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Multivariate analysis of *Xiphinema diversicaudatum* and some related species (Nematoda: Longidoridae)

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Summary - The "morphometric characters of 22 *Xiphinema* populations from Portugal, which were assumed to belong to *X. belmontense* Roca & Pereira, 1992 and *X. dissimile* Roca, Pereira & Lamberti, 1987, the respective type populations, and published values of a Portuguese population of *X. pseudocoxi* Sturhan, 1984, the type population of *X. pseudocoxi*, as well as the type populations of *X. cadavalense* Bravo & Roca, 1995, *X. diversum* Roca, Lamberti, Santos & Abrantes, 1988, *X. gersoni* Roca & Bravo, 1993 and *X. lusitanicum* Sturhan, 1983 were studied by principal component analysis (PCA) and hierarchical cluster analysis (HCA). The study included also the published values of *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, a British population of *X. diversicaudatum* and two populations from Italy. All of these species are morphologically similar in having a well differentiated pseudo-Z-organ, but they differ in their morphometrical characters. Populations were placed by the analyses into three groups; those included in the group of *X. belmontense* were separated at the first level of analysis. The others were included in a large group that was divided into two smaller groups separating *X. dissimile* from *X. diversicaudatum*. The *X. dissimile* group is composed of two sub-groups, one regarded as "typical" and the other as "small" *X. dissimile*, on the basis of morphometrical characters. Observations on the morphological variability between populations of *X. dissimile* in Portugal are also given, together with considerations on the use of PCA and HCA in taxonomy.

Résumé - ***Analyse multivariée de* Xiphinema diversicaudatum *et d'espèces voisines (Nematoda: Longidoridae)*** - Les caractères morphométriques de 22 populations attribuées à *X. belmontense* Roca & Pereira, 1992 et *X. dissimile* Roca, Pereira & Lamberti, 1987 de même que ceux d'une population de *X. pseudocoxi* Sturhan, 1984 provenant du Portugal, et des populations types de ces espèces ainsi que de *X. cadavalense* Bravo & Roca, 1995, *X. diversum* Roca, Lamberti, Santos & Abrantes, 1988, *X. gersoni* Roca & Bravo, 1993 et *X. lusitanicum* Sturhan, 1983, ont été étudiées par analyse en composante principale (PCA) et par analyse des groupes hiérarchiques (HCA). L'étude inclue les données morphométriques publiées sur différentes populations de *X. diversicaudatum* dont deux provenant d'Italie et une de Grande-Bretagne. Ces espèces se ressemblent morphologiquement et possèdent toutes un pseudo-organe Z bien différencié. Les populations se disposent en trois groupes. Celles du groupe de *X. belmontense* sont séparées au premier niveau; les autres populations se placent dans un grand groupe composé de deux sous-groupes, celui de *X. dissimile* et celui de *X. diversicaudatum*. Le sous-groupe de *X. dissimile* est composé de deux ensembles de populations: l'un "typique" et l'autre considéré, sur la base des caractères morphométriques, comme un "petit" *X. dissimile*. Des observations sont faites sur la variabilité morphologique des populations de *X. dissimile*, et des considérations avancées sur l'utilisation en taxinomie des analyses en PCA et HCA.

Key-words: nematodes, Portugal, taxonomy, variability, *Xiphinema*.

Populations with similar morphological characters but differing biometrically were collected in Portugal. Most of them were regarded as belonging to the *Xiphinema diversicaudatum*-complex (Lampreia & Brown, 1992). Their morphometrics were statistically studied by a principal component (PCA) and hierarchical cluster (HCA) analyses. The study included values from the populations previously identified as *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939 from different countries and continents (Brown & Topham, 1985), as well as 22 Portuguese populations which were assumed to belong to *X. belmontense* Roca & Pereira, 1992 and *X. dissimile* Roca, Pereira & Lamberti, 1987 and the respective type populations. Two populations of *X. diversicaudatum* from Italy (Roca *et al.*, 1987; 1990) and one from Brítain (Goodey *et al.*, 1960), the type population of this species and a Portuguese population of *X. pseudocoxi* Sturhan, 1984 (Pereira & Roca, 1992), and the type populations of *X. cadavalense* Bravo & Roca, 1995, *X. diversum* Roca, Lamberti, Santos & Abrantes, 1988, *X. gersoni* Roca & Bravo, 1993 and *X. lusitanicum* Sturhan, 1983 were also included in this study.

Material and methods

Nematodes were extracted from soil samples by the Cobb wet sieve technique, killed and fixed in 5%

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Table 1. *Populations of* Xiphinema *used in the statistical analyses.*

Species	Population, Origin	Host	Reference
1 *X. dissimile*	Type population	*Cupressus* sp.	Roca *et al.*, 1987
2 *X. dissimile*	Quinta dos Anjos, Palmela, Setubal, Portugal	Peach	
3 *X. dissimile*	Viveiro do Escauropim, Salvaterra, Portugal	Grapevine	
4 *X. dissimile*	Monte dos Alhos, S. Tiago do Cacém, Portugal	Cereals	
5 *X. dissimile*	Requiao, Vila Nova de Famalição, Portugal	Grapevine	
6 *X. dissimile*	Castromil, Paredes, Portugal	Strawberry	
7 *X. dissimile*	Viveiro Florestal, Amarante, Portugal	*Cupressus* sp.	
8 *X. dissimile*	Viveiro Florestal, Amarante, Portugal	*Acer* sp.	
9 *X. dissimile*	Viveiro Florestal, Amarante, Portugal (a)	Lime, plane & oak	
10 *X. dissimile*	Teloes, Vila Pauca de Aguiar, Portugal	Strawberry	
11 *X. dissimile*	Vilar de Frades, Braga, Portugal	Hop	
12 *X. dissimile*	Vairao, Vila do Conde, Portugal (a)	Raspberry	
13 *X. dissimile*	Jardim da Serra, Madeira Island, Portugal (a)	Grapevine	
14 *X. dissimile*	Quinta dos Marmelinhos, Setubal, Portugal	*Citrus* sp.	
15 *X. dissimile*	Mata d'El Rei, Salvaterra de Magos, Portugal	*Citrus* sp.	
16 *X. diversicaudatum*	British population	Strawberry	Goodey *et al.*, 1960
17 *X. belmontense,*	Type population	Apple	Roca & Pereira, 1992
18 *X. belmontense,*	Jardim da Serra, Madeira Island, Portugal (b)	Grapevine	
19 *X. belmontense,*	Viveiro Florestal, Amarante, Portugal (b)	Lime, plane & oak	
20 *X. dissimile*	Madeira Island, S. Jorge, Portugal	Grapevine	
21 *X. belmontense,*	Quinta do Seixo, Tabuaco, Portugal	Grapevine	
22 *X. belmontense,*	Vairao, Vila do Conde, Portugal (b)	Raspberry	
23 *X. dissimile*	Serradela, Vieira do Minho, Portugal	*Quercus* sp.	
24 *X. belmontense,*	Beirado das Burras, Zebras, Fundao, Portugal	Peach	
25 *X. dissimile*	Sedielos, Régua, Portugal	Maize	
26 *X. diversicaudatum*	Dundee, Scotland (field)	*Sambucus nigra*	Brown & Topham, 1985
27 *X. diversicaudatum*	Dundee, Scotland (glasshouse)	*Rosa* sp.	
28 *X. diversicaudatum*	Cupar, Scotland	*Fragaria* × *Ananassa*	
29 *X. diversicaudatum*	Kilsyth, Scotland	Deciduous woodland	
30 *X. diversicaudatum*	Ilkley, England	*Lolium perenne*	
31 *X. diversicaudatum*	Bury St. Edmunds, England	Deciduous woodland	
32 *X. diversicaudatum*	Harpenden, England	*Lolium perenne*	
33 *X. diversicaudatum*	Aylesford, England	Scrubland	
34 *X. diversicaudatum*	High Halstow, England	deciduous woodland	"
35 *X. diversicaudatum*	Treswithian, England	*Lolium perenne*	"
36 *X. diversicaudatum*	Wrekin, Wales	*Lolium perenne*	"
37 *X. diversicaudatum*	Nevern, Wales	*Rosa* sp.	"

End of Table 1 next page.

Table 1. *(cont.)*

Species	Population, Origin	Host	Reference
38 *X. diversicaudatum*	Saint-Katherina-Lombeek, Belgium	*Fragaria* × *Ananassa*	
39 *X. diversicaudatum*	Kostinbröd, Bulgaria	*Ribes nigrum*	
40 *X. diversicaudatum*	Les Adrets, France	*Rosa* sp.	
41 *X. diversicaudatum*	Liguria region, Italy	*Vitis vinifera*	
42 *X. diversicaudatum*	Lombardia region, Italy	*Rubus idaeus*	
43 *X. diversicaudatum*	Piemonte region, Italy	*Prunus persica*	
44 *X. diversicaudatum*	Wageningen, The Netherlands	*Rosa* sp.	
45 *X. diversicaudatum*	Alexandra, New Zealand	*Prunus armeniaca*	
46 *X. diversicaudatum*	Sandefjord, Norway	*Fragaria* × *Ananassa*	
47 *X. diversicaudatum*	Rygge, Norway	*Fragaria* × *Ananassa*	
48 *X. diversicaudatum*	Cazalegas, Spain	*Vitis vinifera*	
49 *X. diversicaudatum*	Holzieken, Switzerland	*Triticum spelta*	
50 *X. diversicaudatum*	San Diego, U.S.A.	*Prunus persica*	
51 *X. diversicaudatum*	Nowy Sacz, Poland	*Fragaria* × *Ananassa*	
52 *X. diversicaudatum*	Lazio region, Italy	Grapevine	Roca *et al.*, 1987
53 *X. diversicaudatum*	Calabria region, Italy	Medlar	Roca *et al.*, 1990
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54 *X. pseudocoxi*	Type population	Wild plants	Sturhan, 1984
55 *X. pseudocoxi*	Ameal, Torres Vedras, Portugal	Wild plants	Pereira & Roca, 1992
56 *X. diversum*	Type population	*Cupressus lusitanica*	Roca *et al.*, 1988
57 *X. lusitanicum*	Type population	Grapevine	Sturhan, 1983
58 *X. gersoni*	Type population	*Lolium* sp.	Roca & Bravo, 1993
59 *X. cadavalense*	Type population	Grapevine	Bravo & Roca, 1995

hot formaldehyde solution, processed by the Seinhorst's (1959) glycerol-ethanol method and mounted in glycerin on Cobb slides. Measurements were taken with the aid of a camera lucida or were obtained from the literature.

PCA was performed on the morphometric measurements of the populations cited above. Table 1 lists the species, localities and hosts of these populations and gives literature references to their respective morphometric measurements, which are presented in Table 2. The variables utilized in PCA were the mean values of lengths of body (L), odontostyle, odontophore and tail, the distance from oral aperture to guiding ring, the ratios a, b, c, c' and V.

The subsequent hierarchical cluster analysis was based on the average linkage method, using the first three principal components of the PCA as input variables. Multivariate statistics were obtained from the program SAS, release 6.03 (Anon., 1987).

A comparative study was carried out using two different input data in PCA and HCA analyses: one including the first 53 and the second all 59 populations listed in Table 1. The type populations are all from Portugal, except the type population of *X. pseudocoxi*. Both analyses used the same variables and methods of the previous analysis.

Results

Referring to the first study using PCA and HCA analyses performed with the first 53 populations (Table 1), the correlation coefficients among the original variables are listed in Table 3. The variance explained by the first three principal components was 85.521% of the total variance (Table 4).

The dendrogram obtained by HCA analysis (Fig. 1) shows the occurrence of three tentative groups: they are delimited on the scatterplots (Figs 2, 3). At the first higher distance level, the populations of *X. belmontense* are separated from all of the remaining populations. Subsequently, *X. dissimile* is separated as a group containing only populations from Portugal and one population from Spain, which had been identified as *X. diversicaudatum* in 1985, since *X. dissimile* had not yet been described at that time. The remaining

Table 2. *Percentage differences from means in morphometric means of* Xiphinema *species from different populations (All measurements in μm except L in mm).*

Populations	L	a	b	c	c'	V	Od. style	Od. phore	Tail
Grand Means	4.32	79.47	9.13	92.60	1.20	42.83	131.82	75.43	47.71
1	15.52	28.58	13.78	20.53	7.91	7.62	-1.91	-1.50	-5.05
2	5.36	32.10	9.40	4.22	21.20	5.76	2.25	-4.55	-0.44
3	11.13	21.66	9.40	17.50	8.74	4.36	-1.00	-6.94	-7.15
4	10.90	23.30	9.40	19.45	5.42	7.86	2.40	-1.90	-8.41
5	-11.96	-0.85	1.75	-5.06	9.57	-2.40	-7.37	-16.22	-8.83
6	-2.95	11.72	2.84	-1.17	9.57	-2.64	-2.44	-13.83	-3.80
7	-5.49	3.67	-2.62	2.17	3.76	1.55	-5.70	-6.01	-8.62
8	-6.88	0.90	-2.62	4.33	1.27	-0.07	-3.88	-6.41	-11.76
9	-12.19	-0.85	-4.81	-5.60	7.91	0.85	-4.94	-6.81	-8.41
10	5.12	11.34	6.12	7.46	7.91	5.99	-4.03	-9.85	-2.54
11	5.12	12.22	8.31	15.88	7.91	8.09	-1.68	-8.79	-10.08
12	-2.72	1.03	2.84	5.95	2.10	-1.00	-3.43	-5.75	-9.67
13	-11.96	-3.12	1.75	1.84	7.08	-1.00	-14.20	-19.93	-15.11
14	-2.49	11.09	7.22	0.98	7.91	-3.81	-2.90	-13.43	-4.64
15	1.20	13.11	6.12	15.88	-1.21	1.55	-6.46	-5.61	-14.49
16	13.21	-6.89	-0.43	-15.75	-8.68	0.39	8.47	12.67	8.98
17	-9.88	-16.96	-11.37	-14.13	-0.38	-8.47	0.58	2.99	2.48
18	-5.96	-22.74	-8.09	-16.62	-1.21	-10.34	9.16	-2.83	11.28
19	-20.97	-23.62	-9.19	-22.34	-1.21	-6.84	1.72	-0.18	-0.02
20	-15.20	-9.41	-10.28	-5.38	-2.04	-1.00	-1.68	-11.05	-10.92
21	-14.74	-21.11	-9.19	-22.88	2.10	-8.24	5.97	-1.77	8.77
22	-14.74	-25.64	-12.47	-20.83	-1.21	-6.37	4.76	2.86	6.04
23	-8.04	13.86	5.03	-1.28	18.71	-1.00	-16.17	-14.63	-7.78
24	-11.73	-18.72	-9.19	-16.83	-0.38	-8.94	6.65	-0.97	4.16
25	-0.41	6.94	3.93	10.37	2.10	-0.54	-2.90	-5.61	-11.34
26	20.61	6.31	13.78	18.91	-15.32	2.72	3.24	4.72	-0.44
27	6.28	1.28	-2.62	-1.71	-1.21	1.79	-0.70	2.99	6.67
28	14.14	1.40	7.22	21.39	-19.47	-4.51	0.28	7.10	-7.15
29	20.14	6.31	8.31	15.13	-15.32	-2.40	3.24	14.53	4.16
30	14.83	-20.98	0.65	-3.12	-20.30	2.02	13.78	18.77	17.36
31	5.59	-4.00	-5.90	11.99	-18.64	-3.34	-3.50	2.07	-7.15
32	3.04	4.68	-5.90	-2.47	-2.87	-1.94	1.04	9.75	3.74
33	-4.11	-10.41	-13.56	-0.85	-18.64	-2.40	-2.14	5.11	-4.84
34	20.37	5.30	6.12	16.42	-12.00	-4.51	4.45	13.07	3.74
35	10.67	-1.86	-1.53	7.35	-10.34	0.85	1.80	5.51	2.27
36	11.59	0.02	-0.43	10.48	-9.51	-2.40	1.57	9.22	-0.65
37	16.22	4.68	2.84	5.62	-4.53	3.89	4.76	12.41	8.35
38	17.37	4.42	6.12	8.21	-5.36	-2.17	3.92	10.15	6.67
39	- 0.87	-2.49	0.65	-12.19	7.08	0.85	-8.59	-0.71	11.49

End of Table 2 next page.

Table 2. *(cont.)*

Populations	L	a	b	c	c'	V	Od. style	Od. phore	Tail
40	-0.64	0.02	2.84	-8.62	9.57	-0.07	-5.78	-0.71	6.67
41	-5.49	-5.13	-2.62	3.57	-15.32	-0.77	-3.65	0.34	-10.29
42	-2.26	-5.25	-1.53	2.49	-11.17	0.62	-4.94	-1.50	-6.52
43	-4.57	-5.25	-1.53	3.79	-15.32	-2.64	-6.69	1.01	-8.83
44	-2.03	-8.15	0.65	-4.95	-13.66	-3.57	-8.59	-0.58	2.90
45	4.43	8.45	-1.53	-0.63	3.76	0.85	-1.68	3.13	3.32
46	8.36	5.30	6.12	5.51	-6.19	-5.67	-1.91	2.99	1.01
47	3.74	0.15	-0.43	2.27	-8.68	-5.44	-2.90	0.74	-0.44
48	14.60	21.54	15.97	26.57	-3.70	2.96	-6.01	-5.22	-11.13
49	14.60	-3.74	3.93	9.40	-11.17	-2.40	3.24	10.15	2.90
50	5.59	4.17	7.22	3.57	0.44	-6.14	-4.49	4.45	1.43
51	10.90	-12.05	-8.09	-6.90	-7.02	-0.54	17.05	-4.55	17.15
52	-7.57	-13.18	7.22	-0.63	-16.98	-1.94	3.16	0.74	-9.87
53	-7.57	-16.58	-9.19	2.81	-25.28	-3.10	6.80	-1.77	-11.97
- - -									
54	-8.96	11.97	3.93	-13.59	32.82	8.09	-17.31	-17.81	2.69
55	-10.81	17.26	5.03	-21.26	49.42	8.09	-18.83	-16.22	11.28
56	-32.99	-8.40	-20.13	-31.74	41.12	8.09	-22.85	-19.80	-3.17
57	22.68	-10.04	-11.37	-1.71	-16.98	23.50	30.47	46.47	21.55
58	50.18	54.75	28.00	17.50	41.12	6.22	17.58	6.04	26.79
59	10.90	-27.02	-14.66	2.17	-25.28	22.57	19.85	30.57	5.83

Table 3. *Correlation coefficient matrix among variables used in PCA.*

	L	a	b	c	c'	V	Od. style	Od. phore	Tail
L	1.0000	0.5000	0.6281	0.6793	-.2676	0.4239	0.3117	0.5729	0.2978
a	0.5000	1.0000	0.7898	0.7275	0.4762	0.6866	-0.3790	-0.2290	-0.3552
b	0.6281	0.7898	1.0000	0.7453	0.2091	0.5506	-0.2352	-0.0424	-0.2256
c	0.6793	0.7275	0.7453	1.0000	-0.1429	0.5536	-0.1906	0.0613	-0.4624
c'	-0.2676	0.4762	0.2091	-0.1429	1.0000	0.2875	-0.3929	-0.6346	-0.1088
V	0.4239	0.6866	0.5506	0.5536	0.2875	1.0000	-0.1477	-0.1359	-0.2427
Od. style	0.3117	-0.3790	-0.2352	-0.1906	-0.3929	-0.1477	1.0000	0.5608	0.6152
Od. phore	0.5729	-0.2290	-0.0424	0.0613	-0.6346	-0.1359	0.5608	1.0000	0.5811
Tail	0.2978	-0.3552	-0.2256	-0.4624	-0.1088	-0.2427	0.6152	0.5811	1.0000

populations are clustered into another group which includes the populations of *X. diversicaudatum*.

X. dissimile group is subdivided into two sub-groups. The first one is constituted by eight populations, including the type population. It is identified as the "typical" sub-group and it is characterized by a body length of 4.6 (3.5-5.8) mm, an "a" value of 95.5 (71.6-117.0), and an odontostyle length of 131.5 (111.0-150.5) µm. Measurements of two populations of the "typical" *X. dissimile* sub-group are reported in Table 5. The eleven populations of the "small" *X. dissimile* sub-group are characterized mainly by a body length of 3.4 (3.3-5.0) mm, an "a" value of 82.6 (57.5-101.0), and an odontostyle length of 124.0 (103.5-139.5) µm. The measurements of three populations of the "small" *X. dissimile* sub-group also are

Table 4. *PCA: Eigenvalues of the correlation matrix.*

	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	3.79973	1.03757	0.422192	0.42219
PRIN2	2.76215	1.62713	0.306906	0.72910
PRIN3	1.13503	0.59940	0.12 6114	0.85521
PRIN4	0.53563	0.17448	0.059514	0.91473
PRIN5	0.3 6115	0.16357	0.040128	0.95485
PRIN6	0.19758	0.05492	0.021953	0.97681
PRIN7	0.14266	0.09514	0.015851	0.99266
PRIN8	0.04752	0.02896	0.005280	0.99794
PRIN9	0.01856	0.00	0.002062	1.00000

reported in Table 5. The *X. belmontense* group, constituted by six populations including the type population, is characterized by a body length of 3.7 (3.1-4.5) mm, an "a" value of 61.7 (51.2-70.7), and an odontostyle of 129.0 (132.5-149.5) µm long.

No relevant morphological differences were observed between the populations of the "typical" and "small" *X. dissimile* sub-groups (Fig. 4). The pseudo-Z-organ shows no significant differences: it is constituted by a variable number of sclerotized bodies of different size, each of them consisting of a central large and almost rounded hyaline portion, surrounded by irregularly shaped refractive granules of variable thickness. The anterior region is identical in shape in all populations of *X. dissimile*, with a lip region expanded, slightly flattened frontally and rounded laterally, separated from the rest of the body by a very slight depression (Fig. 5). Differences in the shape of the lip region are evident between *X. dissimile* and

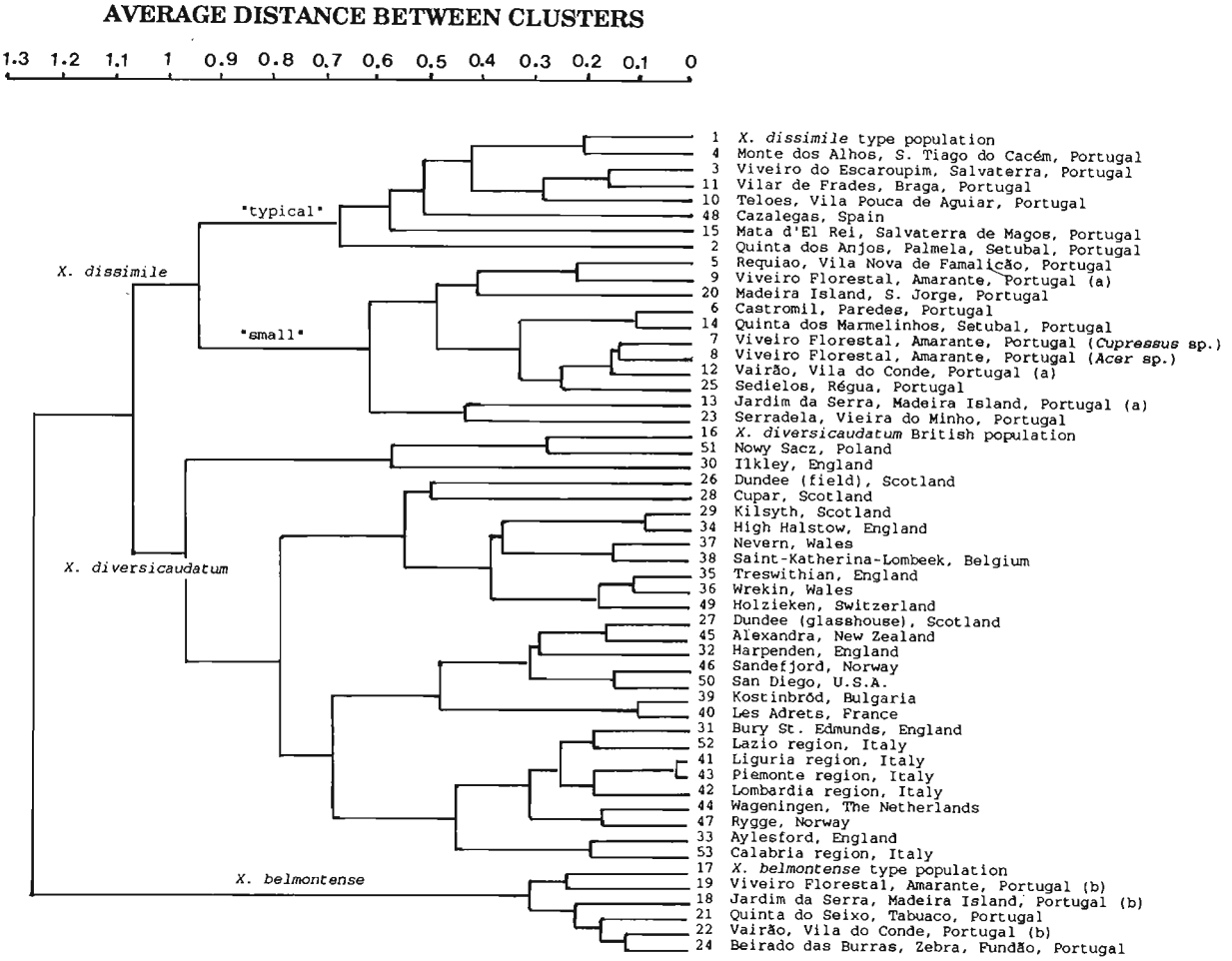

Fig. 1. *Dendrogram of 53 populations of* Xiphinema belmontense, X. dissimile *and* X. diversicaudatum *analyzed by hierarchical cluster analysis of morphometric variables and average distances between clusters.*

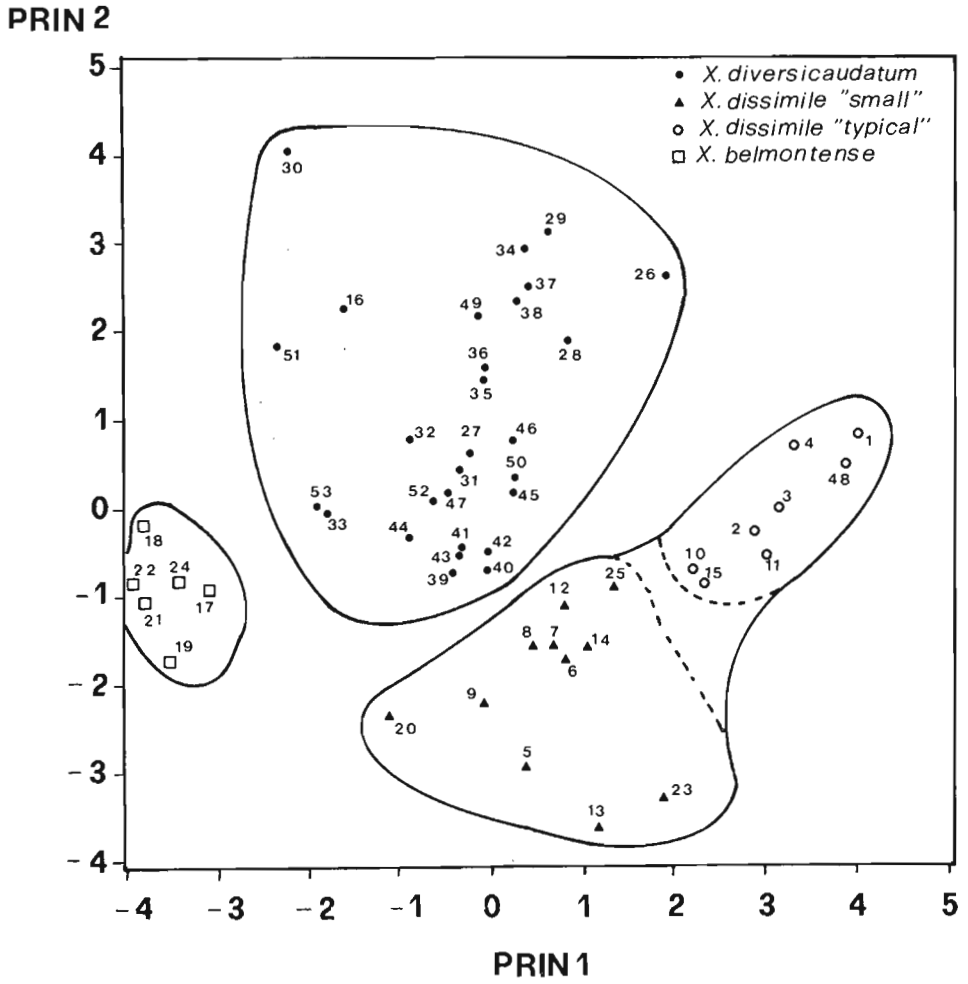

Fig. 2. *Scatterplot of 53 populations of* Xiphinema belmontense, X. dissimile *and* X. diversicaudatum, *on the first and second principal component axis (Population codes as indicated in Table 1).*

X. diversicaudatum: in the latter, the lip region is not separated from the rest of the body as in *X. dissimile*, and it appears almost continuous (Fig. 5). There are no large differences in tail shape between populations of the "typical" and "small" *X. dissimile* sub-groups, taking into account the large individual variability within and between populations (Fig. 4). On the contrary, a substantial difference exists in tail shape between *X. dissimile* and *X. diversicaudatum*; with tails more or less conical with subdigitate terminus in the first, almost rounded with well separated peg in the second species.

Referring to the second study using PCA and HCA analyses performed with morphometric measurements of all 59 populations (Table 1), the dendrogram obtained with HCA analysis is presented in Fig. 6. In this case, the results are completly different since the "small" *X. dissimile* sub-group is included in the group of *X. diversicaudatum*, while populations from Ailesford, England and from Calabria, Italy, are included in the group of *X. belmontense*, as indicated in the dendrogram (Fig. 6). It is also noteworthy that population 15 from Mata d'El Rei, Salvaterra de Magos, Portugal is included in the "typical" *X. dissimile* sub-group in the first dendogram (Fig. 1), whereas it is included in the "small" *X. dissimile* subgroup in the second dendrogram (Fig. 6).

Discussion

Three groups are easily distinguished by the dendrogram and scatterplots (Figs 1, 2, 3), which confirms their biometrical differences. These three groups are constituted by *X. belmontense*, *X. dissimile*,

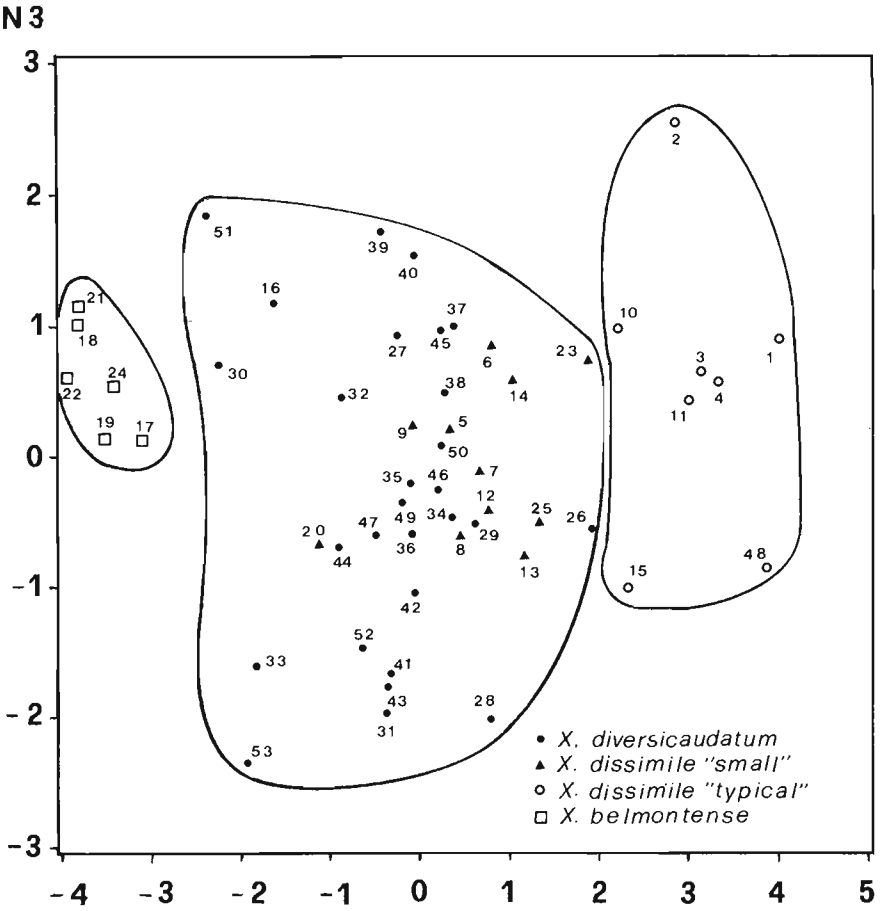

Fig. 3. *Scatterplot of 53 populations of* Xiphinema belmontense, X. dissimile *and* X. diversicaudatum, *on the first and third principal component axis (Population codes as indicated in Table 1).*

and *X. diversicaudatum*. Some morphological differences also exist between these groups. Apart from tail shape (more or less short conical with subdigitate terminus in *X. dissimile*, rounded with terminal peg in *X. diversicaudatum*), a clear difference exists between *X. belmontense* on the one hand, and *X. dissimile* and *X. diversicaudatum* on the other hand, namely the presence of small spiniform structures in the uterus of *X. belmontense*.

Generally, HCA placed populations of the same species into the same group, as the populations belonging to a group including each type population can be identified as members of the species represented by the type population. From the dendrogram, three groups are constituted around each type population, distinguishing the same number of species. Populations belonging to *X. diversicaudatum* are clustered into one group; populations belonging to *X. dissimile* are clustered into another group; all are fused into a larger group containing these two species. The three species are easily distinguishable in the scatterplot of PRNC2 by PRNC1 (Fig. 2) where the separation of the three species is evident and also the separation of the "typical" and "small" *X. dissimile* from *X. diversicaudatum*. In the scatterplot of PRNC3 by PRNC1 (Fig. 3) the separation of "typical" *X. dissimile* from *X. diversicaudatum* is not very clear, and the "small" *X. dissimile* is included into *X. diversicaudatum*. Populations of the first group, *i.e.*, *X. dissimile* are morphologically different from those of *X. diversicaudatum*, although both are clustered in the same larger group. Therefore, we consider that the populations of the group including the type population of *X. dissimile* belong to this species, and that this species includes a "typical" and a "small" one.

Finally, it should also be mentioned that mixed populations were frequently found during the survey in Portugal. They were found at Vairao, Vila do

Table 5. *Morphometrics of "typical" and "small"* Xiphinema dissimile *populations (female specimens), from Portugal (All measurements in µm except L in mm).*

	X. dissimile "typical"		*X. dissimile* "small"		
Locality	Monte dos Alhos, S. Tiago do Cacém.	Teloes, V. P. de Aguiar	Vairao, Vila do Conde	Q. dos Marmelinhos Setubal	Madeira Island, Jardim da Serra
Host	Cereals	Strawberry	Raspberry	*Citrus* sp.	Grapevine
n	36	14	55	24	23
L	4.8 ± 0.43 (3.8 - 5.8)	4.6 ± 0.62 (3.5 - 5.4)	4.2 ± 0.41 (3.4 - 4.9)	4.2 ± 0.26 (3.7 - 4.9)	3.8 ± 0.24 (3.3 - 4.2)
a	97.9 ± 8.25 (76.8 - 112.1)	88.5 ± 8.37 (75.1 - 101.7)	80.3 ± 6.03 (63.5 - 93.8)	88.3 ± 6.38 (72.3 - 99.9)	76.9 ± 7.73 (62.5 - 90.7)
b	10.0 ± 1.07 (8.0 - 13.3)	9.7 ± 1.20 (7.2 - 11.5)	9.3 ± 0.80 (7.9 - 11.2)	9.8 ± 0.70 (8.3 - 11.5)	9.3 ± 1.01 (8.0 - 13.0)
c	110.6 ± 13.78 (83.1 - 145.2)	99.4 ± 14.10 (73.2 - 120.3)	98.2 ± 10.98 (71.9 - 119.8)	93.5 ± 11.31 (72.8 - 122.4)	94.3 ± 6.69 (84.3 - 105.5)
c'	1.3 ± 0.13 (1.0 - 1.5)	1.3 ± 0.17 (1.1 - 1.7)	1.2 ± 0.11 (0.98 - 1.6)	1.3 ± 0.14 (1.1 - 1.6)	1.3 ± 0.13 (1.0 - 1.5)
V	46.2 ± 1.53 (43.1 - 49.0)	45.4 ± 2.80 (41.3 - 50.8)	42.4 ± 1.56 (38.8 - 46.7)	41.3 ± 1.50 (38.3 - 44.7)	42.4 ± 1.13 (40.0 - 44.2)
Lip. reg. diam.	13.5 ± 0.47 (12.5 - 14.0)	12.0 ± 0.59 (10.5 - 13.0)	13.5 ± 0.72 (12.5 - 17.0)	13.1 ± 1.51 12.5 - 14.5)	11.5 ± 0.46 (10.0 - 11.5)
Lip. reg. height	3.5 ± 0.34 (3.0 - 4.0)	3.0 ± 0.30 (3.0 - 3.5)	4.5 ± 0.83 (2.9 - 6.5)	3.0 ± 0.39 (2.5 - 4.0)	3.0 ± 0.13 (2.9 - 3.5)
Odontostyle	135.0 ± 6.45 (117.0 - 150.5)	126.5 ± 7.97 (111.0 - 135.5)	127.5 ± 5.16 (116.5 - 139.5)	128.0 ± 3.41 (121.0 - 134.0)	113.0 ± 3.59 (106.5 - 121.0)
Odontophore	74.0 ± 2.59 (69.0 - 79.5)	68.0 ± 3.56 (61.5 - 74.5)	71.0 ± 3.03 (65.5 - 78.0)	65.5 ± 3.25 (60.0 - 70.5)	60.5 ± 2.34 (56.0 - 65.5)
Stylet	209.0 ± 7.81 (186.5 - 230.0)	194.5 ± 10.89 (173.0 - 206.5)	198.5 ± 6.91 (186.0 - 211.0)	193.5 ± 4.63 (185.5 - 203.5)	173.5 ± 5.25 (162.5 - 183.0)
Flanges width	12.5 ± 0.95 (10.5 - 14.5)	12.0 ± 1.31 (10.0 - 14.0)	12.0 ± 0.95 (10.0 - 14.5)	11.5 ± 0.80 (9.5 - 13.0)	11.5 ± 0.83 (10.5 - 13.5)
Guide ring	116.0 ± 6.6 (98.0 - 130.0)	110.0 ± 9.32 (90.0 - 124.0)	115.5 ± 5.04 (99.0 - 126.5)	107.5 ± 5.12 (98.0 - 117.5)	102.0 ± 5.58 (88.0 - 110.5)
Guide sheath	12.0 ± 3.89 (6.5 - 26.5)	11.5 ± 2.01 (7.0 - 14.0)	15.0 ± 2.23 (6.5 - 21.5)	10.5 ± 1.92 (7.5 - 13.5)	13.0 ± 3.92 (7.0 - 23.5)
Phar. bulb. length	106.5 ± 12.13 (60.5 - 128.0)	112.0 ± 7.47 (101.5 - 126.5)	105.5 ± 6.81 (88.0 - 119.0)	96.0 ± 7.21 (84.0 - 106.5)	112.0 ± 5.44 (101.0 - 120.5)
Phar. bulb. diam.	22.5 ± 1.65 (19.5 - 26.5)	20.0 ± 1.73 (16.5 - 23.5)	22.5 ± 1.62 (17.5 - 27.0)	20.0 ± 1.49 (17.0 - 23.5)	19.5 ± 1.94 (16.5 - 24.0)
Ant. gen. br.	515.7 ± 68.18 (382.3 - 611.7)	506.0 ± 116.28 (347.0 - 694.0)	547.5 ± 92.29 (247.0 - 729.5)	469.0 ± 49.47 (364.5 - 559.0)	417.5 ± 58.43 (300.0 - 564.5)
Post. gen. br.	475.0 ± 85.47 (329.4 - 676.5)	524.5 ± 108.67 (388.0 - 747.0)	525.0 ± 72.59 (394.0 - 682.5)	486.0 ± 48.0 (382.5 - 570.5)	453.5 ± 55.26 (323.5 - 553.0)
Ant. gen. br. (%)	10.9 ± 1.51 (8.6 - 14.1)	11.0 ± 2.05 (8.3 - 14.9)	13.0 ± 2.08 (7.0 - 18.0)	11.0 ± 1.33 (7.5 - 13.5)	11.0 ± 1.30 (8.7 - 14.8)
Post. gen. br. (%)	10.0 ± 1.91 (7.4 - 13.6)	11.5 ± 2.13 (8.7 - 16.1)	12.5 ± 1.55 (9.6 - 16.0)	11.5 ± 1.18 (9.0 - 14.0)	11.9 ± 1.59 (9.5 - 15.0)
Body diam. (mid body)	49.0 ± 3.86 (42.5 - 56.5)	52.0 ± 6.20 (39.5 - 60.5)	52.5 ± 3.21 (45.5 - 59.0)	48.0 ± 3.93 (41.0 - 59.0)	50.0 ± 5.20 (39.5 - 57.0)
Body diam. (anus level)	34.5 ± 2.23 (30.5 - 42.5)	34.5 ± 3.59 (30.0 - 41.0)	35.0 ± 2.02 (29.0 - 39.5)	35.5 ± 2.65 (31.0 - 41.0)	31.5 ± 3.45 (26.5 - 39.0)
Rectum	35.0 ± 3.02 (29.5 - 40.5)	34.5 ± 4.52 (27.0 - 41.5)	32.5 ± 3.17 (27.5 - 39.5)	33.5 ± 2.42 (28.0 - 37.0)	34.5 ± 3.36 (25.5 - 41.0)
Tail	43.5 ± 4.47 (36.0 - 56.0)	46.5 ± 4.48 (39.0 - 56.0)	43.0 ± 3.69 (34.5 - 51.0)	45.5 ± 4.20 (40.0 - 53.0)	40.5 ± 2.35 (37.0 - 45.5)
Hyaline tail tip	16.5 ± 1.74 (13.0 - 20.5)	19.0 ± 1.64 (16.5 - 23.0)	16.5 ± 2.09 (12.5 - 20.5)	17.5 ± 1.50 (13.0 - 20.0)	17.5 ± 2.07 (12.5 - 20.5)
Prerectum	570.0 ± 119.86 (223.5 - 817.5)	597.5 ± 152.47 (353.0 - 847.0)	646.5 ± 127.64 (329.5 - 964.5)	495.5 ± 112.6 (311.5 - 676.5)	515.0 ± 115.89 (353.0 - 847.0)

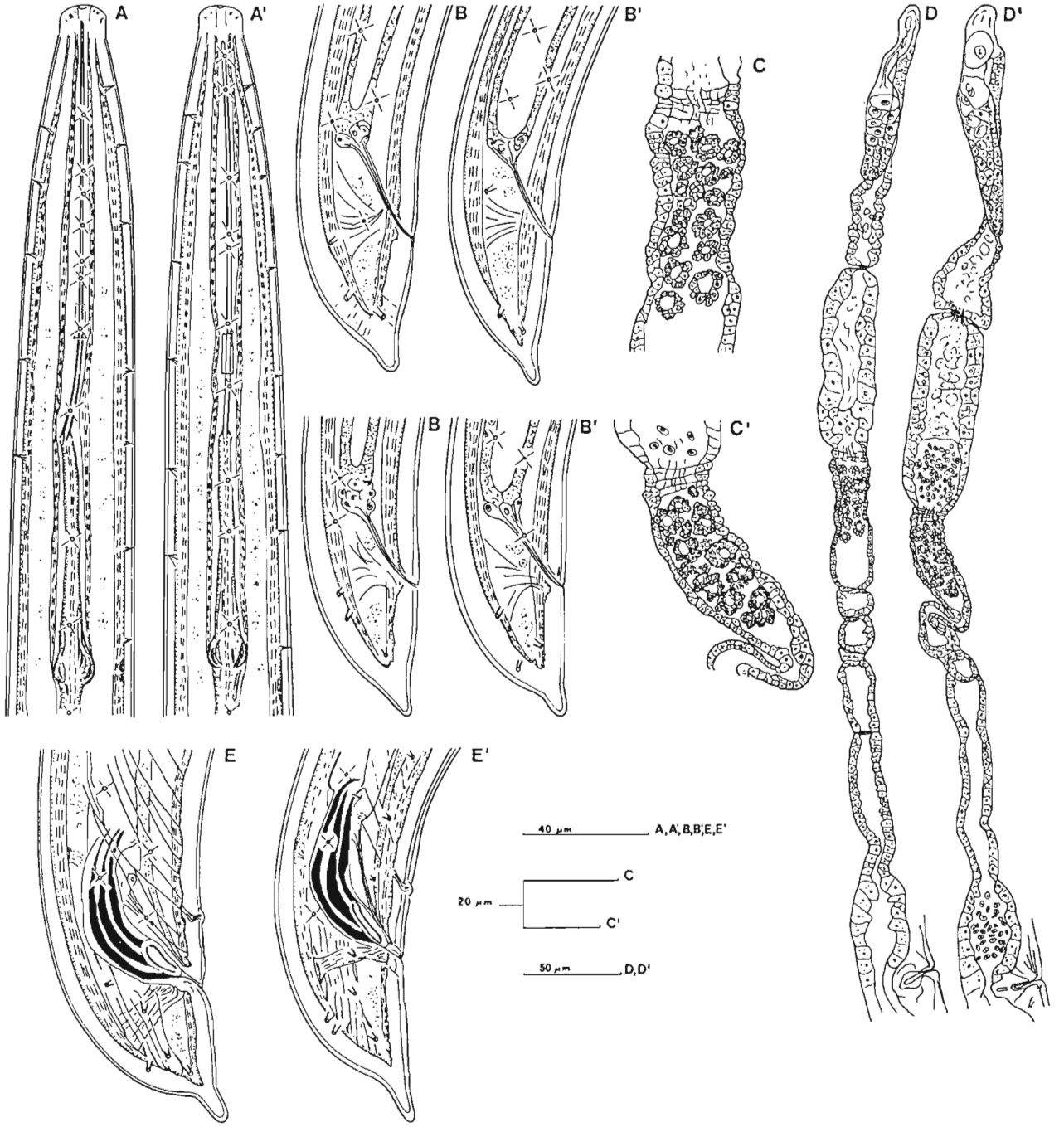

Fig. 4. Xiphinema dissimile *"typical", (A-E) population from Monte dos Alhos, S. Tiago do Cacém, Portugal and "small" (A'-E') population from Vairao, Vila do Conde. A, A': Female, anterior regions; B, B': Female posterior regions; C, C': Pseudo-Z-organ; D, D': Anterior branch of the female genital tract; E, E': Male, posterior region.*

Conde (populations 12, 22), at Amarante, in a forestal nursery (populations 9, 19) and at Madeira Island, Jardim da Serra (populations 13, 18). These mixed populations include both the "small" *X. dissimile*,

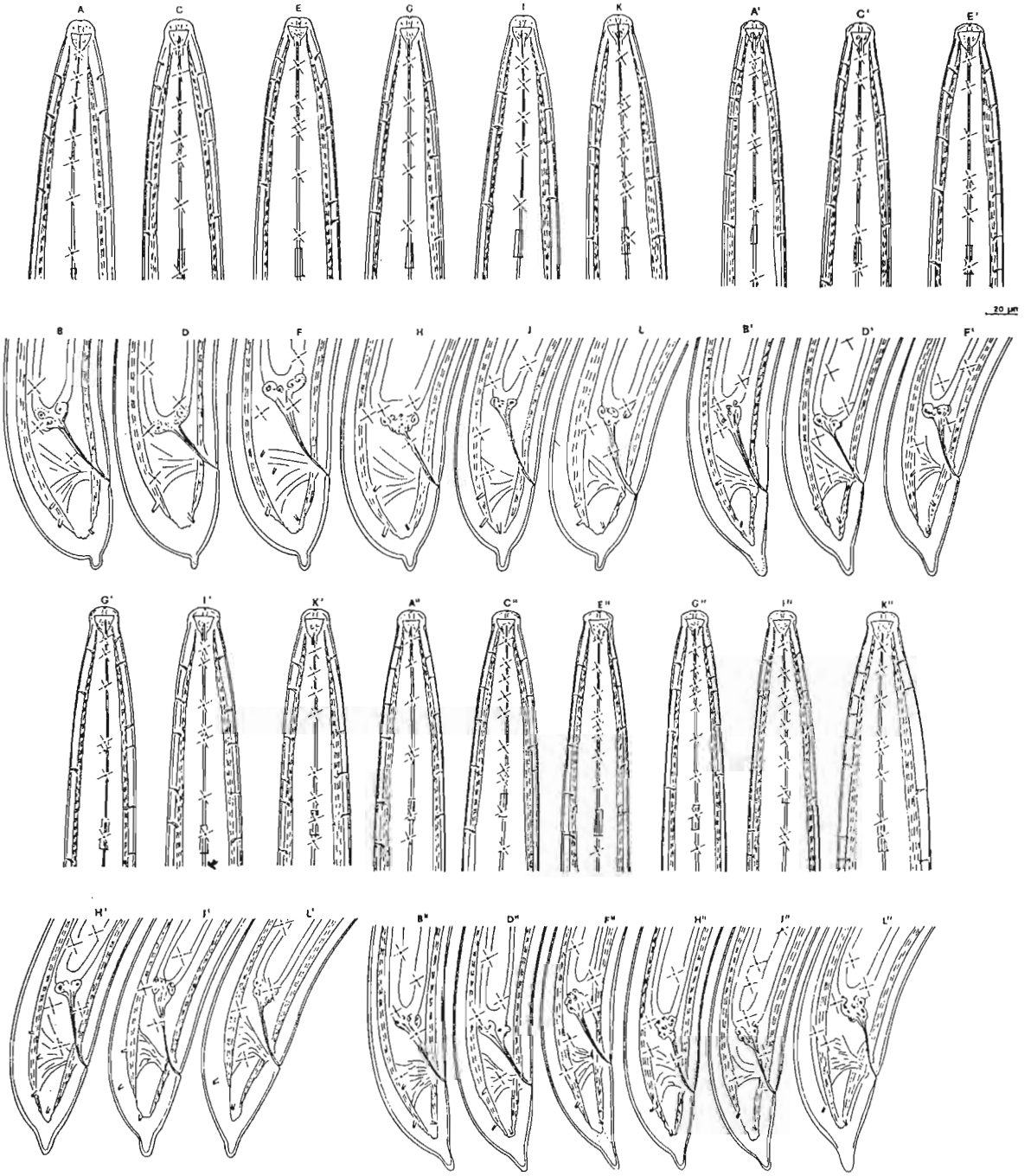

Fig. 5. *Anterior and posterior regions of* Xiphinema diversicaudatum *(A-L),* X. dissimile *"typical" (A'-L') and* X. dissimile *"small" (A"-L"). Female specimens from: A-B: Dundee, Scotland; C-D: France, locality unknown; E-F: Palazzetto, Toscana, Italy; G-H: Borgo d'Ale, Piemonte, Italy; I-J: Bassano, Trentino. Italy; K-L: Catanzaro, Calabria, Italy; A'-B': Viveiro do Escauroupim, Salvaterra, Portugal; C'-D': Monte dos Alhos, S. Tiago do Cacém, Portugal; E'-F': Quinta dos Anjos, Palmela, Setubal, Portugal; G'-H': Teloes, Vila Pouca de Aguiar, Portugal; I'- J': Vilar de Frades, Braga, Portugal; K'-L': Spain, locality unknown (specimens slightly flattened); A"-B": Jardim da Serra, Madeira Island; C"-D": S. Jorge, Madeira Island; E"-F": Requiao, Vila Nova de Famalição, Portugal; G"-H": Quinta dos Marmelinos, Setubal, Portugal; I"-J": Sedielos, Régua, Portugal; K"-L": Viveiro Florestal, Amarante, Portugal.*

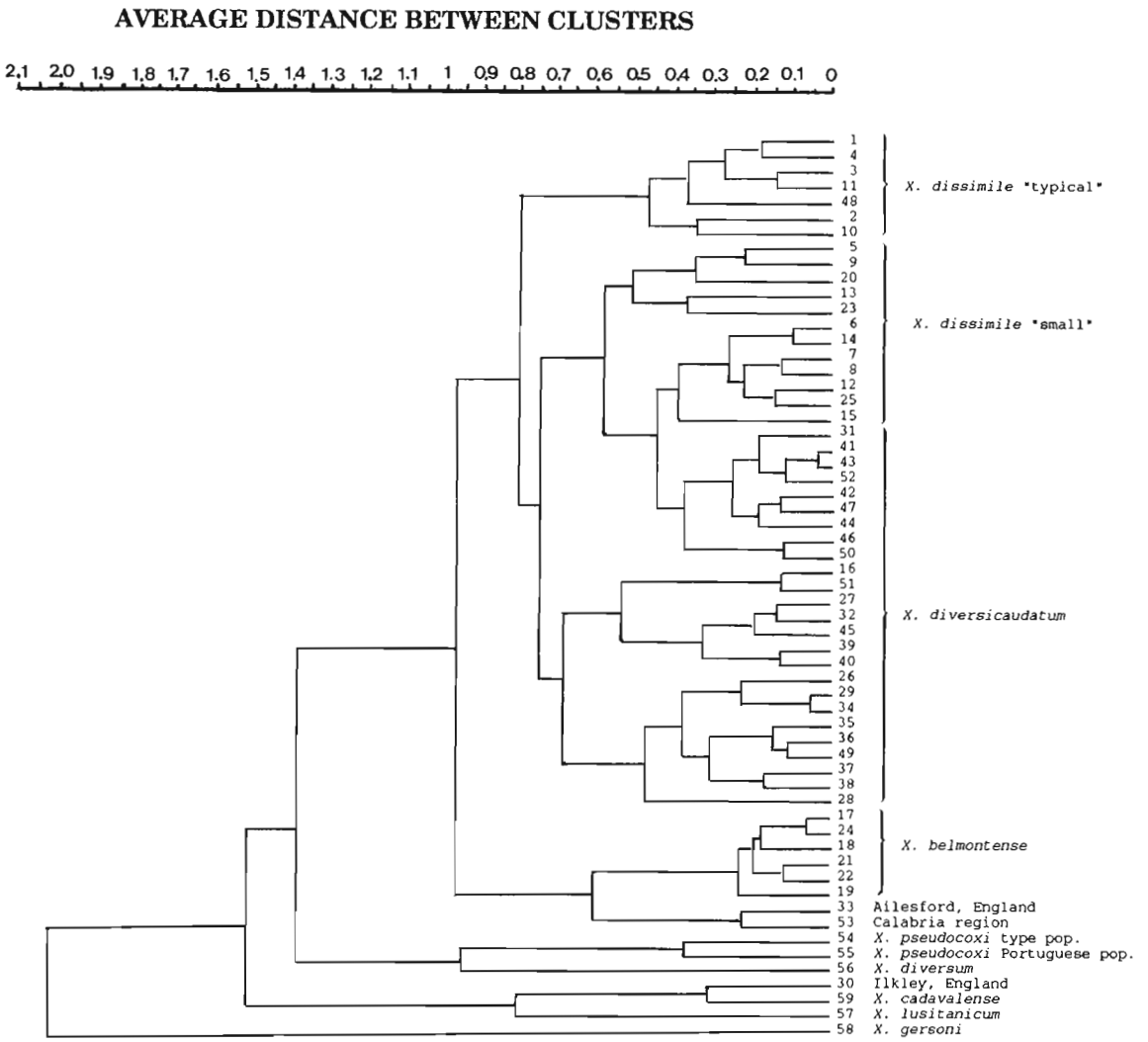

Fig. 6. *Dendrogram of 59 populations of* Xiphinema belmontense, X. cadavalense, X. dissimile, X. diversum, X. diversicaudatum, X. gersoni, X. lusitanicum *and* X. pseudocoxi *analyzed by hierarchical cluster analysis of morphometric variables and average distances between clusters.*

indicated as (a) in the dendrogram, and *X. belmontense*, indicated as (b). The fact that these mixed populations pertaining to two species can be separated under a compound microscope affords the proof that the two species can be easily differentiated from each other.

Conclusions

Statistical studies were performed using dimension values and common ratios as variables in PCA. HCA revealed several groups, each corresponding to an already described species. The present statistical analyses did not include any of the quantitative parameters that could have been used to describe the shapes of tail and lip region or other morphological characters. Brown and Topham (1985) also did not include morphological characters in their study of various populations of *X. diversicaudatum* collected in several countries and continents. Using the same variables as used by Brown and Topham (1985), we obtained similar results in our PCA, although the statistical methods used were different. The population from Cazalegas, Spain, clustered at the 82.5% level of similarity in the Brown and Topham (1985) dendrogram and it was morphometrically very different from the other populations of *X. diversicaudatum*. The same population appears in the group of "typical" *X. dissimile* in our dendrogram. The observations of morpho-

logical characters on specimens from Spain confirm our statistical results (Fig. 5).

Specimens used in the present study have very similar anatomo-morphological characters. Only the Portuguese populations, a group homogeneous in anatomo-morphology differ from Scottish, French and Italian populations of *X. diversicaudatum* in the shape of tail and lip region (Fig. 5). In the present study, the geographical origin of populations, in general, appears to influence the arrangement of populations into morpho-groups: populations belonging to *X. dissimile* found only in the Iberian peninsula, were grouped together as well as populations from Portugal constituting a morpho-group of populations, which belongs to a larger group containing also *X. diversicaudatum.*

However, the use of PCA and HCA in taxonomy, in general, is not easily reliable, particularly in separating the species. The results sometimes confusing as shown by the PCA and HCA analyses performed with all 59 populations reported in Table 1. Obviously, the same population cannot belong to two different species. So, the only conclusion that can be drawn is that, in the present case, HCA and PCA analyses failed to separate the two species. This failure may be due in part to the fact that the last four species included in the second study were each represented by a single population and *X. pseudocoxi* by two as opposed to six populations for *X. belmontense* and a larger number of populations of *X. dissimile* and *X. diversicaudatum* included in both studies (Figs 1, 6). Indeed, the second dendrogram (Fig. 6) does not appear well balanced in comparison with the first one (Fig. 1).

Referring to the dendrogram in Fig. 1, the new position of the population from Cazalegas, Spain, is due to the introduction of new data that act as guide in dragging similar values of populations.

The high sensitivity of PCA and HCA analyses in enhancing small morphometric differences makes them split *X. dissimile* into two sub-groups of populations, to which no taxonomic value can be attributed (Fig.1). This shows that PCA and HCA are not always reliable in separating species, but may only indicate intraspecific morphometric variations due to different ecological conditions or host plants. This means that metric data alone cannot be trusted to separate species, and that morpho-anatomical characters must have priority for the definition and the separation of such species.

In general, the use of PCA and HCA analyses based only on the morphometrics could be useful to separate species only when it is supported by morpho-anatomical observations.

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