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Evolutionary Relationships among the Protostrongylidae (Nematoda: Metastrongyloidea) as Inferred from Morphological Characters, with Consideration of Parasite-Host Coevolution

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EVOLUTIONARY RELATIONSHIPS AMONG THE PROTOSTRONYLIDAE (NEMATODA: METASTRONYLIOIDEA) AS INFERRED FROM MORPHOLOGICAL CHARACTERS, WITH CONSIDERATION OF PARASITE–HOST COEVOLUTION

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ABSTRACT: The phylogeny of nematodes in the family Protostrongylidae (Nematoda: Metastrongylioidea) was reconstructed by cladistic analysis of 28 binary and multistate characters derived from comparative morphology. Analyses were hierarchical, and examined (1) relationships among genera, including 13 ingroup taxa and Metastrongylidae as an outgroup (single tree, 78 steps, consistency index [CI] = 0.705); and (2) relationships among genera and species groups, including 21 ingroup taxa and Metastrongylus aprt as an outgroup (single tree, 76 steps, CI = 0.592). In the species-level tree, Protostrongylidae was divided into 2 major clades, 1 containing the subfamilies Muellerininae (including the recently described Umingmakstrongylus pallikaukenis), Elaphostrongylinae, and the Varestrongylinae (excluding Pneumocaulus kadenazzi). Varestrongylus was paraphyletic as it included Pneumostrongylus calcarius. The second major clade consisted of a paraphyletic group containing Protostrongylus spp. and Spiculocaulus leuckarti and, basal to this subclade, several other individual protostrongylid lineages. The various subclades generally correspond to the subfamilial divisions of the Protostrongylidae. The Neostongylinae, however, is not supported as Neostrongylus and Orthostrongylus are not sister groups. Based on a large number of hypothesized synapomorphies, the elaphostrongylines appear to be a highly derived group of protostrongylids, a feature potentially correlated with their habitat localization in muscular and nervous tissues. The generic-level tree retained most of the primary structure revealed among the species but excluded the varestrongylines from the Muellerininae + Elaphostrongylinae subclade. Artiodactyls of the family Cervidae are considered basal hosts for protostrongylids; secondary colonization in Caprini, Rupicapriini, and among lagomorphs is postulated.

Nematodes of the Protostrongylidae Leiper, 1926 include predominantly lung-inhabiting parasites, many of which are serious pathogens in wild and domestic ruminants and lagomorphs. Adults and first-stage larvae of most protostrongylids form nodules in the lungs that can lead to respiratory distress and secondary bacterial infection resulting in verminous pneumonia (e.g., Demartini and Davies, 1977; Svarc, 1984; Sauerländer, 1988; Costantini et al., 1990; Mansfield et al., 1993; Pajersky, 1995). Lungworm infections with species of Protostrongylus Kamensky, 1905 are among the most important diseases of Rocky Mountain bighorn sheep (Hibler et al., 1982). Protostrongylids in Elaphostrongylus Cameron, 1931 and Parela- phostrongylus Boe and Schulz, 1950 are also serious pathogens in cervids. Members of both genera occur in the skeletal muscles and nervous system, and Parelaophostrongylus tenius (Dougherty, 1945) is responsible for neurologic disease in North American moose and potentially in many wild and farmed ruminants including cervids and camels (e.g., Anderson, 1964, 1971; Nettles et al., 1977; Brown et al., 1978; Guthery et al., 1979; Tyler et al., 1980; Krogdahl et al., 1987; Lankester and Fong, 1989), whereas species of Elaphostrongylus are of similar importance in Eurasian cervids and other hosts (Roneus and Nordkvist, 1962; Frosl and Kutzer, 1980; Handeland and Norberg, 1992; Handeland and Skorping, 1992, 1993). Thus, the protostrongylids have been known for a long time as important disease agents in wild, farmed, and domestic ruminants.

The genera of Protostrongylidae are distinguished from other metastrongylids in having a well developed gubernaculum and telamon apparatus (Boe and Lankester, 1994). All species for which life cycles are known have a gastropod intermediate host in which infective third-stage larvae develop. Differentiation of adults is based primarily on the morphology of the spicules, gubernaculum, telamon, and vulva. Differences in bursal morphology, particularly in the shape and number of branches of the dorsal ray, have also been applied. These characters were used by Boe (1975) in his designation of various subfamilies in the Protostrongylidae (Table 1). This classification was based on comparative morphology among genera but did not explicitly develop hypotheses for phylogenetic relationships in the group.

Little is known about the evolutionary and biogeographic history of the Metastrongylioidea and Protostrongylidae, and these taxa have not been evaluated phylogenetically as have other groups such as the Trichostrongylidae and Trichostrongylidae (e.g., Durette-Desset, 1985; Hoberg and Lichtenfels, 1994). Within the Metastrongylidae, the Protostrongylidae were believed by Dougherty (1949) to be part of a lineage that included the Filarioidea and Pseudalinae. He suggested that these 3 lineages corresponded, respectively, to radiations in carnivores, cetaceans, and artiodactyles. Pryadko (1984) (as in Lichtenfels, 1987) proposed that protostrongylids were archaic and considerably older than reptiles and mammals and that metastrongylids were originally parasites of amphibians.

Within the Protostrongylidae, there have been no prior phylogenetic interpretations for the evolution of the family, and relationships among the genera are unknown. Although phylogenetic analyses exist for elaphrostrongyline nematodes (Platt, 1984; Carreno and Lankester, 1994), the evolution of this clade relative to other protostrongylids has remained unclear. In addition, the recent discovery and diagnosis of a new protostrongylid genus, Umingmakstrongylus Hoberg, Polley, Gunn, and Nishi, 1995 in North American muskoxen, have raised the question of how this parasite is related to other protostrongylids (Hoberg et al., 1995). In this analysis we provide a phylogenetic hypothesis for the Protostrongylidae obtained using morphological characters. The results of this phylogenetic reconstruction...
Table I. Classification of the Protostrongylidae Leiper, 1926.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Genera</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elaphostrongylineae</td>
<td>Elaphostrongylus Cameron, 1931</td>
<td>Boe and Schulz, 1950</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Cystoacaulus Schulz, Orlow, and Kutass, 1933</td>
<td>Boe and Schulz, 1950</td>
</tr>
<tr>
<td>Muellerinae Skrjabin, 1933</td>
<td>Neostongylus Gebauer, 1932</td>
<td>Orlow and Goble, 1946</td>
</tr>
<tr>
<td>Neostongylidae</td>
<td>Neostongylus Kamensky, 1905</td>
<td>Boe and Schulz, 1950</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Spiculocaulus Schulz, Orlow, and Kutass, 1933</td>
<td>Boe and Schulz, 1950</td>
</tr>
<tr>
<td>Skrjabinoacaulinae Boe and Sulimov, 1963</td>
<td>Skrjabinoacaulus sofevi Boe and Sulimov, 1963</td>
<td>Boe and Schulz, 1950</td>
</tr>
<tr>
<td>Varestrongylidae</td>
<td>Varestrongylus Bhalerao, 1932</td>
<td>Mönig, 1933</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Pneumostrongylus Schultz and Andreeva, 1948</td>
<td>Pneumostrongylus Mönig, 1933</td>
</tr>
</tbody>
</table>

* After Boe (1975), with modification of the Muellerinae Skrjabin, 1933 by Hoberg et al. (1995).

are a test of the validity of various taxonomic classifications of the protostrongylids and provide a basis for future systematic studies within the family. Additionally, we explore the putative history for parasite–host relationships for the protostrongylids, arthoactydes, and lamorphs within a phylogenetic context for these nematodes.

**MATERIALS AND METHODS**

**General methods and specimens**

Representative specimens of each of the genera of the Protostrongylidae were examined to acquire an understanding of the diversity and homology for structural characters across the family; at least 1 representative of each genus was studied, and this information was augmented by detailed descriptions in the literature (Table II). Specimens were obtained from the United States National Parasite Collection, the Canadian Museum of Nature, Division of Invertebrates, the University of Alberta parasite collection, and from the authors’ personal collections. It was not possible to obtain specimens of Spiculocaulus leuckarti Schultz, Orloff, and Kutass, 1933, Skrjabinoacaulus sofevi Boe and Sulimov, 1933, Pneumostrongylus kadenazzii Schulz and Andreeva, 1948, and Neostongylus linears (Marotet, 1913). For these species, information was obtained from published descriptions (Table II). A total of 28 binary and multistate characters were defined.

**Phylogenetic analysis**

Higher-level analyses of superspecific taxa in the Metastrongyloidea have not been conducted, and thus it was problematic to determine the sister group and maximally informative outgroups for the Protostrongylidae. Although the Metastrongyloidea are presently unresolved, there is substantial evidence that the Metastrongylidae (and species of Metastrongylus) are basal in the superfamily. Species of *Metastrongylus* have well developed cephalic labia, a simple gubernaculum (sometimes absent), and a unique structure for bursal rays (Dougherty, 1949). Additionally, they are oviparous and the egg-shell is thick (Anderson, 1978). Furthermore, members of this genus occur exclusively in suids and use earthworms as intermediate hosts rather than gastropods as in most other groups including the Protostrongylidae. These features provide sufficient evidence that Metastrongylidae is distinct from other groups, and it is generally believed to be basal to the other major families in the Metastrongyloidea (Dougherty, 1949; Lichtenfels, 1987; Durette-Desset et al., 1994). Consequently, based on this justification, Metastrongylidae and Metastrongylus apr, respectively, were designated as outgroups for hierarchical analysis of genera and species of Protostrongylidae in the present study. Additionally, attempts to resolve relationships of the Protostrongylidae were also examined relative to genera and species of Crenosommatidae Schulz, 1951 and Skrjabinidae (Skrjabin, 1933).

Character polarity was determined relative to the taxonomic outgroups as specified above. Separate data matrices for generic and species-level taxa were written using MacClade 3.05 (Maddison and Maddison, 1992) and included 28 binary and multistate characters (Tables III and IV). In the generic-level matrix certain characters were coded as polymorphic to recognize interspecific variation in an attribute among species in a genus (multistate taxa) (Table IV). The data matrices were analyzed using the software Phylogenetic Analysis Using Parsimony (PAUP), version 3.1.1 (Swofford, 1993) first in a heuristic search mode with variation in options for branch swapping, e.g., TBR, SPR, NNI, with Addition Sequence = Simple and with MULPARS in effect; multistate characters were unordered. Trees were confirmed with Branch and Bound, a more exact algorithm for obtaining the most parsimonious solution. Descriptive statistics include the consistency index (CI), homoplasy index (HI), and retention index (RI). Host associations for protostrongylid genera and species were examined by mapping and optimization of mammalian taxa onto the parasite phylogeny with MacClade 3.05 (Maddison and Maddison, 1992).

**RESULTS**

**Character descriptions**

1. First stage larva, tail: blunt tail = 0; dorsal spine on tail = 1; long and tapering tail ending in a spiked tip = 2.
2. Provagina: absent = 0; small cuticular projection over vulva = 1; large ventral flap extending close to or up to the tail tip attached to body by thin folds of cuticle = 2; cylindrical = 3; several flaps = 4.
3. Gubernaculum, crura: crura absent = 0; crura small, smooth, and round = 1; rod shaped and smooth = 2; rod shaped and with projections or ridges = 3; in the form of plates with large, often sharp (pointed) projections = 4; in the form of plates with small projections = 5.
4. Telamon: absent = 0; telamon consisting of simple basal and transverse plates = 1; consisting of several shieldlike plates = 2; a small distal plate forming a pear-shaped or heart-shaped structure = 3; complex and consisting of many plates = 4; consisting of 2 crescentic, sagitally symmetrical parts = 5.
5. Capitulum: absent = 0; present with 2 spikes or “ears” sensu Boe (1975) = 1; 4 or more spikes present = 2.
Table II. Protostrongylid species examined in this study, listed by putative subfamily according to Boev (1975); Metastrongylidae represents the outgroup for analysis.*

**METASTRONGYLIDAE**

*Metastrongylus apri* (Gmelin, 1790) Vostokov, 1905: NMCP1984-0237, acc. 1981-140; USNPC 76946

**PROTOSTRONGYLIDAE**

Elaphostrongylinae

*Elaphostrongylus cervi* Cameron, 1931: personal collection (R.A.C.)

*Farelaphostrongylus odocollei* (Hobmaier and Hobmaier, 1934) Boev and Schulz, 1950: description in Carreno and Lankester (1993); personal collection (R.A.C.)

Varestrongylinae

*Varestrongylus alpenae* (Dikmans, 1935) Dougherty, 1945: USNPC 34066 (holotype), USNPC 78599 (voucher)

*Varestrongylus pneu monicus* Bhalerao, 1932: USNPC 45199

*Varestrongylus sagittatus* (Mueller, 1890) Dougherty, 1945: USNPC 37855

*Pneumostrongylus calcaratus* Mönig, 1932: USNPC 65889 (voucher)

*Pneumocaulus kadenzii* Schulz and Andreeva (1948): description in Schulz and Andreeva (1948)

Muellerinae

*Muellerius capillator* (Mueller, 1889) Cameron, 1927: USNPC 45386 (voucher)


*Cysto caulus nigrescens* (=oce reatus) (Railliet and Henry, 1907) Mikacic, 1939: USNPC 37845; personal collection (R.A.C.)

Neostrongylinae

*Neostrongylus linearis* (Marotol, 1913) Gebauer, 1932: descriptions in Gebauer (1932), Kreis (1944), Rojo-Vazquez and Cordero del Cam pillo (1974), Castañon Ordoñez et al. (1984)

*Orthostrongylus macrostis* (Dikmans, 1931) Dougherty and Goble, 1946: USNPC 65929, 76738, 43679, 43610, 30418

Protos trongylinae

*Protostrongylus boughtoni* Goble and Dougherty, 1943: University of Alberta Parasite Collection (UAPC 10796); personal collection (R.A.C.)

*Protostrongylus hobmaieri* (Schulz, Orlow, and Kutass, 1933) Cameron, 1934: USNPC 37839

*Protostrongylus pulmonalis* (Frölich, 1802) Goble and Dougherty, 1943: description in Costantini et al. (1990)

*Protostrongylus raillieti* (Schulz, Orlow, and Kutass, 1933) Cameron, 1934: USNPC 37831

*Protostrongylus rufescens* (Leuckart, 1865) Kamensky, 1905: USNPC 46516

*Protostrongylus rushi* Dikmans, 1937: USNPC 66043, 78423

*Protostrongylus stilesi* Dikmans, 1931: USNPC 66044, 66045, 49227, 59749

*Spiculocaulus leuckarti* Schulz, Orlow, and Kutass, 1933: description in Schulz et al. (1933)

Skrjabinocaulinae

*Skrjabinocaulus soiesi* Boev and Sulimov, 1963: description in Boev and Sulimov (1963)

* In addition to morphological descriptions in Boev (1975), the following museum specimens and published descriptions were studied for character information. Specimen lots designated as NMCP and CMNP are from the Canadian Museum of Nature, Ottawa; those designated USNPC are from the United States National Parasite Collection, Beltsville, Maryland.

Table III. Data matrix generated by using Metastrongylidae as an outgroup in assessing characters for generic-level taxa in the Protostrongylidae.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Characters 1–28*</th>
</tr>
</thead>
<tbody>
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<td>Pneumostrongylus</td>
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<tr>
<td>Neos trongylus</td>
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</tr>
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<tr>
<td>Pneumostrongylus</td>
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<td>Elaphostrongylus</td>
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<tr>
<td>Parelaphostrongylus</td>
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<tr>
<td>Varestrongylus</td>
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<tr>
<td>Orthostrongylus</td>
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<tr>
<td>Cysto caulus</td>
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</tr>
<tr>
<td>Umningastrengylus</td>
<td>1033100010010011010012011113</td>
</tr>
<tr>
<td>Spiculocaulus</td>
<td>1335110010000000010012002012</td>
</tr>
</tbody>
</table>

* Missing data indicated by \?; polymorphism in multistate taxa by \&.
Table IV. Data matrix generated by using *Metastrongylus apri* (Gmelin, 1790) as an outgroup in assessing characters of selected species representing *Protostrongylidae* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Characters 1–28*</th>
</tr>
</thead>
<tbody>
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<td><em>Metastrongylus apri</em></td>
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</tr>
<tr>
<td><em>Muellerius capillaris</em></td>
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</tr>
<tr>
<td><em>Pneumocaulus kadenazii</em></td>
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<tr>
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<tr>
<td><em>Elaphostrongylus cervi</em></td>
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<tr>
<td><em>Parelaphostrongylus odocoilei</em></td>
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<tr>
<td><em>Varestrongylus pneumonicus</em></td>
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<td><em>Varestrongylus alpinae</em></td>
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</tr>
<tr>
<td><em>Varestrongylus sagittatus</em></td>
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<td><em>Orthostrongylus macrotis</em></td>
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<td><em>Uningmakstrongylus palliakaukensis</em></td>
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<td><em>Spiculocaulus leueckarti</em></td>
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<tr>
<td><em>Protostrongylus rufescens</em></td>
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<td><em>Protostrongylus boughtoni</em></td>
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</tr>
<tr>
<td><em>Protostrongylus pulmonalis</em></td>
<td>2025211000100100000002002011</td>
</tr>
</tbody>
</table>

* Missing data indicated by ?.

6. Bursa: split = 0; slightly notched dorsally = 1; undivided = 2.
7. Dorsal ray: either absent or, if present, elongate = 0; distinctly spherical = 1.
8. Spicule shaft: distally unbranched = 0; distally branched = 1.
9. Posterolateral rays: equal in length to other rays = 0; shorter than other lateral rays = 1.
10. Ventral rays: common stalk of ventral rays short = 0; joined by a long and thick common stalk = 1.
11. Structure of gubernaculum: crura short relative to corpus, proximal part of corpus unpaired = 0; crura long relative to corpus, proximal part of corpus paired = 1; crura short relative to corpus, proximal part of corpus paired = 2.
12. Excretory pore: at midregion or posterior half of esophagus = 0; at anterior half of esophagus = 1.
13. Nerve ring: surrounding esophagus at a point in its posterior ¼ = 0; at its anterior ¼ = 1.
14. Spicule tips: tip of spicule shaft pointed, sharp = 0; tip blunt = 1.
15. Spicule shaft: unjointed = 0; with a joint at midlevel of shaft = 1.
16. Spicule ribs: spicules without ctenidia-like ribs near tip of main shaft = 0; ctenidia-like ribs present = 1.
17. Shape of eggs: eggs oval or elliptical = 0; eggs spherical = 1.
18. Muscle radiations at bursa: strong visible cuticular or muscular radiations associated with copulatory structures absent = 0; strong cuticular or muscular radiations present = 1.
20. Shape of cephalic extremity: blunt = 0; rounded = 1.
21. First stage larva: tail tip uniform = 0; tip made up of 2 or 3 segments = 1.
22. Crura, attachment to corpus: no crura attached to corpus = 0; crura of different density than corpus or unattached to corpus = 1; crura of similar density and fused to corpus by the proximal ends = 2.
23. Female tail: tip in the form of an acute (sharp) cone = 0; in the form of an obtuse (blunt) cone = 1.
24. Membranes on crura: distal part of crura not surrounded by a thick membrane = 0; surrounded by a colorless, light-refracting membrane = 1.
25. Gubernaculum, corpus: corpus in the form of a simple, compact plate = 0; in the form of an elongate, narrow plate = 1; in the form of 2 cords sometimes united through a less compact tissue = 2.
26. Dorsal ray, branching: dorsal ray not trilobed = 0; trilobed = 1.
27. Unpaired pedunculate dorsal papilla: absent = 0; present = 1.
28. Dorsal ray division: short and divided = 0; short and undivided = 1; long and undivided = 2; long and divided = 3.

**Phylogenetic analysis**

Analysis at the generic level in Protostrongylidae resulted in a single most parsimonious tree (MPT; 78 steps; CI = 0.705; CI, excluding uninformative characters = 0.614; HI = 0.436; RI = 0.676) that diagnoses monophyly for the family based on characters 1, 4, 8, 27, and 28. (Fig. 1); diagnostics for individual characters are shown in Table V. Homoplasy was associated with 11 characters (parallelism/convergence for chars. 2, 3, 5, 6, 8, 9, 11, 21, and 25; reversal for 6, 7, 8, 11, and 18). In the fully resolved MPT, 2 major clades are diagnosed: (1) a sister-
group association for Elaphostrongylinae (with Elaphostrongylus and Parelophostrongylus) + Muelleriinae (with Muellerius Cameron, 1927 and Cystocaulus Schulz, Orlow, and Kutass, 1933 + Umingmakstrongylus); and (2) Pneumostrongylus Mönig, 1932 + Varestrongylus Bhalerao, 1932 in a subclade basal to Pneumostrongylus Schulz and Andreeva, 1948, Neostrostrongylus Gebauer, 1932, Spiculocaulus Schulz, Orlow, and Kutass, 1933, Skrjabinocaulus Boev and Sulimov, 1963, Orthostrongylus Dougherty and Goble, 1946, and Prostostrongylus (Fig. 1).

Analysis at the species level yielded a single MPT (76 steps; CI = 0.582; CI, excluding uninformative characters = 0.577; HI = 0.418; RI = 0.759) (Fig. 2) that diagnoses monophyly for the family based on characters 1, 6, 27, and 28 (Fig. 2); diagnostics for individual characters are shown in Table V. Homoplasy was associated with 15 characters (parallelism/convergent for characters 2–7, 9, 11, 18, 19, 21, 22, and 25; reversal for 2, 5, 8, 14, 18, and 28). Two major clades were diagnosed. The first contained 3 subclades with Varestrongylus spp. + Pneumostrongylus calcaratus (Mönig, 1932) as the sister group for the Elaphostrongylinae + Muelleriinae (Muellerius capillaris (Mueller, 1889), Cystocaulus ocreatus (Raillet and Henry, 1908), and Umingmakstrongylus pallikuukensis). This subclade is diagnosed by 2 synapomorphies including a distally branched spicule shaft (character 8) that was secondarily lost in Elaphostrongylus spp. and a long, divided dorsal ray (character 28). The subclade containing Varestrongylus spp. included an unresolved trichotomy of Varestrongylus pneumonicus Bhalerao, 1932, Varestrongylus sagittatus (Mueller, 1891), and P. calcaratus. Basal to these 3 species was Varestrongylus alpinae (Dikmans, 1935). This subclade of 4 species is diagnosed by having ventral rays joined by a long and thin common stalk (character 10). The elaphostrongyline subclade was characterized by 5 synapomorphies including an anteriorly located excretory pore (character 12), an anteriorly located nerve ring (13), spherical eggs (17), a rounded cephalic extremity (20), and a female tail in the form of a blunt cone (23). The Muelleriine subclade containing M. capillaris, C. ocreatus, and U. pallikuukensis is diagnosed by a jointed spicule shaft (character

![Figure 1. Phylogenetic relationships for genera of the family Protostrongylidae Leiper, 1926 as inferred by parsimony analysis of characters derived from studies of comparative morphology. Represented is the single most parsimonious tree (CI = 0.705). Character support for terminal taxa and internodes is defined below (nodes are numbered) and includes apomorphy (A), homoplasy as convergence or parallelism (H) and reversal (R), and polymorphic change in terminal taxon (P). Terminal taxa are labeled according to generic-level nomenclature: outgroup, Metastrongylinae and Metastrongylidae; ingroup: Cystocaulus (H: 2); Umingmakstrongylus (R: 6); Muellerius (A: 2, 4, H: 25); Elaphostrongylus (H: 1); Parelophostrongylus (H: 1, 22); Pneumostrongylus (A: 3, H: 9); Varestrongylus (without diagnostic characters; P: 2, 4, 6); Pneuocaulus (A: 2); Neostrostrongylus (A: 4, 6, 21; R: 11); Spiculocaulus (H: 3); Skrjabinocaulus (A: 4, 28; R: 7); Orthostrongylus (A: 4; R: 11); Protostrongylus (H: 3; P: 2, 3, 5, 6, 19). Internodes beginning basally are designated 1–12: 1 (A: 1, 4, 8, 27, 28); 2 (A: 6, 18, 28; H: 9); 3 (A: 4, 12, 13, 17, 20, 23); 4 (A: 15, 16, 26; H: 11); 5 (A: 3, 21, 22, 24; H: 5; R: 18); 6 (H: 2, 3, 22); 7 (A: 10); 8 (A: 6, 4; H: 5, 11; R: 8); 9 (A: 3, 7, 22); 10 (A: 19, 25; H: 9); 11 (A: 2, 14); 12 (A: 1, 5).

![Table V. Consistency indices and number of steps on the tree for the 28 characters used in generic- and species-level analyses of Protostrongylidae.*](https://example.com/table_v.png)
boughtoni Goble and Dougherty, 1943 and Prostrongylus pulmonalis (Frölich, 1802) are basal and putative sister species. Two additional groups are diagnosed: (1) Prostrongylus hobmaieri (Schulz, Orlow, and Kutass, 1933) and S. leuckarti are sister species; and (2) Prostrongylus stilesi Dikmans, 1931 is the sister for Prostrongylus rushi Dikmans, 1937, Prostrongylus raiilieti (Schulz, Orlow, and Kutass, 1933), and Prostrongylus rufescens (Leuckart, 1865).

Based on CI values for individual characters (Table V), characters 1, 10, 12, 13, 15–17, 20, 23, 24, 26, and 27 were the most informative (CI = 100%), whereas characters 2, 6, 9, 18, and 22 had low CI values (<50%). These were relatively consistent in both the generic and species-level analyses.

The major clades and subclades were diagnosed in both generic and species-level analyses, although relative position in respective trees was variable (Figs. 1, 2). Both analyses placed the Muelleriniae and Elaphostrongylinae as sister groups. Varastron- 

\[ \text{Protostrongylus} + \text{Pneumostomum} \]

however, were either the sister of the Muelleriniae + Elaphostrongylinae (Fig. 2) or excluded from this subclade to be placed basal to the remaining protostrongylid genera (Fig. 1). Protostrongylus was consistently placed in the crown of both trees.

When the Crenosomatidae or Skrjabingyliidae were used as outgroups either together or independent from the Metastronychidae, results were characterized by a high level of instability and ambiguity. Multiple outgroups did not aid in resolution of the tree(s), and results of these analyses (not shown) were highly inconsistent, both in the numbers of MPTs and in the relationships postulated among genera and species. Additionally, inclusion or exclusion of species from the analysis influenced the structure of the major clades, subfamily groupings, e.g., subclades, and in species relationships as depicted in Figure 2. In contrast, stability in tree topology was observed with the Metastronychidae designated as the sole outgroup in both generic and species-level analyses.

Parasite-host relationships

Host-group taxa at the level of family, subfamily, tribe, or species were mapped onto the parasite phylogeny derived from species-level analysis (Fig. 3). Mapping and optimization (CI = 0.82) indicates the following: (1) Cervidae are basal hosts for protostrongylids; (2) a minimum of 3 independent colonization events from cervids to the Caprinae (including the Caprini and Rupicaprinae) are postulated; and (3) additional host-switching is recognized with respect to the distributions of U. pallikuukensis, P. calcaratus, O. macroti, and P. hobmaieri.

The structure of this tree, relative to the species of protostrongylids examined, suggests that distinct groups of Protostron- 

\[ \text{Protostron-} \]

yulus spp. occur in Caprini and in Lagomorpha, but basal host associations cannot yet be resolved for this assemblage (Fig. 3). These overall relationships were consistent with mapping of host groups onto the generic-level phylogeny (not shown).

DISCUSSION

Phylogeny for Protostrongylidae

Monophyly for the Protostrongylidae is corroborated by the current analysis. The putative subclades diagnosed in the present analysis (Figs. 1, 2) correspond in general to the subfam-

15), ctenidia-like ribs on the spicules (16), and a consistently trilobed dorsal ray (26).

The second major clade (Fig. 2) contained N. linearis, P. kadenazii, and S. sofievii as species basal to a group consisting of Orthostrongylus macroti (Dikmans, 1931) plus a subclade with 7 species of Protostronychus and S. leuckarti and is weakly diagnosed by the structure of the crura and the telamon (characters 3 and 4). The O. macroti + Protostronychus subclade is diagnosed by first-stage larvae with spiked tails (character 1). Within the Protostronychus subclade, Protostronychus

FIGURE 2. Phylogenetic relationships for selected species of Protostrongylidae Leiper, 1926, particularly those in the genera Varastronychus Bhalerao, 1932 and Protostrongylus Kamensky, 1905 as inferred by parsimony analysis of morphological characters. Represented is the single most parsimonious tree (CI = 0.582). Character support for terminal taxa and internodes is defined below (nodes are numbered) and includes apomorphy (A) and homoplasy as convergence or parallelism (H) and reversal (R). Terminal taxa are labeled according to species-level nomenclature: outgroup, Metastronychus apri; ingroup: Muelleria apri (A: 2; H: 25); Cystocaulus ocreatus (H: 2); Umingmakstronychus pallikuukensis (R: 6); Elaphostrongylus cervi (H: 25; R: 8); Par- 

\[ \text{Protostrongylus} + \text{Pneumostomum} \]

elastronychus odocoilei (H: 3, 22); Pneumostrongylus calcaratus (A: 3; H: 9); Varastronychus alpinae (without diagnostic characters); Varastronychus sagittatus (without diagnostic characters); Varastronychus pneumonicus (A: 2); Neostonychus linearis (H: 2, 7, 21); Pneumo- 

\[ \text{Protos-} \]

cuarus kadenazii (A: 3, 22; H: 2); Skrjabinocaulus sofievii (A: 4, 28); Or- 

\[ \text{Or-} \]

thostrongylus macroti (A: 4; R: 11; H: 19); Spiculocaulus leuckarti (R: 14); Protostrongylus hobmaieri (A: 2); Protostrongylus rufescens (R: 2); Protostronychus raiilieti (without diagnostic characters); Protostronychus rushi (without diagnostic characters); Protostronychus stilesi (without diagnostic characters); Protostronychus boughtoni (H: 2, 19); and Protostronychus pulmonalis (without diagnostic characters). Internodes beginning basally are designated 1–19: 1 (A: 1, 6, 27, 28); 2 (A: 8, 28); 3 (A: 10; H: 2, 3, 22); 4 (H: 4; R: 6, 28); 5 (H: 9, 18); 6 (A: 12, 13, 17, 20, 23); 7 (A: 4, 15, 16, 26; H: 11); 8 (A: 3, 24; H: 4, 5, 21, 22; R: 18); 9 (A: 3, 4; H: 5, 22); 10 (A: 4, 6; H: 11); 11 (A: 14, 25; H: 9); 12 (A: 1, 5; H: 7); 13 (A: 3); 14 (H: 2); 15 (A: 3); 16 (A: 2); 17 (R: 6); 18 (R: 5); 19 (H: 18).
though the spicule structure may, as an autapomorphy, distinguish Pneumostrongylus from Varestrongylus, this feature as well as the morphology of the crura are apparently not informative as phylogenetic characters in diagnosing the 2 genera. The synapomorphies for the subclade indicate paraphyly of Varestrongylus if Pneumostrongylus is excluded, suggesting a necessity to modify the current classification and to reduce the latter as a synonym (see Boev, 1975).

Pneumostrongylus has been a confusing taxon in protostrongylid systematics as many nematodes currently classified in other genera have, in the past, been referred to this genus. Varestrongylus alpenae from the lungs of the white-tailed deer Odocoileus virginianus Zimmermann was originally described as a species of Pneumostrongylus. Similarly, P. tenuis was originally described by Dougherty (1945) as a species of Pneumostrongylus. Parelaphostrongylus was not erected until 1950 (Boev and Schulz, 1950), and Pneumostrongylus would have been the most accurate diagnosis at the time of Dougherty’s original description. Dougherty and Goble (1946) erected Lep- tostrongylus to accommodate V. alpenae and Varestrongylus capreoli (Stroh and Schmid, 1938). The classification of Boev (1975) appears to be most commonly used at present. Here, Pneumostrongylus is restricted to 2 species, P. calcarius and Protostrongylus cornigerus Ortlepp, 1962.

The grouping of S. leuckarti with the Protostrongylus subclade indicates that Protostrongylus may also be paraphyletic if Spiculocoecus is excluded. Boev (1975) included Spiculocoecus with Protostrongylus in the Protostrongylinae. The main difference between the 2 genera, as described by Boev and others (Dougherty and Goble, 1946; Anderson, 1978) is the much longer, filiform spicules in Spiculocoecus. The characters used in our analysis indicate that Spiculocoecus may be a synonym of Protostrongylus despite the autapomorphic feature of the longer spicules.

Similarly, Orchostrongylus was erected to separate O. macrotis from other species of Protostrongylus because of the elaborately developed telamon in the former (Dougherty and Goble, 1946). Although we hypothesize that O. macrotis is basal to the Protostrongylus subclade, it is likely that the only species in this genus, O. macrotis, could be classified in Protostrongylus, as it was originally named by Dikmans (1931). Our phylogenetic hypotheses do not correspond to the classification of Boev (1975), who grouped Orchostrongylus with Neostrongylus in the Neostongylinae. The key to subfamilies provided by Boev (1975) indicates that the spicules are distinctly unequal in Neostongylinae. The spicules are not distinctly unequal in O. macrotis. The complex form of the telamon in both genera has also been used to group them into 1 subfam- ily. This is likely a convergent feature as the morphology of the telamon differs greatly between these 2 taxa.

Evidence for the monophyly of the elaphostrongyline subclade is based on an extensive suite of synapomorphic characters. The predilection site for nervous or skeletal muscle tissue rather than the lungs, bronchi, or bronchioles (e.g., Anderson, 1992; Hemmingsen et al., 1993) may have led to selection and structural modification as the elaphostrongylines became adapted to their unique habitat.

It has been postulated by various authors that percutaneous modes of transmission are primitive among nematode parasites of vertebrates; in the Metastrongylidae, when maturation of...
nematodes occurred at an earlier stage in their tissue migration, the parasites were able to colonize deep tissue sites (Adamson, 1986; Durette-Desset et al., 1994). Members of the metastrongylid Skrjabinylidae parasitize the sinuses of mustelids. These have been considered to be a group that became isolated in a specific site in the host, whereas other taxa colonized the lungs (Anderson, 1982). The Elaphostrongylinae have also become specialized in a nonpulmonary site, although the evolutionary effects of competition from other metastrongyloids in the lungs are unclear.

**Additional phylogenetic characters**

The precise functions of the structural characters in the Elaphostrongylinae and in other taxa are presently unknown. Unfortunately, it was not possible to obtain additional suitable morphological data for the cephalic papillae and stoma in each of the ingroup taxa. Although en face observations using light microscopy are useful, more detail is observable by scanning electron microscopy (SEM). Despite the availability of SEM descriptions for 3 genera (Gibbons et al. [1991]; Carreno and Lankester [1993] for Elaphostrongylus and Parelaphostrongylus; Hoberg et al. [1995] for Umingmakstrongylus), there are no data for most other genera. Obvious differences are apparent between Umingmakstrongylus and the Elaphostrongylinae, and our preliminary observations on other taxa (data not shown) indicate considerable differences among some species in Protostrongylus as well as in other genera (e.g., character 19). There is clearly also a need for SEM studies of first-, second-, and third-stage larvae, with emphasis on the morphology of the tail.

There are presently no DNA sequences available for protostrongylid nematodes that can be used in phylogenetic analysis. However, preliminary analyses using protein electrophoresis have indicated differences between Protostrongylus spp. and other lungworms (Cutillas et al., 1995). Similarly, the use of isoelectric focusing has demonstrated similar isoelectric points between adults and larvae of presumed single species (Steen et al., 1994). Recently, internal-transcribed spacer (ITS) sequences were used to distinguish several Metastrongylus spp. in a sympatric population (Leignel et al., 1997). In the latter study, ITS sequences were used to avoid the use of morphological characters to identify phenotypically similar species. Similar sequences would also be useful in analyses of the Protostrongylidae, particularly within the genus Protostrongylus.

**Supraspecific taxa or species in phylogenetic analysis**

The major clades and subclades were diagnosed in both generic and species-level analyses, although their relative position in respective trees was variable (Figs. 1, 2). Matrices used to explore relationships were largely similar (Tables III, IV) but differed in using supraspecific (Fig. 1) versus species-level taxa (Fig. 2) as terminal groups in respective analyses. This influenced character coding, particularly at the generic level where multistate taxa (Protostrongylus and Varestrongylus) in this analysis were coded as polymorphic for a limited number of characters (Table III).

Recent studies have discussed the problems and the range of proposed solutions associated with examining relationships of higher-level or supraspecific taxa (e.g., Yeates, 1995; Binida-Emonds et al., 1998; Wiens, 1998). Although conclusions and recommendations from these studies are not entirely in agreement, it is clear that the strength of phylogenetic hypotheses is dependent on inclusion of data for variable characters and their distribution within taxa (Wiens, 1998).

Whereas a complete species-level analysis might be preferred, it is also clear that this is often not practical or possible. When supraspecific taxa are used as terminals in phylogenetic analysis, methods that infer ancestral states, e.g., the IAS method, for polymorphic (multistate) taxa are preferable to those that employ estimates of primitive or derived states or include polymorphism and may provide more accurate results (Yeates, 1995; Wiens, 1998). Binida-Emonds et al. (1998) found that such an ancestral method provided a strong and justified alternative approach to using exemplars (1 or more species-level representatives of a higher taxon) in estimates of higher-level phylogeny. In contrast, Wiens (1998) advocated using species as terminals (splitting of higher taxa) whenever possible and that methods using higher-level taxa alone performed relatively poorly based on a simulation approach to examining the problems of coding and taxon representation.

The current study used designation of polymorphism in generic-level taxa (Table III) because previous estimates of higher-level phylogeny within the Protostrongylidae were not available. Thus, within the context of ingroup phylogeny, ancestral states could not be estimated (Wiens, 1998). This may have influenced the topology of the MPT recovered (Fig. 1), although the major subclades are largely consistent with the species-level evaluation. Whereas the protostrongylides should eventually represent a group in which complete analysis of species is tractable, as noted below, the availability of high-quality specimens in museum collections is limited. Additionally, inclusion or exclusion of species from the analysis (Table IV; Fig. 2) influenced the structure of the major clades, subfamily groupings, e.g., subclades, and in relationships for species, often yielding highly ambiguous results. Thus, it is clear that an exemplar method that uses single or limited numbers of species to represent large higher-level taxa may result in erroneous estimates of relationship, and phylogenetic structure will vary as a function of the species included or excluded from the analysis (Binida-Emonds et al., 1998; Wiens, 1998).

**Parasite–host coevolution**

Putative patterns of parasite–host coevolution (collectively cospeciation and coadaptation) can be elucidated initially by examining host-distribution relative to a phylogeny for a parasitic group (e.g., Brooks and McLennan, 1993). This has been explicitly examined for the Elaphostrongylinae and their cervid hosts (Platt, 1984; Carreno and Lankester, 1994), but critical coevolutionary studies of other protostrongylids are lacking. Protostrongylids are widespread in Cervidae, Caprini, and Lagomorpha but rare among such ruminant groups as the Rupicaprinae, Alcelaphinidae, and Antelopinae, and are unknown in suids, tragulids, and giraffids.

Mapping of host taxa onto the species-level phylogeny for the Protostrongylidae unequivocally revealed Cervidae as the basal hosts for the family and supports a history of cospeciation involving the elaphostrongylines (see Platt, 1984) and other genera (Fig. 3). Colonization of bovids of the subfamily Ca-
prinai and those in the tribes Caprini and Rupicaprina from a
cervid source is postulated with respect to the Muelleriinae, V.
pneumonicus, and N. lineai. Further, host-switching appears
to be compatible with the occurrence of Muellerius in rupica-
prine hosts, U. pallikauensis in Ovis moschatus (Zimmer-
mann), P. calcarius in African Antelopinae and Alcelaphinae,
O. macrotis in Antilocapra americana (Ord), and P. hobi mari-
eri in Rupicaprina.

Patterns of cospeciation are also suggested by the distribution of
Protostrongylus in either Lagomorpha or in the Caprini, but
total associations for this clade are unresolved in the con-
text of the current study (Fig. 3). The putative sister-group rela-
tionship of P. pulmonalis and P. boughtoni, the only 2 species
examined in this study that parasitize lagomorphs, may be in-
dicative of cospeciation with a distinct group in Protostrongy-
lus. A more inclusive analysis of the genus, including other
species known to parasitize lagomorphs is necessary, e.g., Pro-
ostostrongylus curricularis (Joyeux and Gaud, 1946) Schulz and
Kadenazii, 1949; Protostrongylus orytological Babos, 1955; Pro-
ostostrongylus tauricus Schulz and Kadenazii, 1949; Protostrong-
ylus terminalis (Passerini, 1884) Schulz, Orlov, and Kutass,
1933; and Protostrongylus kamenskyi Schulz, 1930. There are
few suitable museum specimens available for these species as
well as for other protostrongylids, further constraining the pos-
sibility of a more comprehensive analysis.

Cospicuation of protostrongylids following independent col-
ization of the Caprini may have involved both species of
Protostrongylus (including Spiculocaulus) and those in the
Muelleriinae (Fig. 3). Host-specificity appears limited, however,
with many species being reported from a wide range of caprine
hosts (Boev, 1975), an observation that will confound clear res-
olution of a history for diversification of protostrongylids in
wild sheep, goats, and allied bovids.

Although clearly associated basally with the Caprini, the host
associations for constituent genera and species of the Mueller-
iiinae continue to be resolved incompletely (see Hoberg et al.,
1995). The current tree suggests that the occurrence of Muel-
leriina in rupicaprine hosts represents colonization. Understan-
ding the history of Umingmakstrongylus, however, is in part
linked to resolution of phylogenetic relationships among the
Caprini and Rupicaprina and the placement of O. moschatus
(Thenius, 1980; Pasitschniak-Arts et al., 1994; Hoberg et al.,
1995; Groves and Shields, 1996). Muskoxen are not considered
to be close to the Caprini (wild sheep and goats, hosts for Cy-
tocaulus, the sister group of Umingmakstrongylus) based on
the most recent phylogenetic studies of the Caprinae (Groves
and Shields, 1996). Alternative evidence has suggested an associa-
tion with the takin (Budorcas taxicolor Hodgson), considered
by some as the nearest extant relative of muskoxen with place-
ment in the tribe Ovibovini (Pasitschniak-Arts et al., 1994). As
indicated by Hoberg et al. (1995), however, the occurrence of
a species of Varestrongylus in the takin provides no phyloge-
netic information relative to host phylogeny or the occurrence of
Umingmakstrongylus in muskoxen. Hypotheses presented by
Hoberg et al. (1995) for colonization of muskoxen from a cap-
rine source, e.g., wild species of Ovis Linnaeus, during the
Pleistocene are not refuted.

The Protostrongylidae evolved primarily in ruminants
(Dougherty, 1949), and our results further support Dougherty’s
hypothesis that parasitism of lagomorphs was a host-switching
event that occurred from a ruminant ancestor. Caprine hosts
(particularly sheep and goats) are very common for various
distantly related protostrongylids, and 2 independent lineages
appear to have coevolved with this group; cervids are also hosts
of diverse genera. Recognition of a basal association for Cer-
vidae and protostrongylids, however, still does not completely
address the broader coevolutionary history for ruminants and
these lungworms. This situation exists because of the array of
largely dissimilar hypotheses that have been developed for the
phylogeny and interfamilial relationships for the pectora-
ruminants that serve as the primary hosts for genera and species
of protostrongylids (e.g., Groves and Grubb, 1987; Janis and
Scott, 1988; Gentry and Hooker, 1988; Kraus and Miyamoto,
1991). Radiation of the pectoral ruminants is estimated to have
occurred rapidly between 23 and 28 million years ago (Kraus
and Miyamoto, 1991), and origins of the protostrongylids may
be eventually linked to this diversification assuming evidence
for a history of coevolution.

Further confounding development of a comprehensive hy-
pothesis for host–parasite coevolution, however, is the recog-
nition that data for host occurrence and specificity may be
limited in some genera. Few cross-infection experiments have
been undertaken to determine host specificity, and it is not
known whether some species can infect distantly related hosts.
For ex-
ample, the initial description of Protostrongylus coburni Dik-
mans, 1935 was based on parasites found in the lungs of white-
tailed deer (O. virginianus). The illustration of the first-stage
larvae, however, showed dorsal-spined forms not characteristic
of the genus Protostrongylus and were likely those of Vare-
strongylus alpenae. Larvae with the spike-shaped tail character-
istic of Protostrongylus spp. were not shown (Dikmans, 1935).
In a recent examination of parasites described as P. coburni
(USNPC lot 46229 but not the types 34065), we could not dis-
tinguish these parasites from P. boughtoni of snowshoe hares
(unpublished observations).

Thus, the possibility of less strict host specificity than has
previously been known should be further investigated in the
Protostrongylidae. This, and more complete treatment of spe-
cies of Protostrongylus and other genera will promote a refined
understanding of the evolutionary history of these significant
mammalian parasites.

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