4-2002

Identical ITS-1 and ITS-2 Sequences Suggest *Spiculopteragia asymmetrica* and *Spiculopteragia quadrispiculata* (Nematoda: Trichostrongylidae) Constitute Morphologically Distinct Variants of a Single Species (Research Notes)

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J. Parasitol., 88(2), 2002, pp. 417–418

Identical ITS-1 and ITS-2 Sequences Suggest *Spiculopteragia asymmetrica* and *Spiculopteragia quadrispiculata* (Nematoda: Trichostrongylidae) Constitute Morphologically Distinct Variants of a Single Species

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ABSTRACT: Sequences of ITS-1 and ITS-2 rDNA for adult males of *Spiculopteragia asymmetrica* and *Spiculopteragia quadrispiculata* in red deer (*Cervus elaphus*) were determined. They were found to be identical, suggesting that *S. asymmetrica* and *S. quadrispiculata* represent a single species and do not refute the concept of dimorphic species in the *Spiculopteragia*.

Since the hypothesis for polymorphism among male nematodes within species of the Ostertagiinae was proposed (Daskalov, 1974; Dróżdż, 1974, 1995; Lancaster and Hong, 1981), numerous studies have been conducted to examine that assumption. Polymorphism has been demonstrated in *Marshallagia*, *Teladorsagia*, and *Ostertagia* in the following species: *M. marshalli*/*M. occidentalis*, *T. circumcincta*/*T. trifurcata*/*T. davtiani*, *T. boreoarcticus* forma major/*T. boreoarcticus* f. minor, *O. ostertagi*/*O. lyrata*, *O. mossi*/*O. dikmansi*, *O. leptospicularis*/*O. kolchida*, and *O. gruehneri*/*O. arctica*. These conclusions were based on morphological studies (Lichtenfels and Hoberg, 1988; Lichtenfels et al., 1990; Hoberg et al., 1993, 1999; Lichtenfels and Hoberg, 1993; Dróżdż, 1995), cross-breeding experiments (Lancaster et al., 1983; Suárez and Cabaret, 1992), allozyme electrophoresis (Andrews and Beveridge, 1990; Gasnier et al., 1993), and comparisons of DNA sequences (Stevenson et al., 1996; Zarlenga et al., 1998; Hoberg et al., 1999; Dallas et al., 2000). It is considered that continued documentation of polymorphism within Ostertagiinae, as well as standardization of taxonomy for major and minor morphotypes, is of interest for biological and epidemiological studies (Hoberg et al., 1999).

Within *Spiculopteragia*, the sole basis for suspecting polymorphism has been the co-occurrence of 2 morphotypes of male nematodes in the same host (Dróżdż, 1995). For example, *Spiculopteragia asymmetrica* and *S. quadrispiculata* are commonly found in the abomasum of cervids. They were considered separate species based on morphological characters of male nematodes (Dróżdż, 1965). To date, no genetic studies have been performed to investigate polymorphism in any species of *Spiculopteragia*. In this study, the hypothesis that *S. asymmetrica* and *S. quadrispiculata* could be differentiated genetically was tested. For this purpose, the DNA sequences of the first internal transcribed spacer (ITS-1) and second internal transcribed spacer (ITS-2) of rDNA were determined and compared in *S. asymmetrica* and *S. quadrispiculata*.

Abomasa were removed from red deer at Extremadura (Spain), and adult nematodes were preserved in 70 % ethanol. The caudal extremity was cut from each male specimen, and identity of the specimens was determined by examination of structural characters of the bursa and spicules (e.g., Dróżdż, 1965). Genomic DNA was isolated from 5 individual worms corresponding morphologically to *S. asymmetrica* and from 5 representing *S. quadrispiculata* using DNeasy™ (Qiagen, Valencia, California). The ITS-1 was subsequently amplified using primers 241 (5′AAAGGAATTCAAGTCGTAACAAGGTTTCCGTAGG 3′) and 242 (5′ATTGGATCCAACAACCCTGAACCAGACGTAC 3′) (Zarlenga et al., 1998) and the ITS-2 with primers NC1 (5′ACGTCTGGTTCAGGGTTGTT 3′) and NC2 (5′TTAGTTTCTTTTCCTCCGCT 3′) (Gasser et al., 1993). The polymerase chain reaction (PCR) was performed in a 50-μl reaction volume containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 2.5 U of Taq DNA polymerase, 40 μM of each deoxynucleotide triphosphate (Gibco BRL®, Foster City, California). The PCR program was as follows: 35 cycles of 94 C for 1 min, 55 C for 1 min, and 72 C for 2 min, followed by a final extension at 72 C for 7 min. Negative (no-DNA) and positive controls (*Haemonchus contortus* DNA) were included in each set of reactions. PCR products were detected on ethidium bromide-stained 1.5% TAE (0.04 M Tris-acetate, 1 μM EDTA) agarose gels. PCR products were purified using Qiaquick™ spin columns (Qiagen) then sequenced using the same primers as for PCR in 10-μl reactions using BigDye chemistries and a 377 automated sequencer (PE Biosystems, Rockland, Maine). Each of the 10 individuals were bidirectionally sequenced at the ITS-1 and ITS-2 loci. Sequence chromatograms from each strand were aligned and inspected using Sequencer version 3.1 (Gencodes Corp., Ann Arbor, Michigan).

The PCR products represented single fragments of ~500 bp ITS-1 and ~300 bp ITS-2, comparable to those found in other members of the subfamily (Stevenson et al., 1996; Dallas et al., 2000). The sequence

corresponding to the ITS-1 and ITS-2 locus for each of these individuals has been deposited in GenBank™ (GenBank accession nos. AF480615–AF480618, respectively). No fixed differences between *S. asymmetrica* and *S. quadrispiculata* were detected in the ITS-1 or ITS-2 sequence. Instead, all but 1 nucleotide position were invariant. For this sole exception, individual worms exhibited both C and T in position 50 of the ITS-2 sequence. Such dimorphism was not restricted to members of either putative taxon, or was it restricted to either sequencing direction.

The absence of differences in the ITS-1 and ITS-2 rDNA sequences has previously been interpreted as evidence that morphological polymorphism exists among males belonging to single species of *Teladorsagia* and *Ostertagia* (see Stevenson et al., 1996; Zarlenga et al., 1998; Dallas et al., 2000). Additionally, the occurrence of polymorphism within *Teladorsagia* spp. has been assessed based on sequences of mitochondrial DNA (Hoberg et al., 1999). Clearly the possibility of *S. asymmetrica* and *S. quadrispiculata* representing 2 different species cannot be ruled out because variations at other loci not examined could refute the hypothesis that both taxa comprise a single reproductive and evolutionary lineage. However, results obtained are consistent with the concept of polymorphism observed within species among related genera, including *Ostertagia* and *Teladorsagia* (Zarlenga et al., 1998; Hoberg et al., 1999). Their close evolutionary relationship is further suggested by the segregation of the same nucleotide polymorphism in individuals corresponding to each morphological type. The absence of any fixed genetic difference in ITS-1 and ITS-2 between *S. asymmetrica* and *S. quadrispiculata* comprises the first genetic evidence for morphological polymorphism among individuals of species in the genus *Spiculopteragia*.

Mónica Santín-Durán was supported by a predoctoral fellowship from Comunidad de Madrid. The study was conducted while M.S.-D. was a visiting scientist at the Biosystematics Unit, Parasite Biology, Epidemiology and Systematics Laboratory, ARS, and was assisted by Mayee Wong.

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