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

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Summary

Multivariate analyses were made on 28 populations of *Helicotylenchus pseudorobustus*, eleven populations of *H. dihystera*, and type populations of *H. microlobus*, *H. bradyis*, *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. bradyis* 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. dihystera* or may represent other, unidentified species. Paratypes of *H. egyptiensis* and *H. africanus* were described.

Résumé

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


The variability of the taxonomic characters has been studied in *Helicotylenchus dihystera* (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 Quénéhervé (1980) observed the additional variability in such a progeny when cultivated under several different host-plants ; and Fortuner, Merny and Roux (1981) reported the variability in field populations of a species of *Helicotylenchus* which was identified as *H. dihystera* as a result of multivariate analyses.

These studies show that many characteristics in *H. dihystera* 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. dihystera* were also valid for other members of the genus *Helicotylenchus*.

*H. pseudorobustus* (Steiner, 1914) Golden, 1958 was selected for the present study. It is, with *H. multicinctus* and after *H. dihystera*, 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 variability in several characteristics. Rather small process of Thorne (1973) to 30 μm (Thorne & Malek, 1968); the tail is generally described with a large ventral projection, but some illustrations show a somewhat 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 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. bradyi Thorne & Malek, 1968, H. microlobus Golden, 1956, H. phalerus Anderson, 1974, and also H. dihystera. 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. bradyi were included in the study. The eleven field populations of H. dihystera 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 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 CDFA Permanent Slide Reference Collection (Nematology).

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

The measurements of the eleven populations of H. dihystera 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 RAV = DTV x 100 /LON.

Eleven qualitative characters were also observed:

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

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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* (Al) 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. dihystera*. 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. egiyptiensis* 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. dihystera* (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-II-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. dihystera* 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 (brad), 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 egyp. 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. dihystera* 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. dihystera* (see Fig. 4 of Fortuner, Merny & Roux, 1981). In the samples of *H. pseudorobustus* other patterns were also observed (u, 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>
<thead>
<tr>
<th>Sample Code</th>
<th>n</th>
<th>Origin</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. H. pseudoro bustus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>20</td>
<td>Moss, Altmat, Switzerland</td>
<td>Topotypes-Sher (1966)</td>
</tr>
<tr>
<td>A2</td>
<td>24</td>
<td>Pine, Hünxe, Germany</td>
<td>Mancini &amp; Moretti (1976)</td>
</tr>
<tr>
<td>A3</td>
<td>11</td>
<td>Chestnut, Torino, Italy</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>28</td>
<td>Apple, Bergerac, France</td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>17</td>
<td>Tomato, Carpentrás, France</td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>5</td>
<td>Leek and Millet, Šoure, Portugal</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>1</td>
<td>Turkey</td>
<td>Geraert et al. (1975)</td>
</tr>
<tr>
<td>B2</td>
<td>15</td>
<td>Kikuuyu Grass, Israel</td>
<td>Sher (1966)</td>
</tr>
<tr>
<td>B3</td>
<td>3</td>
<td>Orange, Shahsarar, Iran</td>
<td>Sher (1966) — tentative identification</td>
</tr>
<tr>
<td>C1</td>
<td>14</td>
<td>Citrus, Ibadan, Nigeria</td>
<td>Sent by Caveness</td>
</tr>
<tr>
<td>C2</td>
<td>18</td>
<td>Plantain, Ibadan, Nigeria</td>
<td>Sher (1966) — tentative identification</td>
</tr>
<tr>
<td>C3</td>
<td>8</td>
<td>Sugarcane, Kumasi, Ghana</td>
<td>All et al. (1973)</td>
</tr>
<tr>
<td>C4</td>
<td>2</td>
<td>Tobacco, Nioka, Zaire</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>2</td>
<td>Fern, Harding, Natal</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>D2</td>
<td>1</td>
<td><em>Rübus</em> sp., Ixopo, Natal</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>D3</td>
<td>1</td>
<td>Peach, Upington, Cape Province</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>D4</td>
<td>1</td>
<td>Cotton, Longlands, Cape Province</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>D5</td>
<td>1</td>
<td>Grass, Kokstad, Cape Province</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>D6</td>
<td>9</td>
<td>Cape Province</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>D7</td>
<td>1</td>
<td>Agave, Potgietersrus, Transvaal</td>
<td>Van den Berg &amp; Heyns (1975)</td>
</tr>
<tr>
<td>E1</td>
<td>27</td>
<td>Blue Grass, West Point, New York</td>
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</tr>
<tr>
<td>E2</td>
<td>7</td>
<td>Blue Grass, College Park &amp; Beitsville, Maryland</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>25</td>
<td>Corn, Near LaFayette, Indiana</td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>17</td>
<td>Corn, Boone County, Iowa</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>16</td>
<td>Homestead, Florida</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>30</td>
<td>Itchgrass, Sulfur Springs, St. Lucia</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>4</td>
<td>Sugarcane, Venezuela</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>30</td>
<td>Philodendron, San Francisco, California</td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>11</td>
<td>sweet Potato, Segamat &amp; Labis, Malaysia</td>
<td>Sauer &amp; Winoto (1975)</td>
</tr>
<tr>
<td>H2</td>
<td>6</td>
<td>Pine, Jung Pyung, Korea</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>10</td>
<td>Cedar, Kyungpook, Korea</td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>2</td>
<td>Hardy orange, Bosung, Korea</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>3</td>
<td>Persimmon, Taegu, Korea</td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>2</td>
<td>Amorpha, Kyungpook, Korea</td>
<td></td>
</tr>
<tr>
<td>I1</td>
<td>24</td>
<td>Pasture, Kaitoke, New Zealand</td>
<td></td>
</tr>
</tbody>
</table>

2. Paratypes of related species

| micr       | 5  | *Poa pratensis*, Madison, Wisconsin | Paratypes *H. microlobus*                             |
| brad1      | 7  | Soybean, near Viborg, South Dakota | Paratypes *H. bradys*                                |
| brad2      | 7  | Soybean, near Ames, Iowa           | Population *H. bradys*                              |
| egyp       | 4  | Sugarcane, Wabour el Barabra, Egypt| Paratypes *H. egypiensis*                            |
| phal       | 9  | Grass, Lethbridge, Atta, Canada    | Paratypes *H. phaleris*                              |
| afrif      | 6  | Victoria Falls, North Rhodesia     | Topotypes *H. africanus*                             |

3. Populations of *H. dihystera* (see Fortuner et al., 1981)

| HdB        | 20  | Cocoa, Madagascar                | Pop. A in Fortuner et al. (1981)                    |
| Hdb        | 19  | Banana, Canary Island            | Pop. B in Fortuner et al. (1981)                    |
| Hdc        | 17  | Forest, Senegal                  | Pop. C in Fortuner et al. (1981)                    |
| Hdd        | 16  | Millet, Senegal                  | Pop. D in Fortuner et al. (1981)                    |
| Hde        | 17  | Rice, Senegal                    | Pop. E in Fortuner et al. (1981)                    |
| Hdf        | 19  | Peanut, Gambia                    | Pop. F in Fortuner et al. (1981)                    |
| Hdg        | 17  | Corn, Gambia                      | Pop. G in Fortuner et al. (1981)                    |
| Hdh        | 18  | Tobacco, Senegal                 | Pop. H in Fortuner et al. (1981)                    |
| Hdi        | 16  | Peanut, Senegal                   | Pop. I in Fortuner et al. (1981)                    |
| Hdj        | 16  | Papaya, Mauretanía                | Pop. J in Fortuner et al. (1981)                    |
| Hdk        | 15  | Potato, California                | Pop. K in Fortuner et al. (1981)                    |
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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).

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Fig. 2. Correspondance analysis. Position of 51 samples (A1, etc.: see Tab. 1) in relation to axes 1 and 2, with indication of the modalities of quantitative (DTL, STY, QUL, ANQ, and PRO) and qualitative (ARO and INC) criteria.
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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. dihystera 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. dihystera. Only samples C1 and C2 are among the H. dihystera 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 II 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.

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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 four 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. dihystera*: 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 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. dihystera* (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.

In *H. dihystera* 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. dihystera* 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. dihystera* have a y/v pattern, other species, like *H. paracanaxis* (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. dihystera* 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.)

Revue Nématol. 7 (2) : 121-135 (1984)
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 = 142 (9); dorsal gland opening = 8.8 (1.1); excretory pore = 114 (3); 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...
framework 1-3 \( \mu m \) wide, anterior cephalid not seen; posterior cephalid seen in five specimens, 15-16 \( \mu m \) from anterior end. Stylet knobs flattened in sixteen specimens, rarely rounded (Fig. 4E) or indented (Fig. 4F). Excretory pore at about the level of esophageal-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 \( \mu m \) 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 tail region (thirteen specimens). Long (mean = 4 L) and a u pattern on the other side; seven to eleven annules (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, \( s = 1.2 \)), easy to see or indistinct; in the rest of the samples (\( A2 \); \( A4 \); \( A5 \); \( E1 \); \( E2 \); \( E3 \); \( E4 \); \( F1 \); \( F2 \); \( F3 \)) 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 \( \mu m \) long (mean : 2.2 \( \mu m \)), 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 ± 2 × 0.6 = 25.9-28.3 \( \mu m \) against 26-30 \( \mu m \) 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 \( \mu m \)) 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); esophageal glands = 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. 5 P; \( E3 \); \( E4 \)); the lateral field is most often not areolated on body/tail or some transverse striae are present in only a few specimens (\( A2 \); \( A4 \); \( A5 \); \( E1 \); \( E2 \); \( E3 \); \( E4 \); \( F1 \); \( F2 \); \( F3 \); \( F4 \); \( G1 \); \( G2 \); \( G3 \); \( G4 \); \( G5 \); \( G6 \); \( G7 \); \( G8 \); \( G9 \); \( H1 \); \( H2 \); \( H3 \); \( H4 \); \( H5 \); \( H6 \); \( H7 \); \( H8 \); \( H9 \); \( I1 \); \( I2 \); \( I3 \); \( I4 \); \( I5 \); \( I6 \); \( I7 \); \( I8 \); \( I9 \); \( J1 \); \( J2 \); \( J3 \); \( J4 \); \( J5 \); \( J6 \); \( J7 \); \( J8 \); \( J9 \); \( K1 \); \( K2 \); \( K3 \); \( K4 \); \( K5 \); \( K6 \); \( K7 \); \( K8 \); \( K9 \); \( L1 \); \( L2 \); \( L3 \); \( L4 \); \( L5 \); \( L6 \); \( L7 \); \( L8 \); \( L9 \); \( M1 \); \( M2 \); \( M3 \); \( M4 \); \( M5 \); \( M6 \); \( M7 \); \( M8 \); \( M9 \); \( N1 \); \( N2 \); \( N3 \); \( N4 \); \( N5 \); \( N6 \); \( N7 \); \( N8 \); \( N9 \); \( O1 \); \( O2 \); \( O3 \); \( O4 \); \( O5 \); \( O6 \); \( O7 \); \( O8 \); \( O9 \); \( P1 \); \( P2 \); \( P3 \); \( P4 \); \( P5 \); \( P6 \); \( P7 \); \( P8 \); \( P9 \); \( Q1 \); \( Q2 \); \( Q3 \); \( Q4 \); \( Q5 \); \( Q6 \); \( Q7 \); \( Q8 \); \( Q9 \); \( R1 \); \( R2 \); \( R3 \); \( R4 \); \( R5 \); \( R6 \); \( R7 \); \( R8 \); \( R9 \); \( S1 \); \( S2 \); \( S3 \); \( S4 \); \( S5 \); \( S6 \); \( S7 \); \( S8 \); \( S9 \); \( T1 \); \( T2 \); \( T3 \); \( T4 \); \( T5 \); \( T6 \); \( T7 \); \( T8 \); \( T9 \); \( U1 \); \( U2 \); \( U3 \); \( U4 \); \( U5 \); \( U6 \); \( U7 \); \( U8 \); \( U9 \); \( V1 \); \( V2 \); \( V3 \); \( V4 \); \( V5 \); \( V6 \); \( V7 \); \( V8 \); \( V9 \); \( W1 \); \( W2 \); \( W3 \); \( W4 \); \( W5 \); \( W6 \); \( W7 \); \( W8 \); \( W9 \); \( X1 \); \( X2 \); \( X3 \); \( X4 \); \( X5 \); \( X6 \); \( X7 \); \( X8 \); \( X9 \); \( Y1 \); \( Y2 \); \( Y3 \); \( Y4 \); \( Y5 \); \( Y6 \); \( Y7 \); \( Y8 \); \( Y9 \); \( Z1 \); \( Z2 \); \( Z3 \); \( Z4 \); \( Z5 \); \( Z6 \); \( Z7 \); \( Z8 \); \( Z9 \);

Diagnosis
\textit{Helicolenchus} with spiral body, hemispherical lips, stylet of about 27 \( \mu m \) (mean values : 25.5-28 \( \mu m \)), medium body length (mean values : 650-775 \( \mu m \)), 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) \(\mu\)m, shorter than indicated in the description (29-33 \(\mu\)m). The tail terminus "slightly upturned, bluntly rounded" is different from shapes observed in _H. pseudorobustus_. The DGO is 10 (s = 1.3) \(\mu\)m from the base of stylet, which is also consistent with measurements of _H. pseudorobustus_. _H. bradys_ is here proposed as a junior synonym of _H. pseudorobustus_.

_Helicotylenchus phalerus_ Anderson, 1974
(Fig. 5 GG-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 or _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 DG0 - stylet. The differences between _H. microlobus_ (8.4 \(\mu\)m, s = 1.9), _H. phalerus_ (10.4 \(\mu\)m, s = 0.7), and the different samples of _H. pseudorobustus_ (from 8.8 to 13 \(\mu\)m) cannot be accepted as diagnostic in view of the great variability observed among samples of _H. pseudorobustus_.
- Tail with "a smaller and less conspicuously scultured 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_.

_H. phalerus_ is here proposed as a junior synonym of _H. pseudorobustus_.

_Helicotylenchus egypiensis_ Tarjan, 1964
(Fig. 6 A-E)

Measurements (in \(\mu\)m)

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).

* _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.
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 I, 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 anterior to esophag-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 terminal 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-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) Andrássy, 1958 (Fig. 6 F-J)

**Measurements** (in μm)

<table>
<thead>
<tr>
<th>Topotypes (n=6)</th>
<th>L = 886 (s = 70)</th>
<th>stylet = 29.9 (1.2)</th>
<th>esophagus = 127 (7)</th>
<th>esophageal glands = 174 (14)</th>
<th>dorsal gland opening = 9.5 (1.9)</th>
<th>excretory pore = 116 (4)</th>
<th>body diameter = 24.3 (3.5)</th>
<th>tail length = 39.9 (4.3)</th>
<th>anal body diameter = 15.6 (1.3)</th>
<th>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).</th>
</tr>
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**Description**

**Females**: body in G 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 esophag-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. dihystera 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


References


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


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