INTRASPECIFIC VARIATION IN THE rDNA ITS LOCI OF 37-COLLAR-SPINED ECHINOSTOMES FROM NORTH AMERICA: IMPLICATIONS FOR SEQUENCE-BASED DIAGNOSES AND PHYLOGENETICS

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ABSTRACT: The recent finding of the 37-collar-spined Echinostoma revolutum in North America prompted rDNA nucleotide sequence comparisons between this worm and the sympatric Echinostoma trivolvis. Three isolates of E. revolutum from distinct sites and 2 isolates of E. trivolvis collected from a single site were used in this analysis. Sequence data were compared to those from previously sequenced members of the 37-collar-spine group. The 3 North American isolates of E. revolutum were found to be identical, but they differed from Eurasian isolates of E. revolutum at 9 of the 1,006 sites sequenced. Further, 1 of the E. trivolvis isolates studied herein was identical to the published sequence for this species, but 6 nucleotide changes were observed in the second E. trivolvis isolate. Restriction fragment length polymorphisms at this locus support the nucleotide differences found between the E. trivolvis isolates. The degree of intraspecific variation detected raises questions regarding the utility of the internal-transcribed spacer regions of the ribosomal DNA repeat for taxonomic diagnosis and in phylogenetic studies for poorly differentiated groups, such as the 37-collar-spined congeners.

Echinostoma (Trematoda: Echinostomatidae) consists of over 60 species of flatworms that parasitize snail and vertebrate hosts during their complex life cycle. All members of this genus possess a circumoral collar surrounded by a double, uninterrupted crown of spines, and the number of collar spines serves as a defining taxonomic character (Yamaguti, 1971). Echinostoma revolutum Froelich (1802), the type-species, bears 37 collar spines. During the past century, taxonomic descriptions of members of the 37-collar-spine group have led to considerable ambiguity within the literature (Kanev, 1994). More recently, investigators have sought to clarify this confusion by considering both genetic and phenotypic characteristics of 37-collar-spined trematodes.

Significant phenotypic differences exist within and among geographic populations of trematodes, and this phenotypic diversity is reflected in the genetic separation of trematode strains (Minchella et al., 1994). The greatest resolution of trematode genetic diversity can be achieved by directly assessing the variation in DNA sequences found within and among species (Johnston et al., 1993; McManus and Hope, 1993; Rollinson et al., 1997). The internal-transcribed spacers (ITS) of the ribosomal DNA (rDNA) repeat have been a common target for studies using nucleic acid sequencing techniques (Després et al., 1992; Kane and Rollinson, 1994; Bowles et al., 1995) because of their size, copy number, and degree of conservation.

Morgan and Blair (1995) used sequence data from the ITS and 5.8S regions of the nuclear rDNA to postulate a phylogeny for the 37-collar-spine group. They obtained sequence data from the ITS1, 5.8S, and ITS2 of 7 nominal species in this group and of a distantly related 28-collar-spined echinostome. This approach allowed them to discriminate 6 distinct species; 2 of the putative species were identical in sequence to Echinostoma caproni and were thus determined to be synonymous. Whereas their analysis supported a number of relationships that are consistent with the biology of the group, the bootstrap support for the branchpoints was weak due to the limited degree of interspecific variation detected in these closely related species. Nonetheless, Morgan and Blair (1995) state that the resolution obtained through rDNA ITS sequencing can assist reclassification efforts and provide a model for work on sibling species.

The nature of the rDNA ITS regions is such that variation accumulates rapidly enough to distinguish closely related species, though intraspecific variation is more rarely encountered (Hillis and Dixon, 1991; Adlard et al., 1993; Minchella et al., 1997). An ITS2 sequence analysis of Schistosoma species found no nucleotide differences between 3 strains of Schistosoma japonicum; the only intraspecific differences detected occurred between ITS2 regions sequenced in different laboratories (Després et al., 1992; Bowles et al., 1995). Similarly, the ITS sequence analysis of the 37-collar-spined Echinostoma revealed no intraspecific variation between pairs of isolates, even pairs originating from different continents (Morgan and Blair, 1995). Curiously, as described below, our polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis at this same locus detected polymorphism between Echinostoma trivolvis worms isolated from different Helisoma trivolvis snails collected within a single pond in northern Indiana.

Sorensen et al. (1997) documented the presence of 37-collar-spined echinostomatids infecting Lymnaea elodes snails in a number of ponds in northern Indiana. Although recent redescriptions of E. revolutum (Kanev, 1994) and E. trivolvis (Kanev et al., 1995) dictate that only the latter is present in North America, the newly collected worms had the diagnostic characteristics (morphology and host use) of E. revolutum. The presence of a published sequence alignment for the ITS regions of 37-collar-spined echinostomes (Morgan and Blair, 1995) provides us the opportunity to test the utility of these data in determining the affinity of taxonomically challenging specimens.

The specific goals of this study are to determine the nature and extent of the intraspecific variation in rDNA ITS sequences among 5 samples of the 37-collar-spined Echinostoma from North America. In the case of the isolates of E. revolutum, we use these sequences to further confirm the identity of these specimens by sequencing the locus in 3 isolates from 3 different collection sites. We also report the nucleotide sequence of the 2 isolates of E. trivolvis showing evidence of intraspecific variation at this locus and document the amount of variation present therein.

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Table I. Identification of 37-collar-spined Echinostoma specimens sequenced in this study and the sites where they were collected in northern Indiana.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species*</th>
<th>Description of collection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>E. revolutum</td>
<td>Permanent, shallow inlet (86°18′8″W, 41°32′28″N)</td>
</tr>
<tr>
<td>298</td>
<td>E. revolutum</td>
<td>Temporary, wooded pond (85°38′32″W, 41°10′38″N)</td>
</tr>
<tr>
<td>318</td>
<td>E. revolutum</td>
<td>Permanent, shallow bay (85°41′30″W, 41°21′17″N)</td>
</tr>
<tr>
<td>11B, 12B</td>
<td>E. trivolvis</td>
<td>Permanent, open pond (87°3′8″W, 40°27′13″N)</td>
</tr>
</tbody>
</table>

* Specific nomenclature based on host-use and morphology criteria.

**Materials and Methods**

Sample collection and DNA extraction

We studied trematodes from naturally infected snail hosts originating at 4 sites in northern Indiana. The echinostome species were identified according to host-use and key morphological characteristics of the adults and cercariae (Kaney et al., 1995; Sorensen et al., 1997). *Echinostoma revolutum* isolates, 293, 298, and 318, were collected from *L. elodes* in wetlands near the municipalities of South Bend, Larwill, and North Webster, Indiana, respectively, whereas the *E. trivolvis* specimens, according to host-use and key morphological characteristics of the metacercariae from experimentally infected *L. elodes* to day-old domestic chickens (*Gallus gallus dom.*), were obtained from *L. elodes* in wetlands near the municipalities of South Bend, Larwill, and North Webster, Indiana, respectively, whereas the *E. trivolvis* specimens, 11B and 12B, came from *E. trivolvis* in ponds near West Lafayette, Indiana (Table I). Adult worms were obtained by feeding encysted metacercariae from experimentally infected *L. elodes* to day-old domestic chickens (*Gallus gallus dom.*.) according to previously described techniques (Fried and Weaver, 1969). Once removed, adult worms were stored in a 10 mM Tris-HCl, 1 mM EDTA solution at -80°C. To extract DNA from these worms, we placed a single worm from each collection site into a 1.5-ml microcentrifuge tube and pulverized the fluke on dry ice using a chilled microcentrifuge pestle. The pestle was rinsed with a buffer containing 100 mM NaCl, 25 mM sucrose, 10 mM EDTA, and 2% (v/v) sodium dodecyl sulfate in 50 μl of 50 mM Tris, pH 8.0 (Brindley et al., 1989). This mixture was incubated at 65°C for 30 min. Salts were removed by spinning the mixture for 10 min, after which the gels were washed with 10% (v/v) methanol/10% (v/v) acetic acid, then transferred to 3MM Whatman filter paper, and dried under vacuum at 80°C. Kodak X-ray film was placed on the dried gel overnight and then developed. We read sequence data from the autoradiographs manually. Sequences were confirmed with a second clone of each of the 5 isolates.

**DNA amplification**

To amplify the rDNA fragment containing ITS1, 5.8S, and ITS2, we performed PCR between the forward primer ITS5 (5′ GGAAATTTAAAAGTCCGTAACAC 3′) and reverse primer ITS4 (5′ TCCTCCTTGTGGTCAGGTATCAG 3′) because these sequences exhibit strong fidelity with conserved regions of eukaryotic 18S and 28S rDNA, respectively (White et al., 1990). The PCR mixture consisted of 100 ng of template DNA, 25 pmol of each primer, 2.5 mM Mg²⁺, 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% (v/v) Triton X-100, 1% (v/v) dimethyl sulfoxide, and 1.5 units Taq polymerase in a 50-μl solution overlaid with 20 μl of sterile mineral oil. Initially, the reactions were incubated for 4 min at 94°C, and then they were subjected to 30 3-step cycles: 94°C for 1 min, 45°C for 1 min, and 72°C for 90 sec. After the final cycle, the samples were held 4 min at 72°C. A 4-μl aliquot from each of these reactions was visualized by 1% agarose gel electrophoresis to confirm successful amplification. The remainder of the products were spin-filtered with Promega Wizard PCR prep and resuspended in double-distilled H₂O.

**Endonuclease restriction digests**

In our initial assessment of intraspecific nucleotide sequence diversity among *E. trivolvis* worms, we employed PCR-RFLP procedures on isolates of this species from 15 infected snails. To do so, we used the ITS-5 and ITS-4 primers to PCR amplify rDNA containing ITS1, 5.8S, and ITS2 (this reaction was separate from the PCR amplification used in cloning). A 5-μl aliquot of this PCR product was digested with either *HaeIII* or *MseI* according to the manufacturer’s specifications at 37°C for 2 hr. The resulting restriction fragments were electrophoresed on a 1.5% agarose gel and visualized with ethidium bromide. We repeated this PCR-RFLP protocol with *E. revolutum* isolates 293, 298, and 318.

**Cloning and sequencing**

The amplified DNA was inserted into the Promega pGEM-5Zf(+) vector with T4 ligase. We used high-voltage electroporation to transform competent DH5α* Escherichia coli* cells. Positive recombinants from each of the isolates were prepared according to Sambrook et al. (1989) and electrophoresed on a 1% agarose gel to verify incorporation of the PCR product.

Sequencing of the ITS1, 5.8S, and ITS2 was accomplished with direct incorporation of α-³²P-radioisotope by using the Promega fmol DNA sequencing system according to the manufacturer’s protocols. The primers ITS-5, ITS-4, and two internal primers, ITS-300 (5′ TTAACGTTCAAGTGTGGTGT 3′) and ITS-620 (5′ GTCCGCTTAAACTATCAC 3′) were used in sequencing reactions containing 3 pmol of the primer and 1 μg of the template DNA. Products of these reactions were electrophoresed on 4% acrylamide gels at 50°C, after which the gels were washed with 10% (v/v) methanol/10% (v/v) acetic acid, then transferred to 3MM Whatman filter paper, and dried under vacuum at 80°C. Kodak X-ray film was placed on the dried gel overnight and then developed. We read sequence data from the autoradiographs manually. Sequences were confirmed with a second clone of each of the 5 isolates.

**Sequence analysis**

Previously published ITS1, 5.8S, and ITS2 *Echinostoma* sequences (Morgan and Blair, 1995) retrieved from GenBank and sequences from the 5 isolates that we read were aligned by eye using SeqApp version 1.9 (Gilbert, 1992). In cases where the sequences of 2 or more isolates were identical, the sequence from 1 of them was chosen as a representative for that group. Using the multiple sequence alignment, we created a distance matrix using the DNADIST81 algorithm of PHYLIP (Felsenstein, 1993) to determine maximum likelihood estimates of distance under the Kimura 2-parameter model (transversions: transversions = 2:1) (Kimura, 1980; Swofford et al., 1996).

We also conducted a cladistic analysis of the aligned sequences with PAUP v3.0d (Swofford, 1990). The inferred gap states were included as characters, i.e., a fifth base, in this analysis because there are only 3 instances of such gaps (positions 27, 80, and 85 in Table II), and all seem to be clear cases of a deletion in the *E. revolutum* lineage. The only alignment positions excluded from the analyses were those where ambiguities occurred in comparisons with the previously published sequences: positions 367, 553, 554, and 717 (Table II). Trees were produced under the principle of maximum parsimony using the TBR branch-swapping option of PAUP with polytomies allowed. Because it is well established that the 37-collar-spined worms form a cohesive phylogenetic unit, *Echinostoma hortense*, a 28-collar-spined echinostome, served as the outgroup for phylogenetic analyses. An exhaustive search generated all possible trees, and we used the frequency distribution of the lengths of these trees to calculate the g1 statistic that determines the degree of left skewness indicative of hierarchical structure (Swofford et al., 1996). A tree depicting the consensus hypothesis of the most parsimonious trees was created using the strict consensus rule, and bootstrap resampling with 1,000 pseudoreplicates was used with parsimony inference to assess the relative robustness of the relationships hypothesized therein.

**RESULTS**

Endonuclease restriction digests

Digestion with either *HaeIII* or *MseI* restriction endonucleases yielded restriction fragment length polymorphisms among the 5 isolates studied (Fig. 1). The *HaeIII* digest showed 2 distinct banding patterns, with *E. trivolvis* isolate 11B possessing the unique profile, whereas isolate 12B and the *E. revolutum*...
isolates were identical to each other. In the MseI digest, there were 3 distinct profiles, as the 2 E. trivolvis isolates each had unique banding patterns that differed from the common E. revolutum pattern.

Sequence alignment

The sequences of the 2 distinct isolates of E. trivolvis (designated E. trivolvis 11B and E. trivolvis 12B, hereafter) and the 3 isolates from North America were aligned with the published sequences of E. trivolvis and Eurasian E. revolutum, referred to as E. revolutum (EU) hereafter (Morgan and Blair, 1995). The sequence of E. trivolvis isolate 11B is identical to the previously published sequence of E. trivolvis, and the sequences for the North American isolates of E. revolutum were identical to one another, hereafter designated singly as E. revolutum (NA) (Table II). Genbank accession numbers (in brackets) for these isolates are E. revolutum (NA) [AF067850], E. trivolvis 11B [AF067851], and E. trivolvis 12B [AF067852]. We found 9 nucleic acid differences between E. revolutum (EU) and E. revolutum (NA), whereas there are 6 differences between E. trivolvis 11B and E. trivolvis 12B. The alignment includes 415 base pairs (bp) of ITS1, 159 bp corresponding to the 5.8S portion, and 432 bp completing ITS2, for a total of 1,006 bp. For the isolates of these 2 species, the variable sites are similarly distributed between ITS1 (12 variable sites, discounting position 367) and ITS2 (10 variable sites). The only variable positions in the 5.8S rDNA are found at positions 553 and 554. Of these 25 variable sites, 8 (5 in ITS1) are informative for

![Figure 1](image1.png)

**Figure 1.** PCR-RFLPs from North American 37-collar-spined Echinostoma species cut with HaeIII, left panel, and MseI, right panel. In each panel, lanes 1 and 2 are isolates E. trivolvis 11B and 12B, respectively, whereas lanes 3–5 are E. revolutum isolates 293, 298, and 318, in that order. The 1-kb (lane m1) and 100-bp (lane m2) size standards are in the outermost lanes.
TABLE III. Pairwise sequence differences among 37-collar-spined echinostomes.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>11B</th>
<th>12B</th>
<th>(NA)</th>
<th>(EU)</th>
<th>E. par</th>
<th>E. cap</th>
<th>E. sp. 1</th>
<th>E. hor</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. trivolvis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11B</td>
<td>0</td>
<td>0</td>
<td>0.0060</td>
<td>0.0111</td>
<td>0.0141</td>
<td>0.0090</td>
<td>0.0192</td>
<td>0.0234</td>
</tr>
<tr>
<td>12B</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.0141</td>
<td>0.0090</td>
<td>0.0101</td>
<td>0.0192</td>
<td>0.0244</td>
</tr>
<tr>
<td><em>E. revolutum</em> (NA)</td>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td>0.0091</td>
<td>0.0111</td>
<td>0.0182</td>
<td>0.0234</td>
</tr>
<tr>
<td><em>E. revolutum</em> (EU)†</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td></td>
<td>0.0080</td>
<td>0.0151</td>
<td>0.0183</td>
<td>0.1228</td>
</tr>
<tr>
<td><em>E. paraensei</em>†</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td></td>
<td>0.0172</td>
<td>0.0224</td>
<td>0.1253</td>
</tr>
<tr>
<td><em>E. caproni</em>†</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>15</td>
<td>17</td>
<td></td>
<td>0.0296</td>
<td>0.1276</td>
</tr>
<tr>
<td><em>E. Sp. I</em>†</td>
<td>23</td>
<td>24</td>
<td>23</td>
<td>18</td>
<td>22</td>
<td>29</td>
<td></td>
<td>0.1287</td>
</tr>
<tr>
<td><em>E. hortense</em>†</td>
<td>109</td>
<td>106</td>
<td>112</td>
<td>110</td>
<td>112</td>
<td>114</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers above the diagonal are genetic distance (as described in text), whereas the values below the diagonal are the actual number of nucleotide differences found.
† Sequence data for these isolates are from Morgan and Blair (1995).

Phylogenetic analysis

The distance matrix (Table III) includes both the raw number of sequence positions that are different between a pair of isolates and the pairwise genetic distances adjusted according to the Kimura 2-parameter maximum likelihood model. Isolates of *E. trivolvis* are quite similar (*D* = 0.0060), whereas the isolates of *E. revolutum* are more dissimilar (*D* = 0.0091). This amount of intraspecific separation is on the same order as several of the interspecific distances.

The frequency distribution of all 10,395 possible parsimony trees has a g1 value of −0.6, indicating the data set contains significant cladistic structure. Four shortest trees, each with a length of 211 steps and a consistency index of 0.943, were retained by the parsimony analysis. These trees are summarized in the consensus tree (Fig. 2), which includes the bootstrap scores of the branchpoints and branch lengths.

As detailed by Morgan and Blair (1995), the undescribed species, *E. sp. I*, remained the most distant member of the 37-collar-spine group in all 4 of the most parsimonious trees (and 70.3% of the bootstrap replicates). Other features common to all 4 trees were that the Eurasian and North American *E. revolutum* isolates form a pair (also found in 57.5% of the bootstrap replicates), as do the 2 *E. trivolvis* isolates (80.1% of replicates). In 3 of the 4 trees, this latter pair has as its closest relative *Echinostoma paraensei* (48.3% of replicates); in the other case, *E. paraensei* forms a clade with the *E. revolutum* isolates (23.5% of replicates, data not shown). The relationships between the *E. trivolvis*–*E. paraensei* clade, the *E. revolutum* isolates, and *E. caproni* are not resolved, as demonstrated by the polytomy in 2 of the 4 trees (Fig. 2). In 1 tree, *E. caproni* is the closest relative of the *E. trivolvis*–*E. paraensei* clade, and in the other it diverges along with an *E. revolutum*–*E. paraensei* clade.

**DISCUSSION**

We found intraspecific variation among isolates of *E. revolutum* and *E. trivolvis*, but the amount of variation present in these isolates does not invalidate the taxonomic diagnoses of the specimens. The previously sequenced isolates of *E. revolutum* proved to be the closest relatives of their North American conspecifics, although the variation present within this species matches or exceeds that previously reported between the 3 most closely related species of the 37-collar-spine group (Morgan and Blair, 1995), i.e., *E. trivolvis, E. revolutum,* and *E. paraensei*.

The presence of ITS sequence variation between 2 isolates of *E. trivolvis* from a single pond is surprising given the lack of nucleotide differences between conspecific *Echinostoma* isolates from different continents reported by Morgan and Blair (1995), though intraspecific variation in ITS sequences has been reported in trematodes (Adlard et al., 1993) and a number of other organisms (reviewed by Hillis and Dixon, 1991). For *E. trivolvis* 11B and 12B, 2 of these differences can be confirmed through digestion of this ITS locus with the restriction endonuclease HaeIII; the change from an adenine to a guanine at position 149 in the sequence of 12B results in an additional restriction site, whereas the transition to an adenine at position 715 causes the loss of a site. Digestion with the MseI restriction enzyme confirms 2 other differences between the *E. trivolvis* isolates at positions 264 and 265, where isolate 12B contains 2 adenines rather than the cytosine and guanine, of *E. trivolvis* 11B. Although the parsimony analysis provides reasonable support (along with other biological characteristics) that isolates...
11B and 12B are both specimens of *E. trivolvis*, the genetic distance between them approaches that found between sister species in the previous phylogenetic analysis (Morgan and Blair, 1995).

Alternatively, these isolates could represent genetically differentiated cryptic species, indicating that more research is needed to either confirm that these parasites interbreed or to search for other, yet unknown, distinguishing factors if they do not. The previously sequenced isolates that are identical in sequence to *E. trivolvis* 11B originated from Pennsylvania (B. Fried, pers. comm.), but the distribution of this genotype across the northeastern USA remains unknown. Furthermore, although 4 *E. trivolvis* isolates collected from 15 naturally infected *H. pustulatum* as shown by Sorensen et al. (1997). However, it is troubling that, whereas the Eurasian *E. revolutum* is the closest relative for the North American isolates (*D* = 0.0091), the genetic distance is even shorter between the Eurasian isolate and *E. paraensei* (*D* = 0.0080). There are a number of reliable morphological and host-use characteristics that can be used to distinguish *E. revolutum* from *E. paraensei* and *E. trivolvis*, leaving little possibility for synonymy; most notably, the former parasitizes snails in the family Lymnaeidae, whereas the latter use snails in the family Planorbidae. Nevertheless, phylogenetic methods that require ultrametric distances for predicting relationships between species, i.e., UPGMA, would consistently position *E. paraensei* in the midst of the *E. revolutum* isolates (data not shown). Parsimony methods are better able to separate these species, forming a pair of the *E. revolutum* isolates in all of the most parsimonious trees. Whereas it is again possible that they represent cryptic species, we believe that the variation present among the isolates of *E. revolutum*, while being substantial, is not sufficient to counter biological evidence for their classification as a single species.

The large amount of intraspecific variation in these *Echinostoma* isolates (relative to the amount of interspecific variation in the 37-collar-spine group) is problematic when considering the utility of the ITS rDNA loci for taxonomic diagnosis. Morgan and Blair (1995) rightly point out that “there is no yardstick for recognizing species boundaries using DNA sequence differences.” The results of this study demonstrate why there should not be such a yardstick. The shortest genetic distance between species in their analysis was 0.008 (over the entire locus) between *E. revolutum* and *E. paraensei*. This study finds that the North American *E. trivolvis* isolates are separated by a genetic distance of 0.006, and the distance between isolates of *E. revolutum* exceeds 0.009. In the absence of other biologically relevant data, it would then be tempting to conclude that these 2 groups of isolates represent more than 2 species. However, some of these distance-defined species would be cryptic, indistinguishable on the basis of standard morphological and host-use characteristics, or worse, would be members of a single species, able to interbreed. Thus, the use of only genetic data to distinguish between these closely related species is not recommended, particularly with a locus that exhibits the levels of variation found in the ITS sequences of 37-collar-spined *Echinostoma*. Furthermore, the use of nucleotide sequence data for taxonomic diagnosis of a specimen is troublesome with this amount of genetic differentiation. The finding of 6 differences between 2 *E. trivolvis* isolates from a single locale makes an unequivocal diagnosis of the taxonomic affiliation of the North American echinostomes difficult without more traditional characteristics based on the biology of the organism.

The level of intraspecific variation detected in these sequence data made it difficult to resolve the order of divergence among the major lineages of the 37-collar-spined *Echinostoma*. Typically, rDNA similarity among members of a species is higher than that between species as concerted evolution is able to homogenize the rDNA repeats within individuals of a species over evolutionary time (Arneheim, 1983; Hillis and Davis, 1988). It may be that the branches in the phylogenetic tree of the 37-collar-spined echinostomes are not separated by enough time to allow homogenization to occur (Sanderson and Doyle, 1992). This suggests that, relative to the rate at which this locus evolves, these species have recently and rapidly diverged.

The relative similarity between the interspecific variation shown previously and the intraspecific differentiation we found implies that diagnosis of specific affinities based on nucleotide data at this locus are ill advised in the absence of corroborative biological information. Sequence analysis with more rapidly evolving loci should provide a better understanding of evolutionary relationships among the related members of this genus (Morgan and Blair, 1998).

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LITERATURE CITED


Gilbert, D. 1992. SeqApp (Sequence editing and analysis application)


