the TIMETREE of LIFE

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Nematodes (Nematoda)

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Abstract

The Nematoda is a phylum of noncoelomate invertebrates with ~23,000 described species. Traditional taxonomies recognize ~250 families, but these schemes are undergoing substantial revision based on molecular phylogenetic analyses. Nematode fossils are rare, and mostly uninformative as to deeper relationships. While the phylum has an origin in the Precambrian (>543 million years ago, Ma), and the three extant recognized classes likely diverged very early, few dates have been estimated for nematode divergences. The caenorhabditids are estimated to have diverged from the vertebrate-parasitic Strongyloidea ~380 Ma, and their divergence from the parasitic Ascaridomorpha is dated at ~540 Ma.

The phylum Nematoda encompasses small and abundant organisms (Fig. 1) that inhabit most of the available habitats on Earth. A famous image derived from a pioneering, evangelical nematologist, Nathan Cobb, suggested that if all the other matter of the Earth, apart from nematodes, were taken away, it would still be possible to make out the shape of the planet, and of most organisms on it because of the abundance and ubiquity of the group. Commonly known as roundworms or eelworms, they largely escape notice except when they cause damage to humans, domestic animals, and agricultural crops; many species are successful parasites. Over 23,000 species have been described from just over 3000 genera in 250 families, but estimates of true nematode diversity range from 100,000 to 10 million species (*1, 2*).

Nematodes are defined by their generally cylindrical body shape (secondarily modified in some parasites), a collagenous cuticle that is moulted four times in post-embryonic development, and a series of anterior sensory organs that have a particular hexaradially symmetrical distribution (Fig. 1). Here I review the divergence times and evolutionary relationships of selected taxa within the Nematoda. Traditional systematics have placed the

Nematoda in several different places within Metazoa, but recent molecular analyses are converging upon a placement within the Superphylum Ecdysozoa (*3*). This conflicts with opinions based on morphological characters such as the possession of a true coelom, and of metameric segmentation, but is supported by features of moulting and ciliated epithelia.

The internal phylogeny of the Nematoda has been much debated also, and molecular data are being brought to bear (*4–6*). While no complete system has yet been devised, there are some general areas of agreement between different studies that also have morphological support (*7, 8*). The Nematoda has three classes: Enoplia, Dorylaimia, and Chromadoria. The relative branching order of these has not been determined. All three classes contain parasitic groups, and whole-phylum analyses suggest that animal and plant parasitisms have arisen independently at least six and three times, respectively (*9*). Within the Chromadoria, the free-living model species *Caenorhabditis elegans* is part of a radiation of terrestrial

Fig. 1 Scanning electron micrograph of the head of a soil-dwelling cephalobomorph nematode (*Acrobeles complexus*). Credit: M. Mundo-Ocampo.
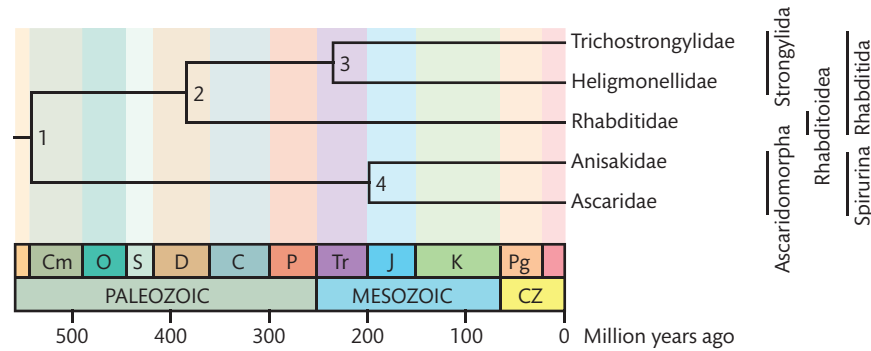
Fig. 2 A timetree of Nematoda. Divergence times are shown in Table 1. *Abbreviations*: C (Carboniferous), Cm (Cambrian), CZ (Cenozoic), D (Devonian), J (Jurassic), K (Cretaceous), O (Ordovician), P (Permian), Pg (Paleogene), S (Silurian), and Tr (Triassic).

microbivores that also includes animal-parasitic clades. Also in the Chromadoria are the Tylenchina (including plant and animal parasites), the Spiruromorpha which are all parasitic (*10*) and a paraphyletic group of free-living marine and terrestrial taxa, the "chromadorids" (*4*). Within the Dorylaimia are marine and terrestrial microbivores, plant parasites, and animal parasites. The Enoplia includes marine and terrestrial microbivores and predators, and plant parasites.

While the largest nematodes (parasites of the cetacean gut or urinary systems) are meters in length, most of the members of this soft-bodied taxon are less than a millimeter, and thus fossilize poorly. Two sources of fossil nematodes have been found. One is in ambers from the Dominican Republic (20–15 Ma; Neogene), Mexico (26 Ma; late Paleogene), the Baltic (~40 Ma; Paleogene), and Myanmar (~110–100 Ma; Cretaceous). The other is in coprolites of subfossil (~1 Ma; Quaternary) and early Cretaceous (~130 Ma) age. Fossils from amber (*11*) have been diagnosed as being from representatives of insect parasites from the Mermithida (*12–15*), and Tylenchina (*16–18*), and from free-living plectids and diplogasterids (*16, 19*). Fossils from coprolites are often poorly preserved, and the only robust identification has been of eggs with morphology typical of the Ascaridomorpha in dinosaur coprolites from Belgian strata (*20*).

Molecular analyses of nematode phylogeny using both nuclear and mitochondrial genes have been published, but few have attempted to place estimates of divergence times on internal nodes. One feature that is evident from many analyses, particularly those using nuclear ribosomal RNAs, is that of extremes of evolutionary rate heterogeneity in different lineages. Thus the lineage that includes the model *C. elegans* is characterized by

an elevated rate of substitution compared to its close relatives (*5*). Despite these caveats, four studies have placed time estimates on the nematode tree. In the publication describing the sequencing of the genome of *Caenorhabditis briggsae* (*21*), Avril Coghlan generated an estimate of 110–80 Ma for the divergence time of this species from the model *C. elegans*. This estimate used 338 sets of protein-coding orthologous genes that appeared not to display elevated rates in the nematodes compared with humans and the mosquito *Anopheles gambiae*, and they assumed a nematode–arthropod divergence date of 1000–800 Ma. While the date of nematode–arthropod divergence is obviously open to debate, these estimates compare favorably with earlier ones (*22*). When a nematode–arthropod divergence of 800 Ma was assumed, the 95% confidence interval for the *C. briggsae–C. elegans* divergence was 90–78 Ma. More recently, Asher Cutter (*23*) has used estimates of neutral mutational accumulation to reassess this estimate and has presented analyses that suggest a much more recent divergence (<30 Ma). This estimate is more in keeping with dates derived from mitochondrial genes (*24*), but the true timing of caenorhabditid evolution is likely to remain an active research issue for some time to come.

The diplogasterid *Pristionchus pacificus* is a satellite model organism, and its genome has been sequenced also. Several estimates of the divergence between *P. pacificus* (Diplogasteromorpha) and *C. elegans* (Rhabditomorpha) have been published (*25–27*), with the most recent being "200–300 million years" (*27*) but these are not explicitly supported by data. An initial estimate of >100 Ma made in 1997 (*25*) had no justification other than that the split must predate between species within the genus *Caenorhabditis*. These estimates

Table 1. Divergence times (Ma) among Nematoda, from ref. (*28*).

Timetree	
Node	Time
1	541
2	383
3	235
4	198

all conflict with that made by Vanfleteren *et al.* (*28*) (see later) for the divergence of *C. elegans* from another group in the Rhabditomorpha, and need close revision.

The only other molecular dating study used cytochrome *c* and nematode-type globin sequences to date the divergences of a number of parasitic groups from each other and from *C. elegans* (*28*). In this study, the unit evolutionary periods (the time represented by a 1% sequence divergence at the protein level) for cytochrome *c* (21 Ma) and globin (5 Ma) were derived from other, mainly vertebrate taxa, and it is thus unclear how accurate these estimates are. However, using the same method to estimate the *C. elegans*–*C. briggsae* divergence yields 112 Ma (Blaxter, unpublished observations), suggesting that if the estimates for the same divergence made using multiple genes or mutation accumulation experiments (*23*) are more correct (*21*), then the globin-dated divergences may be overestimates, particularly in the rapidly evolving chromadorid clades. Using cytochrome *c* or globin, this method yielded estimates of 1200–950 Ma for the divergence of Nematoda from other phyla tested (Arthropoda, Echinodermata, and Chordata). Within Nematoda, globin comparisons yielded a 383 ± 8 Ma (i.e., Devonian) divergence for *C. elegans* and two families of strongylid parasites (Trichostrongylidae and Heligmonemellidae). While traditional systems place Strongylida at ordinal rank, "Strongylida" in molecular analyses is deeply nested within the traditional Order "Rhabditida": both of these families are now considered members of the Superfamily Strongyloidea within Infraorder Rhabditomorpha (*8*). Within the Ascaridomorpha, globin sequences were used to give an estimate of 198 ± 42 Ma (i.e., Jurassic) divergence for members of the Ascarididae and Anisakidae. The estimate for the divergence of Rhabditomorpha and Ascaridomorpha was 541 ± 11 Ma (i.e., Cambrian).

The paucity of informative fossils, and the related issues of identification of molecular markers with verifiable clocklike behaviors, limits knowledge of the dates of divergences of a major component of the biota, the Nematoda. Other routes to dating using external mark points such as examining the congruence of host and parasite phylogenies to transfer robust dates from the hosts (e.g., vertebrates) have not been successful, because of rampant lateral capture of hosts by parasite groups (*29*). However, the emerging genome sequence data sets available for a wide phylogenetic range of nematodes, and the extensive EST data sets being generated for parasitic species (*30*), should yield additional protein-coding genes with which analyses aimed at dating nematode radiations can be performed. These will have to be analyzed with caution, as the most extensive comparisons performed to date, between members of the genus *Caenorhabditis* (*21, 23*), have yielded date estimates that vary by several fold.

Acknowledgments

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