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A method to discriminate species of virus vector trichodorid nematodes exploring 18S rDNA region

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The Trichodoridae nematodes are polyphagous root-feeding ectoparasites, represented by more than 100 species currently included in six genera. *Monotrichodorus*, *Allotrichodorus* and *Ecuadorus* contain 12 monodelphic species and have only been recorded from Central and northern America. The other didelphic genera, *Trichodorus*, *Paratrichodorus* and *Nanidorus*, include 13 species that are natural vectors of specific tobraviruses strains, commonly of Tobacco rattle virus, that have deleterious effect upon economically important crops, such as ornamental bulbs, potatoes and tobacco. In Portugal, 16 species were reported, some known as TRV vectors. When field populations of trichodorids become viruliferous, virus can persist for many years, acting the infected plants as virus reservoirs. Due to the specificity of virus-vector transmission, it is required to identify the virus and the vector at strain and species level, respectively. Accurate techniques to test suspicious soil and plant material are imperative for effective tobravirus management, namely for pre-planting risk assessment. Morphological identification of the vector nematode is time-consuming and requires well-trained specialists. Moreover samples frequently contain few specimens, and at immature stages, impossible to identify. We developed an alternative DNA barcoding method for a clear detection and identification of *Trichodorus*, *Paratrichodorus* and *Nanidorus* species, regardless of their life stage and geographical origin. A 500 bp region, located at the 3' end of the 18S gene exhibiting species-specific nucleotide variability was found suitable to be used as a trichodorid barcode tool. This region was identified based on the alignment of 12 nucleotide sequences of morphologically well-characterised specimens, representing the three didelphic genera. The selected region is flanked by two highly conserved sequences, which were used to design 2 primers for the PCR amplification of a 615 bp fragment. Direct sequencing of the amplicon allows a clear species identification. The typeability, reproducibility and the high discriminatory power of this approach was demonstrated with 21 populations, six of which non-indigenous. The method resolved individuals of different species but did not discriminate different populations of the same species. Our results suggest that this genetic region is adequate and effective for barcoding of virus vector trichodorids and seems very promising with soil environmental samples.

References:
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