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Morphometrics of *Globodera tabacum tabacum, G. t. virginiae,* and *G. t. solanacearum* (Nemata: Heteroderinae)[1]

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Abstract: A morphometric evaluation of second-stage juveniles (J2), males, females, cysts, and eggs of several isolates of the tobacco cyst nematode (TCN) complex, *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS) is presented. Morphometrics of eggs, J2, and males are considerably less variable than of females and cysts. No measurements of eggs and J2 are useful for identification of the three subspecies. Distance from the median bulb and excretory pore to the head end in J2 and males is quite stable. Stylet knob width of males is useful for identifying GTV isolates and tail length in separating males of GTT isolates from GTV and GTS. Body length/width (L/W) ratio of females and cysts discriminates GTT from GTV and GTS; stylet knob width is an auxiliary character for identifying GTV. This subspecies complex has a continuum of values for the other characters. Data suggest a close relationship between GTV and GTS, which also occur in close proximity in Virginia.

Key words: Cyst, *Globodera tabacum tabacum, G. t. solanacearum, G. t. virginiae,* morphometrics, nematode, tobacco cyst nematode, species complex, subspecies, variability.

Morphometrics has been used extensively in the taxonomy of cyst nematodes, subfamily Heteroderinae sensu lato Luc et al. (8), for identification (2,4,6,17,21), and for diagnoses of new genera and species (1,18,20). Several morphometric characters of second-stage juveniles (J2), males, and females were used by Stone to separate *Globodera pallida* Stone, 1972 from *G. rostochiensis* Wollenweber, 1923 (18). Wouts and Weischer (21) used J2 characters to distinguish between these and other heteroderine species.

Morphometric studies of *Globodera tabacum tabacum* (GTT) (Lownsbery & Lownsbery, 1954) Behrens, 1975; *G. t. virginiae* (GTV) (Miller & Gray, 1968) Behrens, 1975; and *G. t. solanacearum* (GTS) (Miller & Gray, 1972) Behrens, 1975 have been based on one isolate of each subspecies and few characters (5,16,19). Furthermore, the isolates were reared on different hosts, under different conditions, and comparisons are not valid.

No major morphological differences were reported among J2 and males of several isolates of this complex (13). Some differences were found in female and cyst characters, but morphological variability among the individuals and isolates was great (14).

This paper measures several characters of eggs, J2, males, white females, and cysts of the TCN complex in detail, to better understand the extent of variability within this group, and to search for useful morphometrical characters that may be used for identification.

MATERIALS AND METHODS

Isolates used in this study are in Table 1. Locations of GTV and GTS isolates are listed according to L. I. Miller, except for GTS-12. Collection methods were described previously (13,14). GTV-1-X was recently collected from the same location as isolate GTV-1, which was collected more than 25 years ago by L. I. Miller from the Horton farm in Suffolk County, Virginia. GTV-1-X was collected from the roots of horsenettle (*Solanum carolinense* L.) in the field, whereas all other isolates were reared in the greenhouse on 'Rugters' tomato (*Lycopersicum esculentum* Mill.).

Specimens were observed with a light microscope and measured with a Leitz drawing tube. Twenty to 30 specimens of

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TABLE 1. Isolates of the tobacco cyst nematode complex, *Globodera tabacum* sspp. *tabacum* (GTT), *virginiae* (GTV), and *solanacearum* (GTS).

Isolate	Location[1]	County, state	Origin
GTT-1 (type locality)	Hazardville	Hartford, CT	P. M. Miller
GTT-2	Windsor	Hartford, CT	P. M. Miller
GTT-3	Windsor	Hartford, CT	J. LaMondia
GTT-4	Windsor	Hartford, CT	J. LaMondia
GTT-5	Enfield	Hartford, CT	J. LaMondia
GTV-1	Horton	Suffolk, VA	L. I. Miller
GTV-1-X	Horton	Suffolk, VA	M. Mota/J. D. Eisenback
GTV-4	93A	Suffolk, VA	L. I. Miller
GTV-6	125A	Suffolk, VA	L. I. Miller
GTV-8	H. N. Williams	Suffolk, VA	L. I. Miller
GTV-11 (type locality)	Standard 24	Suffolk, VA	L. I. Miller
GTS-1	Fisher-Nottoway	Nottoway, VA	L. I. Miller
GTS-2	Fisher-WWO	Nottoway, VA	L. I. Miller
GTS-3	Irby	Amelia, VA	L. I. Miller
GTS-4	5-2-A	Amelia, VA	L. I. Miller
GTS-5	Lynch	Amelia, VA	L. I. Miller
GTS-6	Paulette	Amelia, VA	L. I. Miller
GTS-8	Smith	Amelia, VA	L. I. Miller
GTS-9	Anderson	Amelia, VA	L. I. Miller
GTS-10 (type locality)	Watkins	Amelia, VA	L. I. Miller
GTS-12	D-132	Dinwiddie, VA	Plant Dis. Clinic, Virginia Tech

each isolate were measured, except where noted. All linear measurements are in μm. Tables (2–6) include the mean, standard deviation (±), and range (in parenthesis). The coefficient of variability (CV) was also calculated. Standard analysis of variance (ANOVA) was performed for each character, and the Waller-Duncan k-ratio *t*-test (k = 100) was used for multiple comparisons of the means. Box plots (Figs. 1–8) indicate distribution of data between the 10th and 90th percentile between the box caps, and 25th to 75th percentile in the box. Circles represent outliers.

RESULTS

Egg (Table 2; Fig. 1): Measurements of eggs had a very low coefficient of variability (CV), less than 10% for all characters. The values for length (L) and width (W) overlapped across all isolates (Table 2). The L/W ratio ('a') separated GTT-5 from GTV-1-X according to the Waller-Duncan test. GTT-5 showed the lowest value of L/W of all the isolates (2.2) whereas GTV-1-X the highest (2.7).

J2 (Table 3; Figs. 2,3): CV for the morphometrical characters of J2 (Fig. 2) was generally low. Only body width, body length/width ('a' ratio), the distance of the dorsal esophageal gland opening (DEGO) to the base of the knobs, stylet knobs width (kw) and height (kh), the ratio kw/kh, width of body at anus, and tail terminus had CV greater than 10%. GTS-4 had the highest body length mean. GTS-4 was separated from other isolates by the median bulb to head end mean values. In contrast, GTS-10 and GTT-1 had the lowest values and were placed in separate groups in the means comparisons test. The excretory pore to head end distance discriminated GTS-4 (highest mean) and GTT-1 (lowest mean) from all other isolates. The wide overlapping of means and the relatively high CV (8.7–12.7%) of the distance from base of the knobs to the dorsal esophageal gland opening (DEGO) suggest that this character is not useful for differentiating subspecies. The very low CV (3.1–6.5%), together with the ability to separate groups by comparison of their means, indicates that the stylet length is a stable character, although it is not useful for separating the subspecies. Even though tail terminus length has a high CV (8.2–14.4%), it was the best character to group the isolates, but

TABLE 2. Morphometrics of eggs of the tobacco cyst nematode complex *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS).

Character	GTT-1	GTT-2	GTT-3	GTT-4	GTT-5	GTV-1	GTV-1-X
Length (L)	106.5 ± 6.3 ghi	116.6 ± 7.3 a	110.0 ± 3.9 ef	107.4 ± 5.8 gh	108.9 ± 4.6 fg	111.8 ± 6.2 de	114.1 ± 4.0 bcd
	(89.6 – 116.2)	(103.6 – 130.2)	(100.8 – 120.4)	(95.2 – 117.6)	(100.8 – 117.6)	(99.4 – 123.2)	(109.2 – 126.0)
Width (W)	44.7 ± 2.3 ef	47.0 ± 2.0 bc	43.7 ± 2.2 fg	45.8 ± 2.6 d	49.8 ± 3.3 a	45.7 ± 3.7 de	42.4 ± 1.9 hi
	(40.6 – 50.4)	(43.4 – 50.4)	(39.2 – 50.4)	(39.2 – 50.4)	(43.4 – 56.0)	(40.6 – 51.8)	(39.2 – 49.0)
L/W	2.4 ± 0.2 efghi	2.5 ± 0.2 bcd	2.5 ± 0.1 b	2.4 ± 0.2 ghi	2.2 ± 0.2 j	2.5 ± 0.3 bcdef	2.7 ± 0.1 a
	(2.1 – 2.9)	(2.2 – 2.8)	(2.2 – 2.8)	(1.9 – 2.8)	(1.8 – 2.6)	(2.0 – 2.8)	(2.3 – 2.9)
Character	GTV-4′	GTV-8	GTV-10	GTV-11	GTS-1	GTS-2	GTS-3
Length (L)	104.6 ± 3.0 ijk	105.7 ± 3.6 hij	116.7 ± 6.9 a	103.8 ± 5.3 jk	106.5 ± 4.0 ghi	114.4 ± 6.2 abc	110.4 ± 5.1 ef
	(99.4 – 110.6)	(96.6 – 112.0)	(103.6 – 131.6)	(96.6 – 114.8)	(99.4 – 114.8)	(103.6 – 128.8)	(98.0 – 120.4)
Width (W)	42.9 ± 2.2 ghi	42.3 ± 2.3 i	46.5 ± 2.2 cd	42.1 ± 1.8 i	46.2 ± 3.0 cd	46.4 ± 2.1 cd	47.7 ± 2.1 b
	(39.2 – 47.6)	(37.8 – 47.6)	(42.0 – 50.4)	(39.2 – 46.2)	(39.2 – 51.8)	(42.0 – 51.8)	(43.4 – 50.4)
L/W	2.4 ± 0.1 cdefg	2.5 ± 0.2 bc	2.5 ± 0.2 bc	2.5 ± 0.2 bcde	2.3 ± 0.2 i	2.5 ± 0.2 bcde	2.3 ± 0.2 hi
	(2.2 – 2.7)	(2.2 – 2.9)	(2.1 – 2.9)	(2.2 – 2.9)	(1.9 – 2.8)	(2.1 – 2.8)	(2.0 – 2.6)
Character	GTS-4	GTS-5	GTS-6	GTS-8	GTS-9	GTS-10	GTS-12
Length (L)	116.3 ± 6.2 ab	113.4 ± 6.1 cd	114.6 ± 5.9 abc	103.1 ± 5.8 k	105.0 ± 5.4 hijk	102.8 ± 5.2 k	104.2 ± 4.0 ijk
	(103.6 – 130.2)	(100.8 – 124.6)	(106.4 – 128.8)	(89.6 – 114.8)	(89.6 – 113.4)	(92.4 – 114.8)	(95.2 – 112.0)
Width (W)	47.0 ± 2.8 bc	46.7 ± 2.1 bcd	49.4 ± 2.5 a	42.7 ± 2.1 ghi	42.8 ± 1.9 ghi	43.4 ± 1.8 gh	43.0 ± 1.5 ghi
	(42.0 – 53.2)	(42.0 – 51.8)	(43.4 – 54.6)	(39.2 – 46.2)	(40.6 – 47.6)	(37.8 – 47.6)	(39.2 – 46.2)
L/W	2.5 ± 0.2 bcde	2.4 ± 0.2 bcdef	2.3 ± 0.2 hi	2.4 ± 0.2 defgh	2.5 ± 0.2 bcdef	2.4 ± 0.2 fghi	2.4 ± 0.1 cdefg
	(2.2 – 3.0)	(2.0 – 2.9)	(2.0 – 3.0)	(2.1 – 2.9)	(2.1 – 2.8)	(2.1 – 2.6)	(2.3 – 2.8)

All linear measurements are in μm. Values are means ± standard deviation (range in parenthesis). Values in a row followed by the same letter are not significantly different according to the Waller-Duncan k-ratio *t*-test (k = 100).

TABLE 3. Morphometrics of second-stage juveniles of the tobacco cyst nematode complex *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS).

Character	GTT-1	GTT-2	GTV-1	GTV-1-X	GTV-6
Body length	521.3 ± 20.1 de	516.4 ± 32.1 e	556.1 ± 44.9 b	515.0 ± 21.1 e	541.3 ± 47.4 bc
(L)	(469.8 – 564.3)	(464.0 – 580.0)	(476.0 – 661.2)	(464.0 – 551.0)	(464.0 – 632.2)
Body width	24.8 ± 2.4 abc	23.5 ± 2.3 ed	26.0 ± 3.4 a	23.9 ± 2.0 cd	23.3 ± 2.3 de
(W)	(20.3 – 28.5)	(20.3 – 29.0)	(21.1 – 34.2)	(20.3 – 29.0)	(20.3 – 29.0)
Median bulb to	66.5 ± 3.6 f	71.3 ± 3.9 cd	70.4 ± 2.7 d	73.1 ± 3.9 b	71.2 ± 4.8 cd
head end (MB)	(58.8 – 74.2)	(63.0 – 79.2)	(67.5 – 77.4)	(58.5 – 79.2)	(63.0 – 81.0)
Excretory pore	102.6 ± 4.6 f	113.9 ± 6.7 c	109.8 ± 8.7 de	112.8 ± 6.2 cd	111.6 ± 10.5 cde
to head end (EP)	(94.9 – 111.2)	(99.0 – 126.9)	(96.2 – 128.7)	(100.8 – 124.2)	(99.0 – 133.2)
DEGO	5.8 ± 0.7 cde	5.8 ± 0.7 cde	5.9 ± 0.8 bcd	6.2 ± 0.8 b	6.1 ± 0.7 bc
	(3.8 – 6.8)	(4.5 – 7.2)	(4.3 – 7.2)	(4.5 – 7.2)	(5.0 – 7.2)
Stylet length	22.6 ± 1.4 de	21.2 ± 0.8 g	23.3 ± 1.0 c	22.3 ± 0.7 e	22.5 ± 1.5 de
(STY)	(19.8 – 25.7)	(19.8 – 22.5)	(20.7 – 24.7)	(21.2 – 23.4)	(20.7 – 25.2)
Shaft length	9.6 ± 0.7 b	9.3 ± 0.5 a	10.1 ± 0.7 ab	9.5 ± 0.6 ab	9.7 ± 0.7 b
(SHA)	(8.1 – 11.2)	(8.1 – 9.9)	(9.0 – 11.4)	(8.1 – 10.8)	(9.0 – 10.8)
Knobs width	4.6 ± 0.4 ef	4.2 ± 0.4 g	4.8 ± 0.4 bc	4.6 ± 0.3 def	4.7 ± 0.4 cde
(kw)	(3.8 – 5.4)	(3.6 – 4.5)	(4.0 – 5.7)	(4.1 – 5.3)	(4.5 – 5.4)
Knobs height	2.9 ± 0.3 bcd	2.8 ± 0.2 de	3.1 ± 0.4 ab	2.9 ± 0.3 cde	2.6 ± 0.2 f
(kh)	(2.3 – 3.6)	(2.3 – 3.2)	(2.3 – 3.8)	(2.3 – 3.6)	(2.3 – 2.7)
Tail length	54.3 ± 4.4 b	49.8 ± 3.9 e	56.4 ± 3.8 a	52.1 ± 3.3 cd	53.0 ± 5.0 bcd
(t)	(46.8 – 67.5)	(44.1 – 60.3)	(49.5 – 63.7)	(45.0 – 59.4)	(45.9 – 63.0)
Tail terminus	27.5 ± 3.1 b	20.9 ± 3.0 d	27.8 ± 3.0 b	25.9 ± 2.9 c	28.0 ± 2.9 b
(tt)	(21.9 – 33.3)	(17.1 – 27.0)	(19.9 – 34.2)	(20.7 – 31.5)	(22.5 – 32.4)
Width of body	13.8 ± 1.3 cde	14.0 ± 1.2 bc	13.7 ± 1.3 cde	14.2 ± 1.6 bc	13.3 ± 1.5 de
at anus	(11.7 – 17.1)	(12.6 – 17.1)	(10.8 – 16.2)	(11.7 – 17.1)	(10.8 – 16.2)
'a'	21 ± 1.5 d	22.1 ± 1.9 cd	21.6 ± 1.8 d	21.7 ± 1.7 d	23.3 ± 1.7 b
	(18.4 – 25.1)	(19.1 – 26.6)	(18.3 – 25.8)	(18.2 – 25.1)	(20.0 – 27.7)
Shaft-stylet	0.4 ± 0.02 f	0.4 ± 0.03 g	0.4 ± 0.02 cd	0.4 ± 0.03 fg	0.4 ± 0.02 ef
	(0.4 – 0.5)	(0.4 – 0.5)	(0.4 – 0.5)	(0.4 – 0.5)	(0.4 – 0.5)
kw/kh	1.6 ± 0.2 de	1.5 ± 0.2 e	1.6 ± 0.2 cde	1.6 ± 0.1 cde	1.9 ± 0.2 a
	(1.2 – 2.0)	(1.3 – 1.8)	(1.3 – 2.0)	(1.4 – 2.0)	(1.7 – 2.4)
tt/t	0.5 ± 0.05 ab	0.4 ± 0.04 e	0.5 ± 0.04 bcd	0.5 ± 0.04 bcd	0.5 ± 0.05 a
	(0.4 – 0.6)	(0.3 – 0.5)	(0.4 – 0.6)	(0.4 – 0.6)	(0.4 – 0.6)

Character	GTV-11	GTS-1	GTS-4	GTS-5	GTS-10
Body length	534.0 ± 34.5 cd	540.7 ± 42.8 b	576.1 ± 33.5 a	545.8 ± 25.1 bc	516.0 ± 20.3 e
(L)	(481.4 – 603.2)	(458.9 – 621.3)	(504.6 – 643.8)	(458.2 – 580.0)	(475.6 – 562.6)
Body width	25.2 ± 2.3 ab	24.0 ± 3.0 a	23.1 ± 2.8 de	25.6 ± 3.1 a	22.4 ± 1.8 e
(W)	(20.3 – 29.0)	(17.1 – 28.5)	(17.4 – 29.0)	(20.3 – 31.9)	(17.4 – 26.1)
Median bulb to	72.9 ± 3.3 bc	71.8 ± 3.3 bcd	76.8 ± 3.7 a	72.5 ± 2.8 bc	68.2 ± 3.8 e
head end (MB)	(61.2 – 76.5)	(65.8 – 78.4)	(63.0 – 81.0)	(66.6 – 79.2)	(61.2 – 80.1)
Excretory pore	112.4 ± 5.7 cd	108.9 ± 7.3 e	125.2 ± 6.5 a	118.7 ± 4.9 b	110.5 ± 5.8 de
to head end (EP)	(101.7 – 122.4)	(94.3 – 122.2)	(113.4 – 144.0)	(108.0 – 126.0)	(99.9 – 129.6)
DEGO	5.8 ± 0.7 cde	6.0 ± 0.6 bcd	6.6 ± 0.6 a	5.7 ± 0.7 de	5.5 ± 0.5 e
	(4.5 – 7.2)	(4.8 – 7.1)	(5.4 – 8.1)	(4.5 – 7.2)	(4.5 – 6.3)
Stylet length	22.8 ± 1.0 d	24.9 ± 1.0 a	24.3 ± 0.8 b	23.3 ± 0.8 c	21.7 ± 1.0 f
(STY)	(20.3 – 24.3)	(22.3 – 26.6)	(22.5 – 25.2)	(21.6 – 24.3)	(18.9 – 23.4)
Shaft length	9.9 ± 0.6 ab	10.8 ± 0.6 ab	10.7 ± 0.5 ab	10.4 ± 0.5 a	9.7 ± 0.5 a
(SHA)	(9.0 – 10.8)	(9.5 – 12.4)	(9.9 – 11.7)	(9.0 – 11.3)	(8.1 – 10.4)
Knobs width	4.8 ± 0.5 cd	5.2 ± 0.4 a	5.0 ± 0.6 ab	4.9 ± 0.4 bc	4.5 ± 0.4 f
(kw)	(4.1 – 5.4)	(4.8 – 5.7)	(3.2 – 5.9)	(4.5 – 5.4)	(3.6 – 5.4)
Knobs height	2.7 ± 0.4 e	3.1 ± 0.4 a	3.1 ± 0.4 a	2.9 ± 0.3 abc	2.8 ± 0.2 de
(kh)	(1.8 – 3.6)	(2.4 – 3.8)	(2.7 – 4.1)	(2.7 – 3.6)	(2.7 – 3.2)
Tail length	52.3 ± 3.7 bcd	51.8 ± 5.1 cde	57.6 ± 5.1 a	53.5 ± 3.9 bc	51.3 ± 3.6 de
(t)	(44.1 – 58.5)	(44.2 – 61.8)	(45.0 – 71.1)	(44.1 – 62.1)	(42.3 – 60.3)
Tail terminus	25.2 ± 3.1 c	25.8 ± 2.4 c	29.8 ± 4.2 a	25.4 ± 2.8 c	25.0 ± 2.3 c
(tt)	(18.0 – 31.5)	(20.8 – 29.9)	(20.7 – 37.8)	(16.2 – 28.8)	(21.6 – 31.5)
Width of body	13.9 ± 1.1 bcd	13.2 ± 1.1 e	15.0 ± 1.6 a	14.5 ± 1.0 ab	13.3 ± 0.8 de
at anus	(11.7 – 16.2)	(11.2 – 15.4)	(11.7 – 19.8)	(13.5 – 17.1)	(11.7 – 14.4)
'a'	21.3 – 1.9 d	22.8 – 2.4 bc	25.2 – 2.8 a	21.6 – 2.7 d	23.2 – 2.0 b
	(18.0 – 24.8)	(18.9 – 27.1)	(20.4 – 33.7)	(15.8 – 28.3)	(20.0 – 29.3)
Shaft/stylet	0.4 ± 0.03 de	0.4 ± 0.02 a	0.4 ± 0.02 ab	0.4 ± 0.02 bc	0.4 ± 0.02 ef
	(0.4 – 0.5)	(0.4 – 0.5)	(0.4 – 0.5)	(0.4 – 0.5)	(0.4 – 0.5)
kw/kh	1.8 ± 0.3 ab	1.7 ± 0.2 bc	1.6 ± 0.2 cd	1.7 ± 0.2 bcd	1.6 ± 0.2 cde
	(1.4 – 2.5)	(1.4 – 2.4)	(1.2 – 2.0)	(1.4 – 2.0)	(1.3 – 2.0)
tt/t	0.5 ± 0.05 d	0.5 ± 0.03 cd	0.5 ± 0.05 abc	0.5 ± 0.05 d	0.5 ± 0.03 cd
	(0.4 – 0.6)	(0.4 – 0.5)	(0.4 – 0.6)	(0.3 – 0.5)	(0.4 – 0.5)

All linear measurements are in μm. Values are means ± standard deviation (range in parenthesis). Values in a row followed by the same letter are not significantly different according to Waller-Duncan k-ratio *t*-test (k = 100).

TABLE 4. Morphometrics of males of the tobacco cyst nematode complex *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS).

Character	GTT-1	GTT-2	GTV-1	GTV-1-X	GTV-11	GTS-1	GTS-10
Body length	1186.3 ± 107.3 a	1119.0 ± 113.7 bc	1136.81 ± 115.1 abc	1079.1 ± 106.5 c	1170.1 ± 103.0 ab	1140.7 ± 119.5 abc	1099.0 ± 119.3 c
(L)	(957.0 – 1450.0)	(893.2 – 1316.6)	(870.0 – 1305.0)	(852.6 – 1229.6)	(812.0 – 1334.0)	(899.0 – 1450.0)	(812.0 – 1270.2)
Body width	34.7 ± 4.1 ab	34.4 ± 2.9 ab	33.1 ± 3.7 bc	36.0 ± 2.9 a	32.0 ± 3.4 cd	30.8 ± 3.6 de	30.0 ± 2.7 e
(W)	(23.2 – 43.5)	(26.1 – 40.6)	(26.1 – 40.6)	(31.9 – 40.6)	(26.1 – 37.7)	(23.2 – 37.7)	(26.1 – 34.8)
Median bulb to	95.7 ± 7.0 ab	92.4 ± 4.7 bc	94.2 ± 5.9 bc	92.0 ± 8.1 c	98.5 ± 6.6 a	94.8 ± 6.7 bc	92.4 ± 4.6 bc
head end (MB)	(76.5 – 108.0)	(82.8 – 100.8)	(83.7 – 103.6)	(77.4 – 105.3)	(85.5 – 110.7)	(81.0 – 110.7)	(82.8 – 100.8)
Excretory pore	160.1 ± 11.3 a	156.0 ± 10.1 ab	153.3 ± 8.2 ab	157.1 ± 12.3 ab	159.6 ± 14.5 ab	151.2 ± 16.4 b	158.9 ± 12.0 ab
to head end (EP)	(137.7 – 181.8)	(133.2 – 173.7)	(129.6 – 169.2)	(123.3 – 177.3)	(137.7 – 207.0)	(126.0 – 189.0)	(135.0 – 173.7)
DEGO	3.8 ± 0.7 ab	3.6 ± 0.5 bc	3.5 ± 0.6 c	3.0 ± 0.6 d	4.0 ± 0.7 a	3.2 ± 0.6 d	3.1 ± 0.6 d
	(2.3 – 4.5)	(2.4 – 4.1)	(2.7 – 4.5)	(2.3 – 4.5)	(2.7 – 5.4)	(2.3 – 4.5)	(2.3 – 4.1)
Stylet length	26.7 ± 1.3 ab	26.2 ± 1.1 bc	26.0 ± 0.9 c	24.8 ± 1.3 d	25.0 ± 1.2 d	26.8 ± 1.1 a	25.1 ± 1.1 d
(STY)	(23.4 – 28.8)	(24.3 – 27.9)	(24.3 – 27.9)	(23.4 – 27.9)	(22.1 – 27.5)	(25.2 – 30.6)	(22.5 – 26.5)
Shaft length	11.1 ± 0.8 a	10.8 ± 0.8 ab	10.7 ± 0.7 ab	10.5 ± 0.7 b	10.7 ± 0.6 b	11.1 ± 0.5 a	10.7 ± 0.6 b
(SHAF)	(9.9 – 12.6)	(9.0 – 12.2)	(9.9 – 12.6)	(9.5 – 11.7)	(9.9 – 11.7)	(9.9 – 11.7)	(9.5 – 11.7)
Knobs width	5.0 ± 0.4 c	4.9 ± 0.4 c	5.2 ± 0.4 b	5.5 ± 0.6 a	5.6 ± 0.5 a	5.0 ± 0.4 c	5.0 ± 0.4 c
(kw)	(4.5 – 5.4)	(4.5 – 5.4)	(4.5 – 5.9)	(4.5 – 6.3)	(4.5 – 6.3)	(4.5 – 5.4)	(4.1 – 5.4)
Knobs height	3.2 ± 0.4 a	3.0 ± 0.3 ab	3.1 ± 0.4 a	3.0 ± 0.3 ab	3.1 ± 0.4 a	3.1 ± 0.4 a	2.8 ± 0.3 b
(kh)	(2.7 – 3.6)	(2.7 – 3.6)	(2.7 – 3.6)	(2.7 – 3.6)	(2.7 – 3.6)	(2.7 – 3.6)	(2.3 – 3.2)
Head width	11.0 ± 0.8 bc	11.0 ± 0.6 ab	10.6 ± 0.5 d	10.8 ± 0.4 bcd	11.3 ± 0.6 a	10.8 ± 0.5 bcd	10.6 ± 0.5 cd
(HW)	(9.9 – 12.6)	(9.9 – 12.6)	(9.9 – 11.7)	(9.9 – 11.7)	(10.4 – 12.6)	(9.9 – 11.7)	(9.5 – 11.3)
Head height	5.7 ± 0.6 ab	5.8 ± 0.6 a	5.6 ± 0.5 ab	5.2 ± 0.5 c	5.8 ± 0.5 a	5.4 ± 0.4 bc	5.5 ± 0.5 bc
(HH)	(4.5 – 7.2)	(4.5 – 6.8)	(4.5 – 6.3)	(4.5 – 5.9)	(5.0 – 6.8)	(4.5 – 6.3)	(4.5 – 6.3)
Spicule (SPIC)	34.8 ± 1.9 a	34.4 ± 1.8 ab	34.0 ± 1.7 abc	31.1 ± 1.5 d	33.7 ± 1.7 bc	34.4 ± 1.7 ab	33.4 ± 1.3 c
	(30.6 – 37.8)	(30.6 – 36.9)	(30.8 – 36.9)	(28.8 – 33.3)	(28.8 – 36.0)	(31.5 – 37.8)	(31.5 – 36.0)
Gubernaculum	12.2 ± 1.2 a	12.0 ± 1.1 a	11.8 ± 0.9 a		10.9 ± 0.8 b	10.9 ± 1.0 b	10.8 ± 0.6 b
(GUB)	(9.9 – 14.4)	(9.9 – 14.4)*	(9.9 – 13.5)***		(9.9 – 12.6)	(9.9 – 12.6)	(9.9 – 12.6)
Tail length	4.0 ± 0.9 c	4.1 ± 0.8 c	4.8 ± 0.9 b		5.0 ± 0.5 b	4.9 ± 0.9 b	5.6 ± 0.8 a
	(2.7 – 5.9)	(2.7 – 5.4)**	(3.6 – 7.2)		(4.1 – 5.9)	(3.6 – 6.3)	(4.5 – 7.2)
'a'	34.5 ± 3.9 bc	32.7 ± 4.1 c	34.6 ± 4.1 ab	30.1 ± 3.4 d	36.8 ± 3.8 a	34.8 ± 3.8 ab	36.4 ± 4.4 ab
	(28.6 – 43.0)	(26.5 – 42.9)	(25.7 – 44.4)	(23.3 – 35.3)	(31.1 – 44.0)	(24.3 – 42.0)	(28.0 – 44.4)

All linear measurements are in μm. Values are means ± standard deviation (range in parenthesis). Values in a row followed by the same letter are not considered significantly different according to Waller-Duncan's k-ratio *t*-test (k = 100).

TABLE 5. Morphometrics of females of the tobacco cyst nematode complex *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS).

Character	GTT-1	GTT-2	GTV-1	GTV-11	GTS-1	GTS-10
Body length (L)	635.3 ± 66.7 a (493.0 – 804.0)	539.8 ± 65.3 b (417.6 ± 649.6)	574.2 ± 86.1 b (417.6 ± 754.0)	560.5 ± 99.3 b (406.0 ± 742.4)	573.4 ± 78.3 b (464.0 ± 754.0)	573.0 ± 78.3 b (475.6 ± 684.4)
Body width (W)	620.8 ± 86.7 a (359.6 – 777.2)	533.4 ± 87.5 b (365.4 – 684.4)	525.9 ± 96.7 bc (348.0 – 707.6)	486.8 ± 113.2 c (319.0 – 696.0)	513.1 ± 81.3 bc (394.4 – 678.6)	497.1 ± 56.1 bc (406.0 – 609.0)
Neck length	133.7 ± 25.2 c (87.3 – 175.0)	155.0 ± 28.7 b (112.0 – 245.0)	168.6 ± 20.1 a (126.0 – 210.0)	140.4 ± 22.3 c (105.0 – 182.0)	162.3 ± 18.4 ab (126.0 – 196.0)	158.3 ± 26.8 ab (112.0 – 210.0)
DEGO	5.3 ± 1.4 ab (3.6 – 7.2)	5.9 ± 1.3 a (4.5 – 9.0)	5.0 ± 1.0 bc (3.6 – 6.3)	4.7 ± 0.8 cd (2.7 – 5.9)	4.0 ± 1.1 d (2.7 – 7.2)	5.1 ± 0.6 bc (4.1 – 6.3)
Stylet length (ST)	23. ± 2.3 c (19.8 – 27.9)	24. ± 2.3 bc (17.1 – 26.6)	25.2 ± 1.6 b (20.7 – 27.9)	24.5 ± 1.3 bc (21.6 – 27.0)	26.6 ± 1.1 a (23.9 – 28.4)	24.5 ± 1.7 bc (21.6 – 27.0)
Shaft length (SH)	9.0 ± 1.5 bc (6.3 – 12.6)	9.5 ± 1.2 ab (7.7 – 12.6)	8.0 ± 1.1 c (6.3 – 11.7)	8.6 ± 0.9 c (7.2 – 10.8)	10.0 ± 0.8 a (8.6 – 11.7)	9.0 ± 0.8 bc 10.8
Stylet knobs width (kw)	5.5 ± 0.8 bc (4.1 – 6.8)	5.2 ± 0.7 c (3.6 – 6.3)	5.6 ± 0.8 b (4.5 – 7.2)	6.0 ± 0.5 a (4.5 – 7.2)	5.5 ± 0.5 bc (4.1 – 6.3)	5.4 ± 0.5 bc (4.5 – 6.3)
Stylet knobs height (kh)	2.9 ± 0.4 b (2.3 – 3.6)	3.2 ± 0.5 a (2.7 – 4.5)	3.2 ± 0.4 a (2.7 – 4.5)	2.8 ± 0.4 b (2.3 – 3.6)	3.2 ± 0.3 a (2.7 – 4.1)	2.9 ± 0.3 b (2.7 – 3.6)
Number of ridges (R)	9.7 ± 1.8 ab (7.0 – 14.0)	8.4 ± 1.2 c (6.0 – 11.0)	9.4 ± 1.4 b (7.0 – 14.0)	10.4 ± 1.8 a (8.0 – 14.0)	8.5 ± 1.8 c (7.0 – 15.0)	9.7 ± 2.2 ab (7.0 – 16.0)
Anus to edge of fenestra (AF)	48.4 ± 10.9 a (26.6 – 72.8)	39.2 ± 7.7 c (25.2 – 51.8)	47.8 ± 8.8 a (32.2 – 67.2)	46.2 ± 8.0 ab (33.6 – 68.6)	41.7 ± 7.4 c (30.1 – 58.8)	43.4 ± 8.7 bc (32.2 – 70.0)
Anus to center of fenestra (AFc)	61.8 ± 11.8 a (36.4 – 86.8)	51.0 ± 7.8 c (37.8 – 64.4)	59.8 ± 9.9 a (40.6 – 82.6)	58.0 ± 8.3 ab (42.0 – 82.6)	56.8 ± 12.0 bc (42.0 – 75.6)	55.0 ± 9.6 bc (42.0 – 84.0)
Fenestra length (FenL)	27.0 ± 3.6 a (19.6 – 36.4)	23.4 ± 2.5 b (18.2 – 29.4)	23.4 ± 2.9 b (18.2 – 29.4)	23.5 ± 3.5 b (15.4 – 30.8)	25.6 ± 3.3 a (19.6 – 32.2)	23.9 ± 2.3 b (21.0 – 29.4)
Fenestra width (FenW)	20.9 ± 3.4 a (14.0 – 29.4)	19.2 ± 2.9 b (14.0 – 29.4)	19.0 ± 2.3 b (14.0 – 23.8)	20.4 ± 2.7 ab (16.1 – 25.2)	21.0 ± 2.8 a (16.8 – 26.6)	21.4 ± 2.1 a (16.1 – 25.2)
Length of vulval slit (V)	9.7 ± 1.5 a (7.0 – 12.6)	8.0 ± 1.1 c (6.3 – 9.8)	8.1 ± 1.0 bc (7.0 – 10.5)	8.3 ± 1.1 bc (7.0 – 10.5)	8.6 ± 1.2 b (7.0 – 10.5)	8.4 ± 0.9 bc (7.0 – 9.8)
L/W	1.0 ± 0.1 c (0.8 – 1.4)	1.0 ± 0.1 c (0.9 – 1.2)	1.1 ± 0.1 b (1.0 – 1.3)	1.2 ± 0.1 a (1.0 – 1.4)	1.1 ± 0.1 ab (1.0 – 1.3)	1.2 ± 0.1 a (1.0 – 1.3)
Fenestra L/W (FenLW)	1.3 ± 0.2 a (0.9 – 1.6)	1.2 ± 0.2 ab (0.9 – 1.8)	1.2 ± 0.2 ab (0.9 – 1.8)	1.2 ± 0.2 bc (0.9 – 1.6)	1.2 ± 0.2 ab (0.9 – 1.9)	1.1 ± 0.1 c (0.9 – 1.5)

All linear measurements are in µm. Values are means ± standard deviation (range in parenthesis). Values in a row followed by the same letter are not significantly different according to the Waller-Duncan k-ratio *t*-test (k = 100).

TABLE 6. Morphometrics of cysts of the tobacco cyst nematode complex *Globodera tabacum tabacum* (GTT), *G. t. virginiae* (GTV), and *G. t. solanacearum* (GTS).

Character	GTT-1	GTT-2	GTT-3	GTT-4	GTT-5	GTV-1	GTV-1-X
Length (L)	608.6 ± 79.4 cd	539.6 ± 105.0 fg	642.4 ± 52.1 g	642.3 ± 58.8 h	656.8 ± 67.8 ab	635.5 ± 84.0 bc	555.1 ± 96.4 efg
	(406.0 – 783.0)	(377.0 – 754.0)	(522.0 – 736.6)	(493.0 – 730.8)	(493.0 – 754.0)	(464.0 – 817.8)	(411.8 – 759.8)
Width (W)	598.5 ± 103.8 a	536.5 ± 121.0 bc	610.7 ± 59.6 a	614.0 ± 65.5 a	634.5 ± 75.6 a	557.8 ± 84.2 b	503.6 ± 101.4 cd
	(435.0 – 870.0)	(348.0 – 812.0)	(481.4 – 719.2)	(452.4 – 725.0)	(446.6 – 754.0)	(400.2 – 713.4)	(319.0 – 742.4)
Ridges (R)	8.4 ± 2.0 de	8.8 ± 2.1 ef	8.8 ± 1.9 c	9.4 ± 1.6 de	9.8 ± 2.0 cd	8.3 ± 1.0 efg	9.7 ± 3.0 a
	(4.1 – 5.0)	(5.0 – 15.0)	(6.0 – 14.0)	(6.0 – 13.0)	(6.0 – 15.0)	(6.0 – 10.0)	(5.0 – 20.0)
Anus to edge of	48.7 ± 10.9 bc	44.8 ± 7.9 cde	50.1 ± 12.0 b	45.0 ± 8.6 cde	42.6 ± 8.8 e	47.8 ± 11.2 bcd	59.2 ± 12.5 a
fenestra (AF)	(30.8 – 71.4)	(29.4 – 67.2)	(30.8 – 92.4)	(28.0 – 58.8)	(29.4 – 61.6)	(30.8 – 72.8)	(40.6 – 86.8)
Anus to center of	62.2 ± 11.6 b	59.0 ± 8.7 bcde	60.8 ± 12.4 bcd	56.8 ± 9.7 def	53.5 ± 9.5 f	60.0 ± 12.6 bcde	70.4 ± 13.1 a
fenestra (AFc)	(42.0 – 84.0)	(43.4 – 81.2)	(42.0 – 102.2)	(37.8 – 72.8)	(37.8 – 72.8)	(40.6 – 86.8)	(51.8 – 100.8)
Fenestra length	26.4 ± 4.1 ab	27.6 ± 3.2 a	21.2 ± 2.2 e	23.3 ± 3.9 c	21.8 ± 3.6 de	23.4 ± 4.6 c	21.5 ± 3.5 e
(FEN)	(18.2 – 37.8)	(21.0 – 36.4)	(16.8 – 26.6)	(16.8 – 29.4)	(15.4 – 29.4)	(16.8 – 33.6)	(16.8 – 28.0)
L/W	1.0 ± 0.1 ef	1.0 ± 0.1 f	1.0 ± 0.1 e	1.1 ± 0.1 e	1.0 ± 0.1 ef	1.1 ± 0.1 cd	1.1 ± 0.1 d
	(0.8 – 1.2)	(0.9 – 1.3)	(1.0 – 1.2)	(0.9 – 1.2)	(1.0 – 1.2)	(1.0 – 1.3)	(1.0 – 1.4)
Granek/Hesling	1.9 ± 0.4 def	1.6 ± 0.3 g	2.4 ± 0.6 b	2.0 ± 0.4 cd	2.0 ± 0.4 cd	2.1 ± 0.4 c	2.8 ± 0.6 a
ratio (HES)	(1.1 – 2.8)	(1.0 – 2.4)	(1.4 – 4.7)	(1.3 – 2.8)	(1.2 – 3.2)	(1.4 – 2.9)	(1.5 – 4.0)
M	2.4 ± 0.4 efg	2.2 ± 0.3 h	2.9 ± 0.6 b	2.7 ± 0.7 de	2.4 ± 0.6 de	2.7 ± 0.7 cd	3.3 ± 0.6 a
	(1.6 – 3.3)	(1.4 – 3.0)	(1.9 – 5.2)	(1.4 – 4.2)	(1.5 – 4.2)	(1.7 – 3.9)	(2.0 – 4.5)

GTV-4	GTV-8	GTV-11	GTS-1	GTS-3	GTS-5	GTS-10	GTS-12
460.5 ± 75.3 h	595.7 ± 75.4 d	572.5 ± 72.0 def	579.2 ± 91.1 de	531.7 ± 56.2 g	539.0 ± 94.2 fg	687.1 ± 76.8 a	546.0 ± 84.9 efg
(348.0 – 638.0)	(464.0 – 759.8)	(464.0 – 754.0)	(446.6 – 783)	(394.4 – 638.0)	(377.0 – 783.0)	(464.0 – 812.0)	(382.8 – 725.0)
354.4 – 88.1 g	536.1 ± 82.8 bc	514.5 ± 67.7 cd	519.5 ± 99.3 bcd	436.0 ± 68.0 f	463.0 ± 95.6 ef	624.3 ± 81.9 a	492.6 ± 85.7 de
(266.8 – 609.0)	(388.6 – 667.0)	(382.8 – 667.0)	(377.0 – 730.8)	(301.6 – 580.0)	(290.0 – 661.2)	(435.0 – 754.0)	(348.0 – 667.0)
10.9 ± 1.9 b	10.9 ± 2.5 c	12.0 ± 1.9 b	7.2 ± 1.1 gh	7.4 ± 1.6 h	7.5 ± 1.2 fgh	7.7 ± 1.4 de	8.9 ± 1.8 h
(7.0 – 14.0)	(7.0 – 17.0)	(7.0 – 15.0)	(5.0 – 9.0)	(5.0 – 12.0)	(6.0 – 11.0)	(5.0 – 11.0)	(5.0 – 15.0)
38.1 ± 9.6 f	46.8 ± 12.4 bcde	50.6 ± 9.8 b	48.3 ± 10.2 bc	44.3 ± 7.1 cde	44.6 ± 8.6 cde	43.5 ± 8.0 de	44.4 ± 8.7 cde
(21.0 – 54.6)	(23.8 – 82.6)	(30.8 – 70.0)	(30.8 – 79.8)	(29.4 – 61.6)	(29.4 – 63.0)	(28.0 – 64.4)	(32.2 – 65.8)
46.5 ± 10.5 g	57.1 ± 12.5 cdef	61.3 ± 10.3 bcd	61.6 ± 10.2 bc	57.9 ± 7.9 bcdef	56.8 ± 9.3 def	55.3 ± 9.3 ef	57.3 ± 9.1 cdef
(29.4 – 64.4)	(33.6 – 95.2)	(39.2 – 81.2)	(42.0 – 86.8)	(40.6 – 75.6)	(40.6 – 75.6)	(37.8 – 78.4)	(44.8 – 79.8)
16.7 ± 2.7 f	20.4 ± 2.8 e	21.0 ± 3.9 e	25.3 ± 3.9 b	26.6 ± 3.3 ab	23.8 ± 3.4 c	22.8 ± 3.7 cd	25.7 ± 2.4 b
(12.6 – 22.4)	(15.4 – 26.6)	(14.0 – 28.0)	(16.8 – 33.6)	(19.6 – 33.6)	(16.8 – 32.2)	(12.6 – 30.8)	(22.4 – 30.8)
1.3 ± 0.3 a	1.1 ± 0.1 d	1.1 ± 0.1 d	1.1 ± 0.1 d	1.2 ± 0.1 b	1.2 ± 0.1 c	1.1 ± 0.1 d	1.1 ± 0.1 d
(1.0 – 1.6)	(1.0 – 1.3)	(1.0 – 1.2)	(1.0 – 1.3)	(1.0 – 1.6)	(1.0 – 1.5)	(1.0 – 1.3)	(1.0 – 1.3)
2.3 ± 0.5 b	2.3 ± 0.7 b	2.5 ± 0.5 b	1.9 ± 0.4 cd	1.7 ± 0.3 fg	1.9 ± 0.3 cde	1.9 ± 0.4 cde	1.7 ± 0.3 efg
(1.5 – 3.2)	(1.3 – 4.9)	(1.5 – 3.8)	(1.4 – 2.9)	(1.3 – 2.3)	(1.1 – 2.8)	(1.3 – 3.4)	(1.2 – 2.4)
2.8 ± 0.5 bc	2.3 ± 0.7 b	3.0 ± 0.5 b	2.5 ± 0.4 de	2.2 ± 0.3 gh	2.4 ± 0.3 def	2.5 ± 0.4 def	2.2 ± 0.3 fgh
(1.9 – 3.7)	(0.9 – 4.2)	(2.1 – 4.3)	(1.9 – 3.4)	(1.8 – 2.8)	(1.6 – 3.4)	(1.8 – 4.0)	(1.7 – 2.9)

All linear measurements are in μm. Values are means ± standard deviation (range in parenthesis). Values in a row followed by the same letter are not significantly different according to the Waller-Duncan k-ratio *t*-test (k = 100).

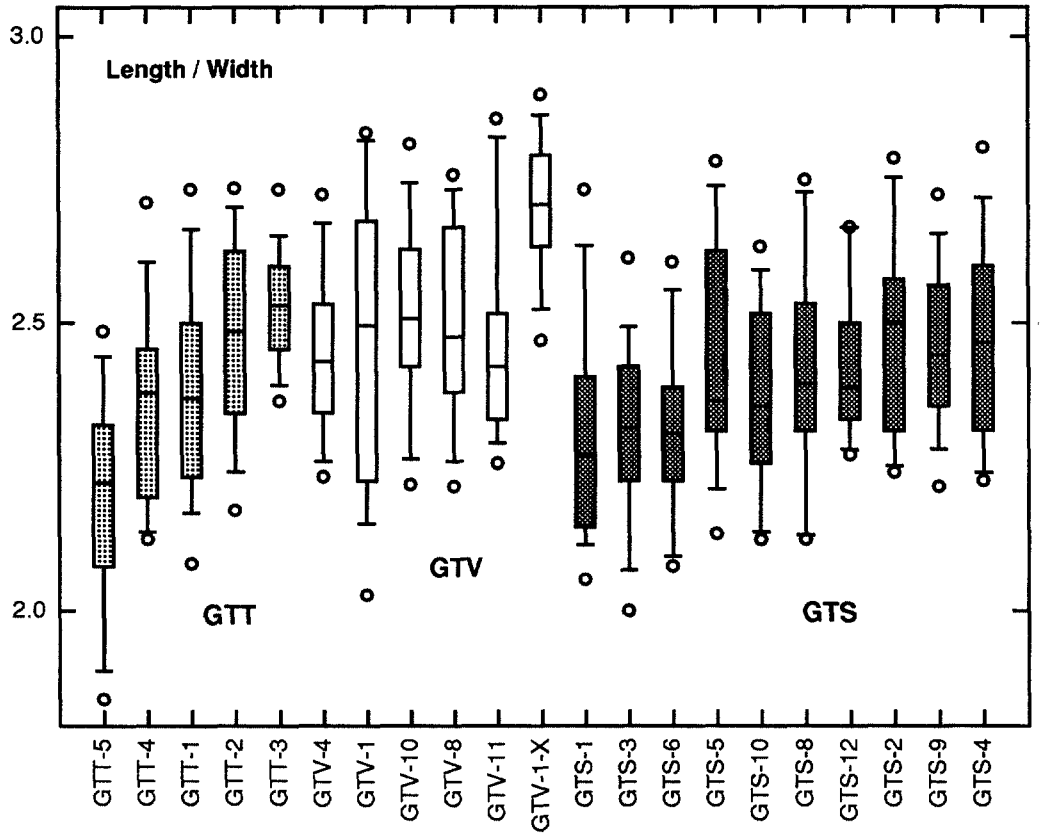

FIG. 1. Morphometrics (body length/width) of eggs of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum*, GTV = *G. t. virginiae*, and GTS = *G. t. solanacearum*.

the grouping did not correlate to subspecific categories. Body length/body width had a good capability to group isolates. The tail terminus/tail length ratio was very homogeneous among the isolates and only GTT-2 was placed in a separate group.

Male (Table 4; Fig. 4): CV was low for most characters, but it was greater than 10% for body length, body width, DEGO, stylet knobs height, tail length, and the 'a' ratio. Ranges of most characters greatly overlapped. DEGO showed a high CV (13.8–20.6%), and is not a useful character. Length of the stylet had very low CV (3.7–5.4), and also discriminated some groups of isolates, but not subspecies. Stylet knob width discriminated three groups of isolates, but two of the groups contained isolates of GTV, and the third contained GTT and GTS. This measurement may be useful as an auxiliary character. GTV-1-X was separated from all other isolates by the lowest mean spicule length. Gubernaculum length discriminated two groups, with GTT and GTV-1 having the highest mean value, and GTV-11 and GTS having the lowest. Tail length was the only character to separate GTT from GTV and GTS. GTT-1 and GTT-2 had the lowest mean tail length. GTV-1-X was the only isolate discriminated from all other isolates by the 'a' ratio, and it had the lowest mean value.

Female (Table 5; Figs. 5,6): Characters generally have a higher CV value than eggs, J2, or males. All characters had a CV higher than 10%, except for the 'a' ratio and stylet length.

GTT-1 was separated from the other isolates usually by higher mean values. Body length separated GTT-1 from the rest. The high CV value among isolates

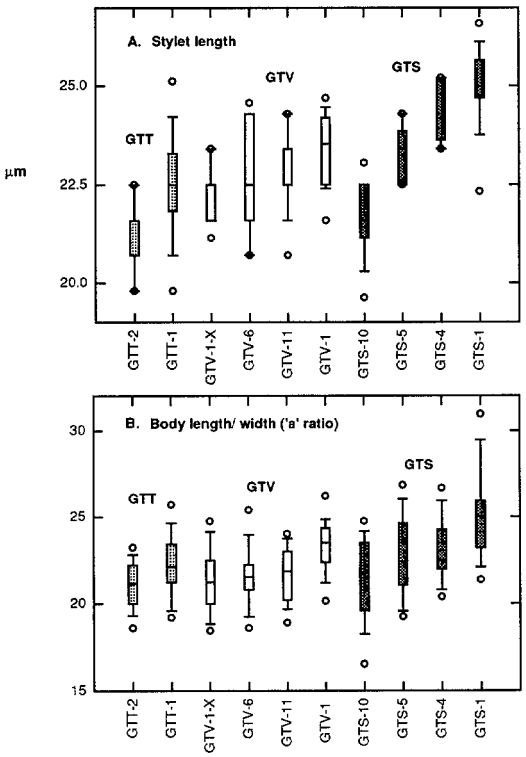

FIG. 2. Morphometrics of second-stage juveniles of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum,* GTV = *G. t. virginiae,* and GTS = *G. t. solanacearum.* A) Stylet length. B) Body length/width ('a' ratio).

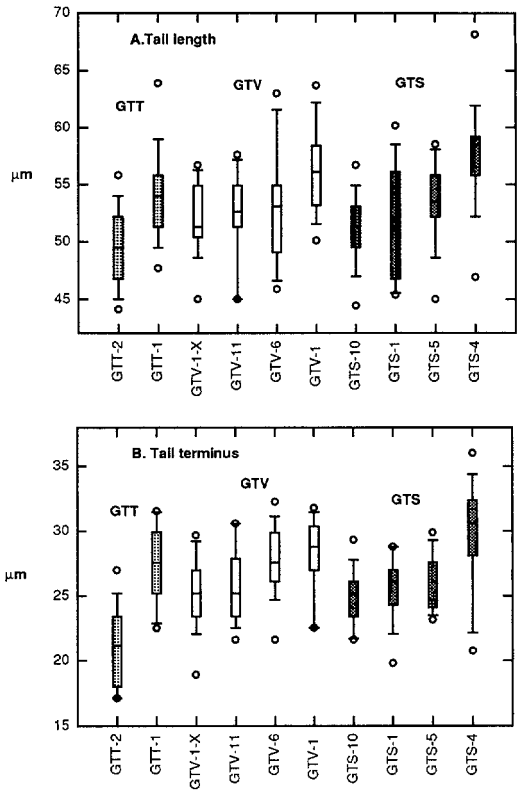

FIG. 3. Morphometrics of second-stage juveniles of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum,* GTV = *G. t. virginiae,* and GTS = *G. t. solanacearum.* A) Tail length. B) Tail terminus.

(17.0–25.3%) of the DEGO revealed that it is very unstable, ranging from 4.4 µm to 5.8 µm. Stylet length was stable (CV = 4.2–9.8%) and separated GTS-1 from the other isolates. Stylet knob width clearly separated GTV-11 from the other isolates. It had the highest mean (6.0 µm), which is correlated from the wide dorsal knob shape characteristic for this subspecies. Stylet knob height discriminated two groups, but isolates of the three subspecies were included in each group. Distance of the anus to center of the fenestra had a smaller CV (14.3–21.1%) than distance of anus to edge of fenestra (17.3–22.5%). Two groups were formed by fenestra length, but the three subspecies were represented in one group. GTT-1 had the highest mean of vulval slit length, and it was separated from the other isolates. GTT-1 and GTT-2 were clearly separated from GTV and GTS by the 'a' ratio, the only female character that distinguished GTT from GTV and GTS.

Cyst (Table 6; Fig. 7–8): Characters showed a relatively high CV similar to females. The only character with a CV less than 10% was the 'a' ratio. Most characters were not useful for subspecies identification because of overlapping.

A new ratio, M, the distance of anus to center of fenestra divided by the fenestra length, was less variable than the Granek–Hesling ratio (3,6), which is calculated from the anus to the edge of the fenestra. GTV-4 had the lowest body length mean value (460.5 µm) and consistently had the lowest mean for several other characters. Isolates GTV-1-X and GTV-4 had extreme values for the anus to edge of the fenestra distance character (59.2 and 38.1 µm, respectively). Similarly, the distance of the anus to center of the fenestra was ex-

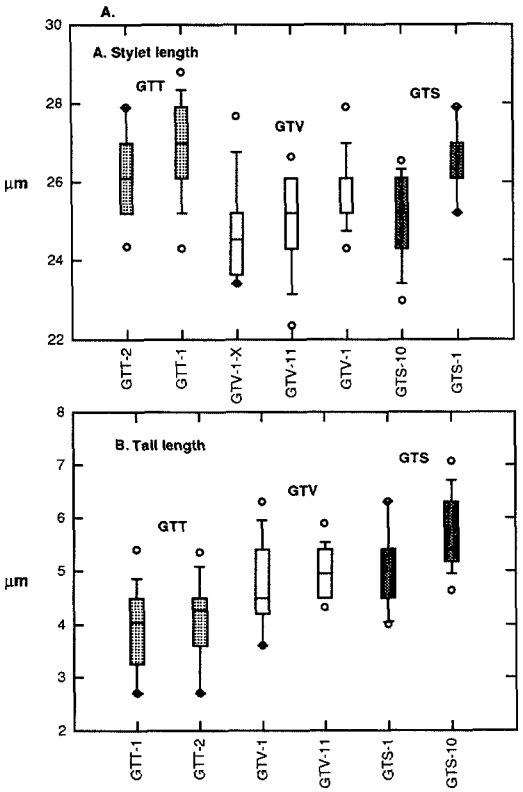

FIG. 4. Morphometrics of males of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum*, GTV = *G. t. virginiae*, and GTS = *G. t. solanacearum*. A) Body length. B) Tail length.

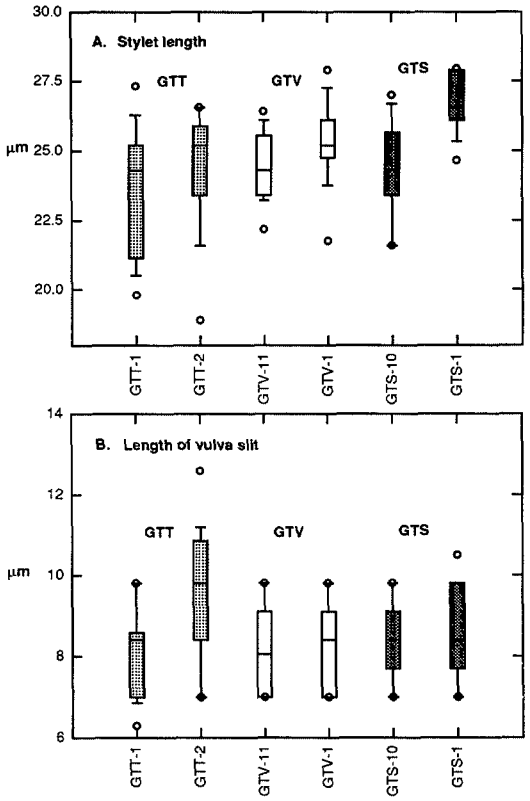

FIG. 5. Morphometrics of females of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum*, GTV = *G. t. virginiae*, and GTS = *G. t. solanacearum*. A) Stylet length. B) Length of vulval slit.

treme for GTV-1-X and GTV-4 (70.4 and 46.5 µm, respectively). Three GTV isolates (GTV-1-X, GTV-4, and GTV-11) had the highest mean for the number of ridges between the anus and fenestra. The five isolates of GTT were clearly discriminated from the isolates of GTV and GTS by the 'a' ratio. The value 1.0 was correlated to the round shape of the cyst compared to 1.1–1.3, which was related to the ellipsoid or ovoid shape of cysts of GTV and GTS. Cyst shape was the only character that distinguished GTT from GTV and GTS. Granek-Hesling and M ratios were generally higher for GTV isolates than in GTT and GTS, and they may be useful supplemental characters.

DISCUSSION

The measurements of eggs fail to discriminate the subspecies of the TCN complex. The low CV of these characters, however, indicates that they may be useful to compare this complex with other species of cyst nematodes.

Second-stage juveniles have been traditionally used for differentiating species of heteroderids (18,21). Some authors consider measurements of J2 to be the most reliable because of the narrow limits of their size range (18). The CV of isolates of the TCN complex are low; however, few characters discriminated groups of isolates or subspecies. As with eggs, J2 measurements overlap extensively. The best discriminating characters are tail terminus and stylet lengths, even though they do not identify subspecies. GTS-4 is frequently discriminated from all other isolates on the basis of several characters, including body length, median bulb to head end, excretory pore to head end, DEGO, stylet

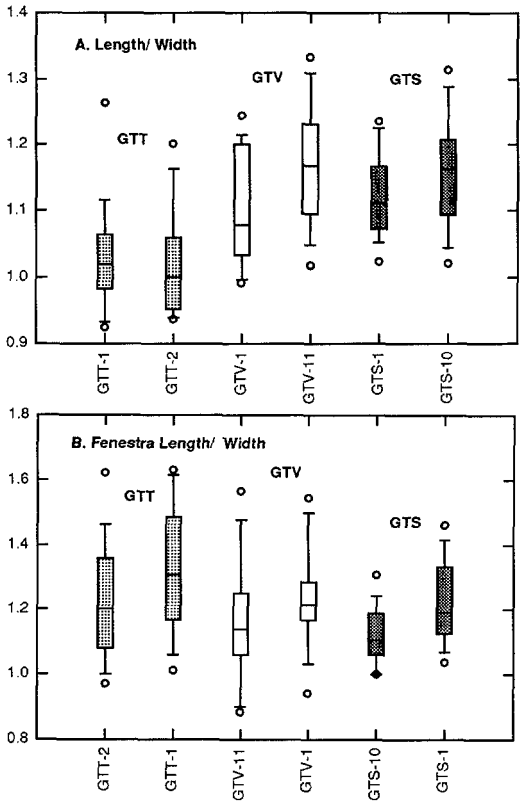

FIG. 6. Morphometrics of females of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum*, GTV = *G. t. virginiae*, and GTS = *G. t. solanacearum*. A) Body length/width. B) Fenestral length/width.

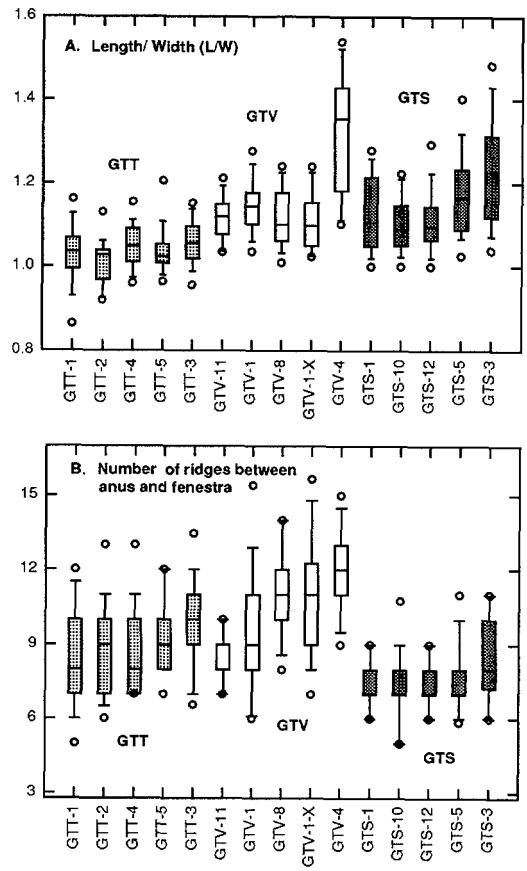

FIG. 7. Morphometrics of cysts of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum*, GTV = *G. t. virginiae*, and GTS = *G. t. solanacearum*. A) Length/width (L/W). B) Number of ridges between the anus and fenestra.

length, tail terminus length, and the 'a' ratio. The distance of the center of the median bulb to the anterior side is stable (CV = 3.2–6.7%), despite concern by some authors (18) that it is not reliable because of the differences in the contraction of the esophagus.

Characters of males are quite stable, and most have a CV below 10%. DEGO, however, has a high CV (13.8–20.6%) and is not useful as a morphometric character. Tail length is useful for separating GTT from GTV and GTS. The measurement of more isolates of GTT may clarify the value of this character. As with J2, the median bulb to head end distance is stable (CV = 6.6). The stylet length (CV = 4.5) and the spicule length (CV = 5.0) are also useful characters. Stylet knob width may be a useful auxiliary character in the taxonomy of the heteroderids. Despite the fact that the gubernaculum cannot discriminate among subspecies, GTT has higher values (11.9–12.1 μm) than the other two subspecies, and its length may be a useful character for identifying geographic isolates of GTT.

Except for the 'a' ratio, there are no useful morphometric characters of females for discriminating the three subspecies. Frequently, a discriminating group contains isolates from all three subspecies. The 'a' ratio is the only character that discriminates GTT from GTV and GTS and that has a low CV of 8.4. The ratio is 1.0 for GTT, which indicates a more rounded shape versus 1.1–1.2 in GTV and GTS, typical of a more ellipsoid or ovoid shape. Cyst shape is very useful for identifying

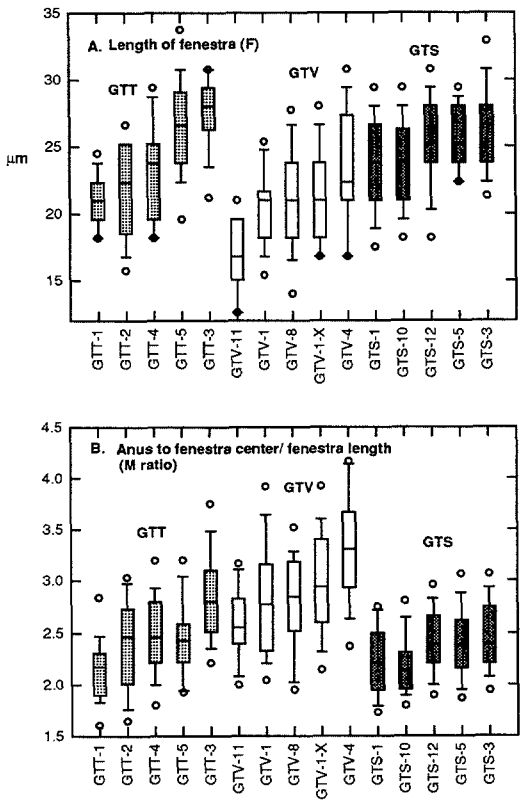

FIG. 8. Morphometrics of cysts of the tobacco cyst nematode complex. GTT = *Globodera tabacum tabacum*, GTV = *G. t. virginiae*, and GTS = *G. t. solanacearum*. A) Length of fenestra. B) Anus to fenestral center/fenestral length (M ratio).

the subspecies. Stylet length is stable (CV = 4.2–9.8%). The anus to the center of the fenestra distance is more useful than the anus to the edge of the fenestra because its CV is lower. The DEGO is not a useful character because it has a high CV (17.0–25.3%). The larger values of knob width of GTV are related to the size and shape of the dorsal knob previously described in the literature (11). GTT-1 stands out frequently as having the highest value for all characters of females. Fenestral length places all isolates into one group.

The CV of most characters of cysts, similar to females, is high (15–20%), and they are not reliable for identification. The Waller-Duncan test fails to separate isolates for most of the characters. Also, as in females, the 'a' ratio (CV = 8) is the only character that discriminates GTT from GTV and GTS. Similarly the 1.0 'a' ratio of GTT indicates a more rounded shape. The M ratio is less variable than the Granek–Hesling ratio. The M ratio may be used in conjunction with other more reliable characters such as female stylet knob shape and terminal area morphology, to confirm identification of GTV isolates. In conclusion, only the 'a' ratio of females and cysts combined with tail length of males are useful in separating the three subspecies.

Behrens (1) used several characters to differentiate GTV from GTT. The J2 stylet knob was wider in GTV (5.0–5.5 µm) than in GTT (4.0–5.0 µm). Our data, based on several isolates of GTT and GTV, failed to support that observation (Table 3). The anus to fenestra distance mean values showed much higher variability (1) than our observations. However, two GTV isolates, GTV-1-X and GTV-11, have higher mean values than all of the GTT isolates, which agrees with Behrens' observations (1).

Stone (19) used principal coordinate analysis of five J2 characters to differentiate GTT from GTV and GTS. Four cyst characters differentiated GTT and *G. pallida* from GTV and GTS (19); thus GTT is more similar to *G. pallida* than the two subspecies of tobacco cyst nematodes that occur in Virginia. Miller (9), using one isolate each, showed significant differences between GTT and the group formed by GTV and GTS based on Granek's ratio (3) as modified by Hesling (6) and J2 stylet length. Our data, using many more isolates, do not corroborate these findings. Our data do not separate GTT, GTV, and GTS for either character. Greet (5) was unable to separate the three subspecies using measurements of certain J2 and male characters.

We consider this complex of three subspecies as having a continuum of values for the majority of the observed characters. The data suggest a closer relationship between GTV and GTS. These two subspecies occur in Virginia in very close proximity to each other. There is a 38-km separa-

tion between the closest adjacent geographical ranges of these two subspecies (12), and they are possibly conspecific (15). GTT occurs 700 km away in the northeastern part of the United States (10).

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