Morphometric evaluation of 20 *Heterorhabditis* isolates from Puerto Rico[1,2]

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ABSTRACT

From November 1992 through January 1993 soil samples were collected from different localities of Puerto Rico to isolate entomopathogenic nematodes. Samples were baited with the greater wax moth, *Galleria mellonella*, under laboratory conditions. Nematodes belonging to the genus *Heterorhabditis* were isolated from 20 of the baited samples. The nematodes could not be properly identified to species but were found closely related to *H. bacteriophora* and *H. indicus*. Thirteen morphometric characters of the infective third-stage juvenile were measured for each isolate and subjected to evaluation by Standard Descriptive Statistics, Stepwise Discriminant Analysis, Canonical Discriminant Analysis and Hierarchical Cluster Analysis. Results suggested the presence of a new species of *Heterorhabditis* with sufficient variation among the isolates to separate three different subgroups.

Key words: *Heterorhabditis*, morphometrics, nematode, Puerto Rico.

RESUMEN

Evaluación morfométrica de 20 aislados de *Heterorhabditis* de Puerto Rico

Entre noviembre de 1992 y enero de 1993 se recogieron muestras de suelo de diferentes localidades de Puerto Rico para aislar nematodos entomopatógenos. Las muestras se inocularon con larvas de la alevilla de los apiarios, *Galleria mellonella*, bajo condiciones de laboratorio. De 20 de las muestras inoculadas se aislaron nematodos del género *Heterorhabditis*. La especie de estos nematodos no se pudo identificar adecuadamente, pero se encontró que estaba relacionada taxonómicamente con *H. bacteriophora* y *H. indicus*. Se midieron trece caracteres morfológicos del tercer estadio juvenil de cada aislado y se sometieron a evaluación por Estadistica Descriptiva Estándar, Análisis Discriminante "Stepwise", Análisis Canónico Discriminante y Análisis "Jerárquico de Grupo". Los resultados sugirieron la presencia de una nueva especie de *Heterorhabditis* con una marcada variación entre los aislados como para separar tres subgrupos diferentes.

[1]Received for publication 30 August 1994.

[2]We thank Mrs. C. Chao, Statistics Section, and Mr. M. Antoni, Department of Agricultural Economics and Rural Sociology, Agricultural Experiment Station, University of Puerto Rico, for help in statistical analyses; Dr. Hedwig Hirschmann, Department of Plant Pathology, North Carolina State University, Raleigh, NC, for help in manuscript review; and Dr. G. Poinar, Department of Entomological Sciences, University of California, Berkeley, CA, for review of the morphometrical data.

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INTRODUCTION

In 1975 Poinar erected the family Heterorhabditidae with *Heterorhabditis bacteriophora* as the type species (Poinar, 1975). At present the family is composed of four species: *H. bacteriophora* Poinar (23 strains); *H. zealandica* Poinar; *H. megidis* Poinar, Jackson, and Klein; and *H. indicus* Poinar (Poinar, 1992). The species *H. heliothidis* (=*Cromonema heliothidis* Khan, Brooks, and Hirschmann) (Khan et al., 1976) was synonymized with *H. bacteriophora* and *H. zealandica* was erected as synonym of a New Zealand population of *H. heliothidis* sensu Wouts by Poinar (Poinar, 1990).

Two keys have been published for the identification of the infective third-stage juveniles of *Heterorhabditis*. In the first key, the total body length and pharynx length were used to separate the species (Poinar, 1990). The second key included two additional discriminating characters: Ratio E (distance from head to excretory pore divided by tail length) and Ratio F (body width divided by tail length) (Poinar, 1992).

Keys based on measurements of morphological characters do not always provide a clear discrimination among species. Nevertheless, morphology and morphometrics have been widely used for identification purposes (Hirschmann and Rammah, 1993; Norton and Hinz, 1992; Poinar, 1979; Román and Hirschmann, 1969). In the search for more relevant information that could define taxonomically useful characters to separate species or species groups, taxonomists have subjected morphometrical data to different statistical analyses, such as standard descriptive statistics (SDS), stepwise discriminant analysis (SDA), cluster analysis (CA), and canonical discriminant analysis (CDA). Some of the multivariate tests study the relationship between a group of variables for assessing relative similarities. Different authors have used CDA and hierarchical cluster analysis (HCA) in an effort to divide genera of nematodes into species or taxonomically related groups (Cho and Robbins, 1991; Georgi, 1988; Mota and Eisenback, 1993; Pantone et al., 1987). Most taxonomists accept that a sound discrimination of species and races can be obtained only when the above tools are used in combination with gel electrophoresis (Akhurst, 1987; Huettle et al., 1983; Hussey, 1979) and DNA fingerprinting (Curran, 1990; Poinar et al., 1987).

The present work was undertaken to determine the species, races or strains of *Heterorhabditis* present in Puerto Rico and to assess the morphological variation among 20 isolates by subjecting the obtained morphometrical data to SDS, SDA, HCA, and CDA.

MATERIALS AND METHODS

Soil samples collected from different localities of Puerto Rico were processed for the isolation of entomopathogenic nematodes. Nematodes obtained were maintained in the laboratory following the methodology described by Figueroa et al. (1993). Twenty of the isolates collected were identified as belonging to the genus *Heterorhabditis* (Poinar, 1990): Isolates 1 and 2 from Catano, 3 and 20 from Piñones, 4 from Río Piedras, 5 from Loiza, 6 and 7 from Río Grande, 8 from Palmer, 9 to 12 from El Yunque, 13 from Luquillo, 14 and 15 from Ceiba, 16 from Patillas, 17 from Manatí, 18 from Adjuntas and 19 from Villalba. Ensheathed, third-stage juveniles (J_3) of these isolates were subjected to a morphometric study. The kinds of characters and ratios used were the same as those employed by Poinar (Poinar, 1990; 1992). In addition, the tail cuticle length (distance between tail tip of third-stage juvenile and second-stage cuticle) was included (Table 1).

Ensheathed infective third-stage juveniles were killed by gentle heat and mounted in water on glass slides. Specimens were examined and measured within a six-hour period by using a light microscope with a calibrated camera lucida attachment.

The data were subjected to different statistical analyses. The mean, standard deviation of the mean, and the range were calculated. Also Duncan's Multiple Range Test, SDA, HCA, and CDA were performed with SAS User's Guide (SAS Institute Inc, 1982).

RESULTS

Standard Descriptive Statistics (SDS):

Table 1 presents morphometrics of 13 characters of 20 isolates of *Heterorhabditis*. Evaluation of these measurements by SDS revealed their limited use for differentiating the isolates. The majority of the characters overlapped. Only two cases were found with no overlapping; isolates 15 and 19 did not overlap in their greatest width, nor did isolates 18 and 19 in their Ratio F value.

Mean values of certain characters were found to be statistically significant. Isolate number 9 was significantly longer than all other isolates. Isolate number 19 had a significantly greater body width, Ratio D and Ratio F than all other isolates but a significantly smaller Ratio A. Isolate 20 had a significantly greater width and Ratio F value than all the other isolates except isolate 19. The smallest Ratio F value was found in isolate 18.

TABLE 1.—*Morphometric comparison (μm) of 20 isolates of third-stage juveniles of* Heterorhabditis *spp.*

Character	Population number (N = 25)				
	1	2	3	4	5
Body Length	537 ± 21.14i (495 - 571)	560 ± 15.75cdefgh (529 - 590)	550 ± 33.40ghi (490 - 605)	543 ± 24.43hi (495 - 586)	550 ± 18.15ghi (510 - 586)
Greatest Width	20 ± 1.21fgh (18 - 23)	20 ± 0.82fgh (19 - 22)	19 ± 1.22ghij (18 - 23)	20 ± 1.31fghi (18 - 24)	20 ± 1.45efg (18 - 24)
Dist. Head to Ex. Pore	93 ± 5.54g (81 - 102)	95 ± 3.39efg (88 - 100)	98 ± 6.57bcdef (86 - 112)	96 ± 5.37defg (88 - 110)	95 ± 3.81efg (86 - 102)
Dist. Head to Nerve Ring	80 ± 4.67fg (69 - 90)	80 ± 3.74efg (71 - 88)	80 ± 5.45fg (68 - 98)	81 ± 591defg (69 - 93)	81 ± 3.57cdefg (74 - 90)
Dist. Head to Pharynx base	111 ± 5.83cdef (100 - 119)	110 ± 5.50ef (100 - 119)	111 ± 6.79def (98 - 124)	113 ± 3.99bcdef (105 - 119)	112 ± 5.38bcdef (98 - 119)
Total Tail Length	94 ± 8.41ghi (62 - 105)	99 ± 3.73bcd (92 - 106)	94 ± 3.97ghi (86 - 103)	90 ± 4.43j (78 - 96)	95 ± 6.97fghi (84 - 112)
Tail Cuticle Length	33 ± 3.80defg (24 - 40)	36 ± 2.59bcd (30 - 41)	33 ± 3.51efgh (26 - 40)	29 ± 3.22i (24 - 36)	32 ± 5.17fgh (24 - 42)
Ratio A[1]	27 ± 1.51def (24 - 30)	28 ± 1.26abc (26 - 31)	28 ± 1.69abc (26 - 33)	28 ± 1.60cd (24 - 30)	27 ± 1.46ed (24 - 31)
Ratio B[2]	4.8 ± 0.23cdef (4.4 - 5.3)	5.1 ± 0.30ab (4.8 - 5.7)	5.0 ± 0.23abcde (4.6 - 5.4)	4.8 ± 0.22def (4.4 - 5.2)	4.9 ± 0.31bcdef (4.5 - 5.8)
Ratio C[3]	5.9 ± 0.82abcdefg (5.2 - 8.6)	5.7 ± 0.19ghi (5.4 - 6.1)	5.8 ± 0.29bcdefg (5.4 - 6.6)	6.0 ± 0.20abc (5.6 - 6.5)	5.8 ± 0.39bcdefg (5.0 - 6.5)
Ratio D[4]	0.84 ± 0.04fgh (0.77 - 0.92)	0.86 ± 0.03bcdef (0.78 - 0.93)	0.88 ± 0.03b (0.82 - 0.94)	0.85 ± 0.04cdefg (0.80 - 0.94)	0.85 ± 0.03defgh (0.77 - 0.90)
Ratio E[5]	1.02 ± 0.14de (0.86 - 1.42)	0.96 ± 0.05fgh (0.88 - 1.09)	1.04 ± 0.07abcd (0.93 - 1.21)	1.07 ± 0.07ab (0.94 - 1.28)	1.01 ± 0.09def (0.85 - 1.17)
Ratio F[6]	0.22 ± 0.03cd (0.19 - 0.32)	0.20 ± 0.01h (0.18 - 0.23)	0.21 ± 0.01efgh (0.18 - 0.24)	0.22 ± 0.01cd (0.20 - 0.26)	0.21 ± 0.02cdefg (0.18 - 0.26)

Values are Means. ± S.D., (Range). Means followed by the same letter within a row are not different according to the Duncan's Multiple Range Test (P = 0.05).
[1]Body length/Greatest width. [2]Body length/Dist. head to pharynx base. [3]Body length/Total tail length. [4]Dist. head to Ex. pore/Dist. head to pharynx base. [5]Dist. head to Ex. pore/Total tail length. [6]Greatest width/Total tail length.

TABLE 1.—*Morphometric comparison (μm) of 20 isolates of third-stage juveniles of* Heterorhabditis *spp. (Cont.)*

Character	Population number (N = 25)				
	6	7	8	9	10
Body Length	569 ± 23.57bcde (524 - 610)	568 ± 34.92bcdef (495 - 638)	560 ± 21.38cdefgh (529 - 605)	595 ± 32.95a (514 - 662)	553 ± 19.42efghi (514 - 586)
Greatest Width	21 ± 1.53de (19 - 24)	21 ± 1.58ef (18 - 24)	20 ± 1.08ef (19 - 23)	22 ± 2.16c (19 - 26)	19 ± 0.87hij (18 - 22)
Dist. Head to Ex. Pore	98 ± 7.22bcdef (83 - 117)	98 ± 4.20bcde (93 - 107)	95 ± 3.95fg (88 - 102)	101 ± 5.64ab (93 - 117)	96 ± 3.78def (90 - 105)
Dist. Head to Nerve Ring	86 ± 6.12a (74 - 102)	82 ± 4.67bcdef (76 - 93)	82 ± 3.35bcdefg (71 - 88)	84 ± 3.15abc (79 - 90)	80 ± 3.29efg (74 - 86)
Dist. Head to Pharynx base	113 ± 9.17bcdef (83 - 131)	115 ± 5.45abc (102 - 126)	110 ± 5.38f (100 - 121)	117 ± 4.69a (107 - 125)	114 ± 3.24abcd (110 - 121)
Total Tail Length	103 ± 5.37a (94 - 118)	99 ± 4.85cd (88 - 106)	102 ± 5.64ab (94 - 115)	101 ± 5.44abc (92 - 116)	93 ± 3.48ij (84 - 98)
Tail Cuticle Length	39 ± 4.36a (30 - 48)	35 ± 3.54cdef (28 - 42)	38 ± 5.24ab (30 - 52)	37 ± 5.18abc (30 - 48)	31 ± 3.03hi (24 - 36)
Ratio A[1]	27 ± 1.51def (25 - 30)	28 ± 1.89bcd (24 - 31)	28 ± 1.71cd (24 - 30)	27 ± 1.89def (25 - 31)	29 ± 1.41ab (25 - 31)
Ratio B[2]	5.1 ± 0.52ab (4.3 - 7.2)	4.9 ± 0.30abcde (4.4 - 5.7)	5.1 ± 0.25ab (4.6 - 5.5)	5.1 ± 0.29a (4.3 - 5.8)	4.8 ± 0.17cdef (4.6 - 5.1)
Ratio C[3]	5.5 ± 0.30hij (5.1 - 6.1)	5.8 ± 0.29defg (5.3 - 6.3)	5.5 ± 0.32ij (4.9 - 6.1)	5.9 ± 0.44abcdefg (5.2 - 6.6)	6.0 ± 0.26abcd (5.5 - 6.5)
Ratio D[4]	0.87 ± 0.07bcd (0.77 - 1.18)	0.86 ± 0.03cdefg (0.80 - 0.93)	0.86 ± 0.03bcde (0.81 - 0.95)	0.84 ± 0.06efgh (0.71 - 0.94)	0.84 ± 0.03defgh (0.80 - 0.89)
Ratio E[5]	0.95 ± 0.08gh (0.81 - 1.17)	1.00 ± 0.04defg (0.91 - 1.08)	0.94 ± 0.06h (0.79 - 1.06)	0.97 ± 0.08fgh (0.86 - 1.19)	1.04 ± 0.04abcd (0.97 - 1.11)
Ratio F[6]	0.20 ± 0.02fgh (0.18 - 0.24)	0.21 ± 0.02defgh (0.18 - 0.25)	0.20 ± 0.01h (0.17 - 0.23)	0.22 ± 0.02cde (0.18 - 0.25)	0.21 ± 0.01efgh (0.19 - 0.24)

Values are Means, ± S.D., (Range). Means followed by the same letter within a row are not different according to the Duncan's Multiple Range Test (P = 0.05).
[1]Body length/Greatest width. [2]Body length/Dist. head to pharynx base. [3]Body length/Total tail length. [4]Dist. head to Ex. pore/Dist. head to pharynx base. [5]Dist. head to Ex. pore/Total tail length. [6]Greatest width/Total tail length.

TABLE 1.—*Morphometric comparison (μm) of 20 isolates of third-stage juveniles of* Heterorhabditis *spp. (Cont.)*

Character	Population number (N = 25)				
	11	12	13	14	15
Body Length	567 ± 26.22bcdefg (529 - 629)	572 ± 22.59bcd (519 - 619)	546 ± 34.38hi (476 - 600)	555 ± 28.38defghi (500 - 610)	544 ± 28.90hi (486 - 581)
Greatest Width	19 ± 0.86ghij (18 - 22)	21 ± 1.71de (19 - 24)	20 ± 1.78ef (18 - 23)	19 ± 0.81ghij (18 - 22)	19 ± 0.65j (18 - 20)
Dist. Head to Ex. Pore	97 ± 3.50cdef (90 - 105)	100 ± 3.99abc (93 - 107)	98 ± 6.68bcdef (86 - 112)	97 ± 4.60def (86 - 105)	97 ± 3.92cdef (88 - 105)
Dist. Head to Nerve Ring	81 ± 2.89defg (74 - 88)	83 ± 3.89bcde (74 - 90)	83 ± 5.80bcde (74 - 98)	83 ± 4.28abcd (71 - 90)	82 ± 3.63bcdefg (71 - 88)
Dist. Head to Pharynx base	115 ± 3.62abc (107 - 121)	114 ± 3.23abcde (107 - 119)	112 ± 6.89cdef (95 - 124)	112 ± 5.37cdef (95 - 119)	113 ± 3.56bcdef (105 - 119)
Total Tail Length	94 ± 4.45hi (86 - 104)	98 ± 3.15de (90 - 104)	95 ± 5.74efghi (84 - 104)	97 ± 5.33defg (90 - 109)	90 ± 5.06j (78 - 98)
Tail Cuticle Length	32 ± 4.18gh (24 - 42)	34 ± 3.06defg (30 - 41)	32 ± 4.22fgh (23 - 40)	34 ± 3.62cdefg (28 - 43)	29 ± 3.47i (24 - 36)
Ratio A[a]	29 ± 1.46a (25 - 33)	27 ± 1.85de (24 - 31)	26 ± 1.56ef (23 - 29)	29 ± 1.42ab (26 - 31)	29 ± 1.35a (26 - 31)
Ratio B[b]	5.0 ± 0.20abcde (4.7 - 5.3)	5.0 ± 0.18abcd (4.7 - 5.4)	5.0 ± 0.37bcdef (4.2 - 6.0)	5.0 ± 0.25abcde (4.4 - 5.4)	4.8 ± 0.21ef (4.3 - 5.3)
Ratio C[c]	6.0 ± 0.25ab (5.4 - 6.5)	5.8 ± 0.22bcdefg (5.4 - 6.4)	5.8 ± 0.30efg (5.0 - 6.5)	5.7 ± 0.27fghi (5.2 - 6.1)	6.1 ± 0.24a (5.6 - 6.8)
Ratio D[d]	0.85 ± 0.03defgh (0.80 - 0.92)	0.88 ± 0.04b (0.80 - 1.00)	0.88 ± 0.03bc (0.80 - 0.94)	0.86 ± 0.04bcd (0.79 - 0.94)	0.86 ± 0.03cdefg (0.77 - 0.89)
Ratio E[e]	1.04 ± 0.05abcd (0.92 - 1.14)	1.02 ± 0.04cde (0.94 - 1.11)	1.03 ± 0.07bcd (0.90 - 1.22)	1.00 ± 0.06defg (0.88 - 1.11)	1.08 ± 0.07a (0.90 - 1.22)
Ratio F[f]	0.21 ± 0.01efgh (0.18 - 0.23)	0.22 ± 0.02cdef (0.19 - 0.25)	0.22 ± 0.02cdefg (0.18 - 0.26)	0.20 ± 0.01h (0.18 - 0.24)	0.21 ± 0.01defgh (0.19 - 0.24)

Values are Means, ± S.D., (Range). Means followed by the same letter within a row are not different according to the Duncan's Multiple Range Test (P = 0.05).
[a]Body length/Greatest width. [b]Body length/Dist. head to pharynx base. [c]Body length/Total tail length. [d]Dist. head to Ex. pore/Dist. head to pharynx base. [e]Dist. head to Ex. pore/Total tail length. [f]Greatest width/Total tail length.

TABLE 1.—*Morphometric comparison (µm) of 20 isolates of third-stage juveniles of* Heterorhabditis *spp. (Cont.)*

Character	Population number (N = 25)				
	16	17	18	19	20
Body Length	568 ± 25.48bcde (529 - 619)	541 ± 29.09i (495 - 586)	550 ± 18.16fghi (514 - 586)	574 ± 45.27bc (457 - 652)	579 ± 26.86b (524 - 629)
Greatest Width	22 ± 1.81cd (18 - 24)	20 ± 1.10fghi (18 - 22)	19 ± 0.78ij (18 - 22)	25 ± 1.84a (21 - 29)	24 ± 1.97b (19 - 29)
Dist. Head to Ex. Pore	96 ± 7.07efg (83 - 119)	95 ± 4.24efg (88 - 105)	95 ± 4.67fg (86 - 107)	103 : 7.13a (86 - 121)	99 ± 4.53bcd (90 - 107)
Dist. Head to Nerve Ring	83 ± 6.53bcde (71 - 105)	79 ± 3.43g (74 - 90)	79 ± 4.23fg (74 - 90)	84 ± 4.93ab (71 - 93)	81 ± 4.32bcdefg (74 - 90)
Dist. Head to Pharynx base	111 ± 7.64cdef (100 - 136)	114 ± 4.42abcde (105 - 121)	115 ± 3.74abc (107 - 121)	112 ± 6.70cdef (95 - 124)	116 ± 4.32ab (110 - 124)
Total Tail Length	98 ± 3.87de (89 - 106)	94 ± 4.57ghi (86 - 103)	101 ± 3.22abc (95 - 108)	97 ± 5.31defgh (86 - 107)	97 ± 4.42def (90 - 106)
Tail Cuticle Length	34 ± 4.17defg (26 - 42)	33 ± 3.58fgh (26 - 40)	35 ± 2.90bcde (30 - 42)	32 ± 3.56fgh (26 - 42)	34 ± 3.58defg (27 - 40)
Ratio A[1]	26 ± 1.88f (23 - 30)	28 ± 1.98cd (23 - 31)	29 ± 1.41a (24 - 31)	23 ± 1.62h (20 - 26)	25 ± 1.80g (21 - 30)
Ratio B[2]	5.1 ± 0.30a (4.6 - 5.7)	4.7 ± 0.27f (4.2 - 5.3)	4.8 ± 0.16ef (4.6 - 5.3)	5.1 ± 0.58a (3.9 - 6.9)	5.0 ± 0.20abc (4.7 - 5.4)
Ratio C[3]	5.8 ± 0.33cdefg (5.3 - 6.6)	5.7 ± 0.26efgh (5.1 - 6.2)	5.4 ± 0.17j (5.0 - 5.7)	5.9 ± 0.34abcdef (5.1 - 6.5)	6.0 ± 0.38abcde (5.4 - 6.7)
Ratio D[4]	0.86 ± 0.03bcdef (0.79 - 0.93)	0.83 ± 0.03gh (0.76 - 0.89)	0.82 ± 0.03h (0.74 - 0.90)	0.92 ± 0.04a (0.86 - 1.00)	0.85 ± 0.05cdefg (0.65 - 0.91)
Ratio E[5]	0.98 ± 0.09efgh (0.86 - 1.23)	1.01 ± 0.06def (0.90 - 1.16)	0.93 ± 0.04h (0.86 - 1.04)	1.06 ± 0.08abc (0.86 - 1.21)	1.01 ± 0.08def (0.77 - 1.17)
Ratio F[6]	0.22 ± 0.02c (0.19 - 0.27)	0.20 ± 0.02gh (0.13 - 0.24)	0.19 ± 0.01i (0.18 - 0.20)	0.26 ± 0.02a (0.23 - 0.29)	0.24 ± 0.02b (0.20 - 0.30)

Values are Means, ± S.D., (Range). Means followed by the same letter within a row are not different according to the Duncan's Multiple Range Test (P = 0.05).
[1]Body length/Greatest width. [2]Body length/Dist. head to pharynx base. [3]Body length/Total tail length. [4]Dist. head to Ex. pore/Dist. head to pharynx base. [5]Dist. head to Ex. pore/Total tail length. [6]Greatest width/Total tail length.

Stepwise Discriminant Analysis (SDA):

The 13 morphometric characters were entered in the SDA (forward and backward selection methods) to determine the variables or diagnostic characters that accounted for most of the variation of the data. The forward version of the SDA selected 10 variables; the backward, 12. For this study the four variables with the highest Eigenvalues and the highest correlation in decreasing significance were selected for further analyses (Tables 2 and 3). These correlations were significant at $P = 0.01$. The canonical variables constituted 1) body length, 2) greatest width, 3) distance from head to pharynx base, and 4) tail length. The first and second canonical variables accounted for 88% of the total variance (Table 2). The third and fourth added little to the variance in the data. Nevertheless, they were selected because of their importance in the taxonomy of the genus.

Hierarchical Cluster Analysis (HCA):

Input variables for HCA were the same four variables as obtained by SDA. Three subgroups were indicated in the dendrogram according to the HCA (Figure 1). Subgroup 1 included 10 isolates in three divisions. Isolates 1, 3, 5, 13, and 14 formed the first division. Isolates 15, 4, 17, and 10 formed the second division, which was clustered with the first. Isolate 11 composed the third division clustered with the former two divisions. Subgroup 2 included seven isolates in two divisions. Isolates 2, 8, and 16 formed the first division clustered with isolates 6, 7, 12, and 18 in the second division. Subgroup 2 was clustered with subgroup 1. Subgroup 3 included isolates 9, 20 and 19 clustered with the other two subgroups.

Canonical Discriminant Analysis (CDA):

Figures 2 through 5 present scatterplots of canonical means of 20 isolates of *Heterorhabditis*.

TABLE 2.—*Eigenvalues of the correlation matrix generated during Stepwise Discriminant Analysis of* Heterorhabditis *isolates.*

Canonical Variable[1]	Eigenvalue	Difference	Proportion	Cumulative
C 1	1.28	0.76	0.62	0.62
C 2	0.53	0.36	0.26	0.88
C 3	0.17	0.09	0.08	0.96
C 4	0.08	—	0.04	1.00

[1]C 1 = Total length, C 2 = Greatest width, C 3 = Distance from head to pharynx base, C 4 = Tail length.

TABLE 3.—*Correlation coefficients among the variables chosen by Stepwise Discriminant Analysis.*

	T L[1]	G W	D H Ph
G W	0.5403		
D H Ph	0.3471	0.1169	
T	0.4789	0.2703	0.0897

[1]T L = Total length, G W = Greatest width, D H Ph = Distance from head to pharynx base, T = Tail length.

Input variables for CDA were the same four variables as obtained by SDA.

When the means of the first and second canonical variables were plotted on canonical axes, and borderlines following results of HCA traced, the three principal subgroups of isolates observed in the HCA were delimited (Figure 2). Subgroup 1 was composed of 10 isolates (1, 3, 4, 5, 10, 11, 13, 14, 15, and 17). Subgroup 2 comprised six isolates (2, 6, 7, 8, 12, and 16) and subgroup 3 comprised three isolates (9, 19, and 20).

Similarly, when means of the first and fourth canonical variables (Figure 3), second and fourth variables (Figure 4) and second and third variables (Figure 5) were plotted on canonical axes, the same three subgroups were delimited. Isolate 18 was found encompassed within

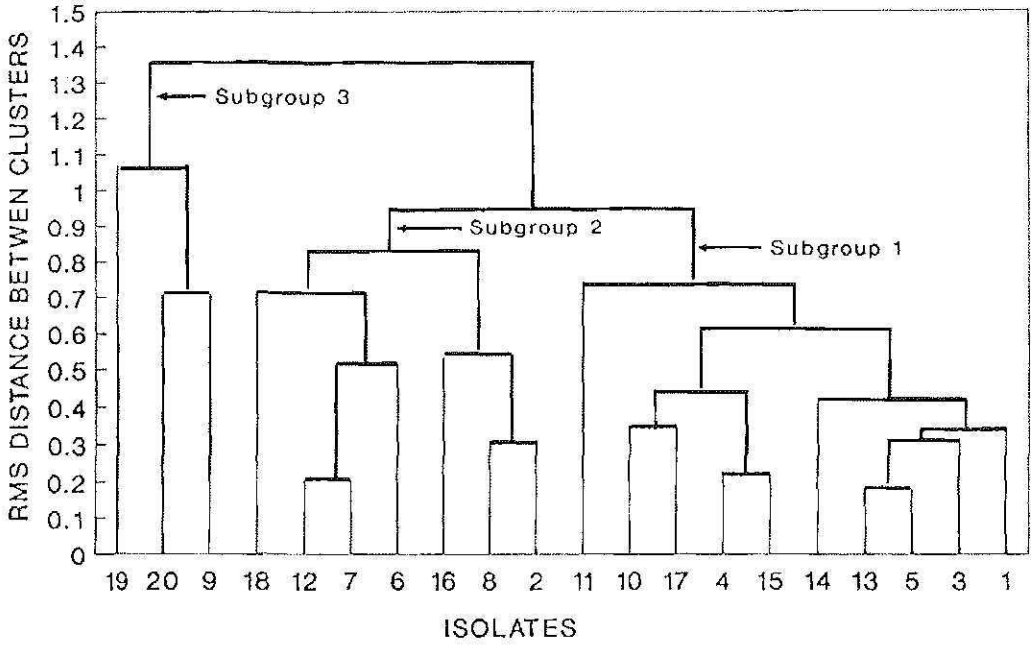

FIGURE 1. Dendrogram showing the clustering of 20 Puerto Rican isolates of *Heterorhabditis* based on mean values of four morphometric variables of J_3.

subgroup 2, when the means of the first and fourth canonical variables and of the second and fourth variables were plotted on canonical axes (Figures 3 and 4). Otherwise, this isolate was observed away from subgroup 2 (Figures 2 and 5).

DISCUSSION

The morphometric data obtained in this study indicate that the 20 isolates of *Heterorhabditis* collected from Puerto Rico belong to a new species closely related to *H. bacteriophora* and *H. indicus*.[5] In spite of the fact that morphometrics supported the existence of a new species, the variability in dimensions among the 20 isolates was great, and much overlapping occurred within each character. The SDS separated isolates 9, 18, 19, and 20 as significantly different from each other on the basis of certain morphometric characters (Table 1). Results of the HCA (Figure 1) and CDA (Figures 2 through 5) support these observations.

The forward and backward versions of the SDA selected 10 and 12 variables, respectively. Such a large number of variables, when used in

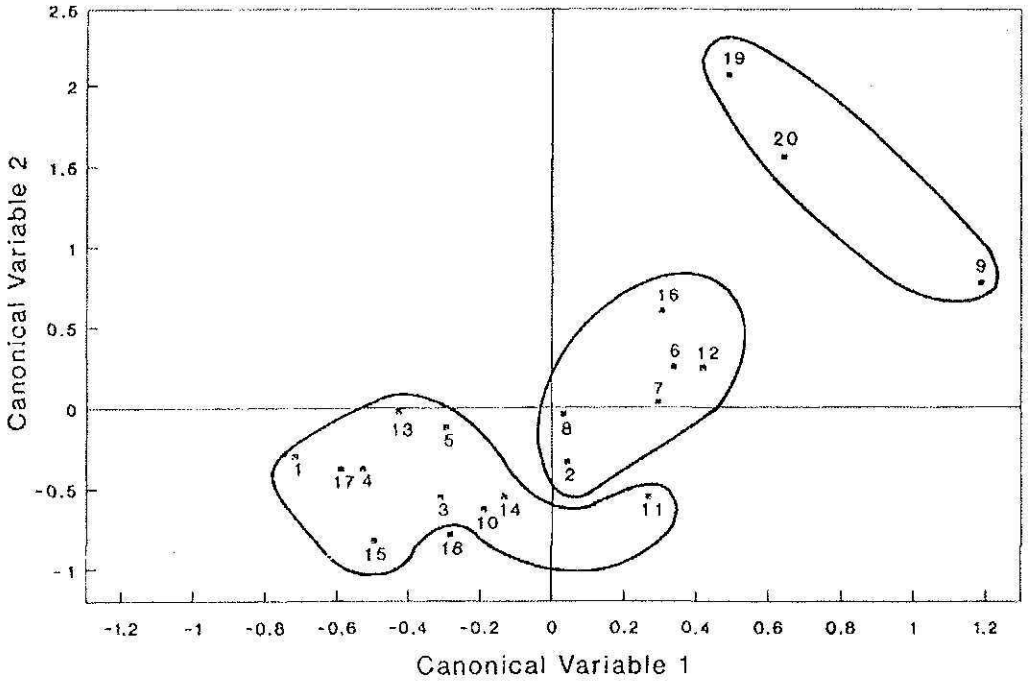

FIGURE 2. Scatterplots of J_3 of 20 isolates of *Heterorhabditis* on the first and second canonical axes with three subgroups circled. Population 18 appears away from subgroup 2.

[5]This species could not be identified by using existing keys, and was also confirmed as new by G. O. Poinar (personal communication).

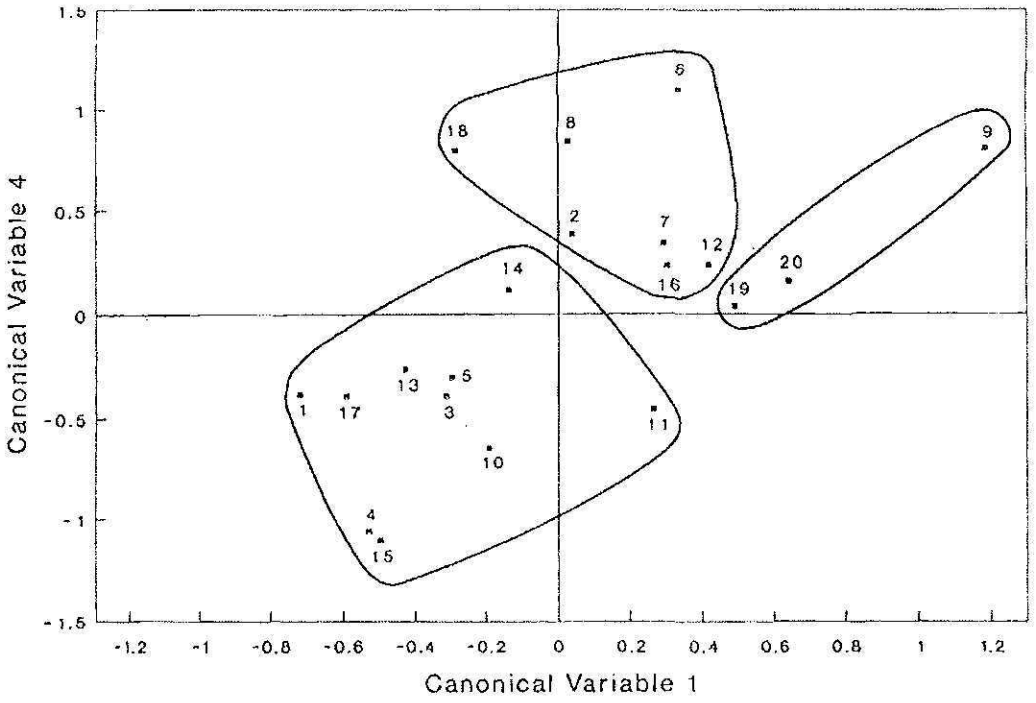

FIGURE 3. Scatterplots of J_3 of 20 isolates of *Heterorhabditis* on the first and fourth canonical axes with three subgroups circled.

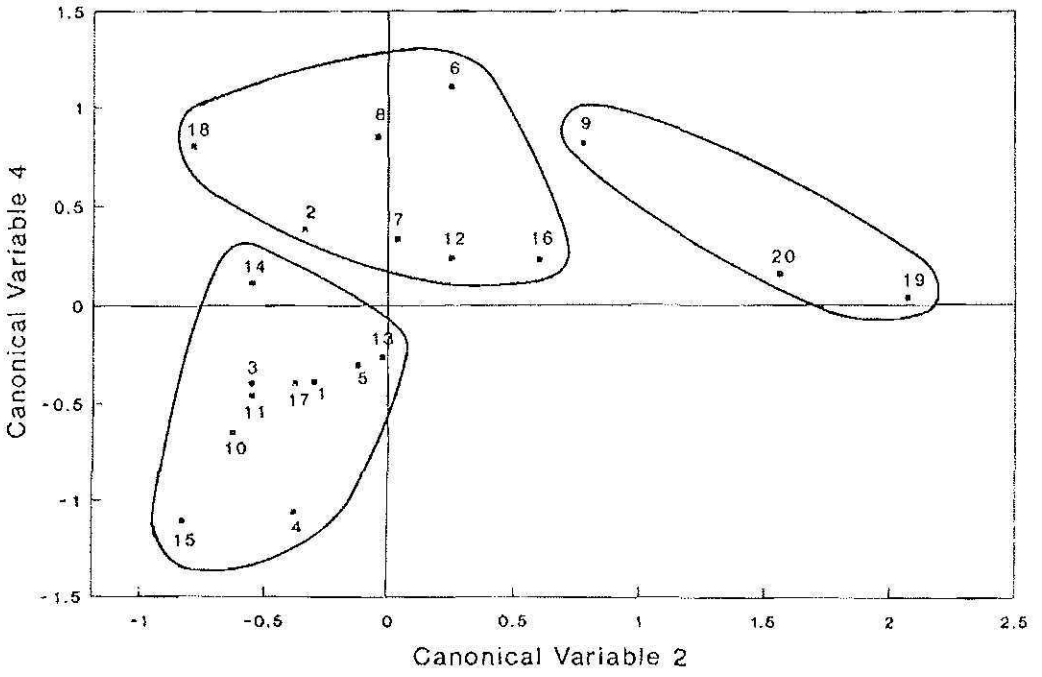

FIGURE 4. Scatterplots of J_3 of 20 isolates of *Heterorhabditis* on the second and fourth canonical axes with three subgroups circled.

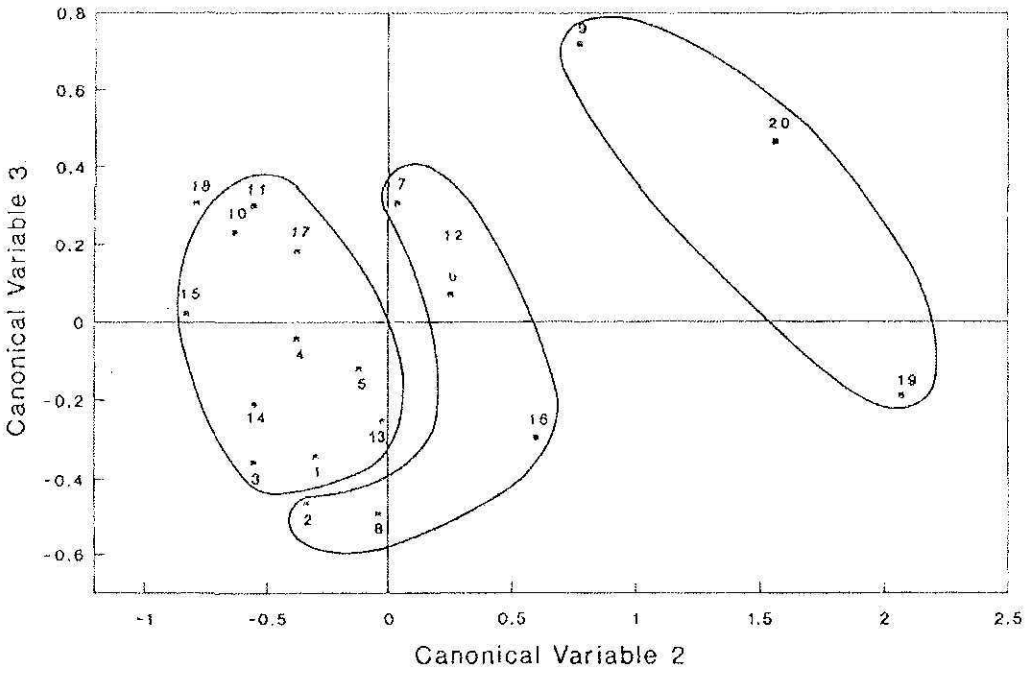

FIGURE 5. Scatterplots of J_3 of 20 isolates of *Heterorhabditis* on the second and third canonical axes with three subgroups circled. Population 18 appears away from subgroup 2.

the canonical analysis, could not be plotted in polygonals to demarcate the range of each isolate as Hirschmann and Rammah (1993) did for eight populations of *Meloidogyne arenaria*. Too much overlapping occurred among the various isolates of *Heterorhabditis*. Plotting polygonals using the four canonical variables selected (Table 2) was also confusing. We found that scatterplots with canonical means could best illustrate the relationship among the isolates. Scatterplots of canonical means have been previously used by other authors to separate populations of certain species (Cho and Robbins, 1991; Georgi, 1988; Lamberti and Ciancio, 1993).

Results of the HCA and the CDA were fairly consistent. The three subgroups delimited by HCA (Figure 1) were also observed in the scatterplots of the CDA (Figures 2 through 5) especially when borderlines were traced following the results obtained by the HCA. Subgroup 3, composed of isolates 9, 19, and 20, was the most consistent of the three. It appeared well demarcated by the HCA (Figure 1). Lamberti and Ciancio (1993) separated 49 populations belonging to the *Xiphinema americanum* group into five subgroups by HCA and Principal Component Analysis. Hirschmann and Rammah (1993) conducted a morphometric study of the females, males, and J_2 of hypotriploid and triploid populations of *M. arenaria* with SDS, SDA, and CA. They dem-

onstrated that in each life stage, the hypotriploid populations were set off to varying degrees from the triploid populations. In our study of *Heterorhabditis*, it appeared that subgroup 3 is distinctly demarcated in all four CDA scatterplots but more clearly so in two of them (Figures 2 and 5). Certain isolates, especially 11 and 18, were usually set off at the borderlines of subgroups 1 and 2, respectively, indicating relationship or dissimilarity with the three subgroups plotted (Figures 1 through 5). The location of the isolates on scatterplots depended on the contribution made by each canonical variable. Cho and Robbins (1991) separated three groups and four populations belonging to four different species from 23 *X. americanum* sensu lato populations by using CDA scatterplots. Georgi (1988) employed CDA to investigate morphological variations in *X. americanum* and correlated them with regional differences. The latter study failed to separate mixed species clearly.

The information obtained in the present study indicates the existence of a new species of *Heterorhabditis* from Puerto Rico which appears closely related to *H. bacteriophora* and *H. indicus*. Our work also showed a large amount of variability among the isolates studied. It is difficult to determine at present whether or not the observed variability is an indication of the presence of other species. Studies underway on polyacrilamide gel electrophoresis should elucidate this problem.

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