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The evolutionary origins of nematodes within the order Strongylida are related to predilection sites within hosts

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Abstract

The evolutionary relationships of the different groups of nematodes within the order Strongylida based on morphological data have been speculative and the subject of conjecture. In this paper, we present a multigene phylogenetic analysis, using sequence data of the 18S and 28S ribosomal RNA genes from representatives of all four suborders and seven superfamilies of the Strongylida, to test existing hypotheses proposed for the relationships of the suborders based on morphological data sets. The results obtained demonstrated that the Strongylida is a monophyletic assemblage, with only the Metastrongylina (but not the other suborders) forming a distinct monophyletic clade. We show that, in contrast to all previous hypotheses, one major lineage comprises taxa which occur exclusively in the pulmonary, circulatory or nervous systems of marsupial and eutherian mammals, whereas a second lineage comprises species occurring in the gastro-intestinal tracts or perirenal tissues of vertebrates, or in the lungs of birds. The findings suggest that the predilection site of adult nematodes and host type reflect the evolutionary origin of the different taxonomic groups within the Strongylida.

Keywords: Strongylida; Phylogenetic relationships; Small and large subunit rRNA genes; Metastrongylina; Strongylina; Trichostrongylina; Ancylostomatina; Parasitic nematodes

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1. Introduction

The phylum Nematoda represents a diverse assemblage of organisms, including free-living bacteriovores, entomopathogens, and parasites of plants and animals. There is often disagreement as to the taxonomy and phylogenetic relationships of some groups based on morphological data because of a lack of informative characters and an absence of a fossil record. Molecular-based studies have, however, provided valuable insights into the evolutionary relationships of different nematode groups (Audebert et al., 2005; Blaxter et al., 1998, 2000; Carreno and Nadler, 2003; Chilton et al., 1997, 2001; Dorris et al., 2002; Fitch, 2000; Gouÿ de Bellocq et al., 2001; Litvaitis et al., 2000) and the origins of parasitism within the phylum (Blaxter et al., 1998, 2000).

The order Strongylida represents one of the major radiations of parasitic nematodes. They are distributed worldwide, occur in every terrestrial class of vertebrate and in some marine mammals, but are absent from fish, except for the infective larvae of *Parafilaroides decorus* which use teleosts (*Girella niricans*) as paratenic (i.e., transfer) hosts ((Dailey, 1970). The Strongylida, which contains many species of paramount medical and veterinary importance (Skrjabin et al., 1952), is biologically diverse, with the predilection sites of adults varying from the gastrointestinal tract, the perirenal tissues, the pulmonary, circulatory, and nervous systems, musculature, and the nasal sinuses (Anderson, 2000). The infective third-stage larvae use oral and/or percutaneous (skin penetration) transmission to infect their vertebrate hosts, of which the latter mode is considered to be more primitive (Adamson, 1986; Anderson, 1988, 2000; Fülleborn, 1929).

According to the CIH keys (Anderson et al., 1974), the Strongylida consists of five well-defined superfamilies: the

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Ancylostomatoidea, comprising hookworms in the small intestine of mammals, the Diaphanocephaloidea, which occur in the intestines of snakes, the Strongyloidea, which occur in the gastrointestinal tracts and occasionally the tracheae or perirenal tissues of mammals and birds, the Metastrongyloidea, which occur in the respiratory, vascular, and nervous systems of mammals, and the Trichostrongyloidea, which occur primarily in the gastrointestinal tracts of amphibians, reptiles, monotremes, Australian marsupials, and eutherian mammals (the one exception being *Dictyocaulus* spp. which occur in the trachea and bronchi of ungulates). However, Durette-Desset and Chabaud (1993) elevated four of the five superfamilies to subordinal status so that the three major evolutionary lineages of trichostrongylid nematodes (i.e., families Trichostrongylidae, Molineidae, and Heligmosomidae) recognized by Durette-Desset (1985) could be raised to superfamily status (i.e., the Trichostrongyloidea, Molineoidea, and Heligmosomoidea). Therefore, according to the taxonomic revision of Durette-Desset and Chabaud (1993), the Strongylida comprises seven superfamilies (in four suborders): the Ancylostomatoidea, Diaphanocephaloidea (Ancylostomatina), Strongylina (Strongyloidea), Metastrongyloidea (Metastrongylina), Trichostrongyloidea, Molineoidea, and Heligmosomoidea (Trichostrongylina). Although recognition of the three primary lineages within the Trichostrongylina and the proposal for their elevated taxonomic rank has not always been accepted (e.g., Lichtenfels et al., 1997), there is some molecular evidence to a support the monophyly of each major trichostrongylid lineage and for a sister-taxon relationship between the Molineoidea and Heligmosomoidea (Audebert et al., 2005; Gouÿ de Bellocq et al., 2001).

It is accepted that the Strongylida evolved from free-living ancestors related to contemporary species within the order Rhabditida (Dougherty, 1949, 1951a; Durette-Desset et al., 1994; Inglis, 1965; Poinar, 1983; Schulz, 1951; Skrjabin, 1941; Skrjabin et al., 1952), either as early as the late Devonian or early Carboniferous, the time when their most primitive vertebrate hosts (amphibians) appeared (Poinar, 1983), or during the Mesozoic, after the appearance of reptiles (Dougherty, 1951a; Inglis, 1965). Rhabdiasoid nematodes, such as *Rhabdias* (family Rhabdiasidae: parasites of the lungs of amphibians and reptiles) and *Strongyloides* spp. (family Strongyloididae: parasites of the gut mucosa of tetrapods, mostly mammals), have often been considered to represent a transition from the free-living rhabditids to the obligate parasitic strongylid nematodes (Durette-Desset et al., 1994; Inglis, 1965; Poinar, 1983; Skrjabin et al., 1952). Rhabdiasoids are exclusively parasitic in vertebrates but retain a free-living generation in their life cycle, and some species (e.g., *R. bufonis*) have a percutaneous mode of transmission, as do some species within the Strongylida (Fülleborn, 1929). However, results of molecular phylogenetic studies (Blaxter et al., 1998, 2000; Dorris et al., 2002; Fitch, 2000) have now shown that *Strongyloides* spp. and *R. bufonis* are more closely related to species within the Panagrolaimoidea and Cephaloboidea (Rhabditida: Cephalobina), while the Strongylida represent a sister taxa to the rhabditids of the *Eurhabditis* group and the genus *Heterorhabditis*. Some eurhabditids (e.g., *Oscheius insectivora*) are facultative parasites of insect larvae but retain the ability or need to reproduce under free-living conditions, whereas the heterorhabditids are obligate parasites of invertebrates which carry a specialized symbiotic bacterium to kill their hosts (Poinar, 1983). Invertebrates are also used as intermediate or paratenic hosts by some species within the Strongylida, however many species do not require an intermediate host to complete their life cycle (Anderson, 2000). Although molecular sequence data suggest a sister-taxon relationship between the Strongylida, *Heterorhabditis*, and the *Eurhabditis* group, the monophyly of the Strongylida has not been tested rigorously using molecular data sets because previous analyses (Blaxter et al., 1998, 2000; Fitch, 2000) have been based on only four species representing only two of the four suborders within the Strongylida.

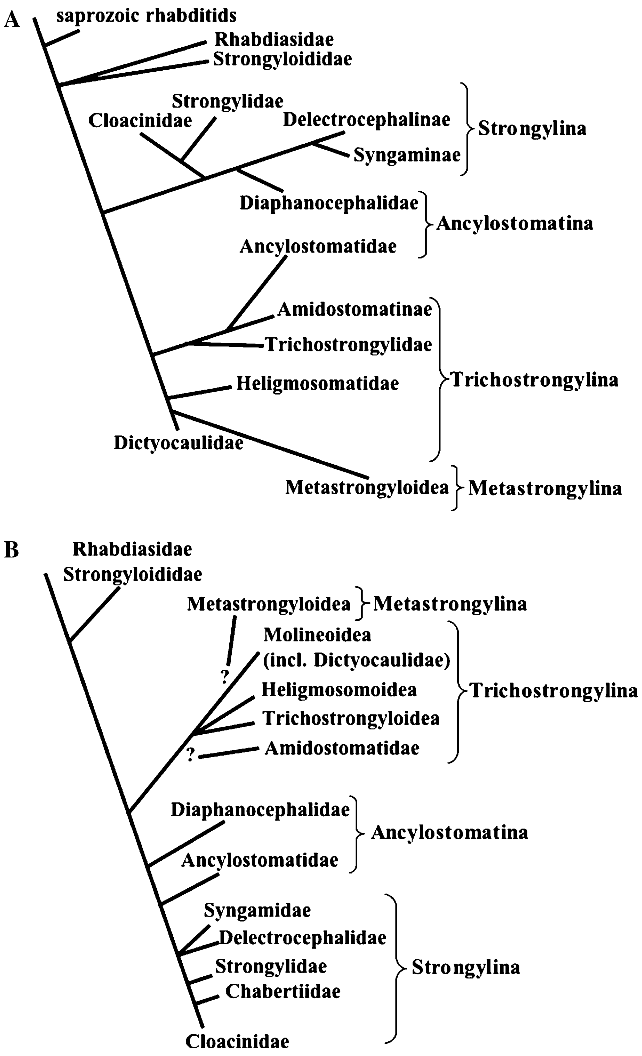

Fig. 1. Hypotheses for the phylogenetic relationships of the major groups within the Strongylida according to (A) Schulz (1951) and (B) Durette-Desset et al. (1994), but with the suborder designations for each taxon according to Durette-Desset and Chabaud (1993).