin dissolved in lactophenol (McBeth *et al*., 1941). The nematode retains the deep red stain more strongly than the root tissue. For dense staining thinly sliced material is essential.

Staining with Cotton Blue or Acid Fuchsine in Lactophenol

Gently washed plant material free from soil is plunged into 100 ml boiling lactophenol containing 0.1% cotton blue or 0.05-0.1% acid fucshin stain for 3 minutes. Lactophenol should be in a deep beaker because it froths when the material is added. Several small samples can be stained in one operation by wrapping them separately in pieces of muslin cloth.

The tissue is removed, excess stain washed off in running water and placed in a Petri dish. Roots are covered with plain lactophenol. Liquid phenol is used for decolourization of leaves or stem. After 2-3 days, they can be examined in lactophenol under a stereomicroscope; nematodes stain blue or red if cotton blue or acid fucshin, respectively are used; whereas the plant tissues, except for meristematic region, remain largely unstained.

Staining in Lactophenol/ Lactoglycerol

Lactophenol method has been widely used in the past; however, it is now recognized that phenol fumes are dangerous for human health; hence the use of lactoglycerol is recommended (Bridge *et al*., 1982). This is a solution of equal volume of glycerol, lactic acid and distilled water plus 0.05% acid fucshin or 0.05% methyl blue stain.

Procedurally, the gently washed plant material free from soil and debris is sliced thinly before staining. The washed infected plant material is gently plunged into boiling lactoglycerol. Lactoglycerol should be in a deep beaker as frothing occurs when material is added. Several small samples can be stained in one operation by wrapping each in a separate piece of muslin cloth.

The material is boiled for 3 minutes; allowed to cool in the stain and washed well in water before it is cleared in equal volume of glycerol and distilled water (acidification with a few drops of lactic acid is also recommended). After 2-3 days, stained nematodes can be seen in largely unstained tissue. *Meloidogyne* egg-masses can be detected on roots by

soaking them in phloxine-B stain (0.15g/L water) for 15-20 minutes, rinsed and examined in water; the gelatinous matrix of the egg sac stains red (Holbrook *et al*., 1983).

Estimation of Nematode Population

Nematode population in a given sample is estimated by the following methods.

Quantitative Analysis

Quantitative sampling is performed to determine the number of nematodes in a sample. The nematode suspension is collected in a beaker and the volume madeup to 100 ml with water. The suspension is vigorously bubbled with a pipette and 5ml of this suspension poured in a counting dish. The counting dish is kept under the stereomicroscope and allowed the nematodes to settle for a few seconds (**Fig.2.3 a,b**).

The magnification of stereomicroscope is adjusted so that one complete square of counting dish can be visible. The counting of nematodes is started from first square and moved to the next square either vertically or horizontally, till nematodes in all the squares have been counted (**Fig.2.3 c,d**). Three readings/counts are done and calculation is done to get the average number of nematodes per ml. In this way the nematode population per 500 g soil is determined.

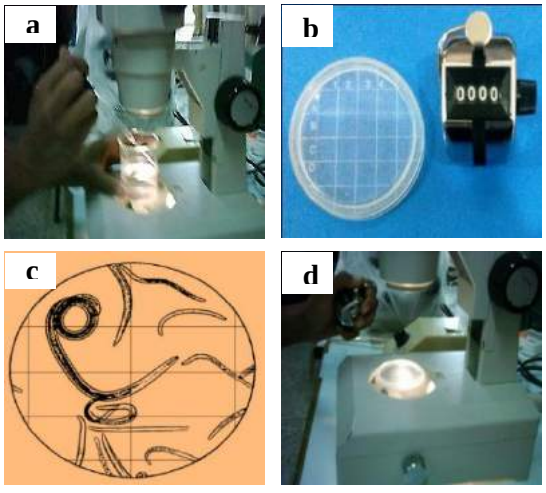


Fig.2.3. a-d. Quantitative analysis of nematodes (from NNRC).

Qualitative Analysis

For qualitative analysis, observations are made on temporary mounts under the stereomicroscope. The nematode suspension is allowed to settle for 2 hours or more. The excess supernatant water is poured off by a dropper. Then the remaining concentrated contents, containing nematodes is transferred into a cavity block. Three small drops of concentrated nematode suspension are placed on a 3x1" glass slide. The glass slide is then placed on a hot plate to gently kill the nematodes till assuming their characteristic body posture/shape; straight in some genera, curved or spiral in others; it is must be ensured not to overheat the specimens.

A clean cover-slip is carefully placed over the drop of water on the glass slide, care is taken not to trap air bubbles. The specimens are sealed by applying zut or nail polish around the cover-slip, the slides are then labelled and examined under the compound microscope. The identification of nematodes is done to generic level for nematode population. By this method, the percentages of nematode genera present in the sample are calculated.

Processing of Nematodes

Handling, Killing, Fixing and Mounting of Nematodes

Handling of Nematodes

The nematodes are picked individually with handling/hair needle from a suspension. Small batches of nematodes can be transferred from a suspension to an observation dish, using a fine pipette. The nematodes so transferred, should be in shallow water, and in the center of cavity block/dish to be examined at the lowest microscope magnification in order to allow the greatest possible depth of focus and working distance. For viewing under microscope, the needle be held underneath the nematode and quickly flicked up so that the nematode is pulled out through the surface of the water. The number of nematodes present in a population of a given sample are then calculated.

Killing and Fixing of Nematodes

After qualitative analysis, the nematode suspension is left for 2 h to let the nematodes settle in the bottom of the beaker. The excess water is decanted with a pipette/dropper, leaving the nematodes in a comparatively small volume of water. The nematodes are killed over small flame and then fixed at the same time. Best results are obtained if nematodes are killed quickly and fixed immediately in one process (Seinhorst, 1966). If live nematodes are put into the cold fixative, they are usually distorted and spoiled. The best way to kill nematodes is on a controlled hot plate at 60-80 °C. It prevents the specimens from the damage caused by overheating. Nematodes can also be killed by transferring them in a cavity block containing water or on a glass slide, then heated on hot plate, water-bath or in an oven (43°C). They should be transferred to fixative immediately.

Killing and fixing at the same time can be carried out with dilute solution of 2-4% formaldehyde or FA 4:1; in both cases the fixative is heated to 80°C and added to the nematodes which are in a small drop of water. Formaldehyde at 4% has been considered better, followed by killing with FA or FP 4:1. Specimens can be immediately fixed and preserved in a TAF solution for 24 h. In TAF fixative, the appearance of nematodes is remarkably lifelike; however, it is not recommended as a long term fixative.

Fixatives: Numerous fixatives have been recommended from time to time. Fixatives which are commonly used are:

T. A. F. (Courtney *et al*., 1955)

Triethanolamine 40 % (C_6H_{15}-NO_3)	= 02 ml
Formalin (37 % formaldehyde)	= 07 ml
Distilled Water	= 91 ml

Formal Acetic (F.A.) or Formal Propanic (F.P.) 4:1

Formalin (40% formaldehyde)	= 10 ml
Glacial acetic acid / Propanic acid	= 01 ml
Distilled water	= 89 ml

2-4 %formaldehyde solution (i.e. 5-10% formalin)

Mounting Process of Nematodes

The fixed specimens (after 24 h) are washed 3 times with distilled water to remove most of the fixative. The excess amount of supernatant water is poured off each time. The fixed nematodes are processed by a slow dehydration method (Seinhorst, 1959). The specimens are placed in a cavity block containing 2 ml of 1.25% glycerine solution. A few drops of picric acid may be added, which helps to prevent the growth of molds. The cavity block is placed in an incubator at 50-55^{o} C for 5-6 days.

Mounting of Nematodes on Slides for Microscopic Observation

Temporary Slide Mounts: Temporary mounts are prepared for the qualitative analysis; whereby the suspension of nematodes is placed on a glass slide in three drops, and cover slips are placed over the drops on the slide and then sealed with zut (Siddiqi, 2000). The specimens can then be examined under the microscope and identified.

Permanent Slide Mounts: For permanent mounts, a small drop of pure glycerin (anhydrous glycerol) is placed in the centre of a clean glass slide and nematodes are transferred to it, using a hair needle. The specimens are arranged neatly in the centre of the drop, so that their heads all point in the same direction, making sure that they are resting on the glass surface and not floating in the drop.

The drop is covered with a 19mm cover slip supported by three small lumps of Paraffin wax (60-65^{o}C melting point). Glass fiber is also used as support under cover slip for permanent mounting of nematodes. Three small and fine pieces of glass fiber about 3 mm each are arranged with a hair needle around the drop of glycerine on the slide before heating. The slide is then gently heated on a hot plate for melting the wax, which should fill the space between the slide and the cover slip.

The permanent slides are finally labeled with the details of the genus, species and stages (female, male and juvenile); date of making slide, host, date of collection and localities etc **(Fig.2.4)**.

Fig. 2.4. Required material for mounting of nematodes (from NNRC).

Mounting Vulval Cone of Cyst Nematodes (Golden, 1978)

For the identification of cyst nematodes, the general shape of the cyst, the structure and size of the vulva, vulval cone, fenestrae, and body wall are used as parameters (Hesling, 1978). The method suggested by Golden (1978) is used for the preparation of cyst cone mounts.

The cyst is placed in 3% formaldehyde drop on a glass slide and the posterior end is cut using an eye scalpel so as the fenestral area is in the centre of the cut piece. The cut end is placed as much as possible without damaging the cyst wall. Vulval cone and body wall are placed in a drop of lactophenol on another slide, and the remaining juveniles are mounted in 3% formalin (formaldehyde). The vulval cone is further trimmed in lactophenol. After 3-5 minutes, the cone top is transferred into distilled water, and then into clove oil to remove water; and then mounted in euparal with the cone top projecting upwards.

Mounting Perineal Pattern of Root-Knot Nematodes (Eisenback *et al*., 1981)

The cuticular markings surrounding the vulva and anus or perineal pattern of females of *Meloidogyne* spp. are used in their identification. Freshly dissected females are preferred as their body contents are more easily removed. Young, egg-laying females dissected from gall roots into a drop of 45% lactic acid are placed in a plastic Petri dish, using fine pointed forceps. The posterior halves of the body are cut off with a scalpel and the posterior pieces of the cuticle having perineal patterns can further be trimmed to a size slightly greater than the pattern. The inner tissue is completely and carefully removed with a flexible bristle. The pieces of perineal patterns are then transferred into a drop of glycerin on microscope slide. The posterior ends are arranged in one or two rows and the cover glass gently placed over; and sealed with zut or nail polish.

Mounting Enface View of Nematodes

A small bit of hard glycerin jelly is placed on a glass slide and melted over a small flame. The processed nematode is transferred to this melted glycerin jelly with head-end pointing towards the spread out portion of the glycerin jelly drop. The glycerin jelly is allowed to become hard. A small piece of a razor blade is mounted onto a needle and with this knife the head end of the nematode is cut about 1 head width from the anterior end of the nematode. The razor blade is held vertically and the nematode is further cut in transverse section. The transverse section of the head is transferred with hair needle onto the drop of the melted glycerol jelly . The cover slip is placed and the section is turned carefully with the handling needle in such a way that the lip region faces up. After half an hour, the cover slip is sealed with zut and can be examined under the microscope.

Culture Techniques

The ability to culture organisms is always of great importance for their scientific study and sometimes simply essential. Cultures also provide all

developmental stages of the organisms for observation, demonstration and experimentation. Live specimens usually provide the best starting point for a wide range of approaches such as; scanning or transmission electron microscopy (SEM/TEM), developmental studies, molecular analyses etc. Methods for culturing nematodes on excised plant tissues and calli was reviewed by Dougherty (1960) and Zuckerman (1971).

A number of terms are generally used to describe the number and known identity of co-occurring organism in a culture system (Southey, 1986). Those include the following:

i) Agnotobiotic or Xenic culture: The culture is said to be agnotobiotic or xenic when the nematode is cultured with an unknown number of associated organisms e.g., a mixture of fungi and/ or bacteria. Green house culture on a whole plant belongs to this category.

ii) Gnotobiotic Culture: In gnobiotic culture the nematodes are cultured with known associated organism(s), usually microbial flora.

iii) Monoxenic Culture: When there is only one such organism, the culture may be called monoxenic. Monoxenic cultures include cultures on callus tissue or excised roots.

iv) Axenic Culture: In axenic culture, there are no associated organisms and the nematodes are cultured on a chemical nutritive medium that contains no living organisms or part of organisms, other than the nematodes themselves. Bolla (1987) gave a comprehensive account of the problems for axenic culture of plant parasitic nematodes. Success was achieved only for Aphelenchids. True axenic cultures for Tylenchids have so far not been established due to some mechanical and biochemical problems.

Identification of Nematodes

Identification of nematodes is primarily based on morphological characters and morphometric measurements. Most plant parasitic nematodes are microscopic and are not visible to the naked eye. Some

morphological traits are readily observed at low magnification of a stereomicroscope e.g., size and shape of nematode, stylet, vulva position, tail shape; while other morphological characters can only be seen at higher magnification with a compound microscope. There are various methods for identification of nematodes; those include measurements and drawing (camera Lucida), light microscopy (automatic camera) and Photography by LCD (Ds-L2 camera).

Measurements and Drawing of Nematodes

Accurate measurements are essential for the description and identification of nematodes, and small but consistent differences can be important for distinguishing species (Stone, 1973). An occular micrometer or eye piece scale is preferably used for taking linear measurement of straight structures, such as; stylet, body width, total body length and position of vulva in straight nematodes. However spirally curved nematodes must be drawn first, then measured carefully to be accurately measured (Hooper, 1986).

Formula for Measurements

Nematode descriptions are always accompanied by measurements and are given in the form of a formula. The most commonly used formula is given by deMan (1884) consisting of letters which designate various body proportions of nematode (Fig.5).

deMan Formula (deMan, 1884)

L	Total body length in µm.
a	Body length / greatest body width
b	Body length / distance from anterior end to junction of oesophagus and intestine
b’	Body length / distance from anterior end to posterior end of oesophageal gland (when glands overlap intestine)
c	Body length / tail length
c’	Tail length / body width at anus or cloaca

G_1	Overall length of anterior ovary from vulva x 100/ body length
G_2	Overall length of posterior ovary from vulva x 100/ body length
V%	Distance of vulva from anterior end x 100/ body length
t	Overall testis length x 100 / body length
n	Number of specimens
ABW	Anal body width / anal body diameter
Cp	Conical part of stylet
L’	Distance from anterior end to anus
M	Length of conical part of stylet (in Tylenchida) x 100 / total stylet length
MB	Distance of median bulb from anterior end x 100 / total esophageal length
O	Distance from stylet knobs to the outlet of the dorsal gland x 100/ length of stylet
Od St	Length of odontostyle
Od-st-ap	Length of odontostyle aperture
Prer	Length of prerectum
R	Number of body annules
Ran	Number of body annules from anal opening to tail terminus
RS	Breadth of one body annule
RV	Number of annules from tail terminus to vulva
Rvan	Annules between vulva and anus
Spic	Spicule length
T	Length of tail
Van bp	Ventral body pores
VL/VB	Distance from terminus to vulva/ body width at vulva
VL/St	Distance from terminus to vulva/ Stylet length
H or h	Length of hyaline (clear) area in the tail b/w body contents and cuticle at tail terminus
P	Distance of phasmid from anus x 100/ tail length
Pa	Distance of anterior phasmid from anterior end x 100/ body length
Pp	Distance of posterior phasmid from anterior end x 100/ body length

Loof & Coomans (1970) proposed a system for locating oesophageal gland nuclei in Dorylaimina with the following abbreviations.

DO	Position of the opening of dorsal oesophageal gland into the oesophageal lumen
DN	Position of the dorsal oesophageal gland nucleus
S_1N	First subventral pair of oesophageal nuclei
S_2N	Second subventral pair of nuclei

Line Drawing of Nematodes

Line drawings are essential in any study describing nematodes; they should clearly show the distinguishing features of the species concerned and at least one drawing of a complete nematode to show its general form, and preferably one of each sex. Details of particular features should be drawn at a higher magnification in the drawing. The entire specimen may not be visible in the microscope field and will have to be drawn in several sections, overlapping a little each time so that they can be joined easily and accurately. Essential structures should be drawn in detail. The drawing paper is joined with sticky tape to make up the whole nematode. When everything has been arranged satisfactorily, the drawing can be transferred to white board sheet or butter paper by tracer. Finally, the line drawing is arranged and clearly made up by completing with roter ink or drafting ink pen, pens with a self-contained ink reservoir and tubular nibs of 0.2 to 0.1 mm diameter are useful because they make dots and lines of standard sizes.

Light Microscopy of Nematodes

Photomicrographs of free living and plant parasitic nematodes are made with an automatic camera attached to a compound microscope using Nomarskiٰs interference contrast.

Scanning Electron Microscope of Nematodes

For scanning electron microscopy (SEM), the nematodes are fixed in 3% glutaraldehyde solution with 0.05 M-phosphate buffer (pH 6.8),

dehydrated in a graded series of ethanol, critical point dried using liquid CO_2, sputter coated with a 20-30 NM layer of gold palladium, and examined with an electron microscope.

Photography of Nematodes

Photomicrographs of special features of new species of nematodes can supplement drawings for authenticity. For still photomicrography, DS-L2 camera is used for nematode photography. In this camera USB mouse is used for various menu settings and operations. Although one can enter photographed data and comments using a mouse, it is easier to enter such information with a key board. For the photography, a monitor would be needed to operate the DS-L2 to display the image data on the PC. When several photographs or a series are to fill a page, they can be laid out on word pad of computer and finally the captions are written on lower side of photograph sheets.

Chapter 3

NEMATODE MORPHOLOGY AND ANATOMY

Nematode Morphology and Anatomy

Morphological characters are the basis for the differential diagnosis of various genera and higher categories as well as for systematics.

Nematodes are highly diversified group within the animal kingdom that occurs worldwide in every conceivable environment and perhaps the most numerous multicellular animals on this earth. Nematodes are bisexual animals; and the male and female mostly look alike except for differences in their sexual organs. Usually, females and males are vermiform. Often the females show sexual dimorphism and males of some nematode species degenerate. Nematodes are usually colorless and their body shapes differ greatly. The head (lip region) and tail are extremely variable **(Fig.3.1)**. Most plant parasitic nematodes are minute, ranging in size from less than 0.2 mm to over 12 mm, tapering toward both ends. In Tylenchida all plant parasitic nematodes characteristically have a feeding apparatus known as a stylet. The stylet enables the nematodes to puncture plant cell walls, secrete enzymes into the cell and suck out the digested cell contents. This feature distinguishes them from the majority of other soil nematodes and non-plant parasitic forms.

Above mentioned are the diverse morphological features and anatomical variations that make the bases for differentiation among the nematode orders, families, sub-families, genera and species etc., and are of great taxonomic significance. Generally, all the nematodes possess the following morphological characters.

Body Shape

In general nematodes are lower invertebrates having cylindrical, bilaterally symmetrical, triploblastic, unsegmented pseudo-coelomatic worms, usually unciliated; tapering at both ends but females of some species of plant parasitic nematodes assume varying size and shape e.g., kidney shaped, lemon shaped, pear shaped and globular.

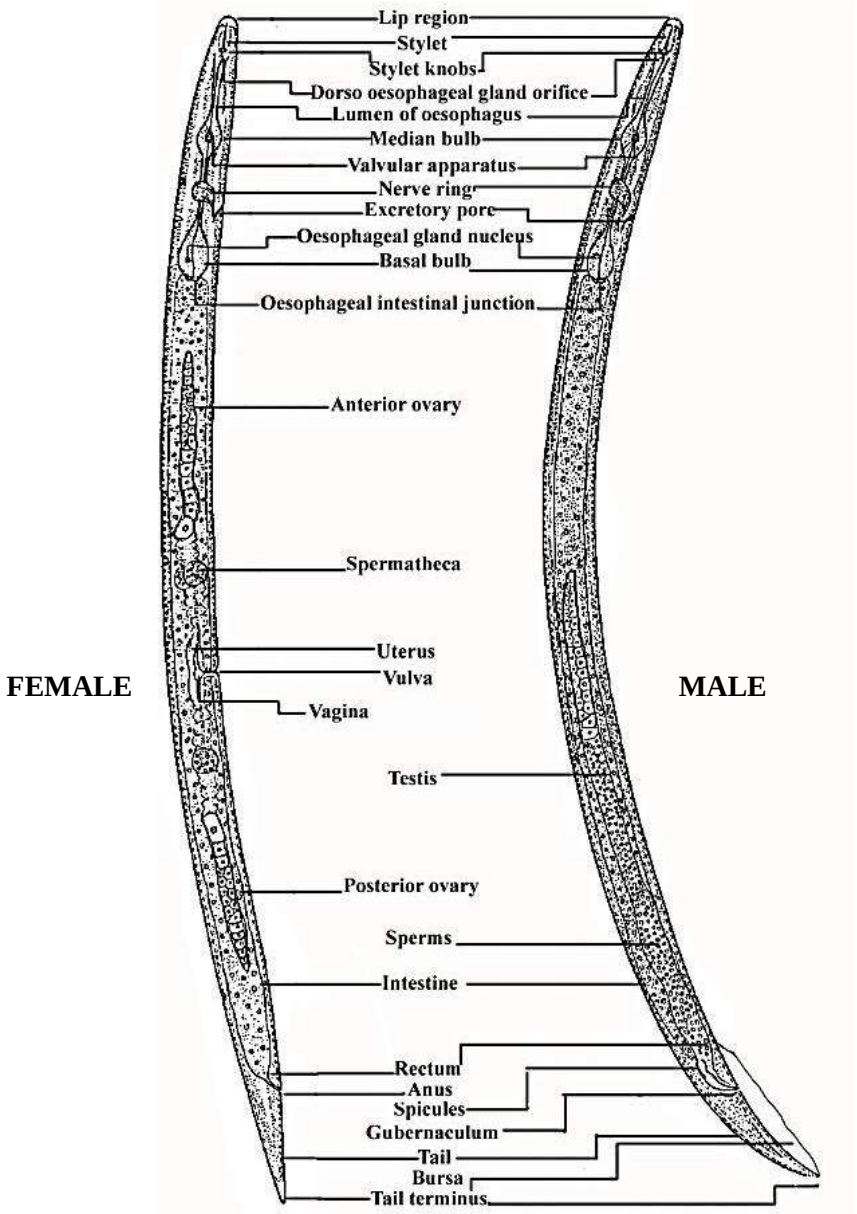


Fig.3.1. Ge neral morphology of plant parasitic nematodes (from NNRC).

They are devoid of respiratory and circulatory systems. The body posture on death or in relaxed conditions is used as a diagnostic character. The habitus is commonly straight to slightly arcuate ventrally; however, spiral coiling is also commonly present. The anterior end of a nematode is usually bluntly rounded with a terminal oral aperture (mouth opening) and is surrounded by lips and papillae while the posterior end may be bluntly rounded or pointed. Sometimes it is tapered to a point or it may be long and filiform **(Fig. 3.2).**

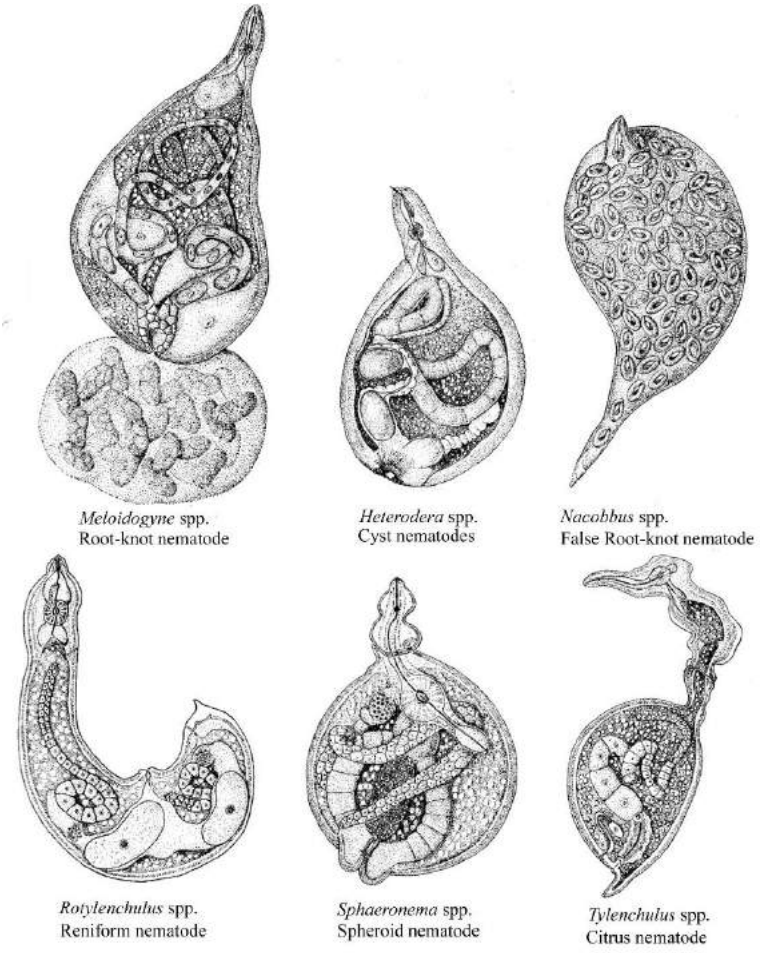


Fig. 3. 2. Variation in body shape of female parasitic nematodes (courtesy Chitwood, 1977).

Body Structure

Body structure of nematodes is tubular consisting of an outer tube which is wide and represents the body wall of the animal; an inner tube which is narrow, lies inside the outer tube and represents the digestive organs. The space in between the two tubes is body cavity or pseudocoel or pseudocoelom, contains a viscous fluid which acts as hydrostatic skeleton. Three major organ systems, the excretory, nervous and reproductive systems of the nematode are suspended within the fluid.

Outer Body Tube

1. The Body Wall

The body wall or outer body tube of nematode consists of three principal layers: Cuticle, Hypodermis and Somatic muscular layer.

a. Cuticle

The protective covering of the nematode body is a tough flexible layer which forms the exoskeleton and is known as the cuticle. It is basically a three layered structure; cortical layer, median layer and basal layer. The internal side of the basal layer forms the basal lamella where the cuticle, the hypodermal cells and cells of somatic musculature come together. Some authors identify four subdivisions of the cuticle – epicuticle, exocuticle, mesocuticle and endocuticle (Meggenti, 1981). The cuticle is made of mainly proteins, with small amounts of lipids and carbohydrates. Cuticle varies in thickness and structure in different species. The cuticle (exoskeleton) of the nematode is semipermeable and plays an important role in its physiology. It plays an important role in movement, environmental protection, and growth and development.

The cuticle is the exoskeleton of nematodes. It is a non-cellular, proteinaceous secretion of the hypodermis and covers the entire body. Externally, it bears longitudinal or transverse striations or both. Besides covering the body of the nematode from outside (external cuticle) organs like the stomatal cavity, oesophagus, body pores, vulva, vagina, anus, cloaca, rectum and excretory duct are also lined with cuticle which is

referred to as internal cuticle. Besides the longitudinal and transverse striations, the cuticle may possess differently modified structures called Cuticular ornamentations **(Fig.3.3)**.

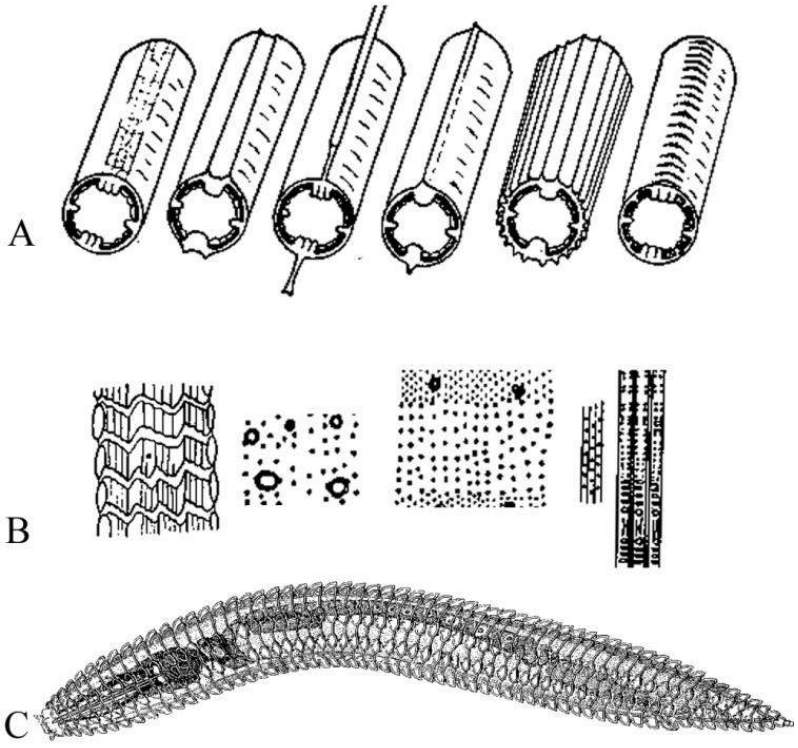


Fig. 3.3. A. Cuticle longitudinal or transverse striations or both; B. Zig-zag pattern and variation in punctuation; C. Ornamentation (adapted from Chitwood, 1977)

Cuticular Modifications: The external cuticle is provided with many structures (external ornamentation) which are of great taxonomic significance, transverse striation (fine and coarse), annulations, longitudinal striations, longitudinal ridges or lamellae, lateral lines, cervical and caudal alae or bursa, spines and scales etc. are also present. The bursa is present only in male nematodes, its shape and size varies in different nematode species and may also be correlated with the length of the tail. These cuticular structures usually involve only the epicuticle and

exocuticle. In plant parasitic nematodes, cuticular ornamentations are important diagnostic features.

Sensory Organs Formed in the Cuticle*:* Sensory structures which keep the nematode aware of its surrounding environment, are present and are taxonomically important. These are amphids, deirids, phasmids, hemizonid, hemizonion, cephalids and caudalids.

Moults: Nematodes usually undergo four moults during their development from egg to adult, and during each of these periods, the cuticle is cast off, taking with it the cuticular linings of the pharynx, vagina and rectum and all are replaced a new. With each moult, the body becomes larger and certain developments occur especially in the reproductive system.

b. Hypodermis

The hypodermis is a thin layer of tissues underneath the cuticle and also responsible for its formation whether external or internal; attached to four longitudinal chords. The two lateral chords are better developed than the ventral and dorsal ones. The chords provide four interchordal zones; on which the somatic muscle cells are arranged. The lateral chords correspond externally to the lateral field which is marked by a number of longitudinal lines or incisures, the region between two incisures being known as a band or ridge. The hypodermis is an active layer of the body wall; it is cellular with cell bodies aggregated in lateral field, resulting in thickening of hypodermal chords under lateral field; it also thickens in ventral and dorsal region. The chords contain nuclei, mitochondria, endoplasmic reticulum and other cell bodies. Hypodermal cells become very active during the moulting of cuticle.

c. Somatic Muscular Layer

The somatic muscular layer, consisting of single layer of elongate spindle-shaped muscle cells, is situated beneath the hypodermis and attached to it throughout their length. The muscle cells are arranged longitudinally in the body and often form four groups being separated by

the four hypodermal chords. Each muscle is elongate spindle-shaped and has a connection with the nervous system. The muscles are connected to the dorsal nerve chord, lateral nerves and ventral nerve chord. During the movement, the dorsal and ventral muscles are alternately contracted and relaxed, and this accounts for the dorso-ventral movement of vermiform nematode. Specialized somatic muscles are scattered throughout the body. They perform the function to operate the stylet muscles in plant parasitic nematodes; as protractors and retractors of male spicules and gubernaculum and female vulval muscles.

Pseudocoelom (Pseudocoel) or Body Cavity

The pseudocoelom or body cavity is a secondary structure lacking mesentery and is lined by the somatic muscles and the basal lamina that covers the epidermal chords. This fluid filled cavity bathes the internal organs and contains some large amoeboid cells called pseudocoelomocytes. These vary in number, size and shape and their function includes osmoregulation, secretion and transport of material. The pseudocoelomic fluid acts as part of the turgor-pressure system, but also has some circulatory function **(Fig.3.4 & 3.5)**.

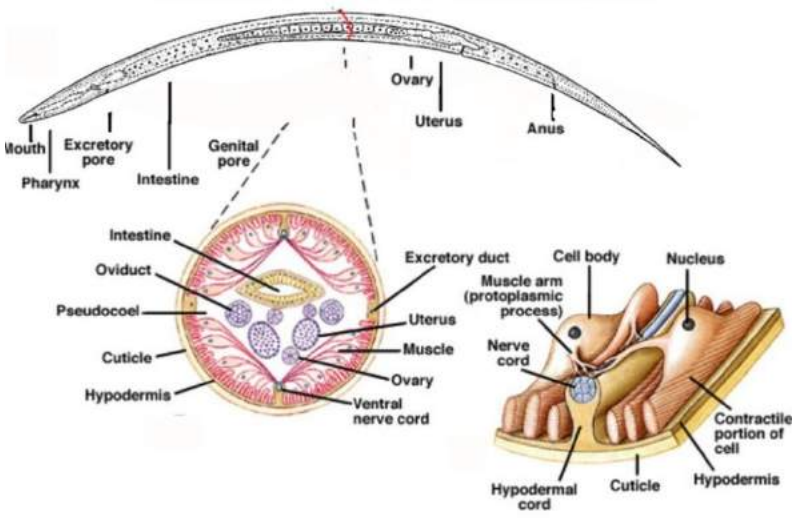


Fig.3. 4. Transverse and longitudinal section of body cavity (courtesy CABI Publishers).

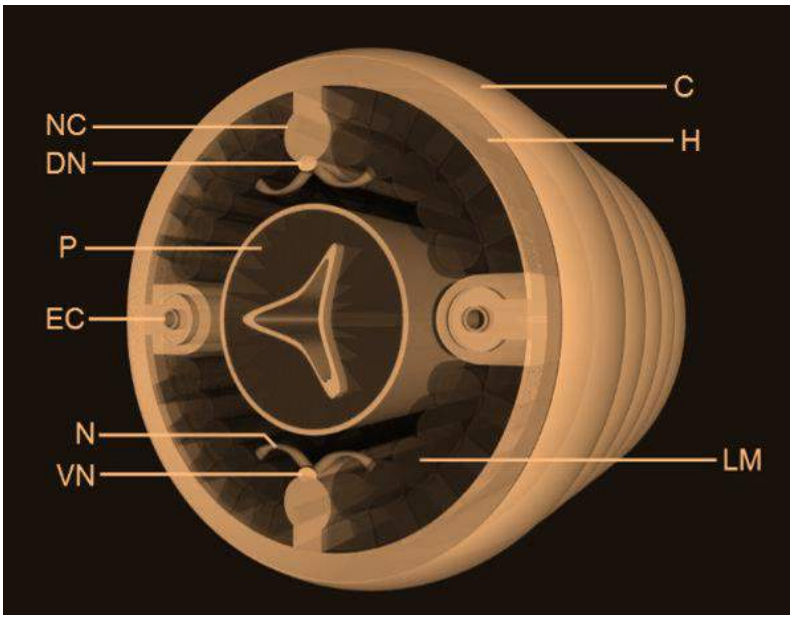


Fig.3. 5. Transverse section through a nematode. C=cuticle; DN= dorsal nerve; EC= excretory canal enclosed by the lateral nerve cord; H=hypodermis; LM= longitudinal muscles; N= nerve extending from muscle cell to main nerve; P= phyrynx; VN= ventral nerve (courtesy CABI Publishers)

Digestive System Organs (Alimentary canal)

The digestive organs of nematodes comprise a spear or stylet (buccal cavity), an oesophagus (pharynx), oesophagus-intestinal junction (cardia), intestine, rectum and anus.

The digestive tract or alimentary canal of nematodes is divided into three main regions: the stomodeum, the mesentron and the proctodeum. The stomodeum and the proctodeum are lined with cuticle which is absorbed or shed at each moult. The stomodeum begins at the oral opening and includes the mouth or buccal cavity/ stoma and the oesophagus/ pharynx. The stomodeum is mainly responsible for feeding and its structure is highly diversified because of the varied feeding habit of nematodes. The mouth or lips are also associated with feeding activity. The mesentron is

the intestine proper which leads to the proctodeum or rectum that ends at the anal or cloacal opening.

Stoma or Mouth

The stoma of a nematode is often referred to as the mouth cavity, buccal capsule or buccal cavity. The buccal cavity extends from the mouth opening to the triradiate lumen of pharynx. In plant parasitic nematodes, which feed on plant cells by piercing with a stylet, the papillae are greatly reduced to facilitate movement in the confined spaces and the teeth are absent. The oral aperture, generally situated at the anterior end directly opens in the stoma or buccal cavity. The shape, size and detailed morphology of the buccal cavity vary widely among nematodes. The shape may be either simple subglobular, cylindrical, triangular, oval, conoid or collapsed **(Fig.3.6)**.

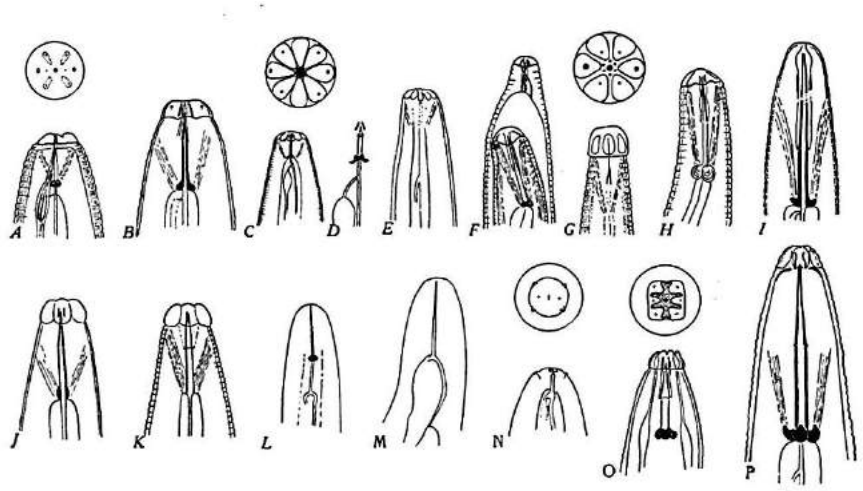


Fig. 3.6. Stoma variation in plant parasitic nematodes (After Chitwood, 1977).

Stomatostylet/ Odontostylet (Feeding Apparatus)

In the plant parasitic nematodes of the orders Tylenchida & Aphelenchida, the spear or stylet is called stomatostylet because it is

supposed to be derived through sclerotization of the stoma; whereas, in case of Dorylaimida, it is an odontostylet. In Dorylaimida it is formed in the wall of the anterior part of the oesophagus and moves into position in the stoma where it is attached to the odontophore.

The stylet of Tylenchida or Aphelenchida is made up essentially of 3 parts: the anterior conical part (conus), the shaft, and the basal knobs. The basal knobs are 3 in number (one dorsal, two subventral), differ in size and shape and serve for the attachment of protractor muscles of the spear; rarely the knobs may be absent. The stylet has a narrow passage called lumen which connects with the lumen of oesophagus and opens ventrally slightly below the stylet tip.

The odontostyle of the Dorylaimida consists of the stylet proper and the stylet extensions (odontophore). The stylet of Tylenchida and Dorylaimida and their components differ in size, shape etc. and provide important characters for identification.

Oesophagus/ Pharynx

Like the feeding apparatus, the oesophagus is also greatly variable in different groups of nematodes. It is simple cylindrical in Mononchida, bottle-shaped (or with an anterior narrow part and posterior expanded part) in Dorylaimida. In Tylenchida it has an anterior cylindrical part (procorpus) followed by a swollen median bulb (metacorpus) with well developed crescentic plates. This is followed by narrow region called isthmus which leads to the basal glandular region. The median bulb may be fusiform and without valvular plates in Neotylenchid nematodes and very well developed with large crescentic plates in Hoplolaimid or Aphelenchid nematodes.

The glandular region which contains 3 unicellular glands (one dorsal and two ventro-sublaterals) may form a basal bulb structure adjoins the intestine or an overlap dorsal or ventral to the intestine, e.g., Hoplolaimids and Aphelenchids etc. The ventro-sublateral glands have their openings in the median bulb posterior to the crescentic plates, but the dorsal gland opens in the procorpus very near to the basal knobs of

the stylet in Tylenchids, but all three glands open within the median bulb anterior to crescentic plates in Aphelenchids.

Oesophago-intestinal Valve (Cardia)

At the junction of the oesophagus with intestine, lies a heart-shaped structure known as the cardia. It is a muscular structure at the base of the oesophagus at the opening into the intestine. It is a valvular structure regulating the passage of food from the oesophagus to the intestine. It may be conoid, rounded or flat disc-like. In some nematodes it is provided with cardiac glands (e.g., Nygolaimina); in Mononchs, it may be tuberculate or non-tuberculate.

Intestine, Rectum and Anus

Intestine (mesenteron) is simple sac like, or hollow tubular structure, consisting of a single layer of epithelial cells. These cells may be similar in shape and size (homocytous) or dissimilar (heterocytous). The number of cells which constitute the intestine is also variable: few cells-oligocytous and numerous cells-polycytous. The intestine leads to the rectum which opens outside through the anus (females) or cloaca (males).

Rectum is a short portion of the digestive tract at the end of the intestine. It opens out through a ventrally situated anus in females and cloaca in males. The digestive and reproductive systems in males join the rectum, forming a cloaca, from the wall of which several copulatory organs develop. Cloaca is a common opening of reproductive and digestive system in males; reproductive system in female opens through vulva which is separated from the anus. The anus is usually a minute pore-like opening in Tylenchida but in other nematode groups it may be large slit-like. Cloaca, anus and rectum are lined with cuticle **(Fig.3.7)**.

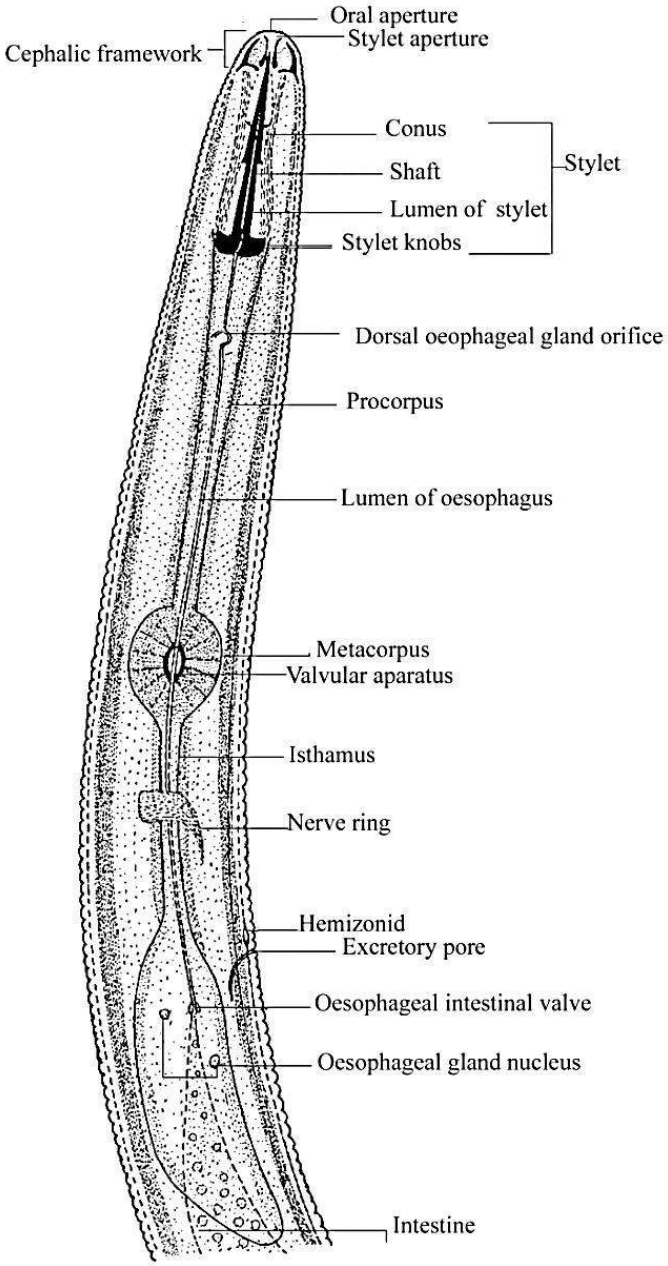


Fig.3.7. Digestive system of plant parasitic nematode (from NNRC).

The Excretory System

The excretory system is represented by an excretory pore situated on the ventral side of the body in the oesophageal region. The excretory pore is connected to an excretory duct which leads to a large cell known as renette cell; excretory duct is cuticularized and easily visible. Lateral excretory canals may or may not be present. The excretory system of Nematoda provides characters of taxonomic and systematic significance. In Tylenchida, the excretory system is asymmetrical consisting of a single excretory cell or renette located laterally or ventro-laterally, usually posterior to the oesophageal region. The excretory pore is mid-ventrally located in the region of oesophagus, usually either in the region of isthmus or basal bulb/basal gland lobe, rarely anteriorly (in the cephalic region) or posteriorly (as vulval region, *Tylenchulus*). The renette cell may become very large to produce gelatinous matrix in which eggs are deposited (*Tylenchulus*) **(Fig.3.8)**.

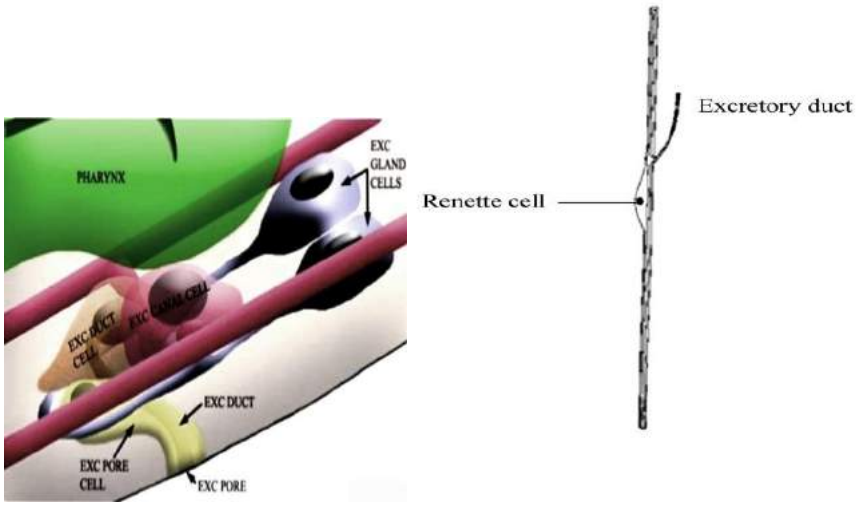


Fig.3.8. Excretory system of plant parasitic nematodes (from NNRC).

The Nervous System

The nervous system consists of the nerve ring (also called circum-oesophageal commissure; represents the brain of the animal) which

encircles the isthmus of the oesophagous and is easily visible in all species of nematodes. It is located around the oesophagus-isthmus in Tylenchida and usually in the middle of anterior slender part in Dorylaimida. Rarely, it may be around the intestine in some Hexatylina. Nerve connections (longitudinal nerves) arise from the nerve ring in anterior and posterior directions and join various organs of the body and sensory structures. These sensory organs are mostly in the labial region (sensillae and amphids), the oesophageal region (cephalids, deirids, hemizonid and hemizonion) and on the tail (phasmids and caudalids). These can be located with special staining techniques only and used in taxonomic descriptions and differentiations. Transverse commissures connecting the nerves are present in different regions of the body.

Amphids are paired lateral sense organs located in the cephalic region, generally close to the oral aperture. They probably act as chemoreceptors. The amphid aperture may be slit-like, transversely, longitudinally or obliquely placed on the head. The deirids are paired cervical papillae near the nerve ring. In Tylenchidae deirids are located in the center of the lateral fields in the form of protuberances. The deirids are sensory structures without an opening to the exterior. Cephalids are highly refractive band-like structures, about one body annule wide and encircle the neck. Anterior cephalids are just behind the cephalic region and posterior one at some distance behind them. Hemizonid is the major latero-ventral commissure, highly refractive, bioconvex structure forming a semicircle ventrally, extending through three body annules just posterior to the nerve ring and close to the excretory pore. Its position relative to the excretory pore is often of taxonomic importance. The hemizonion is structurally identical to the hemizonid but is smaller and located posterior to hemizonid. Phasmids are a pair of lateral sensory organs situated in the posterior region of the body. The phasmids sometimes preanal or situated even more anteriorly or rarely absent. The position of phasmids and their external appearance (small pore-like or large scutella-like) are important taxonomic characters at species and generic levels. They probably serve as chemoreceptors. Caudalid is a smaller inconspicuous structure just anterior to the anal region **(Fig. 3.9 & 3.10)**.

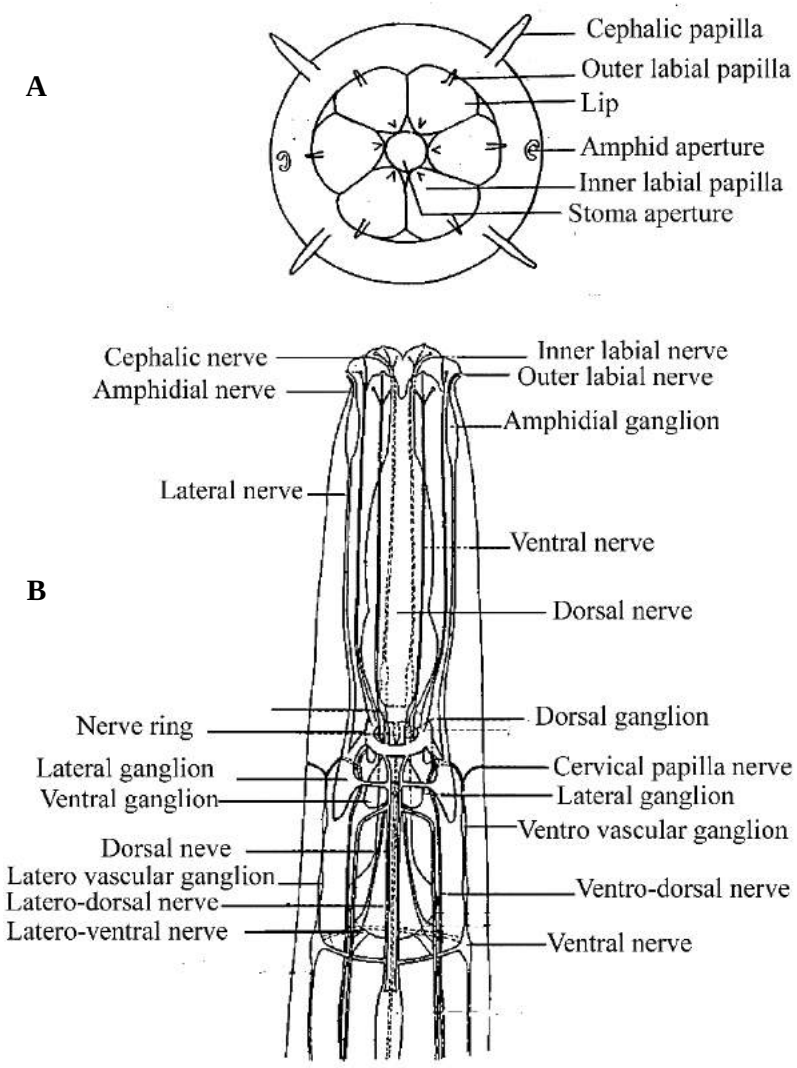


Fig. 3.9. A. Enface view of a nematode head showing different parts; B. Nervous system in the anterior body of the nematode (Courtesy Chitwood, 1977).

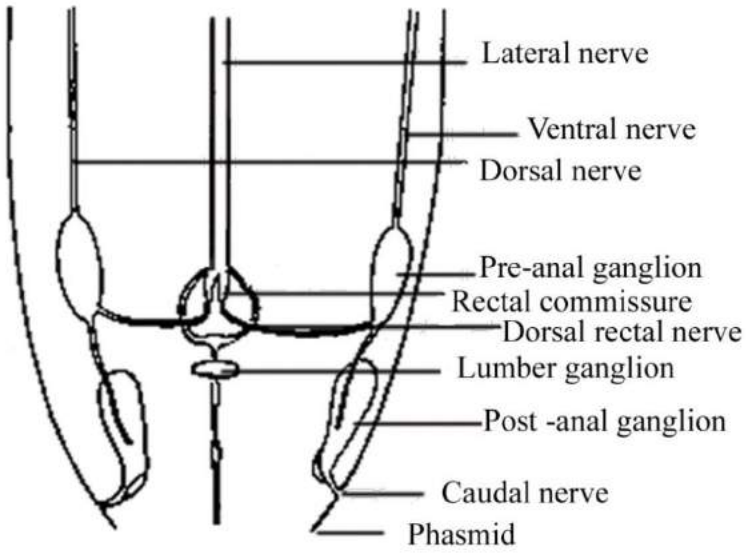


Fig. 3. 10. Nervous system in the posterior body of the nematode.

Reproductive System

Nematodes are commonly dioecious or amphigonous animals, with separate males and females within a species. Sexes can be easily distinguished by primary and secondary sexual characteristics of the body. However, in the majority of species the number of females is far more than the males. This shows a tendency towards hermaphroditism and pathogenesis. In some species intersexes have also been reported. The reproductive system of nematodes is tubular and consists of one or two sets in the two sexes.

- **Female Reproductive Organs**

Females possess one, two or rarely many sets of sexual branches. These are referred to as mono, di and polydelphic condition. In mono-delphy, the posterior branch may be reduced to a post-uterine sac or be entirely absent. In plant and soil nematodes, there are usually only one or two sets of reproductive organs. The arrangement of the sexual branches with respect to vulva position is also important. If the branch(es) are anterior

to the vulva the condition is referred to as prodelphic; likewise, posterior to vulva is opisthodelphic and one branch on either side of vulva is amphidelphic.

Each sexual branch consists of an ovary, oviduct, uterus, vagina and vulva. The ovary contains germinal cells enclosed in an epithelial sac. At the tip of the ovary is a large cap cell which proliferates to give rise to the germinal cells. This condition is known as telogony since germ cells are produced only in the distal end of the ovary. In some animal parasitic nematodes, the germ cell proliferation takes place along the entire length of gonads, and is referred to as hologony. In the telogonic gonads we can differentiate two zones – the small germinal zone, where rapid division takes place leading to the proliferation of the germ cells, and the lager growth zone where the developing oocytes attain maturity.

Vulva or female gonopore is an opening of reproductive tract to exterior; it is a transverse slit-like aperture which may be transversely oval but rarely round, located on the ventral side of the body. In didelphic form it is generally median or submedian but in obese form it may become terminal or subterminal. In monodelphic form it often lies near the anus. Epiptygma is a cuticular, membranous structure located on the vagina or on the vulval lips, commonly found in Hoplolaimoidea. The position of the vulva on the body, and the presence/absence of epiptygma are important diagnostic characters. The vagina, a muscular passage leading from the vulva to the uterus, is thin or thick-walled, leading inwards at right angles to the body axis. The uterus consists of two parts – the proximal part is known as crustaformeria or quadricolumella consisting of tall columnar secretory cells, which are believed to form the egg shell; the distal part of the uterus is pouch like, rich in musculature and may serve as an ovijector.

The uterus joins the vagina which opens to the exterior through the vulva. The oviduct consists of a narrow tube of high columnar epithelial cells. It may or may not be separated from the uterus by a sphincter. Often a part of the oviduct is swollen and may serve as spermatheca. The ovary is either outstretched or reflexed back towards the vulva. The ovary has an apical germinative or multiplication zone and a growth

zone. Oogonial cells multiply in the germinal zone while oocytes increase in size in germinal zone (Fig. 3.11).

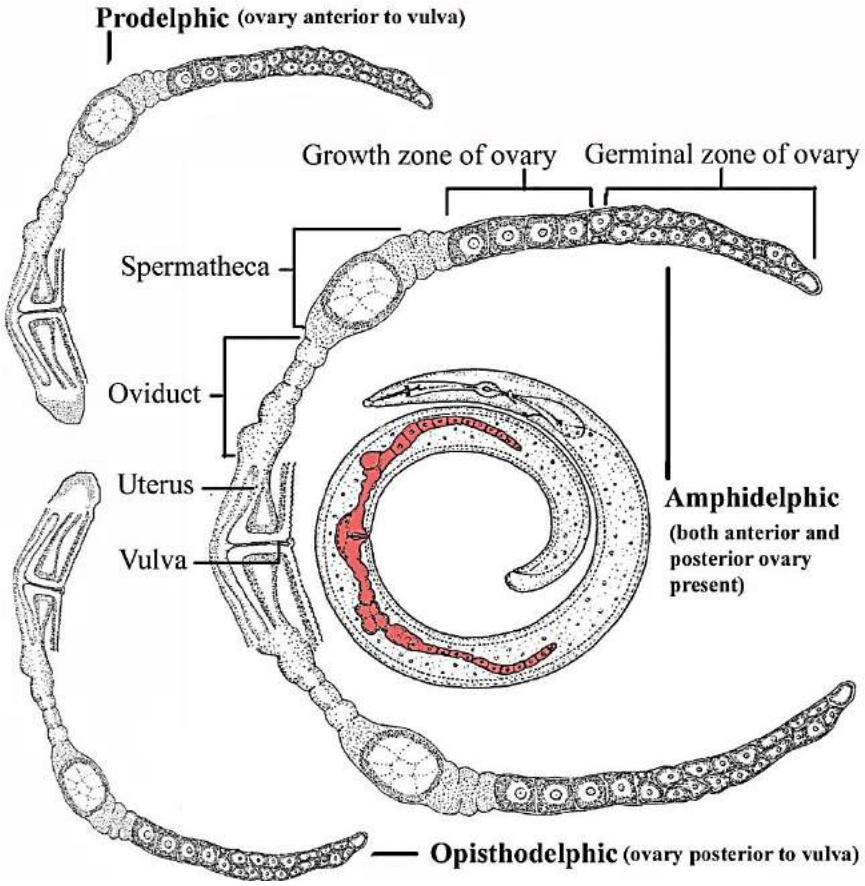


Fig. 3. 11. Female reproductive system of plant parasitic nematode (from NNRC).

- **Male Reproductive System**

The males possess two testes (diorchic) or one (monoarchic). The testis (es) are outstretched or reflexed and are connected to the cloaca by the vas deferens. Part of the vas deferens may be dilated to form the seminal vesicle for the storage of sperm, and the terminal part of the vas deferens nearest to the cloaca may be muscular and serve as the ejaculatory duct.

The secondary sexual organs of the male consist of spicules, gubernaculum, lateral guiding pieces, supplements (genital papillae), bursa etc. All these are very important taxonomically and are of diagnostic value.

The lateral wing-like cuticular expansions around the tail of male nematodes are called bursa or caudal alae. The bursa is a cuticular sheath which encircles the spicules. It is usually present in Tylenchida. It may be adanal, subterminal or terminal, enveloping the entire tail. The bursa margins may be smooth or crenate. It may serve to grasp the female during copulation.

Gubernaculum is a guiding structure during spicule protrusion. Spicules are main copulatory structures, cuticularized and tubular. They are paired, ventrally arcuate or curved or fused at tip. Spicules are generally crescent-shaped. They are sensory structures which also serve as an aid in the transfer of sperm to the female genital tract. The genital papillae (one to four) are grouped around the cloacal aperture, may be absent. There are no male caudal papillae in Tylenchida **(Fig. 3.12)**.

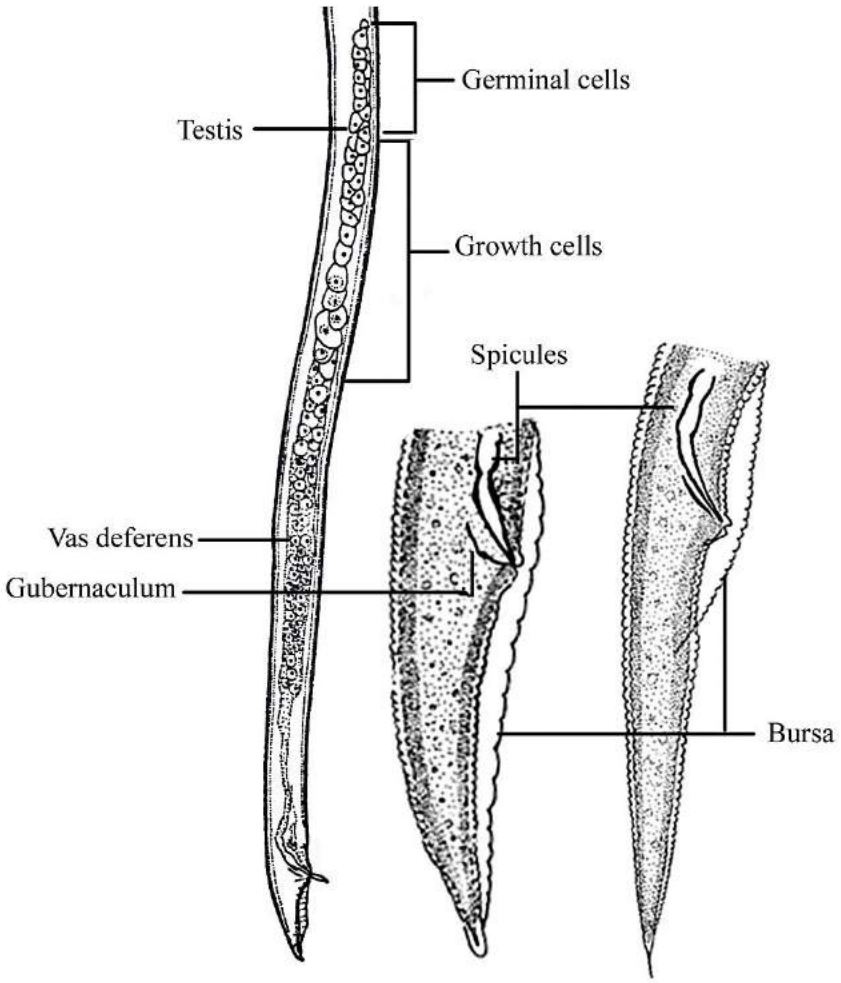


Fig. 3. 12. Male reproductive system of plant parasitic nematode (from NNRC).

Chapter 4

NEMATODE SYSTEMATICS

Systematics is a discipline of biology that examines the natural variation and relationships of organisms which includes the field of taxonomy. Systematics also deals with the relationships of the different groups of organisms as most systematic endeavor to construct natural classification systems reflecting evolutionary relationships, i.e., organisms are grouped together into taxa (singular: taxon) and given a taxonomic rank. Groups of a given rank can be aggregated to form a super group of higher rank and thus create a taxonomic hierarchy. Many biologists use the terms taxonomy and systematics interchangeably. Systematics, in other words is used to understand the evolutionary history of life on Earth.

Outline Classification of Phylum Nematoda

The highest level of classification is the Phylum and the lowest is the species. A species is the basic unit of scientific classification. Nematodes belong to the Animal Kingdom and their taxonomic hierarchy is expressed below. Pearse (1936) system of nomenclature of endings for higher taxa is frequently used in Nematology as follows:

- Phylum - a
- Class - ea
- Subclass - ia
- Order - ida
- Suborder - ina
- Superfamily - oidea
- Family - idae
- Subfamily - inae
- Genus
- Species

The classification of this large group of metazoans is still not stable and the creation of new genera and higher categories' continues (Siddiqi, 2000).

In almost all classification systems the phylum Nematoda Rudolphi, 1808 (Lankester, 1877) was divided into two classes: Secernentea von Linstow, 1905 (Phasmidia Chitwood & Chitwood, 1933) and Adenophorea (Aphasmidia). The Secernentea includes most of the plant and animal parasites with primarily terrestrial and fresh water taxa, whereas Adenophorea was predominantly marine and contain some fresh water and relatively few parasites of animals or plants, occupying a wide range of habitats. Within Secernentea, the order Tylenchida contains the great majority of economically important plant pathogens as well as important parasites of insects and has been revised a number of times.

Classification of plant parasitic, free-living soil and entomopathogenic nematodes was based on the systematics followed by Siddiqi (2000), Jairajpuri and Ahmad (1992), Hunt (1993), and Nguyen and Hunt (2007).

Siddiqi (2000) considered Tylenchida Thorne (1949) as a sole order of the subclass Tylenchia Inglis (1983) of the class Secernentea while Aphelenchida Siddiqi (1980) belongs to the subclass Rhabditia. Siddiqi (2000) placed four suborders viz., Tylenchina, Hoplolaimina, Criconematina and Hexatylina under order Tylenchida. He also proposed two infraorders under suborder Tylenchina viz., Tylenchata and Anguinata on the basis of their separate origins and evolutionary tendencies. Algal and moss feeding is the ancestral trait of the infraorder Tylenchata while fungal feeding is the ancestral trait of the infraorder Anguinata.

The Dorylaimida represent a large and very important group of soil and fresh water nematodes, having a very large number of economically important species. A generic monograph of the Dorylaimida was produced by Jairajpuri and Ahmad (1992). They made an excellent endeavor in summarizing, evaluating and systematizing all the information published to-that date of this diverse and taxonomically difficult order; they recognized three suborders viz., Dorylaimina, Nygolaimina and Campydorina under order Dorylaimida.

Hunt (1993) placed aphelenchs under suborder Aphelenchina of order Aphelenchida as proposed earlier by Siddiqi (1980) with some modification. The longidorid nematodes, containing some virus vector nematode genera, were assigned to the superfamily Dorylaimoidea under suborder Dorylaimina of order Dorylaimida. While the trichodorid nematodes were placed in superfamily Trichodoroidea under suborder Diptherophorina of the order Triplonchida (Hunt, 1993) as earlier proposed by Siddiqi (1983) and Jairajpuri and Ahmad (1992).

Characterization of Phylum Nematoda (Roundworms)

- Habitat: Fresh and salt water; terrestrial.
- Vermiform (worm-like), usually cylindrical in shape.
- Size varies from microscopic to 1m in length.
- Basic elongate body shape often have cuticular markings – annulation/striation
- Triploblastic; three cell layers; ecto, endo and mesoderm. Non-segmented.
- Bilateral symmetry with an anterior and a posterior end.
- Covered by a protective cuticle, having no external cilia.
- Pseudocoelomate: Body cavity a pseudocoelom, which functions as a hydrostatic skeleton (Pseudocoelom is vaguely defined as a body cavity not completely surrounded by mesoderm).
- Muscle layers include longitudinal fibers only.
- Complete digestive system which means their digestive tract has two openings; a mouth to ingest food and an anus to eject waste.
- Circulatory and respiratory organs lacking.
- Excretory system consists of one or more large gland cells opening to an excretory pore or canal system.
- Circular nerve ring with dorsal and ventral nerve cords; sense organs include ciliated pits.
- Alimentary canal: mouth to anus.
- Sexes usually separate; sexual dimorphism – females with separate anus and gonopore, male with common cloaca for intestine and gonad.

- Male usually smaller than the female.
- Fertilization internal.
- Mostly parasites.
- Lifecycle includes: i) the egg stage; ii) Four larval stage; iii) the adult stage.
- Important in decomposition and soil nutrients.

Differences between class Secernentea von Linstow, 1905 and Adenophorea von Linstow, 1905.

S. #	Secernentea (Phasmidia)	Adenophorea (Aphasmidia)
1.	Excretory system with lateral canal	Excretory system without lateral canal
2.	Amphid aperture usually small pore like, located dorso-laterally	Amphid aperture usually well-developed, variable in shape, postlabial
3.	Phasmid present	Phasmid absent
4.	Caudal glands absent	Caudal glands present
5.	Somatic sensory organ usually absent	Somatic sensory organ present

Among the orders of Class Secernentea, four important and most commonly found orders in Pakistan are; Tylenchida, Aphelenchida, Rhabditida and Diplogasterida; while seven orders of class Adenophorea are mostly found here. The order Tylenchida has the convergent evolutionary similarities with the order Aphelenchida, Siddiqi, 1980 due to the development of protrusible stylet and its muscles, modification in the structure of cephalic region, stoma, oesophagus and the parasitism on plants and insects. However, the major differences between the two orders are as follows:

Differences between order Tylenchida and Aphelenchida.

S. #	Tylenchida	Aphelenchida
1	Amphids mostly lateral in position	Amphids latero-subdorsal in position
2	Basal konbs of stylet well developed and marked off	Basal konbs of stylet not a separate entity but represented by thickenings and not marked off
3	Orifice of dorsal oesophageal gland in precorpus at the base of the stylet or a short distance behind it	Orifice of dorsal oesophageal gland in muscular postcorpus anterior to the central valve-like cuticular thickening
4	Median oesophageal bulb, if present, without a muscular valve	Median oesophageal bulb always present, with a muscular valve
5	Anus inconspicuous, minute, pore-like, directed outward	Anus conspicuous, large, crescentric, backwardly directed slit
6	Sperm usually small-sized	Sperm large sized
7	Male caudal papillae absent; bursa lacking papillary ribs or rays, never present only at the tail tip	Male caudal papillae present, bursa with papillary ribs or rays when it is large and envelops the entire tail; a short bursa only at the tail tip in several genera
8	Spicules not thorn-shaped	Spicules mostly thorn- shaped

Taxonomic Position of Nematode Fauna of Pakistan

According to the latest information available the 239 new nematode species have been recorded in Pakistan so far. A comprehensive list of 774 species of all nematode groups prevalent in Pakistan belonging to 274 genera, 85 families and 12 orders, is provided hereunder **(Fig. 4.1)**. The taxonomic positions of species are also incorporated in the list.

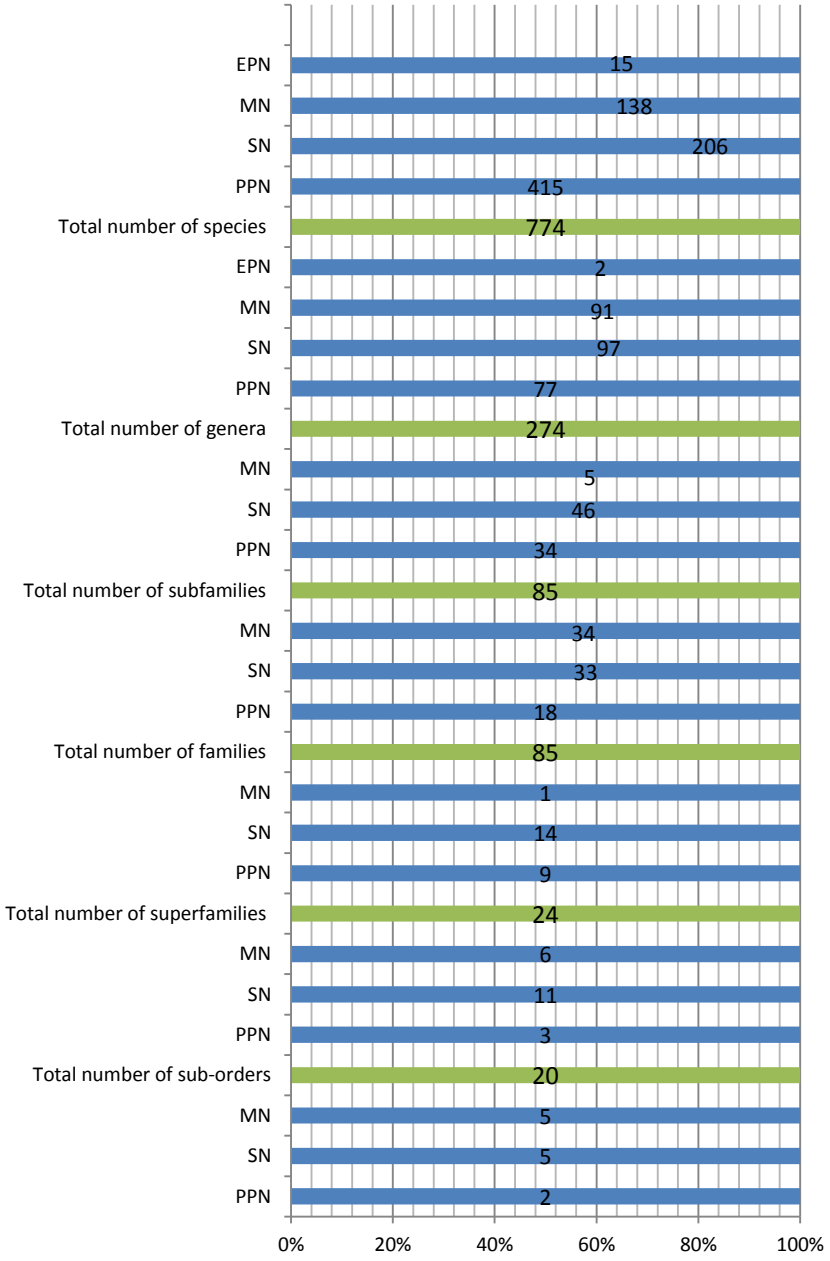
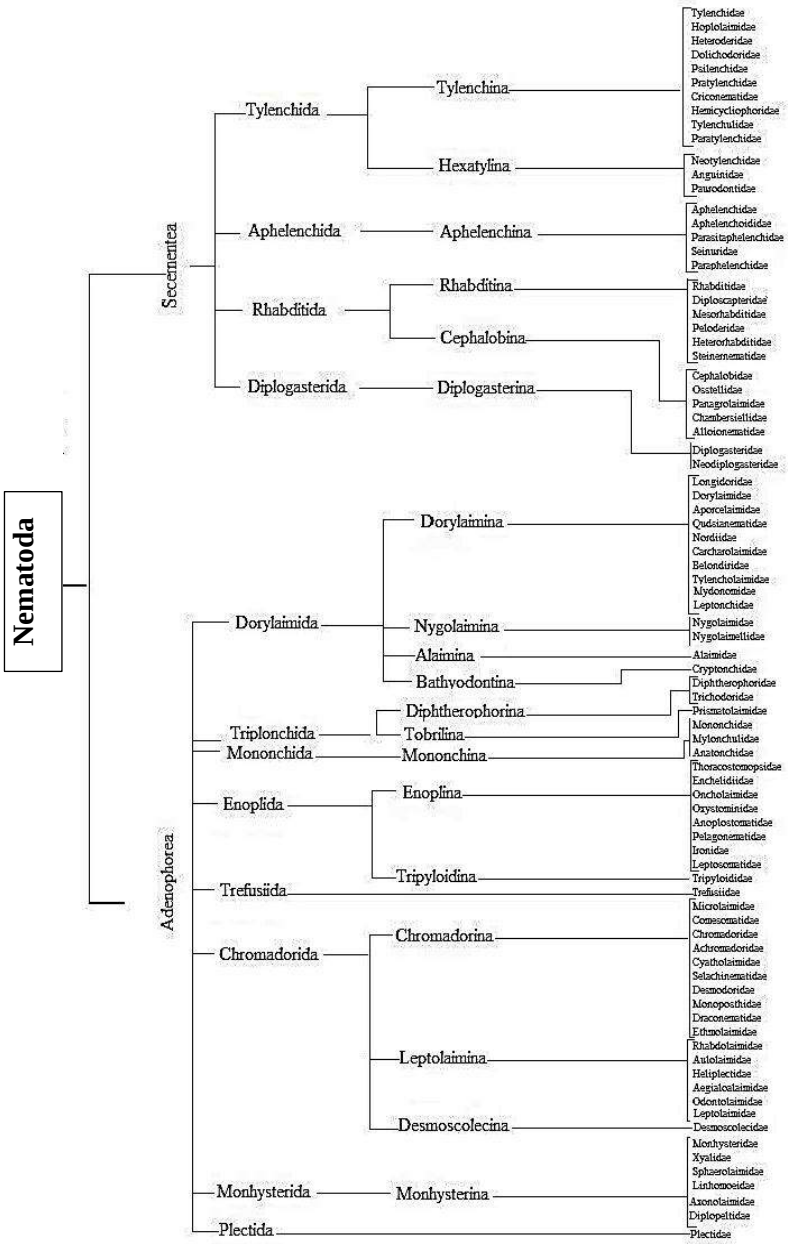


Fig.4.1. Graphical presentation of reported nematode fauna of Pakistan (from NNRC).



Classificaion tree of Nematode fauna of Pakistan (From NNRC)

Phylum: Nematoda Rudolphi, 1808 (Lankester, 1877)

syn. Nemata Cobb, 1919 (1932)

Class: Secernentea von Linstow, 1905

syn. Phasmidia Chitwood & Chitwood, 1933

Tylenchida Thorne, 1949

Tylenchina Chitwood in Chitwood & Chitwood, 1950

Tylenchoidea (Orley, 1880) Chitwood & Chitwood, 1937

Tylenchidae Orley, 1880

Tylenchinae (Orley, 1880) Marcinowsky, 1909

Tylenchus Bastian, 1865

T. bhitai Maqbool & Shahina, 1987

T. butteus Thorne & Malek, 1968

T. hamatus Thorne & Malek, 1968

T. naranensis Maqbool, Zarina & Ghazala, 1987

T. pakistanensis Farooq, Fatima & Khan, 1991

T. sandneri Wasilewska, 1965

syn. *T. cerealis* Kheiri, 1970

T. skarduensis Maqbool & Shahina, 1987

T. uniformis Cobb, 1893

syn. *Anguillulina uniformis* (Cobb) Goodey, 1932

Filenchus (Andrassy, 1954) Meyl, 1961

F. afghanicus (Khan & Khan, 1978) Siddiqi, 1986

syn. *Tylenchus afghanicus* Khan & Khan, 1978

F. cylindricus (Thorne & Malek, 1968) Niblack & Barnard, 1985

F. ditissimus (Brzeski, 1963) Siddiqi, 1986

syn. *Tylenchus ditissimus* Brzeski, 1963

F. filiformis (Butschli, 1873) Meyl, 1961

syn. *Tylenchus filiformis* Butschli, 1873

Anguillulina filiformis (Butschli) Goodey, 1922

T. vulgaris Brzeski, 1963

F. maqbooli Aatika, Nasira & Shahina, 2017

F. microdorus Chawla, Prasad, Khan& Nand, 1969

F. parvissimus (Thorne & Malek, 1968) Siddiqi, 1986

syn. *Tylenchus parvissimus* Thorne & Malek, 1968

F. sheri (Khan & Khan, 1978) Siddiqi, 1986

syn. *Tylenchus sheri*Khan & Khan, 1978

F. sindhicus Shahina & Maqbool, 1994

F. vulgaris (Brzeski, 1963) Lownsbery & Lownsbery, 1985

syn. *Tylenchus vulgaris* Brzeski, 1963

Filenchus conicephalus Siddiqi & Khan, 1983

Aglenchus (Andrassy, 1954) Meyl, 1961
A. agricola (de Man, 1884) Meyl, 1961
syn. *Tylenchus Agricola* de Man, 1884
Anguillulina Agricola (de Man) Goodey, 1932
Tylenchus filiformis apud de Man, 1876
Tylenchus filiformis de Man, 1876 in Goodey, 1932
nec *T. filiformis* Butschli, 1873
Tylenchus paragricola Paetzold, 1958
A. paragricola (Paetzold) Meyl, 1961
A. mardanensis Maqbool, Shahina & Zarina, 1984
A. siddiqii Khan, Khan & Bilqees, 1992

Coslenchus Siddiqi, 1978
syn. ***Paktylenchus*** Maqbool, 1983
C. areolatus (Egunjobi, 1967) Siddiqi, 1978
syn. *Tylenchus* (*Aglenchus*) *areolatus* Egunjobi, 1967
C. costatus (de Man, 1921) Siddiqi, 1978
syn. *Tylenchus costatus* de Man, 1921
T. (Aglenchus)costatus de Man (Andrassy, 1954)
Aglenchus costatus (de Man) Meyl, 1961
Anguillulina costata (de Man) Goodey, 1932
T. buffalorae Altherr, 1950
Anguillulina buffalorae (Altherr) Altherr, 1952
Coslenchus buffalorae (Altherr) Siddiqi, 1986
T. (Aglenchus) neozelandicus Egunjobi, 1967
Coslenchus neozelandicus (Egunjobi) Siddiqi, 1986
C. tuberosus (Maqbool, 1983) Siddiqi, 1986
syn. ***Paktylenchus tuberosus*** Maqbool, 1983

Irantylenchus Kheiri, 1972 (Andrassy, 1976)
I. clavidorus Kheiri, 1972

Boleodorinae Khan, 1964
Boleodorus Thorne, 1941
B. acutus Thorne & Malek, 1968
B. arachis Maqbool & Ghazala, 1986
B. azadkashmirensis Maqbool, Shahina & Firoza, 1990
B. neosimilis Geraert, 1971
syn. *B. similis* Thorne & Malek, 1968
B. pakistanensis Siddiqi, 1963
B. rafiqi Hussain & Khan, 1965
B. thylactus Thorne, 1941

B.volutus Lima & Siddiqi, 1963
B. zaini Maqbool, 1982

Basiria Siddiqi, 1959
*B. bajorensis*Khan & Bilqees, 1993
B. graminophila Siddiqi, 1959
syn. *Tylenchus graminophilus* (Siddiqi) Goodey, 1963
B. incita Szczygiel, 1970
B. ritteri (Baqri & Jairajpuri, 1969) Bernard, 1980
syn. *Tylenchus* (*Clavilenchus*) ritteri Baqri & Jairajpuri, 1969
Clavilenchus ritteri (Baqri & Jairajpuri) Baqri & Jairajpuri, 1969

Basiroides Thorne & Malek, 1968
B.citri Maqbool, Fatima & Shahina, 1984
B. sindhicus Maqbool, Fatima & Shahina, 1984

Neopsilenchus Thorne & Malek, 1968
N. (*Acusilenchus*) Shahina & Maqbool, 1990
N. (*A.*) *bilineatus* Shahina & Maqbool, 1990
N. (*Neopsilenchus*) Shahina & Maqbool, 1990
N. (*N.*) *curvistylus* Shahina & Maqbool, 1990
N. (*N.*) *minor* (Geraert, 1968) Shahina & Maqbool, 1990
syn. *Basiria minor* Geraert, 1968
Neopsilenchus minor (Geraert, 1968) Kheiri, 1972
N. (*N.*) *peshawarensis* Shahina & Maqbool, 1990

ThadinaeSiddiqi, 1986
Neothada Khan, 1973
N. major Maqbool & Shahina, 1989

Duosulciinae Siddiqi, 1979
Duotylenchus Saha & Khan, 1982
D. bilineatus Saha & Khan, 1982

Malenchus Andrassy, 1968
M. andrassyi Merny, 1970
M. exiguous (Massey, 1969) Andrassy, 1980
syn. *Aglenchus exiguous* Massey, 1969
M. fusiformis (Thorne & Malek, 1968) Siddiqi, 1979
syn. *Tylenchus fusiformis* Thorne & Malek, 1968
Ottolenchus fusiformis (Thorne & Malek) Wu, 1970
M. labiatus Maqbool & Shahina, 1985
M. nanellus Siddiqi, 1979

M. platycephalus (Thorne & Malek, 1968) Andrassy, 1981
syn. *Tylenchus platycephalus* Thorne & Malek, 1968
Ottolenchus platycephalus (Thorne & Malek) Siddiqi & Hawksworth, 1982
M. pyri Maqbool & Shahina, 1985

Telomalenchus Siddiqi, 2000
T. williamsi (Geraert & Raski, 1986) Siddiqi, 2000

Ottolenchus (Husain & Khan, 1967) Wu, 1970
O. azadkashmirensis Maqbool & Shahina, 1985
O. facultativus (Szczygiel, 1970) Brzeski, 1982
syn. *Tylenchus facultativus* Szczygiel, 1970
O. facultativus (Szczygiel) Siddiqi & Hawksworth, 1982
Malenchus piahyuensis Monteiro, 1974
Ottolenchus piahyuensis (Monteiro) Siddiqi, 1986
O. longicauda Maqbool & Shahina, 1985

Tylodorinae Paramonov, 1967
Cephalenchus (Goodey, 1962) Golden, 1971
C. longicaudatus Maqbool & Ghazala, 1986
C. sacchari Maqbool, Fatima & Shahina, 1984

EutylenchusCobb, 1913
Eutylenchus spp.

Epicharinematinae Maqbool & Shahina, 1985
Karachinema Maqbool & Shahina, 1985
K. elongatum Maqbool & Shahina, 1985

Hoplolaimidae Filipjev, 1934
Hoplolaiminae Filipjev, 1934
Hoplolaimus Von Daday, 1905
syn. ***Basirolaimus*** Shamsi, 1979
H. aegypti Shafiee & Koura, 1969
syn. *Basirolaimus aegypti* (Shafiee & Kora, 1969) Shamsi, 1979
H. aegypti (Shafiee & Koura, 1969) Luc, 1981
B. aegypti (Shafiee & Koura, 1969) Siddiqi, 1986
H. californicus Sher, 1963
syn. *Hoplolaimoides californicus* (Sher, 1963) Shakil, 1973

Hoplolaimus californicus (Sher, 1963) Siddiqi, 1986

H. columbus Sher, 1963
syn. *B. columbus* (Sher, 1963) Shamsi, 1979
H. columbus (Sher, 1963) Luc, 1981
B. columbus (Sher, 1963) Siddiqi, 1986

H. dimorphicus Mulk & Jairajpuri, 1976
syn. *B. dimorphicus* (Mulk & Jairajpuri, 1976) Shamsi, 1979
H. dimorphicus (Mulk & Jairajpuri, 1976) Luc, 1981
B. dimorphicus (Mulk & Jairajpuri, 1976) Siddiqi, 1986

H. dubius Chaturvedi & Khera, 1979
syn. *B. dubius* (Chaturvedi & Khera, 1979) siddiqi, 1986

H. galeatus (Cobb, 1913) Thorne, 1935
syn. *Nemonchus galeatus* Cobb, 1913
Hoplolaimus coronatus Cobb, 1923

H. indicus Sher, 1963
syn. *B. indicus* (Sher, 1963) Shamsi, 1979
H. indicus (Sher, 1963) Luc, 1981
syn. *H. arachidis* (Maharaju & Das, 1982) Siddiqi, 1986
B. arachidis (Maharaju & Das, 1982) Siddiqi, 1986
B. indicus (Sher, 1963) Siddiqi, 1986

H. pararobustus (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963
syn. *Tylenchorhynchusrobustus* Schuurmans Stekhoven, 1936
T. pararobustus Schuurmans Stekhoven & Teunissen, 1938
Rotylenchus pararobustus (Schuurmans Stekhoven & Teunissen)
Filipjev & Schuurmans Stekhoven, 1941
Hoplolaimus proporicus Goodey, 1957
Gotholddteineria pararobusta (Schuurmans Stekhoven &
Teunissen) Andrassy, 1958
Hoplolaimus angustalatus Whitehead, 1959
H. kittenbergeri Andrassy, 1961

H. seinhorsti Luc, 1958
syn. *B. seinhorsti* (Luc, 1958) Shamsi, 1979
H. seinhorsti (Luc, 1958) Luc, 1981
B. seinhorsti (Luc, 1958) Siddiqi, 1986
H. seshadrii Mulk & Jairajpuri, 1976

syn. *B. seshadrii* (Mulk & Jairajpuri, 1976) Shamsi, 1979

H. seshadrii (Mulk & Jairajpuri, 1976) Luc, 1981

syn. *B. seshadrii* (Mulk & Jairajpuri, 1976 Siddiqi, 1986

H. stephanus Sher, 1963

H. tabacum Firoza, Nasira & Maqbool, 1990

H. tylenchiformis Daday, 1905

syn. *Criconema tylenchiformis* (Daday) Micoletzky, 1917

Scutellonema Andrassy, 1958

S. bradys (Steiner & LeHew, 1933) Andrassy, 1958

syn. *Hoplolaimus bradys* Steiner & LeHew, 1933

Anguillulina bradys (Steiner & LeHew) Goodey, 1935

Rotylenchus bradys (Steiner & LeHew) Filipjev, 1936

R. blaberus Steiner, 1937

Scutellonema blaberum (Steiner, 1937) Andrassy, 1958

S. dioscoreae Lordello, 1959

S. brachyurus (Steiner, 1938) Andrassy, 1958

Rotylenchus Filipjev, 1936

R. alii Maqbool & Shahina, 1986

R. buxophilus Golden, 1956

syn. *R. sheri* Jairajpuri, 1964

R. capsicumi Firoza & Maqbool, 1991

R. cypriensis Antoniou, 1980

R. dalhousiensis Sultan & Jairajpuri, 1979

R. fragaricus Maqbool & Shahina, 1986

R. goldeni Firoza & Maqbool, 1993

R. himprus (Sultan, 1980) Fortuner, 1987

syn. *Orientylus himprus* Sultan, 1980

Varotylushimprus (Sultan, 1980) Siddiqi, 1986

R. jagatpurensis Sultan, 1985

R. karachiensis (Maqbool & Ghazala, 1984) Fortuner, 1987

syn. *Orientylus karachiensis* Maqbool & Ghazala, 1984

R. pakistanensis Maqbool & Shahina, 1986

R. robustus (de Man, 1876) Filipjev, 1936

syn. *Hoplolaimus uniformis* Thorne, 1979

Scutellonema picea Gubina, 1973

R. siddiqii (Mulk & Jairajpuri, 1976) Fortuner, 1987

syn. *Orietylus siddiqii* (Mulk & Jairajpuri, 1976) Jairajpuri & Siddqi, 1970
Varotylus siddqii (Mulk & Jairajpuri, 1976) Siddiqi, 1986

Pararotylenchus Baldwin & Bell, 1981
P. microstylus Maqbool, Ghazala, Fatima & Qasim, 1985

Helicotylenchus Steiner, 1945
H. abuharazi Zeidan & Geraert, 1990
H. abunaamai Siddiqi, 1972
H. arachisi Mulk & Jairajpuri, 1975
H. californicus Sher, 1966
H. canadensis Waseem, 1961
syn. *H. cairnsi* Waseem, 1961
H. cavenessi Sher, 1966
H. certus Eroshenko & Nguen Vu Tkhan, 1981
H. conicephalus Siddiqi, 1972
H. crenacauda Sher, 1966
syn. *H. pteracercus* Singh, 1971
H. paracrenacauda Phukan & Sanwal, 1981
H. pteracercusoides Fotedar & Kaul, 1985
H. digonicus Perry in Perry, Darling & Thorne, 1959
syn. *H. broadbalkiensis* Yuen, 1964
H. dihystera (Cobb, 1893) Sher, 1961
syn. *Tylenchus dihystera* Cobb, 1893
T. olaae Cobb, 1906
T.ylenchorhynchusolaae (Cobb) Micoletzky, 1922
Helicotylenchus olaae (Cobb) Siddiqi, 1986
Aphelenchus dubius var. peruensis Steiner, 1920
Tylenchusspiralis Cassidy, 1930
Helicotylenchusspiralis (Cassidy) Siddiqi, 1986
H. nannus Steiner, 1945
H. crenatus Das, 1960
H. flatus Roman, 1965
H. punicae Swarup & Sethi, 1968
H. paraconcavus Rashid & Khan, 1974
H. discocephalus Firoza & Maqbool, 1993
H. egyptiensis Tarjan, 1964
H. erythrinae (Zimmermann, 1904) Golden, 1956
syn. *Tylenchus erythrinae* Zimmermann, 1904
Tylenchorhynchus erythrinae (Zimmermann) Balley & Reydon, 1931
Anguillulina erythrinae (Zimmermann) Goodey, 1932

Rotylenchus erythrinae (Zimmermann) Goodey, 1951
R. melancholicus Lordello, 1955
H. melancholicus (Lordello) Andrassy, 1958
H. spicaudatus Tarjan, 1964

H. exallus Sher, 1966
syn. *H. regularis* Phillips, 1971

H. falcatus Eroshenko & Nguen Vu Thanh, 1981
H. goodi Tikyani, Khera & Bhatnagar, 1969
H. gulabi Jain, Siddiqui & Aruna Parihar, 2000
H. handooi Khan, Ghazi & Soomro, 2008
H. indicus Siddiqi, 1963
H. jasminii Jain, Siddiqui & Aruna Parihar, 2000
H. lemoni Firoza & Maqbool, 1996
H. lucernis Khan & Ahmad, 1970
H. macronatus Mulk & Jairajpuri, 1975
H. martini Sher, 1966
H. meloni Firoza & Maqbool, 1994
H. microcephalus Sher, 1966
H. microdorus Prasad, Khan & Chawla, 1965
H. microlobus Perry in Perry, Darling & Thorne, 1959
H. microtylus Firoza & Maqbool, 1993
H. multicinctus (Cobb, 1893) Golden, 1956
syn. *Tylenchus multicinctus* Cobb, 1893
Tylenchorhynchus multicinctus (Cobb) Micoletzky, 1922
Anguillulina multicincta (Cobb) Goodey, 1932
Rotylenchus multicinctus (Cobb) Filipjev, 1936
R. iperoiguensis Carvalho, 1956
Helicotylenchus iperoiguensis (Carvalho) Andrassy, 1958

H. obliquus Maqbool & Shahina, 1986
H. oscephalus Anderson, 1979
H. platyurus Perry in Perry, Darling & Thorne, 1959
H. pseudorobustus (Steiner, 1914) Golden, 1956
syn. *Tylenchus robustus* var. pseudorobustus Steiner, 1914

H. seshadrii Singh & Khera, 1980
H. siddiqii Zarina & Akhter, 2016
H. striatus Firoza & Maqbool, 1994
H. thornei Roman, 1965
H.urobelus Anderson, 1978
H. verecundus Zarina & Maqbool, 1991
H. wajihi Sultan, 1981
H. willmottae Siddiqi, 1972

Rotylenchulinae Husain & Khan, 1967
Rotylenchulus Linford & Oliveira, 1940
R. parvus (Williams, 1960) Sher, 1961
syn. *Helicotylenchusparvus* Williams, 1960
R. reniformis Linford & Oliveira, 1940
syn. *Tetylenchus nicotiana* Yokoo & Tanaka in Tanaka & Tsumagori, 1954
Rotylenchulus nicotiana (Yokoo & Tanaka in Tanaka &Tsumagori)Baker, 1962
Rotylenchus elisensis Carvalho, 1957
Helicotylenchus elisensis (Carvalho) Carvalho, 1959
Rotylenchulus elisensis (Carvalho) Siddiqi, 1986
Spyrotylenchus queirozi Lordello & Cesnik, 1958
Rotylenchulus queirozi (Lordello & Cesnik) Sher, 1961
Leiperotylenchusleiperi Das, 1960
Rotylenchulus leiperi (Das) Loof & Oostenbrink, 1961
R. stakmani Husain & Khan, 1965

Heteroderidae Filipjev & Schuurmans Stekhoven, 1941
Heteroderinae Filipjev & Schuurmans Stekhoven, 1941
Heterodera Schmidt, 1871
H. avenae Wollenweber, 1924
syn. *H. schachtii* var. *avenae* Wollenweber, 1924
H. bidera avenae (Wollenweber, 1924) Krall & Krall, 1978
H. bergeniae Maqbool & Shahina, 1988
H. cajani Koshy, 1967
H. cruciferae Franklin, 1945
syn. *H. vigni* Edward & Misra, 1968
H. cynodontis Shahina & Maqbool, 1989
H. fici Kirjanova, 1954
H. mani Mathews, 1971
syn. *Bidera mani* (Mathews, 1971) Krall & Krall, 1978
H. mothi Khan & Husain, 1965
H. oryzae Luc & Berdon Brizuela, 1961
H. pakistanensis Maqbool & Shahina, 1986
H. sacchari Luc & Merny, 1963
H. schachtii Schmidt, 1871
syn. *Tylenchus schachtii* (Schmidt, 1978) Orley, 1880
Heterodera schachtii minor Schmidt, 1930
H. zeae Koshy, Swarup & Sethi, 1971

Globodera Skarbilovich, 1959
G. pallida (Stone, 1973) Behrens, 1975
syn. *Heterodera pallida* Stone, 1973
G. rostochiensis (Wollenweber, 1923) Skarbilovich, 1959
syn. *Heterodera schachtii rostochiensis* Wollenweber, 1923
H. achachtii solani Zimmermann, 1927
G. tabacum tabacum (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959
syn. *H. tabacum* Lownsbery & Lownsbery, 1954

Meloidogyninae Skarbilovich, 1959
Meloidogyne Goeldi, 1892
M. arenaria (Neal, 1889) Chitwood, 1949
syn. *Anguillula arenaria* Neal, 1889
Tylenchus arenarius (Neal, 1889) Cobb, 1890
Heterodera arenaria (Neal, 1889) Marchinowski, 1909
M. arenaria arenaria (Neal, 1889) Chitwood, 1946
*M.arenaria thamesi*Chirwood in Chitwood, Specht & Havis, 1952
M. thamesi (Chitwood in Chitwood *et al*., 1952) Goodey, 1963
M. graminicola Golden & Birchfield, 1965
M. hapla Chitwood, 1949
M. incognita (Kofoid & White, 1919) Chitwood, 1949
syn. *Oxyuris incognita* (Kofoid & While, 1919)
Heterodera incognita (Kofoid & While, 1919) Sandground, 1923
M. incognita incognita (Kofoid & While, 1919) Chitwood, 1949
M. incognita acrita Chitwood, 1949
M. acrita (Chitwood, 1949) Esser, Perry, and Tylor, 1976
M. incognita inornata Lordello, 1956
M. elegans da Ponte, 1977
M. graham Golden &Slana, 1978
M. incognita wartellei Golden & Birchfield, 1978
M. inornata Lordello, 1956
M. javanica (Treub, 1885) Chitwood, 1949
syn. *Heterodera javanica* Treub, 1885, Cobb, 1890

Anguillula javanica (Treub, 1885) Lavergne, 1901
M. javanica javanica (Treub, 1885) Chitwood, 1949
M. javanica bauruensis Lordello, 1956
M. bauruensis (Lordello, 1956) Esser, Perry & Taylor, 1976
M. lucknowica Singh, 1969
M. lordelloi da Ponte, 1969
M. pakistanica Shahina, Nasira, Salma, Mehreen & Bhatti, 2015

Dolichodoroidea (Chitwood in Chitwood & Chitwood, 1950) Siddiqi, 1986
Dolichodoridae (Chitwood in Chitwood & Chitwood, 1950) Skarbilovich, 1959
Telotylenchinae Siddiqi, 1960
Tylenchorhynchus Cobb, 1913
T. annulatus (Cassidy, 1930) Golden, 1971
syn. *Tylopharynx annulatus* Cassidy, 1930
Anguillulina annulata (Cassidy, 1930) Googey, 1932
Chitinotylenchus annulatus (Cassidy, 1930) Filipjev, 1936
Ditylenchus annulatus (Cassidy, 1930) Sher, 1970
Tylenchorhynchus martini Fielding, 1956
T. brassicae Siddiqi, 1961
T. clarus Allen, 1955
syn. *T. tener* Erzhanova, 1964
T. claytoni Steiner, 1937
syn. *Tessellus claytoni* (Steiner, 1937) Jairajpuri & Hunt, 1984
T. crassicaudatus Williams, 1960
T. cylindricus Cobb, 1913
syn. *Tylenchus* (*Tylenchorhynchus*) c*ylindricus* (Cobb) Filipjev,1934
Anguillulina cylindrical (Cobb) Thorne, 1935
T. elegans Siddiqi, 1961
T. ewingi Hooper, 1959
*T. fatimae*Khan, Saeed & Akhter, 2004
T. gossypii Nasira & Maqbool, 1996
T. handooi Khan, 2004
T. intervallatus (Tobar-Jimenez, 1970) Fortunr & Luc, 1987
T. kegasawai Minagawa, 1995
T. leviterminalis Siddiqi, Mukherjee & Dasgupta, 1982

T. madrasensis Gupta & Uma, 1981
syn. *Divittus madrasensis* (Gupta & Uma, 1981) Jairajpuri, 1984
T. manubriatus Litvinova, 1946
T. mashhoodi Siddiqi & Basir, 1959
T. nudus Allen, 1955
T. obscurisulcatus Andrassy, 1959
T. penniseti Gupta & Uma, 1980
T. qasimii Mussarat, Handoo & Shahina, 2008
T. quaidi Golden, Maqbool & Handoo, 1987
T. rosei Zarina & Maqbool, 1991
T. striatus Allen, 1955
T. swatiensis Nasira, Shahina & Maqbool, 1991
T. trilineatus Timm, 1963
T. tritici Golden, Maqbool & Handoo, 1987
T. tuberoses Zarina & Maqbool, 1993

Bitylenchus (Filipjev, 1934) Siddiqi, 1986
B. brevilineatus (Williams, 1960) Siddiqi, 1986
syn. *Tylenchorhynchus brevilineatus* Wiliams, 1960
T. indicus Siddiqi, 1961
Bitylenchus indicus (Siddiqi) Siddiqi, 1986
B. canalis (Thorne & Melak, 1968) Siddiqi, 1986
B. capsicumi Akhter & Zarina, 2014
syn. *Tylenchorhynchus canalis* Thorne & Malek, 1968
B. clavicaudatus (Seinhorst, 1963) Siddiqi, 1986
syn. *Tylenchorhynchus clavicaudatus* Seinhorst, 1963
T. clavicauda Seinhorst, 1968
B. goffarti (Sturhan, 1966) Siddiqi, 1986
syn. *Tylenchorhynchus goffarti* Sturhan, 1966
B. maximus (Allen, 1955) Siddiqi, 1986
syn. *Tylenchorhynchus maximus* Allen, 1955
B. maximus pakistanensis Zarina, Siddiqi &Shahina, 2004
B. parvus (Allen, 1955) Siddiqi, 1986
syn. *Tylenchorhynchus parvus* Allen, 1955
B. vulgaris (Upadhyay, Swarup & Sethi, 1972) Siddiqi, 1986
syn. *Tylenchorhynchus vulgaris* Upadhyay, Swarup & Sethi, 1972

Dolichorhynchus Mulk & Jairajouri, 1974
D. motiaii Zarina & Maqbool, 1998

D. phaseoli (Sethi & Swarup, 1968) Mulk & Jairajpuri, 1974
D. tuberosus Maqbool, Ghazala & Fatima, 1984

Neodolichorhynchus Jairajpuri & Hunt, 1984
N. (*Mulkorhynchus*) *indicus* Erum, Mussarat & Shahina, 2011

Paratrophurus Arias, 1970
P. anomalus Kleynhans & Heyns, 1983

Quinisulcius Siddiqi, 1971
Q. acutoides (Thorne & Malek, 1968) Siddiqi, 1971
syn. *Tylenchorhychus acutoides* Thorne & Malek, 1968
Q. acutus (Allen, 1955) Siddiqi, 1971
syn. *Tylenchorhynchus acutus* Allen, 1955
Q. capitatus (Allen, 1955) Siddiqi, 1971
syn. *Tylenchorhynchus capitatus* (Allen, 1955)
T. acti Hopper, 1959
Q. acti (Hopper) Siddiqi, 1971
T. nilgiriensis Seshadri, Muthukrishnan & Shunmugam, 1967
Q. nilgiriensis (Seshadri, Muthukrishnan & Shunmugam, 1967)
Siddiqi, 1971
Q. himalayae Mahajan, 1974
Q. curvus (Williams, 1960) Siddiqi, 1970
syn. *Tylenchorhynchus curvus* Williams, 1960
Q. quaidi Zarina & Maqbool, 1992
Q. solani Maqbool, 1982

Telotylenchus Siddiqi, 1960
*T. indicus*Siddiqi, 1960

Merliniinae Siddiqi, 1071
Merlinius Siddiqi, 1970
M. adakensis Bernard, 1984
M. alboranensis (Tobar-Jimenez, 1970) Tarjan, 1973
M. bavaricus (Sturhan, 1966) Siddiqi, 1970
syn. *Tylenchorhynchus bavaricus* Sturhan, 1966
M. bilqeesae Khan & Khan, 1995
M. brevidens (Allen, 1955) Siddiqi, 1970
syn. *Tylenchorhynchus brevidens* Allen, 1955
M. indicus Zarina & Maqbool, 1995
M. khuzdarensis Handoo, Khan & Islam, 2007
M. microdorus (Geraert, 1966) Siddiqi, 1970

syn. *Tylenchorhynchus microdorus* Geraert, 1966
M. montanus Maqbool & Shahina, 1987
*M. nagerensis*Sagir & Erum, 2017
M. nanus (Allen, 1955) Siddiqi, 1970
syn. *Thylenchorhynchus nanus* Allen, 1955
M. niazae Maqbool, Fatima & Hashmi, 1983
M. nothus (Allen, 1955) Siddiqi, 1970
M. pistaciei Fatima & Farooq, 1992
M. pseudobavaricus Saltukoglu, Geraert & Coomans, 1976
M. pyri Fatima & Farooq, 1992

Amplimerlinius Siddiqi, 1976
A. parbati Zarina & Maqbool, 1990

Nagelus Thorne & Malek, 1968
N. saifulmulukensis Maqbool & Shahina, 1987
N. varians (Thorne & Malek, 1968) Siddiqi, 1986

Scutylenchus Jairajpuri, 1971
S. baluchiensis Maqbool, Ghazala, Fatima & Qasim, 1985
S. fici Farooq & Fatima, 1994
S. koreanus (Choi & Geraert, 1971) Siddiqi, 1979
syn. *Merlinius koreanus* Choi & Geraert, 1971
S. quettensis Maqbool, Ghazala & Fatima, 1984
*S. rugosus*Siddiqi, 1963

Tetylenchus Filipjev, 1936
Tetylenchus spp.

Belonolaiminae Whitehead, 1960
Belonolaimus Steiner, 1949
B. gracilis Steiner, 1949
B. longicaudatus Rau, 1958

Psilenchidae (Paramonov, 1967) Khan, 1969
Psilenchinae Paramonov, 1967
Psilenchus de Man, 1921
P. aestuarius Andrassy, 1962
P. hilarulus de Man, 1921
P. hilarus Siddiqi, 1963
syn. *P.neoformis* Jairajpuri & Siddiqi, 1963
P. iranicus Kheiri, 1970
P. khuzarensis Khan, Batool & Khatoon, 2004
P. minor Siddiqi, 1963
syn. *P. pratensis* Doucet, 1996

Atetylenchus Khan, 1973
A. amiri (Maqbool & Shahina, 1984) Geraert & Raski, 1987
syn. *Leipotylenchus amiri* Maqbool & Shahina, 1984
A. metoporus Erum & Shahina, 2008

Pratylenchidae (Thorne, 1949) Siddiqi, 1963
Pratylenchinae Thorne, 1949
Pratylenchus Filipjev, 1936
P. alleni Ferris, 1961
P. brachyurus (Godfrey, 1929) Filipjev & Schuurmans Stekhovan, 1941
syn. *P. pratensis* apud Thorne, 1940
P. leiocephalus Steiner, 1949
P. steineri Lordello, Zamith & Boock, 1954
P. coffeae (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941
syn. *P. musicola* (Cobb, 1919) Filipjev, 1936
P. mahogany (Cobb, 1920) Filipjev, 1936
P. pratensis apud Yokoo, 1956
P. crenatus Loof, 1960
syn. *P. clavicaudatus* Baranovskaya & Haque, 1968
P. delatterei(Luc,1958) Handoo & Golden, 1989
P. flakkensis Seinhorst, 1968
P. goodeyi Sher & Allen, 1953
P. hexincisus Taylor & Jenkins, 1957
P. kralli Ryss,1982
P. neglectus (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941
syn. *P. minyus*Sher & Allen, 1953
P. capitatus Ivanova, 1968
*P. neocapitatus*Khan& Singh, 1975
P. similis Khan & Singh, 1975
P. penetrans (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941
syn. *Tylenchus gulosus* Kuhn, 1890
P. globulicola Romaniko, 1960
P. portulactus Zarina & Maqbool, 1998
P. pratensis (de Man, 1880) Filipjev, 1936
syn. *P. helophilus* Seinhorst, 1959
P. irregularis Loof, 1960
P. pseudofallax, Café-Filo & Huang,1989
P. pseudopratensis Seinhorst, 1968
P. roseus Zarina & Maqbool, 1998
P. sacchari (Soltwedel, 1888) Filipjev, 1936

P. scribneri Steiner in Sherbakoff & Stanley, 1943
P. sefaensis Fortuner, 1973
P. similis Khan& Singh, 1975
P. sudanensis Loof & Yassin, 1971
P. thornei Sher & Allen, 1953
P. vulnus Allen & Jensen, 1951
P. zeae Graham, 1951

Zygotylenchus Siddiqi, 1963
Z. guevarai (Tobar-Jimenez, 1963) Braun & Loof, 1966

Hirschmanniellinae Fotedar & Handoo, 1978
Hirschmanniella Luc & Goodey, 1964
H. gracilis (de Man, 1880) Luc & Goodey, 1964
syn. *Tylenchus gracilis* de Man, 1880
Tylenchorhynchus gracilis (de Man, 1880) Micoletzky, 1925
Anguillulina gracillis (de Man, 1880) Goodey, 1932
Chitinotylenchus gracilis (de Man, 1880) Hirschmann, 1955
Radopholus gracilis (de Man, 1880) Allen, 1955
Radopholus gigas Andrassy, 1954
Hirschmanniella gigas (Andrassy, 1954) Sher, 1968

H. magna Siddiqi, 1966
syn. *Hirschmanniella dubia* Khan, 1972
H. mexicana (Chitwood, 1951) Sher, 1968
syn. *Halenchus mexicannus* Chitqood, 1951
Hirschmanniella caudacrena Sher, 1968
H. mucronata (Das, 1960) Khan, Siddiqi, Khan, Hussian & Saxena, 1964
syn. *Radopholus mucronatus* Das, 1960
Hirschmanniella mucronata (Das, 1960) Timm, 1965
Hirschmanniella mangalorensis Mathur & Prasas, 1971
Hirschmanniella indica Ahmad, 1974
H. oryzae (van Breda de Haan, 1902) Luc & Goodey, 1964
syn. *Tylenchus oryzae* van Breda de Haan, 1902
Anguillulina oryzae (van Breda de Haan, 1902
Radopholus oryzae (van Breda de Haan, 1902) Filipjev &
Schuurmans Stekhoven, 1941

Tylenchus apapillatus Imamura, 1931
Anguillulina apapillata (Imamura, 1931) Goodey, 1932
Rotylenchus apapillatus (Imamura, 1931) Filipjev, 1936
Hirschmanniella apapillata (Imamura, 1931) Sher, 1968
Hirschmanniella nana Siddiqi, 1966
Hirschmanniella exigua Khan, 1972

H. spinicaudata (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964
syn. *Tylenchorhynchus spinicaudatus* Schuurmans Stekhoven, 1944
Radopholus lavabri Luc, 1957
Hirschmanniellala vabri (Luc, 1957) Sher, 1968

Radopholinae Allen & Sher, 1967
Radopholus Thorne, 1949
R. allius Shahina & Maqbool, 1996
R. brassicae Shahina & Maqbool, 1996
R. similis (Cobb, 1893) Thorne, 1949
syn. *Tylenchus similis* Cobb, 1893
Anguillulina similis (Cobb, 1893) Goodey, 1932
Rotylenchus similis (Cobb, 1893) Filipjev, 1936
Tylenchus granulosus Cobb, 1893
Anguillulina granulose (Cobb, 1893) Goodey, 1932
Tetylenchus granulosus (Cobb, 1893) Filipjev, 1936
Radopholus granulosus (Cobb, 1893) Siddiqi, 1986
Tylenchus acutocaudatus Zimmermann, 1898
Anguillulina acutocaudata (Zimmermann, 1898) Goodey, 1932
Tylenchorhynchus acutocaudatus (Zimmermann, 1898) Filipjev, 1934
Radopholus acutocaudatus (Zimmermann, 1898) Siddiqi, 1986
Tylenchus biformis Cobb, 1909
Anguillulina biformis (Cobb, 1909) Goodey, 1932
Radopholus biformis (Cobb, 1909) Siddiqi, 1986
R. similis similis in Siddiqi, 1986

Pratylenchoides Winslow, 1958
P. maqsoodi Maqbool & Shahina, 1989

Criconematoidea Taylor, 1936
Criconematidae Taylor, 1936
Criconematinae Taylor, 1936
Criconema Hofmanner & Menzel, 1914
Subgenus *Nothocriconemella* (Ebsary, 1981) Siddiqi, 1986
C. (*N.*) *mutabile* (Taylor, 1936) Raski & Luc, 1985
syn. *Nothocriconema mutabile* (Taylor) de Grisse & Loof, 1965
Nothocriconemella mutabilis (Taylor) Ebsary, 1981
Nothocriconema kovacsi (Andrassy) de Grisse &Loof, 1965
Nothocriconemella kovacsi (Andrassy) Ebsary, 1981
Criconema kovacsi (Andrassy) Siddiqi, 1986
Criconemoides raskii Goodey, 1963
Criconema raskii (Goodey) Siddiqi, 1986
Criconemoides siddiqi Khan, 1964
Criconema siddiqi (Khan) Siddiqi, 1986
Criconemoides magnolia Edward & Misra, 1964
Criconema magnolia (Edward & Misra) Siddiqi, 1986
Cricinemoides californicus Diab & Jenkins, 1966
Criconema californicum (Diab & Jenkins) Siddiqi, 1986
Criconemoides kashmirensis Mahajan & Bijral, 1973
Criconema kashmirense (Mahajan & Bijral) Siddiqi, 1986
Criconemoides mutabilis Taylor, 1936

Ogma Southern, 1914
O. multiannulata Shahina & Maqbool, 1991
O. qamari Shahina & Maqbool, 1991
O. sadabhari Shahina & Maqbool, 1991

Macoposthoniinae Skarbilovich, 1959
Criconemoides Taylor, 1936
Subgenus *Criconemoides* Taylor, 1936
C. (*C.*) *afghanicus* Shahina & Maqbool, 1993
C. (*C.*) *bakeri* Wu, 1965
syn. *Criconemella bakeri* (Wu, 1965) Luc & Raski, 1981
C. (*C.*) *brevistylus* Singh & Khera, 1976

syn. *Criconemella brevistylus* (Singh & Khera, 1976) Luc & Raski, 1981

C. (*C.*) *curvatus* Raski, 1952

syn. *Criconemella curvata* (Raski, 1952) Luc & Raski, 1981

Macroposthonia coomansi de grisse, 1967

C. (*C.*) *kamaliei* Khan, 1971

C. (*C.*) *onoensis* Luc, 1959

syn. *Criconemellaonoensis* (Luc, 1959) Luc & Raski, 1965

C. (*C.*) *parvulus* Siddiqi, 1961

syn. *Criconemella parvula* (Siddiqi, 1961) de Grisse & Loof, 1965

C. (*C.*) *pruni* (Siddiqi, 1961) Raski & Golden, 1966

syn. *Criconemella pruni* (Siddiqi, 1961) Luc & Raski, 1981

Criconema pruni Siddiqi, 1961

C. (*C.*) *sphaerocephalus* Taylor, 1936

syn. *Criconemella sphaerocephala* (Taylor, 1936) Luc & Raski,1981

C. (*C.*) *xenoplax* Raski, 1952

syn. *Criconemella xenoplax* (Raski,1952) Luc & Raski,1981

Pakcriconemeoides Shahina & Maqbool, 1993

P. anastomoides (Maqbool & Shahina, 1985) Shahina & Maqbool, 1993

syn. *Criconemella anastomoides* Maqbool & Shahina, 1985

Macroposthonia anastomoides (Maqbool & Shahina, 1985)
Siddiqi, 1986

Mesocriconema anastomoides (Maqbool & Shahina, 1985)
Loof & de Grisse, 1989

Macroposthonia de Man, 1880

M. (*curvata*) *alpina* Shahina & Maqbool, 1993

M. coomansi De Grisse, 1967

Hemicriconemoidinae Andrassy, 1979

Hemicriconemoides Chitwood & Birchfield, 1957

H. brachyurus (Loos, 1949) Chitwood & Birchfield, 1957

H. cocophillus (Loos, 1949) Chitwood & Birchfield, 1957

syn. *Criconemoides cocophillus* Loos, 1949

Hemicycliophora cocophilla (Loos) Goodey, 1963
Criconema mangiferum Edward & Misra, 1963
Hemicriconemoides mangiferus (Edward & Misra) Siddiqi, 1986
H. communis Edward & Misra, 1964
H. gaddi (Loos, 1949) Chitwood & Birchfield, 1957
syn. *Criconemoides gaddi* Loos, 1949
Hemicycliophora gaddi (Loos) Goodey, 1963
H. ghaffari Maqbool, 1982
H. mangiferae Siddiqi, 1961
syn. *H. litchi* Edward & Misra, 1964
H. birchfieldi Edward, Misra & Singh, 1965
*H. aberrans*Phukan & Sanwal, 1983
H. strictathecatus Esser, 1960
syn. *Hemicycliophora strictathecata* (Esser) Goodey, 1963

Hemicycliophoroidea (Skarbilovich, 1959) Siddiqi, 1980
Hemicycliophoridae (Skarbilovich, 1959) Geraert, 1966
Hemicycliophorinae Skarbilovich, 1959
Hemicycliophora de Man, 1921
H. gracilis Thorne, 1955
H. veechi Maqbool, Shahina & Zarina, 1986

Aulosphora Siddiqi, 1980
A. karachiensis Maqbool, Shahina & Zarina, 1986
A. penetrans (Thorne, 1955) Siddiqi, 1980
syn. *Hemicycliophora penetrans* Thorne, 1955

Tylenchuloidea (Skarbilovich, 1947) Raski & Siddiqi, 1975
Tylenchulidae (Skarbilovich, 1947) Kirjanova, 1955
Tylenchulinae Skarbilovich, 1947
Tylenchulus Cobb, 1913
T. semipenetrans Cobb, 1913

Paratylenchidae (Thorne, 1949) Raski, 1962
Paratylenchinae Thorne, 1949
Paratylenchus Micoletzky, 1922
P. hamatus Thorne & Allen, 1950
P. holdemani Raski, 1975
P. italiensis Raski, 1975
P. karachiensis Zarina & Akhter, 2016
P. manilkarii Erum, Nasir, Nasira & Shahina, 2019
P. nainianus Edward & Misra, 1963
P. nanus Cobb, 1923
P. projectus Jenkins, 1956

syn. *P. amblycephalus* Reuver, 1959
P. salubris Raski, 1975
P. sindhicus Erum, Nasir, Nasira&Shahina, 2019
P. tateae Wu & Townshend, 1973

Gracilacus Raski, 1962
G. musae Shahina & Maqbool, 1993

Hexatylina Siddiqi, 1980
Neotylenchoidea (Thorne, 1941) Jairajpuri & Siddiqi, 1969
Neotylenchidae Thorne, 1941
Neotylenchinae Thorne, 1941
NeotylenchusSteiner, 1931
Neotylenchus spp.

Deladinae Siddiqi, 1986
Deladenus Thorne, 1941
D. cocophilus Nasira, Shahina & Firoza, 2013
D. imperialis Bedding, 1974
D. pakistanensis Shahina & Maqbool, 1992

Fergusobiinae Goodey, 1963
Fergusobia Currie, 1937
F. indica (Jairajpuri, 1962) Siddiqi, 1986
syn. *Boleodorus indicus* Jairajpuri, 1962
Dorsalla indica (Jairajpuri) Jairajpuri, 1966

Anguinoidea Nicroll, 1935
Anguinidae Nicroll, 1935
Anguininae Nicroll, 1935
Anguina Scopoli, 1777
A. tritici (Steinbuch, 1799) Filipjev, 1936
syn. *Vibrio tritci* Steinbuch, 1799
Rhabditis tritici (Steinbuch) Dujardin, 1875
Anguillula tritici (Steinbuch) Grube, 1849
Anguillulina tritici (Steinbuch) Gervais &Van Beneden, 1859
Tylenchus tritici (Steinbuch) Bastian, 1865
Anguillula scandens Schneider, 1866
Tylenchus scandens (Schneider) Cobb, 1890
Anguillulina scandens (Schneider) Goodey, 1932

Subanguina Paramonov, 1967
S. balochia Erum, Shahina & Siddiqi, 2008

Ditylenchus *Filipjev, 1936*
D. anchilisposomus (Tarjan, 1958) Fortuner, 1982

syn. *Pseudhalenchus anchilisposomus* Tarjan, 1958
Safianema anchilisposomus Tarjan, 1958

D. angustus (Butler, 913) Filipjev, 1936
syn. *Tylenchus angustus* Butler, 1913
Anguillulina angusta (Butler, 1913) Goodey, 1932

D. bilqueese Khan, Batool & Khan, 2004

D. clarus Thorne & Malek, 1968

D. destructor Thorne, 1945

D. dipsaci (Khun, 1857) Filipjev, 1936
syn. *Anguillula dipsaci* Kuhn, 1857
Anguillulina dipsaci (Kuhn, 1857) Gervais Van Beneden, 1859
Tylenchus dipsaci (Kuhn, 1857) Bastian, 1965
Anguillulina dipsaci var. *allocates* Steiner, 1934
D. allocates (Steiner, 1934) Filipjev & Schuurmans Stekhoven, 1941
Anguillulina dispsaci var. *communis* Steiner &Scott, 1935
D. dipsaci var. narcissi de Bruyn Ouboter, 1930
Anguillula devastatrix Kuhn, 1869
Tylenchus devastatrix (Kuhn, 1869) Orley, 1880
Anguillulina devastatrix (Kuhn, 1869) Neveu-Lemaire, 1913
D. devastatrix (Kuhn, 1969) tarjan, 1960
Anguillula secalis Nitschke, 1869
Anguillulina secalis (Nitschke, 1869) Goodey, 1932
D. secalis (Nitsxhke, 1869) Siddiqi, 1986
Tylenchus allii Beijerinck, 1883
D. allii (Beijerinck, 1883) Tarjan, 1960
Tylenchus havensteinii Kuhn, 1881
Anguillulina hyacinthi (Prillieux, 1881) Goodey, 1932
D. havensteinii (Kuhn, 1881) Siddiqi, 1986
Tylenchus hyacinthi Prillieux, 1881
Anguillulina hyacinthi (Prillieux, 1881) Goodey, 1932
D. dipsaci var. *hyacinthi* (Prillieux, 1881) Fillipjev &
Schuurmans Stekhoven, 1941
Tylenchus puterfaciens Kuhn, 1879
D. putrefaciens (Kuhn, 1879) Tarjan, 1967
Tylenchus tobaensis Schneider, 1937
D. tobaensis (Schneider, 1937) Kirjanova, 1951
D. phloxidis Kirjanova, 1951

D. fragariae Kirjanova, 1951
D. galeopsidis Paramonov, 1970
D. sonchophilus Paramonov, 1970
D. dipsaci falcariae Poghosyan, 1967
D. emus Khan, Chawla &Prasad, 1969
D. geraerti (Paramonov, 1970) Bello & Geraert, 1972
syn. (*Tylenchus geraerti*) Paramonov, 1970
D. kheirii Fortuner & Maggenti, 1987
syn. *Nothotylenchus geraerti* Kheiri, 1971
D. medicaginis Wasilewska, 1965
D. myceliophagus Goodey, 1958

Nothotylenchinae Thorne, 1941
Nothotylenchus Thorne, 1941
N. basiri Khan, 1965
N. goldeni Maqbool, 1982

Paurodontidae (Thorne, 1941) Massey, 1967
Paurodontinae Thorne, 1941
Paurodontus Thorne, 1941
P. similis Siddiqi, 1961

Paurodontella Husain & Khan, 1968
P. balochistanica Handoo, Erum, Nasira & Shahina, 2010
P. myceliophaga Handoo, Erum, Nasira & Shahina, 2010
P. sohailai Maqbool, 1982

StictylusThorne, 1941
Stictylus spp.

Aphelenchida Siddiqi, 1980
Aphelenchina (Fuchs, 1937) Geraert, 1966
Aphelenchoidea (Fuchs, 1937) Thorne, 1949
Aphelenchidae (Fuchs, 1937) Steiner, 1949
Aphelenchinae (Fuchs, 1937) Schuurmans Stekhoven & Teunissen, 1938
AphelenchusBastian, 1865
A. avenae Bastian, 1865
A. eremitus Thorne, 1961
A. isomerus Anderson & Hooper, 1980
A. sacchari (Akhtar, 1962) Goodey & Hooper, 1965
syn. *Metaphelenchus sacchari* Akhtar, 1962

Aphelenchoidoidea Skarbilovich, 1947 (Siddiqi, 1980)
Aphelenchoididae (Skarbilovich, 1947) Paramonov, 1953
Aphelenchoidinae Skarbilovich, 1947
Aphelenchoides Fischer, 1894
A. acacia Khan, Tabassum, Nasira, Javed & Shahina
A. aligarhiensis Siddiqi, Husain & Khan, 1967
A.asterocaudatus Das, 1960
A. besseyi Christie, 1942
A. bicaudatus (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941
A. blastophthorus Franklin, 1952
A. caprifici (Gasparrini, 1864) Filipjev, 1934
A. dactylocercus Hooper, 1958
A. goodeyi Siddiqi & Franklin, 1967
A. helicosoma Maslen, 1979
*A. involutus*Minegawa, 1992
A. marwataensis Javed, Khan & Nasira
A. macrospica Golhasen *et al*, 2017
A. naurangiensis Samreen Khan, Tabassum Ara, Nasira Kazi, Salma Javed and Shahina Fayyaz
A. parietinus (Bastian, 1865) Steiner, 1932
syn. *Aphelenchus parietinus* Bastian, 1865
A. richardsoni Grewal, Siddiqi & Atkey, 1992
A. ritzemabosi (Schwartz, 1911) Steiner, 1932
syn. *Aphelenchus ritzemabosi* Schwartz, 1911
Tylenchus ribes Taylor, 1917
Aphelenchus ribes (Taylor) Goodey, 1923
Aphelenchoides ribes (Taylor) Goodey, 1933
Aphelenchus phyllophagus Stewart, 1921
A. rutgersi Hooper &Myers, 1971
A. sacchari Hooper, 1958
A. saprophilus Franklin, 1957
A. siddiqi Fortuner, 1970
A. turnipi Israr, Shahina & Nasira, 2017
A. unisexus Jain & Singh, 1984
A. winchesi Paesler, 1957

Parasitaphelenchidae Rhum, 1956 (Siddiqi, 1980)
Rhadinaphelenchinae Paramonov, 1962
Rhadinaphelenchus Goodey, 1960
R.cocophilus (Cobb, 1919) Goodey, 1960

Seinuridae (Hussain & Khan 1967) Baranovskaya, 1981
Seinurinae Hussain & Khan, 1967
Seinura Fuchs, 1931
S. nagini Husain & Khan, 1965
S. oostenbrinki Husain & Khan, 1967

Paraphelenchidae Goodey, 1951
Paraphelenchinae Goodey, 1951
Paraphelenchus Micoletzky, 1922
P. litoralis Akhtar, 1962
P. myceliopthorus Goodey, 1958
*P. sacchari*Husain & Khan, 1967
Paraphelenchus spp.

Ectaphelenchidae Paramonov, 1964
Ectaphelenchinae Paramonov, 1964
***Ectaphelenchoide*s** Banjard, 1984
E. poinari Aliramaji, 2014

Rhabditida Chitwood, 1933
Rhabditina Andrassy, 1974
Rhabditoidea Orley, 1880
Rhabditidae Orley, 1880
Rhabditinae Orley, 1880
Rhabditis Durjardin, 1845
R. karachiensis Khan, Seema, Khan, Qamar & Anwarullah, 1992
R. longicaudatus (Bastian, 1865) Osche, 1952
R. marious Maupas, 1899
R. producta (Schneider, 1866) Linstow, 1878
R. terricola Dujardin, 1845
Rhabditis spp.

Cuticularia Van der Linde, 1938
C. regenfussi (Sudhaus, 1980) Andrassy, 1983

Rhitis Andrassy, 1983
R. luci (Andrassy, 1982) Andrassy, 1983

OscheiusAndrassy, 1976
O. andrassi Tabassum &Shahina, 2008
O. citri Tabassum, Shahina, Nasira & Erum, 2016
syn. O. cobbi Tabassum, Shahina, Nasira & Erum, 2016
O. cynodonti Tabassum, Shahina, Nasira & Erum, 2016
O. esculentus Tabassum, Shahina, Nasira & Erum, 2016
O. punctata Tabassum, Shahina, Nasira & Erum, 2016
O. sacchari Tabassum, Shahina, Nasira & Erum, 2016
O. karachiensis Tabassum & Nasir, 2018

O. maqbooli Tabassum & Shahina, 2002
O. shamimi Tehseen & Nisa, 2006
O. siddiqi Tabassum & Shahina, 2010
syn. O. niazii Tabassum & Shahina, 2010

Metarhabditis Tehseen, Hussain, Tomer, Sluh & Jairajpuri, 2004
M. adenobia (Poiner, 1971) Sudhas, 2011
M. amasectae (Ali, Pervez, Andrabi, Sharma & Verma, 2011)
M. rainai (Carta & Osbrink, 2005) Sudhas, 2011

Pelideridae Andrassy, 1976
Pelodera Schneider, 1866
P. puntata (Cobb, 1914) Dougherty, 1955

Diploscapteridae Micoletzky, 1922
Diploscapter Cobb, 1913
D. cornata (Cobb, 1893) Cobb, 1913

Mesorhabditidae Andrassy, 1976
Bursillinae Andrassy, 2005
Bursilla Andrassy, 1976
B. monhystera Butscili, 1873

Mesorhabditinae Andrassy, 1976
Crustorhabditis Sudhaus, 1974
C. scanica (Allgen, 1949) Andrassy, 1983

Cruzema Artigas, 1927
C. brzeskii (Brzeski, 1989) Sudhaus & Hooper, 1994
C. tripartitum (Linstow, 1906) Zullini, 1982

Distolabrellus Anderson, 1983
D. veechi Anderson, 1983
D. pakistanensis Tabassum, Shahina, Firoza & Siddiqi, 2005

Mesorhabditis (Osche, 1952) Dougherty, 1953
M. cranganorensis (Khera, 1968) Sudhaus, 1976
M. minuta Bostrom, 1991
M. striatica Dassonville & Heyns, 1984

Teratorhabditis (Osche, 1952) Dougherty, 1955
T. andrassyi Tahseen & Jairajpuri, 1988

Peloderidae Andrassy, 1986
Pellioditinae Andrassy, 2005
Pelliodites (Dougherty, 1953) Timm, 1960
P. typica (Stefanchi, 1922) Dougherty, 1955

Dolichorhabditis Andrassy, 1983
D. dolichura (Schneider, 1866) Andrassy, 1983

Heterorhabditidae Pionar, 1976
Heterorhabditis Pionar, 1976
H. bacteriophora Poinar, 1976
H. indica Pionar, Karunakar & David, 1992
H. pakistanense Shahina, Tabassum, Salma, Mehreen & Knoetze, 2016

Steinernematidae Chitwood & Chitwood, 1937
Steinernema Travassos, 1927
S. abbasi Elawad, Ahmad & Reid, 1997
S. affine (Bovien, 1937) Wouts, Mracek, Gerdin & Bedding, 1982
S. asiaticum Anis, Shahina, Reid, & Rowe, 2002
S. balochiense Shahina, Tabassum, Shaukat, Sarwar, Mehreen & Salma, 2015
S. bifurcatum Shahina, Yan, Qui, Han, Mehreen, Tabassum & Salma, 2014
S. carpocapsae (Weiser, 1955) Wouts, Mracek, Gerdin & Bedding, 1982
S. cholashanense Nguyen, Puza & Mracek, 2008
S. feltiae (Filipjev, 1934) Wouts, Mracek, Gerdin & Bedding, 1982
S. litorale Yoshida, 2004
S. maqbooli Shahina, Tabassum, Mehreen & Salma, 2013
S. pakistanense Shahina, Anis, Reid, Rowe & Maqbool, 2001
S. siamkayai Stock, Somsook & Reid, 1998

Diplogasterina Micoletzky, 1922
Diplogesteroidea Micoletzky, 1922
Diplogesteroidae Filipjev, Schuurmans Stekhoven, 1941
Diplogasteroidinae Filipjev, Schuurmans Stekhoven, 1941

Rhabditiodoides Rahim, 1928
Rhabditidoides Rahim, 1928
Rhabditidoides stigmatus (Steiner, 1930) Andrassy, 1984

Demaniella Steriner, 1914
D. basili Pilla and Taylor, 1968

Cephalobina Andrassy, 1974
Cephaloboidea Filipjev, 1934
Cephalobidae Filipjev, 1934
Cephalobinae Filipjev, 1934
Cephalobus Bastian, 1865
syn. *Paracephalobus*Akhter, 1962
C. litoralis (Akhter, 1962) Andrassy, 1984
syn. *P. litoralis* Akhter, 1962
C. nanus de Man, 1880
C. persignis Bastian, 1865
C. sacchari Shahina & Tabassum, 2002

Acrobeles Von Linstow, 1877
A. complaxus Thorne, 1925
A. geraerti Rashid, Heyns & Coomans, 1990
A. mariannae Andrassy, 1968
A. ornatus Thorne, 1925

Acrobeloides Cobb, 1924
A. bodenhemeri (Steiner, 1936) Thorne, 1937
A. enoploides Loof, 1971
A. gossypii Ashfaque, Shahina & Nasira, 2019
A. saeedi Siddiqi, Ley & Khan, 1992

Eucephalobus Steiner, 1935
Eucephalobus aculocaudatus Bostrom and Holovacham, 2011
Eucephalobus spp.

Chiloplacus Thorne, 1937
*C. longiuterus*Rashid & Heyns, 1990
Chiloplacus spp.

Pseudoacrobeles Steiner, 1928
Pseudoacrobeles spp.

Cervidellus Thorne, 1937
Cervidellus spp.

Acrobelinae
Zeldia Thorne, 1937
Z. punctata (Thorne, 1925) Thorne, 1937

Osstellidae Heyns, 1962

Drilocephalobinae Ali, Suryawanshi & Chisty, 1973
Drilocephalobus Coomans & Goodey, 1965
D. alykhani Siddiqi, 2001

Panagrolaimoidea Thorne, 1937
Panagrolaimidae Thorne, 1937
Panagrolaiminae Thorne, 1937
Panagrolaimus Fuchs, 1937
P. hygrophilus Bassen, 1940
P. rigidis (Schneider, 1866) Thorne, 1937
Panagrolaimus spp.

Tricephalobus Steiner, 1936
T. longicaudatus (Butschli, 1873) Steiner, 1936

Propanagrolaimus Andrassy, 2005
P. hygrophilus (Bassen, 1940) Andrassy, 2005

Brevibuccinae Paramonov, 1956
Brevibucca Goodey, 1935
B. saprophaga Goodey, 1935

Chambersiellidae Thorne, 1937
Macrolaiminae Sanwal, 1971
Macrolaimus Maupas, 1900
Macrolaimus spp.

Alloionematidae Chitwood & Mcintosh, 1934
Rhabditophanes Fuchs, 1930
R. brassicae Fuchs, 1930

Diplogasterida Maggenti, 1981
Diplogasterina Micoletzky, 1922
Diplogasteroidea Micoletzky, 1922
Diplogasteridae Micoletzky, 1922
Diplogaster Schultze in Carus, 1857
Diplogaster spp.

Butlerius Goodey, 1929
B. micans Pillar and Taylor, 1968

Demaniella Steiner, 1914
D. cibourgensis Steiner, 1914

Neodiplogasteridae Paramonov, 1952
Neodiplogasterinae Paramonov, 1952
Neodiplogaster Cobb, 1924

Neodiplogaster spp.

***Mononchoides* Rahm, 1928**
M. andrassyi Timm, 1961
M. change Goodrich *et al*., 1968

Adenophorea von Kinstow, 1905
syn. Aphasmidia Chitwood & Chitwood, 1933
Enoplia Pearse, 1942

Dorylaimida Pearse, 1942
Dorylaimina Pearse, 1936
Dorylaimoidea (de Man, 1876) Thorne, 1934
Longidoridae (Thorne, 1935) Meyl, 1961
Xiphinematinae Dalmasso, 1969
Xiphinema Cobb, 1913
X. americanum Cobb, 1913
syn. *Tylencholaimus americanus* (Cobb, 1913) Micoletzky, 1922
X. neoamericanum Saxena, Chabra & Joshi, 1973
X. basiri Siddiqi, 1959
syn. *X. cobbi* Sharma & Saxena, 1981
X.hayati Javed, 1983
X. bergeri Luc, 1973
X. bolandium Coomans & Heyns, 1985
X. brevicolle Lordello & Da Costa, 1961
syn. *X. americanum* apud Carvalho, 1955, 1962
X.saopaoloense Khan & Ahmad, 1975
X. californicum Lamberti & Bleve- Zacheo, 1979
X. chambersi Thorne, 1939
X. cynodontis Nasira & Maqbool, 1994
X. diversicaudatum (Micoletzky, 1927) Thorne, 1939
X. diffusum Lamberti and Bleve-Zacheo, 1979
X. elongatum Schuurmans Stekhoven & Teunissen, 1938
syn. *X. campinense* Lordello, 1951
X. hydrabadense Qureshi & Das, 1984
X.nagarjunense Khan, 1982
X. pretense Loos, 1949
X. uasi Edward & Sharma, 1982
X. incognatum Lamberti & Bleve-Zacheo, 1979
X. index Thorne & Allen, 1950
X. insigne Loos, 1949
syn. *X. indicum* Siddiqi, 1959
X. neodimorphicaudatum Khan, 1982
X. tugewai Darekar & Khan, 1983

X. intermedium Lamberti & Bleve-Zacheo, 1979
X. karachiense Nasira, Firoza & Maqbool, 1992
X. machoni Hunt, 1980
X. oxycaudatum Lamberti & Bleve-Zacheo, 1979
X. pachtaicum (Tulaganov, 1938) Kirjanova, 1951
syn. *X. mediterraneum* Martelli & Lamberti, 1967
X. neoelongatum Bajaj & Jairajpuri, 1077
X. pachydermum Sturhan, 1983
X. pakistanensis Nasira & Maqbool, 1998
X. radicicola Goodey, 1936
syn. *X. australiae* Mcleod & Khair, 1971
X. pararadicicola Phukan & Sanwal, 1982
X. rivesi Dalmasso, 1969
X. rotundatum Schuurmans Stekhoven & Teunissen, 1938
X. thornei Lamberti & Golden, 1986

Longidorinae Thorne, 1935
Longidorus (Micoletzky, 1922) Filipjev, 1934
L. africanus Merny, 1966
L. elongatus (de Man, 1876) Thorne & Swanger, 1936
syn. *Dorylaimuselongatus* de Man, 1876
D. (*Longidorus*) *elongates* (de Man, 1876) Micoletzky, 1922
Trichodoruselongatus (de Man, 1876) Filipjev, 1921
D. tenuis Linstow, 1879
L. pisi Edward, Misra & Singh, 1964
syn. *Brevinema pisi* (Edward, Misra & Singh, 1964) Stegarescu, 1980
L. siddiqi Aboul Eid, 1970
Xiphinema brevicaudaturn Siddiqi, 1959
nec. Schuurmans Stekhoven, 1951
L. lectocephalus Lambarti, Choleva & Agostinelli, 1983
L. trapezoides Nasira & Maqbool, 1995

Paralongidorus Siddiqi, Hooper & Khan, 1963
P. beryllus Siddiqi & Husain, 1965
P. citri (Siddiqi, 1959) Siddiqi, Hooper & Khan, 1963
syn. *Xiphinemacitri* Siddiqi, 1959
L. citri (Siddiqi, 1959) Thorne, 1961
P. lemoni Nasira, Shahina, Firoza & Maqbool, 1993
P. major Verma, 1973

P. xiphinemoides Heyns, 1965

Dorylaimidae de Man, 1876
Dorylaiminae de Man, 1876
Dorylaimus Dujardin, 1845
D. bastiani Butschli, 1873
D. biroi Daday, 1899
D. granuliferus Cobb, 1893
D. stagnalis Dujardin, 1845
D. subsimilis Cobb, 1893

Afrodorylaiminae Andrassy, 1969
Paradorylaimus Andrassy, 1969
P. dorsocaudali Nasira, Israr & Shahina, 2017

Laimydorinae Andrassy, 1969
Mesodorylaimus Andrassy, 1959
M. aberrans Loof, 1969
M. bastiani (Butschli, 1873) Anrassy, 1959
M. clavicaudatus (Thorne & Swanger, 1936) Andrassy, 1959
M. mehraniensis Khan & Saeed, 1986

Prodorylaimus Andrassy, 1959
Prodorylaimus spp.

Laimydorus Siddiqi, 1969
L. pseudostagnalis (Micoletzky, 1927) Siddiqi, 1969
L.wirisi Tabassum, Shahina & Nasira, 2006

Thornenematinae Siddiqi, 1969
Timminema Khan, 1977
T. pakistanicum Khan, 1977

Thornenema Andrassy, 1959
T. maruitianum (Williams,1959) Baqri & Jairajpuri,1969

Aporcelaimidae Heyns, 1965
Aporcelaiminae Heyns, 1965
Aprocelaimellus Heyns, 1965
A. goldeni Khan & Fatima, 1980
A. obscurus (Thorne & Swanger, 1936) Heyns, 1965
A. obtusicaudatus (Bastian, 1865) Altherr, 1960
A. sacchari Khan, 1989
A. taylori Yeates, 1967

Aprocelaimus Thorne & Swanger, 1936
A. cocophilus Loos, 1949
A. digitalis Loos, 1949
A. spiralis (Cobb, 1913) Thorne & Swanger, 1936

Qudsianematidae Jairajpuri, 1965
Qudsianematinae Jairajpuri, 1965
Labronema Thorne, 1939
L. digitatum Sakul, Das & Mitra, 1975
L. mauritiense Williams, 1959

Eudorylaimus Andrassy, 1959
E. andersoni Khan, 1989
E. magestri Andrassy, 1986
E. major
E. minusculus (Loos, 1946) Siddiqi, 1969
E. subjunctus Loof, 1971
E. varians Thorne, 1974
Eudorylaimus spp.

Thonus Thorne, 1974
T. saccatus Thorne, 1974

Ecumenicus Thorne, 1974
E. monohystera (de Man, 1880) Thorne, 1974

Allodorylaimus Andrassy, 1986
A. americanus Andrassy, 1986

Microdorylaimus Andrassy, 1986
M. miser (Thorne & Swanger, 1936) Andrassy, 1986

Talanema Andrassy, 1991
T. mauritiense (Williams, 1959) Andrassy, 1990

Thorniinae De Coninck, 1965
Nygolaimoides Meyl, 1960
Nygolaimoides spp.

Chrysonematinae Siddiqi, 1969
Chrysonema Thorne, 1929
C. mauritiana Williams, 1959

Discolaiminae Siddiqi, 1969
Discolaimium Thorne, 1939
D. australe (Yeates, 1967) Andrassy, 1990
D. brachyurum Husain & Siddiqi, 1967

D. capitulum Shahina, Mussarat & Siddiqi, 2005
D. gracile Thorne, 1939
D. pakistanicum Timm, 1963
D. upum Baqri & Jairajpuri, 1968

Discolaimoides Heyns, 1963
D. spatilabum Khan & Laha, 1982

Discolaimus Cobb, 1913
D. acuticapitatus Furstenberg & Heyns, 1966
D. bulbiferus (Cobb, 1906) Thorne & Swanger, 1936
D. lahorensis Khan, 1998
D. major Thorne, 1939
D. miniodontii Erum, Nasira, Sagir & Firoza, 2021
D. pakistanense Nasira, Shahina & Erum, 2008
D. similus Thorne, 1939
D. texanus Cobb, 1913

Latocephalus Patil & Khan, 1982
L. smithi (Heyns, 1963) Patil & Khan, 1982

Lordellonematinae Siddiqi, 1969
Lordellonema Andrassy, 1960
L. subannulatum Tabassum, Shahina & Siddiqi, 2005

Moshajia Siddiqi, 1982
M.qasmi Zarina & Shahina, 2007
M. teres Tabassum, Shahina & Siddiqi, 2005

Poronemella Siddiqi, 1969
P. divulgata Tabassum, Shahina & Siddiqi, 2005

Nordiidae Jairajpuri & Siddiqi, 1964
Nordiinae Jairajpuri & Siddiqi, 1964
Longidorella Thorne, 1939
L. (*Kantbhala*) *allii* Suryawanshi, 1971
L. (*Saevadorella*) *tharensis* Nasira, Shahina & Firoza, 2010
Longidorella spp.

Actinolaimoidinae Jairajpuri & Ahmad, 1992
Paroriverutus Carbonell & Coomans, 1982
P. timmi Khan, 1994

Pungentinae Siddiqi, 1969
Pungentus Thorne & Swanger, 1936
P. engadinensis Alterr, 1950

Pungentus spp.

Enchodelus Thorne, 1939
E. arcuatus Thorne, 1939
E. macrodorus (de Man, 1880) Thorne, 1939

Heterodorus Altherr, 1952
H. longidens (Jairajpuri& Loof, 1968) Andrassy, 2009

Actinolaimoidea Thorne, 1939
Carcharolaimidae Thorne, 1967
Carcharolaiminae Thorne, 1967
Carcharolaimus Thorne, 1939
Carcharolaimus spp.

Belondiroidea Thorne, 1939
Belondiridae Thorne, 1939
Belondirinae Thorne, 1939
Belondira Thorne, 1939
B. paraclava Jairajpuri, 1964
Belondira spp.

Amphibelondira Rahman, Jairajpuri, Ahmad & Ahmad, 1987
A. sindhicus Ashfaque, Nasira & Shahina, 2019

Axonchium Cobb, 1920
A. gigas Thorne, 1939
A. indicum Siddiqi, 1964
A. mauritiense Williams, 1958
A. oostenbrinki Khan & Ahmad, 1970

Dorylaimellinae Jairajpuri, 1964
Dorylaimellus Cobb, 1913
D. clavicaudatus Williams, 1958
D. vexator Heyns, 1963

Axodorylaimellus Siddiqi, 1983
A. aghai Siddiqi, 1983

Elongidorylaimellus Siddiqi, 1983
E. lauceolatus Siddiqi, 1983

Jamilius Siddiqi, 1983
J. adenophorus Siddiqi, 1983
J. jamali Siddiqi, 1983

Sindellus Siddiqi, 1983
S. sindi Siddiqi, 1983

RashidanemaSiddiqi, 1983
R. cognatum (Siddiqi, 1983) Jairajpuri & Ahmad, 1992

Tylencholaimoidea Filipjev, 1934
Tylencholaimidae Filipjev, 1934
Tylencholaiminae Filipjev, 1934
Tylencholaimus de Man, 1876
T. cynodonti Nasira, Erum & Shahina, 2005
T. gertii Kruger, 1965
T. nagauriensis Baqri & Bohra, 2001
T. proximus Thorne, 1939
Tylencholaimus spp.

Discomyctus Thorne, 1939
Discomyctus spp.

Mydonomidae Thorne, 1964
Dorylaimoides Thorne & Swanger, 1936
D. (*Acridorylaimoides*) *arcuatus* Siddiqi, 1964
D. (*Digidorylaimoides*) *pakistanensis* Siddiqi, 1964
D. (*Dorylaimoides*) *parateres* Siddiqi, 1964

Xiphinemellinae Jairajpuri, 1964
Xiphinemella Loos, 1950
X. ornata (Loos, 1949) Loos, 1950

Leptonchidae Thorne, 1935
Leptonchinae Thorne, 1935
Leptonchus Cobb, 1920
L. granulosus Cobb, 1920
Leptonchus spp.

Belonenchinae Thorne, 1964
Basirotyleptus Jairajpuri, 1964
B. (*Basirotyleptus*) *basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992

Tylencholaimellinae Jairajpuri, 1964
Doryllium Cobb, 1920
D. minor Jairajpuri, 1963
Doryllium spp.

Nygolaimina Ahmad & Jairajpuri, 1979
Nygolaimoidea Thorne, 1935
Nygolaimidae Thorne, 1935
Nygolaiminae Thorne, 1935
Nygolaimus Cobb, 191 3
N. gigantus Shahina, Erum & Siddiqi, 2005
N. neominimus Shahina, Erum & Siddiqi, 2005
N. vulgaris Thorne, 1930

Laevides Heyns, 1968
L. hunderansis Erum, Nasira, Sagir, Firoza, 2021

Solididentinae Ahmad & Jairajpuri, 1982
Solididens Heyns, 1968
S. swatiensis Nasira, Shahina & Maqbool, 1999

Nygolaimellidae Clark, 1961
Nygolaimellinae Clark, 1961
Nygolaimellus Loos, 1949
N. abnormis Loos, 1949

Alaimina Clark, 1971
Alaimidae Micoletzky, 1922
Alaimusde Man, 1880
A. minor Cobb, 1893

Bathyodontina Coomans & Loof, 1970
Cryptonchidae Chitwood, 1937
Cryptonchus Cobb, 1930
Cryptonchus spp.

Triplonchida Cobb, 1920
Diphtherophorina Coomans & Loof, 1970
Diptherophoroidea Micoletzky, 1922
Diphtherophoridae Micoletzky, 1922
Diptherophora de Man, 1880
Diptherophora spp.

Trichodoroidea (Thorne, 1935) Siddqi, 1974
Trichodoridae (Thorne, 1935) Siddiqi, 1961
Trichodorus Cobb, 1913
T. obtusus Cobb, 1913
T. pakistanensis Siddiqi, 1962

Paratrichodorus Siddiqi, 1974
P. faisalabadensis Nasira & Maqbool, 1994
P. minor (Colbran, 1956) Siddiqi, 1974
syn. *Trichodorus minor* Colbran, 1956

P. christiei (Allen, 1957) Siddiqi, 1974
P. obesus (Rasjivin & Penton, 1975) Rodriguez & Bell, 1978
P. mirzai (Siddiqi, 1960) Siddiqi, 1974
syn. *Trichodorus mirzai* Siddiqi, 1960
T. musaambi Edward & Misra, 1970
P. psidii Nasira & Maqbool, 1994
P. renifer Siddiqi, 1974

Tobrilina Tsalolikhin, 1976
Prismatolaimoidea Miocoletzky, 1922
Prismatolaimidae Miocoletzky, 1922
Prismatolaimus de Man, 1980
P. intermedius (Butschli, 1873) de Man, 1980

Mononchida Jairajpuri, 1969
Mononchina Kirjanova & Krall, 1969
Mononchoidea Chitwood, 1937
Mononchidae Chitwood, 1937
Mononchinae Chitood, 1937
Mononchus Bastian, 1865
*M. aquaticus*Coetzee, 1968
M. pappilatus Bastian, 1865
Mononchus spp.

Prionchulinae Andrassy, 1976
Clarkus Jairajpuri, 1970
C. papillatus (Bastian, 1865) Jairajpuri, 1970

Mylonchulidae Jairajpuri, 1969
Mylonchulinae Jairajpuri, 1969
Mylonchulus (Cobb, 1916) Altherr, 1953
*M. amurus*Khan & Jairajpuri, 1979
M. brachyuris Butschli, 1873
M.contractus Jairajpuri, 1970
M. lacustis (Cobb in Cobb, 1915) Andrassy, 1958
M. minor (Cobb, 1893) Andrassy, 1958
M. nainitalensis Jairajpuri, 1970
M. paitensis Yeates, 1992
M. rosensis Khan, 1975
M. sigmaturus (Cobb, 1916) Altherr, 1953
Mylonchulus spp.

Bathyodontina Coomans &Loff, 1970
Mononchuloidea De coninck, 1965
Oionchus Cobb, 1913

Oionchus sindhicus Uzma, Shahnaz, Nasira, Erum & Saboohi, 2021
Oionchus paraobtusus Jairajpuri and Khan, 1982
Oionchus obtusus Cobb, 1913

Pakmylonchulus Khan & Saeed, 1987
P. amurus Khan & Saeed, 1987

Anatonchoidea Jairajpuri, 1969
Anatonchidae Jairajpuri, 1969
Anatonchinae Jairajpuri, 1969
Anatonchus (Cobb, 1916) de Coninck, 1939
A. valitangiensis Khan & Saeed, 1987

Miconchinae Andrassy, 1976
Miconchus Andrassy, 1958
M. dalhousiensis Jairajpuri, 1969

Enoplida Filipjev, 1929
Enoplina Chitwood & Chitwood, 1937
Thoracostomopsidae Filipjev, 1927
Enoplolaimus de Man, 1893
E. karachiensis Maqbool, Nasira & Turpeenniemi, 1999

Enchelidiidae Filipjev, 1918
Eurystomina Filipjev, 1921
*E. indica*Yoshimura, 1980
Eurystomina spp.

Pareurystomina Micoletzky, 1930
P.vaughtae Keppner, 1989

Bathyeurystomina Lambshead & Platt, 1979
B. minima Nasira, Shahina & Shamim, 2014

Belbolla Andrassy, 1973
B. longispiculata Nasira, Shahina & Shamim, 2014

Oncholaimidae Filipjev, 1921
Oncholaimus Dujardin, 1845
O. oxyuris Ditlevsen, 1911
O. paraoxyuris Salma, Nasira, Saima & Shahina, 2017

Dentolaimoides Khan, 1994
D. papillifer Khan, 1994

Metoncholaimus Filipjev, 1918
M. medispiculatum Salma, Nasira, Saima & Shahina, 2017
M. siddiqii Shahina, Nasira & Shamim, 2015

Viscosia de Man, 1890
V. elegans Kreis, 1924

Oxystominidae (Filipjev, 1918) Chitwood, 935
Oxystomina Filipjev, 1921
O. elongata Butschli, 1874

Halalaimus de Man, 1888
H. gidanensis Nasira & Turpeenniemi, 2002

Anoplostomatidae Gerlach & Riemann, 1974
Anoplostoma Butschli, 1874
A. sunderbanae Timm, 1967

Pelagonematidae Andrassy, 1976
Pelogonema Cobb, 1894
Pelagonema spp.

Ironidae de Man, 1876
Ironus Bastian, 1865
I. dentifurcatus Agro & Heyns, 1972

Syringolaimus de Man, 1888
S. brevicaudatus Micoletzky, 1922

Thalassironusde Man, 1889
T. qatarense Nasira, Kamran, Shahina & Kazmi, 2006

Trissonchulus Cobb, 1920
T. benepapillosus Schulz, 1935
T. lichenii Nasira & Turpeenniemi, 2002
T. oceanus Cobb, 1920

Leptosomatidae Filipjev, 1916
Leptosomatum Bastian, 1865
L. ranjhai Timm, 1960

Tripyloidina de Coninck 1965
Tripyloididae Filipjev, 1918
Bathylaimus Cobb, 1894

B. australis Cobb, 1894

Trefusiida Lorenzen, 1981
Trefusiidae Gerlach, 1966
Trefusiade Man, 1893
Trefusia spp.

Chormadoria (Pearse, 1942) Filipjev, 1929
Chromadorida Filipjev, 1929
Chromadorina Filipjev, 1918
Microlaimidae Micoletzky, 1922
Microlaimus de Man, 1880
M. arenicola Schulz, 1938
M. amphidius Kamran, Nasira & Shahina, 2009
M. karachiensis Kamran, Nasira & Shahina, 2009
M. sonmianensis Nasira, Maqbool, Turpeenniemi & Zarina, 2000

Calomicrolaimus Lorenzen, 1976
C. arenarius Blome, 1982

Cinctonema Cobb, 1920
C. papillata Timm, 1962

Comesomatidae Filipjev, 1918
Paracomesoma Hope & Murphy, 1972
P. longispiculum Timm, 1961

Sabatieria Rouville, 1903
S. microsetosa Timm, 1967

Chromadoridae Filipjev, 1927
Hypodontolaimus de Man, 1886
Hypodontolaimus spp.

Graphonema Cobb, 1898
Graphonema spp.

Endeolophos Boucher, 1976
E. minutus Gerlach, 1967

Chromadora Bastian, 1865
C. nudicapitata Bastian, 1865

Spilophorella Filipjev, 1917
*S. candida*Gerlach, 1951

Ptycholaimellus Cobb, 1920
P. sindhicus Turpeenniemi, Nasira & Maqbool, 2001

Trichromadorita Timm, 1961
T. arinus Khan, 1991

Panduripharynx Timm, 1961
Panduripharynx spp.

Achromadoridae Gerlach & Riemann, 1973
Achromadorinae Gerlach & Riemann, 1973
Achromadora Cobb, 1913
A. ruricola (de Man, 1880) Micoletzky, 1925
Achromadora spp.

Cyatholaimidae Filipjev, 1918
Paracanthonchus Micoletzky, 1924
P. sandspitensis Nasira, Kamran & Shahina, 2007
P. hawaiiensis Allgen, 1951

Cyatholaimus Bastain, 1865
Cyatholaimus spp.

Marylynnia Hopper, 1977
M. musharafii Nasira, Kamran & Shahina, 2007

Selachinematidae Cobb, 1915
Choanolaimus de Man, 1880
Choanolaimus spp.

Halichoanolaimus de Man, 1886
H. balochiensis Turpeenniemi, Nasira & Maqbool, 2001

Synonchium Cobb, 1920
S. marina Kamran, Nasira & Shahina, 2009
S. oblongus Kamran, Nasira & Shahina, 2009
S. pakistanense Kamran, Nasira & Shahina, 2009

Desmodoridae Filipjev, 1922
Desmodora de Man, 1889
D. (*Pseudochromadora*) *cliftesnsis* Turpeenniemi, Nasira & Maqbool, 2001

Metachromadora Filipjev, 1918
M. (*Metachromadoroides*) *remanei* Gerlach, 1951

Spirinia Gerlach, 1963
S. (*Perspiria*) *striaticaudata* (Timm, 1962) Wieser & Hooper, 1967

Onyx Cobb, 1891
O. balochiensis Nasira, Rehmat & Shahina, 2011

Monoposthidae Filipjev, 1934
Monoposthia de Man, 1889
Monoposthia spp.

Ethmolaimidae Filipjev & Stekhoven, 1941
Paraethmolaimus Jensen, 1994
P. appendixocaudatus Jensen, 1994

Leptolaimina Lorenzen, 1981
Leptolaimidae Orley, 1880
Diodontolaimus Southern, 1914
D. karachiensis Nasira, Kamran & Shahina, 2005

Leptolaimus de Man, 1876
L. luridus Timm, 1963

Camacolaimusde Man, 1889
C. tardus de Man, 1889
Camacolaimus spp.

Ionema Cobb, 1920
I. cobbi Timm, 1963

Chronogaster Cobb, 1913
C. typica (de Man, 1921) de Coninck, 1937

Stephanolaimus Ditlevsen, 1914
Stephanolaimus spp.

Paraphanolaimus Micoletzky, 1923
P. granuliferus Timm, 1963

Halaphanolaimus Southern, 1914
H. marinus Kamran, Nasira & Shahina, 2010

Rhabdolaimidae Chitwood, 1961
Rogerinae Andrassy, 1976
Rogerus Hoeppli & Chu, 1934
syn. *Greenenema* Andrassy, 1959
Rogerus spp.

Aulolaimidae Jairajpuri & Hooper, 1968
Aulolaimus de Man, 1880

A. mubarakvilli Salma, Saima, Nasira & Shahina, 2018
A. rashidae Shahina, Hunt & Siddiqi, 1996

Heliplectidae Chitwood, 1951
Heliplectus Cobb, 1913
H. dorsalis Cobb in Chitwood, 1956
H. gracilis Shahina, Siddiqi & Nasira, 2014
H. minor Shahina, Siddiqi & Nasira, 2014
H. monodelphis Shahina, Siddiqi & Nasira, 2014
H. pakistanensis Shahina, Siddiqi & Nasira, 2014
H. paradorsalis Shahina, Siddiqi & Nasira, 2014
H. robustus Shahina, Siddiqi & Nasira, 2014
Haliplectus spp.

Aegialoalaimidae Lorenzen, 1981
Aegialoalaimus de Man, 1907
Aegialoalaimus spp.

Odontolaimidae Gerlach & Riemann, 1974
Odontolaimusde Man, 1880
Odotolaimus spp.

Monochromadorinae Andrassy, 1958
Monochromadora Goodey, 1951
Monochromadora spp.

Desmoscolecina Filipjev, 1934
Desmoscolecidae Shipley, 1896
Desmosoclex Claparede, 1863
Desmoscolex spp.

Triconema Cobb, 1893
Triconema spp.

Draconematidae Filipjev, 1918
Dracograllus Allen & Noffsinger, 1978
D. demanii Allen & Noffsinger, 1978

Monhysterida Filipjev, 1929
Monhysterina Chitwood & Chitwood, 1950
Monhysteridae de Man, 1876
Monhystera Bastian, 1865
M. karachiensis Timm, 1963
M. parelegantula de Coninck, 1943

Geomonhystera Andrassy, 1981
G. dubia Siddiqi & Shahina, 2004
G. karuni Siddiqi & Shahina, 2004

Monhystrella Cobb, 1918
Monhystrella spp.

Diplolaimella Allgen, 1929
D. dievengatensis Jacobs, Velde, Geraert & Vranken, 1990

Diplolaimelloides Meyl, 1954
D. delyi Andrassy, 1958

Xyalidae Chitwood, 1951
Arabanema Turpeenniemi, Nasira & Maqbool, 2001
A. pakistanensis Turpeenniemi, Nasira & Maqbool, 2001

Cobbia de Man, 1907
C. macrostoma Timm, 1963

Daptonema Cobb, 1920
Daptonema spp.

Megalolaimus Timm, 1961
Megalolaimus spp.

Paramonohystera Steiner, 1916
P. (*Leptogastrella*) *pellucida* Cobb, 1920
P. (*Paramonhystera*) *longicaudata* Timm, 1963

Rhynchonema Cobb, 1920
R. scutatum Lorenzen, 1971

Gonionchus Cobb, 1920
G. arabica Nasira & Turpeenniemi, 2003

Steineria Micoletzky, 1922
S. simplex Timm, 1963
S. pilosa brevisetosa Timm, 1957

Theristus Bastian, 1865
T. cylindricus Salma, Nasira, Saima & Shahina, 2017
T. flevensis Stekhoven, 1935
T. (*Penzancia*) *karachiense* Salma, Nasira, Saima & Shahina, 2017

T. longisetifer Kito & Aryuthaka, 1998
T. otoplanobius Gerlach, 1951
T. (*Cylindrotheristus*) *poloris* Cobb, 1914
T. (*Cylindrotheristus*) *normandicus* de Man, 1890

Thrichotheristus Weiser, 1956
T. floridanus Wieser & Hopper, 1967
Trichotheristus spp.

Sphaerolaimidae Filipjev, 1918
Sphaerolaimus Bastian, 1865
S. gracilis de Man, 1876
S. maeoticus Filipjev, 1922

Linhomoeidae Filipjev, 1922
Eleutherolaimus Filipjev, 1922
E. inglisi Timm, 1967
E. longus Filipjev, 1922
E. obtusicaudatus Allen, 1947

Terschellingia de Man, 1888
T. longicaudata de Man, 1907
T. communis de Man, 1888
T. lissa Timm, 1962
T. longissimicaudata Timm, 1962
T. magna Timm, 1962
syn. *T. communis* Gerlach, 1955
T. mora Gerlach, 1954

Metalinhomoeus de Man, 1907
M. karachiensis Timm, 1962

Megadesmolaimus Wieser, 1954
M. controtus Timm, 1962
Paralinhomoeus de Man, 1907
P. dubius Timm, 1961

Linhomoeus Bastian, 1865
syn. *Anticyclus* Cobb, 1920
Linhomoeus spp.

Axonolaimidae Filipjev, 1918
Pseudolella Cobb, 1920
P. granulifera Cobb, 1920

Odontophora Butschli, 1874
O. hawksbiensis Turpeenniemi, Nasira & Maqbool, 2001

Parodontophora Timm, 1963
P. cobbi Timm, 1963
P. pacifica (Allgen, 1947) Timm, 1963

Diplopeltidae Filipjev, 1918
Araeolaimus de Man, 1888
A. elegans de Man, 1888
A. taxianus Chitwood, 1951

Plectida Malakhov, 1982
Plectoidea Orley, 1880
Plectidae Orley, 1880
Plectinae Orley, 1880
Plectus Bastian, 1865
Plectus (*Ceratoplectus*) *armatus* (Butschli, 1873) Andrassy, 1984
P. karachiensis Shahina, Tabassum & Maqbool, 2001
P. perietinus Bastian, 1865
P. raabei Brzeski, 1961
P. tenuis Bastian, 1865
Plectus spp.

Willsonematinae Chitwood, 1951
Willsonema Cobb, 1913
W. promissum Khan, Seema & Khan, 1990

Characters of Major Groups from Class to Family

Characterization of order Tylenchida Thorne, 1949

Order Tylenchida contains a majority of the known plant parasitic forms. It is a monophyletic group. Members of Tylenchida share several common characters and features such as:

- Protrusible stylet (stomatostylet) through which fluid food is taken.
- A small pore-like anus in juveniles and females.
- Essentially a non-musculature oesophagus, except for the median oesophageal bulb.
- Orifice of the dorsal oesophageal gland located in the procorpus, usually at the base of the stylet.
- Outstretched gonoducts.
- Oviduct with two rows of cells.
- Paired spicules.
- Genital papillae, if present, located around the cloacal aperture.
- No male caudal papillae.

Order Tylenchida is divided into four suborders viz., Tylenchina, Hoplolaimina, Criconematina and Hexatylina.

1. Suborder Tylenchina Chitwood in Chitwood and Chitwood, 1950

Siddiqi (2000) proposed two infraorders under Tylenchina viz., Tylenchata and Anguinata on the basis of their separate origins and evolutionary lines. Tylenchata are algal and moss feeders and parasites of roots while Anguinata are fungal feeders and parasites of above ground plant parts.

Diagnosis: Tylenchida: Algal and moss feeders, fungal feeders (Anguinata) and parasites of lower and higher plants, not parasitic in animals. Phasmids absent. Cuticle smooth or distinctly annulated, sometimes marked with longitudinal striae. Cephalic region smooth or annulated. Amphidial aperture labial, sometimes just postlabial. Stylet generally small; knobs small and rounded. Procorpus cylindroids or fusiform; postcorpus or median bulb usually muscular with refractive thickenings. Oesophageal glands forming a basal bulb or rarely

extending over intestine. Oesophago-intestinal valve (cardia) three celled, reduced in the forms with overlapping glands or absent, as in Anguinata. Excretory pore in oesophageal region. Anus pore-like, outwardly directed. Tail similar in both sexes, elongate-tapering, filiform. Female reproductive system monodelphic, prodelphic, usually with a postvulval uterine sac. Vulva postmedian or more posterior. Ovary normally outstretched with serially arranged oocytes. Spermatheca round, elongate, lobed, axial or offset. Ovary normally outstretched. Testis single, outstretched. Spicules paired, cephalated, arcuate, distally round to pointed with sensort pore ventrally subterminal. Gubernaculum generally simple, fixed. Bursa simple or lobed, not enveloping entire tail.

i) Infraorder Tylenchata Siddiqi, 2000

Diagnosis: Tylenchina: Small nematodes. Lateral field with one to six incisures, rarely obscure. Cephalic framework poorly developed, rarely sclerotized. Amphidial aperture pore or slit like, extending along the lateral sides of cephalic region. Deirids present, near level of excretory pore. Phasmids absent. Prophasmids present. Stylet generally small and weak; basal knobs generally small, rounded, occasionally absent. Orifice of dorsal-oesophageal gland 1-4µm or more behind stylet knobs. Median bulb present or absent, muscular or non muscular, with or without inner refractive thickenings (valvate or non-valvate), smaller than basal bulb, not occupying entire body width cavity. Basal or terminal oesophageal basal enclosing oesophageal glands, occasionally only the dorsal gland may form a short lobe over the intestine. Tail similar in both sexes. Bursa adanal, simple or lobed. Vulva a transverse slit, usually postmedian. Ovary single, anteriorly outstretched with oocytes mostly in a row. Postvulval uterine sac shorter than body width or absent. Spicules paired, similar, cephalated, ventrally arcuate. Gubernaculum generally simple, trough-shaped, not protrusible. Algal and root feeders, not parasites of above-ground plant parts.

Only one superfamily Tylenchoidea Orley, 1880 (Chitwood & Chitwood, 1937) and four families. Type family is Tylenchidae Orley, 1880 and type genus is *Tylenchus* Bastian, 1865.

ii) Infraorder Anguinata Siddiqi, 2000

Diagnosis: Tylenchina: Small to large sized nematodes, in some genera adults may be obese. No marked sexual diamrphism in anterior region. Cuticle with fine striation, often appearing smooth. Lateral field plain, or with four to six or more incisures. Deirids usually present. Phasmids absent; prophasmids present in postmedian region outside lateral field, in female near vulva. Cephalic region low, cap-like, smooth with indistinct or no annulations, generally continuous with body contour. Amphids indistinct, oval, slit-like, slight dorso-lateral at some distance from oral opening. Stylet small with small rounded knobs. Orifice of dorsal-oesophageal gland close to stylet base. Median oesophageal bulb present or absent, with or without refractive thickenings. Oesophageal glands tend to be enlarged, forming a basal bulb, or rarely, the dorsal gland extends over intestine dorsally and laterally. Cardia absent. Gonads single, anteriorly outstretched may be reflexed or coiled in obese adults. Vulva a large transverse slit, posteriorly located. Spermatheca axial, elongated, sac-like. Sphincter valve between oviduct and uterus may be present, may serve as storage for sperms. Sperm round, large. Spicules robust, anteriorly expended, separate or fused medially, tip often truncate or broadly rounded. Gubernaculum simple, trough-shaped, not protrusible, rarely absent. Bursa moderately large, usually subterminal but may extend to terminus or be adanal. Tail similar in both sexes, usually elongate-conoid, may be cylindrical to filiform. Fungal feeders or parasites of lower and higher plants, attacking stems, leaves, floral parts and seeds, almost always inciting galls, also associates with insects but not parasitic in insects or other animals.

Only one superfamily Anguinoidea Scopoli, 1777 and two families. Type family is Anguinidae Nicoll, 1935 and type genus is *Anguina* Scopoli, 1777.

2. Suborder Hoplolaimina Chizhov & Berezina, 1988

Diagnosis: Order Tylenchida: Small to large nematodes. Sexual diamorphism in cephalic region present or absent. Cuticle with distinct outer and inner layers, strongly annulated. Lateral field with one to six

incisures, reducing towards extremities, occasionally reduced or absent. Cephalic framework well developed, strongly sclerotized. Labial disc distinct in several genera. Amphidial aperture pore or slit like, just below labial disc, rarely post labial. Deirids generally absent, or present. Phasmids present in or near tail region, small with pore-like apertures or large scutellum-like, always in lateral position. Prophasmids absent. Stylet usually well developed; basal knobs prominent (absent in Psilenchidae). Orifice of dorsal-oesophageal gland close to or at some distance from stylet base. Oesophageal glands free in body cavity or enclosed in a basal bulb. Median bulb well developed, muscular with inner refractive thickenings. Female reproductive system basically didelphic, amphidelphic; posterior branch may be reduced. Vulva a transverse slit, median or submedian; epiptygma present or absent. Spermatheca generally axial. Ovaries outstretched in opposite direction, reflexed or coiled in obese form. Tail dissimilar between sexes. Female tail generally short (less than two anal body widths) but may vary to become elongate-conoid or absent in some swollen females. Hypoptygma double. Bursa usually enveloping tail, subterminal, adanal or rarely absent. Testis single, anteriorly outstretched, Spicules paired, similar or rarely dissimilar, cephalated, straight to arcuate, independently protrusible. Gubernaculum simple, trough-shaped or modified rod-like, fixed or protrusible. Obligate parasites of plant roots. No mycetophagy or insect parasitism.

Two superfamilies *viz*., Hoplolaimoidea Filipjev, 1934 (Paramonov, 1967) and Dolichodoroidea Chitwood in Chitwood and Chitwood, 1950.

3. Suborder Criconematina Siddiqi, 1980

Diagnosis: Tylenchida: Exclusive root-parasites; males and some juveniles lack a stylet or degenerated one and cannot feed on roots. Marked sexual diamorphism in anterior region. Cuticle either thin and finely annulated or thick and coarsely annulated, in later case may have retrose annules, scales, spines or an extra cuticular body sheath. Lateral fields present or absent. Deirida absent. Phasmids absent. Female vermiform, sausage-shaped, or obese only in Tylenchuloidea as root ecto, rarely endoparasites. Cephalic region smooth or usually with one to

three coarse annules. Stylet long or short, but shaft always about 8-10µm long. Basal knobs well developed, large knobs may characteristically be anchor-shaped. Orifice of dorsal gland at about 3 µm or more from base of stylet. Oesophagus criconematoid, corpus enormously developed, broad cylindroids with muscular postcorpus amalgamated with precorpus, isthmus either slender and offset from basal bulb (Tylenchuloidea) or broad and amalgamated with it (Criconematoidea, Hemicycliophoroidea), basal bulb small, containing three oesophageal glands. Oesophago-intestinal valve (cardia) small, usually indistinct. Excretory pore oesophageal or post- oesophageal. Excretory system may produce gelatinous matrix in which eggs are deposited (e.g., *Tylenchulus*). Rectum obscure, short; anus a small round pore, rarely absent. Vulva transversely oval or slit-like, located posteriorly, generally at more than 85% of body from anterior end. Vagina anteriorly directed. Postvulval uterine sac absent. Spermatheca small, offset, ventral or ventro-lateral to the axis of the gonoduct. Male vermiform, oesophagus degenerate. Stylet also degenerate or lacking. Monorchic, gonoduct usually filled by minute round or amoeboid sperm. Testis usually obliterated in adult. Bursa weakly developed (except Hemicycliophoroidea), rarely enveloping tail tip (*Tylenchocriconema*), absent in several groups (Tylenchulidae, Sphaeronematidae, most Paratylenchidae). Spicules setaceous, often very long, straight, arcuate, U- or hook-shaped. Gubernaculum simple, linear or cresent-like in lateral view, fixed. Cloacal lips narrow, sometimes drawn out as penial tube. Hypoptygma (cloacal papilla) single, rarely double (*Tylenchocriconema*) or absent (*Tylenchulus*). Terrestrial, not marine in habitat. Females and most juvelines obligate root ectoparasites.

Three superfamilies viz., Criconematoidea Taylor, 1936 (type superfamily), Hemicycliophoroidea Skarbilovich, 1959 and Tylenchuloidea Skarbilovich, 1947 with altogether six families.

4. Suborder Hexatylina Siddiqi, 1980

Diagnosis: Tylenchida: Primarily entomoparasitic, mostly with free-living mycetophagous or plant parasitic (e.g., *Furgusobia*) generation. Female of several genera di-, tri- or tetramorphic according to feeding

habits. Entomoparasitic generation with only adult female parasitic in insect or mite haemocoel, other stages in host non-parasitic; obese adults occurring in arthropod haemocoel and in plant galls (*Furgusobia*). Cuticle smooth or finely annulated. Lateral fields present or absent. Deirids usually present. Phasmids and prophasmids not known. Cephalic region generally low, smooth or finely striated, no labial disc. Amphidial apertures dorso-sublateral pore or oblique slit-like. Stylet generally under 20µm long (hypertrophied in pre-adult insect-parasitic female), with or without basal knobs. Orifice of dorsal gland close to or at some distance behind stylet. Oesophagus in entomoparasitic form not divisible into corpus, isthmus and basal region. Oesophageal glands three but two reported in some genera, contained in a basal bulb or extending over intestine. A cellular cardia absent. Rectum may act as feeding pump (e.g., *Hexatylus*) in free-living stage. Anus pore-like, atrophied in saccate female.

Female: Monodelphic, prodelphic. Free-living female with a short slender stylet, oesophagus with corpus, isthmus and basal region and nerve ring encircling isthmus. Pre-adult entomoparasitic female with hypertrophied stylet (pseudostylet) and oesophagus, small vulva, elongated uterus serving as storage for sperms, in adult female often with several eggs and/or juveniles. Ovary, single, outstretched, reflexed at tip or coiled. Vulva a large transverse slit, oval or small pore-like, located posteriorly, usually at over 85% of body length.

Male: With or rarely without a stylet. Oesophagus as in free-living female, or rarely degenerated. Testis single, outstretched or with tip reflex. Functional or degenerated in adult. Spicules small (usually under 30 µm long), paired, arcuate, cephalated or in Iotonchidae large, robust and angular, never setaceous. Gubernaculum simple, fixed, may be lacking. Bursa simple, neither lobed nor with phasmidial pseudoribs, may be absent. Hypoptygma single, caudal papillae absent.

Two superfamilies *viz*., Sphaerularioidea Lubbock, 1861 (syn. Neotylenchoidea Thorne, 1941, type superfamily) and Iotonchioidea Goodey, 1953 with four families.

Characterization of order Aphelenchida Siddiqi, 1980

Several systems have been proposed for the classification of plant parasitic nematodes of the order Tylenchida by many great scientists. The majority of these classifications include the aphelenchs as a suborder. Siddiqi (1980) erected a new order Aphelenchida and placed them into separate order Aphelenchida. Siddiqi (1986, 2000) and Hunt (1993) followed the classification for aphelenchs as proposed by Siddiqi (1980).

- Aphelenchida is a moderately large order of nematodes. They are cosmopolitan.
- Aphelenchida have a stylet for feeding.
- Have a strongly developed median bulb in the oesophagus.
- They are associated with plant parts such as; root, stem and/or leaves and may or may not be pathogenic to the plants.
- Some are associated with insects, and may be ectoparasites or endoparasites, or merely use the insect as transport.
- Some others are associated with fungi, and some are free-living.
- There may be considerable plasticity of feeding habits within species, involving almost any combination of the categories listed above.
- Sometimes different feeding habits involve morphologically distinct phases, but they may involve only behavioral differences, and sometimes depend only on the immediate availability of different foods.
- Some lifecycles involve a definite progression of particular hosts and depend on the lifecycle of the host.
- Fungal feeders may have lifecycles as short as 5 days.

Diagnosis: Soil dwelling or insect associates, trophic habit mycetophagous, phytoparasitic, predacious or entomoparasitic. Small, vermiform, rarely obese except in some insect parasites. Cuticle thin, finely annulated. Amphidial aperture oval, pore-like, dorso-sublateral on labial region. Stylet always present; basal swellings or knobs usually weakly developed or entirely absent. Strongly developed median bulb

with crescentic valve plates. Well developed oesophageal glands forming a dorsally overlapping lobe in all genera except in *Paraphelenchus* where the glands are small and enclosed in a non-overlapping basal bulb. All three gland orifices (including the dorsal gland orifice) located within the median bulb. Anus a broad transverse slit with an overhanging anterior lip. Vulva posterior. Genital tract monoprodelphic, outstretched. Sperm large, rounded. Spicules rosethorne-shaped. Gubernaculum usually absent, but elongate and cephalated in *Aphelenchus* and *Paraphelenchus*. Bursa usually absent but present in *Aphelenchus* only. Usually three pairs of caudal papillae present.

Order Aphelenchida has only one suborder Aphelenchina Geraert, 1966

Suborder Aphelenchina Geraert, 1966

Diagnosis: Aphelenchida. Characteristics are same as that of the order.

Suborder Aphelenchina: 2 Superfamilies: Aphelenchoidea, Aphelenchoidoidea

I. Superfamily Aphelenchoidea: 2 Families: Aphelenchidae, Paraphelenchidae

1. **Family Aphelenchidae:** Genus= *Aphelenchus*
2. **Family Paraphelenchidae:** Genus= *Paraphelenchus*

II. Superfamily Aphelenchoidoidea: 6 Families:

1. Aphelenchoididae
2. Seinuridae
3. Acugutturidrae
4. Ektaphelenchidae
5. Entaphelenchidae
6. Parasitaphelenchidae

Family Aphelenchoididae: 2 Subfamilies: Aphelenchoidinae and Anomyctinae

Subfamily Aphelenchoidinae: Genus *Aphelenchoides*

Family Seinuridae: 1 Subfamily: Seinurinae

Subfamily Seinurinae: Genus *Seinura*

Family Parasitaphelenchhidae*:* 2 Subfamilies: Bursaphelenchinae and Parasitaphelenchinae

Subfamily Bursaphelenchinae: 2 Genera: *Bursaphelenchus* and *Rhadinaphelenchus*

Differential Characteristics of Families of Plant Parasitic Nematodes

Family Tylenchidae (Tylenchina: Tylenchoidea): Small vermiform nematodes, with annulated cuticle; stylet mostly short, rarely long; weak to moderate median bulb, with or without valve; oval to spheroid; oesophageal glands in a pyriform bulb-like swelling; female prodelphic, usually with short postvulval sac; phasmids absent, short adanal bursa; elongate conoid to filiform tails in both sexes. Terrestrial root feeders, inhabiting soil, moss, algae or rhizosphere of plants.

Family Anguinidae (Tylenchina: Tylenchoidea): Body slender; female obese in some genera; cuticle finely annulated or smooth; stylet mostly short or moderately long, basal knobs present; median bulb muscular or weak, with or without valve; oesophagus terminus ending in a bulb or lobe; female prodelphic, postuterine sac present, bursa adanal or longer, subterminal; tails in both sexes similar, mostly conoid, occasionaly filiform. They live in various terrestrial habitats, fungal feeders or plant parasitic, attacking above- ground parts of higher plants and often inciting galls.

Family Hoplolaimidae (Hoplolaimina: Hoplolaimoidea): Small to moderately large nematodes, cuticle well annulated; cephalic region high with sclerotized framework; often with sexual diamorphism; oesophagus with well developed muscular median bulb, posterior bulb overlapping intestine; female genital tract paired; phasmids either small, pore-like, near anus, or large, scutellum-like near anus or far precaudal in position;

not extending into male bursa; bursa large, enveloping tail (peloderan), tail short in both sexes, rounded or conical in females and only conical in male. Primarily they are ectoparasites, penetrate into root tissues and cause heavy crop losses.

Family Heteroderidae (Hoplolaimina: Hoplolaimoidea): A marked sexual diamorphism present; adult females strongly swollen, saccate or globular with short neck, and sedentary; males vermiform fairy robust; cuticle with strong annulations; modified to a lace-like or zig-zag pattern in swollen females; Labial disc rounded, cephalic framework strong; stylet well developed; median oesophageal bulb ovoid to rounded, oesophgeal glands lobe-like, extending over intestine ventrally; excretory pore generally behind median bulb in mature females; female gonads paired; eggs mostly retained in the female body that transforms into a hard-walled resistant cyst; bursa absent; tail conoid in second stage juvelines, mostly absent in females, very short and rounded or absent in males, posterior end of male predominantly twisted. They are root parasites, may also occur on underground stems or tubers on vascular plants.

Family Meloidogynidae (Hoplolaimina: Hoplolaimoidea): Well expressed sexual diamorphism present; mature females swollen, pear-shaped or spheroid with short or long neck, and sedentary; males vermiform; cuticle moderately thick, striated; cephalic region low, annulated, with weak sclerotization; stylet longer and robust in males than females, median oesophageal bulb oval or spherical; oesophgeal glands extending over intestine ventrally and laterally; excretory pore in mature females generally opposite to or anterior to median bulb; ovaries two, prodelphic, coiled; cuticle in vulval region generally with finger-print like perineal pattern; eggs deposited in gelatinous matrix, no cyst stage; bursa absent; male posterior end twisted, tail short or absent. They are very important plant pathogens, incite galls on the roots.

Family Rotylenchulidae (Hoplolaimina: Hoplolaimoidea): Small nematodes; with distinct dimorphism between females and males, mature female swollen, kidney-shaped, immature female and male vermiform; cuticle annulated; stylet in females well developed, in male weak; orifice

of dorsal oesophageal gland often far from stylet base; median bulb muscular, basal bulb with ventral or dorsal lobe; female amphidelphic, rarely prodelphic; bursa present, advulval or enveloping tail; phasmids pore-like, near anus; tail of mature female short, conoid or reduced in immature females and males. Adult females are sedentary ectoparasites of plant roots; eggs are laid in a gelatinous matrix on the roots.

Family Pratylenchidae (Hoplolaimina: Hoplolaimoidea): Small, vermiform nematodes, rarely swollen; some mature females are obese or spindle-shaped; several genera shows sexual dimorphism in anterior region; cuticle distinctly annulated; amphids pore-like, or dorso-ventral slits; labial frame strongly sclerotized; stylet well developed, short, basal knobs large; median bulb valvate, oesophageal gland lobe-like, extending over intestine; ovary paired; bursa terminal or subterminal; phasmids small, in males rib-like, extending into bursa. They are terrestrial, rarely aquatic, migratory phytonematodes; most species penetrate and feed on internal root tissues.

Family Psilenchidae (Hoplolaimina: Dolichodoroidea): Body small to middle sized; cuticle well annulated; lip region continuous; amphidial aperture a minute pore-like or a transverse slit; stylet moderate, with or without basal knobs; median bulb present, basal bulb offset; female gonads amphidelphic; bursa adanal; phasmids present on tail; tail elongate to filiform in both sexes.

Family Telotylenchidae (Hoplolaimina: Dolichodoroidea): Medium sized nematodes; cuticle well annulated; lateral field with three to six incisures. labial region annulated, framework moderately sclerotized; amphids pore-like; stylet of medium sized, knobs distinct; median oesophageal bulb muscular, valvate, oesophageal glands enclosed in a terminal bulb or extending over intestine; female genital organ paired; bursa enveloping tail; phasmids small, at male rib-like, female tail conoid, cylindroid or rounded, male tail conoid. They are root feeders, migratory phytonematodes, sometimes found inside plant tissues.

Family Criconematidae (Criconematina: Criconematoidea): Small nematodes; females and juveniles spindle shaped with thick cuticle and retrose annules, cuticle of females and juveniles may have out growths such as scales, spines or other elongations; cephalic region with indistinct labial disc; stylet well developed, knobs large and anchor-shaped; oesophageal procorpus broad, posteriorly expanded, bulb-like, isthmus short, broad and amalgamated with basal bulb; one prevulval ovary, vulva far posterior; no postvulval uterine sac, male without stylet and with reduced alimentary tract; spicules elongate, setose; male tail short; bursa small, rarely absent. They are ectoparasites of agricultural crops.

Family Tylenchulidae (Criconematina: Tylenchuloidea): Small body; sexual dimorphism present; female elongate, obese, ventrally curved, with distinct postvulval region, sedentary; male slender, with reduced stylet and oesophagus, migratory; cuticle in obese female thick, annulated; stylet short, knobs rounded; excretorty pore far posterior, behind mid-body; excretory glands highly developed and producing gelatinous matrix; female gonads prodelphic, rectum and anus indistinct; phasmids present; testis degenerate, bursa absent. They are obligate parasites of roots. The genus *Tylenchulus* is an important plant pathogen of citrus plantations.

Chapter 5

TAXONOMY OF IMPORTANT PLANT PARASITIC NEMATODE GENERA

Identification of the Major Genera

Taxonomy is the science of defining groups of biological organisms on the basis of shared characteristics and giving names to those groups. It includes the identification of specimens, publication of descriptive data and the study of diversity and relationships among organisms. Thus taxonomy is the systematic grouping of organisms according to their natural relationships. Identification involves naming of taxa, mostly at generic and species levels, using taxonomic characters. Accurate identification of nematodes is vital for assessment of their populations and effective management programmes. Rapid and accurate classification of the pathogens involved is a prerequisite to strategize appropriate control measures.

Meloidogyne Göldi, 1892
(Root-knot nematode)
Fig. 5.1

Parasitism and Habitat: Sedentary endoparasitic, embedded in root tissues of plants. All stages found in roots or soil. The genus *Meloidogyne* is characterized by the presence of characteristic pattern of striae on the cuticle, the perineal pattern around anus and vulva. Mature females lay eggs in a protective gelatinous matrix outside the body.

Main Morphological Characteristics: Root-knot nematodes (RKN) are sexually diamorphic and sedentary endoparasites. The mature females are white, swollen, round to pear shaped, with short projecting neck. Cuticle striated. Cephalic framework moderately sclerotized, hexaradiate. Stylet moderately strong, with well-developed basal knobs; procorpus cylindrical followed by spherical metacorpus with well-developed musculature and valve plates. Excretory pore anterior to median bulb and near to stylet knobs. Oesophageal glands overlap

intestine mostly ventrally. Genital tracts paired, elongated, anteriorly directed and coiled. Tail absent; anus and vulva terminal surrounded by characteristic pattern of striae on the cuticle (perineal pattern). The 2nd stage juvenile (J_2) is the infective stage. Stylet weak to moderately developed. Tail elongate-conoid with minutely rounded tip and conspicuous terminal hyaline portion. After invading a root, the nematode becomes sessile and initiates the development of specialized trophic cells to nurture the subsequent stages.

Mature female: Sedentary, white, swollen, globular or pear-shaped, cuticle thin, striated. Stylet slender with small basal knobs. Cuticle strongly annulated. Excretory pore anterior to median bulb; near the base of stylet. Stylet< 25µm long. Genital tracts paired, prodelphic, convoluted. Tail absent, anus and vulva terminal, surrounded by characteristic pattern of striae on the cuticle (perineal pattern). Eggs not retained in body but laid in a gelatinous matrix.

Male: Vermiform, migratory, over 1mm long. Stylet well developed, basal knobs prominent.Cephalic framework moderately sclerotized. Tail short, bluntly rounded; tail end twisted, Testis usually one but sometimes two. Spicules robust, paired, gubernaculum simple, bursa absent. Intersexes or sex reversal may occur, particularly in response to nutrient stress.

Juveniles: Second stage juveniles (J_2) slender, vermiform, migratory and infective. Stylet and cephalic region weakly sclerotized. Tail elongate, conoid with conspicuous terminal hyline portion. The 3rd and 4th stage juveniles occur within the roots, swollen, sedentary, with a blunt terminus and without stylet.

Perineal Pattern of *Meloidogyne* Species (Eisenback *et al*., 1981)

The cuticular markings surrounding the vulva and anus or perineal pattern of females of *Meloidogyne* spp. are used in their identification. Freshly dissected females are preferred as their body contents are more easily removed.

Young, egg-laying females dissected from gall roots are place into a drop of 45 % lactic acid in a plastic Petri dish, using fine pointed forceps. Posterior halves of the bodies are cut off with a scalpel. The posterior pieces of the cuticle having perineal patterns are further trimmed to a size slightly greater than the pattern. The inner tissues are removed completely and carefully by flexible bristle. Now the pieces of perineal patterns are transferred into a drop of glycerin on microscope slide and arrange the posterior ends, in one or two rows. Cover glass slip is placed gently and is sealed with zut or nail polish.

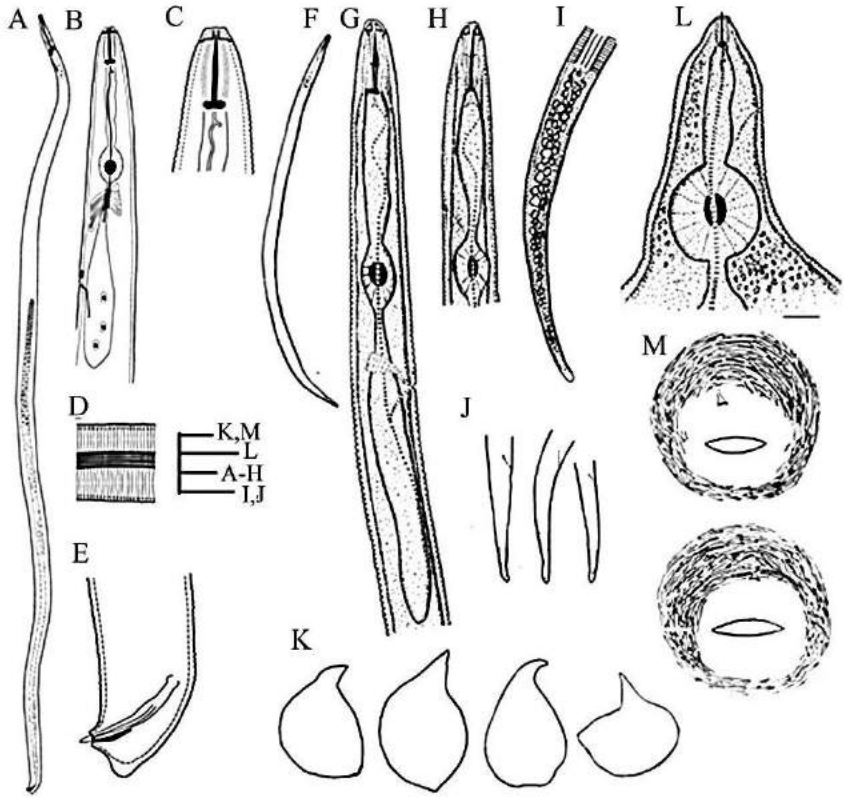


Fig. 5.1. *Meloidogyne* spp. A. Male whole body; B. Male anterior region; C. Male cephalic region; D. Lateral lines in male; E. Male tail; F. Juvenile whole body; G & H. Juvenile anterior region; I .& J. Juvenile tail; K. Body shapes; L. Female anterior end; M. Perineal pattern (from Ye Tao *et al*., 2017).

Heterodera Schmidt, 1871
(Cyst nematode)
Fig. 5.2

Parasitism and Habitat: Sedentary endoparasitic, their enlarged body protruding from the root's surface and their elongate anterior region embedded in the host root. Juveniles, males and cysts found in soil. The genus *Heterodera* is unique among other nematode genera because of the unusual ability of the female cuticle to transform into a tough, brown, cyst like sac, protecting the eggs which have been formed within the body.

Main Morphological Characteristics: Female: Sexually diamorphic. Cyst stage present. Body swollen, globose, lemon shaped, with short anterior neck and terminal posterior protrusion, the vulval cone turns into a hard-walled cyst, brown to black in colour withthick cuticle having zig-zag pattern of ridges. Vulva terminal, near anus on terminal cone; vulval slit of variable length; vulval lips not protruding. Vulval area of cyst ambi or bifenestrate, anal fenestration absent. Underbridge generally present; bullae present or absent. Eggs retained in body; in some cases egg-mass also present. Two genital tracts, convoluted.

Male: Vermiform, body often longitudinally twisted, variable in length.Lateral field with four (rarely three) lines. Stylet and cephalic region robust. Spicules >30µm, robust, slightly curved, directed obliquely, with distal extremity pointed or notched. No bursa. Tail very short, rounded or hemispherical.

Juvenile Stage: Juvenile (J_2) body straight to arcuate, slender; found in soil or readily hatching from cysts on plants. Stylet and cephalic region robust.Lateral field with four (rarely three) lines. Oesophageal glands filling body cavity.Tail conical, pointed; distinctive hyaline part variable, generally half tail length.Phasmids punctiform. Feeding induces a syncytium.

Wall Structure: Cuticular surface pattern and particularly those of the mid-body region, occur in diverse forms among cysts of Heteroderinae. These particular patterns may characterize specific taxa. These mid-body

patterns are broadly striated and zig-zag. Particular patterns may further be specified as fine or coarse, ridged, reticulate, lacelike or punctate.

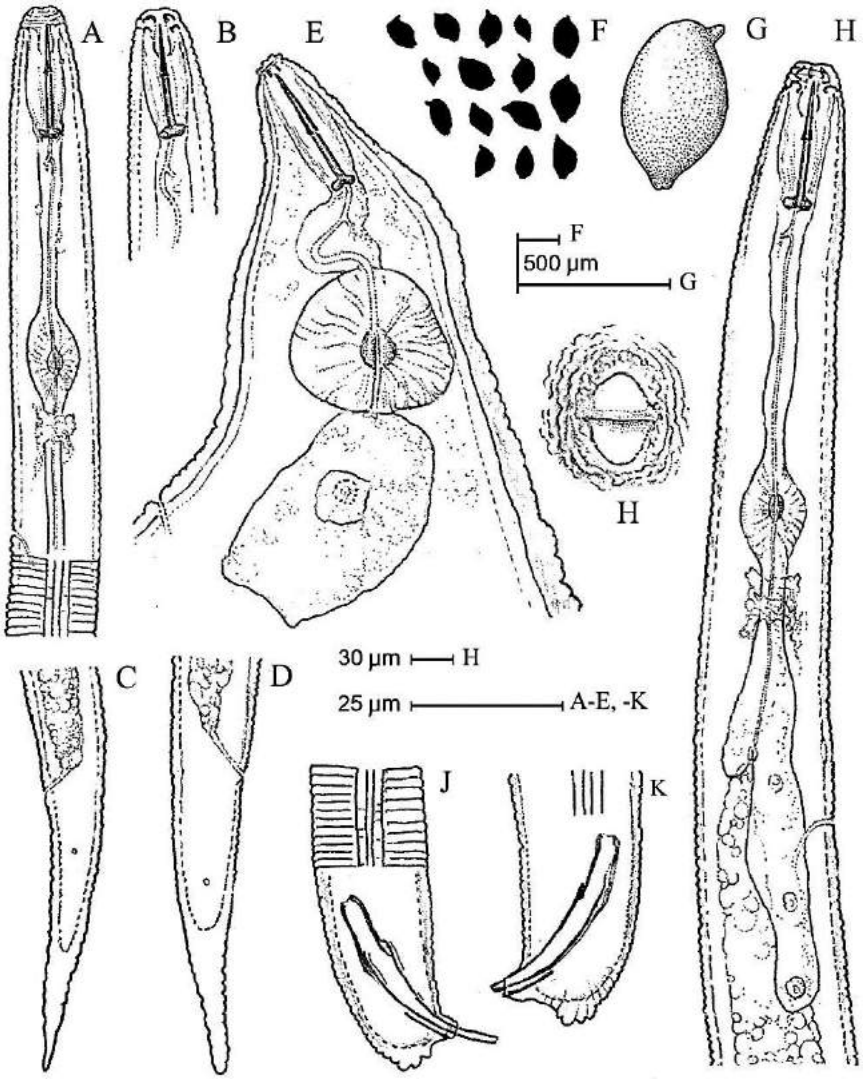


Fig. 5.2. *Heterodera* spp. A. Juvenile anterior end**;** B. Juvenile cephalic region; C & D. Juvenile tail; E. Female anterior end; F. & G. Female whole body; H. Vulval cone; I. Male Anterior end; J. & K. Male tail (From Fanelli *et al*., 2019).

Vulval Cone: Lemon shaped cysts (*Heterodera*, *Cactodera* and *Afenestrata*) have a protuberance or prominence at the posterior end commonly called the vulval cone. The size and shape of the cone is variable, ranging from prominent and rather narrow. The vulva is a slit located at the terminus of the cone, which is bordered by two vulval lips forming the vulval bridge. The bridge extends across the fenestra (the thin walled area of the cone terminus). The fenestra disintegrates and leaves a rounded opening, forming a circumfenestrate. The semifenestrate on each side of the vulval slit and bridge disintegrate, leaving two openings each of about a half circle or less, separated by a narrow to medium vulval bridge is ambifenestrate. The semifenestrate also disintegrate, leaving two openings each a full or more than half circle on each side of a strong, wide vulval bridge is bifenestrate. The fenestra is encircled by a band of cuticle which extends to the cuticular surface of the cyst is basin bullae.

Pratylenchus Filipjev, 1936
(Lesion nematode)
Fig. 5.3.

Parasitism and Habitat: Migratory endoparasitic, feeding in root cortex of many plant roots. All stages found in roots or soil.

Main Morphological Characters: Small nematodes; body length 0.4-0.8 mm in length. Cephalic region low, flattened, continuous with body contour, strongly sclerotized. Lateral field with four to six incisures. Stylet short, moderately sclerotized with rounded, anteriorly flattened or indented basal knobs. Median bulb well developed, oval to round, muscular. Oesophageal gland lobes overlapping intestine ventrally. Ovary one, prodelphic. Vulva in posterior region (70-80%). Postvulval uterine sac present. Spermatheca oval or round, usually filled with sperms in bisexual species. Female tail subcylindrical to conoid with a broad to narrowly rounded or truncate terminus, smooth or annulated. Phasmid pore-like, near middle of tail. Male tail short, dorsally convex-conoid, bursa extending to tail tip. Spicules slender, arcuate. Gubernaculum simple, trough-like and fixed.

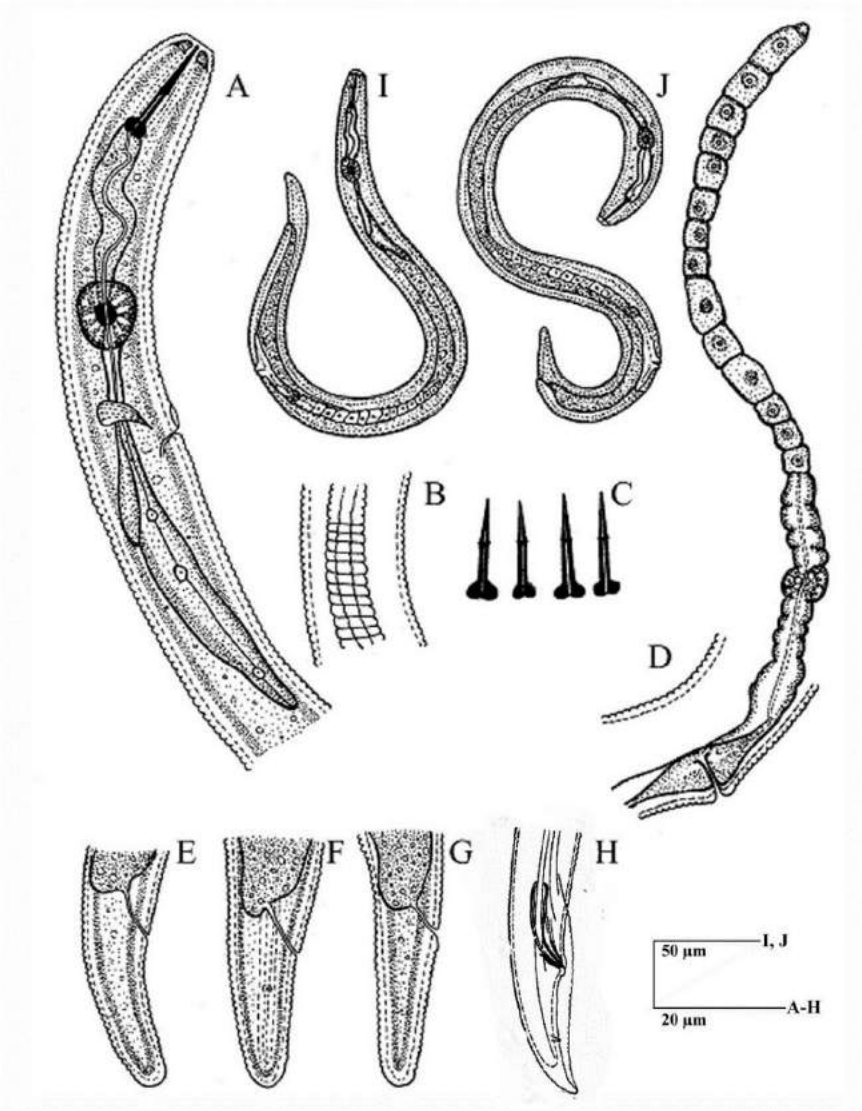


Fig. 5.3. *Pratylenchus* spp. A. Female anterior region; B. lateal lines; C. variation in stylet; D. Female genital branch; E - G. Female tail; H. Male tail; I. & J. Whole body (From NNRC).

Helicotylenchus Steiner, 1945
(Spiral nematode)
Fig. 5.4

Parasitism and Habitat: Ectoparasitic and endoparasitic on many plant roots; all stages found in soil and roots.

Main Morphological Characteristics: Small to medium sized; 04 to 1.2mm in length; female spirally coiled or rarely arcuate in shape when dead or relaxed. Cephalic region low or elevated, conoid-rounded, rarely truncate, continuous or rarely offset, with or without annulations, sclerotization moderate. Stylet robust, moderately long with rounded or cup-shaped knobs. Dorsal oesophageal gland orifice located one-fourth to a little more than one-half stylet length, behind base of stylet Oesophageal gland overlapping intestine ventrally. Ovaries two; didelphic, amphidelphic. Vulva posterior to middle of body. Phasmids small, pore-like near anal region. Female tail short, usually convex-conoid or hemispherical, with or without a ventral projection. Male tail short, conical, bursa enveloping entire tail tip. Spicules slender, arcuate, well developed. Gubernaculum trough or rod shaped. Bursa reaching tail tip.

Hoplolaimus Von Daday, 1905
(Lance nematode)
Fig. 5.5

Parasitism and Habitat: Migratory ectoparasitic on many plant roots. All stages found in soil.

Main Morphological Characteristics: Large sized, body length from 1.0 to 2.0 mm, slightly curved ventrally upon gentle heat. Cephalic region offset, annulated with longitudinal striations, rounded with sclerotized framework, labial disc distinct. Lateral field with two or four incisures. Stylet strongly developed with compact tulip-shaped basal knobs, having anterior tooth-like projections. Dorsal oesophageal gland orifice near the base of the stylet knobs. Oesophagus with well-developed median bulb; oesophageal glands large, overlapping anterior end of intestine dorsally, containing either three or six nuclei. Ovaries two; didelphic, amphidelphic; vulva centrally located. Epiptygma present, often indistinct. Phasmids large scutellum-like, not opposite each other, one

prevulval, another post vulval. Tail short in both sexes. Female tail hemispherical or rounded, annulated, tail conical in males. Spicules well developed, arcuate. Gubernaculum large, protrusible or fixed. Bursa large, enveloping tail.

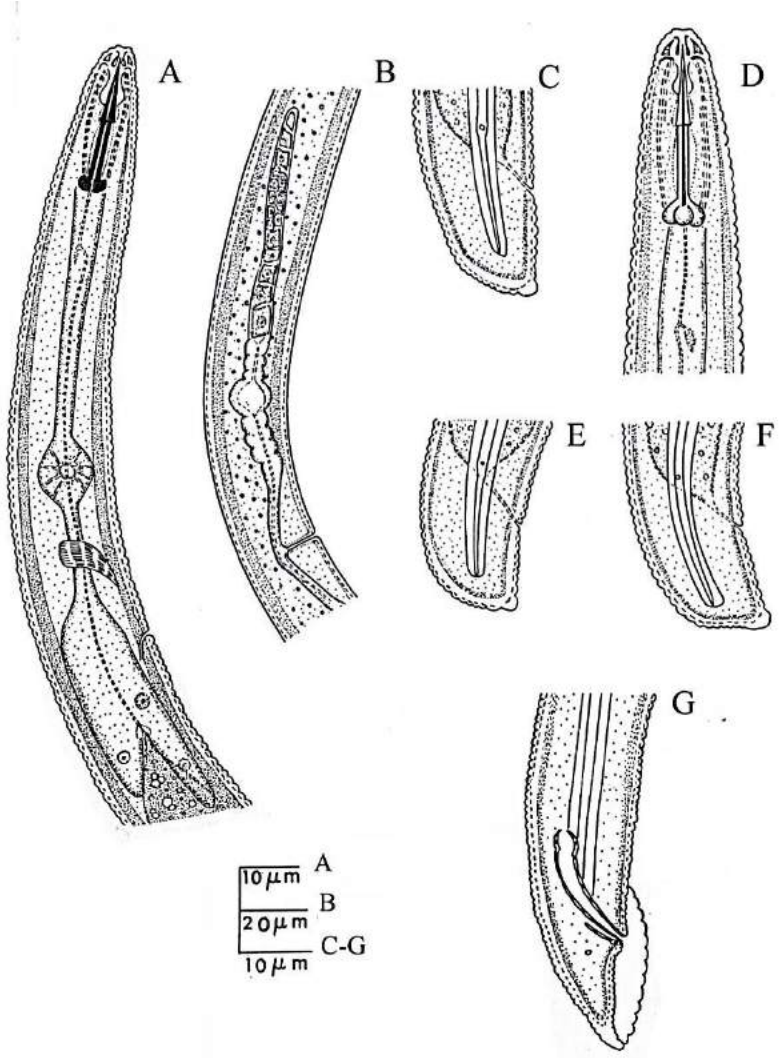


Fig. 5.4. *Helicotylenchus* spp. A. Female anterior region; B. Vulva and anterior genital branch; C, E, F. Female tail; D. Female head and stylet; G. Male tail (From NNRC).

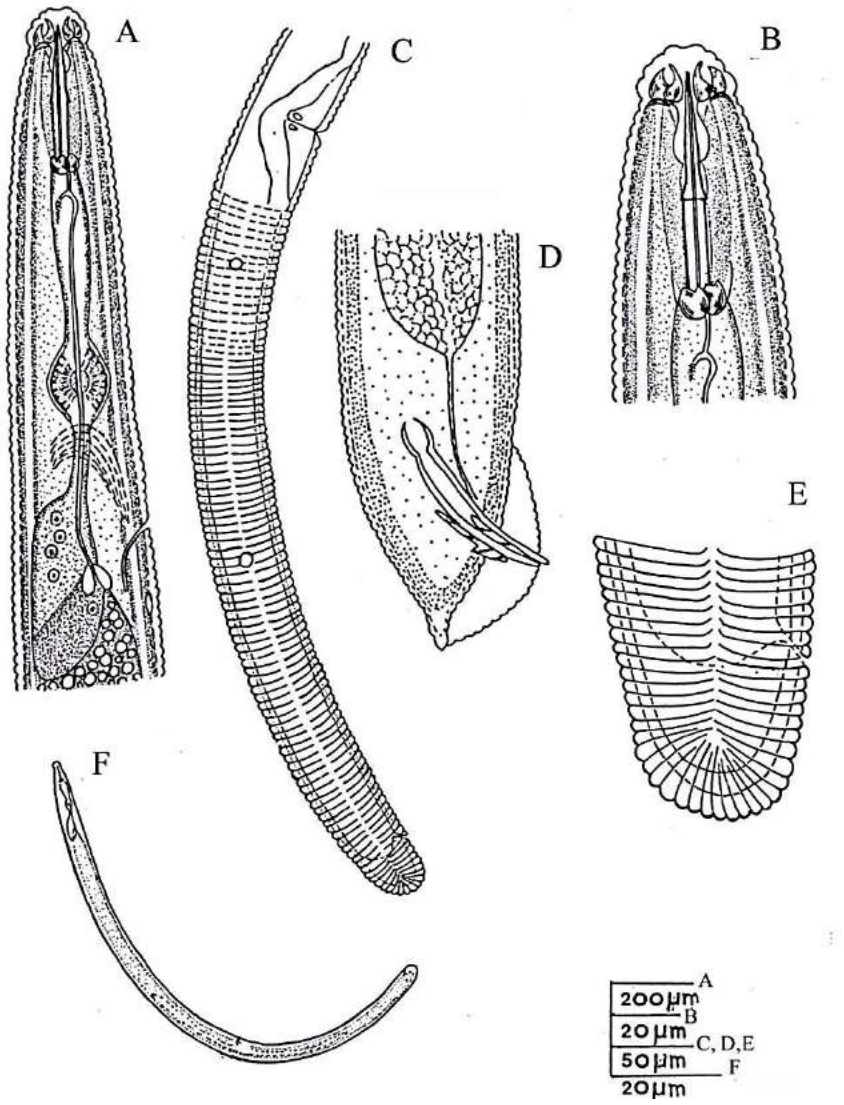


Fig. 5.5. *Hoplolaimus* spp. A. Anterior end; B. Head region; C. Vulva and tail; D. Male tail; E. Female tail ; F. Female whole body (From NNRC).

Tylenchorhynchus Cobb, 1913
(Stunt nematode)
Fig. 5.6

Parasitism and Habitat: Migratory ectoparasitic and sometimes endoparasitic, on many plant roots. All stages found in soil.

Main Morphological Characteristics: Body length 0.6 to 1.0mm in length. More or less straight or slightly curved ventrally on heat relaxation. Cephalic region rounded, offset from body or continuous, annulated or rarely smooth, framework light to moderately sclerotized. Lateral field with three or four incisures. Stylet well developed with rounded backwardly sloping basal knobs. Median bulb round or oval with distinct refractive thickenings. Oesophagus with well-developed posterior basal bulb, offset from intestine. Cardia prominent. Ovaries paired; outstretched, didelphic, amphidelphic. Vulva near middle of body. Spermatheca rounded, axial. Female tail usually conoid, may be subcylindrical, cylindrical or subclavate, terminus smooth, rarely striated. Male tail enveloped by a large, simple bursa. Spicules slightly curved.

Rotylenchulus Linford & Oliveira, 1940
(Reniform nematode)
Fig. 5.7

Parasitism and Habitat: Semi endoparasitic on many plant roots; juveniles, males and young females found in soil.

Main Morphological Characteristics: Small nematodes with distinct dimorphism between females and males. Immature female, male and juvenile vermiform, migratory, arcuate to spiral upon relaxation. Mature female swollen, reniform or kidney-shaped. Cephalic region high, continuous with body contour, rounded to conoid, striated. Lateral field with four incisures. Stylet in females well developed, in male weak with rounded knobs. Orifice of dorsal oesophageal gland often far from stylet base. Median bulb well developed, muscular, basal oesophageal bulb with long ventral overlapping. Ovaries paired, with double flextures. Vulva situated posteriorly. Tail elongate-conoid with rounded terminus.

Eggs deposited in a gelatinous matrix on the roots. Males vermiform with a degenerate oesophagus and weak stylet.

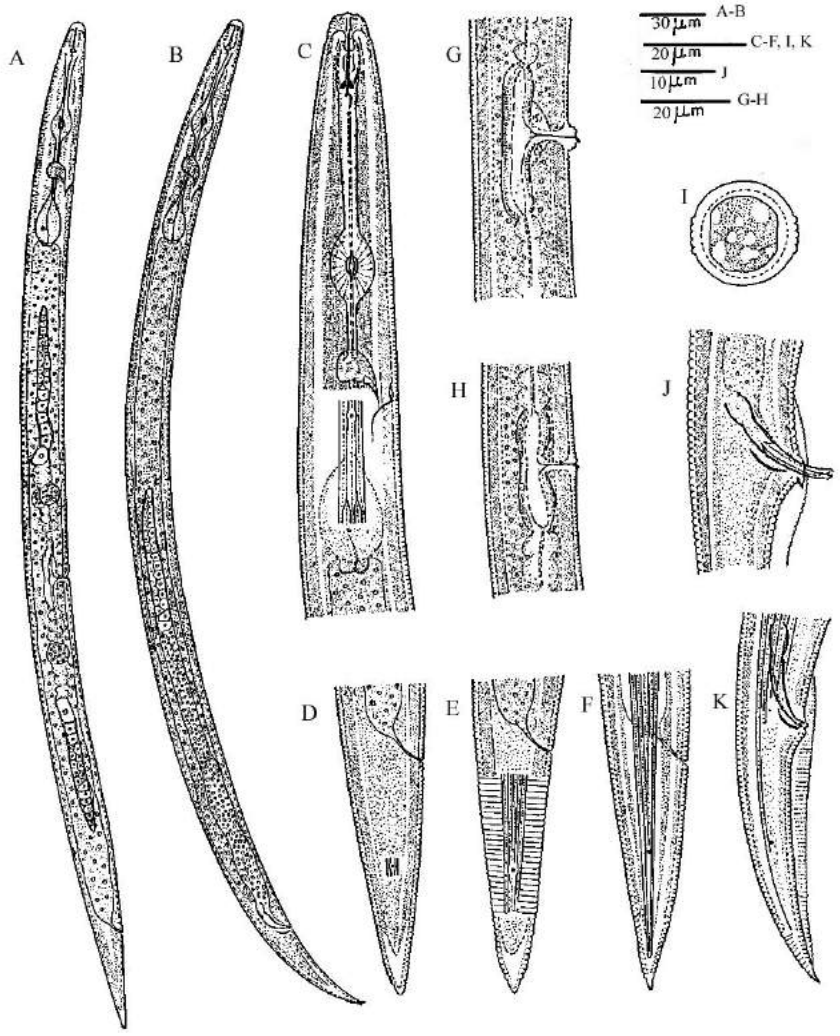


Fig. 5.6. *Tylenchorhynchus* spp. A. Female whole body; B. Male whole body; C. Female anterior region; D-F. Female tail; G & H.Vulval region; I. Transverse section; J & K. Male tail (From NNRC).

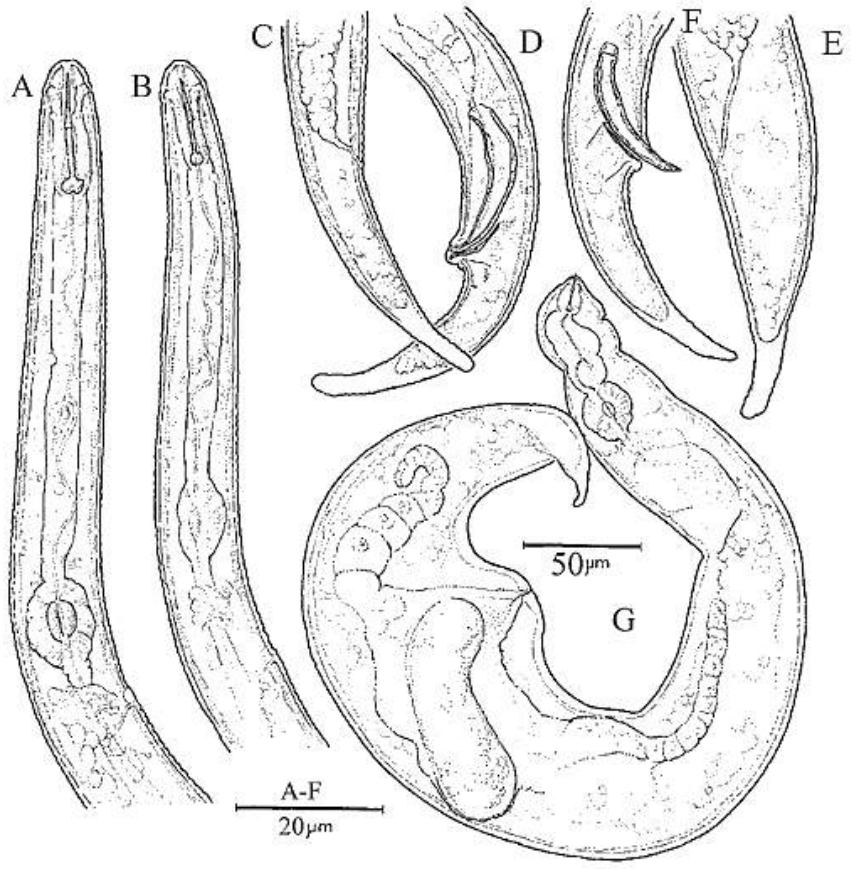


Fig. 5.7. *Rotylenchulus* spp. A & B. Immature female anterior region; C & E. Immaure female tail; D & F. Male tail; G. Female whole body (From P. Castillo *et al*., 2003).

Tylenchulus Cobb, 1913
(Citrus nematode)
Fig. 5.8.

Parasitism and Habitat: Semi-endoparasitic, mainly on citrus plant roots; young females and juvenile found in soil.

Main Morphological Characteristics: Small sized body; sexual dimorphism present. Mature female elongate-obese, ventrally curved, with distinct postvulval region, adult female sedentary, partially

embedded in roots. Stylet well developed with distinct knobs. Excretory pore far posterior, in front of vulva, at 68-85% of body length from anterior end. Basal bulb offset from intestine. Excretory system highly developed, produces gelatinous matrix. Vulva a distinct transverse slit; vulval lips bulging. Ovary coiled, or with one to two flexures, extending to oesophageal region. Female gonads prodelphic, postvulval uterine sac absent. Spermatheca present. Rectum and anus indistinct or obscure, non-functional.Tail tapering, tip rounded or with a peg. Eggs deposited in a gelatinous matrix secreted from the excretory pore. Preadult or immature female migratory, vermiform, straight to arcuate upon relaxation, Ovaries immature with a few oocytes. Male slender, non-feeding, with reduced stylet and oesophagus, migratory. Testis degenerate, bursa absent.

Hemicriconemoides Chitwood & Birchfield, 1957
(Sheath nematode)
Fig. 5.9.

Parasitism and Habitat: Ectoparasitic on many plant roots; females and juvenile found in soil, males seldom or never found.

Main Morphological Characteristics: Strong sexual diamorphism. Body short (0.3 -0.8mm), with large annules in females; annules of males much smaller. Female elongate, cylindrical with double cuticle, outer one sheath like attached to the body at head, vulva and sometimes at tail tip, annules of sheath and body round and flat or rarely retrorse. Cephalic region continuous or offset with two or rarely three annules. Stylet elongate with anteriorly cupped knobs, appearing anchor-shaped; often absent in males. Oesophagus with median bulb and short, narrow posterior bulb. Oesophageal tube lying in coils above median bulb valve when stylet not exerted. Ovary one; prodelphic. Vulva near posterior part of body (annules modified where it opens), open or closed, with or without cuticular flaps. Female tail short, variable, bluntly rounded to pointed. Male tail conoid to subcylindroid, bursa when present, low, sub-terminal or terminal. Spicules setose, arcuate. Gubernaculum simple, small.

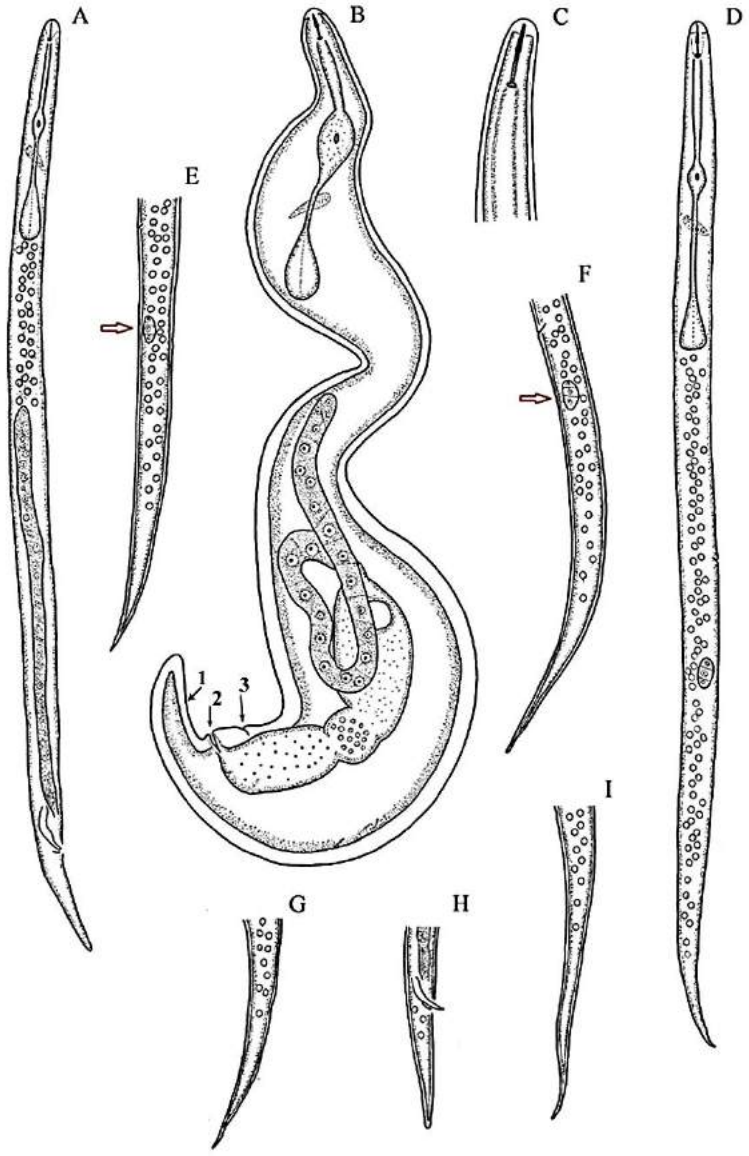


Fig. 5.8. *Tylenchulus* spp. A. Entire male; B. Entire female [1. Anus, 2. Vulva and 3. Secretory Excretory pore (SE pore) indicated by arrow]; C. Anterior end of Juvenile; D. Juvenile (J2); E , F, G,I. posterior end of J2 ; H. Male posterior end (Rashidifard e*t al.*, 2015).

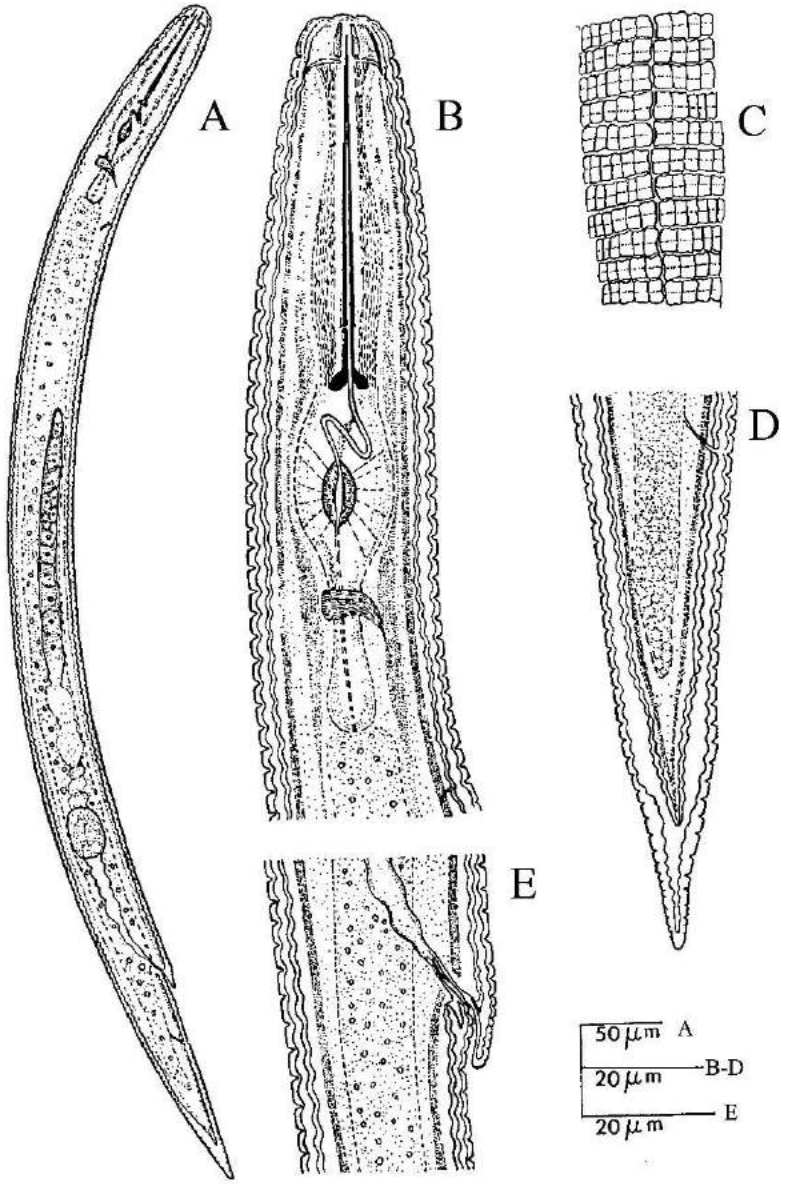


Fig. 5.9. *Hemicriconemoides* spp. A. Whole body; B. Anterior region; C. Cuticle pattern; D. Female tail; E. Male tail (From NNRC).

Anguina Scopoli, 1777
(Wheat gall or seed gall nematode)
Fig. 5.10

Parasitism and Habitat: Semi endoparasitic on galls in seeds of cereals and grasses, stems, leaves and inflorescence of various plants.

Main Morphological Characteristics: Sexually dimorphic. Female: Medium to large nematodes (1.5-5mm), obese. Spirally coiled upon heat relaxation. Median oesophageal bulb muscular. Basal bulb in adults enlarged, continuous or offset from isthmus by a constriction, base usually extending over anterior end of intestine. Ovary one, anteriorly directed, reflexed, twice or more due to excessive growth; post-uterine sac well developed. Oocytes in multiple rows, arranged about a rachis. Male: Testis well developed with one or more flexures. Tail similar in both sexes. Bursa adanal. Gubernaculum present. Second stage juvenile generally resistant and is the infective stage. Obligate plant parasites inciting galls in seeds of cereals and grasses, stems, leaves and inflorescence of various monocotyledonous plants; type species causes wheat seed-galls (ear cockles).

Ditylenchus Filipjev, 1936
(Stem nematode)
Fig. 5.11

Parasitism and Habitat: Ectoparasites on plant stems and leaves, also found within the tissues. Some species of *Ditylenchus* are endoparasites (migratory ecto/endo parasites) of the flower, buds, bulbs, stem, leaves and inflorescences.

Main Morphological Characteristics: Small and slender nematodes, less than 1.5 mm in length; slightly curved ventrally on heat relaxation. Labial region weakly sclerotized. Lateral field with four or six incisures, may be indistinct. Stylet moderately developed with small basal knobs. Oesophagus with a muscular median bulb with or without refractive thickenings, and a basal bulb which may extend as a lobe over the intestine. Genital tract single, anteriorly outstretched, with one or two

rows of oocytes. Spermatheca elongate, axial. Vulva well posterior. Post-uterine sac present. Tail in both sexes elongate, conoid to subcylindrical. Male testis outstretched, bursa adanal to subterminal, not reaching tail tip.

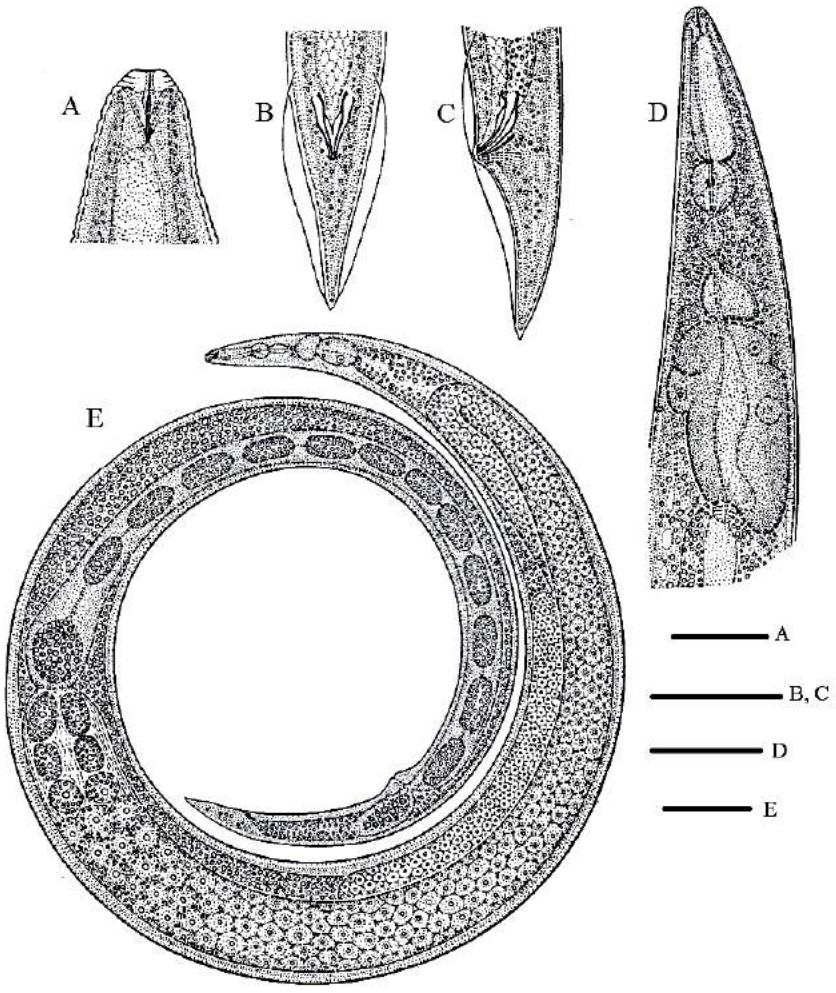


Fig. 5.10. *Anguina* spp. A. Cephalic region; B & C. Male tail; D. Female anterior region; E. Male whole body (Thorne, 1949).

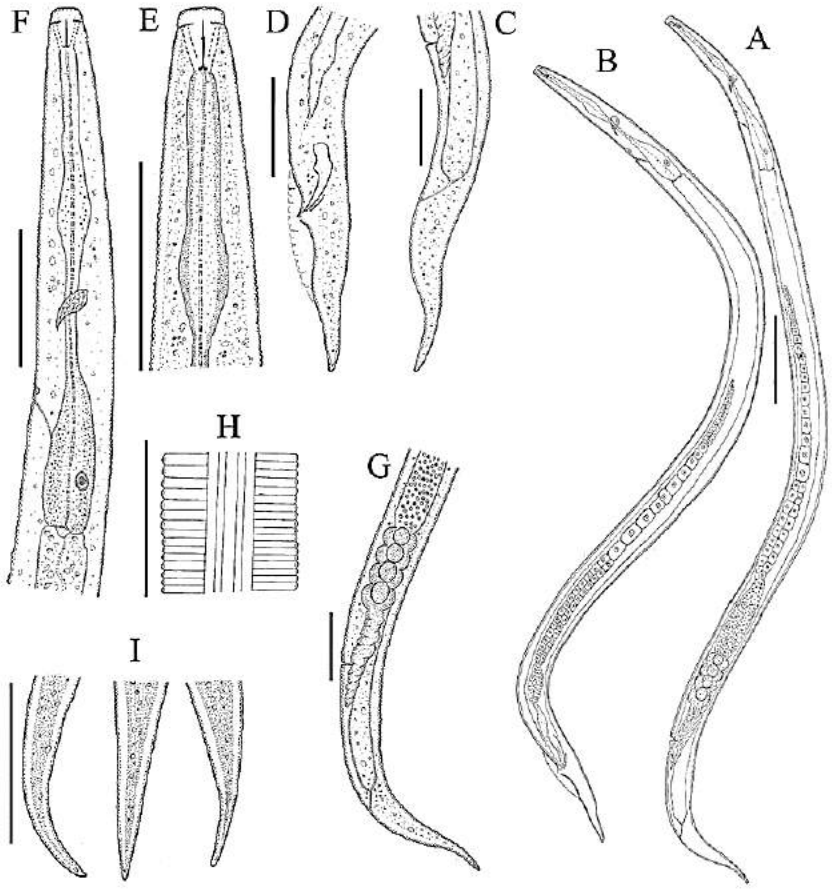


Fig. 5.11. *Ditylenchus* spp. A. Female whole body; B. Male whole body; C. Female tail; D. Male tail; E. Male anterior region; F. Female anterior region; G. Female vulval region and post uterine sac; H. Lateral lines; I. Female tail variation (From NNRC).

Aphelenchus Bastian, 1865
Fig. 5.12

Parasitism and Habitat: Ectoparasitic on many kinds of plants. Males, females and juvenile found in soil.

Main Morphological Characteristics: Medium sized to fairly long nematodes 0.5-1.2mm, tapering anteriorly. Straight to ventrally arcuate on heat relaxation. Cephalic region low, rounded and slightly offset. Cuticle

finely striated. Lateral field broad with numerous incisures, usually in excess of ten. Stylet slender, with slight basal thickening. Procorpus cylindrical, median bulb oval contains prominent crescentic valve plates. Oesophageal glands in a dorsal lobe. Ovary one, prodelphic, outstretched. Vulva posterior, about 70-80% of the body length. Oocytes mostly in a single row. Post vulval sac present. Tail short, cylindroids, sometimes slightly ventrally concave, with a broadly rounded terminus. Male with paired slender spicules, ventrally arcuate. Gubernaculum linear. Bursa well developed, extending to the tail tip, with four (rarely three) pairs of ribs; one preanal and the other three in a subterminal group. Tail tapering to a narrowly rounded tip.

Aphelenchoides Fisher, 1894
(Bulb and leaf nematode)
Fig.5.13

Parasitism and Habitat: Ectoparasites on leaves, stems and other parts of higher plants.

Main Morphological Characteristics: Small to medium sized (0.4-1.2mm), slender nematodes. Females straight to ventrally arcuate on heat relaxation, while male tail curls ventrally assuming a walking-stick shape. Labial region weakly sclerotized. Cuticle finely annulated. Lateral field often with four incisures but may be two or three. Cephalic region usually rounded and slightly offset; cephalic framework weak. Stylet slender, usually with basal knobs or swellings. Procorpus cylindrical; median bulb well developed, ovoid or spherical with central valve plates, and more or less filling the body diameter. Dorsal oesophageal gland orifice within bulb, just anterior to the valve plates. Oesophageal gland lobe well developed, overlapping intestine dorsally. Genital tract single, prodelphic, outstretched, but may reflex, anteriorly directed. Vulva posterior (60-75%) of the body length. Oocytes in one or more rows. Post uterine sac present. Tail medium conoid with a variable terminus which may be bluntly or finely rounded, digitate or bifurcate or with a ventral projection. One or more mucrons of various shapes may be present. Male tail strongly hooked ventrally to form a characteristic ‘walking-stick’ form. Spicules well developed, thorne-shaped, paired and

separate. Typical three pairs of caudal papillae, one pair adanal, one pair subterminal and the other in between. Bursa absent.

Radopholus Thorne, 1949
(Burrowing nematode)
Fig.5.14

Parasitism and Habitat: Migratory endoparasites of root and corm/tuber tissues.

Main Morphological Characteristics: Small nematodes, less than 1mm long. More or less straight or slightly curved ventrally on heat relaxation. Sexual diamorphism in anterior region: male cephalic region higher, rounded and more offset than female, cephalic framework, stylet and oesophagus markedly reduced. Female cephalic region low, rounded, continuous or slightly offset, annulated or smooth, cephlic sclerotization strong, stylet and oesophagus well developed, dorsal gland orifice near the stylet base. Lateral field with four incisures, not areolated. Oesophageal glands elongated, mostly overlapping intestine dorsally. Vulva median, 50-70% of body length. Genital system didelphic, amphidelphic, with oocytes in a single row. Spermatheca rounded or oval, axial, with rod-shaped sperms. Female tail mostly elongate, conoid to subcylindroid. Male tail elongate, conoid, ventrally arcuate, more tapering than female. Bursa subterminal or rarely terminal. Spicules slender, arcuate. Gubernaculum large, protrusible.

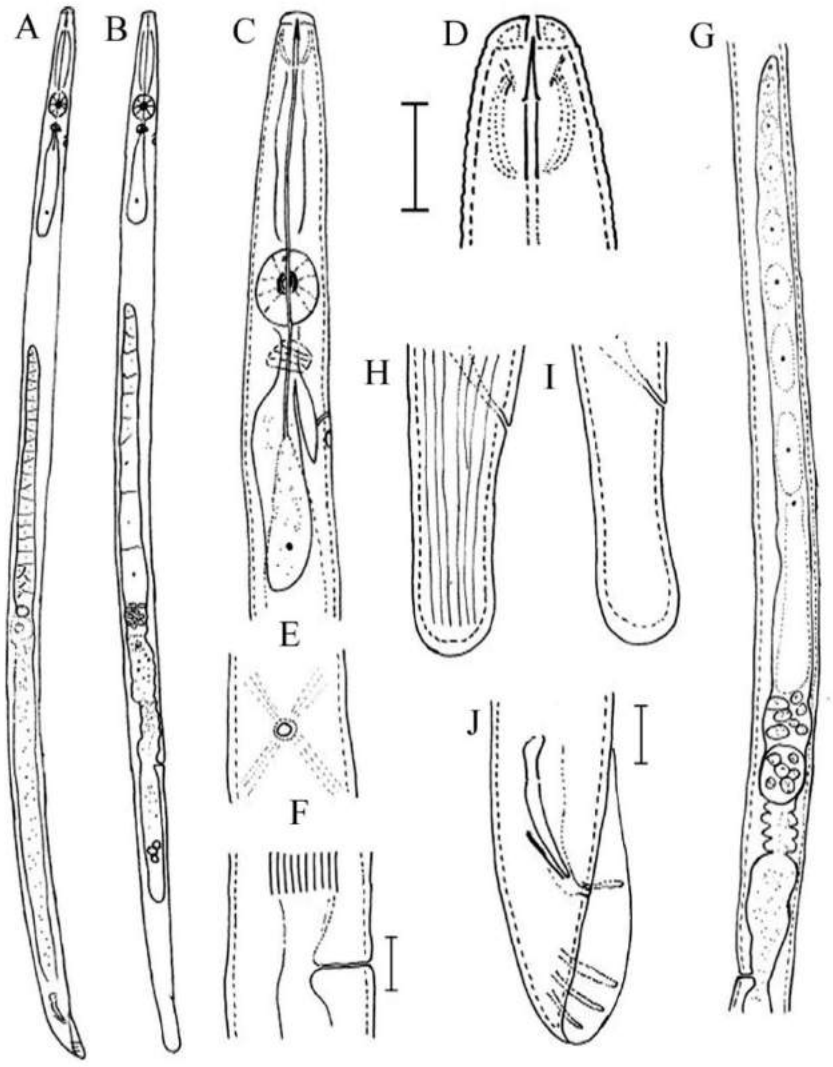


Fig. 5.12. *Aphelenchus* spp. A. Male whole body; B. Female whole body; C. Anterior region; D. Heat; E. Ventral view of vulva; F. Vulval region; G. Ovary; H & I. Female tail; J. Male tail (From NNRC).

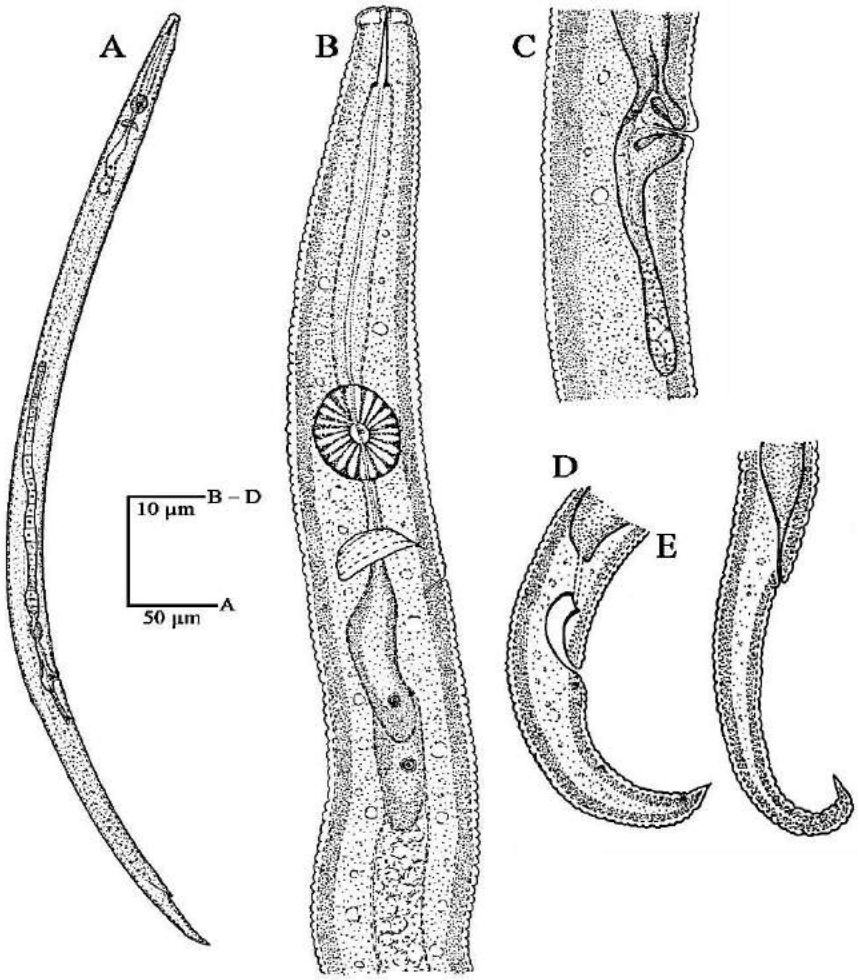


Fig. 5.13. *Aphelenchoides* spp. A. Female whole body; B. Oesopphageal part; C. Vulva and post uterine sac; D. Male tail; E. Female tail (From NNRC).

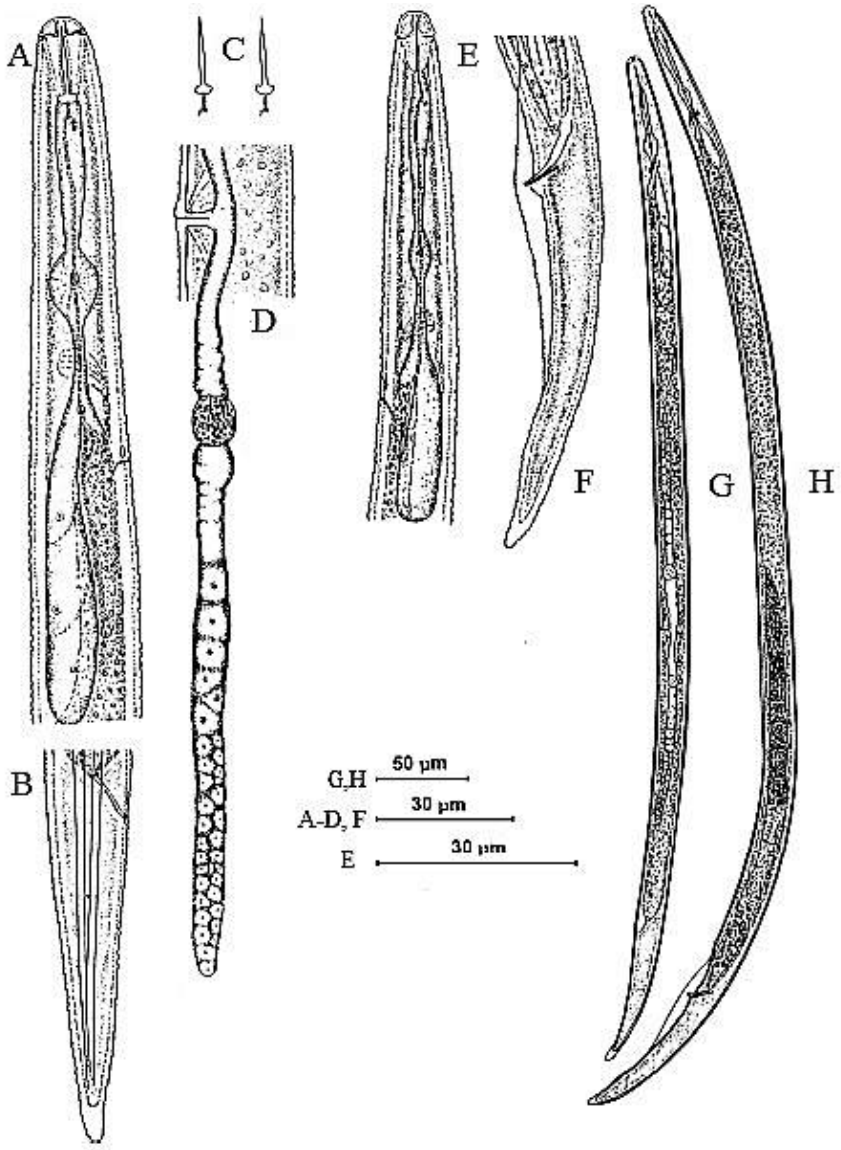


Fig. 5.14. *Radopholus* spp. A. Anterior region of female; B. Tail of female; C. Stylet; D. Ovary; E. Male anterior region; F. Male tail; G. Female whole body; H. Male whole body (From NNRC).

Xiphinema Cobb, 1913
(Dagger nematode)
Fig. 5.15

Parasitism and Habitat: Migratory ectoparasitic on many kinds of plant roots and a number of species are known to be vectors of certain viruses.

Main Morphological Characteristics: Body long to very long (1.5-6.0mm), slender. Straight to ventrally arcuate, C-shaped or an open spiral on heat relaxation. Cuticle smooth. Lateral chords broad with one or two rows of lateral body pores. Cephalic region rounded, continuous or offset. Amphidial aperture a broad slit extending to almost the entire lip width leading to a stirrup or funnel-shaped amphidial fovea. Odontostyle elongate, needle-like, heavily sclerotized. Guiding apparatus tubular with a strongly sclerotized posterior ring and a lightly sclerotized anterior ring at odontostyle/odontophore junction. Proximal end of the odontostyle forked at its junction with the strongly developed odontophore with three massive posterior flanges. Oesophagus consisting of a long, narrow cylindrical anterior region (procorpus) and a wide posterior expanded region (glandular bulb). Genital tract variable, often amphidelphic, reflexed, or mono opisthodelphic. Vulva near the middle of the body or near posterior end of oesophagus when only one ovary is present. Tail very variable from short and rounded to long filiform, hemispheroid with or without projections on ventral side, medium to long conoid in both sexes. Male genital tract diorchic, opposed. Spicules paired, strong, arcuate, dorylaimoid with distal accessory guiding pieces. Ventral supplements form a pre-cloacal row.

Trichodorus Cobb, 1913
(Stubby root nematode)
Fig. 5.16

Parasitism and Habitat: Migratory ectoparasitic on plant roots; some species are known to be virus vectors.

Main Morphological Characteristics: Body plump, cylindrical with rounded ends, female ventrally arcuate on heat relaxation the males, J-shaped with the tail region more sharply curved ventrad. Cuticle not swelling strongly on fixation. One to four pairs of lateral body pores

usually present. One pair of lateral body pores (i.e. one pore on each side) always situated within a body width of the vulva, and usually located posteriorly. Onchiostyle dorsally convex with a simple, anterior, guiding ring. Oesophagus consisting of a narrow anterior section which expands posteriorly to form a spathulate bulb. Bulb usually non-overlapping, but in some species a ventral overlap develops whereas in others the intestine extends dorsally along the bulb to form an overlap. Vulva a median pore or a transverse of longitudinal slit. Vaginal musculature well developed and prominent and sclerotization usually strong. Genital tract amphidelphic reflexed; spermatheca present, weakly developed in a few species. Anus subterminal; tail rounded. Caudal pores paired. Males usually with one to three ventromedian cervical pores, exceptionally absent or as many as four. Lateral cervical pores usually present. Male genital tract monarchic, outstretched. Sperm large, subcylindroid. Spicules more or less ventrally arcuate, never straight; either smooth or with various ornamentations, bristles, etc. A ventral flage, or velum, is present in the eponymous *T. velatus*. Gubernaculum present. Spicule suspensor muscles forming a prominent oval capsule around the spicules. Bursa absent. Three, sometimes four, ventromedian copulatory supplements; the first being within the range of the retracted spicules. Tail short, rounded, with one pair of ventro-sublateral papillae and a pair of caudal pores.

Longidorus Micoletzky, 1922 (Filipjev, 1934)
(Needle nematode)
Fig. 5.17

Parasitism and Habitat: Migratory ectoparasites mainly of roots of herbaceous plants. A number of species have been implicated in the transmission of nepoviruses.

Main Morphological Characteristics: Body long to very long (3 to > 10mm) and slender. Heat relaxed form varying from more or less straight to C-shaped. Lateral chords broad and with one or two rows of lateral body pores. Cephalic region rounded; continuous or offset. Amphidial apertures inconspicuous pores which lead back to well develop pouch-like amphid fovea. Odontostyle elongate, needle-like; not heavily sclerotized. Guiding apparatus with a simple ring usually situated within

a couple of head-widths of the anterior end, but exceptionally further posterior. Junction of odontostyle and odontophore simple. Odontophore about two thirds of the odontostyle in length, moderately sclerotized, thickening slightly in the posterior region, but lacking basal flanges. Oesophagus narrow, cylindrical anterior section, which is looped back on itself when the odontostylet is in the retracted position, and a posterior bulboid expansion which is muscular and glandular with valve plates running for almost the full length. There are three glands: dorsal and two ventrosublateral. Nerve ring located around the narrow anterior section of the oesophagus; a second nerve ring, located more posteriorly, occurs in some species. Intestine simple, prerectum well developed and several anal body widths long. Anus in the form of a transverse slit. Vulva a transverse slit, median in position. Vagina well developed, muscular. Genital tract amphididelphic, reflexed. Tail short, dorsally convex-conoid to a finely rounded terminus, or broadly rounded. Several pairs of caudal pores present. Male genital tract diorchic, opposed, the posterior testis being reflexed. Spicules dorylaimoid, paired, massive, ventrally arcuate and with short accessory guiding pieces located distally. Copulatory supplements consisting of an adanal pair (some species have two or three pairs) and then a ventromedian series of up to 20 extending anteriorly without a gap between the adanal pair and the series. Tail similar in shape to that of the female.

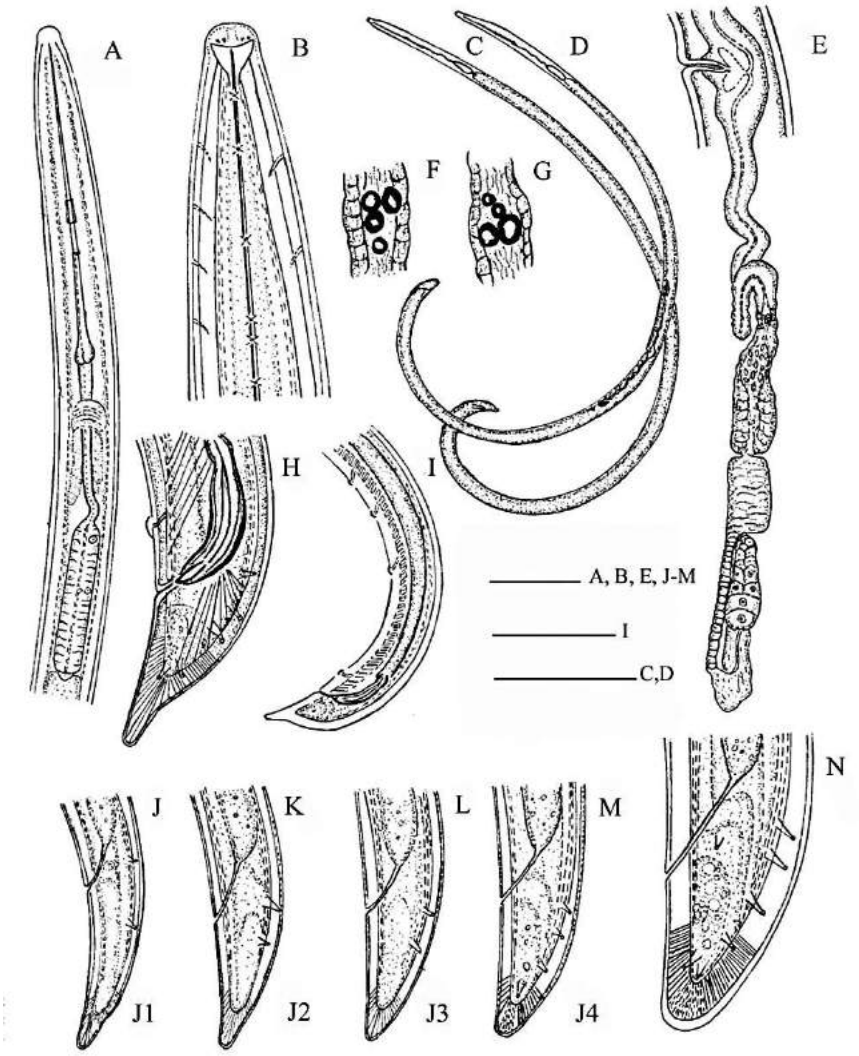


Fig. 5.15. *Xiphinema* spp. A. Oesophageal region; B. Head region; C & D. Female and male whole body; E. Vulva; F & G. Z-pseudo-organ J-M. J1-J4; N. Female tail (From NNRC).

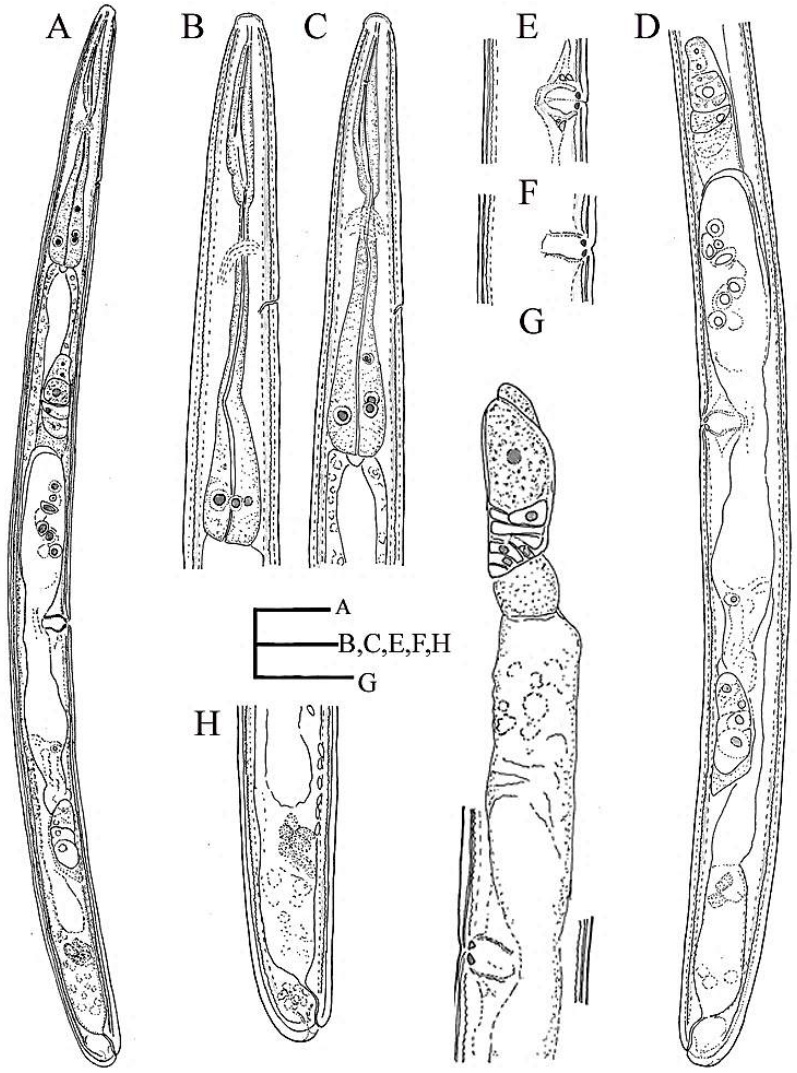


Fig. 5.16. *Trichodorus* spp. A. Female whole body; B. Female anterior region; C. Male anterior region; D. Vulva and ovaries; E & F. Vulval region; G. Anterior gonad; H. Female tail (From NNRC).

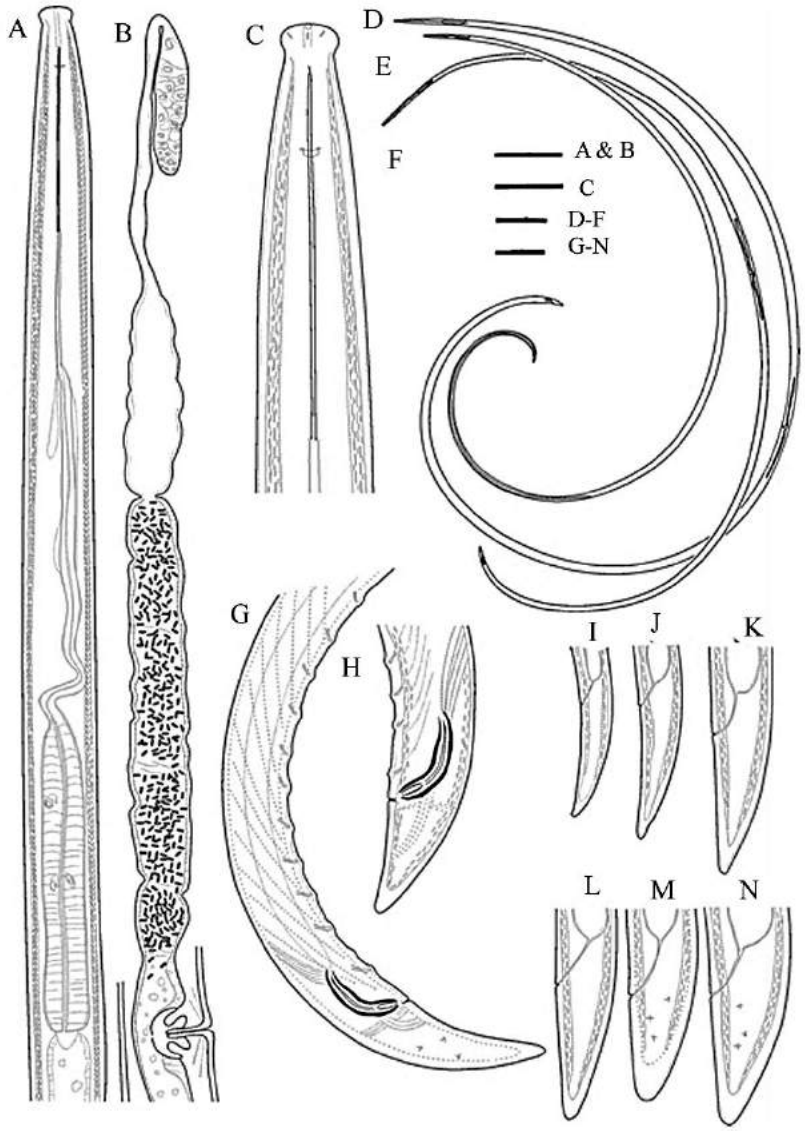


Fig. 5.17. *Longidorus* spp. A. Female anterior region; B. Anterior ovary; C. Odontostyle ; D-F. Whole body; G & H. Male tail; I-N. Female tail (From NNRC).

Chapter 6

ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

Root-knot nematodes (RKN) of the genus *Meloidogyne* (Göldi, 1892) are ubiquitous in nature and associated with nearly every crop. Root-knot nematodes (*Meloidogyne* spp.) rank at the top of list of the most economically important species and have a significant economic impact on host-plants due to their complicated association with the hosts, broad host range, severity and intensity of damage developed by infection and their cosmopolitan distribution. RKN are obligate sedentary endoparasites. They feed and reproduce on plant roots, inducing a permanent feeding site to complete their lifecycle leading to formation of small to large galls or root-knots. Infected roots alter the water and nutrient uptake which cause marked poor growth and decrease in crop yield and product quality. Several generations can reproduce during one cropping season, resulting in severe crop damage.

Root-knot nematode was first reported by Berkeley in 1855 from England on cucumbers. *Meloidogyne exigua*, the type species of the genus was described by Göldi in 1887 from coffee plants in Brazil. Chitwood, 1949 described 4 species and one subspecies (*M. incognita acrita*) of *Meloidogyne*. About 100 species of *Meloidogyne* have been described and host the range exceeds over 3000 plant species. The economically important and globally ubiquitous species are *M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi* and *M. graminicola* (Mitkowski and Abawi, 2003; Hunt and Handoo, 2009).

Root-knot nematode females become obese and sedentary when mature; attain a size of 400 to 1000 µm in length. They establish a permanent feeding site within the plant root. This feeding site is in fact a group of cells known as "giant-cells" infected by a mature root-knot female. Cells adjacent the giant-cells also enlarge and divide quickly resulting in gall formation **(Fig.6.1)**.

Female nematode increases in size and deposits her eggs into a gelatinous egg-mass by breaking the epidermis of the root. Mature root-knot females are pearly white in color and can be observed without magnification while second-stage juveniles (J_2) and males can only be observed with the aid of a microscope. The infective J_2 and males of the root-knot nematode are vermiform and mostly found in soils. Several *Meloidogyne* species are parthenogenic. Therefore, males can be rare in a number of species and are only encountered when the nematode population is subjected to an environmental stress (Mitkowski and Abawi, 2003).

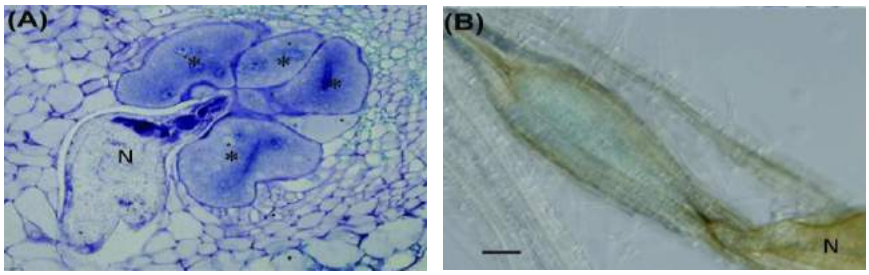


Fig.6.1. A. Formation of "Giant-cells"; B. Light micrograph of a cross section of a root gall (N= nematode) (Sigal Braun Maira *et al*., 2015).

Symptoms

Root-knot nematodes induce morphological and physiological modification in host plant that results in abnormal or stunted growth of plants, lack of vigor, nutrient deficiency symptoms, root galling, forking and secondary infection by other pathogens. The above-ground symptoms of RKN are almost similar to those produced by damaged and broken root system. Heavily infected plant sometimes shows no external symptoms on the harvested products (Moens *et al*., 2009). However, many symptoms can also be observed on above ground parts of the plant. Above-ground symptoms, when present, are often associated with high nematode populations, and may include stunting, chlorosis (yellowing), wilting of plants and yield losses. Presence of high population of RKN in field during growing season can kill the host plants **(Fig. 6.2)**.

The presence of galls on the root system can be easily observed at low magnification in stained roots. Infested plant roots are commonly stunted, abnormally swollen with immense galling **(Fig. 6.3)**. The extent of damage is not necessarily correlated with the number of nematodes present but varies both with the species of *Meloidogyne* and the host plant. The galls are invaded by secondary pathogens-fungi and bacteria that stimulate severe root infection (Mitkowski and Abawi, 2003).

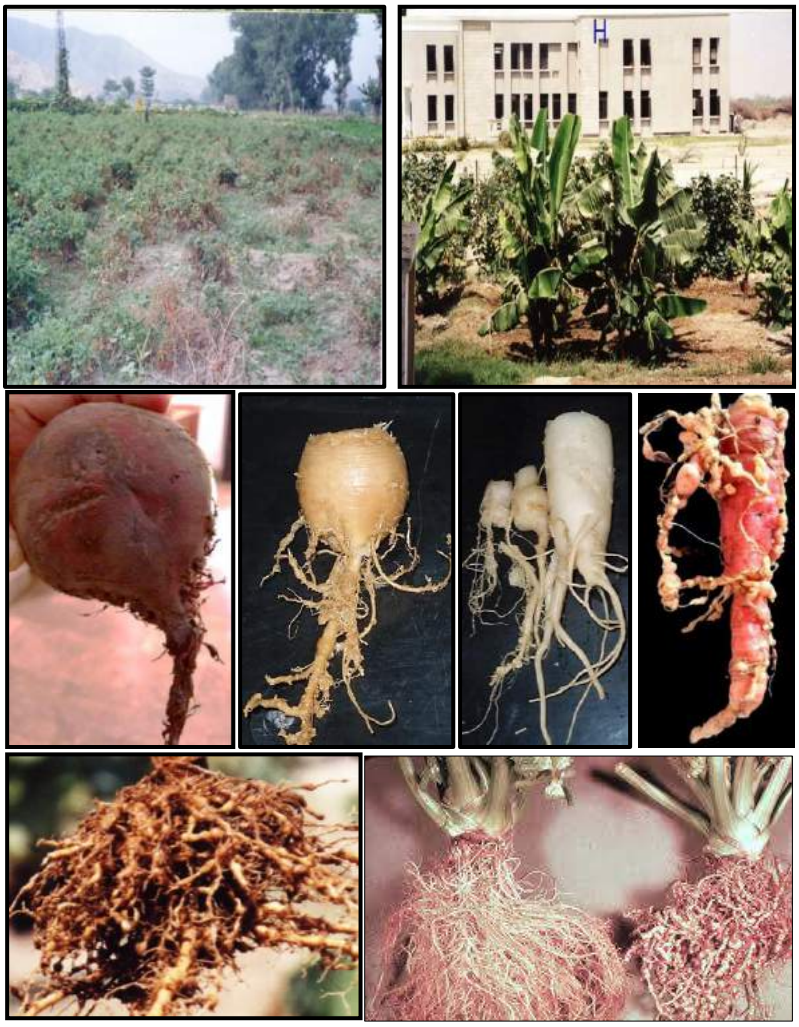


Fig.6.2. Above and below ground symptoms of root-knot disease (From NNRC).

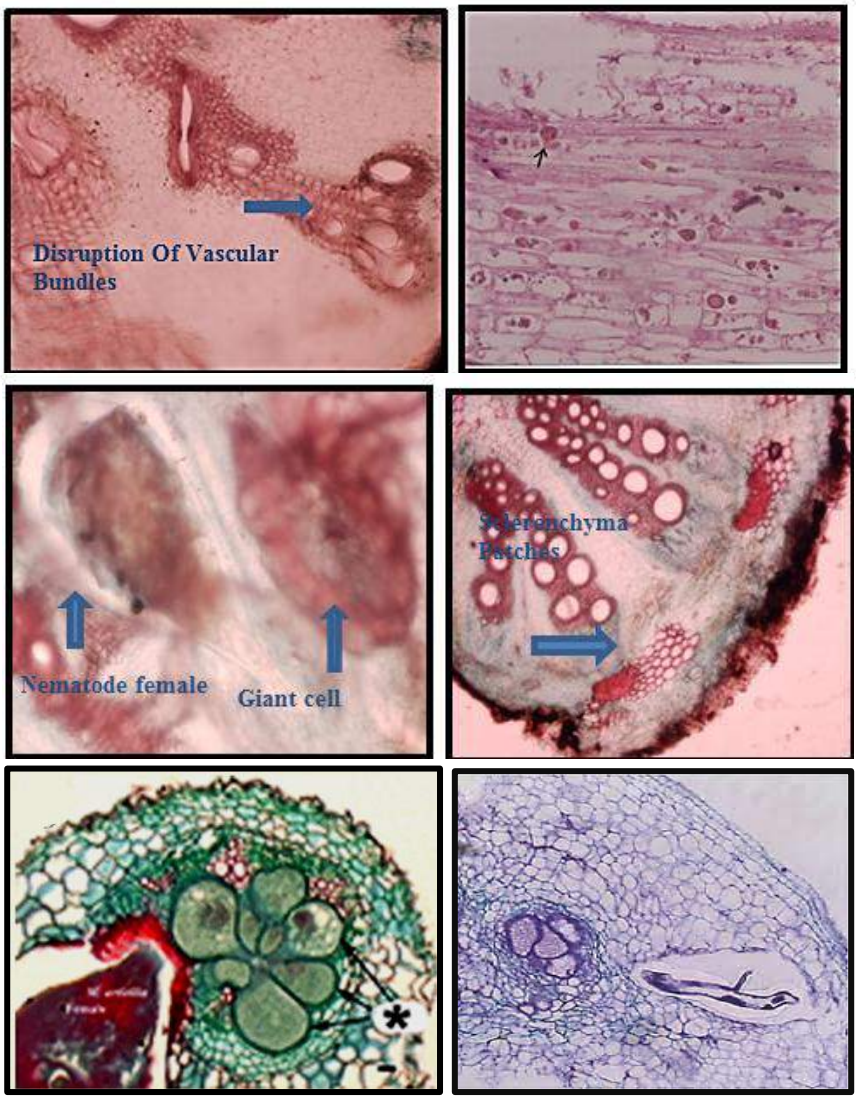


Fig. 6.3. Giant cell formation in root system (From Khan, 2006 and NNRC).

Several investigations have been made by scientists on distribution, taxonomy, susceptibility, pathogenicity, differential host test, histopathology, new host records, abiotic effects, control strategies and other aspects of *Meloidogyne* species in Pakistan **(Fig. 6.4)**.

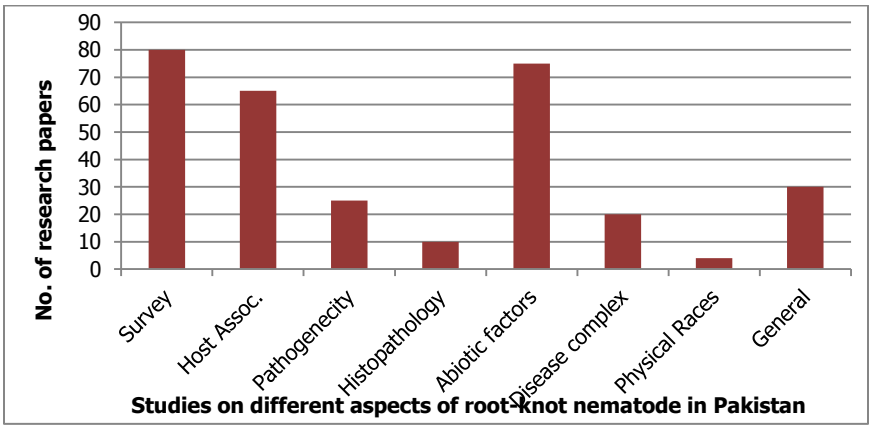


Fig. 6.4. Different aspects of root-knot nematode study (updated 2021, NNRC).

So far six species of root-knot nematodes have been reported from Pakistan including a new species *viz*., *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. graminicola* and *M. pakistanica* (Shahina *et al*., 2019). Their prevalence percentage from Pakistan is given in **Fig. 6.5**.

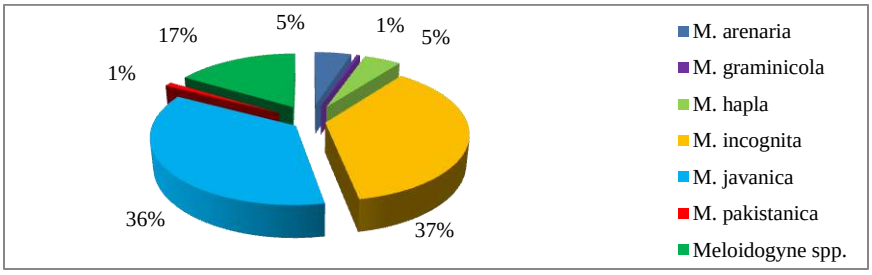


Fig. 6.5. Prevalence percentage of different root-knot nematodes from Pakistan (updated 2021, NNRC).

Morphology of Root-knot Nematode

Recently, the morphology of the root-knot nematodes has been reviewed by Eisenback and Hunt (2009). The morphological details of root-knot nematodes are important for the identification of species (Chitwood, 1949; Karssen and Moens, 2006; Eisenback and Hunt, 2009).

The morphology of root-knot nematodes changes during their lifecycle, the first stage juvenile, formed at the end of the embryogenesis, immediately moults while still in the egg, becoming a second-stage juvenile. After the establishment of a host parasite relationship and initial feeding period of 3-8 weeks, the second-stage juvenile rapidly moults three times and develops into an adult male or female **(Fig. 6.6)**.

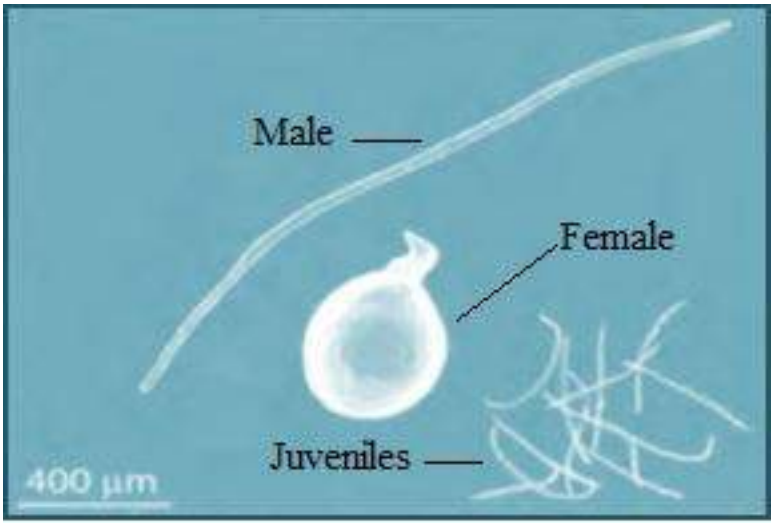


Fig. 6.6. Scanning electron micrograph of root-knot nematode showing general body shape and relative dimensions of the stages: Male (left), mature female (center) and second-stage juveniles, J_2 (right), (Source: Perry *et al*., 2009).

Female: Root gall inciting, sedentary, white, and globular to pear-shaped with protruding neck. Body length 295-450µm, cuticle striated with characteristic terminal striation forming vulva and anus, perineum (vulva-anus region) with characteristic finger print-like cuticular pattern, elevated vulva and anus terminal. Phasmids dot like situated on either side of the anus. Head slightly set off or continuous, cuticular framework distinct, labial cap with six lips, fused with medial and lateral lips. Two slit-like amphidial and ten small sensilla opening present. Stylet slender 10-25µm long, cone half of the stylet length, dorsal curvature, shaft with three knobs, dorsal oesophageal gland orifice 2.5-9.0µm from posterior to stylet knobs. Excretory pore located between the stylet knob and median bulb, procorpus cylindrical and metacorpus spherical, muscular

with cuticular feeding valves, oesophageal gland ventrally overlapping the intestine. Ovaries paired, convoluted, prodelphic, perineal pattern posterior, rectal glands six, large, secrete gelatinous matrix through anus, and eggs laid in gelatinous material, most of the unembryonated eggs deposited in egg sac, not retained in the body **(Fig.6.7)**.

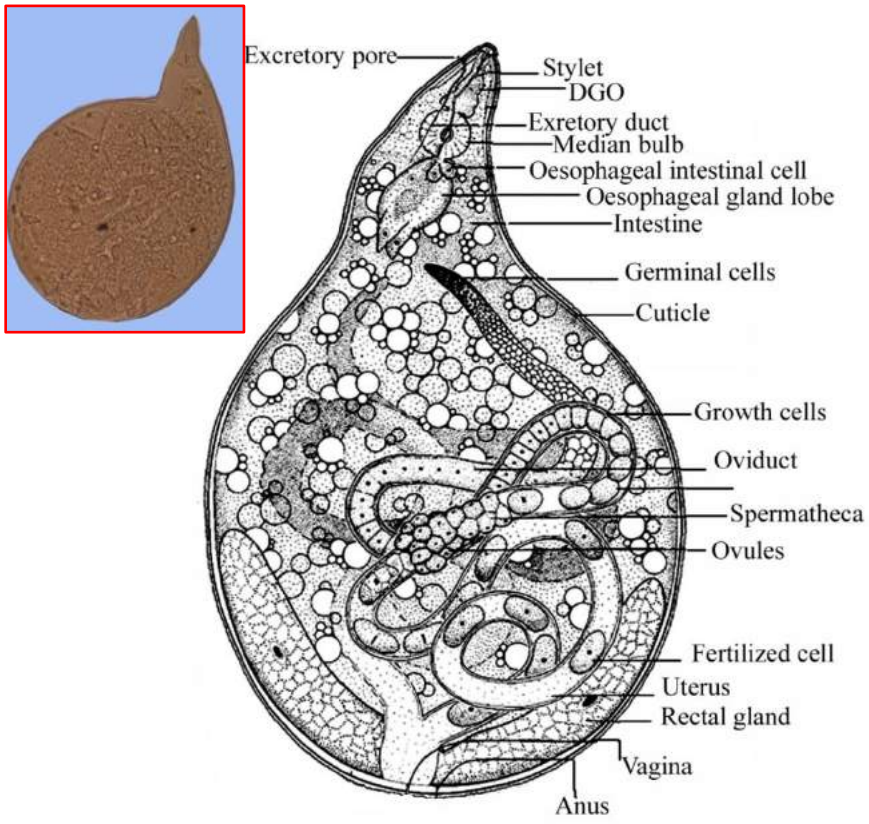


Fig.6.7. Whole body of female (Nickle, 1984 and NNRC).

Male: Vermiform, motile and does not feed. Body length 0.60-2.50mm. Cuticle annulated, lateral field with four incisures, Head slightly offset, head cap with rounded labial disc and four medial lips fused, six inner labial sensilla central around the stylet stoma and one cephalic sensillum present on each medial lip, strong, labial sectors wider than median. Cephalic framework strong, stylet robust 13-33µm long with large knobs. Dorsal oesophageal gland orifice 1.5-13µm, behind the three

stylet knobs. Procorpus cylindrical, metacorpus smaller than in female, oesophageal gland overlaps the intestine ventrally. Excretory pore located between metacorpus and the ventrally overlapping of the oesophageal glands. Hemizonid anterior or posterior. Testis single, rarely two reduced ones. Tail short, bluntly rounded. Spicules slender 20-40 µm long, gubernaculum simple 7-11µm long, phasmids dot like near cloacal aperture, bursa absent. After the fourth moult they leave the root and move freely through the soil. In populations, which reproduce by amphimixis the ability of the male to find a suitable mate is critical for survival of the species **(Fig.6.8)**.

Juvenile: First stage with a blunt tail tip moults within the egg, second and third moult occurring within the cuticle of second stage.

Second Stage Juvenile: Vermiform, migratory infective stage. Lateral field with 4 incisures. Body length is 250-600µm, cuticle annulated. Head with labial cap of six lips, median lips fused in two pair forming dumble-shape, distinct labial disc. Cephalic framework lightly sclerotized, stylet slender about 9-20µm long with three knobs. Dorsal oesophageal gland orifice 2-12µm behind knobs. Procorpus cylindrical and spherical, muscular metacorpus. Oesophageal glands overlap intestine ventrally. Excretory pore posterior to metacorpus and hemizonid. Rectum often inflatted, anus 15-100µm from tail. Tail tapering, towards hyaline tail part.

Third Stage: Sedentary, swollen, inside the root, stylet absent, short blunt tail.

Fourth Stage: Sedentary, swollen, with terminal anus, stylet absent, basic haploid chromosome number 13±10 **(Fig.6.9)**.

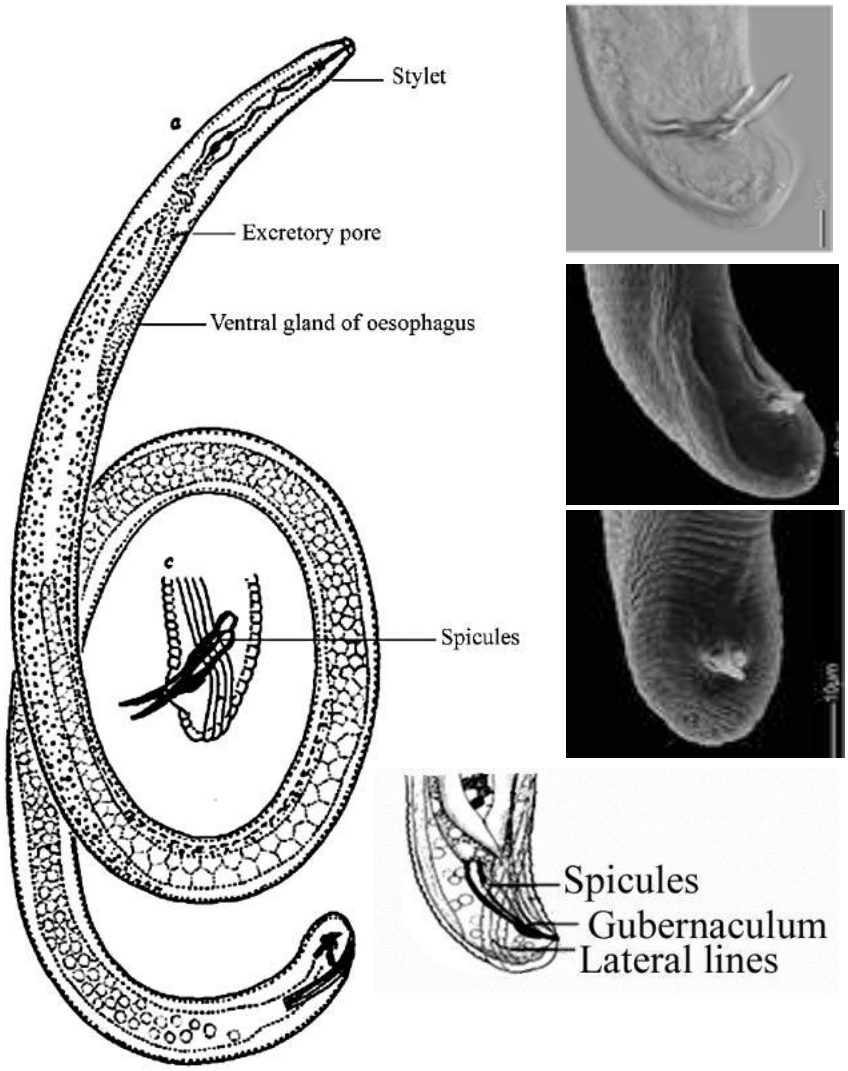


Fig.6.8. Whole body of male and tail showing spicules (Nickle, 1984)**.**

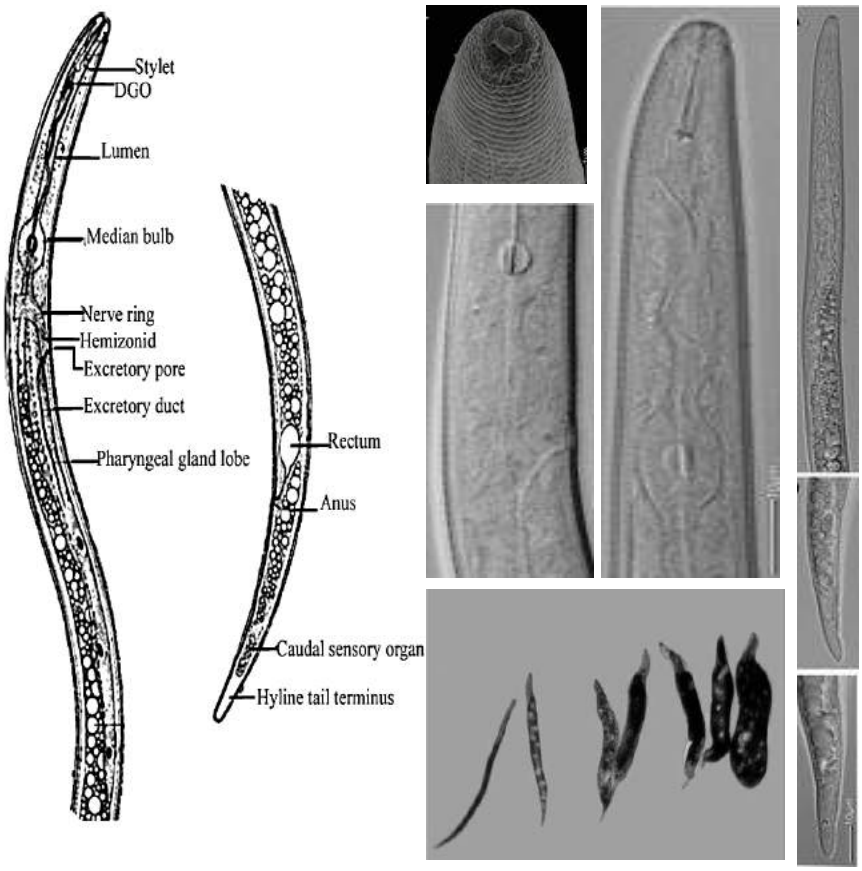


Fig. 6.9. Juvenile: SEM and light-microscopy (Nickle, 1984).

Lifecycle:

Root-knot nematodes begin their lives as eggs that rapidly develop into J_1 (first-stage juvenile) nematodes. The J_1 stage resides entirely inside the translucent egg case, where it moults into an infective J_2 **(Fig. 6.10)**. The motile J_2 stage is the only stage that can initiate infections. J_2 attack growing root tips and enter roots intercellularly, behind the root cap. They move to the area of cell elongation where they initiate a feeding site by injecting oesophageal gland secretions into root cells. These nematode secretions cause dramatic physiological changes in the parasitized cells, transforming them into giant cells.

J_2s do not possess reproductive organs. RKN also undergo four juvenile stages, each progressing through a "moulting" process similar to that of insects. As a result of this process, juvenile root-knot nematodes have little resemblance to adult males and females. In the J_4 stage, the progression from juveniles to globose adult females or vermiform adult males becomes clearly visible. They emerge as adults from the J_4 cuticle. A single female nematode can produce 500 to more than 1000 eggs. The length of a root-knot nematode lifecycle varies among species but can be as short as two weeks. Nematodes in cooler regions typically have longer lifecycles. Eggs may remain inside root tissue or may be released into the gelatinous matrix. Eggs hatch at random, i.e. hatching does not require exposure to root exudates.

Under favorable conditions, root-knot nematode eggs have been reported to survive for at least one year in the soil.

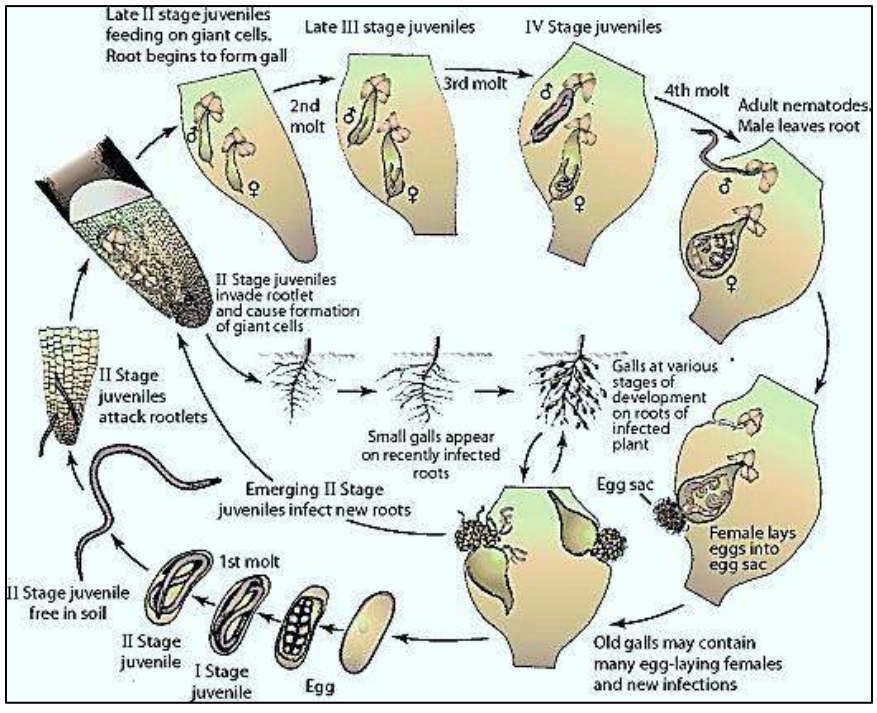


Fig. 6.10. The lifecycle of the root-knot nematode *Meloidogyne* spp (Agrios, 2005).

Identification of Root-knot Nematode

Root-knot nematodes can be identified to species using a number of techniques, but one common method is perineal pattern analysis. The perineum (the region surrounding the vulva and anus) of female nematodes displays a pattern of ridges and annulations for each species. While some variation does exist among individuals, these patterns are quite consistent within a species. The analysis of isoenzyme electrophoretic profiles, using esterase and malate dehydrogenase, is a common method for the diagnosis of *Meloidogyne* species in properly equipped labs. Likewise, DNA analyses can also be used to identify different species of root-knot nematode.

Preparation of Perineal Pattern for Identification of *Meloidogyne* species

Live, young, egg-laying females are dissected from gall roots and place into a drop of 45 % lactic acid in a plastic Petri dish. The posterior half of the body is cut off with a scalpel; the lower pieces of the cuticle having perineal patterns are further trimmed to square and inner tissue are completely removed by flexible bristle. The inner tissue is removed by trimming and the perineal patterns are transferred into a drop of glycerine on microscope slide. The posterior patterns, outside upper most, are arranged in one or two rows. The cover glass is gently placed and sealed with zut (Eisenback *et al*., 1981) **(Fig.6.11)**.

Staining of *Meloidogyne* Egg-mass: Galled roots are placed in an aqueous solution of Phloxine B (0.15 g / liter tap water) for 15-20 minutes. The color of egg-masses becomes pink; they are then easily counted under a stereomicroscope (Eisenback *et al*., 1981).

Maintenance of Root-knot Nematode Culture: The second stage juveniles (J_2) of root-knot nematode, hatched from a single egg-mass from a tomato root serve as the initial inoculums to the seedlings of brinjal (*Solanum melongena* L.) in earthen pots containing sterilized soil-manure mixture. The nematode species is identified by close examination of perineal pattern of the female from the first egg-mass collected. It is

confirmed by similar examination of randomly collected females from the inoculums raised as above.

Root-knot Index (RKI): Infection of roots by root-knot nematode is estimated using the following 0-5 scale as described by Taylor and Sasser (1978).

S. No.	No. of knots	RKI
1	0	0
2	1-2	1
3	3-10	2
4	11-30	3
5	31-100	4
6	>100	5

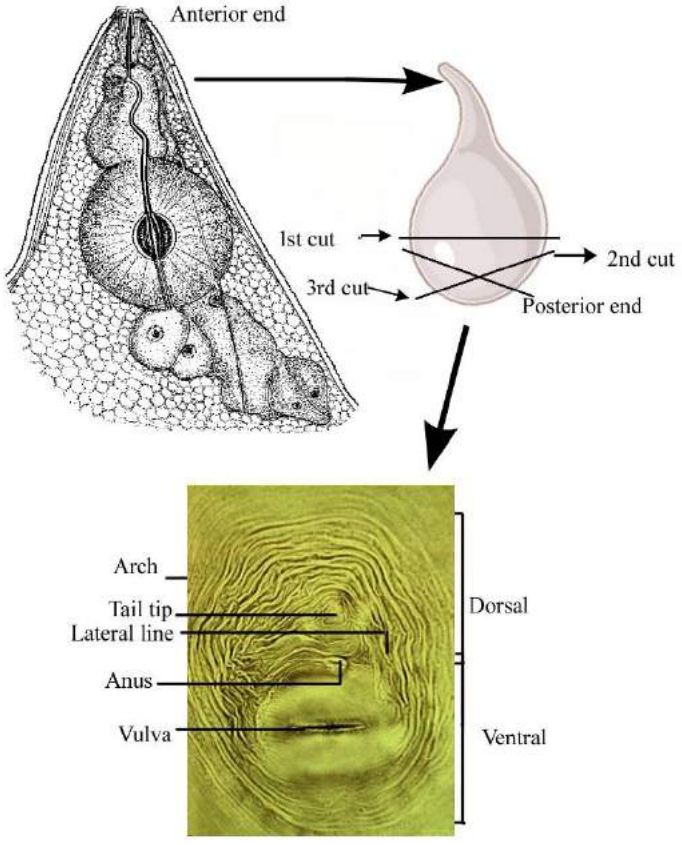


Fig.6.11. Preparation of perineal pattern (From NNRC).

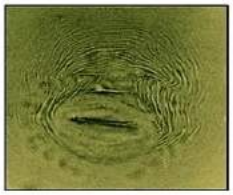
Key to root-knot nematodes *(Meloidogyne* spp.) found in Pakistan (based on morphology of head and stylet of males).

1.	Labial disc usually raised above medial lips	*M. incognita*
	Labial disc and medial lips fused to form one smooth head cap	2
2.	Head annule not in contour with the first body annule	*M. hapla*
	Head annule in contour with the first body annule	3
3.	Stylet knob transversely elongate	*M. javanica*
	Stylet knob not transversely elongate	4
4.	Stylet robust, 20-24µm in length, dorsal oesophageal gland orifice to base of stylet 4-7µm	*M. arenaria*
	Stylet short, less than 20 µm in length, dorsal oesophageal gland orifice to base of stylet less than 7µm	5
5.	Stylet 16.2-17.3µm in length, dorsal oesophageal gland orifice to base of stylet 2-6µm	*M. graminicola*
	Stylet 14-18µm in length, dorsal oesophageal gland orifice to base 4-6 µm of stylet 2-6 µm	*M. pakistanica*

Key to root-knot nematodes (*Meloidogne* spp.) found in Pakistan (based on morphology of the perineal pattern)

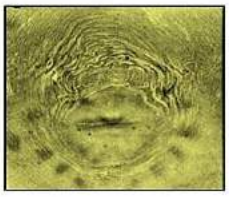
1.	Punctation present in tail terminal area	*M. hapla*
	Punctation absent in tail terminal area	2
2.	Lateral line clearly marked by deep incisures. Usually extending well beyond perineum	*M. javanica*
	Lateral line not clearly marked or ending near perineum	3
3.	Dorsal arch high and square, striae smooth to wavy, lateral field not marked by loop and fold striae	*M. incognita*
	Dorsal arch low and rounded	4
4.	Striae in dorsal arch rounded and forming shoulder .	*M. arenaria*
	Dorsal arch without distinct shoulder	5
5.	Lateral field absent	*M. graminicola*
	Lateral field prominent	*M. pakistanica*

Meloidogyne incognita

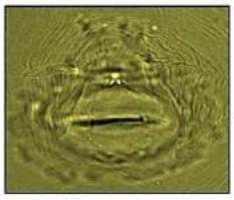


Meloidogyne arenaria

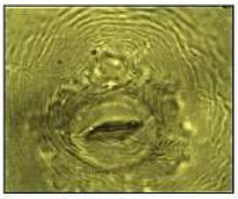
Meloidogyne hapla



Meloidogyne graminicola



Meloidogyne javanica



Meloidogyne pakistanica

Diagnostic characteristics of stylet of female root-knot nematode (*Meloidogyne* spp.) found in Pakistan

RKN species	Stylet cone	Stylet shaft	Stylet knobs	Stylet Length (µm)	DGO (µm)
M. arenaria	Straight, broad and robust	Wider posteriorly	Not set off, sloping posteriorly, merging with shaft	11-13	3-7
M. graminicola	Slightly curved dorsally	Cylindrical to slightly wider posteriorly	Set off, transversely elongate	10.5-11	3-4
M. hapla	Slightly curved dorsally, narrow & delicate	Slightly wider posteriorly	Set off, small and rounded	14-17	5-6
M. incognita	Anterior half distinctly curved dorsally	Slightly wider posteriorly	Set off, rounded to transversely elongate, sometimes indented anteriorly	15-17	2-4
M. javanica	Slightly curved dorsally	Cylindrical	Set off, transversely elongate	14-18	2-5
M. pakistanica	Long, curved dorsally	Cylindrical	Rounded, sloping posteriorly	14-18	2-5

Chapter 7

CYST NEMATODES (*HETERODERA* AND *GLOBODERA* SPP.)

Among the plant parasitic nematodes, the cyst forming nematodes (*Heterodera* and *Globodera* spp.) rank second to root-knot nematode and are among the most economically important pests of agricultural crops all over the world. Generally, these nematodes have been recognized for causing losses to crop plants, mainly in temperate regions of the world. The importance of cyst nematodes in agriculture was recognized in early 1818 when the European sugar industry was threatened by the infestation of *Heterodera schachtii* in sugar beet fields.

The cyst forming nematodes exhibit unique characteristics of retention of eggs and juveniles within the mature female body, which after its death, turns into a brown and hard body termed as cyst. The cyst protects the eggs and juveniles from adverse environmental conditions. The juveniles are released near the host only when favorable conditions like temperature, moisture, are prevalent.

Cyst nematode species are endoparasites which feed on the root cortex. The type and degree of damage depends upon the host plant, nematode population level and environmental conditions. Cyst nematodes cause very little mechanical injury to the roots. Rarely do they cause lesion or galls. In some instances, damage is limited to necrosis surrounding the region of invasion. However, feeding by these nematodes generally results in the formation of "Giant cells". Infested fields frequently contain concentric patches of plants which exhibit poor growth. Below ground symptoms primarily include stunting and wilting which results from an improper functioning root system. Leaves turn pale green to yellowish red and older plants often become necrotic.

Cyst forming nematodes as a family are further classified into 3 subfamilies (Meloidoderinae, Heteroderinae, Ateloderinae), 6 genera (*Afenestrata, Cactodera, Heterodera, Punctodera, Globodera,*

Dolicodera) and 107 species, which have been described from all over the world so far.

General Morphology

This is a branch of biology that deals with the structure and form of an organism at any stage of its life history. At present the morphology is the basis for taxonomy and classification. In cyst nematodes several life stages are important and useful in both morphology and identification.

Female: Body white or pearly white in appearance, generally spherical, subspherical or lemon shaped. All Heteroderinae females have a narrow anterior protuberance or neck and swollen body. The mature female cuticle is not annulated, except at the head where several prominent annules occur and the lateral field is absent. The stylet and oesophagus are strongly developed with a prominent median bulb. The excretory pore lies at the base of the neck; posterior to the excretory pore the swelling of the body is greatly developed.

The vulva lies at the posterior end of the body. In lemon shaped *Heterodera* and *Cactodera* species, the vulva is raised on a greater or lesser projection of the vulva cone. Some genera lack a cone, such as *Meloidodera* and other genera with globose females also have no cone, including the *Globodera* species. At the female stage, some parts of vulva cone are not fully developed but these structures are prominent at cyst stage and useful in species identification.

The body wall of Heteroderinae females is modified from the A and B layers present in vermiform Tylenchida to include a broad C layer; in some genera, including non-cyst-forming *Atalodera* and cyst-forming *Globodera,* an additional D layer occurs. The presence or absence of the D layer among cyst nematodes was used to support separation of round and lemon-shaped cysts into distinct genera. However, *Heterodera,* previously reported to lack a D layer, was recently found to have a very thin D layer 4 weeks after the final moult. Overall thickness of cuticle does not seem to relate to the number of layers. For example, *Punctodera,* with a D layer, may have a very thin cuticle. Similarity of

layers in the female and layers in the cyst have not been established, nor is it known if particular cuticular layers or other parts of the body are especially responsible for the colour that occurs in many dead females and cysts. In some cases the sequence of colour change as a female matures, is distinctive, as in *G. rostochiensis* which forms a golden, followed by a brown cyst, versus *G. pallida* with no golden stage (Baldwin and Mundo, 1991) **(Fig. 7.1)**.

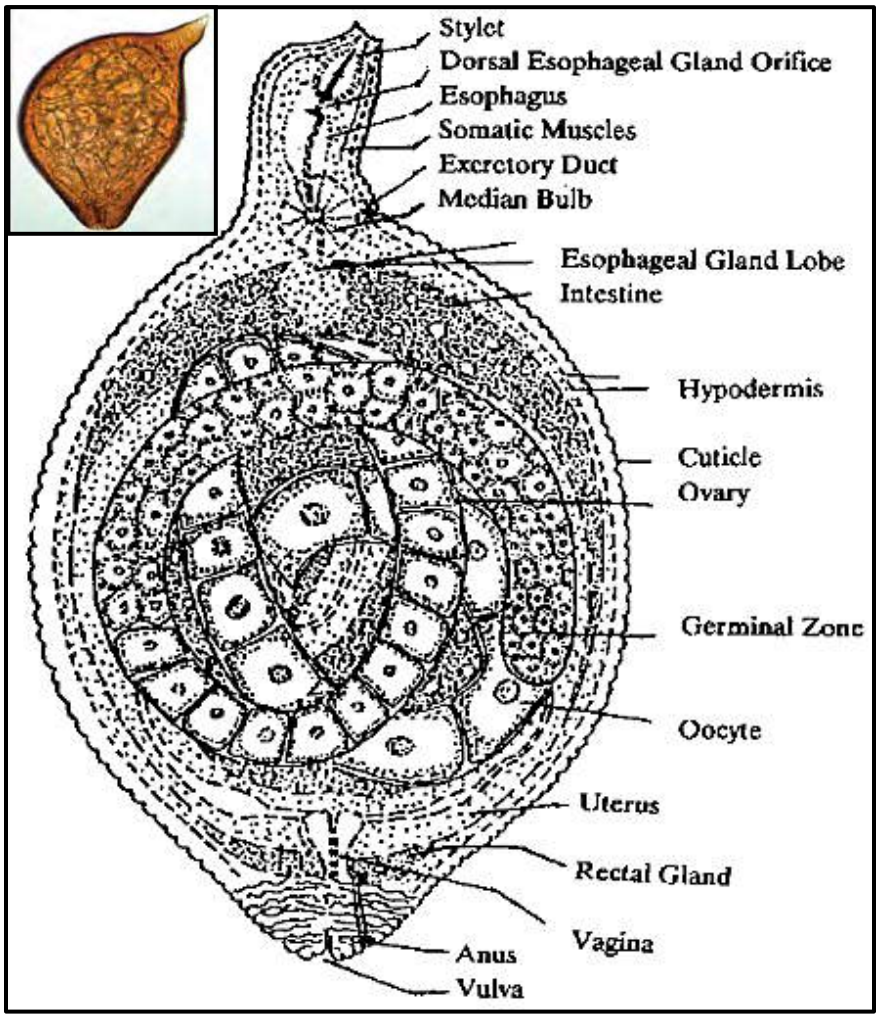


Fig. 7.1. Gross morphology of female of cyst nematodes (From NNRC).

Cyst: Cysts retain the female shape. All cysts have an anterior protuberance representing the neck and many have a posterior

protuberance, the vulval cone. Cysts of the species of *Heterodera, Cactodera,* and *Afenestrata* are are lemon shaped; while the species of *Globodera* and *Punctodera* are basically round, without a posterior protuberance, but the exact shape may be spherical, ovoid or pear shaped **(Fig. 7.2)**.

Color: Cysts range in color from light tan, brown, dark brown, reddish brown to black.

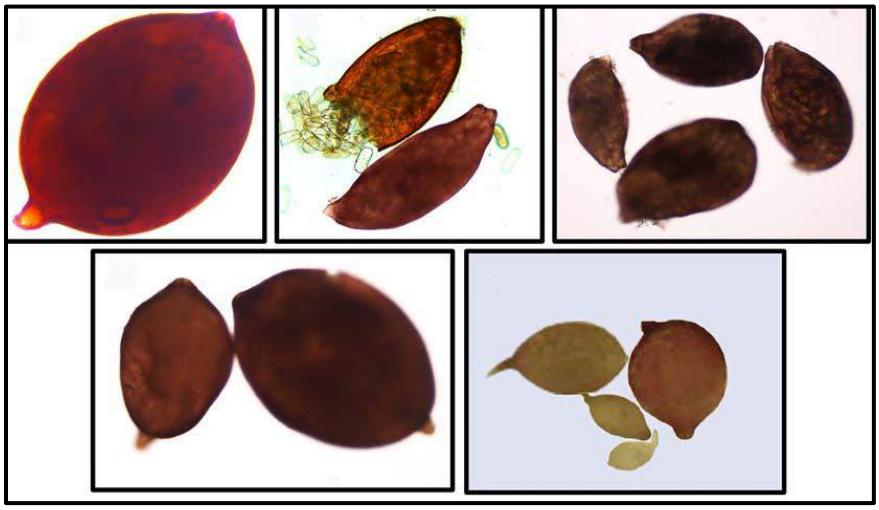


Fig. 7.2. Shapes of cysts of different genera of Heteroderidae (From NNRC).

Wall structure: Cuticular surface patterns, and particularly those of the midbody region, occur in diverse forms among cysts of Heteroderidae. These particular patterns may characterize specific taxa. These midbody patterns broadly include striated and zig-zag pattern. However, particular patterns may be further specified as fine or coarse, ridged, reticulate, lacelike, or punctuate. Striated patterns are common among some noncyst-forming genera including *Meloidodera* and *Cryptodera.* Zig-Zag patterns occur in noncyst genera such as *Atalodera,* as well as most cyst nematodes including *Heterodera.* Zig-zag patterns range from course to fine. They also vary in details of anastomosing and reticulation and they may be associated with punctuations. Punctuations especially characterize *Punctodera* where they occur as distinct rows beneath the

zig-zag surface, but they also occur with varying reliability and clarity in many other genera **(Fig. 7.3)**.

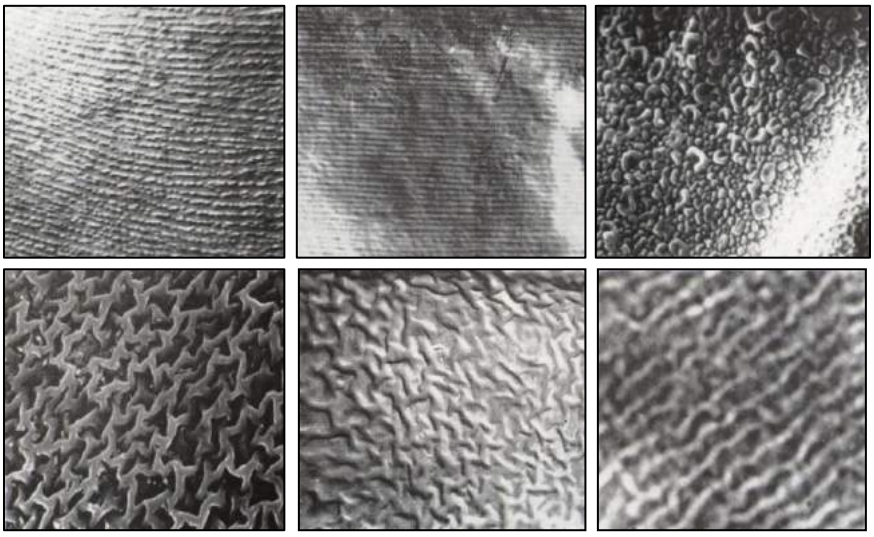


Fig. 7.3. Morphology of cuticulat surface pattern of cyst nematodes (From NNRC).

Cyst Posterior: The nature of the posterior region of cyst is important in both classification and identification of cyst species.

Vulval Cone: Lemon- shaped cyst (*Heterodera*, *Cactodera* and *Afenestrata*) have a protuberance or prominence of the posterior end commonly called the vulval cone. The size and shape of the cone is variable, ranging from prominent and rather narrow in *H. schachtii* and *H. triforlii*, short and nipple like in several *Cactodera* species, to insignificant and button like in some *Cactodera* species, to insignificant and button- like in *C. betulae.*

At the terminal of the cone, is located the vulva slit, which is bordered by the two vulval lips, forming the vulval bridge; the bridge extends across the fenestra. (The thin walled area of the cone terminus) and divides the fenestra into two semifenestrae. However in the monotypic genus *Afenstrata*, fenestrae are not present, and in cysts of *Cactodera* species, the fenestra disintegrates and leaves a round opening, thus

forming a circumfenestrate cyst. In all *Heterodera* species, there are two types of fenestration: ambifenestrate and bifenestrate. In ambifenestrate the semifenestrae on each side of the vulval slit and bridge disintegrate, leaving two openings, each of about one half circles or less, separated by a narrow to medium vulval bridge. In certain species, e.g., *H. carotae* and in some old cysts, the vulval bridge can disappear along with the semifenestrae, giving a false appearance of a circumfenestrate cyst **(Fig. 7.4).**

In bifenestrate the semifenestrae also disintegrate, leaving two openings each; a full or more than half circle on each side of a strong, wide vulval bridge. On cysts of many *Heterodera* species the fenestra is encircled by a band of cuticle which extends to the cuticular surface of the cyst, is called the basin bullae which are prominent, single or branched knob like or finger like structures attached to the cyst wall in the vulval cone. They may be clustered in the distal end of the cone as in *H. avenae,* or rather scattered in the vicinity of the underbridge as in *H. schachtii*. Some species have no bullae.

In a redescription of *H. zeae* Golden and Mulvey (1983) reported four conspicuous finger like bullae always in a distinct arrangement immediately below the underbridge (anteriad), followed by a number of randomized bullae. Vulval denticles were named and described by Golden and Raski (1977) as "small tooth like structures seen below the fenestral surface and within the vulval cone". They are attached to the cone wall; may occur in small clusters or as a partial ring, and were seen in the five *Cactodera* species and in *H. schachtii.*

Externally located on the dorsal surface of the vulval cone is the anus*;* it may be small and inconspicuous to fairly prominent, and in some species e.g., *H. cyperi* and *H. mothi*, it is surrounded by a perineal pattern. A structure often seen attached to or surrounding the vulval cone of females and young cysts is the egg sac or gelatinous matrix in which eggs may be deposited. This is a jelly like substance apparently secreted through the vulva. The above described structures of the vulval cone have much value in species identification.

Terminal Region: This term is used here to refer the vulval anal area and associated structures of all the round cyst species (*Globodera, Punctodera* and *Dolichodera*) and is less restricted than the terminus as defined by Green (1971). All of these species are rounded posteriorly, circumfenestrate, and have a vulva fenestra and small anal pore, except for *Punctodera* species which have an anal fenestra about the same size as the vulval fenestra. Some of the structures discussed below are best seen in females or young cysts as they would not be present in older cysts after fenestration. The vulva is the female genital opening at the external surface and its immediate margin of cuticle, the lips. In *Globodera* species, the vulval slit is 4 to 11µm in length, begins fairly constant for a given species and therefore of diagnostic value in *Punctodera, Dolichodera.* The fenestra is central, thin translucent sheet of tissue with the vulva in its centre; fenestra may be circular or oval and differ in size and shape, which are of diagnostic use.

The fenestral margin is the junction of the fenestra and the cyst wall and can be readily delimited using transmitted light. Adjacent to and outside the dorsal and ventral margins of the fenestra of some species, are the regions containing external oval or rounded protuberances; these regions were designated as anterior and posterior vulva crescents or tubercular crescents; the protuberances, approximately 1µm in width; were named vulval papillae or perineal tubercles. Mulvey (1973) suggested that the tubercles (or papillae) are knob like modification of the cuticular ridges (of the cyst surface) and Hesling (1978) discussed this further and agreed with Mulvey, although he preferred the term "vulval papillae".

These structures also are useful in identification. Fenestral shelves side by the transfenestral bar. The shelves and bars-are especially prominent on *G. pallida* and appear as "D"-shaped shelves of tissue. Near the vulva and parallel to it are two very slender bars called hyaline parapets which resemble structures of the same name associated with the vulva bridge of lemon-shaped species. Cysts of some species have a circumfenestral zone or basin which consists of less pigmented, more translucent tissue than the cyst wall and which may wholly or partially encircle the fenestral area. The perineum or perineal area is the surface between the

vulva and anus; and its length and number of ridges in the cyst pattern is different in different species.

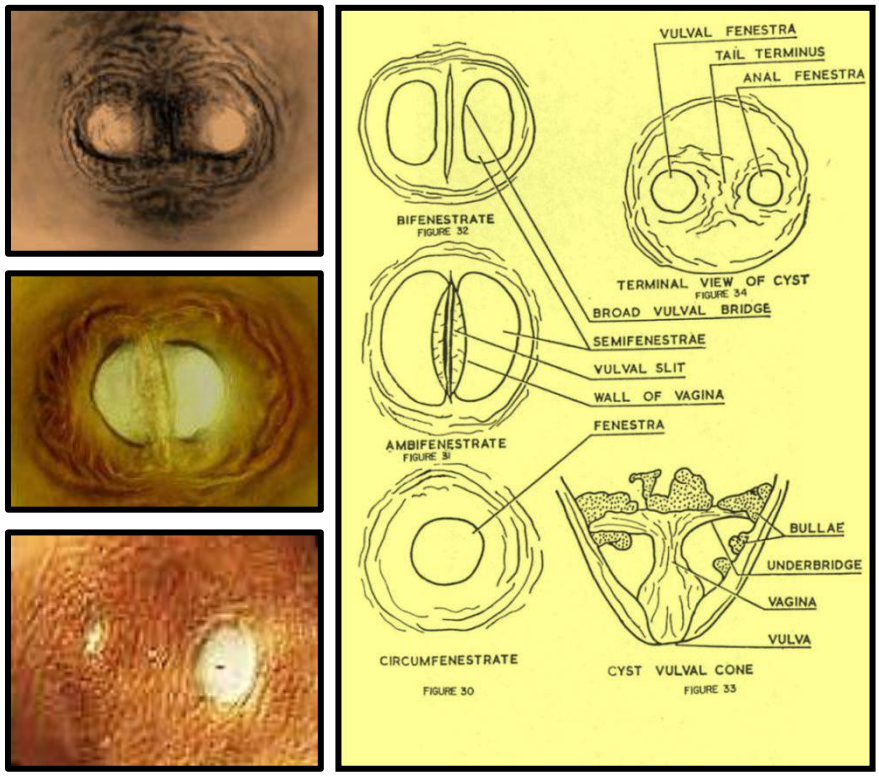


Fig. 7.4. Fenestration of cyst nematodes (From NNRC).

Perineum is useful in identification of certain round cyst species. Also the ridges and furrows of the cyst surface on the perineum form a perineal pattern of two basic types: a maze like or reticulate pattern; and a pattern of parallel ridges. These patterns too are of diagnostic value. Granek's ratio is derived by diving the distance from the anus to the nearest edge of the fenestra by the length of the fenestra. This ratio is of much value in separating certain species where there are rather wide differences in the mean ratio for each species: e.g., *G. rostochiensis* (4.5) and *G. pallida* (1.6). Bullae have been reported in two of the three species of *Punctodera* and the single species of

Dolichodera, but perineal tubercles have not been observed in species of *Dolichodera* and *Punctodera* (Golden, 1986).

Male: Body vermiform with an offset, hemispherical head. The cuticle is annulated and the lateral fields terminate abruptly on the tail. Morphology of the anterior end is similar to that of the 2nd stage juvenile. The median bulb is more slender. The single testis extends anteriorly for 1/3 to 2/3 of body length. Spicules curved, flask shaped. Tail very short, about 1/4 total body widths and bluntly rounded without bursa.

Second Stage Juvenile: Body vermiform, with an offset dome-shaped head and conical tail tapering to a point. The cuticle is annulated with three to four lateral lines, running from near the head on to the tail. The head, stylet and oesophagus are well-developed, median bulb rounded with a prominent valve. The excretory pore is just opposite to theo esophageal glands. Anus prominent and the tail have a clear tip, the hyaline terminus. Phasmids are prominent.

Lifecycle

Eggs of the cyst nematode (*Heterodera*) hatch in the soil, and second-stage juvenile migrate to a host root. There they penetrate completely into the cortex of the root, assume a sedentary position, and establish a feeding site. Sexual dimorphism is also present in this genus. After the female larva moults a third time and becomes fourth stage juvenile, her body swells enough to break through the root cortex and epidermis. By the time she has under gone a fourth moult, her entire body, with the exception of the neck region, is protruding out of the root into the soil. At this point, if the roots are carefully dug and the soil washed away gently, she can be visible to the naked eye as a glistening white speck about the size of a pin head. The number of eggs that are laid depends upon the species. The sugar beet cyst nematode, *H. schachtii* also lays some eggs in a gelatinous matrix. These eggs may hatch under favorable conditions, and another lifecycle can be completed in approximately 30 days. Most eggs, however, are retained within the body of the female. When the female dies, the body wall becomes an encrusted brownish protective

layer (cyst) with the eggs remaining inside. The cyst protects the unhatched eggs from adverse environmental conditions, and parasites and predators for prolonged periods under field conditions. A portion of these eggs or hatched juvenile die each year in the field in the absence of a host, and it has been demonstrated that a 3 to 5 year rotation with a non host crop is necessary to reduce the population to a level where another susceptible host may be grown. Males develop in the same fashion as the females but emerge from the fourth moult as elongated migratory worm that fertilize females. In most species of *Heterodera*, males are required for fertilization of the females and completion of the lifecycle **(Fig. 7.5)**.

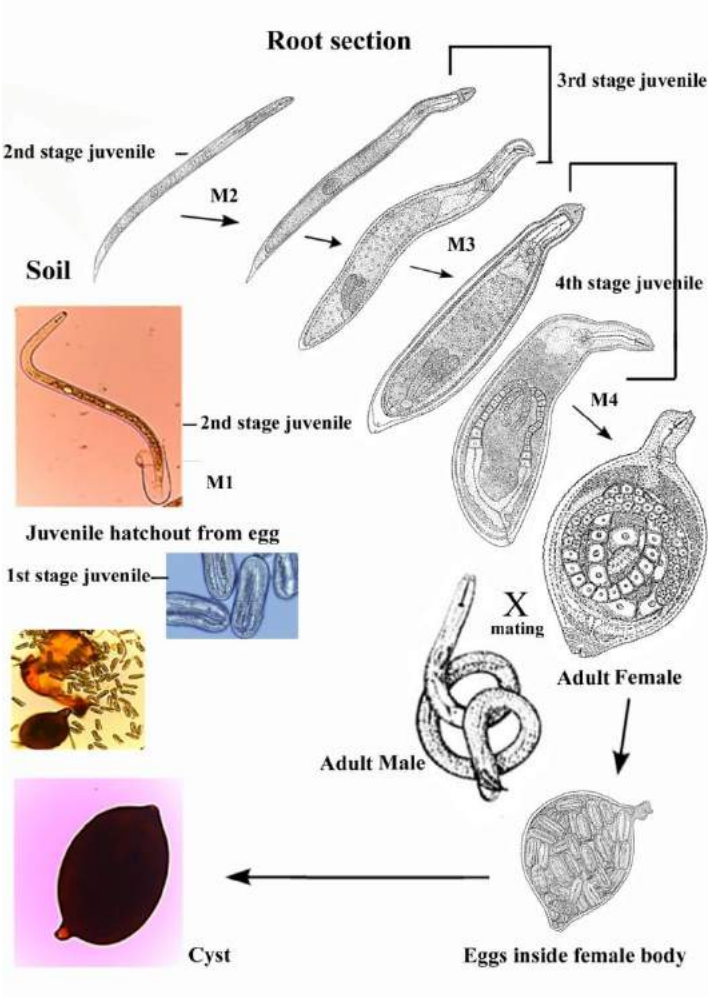


Fig. 7.5. Lifecycle of cyst nematodes (From NNRC).

Morphological characters used for identification

Female / Male

Length, Width, Stylet, Neck length, Dorsal gland opening, Stylet, Lateral field, Spicules, Gubernaculum, Tail

Cyst Second Stage Juvenile

Cyst shape length, Cyst size width, Fenestra length, Fenestra width, Stylet, Dorsal gland outlet, Excretory pore and hemizonid, Median bulb valve, Vulval bridge width, Under bridge length, Under bridge depth, Under bridge width, Vulval slit, Phasmids, Bullae, Tail, Tail terminus, Genital primordium, Lateral field.

Granek's ratio = $\frac{\text{Distance from anus to nearest edge of vulval opening}}{\text{Diameter of vulval opening}}$

Eggs

Length, Width, L/W

Preparation of vulval cone mount for identification

For the identification of cyst nematodes, the general shape of the cyst, the structure, size of the vulva, vulva cone, fenestrae and body wall are used. The method suggested by Golden (1978) is used for the preparation of cyst cone mounts. The cyst is placed in 3% formaldehyde drop on a glass slide and the posterior end is cut, using an eye scalpel, so that the fenestral area is in the centre of the cut piece. The vulval cone and body wall are placed in a drop of lactophenol on another slide and the remaining juveniles are mounted in 3% formalin. The vulva cone is trimmed further in lactophenol. After 3-5 minutes, the cone-top is first transferred into distilled water and then into clove oil to remove water. It is then mounted in Euparal with the cone top projecting upwards.

Key to genera of Heteroderinae

1. Vulval fenestration absent……..……..***Afenestrata***
   Vulval fenestration present……...……..…..……**2**
2. Body with a terminal cone…….……..……..**3**
3. Body without a terminal cone ……...……...……**4**
4. Cuticular surface with roughly parallel ridges; vulval denticles present; circumfenestrate……..…….***Cactodera***
   Cuticular surface with lace-like or zig-zag ridges; vulval denticles generally absent; rarely circumfenestrate…….....…….…….***Heterodera***
5. Anal region with fenestration……..***Punctodera***
   Anal region without fenestration …….……**6**
6. Mature female and cyst spheroidal; perineal tubercles present; bullae usually absent……..…….…….***Globodera***
   Mature female and cyst elongate-oval; perineal tubercles absent; bullae present.....……..……...***Dolichodera***

Identification key to 16 cyst nematode species found in Pakistan (Modified after Golden, 1986)

Key to Genera

1. Cyst without posterior protuberance, spherical, bullae absent, cone circum fenestra……...……..…***Globodera***
2. Cysts, with distinct protuberance, lemon-shaped, bullae present, cone bifenestrate or ambifenestrate…….…..**.....*Heterodera***

Key to Species

1. Granek's ratio, as modified by Hesling (1973), averages 2.0 or less……..**....*G. tabacum***
   Granek's ratio averages 2.5 or more……...……..**2**
2. Number of cuticular ridges between anus and vulval fenestra 12.2 (8-20); Granek's ratio averages 2.5; distance from anus to edge of fenestra 43.9µm (22-67); second stage juvenile sytlet mean length 23.6µm; perineal tubercles small and discrete**...**……...…..***G. pallida***

Number of cuticular ridges between anus and vulval fenestra 21.6µm (16-30); Granek's ratio averages 4.5µm; distance from anus to edge of fenestra 68µm (29-116); second stage juvenile stylet mean length 22µm; perineal tubercles large and clustered……..***G. rostochiensis***

3. Vulval slit very short (6-12µm). Vulval cone bifenestrate. Vulval denticles present or absent, bullae few to many…….**....*Avenae* Group**…....**4**
4. Vulval slit very long, more than 30µm. Vulval cone ambifenestrate, sometime it appears bifenestrate…..…….…….....**5**
5. Cyst dark brown to black. Vulval slit mean length 9.6µm; second stage juvenile stylet knobs shallowly concave anterirorly….***H. avenae***
   Cyst light brown. Vulva slit mean length 6.5µm; second stage juvenile stylet knobs deeply concave anteriorly……..….***H. mani***
6. Bullae strong well developed. Vulval cone ambifenestrate……..***Schachtii* Group**…….**7**
   Bullae absent or few, scattered. Vulval cone ambifenestrate or bifenestrate……***Goettingiana* Group**…...**11**
   Second stage juvenile with three lines in lateral field ……..…**8**
   Second stage juvenile with four lines in lateral field …..……..…..**9**
   Under bridge massive, with finger like projections, bullae few, vulval slit length 50-52µm. Second stage juvenile tail length 55µm (49-60)…..…….**.....*H. sacchari***
   Under bridge weakly to strongly develop without finger like projections, bullae few to many, vulval slit 38-44µm. Second stage juvenile tail length 44µm ……..……...…….……***H. vigni***
7. Bullae located at two levels, level one below under bridge four finger like bullae, level two randomly located long, heavy bullae……..…..……...***H. zeae***
9. Bullae located at one level, typically molar shaped, or small, scattered ……....**10**
10. Fenestra length 32.5µm (24-38), bullae typically molar shaped, second-stage juvenile stylet mean length 25.6µm ……....***H. schachtii***
    Fenestra length 45-68µm, bullae small scattered, second-stage juvenile stylet mean length 23µm.……..…….**.....*H. fici***
11. Second-stage juveniles with three lines in lateral field……...…….**12**
    Second-stage juveniles with four lines in lateral field…….**.....14**

12. Distinct perineal pattern present, cyst elongate, L/W ratio 1.7……...***H. mothi***
    Perineal pattern absent, cysts fairly stout, L/W ratio 1.2-1.3 ……..**13**
13. Bullae present, vulval slit 42-50µm, under-bridge 80-115µm, female stylet length 28-30µm. Second stage juvenile tail length 67-69µm ……..….***H. oryzae***
    Bullae absent, vulval slit 33-40µm, underbridge 60-70µm, female stylet length 19-21.4µm. Second-stage juvenile tail length 40-48 µm ……..…***H. cynodontis***
14. Anus with distinct cuticular pattern. Second-stage juvenile stylet length 16-18 µm, tail length 68-72µm ……...***H. pakistanensis***
    Anus without cuticular pattern. Second-stage juvenile stylet length more than 20 µm and tail length less than 60µm …….....…..……**15**
15. Vulval slit in a cleft of the vulval bridge on the top of the cone; tail length 30-38 µm …….……..……..……...***H. bergeniae***
    Vulval slit simple on the top of the cone, tail length 48-52µm……..….***H. cruciferae***

Chapter 8

NEMATODE LIFECYCLE

All nematodes are very similar in their basic body plan; their genetic diversity is enormous and reflects the long evolutionary route of the phylum. They all possess a hollow protrusible stylet that is used to puncture cell walls, inject secretions, and ingest nutrients from the plant cell. The stylet secretions are synthesized in unicellular pharyngeal glands that are much more developed in PPN than in free living nematodes.

Plant parasitism occurs in both classes of Nematoda: in Tylenchida of class Secernentea and Dorylaimida of class Adenophorea. Plant parasitic nematodes possess a hardened mouth piece, a stylet with which they can puncture plant cells. In Tylenchida (Tylenchina, Hoplolaimina, and Criconematina) the stylet is a stomatostyle developing from tissues of the stoma (mouth). The parasitism in orders Tylenchida and Aphelenchida is either ectoparasitism or endoparasitism as all parts of the plant, above and below ground are attacked by nematodes **(Table 8.1)**.

In the Dorylaimida (Longidoridae and Trichodoridae) the stylet is an odontostyle as it develops from oesophageal tissue; their feeding on plant roots can do direct damage to their host, but they are better known for their ability to transmit plant viruses (Wouts, 2006). The parasitism in Dorylaimida is confined to belowground ectoparasitism.

Plant parasitic nematodes feed on living plant tissues, using an oral stylet, a spearing device like a hypodermic needle, to puncture host cells. Many, probably all, plant nematodes inject enzymes into a host cell before feeding to partially digest the cell contents before they are sucked into the gut. Most of the injury that nematodes cause plants is related in some way to the feeding process.

On the basis of their feeding habits, plant parasitic nematodes can be divided into following categories: ectoparasites, endoparasites and semi-endoparasites **(Fig. 8.1)**.

Ectoparasitic nematodes: Ectoparasitic nematodes generally remain on the surface of the plant roots and feed by inserting the stylet into cells that are within reach. All stages of the migratory ectoparasites feed on the roots.

Ectoparasitic nematodes can be grouped according to their parasitic strategy into:

a. Migratory ectoparasites: Migratory ectoparasitic nematodes remain outside of the roots and stay vermiform throughout their lifecycle. They feed externally for short periods along the root systems on epidermis (*Tylenchorhynchus, Helicotylenchus, Rotylenchus*) or cells deeper in the root (*Belonolaimus, Dolichodorus*). Longidoridae and Trichodoridae are migratory root ectoparasites. They cause direct damage to the wide variety of plant crops and also act as vectors of plant viruses (*Longidorus, Paralongidorus, Xiphinema, Trichodorus, Paratrichodorus*).

b. Sedentary ectoparasites: Sedentary ectoparasitic nematodes may feed for several days on the same cell (cortical or epidermal) often on the root tip. Members of the superfamily Criconematoidea are sedentary ectoparasities (*Paratylenchus, Criconemoides, Criconema, Hemicriconemoides, Hemicycliophora*).

Endoparasitic nematodes: Endoparasitic nematodes conduct most of their vital processes inside the roots, their motility and feeding habits resulting in death of cells. Some species of the genera *Anguina, Aphelenchoides* and *Ditylenchus* are endoparasites (migratory ecto/endo) of the flower, buds, bulbs, stem, leaves and inflorescences. They are divided into two groups:

a. Migratory endoparasites: Migratory endoparasitic nematodes invade the roots, completely penetrate the plant tissues, migrate intracellular within the cortex, remain mostly vermiform as they move through and feed on the plant cells. The nematodes of this group do not induce permanent feeding sites (*Pratylenchus, Radopholus, Hircshmanniella*).

b. Sedentary endoparasites: In sedentary endoparasites, the stylet is small and delicate, the immature female or juvenile nematodes completely enter the plant root tissues where they develop a permanent feeding site, become immobile, swell and become obese. Sedentary endoparasite females may be transformed into a cyst containing J_2 within eggs (*Heterodera*, *Globodera*) or the females may be non-cyst forming (*Meloidogyne* spp.).

Semiendoparasitic nematodes: Sedentary semiendoparasitic nematodes only partially penetrate the roots, leaving the posterior half to two thirds of the body projecting into the soil. These nematodes become immobile at a fixed feeding site and the projecting posterior of the female body swells (*Tylenchulus*, *Rotylenchulus*).

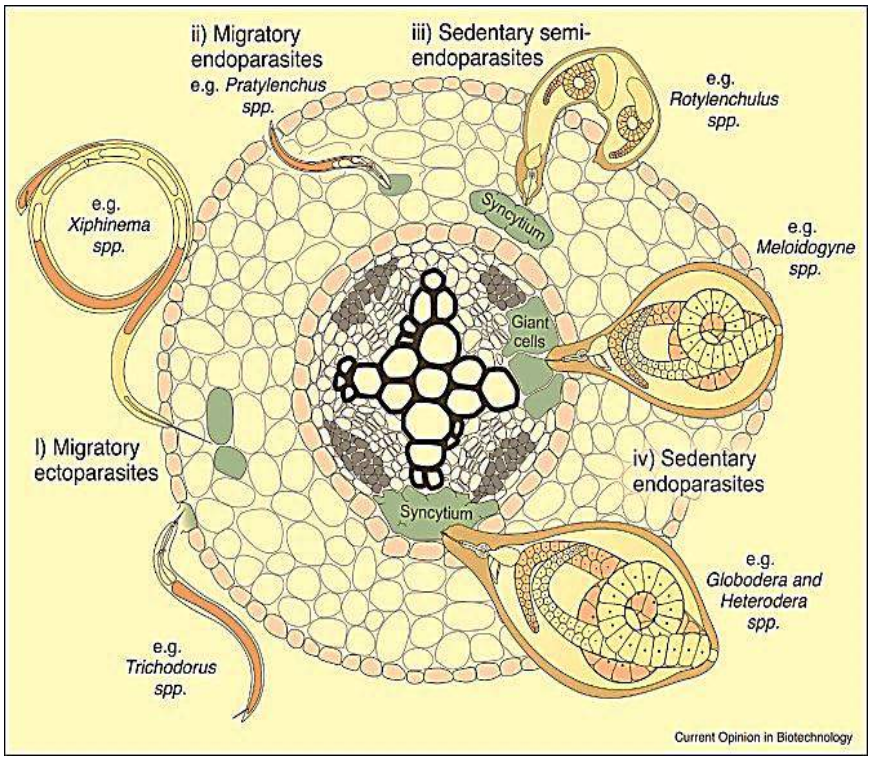


Fig. 8.1. Nematode parasitism patterns (Sebastian E.A. *et al*., 2021).

A plant-parasitic nematode has six phases in its lifecycle: egg, four juvenile stages, and adult. Most species have both male and female nematodes, however reproduction without males is frequent, and some species are hermaphroditic (Females capable to produce both sperm and eggs). The cycle is completed when the individual produces eggs. Depending on the nematode species and their surroundings, most females produce between 50 and 500 eggs, although others can generate over 1,000 eggs. The length of the lifecycle differs significantly depending on the nematode type, host plant, and environment temperature. Many plant nematodes complete their lifecycle in about four weeks during the summer months, when soil temperatures reach 80 to 90°F.

Lifecycle

Ectoparasitic Nematodes: This is the initial feeding mode, in which the nematode remains outside of the plant and feeds from the cells of the plant roots with its stylet. This strategy allows nematodes to graze on a variety of plants, making it easier for them to move hosts, but it also makes them more vulnerable to environmental changes and predators. Ectoparasitic nematodes can have extraordinarily lengthy stylets, which help them feed on nutrient-rich plant cells deep within the plant root. Some of these nematodes cause the plant to produce larger cell(s) from which the nematode feeds for a long time. The term J=juvenile and the number refers to the worm's stage, while M=moult refers to how many moults the nematode has performed throughout all lifecycle.

The lifecycle shown **(Fig. 8.2)** is typical for nematodes in the Enoplea class, although most nematodes in the Chromadorea class, moult in the egg and hatch as J_2. The plant is capable of feeding all motile nematode stages. The nematodes eat, moult four times to become adults, mate, and lay eggs. Some nematodes that use this feeding strategy can form terminal galls in the roots and cause severe damage. Some nematodes that adopt this feeding method can cause severe root stunting by forming terminal galls in the roots. *Xiphinema* (dagger nematode) feeding on a fig root as an example of an ectoparasite. It can host and transmit plant viruses; this enoplean nematode is extremely dangerous. Only ectoparasitic nematodes in the Enoplea class transmit viruses, however even in

low numbers, this nematode can be exceedingly destructive to plants due to virus transmission (Fisher and Raski, 1967; Hewitt *et al*., 1958) causing stunting of the root system.

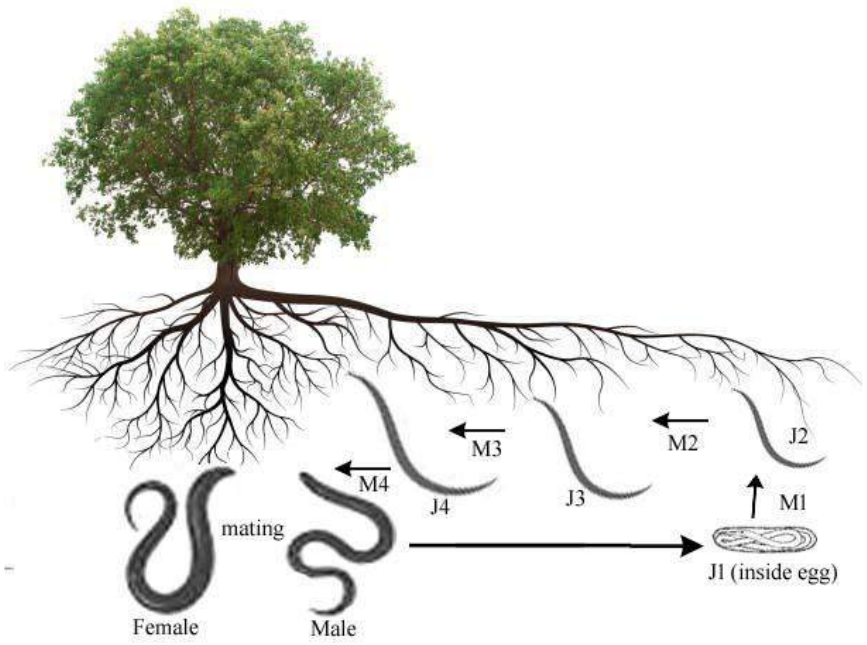


Fig. 8.2. Lifecycle of ectoparasitic nematodes (From NNRC).

Semi-endoparasitic Nematodes: Semi-endoparasite nematodes have the ability to partially penetrate the plant and feed at some time throughout their lifecycle **(Fig.8.3)**. The nematode's head usually enters the root, allowing the nematode to establish a permanent feeding cell. Once they enter the endoparasitic phase of their lifecycle, these nematodes enlarge and stop moving. The nematodes risk mortality if their host plant dies by giving up their mobility, but they gain a permanent feed location, which boosts their nutrient intake and reproductive capacity. *Rotylenchulus reniformis*, the reniform (kidney-shaped) nematode, is an example of a nematode having this lifecycle. This nematode hatches as a J_2 from the egg, then moults to the adult stage in the soil without feeding. An adult female's anterior end enters the plant root and develops a feeding cell. After mating, the female lays her eggs in a gelatinous egg-mass outside of the root.

The citrus nematode, *Tylenchulus semipenetrans*, has a similar feeding pattern, though the juvenile stages of this nematode do feed as ectoparasites. Due to variety in behavior, it is often difficult to exactly classify animals, as is usual in biological systems. Several kinds of ectoparasitic nematodes (e.g. *Helicotylenchus*) can partially penetrate the root and feed as a result of this rule. However, because these nematodes do not demonstrate a continuous endoparasitic feeding habit, we do not designate them as semi-endoparasites (Maggenti, 1981) **(Fig. 8.3)**.

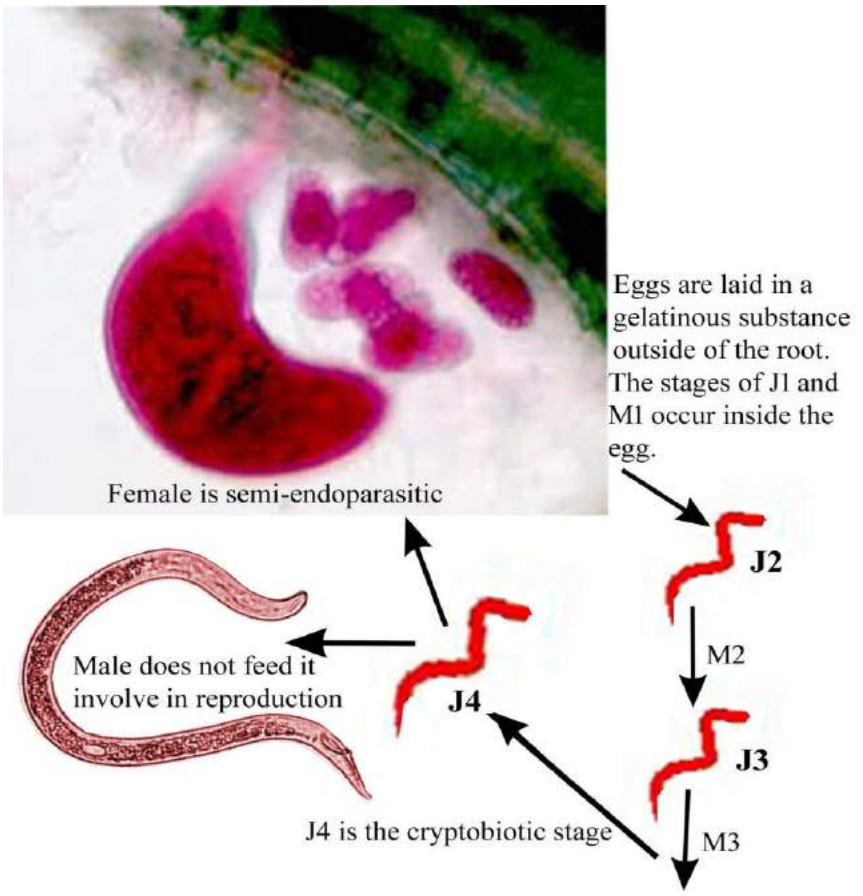


Fig. 8.3. Lifecycle of semi-endoparasitic nematodes (From NNRC).

Sedentary Endoparasitic Nematodes: These are the most harmful nematodes among all parasitic ones. The cyst nematodes (*Heterodera*

and *Globodera*) and the root-knot nematodes are the two dominant nematodes in this group (*Meloidogyne*). The J_2 invades the plant near the root tip and migrates through the tissue to the developing vascular cells. During their early stages of growth, these nematodes are totally lodged in the root, but later on, the cyst nematodes protrude from the root. J_2 nematodes inject secretions into and around plant cells to promote the growth of giant feeder cells, which they feed on in a nondestructive manner throughout their lifecycle. In the absence of cell division, root-knot nematode feeding cells (giant cells) form by repetitive nuclear division. Cyst nematode feeding cells are created by the fusion of adjoining cells into a syncytium formed by the disintegration of neighbouring cell walls.

Because their somatic muscles atrophy once the feeding cells are developed, the juvenile nematode quickly becomes sedentary. The juveniles feed, grow, and moult three times before reaching adulthood. These nematodes block the plant's vascular tissue with enormous feeding cells, making it vulnerable to water stress. Female sedentary endoparasites grow to a sac-like structure and can lay a large number of eggs. In most nematodes, eggs are laid in a gelatinous egg mass outside the nematode, however, in cyst nematodes, the majority of eggs are maintained inside the female body. Both types of nematodes eat in the same way, however, many cyst nematodes have an obligate sexual cycle, whereas most root-knot nematode species reproduce primarily through parthenogenesis.

Cyst nematodes are particularly troublesome since they can survive in a field for lengthy periods of time. Their capacity to develop a cyst, which is the hardened dead body of the female nematode, that covers the eggs, accounts for their exceptional tenacity. The cyst's eggs are latent, and they develop in the field over after years, usually in response to signals from the host roots. Nematodes with a resistant or dormant stage can easily survive as compared to those nematodes which require ideal conditions and hosts. Root-knot nematodes lack an environmentally resistant stage; they do have a wide host range that allows them to survive on a different host. Plant nematodes have two essential tactics to

survive in the environment: environmentally resistant nematode stages and a wide host range **(Fig. 8.4)**.

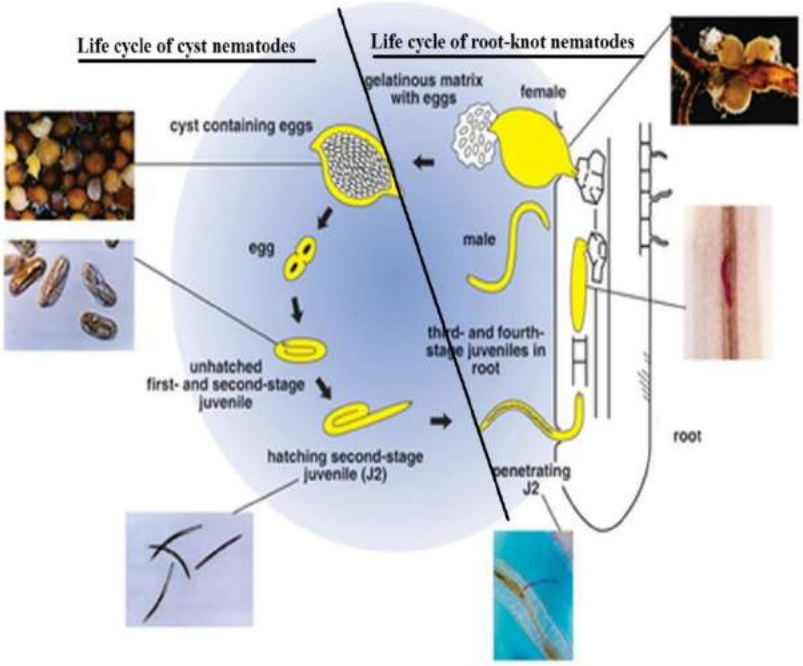


Fig. 8.4. Lifecycle of sedentary endoparasitic nematodes (From University of Minnesota Extension).

Stem and Bulb Nematodes: Stem and bulb nematodes (*Ditylenchus* spp.) are nematodes that attack the top and lower sections of plants, respectively. They travel up the stem of the plant, using water films, making them more harmful under wet situations. In the lifecycle of the stem and bulb nematodes, fourth stage juvenile is the "infectious stage". This stage usually enters emerging plant tissues below ground, although it can also climb up stems in a layer of water and enter shoots through buds, petioles, or stomata. They feed destructively as migratory endoparasites on the host plant, moult into adults, and reproduce. The J_2 nematodes hatch from the egg and feed, moult, and reproduce, macerating and warping the plant tissue significantly. The stem and bulb nematode juveniles stop developing at the environmentally resistant J_4

stage and overwinter after the plant is destroyed or winter arrives. "Nematode wool" is a fluffy pile of dried (cryptobiotic) *Ditylenchus* spp. which can be visible on the surface of bulbs. This is the cryptobiotic J_4 which become active once environmental conditions are favorable, and then their lifecycle resumes again (Hooper, 1972) **(Fig. 8.5)**.

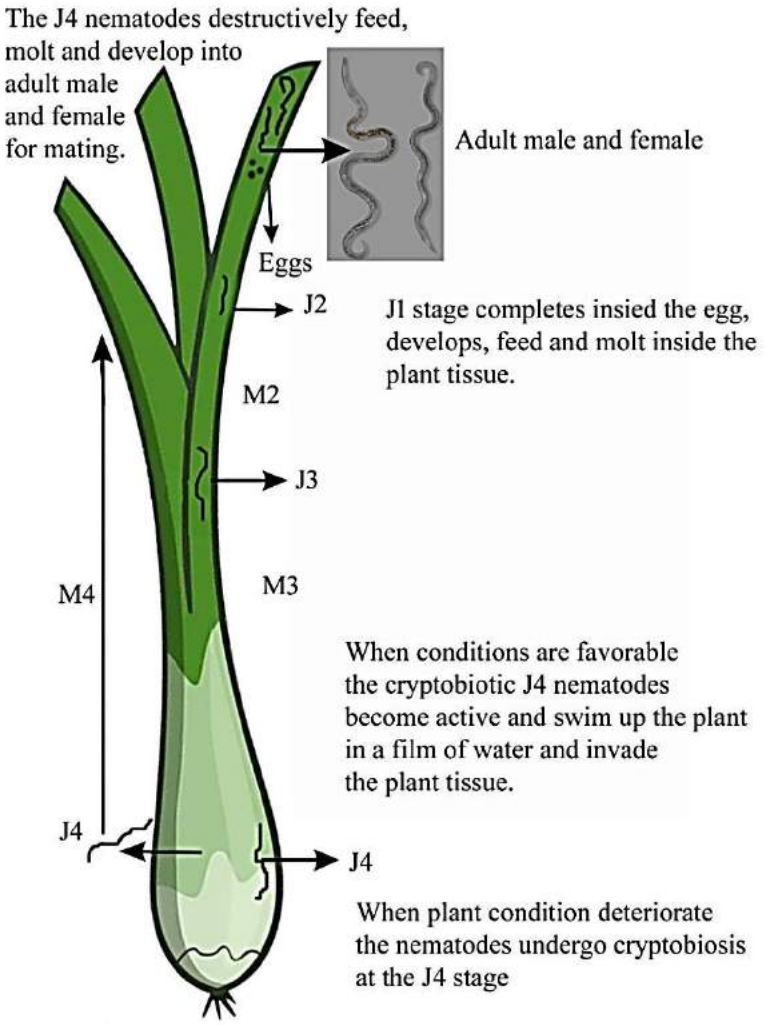


Fig. 8.5. Lifecycle of stem and bulb nematodes (From NNRC).

Pine Wilt Nematodes: The nematode *Bursaphelenchus xylophilus* (pine wood nematode) infects pine trees and has a unique and fascinating

lifecycle. In the tracheae of bark beetles, resistant stages of these nematodes are transmitted into pine trees. The nematodes are freed from the beetle once within the tree, and they travel through the tree's resin canals. Pine wilt disease is caused by the pine wood nematode, which feeds destructively on cells in resin canals and the vascular structure by blocking them. The nematodes feed, moult, and reproduce sexually at a breakneck speed. The population of pine wood nematodes grows quickly and kills the host tree quickly. After the plant cells have died, the nematodes can feed on fungi in the tree. The J_3s dispersed through the winter and then are attracted to beetle juvenile.

The nematodes moult to the resistant J_4 stage (cryptobiotic) in the spring and enter the beetle, dispersing to other trees. Because nematodes dispersed by insects, they can spread quickly from tree to tree, causing a forest to be destroyed quickly (Mamiya, 1983). This nematode is assumed to be endemic to the United States, where all native trees are resistant to its harmful effects; nevertheless, pine tree species in Asia and Europe are extremely sensitive, resulting in the annual destruction of millions of trees **(Fig. 8.6)**.

Seed Gall Nematodes: In 1743, seed gall nematodes (*Anguina* spp.) were described as the first plant-parasitic nematodes. These nematodes travel as J_2s through water films to plant leaves, where they feed as ectoparasites at the tips, producing leaf deformation. J_2 penetrates the floral primordia and begins feeding on the developing seed as the plant begins to blossom. The nematode moults once within the seed, continues to feed, and eventually kills the seed to produce a blackened "cockle" (seed gall).

The adults reproduce sexually, the eggs hatch as J1s, and the juvenile soon moult into the J_2 survival stage. Within the seed gall, the environmentally resistant J_2 desiccates and overwinters. If kept dry, the nematodes in the seed gall can live for up to 30 years. The cryptobiotic J_2 becomes active when the necessary moisture and temperature conditions are met. When proper moisture and temperature conditions arise, the cryptobiotic J_2 becomes active and starts the lifecycle over again (Southey, 1972) **(Fig.8.7)**.

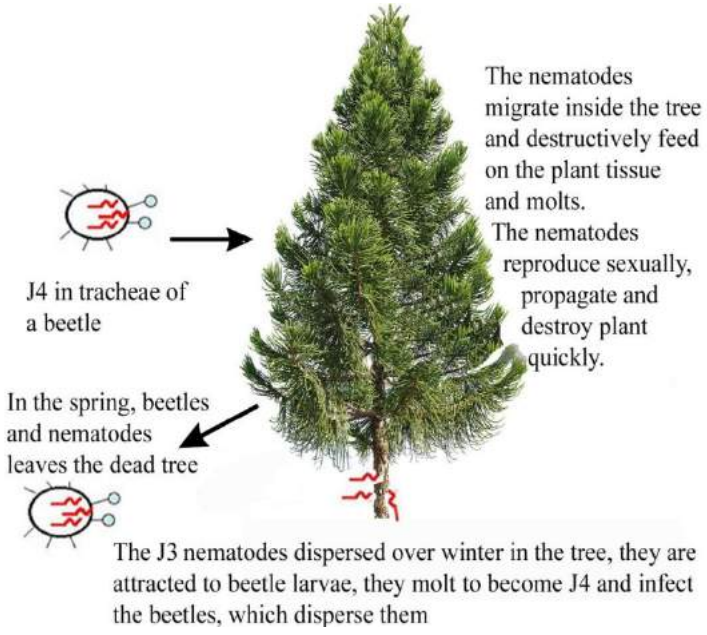


Fig. 8.6. Lifecycle of Pine wood nematodes (From NNRC).

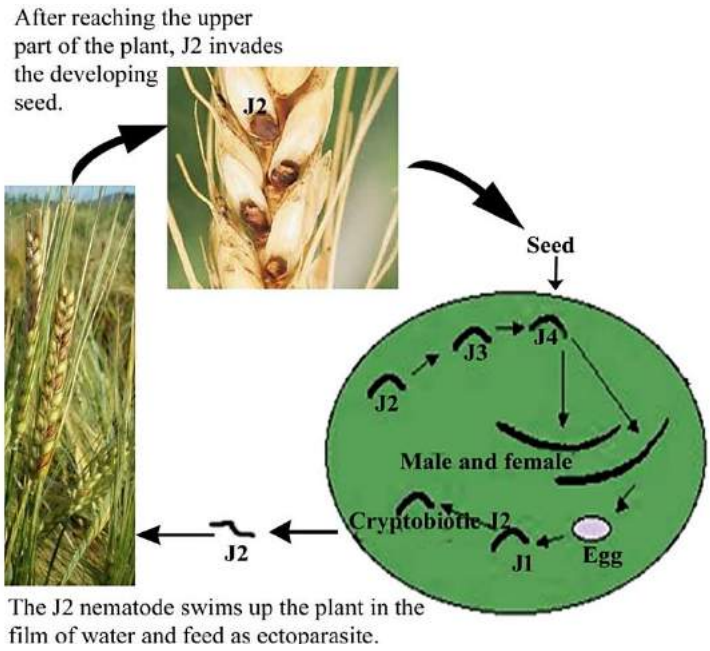


Fig. 8.7. Lifecycle of Seed gall nematodes (From NNRC).

Foliar Nematodes: Foliar nematodes are members of the *Aphelenchoides* genus. Adult nematodes move to the leaves of their host plant by water films on the stems and penetrate the leaves through natural holes (stomata). The nematodes move to the leaves, eat destructively, moult, and lay eggs. The nematodes' feeding activity causes interveinal chlorosis and necrosis of the leaf, which eventually kills it. If the favourable (wet) climatic conditions exist, the nematodes can spread from leaf to leaf, causing serious damage to the plant. Adult nematodes survive the winter in the dead leaves, waiting for optimal conditions to emerge in the spring. The nematode will be dispersed near new host plants if the dead, nematode-infested leaves are disturbed or blown around (Hesling and Wallace, 1961) **(Fig. 8.8)**.

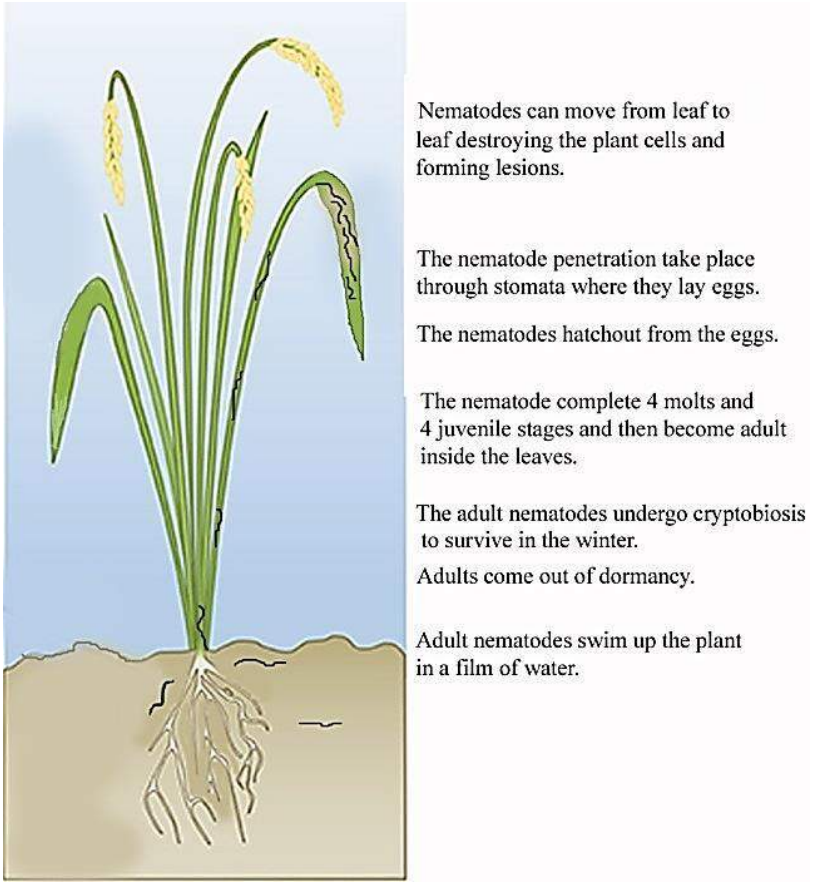


Fig. 8.8. Lifecycle of foliar nematodes (From NNRC).

Table 8.1. Summary of Plant Parasitic Nematode Feeding Strategies.

Feeding Strategy	Example Genera	Order	Infective Stage	Resistant Stage	Notes
Ectoparasite	*Belonolaimus* *Xiphenema* *Trichodorus*	Tylenchida Dorylaimida Triplonchida	J2-adult J2-adult J2-adult		Vector viruses Vector viruses
Semi-Endoparasites	*Rotylenchulus* *Tylenchulus*	Tylenchida Tylenchida	J4 J2	J4 J2	
Migratory Endoparasites	*Pratylenchus* *Radopholus*	Tylenchida Tylenchida	J2-adult	*	
Sedentary Endoparasites	*Meloidogyne* *Heterodera* *Naccobus*	Tylenchida Tylenchida Tylenchida	J2 J2 J2	Egg/cyst	
Stem and Bulb Nematodes	*Bursaphelenchus* *Ditylenchus*	Tylenchida Tylenchida	J4 J4	J3 J4	J4 vectored by insects
Seed Gall Nematodes	*Anguina*	Tylenchida	J2	J2	
Foliar Nematodes	*Aphelenchoides*	Tylenchida	J2-adult	Adult	

* eggs, all juvenile stages and adults can survive in the winter, but not egg producing females.

Chapter 9

DISEASES CAUSED BY NEMATODES, THEIR SYMPTOMS, ETIOLOGY AND MANAGEMENT

Nematodes parasitic on crop plants can be very damaging but due to their microscopic size, they are invisible to the naked eyes; their association with crop damage is therefore mainly established by determining the symptoms of their effect on plant growth and health. Some nematodes cause distinctive diagnostic symptoms; while others produce non-specific symptoms. Symptoms of nematode infestation vary according to the nematode species involved and the crop type. The symptoms can be visible on above ground plant parts; however, the specific nematode induced damage symptoms can often be seen only on the below ground plant organs e.g., roots, bulbs, tubers and corms. Therefore, a sound diagnosis should be based on symptoms above and below ground. Some of the nematode diseases named on the basis of their symptomology are enlisted in **Table 9.1 & Fig. 9.1**.

Table 9.1. Important diseases caused by nematodes.

Diseases	**Nematodes**
Root-knot disease	*Meloidogyne* spp.
Ear cockle disease	*Anguina tritici*
Potato disease	*Globodera* spp.
Dry rot disease	*Ditylenchus* spp.
Lesion disease	*Pratylenchus* spp.
Slow decline disease	*Tylenchulus semipenetrans*
Spreading decline disease	*Radopholus similis*
Red ring disease	*Rhadinaphelenchus cocophilus*
Molya disease	*Heterodera avenae*
Ufra disease	*Ditylenchus angustus*
White tip disease	*Aphelenchoides besseyi*
Pine wilt disease	*Bursaphelenchus xylophilus*
Reniform nematode	*Rotylenchulus reniformis*

The importance of nematodes in agriculture can be anticipated by their devastating damage to major crops. Some of the important nematode species causing plant diseases and severe damage to economically important crops are given below:

Root-knot Disease (*Meloidogyne* Göldi, 1887)

The species of the genus *Meloidogyne* Göldi, 1887, commonly known as "root-knot nematodes (RKN)" are obligate endoparasites of great economic importance. They are the most destructive, damaging and economically important group of plant parasitic nematodes having significant impact on major crops worldwide (Hunt & Handoo, 2009). They are among the major limiting factors in the production of field and plantation crops. RKN species are especially prevalent in warm temperate and tropical regions. They have worldwide distribution and have broad host range; they parasitize nearly every species of cultivated and higher plants. They produce galls or root-knots on plant roots of varying sizes, hence acquired the name; they cause great damage to agricultural production all over the world. Species of major economic importance worldwide are: *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (Sasser and Carter, 1985; Jepson, 1987; Perry *et al*., 2009). In Pakistan vegetable crops are found highly susceptible to these nematodes with overall 50-60% incidence of the disease (Maqbool, 1990; Zarina and Shahina, 2012).

Disease: The species of the genus *Meloidogyne* Göldi, 1887 cause the root-knots or galls on plant roots.

Symptoms: Symptoms associated with RKN can be easily diagnosed with the presence of distinctive galls or knots on the root systems. RKN infected plants show yellowing of leaves, stunting growth, abnormalities in root formation, nutrient deficiencies symptoms and may lead to secondary infections by other pathogens (Hunt and Handoo, 2009). Galled roots show rotting symptoms afterwards. Yield losses depend on the nematode species involved, initial population of nematodes, and the cultivated crop species (Ornat and Sorribas, 2008).

Etiology and Lifecycle: Root-knot nematodes are endo-sedentary parasitic nematodes. The second-stage juvenile (J_2) is the infective stage. After RKN hatch from eggs, the J_2 migrates through the soil towards suitable root and uses special enzymes and the stylet to force penetration into the vascular cylinder, where RKN establishes its feeding site by inducing hypertrophy and hyperplasia of a group of cells leading to swelling and formation of giant cells. On this site, nematode goes through three more ecdysis (moulting) to become a swollen young female. Mature females begin laying eggs in the root, forming egg-masses wrapped in a gelatinous matrix. Each egg-mass contains 400–500 eggs on average, and it is formed in the midst of cortical parenchyma or on the surface of the roots (Lima *et al*., 2018).

Management

- Deep ploughing 2-3 times during May-June.
- Deep summer ploughing of nematode infested fields 2-3 times at 10-15 days interval.
- Solarization of nursery beds with 25 μm clear plastic film for 15 days during May/June.
- Rotation with non-host crops like cereals.
- Use of nematode-free transplants and raising nurseries in root-knot nematode-free soil.
- Seed treatment with carbosulfan (25ST) or Bacillus macerans at 3% w/w.
- Application of neem cake 1 t/ha at sowing in the main-field.
- Application of carbofuran (3G) @ 10 g/m2 in the nursery beds prior to sowing and neem cake @ 500 kg/ha 10 days before transplanting in the field.
- Soil application of carbofuran (3G) @ 1-2 kg a.i./ha at sowing.

Cyst Nematodes (*Heterodera/Globodera*)

Cyst forming nematodes or cyst nematodes (*Heterodera/Globodera* spp) rank second to root-knot nematodes in agricultural and economic importance. Cyst nematodes cause plant diseases mostly in the temperate regions of the world but several species of this group (mainly *Heterodera*

spp.) have been found in tropical and subtropical environments. Some species of cyst nematodes are host specific with limited geographical occurrence, whereas other species attack a large number of plant species and are worldwide in distribution. They derive their common name from the swollen (cyst like) endoparasitic female, which contains fully embryonated eggs inside its body, protected by the hardened cuticle. Cyst nematodes have the ability to persist for many years in the soil in the absence of a host (Sharma, 1998). In agriculture, the most significant cyst nematode species are the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*, the soybean cyst nematode (*Heterodera glycines*) and cereal cyst nematodes (CCNs) including *Heterodera avenae* and *H. filipjevi* (Bernard *et al*., 2017).

Cereal Cyst Nematodes (*Heterodera avenae*)

Cereal cyst nematodes (CCNs) are the most important pathogens of cereal crops in many parts of the world, with particularly adverse effects on the production of wheat and barley. Three species of CCN belonging to the *Heterodera avenae* group have been identified in cereal fields. *H. avenae* is widely distributed in temperate wheat-producing regions worldwide.

Disease: Molya disease of wheat and barley.

Symptoms: The plants heavily infested with the cyst nematodes are stunted with uneven patchy growth, reddish yellow leaves with narrow leaf blades, and develop bushy root system. Plant size and number of patches are directly related to nematode population level and their distribution in the field.

Etiology: Cyst nematodes enter root tips and induce specialized feeding structures in the infected plant roots called syncytia via esophageal gland secretions released through the stylet. These secretions promote cell wall degradation and protoplast fusion of numerous adjacent cells to form the syncytium.

Lifecycle: There is only one generation of *H. avenae* during a cropping season. Sexual diamorphism is present; males are vermiform while females become lemon shaped and spend their life inside or attached to a root.

The nematode is active only during the growth period of crops while it remains in cyst form containing eggs and larvae during the rest of the year. During the sowing season of the crop, the juveniles emerged from the cysts in the soil; penetrate into the host root tips, take position in the stellar region and start feeding on the specialized cells called syncytia surrounding the head. Some juveniles undergo 3 moults and after 3-4 weeks, young white swollen females are formed on the roots; while those juveniles which become males, remain vermiform and move out of the root tissue in the soil. Each fertilized female lays approximately 200-500 eggs which are retained in the body. The young white females change color from yellow to brown or dark brown. The mature brown cysts detached from the roots at crop harvest and remain in dormant state until the conditions become favorable.

Management

- Two-three deep summer ploughings during May/June.
- Crop rotation with non-host plants such as; mustard, chickpea, seed spices for 2-3 years.
- Growing resistant varieties.
- Early sowing of wheat by a fortnight.
- Application of carbofuran (3G) @ 1 kg a.i./ha at sowing.
- Seed treatment with *Azotobacter chroococcum* (strain HT 54).

Potato Cyst Nematodes (*Globodera* spp. Skarbilovich, 1959**)**

The potato cyst nematodes (PCN), *Globodera* Skarbilovich, 1959, are one of the most limiting plant parasitic nematodes around the world. Within the genus, *G. rostochiensis and G. pallida* are very important species in agriculture. The PCN, *Globodera rostochiensis* (golden or yellow potato cyst nematode) and *Globodera pallida* (pale potato cyst nematode) cause major losses in potato (*Solanum tuberosum* L.) crop and

are also considered as official quarantine pests in many countries. If PCN species are left uncontrolled, they may reduce potato yield up to 80%, representing major economic losses in the potato industry worldwide. Due to the small size and cryptic nature of these nematodes within large volumes of soil, their intimate association with host and their adaptation for long-term survival in the soil in the absence of a suitable host is the main devastating character of this nematodes. PCN is recognized throughout the temperate regions of the world as one of the most difficult crop pests to control.

Disease: The potato cyst nematodes, *Globodera rostochiensis* (golden or yellow potato cyst nematode) and *Globodera pallida* (pale potato cyst nematode) cause potato diseases worldwide. Potato cyst nematodes are obligate sedentary endoparasites.

Symptoms: Above-ground symptoms due to PCNs are not specific and often go undetected. General symptoms include patches of poor growth in the crop, with plants sometimes showing yellowing, wilting or death of foliage; tuber size is reduced and roots are extensively branched with soil stuck to them. PCN can cause stunting of plants, reduce yields, and sometimes lead to complete crop failure. However, there are many other causes of these symptoms; plants should therefore be lifted up for a visual check for the presence of cysts and young females on the roots. Young females and cysts are just visible to the naked eye as tiny white, yellow or brown pin-heads on the root surface. Infested potato plants have a reduced root system and, because of the decreased water uptake, death of the plant can eventually occur.

Lifecycle: PCN requires 38-48 days to complete its lifecycle, depending on soil temperature. They reproduce sexually. After mating, each female produces approximately 200-500 eggs, dies, and the cuticle of the dead female forms a cyst. Eggs mostly remain dormant within the cyst until receiving a hatching stimulus by host plant roots. PCN eggs can remain dormant and viable within the cyst for at least 30 years and are resistant to nematicides. When soil temperature rises above 10° C, second-stage juveniles hatch from the eggs, escape from the cyst, and migrate towards the host plant roots. Juveniles penetrate roots; begin feeding on cells in

the pericycle, cortex or endodermis. The nematode induces enlargement of the root cells and breakdown of their walls to form a large, syncytial transfer cell. This syncytium provides nutrients for the nematode. After feeding, the juvenile grows and undergoes three more moults to become an adult. Females grow and become round, break through the roots and expose the posterior portion of their body to the external environment. Male juveniles remain active, feed on the host plant until maturity, and then they stop feeding, become vermiform, and seek females. Adult males do not feed. Sex is determined by food supply; more juveniles develop into males under adverse conditions and heavy infestations.

Management

- The potato cyst nematodes are among the most difficult pests to control.
- In countries that are free of the potato cyst nematodes, quarantine measures can help prevent their introduction; in countries where their occurrence is localized, local quarantine can help prevent further spread.
- When a non-host crop is grown, population levels of the potato cyst nematodes can be reduced.

Soil solarization has been used to control *G. rostochiensis* in some countries where populations were reduced by 96-99% within the top 10 cm of soil.

Root Lesion Nematodes (*Pratylenchus* Filipjev, 1936)

Pratylenchus spp., are migratory endoparasites. They have a broad host range and are widely distributed in tropical and subtropical regions. The root lesion nematodes are ranked third after root-knot and cyst nematodes as the nematodes of greatest economic impact in crops worldwide. This is not only due to their wide host range (more than 400 crop plant species), but also because of their distribution in almost every temperate and tropical environment around the globe.

Pratylenchus species feed on a wide range of important crops of primary importance including cereals, legumes, vegetables, fruit trees, ornamentals, coffee, peanut, ramie, etc. Root lesion nematodes compromise approximately 100 valid species, distributed around almost every cool, temperate and tropical environment. They are migratory endoparasites, infecting and developing mainly in the cortical parenchyma which obstructs the absorption of water and nutrients from the soil, thereby causing severe root damage and reduced plant growth. Major and economically important species are *P. brachyurus*, *P. coffeae*, *P. goodeyi*, *P. penetrans*, *P. vulnus* and *P. zeae*. These species are responsible for substantial yield losses in many agronomic and horticultural crops. Lesion nematode damage to roots not only causes losses in yield, but also can cause losses in marketable quality of infected plant products.

Disease: *Pratylenchus* species cause lesion disease.

Symptoms: *Pratylenchus* species are commonly referred to as the root lesion nematodes due to the characteristic symptoms of necrotic lesions (darkened areas of dead tissue) they cause on the roots. All stages of root lesion nematodes are found in the root cortex. They do not induce permanent feeding sites, but feed and reproduce while migrating between or through plant cells which cause mechanical destruction of root cells. They produce small lesions on roots, initially the symptoms on infected roots are light-to-dark brown lesions which turn pale yellow to black in severe cases of infection; lesions tend to expand and to merge as the growing season progresses, giving the roots a discolored appearance overall, which results in significant plant growth reduction and often invaded by other soilborne plant pathogens. Interaction with pathogenic fungi causes greater damage than by nematodes or fungi alone. Above-ground symptoms of attack include chlorosis, stunting, premature wilting and unthrifty growth of plants, which often die. A crop field may be patchy as plants wither and die.

Etiology: *Pratylenchus* nematodes (juveniles and adults) enter roots by a persistent thrusting of the stylet which softens and breaks the cell wall. The cell walls and the cytoplasm turn light brown due to the nematode

feeding. The nematodes move into the cortex, where they feed and reproduce. The necrosis of cortical cells follows the path of nematodes. Continuous feeding of the nematode on cortical cells causes break down of cell walls and appearance of cavities in the cortex.

The females lay their eggs in the cortex; as the eggs hatch, the young nematodes feed on the parenchyma cells and move mostly lengthwise within the cortex, which results in enlarging the lesion. Some of the nematodes leave the lesion and travel to other points of the root or other roots, where they cause new infections. Necrotic cortical tissues are invaded by secondary fungi and bacteria, resulting in rotting and sloughing off of the root tissues around the point of infection and subsequent death of the distal part of the root. Thus, the reduced number of functioning roots results in reduced absorption of water and nutrients that makes the plants stunted and chlorotic.

Lifecycle: The lifecycle of a lesion nematode is rather simple. Like all nematodes, lesion nematodes have six life stages: egg, four juvenile stages, and the adult stage. Adult males are numerous in some species and rare in others. In sexual reproduction (amphimixis) after mating, the female lays eggs singly or in small groups in the host root or in the soil near the root surface. The first larval stage and moult occurs within the egg. The egg hatches within 3 to 8 weeks, depending on the environmental conditions such as adequate soil temperature and moisture. The second-stage larva emerges from the egg and undergoes three more moults before becoming an adult. Where males are rare or absent, they reproduce asexually (parthenogenesis). The length of the lifecycle depends on the species and the soil temperature. All juvenile and adult stages are capable of feeding on and entering plant roots. The number of nematodes in root tissue increases greatly during the growing season.

Management

- Crop rotation, host-plant resistance, chemical control, soil solarization, and biological control.
- Application of carbofuran (3G) @ 2 kg a.i./ha .

- Summer ploughing along with seed soaking with carbosulfan (25DS) @ 0.1%.

Reniform Nematode (*Rotylenchulus reniformis* Linford & Oleivera, 1940)

Rotylenchulus nematodes are sedentary semi-endoparasitic (partially inside roots) species with a worldwide distribution in the tropical and subtropical regions. This nematode has a wide range of food, fibre, oilseed, fruits and plantation crops as hosts. It is called the reniform nematode because of the distinct kidney-shaped body of the mature females. The immature females establish permanent feeding sites in roots. The anterior portion (head region) of the body remains embedded in the root; whereas the posterior portion protrudes outside the root and swells during maturation.

Disease: *Rotylenchulus* species cause root necrosis.

Symptoms: The type of damage incurred by *R. reniformis* often depends on the host species and/or cultivar as well as the nematode population. General symptoms include reduced root systems, leaf chlorosis and stunted growth of host plants. Damage by *R.* renifomis may lead to reduced crop yields and plant longevity. It feeds on cortical tissue, phloem and pericycles and its infection may cause formation of necrosis on roots of certain crops. Symptoms appear as root discolouration, shedding of the leaves and formation of malformed fruits and seeds. In addition to causing direct damage to plants roots, the nematode, if joined by other pathogens like *Fusarium* spp., *Verticillium* spp., *Sclerotium rolfsii* and *Rhizoctonia solani* they together can develop diseases complexes.

Etiology: Only females infect plant roots. After infection, a feeding site composed of syncytial cells is formed. A syncytial cell is a multinucleated cell resulting from cell wall dissolution of several surrounding cells. These syncytia are mostly confined to the pericycle.

Lifecycle: The species is bisexual and reproduction is amphimixis. However, some populations of the reniform nematode reproduce parthenogenetically (egg production without fertilization). The male of *R. reniformis* has a weak stylet and does not feed. The adult vermiform female is the infective stage, has a robust stylet and penetrates the cells of root cortex, eliciting formation of a feeding cell and finally establishment of a multinucleate syncytium without migrating longitudinally through the root. Seven to nine days after infecting the roots, posterior part of the female body swells to form the typical reniform (kidney) shape. A female deposits approximately 60-200 eggs into the soil, surrounded by a gelatinous matrix. This nematode goes through four moults before becoming an adult. The first moult occurs within the egg. After the eggs hatch, the larvae develop to the preadult stage without feeding or growing. Nematodes differentiate into adult males and females after the fourth moult. The complete cycle takes 24 to 29 days at optimum conditions; however, the duration depends on soil temperature. It can survive at least two years in the absence of a host in dry soil through anhydrobiosis.

Management

- Cultural practices: Any cultural practice which reduces nematode densities is beneficial.
- Crop rotation: Non-host crops or resistant crops should be planted when nematode population is high.
- Use of organic amendments: Use of trap and antagonistic crops. Planting *Tagetes erecta* and *Crotolaria spectabilis* in nematode infested soil has been found effective against the nematode.
- Biological control: *Paecilomyces lilacinus*, a fungal egg parasite has been found effective against the reniform nematode.
- Chemical control: Several nematicides have been reported to be effective against the reniform nematode. Any systemic nematicide available may help; however, local trials are always advisable befor any recommendation.

Citrus Nematode (*Tylenchulus semipenetrans* Cobb, 1913)

Tylenchulus species- the citrus nematode is a sedentary semi-endoparasite. It causes "slow decline of citrus trees". The citrus nematode is the major nematode pathogen of citrus orchards throughout the world. Originally from Asia, it spread worldwide with infested planting stock.

Females are most commonly found on thick stunted rootlets and lay eggs in a gelatinous matrix to which a layer of soil particles clings. The particles held by a gelatinous mucus secreted by the female and protect the female and eggs deposited by them from natural enemies. The egg laying and young female bury their anterior end (head and neck) deep inside the roots, causing infection. The remaining body of the nematode swells into a characteristic asymmetrical shape on the surface of the root.

Disease: *Tylenchulus* species cause slow decline disease of citrus trees.

Symptoms: The symptoms of *T. semipenetrans* infection on citrus trees develop slowly. The aerial parts of the infected plants show drought and malnutrition symptoms comprising of yellowing and shedding of leaves as well as dieback of twigs resulting in heavy losses and slow decline of plants. Normally, the trees are not killed but their productivity goes on decreasing year after year and finally trees become non-productive. This syndrome is thus named as the slow decline of citrus. The leaves of infected trees are smaller than normal and are commonly chlorotic. Wilting is more pronounced in infected trees than in healthy trees during conditions of water stress. Affected trees show reduced terminal growth, die-back of branches and considerable reduction in number and size of fruits. Die-back symptoms first occur on the upper portion of the tree but later extend to the lower portion. Heavily infected fibrous roots of such trees appear thicker than healthy roots because soil particles adhere to the gelatinous egg-masses of the nematode and are retained on the root surface. Infected fibrous roots decay because of the lesions and secondary organisms infect them at the sites of nematode penetration and feeding. Heavy nematode root infections result in root lesions and cortical sloughing.

Etiology: Only females infect plant roots. The egg laying and young female bury their anterior end deep inside the roots, causing infection. They initiate a feeding site with several nurse cells. These nurse cells are uninucleate, not enlarged parenchyma cells in the cortex which is the characteristic for this nematode. Syncytium is not formed. Feeding of the citrus nematode in cortical cells, results in necrosis. Young adult females burrow the anterior portion of their bodies several cell layers deep into the root cortex and initiate the development of numerous nurse (feeding) cells around the head. At this stage, the females are sessile semi-endoparasites and the posterior portion of the female swells with maturation of the gonads.

Lifecycle: Marked sexual diamorphism is present. Reproduction occurs both sexually and asexually. Each female can produce about 100 eggs that are embedded in a protective gelatinous matrix. The female lifecycle duration from egg to egg, ranges from four to eight weeks. The nematode is an obligate parasite, which lives in modified cortical cells in the roots of a limited number of woody plants. Sedentary, swollen female (*T. semipenetrans*) are permanently attached to the fibrous roots of the host. Motile juvenile stages and males are present in the host rhizosphere. The eggs are embedded in a gelatinous matrix surrounding the posterior portion of the female body on the surface of the host root. As with all plant-parasitic nematodes, the first juvenile moult occurs in the egg, so that exclusion involves second-stage juveniles.

On citrus at 25°C, the female second-stage juveniles migrate along the surface of fibrous roots for up to 14 days, before feeding begins on epidermal cells. Thereafter, the juvenile moults three times until it reaches the adult stage within 7 days. Five weeks after hatching, egg laying begins. Males generally moult to the third stage before leaving the egg-mass, and development to the adult stage can occur within one week without feeding. Adult males are non-parasitic.

Management

- Nursery soil should be fumigated before planting of citrus rootstock. Suspected rootstocks should be de-nematized by hot water treatment.

- Use of organic amendments (chicken manure, cotton waste, castor cake, sugarbeet pulp) reduce the population of citrus nematodes.
- DBCP (Nemagon) has been used extensively for the control of citrus nematodes. The nematicide is effective in reducing the nematode population with increase in fruit yields.
- Use of resistant or tolerant varieties against the citrus nematode is the most practical method of control.

Burrowing nematodes (*Radopholus similis* (Cobb, 1893) Thorne, 1949)

Radopholus species is an important group of migratory endoparasitic root nematodes inhabiting a wide variety of plants. These nematodes occur in the tropical and subtropical regions of the world. *Radopholus similis* is the most common, widespread and economically important species of this genus; a serious limiting factor in the production of many crops. The worldwide occurrence of burrowing nematodes is due to the spread of contaminated plant material over many years; whereas use of infected plant material, movement of soil or agricultural machinery, usually spread the nematode within regions.

Disease: *Radopholus similis* causes several diseases viz., severe "spreading decline" disease of citrus trees; yellow disease of black pepper vines; blackhead or toppling disease; banana decline or root rot diseases in banana.

Symptoms: Early symptoms caused by root feeding nematodes are due to impaired water and nutrient uptake. These symptoms include stunted plant growth, decreased vigor and yield, premature leaf drop and an increased tendency to wilt or dieback during dry periods.

Symptoms of burrowing nematode in banana are most readily observable as dark and necrotic lesions on the root system, similar to those caused by pathogenic fungi, and other endoparasitic nematodes that may infect banana roots. The roots develop cavities by destruction of cells; root systems may become stunted, unthrifty and necrotic; lesions may be present in both the roots and outer layer of the rhizome. Symptoms of

root damage by *Radopholus* are reddish, brownish to black lesions caused by cell wall collaspe as nematode moves inter and intra-cellularly. Heavily infected plants show stunting, wilting and chlorosis symptoms. Tree damage includes smaller and sparser foliage and fruit. Disease incidence is greatest in dry seasons in affected tree crops.

The diseased trees of banana may topple over in severe cases of infection during periods of heavy rain or wind due to their severely reduced and damaged root system, which is unable to anchor the plant into the ground. Toppling of plants is more so in case of tall banana varieties with heavy fruit bunches.

Citrus roots infected with burrowing nematodes have few to no small feeder roots and are severely damaged by the nematode feeding and tunneling. Aboveground symptoms of citrus and other plants infected by burrowing nematode include; yellowing, stunting, dieback, reduced fruit size, and thinning of the canopy. Spreading decline of citrus can begin in small areas of citrus groves where a few trees demonstrate reduced thriftiness, canopy thinning near the crown and a reduction in fruit size. Pepper fields suffering from burrowing nematode infestation display yellowing symptoms coupled with root necrosis and canopy dieback.

Etiology: Burrowing nematodes infect at or near the root tip and migrate to the cortical region. The nematode usually penetrates the region of elongation but if it is terminal, the root tips can become swollen or stubby. Tissue rot occurs following secondary infections. Primary and secondary roots and corms may suffer serious damage in banana crop; whereas non-lignified fibrous roots of woody plants are damaged superficially.

Lifecycle: Burrowing nematodes normally reproduce sexually but can be hermaphrodites (without males). The nematode (*Radopholus* spp.) completes its lifecycle within the root cortex; however, juveniles and adults are also present in rhizosphere soil. Mature females lay eggs at the rate of two to four per days for about seven to eight days. At optimum temperature, the juveniles may hatch in 2-3 days in some hosts or little longer in others. The lifecycle on citrus can be completed in 18-

20 days under optimum conditions. Mostly the *R. similis* population reproduces best at temperatures between 25ºC and 30ºC, but not below 15-20º C or above 30ºC temperature. Soil texture affects population growth and virulence of the nematode. Population growth on banana is greater in coarse sand than in finer soils; whereas in citrus, it reproduces well in sandy than loamy soils in controlled pot studies and in the field.

Management

- Sanitation is one of the most important methods to manage burrowing nematodes.
- Infected plant material such as banana corms can be treated with hot water or by paring the lesions before planting.
- Crop rotation with non-host crops reduces the population of burrowing nematode as this nematode does not survive for longer periods in the absence of host roots.
- Systemic nematicides are generally effective in controlling endoparasitic nematodes but they are expensive, highly toxic and have a negative impact on the environment and public health; therefore, alternative methods of control may be used.
- *Paecilomyces lilacinus* (fungus) parasitizes eggs, juveniles and adults of *R. similis*. It can be applied as dip, soil drench or incorporated into the soil. It is marketed commercially.
- *Pseudomonas fluorescens* (bacterium) also inhibits invasion by *R. similis* and can be beneficial to manage the nematode.

Stubby-root nematodes (*Trichodorus* spp.)

Longidoridae and Trichodoridae are the only families of plant parasitic nematodes of the order Dorylaimida that can transmit plant viruses. Species of *Trichodorus* and *Paratrichodorus* (Trichodoridae) transmit Tobraviruses or NETU (nematode transmitted tubular viruses); whereas *Longidorus* and *Xiphinema* (Longidoridae) transmit the Nepoviruses (nematode transmitted polyhedral viruses).

Trichodorid nematodes, also known as stubby root nematodes, are migratory ectoparasites of the roots of perennial and woody plants and

occur worldwide. The stubby root nematodes have been found to infect citrus, mulberry and sugarcane. *Trichodorus* species attack the roots and deposit eggs in the surrounding soil. They cause direct damage to the root system of various crops. Several *Trichodorus* species are capable to transmit plant viruses.

Disease: Lateral roots when heavily attacked by the *Trichodorus* species give rise to the characteristic stubby root symptoms, hence the name.

Symptoms: *Trichodorus* species cause direct damage to the root system of various crops. The infected plants produce short, stunted, stubby and necrotic roots while the leaves become chlorotic and yellow.

Etiology: The members of Trichodoridae usually aggregate near the tips of growing roots where they often feed together on epidermal cells and root hairs. When root growth is retarded due to massive destruction of the epidermal layer, the nematodes move to new sites for feeding usually on lateral roots. These lateral roots when heavily attached, attain the stubby root appearance.

Lifecycle: Reproduction is amphimictic and under optimum conditions the lifecycle is completed in 45 days in *T. similis*. Rate of reproduction is determined by host, soil temperature and soil type.

Management:

- Soil treatments with fumigants or oxime carbamates (e.g., aldicarb or oxamyl) are known to be effective.

Wheat-gall nematodes (*Anguina tritici* (Steibuch, 1799) Filipjev,1936)

Anguina tritici was the first plant parasitic nematode mentioned in the literature to cause plant disease. It was described from wheat by Needham in 1743. The nematode is reported from all the important wheat growing regions of the world including Pakistan. Seed-gall nematode (*Anguina tritici*), also known as ear-cockle, is commonly found on small grain cereals. It has also been recorded from barley in

many countries. A single gall may contain over 10,000 dormant juveniles. Nematodes can remain dormant in the galls over a period of ten years; capable of causing infection when sown with the seed. Threshold of 10,000 juveniles/kg soil develop disease. The nematode also causes yellow ear rot or tundu disease in wheat in association with the bacterium *Clavibacter tritici.* In Pakistan ear-cockle is a known pest on wheat and barley and is found in nearly all parts of the country.

Disease: It causes a disease in wheat and rye called "ear-cockle disease" or seed gall disease, respectively.

Symptoms: Symptoms are basal swelling of stem, wrinkling, curling and twisting of leaves, stunted growth; and formation of cockles or seed galls instead of normal grains. Infected grains are blackish, round, hard and short sized as seen.

Etiology: The nematode is normally first detected as the brown or black galls (ear-cockles)in threshed grain. The galls contain the quiescent second satge juveniles (very resistant to desiccation) which emerge in mosit soils to invade host seedlings which are fed on ectoparasitically: quite severe stuning of the wheat plant may occur, the leaves often being twiated crinkled and distorted. Nematode development only occurs when the larvae entre the young inforescence and it produce juveniles.

Management

- Use of certified seeds
- Separation of cockles from contaminated seed by water floatation
- For more effective separation of cockles, 10% salt solution may be used in place of plain water. However, seed must be washed thoroughly to remove salt, dried in shade before sowing.

Potato rot nematode (*Ditylenchus destructor* Thorne, 1945)

Potato rot nematode or potato tuber nematode, *Ditylenchus destructor* Thorne, 1945, is a serious nematode pest in a number of root and tuber crops, primarily in potatoes, and is an internationally quarantine pest. It

is a migratory endoparasitic nematode, invades mainly below ground parts of the plant, generally with no evident above-ground symptoms and may cause severe losses in infested fields. *D. destructor* is a very polyphagous species; all stages of this nematode can be found either in the host plant tissues or in the surrounding soil. The nematode can move into, out of, or within the host tissue. They can continue to live and reproduce within harvested tubers in storage.

Disease: It causes rot of potato tubers. It can also attack other crops like tulip, carrot, iris, dahlia, garlic, onion, sugar beet and sweet potato damaging their fleshy organs.

Symptoms: *Ditylenchus destructor*, enters tubers through lenticels and multiplies rapidly. The nematode produces enzymes that cause cell disintegration and destroy potato tubers, causing diagnostic "dry rot" symptoms of the potato rot disease; symptoms in its other hosts are the rotting and discoloration of subterranean plant tissue. In potatoes, early infection can be detected by small shining white spots underneath the potato's skin. After sometimes, these spots join together and their colour changes to brown. Secondary invasion of bacteria and fungi accelerates the rotting which changes the colour to black. Above ground symptoms are rare. Most infestations remain undetected until symptoms develop in mature potato tubers. Heavily infested potato plants may be smaller and have curling, smaller and discoloured leaves.

Lifecycle: The lifecycle of this nematode species occurs inside the host tissue. After feeding and after fertilisation by a male, females lay eggs throughout the plant tissue while moving from cell to cell. Eggs hatch and larvae are able to invade hosts immediately. There are four moults in *D. destructor* during its lifecycle. After hatching from an egg (first juvenile stage-first moult occurs within the egg), the emergent second stage juvenile (J_2) can immediately invade the host and undergos a series of three moults through the third (J_3) and fourth (J_4) juvenile stages to reach the adult stage. Once hatched, the juveniles either move throughout the surrounding plant tissue or leave the plant to a nearby, healthy host. Potato rot nematodes overwinter on leftover plant debris or in soil as adults or juveniles and may even multiply during a warmer winter by

feeding on alternative weed hosts, unharvested potato tubers or soil inhabiting fungi. *D. destructor* is mycophagous and can survive in soil in the absence of host plants by feeding on many soil borne fungi. It can also live and reproduce in harvested tubers in storage.

Etiology: *D. destructor* entres potato tubers through lenticles and initially causes small white mealy spots just below the surface that are only visible if the skin is removed. Infested areas enlarge and coalesce and light brown lesions, consisting of dry granulat tissue, may be visible beneath the skin. As the infestation progresses, the tissues dry and shrink and the skin becomes cracked and papery. Internal tissues gradually darken and there are often secondary invasions of fungi and bacteria etc.

Management

- The use of nematode free seed potatoes is an essential component of any control programme.
- Avoid moving potentially infested plant debris and soil onto other agricultural land
- Control by crop rotation is possible using non-host crops such as sugarbeet, but it is important to control weeds carefully because of the polyphagous habit of *D. destructor*.
- Chemical control of *Ditylenchus destructor* can be achieved with soil-applied nematicides such as carbofuran, ethylene dibromide, VAPAM HL, and TELONE. Fumigation with these nematicides is often paired with mechanical measures to attain optimal control.

Red ring nematode (*Rhadinaphelenchus cocophilus* (Cobb, 1919) Goodey, 1960)

Rhadinaphelenchus cocophilus causes red ring disease of palms. The common name, the red ring nematode, is derived from its distinguishing symptom. Red ring disease can appear in several species of tropical palms, but is most common in oil and coconut palms. The red ring nematode parasitizes the palm weevil *Rhynchophorus palmarum* L., which is attracted to fresh trunk wounds and acts as a vector for *B. cocophilus* to uninfected trees.

Disease: *Rhadinaphalenchus cocophilus* causes red ring disease of coconut and other palms by the infestation of roots, trunks and leaves. Nematode is transmitted by palm weevil, *Rhyncophorus palmarum.*

Symptoms: Characteristic symptoms are the red ring of diseased tissue full of *R. cocophilus*, 2-5 cm beneath the trunk surface. Internal damage can be seen within two to three weeks after *R. cocophilus* enters the tissue of a healthy palm. External symptoms can take up to two months to appear. A crosswise cut through the trunk of an infected palm reveals a circular, colored band approximately 3 to 5 cm wide. The surface of the cut in a healthy tree appears as solid, creamy white. The most common color of the band is bright red, although the shade can vary from light pink or cream to dark brown in infected African oil palms. In external symptoms, leaves become short and deformed and turn yellow-bronze, then deep reddish-brown. The color change usually begins at the tip of each leaf and starts in the older leaves before moving to the younger ones. As the leaves change color and dry up, they wilt and die. In coconut palms, red ring nematodes most often attack trees between three and seven years age. These young trees usually die within six to eight weeks after the appearance of symptoms. Trees that have been affected by red ring disease for more than three years are noticeably stunted compared with healthy trees of a similar age.

Lifecycle: The red ring nematode follows a typical plant parasitic lifecycle, having 4 moults before becoming an adult. The lifecycle of *R. cocophilus* lasts nine or ten days. It is the third larval stage that is typically deposited in a palm by *R. palmarum* as it lays its own eggs there. Inside the tree, the red ring nematodes feed, grow, and reproduce. When the weevil eggs hatch, immature nematodes associated with the insect larvae and can remain inside them as the weevils undergo metamorphosis. When the weevils mature, they leave the palm with new batches of third-stage larval nematodes ready to infest a new tree. The nematodes do not reproduce inside the weevils. The red ring nematodes primarily invade the parenchymatous tissue of the palm in a band where the red ring develops.

Etiology: The main vector of red ring disease is the palm weevil, *Rhyncophorus palmarum.* At an infected palm, a weevil ingests the red ring nematodes or picks them up on the surface of its body. Those nematodes are then left behind at the next palm, usually transmitted as the weevil lays its eggs. Red ring nematodes invade both palm tissue and roots. In leaves, stem and roots, they block water pathways, reducing the palm's water absorption.

Management

- The most useful and most important method for management of red ring nematode is the early removal and destruction of red-ring infested palms.
- Trapping the vector is also a useful strategy, reducing the disease incidence from 10% to 1%.
- Controlling the vector *R. palmarum* can help reduce red ring nematode infestation.
- Treating infested palms with nematicides is difficult because the nematicides do not penetrate the area of the trunk usually inhabited by the nematodes.

Dagger Nematodes (*Xiphinema* spp. (Cobb, 1913) Inglis, 1983)

Nematodes of the genus *Xiphinema*, commonly called dagger nematodes, parasitize plants. Many of these nematodes, the majority of them belonging to the *Xiphinema americanum*-group, can transfer viruses to plants during feeding. Dagger nematodes can cause economic damage and death of host plants through feeding on the roots and also by spreading viral mosaic and wilting diseases. Species of the genus *Xiphinema* are widely distributed in both temperate and tropical areas. *Xiphinema* spp. are the eighth most economically important plant parasitic nematode group to agricultural crops worldwide.

Dagger nematodes feed as ectoparasites (their bodies remain in the soil and only their stylets penetrate the roots). Primary symptoms are root death and a proliferation of small club-shaped lateral roots emerging behind the root cap. They are pathogens of many plant species, but they

are most notorious for spreading the plant viruses such as tobacco and tomato ringspot viruses and peach rosette mosaic virus.

Disease: *Cherry rasp leaf virus*, *Tomato ringspot virus*, and *Tobacco ringspot virus* are some of the major viruses transmitted by the dagger nematodes during feeding. Bermuda grass is a potential reservoir for GFLV (*Grapevine fanleaf virus*), which is transmitted by dagger nematodes.

Symptoms: *Xiphinema* species feeding along roots produces symptoms similar to other cortical feeders (disintegration of cortex); those that feed at root tips produce very different symptoms, viz-a-viz. root stunting. The feeding at the meristematic root-tips destroys root cells and reduces root volume. Terminal galling of roots of woody plants is common. There is also ample evidence that the nematode injects substances into roots which causes root swelling. Dagger nematodes transmit numerous viruses to plants. The feeding stylet of *Xiphinema* causes large holes in plant roots, often leading to secondary bacterial and fungal infections. An association between *Fusarium* and *Xiphinema* has been reported from several crops. The above ground effects of damaged roots are stunted growth of crops and patchy fields.

Lifecycle: Dagger nematodes have six lifecycle stages. Parthenogenesis, a form of reproduction that does not require males, is common in many, but not all species. Females lay eggs in soil. The lifecycle of a dagger nematode is similar to other ectoparasitic, vermiform nematodes. Juveniles hatch from eggs and moult four times, increasing in size with each moult until they become adults. As vector-capable juveniles feed on virus-infected plants and mature into adults, they can acquire plant pathogenic viruses, commonly known as nepoviruses (nematode polyhedral viruses). The viruses form a lining in the pharynx-stylet tube and are injected into root tissues during feeding.

Etiology: Dagger nematodes are ectoparasitic, which means that all stages, except eggs, attack and feed on the roots of the host plants. The nematode inserts its long stylet deep into the root while the body remains outside the root, in the soil. The stylet punctures cell walls as it

penetrates plant tissues. During feeding, enzymes are secreted to digest plant cell contents; that destroy root cells, resulting in malformed root tissues. Root cells eventually collapse due to feeding. *Xiphinema* causes darkening of tissues, cortical hyperplasia, lateral root proliferation and tip galling.

Management

- Control of *Xiphinema* spp. on many annual crops may not be of major importance since species of this nematode seldom attain high population levels when soil is frequently tilled.
- Field studies have shown that some control measures, such as biofumigation and rotation of crops, targeting reduction in population of virus vectors- the dagger nematodes, can be effective to some extent.
- Dagger nematodes are spread to fields through the use of contaminated equipment, planting of infested plants and contaminated irrigation water or run-off. Thus, clean equipment such as tractors, planters, and ridgers should be used in order to minimize transfer of nematodes to fields.
- Weed control is important because some weeds are also good hosts to dagger nematodes and the plant virus they transmit.

White tip disease (*Apelenchoides besseyi* Christie, 1942)

Aphelenchoides besseyi Christie, 1942 or rice leaf nematode, is best known as the causal agent of white tip disease of rice. It is a facultative ecto and endoparasite of the leaves and young tissues of rice. The nematode is seed borne and can survive in a state of anhydrobiosis for several years on stored grains, but much less under field conditions.

The rice white tip nematode *Aphelenchoides besseyi*, which is widely distributed throughout almost all the rice growing regions of the world, is considered to be a major contributor to the seed-borne pathogens of rice. *A. besseyi* is a global burden, causing huge losses in rice crop yields.

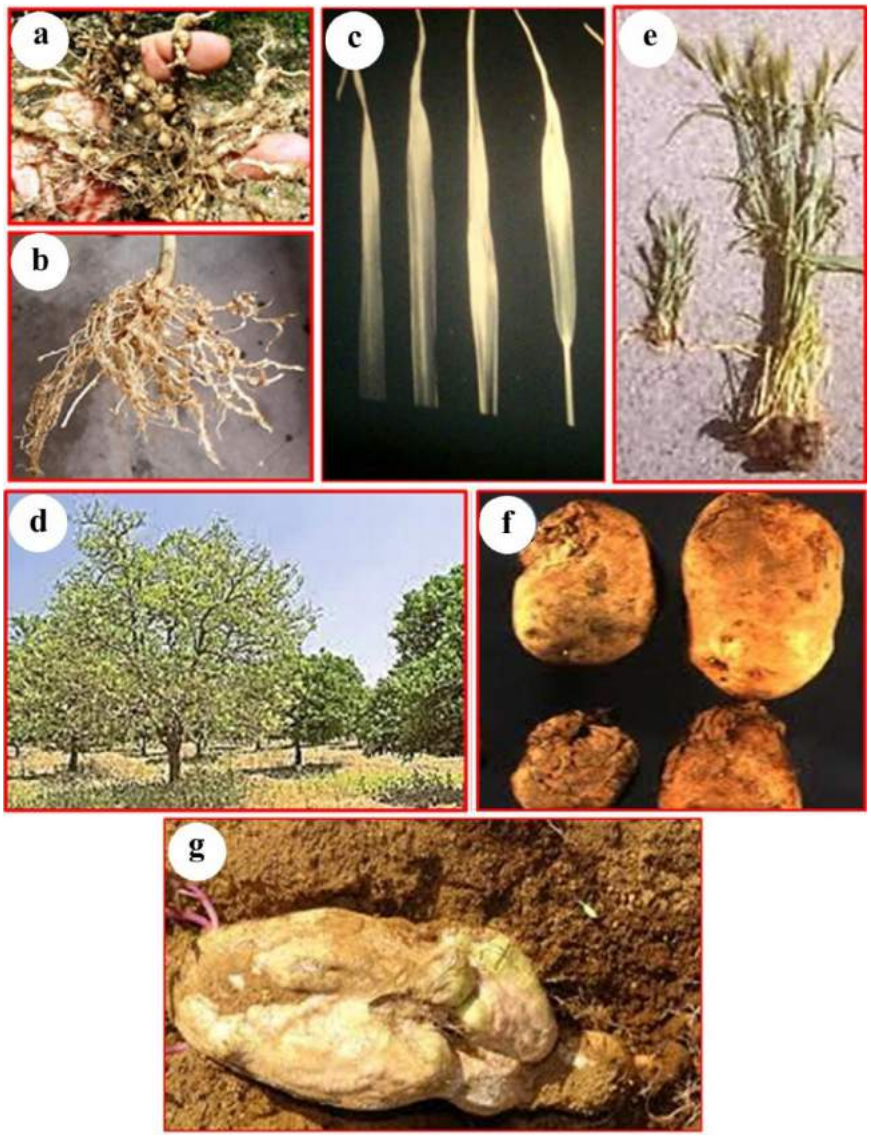
Disease: White tip disease is caused by *Aphelenchoides besseyi*

Symptoms: *A. besseyi* may inflict damage without producing symptoms on susceptible plants. At early growth stages, infection of *A. besseyi* manifests as chlorotic of newly emerged leaves. Later the leaf, 2-5cm from the tip, turns bronze with crinkled margins and there is browning, followed by death and shredding of the leaf tip. Chlorotic patches are also observed in the interveinal area of leaf sheath and leaf blade and sometimes the sheath appears abnormally dark green with greater chlorophyll content as compared with the healthy ones. The flag leaf characteristically becomes shorter and often twisted and curled, with the leaf margin corrugated and distorted at booting stage. Initiation of panicle and development is arrested and the flag leaf emerges only in a shortened and distorted form. Affected panicles are shorter and often swollen at the tips, bearing fewer grains.

Lifecycle: Reproduction is usually amphimictic, the lifecycle takes 8 days at 23°C to complete. No development occurs below 13°C.

Management

- Hot water treatment of seed is probably the most effective and the cheapest control measure.
- Growing resistant or tolerant cultivars.
- Early planting if rice season is preceded by a cooler period.
- Low seed bed planting densities.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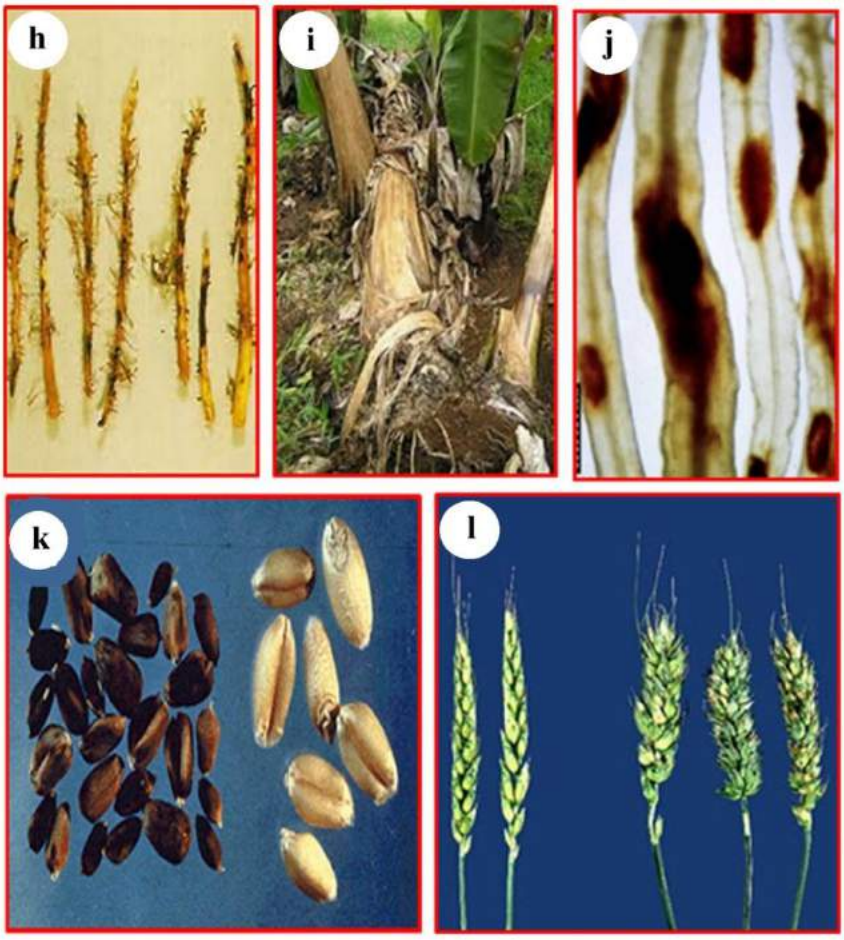

Fig. 9.1. a & b. Root-knot or root gall disease; c. White tip disease of rice; d. Citrus decline disease/slow disease of citrus; e. Molya disease of wheat and barley; f & g. Ear-cockle disease of wheat; h. Lesion disease of root; i. j. Blackhead/topping disease of banana; k & l. Potato rot disease (From different source).

Chapter 10

MODE AND MECHANISM OF NEMATODE INFECTION

All nematodes are very similar in their basic body plan; their genetic diversity is enormous and reflects the long evolutionary route of the phylum. They all possess a hollow protrusible stylet that is used to puncture cell walls, inject secretions, and ingest nutrients from the plant cell. The stylet secretions are synthesized in unicellular pharyngeal glands that are much more developed in PPN than in free living nematodes.

Ectoparasitic nematodes do not enter the host tissues with their body, but rather puncture plant cells using their stylet and feed on the content of the cells. Depending on the species, feeding can prolong for a few hours till several days. The size of the stylet determines where and how the nematodes in this group feed. Ectoparasitic nematodes with a short stylet (e.g., Trichodoridae, *Tylenchorhynchus* spp.) often feed on root hairs and epidermal cells, while those with a long stylet (e.g., Longidoridae, *Belonolaimus*, *Helicotylenchus* spp.) feed on cortical or even endodermal cells. They insert their stylet into the host cell, inject glandular secretions that dissolve the cell content, and ingest the cytoplasmic contents of plant. Depending on the species, these actions lead to wounding, extensive necrosis, or even gall formation of the root tissue.

Migratory endoparasites are equipped with a robust stylet, which renders them the ability to penetrate and continuously migrate through the root while feeding on the cytoplasm of cortical cells. With the exception of some shoot parasites (Anguinidae and Aphelenchoididae); they all belong to the family of Pratylenchidae (e.g., *Pratylenchus* spp., *Radophulus* spp.). Migration inside the roots is aided by the release of cell wall degrading enzymes via the stylet. Extensive necrosis and sometimes galling or swelling of the root tissue is typical symptoms that develop as a result of infection with such nematodes.

Formation of giant cell and syncytia

Sedentary endoparasitic nematodes have the most evolved interactions with their host. After root penetration and migration, they induce permanent feeding cells inside the vascular cylinder. The best studied are the cyst nematodes and root-knot nematodes. Freshly hatched juveniles penetrate the roots close to the root tip, and migrate intracellularly (cyst nematodes) or intercellularly (root-knot nematodes) toward the vascular cylinder. Also here, migration is performed by vigorous stylet thrusting and secretion of a mix of cell wall degrading enzymes. After arrival at the vascular cylinder, they puncture the cell wall of a certain cell and start repeated cycles of stylet secretion release into the cytoplasm and ingestion of cytoplasmic content. The initial feeding cell responds with an extensive change in gene expression and morphology. The cells become hypertrophic and show a huge proliferation of all organelles. Root-knot nematodes induce six to seven giant cells, which become multinucleated by repeated mitosis without cytokinesis. In contrast, cyst nematodes induce a syncytium, which is formed due to the dissolving of cell wall of the initial feeding cell and fusion with the neighboring cells. In clear contrast to the migratory endoparasitic and ectoparasitic nematodes that mostly kill the cells on which they feed, the sedentary endoparasitic nematodes maintain their feeding cells healthy and metabolically active throughout their lifecycle. Once they started feeding, they even lose their locomotory muscles, and become completely dependent on the hypertrophic cells for further development. Though the giant cells and the syncytia are distinct in their development, functionally they are similar in that they serve as transfer cells of nutrients derived from the phloem. While cyst nematodes species show specificity for certain plant families, root-knot nematodes such as *Meloidogyne incognita* for instance have an extremely wide host range comprising almost all families of flowering plants.

Molecular mechanisms of plant resistance

In order to respond to diseases, plants have various predefined physical (e.g., wax layer, trichomes) and chemical (toxins) barriers. Plants trigger a multilayered innate immune system to reduce infection if these barriers

are overcome by invaders. The majority of what we know about the plant immune system comes from research on plant-pathogen interactions (bacteria, viruses, fungi). As more information about nematode–plant interactions becomes available, it becomes obvious that, while these interactions have certain unique characteristics, they also share many parallels with pathogen-induced plant responses. As a result, understanding the molecular mechanisms underlying plant responses to diseases not only aids in the interpretation of observations on nematode and herbivore plant interactions, but also inspires new research. Thus, before getting into the nematode–plant interactions, a look at the basic principles of plant pathogen immunity is necessary.

1. The first layer of plant immunogenicity is made up of a system that fights pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs), are conserved molecules that are shared by a large phylogenetic group of pathogenic and non-pathogenic microbes and are frequently found on the outside of the plant (e.g., fungal chitin or bacterial lipopolysaccharides).
2. The PAMP-triggered immunity (PTI) begins with pattern recognition receptors, which are transmembrane proteins found on the cell surface, detecting these PAMPs. An extracellular leucine-rich repeat, a transmembrane domain, and a cytoplasmic kinase domain are typical components.
3. When these immunological receptors bind to PAMPs, a cellular signal cascade involving ion influxes and mitogen-activated protein (MAP) kinase activation occurs, terminating in the formation of reactive oxygen species, gene expression modifications, and cell wall supports.
4. Pathogens have evolved many strategies to avoid or suppress the plant's innate immune system in response to plant defenses.
5. Proteins, known as "effectors," are secreted to do this. Effectors can also be involved in manipulating the host developmental programme, such as when galls are produced, in addition to evading or suppressing the host defensive system.
6. In the course of evolution, plants have developed a second layer of immunity that responds to the presence of these effectors and is called Effector-triggered immunity (ETI).

7. The immune receptors for ETI, called resistance (R) proteins, consist of a leucine-rich-repeat domain attached to a nucleotide binding domain with a coiled–coiled or toll-interleukin receptor N-terminal domain. They are mostly located inside the cytoplasm, but a few also reside on the plasma membrane with their leucine-rich-repeat facing the apoplast.
8. The ETI results in a very fast defense response at the site of invasion, which is marked by a rapid calcium and potassium entry, for the activation of MAP kinase pathways, formation of reactive oxygen species, and ultimately a local programmed cell death, also known as the hypersensitive response.
9. Neighboring cells respond by producing toxic compounds and strengthening of their cell walls. An ETI response is much stronger than a PTI response and often blocks the pathogen at the site of invasion **(Fig. 10.1).**

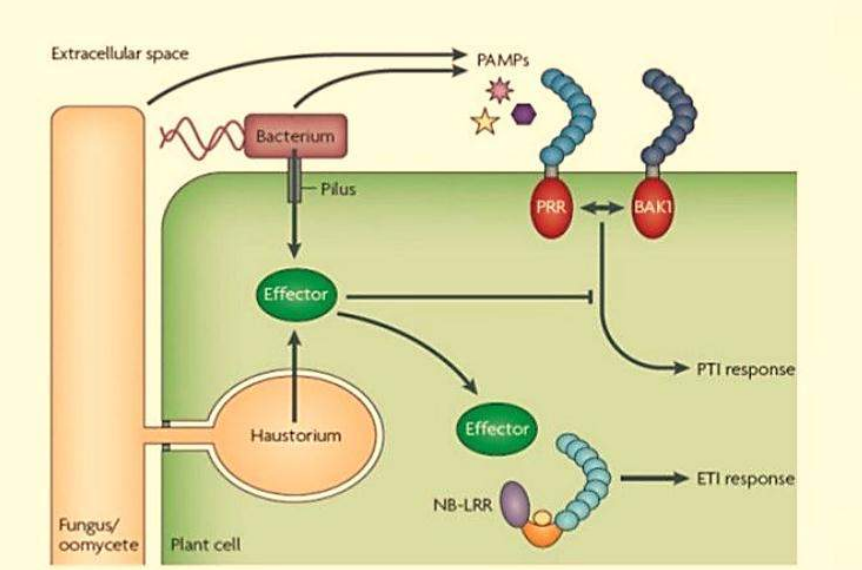

Fig.10.1. Plant natural immunity system (Peter N. Dodds & John P. Rathjen, 2010).

The "R" resistance proteins were once considered to bind directly with a specific effector. ETI would only be successful against closely related

pathogen strains since effectors are species specific, but PTI is directed against a broader phylogenetic range of pathogens with conserved PAMPs. Although certain R proteins have been found to interact directly with effectors, new evidence suggests that the bulk of R proteins monitor the effects of effectors on their own proteins. This self-protein protection has the advantage of allowing the activity of many effectors to be sensed with a small number of R proteins. Indeed, complete genome sequencing of multiple plant species revealed that the number of distinct R proteins is far less than the number of effectors that they may encounter after being attacked by various pathogens. Effectors are typically directed against the same "hubs" in the immune reaction signalling system, according to protein interaction studies, therefore guarding the modification of a small number of these hub self-proteins by the R proteins is enough to offer resistance against a wide range of invaders.

The activation of hormonal signalling pathways such as the salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) pathways is also triggered by the induction of PTI and ETI. In general, biotrophic pathogens trigger the SA pathway, whereas wounding or necrotrophic pathogens trigger the JA and ET pathways.

Systemic imediated responses after nematode infestation

While various gene expression studies on PPN-infected plants have been conducted, the majority of them were designed to examine the local response, specifically the formation of sedentary endoparasitic nematode feeding sites. Recently little research has been conducted that detail the systemic generated resistance after PPN infection.

1. At day 3 after nematode inoculation, a microarray analysis of *Arabidopsis thaliana* after infection with the cyst nematode *Heterodera schachtii* revealed a substantial upregulation of VSP2, a marker gene of the JA defence pathway, in the entire root system. Juveniles had entered the roots and reached the vascular cylinder at that moment, where they had just begun stimulating the feeding site. Analysis of the transcriptome, during a time course experiment with the soybean cyst nematode, *Heterodera glycines*, researchers

discovered a clear induction of the JA pathway throughout the entire root system at all time periods (6 hours to 8 days after infection). At days 2, 5, and 10 after *H. glycines* infection, a comparable systemic activation of the JA pathway was found in soybean roots; however, the JA-controlled defence was inhibited locally in the growing syncytia.

2. After rice infection with a root-knot nematode and a migratory endoparasitic nematode, systemic defensive signalling was compared. At day 3, infection with the migratory endoparasitic nematode *Hirschmanniella oryzae* promotes systemic JA and ET signalling while suppressing the SA pathway. However, by day 7, JA and ET signalling have been inhibited. The root-knot *Meloidogyne graminicola* stimulates SA and JA in the systemic root tissue on day 3, but suppresses ET. By day 7, the JA pathway has been largely inhibited as well. This nematode, on the other hand, suppresses all three hormonal defence pathways in the shoot tissue as early as day 3. *M. graminicola* is mostly sensitive to a JA- and ET-induced defence, but only slightly to an SA-induced defence, according to foliar application of the hormones SA, JA, or ET and mutant analysis, whereas *H. oryzae* is sensitive to all three hormone-controlled defences. Spraying tomato plants with methyl jasmonate reduces the number of *M. incognita* infections.
3. In *A. thaliana*, similar observations of early shoot defence suppression after *M. incognita* infection were made by measuring numerous marker genes for the SA and JA pathways from 5 to 14 days. At day 9 and 14, *M. incognita* infection of *A. thaliana* roots strongly promotes SA-controlled defence, but not at day 5. At day 9, there was also a weak response of JA-controlled defensive markers. Starting on day 5, *A. thaliana* roots infected with the cyst nematode *H. schachtii* substantially stimulate the SA but not the JA marker genes. However, several JA marker genes are activated in the shoots. Furthermore, SA-deficient *A. thaliana* mutants are more susceptible to *H. schachtii*, whereas wild type plants are less susceptible when SA is applied ectopically. To summarise, sedentary endoparasitic nematodes appear to stimulate the JA, ET, and SA pathways at first, but sections of these pathways are swiftly suppressed, particularly following *M. incognita* infection.

Chapter 11

ECOLOGY OF PLANT PARASITIC NEMATODES

The word ecology is derived from Greek word “Oikos” meaning house, habitat or place of living and “Logos” meaning to study. Ecology is defined as the scientific study of interrelationship of different organisms with each other and with their environment. It is concerned with the general principles that apply to both animals and plants. In ecology, ecosystems are composed of dynamically-interacting parts, which include organisms, the communities they comprise, and the non-living (abiotic) components of their environment.

Nematode communities

Most plant-parasitic nematodes live in polyspecific communities. They are abundant in most habitats. Their numbers and diversities are influenced greatly by the host and diversity of the habitats. Although populations are affected by interactions with other organisms working through the host plant, it is doubtful that there is much direct competition among plant-parasitic nematodes. There usually is an abundance of food, except where damage is severe as a result of parasitism. Damage to plants may be additive or partly so by two or more species of nematodes (Norton, 1987). Nematodes are heterotrophic organismsand are ultimately dependent on food resources provided by autotrophic organisms for their energy supply. Nematode populations depend onphysical access to these resourcesthat may be limited by temperature and moisture conditions. An integrated approach to nematode ecology means that relevant information is drawn from nematodes of plants,invertebrates, vertebrates and from those in soil, freshwater and marine substrates.Nematodes exhibit a great diversity in their food sources which is fundamental to trophic interaction and provides the basis of the essential feeding types (Yeates *et al*., 1993). Nematodes are separated into the following different feeding/trophic groups, based primarily on the morphology of the stoma and oesophagus and known feeding habits of recognizable groups (Yeates *et al*., 1993) such as:

Herbivores (Plant feeders)
Bacterivores (Bacterial feeders)
Fungivores (Fungal/ hyphal feeders)
Algal feeders (Unicellular eukaryote feeders)
Carnivores (Predators/Flesh eaters)
Omnivores (Feed on multiple sources)

Herbivores (Plant feeders)

These are the plant parasites obtaining their nourishment directly from plants. This group includes many members of the order Tylenchida, as well as a few genera in the orders; Aphelenchida and Dorylaimida. Their mouth part is a needle like stylet/odontostyle which is used to puncture the plant cell during feeding.

In terms of habitat, these nematodes are either ectoparasites i.e., species that do not normally enter root tissue but feed only from the outside on the cells near the root surfaces, or endoparasites i.e., species that enter the host and feed within the roots. Both of these can be either migratory i.e., they live freely in the soil and feed on plants without becoming attached or move around inside the plant, or sedentary i.e., species that once within a root do not move about.

Bacterivores (Bacterial feeders)

Many kinds of free-living nematodes feed only on bacteria, which are always extremely abundant in soil. In these nematodes, the mouth or stoma is a hollow tube for ingestion of bacteria. This group includes many members of the order Rhabditida as well as several other orders which are encountered less often. These nematodes are beneficial in the decomposition of organic matter.

Fungivores (Fungal feeders)

This group of nematodes feeds on fungi and uses a stylet to puncture fungal hyphae. Many members of the order Aphelenchida are in this

group. Like the bacterivores, fungivores are very important in decomposition of organic matter.

Algal feeders (Unicellular eukaryote feeders)

These nematodes feed on diatoms or other algae. These feeding types include ingestion of fungal spores and whole yeast cells.

Carnivores (Predators/Flesh eaters)

These nematodes feed on other soil nematodes and on other animals of comparable size. They feed indiscriminately on both plant parasitic and free-living nematodes. One order of nematodes, the Mononchida, is exclusively predacious, although a few predators are also found in the Dorylaimida and some other orders. Compared to the other groups of nematodes, predators are not common, but some of them can be found in most soils.

Omnivores (Feed on multiple sources)

The food habits of most nematodes in soil are relatively specific. For example, bacterivores feed only on bacteria and never on plant roots and the opposite is true for plant parasites. A few kinds of nematodes may feed on more than one type of food material and therefore are considered omnivores (feed on a diversity of substrates). For example, some nematodes may ingest fungal spores as well as bacteria. Some members of the order Dorylaimida may feed on fungi, algae and other animals, subsisting on all types of food, especially feeding on both animal and vegetable material.

Methods of Community Analysis

Parameters for community analysis of various nematode species were calculated using formulae given by Norton (1978) in presenting data in ecological work.

Frequency

Frequency is how often a species occurs among samples. It is a measure of distribution uniformity or rate of occurrence, not abundance.

Frequency (N): Frequency of a nematode species (i.e., the number of samples in which a species was present).

Absolute Frequency

Absolute frequency is the rate of occurrence of a species in collected samples divided by total number of samples collected. Absolute frequency is usually expressed as percentage.

$$\text{Absolute Frequency (\%)} = \frac{\text{No. of samples containing a species}}{\text{Total no. of samples collected}} \times 100$$

Relative frequency

Relative frequency is the ratio of the frequency of individual species in a sample to the total frequencies of all species in a sample.

$$\text{Relative frequency (\%)} = \frac{\text{Frequency of individual species}}{\text{Sum of frequencies of all species}} \times 100$$

Density

Density is a quantitative measure of species in a sample.

Absolute density

Absolute density is the number of a species per unit mass or volume of the sample. Absolute density is usually expressed as a percentage.

$$\text{Absolute density (\%)} = \frac{\text{No. of individuals of a species in a sample}}{\text{Volume or mass per unit of the samples}} \times 100$$

Relative density

Relative density is the ratio of the individuals of a species in a sample to the total of all individuals in a sample.

Relative density (%): $\frac{\text{No. of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$

Prominence Value

Prominence value is the relation of population density and frequency and expressed as

Prominence value: $\frac{\text{Absolute density} \times \sqrt{\text{Absolute frequency}}}{100}$

Biomass

Biomass is the weight of living organisms in a given area; often applied as the total weight of organisms participating in a specific ecosystem function.Nematode biomass was calculated from measurements of adult females. Measurements were taken from preserved specimens or from previous calculations. It is calculated by the following formula given by Andrassy (1956).

$$G = \frac{a^2 b}{16 \times 100,000}$$

where "G" equals to biomass in micrograms, "a" is the greatest body width, "b" equals to body length and 16 is a previously determined empirical value.

Importance Value

Importance value (IV) is the sum of relative density, relative frequency and relative biomass of each nematode species. Frequency, density and biomass are among the parameters that encompass the importance of a nematode species in the community.

Importance value is a measure of how dominant a species is in a given community. Three kinds of data are often used: relative density, relative frequency and relative biomass. Each of these values is expressed as a percentage, and ranges from 0 to 100.

Diversity Analysis

Nematode communities are sensitive to changes in food web (Yeates, 1987), environment and temperature (Samoiloff, 1987; Wasilewska, 1989) and can be quantified through Euclidean Distance and Diversity index. Diversity consists of two components - species richness, i.e. the number of species in the community, and evenness, i.e. the fact that some species in community are common and others are rare.

Different parameters used to measure the diversity are:

Dominance (D)

Dominance = 1-Simpson index ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). Where *ni* is number of individuals of taxon *i*. If the "Unbiased" option is selected, an alternative form of *D* is computed:

$D = \Sigma\ (ni - 1)\ (n - 1)$.

Simpson's Diversity Index (SDI) (Simpson, 1949)

Simpson's Diversity Index is a measure of diversity (Simpson, 1949). In ecology, it is often used to quantify the biodiversity of a habitat. It takes into account the number of species present, as well as the abundance of each species.

$D=1- \sum n\ [(n-1)/N]\ (N-1)$

where:

- n = number of individuals of each species
- N = total number of individuals of all species

Shannon Weiner Diversity Index (H') (Shannon and Weaver, 1949)

The diversity index (H') was computed after Shannon and Weaver (1949) information theory function. It is commonly used to characterize

species diversity in a community. Shannon's index accounts for both abundance and evenness of the species present.

H'= -∑ pi log pi

where pi is the proportion of total number N belonging to the i-th species.

Evenness or Equitability (J) (Pielou, 1975)

Species evenness refers to how close in numbers each species in an environment is. It is a synthetic measure describing pattern of relative species abundances in a community. It was calculated according to Pielou (1975) as:

Equitability J'= H'/H max = H'/log S

where H' is the number derived from the Shannon diversity index and H max equals the maximal diversity (log S).

Brillouin Diversity index (HB) (Brillouin, 1962)

The Brillouin index (HB), is calculated using:

$$HB = \frac{\ln N! - \sum_{i=1}^{s} \ln n_i!}{N}$$

whereN is the total number of individuals in the sample, ni is the number of individuals belonging to the ith species and s the species number.

The Brillouin index measures the diversity of a collection, as opposed to the Shannon index which measures a sample.

Menhinick Diversity Index (Menhinick, 1964)

A diversity index, taking into account the number of individuals as well as number of taxa. Menhinick's richness index - the ratio of the number of taxa to the square root of sample size.

Margalef's richness index (Margalef, 1958).

(S-1)/ln(n),

where S is the number of taxa, and n is the number of individuals.

Fisher's alpha

A diversity index, defined implicitly by the formula $S=a*\ln(1+n/a)$ where S is number of taxa, n is number of individuals and a is the Fisher's alpha.

Chapter 12

NEMATODE-MICROBE INTERACTION

Plant-parasitic nematodes are considered as pathogens, capable of producing recognizable disease on the host plant. Plant-parasitic nematodes can often play a major role in disease interactions. In the soil environment, plants are constantly exposed to various microorganisms, which are able to influence each other as they occupy the same habitat. Thus there exists an interrelationship among them which ultimately affects upon host plants. A plant infected by one pathogen may also be affected by another which may alter the host response to additional invaders. These alterations may have significant influences upon disease development within a particular host, epidemiologically of all pathogens involved, and ultimately, on disease management. Infection by nematodes may also alter the host response to subsequent infection by another pathogen.

All the interactions considered here have three major components in common: the host plant, nematode(s) and the other pathogen(s). The most familiar type of interaction is that wherein the pathogen is able to parasitize a host which it would otherwise be incapable of attacking, or to make a more damaging attack than it could have done in the absence of the nematode. However, in a few cases the nematode's activities decrease the damage done by the pathogen, and probably more often, the nematode is affected by the pathogen, either to its benefit or to its detriment.

Most nematodes are capable of elevating a normally minor pathogen to a major status. Plant-parasitic nematodes favor the establishment of secondary pathogens viz., fungi, bacteria, viruses etc. They alter the host in such a way that the host tissue becomes suitable for colonization by the secondary pathogens. Even though the nematodes are themselves capable of causing considerable damage to the crops, their association with other organisms aggravates the disease. The nematodes cause mechanical wound on host surfaces, which favors the entry of micro-organisms. In some cases, the association of nematode and pathogen

breaks the disease resistance in resistant cultivators of crops plants. Plant-parasitic nematodes commonly interact in soil with fungi, bacteria and viruses.

Nematode-Fungus Interactions

Many organism species, including bacteria, archaea, protozoa, fungi, and small animals like nematodes, make up the ecosystems. To execute ecological activities, these species interact with one another and with macroscopic organisms such as plants and large animals. Their relationships take many forms, are direct or indirect, involve two or more partners, and are mediated by a variety of mechanisms including predation, parasitism, mutualism, and competition.

These interactions are necessary for the ecosystem's equilibrium to be maintained.

In the terrestrial ecosystem, fungi and nematodes are among the most abundant creatures. With an estimated 500,000 species, the phylum Nematoda, popularly known as round worms or nematodes, is the second biggest in the animal kingdom. Ninety percent of terrestrial nematodes live in the top 15 cm of soil, where they play a significant role in nitrogen mineralization and the nitrogen cycle. Nematodes are parasitic or free-living creatures that feed on living materials rather than decomposing organic substances (Burros, 1998).

Fungi, on the other hand, are the primary decomposers of decaying organic materials and play critical roles in ecosystem nutrient cycle. The coexistence and interactions of nematodes and fungi, whether antagonistic or mutualistic, direct or indirect, are critical to understanding their ecosystem consequences and potential agricultural interventions.

Identification of innovative control measures against phytophagous nematodes and soil borne fungal infections is an essential long-term goal in agriculture pest and pathogen management, since it can assist in raising both; the quality and quantity of agricultural products.

We are particularly interested in the interactions between these two categories of creatures, which have demonstrated both; hostile and mutualistic interactions with one another, either directly or indirectly **(Fig. 12.1).**

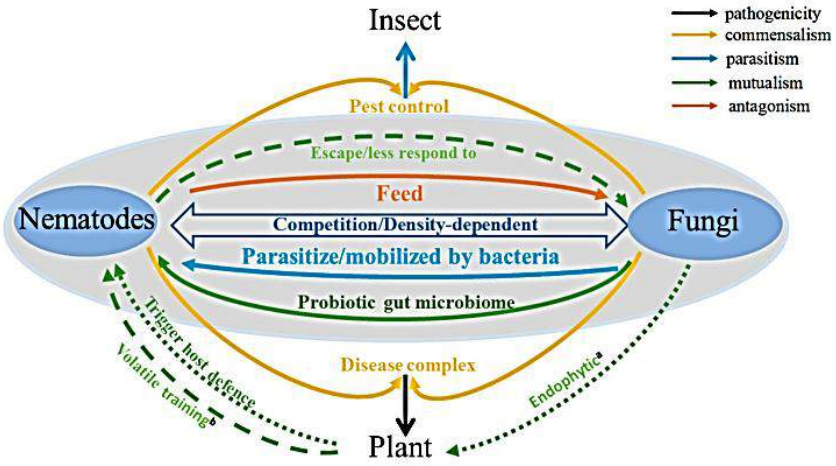

Fig. 12.1. Fungi–nematode interactions in soil. a: Entophytic fungi trigger host plant defense against plant pathogenic nematodes. b: Plant helps nematodes escape fungal attacks through volatile training (Zhang *et al*., 2020).

The greatest number of complexes involving nematodes have included a fungus as the other component mainly wilt-inducing fungi as reported in literature. Nematode-fungus interaction was first observed by Atkinson (1892) in cotton. *Fusarium* wilt was more severe in the presence of *Meloidogyne* spp. Since then, the nematode-fungus interactions have received considerable attention on important crops like banana, cotton, cowpea, brinjal/eggplant, tobacco and tomato.

Antagonistic Interactions

Fungi and nematode antagonistic interactions are as diverse as they are numerous. *Aphelenchus avenae*, *Aphelenchoides* spp., and *Paraphelenchus acontioides*, for example, can feed on a wide variety of fungi. Fungivorous nematodes are the common name for these nematodes. Several fungal species, such as *Arthrobotrys oligospora*, can

prey on nematodes and their eggs and consume them as food. Nematophagous fungi are the fungi that eat nematodes.

Fungi are found in the diets of many nematodes, and some feed exclusively on them (Hasna *et al*., 2007). As a fundamental component of the soil food web, nematodes have the ability to influence both, fungal variety and abundance as well as community structure, including crop development and soil pollution tolerance. Fungivorous nematodes are widespread in soil that contains a variety of fungal species. *Aphelenchus*, *Aphelenchoides*, *Ditylenchus*, and *Tylenchus* nematodes are some of the most frequent fungivorous nematodes (Wall & Caswell, 2003). Despite the fact that fungivorous nematode population densities in soil are lower than those of phytoparasitic and bacterivorous nematodes, in the presence of adequate fungal food, population densities of fungivorous nematodes can rapidly grow (Hua *et al*., 2014). Nematode feeding on soil fungus could have a big impact on soil ecology and agricultural yield, depending on the soil microbiome.

Synergistic Interactions

The phytopathogen population in the soil could be decreased, for example, if fungivorous nematodes feed on plant-pathogenic fungi. Root-knot nematode infection on both cotton and tomato cultivars normally resistant to fungal wilt can predispose these plants to severe *Fusarium* wilt infection. Combine infection by *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* can severely damage cotton. Galled okra and tomato roots infected with *M. incognita* are highly susceptible to infection by *Rhizoctonia solani*, a disease complex that results in root decay caused by the fungus within one month after nematode infection. This interaction is common in the irrigated soils. The fungus, *Rhizoctonia solani* and the root lesion nematode *Pratylenchus minyus* have been closely and consistently associated with natural infections of winter wheat that results in root rot, yellowing, stunting, and yield reduction of the crop. Similar associations between fungi and nematodes have been found to cause brown rot of tobacco.

Disease associations between *Verticillium* species and *Pratylenchus penetrans* have been recorded on eggplant, mint, and tomato. Association of nematodes with bacteria is rare e.g., the foliar nematode *Aphelenchoides ritzemabosi* and *Corynebacterium tumefasciens* - a joint infection that causes "cauliflower" disease on strawberries. The *Trichoderma* fungus has a very poor pathogenic reputation on any host, however, in tobacco roots infected with root-knot nematode *Trichoderma* is able to move into such roots. This may illustrate its potentiality as a bioagent in the management of nematodes.

Species of *Curvularia*, *Botrytis*, *Aspergillus*, and *Penicillium* can invade and cause the decay of tobacco roots if roots have been predisposed earlier by root-knot nematode infection. Without this predisposition, these fungi appear incapable of establishing a parasitic relationship in tobacco. The burrowing nematode, *Radopholus similis*, has been implicated in root rot complexes on banana. *Fusarium solani* is more commonly associated in the deep root lesions formed by this nematode, whereas *Rhizoctonia solani* is found in shallow lesions caused by *Helicotylenchus* spp. on banana roots. *Tylenchulus semipenetrans* increases root decay by *Fusairum* spp. in lemon. *Chrysanthemum* roots inoculated with *Belonolaimus longicaudatus* and *Pythium aphanidermatum* develop symptoms of *Pythium* root rot earlier and more extensively than those inoculated with fungus alone. *Tylenchus agricola* contributes to an increase in the root rot of corn caused by *F. roseum*, but fails to influence disease development by *Pythium ultimum*. *Heterodera schachtii* and *R. solani* combine to promote root and seedling damage in sugar beet.

Giant cells resulting from nematode activity are very suitable substrates for fungus growth, and mycelium readily invades adjacent areas as well. *G. rostochiensis* interacts with both *R. solani* and *Colletotrichum atramentariumm* (the causal agent of brown root rot in tomato). Few recent examples of nematode–fungi pathogen disease complexes reported in crops and insects are given in **Table 12.1.**

Table 12.1. Nematode–fungi pathogen disease complexes reported in crops and insects.

Nematode	Pathogen	Crop/Insect	Reference
Steinernema diaprepesi	*Fusarium solani*	Wax moth, Weevil	Wu, *et al*., 2018
Heterorhabditis bacteriophora, Steinernema feltiae, S. kraussei	*Metarhizium anisopliae*	Black vine weevil	Ansari *et al*., 2008
S. feltiae, S. carpocapsae, H. bacteriophora	*Aspergillus* spp., *Penicillium* spp.	Carob moth	Memari *et al*., 2016
H. sonorensis	*F. oxysporum*	Corn earnematode	Navarro *et al*., 2013
Meloidogyne incognita	*F. oxysporium f.* sp. *phaseoli*	Bean	Carneiro *et al*., 2010
M. incognita	*F. oxysporium f.* sp.	Potato	El-Shennawy *et al*., 2012
M. incognita	*Rhizoctonia solani*	Green bean	Alhazmi *et al*., 2015
M. incognita	*Phytophthora capsici*	Pepper	Parkunan *et al*., 2016
M. spp.	*F. oxysporum f.* sp. *lycopersici*	Tomato	Wanjohi *et al*., 2018
M. spp.	*F. oxysporum, F. solani*	Tomato	Hajji *et al*., 2019
M. javanica	*F. oxysporum f.* sp. *lycopersici*	Tomato	Beyan, 2019
M. incognita	*F. solani*	Fig	Alfadhl *et al*., 2019
M. incognita	*F. oxysporum f.* sp. *niveum*	Watermelon	Keinath *et al*., 2019
M. incognita	*Ralstonia solanacearum, Phomopsis vexans*	Eggplant	Khan, & Siddiqui, 2017
M. incognita	*Alternaria dauci, Rhizoctonia solani*	Carrot	Ahmad *et al*., 2019
Pratylenchus spp.	*Rhizoctonia solani*	Potato	Björsell *et al*., 2017

If mycopathogenic fungi antagonistic to plant-pathogenic fungi (e.g., species in the genus *Trichoderma*) are discovered to be the food of nematodes, the beneficial effects of these antagonist fungi on plants will be lessened due to the actions of these nematodes. All of these fungi can

coexist in the same ecological niches despite having diverse connections with nematodes and with each other. Furthermore, nematode food fungi are not all identical. Different food fungus may attract fungivorous nematodes in different ways, and that attractiveness may change depending on the environment. Fungi and nematodes are also mobile in distinct ways, allowing them to disseminate across ecological niches (Hasna *et al*., 2007).

Fungivorous nematodes have the potential to be multifunctional. The fungivorous nematode *Aphelenchus avenae* is a non-parasitic nematode that can suppress plant-pathogenic fungus (Lamondia & Timper, 2016). Both *Aphelenchus avenae* and *Aphelenchoides* spp., for example, decreased *Rhizoctonia solani* and reduced the damping-off disease in cauliflower seedlings (Lagerlöf *et al*., 2011). Furthermore, *A. avenae* has been shown to inhibit the propagation of the plant parasitic nematode *Ditylenchus destructor*, implying that it could be used as a biocontrol agent against both plant-pathogenic fungi and plant parasitic nematodes (Haraguchi and Yoshiga, 2020).

The roles of cell wall-degrading enzymes from *Aphelenchus avenae* in feeding on both plant pathogenic fungus and a plant parasitic nematode have been supported by genetic investigations (Karim *et al*., 2009). A cellulose comparable to those found in fungi and connected with the ability to parasitize living plants was discovered in the pinewood nematode *Bursaphelenchus xylophilus* as the product of horizontal gene transfer acquired during the evolution of plant parasitism (Kikuchi *et al*., 2004). Like other types of prey–predator partnerships, the fungal prey can develop resistance mechanisms against nematode predation in the fungal prey–nematode predator relationship. The synthesis and secretion of harmful secondary metabolites and toxic proteins is one type of fungal prey defense (Tayyrov *et al*., 2018). The model mushroom *Coprinopsis cinerea*, for example, produces a poisonous material on its mycelial surface that can kill nematodes when they come into touch with it (Schmieder *et al*., 2019). Upon nematode predation, *C. cinerea* produced a bacterial cytolysin-like toxin and demonstrated a large number of differentially expressed genes (DEGs). Some of these DEGs in *C.*

cinerea are a new type of nematode-fighting fungal effector protein (Tayyrov *et al*., 2019).

Fungal Predation Structures: Diversity and Evolution

Based on the processes, that nematophagous fungi use to fight nematodes, they have traditionally been categorized into four groups: (i) nematode-trapping fungi, which produce extensive hyphal networks, knobs, and constricting rings as trapping devices to catch and hold live nematodes; (ii) endoparasitic fungi, which exist as conidia in the environment and infect nematodes by adhering to the prey's surface or directly being ingested by the nematodes, followed by germination, growth, and nematode killing, (iii) egg- and cyst-parasitic fungi, which grow on and parasitize nematode eggs as facultative parasites; and (iv) toxin-producing fungus, which produces poisonous chemicals that are active against nematodes (Liu *et al*., 2009).

Except for the egg stage, most nematodes at other life stages can move across their habitats, posing a challenge for fungal parasites that are slow-growing and sedentary. Many fungi, on the other hand, have evolved to parasitize nematode mobile stages by deploying intricate and sophisticated predatory mechanisms, which include four representative types of traps known among the nematode-trapping fungi, including constricting ring (CR), adhesive network (AN), adhesive column (AC), and adhesive knob (AK). In CR, when triggered, the three curved ring cells swell rapidly inward and lasso the victim quickly using mechanical force (Fig. 12.2.A).

Instead of mechanical forces, fungi with AN form interlocking loops by growing branching hyphae and fusing with the parent hyphae to develop adhesive networks to capture nematodes (Fig. 12.2.B). Fungi with the AC device form a string of cells with adhesive surfaces (Fig.12.2.C). Lastly, AK is an erect stalk with an adhesive bulb at the end (Fig.12.2.D).

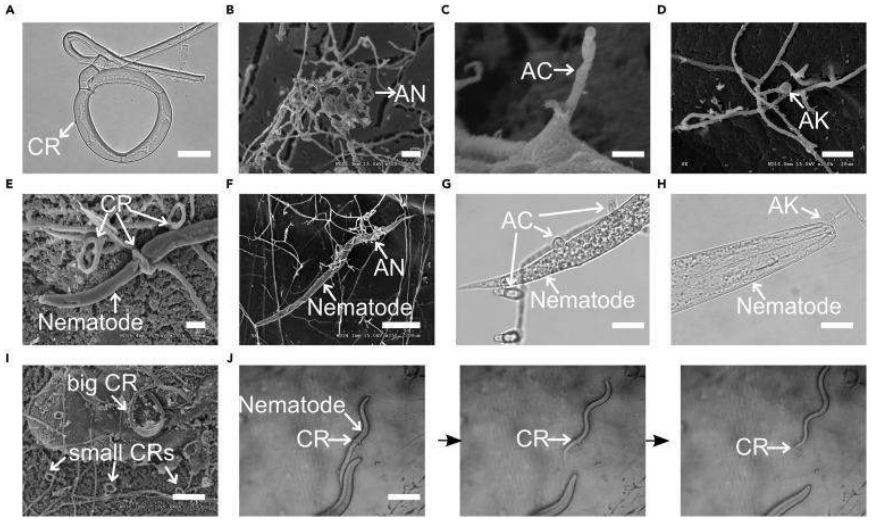

Fig. 12.2. Various trapping devices have been developed to capture nematodes (Su *et al*., 2015).

(A) Constricting ring developed by *Drechslerella brochopaga* (Scale bar, 10 μm).
(B) 3-D adhesive networks developed by *Arthrobotrys oligospora* (Scale bar, 20 μm).
(C) Adhesive columns developed by *Dactylellina cionopagum* (Scale bar, 5 μm).
(D) Adhesive knob developed by *Dactylellina entomopaga* (Scale bar, 10 μm).
(E) Nematode being captured by constricting ring (Scale bar, 10 μm).
(F) Nematode being captured by adhesive networks (Scale bar, 100 μm).
(G) Nematode being captured by multiple adhesive columns (Scale bar, 10 μm).
(H) Nematode being captured by one single adhesive knob (Scale bar, 10 μm).
(I) Constricting rings of various sizes were developed (Scale bar, 50 μm).
(J-L) Nematode escaped from the constricting rings (Scale bar, 50 μm).

Nematode-bacterium interactions

Microorganisms that can develop in the rhizosphere are ideal for use as biocontrol agents because they provide the first line of defence for roots against pathogen attack (Weller, 1988). Fungi and bacteria are two important categories of soil-dwelling microorganisms, and some of them have shown considerable promise as nematode biocontrol agents (Jatala,

1986; Weller, 1988; Sayre and Walter, 1991; Stirling, 1991; Siddiqui and Mahmood, 1993, 1995). Bacteria are the most prevalent organisms in the field soil in terms of numbers, but their biomass is smaller than that of fungus, although exceeding that of algae, protozoa, and nematodes combined (Clark, 1967).

Associations between nematodes and bacteria can be beneficial (mutualistic) or destructive (pathogenic/parasitic), and they can range from facultative, transient interactions to long-term symbioses. Bacteria can be a source of food for nematodes. In specialized interactions, nematodes selectively rely on specific genera or species of bacteria, which may be introduced or produced by the nematode on purpose (Goodrich-Blair, 2007). Parasitic bacteria and non-parasitic rhizobacteria are the two types of bacteria utilized in nematode biocontrol, and each is discussed below separately.

Parasitic Bacteria

Pasteuria penetrans has been researched extensively as a nematode parasite (Sayre, 1980), but *Pseudomonas denitrificans* was also discovered parasitizing *Xiphinema americanum* populations (Adams and Eichenmuller, 1963). *P. penetrans*, in particular, has a lot of potential as a biocontrol agent, notably against *Meloidogyne* species (Brown *et al*., 1985). Several investigations on the taxonomy of *Duboscqia penetrans* have been carried out since Thorne (1940) identified it as a protozoan. *Pasteuria penetrans* was given a new name after electron microscope studies confirmed its prokaryotic and bacterial nature. This bacterium is a nematode obligate parasite with a wide host range. It has been reported from at least 51 countries throughout the world. *P. penetrans* is most likely an assembly of several 'forms' or separate species, each with a specialized and limited host range, according to observations (Sturhan, 1988). *P. penetrans sensu stricto*, which primarily parasitizes *Meloidogyne* spp., and *P. thornei*, which parasitizes root lesion nematodes such as *Pratylenchus brachyurus*, are the two principal *P. penetrans* species.

Endospore size and form, development cycles, and host specificity are now used to identify them (Starr and Sayre, 1988). Adult females of *Heterodera* and *Globodera* species are parasitized by these mycelial and endospore-forming bacteria. *P. penetrans* is distinguished by its ultrastructural characteristics and unique host range.

Several researchers have speculated on the possibility of the existence of strains of *P. penetrans* biotypes. Each isolate appears to have a limited host range, and some are quite selective. The number of spores that attach, varies between populations of the same nematode species, as does the size of the spore. Diverse spore size categories, on the other hand, failed to keep their sizes in succeeding generations, and different size bacterial spores can be discovered linked to females of one root-knot nematode species. Because varied growth rates of *P. penetrans* mycelium colonies might result in different spore sizes, spore size recognition of *P. penetrans* isolates, does not appear to be possible. The width of the central core of endospores was shown to be related to the thickness of the host nematode's body wall. Researchers have used host range, spore morphometrics and serology to characterise *Pasteuria* isolates.

P. penetrans completes its lifecycle on the nematodes it parasitizes in a short period of time (18-20 days). Temperature affects how long it may take *P. penetrans* to complete its lifecycle. When *P. penetrans* spores travel through soil, they connect to the cuticle of second-stage juveniles (J_2). After the spores are connected/attached to the juveniles and the juveniles have entered the roots and begun feeding, the germination of the spores begins. A germ tube arises from the spore and penetrates the cuticle, forming micro-colonies that spread throughout the developing female's body. Micro-colonies then proceed to sporulate, filling the nematode's body with spores and preventing nematode reproduction.

The scientists have revealed that infection needed five spores per nematode, and females, males, and juveniles of *M. javanica* were all infected with spores. The parasitism of *P. penetrans* on second-stage juveniles harmed the growth of root-knot nematodes as well. Under various agro-climatic circumstances, different nematodes showed

varying degrees of infection. *P. penetrans* was found to be effective in suppressing nematode multiplication, thereby enhancing plant development when combined with other nematode opportunistic fungus.

The combination of *P. penetrans* and other microorganisms seems to be a promising method for biocontrol of plant parasitic nematodes. *P. penetrans* possesses various characteristics that make it a promising nematode biocontrol agent. This bacterium not only parasitizes nematodes and stops them from reproducing; it also lowers the infectivity of spore-encumbered juveniles.

Non-parasitic Rhizobacteria

Agrobacterium, *Alcaligenes*, *Bacillus*, *Clostridium*, *Desulfovibrio*, *Pseudomonas*, *Serratia*, and *Streptomyces* are non-parasitic rhizobacteria that have been researched for nematode biocontrol. Bacteria with the ability to colonize roots aggressively are referred to as rhizobacteria. Some of these bacterial strains have been called 'plant-growth-promoting rhizobacteria', and they boost plant growth by colonizing the root system and preventing or suppressing the establishment of harmful rhizosphere microorganisms. The bacteria involved are a diverse collection of species with a variety of mechanisms of action. Rather than parasitism, most rhizobacteria that are known to be harmful to plant parasitic nematodes work through metabolic by-products, enzymes, and toxins. The use of non-parasitic rhizobacteria in combination with other microorganisms such as root-nodule bacteria, arbuscular mycorrhizae, saprophytic fungi, and nematode opportunistic fungus has been proven to be effective in lowering nematode populations on a variety of crops.

Mechanism of Nematode Suppression

The behavior, production, and biocontrol tactics of these two kinds of bacteria (nematode parasitic bacteria and non-parasitic rhizobacteria) are vastly different. Females and juveniles of nematodes are parasitized by parasitic bacteria like *P. penetrans*, which reduces nematode populations. The introduction of non-parasitic rhizobacteria that inhabit the rhizosphere of the host plant is another technique for controlling

nematodes. By regulating nematode behavior during the early root-penetration phase of parasitism, these plant-health-promoting rhizobacteria have a negative impact on the intimate interaction between the plant-parasitic nematode and its host. The syntheses of metabolites that inhibit 'hatch and attraction', as well as the breakdown of certain root exudates that influence nematode behaviour, are thought to be important for reducing nematode infection of roots. The effect of non-parasitic rhizobacteria on nematode penetration could be due to bacteria attaching to lectins on the root surface, interfering with nematode host recognition. Ammonia is created by ammonifying bacteria in aerobic soil during the decomposition of nitrogenous organic materials, which has resulted in lower nematode populations. *Clostridium butyricum* produced butyric acid, whereas *Desulfovibrio desulfuricans* produced hydrogen sulphide, resulting in reduced nematode growth. Another plant-growth-promoting bacterium, *Pseudomonas fluorescens*, works by directly opposing diseases, producing antibiotics, competing with pathogens for key minerals such as iron, or more directly by boosting plant growth. Increased activity of chitinases, β- 1.3 gluconases, peroxidases, and other pathogenesis-related proteins, accumulation of phytoalexin and formation of protective biopolymers such as lignin, callose, and hydroxyproline-rich glycoproteins are all possible mechanisms for induced systemic resistance.

Few examples of bacteria and nematode interactive effect are as follows:

1. Bacterial wilt of several host plants caused by *Pseudomonas solanacearum* (= *Ralstonia solanacearum*) is one of the best-known diseases, which have been commonly reported to be associated with nematodes. In the presence of nematodes, incidence of this wilt is higher. In tomato, both *Meloidogyne hapla* and *Helicotylenchus nannus* contribute to an increase in wilt development, while *Rotylenchus* spp., a non-parasite on tomato, does not influence the rate or severity of wilt symptoms, *M. incognita* influences bacterial wilt development in tobacco. Plants exposed to nematodes 3 to 4 weeks before exposure to *R. solanacearum* develop more severe wilt symptoms earlier than when plants are exposed only to bacteria or both pathogens simultaneously. Giant cells, in jointly infected roots, harbor bacteria like inclusions and degenerate rapidly.

2. Bacterial wilt of alfalfa (*Corynebacterium insidiosum*) increases by the stem nematode, *Ditylenchus dipsaci*. The varieties, which have higher resistance to wilt, become diseased in the presence of *D. dipsaci*. On the other hand, varieties with nematode resistance remain relatively free from wilt when exposed to both pathogens.

3. Hairy roots of roses caused by *Agrobacterium rhizogenes* is usually of minor importance, but turns serious when the plants are infected with *Pratylenchus vulnus*.

4. Crown gall of peach caused by *A. tumefaciens* is increased by high populations of *M. javanica*. At low nematode population levels, however, crown gall symptoms are no more severe than those occurring in plants inoculated with bacteria alone after wounding.

5. The foliage and meristem disorder in strawberry known as cauliflower involving *Aphelenchoides ritzemabosi* and *Corynebacterium fascians* is another striking example of nematode-bacterium complex. This complex requires both the pathogens for expression of the complete disease syndrome.

6. The association between nematode, *Anguina tritici* and the bacterium, C*lavibacter tritici* cause a disease called tunda or yellow slime or ear-rot disease of wheat. Grains are not formed in the spikelets but only a mass of bacteria. The nematode causes ear-cockle or seed-gall disease (blackened seed-galls) in the absence of bacteria.

Symbiotic Bacteria

At least two genera of nematodes, *Steinernema* and *Heterorhabditis,* have evolved symbiotic gammaproteobacteria, *Xenorhabdus* and *Photorhabdus* respectively, that allow them to kill insects and utilize the cadavers as food sources (Dillman *et al.*, 2012). A specialized infective stage of EPNs carries a population of the symbiont within the intestine, and releases them upon invasion of an insect host. There, the bacteria contribute to killing the insect, help degrade the insect cadaver for nutrients, and protect the cadaver from opportunists. Once the insect resources are consumed, the EPN progeny nematodes develop into the colonized infective stage and emerge to hunt for a new insect host (Herbert and Goodrich-Blair, 2007; Clarke, 2008) **(Fig. 12.3)**. There are three species recognized within the *Photorhabdus* genus: *P. temperata*,

P. luminescens, and *P. asymbiotica*. The last was isolated originally from human wounds, but recently was discovered to colonize, like the other species, a heterorhabditid nematode host, of which there are 18 recognized species (Stock and Goodrich-Blair, 2012). In contrast, there are 22 species of *Xenorhabdus* that colonize one or more of the >70 known species of *Steinernema* nematodes (Stock and Goodrich-Blair, 2012). In both types of associations, the bacteria and nematodes can be cultivated independently or together, and molecular genetic techniques are available for the bacteria and, in some cases, for the nematodes (Clarke, 2008). This technical tractability has enabled the use of EPNs and bacteria as models of mutualism, virulence, evolution, behavior, ecology, and drug discovery (Adhikari *et al.*, 2009; Bode, 2009; Richards and Goodrich-Blair, 2009; Bashey *et al.*, 2012). Furthermore, since these nematode-bacterium complexes are pathogenic toward a wide but varying range of insects, an additional goal in studying EPNs is improving their use in biological control of insect pests (Stock, 2004). In particular, investigators have focused on identifying nematode traits associated with host range and successful parasitism to help improve the field efficacy of EPNs, and on identifying products of the entomopathogenic bacteria with insecticidal properties, efforts facilitated by sequencing of both bacterial and nematode genomes (Chaston *et al.*, 2011).

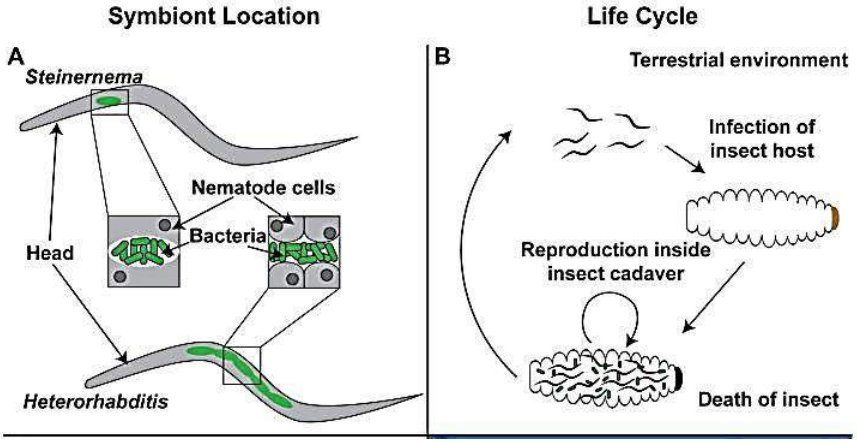

Fig.12.3. Schematic overview of model nematode-bacterium symbioses: Symbiont location and Lifecycle (Kristen E. Murfin, 2012).

A: *Xenorhabdus* and *Photorhabdus* (green) are located within infective juveniles (environmental stage) of *Steinernema* and *Heterorhabditis* nematodes respectively. The bacteria are located in the lumen between intestinal epithelial cells (grey with dark grey nuclei) (insets).
B: The infective juveniles of *Sterinernema* and *Heterorhabditis* parasitize insect hosts. The nematodes and bacteria kill the insect and reproduce within the insect cadaver. The nematodes then re-associate with their bacterial symbionts and migrate away from the cadaver into the environment to seek new hosts.

Nematode-virus Interactions

Virus-nematode vector relationships are a significant aspect of plant pathology that has raised curiosity in possible relationships between nematodes and viruses. *Xiphinema*, *Longidorus*, *Trichodorus* and *Paratrichodorus* are of great importance as they transmit a number of plant viruses. There are 22 longidorid and 14 trichodorid nematodes that have been reported as vectors of plant viruses. Among Longidoridae, 11 species belong to the genus *Xiphinema*, 10 to the genus *Longidorus*, and one to *Paralongidorous*. Of the Trichodoridae, 5 species belong to the genus *Trichodorus* and 9 to *Paratrichodorus*.

Nematode-virus complexes have been identified by Hewitt *et al*., (1958), who observed that *Xiphinema index* was the vector of grapevine fan leaf virus. *Xiphinema Longidorus* and *Paralongidorus* spp., transmit the ring spot viruses, which are called NEPO virus (nematode transmitted polyhedral-shaped particles), *Trichodorus* and *Paratrichodorus* transmit the rattle viruses and are called Tobraviruses or NETU (nematode transmitted tubular shaped particles). All these nematodes have modified bottle-shaped oesophagus with glands connected by short ducts directly to the lumen of the oesophagus. This actually helps in the transmission of viruses, which is different in other genera of nematodes.

Soybeans infected by both root-knot nematode and tobacco ring spot virus - a strain of which causes a bud blight diseases in tobacco – suffer from extensive galling and greatly reduced root systems. Root-knot nematodes grow more rapidly in the roots of tomato plants infected with tobacco mosaic virus than in virus-free roots. In the latter case, the virus

has no observable effect on number of nematodes entering the root. No vector relationship has been reported in either instance.

Tobacco plants infected with tobacco mosaic virus revealed decreased populations of both *A. ritzemabosi* and *D. dipsaci* and high growth retardation of plant under combined attack was observed. This clearly indicates that the damage by the combination of the virus and nematode pathogens is greater than would be evident when either pathogen is present alone. Even in the absence of a vector relationship, interactions between nematode and virus seem to occur. Accordingly, both pathogens, being obligate parasites, must be influenced by some physiological change in the host.

The ability of different nematode species of the same or different genera transmit the same virus, or on the other hand, the capacity of two different viruses to be transmitted by the same nematode species indicates that the vector specificity is less developed in trichodorids than in longidorids. Among the factors affecting the efficiency of trichodorids indicates that 15°C is the optimum temperature for the transmission of Tobacco Ring Spot Virus (TRV) and that below 10% soil moisture content, virus transmission is unlikely. Trichodorid nematodes can retain the virus as long as 10 months and exponentially, even a year, and single nematode has been shown to transmit TRV to several plants when allowed to feed for 1 day on the roots of each plant separately. The distribution of these nematodes in soil is very erratic and affected by several physical and chemical components. But even low number of trichodorids can efficiently transmit viruses. Establishment and/or reproduction of trichodorids in the soil is more difficult than for many longidorid nematodes, but the virus-vector-plant relationship is more stable among trichodorids than longidorids.

There are often changes in root-knot nematode reproduction rates when plants are jointly infected with virus and nematode. These changes are usually reflected as increase in the number of nematode eggs in roots of plants, inoculated with both pathogens in combination.

Interactions with other Nematodes

Interaction between different species of nematodes is usually antagonistic rather than beneficial. Two species can cohabit in the same host, but competition is severe when both species have similar feeding behavior. Interaction between ectoparasites can be stimulatory, but competitive advantage increases as the host parasite relationship becomes more complex. Combined infections of ecto and endoparasitic nematodes are, in most cases, suppressive for ectoparasites as sedentary endoparasites are most advanced parasites. In any case, two or more species of nematodes may interact and the outcome of the interaction is density and time dependent. The combined effect usually leads to increase the disease, which becomes more severe when two or more species of nematode interact with another pathogen (Eisenback, 1985).

Chapter 13

NEMATODE POPULATION DYNAMICS AND CROP / YIELD LOSSES

Nematode Population Dynamics

Population dynamics is the study of changes in nematode population size and structure over time. Knowledge of population dynamics is essential to predict nematode population growth and to anticipate nematode damage. Nematodes cannot migrate readily from site to site during a growing season. Therefore it is possible to predict the final nematode population size (P_f) from the initial population size (P_i) present within a site. P_f can be measured at harvest of a crop, or after some specified time interval. Patterns in the population dynamics of nematodes are determined by the essential characteristics that regulate rates of births and deaths of individuals and modified by conditions of the environment in which the population functions. Essential factors include the productive capacity of the gonad in relation to resource demands of somatic tissues, the rate and length of the reproductive period, and the life history strategy. Modifying factors include availability of food, and environmental conditions.

Trudgill Theory

1. Lifecycles or reproductive strategies of plant feeding nematodes vary greatly. Some grow large and have long lifecycles with low rates of population increase (K strategists); others are relatively small, have short lifecycles and potentially higher reproductive rates (r strategists).
2. A reduction in the number of active juvenile stages further decreases the development time, thereby reducing generation time and increasing the potential for multiple generations in a season.
3. A wide host range completes the adaptation of pathogens such as some *Meloidogyne* spp., an example of **r strategists**. Many *Longidorus* spp., are examples of **K strategists (Fig. 13.1).**

The r strategists increase rapidly where the environment is favorable, often overshooting the equilibrium density. Severe damage to the host occurs and the population crashes. This can occur with repeated cropping of hosts. The K strategists do best in stable environments, where populations are usually close to the equilibrium density (the population density that can be sustained).

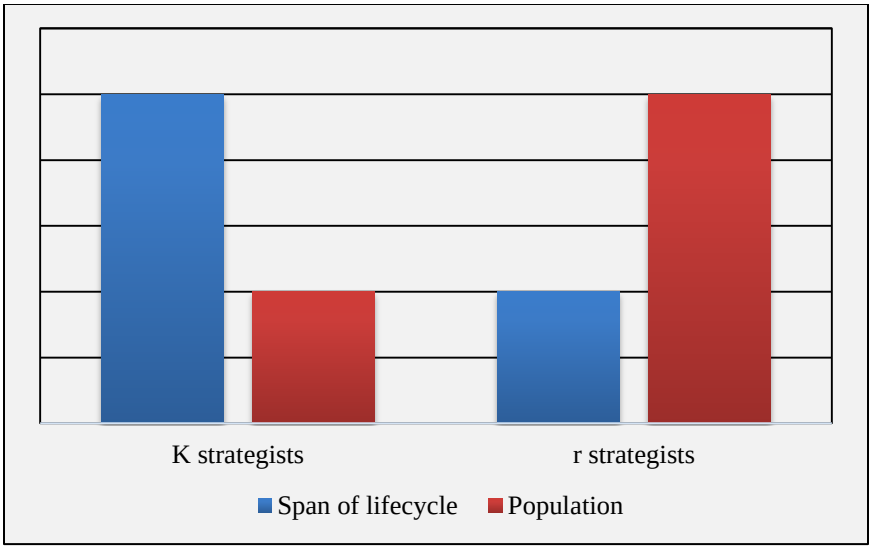

Fig.13.1. Graphical presentation of Trudgill theory.

Nematode Population and Density Relation

Nematode multiplication rates are strongly density dependent. Density is defined or expressed as the number of nematodes per gram of soil. However, the units that directly affect the nematodes are those that are root related; e.g., number of root tips and/or length or weight. Hence, a cultivar with twice the root mass of another will, except at low densities where the multiplication rate is maximum, support a higher multiplication rate. Similarly, tolerant cultivars that maintain a greater root mass than intolerant cultivars as Pi increases, will have a greater equilibrium density and maintain a greater multiplication rate at high pre-planting population densities.

Population Density in Relation to Plant Response

There are three models proposed by Seinhorst, in their most basic form, show maximum rates of multiplication at low initial densities.

1. The P_i increases due to the increasing damage imposed and the loss of roots. Furthermore, increases in P_i can inflict so much root damage that the population increase becomes negative and the population size is ultimately reduced (**Fig. 13.2)**.

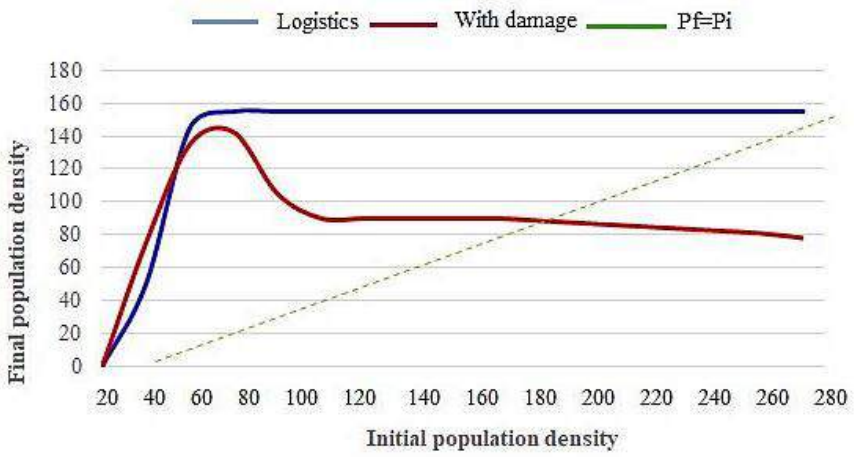

Fig. 13.2. The theoretical logistic relationship between initial population density and final population density and the relationship when roots are damaged (After Trudgill, NNRC).

2. An important method of expressing and comparing the effects of different cultivars or cropping regimes is to consider the equilibrium density, i.e. the point at which $P_f = P_i$. This density is usually observed at a P_i which is larger than that which gives the largest P_f **(Fig. 13.3)**.
3. In practice, this equilibrium density is reached after a period of oscillation about the equilibrium density. The size of the oscillations is determined by the tolerance and resistance of the host. Tolerance and resistance produces small oscillations; while susceptibility and intolerance can result in large oscillations. Indeed, these two factors can interact to the extent that a tolerant

but partially resistant cultivar can produce a higher equilibrium density than an intolerant susceptible cultivar **(Fig. 13.4)**.

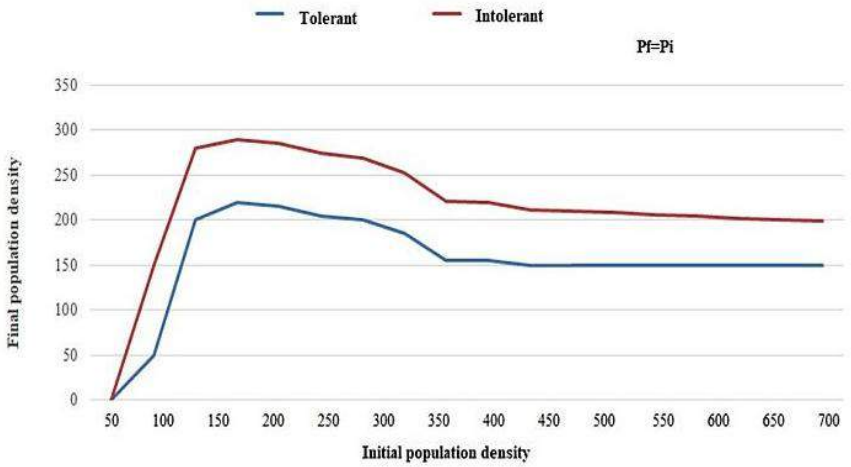

Fig. 13.3. The relationship between P_f and P_i when a tolerant and an intolerant host are grown (After Trudgill, NNRC).

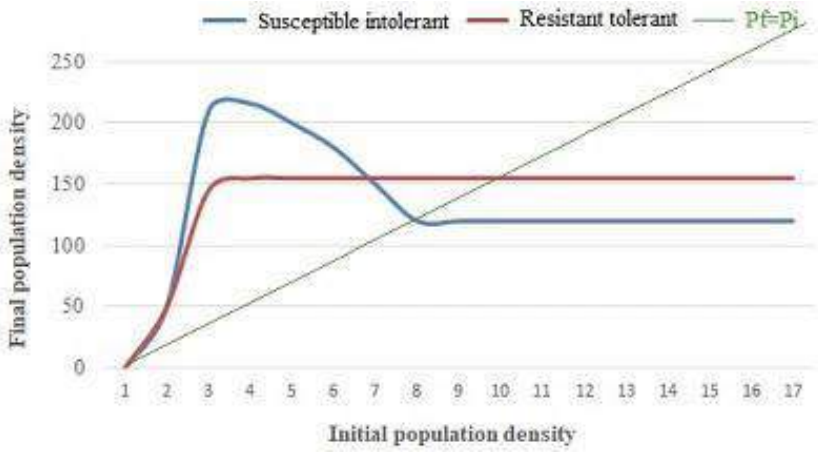

Fig. 13.4. The relationship between P_f and P_i contrasting the response when an intolerant susceptible host is grown as compared to that which occurs when a tolerant but partially resistant host is grown (After Trudgill, NNRC).

Care needs to be taken in devising management strategies for the control of nematodes to balance the benefits of tolerance against the benefits of resistance, to ensure that while yields are maximized, nematode populations are not raised to levels that are damaging to other cultivars.

Models can be used to examine and explore nematode management strategies, but need to take into account the effective population if this is less than the actual population, and the decline in the numbers of nematodes in the absence of a host crop.

Economic Thresholds

Economic thresholds are management tools for minimizing economic losses due to nematodes. They are based on projections of expected crop performance in relation to population levels at a critical point in time or at multiple points in time. The economic threshold is that level to which the population of the target nematode species should be managed under prevailing economic and environmental conditions. In its most comprehensive sense, the economic threshold is based on the integral of expected returns from the current crop and from future crops, given the expected line of the nematode population at this level of management.

Crop / Yield Losses

Crop losses are determined by a variety of parameters, including the pathogenicity of the nematode species involved, nematode population density at planting, host sensitivity and tolerance, and a variety of environmental factors. As a result, current models can only estimate yield losses as a percentage of the nematode-free yield. Various economic factors are also involved in determining threshold values. As a result, forecasting yield losses and setting economic thresholds for most nematode/crop problems is still a work in progress. More field-based data on the association between nematode population densities and crop performance is needed, and several methods for gathering such data are outlined. The measurement of population density, particularly for *Meloidogyne* species, is a key issue that must be addressed.

Crop loss estimations must be accurate in order to set research, extension, and financial priorities. Obtaining such estimations is, unfortunately, challenging. Nematode population dynamics are also density dependent, impacted by host growth, the species' reproductive potential, and a variety of environmental conditions. As a result, modelling nematode population dynamics is a science that is equally impressive. Good field data are required for this, but the complicating effects of biological control agents, host susceptibility differences and environmental factors, as well as errors associated with measuring initial population densities, may make predicting the multiplication rates of most nematodes, especially those with multiple generations per season, practically impossible.

Damage Models

When modelling the damage caused to plants by root-feeding nematodes certain basic principles may apply. These are:

- Damage is proportional to the nematode population density.
- The degree of damage is influenced by environmental factors.
- The yield harvested is determined by the amount of light intercepted by the crop, by how efficiently the intercepted light is converted into dry matter, and how that dry matter is partitioned into non-harvested and harvested yield. For some crops significant variations in moisture content will also affect final yield.

The above principles seem very simple as a statement but practically are more complicated in use.

Damage may be proportional to the nematode population density, but there are several qualifications of this statement. The relationship is usually curvilinear, increasing numbers of nematodes having proportionally diminishing effects. There is some evidence that at low densities the host plant can repair the damage and that growth may even be slightly stimulated. Seinhorst (1965) termed the population density (Pi) at which damage first became apparent as the tolerance limit (T).

Equally, at very high values of P_i, increasing numbers of nematodes may not further reduce dry matter productivity. Seinhorst termed this the minimum yield (m). There are various reasons why minimum yield may occur; there may be some growth before attack starts or after it finishes, and a significant biomass may be planted (e.g. potato tubers). However, "m" applies to total dry matter and because of effects on partitioning, the harvest value of "m" may be greater or less than that for total dry matter.

The third parameter in the Seinhorst equation is z, a constant slightly less than one. The equation is:

$y = m + (1-m)\, z^{(Pi-T)}$
for $P_i > T$
$y = 1$ where $P_i > T$

where y is the yield.

An important qualification is that "y" is expressed as a proportion of the nematode-free yield. Hence, according to Seinhorst, the greater the yield potential the greater the loss in tonnes per hectare for any value of P_i.

Mechanisms of Damage and Environmental Effects on Damage

Damage is proportional to the intensity of attack; this is often proportionally greater in sandy soils where nematodes can move more freely, than in heavier soils where movement is impeded. Adequate soil moisture is essential for free movement; hence the attack is often limited as soils dry out later in the season. Temperature also influences the rate of nematode movement, but plant growth is usually equally affected.

Primary damage to the attacked roots can be attributed to mechanical damage associated with feeding or invasion, to withdrawal of nutrients, and/or to more subtle physiological effects. Generally damage reduces the rate of root extension. This reduces the rate of uptake of nutrients and water, and if any factor becomes limiting (and they usually do, even for crops without nematode damage), top growth rates are reduced. This reduces the rate of increase in light interception and carbohydrate

synthesis and hence the capacity of the plant to generate more roots to overcome the limitations imposed by nematode damage. Such a process appears to be the main mechanism of damage by potato-cyst nematodes (*Globodera* spp.) whose effect is further increased by reductions in root efficiency, revealed in a decrease in root: shoot ratio. Further damage is associated with withdrawal of nutrients by the developing females (resistant cultivars of potato are often less damaged than susceptible cultivars) and by secondary pathogens such as *Verticillium dahliae*. The central role of nutrient uptake is revealed, however, by the substantial ameliorating effect on damage of additional fertilizer.

With *Meloidogyne* spp., impaired water relations appear to contribute substantially to reduced rates of top growth. This is probably because the developing giant cell systems interfere with and disrupt the developing xylem. Clearly, with such damage, effects on growth and yield are likely to be greater where the plants are on the threshold of becoming moisture stressed. Other effects include reduced photosynthetic efficiency.

Effects on Light Interception and Utilization

There is a good correlation in many crops between percent ground cover (i.e. the percentage of ground occupied by a plant or a crop, when viewed from above, which is covered by green leaves) and percent light interception. Most annual crops start as individual, separate plants and a reduction in growth rate is directly reflected in ground cover and hence light interception. As they grow, the leaves of neighbouring plants merge to form a continuous canopy. Nematode damage that only delays the production of a continuous canopy, and hence 100 percent light interception, will have a smaller effect on final yield than damage which prevents the crop from achieving such full cover. Premature crop death will also proportionally reduce yield.

Methods of Estimating Yield Losses

Pot studies can be used to determine some of the basic information on yield-loss relationships, but because of environmental differences and interactions, field studies are also needed. There are two approaches: one

is to use nematicides at relatively uniformly infested sites; the other is to work at sites with a range of population densities but which are uniform in other respects. A combination of both approaches is often a happy compromise. The former gives practical information on the effectiveness and potential value of a particular treatment but tells little about the nature of the relationship. It also suffers from the criticism that nematicides have a range of side-effects. The latter has the benefit of producing information on the relationship between P_i and yield, but it requires experimental errors to be minimized. Because P_i estimates have large errors, accuracy is improved by reducing plot size and by taking and processing multiple samples from each plot. However, plot size must be large enough to obtain a realistic yield and adequate guard plants are essential.

Another option is to establish many small plots in large but otherwise uniform fields. These can be at random, in a grid pattern or along known trends in P_i. The plots can be split and a nematicide applied to one half. For each plot the P_i and yield are determined. The results will produce a scatter of points, hopefully with yield decreasing as P_i increases. Much of the scatter is due to errors in estimating Pi and yield, and it can be minimized by taking the average of all the results within each error band. Such an approach needs:

i) a wide range of initial populations;
ii) a uniform field;
iii) a large number of plots (100 or more);
iv) the plots to be part of an otherwise uniform crop.

Control Measures

Control measures aim to protect the treated crop from damage, and to prevent nematode multiplication and reduce the threat to the next susceptible crop in the rotation. Most cost-effective and successful measure is the growing of resistant varieties. However, while these may prevent nematode multiplication, they are often as vulnerable to damage as a susceptible variety. In yield-loss studies resistant varieties can be a very useful tool for preparing plots with reduced populations without the

side-effects associated with other treatments. Rotations between vulnerable crops and non-hosts are almost essential. Nematicides, whether natural or artificial, are a last resort and should not be used as a crutch to compensate for poor management. They are always costly and frequently toxic and environmentally damaging. However, their side-effects can make them attractive in some situations; the oxime-carbamates control a broad range of pests, until they develop resistance, while the fumigant nematicides release nitrogen, thereby increasing yields.

Chapter 14

NEMATODES AS VECTORS OF PLANT VIRUSES

There are several landmarks on the pathway of our expanding knowledge of nematode transmission of plant viruses. The initial discovery of *Xiphinema index* as vector of grapevine fan leaf virus (GFLV) (Hewitt *et al*., 1958) stimulated the search for nematode vectors of other soil-borne viruses, and this was accompanied by research on many aspects of the biology, ecology, and taxonomy of both nematodes and viruses. Early investigations established that plant viruses specifically associate with their nematode vectors, and the mechanism of this association began to emerge when it was discovered that the virus coat protein was a key factor in the adsorption of particles at virus retention sites within the nematodes.

In recent years, improved technology has provided detailed information on the characteristics of viruses of the Nepovirus and Tobravirus groups. Interest in the taxonomy of the virus vectors- longidorids and trichodorids continues, as the number of research increased. There have been numerous reviews on many aspects of nematode transmission of viruses. In the recent review, the geographical distribution of the nepoviruses and tobraviruses has been considered as an approach to understanding the ecological and biological association between these viruses and their vectors.

Longidorid nematodes of the genera *Xiphinema*, *Longidorus* and *Paralongidorus* belong to the family Longidoridae. Trichodorid nematodes of the genera *Trichodorus and Paratrichodorus* belong to the family Trichodoridae. These forms are amongst the most important phytoparasitic nematodes. A large number of these species are known to feed on the roots of fruit trees and other plants causing direct damage; while many species have also been found responsible for transmission of serious virus diseases from one plant to another in various countries. These virus vector nematodes cause damage to many economically important crops both by direct feeding on the roots and by vectoring plant viruses (Hewitt *et al*., 1958; Sol and Seinhorst, 1961).

Nematode Transmission of Viruses

It is also a well established fact that longidorids transmit nepo viruses and trichodorids transmit tobra viruses. Viruses are named on the basis of particle shape, and those with polyhedral particles are called NEPO viruses, i.e., nematode transmitted viruses with polyhedral particles. Viruses in this group are frequently referred to as "*ring spot viruses"* (Harrison, 1961) and are transmitted by *Xiphinema* and *Longidorus* nematode species. The other nepo viruses are arabis mosaic, strawberry latent ring spot, cherry leaf roll, tobacco ring spot, tomato ring spot, raspberry ring spot and tomato black ring viruses. They all possess polyhedral particles about 27-30nm diameter.

The second group includes the viruses with rod-shaped or tubular particles for which Harrison (1964) derived the name NETU, i.e., nematodes transmitted viruses with tubular particles. The three viruses in this group: tobacco rattle virus, pea early browning virus and pepper ring spot virus, are transmited by *Trichodorus* and *Paratrichodorus.* The tobra viruses have tubular particles of predominantly two lengths, 190 nm and 45-115nm depending on the isolate.

At present there are 11 species of *Xiphinema*, 10 species of *Longidorus* and one species of *Paralongidorus* and in Trichodoridae 5 species of *Trichodorus* and 9 of *Paratrichodorus* that have been reported as vectors of plant viruses throughout the world (Lamberti & Roca, 1987). Overall there are 22 longidorid **(Tables 14.1 and 14.2)** and 14 trichodorid **(Table 14.3)** nematodes that have been reported as vectors of plant viruses. The tables show alphabetically arranged lists of those species that have been implicated as virus vectors. *Xiphinema americanum* sensu lato is reported to be a vector of tomato ringspot virus (TomRV) (Converse and Ramsdell, 1982; Teliz *et al*., 1966). This virus vector relationship should, however, be reconsidered after numerous descriptions and redescriptions of species and populations which fall in the *X. americanum* group.

Table 14.1. Species of *Xiphinema* implicated in transmission of plant viruses.

S. #	Nematode species	Virus
1.	*X. americanum* Cobb	TomRV (tomato ring spot)
		TobRV (tobacco ring spot)
		PRM V (peach rosette mosaic)
		CRLV (cherry rasp leaf)
2.	*X. bakeri* Williams	AMV (arabis mosaic)
3.	*X. brevicolle* Lordello *et* Da Costa	TomRV (tomato ring spot)
4.	*X. californicum* Lamberti *et* Bleve-Zacheo	TomRV (tomato ring spot)
5.	*X. coxi* Tarjan	BMV (brome mosaic)
		AMV (arabis mosaic)
		CLRV (cherry leaf roll)
		SLRV (strawberry latent ring spot)
		TobRV (tobacco ring spot)
6.	*X. diversicaudatum* (Micoletzky) Thorne	AMV (arabis mosaic)
		SLRV (strawberry latent ring spot)
		BMV (brome mosaic)
		CLRV (cherry leaf roll)
		CRV (carnation ring spot)
		RRV (raspberry ring spot)
7.	*X. ifacolum* Luc (nec *X. basiri* Siddiqi)	CPMV (cowpea mosaic)
8.	*X. index* Thorne and Allen	GFV (grapevine fan leaf)
		GCMV (grapevine chrome mosaic)
		AMV (arabis mosaic)
9.	*X. italiae* Meyl	GFV (grapevine fan leaf)
10.	*X. rivesi* Dalmasso	TomRV (tomato ring spot)
11.	*X. vuittenezi* Luc, Lima, Weischer and Flegg	CLRV (cherry leaf roll)

Table 14. 2. Species of *Longidorus* and *Paralongidorus* implicated in transmission of plant viruses.

S. #	Nematode species	Virus
1.	*L. apulus* Lamberti and Bleve-Zacheo	AILV (artichoke Italian latent) Apulian strain
2.	*L. attenuates* Hooper	TBRV (tomato black ring) English strain
3.	*L. caespiticola* Hooper	AMV (arabis mosaic)
		RRV (raspberry ring spot)
4.	*L. diadecturus* Eveleigh and Allen	PRMV (peach rosette mosaic)
5.	*L. elongatus* (de Man) Thorne and Swanger	TBRV (Scottish strain)
		RRV
		CLRV
		CRV
6.	*L. fasciatus* Roca and Lamberti	AILV (artichoke Italian latent) Greek strain
7.	*L. leptocephalus* Hooper	RRV (raspberry ring spot)
		CLRV
8.	*L. macrosoma* Hooper	RRV (English strain)
		Scottish strain
		CLRV
		BMV
		CRV
		PNRV (prunus necrotic ringspot)
9.	*L. martini* Merny	MRV (mulberry ringspot)
10.	*L. profundorum* Hooper	RRV
11.	*P. maximus* (Butschli) Siddiqi	AMV
		RRV
		SLRV
		CLRV

Table 14.3. Species of *Paratrichodorus* and *Trichodorus* implicated in transmission of plant viruses.

S.#	Nematode species	Virus
1.	*P. allius* (Jensen) Siddiqi	TRV (tobacco rattle) Oregon
		TRV California
2.	*P. anemones* (Loot) Siddiqi	PEBV (pea early browning) English strain
		TRV
3.	*P. christiei* (Allen) Siddiqi	TRV Wisconsin California
4.	*P. minor* (Colbran) Siddiqi	TRV Japan
5.	*P. nanus* (Allen) Siddiqi	TRV Dutch strain
6.	*P. pachydermus* (Seinhorst) Siddiqi	TRV Dutch strain
		English strain PEBV
		Dutch strain
		English strain
7.	*P. porosus* (Allen) Siddiqi	TRV California
8.	*P. teres* (Hooper) Siddiqi	PEBV Dutch strain
		TRV Dutch strain
		Oregon
9.	*P. tunisiensis* (Siddiqi) Siddiqi	TRV
10	*T. cylindricus* Hooper	TRV Dutch strain
11.	*T. hooperi* Loof	TRV
12.	*T. primitivus* (de Man) Micoletzky	TRV English strain
		German strain
		Scottish strain
		PEBV English strain
13.	*T. similis* Seinhorst	TRV
14.	*T. viruliferus* Hooper	PEBV English strain
		Italian strain N 6
		TRV

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There are several published reports of the experimental transmission of tobacco ringspot virus (TobRV) by populations of *X. americanum.*

Xiphinema diversicaudatum is one of the most studied nematode vectors of plant viruses. It is well established that the species is a very efficient vector of AMV and of its hop- strain (Jha and Posnette, 1961; Valdez *et al*., 1974). In glasshouse and field studies it has been shown that adult and juvenile stages of the nematode differ in their vector efficiency and that infectivity is lost during the moulting process. *X. diversicaudatum* is also the vector of SLRV (Harrison, 1967). The differential transmission of strains of the virus and the variability of the vector efficiency between field populations of *X. diversicaudatum* has been amply demonstrated by Lamberti *et al*., 1986; Brown, 1985.

Xiphinema index was the first nematode to be conclusively demonstrated as a vector of a plant virus Hewitt *et al*., 1958. Since then the nematode has been shown to be a very efficient vector of grapevine fanleaf virus (GFV) in all the grapevine growing countries of the world.

Paratrichodorus pachydermus is the first trichodorid species reported to transmit a plant virus i.e. tobacco rattle virus (TRV) (Sol and Seinhorst, 1961). Later it was also shown that this species and *Paratrichodorus teres* are also vectors of pea early browning virus (PEBV) that damages pea crop and infect other plant also. These are the only two viruses transmitted by nematodes belonging to the family Trichodoridae.

There are five species of *Trichodorus* and eight of the nine species of *Paratrichodorus* (*Paratrichodorus anemones* is the exception) implicated in virus transmission as vectors of TRV. Conversely, only two species of *Trichodorus* and three *Paratrichodorus* are said to be vectors of PEBV.

Studies on the factors affecting the vector efficiency of Trichodorids indicate that 15° C is the optimum temperature for transmission of TRV and that below 10% soil moisture content, virus transmission is unlikely (Van Hoof, 1976). Trichodorid nematodes can retain the virus as long as 10 months and exceptionally even a year and single males have been

shown to transmit TRV to several plants when allowed to feed for 1 day on the roots of each plant separately. The epidemiology and the spread of TRV in the field with relation to trichodorid vectors were investigated by various authors and they indicate that the distribution of trichodorids in the soil is very erratic and affected by several physical and chemical components. But whenever the nematode is present, even in low numbers, virus transmission is efficient. Establishment and (or) reproduction of trichodorids in the soil is more difficult than for many longidorid nematodes, but the virus-vector-plant relationship seems to be more stable among trichodorids than among longidorids.

Characterization of Longidorid Nematodes

Longidorid nematodes are characterized by an axial stylet measuring up to 200 µm consisting of an elongated spear (odontostyle) plus an extension (odontophore) usually half as long as the odontostyle; by a typical dorylaimid oesophagus with the stylet connected to the cylindrical, muscular and glandular bulb by a slender food canal; and by their 1.5-13 mm long slender body. Juveniles are recognized by having both a functional and a replacement odontostyle, for the next stage, situated in the wall of the anterior oesophagus. The tail is usually short, hemispheroid or conoid, rarely elongate or filiform. Of the five genera comprising the family, only some members of the genera *Xiphinema*, *Longidorus* and *Paralongidorus* are virus vectors. Species of *Longidorus* are characterized by a single guiding ring around the anterior part of the odontostyle; by a simple junction between odontostyle and odontophore; and by an odontophore usually without basal flanges. In *Xiphinema* the guiding sheath is around the posterior part of the odontostyle. The base of the odontostyle is forked at the junction with the odontophore and the odontophore has prominent basal flanges.

Characterization of Trichodorid Nematodes

Trichodorid nematodes are readily recognized by their ventrally curved mouth spear (onchiostyle), which is an elongated dorsal tooth without a lumen and without basal knobs or flanges. The oesophagus consists of a narrow anterior part swelling posteriorly to form an elongate or pear-

shaped basal bulb. The body length in adults is range from 0.5 to 1 mm, the females appearing plump and cigar shaped. The cuticle appears thick, particularly in dead individuals. Virus vectors occur in the genera *Trichodorus* and *Paratrichodorus*. Both are similar in appearance, the oesophagus of a narrow anterior section which expands posteriorly to form a spathulata bulb. Bulb usually non overlapping in *Trichodorus* whereas oesophageal bulb usually overlaps the intestine ventrally in *Paratrichodorus*. Furthermore, males of *Paratrichodorus* have a bursa, which is absent in *Trichodorus*.

Occurrence of Virus Vector Nematodes in Pakistan

In Pakistan, four known species of *Xiphinema* and one each of the genus *Longidorus* and *Paratrichodorus* as virus vector nematodes *viz*., *Xiphinema americanum*, *X. brevicolle*, *X. index*, *X. rivesi*, *Longidorus elongatus* and *Paratrichodorus minor* have been found associated with various crops from Pakistan (Table 4). Akhtar (1962) first reported the presence of *X. americanum* associated with the roots of sugarcane. *X. brevicolle* was reported from coconut palm plantation in Balochistan by Bilquees and Khan (1982). Maqboo1 (1984) detected *X. index* and *X. rivesi* associated with grapevine and citrus from Quetta and Punjab, respectivcly.

Longidorus elongatus and *Paratrichodorus minor* were reported by Anwer & Sarwar (1981) associated with citrus roots and rhizosphere in the Punjab. It is evident that plant parasitic nematodes, especially those of the genus *Xiphinema* are widely distributed in cultivated soils in all districts of Pakistan surveyed till 2021. Though *Xiphinema* species were collected from vegetable, cereal and other crops also, they were mostly confined to fruit trees. The species of this genus are polyphagous and are predominant in soil around woody and perennial plants, although numerous soil samples from annual crops have also been examined.

The common species of virus vector nematode is *X. americanum* which is widely distributed throughout the country and was present around the roots of a majority of the crops surveyed. It is more common in fruit trees (54%), less around cereals (25%), ornamentals (12.5%), and

vegetables and other crops (4.1%). It is also occasionally found associated with the roots of flowering plants, grasses and other seasonal crops. *X. americanum* is reported to be a vector of tomato and tobacco ring spot virus (Converse & Ramsdell, 1982; Dias, 1977). It was collected from 24 different hosts in 36 localities of all provinces of Pakistan. Of these, 15 were from Punjab, 8 from Sindh, 7 from NWFP (since renamed as Khyber Pakhtunkhwa or KP) and 6 from Balochistan. Other *Xiphinema* species of known virus vector nematodes in Pakistan are *X. brevicolle*, *X. index* and *X. rivesi*. Of these, *X. brevicolle* and *X. index* are frequently found in different crop soils. *X. index* and *X. rivesi* are common in soil around the roots of fruit trees (45%).

It was reported from *Citrus* spp. in Swat, Rawalpindi, Islamabad, Sargodha, Faisalabad, Sahiwal and Multan and from soil around the roots of *Ficus carica* in Dadu, Nawabshah and Badin. *Xiphinema brevicolle* was found from all the fruit trees surveyed in low to high populations in all the four provinces. According to Fritzsche & Kegler (1968) *X. brevicolla* transmitted tomato ring spot virus (TomRV). *X. rivesi* was found only from soil around the roots of citrus from four localities of Punjab. It has been recorded as a vector of tomato ring spot virus (Forer *et al*., 1981; Mountain *et al*., 1983; Powell & Forer, 1982). *Longidorus elongatus* was the only species found in the genus *Longidorus* and was reported from soil around the roots of *Arachis hypogaea* in Rawalpindi, Jehlum and Attock. It was also recovered from soil around the roots of *Citrus* species in Mardan, Peshawar, Nowshera, Charsadda, Faisalabad, Jhang and Sahiwal. *Gossypium hirsutum* is the host from Rawalpindi, Islamabad, Lahore and Faisalabad.

It was also reported from soil around the roots of *Solanum tuberosum* in Rawalpindi, Okara and Attock. *Longidorus elongatus* is the vector of the Scottish strains of TBRV and RRV (Taylor, 1962). It has also been associated with the transmission of CLRV and of CRV in laboratory experiments (Jones *et al*., 1981; Fritzsche *et al*., 1979). *Parotrichodorus minor* (syn= *T. christiei*) is the only trichodorid species reported from Pakistan. It was found in soil around the roots of *Citrus* species and *Saccharum officinarum* from five localities of Punjab province. *Paratrichodorus minor* is the vector of TRV (Walkinshaw *et al*., 1961;

Ayala & Allen, 1966). It is also a vector of pepper ring spot virus in South America (Walkinshaw *et al*., 1961).

Sites of Retention of Viruses in Nematodes

The sites of retention of viruses in nematodes have been discovered by means of electron microscopy of ultrathin sections of virus-carrying nematodes. Taylor & Robertson (1969) found that in *Longidorus elongates* particles of raspberry ring spot or tomato black ring viruses accumulate as a single layer on the inner surface of the guiding sheath **(Fig.14.1)**. In *Xiphinema diversicaudatum* carrying arabis mosaic virus and *X. index* carrying grapevine fan leaf virus, they found virus particles only in association with the cuticular lining of the oesophageal lumen, occurring as a monolayer from the anterior part of the odontophore to the posterior end of oesophageal basal bulb (Taylor & Robertson, 1970a); similar observations have been made on *X. americanum* carrying tobacco ringspot virus (McGuire *et al*., 1970). It is evident that virus particles are adsorbed more readily to the lining of the odontophore than to that in the remainder of the oesophagus. Tobacco rattle virus is found in a similar position to that of the viruses in *Xiphinema* spp., namely in association with the cuticular lining of the lumen of the oesophagus (Taylor & Robertson, 1970).

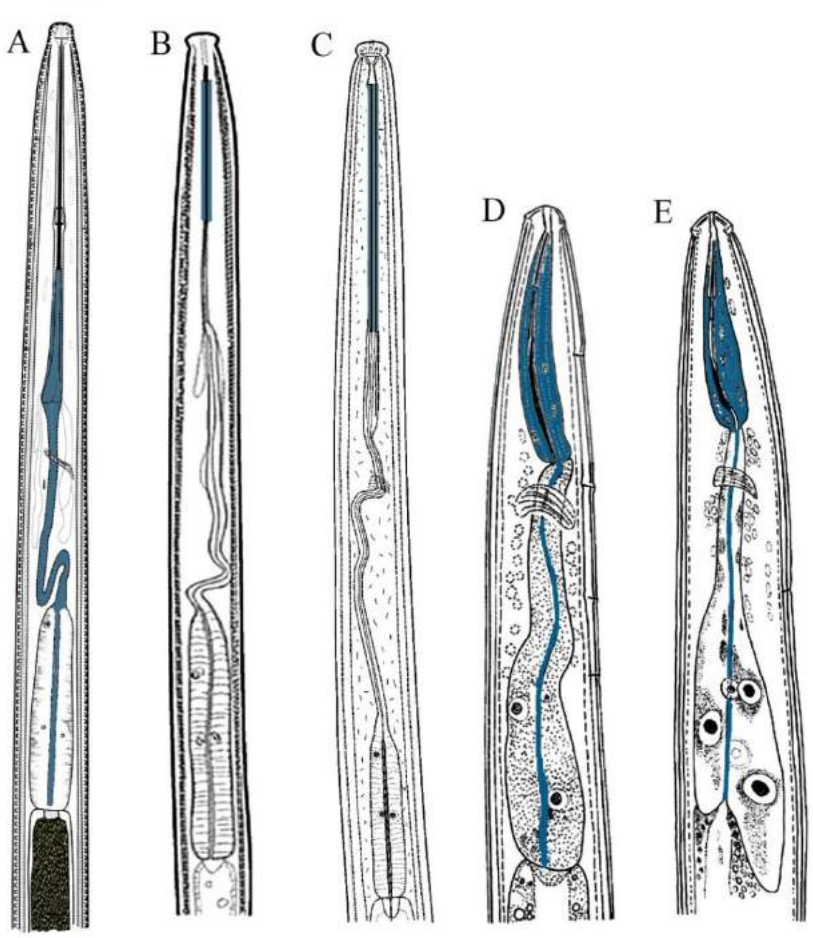

Fig. 14.1. Sites of virus retention (marked in blue): A. *Xiphinema*; B. *Longidorus*; C. *Paralongidorus*; D. *Trichodorus*; E. *Paratrichodorus* (From NNRC).

Chapter 15

ENTOMOPATHOGENIC NEMATODES

Nematodes of the family Steinernematidae (Chitwood and Chitwood, 1937) and Heterorhabditidae (Poinar, 1976) are known as entomopathogenic nematodes (EPNs). EPNs are potential biological control agent nematodes, as they are highly virulent because of their symbiotic association with bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. (Akhurst and Boemare, 1990). These nematodes are obligate parasites of insects and have many attributes that make them good and promising biocontrol agents, such as; they have short lifecycle, a wide host range, are capable of resisting under unfavourable conditions, and easy to mass produce and apply under field conditions.

The parasitism by these beneficial nematodes results in suppressing the insect host immune system (Lewis and Clarke, 2012). Many insect antagonists are found within the phylum Nematoda, but only species within the genera *Steinernema* and *Heterorhabditis* (Rhabditida) have gained major importance as biocontrol agents in plant protection. Many species of these EPNs have been commercially developed as bioinsecticides. EPNs have been a growing concern globally mainly because of their potential efficiency, efficacy, exemption from registration, and are attractive and excellent candidates for commercial utilization. They are popular, also because several species can be mass produced economically, formulated and applied for control of a number of soil-dwelling insect pests and are compatible with most agricultural chemicals.

In Pakistan the pioneering work on EPNs has mainly been done by Shahina and co-workers. So far two genera and fifteen species belonging to the families Steinernematidae and Heterorhabditidae have been indigenously discovered in Pakistan (Shahina *et al*., 2019).

EPN Extraction from Soil Samples

For using EPNs as biocontrol agents for insect pests, they need to be collected from the natural fields using insect baiting techniques and

cultured/reared in control conditions for bulk production as explained below.

***Galleria mellonella* L. Rearing:** Entomopathogenic nematodes can be extracted from soil using the Galleria soil trap method. The greater wax moth *Galleria mellonella* L. is used as an excellent bait for collection and production of EPNs; thus rearing of the wax moth is a prerequisite. Rearing diet of *G. mellonella* is prepared by mixing bran (150 g), yeast granules (10 g), ground grains of wheat (65 g), maize (65 g) and rice (65 g) in a solution of 80 ml warm honey and 100 ml glycerol (Shahina *et al*., 2011).

***Galleria* Soil Trap Method:** "*Galleria* soil trap" method is used for the extraction of entomopathogenic nematodes. Moist soil samples are distributed into plastic pots (90 cm diam) @ 200-250 g/pot (Bedding and Akhurst, 1975). Five wax moths *G. mellonella* larvae (insect bait) are placed in a pot covered with a lid; pots are turned upside down. Pots are incubated for 5-7 days at room temperature. From the 2nd day onwards, pots are checked daily and the dead larvae removed.

They are rinsed thoroughly with water and dissected in Ringer's solution. Dead larvae are placed in a White trap for recovery of nematode progeny. Dead larvae with a brown or ochre coloration would usually be parasitized by steinernematids, whereas brick reds to dark purple cadavers indicate having been parasitized by heterorhabditids. The insect baiting technique is the most commonly used method for recovery of EPN from soil samples. It is a convenient strategy that allows the retrieval of EPN in the lab.

White Trap Method: Infective juveniles are recovered from infected insect (dead cadaver) by the method given by White, 1927. Plastic containers are used as a White trap. Plastic cavity block or small Petri dish is placed inverted at the bottom of the larger Petri dish; filter paper is placed on the top of cavity block or small Petri dish. Larger dish is filled with distilled water. Plastic containers are covered with lid and incubated at room temperature until emergence of infective juvenile stages (IJs). Infective juveniles emerge from cadaver and migrate to the

surrounding water. IJs are then collected every day until all nematodes have been recovered. Infective juveniles are placed in a storage chamber at 10-20 ºC in 100 ml tissue culture flask having distilled water with a drop of Triton X-100. The White trap technique utilizes the attraction of nematodes to water and successfully recovers infective stages of EPN free of insect tissues and/or debris.

EPN Lifecycle

The third stage larvae (or dauer larva), is the only stage of EPN lifecycle that can survive outside the host. It is the stage that is applied in control programmes. It does not feed but searches or waits for an insect host that it can infect. In nature, the infective juvenile (IJ) stage of the EPNs is found in soil. The infective larvae infect its host by entering through natural openings viz., mouth, anus or spiracles and penetrating into the insect's haemocoel. Infective larvae of *Heterorhabditis* species may also penetrate directly through cuticle. The nematodes enter the haemocoel and release their symbiotic bacterial cells. The bacteria (*Xenorhabdus* spp. for steinernematids; and *Photorhabdus* spp. for heterorhabditids) are found in the anterior part of the intestine of the third stage infective larvae; the bacteria proliferate and release a wide range of toxins and many secondary metabolites that cause massive septicaemia and death of the host within 24–72 h. The infective larvae develop and reproduce in the insect cadaver by feeding on the bacterial cells and decayed host tissues, and mature into males and females (*Steinernema* spp.) or hermaphrodites (*Heterorhabditis* spp.). One or more generations of offsprings develop, reproducing continually until the resources of the host are exhausted. The third stage larvae retain their second stage cuticle and are released from the cadaver as the insect disintegrates. The cycle may continue in the presence of sufficient insect hosts (Kerry and Homonick, 2002) **(Fig. 15.1)**.

Commercial Use of EPN in Biocontrol

EPNs have several advantages that qualify them as commercially valuable biocontrol agents. They are highly effective than chemical compounds. EPNs are mobile and persistent. They recycle inside the host

insect, thereby causing long-term, sustainable effects on the pest populations (Peters, 1996). The use of EPN is safe for both the user and the environment. They have little detrimental effects on non-target insect populations; and neither the nematodes, nor their bacterial associates cause any detrimental effect to mammals or plants (Ehlers and Hokanen, 1996).

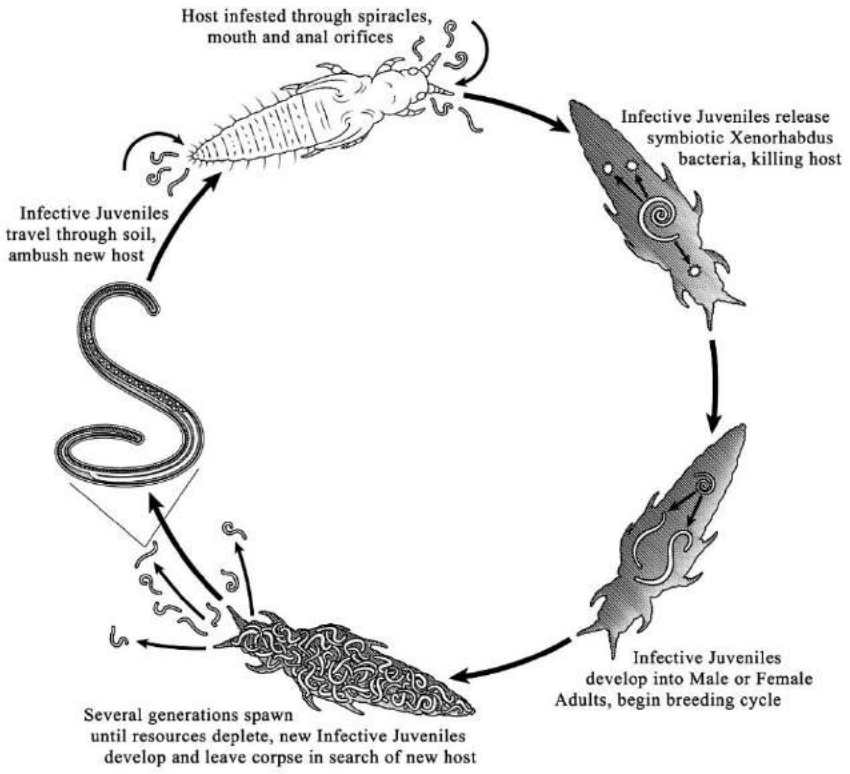

Fig. 15.1. Lifecycle of entomopathogenic nematodes (Courtesy of Sound Horticulture).

Steinernema Travassos, 1927

Parasitism and Habitat: *Steinernema* species are parasites of insects and other arthropods. EPNs (from the families Steinernematidae and

Heterorhabditidae) are found under diverse ecological conditions including cultivated fields, forests, grasslands, deserts and beaches of oceans (Hominick *et al*., 1996).

Main Morphological Characteristics

Female: Large, size variable. C-shaped or spirally coiled on heat relaxation. Cuticle smooth or annulated. Lateral fields absent. Excretory pore distinct. Head rounded or truncate, rarely offset. Six lips present, partly or completely fused, each lip with one labial papilla, sometimes additional papilla-like structures present near labial papillae. Four cephalic papillae. Amphids present, small. Cheilorhabdions pronounced, forming a ring resembling two large sclerotized dots in lateral view. Other parts of stoma forming an asymmetrical funnel with thick anterior end. Oesophagus rhabditoid with metacorpus slightly swollen, narrow isthmus surrounded by nerve ring, and large basal bulb with reduced valve. Excretory pore distinct, located anterior to nerve ring. Oesophago-intestinal valve usually pronounced. Vulva at mid-body, sometimes on a protuberance with or without epiptygma. Genital system didelphic, amphidelphic, reflexed, oviparous or ovoviviparous with juveniles developing up to the infective stage (IJ) before emerging from the body of the female. Tail shorter, one or less than anal body width in first generation but longer in second generation. Phasmids may be inconspicuous.

Male: Medium sized, smaller than female. J- or C-shaped on heat relaxation. Cephalic region with six labial papillae and four cephalic papillae usually with perioral disc. Oesophagus similar to that of the female. Testis single, reflexed. Spicules paired, robust. Gubernaculum large, sometimes as long as spicule; bifurcate in ventral view. Bursa absent. Tail short, terminus rounded, digitate or mucronate. One single and 10 to 14 pairs of genital papillae present with 7 to 10 pairs precloacal.

Infective Juvenile (third-stage infective juvenile): Body slender, vermiform, non-feeding, with or without a sheath (cuticle of second-stage juvenile). Straight or slightly ventrally arcuate on heat

relaxation. Cuticle finely annulated. Mouth and anus closed, stoma collapsed. Lateral fields well developed with 4-9 incisures. Oesophagus and intestine non-functional, reduced. Excretory pore distinct, anterior to nerve ring. Symbiotic bacteria usually visible in intestinal pouch just posterior to basal bulb. Tail conoid or filiform. Phasmids located about mid-tail, inconspicuous **(Fig. 15.2)**.

Fig. 15.2. A, D & F. First generation male: A. Entire body; D. Oesophageal region; F. Tail region; B, E & G. Second generation male: B. Entire body; E. Oesophageal region; G. Tail region; C. First generation female (From NNRC).

Heterorhabditis **Poinar, 1976**

Parasitism and Habitat: *Heterorhabditis spp.* are obligate parasites of insects and other arthropods. EPNs (from the families Steinernematidae and Heterorhabditidae) are found under diverse ecological conditions, including; cultivated fields, forests, grasslands, deserts and beaches of oceans (Hominick *et al*., 1996).

Main Morphological Characteristics

Hermaphroditic Female: After entry into an insect host, infective juveniles develop into hermaphroditic females. Head truncate to slightly rounded; six conical lips well developed, separate, each with a terminal papilla; one or two small raised structures, sometimes visible at the base of each lip; amphidial opening small. Stoma wide but shallow; cheilorhabdions present, forming a cuticularised ring in lateral view resembling two refractile elongate structures. Other parts of the stoma fused to form a collapsed posterior portion. Posterior part of stoma covered by oesophagus. Oesophagus without metacorpus; isthmus slender; basal bulb swollen; valve in basal bulb reduced. Nerve ring at middle of isthmus. Excretory pore usually posterior to oesophagus. Vulva slight anterior to mid-body (V% = 43-48), slit-like, surrounded by elliptical rings; ovotestis amphidelphic, reflexed. Oviparous, later becoming ovoviviparous. Tail pointed, longer than anal body width, postanal swelling usually present.

Amphimictic Female: Similar to, but usually smaller than hermaphroditic female. Labial papillae prominent. Genital system amphidelphic, reflexed. Vulva not functional for egg deposition (juvenile hatch in body), but functional for mating.

Male: Testis one, reflexed. Spicules paired, separate, slightly curved ventrally. Spicule head short, offset from lamina by a constriction. Gubernaculum usually about half as long as spicule length. Bursa well developed peloderan with nine pairs of genital papillae.

Infective Juvenile: Third-stage infective juvenile usually with sheath (cuticle of second-stage juvenile). Sheath with anterior tessellate pattern and longitudinal ridges; IJ cuticle striated with one smooth band marginated by two ridges in lateral fields. Head with prominent dorsal tooth. Mouth and anus closed, non-functional. Stoma appearing as a closed chamber with parallel walls. Oesophagus and intestine reduced. Excretory pore posterior to nerve ring. Symbiotic bacterial cells found in intestine. Tail conoid, pointed **(Fig. 15.3)**.

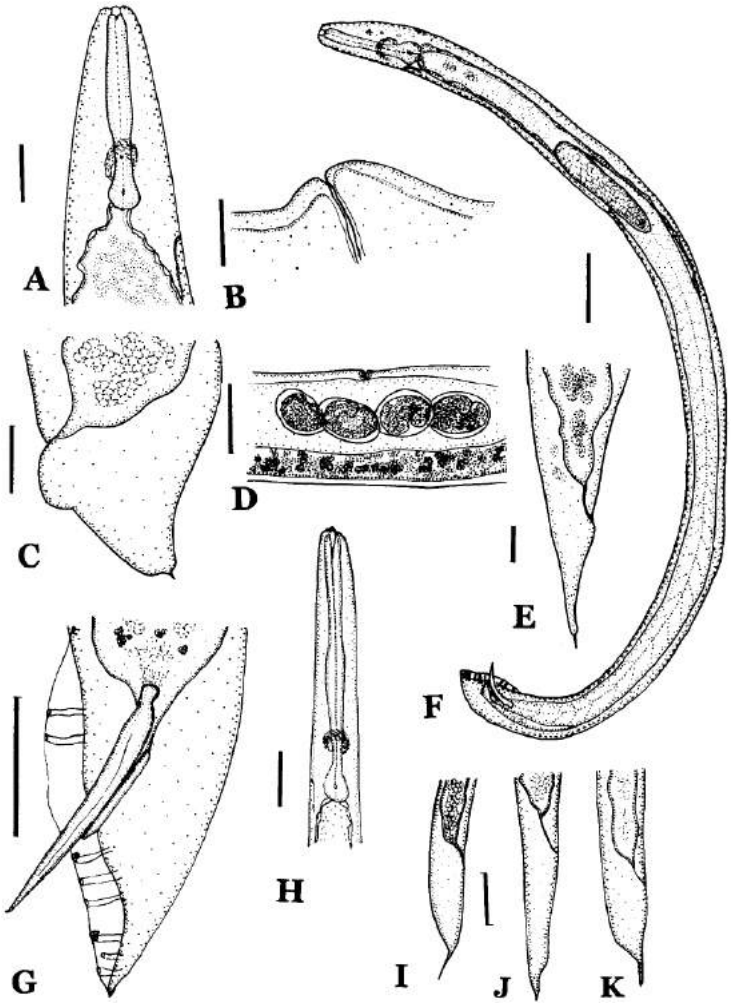

Fig. 15.3. A. Anterior end of hermaphrodite; B. Vulva of hermaphrodite; C. Tail of hermaphrodite in lateral view; D. Vulva of female; E. Ttail of

female in lateral view; F. Male entire body; G.Male tail in lateral view; H. Anterior end of third stage infective juvenile; I.–K. Tail of third stage infective juvenile (Patrick S, 2002).

Entomopathogenic Nematode Research Studies in Pakistan

Shahina and Maqbool in 1996 initiated the EPN research at National Nematological Research Centre (NNRC) of the University of Karachi and reported *Heterorhabditid* and *Steinernematid* nematodes for the first time in Pakistan. Thereafter, intensive research has been carried out on different aspects of these nematodes viz., their occurrence, geographical distribution, morphological and molecular identification, virulence, pathogenicity, mass culturing, formulations, and application as biopesticides on many insect pests of several crops, fruits and trees etc. The molecular identification of entomopathogenic nematode was initiated by Shahina *et al*., (2010) at NNRC. Moreover, one US International patent and 10 National patents have been obtained so far.

Up till 2021, six new species and nine new records of EPN have been described and re-described along with their symbiotic bacteria from different localities in Pakistan (Shahina *et al*., 2019). Currently, a total of the fifteen (15) EPN species (12 steinernematids and 3 heterorhabditids) have been detected from Pakisan. The detailed morphological and molecular studies undertaken include; the description of six new species viz., *Steinernema pakistanense* Shahina *et al*., 2001, *S. asiaticum* Anis *et al*., 2002, *S. maqbooli* Shahina *et al*., 2013, *S. bifurcatum* Shahina *et al*., 2014, *S. balochiense* Shahina *et al*., 2015 and *Heterorhabditis pakistanense* Shahina *et al*., 2016 while nine species viz., *S. abbasi* Elawad *et al*., S. *affine* (Bovien, 1937) Wouts *et al*., 1982, *S. carpocapsea* (Weiser, 1955) Wouts *et al*., 1982, *S. feltiae* (Filipjev, 1934) Wouts *et al*., 1982, *S. cholashanense* Nguyen *et al*., 2008, *S. litorale* Yoshida, 2004, *S. siamkaiya, H. bacteriophora* Poinar, 1976 and *H. indica* Poinar *et al*., 1992. All these species have been reported as new records from Pakistan. Moreover, three species of symbiotic bacteria of the EPN viz., *Photorhabdus luminescens* Boemare *et al*., 1993, *Xenorhabdus nematophila* (Poinar and Thomas, 1965) Thomas and Poinar, 1979 and *X. bovenii* (Akhurst, 1983) Akhurst and Boemare,

1993 have been reported from Pakistan (Shahina *et al*., 2004; Kazmi *et al*., 2013).

Chapter 16

MOLECULAR TECHNIQUES IN NEMATOLOGY

Nematodes are one of the most diverse species. Despite this, nematodes are one of the least studied organisms, with only 0.01 percent of their species variety described. The traditional morphology-based identification method has been found to be insufficient for studying nematode identity and diversity, owing to a lack of morphological variation across closely related taxa. Various molecular methods have been employed to supplement morphology-based methods.

The molecular techniques in nematology include fingerprinting and sequence studies of DNA and/or protein-based data. This accomplishment has also been aided by visual analysis tools. Each of these methods involves and presents example of how these techniques have been used in nematode identification in recent years. These alternate methodologies have facilitated nematode identification and improved our understanding of nematode diversity and phylogeny. Review of the advantages and disadvantages of these methods lead to conclusion that no single method can provide all of the answers; the method to use is determined by the topic at hand, the nature of the samples, and the resources available.

Plant parasite species account for around 4100 of the 26,000 species identified, causing severe economic damage to all crops. Nematodes are also important in medicine and veterinary medicine, and free-living nematodes are essential for environmental nutrient recycling. Therefore correct identification is critical for understanding the nematode diversity, their function in the ecosystem and for developing effective control and management techniques.

Body length, mouth and tail parts, sexual organ morphology, and other physical traits have traditionally been used to identify animals. Because of the absence of clear variation among closely related species and the need for highly qualified taxonomists, this morphology-based classification may prove unsatisfactory. Morphology-based identification

is likewise a difficult task, especially when dealing with huge numbers of samples. Several molecular (protein- and DNA-based) approaches have been used to enhance or overcome the limits of morphology-based nematode categorization.

Nematologists have used nematode ribosomal DNA (rDNA) sequencing and identification for better understanding of nematode evolutionary relationships and identification (Abebe *et al*., 2011). Without going into the details on nematode evolution or phylogenetic relationships, it is critical to ensure accurate nematode identification, and more importantly, to understand how we identify a nematode species.

In this chapter the existing nematode taxonomy approaches as well as prospective advancements are discussed.

Shortcomings of Classical Taxonomy

Using microscopic image analysis, nematode identification is traditionally based on morphological and anatomical differences. Morphological identification is one of the less expensive ways of identification, and it aids in the correlation of morphology with potential functions.

While significant distinctions in nematodes' morphology are the most effective too, the nematodes with minor morphological and morphometric changes, such as body length, the form of a stylet, the shape of the tail, and so on, are difficult to identify morphologically. RKN (*Meloidogyne* spp.) were previously diagnosed based on a set of characteristics that was originally proposed to distinguish *Meloidogyne incognita*, *M. javanica*, etc., such as; adult female perineal patterns i.e., the posterior region of the vulva-anus area (perineum), tail terminus, phasmids, lateral lines, and surrounding cuticular striae; However, the perineal patterns (and other morphometric features) overlapped between species, particularly with the discovery of additional species; hence they became insufficient tools to differenciate the *Meloidogyne* species. Previously described new species of RKN are now identified using a combination of morphological and molecular features.

Cyst nematodes (*Heterodera* spp. and *Globodera* spp.) are another example of harmful plant parasitic nematodes with a global distribution. The morphology of their cysts distinguishes *Heterodera* and *Globodera*. Cysts of *Heterodera* are lemon-shaped, while *Globodera* are round. The vulval cone, cone top, vaginal, and lip features are used to identify species within *Heterodera*. The shape of cysts and second stage juveniles is used to make taxonomic distinctions within *Globodera*.

The presence of a host plant can also indicate the cyst nematode species; however this can be misleading at times, as in the case of *Heterodera* cereal cyst nematode group. Cyst nematode morphological identification necessitates taxonomic competence and can be difficult if samples contain mixed species. Furthermore, both genera contain species complexes with individuals that are difficult to differentiate solely on the basis of morphology.Similarly, the shape of head, number of annules, body length, length of stylet, shape of stylet knob, structure of lateral fields, presence/absence and shape of spermatheca, shape of female tail terminus, shape and length of spicule, and gubernaculum, are all important morphological identification characters in nematodes. Measurement of these features and sample processing for this purpose requires the availability services of experienced taxonomists, whose numbers are declining.

Moreover, nematode morphology can be influenced to certain extent by geographic location, host plant, nutrition, and other environmental conditions, as observed in case of free-living and some plant parasitic nematodes. It is therefore very difficult for non-specialists to correctly and confidently identify a nematode species based on morphology alone, and evidence such as DNA sequence must be required for correct identification and confirmation.

Advance Techniques of Nematode Identification

Auto-florescence

Natural auto-fluorescence of microorganisms could be used as a supplement to regular light microscopy. The emission and excitation

spectra of the bacterial genera *Lactobacillus* and *Saccharomyces* were proved to be different as these spectroscopic fingerprints may be used to distinguish between various *Saccharomyces* species without the need of fluorescent labelling. Researchers have done extended work on this by demonstrating that; when eggs of different helminths were irradiated at different wavelengths spanning from white light to infrared light, they displayed distinct florescence. They also demonstrated that differences in florescence lifetime values (florescence intensity decay) were diagnostic of the two species studied, *Ascaris lumbricoides* and *Ascaris suum*. It was concluded that spectroscopic features and lifetime value measurements of auto-fluorescence in nematodes are promising tools in the taxonomy of these organisms (Bhatta *et al*., 2006).

DNA-Based Methods

For the identification of nematodes, a variety of DNA-based approaches have been developed. These can be divided into two categories: fingerprint-based and nucleotide-based approaches.

Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplification of Polymorphic DNA (RAPD), and the use of species-specific primers are examples of fingerprint-based approaches that rely on the presence or absence of a polymerase chain reaction (PCR) amplification result. All fingerprint-based procedures use PCR followed by electrophoresis, with the exception of RFLP, which may not require PCR. The resulting DNA fingerprint, or pattern of resolution of the DNA fragments, is utilized to identify and/or phylogenetically analyze the nematode taxa in question.

Nucleotide-based approaches, on the other hand, entail PCR amplification, particular probe hybridizations and sequencing of a region(s) of DNA, which is subsequently employed in phylogenetic analysis. When compared to other nematode identification methods, DNA-based or not, each of these methods has its own set of benefits and drawbacks (Blaxter, *et al.*, 1998). However, it is worth noting that nematode genomes have significantly impacted our knowledge of taxonomic relationships.

Fingerprint-Based Methods

Fingerprints formed from genomic DNA (gDNA) digested with one or more endonucleases can be used in RFLP analysis. Alternatively, PCR-amplicons (PCR-RFLPs) can be used to create fingerprints. Because of the size of the gDNA template, gDNA-RFLPs are more complex, but they may disclose more polymorphisms. Furthermore, unlike PCR-RFLPs, gDNA-RFLPs do not require prior knowledge of sequence information. However, in all circumstances, it is important to make sure that restriction digestions are completed; because incomplete digestions can result in non-reproducible fingerprints.

By preferentially amplifying fewer restriction products and yielding less-complex fingerprints, the AFLP approach outperforms gDNA-RFLP. Two restriction enzymes degrade gDNA, resulting in sticky ends to which ligated adaptors are attached. The adaptors, sticky ends, and one to three nucleotides inside the restriction sites are all recognized by primer sets that preferentially amplify a fraction of these adaptor-ligated segments. AFLPs, like gDNA-RFLPs, do not require prior sequence knowledge, but completion of restriction digestions is required for repeatable fingerprints.

RAPD entails amplification of gDNA fragments by PCR using short (typically 10 bp) arbitrary sequence primers. Amplification occurs when two primers bind on opposite strands of DNA with their 3’ends facing each other at a distance that the polymerase can travel. As a result, fragments of varying sizes may be formed, with the size of the bigger fragments being determined by the efficiency of polymerase. For this reason, it's critical to use a large, undamaged gDNA template. Because RAPDs are performed at lower temperatures, there is less stringency for primer annealing, which limits reproducibility, especially between laboratories. This approach has the advantage of not requiring prior knowledge of the template DNA's sequence information. Primers sets that only amplify a PCR product in a taxon of interest are now widely used. These primer sets can be based on taxon-specific nucleotide sequence variations in matched sequence data or on fragments that uniquely identify the taxon in fingerprint investigations. To ensure

specificity of the primer set, attention must be given to include as much genetic diversity within the taxon of interest as well as that of its close phylogenetic relatives.

Species-specific primers have diagnostic relevance since they only amplify a product in the species for which they were designed. As a result, an internal control is required for a successful PCR, and false negatives can be avoided by multiplexing the reaction with a second set of primers that amplify a product nonspecifically; after electrophoresis, two bands corresponding to the species of interest would be diagnostic, while single bands corresponding to the internal control indicate otherwise.

Miroarrays and Probe Based Methods

A DNA microarray is a collection of small DNA fragments set in predetermined places on a solid surface, such as a glass slide, in pico-moles. These DNA fragments can be created from sequence characterized amplified regions (SCARs) and utilized as probes in high-throughput diagnostics to which test samples containing fluorescent-labeled PCR products or gDNA are made to hybridize. At the emission wavelengths of the fluorescent dyes utilized, data from hybridized slides is captured using an array scanner. Using *M. chitwoodi*-specific oligonucleotides as probes, the suitability of the DNA microarray approach for nematode identification was evaluated. The probes were created using nucleotide sequences found inside the binding sites of primer sets used to amplify SCAR and satellite DNA segments in *M. chitwoodi* but not in *M. arenaria*, *M. javanica*, *M. fallax*, or *M. hapla*. Both SCAR- and satellite DNA-based probes recognized *M. chitwoodi* regardless of the nematode's geographic origin, confirming the specificity of the primer sets used in routine PCRs.

When satellite DNA-based probes developed from the pMfFd satellite DNA family of *M. fallax*, a closely similar species, were utilized, cross-hybridization with *M. chitwoodi* targets was found. This demonstrates the importance of probe selection. We found only one paper in which DNA microarray technology was employed in nematode diagnoses.

For the identification and quantification of nematodes, TaqMan qPCR uses labelled DNA probe(s). The tagged probe binds to the template DNA within the location defined by the primers at the commencement of TaqMan qPCR. As the process advances and the polymerase reaches the probe, the probe is cleaved by its endogenous 50 nuclease activity, separating the dye from the quencher at the probe's 3' end (Blaxter *et al*., 1998).

More dye molecules are produced with each PCR cycle, resulting in fluorescence intensity proportional to the amount of amplicon created. The addition of probes improves the technique's specificity over traditional PCRs, and the quantity of florescence found can be used to estimate the number of nematodes in the sample. Primers and probes can be made using aligned sequence data, or from SCARs, as discussed above for species-specific primers. Sapkota *et al*., 2016, developed a real-time PCR assay for the identification of *M. hapla* in soil and root galls using this technique.

Sequence-Based Methods

Nucleotide sequence information from specific segments of nuclear DNA, mitochondrial DNA (mtDNA), or the entire genome can be analyzed using sequence-based approaches (for examples of gene areas and matching primer sets). Most investigations choose the rDNA and mitochondrial cytochrome c oxidase subunit I (COX1) genes for diagnostic purposes since they have variable areas circumscribed by conserved ones (De Oliveira, *et al*., 2011).

The higher level of sequence diversity in the variable region makes COX1 preferable for resolution at lower taxonomic levels such as species and subspecies groups, whereas the higher level of sequence conservation in the flanking regions has made the rDNA more suitable for use at higher taxonomic levels. ITS2 alone has been used for species diagnosis in *Caenorhabditis* involving genetic crosses of newly collected isolates with known biological species, though the authors do not advocate for the use of ITS2 as an absolute criterion for species diagnosis

because of the potential that distinct species may share identical ITS2 sequences (Carneiro *et al*., 2017).

The internal transcribed spacer (ITS), which is interrupted by the 5.8S coding region in the rDNA cistron into ITS1 and ITS2, contains the majority of the sequence diversity in the rDNA, making it valuable in molecular systematics of closely related nematode species. In *Caenorhabditis*, ITS2 alone has been used for species diagnosis in genetic crosses of newly collected isolates with known biological species, though the authors do not recommend using ITS2 as an absolute criterion for species diagnosis because distinct species may share identical ITS2 sequences.

The COX1 and rDNA have the added benefit of being found in numerous copies in worm genomes, allowing for PCR amplifications using modest amounts of DNA templates such as those acquired from single nematodes. The sequence data is subsequently employed in character-based or phylogenetic analyses to resolve and/or identify the taxa in question, with the latter allowing for evolutionary inferences. The rDNA molecule is made up of tandem repeats of conserved coding regions (28S, 18S, and 5.8S subunits) and variable non-coding portions (ITS and ETS; the external-transcribed region) separated by intergenic spacers.

As previously stated, rDNA gives phylogenetic resolution at a wide variety of taxonomic levels, allowing for the development of ‘universal' primers for usage in these taxa. Thus the researchers have proposed that distinct portions of the rDNA be utilized as DNA barcodes in various creatures; unique nucleotide sequences that may be used to identify each species. For fungi, bacteria, and nematodes, proposed DNA regions include ITS, 16S, and 18S. The COX1 region is the barcode region used in animals (Floyd *et al*., 2002). DNA barcodes identify species by employing sequence information from specific sections of DNA and primers that are relevant to the broadest taxonomic group feasible. In the barcode region, intraspecific differences should be fewer than interspecific differences.

Molecular Operational Taxonomic Units

The researchers have employed 18S (small subunit; SSU) sequencing information to classify soil nematodes into molecular operational taxonomic units (MOTUs). Over matched sequence data, each of these MOTUs was made up of a cluster of sequences that differed by less than three bases. After gaps, ambiguous characters, and unresolved base calls were removed from 450–500 nucleotide-long raw sequences generated using primer SSU94, the aligned data contained 349 to 396 nucleotides. MOTU content was anticipated using neighbouring trees built with absolute character differences as a distance metric. MOTUs largely belonged to morphologically identified species or taxa. (Powers *et al*., 2011).

The value of a barcode is proportional to the taxonomic rank to which it can be applied. The fact that sequence information is kept in publicly accessible databases such as Gene Bank (ncbi.nlm.nih.gov) and NEMBASE, is a significant advantage of sequence-based techniques (nematodes.org). This makes it easier to identify nematodes based on sequencing information by comparing it with information in these databases. However, the accuracy of identification is dependent on the quality of sequences published in databases and the authenticity of the taxa from which the sequences were derived.

At lower taxonomic levels, gene-specific sequence information is typically used; however, since that sequencing has become more inexpensive, there is a rising attempt to include whole mitochondrial or whole genome sequence information at all taxonomic levels. In addition to assisting a more advanced understanding of nematode biology, comparative genomics allows for the recovery of additional information such as gene order for the research of underlying evolutionary mechanisms such as; inversion, translocation, fusion, and so on. At present, 1159 nematode species have been sequenced and increasing number of whole nematode genomes are in progress (Davis & Nixon *et al.*, 1992).

Protein-Based Methods

Protein sequences, mass-to-charge ratios, and immunological techniques, like DNA-based methods, use distinct protein composition and structures to distinguish nematode species. Proteins have a smaller vocabulary than DNA due to the genetic codes redundancy; nevertheless, the alphabet utilized is far more sophisticated, with over 20 characters compared to the four DNA bases.

Isozyme Analyses

Enzyme phenotypes were one of the first non-morphology-based nematode identification approaches that have been used. In a nutshell, this method entails extracting soluble proteins from entire nematodes in buffer solutions, resolving the extracts using starch or polyacrylamide gel electrophoresis, and labelling for certain enzymes. This electrophoretic approach, also known as Multi-locus Enzyme Electrophoresis (MEE), is based on the migration patterns of isozymes, which are caused by variances in electrical charge, molecular weight, and conformation caused by minor alterations in amino acid compositions.

Esterases were the most widely used enzymes, however malate dehydorgenase, superoxide dismutase, and glutamate-oxaloacetate transaminase have also been used to varying degrees. This method complimented morphological methods and offered insights in evolutionary relationships, particularly among the genus *Meloidogyne* species. The procedure is however, still complex and time-consuming, with one of its constraints being the requirement to add known samples for reference purposes (DeSalle *et al*., 2005).

Two-Dimensional Gel Analyses

Nematode taxonomy investigations have made use of the two-dimensional gel electrophoresis (2-DGE). Charge-based isoelectric focusing in one dimension is followed by mass-based resolution in a dimension perpendicular to the first, allowing complicated protein mixtures to be resolved. The resolution pattern is then compared amongst

isolates to find similarities and differences, which can then be scored as presence/absence for the resulting data matrix. 2-DGE was employed to show proteome differences among 18 root-knot nematodes representing four species. Researchers found that some of the changes were species-specific, while others highlighted evolutionary links between various species.

When it comes to nematode taxonomy, the approach has a lot of advantages and disadvantages. One of the advantages of 2-DGE is that it allows for evolutionary assumptions about the species being studied. Polypeptides peculiar to a species can also be extracted and examined using mass spectrometry, allowing assumptions about the encoding genes to be established. The quantity of polypeptides resolved and the polymorphism seen are both dependent on the technique used and the number of samples tested, which are both disadvantages.

The number of polypeptides found among the 18 isolates, for example, ranged from 73 to 203. Scoring the spots was difficult at times, according to the scientists, because it was impossible to determine if any of the observed variations were true or due to gel deformations. As a result, they only assessed 95 locations that were consistently expressed in both of the nematode's duplicates. Thirty-seven of the spots were monomorphic, making them useless. Given that two of the species in their analysis were represented by single isolates, it can be assumed that if they had employed a higher number of isolates, both the overall number and the number of informative spots would have been different.

Mass Spectral Analyses

An ionization technology, MALDI (matrix-assisted laser desorption/ ionization) is that generates gaseous ions from big molecules in the solid state using a laser energy-absorbing matrix. The sample is embedded in a suitable matrix, then put to a plate and irradiated with a pulsed laser, causing the sample and matrix material to vaporize. Molecules are ionized in a hot cloud of ablated gases by proton loss/gain and accelerated into a mass spectrometer for detection.

The mass/charge (m/z) values direct the time it takes for these ions to reach the detector in a time of flight mass spectrometer (ToF-MS), with smaller and/or more charged ions travelling quicker. Because MALDI produces minimal fragmentation, the ions produced are mostly non-fragmented and single-charged, making parental ion masses straightforward to calculate from mass spectra. The capacity to detect protein/peptide ions or protein profiles that are diagnostic to the taxa under consideration is the foundation of MALDI-ToF-MS taxonomic identification. Research has also been done to intact second stage juveniles (J_2s) and/or proteins isolated from them using various organic solvents to distinguish between *Anguina tritici*, *Anguina funesta*, and *M. javanica* using unique peaks in their spectra and/or spectral profiles. However, the scientists cautioned that while choosing a solvent for protein extraction and a matrix material for MALDI, care should be taken because the reproducibility and quality of the spectra vary depending on the materials employed.

It was also proved that single *M. incognita* nematode (an adult female or a J_2), cleaned or unwashed, crushed or intact, can be used for MALDI-ToF-MS diagnoses. Protein profiles differed between adults and J_2s, with each having its own diagnostic peaks; when cleaned and/or crushed samples were employed, more masses and stronger peaks were found. Both investigations concluded that fine-tuning instrument settings is also critical.

The identification of proteins for usage as biomarker molecules, that produced by MALDI-ToF-MS spectrum profiles for species-specific proteins have obtained from excised 2-DGE gels. However, due to a lack of information in the databases at the time, the attempts to identify the proteins using similarity matches yielded no results. A similar study was conducted, involving 2-DGE and MALDI-ToF-MS proteome analyses of two nematomorph species, *Paragordius tricuspidatus* and *Spinochordodes tellinii*. While 36.2% of total protein spots on 2-DGE analyses were shared between the two hairworm species, 38.0% were specific to *P. tricuspidatus* and 25.8% to *S. tellinii*. A genetic distance of 0.47 separated the two species, confirming the previously reported distant relationship.

These investigations show that 2-DGE combined with MALDI-ToF-MS is an effective technique for nematode taxonomy. The methods can be used to make conclusions about evolutionary relationships between taxa as well as to develop species-specific markers. The protein extraction procedure, the quality of 2-DGE runs and the instrument configuration, among other things, can all affect the results. Protein expression profiles are known to vary based on nematode developmental stage and growth circumstances.

Serological Analyses

Several researchers have investigated the use of polyclonal and monoclonal antibodies (mAbs) against nematodes. It was initially reported on the feasibility of creating antisera against nematodes, with mixed results. It has been observed that antiserum generated against *M. incognita* that did not create precipitation band when combined with antigens from another species of the same genus, *M. hapla*, indicated a lack of cross-reactivity. The apparent specificity, however, may be owing to the tiny number of nematodes utilized in the assay. Further research revealed the absence of specificity in *Meloidogyne* spp. antisera reactivity. *Heterodera* and *Globodera* species of cyst nematodes have shown similar mixed findings. It is natural for polyclonal antisera generated against complete infused nematodes, including the associated microbiota and metabolites, to react with each other in their bodies.

The nematology community hoped to produce mAbs for diagnostic reasons by developing the hybridoma method. The method involved extracting mature B-cells from animals immunized with nematode antigens and fusing them with mouse lymphoid tumour cells to create hybridomas that can be kept *in vitro* indefinitely for continuous antibody production. Depending on the immunogen against which the antibodies were generated, mAbs provide better specificity. The hybridoma approach was used to generate mAbs against a range of agriculturally significant nematodes, including *H. glycines, M. incognita , G. rostochiensis* and *G. pallida*.

Output

The goal of taxonomy is to better understand biodiversity, categorize organisms, and facilitate the exchange of biological data. Scientific name is required for taxonomic communication, and valid naming is only achievable with type specimens and associated morphological data. This isn't always achievable, especially when working with environmental materials (eDNA). Furthermore, it is now widely acknowledged that morphological traits are insufficient to represent biological diversity, and the use of molecular data to enhance and/or overcome this constraint is routine.

However, rather than representing a collection of individuals with similar physical or genetic characteristics, a taxon is more significant if its members have unique biological characteristics. Taxonomy is built on the foundation of morphology-based classification. Recent breakthroughs in image analysis have aided it. AI (Artificial Intellegence) aids in overcoming the limits posed by a scarcity of highly qualified taxonomists by enabling objective decision-making as well as quick and precise identification. Additional attributes that can be used for identification include spectroscopic properties and lifetime value measurements of auto-fluorescence.

The relative ease of molecular approaches **(Table 16.1)** has resulted in the identification of numerous novel taxa, some of which are based only on sequence information. These taxa would have been impossible to characterize morphologically due to a lack of taxonomists and adequate morphological distinctions, as well as the difficulty to culture members of these species. Taxa found using distinct molecular techniques, on the other hand, are not necessarily consistent; for example, when sequence data generated from the same DNA area is evaluated differently between researchers, or when sequence data generated from the same DNA region is used in multiple studies. Similarly, taxa defined by morphological characteristics do not always correspond to those defined by molecular data, and vice versa. As a result, no single approach can provide all of the answers all the time, and the method(s) to use are determined by the

question being asked, the nature of the samples and the resources available.

If the goal is to identify a nematode sample, the most direct method is to inspect the sample under a microscope and assign the nematode to the lowest taxonomic rank feasible. In this case, the sample's origins may also be helpful. This, however, may necessitate some taxonomic knowledge. A molecular approach can subsequently be used to identify the nematode to species or even subspecies level using this information. If the issue is quarantine, molecular approaches specific to the quarantined nematode species can be used to determine whether or not the nematode in question is quarantined. Any of the fingerprinting approaches and/or sequence analysis based on one or a few genes may suffice if the goal is to quantify diversity in a field population(s). To explore the variety of nematodes in an environmental sample, high-throughput sequencing using second or third generation technology and appropriate bioinformatics approaches are useful (eDNA).

Table 16 1. Comparison of different nematode identification methods.

Technique	Expertise			Cost			Effectiveness		
	High	Medium	Low	High	Medium	Low	High	Medium	Low
Morphological and Image-Based									
Classical Morphometrics	√	-	-	-	√	-	-	-	√
Machine Learning	√	-	-	-	√	-	-	-	√
Autoflorescence	√	-	-	-	√	-	-	-	√
DNA-Based					-	-	-	-	-
Fingerprint	-	√	-	-	√	-	-	√	-
Microarray / Probe-Based	-	√	-	-	-	√	-	√	-
Sequencing	-	√	-	√	-	-	√	-	-
Protein-Based					-	-	-	-	-
Isozyme Analyses	-	√	-	-	√	-	-	√	-
2-D Gel Analyses	-	√	-		√	-	-	√	-
Mass Spectrometry	-	√	-	-	√	-	-	√	-
Serological Analyses	√	-	-	√	-	-	√	-	-

GLOSSARY OF TERMS

Abaxial Not situated in the line of the axis. Directed away from the axis.
abd Anal body diameter, used as a measure of distances between structures, or size of structures, in posterior region.
Abductor Muscle Any muscle that draws away from the main axis or extends or draws parts from the body.
Abiotic Pertaining to or characterized by non- living, inanimate phenomenon or objects.
Abnormal Deviating from the usual type or form. Aberrant.
Aboral Remote from or opposite to the mouth.
Absent Buccal Capsule When an embedded buccal capsule has walls of the same consistency as the lining of the esophagus the buccal capsule is said to be absent.
Absorb To take something within the body.
Abullate Lacking bullae.
Aciform-shaped like a needle.
Adcloacal Situated in the proximity of the cloaca.
Adanal Situated in the proximity of the anus.
Adanal Bursa A bursa which does not enclose the tail terminus.
Adanal Copulatory Papillae The adanal supplements.
Adanal Supplements Organs of secretion and attachment adjacent to the anus of some male nematodes.
Adaptation Modification of an organism or its parts or organs to make it more fit for existence under the conditions of its environment.
Aerial Inhabiting the air. Growing or existing above ground.
Aerobic Requiring the presence of oxygen to live.
Aerolated A situation where the transverse striae enter the lateral fields.
Allotype A paratype of the opposite sex of the specimen designated as the holotype.
Ambifenestrate In some species of the genus *Heterodera* where the vulval bridge is slender and the hatching pore is typically an hourglass shape.
Amphid (pl. **Amphids**) Paired lateral sense organs which generally open to the exterior on or near the lip region.
Amphid Aperture The opening leading to the pouch of the amphid.

Amphidelphic Having two ovaries, generally one extending anteriad and the other posteriad of the vulva.
Amphidial Duct The connecting passage between the amphidial opening and the amphidial pouch.
Amphidial Gland An organ located posterior to the nerve ring and connects with the central nervous system through the lateral ganglion.
Amphidial Nerve The nerve extending anteriad from the nerve ring to the amphid.
Amphidial Opening The amphid aperture.
Amphidial Orifice The amphid aperture.
Amphidial Pore The amphid aperture.
Amphidial Pouch The cavity or chamber of the amphid which contains the sensilla. A dilation of the amphidial gland. Sensilla pouch.
Amphidial Tubes The passages containing the amphidial nerves which connect the fibrillar terminals and the sensilla. An extension of the amphidial gland.
Amphimixis The union of germplasm of two organisms in sexual reproduction.
Anal Aperture The anal opening.
Anal Body Diameter (Width) The body diameter at the level of the anus; sometimes abbreviated as abd or abw.
Anal Diameter The body diameter at the level of the anus.
Anal Fenestra In the genus *Heterodera*, the opening resulting from the breakdown of the thin walled, transparent cuticular region of the anus.
Anal Muscles Muscle cells/tissue which function to make the anus operative.
Anal Opening The orifice to the exterior at the terminus of the rectum and delimited by the anus.
Androtype A male type.
Aneuploid Having a chromosome number that is not a multiple of the haploid number.
Annulate Comprising or furnished with rings. Ringed.
Anterior In front. Before. The front position as opposed to the posterior.
Anterior Cephalid The anteriad cephalid at which the two lateral cords arise.
Anterodorsal Toward the front and dorsum.

Anteroposterior Axis The long axis from head tail. Longitudinal axis.
Anteroventral Toward the front and the ventral.
Antibiosis An antagonism between two or more organisms, especially microorganisms in soil, to the detriment of one of them.
Aperture An opening. Hole. Orifice. The diameter of the opening.
Apex (pl. **Apices**) A proximal continuation of the aphelenchoid spicule shaft. The tip. The point of culmination.
Aphelenchoid Esophagus Having a narrow procorpus with a strongly formed median bulb followed by a narrow tube which extends to the intestine. A basal swelling is lacking and the three esophageal glands lie outside the esophagus proper.
Apical Cell A cell of the epithelium that forms the gonoduct wall and is situated at the distal end of the ovary and to be differentiated from the terminal cell.
Autapomorphy A situation where all the species of a taxon descended from the same ancestor share the same "innovative" or recent apomorphic trait.
Autotype A specimen identified by the author as an illustration of his species and compared with the type or cotype.
Axil The angle formed between a branch or petiole and the stem from which it arises; the cleft between two lips.
Axis (pl. **Axes**) A straight line passing through a body about which the parts are symmetrically arranged.
Axon, Axone The long process of a nerve cell conducting impulses way from the cell body.
Bacteriophagous Feeding on or consuming bacteria.
Bacterivores Organisms that feed on bacteria.
Baermann Apparatus The funnels and accessories used to implement the Baermann funnel technique.
Baermann Funnel Technique A method of isolating nematodes from soil, screening residue, plant tissue, or other matter where the material is placed in water and the nematodes by their own action move out into the water, settle and are drawn off from tubing attached to the funnel stem.
Barcode A machine-readable representation of data by lines of different widths and spacings; for example, the DNA sequence data that characterize a species.

Basal Related to, located at, or forming the base.
Basal Bulb An enlargement of the esophageal wall, muscular or glandular, at the posterior of the esophagus.
Basal Knobs The posterior knobs of the stylet.
Bifenestrate In some species of the genus *Heterodera* the vulval bridge is stout and divides the fenestra so that the semifenestrae appear to be two nearly separate holes.
Bifurcate Divided into two branches. Forked.
Bilaterally Symmetrical An organism in one plane with each side being approximately a mirror image of the other. This plane usually lies antero-posteriorly and dorsoventrally.
Bioassay Quantitative estimation of a biologically active substance by testing its effect under standardized conditions on living organisms or the effect of one organism upon another.
Biodiversity The variety and variability of life in an ecosystem based on variation at the genetic, species, functional or ecosystem levels.
Biological Control The limiting of pathogenic nematode population through depredation by natural enemies.
Biotype A subgroup of organisms which possess the same genetic characteristics. A subdivision of a race.
Body The nematode frame with its organs. The important and largest part of an organ as the body of the spicule.
Body Cavity The hollow within the body which contains the internal organs.
Body Pores A series of minute depressions slightly submedian or lateral and apparently connected with the lateral cords via small canals.
Body Wall The structural frame consisting of cuticle, hypodermis and muscle layer.
Buccal Cavity A buccal capsule in the broad sense.
Bursa (pl. **Bursae**) Wing-like extensions of the lateral cuticle at the caudal end of the male. Caudal alae.
Bursa Copulatrix The bursa.
Bursa Enveloping Tail Peloderan bursa.
Bursal Musculature The arrangement of the muscles pertaining to the bursa.
Bursal Nerve Innervated from bipolar sensory nerve cells forming a longitudinal strand on either side in or near the lateral cords.

Cardiac Esophageal Bulb The basal bulb of the esophagus.
Cardiac Glands Three glandular bodies located at the base of the esophagus.
Cardiac Region of the Intestine The anterior portion of the intestine as distinguished from the prerectum.
Cardiac Valve A thickening or complication of the esophageal lumen lining in the basal bulb.
Cardiac **Bulb** The basal bulb.
Carina (pl**. Carinae**) An elevated ridge or keel-like structure not necessarily pronounced or acute.
Caudal Belonging to, or like, a tail. Situated on or near the tail.
Caudal Alae The bursa. Alae confined to the posterior part of the male nematode body.
Caudal Appendage In mermithids, the terminal portion of the larval tail.
Caudal Bursa A bursa which completely encloses the tail. Peloderan.
Caudal Flagellum A more or less filiform, long and thin elongation of the tail of uniform diameter, or becomes a caudal thread which is pointed at its tip.
Caudal Glands Usually three elongate unicellular cells in or near the tail, discharging by separate ducts into a common ampulla at the spinneret.
Caudal Papillae The terminal portion of the larval tail.
Caudal Pore The spinneret. The outlet of the caudal glands.
Caudal Wing The bursa.
Cell The structural and functional unit of all plant and animal life. It consists of protoplasm, a nucleus and is surrounded by a membranous wall.
Cell Body The portion of a cell that contains the nucleus. In a nerve cell it is different from the region comprising the axon or dendrites.
Cell Membrane A differentially permeable membranous surface which surrounds the cell.
Cephalids Structures of a highly refractive nature, biconvex in longitudinal section and extending around the nematode body in the cephalic region.
Cervical Papillae Paired lateral tactile receptors situated near the nerve ring. Deirids.

Cervical Pore The excretory pore.
Cervical Vesicle Inflated cuticle anterior to the cervical groove.
Cheilorhabdions The dense cuticular walls of the cheilostom.
Cheilostom The lip cavity of the stoma, delimited anteriorly by the oral aperture posteriorly by the protostome; the anterior region of the stoma.
Cheilostome Alternative spelling of cheilostom.
Chemoreceptors Anteriorly the amphids, posteriorly the phasmids.
Chemotropism A movement of turning or curvature induced by a chemical stimulus.
Chemotaxis A change of position toward or away from a source of chemical stimulation.
Chitin The substance composing the exoskeleton of insects which is not equivalent to the cuticle of nematodes but is present in egg shells of some forms.
Chitin Plates The valvular apparatus - probably a misnomer in terms of the constituent material of the plates.
Chords Var. of cords.
Circumfenestrate In the genera *Heterodera* and *Globodera* where the vulva is lost when fenestration occurs by the breakdown of the thin cyst wall around the vulva leaving a circular hatching pore.
Circumenteric Ring The nerve ring.
Clavate Club-shaped.
Clavate Cells Modifications of the hypodermis which contribute to the formation of the lips, being two in each lip and they are long cells with a hollow interior, lamellate with expanded distal ends that fill the convexity of the lips.
Claviform Club-shaped. Clavate.
Clay As a soil separate, the mineral soil particles less than 0.002 mm. in diameter. As a soil textural class, soil material that contains 40 percent or more of clay, less than 45 percent of sand, and less than 40 percent of silt.
Clay Loam Soil material that contains 27 to 40 percent of clay and 20 to 45 percent of sand.
Cloaca In the male a common chamber lined with cuticle which receives the products of the intestinal and reproductive tracts and empties to the exterior via the cloacal orifice. The hind gut.

Cloacal Cuticle The cuticular linings of the cloaca which differs in some respects from the external cuticle.
Cords Four longitudinal lines of hypodermal thickening lying on the inner side of the hypodermis and variously termed the dorsal, lateral and ventral cords. The cords contain the nuclei of the hypodermis.
Cuticle The noncellular external covering of the nematode and apparently connective tissue of the hypodermis. The cuticle lines the natural opening of the body i.e. the oral cavity, the anal aperture, the vulva, the vagina, the lumen of the esophagus, the rectum, the cervical pore, and the cuticular ampulla of the cervical pore.
Cylindrical Esophagus Of a large diameter and uniform in width throughout. Characteristic of rapacious nematodes.
Cyst At maturity the body wall of the *Heterodera* female thickens, becomes resistant to decay, and turns brown as the worm dies, resulting in a protective shell for the eggs and is termed a cyst.
Cyst Cone The conical perineal region of cyst- forming nematodes.
Cyst-Forming Nematode Any species of the genera *Globodera* and *Heterodera.*
Cytogenetics The study of the structural basis of heredity and variation.
Cytokinesis The changes affecting cytoplasm of a cell undergoing mitosis, meiosis and fertilization.
Cytology The study of the structure, organic processes, functions, etc., of cells.
Cytoplasm The protoplasm of a cell exclusive of the nucleus and cell wall.
Deirids Exceptionally large cervical papillae. Lateral tactile receptors situated in the lateral field near the nerve ring.
Delicate Of slight or thin characteristics.
Deltoid Triangular in shape.
Dentoid Tooth-shaped.
Deoxyribonucleic Acid DNA.
Depauperate Lacking in abundance or variety of species in an ecosystem.
Dependent Hatching The need for an external stimulus for hatching to be affected.
Depresssion Said of the junction of the head and the cervical region when delimited by a more or less broad hollow.

Desiccate To dry up.
Desiccation The state or condition of being dried up.
Devolution Retrograde development. Degeneration.
Didelphic Having two ovaries.
Didymous In pairs. Two fold.
Dieback A condition of progressive necrosis of branch terminals of trees and other plants.
Differentiation The act or process of characterizing or making different. Changing from general to special characters. Specialization.
Diffusate A compound, especially a stimulatory one, which spreads through the substratum from the point of origin.
Digestion The process of preparing food by chemical breakdown for absorption and assimilation.
Digitate Finger-like in shape or divided into finger-like processes.
Digitiform Having the shape of a finger.
Digonic Female A digonic hermaphrodite.
Digonic Hermaphrodite A situation where sperm and ova are produced in different gonads of the same individual.
Diluent A diluting agent.
Dimorphic Individuals of the same species occurring in two distinct forms i.e. male and female.
Dioecious Existing as separate and distinct male and female.
Diploid Having a double (2n) number of chromosomes.
Direct Cycle Eggs of parasitic parents hatch into free-living juvenile which develop directly into parasitic forms. Homogonic cycle.
Disc Plate-like with a flat circular surface.
Disciform Having the shape of a plate or disc.
Discoid Having a shape like a round plate. Disk-like.
Discoloration Changed to a different color. A condition produced by some factor in which any part of a living organism is abnormal.
Dissemination The spread of infectious agents by any means.
Distad Toward the distal end.
Distal Remote from the point of origin or attachment.
Distal Tubes The marginal tubes of esophageal radii.
Distention The act or state of extending or stretching.
Divergent Extended from a point in different directions or the trends of development.

Diversity The condition of being comprised of different types of entities, such as a species. Diversity is greatest when all species present are equally abundant.
Diversity Indices Measures or indicators of the degree of diversity.
Diversity-weighted Abundance A product of diversity and abundance which recognizes that both may contribute to ecosystem services.
Diverticulum (pl. **Diverticula**) A blind tube or sac off from a cavity or canal.
Divided Parted. Disunited.
DNA Deoxyribonucleic acid. A nucleic acid containing the genetic information for development and functioning of living organisms. Consists of two strands of nucleotides which have sugar and phosphate backbones. One of four bases is attached to each sugar. The sequence of bases is copied into RNA through transcription and determines the sequence of amino acids in proteins.
DNA Barcoding A taxonomic method utilizing a short genetic marker in an organism's DNA, represented in barcode format, to identify the organism as belonging to a particular species.
DNA Polymerase An enzyme that catalyzes production of DNA molecules from nucleoside triphosphates.
Dormancy The dormant state.
Dormant Inactivation as if in sleep.
Dorsad In the direction of the dorsum. Toward the dorsal aspect.
Dorsal The back side aspect of a nematode. Belonging to the back side.
Dorsal Cord The dorsal line of the four longitudinal lines of thickening on the inner side of the hypodermis.
Dorsal Cone A prominent projection on the roof of the buccal capsule which contains the terminal orifice of the dorsal esophageal gland.
Dorsal Esophageal Gland The dorsal most of the three esophageal glands. Note: esophagus as alternate terminology for pharynx.
Dorsal Esophageal Gland Orifice The aperture of the duct from the dorsal esophageal gland into the lumen of the esophagus.
Dorsal Rays The paired genital papillae of the dorsal lobe of the bursa.
Dorsal Somatic Nerve The dorsal nerve.
Dorsolateral The position on the nematode body situated laterally from the dorsomedian line and perpendicular to the anteroposterior axis Subdorsal.

Dorsolateral Muscle Fields A segment of the dorsosubmedian muscle field being delineated by a lateral cord and a submedian thickening of the hypodermis.
Dorsolateral Nerves Nerves that arise between the dorsal and lateral nerves.
Dorsomedian The true middle line on the back side of an individual.
Dorsoventral An imaginary line extending from the dorsal to ventral side. The dorsoventral axis. The median line.
Dorylaimoid Esophagus Having the shape of a long- necked bottle, being slender and thin at the anterior end then expanding towards the cardia.
Double Eggs Nematode ova of an abnormally large size and generally nonviable.
Double Guiding Ring A condition where the guiding sheath of the stylet has two thickened rings of cuticle.
Eclosion The act or process of hatching from the egg.
Ecology The relations between organisms and their environment. The reactions of organisms to the conditions of their existence.
Ecosystem An assemblage of living organisms interacting as a system in conjunction with each other and with their abiotic environment.
Effector A molecule that selectively binds to a protein and regulates its biological activity. It may increase or decrease enxyme activity, gene expression or cell signalling. Molecules secreted by the esophageal glands of plant-feeding nematodes that affect the development or function of plant cells are examples of effectors.
Efferent Bearing or conducting away from an organ or position. See afferent.
Embryonic Found in, or belonging to the embryo. Incipient and rudimentary.
Embryonic Development Growth and maturation of the egg.
Encyst To become enclosed in a cyst, capsule, moulted cuticle or by structures which function as such.
End Bulb The basal bulb.
Endemic Native to a certain region. Indigenous. See exotic.
Endocuticle A section of homogenous material internal to the exocuticle. A 5-banded layer in some H*eterodera*.
Entomopathogenic Nematode A nematode that, either by direct feeding

or by vectoring toxic bacteria, cause harm or death to an insect.
Entoparasite An internal parasite. Endoparasite.
Entrails Viscera, Internal organs.
Environment All external conditions that may act upon an organism or thing to influence its development or survival.
Epaulets Specialized, ribbon-shaped, paired bands of cephalic cuticle.
Epiblast The outer layer of the blastoderm.
Epidermal Fields The cords.
Epidermis The outer cylinder of cells consisting of a single layer of epithelium which secretes the cuticle. The hypodermis.
Epiptygma A vulval flap.
EPN Entomopathogenic nematode.
Equatorial Situated at the middle or central region of a body or part.
Eradicate Complete destruction especially of a population.
Eructation The ejection of contents from the intestine via the mouth.
Esophageal Collar Muscle tissue which surrounds the stoma.
Esophageal Cuticle The cuticular lining of the esophagus lumen which differs in some respects from the external cuticle.
Esophageal Glands Elongated glands of simple or branched tubules located in the esophageal sectors, the secretions of which are apparently of an enzymatic nature, one gland being situated dorsal and two submedian or modified.
Esophageal Nerve Ring The nerve ring.
Esophago-Intestinal Cells The esophageal-intestinal valve.
Esophago-Intestinal Junction The esophageal-intestinal value.
Esophago-Intestinal Valve Var. of esophageal-intestinal valve.
Esophagus The muscular tube that leads from the stoma or stylet base to the intestine.
Etiology Inquiry into the causes of a disease.
Etymology The origin and derivation of the genus and species name of an organism.
Excretory System All the structures concerned in ridding the body of waste products other than the intestine.
External Cuticle The cuticle covering the exterior of the nematode body which differs in some respects from that which lines parts of the digestive tract and vagina.

Fecundation Fertilization, as of eggs by spermatozoa.
Fenestra (pl. **Fenestrae**) A window. A transparent spot. In the genus *Heterodera* a thin walled transparent region of the vulval cone which may breakdown to form the hatching pore. The thin- walled region about the anus in some *Heterodera.*
Fenestration The process of forming the hatching pore by the breakdown of the thin areas of cuticle of the anal or vulval cone.
Fiber Cells Modifications of the hypodermis which contributes to the formation of the lips, two in the dorsal lips and one in the lateral and ventral lips.
Fiber Layers Three cuticular strata of dense connective tissue which is oblique, ribbon-like, possibly spiral and delimited by the boundary layer and the basal lamella.
Gamogonic Pertaining to sexual reproduction.
Ganglion (pl. **Ganglia**) A well defined concentration of nerve cell bodies forming a nerve center.
Genotype The species which is designated as the type species of a genus.
Genus (pl. **Genera**) In the systematic arrangement of organisms into groups or categories denoting natural relationships a category ranking above a species and below a family. In binominal nomenclature the first word of the scientific name of a species is the generic name and is capitalized. The second term is the trivial name and is not capitalized.
Geographical Distribution The inhabited range of a species.
Growth Zone That part of the ovary where the oogonia develop.
Gubernacular Muscles Specialized muscles for the functioning of the gubernaculum.
Gubernaculum In male nematodes a grooved cuticularized structure, sometimes paired, which guides the spicule and is formed by sclerotization of the dorsal wall of the spicular pouch.
Guide Collar The guiding ring of the stylet.
Guide Ring The guiding ring of the stylet.
Guiding Ring of Stylet The anterior end of a guiding sheath of the stylet cuticularized or muscular thus appearing to be more dense than surrounding tissue.
Gynogenesis The process by which progeny are developed solely from the maternal genome; i.e. sperm DNA is not utilized.

Gynotype A female type.
Habitat The natural environment of an organism, specifically, the locale in which it grows and lives.
Haemolymph The watery lymph-like nutritive fluid of the nematode pseudocoel.
Hatching The breaking of the egg shell by a larva during the process of emergence. Eclosion.
Hatching Factor An external hatching stimulus which acts on eggs.
Hatching Pore The fenestra.
Helminthic Relating to, or belonging to, worms.
Helminthology The branch of zoology dealing with worms, especially parasitic worms.
Hemizonid A nerve commissure of a highly refractive nature, generally biconvex in longitudinal section and which extends ventrally from lateral cord to lateral cord.
Hemizonion A small nerve commissure with many characteristics of the hemizonid and apparently always shortly posterior to the hemizonid.
Herbivores Organisms that derive their sustenance entirely from vegetable matter.
Herbivorous Feeding on vegetable matter.
Heredity Transmission of genetic characters of parents to their progeny.
Hermaphrodite An individual with both functional male and female reproductive organs.
Holotype The individual specimen selected as type of a species by the author.
Homogeneous Similar in kind, qualities or nature.
Homogenous Layer The matrix layer of the cuticle.
Homogonic Cycle A situation where eggs of parasitic parents hatch into free-living juvenile which develop directly into parasitic forms. Direct cycle.
Homologue A part or organ of the same relative structure, position or origin as another. The same in different individuals varying in form and function.
Homologous Similarity of structure, but independent of function.
Homology Structure of fundamental similarity, but independent in function, derived from descendence through some common ancestral form.

Homonym A generic or specific name already occupied and therefore rejected due to the law of priority which requires the use of the earliest published name. Two or more entities having the same name.
Homotype A specimen compared with the type and determined to be conspecific with the type.
Horizontal Gene Transfer (HGT) The transfer of genetic material between organisms in a manner other than sexual or asexual reproduction, e.g. by transformation or viral infection.
Horizontal Resistance Resistance to races or biotypes of the nematode species is uniform, not race specific - reduces selection pressure. Usually controlled by several genes.
Host The organism which is invaded or parasitized by a disease-producing agent and from which the parasite obtains its sustenance.
Host List The range of organisms infected by a specific parasite.
Host Plant The plant which affords sustenance to a nematode parasite and allows reproduction.
Host Preference The selection of a host most suitable for the survival of the parasite.
Host Race Nematodes of the same species differing only in their preference of host plants.
Host Range The number of organisms parasitized by a specific nematode.
Host Selection The food preferences of parasitic nematodes.
Hot water Treatment The steeping of bulbs, seeds and other plant parts in a water bath at a temperature lethal to the infecting nematodes and leaving the plant material undamaged.
Humidity The weight of water vapor in a given quantity of air, compared with the total weight of water vapor which the air is capable of holding at a given temperature.
Humus The well decomposed organic matter in mineral soils.
Hyaline Clear. Transparent. Without color.
Hydrogen-ion Concentration. A measure of the acidity of a chemical in solution. The greater the concentration of hydrogen ions, the more acid the substrate. The hydrogen-ion concentration is expressed in terms of the pH of the substrate.
Hydrolysis A chemical decomposition process of a compound which involves the addition of the elements of water.

Hypersensitive A violent reaction to parasitic attack resulting in sudden death of invaded tissues providing a barrier against further invasion.
Hypertonic A solution having an osmotic pressure higher than an isotonic fluid such that it gains water by osmosis across a membrane.
Hypertrophy The abnormal enlargement of cells generally by dissolution of common cell walls.
Hypoblast The inner layer of the blastoderm.
Hypodermis A thin cell layer beneath the cuticle with longitudinal thickenings protruding between the longitudinal muscles to form the cords which contain the nuclei of the hypodermal cells.
Hypothesis (pl. **Hypotheses**) A tentative explanation of a phenomenon.
Hypotonic A solution having an osmotic pressure lower than an isotonic fluid such that it loses water by osmosis across a membrane.
Hypotygma Papilla-like processes on cloacal aperture of male.
Hypotype A specimen, other than the type, upon which a subsequent or emended description or figure is based. Apotype. Plesiotype.
Immobile Larva The development of the male of cyst-forming nematodes within the cuticle of the third stage larva.
Immunity The ability of an organism to remain free from a disease or of parasitism by virtue of inherent properties of that organism. An immune organism is exempt from the particular disease.
Impermeable Not permitting passage. Impervious.
Incubation The period of time and conditions of environment between inoculation of an organism by a disease-producing agent and the appearance of symptoms.
Indentate Having an irregular margin.
Indicator Plant A suitable suscept plant used to signify the survival or reproduction of plant-parasitic nematodes after some treatment or condition of population stress.
Indigenous Living in its natural or original locality.
Indirect Cycle Eggs of parasitic parents develop into free-living males and females, the offspring then proceed to the parasitic phase. Heterogonic cycle.
Indirect Development Complete metamorphosis.
Infect To invade and establish a parasitic relationship within the host proper.

Infection Incidence The population density of attacking parasitic nematodes.
Infectious Juvenile Infective juvenile.
Infective Having the qualities necessary to enter a host and produce a disease condition.
Infective Juvenile Nematode larva at a stage of development capable of penetrating and infecting a host.
Infective Juveniles Infective juvenile.
Infective Stage The period of development in the lifecycle of a parasitic nematode in which it possesses the qualities enabling infection of a host.
Infective State The infective stage.
Infest To attack externally. To contain the parasites, said of nonliving material.
Isthmus The segment of musculature between the medium bulb and basal bulb of the esophagus.
Juvenile A nematode in a developmental stage which does not yet have functional gonads. Any immature nematode.
Juvenile Female A fourth stage female in which the vagina and uterus are functional but the ovaries have yet to mature.
Karyoplasm The protoplasm of the nucleus. Nucleoplasm.
Keratin A segregate form of the cuticle corresponding to the external cortical layer.
Kinesis Movement induced by a stimulus and is not necessarily orienting.
Koriogamy The impregnation of a female nematode which possesses a fully developed vagina and uterus but an immature ovary.
Labial Annule The labial disc.
Labial Disc (or Labial Disk) The more or less circular font of cuticle about the oral opening and delimited posteriorly by the first transverse striation.
Labial Papillae Papillae located on the lips.
Labial Muscles Specialized lip muscles apparently of the same origin as somatic muscles.
Labial Setae The setae of the inner circlet and located on the lips or close to the mouth.
Lateral Alae Lateral or sublateral extensions of the cuticle which extend along the body in both males and females.

Lateral Cords Longitudinal hypodermal thickenings lying in the lateral position.
Lateral Fields A form of cuticular configuration above the lateral cords.
Lateral Glands Lateral hypodermal glands.
Lateral Grooves Involutions.
Lateral Membrane A cuticular flap situated on both sides of the vulval slit in some nematodes.
Lateral Nerves Nerves originating mostly at the lateral ganglia. Of a sensory nature with ganglionic swellings along the lateral cords, supplying a sensory branch to the cervical papillae when present and enter the lumbar ganglia posteriorly.
Lateroanal In the lateral position at the level of the anus.
Latitude A site in a nematode measured on the meridian of a cross section.
LC_{50} The concentration of a chemical at which 50% of the target organisms are killed; for example, 10 mg of chemical per liter of air or water.
LD_{50} The dosage of a chemical, based on the mass of the affected organism, at which 50% of the target organisms are killed; for example, 10 mg of chemical per Kg of target organism tissue.
Lesion An injury, wound or morbid structural change. A localized spot of diseased tissue.
Lethal Concentration The concentration of a chemical at which the target organisms are killed; for example, 10 mg of chemical per liter of air or water.
Lifecycle The successive series of changes through which an organism passes in the course of its development.
Life History The record of events in the development of an individual.
Linear Resembling a line. Having a form long and uniform in width.
Lip Pulp The interior tissue of the lips composed of several large, elongate cells.
Lip Region The cuticular area from the basal ring forward.
Lip Sclerotizations The cephalic framework.
Longitudinal Fields The longitudinal ridges.
Longitudinal Lines The longitudinal ridges of some authors and the longitudinal cords of others.
Lumen The cavity delimited by the walls of a tubular vessel.

Lumen Rays The esophageal radii.
Lysis Cell destruction. A tissue dissolution process.
Macerate To waste away. To soften, separate and wear away.
Macrophagous Feeding on objects of a relatively large size.
Macroscopic Visible to the unaided eye.
de Man Indices The De Man formula.
Marginal Tubes The terminal cylindrical endings of some esophageal radii; the radii of other forms may have convergent terminals.
Marine Living in the sea.
Mass Invasion A situation where large numbers of nematode parasites attack a host simultaneously.
Mass Hatching The phenomenon of large number of nematode eggs hatching within a short period of time in response to some stimulus.
Matrix The enveloping substance within which something originates or develops.
Medial Pertaining to, directed toward, situated at, or occurring in the middle.
Median A midway point in position. Situated in the middle position. A plane of division dividing a bilateral organism into right and left halves.
Median Bulb The metacorpus.
Median Esophageal Bulb The metacorpus.
Median Pseudobulb The metacorpus.
Medioventral Ventromedian.
Medium (pl. **Media**) Substratum.
Meiofauna Organisms that pass through a 1mm mesh sieve and are retained on a 42 or 63µm mesh sieve.
Moult, Moult To cast off the cuticle.
Monograph A treatise in detail on a particular subject.
Morphogenesis Development or evolution of morphological characters.
Morphology The study of form and structure of organisms.
Morphometry Measurement of external form.
Morphometric Parameters Measurement of external form.
mRNA Messenger Ribonucleic Acid. The molecule of ribonucleic, that is transcribed from a DNA template and carries the coding information for the structure of a protein to the ribosomes, the sites of protein synthesis.

Mural Tooth A cutting or piercing structure situated on the pharyngeal wall but formed further back in the esophagus.
Mutation A genetic variation with the progeny differing from their parents in one or more characters.
Mycophagous Feeding on fungi.
Necrobiotic Food Tendencies The preference of plant cells for food at an early stage of necrosis by pathogenic nematodes.
Necrosis The death of cells surrounded by living tissue, specifically death to cells in mass in contrast to necrobiosis.
Necrotic In a dead and decaying condition. Affected with or characterized by necrosis.
Nematode wool A mass of resistant, desiccated *Ditylenchus* juvenile frequently found on bulb crops and others. Wool.
Nematotoxin Any substance lethal to nematodes.
Nerve Ring A belt, broad and flat in slender nematodes but narrow in forms with a large esophagus, containing cell bodies of neurons distributed around it. The nerve cells are generally too diffuse to be termed true ganglia. The nerve ring represents the dorsal and ventral connections between the lateral ganglia.
Obligate Parasite An organism only capable of deriving its food from living organisms.
Obligate Saprophyte Organisms which subsist on dead organic matter or from available inorganic material and have no relationship with living cells.
Obligatory Aerobic Requiring the presence of atmospheric oxygen in order to live.
Obligatory Anaerobic Growing only in the absence of atmospheric oxygen.
Oblique Slanting. Inclined.
Oblique Cuticular Markings Conspicuous oblique beneath the cortical layer of the cuticle.
Oblong Elongated.
Obovate Inversely ovate.
Obtuse Not pointed. Blunt.
Offset Not in line with the body contour or not contioguous with the axis of an organ, for example, an offset head or an offset spermatheca.

Oligonucleotide Short DNA or RNA molecules that function in the regulation of gene expression.
Oligophagous Feeding on a few kinds of food, especially nematodes with a limited host range.
Ontogeny The life history or development of an individual as distinguished from that of the species and higher groups.
Oocyte A female gamete prior to maturation.
Oogenesis The formation of the egg, its preparation for fertilization and development.
Oral Aperture The anterior entrance into the stoma.
Organs of Sense Tactile organs, amphids, phasmids, ocellus.
Papilla (pl. **Papillae**) Minute elevations of the cuticle. Any small nipple-like projection or part. In general sensory organs.
Paplliform Having the shape of a papilla.
Parasite An organism that obtains its sustenance wholly or in part from another living organism.
Parasiticide Any agent lethal to parasites.
Parasitism An association where one individual lives at the expense of another, makes no return and is destructive to its host. The state of being parasitic.
Paratype All specimens remaining after the selection of the holotype and allotype.
Parthenogenesis The development of eggs without benefit of fertilization by spermatozoa.
Perineal Pattern Configurations, especially of *Meloidogyne* spp., on the cuticle surface of the perineum; specific designs are common to each species.
Perineum The superficial region about the anus.
Perioral Around, enclosing or surrounding the oral aperture.
Pharyngeal Bulb A muscular swelling of the esophageal wall around the buccal capsule.
Pharyngeal Caecum The esophageal caecum.
Pharyngeal Glands The esophageal glands.
Phasmids The lateral caudal papillae connected with the lateral precaudal glands. Paired postanal lateral chemoreceptor sensory organs.

Phenotype The visible characters of an organism resulting from the interaction of genotypic characters and environment.
Phoresy A symbiotic relationship in which one organism transports another organism of a different species. Often exhibited in arthropods and also in some fishes.
Phylogeny The lineage history of the development of a genus, family, class or other natural group.
Phylum (pl**. Phyla**) In the systematic arrangement of organisms into groups or categories denoting natural relationships a category ranking above a class and below a kingdom.
Phytoparasite Nematodes capable of obtaining sustenance from plants.
Piriform Pear-shaped.
Pisiform Having the size and shape of a pea
Plankton Organisms that float and move passively with winds and currents, generally of microscopic size.
Population Density The number of individuals per unit of substratum.
Posterior Bulb The basal bulb.
Posterior Uterine Branch The post-vulval uterine branch.
Postvulval Ganglion A ganglion which receives the paired ventral nerve cords at a point posterior of the vulva where fusion into a single nerve cord occurs.
Predaceous Living by preying on other organisms.
Prerectum The segment of the alimentary tract between the intestine and the rectum, separated from the intestine by a stricture in the lumen and from the rectum by a sphincter muscle.
Primer Short single-strand DNA fragments called oligonucleotides that are a complentary sequence to the traget DNA region to be amplified in the polymerase chain reaction.
Prodelphic Having a single ovary anterior to the vulva.
Protoplasm The basic substance of which all living matter
is made, being grayish, semitransparent, viscid and a complex colloidal physiochemical system that constitutes tile living matter of plant and animal cells.
Protuberance An elevation above the surface. A protrusion or bulge.
Punctations Small pits or deep depressions on the surface of the cuticle; the shape is usually rounded but may vary.

Pure Culture A nematode population containing a single species only; it differs from aseptic culture by not being free of other organisms.
Pyriform Pear-shaped.
Quadricolumella In the female gonad probably the gland region that secretes the egg shell.
Quarantine A restraint upon goods, animals, plants or other materials which may bear pathogenic organisms.
Race A hiotype or groups of biotypes which differ from one another in certain physiological characters which function to segment the species into subspeciflc categories. A population which reacts differently than other populations of apparently the same species.
Rachis An axial structure or column.
Random Movement Having a haphazard course or direction in contrast to a tactic response.
r**DNA** Ribosomal DNA are sequences encoding for ribosomal RNA and which regulate transcription of DNA to RNA. They contain transcribed and nontranscribed spacer segments. 18S rDNA is the ribosomal DNA sequence containing genes that encode for ribosomal RNA.
Reticulate Having cross markings like a net.
Saccate Sac-shaped. Contained in a sac.
Sacciform Having the shape of a sac.
Salivary Gland The esophageal glands.
Sandy Clay Soils of this textural class contain 35 percent or more of clay and 45 percent or more of sand.
Sandy Clay Loam Soils of this textural class contain 20 to 35 percent clay, less than 28 percent silt and 45 percent or more of sand.
Sandy Loam Soils of this textural class have 50 percent sand and less that 20 percent clay.
Saprophage An organism which derives its sustenance from decaying organic matter.
Seinhorst Technique An elutriation method for quantitative extraction of nematodes from soil.
SEM Scanning electron microscope or scanning electron microscopy.
Semifenestrate A situation in some species of the genus *Heterodera* where the vulval bridge divides the fenestra into two parts. *See* fenestrate, bifenestrate, ambifenestrate, circumfenenstrate.
Sensory Organ A body structure which functions as a preceptor of

stimuli.

Sessile Obligate feeding and reproducing at one fixed site. Not free to move from one site to another.

Seta (pl. **Setae**) Tactile sensory organs of elongate cuticular structure and articulate with the cuticle proper. A bristle-like protuberance. An aciculum.

Sexual Dimorphism A difference in form or stricture as males and females of the same species.

Species Diversity The condition of being comprised of different types of apecies. Diversity is greatest when all species present are equally abundant.

Species Richness The number of species present or the number of species in a particular guild.

Specimen A unit of sample. A single example of nematode. The individual unit of a collection.

Spermatheca The enlarged portion of the female reproductive system which functions as a reservoir in receiving and holding spermatozoa from the male.

Spicule (pl. **Spicules**) Male intromittent organs functioning during copulation for the transfer of sperm, often paired; each an elongate cuticularized piece, extrusible through the cloacal opening.

Spindle-Shaped A cylindrical body tapering towards both ends.

Stomatal Pertaining to or belonging to the stoma.

Stunted Plant A plant reduced and deformed in size and shape.

Stylet A relatively long, rather slender, hollow feeding structure; usually axial in the adult. A spear.

Stylet Aperture The anterior opening into the stylet. In Tylenchida the stylet aperture is obliquely ventral and short, in Dorylaimida the stylet aperture is obliquely dorsal and up to approximately half the stylet length.

Stylet Knobs Three swellings, derived from the telorhabdions, in triradiate positions at the base of the stylet.

Stylet Protractor The muscles which function to extend the stylet.

Stylet Shaft The subulate portion of a stylet, derived from the metarhabdions.

Styliform Having the shape of a stylet. Having a subulate terminus.

Subdorsal The position on the nematode body situated 30

degrees laterally from the dorsomedian line and perpendicular to the anteroposterior axis; the sector between the lateral and dorsal surfaces. Dorsolateral.
Sublateral The position on the nematode body 30° ventral of the lateral position and perpendicular to the anteroposterior axis.
Suborder A grouping less inclusive than an order but more than a family.
Subordinal Belonging to or characteristic of, a suborder.
Subphylum A grouping less inclusive than a phylum but more than a class.
Subspecies A grouping less inclusive than a species but more than a variety or strain.
Tactile Organs Sensory organs of touch.
Tail Terminal The hyaline portion of the tail peculiar to some nematode juvenile, especially *Heterodera* and *Meloidogyne* spp., in which no internal bodies, structures or arrangements can he seen.
Tail Whorl The cuticular configurations in the perineal pattern of *Meloidogyne* spp., at the site of the tail terminus.
Taxonomy The systematic grouping of organisms according to their natural relationships.
Teardrop Having an oblanceolate shape.
Tease To shred plant or animal material under magnification in search of nematodes.
Terminal Bodies Phagocytic structures of some coelomocytes.
Terminal Bulb The basal bulb of the esophagus.
Threshold The minimum point or level at which a response or affect is produced on an organism or system.
Toxic Pertaining to a poison. Poisonous.
Toxicity The quality or degree of being poisonous.
Toxin A poisonous product of secretion derived through the metabolism of organisms.
Tract Some special purpose system of parts or organs, i.e. the digestive tract.
Transfer Host A host that provides resources until the defensive host is reached but that is not necessary for completion of the parasite lifecycle.
Trichoid Hair-like.
Trichotomous Bearing three branches or forks.

Tricuspid Divided into three points.
Tricuspidate Divided into three points. Tricuspid.
Tripartite Consisting of three parts.
Triploblastic Composed of three germ layers, the ectoderm, mesoderm and endoderm, from which develop complex organ-systems.
tRNA Transfer ribonucleic acid. Molecules that adapt the 20 letter code of amino acids in proteins to the four letter (ACGU) genetic code of messenger RNA (See mRNA) with the 20 letter code of amino acids in proteins. tRNAs are essential components of the biological synthesis of proteins.
Tylenchoid Esophagus Having a narrow procorpus, a strongly formed median bulb, typical isthmus and terminating with a glandular basal bulb.
Type Specimen A single example of a nematode designated as a type individual with the characteristics of the species description.
Ubiquitous Existing in all areas. Cosmopolitan.
Ufra Disease A disease of rice incited by *Ditylenchus angustus*.
Unsegmented The condition of lacking metameric segmentation.
Unsuitable Host An immune or resistant plant.
Uterine Pertaining to or belonging to the uterus.
Uterine Chamber That proportion of the reproductive tract connecting the uterus and the vagina.
Uterine Sac A thin-walled, muscular sac to which the uterus is joined and into which the vagina opens. It may be obscurely separated from the uterus by a slight constriction or merely by a differentiation of tissue.
Uterine Tract The uterus.
Uterus (pl. **Uteri**) A region of the oviduct modified to function as a place of development (sometimes maturation) of the egg.
Vagina (pl. **Vaginae, Vaginas**) A canal lined with cuticle connecting the uterus or the ovijector with the genital orifice, the vulva. The vagina is considered to have three sections, proximal, intermediate and distal.
Vaginal Cuticle The cuticular lining of the vagina which differs in some respects from the external cuticle.
Values A term used to denote the measurables of the de Man formula.
Valve Flaps The valvular apparatus.
Valve Plates The valvular apparatus.
Valvular Apparatus A refractive structure situated in the median bulb

or basal bulb of some nematode forms and probably functions as a pump during feeding.
Valvulate Possessing a valvular apparatus.
Variant A departure from the normal form or condition.
Variation Any divergence from the normal form or condition.
Vas Deferens (pl. **Vasa Deferentia**) A slender muscular tube extending from the ejaculatory duct to the testis.
Vector An organism or other agency which transmits or disseminates pathogens or other identities.
Velum A delicate cuticular membrane on the inner side of the spicule.
Venter The belly. Underside. Any part corresponding to the belly.
Ventrad In the direction of the venter.
Ventral The bottom side aspect of a nematode. Pertaining to the lower side.
Ventral Cord The ventral line of the four longitudinal lines of thickening on the inner side of the hypodermis.
Ventral Gland The excretory gland.
Ventral Nerve The main body nerve which is actually a ganglionated cord.
Ventricular Esophagus An esophagus with a glandular basal bulb.
Ventromedian The true middle line on the lower side of an individual.
Viviparous Producing living young from within the body in contrast to eggs.
Voracious Ravenous. Rapacious in feeding.
Vulval Bridge In some species of *Heterodera* the vulval slit persists cross the fenestra as a bridge forming two semifenestrae.
Vulval Body Diameter (Width) The diameter of the body at the level of the vulva; sometimes abbreviated as vbd or vbw.
Vulval Cone A relatively moderate protuberance at the posterior end of *Heterodera* cysts.
Vulval Fenestra In *Heterodera* a thin-walled transparent region at the top of the vulval cone which may breakdown to form the hatching pore.
Vulval Flap Cuticular membranes sited at both ends of the vulva.
Vulva Glands Glands emptying through the vulva probably functioning during copulation.
Vulval Muscles Specialized muscles of function for the dilation and constriction of the vulva.

Vulval Pad The subcuticular clear zone surrounding the vulva consisting of vulval musculature.
Vulval Slit The shape or border of the female external genital opening.
Vulviform Resembling the vulva.
Wart A cuticular excrescence somewhat potato-shaped.
White Bud A disease of strawberry incited by *Aphelenchoides fragariae.*
White Cysts Newly matured females of the genus *Heterodera.*
White Tip A disease of rice incited by *Aphelenchoides besseyi.*
Wild Plant A disease of strawberry incited by *Aphelenchoides fragariae.*
Wilt Loss of freshness and drooping of plant leaves due to inadequate water supply, excessive transpiration, a vascular disease or a toxin produce by an organism
Wilty Soil Light, sandy, well-drained soil which harbors the wilt disease organism of cotton and plant pathogenic nematodes.
Wing An anteriad configuration *Meloidogyne* perineal patterns into a lateral lingulate shape.
Wool A mass of desiccated *Ditylenchus* juvenile in a resistant state; frequently found in bulb crops. Nematode wool.
Worm Nematode in the very broad sense.
Worm-shaped Vermiform.
Wrinkle A small ridge or furrow on a surface.
X-Bodies Binucleate, rounded and unbranched coelomocytes.
Xerophilous or **Xerophytic** Inhabiting dry places.
Yellows Disease A disease of black pepper vines incited by *Radopholus similis.*
Zooparasitic Infective in a host organism of the animal kingdom.
Zygote A fertilized egg resulting from the union of an ovum and a spermatozoon. A diploid nucleus resulting from the union of two gametes of opposite kind.

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