Journal of Nematology 23(1):134–144. 1991.

Morphological Variation among 23 *Xiphinema americanum* Populations[1]

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Abstract: Morphometrics of 23 United States populations of *Xiphinema americanum* sensu lato, sharing the characteristics of an offset lip region and conoid tail, were examined and analyzed statistically by canonical discriminant analysis (CDA). Specimens were collected from Arkansas, Georgia, Tennessee, Mississippi, Florida, Oklahoma, California, and North Dakota. Eleven measurements and body ratios obtained from female specimens were used in the analysis. *Xiphinema americanum, X. bricolensis, X. californicum, X. citricolum, X. intermedium, X. tarjanense,* and *X. thornei,* and one undescribed species were identified among the 23 populations. Three groups—*X. americanum*-group, *X. californicum*-group, and *X. intermedium*-group (*X. intermedium* and *X. tarjanense*)—were formed and four populations belonging to four different species were separated consistently from these groups in CDA scatterplots of the 23 populations. Composition of the groups was somewhat related to the geographical origins of the populations in the groups. A population from California had morphometrics intermediate between *X. americanum* and *X. californicum.* Separation between the *X. americanum*-group and *X. californicum*-group in the CDA scatterplots was not as distinct as that between them and the *X. intermedium*-group or between any of the three groups and the four single outlying populations.

Key words: canonical discriminant analysis, morphology, morphometrics, *Xiphinema americanum, X. bricolensis, X. californicum, X. citricolum, X. intermedium, X. tarjanense, X. thornei.*

Xiphinema americanum Cobb was described in 1913 (5), with brief illustrations given of the anterior end and a male tail, and supplemented by Cobb in 1915 with a more detailed description of the female (6). Subsequent descriptions of the species show significant variation from the original description (21).

Lima (19) proposed an hypothesis that *X. americanum* was a complex of at least seven different species: *X. americanum, X. brevicolle, X. opisthohysterum,* and four undescribed species. Tarjan (26) suggested that morphological differences among 75 worldwide populations of *X. americanum* complex interpreted as geographical variants correlated with climatic influences.

Lamberti and Bleve-Zacheo (14) reviewed the taxonomy of the group and recognized 25 species within *X. americanum* sensu lato. They described 15 new species and defined the distribution of *X. americanum* sensu stricto as the eastern part of the North American continent. Seventeen species of *Xiphinema americanum* sensu lato have been described from North America, of which 10 were described by Lamberti and Bleve-Zacheo (14). Their work led to other descriptions and redescriptions of closely related species in the group (7,8,15,16,28).

Heyns (12) postulated that *X. americanum* sensu lato consists of a conglomerate of monosexual forms and does not represent a true biological species or group of species. In a study of South African populations of *X. americanum* sensu Heyns, 1974, Loots and Heyns (21) concluded that there was considerable intraspecific variation. They could not agree completely with either Tarjan (26) or Lamberti and Bleve-Zacheo (14). Tarjan's definition of *X. americanum* was too broad to explain the gaps between the European and American populations, and the species demarcation by Lamberti and Bleve-Zacheo was restricted to such a degree that it was impossible to discern intraspecific variation from valid specific differences. These problems of species demarcation were ascribed to the fact that *X. americanum* reproduces parthenogenetically (21).

Correctly identifying nominal species in the *X. americanum*- group is important be-

Received for publication 2 January 1990.

[1] Published with the approval of the director of the Arkansas Agricultural Experiment Station. Supported in part by grant #0403-05414-24-1199 from the US-USDA-CSRS-84-14384/Robbins.

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cause several are vectors of plant viruses (13,14,18). The discordance on the identity of species in the *X. americanum*-group has caused problems in communication between nematologists and extension workers and growers (4,17).

In plant nematology, canonical discriminant analysis (CDA) and similar statistical methods have been used as objective methods for assessing relative similarities among populations on the basis of selected combinations of variables (3,9,22). The objectives of this study were to assess morphological variation among populations of *X. americanum* sensu lato in the United States and to determine if morphological clines could be detected statistically by CDA.

Materials and Methods

Xiphinema americanum sensu lato specimens were obtained from several different sources. One was a collection of 20 populations from the southeastern United States made by Dr. Dayle H. Zanzinger in 1985–86 (Table 1). These populations were subjected to a tentative identification process in which specimens were heat killed and fixed in 2% formalin. Specimens were mounted in 2% formalin on temporary slides for examination, and direct measurements of length of odontostyle and odontophore, distance of guide ring from the anterior end, tail length, body width at the tail, hyaline length and width, tail shape, and head shape were made. On the basis of these observations, eight populations with measurements that varied the most from those of *X. americanum* sensu stricto were selected for statistical analysis. A second group of nematodes included 12 California populations collected by the second author between 1971 and 1978 and kept in 2% formalin; populations from Arkansas and Oklahoma collected by the first author in 1987 and 1989, respectively; and a North Dakota population sent by Dr. J. G. Baldwin. These 23 populations are listed in Table 2, and their geographical origins are shown in Figure 1.

Nematodes were extracted from the soil by the combined roiling–sieving and Baermann technique or by the roiling–sieving and mist extraction techniques. Nematodes were heat killed, fixed in 2–4% formaldehyde, and processed to pure glycerin by a modified Seinhorst technique (24). Permanently mounted specimens were measured with an ocular micrometer.

For each specimen, lip width, distance of guide ring from the anterior end, length of odontostyle and odontophore, total stylet length, esophagus length, distance of vulva from the anterior end, body width at the vulva, depth of vagina, tail length, body width at the anus, hyaline length and width, and total body length were measured. All measurements are given in micrometers unless noted otherwise. The ratios of a, b, c, c', J' (hyaline length/hyaline width), and V% were calculated.

The results were analyzed statistically using the SAS computer statistical package (25). Eleven of the twenty variables measured or calculated, representing most of the variation between populations, were selected for canonical discriminant analysis (CDA) based on the result of a STEPDISC procedure of SAS (25) and earlier works on the variability in *Xiphinema* species (3,9,21–23).

Results

All the populations included in the analysis had the general characteristics of *Xiphinema americanum* sensu lato; i.e., set off lip region, vulva at midbody, two equal genital tracts, conical tail, and males occurring rarely. The following were identified from the 23 populations included in the analysis (Table 2): *X. americanum* sensu stricto, *X. bricolensis* Ebsary, Vrain, & Graham, 1989, *X. californicum* Lamberti & Bleve-Zacheo, 1979, *X. citricolum* Lamberti & Bleve-Zacheo, 1979, *X. intermedium* Lamberti & Bleve-Zacheo, 1979, *X. tarjanense* Lamberti & Bleve-Zacheo, 1979, *X. thornei* Lamberti & Golden, 1986, one undescribed species, and a population with morphological characteristics intermediate between *X. americanum* and *X. californicum*. The means and standard deviations of the 11 variables used in the CDA are in Table 3.

TABLE 1. Location, host plant, and number of nematodes (N) measured for the tentative identification of *Xiphinema* species in 20 populations collected in the southeastern United States, 1985–86.

Location	Host plant	N	Tentative ID
Bay Minette, AL	Muscadine grape	100	*X. americanum*
Beaver Lake, AR	Juniper	69	*X. rivesi*
		76	*X. americanum*
Clarksville, AR	Apple	319	*X. americanum*
Clarksville, AR	Blackberry	238	*X. americanum*
Clarksville, AR	Dewberry	117	*X. americanum*
Clarksville, AR	Grape	145	*X. americanum*
Clarksville, AR	Peach	97	*X. americanum*
Eubanks, AR	Strawberry	42	*X. americanum*
Marianna, AR	Blackberry	17	*X. americanum*
White River, AR	Box elder	21	*X. americanum*
		81	*X. rivesi*
		3	*X. bakeri*
White River, AR	Sweet gum	50	*X. americanum*
		88	*X. rivesi*
		3	*X. bakeri*
DeFuniak Springs, FL	Muscadine, grass	98	*X. americanum*
Gainesville, FL	Alfalfa	79	*X. coxi*
		20	*X. americanum*
Gainesville, FL	Blueberry	98	*X. americanum* ?
		2	*X. krugi*
Jay, FL	Blueberry	50	*X. americanum* ?
Blue Ridge, GA	Apple	76	*X. americanum*
Pascagoula, MS	Grass	100	*X. americanum* ?
Newberry, SC	Apple	108	*X. americanum*
Clemson, SC	Strawberry	100	*X. americanum*
Spring Hill, TN	Strawberry	97	*X. americanum*

Characteristics of populations

Populations A–C: Morphometrics of these *X. americanum* populations fit very closely with those of the Canadian and Maryland populations of *X. americanum* by Lamberti and Bleve-Zacheo (14). These three populations had slightly longer odontostyles than the four populations included in the redescription of the species by Lamberti and Bleve-Zacheo (14).

Population D: Xiphinema intermedium having slightly rounded tail tips and a weakly set off lip region. Morphometrics fit closely with those of the *X. intermedium* types (14). Minor differences found were a higher c ratio and a lower c' ratio.

Population E: Xiphinema tarjanense was found together with *X. coxi* Tarjan, 1964. Lip region was distinctly set off from the body with widely expanded lips. The tail was conoid with a subdigitate tip. Relative to types of *X. tarjanense* (14), this population had a higher a ratio, narrower lip region, and a more anterior vulva.

Populations F–H: Xiphinema intermedium with morphometrics very similar to the species description, but the vulva was slightly more anterior than that of the type population.

Population I: Xiphinema citricolum with the button-shaped lip region distinctly separated from the body and a conoid tail with a slightly subdigitate tip. Minor differences from the type population were smaller lip widths and shorter hyaline length.

Population J: Morphometrics of this population were unlike those of any *Xiphinema* species, and it probably represents an undescibed species. Specimens had the lip region weakly separated from the body, the tail was slightly rounded at the tip, and the total stylet length was shorter than stylets of other species in the *X. americanum*-group.

Population K: Xiphinema californicum with

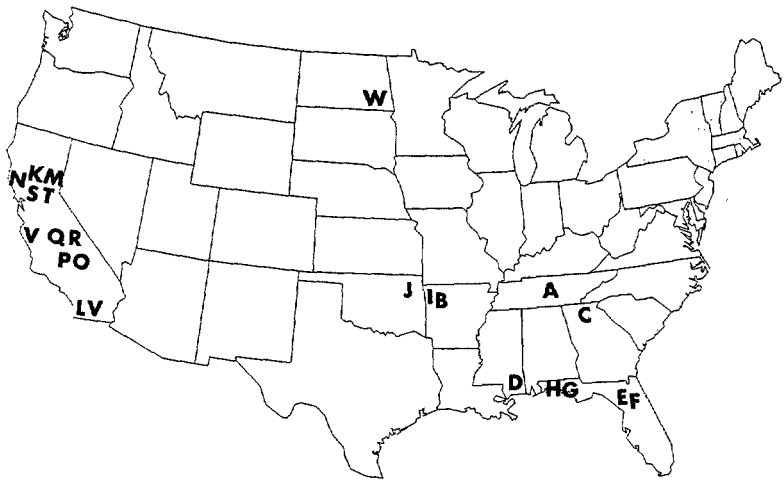

FIG. 1. Geographical origins of the 23 *Xiphinema americanum*-group populations included in the canonical discriminant analysis.

morphometrics similar to the Mexican populations (14), but the anal body width was larger.

Population L: Xiphinema californicum with the lip region distinctly set off, tail slightly rounded at the tip, and total body length shorter than the type population (14).

Population M: Xiphinema californicum with a wider anal body width and a shorter total body length relative to the type and two other populations (14).

Population N: Xiphinema bricolensis with lip region distinctly set off and morphometrics similar to the type population from British Columbia (7). Anal body width is wider than any population in the description (7) or the *X. americanum*-group.

Populations O, Q, S: Xiphinema californicum with populations Q and S having longer odontostyles than the type and two other populations (14). Population S had a wider anal body width and more posteriorly positioned vulva than the described populations (14).

Population P: Morphometric characters were intermediate between *X. americanum* and *X. californicum.* Body measurements were closer to *X. californicum* (14) except for shorter body length and slightly longer odontostyle; however, body ratios were closer to those of *X. americanum* (14).

Population R: Xiphinema americanum with the vulva slightly more anterior than in the four populations of Lamberti and Bleve-Zacheo (14).

Populations T–V: Xiphinema californicum with populations T and V having more posterior vulvas and population V a longer odontostyle than in the type population (14). Lip region of specimens in these populations were all distinctly set off from the body with population T having button-shaped lips and populations U and V having flat expanded lips.

Population W: Xiphinema thornei with a slightly more posterior vulva and a shorter odontophore and odontostyle than the type and four other populations (16).

Canonical discriminant analysis

Of the 11 canonical variables calculated, the first five accounted for 93% of the variance (Table 4). The first canonical variable was interpreted as a ratio between the combination of total stylet length and a and the combination of hyaline length, c, and J′. The second canonical variable was interpreted as the ratio between the combination of body width at the vulva, tail length, and a and the combination of anal body width, total body length, and c′. The third canonical variable was interpreted as the ratio between the combination of anal body width, a, and c′ and the combination of tail

TABLE 2. Populations of *Xiphinema americanum* sensu lato (Pop.), number of nematodes measured (N), location, host, shape of head and tail tip, and species included in the canonical discriminant analysis.

Pop.	N	Location	Host	Shape†		Species
				Tail	Head	
A	23	Spring Hill, TN	Strawberry	S	+	*X. americanum*
B	19	Clarksville, AR	Peach	S	+	*X. americanum*
C	19	Blue Ridge, GA	Apple	S	+	*X. americanum*
D	24	Pascagoula, MS	Grass	Sr	−, +	*X. intermedium*
E	13	Gainesville, FL	Alfalfa	Csd	++	*X. tarjanense*
F	23	Gainesville, FL	Blueberry	S	+	*X. intermedium*
G	23	DeFuniak Sp., FL	Grass	S	+	*X. intermedium*
H	24	Jay, FL	Blueberry	S	+	*X. intermedium*
I	27	Fayetteville, AR	?	Csd	++	*X. citricolum*
J	30	Tulsa, OK	Grass	Sr	−, +	*X.* ?
K	23	Napa Co., CA	Vineyard	S	+	*X. californicum*
L	30	San Diego Co., CA	Citrus	Sr	++	*X. californicum*
M	24	Napa Co., CA	Grape	Sr	+	*X. californicum*
N	23	Marin Co., CA	Juncus	Cr	+, ++	*X. bricolensis*
O	24	Ventura, CA	Citrus	S	++	*X. californicum*
P	25	Kern Co., CA	Composite	S	+	*X.* ?
Q	27	Fresno Co., CA	Alfalfa	S	++	*X. californicum*
R	29	Fresno Co., CA	Plum	S	+	*X. americanum*
S	8	Napa Co., CA	Grape	S	+	*X. californicum*
T	5	Napa Co., CA	Grape	S	++	*X. californicum*
U	5	Imperial Co., CA	Bursage	S	++	*X. californicum*
V	8	Santa Cruz, CA	Rose	S	++	*X. californicum*
W	9	Wahpeton, ND	Cottonwood	S	+	*X. thornei*

† S = sharp tip, Sr = sharp with roundish tip, Cr = conoid with round tip, Csd = conoid with subdigital tip, − = slightly set off, + = moderately set off, ++ = distinctly set off.

length and c. Total body length, a, and body width were the factors contributing most to the fourth canonical variable, whereas anal body width, c', a, and tail length were the factors contributing most to the fifth canonical variable.

Two dimensional scatterplots of population means of canonical variables of all combinations of the first five canonical axes were generated. Figures 2–5 were selected to show the relative positions of the population means on two axes of canonical variables to compare their grouping with the morphological identification of each populations. Three groups (groups of populations not distinctly separated in the scatterplots), four species represented by single populations, and one population (P) intermediate between two groups could be distinguished based on the scatterplots of the first four axes (Figs. 2–4).

Populations D–H formed the *H. intermedium*-group. Population E, identified as *X. tarjanense,* was included also in this group (Figs. 2, 3, 5) and was separated to some degree only in the scatterplot of the first and fourth axes (Fig. 4). The populations A–C, R formed the *X. americanum*-group, whereas populations K–M, O, Q, S–V formed the *X. californicum*-group. Population P was always located between the *X. americanum*-group and the *X. californicum*-group. Populations I, J, N, W were identified as distinct species and always distinctly separated from the three groups. Compositions of the groups were fairly consistent in all the scatterplots except for the plot of the second and the third canonical axes (Fig. 5). In Figure 5, *X. intermedium*-group, *X. americanum*-group, and *X. californicum*-group amalgamated into a still larger group well apart from the distinct species represented by populations I, J, N, W.

Populations in the *X. intermedium*-group were all from Florida except D, which was from Mississippi. All populations in the *X. americanum*-group were identified as *X.*

TABLE 3. Means and standard deviations (in parentheses) of 11 variables used in canonical discriminant analysis of 23 *Xiphinema* populations.

Pop.	N	Lip width	Total stylet	Body width	Tail length	Hya-line length	Anal body width	Total body length	a	c	c′	J′
A	23	9.8	126.9	34.0	33.4	7.9	20.0	1,668	49.6	50.6	1.7	1.1
		(0.4)	(3.6)	(2.1)	(1.6)	(0.8)	(1.4)	(123.2)	(2.4)	(3.9)	(0.1)	(0.1)
B	19	9.8	125.1	33.6	33.1	7.5	20.6	1,632	48.6	49.5	1.6	1.0
		(0.5)	(4.3)	(1.5)	(2.1)	(0.7)	(1.0)	(90.0)	(2.5)	(3.9)	(0.1)	(0.1)
C	19	10.2	128.1	35.1	32.2	6.8	20.6	1,697	48.4	52.9	1.6	0.9
		(0.4)	(3.2)	(1.7)	(2.5)	(1.0)	(1.2)	(121.0)	(2.5)	(4.0)	(0.2)	(0.1)
D	24	10.7	127.0	38.1	29.6	10.2	22.2	1,579	41.4	53.5	1.3	1.0
		(0.5)	(4.1)	(1.7)	(1.7)	(1.1)	(1.1)	(102.1)	(1.9)	(4.6)	(0.1)	(0.1)
E	13	10.8	123.2	32.6	31.8	10.9	19.1	1,448	44.4	45.8	1.7	1.4
		(0.4)	(4.5)	(1.5)	(1.9)	(1.9)	(1.0)	(97.9)	(2.4)	(4.5)	(0.1)	(0.2)
F	23	10.5	123.3	37.9	31.6	10.4	22.1	1,557	41.1	49.5	1.4	1.1
		(0.5)	(2.4)	(2.2)	(1.8)	(0.9)	(1.3)	(114.0)	(2.3)	(5.0)	(0.1)	(0.1)
G	23	10.2	120.8	37.0	33.1	10.6	21.2	1,605	43.6	48.6	1.6	1.3
		(0.5)	(4.3)	(2.6)	(1.9)	(1.2)	(1.5)	(84.8)	(3.3)	(3.6)	(0.1)	(0.2)
H	24	10.0	120.1	36.8	32.8	10.1	20.9	1,596	43.5	48.7	1.6	1.2
		(0.2)	(2.4)	(2.7)	(1.8)	(0.7)	(1.1)	(80.9)	(2.6)	(2.8)	(0.1)	(0.1)
I	27	10.2	136.4	36.8	33.4	10.3	21.1	1,686	46.0	50.7	1.6	1.3
		(0.4)	(3.0)	(2.4)	(1.7)	(0.9)	(0.8)	(98.6)	(3.0)	(3.8)	(0.1)	(0.1)
J	30	10.3	112.5	36.6	29.7	6.8	24.0	1,809	49.5	61.1	1.2	0.7
		(0.5)	(4.0)	(2.1)	(2.3)	(1.0)	(1.7)	(129.5)	(2.7)	(5.4)	(0.1)	(0.1)
K	23	10.0	136.1	33.7	33.5	7.2	22.1	1,864	55.4	55.7	1.5	0.8
		(0.2)	(3.9)	(1.8)	(1.7)	(0.8)	(1.1)	(78.0)	(3.2)	(3.3)	(0.1)	(0.1)
L	30	10.7	132.8	33.2	31.0	5.7	20.4	1,788	54.0	57.9	1.5	0.7
		(0.7)	(3.2)	(1.9)	(1.9)	(0.7)	(1.0)	(80.2)	(2.7)	(4.2)	(0.1)	(0.1)
M	24	10.0	141.0	32.8	32.6	(6.9)	22.3	1,829	55.8	56.2	1.5	0.8
		(0.2)	(4.5)	(1.9)	(1.7)	(0.9)	(1.5)	(101.0)	(2.4)	(3.8)	(0.1)	(0.1)
N	23	11.2	132.7	39.9	33.5	6.4	26.9	2,031	51.0	60.7	1.3	0.7
		(0.5)	(3.9)	(2.0)	(2.2)	(1.0)	(1.6)	(101.9)	(2.0)	(3.5)	(0.1)	(0.1)
O	24	10.6	137.7	34.8	32.5	6.0	22.1	2,013	58.1	62.1	1.5	0.7
		(0.6)	(4.8)	(2.5)	(2.1)	(0.6)	(1.0)	(96.7)	(4.5)	(4.3)	(0.1)	(0.1)
P	25	10.0	128.4	32.6	32.4	6.0	19.9	1,675	51.4	51.7	1.6	0.8
		(0.0)	(3.9)	(1.4)	(1.0)	(0.6)	(0.8)	(57.0)	(2.7)	(2.4)	(0.1)	(0.1)
Q	27	10.0	144.1	30.7	31.5	5.7	19.3	1,940	63.3	61.7	1.6	0.7
		(0.4)	(3.9)	(1.8)	(1.5)	(0.5)	(1.0)	(121.2)	(3.7)	(4.5)	(0.1)	(0.1)
R	29	10.5	123.5	32.3	33.2	6.8	19.5	1,575	48.7	47.6	1.7	1.0
		(0.6)	(3.7)	(1.2)	(2.2)	(0.8)	(1.1)	(81.8)	(1.5)	(4.1)	(0.1)	(0.1)
S	8	9.9	144.3	32.6	32.3	6.6	22.1	1,851	56.9	57.4	1.5	0.8
		(0.4)	(5.0)	(2.7)	(1.3)	(1.2)	(1.4)	(114.6)	(2.9)	(3.0)	(0.1)	(0.1)
T	5	9.4	132.6	30.2	31.4	6.8	20.2	1,790	59.4	57.2	1.6	0.9
		(0.9)	(5.4)	(2.4)	(2.1)	(0.5)	(1.6)	(63.6)	(3.1)	(4.9)	(0.1)	(0.2)
U	5	10.4	140.4	29.8	31.6	6.8	18.8	1,888	63.4	60.1	1.7	0.9
		(0.5)	(2.1)	(2.4)	(2.1)	(1.1)	(0.8)	(144.1)	(3.2)	(7.4)	(0.2)	(0.2)
V	8	10.3	145.4	31.3	33.1	6.4	20.0	1,919	61.6	58.0	1.7	0.8
		(0.5)	(2.2)	(2.3)	(1.4)	(0.5)	(0.5)	(54.4)	(3.8)	(2.4)	(0.1)	(0.1)
W	9	10.1	126.9	40.0	30.3	6.4	23.7	2,033	50.9	67.2	1.3	0.7
		(0.3)	(3.1)	(2.1)	(1.7)	(0.7)	(1.1)	(86.7)	(1.7)	(5.6)	(0.1)	(0.1)
Grand mean	465	10.3	129.7	34.8	32.2	7.7	21.4	1,746	50.5	54.5	1.5	0.9
Total SD		(0.6)	(9.3)	(3.3)	(2.2)	(2.0)	(2.2)	(184.9)	(6.7)	(6.7)	(0.2)	(0.2)

All measurements are in μm.

TABLE 4. Canonical discriminant analysis of 11 variables of *Xiphinema* species females from 23 populations.

	Standardized canonical coefficients				
	Can. 1	Can. 2	Can. 3	Can. 4	Can. 5
Lip width	−0.0717	−0.1127	0.1037	0.2355	1.0390
Total stylet	1.2274	1.5577	1.2570	0.9675	0.1392
Body width	0.0362	2.2981	1.9779	−6.0331	1.9539
Tail length	−0.1057	2.4438	−2.6411	−1.4079	−4.0465
Hyaline length	−0.8205	0.5150	1.0640	−1.4945	−0.0710
Anal body width	−0.4005	−4.6085	3.1509	2.6335	3.8911
Total body length	0.5751	−2.5203	−0.7428	6.7867	−1.6747
a	1.4588	2.8876	2.4104	−8.8771	2.9816
c	−0.8863	−0.7799	−1.7119	−0.7346	−1.4321
c′	−0.2252	−4.4594	2.3975	2.2723	5.0265
J′	−0.6173	0.3436	−0.3950	1.1980	−0.1343
Canonical correlation	0.9549	0.9093	0.8382	0.7496	0.6658
Cumulative (%)	0.4917	0.7189	0.8311	0.8921	0.9300

americanum. Populations A–C, R were from Tennessee, Arkansas, Georgia, and California, respectively. Populations in the *X. californicum*-group were all from California and identified as *X. californicum*. Populations N and W were identified as *X. bricolensis* and *X. thornei*, respectively, and placed close together in Figure 2; however, these populations separated clearly in other plots (Figs. 3–5). Population I was identified as *X. citricolum* and was separated from other groups in Figures 2, 3, and 5 but was close to the *X. americanum*-group in Figure 4. Population J is an undescribed species and was distinctly separated from all populations and groups in all the scatterplots.

A scatterplot of individual nematodes belonging to the three groups, after removal of the distinct species I, J, N, W, was obtained on the first two canonical axes (Fig. 6). Significant overlap was observed between the *X. americanum*-group and the *X. californicum*-group with populations P and L connecting them. The *Xiphinema intermedium*-group and the *X. californicum*-group were separated with only a very slight overlap (Fig. 6). No significant differences were present between a CDA in which all 20 available variables were used and the CDA with 11 selected variables.

DISCUSSION

Results from CDA supported the morphometric identification of each population. The CDA derives a linear combination of the variables that have the highest

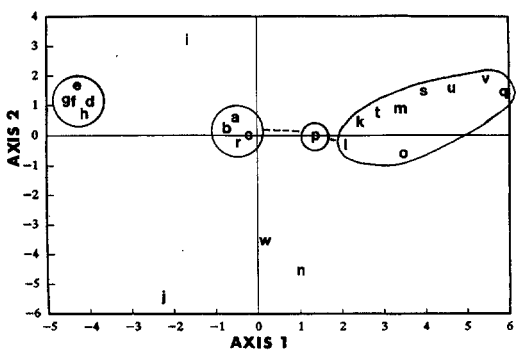

FIG. 2. Scatterplot of 23 *Xiphinema americanum*-group population means on the first and the second canonical axes with three groups circled and the intermediate population circled and possible relationships indicated with dashed lines.

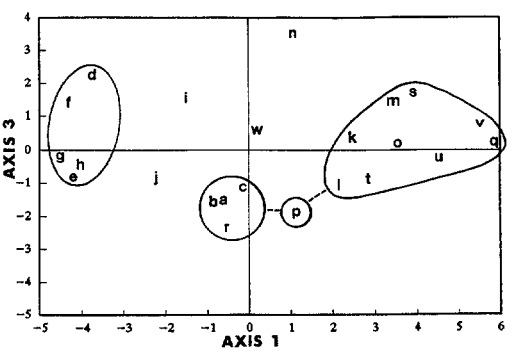

FIG. 3. Scatterplot of 23 *Xiphinema americanum*-group population means on the first and the third canonical axes with three groups circled and the intermediate population circled and possible relationships indicated with dashed lines.

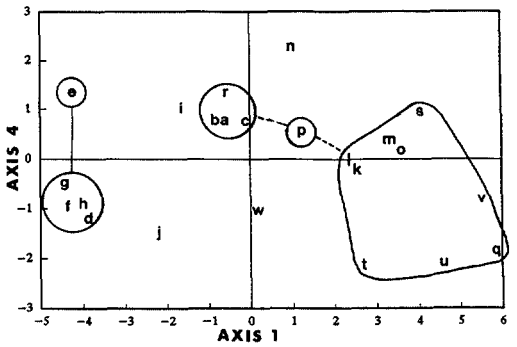

FIG. 4. Scatterplot of 23 *Xiphinema americanum*-group population means on the first and the fourth canonical axes with three groups circled and the intermediate population circled and possible relationships indicated with dashed lines.

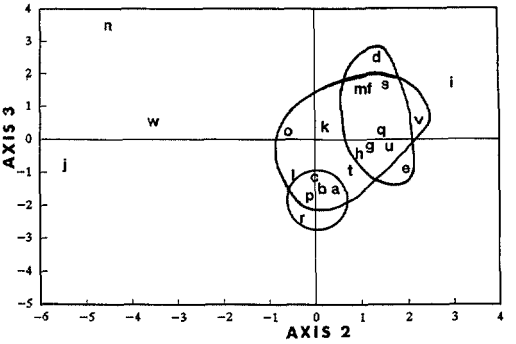

FIG. 5. Scatterplot of 23 *Xiphinema americanum*-group population means on the second and the third canonical axes with three groups circled.

possible multiple correlation with the populations.

The results obtained with the CDA and morphometric identification of each population indicate that morphological clines are present. The CDA formation of three groups and the separation of several species from these groups and from each other agreed well with the morphological identification of the populations.

The three groups were closely related to the geographic origin of the populations. All populations from Florida and one Mississippi population clumped into the *X. intermedium*-group. The geographical origin of the Mississippi population is in close proximity to the Florida populations (Fig. 1). Populations from Tennessee (A), Arkansas (B), Georgia (C), and California (R) merged into the *X. americanum*-group. All California populations except N, P, R clumped into the *X. californicum*-group. Population R was collected from ornamental plum trees, which in California often originate from the southeastern states; that may explain the merging of population R with the three southeastern U.S. populations in the *X. americanum*-group (i.e., the nematodes possibly were introduced from a southeastern state to California with plum saplings). Results of this study suggest that the geographical origin and the distribution of the three groups are closely related. However, geographical location alone was not the only factor correlated with morphometrics of populations of *X. diversicaudatum* (Micoletzki, 1922 & 1927) Thorne, 1939 (3) as biotopic differences had similar effects (1).

Luc and Southey (22) separated the closely related species, *X. insigne* Loos, 1949, *X. elongatum* Schuurmans Stekhoven & Teunissen, 1938, and *X. savanicola* Luc & Southey, 1980, using canonical variate analysis when morphometrics of individual nematodes were scatterplotted on the first two canonical axes. In this study, four species were distinctly separated from one another and the three groups (Figs. 2–5). The scatterplot of individual nematodes of the *X. americanum*-group and *X. californicum*-group, together with those of the intermediate population P, showed significant overlap, whereas the *X. intermedium*-group was separated somewhat from these groups (Fig. 6).

Morphometric characters separating the *X. intermedium*-group from the *X. americanum*-group and *X. californicum*-group were shorter body length, longer hyaline length, and larger J′ ratio. The morphometric differences separating the *X. californicum*-group from the *X. americanum*-group were greater body length, longer stylet, higher a and c ratios, and smaller J′ ratio. The variable J′ used in the CDA proved useful in the separation of the three groups and four species because less overlap was present between the populations than with the hyaline length and width. Georgi (9) also

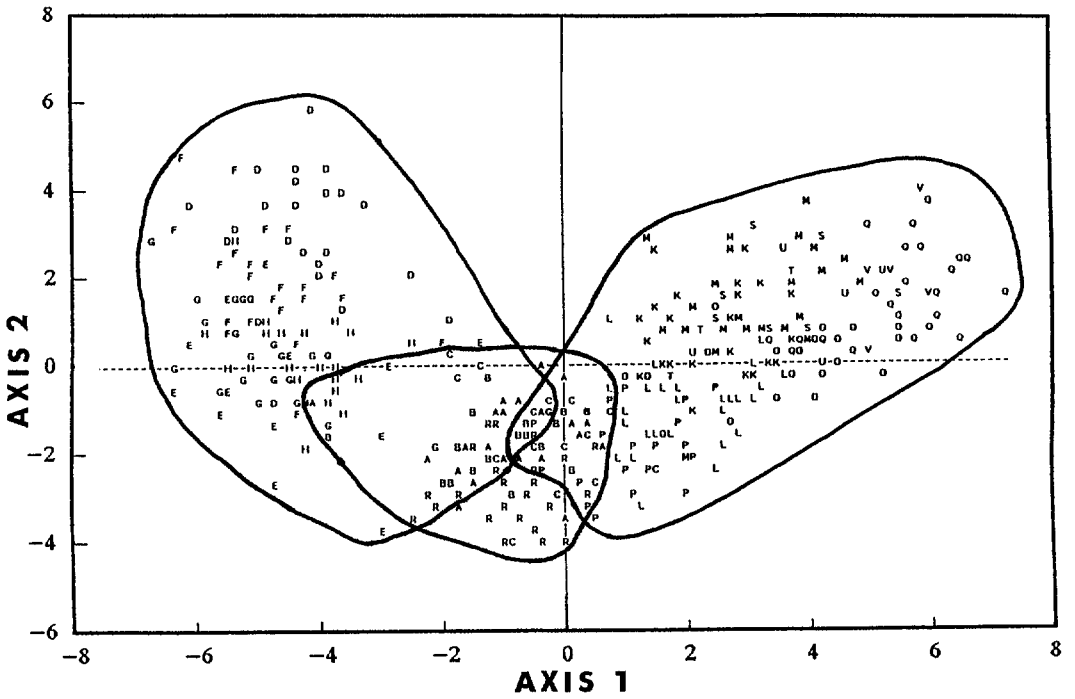

FIG. 6. Scatterplot of all individuals of the three *Xiphinema americanum*-groups on the first and the second canonical axes with the three groups circled.

found this ratio useful in distinguishing *X. rivesi, X. americanum,* and *X. californicum.*

Presence of the population P, intermediate between the *X. americanum*-group and *X. californicum*-group (Fig. 6), raises the question of separate species identity for *X. californicum* and *X. americanum.* Griesbach and Maggenti (10) also reported a *X. americanum* sensu lato population intermediate in morphology between *X. californicum* and *X. americanum* sensu stricto from California. The separation between the *X. intermedium*-group and the *X. americanum*-group was more distinct than that between the *X. americanum*-group and the *X. californicum*-group. Because only 8 of 22 populations were used in this study (Table 1), the possibility cannot be excluded that other populations exist with intermediate morphometric characteristics. The second canonical axis generally failed to separate the three groups which were clearly separated on the first canonical axis (Figs. 2–5). The amalgamation of the three groups into a single homogenous group in the scatterplot of the second and third canonical axes demonstrates their morphological similarity (Fig. 5). This amalgamation may be due to low variability among population morphometrics, especially in tail length, anal body width, and ratios of a, c, and c′, which were the main contributors for the second and third canonical variables. A study of variation in *Xiphinema* species from New York using CDA (9) showed that individual nematodes plotted on the first two canonical axes formed a continuum from eastern to western populations of *X. americanum,* through *X. californicum,* to *X. rivesi.*

The consistent separation of the four different species from the three groups (Figs. 2–5) provided additional objective evidence of the accuracy of species identifications made previously using morphometrics. However, the closeness of populations N and W (Figs. 2, 3, 5), identified as *X. bricolensis* and *X. thornei,* respectively, indicated that they are morphometrically close. Lamberti and Golden (16) suspected that the British Columbian populations described by Ebsary et al. (7) were *X. thornei* and not *X. occiduum.* Ebsary

et al. (8) later reported that the populations were *X. bricolensis,* which is widespread throughout the Okanagan and Similkameen valleys of British Columbia.

In a study of *Xiphinema* species from Surinam, Loof and Maas (20) observed both quantitative and qualitative variations in a species as well as continuous and discontinuous variations in dimensions. They concluded that body dimensions are unsatisfactory for distinguishing species, and qualitative characters should be given at least equal weight. Loots and Heyns (19) proposed that species separation becomes important only when other biological aspects such as host specificity, pathogenicity, or virus transmissibility can be correlated with morphological differences. Brown (2) demonstrated differences in the efficiency and ability of populations of *X. diversicaudatum* to transmit viruses. Tomato ringspot virus is reputed to be transmitted by six species in the *X. americanum*-group including *X. americanum* and *X. californicum.* Virus transmissibility cannot be used alone to identify species in the *X. americanum*-group (4).

Luc and Southey (22) discussed the taxonomic problem of thelytokous (parthenogenetic) species concluding that "thelytokous species which can be clearly defined by morphological and morphometric characters should be accepted as valid." However, the problem remains of defining when intraspecific variation is sufficiently large as to suggest interspecific difference. Griesbach and Maggenti (10) questioned the practice of separating thelytokous populations into species based solely on minor morphometric differences. Furthermore, their virus transmission study with seven California populations of *X. americanum* sensu lato, three strains of tomato ringspot virus, and a strain of tobacco ringspot virus showed that specimens of two California *X. americanum* populations were able to transmit tobacco ringspot virus (10). This result does not support the proposition by others (17) that the eastern and western U.S. populations of *X. americanum* sensu lato can be separated into species on the basis of the biological properties of virus transmission. Griesbach and Maggenti (11) synonymized *X. californicum* to *X. americanum* on the basis of a morphometric analysis of 12 specimens from each of one Pennsylvania, one New York, and 10 California populations in the *X. americanum*-group. This study does not support the synonymy made by Griesbach and Maggenti (11).

In this study, three groups were detected by the CDA when population means were used. Clear distinction between total populations within and between the groups could not be made because of overlap. More objective means, such as DNA probe analysis (27), might better be used to determine species in the *X. americanum*-group.

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