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Research Note

A single bivulval female of *Xiphinema diversicaudatum* and two bivulval females of *Xiphinema vuittenezi* (Nematoda: Longidoridae)

S. KUMARI¹, W. DECRAEMER^{2,3}

¹Crop Research Institute, Division of Plant Health, Drnovská 507, Ruzyně, 16106 Prague 6, Czech Republic, E-mail:

kumari@vurv.cz; ²Royal Belgian Institute of Natural Sciences, Department of Invertebrates, Vautierstraat 29, 1000

Brussels, Belgium; ³Department of Biology, Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium

Summary

A population of *Xiphinema vuittenezi* from an apple orchard and a population of *X. diversicaudatum* from a peach orchard yielded bivulval females. Morphometrics of these females are presented.

Keywords: *Xiphinema vuittenezi*; *Xiphinema diversicaudatum*; bivulval female

Introduction

Plant-parasitic nematodes belonging to genus *Xiphinema* are economically important pests. Therefore, an extensive study of these nematodes is being carried out in the Czech Republic. During this study Kumari and Decraemer (2006) reported a single bivulval female of *X. vuittenezi* from an apple orchard at Slaný, Bohemia among thousands of normal females and males. This research note deals with a first record of a bivulval female of *X. diversicaudatum* from the Czech Republic and two more bivulval females of *X. vuittenezi*.

Materials and methods

Soil samples were taken at a depth of 0–60 cm from an apple orchard at Slaný and a peach orchard at Bílé Podolí, Czech Republic. Nematodes were extracted from soil by sieving and decanting method. Bivulval females were killed in a drop of water over a flame and temporary mounts were made on glass slides. Measurements and photographs were taken from the temporary mounts and slides were dismantled immediately. DNA was prepared from one specimen (Kumari *et al.*, 2009) of *X. vuittenezi* and another specimen of *X. vuittenezi* and a single specimen of *X. diversicaudatum* were preserved in water at -70 °C for future molecular study. Identification was made using an Olympus BX-51 light microscope, equipped with a digital camera C4040 and differential interference con-

trast (DIC, Nomarski). Measurements were made with the aid of imaging software (Olympus DP-soft).

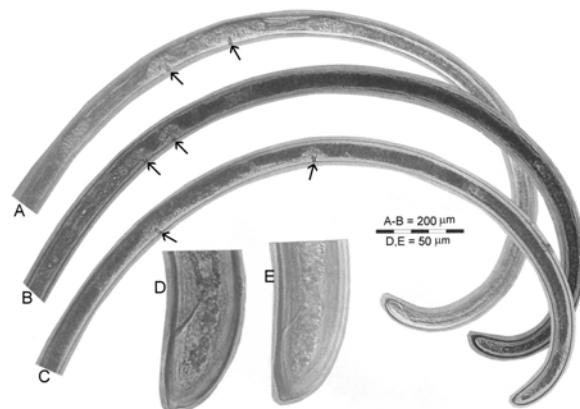


Fig. 1. Three bivulval females of *Xiphinema vuittenezi* from a single population. A: Posterior region of ♀1 (described by Kumari and Decraemer, 2006); B,C: Posterior region of ♀2 and ♀3; D: Tail of ♀2; E: Tail of ♀3. Arrows indicate vulvae.

Results and Discussion

Nematode extraction from soil samples from an apple orchard at Slaný yielded a single bivulval female of *X. vuittenezi* (♀1) (Kumari & Decraemer, 2006). Further extraction of soil samples from the same locality yielded two more bivulval female specimens (♀2, ♀3) (Table 1 and Fig. 1). Measurements and photomicrographs taken from temporary mounts of these specimens were compared with the previously recorded specimen (Kumari & Decraemer, 2006). Morphometrics of both bivulval ♀2 and ♀3 were similar except for few minor differences (e. g. the odontostyle of ♀3 is shorter compared to ♀2 and previously recorded ♀1). Intestine of both ♀2 and ♀3 prevented clear observation of the reproductive system from tempo-

Table 1. Morphometrics of three bivulval females of *Xiphinema vuittenezi* Luc, Lima, Weischer & Flegg, 1964 and a single bivulval female of *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939. Measurements in μm .

| Species | <i>X. vuittenezi</i> | | | <i>X. diversicaudatum</i> |
|-------------------------------------|----------------------|-------------|-------------|---------------------------|
| Specimens | $\varphi 1^*$ | $\varphi 2$ | $\varphi 3$ | $\varphi 1$ |
| L | 3215 | 3186 | 2933 | 3760 |
| a | 61.8 | 59 | 59.9 | 75.2 |
| b | 6.74 | 6.19 | 7.6 | 8.0 |
| c | 82.4 | 72.4 | 79.3 | 81.7 |
| c' | 1.05 | 1.19 | 0.76 | 1.1 |
| V1 | 50.0 | 50.5 | 52.5 | 43.4 |
| V2 | 54.8 | 52.9 | 65.9 | 44.7 |
| Distance between V1 and V2 | 156 | 81 | 396 | 47 |
| Odontostyle | 126 | 127 | 114 | 130 |
| Odontophore | 75 | 75 | 73 | 75 |
| Total stylet length | 201 | 202 | 187 | 205 |
| Greatest flange width | 14 | 13 | 11 | 13 |
| Oral aperture to guide ring | 109 | 114 | 103 | 120 |
| Pharyngeal bulb length | 122 | 122 | 110 | 108 |
| Pharyngeal bulb diam. | 23 | 23 | 27 | 27 |
| Tail length | 39 | 44 | 37 | 46 |
| Length of hyaline tip | 11 | 13 | 10 | 16 |
| Mucro length | 5 | 4 | 3 | 9 |
| Anterior reproductive branch of V1 | 511 | — | — | 638 |
| Posterior reproductive branch of V2 | 466 | — | — | 656 |
| Body diam. at lip region | 13 | 12 | 13 | 13 |
| at guiding ring | 37 | 37 | 38 | 39 |
| at base of pharynx | 43 | 48 | 44 | 49 |
| at mid body | 52 | 54 | 49 | 50 |
| at anus | 37 | 37 | 36 | 42 |
| at beginning of hyaline tip | 23 | 21 | 20 | 19 |

* According to Kumari and Decraemer, 2006

rary mounts. The distance between the two vulvae of *X. vuittenezi* differs among both specimens (81 vs 396 μm) and also from previously observed specimen $\varphi 1$ (156 μm) from the same population. The posterior vulva of third specimen lies far posteriorly (at 65.9 % of total body length from anterior end) but within the maximum value (74.4 %) of the range observed for *X. vuittenezi* (Kumari, 2003). Ribosomal and mitochondrial DNA sequences (accession numbers EF614265, EF614266, EF614267 and EU444017) of $\varphi 2$ (see Fig. 1) are studied by Kumari *et al.* (2009). Lately, six normal females were sequenced from three populations from the Czech Republic. Sequences of ribosomal and mitochondrial DNA of bivulval and normal females are 100 % identical. Previously bivulval females of *X. vuittenezi* were described from Serbia (Barsi, 1994), Italy (Coiro & Lamberti, 1980) and Czech Republic (Kumari & Decraemer, 2006).

A population of *X. diversicaudatum* extracted from a peach orchard at Bílé Podolí also yielded a single bivulval female, a new record for the Czech Republic (Table 1 and Fig. 2). Both gonads and genital ducts are normally devel-

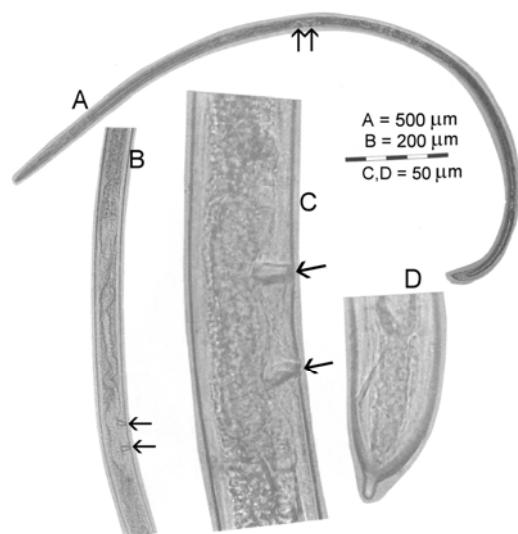


Fig. 2. A bivulval female of *Xiphinema diversicaudatum*. A: Entire female; B: Anterior reproductive branch and vulvae; C: Vulval region of female with two vulvae; D: Tail region. Arrows indicate vulvae.

oped. Both vulvae are close to each other and the vaginas are connected by a common uterus portion (Fig.2). The bivulval female has normal general morphology, but compared to the previously described bivulval specimens (Barsi, 1994; Brown & Coiro, 1984) the body of the Czech bivulval female is shorter.

Within the genus *Xiphinema* bivulval specimens have also been observed in other species, e.g., in *X. dentatum* (Radivojević, 1991; Radivojević, 2005) and *X. index* (Catalano, 1991). Bivulval female of *X. turcicum* (Radivojević, 1991; Kumari & Decraemer, 2006) also belongs to *X. dentatum* (see Radivojević & Baujard, 1998; Radivojević, 2005).

Radivojević (2005) noted a frequency of one bivulval nematode per some 12000 females in *X. dentatum* and it is probably the only reliable estimate for the anomalies reported. Kumari and Decraemer (2006) also found the first bivulval specimen of *X. vuittenezi* among 12460 normal females, but further observation of the same population lead to two more bivulval specimens among 7078 females; no bivulval specimens observed in further 33387 female specimens of the same species. In total 52925 females, 80 males and 3 bivulval female specimens were observed in the same population i.e. an average of one bivulval specimen per 17641 females in *X. vuittenezi*. However, for *X. diversicaudatum*, a single bivulval female was observed among 2809 female and 1826 male specimens.

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