Variation among Populations of 
Belonolaimus longicaudatus

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Abstract: Three North Carolina populations of Belonolaimus longicaudatus differed significantly from three Georgia populations in stylet measurements, the c ratio, the distance of the excretory pore from the anterior end for both sexes; the a ratio for females only; and the total body length, tail length, and spicule length for males only. The Georgia nematodes were stouter, and the females possessed sclerotized vaginal pieces. The distal portion of the spicules of North Carolina males had an indentation and hump lacking in those of the Georgia males. The haploid number of chromosomes was eight for males from all populations of B. longicaudatus and a North Carolina population of B. maritimus. Interpopulation matings of the Tarboro, N.C. and Tifton, Ga. populations indicated that the offspring produced were infertile. Morphological differences and reproductive isolation suggest that the North Carolina and the Georgia populations belong to different species. Key Words: morphology, cytology, hybridization, Belonolaimus maritimus, populations.

The sting nematode, Belonolaimus longicaudatus Rau, an important plant pathogen, has an extensive host range and inflicts economic losses on a wide variety of crops (3, 6, 8, 9, 15). Previous investigations suggest that there are several pathotypes or physiological races (1, 14, 15). This nematode has been reported to be pathogenic on peanut in North Carolina and Virginia (9, 17, 18). In Georgia, however, it did not cause injury or reproduce well on peanuts (5). A population of B. longicaudatus from Gainesville, Florida did not cause damage or reproduce well on peanut, whereas a population from Sanford, Florida did (10).

Morphological differences reported between B. longicaudatus populations involve the following characters: average stylet length, degree of lip constriction, tail shape, presence or absence of sclerotized pieces in the vagina, stylet length/tail length ratio, a ratio, b ratio, and number of tail annules (1, 7, 11, 12, 13, 14).

The purpose of this study was to determine whether significant differences in morphology exist between the North Carolina and Georgia populations of B. longicaudatus. In addition, possible differences in chromosome number, interspecies and interpopulation hybridization, as well as fecundity of the resulting offspring were investigated. The North Carolina and Georgia populations were chosen because of their reported difference in pathogenicity on peanuts. B. maritimus was included in these studies for comparative purposes.

MATERIALS AND METHODS

The six Belonolaimus longicaudatus populations studied were from the following sources: a greenhouse population originally obtained from a cotton field near Tarboro, N.C. (designated “NC-1”), a corn field near Dudley, N.C. (“NC-2”), a peanut field near Sevier, N.C. (“NC-3”), a greenhouse population provided by Dr. A. W. Johnson, and obtained from Tifton, Ga. (“G-1”), a population from agronomy field number 15, Tifton, Ga. (“G-2”), and a field population from Fulwood, Ga. (“G-3”). The population of B. maritimus Rau (designated “Bm”) was obtained from American beach grass, Ammophila breviligulata Fernald, collected on Ocracoke Island, N.C.

All nematode populations were greenhouse-propagated at approximately 25 C. B. longicaudatus was increased on strawberry, Fragaria virginiana Duch. 'Earlibelle' for quantitative morphological studies, on soybean, Glycine max (L.) Merr. 'Lee' for cytological and qualitative morphological studies. B. maritimus was propagated on pearl millet, Pennisetum glaucum (L.) R. Br. 'Starr' for morphological studies.

Isolation of nematodes from soil was by a sugar-flotation-sieving method (2). Morphology: Selected quantitative morphological characters of females, males, second-stage larvae, and eggs were measured from each of the six populations of B.
longicaudatus and one population of B. maritimus. Females and males were killed by gentle heat, fixed in FAA (20), transferred to glycerin by the rapid method of Seinhorst (19) and permanently mounted in glycerin before they were measured. Viable eggs (one- to eight-celled stages), obtained from hand-picked, gravid females, and freshly hatched second-stage larvae killed by gentle heat, were measured in water mounts. Separate analyses of variance were performed for each character measured; Duncan's multiple range test values were also calculated.

For qualitative morphological comparisons between the different populations of B. longicaudatus, specimens were killed by gentle heat, mounted in water and examined immediately.

Cytology: Chromosomal counts were made of each of the six populations of B. longicaudatus and of B. maritimus. Late fourth-stage males and males undergoing the fourth molt were hand-picked from freshly extracted samples. Entire nematode body contents, including reproductive systems, were smeared on slides. Smears were subsequently fixed and stained using the propionic orcein method (21). Specimens were made permanent by the quick-freeze method (4) and mounted in Euparal (21).

Matings: The ability of the NC-I and G-I populations of B. longicaudatus to reproduce without males was tested as follows: NC-I population — 10 late fourth-stage females and 10 males, 10 late fourth-stage females alone; G-I population — 10 late fourth-stage females and 10 males, 10 late fourth-stage females alone. The selected nematodes were placed in 65-mesh silica sand in 5.5-cm diam clay pots each containing a 7-day-old Lee soybean seedling. These pots were placed in 15-cm diam pots filled with the same type of sand to prevent excessive drying and temperature changes. Pieces of 325-mesh screen were glued over the drainage holes of the smaller pots to prevent nematode loss. Each treatment was replicated four times. After 35 days, reproduction was determined by the presence of second-stage larvae (2).

To test whether interbreeding is possible between B. longicaudatus and B. maritimus or between the NC-I and G-I populations of B. longicaudatus, hybridization tests were conducted (Exp. 1; Table 3). Ten late fourth-stage females of B. maritimus, the NC-I and G-I populations of B. longicaudatus were combined with 10 males of each population, respectively, giving a total of nine combinations. Inoculation procedure and test conditions were as in the previously described test, except that a 7-day-old millet seedling was included with the 7-day-old soybean seedling. Each treatment was replicated three times. After 40 days, the number of larvae present was determined (2).

Two other experiments (Exp. 2, 3; Table 3)

### TABLE 1. Morphological data of females of six populations of Belonolaimus longicaudatus and one population of B. maritimus

<table>
<thead>
<tr>
<th>Measurements (µm) and ratios</th>
<th>NC-1</th>
<th>NC-2</th>
<th>NC-3</th>
<th>G-1</th>
<th>G-2</th>
<th>G-3</th>
<th>B. maritimus&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length</td>
<td>2096.0 w</td>
<td>2161.0 wx</td>
<td>2334.0 y</td>
<td>2324.0 y</td>
<td>2337.0 y</td>
<td>2288.0 xy</td>
<td>2337.0 y</td>
<td>1%</td>
</tr>
<tr>
<td>a</td>
<td>66.0 yz</td>
<td>64.4 y</td>
<td>68.6 z</td>
<td>52.7 w</td>
<td>57.6 x</td>
<td>53.6 w</td>
<td>58.3 x</td>
<td>1%</td>
</tr>
<tr>
<td>b</td>
<td>12.6 x</td>
<td>11.9 wx</td>
<td>12.7 x</td>
<td>10.5 w</td>
<td>11.2 wx</td>
<td>11.3 wx</td>
<td>10.5 w</td>
<td>1%</td>
</tr>
<tr>
<td>c</td>
<td>18.8 x</td>
<td>18.7 x</td>
<td>18.4 x</td>
<td>17.3 w</td>
<td>17.3 w</td>
<td>17.0 w</td>
<td>18.6 x</td>
<td>5%</td>
</tr>
<tr>
<td>V%</td>
<td>49.5 xy</td>
<td>48.2 w</td>
<td>48.5 wx</td>
<td>50.1 y</td>
<td>50.0 y</td>
<td>50.2 y</td>
<td>52.4 z</td>
<td>5%</td>
</tr>
<tr>
<td>Styler length</td>
<td>107.3 w</td>
<td>110.0 wx</td>
<td>114.3 x</td>
<td>128.1 y</td>
<td>130.0 y</td>
<td>129.2 y</td>
<td>137.3 z</td>
<td>1%</td>
</tr>
<tr>
<td>Styler cone length</td>
<td>79.0 w</td>
<td>82.1 wx</td>
<td>85.5 x</td>
<td>92.8 y</td>
<td>93.5 y</td>
<td>91.8 y</td>
<td>99.1 z</td>
<td>1%</td>
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<tr>
<td>Stylet shaft and knobs length</td>
<td>28.3 w</td>
<td>27.8 w</td>
<td>28.8 w</td>
<td>35.3 x</td>
<td>36.5 xy</td>
<td>37.3 xy</td>
<td>38.3 y</td>
<td>1%</td>
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<tr>
<td>Distance to excretory pore</td>
<td>182.9 w</td>
<td>188.6 w</td>
<td>200.8 x</td>
<td>237.3 z</td>
<td>237.6 z</td>
<td>226.3 y</td>
<td>245.7 z</td>
<td>1%</td>
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<tr>
<td>Tail length</td>
<td>111.9 w</td>
<td>115.8 w</td>
<td>127.8 x</td>
<td>136.9 y</td>
<td>135.9 y</td>
<td>135.0 xy</td>
<td>128.2 xy</td>
<td>5%</td>
</tr>
<tr>
<td>Stylet length/tail length</td>
<td>0.97 w</td>
<td>0.95 w</td>
<td>0.90 w</td>
<td>0.95 w</td>
<td>0.96 w</td>
<td>0.96 w</td>
<td>1.10 x</td>
<td>1%</td>
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<sup>1</sup> Numerical data represent the mean of 24 replicates (females); unlike letters depict significant differences among populations (Duncan's new multiple range test).
<sup>2</sup> Reared on strawberry.
<sup>3</sup> Reared on millet; origin, Ocracoke Island, North Carolina.
were conducted to test the fecundity of the F₁ offspring of crosses between populations NC-1 and G-I. Ten late fourth-stage females and 10 males were used for each test in the second experiment while 25 late fourth-stage females and 25 males were used in the third experiment. Test conditions were the same as for the first mating experiment with each treatment being replicated eight and four times, respectively. After 5 wk, second- or third-stage larval offspring obtained from each crossing were collectively inoculated onto single soybean seedlings to test their ability to produce offspring. After 35 or 62 additional days, the number and stage of nematodes recovered were determined (2).

RESULTS

Morphology: Measurements of permanently mounted adult specimens of all Georgia populations of *B. longicaudatus* were significantly different from those of all North Carolina populations for the following character means: 1) females—*a* ratio, *c* ratio, stylent length, stylet cone length, stylet shaft and knobs length and distance of excretory pore from anterior end; 2) males—total length, *c* ratio, stylet length, stylet cone length, stylet shaft and knobs length, distance of excretory pore from anterior end, tail length and spicule length (Tables 1 and 2). The mean total length of females from the NC-3 population was greater than in either the NC-2 or NC-1 populations, but was not different from any of the Georgia populations (Table 1). North Carolina populations did not differ significantly from Georgia populations in linear measurement or ratio means of eggs or second-stage larvae (15).

Additional morphological observations of head, tail, and vulva regions of freshly heat-relaxed, water-mounted adults revealed differences between the North Carolina and Georgia populations of *B. longicaudatus*. Adults from the Georgia populations typically have eight or nine annules in the lip regions, and their cephalic region is more massive than that of adults from North Carolina, which normally have seven or eight annules in the lip region (Fig. 1-A to H). Females of the Georgia populations have opposed sclerotized vaginal pieces lacking in the North Carolina females (Fig. 1-I, J). The spicules and gubernacula of Georgia males are larger than those of North Carolina males (Fig. 1-K to R). The distal portion of the spicule of North Carolina males has a typical indentation and hump, when viewed laterally that is lacking in Georgia males. The proximal flexure of the gubernaculum is longer in Georgia males. Tails of Georgia females are typically wider, less tapered and more bluntly rounded than those of North Carolina females (Fig. 2).

| TABLE 2. Morphological data of males of six populations of *Belonolaimus longicaudatus* and one population of *B. maritimus*²  
Measurements (µm) | North Carolina | Georgia | B. maritimus³ | Significance level |
<table>
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<tbody>
<tr>
<td>and ratios</td>
<td>NC-1</td>
<td>NC-2</td>
<td>NC-3</td>
<td>G-1</td>
</tr>
<tr>
<td>Total length</td>
<td>1648.0 v</td>
<td>1698.0 v</td>
<td>1736.0 v</td>
<td>1914.0 w</td>
</tr>
<tr>
<td>a</td>
<td>61.1 w</td>
<td>61.7 w</td>
<td>63.8 wx</td>
<td>55.4 v</td>
</tr>
<tr>
<td>b</td>
<td>10.1 wx</td>
<td>10.3 wx</td>
<td>10.5 x</td>
<td>9.6 v</td>
</tr>
<tr>
<td>c</td>
<td>15.9 x</td>
<td>15.9 x</td>
<td>15.8 x</td>
<td>14.2 v</td>
</tr>
<tr>
<td>Stylet length</td>
<td>100.8 v</td>
<td>101.5 v</td>
<td>100.6 v</td>
<td>119.8 w</td>
</tr>
<tr>
<td>Stylet cone length</td>
<td>76.0 v</td>
<td>76.5 v</td>
<td>76.8 v</td>
<td>89.2 w</td>
</tr>
<tr>
<td>Stylet shaft and knobs length</td>
<td>24.8 v</td>
<td>25.0 v</td>
<td>23.9 v</td>
<td>30.7 w</td>
</tr>
<tr>
<td>Distance to excretory pore</td>
<td>172.8 wv</td>
<td>170.8 w</td>
<td>180.6 w</td>
<td>220.8 y</td>
</tr>
<tr>
<td>Tail length</td>
<td>103.9 v</td>
<td>107.3 v</td>
<td>110.1 v</td>
<td>134.8 w</td>
</tr>
<tr>
<td>Spicule length</td>
<td>41.0 v</td>
<td>42.7 wv</td>
<td>43.6 w</td>
<td>51.7 y</td>
</tr>
<tr>
<td>Spicule length/tail length</td>
<td>0.97 wx</td>
<td>0.95 wx</td>
<td>0.92 v</td>
<td>0.89 v</td>
</tr>
</tbody>
</table>

¹ Numerical data represent the mean of 24 replicates (males); unlike letters depict significant differences among populations (Duncan's new multiple range test).
² Reared on strawberry.
³ Reared on millet; origin, Ocracoke Island, North Carolina.
FIG. 1-(A to R). Cephalic region and reproductive structures of adults of two populations of *Belonolaimus longicaudatus*—A-D. Female cephalic region: A. Tifton, Ga., A. W. Johnson greenhouse population (G-1) (lateral); B. G-1 (dorsal); C. Tarboro, N.C. population (NC-1) (lateral); D. NC-1 (dorsal); E-H. Male cephalic region: E. G-1 (lateral); F. G-1 (dorsal); G. NC-1 (lateral); H. NC-1 (dorsal); I. NC-1, vulval area; J. G-1, vulval area; K. NC-1, gubernaculum (dorsal); L. NC-1, gubernaculum (lateral); M. NC-1, spicules (lateral); N. NC-1, spicules (dorsal); O. G-1, gubernaculum (dorsal); P. G-1, gubernaculum (lateral); Q. G-1, spicule (lateral); R. G-1 spicule (dorsal).
The stylet knobs of the North Carolina nematodes are typically teardrop or kidney-shaped, whereas those of the Georgia populations are typically rounded or oval (Fig. 3). The stylet knobs of the North Carolina populations are approximately twice as wide as high (height/width = 0.507, number = 48), whereas those from the Georgia populations are about one and one-half times as wide as high (height/width = 0.651, number = 48).

The length/width ratio of the female median bulb is approximately 1.25 for all populations. However, the median bulbs of Georgia females are larger (25.4 by 31.0 μm, number = 48) than those of North Carolina females (20.1 by 25.4 μm, number = 48). The ratio of tail length/anal body width is 4.7 and 4.1 (number = 48) for the North Carolina and Georgia females, respectively.

The permanently mounted adults of *B. maritimus* have longer stylets than any of the *B. longicaudatus* populations. This species also differs from *B. longicaudatus* by having a greater stylet length/tail ratio and a greater vulva % (Tables 1 and 2). Eggs and larvae of *B. maritimus* are larger than those of all *B. longicaudatus* populations (15).

Cytology: No differences in chromosome number were detected between the Georgia and North Carolina populations of *B. longicaudatus* (Fig. 4). The haploid chromosome number observed in primary spermatocytes was eight for all *B. longicaudatus* populations studied as well as for *B. maritimus*.

Matings: No reproduction was detected when females of the NC-I and G-I populations were kept alone, whereas, when females were combined with males of the same populations, offspring were produced. Thus males are necessary for reproduction.

In the hybridization tests with the NC-I and G-I populations of *B. longicaudatus* and with *B. maritimus*, F₁ offspring were produced from all but one (G-I × Bm) mating combination (Table 3). Interpopulation matings of the NC-I and G-I nematodes, however, resulted in significantly fewer offspring than matings within populations. No F₂ was obtained from any of the NC-I and G-I intercrosses, although F₁ adults of both sexes were present. On the other hand, a large number of F₂ offspring resulted from matings within NC-I and G-I populations.

The F₁ larvae of interpopulation matings appeared sluggish and few reached the adult stage. In many respects the adult females that were recovered appeared to resemble the Georgia population. They possessed the opposed sclerotized pieces in the vagina and their stylets were as long as those of the Georgia females. The F₁ males which were recovered lacked the indentation and hump of the spicules of the North Carolina males and the spicules were of approximately the same length as those of males from Georgia.

**DISCUSSION**

Two methods of statistically comparing different populations of *B. longicaudatus* have been reported. Rau and Fassuliotis (14) compared populations by using the bivariate characters of stylet length and tail length in 95% equal-frequency ellipses. Abu-Gharbieh
FIG. 3. Stylet knobs of six populations and stylets of two populations of *Belonolaimus longicaudatus*—A-F. Stylet knobs (dorsal): A. Tifton, Ga., A. W. Johnson greenhouse population (G-1); B. Tifton, Ga. Agronomy Field Number 15 population (G-2); C. Fulwood, Ga. population (G-3); D. Tarboro, N.C. population (NC-1); E. Dudley, N.C. population (NC-2); F. Severn, N.C. population (NC-3); G-L. stylet knobs (lateral); G. G-1 population; H. G-2 population; I. G-3 population; J. NC-1 population; K. NC-2 population; L. NC-3 population; M-N. entire stylet (lateral): M. G-1 population; N. NC-1 population.
and Perry (1) compared three Florida populations by separate statistical analysis of each of several characters. The latter method was chosen for this investigation because a broad range of comparisons was sought. Also, it became apparent that there would be little agreement of ellipses for the bivariate characters used above for the populations from North Carolina and Georgia. As expected, *B. maritimus* was easily separated from all *B. longicaudatus* populations by statistical analysis.

These morphological investigations indicate that the three North Carolina populations are very similar. The three Georgia populations are also similar, but fall into a second group. Morphological differences exist not only between the nematode populations from the two states, but also between populations within a state. Furthermore, none of the populations fit the original and amended descriptions of *B. longicaudatus* (11, 12, 13). The Georgia populations differ from the descriptions of *B. longicaudatus* by being stouter (greater body diam), having longer stylets, longer spicules, and a greater distance of the excretory pore from the anterior end. These differences seem similar to those distinguishing the Gainesville, Fla. population from the Sanford and Fuller’s Crossing, Fla. populations (1). The three North Carolina populations deviate from Rau’s descriptions of *B. longicaudatus* by slightly shorter stylets, lack of opposed sclerotized pieces of the vaginal region, and the distinct hump and indentation of the spicules. The three North Carolina and three Georgia populations differ from *B. euthychilis* Rau, *B. gracilis* Steiner and *B. maritimus* (13) by having shorter stylets and an average stylet length/tail length ratio less than one. They differ from both *B. nortoni* Rau (13) and *B. lineatus* Roman (16) by having longer stylets, from the former by lacking the protruding vulval lips, and from the latter by having one lateral line instead of four.

No differences were detected in the haploid number of chromosomes in males. All populations of *B. longicaudatus* and *B. maritimus* investigated possessed eight haploid chromosomes in the males, indicating that these species apparently are closely related.

Interpopulation matings of the NC-1 and G-1 populations resulted in only a limited...
number of offspring which failed to reproduce under the conditions where intrapopulation offspring did reproduce. Differences in host range (15), morphology, and apparent infertility of interpopulation offspring suggest that the North Carolina and Georgia nematodes are of different species, neither being *B. longicaudatus* as described by Rau (11, 12, 13). As it was beyond the intended scope of this investigation, no direct microscopic comparisons were made between type specimens of *B. longicaudatus* and the North Carolina or Georgia nematodes. Thus, no attempt was made to describe new species. These comparisons are under investigation and are to be included in a review of the genus by the senior author.

**LITERATURE CITED**

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