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Comparisons of Two Polymorphic Species of *Ostertagia* and Phylogenetic Relationships within the Ostertagiinae (Nematoda: Trichostrongyloidea) Inferred from Ribosomal DNA Repeat and Mitochondrial DNA Sequences

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COMPARISONS OF TWO POLYMORPHIC SPECIES OF *OSTERTAGIA* AND PHYLOGENETIC RELATIONSHIPS WITHIN THE OSTERTAGIINAE (NEMATODA: TRICHOSTRONGYLOIDEA) INFERRED FROM RIBOSOMAL DNA REPEAT AND MITOCHONDRIAL DNA SEQUENCES

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ABSTRACT: The first internal transcribed spacer DNA (ITS-1) (rDNA) and the mitochondrial (mt) DNA-derived cytochrome oxidase I gene (COX-1) were enzymatically amplified, cloned and sequenced from 6 nominal species of Ostertagiinae as well as *Haemonchus contortus* and *Haemonchus placei*. The portion of the COX-1 gene analyzed was 393 base pairs (bp) in length and contained 33 within species polymorphic base changes at 28 synonymous sites. The ITS-1 rDNA consensus sequences ranged from 392 bp (*Ostertagia ostertagi/Ostertagia lyrata*, *Teladorsagia circumcincta*) to 404 bp (*H. contortus*, *H. placei*). These data were used both in a distance analysis to assess the concept of polymorphic species within the genus *Ostertagia* and in parsimony analysis to assess phylogenetic relationships within a limited group of Ostertagiinae. Pairwise similarity scores of both ITS-1 and COX-1 data showed the highest number of conserved sites between the proposed dimorphic species of *Ostertagia*. The level of similarity was lower in the COX-1 data due to the high number of synonymous base changes. Analysis by maximum parsimony of the same data did not refute *O. ostertagi/O. lyrata* and *Ostertagia mossi/Ostertagia dikmansii* as dimorphic species and supported monophyly of these ostertagiines relative to representatives of the haemonchine outgroup. In the single most parsimonious tree from ITS-1 rDNA data, a subclade of *Ostertagia* spp. included forms possessing parallel synlophes and long esophageal valves that typically occur in cervid hosts.

A major advancement in the systematics of the Ostertagiinae was the recognition of polymorphism in males (Daskalov, 1974; Drózd, 1974; Lancaster and Hong, 1981). The occurrence of polymorphism among genera and species within the Ostertagiinae was reviewed recently by Drózd (1995). A partial list of those he had studied from the Palearctic included 19 polymorphic species in 5 genera. Studies on parasite hybridization (Suarez and Cabaret, 1992), synlophes structure (Lichtenfels and Hoberg, 1993), environmental effects (Suarez et al., 1995), allozymes (Gasnier et al., 1993), and random amplified polymorphic DNA (Humbert and Cabaret, 1995) have not refuted the hypothesis that members of the “species pairs” are dimorphic males of a single species. One study utilizing DNA sequence data to study polymorphic species within the genus *Teladorsagia* Andreeva and Satubaldin, 1954 has been undertaken (Stevenson et al., 1996); however, the application of both nuclear and mitochondrial-derived sequence data to study dimorphic species within the Ostertagiinae are lacking.

DNA sequence data has long been used to assess relationships among organisms. Specifically, various regions of the ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) have received the most attention when studying more divergent taxa (rDNA) or more recently derived species (mtDNA). Studies by Zarlenga, Stringfellow, and Lichtenfels (1994) and Zarlenga, Stringfellow, Nobary, and Lichtenfels (1994) suggested that within genera of trichostrongyles, the small subunit rDNA molecule (ssrDNA) is unlikely to provide adequate information to assess phylogenetic relationships because of the high level of sequence conservation among this group of parasites. However, the internal transcribed spacer (ITS) region has received substantial attention as a sequence that bridges the gap of utilizing nuclear sequences to assess more recently evolved species because of the higher degree of sequence variability. Studies in-

volving sequence analysis of external and internal transcribed spacers of the rDNA repeat of other nematodes (Zarlenga, Stringfellow, and Lichtenfels, 1994; Zarlenga, Stringfellow, Nobary, and Lichtenfels, 1994; Hoste et al., 1995) have shown them to be of value for species level systematics of nematodes, including species of *Haemonchus* Cobb, 1898 and *Trichostrongylus* Looss, 1905, and for assessing divergence among polymorphs of *Teladorsagia circumcincta* (Stadelmann, 1894) (see Stevenson et al., 1996).

This report presents sequence data for the first internal transcribed spacer (ITS-1) of the rDNA repeat and partial data from the mitochondrial-derived, cytochrome oxidase I (COX-1) gene of 2 putative polymorphic species, *Ostertagia ostertagi* (Stiles, 1892)/*Ostertagia lyrata* Sjöberg, 1926 and *Ostertagia mossi* Dikmans, 1931/*Ostertagia dikmansii* Becklund and Walker, 1968. For additional within group comparison, similar data are provided for *Ostertagia leptospicularis* Assadov, 1953, and *T. circumcincta*. *Haemonchus contortus* (Rudolphi, 1802), and *Haemonchus placei* (Place, 1893), representing the putative sister-group, Haemonchinae, are included as the basis of outgroup comparison (Hoberg and Lichtenfels, 1994). Utilizing these data we provide first, a test of the polymorphism hypothesis based on pairwise similarity scores from molecular sequences of 2 independent DNA regions and second, examine putative evolutionary relationships of the taxa based upon cladistic analysis of the sequence data.

MATERIALS AND METHODS

Specimens

Adult nematodes were obtained from hosts experimentally infected at Beltsville or collected elsewhere from naturally infected hosts, quick frozen and shipped to Beltsville for identification (Table I). Specimens were identified to species using characteristics of spicules, copulatory bursa, genital cone, esophagus and synlophes (males) or characteristics of the ovejectors, tail, esophagus and synlophes (females) (Durette-Desset, 1982; Lichtenfels and Hoberg, 1993; Lichtenfels et al., 1994).

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TABLE I. Source of nematodes studied.

Nematode species	Host	Locality and source
<i>Ostertagia ostertagi</i>	<i>Bos taurus</i>	Beltsville, Maryland (L. C. Gasbarre)*
<i>Ostertagia lyrata</i>	<i>B. taurus</i>	Weybridge, England (M. B. Lancaster)*
<i>Ostertagia mossi</i>	<i>Odocoileus virginianus</i>	Mississippi (C. E. Couvillion)†
<i>Ostertagia dikmansii</i>	<i>O. virginianus</i>	Mississippi (C. E. Couvillion)†
<i>Ostertagia leptospicularis</i>	<i>B. taurus</i>	Weybridge, England (M. B. Lancaster)*
<i>Teladorsagia circumcincta</i>	<i>Ovis aries</i>	Beltsville, Maryland (F. Stringfellow)*
<i>Haemonchus contortus</i>	<i>B. taurus</i>	Beltsville, Maryland (L. C. Gasbarre)*
<i>Haemonchus placei</i>	<i>B. taurus</i>	Australia (W. L. Shoop)*

* Larvae from various sources used to infect experimental host at Beltsville, Maryland.

† Adults collected and shipped frozen to Beltsville, Maryland.

Polymerase chain reaction (PCR) amplification and cloning

Genomic DNA was isolated by proteinase K–sodium dodecyl sulfate (SDS) digestion from a pooled sample of adult parasites as previously described (Dame et al., 1987) without prior homogenization. The COX-1 gene was enzymatically amplified using evolutionarily conserved *Fasciola hepatica*-derived primers 239 (5'AAAGGAATTCTTTTTGGGCATCCTGAGGTTTAT) and 240 (5'ATTGGATCCTAAAGAAAGAACATAATGAAAATG) (Garey and Wolstenholme, 1989) to which EcoRI and BamHI restriction sites (underlined) had been added, respectively. The data presented represent partial COX-1 sequence information. The sequence encoding ITS-1 was amplified from genomic DNA using primers 241 (5'AAAGGAATTCAAGTCGTAACAAGGTTCCGTAGG) and 242 (5'ATTGGATCCTAAACAACCCTGAACCAACGCTAC) that also contain EcoRI and BamHI restriction sites (underlined) for cloning. The primers are complementary to the 3' end of the ssrRNA and the 3' end of the 5.8S rRNA, respectively. Amplification was performed in a 100- μ l reaction mixture containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 10 nmol each deoxynucleotide triphosphate, 0.2 μ M of each primer, and 2.5 U of Taq DNA polymerase for 35 cycles as follows: 1 min at 94 C, 1 min at 55 C, and 2 min at 72 C, followed by a final 7 min at 72 C extension. PCR products were restriction enzyme-digested with EcoRI and BamHI, agarose gel purified, and ligated overnight to similarly digested pUC18 plasmid DNA in the presence of T4 DNA ligase. The next day, recombinant plasmids were used to transform electrocompetent DH5 α bacterial cells. For each sequence, 2 clones were picked, verified for correctness, and independently sequenced using universal forward and reverse pUC primers.

Sequence analysis

Sequence analysis was performed using Sequenase™ (Amersham, Arlington Heights, IL) according to the manufacturer's recommendations. Homologous sequences from each species of parasite were aligned both visually and through the Intelligenetics PCGENE version of the Clustal alignment program by Higgins and Sharp (1988). Transitions were weighted twice as likely as transversions and the gap pen-

alty was 5. Computation of pairwise similarity scores were based upon the method of Wilbur and Lipman (1983) and tabulated for the ITS-1 data (Table II) and the COX-1 data (Table III). Due to the high level of synonymous base changes and polymorphic sites observed in the COX-1 sequences, the DNA data were analyzed without modification (Table III), by eliminating the third base of the amino acid codon (Table III) and as predicted amino acid sequences (not shown).

Relationships among all parasite groups presented herein were analyzed by maximum parsimony with PAUP 3.1 (Swofford, 1993). Polarity was determined with reference to the haemonchine outgroup (*H. contortus* and *H. placei*), the putative sister-group for the Ostertagiinae, based on phylogenetic analysis and comparative morphology (Hoebig and Lichtenfels, 1994). Characters were equally weighted and an exhaustive search was conducted. Unambiguous gaps were treated as a fifth character. Bootstrap resampling with 1,000 replicates was performed to examine relative support for clades recovered in the respective analyses. Results are shown as phylogenetic trees with accompanying descriptive statistics including the consistency index (CI), homoplasy index (HI), retention index (RI), and rescaled consistency index (RC) as defined by Swofford (1993). Results from bootstrap analyses are shown as a 50% majority-rule consensus tree.

RESULTS

Pairwise similarity scores

Enzymatic amplification of the ITS-1 rDNA generated 2 distinct size fragments within this group of parasites. The ITS-1 rDNA for *O. ostertagi* and *O. lyrata* was approximately 1,000 base pairs (bp), whereas the homologous sequence in all other trichostrongyles was only 600 bp (approximate). This size difference was the result of a 408-bp insertion within the *O. ostertagi* and *O. lyrata* genomes (data not shown).

The ITS-1 rDNA data (Fig. 1) consisted of a contiguous stretch of 392–404 bp (with the exception of *O. ostertagi*/

TABLE II. Pairwise similarity scores for ITS-1 DNA sequences of 6 nominal species of Ostertagiinae and *Haemonchus* spp.

Nematode species	1	2	3	4	5	6	7	8
1. <i>O. ostertagi</i> *	(392)†	—	—	—	—	—	—	—
2. <i>O. lyrata</i> *	391	(392)	—	—	—	—	—	—
3. <i>O. mossi</i>	315	315	(400)	—	—	—	—	—
4. <i>O. dikmansii</i>	315	315	400	(400)	—	—	—	—
5. <i>O. leptospicularis</i>	319	319	366	366	(401)	—	—	—
6. <i>T. circumcincta</i>	323	322	314	314	323	(392)	—	—
7. <i>H. contortus</i>	269	269	275	275	276	296	(404)	—
8. <i>H. placei</i>	269	269	272	272	276	295	400	(404)

* Table excludes sequence inserts in *O. ostertagi* and *O. lyrata*.

† Numbers in parentheses are total number of bases. Within the ITS-1 region, sequence length was not conserved.

TABLE III. Pairwise similarity scores for COX-1 DNA sequences among 6 nominal species of Ostertagiinae and *Haemonchus* spp.

Nematode species	1	2	3	4	5	6	7	8
1. <i>O. ostertagi</i>	—	376*	337	336	340	332	335	327
2. <i>O. lyrata</i>	261†	—	339	339	339	335	337	329
3. <i>O. mossi</i>	259	258	—	390	333	328	337	332
4. <i>O. dikmansii</i>	259	258	262	—	334	329	336	331
5. <i>O. leptospicularis</i>	261	260	260	260	—	339	338	335
6. <i>T. circumcincta</i>	260	259	256	256	259	—	337	337
7. <i>H. contortus</i>	260	259	256	256	259	259	—	341
8. <i>H. placei</i>	258	257	254	254	257	257	260	—

* Numbers above diagonal include all COX-1 sequence data; consensus length = 393 bp.
 † Numbers below diagonal include only bases 1 and 2 of amino acid codons; consensus length = 262 bp.

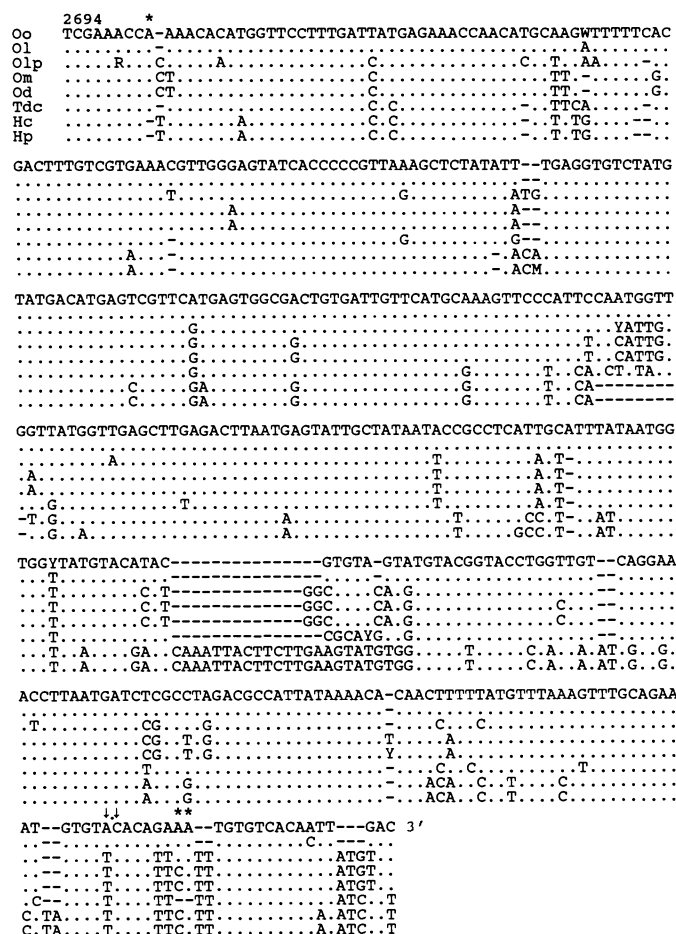


FIGURE 1. Comparison of the ITS-1 rDNA sequences from 5 species of *Ostertagia*, 1 species of *Teladorsagia* and 2 species of *Haemonchus*. The site of sequence discontinuity within *O. ostertagi* and *O. lyrata* due to the presence of a 408-bp insert is indicated by ↓↓. Gaps denoted by * were not used as fifth characters in arriving at the tree shown in Figure 3. Base pair numbering is in accordance with that for *Caenorhabditis elegans*. M = A,C; R = A,G; W = A,T; Y = C,T. Oo, *O. ostertagi*; Ol, *O. lyrata*; Om, *O. mossi*; Od, *O. dikmansii*; Omp, *O. leptospicularis*; Tdc, *T. circumcincta*; Hc, *H. contortus*; Hp, *H. placei*. Sequences shown in Figure 1 have been assigned Genbank accession numbers AF044927–AF044934.

O. lyrata). Sequence alignment for the dimorphic species showed a consensus of 391 of 392 base pairs between *O. ostertagi* and *O. lyrata* with 2 additional polymorphic sites within the ITS-1 region. No differences were observed between *O. mossi* and *O. dikmansii*; however, 1 polymorphic site was identified. All polymorphic sites contained one base consistent with the consensus sequence. Pairwise similarity scores indicated that sequences of morphs within each dimorphic species were most similar and substantially distinct from all other parasites analyzed (Table II).

When the COX-1 gene was similarly amplified using *F. hepatica*-derived COX-1 primers, a single fragment was produced for all taxa examined that was approximately 450 bp in size. The aligned region of the COX-1 gene was 393 bp in length and contained 33 polymorphic bases within 28 polymorphic sites among all 6 taxa (Fig. 2). All polymorphic sites occurred within the third base positions of amino acid codons. Pairwise similarity scores (Table III) were consistent with that derived from the ITS-1 data; however, due to the sequence polymorphism in this gene, the level of similarity between polymorphic species was substantially lower than that derived from the ITS-1 data. Analysis of all COX-1 sequence data indicated a consensus of 376 of 393 bp for *O. ostertagi*/*O. lyrata* and 390 of 393 bp for *O. mossi*/*O. dikmansii*. Results from analyzing the first 2 bases of the amino acid codons revealed a consensus of 261 of 262 bp between *O. ostertagi* and *O. lyrata* and no differences between *O. mossi* and *O. dikmansii*.

Maximum parsimony

Parsimony analysis of the ITS-1 rDNA data produced 1 tree of 140 steps (CI = 0.92, HI = 0.079, RI = 0.92, and RC = 0.85) consistent with monophyly for the Ostertagiinae. Within Ostertagiinae, *T. circumcincta* is basal and *O. ostertagi*/*O. lyrata* is presented as the putative sister species of *O. leptospicularis* + *O. mossi*/*O. dikmansii*. The bootstrap consensus tree (Fig. 3) was well supported and identical to the single most parsimonious tree.

Because of the high level of polymorphic sites and synonymous base changes within this gene, parsimony analysis of the COX-1 DNA sequences did not fully resolve relationships among the 6 taxa. Parsimony analyses using all base pair sites, the first 2 bases of amino acid codons only, or predicted amino acids (data not shown) were all unresolved. Congruence among the analyses was limited to support of monophyly for the Ostertagiinae where *O. mossi* and *O. dikmansii* were consistently

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Oo ATTTTAATTTTACCTGCWTTTGGTATCATTAGTCAATCTTCGTTGTATTTAACWGGAAAAA
Ol .....C.....T.....T.....T.....T.....T.....T.....T.....T.....
Olp .....T.....T.....T.....T.....T.....T.....T.....T.....T.....T.....
Om .....G..T.....T.....T.....C.W.....Y.....T.....T.....T.....
Od .....G..T.....T.....T.....C.T.....T.....T.....T.....T.....
Tdc .....A..C.....T.....T.....A..A.....T.....C.....T.....T.....
Hc .....T.....G..TG.A.A.....A.....A.....A.....A.....A.....A.....
Hp .....A.....TG.....T.....T.....T.....T.....T.....T.....T.....

AGAGGTGTTGGTTCATTGGGTATAGTATGCTATTTTAAAGAAATGGTTAATTGGTTGCTGTGCTG
.....C.....C.....C.....C.....C.....C.....C.....C.....C.....C.....
.....A.....G.....R.A.....T.Y.....G.....T.....T.....T.....T.....T.....
.....A..T.....A..T.....T.....T.....T.....T.....T.....T.....T.....T.....
.....A..T.....A..T.....T.....T.....T.....T.....T.....T.....T.....T.....
.....A..A.....T.....G..A..Y.....T.....T.....T.....T.....T.....T.....
G..A..A.....G.....A..C.....T.....T.....A.....T.....T.....T.....T.....
.....A.....GC.T.A.G.T.....A.....T.....C.....C.....C.....A.....

TGAGCTCAYCAYATGTATACAGTRGGRATAGATTGGATTACCGTCTTATTTTACGGCGGCTACTA
.....T.....T.....T.....T.....T.....T.....T.....T.....T.....T.....T.....
.....A.....T.....Y..W.....A.....A.....T.....T.....T.....T.....T.....T.....
.....C.....T.....T.....T.....G.....A..C.....G.....T.....T.....C.....A.....
.....C.....T.....T.....T.....G.....A..C.....G.....T.....T.....C.....A.....
.....T.....T.....C.....A..T..G.....A.....T.....T.....T.....T.....T.....
.....G..C..C..T.....A..T.....A.....G.....T.....T.....T.....T.....T.....
.....C.....T.....C.....A.....T.....A.....G.....T.....T.....T.....T.....A.....

TGGTAATTCAGTACCYACAGGGTTAAGGTTTTAGTGGTTRGCAACTTTGTCGGAATAAAGAT
.....G.....C.....C.....C.....C.....C.....C.....C.....C.....C.....C.....
.....A.....T.....G..T..G..T..A..A..R.....A..A.....T.....T.....T.....A.....
.....A.....T.....C.....C.....A.....A.....A..T.....A..T.....T.....A.....
.....A.....T.....C.....C.....A.....A.....A..T.....A..T.....T.....A.....
.....A..T.....T..R..C..Y..T..R..A.....A..A..T.....T..G.....A.....
.....A.....T.....T..T.....T.....A..A..A.....A..A..T.....T.....T.....A.....
.....T.....T.....T.....T.....C.....T.....A.....G.....AC.A.....T.....T.....A.....

AAATTTTCAACCTTTGTTGTTGAGTGTAGGTTTTATTTTTTATTACTATTGGGGTTTRACR
.....A.....A.....A.....A.....A.....A.....A.....A.....A.....A.....A.....
.....R.....A.....R.....T..G.....G.....G.....G.....G.....G.....G.....A.....
.....G.....C..A..A.....T..G.....G.....G.....T..G..A..T.....
.....G.....C..A..A.....T..G.....G.....G.....T..G..A..T.....
.....C..T.....G..AA.....G.....G.....A..C..T.....A..G.....
.....GG.....T.....T..G.....A.....A..A..A..T.....
.....AG.....A..A.....T.....G.....G.....A.....T..A..A..T.....

GGTGAATTTGTCCAATTCTAGTTGGATATTTTGCATGATACTTATTATGTTGGTTAGY
.....C.....C.....C.....C.....C.....C.....C.....C.....C.....C.....C.....
.....G.....G.....T.....A..A.....Y..Y.....C.....T.....T.....T.....T.....
.....TG.....A..T.....A.....A.....C.....C.....A.....A.....A.....A.....
.....TG.....A..T.....A.....A.....C.....C.....A.....A.....A.....A.....
.....T.....T.....R..A..A.....C.....C.....A.....Y..C.....
.....T.....A..T.....A..A.....A.....A.....A.....A.....A.....A.....T.....
.....A.....A.....A.....A.....C.....C.....A.....C.....A.....C.....
    
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FIGURE 2. A comparison of the mtDNA-derived cytochrome oxidase I gene sequences from 5 species of *Ostertagia*, 1 species of *Teladorsagia*, and 2 species of *Haemonchus*. R = A,G; W = A,T; Y = C,T. Oo, *O. ostertagi*; Ol, *O. lyrata*; Om, *O. mossi*; Od, *O. dikmansii*; Olp, *O. leptospicularis*; Tdc, *T. circumcincta*; Hc, *H. contortus*; Hp, *H. placei*. Sequences shown in Figure 1 have been assigned Genbank accession numbers AF044935–AF044942.

grouped together; however, *O. ostertagi* and *O. lyrata* were periodically joined in a polytomy with other ostertagiines.

Morphological characters representative of the taxa as well as the ITS-1 sequence repeat of *O. ostertagi/O. lyrata* were mapped onto the single most parsimonious tree generated from ITS-1 rDNA data (Fig. 4). Phylogenetic groupings inferred from recent morphological analyses are supported by the ITS-1 rDNA-based consensus tree.

DISCUSSION

We have analyzed sequence data from the ITS-1 and the mtDNA-derived COX-1 gene in order to first test the hypothesis of polymorphism for *O. ostertagi/O. lyrata* and *O. mossi/O. dikmansii* and second to infer phylogenetic relationships of the Ostertagiinae studied herein. Although a subfamily analysis requires the study of more taxa than included in this study, the examination of nuclear DNA sequences for 6 nominal species of the Ostertagiinae including species from cervids and bovids has, nonetheless, provided a preliminary opportunity to assess putative phylogenetic relationships among these species and compare newly generated genetic data.

Pairwise similarity scores

Mitochondrial DNA sequence data, though believed to be more informative in generating gene trees congruent with species trees for recent speciation events (Moore 1995), produced essentially unresolved trees in this case. This occurred regardless of the sequence basis for the analysis. Presumably, this resulted from the high numbers of synonymous base changes observed within and between species. This is supported by work performed by Blouin et al. (1992, 1995) and by Tarrant et al. (1992) who demonstrated high sequence variability between mtDNA from individual *O. ostertagi* as well as other trichostrongylids both within and between populations. Pairwise sequence divergence between mtDNA for *H. contortus* and *H. placei*, for example, was 14.4%, whereas the predicted amino acid data demonstrated a substantially smaller sequence divergence of 3.1%. A similar pattern was observed throughout the matrix. Given that only 7 ambiguous sites were identified within all the ITS-1 rDNA sequences analyzed, and all the polymorphic sites in the mtDNA sequences were observed within the third base of the amino acid codons, we concluded that the divergence seen within the mtDNA data was not an artifact of PCR amplification.

The identical sequences for *O. mossi/O. dikmansii* and the nearly identical sequences for *O. ostertagi/O. lyrata* in the ITS-1 rDNA repeat strongly support the concept of dimorphic species. The minor differences between *O. ostertagi* and *O. lyrata*, compared to those seen between other ostertagine species in this study, may be attributed to the different geographical and experimental sources of these specimens. Those of *O. lyrata* were an experimental strain from Europe that had been selected over several generations to increase the percentage of the minor morphotype males in the population. These data in conjunction with population studies performed by Blouin et al. (1992, 1995) and isolate differences investigated by Stevenson et al. (1996) suggest that the similarity scores are representative of interspecific variation and not substantially influenced by diversities among worm populations. These data further support the work of Stevenson et al. (1996) showing strong similarities among morphotypes of polymorphic species; however, care must be taken when using only sequence data to imply species status.

Phylogenetic relationships

Monophyly for this group of Ostertagiinae was supported by the analysis of both ITS-1 and COX-1 sequence data sets. In the fully resolved ITS-1 tree, *Teladorsagia* is the sister-group for *Ostertagia* spp. and the concept of dimorphic species that was supported by pairwise similarity scores for *O. ostertagi/O. lyrata* and *O. mossi/O. dikmansii* is not refuted.

Results of the current study, though lacking the broader context of ostertagiine diversity (e.g., Durette-Desset, 1982, 1989; Gibbons and Khalil, 1982; Jansen, 1989; Drózd, 1995), support monophyly of the subfamily, relative to the Haemonchinae (Hoberg and Lichtenfels, 1994). In a limited manner, the phylogenetic tree for this restricted group of *Ostertagia* spp. and *Teladorsagia* can be used to examine hypotheses for character polarity and evolution, particularly for the synophe, bursa and genital cone (e.g., Hoberg et al., 1993; Hoberg and Lichtenfels, 1994) and for host-association (e.g., Drózd, 1967). As a result, we have mapped and optimized morphological characters onto

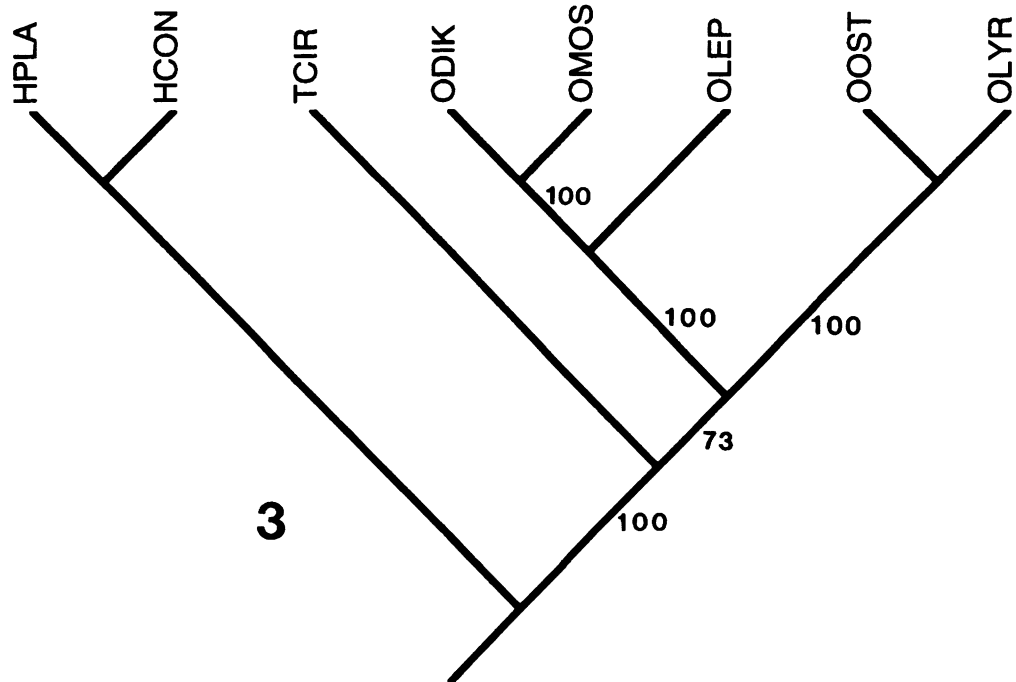


FIGURE 3. Phylogenetic hypothesis based on ITS-1 rDNA sequences for some ostertagiine nematodes. This single most parsimonious tree was derived from maximum parsimony (CI = 0.92) and is congruent with bootstrap analysis. Bootstrap values are shown for each node. Taxa include *Haemonchus contortus* (HCON) and *H. placei* (HPLA) in the outgroup and *Teladorsagia circumcincta* (TCIR), *Ostertagia dikmansii* (ODIK), *O. mossi* (OMOS), *O. leptospicularis* (OLEP), *O. ostertagi* (OOST), and *O. lyrata* (OLYR) in the in group.

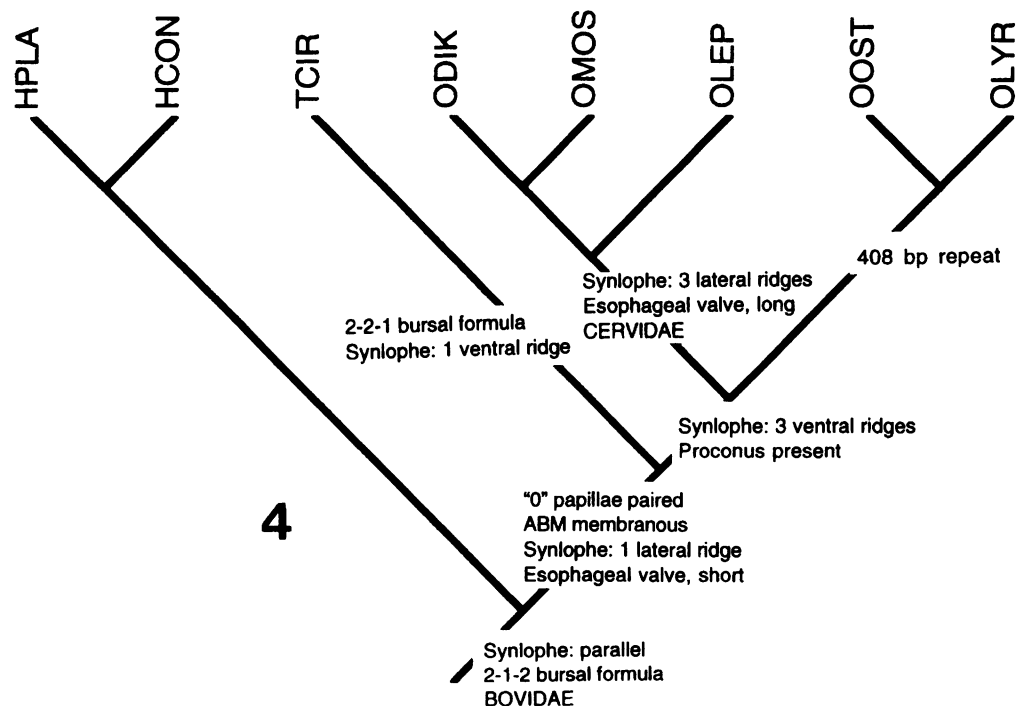


FIGURE 4. Morphological and genetic characters for Ostertagiinae mapped and optimized onto the tree derived from the ITS-1 rDNA sequence data analysis (Fig. 2). Taxa are labeled as in Figure 3. This hypothesis is consistent with the following as plesiomorphic in the subfamily: (1) parallel synlophe; (2) 2-1-2 bursa; and (3) bovid hosts as ancestral for ostertagiines. Other interpretations are outlined in the text.

the phylogenetic hypothesis independently derived from ITS-1 sequence data. It is apparent that the 2-1-2 form of the ventral and lateral rays of the copulatory bursa and a parallel synlophe are plesiomorphic and the "ostertagiines" can be defined by a membranous accessory bursal membrane, and paired "O" papillae relative to other trichostrongylids and the haemonchine sister group (see Hoberg and Lichtenfels, 1994).

The presence of a short esophageal valve, and a "Type I" lateral tapering synlophe with a single lateral ridge (see Lichtenfels et al., 1988a, 1988b), further define *Teladorsagia* + *Ostertagia*. At this level of analysis, *Teladorsagia* is distinguished from *Ostertagia* by the 2-2-1 form of the bursa and the presence of a single ventral ridge in the cervical zone (Lichtenfels et al., 1988b); however, the form of the bursa is not a synapomorphy for the genus (Hoberg et al., 1993; Hoberg and Lichtenfels, 1994; Lichtenfels et al., 1996). The species of *Ostertagia* analyzed in the present study share the presence of a proconus in the ventral aspect of the genital cone and 3 ventral ridges in the synlophe. The system of 3 lateral ridges, a "Type II" lateral synlophe, an elongate esophageal valve, and cervid hosts, on the other hand, are characteristic of *O. mossi/O. dikmansii* + *O. leptospicularis*, which are also grouped as sister species with respect to the DNA data. Additionally, the DNA and morphological data are consistent with the occurrence of *Ostertagia* among cervids resulting from a host-switch by an ancestral species from bovids.

We previously presented data (Zarlenga, Stringfellow, and Lichtenfels, 1994; Zarlenga, Stringfellow, Nobary, and Lichtenfels, 1994) indicating that the ssrDNA sequence will likely be uninformative for phylogenetic inference among this group of parasites because of the lack of sufficient diversity within the gene. It is equally possible that mtDNA sequence data will be uninformative in resolving phylogenetic issues within this genus given the high level of sequence polymorphism within this molecule. Though it may be argued that the mitochondrial-derived COX-1 sequence is inappropriate for this investigation, or that a larger mitochondrial-derived data set is necessary for a more thorough analysis, prior studies suggest that the level of polymorphism within this molecule is ubiquitous (Blouin et al., 1992) and that this polymorphism exists at the level of individual parasites. Thus, one might expect a similar level of unresolved sequence characters within other regions of this mtDNA as well. Nonetheless, the pairwise similarity scores presented herein strongly support and offer yet another level of resolution to the hypothesis for male dimorphism in the single species represented by *O. ostertagi/O. lyrata* and *O. mossi/O. dikmansii*. On the other hand, the application of additional genetic data to the phylogenetic analyses of the Ostertagiinae is necessary to more completely delineate issues such as character evolution, the basis for recognition and differentiation of generic-level taxa, and the history of host-parasite associations. It remains questionable whether mtDNA will provide the additional data source to resolve these issues.

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