

Molecular confirmation of *Xiphinema brevicollum* (*X. americanum* complex) from New Zealand

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Introduction

- The identity, taxonomy and worldwide distribution of nematodes in the *Xiphinema americanum* complex remain unclear, because of their close morphological similarities.
- Species of the *X. americanum* complex have been subject to much study, because they transmit plant-pathogenic nepoviruses.
- Virus-vectoring nematodes pose biosecurity and market access threats for New Zealand.
- At least three *X. americanum* species have been described previously from New Zealand, but molecular techniques have not been used in these identifications.
- We report the first molecular identification of *X. brevicollum*, a nematode from the *X. americanum* complex, in New Zealand.



Figure 1
(a) Stylet and head and (b) tail of *X. brevicollum*.

Results

The nematodes had morphological characteristics typical of previously described *Xiphinema* spp. (Figures 1-3).

Following digestion with prepGEM enzyme, we were easily able to amplify the ~830bp ribosomal LSU DNA fragment. After digested nematode material had been stored at -20°C for several weeks, we were still able to amplify further DNA products. This technique was much more reliable than SDS/proteinase K based protocols.

BLASTN comparison indicated that the LSU and ITS1 fragments were most similar to sequences from *X. brevicollum* nematodes. Phylogenetic trees based on LSU fragments inferred from collected nematode sequences show this close relationship as well as the clustering of *X. brevicollum* with *X. diffusum* and *X. taylori* sequences (Figure 4).

Materials and methods

Nematode isolation

Nematodes were isolated from soil collected from beneath *Pseudopanax arboreus* (five finger) trees growing in Palmerston North, New Zealand, using a centrifugal flotation method.

Body shape, head end, tail end, long stylet, stylet base and guiding apparatus were used for morphological identification.

DNA techniques

Nematodes were transferred to 0.2 mL PCR tubes containing 30 µL 1X DNA extraction buffer and 4 µL prepGEM enzyme (ZyGEM Corporation Ltd., Hamilton, New Zealand). DNA fragments were amplified with the LSUD3B/LSUD2A and



Figure 2 Part of the *X. brevicollum* female reproductive system.

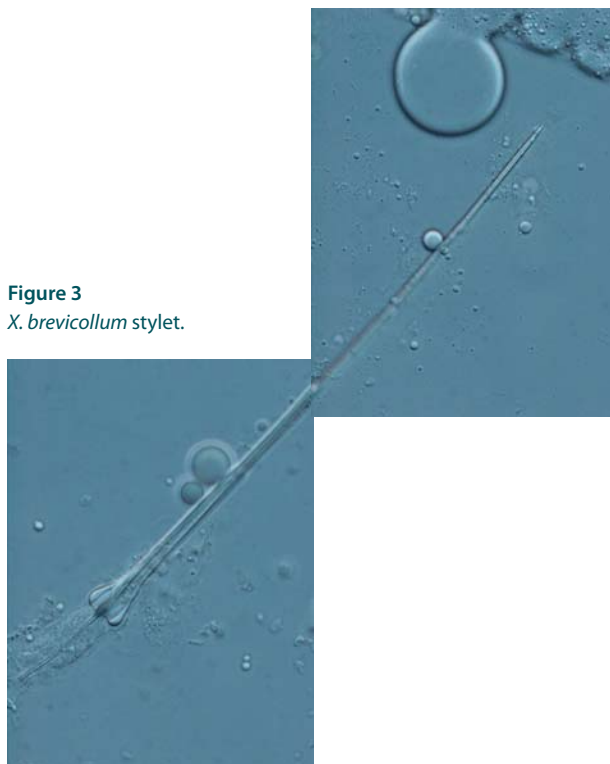


Figure 3
X. brevicollum stylet.

Conclusions

- This is the first molecular identification of a nematode from the *X. americanum* group in New Zealand.
- DNA-based phylogenies provide a robust framework for understanding relationships within groups such as the *X. americanum* complex.
- The prepGEM enzyme system for nematode DNA extraction is quick and simple.
- Further, wider surveys will be required to confirm whether this *X. brevicollum* is the same as “species b” previously reported in New Zealand².

NEMBL18F/NEM5818R primer pairs.

Amplified products were gel purified (QIAquick Gel Extraction Kit; Qiagen) before the LSU fragments were directly sequenced. ITS DNA fragments were cloned into the TOPO TA cloning Kit (Invitrogen), and sequenced with PCR primers situated in the vector.

The DNA sequences were used for BLASTN¹ comparison against GenBank sequences. DNA sequences from the top BLASTN matches, plus selected other *Dorylaimida* nematode sequences, were downloaded from GenBank and aligned using Clustal X.³

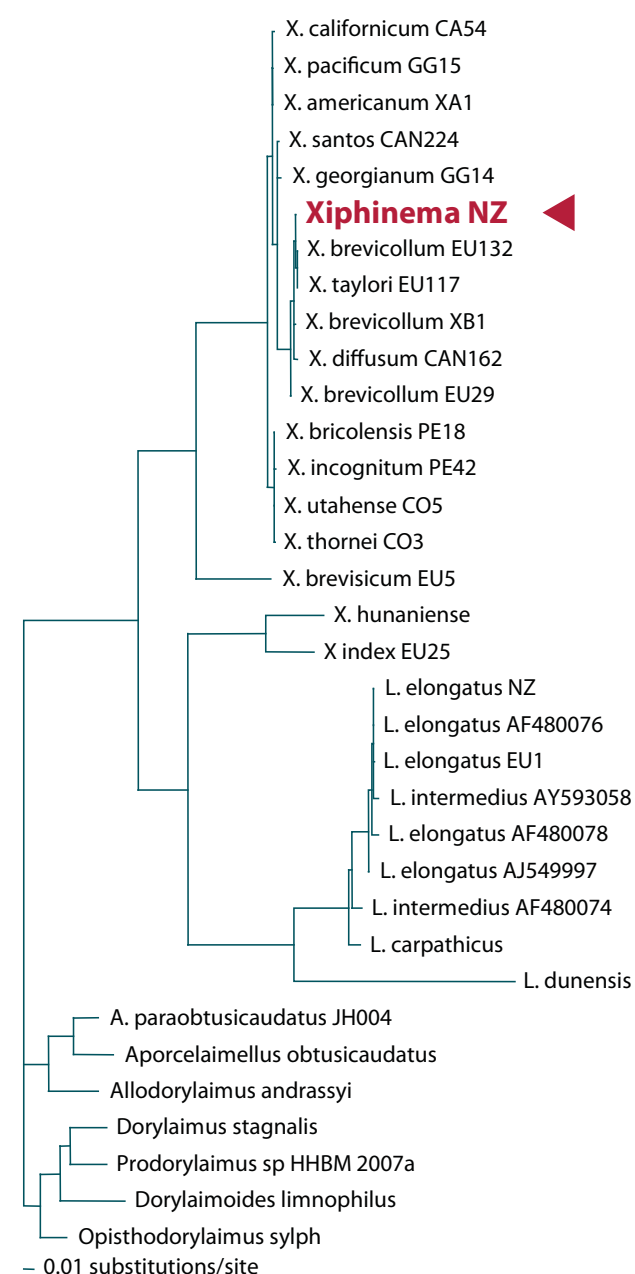


Figure 4 Phylogenetic tree showing position of *X. brevicollum* from New Zealand.

References

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