

Molecular characterization

For phylogenetic analysis, three molecular markers were used: partial sequence of 28S, D2D3 domain, internal transcribed spacer (ITS) regions of rDNA, and mitochondrial gene encoding cytochrome C oxidase subunit I (*COI*).

Nematode DNA was extracted from several thousand IJs using JETQUICK Tissue DNA Spin Kit (GENOMED) according to supplier information.

Ribosomal and mitochondrial regions were amplified by PCR from the previously extracted genomic DNA. The nuclear large subunit rDNA gene region (28S), corresponding to positions 3745-4700 in *Caenorhabditis elegans* Maupas, and containing the D2 and D3 divergent domains, was amplified using forward primer 391 (D2F) 5'-AGC GGA GGA AAA GAA ACT AA-3' and reverse primer 501 (D3R) 5'-TCG GAA GGA ACC AGC TAC TA-3' (Nadler *et al.*, 2006). For amplification of the ITS region we used primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') (forward primer) and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') (reverse primer) (Joyce *et al.*, 1994).

Approximately 45% of the mitochondrial *COI* gene, corresponding to positions 7893-8596 in *C. elegans*, was amplified using forward primer 507 (COXIF) 5'-AGT TCT AAT CAT AAR GAT ATY GG-3' and reverse primer 588 (COXIR) 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Nadler *et al.*, 2006). PCR cy-

cling conditions varied according to the region amplified. For the 28S (D2D3) rDNA region, the parameters included denaturation at 94 °C for 3 min, followed by 33 cycles of 94 °C for 30 sec, 52 °C for 30 sec and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. For the ITS rDNA region, PCR conditions included initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min 30 sec and 72 °C for 2 min, followed by a final extension at 72 °C for 5 min. For the *COI* mtDNA region, the PCR protocol included denaturation at 94 °C for 3 min, followed by 37 cycles of 94 °C for 30 sec, 50 °C for 30 sec and 72 °C for 45 sec, followed by a final extension at 72 °C for 7 min. The products were analysed on 1% agarose gels with TBE buffer. The PCR products were purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) following the manufacturer's instructions. PCR products were sequenced in both directions by a contract sequencer (STABVIDA Inc).

Sequence alignment and phylogenetic analysis of ITS, D2D3 and *COI* sequences

The sequences obtained (Table I) were compared with those from GenBank using the BLASTN program (Altschul *et al.*, 1990) and were aligned with BioEdit ver. 7.0.5 (Hall, 1999) with other *Steinernema* species sequences (Table II) under default alignment parameters. Sequences were deposited under the accession numbers

Table II. Details of *Steinernema* spp. used in this study. Site of sequencing: NCBI, BLAST (Spaces shown as '-' means the absence of information).

Species	Accession number to ITS rDNA, 28S domain D2D3, cytochrome oxidase c subunit I (<i>COI</i>)
<i>S. abbasi</i> Elawad, Ahmad, and Reid, 1997	AY248749, GU569060, HQ406728
<i>S. affine</i> (Bovien, 1937)	AF331912, AF331899, GU569061
<i>S. arenarium</i> (Artyukhovsky, 1967)	AY230160, AF331892, AY943979
<i>S. beddingi</i> Qiu, Hu, Zhou, Pang and Nguyen, 2005	AY603397, AY603396, -
<i>S. boemarei</i> , Lee, Sicard, Skeie and Stock, 2009	FJ152414, GU569046, GU569065
<i>S. bicornutum</i> Tallosi, Peters, and Ehlers, 1995	AY230163, GU569045, GU569064
<i>S. carpocapsae</i> (Weiser, 1955)	GQ421615, EU598241, AY943981
<i>S. ceratophorum</i> (Jian, Reid, and Hunt, 1997)	AY230165, AF331888, AY943982
<i>S. cubanum</i> Mracek, Hernandez, and Boemare, 1994	AY230166, AF331889, AY943983
<i>S. diaprepsi</i> Nguyen and Duncan, 2002	GU173997, GU177830, GU569067
<i>S. feltiae</i> (Filipjev, 1934)	EU598240, EU598250, AY943985
<i>S. glaseri</i> (Steiner, 1929)	AF122015, AF331908, AY943986
<i>S. hermaphroditum</i> Stock, Griffin and Chaenari, 2004	- , AY598358, AY943987
<i>S. intermedium</i> (Poinar, 1985)	AY230172, AF331909, AY943988
<i>S. loci</i> Phan, Nguyen and Moens, 2001	GQ497740, - , -
<i>S. kraussei</i> (Steiner, 1923)	EU914856, GU569053, GU569070
<i>S. longicaudum</i> Shen and Wang, 1992	AY230177, GU569054, AY943993
<i>S. monticolum</i> Stock, Choo and Kaya, 1997	AF331914, AF331895, AY943994
<i>S. neocurtillae</i> Nguyen and Smart, 1992	AF122018, FJ263674 , -
<i>S. oregonense</i> Liu and Berry, 1996	AY230180, GU569055, GU569072
<i>S. pakistanense</i> Shahina, Anis, Reid, Rowe and Maqbool, 2001	AY230181, - , -
<i>S. riobrave</i> Cabanillas, Poinar and Raulston, 1994	GU174001, GU177834, AY943998
<i>S. robustispiculum</i> Phan, Subbotin, Waeyenberge & Moens, 2005	AY355444, - , -
<i>S. silvaticum</i> Sturhan, Spiridonov and Mráček, 2005	- , DQ399663, -
<i>S. websteri</i> Cutler and Stock, 2003	- , JF503100, GU569074
<i>S. weiseri</i> Mráček, Sturhan and Reid, 2003	- , GU569059, GU569075