of isolates from North America and throughout Eurasia. These isolates vary significantly in morphology, even sharing characters with *S. affine* (Yoshida *et al*., pers. com.). The isolates of the *S. affine/S. intermedium* complex need a serious morphological and DNA revision. Stock (2002) points to the difficulties in using just molecular identification for EPN.

Several studies on molecular markers show that the 28S and ITS regions from ribosomal DNA and mitochondrial cytochrome C oxidase subunit I gene (*COI*) can be considered the best DNA regions to study phylogenetic relationships among EPN (Liu *et al*., 1999; Stock *et al*., 2001; Nguyen *et al*., 2007). The D2D3 segment is a region that seems to evolve slower than ITS and *COI,* so it is an interesting tool for species delimitation (Nadler, 2002). Morphological characters in phylogenetic analyses of EPN remain robust, but their use requires taxonomic expertise. For a more accurate identification, phylogenetic trees should incorporate molecular and morphological data, because with molecular data alone it is sometimes impossible to make inferences from homologous characters (Liu and Berry 1996; Qui *et al*., 2004; Nguyen *et al*., 2007; Lee *et al*., 2009).

During the survey undertaken after 2006 in continental Portugal, *H. bacteriophora* and *S. feltiae* were recovered (Valadas *et al*., 2007; Valadas *et al*., 2009). The present work describes three additional species that were identified as *S. kraussei, S. intermedium* and *Steinernema* sp. *glaseri*-group. *Steinernema intermedium* is a common species in Spain (Garcia del Pino, 2005), a neighbouring country with climatic conditions similar to Portugal. *Steinernema intermedium* appears to have a global distribution, having been originally described from South Carolina, USA (Nguyen *et al*., 2007). In Europe this species is known from Germany (Sturhan, 1999), Czech Republic (Mráček *et al*., 1999), Spain (Garcia del Pino, 2005) and Switzerland (Steiner, 1996). *Steinernema kraussei* is widely distributed in Europe, having been reported from Belgium, Czech Republic, Germany, Sweden, Netherlands, UK and Italy (Tarasco *et al*., 2009). This species is common in European woodlands and forest soils (Sturhan *et al*., 2005). Also, it has been found in Canada (Mráček and Webster, 1993) and USA (Stock *et al*., 2000); it is prevalent in woodland habitats (Mráček *et al*., 2005) and is rarely found in open habitats.

The current study on EPN identification was based on three regions from ribosomal and mitochondrial DNA combined with morphological and morphometric identification. Thus, the comparison of ITS and 28S rDNA and cytochrome C oxidase subunit I (*COI*) mtDNA provides information to assess the relatedness among *Steinernema* spp. In the present study the use of DNA sequences provided limited information concerning the closest species of our isolates, using two regions of ribosomal DNA (ITS and 28S, D2D3 domain) and one region of mtDNA (cytochrome C oxidase subunit I) for isolate 2B. Based on morphological and morphometric data, isolate 2B was identified as *S. intermedium* due to the presence of bluntly tipped spicules and lack of a spine in the tail of infective juveniles (Fig. 1), which is characteristic of the IJ of *S. affine*. In Portugal, *S. intermedium* occurred in *Pinus pinea* forests and in a soil with low pH (5.34). *Steinernema affine* and *S. intermedium* probably derived from the same ancestor, the differences between the two being due to their adaptations for survival in different geographic conditions, and so there is a clear disadvantage of using just a molecular and not a morphological approach to identify the species. Using just molecular data, isolate 2B groups with *S. affine* in ITS and *COI* markers with bootstraps of 68% and 99%, respectively. These groups then group with *S. intermedium*, with bootstraps between 74-100%. Without morphological characterization, it would not be possible to identify isolate 2B as *S. intermedium*. Isolate 20F was morphologically identified as *S. kraussei*. This species can be distinguished by spicule shape, usually with a finely oblongate manubrium, moderately developed rostrum and short hyaline layer. Molecularly, isolate 20F groups with *S. kraussei*, for all the three markers with strong bootstrap values between 52 and 99%. Isolates 15G and 59F, represent the same species because, in phylogenetic analysis using ITS and *COI* markers, they always group together. They were identified as *Steinernema* sp. belonging to the *glaseri*-group, with which they share morphological characteristics. The only information that we obtained from the morphology and sequence analysis of these two isolates was that they share high degrees of identity, and group with species of the *glaseri*-group. The clarification of whether these isolates are conspecific either with *S. glaseri*, found in the Azores (Rosa *et al*., 2000), or *S. arenarium*, found in Spain (Garcia del Pino, 2005), or are even a new species, requires more detailed study. Moreover, a comparison with *S. boemarei* Lee, Sicard, Skeie *et* Stock, belonging to the *glaseri*-group and described from southern France (Lee *et al*., 2009), is needed.

This study has identified three more species new to continental Portugal, namely *S. kraussei, S. intermedium* and a species which we have so far identified as *Steinernema* sp. *glaseri*-group. Previously, only *S. feltiae* (Valadas *et al.,* 2007) and *H. bacteriophora* (Valadas *et al.,* 2009) had been reported from continental Portugal. The habitats where *S. kraussei* was found was moors and heathlands and *S. intermedium* was detected in mixed forest, both considered acid soils (5.34 for *S. intermedium* and 4.17 for *S. kraussei*). Also, it seems that these two previous species are not so abundant in continental Portugal as *S. feltiae* and *H. bacteriophora,* that were found in 36 of the soil positive samples, whereas *S. intermedium* and *S. kraussei,* as well as *Steinernema* spp. were just detected in four soil samples, respectively, out of the 320 samples collected in Alentejo and central continental Portugal.