MORPHOLOGICAL CHARACTERIZATION OF THE TOBACCO CYST NEMATODE COMPLEX, GLOBODERA TABACUM SSPP. TABACUM, VIRGINIAE, AND SOLANACEARUM (NEMATA: HETERODERINAE).

by

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Morphological characterization of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum, virginiae, and solanacearum (Nemata: Heteroderinae).

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(Abstract)

A morphological and morphometrical study was made of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum (GTT), virginiae (GTV), and solanacearum (GTS) including observations of eggs, second-stage juveniles (J2), males, females, and cysts. Observations focused on the anterior region including head shape, lip pattern, stylet morphology, and the tail region including tail shape in J2 and spicules in males. The head region of J2 was set off from the body and consisted of three head annules, six lips, and an oral disk. The shape of the head seen in lateral view showed little variability within or among the subspecies. Stylets of J2 were robust and had three anchor-shaped, rounded knobs. They varied slightly in width and height within each subspecies. The lip region consisted of a central oral disc surrounded by two lateral and two submedial lips. The oral disc varied from rectangular to elliptical. The submedial lip pairs were not fused in all specimens. The tail of J2 was finely pointed and the tip was rounded in all subspecies. The head of the male was set off and contained five head annules, six lips, and an oral disc. The head region was similar in lateral view in all three subspecies. The stylet was robust and had three rounded knobs that slope posteriorly. The dorsal knob of males of GTV appeared to slope more posteriorly than in the other two subspecies. In the SEM, the head region had a large central rounded to elliptical oral disc with two lateral and two submedial lip pairs. The submedial lips were rounded or rectangular. The submedial lip pairs were fused in most specimens, but not all. The spicules of GTT showed a slightly more enlarged head region than the other two subspecies. The three subspecies could not be separated on the basis of any character or group of characters of J2 or males.The anterior region including body shape, head shape, lip pattern, stylet morphology, and the terminal area in females; and body shape and terminal area of cysts were observed. The most useful characters to separate the three

subspecies were female body shape, stylet knobs, perineal tubercles, cyst shape, anal-fenestral ridge pattern, anus, and tail region. GTT was characterized by rounded females and cysts with sharply back sloped stylet knobs, clumped perineal tubercles in the vulval region, tight parallel ridges in the anal-fenestral region of the cyst, and a distinct tail region not shaped like a crescent. GTV was characterized by its ovoid to elipsoid female and cyst shape, the "Dutch shoe" shape of the dorsal stylet knob, the more individualized perineal tubercles, a maze-like pattern of ridges in the anal-fenestral region, and an indistinct anus. GTS was characterized by its ovoid to elipsoid female and cyst shape, moderately backward sloped stylet knobs, more widely separated ridges, a distinct anus, and a tail region usually shaped like a crescent. Much variability in shape and patterns was visible among all the isolates of the different subspecies. Tubercles in the neck as well as bullae were reported and discussed. A morphometric evaluation of second-stage juveniles (J2), males, females, and cysts was performed for several characters. Morphometrics of eggs, J2, and males were considerably less variable than in females and cysts. No measurements of eggs and J2 were useful for identification of the three subspecies. The distance of the median bulb to the head end and the distance of the excretory pore to the head end in J2 and males were quite reliable and useful. The stylet knob width of males was useful for identifying GTV isolates. Tail length was useful in separating males of GTT isolates from GTV and GTS. The body length/ width (L/W) ratio of females and cysts discriminated GTT from GTV and GTS. Stylet knob width of females was an auxiliary character useful for identifying GTV. This subspecies complex has a continuum of values for the majority of the characters observed. The data suggests a closer relationship between GTV and GTS which is likely because these two subspecies occur in very close proximity in Virginia, 38 km separation between closest adjacent geographical ranges; whereas GTT occurs 700 km away in the northeastern U.S.

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Part I. Morphology and identification of cyst nematodes of the genera Heterodera and Globodera (Nemata: Heteroderinae). A literature review.

ABSTRACT

The major contributions to the morphology and identification of cyst forming species of nematodes of the genera Heterodera and Globodera are reviewed. After discussing the early research, aspects of cyst nematode morphology including the cuticle, cyst wall, anterior region, vulval cone and terminal area, sperm, and spicules are described. Potato cyst, cereal cyst, soybean cyst, tobacco cyst, and the Heterodera trifolii "complex" are evaluated for morphological differences. Because of their agricultural importance and because morphology is still the main tool for identification, emphasis is placed on specific problems such as the integration of morphology with recent biochemical and molecular biology approaches, as well as utilization of the maximum potential of techniques such as the scanning electron microscope including the correct preparation and viewing of specimens. A general reappraisal of the subfamily Heteroderinae, with utilization of recent advances in computer programs is suggested, due to the confusion still existing in many groups of species .

INTRODUCTION

The purpose of this paper is to review the major contributions on the morphology of the cyst nematodes, Heterodera and Globodera spp., to provide a guide for both the general reader and the student trying to identify species or pursuing research on this topic. Species descriptions (more than 60 for Heterodera) are not included unless a new and important morphological character was described. However, a list of the species of Heterodera and Globodera (Tables 1-3), a compendium for identification of Globodera spp. (Table 4) and Heterodera spp. (Table 6), as well as a key to the family Heteroderinae (Table 5) are provided. The genera Punctodera, Cactodera, and Dolichodera are mentioned only because their species were previously placed in the genus Heterodera.

Morphology is still the most reliable and widespread method for identification (Golden, 1986; Baldwin and Mundo-Ocampo, 1991) even though nematodes are known for their paucity of observable characters. Scanning electron microscopy (SEM), transmission electron microscopy, and interference contrast microscopy have expanded the limits of our observations (Hirschmann, 1983; Eisenback, 1985; 1991). However, good photographs are still needed to clarify particular aspects of cyst nematode morphology. Although most descriptions provide good drawings, many do not include high quality light and scanning micrographs. Some structures have not been adequately illustrated; for example, details of the vulval cone of Heterodera spp. and terminal area of Globodera spp. have not been thoroughly examined with SEM. Sometimes specimens examined by SEM and light microscopy (LM) are not adequately preserved by the fixation and preparation techniques. New approaches utilizing computer programs to clarify the phylogeny of cyst nematodes promise a better system for identification (Baldwin, 1988; Baldwin and Schouest, 1990).

New and exciting methods are being developed for species identification such as 2-D protein analysis (Bakker et al., 1988), esterase polymorphism (Esbenshade and Triantaphyllou, 1988; Radice et al., 1988), monoclonal antibodies (Schots, 1990) and DNA-RFLP analysis (Kalinski et

al., 1988; Burrows, 1990). However, identification of species is based primarily on morphological observations and no practical biochemical kit for use in routine diagnostic labs is currently available. In most instances biochemical and DNA methods have confirmed the status of species that were established by morphological observations. For example, G. pallida and G. rostochiensis have been differantiated by two cloned DNA fragments (Burrows and Perry, 1988). The biochemical and DNA characterizations have been most useful at the subspecific level, and have provided additional information on intraspecific variability (Burrows, 1990) because of small morphological variability within Heterodera and Globodera spp. Since dispersal of cyst nematodes in the field is relatively limited, some species have become genetically isolated thus facilitating speciation. This is evident, for example, in the existence of some reproductive incompatibility among races of H. glycines (Price et al., 1978).

Whenever the three taxa "pathotype", "race", and "subspecies" are used, they create much controversy (Sturhan, 1985; Huettel et al., 1990). "Pathotype" refers to a pathogen population capable of reproducing on a plant host with genetic resistance to other populations of the same pathogen species (Dropkin, 1988). In this review pathotypes are used in potato cyst and cereal cyst nematodes. "Race" is a population of a certain pathogen species with distinct characters, morphological and/ or physiological (Dropkin, 1988). In practical terms races or pathotypes are distinguished with differential hosts. More recently race has been defined as an infrasubspecific term for a group of nematodes whose members are distinguishable by a single characteristic of composition, appearance, behavior, or host range, (Huettel et al., 1990). Race, population, and isolate, are acceptable infrasubspecific categories used for nematodes. While British nematologists commonly use the term pathotype, Americans prefer the designation race (e.g. root-knot and soybean cyst nematode races). In some instances different pathotypes have been distinguished morphologically , resulting in new species. The term "subspecies", an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species

(Mayr and Ashlock, 1991), has been utilized only for the tobacco cyst nematode (TCN) complex (Stone, 1983).

In summary, this review is intended to evaluate the most important morphological characters that are useful for identification of cyst nematodes. These characters are helpful for constructing keys and phylogenetic trees. This review will provide the student and the general reader with an understanding of the basic morphology of cyst nematodes and a discussion of the characters that are most useful for identifying the subspecific categories of the economically most important species of cyst nematodes.

EARLY CONTRIBUTIONS

The first recorded observation of a cyst nematode was made by Schacht (1859) on sugarbeet roots in Germany. It was named Heterodera schachtii A. Schmidt, 1871 to honor its discoverer (Schmidt, 1871). Chatin (1887) designated the brown round/lemon-shaped bodies as "cysts" and described them in a communication to the French Academy of Sciences, presented by Louis Pasteur.

Wollenweber (1923) described a new species of cyst nematode infesting potatoes, H. rostochiensis Wollenweber, 1923, on the basis of the unique round shape of the cyst (Fig.1) compared to the lemon shape of H. schachtii. Later, this morphological feature was considered sufficient to establish a sub-genus (s.g.), H. (s.g. Globodera) (Skarbilovich, 1959), and finally a separate genus, (Behrens, 1975). Mulvey and Stone (1976) had also established the genus Globodera independently from Behrens, however, the authority belongs to this last author (Decker and Loof, 1979).

During the first half of this century many new cyst nematodes were recognized that were parasitizing different crops. They were always considered as "strains" of H. schachtii because they were thought to be morphologically identical and differ only in host range. Meanwhile, new morphological characteristics were recognized such as the morphology of the esophageal

glands and the shape of the stylet among several important genera including Aphelenchus, Tylenchus, and Heterodera (Goodey, 1929). Investigators differed in their opinion of the importance of morphological characters in the recognition of a new taxon. Triffitt (1928) concluded that the so called "potato strain", described by Wollenweber as H. (=G.) rostochiensis, should not be recognized as a new species because the major differences were only in the shape of the cyst (round vs. lemon) (Fig. 1) and the size of the male spicules (Fig. 9). The shape of the cyst, however, is now accepted as the major character for separating the two genera, Globodera and Heterodera. In a later publication (Trifitt, 1929) she provided more details on the dimensions of cysts, eggs, second-stage juveniles, and males. This paper was the first major report on cyst nematode morphology utilizing statistical analysis.

Mary Franklin (1939) initiated a series of studies which provided a basis for the independent status of several new species of cyst nematodes. She reported on the occurrence of the subcrystaline layer, a palisade-like, fatty acid-rich structure overlaying the cuticle of some cyst nematodes and apparently secreted by them (Fig. 2A). The importance of the gelatinous sac secreted by females of certain species to protect the eggs was recognized by Franklin, and differences in the structure of the cyst wall were also noted (zig-zag and punctations) (Fig. 2C-F). Later, the punctations in the cyst wall became an important character and were used to describe a new species, H. (=Punctodera) punctata (Thorne, 1928), which was later recognized as a new genus, Punctodera (Mulvey and Stone, 1976).

New morphological characters, such as dimensions of second-stage juveniles, were used in combination with host range and the effect of different chemicals on hatching to diagnose new species (Franklin, 1940a,b). As a result of these studies four new species were recognized: H. schachtii Schmidt, 1871; H. rostochiensis Wollenweber, 1923; H. goettingiana Liebscher, 1890 and H. major (O. Schmidt, 1930), later synonymized with H. avenae (Wollenweber, 1924). This report was complemented by additional work (Fenwick and Franklin, 1942; 1951) that used analysis of variance to compare the lengths of second-stage juveniles. Franklin (1951)

summarized the main concepts concerning morphology and species nomenclature in an important book: "The cyst-forming species of Heterodera". Two species, H. (= Cactodera) weissi and H. carotae Jones, 1950 were added to the five recognized species. Franklin's major contribution was to elevate most of the so-called "strains" of H. schachtii to species level based on characters such as the length of the J2 and the size and shape of male spicules (Franklin, 1940b).

Shortly after Franklin's work, the soybean cyst nematode (SCN) H. glycines Ichinoe, 1952, was described with much biological and morphological detail (Ichinoe, 1952; 1961). Ichinoe placed H. glycines within the "schachtii" group of Heterodera. The SCN was distinguished from the very similar H. trifolii by the abundance of males and maturation on soybean and not clover. Hirschmann (1956) made a detailed comparison between H. glycines and H. trifolii and reported major differences between J2 in body length, shape and size of the stylet, distance from base of the stylet knobs to the dorsal esophageal gland opening (DEGO), shape and length of the tail, and length of the tail terminus (Fig. 11E-F).

Cooper (1955) emphasized the morphology of the vulval cone, and was the first to use the term "bullae" to designate the brown bodies found inside the cone, previously designated by Franklin (1940) as "knob-like projections" (Fig. 4). He divided cyst nematodes as "bullata" (with bullae in the vulval cone) and "abullata" (without bullae), which was important in distinguishing H. glycines (with bullae) from the closely related H. trifolii and H. goettingiana (without bullae).

Granek (1955) established the ratio B/A (B=distance from anus to closest edge of the vulval opening and A=diameter of the vulval opening). This ratio is quite important in distinguishing Globodera species and has been redefined by Jones (1962) and Hesling (1973). It can not be applied to Heterodera species because the anus and vulva are situated on a cone rather than on a relatively flat surface (Fig. 6). In both genera, an opening (=fenestra) for the release of J2 may be formed as a result of the rupturing of the vulval tissues.

The cyst wall was extensively investigated by Wieser (1953) and by Ferris and Siegel (1957).

Oostenbrink and den Ouden (1954) utilized the vulval cone to distinguish H. schachtii from H. trifolii. Mulvey (1957) established differences in the depth of the underbridge, a muscular structure inside the vulval cone (Figs. 4, 5) of H. schachtii, H. trifolii and H. glycines. He also considered cyst volume, position of bullae, and fenestral length to be differentiating characters. Kämpfe (1960) examined tails of J2 of more than 200 000 specimens and reported much variability. Other authors described abnormalities of J2 as sources of variability (Onions, 1953; Mulvey, 1960a; 1960b). Collection and preparation methods were also considered to cause variation (Stone, 1971).

THE CUTICLE AND THE CYST WALL

Three major morphological characters, color changes, surface morphology, and layering, have been evaluated for their utility as morphological characters. Very few researchers have studied changes in color (chromogenesis). Guile (1966; 1967; 1970) related the color changes in females and cysts to the different pathotypes of potato cyst nematode (PCN) even though they were thought to be morphologically identical (Shepherd, 1965). Guile noted that cysts of pathotype A of H. (= G.) rostochiensis had an intermediate golden color, wheras pathotypes B and E changed directly from a pale yellow to a dark brown color, typical of the mature cyst. Pathotypes B and E were later described as a new species, Heterodera pallida (Stone, 1972a). Tanning of the cyst wall is of primary importance. The cyst is defined as: " ... a persistent tanned sac which retains eggs and is derived from some or all components of the mature female body wall" (Luc et al., 1986).

The first observations of the cyst wall surface of several cyst nematode species were made by Franklin (1939) who suggested that the striated and zig-zag patterns visible in the mid-region of the cyst, resulted from the annules of the J2. This developmental process in the different genera of Heteroderinae is still not clearly understood (Baldwin and Mundo-Ocampo, 1991). The utility of cuticular patterns in the mid-region of the cyst for identification and taxonomic

purposes has been studied by several authors (Wieser, 1953; Taylor, 1957; Wilson, 1969; Lamberti, 1971). They reported fine, coarse, excavated, reticulated, lace-like, and punctuated variants of the major striated and zig-zag patterns (Fig. 2C-F). The arrangement of punctations may have value for identification of Punctodera and Globodera. The genus Punctodera was split from Heterodera because, among other things, it has a very characteristic and noticeable punctation (Fig. 2G) (Thorne, 1928; Mulvey and Stone, 1976).

The neck region of females and cysts has received some attention because it attaches the nematode to the root (Shepherd and Clark, 1978). The surface of the neck region has protuberances (= tubercles) that are typical for the genus Globodera (Fig. 2I-J) (Baldwin, 1988; Othman et al., 1988). These protuberances appear to result from the fragmentation of the annules (Fig. 2J) (Mota and Eisenback, unpublished).

Stratification of the female cuticle of heteroderines has been well studied. The cuticle is highly resistant to adverse environmental conditions, including other competing pathogens, and provides cyst nematodes with an extraordinary means of survival (Shepherd et al., 1972; Cliff and Baldwin, 1985). The stratification of the cuticle is also useful in their taxonomy. Transmission electron microscopy (TEM) has revealed two typical Tylenchida layers, A and B, and a relatively thick fibrous C layer present in most cyst nematodes. Globodera possesses an extra D layer (Fig. 2H; Table 10) (Gunther and Kämpfe, 1966; Wisse and Daems, 1968; Shepherd et al., 1972). The cyst wall of several species of Heteroderinae displays a subcrystaline layer (SCL) (Fig. 2A-B), a pallisade-like layer external to the cyst wall (Kirjanova, 1969; Brown et al., 1971) first noticed by Chatin (1887). This layer may be of taxonomic value (Baldwin and Mundo-Ocampo, 1991).

Cyst wall structure has also been used to evaluate the phylogeny of the genera of Heteroderinae (Cliff and Baldwin, 1985; Baldwin, 1986; Baldwin and Schouest, 1990). As a result of these tests, Cactodera, Punctodera, and Globodera was considered to form a monophyletic group based on the presence of the D layer and a superficial layer impregnated

with electron dense substance (Table 10). The D layer was suggested to have been lost in Heterodera, although some authors provide evidence to the contrary (Cordero and Baldwin, 1990). The same authors have recently described an additional E layer (with subdivisions E_1, E_2) in aging H. schachtii females (Cordero and Baldwin, 1990) which is absent in C. cacti (Cordero et al., 1991). The E layer may be responsible for the formation of bullae (Cordero and Baldwin, 1990).

THE ANTERIOR REGION

Studies on the morphology of the anterior cephalic region were limited because of the difficulty in observing "en face" patterns of J2, males, and females, but the SEM has made these observations possible. Rivoal (1974) was one of the first to show differences among lip patterns of J2 of H. avenae , G. rostochiensis, and G. pallida. Stone (1972b) was the first to use the lip pattern of J2 to separate Heterodera and Globodera (Fig. 3): Heterodera J2's have sub-lateral lips that are fused with a long oral disk, whereas Globodera J2's have two pairs of sub-lateral and a separate eliptical oral disk. Stone (1972b) separated H. (= G.) rostochiensis pathotype A from B and E based on the oval shape of the lips and oral disc in A versus the more rectangular shape in B and E. This information was used to validate the occutence of two separate potato cyst nematode (PCN) species. Observations of head morphology of J2 using the SEM were the basis for Stone (1975) to subdivide cyst nematodes into six groups (Table 8).

Stone (1975) compared his groupings using lip pattern of J2 with cyst shape (Mathews, 1971; Mulvey, 1972; 1974) (Tables 8, 9). Other authors have proposed an additional group to Mulvey's scheme (=group VI) because of the variability of the bullae and underbridge (Sharma and Swarup, 1983). This new group accomodates H. graminophila Golden and Birchfield, 1972, H. canadensis Mulvey, 1979, and H. graminis Stynes, 1971.

Momota and Ohshima (1976) utilized SEM to distinguish J2 and males of H. elachista, H. avenae, H. glycines,and G. rostochiensis on the basis of lip morphology. Intraspecific variation

of lip pattern was shown with SEM among the different genera of Heteroderinae, in particular in J2 and males (Othman et al., 1988). Recently, SEM of cephalic morphology helped distinguish C. milleri Graney and Bird, 1990 from C. cacti (Filipjev and Schuurmans Stekhoven, 1941) Krall and Krall, 1978 (Graney and Bird, 1990).

Very few observations have been made of isolated stylets of cyst nematodes despite the reliability and usefulness of this technique in understanding morphological diversity (Eisenback and Rammah, 1987). The stylet tip of G. rostochiensis was observed by SEM (Ellenby and Wilson, 1969). Small morphological differences were apparent in stylets of J2 and males of the three different subspecies of the tobacco cyst nematode complex (Mota and Eisenback, 1990; 1991; 1992) The dorsal knob of G. tabacum virginiae males appears to slope more than in the other two subspecies, G. t. tabacum and G. t. solanacearum (Mota and Eisenback, 1992).

The ultrastructure of the anterior region of cyst nematodes has been studied in H. glycines, although not for purposes of identification (Baldwin and Hirschmann, 1975a,b; Endo, 1980; 1983; 1984; 1985; 1986; 1987). Phasmids of J2 and males have also been examined (Baldwin, 1985; Carta and Baldwin, 1990). The pore type or lens-like configuration of the phasmid is useful in J2 to differentiate genera (Baldwin, 1988). Phasmids in males are either absent, as in H. schachtii (Carta and Baldwin, 1990), or are very difficult to observe. The width of the esophageal gland in relation to total body width may also be a useful character in describing J2 (Baldwin, 1988).

VULVAL CONE AND TERMINAL AREA

The morphology of the posterior region of cyst nematodes, the vulval cone in Heterodera and the terminal area in Globodera, is second only to the general cyst shape in importance for identification. Franklin (1940a;1951) first studied details of this region and identified or named the different structures such as the vulva, anus, bullae, etc.

Cobb and Taylor (1953) included details of the posterior region in a description of a new

species, H. (= G.) leptonepia. Currently terminology was established by Cooper (1955), who constructed a key based on vulval cone characters. Other authors have utilized characters of the vulval cone for constructing keys for species or "groups" of species (Taylor, 1957; Oostenbrink, 1960; Granek, 1968) (Table 7), or to make identification charts (Fig. 7) (Jones and Jones, 1984).

The major contribution to the identification of species based on the posterior region of cysts was made by Mulvey (1957, 1972, 1973, 1974). In his initial study (Mulvey, 1957), the author differentiated three species, H. schachtii, H. trifolii, and H. glycines, by the position of the bullae, depth of the underbridge, fenestral length, and vulval slit length. Cyst shape, structures of the vulval cone, and the terminal area were utilized to construct a key of the species of Heterodera (Mulvey, 1972). The morphological characters used in Mulvey's key were the fenestra, underbridge, bullae, basin, vulval bridge and slit, cyst size and shape, and Granek's ratio (Figs. 4, 5).

The fenestra may be circumfenestrate, ambifenestrate, or bifenestrate (Figs. 4A-D; 6B-C). A circumfenestrate cyst has only one opening, whereas an ambifenestrate or a bifenestrate cyst displays two semifenestrae (Figs. 4B-D; 5A-B). The underbridge, when present, varies in length and shape. Some species display a second bridge, Mulvey's bridge, perpendicular to the underbridge (Fig. 4E). The presence or absence of bullae may distinguish some species. Besides bullae, other bodies associated with the terminal area or vulval cone may be present (Wilson, 1968). The width of the vulval bridge and the vulval slit may also distinguish some species. Lemon shaped cysts with a prominent vulval cone distinguish Heterodera spp. from the round shaped cysts of Globodera (Figs. 1; 6A). Granek's ratio is an important measurement in distinguishing some Globodera spp.

Green (1971) and Mulvey (1973) examined the perineal area of round cyst nematodes utilizing SEM. Green observed the area between the anus and the vulva of certain round cyst, and pointed out several useful characters including size, the number of ridges, and type of

branching of the ridges. He used the size and form of the papillae in the vulval area to characterize species of the tobacco cyst nematode complex, as well as H. (=G.) rostochiensis. Green's SEM analysis (1975) indicated that the vulval cone surface morphology is a stable character within species of Heterodera.

Vulval cone morphology was compared among members of the H. trifolii complex by Hirschmann and Triantaphyllou (1979). Stanger and Noel (1988) utilized the SEM to detect differences in the vulval cone of several cyst nematode species and among soybean cyst nematode races. Combined LM, TEM, and SEM observations of the posterior cone of H. schachtii and C. cacti have proved useful to understanding the phylogeny of Heteroderinae (Cordero and Baldwin, 1991; Cordero et al., 1991).

SPERM AND SPICULES

Spicules in cyst nematode males were first observed and recorded by Triffit (1929). Franklin (1951) recorded the mean length to be 25μm. Skarbilovich (1959) utilized the shape of the spicule tip to differentiate between H. (s.g. Heterodera) and H. (s.g. Globodera), the former having bifid tips and the latter having single tips (Fig. 9E-F).

Spicules are highly innervated cuticular male copulatory organs (Fig. 9A-B). Clark et al. (1973) were the first to study spicule and sperm ultrastructure with the TEM and the SEM. The pores near the tip apparently have sensory functions in determining the location of the female vulva (Clark, et al., 1973).

Although most of the work related to sperm and spermatogenesis has used TEM to view their ultrastructure (Shepherd et al., 1973; 1983), the SEM may be useful for understanding relationships among species and for identification of species on the basis of the morphology of sperm. A cyst nematode sperm is of a non-flagellate ameboid type with no granulation, similar to that of other nematode genera (Fig. 9C-D). According to Shepherd et al. (1973), a sperm of Heterodera does not undergo the different stages of chromatin transformation as in Globodera .

Momota and Oshima (1976) provided evidence with SEM to separate H. glycines and H. elachista from H. avenae on the basis of the position of the two pores located at the spicule tip of males: they are in an exterior position in the first two species and internally in the latter.

Descriptions of cyst nematode spicules usually refer only to the spicule length. Geraert and De Grisse (1981), in a comparative study of spicules of different nematode genera, indicated that Heteroderidae spicule length was 2.5-4% of the total male body length, which is considered relatively low when compared to 11% in Criconematidae. Some male characters such as its body length, and shape of spicule tip may be used in conjunction with other life stages for species identification (Fig. 8).

Behrens (1975) and Olsson (1985) have proposed use of the shape of spicules to differentiate among the two PCN species, G. rostochiensis and G. pallida, as well as among the three subspecies of the TCN complex. However, recent observations do not support the utility of spicule shape for analyzing the TCN complex (Mota and Eisenback, 1991).

Spicules and sperm may be extracted from the male body in order to better observe and photograph them (Eisenback, 1985). This technique was used by Rammah and Hirschmann (1987) to compare spicules of different nematode genera, including Heterodera and Globodera. The bifid nature of Heterodera spicule tip was clearly observed (Fig. 9E-F). The authors used the following terminology for the different portions of the spicule: the head with a cytoplasmic core opening; the shaft; and the blade with two projections or wings (Fig. 9A).

SPECIES AND SUBSPECIES COMPLEXES

Morphology is the essential basis for species identification of cyst nematodes (Golden, 1986; Baldwin and Mundo-Ocampo, 1991). For many years several authors constructed keys to separate different "groups" (avenae, rostochiensis, etc.) because of the difficulty in clearly identifying species. Some species, however, are so similar that they are refered to "species complexes" (e.g. "trifolii complex"). Morphological differences among them are lacking or very

small. Cyst nematodes, and in particular Globodera spp., display a very narrow host range (mostly in the families Solanaceae and Compositae) and have apparently coevolved with their plant hosts (Stone, 1979). Races of H. glycines as well as pathotypes in Globodera and Heterodera spp. may be considered field populations of different genetic constitution (Triantaphyllou and Hirschmann, 1980).

The remaining portion of this chapter examines the morphological differences, if any, that exist among cyst nematode races, pathotypes, and subspecies, as well as within species complexes. Resistance-breaking pathotypes of potato cyst nematodes (PCN) and races of soybean cyst nematode (SCN) increase the need to clarify the criteria utilized to identify these subspecific categories.

A. Potato cyst nematodes (PCN) species.

Before the description of G. pallida, it was impossible to distinguish morphologically several pathotypes of G. rostochiensis (Shepherd, 1965).These different pathotypes were physiologically distinguished by the ability to reproduce on different potato cultivars. However, several authors observed morphological differences among pathotypes. Guile (1967, 1970) separated pathotypes A from B and E on the basis of cyst color. Webley (1970) noticed that J2's of pathotype A had a shorter body length, a shorter stylet, and a shorter distance of the median bulb to the excretory pore than did pathotypes B and E. Evans and Webley (1970) also noted differences in the shape of the J2 stylet and stylet knobs and suspected that pathotype A was one species and pathotypes B and E another (Jones et al., 1970). Additional measurements of J2 and males provided support for the separation of species (Trudgill et al., 1970), especially when combined with electrophoretic data of soluble proteins (Trudgill and Carpenter, 1971). Bowman and Ross (1972) argued that differences in female color and morphometrics justified the division of H. (=G.) rostochiensis into two species. Finally Stone (1972a) described H. (= G.) pallida (Fig. 10). This new species was validated by other authors (Øydvin, 1973; Hesling and

Ellis, 1974; Evans and Franco, 1977). At present, five pathotypes of G. rostochiensis (Ro 1-5) and three of G. pallida (Pa 1-3) are recognized. It is currently impossible to differentiate these pathotypes by morphology. Identification of pathotypes is based on host tests (Kort et al., 1978), serology (Schots et al., 1987), or DNA analysis (Schnick et al., 1990).

B. Tobacco cyst nematode (TCN) complex.

The first cyst nematode parasitic on tobacco was described by Lownsbery and Lownsbery (1954) in Connecticut, USA. The tobacco cyst nematode (TCN), H. (= G.) tabacum, was distinguished from H. (= G.) rostochiensis on the basis of differences in the perineal area of the cysts, the female lip region with three annules instead of two, and shape of head, tail shape, and distance of the base of the stylet knobs to the dorsal esophageal gland opening (DEGO) in the male. These characters were in addition to the fact that H. (= G.) tabacum does not reproduce on potato and H. rostochiensis does not reproduce on tobacco. Another cyst nematode was found parasitizing horsenettle weed, Solanum carolinense, L. in Virginia, and was described as H. (= G.) virginiae (Miller and Gray, 1968). This nematode was shown to be also pathogenic to several cultivars of tobacco, eggplant, and tomato (Miller, 1970b). The morphological differences between this new species and H. tabacum and H. rostochiensis were the shape of the female stylet knobs, the turbinate/ globose shape of the cysts, and the "maze-like" wall pattern in the perineal area of the cyst (Fig. 10; Table 11) (Miller and Gray, 1968). Miller and Gray (1972) also described a new species that had previously been found parasitizing 'Hicks' tobacco in the Piedmont region of Virginia. The new species H. (= G.) solanacearum was distinguished from H. (= G.) virginiae based on the morphology of the dorsal stylet knob of the female, the barrel-shaped fenestra with convex ends of the cyst, and Granek's ratio (Fig. 10; Table 11). Because of the very small differences among the three species mentioned above, and the fact that they very easily interbreed (Miller, 1983), Stone (1983) proposed their ranking as subspecies: G. tabacum tabacum (= H. tabacum); G. t. virginiae (= H. virginiae) and G. t.

solanacearum (= H. solanacearum). Although not all authors accept this classification (Siddiqi, 1985), recent morphological studies of these three subspecies support the present status established by Stone (Mota and Eisenback, 1990; 1991; Mota and Eisenback, unpublished).

C. Cereal cyst nematodes (CCN)

Cereals constitute hosts for several cyst nematodes species; however, only H. avenae Wollenweber, 1924 (= H. major O. Schmidt, 1930), is considered a serious problem to small grains and maize (Stone and Hill, 1982). Nevertheless, because of their morphological similarity it is very important to distinguish the different species for appropriate control measures. H. mani Mathews, 1971 and H. iri Mathews, 1971 are not pathogenic to cereals, but may be confused with H. avenae (Mathews, 1971). According to principal coordinate analysis, H. arenaria Cooper, 1955 (another species of this complex) is morphologically distinct from H. mani and H. avenae (pathotypes 1, 2 and 3) (Stone and Hill, 1982). The pathotypes of H. avenae are usually distinguished by differential hosts (Dropkin, 1988). Stone and Williams (1974) examined J2, females, and cysts of pathotypes 1 and 2 of H. avenae and concluded that there were no consistent differences. The observations included SEM micrographs of the anterior region of J2. Together with electrophoretic data, the authors concluded that there was no justification for a different species status; however, pathotype 3 was morphologically different (Cook and Williams 1972; Cook, 1976). No morphological differences were found between the Australian populations of cyst nematodes found parasitizing cereals and H. avenae (McLeod and Khair, 1977). Among other cyst nematode species, J2 characters were useful in identifying cereal and the other closely related grass cyst nematodes (Wouts and Weischer, 1977; Sturhan, 1982).

D. Soybean cyst nematode (SCN).

The present status of the SCN, H. glycines Ichinoe, 1952, includes 16 races based on differential cultivars (Riggs and Schmitt, 1988). Golden and Epps (1965) were first to recognize

morphological differences among SCN races. The characters used to differentiate three proposed "groups" were the J2 tail length, the length of the hyaline portion of the tail, and the general tail shape. Groups I and II included U.S. populations, whereas Group III consisted of a Japanese population.

Miller and Duke (1967) examined eggs, J2, males, and cysts of 11 populations from various regions in the United States and concluded that each isolate was morphologically different. A more detailed study of a large number of different morphological characters of the same 11 isolates concluded that the ratios between some characters were of limited value in distinguishing the isolates since only few pairs showed a high degree of correlation (Miller, 1969; 1970a). Tail length of J2 was suggested to help distinguish the four known host races in 1970 (Golden et al., 1970). Koliopanos and Triantaphyllou (1971) were also able to observe morphological differences in body length, tail, and tail terminus length among the four races, but provided no clue as to a possible classification scheme. Riggs et al. (1982) were equally unable to differentiate races within 34 SCN populations using J2 characters such as stylet length, body width, length of hyaline portion of tail, and distance of DEGO to base of knobs. These results are somewhat contradictory to results using Northern isolates of SCN in Indiana (Faghihi et al., 1986); significant differences were found among Northern SCN populations in the length of the esophagus, tail length, and hyaline portion of tail of J2. Morphometrics of males and eggs, on the other hand, proved to be of little value and measurements of cysts showed great variability. The cephalic morphology of J2 of five races of SCN (Noel and Stanger, 1986) was not of differential value among races. However, the authors suggested that differences may occur among polyploid populations. In summary, it is not currently possible to distinguish SCN races by morphology. Biochemical and DNA techniques may be able to acomplish this goal (Kalinski et al., 1988).

E. The Heterodera trifolii "complex".

The H. trifolii "complex" comprises a series of cytogenetically different parthenogenetic populations of H. trifolii Goffart, 1932, as well as two closely related species, H. lespedezae Golden and Cobb, 1963, and H. galeopsidis Goffart,1936. Several species of the "schachtii" group attacking legumes such as H. glycines and H. daverti Wouts and Sturhan, 1978 have some very similar morphological characteristics to the "trifolii complex" (Maas et al., 1982). Hirschmann (1956) was the first to make a detailed comparative study between H. glycines and H. trifolii. Differences were found in the J2, namely in the body length, shape, and size of the stylet, DEGO, shape, and length of the tail and the tail terminus (Fig. 11). In a later study (Hirschmann and Triantaphyllou, 1979), several parthenogenetical populations of H. trifolii were discovered with different chromosome numbers. H. galeopsidis and H. lespedezae were also included in the study (Table 12). In conclusion, the authors could distinguish the different chromosomal forms and the other two species morphologically, but they recommended subspecific status, because of their close relationship. Other authors have also analyzed the H. trifolii "complex". Maas et al. (1982), studying populations from the Netherlands suggested the designation of "forma specialis" instead of subspecies. In this same work, H. galeopsidis was synonymized with H. trifolii and a special population found parasitizing sugarbeet (the yellow beet cyst nematode), was named H. trifolii "f.sp." beta. A more complete discussion of the biology of this "complex" is presented by Sikora and Maas (1986).

F. Comparative studies.

Several comparative morphological studies have been made among cyst nematodes and some have been discussed previously. Wouts and Weischer (1977) separated15 species of Heteroderinae using body length, distance of median bulb to anterior end, tail length, hyaline portion of tail, stylet shape and length, lip annules, lines in lateral field and size of phasmid. The authors presented a key with these characters and an extensive table of characters and

species.

H. glycines has been suggested by some authors as possibly a subspecies of H. schachtii (Miller, 1983). Graney and Miller (1982) justified the specific status of H. glycines and H. schachtii based on morphological differences. On the other hand, morphology was not useful in distinguishing isolates of each of the species (Graney, 1981).

More recently, Othman (1988) compared several species of Globodera, Cactodera, and Punctodera with SEM. Lip pattern of J2 and males were considered useful for the distinction of genera, though somewhat variable. P. punctata populations from Europe were also compared (Wouts et al., 1986). Six populations were placed into two different groups based on multivariate analysis and the authors speculated about the possibility of two species.

CONCLUSIONS

Morphology is at present the most common tool available for identification of cyst nematodes. The SEM, in conjunction with LM and TEM, has enormous potential to clarify details and provide information for improved phylogenetic analysis. Biochemical techniques and DNA analysis will complement information already acquired during the last 150 years. Some morphological characters remain to be utilized to their fullest potential (e.g. stylets and sperm). Techniques for fixing, observing, and obtaining good photographs of cyst nematodes are still needed.

New methods involving computer software may provide nematologists with quick reference keys which may remove the difficulty and boredom of using old cumbersome keys (Fortuner, 1988).

Some groups need to be clarified, in particular those with closely related species or subspecies. Perhaps a general reappraisal of the subfamily Heteroderinae, with the integration of all the available techniques is needed. We hope that the excitement of the new biochemical methods will not cause nematologists to abandon the classical approaches that are still very useful for identification and phylogenetic analysis.

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Table 1. **Genera of Heteroderinae and Numbers of Recognized Species**

Genera	Authority	Number of species
Heterodera	Schmidt, 1871	57
Afenestrata	Baldwin and Bell, 1985	1
Cactodera	Krall and Krall, 1978	9
Globodera	Skarbilovich, 1959	12
Punctodera	Mulvey and Stone, 1976	3
Dolichodera	Mulvey and Ebsary, 1980	1
Atalodera	Wouts and Sher, 1971	4
Thecavermiculatus	Robbins, 1978	4
Camelodera	Krall, Shagalina, and Ivanova, 1988	1
Bellodera	Wouts, 1985	1
Sarisodera	Wouts and Sher, 1971	1
Rhizonema	Cid Del Prado Vera, Lownsbery, and Maggenti, 1983	1
Hylonema	Luc, Taylor, and Cadet, 1978	1
Ekphymatodera	Baldwin, Bernard, and Mundo, 1989	1
Cryphodera	Colbran, 1966	4
Meloidodera	Chitwood, Hannon, and Esser, 1956	6
Verutus	Esser, 1981	3

(adapted from Baldwin and Mundo-Ocampo, 1991)

Table 2. **Species of Heterodera**

'Schachtii' Group

- Heterodera schachtii A. Schmidt, 1871
  - syn. Tylenchus schachtii (A. Schmidt, 1871) Orley, 1880
  - syn. Heterodera schachtii minor 0. Schmidt, 1930
- H. amygdali Kirjanova and Ivanova, 1975
- H. cajani Koshy, 1967
  - syn. H. vigini Edward and Misra, 1968
- H. ciceri Vovlas, Graco and Di Vito, 1985
- H. daverti Wouts and Sturhan, 1979
- H. elachista Oshima, 1974
- H. fici Kirjanova, 1954
- H. galeopsidis Goffart, 1936
  - syn. H. schachtii galeopsidis Goffart, 1936
- H. gambiensis Merny and Netscher, 1976
- H. glycines Ichinohe, 1952
- H. lespedezae Golden and Cobb, 1963
- H. leuceilyma Di Edwardo and Perry, 1964
- H. limonii Cooper, 1955
- H. medicaginis Kirjanova in Kirjanova and Krall, 1971
- H. oryzae Luc and Berdon Brizuela, 1961
- H. oryzicola Rao and Jayaprakash, 1978
- H. oxiana Kirjanova, 1962
- H. rosii Duggan and Brennan, 1966
- H. sacchari Luc and Merny, 1963
- H. salixophila Kirjanova, 1969
- H. sonchophila Kirjanova, Krall, and Krall, 1976
- H. sorghi Jain, Sethi, Swarup, and Srivastava, 1982
- H. tadshikistanica Kirjanova and Ivanova, 1966
- H. trifolii, Goffart, 1932
  - syn. H. schachtii var trifolii Goffart, 1932
    - H. paratrifolii Kirjanova, 1961
    - H. rumicis Poghossian, 1961
    - H. scleranthii Kaktina, 1957
- H. zeae Koshy, Swarup, and Sethi, 1971

'Goettingiana' Group

- H. bergeniae Maqbool and Shahina, 1988
- H. canadensis Mulvey, 1979
- H. cardiolata Kirjanova and Ivanova, 1969
- H. cruciferae Franklin, 1945
- H. cyperi Golden, Rau, and Cobb, 1962
- H. delvii Jairajpuri, Khan, Setty, and Govindu, 1979
- H. goettingiana Liebscher, 1892
- H. graminis Stynes, 1971
- H. graminophila Golden and Birchfield, 1972
- H. humuli Filip'ev, 1934
- H. longicolla Golden and Dickerson, 1973
- H. mediterranea Volvas, Inserra, and Stone, 1981
- H. menthae Kirjanova and Narbaer, 1977

Table 2. (cont.).

H. methwoldensis Cooper, 1955
H. mothi Khan and Husain, 1965
H. pakistanensis Maqbool and Shahina, 1986
H. phragmitidis Kazachenko, 1986
H. plantaginis Narbaev and Sidikov, 1987
H. polygone Cooper, 1955
H. graduni Kirjanova, 1971
H. raskii Basnet and Jayaprakash, 1984
H. urticae Cooper, 1955
H. uzbekistanica Narbaev, 1980

'Avenae' Group

H. arenaria Cooper, 1955
syn. Bidera arenaria (Cooper, 1955) Krall and Krall, 1978
H. avenae Wollenweber, 1924
syn. H. schachtii var. avenae Wollenweber, 1924
H. Bidera avenae (Wollenweber, 1924) Krall and Krall, 1978
H. schachtii major 0. Schmidt, 1930
H. major O. Schmidt, 1930
H. ustinovi Kirjanova, 1969; Krall and Krall, 1978
H. bifenestra Cooper, 1955
syn. H. Bidera bifenestra (Cooper, 1955) Krall and Krall, 1978
H. longicaudata Seidel, 1972
H. Bidera longicaudata (Seidel, 1972) Krall and Krall, 1978
H.filipjevi (Madzhidov, 1981) Stone, 1985
syn. Bidera filipjevi Madzhidov, 1981
H. hordecalis Andersson, 1975
syn. Bidera hordecalis (Andersson, 1975) Krall and Krall, 1978.
H. iri Mathews, 1971
syn. Bidera iri (Mathews, 1971) Krall and Krall, 1978
H. latipons Franklin, 1969
syn. Bidera latipons (Franklin, 1969) Krall and Krall 1978
Ephippiodera latipons (Franklin, 1969) Shagalina and Krall, 1981
H. mani Mathews, 1971
syn. Bidera mani (Mathews, 1971) Krall and Krall, 1978
H. turcomanica Kirjanova and Shagalina, 1965
syn. Bidera turcomanica (Kirjanova and Shagalina, 1965) Krall and Krall, 1978
Ephippiodera turcomanica (Kirjanova and Shagalina, 1965) Shagalina and Krall, 1981

(adapted from Luc et al., 1988, in Baldwin and Mundo-Ocampo, 1991)

Table 3. **Species of Globodera**.

Globodera Skarbilovich, 1959
syn. Heterodera Globodera Skarbilovich, 1959
Globodera rostochiensis (Wollenweber, 1923) Behrens, 1975
syn. Heterodera schachtii rostochiensis Wollenweber 1923
H. schachtii solani Zimmermann, 1927
G. achilleae (Golden and Klindic',1973) Behrens, 1975
syn. Heterodera achilleae Golden and Klindic', 1973
G. artemisiae (Eroshenko and Kazachenko, 1972) Behrens, 1975
syn. H. artemisiae Eroshenko and Kazachenko, 1972
G. hypolysi Ogawa, Ohshima, and Ichinohe, 1983
G. leptonepia (Cobb and Taylor, 1953) Behrens, 1975
syn. H. leptonepia Cobb and Taylor, 1953
G. millefolii (Kirjanova and Krall, 1965) Behrens, 1975
syn. H. millefolii Kirjanova and Krall, 1965
G. mirabilis (Kirjanova, 1971) Mulvey and Stone, 1976
syn. H. mirabilis Kirjanova, 1971
G. pallida (Stone, 1973) Behrens, 1975
syn. H. pallida Stone, 1973
G. pseudorostochiensis (Kidanova, 1963) Mulvey and Stone, 1976
H. pseudorostochiensis Kirjanova, 1963
G. tabacum tabacum (Lownsbery and Lownsbery, 1954) Behrens, 1975
syn. H. tabacum Lownsbery and Lownsbery, 1954
G. tabacum solanacearum (Miller and Gray, 1972) Behrens, 1975
syn. H. solanacearum (Miller and Gray, 1972)
G. solanacearum (Miller and Gray, 1972)
G. tabacum virginiae (Miller and Gray, 1968) Behrens, 1975
syn. H. virginiae (Miller and Gray, 1968)
G. virginiae (Miller and Gray, 1968)
G. zelandica Wouts, 1984

(adapted from Luc et al., 1988, in Baldwin and Mundo-Ocampo, 1991)

Table 4. **Compendium for Identification Among Select Globodera spp.**

Species	J2 stylet knob shape	No. cuticular ridges between vulva-anus	Granek's ratio of cysts	Host [c]
G. rostochiensis	Rounded: dorsal may slope posteriad	16-31 ridges (about 22) [a]	1.3-9.5 (4.5) [a]	Potato Not tobacco
G. pallida	Pointed anteriorly; anterior surface of dorsal slightly concave	8-20 ridges (about 12) [a]	1.2-3.5 (2.3) [a]	Potato Not tobacco
G. tabacum complex	Pointed to rounded anteriorly; dorsal slightly concave	5-15 ridges	1.0-4.9 (2.8) [a b]	Tobacco not potato

	J2 stylet length (μm)	Female stylet length (μm)
G. rostochiensis	(21-23) 21.8-22 [a]	(21-25) 23 [a]
G. pallida	(21-26) 22.8-24 [a]	(23-29) 27 [a]
G. tabacum	(19-28) 22.8-24 [a]	(18-30) 25 [a]

[a] most typical measurements.

[b] generally less than 2.1 in G. t. tabacum.

[c] see Baldwin and Mundo-Ocampo for exceptions; according to Miller (pers. communication) potato cyst nematodes are able to reproduce on certain tobacco cultivars and conversely tobacco cyst nematodes are also able to reproduce on certain varieties of potato.

(modified from Baldwin and Mundo-Ocampo, 1991)

Table 5. **Key to genera of Heteroderinae**.

1. Vulva subequatorial 2
   Vulva terminal 3

2 Female reniform ..... Verutus
   Female not reniform ..... Meloidodera

3. Cyst absent; eggs not retained in
   body of dead female 4
   Cyst present; at least some eggs retained in
   tanned body of dead female 13

4. Vulval-anal distance 15-35 μm 5
   Vulval-anal distance geater than 35 μm 10

5. Esophageal gland lobe of J2 nearly fills
   diameter of body cavity 6
   Esophageal gland lobe of J2 narrow;
   about one-third body diameter 7

6 Female with rounded terminus ..... Hylonema
   Female with distinct cone 8

7. Little or no terminal cone ..... Thecavermiculatus
   Distinct terminal cone ..... Atalodera

8. Cuticle pattern of female striated at midbody ..... Rhizonema
   Cuticle pattern of female not striated at midbody 9

9. Cuticle pattern of female zig-zag ..... Sarisodera
   Cuticle pattern of female not zig-zag
   but with rough surface and longitudinal furrows ..... Ekphymatodera

10. Female cuticle with prominent striae at midbody 11
    Female cuticle without prominent striae at midbody 12

11. Mature female lemon-shaped;
    prominent vulval cone ..... Bellodera
    Mature female nearly spherical
    little or no vulval cone ..... Cryphodera

12. Mature female nearly spherical,
    little or no terminal prominence ..... Thecavermiculatus andinus
    Mature female with distinct terminal prominence ..... Camelodera

13. Cyst lacking fenestration of vulval region ..... Afenestrata
    Cyst with fenestration of vulval region 14

Table 5 (cont.).

14.	Cyst with fenestration of anal region	Punctodera
	Cyst lacking fenestration of anal region	15
15.	Cyst with two semifenestrae in vulval region	Heterodera
	Cyst with one fenestra (circumfenestrae) in vulval region	16
16.	Terminal cone of female prominent to slightly reduced	Cactodera
	Terminal cone of female absent, rounded terminus	17
17.	Cuticle of female zig-zag	Globodera
	Cuticle of female striated	Dolichodera

(adapted from Baldwin and Mundo-Ocampo, 1991)

Table 6. **Compendium for Identification Among Select Heteroderidae of Particular Economic Significance.**

'Schachtii' Group: Bullae and underbridge well developed; vulval slit > 35 μm, ambifenestrate.

Species	Bullae	J2 stylet length(μm)	J2 tail length(μm)	J2 stylet knobs shape	Hosts
H. schachtii	Scattered	25-27	48-55	Anchor	Wide
H. glycines	Scattered rounded	22-25	40-49	Subventral	Wide
H. trifolii	Scattered	27	55	Anchor	Wide
H. zeae	Four distinct "fingers"	< 22	40-49	Concave on top	Gramineae

'Goettingiana' Group: No bullae, underbridge poorly developed or absent; vulval slit > 30 μm; ambifenestrate or bifenestrate

Species	Underbridge	Egg mass	J2 hyaline part tail(μm)	Hosts
H. goettingiana	Slender but present	Small	> 34	Legumes
H. crucifera	Slender but present	Small	< 26	Crucifers (especially Brassicae)
H. carotae	Very thin; rarely persists in cysts	Large, often size of female	26-32	Carrots

'Avenae' group: Bullae and underbridge present or absent; short vulaval slit (< 16 μm), bi-fenestrate

Species	Bullae	Underbridge	J2 lateral field	Width vulval bridge(μm)
H. avenae	Well-developed	present	Four lines outer two faint	Wide; 18-39

(adapted from Baldwin and Mundo-Ocampo, 1991)

Table 7. **Heterodera "groups"**

1. Cysts spherical to pear-shaped, vulva on same plane as cyst wall or only slightly protruding linear punctation present in cyst wall .... H. rostochiensis group

1'. Cysts lemon-shaped (protrusion of vulva well defined) **2**

2. Vulval split extends below apex of vulval cone, cyst with zigzag line pattern, punctation, when present, not linear..... **3**

2'. Vulval split does not extend below apex of vulval cone, punctation absent, cyst wall pattern with angulated, broken, straight or wavy lines at right angles to long axis of cyst, bullae (brown knobs) absent in vulval cone ..... H. cacti group

3. Bullae present ..... H. schachtii group
3'. Bullae absent .....H. goettingiana group

H. rostochiensis "group"

1. Anal and vulval openings (light thin areas of fenestrae in cyst walls) about equal in size ..... H. punctata

1'. Anal and vulval openings unequal, anal opening very small, and located at apex of a V-shaped sheath-like structure..... **2**

2. Distance from anus to vulva more than 2.7 times the diameter of the vulval opening, apex of V- sheath of the anus pointing in direction of the shorter diameter of vulval opening, linear cuticular punctation at right angles to vulva-anus axis.....H. rostochiensis

2'. Distance from anus to vulva less than 2. 8 times the diameter of the vulval opening, apex of V-sheath of the anus pointing in direction of the longer diameter of vulval opening, linear cuticular punctation, when visible, is parallel to the vulva-anus axis ..... H. tabacum

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(adapted and modified from Granek, 1968)

Table 8. **Classification of Heterodera species by lip pattern**

Lip pattern	H. (Globodera)	
Type I	virginiae	Miller & Gray, 1968
	solanacearum	Miller & Gray,1972
	tabacum	Lownsbery &Lownsbery, 1954
	"Mexican cyst nematode"	Campos Vela (1967)
	rostochiensis	Wollenweber, 1923
	pallida	Stone, 1973
	achilleae	Golden & Klindic, 1973
	H. (Heterodera) (= Cactodera)	
Type 2	betulae	Hirschmann & Riggs, 1969
	weissi	Steiner, 1949
	cacti	Filipjev & Schuurmans Stekhoven, 1941
Type 3	**H. (Heterodera)**	
	goettingiana	Liebscher, 1892
	carotae	Jones, 1950
	cruciferae	Franklin, 1945
	urticae	Cooper, 1955
Type 4	avenae	Wollenweber, 1924
	mani	Mathews, 1971
	latipons	Franklin, 1969
	hordecalis	Andersson, 1975
	leuceilyma	Di Edwardo & Perry, 1964
	sacchari	Luc & Merny, 1963
	cajani	Koshi, i967
	oryzae	Luc & Brizuela, 1961
	zeae	Koshi, Swarup & Sethi, 1971
	fici	Kirjanova, 1954
	cyperi	Golden, Rau & G. S. Cobb, 1962
	mothi	Khan & Husain, 1965
	graminophila	Golden & Birchfield, 1972
	graminis	Stynes, 1.971
	humuli	Filipjev, 1934
Type 5	schachtii	Schmidt, 1871
	limonii	Cooper, 1955
	galeopsidis	Goffart, 1936
	rosii	Duggan & Brennan, 1966
	glycines	Ichinohe, 1952
	lespedezae	Golden & G. S. Cobb, 1963
	trifolii	Goffart, 1932
	H. (Globodera) (= Punctodera)	
Type 6	punctata	Thorne, 1928

(adapted from Stone, 1975)

Table 9. **Comparison of classifications of Heterodera species by lip morphology after Stone (1975) and by lip morphology and cyst characters after Mathews (1970) and Mulvey (1972 and 1974)**

Heterodera	Lip pattern type (Stone, 1975)	Mathews,1971	Mulvey,1972,1974
virginiae [a]	1	Rostochiensis	1
solanacearum [a]	1		1
tabacum [a]	1	Rostochiensis	1
"Mexican cyst nematode"	1		1
rostochiensis [a]	1	Rostochiensis	1
pallida [a]	1		
achilleae [a]	1		
betulae [b]	2	Cacti	2
weissi [b]	2	Cacti	2
cacti [b]	2	Cacti	2
goettingiana	3	Goettingiana	5
carotae	3	Goettingiana	5
cruciferae	3	Goettingiana	5
urticae	3	Goettingiana	5
avenae	4	Avenae	3
mani	4		3
latipons	4	Humuli	3
hordecalis	4		
leuceilyma	4	Schachtii	4
sacchari	4	Schachtii	4
cajani	4		4
oryzae	4	Schachtii	4
zeae	4		4
fici	4	Humuli	4
cyperi	4	Goettingiana	5
mothi	4	Schachtii	5
graminophila	4		5
graminis	4		5
humuli	4	Humuli	5
schachtii	5	Schachtii	4
limonii	5		4
galeopsidis	5	Schachtii	4
rosii	5	Schachtii	4
glycines	5	Schachtii	4
lespedezae	5	Schachtii	4
trifolii	5	Schachtii	4
punctata [c]	6	Rostochiensis	1

[a] = Globodera; [b] = Cactodera; [c] = Punctodera

(adapted and modified from Stone, 1975)

Table 10. **Layers and zones of the body wall cuticle of females of Heteroderidae.**

Layer	Zone	Characteristics
A	A_1	Homogeneous, thin ($\leq$ 0.05 μm), moderately dense.
	A_2	Fine fibers, electron lucent.
	A_3	Electron-dense chambers among fibrous strands.
B		Patches of striations; striations with a periodicity of ±18 nm.
C	C_1	Randomly arranged fibers.
	C_2	Randomly arranged fibers embedded in an electron-dense matrix.
	C_3	Randomly arranged fine-textured fibers.
D		Clearly defined fibers arranged in a repeating helicoidal pattern.

(modified from Shepherd et al. , 1972, in Cliff and Baldwin, 1985)

Table 11. **Major morphological differences among the three TCN subspecies (GTT, GTV and GTS) and the PCN species (GR, GP) based on female and cyst characters**

	GTS	GTV	GTT	GP	GR
CYST SHAPE	Turbinate/sub-globose			Spherical/globose	
CYST COLOR (Munsell)[a]	2.5 YR 6/12	10.0 YR 5/10	10.0 YR 5/10	– –	2.5 YR 5/10
CYST WALL PATTERN	Greatest groove breadth≥breadth of adjacent ridge			Groove < ridge	
STYLET KNOBS ANGLE Miller's (ϕ_d)	108°	80°	136°	–	131°
GRANEK'S RATIO	2.2±0.4 (1.3-3.8	2.8±0.2 (1.5-4.2)	1.5±0.4 (0.9-2.8)	2.3 (1.2-3.5)	4.6±1.3 (2.7-8.9)
SHAPE OF FENESTRA	Barrel with convex ends	Circular/ eliptical	Eliptical w/ obtuse ends	Circular	

GTT = Globodera tabacum tabacum
GTV = G. t. virginiae
GTS = G t. solanacearum

GR = Globodera rostochiensis
GP = G. pallida

[a] According to Munsell's color scale, YR is yellow/red; the number before YR represents hue; the following numbers are light strength/ chroma notations.

(adapted from Miller and Gray, 1972)

Table 12. **Morphometric characters examined in the study of life stages of populations of the Heterodera trifolii species complex**

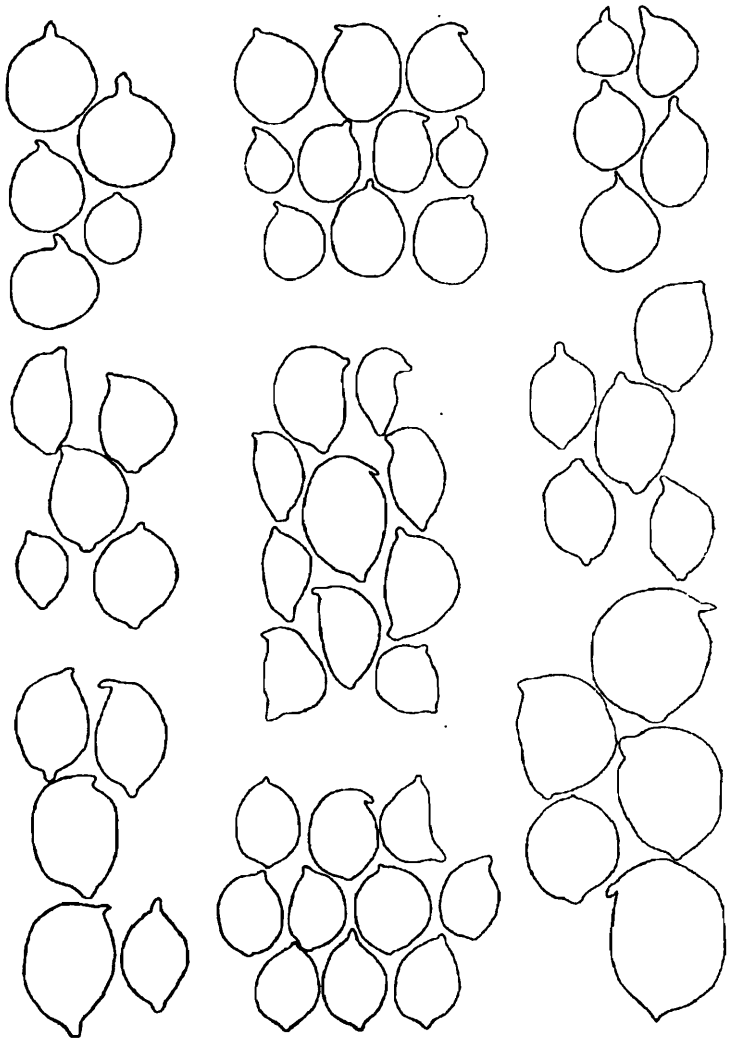
JUVENILES	FEMALES	CYST CONE TOPS
Body length	Stylet length	Fenestra length
Stylet Length	Dorsal esophageal gland orifice distance	Dorsal semifenestra width
Head end to stylet knob base	Valve length	Ventral semifenestra width
Stylet shaft and knobs length	Valve width	Cone top outline length
Stylet knob height	EGGS	Cone top outline width
Stylet knob width	Length	Vulval slit length
Dorsal esophageal gland orifice distance	Width	Vulval bridge width
Body width at anus	Length/width	Underbridge length
Tail length		Underbridge width
Hyaline tail terminus length		Underbridge depth-top view
Hyaline tail terminus width at beginning	CYSTS	Underbridge depth-side view
Hyaline tail terminus width 5µm from tip	Length	Vulva - anus distance- top view
Knob width/height	Width	Vulva - anus distance- side view
Tail length/tail terminus length	Length/width	Fenestra length/dorsal semifenestra width
Tail teminus length/ stylet length		
c = body length/tail length		
d = tail length/body width at anus		
Caudal ratio A		
Caudal ratio B		

(adapted from Hirschmann and Triantaphyllou, 1979)

Fig. 1. **A**. Typical shape of cysts: round = Globodera; elipsoid = Punctodera; 'lemon'-shape = Heterodera. **B**. Variability of shapes of different species of cyst nematodes (**A** from Hesling, 1982; **B** from Filipjev and Schuurmans-Stekhoven, 1941).

round elipsoid "lemon"

A



B

Fig. 2. Surface patterns and cuticular layering of females and cysts of Heteroderinae. (SEM except for **G** and **H**). **A**. Subcrystalline layer of cyst of Heterodera avenae. **B**. Enlargement from A showing boundary of subcrystalline layer. **C.** Striated cuticle, Rhizonema sequoiae. **D**. Zig-zag cuticle, Atalodera lonicerae. **E**. Tuberculate cuticle with longitudinal striae, Ekphymatodera thomasoni. **F**. Cuticle of tuberculate neck region of Globodera rostochiensis. **G**. Subsurface cuticular punctations, Punctodera chalcoensis. **H**. Diagramatic cross-sections of female cuticles showing A and B layers alone as they occur in males and second-stage juveniles, as well as presence of layers C and C+D as they occur in adult females of Heteroderinae. **I**. Lateral view of neck region of cyst of Globodera tabacum solanacearum. **J**. Ventral view of neck region of cyst of Globodera tabacum solanacearum, showing excretory pore (**A-H** from Baldwin and Mundo-Ocampo, 1991; **I-J** original).

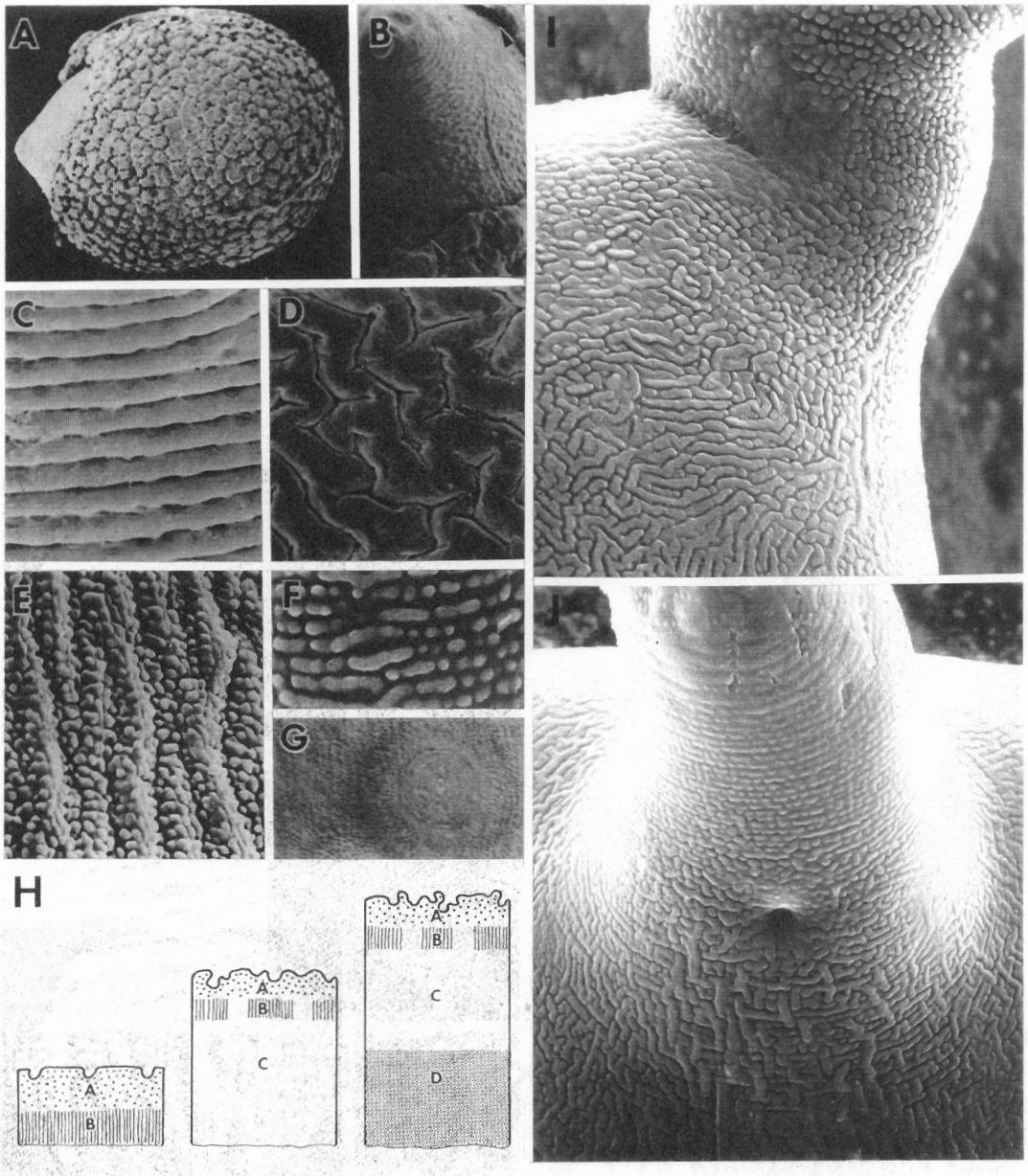

A
B
C
D
E
F
G
H
A
B
C
A
B
C
A
B
C
D
I
J

Fig.3. Lip patterns of second-stage juveniles (J2) of Heteroderinae (see also Tables 8 and 9 for list of species from each type). **A**. Stone's six basic types of lip patterns of J2. **B**. SEM 'en face' view of Globodera sp. (type 1). C. SEM 'enface' view of Heterodera sp. (type 4). **D**. SEM 'enface' view of Heterodera glycines (type 5). (**A** from Stone, 1975; **B-D** original)

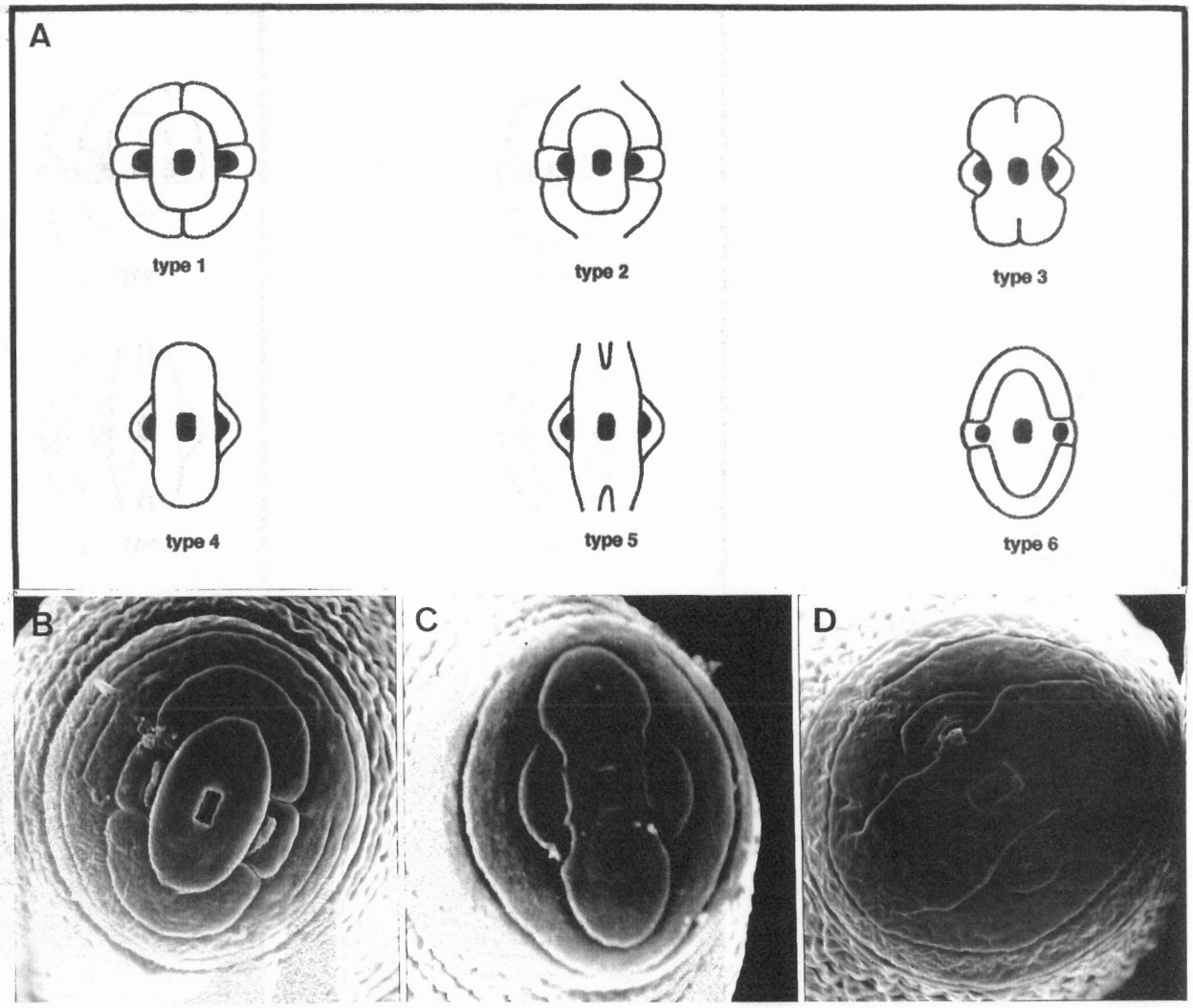
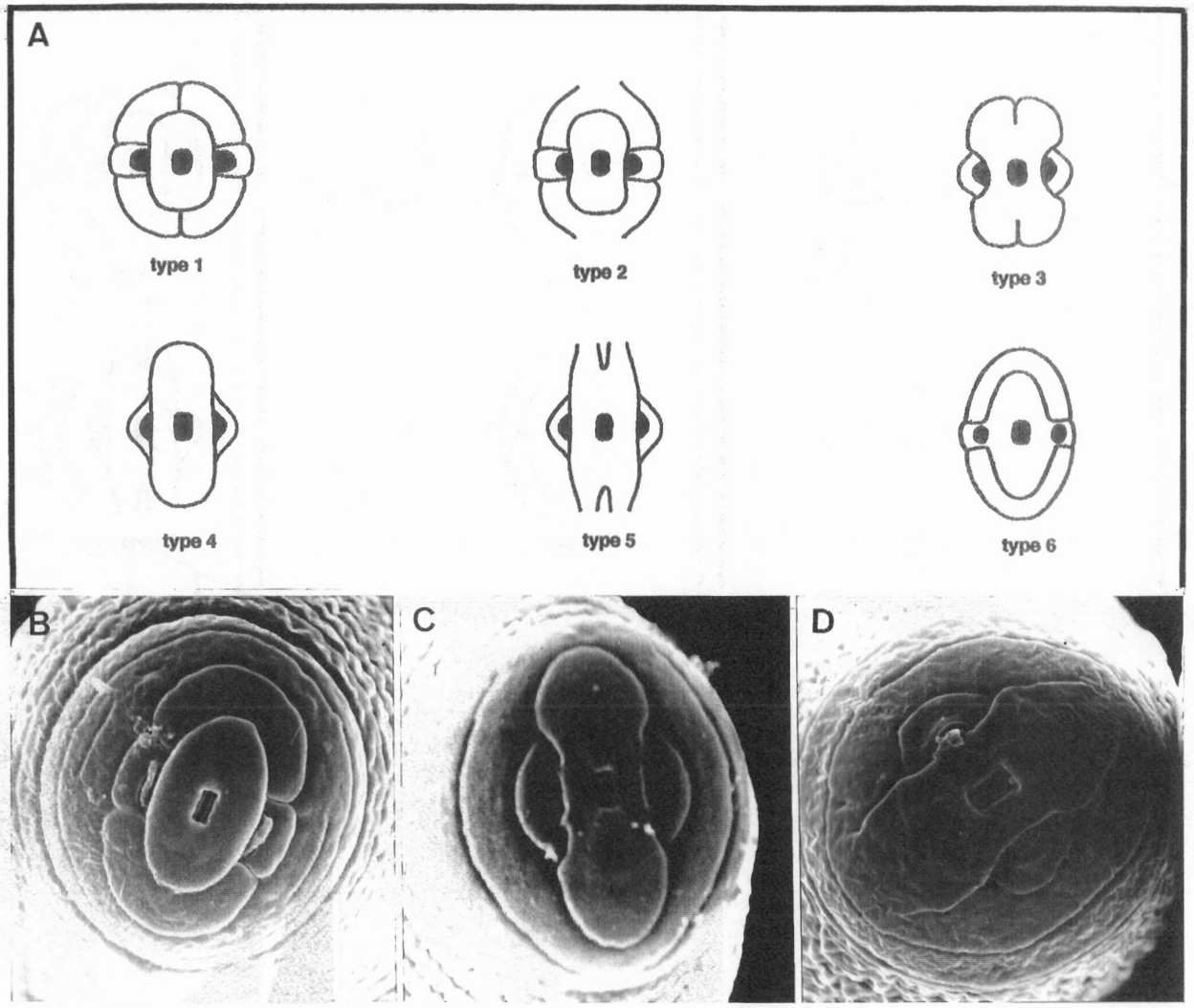
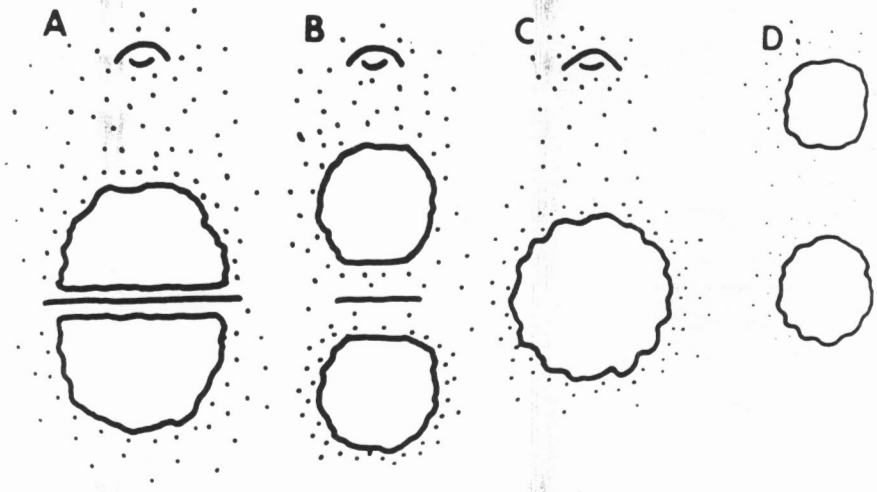

A
type 1
type 2
type 3
type 4
type 5
type 6
B
C
D

Fig.4. Diagram of of fenestration types occuring in cyst nematodes. **A**. Ambifenestrate type. **B**. Bifenestrate type. **C**. Circumfenestrate with non-fenestrated anal region. **D**. Circumfenestrate with separate anal fenestration . **E**. Lateral view of vulval cone of species of Heterodera showing external and internal structures: **a**. vulval slit on vulval bridge; **b**. basin; **c**. external cuticular pattern; **d**.cervix; **e**. underbridge; **f**. Mulvey's bridge; **g**. bullae; **h**. vaginal remnant; **j**. semifenestrae (**A-D** from Baldwin and Mundo-Ocampo, 1991; **E** from Hesling, 1982).


A
type 1
type 2
type 3
type 4
type 5
type 6
B
C
D


A
B
C
D

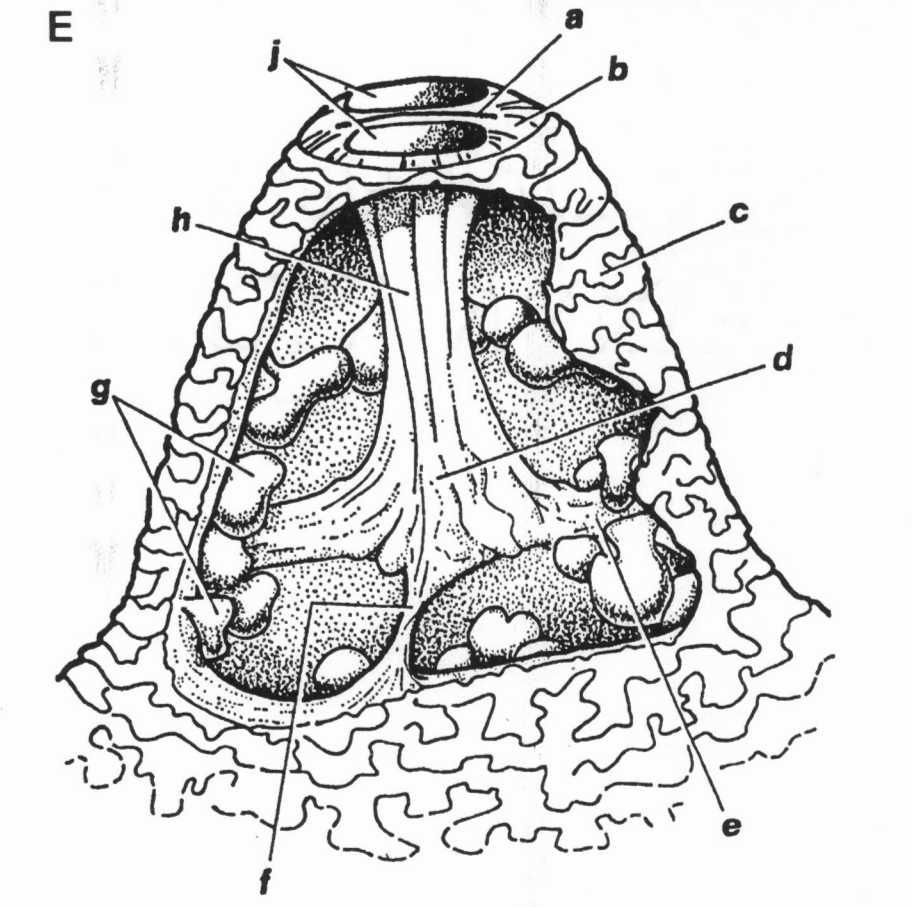

E
a
j
b
h
c
d
g
e
f

Fig. 5. Vulval cone and fenestration types in Heterodera spp. **A**. Bifenestrate vulval cone of Heterodera humuli, seen from top with light microscopy (LM). **B**. Ambifenestrate vulval cone of Heterodera glycines, seen from top with LM. **C**.Lateral view of vulval cone of Heterodera schachtii seen with LM. **D**. Vulval cone of Heterodera sp. as seen with SEM (**A** from Hesling, 1982; **B-C** from Graney, 1981; **D** original).

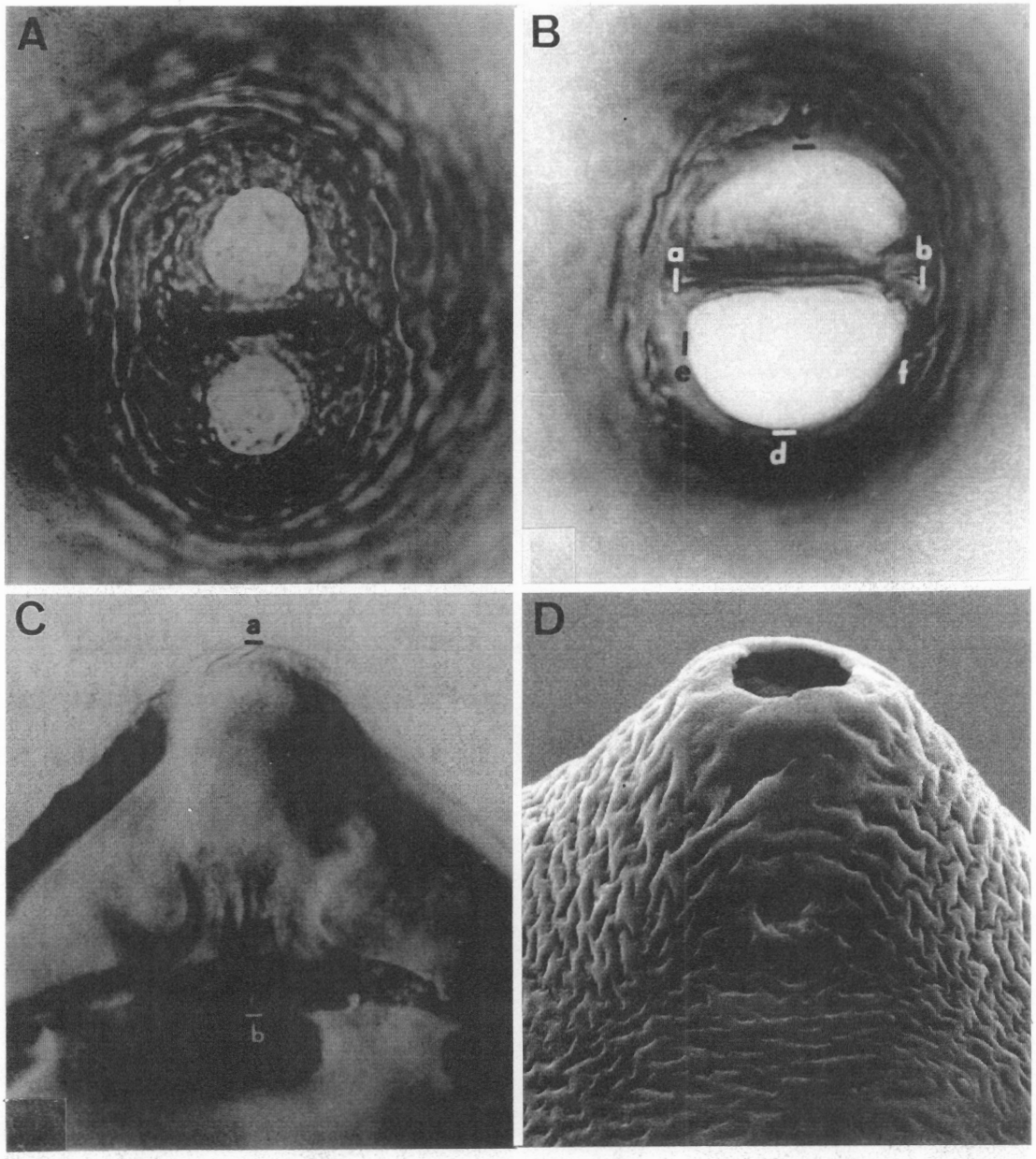

A
B
a
b
e
f
d
C
a
b
D

Fig.6. Terminal area of 'round' cyst nematodes, genus Globodera. **A**. Total cyst of Globodera tabacum solanacearum, viewed with SEM, showing terminal area, containing anus and vulva. **B**. Enlarged terminal area of young cyst of G .t. solanacearum displaying incomplete fenestration, as seen by SEM; notice the two conspicuous crescents of tubercles. **C**. SEM of completely fenestrated cyst of G. t. solanacearum. (original).

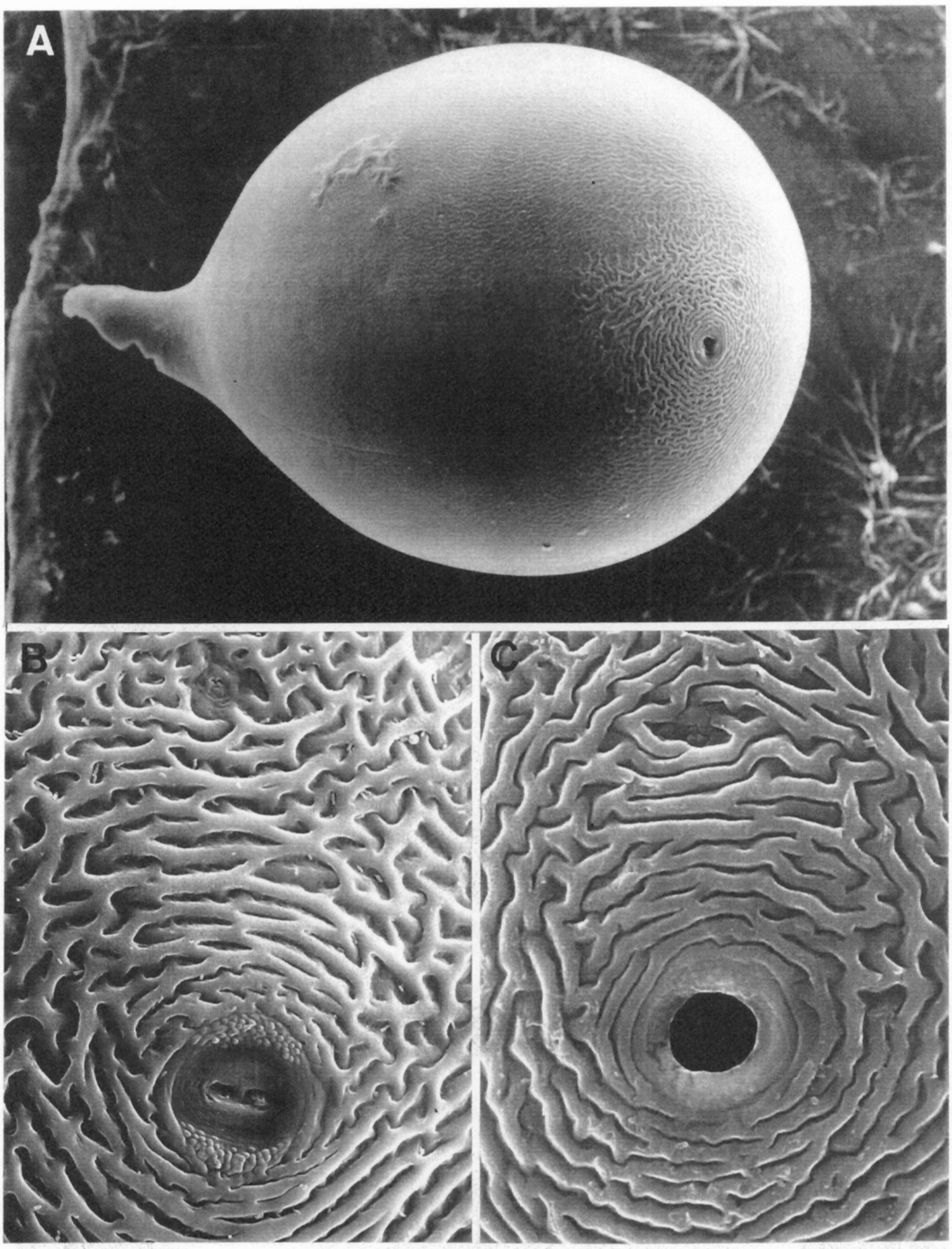

A
B
C

Fig. 7. Distinguishing characters of some common species of Globodera, Heterodera and Punctodera, based on cyst morphology (from Jones and Jones, 1984).

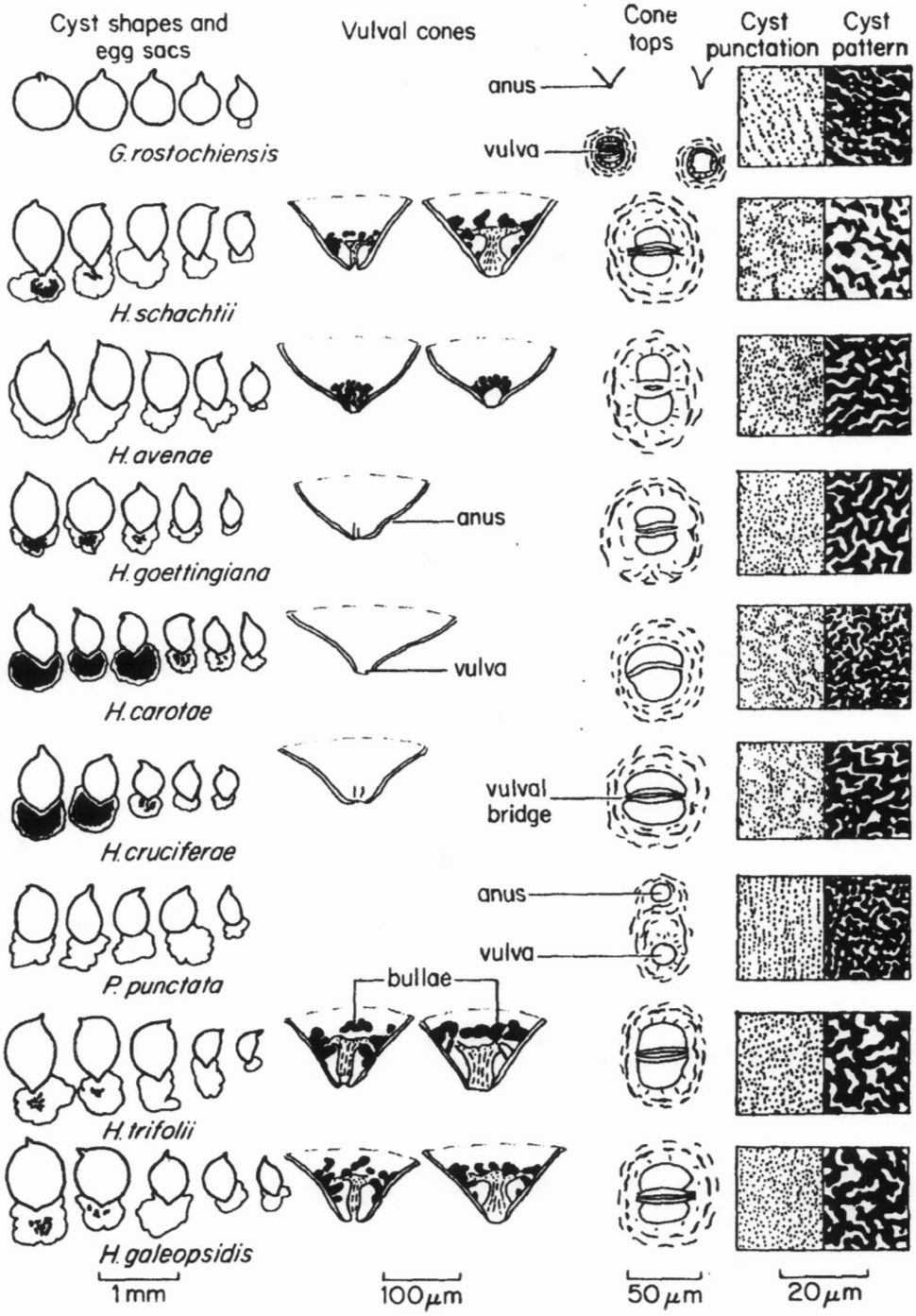

Cyst shapes and egg sacs
Vulval cones
Cone tops
Cyst punctation
Cyst pattern
anus
vulva
G. rostochiensis
H. schachtii
H. avenae
H. goettingiana
anus
H. carotae
vulva
H. cruciferae
vulval bridge
anus
vulva
P. punctata
bullae
H. trifolii
H. galeopsidis
1 mm
100 µm
50 µm
20 µm

Fig. 8. Distinguishing characters of some common species of Globodera, Heterodera and Punctodera, according to male morphology (tail and spicules) (from Jones and Jones, 1984).

	♂ tail	Spicule tip	Cyst length μm	Cyst breadth μm	Male length μm	Larval length μm	Colour of large ♀♀	Colour of cysts	Subcrystalline layer
rostochiensis			1000-400	860-180	1100	471	golden	chestnut	No
schachtii			1100-450	750-200	1500	459	white	brown	Yes
avenae			1000-450	800-150	1400	582	white	dark brown	marked
goettingiana			940-400	780-250	1300	474	white	brown	No
carotae			760-300	600-120	1200	451	white	brown	Yes
cruciferae			830-400	570-180	1200	414	white	brown	Yes
punctata			830-470	450-230	1000	brown	white	brown	Yes
trifolii	Absent		1000-450	750-200	-	502	cream	brown	Yes
galeopsidis	Absent		1000-450	850-220		518	cream	brown	Yes

SD 5% for larvae

Fig. 9. Morphology of male spicules, tail and sperm. **A**. Diagram of the different parts of a spicule as viewed externally and internally. **B**. Light micrograph of the tail of G. t. solanacearum male (lateral view); note cloaca opening very near tail tip. **C**. **D**. SEM of Heterodera glycines sperm (**C** from uterus of female; **D** in spermatheca of male). **E**. Single-tip spicule characteristic of Globodera (G. t. tabacum). **F**. Bifid spicule, characteristic of Heterodera (H. glycines) (**A** and **F** from Rammah and Hirschmann, 1987; **B-E** original).

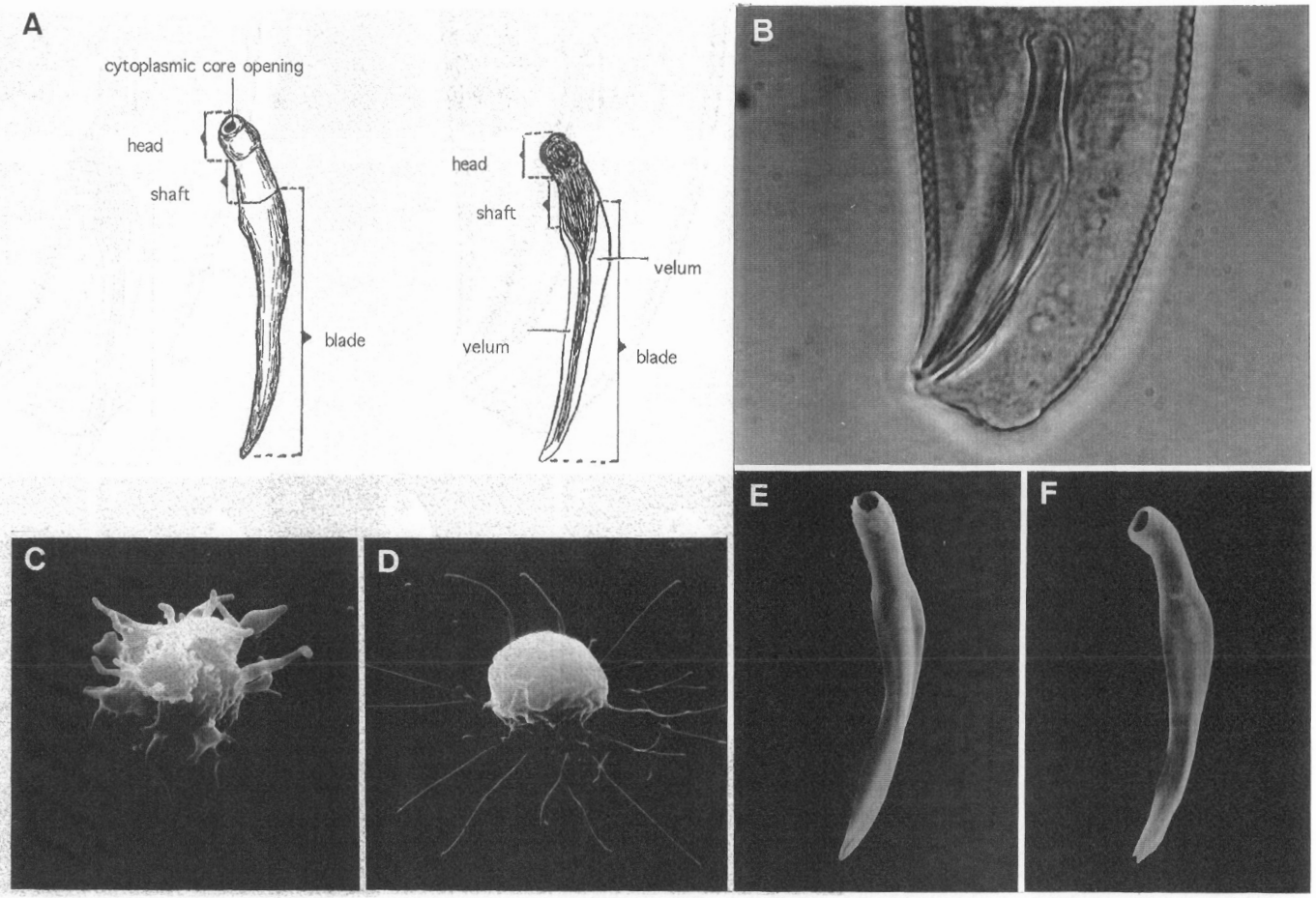
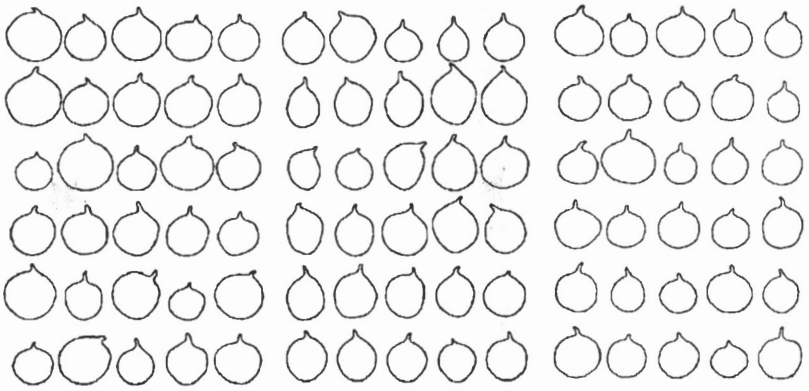
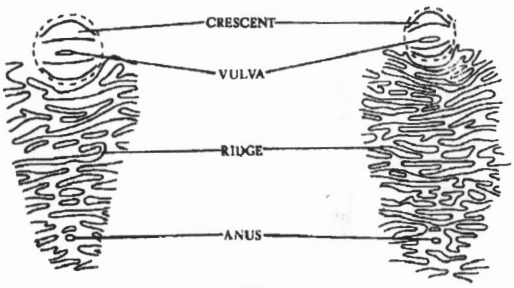

A
cytoplasmic core opening
head
shaft
blade
head
shaft
velum
velum
blade
B
C
D
E
F

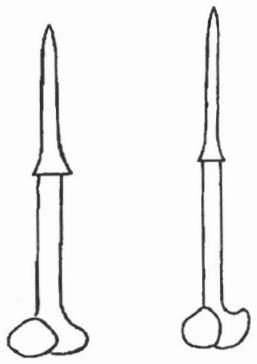
Fig. 10. Comparisons between potato cyst nematodes (PCN), Globodera rostochiensis and G. pallida and tobacco cyst nematodes (TCN), G. tabacum spp. tabacum, virginiae and solanacearum, based on cyst, terminal area and stylets. **A**. Shape of cysts of Globodera rostochiensis. **B**. Shape of cysts of Globodera tabacum virginiae. **C**. Shape of cysts of G. t. tabacum. **D**. Comparison between terminal areas of Globodera pallida (left) and G. rostochiensis (right). **E**.Stylet of J2 of G. rostochiensis. **F**. Stylet of J2 of G. pallida. **G**. Stylet of J2 of G. t. virginiae. **H**. Stylet of J2 of G. t. solanacearum. **I**. Knobs of stylet of G. rostochiensis. **J**. Stylet of G. pallida. **K**. Knobs of stylet of G. t. tabacum. **L**. Stylet of female of G. t. virginiae. **M**. Stylet of female of G. t. solanacearum (**A-C**, **G-I**, **K-M** from Miller and Gray, 1968,1972; **D-F**, **J** from Stone, 1972).



A B C

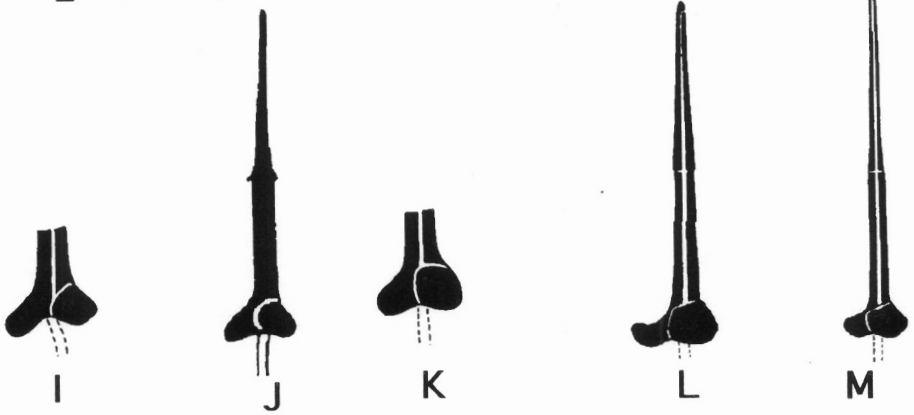


D



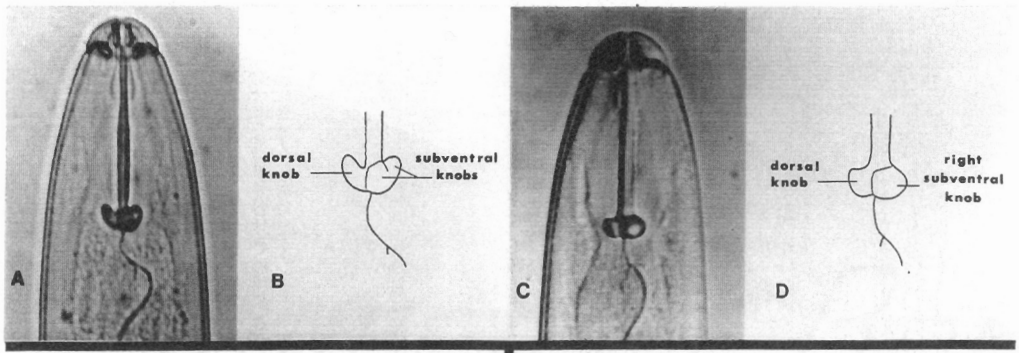
E F

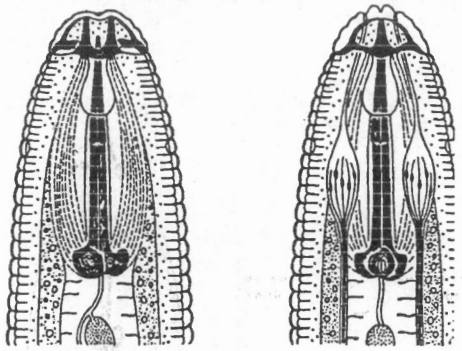
G H



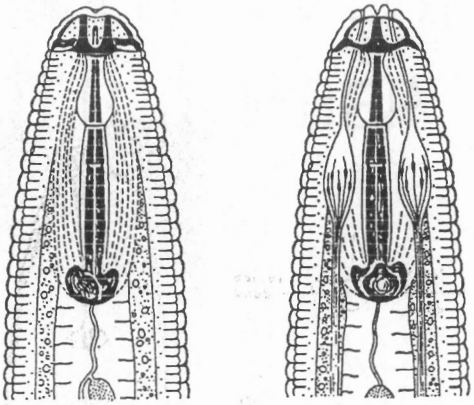
I J K L M

Fig. 11. Comparisons among Heterodera glycines, H. schachtii and H. trifolii J2 based on head morphology, stylets and tails. **A.B**. Anterior region of J2 of H. schachtii. **C.D**. Anterior region of J2 of H. glycines. **E**. Anterior region, stylets and tails of H. glycines. **F**. Anterior region of J2 of H. trifolii (**A-D** from Graney, 1982; **E-F** from Hirschmann, 1956).

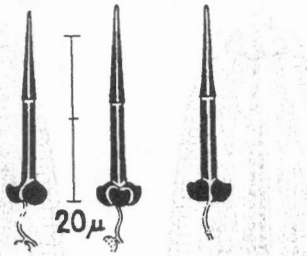

A
dorsal knob
subventral knobs
B
C
dorsal knob
right subventral knob
D

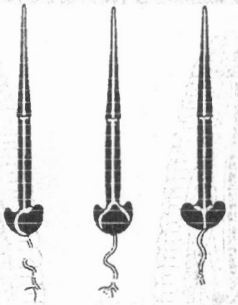


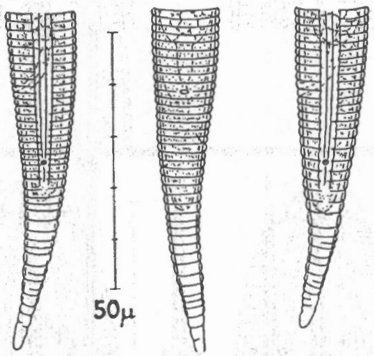
E

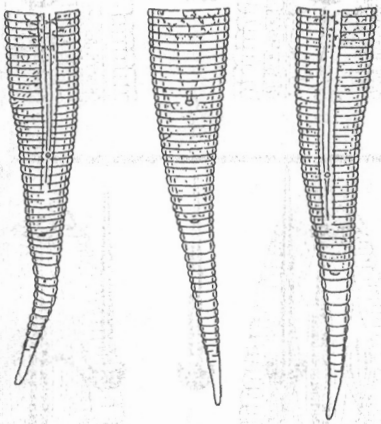


F


20μ




50μ



Part II. Morphology of second-stage juveniles and males of the tobacco cyst nematode complex, Globodera tabacum tabacum, G. t. virginiae and G. t. solanacearum (Nemata: Heteroderinae).

ABSTRACT

Morphological comparisons with light microscopy (LM) and scanning electron microscopy (SEM) were made among second-stage juveniles (J2) and males of several isolates of the three subspecies of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum (GTT), virginiae (GTV) and solanacearum (GTS). Observations focused on the anterior region including head shape, lip pattern, stylet morphology and the tail region including tail shape in J2 and spicules in males. The head region of J2 was set off from the body and consisted of three head annules, six lips, and an oral disk. The shape of the head seen in lateral view showed little variability within or among the subspecies. Stylets of J2 were robust and had three anchor-shaped, rounded knobs. They varied slightly in width and height within each subspecies. The lip region consisted of a central oral disc surrounded by two lateral and two submedial lips. The oral disc varied from rectangular to elliptical. The submedial lip pairs were not fused in all specimens. The tail of J2 was finely pointed and the tip was rounded in all subspecies. The head of the male was set off and contained five head annules, six lips, and an oral disc. The head region was similar in lateral view in all three subspecies. The stylet was robust, with three rounded knobs sloping posteriorly. The dorsal knob of GTV appeared to slope more posteriorly than the other two subspecies. In the SEM, the head region had a large central rounded/ elliptical oral disc with two lateral and two submedial lip pairs. The submedial lips were rounded or rectangular. The submedial lip pairs were fused in most specimens, but not all. The spicules of GTT showed a slightly more enlarged head region than the other two subspecies. The three subspecies could not be separated on the basis of any character or group of characters of J2 or males.

INTRODUCTION

The first report of a cyst nematode parasitizing tobacco plants was made by Lownsbery in 1951(15). The tobacco cyst nematode was described as Heterodera (= Globodera) tabacum and distinguished from the closely related potato cyst nematode (PCN), H. (= G.) rostochiensis Wollenweber, on the basis of host range. TCN did not reproduce on potato whereas PCN did not reproduce on several tobacco varieties (35). Some morphological differences between the two species were reported. The tail was shorter, the distance of the dorsal esophageal gland opening (DEGO) to the base of the stylet of the male was shorter, and the head shape was more set off in males of G. tabacum than in G. rostochiensis (15). The lip region of G. tabacum females had three prominent annules, while those of G. rostochiensis had only two. The display of cuticular punctations between anus and vulva of adult females was parallel or with no alignment in G. tabacum, but perpendicular to the vulva/ anus axis in G. rostochiensis (15).

A cyst nematode belonging to the genus Hetrodera (= Globodera) was found parasitizing Nicotiana tabacum L. var. 'Hicks', in Amelia County, Virginia, by M.H. Holmes and W.W. Osborne in 1961 (28). This nematode, as well as another cyst nematode discovered parasitizing roots of the horsenettle weed, Solanum carolinense L., remained undescribed for several years. Miller and Gray (21) described the 'horsenettle cyst nematode' (HCN) as H. (= G.) virginiae in 1968.

The HCN was found parasitizing horsenettle plants in a field that had not grown potato or tobacco in the previous 30 years. It was found to be pathogenic to certain varieties of burley tobacco, tomato, and eggplant, although it was not known to infest soil where those crops were grown commercially. Miller and Gray (21) distinguished G. virginiae from G. rostochiensis and G. tabacum on the basis of general cyst shape, posterior cyst wall pattern, shape of the female dorsal stylet knobs, Granek's ratio, and the shape of the fenestra . G. virginiae reproduced on Nicotiana acuminata (R. Grah.) Hook., whereas G. tabacum did not.

H.(= G.) solanacearum was initially reported by Osborne (28) in 1961, and was distinguished from the other two TCN species, G. tabacum and G. virginiae, based on slight differences in

general cyst shape, color of young cysts, posterior cyst wall pattern, shape of female stylet knobs, and shape of fenestra (22). G. solanacearum did not reproduce on N. sanderae W. Wats., whereas the other TCN species did. The Virginia TCN species were detected within 48 km of each other. They possibly overlap in their geographic distribution and may interbreed naturally (22, 34).

The tobacco cyst nematode complex was reavaluated by Stone to consist of three subspecies, G. tabacum tabacum (Lownsbery and Lownsbery, 1954) Behrens, 1975 (GTT), G. t. virginiae (Miller and Gray, 1968) Behrens, 1975 (GTV), and G. t. solanacearum (Miller and Gray, 1972) Behrens, 1975 (GTS)(34). GTT is restricted to two counties in Connecticut and two counties in Massachusetts. GTV has been reported in four counties in eastern Virginia, and GTS has been found in 13 counties in the central Piedmont region of Virginia (12,20), as well as one county in North Carolina (16). Reports of their presence in other countries have been published (1, 6).

Tobacco cyst nematodes are responsible for serious yield losses in tobacco, sometimes exceeding 50% (2, 20). Losses caused by cyst nematodes on tobacco in 1984 in Virginia were estimated at nearly 1.8 million dollars, and an additional 2.8 million dollars were spent on chemicals for their control (3, 20). In some cases, growers in Virginia spend $250/ ha to control tobacco cyst nematode (TCN) (11; Johnson, C.S., pers. commun.). Although TCN-resistant cultivars are available, they are intolerant to nematode parasitism and must be used in combination with a nematicide. In Connecticut, tobacco cyst nematode has been responsible for a $50 000/ year loss in broad-leaf tobacco (13) and interacts synergistically with Fusarium wilt (14). GTT and GTS are serious pathogens of tobacco and GTV is pathogenic to several cultivars (19). All three subspecies are pathogenic to certain cultivars of tomato and eggplant (21, 22).

Morphology is still the most commonly used method for identification of nematodes (2). The morphology of second-stage juveniles (J2) or males of Globodera spp. have received little attention. Studies on the morphology of these stages may contribute new and more reliable

characters for identifying Globodera spp.

The purpose of this research is to evaluate new characters for identification of GTT, GTV and GTS and to describe the variability within the tobacco cyst nematode complex. Preliminary observations have been reported (24, 25). Future papers will compare the morphology of females and cysts, and present morphometrics of all life stages.

MATERIALS AND METHODS

Isolates of TCN used in this study are listed in Table I. All populations were reared on 'Rutgers' tomato Lycopersicum esculentum L., in the greenhouse maintained at 25-30° C in either 10- or 15- cm clay pots with a 2:1 steam sterilized top soil to sand mix. After 60-70 days nematodes were extracted by a combination of Cobb's decanting and sieving and a Baermann funnel.

Light microscopy (LM). Specimens prepared for LM were heat-killed and mounted in water on a microscope slide with a thin ring of nail polish. The posterior portion of the male body was cut to overcome difficulties in orientation and for better observation. Observations were made immediately after preparation and specimens were photographed through a planapochromatic, bright field, compound microscope with Polaroid type 55 or 35 mm Agfa Copex film.

Scanning electron microscopy (SEM). Specimens were prepared for SEM by placing them in a BPI dish with 10 drops of buffer (0.1M sodium cacodylate pH 7.2) at 4° C for approximately 15 min. (7). A sequential fixation previously described (26) was modified with various fixatives (4% glutaraldehyde, 2% formalin, 2% acrolein) and mixtures of two or more fixatives (8). After fixation was completed, specimens were left in a refrigerator (4-5° C) for 24-48 hours, followed by three rinses with buffer within a 15-minute period. They were postfixed under a fume hood in 2% osmium tetroxide for 8-48 hours in a refrigerator, and rinsed three times with buffer within a 15-minute period. Specimens were dehydrated in a seven-step ethanol series, including three changes of 100% ethanol. They were either critical point dried or freeze dried. Stylets were extracted (7) by cutting off the anterior portion of the nematode in 0.01- 0.05% sodium

hypochlorite (23) which allowed the stylet to remain intact while the surrounding tissues were dissolved. The stylet was cleaned and placed in the central area of the cover slip using a dental root canal file with the aid of a stereoscope at x60. The stylet was attached and fixed to the cover slip with one drop of 2.5% formalin every 2 minutes until the sodium hypochlorite was removed. Alternatively, tap water was used in place of the formalin which removed the sodium hypochlorite crystals better than did the formalin. The excess formalin or water was drained from the coverslip with either filter paper or a micropipette, and the stylet was air-dried. The specimens were stored in a desiccator overnight, mounted on SEM stubs with double sticky tape, sputter-coated with 20 nm of gold/ palladium, and observed with a Philips 505 SEM operating at 20 kV with a 20-50nm spot size. Images were recorded with Polaroid type 55 film.

OBSERVATIONS

Second-stage juveniles (Figures 1A-L). No clear-cut morphological differences were observed among isolates of the three subspecies and only slight variability was observed.

Anterior region and stylet (Fig. 1A-C; D,F,H): The body ofJ2 tapers anteriorly. The head region is slightly set off and is formed by four head annules, the lip region, and an oral disk. In lateral view it has a slight central depression that corresponds to the oral opening. The cephalic framework is heavily sclerotized. The stylet is robust with three stout, rounded knobs (Fig. 1D, E, F). The dorsal knob is curved anteriorly and appears anchor-shaped. The stylet knobs of some specimens of GTT were stouter than others. The lip region, in SEM (Fig. 1J-L), has an elongated elliptical oral disc containing a rectangular prestoma. In some specimens, the oral disc was more rectangular than elliptical (Fig. 1J). The oral disc is surrounded by submedial lips formed by fusion of the dorsal and ventral lip pairs which may not be fused in some specimens. The smaller lateral lips are separated from the oral disc by the amphidial openings that are frequently obstructed with secretions. The shape of the submedial lips is rectangular to rounded (Fig. 1J-L) and this lip pattern is Stone's type 1 (33). There were no observable

differences among the three subspecies, and the variability described above is present in GTT, GTV, and GTS.

Tail region (Fig. 1E, G, I). The tail of second-stage juveniles of all members of the TCN complex is pointed and ends in a fine rounded tip. There is only slight variability in the width of the tip. The hyaline portion of the tail is approximately one half the tail length. Frequently, lipid inclusions are observed near the tail tip (Fig. 1I). No observable morphological differences occurred among the isolates other than natural variability.

Males (Figures 2A-I; 3A-F). Few morphological differences were found among isolates of the three subspecies. The shape of the stylet knobs was slightly different. The dorsal knob of GTV males slopes posteriorly more than in GTT and GTS (Fig. 2A-C; D-F). The head of the spicule of GTT was slightly larger than in GTV and GTS.

Anterior region and stylet. (Fig. 2A-I). The anterior region is slightly set off from the body annules and consists of six annules, six lips, and an oral disc. The center of the head region is slightly depressed, in lateral view, corresponding to the oral opening. The cephalic framework is heavily sclerotized. The stylet is robust and the cone is slightly shorter than the shaft. The cone narrows slightly in the middle. The knobs are rounded and sloped posteriorly. In GTV, the dorsal knob slopes more than in the other two subspecies (Fig. 2B, E). When viewed with the SEM (Fig. 2G-I), the lip region consists of a large, elliptical oral disc containing a centrally located rectangular prestoma. The oral disc is surrounded by four large submedial lips which are rectangular or rounded and by two small lateral lips. The amphids open between the lateral lips and oral disc. Morphological differences of males of the isolates of the three subspecies were not detected.

Tail region (Fig. 3A-F). The tail of males of the TCN complex is twisted 90° in relation to the rest of the body. Two conspicuous spicules that slightly curved ventrally are present. The spicules usually protrude in live specimens but they are usually retracted in fixed specimens (Fig. 3A-C). The head of the spicule is more enlarged in GTT (Fig. 3A, D). However, in some

specimens, the head region appeares to be the same diameter as the shaft. The cylindrical shaft and blade end in a single tip, characteristic of males of the genus Globodera. The tip of the spicule may be pointed (Fig.3D, F) or more rounded (Fig.3E) and varies with individuals. No clear-cut differences in spicule morphology were detected among the three subspecies.

DISCUSSION

Members of the TCN complex were analyzed by Stone (32, 34), Greet (10), and Green (9). Stone compared the morphometrics of second-stage juveniles and males of the three TCN species, three pathotypes (A, B and E) of PCN, and another cyst species, H. mexicana nomen nudum (5). Comparisons were made also using the lip patterns of J2. Stone (32) recognized three major groups of round cyst nematodes on the basis of lip patterns of J2. One group included PCN pathotype A, another included pathotypes B and E, and a final group included the TCN complex and H. mexicana . Greet considered the TCN complex and H. mexicana to be indistinguishable based on polyacrylamide gel electrophoretic patterns of total proteins (10). Only G. tabacum had a faint grayish band that was distinct from the others. PCN pathotype A was distinct from pathotype E, and both differed from the TCN species. Miller and Gray (21, 22) did not report morphological differences among J2 and males of G. virginiae, G. solanacearum, and G. tabacum.

Roberts and Stone (30) concluded that host range data within Solanum spp. failed to differentiate G. solanacearum, G. virginiae, and G. tabacum. Furthermore, Miller (19) reported that all crosses between combinations of the three species produced fertile hybrids. Because the three TCN species have very small morphological differences, similar polyacrylamide gel patterns, host range, and ability to produce viable hybrids, Stone (34) proposed ranking them as subspecies: GTT, GTV and GTS. Although not all authors accept this scheme (3, 31), our observations support the present status, as established by Stone.

Olsson (27) used the morphology of male spicules to distinguish between PCN species and

two TCN species (G. virginiae and G. tabacum). According to this author, G. tabacum has a shorter and thicker spicule as compared to G. virginiae. Our studies, however, failed to show observable morphological differences in spicule morphology. Extracted stylets of J2 and males of the three subspecies were similar.

The lip patterns ofJ2 and males were not different. Othman, et al. (29) reported that the submedial lip pairs of J2 were fused in Globodera (contrary to Stone's type 1 pattern, in which the submedial lip pairs are separate), except for G. rostochiensis. Our observations indicate that the submedian lip pairs of J2 are occasionally fused, and this character is not useful to distinguish subspecies. Wouts (36) differentiated several species of Globodera by the shape of the J2 stylet knobs but he did not include TCN. Our studies revealed no clear-cut differences in stylet knob morphology among the three subspecies.

In conclusion, J2 and males of several isolates of the three subspecies of the tobacco cyst nematode complex, GTT, GTV and GTS, reared on the same plant host to avoid ecophenotypic variation were morphologically very similar, suggesting that these subspecies can not be identified on the basis of morphology of J2 and males. These results are consistent with Stone's placement of these organisms as a group of subspecies.

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Table I. **Isolates of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum (GTT), virginiae (GTV) and solanacearum (GTS) used in this study.**

ISOLATE	LOCATION	COUNTY, STATE	ORIGIN
GTT-1 (type locality)	Hazardville	Hartford, CT	P.M. Miller
GTT-2	Windsor	Hartford, CT	P.M. Miller
GTV-1	Horton	Suffolk, VA	L.I. Miller
GTV-1-X	Horton	Suffolk, VA	M. Mota
GTV-11(type locality)	Standard 24	Suffolk, VA	L.I. Miller
GTS-1	Fisher-Nottoway	Nottoway,VA	L.I. Miller
GTS-10 (type locality)	Watkins	Amelia, VA	L.I. Miller

Figure 1. Anterior region (**A-C; D, F, H; J-L**) and tails (**E, G, I**) of second-stage juveniles of certain isolates (see Table I) of the tobacco cyst nematode complex, Globodera tabacum tabacum (GTT), G. t. virginiae (GTV) and G. t. solanacearum (GTS). (**A**). LM of GTT-1. (**B**). LM of GTV-1. (**C**). LM of GTS-10. (**D**). SEM of stylet of GTT-1. (**E**). LM of tail of GTT-1. (**F**). SEM of stylet of GTV-1. (**G**). LM of tail of GTV-1. (**H**). SEM of stylet of GTS-1. (**I**). LM of tail of GTS-1; arrows indicate lipidic inclusions (**J**). SEM of "en face" view of GTT-1. (**K**). SEM of "en face" view of GTV-1. (**L**). SEM of "en face" view of GTS-1. (**A**), (**B**),(**C**), (**E**), (**G**), and (**I**) are lateral observations.

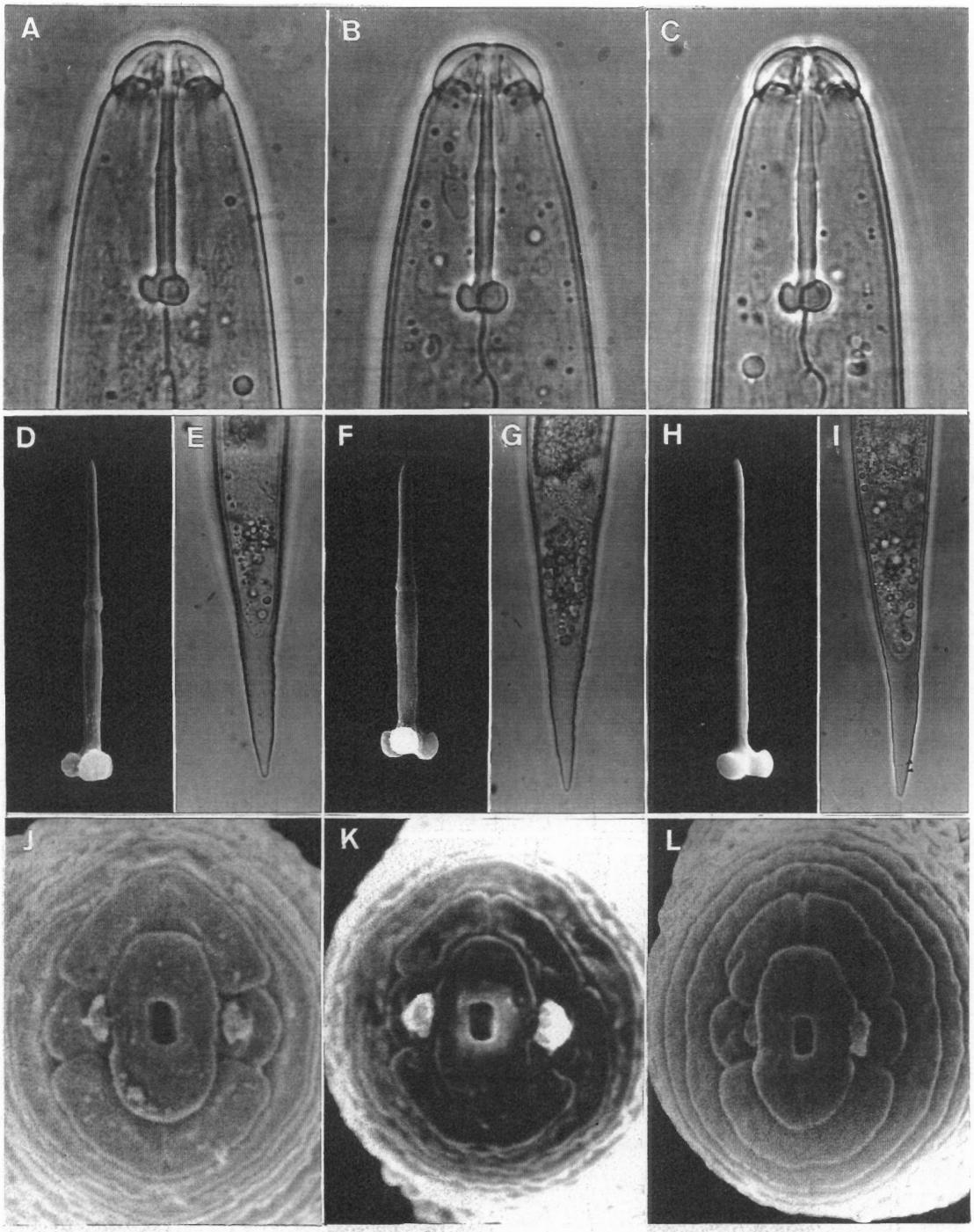
A
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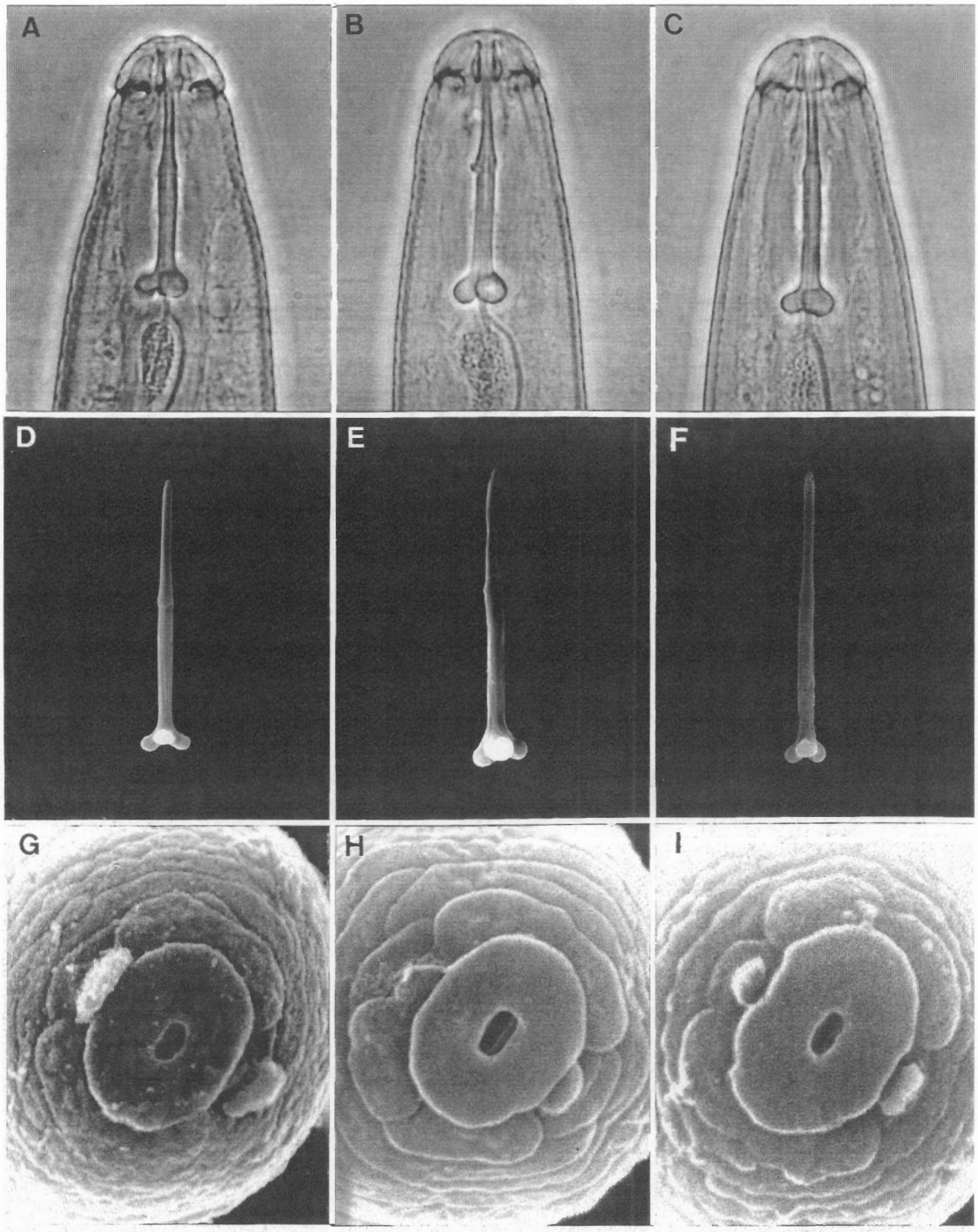
Figure 2. Anterior region of males of certain isolates (see Table I) of the tobacco cyst nematode complex, Globodera tabacum tabacum (GTT), G. t. virginiae (GTV) and G. t. solanacearum (GTS). (**A**). LM of GTT-1. (**B**). LM of GTV-1. (**C**). LM of GTS-1. (**D**). SEM of stylet of GTT-1. (**E**). SEM of stylet of GTV-1. (**F**). SEM of stylet of GTS-1. (**G**). SEM of "en face" view of GTT-2. (**H**). SEM of "en face" view of GTV-1. (**I**). SEM of "en face" view of GTS-1. (**A**), (**B**) and (**C**) are lateral observations.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I

Figure 3. Tail region of males of certain isolates (see Table I) of the tobacco cyst nematode complex, Globodera tabacum tabacum (GTT), G. t. virginiae (GTV) and G. t. solanacearum (GTS), observed in lateral position. (**A**). GTT-2. (**B**). GTV-11. (**C**). GTS-1. (**D**). SEM of spicule of GTT-1. (**E**). SEM of spicule of GTV-1. (**F**). SEM of spicule of GTS-10.

A
B
C
D
E
F

Part III. Morphology of females and cysts of the tobacco cyst nematode complex, Globodera tabacum tabacum, G. t. virginiae, and G. t. solanacearum (Nemata: Heteroderinae).

ABSTRACT.

Detailed morphological comparisons with light and scanning electron microscopy were made of white females and cysts of several isolates of the three subspecies of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum (GTT), virginiae (GTV), and solanacearum (GTS). Observations focused on the body shape, anterior region including head shape, lip pattern, stylet morphology, and the terminal area in females; and body shape and terminal area of cysts. The most useful characters to separate the three subspecies were female body shape, stylet knobs, perineal tubercles, cyst shape, anal-fenestral ridges pattern, anus, and tail region. GTT is characterized by having round females and cysts with sharply back sloped stylet knobs, clumped perineal tubercles in the vulval region, tight parallel ridges in the cyst anal-fenestral region, and a distinct non crescent shaped tail region. GTV is characterized by its ovoid to elipsoid female and cyst shape, the "Dutch shoe" shape of the dorsal stylet knob, the more individualized perineal tubercles, a maze like pattern of ridges in the anal-fenestral region, and an indistinct anus. GTS is characterized by its ovoid to elipsoid female and cyst shape, moderately backward sloped stylet knobs, more widely separated ridges, and a distinct anus with a usually crescent shaped tail region. Much variability in shape and patterns is visible among all the isolates of the different subspecies. Tubercles in the neck as well as bullae are reported and discussed.

The tobacco cyst nematodes (TCN) are grouped into three sub-species: Globodera tabacum tabacum (Lownsbery and Lownsbery, 1954) Behrens, 1975, (6) (GTT), G. t. virginiae (Miller and Gray, 1968) Behrens, 1975, (9) (GTV), and G. t. solanacearum (Miller and Gray, 1972) Behrens, 1975 (10) (GTS). GTV and GTS primarily occur in Virginia (8) and GTT is limited to Connecticut and Massachusetts (8).

Efficient management of these cyst nematodes requires accurate diagnosis, first at the genus level, between Globodera and Heterodera; at the species level, among Globodera, and also among the subspecies of the TCN. For example, a single field may have soybean cyst (Heterodera glycines) and/ or tobacco cyst (Globodera tabacum) nematode or some other common cyst nematode such as Cactodera weissi or H. glycines. Likewise, G. t. solanacearum is damaging to flue-cured tobacco but G. t. virginiae is not. Furthermore, changes in farming practices and crop diversification may cause shifts in the population dynamics and affect species diversity and distribution. Detailed morphological observations of various life stages may provide additional characters that are useful for the identification of these forms.

Recent observations by light (LM) and scanning electron microscopy (SEM) of second-stage juveniles (J2) and males of the TCN complex revealed no morphological characters that were useful to separate the three subspecies (13). Morphological variability among isolates and subspecies was extremely low for all features examined. Morphometrics of J2 and males were also useless for identification of subspecies, with the possible exception of male tail length (Mota and Eisenback, unpublished).

The cyst is the most readily available stage in the soil and has traditionally been used for identification and taxonomy (1). The terminal area containing the anus and vulva was used to classify Globodera species (3, 4,15); however, females have not been utilized frequently. The only morphological studies of females of the TCN complex, besides the original descriptions, were done by Green (3) and Mulvey (15).

The purpose of this paper is to evaluate the morphological variability of the white females and

cysts, and to search for new and reliable characters of the three subspecies, GTT, GTV, and GTS. Preliminary observations have shown some variability within each subspecies (12). A separate paper will report on the morphometrics of eggs, J2, males, females, and cysts of various isolates of the TCN complex.

MATERIALS AND METHODS

Isolates of TCN used in this study are listed in Table 1. All populations were reared on 'Rutgers' tomato (Lycopersicum esculentum L.) in the greenhouse in either 10- or 15- cm clay pots with a 2:1 steam sterilized top soil to sand mix. After 60-70 days females and cysts were extracted by Cobb's decanting and sieving method. Females were immediately processed for LM and SEM to minimize cement formation around the head. All images were recorded on Polaroid type 55 film.

Light microscopy (LM). Females were sequentially fixed in a 1:1 (v:v) mixture of 2% glutaraldehyde and 1% formalin for at least 24 h. The anterior portion of the body was severed and placed in a drop of water on a thickly ringed slide and covered with a coverslip. Properly oriented specimens were photographed through a planapochromatic, bright field, compound microscope. The cyst terminal area was cut in 45% lactic acid, transferred to glycerin jelly on a microscope slide, and topped with a coverslip. The mount was sealed with nail polish.

Scanning electron microscopy (SEM).

Females. Specimens were placed in a Bureau of Plant Industry (BPI) dish with 10 drops of tap water at 4° C for approximately 15 min. (2). Four percent glutaraldehyde was added, two drops every 10 min. After fixation was completed, specimens were kept in a refrigerator (4-5° C) for 24-48 hours, followed by three rinses with 0.1M sodium cacodylate buffer pH 7.2 within a 15 min. period. They were postfixed under a fume hood in 2% osmium tetroxide, kept for 8-48 hours in a refrigerator, and rinsed three times with buffer within a 15 min. period. The specimens were stored in a desiccator overnight, mounted on SEM stubs with double sticky tape with the

head region straight up for observation of the anterior region, sputter-coated with 20 nm of gold/ palladium, and observed with a Philips 505 SEM operating at 20 kV with a 20-50 nm spot size.

Stylets were extracted by cutting off the anterior portion of the female body in 0.01- 0.05% sodium hypochlorite (11) in which the stylet remained intact while the surrounding tissues dissolved. Some specimens required higher concentrations of bleach. The stylet was cleaned and placed in the central area of the dissecting chamber with a dental root canal file, using a stereoscope at x60. The stylet was attached and fixed to the cover slip with one drop of 2.5% formalin every 2 min. until the sodium hypochlorite was removed. Alternatively, tap water was used in place of the formalin to remove the sodium hypochlorite. The excess formalin, or water, was drained from the coverslip with either filter paper or a micropipette, and the stylet was air-dried. The specimens were stored in a desiccator overnight, mounted on SEM stubs with double sticky tape, sputter-coated and observed as previously described.

Cysts. A simplification of a previously described method (5) was used to prepare cyst terminal areas for SEM. The terminal areas were cut in 45% lactic acid and placed directly on a SEM stub with double sided sticky tape, convex side up. To observe the internal morphology of the cyst wall, some specimens were mounted concave side up. Specimens were sputter-coated, as previously described.

OBSERVATIONS

Females (Figs. 1-5). The female body shape of the tobacco cyst nematode varies from round to ovoid or elliptical, the same way as in cysts. The round shape is more frequent among the GTT isolates, whereas GTV and GTS are mostly ovoid (Fig. 1 A) or ellipsoid. The surface of the female body is covered by cuticular ridges. These ridges are transverse or annulated in the neck and head regions in a similar fashion as in cysts (Figs. 1 E,F), zig-zag in the midbody of most specimens, and whorled around the terminal region containing the anus and vulva similar

to cysts (Fig. 1 D).

Anterior region. The conical neck region is quite variable in size (Figs. 1 E, 2 C). Two or three annules are prominent in the head when viewed laterally with LM. These annules correspond to the oral disk, the fused lips, and perhaps an additional body annule (Fig. 2 D). The stomatal opening is located in the center of the oral disk (Fig. 3 C). The oral disk is typically an oblong rectangle in all three subspecies and usually has sharp edges; however in some specimens the margins are rounded (Fig. 3 A). Below the oral disk, the lateral and submedial lips are typically fused. The fusion of the lips may result in the formation of a single hexagonal annule (Fig. 4 D). The amphidial openings are apparent in many specimens as slits in the lateral lips. Frequently amphidial exudates obscure the openings (Fig. 3 B, D). The lateral and submedial lips may also have irregular edges or cusps (Fig. 4 A, C; 5 A, B). These cusps may be rounded (Fig. 5 A, B) or pointed (Fig. 4 A, B). Neck annules may be continuous (Fig.5), fragmented or transformed into tubercles (Fig. 3 B, D). These tubercles vary in size but are not present in all specimens. The excretory pore is clearly at the base of the neck (Fig.1 F, arrow). It is contained within a subglobular depression within the cuticle.

The stylet is robust with a cone approximately 1/2 the total length, a cylindrical shaft, and three stout basal knobs. The stylet cone is very elastic (Fig.1 K). The dorsal esophagheal gland opening (DEGO) is visible in LM 4-8 μm below the basal knobs (Fig. 1 K). The basal knobs in GTT are sharply sloped posteriorly (Fig. 2 B) but in GTV the dorsal knob is typically curved anteriorly like a "Dutch shoe" (Fig. 2 F). The basal knobs in GTS are similar to those of GTT but they are not as sharply sloped posteriorly (Fig. 2 J). In each subspecies variation in the shape of the stylet knobs occurred that differed from the pattern typical for the subspecies and resembled that of other subspecies (Fig. 2 C, D, G, H, and K, L). The lumen lining of the median bulb is triradiate (Fig. 1 E, I, J). Several small vesicles surrounded the lumen lining between the DEGO (Fig. 1 K) and the median bulb in a few specimens of GTT (Fig. 1 L). Grooves were frequently observed on the stylet shaft of all subspecies in SEM (Fig. 2 A, E, I)

Terminal area (Fig. 6). The posterior region of the female, the terminal area, contains the vulva and the anus. The cuticular ridges of this region assume a circular whorled pattern (Fig. 1 D). The vulval slit is clearly visible and the vulval region is flanked on each side by two vulval crescents made up of perineal tubercles (Fig. 6). These tubercles appear to be more clumped in the GTT isolates (Fig. 6 A) and more discrete or individualized in GTV and GTS (Figs. 6 B, D). The vulval region is variable in shape but it has a general ovoid to elliptical shape in all three subspecies. It may be more rounded and compressed (Fig. 6 A) or more elongated (Fig. 6 B). No clear differences are visible in the terminal area of the female that permit differentiation of the three subspecies except for the more clumped tubercles in GTT.

Cysts (Figs. 7, 8). Similar to the female, the cyst shape may be rounded in GTT or elliptical to ovoid in GTV and GTS (Fig. 1, B-D). The cuticular ridges also display similar patterns as in the females, but are more clearly distinct. There are transverse annulations in the neck region (Fig. 1 F), zig-zag markings in the midbody (Fig. 1 C), and a whorled pattern in the terminal area (Fig. 1 D). At the base of the neck, a transition from zig-zag to the annulated pattern may be visible. Transverse striation in the midbody is present in some rare instances (Fig. 1 D, 3 A).

Most observations of the terminal area were made with SEM because in LM the ridges and grooves or valleys produce an optical illusion which makes it difficult to determine which is which. The terminal area is circumfenestrated with a round to elliptical fenestra. Young cysts may have remnants of vulval tissue as well as perineal tubercles. Fully matured cysts show none of these structures, the fenestra is a simple hole from which the J2 emerge. Dark bodies resembling the bullae found in some species of Heterodera were rarely present in all three subspecies (Fig. 1 M-P). Both round (Fig. 1 N,O) and finger-shaped bullae (Fig. 1 M) were observed inside the terminal area and surrounding the fenestra. A thick ring of cuticle which may have been formed by the coalescence of the bullae was present in some specimens (Fig. 1 P). Inside the cyst wall, a conspicuous V-shaped groove points to the anus (Fig. 1 P).

The morphology of the ridges between the anus and the vulva is fairly characteristic for each

subspecies. However, this character is variable among and within isolates of the same subspecies. In GTT the ridges are tightly packed and usually oriented in parallel rows perpendicular to the vulva-anus axis (Fig. 7 A, D). In some specimens, however, the ridges are not parallel and are wider apart (Fig. 8 A). Usually, the anus is closer to the fenestra in GTT than in GTV and GTS, and it is also flanked by parallel ridges (Fig. 7 A, D). In several specimens the pattern of ridges around the anus is more circular (Fig. 8 A, G). Also some specimens have a large crescent shaped tail region just above the anus (Fig. 8 D), although it was not characteristic for the subspecies.

In GTV the ridges between the fenestra and anus are usually compacted and typically form a maze-like pattern (Fig. 7 B, E). The pattern often extends to adjacent areas around the anus and the fenestra. In some variant specimens the pattern of ridges may be wider apart, as in GTS (Fig. 8 B). Some specimens of GTV also have parallel ridges in the anus-fenestra region similar to that of GTT (Fig. 8 B). The anus of GTV is usually further from the fenestra than in GTT and is sometimes difficult to locate because it is obscured by adjacent grooves and ridges. Usually the tail region is vague and not shaped like a crescent (Fig. 8 E).

The typical characteristic cyst pattern for GTS has large, wide grooves between the ridges and no maze-like pattern of ridges between the anus and fenestra (Fig. 7 C, F). Variants of this pattern, however, occur in all isolates. In some, parallel, tight ridges are present in the anus-fenestra region similar to that of GTT. In a few specimens the ridges even run in the same direction of the anus-fenestra axis (Fig. 8 I). The anus is usually separated from the adjacent grooves in the center of a small anal basin (Figs. 7 C, F; 8 F), and the tail region is ususally crescent-shaped (Fig. 7 C). None of the specimens of any of the isolates of GTS had parallel ridges running across the anal region.

DISCUSSION

Miller and Gray (10) used the area between the anus and fenestra to distinguish among the three subspecies Heterodera tabacum (=GTT), H. virginiae (=GTV), and H. solanacearum (=GTS). The authors distinguished GTS from the other two subspecies by the presence of widely spaced grooves in the terminal area. Although very common in this subspecies, variants with more closely spaced cuticular ridges are often found. This variability is implicitly accepted by the expression "presence of highly variable grooves in the terminal area" (10). The authors in a previous report distinguished GTV from GTT by a maze-like pattern of grooves and ridges between the anus and fenestra in GTV compared to a non maze-like pattern in GTT (10). Our observations support the absence of a maze-like pattern of ridges in GTT, but in some variants of GTV the parallel ridges occurred in the anus-vulva area (Fig.8 B, E). Miller and Gray (10) also distinguished the three subspecies based on cyst and fenestral shape. Cysts are globose in GTT and turbinate to subglobose in GTV and GTS. The fenestra is barrel-shaped with convex ends in GTS, circular to elliptical in GTV, and elliptical with obtuse ends in GTT. Our observations indicate that the globose shape of the cyst is quite typical and consistent among all the GTT isolates in comparison to the more elongated (turbinate to subglobose) shape in GTV and GTS. The shape of the cyst may be the most reliable character in distinguishing GTT from GTV and GTS.

Green (3) observed several Globodera species with the SEM. He found that the anus was near the middle of the terminal area in GTT which resulted in relatively small anus to fenestra distance. Also, the ridges between anus and vulva grooves are parallel with relatively few ridges (6-7) that are forked but rarely joined. The anal grooves are parallel. In GTS and GTV, the anal grooves are spiral and the anus is closer to the tail tip. GTS has an anal area usually separated from adjacent grooves, whereas in GTV it is continuous. The tail tip in GTS is large and crescent-shaped, but in GTV it was indistinct or small and circular. Green only compared one isolate of each subspecies (3) and our observations of several isolates indicate considerable variability in

the typical situations for the three subspecies mentioned by Green. The position of the anus relative to the fenestra is variable in comparisons of several isolates of the same subspecies. In GTT isolates, the anus is closer to the fenestra than some isolates of GTV and GTS, but not all. The anus to fenestra distance and other morphometrics will be discussed in a separate paper on the TCN complex . The ridges are typically parallel in the anus region of GTT, but are variable. In GTS, the anus is generally separated from the surrounding grooves, whereas in GTV the anus is more continuous with the grooves; in some cases it is even difficult to locate. We prefer the designation "tail region" instead of "tail tip", since the region is flat and no protuberances were visible. In GTS the tail region is often crescent shaped but not in GTV and GTT.

Mulvey (15) distinguished GTS from GTV and GTT by a distinct circumfenestral area in most cysts; GTV has a maze-like pattern of lines between anus and vulva unlike GTT. In addition GTT has more clumped tubercles, and in GTV and GTS they are more discrete. We found that the circumfenestral area is distinct in all isolates of all subspecies and does not seem to be characteristic of only GTS. We agree that the perineal tubercles are more clumped in GTT, particularly in white females, even though sometimes it is difficult to distinguish GTS and GTV from GTT by this character alone. The tubercles were distinct in many specimens of GTT. These tubercles may originate from transformation of the nearby ridges. Othman and Baldwin (16) reported the existence of tubercles on the neck region of females of all Globodera species. We have noticed that they occur frequently, but not always. The tubercles appear to form in the basal region of the neck from annules that fragment.

Miller and Gray (9) reported vesicle-like structures arround the lumen lining of the esophagus of females of GTV, just above the median bulb. We found similar structures in GTT females further anterior on the lumen lining (Fig. 1 L), close to the DEGO. They were not observed in GTV or GTS, but they may be present since they were observed in GTT only incidentally. Transmission electron microscopy combined with video techniques observing the development and function of these structures may help clarify their genesis and purpose. The

V-shaped structure inside the cyst wall is refered "as possibly a discontinuity in one of the inner cuticular layers" (18). It is more likely to be the place of attachment of anal and/ or vaginal dilator muscles. The presence of bullae, rare in this genus but common in Heterodera, may be useful to understand the relationships and phylogeny of Globodera and Heterodera.

In conclusion the best characters for identification of these subspecies are the shape of the female stylet knobs, the maze-like pattern in the terminal area, and the small almost inconspicuous anus in GTV (Table 2). In GTT, the shape of the cyst is round, the ridges in the anal region are parallel, and the perineal tubercles are more clumped. In GTS, ridges in the terminal area are more widely separated, the anal region is distinct, and the tail region is crescent shaped. Our observations were made with cysts collected on a 60-mesh screen and were at different stages of maturation. Future research on the developmental biology of these nematodes may clarify how the observed morphological characters change over time.

The taxonomic status of this complex has not been totally clarified. Even though Stone (19) proposed their status as subspecies, which was recently confirmed by Mugniery et al. (14), not all authors agree. The three subspecies interbreed quite easily and produce viable hybrids (7). Recent developments on the morphology of some of the hybrids formed between GTV and GTS confirm their subspecific status (14). GTS has been suggested to be a junior synonym of GTV (14) but GTT was not included in the study. The morphology as well as the morphometrics of J2 and males failed to provide useful characters to distinguish these subspecies (13; Mota and Eisenback, unpublished). The differences in the white females and cysts of the TCN complex observed in this study are quite subtle, except for the general shape which also correlates with the morphometrics of the length/ width ratio (Mota and Eisenback, unpublished). Also, variants within the isolates of a particular subspecies have characters that are typical of other subspecies.

Globodera species appear to have a close evolutionary relationship with the solanaceous plants that they parasitize (17), and thus create opportunities for the recombination of the

genotypes of different species and make the biological concept of species difficult to apply.

Host range studies using several different Solanum and Globodera isolates also proved unsuccessful in differentiating the three subspecies (17). DNA analysis is a promising tool to help clarify variability in intraspecific populations of plant parasitic nematodes. The analysis of the TCN complex genome may provide more insight into the extent of the variability of this group. Until further research is accomplished, we suggest the utilization of the subspecific status for the TCN complex, based on the occurrence of subtle morphological differences and the fact that they interbreed easily.

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Table 1. **Isolates of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum (GTT), virginiae (GTV) and solanacearum (GTS) used in this study.**

ISOLATE	LOCATION	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 farm	Suffolk, VA	L.I. Miller
GTV-1-X	Horton farm	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-3	Irby	Amelia, VA	L.I. Miller
GTS-5	Lynch	Amelia, VA	L.I. Miller
GTS-8	Smith	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

Table 2. Most important female and cyst characters for distinguishing Globodera tabacum tabacum (GTT), G. t. virginiae (GTV) and G. t. solanacearum (GTS).

	Females		Cysts		
	Perineal tubercles	Stylet knobs	Shape	Anal-fenestra ridges	Anus
GTT	clumped	sloping backwards	round	parallel ridges	distinct
GTV	individual	dorsal knob shaped as a "Dutch shoe"	ovoid/ ellipsoid	maze-like pattern	small indistinct
GTS	individual	moderately sloping backwards	ovoid/ ellipsoid	more widely separated	distinct

Fig. 1. General morphological characters of Globodera tabacum sspp. females and cysts. GTT= G. t. tabacum; GTV= G. t. virginiae; GTS= G. t. solanacearum. LM= light microscopy; SEM= scanning electron microscopy. **A**. GTT-1 female, LM. **B**. GTV-6 cyst, SEM. **C**. GTT-1 cyst, SEM. **D**. GTS-1 cyst, SEM. **E**. GTT-1, female anterior region, LM. **F**. GTS-6, cyst neck and excretory pore, SEM. **G**. Excretory pore, detail magnified from F. **H**. GTS-9, cyst, SEM, detail of base of neck shoing cuticular ridges and tubercles. **I**. GTS-1, stylet and median bulb lumen lining, SEM. **J**. Median bulb lumen lining, magnified from I. **K**. GTS-1 stylet showing DEGO (arrow), LM. **L**. GTT-2 vesicles attached to lumen lining, LM. **M**. GTS-15, finger-shaped bullae inside fenestra, LM. **N**. GTS-5, round bullae inside fenestra, SEM. **O**. Same as N, but LM. **P**. GTV-5, V-shape structure pointing to anus and Thickening surrounding inside of fenestra, SEM.

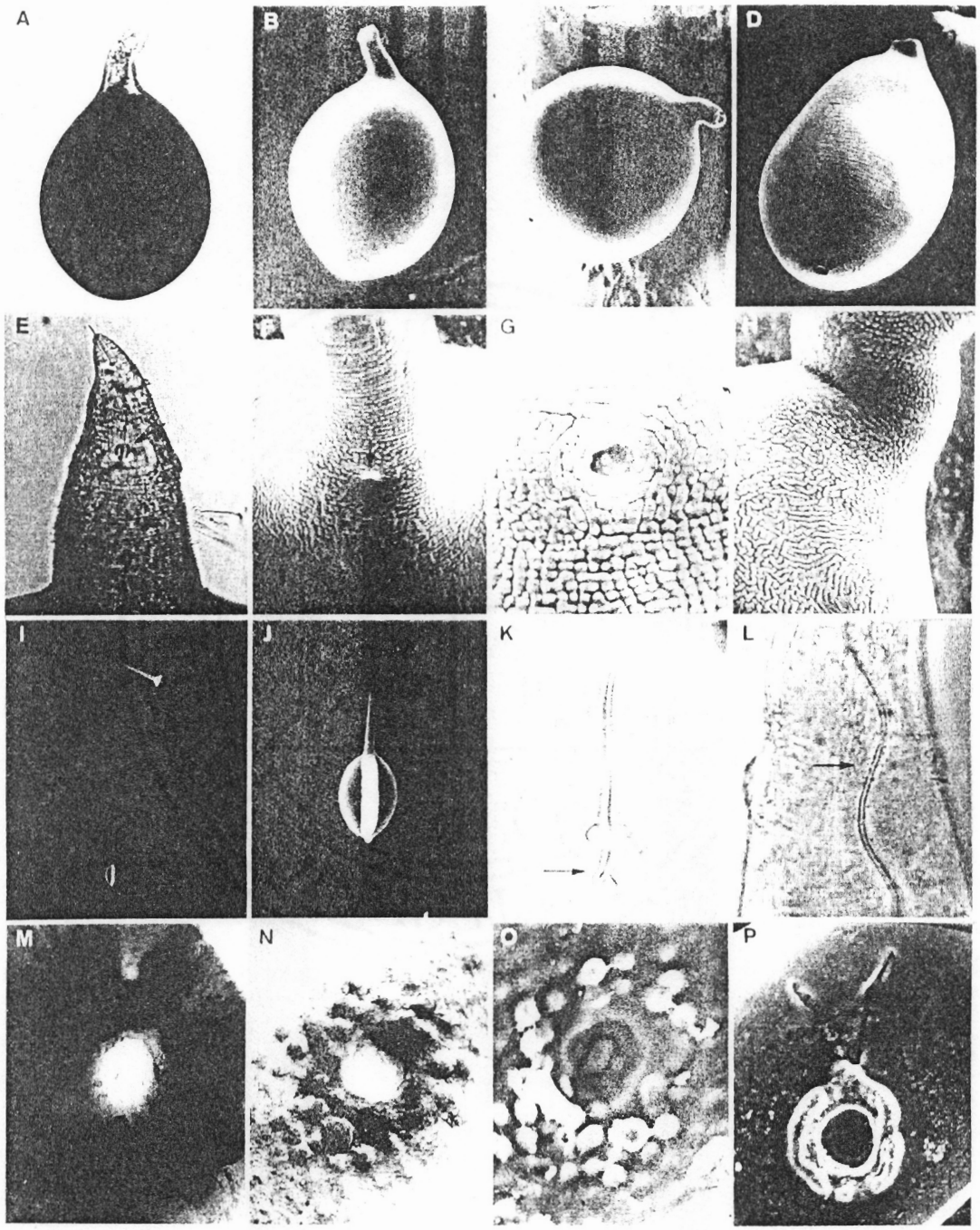
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Fig. 2. Morphology of the anterior region of females of Globodera tabacum sspp. GTT= G. t. tabacum; GTV= G. t. virginiae; GTS= G. t. solanacearum. LM= light microscopy; SEM= scanning electron microscopy. B-D; F-H; and J-L, lateral view, LM. **A**. GTT-1 exctracted stylet, SEM. **B**. GTT-2. **C**. GTT-1. D. GTT-2. **E**. GTV-1, extracted stylet, SEM. **F**. GTV-11. **G**. GTV-1. **H**. GTV-11. **I**. GTS-1, extracted stylet, SEM. **J**. GTS-10. **K**. GTS-1. **L**. GTS-1.

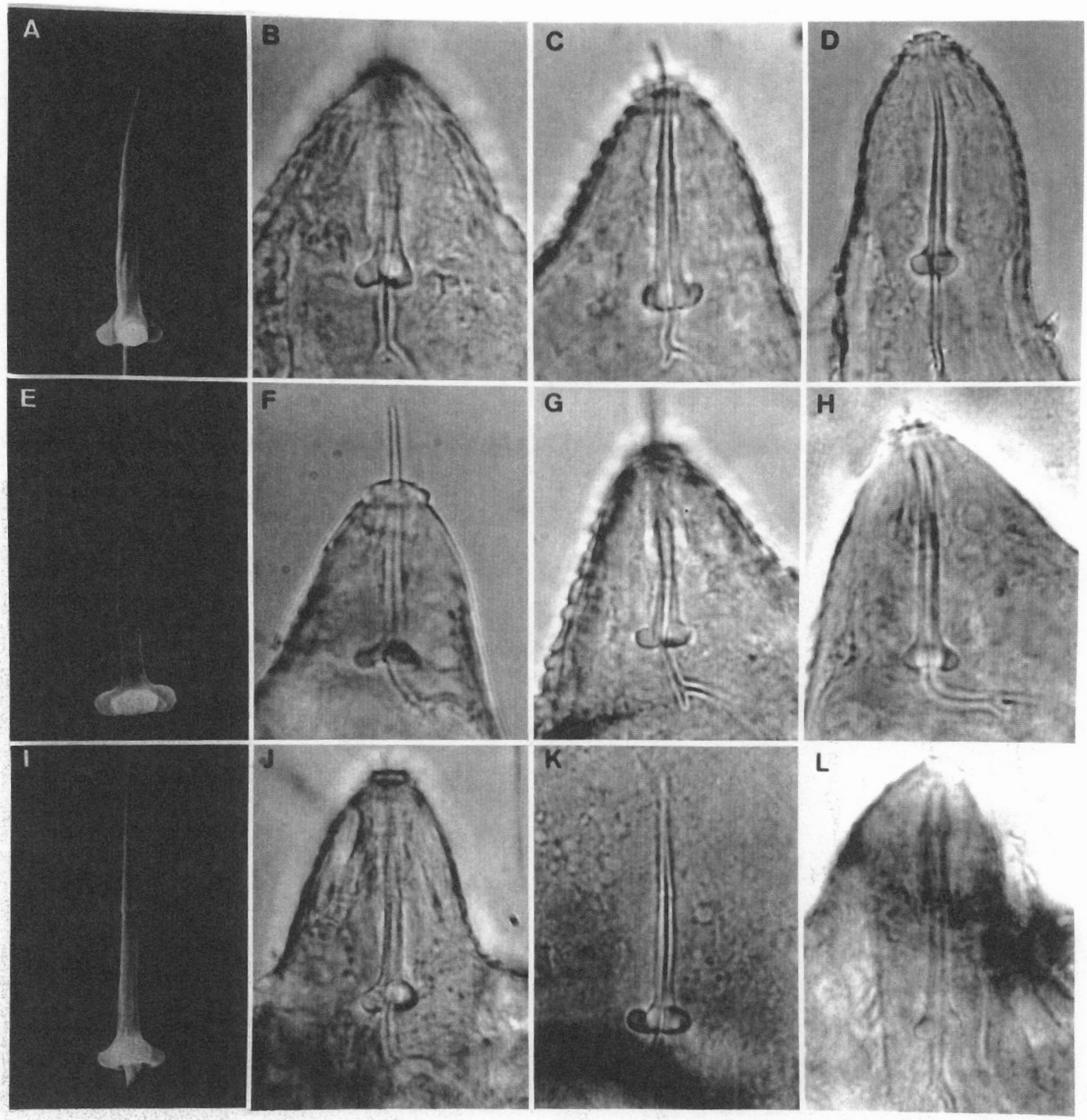
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Fig. 3. Anterior region of females of Globodera tabacum tabacum, as seen in oblique (A, B) and face (C, D) SEM views. **A, C**. GTT-5. **B, D**. GTT-5.

A
B
C
D

Fig. 4. Anterior region of females of Globodera tabacum virginiae, as seen in oblique (A, B) and face (C, D) SEM views. **A, C**. GTV-11. **B, D**. GTV-11.

A
B
C
D

Fig. 5. Anterior region of females of Globodera tabacum solanacearum, as seen in oblique (A) and face (B) SEM views. **A.** GTS-10. **B.** GTS-10.

B
A

Fig. 6. SEM observations of the terminal area of females of Globodera tabacum sspp. GTT= G. t. tabacum; GTV= G. t. virginiae; GTS= G. t. solanacearum. **A**. GTT-1. **B**. GTV-1. **C**. GTS-1. **D**. Detail of the fenestra, magnified from C.

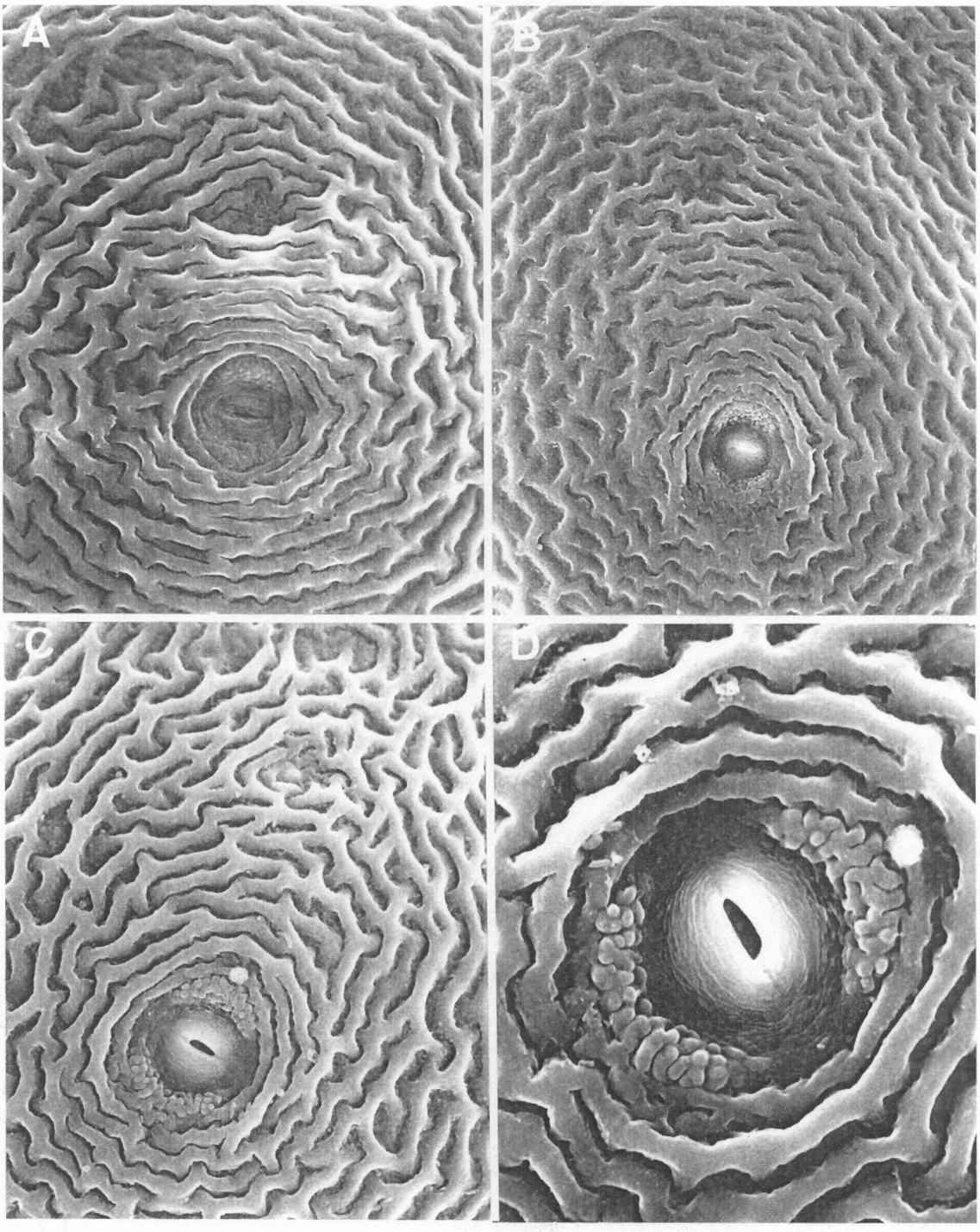
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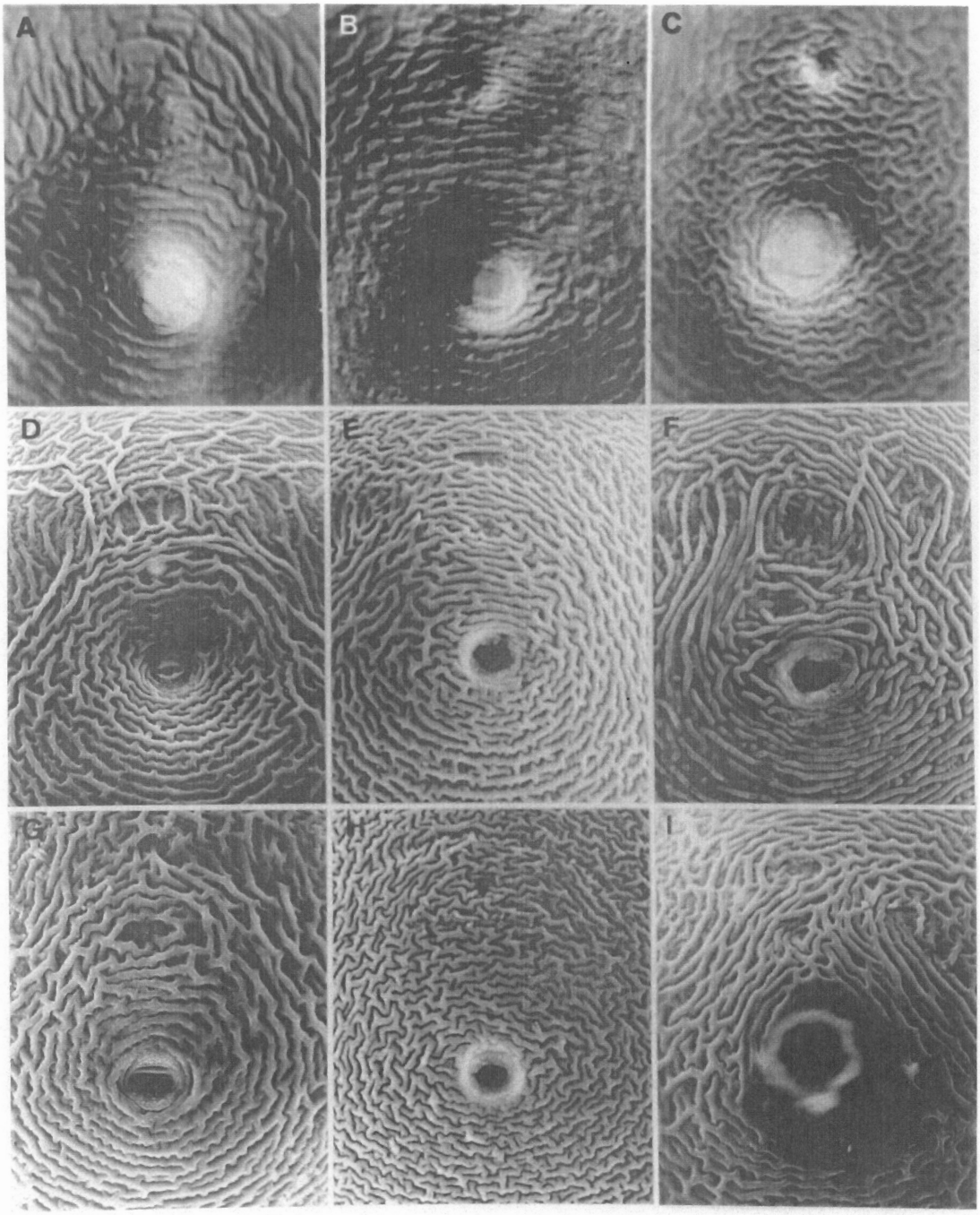
Fig. 7. LM (A-C) and SEM (D-F) observations of the terminal area of cysts of Globodera tabacum sspp (typical cases). GTT= G. t. tabacum; GTV= G. t. virginiae; GTS= G. t. solanacearum. **A**. GTT-1. **B**. GTV-11. **C**. GTS-1. **D**. GTT-3. **E**. GTV-6. **F**. GTS-1.

A
B
C
D
E
F

Fig. 8. LM observations of the terminal area of cysts of Globodera tabacum sspp (variant cases). GTT= G. t. tabacum; GTV= G. t. virginiae; GTS= G. t. solanacearum. **A**. GTT-2. **B**. GTV-1-X. **C**. GTS-10. **D**. GTT-1. **E**. GTV-1-X. **F**. GTS-9. **G**. GTT-3. **H**. GTV-11. **I**. GTS-8.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Part IV. Morphometrics of the tobacco cyst nematode complex, Globodera tabacum tabacum, G. t. virginiae, and G. t. solanacearum (Nemata: Heteroderinae).

ABSTRACT

A morphometric evaluation of second-stage juveniles (J2), males, females, and cysts of several isolates of the tobacco cyst nematode (TCN) complex, Globodera tabacum tabacum (GTT) (Lownsbery and Lownsbery, 1954) Behrens, 1975, G. t. virginiae (GTV) (Miller and Gray, 1968) Behrens, 1975, and G. t. solanacearum (GTS) (Miller and Gray, 1972) Behrens, 1975, was performed for several characters. Morphometrics of eggs, J2, and males were considerably less variable than in females and cysts. No measurements of eggs and J2 were useful for identification of the three subspecies. The distance of the median bulb to the head end and the distance of the excretory pore to the head end in J2 and males were quite stable. The stylet knob width of males was useful for identifying GTV isolates. Tail length was useful in separating males of GTT isolates from GTV and GTS males. The body length/ width (L/W) ratio of females and cysts discriminated GTT from GTV and GTS. Stylet knob width of females was an auxiliary character for identifying GTV. This subspecies complex has a continuum of values for the majority of the characters observed. The data suggest a closer relationship between GTV and GTS which is likely because these two subspecies occur in very close proximity in Virginia. There is a 38 km separation between closest adjacent geographical ranges. GTT occurs 700 km away in the northeastern U.S.

Morphometrics have been used quite extensively in the taxonomy of cyst nematodes, subfamily Heteroderinae sensu Luc et al. (8), either for producing tables and keys (2, 4, 6, 17, 21), or for diagnosis of new genera and species (1, 18, 20). Several morphometric characters of second-stage juveniles (J2), males, and females were utilized by Stone to separate Globodera pallida Stone, 1972 from G. rostochiensis Wollenweber, 1923 (18). Wouts and Weischer (21) also utilized J2 characters to distinguish several heteroderine species, including G. rostochiensis and G. pallida.

The papers that have analyzed the morphometrics of the tobacco cyst nematode (TCN) complex Globodera tabacum tabacum (GTT) (Lownsbery and Lownsbery, 1954) Behrens, 1975; G. t. virginiae (GTV) (Miller and Gray, 1968) Behrens, 1975; and G. t. solanacearum (GTS) (Miller and Gray, 1972) Behrens, 1975 used only one isolate of each subspecies and few characters (5, 16, 19). Original descriptions also provide morphometrics of isolates from their respective type localities (7, 11, 12). However, each was reared on different hosts, under different conditions, and the subspecies cannot be compared from the original descriptions.

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).

The purpose of this paper is to measure several characters of eggs, J2, males, white females, and cysts of the TCN complex in more detail, in order to better understand the extent of the variability within this group, and search for useful morphometrical characters that might be used for distinguishing the three subspecies.

MATERIALS AND METHODS.

All isolates used in this study are presented in Table 1. The locations of GTV and GTS isolates are listed according to Dr. L.I. Miller's terminology, 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 Dr. L.I. Miller from the Horton farm in Suffolk County, VA. 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 'Rutgers' tomato (Lycopersicum esculentum Mill.).

Specimens were observed with a bright field compound microscope and measured with a Leitz drawing tube. At least twenty specimens of each isolate were observed and measured, except where noted. In most cases thirty specimens were measured. All linear measurements are in μm. Tables (2-6) indicate mean ± standard deviation (SD) with the range in parenthesis. The coefficiant of variability (CV), which is the SD/ mean x 100, 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

Morphometrics of eggs, J2, males, females, and cysts are listed in Tables 2-6. Box plots of selected characters are shown in Figs.1-8.

Eggs (Table 2; Fig. 1). Length (L) and width (W) were measured and used to calculate the ratio L/W (Fig. 1). Measurements of eggs had a very low coefficient of variability (CV), less than 10% for all characters. The values for L and W overlapped across all isolates (Table 2). The L/W ratio 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). The 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), stylet knobs 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, and was also the only isolate to be separated from the others by this character. GTS-4 was separated from all other isolates by the median bulb to head end and had the highest mean value. GTS-10 and GTT-1 were also placed in separate groups. The excretory pore to head end distance was able to discriminate 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, indicate the stylet length as a stable character, although it is not useful for separating the three subspecies. Even though the tail terminus length has a relatively high CV (8.2-14.4%) it was the best character to group the isolates, but the grouping did not correlate to subspecific categories. GTS-4 had the highest mean for this character as well. The body length/ body width ratio 'a' had a good capability to group isolates. The tail terminus/ tail length ratio was very homogeneous among the isolates and only one isolate was placed in a separate group (GTT-2).

Males (Table 4; Fig. 4). The 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.

The ranges of most characters greatly overlapped. The DEGO showed a high CV (13.8-20.6%), and is not a useful character. The length of the stylet had very low CV (3.7-5.4), and was also able to discriminate some groups of isolates, but not subspecies. Stylet knob width was capable of discriminating 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 lengths. GTV-1-X was the only isolate discriminated from all other isolates by the a' ratio, and it had the lowest mean value.

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

GTT-1 was frequently separated from the other isolates usually with higher mean values. Body length separated GTT-1 from the rest. The high CV value (17.0-25.3%) of the DEGO revealed that it is very unstable. Among the isolates, the means ranged from 4.4 μm to 5.8 μm. The stylet length was a stable character (CV= 4.2-9.8%) and separated GTS-1 from the others. Stylet knob width clearly separated GTV-11 from the other isolates. It had the highest mean (6.0 μm) which is correlated with the wide "Dutch shoe" 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 present 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 L/ W ratio. It was the only female character capable of distinguishing GTT from GTV and GTS.

Cysts (Table 6; Fig. 7-8). Cyst characters showed a relatively high CV similar to females. The only character with a CV less than 10% was the ratio length/ width (L/W). Most characters were not useful for subspecies identification because they were overlapping.

A new ratio, M, which is the distance of anus to center of fenestra divided by the fenestra length, was less variable than the Granek- Hesling ratio (3, 6) which uses the edge of the fenestra instead. GTV-4 had the lowest body length mean value (460.5 μm). This isolate consistently had the lowest mean for several other characters. 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. Two GTV isolates (GTV-1-X, and GTV-4) had extreme values for the anus to edge of the fenestra distance character (59.2 and 38.1μm, respectively). Similarly, the distance of the anus to center of the fenestra was extreme for GTV-1-X and GTV-4 (70.4 and 46.5 μm, respectively). The five isolates of GTT were clearly discriminated from the isolates of GTV and GTS by the L/W ratio. The value 1.0 was correlated to the round shape of the cyst compared to 1.1-1.3 that was related to the elipsoid 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

Eggs. The measurements of eggs failed 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.

J2. 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 overlapped extensively. The best discriminating characters of J2 were tail terminus and stylet length, even though they did not separate the isolates into subspecies. However, the differences in stylet length among isolates are in fractions of μm and may not be biologically significant.

GTS-4 was 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 length, tail terminus length, and the a ratio. GTV-4 and GTV-11 were recently used for measurements of certain characters of J2 (15).

The median bulb was found to be a stable character (CV= 3.2-6.7%) despite concern by some authors (18) that it was not reliable because of the differences in the contraction of the esophagus.

Males. Characters of males were quite stable and most had a CV below 10%. The DEGO, however, had a high CV (13.8-20.6%) and was not useful as a morphometric character. The tail length was 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 was stable (CV= 6.6). The stylet length (CV =4.5), and the spicule length (CV=5.0) were also useful characters. Stylet knob width may be a useful auxiliary character in conjunction with other more reliable characters. Despite the fact

that the gubernaculum cannot discriminate among subspecies, GTT had higher values (11.9-12.1 μm) than the other two subspecies, and may be a useful character for identifying isolates of GTT.

Females. Except for the L/W ratio, there were no useful morphometric characters for discriminating the three subspecies. Frequently, a discriminating group contained isolates from all three subspecies. The L/W ratio was the only character that discriminated GTT from GTV and GTS and that had a low CV of 8.4. The ratio was 1.0 for GTT, which indicated a more rounded shape versus 1.1-1.2 in GTV and GTS typical of a more elipsoid or ovoid shape. Cyst shape was very useful for the diagnostics of the subspecies. The stylet length was stable (CV= 4.2-9.8%). The anus to the center of the fenestra distance was more useful than the anus to the edge of the fenestra because its CV was lower. The DEGO was not a useful character because it had a high CV (17.0-25.3%). The larger values of knob width of GTV were related to the observed "Dutch shoe shape" previously described in the literature (11). GTT-1 stood out frequently as having the highest value for all characters of females. Fenestral length placed all isolates into one group.

Cysts. Similar to females, the CV of most characters was very high (15-20%), and they were not very reliable for identification. The Waller-Duncan test failed to separate isolates for most of the characters. Also as in females, the L/W (CV= 8) was the only character that discriminated GTT from GTV and GTS. Similarly the 1.0 L/W ratio of GTT indicates a more rounded shape. The M ratio (anus to fenestra center/ fenestra length) was less variable than the traditionally used Granek-Hesling ratio. The M ratio may be used in conjunction with other more reliable characters to confirm identification of GTV isolates. In conclusion, only the L/W ratio of females and cysts combined with tail length of males were 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 the GTT isolates, which agrees with Behrens observations (1). She does not mention which isolates were used in her study, therefore a comparison becomes difficult.

Stone (19) used principal coordinate analysis of five J2 characters to show differentiation of GTT from GTV and GTS. Principal coordinate analysis of four cyst characters, distance from anus to edge of the fenestra, length of the fenestra, number of ridges between anus and fenestra, and Granek's ratio (19) differentiated GTT and G. pallida from GTV and GTS.

Miller (9), using one isolate each, showed significant differences between GTT and the group formed by GTV and GTS based upon 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 very close to each other. There is a 38 km separation 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 U.S (10).

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Table 1. **Isolates of the tobacco cyst nematode complex, Globodera tabacum sspp. tabacum (GTT), virginiae (GTV) and solanacearum (GTS) used in this study.**

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

Table 2. Morphometrics of eggs of the tobacco cyst nematode complex Globodera tabacum tabacum (GTT), G. t. virginiae (GTV), and G. t. solanacearum (GTS). All linear measurements are in μm. Values are means ± standard deviation and 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 2. Morphometrics of eggs of the tobacco cyst nematode complex.

Character	GTT-1	GTT-2	GTT-3	GTT-4	GTT-5
Length (L)	106.5±6.3 ghi (89.6-116.2)	116.6±7.3 a (103.6-130.2)	110.0±3.9 ef (100.8-120.4)	107.4±5.8 gh (95.2-117.6)	108.9±4.6 fg (100.8-117.6)
Width (W)	44.7±2.3 ef (40.6-50.4)	47.0±2.0 bc (43.4-50.4)	43.7±2.2 fg (39.2-50.4)	45.8±2.6 d (39.2-50.4)	49.8±3.3 a (43.4-56.0)
L/W	2.4±0.2 efghi (2.1-2.9)	2.5±0.2 bcd (2.2-2.8)	2.5±0.1 b (2.2-2.8)	2.4±0.2 ghi (1.9-2.8)	2.2±0.2 j (1.8-2.6)

Character	GTV-1	GTV-1-X	GTV-4	GTV-8	GTV-10	GTV-11
Length (L)	111.8±6.2 de (99.4-123.2)	114.1±4.0 bcd (109.2-126.0)	104.6±3.0 ijk (99.4-110.6)	105.7±3.6 hij (96.6-112.0)	116.7±6.9 a (103.6-131.6)	103.8±5.3 jk (96.6-114.8)
Width (W)	45.7±3.7 de (40.6-51.8)	42.4±1.9 hi (39.2-49.0)	42.9±2.2 ghi (39.2-47.6)	42.3±2.3 i (37.8-47.6)	46.5±2.2 cd (42.0-50.4)	42.1±1.8 i (39.2-46.2)
L/W	2.5±0.3 bcdef (2.0-2.8	2.7±0.1 a (2.3-2.9)	2.4±0.1 cdefg (2.2-2.7)	2.5±0.2 bc (2.2-2.9)	2.5±0.2 bc (2.1-2.9)	2.5±0.2 bcde (2.2-2.9)

Character	GTS-1	GTS-2	GTS-3	GTS-4	GTS-5	GTS-6	GTS-8	GTS-9	GTS-10	GTS-12
Length (L)	106.5±4.0 ghi (99.4-114.8)	114.4±6.2 abc (103.6-128.8)	110.4±5.1 ef (98.0-120.4)	116.3±6.2 ab (103.6-130.2)	113.4±6.1 cd (100.8-124.6)	114.6±5.9 abc (106.4-128.8)	103.1±5.8 k (89.6-114.8)	105.0±5.4 hijk (89.6-113.4)	102.8±5.2 k (92.4-114.8)	104.2±4.0 ijk (95.2-112.0)
Width (W)	46.2±3.0 cd (39.2-51.8)	46.4±2.1 cd (42.0-51.8)	47.7±2.1 b (43.4-50.4)	47.0±2.8 bc (42.0-53.2)	46.7±2.1 bcd (42.0-51.8)	49.4±2.5 a (43.4-54.6)	42.7±2.1 ghi (39.2-46.2)	42.8±1.9 ghi (40.6-47.6)	43.4±1.8 gh (37.8-47.6)	43.0±1.5 ghi (39.2-46.2)
L/W	2.3±0.2 i (1.9-2.8)	2.5±0.2 bcde (2.1-2.8)	2.3±0.2 hi (2.0-2.6)	2.5±0.2 bcde (2.2-3.0)	2.4±0.2 bcdef (2.0-2.9)	2.3±0.2 hi (2.0-3.0)	2.4±0.2 defgh (2.1-2.9)	2.5±0.2 bcdef (2.1-2.8)	2.4±0.2 fghi (2.1-2.6)	2.4±0.1 cdefg (2.3-2.8)

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). All linear measurements are in μm. Values are means ± standard deviation and 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 3. Morphometrics of second-stage juveniles (J2) of the tobacco cyst nematode complex.

Character	GTT-1	GTT-2	GTV-1	GTV-1-X	GTV-6	GTV-11	GTS-1	GTS-4	GTS-5	GTS-10
Body length (L)	521.3±20.1 de (469.8-564.3)	516.4±32.1 e (464.0-580.0)	556.1±44.9 b (476.0-661.2)	515.0±21.1 e (464.0-551.0)	541.3±47.4 bc (464.0-632.2)	534.0±34.5 cd (481.4-603.2)	540.7±42.8 b (458.9-621.3)	576.1±33.5 a (504.6-643.8)	545.8±25.1 bc (458.2-580.0)	516.0±20.3 e (475.6-562.6)
Body width (W)	24.8±2.4 abc (20.3-28.5)	23.5±2.3 ed (20.3-29.0)	26.0±3.4 a (21.1-34.2)	23.9±2.0 cd (20.3-29.0)	23.3±2.3 de (20.3-29.0)	25.2±2.3 ab (20.3-29.0)	24.0±3.0 a (17.1-28.5)	23.1±2.8 de (17.4-29.0)	25.6±3.1 a (20.3-31.9)	22.4±1.8 e (17.4-26.1)
Median bulb to head end (MB)	66.5±3.6 f (58.8-74.2)	71.3±3.9 cd (63.0-79.2)	70.4±2.7 d (67.5-77.4)	73.1±3.9 b (58.5-79.2)	71.2±4.8 cd (63.0-81.0)	72.9±3.3 bc (61.2-76.5)	71.8±3.3 bcd (65.8-78.4)	76.8±3.7 a (63.0-81.0)	72.5±2.8 bc (66.6-79.2)	68.2±3.8 e (61.2-80.1)
Excretory pore to head end (EP)	102.6±4.6 f (94.9-111.2)	113.9±6.7 c (99.0-126.9)	109.8±8.7 de (96.2-128.7)	112.8±6.2 cd (100.8-124.2)	111.6±10.5 cde (99.0-133.2)	112.4±5.7 cd (101.7-122.4)	108.9±7.3 e (94.3-122.2)	125.2±6.5 a (113.4-144.0)	118.7±4.9 b (108.0-126.0)	110.5±5.8 de (99.9-129.6)
DEGO	5.8±0.7 cde (3.8-6.8)	5.8±0.7 cde (4.5-7.2)	5.9±0.8 bcd (4.3-7.2)	6.2±0.8 b (4.5-7.2)	6.1±0.7 bc (5.0-7.2)	5.8±0.7 cde (4.5-7.2)	6.0±0.6 bcd (4.8-7.1)	6.6±0.6 a (5.4-8.1)	5.7±0.7 de (4.5-7.2)	5.5±0.5 e (4.5-6.3)
Stylet length (STY)	22.6±1.4 de (19.8-25.7)	21.2±0.8 g (19.8-22.5)	23.3±1.0 c (20.7-24.7)	22.3±0.7 e (21.2-23.4)	22.5±1.5 de (20.7-25.2)	22.8±1.0 d (20.3-24.3)	24.9±1.0 a (22.3-26.6)	24.3±0.8 b (22.5-25.2)	23.3±0.8 c (21.6-24.3)	21.7±1.0 f (18.9-23.4)
Shaft length (SHA)	9.6±0.7 b (8.1-11.2)	9.3±0.5 a (8.1-9.9)	10.1±0.7 ab (9.0-11.4)	9.5±0.6 ab (8.1-10.8)	9.7±0.7 b (9.0-10.8)	9.9±0.6 ab (9.0-10.8)	10.8±0.6 ab (9.5-12.4)	10.7±0.5 ab (9.9-11.7)	10.4±0.5 a (9.0-11.3)	9.70.5 a (8.1-10.4)
Knobs width (kw)	4.6±0.4 ef (3.8-5.4)	4.2±0.4 g (3.6-4.5)	4.8±0.4 bc (4.0-5.7)	4.6±0.3 def (4.1-5.3)	4.7±0.4 cde (4.5-5.4)	4.8±0.5 cd (4.1-5.4)	5.2±0.4 a (4.8-5.7)	5.0±0.6 ab (3.2-5.9)	4.9±0.4 bc (4.5-5.4)	4.5±0.4 f (3.6-5.4)
Knobs height (kh)	2.9±0.3 bcd (2.3-3.6)	2.8±0.2 de (2.3-3.2)	3.1±0.4 ab (2.3-3.8)	2.9±0.3 cde (2.3-3.6)	2.6±0.2 f (2.3-2.7)	2.7±0.4 e (1.8-3.6)	3.1±0.4 a (2.4-3.8)	3.1±0.4 a (2.7-4.1)	2.9±0.3 abc (2.7-3.6)	2.8±0.2 de (2.7-3.2)
Tail length (t)	54.3±4.4 b (46.8-67.5)	49.8±3.9 e (44.1-60.3)	56.4±3.8 a (49.5-63.7)	52.1±3.3 cd (45.0-59.4)	53.0±5.0 bcd (45.9-63.0)	52.3±3.7 bcd (44.1-58.5)	51.8±5.1 cde (44.2-61.8)	57.6±5.1 a (45.0-71.1)	53.5±3.9 bc (44.1-62.1)	51.3±3.6 de (42.3-60.3)
Tail terminus (tt)	27.5±3.1 b (21.9-33.3)	20.9±3.0 d (17.1-27.0)	27.8±3.0 b (19.9-34.2)	25.9±2.9 c (20.7-31.5)	28.0±2.9 b (22.5-32.4)	25.2±3.1 c (18.0-31.5)	25.8±2.4 c (20.8-29.9)	29.8±4.2 a (20.7-37.8)	25.4±2.8 c (16.2-28.8)	25.0±2.3 c (21.6-31.5)
Width of body at anus	13.8±1.3 cde (11.7-17.1)	14.0±1.2 bc (12.6-17.1)	13.7±1.3 cde (10.8-16.2)	14.2±1.6 bc (11.7-17.1)	13.3±1.5 de (10.8-16.2)	13.9±1.1 bcd (11.7-16.2)	13.2±1.1 e (11.2-15.4)	15.0±1.6 a (11.7-19.8)	14.5±1.0 ab (13.5-17.1)	13.3±0.8 de (11.7-14.4)
a	21.±1.5 d (18.4-25.1)	22.1±1.9 cd (19.1-26.6)	21.6±1.8 d (18.3-25.8)	21.7±1.7 d (18.2-25.1)	23.3±1.7 b (20.0-27.7)	21.3±1.9 d (18.0-24.8)	22.8±2.4 bc (18.9-27.1)	25.2±2.8 a (20.4-33.7)	21.6±2.7 d (15.8-28.3)	23.2±2.0 b (20.0-29.3)
Shaft/ stylet	0.4±0.02 f (0.4-0.5)	0.4±0.03 g (0.4-0.5)	0.4±0.02 cd (0.4-0.5)	0.4±0.03 fg (0.4-0.5)	0.4±0.02 ef (0.4-0.5)	0.4±0.03 de (0.4-0.5)	0.4±0.02 a (0.4-0.5)	0.4±0.02 ab (0.4-0.5)	0.4±0.02 bc (0.4-0.5)	0.4±0.02 ef (0.4-0.5)
kw/ kh	1.6±0.2 de (1.2-2.0)	1.5±0.2 e (1.3-1.8)	1.6±0.2 cde (1.3-2.0)	1.6±0.1 cde (1.4-2.0)	1.9±0.2 a (1.7-2.4)	1.8±0.3 ab (1.4-2.5)	1.7±0.2 bc (1.4-2.4)	1.6±0.2 cd (1.2-2.0)	1.7±0.2 bcd (1.4-2.0)	1.6±0.2 cde (1.3-2.0)
tt/t	0.5±0.05 ab (0.4-0.6)	0.4±0.04 e (0.3-0.5)	0.5±0.04 bcd (0.4-0.6)	0.5±0.04 bcd (0.4-0.6)	0.5±0.06 a (0.4-0.6)	0.5±0.05 d (0.4-0.6)	0.5±0.03 cd (0.4-0.5)	0.5±0.05 abc (0.4-0.6)	0.5±0.05 d (0.3-0.5)	0.5±0.03 cd (0.4-0.5)

Table 4. Morphometrics of males of the tobacco cyst nematode complex Globodera tabacum tabacum (GTT), G. t. virginiae (GTV), and G. t. solanacearum (GTS). All linear measurements are in μm. Values are means ± standard deviation and 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 4. Morphometrics of males of the tobacco cyst nematode complex.

Character	GTT-1	GTT-2	GTV-1	GTV-1-X	GTV-11	GTS-1	GTS-10
Body length (L)	1186.3±107.3 a (957.0-1450.0)	1119.0±113.7 bc (893.2-1316.6)	1136.8±115.1 abc (870.0-1305.0)	1079.1±106.5 c (852.6-1229.6)	1170.1±103.0 ab (812.0-1334.0)	1140.7±119.5abc (899.0-1450.0)	1099.0±119.3 c (812.0-1270.2)
Body width (W)	34.7±4.1ab (23.2-43.5)	34.4±2.9 ab (26.1-40.6)	33.1±3.7 bc (26.1-40.6)	36.0±2.9 a (31.9-40.6)	32.0±3.4 cd (26.1-37.7)	30.8±3.6 de (23.2-37.7)	30.0±2.7 e (26.1-34.8)
Median bulb to head end (MB)	95.7±7.0 ab (76.5-108.0)	92.4±4.7 bc (82.8-100.8)	94.2±5.9 bc (83.7-103.6)	92.0±8.1 c (77.4-105.3)	98.5±6.6 a (85.5-110.7)	94.8±6.7 bc (81.0-110.7)	92.4±4.6 bc (82.8-100.8)
Excretory pore to head end (EP)	160.1±11.3 a (137.7-181.8)	156.0±10.1 ab (133.2-173.7)	153.3±8.2 ab (129.6-169.2)	157.1±12.3 ab (123.3-177.3)	159.6±14.5 ab (137.7-207.0)	151.2±16.4 b (126.0-189.0)	158.9±12.0 ab (135.0-173.7)
DEGO	3.8±0.7 ab (2.3-4.5)	3.6±0.5 bc (2.4-4.1)	3.5±0.6 c (2.7-4.5)	3.0±0.6 d (2.3-4.5)	4.0±0.7 a (2.7-5.4)	3.2±0.6 d (2.3-4.5)	3.1±0.6 d (2.3-4.1)
Stylet length (STY)	26.7±1.3 ab (23.4-28.8)	26.2±1.1 bc (24.3-27.9)	26.0±0.9 c (24.3-27.9)	24.8±1.3 d (23.4-27.9)	25.0±1.2 d (22.1-27.5)	26.8±1.1 a (25.2-30.6)	25.1±1.1 d (22.5-26.5)
Shaft length (SHAF)	11.1±0.8 a (9.9-12.6)	10.8±0.8 ab (9.0-12.2)	10.7±0.7 ab (9.9-12.6)	10.5±0.7 b (9.5-11.7)	10.7±0.6 b (9.9-11.7)	11.1±0.5 a (9.9-11.7)	10.7±0.6 b (9.5-11.7)
Knobs width (kw)	5.0±0.4 c (4.5-5.4)	4.9±0.4 c (4.5-5.4)	5.2±0.4 b (4.5-5.9)	5.5±0.6 a (4.5-6.3)	5.6±0.5 a (4.5-6.3)	5.0±0.4 c (4.5-5.4)	5.0±0.4 c (4.1-5.4)
Knobs height (kh)	3.2±0.4 a (2.7-3.6)	3.0±0.3 ab (2.7-3.6)	3.1±0.4 a (2.7-3.6)	3.0±0.3 ab (2.7-3.6)	3.1±0.4 a (2.7-3.6)	3.1±0.4 a (2.7-3.6)	2.8±0.3 b (2.3-3.2)
Head width (HW)	11.0±0.8 bc (9.9-12.6)	11.0±0.6 ab (9.9-12.6)	10.6±0.5 d (9.9-11.7)	10.8±0.4 bcd (9.9-11.7)	11.3±0.6 a (10.4-12.6)	10.8±0.5 bcd (9.9-11.7)	10.6±0.5 cd (9.5-11.3)
Head height (HH)	5.7±0.6 ab (4.5-7.2)	5.8±0.6 a (4.5-6.8)	5.6±0.5 ab (4.5-6.3)	5.2±0.5 c (4.5-5.9)	5.8±0.5 a (5.0-6.8)	5.4±0.4 bc (4.5-6.3)	5.5±0.5 bc (4.5-6.3)
Spicule (SPIC)	34.8±1.9 a (30.6-37.8)	34.4±1.8 ab (30.6-36.9)	34.0±1.7 abc (30.8-36.9)	31.1±1.5 d (28.8-33.3)	33.7±1.7 bc (28.8-36.0)	34.4±1.7 ab (31.5-37.8)	33.4±1.3 c (31.5-36.0)
Gubernaculum (GUB)	12.2±1.2 a (9.9-14.4)	12.0±1.1 a (9.9-14.4)*	11.8±0.9 a (9.9-13.5)***	_ _	10.9±0.8 b (9.9-12.6)	10.9±1.0 b (9.9-12.6)	10.8±0.6 b (9.9-12.6)
Tail length	4.0±0.9 c (2.7-5.9)	4.1±0.8 c (2.7-5.4)**	4.8±0.9 b (3.6-7.2)	_ _	5.0±0.5 b (4.1-5.9)	4.9±0.9 b (3.6-6.3)	5.6±0.8 a (4.5-7.2)
a	34.5±3.9 bc (28.6-43.0)	32.7±4.1 c (26.5-42.9)	34.6±4.1 ab (25.7-44.4)	30.1±3.4 d (23.3-35.3)	36.8±3.8 a (31.1-44.0)	34.8±3.8 ab (24.3-42.0)	36.4±4.4 ab (28.0-44.4)

Table 5. Morphometrics of females of the tobacco cyst nematode complex Globodera tabacum tabacum (GTT), G. t. virginiae (GTV), and G. t. solanacearum (GTS). All linear measurements are in μm. Values are means ± standard deviation and 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.

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.±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)
N° 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)

Table 6. Morphometrics of cysts of the tobacco cyst nematode complex Globodera tabacum tabacum (GTT), G. t. virginiae (GTV), and G. t. solanacearum (GTS). All linear measurements are in μm. Values are means ± standard deviation and 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 6. Morphometrics of cysts of the tobacco cyst nematode complex.

Character	GTT-1	GTT-2	GTT-3	GTT-4	GTT-5	GTV-1	GTV-1-X	GTV-4	GTV-8	GTV-11
Length (L)	608.6±79.4 cd (406.0-783.0)	539.6±105.0 fg (377.0-754.0)	642.4±52.1 g (522.0-736.6)	642.3±58.8 h (493.0-730.8)	656.8±67.8 ab (493.0-754.0)	635.5±84.0 bc (464.0-817.8)	555.1±96.4 efg (411.8-759.8)	460.5±75.3 h (348.0-638.0)	595.7±75.4 d (464.0-759.8)	572.5±72.0 def (464.0-754.0)
Width (W)	598.5±103.8 a (435.0-870.0)	536.5±121.0 bc (348.0-812.0)	610.7±59.6 a (481.4-719.2)	614.0±65.5 a (452.4-725.0)	634.5±75.6 a (446.6-754.0)	557.8±84.2 b (400.2-713.4)	503.6±101.4 cd (319.0-742.4)	354.4±88.1 g (266.8-609.0)	536.1±82.8 bc (388.6-667.0)	514.5±67.7 cd (382.8-667.0)
Ridges (R)	8.4±2.0 de (4.1-5.0)	8.8±2.1 ef (5.0-15.0)	8.8±1.9 c (6.0-14.0)	9.4±1.6 de (6.0-13.0)	9.8±2.0 cd (6.0-15.0)	8.3±1.0 efg (6.0-10.0)	9.7±3.0 a (5.0-20.0)	10.9±1.9 b (6.0-14.0)	10.9±2.5 c (7.0-17.0)	12.0±1.9 b (7.0-15.0)
Anus to edge of fenestra (AF)	48.7±10.9 bc (30.8-71.4)	44.8±7.9 cde (29.4-67.2)	50.1±12.0 b (30.8-92.4)	45.0±8.6 cde (28.0-58.8)	42.6±8.8 e (29.4-61.6)	47.8±11.2 bcd (30.8-72.8)	59.2±12.5 a (40.6-86.8)	38.1±9.6 f (21.0-54.6)	46.8±12.4 bcde (23.8-82.6)	50.6±9.8 b (30.8-70.0)
Anus to center of fenestra (AFc)	62.2±11.6 b (42.0-84.0)	59.0±8.7 bcde (43.4-81.2)	60.8±12.4 bcd (42.0-102.2)	56.8±9.7 def (37.8-72.8)	53.5±9.5 f (37.8-72.8)	60.0±12.6 bcde (40.6-86.8)	70.4±13.1 a (51.8-100.8)	46.5±10.5 g (29.4-64.4)	57.1±12.5 cdef (33.6-95.2)	61.3±10.3 bcd (39.2-81.2)
Fenestra length (FEN)	26.4±4.1 ab (18.2-37.8)	27.6±3.2 a (21.0-36.4)	21.2±2.2 e (16.8-26.6)	23.3±3.9 c (16.8-29.4)	21.8±3.6 de (15.4-29.4)	23.4±4.6 c (16.8-33.6)	21.5±3.5 e (16.8-28.0)	16.7±2.7 f (12.6-22.4)	20.4±2.8 e (15.4-26.6)	21.0±3.9 e (14.0-28.0)
L/W	1.0±0.1 ef (0.8-1.2)	1.0±0.1 f (0.9-1.3)	1.0±0.1 e (1.0-1.2)	1.1±0.1 e (0.9-1.2)	1.0±0.1 ef (1.0-1.2)	1.1±0.1 cd (1.0-1.3)	1.1±0.1 d (1.0-1.4)	1.3±0.3 a (1.0-1.6)	1.1±0.1 d (1.0-1.3)	1.1±0.1 d (1.0-1.2)
Granek/ Hesling ratio (HES)	1.9±0.4 def (1.1-2.8)	1.6±0.3 g (1.0-2.4)	2.4±0.6 b (1.4-4.7)	2.0±0.4 cd (1.3-2.8)	2.0±0.4 cd (1.2-3.2)	2.1±0.4 c (1.4-2.9)	2.8±0.6 a (1.5-4.0)	2.3±0.5 b (1.5-3.2)	2.3±0.7 b (1.3-4.9)	2.5±0.5 b (1.5-3.8)
M	2.4±0.4 efg (1.6-3.3)	2.2±0.3 h (1.4-3.0)	2.9±0.6 b (1.9-5.2)	2.7±0.7 de (1.4-4.2)	2.4±0.6 de (1.5-4.2)	2.7±0.7 cd (1.7-3.9)	3.3±0.6 a (2.0-4.5)	2.8±0.5 bc (1.9-3.7)	2.3±0.7 b (0.9-4.2)	3.0±0.5 b (2.1-4.3)

Table 6 (cont.). Morphometrics of cysts of the tobacco cyst nematode.

Character	GTS-1	GTS-3	GTS-5	GTS-10	GTS-12
Length (L)	579.2±97.1 de (446.6-783)	531.7±56.2 g (394.4-638.0)	539.0±94.2 fg (377.0-783.0)	687.1±76.8 a (464.0-812.0)	546.0±84.9 efg (382.8-725.0)
Width (W)	519.5±99.3 bcd (377.0-730.8)	436.0±68.0 f (301.6-580.0)	463.0±95.6 ef (290.0-661.2)	624.3±81.9 a (435.0-754.0)	492.6±85.7 de (348.0-667.0)
Ridges (R)	7.2±1.1 gh (5.0-9.0)	7.4±1.6 h (5.0-12.0)	7.5±1.2 fgh (6.0-11.0)	7.7±1.4 de (5.0-11.0)	8.9±1.8 h (5.0-15.0)
Anus to edge of fenestra (AF)	48.3±10.2 bc (30.8-79.8)	44.3±7.1 cde (29.4-61.6)	44.6±8.6 cde (29.4-63.0)	43.5±8.0 de (28.0-64.4)	44.4±8.7 cde (32.2-65.8)
Anus to center of fenestra (AFc)	61.6±10.2 bc (42.0-86.8)	57.9±7.9 bcdef (40.6-75.6)	56.8±9.3 def (40.6-75.6)	55.3±9.3 ef (37.8-78.4)	57.3±9.1 cdef (44.8-79.8)
Fenestra length (FEN)	25.3±3.9 b (16.8-33.6)	26.6±3.3 ab (19.6-33.6)	23.8±3.4 c (16.8-32.2)	22.8±3.7 cd (12.6-30.8)	25.7±2.4 b (22.4-30.8)
L/W	1.1±0.1 d (1.0-1.3)	1.2±0.1 b (1.0-1.6)	1.2±0.1 c (1.0-1.5)	1.1±0.1 d (1.0-1.3)	1.1±0.1 d (1.0-1.3)
Granek/ Hesling ratio (HES)	1.9±0.4 cd (1.4-2.9)	1.7±0.3 fg (1.3-2.3)	1.9±0.3 cde (1.1-2.8)	1.9±0.4 cde (1.3-3.4)	1.7±0.3 efg (1.2-2.4)
M	2.5±0.4 de (1.9-3.4)	2.2±0.3 gh (1.8-2.8)	2.4±0.3 def (1.6-3.4)	2.5±0.4 def (1.8-4.0)	2.2±0.3 fgh (1.7-2.9)

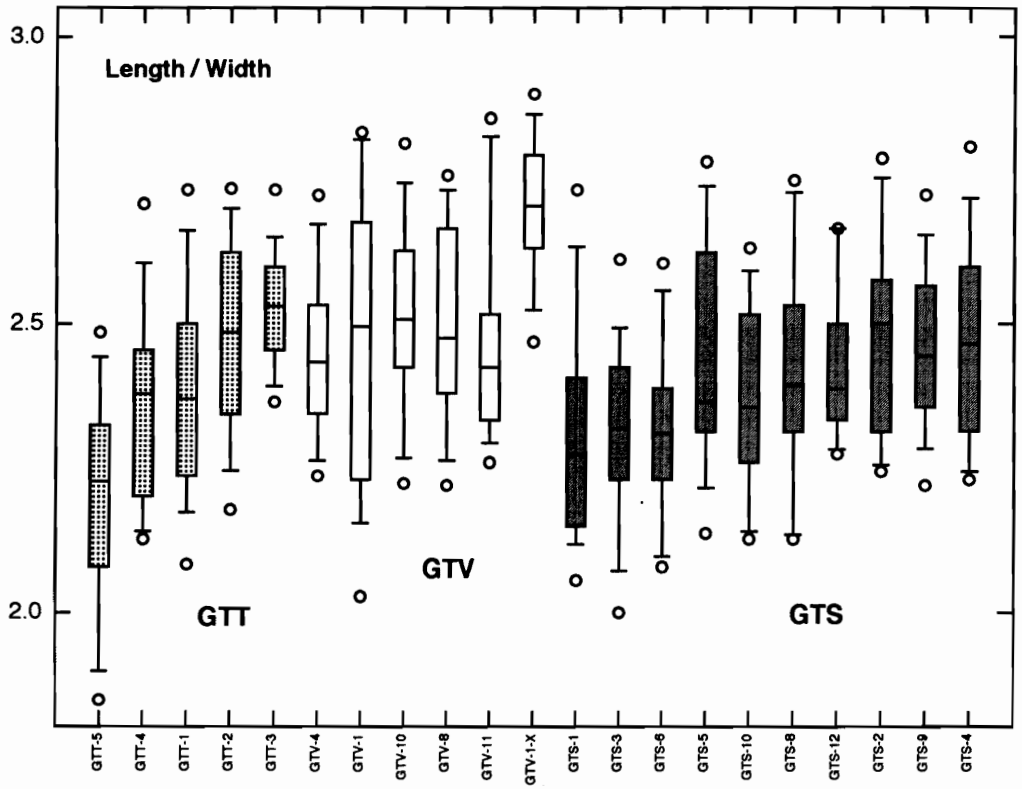

Fig. 1. Morphometrics (Body length/ width) of eggs of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum .

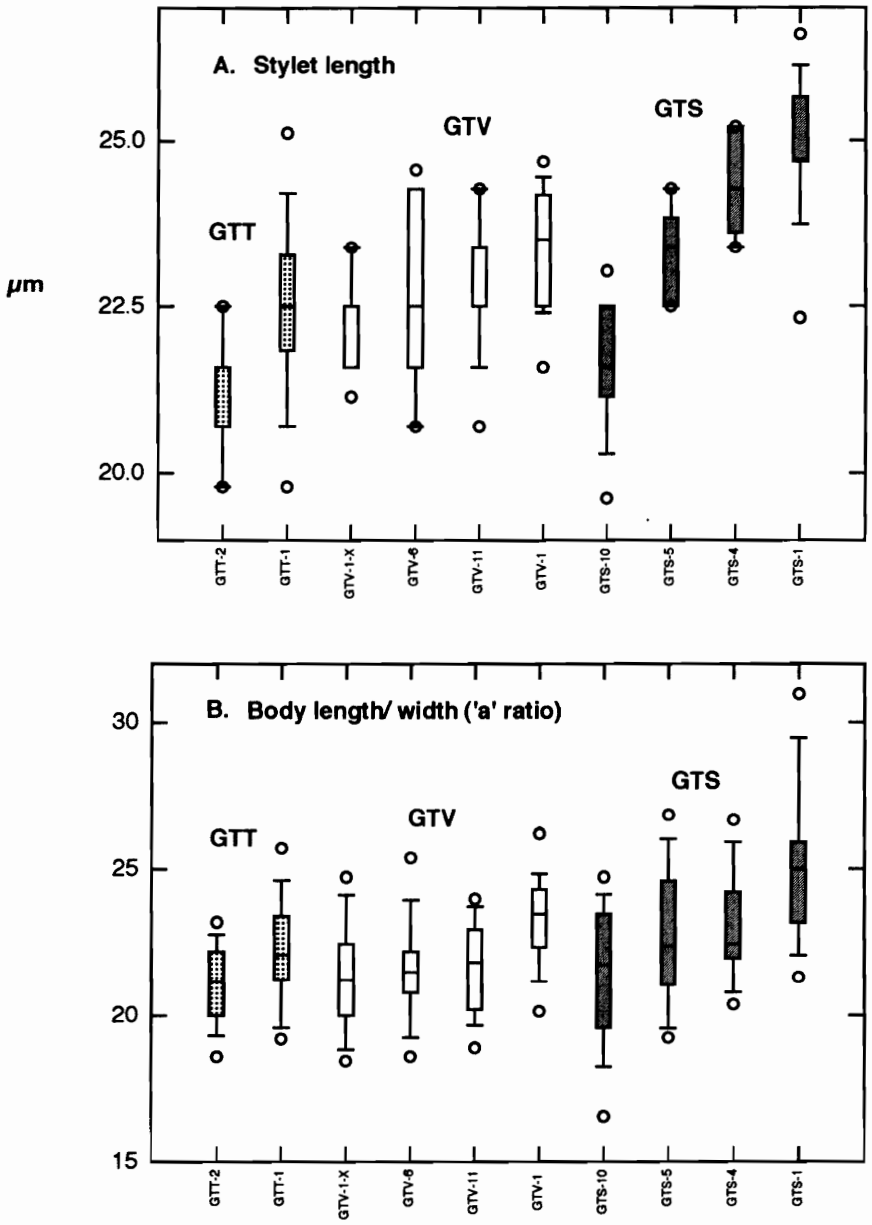

Fig. 2. Morphometrics of second-stage juveniles of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum. A= Stylet length. B. Body length /width ('a' ratio).

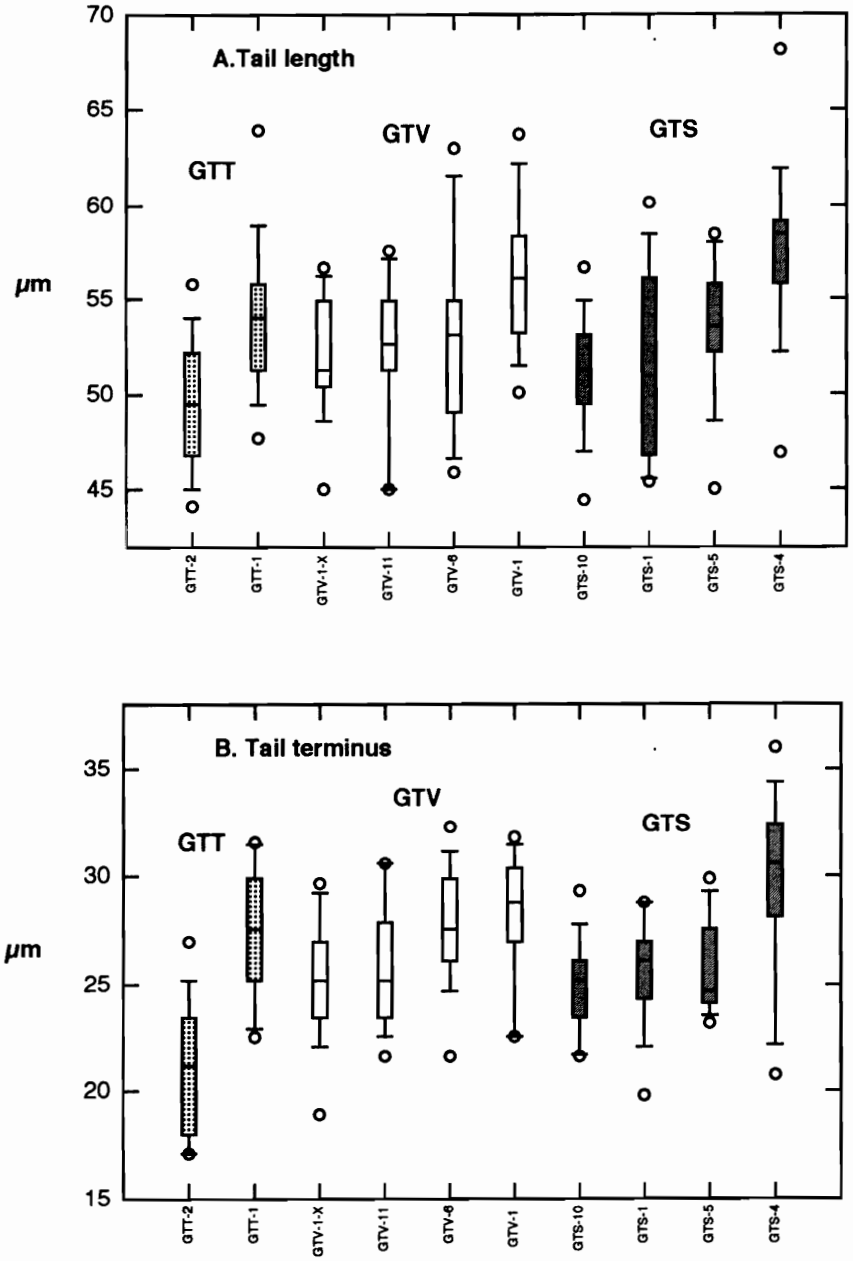

Fig. 3. Morphometrics of second-stage juveniles of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginae. GTS= G. t. solanacearum. A= Tail length. B= Tail terminus.

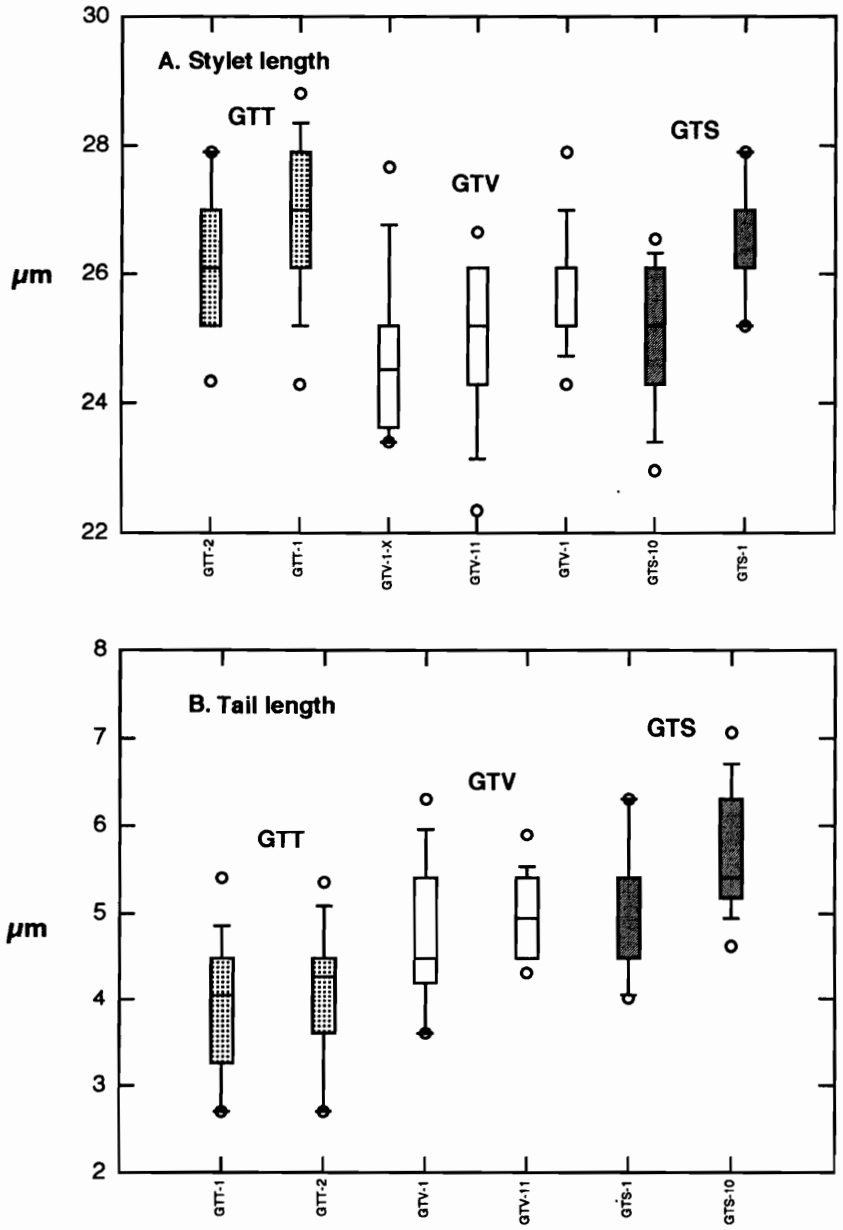

Fig. 4. Morphometrics of males of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum. A= Body length. B. Tail length.

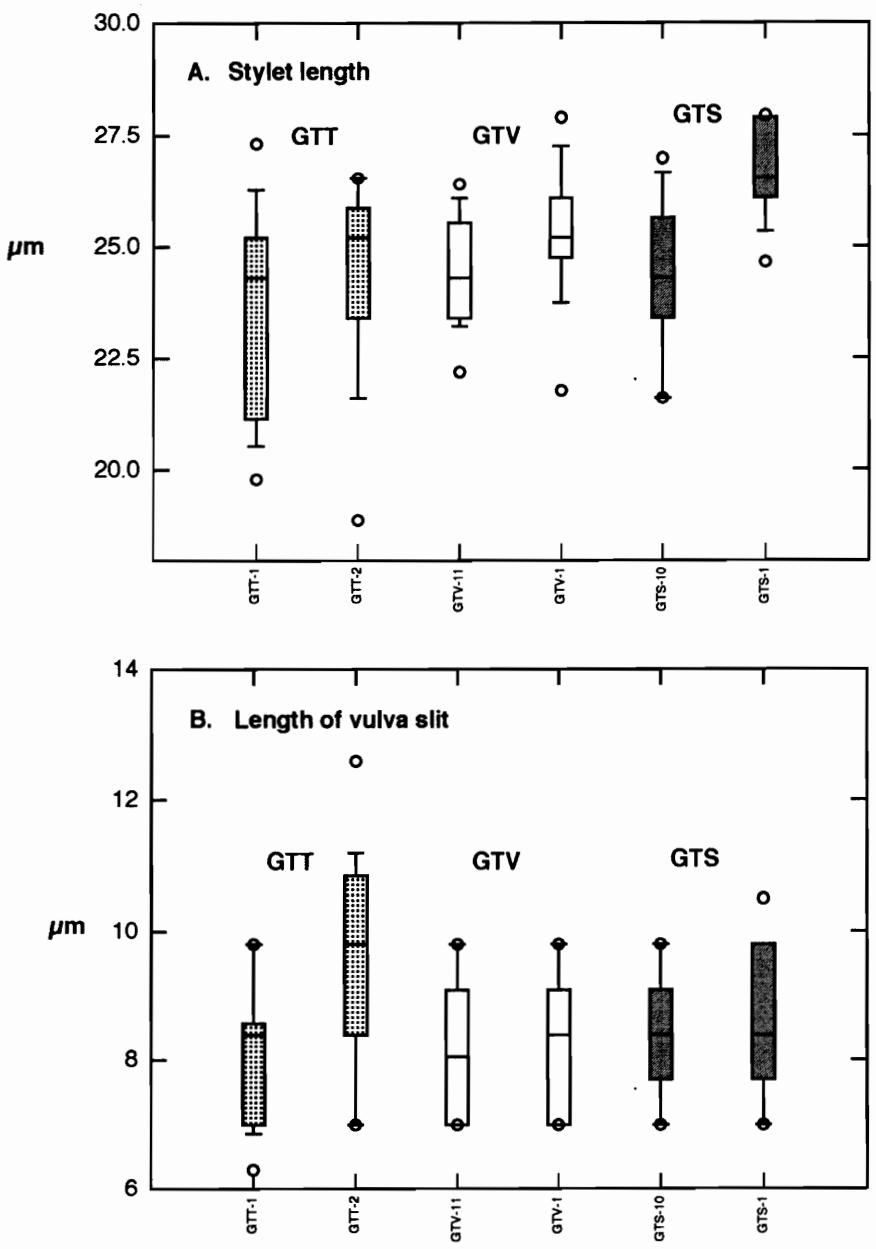

Fig. 5. Morphometrics of females of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum. A= Stylet length B= Length of vulva slit.

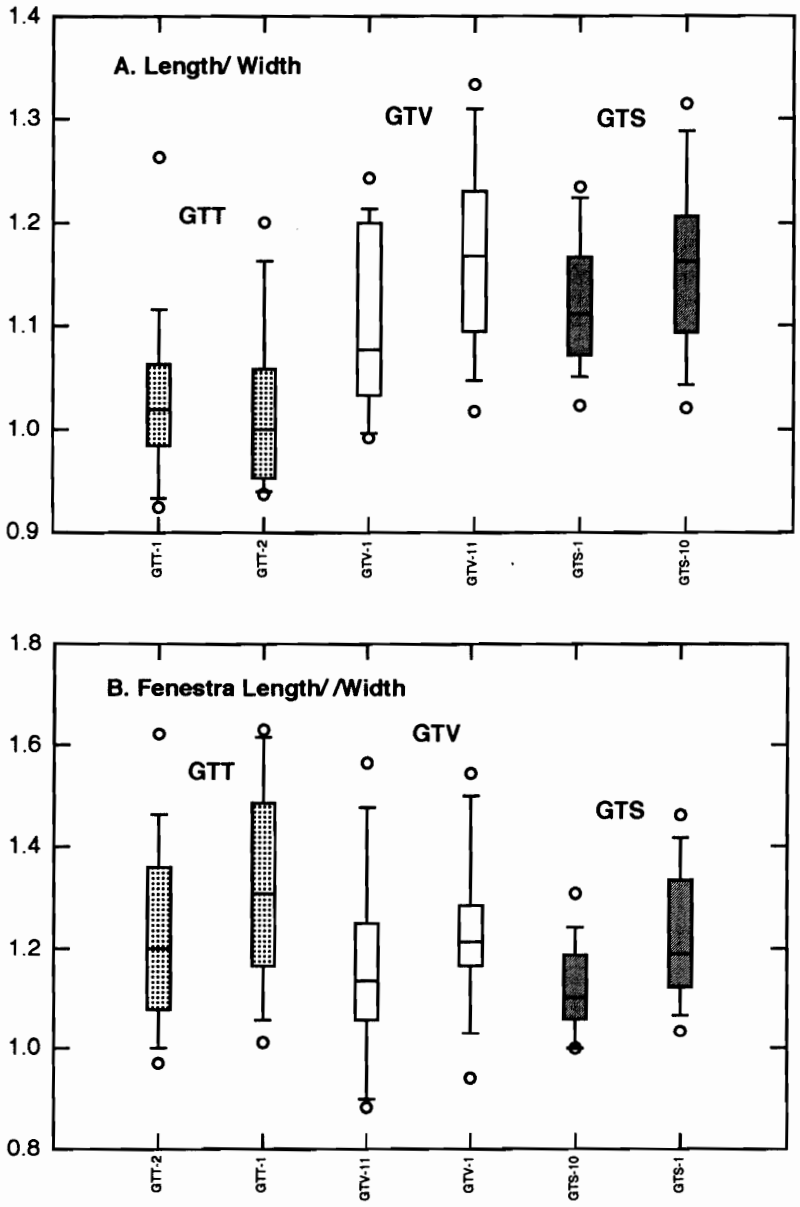

Fig. 6. Morphometrics of females of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum.
A. Body length/ width. B. Fenestra length/ width.

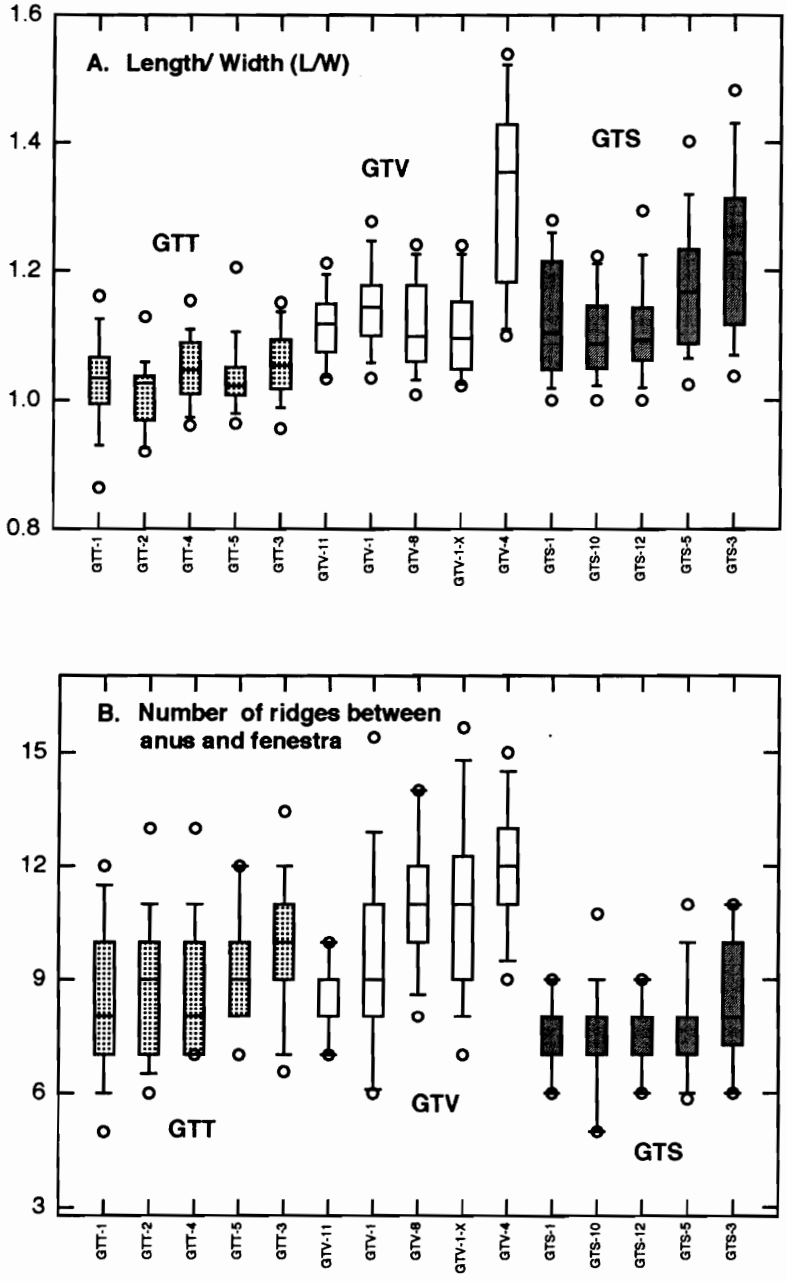

Fig. 7 Morphometrics of cysts of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum. A= Length/ width (L/W). B. Number of ridges between anus and fenestra.

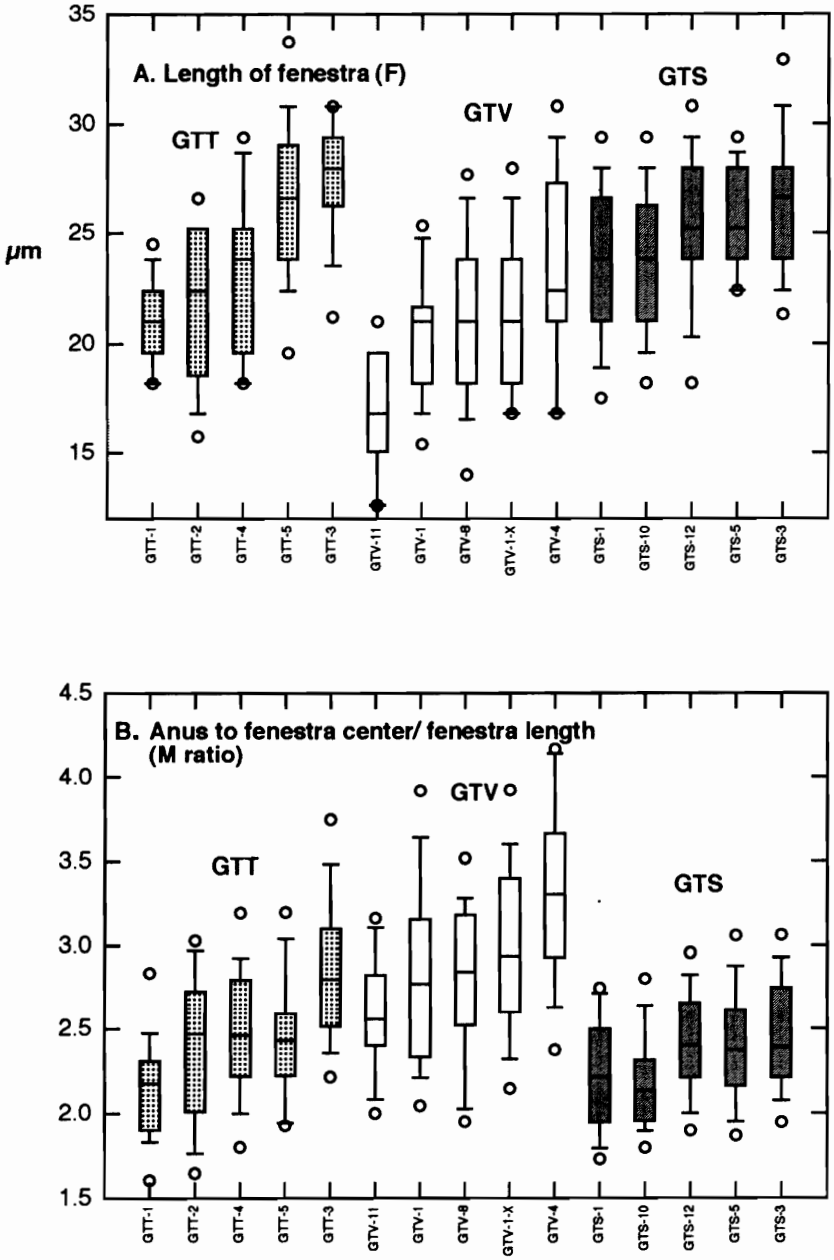

Fig. 8. Morphometrics of cysts of the tobacco cyst nematode complex. GTT= Globodera tabacum tabacum. GTV= G. t. virginiae. GTS= G. t. solanacearum. A= Length of fenestra. B= Anus to fenestra center /fenestra length (M ratio).

VITA

Manuel Galvão de Melo e Mota , son of Ruth and Miguel Eugénio Galvão de Melo e Mota, was born in Wales, Great Britain, on July 15, 1953. He attended several U.S. schools and graduated from the "Liceu Nacional de Oeiras" (high school) in 1971; attended the "Instituto Superior de Agronomia" (College of Agriculture) at the University of Lisbon, Portugal, from 1971 to 1976. In 1978-79 he served the Portuguese Army as a second-lieutenant. After teaching high school biology, he returned to finish his University degree "Licenciatura" in Biology, Faculty of Sciences, University of Lisbon, in 1983. In 1986 he applied and obtained a position as a teaching assistant in the Biology Department of the University of Évora, Portugal, thus initiating his research in Nematology. In 1987, at the invitation of Dr. J.D. Eisenback, he initiated his PhD degree program in Plant Pathology, in the Department of Plant Pathology, Physiology, and Weed Science, at the Virginia Polytechnic Institute and State University, in Blacksburg, VA, USA. He has been awarded several scholarships such as the "Bolsa Ciência" (a doctoral scholarship) from the Junta Nacional de Investigação Científica e Tecnológica and a Fulbright scholarship. He has completed his PhD in September 1992, and is presently an Assistant Professor at the Biology Department of the University of Évora.