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## *Steinernema poinari* sp. n. (Nematoda: Steinernematidae) a new entomopathogenic nematode from the Czech Republic

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### Abstract

A new steinernematid nematode, herein described as *Steinernema poinari* sp. n., was recovered by baiting soil samples with the last instar wax moth larvae *Galleria mellonella* in three localities of southwest Bohemia, Czech Republic. Morphological and molecular data confirmed this nematode as a new species which belongs to the *affine/intermedium* group. *S. poinari* sp. n. was characterized by male, female and infective juvenile morphological observations. Male spicules are robust with a pointed tip, SW% = 109 (98–123) and GS% = 70 (58–87). The tail terminus of the first generation have a papillated mucron, whereas in the second generation a short filamentous mucron sometimes develops. Females of the first generation have a moderately protruding vulva, postanal swelling absent or slightly developed. Second generation females have a long filamentous mucron on the tail tip. Infective juvenile have a body length of 768 µm, lateral field formula 2, 5, 6, 5, 2, 1, ratio D% of 46 (40–55), E% of 84 (76–95) and H% of 50 (43–56). The new species was characterized by sequences of ITS and D2D3 regions of ribosomal DNA. *S. poinari* sp. n. can also be separated from *S. affine* and *S. intermedium* by cross-breeding tests.

**Key words:** new species, *affine/intermedium* group, ITS, D2D3, phylogeny, morphology

### Introduction

Entomopathogenic nematodes (EPNs) of the family Steinernematidae belong to important parasites of many insect species. During a long-lasting survey of these nematodes in the Czech Republic several hundred strains from nine steinernematid and two heterorhabditid species were recovered, (Mráček *et al.* 2005). In 2012 a new steinernematid nematode was recovered by soil sampling along the bank of an unnamed creek covered by willow and alder bush in west Bohemia. Laboratory *Galleria* baiting revealed a nematode isolate belonging to the *affine/intermedium* group which was morphologically and genetically different from previously described species *S. affine* (Bovien 1937) Wouts, Mráček, Gerdin and Bedding, 1982, *S. intermedium* (Poinar 1985) Mamiya, 1988, *S. beddingi* Qiu, Hu, Zhou, Pang and Nguyen, 2005, *S. sichuanense* Mráček, Nguyen, Tailliez, Boemare and Chen, 2006 and *S. arasbaranense* Nikdel, Niknam and Ye, 2011. The new species is described herein as *S. poinari* sp.n. named after professor George O. Poinar, Jr.

### Materials and methods

**Nematode collection and isolation.** Soil samples containing *S. poinari* sp. n. were collected from three localities in southwest Bohemia, Czech Republic. Nematodes were recovered from sandy soil samples using the *Galleria* baiting technique (Bedding & Akhurst 1975).

Soil samples (approximately 2dm<sup>3</sup>) were collected and placed into plastic bags (Mráček 1980). Two steel mesh pockets containing 5 late instar *Galleria mellonella* (L.) (Lepidoptera, Galleriidae) larvae were placed in each bag. These samples were kept for seven days at laboratory temperature of 20°C and in a refrigerator at 15°C at the

The PCR-product of part of the 28S rRNA gene containing the D2D3 expansion fragments had a length of 873 bp and from its closest relatives it is separated by 6–21 bp (Table 4).

The phylogenetic relationships between species of *Steinernema* are presented in Fig 4 and Fig 5. For Bayesian analysis of the ITS sequence, 1428 characters were included (tree length = 5527, CI = 0.363, RI = 0.590, RCI = 0.214 and HI = 0.637) for D2D3 region, 933 characters were included (tree length = 1333, CI = 0.464, RI = 0.659, RCI = 0.306 and HI = 0.536).

Both trees support *S. poinari* **sp. n.** as a new species belonging to the *affine/intermedium* group. In agreement with some previous studies (e.g. Spiridonov *et al.* 2004) the ITS tree further shows a basal position of the *affine/intermedium* group within the genus *Steinernema*. Morphological and molecular characteristics presented above clearly show *S. poinari* **sp. n.** as a new species of *affine/intermedium* group.

**Distribution.** Three recent findings of *S. poinari* **sp. n.** show that the species can be quite abundant in the Czech Republic. Its omission in previous surveys (Mráček *et al.* 1999, Mráček *et al.* 2005) could be due to misidentification with morphologically similar *S. intermedium*. Another strain of *S. poinari* **sp. n.** “Tomsk” originates from a distant area of the Tomsk region, Siberia. A Genbank search of the ITS region of *S. poinari* revealed close similarity (99–100%) to several steinernematid strains referred to as “*Steinernema* sp. 6” (Spiridonov *et al.* 2004) that were isolated in UK, Belgium, Estonia, and Moscow and Altai regions of Russia. These strains are most likely conspecific with *S. poinari* **sp. n.** and thus the geographic distribution of *S. poinari* **sp. n.** seems to be quite wide, probably covering a large area of the Palearctic region.

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