

ACC-3') (reverse) (Nguyen *et al.*, 2004). Nucleotide sequences were analysed and edited using BioEdit Sequence Alignment Editor (version 7.0.4.1). The DNA sequences of the Portuguese isolates were compared with other sequences deposited in the GenBank database. Multiple alignments of the ITS rDNA sequences were obtained using Clustal W (Thompson *et al.*, 1994). Phylogenetic analyses were performed by the Neighbour-Joining method, with bootstrap analysis based on 1000 resamplings using MEGA4 software (Tamura *et al.*, 2007).

Results and discussion

EPNs were recovered from three samples from the Alentejo region, corresponding to three different sampled habitats (Table 1). The baited larvae infected from these samples displayed the typical red coloration, lack of putrefaction and retention of shape that is characteristic for *G. mellonella* cadavers infected with heterorhabditids. All the specimens found associated with the different sampled areas displayed the same main morphological characters (Fig. 1).

Table 2. Positive samples: geographical region and characteristics from soil with *H. bacteriophora*

Sample	Seasons	Soil Characteristics			Geographic location			Atmospheric conditions	
		PH	Texture	Total Nitrogen	Latitude	Longitude	Altitude	Tm anual (°C)	Pp (mm)
I3	Autumn	6.58	Medium	0.2 %	38.77 N	8.68 W	77 m	21.15	3.0
R7	Winter	6.35	Medium	0.2 %	37.34 N	8.79 W	90 m	13.35	0
X7	Spring	5.48	Coarse	0.13 %	39.29 N	7.46 W	394 m	7.65	0

In the first generation, hermaphroditic females display a truncated head, with six well-developed conical lips (Fig. 1A), and median vulva without epitygma (Fig. 1B). Second-generation amphimictic females are similar to the hermaphroditic females. Males displayed paired, separate spicules; bursa with nine pairs of papillae, a pair anterior to cloaca, two pairs adjacent to the spicules and six pairs distal to the anal opening, the latter six distributed in two sets of three (Fig. 1C).

The sequence flanked by the two primers yielded a total of 809 bp, composed of the partial 18S (nucleotides 1 – 14 in the alignment), ITS1 (15 – 403), 5.8S gene (404 – 557), ITS2 (558 – 785), partial 28S (786 – 809). The ITS1-5.8S-ITS2 region of the three Portuguese isolates displayed 100 % similarity to each other [accession numbers EU435138 (isolate I3), EU435139 (isolate R7), EU435140 (isolate X7)]. BLAST searches suggested that the isolates belong to the species *Heterorhabditis bacteriophora* Poinar, 1976. Phylogenetic relationships based on the sequence alignment of the ITS rDNA region confirmed that the Portuguese isolates group with other isolates of *H. bacteriophora*, when compared with other species of the genus (Fig. 2).

H. bacteriophora, the most geographically widespread species of this genus, is a common species in regions with continental and Mediterranean climates and it has been reported from different areas of Africa, Asia, Europe, and America (Hominick, 2002). In Southern Europe, this species has an abundant distribution, and it was found associated with several types of habitats in Northern Spain (Garcia del Pino and Palomo, 1996; Campos-Herrera *et al.*, 2007) and in Southern France (Emelianoff *et al.*, 2008). In the last case, two strains of *H. bacteriophora* were reported, based on small differences (4 nucleotides) in the ITS sequence, both strains being associated with the different types of sampled habitats (Emelianoff *et al.*, 2008). Although several habitats with the same soil and climate characteristics as those in Southern France were sampled in Alentejo and the Algarve, only specimens belonging to one of the strains (strain 2) were found, and only in non-coastal habitats (Table 2) as reported in southern France (Emelianoff *et al.*, 2008).

Based on the biodiversity and distribution of EPN's from the Iberian Peninsula and other Mediterranean regions, the presence of *H. bacteriophora* should be expected in conti-

ental Portugal. However, no species of this genus had been reported until now for continental Portugal, therefore this work represents the first report of the genus in this geographic area.

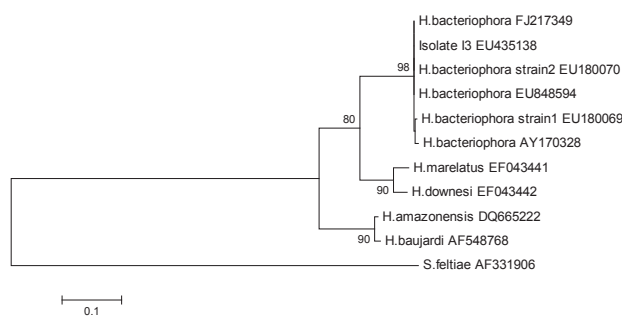


Fig. 2. Phylogenetic relationships of *Heterorhabditis bacteriophora* isolates from Portugal and other geographical regions, including other species of the genus, based on the sequence alignment of the ITS regions from ribosomal DNA. The dendrogram was generated by Neighbor-Joining analysis with 1000 bootstrap replication. Bootstrap values (%) are indicated at the nodes. The scale bar indicates 1% substitutions per site.