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Steinernema tophus sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from South Africa

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Abstract

A new entomopathogenic nematode, *Steinernema tophus* **n. sp.** is described from South Africa. Morphological, molecular (ribosomal gene sequence data) together with cross-hybridization studies were used for diagnostics and identification purposes. Both molecular and morphological data indicate the new species belongs to the '*glaseri*-group' of *Steinernema* spp. Key morphological diagnostic traits for *S. tophus* **n. sp.** include the morphology of the spicules and gubernaculum. Morphometric traits of third-stage infective juveniles, including total body length (average 1,046μm), tail length (average 70μm), location of the excretory pore (average 92 μm), D% (average 63), E% (average 132) and H% (average 32) values are definitive. In addition to these morphological characters, analysis of rDNA (28S and ITS) gene sequences depict this *Steinernema* species as a distinct and unique entity.

Key words: Steinernema, South Africa, rDNA genes, mitochondrial genes, morphology, cross hybridization

Introduction

Steinernema nematodes Travassos, 1927 are obligate and lethal endoparasites that have a symbiotic relationship with Gram-negative γ-Proteobacteria in the genus *Xenorhabdus* Thomas & Poinar, 1979. This nematode-bacteria complex represents a mutualistic association, where the nematodes (third stage IJs[1]) vector the symbiotic bacteria between insects in a specialized intestinal receptacle (Stock & Goodrich-Blair, 2008). Once the bacteria are released in the insect's hemocoel, the bacteria kill the insect host and create a favorable environment within the host cadaver for nematode growth and development.

This pairing is pathogenic for a wide range of insects and has successfully been implemented in biological control and integrated pest management programs worldwide (Gaugler & Kaya, 1993; Gaugler, 2002). Surveys for EPNs[2] have been conducted in temperate, subtropical and tropical regions (summarized by Hominick, 2002; Adams *et al*., 2006). In South Africa, several surveys have been conducted to document the diversity of this group of nematodes, with the goal of finding locally-adapted species and/or isolates that can be assayed for control of native insect pests (Malan *et al*., 2006, 2011, 2012; Hatting *et al*., 2009; Pillay *et al*., 2009).

Until now, five *Steinernema* and four *Heterorhabditis* species have been isolated and/or described in South Africa, and several of these species are being implemented in successful agricultural pest management programs (De Waal *et al*., 2010; Malan *et al.,* 2009; Van Niekerk & Malan, 2012; Çimen *et al.* 2014).

This study describes a new *Steinernema* sp. originally recovered from a vineyard in Clanwilliam (Western Cape), South Africa. Differential interference contrast optics (DIC), DNA sequence analysis and cross-hybridisation assays were conducted to describe and illustrate this new *Steinernema* species.

1. Infective juvenile
2. Entomopathogenic nematode

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related species by the morphology of the spicules and gubernaculum, the arrangement of the genital papillae and the values of ratios SW (average: 1.2, range: 1.0–1.6) and GS (average: 0.7, range: 0.6–0.9).

Phylogenetic analyses placed *S. tophus* **n. sp.** in clade V (as depicted by Spiridinov *et al.* 2004). Within this clade, the new species was found most closely related to a newly described South African species, *S. innovationi* followed by *S. khoisanae* (Nguyen et al. 2006) in the 28s topology. However, the new species can be differentiated from *S. khoisanae* and *S. innovationi* by morphological and morphometric differences of the IJ and first generation male (See Table 1). Furthermore, phylogenetic analysis of ITS and 28s datasets revealed distinct base pair differences between *S. tophus* **n. sp.** and members of the clade V *glaseri*-group (Tables 3 and 4). In particular, for the more variable gene, ITS dataset, there were 59 base pairs difference between *S. tophus* **n. sp.** and its sister species, *S. innovationi*.

Steinernema tophus **n. sp.** can be differentiated from *S. khoisanae* by the morphology of both infective juveniles and first generation adult males. For example *S. tophus* **n. sp.** infective juveniles are wider (average 38 µm *vs* 33 µm) and slightly shorter than those *S. khoisanae* (average 1,046 µm *vs* 1,075µm). The excretory pore in the new species is generally located more posteriorly, and the tail is usually shorter than that of *S. khoisanae* (average 70 µm *vs* 85 µm), though given the overlap in ranges for these features, they should not be considered key diagnostic traits, but only as general trends. Males of *S. tophus* **n. sp.** can be distinguished from *S. khoisanae* by the D% (average: 92 *vs* 88) and SW ratio (average: 1.2 *vs* 1.99). First-generation females of *S. tophus* **n. sp.** are characterized by having a conoid tail with a mucro, whereas in *S. khoisanae* the tail is digitated.

Third-stage infective juveniles of *S. tophus* **n. sp.** differ from those of *S. innovationi* by the location of the excretory pore (average 90 µm *vs* 88 µm), the tail length (average 70 µm *vs* 76 µm) and the values of D% (63 *vs* 58) and E% (132 *vs.* 115). First generation females of the new species and *S. innovationi have* a mucronated tail, however the new species does not have a digitate tail which is present in *S. innovationi.* Males of *S. tophus* **n. sp.** can be separated from those of *S. innovationi* by the morphology the spicules. Specifically, the spicule calomus is rhomboidal in *S. innovationi* but it is elongated in the new species. The curvature of the lamina is less pronounced in *S. tophus* **n. sp.** when compared to *S. innovationi*. Furthermore, the number and arrangement of postcloacal papillae is different when compared to *S. innovationi*. The new species has four pairs of postcloacal papillae of which one subventral, one subdorsal and two terminal) whereas S. innovationi has five postcloacal pairs (one subventral and two subdorsal and two terminal). First generation males of the new species differ from *S. tophus* n. sp. males also differ in the value of SW when compared to that of *S. innovationi* (average 1.2 *vs.* 1.4)

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