PARASITISM OF TIMBER BAMBOO ROOTS BY *GRACILACUS LATESCENS* RASKI, 1976 AND MORPHO-BIOLOGICAL NOTES ON MATURE AND IMMATURE LIFE STAGES

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ABSTRACT

Troccoli, A., N. Vovlas, and R. N. Inserra. 2002. Parasitism of timber bamboo roots by *Gracilacus latescens* Raski, 1976 and morpho-biological notes on mature and immature life stages. Nematropica 32:87-102.

Colonies of *Gracilacus latescens* consisting of swollen females, males, second-stage juveniles (J2) and eggs were observed on timber bamboo (*Phyllostachys bambusoides*) roots collected in central Florida. These nematode life stages were embedded in a hardened gelatinous matrix adhering to the root surface. Microscopical examination of the roots indicated that the vermiform female inserts its long stylet into root tissues and remains attached to the root surface by the stylet. The female body swells with gonad maturation. Eggs are deposited in a gelatinous matrix, which embeds and protects the female and newly hatched J2s. Third (J3) and fourth stage juveniles (J4) are inactive, coiled, and lack a developed stylet. They retain the cuticle after molting and produce motile males and vermiform females, which are enclosed in the juvenile cuticles. Newly hatched J2, kept in water at 25°C, completed the postembryogenic development without feeding in 8-9 days. Histological examination of bamboo root sections infected by the nematode showed nematode feeding on the epidermis, cortical parenchyma and sclerenchyma. Feeding induces cell wall thickening of the epidermis at the feeding site. Up to 3-4 layers of cells are perforated by the stylet, which becomes encased in a cytoplasmic feeding tube. The feeding tube has been observed only in the epidermal, sclerenchymal and peripheral cortical parenchymal cells, which are usually the tissues explored and reached by the nematode stylet. Continuous feeding on these tissues results in the formation of a syncytium, which expands in the cortical parenchyma and extends into the central cylinder incorporating endodermis, pericycle, phloem and vascular parenchyma cells. Densely stained, discrete cells with hypertrophied nuclei and nucleoli, and granular cytoplasm are visible in the syncytium. Endodermal cells incorporated in the syncytium lose cell wall thickening. The morphological examination of the Florida population of *G. latescens* provides further indications that specimens described originally as putative J4s are actually J2s. The J2s of the Florida population have shorter stylets than reported in the original description (10 µm vs. 15 µm).

Key words: Biology, feeding tube, Florida, *Gracilacus latescens,* histopathology, host response, morphology, nematode development, pin nematodes, syncytium.

RESUMEN

Troccoli, A., N. Vovlas, and R. N. Inserra. 2002. Parasitismo de las raíces del bambú maderero por *Gracilacus latescens* Raski, 1976 y notas morfo-biológicas sobre estados de vida maduros e inmaduros. Nematrópica 32:85-100.

Colonias de *Gracilacus latescens* compuestas de hembras hinchadas, machos, segundo estado juvenil (J2) y huevos, se observaron en raíces de bambú maderero (*Phyllostachys bambusoides*) colectadas en la región central de Florida. Estos estados de vida se encontraron incrustados en una matriz gelatinosa, adherida a la superficie de las raíces. Examinación microscópica de las raíces indicó.que la hembra vermiforme inserta su largo estilete dentro de los tejidos de la raíz y permanece sujeta a la superficie de la raíz a través del estilete. El cuerpo de la hembra se inflama con la maduración de la

gonada. Los huevos son depositados en una matriz gelatinosa, la cual cubre y proteje la hembra y los recién emergentes J2s. Los tercer (J3) y cuarto (J4) estados juveniles son inactivos, enrollados, y carecen de un estilete desarrollado. Ellos retienen la cutícula después de la muda y producen machos móviles y hembras vermiformes, los cuales estan encerrados en en la cutícula juvenil. Recién emergentes J2 completaron el desarrollo post-embriónico sin alimentación en 8-9 días, cuando se mantuvieron en agua a 25°C. Examinación histológica de secciones de raíces de bambú infectadas por el nematodo mostraron que el nematodo se alimenta de la epidermis, parénquima cortical y esclerénquima. La penetración induce engrosamiento de la pared celular de la epidermis del sitio de alimentación. El estilete penetra hasta 4-5 capas de células, el cual es envuelto en un conducto de alimentación citoplasmica. El conducto alimenticio ha sido observado solamente en células del parénquima, esclerénquima y parénquima cortical periférico, estos tejidos son usualmente explorados y alcanzados por el estilete. Continua alimentación sobre esos tejidos resulta en la formación de un syncytium, el cual se expande en el parénchima cortical y se extiende en el cilíndro central, incorporando la endodermis, periciclo, floema y células parénquimatosas de los haces vasculares. Células discretas, densamente teñidas, con núcleos y nucleolos hipertrofiados y citoplasma granular son visibles en el syncytium. Células endodermales incorporadas en el syncytium pierde grosor de la pared celular. La examinación morfológica de la población de Florida de *G. latescens* brinda más soporte para establecer que los especímenes descritos como J4s son de hecho J2s. El estilete de los J2s de la población de Florida es más cortos que el reportado en la descripción original (10 µm vs. 15 µm).

Palabras claves: Biología, conducto alimenticio, desarrollo del nematodo, Florida, *Gracilacus latescens,* histopatología, morfología, nematodo alfiler, respuesta del hospedero, syncytium.

In November 2000, high numbers of pin nematodes were detected in regulatory samples collected from timber bamboo (*Phyllostachys bambusoides* Siebold & Zucc.) in central Florida. The population consisted of active vermiform stages of juveniles and adults, and also of inactive coiled juveniles (Fig. 1). Remnants of saccate female bodies were also present. Morphological characters of vermiform females, which have important diagnostic value, matched those reported for *Gracilacus latescens* Raski, 1976. This species was described from the rhizosphere of mesquite (*Prosopis* sp.) in Weslaco, Texas, and also reported in association with *Mesembryanthemum* sp. and strawberry (*Fragaria* × *Ananassa)* in Marin and Mendocino Counties, respectively, in California. Some taxonomists (Brzeski and Háněl, 1999; Van den Berg and Quénéhervé, 1999) accept the synonymy of *Gracilacus* with *Paratylenchus* by Siddiqi and Goodey (1963) who rejected Raski's proposal (1962) to separate these two genera on the basis of the stylet length. We prefer to follow Raski's classification, which includes all pin nematodes with stylet length >48 µm in the genus *Gracilacus.* A major advantage of Raski's classification is the separation of pin nematodes with long stylets, obese females and sedentary habits from those pin nematodes with short stylets, slender females and migratory habits. Many (>30) *Gracilacus* species (Raski, 1991) have been described, but parasitism and postembryonic development of pin nematodes with long stylets and sedentary swollen females are not well characterized.

Brzeski and Háněl (1999) provided information on the postembryonic development of *G. straeleni* (de Coninck, 1931) Raski, 1962 which lacks swollen females and *G. steineri* (Golden, 1961) Raski, 1962 which has sedentary swollen females. According to this study, all the *G. straeleni* postembryonic developmental stages, except adult males, have a well developed stylet, whereas *G. steineri* fourth stage-juveniles (J4s) lack a stylet and are encased in the

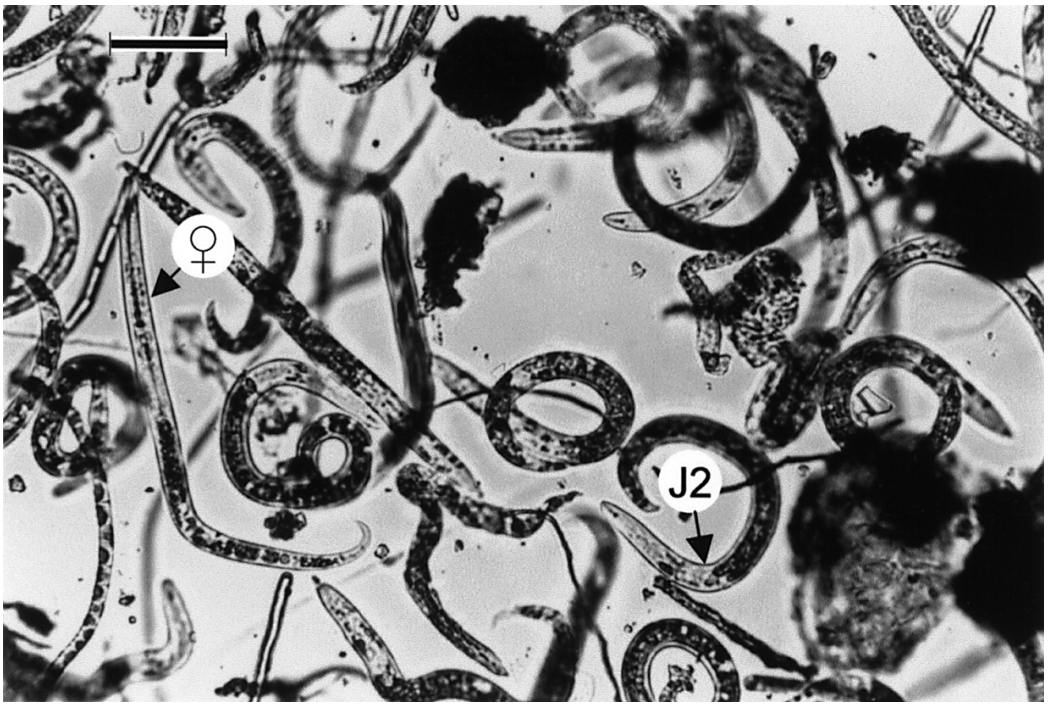

Fig. 1. Vermiform life-stages of *Gracilacus latescens*. Note a female (♀) with a distinct long stylet compared to that of the second-stage juveniles (J2). These juveniles resemble those of the reniform nematode *(Rotylenchulus reniformis)*. Scale bar = 50 µm.

cuticle of the third-stage juveniles (J3s). There is a general agreement among nematologists about the occurrence of quiescent stages in all pin nematode species belonging to both *Gracilacus* and *Paratylenchus sensu* Raski, (1962). However, there are different opinions about *Gracilacus* juvenile stages that become quiescent under adverse environmental conditions. Brzeski and Háněl (1999) pointed out that in most pin nematodes with long stylets (= *Gracilacus* spp.) J4 is the quiescent stage, which lacks a developed stylet whereas in other species such as *G. idalima* and *G. macrodora* Raski, 1962, and *G. steineri* the resting stage is J3. Because of this variability in the life cycle reported in the literature, further study to elucidate the biology of these nematodes is needed. Information on host response is available on only two *Gracilacus* species, *G. peratica* Raski, 1962, which induces feeding tubes in the host root tissues and *G. hamicaudata* Cid del Prado Vera & Maggenti, 1988, which induces specialized feeding cells (Inserra and Vovlas, 1977; Cid del Prado Vera and Maggenti, 1988). Variability is also reported on the morphology of J3s and J4s of *Gracilacus* species (Raski, 1976, 1991; Esser 1992; Brzeski, 1995; Brzeski and Háněl, 1999). The objective of this study was i) to provide information on the ontogeny and parasitic habits of *G. latescens* and ii) to relate this information with data published in the literature on the postembryonic developmental stages of *G. straeleni* and *G. steineri* and the parasitic habits of other *Gracilacus* species, specifically *G. hamicaudata* and *G. peratica*.

MATERIALS AND METHODS

Morphological observations: Soil and root samples were collected from timber bamboo plantings for regulatory examination. Juveniles and vermiform adults were extracted from soil by sugar flotation centrifugation (Jenkins, 1964). Bamboo roots were separated from soil, observed with the aid of a stereomicroscope, and nematodes were removed from the root surface and transferred to water agar (Esser, 1986) to determine life stages and for photographs. Specimens extracted from soil were also transferred to water agar, and life stages identified with a compound microscope on the basis of body size and retained molting cuticles. Other specimens for drawings were killed and fixed in hot aqueous 2% formaldehyde + 1% propionic acid, dehydrated in ethanol vapor, and mounted in dehydrated glycerin (Hooper, 1970). Nematodes were measured and morphometrics of diagnostic value for the genus *Gracilacus* (Raski, 1991) were calculated. Specimens were prepared for scanning electron microscopy (SEM) examination using Spurr's resin (Clark and Stone, 1975). Fixed nematodes were coated with gold and observed at 15 kV accelerating voltage.

Nematode postembryogenesis: Since it is known that the embryogenesis of pin nematodes is similar to that of other tylenchids (Rhoades and Linford, 1961), our observations were directed only at the postembryogenic development of *G. latescens*. We used second-stage juveniles (J2s) freshly emerged from eggs, kept in water, in Syracuse watch glasses. Two groups of 20 newly emerged J2s were maintained separately, at 25°C, in tap water, in Syracuse watch glasses enclosed in petri dishes to avoid evaporation. Juveniles were observed with either a stereomicroscope or a compound microscope every 12 hours to determine molting and degree of development based on the number of retained cuticles.

Parasitism: Nematode parasitism was determined by observing segments of timber bamboo roots infected by the nematode with a stereomicroscope. Nematode life stages attached to the roots were removed and the life stage identified using a compound microscope.

Histopathology: Histological examination of nematode-infected roots was conducted on selected timber bamboo roots with attached swollen female nematodes. The roots were gently washed free of soil, cut into 4-5 mm long pieces, fixed in Randolph's solution, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin under vacuum. Embedded root segments were sectioned 10-12 µm thick, stained with safranin fast green, mounted in Dammar xylene and examined with the aid of a compound microscope (Johansen, 1940).

RESULTS

Morphological observations: Morphological examination of *G. latescens* immature stages collected from soil indicated that only the active vermiform J2s produce a stylet (Figs. 1; 2A,B; 5F). The inactive J3s and J4s are coiled, without a developed stylet, and retain the molting cuticles (Figs. 2E,F; 3A,B). These stages are present in the soil surrounding the bamboo roots. The active vermiform females produce a long stylet. They are often encased in the cuticles of the J2, J3, and J4 (Figs. 2H; 3C; 4A, 5A). These slender females have ectoparasitic sedentary habits, but after gonad maturation become obese with an elongated posterior portion of the body (Figs. 2G; 5C,D). SEM observations of the cuticle of swollen females show it to be coarsely annulated with a lateral field of three lines or two prominent bands (Figs. 2G; 4B).

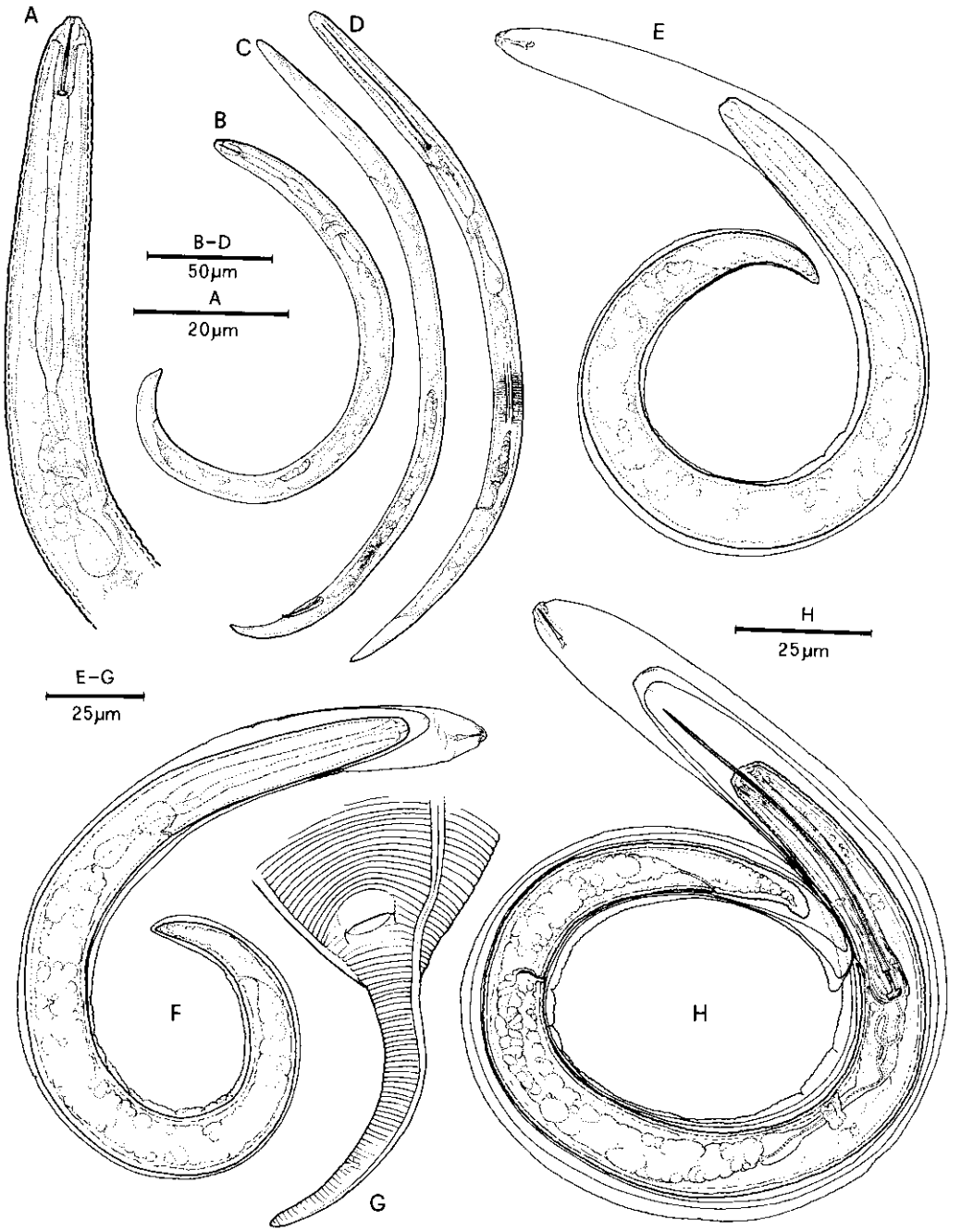

Fig. 2. *Gracilacus latescens* selected life stages. A) Anterior portion of the body of a second-stage juvenile (J2). B) Entire body of a J2. C) Male. D) Vermiform female. E) Third-stage juvenile (J3) encased in the molted cuticle of the J2. F) Fourth-stage juvenile (J4) encased in the J3 and J2's molted culticles. G) Ventral view of the posterior portion of the body of a saccate female. Note the vulva aperture and the elongate terminal portion of the body. H) Vermiform adult female encased in the J4, J3 and J2's molted cuticles. Note the extruded stylet.

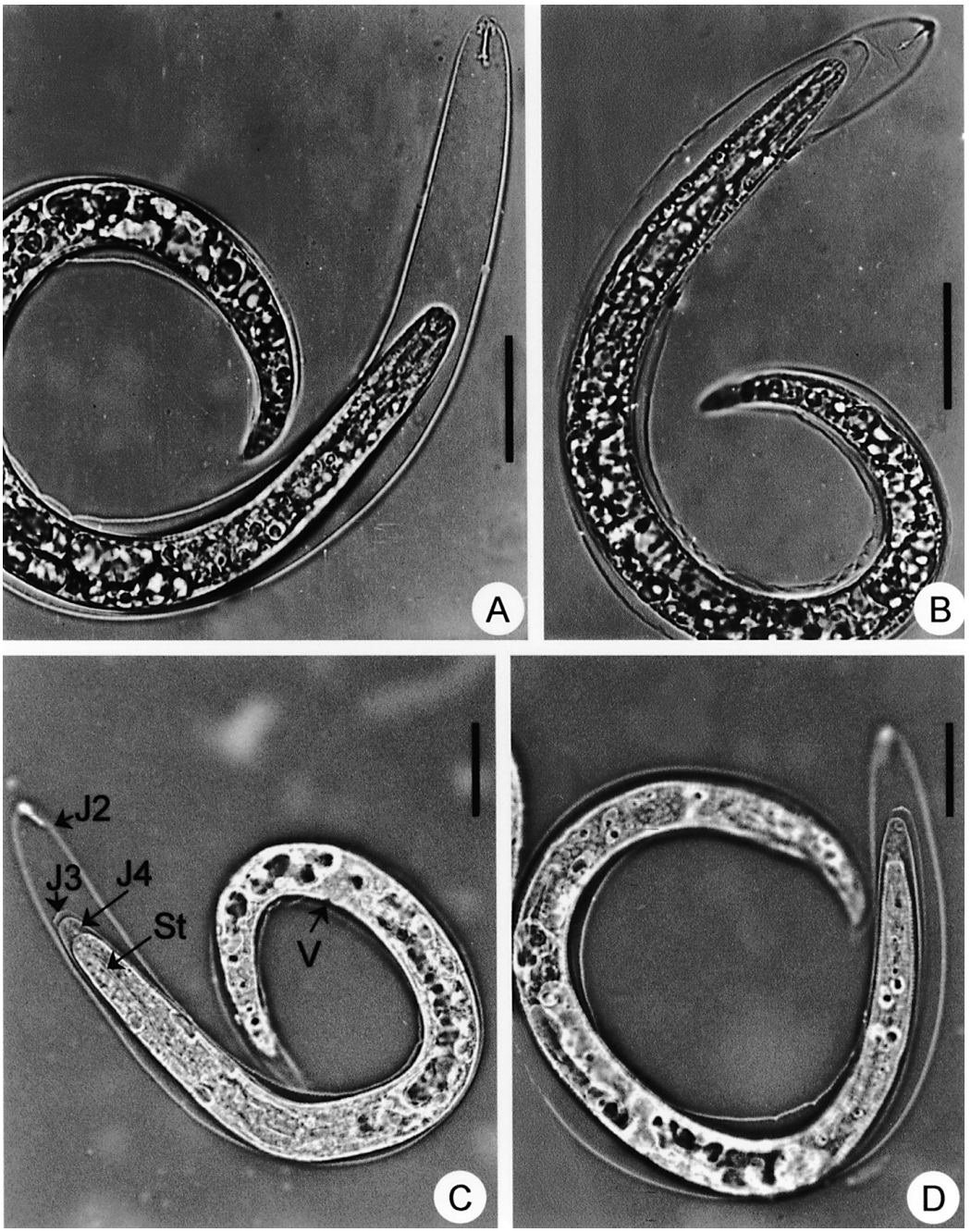

Fig. 3. Photomicrographs of selected life stages of *Gracilacus latescens*. A-C) Life stages drawn in figure 2 E,F,H, respectively. Note cuticles of J2, J3, and J4. St = stylet. V = vulva. D) A forming male encased in the cuticles of the juvenile stages. Scale bar = 25 µm.

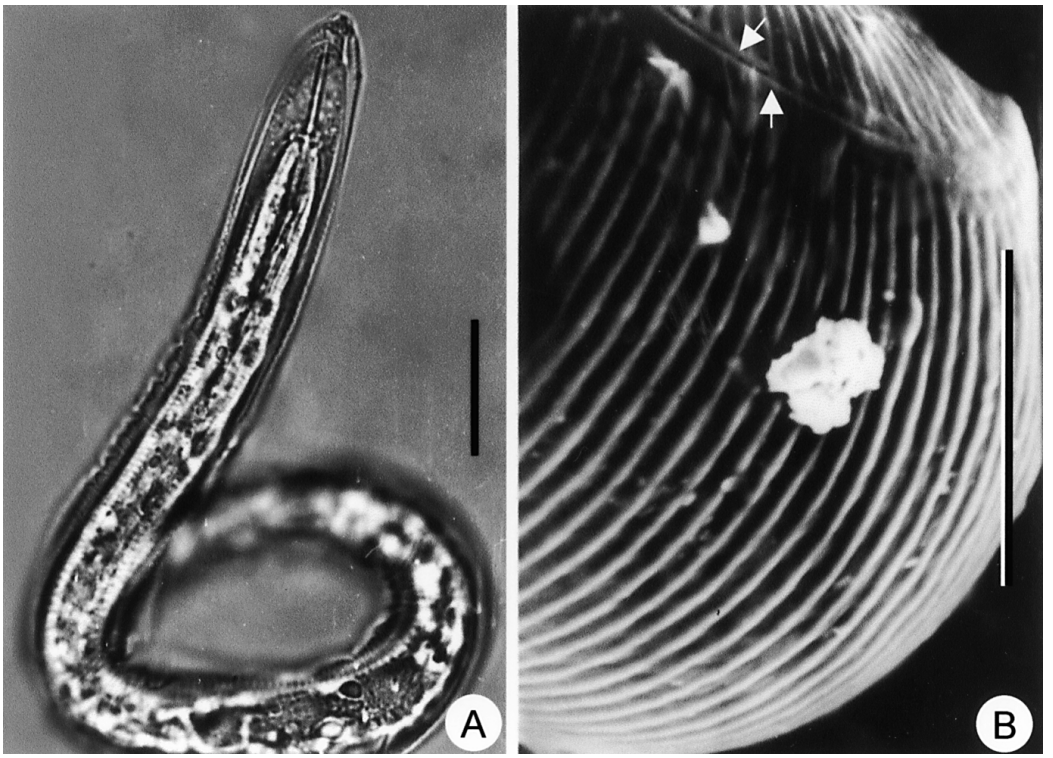

Fig. 4. A) A female encased in the cuticles of juvenile stages. Note the extruded stylet and the indistinct J3 and J4 cuticles which are adhering together. B) SEM micrograph of a swollen female showing the coarse body annulation and the lateral field marked by two bands (arrows). Scale bars = 25 µm.

The widths of the annulus and the lateral field range 1.1-1.4 µm and 2.0-2.5 µm, respectively. Males are vermiform without stylets and are not parasitic (Figs. 2C; 5A). Morphometrics of the life stages of the Florida bamboo population are listed and compared with those reported in the original description in Table 1. Morphometrics of slender, obese females and males of the Florida bamboo population were similar to those reported for the paratypes. However, stylet length of fixed Florida J2s was shorter (8.5-11.5 µm) than that (13-16 µm) of the J2 paratypes. Florida J2s were also more slender than J2 paratypes. Ratio a values of Florida J2s were 18.4-22.8 *vs.* 14-15 of J2 paratypes (Table 1). These differences were confirmed by measurements of a poorly preserved J2 paratype deposited in the University of California Davis Nematode Collection (slide # UCNC 1740). The paratype stylet length was 12.7 µm and the body width was 17.6 µm. In contrast, the maximum stylet length and body width values observed in live J2s of the Florida population were 11.7 µm and 15.6 µm, respectively. These morphological differences between the J2 paratypes and those of the Florida population cast some doubts about the real identity of the Florida population. However, until more specimens of *G. latescens* J2 from the type locality in Texas are available, we prefer to consider the Florida population *G. latescens*.

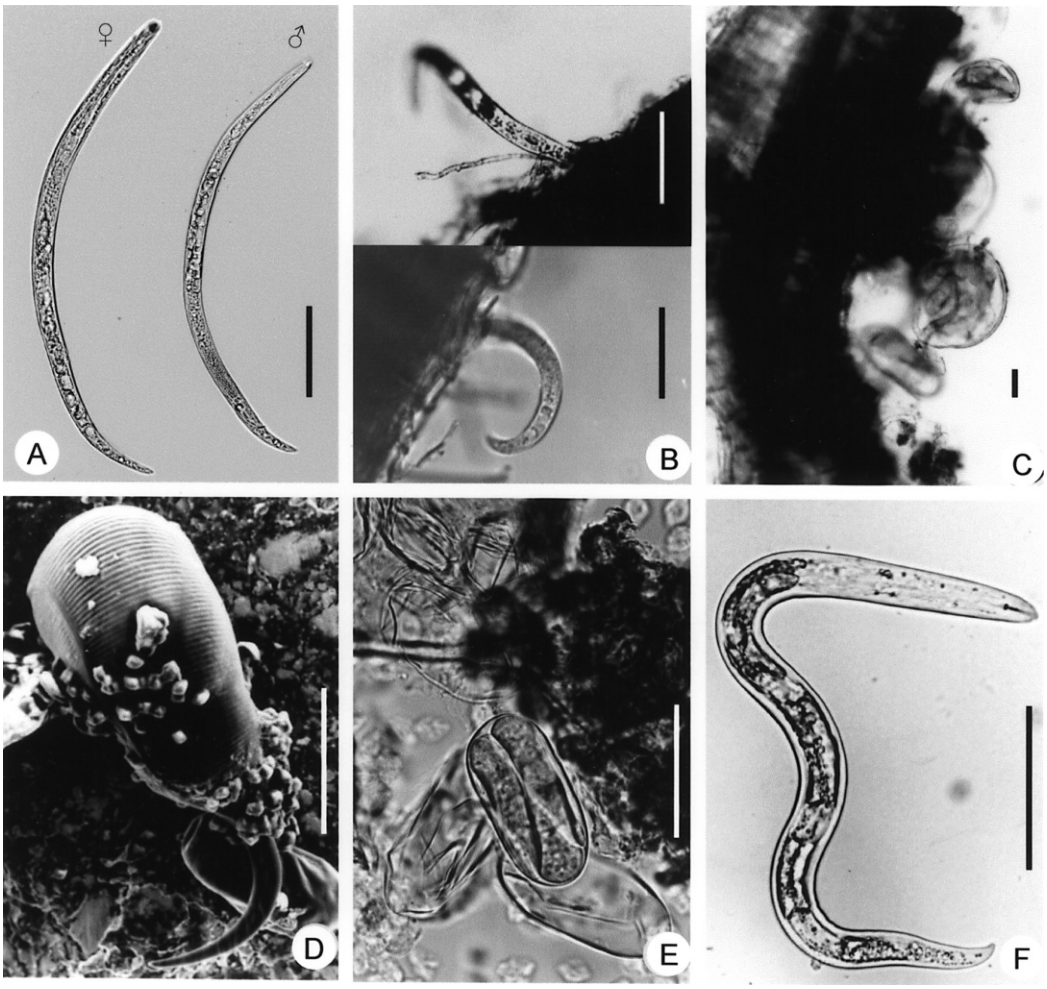

Fig. 5. Photomicrographs of *Gracilacus latescens*. Ectoparasitic sedentary habits of vermiform and swollen females on *Phyllostachys bambusoides* roots. A) Vermiform adult female and male. B) Vermiform females attached to the root surface. C) Colony embedded in the gelatinous matrix showing two swollen females. D) Dorsal view of a swollen female with an egg. E) Eggs. F) Newly emerged second-stage juvenile. Scale bar = 50 µm.

Nematode postembryogenesis: Observations on newly emerged J2s in water at 25°C indicated that the motile J2s become inactive and initiate molting 2-3 days after emerging from the egg. J2s molt, forming coiled, non motile and stylet-less J3s, which remain encased in the J2's cuticle (Fig. 3A). The J3s molt within 1 day forming the stylet-less, coiled and inactive J4s, which remain encased in the J2 and J3's cuticles (Fig. 3B). The J4s initiate molting within 1 day. During this molting process which lasts 3 days, the non-motile adult stages, in the process of being formed and encased in the three juvenile cuticles, are observed after the second day from molting initiation, whereas sluggish females with stylets and males with spicules are visible after the third day (Fig. 3C,D). These females and males encased in juvenile cuticles become

Table 1. Morphometrics and derived variables for vermiform and swollen females, males and second-stage juveniles (J2s) of a Florida bamboo population and Texas paratypes of *G. latescens*.

Character	Vermiform females		Swollen females		Males		(J2)	
Linear (µm)	Florida	Texas	Florida	Texas	Florida	Texas	Florida	Texas
N	15	19	8	5	7	8	10	5
L	279 ± 10.9	270 ± 10.9	386 ± 37.9	370	298 ± 24.9	290	265 ± 11.3	(260)
	(264-300)	(220-290)	(333-460)	(360-390)	(278-348)	(230-350)	(248-281)	(230-290)
Stylet	72.0 ± 2.8	75	—	—	—	—	10 ± 0.8	15
	(67.5-76.5)	(63-80)	—	—	—	—	(8.5-11.5)	(13-16)
Conus	66 ± 3.3	67	—	—	—	—	5.0 ± 0.7	10
	(59.5-70)	(58-74)	—	—	—	—	(4.0-5.5)	(9-11)
St. shaft + knobs	6.0 ± 0.9	7	—	—	—	—	—	—
	(4.5-7.5)	(5-10)	—	—	—	—	—	—
Knobs width	2.5 ± 0.4	—	—	—	—	—	—	—
	(2-3.5)	—	—	—	—	—	—	—
MB valve height	9.0 ± 0.7	9	9.8 ± 1.9	—	—	—	—	—
	(7.5-10)	—	(8.5-13)	—	—	—	—	—
MB max. diam.	8.0 ± 0.4	—	26.5 ± 1.5	—	—	—	4.5 ± 0.6	—
	(7.5-8.5)	—	(24.5-27.5)	(20-28)	—	—	(3.5-5.5)	—
Isthmus length	16 ± 1.7	11	13.5 ± 2.9	—	—	—	17 ± 2.1	—
	(12-18.5)	—	(10.5-16)	—	—	—	(13-20)	—
Oesoph. length	127 ± 4.3	—	104 ± 6.5	—	—	—	75 ± 2.6	—
	(120-132)	—	(99-113)	—	—	—	(69-77)	—
Excretory pore	68 ± 3.5	71	—	111	65 ± 6.9	65	62 ± 2.6	64
	(64-78)	(64-82)	—	—	(56-76)	(58-77)	(57-65)	—

Table 1. (Continued) Morphometrics and derived variables for vermiform and swollen females, males and second-stage juveniles (J2s) of a Florida bamboo population and Texas paratypes of *G. latescens*.

Character	Vermiform females		Swollen females		Males		(J2)	
Linear (µm)	Florida	Texas	Florida	Texas	Florida	Texas	Florida	Texas
Max. body width	11.5 ± 0.6	—	90.5 ± 15.8	—	10.5 ± 0.5	—	13 ± 0.6	—
	(11.0-13.0)	—	(64-112)	—	(10.0-11.5)	—	(12.0-13.5)	—
Annuli width	1.2 ± 0.1	—	1.7 ± 0.3	—	1.3 ± 0.1	—	1.1 ± 0.2	—
	(1.1-1.4)	(1.0-1.6)	(1.5-2.3)	—	(1.1-1.4)	(1.0-1.4)	(1.0-1.4)	—
Lat. field width	2.2 ± 0.3	—	3.5 ± 0.7	—	—	—	—	—
	(2-2.5)	—	(2.5-4.5)	—	—	—	—	—
VL	198 ± 9.2	—	290 ± 39	—	—	—	—	—
	(185-218)	—	(241-356)	—	—	—	—	—
Genital tract	37 ± 3.5	—	—	—	118 ± 4.9	—	14 ± 3.3	—
	(31-42)	—	—	—	(111-124)	(40-49)	(11-21.5)	(9-10)
Vulva-anus dist.	55 ± 3.1	—	—	—	—	—	—	—
	(47-59)	—	—	—	—	—	—	—
Tail length	24.5 ± 2.5	—	24 ± 1.3	—	26.5 ± 2.2	—	23.5 ± 3.1	—
	(21.5-31.5)	—	(23-25.5)	—	(24.0-30.5)	—	(20-29)	—
Anal body width	6.0 ± 0.4	—	6.5 ± 1.1	—	7.5 ± 0.3	—	8.2 ± 0.4	—
	(5.5-6.5)	—	(5.0-8.0)	—	(7.5-8.0)	—	(7.5-9.0)	—
Spicules	—	—	—	—	18.5 ± 0.7	20	—	—
	—	—	—	—	(18.0-19.5)	(18-23)	—	—
Gubernaculum	—	—	—	—	4.5 ± 0.2	4	—	—
	—	—	—	—	(4.5-5.0)	—	—	—

Percentages

Table 1. (Continued) Morphometrics and derived variables for vermiform and swollen females, males and second-stage juveniles (J2s) of a Florida bamboo population and Texas paratypes of *G. latescens*.

Character	Vermiform females		Swollen females		Males		(J2)	
Linear (µm)	Florida	Texas	Florida	Texas	Florida	Texas	Florida	Texas
V or T (%)	71 ± 1.4	71	75 ± 2.2	—	39.3 ± 2.8	37	—	—
	(69-74)	(68-73)	(72-78)	(78-79)	(35.6-42.8)	(27-47)	—	—
G (%)	13 ± 1.4	—	—	—	—	—	5.4 ± 1.2	—
	(11-15)	—	—	—	—	—	(4.2-7.9)	—
Ratios								
a	23.9 ± 1.8	19	4.5 ± 1.0	5.5	28.6 ± 0.9	26	20.3 ± 1.2	15
	(21.5-27.3)	(14-23)	(3.7-6.4)	(4.1-9.3)	(27.6-30.3)	(20-29)	(18.4-22.8)	(14-15)
b	2.2 ± 0.1	2.2	3.5 ± 0.4	3.8	—	3.5	3.5 ± 0.1	3.7
	(2.1-2.4)	(1.7-2.5)	(2.9-3.8)	(3.6-4.1)	—	(3.3-3.7)	(3.3-3.7)	(3.5-4.1)
c	11.7 ± 1.2	13	15.8 ± 1.6	—	11.5 ± 0.7	12	10.4 ± 1.4	—
	(8.4-14.0)	(10-13)	(14.0-17.2)	—	(10.5-12.6)	(11-13)	(9.7-14)	—
c’	4.1 ± 0.4	—	3.9 ± 0.6	—	3.4 ± 0.3	—	2.9 ± 0.4	—
	(3.5-4.8)	—	(3.6-4.6)	—	(3.0-3.8)	—	(2.4-3.6)	—

active within 2 days. The total length of the postembryonic development took 8-9 days after eclosion. The molting processes of the J3s into J4s and J4s into adults are difficult to detect because often the J3 and J4 cuticles remain attached and are not clearly discernable (Fig. 4A). The J2 bodies are larger [14.9 µm (14.7-15.6); n = 18] than those of adults [13 µm (12.5-14.2); n = 13 females] because of the large quantity of stored fat globules, which permit development without feeding.

Parasitism: Examination of nematode-infected bamboo roots indicated that *G. latescens* slender females initiate root infection. These slender females remain attached to the root surface by the stylet (Fig. 5B). Soil particles and cell debris accumulate around the anterior portion of the female body outside the root. As females reach sexual maturity, they become swollen and secrete a gelatinous matrix, which covers and protects their bodies (Fig. 5C). The gelatinous matrix hardens around females, males, newly hatched J2s, and eggs (63-69 µm long and 31-38 µm wide) (Fig. 5E). Multiple infection by four or five females packed together in the same gelatinous matrix was common (Fig. 5C). J2s leave the gelatinous matrix and move to the soil and molt to initiate another cycle as mentioned above.

Histopathology. Histological examination of *G. latescens*-infected bamboo roots shows that only females are able to establish permanent feeding sites. J2s are able to develop and molt without feeding. However, we did not investigate whether they can feed on epidermal tissues as reported for other pin nematode J2s (Rhoades and Linford, 1961). Examination of root cross sections with attached nematode colonies indicated that swollen female stylets penetrate into the cells of the epidermis, sclerenchyma, and cortical parenchyma. Up to 3-4 layers of cells are perforated by the stylet, which is encased in a feeding tube (Fig. 6A-C). Epidermal cell wall thickening, at the point of stylet penetration, is commonly observed (Fig. 6A-D). Continuous nematode feeding results in the formation of specialized syncytial cells, which originate in the cortical parenchyma and extend from the cortical parenchyma into the central cylinder involving vascular tissues, such as endodermis, pericycle, phloem, and vascular parenchyma (Fig. 6D-F). In infected root sections examined there was no evidence of stylet penetration into stelar tissue cells. The length of the stylet limits the range of root tissues explored by the nematode to the peripheral layers of the cortex (Fig. 6A-D). However, nematode feeding in this tissue induces a syncytium that expands to cortical parenchymal and stelar cells (Fig. 6D). Syncytial cells have thickened walls, enlarged nuclei, and densely stained cytoplasm (Fig. 6B,E). The densely stained and granular cytoplasm suggests high metabolic activity of the syncytial cells. Cell wall fragmentation between adjacent cells is also observed (Fig. 6D-F). The incorporation of endodermal cells in the syncytium causes loss of the characteristic cell wall thickening of the fused cells (Fig. 6D-F) Our light microscope observations were not able to detect ultrastructural features of the cell walls such as pit fields or ingrowths in the syncytial cell walls. Transmission or scanning electron microscope observations are necessary to detect these cell wall ultrastructures.

DISCUSSION

Pin nematode species with a short stylet (*Paratylenchus sensu* Raski) have non-feeding and inactive J4s lacking a well developed stylet, whereas the J2s and J3s are active and have a well developed stylet (Corbett, 1978; Maggenti, 1981; Raski, 1991; Rhoades and Linford, 1961; Siddiqi,

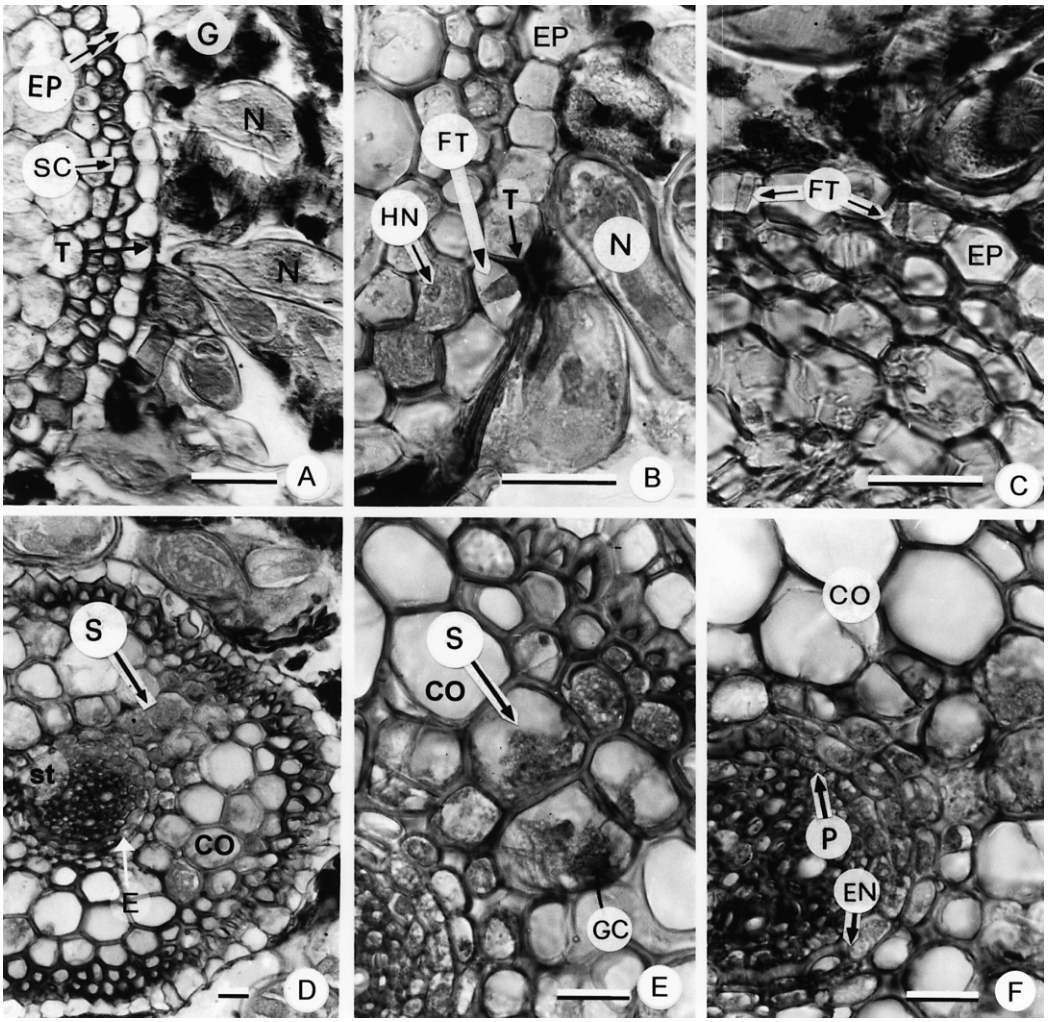

Fig. 6. Cross sections of *Phyllostachys bambusoides* roots infected by *Gracilacus latescens*. A) Feeding swollen females (N) attached to the root surface. Note thickening (T) of the epidermis (EP) at the point of stylet penetration. G = gelatinous matrix. SC = sclerenchyma. B) Feeding tube (FT) in the epidermis (EP) induced by the feeding of a swollen female (N). Note cell wall thickening (T) of the epidermis at the point of stylet penetration and enlarged nucleus (HN) in sclerenchyma cells showing densely stained and granular cytoplasm. C) Feeding tubes (FT) in the epidermis (EP) and sclerenchyma cells. D) Syncytium (S) with densely stained cytoplasm extending from the cortical parenchyma (CO) into the stele (St). Note the disruption of the stelar tissues caused by the expanding syncytium. E = endodermis. E) Syncytial cells (S) with thickened walls and granulated cytoplasm (GC) in the cortical parenchyma (CO). F) Syncytium extending from the cortical parenchyma (CO) into the stele and incorporating endodermal (EN), pericyclic (P), phloem and vascular parenchymal cells. Note the lack of thickened wall in the endodermal cells fusing with pericyclic and cortical parenchymal cells in the syncytium. Scale bars = 100 µm.

1986). Inconsistent reports are available in the literature about the morphology of the juvenile stages of pin nematodes with long stylet (>48 µm) (*Gracilacus*). The J2s of all species in this genus have a well developed stylet, which is not consistently present in the J3s and J4s. Some *Gracilacus* species are reported having stylet-bearing J3s and non-

feeding and non-stylet J4s, whereas other species are reported having non-feeding and non-stylet J3s, but stylet bearing J4s (Raski, 1962, 1991; Brzeski, 1995; Brzeski and Háněl, 1999). The authors of these studies observed the presence of retained molted cuticles in some vermiform life stages of these species. Our study provides evidence that *G. latescens* J2s are active and with a stylet, while J3s and J4s are non-motile and lack a developed stylet. With the exception of the J2s, all juvenile stages and the slender immature females and males of this species retain molted cuticles. The morphology of *G. latescens* J3 differs from that reported for *G. straeleni* and *G. steineri* because these two species have stylet-bearing J3s, which is stylet-less in *G. latescens* (Brzeski and Háněl, 1999). *Gracilacus straeleni* J4 has a stylet, whereas *G. latescens* and *G. steineri* J4s are both stylet-less and non-feeding. No retained cuticles were reported in the postembryonic developmental stages of *G. straeleni*, but retained cuticles were observed with difficulty and detected only in some vermiform stages of *G. steineri* (Brzeski and Háněl, 1999). Our observations show a distinct J2s'retained molted cuticle, but J3 and J4 cuticles were often indistinguishable because these cuticles remain attached together and adhere to the J2's cuticle. The difficulty in the detection of molted cuticles makes the identification of J3s and J4s problematic and explains the uncertain determination of these stages reported during the description of *Gracilacus* species (Raski, 1962, 1976). Juvenile morphology of other *Gracilacus* species needs careful re-examination in order to verify if other species have stylet-less and non feeding J3s and J4s like those of *G. latescens*. The unreliable identification of J3s and J4s in the taxonomic literature concerning the description of many *Gracilacus* species was pointed out by Esser (1992). No swollen sedentary females were observed in *G. straeleni* and *G. steineri* populations studied by Brzeski and Háněl (1999), who stated that swollen stages were probably undetected because of extraction techniques used. The results of our and Brzeski and Háněl's studies point out the need for the use of appropriate techniques during the extraction of *Gracilacus* from soil and roots in order to obtain all life stages of these nematodes and to avoid incomplete descriptions.

Gracilacus latescens juveniles differ morphologically and biologically from those of *Paratylenchus sensu* Raski. The ability of *G. latescens* juveniles to molt and to attain the adult stage without feeding has not been previously reported and represents a distinct biological characteristic from that of *Paratylenchus sensu* Raski juveniles, which require feeding to develop and reach the adult stage (Rhoades and Linford, 1961). However, it is necessary to verify whether this biological characteristic is shared by other *Gracilacus* species with swollen sedentary females.

The parasitic habits of *G. latescens* are similar to those reported for *G. hamicaudata* and *G. peratica* (Inserra and Vovlas, 1977; Cid del Prado Vera and Maggenti, 1988). However, *G. latescens* and *G. peratica* colonies adhere to the root surface whereas those of *G. hamicaudata* are located beneath the root cortex of the host plant. The colonies of *G. peratica* observed lacked a gelatinous matrix, which may have been lost during removal of infected roots from the soil. Gelatinous matrix was always observed with *G. hamicaudata* and *G. latescens*. The presence of a gelatinous matrix was also reported in colonies of *Cacopaurus pestis* Thorne, 1943, which is closely related to *Gracilacus* spp. (Inserra and Vovlas, 1981). No attempt was made, in our study, to determine the origin of the gelatinous matrix in *G. latescens* females. We don't know whether the gelatinous

matrix is secreted through the excretory pore as in tylenchulids or through the vulva as in reniform nematodes.

The anatomical alterations induced by *G. latescens* are similar to those reported for *G. hamicaudata* and *G. peratica*. The feeding tubes observed in *G. latescens*-infected bamboo roots also occur in the epidermis and cortical parenchyma of olive roots infected by *G. peratica*. Our observations, made with a compound microscope, were unable to determine the role played by the feeding tubes. It is reasonable to assume that they consist of callose-like deposits as those reported for the feeding tubes induced by the taxonomic related ring nematode *Mesocriconema xenoplax* (Raski, 1952) Loof & de Grisse, 1989 (Hussey *et al.*, 1991). These callose-like deposits may prevent the stylet's retraction and keep the nematode anchored to the root surface. Studies on the ultrastructure of the specialized feeding sites of *G. latescens* are needed to clarify their role. The specialized syncytial cells with dense granular cytoplasm and enlarged nuclei induced by *G. latescens* in bamboo roots were similar to those observed in *Sequoia sempervirens* roots infected by *G. hamicaudata*. As a conclusive statement, we would like to emphasize that specialized cells are usually induced by endo- or semiendoparasitic sedentary species. In spite of the fact that *G. latescens* is an ectoparasitic nematode, the sedentary habits and continuous feeding of the obese females result in the formation of specialized feeding sites in root tissues of host plants as it has been reported for other ectoparasitic nematodes, such as some dagger nematodes (Weischer and Wyss, 1976).

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