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Cluster Analysis of *Longidorus* Species (Nematoda: Longidoridae), a New Approach in Species Identification[1]

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Abstract: Hierarchical cluster analysis based on female morphometric character means including body length, distance from vulva opening to anterior end, head width, odontostyle length, esophagus length, body width, tail length, and tail width were used to examine the morphometric relationships and create dendrograms for (i) 62 populations belonging to 9 *Longidorus* species from Arkansas, (ii) 137 published *Longidorus* species, and (iii) 137 published *Longidorus* species plus 86 populations of 16 *Longidorus* species from Arkansas and various other locations by using JMP 4.02 software (SAS Institute, Cary, NC). Cluster analysis dendograms visually illustrated the grouping and morphometric relationships of the species and populations. It provided a computerized statistical approach to assist by helping to identify and distinguish species, by indicating morphometric relationships among species, and by assisting with new species diagnosis. The preliminary species identification can be accomplished by running cluster analysis for unknown species together with the data matrix of known published *Longidorus* species.
Key words: hierarchical cluster analysis, identification, *Longidorus,* morphometrics.

The first *Longidorus* species, *L. elongatus* de Man, was described in 1876. The genus now includes 139 nominal species. These nematodes are ectoparasites of many crops and are widely distributed throughout the world. Some are important vectors of nepoviruses, and their association is species specific (Taylor and Brown, 1997). Therefore, correct identification of *Longidorus* species is economically important. Currently, species discrimination in *Longidorus* is based primarily on morphometrics. A high degree of variability within morphometrics leads to considerable overlap among species and increases the potential for mis-identification. Some nematologists have expressed doubts on the necessity and nominality of some proposed species, in the absence of other data other than that based on morphometrics (McHenry, 1987; Thorne, 1961).

Lamberti (1975) published a dichotomous key to the species of *Longidorus* that is considered outdated and difficult to update to include new species descriptions. Rey et al. (1988) proposed a computer method for identifying *Longidorus* species; however, no subsequent paper has used their approach. Identifying species by means of a polytomous key permits a range of characters to be used simultaneously, which makes it more effective for identifying closely related species with overlapping features. Romanenko (1978) published such a polytomous key for identifying *Longidorus* species, and it was revised by Chen et al. (1997) and Loof and Chen (1999). More recently, computer software was developed to aid the species identification in *Longidorus* (Tiefenbrunner et al., 2002). Unfortunately, in practice, the usefulness of keys in the identification of *Longidorus* species is limited due to high intraspecific variability and minor interspecific differences that result in great overlap of morphometrics. Moreover, none of the above methods lead directly to species identification.

Taxonomists who wish to use numerical methods in the study of similarities and differences among organisms and for the construction of classifications now have a variety of methods of measuring similarity and analyzing matrices of similarity values. A number of statistical techniques have been employed including factor analysis, principal component analysis, and multiple regression analysis. But more recently the problem has been addressed with some success by cluster analysis techniques (Roca, 1996; Rubtsova et al., 1999). Such techniques are generally used for the grouping of objects or individuals under investigation. Ideal data for such analysis would yield clusters so obvious that they could be picked out, at least in small-scale cases, without the need for complicated mathematical techniques. A dendrogram generated by cluster analysis may simply represent a convenient method for organizing a large data set so that it can be more easily understood and information can be retrieved more efficiently. If the data can nominally be summarized by a small number of groups of objects, then group labels may provide a concise description of patterns of similarities and differences in the data. They may be used to search for natural groupings in the data, to simplify the description of a large set of data, and to generate hypotheses to be tested on future samples. The need to summarize data sets in this way is becoming increasingly important because of the growing number of nematode species being described.

Lamberti and Ciancio (1993, 1994) used principal components and hierarchical cluster analysis to separate 49 populations of the *Xiphinema americanum*-group into five groups but were not successful in simplifying species identification. Lamberti et al. (2002) used hierarchical cluster analysis for 117 populations representing 39 putative species in *Xiphinema americanum*-group into four groups. Cho and Robbins (1991) studied mor-

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phological variation among 23 *X. americanum*-group populations by canonical discriminant analysis and three groups were detected, but clear distinction between total populations within and between the groups could not be made because of overlap. Griesbach and Maggenti (1990) proposed *X. californicum* as a junior synonym of *X. americanum* Cobb, 1913 based on descriptive statistics and a stepwise discriminant analysis. Brown et al. (1997) examined the morphometric variability between populations of *L. vineacola* and morphologically related species by canonical analysis using five morphometric characters. The clusters formed were proven to be a reliable means for distinguishing members of the *L. vineacola* complex of morphologically similar species. In the above study, *L. apuloides* was regarded as a junior synonym of *L. vineacola.* Cluster analysis was also used in new species description to compare the new species with other closely related species, for example *L. apuloides* (Roca, 1996) and *L. artemisiae* (Rubtsova et al., 1999). Bravo and Roca (1998) used multivariate analysis including principal component analysis and hierachical cluster analysis to separate the four juvenile stages of *L. vinearum* and *L. belloi* from Portugal. However, none of the previous studies have attempted to examine the relationships of all the species in either *Longidorus* or *Xiphinema.*

The objectives of this study were to investigate the morphometric relationships by generating dendrograms for (i) the *Longidorus* species occurring in Arkansas, (ii) the 137 published *Longidorus* species, (iii) the published *Longidorus* species plus 86 populations of 16 *Longidorus* species from Arkansas and various other locations, and (iv) to assess hierachical cluster analysis usefulness as a *Longidorus* species identification tool.

Materials and Methods

Arkansas nematode samples and measurements: Arkansas nematode specimens were obtained from several different sources. Most of them were collected from sandy soil around hardwood trees growing on Arkansas stream banks from 1999 to 2001. The others were from either the junior author's slide collection or from various other Arkansas locations (Ye and Robbins, 2003a, 2003b, 2004). Specimens were examined using a Nikon Optiphot II compound microscope with Nomarski differential interference contrast. Measurements were made using a Nikon drawing tube or an ocular scale and micrometer. All measurements are in micrometers and processed using Excel (Ye, 1996).

Hierarchical cluster analysis: For each population, nine characters were used in cluster analysis, e.g., body length (L), distance of vulva from the anterior end (VL), lip width, odontostyle length, distance of guide ring from anterior end (DGR), esophagus length, body width, tail length, and anal body width (ABW). These nine characters covered most of the morphological features of the species but did not cover aspects of head shape, amphid shape, tail shape, and the presence or absence of males. Since a single value for each population of a data matrix is required for cluster analysis, the mean of the values measured was adopted as the most satisfactory entry. Hierarchical cluster analysis was performed using Average method with the JMP 4.02 program (SAS Institute Inc., Cary, NC). Table 1 lists the species, study population number, associated plants, locations, and measurements. Table 2 lists the measurements and authority of all the published *Longidorus* species and, for ease of reading, species authority's are not cited in the following text. Authority references are omitted in the literature cited to save space as they are readily available in the polytomous keys of Chen et al. (1997) and Loof and Chen (1999). Morphometric values were obtained from paratype means or the holotype of the original species descriptions. The lectotype of *L. crassus* (Robbins and Brown, 1995) was used instead of the data from the brief description by Thorne (1974). Because of insufficient data, *L. heynsi* and *L. tardicauda* were not included in this study. The similarity level of the species is determined by the distance of the horizontal line in the dendrogram, but no numerical similarity level is provided by JMP software. The graph at the bottom of each dendrogram indicates that the distance of the horizontal line is proportional to the increasing rate of the curve from left to right.

Results

Arkansas Longidorus species: Hierachical cluster analysis generated eight groups (Fig. 1) in the 68 populations (Table 1). The groups are as follows: Group 1, 9 populations of *L. biformis* and 1 population of *L. crassus;* Group 2, 1 population of *L. crassus,* 1 population of *L. glycines,* and 3 populations of *L. grandis;* Group 3, 5 populations of *L. paravineacola* and 2 populations of *L. crassus;* Group 4, 6 populations of *L. breviannulatus;* Group 5, 19 populations of *L. crassus;* Group 6, 4 populations of *L. fragilis;* Group 7, 14 populations of *L. paralongicaudatus;* and Group 8, 3 populations of *L. diadecturus.* Populations from the same species are grouped together by cluster analysis, except for populations of *L. crassus* found in 3 different clusters.

All 137 published Longidorus species: Hierarchical cluster analysis of 137 published *Longidorus* species in Table 2 grouped the species according to morphometric similarity (Fig. 2). For example: *L. diadecturus, L. himalayensis, L. jonesi, L. fursti, L. macromucronatus, L. jagerae, L. martini, L. mobae, L. doonensis, L. fangi, L. naganensis, L. laricis,* and *L. litchi,* all with posteriorly located guide ring and many other similar features, were grouped together. Two New Zealand species also with posterior guide ring position, *L. orongorongensis* and *L. waikouaitii,* are in a separate cluster due primarily to their dissimilar bodies (Table 2). Some species with a high degree

TABLE 1. Average morphometrics of *Longidorus* species populations from Arkansas locations used for cluster analysis (all measurements in µm).

Species	Population number	*n*	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW	Associated plant	Locality
L biformis	Long-4	25	6,276	2,984	23.8	103.3	33.0	443.5	54.6	56.0	39.6	Elm, hackberry, maple, shrub	Middle Fork of White River, near Elkins, Washington County
L. biformis	Long-76	6	7,455	3,403	23.7	107.2	31.2	434.2	51.2	54.3	37.3	Birch, sweet gum, sycamore	South Fork of Little Red River, Clinton, Van Buren County
L. biformis	Long-105	8	6,515	3,026	22.9	103.6	31.5	346.9	48.4	63.9	35.0	Elm, hackberry	Crooked Creek, Yellville, Marion County
L. biformis	Long-131	6	7,628	3,755	23.3	108.2	32.2	388.3	52.3	60.5	38.5	Grape, oak	County Road 62 Bridge, Illinois River, Washington County
L. biformis	Long-133	3	7,127	3,453	22.3	108.3	30.7	398.3	46.0	56.3	37.7	Box elder, cottonwood, locust, maple	War Eagle Mill, near Rogers, Benton County
L. biformis	Long-136	8	5,901	2,840	20.6	100.8	32.8	378.3	44.3	56.9	33.9	Box elder, hackberry, maple, pine	Little Missouri River by Highway 195, Hempstead County
L. biformis	Long-149	7	7,324	3,544	24.0	107.9	35.1	381.4	54.0	60.9	38.7	Elm, Osage orange, sycamore, willow	Osage Creek, Highway 412, Carrol County
L. biformis	Long-158	10	7,333	3,467	23.9	113.5	34.2	452.0	55.8	55.3	41.3	Birch, black cherry, river cane	Big Piney Creek Access Area, Highway 164, Pope County
L. biformis	Long-264	3	6,303	2,917	22.0	104.3	30.3	353.3	42.0	62.0	34.7	Birch, black walnut, hickory, maple, sycamore, tree of heaven	Frog Bayou, Highway 162, south of Alma, Crawford County
L. breviannulatus	Long-66	20	4,798	2,332	17.4	82.1	25.6	348.8	41.4	36.7	29.1	*Amorpha* sp.	Beulah near Des Arc, Prairie County
L. breviannulatus	Long-69	3	5,030	2,543	18.3	85.3	26.7	298.3	47.7	41.7	31.0	Bentgrass	Branchwood Golf Club, Bella Vista, Benton County
L. breviannulatus	Long-116	12	5,045	2,323	19.5	87.6	27.4	336.0	48.5	36.3	34.9	Black cherry, box elder, cottonwood, maple, sycamore, willow	War Eagle Mill, near Rogers, Benton County
L. breviannulatus	Long-124	10	5,149	2,568	19.2	82.5	25.1	338.0	45.4	37.6	32.6	Elm, persimmon, willow	Arkansas River, Haroldton Access, Van Buren, Crawford County
L. breviannulatus	Long-140	9	4,812	2,293	17.0	79.8	24.1	268.0	40.0	36.0	27.7	Cottonwood	Toad Suck Park, Perry County
L. breviannulatus	Long-156	25	5,730	2,828	20.5	94.0	30.5	338.8	48.8	35.8	37.5	Birch, sweet gum	South Fork of Little Red River, Clinton, Van Buren County
L. crassus	Long-10	35	6,929	3,573	20.2	87.1	26.3	374.7	54.4	40.1	36.8	Soybean	Kibler, Arnolds farm, Crawford County
L. crassus	Long-12	19	4,630	2,264	17.5	97.6	27.6	359.1	54.3	35.3	37.2	Japanese holly	Little Rock, Pulaski County
L. crassus	Long-13	6	4,628	2,226	18.8	104.1	26.7	334.3	49.9	40.2	35.9	Centipide grass	Lowe Lawn, Texarkana, Miller County
L. crassus	Long-14	9	4,434	2,176	18.6	103.4	25.5	399.1	47.7	34.2	32.4	St. Augustine grass	Lowe Lawn, Texarkana, Miller County
L. crassus	Long-40	14	4,180	2,002	19.5	103.2	27.4	329.8	50.9	39.6	33.1	Water oak	Booneville, Logan County
L. crassus	Long-42	4	4,405	2,243	17.5	99.5	24.8	290.5	51.4	37.5	38.0	Grass	Rebsaman Golf Course, Little Rock, Faulkner County
L. crassus	Long-63	11	5,431	2,809	19.3	107.9	32.7	425.8	67.3	36.4	46.8	Wisteria	Beaver Lake Dam, Eureka Springs, Carrol County
L. crassus	Long-68	4	4,680	2,413	17.5	95.3	26.3	290.0	54.3	35.7	38.7	Centipide grass	Royce Martin Lawn, Mavern, Hot Springs County

TABLE 1. *Continued.*

Species	Population number	*n*	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW	Associated plant	Locality
L. crassus	Long-75	3	4,033	2,083	16.7	99.0	28.3	366.7	53.3	34.3	45.0	Grass	War Memorial Golf Course, Little Rock, Pulaski County
L. crassus	Long-79	8	4,450	2,158	20.5	104.3	29.8	382.0	53.0	40.6	37.8	Unidentified plant	Ozarks, Washington County
L. crassus	Long-80	4	5,543	2,925	21.3	115.8	36.0	478.8	51.5	57.8	44.8	Unknown	Ozarks, Washington County
L. crassus	Long-84	9	4,448	2,122	19.0	102.8	27.9	407.1	55.8	36.8	36.9	Oak, Osage orange	Combs Park, Fayetteville, Washington County
L. crassus	Long-86	3	4,113	2,000	22.0	107.0	31.0	403.3	49.0	37.3	36.7	Grape	Crowley's Ridge State Park, Greene County
L. crassus	Long-88	3	5,586	2,736	18.8	116.9	33.8	465.0	65.5	39.7	46.8	Cypress, elm, maple, oak	Shirey Bay—Rainey Brake Wildlife Management Area, Lawrence County
L. crassus	Long-90	12	3,998	1,985	17.3	100.8	26.9	317.2	46.8	37.8	34.3	Elm, maple, white oak	Wilbur Botts Access Area, St. Charles, Arkansas County
L. crassus	Long-94	10	5,323	2,722	21.1	103.7	31.1	380.9	53.8	41.6	40.0	Elm, oak	Wyman Bridge, White River, Fayetteville, Washington County
L. crassus	Long-112	3	4,910	2,367	17.3	99.7	30.7	410.0	49.3	39.3	35.7	Blackberry	Bayou Meto Wildlife Management Area, Arkansas County
L. crassus	Long-115	20	4,931	2,472	19.0	107.3	31.3	418.7	58.7	38.9	42.4	Box elder, elm, grape, oak, Osage orange, red bud, sycamore	Illinois River, County Road 62 Bridge, Washington County
L. crassus	Long-147	5	4,500	2,276	18.6	109.0	29.0	384.0	52.4	36.0	37.6	Box elder	Kings River, Highway 412, Marble, Madison County
L. crassus	Long-157	9	4,156	1,994	21.0	106.6	28.4	352.9	54.7	36.3	35.9	Hickory	Illinois Bayou, Highway 27, Pope County
L. crassus	Long-206	6	4,601	2,163	20.0	104.3	29.2	367.5	50.3	36.2	35.0	Birch, grape, river cane	Caddo River below Lake De Gray, Hot Spring County
L. crassus	Long-214	3	3,940	1,823	20.7	103.3	26.3	409.0	49.3	34.3	35.0	Birch, black cherry, river cane, white oak	Big Piney Creek Access Area, Highway 164, Pope County
L. crassus	Long-223	4	4,633	2,200	19.3	105.3	29.0	405.0	58.7	34.0	41.0	Box elder, ivy	Haroldton Access, Arkansas River, Van Buren, Crawford County
L. diadecturus	Long-121	12	3,973	1,833	16.1	115.4	64.1	385.5	42.7	28.7	30.5	Birch, black cherry, box elder	Springhill Park, Arkansas River, Sebastian County
L. diadecturus	Long-23	25	3,942	1,802	15.3	108.2	61.4	379.2	43.2	27.5	32.6	Cottonwood, elm, Osage orange, sweet gum, hackberry, maple	Middle Fork of White River, near Elkins, Washington County
L. diadecturus	Long-64	17	4,371	2,002	16.5	122.8	65.7	367.6	49.4	26.9	33.5	Osage orange	Mud Creek, Old Missouri Road, Fayetteville, Washington County
L. fragilis	Long-97	17	5,117	2,455	12.6	91.1	30.1	373.9	39.9	73.5	28.6	Cottonwood, sycamore	Mississippi River, 2 miles east of Wapanocca National Wildlife Refuge, Crittenden County
L. fragilis	Long-127	5	5,476	2,590	12.6	94.8	29.8	366.4	42.0	75.4	27.0	Box elder, cottonwood, maple	Toad Suck Park, Perry County

Table 1. *Continued.*

Species	Population number	*n*	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW	Associated plant	Locality
L. fragilis	Long-224	10	5,347	2,416	12.8	93.7	29.9	387.0	43.9	78.8	29.2	Grape, willow	Haroldton Access, Arkansas River, Van Buren, Crawford County
L. fragilis	Long-225	5	5,322	2,496	13.0	88.6	30.8	370.6	47.8	67.4	27.2	Cottonwood, sycamore	Fort Smith Park, Fort Smith, Sebastian County
L. glycines	Long-9	23	7,550	3,917	22.4	89.9	24.3	361.0	46.3	37.2	32.6	Soybean	University of Arkansas main research station, Fayetteville, Washington County
L. grandis	Long-148	14	7,244	3,473	22.7	94.4	29.6	406.8	52.3	36.8	36.9	Elm, Osage orange, sycamore, willow	Osage Creek, Highway 412, Carrol County
L. grandis	Long-151	3	6,870	3,353	26.0	93.3	28.7	393.7	49.0	37.3	41.3	Elm, hackberry, red bud	Crooked Creek, Yellville, Marion County
L. grandis	Long-201	11	6,575	3,178	26.5	90.9	27.5	391.0	47.5	39.2	40.3	Black cherry, river cane	Big Piney Creek Access Area, Highway 164, Pope County
L. paralongicaudatus	Long-17	27	3,649	1,608	14.0	98.4	24.9	321.8	40.8	52.2	25.6	Japanese holly	Little Rock, Pulaski County
L. paralongicaudatus	Long-72	13	3,198	1,518	14.5	110.0	24.8	271.0	37.5	55.1	25.1	White oak	Beaver Lake, Hickory Creek Park, Benton County
L. paralongicaudatus	Long-78	4	4,160	1,908	15.0	123.8	28.5	348.3	42.5	51.8	26.3	Unidentified plant	Ozarks, Washington County
L. paralongicaudatus	Long-110	6	3,233	1,545	15.3	111.0	26.8	332.3	38.5	63.0	27.5	Grape	Crowley's Ridge State Park, Greene County
L. paralongicaudatus	Long-132	3	3,853	1,783	16.3	113.0	28.3	338.3	46.0	63.3	29.0	Grape	Caddo River below Lake De Gray, Hot Spring County
L. paralongicaudatus	Long-135	15	2,886	1,389	14.5	104.8	24.7	328.1	39.7	51.3	24.1	Ash	Ouachita River, Hwy 270 Bridge, Montgomery County
L. paralongicaudatus	Long-137	26	3,620	1,700	15.7	105.0	25.5	309.9	43.3	47.2	26.8	Elm, maple, oak	Illinois River, County Road 62 Bridge, Washington County
L. paralongicaudatus	Long-143	16	3,259	1,526	14.9	106.1	26.7	368.8	40.4	49.1	25.6	Birch, black walnut, blackberry, elm, hickory, maple	Frog Bayou, Highway 162, South of Alma, Crawford County
L. paralongicaudatus	Long-153	4	3,435	1,570	15.0	108.3	25.5	287.8	43.0	53.0	26.8	Elm, oak	Des Arc Bayou, near Floyd, White County
L. paralongicaudatus	Long-155	6	3,705	1,632	15.0	108.0	27.2	368.0	43.0	51.3	27.0	Birch, oak, sweet gum, sycamore	South Fork of Little Red River, Clinton, Van Buren County
L. paralongicaudatus	Long-207	3	3,670	1,667	15.7	107.0	25.7	443.3	40.3	48.7	26.0	Hackberry, Ivy	Haroldton Access, Arkansas River, near Van Buren, Crawford County
L. paralongicaudatus	Long-208	4	3,475	1,638	15.0	103.3	27.5	341.3	41.8	46.5	25.0	Birch	East Cadron Creek, Highway 107 Bridge, Faulkner County
L. paralongicaudatus	Long-210	4	3,628	1,679	13.9	108.8	27.0	351.0	47.0	48.0	27.5	Unidentified plant	Beaver Lake, Fulton's cabin, Benton County
L. paralongicaudatus	Long-220	3	3,390	1,587	15.0	101.3	24.3	327.7	41.7	47.7	27.7	Grape, pecan	Toad Suck Park, Perry County
L. paravineacola	Long-108	8	7,645	3,739	25.3	101.0	32.3	455.8	65.6	32.9	45.8	Elm, Osage orange, sycamore, willow	Osage Creek, Highway 412, Carrol County

Table 1. *Continued.*

Species	Population number	*n*	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW	Associated plant	Locality
L. paravineacola	Long-123	12	8,824	4,164	24.2	106.5	33.9	457.3	65.3	38.8	46.8	Box elder, elm, grape, maple, oak, Osage orange, red bud, sycamore	Illinois River, County Road 62 Bridge, Washington County
L. paravineacola	Long-216	4	7,635	3,705	22.8	104.5	31.0	430.3	61.3	36.5	43.0	Osage orange	Old Missouri Road, Mud Creek, Fayetteville, Washington County
L. paravineacola	Long-154	1	7,310	3,630	25.0	104.0	28.4	435.0	63.0	37.0	45.0	Birch, sweet gum	South Fork of Little Red River, Clinton, Van Buren County
L. paravineacola	Long-266	2	8,085	3,960	24.5	107.0	34.5	477.5	64.5	36.0	45.5	Red bud	Middle Fork of White River, near Elkins, Washington County

of similarity were: *L. aetnaeus* with *L. juvenilis; L. conicaudoides* with *L. mirus; L. conicaudoides* and *L. mirus* with *L. bernardi; L. concavus* with *L. conicephalus; L. juveniloides* with *L. reneyii; L. silvae* with *L. uroshis; L. cretensis* with *L. nevesi; L. grandis* with *L. glycines; L. closelongatus* with *L. seinhorsti; L. apulus* with *L. dunensis; L. apulus* and *L. dunensis* with L. *euonymus; L. crassus* with *L. pseudoelongatus; L. iranicus* with *L. trapezoides; L. fasciatus* with *L. pauli;* and *L. diadecturus* with *L. himalayensis.*

Test populations with all published Longidorus species: Hierachical cluster analysis demonstrated the grouping and morphometric relationships among 86 test populations and 137 published *Longidorus* species (unpubl. data). Same-species test populations grouped together with morphologically similar species, generally with the identified species. Some of the test populations of *L. africanus, L. crassus,* and *L. breviannulatus* did not group exactly with the identified species. This unpublished dendrogram also demonstrated which existing described species were closely related to our 86 test populations.

Discussion

This study has evaluated cluster analysis as a method for grouping and distinguishing *Longidorus* species by morphometric parameters. Hierarchical cluster analysis based on average female morphometric characters, including L, VL, DGR, lip width, odontostyle length, esophagus length, body width, tail length, and tail width, generated eight clusters for 62 populations of *Longidorus* species from Arkansas. The populations of the same species generally grouped together and were separated from the other species. Some populations identified as *L. crassus* were in different clusters due to high morphometric variability of the populations. This is discussed later. Thus, species identification of *Longidorus* should never be based solely on cluster analysis using morphometric characters. Other features used for species identification in our study include qualitative characters like head shape, tail shape, amphid shape, presence or absence of males, male morphology, number of juvenile stages, juvenile morphology, and DNA sequencing of the ITS1 region and the 18S rDNA gene. A possible shortcoming of cluster analysis may be that qualitative characters such as head shape, tail shape, amphid shape, male presence or absence, etc. are not used in the analysis. However, these qualitative characters were useful in diagnosis and relationship elucidation of species within a cluster. It must be noted and emphasized that the cluster analysis approach is based on morphometric data; it does not reflect phylogenetic relationships as do the species defined by Adams (1998).

Choosing the correct variables is critical in discriminant analysis. In *Longidorus* species, the length of ovaries depends on the age (reproductive history) of fe-

TABLE 2. Average morphometrics of 137 published *Longidorus* species used for cluster analysis (all measurements in μm).

Species	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW
L. aetnaeus Roca, Lamberti, Agostinelli & Vinciguerra, 1986	3,250	1,495	9	76	25	349	38	49	24
L. africanus Merny, 1966	3,490	1,571	11	88	27	401	39.2	32.9	22
L. alaskaensis Robbins & Brown, 1996	5,452	2,641	17.9	113.6	34.8	486.1	73.9	47.1	48.5
L. alvegus Roca, Pereira & Lamberti, 1989	6,300	2,993	13.1	83	26	444	34	63	28
L. ampullatus Jacobs & Heyns, 1987	2,880	1,420	13	76	25	282	29	41	19
L. apuloides Roca, 1996	8,400	4,158	15.5	119	33.5	400	55	45	44
L. apulus Lamberti & Bleve-Zacheo, 1977	6,700	3,484	15	103	31	444	48	40	40
L. arenosus Kankina & Ivanova, 1986	8,290	4,137	15.5	103	24.8	391	39	35	32
L. artemisiae Rubtsova, Chizhov & Subbotin, 1999	5,900	2,891	15.3	92	29	412	46	40	32
L. arthensis Brown, Grunder, Hooper, Klingler & Kunz, 1994	5,920	3,019	16	108	35	442	67	40	42
L. athesinus Lamberti, Coiro, & Agostinelli, 1991	4,900	2,499	17	90	34	430	67	42	45
L. attenatus Hooper, 1961	6,100	2,989	12	78	28	401	38.6	52	29.3
L. auratus Jacobs & Heyns, 1987	3,650	1,664	10	86	29	287	31.5	37	22
L. balticus Brzeski, Peneva & Brown, 2000	7,738	4,024	21	97	27	433	63	33	41.3
L. belloi Andres & Arias, 1988	6,700	3,551	12.6	93	31	424	71	40	54
L. belondiroides Heyns, 1966	3,800	2,071	9	95	36.5	447	63	25	50
L. bernardi Robbins & Brown, 1996	4,071	1,948	10.9	74.8	24.9	315.6	39.4	45.4	27.1
L. biformis Ye & Robbins, 2004	6,276	2,984	23.8	103.3	33	443.5	54.6	56.0	39.6
L. breviannulatus Norton & Hoffman, 1975	4,755	2,220	18	83.2	23	281	50	38.7	31
L. brevis Swart, Cadet & N'Diaye, 1996	1,900	912	8.5	42.4	24	226	44	50.5	22
L. caespiticola Hooper, 1961	6,700	3,551	18	110	37	532	93	65	65
L. camelliae Zheng, Peneva & Brown, 2000	2,740	1,315	7.8	85	33	282	46	36	30
L. carpathicus Liskova, Robbins & Brown, 1997	6,360	3,358	16.2	154	44	441	71	41	51
L. carpetanensis Arias, Andres & Navas, 1986	4,200	2,050	12.5	59	23.8	323	38	52	28.9
L. chikmagalurensis Dhanam & Jairajpuri, 1997	2,800	1,428	9	99	30	397	41	34	33
L. closelongatus Stoyanov, 1964	6,300	3,068	21	114	31.4	432	46.5	43	39
L. cohni Heyns, 1969	8,390	4,237	17	114	33	450	44	49	36
L. concavus Singh & Khan, 1996	3,600	1,764	11	95	30	434	49	29.5	29.5
L. congoensis Aboul-Eid, 1970	3,000	1,350	12.5	74	25	280	57	33	26
L. conicaudatus Khan, 1986	5,500	2,833	13.4	90	37.6	444	63	53	52
L. conicaudoides Jacobs & Heyns, 1987	3,580	1,683	10	88	28	335	37	40	25
L. conicephalatus Singh & Khan, 1996	3,580	1,661	11.3	95	36	393	45	29.8	30
L. crassus Thorne, 1974 (Lectotypes Robbins & Brown, 1995)	5,500	2,640	15	110	32.5	500	60	35	48
L. crassus Thorne, 1974 (Lectotype, from Robbins & Brown, 1995)	6,789	3,516	17	110	29	499	64	39	44
L. crataegi Roca & Bravo, 1996	7,300	3,687	16.5	104	33.5	417	90.5	47.5	58
L. cretensis Tzortzakakis, Peneva, Terzakis, Neilson & Brown, 2001	7,650	3,901	21	140	43	588	85	48	53
L. curvatus Khan, 1986	3,250	1,560	12	90	34	322	40	53	18
L. cylindricaudatus Kozlowska & Seinhorst, 1979	5,200	2,496	12	137	34	547	47	44	34.6
L. dalmassoi Peneva, Loof & Brown, 1999	7,390	3,473	21	158	36	496	65	45	41
L. diadecturus Eveleigh & Allen, 1982	3,710	1,707	16	115	58	364	44	27	31
L. distinctus Lamberti, Choleva & Agostinelli, 1983	4,600	2,116	12	80	30	338	46	58	30
L. doonensis Singh & Khan, 1996	3,600	1,656	12.5	120	76	461	67	28	17
L. dunensis Brinkman, Loof & Barbez, 1987	6,510	3,314	15.1	100	31.2	428	47	41.5	34.8
L. edmundsi Hunt & Siddiqi, 1977	5,460	2,681	30	104	24	417	34	27	34
L. elongatus (de Man, 1876) Thorne & Swagger, 1936 (Hooper, 1961)	5,500	2,695	17	94	33	414	60	56	47
L. eridanicus Roca, Lamberti & Agostinelli, 1984	4,800	2,064	11	163	38	558	52	26	39
L. euonymus Mali & Hooper, 1974	6,910	3,524	14	86	30	424	45	45	35
L. fagi Peneva, Choleva & Nedelchev, 1997	5,620	2,883	12	105	37	446	59	65	34
L. fangi Xu & Cheng, 1991	5,090	2,647	17	136	79	530	57	37	41
L. fasciatus Roca & Lamberti, 1981	7,700	3,542	14	112	28	410	61	36	43
L. fragilis Thorne, 1974 (Lectotypes Robbins & Brown, 1995)	5,551	2,548	13	96	30	382.8	44	64	28
L. fursti Heyns, Coomans, Hutsebaut & Swart, 1987	4,570	2,404	14.7	104	67.5	408	38.2	27	27
L. globulicauda Dalmasso, 1969	5,300	2,581	12	77	31	390	53	61	34
L. glycines Ye & Robbins, 2004	7,550	3,917	22.4	89.9	24.3	361	46.3	37.2	32.6
L. goodeyi Hooper, 1961	6,700	3,484	8	101	35	453	42	50	50
L. grandis Ye & Robbins, 2004	7,244	3,473	22.7	94.4	29.6	406.8	52.3	36.8	36.9
L. hangzhouensis Zheng, Peng, Robbins & Brown, 2001	4,500	2,205	10.9	118	43	421	70	33	47
L. henanus Xu & Cheng, 1992	6,080	2,979	16	98	42	461	54	36.5	39
L. helveticus Lamberti, Kunz, Grunder, Molinari, de Luca, Agostinelli & Radicci, 2001	7,600	3,800	21.8	145.3	44	558.8	107	47	67.6
L. himalayensis (Khan, 1986) Xu & Hooper, 1990	3,600	1,746	14.5	120	57.5	367	34	24	32
L. igoris Krnjaic, Lamberti, Krnjaic, Agostinelli & Radicci, 2000	5,600	2,800	11	96	32.5	418	49	34	36

TABLE 2. *Continued.*

Species	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW
L. indicus Prabha, 1973	4,000	1,960	11	91	33	459	56	46	46
L. intermedius Kozlowska & Seinhorst, 1979	4,250	1,955	11.5	113	29	472	48	43	34
L. iranicus Sturhan & Barooti, 1983	5,990	3,031	12	112	35	472	58	34	40.5
L. ishrati Javed, 1983	4,600	2,162	8.5	90	32	354	40	46	31
L. israelensis Peneva, Orion, Shlevin, Bar-Eyal & Brown, 1998	8,170	4,110	18.6	130	37	579	71	41	48
L. iuglandis Roca, Lamberti & Agostinelli, 1984	7,100	3,905	15	120	36	530	82	36	55
L. jagerae Heyns & Swart, 1998	3,480	1,872	12	103	67	341	35	22.5	21.5
L. jiangsuensis Xu & Hooper, 1990	3,380	1,622	7.3	87	32	307	34	20	25
L. jonesi Siddiqi, 1962	3,430	1,742	13.5	113	61.5	399	52	21	28
L. juglandicola Liskova, Robbins & Brown, 1997	6,930	3,811	19	89	32	459	64	47	49
L. juvenilis Dalmasso, 1969	3,320	1,587	11	66	22	311	40.3	51	23
L. juveniloides Jacobs & Heyns, 1987	2,870	1,360	11	46	18	278	34	29	21
L. kakamus Jacobs & Heyns, 1987	6,540	3,263	15	70	28	344	28	33	21
L. kuiperi Brinkman, Loof & Barbez, 1987	7,470	3,937	28.6	107	27.6	455	51	28.3	36.9
L. laevicapitatus Williams, 1959	2,500	1,175	10	58	23	366	44	37	32
L. laricis Hirata, 1995	5,290	2,551	17.2	171	94	573	56	30	40
L. leptocephalus Hooper, 1961	4,200	2,268	9.2	64	30	316	44	44.7	37.3
L. lignosus Chizov, Subbotin, Romanenko & Kruchina, 1991	4,800	2,496	19	114	35	516	81	38	54
L. litchii Xu & Cheng, 1992	4,560	2,371	13.5	155	90	536	58	28	40
L. longicaudatus Siddiqi, 1962	2,640	1,188	12	95	22.5	343	36	59.2	19.7
L. lusitanicus Macara, 1985	6,200	3,100	20	85	29	395	57	33	40
L. macromucronatus Siddiqi, 1962	4,340	2,009	14	121	63	472	44	21.3	29.5
L. macrosoma Hooper, 1961	10,500	5,460	24	134	42	565	105	41.4	69
L. macroteromucronatus Altherr, 1974	7,560	3,856	20	133	37.5	552	94.5	27	46
L. magnus Lamberti, Bleve-Zacheo & Arias, 1982	9,500	4,655	19	114	46	605	112	51	77
L. major Roca & D'Errico, 1987	10,500	5,460	25	133	43	669	117	52	81
L. makatinus Jacobs & Heyns, 1987	3,830	1,846	11	62	26	295	33.6	28	24
L. martini Merny, 1966	4,290	2,402	12	89	66	352	28	25	22.7
L. mirus Khan, Chawla & Seshadri, 1972	3,400	1,530	10	78	25	309	39	42.5	28.3
L. mobae Jacobs & Heyns, 1987	5,520	2,848	13	116	49	386	31	32	22
L. moesicus Lamberti, Choleva & Agostinelli, 1983	7,200	3,816	12	119	34	456	61	43	42
L. monile Heyns, 1966	4,190	2,179	10.5	54	21.5	233	38	30	26.8
L. moniloides, 1966 Heyns	3,840	1,882	15	59	28.5	325	48.6	32	27.8
L. naganensis Hirata, 1995	4,360	2,224	16.6	153	82	513	60.5	33	43.2
L. nevesi Macara, 1985	8,000	4,080	20	142	43	552	91	43	56
L. nirulai Siddiqi, 1965	4,100	1,898	12	103	33.3	380	45.6	78	31.1
L. olegi Kankina & Metlitskaya, 1983	8,410	4,424	14	114	32.5	501	74	48.5	56
L. orientalis Loof, 1982	4,930	2,465	10.5	100	33	325	53	32	25.4
L. orongorongensis Yeates, Van Etteger & Hooper, 1992	7,240	3,743	22.6	160	68	548	79	39	59
L. paraelongatus Altherr, 1974	7,350	3,602	17.4	140	35	459	67	36.1	42.5
L. paralaskaensis Robbins & Brown, 1996	6,386	3,320	19	128	37	546	76	42	44
L. paralongicaudatus Ye & Robbins, 2003	3,620	1,700	15.7	105	25.5	309.9	43.3	47.2	26.8
L. paramirus Darekar & Khan, 1982	3,700	1,628	10.5	80	45	352	40	44	37
L. paramonile Jacobs & Heyns, 1982	4,650	2,209	10.5	53	25.5	316	31	31	43.4
L. paravineacola Ye & Robbins, 2003	8,824	4,164	24.2	106.5	33.9	457.3	65.3	38.8	46.8
L. pauli Lamberti, Molinari, De Luca, Agostinelli and Di Vito, 1999	7,600	3,884	15.2	109	30.6	447	57.5	37.8	41.4
L. pawneensis Luc & Coomans, 1988	5,030	2,530	14.5	70	23.5	338	45	34.5	30.5
L. piceicola Liskova, Robbins & Brown, 1997	5,140	2,364	16	166	42	559	53	43	39
L. picenus Roca, Lamberti & Agostinelli, 1984	6,800	3,604	20	137	39	586	81	38	59
L. pini Andres & Arias, 1987	4,700	2,397	9.5	68	26	328	39	63	26
L. pisi Edward, Misra & Singh, 1964	3,140	1,592	7.5	58	32.9	266	24	34	13
L. pius Barsi & Lamberti, 2001	5,320	2,660	22.5	134.6	37.5	530	89.4	44.7	67.5
L. poessneckensis Altherr, 1974	8,700	4,829	21	127.5	36.5	561	81	38.5	58
L. profundorum Hooper, 1966	7,000	3,710	14.4	97	37	504	67	45.5	41
L. protae Lamberti & Bleve-Zacheo, 1977	6,700	3,216	12	79	27	424	43	35	32
L. proximus Sturhan & Argo, 1983	7,330	3,489	18	107	34	476	59	38	45
L. pseudoelongatus Altherr, 1976	5,300	2,915	12	120	30	530	58	36	43
L. psidii Khan & Khan, 1972	3,210	1,518	12	97	34	353	50	35	35
L. raskii Lamberti & Agostinelli, 1993	7,400	3,774	17.1	98	36	525	90	43	59
L. reisi Roca & Bravo, 1993	9,200	4,572	17	107	30.5	449	43	84	30
L. reneyii Raina, 1966	2,300	1,150	10	54	23	256	31	31	18
L. rotundicaudatus Jacobs & Heyns, 1987	4,360	2,289	11	76	34	415	46.4	27	30
L. rubi Romanenko, 1998	4,530	2,174	12	78	30	423	41	57	29
L. saginus Khan, Seshadri, Weischer & Mathen, 1971	5,900	2,596	25	154	34	655	65	30	24

TABLE 2. *Continued.*

Species	L	VL	Lip width	Odontostyle	DGR	Esophagus	Body width	Tail length	ABW
L. seinhorsti Peneva, Loof & Brown, 1998	5,500	2,695	21	121	32	420	45	42	33
L. silvae Roca, 1993	6,900	3,353	15	123	40	543	69	42	53
L. socialis Singh & Khan, 1997	3,910	2,017	14	104	32	301	50	67	30.5
L. sturhani Rubtsova, Subbotin, Brown & Moens, 2001	5,500	2,860	15	89	30	305	49	36	36
L. sylphus Thorne, 1939 (lectotypes Robbins & Brown, 1995)	3,841	1,782	11	75	25	366	50	44	30
L. taniwha Clark, 1963	4,640	2,390	18	116	52	498	31	31	58.5
L. tarjani Siddiqi, 1962	6,800	3,536	34	180	35	523	68	50	45
L. trapezoides Nasira & Maqbool, 1995	6,500	3,029	12.2	109	32	428	51	38	42
L. unedoi Arias, Andres & Navas, 1986	5,400	2,916	12.5	59	25	360	40	40	25
L. uroshis Krnjaic, Lamberti, Krnjaic, Agostinelli & Radicci, 2000	6,500	3,296	17	134	42.5	551	64	46	49
L. vineacola Sturhan & Weischer, 1964	8,160	4,260	20.3	97	32	464	60	39	50
L. vinearum Bravo & Roca, 1995	9,300	4,297	21	119	40	517	102	48	69
L. waikouaitii Yeates, Boag & Brown, 1997	6,790	3,510	16.5	115	58.5	530	82	39	62

male. Thus, anterior ovary length, posterior ovary length, G1%, and G2% have high variability within the same population and should not be used. The odontophore length is often difficult to measure because the odontophore base is usually not flanged and is barely distinguishable from adjacent tissue within the corpus of the esophagus. The length and width of the basal bulb of the esophagus can be easily affected by fixation and mounting procedures. Moreover, the length measurement of the basal bulb is often uncertain due to its flask shape and lack of a delimiting anterior feature. Therefore, these parameters were not used in species discrimination. The esophagus length was used because the basal bulb is clearly separated from the intestine. In our study, only linear morphometrics were used in our cluster analysis. Brown et al. (1997) and Georgi (1988) also used only the linear morphometrics in their analysis. However, in some studies (Griesbach and Maggenti, 1990; Lamberti and Ciancio, 1993, 1994; Roca, 1996; Rubtsova et al., 1999) de Man's ratios (a, b, c, V) were frequently used. All those ratios are calculated from one of the parameters, namely body length. Therefore, body length was used multiple times in analysis and could affect the result.

The growing number of species in *Longidorus* has increased the difficulty of new species diagnosis and the determination of close relationships. Using cluster analysis as a computer-based approach, morphometric relationships among species were elucidated. Therefore, the diagnosis for new species becomes less arbitrary. This study constructed a dendrogram for all 137 published *Longidorus* species by hierachical cluster analysis using L, VL, lip width, odontostyle length, DGR, esophagus length, body width, tail length, and ABW. Thus, the species were grouped and separated by only these morphometric characters. The dendrograms generated in this study do not reflect the phylogenetic relationships of *Longidorus* species. Five species described from Arkansas and aided by cluster analysis were diagnosed and relationships elucidated (Ye and Robbins, 2003a, 2003b, 2004).

Roca (1996) described a new species *L. apuloides* because it was thought to be close to *L. apulus* by hierarchical cluster analysis, and less close to *L. vineacola* and *L. proximus*. Brown et al. (1997) synonymized *L. apuloides* with *L. vineacola* due to its closer relationships with *L. vineacola* as shown by their cluster analysis. From our dendrogram (Fig. 2), *L. apuloides* is closest to *L. cohni.* Other similar species include *L. arenosus, L. balticus, L. vineacola, L. paravineacola, L. lusitanicus, L. grandis,* and *L. glycines,* whereas *L. apulus* is located in another cluster with *L. dunensis, L. euonymus, L. artemisiae,* and *L. protae.* The conflicting results among various sources are probably caused by using different data sets.

Some recently described new species were investigated for their diagnosis. Zheng et al. (2001) found *L. hangzhouensis* and *L. belondiroides* to be most similar, and they are in the same cluster in our dendrogram and are thus quite similar. Our dendrogram confirms that *L. balticus* closely resembles *L. vineacola* in the diagnosis as indicated by Brzeski et al. (2000). Rubtsova et al. (1999) considered *L. artemisiae* to be closest to *L. elongatus, L. attenuatus, L. proximus, L. apulus, L. euonymus,* and *L. dunensis.* In our dendrogram *L. artemisiae* was found in the same cluster with the last three species but was different from the first three species. Rubtsova et al. (2001) considered *L. sturhani* most similar with *L. seinhorsti* and *L. artemisiae,* but in our dendrogram *L. breviannulatus, L. kakamus, L. pawneensis,* and *L. unedoi* are in the same cluster with *L. sturhani.* Lamberti et al. (2001) compared *L. helveticus* with *L. macrosoma, L. poessneckensis, L. picenus,* and *L. neves;* however, in our dendrogram *L. helveticus* is closer to *L. magnus, L. vinearum, L. macrosoma,* and *L. major. Longidorus camelliae* is much closer to *L. laevicapitatus, L. congoensis,* and *L. moniloides* in our dendrogram than it is to *L. jiangsuensis, L. belloi,* and *L. belondiroides* as given in the description (Zheng et al., 2000). Krnjaic et al. (2000) com-

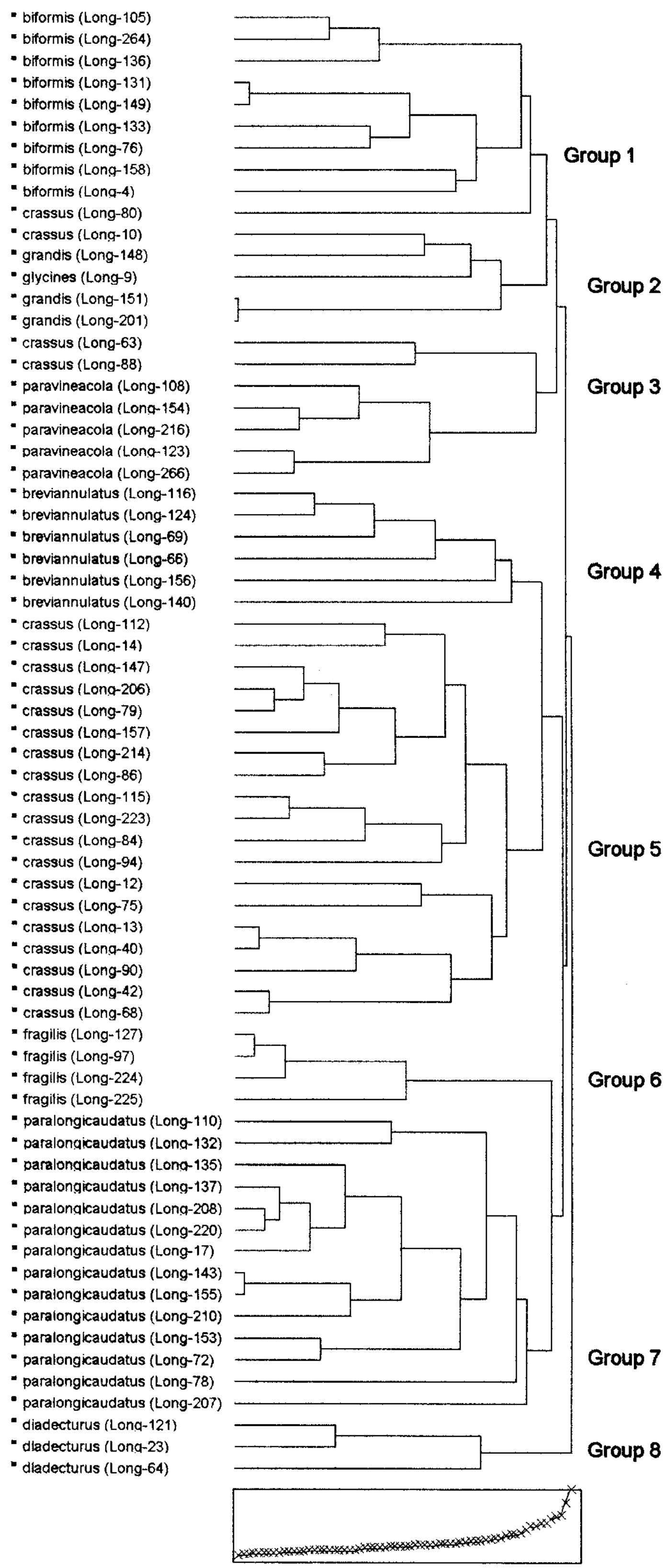

FIG. 1. Hierachical cluster dendrogram of 68 populations of nine Arkansas *Longidorus* species.

pared only *L. igoris* with *L. moesicus,* but in our dendogram it is grouped with *L. iranicus* and *L. trapezoides.* Krnjaic et al. (2000) compared *L. uroshis* with *L. saginus* and *L. apulus,* but it is closer to *L. silvae* in our dendogram. Barsi and Lamberti (2001) reported *L. pius* dissimilar to all other species, but in our dendrogram it is

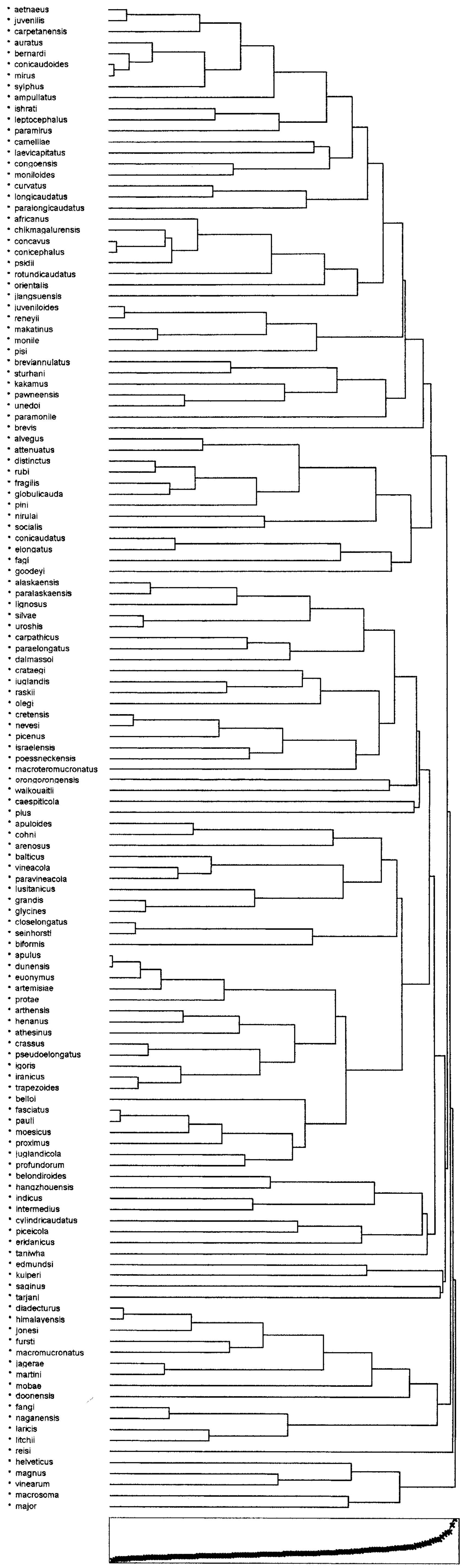

FIG. 2. Hierachical cluster dendrogram of 137 published *Longidorus* species.

closer to *L. caespiticola* than to *L. picenus* and *L. nevesi,* the species they suggested. New species diagnoses are often primarily based on geographical distribution and the author's experience. Therefore, cluster analysis provides a computerized statistical approach to aid in selecting which species to compare in new species diagnosis.

Longidorus diadecturus was described as a vector of peach rosette mosaic virus in Ontario, Canada (Eveleigh and Allen, 1982). It is the most widely distributed species in Arkansas, with 36 populations found. A total of 5 *L. diadecturus* populations from Oklahoma, Iowa, Indiana, and Minnesota also were found in our study. In our species identification of populations, our specimens are similar with *L. diadecturus, L. macromucronatus, L. martini, L. fursti,* and *L. jagerae* in having the guide ring located posteriorly, a moderately flanged odontophore base, small body size, a slightly expanded head, similar odontostyle length, a short rounded tail and parthenogenetic reproduction. Our specimens also come close to *L. jonesi, L. himalayensis,* and *L. doonensis* but differ in the head shape, slightly expanded head in our populations vs. rounded, continuous head in the latter species. However, this difference could be intraspecies variation. Two species described from South Africa, *L. fursti* and *L. jagerae,* were not appropriately diagnosed. *Longidorus fursti* was compared with *L. pisi, L. mobae,* and *L. latocephalus* (Heyns et al., 1987). *Longidorus jagerae* was compared with *L. fursti, L. pisi, L. mobae,* and *L. latocephalus* (Heyns and Swart, 1998). Instead of comparing with those much closer species *L. macromucronatus, L. martini, L. jonesi, L. diadecturus, L. himalayensis,* and *L. doonensis.* Heyns et al. (1987) studied the ultrastructure of the flanged odontophore base of *L. fursti.* The same structure was also observed by Cho and Robbins (1990) in studying *L. diadecturus* from Arkansas. *Longidorus diadecturus* is similar to *L. macromucronatus.* Eveleigh and Allen (1982) distinguished the two species by the shape and length of the corpal mucro (sagittate, 1–2 µm in *L. diadecturus* vs. conoid, which is more than 5 µm in *L. macromucronatus*), length of odontophore (55–66 µm in *L. diadecturus* vs. 67–77 µm in *L. macromucronatus*). The corpal mucro was usually not found in our specimens and is a difficult structure to observe due to its small size and the clouding of the esophagus in fixed specimens. The odontophore length of our various populations ranges from 55 µm to 78 µm, in the combined range of both species. Therefore, could *L. diadecturus* described from Canada possibly be synonymous with *L. macromucronatus,* described from halfway around the world in India? Cluster analysis of all *Longidorus* species showed that *L. diadecturus, L. martini, L. fursti, L. jagerae, L. macromucronatus, L. mobae, L. himalayensis,* and *L. doonensis* are in one cluster. Moreover, paratypes of *L. macromucronatus* from India measured by us had a close relationship with *L. diadecturus* based on our unpublished dendrogram. Whether *L. diadecturus, L. martini, L. fursti,* and *L. jagerae* are synonymous with *L. macromucronatus,* and whether *L. himalayensis,* and *L. doonensis* are synonymous with *L. jonesi,* or whether they are all synonymous with *L. macromucronatus* is not clear and will remain unclear without examining all the type specimens or comparing DNA sequences.

Longidorus crassus was frequently found in Arkansas. The specimens of various populations generally conform to the original description (Thorne, 1974) and redescription of this species (Robbins and Brown, 1995) but show a large morphometric variation between populations. Specimens of population Long-10 have a longer body and shorter odontostyle compared with the other populations in Arkansas. Specimens of population Long-80 have longer odontostyle, longer tail, and more posteriorly located guide ring. The body length of one female of population Long-63 is 8,074 µm, but the body length of the other 34 females range from 4,370 µm to 6,315 µm. Some females of many populations (Long-12, Long-14, Long-40, Long-75, Long-90, Long-157, Long-214) are less than 4,000 µm—much smaller than the type specimens (5,000–6,000 µm). Without observing any other morphological difference, we considered those differences as intraspecific variation and identified them as *L. crassus.* Due to the high intraspecies morphometric variation, *L. crassus* populations are in several clades, as shown in Fig. 1.

The cluster analysis dendrogram illustrated the grouping and morphometric relationships of all 137 published *Longidorus* species. Morphometrically similar species are grouped in the same cluster and separated from the other species. This approach has proven useful in establishing relationships among species by examining our populations and published species. The hierachical cluster analysis on test samples together with all the published *Longidorus* morphometric data appears to provide a reliable means for distinguishing and identifying *Longidorus* species, for establishing relationships among species, and for assisting new species diagnosis. However, the conclusive identification must also refer to quantitative morphology (head shape, amphid shape, tail shape, etc.), presence or absence of males, geographical distribution, DNA sequences, and comparison with type specimens, if possible. The cluster analysis approach is better than using keys in the following ways: (i) it has no need to transform the data into code, (ii) resulting dendrograms clearly demonstrate the relationships of the species without user prejudice, and (iii) JMP software has a Windows-based interface and is easy to use without any complicated programming.

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