Micro-nematodes

Mum: why does Fido need those de-worming tablets?

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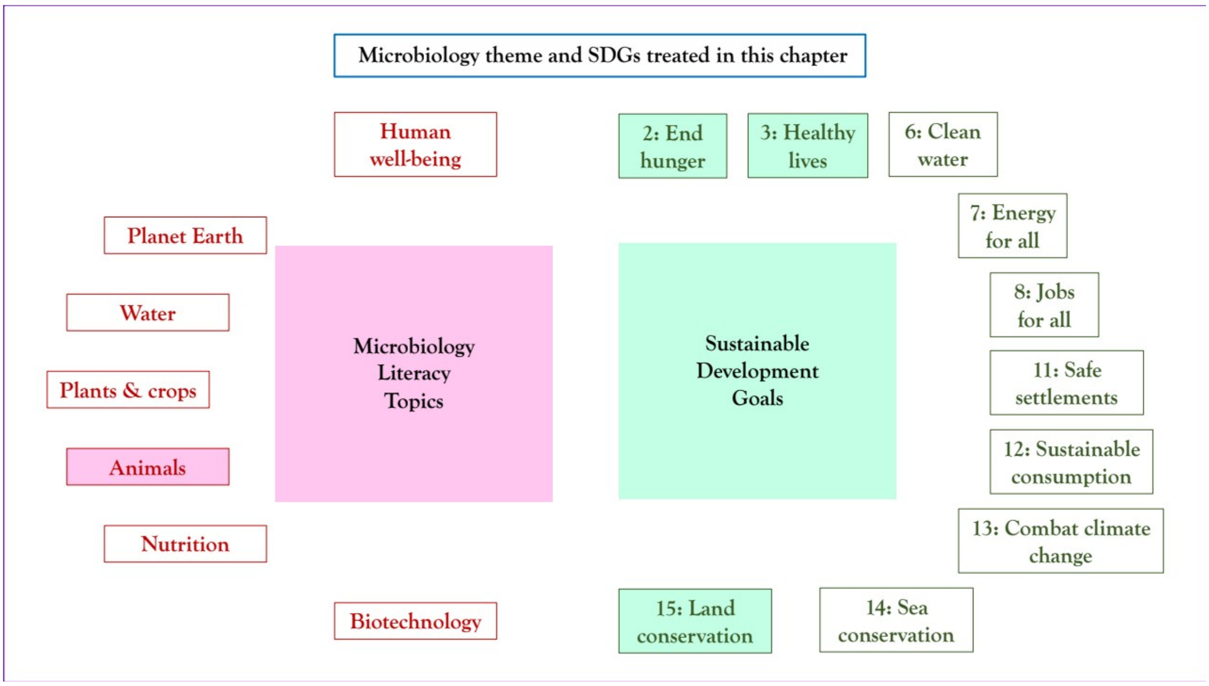
Storyline

Not only bacteria, also animals can be invisible to the naked eye. This includes the nematodes, microscopic animals that can occur as free-living organisms in marine, fresh-water or soil habitats. In addition, they can also be parasites of plants, animals and humans and can harm agriculture and food supply or cause devastating diseases. Our dogs and cats suffer as much from nematode parasites as we do. Therefore, de-worming tablets help improve the quality of life of our pets. The rich diversity of nematodes with a total species number that might be more than a million, makes some of them important model organisms for scientists to study all types of problems in biology and medicine. Thus, small animals, such as nematodes, are important for ecosystem functioning but we need a better awareness and more detailed investigations to fully understand their role in health, ecology and evolution.

The Microbiology and Societal Context

The microbiology: nematodes; microbial diversity; microbial interactions; symbioses; infections of plants and animals; biological control of insect pests. *Sustainability issues:* end hunger; healthy lives; protect terrestrial ecosystems.



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1. ***Nematodes are the major group of microscopic animals.*** Microbial diversity is not restricted to the unicellular bacteria and protists. In animals, microscopic multicellular species are found abundantly in soil and water habitats alike. Common to all these organisms is the poor understanding of their taxonomy and ecosystem functioning. This largely results from the fact that professional zoology has long focused on macroscopic vertebrates, insects and other arthropods. Among microscopic animals, one phylum is the most numerous and widespread: the *nematodes* or *roundworms*. Nematodes are thought to represent the dominant group of animals, both with regard to abundance and species numbers, and in all ecosystems. However, while species numbers are estimated to be in the range of 500,000 to 10 million, the number of scientifically described nematodes is less than 30,000, reflecting our limited understanding of micro-nematodes and their ecosystem functioning.

A recent macroecological study investigated the abundance of soil nematodes at a global scale. Based on more than 6,700 referenced samples, it was suggested that $4.4 + 0.64 \times 10^{20}$ nematodes inhabit surface soils across the world. This equals a total biomass of 0.3 gigatonnes. It is believed that approximately 80% of all land animals are nematodes and that they are major representatives in all trophic levels.

2. ***Reproduction.*** Nematodes have an external skeleton and in the egg stage, a chitinous shell that provides strong protection. All nematodes go through four juvenile stages before reaching adulthood. Species vary in generation time. Some are rapidly reproducing with a generation time as little as 3-4 days under laboratory conditions (i.e. 20 °C), which make some of them attractive model organisms (see below).

Three different modes of reproduction can be distinguished in nematodes.

a. First, most species are outcrossing males and females, similar to humans.

b. Second, hermaphroditism has evolved multiple times independently. Such species are self-fertilizing: as such animals are first males by producing a limited number of sperm during postembryonic development. When adult, these individuals switch to become females and use their stored sperm to fertilize their own eggs. This unique mode of reproduction has major advantages for biomedical studies, as it simplifies husbandry and results in isogenic cultures – animal lines that are genetically identical. However, many hermaphroditic species have additional males as a second sex and hermaphrodites can outcross to male individuals.

c. Finally, some nematodes reproduce by parthenogenesis, a mode of asexual reproduction of embryos without fertilization by sperm.

3. ***Free-living nematodes are small: the micro-nematodes.*** The overwhelming majority of nematodes are free-living and are found in marine, freshwater and soil ecosystems. Sediments of marine and freshwater systems, but also surface soil are thought to contain millions of nematode specimens. Many of the free-living species are around 1 mm in length as adults, but they can also be smaller and rarely larger.

4. ***Parasitic nematodes are often large.*** A perplexing difference in body size is observed between free-living and parasitic nematodes. While the free-living species are overwhelmingly small, many of the parasitic groups evolved a large-sized body. The record holder is the giant nematode *Crassicauda magna,* parasite of the sperm whale *Kogia breviceps,* that is more than three meters long. The nematode parasites of men and other mammals are also of large size. It

is now clear that parasitism in nematodes has evolved at least seven times independently, with phylogenetically unrelated parasites of animals and plants.

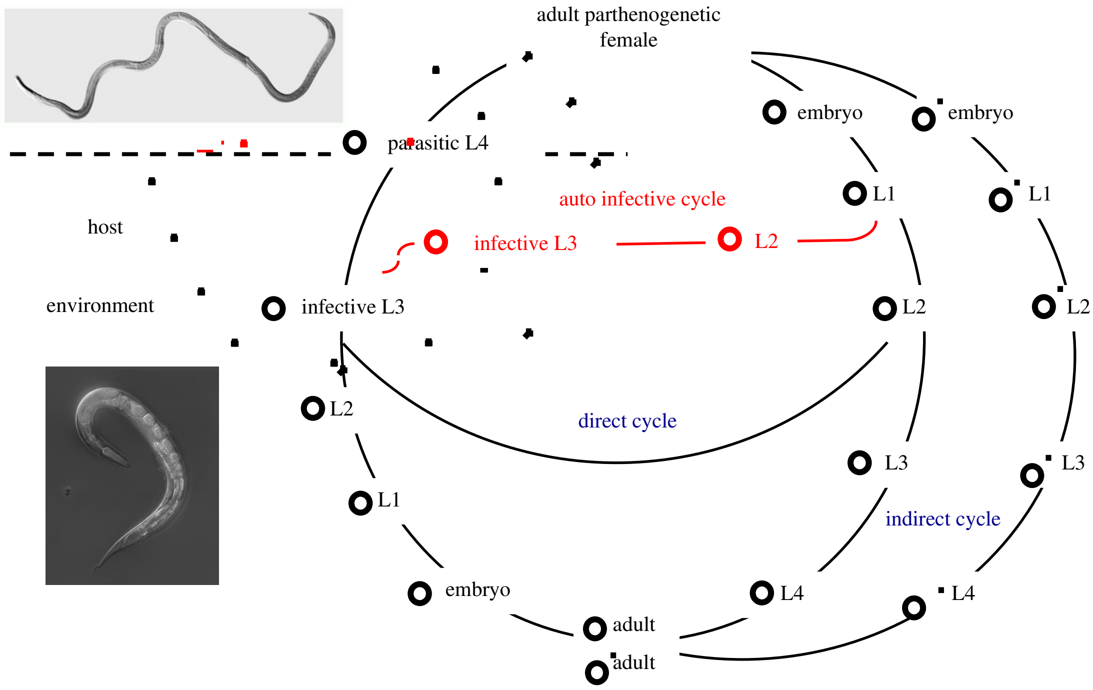
5. ***Nematode parasites of plants.*** Three groups of nematodes have evolved to become parasites of plants with currently more than 4,000 described species. Plant-feeding nematodes can be inside or outside of the plant host (ecto – or endoparasites). Usually, they attack the plant root, puncture the cell wall to extract cellular content and, in some cases, redirect the metabolism of plant cells. The major groups of plant parasites are endoparasitic and are of economic importance, including among others i) cyst nematodes (*Heterodera* ssp.; *Globodera* ssp.), ii) root-knot nematodes (*Meloidogyne* ssp.), and iii) lesion nematodes (*Pratylenchus* ssp.). In addition, the aerial parts of plants can be attacked by nematodes. For example, the pine wild or pine wood disease caused by *Bursaphelenchus* ssp. resulted in tremendous problems for pine trees in Asia and other areas.

6. ***Nematode parasites of animals.*** The majority of insects and vertebrates, including livestock and humans, are also infected by nematode parasites, often in a species-specific manner. In humans, around 30 nematode parasites are known, which can cause severe health problems and even death. There is growing awareness of zoonotic transmissions, similar to bacterial and viral transmissions between mammalian hosts.

A major challenge for human health is due to the fact that nematode parasites belong to four unrelated phylogenetic groups of nematodes, and thus the mechanisms of infection and also treatment options are fundamentally different. Only ivermectin is a general antiparasitic drug that, after its discovery in 1975, was also approved for humans in 1987 for treatment of various nematode-caused diseases.

The most important nematode parasites of humans are: i) hookworms (*Ancylostoma duodenale* and *Necator americanus*), intestinal parasites found in large parts of the world, ii) intestinal roundworms (*Ascaris lumbricoides*), iii) the related *Wuchereria bancrofti* that causes elephantiasis, iv) the completely unrelated *Trichinella spiralis*, the causal agent of trichinosis, and v) the threadworm *Strongyloides stercoralis* that has begun to cause severe health issues including death in recent years (see life cycle below).

Some species have different intermediate hosts and/or free-living stages with humans being infected by specific infective stages. Infections often occur through contaminated waters and surface areas. Increased hygiene standards and treatment with several antihelminths including ivermectin resulted in a decrease in parasitic burden globally in the middle of the 20th century. However, the last decades see again a severe increase at a global scale. There are multiple reasons for these developments, including a lack of awareness of the diseases, but also the increase in tourism and migration.



Life cycle *Strongyloides stercoralis.* Adult parasites are parthenogenetic females and live in the intestine of mammals, for *S. sterocoralis* the intestine of humans. Eggs are release with the feces into the environment and three larval stages, L1, L2 and L3 are formed with two different sexes, females and males. In the 'indirect cycle', both sexes remain free-living, and adults mate to form progeny. These progenies are all female and free-living until the second larval stage (L2). The L3 larvae become infective and must find their host. After a complex migration the adult females end up in the intestine again to complete the life cycle. Note the different morphology and size of the parasitic and free-living adult females. There is a 'direct cycle' with the female progeny of parthenogenic females becoming infective larvae after the L2 females were released from the host. Only for the human parasite, but not for other *Strongyloides* parasites in other mammals, there is also an 'auto infective cycle' (indicated in red), where the progeny of the parasitic females do not leave the host at all. This is one of many examples of complex life cycles of nematode parasites. © Prof. Adrian Streit, Max Planck Institute for Biology Tübingen.

7. ***Model nematodes provide insights into all aspects of biology.*** Progress in modern biology largely dependents on 'model organisms', species that can be easily cultured and that reproduce rapidly under laboratory conditions, with limited associated costs. Given their small body size and the large number of species, it might not be surprising that one of the animal counterparts to the famous bacterial and fungal model species, such as *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae*, is a nematode.

Caenorhabditis elegans was introduced as a model organism by Sydney Brenner in the 1960s. This species combines a number of technical features that made it very attractive for basic research:

a. it can be grown using as food monoxenic cultures of *E. coli*, usually on 6 cm agar plates (cover image).

b. it is one of the self-fertilizing hermaphrodites that generates up to 300 self-progenies.

c. it has a generation time of 3,5 days and 2,5 days at 20 and 25 °C, respectively. Thus, it can produce thousands of animals in less than a week, starting from a single hermaphroditic adult.

d. While the generation time is short, these worms live for up to three weeks, which made them an important model for aging research.

e. Finally, worms can be stored in liquid nitrogen indefinitely and can be 'revived' within minutes after thawing a small vial of worms.

Together, these features have attracted a large number of researchers to study all aspects of *C. elegans* biology.

8. ***Sophisticated interactions with insects and bacteria.*** Nematodes can also provide important insights into sophisticated ecological interactions in nature, many of which are cross-kingdom. One such system is the entomopathogenic nematodes (EPNs) that represent an astonishing example of insect-nematode-bacterial interactions. Two unrelated nematode genera, *Heterorhabditis* and *Steinernema*, cause the death of insects by means of symbiotic bacterial pathogens they carry. Specifically, *Heterorhabditis* nematodes carry bacteria of the genus *Photorhabditis,* whereas *Steinernema* carry related bacteria of the genus *Xenorhabdus*. Importantly, nematodes alone are largely not pathogenic to insects. However, when an infective juvenile of *Heterorhabditis* or *Steinernema* infects a new insect host by penetrating the insect´s body cavity, the bacteria are released from the nematode gut and become toxic to the insect. The insect usually dies within 24-48 hours, following rapid multiplication of the bacteria.

It is important to note that EPNs can target both insect larvae that live in the soil (i.e. beetles, flies, moths), and adult insects, including beetles, crickets, locusts and flies. After the death of the insect, the flourishing culture of *Photorhabdus* or *Xenorhabdus* in the carcass provides lots of food for nematode growth. Once the microbial food is exhausted, the nematodes form infective juveniles again to initiate foraging and infection of a new host.

9. ***Nematodes as biological control agents.*** Insect pests inflict major reductions on crop yields in agriculture and hence play a pivotal role in food security. While chemical insecticides help mitigate insect damage, because of their toxicity they cause environmental pollution and may kill beneficial non-target insects. Biological insect control agents are both non-toxic, and hence non-polluting, and may have high levels of target specificity so do not affect beneficial insects. Several features of the sophisticated life cycle of nematodes make them unique and important for biological control:

a. First, EPNs have evolved independently twice because *Heterorhabditis* and *Steinernema* are phylogenetically unrelated.

b. Second, both genera have evolved to form large taxa with more than 50 species each, some of which are specialists that target certain insects, whereas others are generalists.

c. Finally, the high specificity of the pathogenic interaction makes these nematodes important biological control agents for many agricultural insect pests.

10. ***Nematodes and ecosystem function: Complex interactions in a microcosm.*** The small body size allows nematodes to interact with nearly all macroscopic animals, such as gastropods, annelids and arthropods. Our knowledge is most detailed for insects and includes as one of the most extreme types, the entomopathogenic interactions described above. In general, however, one can consider the interactions between insects and nematodes as a continuum from unspecific and random to parasitic or pathogenic interactions.

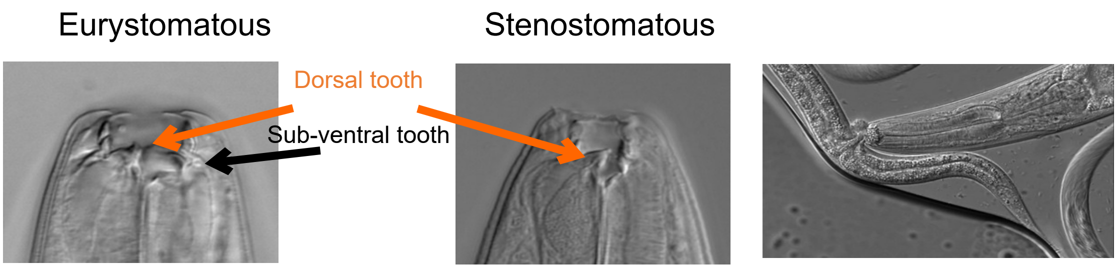
a. Saprobionthic nematodes. So-called 'saprobionthic' nematodes feed on dead organic matter mostly in the surface layers of diverse soil systems. As such, they can be completely free-living, but can also associate themselves with certain carriers for transportation.

b. Phoretic nematodes. 'Phoresy' describes the phenomenon in which a nematode or other small invertebrate (such as mites) attach to the external surface of a macroscopic animal for a limited time period. Such interactions can be unspecific but can also involve preferential vectors that are more likely to result in dispersal into a new area that will provide sustainable food supplies.

c. Necromenic nematodes. While strictly phoretic organisms will disembark from the living vector, nematodes might 'wait' until the carrier´s (insect´s) death, in order to feed on the microbes developing on the carcass. This phenomenon is called 'necromeny' and is just one step ahead of phoresy. Indeed, several authors argued for the transitions from saprobiontic, phoretic to necromenic as important intermediate steps towards real parasitic or pathogenic interactions.

d. Mouth-form polyphenism. One other nematode that is intensively studied in this context and that highlights the complex interactions in soil microcosms are nematodes of the genus *Pristionchus*, with *P. pacificus* as a hermaphroditic model organism that allows molecular insight, similar to *C. elegans*. *Pristionchus* nematodes are found in soil, but are most reliably isolated from scarab beetles, *i.e.*, cockchafers, stag beetles or dung beetles. These nematodes can use adult beetles as vectors for dispersal (phoresy), but also occupy the carcass after the short-lived adult beetles died naturally (necromeny).

Pristionchus nematodes are highly adapted to such ecological settings as they exhibit a mouth-form polyphenism, a special form of phenotypic (developmental) plasticity. That is, worms form either a strict bacterial feeding mouth-form (called 'stenostomatous'), or an omnivorous mouth with two strong teeth that allow predation on other nematodes (called 'eurystomatous). Such eurystomatous animals can kill potential competitors on the insect carcass and thereby influence the niche of the short-lived ecosystem. This is just another example of the sophisticated interactions in soil ecosystems involving microscopic interactions that escape the attention of the naked eye.



P. pacificus **feeding structures.** This nematode can form two alternative feeding structures in genetically identical organisms, an example of phenotypic plasticity. Adult animals are either 'eurystomatous' (left picture) with two teeth and a wide buccal cavity or 'stenostomatous' (middle picture) with a single tooth and a narrow buccal cavity. Both forms can feed on bacteria, but the eurystomatous form can also predate and kill other nematodes (right picture). © Prof. Ralf Sommer, Max-Planck institute for Biology Tübingen.

11. **Conclusions: small, everywhere and sometimes dangerous.** In conclusion, nematodes are mostly microscopic animals that can be found in all ecosystems on the planet, but the current understanding of their diversity and ecosystem functioning is far from complete as only

30,000 of the more than one million expected species is known to science. In an overpopulated world in need of massive agriculture and a rapid expansion of human populations, parasites of food crops, livestock and men are causing severe problems. As is the case for other parasites and pathogens, zoonotic transmissions are increasingly recognized to play an important role for infection and transmission.

Besides these alarming parasitic associations, the world of nematodes is full of exciting organismal interactions, often cross-kingdom. They provide important model organisms that helps biologists to understand the general principles and the diversity of life. But as for bacterial and fungal microbes, we need concentrated efforts to better understand their biodiversity and their service to ecosystem functioning.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 2. End hunger, achieve food security.** Insect pests and plant pathogenic nematodes inflict serious damage to plant crops and reduce yields, therefore contributing to food insecurity. On the other hand, nematodes pathogenic for insects reduce insect loads in agricultural settings and are being weaponized as biological control agents. Nematodes are thus negative and positive forces in food security.
- **Goal 3. Ensure healthy lives and promote well-being for all at all ages**. Pathogenic nematodes cause serious diseases in humans, including filariasis (river blindness) and elephantiasis.
- **Goal 15. Protect terrestrial ecosystems.** Insect pests wreak havoc on crop plants and, to combat this, chemical pesticides are applied in enormous quantities to agricultural systems. These compounds pollute farmlands and surface waters worldwide, and kill beneficial non-target insects, including all-important plant pollinators. The use of nematodes as biocontrol agents to kill insect pests reduces inputs of chemical pesticides and hence protects terrestrial ecosystems.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Annelids. Annelids are ‘ringed worms’ and as such, are unrelated to nematode roundworms. They include the earthworms and leeches. Since 1997 it is known that they are unrelated to insects and are members of a large animal supergroup, called ‘Lophotrochozoa’, which also includes the gastropods (see below).

Antihelminths. A type of drugs that expels parasitic worms from the body. As the term says, these drugs can act against a diversity of 'helminths', which is a term that also includes worms other than nematodes, such as flatworms.
Arthropods. Arthropods are a 'supergroup' of animals with an i) external skeleton, ii) a segmented body, iii) and paired joined appendages. They include the insects, crustaceans, millipedes and spiders.
Chitinous shell. Chitin is a macromolecular component formed from sugars, such as glucose, and its derivatives. It forms part of the shell and many other structures of insects and other arthropods, but also fungi and gastropods. The success of these organisms is largely due to the rigorous structure of their external skeleton with chitin as its major component.
Ecosystem functioning. This term describes the sum of all life activities of bacteria, plants and animals and the role that these activities play for the environment and ecosystem in which these organisms live. Importantly, organisms living in the same environment might interact with each other and thereby, collectively shape the ecosystem function.
Gastropods. Gastropods is the scientific name for snails and slugs, and represents the second major group of the Lophotrochozoa, to which also the annelids belong.
Insecticides. These are compounds that can be used to kill insects. Long used in agriculture, many insecticides are now banned because they harm the environment systemically. Modern research aims to replace insecticides by biological pesticides.
Macroecology. Macroecology is the study of broad scale ecological patterns and processes in any group of organisms and/or any ecosystem.
Mammals. One of the major groups of vertebrates (see below) that is characterized by i) hair, ii) mammary glands and iii) a constant body temperature, and iv) three middle ear bones. The mammals include human, chimpanzees and many other groups.
Taxonomy. Taxonomy is the broad science of ordering and classifying living or extinct organisms.
Vertebrates. Vertebrates are the animals that have a backbone inside their body and include five major groups; fishes, amphibians, reptiles, birds and mammals.