TROPICAL PLANT AND SOIL NEMATODES: DIVERSITY AND INTERACTIONS

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Contents

Summary

Nematodes are amongst the most abundant and diverse invertebrate phyla of the animal kingdom. A high proportion of plant and soil nematode species occur in the tropics and broad, but different, approaches such as species number and trophic groups, are used to assess diversity and their interactions with plants, plant pathogens and other organisms. As inhabitants of the soil rhizosphere nematodes are involved in energy flux, carbon, mineralization and other nutrient cycles, and as plant parasites, either alone or in

synergism with other pathogens, are responsible for plant disease complexes and major crop losses. Nematodes have more generations per crop season at higher temperatures, thereby placing crops in the tropics under much greater pressure than those in temperate regions. This phenomenon is usually aggravated by simultaneous infection by more than one species of plant nematode. A general description is provided of the morphology, trophic groups, parasitic habits, and interactions of nematodes within the soil and rhizosphere environment. Diversity in the tropics, host response to the various plant parasitic nematodes, as well as relevance to some ecological processes where soil and plant nematodes participate, are discussed.

1. Introduction

From the evolutionary and biodiversity perspectives, the Nematoda is one of the most successful of the non-segmented invertebrate phyla. Nematodes occur in both aquatic and terrestrial ecosystems, some adopting a parasitic habit in vertebrates, invertebrates and plants. They include microscopic plant nematode species, some of them measuring less than 200 µm and parasites of vertebrates that can reach several meters in length (e.g. *Placentonema gigantissima*, a parasite of sperm whales). Nematodes occur on all continents, including Antarctica, and are abundant in marine and freshwater habitats. Almost every plant, invertebrate and vertebrate organism (including humans) can be parasitized during its life span by one or more parasitic species of nematode. Nematodes are common in arable and non-arable soils, sediments and estuarine muds, where they often occur in truly colossal numbers, It has been estimated that their total number ranged from 6-18,000 million individuals per hectare of arable land.

Many nematodes are microbivores, feeding on bacteria, yeasts and/or other fungal spores; others feed on the contents of fungal hyphae, root hairs or plant roots, or are predatory on other soil organisms or parasitic in arthropods, mollusks, annelids, etc. As a result they form an important component of the carbon and other soil nutrient cycles. In turn, they are parasitized by bacteria and fungi and preyed upon by mites, annelids, tardigrades and other nematodes. Although most species are vermiform and relatively mobile in the soil phase, many plant-parasitic species are endoparasitic or semi-endoparasitic in plant roots and, in highly evolved forms, may become obese and lose their mobility in all stages except for the infective juvenile and adult male, if present. The almost ubiquitous occurrence, high diversity and great abundance of the soil nematofauna are all essential criteria that qualify nematodes as model bioindicators of environmental stability and of other edaphic factors, including pollution by heavy metal ions.

2. What are the Characteristics of the Phylum Nematoda?

There is a range of morphological and physiological characters that identify an invertebrate organism as a nematode and, in combination, these criteria form the diagnosis of the Phylum. The diagnosis presented below includes features frequently used in the identification of soil and plant nematodes (see Figure 1 for the basic structure of a nematode).

Nematodes, known colloquially as 'round worms', are invertebrate metazoan (multicellular), unsegmented pseudocoelomate organisms. The body size of plant nematodes varies from the minute (200 µm in *Paratylenchus*) to well in excess of a centimeter (over 15 mm in some *Paralongidorus*), although most are less than a millimeter in size. The body shape is typically vermiform and tapers to the pointed anterior and posterior ends, but many saccate forms (e.g. lemon-shaped, pear-shaped, reniform) associated with specialized, sedentary life styles are found. Nematodes are usually colorless or white in appearance, although the female cuticle of specialized forms that produce cysts (e.g. *Heterodera*, *Globodera*) tans to a yellow or brownish color.

Nematodes lack specific respiratory and circulatory organ systems and the body wall is involved in direct gaseous exchange with the environment. It consists of the cuticle (formed by the epicuticle, exocuticle, mesocuticle and endocuticle), epidermis (or hypodermis), four epidermal chords (two lateral, one dorsal and one ventral) that divide the pseudocoelomic cavity into four quadrants, and muscular layers (both longitudinal and oblique). The pseudocoelomic cavity, within which the tubular body organs lie, is filled with fluid and acts as a hydrostatic skeleton. The external cuticle can be smooth or divided into superficial rings or annules and may bear various ornamentations such as punctations, ridges, or pectinate fringes. The natural orifices of the body (mouth, excretory pore, anus, cloaca, vulva) are lined with cuticle.

The anteriormost region of the body, known as the head, contains the mouth which is typically surrounded by six lips (Figure 1). The digestive system begins with the mouth which opens into the stoma (also known as the buccal cavity), a structure exhibiting a myriad of forms and often containing specialized feeding structures, such as jaws, teeth or a stylet (Figures 2, 3). The pharynx (which is also known as the esophagus) is highly variable in form but typically has one or more muscular or glandular bulbs that may be equipped with cuticularized plates of various forms. It is divided into three parts: corpus, isthmus and basal bulb. The corpus can be subdivided into the procorpus and metacorpus and the basal bulb includes a cuticularised grinder and three to five digestive glands embedded amongst the muscles. Secretions produced in the pharyngeal glands debouche via ducts into the procorpus and metacorpus (Tylenchida and Aphelenchida). The pharynx connects with the intestine via a pharyngo-intestinal valve. The intestine, a long, tube-like structure, occupies most of the body length and leads to the rectum which opens to the exterior via the ventrally located anus in the case of the female or a joint alimentary/reproductive opening, the cloaca, in the male. The excretory system in Adenophorea (= Enoplea) is a single ventral cell (renette). In Secernentea (= Chromadorea), it has two lateral excretory canals embedded in the lateral chords of the hypodermis, the longitudinal canals being connected anteriorly and ventrally by a transverse canal and a terminal excretory duct which opens to the exterior via the excretory pore located in the ventral body wall. The central nervous system is formed by a nerve ring (or commissure), ganglia and a variety of other commissures. Nematodes have sensory papillae plus mechanoreceptors, those chemoreceptors located on the head (amphids) and on the tail (phasmids) being especially important.

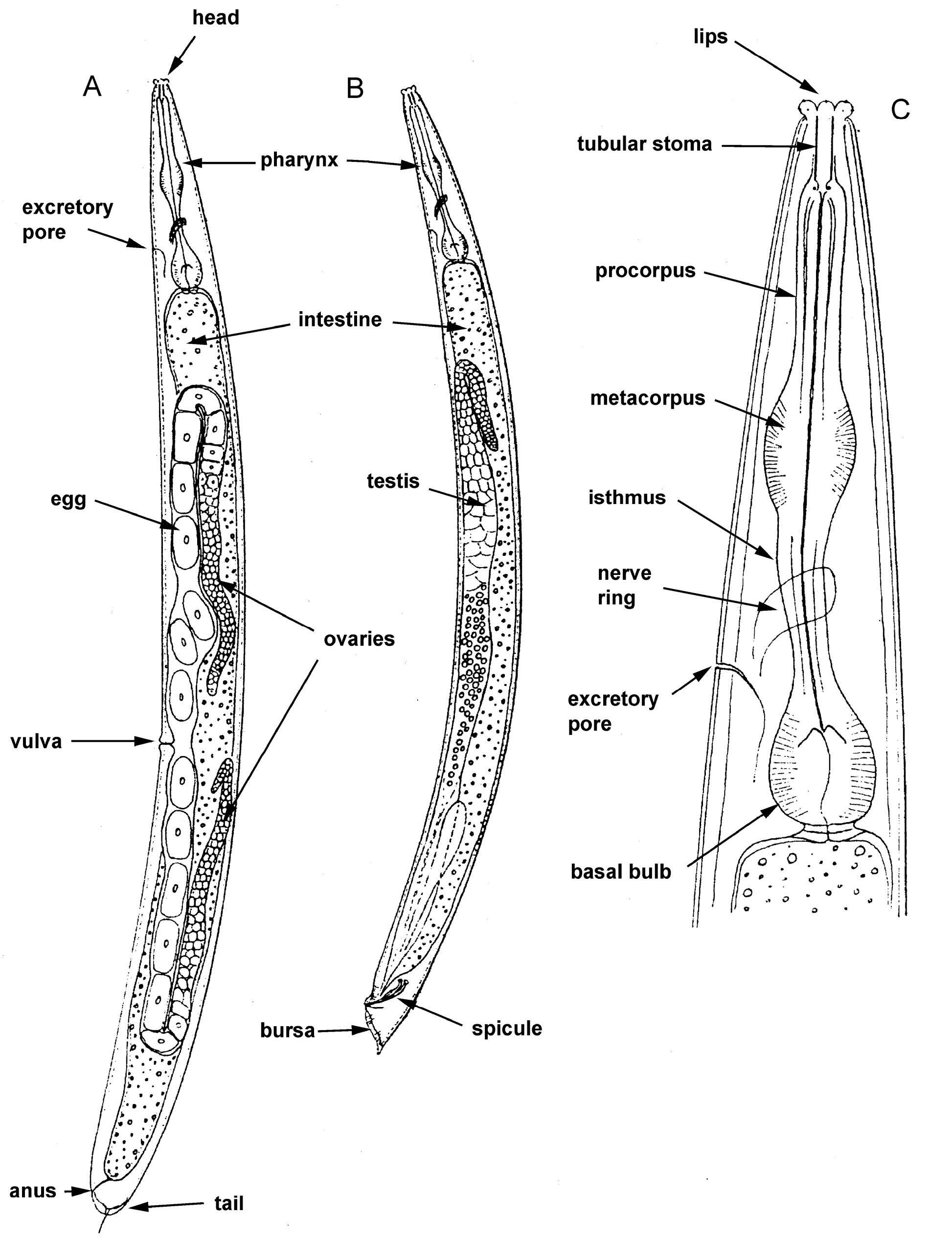

Figure 1. Basic anatomy of a soil nematode. A: Female; B: Male; C: Anterior region showing pharynx and associated structures. Figures modified after T. Goodey (1951) and not to scale.

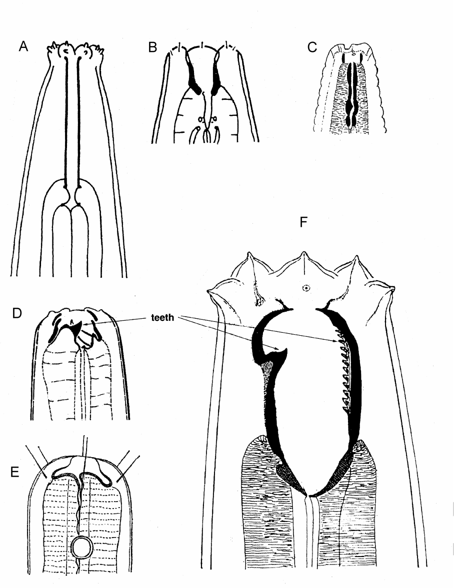

Figure 2. Variations in form of stoma in non-stylet bearing soil nematodes. A, B: Rhabditida (bacteriophagous); C: Cephalobida (bacteriophagous); D: Diplogastrida (predatory); E: Monhysterida (bacteriophagous); F: Mononchida (predatory). Figures taken from various sources and not to scale.

The sexes are typically separate (dioecious) with the male and female showing strong sexual dimorphism. Reproduction is often by amphimixis although many nematodes are either hermaphroditic or parthenogenetic (amictic). The female reproductive system consists of one or two branches, each branch typically being formed from a tubular ovary, oviduct, spermatheca and uterus, the latter opening via the muscular vagina through the vulva. The male system consists of one or two tubular testes which empty into the vas deferens. The vas deferens unites posteriorly with the rectum to form the cloaca. Within the cloaca are located a pair of cuticularized and protrusible spicules that are used to transfer sperm into the female reproductive tract during mating. During intromission, the spicules of many species are guided by a cuticularized piece, sometimes of complex form, known as the gubernaculum. The male tail may carry thin cuticular extensions or 'wings' that form a bursa, a structure used to enfold the female vulval area during copulation. The vagina, anus and cloaca all open to the exterior on the ventral side of the body.

The life cycle from egg to egg comprises three or four motile juvenile stages each separated by a molt. In some nematodes (e.g. Rhabditida, Dorylaimida) it is the first-stage juvenile (J1) that hatches from the egg, but in others (Tylenchida, Aphelenchida) the molt to the second stage (J2) occurs prior to eclosion. In the absence of a host, nematodes may survive in the soil or in plant residues. In a number of genera, the egg acts as the survival stage, but provided that the environment dries slowly, many nematodes are able to enter a reversible anhydrobiotic state when they are less susceptible to desiccation, temperature extremes and noxious chemicals. The record for longevity in the anhydrobiotic state is held by the gall forming nematode *Anguina tritici* whose infective juveniles have been recorded surviving for at least 32 years in a desiccated state before being revived by the addition of water.

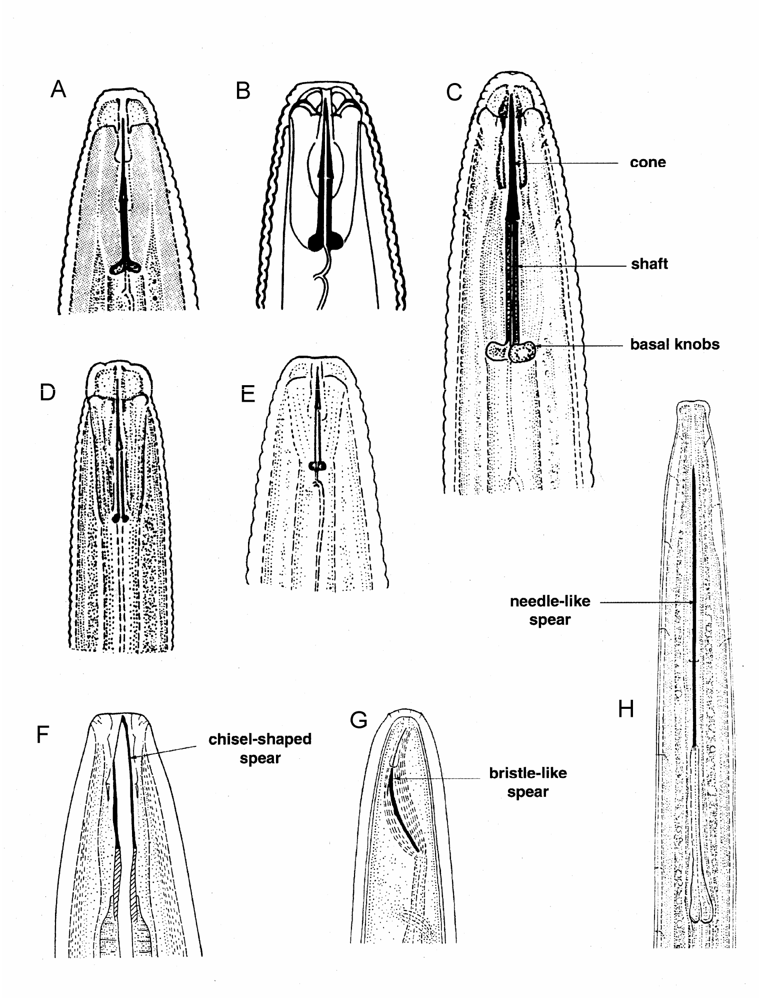

Figure 3. Anterior end showing stylet form in plant and soil nematodes. A-C, E: Tylenchida; D: Aphelenchida; F: Dorylaimida (non plant-parasitic omnivores); G: Triplonchida (Trichodoridae); H: Dorylaimida (plant-parasitic Longidoridae). Plant parasitism in nematodes has evolved at least three times, represented by A-D, G and H. Figures taken from various sources and not to scale.

Variations in morphological features and combinations of selected characters of diagnostic importance are used for classification (systematic) or identification (taxonomic) purposes. For example, the presence or absence of phasmids can be a useful character to separate classes within the Phylum whilst variation in size and shape of tail has utility to discriminate at species level.

3. Biodiversity and Nematode Diversity

The term 'diversity' has different meanings. Its definition is not restricted to differences in shape, form or species numbers as it may also refer to more subtle variation (molecular and genetic) or ecological versatility (e.g. habitats and trophic groups), this broader remit being relevant when considering concepts regarding biodiversity and the means by which it is measured.

In its simplest form, 'biodiversity' refers to the number of species in a community of organisms, although it can be broadened to encompass more than just the total number of species in an area. A more integrative and complex approach refers to biodiversity as the variability found amongst living organisms from all sources including terrestrial, marine and other aquatic systems, and the ecological complexes of which they are a part, including diversity within and between species ecosystems. Three levels can be considered in a global biodiversity assessment: 'genetic', 'organismal' and 'ecological'. Sometimes the term 'organismal' is preferred in order to embrace taxonomic categories above species rank, and 'community' or 'ecological diversity' may be preferred to the term 'ecosystem'. Biological diversity can be quantified in many different ways and at many levels of organization. Often, species are the preferred operational unit rather than units based on gene pools, higher taxa or ecosystem. Common approaches to measure or assess diversity include: *i*) molecular, based on utilization of divergences in molecular characters, especially the use of differences in nucleic acid homology and base sequences; *ii*) intraspecific genetic variation (e.g. chromosomes, genes); *iii*) cladistic and phylogenetic data, which provide an objective measure of taxonomic distance or 'independent evolutionary history' and information on taxa relatedness and abundance, respectively; and *iv*) trophic biodiversity, which is represented by the number of trophic levels, as well as by the number of guilds, the variety of life cycles, and the diversity of biological resources. The criteria most commonly used to assess plant and soil nematode diversity include the number of species and the trophic group profile, although more recently molecular methodologies, such as bar-coding, have become increasingly important.

4. The Tropics and Diversity

Relationships between species, species richness and energy fluxes in ecosystems have been associated in biogeography with latitudinal and depth gradients. Environmental heterogeneity provides species with diverse habitats and niches that are (and have been) filled by different species through adaptive radiation, convergence, divergence, selection and speciation in evolutionary processes. A high proportion of terrestrial and fresh water species occur in the tropics, the tropics having a larger total surface area than any other ecoclimatic zone. Nematodes are poikilothermic organisms (i.e. they cannot regulate their body temperature). Most nematode life processes have thermal optima that also determine nematode distribution at a geographical scale and it can be reasonably predicted that there are ranges of appropriate temperatures for each nematode species that would be contiguous and would meet at the equator for true tropical species. Geographically, the tropics and subtropics embrace several (e.g. tropical rain forest, deserts, prairies and oceans) of the eight biomes of the planet, nematodes being among their most abundant inhabitants.

There is a greater diversity of nematode genera and species in subtropical and tropical regions than in the temperate zone yet few large scale and long-term surveys on nematode biodiversity are available for the tropics. For example, only 10 out of 134 sites studied for nematode diversity have been located in tropical zones. Nematodes are extremely abundant, but their irregular distribution in soil is a major obstacle to obtaining reliable data on actual numbers. As with other organisms, abundance is determined by birth, death, and immigration/emigration rates of species in an area, these rates in turn being dependent on abiotic and biotic factors. Population densities can differ by a factor of 10 across a few meters and this variability is often directly related to host plant, soil and/or biological factors.

5. Nematode Diversity

Any estimate of worldwide nematode species diversity is highly speculative since available figures are so variable. It is generally considered that about 20,000 species have been described to date although there may be as many as 500,000 species in total, a number only exceeded by the highly speciose arthropods and mollusks. High diversity is often attributed to an elevated level of niche specialization, food being an important driving factor, but functional redundancy does occur. In order to assess nematode diversity, several approaches (similar to those already described for assessing biodiversity) have been used, including: *i*) identification and classification (taxonomy) of individuals (e.g. classical morphometrical methods) belonging to a population; *ii*) trophic group analysis where nematodes are arranged (community level) according to feeding habits rather than by exclusively taxonomic traits; and *iii*) genomic and metagenomic level where DNA samples are isolated from individuals (genomic DNA) and subjected to PCR (polymerase chain reaction) or, alternatively, host tissues or soil samples are treated using metagenomic approaches (i.e. archiving environmental DNA in genomic libraries without culturing or PCR). In both approaches DNA samples are sequenced, and then compared (using BLAST search) against gene bank data to identify related taxa using statistical algorithms based on sequence similarity.

6. Taxonomically Based Diversity

Until relatively recently, morphological features have been primarily used to identify and place species in different taxonomic categories (taxa) although molecular methodologies are now of increasing importance. It is clear, however, that delineation of nematode taxa based only on morphological characters is not always congruent with their genetic and phylogenetic (common ancestry) affiliation. A classification of the phylum based primarily on molecular phylogeny has been recently proposed in which the classes Chromadorea and Enoplea replace the former Secernentea and Adenophorea. Following this classification scheme, the order Dorylaimida (= Subclass Dorylaimia in the molecular based scheme) mostly comprises omnivorous species but with some important plant nematodes that are vectors of viruses, whilst the suborder Tylenchina (= Subclass Chromadoria) contains most of the plant nematodes, including the most highly specialized plant-parasitic species. However, in subsequent sections we will, for practical purposes, follow the traditional systematic scheme and refer to the orders better known as: Tylenchida, Aphelenchida, Dorylaimida, and Triplonchida. All four orders can be readily distinguished by their stylet and pharynx types (see Figures 3, 4; Table 1).

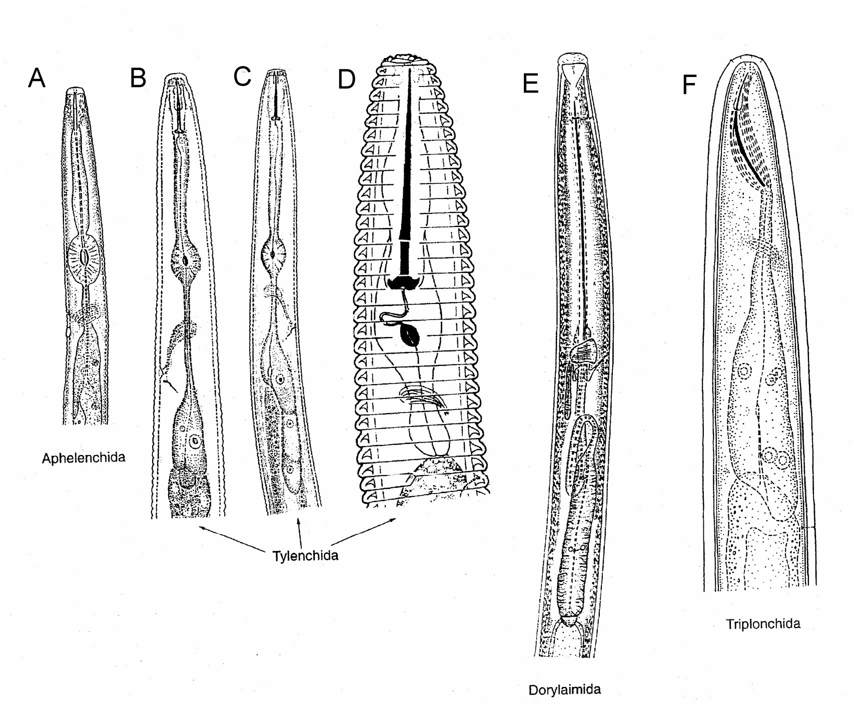

Figure 4. Variation in form of pharynx in plant-parasitic nematodes. A: Aphelenchida; B-D: Tylenchida; E: Longidoridae (Dorylaimida); F: Trichodoridae (Triplonchida). Figures taken from various sources and not to scale.

Character	**Tylenchida**	***Aphelenchida***	**Dorylaimida**	**Triplonchida**
Spear type	Stomatostyle	Stomatostyle	Odontostyle	Onchiostyle
Pharynx form and pharyngeal gland number	Three parts: corpus, isthmus, and posterior bulb. Three glands	Three parts: corpus, isthmus, and posterior bulb. Three glands	Two strongly demarcated parts: corpus and postcorpus. Three to five glands	Two parts, anterior part gradually expanding into posterior part. Five glands
Position of gland outlets	Dorsal pharyngeal gland in procorpus; close to stylet knobs	Dorsal pharyngeal gland in metacorpus, anterior to valve	All close to body of glands	All close to body of glands
Metacorpus	*Width less than 75% of body width*	Width more than 75% of body width	Absent	Absent
Isthmus	*Present*	Absent in Aphelenchoididae	Absent	Absent
Pharyngeal glands	In a bulb or overlapping lobe	In a bulb (Paraphelenchidae) or overlapping lobe	In posterior bulboid expansion	In posterior bulboid expansion

Table 1. Characteristics of stylet and pharynx of plant-parasitic nematodes.

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Biographical Sketches

Rosa H. Manzanilla-López was born in Mexico where she got her first degree and MSc in Biology (1984 and 1990 respectively) from the Autonomous National University of Mexico (UNAM); and later on her PhD from Reading University (UK, 1997) studying the bionomics of the false root-knot nematode *Nacobbus aberrans*. In her native Mexico she worked as research assistant and associate professor of invertebrate zoology, animal biology, animal parasitology and agricultural nematology at the UNAM, Escuela Normal Superior de Mexico (ENS), Autonomous University of Puebla (BUAP), and Postgraduates College of Agriculture (CP). She joined Rothamsted Research in 2001 where is a research scientist participating in projects on diverse subjects such as attachment tests of *Pasteuria penetrans* on *Meloidogyne* spp., *Lotus japonicus* as a model for plant-nematode interactions; plant hypersensitive reaction to nematodes; assessment of genetic variation of *Pochonia chlamydosporia*, a biological control agent of root-knot nematodes (*Meloidogyne* spp.) using molecular tools. *Nacobbus aberrans* has been her main subject of research and she has co-authored a comprehensive review of the genus and promoted the use of molecular tools to separate the groups and/or species of this species complex. She also has co-authored book chapters and research papers on nematology. She is an active member of the Organization of Nematologists of Tropical America (ONTA) a scientific organization committed to knowledge dissemination of plant-parasitic, entomopathogenic and free-living nematodes in the tropical Americas and other tropical and subtropical regions of the world.

David Hunt was born in England and attended Nottingham and Reading universities before working for several years on nematodes of banana and other crops in the West Indies. In 1979 he joined the Commonwealth Institute of Helminthology as a nematode taxonomist, a post he has held through a bewildering number of name changes endured by the organization. He has described over 120 new species and genera of nematode and has published numerous peer-reviewed papers and book chapters plus a monograph on plant-parasitic nematodes. He is co-editor of a book on entomopathogenic nematodes and has been Editor-in-Chief of *Nematology* since 2002 and Series Editor of *Nematology Monographs & Perspectives* since 2004. David has also organized many international training courses in nematode morphology/identification, most recently in Kenya, Estonia and Honduras.