

TYLENCHIDA

Parasites of Plants and Insects, 2nd Edition

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Parasites of Plants and Insects, 2nd Edition

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Foreword

'Exegi monumentum aere perennius . . .' (Horatius)

In the Foreword to the first edition of this book, I wrote: 'Undoubtedly, the representatives of the order Tylenchida are of the greatest importance among the free-living and plant-parasitic nematodes both in science and practice. This is why Dr. Siddiqi's book will be of such significance in the zoological publications of our time and indispensable for everyone working with nematodes. . . . There is no doubt that the present book serves as a milestone in the history of nematology.' This is true for the second edition as well.

On the order Tylenchida, taxonomically and economically so important and, in the Nematoda as a group, so rich in genera and species, two outstanding fundamental works have lately been published. One is *Tylenchida: Parasites of Plants and Insects* by Siddiqi (1986), the other *A Reappraisal of Tylenchina (Nemata)* is by Fortuner, Geraert, Luc, Maggenti and Raski (1987-1988). Both works are of special significance in the history of nematology. However, whereas the latter was composed by the collaboration of an international team of nematologists, the former was truly a one-man work. About this edition of *Tylenchida*, I can say that Dr Mohammad Rafiq Siddiqi, having the widest knowledge and expertise in the taxonomy of this group, has produced one of the grandiose monographs of nematodes for all time.

This second edition of *Tylenchida* is by no means a simple reproduction of the original one, but, although based on it, is essentially a new book. Having accepted several new results of other authors and added the best ideas of his own, Dr Siddiqi has summarized, valued and systematized whatever science could tell us about tylenchid nematodes.

Works that have merited a second edition are very few in our science. The book of Dr Siddiqi is one of those rarities. I was very lucky to have been acquainted with both editions in their manuscript stages, and I am absolutely certain that the *Tylenchida-II* will be as impressive and well-accepted as the *Tylenchida-I* was, or

possibly it will surpass that. It will serve as a 'Bible' for all those who have bound their scientific career to Nematoda, this fascinating group of animals.

The author of the present book, Dr M.R. Siddiqi, has written his name with golden letters in the history book of our science of nematology.

István Andrásy
Budapest, Hungary
January 2000

Preface

The first edition of this book was published in January 1986. Since then, a large number of new taxa, from species to family rank, have been proposed in Tylenchida. The usefulness of the first edition can be judged by the fact that almost 1000 copies of *Tylenchida: Parasites of Plants and Insects* that were printed have been sold and that edition is now out of print. Copies used by nematologists have now almost worn out (I am pleased to know from many nematologists that the book was used extensively). Thus the need for the new print/edition of this book has been felt for many years.

Now the second edition is here, thanks to God who gave me the strength and dedication to complete the script, add new data and numerous plates of figures and submit it to CAB *International* for publication. I know that the first edition was very useful for identification purposes and that opinions differed on my system of classification which, by some, was considered inflationary. On my speculative phylogeny, much interest and research were generated. Some accepted my system and enlarged on it while others offered criticisms both constructive and not constructive. Tylenchida is inflationary (many more new tylenchid taxa will be described in the future) as one can see by the enormously large number of new taxa incorporated in the second edition. Furthermore, new data on terminology, morphology, systematics and biology have been added. Some intricate taxonomical problems have been dealt with and (almost) solved and it is hoped that the reader will find this edition extremely useful in identification of taxa in this large group of animals and stimulating for further research.

Among the important changes made in this edition are the upgraded classification, deletion of photographs of pioneer nematologists (due to lack of space in the book), and details of Myenchina, the suborder later raised to an order and which I have now excluded from the Tylenchida. Tylenchida now has four suborders - Tylenchina (with infraorders Tylenchata and Anguinata), Hoplolaimina, Criconematina and Hexatyliina. On the matter of classification, the importance of the methodology used, the phylogenetical discussions and my personal views on

relationships based on working experience on these tiny but not insignificant worms have to be taken into account. I have my own perception of these nematodes and there is every possibility that opinion will differ on the phylogeny and classification of this large group. I have added frequent comments on the diversity, inter-relatedness, biological characteristics and systematics of several genera. I accept the blame if I am called a splitter (recognizing *Bitylenchus*, *Quinisulcius*, *Neodolichorhynchus*, *Telotylenchus*, *Saueritylenchus*, *Paratrophurus*, *Merlinius*, *Geocnamus* as separate genera different from *Tylenchorhynchus* is not splitting but strengthening taxonomy) since my aim in writing this book is to help fellow nematologists as well as all those interested in nematodes to understand the Tylenchida and to have at hand a large amount of information about them. To this end, I have made use of the taxonomic categories of subgenus and subfamily in the classification of several groups. This should help the users of the book who are mostly concerned with the identification rather than the systematics of the genera. Identification and systematics go hand-in-hand, the former is an essential tool for researchers, experimentalists and field workers, while the latter is a toy of mental exercise to play with, for a philosopher or a desk-worker who tries to discover the likely pathways along which species have evolved.

No nematode taxonomic work can be the last word, since our knowledge of these little worms is far from complete. In this field of classification of organisms, systematists have never been in total agreement with each other. The difference of opinion is the way of the progress of taxonomy. When it comes to the placement of taxa in a hierarchic system of classification, the slippery slope of inter-relatedness and establishing evolutionary pathways becomes more difficult to tread. Speculation and personal judgement have to be used in all such matters.

A large number of new tylenchid taxa have been described since the publication of the first edition, and some progress has been made in the study of the morphology, ultrastructure and systematics of the Tylenchida. This has helped greatly in the understanding of the diversity and inter-relationship of various taxa and thus improving upon their classification.

It is my desire that this edition serves its purpose of providing available information on the Tylenchida to laboratory and field workers and as a teaching manual as well as a reference work. The book should be useful in exploring the existing biodiversity in nematodes and in the identification, description and classification of future new genera and species. It is not only a compilation of available information, but the result of my study, analysis and synthesis of Tylenchida as a group on which I have worked for more than 40 years and in which I have the honour of adding a large number of new taxa from suborder to subspecies level (in fact, the largest number of new genera in Secernentea have been proposed by me (see p. 30 and Andr assy's (1999): A census of genera and subgenera of free-living nematodes, *J. Nem. Morph. Syst.* 2, 45–68)).

Correct identification is important in working on any other biological aspect of the nematodes. It is particularly so in advisory and regulatory services and in programming control strategies including developing resistant varieties. I have tried to present the scientific names of various species and genera and their synonymies as accurately as possible, but if there remain some errors or omissions, I bear full responsibility for them, and I would appreciate it if these are brought to my notice.

I remember the story of a painter who displayed his beautiful painting in a market place with a foot-note: 'Please mark any mistakes', only to find the next day that the entire painting was blackened by correction marks. Next time, the painter displayed another painting with the caption: 'Please improve upon this painting', and it was left spotless and was appreciated by every passer-by who saw it. So please improve upon my ideas expressed in this book and oblige the scientific community. As regards the recognition and placement of various tylenchid taxa in a system of classification as given in this book, any improvement would be most welcome.

I take this opportunity to thank all those who encouraged and helped me in writing this second edition of *Tylenchida: Parasites of Plants and Insects*, particularly the staff of CABI Bioscience UK Centre (Egham) (formerly International Institute of Parasitology, St Albans). I am grateful to the staff of CABI *Publishing*, particularly Tim Hardwick and Emma Critchley, for editing the manuscript and organizing its publication. I am deeply indebted to Dr I. Andr assy for going through the manuscript and making corrections and suggestions for improvement. I offer my appreciation to my wife Rashida for her encouragement and forbearance with my preoccupation with the book since most of the work was done at my home at 24 Brantwood Road, Luton, England, and to my daughter Safia F. Siddiqi of CAB *International*, who has been very helpful, particularly in finding the obscure literature.

Mohammad Rafiq Siddiqi
Luton, England
January 2000

Preface to the First Edition

Tylenchida affect human well-being in several ways, principally by inflicting heavy losses on crops and yields of oil, fibre and timber. There is hardly any crop free of these nematodes, which abound in millions in agricultural fields, groves, grasslands and forested areas. It is their large population and not the isolated groups of individuals which harm the plants. They also kill useful and friendly insects, such as aphidiophagous ladybirds and pollinator bumblebees. They can sterilize and kill females of several insect pests of agricultural crops and forest trees, and thus have considerable potential in biological control.

The problems these nematodes pose are enormous, but their importance as a significant limiting factor in agricultural production has only recently received worldwide attention. During the last two decades, the increase in published information on Tylenchida has been exponential, and this has led to problems of assimilation, even for the best equipped nematologists. Although a number of primary core journals are devoted to nematological research, much information remains scattered throughout the less easily accessible peripheral journals so that a comprehensive awareness of the field becomes difficult, especially for workers in the less developed countries. Taxonomic information, being highly specialized in nature, is all the more difficult to obtain.

Tylenchida now comprises 216 valid genera and 2200 valid species (76 genera and 222 species are considered in this book as invalid synonyms and 120 species as inquirendae, dubiae or insertae sedis). New genera and species are constantly being described and taxonomic changes are disquietingly common. In addition, the actual identification of these nematodes is difficult, largely because they are minute (the smallest being only 100 μm in length) and the diagnostic characters are difficult to observe and interpret.

The classification of the Tylenchida has to be inferred from relationships based on morphological, physiological, cytogenetic, embryological and ecological characteristics and character states, since evolutionary change is too slow to be apparent and there are no fossils of Tylenchida to provide evidence of its direction. Inevitably,

personal intuitive judgement and speculation must play a significant part in such a classification.

Since Goodey (1963), the need for an up-to-date text on the taxonomy of the Tylenchida has been greatly felt by students, researchers and professional nematologists alike. The present work is an attempt to fulfil this need. For this purpose, I have given diagnoses of all the valid genera and higher categories of Tylenchida and have listed all the nominal species under their respective genera. I have also tried to give identification keys in a simple dichotomous manner and have emphasized the important diagnostic characters in semi-bold. I have intentionally not given the descriptions of type or representative species of valid genera because it would have made the book unwieldy and probably too expensive. However, I have enlarged the generic diagnoses and have frequently given details of the type-species and etymologies of generic names. Remarks on the biological characteristics and the economic importance of members of family groups are frequently made and, where appropriate, the taxonomy of various genera and families is discussed.

The first three chapters of the book give a general account of the Tylenchida, a historical review, techniques commonly used, analyses of morphological characters, taxonomic methods and the origin, phylogeny, outline classification and characteristics of the Tylenchida. The next four chapters deal with the taxonomy of the suborders Tylenchina, Criconematina, Hexatylinea and Myenchina, respectively. A comprehensive bibliography of 786 references, including literature cited in the text and references to names of all the genera and higher categories of Tylenchida, has been provided: for references to names of species and subspecies, *Helminthological [Nematological] Abstracts* and the checklist of Tarjan & Hopper (1974) should be consulted. An index of all the taxa in Tylenchida is given at the end of the book. It is my hope that this book should serve as a basic text for students and all field and research workers in plant and insect nematology, plant pathology, plant quarantine and general zoology.

I thank Drs I. Andrassy, A. Coomans, D.J. Hunt, E. Geraert, F.G.W. Jones, P.K. Koshy, A.R. Stone and J.F. Southey for critical reading of portions of the manuscript and for their constructive discussions. I gratefully acknowledge the expertise and patience of Barbara Gibson, who, with the help of Beryl Cunningham, Eileen Gordon and Sheila Eames, typed a very difficult manuscript. I am also grateful to the Commonwealth Institute of Parasitology, literature and technical resources of which have provided the basis for my work; to the ex-Director of the Commonwealth Institute of Parasitology, Dr Sheila Willmott, and to the present Director, Dr Ralph Muller, for their encouragement, and particularly to the Assistant Director, Peter Gooch, who organized the publication.

I take this opportunity to thank all my friends and co-nematologists, too many to mention by name, who generously helped me by sending reprints of papers, specimens of nematodes, photographs, permissions to reproduce printed works and especially persons in charge of the nematode collections at the Indian Agricultural Research Institute, New Delhi, the Nematology Departments at the University of California at Davis and Riverside, and the USDA Nematology Laboratory, Beltsville, Maryland, from whom type-specimens were freely available on loan for study.

I am grateful to the UK Natural Environment Research Council for a generous grant towards the cost of some of the illustrations in this book.

Since the major portion of this work was completed at my home in Luton, UK, I would like to put on record my deep sense of gratitude to my wife Rashida for her encouragement, and, perhaps more importantly, her forbearance with my long preoccupation with the preparation of the book, and to my daughters Safia, Salma, Somaiya, Sayeeda and Saboohi and son Soheb, who helped me in various ways including reading the typescript and compiling the references.

Mohammad Rafiq Siddiqi
St Albans, England
May 1985

I Introduction, Historical Review and Techniques

1. INTRODUCTION

Tylenchida Thorne, 1949 is an Order of nematodes or nemas (Greek *nema*, *nematos* = thread, *eidos* = likeness, resembling) of the Subclass Tylenchia Inglis, 1983 (*nec* Rhabditia Maggenti, 1982), Class Secernentea von Linstow, 1905 (= Phasmidia Chitwood & Chitwood, 1933; Rhabditea Inglis, 1983), Phylum Nematoda Rudolphi, 1808 (= Nematodea Rudolphi, 1808; Nemathelminthes Gegenbaur, 1859; Aschelminthes Grobben, 1909; Nemata Cobb, 1919).

The terms nema (for nematode) and nematology were introduced by Cobb (1932). Chitwood (1957) supported Cobb, arguing that, zoologically speaking, the vernacular word nematode was a corruption of the ordinal name Nematodea of Rudolphi (1808) which was in use in Germany as the plural equivalent 'Nematoden'. Von Siebold (1848, p. 112) used the ordinal word 'Nematodes' which was modified by Diesing (1861, p. 598) as Order Nematoda. Maggenti (1981, 1982) and Goodey (1963) used the names Nemata and Nematodea for Phylum and Class, respectively, instead of Nematoda, but the word nematode is more commonly used than nema. Maggenti *et al.* (1987) stressed that Cobb's (1919) Nemata should be used as a phylum name since Cobb (1919) was the first to exclude all but nematodes from the group. They stated: 'Rudolphi (1808) proposed Leder's "Rundwürms" (gordians and nematodes) as the group "Nematodea" along with Acanthocephala, Trematoda and Cestoidea in the group Entozoa. Therefore there is no reason to credit Rudolphi for the phylum, as Nematodea included all "thread-like" forms of roundworms, no taxonomic distinction being made.'

Rudolphi's Nematodea cannot be rejected on the ground that it included gordians. In fact, the term Nematoden and Nematoda have been continuously in use to-date and gordians had been included within the Nematoda for a very long time. Rudolphi (1808) recognized Nematodea as different from Acanthocephala, Trematoda and Cestoidea. Gegenbaur (1859) placed Nematodea, Acanthocephala and Gordiacea in a new phylum Nemathelminthes. Nemathelminthes as a phylum

name has been used as recently as 1951 by Hyman, and Maggenti *et al.* (1987) had accepted that 'Nemathelminthes, still remains since it was the only one clearly defined by Vejdovsky (1866).' The choice of name for the phylum, therefore, is between Nematoda and Nemathelminthes/Aschelminthes and I prefer to use the former because it is more ancient and has been widely used. Hence the phylum name Nemata is rejected. Why should one use the vernacular names nemas or nematas/Nematoden when nematodes/Nematoden are available and have been used since the 19th century?

Tylenchida are popularly called tylenchs or tylenchids comparable with aphelelchs or aphelenchids, the latter representing a separate order, Aphelenchida Siddiqi, 1980 (see Fig. 1). The term tylench is now restricted to *Tylenchina sensu* Geraert, 1966 and *Tylenchida sensu* Siddiqi, 1980. The Tylenchida are the largest and economically the most important group of plant-parasitic nematodes. The order also includes a large group, Hexatylinea, which parasitize insect and mite haemocoels. As plant parasites, they have exploited all plant organs including flowers and seeds, but mostly they attack roots (Fig. 2).

Several Tylenchida, particularly the families Pratylenchidae, Meloidogynidae and Heteroderidae, are of great economic importance as parasites of agricultural crops and forest trees. They cause substantial crop losses in many countries, for example in Rajasthan, one of the Indian States, it has been estimated that 60 million rupees (approx. US\$1.5 million) are lost in wheat production due to a single nematode species, *Heterodera avenae*. Sasser's (1979) estimates of crop losses due to *Meloidogyne* spp. in tropical countries show on average losses of 15%. Yield losses in vegetable crops from these nematodes of 50–80% are not uncommon. In South Africa, annual loss of vegetables, cereals and fruit crops of about 14% due to plant-parasitic nematodes (comprising mostly of Tylenchida) has been estimated (Keetch, in Kleynhans *et al.*, 1996).

The plant-parasitic Tylenchida are also called eelworms, phytonematodes, phytohelminths or simply plant nematodes. Their study constitutes a major part of nematology, more appropriately, agricultural nematology. The entomoparasitic forms are often referred to as entomogenous (Greek *entomon* = insect, *genés* = born), entomophagous (Greek *phagein* = to eat) or entomophilic (Greek *philein* = to love) nematodes. These words are misnomers for Tylenchida which are truly parasitic in habit and therefore I prefer to call them entomoparasitic.

In entomoparasitic forms (Hexatylinea), only the gametogenetic female is parasitic, other stages in the host are transient and males rarely occur inside the host to fertilize the females of the second generation (*Parasitylenchus*). Occasionally, males and females may occur and mate inside the body of the mother nematode (*Scatonema*). Most of the entomoparasitic Tylenchida leave their hosts as juveniles or eggs to develop in the outside environment. The fertilized female seeks out and penetrates a host's larva or pupa to establish itself in the haemocoel. Several entomoparasitic genera have free-living mycetophagous (fungus-feeding) generations as well (see Fig. 3(a)).

Anguinoidea, a large group of Tylenchina, are close relatives of Hexatylinea but they lack the entomoparasitic phase and are totally mycetophagous and/or phytoparasitic; only the subfamily Sychnotylenchinae is found in association with insects. Another family, Halenchidae, has representatives exclusively parasitizing

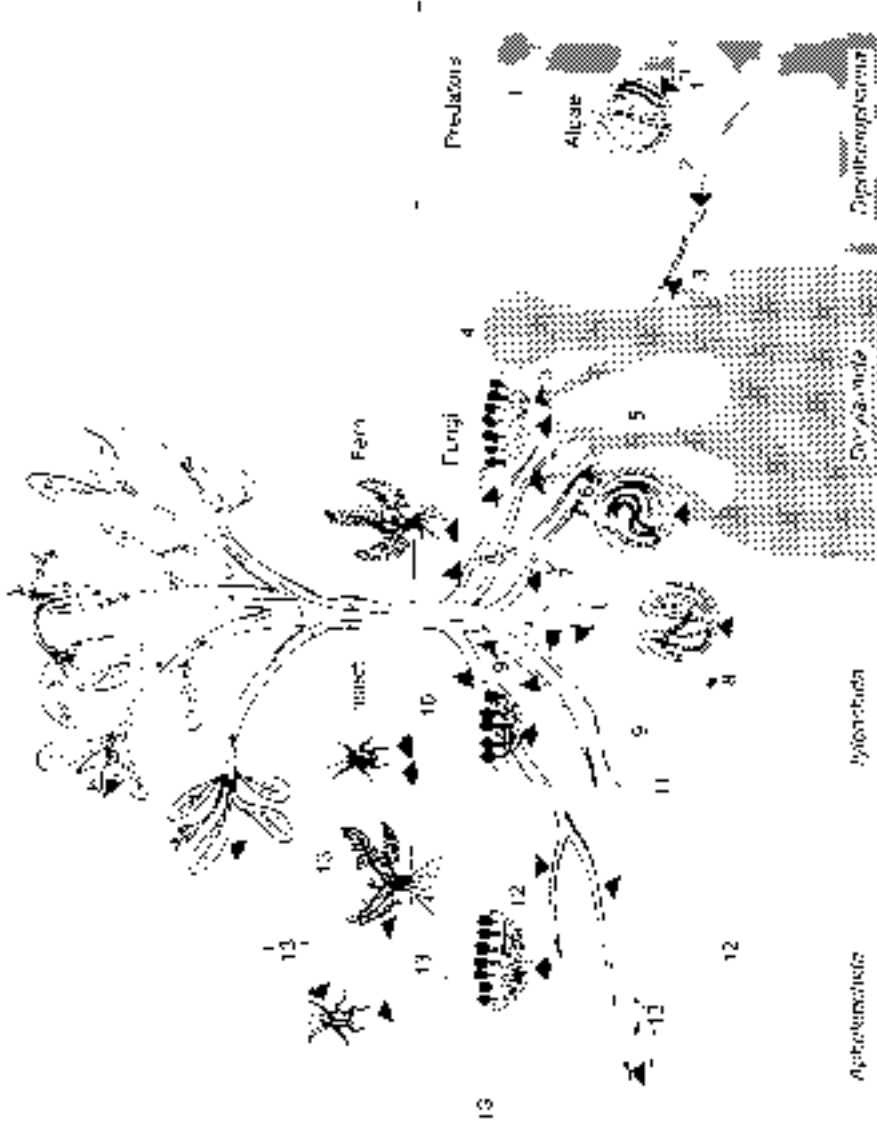


Fig. 1. Evolution of plant-parasitism in nematodes. Knobbed ends represent predators. Numbered evolutionary lines refer to the following taxa. 1. Diphtherophoroidea. 2. Trichodoroida. 3. Dorylaimoidea (*partim*). 4. Nygolaimoidea, Aporclaimoidea, Discolaimidae. 5. Tylencholaimoidea. 6. Longidoroida, Dorylaimoidea (*partim*). 7. Tylenchoidea. 8. Dolichodoroida, Hoplolaimoidea, Criconematina. 9. Anguinoida. 10. Neotylenchoidea. 11. Sphaerularioidea, Iotonchoidea. 12. Aphelenchoidea, Aphelenchoideoidea. 13. Aphelenchoideoidea. (After Siddiqi (1983), courtesy The Systematics Association.)

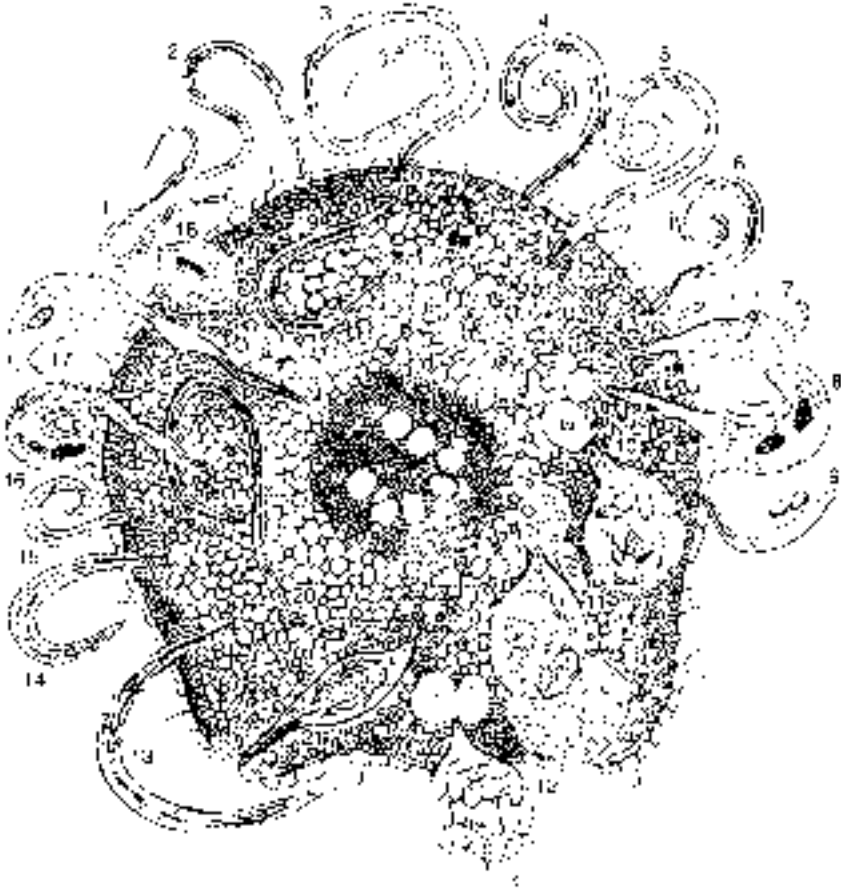


Fig. 2. Diagrammatic presentation of various types of tylenchid nematode feeding on root tissues. 1. *Cephalenchus*. 2. *Tylenchorhynchus*. 3. *Belonolaimus*. 4. *Rotylenchus*. 5. *Hoplolaimus*. 6. *Helicotylenchus*. 7. *Verutus*. 8. *Rotylenchulus*. 9. *Acontylus*. 10. *Meloidodera*. 11. *Meloidogyne*. 12. *Heterodera*. 13. *Hemicycliophora*. 14. *Macroposthonia*. 15. *Paratylenchus*. 16. *Trophotylenchulus*. 17. *Tylenchulus*. 18. *Sphaeronema*. 19. *Pratylenchus*. 20. *Hirschmanniella*. 21. *Nacobbus*.

seaweeds and which are the only known plant-parasitic Secernentea flourishing in the sea (see Fig. 3(b)).

Tylenchida occur in all possible habitats in soil, water and plants. Their greatest diversity of form occurs amongst parasites of roots. A handful of moist soil from the rhizosphere of any plant should normally yield more than one species. A grain of galled wheat might contain up to 30,000 nematodes. One gram of coconut roots could yield about 4000 *Radopholus similis* (including eggs) (Koshy *et al.*, 1976). Andr assy (1992) compared the species of Tylenchida with other soil-inhabiting nematode orders, and added: 'In free-living continental [vs. marine] nematodes the richest orders are the Tylenchida (2240 species, 40%), the Dorylaimida (1880

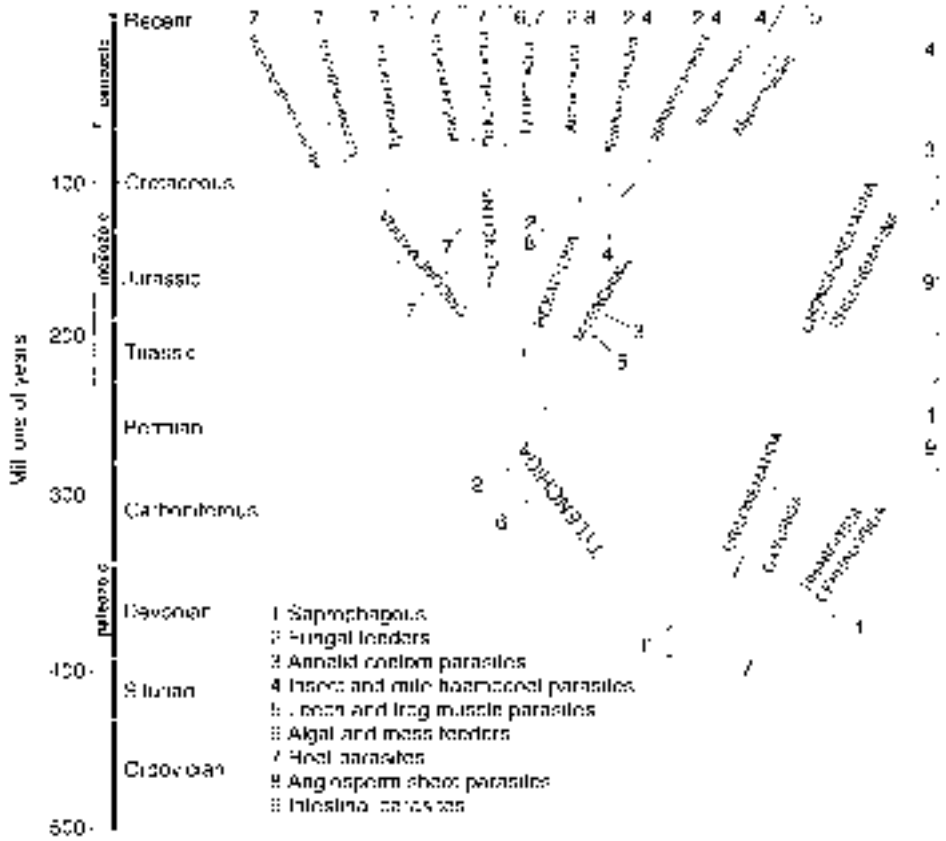


Fig. 4. Origin and evolution of Tylenchida. (After Siddiqi, 1986.)

plant-feeding nematodes would, therefore, include only some nematodes (e.g. Heteroderidae, Tylenchulidae (see Fig. 6)), but not all stages of such nematodes are parasitic. Siddiqi (1983a) referred to this problem and preferred to call all plant- or fungus-feeding Tylenchida 'plant parasites'. In many groups males lack a stylet or have only a degenerated one and cannot feed, hence the female stage is the parasitic stage and is the basis for most descriptions and diagnoses of taxa in this book.

There is some evidence that several highly adapted root parasites (e.g. Heteroderidae) have co-evolved with their hosts (Krall & Krall, 1970; Stone, 1979). Tylenchida have a long history of about 400 million years, having supposedly originated in the Devonian Period of the Palaeozoic Era (Maggenti, 1971; Siddiqi, 1980, 1983a). Early Tylenchida must have been bulk feeders on bacteria, fungi and algae with their stylet-like stoma. Later, with the advent of the protrusible stylet, they evolved as parasites of rhizoids and roots (Tylenchina, Criconematina), as fungal feeders (Anguinata, some stages of Hexatyliina) and as parasites of insect and mite haemocoels (Hexatyliina) (see Fig. 4).

Tylenchida are also a source of food for soil microfauna, fungi and microorganisms. Protozoa (amoebae), nematodes (diplogasterids, dorylaims, mononchs), tardigrades, copepods and mites are predators on members of Tylenchida. About 150 species of fungi feed on these nematodes and an equal number of fungi are food for them. Several plant-pathogenic bacteria and fungi interact with the Tylenchida to produce severe symptoms of disease in plants, nematodes often predisposing normally resistant plants to bacterial or fungal attacks. Obligate parasites of nematodes, such as *Pasteuria penetrans* (= *Dubosquia penetrans* Thorne; *Bacterium penetrans* (Mankau)), have great potential in the management of plant-parasitic nematodes.

In zoological terms, Tylenchida are bilaterally symmetrical (even though some parts are radially symmetrical), elongate-cylindroid, unsegmented pseudocoelomate animals covered with a cuticular exo-skeleton secreted by the hypodermis (= epidermis). They have longitudinal muscles for locomotion, a terminal oral opening surrounded by bilaterally or radially arranged sensilla, protrusible stomatal stylet (see Fig. 7), a substylet orifice of the dorsal oesophageal gland, a circum-oesophageal or circum-intestinal nerve ring, an excretory system with a single duct and renette cell, a pore-like anus directed outward (Fig. 5), a true tail (postanal continuation of the body), and they lack a circulatory system and motile cilia. They are phasmidians but the suborders Tylenchina, Hexatylinea and Criconematina do not have detectable phasmids.

As in other nematodes, the sexes are separate and reproduction is by amphimixis, but autotoky (mainly parthenogenesis) is not uncommon; regeneration or asexual reproduction does not occur in nematodes. The gonads are tubular and outstretched (only secondarily reflexed or coiled). The male accessory genital structures include a pair of cuticularized spicules, a gubernaculum with or without telamon and titillae, a non-papillary (non-ribbed) bursa and genital papillae, 1–4 being grouped around the cloacal aperture; the male tail is devoid of caudal papillae.

The eggs are typically oval without spines, plugs or excrescences. The uterine egg is semifluid and can pass through a small hole. Advanced tissue parasites produce a much larger number of eggs than the surface root browsers and fungal feeders. Most Tylenchida are oviparous but a number of entomoparasitic genera have ovoviviparous species in which the eggs hatch in the uterus. Occasionally, in oviparous females the eggs hatch in the uterus and kill the mother, a phenomenon called *endotokia matricida*. The juvenile may hatch by cutting the egg-shell with its stylet (Doncaster & Shepherd, 1967) or by rupturing the egg-shell with its tail tip as in *Heterodera iri* (Laughlin *et al.*, 1974) or through normal rupture of the egg-shell due to the juvenile growth and movement. The juvenile develops to adult through four moults, the first moult normally occurring within the egg.

The nematodes which live in soil and are mobile in every stage are **necrotrophic**, whereas those that live in roots as sedentary parasites are **biotrophic**. The biotrophic nematodes such as *Heterodera* and *Meloidogyne* live as parasites within the root tissue. A special physiological relationship develops between them and the host tissue. As a response to their feeding on cells, the host plants induce the formation of nurse cells, giant cells or syncytia (Fig. 6). Such nematodes use special fine tubes formed by their salivary secretion/host response to feed from the nurse cells.

Some Tylenchida show a great ability to withstand desiccation and extreme adverse environmental conditions. The phenomenon of **anabiosis** (cryptobiosis) is

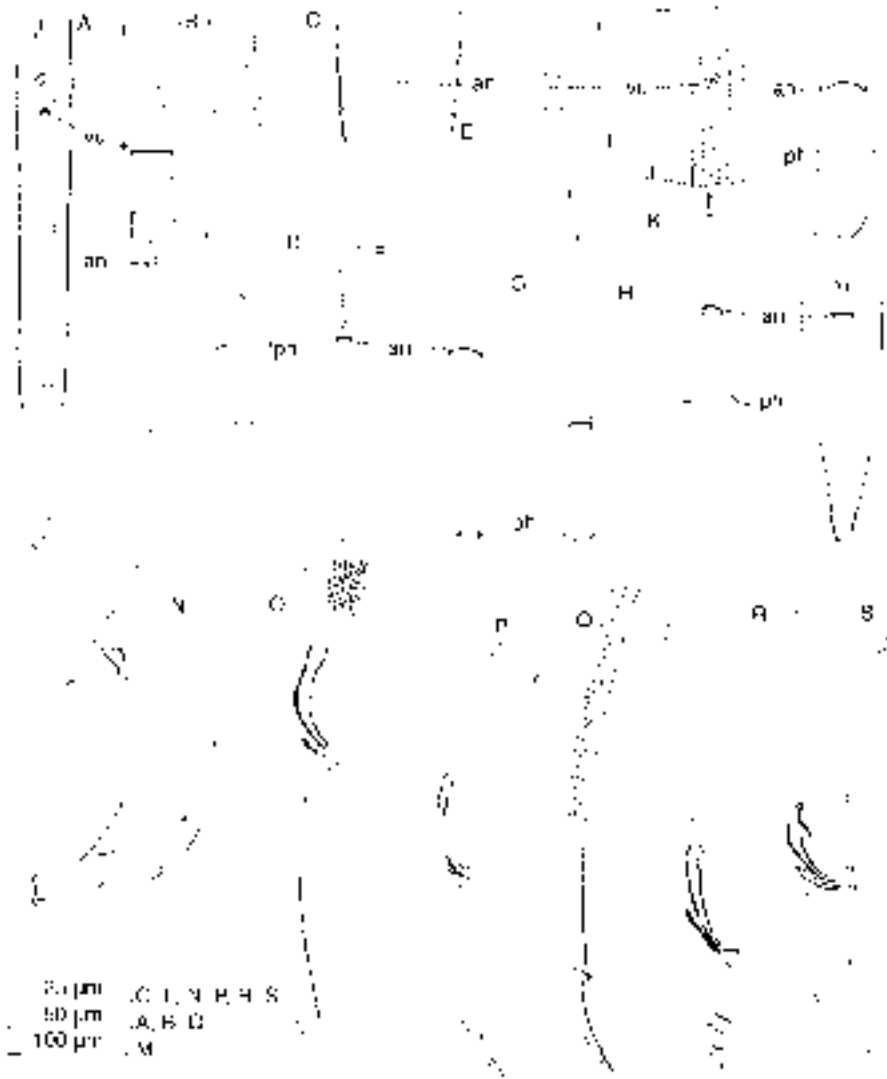


Fig. 5. Female anal apertures and male accessory genital structures in Tylenchida and other orders. A–M. Females. N–S. Males. A and B. Posterior ends showing vulva and anus. C–H and K–M. Tail ends showing anal aperture in ventral view. I and J. Vulva in ventral view. Hexatyliina: A and O. *Sphaerularia bombi*. B. *Hexatylus viviparus*. Tylenchina: C. *Tylenchus davainei*. Q. *Ditylenchus* sp. Hoplolaimina: D. *Pratylenchus brachyurus*. Criconematina: E. *Hemicycliophora conida*. P. *Tylenchocriconema alleni*. Aphelenchida: F. *Aphelenchoides besseyi*. G and S. *Paraphelenchus myceliophthorus*. I, H and R. *Aphelenchus avenae*. Diplogasterida: J and K. *Tylopharynx* sp. Cephalobida: L. *Acrobeloides* sp. Oxyurida: M. *Labiostrumum* sp. N. Unidentified Oxyurida. an = anus, ph = phasmid, vu = vulva. (After Siddiqi, 1980.)

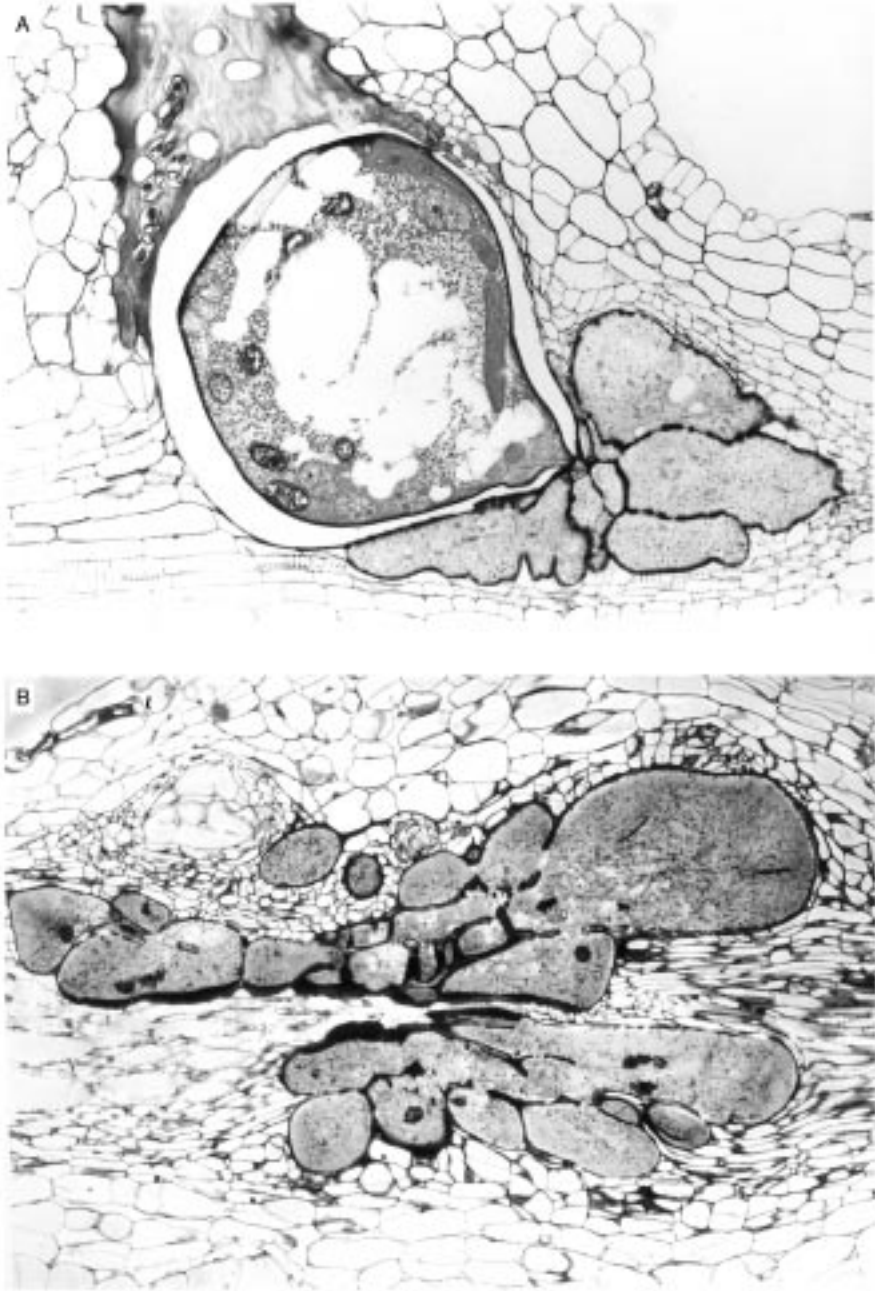


Fig. 6. A. Syncytia (giant cells) formation in tomato root due to feeding by *Meloidogyne incognita*. B. Syncytia formation in potato root due to feeding by *Globodera rostochiensis*. (Courtesy U. Wyss, Germany.)

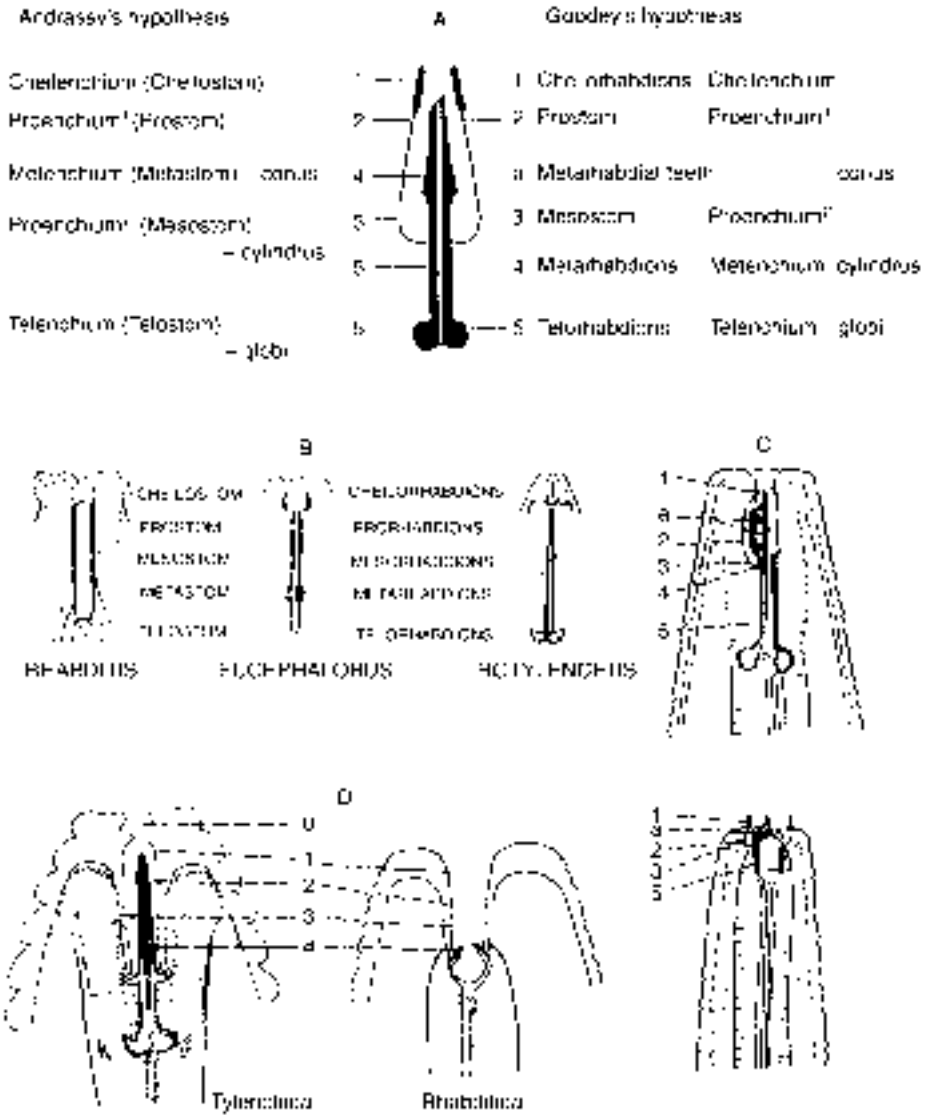


Fig. 7. Homologies of the stylet parts. A. Comparison of the hypotheses of Andrassy (1962) and Goodey (1964), after J.B. Goodey (1964). B. After Thorne (1961). C. Stoma parts of *Tylopharynx* sp. (upper) and *Monochoides* sp. (lower), after Siddiqi (1980). D. After De Grisse (1972). U, prestoma; 1, cheilorhabditans; 2, prorhabditans; 3, mesorhabditans; 4, metarhabditans; 5, telorhabditans; a, metarhabdial teeth.

well illustrated by members of the Anguinoidea. Fielding (1951) found that dormant *Ditylenchus dipsaci* and *Anguina tritici* could be revived after 20 and 28 years, respectively. The Tylenchida also provide good teaching and demonstration material for plant and insect parasitism, the process of metamorphosis (e.g. the development of

the *Heterodera* male, or *Hemicriconemoides* female) dimorphism and sex reversal. Morphology, organ systems, embryology, moulting and life cycle can easily be demonstrated to students (Siddiqi, 1966). Some Hexatylinea are remarkable for the alteration of mycetophagous and entomoparasitic generations, and for the polymorphism with di-, tri- or tetramorphic females. Hexatylinea and Anguinata have the advantage that they are easily cultured on fungi in agar medium and stocks are readily available for demonstrations and experiments.

The plant-parasitic genera, particularly *Heterodera*, *Globodera*, *Meloidogyne*, *Pratylenchus*, *Radopholus*, *Rotylenchulus*, *Ditylenchus*, *Anguina* and *Tylenchulus*, cause significant losses to crop production. Rice, wheat and potatoes, which are the world's most important staple food crops, lose 10, 7 and 12% of production, respectively, due mainly to Tylenchida species (Sasser & Freckman, 1987). Forty species of Tylenchida have been listed as major pests of major mandatory crops (e.g. wheat, maize, sorghum, potato, banana, soybean, chickpea, pigeonpea, groundnut and beans) of the International Agricultural Research Centres of CGIAR (Consultative Group on International Agricultural Research) (Sharma *et al.*, 1997). These Centres – International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), International Institute of Tropical Agriculture (IITA), International Rice Research Institute (IRRI), International Centre for Agricultural Research in Dry Areas (ICARDA), Centro Internacional de Agricultura Tropical (CIAT), Centro Internacional de la Papa (CIP) – have made significant contributions to survey, evaluation of the nematode potential as crop pests, screening and making available resistant or tolerant species germplasms, suggesting effective control measures and making governmental and funding agencies aware of the nematode threat to their mandate crops.

The potential of Tylenchida as research models is considerable for work on biodiversity, ecology, physiology, embryology, neurobiology, cytogenetics, ageing, etc. So far, the nematodes most commonly used as models are either free-living Rhabditida (*Caenorhabditis elegans*, *C. briggsae*, *Turbatrix aceti*) and Cephalobida (*Panagrolaimus redivivus*, *P. silusiae*) or animal parasites.

Sohlenius *et al.* (1997) estimated mean nematode abundance of $9.4 \times 10^6 \text{ m}^{-2}$ in an ombrotrophic mire at Abisko in northern Sweden. The nematode community comprised 34 nematode taxa and was typical for polar tundra soils. In a tropical rainforest at Mbalmayo, Cameroon, carbon fluxes (CO_2 and CH_4) from the forest floor, could account for a high nematode abundance averaging $2.04 \times 10^6 \text{ m}^{-2}$ (Lawton *et al.*, 1996). In Korup National Park, Cameroon, Price & Siddiqi (1994) found on average 4.4 million nematodes m^{-2} of which 33% were rhabditids and cephalobids, 23% tylenchids and 13% dorylaimids; the most diverse orders were the Dorylaimida (65 species-types) and the Tylenchida (35 species-types).

Since Tylenchida maintain a somewhat balanced community structure in the rhizospheres in non-agricultural lands, grasslands, forests and aquatic habitats, and are affected by changes in the environmental conditions, they serve as useful tools in ecological studies and can be used as models in environmental research for monitoring pollution. Price & Siddiqi (1994) stated: 'This suggests a role for the study of plant and soil nematodes in investigating, quantifying and monitoring past, present and future environmental changes. Such works could be restricted to species of the Tylenchida (due to their diversity and potential economic importance) and of the

Dorylaimida (due to their potential ecological significance). Both groups are well investigated taxonomically (Siddiqi, 1986; Jairajpuri & Ahmad, 1992) and nematology has the potential to make significant contributions of immediate relevance. The plant and soil nematode community of the Korup [forest] shows abundance, diversity and complexity features so characteristic of tropical rainforests. Only more research can establish both the ecological role and importance of nematodes and the contributions nematodes can make to our further understanding of the rainforests.'

Many important plant-parasitic nematodes have been spread by man (e.g. *Radopholus similis*, *Heterodera avenae*, *Globodera rostochiensis*, *Tylenchulus semipenetrans*, *Ditylenchus dipsaci*, etc.), but many are still confined to their areas of distribution (*Nacobbus aberrans*, *Meloidodera* spp., *Meloidogyne naasi*, *Pratylenchus goodeyi*, *Rotylenchulus* spp. (other than *R. reniformis* and *R. parvus*), *Ditylenchus angustus*, *Pterotylenchus cecidogenus*, *Heterodera glycines*, *H. oryzae*, etc.). The introduction, establishment and spread of dangerous parasites, predators, pests, and pathogens which are injurious to plants and plant products should be properly regulated and subjected to plant quarantine measures. Pest risk analysis of nematodes is the first step in the application of plant quarantine to promote plant protection (Siddiqi, 1986a). The nematode species dangerous to plants are called A1 category pests if they are not present in a country, and A2 category pests if present and localized in the country. A1 and A2 category plant nematode pests for European region have been listed and nematode data sheets produced by European and Mediterranean Plant Protection Organization (EPPO) and ASEAN (Association of South East Asian Nations) countries. CABI/EPPO (1997) have produced data sheets on quarantine pests including several Tylenchida species, for the European Union and for EPPO. Another example of international collaboration, cooperation and coordination in plant quarantine is the Inter-African Phytosanitary Commission which has a mandate from the Organization of Africa Unity.

Plant-pathogenic nematodes justify post-entry quarantine procedures as well as equivalent checks before export (OEPP/EPPO, 1990). Only material for scientific purposes should normally be imported. The simplest practical measure is to restrict the importation of soil (as such or accompanying plants, tools or packing material). UK plant health measures are based principally on the Plant Health (Great Britain) Order 1993 implementing European Union Directive 77/93/EEC, as amended. Several Tylenchida of quarantine status, e.g. *Ditylenchus dipsaci*, *D. destructor*, *Globodera pallida*, *G. rostochiensis*, *Radopholus similis*, etc. are covered by it. EU marketing schemes require visual freedom from quality-affecting nematodes. Soil samples normally comprising 500 g of soil from 100 cores per 4 ha (or less) are tested for freedom from *Globodera* spp. and virus vector nematodes (*Xiphinema*, *Longidorus* and *Trichodorus*) under certain certification schemes and, where necessary, for export crops (Ward & Hockland, 1996).

To control plant-parasitic nematodes, crop management, cultural practices and organic soil amendments are being favoured over chemical control on pollution strategy and high cost. Non-chemical control of plant-parasitic nematodes involves cultural practices such as crop rotation, fallowing, ploughing after irrigating, and developing resistance in plants, growing non-host and trap plants, growing seedlings in clean soil, paring infested corms, rhizomes and using clean seeds, applying organic

soil amendments such as oilcakes, chopped leaves and other plant material, soil and marine algae, solarization, immersing planting material in hot water to kill infesting nematodes, control of weeds and other host plants on which the plant nematodes may survive, etc. Applying regulatory control for exotic nematodes not reported from the area, health certification of all plant materials imported or exported from the country and monitoring and alerting for the dangerous nematode pests are of utmost importance.

The biological control agents such as nematode-trapping fungi, rhizosphere bacteria, egg-parasitic bacteria, predatory nematodes, insects and mites are useful to check crop damage, particularly over long periods, as they build up their populations in the soil. Considerable work has been done on *Pasteuria penetrans*, nematode-trapping fungi, such as *Arthrobotrys oligospora*, *Dactylaria* spp. and *Dactylella* spp., and antagonistic fungi, *Verticillium chlamydosporium*, *V. udum*, *Trichoderma harzianum* and *Paecilomyces lilacinus* but biopesticides using such agents have not yet been successfully applied in the field on a large scale. The main drawbacks are that the biopesticides or biological nematicides are difficult to produce and maintain long enough for economic effect (see Stirling's (1991) book for details on biological control). Several nematologists are now researching biological control of insect pathogens using species of *Deladenus*, *Steinernema*, *Heterorhabditis* and Mermithidae by studying their biology, ecology, distribution, multiplication, storage and formulations to be used in the fields as biopesticides. These nematodes have capabilities of evading insect defences and penetrating into the insect haemocoel. Steinernematids introduce entomopathogenic bacteria, e.g. *Xenorhabdus* spp., into insect pests, which ultimately kill them.

Because of their ability to sterilize or kill insect pests of agricultural importance, entomoparasitic Tylenchida have the potential to control insect pests of agricultural importance. The siricid wood wasp, *Sirex noctilio*, parasitic on pine trees (*Pinus radiata*), has been controlled and tree mortality reduced by the field application of *Deladenus siricidicola* as a biological control agent (Bedding, 1984). *Paraiotonchium autumnale* is another entomoparasite which has biocontrol potential for controlling *Musca autumnalis*, the face fly of range cattle, in North America (Kaya, 1993). *Orrina phyllobia* is showing promise in controlling *Solanum elaeagnifolium* (silver-leaf nightshade), a noxious weed infesting more than 1.2 million hectares of crop land in the USA.

The cruciferous host plant, *Arabidopsis thaliana*, is considered an ideal plant to study host-parasite relationships particularly at the genome level. The plant has a haploid chromosome number of 5 and its nuclear genome of about 70,000 kb is the smallest known among flowering plants (cf. wheat genome with 5,900,000 kb). A multinational coordinated genome project using *A. thaliana* has been set up to identify and characterize the structure, function and regulation of genes, to develop technologies for plant genome studies and to establish biological resource centres (Wyss & Grundle, 1992).

The diversity of form exhibited by Tylenchida demands a careful study of their morphological characters for the separation of genera and species. Good diagnostic characters should vary little within a taxon, but more between the taxa. The task of a taxonomist is not only to identify and describe new and known genera and species but also to try to explain how these taxa are related to each other and how they fit

into a system of classification. The species are based on populations of living or dead individuals, but genera and higher categories are abstract, with vague boundaries and limits set by the individual experience and concept of taxonomists. Due care must be taken when proposing new genera and higher categories.

When a large number of species occur in a genus, and more is known about their structural variability, generic boundaries become diffuse and, sometimes, it is necessary to shift or regroup species. This may involve removing some species to other genera (transfers) or to new genera (by splitting the old ones), as has happened with the genera *Tylenchus*, *Tylenchorhynchus* and *Criconemoides*, or enlarging the genus by emending its generic diagnosis (lumping) so that different groups (sub-generic) of species can be accommodated under it, as is done in this book with *Neodolichorhynchus*, *Criconema*, *Ogma* and others. Even so, large generic groups with many species still remain, in which splitting has not been tried, e.g. *Helicotylenchus*, or has proved unsuccessful, e.g. *Basiria*.

In this book, several genera have been recognized at the subgeneric rank rather than listing them under their senior genus name. This should help the users of the book who are mostly concerned with the identification and systematic position of genera and species. Identification and systematics go hand-in-hand. It is hoped that the subgeneric categories, as used in this book, will help in understanding the diversity and inter-relatedness of taxa to be useful in identification, as well as in indicating the probable lines of evolution of various species.

The generic and subgeneric categories do help in building a sound system of classification of species. Although genus and subgenus names are of coordinate status, the citation of a subgenus name within brackets between the genus and the species name neither affects the binomen nor makes the name a trinomen. For example, the name *Malenchus (Neomalenchus) ovalis* is the same as *Malenchus ovalis*. However, the name *Malenchus (Neomalenchus) ovalis* does indicate that the species epithet *ovalis* has been combined with *Neomalenchus* and that it lacks a muscular median oesophageal bulb which helps in active feeding. Other examples of sub-generic names are found in the genera *Rotylenchus*, *Hoplolaimus*, *Ogma*, etc.

Some genera (*Aerotylenchus*, *Afrina*, *Basiroides*, *Bidera*, *Mulveyotus*, *Sherodera*, *Zelandodera*, etc.) considered here as junior synonyms may in future be shown to be valid. Similarly, some of the genera treated here as valid may later prove to be junior synonyms, on the basis of new ideas and interpretations of distinguishing characters. There are taxa of uncertain position, e.g. Paurodontidae, and genera dubia of Hexatylinea, which may prove to be senior synonyms. There are also anomalous genera (e.g. *Meloidoderella*) whose status can be determined only after the type-material is refound or topotypes are collected and restudied.

The number of valid genera in Tylenchida considered in this book is 225 including 21 considered as subgenera, three considered as *genera dubia* of uncertain position and another three as *genera inquirenda* needing further study. There are 105 genera listed as invalid synonyms. The number of valid species in Tylenchida considered in this book is 2828, the number of synonym species is 413 and the number of *species inquirendae/dubiae* is 163.

Diagnoses and differential keys are provided for all valid genera. In most cases diagnoses have been enlarged. To avoid confusion with related genera in other sub-families, some familial characters are repeated in generic diagnoses. Data for the type species, especially the type host, habitat and locality, are frequently mentioned

and etymologies of the generic names provided. In the diagnoses of taxa, if the sex or developmental stage is not mentioned, the morphological or ecological characters refer to the female since the female is the parasitic stage both as plant and insect parasite; males and juveniles of some groups have a degenerated oesophagus and cannot feed.

The ICZN Article 23(c) dealing with the Principle of Priority states: 'The priority of the name of a taxon of the family group, genus group, or species group is not affected by elevation or reduction in rank within its group'. Accordingly, in this book, names of authority and year of taxa have been cited as required by the Article, but the authority and year of the first elevating or lowering of the ranks have also been added within the brackets immediately after the citations.

Since new species and genera are constantly being described, most systems of classification soon become unstable due to the variability in structure, and also because their proposers may have failed to consider the likely phylogenetic relationships, may have interpreted homologies wrongly or may not have had a wide enough spectrum of data. Such systems invariably lack predictability, so that the characters used to specify groups will not remain congruent and parsimonious when new characters and taxa are discovered. Nevertheless, the various systems of classification proposed thus far for the Tylenchida have created new knowledge and interest which are so necessary for the progress of systematics.

As for other animals, the phylogenetic histories of Tylenchida can never be correctly stated because there are no fossils and also because the evolutionary pathways of animals can only be inferred. Yet, to achieve a sound and stable classification, modern methodologies of systematics, i.e. evolutionary, cladistic and phenetic, have to be properly used. Homologies (both structural and behavioural) as correspondences in characters and character states should be determined and interpreted to show the possible origin and evolutionary line of the taxa concerned. In addition, the chemical, cytogenetic, embryological and numerical taxonomic data have to be analysed before we can truly understand the diversity of Tylenchida and have a meaningful and stable classification.

It is now more than 50 years since Thorne (1949) proposed the order Tylenchida and gave a system of classification for it. Since then, hundreds of new species and scores of new genera have been discovered and a great deal of information on nematode morphology and characterization has been accumulated, necessitating changes in this system. In fact, several new systems have since been proposed. Important ones are those by Allen & Sher (1967), Golden (1971), Siddiqi (1972, 1986), Andr assy (1976), Fotedar & Handoo (1978), Maggenti (1981), Ryss & Krall (1981), Luc *et al.* (1987) and Chizhov & Berezina (1988).

Although these systems have greatly augmented our knowledge of Tylenchida, a sound, stable and close to natural classification and one which is acceptable to most nematologists is still difficult to achieve. This is largely because disciplined clad-evolutionary methods have not been applied. Nevertheless, systems of classification have been changing for the better, and in many groups we have arrived at a point where many taxonomists are in some agreement.

Taxonomy at the grassroots is still somewhat unsatisfactory: the tug-of-war between the splitters and lumpers continues. I have tried to strike a balance between the conservatism of lumpers, who hate the so-called inflation in the num-

bers of higher categories, and the enthusiasm and liberalism of the splitters, who do not hesitate to propose families and genera based on characters which may be of generic and specific value only. My aim in writing this book is, therefore, twofold: firstly, to give diagnoses and differential keys for all the valid genera and higher categories of Tylenchida, including a list of all the nominal species under their respective genera; and secondly, to provide a system for their classification on as sound a basis as possible which is practicable, stable and has most predictive value. I believe that the system presented here is the best possible expression of the similarities of various taxa both phenetically and phylogenetically.

2. ABBREVIATIONS, SYMBOLS AND GLOSSARY

Abbreviations and Foreign Words and Phrases Used in the Taxonomy of the Tylenchida

ap., apud	in the work of
Art.	Article (of the International Code of Zoological Nomenclature)
auct.	of author(s)
cf.	<i>confer</i> , compare
DO	dorsal oesophageal gland orifice
DN	dorsal oesophageal gland nucleus
DNA	deoxyribonucleic acid
mtDNA	mitochondrial DNA
ELISA	enzyme-linked immunosorbent assay
emend.	emended, emendation
<i>et al.</i>	<i>et alia</i> or <i>et alii</i> , and others
fam.	family
gen.nov., gen.n., n.g.	<i>genus novum</i> , new genus
gub.	gubernaculum
ibid.	<i>ibidem</i> (in the same place as above cited reference)
ICZN	International Code of Zoological Nomenclature, not used here for the International Commission on Zoological Nomenclature (I.Com.Z.N.)
incertae sedis	wrongly inserted, of uncertain position
<i>in litt.</i>	in correspondence
L	body length
L.	Latin or Linnaeus
lapsus calami	typists' or printers' error, an error in spelling in nomenclature
loc. cit.	<i>locus citatus</i> , local citation, as cited above
MAbs	monoclonal antibodies
no., n	number

nomen conservandum	conserved name. A name conserved by the use of the plenary powers of the Commission for Zoological Nomenclature, which otherwise would have been invalid using ICZN
nom. dub.	<i>nomen dubium</i> , dubious name which cannot be applied with certainty to any taxa, e.g. when the description is inadequate and the type specimens are unavailable
nom. nov.	<i>nomen novum</i> , new name, equivalent to new replacement name required for example by homonymy
nomen nudum (pl. nomina nuda)	naked name(s), invalid name(s) which is not an available name, but can be made available for the same or a different concept with the authorship and date from that act of establishment
nomen oblitum	forgotten name, which remained unused as senior synonym in primary zoological literature for more than 50 years, as per ICZN Article 23(b) of the I and II editions, but later omitted from the III edition
p. (pl. pp.)	page(s)
PAGE	polyacrylamide gel electrophoresis
part, partim	part, in part
PCR	polymerase chain reaction
pers. comm.	in personal communication
RAPD	random amplification of polymorphic DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	ribosomal RNA
SD	standard deviation
SE	standard error
SEM	scanning electron microscope/microscopy
<i>sensu</i>	according to
sic	thus (an exact transcription)
s.l.	<i>sensu lato</i> , in a broad sense (cf. <i>sensu stricto</i>)
sp. (pl. spp.)	species
spic.	spicule
sp. inq.	<i>species inquirenda</i> , demanding further inquiry or study as the characters are insufficient for recognition
sp. n., sp. nov., n. sp.	<i>species nova</i> , new species
s.s., s.str.	<i>sensu stricto</i> , in the strict sense (cf. <i>sensu lato</i>)
st.	stylet
subfam.	subfamily

subsp., ssp.	subspecies
SVN	subventral oesophageal gland nucleus
SVO	subventral oesophageal gland orifice
taxa (sing. taxon)	taxonomic categories from subspecies to kingdom
TEM	transmission electron microscope/microscopy
<i>vide</i>	see

Symbols

For symbols used in body measurements see pages 37 and 38.

Explanation of Terms used in Taxonomic Works

α -, β -, and γ -**taxonomy** = in short, descriptive, synthetic and evolutionary taxonomy, respectively. Alpha taxonomy is the process of naming and describing species, and providing diagnosis, emendation, etc. of taxa, etc. Beta taxonomy includes revising and arranging taxa in higher categories that reflect evolutionary history. Gamma taxonomy deals with tracing intraspecific variation and establishing the evolutionary systematic lines, critical analysis of classification and publication of monographs and books on taxonomy.

Agamospecies, morphospecies = species that reproduce asexually, e.g. by parthenogenesis and are distinguished on morphological and not on gene-flow basis.

Allopatry and sympatry = taxa or populations that occupy geographically separate areas, and those that occupy the same geographical areas, respectively.

Allozyme = any variant of an enzyme coded by different alleles at the same gene locus (refers to motility variants identified by electrophoresis).

Binomen, trinomen or scientific names = binomen is the name of a species with two words, first of a genus name beginning with a capital letter and second of species name which is not capitalized, and trinomen is the subspecies name with three words, the last one of which is subspecific and is not capitalized. Specific epithet includes both species and subspecies names. Citation of subgenus name within brackets is not a part of either a binomen or a trinomen, but it does imply that the species name has been combined with the subgeneric name. Binomen and trinomen are called scientific names.

Biodiversity = diversity in number and kind of taxa and/or their combined genetic variations.

Biological species = a species in which gene flow occurs readily and frequently between its members, and not between its members and those of other species, i.e. such species are reproductively isolated from other species. In other words, a biological species is a group of interbreeding natural populations which have a similar genotype but which are reproductively isolated from other such groups.

Biotypes, pathotypes and host races = biological or physiological races of a species which can be differentiated on host reactions; they have no taxonomic status since ICZN does not recognize any taxa below the subspecies level.

Character and character state = any structure or behavioural system that is used to characterize a taxon, and any condition that a character can display, respectively.

- Clade and polyclade** = any supposedly monophyletic group in a phylogenetic analysis. Polyclade is used as a multiple-entry identification key in a computer or in punched cards.
- Cladogram, dendrogram and phylogram** = a cladogram is a dendrogram or tree diagram based on synapomorphies depicting a phylogenetic hypothesis. It only depicts the branching pattern of the evolutionary history and, unlike a phylogram, it does not indicate, by means of branch length, the degree of evolutionary change that occurred along each lineage.
- Clone, cloning** = a group of genetically identical organisms, resulting from non-sexual cell division. A number of copies of a fragment of DNA produced by cloning or amplifying the number of copies of DNA.
- Cluster analysis, OTUs and HTUs** = procedure that links operational taxonomic units (OTUs) into clusters on the basis of their attributes or overall similarities, as used in phenetics. HTUs are the hypothetical ancestral characters or character states used in a cladogram.
- Commonality principle, plesiomorphy and apomorphy** = the principle that plesiomorphies (primitive or ancestral characters or character states) will be commoner on average than apomorphies (derived character or character states).
- Compatibility analysis** = procedure for eliminating characters from a phylogenetic analysis on their not being consistent with other characters.
- Congeneric** = two or more species belonging to the same genus.
- Congruence** = degree of similarity between two phylogenetic systems or phylogenetic trees derived from different data sets.
- Cotype** = a paratype or syntype, not in usage now as it is not recognized by the ICZN.
- Dichotomy** = in a differential key where two new branches arise from one stem.
- Diploid, haploid** = during meiosis, the chromosome number of a diploid cell is halved, $2n$ becomes n .
- Distance matrix** = a taxon-by-taxon matrix in which distance or dissimilarities are measured.
- DNA fingerprinting** = technique based on restriction fragment analysis of DNA that reveals polymorphisms at dispersed loci of tandemly repeated DNA, used for evaluating relatedness between closely related individuals.
- DNA hybridization** = process by which single-stranded DNA will pair with other homologous strands.
- Electrophoresis and electromorphs** = a process of separating molecules in a supporting medium on their electric charge in combination with other factors. Electromorphs are variants of a protein characterized by their motility properties during electrophoresis.
- Entomoparasites and phytoparasites** = parasites of insects (and, for practical purposes, mites) and of plants (and fungi), respectively.
- Family-group names** = any recognized taxonomic category name between genus and infraorder (tribe, subfamily, family and superfamily names). ICZN recognizes categories between superfamily and species only and does not cover taxa above superfamily rank.
- Genotype and type species** = the type species of a genus, genotype, is now obsolete since it causes confusion with genetic make-up.

- Genus-group names** = names of genus and subgenus categories; changing rank of a taxon within a genus group does not affect the authorship or year of publication.
- Gradualism vs. punctuated equilibrium** = a Darwinian evolution concept in which species are believed to have evolved through gradual change over time, as against punctuated equilibrium which postulates that new species appear by sudden jumps or saltations due to mutations during periods of rapid evolution separated by periods of relative constancy, and which gradually dominate or replace the mother species.
- Hennigian method** = using cladistic approach for defining taxa (clades) by synapomorphies as proposed by Hennig (1950, 1957, 1966).
- Heuristic** = method of progressive improvement of estimates by trial-and-error search rather than by following a set method. Also a method of algorithms for finding shortest trees, which are not guaranteed to represent the most parsimonious ones.
- Holotype, allotype and paratype** = a holotype is a member of a type series (the best example on which the concept of a species or type species is based, and it should be well preserved and preferably illustrated in the description); the remainder of the type series are paratypes; an allotype represents the opposite sex to that of the holotype, not in use since a type series includes only the holotype and paratypes.
- Homology and analogy** = similarities in characters or character states in two or more taxa that are the result of their inheritance from a common ancestor; analogy refers to the similarities which are not due to their inheritance from a common ancestor.
- Homonymy, primary and secondary homonyms** = either of two or more identical scientific names. When two identical names occur in a genus, the junior homonym has to be given a new name, called the nomen novum. Primary or secondary homonyms are the identical names that were proposed for different taxa in the same genus (primary homonym) or brought to the same genus (secondary homonym); secondary homonymy breaks by the transfer of a homonym to another genus.
- Homoplasy** = in phylogenetic analysis, when an additional number of character states resulting from reversals and parallel or convergent evolution are also considered, besides the minimum number of changes that could theoretically have taken place.
- Ingroup and outgroup** = in phylogenetic analysis, comparison with members of the same monophyletic group or with members of other such groups, respectively.
- Isoelectric focusing, isoelectric point** = electrophoretic separation of protein variants based on their migration within a pH gradient to their isoelectric points, where they accumulate (focus point).
- Isozyme, allozyme** = usually in electrophoresis, a form of an enzyme coded for by a gene at a different locus from that of another form of the same enzyme. Isozymes may arise, e.g. through gene duplication. An allozyme represents one or more variants of an enzyme coded by different alleles at the same gene locus.
- Karyotype** = chromosome complement of a cell or organism.
- Metaspecies, metataxon** = a species or taxon, respectively, which is only defined by symplesiomorphy and is not known to be paraphyletic.

- Metatype** = a specimen which has been compared with the holotype and believed to belong to the same species.
- Monoclonal antibody** = a pure antibody which is specific to a single antigen determinant and is produced from a specific clonal line of hybridoma cells.
- Monophyletic, holophyletic** = terms used for a taxon or group of taxa whose members are supposed to have descended from a common ancestor.
- Monothetic, polythetic** = to differentiate on one or many sets of characters, respectively, e.g. in a key.
- Monotypy** = monotypic taxon must be designated as type, e.g. when a species is described on a single specimen, it becomes the holotype.
- Name-bearing type** = in 1985 ICZN introduced this term to indicate either a type genus, a type species or any accepted type specimen which provides an objective standard for the application of a scientific name.
- Numerical taxonomy** = a numerical approach to taxonomy, generally applied to refer to phenetic methods involving clustering and classifying purely on similarity rather than phylogenetic grounds.
- Objective synonym** = when two or more named genus or species names are based on the same species or type specimens, respectively.
- Original designation** = designation or fixation of the type species of a genus in an unambiguous way in the original description.
- Paraphyletic, paraphyly** = a taxon or group of taxa supposed to have a common ancestor and defined by a unique apomorph (derived character state), but not all of whose members are included because some may have undergone one or more reversals of that apomorph.
- Parasites, pathogens and predators** = those (nematodes) that feed, develop and reproduce on their hosts and live at their expense; those that produce disease; and those that prey naturally on others and devour their body contents, respectively.
- Parsimony** = in cladism, the most likely phylogenetic explanation requiring the least number of evolutionary steps (i.e. the most parsimonious explanation).
- Patronym** = a scientific name based on a person, in his honour or in recognition of his work.
- Phenetics, phenon** = now used mainly to describe numerical taxonomy using clustering of taxa (phenons) based on similarity.
- Phylogeny** = the evolutionary history of a group of taxa.
- Polarity** = evolutionary direction of a character state transition, determining a plesiomorphic (primitive) or apomorphic (advanced, derived) state.
- Polymorphism** = occurrence of two or more phenotypic states of a character among the members of a taxon.
- Polyploidy, aneuploidy** = increasing of chromosome number above the normal diploid number ($2n$) by an integral number of n , but this might have been modified subsequently to have a different (lower) number by aneuploidy.
- Restriction fragment length polymorphism (RFLP) and restriction enzyme** = the occurrence in a taxon of more than one morph defined by the presence or absence of a particular restriction enzyme recognition (cleaving) site. A restriction enzyme is one that cleaves double-stranded DNA.
- Sibling species** = very closely related species which differ only in minute or cryptic

morphological characters but which have different biological characteristics and are reproductively isolated.

Species-group names = names of species and subspecies; changing the rank of a taxon within a species group does not affect the authorship or year of publication.

Subjective and objective synonyms = synonyms are two or more generic or species names that can be applied to a single taxon. Subjective synonyms are those that result from the opinion of a person, seeing that they are in fact the same taxon described under different names. Objective synonyms result from two or more taxa having the same type (species or specimen) and over which there can be no difference of opinion.

Symplesiomorphy and synapomorphy = sharing of plesiomorphs and apomorphs, respectively.

Syntype lectotype, topotype and neotype = in a type series, if the holotype is lost, all the paratypes become syntypes, from which any one syntype can be chosen and named the lectotype, after which action all the syntypes become paralectotypes (or lectoparatypes). If the type series is lost, specimens of the species collected from its type host and locality are called topotypes, from which a neotype is selected and designated.

Tautonymy = identical spelling of two generic or species names.

Taxon, plural taxa = a group of organisms or a formal taxonomic unit at any level of hierarchic classification from subspecies to kingdom categories.

Taxonomy and systematics = these terms are largely synonymous. Taxonomy deals with identifying, naming and classifying organisms, whereas systematics has a broader definition and includes aspects of the phylogeny, evolution, biogeography, genetics and physiology of the organisms.

Transformation series = in phylogenetic analysis, a hypothesized set of likely transitions between multiple character states.

Type series = all the specimens of a species used at the time of its description.

Voucher specimens = specimens used in a study, which are not from a type series, and which are deposited in a permanent collection for later use or reference.

Weighting = assigning different importance or value to different character systems in phylogenetic analyses or identification systems (e.g. in keys).

3. HISTORICAL REVIEW

Historical perspectives of a science give an insight into man's eternal quest for knowledge and reveal how defective observations, interpretations and judgements are constantly being replaced by better ones. A scientific theory (a hypothesis when tested becomes a theory, which carries more weight) by its very definition is a falsifiable theory since it depends on many variables. The least falsifiable theory is the best theory, but a poor theory is always better than none, since it generates research. Divine revelations and scriptures as eternal truths (only their human interpretations are falsifiable), metaphysical concepts and philosophical idealism have greatly helped scientific pursuits over the ages, simply by providing the basic stimulus for using the most valuable of human assets – the imagination.

The science of nematology began in the 17th century, when the compound

microscope was discovered and the 'first nematologist', Petrus Borellus, looked with utter amazement on the 'little serpents' (= *Anguillula*) in table vinegar. The name *Anguillula* was followed by *Anguillulina* and the two names appeared in the literature for quite a long time but then suddenly disappeared from the scene with few nematologists knowing what actually happened to them. Today the 'little serpents' are made to look as large as a whale with the recent invention of the electron microscope.

The earliest known Tylenchida were highly adapted parasites, which were identified either by the disease symptoms they produced (*Anguina* in wheat-gall and *Meloidogyne* in root-gall) or by their miraculous parasitic adaptations (the everted uterus of *Sphaerularia* and the cyst of *Heterodera*).

The first member of the Tylenchida seen and reported was the wheat-gall nematode, *Anguina tritici*, by John Turbeville Needham (1743, published 1744) who wrote to the President of The Royal Society, London, stating that small black grains of smutty wheat had soft fibrous substances which, upon soaking in water, took life and yielded a large number of motile worms. Linnaeus (1767, *Systema Naturae*, p. 1326) under the genus *Chaos* wrote 'TRITICI Grana abbreviata illa & rotundata, exsiccata etiam post annos, in aqua tepidiuscula intra horulam egerminant in ascaridiformem quasi vermicucum; animatum vix dixerò'.

Scopoli (1777) proposed the genus *Anguina* (original spelling *Angvina*; note that in Latin inscriptions u is often given as v) for the wheat-gall nematode, and 55 years after its discovery by Needham, the species was named *Vibrio tritici* by Steinbuch (1799) who also described *Vibrio agrostis* parasitizing seeds of bentgrass. In 1850 Hardy had recorded a species causing galls on grass leaves and called it *Vibrio graminis*. These *Vibrio* species were finally transferred to *Anguina*. A closely related species, *Anguillula dipsaci* (now *Ditylenchus dipsaci*) was reported from teasel, *Dipsacus fullonum* L. by Kühn (1857).

That Shakespeare's words 'sow'd cockle reap'd no corn' in *Love's Labour's Lost* (Act 4, Scene 3, line 379) refer to the wheat-gall nematode, as suggested by Thorne (1961), has been disputed by Southey (1972) who argued that Shakespeare used 'cockle' to mean a weed of the corn field (e.g. the corn cockle, *Agrostemma githago* L.), and that 'cockle' or 'ear cockle' as a description of *A. tritici* galls had not been in use before the middle of the 19th century.

The first insect-parasitic member of the Tylenchida was reported by Reaumur in 1742 which was probably *Sphaerularia bombi* (vide Nickle & Welch, 1984). The first Criconematina was described by de Man (1880) as a male *Macroposthonia annulata*; the generic name is feminine in gender but means 'large penis'. The first female Criconematina was named as *Dorylaimus giardi* (now *Criconema giardi*) by Certes (1889) who, in the same paper, described *Eubostriachus geurmei* (= *Criconema geurmei*, the type species of the genus) as having a sting (= stylet).

During the middle of the 19th century, the root-parasitic Tylenchida were beginning to receive attention. In England, Berkeley (1855) observed 'Vibrios' (root-knot nematodes) in galls of a glasshouse cucumber. Similar nematodes were found in root-galls in *Dodartia orientalis* by Müller (1884) who called them *Heterodera radicola* by transferring the species described by Greeff (1872) as *Anguillulina radicola* to the genus *Heterodera*. Cornu (1879) in France described a new nematode, *Anguillulina marioni*, from root-knots on sainfoin (*Onobrychis sativus* Lam.). Goodey (1932) transferred this species to the genus *Heterodera* as *H. marioni*. The name *H.*

marioni was in use for a long time, and appears as such for the root-knot nematodes in Franklin's (1951) review of the genus *Heterodera*. Goeldi (1887, published 1892) had called the root-knot nematodes found on coffee in Brazil as *Meloidogyne exigua*. Chitwood (1949) clarified the status of the root-knot nematodes by reinstating *Meloidogyne* as the generic name for them. The genus *Caconema* Cobb, 1924 became a synonym of *Meloidogyne* but here it has been treated as an invalid senior objective synonym of *Subanguina* (see discussions under *Meloidogyne* and *Subanguina*).

In 1859, Schacht reported a serious disease of sugarbeet (Rübenmüdigkeit) in Germany. This was found to be caused by a nematode, *Heterodera schachtii*, named by Schmidt (1871). The genus *Heterodera* Schmidt, 1871 was thought to be preoccupied by the name *Heteroderes* Latreille, 1834 (Coleoptera) by Railliet (1896) who proposed a replacement name *Heterobolbus*, but this was rejected by Baylis & Daubney (1926) and cannot justifiably be used due to lack of homonymy.

After the discovery of wheat-gall nematode by Needham in 1743, Tylenchid nematodes received almost no attention from scientists for the next 150 years and, as put by Maggenti (1988), 'Rudolphi in 1809 discarded them from his classification because they were not intestinal parasites. They were moved and pushed for the next hundred years without thought or reason. For most of their history they were buried within the bacterial feeding rhabditids; a cemetery only recently revisited by Siddiqi (1986).'

By the turn of the 19th century, the taxonomy of Tylenchida had progressed far enough to stimulate scientists to attempt the construction of a hierarchic system of classification of known forms. By 1913, there were seven genera and a large number of known species of plant-parasitic nematodes. The early classification works were ill-coordinated and usually based on a single character for differentiation (the monothetic concept) which produced unstable classification. For example, the free-living nematodes were classified by Diesing (1861) under two families – Cirrhostomae and Anguillulidae – on the presence or absence of cirri or setae in the head region only. Eberth (1863) found this system unsatisfactory and proposed that Anguillulidae be recognized as lacking caudal glands, and that those possessing them be assigned to Urolabes. Rauther (1930) also used a single character, the presence of a rachis in the ovary, to characterize Rachidophora which proved to be a group of unrelated nematodes.

Early workers on Tylenchida studied either free-living and plant-parasitic nematodes, or insect parasites. The former produced excellent monographs, e.g. those by Bastian (1865), Bütschli (1873), Örley (1880), de Man (1880, 1884) and Cobb (1893), while the latter gave us such important genera as *Sphaerularia* Dufour, 1837; *Allantonema* Leuckart, 1884 and *Bradynema* zur Strassen, 1892. Fuchs (1914, 1915) initiated work on the taxonomy of the tylenchid parasites of bark beetles and published excellent papers on this group in 1929 and 1938. The dedication and hard work of Bastian, Bütschli, Örley, de Man, Filipjev, Micoletzky and Cobb laid strong foundations for the science of nematology through their major publications containing the descriptions, fine illustrations and well-attempted classifications of tylenchid and other nematodes.

Bastian (1865) produced an excellent monograph on Anguillulidae, describing 100 new species. Thorne (1961, p. 5) wrote that this publication marked the beginning of the science of nematology. Bastian (1865) realized that the collection and

naming of nematodes up to that time were insufficient for a philosophical classification to be attempted. Instead he gave tables to assist in their characterization and identification. He placed *Tylenchus* (= *Tylenchus*), *Cephalobus* and *Rhabditis* together because they had a striated cuticle, a ventral excretory gland and lacked a caudal sucker, but *Aphelenchus* was grouped with *Diplogaster*, *Plectus* and *Tripyla* because it had a sucker!

Otto Bütschli (1873, 1876) produced comprehensive illustrated descriptions of the free-living soil nematodes including several Tylenchida. In the words of Thorne (1961), 'Perhaps the credit for founding the science of nematology should belong to him, rather than to Bastian.' Örley (1880) published a system of classification for 202 nematode species in 27 genera and proposed the family Tylenchidae.

J.G. de Man produced excellent monographs on soil, plant and freshwater nematodes, which appeared in 1876, 1880, 1884 and 1921. His type specimens are deposited in the Zoological Museum of the University of Amsterdam, and these were restudied by Loof (1961). It is over 100 years since de Man (1884) first used the formula of α , β , γ (now a, b, c) for measuring nematodes. The de Manian formula is universally used in the taxonomy of the Tylenchida today. de Man (1921) gave us the new tylenchid genera *Ecphyadophora*, *Hemicycliophora* and *Psilenchus*, and new species which later became types of new genera, namely, *Hoplolaimus annulifer* for *Nothocriconema* and *Tylenchus costatus* for *Coslenchus*.

I.N. Filipjev (1934, 1934a) based his system of classification on amphid shape, although he considered other characters as supplementary, i.e. he used a polythetic concept in classification. He underlined the importance of embryology and physiology in the relationships of nematodes with other groups – Acanthocephala, Echinodera, Gastrotricha, Gordiacea and Rotatoria. In 1934 he presented a comprehensive classification of the Class Nematoda, recognizing 11 orders: Chromadorata, Desmoscolecata, Enoplata and Monhysterata for free-living forms; Anguillulata for partly free-living, partly parasitic forms; Ascaridata, Dioctophymata, Filariata, Oxyurata, Spirurata and Trichurata for the animal-parasitic forms. Under Anguillulata, he recognized three families – Anguillulidae, Tylenchidae and Strongylidae – and under Tylenchidae he classified Tylenchinae, Hoplolaiminae, Sphaerulariinae, Diplogasterinae and Tylopharynginae by regarding the oesophagus with a median bulb as the unifying character of the group.

Filipjev (1934) argued that, in the plant-parasitic members of this group, the median bulb was the only muscular part of the oesophagus. It was reduced in males of certain species because they did not feed, and more so in Sphaerulariinae, in which the oesophagus and intestine did not function, as feeding was through the skin. He remarked that the Tylenchinae were characterized by a triple spear very like that of *Tylencholaimus* (Dorylaimida) but mostly with a strongly marked triple enlarged base. Hoplolaiminae with the criconematids were recognized as a highly specialized terrestrial group having cuticle with peculiar rings, sometimes subdivided to give a scale-like appearance and a peculiar, huge spear with basal knobs.

Filipjev (1934a) produced an important book on systematics in Russian, *Harmful and Useful Nematodes in Rural Economy*. In this work he assigned Tylenchidae, Anguillulidae and Strongylidae to the order Anguillulata. Within the Tylenchidae he recognized Tylenchinae, Hoplolaiminae, Sphaerulariinae, Diplogasterinae and

Tylopharynginae. Under Hoplolaiminae he considered the genera *Hoplolaimus*, *Atylenchus*, *Eutylenchus* and the criconematid genera *Criconema*, *Iota*, *Paratylenchus* and *Procriconema*. The criconematid genera *Hemicycliophora* and *Macroposthonia* were assigned to the Tylenchinae because they were based only on males lacking a stylet. *Dolichodorus* and *Nemonchus*, although having a large stylet, were also assigned to Tylenchinae, apparently because they had finer body annules than most Hoplolaiminae (*sensu* Filipjev). He proposed a new subgenus *Bitylenchus* under the genus *Tylenchus* and designated *Tylenchus dubius* Bütschli, 1873 as its type. He recognized five subgenera under *Tylenchus*, namely, *Anguillulina* (type indicated as *Vibrio tritici* Bauer, 1823 (= *Tylenchus tritici* auct.)), *Bitylenchus*, *Chitinotylenchus*, *Tylenchorhynchus* and *Tylenchus*, and gave a differentiating key. Filipjev's (1934a) book was later incorporated and enlarged into *A Manual of Agricultural Helminthology* by Filipjev & Schuurmans Stekhoven published in 1941. Filipjev did not see the publication or even its final version since his contact with Schuurmans Stekhoven was lost in 1937 and, according to Dr E.S. Kirjanova, Filipjev died on 22 October, 1940 (*vide* Mjuge, 1977).

In 1922 Heinrich Micoletzky of Austria produced a voluminous monograph *Die freilebenden Erd-Nematoden* listing 142 valid genera and 931 species of free-living (soil, freshwater and marine) and plant- and insect-parasitic nematodes under five families: Alaimidae, Odontopharyngidae, Rhabditidae, Trilobidae and Tylenchidae. To the family Tylenchidae, Micoletzky assigned Diphtherophorinae, Dorylaiminae and Tylenchinae, members of which are now placed under three different orders, Triplonchida, Dorylaimida and Tylenchida. In the subfamily Tylenchinae, he placed the following genera: *Tylenchus* (with *Chitinotylenchus* n.subgen.), *Allantonema*, *Aphelenchus* (with *Paraphelenchus* n.subgen.), *Dolichodorus*, *Eutylenchus*, *Heterodera*, *Hoplolaimus* (syn. *Criconema*, *Iota*), *Nemonchus*, *Parasitylenchus* n.g., *Paratylenchus* n.g., *Triplonchium*, *Tylenchorhynchus* and *Tylenchulus*. Although his familial groups were heterogeneous and his classification did not go beyond the family level, he did publish genealogical tables and family trees. Micoletzky was a 'lumper' for he believed that species varied greatly. He grouped diversified species under one genus and synonymized several otherwise valid species. In the words of Thorne (1961), 'He lumped together all species which were in any way similar to each other and thus produced an impossible conglomeration of trinomial nomenclature which other workers refused to accept.' But Micoletzky was a dedicated scientist with keen powers of observation and his monograph is a landmark in the history of nematology. He prepared excellent slides of nematodes, most of which are in excellent condition today in the Zoological Museum of the Humboldt University in Berlin, where the writer had the opportunity of studying them in 1975. The science of nematology is indebted to Micoletzky for making those long-lasting permanent slides by using dehydrated glycerine, and to an unknown person for their safety during World War II, who 'dumped' the slides, properly wrapped up in a bag, at the Zoological Museum of Berlin. The bag, lying in a corner, was discovered by Dr G. Hartwich (personal communication), who has looked after them ever since.

Baylis and Daubney (1926), in their synopsis of the families and genera of Nematoda, criticized Micoletzky's division of Tylenchidae into Diphtherophorinae, Dorylaiminae and Tylenchinae because the subfamilies were chiefly based on the stylet structure. These authors remarked that the system 'does not, however, appear

to us to offer a natural classification of the group, and it seems to us that a somewhat more satisfactory subdivision is arrived at by considering primarily the characters of the oesophagus'. Consequently, they proposed that Anguillulidae (their equivalent of Tylenchidae) should contain only Anguillulinae and Dorylaiminae! They placed Anguillulinidae under the order Ascaroidea (suffix -oidea was being used for ordinal rank at that time) and listed the following genera under Anguillulinidae: *Anguillulina*, *Aphelenchus*, *Heterodera*, *Hoplolaimus*, *Isonchus*, *Nemonchus*, *Psilenchus*, *Tylopharynx* and *Tylenchulus*; *Aphelenchoides* was listed under genera incerta sedis and *Myenchus* and *Myoryctes* were included in an appendix to the Anguillulinidae with a note that the genera were probably considerably modified due to their parasitic habits, but the presence of a buccal stylet showed their affinity to the members of the Anguillulinae. Baylis & Daubney took a drastic step in proposing the following genera as synonyms of *Anguillulina*: *Anguina*, *Aphelenchulus*, *Atylenchus*, *Chitinotylenchus*, *Dolichodorus*, *Eutylenchus*, *Iotonchium*, *Parasitylenchus*, *Paratylenchus*, *Tylenchorhynchus*, *Tylelenchus* and *Tylenchus*. T. Goodey (1932) proposed a large number of new combinations with *Anguillulina* because he thought *Tylenchus* was a synonym of *Anguillulina*.

The inclusion of free-living and animal-parasitic nematodes in a combined classification was done by Wülker (1924), who believed (see Wülker, 1929) that Ascaroidea were direct descendants of free-living marine forms.

Cobb's (1893) monograph, *Nematodes, mostly Australian and Fijian* contained several tylenchid nematodes, including *Radopholus similis*, which he proposed on the basis of the male as *Tylenchus similis*. The female of this species was described by him in the same paper as another species, *Tylenchus granulatus*. In 1913, he published *New Nematode Genera Found Inhabiting Freshwater and Non-brackish Soils* and in 1920, *One Hundred New Nemas (Type Species of 100 New Genera)*. His collected papers, *Contributions to a Science of Nematology*, is a valuable book containing articles on marine, soil, fresh-water, plant and insect nematodes with excellent illustrations made by W.E. Chambers. Cobb's (1920) system of classification of higher categories of nematodes based on the monothetic concept using the shape and structure of the stoma was never recognized. On this character, two subphyla were proposed by him – Laimia and Alaimia. Laimia was divided into two classes – Onchia and Anonchia, the former having onchia, teeth and/or the stylet, and the latter lacking them. Onchia, had two subclasses – Heteronchia with orders Axonchia and Anaxonchia, and Homonchia with orders Aponchia, Mesonchia, Synonchia and Triplonchia. Each of these categories became an assemblage of unrelated forms and the entire system proved artificial.

In 1919, Cobb proposed the Phylum Nemata and in 1932 gave it a diagnosis. Chitwood (1957) commented on its validity thus: 'The fact that Potts (1932) and the writer [Chitwood] in 1950 did not recognize Nemata Cobb, 1919, but rather synonymized it with the Nematoda (as a phylum) is lamentable. We can only say that the writer was young, foolish, and ignorant and did not realize the far-reaching importance and soundness of Cobb's work.' Such words of humility can only be expressed by one who is a really great person, as Chitwood undoubtedly was. His joint contribution with his wife, M.B. Chitwood, *An Introduction to Nematology*, is a milestone in the history of nematology. His work was mainly on the morphology and taxonomy of human, animal and marine nematodes, but in Tylenchida his contribu-

tions on *Meloidogyne*, *Hemicriconemoides* and *Criconema* and his new suborder Tylenchina had made him a 'plant nematologist'. Chitwood & Chitwood (1937) had considered the superfamily Tylenchoidea as a member of the suborder Rhabditina, and recognized three families – Tylenchidae (with Tylenchinae, Criconematinae and Hoplolaiminae), Allantonematidae (with Allantonematinae and Sphaerulariinae) and Myenchidae – under it.

After Cobb's death in 1932, Gotthold Steiner (8 April, 1886–21 August, 1961) became the leading nematologist in the USA. Steiner's (1949) publication *Plant Nematodes the Grower Should Know* not only introduced nematodes to the growers but did much to promote applied nematology. Similarly, T. Goodey's (1933) book *Plant Parasitic Nematodes and the Diseases They Cause* aroused great interest in the study of plant nematodes. In 1951, T. Goodey wrote the text *Soil and Freshwater Nematodes*, which was rewritten and enlarged by his son, J. Basil Goodey, and was published in 1963.

In the second half of the 20th century, nematology grew up fast and became a fully-fledged discipline of science with its own techniques, fields of research, textbooks and academic programmes. Although large-sized publications with 100 new species, such as those of Bastian (1865) and Cobb (1920), are relics of the past, present Tylenchida taxonomists have produced quite large publications with descriptions of numerous new taxa (Andrássy, 1954, 1979; Allen, 1955; Thorne, 1955; Das, 1960; Siddiqi, 1961, 1979a, 1980b; Sher, 1966, 1968; Thorne and Malek, 1968; Whitehead, 1968; Khan *et al.*, 1976, etc.). Several books and monographs dealing with Tylenchida have been published, namely, by Wachek (1955), Rühm (1956), Meyl (1961), Thorne and Malek (1961, 1968), Paramonov (1962, 1968, 1970), Decker (1969), Kirjanova & Krall (1969), Heyns (1971), Andrássy (1976), Dropkin (1980), Maggenti (1981), Siddiqi (1986), Bongers (1988), Ebsary (1991) and Dasgupta (1998) and those edited by Zuckerman *et al.* (2 volumes, 1971), Southey (1978), Zuckerman & Rohde (1981), Nickle & Welch (1984) and Nickle (1991). Checklists by Baker (1962) and Tarjan & Hopper (1974), pictorial keys to genera by Mai & Lyon (1975) and Mai & Mullin (1996) and *CIH Descriptions of Plant-parasitic Nematodes* edited by Willmott, Gooch, Siddiqi and Franklin are useful aids for taxonomic and identification work. At present, classification and phylogenetic relationships are being given due consideration, while new species and genera continue to be described increasingly. However, we are only part of the way in achieving a stable classification of Tylenchida. The effort which has begun must be sustained so that a more orderly basis for nematology can be provided.

Andrássy (1999) states that the total number of genera of free-living nematodes at present is 1940 (1788 established as genera, 152 as subgenera). Of these, 650 are in Torquentia, 570 in Secernentia (includes Tylenchida) and 705 in Penetrantia (plus 15 which are of uncertain position). Among free-living Secernentia, rhabditids comprise 241 and tylenchids (including aphelenchs) 329 genera, representing, respectively, 12% and 17% of all the free-living genera of nematodes. Andrássy (1999) lists the eight authors who have proposed more than 60 genera each, given here in alphabetical order in Table 1.

Table 1. Leading proposers of genera of free-living nematodes.

Author	Torquentia	Secernentia	Penetrantia
Allg�en (1929–1959)	51	1	12 (+5 incerta)
Andr�ssy (from 1954)	10	47	67
Cobb (1891–1933)	127	21	60
Filipjev (1916–1946)	30	5	28
Jairajpuri (from 1964)	0	13	67
de Man (1876–1922)	32	8	21
Siddiqi (from 1959)	0	52	64
Thorne (1929–1974)	0	22	46

Source: Andr ssy (1999).

4. TECHNIQUES

For identification and taxonomic study, good, clean specimens showing most anatomical details are essential. The proper killing and fixing of nematodes are the most important steps in obtaining good results. Permanent mounts are essential for long-term preservation. Details on the extraction, killing, fixing and mounting procedures of specimens are given by several authors (Oostenbrink, 1960; Thorne, 1961; Hooper, 1969; Southey, 1970; Taylor, 1971; Ayoub, 1977).

Collection and Storage

Soil from the rhizosphere is collected after removing the top 3–5 cm of soil and litter layer. Soil and fine feeder roots are collected ideally in polythene bags which are then tied up and tagged with a label bearing details of the habitat, host, locality and other data such as soil type, associated vegetation and date of collection. To obtain interesting and new species, one should not forget to include rhizospheres of weeds, indigenous plants, grasslands, bushes and forest flora. Macrofungi, beetle frass, mosses and lichens can all harbour interesting Tylenchida fauna, especially that of Anguinoidea and Tylenchoidea, and should therefore also be examined. The samples should be processed as soon as possible, but if necessary they can be stored in polythene bags at about 4°C.

Extraction

A number of methods exist for the extraction of nematodes. The choice of method depends on various factors, e.g. nematode activity (wet-funnel methods and use of filters), specific weight (flotation, elutriation, centrifugation and decanting techniques) and ultimately convenience. Plant tissues, fungi, mosses and lichens can be macerated and immersed in water. A food blender is useful for speeding the process up. The water is sieved after 24 h and the nematodes are then concentrated and collected. Insects and mites should be dissected in Ringer's solution or 1% NaCl to avoid the rupturing of nematode bodies that may occur in pure water.

Baermann's (1917) funnel technique is a primitive way of isolating nematodes from soil and plant tissues and should not be used in its original form, as it offers a recovery of less than 20% of other methods (Oostenbrink, 1970). Baermann's method has been modified in many ways, e.g. by Esser (1957) and Whitehead &

Hemming (1965). The Whitehead & Hemming method involves the use of large trays upon which a wire or plastic mesh supports a piece of tissue paper (such as Kleenex). The soil or plant material is thinly spread over the tissue, and the water is added so that the material on the tissue is just submerged. The active nematodes make their way through the tissue and collect at the bottom within 24 h. The water suspension is then concentrated by Cobb's (1918) sifting and gravity method. This method is good for motile nematodes but unsatisfactory for less active (such as criconematids) or inactive nematodes which are unable to pass through the tissue paper. The procedure should be carried out in a cool environment to avoid bacterial growth and hence depletion of oxygen and the trays should not be over-saturated with water.

The 'bucket-sieving' method described below, although crude, is widely used as it enables the extraction of a large number of both active and inactive nematodes in a relatively short time. About 300 ml of soil (more if sandy) is put in a bucket containing water. The mixture is vigorously stirred to give a suspension which is allowed to settle for a couple of minutes. The floating debris is removed by a very coarse sieve. The remaining suspension is slowly poured over a fine mesh sieve with 45–53 μm aperture which is continuously tapped by hand to avoid blocking. The deposit on the sieve is washed with a very gentle jet of water into a beaker and this can be examined directly or further processed using a miniature version of the above Whitehead & Hemming tray method.

The centrifugal sugar-flotation technique of Caveness & Jensen (1955) is good for quick separation of nematodes (both motile and inactive) from the debris in a suspension. The sugar solution is prepared by dissolving 454 or 484 g of sucrose in 1 litre of water to give a solution of specific gravity 1.13 or 1.14. A 4–5 min spin at about 1750 rpm gives a satisfactory result. After spinning, the syrup containing nematodes is poured onto a fine mesh sieve and the nematodes are washed off into a dish for examination. To avoid the cost of sucrose, Rodriguez-Kábana & King (1975) advocate the use of molasses solution of sp.gr. 1–1.1 at 27°C which, due to its higher viscosity, gives a better recovery of nematodes than the sucrose solution.

An apparatus which uses a controlled water current passing through a sintered plate to separate nematodes from soil particles was described by Trudgill *et al.* (1973). Nematodes of all types and sizes can be extracted using this fluidizing column. Cyst nematodes can be isolated by this method. The Erlenmeyer flask method for isolating cyst nematodes from air-dried soils involves the differential sedimentation of nematodes and soil particles. The soil sample is placed in a conical flask and shaken with water. The cysts float and come to lie at the top within 15 min. The supernatant is poured onto a 100 μm aperture sieve and washed. The debris containing the cysts is then poured off onto a filter paper held in a wide funnel and the cysts are picked individually off the paper under a dissecting microscope. There is no universal method for the quantitative extraction of all nematodes, the final choice being a personal one.

After comparing results of two centrifugal flotation techniques (direct centrifugation and sieving-centrifugation) with Seinhorst elutriation (1956, 1962a), Dunn (1971) recommends direct centrifugation as the least laborious and time-consuming. This involves placing about 50 ml of soil directly into a 250 ml centrifuge bottle, shaking vigorously with 200 ml of water and centrifuging for about 4 min at 1280 g

(sufficient to allow soil particles larger than 0.5 μm to sediment). The supernatant is discarded and the pellet re-suspended in 200 ml of 1.1 M sucrose solution (500 g sucrose in 1 litre tap water). It is centrifuged again for 4 min at 1280 g and the nematodes in the supernatant solution are collected after passing them thrice through a 45–53 μm aperture sieve.

For the extraction of plant and soil nematodes, Persmark *et al.* (1992) compared Seinhorst elutriation, Cobb's decanting and sieving, the Whitehead tray method and the centrifugal-flotation method using silica, sucrose and MgSO_4 . Colloidal silica ('Ludox LS'), sucrose and MgSO_4 solutions with a specific gravity of 1.16 were used. For centrifugation, 600 g soil was dispersed in 3000 ml water and from it a 500 ml aliquot was taken. Plant-parasitic nematodes were recovered in largest numbers with the elutriator and the three centrifugal-flotation methods, and the largest *Pratylenchus* specimens were recovered by the Whitehead tray method. Centrifugal flotation with silica was superior for the extraction of trichodorids.

After extraction, nematodes can be examined directly under the microscope and counted using Winslow's or Fenwick's multichamber counting slides or counting trays. There are various types of handling needles for nematodes. A small piece of hair, fixed to the tip of a long needle on a glass or wooden rod is sufficient and useful. If nematodes are to be kept permanently, they must first be killed, fixed and properly mounted.

Killing, Fixing and Processing

For obtaining good specimens for taxonomic studies, the method of killing is most important and critical. Some nematologists kill their nematodes in water by the gradual application of heat, either placing the dish containing nematodes in a constant-temperature oven at 65 or 70°C or slowly heating the nematodes in water in a test tube or on a slide over a naked flame, avoiding over-heating. A better method is to kill nematodes by applying sudden heat, i.e. by pouring hot water (90–95°C) over them after removing almost all the water from the container. Seinhorst's (1966) method which gives excellent specimens, involves pouring hot (90–100°C) formaldehyde–acetic (FA) or formaldehyde–propionic (FP) acid solution (4:1, i.e. ten parts 40% formalin, one part acetic or propionic acid and 89 parts distilled water) over nematodes.

To avoid deterioration and distortion during mounting, the nematodes are fixed soon after killing. Nematodes are best fixed in FP 4:1 or FA 4:1. A 3–5% solution of formaldehyde is good for fixing and storing nematodes. Courtney *et al.* (1955) advocate TAF (triethanolamine 2 ml, (40%) formalin 7 ml and distilled water 91 ml), but nematodes should not be left in it for more than a year. Esser's (1973) 4-min killing and fixing device uses a timer-operated, calibrated hot-plate to heat nematodes in water in a watch glass to 35°C. Stained lactophenol is added immediately and the heat is continued for a further 2 min.

For entomoparasitic nematodes, killing is best done by adding hot (80°C) Ringer's solution to nematodes. Mendola blue and Nile blue A and New blue R are used for distinguishing dead from living nematodes (*vide* Shepherd, 1962; Ogiga & Estey, 1975). Nematode structures are stained by acid fuchsin, gold chloride, picric acid–iodine, etc. Secretions from amphids, phasmids and excretory pores were induced and stained when nematodes were incubated in 0.1% Coomassie brilliant

blue G-250, in 40% aqueous methanol containing 10% acetic acid on a slide sealed with nail polish or Zut (Premachandran *et al.*, 1988). Nematode structures can be stained by several biological dyes, viz. Coomassie brilliant blue R, Evans blue, naphthol blue black, azure blue, toluidine blue, methyl orange, carminic acid, iodine green, haematoxylin and rosaniline hydrochloride.

Lactophenol (Amann, 1896: lactic acid 20 ml; phenol 20 ml; glycerine 40 ml and distilled water 20 ml) is a good clearing agent and widely used to process nematodes to glycerine. Siddiqi (1964) described a quick method of processing dorylaimid nematodes to glycerine; the same can be used with some modification for tylenchids. Fixed nematodes are transferred to hot lactophenol and left in it until the liquid becomes thick and syrupy. They are then transferred to a warm mixture of 75% glycerine and 25% lactophenol and kept warm for 5 min. The nematodes are finally transferred to pure glycerine and mounted in the same medium. For tylenchid nematodes with a thick cuticle, it is important that the nematodes are properly stretched in hot lactophenol by increasing heat or time until they are fully stretched. It may be necessary to leave them in lactophenol or glycerine–lactophenol mixture for 1–2 h before transferring to pure glycerine. Glycerine should be dehydrated and contain traces of menthol or picric acid to prevent bacterial or fungal growth. For staining methods, lactophenol may be tinted with cotton blue, aniline blue or acid fuchsin (0.0025% solution; 0.01% solution for staining nematodes in roots).

Propionic acid/orcein has been used for staining nematode chromosomes. Grisi *et al.* (1995) prepared the stain by mixing 2 g of orcein and 100 ml of 45% propionic acid. The mixture was boiled for 1 h, cooled and filtered before use. Slides with excised gonads of *Globodera pallida* were stained for 3 h and then washed in 45% propionic acid, mounted and sealed with glyceel.

Processing to glycerine takes longer but specimens so prepared will keep for a very long time. Fixed nematodes are placed in 2 ml of a solution of 1.5% glycerine in distilled water, in a small watch glass. This is then put in a desiccator chamber at 25–30°C, for about 4 weeks or until the water has been fully absorbed. A drop of copper sulphate or thymol can be added to the solution before desiccation in order to prevent the growth of moulds (Thorne, 1961). In Seinhorst's (1962) method, fixed nematodes are placed in a small watch glass containing 0.5 ml of the following solution: 96% ethanol 20 ml, glycerine 1 ml, distilled water 79 ml. The watch glass is then placed on a tripod above a dish containing 96% ethanol (1/10 volume of vessel) in a closed glass vessel. It is left for about 12 h in an oven at 40°C, by which time the specimens should be in a mixture of glycerine with some ethanol. The dish is removed and filled with a solution of five parts glycerine and 95 parts of 96% ethanol and then placed in an oven at 40°C. The ethanol should evaporate within 4 h, after which the nematodes, now in pure glycerine, can be mounted. It is possible to process nematodes from fixative to a permanent mount in glycerine within 1 h using Baker's (1953) rapid method to glycerine. The method involves staining nematodes in lactophenol cotton blue and then processing through a series of solutions for about 10 min each while maintaining at about 55°C. The solutions contain glycerine in increasing concentrations (55% to 100%) and lactic acid, phenol, distilled water and 40% formalin in decreasing concentrations.

The nematodes are best mounted on thin glass slides using 19 mm diameter

cover slips. The nematode(s) are placed in the centre of the slide in a very small drop of mountant. Glass rods (three pieces from one glass fibre about as thick as the nematode) or beads are placed around the nematode(s). Paraffin wax of 60–65°C melting point is placed as three lumps around the drop, each lump about the size of the drop or a little larger, and the cover slip is placed on the wax lumps. The slide is then heated on a hot-plate or over a flame just enough to melt the wax, which spreads and fills the space between the slide and cover slip, holding the nematodes in mountant in the centre.

To prevent drying, it is necessary to seal glycerine + wax slides by glyceel, Zut or Corseal. Corseal can be prepared by dissolving 10 g of Thermocol, a waste packing material, in 15 ml of chloroform, to which 20 ml of butyl acetate, 2 ml of alcohol-soluble linseed oil and 1.5 g of any hard plastic waste is added (Sabir, 1997). Clear nail polish is recommended as a sealant when glyceel or Zut is not available. Esser (1974) uses Zut (glyceel) to provide support in place of glass rods, glass beads or wax. A small drop of mountant containing nematodes is ringed closely by Zut and then the cover slip is placed on top and, if necessary, the Zut can be allowed to dry out a bit to make it more viscous. Slides can be sealed finally by ringing the coverslip using Zut.

Esser (1988) described a simple method for the examination of the vulval cone of mature cysts of *Heterodera* spp. A block of 1.7% water agar measuring about 1.5 cm × 1.5 cm × 2 mm high is put on a slide. A small 1 mm deep cavity slightly less than the diameter of the cyst is made on the agar block with a fine needle. A cyst is gently pushed into the cavity with the anterior end down until the vulva region of the cyst is level with the agar surface. A small drop of water is added to a 15 mm cover slip which is inverted and dropped over the embedded cyst. The slide is ready for viewing the vulval cone structures.

Counting nematodes in a suspension was made easy by using a GOP-302 image analysis system, manufactured by Context Vision, Sweden. The automated computerized image analysis of digitized images is described by Been *et al.* (1996). For clearing adhering tissues, the perineal patterns of *Meloidogyne* are treated with 45% lactic acid.

Monoxenic Cultures

Monoxenic cultures of Tylenchida provide large populations for studies on taxonomy, biology and host–parasite relationships. Bingefors & Bingefors (1976) have cultured *Ditylenchus dipsaci* on callus tissue for longer than 14 years without change in the nematode's host specificity or aggressivity, and have distributed nematodes from a 'nematode bank' for use in breeding resistant plants.

Heterodera oryzae is reared on rice roots cultured axenically in test tubes (Reversat, 1975). *Pratylenchus* and *Heterodera* spp. are easily cultured axenically on excised roots. Seeds are surface-sterilized in 1–1.5% sodium hypochlorite solution for 10 min, germinated under aseptic conditions on 1.5% water-agar in Petri dishes and incubated at 20–25°C. Upon germination, radicles are aseptically incised and transferred to Petri dishes containing sterile tap water (STW) agar medium. The nematodes are surface-sterilized in 50 ppm streptomycin sulphate in aqueous solution, washed in distilled water, and added to the Petri dishes with root radicles which are incubated at optimum temperature for host development (Lauritis *et al.*, 1982).

Interference and Scanning Electron Microscopy

Interference microscopy utilizes polarizing beam splitters and interference of optical paths to give a sharper image, particularly of surface structures. Nomarski's interference unit is fitted to a compound microscope and is used in conjunction with bright-field microscopy. For the former more light is needed but, unlike bright-field microscopy, the image is soothing to the eyes.

Scanning electron microscopy (SEM) is used to study surface structures, excised stylets, spicules and other tissues. Nematodes with a thin cuticle require proper fixation and dehydration to avoid shrinkage. They are washed in phosphate buffer (pH 6.8–7.4), fixed in 3% glutaraldehyde and/or 1% osmium tetroxide, washed in distilled water and dehydrated in a gradual series from 10% to absolute ethanol. Raski *et al.* (1980) passed nematodes in absolute alcohol through a graded series from 30% of amyl acetate in alcohol to absolute amyl acetate. McClure & Stowell (1978) described a simple method of processing nematodes in nylon mesh chambers during fixation, dehydration and critical-point drying or impregnation with embedding resin. In absolute alcohol or amyl acetate the nematodes are critical-point dried by using liquid CO₂. The dried nematodes are mounted on stubs after the surface is first coated with a thin layer of glue (e.g. pieces of transparent sellotape dissolved in benzene or chloroform). The stubs are sputter-coated with gold (400–750 Å) and examined under an electron microscope. Högger & Esty (1977) described a method of cryofracturing to expose internal structures of nematodes for use under the scanning electron microscope.

Lippens & Grootaert (1974) described a routine method for mounting nematodes in ERL, an epoxy resin with low viscosity and high refractive index for light or electron microscopy. Nematodes are fixed for 2 h in 4% paraldehyde in Sorensen buffer at 4°C, rinsed in buffer and then post-fixed in 1% osmic acid in buffer for 1–2 h. The nematodes are dehydrated in a gradual series of acetone solutions. From acetone they are transferred to a 1:4 mixture of ERL and acetone, left for 20 h and then transferred and kept in 100% ERL for 2 h. After mounting in ERL, they are kept for at least 24 h at 60°C. De Grisse (1974) and Clark & Stone (1975) used Spurr's low-viscosity epoxy resin to avoid tissue collapse and surface tension damage.

Abrantes & Santos (1989) described a method of preparing perineal patterns of *Meloidogyne* for SEM study. Females were placed in a large drop of 45% lactic acid on a plastic slide or slab and the perineal patterns were cut and cleaned. Up to ten patterns were then transferred to a small drop of 45% lactic acid on a cover slip rimmed with a thick ring of glyceel and oriented so that the surface side was facing up. The cover slip with the chamber containing patterns was placed on a glass slide and fixed by applying a drop of glyceel to the edge. One drop of 2% formalin was then added every 2–3 min to the chamber to wash out the lactic acid. After 10 min the formalin solution was absorbed by a filter paper and the patterns were allowed to dry in a desiccator at room temperature. The cover slip was then detached from the glass slide and mounted on an SEM stub with a double-sided adhesive tape, coated with 200 Å of gold, and examined with SEM.

Successful cryopreservation by freezing and storing in liquid nitrogen at –150 to –200°C of free-living and plant-parasitic nematodes is now possible. Cryopreservation of plant-parasitic nematodes is useful for long-term storage. Freezing and storing of *Ditylenchus dipsaci* in liquid nitrogen is described by Sayre &

Hwang (1975). Second-stage juveniles of *Meloidogyne graminicola* were successfully preserved in liquid nitrogen using ethanediol (ethylene glycol) as a cryoprotectant. Juveniles were first incubated in 10% (v/v) ethanediol at 37°C for 15 min and then in cold 40% ethanediol for 30–45 min before freezing in liquid nitrogen to –196°C. The recovered juveniles after thawing infected rice roots and multiplied, producing large populations of females and eggs after 40 days (Bridge & Page, 1986).