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A COMPARISON OF REPORTED VARIATION IN THE MORPHOMETRICS OF *XIPHINEMA DIVERSICAUDATUM* (NEMATODA: DORYLAIMIDA) AND THE EFFECTS OF SOME METHODS OF PREPARING SPECIMENS FOR EXAMINATION BY OPTICAL MICROSCOPY

by
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Since Micoletzky (1927) first described *X. diversicaudatum* the morphometrics of adults from different populations have been reported by several authors, including a redescription of the species (Goodey *et al.*, 1960; Erbenova, 1975; Hrzic, 1978; Martelli and Lamberti, 1967; Szczygiel, 1974; Teploukhova, 1975; Terlidou, 1967). The large differences reported in the morphometrics of *X. diversicaudatum* populations may be true differences due to biogeographical factors affecting the various populations (Brown and Topham, 1984) or may be artificats due to the effects of different methods of preparing the specimens for taxonomic examination (Goodey, 1959; Maggenti and Viglierchio, 1965; Stone, 1971; Curran and Hominick, 1981).

Few studies have been reported of the effects of preparation on members of the Longidoridae. Lamberti and Sher (1969) compared the effects of different preparation techniques on *L. africanus* females and reported that significant increases (+ 26%) or decreases (– 18%) occurred in several taxonomic ratios when compared with specimens prepared by a standard method. As no similar study had been reported for the genus *Xiphinema* it is not known if the use of different preparation techniques could account for the morphometrical variability reported between populations of *X. diversicaudatum.* Therefore, 28 combinations of killing, fixing and mounting specimens were tested and their effect on the morphometrics and taxonomic ratios of *X. diversicaudatum* examined and compared with those obtained from live specimens.

Table I - *Published means of morphometrics of* Xiphinema diversicaudatum *from different populations.*

		X. diversicaudatum populations										
		A	B	C	D	E	F	G	H	I	J	K
n	female	1	5	na	43	8	11	5	1	19	5	6
	male	1	2	na	33	3	14	6	1	7	0	4
L	female	4.0	4.4	4.4	4.9	3.6*	4.0	4.2	4.6	4.2	4.5	4.5
	male	4.3	4.2	4.3	4.9	4.5	4.1	4.2	4.7	4.2	—	4.5
a	female	72	78	76	74	66*	70	68	54	73	71	65
	male	81	78	70	76	79*	72	71	58	74	—	71
b	female	10.1	9.0	8.2	9.1	8.9*	8.9	8.3	9.1	8.2	9.0	8.9
	male	8.6	9.4	8.8	8.8	11.0*	8.6	8.7	8.8	8.5	—	8.6
c	female	96	85	88	78	87*	94	90	83	88	97	100
	male	93	97	87	78	102	92	76	89	87	—	82
c'	female	1.1	na	0.9*	1.0*	1.3+	1.2	1.1*	0.8	na	1.1	0.9
	male	1.1	1.2*	0.9	1.1*	1.2+	1.0	1.2*	1.0	na	—	1.1
V	female	48	46	43	43	43*	43	45	39	43	45	44
T	male	59	na	na	58	na	57	58	61	60	—	na
Odontostyle	female	133	132	140	143	na	133	131	146	137	142	134
	male	134	130	132	145	136	128	128	131	137	—	139
Odontophore	female	78	na	82	85	na	80	86	87	83	83	82
	male	75	na	79	83	65*	80	83	80	81	—	82
Spear	female	211	na	222	228	na	212	217	234	220	219	216
	male	209	na	211	226	201*	212	211	212	217	—	221
Width greatest	female	56*	56*	58*	66*	55*	58*	62*	85*	58*	63*	70*
	male	53*	52*	50*	52*	54*	43*	59*	81*	57*	—	63*
Width at anus	female	38*	na	56*	50	43+	36*	47	69*	na	35	50*
	male	42*	36*	59*	50	43+	44*	45	55*	na	—	49*
Tail	female	42*	52*	50*	52	54+	43*	50	55*	50	41	45*
	male	46*	43*	50*	56	52+	44*	53	53*	48	—	54*
Spicula	male	69+	na	78	76	78+	79	72	82	na	—	76

X. diversicaudatum populations.

A USSR female (Micoletzky, 1923); male (Lectotype; Pitcher *et al.*, 1974).
B USSR female (Teploukhova, 1974); male (Micoletzky, 1927).
C Czechoslovakia (Erbenova, 1975).
D England (Goodey *et al.*, 1960).
E Greece (Terlidou, 1967).
F Italy (Martelli and Lamberti, 1967).
G USA (Goodey *et al.*, 1960).
H West Germany (Martelli and Lamberti, 1967).
I West Germany (Sturhan, 1963).
J Yugoslavia (Hrzic, 1978).
K Poland (Szczygiel, 1974).

* Values derived from published data.
\+ Values derived from drawings of specimens in publications.
na Not available.

Materials and Methods

Xiphinema diversicaudatum were extracted, using the method of McElroy *et al.* (1977), from soil collected from a mixed woodland near Dundee. Suspensions of nematodes in water were collected from Baermann funnels after 15 hours, combined and mixed. Ten female and five male specimens were hand-picked from each of 29 sub-samples and these nematodes were used for the various treatments.

The four methods of killing, seven fixatives, three methods of mounting specimens in glycerol and combinations of killing, fixing and processing used in the study are given in Table II. Details of the methods are given in Hooper (1970) and are not repeated here. The rapid glycerol-ethanol method of Seinhorst (1959) for processing nematodes to glycerol was used in the study. For examination all nematode specimens were mounted in the appropriate fixative glycerol or water on slides using a wax ring technique (de Grisse, 1969).

The structures measured, and the ratios derived from them are given in Table III. Measurements were obtained using a Reichert Diapan microscope, with drawing arm attached and with 6.3 fold eyepieces and 2.5, 4, 10, 40, 63 and 100 fold objectives. Structures with measurements given in millimetres and the spicules of males were measured from drawings made of each specimen. The other structures were measured directly with the aid of an eyepiece graticule. Body diameters were checked and corrected if necessary following the procedure by Geraert (1961).

Statistical analysis of the results was made using the GENSTAT computer package (Alvey *et al.*, 1982).

Results

(See Table II for abbreviations of structures used in text)

PUBLISHED MORPHOMETRICS

The morphometrics published for 11 populations of *X. diversicaudatum* are given in Table I. Where necessary, and possible, missing values were derived from the published data, e. g. the value of 42 μm for tail length for population A was obtained by dividing the published mean for L by the mean for ratio c. Also, some data

were calculated from direct measurements made from drawings of specimens presented in the various publications.

Considerable differences are apparent between the populations of *X. diversicaudatum*, some characters being more variable than others. The percent differences in the means of the measurements and ratios for females of *X. diversicaudatum* from different popula-

Table II - *Combinations of methods used to kill, fix and mount* X. diversicaudatum *specimens.*

Method of killing	Fixative	Method of mounting	Abbreviations used in text
Heat killed in water (60 °C for 5 min)	H20	H20	H/H20/H20 (= SM)
	H20	slow glycerol	H/H20/SG
	H20	glycerol-ethanol	H/H20/GE
	TAF	TAF	H/TAF/TAF
	TAF	slow glycerol	H/TAF/SG
	TAF	glycerol-ethanol	H/TAF/GE
	FAA	FAA	H/FAA/FAA
	FAA	slow glycerol	H/FAA/SG
	FAA	glycerol-ethanol	H/FAA/GE
	FA4:1	FA4:1	H/FA4:1/FA4:1
	FA4:1	slow glycerol	H/FA4:1/SG
	FA4:1	glycerol-ethanol	H/FA4:1/GE
	FP4:1	FP4:1	H/FP4:1/FP4:1
	FP4:1	slow glycerol	H/FP4:1/SG
	FP4:1	glycerol-ethanol	H/FP4:1/GE
	FG	FG	H/FG/FG
	FG	slow glycerol	H/FG/SG
	FG	glycerol-ethanol	H/FG/GE
Hot FA4:1 (Seinhorst, 1966)	FA4:1	FA4:1	FA4:1/FA4:1/FA4:1
	FA4:1	slow glycerol	FA4:1/FA4:1/SG
	FA4:1	glycerol-ethanol	FA4:1/FA4:1/GE
Hot FP4:1 (Netscher and Seinhorst, 1969)	FP4:1	FP4:1	FP4:1/FP4:1/FP4:1
	4%F	4%F	FP4:1/4%F/4%F
	4%F	slow glycerol	FP4:1/4%F/SG
	4%F	glycerol-ethanol	FP4:1/4%F/GE
Vapor-phase using Formalin (Maggenti and Viglierchio, 1965)	FH20 3:1	FH20 3:1	Fv/FH20 3:1/FH20 3:1
	FH20 3:1	slow glycerol	Fv/FH20 3:1/SG
	FH20 3:1	glycerol-ethanol	Fv/FH20 3:1/GE
Live nematodes observed and measured in 0.7% water agar.			Live specimens

(= SM), Standard method.

tions ranged from 9% for odontostyle length to 49% for body width at the anus with the average percent difference, for all paramenters measured, being 24%. Similarly, for male *X. diversicaudatum* the percent differences in the means ranged from 7% for ratio T to 39% for body width at the anus and the average for all paramenters measured was 20%. Therefore, a comparison of the published mor-

Table III - *Structures and ratios measured in* X. diversicaudatum *specimens prepared by different methods.*

Structure		Abbreviation used in text
Length of body	mm	L
Length of anterior end to the anus	mm	L'
Length of anterior end to the vulva *	mm	anterior to vulva
Length of anterior end to the oesophageal-intestinal junction	µm	anterior to oesoph-intest junction
Length of body occupied by the anterior gonad *	µm	anterior gonad
Length of body occupied by the posterior gonad *	µm	posterior gonad
Length of tail	µm	tail length
Length of odontostyle	µm	odontostyle
Length of odontophore	µm	odontophore
Length of odontostyle plus odontophore	µm	spear
Body width at the spear base	µm	width at spear base
Greatest body width	µm	greatest width
Body width at the anus	µm	width at anus
Spicula +		spicula
Length of body occupied by the testes +	mm	testes
L /greatest width		a
L / anterior to oesoph-intest junction		b
L / tail length		c
Tail length / width at anus		c'
Anterior to vulva x 100 / L *		V
Anterior to vulva x 100 / L' *		V'
Spear / width at spear base		S
Testes x 100 / L +		T

* Female specimens only.
\+ Male specimens only.

phometrics of different populations of *X. diversicaudatum* shows that, in general, there is a variability in the measurements of 20% to 25%.

The smallest mean female body length (4.4 mm) was for a Greek population (Terlidou, 1967) which was 27% shorter than the largest (4.9 mm) for an English population (Goodey *et al.*, 1960). The least variable characters were the length of the odontostyle, odontophore and spear in the females and the length of the odonstostyle and the ratio T in the males. Generally, most variation was in means for body width and the body width at the anus. However, results for a West German population reported by Martelli and Lamberti (1967) were mainly responsible for this being the most variable characters; possibly because the specimens that they measured had been partially flattened during mounting. Alternatively, the specimens may represent a *Xiphinema* species different from *X. diversicaudatum.*

EFFECT OF PREPARATION TECHNIQUES ON MORPHOMETRICS

Morphometrics of nematodes are most conveniently obtained from specimens which have been killed. Therefore, although morphometrics were obtained from live specimens, the method chosen as standard for this study was to heat-kill specimens in water for 5 min. at 60 °C and then to make temporary water mounts of them.

An examination of the variance ratios calculated from the combined results for the female and male specimens showed that different combinations of killing, fixing and mouting specimens had a significant effect on all measurements except the length of the odontophore. The length of the spear and of the odontostyle were significantly affected by the treatments but their coefficients of variation (CV %) were smaller than that of the odontophore indicating that the spear and odontostyle lengths were less variable than those of the odontophore. These structures had the smallest CV % values recorded; the largest values were those of the female anterior and posterior gonads respectively (15.1% and 16.8%) and the testes (13.6%; Tab. IV).

The sex of the specimens did not significantly affect L, odontophore, anterior to anus lengths nor the ratio S, irrespective of the methods used, but all other morphometrics common to both sexes

Table IV - *The effect of different killing, fixing and mounting techniques on some morphometrics recorded from female and male* X. diversicaudatum.

		Means		Variance ratio	Significance+	CV%
		female	male			
Females and males						
Odontophore	µm	80	80	1.150	NS	4.5
L	mm	4.74	4.71	1.803	**	7.2
Anterior to anus	mm	4.69	4.66	1.820	**	7.3
b		9.91	9.65	1.872	**	10.7
Odontostyle	µm	129	127	2.030	**	3.7
Spear		209	207	2.058	**	2.9
Tail		46.7	49.5	2.240	***	9
c'		1.09	1.14	2.624	***	10.4
Anterior to oesoph-intest junction	µm	481	489	2.643	***	6.4
c		102	96	2.815	***	11
Width at anus	µm	42.9	43.6	5.244	***	6.6
a		80	89	12.414	***	7.7
S		4.62	4.67	15.258	***	6
Width at spear base	µm	45.5	44.6	16.340	***	6.7
Greatest width	µm	59.3	53.3	17.877	***	7.5
Females only						
Anterior gonad	µm	740		1.663	*	15.1
V	%	42.8		1.863	**	6.1
V'	%	43.1		1.872	**	6.1
Anterior to vulva	mm	2.03		1.919	**	8.3
Posterior gonad	µm	793		2.144	**	16.8
Males only						
Spicula	µm		63.3	1.608	*	9
Testes	mm		2.48	1.992	**	13.6
T	%		52.7	3.218	***	11.2

+ NS, not significant.
* P less than 0.05.
** P less than 0.01.
*** P less than 0.001.

were significantly affected by sex (Tab. V). Few interactions between sex and treatment occurred and 11 morphometrics, common to both sexes, were not significantly affected; but width at anus, greatest width and ratio a were affected significantly by the methods used depending on the sex of the specimens. With females the width at anus differed significantly from the standard in one method and in 10 methods with males; greatest width was significantly affected by several methods with males but with females only those methods where the specimens were mounted in fixative significantly affected the measurements.

Table V - *The effect of the sex of the specimens and the interaction between sex and treatments on some morphometrics recorded from female and male* X. diversicaudatum *killed, fixed and mounted using several methods.*

Character	Effect of sex		Interaction with treatment	
	Variance ratio	Significance+	Variance ratio	Significance+
L	0.787	NS	1.005	NS
L'	0.939	NS	1.004	NS
Anterior to oesoph-intest junction	6.365	*	1.215	NS
Tail	41.2	***	0.949	NS
Odontostyle	11.6	***	0.929	NS
Odontophore	0.032	NS	1.148	NS
Spear	7.811	**	0.957	NS
Width at spear base	7.446	**	1.495	NS
Greatest width	190	***	2.185	**
Width at anus	6.877	**	2.204	**
a	186	***	2.149	**
b	6.371	*	0.790	NS
c	32.3	***	0.933	NS
c'	16.1	***	1.246	NS
S	3.215	NS	1.940	NS

+ NS, not significant.
* P less than 0.05.
** P less than 0.01.
*** P less than 0.001.

Much variation was found in morphometric means from male and female *X. diversicaudatum* which had been prepared by the different methods used in the study (Tabs. VI and VII). Significant differences were also recorded in the morphometric means of male and female specimens, prepared by different methods, when the means were compared with the mean values obtained for the standard method. Generally, the method of killing and the method of mounting the specimens in glycerol had similar effects but when the specimens were mounted in fixative i.e. not processed with glycerol, the mean body widths of the specimens were significantly increased when compared with the standard method. The increase in mean body widths was as much as 46% in males (H/FG/FG, greatest width) and 27% in females (FA4:1/FA4:1/FA4:1, width at spear base). The general effect of mounting specimens in glycerol was a reduction in size compared with the standard method except the anterior to oesoph-intest junction which increased in length, often significantly. Also, the morphometrics of males generally were more often affected by the methods used than were those of the females.

Live females differed from those treated by the standard method in their anterior to oesoph-intest junction and width at spear base. These larger values caused the ratios b and S to differ significantly from those obtained using the standard method. In live males, only the morphometric means for L, spicula, odontostyle, odontophore, spear and ratio b were not significantly different from the means obtained with the standard method.

With female specimens the morphometrics recorded were least affected by the method H/FG/SG which affected only the odontostyle. Similarly, the methods H/FG/GE and H/H20/GE each significantly affected only three of the morphometrics compared with the standard method. The method FA4:1/FA4:1/FA4:1 most affected females, significantly affecting over half of the morphometrics. With males, four methods each caused five morphometrics to differ from those recorded using the standard method but specimens subjected to the method H/TAF/GE appeared most similar to those of the standard method. The morphometrics of live male specimens differed more from those from the standard method than did those of any other treatment (Tab. VIII).

Table VI - *Percent differences from the Standard Method, in morphometric means of* X. diversicaudatum *females (n=10) processed by different methods.*

Treatments*	L	L'	Anterior		Gonads		Tail	Odonto	
			o/i junc.	vulva	ant	post		style	phore
1) H/H20/H20 (= SM)**	4.8	4.75	457	2.12	738	800	49	132	80
2) Live specimens	0.4	0.5	−11.1	4.2	−11.0	−7.0	−2.4	−2.2	0.5
3) H/TAF/TAF	2.3	2.4	5.7	−4.1	8.5	3.1	0.8	−1.2	0.2
4) H/FAA/FAA	−0.3	−0.3	9.2	4.6	8.5	14.1	−7.3	0.7	−0.9
5) H/FA4:1/FA4:1	−0.6	−0.6	5.7	−7.1	−5.1	0.8	−6.5	−2.3	−0.4
6) H/FP4:1/FP4:1	−1.8	−1.8	5.5	−3.4	6.8	0	−2.9	1.7	2.1
7) H/FG/FG	2.9	2.9	6.5	4.0	0	−9.4	−0.6	−0.8	−2.0
8) FA4:1/FA4:1/FA4:1	−3.1	−3.1	8.8	−8.8	−7.6	−8.6	−3.5	−2.3	−0.4
9) FP4:1/FP4:1/FP4:1	−4.6	−4.7	9.3	−5.3	−11.9	−8.6	1.8	−1.4	−3.1
10) FP4:1/4%F/4%F	−4.5	−4.6	6.0	−4.6	−1.7	−11.0	0.2	−1.9	−2.1
11) Fv/FH20 3:1/FH20 3:1	−3.6	−3.7	9.4	−6.1	3.4	4.7	1.0	0.2	−0.4
12) H/H20/SG	0.7	0.8	1.6	−5.0	1.5	2.3	−10.0	−4.6	1.5
13) H/TAF/SG	−0.5	−0.4	5.4	−4.1	13.0	10.2	−14.9	−2.5	−3.0
14) H/FAA/SG	1.7	1.8	3.1	−2.5	3.4	3.1	−5.1	−4.1	−0.9
15) H/FA4:1/SG	−5.5	−5.4	5.0	−10.5	−5.9	−15.6	−8.6	−3.4	−2.1
16) H/FP4:1/SG	−4.9	−4.9	1.5	−10.3	−5.1	−13.3	−5.9	−0.8	1.5
17) H/FG/SG	−2.0	−2.0	6.0	−4.0	−3.4	−5.5	−7.1	−3.3	0.7
18) FA4:1/FA4:1/SG	−1.6	−1.6	7.8	−3.7	2.6	−3.1	−7.3	−2.0	−1.9
19) FP4:1/4%F/SG	−3.9	−3.9	5.5	−6.5	0.8	10.2	−4.9	−2.9	−1.1
20) Fv/FH20 3:1/SG	−4.5	−4.5	3.5	−10.5	3.4	1.6	−5.9	−6.2	−0.4
21) H/H20/GE	0.5	0.5	2.4	−4.8	0	2.3	−2.2	−1.1	−0.6
22) H/TAF/GE	1.1	1.2	6.4	0.2	10.2	12.5	−5.3	−5.2	−0.6
23) H/FAA/GE	0.7	0.8	4.2	0.4	7.6	8.6	−6.1	−2.1	−1.9
24) H/FA4:1/GE	−4.9	−4.9	2.2	−8.2	−4.2	−3.1	−6.1	−4.1	−4.0
25) H/FP4:1/GE	2.4	2.4	3.5	−4.1	2.5	−2.3	−4.3	−2.8	−2.2
26) H/FG/GE	−2.0	−1.9	−3.3	−7.0	−6.8	−4.7	−7.8	−2.4	−0.1
27) FA4:1/FA4:1/FA4:1	2.1	2.2	7.0	−1.2	2.5	−3.1	−7.3	−2.5	−0.6
28) FP4:1/4%F/GE	−3.4	−3.4	8.0	−6.8	2.5	3.9	−1.8	−4.0	−1.9
29) Fv/FH20 3:1/GE	2.1	2.2	6.8	−0.5	1.7	−7.8	−2.4	−2.3	−0.6
LSD***									
P less than 5% ±	6.1	6.3	6.3	7.0	13.3	14.6	7.4	3.0	3.8
P less than 1% ±	8.1	8.3	8.3	9.2	17.5	19.1	9.7	4.0	5.1
P less than 0.1% ±	10.3	10.6	10.6	11.7	22.3	24.5	12.4	5.1	6.5

* For explanation of abbreviations see Table III.
** Standard method.
*** Least significant differences as percentages of the standard method.

Table VI continued.

	Spear	Body widths			Ratios						
		s/base	great	anus	a	b	c	c'	V	V'	S
1)*	212	42	56	43	86	10.5	98	1.16	44	45	5
2)	−1.2	9.2	0.9	2.8	−0.1	−9.5	2.7	−5.2	3.4	3.4	−9.5
3)	−0.7	19.6	21.0	5.4	−15.7	−3.3	1.5	−4.7	−6.4	−6.6	−16.5
4)	−0.8	16.0	18.4	3.5	−16.2	−8.6	8.0	−11.3	−4.4	−4.5	−14.1
5)	−1.6	11.3	15.0	0.9	−13.9	−6.0	6.8	−8.1	−6.5	−6.6	−11.5
6)	1.9	10.1	10.7	−1.4	−11.3	−7.0	1.7	−2.2	−1.6	−1.7	−7.4
7)	−1.2	25.2	24.4	4.2	−17.4	−3.5	3.3	−5.1	−6.9	−6.9	−19.8
8)	−1.3	27.1	26.7	11.7	−23.9	−11.0	0.1	−13.3	−6.0	−6.0	−21.2
9)	−2.1	3.3	−0.2	−3.5	−4.7	−12.7	−6.6	5.3	−0.5	−0.4	−5.3
10)	−2.0	5.2	−2.1	−0.9	−3.1	−10.1	−4.4	0.5	−0.2	−0.2	−6.8
11)	0	6.8	6.2	0.9	−9.5	−11.8	−4.7	−0.3	−2.7	−2.7	−5.8
12)	−2.3	5.7	2.3	−0.7	−2.2	−0.3	11.9	−10.2	−5.8	−5.9	−7.5
13)	−2.7	0.5	2.0	−3.7	−2.8	−5.7	17.8	−12.2	−3.7	−3.8	−2.9
14)	−2.9	5.2	2.5	0.5	−1.2	−1.3	7.5	−6.2	−4.3	−4.3	−7.5
15)	−2.9	1.9	0.4	−3.3	−6.4	−9.8	3.0	−6.2	−5.3	−5.4	−4.7
16)	0.1	7.3	5.3	1.6	−10.1	−6.3	1.1	−8.0	−5.8	−5.8	−6.7
17)	−1.8	0.7	−2.3	−0.5	−0.1	−7.5	5.3	−7.3	−2.2	−2.2	−2.5
18)	−1.9	8.0	3.6	−1.2	−5.4	−8.2	6.1	−7.0	−2.2	−2.3	−9.2
19)	−2.2	7.5	3.4	−0.9	−7.9	−8.5	1.2	−4.0	−2.7	−2.7	−8.9
20)	−4.0	2.4	4.6	−1.4	−9.0	−7.5	1.2	−5.3	−6.6	−6.6	−6.3
21)	−0.9	5.2	−2.3	−2.1	2.3	−1.1	3.5	−0.9	−5.3	−5.4	−5.8
22)	−3.5	−1.2	1.1	1.2	−0.5	−5.0	7.5	−7.1	−1.0	−1.1	−1.7
23)	−2.0	7.8	3.7	2.3	−3.3	−3.4	8.1	−9.2	−0.1	−0.2	−9.0
24)	−4.1	−0.7	1.1	−2.1	−6.5	−7.0	1.4	−4.8	−3.5	−3.5	−3.3
25)	−2.6	3.1	4.1	0	−2.1	−1.2	6.9	−4.9	−6.4	−6.5	−5.5
26)	−1.6	−4.0	−2.7	−4.7	0.2	9.3	6.5	−4.1	−4.9	−5.0	−2.5
27)	−1.8	7.5	2.7	1.2	−1.2	−4.5	10.0	−9.2	−3.2	−3.3	−8.7
28)	−3.2	8.3	3.9	1.2	−7.5	−10.3	−1.8	−3.6	−3.6	−3.6	−10.5
29)	−1.2	11.1	11.4	0.2	−8.9	−3.7	7.3	−4.9	−2.5	−2.6	−11.1
LSD											
5% ±	2.5	6.7	6.5	6.1	6.6	8.8	9.8	8.7	5.2	5.2	4.8
1% ±	3.2	8.9	8.5	8.0	8.6	11.5	12.9	11.5	6.8	6.8	6.4
0.1% ±	4.1	11.3	10.9	10.2	11.1	14.7	16.4	14.7	8.7	8.7	8.1

* For explanacion of code see previous page.

Table VII - *Percent differences from the Standard Method, in morfometric means of* X. diversicaudatum *males (n=5) processed by different methods.*

Treatments*	L	L'	Anterior o/i junc.	Testes	Tail	Spicula	Odonto- style	Odonto- phore
1) H/H20/H20	4.64	4.58	458	2.71	55.2	65.2	126	82
2) Live specimens	7.0	7.3	9.6	—20.5	—17.8	—8.6	3.2	—3.2
3) H/TAF/TAF	4.3	4.4	15.4	—10.0	—10.2	—8.6	3.3	0
4) H/FAA/FAA	0.8	1.0	5.1	—1.3	—10.5	6.1	0.5	—2.2
5) H/FA4:1/FA4:1	3.8	3.9	5.5	—5.6	—10.9	—7.7	1.4	—3.9
6) H/FP4:1/FP4:1	2.2	2.3	6.0	—23.6	—7.2	—2.8	1.7	—3.4
7) H/FG/FG	8.9	9.1	5.4	4.4	—4.4	2.4	0.6	—2.7
8) FA4:1/FA4:1/FA4:1	—0.8	—0.8	7.6	—4.7	—3.6	7.4	2.1	—3.9
9) FP4:1/FP4:1/FP4:1	—5.6	—5.6	4.0	—14.6	—10.2	—2.5	0	—5.1
10) FP4:1/4%F/4%F	—8.1	8.1	5.5	—12.8	—9.8	—4.9	1.7	—1.7
11) Fv/FH20 3:1/FH20 3:1	—3.0	—2.9	7.3	—12.8	—8.7	—5.2	5.6	—5.4
12) H/H20/SG	0.2	0.4	5.8	—9.1	—17.4	1.2	1.1	—7.6
13) H/TAF/SG	1.5	1.7	6.9	—6.7	—11.2	—2.5	0.5	—5.6
14) H/FAA/SG	0.9	1.0	1.5	—2.7	—12.3	—3.7	—0.3	—6.6
15) H/FA4:1/SG	1.9	2.0	7.0	—13.1	—6.2	—4.9	0.2	—4.6
16) H/FP4:1/SG	—3.5	—3.4	—2.0	—19.0	—7.2	—8.3	—0.3	—2.2
17) H/FG/SG	3.2	3.4	9.0	—11.9	—9.1	—4.9	1.6	—3.2
18) FA4:1/FA4:1/SG	5.9	6.1	7.0	—4.1	—12.7	4.9	2.8	—1.5
19) FP4:1/4%F/SG	3.4	3.5	7.0	—3.7	—6.5	—2.5	0.2	—2.2
20) Fv/FH20 3:1/SG	8.6	8.9	15.0	3.8	—14.1	4.6	1.4	—2.2
21) H/H20/GE	—4.8	—4.8	0.9	6.5	—10.9	—3.7	1.1	—6.4
22) H/TAF/GE	3.0	3.1	6.9	—10.5	—10.2	—3.7	0	—2.0
23) H/FAA/GE	1.7	1.8	8.0	—15.6	—11.6	—4.9	—0.8	—1.0
24) H/FA4:1/GE	4.0	4.2	7.5	—18.2	—13.4	—7.4	0.2	—5.6
25) H/FP4:1/GE	1.9	2.1	10.6	—9.2	—13.8	—7.4	2.1	0
26) H/FG/GE	0.5	0.7	4.0	—11.7	—15.6	—12.3	—1.1	—2.4
27) FA4:1/FA4:1/FA4:1	0	0.2	9.0	—5.2	—15.6	0	—0.2	—3.4
28) FP4:1/4%F/GE	2.2	2.3	7.6	3.7	—6.5	3.4	1.0	1.0
29) Fv/FH20 3:1/GE	4.9	5.1	13.6	—15.6	—11.2	—7.4	0.3	—1.2
LSD***								
P less than 5% ±	9.7	5.4	7.3	15.7	10.4	11.0	5.0	5.7
P less than 1% ±	12.8	7.1	9.6	20.7	13.7	14.5	6.6	7.6
P less than 0.1% ±	16.5	9.2	12.4	26.6	17.7	18.8	8.5	9.8

* For explanation of abbreviations see Table III.
** Standard method.
*** Least significant differences as percentages of the standard method.

Table VII continued.

	Spear	Body widths			a	b	c	c'	S	T
		s/base	great	anus						
1*	208	38.6	47	40.6	99	10.1	84	1.36	5.4	58
2	0.7	19.2	19.6	8.6	—9.6	—1.9	30.8	—24.3	—15.6	—25.6
3	2.0	12.4	11.5	2.5	—6.7	—9.4	17.7	—12.5	—9.3	—13.5
4	—0.6	38.3	42.1	28.1	—29.1	—3.7	13.1	—29.5	—28.2	—2.2
5	—0.7	18.1	16.6	10.8	—20.8	—1.4	16.9	—19.1	—15.8	—9.1
6	—0.3	20.7	29.4	13.3	—20.3	—3.3	10.1	—18.4	—16.9	—25.2
7	—0.7	40.9	46.4	25.1	—25.6	3.6	13.9	—22.8	—29.4	—3.9
8	—0.3	39.9	41.3	22.7	—29.3	—7.5	3.1	—20.6	—28.3	—3.9
9	—2.0	13.5	11.1	3.4	—15.2	—9.0	6.4	—13.2	—13.5	—9.0
10	0.4	14.5	4.7	8.4	—12.4	—12.8	2.4	—16.2	—12.6	—5.1
11	1.0	12.4	4.2	3.0	—6.4	—9.3	6.4	—11.0	—9.8	—10.6
12	—2.3	14.5	3.0	3.9	—2.7	—5.0	22.0	—20.6	—14.8	—9.2
13	—1.9	8.8	3.8	2.5	—1.6	—4.4	14.6	—13.2	—10.0	—8.2
14	—2.8	10.4	7.6	5.4	—6.4	—0.3	15.9	—16.9	—12.0	—3.6
15	—1.7	13.5	12.3	1.5	—9.5	—4.4	9.5	—7.4	—13.5	—14.7
16	—1.1	13.0	10.2	6.9	—12.4	—1.2	4.2	—13.2	—12.6	—16.4
17	—0.3	9.8	3.4	—2.0	—0.5	—5.0	15.1	—10.3	—9.3	—14.6
18	1.2	18.6	14.0	6.4	—7.2	—0.3	22.0	—17.6	—14.8	—9.5
19	—0.8	18.6	14.9	6.9	—10.2	—2.7	11.5	—12.5	—16.5	—7.0
20	0	18.6	18.3	10.8	—7.2	—5.2	27.0	—22.1	—15.8	—4.5
21	—1.8	8.3	2.6	0.5	—7.4	—5.2	7.2	—11.0	—9.4	—12.1
22	—0.8	3.1	2.6	7.9	0.1	—3.4	15.4	—16.9	—3.9	—12.8
23	—0.9	10.9	13.6	6.9	—10.6	—5.6	15.6	—16.9	—10.6	—17.1
24	—2.1	9.8	12.3	4.4	—7.8	—3.0	20.9	—16.9	—10.1	—21.3
25	1.2	9.8	3.4	3.9	—1.8	—7.6	18.3	—16.9	—8.0	—10.9
26	—1.6	6.2	0.4	2.0	—0.2	—3.0	19.5	—16.9	—7.6	—12.1
27	—1.4	16.6	12.8	2.0	—11.6	—8.1	19.1	—16.9	—15.6	—4.8
28	1	15.5	11.9	6.9	—8.8	—4.7	10.0	—12.5	—12.6	1.9
29	—0.3	16.6	13.6	8.9	—7.6	—6.9	19.3	—18.4	—14.6	—19.2
LSD										
5% ±	2.1	8.1	12.3	7.9	4.7	7.6	9.5	6.2	3.7	12.7
1% ±	2.8	10.7	16.2	10.3	6.2	9.9	12.5	8.1	4.9	16.8
0.1% ±	3.5	13.8	20.9	13.3	8.1	12.8	16.2	10.0	6.3	21.6

* For explanacion of code see previous page.

Table VIII - *The number and level of significance of morphometric differences caused by different methods of killing, fixing and mounting* X. diversicaudatum *when compared with a standard method.*

Method of killing, fixing and mounting nematodes	Number of morphometrics significantly different from the mean of the standard method when:						Ranking	
	P less than 5%		P less than 1%		P less than 0.1%			
	♀	♂	♀	♂	♀	♂	♀	♂
H/H20/H20	(standard method)							
H/FG/SG	1	6	0	3	0	2	1	3
H/H20/GE	3	6	0	3	0	2	2	4
H/FG/GE	3	5	0	4	0	3	3	8
H/FP4:1/GE	4	7	0	5	0	3	4	11
FP4:1/4%F/4%F	2	7	1	6	0	5	5	18
H/FA4:1/SG	8	7	1	3	0	2	6	5
FP4:1/FP4:1/FP4:1	3	6	2	4	0	3	7	10
H/FAA/SG	3	8	2	5	0	2	8	7
Fv/FH20 3:1/FH20 3:1	5	7	3	4	0	2	9	6
H/FP4:1/SG	6	5	3	4	0	3	10	9
H/H20/SG	7	7	3	6	0	5	11	17
FA4:1/FA4:1/SG	3	8	1	5	1	4	12	16
FP4:1/4%F/SG	3	6	1	4	1	4	13	14
H/FAA/GE	3	9	1	6	1	3	14	13
FA4:1/FA4:1/GE	5	9	1	6	1	5	15	20
H/TAF/GE	3	5	2	2	1	1	16	1
H/FA4:1/GE	3	11	2	5	1	3	17	12
FP4:1/4%F/GE	7	6	3	4	1	4	18	15
Fv/FH20 3:1/SG	7	10	4	10	1	5	19	23
Live specimens	4	12	3	10	2	7	20	28
H/TAF/SG	4	5	3	3	2	2	21	2
H/FP4:1/FP4:1	4	9	4	8	2	7	22	27
Fv/FH20 3:1/GE	5	10	4	7	2	5	23	22
H/TAF/TAF	6	8	6	6	4	5	24	19
H/FA4:1/FA4:1	7	8	4	7	4	5	25	21
H/FAA/FAA	6	8	5	7	4	6	26	25
H/FG/FG	7	8	6	8	4	6	27	26
FA4:1/FA4:1/FA4:1	11	7	7	6	5	6	28	24

Discussion

Differences in the published morphometrics of populations of *X. diversicaudatum* (Tab. I) may be attributed to the biotopes from

which the nematodes came. Brown and Topham (1985) reported more variability present between the morphometrics of populations of *X. diversicaudatum* than had previously been published, the differences being affected by biotopic influences. The present study has shown that using different methods to prepare specimens for optical microscopy will also significantly affect the morphometrics of *X. diversicaudatum.* It is likely, therefore, that some of the differences in the published morphometrics of *X. diversicaudatum* are due to the methods used to process the specimens for microscopy. For example, Sturhan (1963) measured heat-killed specimens in water whereas Martelli and Lamberti (1967) measured specimens fixed in FAA and mounted by a rapid glycerol method. Unfortunately, most authors give few, if any, details of methods used to prepare their specimens of *X. diversicaudatum* for microscopy making it difficult to accurately compare their results. In general, morphometric differences caused by processing specimens were less than those attributed to biotopic influence or present between published morphometrics.

All of the methods used in the present study altered significantly at least one morphometric mean when compared with a standard method; males being more affected than females. Although several reports have been published describing « satisfactory » methods for preparing specimens of a particular nematode species for morphometrical study or anatomical examination none was found to be « satisfactory » for all of the characters studied (Curran and Hominick, 1980; Lamberti and Sher, 1969; Maggenti and Viglierchio, 1965; Stone, 1971). Similarly, results from the present study cannot be used to identify a method that may be adopted for general use with plant parasitic nematodes, or even for the genus *Xiphinema.* The data in Tabs. VI and VII may be used for comparative purposes when deciding which method might be appropriate when examining and measuring a particular structure. Also, the methods which cause the fewest and least significant differences in the morphometrics of the nematodes, although not necessarily the least total amount of variability, may be chosen when a general morphometrical study is to be done. The influence of the different methods of processing specimens on the total variability in the nematodes morphometrics has yet to be investigated fully.

Where two nematode species are distinguished by differences in their respective morphometrics e. g. *X. opisthohysterum* and *X.*

pachtaicum; *X. insigne* and *X. attorodorum*; *X. ensiculiferum* and *X. costaricense* (Luc and Dalmasso, 1975) it should be ensured that the differences do not result from different methods having been used to process the specimens. Similarly, to identify correctly specimens to the specific rank, especially when morphometric criteria are being used, it may be necessary to process some specimens using the methods employed for type specimens of the most similar species. However, this may not always be possible because many original descriptions of nematodes, including some in the genus *Xiphinema*, do not contain such details.

Methods used to process nematode specimens for examination by optical microscopy are frequently a matter of convenience and tradition within a laboratory. The results obtained in the present study show that the method chosen for processing specimens for morphometrical study can significantly affect the final results, possibly resulting in erroneous identification of specimens. Furthermore, the study demonstrates the importance of describing the method used for processing specimens for examination by optical microscopy, particularly when specimens are used to describe a new species.

We thank R. J. Clark for help with computing.

SUMMARY

The effect was examined of 28 combinations of different methods of killing, fiixing and mounting specimens in glycerol on the morphometrics of *Xiphinema diversicaudatum*. Fixation of specimens, after killing, caused significant morphometrical differences when compared with specimens prepared by a standard method (= heat killed in water at 60 °C for 5 min). The fixatives caused significant swelling of the body diameters and a shrinkage in length. Mounting specimens in glycerol frequently reduced the effects caused by fixation, but many significant morphometric differences remained after mounting specimens in glycerol when compared with specimens prepared by a standard method. Only the length of body of nematodes appeared not to be affected significantly by killing, fixation or mounting specimens in glycerol. This seemed to be the result of an extension of the anterior to oesophageal-intestinal length counteracting shrinkage occurring in the remaining body length.

It is not practical to recommend the adoption of a procedure for killing, fixing and mounting specimens in glycerol as all methods appeared to affect at least one, if not several, morphometrics. However, to identify correctly specimens to the specific rank, especially when morphometric criteria are being used, some specimens should be processed using the methods employed for type specimens of the most similar species.

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