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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Identical ITS-1 and ITS-2 Sequences Suggest *Spiculopteragia asymmetrica* and *Spiculopteragia quadrispiculata* (Nematoda: Trichostrongyliidae) 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* were determined and compared in *S. asymmetrica* and *S. quadrispiculata*. These conclusions were based on morphological and morphometrical studies (Hoberg et al., 1999). The ITS-1 was subsequently amplified using primers 241 (5′AAAGGAATTCAGTCGTAACAGCCCTGACACATGTA 3′) and 242 (5′ATTTGATCCAACACCTGAACAGACGTAC 3′) (Zarlenga et al., 1998) and the ITS-2 with primers NC1 (5′ACGTCTG- GTTCAGGGTTGTTT 3′) and NC2 (5′TATGTTTCTTTCTCCCGTCG 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 MgCl₂, 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 M EDTA) agarose gels. PCR products were purified using Qiagen® 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 was 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; Zarlena 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 (Zarlena 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.

LITERATURE CITED


