Development of species-specific primers for the ectoparasitic nematode species *Xiphinema brevicolle, X. diffusum, X. elongatum, X. ifacolum* and *X. longicaudatum* (Nematoda: Longidoridae) based on ribosomal DNA sequences

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Introduction

Xiphinema are amongst the ten most economically important nematode genera. They cause damage to an extensive range of crop plants by their direct feeding on root tips and a few *Xiphinema* species have the ability to transmit nepovirus to a wide range of fruit and vegetable crops. There is a paucity of information regarding the direct damage to host plants caused by individual *Xiphinema* species. However, a few studies have demonstrated the pathogenicity of *X. brevicolle* (Cohn & Orion, 1970), *X. elongatum* (Spaull, 1981), *X. ifacolum* (Lamberti *et al*., 1987, 1991) and *X. longicaudatum* (Lamberti *et al*., 1992). The objective of this study was to develop single-step PCR species-specific molecular diagnostic primers for five *Xiphinema* species (Table 1) that are either known agricultural pests or morphologically similar species.

Species	Population Code	Host	Locality (City, State)	Size (bp) Fragment[1]	Size (bp) ITS-1[2]
X. brevicolle	PX 19	*Coffea arabica*	São Paulo, SP	462	725
X. diffusum	PX 01	*Prunus persica*	Pelotas, RS	718	680
X. elongatum	PX 77	*Saccharum officinarum*	Arez, RN	1057	1090
X. ifacolum	PX 79	Natural vegetation	Castanhal, PA	816	1018
X. longicaudatum	PX 41	*Brachiaria decumbens*	Amapá, AP	395	804

1 – fragment obtained when amplified with the universal forward primer BL18 (5' CCCGTCGMTACTACCGATT 3') and species-specific primer.
2 – obtained when amplified with the universal primers BL18 and 5818 (5'ACGARCCGAGTGATCCAC 3') excluding 168 bp from 18S and 29 bp from 5.8S rDNA, respectively.

Table 1. Selected populations of the five target Xiphinema species from Brazil (Oliveira et al., 2003) used in this study for sequencing the ITS-1 region of ribosomal DNA and designing species-specific primers.

Results

Multiplex PCR was effective and reproducible for two (*X. longicaudatum* and *X. ifacolum*) or three (*X. brevicolle, X. diffusum* and *X. elongatum*) of the target nematode species (Fig.1). Specificity was demonstrated by the absence of cross-reactions with 14 non-target *Xiphinema* species (Fig.2). Primer reliability was confirmed by screening different populations of the target species (Fig.3)

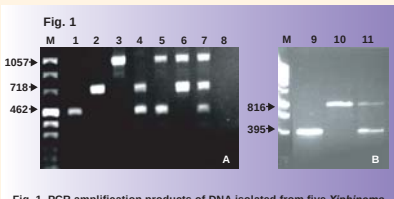

Fig. 1. PCR amplification products of DNA isolated from five *Xiphinema* species using a multiplex test with combinations of species-specific primers (Table 1) and an universal primer BL18. A, Lane 1: *X. brevicolle;* lane 2: *X. diffusum*; lane 3: *X. elongatum*; lane 4: *X. brevicolle* + *X. diffusum*; lane 5: *X. brevicolle* + *X. elongatum*; lane 6: *X. diffusum* + *X. elongatum*; lane 7: *X. brevicolle* + *X. diffusum* + *X. elongatum*; lane 8: negative control (M = marker VIII - Boehringer). B, Lane 9: *X. longicaudatum*; lane 10: *X. ifacolum*; lane 11: *X. longicaudatum* + *X. ifacolum* (M = 1kb marker - Promega).

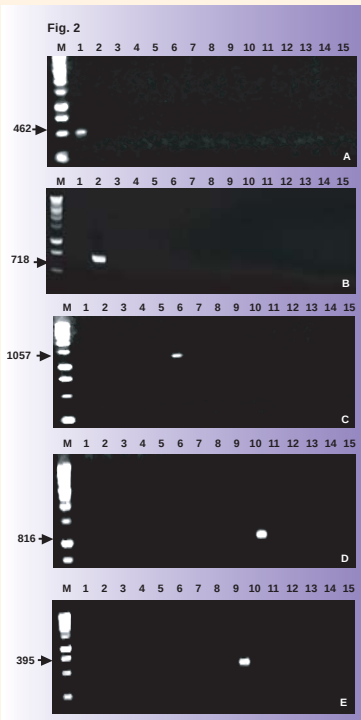

Fig. 2. Specificity tests. Electrophoresis of DNA extracted from single individuals of: 1) *X. brevicolle*, 2) *X. diffusum*, 3) *X. oxycaudatum*, 4) *X. peruvianum*, 5) *X. brasiliense*, 6) *X. elongatum*, 7) *X. ensiculiferum,* 8) *X. krugi* (tail digitate), 9) *X. krugi* (tail subdigitate), 10) *X. longicaudatum,* 11) *X. ifacolum,* 12) *X. paritaliae*, 13) *X. surinamense*, 14) *X. setariae/vulgare* and 15) *X. variegatum*, using the forward primer BL18 coupled with reverse species-specific primers located in the ITS-1 region for: A, *X. brevicolle*; B, *X. diffusum*; C, *X. elongatum*; D, *X. ifacolum* and E, *X. longicaudatum*. M = 1kb marker (Promega).

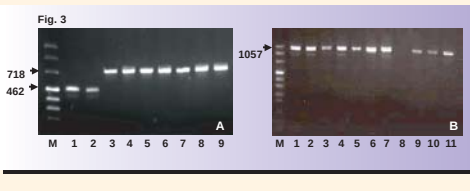

Fig. 3. Primer reliability tests. PCR amplification products of DNA isolated from different populations of the target species using the appropriate species-specific primers (Table 1) combined with the universal primer BL18. A: lanes 1 and 2: *X. brevicolle*; lanes 3-9: *X. diffusum*. B: Lanes 1-7 and 9-11: *X. elongatum*, lane 8 *Xiphinema* sp. (PX 73). M = marker VIII (Boehringer).

Discussion

Primer reliability was confirmed by screening, where possible, different populations of the target species and the specificity was demonstrated by the absence of cross-reactions with non-target *Xiphinema* species. Multiplex PCR was effective and reproducible for DNA mixtures of two (*X. longicaudatum* and *X. ifacolum*) or three (*X. brevicolle, X. diffusum* and *X. elongatum*) of the target nematode species, thus improving the practicability and efficiency of the diagnostic tests. The species-specific primers for *X. brevicolle* and *X. diffusum* yielded the expected PCR products for all screened populations. The taxonomic status of the *X. americanum*-group is controversial, with the two latter species distinguished by only a few minor morphometric or morphological differences. Here, it was possible to clearly distinguish the populations of *X. brevicolle* from *X. diffusum* using the designed species-specific primers.

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