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Molecular and morphological consilience in the characterisation and delimitation of five nematode species from Florida belonging to the *Xiphinema americanum*-group

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Summary – Taxonomic keys and original descriptions were used to identify 26 *Xiphinema americanum*-group populations from Florida comprising *X. georgianum* (eight populations), *X. citricolum* (six), *X. floridae* (six), *X. laevistriatum* (five) and *X. tarjanense* (one). Principal component analysis of a subset of 19 morphometric characters accorded with the species designations; discriminant analysis of six characters assigned 93% (111 of 119) of the specimens to the correct putative species. A phylogeny of these populations estimated from analyses of rDNA sequences (ITS and D2D3) was also congruent with species designations from taxonomic keys and PCA. The D2D3 sequences revealed very little intraspecific variation whereas each population sampled produced a unique ITS sequence. Intraspecific variation in the suites of character code values from polytomous keys resulted mainly from minor discrepancies between population character means and reported character ranges for the species. We show that, for these taxa, species delimitation based on the requirement that sister taxa evolve autapomorphies distinguishes intraspecific variation from phylogeny and, as applied to molecular characters, delimits the same taxa as those predicted by morphological keys and PCA.

Keywords – D2D3, dagger nematode, ITS, phylogeny, rDNA, taxonomy, *Xiphinema citricolum*, *Xiphinema floridae*, *Xiphinema georgianum*, *Xiphinema laevistriatum*, *Xiphinema tarjanense*.

Members of Longidoridae in the genus *Xiphinema* are important plant parasites as well as vectors of plant nepoviruses (Taylor & Brown, 1997). Correct identification of *Xiphinema* species is economically important due to their specificity with regard to plant hosts and virus transmission capability. However, species diversity and taxonomic validity of species in the *X. americanum* Cobb, 1913 group has been somewhat controversial, with the number of recognised species ranging from 34 (Luc *et al.*, 1998; Coomans *et al.*, 2001) to 51 (Lamberti *et al*., 2000, 2002). Lack of agreement about the taxonomy of the group results from few differences reported between many of the species, lack of data on intraspecific variation, and insufficient illustrations for many putative species (Loof & Luc, 1990). Although morphometric variation is abundant, identification is made difficult by overlapping measurements and the use of suites of character combinations as opposed to unambiguous autapomorphies.

Luc and Baujard (2001) proposed the need for a careful re-evaluation of the type and other species in this

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group as a prelude to developing reliable taxonomic keys. They noted that *X. citricolum* Lamberti & Bleve-Zacheo, 1979 and seven other species were indistinguishable from *X. americanum s. s*. on the basis of a published polytomous key to the group (Lamberti *et al*., 2000) and that 17 additional species, including *X. laevistriatum* Lamberti & Bleve-Zacheo, 1979*, X. floridae* Lamberti & Bleve-Zacheo, 1979 and *X. tarjanense* Lamberti & Bleve-Zacheo, 1979, differ from *X. americanum s. s*. by just a single character.

In order to investigate morphological variation as a component of species diversity in the *X. americanum*-group, we examined molecular (ITS and D2/D3 ribosomal DNA sequences) and morphometric characters from 26 populations comprised of five closely related species of the *X. americanum*-group in Florida: *X. citricolum*, *X. floridae*, *X. georgianum* Lamberti & Bleve-Zacheo, 1979, *X. laevistriatum* and *X. tarjanense.* Our objectives were to validate these species based on phylogenetic relationships (Adams, 1998) and to characterise the usefulness of morphological characters for species diagnosis.

Materials and methods

Isolates from populations of putative *X. citricolum*, *X. floridae*, *X. georgianum*, *X. laevistriatum* and *X. tarjanense* were collected from various host plants and locations in Florida (Table 1). Nematodes were extracted from soil by sucrose centrifugation (Niblack & Hussey, 1985). For morphological studies, specimens were killed by gentle heating, fixed in 4% formaldehyde, and processed and mounted in glycerin on glass slides by a modification of Seinhorst's (1959) method. Specimens were measured using light microscopy and a camera lucida. Populations were assigned species designations based on their morphology (Lamberti & Bleve Zacheo, 1979; Lamberti *et al.*, 2004). Species designations were made independently of (*i.e*., prior to) multivariate and phylogenetic analyses.

Up to 19 morphometric variables from between four to eight females from each population were analysed using principal component analysis (Minitab Software, State College, PA, USA; Duncan *et al*., 1999). Discriminant analysis (Minitab Software) was also employed using the polytomous key characters (lengths of odontostyle, body, and tail, c, c′, V) given by Lamberti *et al.* (2004).

DNA was extracted from an individual female nematode from each population using DNeasy tissue extraction kits (QIAGEN, Santa Clarita, CA, USA). Ribosomal DNA of the internally transcribed region was PCR amplified using the 18S (forward) and 26S (reverse) primers designed by Vrain *et al*. (1992) which bind to the posterior 3′ portion of the 18S small ribosomal subunit (forward) and at the 5′ end of the 28S subunit region (reverse). In addition, the D2D3 rDNA was partially amplified using two sets of primers, D3A (forward) and D3B (reverse) and 502 (forward) and 536 (reverse) (Baldwin *et al*., 1997). Polymerase chain reactions were carried out in 25 μl volumes. PCR mix was added to each tube: 2.5 μl 10 × PCR buffer, 1.5 μl $MgCl_2$, 1μl dNTP mixture (10 mM each), 1 μl of 10 pM forward primer, 1 μl of 10 pM reverse primer, 0.25 μl of *Taq* polymerase (CLP), 19.55 μl of distilled water and 5 μl of DNA. All PCR reactions were run in a PTC-100 Thermocycler (MJ Research, Waltham, MA, USA) with the cycling profile: 1 cycle of 94°C for 7 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. The last step was 72°C for 10 min. The resultant PCR products of ITS region were cloned into the vector PCR2.1 Topo-TA cloning kit (Invitrogen, Carlsbad, CA, USA). Plasmid DNA was purified from bacterial cultures using QIAprep Spin Miniprep kit (QIAGEN). For direct sequencing, PCR products were purified using QIAquick PCR purification kit (QIAGEN) and sequenced at the University of Florida ICBR sequencing core facility on Perkin Elmer/Applied Biosystems automated DNA sequencers. The primers used for sequencing were the same that were used for amplification. The ITS rDNA and D2D3 region sequences of five species of *X. americanum*-group were deposited in GenBank (Accessions DQ 299490-DQ 299536).

Sequences were edited using sequencer (4.1.2 Gene codes Corporation 1991-2000). For the analysis of the 28S ribosomal RNA gene (LSU), ingroup sequences, including two *X. americanum* sequences available in GenBank (AY601591, AY601599), were aligned with the outgroup taxa *Longidorus euonymus* Mali & Hooper, 1974 and *L. apulus* Lamberti & Bleve-Zacheo, 1977 (GenBank accession numbers AY601571 and AY601573, respectively) chosen on the basis on sequence similarity expect scores (BLASTnr; Altschul *et al*., 1990) and the sister group relationship to the ingroup taxa by prior inference (He *et al*., 2005). The multiple sequence alignment was constructed using the default parameters of Clustal X 1.83 (Thompson *et al*., 1997) and adjusted manually in MacClade 4.0 (Maddison & Maddison, 2002). Duplicate sequences were removed from the alignment prior to analysis, resulting in 15 taxa (13 ingroup, 2 outgroup).

Table 1. *Sample locations, associated plant species, survey and analysis codes, analyses conducted, and identities of 30* Xiphinema *spp. populations used in this study.*

Identification and survey code	Analysis code	Location	Plant species	D2		
				PCA	D3	ITS
X. citricolum						
315	C1	Umatilla	Citrus	x	x	
408	C2	Winter Haven	Oak	x	x	x
461	C3	Haines City	Oak	x	x	x
472	C4	Cape Canaveral	Hackberry tree	x	x	x
478	C5	Cape Canaveral	Brazilian pepper	x	x	x
473	C6	Cape Canaveral	Oak	x		
X. georgianum						
124	G1	Lake Alfred	Oak	x	x	x
403	G2	Frostproof	Oak	x	x	x
436	G3	Ft. Pierce	Oak	x	x	x
441	G4	Ft. Drum	Oak	x	x	x
446	G5	Auburndale	Oak	x	x	x
447	G6	Auburndale	Oak	x	x	x
486	G7	Titusville	Pine	x	x	x
487	G8	Titusville	Oak	x	x	x
X. laevistriatum						
398	L1	Frostproof	Oak	x	x	x
410	L2	Winter Haven	Oak	x	x	x
458	L3	Polk City	Oak	x	x	x
465	L4	Haines City	Oak	x	x	x
395	L5	Babson Park	Pine	x		
X. floridae						
317	F1	Altoona	Citrus	x	x	x
480	F2	Cape Canaveral	Sea oat	x	x	x
481	F3	Cape Canaveral	Sea grape	x	x	x
488	F4	Titusville	Oak	x	x	x
393	F5	Babson Park	Pine	x		
484	F6	Titusville	Oak	x		
X. tarjanense						
379	T1	Winter Haven	Oak	x	x	x
X. vulgare						
434	V1	Ft. Pierce	Pine		x	
416	V2	Eagle Lake	Oak		x	
X. chambersi						
414	CH1	Eloise	Pines		x	
X. naturale						
440	N1	Ft. Pierce	Oak		x	

Alignment of the ITS sequences to outgroup taxa was not considered because the variation among the ingroup sequences was so high that homology statements between the ingroup and outgroup taxa could not be inferred with confidence. Alignment of the ITS sequences proceeded from inferred relationships based on the LSU sequence data. Accordingly, each of the monophyletic populations were individually aligned and optimised manually in MacClade. Then, the populations (clades) were aligned sequentially to each other according to their inferred relationships from the LSU phylogeny using the profile alignment option in ClustalX.

Because the ITS alignments did not include outgroup taxa, no effort was made to concatenate the ITS and

D2D3 datasets. Maximum parsimony (MP), maximum likelihood (ML), and minimum evolution of log determinant transformed distances (ME) analyses were performed on each dataset separately using PAUP* (Swofford, 2002). Maximum parsimony analyses were performed considering indels as either missing data or as a fifth character state. For the D2D3 dataset, tree and bootstrapping proceeded *via* branch and bound searches. The ITS dataset employed a heuristic search with starting trees acquired *via* stepwise addition (ten replicates of random addition sequence) with tree-bisection-reconnection (TBR) branch swapping. Bootstrap analyses proceeded similarly, but with the maximum number of trees retained at each replicate constrained to 3043, the number of MP solutions found during the initial heuristic search. Models of sequence evolution for maximum likelihood analysis were chosen *via* ModelTest 3.5 (Posada & Crandall, 1998; Posada & Buckley, 2004). Maximum likelihood searches, including bootstrapping, of the ITS and D2D3 alignments were heuristic with starting trees acquired *via* stepwise addition (ten replicates of random addition sequence) with tree-bisection-reconnection (TBR) branch swapping. Minimum evolution solutions employed LogDet transformed genetic distances (Steel *et al*., 2000); bootstrap support for this solution was estimated using 1000 resampling replicates. Comparison tests of alternative topologies were carried out using PAUP* under the parsimony optimality criterion using Templeton (non-parametric) and Kishino-Hasegawa tests, and under the likelihood optimality criterion using the Kishino-Hasegawa test (two tailed) and Shimodaira-Hasegawa test, both utilising RELL test distribution optimisation. Command files for all analyses were assembled into nexus PAUP blocks and batch run on PAUP* portable version 4.0b10 for Unix on a RackSaver 64 node dual Opteron processor supercomputing cluster and are available upon request.

Results

Morphological relationships

Five species of the *X. americanum*-group were identified among 26 of the dagger nematode populations, based on morphology and morphometrics (Tables 2, 3), *viz*., *X. citricolum*, *X. floridae*, *X. georgianum*, *X. laevistriatum* and *X. tarjanense* (Lamberti & Bleve-Zacheo, 1979; Lamberti *et al*., 2004). We also identified two populations of *X. vulgare* Tarjan, 1964 and single populations of *X. chambersi* Thorne, 1939 and *X. naturale* Lamberti *et al*., 2002, none of which belongs to the *X. americanum*-group.

The averages for a number of morphometric characters of the *X. americanum*-group populations were outside the reported ranges for those species (Tables 2, 3). For example, the mean tail lengths of all five populations of *X. laevistriatum* (range = 24.4-26.9 μm) were significantly less than originally described (mean = 34, range = 29-36 μm) and were all inferior to 27 μm which is considered diagnostic and the lower limit of the species (Lamberti *et al*., 2004). *Xiphinema tarjanense* was distinguished from populations of the similar *X. floridae* by relative size and the degree of lip offset; however, the means of diagnostic characters such as body length, tail length, and values of c and c′ for *X. tarjanense* were significantly different than previous reports and were closer to those given for *X. inaequale*, *X. incognitum* and *X. floridae* (Table 3; Lamberti *et al*., 2004). The identities of populations of *X. citricolum*, *X. floridae*, *X. laevistriatum* and *X. georgianum* were generally supported by the polytomous key, but not the more restrictive dichotomous key.

The first two principal components from PCA of the *X. americanum*-group species explained 62% of the variation in 19 measured and derived morphometric characters and revealed three clusters of populations (Fig. 1A). The eight *X. georgianum* populations segregated from all others, whereas a cluster of the five *X. laevistriatum* populations overlapped a cluster of the five *X. citricolum* populations and the single population of *X. tarjanense* was associated with a cluster of the six *X. floridae* populations. PCA of arbitrarily selected characters (length of odontostyle, tail length, lip diameter, distance of the guide ring from the lip anterior, and the ratios c and J) resulted in better discrimination of the designated species and the first two principal components accounted for 75% of the variation in those characters (Fig. 1B). One hundred percent of the individual specimens from populations of *X. laevistriatum*, *X. georgianum* and *X. tarjanense* were assigned to the correct putative species by discriminant analysis (Table 4). Twenty-one of 27 specimens of *X. floridae* and 22 of 24 specimens of *X. citricolum* were correctly identified by this method.

Three of the characters, stylet length, tail length, and lip diameter, were adequate to discriminate the four clusters of populations from principal component analysis (Fig. 1B). *Xiphinema georgianum* was the only species with an average stylet length of all populations >100

Table 2. *Nineteen morphometric characters from 26 populations of five species belonging to the* Xiphinema americanum*-group.*

Pop	Length								
	Body	Lip diam.	Anterior to pharynx	Anterior to vulva	Anterior to guide ring	Odontostyle	Odontophore	Tail	J
C1	1658 ± 43	9.7 ± 0.31	299 ± 21	855 ± 40	67.4 ± 4.1	78 ± 4	45.7 ± 5.0	30.6 ± 1.4	13.8 ± 1.08
	(1590-1702)	(9.2-10.18)	(268-321)	(788-898)	(63.4-76.2)	(73-84)	(39.0-53.5)	(28.5-32.3)	(12.5-15.35)
C2	1698 ± 74	9.7 ± 1.03	311 ± 37	899 ± 41	63.8 ± 2.0	75 ± 3	45.7 ± 1.6	30.7 ± 3.1	9.6 ± 1.45
	(1602-1802)	(8.2-10.91)	(261-345)	(842-949)	(61.0-66.5)	(73-80)	(43.4-47.9)	(27.8-35.6)	(8.1-11.43)
C3	1502 ± 68	10.0 ± 0.37	227.6 ± *	796 ± *	60.9 ± 2.2	74.1 ± 1	43.1 ± 1.7	29.1 ± 1.6	10.6 ± 2.40
	(1393-1561)	(9.5-10.5)	(*)	(*)	(57.1-62.2)	(73-75)	(41.4-45.1)	(27.6-31.1)	(8.7-14.3)
C4	1520 ± 22	9.3 ± 0.26	260 ± 27	793 ± 25	59.6 ± 3.6	74 ± 2	42.3 ± 1.7	30.5 ± 2.6	10.2 ± 1.36
	(1501-1542)	(9.0-9.65)	(227-287)	(765-821)	(54.8-63.2)	(71-75)	(41.1-44.8)	(27.6-32.7)	(9.2-12.05)
C5	1790 ± 68	10.4 ± 0.50	269 ± 12	920 ± 16	62.2 ± 1.1	73 ± 3	46.3 ± 1.0	33.6 ± 1.0	10.4 ± 0.28
	(1725-1881)	(9.7-10.87)	(258-286)	(909-943)	(61.1-63.7)	(70-76)	(45.2-47.6)	(32.2-34.4)	(10.1-10.70)
C6	1713 ± 66	9.6 ± 0.46	278 ± 36	874 ± 31	61.4 ± 1.8	74 ± 2	43.8 ± 3.0	30.6 ± 0.8	10.1 ± 1.06
	(1623-1777)	(8.9-9.99)	(242-325)	(843-905)	(59.7-63.5)	(71-76)	(40.4-47.7)	(29.5-31.3)	(8.8-11.20)
F1	1708 ± 60	12.2 ± 0.59	314 ± 32	865 ± 31	77.2 ± 3.3	96 ± 6	64.1 ± 6.8	29.4 ± 1.0	8.7 ± 1.77
	(1654-1792)	(11.6-12.71)	(284-359)	(831-906)	(75.1-82.0)	(90-104)	(54.2-69.2)	(28.5-30.7)	(7.0-10.51)
F2	1817 ± 52	12.8 ± 0.29	282 ± 25	941 ± 31	75.0 ± 2.5	88 ± 2	51.9 ± 1.9	28.3 ± 1.3	7.9 ± 0.78
	(1729-1852)	(12.4-13.03)	(253-306)	(891-970)	(72.3-77.4)	(85-90)	(48.7-53.4)	(27.3-30.4)	(6.7-8.72)
F3	1793 ± 174	12.6 ± 0.47	261 ± 17	906 ± 85	72.3 ± 1.8	85 ± 4	52.2 ± 2.0	26.0 ± 2.2	8.9 ± 1.54
	(1508-1949)	(12.0-13.19)	(246-285)	(766-993)	(69.6-74.1)	(79-88)	(49.1-54.3)	(23.5-29.0)	(6.5-10.39)
F4	1817 ± 123	12.8 ± 0.22	275 ± 46	905 ± 68	71.9 ± 3.4	86 ± 4	52.9 ± 2.4	29.2 ± 1.9	8.9 ± 0.96
	(1688-2017)	(12.5-13.05)	(238-352)	(842-982)	(68.2-77.3)	(83-92)	(50.3-56.6)	(27.4-31.7)	(7.4-9.75)
F5	1816 ± 62	12.8 ± 0.41	306 ± 20	938 ± 34	75.2 ± 1.9	86 ± 1	51.9 ± 1.8	28.9 ± 1.0	7.9 ± 1.10
	(1759-1904)	(12.2-13.21)	(282-328)	(902-979)	(73.2-77.3)	(84-87)	(49.7-53.9)	(27.7-30.0)	(6.9-9.39)
F6	1853 ± 145	12.4 ± 0.29	278 ± 46	944 ± 90	70.5 ± 2.5	84 ± 2	51.2 ± 3.3	27.6 ± 2.5	9.5 ± 0.35
	(1654-1965)	(12.1-12.66)	(213-315)	(855-1066)	(68.1-73.7)	(82-86)	(49.0-56.0)	(25.3-30.9)	(9.0-9.86)
G1	1971 ± 102	12.2 ± 1.42	383 ± 12	1043 ± 73	83.9 ± 12.7	122 ± 4	56.9 ± 2.1	30.3 ± 2.7	15.4 ± 1.12
	(1895-2087)	(11.4-13.86)	(370-394)	(985-1125)	(69.5-93.6)	(117-125)	(54.6-58.4)	(27.3-32.2)	(14.6-16.21)
G2	1707 ± 78	11.2 ± 0.48	369 ± 29	931 ± 40	91.8 ± 3.4	107 ± 5	59.0 ± 3.8	29.4 ± 1.4	11.6 ± 0.97
	(1616-1793)	(10.8-11.90)	(341-408)	(876-969)	(87.2-95.6)	(101-113)	(55.1-63.1)	(28.0-30.7)	(10.3-12.58)
G3	1774 ± 40	10.6 ± 0.33	281 ± 9	933 ± 38	88.2 ± 2.4	105 ± 2	55.5 ± 0.8	29.6 ± 1.5	13.3 ± 1.15
	(1721-1808)	(10.1-10.89)	(270-294)	(895-991)	(86.1-91.7)	(103-108)	(54.5-56.6)	(27.9-31.5)	(12.5-15.36)
G4	1949 ± 106	11.2 ± 0.54	341 ± 16	992 ± 46	93.3 ± 1.8	108 ± 2	54.4 ± 0.7	27.8 ± 1.3	12.8 ± 0.38
	(1866-2120)	(10.3-11.76)	(326-365)	(940-1056)	(91.6-96.1)	(106-110)	(53.2-54.8)	(25.9-29.1)	(12.3-13.31)
G5	1867 ± 101	11.2 ± 0.35	325 ± 41	1003 ± 62	89.3 ± 1.6	103 ± 3	55.6 ± 3.0	26.3 ± 0.6	11.6 ± 0.88
	(1778-1983)	(10.7-11.54)	(256-362)	(912-1060)	(86.5-90.8)	(99-107)	(52.5-59.2)	(25.8-27.3)	(10.7-12.79)
G6	1965 ± 102	11.1 ± 0.32	347 ± 23	1063 ± 59	90.4 ± 1.6	107 ± 1	53.5 ± 1.5	27.9 ± 1.6	10.4 ± 1.02
	(1818-2070)	(10.7-11.48)	(321-374)	(963-1110)	(88.5-92.3)	(105-108)	(51.5-55.4)	(25.9-29.4)	(9.3-11.68)
G7	2020 ± 158	11.2 ± 0.59	346 ± 36	1082 ± 80	90.3 ± 3.2	110 ± 3	59.1 ± 1.2	29.4 ± 2.0	11.2 ± 0.94
	(1849-2278)	(10.5-12.09)	(296-377)	(991-1190)	(87.9-95.7)	(107-113)	(57.7-60.9)	(27.7-32.8)	(9.5-11.77)
G8	1764 ± 40	11.2 ± 0.16	313 ± 60	962 ± 22	91.5 ± 2.6	109 ± 3	59.9 ± 2.4	29.3 ± 1.9	13.3 ± 1.83
	(1708-1801)	(11.0-11.40)	(260-397)	(940-982)	(88.0-94.0)	(106-112)	(56.6-62.1)	(27.1-30.9)	(11.8-15.60)
L1	1562 ± 105	8.1 ± 0.33	293 ± 22	823 ± 56	65.1 ± 3.2	79 ± 2	42.9 ± 2.8	25.3 ± 1.6	7.5 ± 0.48
	(1382-1648)	(7.6-8.47)	(254-308)	(747-878)	(61.8-69.8)	(77-82)	(39.2-46.0)	(22.7-27.0)	(6.9-8.06)
L2	1572 ± 128	8.4 ± 0.52	284 ± 24	817 ± 80	69.8 ± 1.9	77 ± 2	43.4 ± 0.9	26.4 ± 0.9	8.4 ± 0.99
	(1420-1702)	(7.7-8.84)	(262-315)	(724-899)	(67.1-72.1)	(75-79)	(42.3-44.4)	(25.2-27.4)	(7.1-9.72)
L3	1536 ± 55	8.2 ± 0.23	253 ± 22	812 ± 31	69.3 ± 1.6	78 ± 2	43.1 ± 1.4	26.9 ± 1.7	8.4 ± 0.53
	(1480-1627)	(7.8-8.35)	(224-284)	(779-856)	(67.4-71.9)	(75-80)	(42.2-45.4)	(24.2-28.3)	(7.6-8.91)
L4	1596 ± 48	8.6 ± 0.35	263 ± 16	852 ± 40	63.5 ± 5.4	75 ± 3	42.8 ± 1.2	24.4 ± 1.6	8.6 ± 0.63
	(1553-1651)	(8.3-9.12)	(249-282)	(806-903)	(57.5-68.6)	(72-79)	(41.2-44.0)	(22.6-26.3)	(7.8-9.31)
L5	1670 ± 99	8.5 ± 0.34	294 ± 27	880 ± 37	68.8 ± 2.1	79 ± 3	44.5 ± 1.8	25.2 ± 1.4	7.9 ± 1.13
	(1517-1765)	(8.1-8.75)	(262-330)	(823-915)	(65.8-71.0)	(76-83)	(42.0-46.5)	(23.7-26.5)	(7.1-9.86)
T1	1672 ± 37	12.2 ± 0.73	277 ± 25	858 ± 30	69.4 ± 4.0	86 ± 4	50.4 ± 3.1	27.2 ± 1.0	7.8 ± 0.70
	(1600-1716)	(11.4-13.18)	(237-313)	(811-898)	(61.1-73.4)	(80-92)	(48.2-57.7)	(26.0-28.6)	(6.5-8.70)

All measurements are in μm and in the form: mean ± standard deviation (range). Means for which replicate data are no longer available are denoted by an asterisk (∗).

Table 2. *(Continued).*

Pop	Body diam.					Ratios				
	at guide ring	at pharynx base	at vulva	at anus	at beginning of J	a	b	c	c′	V
C1	23.4 ± 1.22	29.7 ± 1.46	31.1 ± 1.31	17.5 ± 0.71	8.1 ± 0.63	53.4 ± 1.6	5.6 ± 0.53	54.2 ± 3.2	1.8 ± 0.04	0.52 ± 0.01
	(21.2-25.1)	(27.6-32.1)	(29.5-33.6)	(16.6-18.3)	(7.3-9.0)	(50.3-55.0)	(5.0-6.34)	(50.4-58.5)	(1.7-1.80)	(0.50-0.53)
C2	22.5 ± 1.75	29.0 ± 1.74	33.4 ± 2.29	19.5 ± 1.79	8.3 ± 0.79	51.0 ± 2.5	5.5 ± 0.73	55.6 ± 4.3	1.6 ± 0.06	0.53 ± 0.01
	(19.4-23.7)	(26.7-31.2)	(30.2-35.7)	(17.4-21.8)	(7.5-9.3)	(48.2-54.9)	(4.7-6.55)	(50.6-60.2)	(1.5-1.64)	(0.52-0.54)
C3	24.4 ± 0.87	28.5 ± 0.30	33.1 ± 1.38	18.1 ± 0.83	8.2 ± 0.77	45.4 ± 1.3	6.6 ± 0.53	51.8 ± 3.5	1.6 ± 0.08	0.53 ± 0.02
	(23.5-25.4)	(28.0-28.8)	(31.4-34.6)	(16.9-18.8)	(*)	(43.7-46.8)	(5.8-7.0)	(47.9-56.4)	(1.5-1.7)	(0.49-0.56)
C4	23.1 ± 1.80	29.7 ± 1.78	32.5 ± 0.56	18.9 ± 0.80	8.6 ± 0.96	46.8 ± 0.9	5.9 ± 0.61	50.0 ± 4.4	1.6 ± 0.11	0.52 ± 0.01
	(20.9-25.2)	(27.3-31.6)	(32.0-33.3)	(18.4-20.1)	(7.6-9.4)	(46.2-48.2)	(5.2-6.61)	(45.9-55.7)	(1.5-1.75)	(0.51-0.54)
C5	24.9 ± 1.26	31.3 ± 1.74	35.8 ± 1.00	20.5 ± 0.50	8.0 ± 0.68	49.9 ± 1.0	6.6 ± 0.23	53.3 ± 2.2	1.6 ± 0.04	0.51 ± 0.01
	(23.2-26.0)	(29.5-33.2)	(35.0-36.7)	(19.9-21.0)	(7.5-9.0)	(49.0-51.3)	(6.4-6.97)	(50.2-55.1)	(1.6-1.70)	(0.50-0.53)
C6	23.8 ± 0.89	29.1 ± 0.71	33.5 ± 1.73	19.8 ± 0.70	8.2 ± 0.85	51.2 ± 2.9	6.2 ± 0.90	56.0 ± 1.6	1.5 ± 0.07	0.51 ± 0.01
	(23.0-25.0)	(28.4-30.1)	(32.3-36.1)	(19.0-20.6)	(7.1-9.0)	(48.3-54.3)	(5.3-7.20)	(54.6-58.2)	(1.5-1.60)	(0.49-0.52)
F1	30.3 ± 1.48	35.8 ± 1.62	38.2 ± 2.60	23.7 ± 1.51	10.6 ± 1.44	44.8 ± 1.8	5.5 ± 0.42	58.1 ± 0.7	1.2 ± 0.04	0.51 ± 0.00
	(28.9-31.9)	(34.1-37.8)	(34.9-41.2)	(22.6-25.9)	(9.2-12.2)	(43.4-47.4)	(5.0-5.99)	(57.3-59.0)	(1.2-1.26)	(0.50-0.51)
F2	31.3 ± 1.60	37.1 ± 0.85	40.9 ± 1.12	25.9 ± 2.02	11.0 ± 0.85	44.4 ± 1.0	6.5 ± 0.65	64.3 ± 2.7	1.1 ± 0.08	0.52 ± 0.00
	(29.7-33.6)	(36.0-38.3)	(39.2-42.1)	(24.4-28.3)	(10.0-12.0)	(43.0-45.8)	(5.7-7.16)	(60.9-67.5)	(1.0-1.18)	(0.52-0.52)
F3	30.2 ± 1.47	35.3 ± 2.00	38.1 ± 2.77	22.7 ± 1.58	10.9 ± 0.70	47.1 ± 3.5	6.9 ± 1.00	68.9 ± 3.6	1.1 ± 0.07	0.51 ± 0.02
	(28.0-31.9)	(32.1-37.5)	(33.7-40.9)	(20.0-23.8)	(9.9-11.5)	(44.3-51.1)	(5.5-7.93)	(64.3-72.5)	(1.1-1.22)	(0.48-0.52)
F4	30.0 ± 0.65	33.0 ± 0.65	36.9 ± 0.84	22.4 ± 1.31	9.1 ± 0.37	49.3 ± 3.7	6.8 ± 1.23	62.3 ± 4.4	1.3 ± 0.11	0.50 ± 0.02
	(29.3-30.8)	(32.0-33.7)	(36.1-38.2)	(20.5-23.7)	(8.7-9.7)	(46.7-55.6)	(5.2-8.48)	(54.6-65.3)	(1.2-1.41)	(0.48-0.54)
F5	30.5 ± 0.72	36.3 ± 1.70	40.0 ± 1.13	25.4 ± 0.44	10.3 ± 0.84	45.5 ± 1.9	6.0 ± 0.44	63.0 ± 3.3	1.1 ± 0.05	0.52 ± 0.01
	(29.6-31.4)	(34.0-38.1)	(38.6-41.3)	(24.8-25.9)	(9.3-11.3)	(43.6-48.1)	(5.4-6.39)	(60.0-66.5)	(1.1-1.18)	(0.51-0.53)
F6	29.0 ± 1.28	33.9 ± 1.66	36.5 ± 1.56	22.9 ± 1.35	9.3 ± 0.36	50.8 ± 2.6	6.9 ± 1.60	67.6 ± 7.9	1.2 ± 0.07	0.51 ± 0.03
	(27.9-30.6)	(32.4-36.3)	(34.7-38.5)	(21.3-24.2)	(9.0-9.7)	(47.7-54.1)	(5.8-9.22)	(59.6-77.7)	(1.1-1.29)	(0.48-0.54)
G1	37.9 ± 2.35	45.3 ± 2.68	49.8 ± 5.59	25.6 ± 1.20	15.4 ± 1.03	39.8 ± 2.5	5.2 ± 0.42	65.6 ± 9.5	1.2 ± 0.07	0.53 ± 0.01
	(36.5-40.7)	(42.2-47.1)	(44.7-55.8)	(24.6-26.9)	(14.8-16.6)	(37.4-42.4)	(4.9-5.63)	(60.0-76.6)	(1.1-1.25)	(0.52-0.54)
G2	30.6 ± 1.52	35.4 ± 3.42	36.9 ± 2.11	22.5 ± 1.26	8.7 ± 0.54	46.3 ± 1.5	4.7 ± 0.48	58.1 ± 1.3	1.3 ± 0.02	0.55 ± 0.01
	(28.6-31.8)	(31.7-38.7)	(34.8-39.5)	(21.1-23.8)	(8.2-9.3)	(44.2-47.6)	(4.1-5.26)	(56.7-59.8)	(1.3-1.32)	(0.53-0.56)
G3	29.0 ± 0.57	32.6 ± 2.22	35.9 ± 1.70	21.4 ± 0.84	11.4 ± 0.57	49.4 ± 1.9	6.3 ± 0.28	60.0 ± 3.7	1.4 ± 0.05	0.53 ± 0.02
	(28.3-29.8)	(29.1-34.7)	(33.7-38.3)	(20.5-22.6)	(10.7-12.2)	(47.2-51.6)	(6.1-6.70)	(55.3-64.2)	(1.3-1.43)	(0.51-0.55)
G4	26.9 ± 1.32	31.3 ± 1.92	35.6 ± 1.66	18.3 ± 1.01	8.9 ± 0.38	54.8 ± 2.9	5.7 ± 0.39	70.3 ± 5.3	1.5 ± 0.15	0.51 ± 0.01
	(25.2-28.8)	(29.0-34.3)	(34.3-38.4)	(16.5-19.1)	(8.5-9.5)	(51.6-59.6)	(5.4-6.38)	(64.6-76.2)	(1.4-1.76)	(0.50-0.53)
G5	29.5 ± 1.62	34.5 ± 1.09	37.1 ± 1.96	20.3 ± 1.33	9.8 ± 0.92	50.3 ± 1.9	5.8 ± 1.04	70.9 ± 3.1	1.3 ± 0.07	0.54 ± 0.02
	(26.8-31.2)	(33.7-36.4)	(35.5-40.3)	(18.5-22.1)	(8.7-10.9)	(48.5-53.2)	(5.2-7.70)	(68.8-75.8)	(1.2-1.40)	(0.51-0.56)
G6	30.2 ± 1.07	36.4 ± 2.15	42.4 ± 3.05	22.5 ± 2.39	8.8 ± 0.36	46.4 ± 1.6	5.7 ± 0.60	70.6 ± 6.1	1.2 ± 0.07	0.54 ± 0.01
	(29.2-31.8)	(33.7-39.5)	(37.7-45.8)	(20.0-24.9)	(8.5-9.3)	(44.3-48.2)	(4.9-6.23)	(63.0-78.0)	(1.2-1.32)	(0.53-0.55)
G7	29.8 ± 1.77	39.9 ± 12.02	38.8 ± 3.18	21.0 ± 1.53	9.3 ± 0.90	52.1 ± 2.9	5.9 ± 0.51	68.6 ± 2.0	1.4 ± 0.07	0.54 ± 0.02
	(27.7-31.9)	(31.5-60.9)	(35.0-42.0)	(18.7-22.9)	(8.0-10.4)	(47.7-54.9)	(5.2-6.27)	(66.1-71.4)	(1.3-1.49)	(0.52-0.57)
G8	30.8 ± 1.41	39.1 ± 2.81	43.8 ± 1.50	21.7 ± 0.73	10.0 ± 1.16	40.3 ± 1.5	5.8 ± 0.97	60.4 ± 2.8	1.3 ± 0.06	0.55 ± 0.02
	(28.7-31.9)	(35.9-42.3)	(41.8-45.4)	(21.1-22.8)	(8.7-11.2)	(38.6-42.2)	(4.5-6.80)	(57.6-63.0)	(1.3-1.42)	(0.52-0.56)
L1	21.3 ± 2.33	27.0 ± 1.25	30.1 ± 1.40	16.4 ± 0.93	7.3 ± 0.29	51.8 ± 1.4	5.3 ± 0.17	61.9 ± 6.2	1.5 ± 0.12	0.53 ± 0.03
	(17.6-23.0)	(25.0-28.4)	(27.8-31.4)	(15.4-17.5)	(6.9-7.6)	(49.8-53.5)	(5.1-5.54)	(54.7-71.2)	(1.4-1.66)	(0.47-0.55)
L2	22.8 ± 0.73	26.6 ± 0.81	29.8 ± 1.61	17.2 ± 0.95	7.1 ± 0.82	52.7 ± 3.7	5.6 ± 0.63	59.6 ± 5.4	1.5 ± 0.09	0.52 ± 0.01
	(22.2-24.0)	(25.2-27.3)	(28.4-32.3)	(16.2-18.5)	(6.2-7.9)	(50.1-59.1)	(4.5-6.09)	(53.6-64.8)	(1.4-1.67)	(0.51-0.53)
L3	22.1 ± 0.89	25.2 ± 0.89	29.5 ± 1.95	16.1 ± 0.48	7.1 ± 0.58	52.2 ± 2.8	6.1 ± 0.35	57.2 ± 2.8	1.7 ± 0.10	0.53 ± 0.02
	(21.6-23.7)	(24.1-26.6)	(27.9-32.5)	(15.7-16.9)	(6.6-8.0)	(48.6-55.2)	(5.7-6.59)	(53.9-61.2)	(1.5-1.77)	(0.52-0.56)
L4	22.9 ± 0.94	27.8 ± 0.58	31.7 ± 1.15	17.7 ± 0.99	8.1 ± 0.55	50.3 ± 2.6	6.1 ± 0.33	65.6 ± 2.5	1.4 ± 0.11	0.53 ± 0.02
	(21.6-23.9)	(27.3-28.6)	(30.5-33.1)	(16.6-18.7)	(7.6-8.7)	(48.2-54.1)	(5.8-6.46)	(62.7-68.9)	(1.3-1.53)	(0.52-0.55)
L5	22.1 ± 0.63	26.4 ± 0.88	32.3 ± 0.94	17.2 ± 0.72	7.6 ± 0.47	51.7 ± 2.7	5.7 ± 0.38	66.4 ± 5.5	1.5 ± 0.08	0.53 ± 0.01
	(21.6-23.2)	(25.0-27.2)	(31.1-33.6)	(16.3-18.3)	(7.0-8.2)	(48.7-55.3)	(5.2-6.23)	(57.4-72.2)	(1.4-1.57)	(0.51-0.54)
T1	28.8 ± 0.96	36.0 ± 1.94	38.8 ± 1.82	22.5 ± 1.12	9.7 ± 0.68	43.2 ± 1.8	6.1 ± 0.60	61.5 ± 2.7	1.2 ± 0.03	0.51 ± 0.01
	(27.8-30.1)	(33.2-39.2)	(35.6-41.0)	(21.1-24.4)	(8.7-10.6)	(40.2-46.7)	(5.1-7.12)	(57.6-65.9)	(1.2-1.25)	(0.49-0.53)

Table 3. *Polytomous key (Lamberti* et al., *2004) code values for 26 populations of five species in the* Xiphinema americanum*-group.*

Species and population	Odontostyle	V	c′	c	L	f	Lip offset	Tail shape	Tail
X. citricolum	**2/3**	**2**	**3**	**1**	**2**	**1**	**2**	**1**	**3/2**
C1	2	2	3	1	2				2
C2	2	2	3	1	2				2
C3	2	2	3	1	2				2
C4	2	2	3	1	2				2
C5	2	2	3	1	2				3
C6	2	2	3	1	2				2
Individual	**(100)**	**(88)**	**(100)**	**(96)**	**(100)**				**(100)**
X. georgianum	**5**	**2**	**2**	**2**	**2**	**1**	**2**	**1**	**1/2**
G1	6	2	2	2	2				2
G2	5	3	2	1	2				2
G3	5	2	2	2	2				2
G4	5	2	3	2	2				1
G5	5	2	2	2	2				2
G6	5	2	2	2	2				2
G7	5	2	2	2	3				2
G8	5	3	2	2	2				2
Individual	**(89)**	**(67)**	**(92)**	**(75)**	**(83)**				**(94)**
X. laevistriatum	**2/3**	**2**	**3**	**1**	**2**	**1**	**1**	**1**	**3/2**
L1	2	2	3	2	2				1
L2	2	2	3	1	2				1
L3	2	2	3	1	2				1
L4	2	2	2	2	2				1
L5	2	2	3	2	2				1
Individual	**(100)**	**(96)**	**(67)**	**(38)**	**(92)**				**(21)**
X. floridae	**3/4**	**2**	**2**	**2**	**2**	**1**	**2**	**1**	**2/3**
F1	4	2	2	1	2				2
F2	3	2	2	2	2				2
F3	3	2	2	2	2				1
F4	3	1	2	2	2				2
F5	3	2	2	2	2				2
F6	3	2	2	2	2				2
Individual	**(93)**	**(70)**	**(78)**	**(96)**	**(96)**				**(81)**
X. tarjanense	**3**	**2**	**3**	**1**	**1**	**1**	**2**	**1**	**3**
T1	3	2	2	2	2				2
Individual	**(75)**	**(75)**	**(63)**	**(100)**	**(0)**				**(50)**

Code values from Lamberti *et al.* (2004) are in bold adjacent to the species name. Population code values are from character means of 4-8 specimens. The percentages of individual specimens from all populations of each species with characters that conform to the appropriate code are given in bold parentheses.

μm (Fig. 2A). Of the remaining species, *X. floridae* and *X. tarjanense* were the only ones with an average lip diameter in all populations >11 μm (Fig. 2B). Tail length differed for the remaining two species, with all populations of *X. laevistriatum* <28 μm and those of *X. citricolum* >28 μm (Fig. 2C).

PHYLOGENETIC RELATIONSHIPS

Alignment of the D2D3 region resulted in homology statements for 792 characters, of which 553 were constant and 208 informative for parsimony analysis. Thirty-one other positions in the alignment were variable, but uninformative for parsimony analysis (*e.g.*, autapomorphic).

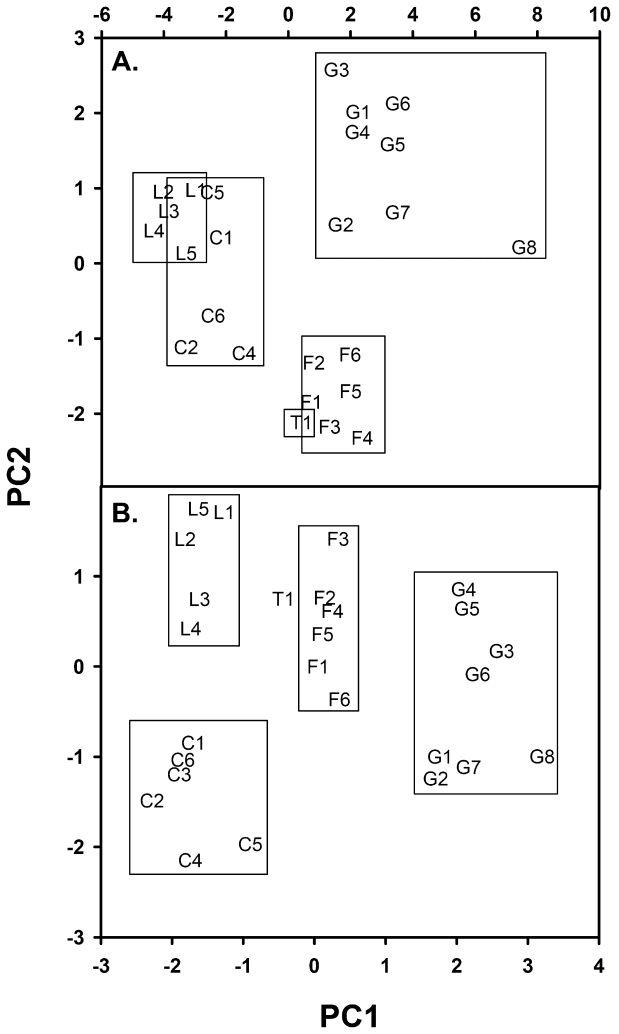

Fig. 1. *Morphological relationships between 26 populations of five species in the* Xiphinema americanum*-group derived from principal component analysis of A: 19 morphometric characters; B: Six arbitrary characters (lip diameter, length of odontostyle and tail, distance between the odontostyle guide ring and the lip anterior, and the de Man ratios c and J).*

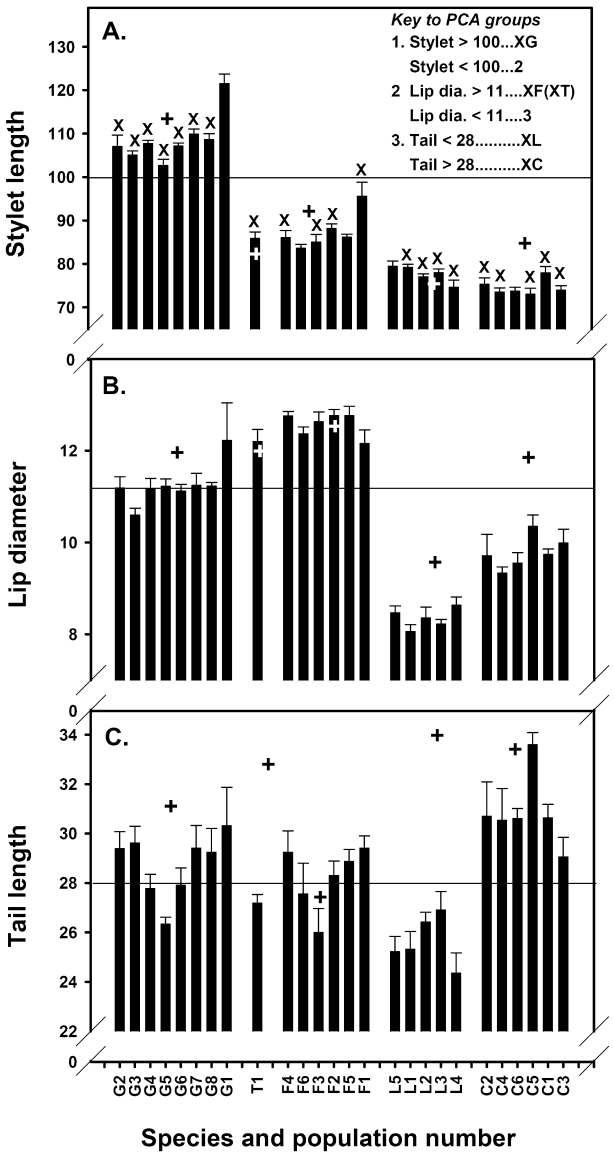

Fig. 2. *Means of three morphometric characters that distinguish the four groups from principal component analysis of populations of five species in the* Xiphinema americanum*-group. A: Mean stylet lengths of* X. georgianum *exceed those of the other populations; B: Mean lip diameters for* X. floridae *and* X. tarjanense *are greater than those of the other populations (except G1); C: Mean tail lengths of* X. laevistriatum *are less than those of* X. citricolum. *Standard errors of means are shown. Populations for which D2D3 DNA was sequenced are shown by 'X' above the bars in A. Character means in the original species descriptions are shown by '+' in each figure.*

When indels were treated as a fifth base, only 470 characters were constant. Forty-one remained uninformative under parsimony, but the number of parsimony informative characters increased to 281. The ITS alignment contained 1514 total characters; 1448 were constant, 56 were parsimony informative. When indels were considered a fifth base, the number of constant characters decreased to 1115 while the number of parsimony informative characters increased to 285. Chi square tests of homogeneity of base frequencies across taxa of both datasets re-

Table 4. *Discriminant analysis of 119 specimens from 26 populations of five species belonging to the* Xiphinema americanum*-group* (*XL*, X. laevistriatum; *XF*, X. floridae; *XG*, X. georgianum; *XC*, X. citricolum; *XT*, X. tarjanense).

	Species				
ID	XL	XF	XG	XC	XT
XL	24	1	0	2	0
XF	0	21	0	0	0
XG	0	1	36	0	0
XC	0	0	0	22	0
XT	0	4	0	0	8
N	24	27	36	24	8
N correct	24	21	36	22	8
Proportion correct	1.000	0.778	1.000	0.917	1.000

Specimens were assigned to groups based on the morphometric character values given in the polytomous key of Lamberti *et al*. (2004).

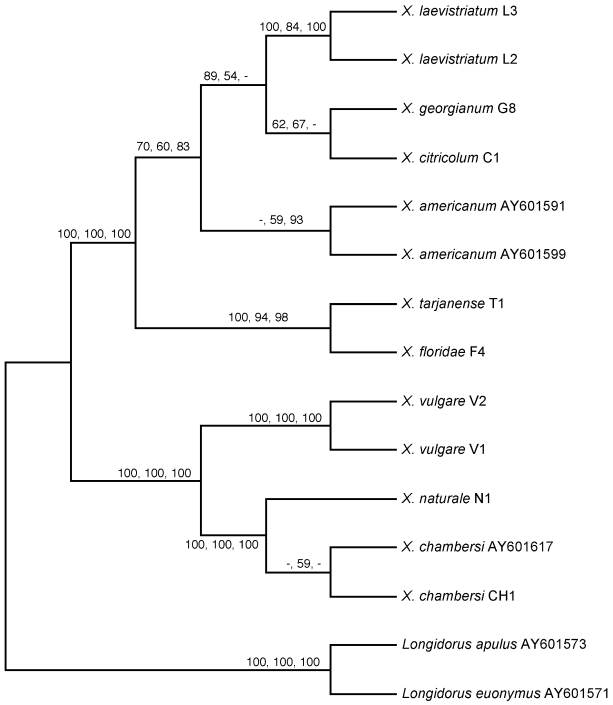

Fig. 3. *Phylogenetic relationships among 26 sampled populations of* Xiphinema *spp. based on D2D3 LSU rDNA sequences. Population identifier (see Table 1) follows species epithet. GenBank accession numbers indicate additional taxa included in the analysis. Values at nodes are bootstrap support indices for neighbour-joining (log determinant transformed distances), maximum likelihood (GTR + G) and maximum parsimony (indels treated as missing data) analyses, respectively. Topological differences between minimum evolution, maximum parsimony (with indels treated as missing characters or as a fifth base) and maximum likelihood approaches differed only at the nodes where bootstrap values are indicated as "-". Redundant (non-unique) sequences from the sampled populations were not included in the analysis.*

vealed no deviation from stationarity: D2D3 base frequencies chi-square = 28.786 (df = 42), $P = 0.94$; ITS Chi-square = 1.986 (df = 60), $P = 1.00$. The optimal models of sequence evolution based on the Akaike information criterion (Posada & Buckley, 2004) for the D2D3 and ITS datasets were the GTR + G (general time reversible, Tavaré, 1986, plus estimated rate heterogeneity among sites) and HKY + I models (Hasagawa *et al*., 1985, including invariable sites).

Phylogenetic relationships based on the D2D3 dataset are summarised in Figure 3. Maximum parsimony, with gaps treated as missing data, yielded a single tree (322 steps; CI = 0.919, RI = 0.947, RC = 0.870, HI = 0.081). With gaps treated as a fifth base, the number of synapomorphies increased, as did homoplasy, but the resulting tree topology was congruent with the MP solution that was based on indels treated as missing data (484 steps, CI = 0.899, RI = 0.948, RC = 0.852, HI = 0.101). The MP solutions differed from the ML and ME solutions in the position of *X. americanum* where it appears as sister taxon to *X. georgianum*. The ME tree depicts the former taxon as paraphyletic, with *X. americanum* AY601599 as sister taxon to *X. americanum* AY601591, and *X. georgianum* + *X. laevistriatum*. The ML solutions were congruent and inferred a monophyletic *X. chambersi*. This solution differs from the MP and ME solutions, which infer that *X. chambersi* AY601573 is more closely related to *X. naturale* N1 than to *X. chambersi* CH1. The ME and ML solutions were two steps longer than the MP solutions, but nonparametric (MP criterion, indels treated as missing data) and Shimodaira-Hasegawa tests of tree topology revealed no significant differences in tree topology. Although MP bootstrap support for a monophyletic *X. georgianum* was less than 50%, all solutions under their respective optimality criterion (ME, ML, MP) favoured this relationship.

The ME heuristic search of the ITS data yielded two solutions that differed only as to placement of the population of G1; either as sister taxon to G6, or as sister to G3. The ML searches produced two identical trees. The MP search yielded 3043 solutions (548 steps; CI = 0.953, RI = 0.903, RC = 0.86, HI = 0.047. When *X. chambersi* (AF51128) was removed from the

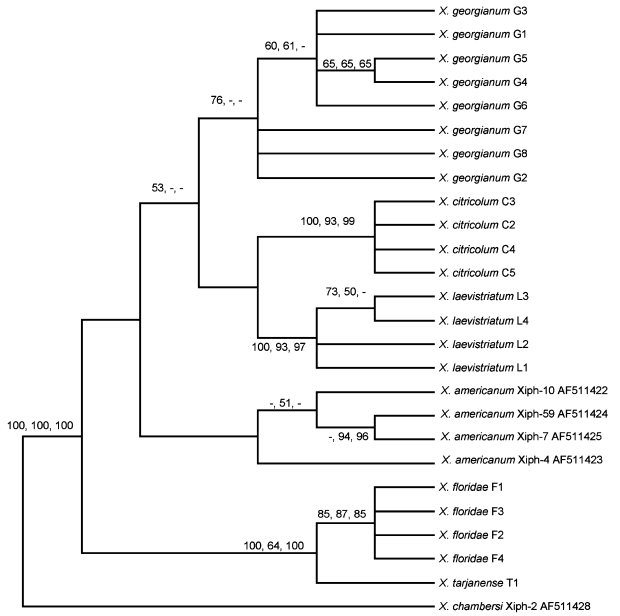

Fig. 4. *Phylogenetic relationships among 21 sampled populations of* Xiphinema *spp. based on ITS rDNA sequences. Population identifier (see Table 1) follows species epithet. GenBank accession numbers indicate additional taxa included in the analysis. Values on branches depict congruent nodes among ME, ML and MP bootstrap analyses where greater than 50%, respectively. Tree is rooted based on relationships established by the D2D3 LSU rDNA analysis (Fig. 3). All populations sampled produced unique ITS rDNA sequences; all were included in the analysis. See Table 1 for population number, sample locations, and associated plant species.*

tree, the length decreased to 109 (CI = 0.89, RI = 0.951, RC = 0.846, HI = 0.11). The MP tree constructed considering gaps as a fifth character state was longer and had a marginally higher incidence of homoplasy (*X. chambersi* removed from the tree; length = 1273, CI = 0.716, RI = 0.870, RC = 0.673, HI = 0.284). All trees were similar in that populations of nominal species were monophyletic (Fig. 4), with the exception of the MP analysis where gaps were included as an extra character state, which suggested that *X. tarjanense* formed a clade with *X. floridae* populations F4 (F2 + F3) to the exclusion of *X. floridae* population F1. All other analyses (ME, MP, ML) depict *X. tarjanense* as sister taxon to a monophyletic *X. floridae*. The *X. americanum* populations identified by Ye *et al*. (2004) comprise a monophyletic group, with slightly more intraspecific variation than those of the Florida species reported here.

Kishino-Hasegawa and Templeton tests, both under parsimony, failed to detect significant differences between any of the alternative topologies except for the topology of the MP tree that considered gaps as a fifth base, which was rejected by both tests ($P = 0.006$, $P = 0.007$, respectively). When gaps were not considered as a fifth base, all other topologies were considerably worse than the optimal solution ($P = 0.001$ for both tests, for all alternative hypotheses). Under the maximum likelihood optimality criterion, the Shimodaira-Hasegawa test failed to detect significant differences between any of the competing hypotheses, except for the MP solutions that considered indels as a fifth base (both significantly worse, $P = 0.044$).

Discussion

The inferred phylogenetic relationships among the 25 *X. americanum*-group populations in this study revealed five species that conformed with the relationships derived through PCA and discriminant analysis of morphometric characters and with identifications made from published keys and species descriptions. The use of keys to categorise the populations was the most subjective and presumably the least reliable of these methods, because the character means in our populations were occasionally outside of the reported ranges.

The single population of putative *X. tarjanense* was collected from its type locality, whereas molecular sequences from topotype material are needed to support our identification of the remaining species. Assuming that the species in this study are correctly identified, a comparison of our data with the most recent keys to the *X. americanum*-group indicates that the morphometric variation within these species is greater than previously recognised. Indeed, the many discrepancies between the morphometrics reported for *X. tarjanense* and those in this study suggest the need to examine variation among populations of the species even within its type locality. Among the remaining species, only *X. citricolum* accorded with the polytomous key code values given in Lamberti *et al*. (2004) for all populations. Code value discrepancies in five of the eight *X. georgianum* populations resulted from minor differences in reported and observed values; however, the code suites resulting from atypical code values for those populations corresponded to no other species in the *X. americanum*-group. Code discrepancies for populations of *X. laevistriatum* and *X. floridae* resulted in code suites typical for *X. microstilum* and for *X. franci* or *X. peruvianum*, respectively.

Loof and Luc (1990) and Luc and Baujard (2001) noted the need to re-evaluate the taxonomy of the *X. americanum*-group because of character discrepancies and character code suites that are common to more than a single species in the published keys (Lamberti, 1980; Lamberti *et al.*, 1991; Lamberti & Carone, 2000). This study underscores a need to further sample the intraspecific morphological variation of *X. americanum*-group species and illustrates the utility of molecular phylogenetics to aid in distinguishing character variation within and between these species (Duncan *et al*., 1999).

The D2D3 dataset produced a robust topology strongly supporting the monophyly of nominal species, especially considering the numerous identical sequences from multiple populations. Areas of conflict in the ITS data exclusively involved subspecific lineages. This conflict is predicted by the fact that their inferred relationships may still be tokogenetic, as opposed to phylogenetic (Adams, 1998, 2001), mirroring other studies that have used this marker for phylogenetic analysis (*i.e*., Nguyen *et al*., 2001). Both markers performed well at identifying lineages that correspond to morphologically defined species. Comparison of the monophyletic lineages (as recovered by phylogenetic analysis) to the identified clusters of the PCA analyses reveals correspondence between molecular and morphological divergence, with few notable exceptions. For example, PCA analyses of populations of *X. floridae* and *X. tarjanense* fail to clearly discriminate between the two morphologically similar species, which differ by a single base in the D2D3 region, and from six to nine bases in the ITS region. On the other hand, populations of *X. georgianum* had the greatest amount of intraspecific morphological variation (Fig. 1A) but had relatively low intraspecific molecular variation (0 D2D3 positions, 0-3 ITS nucleotides). Each of the nominal species is delimited by numerous autapomorphies. However, some intraspecific lineages have evolved autapomorphies where corresponding autapomorphies on sister lineages exist only for the nominal taxa, suggesting that species described from morphological characters would be treated similarly based on analysis of molecular characters. This also suggests the utility of using molecular markers in the delimitation of species. For example, employing a phylogenetic species concept based solely on the diagnosis of lineages (*i.e*., Wheeler, 1999) the L2 and L3 populations of *X. laevistriatum* would be considered different species as they are diagnostically different, (L3 has undergone a transversion at position 621 in the D2D3 alignment). However, morphologically these two populations are more similar to each other than to other populations of the same species (Fig. 1A). Thus, the requirement that sister taxa evolve autapomorphies in recognition of lineage independence works to minimise the confusion of tokogeny with phylogeny. Although morphological characters were not polarised for use in phylogeny reconstruction due to limitations surrounding their properties (ratios are combinations of characters and do not evolve independently; there is not yet a robust method for scoring correlated, continuously varying characters for phylogenetic analysis), comparison with morphological discrimination within a molecular phylogenetic framework reveals robust correspondence between morphology and molecules at a critical juncture – the fundamental unit of biodiversity – the species.

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