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Research Note

First report of the lesion nematodes: *Pratylenchus brachyurus* and *Pratylenchus delattrei* on tomato (*Solanum lycopersicum* L.) plants in Cape Verde

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Summary

Roots of *Solanum lycopersicum* L. were collected in growing season of year 2015, on the island of Santiago in Cape Verde. Morphological, morphometric and molecular (18S rDNA and 28S rDNA) studies revealed the presence of *Pratylenchus brachyurus* and *P. delattrei* in root systems and root zones of tomato plants. To our knowledge, this is the first record of the occurrence of these nematode species in Cape Verde.
Keywords: Cape Verde; new geographic record; *Pratylenchus brachyurus*; *Pratylenchus delattrei*; 18S rDNA; 28S rDNA

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the vegetable crops most widely grown on irrigated land in the Republic of Cape Verde. Little is known about the plant-parasitic nematodes responsible for pest infestations of this economically important crop in this area. So far only *Rotylenchulus reniformis* Linford & Oliveira, 1940 (Germani, 1978), *Heterodera schachtii* Schmidt, 1871 (Sturhan, 1993) as well as some representatives of the genus *Meloidogyne* Göldi, 1892 (Netscher & Taylor, 1976) and *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Flis *et al*., 2018) have been identified as nematodes parasitising tomato roots in Cape Verde. During the study of plant parasitic nematodes associated with tomato cultivation in Cape Verde, representatives of lesion nematodes *Pratylenchus* (Filipjev, 1936) were found. Morphometric analysis and molecular study allowed to identify the two investigated species as *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev et Schuurmans Stekhoven, 1941 and *P. delattrei* Luc, 1958. *Pratylenchus* spp. have significant economic impacts. Crop damage and yield losses by these nematodes have been previously observed (Egunjobi, 1974; Talwana *et al*., 2016). Those migratory endoparasites move within host root tissues causing necrosis and creating wounds, thus providing openings for soil-borne plant pathogens to enter and cause disease. In tropical and subtropical regions *Pratylenchus* spp. infect roots of several crops. *P. brachyurus* has been recorded parasitising pinapple roots in Hawaii (Godfrey, 1929), Uganda (Bafokuzara, 1982) and Brazil (Monteiro & Lordello, 1972); citrus and forest trees in North America (Brooks & Perry, 1967; Ruehle, 1971), coffee in Brazil (Lordello *et al.,* 1968), rubber in India (Mukherjee *et al.,* 2000) and many other species of economically important plants (Luc *et al.,* 2005; Castillo & Vovlas, 2007; De Araujo Filho *et al.,* 2014). *P. brachyurus* has also been reported from a few countries in Europe, in Bulgaria on tobacco (Katalan-Gateva & Nedechlev, 1983), on several crops in Russia (Ryss, 1988) and Australia (Riley & Kelly, 2002). *P. delattrei* primarily described from Madagascar (Luc, 1958) has also been found on several crops in Vietnam (Ryss, 1988), on sugarcane in Sudan (Saadabi, 1988), in ornamental plants, like rose and rhapis, grown in Korea (Kim & Minagawa, 1996), in date palm in Oman (Mani *et al.,* 2005) and in several hosts and localities in India (Jothi *et al*., 2004). In this article we provide morphological description and morphometric data of *P. brachyurus* and *P. dellatrei* from Cape

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Table 1. Morphometrics of *Pratylenchus* spp. from tomato in Cape Verde. All measurements are in µm and in format: mean ± S.D. (range).

Character/ratios	*Pratylenchus brachyurus* (Godfrey 1929)	*Pratylenchus delattrei* Luc 1958
n	14	20
L	517 ± 45 (414 – 573)	532 ± 33 (498 – 586)
a	24.2 ± 1.9 (20.1 – 26.7)	26.6 ± 2.2 (22.1 – 31.3)
b	6.3 ± 0.5 (5.1 – 6.9)	6.6 ± 0.5 (6.1 – 7.7)
b'	3.7 ± 0.4 (2.9 – 4.1)	4.5 ± 0.4 (4.0 – 5.3)
c	20.1 ± 2.4 (15.3 – 25.5)	21.9 ± 2.1 (18.5 – 25.1)
c'	2.2 ± 0.2 (1.7 – 2.5)	2.2 ± 0.2 (1.9 – 2.8)
V	86 ± 1 (84 – 88)	76 ± 1 (75 – 78)
Stylet length	18.3 ± 0.4 (17.8 – 18.9)	16.4 ± 0.4 (15.4 – 16.9)
Dorsal gland opening	2.2 ± 0.3 (1.6 – 2.6)	2.9 ± 0.3 (2.4 – 3.1)
O	12.0 ± 1.9 (8.6 – 14.6)	17.4 ± 1.7 (14.3 – 21.3)
Pharynx length	82.1 ± 1.7 (79.2 – 85.2)	80.1 ± 3.2 (73.1 – 84.2)
Pharyngeal overlap	47.7 ± 7.9 (37.0 – 62.3)	39.0 ± 7.2 (29.8 – 49.0)
Maximal body diameter	21.5 ± 1.9 (17.7 – 26.0)	20.2 ± 2.2 (16.8 – 23.7)
Anal body diameter	12.2 ± 1.1 (15.9 – 20.9)	11.2 ± 1.2 (8.9 – 13.2)
Tail length	25.9 ± 1.9 (22.0 – 28.3)	24.8 ± 2.3 (21.0 – 27.1)
Tail annuli	18 ± 2 (16 – 23)	19 ± 2 (16 – 24)
Phasmid to terminus	14.3 ± 1.5 (11.6 – 16.9)	10.6 ± 2.7 (6.0 – 14.9)

Abbreviations are defined in Siddiqi (2000)

Verde. Genetic characteristics of LSU (28S rDNA) and SSU (18S rDNA) from analysed populations and results of comparative study of these sequences are also supported.

Material and Methods

Eight tomato plants with soil surrounded root systems were harvested in June 2015 in the village of Achada Colaço (located at 15°06'35.3"N, 23°31'31.9"W). Nematodes from roots were isolated by the modified Baermann method, whereas specimens from soil samples were extracted using the decanting and sieving method. Females assigned for morphological analysis were fixed in a triethanolamine formalin water solution (TAF) and mounted in anhydrous glycerine (Seinhorst, 1959) on slides using the paraffin-ring method. Morphological observations and morphometric analyses were performed using a Leica light microscope with the Nomarski differential interference contrast. Females selected for the genetic analysis (three specimens for each species) were fixed in a DESS solution (Yoder *et al.,* 2006). After washing in sterilized milli-Q water, single nematode individuals were used for DNA extraction according to the nematode lysis procedure, as described by Holterman *et al*. (2006). 18S rDNA gene fragments were amplified in PCR reactions with the aid of 988F and 1912R, as well as 1813F and 2646R primer combinations (Holterman *et al.,* 2006). Amplification of the partial 28S rDNA sequence was obtained using primers 61F (Holterman *et al.,* 2008) and MCB1R (Dobosz *et al.,* 2013). The 18S and 28S rDNA regions were sequenced by the Sanger method on ABI 3500L genetic analyzer (Applied Biosystems, Foster City, CA, USA). The newly obtained 28S rDNA sequences were implemented in a phylogenetic analysis concerning relationships within clades IV ('*P. penetrans* group') and VI ('*P. zeae* group') of the genus *Pratylenchus* as defined by Subbotin *et al.* (2008) and Palomares-Rius *et al.* (2014). Initial multiple sequence alignment was performed using the BioEdit program (v. 7.2.5; Hall, 1999) and included publically available (GenBank) representatives of '*P. penetrans* and *P. zeae* groups'. All available unique *P. brachyurus* and *P. delattrei* sequences were used. In order to root the tree two outgroup sequences (FN433867 and JN967754) were chosen based on the total nematode phylogeny by Van Megen *et al.* (2009). The final multiple-sequence alignment contained 663 positions. Substitution models were tested using "FindModel", an online implementation of the MODELTEST program (Posada & Crandall, 1998). The General Time Reversible plus Gamma substitution model was selected. The Bayesian phylogeny was constructed with the program MrBayes (v. 3.1; Ronquist & Huelsenbeck, 2003). Four independent runs were performed with four Markov chains per run. The program was run for 800,000 generations with a sample frequency of 200 generations. The sampled trees from each run were combined in a single 50 % majority-rule tree. Stabilisation of the likelihood and parameters was checked with the program Tracer (v. 1.6; Rambaut *et al.*, 2014).

Results and Discussion

Morphological and morphometric analyses of the investigated females confirmed their affiliation to *P. brachyurus* and *P. delattrei*. The morphology of *P. brachyurus* from Cape Verde (14 females) was characterised by: low and angular lip region with two

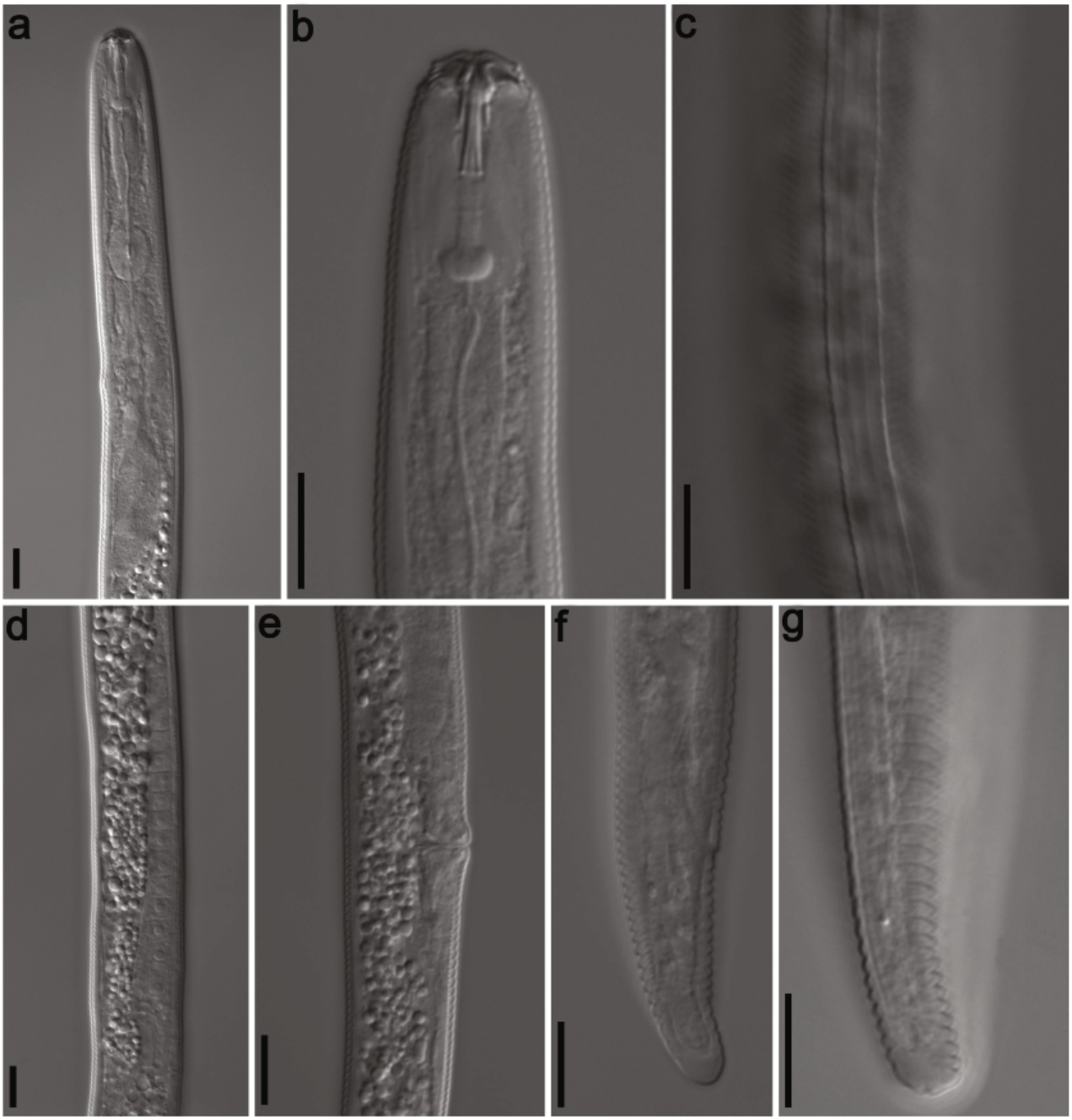

Fig. 1. *Pratylenchus brachyurus* (Godfrey 1929) female from Cape Verde. **a.** pharyngeal region; **b.** anterior end; **c.** lateral field at mid-body; **d.** ovary with single row of oocytes; **e.** vulval region; **f.** tail; **g.** tail, phasmid. (All scale bars = 10 µm)

lips annuli, which is separated from body contour; long spear with rounded, laterally directed knobs; lateral field with four lines; a single row of oocytes; empty and rounded spermatheca; posterior vulva; undifferentiated and well developed postvulval uterine sac; conoid tail with rounded (to truncate), smooth or broadly annulated terminus. The obtained results agreed with the previous descriptions of *P. brachyurus* (Loof, 1978; Castillo & Vovlas, 2007) (Fig. 1, Table 1). The morphology of *P. delattrei* from Cape Verde (20 females) was characterised by continuous body contour, more or less truncate lip region with three annuli; medium spear, with rounded and slightly anteriorly directed knobs; lateral field with four lines; two rows of oocytes; empty and rounded spermatheca, posterior vulva; undifferentiated and well developed postvulval uterine sac; subcylindrical tail with rounded and smooth terminus

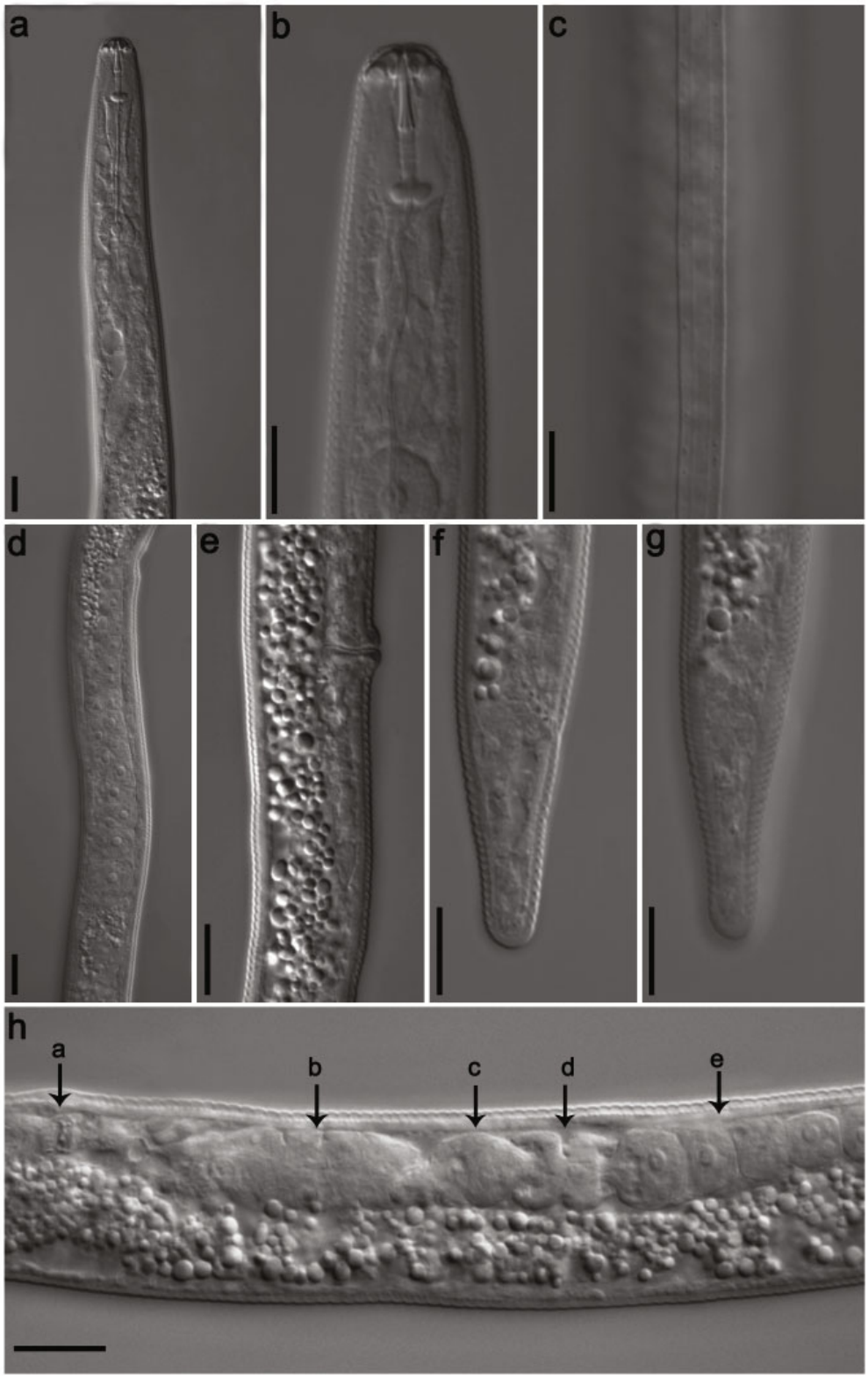

Fig. 2. *Pratylenchus delattrei* Luc 1958 female from Cape Verde. **a.** pharyngeal region; **b.** anterior end; **c.** lateral field at mid-body; **d.** ovary with double rows of oocytes; **e.** vulval region; **f.** tail; **g.** tail, phasmid; **h.** genital system: a - vulva, b - crustaformeria, c - spermatheca, d - oviduct, e - ovary. (All scale bars = 10 µm)

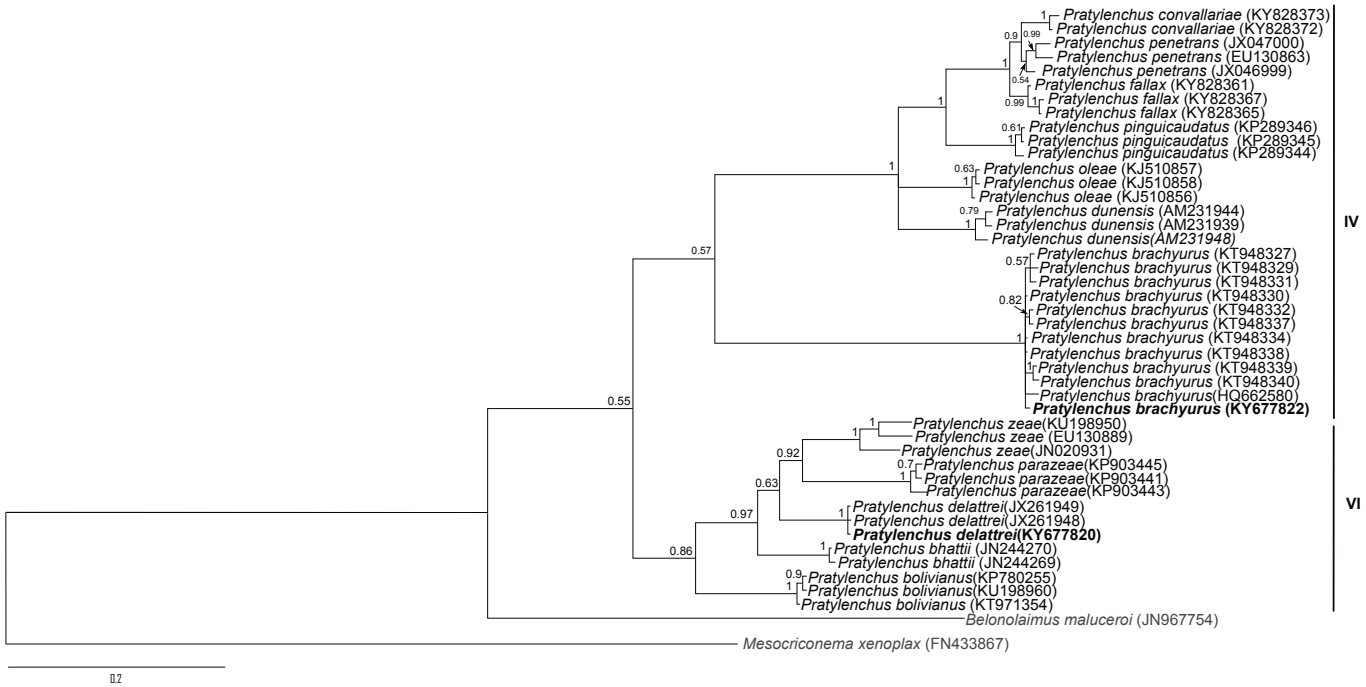

Fig. 3. *Pratylenchus* spp., 28S rDNA-based Bayesian phylogenetic tree revealing relationships within clades IV ('*P. penetrans* group') and VI ('*P. zeae* group'). Numbers near nodes stand for posterior probabilities. The newly obtained 28S rDNA sequence of *P. brachyurus* and *P. delattrei* from Cape Verde are indicated in bold.

(Fig. 2, Table 1). Morphological characteristics of *P. delattrei* from Cape Verde are in line with descriptions of previously investigated materials (Luc, 1958; Loof, 1978; Castillo & Vovlas, 2007; Majd Taheri *et al*., 2013). A comparison of morphometric analysis findings between the specimens concerned and those previously described showed cross-population quantitative differences in tail annulation in females. Studies so far have indicated that the numbers of tail annuli in *P. delattrei* females varies from 17 to 25. According to Ryss (1988) and Loof (1978) the number of annuli does not exceed 20. However, our analysis as well that by Bahmani *et al.* (2013) and Majd Taheri *et al.* (2013) has pointed out the occurrence of specimens exhibiting an even higher number of tail annuli.

The amplification of the almost full-length 18S rDNA fragment was successful only in the case of *P. delattrei* (1672 bp, KY677819) while for *P. brachyurus* we were able to amplify only the second part of the 18S rDNA gene (810 bp, KY677821). To our knowledge this is the first report of a 18S rDNA sequence of *P. delattrei* while the GenBank available 18S rDNA partial sequences of *P. brachyurus* concern the first part of the gene (EU130795-EU130797, EU13084). Therefore, it was not possible to align the GenBank derived 18S rDNA sequences with the second part of the 18S rDNA gene partial sequences, obtained from the *P. brachyurus* from Cape Verde. The Basic Local Alignment Search for 28S rDNA from *P. brachyurus* (521bp) (KY677822) showed a 98 % similarity to the sequences of *P. brachyurus* deposited in GenBank from Brazil (KT948327, KT948329-KT948332, KT948334, KT948337-KT948340, HQ662580). The 28S rDNA sequence alignment from *P. delattrei* (718 bp) (KY677820) showed a 99 % similarity to another sequence of *P. delattrei* deposited in the GenBank from Iran (JX261948, JX261949). As expected in the resulted Bayesian tree (Fig. 3), the newly acquired sequences from Cape Verde had been positioned together with corresponding, GenBank available *P. brachyurus* and *P. delattrei* representatives. As observed in previous works by Palomares-Rius *et al.* (2014) and Janssen *et al.* (2017) *P. brachyurus* belongs to *'P. penetrans* group' and has been localised as a separate, basal branch in the clade IV. *P. delattrei* has been positioned in clade VI in close relationships to *P. parazeae*, *P. zeae*, *P. bhatti* and *P. bolivianus*.

Representatives of *P. brachyurus* and *P. delattrei* species are considered economically important plant-parasitic nematodes that are widespread mainly in tropical environments (Godfrey, 1929; Loof, 1978; Castillo & Vovlas, 2007). It is noteworthy that the distribution range of *P. brachyurus* is broader. This species has been detected on all continents except Antarctica (Tarjan & O'Bannon, 1969; Castillo & Vovlas, 2007). This report broadens our knowledge of nematode biodiversity of Cape Verde as well of the morphometry and molecular characteristics of two economically important species *P. brachyurus* and *P. delattrei*.

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