1 **First report of *Pratylenchus penetrans* (Nematoda: Pratylenchidae) associated with**

2 **amaryllis (*Hippeastrum x hybridum*), in Portugal**

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16 The world flower business is a highly dynamic industry, and in Portugal yields around 500

17 million EUR a year (INE, 2013). Root lesion nematodes (RLN), *Pratylenchus* spp., are

18 considered the third most important group of plant parasitic nematodes (PPN) worldwide due

19 to its large distribution, wide host range, and impact on economically important crops

20 (Castillo and Vovlas, 2007). The most recent report of RLN parasitizing amaryllis

21 (*Hippeastrum* spp.) was the amaryllis lesion nematode, *P. hippeastri*, in Florida (USA)

22 (Inserra et al., 2007). In 2016 soil and root samples of an amaryllis hybrid *Hippeastrum* x

23 *hybridum* CV. Benfica were collected from potted plants produced in greenhouses in Montijo

24 (Setubal Peninsula, Central Portugal), 50 km from Lisbon. Nematodes were extracted from

25 soil samples according to the protocol PM 7/119 (1) (EPPO, 2013) and identified to genus-

26 level based on morphometrical traits of 10 females and 11 males. DNA extraction was

27 conducted from 10 nematodes separately, PCR amplification and sequencing of D2/D3

28 expansion segment of the 28S rRNA and 18S rRNA genes using the following primers D2A

29 and D3B (De Ley et al., 1999) and 1813F and 2646R (Holterman et al., 2006). The nucleotide

30 sequences were deposited as MN756841 and MN756509 at the NCBI. RLN were detected in

31 all 5 samples in a concentration of around 100 nematodes/100cm^3 soil. Some primary roots of

32 affected plants showed small, elliptic reddish-brown lesions and poor growth. More severely

33 affected plants presented stunted, chlorotic foliage and had the cortical lesions enlarged to the

34 point where rotting was severe and many roots were easily detached from the bulb, in some

35 cases, there was not emergence from bulbs (Figure 1). The female body was moderately

36 slender, almost straight when killed by gentle heat, lateral field with four straight lines, body

37 annules distinct, lip with three annules, stylet knobs round, median bulb round, spermatheca

38 round and filled with sperm, tail tapering to rounded terminus, sometimes with two striae.

39 Body length was 558.99 μm ± 48.50, body width 22.45 μm ± 2.02, stylet length 18.52 μm ±

40 1.02, and tail length 32.91 μm ± 7.50. Values of a, c and c' ratios were 25.95 ± 2.53, 17.62 ±

41 3.45 and 38.49 ± 4.42, V (%) value was 80.92 ± 1.69. Males were smaller than females with

42 body length 511.37 μm ± 29.90, stylet length 17.55 μm ± 1.04 and spicule length of 18.53 μm

43 ± 1.17. No other PPN was found in the suspensions extracted from the soil samples. BLAST

44 hits showed that partial sequence of D2/D3 rRNA loci (540 bp) was 96.2% identical (e-value

45 0.0) to *P. penetrans* isolate MU2 (KP161612) and voucher T143 (KY828357). Partial

46 sequence of 18S rRNA (650 bp) was 100% identical to *P. penetrans* isolate ILVO-Pp

47 (MH983023) and 99.81% with *P. penetrans* Praty-A (AB661627). In the corresponding

48 phylogenetic trees, both *P. penetrans* sequences clustered together with other species from the

49 penetrans group (*P. fallax*, *P. convallariae* and *P. oleae*) with good support (Figure 2)

50 (Janssen et al., 2017). Nevertheless, while all species within this group share many

51 morphological characteristics (Subbotin et al., 2008), our specimens presented higher

52 similarity, both morphological and molecular, with *P. penetrans*. So far, this RLN has been

53   found associated mainly with potato fields in Portugal (Abrantes et al., 1987; Esteves et al.,

54   2015) thus, to our knowledge, this is the first report of *P. penetrans* parasitizing amaryllis, in

55   Portugal.

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57   **Literature cited**

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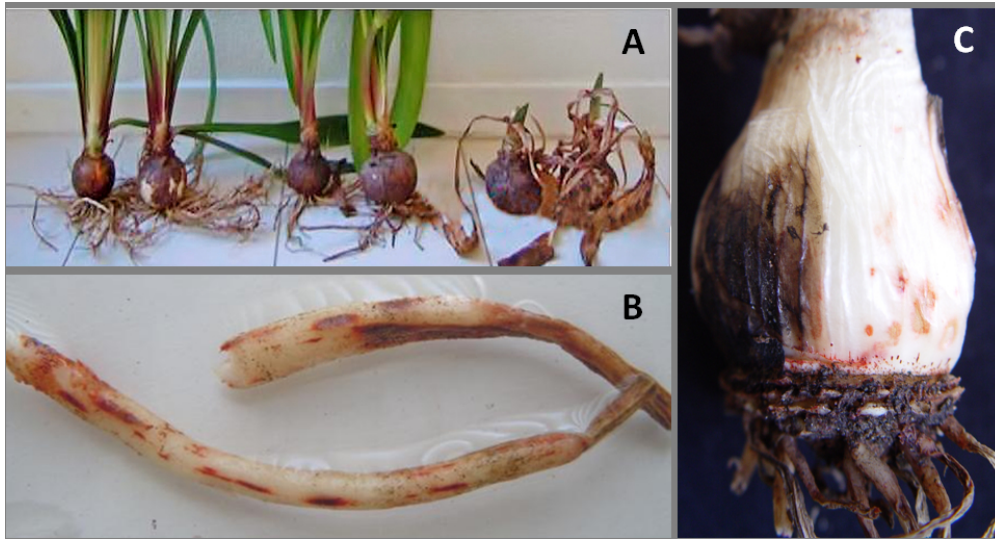69   Subbotin et al. 2008. Mol. Phylogenet. Evol. 48:491-505.

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Figure 1. Symptoms caused by Pratylenchus penetrans in plants of Amaryllis (Hippeastrum x hybridum CV. Benfica), Montijo – Portugal; A) healthy (left) and infected plants (center and right); B) and C) typical reddish lesions on roots and bulbs, respectively.
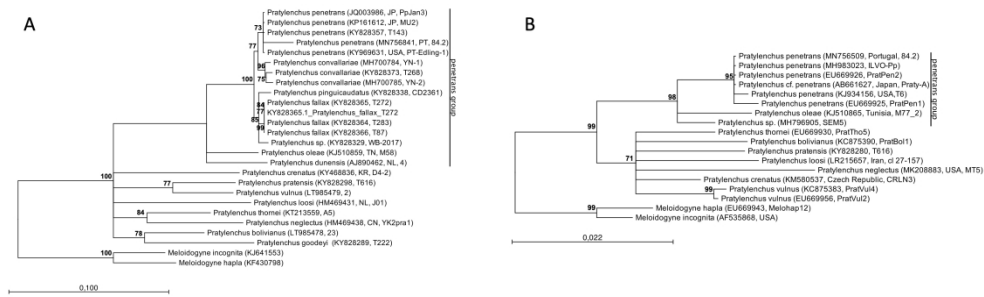
Figure 2. Phylogenetic relationships of Pratylenchus penetrans collected from Portugal. A: Sequence (MN756841) based on the alignment of the D2/D3 rDNA loci. B: Sequence (MN756509) based on the alignment of 18SrRNA gene. Meloidogyne hapla and M. incognita were used as outgroups. The phylogram was generated using the maximum likelihood method based on the General Time Reversible + Gamma model with 1,000 bootstrap replications. Bootstrap values of over 70% are given in the appropriate nodes. Evolutionary analyses were conducted in CLC Workbenck v8.

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