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A survey of the Cape Floristic Region of South Africa for the presence of cyst nematodes (Nematoda: Heteroderidae)

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Abstract

A survey was performed to detect the presence of cyst nematodes in the Cape Floristic Region of South Africa. Soil was collected in the rhizosphere of the dominant plant species within blocks of indigenous vegetation and cysts were extracted from them. A total of 81 blocks of indigenous vegetation were sampled as described. Cysts were detected in 7 of these samples, representing 6 different vegetation types. One set of primers was used to amplify the ITS regions from these cysts, including the 5.8S ribosomal gene, as well as short parts of the 18S and 28S ribosomal genes. ITS-rDNA sequences from the indigenous isolates were aligned with selected sequences of other species from the Heteroderidae. Phylogenetic analyses to resolve the relationships between indigenous isolates and selected representatives of the Heteroderidae were conducted using the Maximum Parsimony method. The consensus tree resulting from alignment of the circumfenestrate cysts revealed that isolates SK18, WK1 and WK26 are included in a clade of *Globodera* species that parasitise non-solanaceous plants, forming a monophyletic group with *G. millefolii, G. artemisiae*, and an unidentified *Globodera* sp. from Portugal. In a tree resulting from the alignment of the *Heterodera* spp., isolates OK14 and WK2 are included in the *Afenestrata* group, forming a monophyletic group with *H. orientalis.* This survey unearthed at least four potentially new species of cyst nematodes, which may prove invaluable for the study of the evolution and biogeography of the group.

Key words: *Globodera*, *Heterodera*, Fynbos, sequencing, phylogenetics

Introduction

The genus *Globodera* can be divided geographically into three main groups: The *G. tabacum* group of species from North America, the potato cyst nematodes, *G. rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone, from South America and a small group of *Globodera* species which are found in the old world and parasitizes the Asteraceae (Evans & Rowe, 1998). Association by descent can be postulated for *Globodera* spp. from the New World, parasitizing Solanaceae and for those from the Eurasian palearctic parasitizing Asteraceae, because Solanaceae and Asteraceae are both placed in the highly natural dicotylendon sub-class Asteridae. Stone (1979) suggested that *Globodera* might have originated in Gondwanaland, on the part of the landmass that later became South America. The ancestors of the *Globodera* species parasitizing the Asteraceae in Europe were suggested to have been carried northwards when fragments of Gondwanaland encountered Laurasia (Subbotin *et al.*, 2010), creating an isolated evolutionary niche, where these species might have co-evolved with their hosts (Stone, 1983).

The use of molecular data for phylogenetic inference is well established for many groups of organisms. Applications of molecular approaches with analysis of ribosomal RNA gene (rRNA) sequences have added new reliability to understanding relationships within cyst nematodes. The Internal Transcribed Spacer (ITS) has been widely used to analyse the phylogenetic relationships between cyst nematodes (Ferris *et al*., 1995; Thiery & Mugniery, 1996; Ferris *et al*., 1999; Subbotin *et al.,* 2000; Subbotin *et al.,* 2001; Sabo *et al.*, 2001; Sturhan, 2002;

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cyst nematode species. This present survey unearthed at least four potentially new species of cyst nematodes. The description of the morphological characteristics, host relationships and phylogenetic analysis of molecular data of these specimens may prove invaluable for the study of the evolution and biogeography of the group. The CFR displays exceptionally high diversity and endemism of vascular plants and invertebrates. Cape Fynbos cover over 41000 km^2 of the CFR, but other vegetation types like renosterveld, karroid shrubland, various thicket types and forest are also present (Cowling *et al*, 2004). The alpha diversity measurement (the number of species in a single plot of one square kilometre) for Cape Fynbos averages around 65 plant species per km^2 (Cowling *et al*, 2004), which complicates the recognition of specific plant-nematode interactions from soil samples from this region. The determination of the host plants of these cysts therefore proved to be quite difficult due to the proliferation of plant species at each sampling point and further investigation is warranted.

This study establishes the distinct phylogenetic positions of the cyst nematode isolates from South Africa relative to an array of other cyst nematode species. Characterisation and phylogenetic analyses of sequences from the isolates SK18, WK1 and WK26 confirms their membership of the genus *Globodera*. The clustering of isolates WK1 and WK26 in the phylogenetic tree, the pairwise distances between their sequences, as well as morphological similarities, led us to believe that these two isolates belong to the same species. Further investigation into the morphology and morphometrics of these nematodes are necessary to confirm if these nematodes are indeed new species of *Globodera*.

Subbotin *et al.* (2011) speculated that South America or Africa appears to be a centre of origin of *Globodera*, supporting Stone's (1979) hypothesis of a Gondwanaland origin of *Globodera* with subsequent dispersal of the species of this genus to Europe, North America, Asia and Oceania. The discovery of these new *Globodera* species in Southern Africa supports the theory of a Gondwanaland origin of the genus and the theory that divergence of the two main *Globodera* lineages might have occurred subsequent to the break-up of Africa and South America. The ancestors of the *Globodera* species parasitizing the Asteraceae in Europe were suggested to have been carried northwards when fragments of Gondwanaland encountered Laurasia creating an isolated evolutionary niche, where these species might have co-evolved with their hosts (Stone, 1983). Although the host plant of the SA *Globodera* species have not yet not yet been confirmed, preliminary tests suggest that these cysts do not parasitize the Solanaceae.

Isolate OK14 displays morphological characteristics of the *Afenestrata*-group of the *Heterodera* genus. One other species of this group, *H. africana*, have been reported from South Africa (Kleynhans, 1991). To confirm if this isolate belongs to the same species, further morphological studies and comparison of molecular data would be necessary. Unfortunately no sequence data for *H. africana* is available in the public databases at this moment.

Phylogenetic analyses of sequences from isolate WK2 also group this nematode in the *Afenestrata*-group, although the morphology of this population is not consistent with that group. WK2 also clusters robustly with *H. bifenestra* and *H. graminis.* Of interest is the fact that the cysts of *H. bifenestra* also are more rounded, with a faint vulval protuberance (Subbotin *et al.*, 2010) as is the case with WK2. Previous authors have expressed concerns about the placing of *H. bifenestra* in the *Heterodera* group (Ferris, 1998; Subbotin *et al.*, 2001). An in-depth phylogenetic analysis including all the *Heterodera* species, as well as a comparison of the morphology of specimens from WK2 with that of *H. bifenestra* is necessary to resolve this ambiguity.

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