Table III. Abiotic/biotic analysis of soil samples positive for *S. intermedium*, *S. kraussei* and *Steinernema* sp., from continental Portugal.

Factor considered	*S. intermedium* isolate 2B	*S. kraussei* isolate 20F	*Steinernema* sp. isolates 59F	*Steinernema* sp. isolate 15G
County	Mora	Castro d´Aire	Figueira da Foz	Figueiró dos Vinhos
Vegetation	Mixed forest	Moors and heathland	Mixed forest	Broad-leaved forest
GPS coordinates	38º 97´N 8º 13´W	40º 91´N 7º 85´W	40º 14´N 8º 83´W	39º 97´N 8º 33´W
Elevation (m)	136	745	47	76
Average annual temperature (ºC)	16.7	15.2	15.2	18.1
Total anual rainfall (mm)	346.1	1152.8	1152.8	987.3
P_2O_5	16.00	30.00	72.00	128.00
NO_3	35.50	214.00	21.50	248.00
Organic matter (%)	3.20	6.10	6.3	6.10
pH (H_2O)	5.34	4.17	4.90	6.23
% Nitrogen (Kjeldahl)	0.11	0.26	0.25	0.27

reported in Table II. Using Mega 5 (Tamura *et al*., 2007), phylogenetic relationships among isolates were reconstructed by the Maximum Parsimony method (Nei and Kumar, 2000). Clades from trees of MP were supported by bootstrap analysis (Felsenstein, 1985) with 1000 replicates. The distances were calculated according to Close-Neighbor-Interchange (CNI) on Random Trees model (Nei and Kumar, 2000) for both nucleotide and amino acid sequence alignments. Sequences of *Bursaphelenchus xylophilus* Steiner *et* Buher were used as out-group in the different phylogenetic trees.

RESULTS

Nematodes extracted from the collected samples belonged to the genera *Steinernema* and *Heterorhabditis*. They were *H. bacteriophora* Poinar (1.9%), *S. feltiae* (Filipjev) Wouts, Mráček, Gerdin *et* Bedding (11.5%), *S. kraussei* (Steiner) Travassos (0.32%), *S. intermedium* (Poinar) Mamiya (0.32%) and *Steinernema* sp. (0.63%) *glaseri*-group. Isolates 15G and 59F belong to *Steinernema* sp., isolate 20F was identified as *S. kraussei* and isolate 2B as *S. intermedium*. The soil samples from which these nematodes were obtained were analysed for pH, organic matter and soil texture (Table III) at the Soil Analysis Laboratory, ICAAM, University of Evora. Isolate 2B was recovered from a forest habitat composed of stone pine (*Pinus pinea* L.) and cork oak (*Quercus suber* L.) stands. Isolate 20F was recovered from a region with heather (moors and heathland, NUTs "Nomenclatura para unidades territoriais para fins estatísticos" classification, accepted by European Union). Isolates 15G and 59F were recovered from olive groves and eucalyptus stands, respectively [NUT classification of mixed forest for isolate 59F, and broad-leaved forest for isolate 15G (Table III)].

Morphological and morphometric analysis

Morphology and morphometric analysis (Table IV) showed that isolate 2B possessed features characteristic of *S. intermedium* (IJ – tail dorsal constriction, male – robust and curved spicules with a prominent rostrum and mucronless tail) (Fig. 1C, D). Isolates 15G and 59F were identified as belonging to the *S. glaseri* (Steiner) Wouts, Mráček, Gerdin *et* Bedding group, in which the IJs were over 1000 µm long, with eight equally spaced and developed ridges in the lateral field and with a pos-

Table IV. Comparison of some morphologically important characters of the Portuguese entomopathogenic nematodes (n = 10).

Species (isolates)	IJ BL	IJ c´	IJ %D	IJ %Hy
S. intermedium (2B)	727-808	4.0-4.4	50-54	48-52
S. kraussei (20F)	909-970	4.0-4.5	43-46	27-30
S. glaseri group (59F)	1030-1232	3.6-3.8	55-60	45-50
S. glaseri group (15G)	1010-1111	3.5-3.8	54-60	45-50

IJ-infective juvenile, BL- body length, c´-tail length/tail width, IJ %D- length to excretory pore/pharynx length x100, IJ % Hy - hyaline layer length/tail length x100.