

Morphometrical variability in *Helicotylenchus* Steiner, 1945. 4 : Study of field populations of *H. pseudorobustus* and related species

Renaud FORTUNER * Armand R. MAGGENTI ** and Laurel M. WHITTAKER *

* California Department of Food and Agriculture, Nematology Lab, Room 340, 1220 N Street,
Sacramento, Ca 95814, U.S.A.

** Department of Nematology, University of California, Davis, Ca 95616, U.S.A.

SUMMARY

Multivariate analyses were made on 28 populations of *Helicotylenchus pseudorobustus*, eleven populations of *H. dihystra*, and type populations of *H. microlobus*, *H. bradys*, *H. phalerus*, *H. egyptiensis*, and *H. africanus*. Some morphological differences were observed among the populations of *H. pseudorobustus*, mostly between samples from North America and from Western Europe. The differences were most apparent in the pattern of junction of the inner lines of lateral field on tail, the position of the phasmids and of the dorsal gland opening. However, these characters vary within some of the populations studied. Most populations originally identified as *H. pseudorobustus* were conspecific with the type population. *H. microlobus* was confirmed as synonym of *H. pseudorobustus*; *H. bradys* and *H. phalerus* were proposed as new synonyms of this species. A few samples originally proposed as *H. pseudorobustus* were in fact closer to *H. dihystra* or may represent other, unidentified species. Paratypes of *H. egyptiensis* and *H. africanus* were described.

RÉSUMÉ

Variabilité morphométrique chez *Helicotylenchus* Steiner, 1945. 4 : Etude de populations naturelles
de *H. pseudorobustus* et d'espèces voisines

Des analyses multifactorielles furent effectuées sur 28 populations de *Helicotylenchus pseudorobustus*, onze populations de *H. dihystra*, et des populations types de *H. microlobus*, *H. bradys*, *H. phalerus*, *H. egyptiensis* et *H. africanus*. Quelques différences morphologiques furent observées entre les populations de *H. pseudorobustus*, principalement entre les échantillons provenant d'Amérique du Nord et d'Europe Occidentale. Les différences concernent surtout l'aspect du raccordement des lignes internes du champ latéral sur la queue, la position des phasmides et celle du débouché de la glande oesophagienne dorsale. Cependant ces caractères varient à l'intérieur de certaines des populations étudiées. La plupart des populations identifiées à l'origine comme *H. pseudorobustus* sont bien conspécifiques de la population type. *H. microlobus* est confirmé comme synonyme de *H. pseudorobustus*; *H. bradys* et *H. phalerus* sont proposés comme nouveaux synonymes de cette espèce. Quelques-unes des populations originellement proposées comme *H. pseudorobustus* sont en fait plus proches de *H. dihystra* ou peuvent appartenir à d'autres espèces, qui n'ont pas été identifiées. Les paratypes de *H. egyptiensis* et de *H. africanus* inclus dans la présente étude sont décrits.

The variability of the taxonomic characters has been studied in *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961 and published in three previous articles : Fortuner (1979) considered the variability in the progeny of a single parthenogenetic female ; Fortuner and Quénehervé (1980) observed the additional variability in such a progeny when cultivated under several different host-plants ; and Fortuner, Merny and Røux (1981) reported the variability in field populations of a species of *Helicotylenchus* which was identified as *H. dihystra* as a result of multivariate analyses.

These studies show that many characteristics in *H. dihystra* vary under external conditions, such as

the nature of the host-plant or the geographical origin of the sample.

It is tempting to consider these observations as valid for the entire genus *Helicotylenchus*, and to use them to decide which characteristics are constant enough within a given species to be used as differentiating criteria in taxonomic studies. However, it was considered best to make similar observations on another species to ascertain that the conclusions obtained from *H. dihystra* were also valid for other members of the genus *Helicotylenchus*.

H. pseudorobustus (Steiner, 1914) Golden, 1956 was selected for the present study. It is, with *H. multicinctus* and after *H. dihystra*, the second most

reported species of *Helicotylenchus* in the world literature. Since Sher (1966) redescribed *H. pseudorobustus* from topotypes, several populations of this species have been described from various countries : South Dakota (Thorne & Malek, 1968), Zaire (Ali Geraert & Coomans, 1973), Canada (Anderson, 1974), Turkey (Geraert, Zepp & Borazanci, 1975), Malaysia (Sauer & Winoto, 1975), South Africa (Van den Berg & Heyns, 1975), Italy (Mancini & Moretti, 1976) and Fiji Islands (Van den Berg & Kirby, 1979). These various populations of *H. pseudorobustus* show great variability in several characteristics : the average length of the stylet, which was the most constant characteristic in *H. dihystra*, varies from 23.5 (Van den Berg & Heyns, 1975) to 30 μm (Thorne & Malek, 1968) ; the tail is generally described with a large ventral projection, but some illustrations show a rather small process (*i.e.*, Fig. 36 of Van den Berg & Kirby, 1979 ; Fig. 3 A of Ali, Geraert & Coomans, 1973 ; Fig. 1 O of Siddiqi, 1972) ; this projection is often described as annulated but is not annulated in some illustrations (Fig. 1 P of Sher, 1966 ; Fig. 25 D of Thorne & Malek, 1968 ; Fig. 3 D, E of Ali, Geraert & Coomans, 1973) ; the phasmids are observed from two to seven annules anterior to the anus level by Sher (1966), but from four annules posterior to eleven annules anterior to the anus level by Van den Berg and Heyns (1975) ; the fusion of the inner lines of the lateral field is generally not described, it is shown as U-shaped in Sher (1966), but other shapes occur in Siddiqi (1972, Fig. 1 O-P), Ali, Geraert & Coomans (1973, Fig. 3 E) and Anderson (1974, Fig. 6 B, C, E). These variations may be interpreted as the result of a greater intraspecific variability, or may be seen as the proof that there exist several species within *H. pseudorobustus*.

As a consequence of this great variability, it was difficult to differentiate *H. pseudorobustus* from several other nominal species of *Helicotylenchus* : *H. bradys* Thorne & Malek, 1968, *H. microlobus* Golden, 1956, *H. phalerus* Anderson, 1974, and also *H. dihystra*. The latter species was differentiated from *H. pseudorobustus* by Fortuner, Merny and Roux (1981) because of a shorter stylet, a higher coefficient V, and differently shaped fusion of the inner lines of the lateral field, but the taxonomic position of the other nominal species remains to be clarified.

The study of field populations tentatively identified in the literature as *H. pseudorobustus* was initiated to : *i*) verify their identity ; *ii*) record the intraspecific variability of *H. pseudorobustus* and include it in a redescription of the species ; *iii*) gather additional information on the variability of taxonomic characters ; and *iv*) clarify the status of some related species.

Material and methods

Forty-three samples, of one to thirty specimens each, representing field populations identified as *H. pseudorobustus*, were received from twenty-five countries or states (Tab. 1). Paratypes of the related species *H. microlobus*, *H. phalerus* and *H. bradys* were included in the study. The eleven field populations of *H. dihystra* studied in Fortuner, Merny & Roux 1981, and paratypes of *H. africanus* (Micoletzky, 1916) Andrassy, 1958 and of *H. egyptiensis* Tarjan, 1964 were also included in the analyses for purposes of comparison. The specimens used for the study were returned to their respective owners except for the specimens from Germany, Portugal, Iowa (in part), California, St Lucia, Nigeria (in part), and New Zealand which were deposited in the CDFP Permanent Slide Reference Collection (Nematology).

396 specimens of nematodes from the *H. Pseudorobustus* material and 38 specimens belonging to the other species (Tab. 1) were observed under a Leitz Ortholux II microscope with an interference contrast device of Nomarsky, at 450 \times and 1000 \times magnifications. Drawings were made using a camera lucida or a drawing tube at 900 \times (total body length and position of vulva) and 2000 \times (all other observations).

The measurements of the eleven populations of *H. dihystra* previously recorded by Fortuner, Merny & Roux, (1981), were added to the gathered data and included in the analyses. For every specimen, fifteen quantitative characters were recorded :

- LON : body length
- STY : stylet length
- STA : length of anterior part of stylet
- DGO : distance between dorsal gland opening and stylet base
- OVI : distance head to esophago-intestinal junction
- OGO : distance head to end of esophageal glands
- PEX : distance head to excretory pore
- QUE : tail length
- DAN : body diameter at anus
- DIV : distance head to vulva
- DVU : body diameter at vulva
- ANQ : number of tail annules
- ANP : number of annules from phasmid to anus level
- ANW : width of one body annule
- PRO : length of terminal process on tail

Body length and distance from head to vulva were replaced by their ratio $\text{RAV} = \text{DTV} \times 100 / \text{LON}$.

Eleven qualitative characters were also observed :

- HAB : habitus
- LIP : shape of lip region
- DIS : presence of a labial disc
- ANL : lip annulation

- KBT : shape of stylet knobs
- ARO : presence of transverse lines on lateral fields
- INC : shape of the junction of the inner lines of the lateral field
- TSH : shape of the tail
- ANT : shape of the dorsal tail annules
- ANV : shape of the ventral tail annules
- APR : annulation of the terminal process

Data analysis consisted of stepwise discriminant analysis (Jennrick & Sampson, 1979) for the quantitative characters. Only 31 samples were used for the definition of the canonical variables : seventeen samples of *H. pseudorobustus* with ten or more specimens per sample ; six samples of *H. dihystra* ; and six samples of the other species. Then, the position of the rest of samples was calculated in relation to the axes so defined. Mahalanobis distances were calculated for each pair of samples. A correspondence analysis (Benzecri & Benzecri, 1980) was made for selected quantitative and qualitative characters. The quantitative characters were recoded into classes, generally four classes per character, and several correspondence analyses including both kinds of characters were performed.

Results

ANALYSIS OF THE QUANTITATIVE CHARACTERS

The positions of the 52 samples (Tab. 1) are shown on the graph of Fig. 1, in relation to axes 1 and 2 which proved to be the more effective for discriminating between the samples. For every sample, the Mahalanobis distance to the nearest sample was noted on the graph of Fig. 1 as a solid line if there was no significant difference (1% level) between the two samples, and as a dotted line if there was a difference.

Axis 1 is positively correlated mostly with variables OGO, PRO, STY, and QUE. Samples with higher values in these variables will be located towards the right of the graph.

Axis 2 is negatively correlated mostly with variables DGO, QUE, RAV, OGO (samples with higher values for these variables to the bottom) and positively correlated mostly with STA and DAN (higher values on top of graph).

66.7% of the specimens were correctly classified into their own group by the analysis. Fig. 1 shows that :

i) the topotype sample of *H. pseudorobustus* (A1) is closest to some of the other Europeans samples : Germany (A2), Italy (A3), and France (A4) ;

ii) however, there is no separation between : a) these four samples ; and b) most of the samples which were identified *a priori* as *H. pseudorobustus* (upper right shaded area in Fig. 1) ;

iii) only the samples from Nigeria (C1, C2), Natal (D1, D2), Cape province (D3, D4, D6) and Transvaal (D7) are well separated from the other samples of *H. pseudorobustus*, and are more related to the samples of *H. dihystra*. Samples from Zaire (C4), St. Lucia (F2), Malaysia (H1), Korea (H4), Cape Province (D5), Turkey (B1), Florida (F1), and California (G1) are at the edge of the cloud of points representing the other samples of *H. pseudorobustus* ;

iv) *H. microlobus*, *H. bradys*, *H. phalerus*, and *H. egyptiensis* are well inside the cloud of samples of *H. pseudorobustus*. *H. africanus* is far distant from all other samples ;

v) the eleven samples of *H. dihystra* (bottom left shaded area) are well separated from the cloud of points representing typical samples of *H. pseudorobustus*. The groupings observed in Fortuner, Merny and Roux (1981) are again recognized here : A-G, D-E, B-K-J, and F-H-G-I ;

vi) the position of *H. pseudorobustus* in relation to axes 1 and 2 is related to the higher values of lengths of stylet, tail, and tail process, while the position of *H. dihystra* depends on greater ratio V and distance from the dorsal gland opening to the stylet.

ANALYSIS OF THE QUALITATIVE AND QUANTITATIVE CHARACTERS

A first analysis was done with all the variables in all 51 samples, bra 1 and bra 2 were grouped into a single sample (bra), but failed to differentiate groups of samples. Several other analyses were done with reduced lists of variables. Of the eleven qualitative characters recorded, four (HAB, LIP, DIS, and ANL) were discarded because they presented the same aspect in every specimen in all the samples except afri and egypt. Four other characters (KBT, ANT, ANV, and APR) were discarded because of their variability, greater within sample than between samples. The quantitative characters LON (body length) and QUE (tail length) were introduced as the ratio $QUL = QUE / LON$. Eventually, a good separation between the *H. dihystra* samples and some of the *H. pseudorobustus* samples was observed when using only five quantitative characters : DTL, STY, QUL, ANQ, PRO, and two qualitative characters : ARO and INC. ARO is the presence (1) or the absence (0) of transverse striae in the lateral field on the body and/or tail. INC is the junction pattern of the two inner lines of the lateral field on the tail. This pattern is typically y and v shaped in *H. dihystra* (see Fig. 4 of Fortuner, Merny & Roux, 1981). In the samples of *H. pseudorobustus* other patterns were also observed (u, μ , m, v : see description and Fig. 4 below). The y and v patterns are here coded : INC 1 ; the other patterns are coded : INC 2.

Table 1
Description of the samples studied

Sample Code	n ♀♀	Origin	Observations
1. <i>H. pseudorobustus</i>			
A1	20	Moss, Altmatt, Switzerland	Topotypes-Sher (1966)
A2	24	Pine, Hünxe, Germany	
A3	11	Chestnut, Torino, Italy	Mancini & Moretti (1976)
A4	28	Apple, Bergerac, France	
A5	17	Tomato, Carpentras, France	
A6	5	Leek and Millet, Soure, Portugal	
B1	1	Turkey	Geraert <i>et al.</i> (1975)
B2	15	Kikuyu Grass, Israel	Sher (1966)
B3	3	Orange, Shahsalar, Iran	
C1	14	Citrus, Ibadan, Nigeria	Sher (1966) — tentative identification
C2	18	Plantain, Ibadan, Nigeria	Sent by Caveness
C3	8	Sugarcane, Kumasi, Ghana	Sher (1966) — tentative identification
C4	2	Tobacco, Nioka, Zaire	Ali <i>et al.</i> (1973)
D1	2	Fern, Harding, Natal	Van den Berg & Heyns (1975)
D2	1	<i>Rubus</i> sp., Ixopo, Natal	Van den Berg & Heyns (1975)
D3	1	Peach, Upington, Cape Province	Van den Berg & Heyns (1975)
D4	1	Cotton, Longlands, Cape Province	Van den Berg & Heyns (1975)
D5	1	Grass, Kokstad, Cape Province	Van den Berg & Heyns (1975)
D6	9	Cape Province	Van den Berg & Heyns (1975)
D7	1	Agave, Potgietersrus, Transvaal	Van den Berg & Heyns (1975)
E1	27	Blue Grass, West Point, New York	
E2	7	Blue Grass, College Park & Beltsville, Maryland	
E3	25	Corn, Near LaFayette, Indiana	
E4	17	Corn, Boone County, Iowa	
F1	16	Homestead, Florida	
F2	30	Itchgrass, Sulfur Springs, St. Lucia	
F3	4	Sugarcane, Venezuela	
G1	30	Philodendron, San Francisco, California	
H1	11	Sweet Potato, Segamat & Labis, Malaysia	Sauer & Winoto (1975)
H2	6	Pine, Jung Pyung, Korea	
H3	10	Cedar, Kyungpook, Korea	
H4	2	Hardy orange, Bosung, Korea	
H5	3	Persimmon, Taegu, Korea	
H6	2	Amorpha, Kyungpook, Korea	
I1	24	Pasture, Kaitoke, New Zealand	
2. Paratypes of related species			
micr	5	<i>Poa pratensis</i> , Madison, Wisconsin	Paratypes <i>H. microlobus</i>
brad1	7	Soybean, near Viborg, South Dakota	Paratypes <i>H. bradyi</i>
brad2	7	Soybean, near Ames, Iowa	Population <i>H. bradyi</i>
egyp	4	Sugarcane, Wabour el Barabra, Egypt	Paratypes <i>H. egyptiensis</i>
phal	9	Grass, Lethbridge, Atta, Canada	Paratypes <i>H. phalerus</i>
afri	6	Victoria Falls, North Rhodesia	Topotypes <i>H. africanus</i>
3. Populations of <i>H. dihystra</i> (see Fortuner <i>et al.</i> , 1981)			
HdA	19	Cocoa, Madagascar	Pop. A in Fortuner <i>et al.</i> (1981)
HdB	20	Banana, Canary Island	Pop. B in Fortuner <i>et al.</i> (1981)
HdC	17	Forest, Senegal	Pop. C in Fortuner <i>et al.</i> (1981)
HdD	16	Millet, Senegal	Pop. D in Fortuner <i>et al.</i> (1981)
HdE	17	Rice, Senegal	Pop. E in Fortuner <i>et al.</i> (1981)
HdF	19	Peanut, Gambia	Pop. F in Fortuner <i>et al.</i> (1981)
HdG	17	Corn, Gambia	Pop. G in Fortuner <i>et al.</i> (1981)
HdH	18	Tobacco, Senegal	Pop. H in Fortuner <i>et al.</i> (1981)
HdI	16	Peanut, Senegal	Pop. I in Fortuner <i>et al.</i> (1981)
HdJ	16	Papaya, Mauretania	Pop. J in Fortuner <i>et al.</i> (1981)
HdK	15	Potato, California	Pop. K in Fortuner <i>et al.</i> (1981)

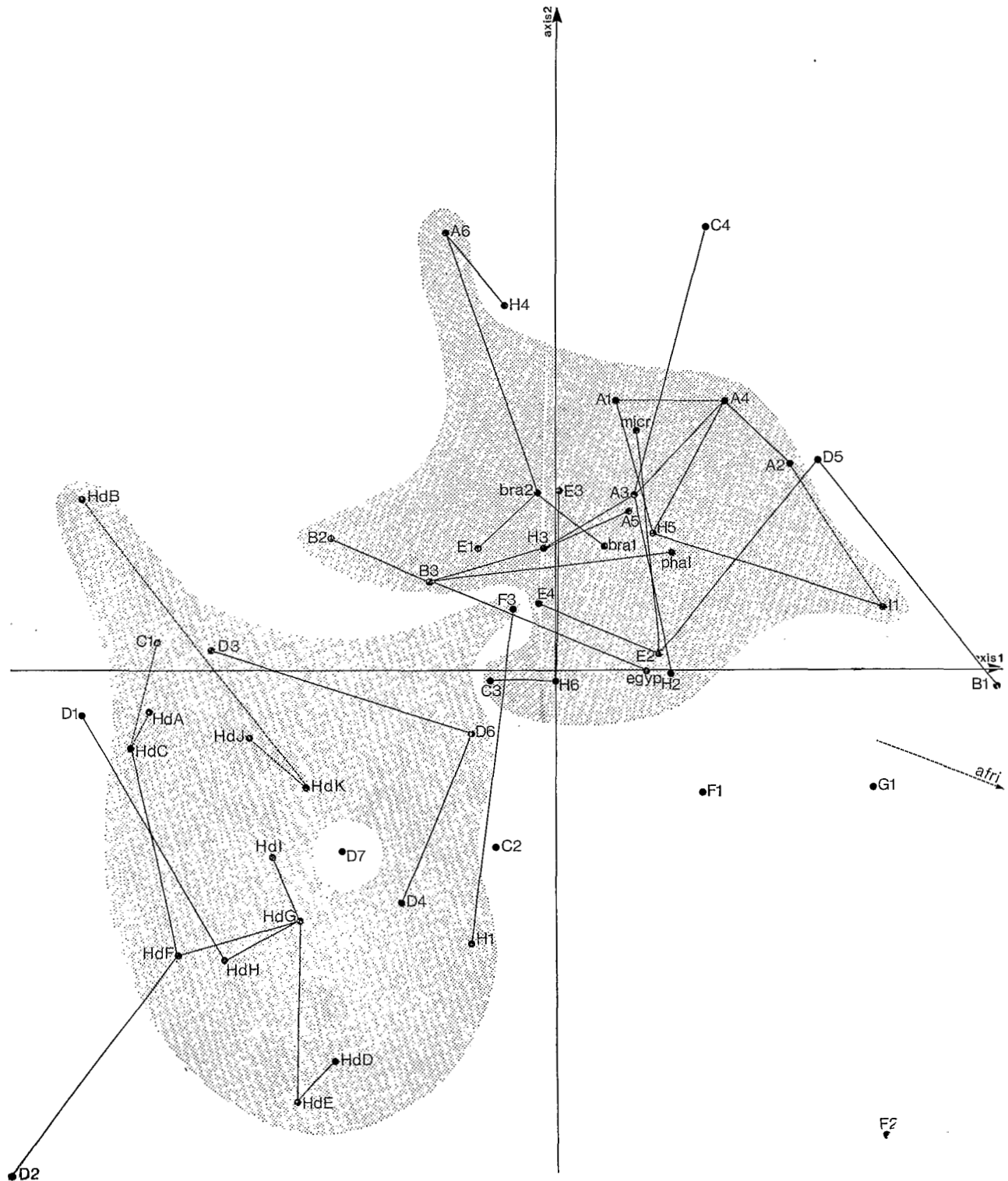


Fig. 1. Stepwise discriminant analysis. Position of 52 samples (A1, etc. : see Tab. 1) in relation to axes 1 and 2, with indication of the lowest distances in solid lines (no significant difference) or dotted lines (difference significant at 1% level).

Tail shape (TSH) was also considered for the analysis but this character is redundant with PRO : tails rounded or with dorsal and ventral sides joining at a straight angle have no ventral projection : PRO = 0. Tails with a ventral projection have PRO = 1 to 5 μm . It was found unnecessary to use both TSH and PRO. PRO was preferred because it discriminates between short and long projections.

Fig. 2 shows the position of 51 samples in relation to axes 1 and 2, found to be the most discriminant to differentiate the samples. Axis 1 is positively correlated with INC 2, and negatively correlated with PRO 1 and STY 1. Axis 2 is negatively correlated with QUL 1 and PRO 1.

Fig. 3 shows a classification tree (dendrogram) made between some samples chosen among those with the highest number of specimens. The topotypes (A1) and some samples from Europe (A2, A3, A4) are grouped because of the characters INC 2 and STY 4. At the opposite end the dendrogram, *H. dihystrera* samples are characterized by INC 1 and STY 1. Other

H. pseudorobustus samples are placed between the two extremes but closer to the European *H. pseudorobustus* than to *H. dihystrera*. Only samples C1 and C2 are among the *H. dihystrera* samples. The samples of *H. microlobus*, *H. bradys*, and *H. phalerus* are not differentiated from the non-European *H. pseudorobustus*.

DISCUSSION

Identity of the samples studied

The analyses separated three groups of samples among the samples studied.

— The group “*pseudorobustus*” : This group is organized around the topotype sample A1. It includes most European samples (A2, A3, A4), samples C3 and C4 from Africa, and sample I1 from New Zealand. It is characterized by stylet length 26-27 μm , ratio V = 61%, L = 700-750 μm , DGO at 9 μm , phasmids seven to eight annules above anus, inner lines of lateral field joining mostly in u pattern (but

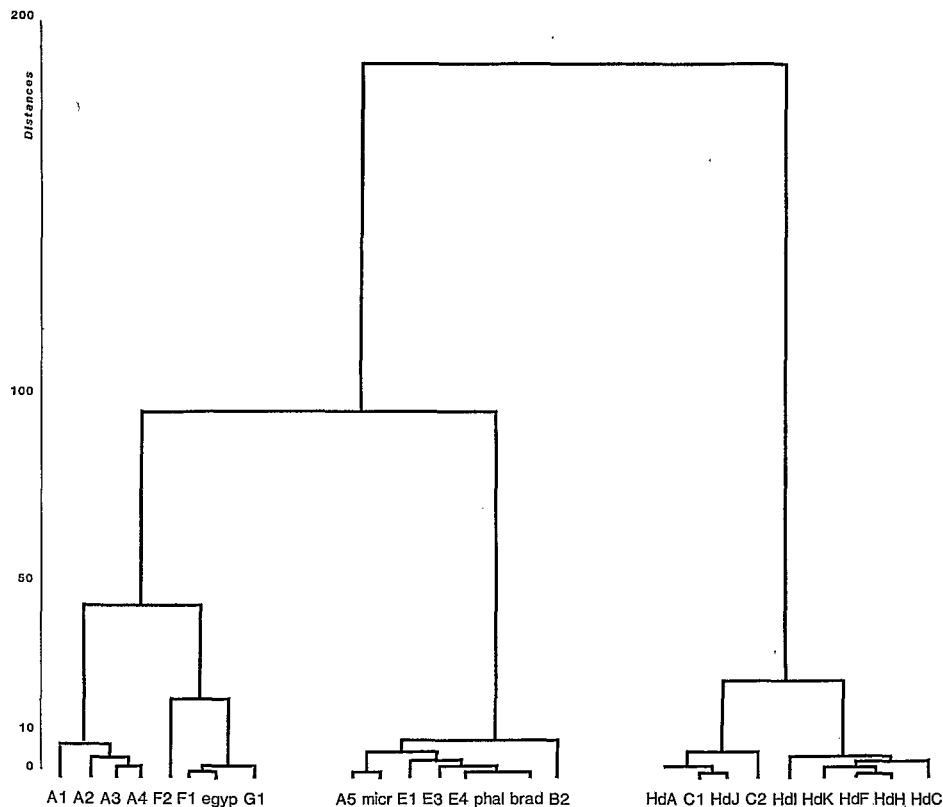


Fig. 3. Classification tree (dendrogram) of some characteristic samples, with indication of the distances between constellations.

some y patterns are present), and areolations on body/tail in some specimens. The tail always ends in a ventral projection of variable length.

— The group “*microlobus*”: This group includes the paratypes of *H. microlobus*, *H. bradys*, *H. phalerus*, and the North American samples of *H. pseudorobustus* E1, E2, E3, and E4. It differs from the first group mostly in the phasmids being closer to the anus (four annules), DGO farther from the stylet (11 μm), and the junction of the inner lines always in the y pattern.

Some samples of *H. pseudorobustus* are intermediate between the two groups: A5 from France is closer to the group “*microlobus*” but has phasmids at six annules above anus; B3 from Iran and F1 from Florida are closer to the group “*pseudorobustus*” but have phasmids at 4 annules. G1 from California and F3 from Venezuela probably belong to the group “*pseudorobustus*” but have a vulva slightly more posterior ($V = 62.2$ and 62.4%). Because of the general variability of these characters, it was considered best not to propose the group “*microlobus*” as a valid species, but to accept it as a geographical variant of *H. pseudorobustus*, characterized by morphological differences too slight to warrant the creation of a subspecies.

— The samples of *H. dihystra*: These samples form a third group clearly separated from the first two by shorter stylet, 24–26.5 μm , more posterior vulva, 62.5–65%, often shorter body, 600–750 μm , DGO sometimes more posterior, 10–15 μm . The junction of the inner lines of the lateral field is always of the y pattern. The tail may or may not have a ventral projection. The samples C1 and C2 from Nigeria belong to this group.

Samples A6 from Portugal and B2 from Israel include some specimens without ventral tail projection. However, all other characters correspond to the description of *H. pseudorobustus* and they can be accepted as members of this species.

Sample F2 from St. Lucia has some characteristics of *H. pseudorobustus* (stylet 27 μm long, tail with long projection, inner lines of the lateral field of the u pattern), and some characteristics of *H. dihystra* ($V = 63.4\%$, DGO = 13 μm). This and its longer tail (20 μm) and esophageal glands (165 μm) explain the position of sample F2 in Fig. 1. It may belong to a different unidentified species.

Samples from Turkey (B1), Malaysia (H1), Korea (H2 to H6) and South Africa (D1 to D7) are too small to be identified.

Intra-specific variability of some characters

— The junction of the inner lines of the lateral field.

In *H. dihystra* it was observed (Fortuner, 1979; Fortuner & Quénéhervé, 1980; Fortuner, Merny & Roux, 1981) that the lines join together in a pattern which looks like a y, or more rarely like a v. In the y pattern, the leg of the y can reach the outer lines or stop short of them.

In the topotypes of *H. pseudorobustus*, several different patterns were observed. Generally the inner lines join together without a sharp angle, but in a rounded pattern resembling the letter u (Fig. 4, H, I).

Sometimes there is a short leg posterior to the u, and the pattern resembles the greek letter μ (Fig. 4, J) or a diapason (Fig. 4, K). This leg can reach the outer lines (Fig. 4, M) or stop short of them. In one specimen the u pattern is at the level of the outer lines and the end of the lateral field looks like an upside-down m with an extra leg (Fig. 4, L).

Fortuner, Merny and Roux (1981) proposed to use these junction patterns to differentiate *H. dihystra* with y and v patterns, from *H. pseudorobustus* with u, μ , or m patterns. However, in every sample of *H. pseudorobustus*, including the topotypes, some specimens present the y/v pattern (Fig. 4, N, O). In the North American samples E1 to E4, all the specimens have a y/v pattern. The character can be used to differentiate species but, as is generally the case for taxonomic criteria in *Helicotylenchus*, it will not permit a clear dichotomy of the genus. Some species, like *H. dihystra* have a y/v pattern, other species, like *H. paracanalisis* (see Fortuner, Merny & Roux, 1981) have a u/m pattern, but many species, like *H. pseudorobustus*, include individuals with one or the other pattern.

— Tail shape

All specimens of *H. pseudorobustus*, with the exception of some individuals in samples A6 and B2, have a ventral tail projection. The length of this projection was very variable, from 1 to 5 μm . This character has been used in several identification keys (Sher, 1966; Siddiqi, 1972), but its variability in *H. pseudorobustus* and *H. dihystra* casts serious doubt on the validity of the species so differentiated.

Descriptions of the species

Helicotylenchus pseudorobustus

(Steiner, 1914) Golden, 1956

(Fig. 4)

Syn. = *H. microlobus* Perry, in Perry, Darling & Thorne, 1959

H. bradys Thorne & Malek, 1968 (new syn.)

H. phalerus Anderson, 1974 (new syn.)

REDESCRIPTION OF THE TOPOTYPES OF SHER (1966)

Measurements (in μm)

Topotypes ($n = 20$), $L = 764$ ($s = 58$)* ; stylet = 27.1 (0.6) ; esophagus = 116 (6) ; esophageal glands =

* The standard deviation of the mean is given between parentheses for every mean. The range in the sample is not given. The range in the population can be estimated for 95% of the individuals as mean ± 2 s ; or for 99% of the individuals as mean ± 3 s.

142 (9) ; dorsal gland opening = 8.8 (1.1) ; excretory pore = 114 (5) ; body diameter = 27.8 (3.8) ; tail length = 15.9 (1.7) ; anal body diameter = 15.6 (1.8). Ratios : a = 28 (3.8) ; c = 48.4 (4.4) ; c' = 12 (3.4) ; m = 46.7 (1.4) ; V = 61.6% (1.8).

Description

Females : body spiral, body annules 1-2.5 μm wide at mid-body. Lip region hemispherical, with four to five annules, sometimes difficult to see ; labial disc not visible in transverse view. Basal ring of cephalic

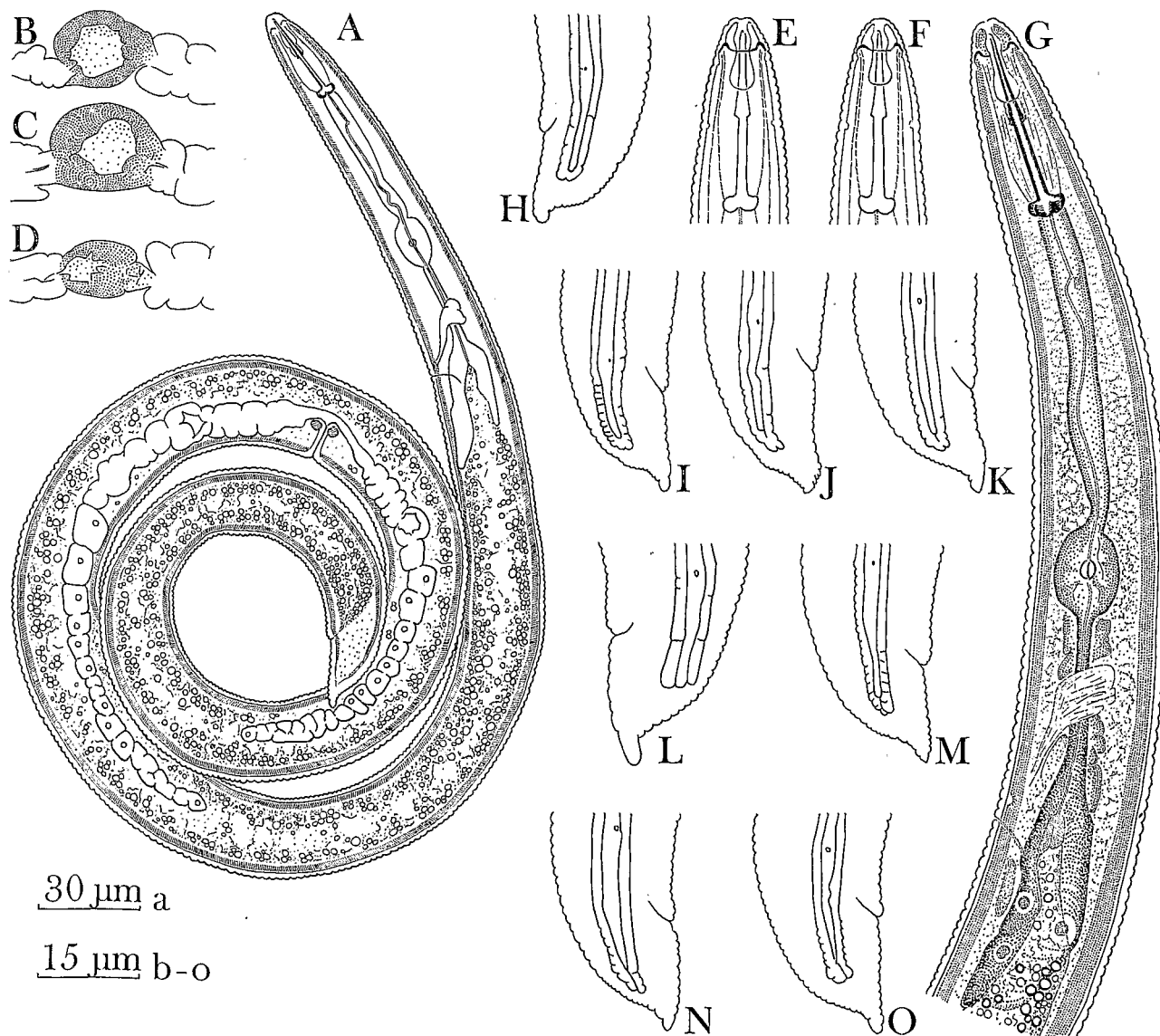


Fig. 4. *Helicotylenchus pseudorobustus*, topotypes (A1). A : whole female ; B-D : spermathecae ; E and F : heads ; G : anterior end ; H-O : tails.

framework 1-3 μm wide, anterior cephalid not seen; posterior cephalid seen in five specimens, 15-16 μm from anterior end. Stylet knobs flattened in sixteen specimens, rarely rounded (Fig. 4E) or indented (Fig. 4F). Excretory pore at about the level of esophago-intestinal junction, sometimes slightly anterior or posterior to it. Hemizonid often difficult to see; when seen, level with to two annules anterior to, excretory pore. Hemizonion not seen. Fasciculi absent. Spermatheca empty, offset (Fig. 4 B, C) but sometimes appears in line (Fig. 4 D) with the genital tract. Lateral field 5-7 μm wide, with transverse lines in the esophageal region (all specimens) and often in the tail region (thirteen specimens); one specimen with a few lines in the vulval region; longitudinal inner lines join together on tail in a u-shaped pattern; in fifteen specimens the u junction is followed by a short line bisecting the outer band of the lateral field as in the Greek letter "u"; one of these specimens presents a m-shaped pattern on one side of the body (Fig. 4 L) and a u pattern on the other side; two other specimens present the typical u shape (Fig. 4 H) without an additional line. In the last three specimens, the junction is rather v-shaped (Fig. 4 N, O) but different from the long tailed y pattern of *H. dihystra* (Fig. 4 in Fortuner Merny & Roux, 1981). Phasmids are three to eleven annules anterior to anus (mean: 7.8, $s = 1.9$), easy to see or indistinct, in the center of the lateral field or closer to the ventral line. Tail with seven to eleven annules (mean: 9.1, $s = 1.2$), with or without a nonannulated ventral section; dorsal terminal tail annules smaller than the other tail annules, rarely of the same size. Tail more curved dorsally with a rounded terminal projection, 1-4 μm long (mean: 2.2 μm), annulated in seventeen specimens.

Males: unknown.

Discussion

The present observations and measurements generally agree with the description of Sher (1966) except for a slightly shorter stylet. The range in 95% of the population can be estimated as $27.1 \pm 2 \times 0.6 = 25.9-28.3 \mu\text{m}$ against 26-30 μm in Sher, 1966. The body is always spiral ("usually in spiral shape" according to Sher); the stylet knobs may be rarely rounded ("flattened or slightly indented anteriorly"—Sher); the tail projection is sometimes short ("pronounced ventral projection"—Sher); and is always terminally rounded ("usually hemispherical"—Sher).

GENERAL VARIABILITY OF THE SPECIES (Fig. 5)

The samples A1 to II (Tab.1) are considered to belong to *H. pseudorobustus* excepted B1, C1, C2, D1

to D7, F2, H1 to H6 which may belong to other species. A species is the sum of all the populations which belong to it. We can consider the accepted samples of *H. pseudorobustus* as successive draws taken at random from the ensemble of populations constituting the species. The mean of the sample means for a measurement is an estimate of the value of this measurement for the species. The means of the different measurements and their standard errors were calculated (in μm) from the sample with $n > 10$: (A1 to A5, B2, E1, E3, E4, F1, G1, and II).

Body = 715 (38.5); stylet = 26.9 (0.73); esophagus = 116 (6); esophageal glands = 146 (8); dorsal gland opening = 10.4 (1.3); excretory pore = 111 (5); body diameter = 26.4 (1.9), tail length = 17.5 (1.1); anal body diameter = 15.2 (0.8). Ratios: $a = 27.4$ (2.1); $c = 41.5$ (3.7); $c' = 1.2$ (0.1); $m = 48$ (1.1); $V = 61.1\%$ (0.8).

The description is identical to that of the topotypes except for the following characters: the first labial annule is sometimes elevated above the general lip outline (E2: Fig. 5P; E3; E4); the lateral field is most often not areolated on body/tail or some transverse striae are present in only a few specimens (A2; A4: Fig. 5C; E2: Fig. 5S; E3; E4: Fig. 5JJ; F3; G1: Fig. 5O; II: Fig. 5I). In some samples (A2; A4: Fig. 5 GG-JJ; etc.), all the specimens possess a u pattern; in other samples (A5: Fig. 5 E-H; A6; B1; B2; E1; E2: Fig. 5R, S; E3; E4: Fig. 5 GG-JJ; etc.), all the specimens possess a y pattern; and in the rest of the samples (A3; F1; G1: Fig. 5 L-O; II: Fig. 5 I-K; etc.) both patterns are present. The tail shape is always typical of *H. pseudorobustus* with a long projection, but the projection is more pointed in some specimens of A3; F1; II: Fig. 5 I. The relative size of the tail annules and the presence of a non-annulated ventral section at the tail end is extremely variable within each sample. California (G1: Fig. 5 L-O) specimens often have irregular mucros at the end of the projection. Mucros were also seen in some individuals of A4 (Fig. 5 D) and A5 (Fig. 5 F).

DIAGNOSIS

Helicolenchus with spiral body, hemispherical lips, stylet of about 27 μm (mean values: 25.5-28 μm), medium body length (mean values: 650-775 μm), vulva not too far posterior (mean V values: 59-62%), phasmids anterior to anus, inner lines of the lateral field joining on tail in variable patterns, tail about as long as wide, more curved dorsally and with rounded medium length projection, no males and empty spermatheca.

SYNONYM SPECIES

Helicotylenchus microlobus Perry,
in Perry, Darling & Thorne 1959*
(Fig. 5 T-X)

Sher (1966) synonymized *H. microlobus* with *H. pseudorobustus* because he could not distinguish the paratypes of the two species. Siddiqi (1972) recognized both species as valid and proposed several characteristics to differentiate them. Sauer and Winoto (1975), considering the variability in populations of *H. pseudorobustus* from Malaysia refused to follow the conclusions of Siddiqi (1972) and accepted the synonymization of Sher (1966).

The differentiating characters proposed by Siddiqi, 1972 (presence of transverse striae in the lateral field, distinctness of the phasmids, their position in the central band of the lateral field, shape of the tail projection, relative size of the dorsal tail annules, ventral tail annulation) are too variable, both in *H. microlobus* and in *H. pseudorobustus* to have any taxonomic value. The pattern of the fusion of the inner lines of the lateral field on the tail, said by Siddiqi (1972) to be different between the two species, is y- or rarely v-shaped in *H. microlobus*, y-, u-, or m-shaped in different samples of *H. pseudorobustus*. Because of the variability of these characters in the samples studied, *H. microlobus* is here accepted as a junior synonym of *H. pseudorobustus*.

Helicotylenchus bradys Thorne & Malek, 1968
(Fig. 5 Y-BB)

This species was originally distinguished from *H. pseudorobustus* by "the coarsely annulated lip region, long spear and tail form." Siddiqi (1972) used the position of the DGO (less or more than 1/3 the spear length from the base of the spear) to separate the two species at line 63 of his key. In lateral view, the lip annules of paratypes of *H. bradys* are no wider nor coarser than those of some specimens of *H. pseudorobustus*. The mean stylet length is 26.4 (s = 0.8) μm , shorter than indicated in the description (29-33 μm). The tail terminus "slightly upturned, bluntly rounded" is no different from shapes observed in *H. pseudorobustus*. The DGO is 10 (s = 1.3) μm from the base of stylet, which is also consistent with measure-

* *H. microlobus* and three other species were first proposed by Perry in a thesis. This is in contravention with the Code of Nomenclature as noted in *Helminthological Abstracts* 28 (1959), no. 35a. The descriptions of the four species were later incorporated into a published article which made their names available.

ments of *H. pseudorobustus*. *H. bradys* is here proposed as a junior synonym of *H. pseudorobustus*.

Helicotylenchus phalerus Anderson, 1974
(Fig. 5 CG-FF)

This species was originally differentiated from *H. pseudorobustus* and *H. microlobus* (considered by Anderson as a valid species) by a combination of five characters, all of which were observed in the present study to be quite variable among samples of *H. pseudorobustus*:

— Presence of a "prominent labial disc with elevated margins" in *H. phalerus*. This structure is noticeable only in some paratypes of *H. phalerus* (Fig. 5 FF). Other specimens (Fig. 5 EE) are not distinguishable from topotypes of *H. pseudorobustus*.

— Terminal tail process marked by transverse and lateral striae. This annulation is not always visible in specimens of *H. phalerus* and exists sometimes in specimens of *H. pseudorobustus*.

— Phasmids easier to see and always in the center of the lateral field. These characters are quite variable in types of *H. pseudorobustus*.

— Shorter distance DGO-stylet. The differences between *H. microlobus* (8.4 μm , s = 1.9), *H. phalerus* (10.4 μm , s = 0.7), and the different samples of *H. pseudorobustus* (from 8.8 to 13 μm) cannot be accepted as diagnostic in view of the great variability observed among samples of *H. pseudorobustus*.

— Tail with "a smaller and less conspicuously sculptured ventral projection." This structure is small in the paratypes examined, but no smaller than in many specimens of *H. pseudorobustus*. The annulation of the tail projection is quite variable in *H. pseudorobustus*.

The inner lines of the lateral field join together in y or v pattern, as in *H. microlobus* and many specimens of *H. pseudorobustus*.

H. phalerus is here proposed as a junior synonym of *H. pseudorobustus*.

Helicotylenchus egyptiensis Tarjan, 1964
(Fig. 6 A-E)

Measurements (in μm)

Paratypes (n = 4). L = 719 (s = 49); stylet = 26 (s = 0.4); esophagus = 121 (s = 1.3); esophageal glands = 150 (s = 5); dorsal gland opening = 10.75 (s = 0.96); excretory pore = 118 (s = 4); body diameter = 25.6 (s = 1.7); tail length = 22.4 (s = 3.9); anal body diameter = 15.9 (s = 1.8). Ratios: a = 28 (s = 2.7); c = 32.5 (s = 3.5); c' = 1.4 (s = 2.2); m = 49.5 (s = 1.3); V = 60.0% (s = 1.2).

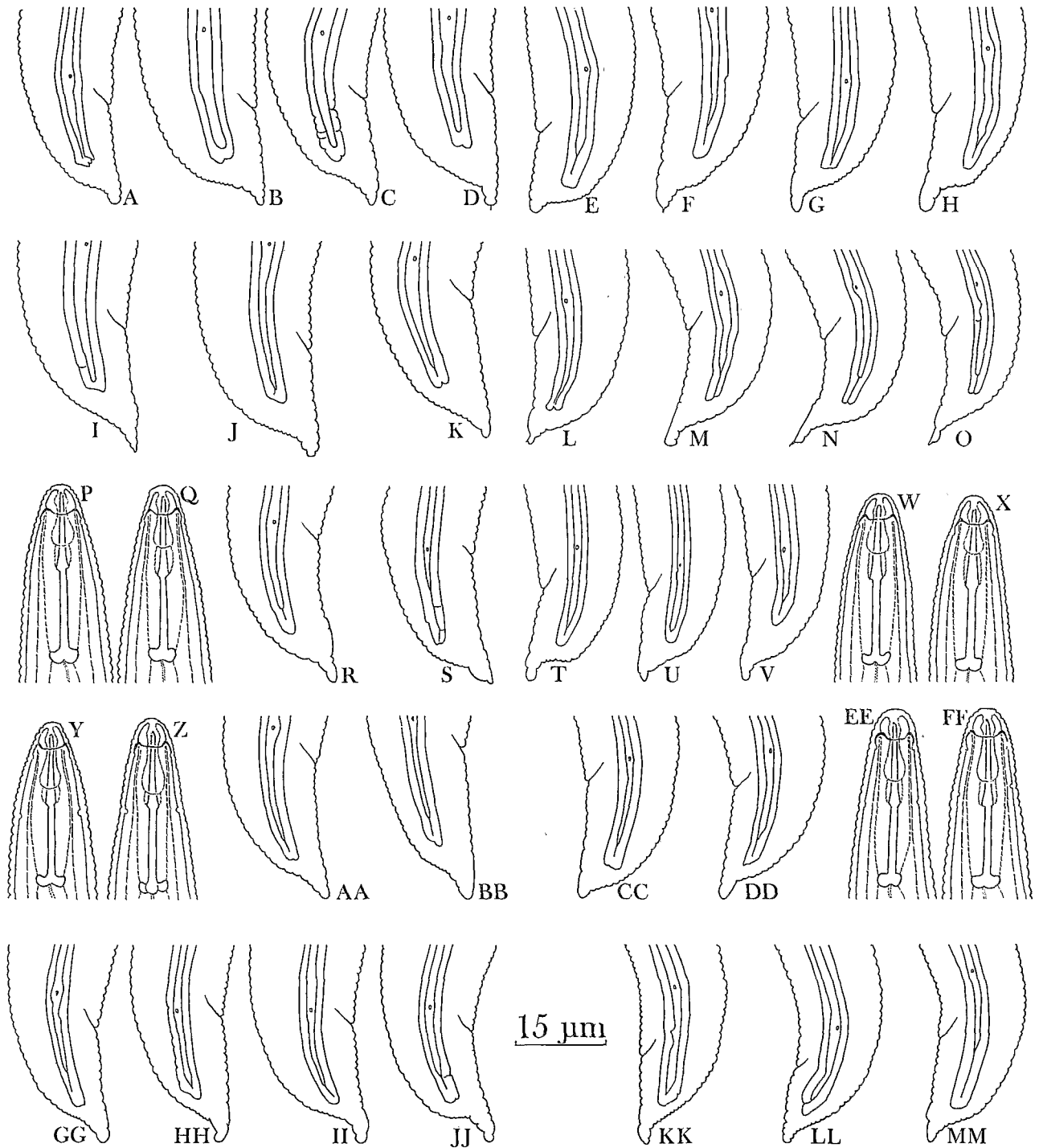


Fig. 5. *Helicotylenchus pseudorobustus*, other populations. A-MM : heads or tails ; A-D : sample A4, France, apple ; E-H : sample A5, France, tomato ; I-K : sample I1, New Zealand ; L-O : sample G1, California, Philodendron ; P-S : sample E2, Maryland, Blue grass ; T-X : paratypes of *H. microlobus* ; Y-BB : paratypes of *H. bradys* ; CC-FF : paratypes of *H. phalerus* ; GG-JJ : sample E4, Iowa, corn ; KK-MM : sample H3, Korea, orange.

Description

Females : Body spiral, body annules 1.5-2 μm wide at mid-body. Lip region flattened to slightly rounded, with four to five annules, well marked ; labial disc not visible in transverse view. Basal ring of the cephalic framework 2 μm deep. Anterior cephalid difficult to see, 2 μm below the basal ring ; posterior cephalid 14-15 μm from the anterior end. Stylet knobs flattened to slightly rounded or indented. Excretory pore level to anterior to esophago-intestinal junction. Hemizonid 1-2 annules anterior to the excretory pore ; hemizonion six annules posterior to the excretory pore. Fasciculi absent. Spermatheca offset, roundish, without sperms. Lateral field 4-6 μm wide without transverse striae on body or tail ; longitudinal inner lines join on tail in a u- (Fig. 6 B, C, E) or a m-shaped (Fig. 6 D) pattern. Phasmids distinct, level with anus or one annule posterior, in the center of the lateral field ; the punctuations in the lateral field reported by Tarjan (1964) are artifacts (Sher, 1966). Tail with eight to fourteen ventral annules with a non-annulated terminal ventral section ; dorsal terminal annules similar to or smaller than the other tail annules. Tail more curved dorsally with a pointed or rounded ventral projection, 1-3 μm long, annulated or not annulated.

Males : absent.

Diagnosis

Helicotylenchus with spiral body, labial region flattened to slightly hemispherical, stylet of medium length (26 μm), medium body length (mean value

720 ; 780 in original description), vulva anterior (mean V value 60%) phasmids level with anus (from one or two annules posterior to five annules anterior to anus according to Tarjan's (1964), Sher's (1966) and Van den Berg and Kirby's (1979) descriptions), inner lines of the lateral field joining on tail in a u/m-shaped junction, tail about as long as wide, with greater dorsal curvature, with variable terminal projection, no males and empty spermatheca.

H. egyptiensis is very close to *H. pseudorobustus*. There is no difference between the measurements of the two species (Fig. 1). In fact the only marked difference is the shape of lips, flattened to slightly rounded in *H. egyptiensis*, markedly rounded in *H. pseudorobustus*. Also the ventral projection is often smaller and more pointed in *H. egyptiensis*.

Helicotylenchus africanus

(Micoletzky, 1916) Andr assy, 1958

(Fig. 6 F-J)

Measurements (in μm)

Topotypes (n-6). L = 856 (s = 70) ; stylet = 29.9 (1.2) ; esophagus = 127 (7) ; esophageal glands = 174 (14) ; dorsal gland opening = 9.5 (1.9) ; excretory pore = 116 (4) ; body diameter = 24.3 (3.5) ; tail length = 30.9 (4.3) ; anal body diameter = 15.6 (1.3). Ratios : a = 35.8 (4.3) ; c = 28.2 (3.3) ; c' = 1.98 (2.5) ; m = 44 (0.6) ; V = 59.0 (1.42).

Description

Females : body in C shape ; annules 1.5-2 μm wide at mid-body. Lip region hemispherical, with 4/5 well-marked annules ; labial disc not visible in lateral view. Basal ring of cephalic framework 2 μm deep. Anterior cephalid not seen, posterior cephalid 11-16 μm from the anterior end. Stylet knobs variable in shape, anteriorly indented, flattened, or rounded. Excretory pore anterior to esophago-intestinal junction. Hemizonid just anterior to excretory pore, hemizonion not seen. Fasciculi absent. Spermatheca apparently in-line with the genital tract, full of rounded sperms. Lateral field 4.5-6.5 μm wide with scattered transverse striae on body and tail ; longitudinal inner lines join on tail generally in a u-shaped pattern, v-shaped in one specimen (Fig. 6 J). Phasmids 1-8 annules anterior to anus (mean : 3.8, s = 2.7), distinct and in the center of the lateral field. Tail with 12-18 annules (mean : 14.8 ann., s = 2.6), about two body diameters long, with a short non-annulated ventral section, and dorsal terminal annules smaller than other tail annules. Tail dorsally curved with a rounded terminal projection 2-4 μm long, annulated, rarely a pointed projection.

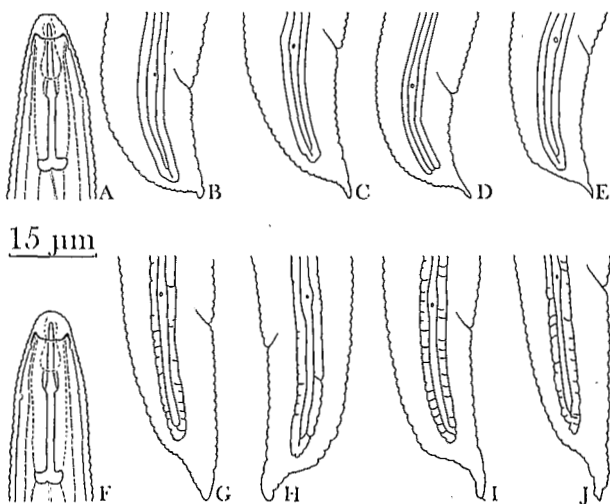


Fig. 6. A-E : *Helicotylenchus egyptiensis* ; F-J : *Helicotylenchus africanus* ; A and F : heads ; B-E, G-J : tails.

Males : present but not included in the present study.

Discussion

Our description agrees with Sher's (1966) except for the shape of the ventral tail projection. Sher described a "distinct pointed ventral projection," but illustrated (Fig. 2 B of Sher, 1966) a rounded projection. Of the six topotypes examined here, five have a distinctly rounded projection, only one has a more pointed projection (Fig. 6 J). One specimen presents a rounded projection and a short mucro identical to some structures observed in *H. pseudorobustus* (Fig. 6 I).

H. africanus is easily distinguished from *H. pseudorobustus* by its C shape, relatively long tail, and presence of males. Its stylet is slightly longer and its vulva slightly more anterior than those of *H. pseudorobustus*.

Conclusions

Multivariate analyses proved once again to be a valuable taxonomic tool to compare populations of related species. The present analyses show that several criteria could differentiate *Helicotylenchus pseudorobustus* from *H. dihystra* in spite of the high intraspecific variability of all characters in both species. The analyses also cast a new light on the relationships between *H. pseudorobustus* and *H. microlobus*, in dispute for the last twenty years. Multivariate analyses have in the past, and will in the future, help solve specific problems in taxonomy. It may be possible to use similar techniques at the generic level, to clarify the relationships of taxa within and between certain genera. However, it is doubtful that multivariate analyses can be used for specific differentiation and identification in the genus *Helicotylenchus*. There are now about 180 species in the genus, differentiated by their original authors from more than forty measurements or qualitative characters, most of which are highly variable. A multivariate analysis including all the species and all the characters may or may not show some grouping of species in the genus. The identification of the criteria responsible for these groupings, and the evaluation of the taxonomic value of the groups so defined, would require a careful study by both a statistician and a taxonomist. For practical identification of species, other methods, for example the evaluation of the similarity between pairs of species, will have to be investigated.

ACKNOWLEDGMENTS

The authors thank I. de Oliveira Abrantes, R. V. Anderson, A. Bell, F. E. Caveness, Y. E. Choi, P. Donald, E. Geraert, A. M. Golden, D. J. Hunt, R. McSorley, G. Mancini, R. Mankau, D. C. Norton, M. R. Sauer, C. Scotto La Massese, M. R. Siddiqi, J. D. Smolik, A. C. Tarjan, E. Van den Berg, B. Weischer, R. Winoto Suatmadji, and G. W. Yeates who supplied the specimens; J. Holmes, G. Merny, P. Mullon, and P. Schneeman who helped with the statistics; and A. French, J. M. Legay, and J. F. Southey who reviewed the manuscript.

REFERENCES

- ALI, S. S., GERAERT, E. & COOMANS, A. (1973). Some spiral nematodes from Africa. *Biol. Jaarb. Dodonaea*, 41 : 53-70.
- ANDERSON, R. V. (1974). Canadian species of the genus *Helicotylenchus* Steiner, 1945 (Nematoda : Haplolaimidae), their identifying characteristics and descriptions of three new species. *Can. J. Zool.*, 52 : 1365-1381.
- ANDRÁSSY, I. (1958). *Hoplolaimus tylenchiformis* Daday, 1905 (syn. *H. coronatus* Cobb, 1923) und die Gattungen der Unterfamilie Haplolaiminae Filipjev, 1936. *Nematologica*, 3 : 44-56.
- BENZECRI, J. P. & BENZECCI, F. (1980). *Pratique de l'analyse des données. 1. L'analyse des correspondances, exposé élémentaire*. Paris, Dunod, 424 p.
- FORTUNER, R. (1979). Morphometrical variability in *Helicotylenchus* Steiner, 1945. 1. The progeny of a single female. *Revue Nématol.* 2 : 197-202.
- FORTUNER, R. (1981). Les nématodes associés au riz pluvial en Côte-d'Ivoire. *Agron. trop.*, Nogent, 36 : 70-77.
- FORTUNER, R. & QUÉNÉHERVÉ, P. (1980). Morphometrical variability in *Helicotylenchus* Steiner, 1945. 2. Influence of the host on *H. dihystra* (Cobb, 1893) Sher, 1961. *Revue Nématol.* : 291-296.
- FORTUNER, R., MERNY, G. & ROUX, C. (1981). Morphometrical variability in *Helicotylenchus* Steiner, 1945. 3 : Observations on African populations of *Helicotylenchus dihystra* and considerations on related species. *Revue Nématol.* 4 : 235-260.
- GERAERT, E., ZEPP, A. & BORAZANCI, N. (1975). Some plant nematodes from Turkey. *Meded. Landb-Hooges. Rijksuniv., Gent*, 40 : 511-515.
- GOLDEN, A. M. (1956). Taxonomy of the spiral nematodes (*Rotylenchus* and *Helicotylenchus*) and the developmental stages and host-parasite relationships of *R. buxophilus* n. sp., attacking boxwood. *Univ. Maryland agric. Exp. Stn Bull.* A-85, 28 p.

- JENNRICH, R. I. & SAMPSON, P. F. (1979). Stepwise discriminant analysis. In : Dixon, W. J. & Brown, M. B. (Eds) *BMDP-79 Biomedical Computer Programs P-Series*, Berkeley, Univ. California Press : 711-733.
- MANCINI, G. & MORETTI, F. (1977). Il genere *Helicotylenchus* Steiner, 1945 in Piemonte e Valle d'Aosta, nota 1. *Redia*, 59 (1976) : 225-228.
- MICOLETZKY, H. (1916). Ergebnisse einer botanischen Forschungsreise nach Deutsch-Ostafrika und Südafrika (Kapland, Natal und Rhodesien). Süßwasser-Nematoden aus Südafrika. *Denkschr. Kaiserl. Akad. Wissensch., Wien*, 92 : 149-171.
- SAUER, M. R. & WINOTO, R. (1975). The genus *Helicotylenchus* Steiner, 1945 in West Malaysia. *Nematologica*, 21 : 341-350.
- SHER, S. A. (1966). Revision of the Hoplolaiminae (Nematoda) VI. *Helicotylenchus* Steiner, 1945. *Nematologica*, 12 : 1-56.
- SIDDIQUI, M.R. (1972). On the genus *Helicotylenchus* Steiner, 1945 (Nematoda : Tylenchida), with descriptions of nine new species. *Nematologica*, 18 : 74-91.
- STEINER, G. (1914). Freilebende Nematoden aus der Schweiz. *Arch. Hydrobiol.*, 9 : 259-276.
- TARJAN, A. C. (1964). Two new mucronate-tailed spiral nematodes (*Helicotylenchus* : Hoplolaiminae). *Nematologica*, 10 : 185-191.
- THORNE, G. & MALEK, R. B. (1968). Nematodes of the Northern Great Plains. Part I. Tylenchida (Nemata : Secernentea). *Bull. S. Dak. agric. Exp. Stn*, 31 : 1-111.
- VAN DEN BERG, E. & HEYNS, J. (1975). South African Hoplolaiminae. 4. The genus *Helicotylenchus* Steiner, 1945. *Phytophylactica*, 7 : 35-52.
- VAN DEN BERG, E. & KIRBY, M. F. (1979). Some spiral nematodes from the Fiji Islands (Hoplolaimidae : Nematoda). *Phytophylactica*, 11 : 99-109.

Accepté pour publication le 27 mai 1983.