SOD polymorphism in the *Xiphinema americanum*-group (Nematoda: Longidoridae)

Sergio MOLINARI [1,*], Franco LAMBERTI [1,†], Larry W. DUNCAN [2], John HALBRENDT [3], Michael MCKENRY [4], George S. ABAWI [5], Juan CARLOS MAGUNACELAYA [6], Renato CROZZOLI [7], Rui M. LEMOS [8], Andrew P. NYCZEPIR [9], Peter NAGY [10], Robert T. ROBBINS [11], James KOTCON [12], Maurice MOENS [13] and Derek J.F. BROWN [14]

[1] *Istituto per la Protezione delle Piante, CNR, Via G. Amendola 165/A, 70126 Bari, Italy*
[2] *Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA*
[3] *The Fruit Research and Extension Center, Biglerville, PA, USA*
[4] *Kearney Horticultural Station, Parlier, CA, USA*
[5] *Department of Plant Pathology, Cornell University, Geneva, NY, USA*
[6] *Escuela de Agronomia, Facultad de Ciencias Agrónomicas, Universidad de Chile, Santiago, Chile*
[7] *Facultad de Agronomia, Universidad Central de Venezuela, Maracay, Venezuela*
[8] *Laboratório de Sanidade Vegetal, Direção Regional de Agricultura da Beira Litoral, Coimbra, Portugal*
[9] *US Department of Agriculture, Agricultural Research Service, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA, USA*
[10] *Szent István University, Department of Zoology and Ecology, Gödöllõ, Hungary*
[11] *Department of Plant Pathology, Nematology Laboratory, University of Arkansas, Fayetteville, AR, USA*
[12] *Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA*
[13] *Crop Protection Department, Agricultural Research Centre, Merelbeke, Belgium*
[14] *Central Laboratory of General Ecology, Sofia, Bulgaria*

Received: 25 May 2004; revised: 17 September 2004
Accepted for publication: 29 September 2004

Summary – Isoelectrofocusing of superoxide dismutase (SOD) isoforms was carried out on the extracts of 117 nematode populations belonging to the so-called *Xiphinema americanum*-group. These populations came from the USA (77), Chile (5), Argentina (1), Venezuela (5), Portugal (15), Italy (2), Crete (1), Montenegro (1), Slovakia (4), Hungary (3), Egypt (1) and India (2). A total of 17 bands of enzyme activity were observed in the screening, whilst single enzyme phenotypes showed from two to eight bands. The high degree of SOD polymorphism of this nematode collection was grouped by cluster analysis into seven distinct homogeneous groups characterised by specific combinations of SOD markers. Sub-groups could be discriminated for larger groups. The small Groups 3 and 5 were constituted mostly by populations from USA east coast states (*i.e.*, NY and PA, respectively). The larger Group 1 resulted from the association of populations coming from various and distant North American States. In other large groups, North American populations were associated with South American and European populations. Overall, the data presented here suggest that geographic separation and different hosts do not seem to be the source of genetic diversity for the *X. americanum*-group. When an adequate number of populations were collected from the same country, the variability expressed by such a sub-sample was comparable to that of the whole nematode collection. For the first time, homogeneous populations of a large collection of *X. americanum*-group populations were associated by molecular means in order to explore further approaches that may help resolve the recalcitrant taxonomy and phylogeny of this much debated group.

Keywords – antioxidants, genetic variability, isozymes, phenotypes, zymograms.

* Corresponding author, e-mail: nemasm04@area.ba.cnr.it

† Franco Lamberti died 16 August 2004.

Separation of superoxide dismutase (SOD) isozymes by electrophoresis has already been used to detect polymorphism in sedentary endoparasitic nematodes (Molinari *et al.*, 1996). Moreover, SOD isozyme profiles have been proved to be a reliable tool for species identification of longidorid nematodes (Molinari *et al.*, 1997; Lamberti *et al.*, 1999a, b, 2001a, b). Within the family Longidoridae, the genus *Xiphinema* Cobb, 1913 attracts considerable interest from nematologists because of its great variation in morphology (more than 200 valid species were listed by Hunt (1993) and many more have been described since) and because of the ability of some species to transmit plant nepoviruses. Determination of the variation within the so-called *X. americanum*-group, which contains potential virus vector and non-virus vector populations, is particularly complex using the usual morphological methodologies. Many characters used to separate species of the group often overlap, making diagnostics difficult and subjective to the point that even the total number of the accredited species is still debated (Luc *et al.*, 1998; Lamberti *et al.*, 2000). Recently, a hierarchical cluster analysis of 117 populations representing 39 putative species, and based on all the 17 characters commonly used in *Xiphinema* morphometrics, was able to discriminate only four main clusters which were divided into a total of 20 subgroups (Lamberti *et al.*, 2002b). Morphological analysis and morphometrics on their own may be inadequate to resolve the diagnostics of *X. americanum* populations as these nematodes appear to reproduce by obligate meiotic parthenogenesis and display a frustrating tendency to radiate into clusters of closely similar forms and morphospecies (Hunt, 1997).

Much work remains to be done on this group, but molecular biology, with comparative analysis of coding and non-coding regions of ribosomal DNA (rDNA), may support diagnostics emanating from 'classical' taxonomy and isozyme analysis. Variation in the rDNA sequence of 16 field populations of *X. americanum* from North America was measured by Vrain *et al.* (1992) using PCR to amplify the 5.8S gene and the internal transcribed spacer region (ITS). Although it is claimed that this molecular approach is capable of separating species within the *X. americanum*-group, the number of populations and putative species tested so far is too limited to have general validity. Moreover, when Lamberti *et al.* (2002a) attempted to separate 13 populations from Florida by restriction digestion of the PCR amplification product, the DNA analysis did not discriminate clearly between the putative species.

Even if specific SOD isozyme profiles have been associated in a few cases with morphospecies of the *X. americanum*-group (Lamberti *et al.*, 1999b), it seems most unlikely that every putative morphospecies may be identified by a single SOD pattern, as indeed was shown with the Florida populations studied by Lamberti *et al.* (2002a).

The approach of the present study was purely to detect the general polymorphism of the SOD loci within this debated group. It was not intended to demonstrate any relationship to morphological identification of the populations. Consequently, the species identity of most of the 117 populations tested was not attempted and their characterisation was based mainly on their geographic origin and the plants from the rhizosphere of which the population was collected.

Materials and methods

Nematode populations

Soil samples were collected from the rhizosphere of cultivated plants and in natural habitats from countries all over the world. A total of 117 populations was analysed from the USA (77), Chile (5), Argentina (1), Venezuela (5), Portugal (15), Italy (2), Crete (1), Montenegro (1), Slovakia (4), Hungary (3), Egypt (1), and India (2). The individuals of these populations showed dimensions comparable with those pertaining to the *X. americanum*-group, but only a few were identified at species level (Lamberti *et al.*, 1999b, 2002a). The locality and associated 'host' of the studied populations are listed in Table 1.

Sample preparation

Nematodes were extracted from soil by the Cobb wet-sieving technique. *Xiphinema* individuals were handpicked and collected. The assayed populations consisted of no fewer than 15 specimens. Nematodes were rinsed with distilled water to remove any soil debris and then transferred with a minimal volume of water into a plastic, Eppendorf-shaped, miniature homogeniser (Biomedix, UK). The distilled water was replaced with 10 μl of the extraction buffer, consisting of 20% sucrose, 0.1 M Trizma-Base, 0.08 M boric acid, pH 8.4, 2.5 mM EDTA, 5 μg of bromophenol blue, in which the inhibitors proteases PMSF (1 mM), pepstatin (1 μM) and leupeptin (1 μM) were added. Samples were kept in an ice-bath and firstly hand-homogenised with a pipette tip and then with

Table 1. *Populations of the* Xiphinema americanum*-group assayed by SOD isozyme isoelectrofocusing.*

Acronym	Putative species	Continent	State or Country	Locality	Host
Flo284	*X. laevistriatum*	North America	Florida	Merrit Island	*Casuarina* sp.
Flo183	*X. laevistriatum*	North America	Florida	Moore Haven	*Casuarina* sp.
Flo201	*X. floridae*	North America	Florida	Altoona	*Citrus aurantium* L.
Flo205	*X. citricolum*	North America	Florida	Altoona	*Citrus aurantium* L.
Flo192	*X. citricolum*	North America	Florida	Oklawaha	*Citrus paradisi* Macf.
Flo218	*X. citricolum*	North America	Florida	Eustis	*Citrus reticulata* Ten.
Flo158	*X. laevistriatum*	North America	Florida	Labelle	*Citrus* sp.
Flo227	*X. citricolum*	North America	Florida	Quincy	*Cynodon dactylon* L.
Flo156	*X. laevistriatum*	North America	Florida	Labelle	*Pinus palustris* Mill.
Flo124	*X. laevistriatum*	North America	Florida	Lake Alfred	*Quercus* sp.
Pe4	not known	North America	Pennsylvania	Adams County	*Acer* sp.
Pe32	not known	North America	Pennsylvania	Adams County	*Malus sylvestris* Mill.
Pe7	not known	North America	Pennsylvania	Arendtsville	*Malus sylvestris* Mill.
Pe7a	not known	North America	Pennsylvania	Arendtsville	*Malus sylvestris* Mill.
PeAx	not known	North America	Pennsylvania	Biglerville	*Malus sylvestris* Mill.
Pe12	not known	North America	Pennsylvania	Biglerville	*Malus sylvestris* Mill.
Pe2	not known	North America	Pennsylvania	Cashtown	*Malus sylvestris* Mill.
Pe23	not known	North America	Pennsylvania	Clear View	*Malus sylvestris* Mill.
Pe40	not known	North America	Pennsylvania	Piney Apple	*Malus sylvestris* Mill.
Pe1	not known	North America	Pennsylvania	Cashtown	*Pinus strobus* L.
Pe33a	not known	North America	Pennsylvania	Fairfield	*Populus alba* L.
Pe22	not known	North America	Pennsylvania	Arendtsville	*Prunus avium* L.
Pe9	not known	North America	Pennsylvania	Biglerville	*Prunus avium* L.
Pe29	not known	North America	Pennsylvania	Lerew	*Prunus avium* L.
Pe15	not known	North America	Pennsylvania	Wenkesville	*Prunus avium* L.
Pe39	not known	North America	Pennsylvania	Wenkesville	*Prunus avium* L.
Pe15a	not known	North America	Pennsylvania	Wenkesville	*Prunus avium* L.
Pe6	not known	North America	Pennsylvania	Arendtsville	*Prunus communis* L.
Pe5	not known	North America	Pennsylvania	Arendtsville	*Prunus persica* L.
Pe43	not known	North America	Pennsylvania	Means Hollow	*Prunus persica* L.
Pe24	not known	North America	Pennsylvania	York Springs	*Prunus persica* L.
Pe26	not known	North America	Pennsylvania	York Springs	*Rosa* sp.
Pe26a	not known	North America	Pennsylvania	York Springs	*Rosa* sp.
Pe20	not known	North America	Pennsylvania	Arendtsville	unknown
Pe20a	not known	North America	Pennsylvania	Arendtsville	unknown
Pe18	not known	North America	Pennsylvania	Fairview	*Zea mays* L.
Pe42	not known	North America	Pennsylvania	Means Hollow	*Zea mays* L.
MD5	not known	North America	Maryland	Thurmont	*Malus sylvestris* Mill.
MD1	not known	North America	Maryland	Thurmont	*Populus alba* L.
MD7	not known	North America	Maryland	Thurmont	*Prunus avium* L.
MD2	not known	North America	Maryland	Thurmont	*Prunus persica* L.
WV10	not known	North America	West Virginia	Kingwood Pive	*Acer* sp.
WV5	not known	North America	West Virginia	Morgantown	*Malus sylvestris* Mill.
WV6	not known	North America	West Virginia	Kingwood Pive	*Populus alba* L.
WV17	not known	North America	West Virginia	Kearnysville	*Prunus persica* L.
NY2	not known	North America	New York	Hudson Valley	*Acer* sp.
NY52	not known	North America	New York	Lakemount	*Acer* sp.
NY62	not known	North America	New York	Arbour Road	*Malus sylvestris* Mill.
NY36	not known	North America	New York	Lyndonville	*Malus sylvestris* Mill.
NY60	not known	North America	New York	Penn Yan	*Malus sylvestris* Mill.
NY24	not known	North America	New York	German Town	*Malus sylvestris* Mill.

Table 1. *(Continued).*

Acronym	Putative species	Continent	State or Country	Locality	Host
NY1	not known	North America	New York	Hudson Valley	*Malus sylvestris* Mill.
NY5	not known	North America	New York	Hudson Valley	*Malus sylvestris* Mill.
NY3	not known	North America	New York	Hudson Valley	*Malus sylvestris* Mill.
NY47	not known	North America	New York	Dresden at Route 14	*Malus sylvestris* Mill.
NY65	not known	North America	New York	Penn Yan	*Malus sylvestris* Mill.
NY78	not known	North America	New York	Albion	*Prunus avium* L.
NY37	not known	North America	New York	Lyndonville	*Prunus avium* L.
NY27	not known	North America	New York	Geneva	*Prunus avium* L.
NY29	not known	North America	New York	Geneva	*Prunus avium* L.
NY22	not known	North America	New York	German Town	*Prunus persica* L.
AR4	not known	North America	Arkansas	Greek State	*Ulmus* sp.
AR7	not known	North America	Arkansas	Toad Suck Park	*Acer* sp.
Ms	not known	North America	Mississippi	Scuby	grasses
GG3	not known	North America	Georgia	Byron	*Vaccinium* sp.
GG15	not known	North America	Georgia	Concord	*Prunus persica* L.
UT28	not known	North America	Utah	Ogden	unknown
UT24	not known	North America	Utah	Holladay	*Populus alba* L.
UT25	not known	North America	Utah	Holladay	*Populus deltoides* Marsh.
Co3	not known	North America	Colorado	Mesa	*Malus sylvestris* Mill.
Co5	not known	North America	Colorado	Mesa	*Malus sylvestris* Mill.
OR4	not known	North America	Oregon	Molalla	*Vitis* sp.
NV5	not known	North America	Nevada	Wells	*Juniperus* sp.
Ca55	not known	North America	California	Kearney	*Malus sylvestris* Mill.
Ca40	not known	North America	California	Chowchilla	*Vitis vinifera* L.
Ca45	not known	North America	California	Kearney	*Vitis* sp.
Ca43	not known	North America	California	Kearney	*Vitis* sp.
Ch2	not known	South America	Chile	Buin	*Prunus domestica* L.
Ch5	not known	South America	Chile	Buin	*Vitis vinifera* L.
Ch39	not known	South America	Chile	Copiapò	*Vitis vinifera* L
Ch36	not known	South America	Chile	Copiapò	*Vitis vinifera* L.
Ch14	not known	South America	Chile	Casablanca	*Vitis vinifera* L.
Ar53	not known	South America	Argentina	Mendoza	*Populus alba* L.
Ve187	not known	South America	Venezuela	Barinas	grasses
Ve168	not known	South America	Venezuela	San Fernando	*Mangifera indica* L.
Ve182	not known	South America	Venezuela	San Nicolas	*Musa* sp.
Ve177	not known	South America	Venezuela	Sosa	*Prosopis* sp.
Ve178	not known	South America	Venezuela	La Rojera	*Psidium guajava* L.
P305	not known	Europe	Portugal	Loulè, Algarve	*Ficus carica* L.
P247	not known	Europe	Portugal	Travanca de S. Tomè	*Pinus* sp.
P263	not known	Europe	Portugal	Montemor-o-Novo	*Quercus* sp.
P227	not known	Europe	Portugal	Lazirão	*Rubus* sp.
P243	not known	Europe	Portugal	Alcafache	*Vitis* sp.
P244	not known	Europe	Portugal	Baco	*Vitis* sp.
P324	not known	Europe	Portugal	Cantanhede	*Vitis* sp.
P325	not known	Europe	Portugal	Cantanhede	*Vitis* sp.
P235	not known	Europe	Portugal	Olivais	*Vitis* sp.
P289	not known	Europe	Portugal	Silves, Algarve	*Vitis* sp.
P264	not known	Europe	Portugal	Silves, Vicente	*Vitis* sp.
P276	not known	Europe	Portugal	Tavira, Algarve	*Vitis* sp.
P278	not known	Europe	Portugal	Tavira, Algarve	*Vitis* sp.
P267	not known	Europe	Portugal	Tavira, Algarve	*Vitis* sp.

Table 1. *(Continued).*

Acronym	Putative species	Continent	State or Country	Locality	Host
P1	not known	Europe	Portugal	Setubal	*Zea mays* L.
Vg30	not known	Europe	Italy	Venezia-Giulia	*Vitis* sp.
Vg29	not known	Europe	Italy	Venezia-Giulia	*Vitis* sp.
Cr7	not known	Europe	Greece	Crete, Stilos	*Olea europea* L.
Y2	not known	Europe	Montenegro	Kruće	*Juniperus oxycedrus* L.
Sl116	*X. simile*	Europe	Slovakia	Velki Lapas	*Populus alba* L.
Sl67	*X. taylori*	Europe	Slovakia	Zavada	*Prunus avium* L.
Sl191	*X. simile*	Europe	Slovakia	Aleksince	*Prunus domestica* L.
Sl165	*X. pachtaicum*	Europe	Slovakia	Moća	*Vitis* sp.
H15	not known	Europe	Hungary	Gödöllö	*Prunus avium* L.
H22	not known	Europe	Hungary	Gödöllö	*Acer* sp.
H19	not known	Europe	Hungary	Gödöllö	*Prunus armeniaca* L.
Eg1	not known	Africa	Egypt	Nubaria	*Vitis vinifera* L.
In31	not known	Asia	India	Himalaya, Ranzi	*Pinus* sp.
In9	not known	Asia	India	Andhra Pradesh, Patancheru	*Bambusa vulgaris* Schrad.

a small plastic pestle connected to a rotor. They were then centrifuged at 2432 *g* for 10 min and clarified supernatants were either used immediately for electrophoresis or stored at −80°C.

Electrophoresis

SOD isozymes were separated by isoelectric focusing on mini-gels (3.6 cm separation zone) inserted into a PhastSystem equipment (Pharmacia Biotech, Piscataway, NJ, USA), as previously described (Molinari *et al.*, 1996, 1997). Mini-gels were loaded with six samples, each sample consisting of a 8 μl nematode extract. Two gels per run could be inserted in the equipment. Gels were maintained at 15°C and runs were stopped at 600 Vh, a procedure which corresponded to a run time of *ca* 30 min. Pre-programming of the runs allowed reliable reproduction of the band patterns of each population.

Gels were calibrated by using a broad pI calibration kit (Pharmacia Biotech) containing amyloglucosidase (3.50), soybean trypsin inhibitor (4.55), β-lactoglobulin A (5.20), bovine carbonic anhydrase B (5.88), human carbonic anhydrase B (6.55), myoglobin-acidic band (6.85), myoglobin-basic band (7.35), lentil lectin-acidic band (8.15), lentil lectin-middle band (8.45), lentil lectin-basic band (8.65), and trypsinogen (9.30). Relative electrophoretic mobilities (Rm in cm) were plotted against pI values and a straight line with a high correlation coefficient (*a.* −0.99) was obtained.

SOD activity was determined by incubating the gels in the dark at 28°C for 20 min in a solution containing 0.12 mM nitrobluetetrazolium (NBT). Thereafter, NBT was removed and the gel immersed in a solution containing 15 mM N,N,N′,N′-tetramethylethylenediamine (TEMED) and 0.26 mM riboflavin in 50 mM Tris-HCl, pH 7.6. Gels were developed on a white light transilluminator until white bands on a dark background appeared. When bands were visually clear, the gels were dried and scanned by means of a Hewlett Packard ScanJet IIcx. For easier detection on prints, the images of the gels were arranged by computer into negatives with dark bands on grey backgrounds.

Statistical analysis

The 17 SOD bands detected were used as variables to construct a data matrix in which the presence or absence of a band was indicated by 1 or 0, respectively. The 117 *X. americanum* populations available were considered as cases. An analysis of the groups forming by the linkage method of Ward, and Euclidean distances as distance units, was carried out using the software STATISTICA (StatSoft Inc., Tulsa, OK, USA). On the dendrogram obtained, groups were determined when they differed by more than half of the maximum Euclidean distance registered (*ca* 13).

Results

Seventeen different bands of SOD activity were observed after the isoelectrofocusing of the extracts from

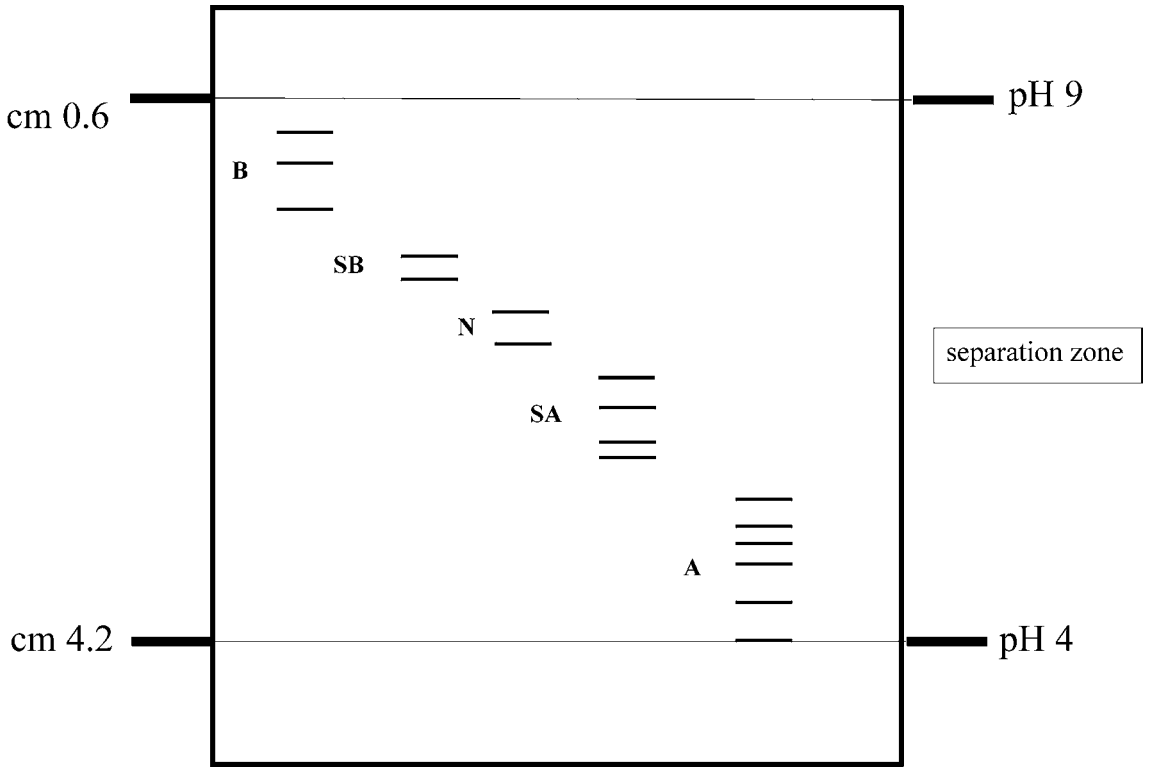

Fig. 1. *Schematic representation of the gels used for isoelectrofocusing in PhastSystem equipment with the 17 SOD isozyme bands detected in the survey of 117* Xiphinema americanum *populations. The separation zone (3.6 cm) ranged between pH 9 and 4. On the basis of their isoelectric points (pI), SOD bands are divided into the following five groups: B = Basic bands (pI for: B1 = 8.6; B2 = 8.4; B3 = 8.1); SB = Slight Basic bands (pI for: SB1 = 7.6; SB2 = 7.4); N = Neutral bands (pI for: N1 = 7.1; N2 = 6.8); SA = Slight Acidic bands (pI for: SA1 = 6.5; SA2 = 6.0; SA3 = 5.8; SA4 = 5.7); A = Acidic bands (pI for: A1 = 5.5; A2 = 5.3; A3 = 5.1; A4 = 4.7; A5 = 4.3; A6 = 4.0).*

the 117 *X. americanum* populations (Fig. 1). These bands were divided into five classes depending on their isoelectric points (pIs), *viz*, three basic bands (Class B), two slightly basic bands (Class SB), two neutral bands (Class N), four slightly acidic bands (Class SA) and six acidic bands (Class A). The number of bands per pattern varied from a minimum of two to a maximum of eight. Most of the populations showed bands on the gels belonging to class B and A, but additional bands from the other classes were frequently observed.

Populations were clustered on the dendrogram (Fig. 2), seven groups, indicated by numbers, being clearly distinguished. Large groups were divided into sub-groups and these were indicated by capital letters. Figure 3 illustrates the typical SOD phenotype of sample populations of each group and sub-group. Groups are generally characterised either by the association of two or three SOD markers, or by the absence of specific markers, as in Group 7. The 17 SOD bands observed in the present analysis revealed a high phenotype polymorphism within the 117 populations attributed to the *X. americanum*-group. The number of enzyme isoforms with different charge possibly reflects the variability in the number of the correspondent genetic loci and the structure of the relative alleles, although it is difficult to predict the effect of extra-genetical sources of variation, such as post-transcriptional changes, or enzyme preparation and electrophoresis-run artefacts.

Essentially, populations from the USA are distributed among the first six groups. They entirely form the small Groups 3 and 5 and comprise most of Group 1 (Table 2). Actually, Group 3 is almost completely constituted by populations collected in New York State and Group 5 by nematodes from Pennsylvania, thus suggesting that those groups may have a local characterisation. It should be noted that the largest portion of populations came from the east coast states of the USA (*i.e.*, NY, PA,

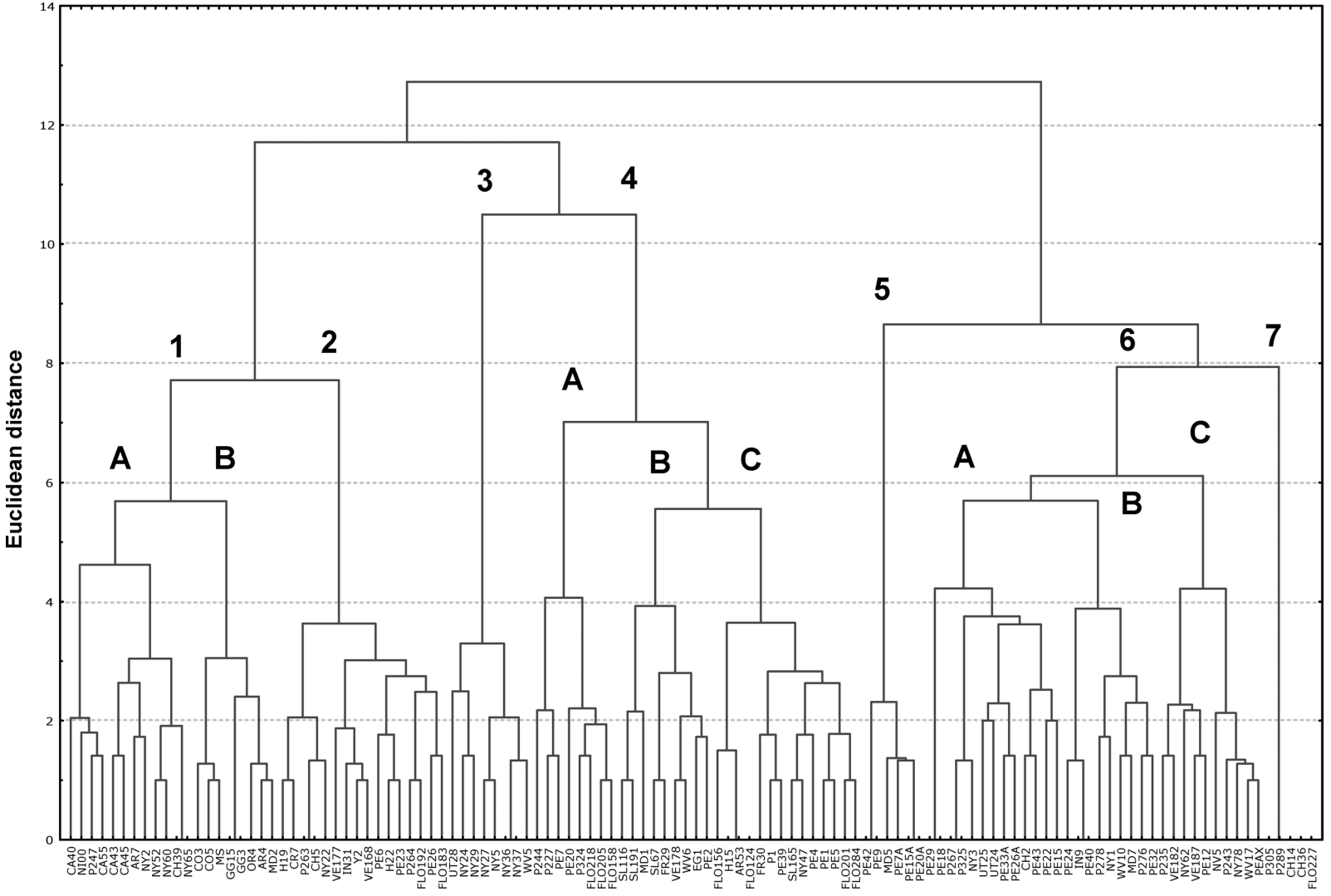

Fig. 2. *Dendogram obtained by cluster analysis of the SOD isoelectrofocusing patterns of 117* Xiphinema americanum *populations. The 17 SOD markers detected were considered as variables (presence 1, absence 0) and the 117 populations as cases (indicated on the x-axis). The linkage method used is Ward's Method and the unit on the y-axis is the Euclidean distance. The seven groups tentatively proposed are indicated by a number; sub-groups within groups by a capital letter.*

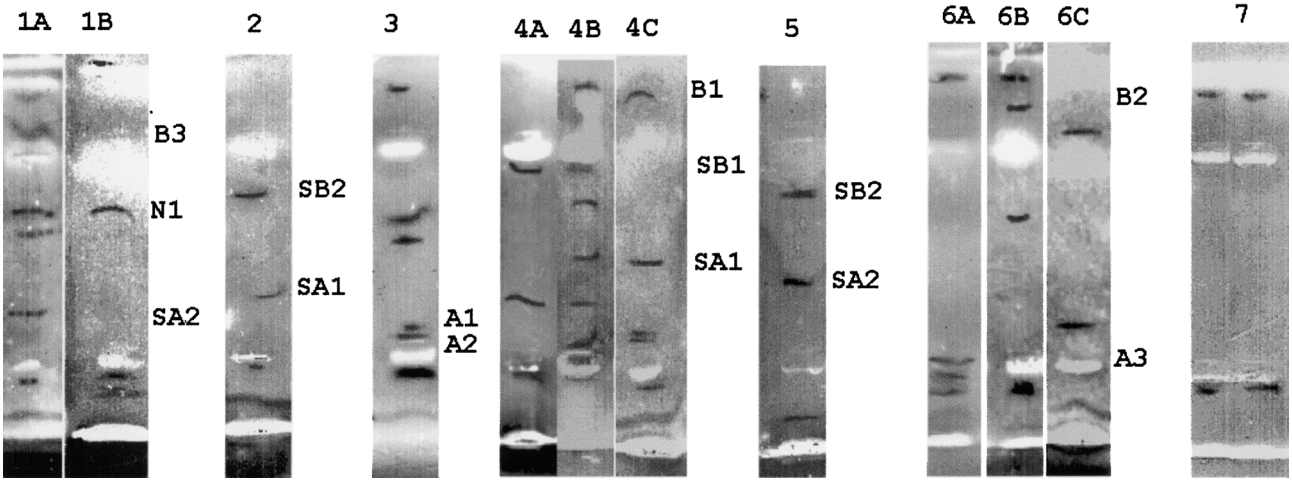

Fig. 3. *SOD isoelectrofocusing patterns of sample* Xiphinema americanum *populations representing the proposed groups and sub-groups: 1A = Ca40; 1B = GC3; 2 = Pe26; 3 = NY24; 4A = P324; 4B = Eg1; 4C = Pe4; 5 = MD5; 6A = NY3; 6B = IN9; 6C = Pe12; 7 = Ch14-36. Specific combinations of SOD markers are associated with each group. SOD bands appear black on a grey background. Population codes are defined in Table 1.*

Table 2. *Groups and sub-groups of* Xiphinema americanum *populations* as determined by SOD isozyme bands and according to the dendrogram shown in Figure 2.*

Group 1		Group 2	Group 3	Group 4			Group 5	Group 6			Group 7
Sub-group				Sub-group				Sub-group			
A	B			A	B	C		A	B	C	
Ca40	Co3	Pe6	UT28	Flo158	Pe2	Flo124	Pe7a	UT24	Pe24	NV5	Flo227
Ca43	Co5	Pe23	NY5	Flo205	WV6	Flo156	Pe9	UT25	Pe32	PeAx	Ch36
Ca45	Ms	Pe26	NY24	Flo218	MD1	Flo201	Pe15a	Pe15	Pe40	Pe12	Ch14
Ca55	GG3	NY22	NY27	Pe7	Ve178	Flo284	Pe20a	Pe18	NY1	NY62	P289
NY2	GG15	Flo183	NY29	Pe20	Vg29	Pe1	Pe42	Pe22	WV10	NY78	P305
NY52	OR4	Flo192	NY36	P227	Sl67	Pe4	MD5	Pe26a	MD7	WV17	
NY60	AR4	Ve168	NY37	P244	Sl116	Pe5		Pe29	P276	Ve182	
NY65	MD2	Ve177	WV5	P324	Sl119	Pe39		Pe33a	P278	Ve187	
AR7		Ch5			Eg1	NY47		Pe43	In9	P235	
Ch39		P263				Ar53		NY3		P243	
P247		P264				P1		Ch2			
		Y2				Vg30		P267			
		H19				Sl165		P325			
		H22				H15					
		Cr7									
		In31									

* See Table 1 for descriptions of the populations indicated by the acronyms.

MD, WV, FL: 61 populations). Such populations are evenly distributed among Groups 2, 4 and 6 and are always in association with populations from Portugal (the largest European group tested). Moreover, Group 2 contains one Indian and several South American and East European populations and therefore seems to assemble together populations from almost all of the globe. The remainder of the East European populations clustered into Group 4, sub-groups B and C, together with American and Mediterranean populations. All the populations from the western states of the USA (*i.e.*, CA, CO, OR) were clustered into Group 1, together with populations coming from other, more distant, US states.

Discussion

Generally, the data presented here suggest that geographic separation and different hosts do not appear to be the source of genetic diversity for the *X. americanum*-group as populations coming from extremely distant geographical sites share analogous SOD phenotypes. Moreover, the variation found in a determined geographical area increased as more populations were collected – the largest samplings carried out in a country produced populations which were practically of ubiquitous occurrence in the clusters obtained. Therefore, it is likely that the apparent phenotype homology found in some geographic zones is due to the low number of populations tested. If further investigation confirms the existence of groups constituted only by American populations, attention should be paid to the ability of those populations to transmit viruses.

As most of the populations tested were not identified to species level, very little can be affirmed on the relationship between the SOD isozyme groups and the morphologically identified nominal species. However, it seems unlikely that a hierarchical cluster analysis carried out by population morphometrics as, for example, the one reported in Lamberti *et al.* (2002b), may produce results similar to those presented here based on SOD phenotype diversity. Such phenotypes may become additional tools to define genetically homogeneous groups of *X. americanum* populations, although they will not be conclusive in resolving the troublesome issue of classical species determination based on the minimal morphological variations observed amongst the populations belonging to this group.

The combination of the present different approach, together with morphometric studies, may help to resolve the much debated taxonomy and phylogeny of this group. Furthermore, it also seems worthwhile to search for a possible relationship between specific SOD markers and the capability of a determined population to transmit plant viruses.

References

HUNT, D.J. (1993). *Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics*. Wallingford, UK, CAB International, 352 pp.

HUNT, D.J. (1997). Nematode species: concepts and identification strategies exemplified by the Longidoridae, Steinernematidae and Heterorhabditidae. In: Claridge, M.F., Dawah, H.A. & Wilson, M.R. (Eds). *Species: The units of biodiversity.* London, UK, Chapman & Hall, pp. 221-245.

LAMBERTI, F., MOLINARI, S., DE LUCA, F., AGOSTINELLI, A. & DI VITO, M. (1999a). Longidorids (Nematoda, Dorylaimida) from Syria with description of *Longidorus pauli* sp. n. and *Paralongidorus halepensis* sp. n. with SOD isozymes and PCR-RFLP profiles. *Nematologia Mediterranea* 27, 63-78.

LAMBERTI, F., SABOTA, M., DE LUCA, F., MOLINARI, S., AGOSTINELLI, A., COIRO, M.I. & VALOCKA, B. (1999b). Phenotypic variations and genetic characterization of *Xiphinema* populations from Slovakia (Nematoda, Dorylaimida). *Nematologia Mediterranea* 27, 261-275.

LAMBERTI, F., MOLINARI, S., MOENS, M. & BROWN, D.J.F. (2000). The *Xiphinema americanum* group. I. Putative species, their geographical occurrence and distribution, and regional polytomous identification keys for the group. *Russian Journal of Nematology* 8, 65-84.

LAMBERTI, F., DUNCAN, L.W., DE LUCA, F., MOLINARI, S., AGOSTINELLI, A., DUNN, D., COIRO, M.I. & RADICCI, V. (2001a). Biometric and molecular characterization and juvenile development stages of *Xiphinema vulgare* Tarjan, 1964 (Nematoda, Dorylaimida). *Nematologia Mediterranea* 29, 137-144.

LAMBERTI, F., KUNZ, P., GRUNDER, J., MOLINARI, S., DE LUCA, F., AGOSTINELLI, A. & RADICCI, V. (2001b). Molecular characterization of six *Longidorus* species from Switzerland with the description of *Longidorus helveticus* sp. n. (Nematoda, Dorylaimida). *Nematologia Mediterranea* 29, 181-205.

LAMBERTI, F., DE LUCA, F., MOLINARI, S., DUNCAN, L.W., AGOSTINELLI, A., COIRO, M.I., DUNN, D. & RADICCI, V. (2002a). Studies on some species of the *Xiphinema americanum*-group (Nematoda, Dorylaimida) occurring in Florida. *Nematologia Mediterranea* 30, 31-44.

LAMBERTI, F., MOLINARI, S., MOENS, M. & BROWN, D.J.F. (2002b). The *Xiphinema americanum*-group. II. Morphometric relationships. *Russian Journal of Nematology* 10, 99-112.

LUC, M., COOMANS, A., LOOF, P.A.A. & BAUJARD, P. (1998). The *Xiphinema americanum*-group (Nematoda:

Longidoridae). 2. Observations on *Xiphinema brevicollum* Lordello & da Costa, 1961 and comments on the group. *Fundamental and Applied Nematology* 21, 475-490.

MOLINARI, S., EVANS, K., ROWE, J. & RUSSELL, M. (1996). Rapid diagnosis of *Heterodera* cysts by SOD isozyme electrophoresis profiles. *Annals of Applied Biology* 129, 361-368.

MOLINARI, S., DE LUCA, F., LAMBERTI, F. & DE GIORGI, C. (1997). Molecular methods for the identification of longidorid nematodes. *Nematologia Mediterranea* 25, 55-61.

VRAIN, T.C., WAKARCHUK, D.A., LEVESQUE, C.A. & HAMILTON, R.I. (1992). Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum*-group. *Fundamental and Applied Nematology* 15, 563-573.