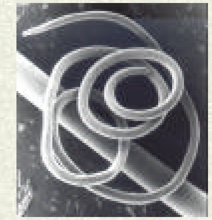
Phylogenetic analysis of *Longidorus* and *Xiphinema* species (Nematoda: Longidoridae) using ITS1 sequences of nuclear ribosomal DNA



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Abstract

Members of Longidoridae in the genera *Longidorus* and *Xiphinema* are important plant parasites as well as vectors for plant nepoviruses. Species discrimination based entirely on morphology is often questionable and controversial because of the morphometric overlap and the lack of a phylogenetic framework for this group. Phylogenetic analysis using the DNA sequencing of nuclear ribosomal DNA ITS1 was conducted to construct an evolutionary tree. Extensive interspecies sequence variation and minor intraspecies sequence variation were observed. The aligned sequences of the ITS1 region including the 3' end of the 18S rDNA gene, 5' end of the 5.8S rDNA gene from each species ranged from 870 bp to 1354 bp for all longidorids. This fragment is highly variable, only 15.9% sequence was constant within the longidorids examined. The analyses suggest two groups within Arkansas Longidoridae species: (1). *Xiphinema* group including *X americanum* Cobb, 1913, *X. bakeri* Williams, 1961, *X chambersi* Thorne, 1939, and *Longidorus diadecturus* Eveleigh & Allen, 1982. *L. diadecturus* shares some morphological characters of both *Xiphinema* and *Longidorus*. (2). *Longidorus* group including *L. breviannulatus* Norton & Hoffman, 1975, *L. crassus* Thorne, 1974, *L. fragilis* Thorne, 1974, 5undescribed species, and one non-Arkansas species *L. elongatus* (De Man, 1876) Thorne & Swanger, 1936 from Scotland. The phylogenetic analysis largely corresponded to the genera and species defined by morphology. Results demonstrated the potential for the use of rDNA ITS1 to infer phylogenetic relationships and PCR-RFLP based indentification among longidororids.

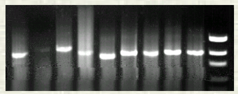
Materials

Table 1. *Longidorus* and *Xiphinema* populations and species used in ITS1 DNA sequencing

Species	Population number	Host	Locality
L. biformis n. sp.	Long-4	Elm	White River, Middle Fork, Elkins, Washington County, Arkansas
L. biformis n. sp.	Long-105	Hackberry	Crooked Creek, Yellville, Marion County, Arkansas
L. biformis n. sp.	Long-149	Osage orange	Osage Creek, Highway 412, Carrol County, Arkansas
L. biformis n. sp.	Long-264	Hickory	Frog Bayou, Highway 162, south of Alma, Crawford County, Arkansas
L. breviannulatus	Long-124	Willow	Haroldton Access, Arkansas River, Van Buren, Crawford County, Arkansas
L. crassus	Long-115	Oak	County Road 62 Bridge, Illinois River, Washington County, Arkansas
L. diadecturus	Long-87	Box elder	War Eagle Mill, near Rogers, Benton County, Arkansas
L. diadecturus	Long-146	Red bud	Crooked Creek, Yellville, Marion County, Arkansas
L. elongatus	Long-198	Greenhouse culture	Scottish Crop Research Institute, Scotland, UK
L. fragilis	Long-224	Willow	Haroldton Access, Arkansas River, Van Buren, Crawford County, Arkansas
L. grandis	Long-148	Osage orange	Osage Creek, Highway 412, Carrol County, Arkansas
L. grandis	Long-201	River cane	Big Piney Creek Access Area, Highway 164, Pope County, Arkansas
L. paralongicaudatus n. sp.	Long-143	Elm	Frog Bayou, highway 162, south of Alma, Crawford County, Arkansas
X. americanum	Xiph-4	Crabapple	University of Arkansas Farm, Fayetteville, Washington County, Arkansas
X. americanum	Xiph-7	Grape	San Diego County, California, USA
X. americanum	Xiph-10	Unknown	Australia
X. americanum	Xiph-59	Elm	County Road 62 Bridge, Illinois River, Washington County, Arkansas

Results

Fig. 1. PCR of ribosomal DNA ITS1. 1.*Longidorus fragilis*; 2. *L. crassus*; 3. *L. breviannulatus*; 4. *L. biformis*; 5. *Xiphinema bakeri*; 6. *L. grandis*; 7. *L. fragilis*; 8. *L.* sp. (L-202); 9. *L. diadecturus*; 10. Low mass ladder



Low mass ladder
2kb,25ng/ ul
1.2kb,15ng/ul
0.8kb,10ng/ul
0.4kb,5 ng/ul

Fig. 2. Blue-white screening on ITS1 clones hosted in competent *E.coli* JIM109 on selective and differential agar (LB/Ampicillin/X-gal) plate to identify recombinant plasmid.

Table 2. Statistics of nucleotides of ITS1 DNA sequences compared among species of *Longidorus* and *Xiphinema*.

Species and population No.		Size	A	C	G	T	A+T	C+G
L. biformis	Total	1244	298	370	253	323	668	576
Long-4	Percentage		24.00%	29.70%	20.30%	26.00%	53.70%	46.30%
L. biformis	Total	1220	310	357	232	321	667	553
Long-105	Percentage		25.40%	29.30%	19.00%	26.30%	54.70%	45.30%
L. biformis	Total	1220	310	357	232	321	667	553
Long-149	Percentage		25.40%	29.30%	19.00%	26.30%	54.70%	45.30%
L. biformis	Total	1220	291	360	249	320	651	569
Long-264	Percentage		23.90%	29.50%	20.40%	26.20%	53.40%	46.60%
L. breviannulatus	Total	1136	294	313	230	299	607	529
Long-124	Percentage		25.90%	27.60%	20.20%	26.30%	53.40%	46.60%
L. crassus	Total	1229	316	345	253	315	661	568
Long-115	Percentage		25.70%	28.10%	20.60%	25.60%	53.80%	46.20%
L. diadecturus	Total	1193	321	291	250	331	612	581
Long-87	Percentage		26.90%	24.40%	21.00%	27.70%	51.30%	48.70%
L .diadecturus	Total	1188	319	292	250	327	611	577
Long-146	Percentage		26.90%	24.60%	21.00%	27.50%	51.40%	48.60%
L. elongatus	Total	1020	221	278	223	298	499	521
Long-198	Percentage		21.70%	27.30%	21.90%	29.20%	48.90%	51.10%
L. fragilis	Total	1096	291	297	229	279	588	508
(Long-224)	Percentage		26.60%	27.10%	20.90%	25.50%	53.60%	46.40%
L. grandis	Total	1239	308	350	261	320	658	581
Long-148	Percentage		24.90%	28.20%	21.10%	25.80%	53.10%	46.90%
L. grandis	Total	1239	308	350	261	320	658	581
Long-201	Percentage		24.90%	28.20%	21.10%	25.80%	53.10%	46.90%
X. americanum	Total	880	226	226	191	237	452	428
Xiph-4	Percentage		25.70%	25.70%	21.70%	26.90%	51.40%	48.60%
X. americanum	Total	877	217	229	194	237	446	431
Xiph-7	Percentage		24.70%	26.10%	22.10%	27.00%	50.90%	49.10%
X. americanum	Total	870	224	226	188	232	450	420
Xiph-10	Percentage		25.70%	26.00%	21.60%	26.70%	51.70%	48.30%
X. americanum	Total	878	217	231	195	235	448	430
Xiph-59	Percentage		24.70%	26.30%	22.20%	26.80%	51.00%	49.00%
X. bakeri	Total	1185	295	317	263	310	612	573
Xiph-23	Percentage		24.90%	26.80%	22.20%	26.20%	51.60%	48.40%
X. bakeri	Total	1185	295	317	263	310	612	573
Xiph-47	Percentage		24.90%	26.80%	22.20%	26.20%	51.60%	48.40%
X. chambersi	Total	1354	339	305	309	401	644	710
Xiph-2	Percentage		25.00%	22.50%	22.80%	29.60%	47.60%	52.40%
Average			25.15%	27.03%	21.12%	26.72%	52.15%	47.85%

Fig. 3. Best maximum likelihood tree for ITS1 of *Longidorus* and *Xiphinema*

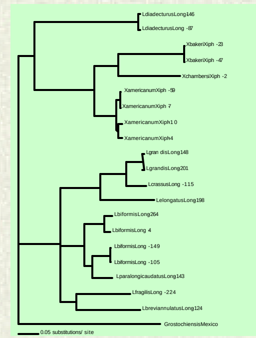


Fig. 4. Consensus tree from the maximum parsimony bootstrap analysis for ITS1 of *Longidorus* and *Xiphinema*. The percentages of bootstrap relicates supporting the clades are indicated at the branch points.

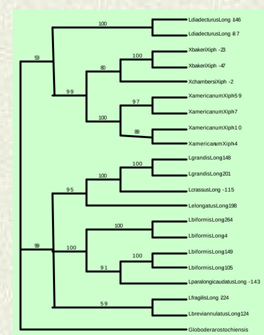
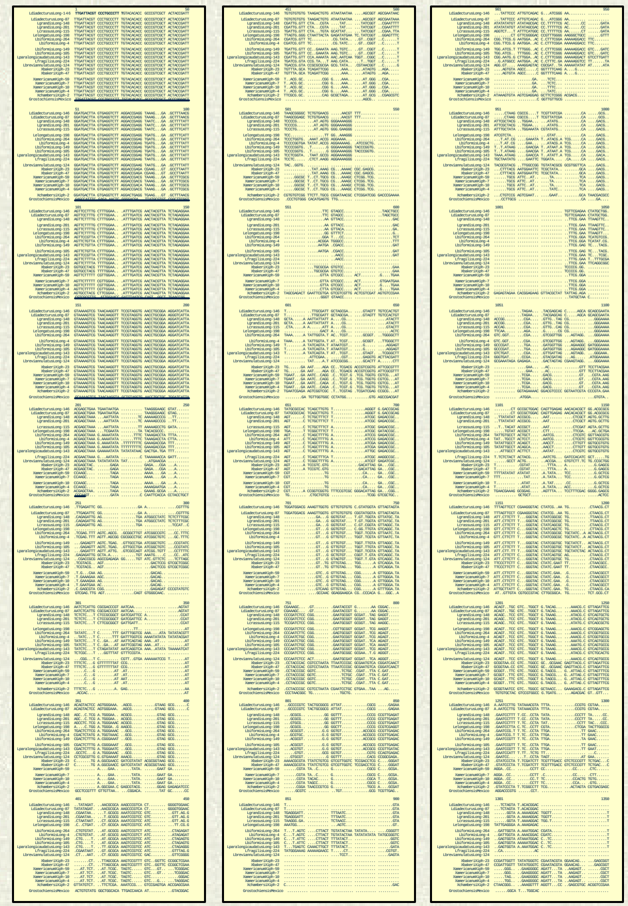


Table 3. Pairwise dissimilarity values in ITS1rDNA nucleotide sequence among species of *Longidorus* and *Xiphinema*.

No.	Species	1	2	3	4	5	6	7	8	9
1	*L.diadecturus* Long-146	-								
2	*L.diadecturus* Long-87	0.0076	-							
3	*X. bakeri* Xiph-23	0.3714	0.3729	-						
4	*X. bakeri* Xiph-47	0.3714	0.3729	0.0000	-					
5	*X. americanum* Xiph-59	0.3007	0.3021	0.1978	0.1978	-				
6	*X. americanum* Xiph-7	0.2950	0.2964	0.1982	0.1982	0.0023	-			
7	*X. americanum* Australia	0.2951	0.2965	0.2025	0.2025	0.0185	0.0163	-		
8	*X. americanum* Xiph-4	0.2983	0.2996	0.2056	0.2056	0.0185	0.0174	0.0184	-	
9	*X. chambersi* Xiph-2	0.3913	0.3944	0.2454	0.2454	0.1754	0.1722	0.1807	0.1788	-
10	*L.africanus* Long-162	0.3062	0.3086	0.3694	0.3694	0.2656	0.2652	0.2642	0.2715	0.3735
11	*L.fragilis* Long-224	0.3063	0.3086	0.3672	0.3672	0.2631	0.2626	0.2616	0.2689	0.3724
12	*L.grandis* Long-148	0.3071	0.3106	0.3777	0.3777	0.2793	0.2749	0.2732	0.2808	0.3823
13	*L.grandis* Long-201	0.3071	0.3106	0.3777	0.3777	0.2793	0.2749	0.2732	0.2808	0.3823
14	*L.crassus* Long-115	0.2960	0.2965	0.3610	0.3610	0.2614	0.2569	0.2588	0.2616	0.3708
15	*L.elongatus* Long-198	0.3064	0.3094	0.3649	0.3649	0.2807	0.2756	0.2778	0.2764	0.3590
16	*L.ventroporus* Long-264	0.3170	0.3191	0.3866	0.3866	0.2814	0.2803	0.2831	0.2831	0.3822
17	*L.ventroporus* Long-4	0.3289	0.3299	0.3878	0.3878	0.2845	0.2834	0.2877	0.2862	0.3883
18	*L.ventroporus* Long-105	0.3210	0.3231	0.3827	0.3827	0.2905	0.2882	0.2892	0.2911	0.3932
19	*L. breviannulatus* Long-124	0.3152	0.3157	0.3822	0.3822	0.2694	0.2665	0.2622	0.2723	0.3755
20	*G. rostochiensis*	0.3983	0.4027	0.4416	0.4416	0.3537	0.3540	0.3532	0.3593	0.4408

No.	Species	10	11	12	13	14	18	19	20	21	22	23
10	*L.africanus* Long-162	-										
11	*L.fragilis* Long-224	0.0037	-									
12	*L.grandis* Long-148	0.2033	0.2032	-								
13	*L.grandis* Long-201	0.2033	0.2032	0.0000	-							
14	*L.crassus* Long-115	0.1862	0.1842	0.0886	0.0886	-						
15	*L.elongatus* Long-198	0.2332	0.2309	0.1922	0.1922	0.1846	-					
16	*L.ventroporus* Long-264	0.2396	0.2385	0.2200	0.2200	0.2175	0.2463	-				
17	*L.ventroporus* Long-4	0.2378	0.2368	0.2182	0.2182	0.2184	0.2537	0.0355	-			
18	*L.ventroporus* Long-105	0.2359	0.2348	0.2163	0.2163	0.2147	0.2477	0.1113	0.1040	-		
19	*L. breviannulatus* Long-124	0.2281	0.2279	0.2378	0.2378	0.2364	0.2464	0.1951	0.1943	0.1926	-	
20	*G. rostochiensis*	0.3773	0.3778	0.3440	0.3440	0.3403	0.3671	0.3537	0.3603	0.3670	0.3838	-

Fig. 5. Alignment of nucleotide bases of ITS1 rDNA nucleotide sequence for *Longidorus* and *Xiphinema* (primers in bold, 18S, 5.8S sequence underlined)



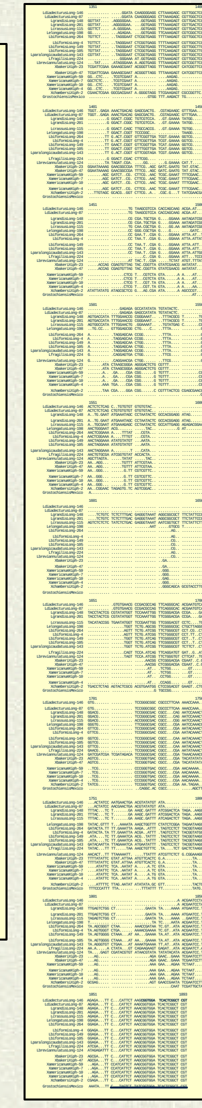


Fig. 6. Some *Longidorus* species from Arkansas