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Research Note

Description of *Paratrichodorus pachydermus* (Nematoda: Trichodoridae) from the Czech Republic

S. KUMARI

Crop Research Institute, Division of Plant Health, Drnovská 507, Ruzyně, 16106 Prague 6, Czech Republic,
E-mail: *kumari@vurv.cz*

Summary

A survey has been carried out to study the occurrence and distribution of *Paratrichodorus pachydermus* nematodes in the Czech Republic in a range of habitats (orchards, forests, vineyards, strawberry and river bank). A total 208 sites were surveyed and 19 sites were found positive for *P. pachydermus*. This species is a new record for Czech nematofauna.

Keywords: *Paratrichododus pachydermus*; PCR; Czech Republic; Nematode

Introduction

Members of the Trichodoridae can cause substantial crop losses by their direct feeding on plant roots. Interest in these nematodes stems largely from the discovery that *Paratrichododus pachydermus* (Seinhorst, 1954) Siddiqi, 1974 transmit tobacco rattle tobravirus (Sol *et al*., 1960; Sol & Seinhorst, 1961). Viruses transmitted by *P. pachydermus* belong to the genus *Tobravirus*, family *Tubiviridae*, which comprises *Tobacco rattle virus* (TRV) and *Pea early-browning virus* (PEBV) (Taylor and Brown, 1997). Both viruses are of considerable economic importance in a number of agricultural crops. Since no research has been carried out in the Czech Republic to determine the presence or extent of *P. pachydermus*, the objective of this research was to identify *P. pachydermus* by morphological characters and verify morphological identification by polymerase chain reaction.

Materials and methods

The occurrence of *P. pachydermus* was studied in orchards (apple, apricot, peach, plum, sour cherry, and sweet cherry), vineyards, forest, strawberry and river bank was examined in a survey carried out in the Czech Republic from 2003 to 2009. Specimens for the morphometric and morphological analysis from the field populations were extracted by sieving on 1 mm, 150 µm and 75µm and placing the residual on a tissue paper on a Baermann funnel from 24 – 48 hours (Brown and Boag, 1988). Nematodes were heat killed, fixed in TAF, processed in slow glycerin process and mounted in anhydrous glycerin on slides. Photomicrographs were recorded with a digital camera linked to a computer and measurements were made with the aid of imaging software (Olympus DP-soft version 3.2).

Total genomic DNA was extracted according to a rapid method by Stanton *et al.* (1998) and DNA template was amplified by polymerase chain reaction (PCR) using species specific primer PACHYREV2 (5′-GCGTACGGGCA ATACGATAC-3′) and antisense primer UNIVERSAL (5′-CCCGTCGCTACTACCGATT-3′) (Boutsika *et al*., 2004). All PCR reactions were performed on a DNA Engine PTC-1148 thermal cycler (Bio-Rad) with heated lid. The DNA was subjected to a PCR with the following specifications: first denaturation for 3 min at 95°C, 35 cycles with 45 s at 94°C, 45 s at 55°C, 45 s at 72°C and final extension at 72°C for 5 min. An aliquot (4 µl) of each amplification reaction was mixed with 1µl of 6x loading dye (Fermentas, MBI) and electrophoresed in high resolution 1.5 % agarose gel and run in TAE buffer. The bands were visualized and photographed under UV (312 nm) after syber safe (1 µg/ml) binding to the DNA fragments. A 100 base pair marker (Fermentas, MBI) was included on the gel.

Results and Discussion

A total 208 sites were surveyed and only 19 sites were found positive for the presence of *P. pachydermus.* The

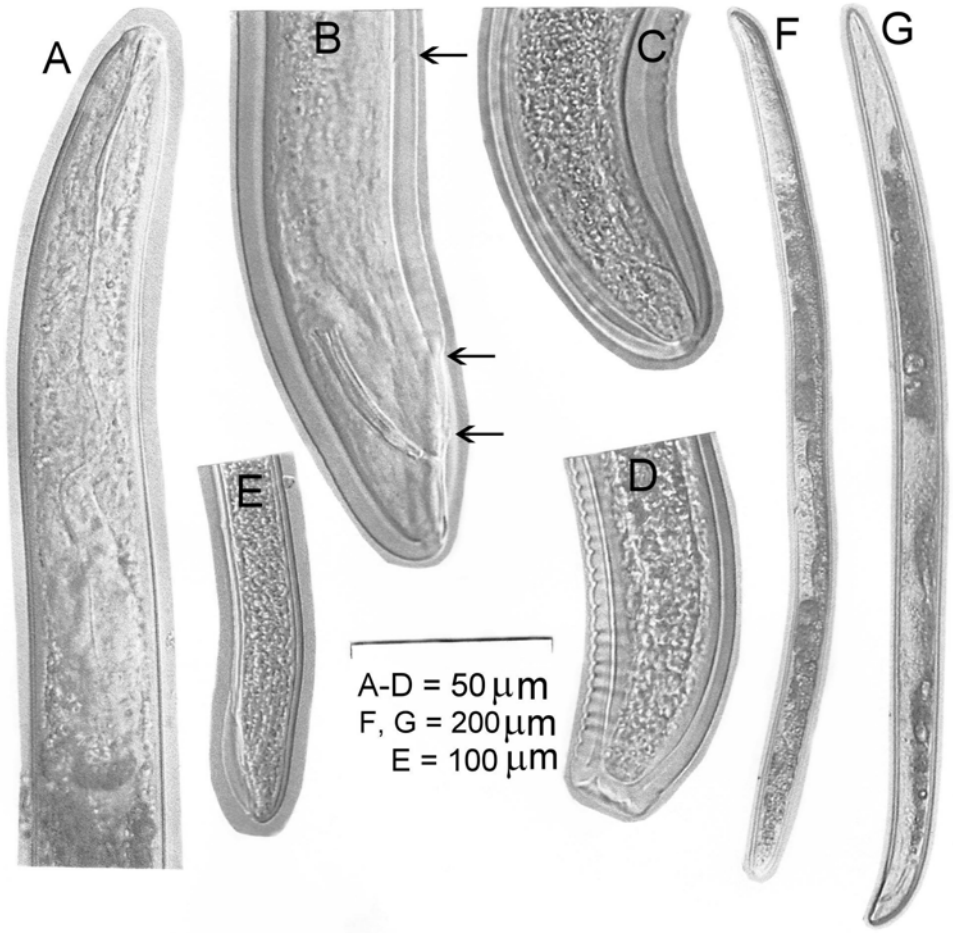

Fig.1. *Paratrichododus pachydermus* (Seinhorst, 1954) Siddiqi, 1974. A. male anterior region; B, E: male tail (arrows showing precloacal supplements); C, D: female tail; F: Entire female; G: Entire male.

species was found associated with fruit orchards (apple, apricot, peach, plum, sour cherry, sweet cherry) and forest trees (*Acer, Aesculus, Alnus, Fagus*, *Quercus*) and occurred with population densities of 2 to 19 specimens per 500 g soil. During the survey three species of genus *Trichodurs* (*T. primitivus*, *T. sparsus, T. virulifeorus*) were also found which are part of another study. Male body habitus straight (posterior end slightly curved) when heat killed. Males with a single ventromedian cervical papillae (CP) just anterior to excretory pore. Excretory pore along the two-thrids of the pharynx. Spicule distal part slightly ventrally curved; manubrium not offset from shaft. Large sperm cells with large sausage-shaped nucleus. A narrow caudal alae present (Fig.1). Three ventromedian precloacal supplementary papillae (SP) are present. SP1 just anterior to cloacal opening, SP2 near anterior end of caudal alae, SP3 about two body widths anterior to SP2 (Fig.1). Females when head killed almost straight. Reproductive system dilephic. Vaginal sclerotization small (less than 1µm) and slightly separated, shape elongated-rounded. Vulva pore shaped. Orientation of vaginal sclerotized pieces oblique. Large sperm cells with sauage-shaped nucleus dispersed throughout the uterus.

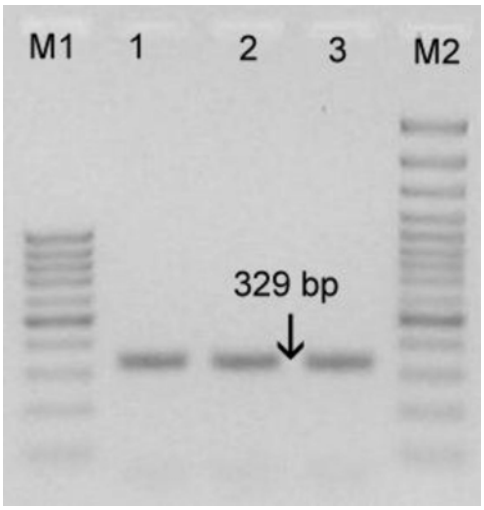

Fig. 2. Electrophoresis of the amplified products from single females of *Paratrichodorus pachydermus* from randomly selected three localities: lane M1 - 100bp DNA ladder (Fermentas); lane 1 - Maroltov; lane 2 - Rájov; lane 3 - Dobříš; M2 - 100bp plus DNA ladder (Fermentas).

Morphometrics of male and females of all populations of *P. pachydermus* from the Czech Republic were in close agreement with the morphometrics ranges described in Decraemer (1995). Males and females occurred in equal numbers. Morphometrics of three females from locality Břasy: L = 895 ± 105 (775 – 969) µm; a = 25.8 ± 2.63 (22.8 – 27.7); b = 5.73 ± 0.50 (5.2 – 6.2); V = 56.3 ± 0.36 (56.0 – 56.7); neck length = 157 ± 23 (134 – 180) µm; onchiostyle = 44 ± 1.73 (42 – 45) µm; anterior end to EP = 92.5 ± 3.54 (90 – 95) µm; anterior genital branch = 165, 182 (2♀) µm ; posterior genital branch = 165, 186 (2♀) µm. Morphometrics of four male from Břasy: L = 970 ± 93 (884 – 1057) µm; a = 28.8 ± 2.17 (26.0 – 30.8); b = 6.15 ± 0.87 (5.1 – 7.2); T = 58.2 ± 5.45 (50.3 – 62.7); onchiostyle = 42 ± 1.41 (40 – 43) µm; enterior end to EP = 89.5 ± 10.61 (82 – 97) µm; spicule = 42 ± 2.94 (39 – 45) µm; cloaca to SP1 = 7 ± 0.82 (6 – 8) µm; SP1 to SP2 = 25.2 ± 5.12 (20 – 32) µm; SP2 to SP3 = 99.3 ± 16.07 (78 – 114) µm. *P. pachydermus* was found at the following sites: Bílé Podolí, Břasy, Dobříš, Jeseň, Kojice, Ktrely, Lhenice, Maroltov, Obroa, Rájov, Temelín a Třebanice. The results of the survey in the Czech Republic confirm the general preference of *P. pachydermus* for sandy soils.

Morphological identification of all populations was reliably verified by PCR using primers of ribosomal DNA from single specimens (Boutiska *et al*., 2004). A single fragment of approximately 329 bp was amplified for all studied individuals (Fig. 2). No PCR products were obtained in the negative control lacking DNA template or in the negative control containing DNA of *T. pakistanensis*, *T. variopapillatus*, *T. similis* and *T. viruliferous*.

Acknowledgements

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