Journal of Nematology 36(4):449–457. 2004.

Stepwise and Canonical Discriminant Analysis of *Longidorus* Species (Nematoda: Longidoridae) from Arkansas[1]

Weimin Ye and R. T. Robbins[2]

Abstract: During a 1998-to-2001 survey from Arkansas, nine distinct species of *Longidorus* were found including five new species. Morphometrics of these nine species were used in a stepwise and canonical discrimination to select a subset of characteristics that best identified each species. Student's *t* test was applied to compare *Longidorus breviannulatus* Norton & Hoffman, 1975; *L. crassus* Thorne, 1974; *L. diadecturus* Eveleigh & Allen, 1982; *L. fragilis* Thorne, 1974; *L. biformis* Ye & Robbins, 2004; *L. glycines* Ye & Robbins, 2004; *L. grandis* Ye & Robbins, 2003; *L. paralongicaudatus* Ye & Robbins, 2003; and *L. paravineacola* Ye & Robbins, 2003 to examine interspecies variation and test for the most useful morphometric characters in species discrimination. Most of the morphometric characters were useful to differentiate species, but species identification could not be based on a single character because the morphometric character ranges often overlap. Stepwise discriminant analysis indicated that the guide ring position, head width, tail length, body length, odontostyle length, and anal body width were the most important variables. These were used to generate canonical variables in discriminating the species. The first three canonical variables accounted for 95% of the total variance. The scatterplots by the first three canonical variables grouped and separated the *Longidorus* species from Arkansas. Stepwise and canonical discriminant analyses were useful for examining the groupings and morphometric relationships of the nine *Longidorus* species.
Key words: Arkansas, canonical discriminant analysis, identification, *Longidorus,* morphometrics, stepwise discriminant analysis.

The genus *Longidorus* damages plant roots and has the potential to transmit viruses. The genus includes 139 nominal species. 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 difficulty of identification. There is a need to group *Longidorus* species using a statistical approach based on morphometrics.

Luc and Southey (1980) examined differences between populations for two species—*Xiphinema insigne* Loos, 1949 and *X. elongatum* Schuurmans Stekhoven & Teunissen, 1938—by morphological discriminant analysis. Lamberti and Ciancio (1993, 1994) used principal components and hierarchical cluster analysis to separate the *X. americanum* group into five subgroups for 49 populations, but were not successful in simplify-

Received for publication 11 March 2004.
[1] A portion of a Ph.D. dissertation by the first author. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.
[2] Former Graduate Research Assistant and Professor, Department of Plant Pathology, Nematology Laboratory, University of Arkansas, Fayetteville, AR 72704. Senior author current address: Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Avenue, Davie, FL 33314.
E-mail: rrobbin@uark.edu.
This paper was edited by K. G. Davies.
The authors thank L. Duncan and Y. Zhang from the University of Arkansas Statistics Department for providing statistical help.

Table 1. Associated plants and sample location of nine *Longidorus* species from Arkansas

Species	Associated plant	Location
L. biformis[1]	Elm (*Ulmus americana* L.), hackberry (*Celtis occidentalis* L.), maple (Acer sp.), unidentified shrub	Middle Fork of the White River, near Elkins, Washington County
L. breviannulatus	Black cherry (*Prunus serotina* Ehrh.), box elder (*Acer negundo* L.), cottonwood (*Populus deltoides* Marsh), maple (*Acer* sp.), sycamore (*Platanus occidentalis* L.), willow (*Salix* sp.)	War Eagle Mill Creek, near War Eagle Mill, Benton County
L. crassus	Wisteria (*Wisteria floribunda* Willd.), DC	Beaver Lake Dam, near Eureka Springs, Carroll County
L. diadecturus	Cottonwood (*Populus deltoides* Marsh), elm (*Ulmus americana* L.), Osage orange (*Maclura pomifera* (Raf.) Schneid), sweet gum (*Liquidambar styraciflua* L.), hackberry (*Celtis occidentalis* L.), maple (Acer sp.)	Middle Fork of the White River, near Elkins, Washington County
L. fragilis	Cottonwood (*Populus deltoides* Marsh), sycamore (*Platanus occidentalis* L.)	Mississippi River Levee, Wapanocca National Wildlife Refuge, Crittenden County
L. glycines[1]	Soybean (*Glycines max* (L). Merr.)	University of Arkansas Farm, Fayetteville, Washington County
L. grandis[1]	Elm (*Ulmus americana* L.), Osage orange (*Maclura pomifera* (Raf.) Schneid.), sycamore (*Platanus occidentalis* L.), willow (*Salix* sp.)	Osage Creek, near Highway 412, Carrol County
L. paralongcaudatus[1]	Elm (*Ulmus americana* L.), maple (*Acer* sp.), oak (*Quercus* sp.)	Illinois River, County Road 62 Bridge, Washington County
L. paravineacola[1]	Box elder (Acer negundo *L.*), elm (*Ulmus americana* L.), grape (*Vitis* sp.), maple (*Acer* sp.), oak (*Quercus* sp.), Osage orange (*Maclura pomifera* (Raf.) Schneid.), red bud (*Cercis canadensis* L.), Sycamore (*Platanus occidentalis L.*)	Illinois River, County Road 62 Bridge, Washington County

[1] = Type population.

ing species identification. Cho and Robbins (1991) studied morphological variation among 23 *X. americanum*-group populations by canonical discriminant analysis. Three groups were detected, but clear distinction among total populations within and between the groups could not be made because of overlap. Griesbach and Maggenti (1990) proposed *X. californicum* Lamberti & Bleve-Zacheo, 1979 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 among populations of *L. vineacola* Sturhan & Weischer, 1964 and morphologically related species by canonical analysis using five morphometric characters. The clusters formed were proven to be reliable for distinguishing members of the *L. vineacola* complex of morphologically similar species. In the above study, *L. apuloides* Roca, 1996 was regarded as a junior synonym of *L. vineacola*.

In a 1998-to-2001 Arkansas survey of longidorid

TABLE 2. The mean and range (in parentheses) of morphometric characters of nine *Longidorus* species from Arkansas.

	Longidorus species								
Variable*	*biformis*	*breviannulatus*	crassus	*diadecturus*	*fragilis*	*glycines*	*grandis*	*paralongicaudatus*	*paravineacola*
N	25	11	35	25	17	23	14	26	12
L (mm)	6.19 c	5.21 d	5.43 d	3.96 e	5.34 d	7.45 b	7.15 b	3.59 e	8.67 a
	(5.60–7.69)	(4.03–5.97)	(4.37–8.07)	(3.55–4.56)	(4.250–6.60)	(6.47–8.43)	(6.33–9.40)	(2.98–4.35)	(7.08–10.50)
VL	2.94 c	2.47 d	2.81 c	1.81 e	2.50 d	3.89 a	3.42 b	1.69 e	4.10 a
	(2.16–3.69)	(2.24–2.98)	(2.24–4.10)	(1.61–1.98)	(2.66–3.13)	(3.24–4.50)	(2.85–4.50)	(1.38–2.00)	(3.35–4.82)
HW	23.8 a	17.5 d	19.3 c	15.3 e	12.9 f	22.2 b	22.5 b	15.7 e	24.3 a
	(23.0–25.4)	(18.0–22.0)	(18.0–23.0)	(14.0–16.0)	(11.0–15.0)	(21.0–24.0)	(22.0–27.0)	(14.0–18.0)	(23.0–26.0)
ODS	103.4 b	82.5 e	107.9 a	108.2 a	93.3 c	89.9 d	93.6 c	104.4 b	106.2 ab
	(96.7–111.5)	(78.0–98.0)	(96.0–116.0)	(102.0–114.0)	(80.0–99.0)	(84.0–96.0)	(88.0–108.0)	(96.0–110.0)	(93.0–115.0)
ODP	69.1 b	45.3 f	70.7 b	64.9 c	57.0 d	52.4 e	65.1 c	64.2 c	76.7 a
	(62.3–75.4)	(34.0–78.0)	(65.0–76.0)	(58.0–70.0)	(50.0–83.0)	(47.0–60.0)	(53.0–80.0)	(56.0–74.0)	(65.0–88.0)
DGR	33.0 b	24.0 e	32.7 b	61.8 a	30.3 c	24.3 e	29.2 c	25.7 d	33.8 b
	(30.3–36.9)	(26.0–30.0)	(30.0–35.0)	(56.5–66.0)	(27.0–32.0)	(22.0–27.0)	(29.0–35.0)	(21.0–28.0)	(32.0–36.0)
BBL	114.3 b	91.0 e	109.9 bc	78.0 f	105.9 cd	103.6 d	115.9 b	87.1 e	124.2 a
	(96.7–124.6)	(88.0–124.0)	(80.0–128.0)	(72.0–84.0)	(80.0–124.0)	(94.0–119.0)	(98.0–136.0)	(70.0–98.0)	(110.0–136.0)
BBW	24.2 b	21.8 c	26.6 a	16.7 e	22.1 c	20.2 d	25.5 ab	20.2 d	26.4 a
	(23.0–26.2)	(18.0–24.0)	(20.0–33.0)	(15.0–19.0)	(19.0–28.0)	(18.0–26.0)	(24.0–34.0)	(17.0–25.0)	(23.0–29.0)
ESOP	444.5 ab	360.0 d	425.8 b	381.2 cd	385.2 cd	362.7 d	408.9 bc	318.7 e	467.1 a
	(382.7–496.7)	(280.0–425.0)	(311.0–530.0)	(309.0–452.0)	(235.0–475.0)	(264.0–480.0)	(370.0–500.0)	(200.0–440.0)	(290.0–510.0)
BW	54.4 b	45.2 de	67.3 a	43.3 ef	40.6 f	46.3 d	50.7 c	43.5 de	65.5 a
	(47.5–60.7)	(42.0–53.0)	(56.0–76.0)	(40.0–46.0)	(37.0–44.0)	(40.0–53.0)	(50.0–76.0)	(39.0–48.0)	(55.0–70.0)
AO	534 b	344 c	343 c	228 d	251 d	645 a	664 a	271 cd	555 b
	(457–684)	(260–630)	(250–724)	(175–271)	(153–455)	(226–996)	(370–920)	(165–540)	(265–790)
PO	550 b	367 c	329 cd	212 e	261 de	659 a	615 ab	266 de	559 b
	(421–726)	(90–800)	(180–690)	(159–246)	(114–405)	(310–1,080)	(310–950)	(160–430)	(237–960)
TL	55.8 b	36.7 d	36.4 d	27.6 e	75.9 a	37.2 d	37.3 d	47.2 c	39.1 d
	(49.2–63.9)	(31.0–42.0)	(26.0–45.0)	(24.0–32.0)	(62.0–84.0)	(30.0–44.0)	(28.0–50.0)	(34.0–60.0)	(30.0–46.0)
ABW	39.6 b	32.0 d	46.8 a	32.8 d	28.9 e	32.4 d	36.4 c	26.9 f	46.7 a
	(36.1–43.4)	(33.0–38.0)	(42.0–52.0)	(28.0–40.0)	(26.0–30.0)	(30.0–34.0)	(36.0–50.0)	(20.0–30.0)	(38.0–50.0)
HYL	20.8 b	13.2 cd	12.6 d	7.7 e	22.3 a	13.5 cd	12.5 d	14.0 c	13.0 cd
	(17.2–23.0)	(10.0–15.0)	(8.0–12.0)	(7.0–8.5)	(16.0–29.0)	(11.0–16.0)	(11.0–18.0)	(10.0–17.0)	(9.0–18.0)
TSL	172.5 b	127.8 f	178.5 a	173.1 b	150.3 d	142.3 e	158.7 c	168.7 b	182.9 a
	(164.7–181.2)	(0.0–168.0)	(168.0–190.0)	(164.0–184.0)	(132.0–172.0)	(138.0–151.0)	(143.0–183.0)	(158.0–182.0)	(158.0–193.0)
a	114 d	116 d	81 f	92 e	132 c	162 a	141 b	83 f	133 c
	(104–135)	(90–122)	(69–128)	(85–101)	(106–157)	(150–188)	(104–157)	(74–100)	(115–169)
b	13.9 cd	14.6 c	12.9 de	10.5 f	13.9 cd	21.0 a	17.5 b	11.6 ef	18.9 b
	(12.7–18.3)	(11.5–17.9)	(10.4–17.7)	(8.8–12.5)	(10.5–22.9)	(15.5–28.9)	(15.4–22.1)	(8.4–17.6)	(15.9–30.0)
c	111 d	1435 c	151 c	144 c	71 e	202 b	194 b	78 e	225 a
	(97–138)	(113–154)	(118–311)	(123–166)	(57–84)	(159–248)	(162–291)	(54–103)	(185–292)
c'	1.4 c	1.2 d	0.8 f	0.9 f	2.6 a	1.2 d	1.0 e	1.8 b	0.8 f
	(1.2–1.8)	(0.9–1.2)	(0.5–0.9)	(0.7–1.0)	(2.1–3.2)	(0.9–1.4)	(0.6–1.2)	(1.2–2.3)	(0.6–1.0)
V	47.5 b	47.5 b	51.7 a	45.7 c	46.9 bc	52.1 a	47.9 b	46.9 bc	47.3 b
	(38.5–51.3)	(41.3–50.9)	(48.7–54.4)	(43.0–47.3)	(41.3–53.2)	(49.1–56.3)	(45.8–50.9)	(44.8–52.4)	(40.4–50.1)
H%	37.3 a	35.9 abc[c]	35.0 abc	28.1 d	29.4 d	36.4 ab	33.7 bc	30.0 d	33.3 c
	(30.9–42.5)	(31.0–40.0)	(26.7–53.8)	(17.5–23.3)	(24.0–37.7)	(30.6–42.4)	(28.2–60.0)	(96.0–110.0)	(28.3–43.9)
G1%	8.6 ab	6.6 cd	6.3 cd	5.8 de	4.8 e	8.7 ab	9.4 a	7.7 bc	6.5 cd
	(7.7–9.9)	(5.1–13.4)	(4.6–11.5)	(4.6–6.7)	(2.6–10.7)	(2.9–13.6)	(4.5–14.5)	(4.5–15.9)	(3.2–10.0)
G2%	8.9 a	7.1 cd	6.0 def	5.3 ef	4.8 f	8.8 ab	8.8 ab	7.5 bc	6.5 cde
	(6.8–11.8)	(1.7–16.4)	(3.7–11.0)	(4.5–6.3)	(2.4–6.1)	(3.9–14.5)	(4.4–12.5)	(4.4–12.6)	2.9–12.0)

* N = number of specimens; L = body length; VL = distance to vulva from anterior end; HW = head width; ODS = odontostyle length; ODP = odontophore length; DGR = distance to guide ring from anterior end; BBL = basal bulb length; BBW = basal bulb width; ESOP = esophagus length; BW = body width midbody; AO = anterior ovary length; PO = posterior ovary length; TL = tail length; ABW = body width at anus; HYL = hyaline tail tip length; TSL = total stylet length; a = body length/midbody width; b = body length/esophagus length; c = body length/tail length; c' = tail length/body width at anus; H% = haline length/tail length × 100; G1% = anterior reproductive system length/body length; G2% = posterior reproductive system length/body length.

Means within a row followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

TABLE 3. Coefficient of variation of *Longidorus* morphometrics.

	Longidorus species									
Variable*	*biformis*	*breviannulatus*	crassus	*diadecturus*	*fragilis*	*glycines*	*grandis*	*paralongicaudatus*	*paravineacola*	Average
L (mm)	11.8	10.3	19.8	8.1	9.1	6.0	9.7	11.8	10.2	10.8
VL	12.9	13.9	22.0	8.3	9.7	6.7	11.1	11.1	12.3	12.0
HW	6.2	8.9	7.7	5.6	6.3	2.8	8.7	6.7	3.5	6.3
ODS	5.6	6.5	8.2	6.7	4.9	4.0	7.1	6.1	5.1	6.0
ODP	8.6	14.2	12.9	8.5	12.0	6.3	16.2	10.9	9.1	11.0
DGR	6.4	8.6	10.8	6.4	4.2	5.0	13.0	6.6	3.4	7.2
BBL	11.4	13.4	13.7	5.2	10.0	6.6	11.1	9.8	6.4	9.7
BBW	12.0	10.4	13.8	14.5	10.5	10.2	17.8	13.6	7.1	12.2
ESOP	16.9	14.6	16.4	10.5	11.7	14.4	15.1	17.2	15.3	14.7
BW	11.1	11.2	14.0	9.7	8.6	6.3	16.8	9.1	8.2	10.6
AO	26.2	44.3	31.0	16.4	28.0	30.3	29.6	40.5	25.4	30.2
PO	23.7	42.0	32.1	26.3	27.9	33.1	31.5	49.7	39.4	34.0
TL	11.1	9.6	12.8	9.3	10.1	9.9	11.4	11.8	12.0	10.9
ABW	8.2	11.9	14.6	9.0	5.3	3.7	13.9	7.7	7.0	9.0
HYL	15.3	14.9	16.7	15.3	13.7	11.1	13.6	13.9	17.4	14.7
TSL	5.3	7.7	9.2	6.4	5.4	2.7	9.4	6.1	5.6	6.4
a	13.4	10.1	18.7	6.6	9.5	7.4	14.7	10.0	12.1	11.4
b	20.6	15.5	21.0	11.7	16.2	17.4	18.0	21.6	22.9	18.3
c	15.2	12.5	20.6	12.0	11.8	11.4	16.7	17.3	15.3	14.8
c'	15.4	14.0	16.1	10.5	10.4	9.8	18.2	11.9	13.9	13.4
V	5.3	5.7	8.3	2.4	5.0	3.9	4.8	4.6	7.0	5.2
H%	17.6	15.3	19.1	18.6	10.9	9.5	19.2	16.6	14.5	15.7
G1%	25.5	33.7	28.2	15.3	32.6	31.3	31.0	43.1	27.1	29.8
G2%	23.9	38.3	29.1	23.9	27.6	32.8	31.3	50.2	43.9	33.4

* L = body length; VL = distance to vulva from anterior end; HW = head width; ODS = odontostyle length; ODP = odontophore length; DGR = distance to guide ring from anterior end; BBL = basal bulb length; BBW = basal bulb width; ESOP = esophagus length; BW = body width midbody; AO = anterior ovary length; PO = posterior ovary length; TL = tail length; ABW = body width at anus; HYL = hyaline tail tip length; TSL = total stylet length; a = body length/midbody width; b = body length/esophagus length; c = body length/tail length; c' = tail length/body width at anus; H% = haline length/tail length × 100; G1% = anterior reproductive system length/body length; G2% = posterior reproductive system length/body length.

nematodes nine species of *Longidorus* were found: *Longidorus biformis* Ye & Robbins, 2004; *L. breviannulatus* Norton & Hoffman, 1975; *L. crassus* Thorne, 1974; *L. diadecturus* Eveleigh & Allen, 1982; *L. fragilis* Thorne, 1974; *L. glycines* Ye & Robbins, 2004; *L. grandis* Ye & Robbins, 2003; *L. paralongicaudatus* Ye & Robbins, 2003; and *L. paravineacola* Ye & Robbins, 2003. Those species were not easily discriminated by morphometric characters due to high variability. The objectives of this study were to: (i) examine the interspecies variation in morphometrics by student's test, (ii) screen for the most useful morphometric characters in species discrimination by stepwise discriminant analysis, and (iii) group and separate the species occurring in Arkansas by generating scatterplots using canonical discriminant analysis.

Materials and Methods

Nematode samples and measurements: Most *Longidorus* specimens were obtained from sandy stream bank soil around hardwood trees growing in Arkansas and col-

TABLE 4. Stepwise selection summary.

Step	Number in Character*	Partial R-square	F value	Pr > F	Wilks' Lambda	Pr < Lambda	Average squared canonical correlation	Pr > ASCC
1	1 DGR	0.953	1349.3	<.0001	0.047056	<.0001	0.119	<.0001
2	2 HW	0.882	497.1	<.0001	0.005552	<.0001	0.227	<.0001
3	3 TL	0.821	304.3	<.0001	0.000994	<.0001	0.326	<.0001
4	4 L	0.667	132.7	<.0001	0.000331	<.0001	0.389	<.0001
5	5 ODS	0.626	110.8	<.0001	0.000124	<.0001	0.451	<.0001
6	6 ABW	0.492	63.9	<.0001	0.000063	<.0001	0.504	<.0001
7	7 HYL	0.237	20.4	<.0001	0.000048	<.0001	0.509	<.0001
8	8 BW	0.178	14.2	<.0001	0.000039	<.0001	0.517	<.0001
9	9 VL	0.174	13.8	<.0001	0.000033	<.0001	0.534	<.0001
10	10 ODP	0.084	6.0	<.0001	0.000030	<.0001	0.540	<.0001
11	11 ESOP	0.048	3.3	0.0011	0.000028	<.0001	0.541	<.0001

* DGR = distance to guide ring from anterior end; HW = head width; TL = tail length; L = body length; ODS = odontostyle; ABW = abody width at anus; HYL = hyaline length of tail; BW = body width; VL = distance from anterior end to vulva; ODP = odontophore; ESOP = esophagus length.

TABLE 5. Error rate of stepwise discriminant analysis using selected variables.

Variable combination*	Error rate
DGR, HW, TL, L, ODS, ABW, HYL, BW, VL, ODP, ESOP	0.040
DGR, HW, TL, L, ODS, ABW, HYL, BW, VL, ODP	0.043
DGR, HW, TL, L, ODS, ABW, HYL, BW, VL	0.043
DGR, HW, TL, L, ODS, ABW, HYL, BW	0.048
DGR, HW, TL, L, ODS, ABW, HYL	0.045
DGR, HW, TL, L, ODS, ABW	0.044
DGR, HW, TL, L, ODS	0.066
DGR, HW, TL, L	0.110
DGR, HW, TL	0.143
DGR, HW	0.246
DGR	0.547

* DGR = distance to guide ring from anterior end; HW = head width; TL = tail length; L = body length; ODS = odontostyle; ABW = abody width at anus; HYL = hyaline length of tail; BW = body width; VL = distance from anterior end to vulva; ODP = odontophore; ESOP = esophagus length.

lected from 1999 to 2001. The species, associated plants, and locations are listed in Table 1. Specimens were examined using a compound microscope with Nomarski interference contrast optics (Nikon, Melville, NY). Measurements were made by use of an ocular micrometer and a Nikon drawing tube. All measurements were in micrometers unless otherwise noted. Voucher specimens were deposited in the collection of the Nematology Laboratory, USDA, ARS, Beltsville, Maryland as either voucher specimens or as type material.

The morphometric data were processed and analyzed (Ye, 1996). For each species population, 15 morphological characters were measured, i.e. body length (L), distance of vulva from the anterior end (VL), head width (HW), odontostyle length (ODS), odontophore length (ODP), distance of guide ring from anterior end (DGR), basal bulb length (BBL), basal bulb width (BBW), esophagus length (ESOP), body width (BW), anterior ovary length (G1), posterior ovary length (G2), tail length (TL), anal body width (ABW), hyaline tail tip length (HYL). The ratios a (body length/body width), b (body length/esophagus length), c (body length/body width), and c' (tail length/anal body width) as well as the percentages V (distance of vulva from the anterior end/body length × 100), H% (hyaline tail length/tail length × 100), G1% (G1/body length × 100), and G2% (G2/body length × 100); and TSL (odontostyle length + odontophore length) were generated within the routine. These morphometrics covered many of the morphometric features of the species but did not cover such morphological aspects as head shape, amphid shape, tail shape, or the frequency of males.

Interspecies variation of Longidorus species from Arkansas: The aforementioned nine *Longidorus* species were studied for interspecies variation. The Student's *t* test was conducted (SAS 8.02, SAS Institute Inc, Cary, NC). The results were used to define the interspecies variation of these nine species and to screen for the most useful variables for further discriminant analysis.

Stepwise discriminant analysis and canonical discriminant analysis: Stepwise discriminant analysis was used to determine the best combination of variables that would separate the nine species of *Longidorus* collected in Arkansas. Canonical discriminant analysis is a dimension-reduction technique related to stepwise discriminant analysis. Given a set of classification variables, canonical discriminant analysis derives canonical variables (linear combinations of the variables) that summarize between-class variation. The species from Arkansas and their measurements, including those selected for this analysis, are listed in Table 2. The variables included in the initial function were DGR, HW, TL, L, ODS, ABW, HYL, BW, VL, ODP, and ESOP. The variables used to generate canonical variables are based on error rate by using different combination of variables. The pooled within canonical structure and pooled within class standardized canonical coefficients were used to determine each variable's contribution to the discriminant function. Three canonical variables were generated to illustrate how the species are grouped and separated. Stepwise and canonical discriminant analyses were performed with the program SAS 8.02 STEPDISC and DISCRIM procedures.

RESULTS

Interspecies variation of Longidorus species: All the variables were useful to some extent in discriminating the nine species examined by Student's *t* test (Table 2).

TABLE 6. Canonical structure and standardized canonical coefficients.

	Pooled within canonical structure			Pooled within-class standardized canonical coefficients		
Variable*	Can1	Can2	Can3	Can1	Can2	Can3
DGR	0.7520	0.3978	0.2000	1.1556	0.2502	0.3110
HW	−0.1818	0.6559	−0.0149	−0.3188	0.5816	−0.0324
TL	−0.1466	−0.3600	0.7347	−0.1249	−0.4538	0.7577
L	−0.1609	0.5819	0.4637	−0.3897	0.5116	0.5880
ODS	0.1304	−0.0521	−0.1316	−0.1358	−0.4782	−0.4534
ABW	−0.0531	0.4133	−0.1022	−0.1836	0.0799	−0.4729

* DGR = distance to guide ring from anterior end; HW = head width; TL = tail length; L = body length; ODS = odontostyle; ABW = abody width at anus.

TABLE 7. Canonical discriminant analysis of the DISCRIM procedure.

Canonical variable	Canonical correlation	Adjusted canonical correlation	Approximate standard error	Squared canonical correlation	Eigenvalue	Difference	Proportion	Cumulative
1	0.98524	0.984907	0.001233	0.970698	33.1277	21.6212	0.6264	0.6264
2	0.959188	0.958425	0.003364	0.920041	11.5065	6.1289	0.2176	0.8439
3	0.918259	0.917282	0.006597	0.8432	5.3775	3.5929	0.1017	0.9456
4	0.800552	0.798265	0.015108	0.640883	1.7846	1.0684	0.0337	0.9793
5	0.646016	0.642838	0.024513	0.417336	0.7163	0.3392	0.0135	0.9929
6	0.523278		0.030551	0.273819	0.3771		0.0071	1

The means of each variable between some species were significant (P = 95%), but usually these differences greatly overlap (except the DGR in *L. diadecturus,* which is distinctly different from that of the other eight species). The variables L, VL, DGR, HW, BBL, BBW, ABW, TL, and HYL proved to be good characters to distinguish these nine species. The variables BW, ODS, ODP, a, b, and c are less important. The least important variables were V, H%, G1, G1, G1%, and G2%. Most of the variables had ca. 5% to 15% coefficients of variation (CV) to the means, but G1, G2, G1% and G2% had ca. 30% CV. The variables HW, ODS, DGR, and V have the least variation (Table 3).

Stepwise discriminant analysis of Longidorus species: Stepwise discriminant analysis found all the variables to be significant ($P < 0.0001$) with respect to their partial r^2 (Table 3). The most important variable for discriminating the species was DGR with the partial r^2 0.9529, followed by HW, TL, ODS, ABW, HYL, BW, VL, ODP, and ESOP (Table 4). Thus, the variables in stepwise discriminant analysis were selected in this order. Table 5 lists the error rates for each group of selected variables. The error rate for the top six variables including DGR, HW, TL, L, ODS, and ABW was 0.0444, which was similar to the error rate for all 11 variables (0.0401). Therefore, only DGR, HW, TL, L, ODS, and ABW were used to generate canonical variables. Six canonical variables were generated to find the optimal combinations of

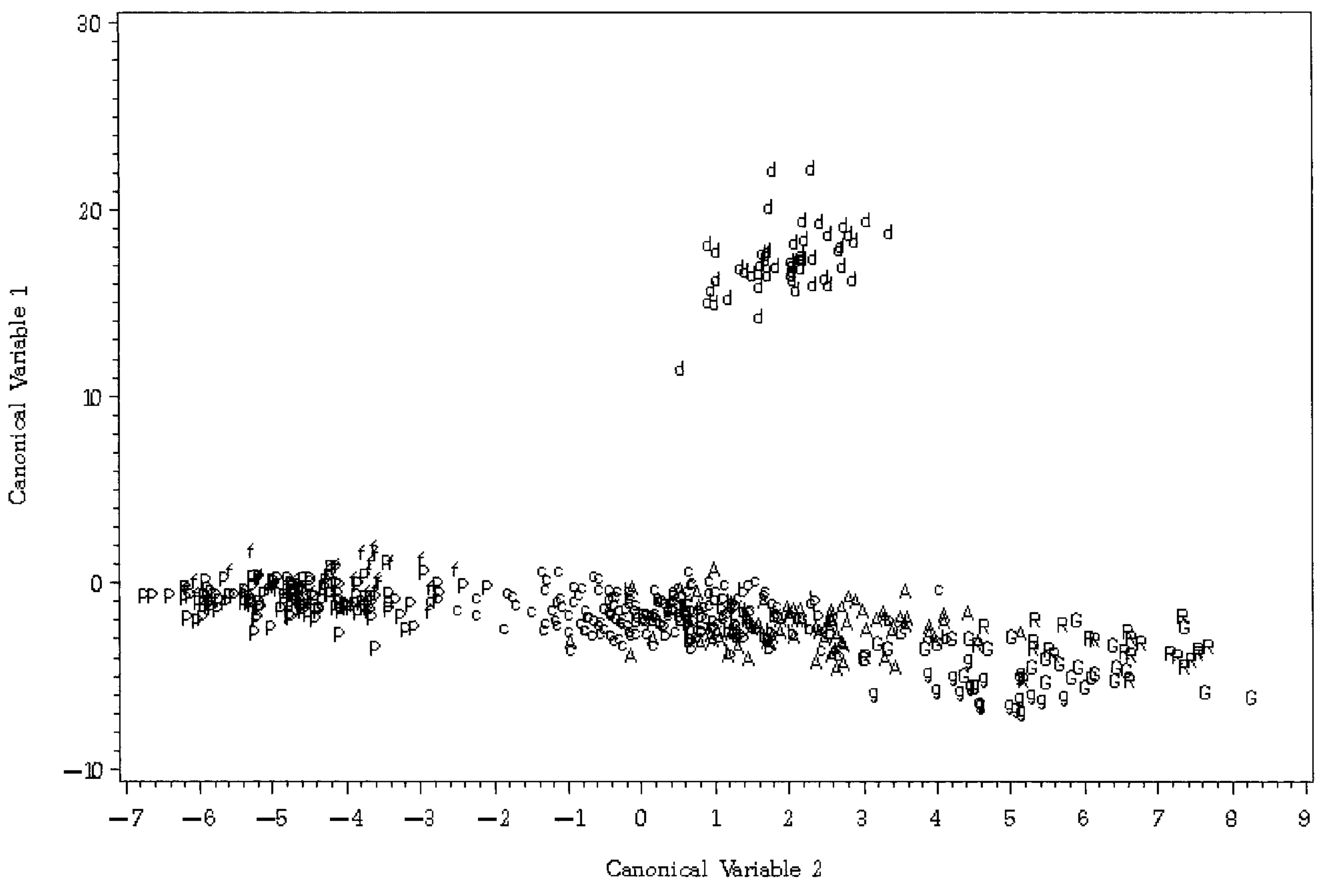

FIG. 1. Plot of the canonical variables 1 and 2 of nine *Longidorus* species from Arkansas. A = *L. biformis,* b = *L. breviannulatus,* c = *L. crassus,* d = *L. diadecturus,* f = *L. fragilis,* g = *L. glycines,* G = *L. grandis,* P = *L. paralongicaudatus,* R = *L. paravineacola.*

variables needed to discriminate between species. Table 6 lists the pooled within canonical structure and pooled within-class standardized canonical coefficients. Canonical variable 1 had the highest correlation with DGR (0.7520) followed by ODS (0.1304); therefore, separation of the groups on this axis was due mainly to differences in the guide ring position. Canonical variable 2 had the greatest correlation with HW (0.6559), followed by L (0.5819), ABW (0.4133), and DGR (0.3978). Canonical variable 3 was most correlated with TL (0.7347), followed by L (0.4637) and DGR (0.2000). The variance associated with the selected first three canonical variables was 95% of the total variance (Table 7). How the species were grouped and separated using these three canonical variables is illustrated in Figures 1, 2 and 3. Individuals of the same species are grouped together; however, overlap often makes species separation difficult. Canonical variables 1 and 2 (Fig. 1) distinctly separated *L. diadecturus* from the other species, of which *L. fragilis* and *L. paralongicaudatus* are in one group; *L. paravineacola, L. grandis,* and *L. glycines* are in a second group; and *L. crassus, L. breviannulatus,* and *L. biformis* are in a third group. Canonical variables 1 and 3 (Fig. 2) also distinctly separated *L. diadecturus* from all other species, of which *L. fragilis* and *L. biformis* were in one group, and the remaining species were in a second group. Canonical variables 2 and 3 (Fig. 3) separated *L. biformis, L. fragilis, L. paralongicaudatus* and *L. crassus* into separate groups, whereas *L. diadecturus* and *L. breviannulatus* grouped together. *Longidorus glycines, L. paravineacola* and *L. grandis* were in another group.

Discussion

This study evaluated stepwise and canonical analysis as a method for grouping and distinguishing nine *Longidorus* species from Arkansas by morphometric parameters. Canonical analysis based on female average morphometric characters including DGR, HW, TL, L, ODS, ABW, HYL, BW, VL, ODP, and ESOP allowed examination of the grouping and morphometric relationships of these *Longidorus* species. The individuals of the same species were grouped together and often were separated from the other species.

Interspecies variation based on these nine species of *Longidorus* found in Arkansas indicated that all characters measured were useful in differentiating these species. However single characters were usually insufficient to differentiate species because of the high degree of variability, even within the same population. Means of

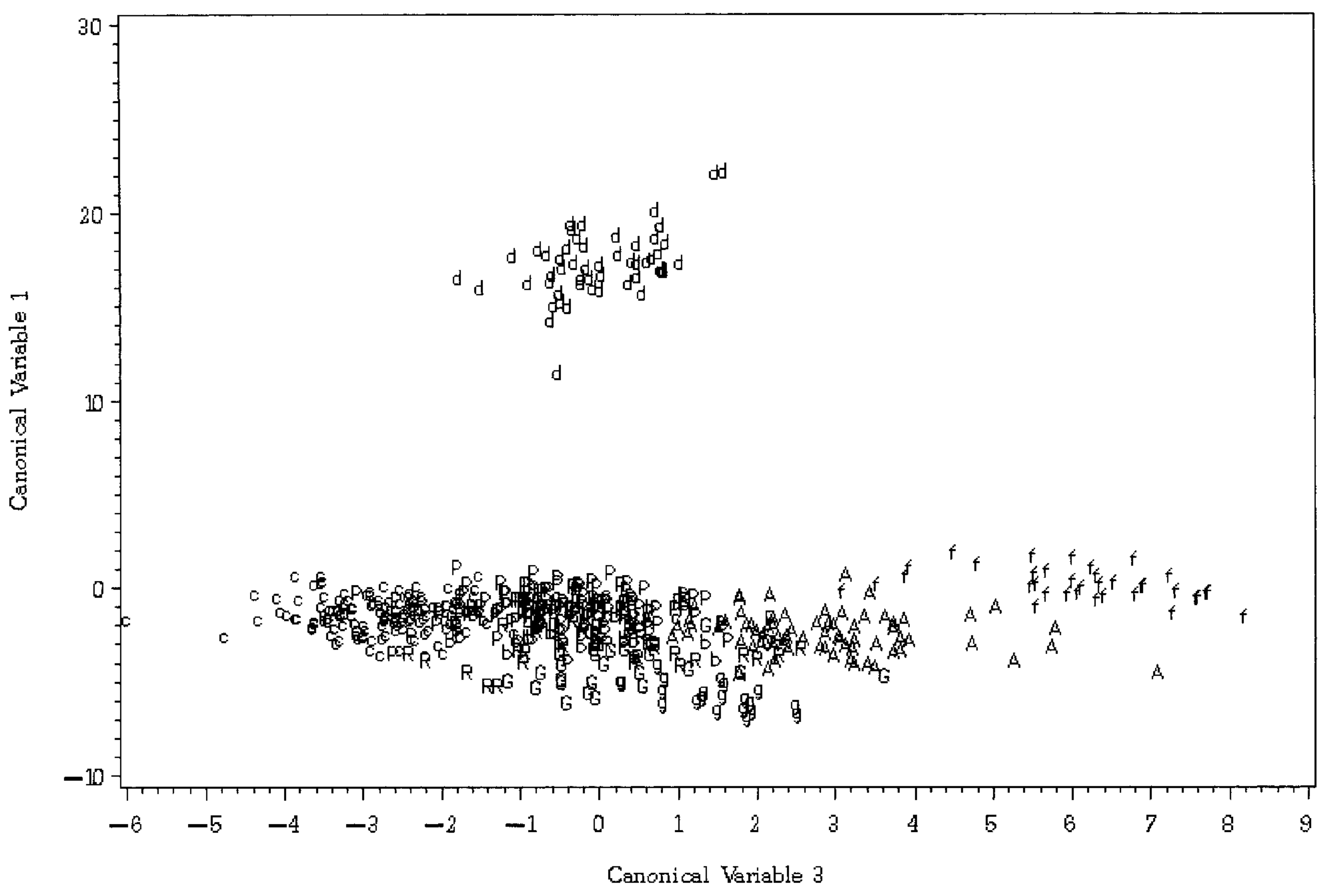

FIG. 2. Plot of the canonical variables 1 and 3 of nine *Longidorus* species from Arkansas. A = *L. biformis,* b = *L. breviannulatus,* c = *L. crassus,* d = *L. diadecturus,* f = *L. fragilis,* g = *L. glycines,* G = *L. grandis,* P = *L. paralongicaudatus,* R = *L. paravineacola.*

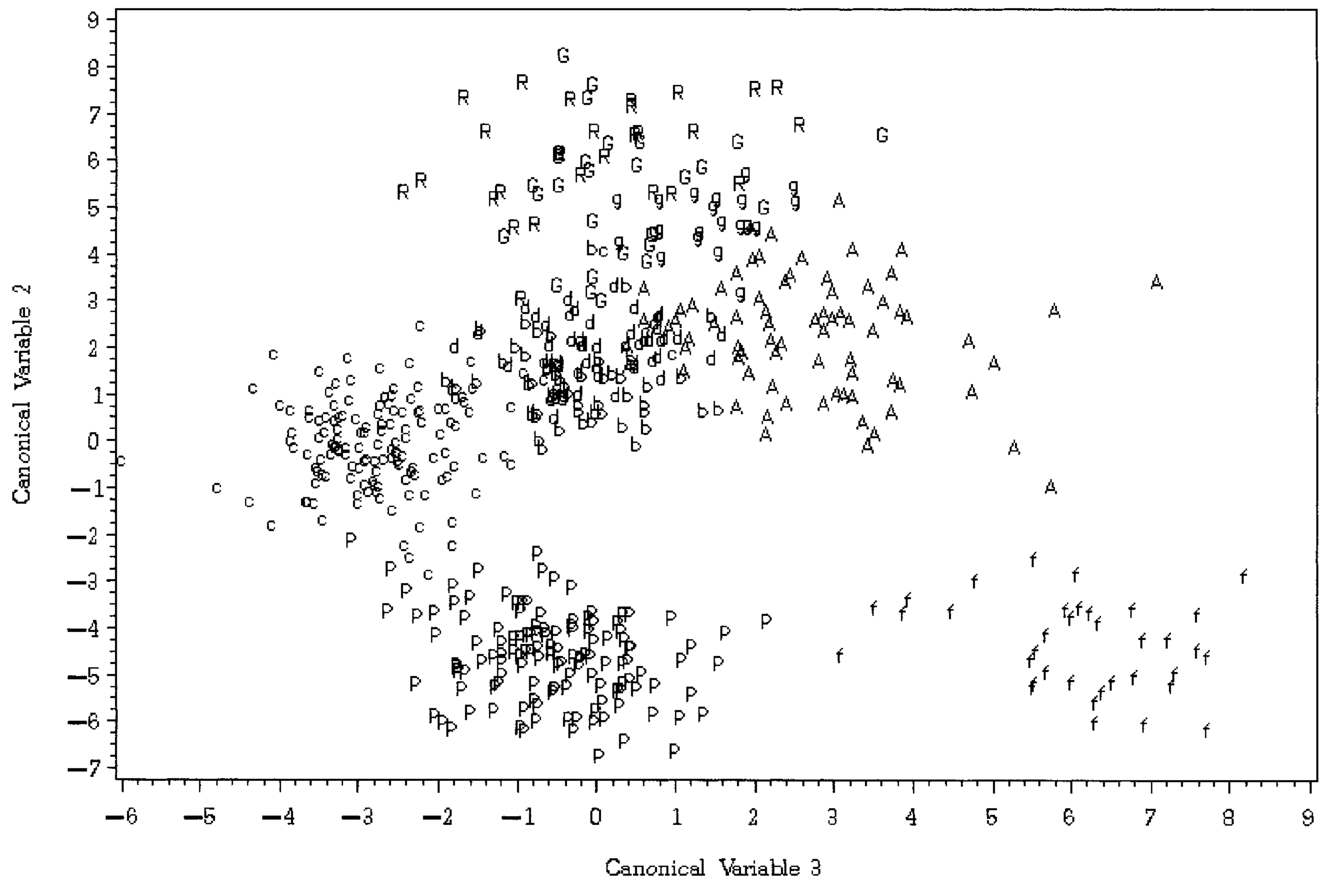

FIG. 3. Plot of canonical variables 2 and 3 of nine *Longidorus* species from Arkansas. A = *L. biformis,* b = *L. breviannulatus,* c = *L. crassus,* d = *L. diadecturus,* f = *L. fragilis,* g = *L. glycines,* G = *L. grandis,* P = *L. paralongicaudatus,* R = *L. paravineacola.*

the above variables were different between some species; however, a high degree of overlap often exists except in the DGR in *L. diadecturus,* which was distinctly different from that of the other eight species. Thus, this species can be easily identified by this single character from all the other Arkansas *Longidorus* species. The characters DGR, HW, TL, L, ODS, ABW, HYL, BW, VL, ODP, ESOP, BBL, and BBW were useful for distinguishing these nine species, whereas a, b, and c were less useful. The least important characters were V, H%, G1, G2, G1%, and G2%, which showed great overlap between species. Most of the variables have about a 5% to 15% CV, however G1, G2, G1%, and G2% have about a 30% CV and thus were not used in the stepwise discriminant analysis.

Stepwise discriminant analysis indicated that the six variables DGR, HW, TL, L, ODS, and ABW were the most important parameters for discriminating the nine Arkansas species. The scatterplots for the first three canonical variables (accounting for 95% of the total variation) successfully grouped and separated most of the *Longidorus* species from Arkansas; however, *L. grandis, L. glycines* and *L. paravineacola* could not be completely separated by these six variables only. Therefore, for these morphometrically similar species further discrimination using additional characters such as head shape, tail shape, incidence of males, and DNA sequences are necessary.

Stepwise discriminant analysis ranks the most useful morphometric characters for use in species discrimination. The optimum variables used to produce canonical variables were determined by comparing the error rates. The scatterplots displaying canonical variables show all the individual observations; thus, any overlap between populations or species can readily be examined. Stepwise and canonical analysis are recommended as useful tools for species discrimination of *Longidorus* species.

LITERATURE CITED

Brown, D. J. F., R. Neilson, T. Connolly, and B. Boag. 1997. An assessment of morphometric variability between populations of *Longidorus vineacola* Sturhan & Weischer, 1964 (Nematoda: Longidoridae) and morphologically related species. Systematic Parasitology 37:93–103.

Cho, M. R., and R. T. Robbins. 1991. Morphological variation among 23 *Xiphinema americanum* populations. Journal of Nematology 23:134–144.

Griesbach, J. A., and A. R. Maggenti. 1990. The morphometrics of *Xiphinema americanum sensu lato* in California. Revue de Nematologie 13:93–103.

Lamberti, F., and A. Ciancio. 1993. Diversity of *Xiphinema america-*

num-group species and hierarchical cluster analysis of morphometrics. Journal of Nematology 25:332–343.

Lamberti, F., and A. Ciancio. 1994. The relationship between species within the *Xiphinema americanum*-group (Nematoda: Dorylaimida). EPPO Bulletin 24:475–484.

Luc, M., and J. F. Southey. 1980. Study on biometrical variability in *Xiphinema insigne* Loos, 1949 and *X. elongatum* Schuurmans Stekhoven and Teunissen, 1938; description of *X. savanicola* n. sp. (Nematoda: Longidoridae) and comments on the thelytokous species. Revue de Nematologie 3:243–269.

Ye, W. M. 1996. Applying Microsoft Works Spreadsheet in statistics for morphometric data of nematode identification. Afro-Asian Journal of Nematology 6:203–211.

Ye, W. M., and R. T. Robbins. 2003a. *Longidorus grandis* n. sp. and *L. paralongicaudatus* n. sp. (Nematoda: Longidoridae), two parthenogenetic species from Arkansas. Journal of Nematology 35:375–387.

Ye, W. M., and R. T. Robbins. 2003b. *Longidorus paravineacola* n. sp. (Nematoda: Longidoridae), a new species from Arkansas. Journal of Nematology 35:388–394.

Ye, W. M., and R. T. Robbins. 2004. *Longidorus biformis* n. sp. and *L. glycines* n. sp. (Nematoda: Longidoridae), two amphimictic species from Arkansas. Journal of Nematology 36:1–13.