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Research Note

A Molecular View of the Superfamily Dioctophymatoidea (Nematoda)

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ABSTRACT: Monophyly of the superfamily Dioctophymatoidea was assessed based on analyses of DNA sequence variation among 3 of 4 constituent genera (5 species). Represented is the first molecular phylogenetic evaluation of the Dioctophymatoidea using maximum parsimony, maximum likelihood, and Bayesian inference of 18S nuclear DNA (786 base-pair [bp] segment) and mitochondrial cytochrome oxidase 1 (293 bp) genes. Dioctophymatoidea is monophyletic and includes a clade with Dioctophyme renale and Eustrongylides ignotus (Dioctophymatoidea) as the sister of Soboliphyme baturini, Soboliphyme jamesoni, and Soboliphyme abei (Soboliphymatidae). Within Soboliphymatidae, S. baturini is the sister of S. jamesoni and S. abei.

KEY WORDS: nematode, Dioctophymatoidea, Dioctophyme renale, Eustrongylides ignotus, Soboliphyme baturini, Soboliphyme jamesoni, Soboliphyme abei, phylogenetic, cytochrome oxidase 1, 18S.

The dioctophymatoids (order Dioctophymatida, superfamily Dioctophymatoidea) are an enigmatic group of nematodes morphologically by well-developed multipolar cells connecting the body wall and the intestine, 8 longitudinal striae, and the presence of a caudal bursa in males (Karmanova, 1986). All dioctophymatoids are gastrointestinal parasites of birds and mammals as adults, and they utilize oligochaetes as intermediate hosts (Anderson, 2000). Molecular tests of monophyly of dioctophymatoids have been lacking. Dioctophymatoidea is regarded as a basal group within the Nematoda based primarily on morphological criteria (Karmanova, 1986). Among the dioctophymatoids (Rusin et al., 2003), the phylogenetic relationship of a single species, Soboliphyme baturini Petrov, 1930, has been explored using the nuclear small-subunit ribosomal RNA gene (18S) and morphological characters. That study supported a sister-group relationship with the Trichocephaloidea (Trichinella spiralis (Owen, 1835) and Trichurus muris Schrank, 1788).

As currently constituted, the Dioctophymatoidea is composed of 2 families, Soboliphymatidae, including 9 species in the genus Soboliphyme Petrov, 1930, and the Dioctophymatidae including the monotypic genus Dioctophyme renale (Goeze, 1782), 11 species in the genus Eustrongylides Jagerskiold, 1909, and at least 6 species in the genus Hystrichis Dujardin, 1845. Species of Soboliphyme are primarily stomach-dwelling nematodes of insectivores throughout Eurasia and North America, and 1 species, S. baturini, is found chiefly in mustelids (Ribas and Casanova, 2004). Dioctophyme renale (giant kidney worm) is found primarily in the kidneys of mink (Neovison vison [Schreber]), and other carnivores throughout the world (Measures, 2001). Species of Eustrongylides and Hystrichis are inhabitants of the proventriculus in avian hosts and are known to be responsible for large mortality events throughout Eurasia and North America (Cole, 1999).

In this phylogenetic study, we use DNA sequences of 5 dioctophymatoids and 3 Trichocephaloidea and include the outgroup Xiphinema americanum as identified in broader analyses (e.g., Blaxter et al., 1998; Rusin et al., 2003; Meldal et al., 2007). We test whether the Soboliphymatidae (Soboliphyme spp.) and Dioctophymatidae (Dioctophyme and Eustrongylides) are reciprocally monophyletic within the Dioctophymatoidea. Molecular data are used for the first time to test current assumptions of monophyly of Dioctophymatoidea (Dioctophyme, Eustrongylides, and Soboliphyme) derived from previous interpretations of morphological data (Karmanova, 1986).

Specimens of nematodes are as follows: (1) Adult D. renale (n = 1), collected by G. H. Parker, Laurentian University, Ontario, and preserved in 95% ethanol after extraction from the kidney of an American mink (N. vison). (2) Larval Eustrongylides ignotus (n = 1) from western mosquito fish (Gambusia affinis [Baird and Girard, 1853]) supplied by E. Marsh-Matthews of the Sam Noble Oklahoma...
Table 1. Nematode specimens obtained through the Beringian Coevolution Project (BCP), collaborators, and GenBank used in the assessment of the superfamily Dioctophymatoidea. Included are the host, locality, collection year (if known), MSB accession number, and GenBank accession numbers.

<table>
<thead>
<tr>
<th>Taxon ID</th>
<th>Host</th>
<th>Location</th>
<th>Collection year</th>
<th>MSB #</th>
<th>18S Accession #</th>
<th>COI Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soboliphyme baturini 1</td>
<td>Martes caurina</td>
<td>Vancouver Island, Canada</td>
<td>2005</td>
<td>NK128176</td>
<td>EU394725</td>
<td>EU128176</td>
</tr>
<tr>
<td>S. baturini 2</td>
<td>M. americana</td>
<td>Fairbanks, Alaska</td>
<td>unknown</td>
<td>NK128108</td>
<td>EU394726</td>
<td>EFS19532</td>
</tr>
<tr>
<td>S. baturini 3</td>
<td>M. zibellina</td>
<td>Kamchatka, Russia</td>
<td>unknown</td>
<td>NK159573</td>
<td>EU394727</td>
<td>EFS194161</td>
</tr>
<tr>
<td>Soboliphyme jamesoni</td>
<td>Sorex tundrensis</td>
<td>Yakutsk, Russia</td>
<td>2006</td>
<td>NK139168</td>
<td>EU394728</td>
<td>EFS19533</td>
</tr>
<tr>
<td>S. jamesoni</td>
<td>S. roboratus</td>
<td>Yakutsk, Russia</td>
<td>2006</td>
<td>NK139584</td>
<td>EU394729</td>
<td>EFS19534</td>
</tr>
<tr>
<td>Soboliphyme abei</td>
<td>S. unguiculatus</td>
<td>Hokkaido, Japan</td>
<td>2003</td>
<td>NK159581</td>
<td>EU394730</td>
<td>EFS19535</td>
</tr>
<tr>
<td>Dioctophyme renale</td>
<td>Neovison vison</td>
<td>Sudbury, Ontario, Canada</td>
<td>2005</td>
<td>NK159579</td>
<td>EU394731</td>
<td>EU394733</td>
</tr>
<tr>
<td>Eustrongylides ignotus</td>
<td>Gambusia affinis</td>
<td>Norman, Oklahoma</td>
<td>2005</td>
<td>NK159580</td>
<td>EU394732</td>
<td>NA</td>
</tr>
<tr>
<td>Trichinella britovi</td>
<td>Rattus norvegicus</td>
<td>Isola del Dran Sasso, Italy</td>
<td>1985</td>
<td>NA</td>
<td>AY851257</td>
<td>DQ007892</td>
</tr>
<tr>
<td>Trichinella murrelli</td>
<td>Ursus americanus</td>
<td>Pennsylvania</td>
<td>1982</td>
<td>NA</td>
<td>AY851259</td>
<td>DQ007894</td>
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<tr>
<td>Trichinella nativa</td>
<td>U. maritimus</td>
<td>Svalbard, Norway</td>
<td>1984</td>
<td>NA</td>
<td>AY851256</td>
<td>AB252966</td>
</tr>
<tr>
<td>Xiphinema index</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>AM086679</td>
<td>AY382608</td>
</tr>
</tbody>
</table>

Museum of Natural History. (3) Adult S. baturini (n = 3) from North American marten (Martes caurina, Martes americana) and Asian sable (Martes zibellina) through the efforts of the Beringian Coevolution Project (BCP) (Hoberg et al., 2003; Cook et al., 2005) and N. Tranbenkova of the Kamchatka Institute of Ecology and Nature Management. (4) Adult Soboliphyme abei (Asakawa et al., 1988) (n = 1) from the stomach of a long-clawed shrew (Sorex unguiculatus Dobson), provided by M. Asakawa of Rakuno Gakuen University, Japan. (5) Adult Soboliphyme jamesoni Read, 1952 (n = 2) from Sorex tundrensis Merriam and Sorex roboratus Hollister near Yakutsk, Russia, in the summer of 2006. Each nematode was subsampled from the midsection of the body for molecular sequencing, whereas the head and tail of individuals were archived as physical vouchers deposited in the Museum of Southwestern Biology (MSB) (Table 1). Specimens were frozen or stored in either 70% or 95% ethanol.

Ethanol-preserved specimens were prepared for extraction by soaking in a water bath for 30 min followed by a 10 min spin in a vacuum centrifuge. Total genomic DNA was extracted from individual worms using a commercial kit (AquaPure Genomic DNA isolation kit, Bio-Rad Laboratories, Hercules, California). A 768 base-pair (bp) region of 18S was amplified using primers Soboliphyme baturini (modified from Rusin et al., 2003). A 293 bp region of cytochrome oxidase 1 (COI) was amplified with the primers Soboliphyme baturini (modified from Rusin et al., 2003). We were unable to sequence COI for E. ignotus.

Total volume for polymerase chain reaction (PCR) was 25 μl, consisting of: 14.25 μl of H₂O, 1 μl of 10 μM primer each, 1 μl of DNA template (~5 ng/μl), 2.5 μl each of 25 mM MgCl₂, 10 mM deoxynucleotide triphosphates dNTPs, and 10X PCR Buffer II, and 0.25 μl Taq (5 units/μl, Amplitaq). PCR analyses were run on PTC-200 thermocyclers (MJ Research) with the following parameters: initial denaturation at 94°C for 60 sec, subsequent denaturation for 30 sec, annealing at 53°C for 15 sec, and extension of 72°C for 30 sec. These steps were repeated for an additional 34 cycles, followed with a final extension of 72°C for 10 min. Product was visualized through electrophoresis on a 0.8% agarose gel and cleaned using 30% polyethylene glycol (PEG) and a QiAQuick Wash (Qiagen Inc.) cleanup kit. BigDye® Terminator v. 3.1 (Applied Biosystems) was used for cycle sequencing reactions. Excess dyes and primer were removed using Sephadex® G-50 spin columns or sodium acetate ethanol wash (Applied Biosystems). Forward and reverse strands were sequenced using an ABI PRISM® 3100 Genetic Analyzer. Sequences were aligned using ClustalW (Chenna et al., 2003). Sequences were deposited in GenBank (Table 1).

Xiphinema americanum Thorne and Allen, 1950 (Longidoridea) (GenBank AM086679 for 18S, AY382608 for COI) was used to root the trees because the genus has been recovered in a sister clade to the Trichocephaloidea and the Dioctophymatoidea (Rusin et al., 2003; Meldal et al., 2006). Since Trichocephaloidea is regarded as the putative sister of the dioctophymatoids (Rusin et al., 2003), species of Trichinella (GenBank accession number for 18S, COI), Trichinella britovi Pozio, La Rosa, Murrell, and Lichtenfels, 1992 (AY851257, DQ007892), Trichinella nativa Britov and Boev, 1972

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(AY851256, AB252966), and Trichinella murrelli Pozio and LaRosa, 2000 (AY851259, DQ007894) were included to test monophyly of the ingroup.

Both maximum parsimony (MP) and maximum likelihood (ML) optimality criteria were used for phylogenetic reconstruction using PAUP* (Swofford, 2002), considering all characters as unordered with 4 possible states (A, C, G, T). Under both optimality criteria, a branch and bound search was performed using concatenated 18S and COI sequences. A partition homogeneity test (PAUP) resulted in a $p$-value $> 0.05$, suggesting that it was appropriate to concatenate the 18S and COI sequences. Node support was evaluated with nonparametric bootstrap methodology using 5,000 replicates for MP (MPB) and 1,000 for ML (MLB) (Felsenstein, 1985). Modeltest v. 3.06 (Posada and Crandall, 1998) was used to determine the appropriate nucleotide substitution model for the concatenated ML matrix, using the Akaike corrected (AICc) option. The model (TIM + I + G) plus invariant sites (I = 0.4041) and gamma distribution of variable sites (0.3256) was selected as the best model.

The Markov Chain Monte Carlo (MCMC) sampling procedure was performed using the program BayesPhylogenies (Pagel and Meade, 2004) to estimate the posterior probability (PP, expressed as a percentage) of phylogenetic trees. We used a general likelihood–based “mixture model” (MM) based on the general time-reversible model (GTR) of gene-sequence evolution to estimate the likelihood of each tree. To find the best “mixture model” of gene-sequence evolution, we determined the likelihood of the trees by first using a simple GTR matrix, then using a GTR matrix plus the gamma-distributed rate heterogeneity model (GTR + G), and then continuing to add up to 6 GTR + G matrices. We ran $5 \times 10^6$ generations and 4 Markov chains, sampling every thousandth tree to assure that successive samples were independent. The first 500 trees were removed to avoid including trees sampled before convergence of the Markov Chain.

Figure 1. Maximum likelihood tree estimated from partial sequences of the combined 18S and COI genes (1,060 bp) indicates monophyly of 3 genera of the dioctophymatoids and suggests that Soboliphymatidae is sister to Diocastromatidae. Nodal support values are (left to right): ML bootstrap (1,000 replicates), MP bootstrap (5,000 replicates), and BayesPhylogenies posterior probabilities expressed as percentages. The outgroup is Xiphinema americanum.
Similar topologies were found for each of the phylogenetic analyses (MP, ML, and Bayesian). High support values (MPB = 100, MLB = 100, PP = 100) were found for a clade composed of species of Dioctophyme, Eustrongyloides, and Soboliphyme, which is consistent with monophyly of Dioctophymatoidea (Fig. 1). The relationship of the genus Hystrichis has yet to be determined with molecular data, but traditional morphological analyses have placed it with the Dioctophymatoidea (Karmanova, 1986). Within the Dioctophymatoidea, Soboliphymatidae (species of Soboliphyme) is monophyletic (MPB = 92, MLB = 91) and the sister group of the Dioctophymatidae (Dioctophyme and Eustrongyloides; MPB = 85, MLB = 89). These molecular phylogenetic results corroborate prior hypotheses based on comparative morphology (Railliet, 1915; Anderson and Bain, 1982; Karmanova, 1986; Anderson, 2000). Species of Trichinella (superfamily Trichinelloidea) included in this study were monophyletic (MPB = 100, MLB = 100, PP = 100) and sister to Diocophymatoidea, which is congruent with the topology recovered for both clades in the Nematoidea phylogeny (Meldal et al., 2007). A comprehensive assessment of diversification that includes broader taxon sampling for all extant species of the Dioctophymatoidea and additional independent genes should be completed. Additional independent genes should also be utilized to resolve relationships at this level.

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LITERATURE CITED


