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A statistical approach to the objective differenciation of *Hirschmanniella oryzae* from *H. belli* (Nemata : Pratylenchidae)

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SUMMARY

California populations attributed to *Hirschmanniella belli* were compared *i)* to paratypes of this species, *ii)* to topotypes of *H. oryzae*, and *iii)* to other populations of the same genus from other parts of the world. Comparisons were made using discriminant function analyses. Some small differences between California populations and paratypes of *H. belli* were attributed to artifacts caused by the long storage of these paratypes. Seven characters were selected that were not affected by these artifacts, but were successful in differentiating all California specimens (including paratypes of *H. belli*) from topotypes of *H. oryzae*. It was verified that a discriminant function analysis using these seven characters was able to separate other California specimens from *Hirschmanniella* specimens from other origins. The discriminant functions defined by this analysis can be used for practical identification of *Hirschmanniella* from California. The significance of these results for the taxonomic validity of *H. belli* is discussed.

RÉSUMÉ

Une méthode statistique pour la différentiation objective de Hirschmanniella oryzae *et de* H. belli *(Nemata : Pratylenchidae)*

Des populations californiennes attribuées à l'espèce *Hirschmanniella belli* ont été comparées à : 1) des paratypes de cette même espèce; 2) des totopypes de *H. oryzae;* et 3) d'autres populations du même genre provenant d'autres régions du monde. Ces comparaisons ont été faites à l'aide d'analyses discriminantes. De petites différences observées entre les populations californiennes et les paratypes de *H. belli* ont été attribuées à des artefacts résultant du grand âge des paratypes. Sept caractères ont été sélectionnés qui n'étaient pas soumis à l'action de ces artefacts mais qui réussissaient à différencier tous les spécimens californiens (y compris les paratypes de *H. belli*) des topotypes de *H. oryzae*. Il a été vérifié qu'une analyse discriminante utilisant ces sept caractères était capable de séparer d'autres spécimens californiens de spécimens d'*Hirschmanniella* d'autres origines. Les fonctions discriminantes définies par cette analyse peuvent être employées pour l'identification pratique des *Hirschmanniella* californiens. La signification de ces résultats en ce qui concerne la validité de *H. belli* est discutée.

Hirschmanniella oryzae (van Breda de Haan, 1902) Luc & Goodey, 1964 is a well-known pathogen of rice. It causes heavy yield reductions in many paddy rice areas of the world (Fortuner & Merny, 1979), but it is unknown from rice growing areas in Mediterranean climates such as the south of France (Camargue), Italy, South Africa, and California. It has not become established in California, but it is considered to be a potential threat to agriculture in the region. *H. belli* Sher, 1968 is another species of the same genus that has been reported only from California, found in association with rice and cat-tail (*Typha* spp.). Pathogenicity to rice has not been demonstrated, and it is doubtful that *H. belli* causes any economically significant damage, because populations in California rice fields are low and far apart.

Sher (1968) differentiated *H. belli* from *H. oryzae* by the shape of the stylet knobs; rarity of males; females with small, empty, spermatheca; and differences in measurements of body, stylet, and tail. The validity of these criteria is doubtful. Shape of stylet knobs is often intra-specifically variable; there are both theoretical and practical objections to the use of parthenogenesis as a diagnostic criterion; and measurements often vary under many external factors. As a consequence, practical differentiation of *H. oryzae* from *H. belli* using each diagnostic character separately is always difficult and in many cases, impossible.

The morphometrics of several populations of rice-root nematodes from California and from other origins were studied by a multivariate technique in order to select a set of criteria that allows an objective and accurate identification of the two species.

Materials and methods

SPECIMENS

Several populations of rice-root nematodes have been studied (Table 1), including the type populations of *H. oryzae* and *H. belli* kept at the nematode collection of University of California, Riverside, local populations of rice-root nematode collected during a survey of California rice fields, and three populations from various origins in Africa and Asia.

The population from Madras, India, was identified as *H. oryzae* by Sher, the original author of the species *H. belli*. The two populations from Senegal and Ivory Coast were identified as *H. oryzae* by ORSTOM nematologists in these countries, including G. Merny, M. Luc, and R. Fortuner.

Table 1

Origin of the specimens

Code	*Sample Size*	*Locality*	*Host*	*Museum*	*Year Mounted*
1	18	Riverside (types, *H. belli*)	Cat-tail	UCR	1965
2	20	Zamora, CA	Rice	CDFA	1987
3	6	Thornton, CA	Rice	CDFA	1987
4	7	Live Oak, CA	Rice	CDFA	1987
5	11	Sacramento, CA	Wild Rice	CDFA	1985
6	3	Yuba City, CA	Water lilies	CDFA	1986
7	18	Bogor, Java (topotypes *H. oryzae*)	Rice	UCR	1959
8	20	Bouake, Ivory Coast	Rice	ORSTOM	1979
9	20	St Louis, Senegal	Rice	UCD	1988
a	4	Madras, India	Rice	UCR	?
b	5	Bruceville, CA	Rice	CDFA	1989
c	5	Elverta, CA	Rice	CDFA	1987

RECORDING OF THE DATA

The specimens were observed with a Leitz Ortholux II® research microscope equipped with Normarski interference contrast. Observations were made at 400 × (body length, position of vulva) and oil immersion 1 000 × (all other features). Measurements were made with the computerized system Bioquant® System IV. Results were exported out of this system in the form of a square data matrix.

LIST OF CHARACTERS CONSIDERED FOR THE STUDY

In addition to the criteria used by Sher (1968) in the diagnosis of *H. belli*, and in the diagnoses of other species in the genus, other characters were considered for possible use in the differentiation of the species being studied. In particular, the coefficient of oesophageal overlap (length of overlap/distance from center of median bulb to the end of the oesophageal glands), and the coefficient measuring the relative size of the posterior genital branch compared to the anterior branch may be biologically and evolutionary significant.

Shape of the stylet knobs was used as a diagnostic character for *H. belli* by Sher (1968). In order to be included in the discriminant function analyses, this nominal character was transformed into a numerical character measured at the interval level. A rating of the stylet knobs shapes was used, depending on the angle made by the stylet axis and a tangent to the anterior face of the knob, from very slopping knobs, rated 1, to knobs anteriorly indented, rated 5.

The following characters were not included in the study, either because they were unreliable, or not discriminant for the populations studied.

The annuli in the lip region were very difficult to count. We could see four or five annuli in the type specimens of *H. belli* while Sher (1968) reported three or four. The separation between the lip region and the rest of the body was not well marked and the number of annuli depends on where this limit was set by the observer. No difference was seen in specimens of the various populations studied. Scanning electron microscope (SEM) photographs were made of specimens from Africa (Fig. 1 B) and California (Fig. 2 A). The labial disc was almost completely fused with the first cephalic annulus, its edge marked only by a few small depressions, four dorsal and four ventral. The first annulus did not appear to be divided into sectors, but the underlying cephalic framework was faintly visible, more visible in the collapsed anterior end of the African specimens. The framework appeared to have six arms : two lateral, two sub-dorsal, and two sub-ventral arms. The areas between two adjacent arms was depressed in the collapsed African specimens, slightly bulging in the California ones, but these were fixation artifacts. The five cephalic annuli were plain and unmarked.

Shape of the tail end was used by Sher (1968) in the diagnoses of several species in the genus *Hirschmanniella*. All specimens observed in the various samples studied had the same general shape with the same variations in the aspect of the tail end. The tail is narrowly rounded, and has one terminal/ventral finger-like projection and two smaller triangular lateral projections. The two lateral projections were seen only under the best circumstances, and can very easily be overlooked. In fact, they were not reported by Sher, and were first recognized as constant features in *H. oryzae* by Mae Noffsinger (pers. comm.). In old and/or poorly fixed specimens the main mucro is difficult or even impossible to observe. All possible states of this character, from three clearly marked projections to obscure tail ends were observed in specimens from all samples studied. Therefore, this character could not differentiate between

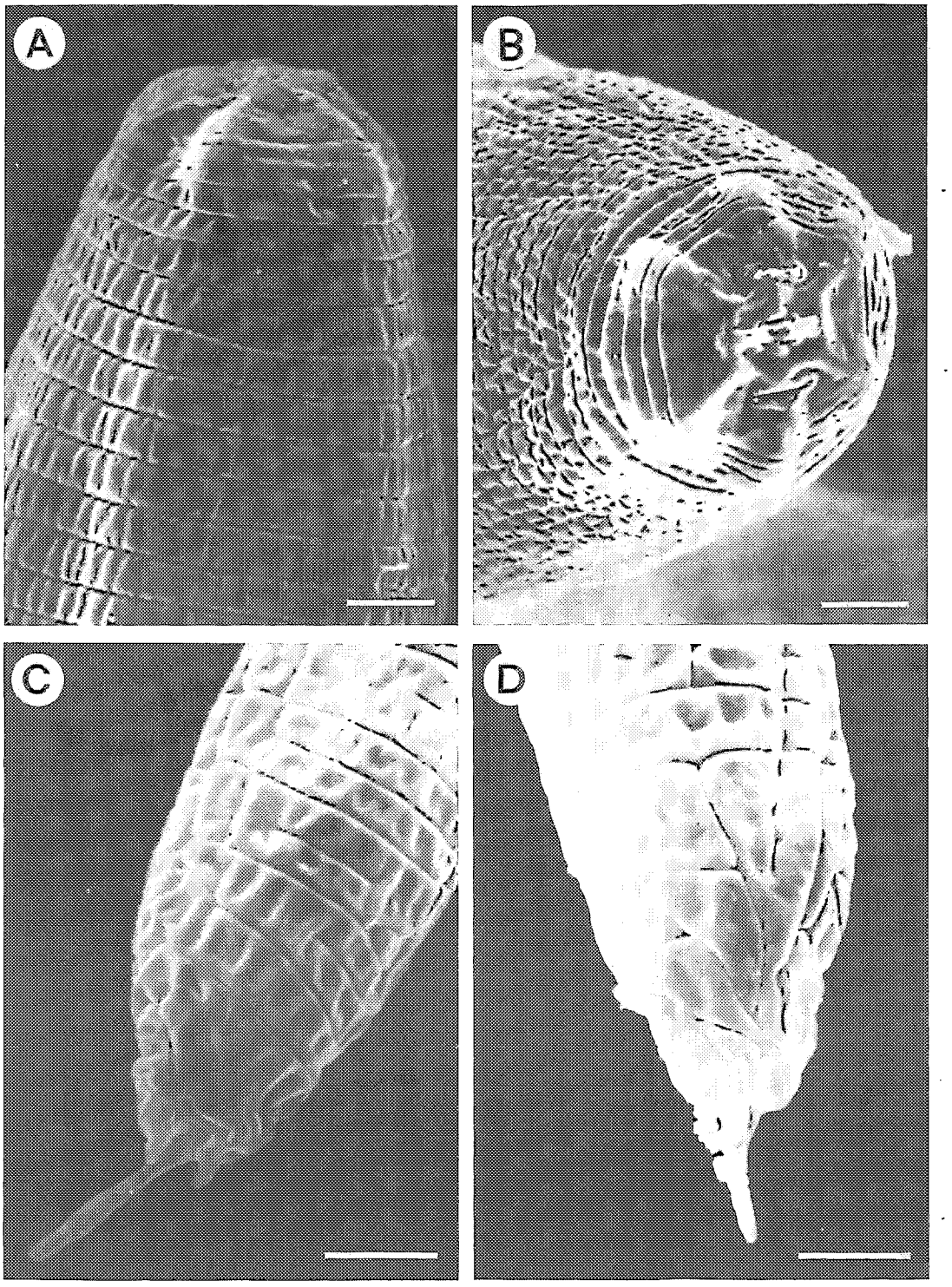

Fig. 1. *Hirschmanniella oryzae* (van Breda de Haan, 1902) Luc & Goodey, 1964. Scanning electron micrographs of specimens from Senegal. A : Anterior end; B : Face view; C, D : Posterior extremity (*Bars = 2 µm).*

these populations, and it has not been included in the analyses. SEM photographs of the same specimens as studied for face view showed a rather long terminal digitation with the faint trace of about three annuli (Figs 1 C; 2 D). Near the base of this digitation, the tail end bears another two smaller processes, either triangular (Fig. 1 C), or finger like (Fig. 2 C), or with two short stubs (Fig. 2 D), or almost completely regressed (Figs 1 D; 2 B).

All specimens observed had no intestinal overlap of the rectum, they all have annulated tails, with annuli visible to the very end, and a low half-dome cephalic region.

Areolation of lateral field was not considered because of its extreme dependance to the circumstances under which it is observed. Areolations are relatively easy to see with interference contrast in specimens where they would be invisible under plain optical microscopy. They

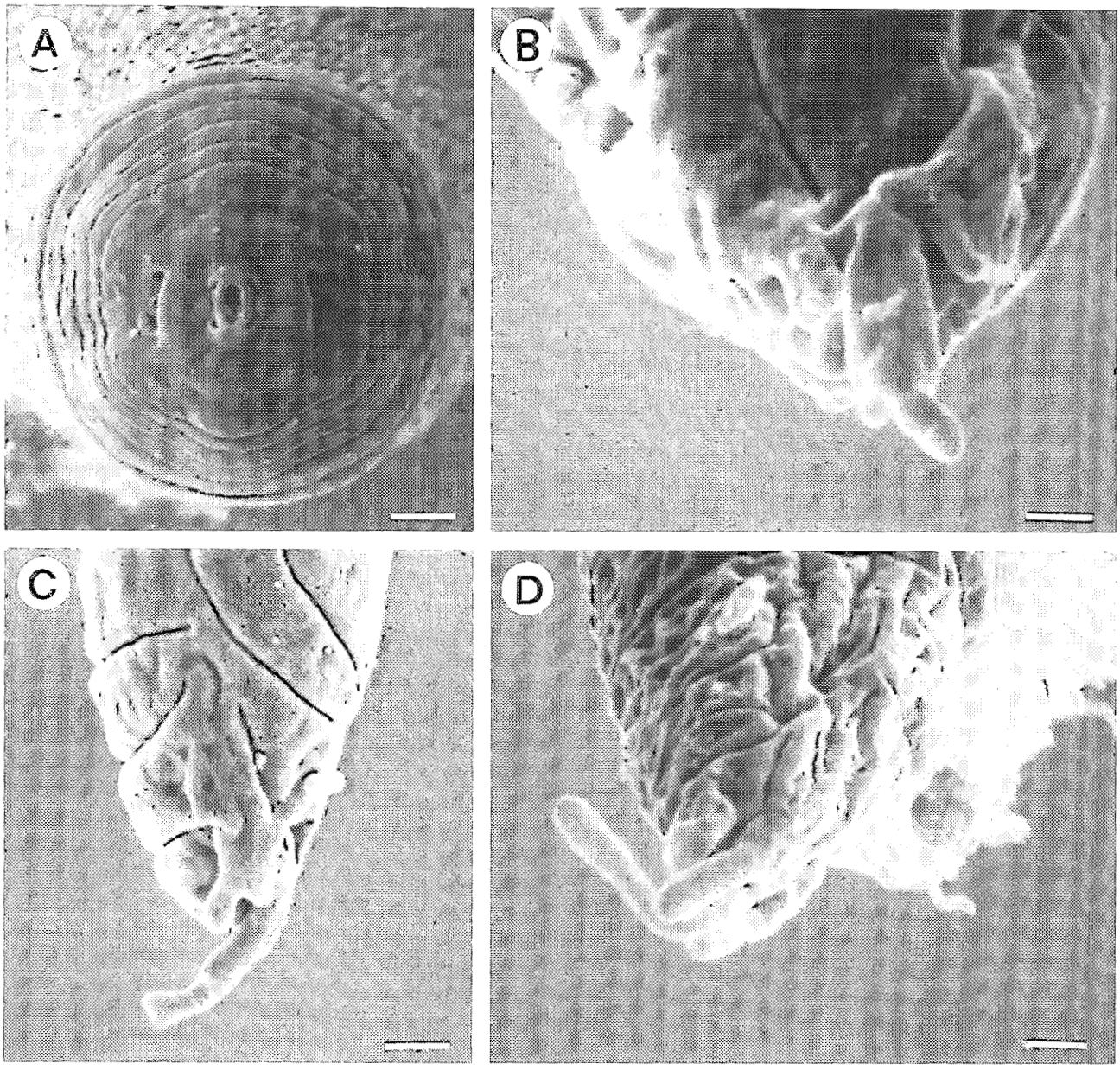

Fig. 2. *Hirschmanniella belli* Sher, 1968. Scanning electron micrographs of specimens from California. A : Face view; B-D : Posterior extremity *(Bars : A = 1.3 µm; B = 0.47 µm; C = 0.93 µm; D = 0.68 µm).*

may disappear in fatter females, or in flattened specimens where the cuticle is stretched.

Inconspicuous spermatheca without sperms and rarity of males will be discussed separately in conclusion of this study.

Stastistical analyses

The data was analyzed with the statistical package SAS® PC on an IBM AT. Univariate statistics were used for the description of the data, then several canonical discriminant function analyses were performed, using the SAS procedures CANDISC for the analyses proper, and CHART and PLOT for the display of the data.

Verification of the data

Comparison of present data with the description by Sher (1968)

The effect of observer error on measurements has been well documented (Brown & Boag, 1989). Long term storage also is known to cause shrinkage of some measurements, while specimens may be flattened by an improperly supported coverslip. The results of the present observations of the types of *H. oryzae* (Table 2) and *H. belli* (Table 3) were compared to the measurements given twenty years ago by Sher, when he first described the same specimens.

In *H. oryzae,* the mean values are very close in Sher and in the present study. The body length is a little longer, the indexes a and b′ a little lower, and b a little higher. Some extreme values are different, but this was to be expected because only some of Sher's paratypes were well enough preserved to be included in the present study.

Table 2

Comparison present data with description of *H. oryzae* by Sher (1968)

Variables	*Sher*	*Present data*
Body length	1.44 mm (1.14-1.63)	1.52 mm (1.37-1.70)
Ratio a	60 (50-67)	58.0 (39.7-79.9)
Ratio b	10.7 (8.8-12.1)	12.1 (9.9-13.4)
Ratio b′	5.7 (4.5-7.2)	4.9 (4.2-5.8)
Ratio c	17 (15-19)	17.6 (15.7-20.2)
Ratio c′	4.6 (4.3-5.5)	4.7 (3.6-6.8)
Ratio V	52 (50-55)	51.8 (48.4-54.7)
Stylet length	17 µm (16-19)	16.9 µm (16-18.3)
Ratio m	48 (47-50)	47.7 (43.9-50.3)
Ratio o	17 (15-19)	not calculated
Lip annuli	3 to 4	4 to 5

Table 3

Comparison present data with description of *H. belli* by Sher (1968)

Variables	*Sher*	*Present data*
Body length	1.87 mm (1.61-2.22)	1.73 mm (1.46-1.97)
Ratio a	67 (58-78)	63 (45-84)
Ratio b	13.8 (11-16)	12.4 (11-17)
Ratio b′	6.0 (5.3-7.8)	5.4 (4.3-7.7)
Ratio c	17.8 (15-19)	16.6 (14.3-21.5)
Ratio c′	5.5 (4.7-6.8)	5.4 (3.7-6.3)
Ratio V	52 (50-55)	52 (50-54)
Stylet length	21 µm (20-22)	19.5 µm (18-21)
Ratio m	48 (45-50)	48 (45-51)
Ratio o	17 (11-21)	not calculated
Lip annuli	3 to 4, indistinct	4 to 5, indistinct

The specimens of *H. belli,* kept in the museum collection for twenty years, have shrunk, which explains the smaller body and stylet dimensions, and many were flattened, which explains the lower index a. The indexes c, b, and b′ also are smaller, which can be explained if the tail and oesophagus remained constant, or at least shrunk less, than the body. Indexes V and m as measured by Sher (1968) and by the present authors are almost identical, and index c′ differs only in the lower minimum value.

Study of correlations

The matrix of correlations for all the characters in all the samples was studied, but it is not shown here. Most characters describing the size of the nematodes are highly correlated to each other. Body length, lengths of tail, stylet, oesophagus proper, oesophagus including the glandular overlap, body diameters anterior to vulva level and at anus level, are all very highly significantly correlated ($P < 0.001$). It will be possible to discard some of these characters without changing the results of discriminant function analyses. The indexes b, b′, and c also are highly correlated with most other characters. These indexes are not biologically significant (Fortuner, 1984), and they can be eliminated from the DFA.

Tail length and number of tail ventral annuli are very highly correlated ($P < 0.0001$). The exact number of tail annuli is difficult to record, because the annuli become smaller and narrower on the tail, and they become difficult to distinguish from each other. In older specimens, the cuticle may have separated from the rest of the body, making counting annuli even more difficult. Tail length will be used instead of number of tail annuli.

Normality

A first examination of the data, and the results of

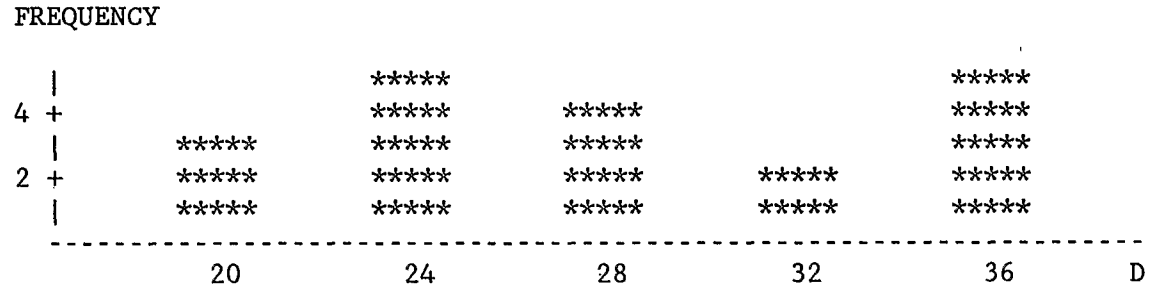

Fig. 3. *Hirschmanniella belli.* Frequency of values of body maximum diameter (D) in paratypes.

preliminary statistical analyses, showed that the body maximum diameter, particularly in type specimens of both species, departed significantly from normality (Fig. 3).

The higher values of D are found in specimens obviously flattened during storage. An attempt was made (Table 4) to correct the error by using the three formulas in Geraert (1961) :

$$d1 = (h + v)/2 \qquad d2 = 2/3\ h + 1/3\ v$$
$$d3 = 1/3\ (4\ hv - v)$$

where *d1*, *d2*, and *d3* are the corrected body diameters, *h* is the apparent diameter as measured from the lateral view of the specimen, and *v* is the approximate thickness as estimated with the graduations of the fine focus knob of the microscope, by focusing first on the top, then on the bottom of the body.

One of the specimens (No. 12 in Table 4) is not flattened at all, and the values given by all three formulas in Geraert (1961) are within observational error of the original measurement. The specimens 17 and 18 are slightly flattened, and the values given by the three formulas are within a micrometer of each other, for each specimen. However, in the specimens most flattened, particularly specimen number 16, the three formulas give corrected values that differ by up to six micrometers. Geraert stated that, even though *d2* is probably the more correct, it is uncertain which formula should be used. The present data does not allow to decide which formula is preferable. Furthermore, even after correction with either one of the three formulas, the distribution of D is still far from normal.

Table 4

Raw and corrected values for body diameter in paratypes of *H. belli*

Specimen number	*h*	*v*	*d1*	*d2*	*d3*
1	36.2	16.4	26.3	29.4	26.5
2	34.8	16.4	25.6	28.7	25.9
9	31.4	21.9	26.7	28.2	27.5
12	26.1	25.5	25.8	25.9	25.9
13	33.0	10.9	22.0	25.4	21.0
14	35.4	12.8	24.1	27.6	23.4
16	37.4	10.9	24.2	28.3	22.4
17	29.7	25.5	27.6	28.1	28.1
18	28.9	21.9	25.4	26.4	26.2

The body diameter is highly correlated to the body length in all samples, but particularly in the samples recently processed (i.e., specimens not flattened). For the discriminant analyses, use of both length and accurate body diameter would be redundant. The body diameter was not given in the original descriptions by Sher (1968). The index a (body length/body diameter) was not used as a diagnostic character for *H. belli.* In view of the error caused by the flattening of old specimens, both body diameter and index a will not be used in the statistical analyses.

The final list of characters retained for the analyses is given in Table 5.

Table 5

List of characters used in the analyses, codes and character names

Kbw :	Knob width	hem :	Distance to hemizonid
DGO :	Distance stylet-DGO	ExPOre :	Distance to excret. pore
Tlgth :	Tail length	oes :	Length of oesophagus
ADiam :	Diameter at anus level	Pos ExPo :	Position excret. pore
KBshape :	Shape of knobs	glands :	Length to end of glands
L :	Body length	Coverl :	Coefficient of overlap
sty :	Stylet length	Covary :	Post./Anterior genital br.
m :	Index m (cone/stylet)	c' :	Index c'
bulb :	Median bulb shape	phas :	Position phasmids
NRing :	Distance to nerve ring	V :	Index V

Multivariate analyses

Comparison between type specimens of *H. oryzae* and *H. belli*

A first canonical Discriminant Function Analysis (DFA) was made with the two type samples, using all the

selected variables. With only two groups, only one discriminant function could be calculated. It represents 94 % of the variance, and it is very highly significant.

The standardized coefficients in Table 6 give the relative contribution of each variable, i.e. its contribution if all variables had standard deviation equal to 1.0. Tail length, stylet length, oesophagus length, and distance from anterior end to hemizonid have the highest positive contribution, while body diameter at anus level (index c'), and distance from anterior end to excretory pore have the highest negative contribution.

The standardized coefficients offer a somewhat distorted view of the variable contributions when two variables are highly correlated, because they must then share their contribution to the discriminant score. Conversely, total canonical structure gives bivariate correlations between each successive variable and the discriminant function, independently of any correlations between the variables.

With the total canonical structure (Table 6) it becomes evident that the function is highly correlated with the general body size represented by tail length, body length, stylet length, distances from anterior end to nerve ring, hemizonid, and excretory pore, and length of oesophagus.

The function is also correlated to the positions of the dorsal oesophageal gland opening (DGO) and the phasmids, and to the tail shape index (c') and bulb shape index (length/width). The measures of knob shape and width have a rather high negative correlation with the function. The other variables have a very low influence on the function.

This first analysis shows that there is a significant difference between the type specimens of *H. oryzae* and *H. belli* as described by the twenty characters in Table 5. It is now necessary to determine whether the same characters are constant among several populations of *H. belli*, or if there are local variations of these characters among different populations of this species. The answer to this question will help us decide whether the difference observed between the two type populations is significant at the species level, or whether it represents limited variations that affect local populations within the same species.

Table 6

Comparison between type specimens of *H. oryzae* and *H. belli*; canonical discriminant function analysis (DFA)

	Standardized Canonical Coefficients		*Total Canonical Structure*
KBW	− 0.517661994	KBW	− 0.348188
DGO	0.422517780	DGO	0.637738
TLGTH	2.234062707	TLGTH	0.764832
ADIAM	− 1.660925944	ADIAM	0.124278
KBSHAPE	− 0.281640621	KBSHAPE	− 0.358722
L	− 0.472538590	L	0.710220
STY	2.060395917	STY	0.903297
M	− 0.389434630	M	− 0.062969
BULB	− 0.123292100	BULB	0.437619
NRING	− 0.164795934	NRING	0.717179
HEM	0.979133324	HEM	0.757283
EXPLORE	− 1.021463491	EXPLORE	0.650907
OES	2.158562181	OES	0.558103
POSEXPO	0.853980819	POSEXPO	− 0.033457
COVERL	0.984856344	COVERL	− 0.152149
COVARY	− 0.119907074	COVARY	0.078751
CC	− 1.147903335	CC	0.473312
PHAS	0.366680362	PHAS	0.307162
V	− 0.086325074	V	0.032099

Comparison of different samples of *H. belli*

In a second DFA, using the same characters (Table 5), several samples from California, identified *a priori* as *H. belli* because of origin, and because they fit the description of this species given by Sher (1968), are compared to one another. The samples 1 to 6 (Table 1) were included. Sample 1 includes the original paratypes of the species, processed twenty years ago. Samples 2 to 6 come from recently processed California populations.

With six groups, five functions could be calculated. The first function represents 54 % of the variance and it is very highly significant (P = 0.0002). The second function represents 21 % of the variance and it is significant only at the 10 % level (P = 0.1040). The other three functions are not significant.

The total canonical structure (Table 7) shows a high correlation of the first function with some of the size related variables, particularly tail length, body length, distance to nerve ring, but a lower correlation with other size variables such as distance to hemizonid, and particularly to excretory pore.

Some of the variables describing shape or position of features also differentiate between the samples of *H. belli*, particularly index V, shape of median bulb, and position of the phasmids.

In opposition, shape of knobs, coefficient of overlapping, and coefficient of regression of posterior genital branch have a very low correlation and do not separate the samples. Some of these variables have a higher correlation with the second function, but we saw that it was significant only at the 10 % level.

Figure 4 shows the repartition of the specimens studied in a plan defined by the first two functions (CAN 1 and CAN 2).

Axis 1 separates the types of *H. belli* (1) from the recently processed specimens (2 to 6). This result is unexpected as we were working under the assumption that all the samples in this second analysis belong to the same species, and that they should not have been separated.

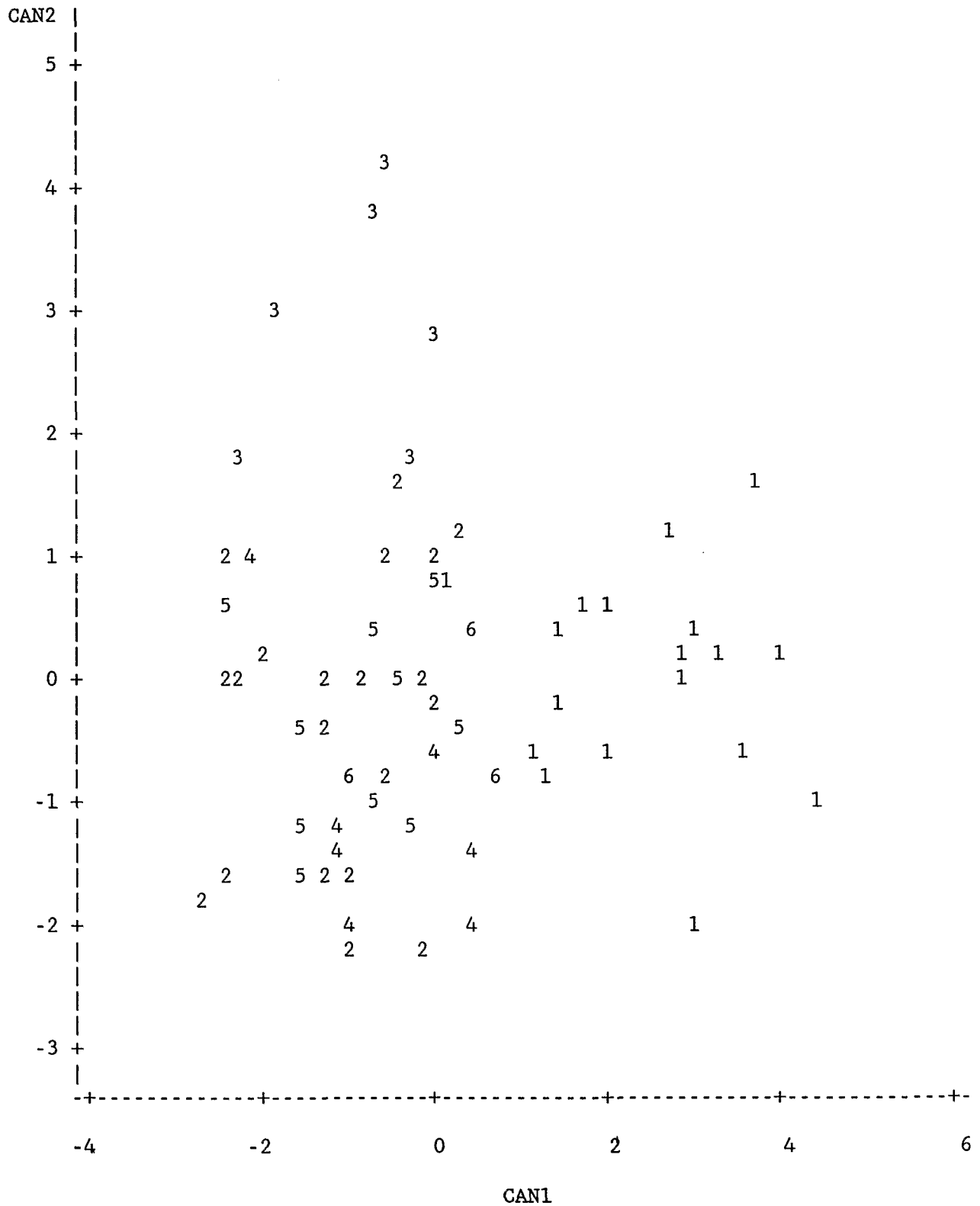

Fig. 4. *Hirschmanniella belli.* Comparison of different samples; 1 observation hidden; symbol represent the samples 1 to 6; CAN1, CAN2 : discriminant functions 1 and 2.

Table 7

Comparison of different samples of *H. belli*

Standardized Canonical Coefficients			*Total Canonical Structure*		
	CAN1	CAN2		CAN1	CAN2
KBW	– 0.018282813	0.501429525	KBW	– 0.241373	0.278241
DGO	0.081245976	0.300115711	DGO	0.322855	0.325419
TLGTH	– 0.366444693	0.727044229	TLGTH	0.706735	0.007058
ADIAM	1.765596151	– 1.544281146	ADIAM	0.418486	0.054902
KBSHAPE	– 0.117266168	– 0.286947435	KBSHAPE	0.074874	– 0.196829
L	– 0.487967306	– 0.124546352	L	0.644682	0.147328
STY	– 0.610491797	0.407953312	STY	– 0.227863	0.389067
M	0.049757831	0.224863941	M	– 0.082092	– 0.025109
BULB	0.572217406	– 0.421917296	BULB	0.477835	– 0.124057
NRING	0.951855425	0.448120486	NRING	0.660963	0.321593
HEM	1.282255481	– 0.460165954	HEM	0.360939	– 0.018430
EXPORE	– 1.693256504	– 4.203717427	EXPORE	0.201883	– 0.090826
OES	– 0.138756163	5.602867881	OES	0.303232	0.302124
POSEXPO	0.829952439	3.679091212	POSEXPO	– 0.122665	– 0.435835
COVERL	– 0.495801035	– 0.039803646	COVERL	– 0.011549	– 0.306295
COVARY	0.145195294	– 0.220625434	COVARY	0.090127	– 0.099721
CC	1.533285232	– 1.462775723	CC	0.121641	– 0.067416
PHAS	0.229051436	– 0.126912390	PHAS	0.422254	– 0.143799
V	– 0.554357805	– 0.490153966	V	– 0.598191	– 0.373351

A first explanation of the differentiation of sample 1 from the other samples could be that the samples 2 to 6, that were all taken from rice fields in Northern California, represent a species different from *H. belli* that was described from the Riverside region. Another explanation would be that long storage of the type specimens has caused some shrinkage and distortions of the specimens. A study of freshly sampled topotypes would allow to decide between these two possibilities, but the type locality of *H. belli* has been destroyed and attemps to obtain specimens from its vicinity have been unsuccessful.

The second explanation will be assumed to be true, at least for the present study, because we did find differences between the current measurements and the values obtained by Sher twenty years ago from the same specimens (Table 3). Another reason is that the main goal of the study is to find a way to differentiate Californian *Hirschmanniella* populations (assumed to belong to *H. belli*) from exotic populations of the same genus that may belong to *H. oryzae*. In this optic, we need to select a set of characters able to separate *H. oryzae* from all samples of *H. belli*, while not separating the various samples of *H. belli* from each other.

Search for a better set of differentiating criteria

In an attempt to identify such variables, the total canonical structure from the first analysis (Comparison between type specimens of *H. oryzae* and *H. belli*) and that from the second analysis (Comparison of different samples of *H. belli*), were compared (Table 8) in order to select variables that have a high correlation in the first column (i.e. they participate in the discrimination between *H. oryzae* and *H. belli*) but a low correlation in the second column (to avoid separating old and new *H. belli*).

Some variables must be rejected outright, because they are just the opposite of what is needed. The body diameter at anus level (ADIAM), the distance to the hemizonid (HEM), the position of the excretory pore in relation to the intestinal valve (POSEXPO), the position of the phasmids on tail (PHAS), and the index V (V) have higher coefficients on the second column.

Some variables are almost useless in both analyses, and may as well be deleted : index m, and coefficient of regression of the posterior genital branch (COVARY).

Some variables have high correlations in both columns : tail length (TLGTH), body length (L), median bulb shape (BULB), distance to nerve ring (NRING), and oesophagus length (OES) participate in the separation of all types of samples. They must be deleted to avoid differentiating the various *H. belli* samples.

The remaining variables, width of stylet knobs (KBW), distance of the dorsal gland opening to stylet base (DGO), knob shape (KBSHAPE), stylet length

Table 8

Selection of differentiating criteria between *H. oryzae* and *H. belli*

	Structure coefficients		
Types of *H. oryzae*, H. belli		All samples of *H. belli*	
KBW	– 0.348188	KBW	– 0.241373
DGO	0.637738	DGO	0.322855
TLGTH	0.764832	TLGTH	0.706735
ADIAM	0.124278	ADIAM	0.418486
KBSHAPE	– 0.358722	KBSHAPE	0.074874
L	0.710220	L	0.644682
STY	0.903297	STY	– 0.227863
M	– 0.062969	M	– 0.082092
BULB	0.437619	BULB	0.477835
NRING	0.717179	NRING	0.660963
HEM	0.757283	HEM	0.360939
EXPORE	0.650907	EXPORE	0.201883
OES	0.558103	OES	0.303232
POSEXPO	– 0.033457	POSEXPO	– 0.122665
COVERL	– 0.152149	COVERL	– 0.011549
COVARY	0.078751	COVARY	0.090127
CC	0.473312	CC	0.121641
PHAS	0.307162	PHAS	0.422254
V	0.032099	V	– 0.598191

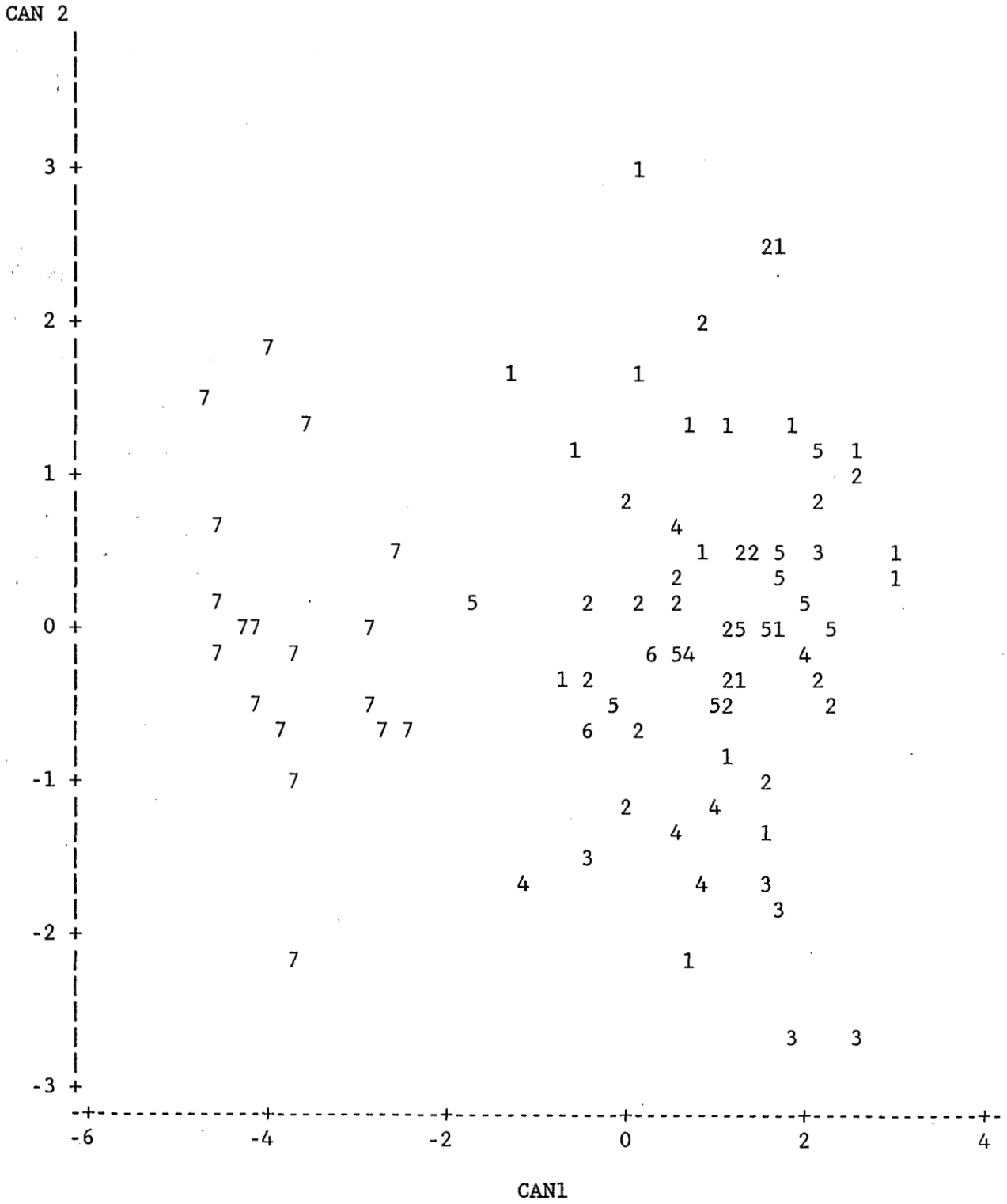

Fig. 5. *Hirschmanniella belli.* Comparison of different samples (1 to 6) with the type specimens of *H. oryzae* (7) using seven selected variables; one observation hidden; symbols represent the samples 1 to 7; CAN1, CAN2 : discriminant functions 1 and 2.

(STY), distance to the excretory pore (EXPORE), coefficient of overlap (COVERL), and index c' (CC) are retained. It is interesting to note that three of the original diagnostic criteria of Sher are retained, knob shape, stylet length and ratio c'.

A new canonical DFA was performed on samples 1 to 7, using only the seven variables selected.

Only the first two functions are significant. The first function represents 85 % of the variance (P = 0.0001), and the second represent 8 % of the variance (p = 0.269). The standardized coefficients and the total canonical structure are given in Table 9.

Table 9

Comparison between several samples of *H. belli* and type specimens of *H. oryzae* Using seven selected variables

Standardized Canonical Coeff.			*Total Canonical Structure*		
	CAN1	*CAN2*		*CAN1*	*CAN2*
KBW	− 0.447184	− 0.470670	KBW	− 0.207024	− 0.265362
DGO	0.131261	− 0.183588	DGO	0.527417	− 0.094049
KBSHAPE	− 0.108720	0.234457	KBSHAPE	− 0.412152	0.220909
STY	1.825104	− 0.613982	STY	0.968498	− 0.165920
EXPORE	0.265452	1.147509	EXPORE	0.487610	0.646000
COVERL	− 0.056651	0.150239	COVERL	− 0.156640	0.014195
CC	0.217857	0.398000	CC	0.388579	0.228778

Figure 5 shows that the separation of *H. oryzae* from the other samples is maintained, while the various samples of *H. belli* are no longer separated.

Table 10 shows that there are no classification errors for the type specimens of *H. oryzae*, that are all correctly classified in sample 7. Among all the specimens in the *H. belli* samples, only one (from sample 5) is incorrectly placed with *H. oryzae*. The many classification " errors " among the different *H. belli* samples (1 to 6) shows that the discriminant functions do not discriminate among the various populations of this species, which is the result we were trying to obtain.

The variables we selected are valid criteria for the differentiation of the two species under discussion. Their list can be compared with the list of diagnostic criteria used by Sher (1968) for the original differentiation of *H. belli*.

Both lists include stylet length and stylet knob shape. Sher noted a longer tail, but he did not give tail length in the description of the new species. This criterion had to be eliminated here because it participated in the differentiation of the various samples of *H. belli*. However, we are using index c' which is somewhat related to the relative length of the tail.

Sher's diagnosis noted the " usually longer body length " of *H. belli*, (1.61-2.22 mm *vs* 1.14-1.63 mm for *H. oryzae*). This measurement had to be eliminated in the present study, and it was replaced by the distance from anterior end to excretory pore. This measurement is also size related and has a very high correlation with total body length, but its contribution to the separation of the various samples of *H. belli* is low.

We are using a few other characters, i.e. width of knobs, position of dgo, and coefficients of overlap, that were not considered by Sher (1968).

The coefficient of regression of the posterior genital branch has not been retained, probably because it does not differentiate the various samples used.

The seven characters selected can differentiate type specimens of *H. oryzae* from several samples of *H. belli*. The analyses so far have used samples from two geographical origins, California and Java, corresponding to the regions from where the two species were originally described.

To test the robustness of the differentiating criteria, they were tested with other samples from other geographical origins, i.e. West Africa and India, as well as other California samples, from localities different from those used in the main study.

Test

Comparison of samples from different geographical origins

A canonical DFA was performed with the same seven characters as previously selected and with five samples : the types of *H. belli* (1), one recently processed sample from California (2), the types of *H. oryzae* (7), and two samples from West Africa : Ivory Coast (8) and Senegal (9).

The first three functions are very highly significant (P = 0.0001). The first represents 73 %, and the second 21 % of the variance. The third function represents only 5 % of the variance.

In Figure 6, axis 1 separates the 2 samples of *H. belli* from the other 3 samples, while axis 2 somewhat separates the other 3 samples from one another. The specimens of *H. belli* in samples 1 and 2 are left in a single cloud of points. This verifies that the variables selected do not separate the various samples of this species.

To confirm these conclusions, two other samples from California (b, c), and a sample from Madras, India (a), identified as *H. oryzae* by Sher, were positioned against the axes defined above (Fig. 7).

Figure 7 shows that the samples from California (b and c), are positioned among the samples 1 and 2, representing *H. belli*, and that they are clearly separated from the non-California samples. The sample from India (a) appears closer to the African samples (8, 9) and

Table 10

Comparison between several samples of *H. belli* and type specimens of *H. oryzae* using selected variables; classification errors

From sample	*Number of observations and percent classified into sample*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*Total*
1	8	3	1	2	2	2	0	18
	44.44	16.67	5.56	11.11	11.11	11.11	0.00	100.00
2	3	6	1	0	5	5	0	20
	15.00	30.00	5.00	0.00	25.00	25.00	0.00	100.00
3	1	0	4	0	0	1	0	6
	16.67	0.00	66.67	0.00	0.00	16.67	0.00	100.00
4	1	0	0	4	2	0	0	7
	14.29	0.00	0.00	57.14	28.57	0.00	0.00	100.00
5	2	3	0	1	4	0	1	11
	18.18	27.27	0.00	9.09	36.36	0.00	9.09	100.00
6	0	0	0	0	1	2	0	3
	0.00	0.00	0.00	0.00	33.33	66.67	0.00	100.00
7	0	0	0	0	0	0	18	18
	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00
Total	15	12	6	7	14	10	19	83
Percent	18.07	14.46	7.23	8.43	16.87	12.05	22.89	100.00

from *H. oryzae*, while it is clearly separated from *H. belli* and all the California samples.

Presence of males

Sher (1968) also differentiated *H. belli* from *H. oryzae* by the character " inconspicuous spermatheca without sperms, rarity of males ". This character was not included in the DFA above for an obvious reason (DFA only analyses quantitative characters).

In paddy rice growing areas, rice root nematodes most frequently are represented by an amphimictic species *(H. oryzae)* that is present throughout the Far East and South East Asia.

In Africa, there exist local populations of an *Hirschmanniella* sp. very close to *H. oryzae* in morphology and measurements. Some of these populations are amphimictic, others are parthenogenetic. It is not proved but quite possible that amphimictic populations can easily become parthenogenetic.

In California, the populations described under the name *H. belli* are consistently parthenogenetic, but Sher (1968) did observe five males in the type population. The apparition of males in seemingly parthenogenetic populations may be due to ecological factors. It is quite common to observe both types of reproduction among the same species of plant parasitic nematode. In *Pratylenchus*, some species are always amphimictic (*P. penetrans*), males are extremely rare in others *(P. brachyurus)*, and they are quite unknown or are seen only exceptionally in some species *(P. neglectus, P. zeae)*.

Absence of males in an *Hirschmanniella* population from California certainly is an indication that this population belongs to the species *H. belli*. However, this character should never be accepted as a primary criterion for the identification of this species.

Discussion

Practical identification

The morphometrics of California specimens are given in Table 11.

The study has demonstrated that it is possible to characterize representative samples of local populations in California by a combination of the following seven characters (given with mean values) :

KBW : width of stylet knobs (4.1 to 4.3);

DGO : distance of the dorsal gland opening to stylet base (3.9 to 4.5);

KBSHAPE : shape of the stylet knobs (shapes 2 to 3, see Table 2);

STY : stylet length (19 to 19.5 µm);

EXPORE : distance from anterior end to the excretory pore (137 µm);

COVERL : coefficient of oesophageal overlap (77 to 78 %); and

CC : index c' (5.3 to 5.5).

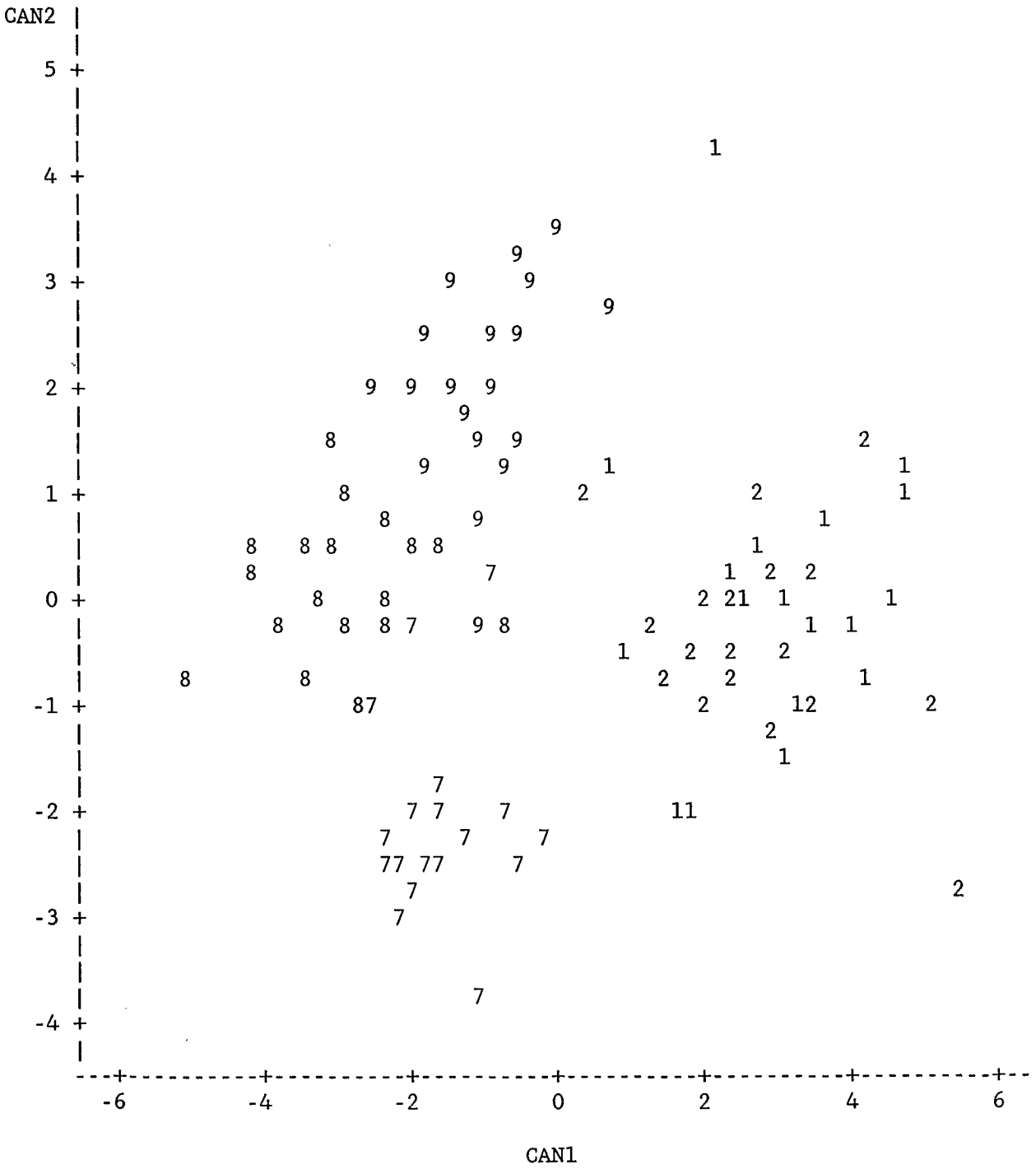

Fig. 6. *Hirschmanniella belli.* Comparison of two samples (1, 2) with the type specimens of *H. oryzae* (7) and two samples from Africa (8, 9) using selected variables; three observations hidden; symbols represent the samples; CAN1, CAN2 : discriminant functions 1 and 2.

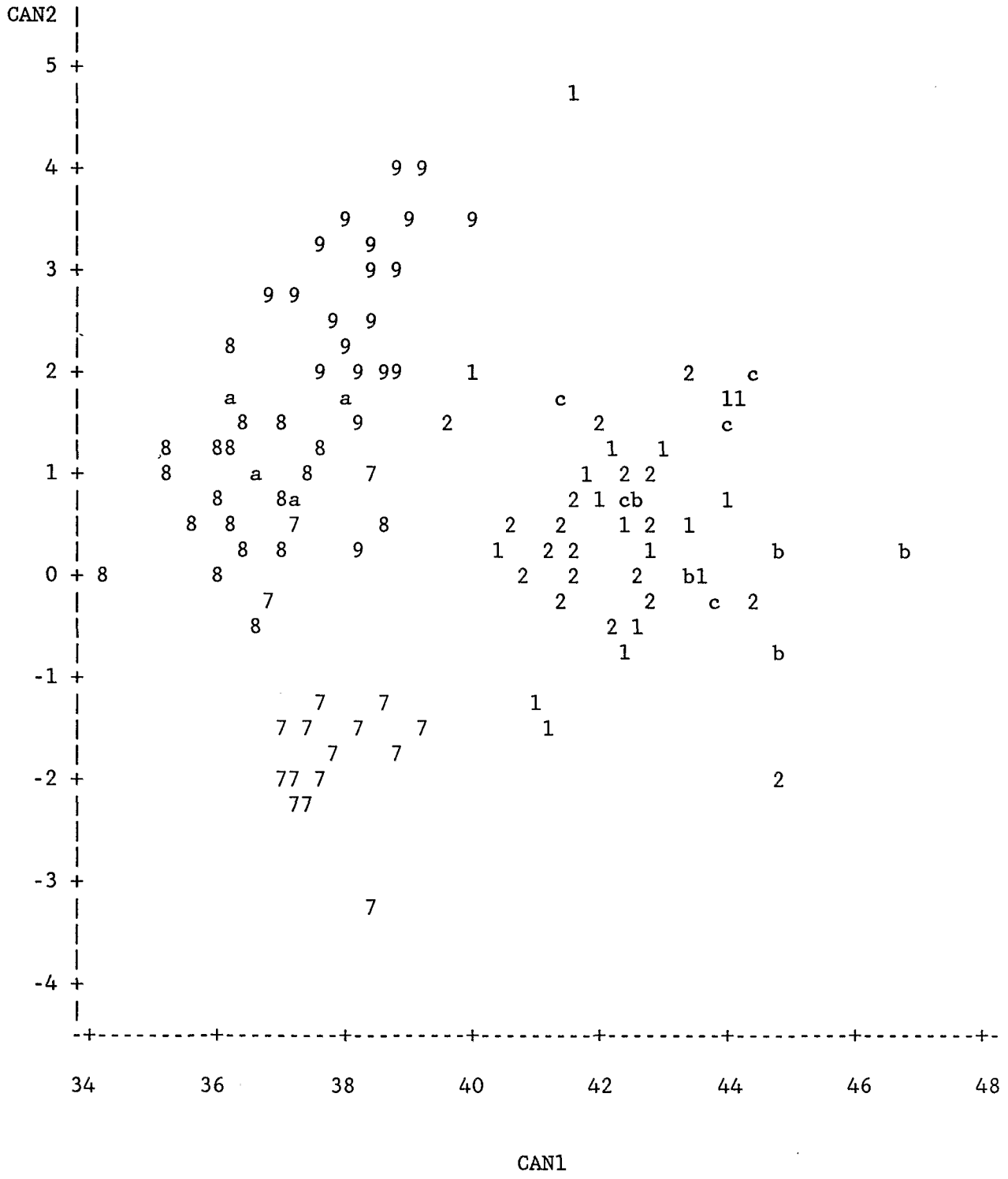

Fig. 7. Position of specimens from California (b, c) and from India (a) (see Table 1) on the axes defined by a canonical DFA using samples 1, 2, 7, 8, 9 and seven selected variables; CAN1, CAN2 : discriminant functions 1 and 2.

For practical identification, these characters can be assessed by comparing the data from the unknown sample with the values indicated above.

It would be more accurate to perform a DFA from the values of these characters in the unknown sample and the data in the present study.

Alternatively, the position of the unknown specimens can be ascertained by using the following two functions, that were used for plotting (Fig. 10).

CAN 1 = (KBW × 0.6367207) + (DGO × 0.1041316) - (KBSHAPE × 0.0997309) + (STY × 1.0765882) + (EXPORE × 0.1044914) + (COVERL × 0.0885512) + (CC × 0.4983746).

CAN 2 = - (KBW × 0.5324927) + (DGO × 0.6622350) + (KBSHAPE × 0.2847247) + (STY × 0.8268202) - (EXPORE × 0.0960910) - (COVERL × 0.0892046) + (CC × 0.6100895).

Absence of males and spermathecae empty of sperms are other indications that help identifying *H. belli.*

It is hoped that the method used in the present study will be applied to other cases where the identification of two species morphologically very close to each other is difficult or impossible by traditional (univariate) methods.

Systematic position of *H. belli*

A question that was left unanswered is the significance of the differences observed between the California populations of *H. belli* and other populations of *Hirschmanniella.* These differences can be seen either as specific differences or as intra specific variations caused by different local geographical conditions within a single species.

One can recognize as a distinct species every geographical isolate that shows slight morphological differences, but such an informal arrangement " appeals to no underlying process or theory other than some undisclosed, subjective (and arbitrary), measure of general similarity " (Frost & Wright, 1988).

Conversely, one can reject all parthenogenetic species because their mode of reproduction places them out of the realm of Mayr's (1942) definition of the biological species as groups of actually or potentially interbreeding natural populations which are reproductively isolated

Table 11

Description of the six California sample of *Hirschmanniella belli*
(SA, sample number, see Table 1; all measurements in micrometers)

Variable	*SA = 1 (n = 18)*	*SA = 2 (n = 20)*	*SA = 3 (n = 6)*	*SA = 4 (n = 7)*	*SA = 5 (n = 11)*	*SA = 6 (n = 3)*
Knob width	3.99 ± 0.54	4.27 ± 0.35	4.47 ± 0.48	3.90 ± 0.45	4.11 ± 0.50	4.37 ± 0.76
DGO	4.47 ± 0.92	3.89 ± 0.62	4.65 ± 0.64	4.10 ± 0.44	3.90 ± 0.62	4.23 ± 0.64
Diameter	24.27 ± 2.40	23.92 ± 2.76	26.90 ± 3.23	24.00 ± 3.04	24.34 ± 2.05	32.50 ± 1.01
Tail length	106.56 ± 9.16	91.94 ± 7.66	93.20 ± 6.92	91.66 ± 5.20	95.47 ± 8.33	107.03 ± 6.36
Anal Diameter	19.77 ± 3.20	17.28 ± 2.19	18.17 ± 1.78	17.67 ± 2.23	18.04 ± 2.09	21.40 ± 2.89
Tail annuli	69.50 ± 4.99	67.55 ± 4.33	67.67 ± 2.07	65.86 ± 3.58	69.36 ± 5.30	79.00 ± 4.58
Knob shape	2.83 ± 1.15	2.80 ± 0.83	2.33 ± 0.52	2.71 ± 0.49	2.73 ± 0.65	3.00 ± 1.00
L	1728 ± 128.96	1548 ± 102.45	1584 ± 135.40	1487 ± 85.27	1579 ± 138.10	1680 ± 38.41
Stylet lgth	19.22 ± 0.71	19.43 ± 0.54	19.98 ± 0.55	19.27 ± 0.51	19.48 ± 0.70	19.20 ± 0.41
Index m	47.50 ± 1.65	48.08 ± 1.04	47.75 ± 2.16	48.18 ± 1.91	46.91 ± 1.85	46.88 ± 0.49
Bulb shape	0.77 ± 0.08	0.69 ± 0.07	0.67 ± 0.06	0.71 ± 0.10	0.69 ± 0.05	0.65 ± 0.05
Dist. to N. Ring	113.76 ± 7.41	102.69 ± 5.69	106.55 ± 7.26	95.23 ± 8.84	103.63 ± 6.36	103.07 ± 4.34
Dist. to Hemiz.	133.41 ± 7.59	129.78 ± 6.65	125.05 ± 8.45	120.77 ± 9.74	128.58 ± 5.54	127.73 ± 2.76
Dist. to Ex. Pore	137.56 ± 10.35	136.69 ± 7.86	129.07 ± 10.17	125.26 ± 8.52	134.56 ± 6.78	134.67 ± 1.93
Oesophagus lgth	140.76 ± 12.85	135.46 ± 7.29	138.20 ± 14.00	122.37 ± 10.24	135.09 ± 7.56	136.30 ± 2.43
Index b	12.37 ± 1.47	11.43 ± 0.54	11.52 ± 1.05	12.18 ± 0.52	11.69 ± 0.86	12.32 ± 0.13
Position Ex. Pore	0.98 ± 0.10	1.01 ± 0.05	0.94 ± 0.05	1.02 ± 0.03	0.99 ± 0.05	0.99 ± 0.02
Gland lgth	327.03 ± 33.56	323.27 ± 34.08	316.17 ± 16.69	322.56 ± 31.26	336.97 ± 27.10	315.67 ± 35.44
Index b′	5.35 ± 0.78	4.82 ± 0.45	5.03 ± 0.58	4.63 ± 0.34	4.69 ± 0.35	5.37 ± 0.69
Coef. Overlap	78.05 ± 4.21	77.65 ± 3.10	76.20 ± 4.32	80.93 ± 1.76	78.92 ± 3.90	75.98 ± 5.11
Index a	71.70 ± 7.34	65.15 ± 4.37	59.33 ± 6.08	62.50 ± 5.13	64.98 ± 5.07	51.73 ± 2.57
Post Gen. Branch	99.20 ± 7.80	99.95 ± 6.96	94.52 ± 24.77	96.64 ± 6.25	94.40 ± 6.94	92.99 ± 6.25
Index c	16.29 ± 1.37	16.88 ± 0.82	17.08 ± 1.14	16.24 ± 0.86	16.54 ± 0.46	15.71 ± 0.59
Index c′	5.49 ± 0.73	5.37 ± 0.54	5.18 ± 0.72	5.26 ± 0.80	5.35 ± 0.73	5.10 ± 1.07
Posit. phasmid	74.47 ± 4.73	70.49 ± 5.43	69.64 ± 4.14	72.88 ± 2.79	69.84 ± 3.35	73.49 ± 8.54
Index V	51.90 ± 1.34	53.88 ± 1.02	52.30 ± 1.69	53.52 ± 0.75	53.62 ± 1.47	53.33 ± 0.74

from other such groups. However, this definition was never meant to be operational.

A middle of the road approach would be to give specific status to parthenogens that originated from a single amphimictic species, but that have experienced enough post-divergence mutations as to become a distinct species. This immediately raises the question, how far is far enough?

In a somewhat arbitrary manner, we can recognize a population or a group of populations to represent a distinct species when the distance to another such group in a correctly executed DFA is significant at the 95 % level, in other words, when the two group centroids are separated by more than 2 standard deviation units. In this optic, we would have to accept *H. belli* as a valid species.

A more objective approach would be to study how far apart are valid species in the genus considered. The distance in a DFA between several amphimictic species that are obsiously different from *H. oryzae* (e.g. *H. spinicaudata, H. gracilis,* etc.) and *H. oryzae* would be compared to the distance between *H. belli* and *H. oryzae.* However, the results would depend on how different the bench mark species are from each other. Also, it would be very difficult to separate genetic and environmental causes for the morphological differences observed. To avoid the environmental errors, we should consider the differences that may exist between the genome of the various populations involved, because the genome is independent of any external factor.

It is proposed to study and compare the genetic (DNA) material of several parthenogenetic species and that of their amphimictic parent. If the genetic distance between the parthenogens is as large as that between true amphimictic species, then there will be a strong case to recognize each parthenogenetic variant as a true species.

It seems preferable to maintain the systematic status of *H. belli* in its present status until the conclusion of the genetic studies.

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REFERENCES

BROWN, D. J. F. & BOAG, B. (1989). Morphometrics variability and aberrations in nematode identification. In : Fortuner, R. (Ed.), *Nematode identification and expert-system technology,* New York, Plenum Publishing Corp. : 185-200.

FORTUNER, R. (1984). Morphometrical variability in *Helicotylenchus* Steiner, 1945. 5 : On the validity of ratios. *Revue Nématol.,* 7 : 137-146.

FORTUNER, R. & MERNY, G. (1979). Root-parasitic nematodes of rice. *Revue Nématol.,* 2 : 79-102.

FROST, D. R. & WRIGHT, J. W. (1988). The taxonomy of uniparental species, with special reference to parthenogenetic *Cnemidophorus* (Squamata : Teiidae). *Syst. Zool.,* 37 : 200-209.

GERAERT, E. (1961). Corrections of measurements of nematode diameters. *Nematologica,* 6 : 258-259.

MAYR, R. (1942). *Systematics and the origin of species.* New York, Columbia University Press, 334 p.

SHER, S. A. (1968). Revision of the genus *Hirschmanniella* Luc & Goodey, 1963 (Nematoda : Tylenchoidea). *Nematologica,* 14 : 243-275.