Morphometrical variability in *Helicotylenchus* Steiner, 1945
I. The progeny of a single female (1)

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SUMMARY

Morphometrical variability in a species of *Helicotylenchus* was studied in the progeny of a parthenogenetic female, probably belonging to *Helicotylenchus dihystera*. Several criteria currently used in specific diagnoses, and particularly those describing the tail, were proved to be highly variable. Their use in taxonomical studies is discussed.

RÉSUMÉ

Variabilité morphométrique chez Helicotylenchus *Steiner, 1945. I. La descendance d'une seule femelle*

La variabilité morphométrique intraspécifique chez *Helicotylenchus* a été évaluée dans la descendance d'une femelle parthénogénétique du genre, appartenant probablement à l'espèce *Helicotylenchus dihystera*. Plusieurs critères couramment utilisés dans la caractérisation des espèces, et en particulier ceux qui se réfèrent à la queue, se sont révélés extrêmement variables. Leur usage en taxonomie est discuté.

Morphological study of nematodes of the genus *Helicotylenchus* in the ORSTOM (Dakar) slide collection, representing specimens from numerous different hosts and countries in Africa, demonstrated that many have the same general features : spiral body, more or less hemispherical lip region, body length of 500-900 µm, stylet length of 24-26 µm, V-value of 59-65%, empty offset spermathecae and no males. They probably all belong to the same species, close or identical to *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961. However, in most specimens observed, some other criteria, notably those of the tail region, e.g. tail shape, used in 70% of specific diagnoses in the genus, position of phasmids (44% of diagnoses), fusion of inner incisures, used to differentiate *H. dihysteroides* Siddiqi, 1972 from *H. dihystera*, for example, might suggest that these specimens belong to various described species.

Although the populations from which came the observed specimens might have consisted of a mixture of species, to find mixtures of the same closely related species in almost every soil sample from Africa seems most unlikely.

It was then suggested that some dimensions

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(1) A portion of this paper was presented at the XIIIth International Nematology Symposium, Dublin, Ireland, 5-11 September 1976.

and features used in the diagnoses of new species could represent individual variations within the same species. In order to test this hypothesis, variability of those characteristics was studied from specimens selected from the progeny of a single parthenogenetic female.

Material and methods

Three to four rice (cv. I-Kong-Pao) seeds were sown in each of 45 pots containing heat sterilized soil, protected from contamination by plastic shields. After two weeks, each of 20 pots were inoculated with one female of *Helicotylenchus* originating from a field in Senegal with a maize — upland rice — peanut rotation (Inor Mandingue ; sample number 162 in Fortuner, 1975). The other 25 pots, left non-inoculated as controls, were intermixed with the inoculated ones. After six months, the nematodes present in the soil of the pots were extracted by elutriation and examined. Some pots were discarded because of contamination of controls, others for inoculation failure, but one pot was found containing numerous specimens of *Helicotylenchus* and was probably free of contamination as the adjacent controls contained no nematodes. From this pot 45 females were selected at random, killed and fixed by FP 4:1 (Netscher & Seinhorst, 1969) and mounted in glycerin on Cobb slides.

Results

QUANTITATIVE MEASUREMENTS

Range, mean, standard deviation and coefficient of variability were calculated for the following criteria : lengths of body, stylet, anterior part of stylet, oesophagus (to oesophago-intestinal junction and to base of glands), tail ; distances from head to vulva and from dorsal gland opening to stylet knobs ; anal and vulval body diameters ; number of tail annules and positions of inner incisures fusion and of phasmids. Results are given in Table 1.

Stylet length was the most constant measurement (C.V. = 1.7%) ; total length, length of oesophagus measured to oesophago-intestinal junction, anal and vulval body diameters were also reliable (C.V. = 3.2 to 4.5%). On the other hand, criteria related to tail, *viz.* tail length, number of tail annules, position of fusion of inner lateral field incisures and position of phasmids had rather high coefficients of variability (C.V. = 9.6 to 70%).

Table 1

Morphometric characteristics in the progeny of a single *Helicotylenchus* female (n = 45 ♀)

Criteria	*Range* (µm)	*Mean* (µm)	*Standard deviation*	*Coefficient of variation*
Stylet length	23.5-25	24.5	0.4	1.7
Anterior part of stylet	9.5-10.5	10.2	0.3	3.2
Total length	623-751	671	25	3.7
Oesophagus length (to valve)	103-121	111	4.2	3.7
Head to vulva distance	389-461	417	16	3.9
Anal diameter	13.5-16.5	15	0.67	4.5
Vulval diameter	21-25.5	23.5	1.05	4.5
Oesophagus length (to base of glands)	110-147	129	7.5	5.8
Tail length	17.5-26.5	20.9	2	9.6
Number of tail annules	11-17	13.5	1.5	11.4
Dist. d. gland opening to knobs	6-11.5	9.3	1.4	15
Inner incisures fusion (in tail annules from anus)	1-11	7.5	2	26.7
Phasmids (in tail annules from anus ; + if anteriad)	—3 +6	+2.8	1.9	70

RATIOS

The validity of the usual ratios (a, b, b', c, c', V, o and m) was tested by a t-test (Roggen & Asselberg, 1971) with :

$$t = \frac{CVy - CVx}{\sqrt{\dfrac{CVy^2 + CVx^2}{2n}}}$$

where CVx and CVy are the coefficients of variability of the terms of the ratio and n the number of observations (here $n = 45$). The degrees of freedom are $2n - 2 = 88$. Results are given in Table 2.

Ratios b', c, c', m and o are formed with measurements the coefficients of variability of which are significantly different (at 1% level) and thus are statistically unjustified. Ratios a, b and V are justified and were calculated (Table 3).

Table 2

t-test of the significance of the difference between coefficients of variability (n = 45)

Ratios	*Coeff. var.*	*t*	*Significance* (+ *or* —)
a = body length/vulval diameter	3.7/4.5	1.303	—
b = body length/oesophagus (to valve)	3.7/3.7	0	—
b' = body length/oesophagus (to glands)	3.7/5.8	2.896	+
c = body length/tail length	3.7/9.6	5.440	+
c' = tail length/anal body diameter	9.6/4.5	4.563	+
V = dist. head to vulva/body length	3.9/3.7	0.353	—
m = ant. part stylet/stylet length	3.2/1.7	3.927	+
o = dist d.g.o. to knobs/stylet length	15.0/1.7	8.358	+

Table 3

Variability of ratios in the progeny of a single *Helicotylenchus* female

Ratios	*Range*	*Mean*	*Standard deviation*	*Coefficient of variation*
a	26.2-31.8	28.6	1.36	4.8
b	5.6- 6.7	6	0.24	3.9
V	58.6-65.6	62.1	1.4	2.3

It can be noticed that no reduction of variability was obtained with ratios a and b. Only ratio V is both justified and useful : its C.V. is 2.3%, better than the C.V. for body length (3.7%) and for head to vulva distance (3.9%).

QUALITATIVE CRITERIA

This species of *Helicotylenchus* appears to be exclusively parthenogenetic : no male having been observed among the thousands of individuals obtained from field samples, and only empty spermathecae having been seen in the hundreds of fixed females studied for specific determinations.

Other characters were also very constant : body shape spiral in fixed specimens, offset spermatheca, hemispherical lips with labial disc non-visible with transmitted light microscope, oval median oesophageal bulb never filling the body cavity, position of hemizonid and excretory pore always anterior to oesophago-intestinal junction, and absence of fasciculi ('canals'). All of these criteria were observed in each of the 45 females studied.

Shape of stylet knobs was more variable, from anteriorly indented to flattened or rounded. Lip

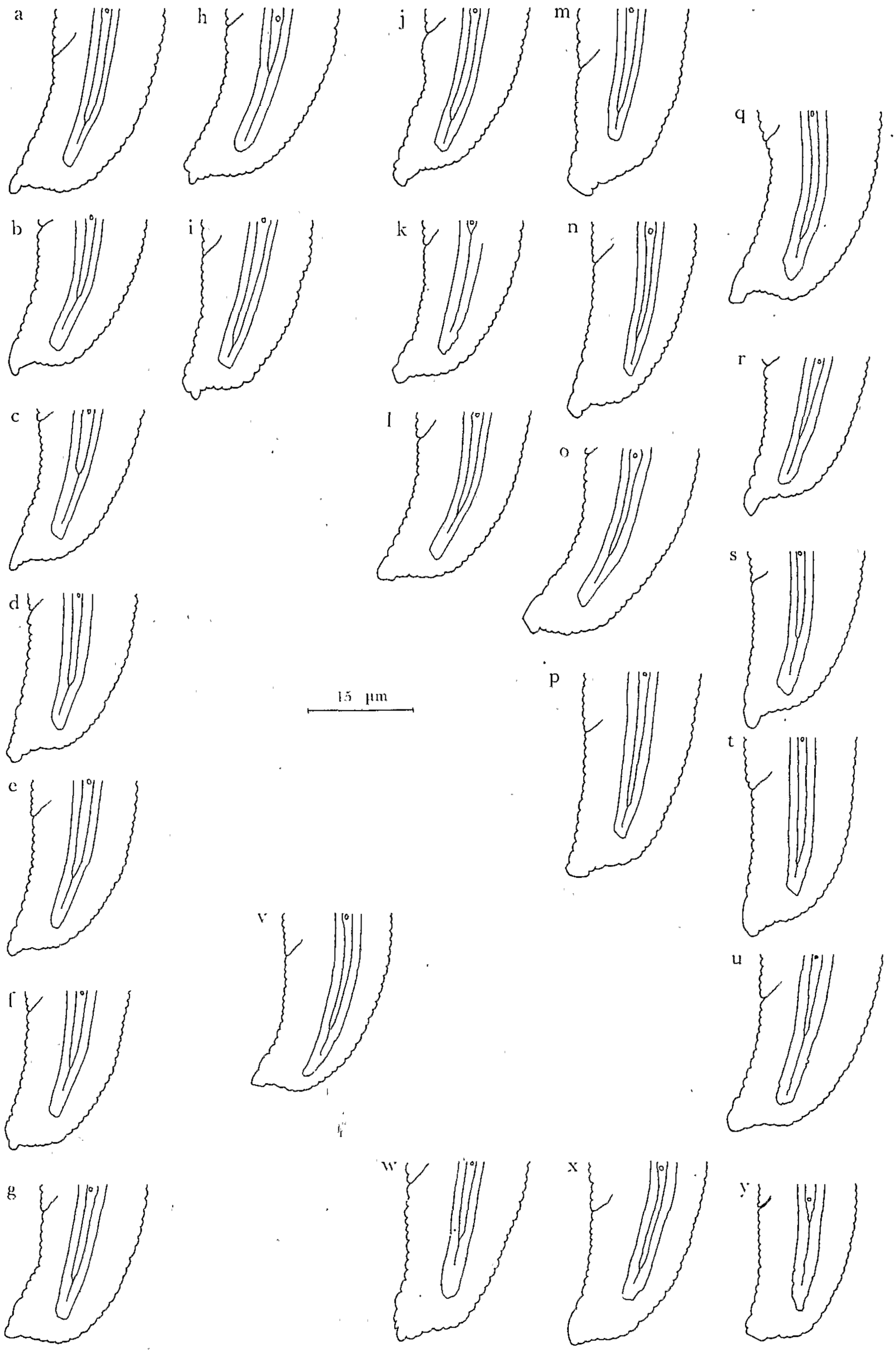

Fig. 1. Variability of tail morphology within the progeny of a single *Helicotylenchus* female.

annulation, generally present, could not be detected on some specimens.

However the most variable criterion was the shape of the tail and related features. Figure 1 illustrates various tail shapes observed within the females studied. Tail 1g is identical to the typical tail of *Helicotylenchus dihystera* as illustrated in Fig. 1B of Sher (1966), Fig. 3C of Román (1965), Fig. H and I of Siddiqi (1972 *b*), Fig. 6G of Anderson (1974), Fig. 1F of Sauer and Winoto (1975) and Fig. 8f of Van den Berg and Heyns (1975).

Other tails in the present figure resemble *H. dihystera* tail variations as described by these authors : the sequence w-x-y is similar to tails D, E, F, and G of Siddiqi (1972 *b*), Fig. 6H and 6I of Anderson (1974), Fig. 1E and 1G of Sauer and Winoto (1975) and Fig. 8d, 8c and 8i of Van den Berg and Heyns (1975) ; series l-p-t-u is similar to tails in Fig. 2F and 2H of Ali, Geraert and Coomans (1973), and tail y to Fig. 3B and 3D of Román (1965).

Some tail shapes illustrated in Figure 1 are also quite similar to those of other described species. For example, the series v-w-x-y ressembles *H. cavenessi* Sher, 1966 (Fig. 6E), *H. elegans* Román, 1965 (Fig. 6D), *H. caribensis* Román, 1965 (Fig. 2D), *H. digonicus* Perry, 1959 (as redescribed by Sher, 1966, Fig. 2G, 2H), *H. paraconcavus* Rashid & Khan, 1974 (Fig. 2C, 2D), *H. aerolatus* Van den Berg & Heyns, 1975 (Fig. 1c). Series r-s is similar to *H. flatus* Román, 1965 (Fig. 7D), *H. borinquensis* Román, 1965 (Fig. 7F), *H. glissus* Thorne & Malek, 1968 (Fig. 26F, 26G), and *H. bambesae* Elmiligy, 1970 (as redescribed by Ali and Geraert, 1975, Fig. 1I, 1J, 1K and 1L). Series j-k-l resembles *H. talonus* Siddiqi, 1972 *a* (Fig. 2R, 2S, 2T). *H. talonus* in Fig. 2P, 2Q is also identical with series r-s. Series j-k-l resembles *H. dihysteroides* (Fig. 1A, 1F) and tail c is similar to *H. pseudorobustus* (Steiner, 1914) Golden, 1956 (as redescribed by Sher, 1966, Fig. 10, 1P).

In addition to variations of tail shape, length, thickness, shape and position of the terminal process, curvature of the ventral side of tail, length of inner incisure fusion and position of phasmids varied greatly from one specimen to the other. The dorsal tail annules were generally identical to the body ones, but sometimes smaller or larger.

Discussion

Study of a strain from a single female cultivated in one pot (with minimum variations between specimens concerning environmental factors) showed that many criteria currently used in taxonomy of *Helicotylenchus* are highly variable.

This is particularly true for tail shape. Intraspecific variability of this feature has already been demonstrated for many genera : eg. *Cylindrocorpus* (Chin, 1975), *Xiphinema* (Luc & Hunt, 1978), *Paratylenchus* (Geraert, 1965) and *Pratylenchus* (Taylor & Jenkins, 1957). Among species of *Helicotylenchus*, tail variability was mentioned by several authors (eg. Sher, 1966 ; Siddiqi, 1972 *a* ; Anderson, 1974, Van den Berg & Heyns, 1975). However I believe that this is the first time that the single female inoculation method (used by Van Weerdt (1958) for *Radopholus similis;* Loof (1960) for *Pratylenchus neglectus;* Goodey and Hooper (1965) for *Aphelenchus avenae*, and others) has been used for assessment of variability in *Helicotylenchus.* It must be stressed that every tail shape in Fig. 1 was observed in the progeny of a single female and that it is possible to find a graded series of tail shapes for example from the typical *H. dihystera* shape (g) to an *H. flatus*-type tail (r,s) : g-f-e-d-c-b-i-k-n-r-s. The wide range of shapes observed here is then all the more meaningful and suggests strongly that differences of tail shape should not be used for differentiating between most of the species of *Helicotylenchus*, and particularly those mentioned in the present article.

Additional studies of this type should be made comparing closely related described species of *Helicotylenchus*, to ascertain the extent of variability of diagnostic characters, their degree of overlap, and ultimately the validity of the species involved.

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Accepté pour publication le 21 février 1979.