nematodes using the Tissue DNA Isolation Kit (Amersham Biosciences). PCR assays were set up in order to amplify the complete ITS-rDNA region, using the forward primer ITS1 [5´-TCCGTAGGTGAACCTGCGG-3'] and the reverse primer ITS4 [5´-TCCTCCGCTTATTGATATGC-3´] (Nasmith *et al.*, 1996). The PCR products were cloned and sequenced using the TOPO TA Cloning®Kit for Sequencing (Invitrogen). Specifically, PCR fragments were ligated into the pCR®4-TOPO plasmid, and transformed into TOP10 chemically competent *E. coli*. Successful recombinant colonies were cultured overnight in LB broth (Lab M), with 100µg/ml ampicillin (Sigma), at 37°C with gentle agitation. Plasmids were extracted from subsequent cultures using the StrataPrep® Plasmid MiniPrep Kit (Stratagene). The presence of ligated inserts was confirmed by *EcoR* I (Promega) digestions of plasmids followed by size comparisons between plasmid and insert using agarose gel electrophoresis. PCR inserts were sequenced directly from plasmids by a contract sequencer (Qiagen Inc.). Nucleotide sequences were determined in both directions using PCR M13 primers. The DNA base sequences obtained for the Portuguese isolates [accession numbers EF595633 (isolate I1), EF595634 (isolate I8), EF595635 (isolate H9)] were compared with other sequences deposited in the GenBank database.

Bait larvae infected with isolates from three sampled sites (I1 and H9 from Alentejo region, and I8 from southern Tejo valley) displayed the grey-brown colouration, lack of putrefaction and retention of shape that is characteristic of *Galleria* cadavers infected with steinernematids. The three strains isolated show the typical morphological characters within the genus *Steinernema* Travassos, 1927 (Adams & Nguyen, 2002).

The ITS1-5.8S-ITS2 region, including the partial 18S and 28S rDNA genes (flanked by the above primers) of the three Portuguese isolates are almost identical (2 – 6 nucleotide substitutions), and are 773 bp long. BLAST searches (Altschul *et al*., 1990) in GenBank showed that the three Portuguese isolates have a high similarity (96 – 99%) with those sequences available for *S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 populations (e.g. accession numbers AF121050, AY1711247, AY230170, AB243439). Sequences of other species from the *feltiae* group, namely *S. oregonense* Liu & Berry 1996 and *S. krauseei* (Steiner, 1923) Travassos, 1927, were obtained from GenBank searches that exhibited a lesser degree of similarity with the Portuguese isolates and other *S. feltiae* populations (e.g. accession numbers AF122019, AB243442) (Fig. 1).

Alignment of the ITS 1 regions from the Portuguese isolates with *S. feltiae* ITS 1 sequences obtained from GenBank, suggests that the Portuguese isolates share a high degree of homology to a UK population (accession number AY230170) as characterised by six shared nucleotide deletions (Fig.1) classified previously by Spiridonov *et al*. (2004).

Unlike other European countries Portugal has not fully engaged with its soil biodiversity for the discovery of EPN. The present study constitutes the first report of an EPN genus for continental Portugal. *S. feltiae* has a wide distribution in temperate regions, being one of the most common species found in Europe, and in many other parts of the world (for a detailed EPN species distribution see Hominick, 2002). The preliminary results presented herein show that *S. feltiae* is distributed in a geographic medium-scale in central-south areas of Portugal, associated with distinct types of habitats (oak stands and rice paddies). In addition, this is the first time that *S. feltiae* is recorded for the Portuguese territory.

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