

Intraspecific Morphological Variation Among Populations of *Pratylenchus brachyurus* and *P. coffeae*¹

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Abstract: Three populations of *Pratylenchus coffeae* and two of *P. brachyurus*, each originating from a single female, were maintained on *Citrus* spp. or *Solanum nigrum* L. for several years under greenhouse conditions. Nematodes were extracted from roots, and adult female specimens were killed, fixed, and mounted in glycerine for microscopic study. Variables measured were distance between vulva and anus and lengths of the stylet, posterior uterine sac, and tail. The mean data and coefficients of variability suggest that stylet length had the least variability, and length of posterior uterine sac the most. When males and distinct spermathecae are not evident in *P. coffeae* populations, the species can be distinguished from *P. brachyurus* by a shorter mean stylet length, longer mean posterior uterine sac length, and much longer distance between the vulva and anus. **Key Words:** taxonomy, morphometrics, variation, intraspecific variation.

The problem of intraspecific variability among plant-parasitic nematodes is well known and has been adequately documented (3, 5, 11, 12, 14, 15). The nomenclatural literature abounds with synonymies of taxa which were once considered discrete species but later found to be conspecific. Such confusion results, in most cases, from the variability exhibited by a discrete species as affected by food, environment, and other factors.

The species of *Pratylenchus* most frequently found infecting citrus roots in Florida is *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941. *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941 is encountered also, though to a much lesser extent (13). Both species have been found pathogenic to citrus (2, 8), although *P. brachyurus* was later deemed a weak pathogen (7). *Pratylenchus coffeae* populations usually have numerous males and females with distinct spermathecae filled with

spermatozoa. *Pratylenchus brachyurus* males are extremely rare and spermathecae are empty and usually indistinct. Essentially, those are the characters differentiating the two species in the keys to *Pratylenchus* by Loof (6) and Corbett (4). Loof further designated *P. coffeae* as having a broadly rounded or indented tail tip, as did Corbett, and *P. brachyurus* as having an angular lip region. Sher and Allen (10) used only the criterion of angular lateral margin of lips for *P. brachyurus* and rounded for *P. coffeae* in their key, although, in the species descriptions, they further differentiated the two by vulva position, presence of males and spermathecae, and tapering tail shape (for *P. coffeae*). Van den Berg (1) referred to the tail terminus of *P. brachyurus* as being bluntly rounded, and that of *P. coffeae* as broadly rounded. A detailed study of the morphology and morphometrics of six *Pratylenchus* species, including the aforementioned two species, was made by Román and Hirschmann (9). They concluded that the shape of the tail termini of *P. brachyurus* and *P. coffeae* showed only slight variation but admitted that considerably more variability might exist within different "natural" populations (not experi-

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mentally bred). They further found that, among other characters, female tail length, stylet length, and vulva-anus distance were useful in distinguishing between species whereas length of postvulvar uterine branch was of doubtful value. Tarté and Mai (14) studied populations of *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, and found that the variability exhibited by a population originating from a single gravid female was so great that a percentage of the progeny, despite the environment in which cultured, possessed all of the characters of various other *Pratylenchus* species.

Although females of *P. brachyurus* can be readily distinguished morphologically from those of *P. coffeae*, the occurrence of isolated females in mixed populations, sometimes with indiscernible characters, can strain the perceptiveness and discrimination of the observer.

The objective of the present research was to determine the extent of variability in individuals within populations derived from single gravid females of *P. brachyurus* and *P. coffeae*. It further sought to determine specific major morphological differences between females of the two species.

MATERIALS AND METHODS

Single gravid females of *P. brachyurus* obtained from rough lemon roots (*Citrus jambhiri* Lush.) in Lutz, Florida, were inoculated to rough lemon seedlings, whereas single females from lemon roots in Indiantown, Florida, were inoculated to black nightshade (*Solanum nigrum* L.). Progenies of the females were maintained without disturbance for 42 and 26 months, respectively. A single plant of rough lemon and of nightshade was sacrificed, and adult female nematodes obtained from the roots were killed, fixed in 2.5% formaldehyde, and mounted in glycerine for study.

Sour orange seedlings (*Citrus aurantium* L.) were inoculated with single fertilized females of *P. coffeae* from two populations obtained from citrus groves near Orlovista and Lake Alfred, Florida. Progenies of the females were maintained for 44 and 48 months, respectively. Nematodes were isolated from the roots of one plant originally infected with a female from Lake Alfred

and from two plants inoculated with single females from Orlovista. Adult female specimens were processed as described previously.

Plants were grown in Astatula fine sand in 15-cm-diam clay pots at greenhouse temperatures ranging from 28 (21-32) C to 21 (11-26) C.

All measurements were made with a Wild microscope fitted with Zeiss apochromatic objectives. The microscope camera lucida used for measurements was calibrated daily. Variables measured were: lengths of stylet (ST), postvulvar uterine sac (PU), and tail (TA); and distance between vulva and anus (VA). The arithmetical mean (\bar{x}), standard deviation (SD), and coefficient of variation (CV) were calculated for all measurements.

RESULTS

Pratylenchus brachyurus: Among the measurements obtained from the two *P. brachyurus* populations, the character of post-uterine sac length had the highest coefficient of variation (CV) and thus the least reliability of the criteria used (Table 1). This problem is reflected in Plate I-A, where the sac in Fig. 2 is short, while the sac in Fig. 3 is longer, even though the body length posterior to the vulva is shorter than that in Fig. 2. The vulva-anus distance was also quite variable (Pl. I-A, Fig. 3, 6; Pl. II-A, Fig. 3, 5), resulting in CV values of 9.65% and 12.22% with a range of 42-75 μm (Table 1). Tail lengths were likewise variable (range 20-40 μm), with resultant CV of 8.71% and 13.32%. Tail termini were of several shapes, including blunt and flattened (Pl. I-A, Fig. 1; I-B, Fig. 1, 3; Pl. II-A, Fig. 2; II-C, Fig. 1-4), rounded (Pl. I-A, Fig. 5; I-B, Fig. 4, 5, 8-10), digitate (Pl. I-A, Fig. 2, 4; I-B, Fig. 24-28; Pl. II-A, Fig. 1, 3; II-C, Fig. 25, 27, 30, 31, etc.), and even indented (Pl. I-A, Fig. 6; I-B, Fig. 36-43). The most reliable measurement was stylet length, which resulted in a CV of 2.4% (range 17-19 μm). Labial-region shape was irregular, being angular in some specimens (Pl. I-C, Fig. 1, 4; Pl. II-B, Fig. 1, 2, 8) and rounded in others (Pl. I-C, Fig. 2, 6; Pl. II-B, Fig. 3, 7). A distinct recognizable spermatheca was observed in the female gonad (Pl. II-D, Fig. 1-5). The area anterior to the tri-

TABLE 1. Morphometrics on females from two populations of *Pratylenchus brachyurus* reared from single females.

Source	N ^a	Part measured (μm)	\bar{x}^{\dagger} (μm)	Range (μm)	SD [*]	CV ^b
Indiantown, Florida (reared on black nightshade)	129	ST ^b	18.0	17-19	0.45	2.48
		PU ^c	13.7	10-19	2.15	15.70
		TA ^d	28.7	21-35	2.50	8.71
		VA ^e	57.0	42-75	5.50	9.65
Lutz, Florida (reared on Rough lemon)	87	ST	17.8	17-19	0.44	2.45
		PU	15.9	9-26	3.07	19.29
		TA	30.0	20-40	3.99	13.32
		VA	56.0	44-72	6.84	12.22

^aN = number of females measured.

^bST = stylet length.

^cPU = posterior uterine sac length.

^dTA = length of tail.

^eVA = length of body between vulva and anus.

[†] \bar{x} = arithmetical mean.

^{*}SD = standard deviation from the mean.

^bCV = coefficient of variation in percent.

columella appeared as nondescript tissue, as expected, since no spermatozoa were present to give function and identity to the spermatheca.

There were no extant morphological differences between the populations reared on rough lemon and on black nightshade. However, females within the *P. brachyurus* population raised on lemon seedlings had tail termini more generally rounded and less indented (Pl. II-C) than those in the population raised on black nightshade (Pl. I-B). The post-uterine sac was also longer in females of the citrus population. The CV, however, was excessively high, thus discounting the reliability of the difference. There were also considerably fewer indented tail termini on females from the rough lemon population than from black nightshade.

Pratylenchus coffeae: Similar to the findings from the study of *P. brachyurus*, which was conducted concurrently, the postvulvar uterine sac of *P. coffeae* had the highest CV of the characters measured (Table 2). Plate III-F, Figs. 1 & 2, and Pl. IV-A, Figs. 3 & 5, depict long and short sacs (range 8-47 μm) observed within the two populations. Tail lengths (range 20-40 μm) also had high CV values (12.8-15.0%), whereas vulva-anus distance (range 65-127 μm) had somewhat lower CV values (8.74-

13.45%). Even so, both were high enough to discourage reliance on the length of those morphological characters as adequately uniform.

Tail termini were sometimes flattened (Pl. III-C, Fig. 1, 2; III-E, Fig. 1, 2; III-F, Fig. 4; Pl. IV-C, Fig. 3, 19, 27), rounded (Pl. III-A, Fig. 4; III-C, Figs. 13, 14; III-E, Fig. 5, 8, 10, 17; III-F, Fig. 2, 3; Pl. IV-A, Fig. 1; IV-C, Fig. 23, 30), conical to bluntly conical (Pl. III-C, Fig. 18; III-E, Fig. 16; Pl. IV-C, Fig. 39), digitate (Pl. III-C, Fig. 22; III-E, Figs. 11, 13; Pl. IV-A, Fig. 6; IV-C, Fig. 33, 45, 48), indented (Pl. III-E, Fig. 23-25; Pl. IV-C Fig. 14), and even cleft (Pl. III-C, Fig. 25; Pl. IV-C, Fig. 13).

Stylet length proved to be the most reliable measurement, with the lowest CV (3.33-3.65%) and a range of 15-17 μm (Table 2). Labial region shape was slightly irregular, varying from low, somewhat flattened (Pl. IV-B, Figs. 3-4) to rounded (Pl. III-D, Fig. 1; Pl. IV-B, Fig. 8). Two annules comprise the labial region although, rarely, a specimen exhibiting three annules on one side and two on the other was found (Pl. IV-B, Fig. 7).

The female reproductive system was distinct, showing the degenerate branch of the posterior gonad (postvulvar uterine sac), vulva (often with prominent lips, especially on older females), well-developed

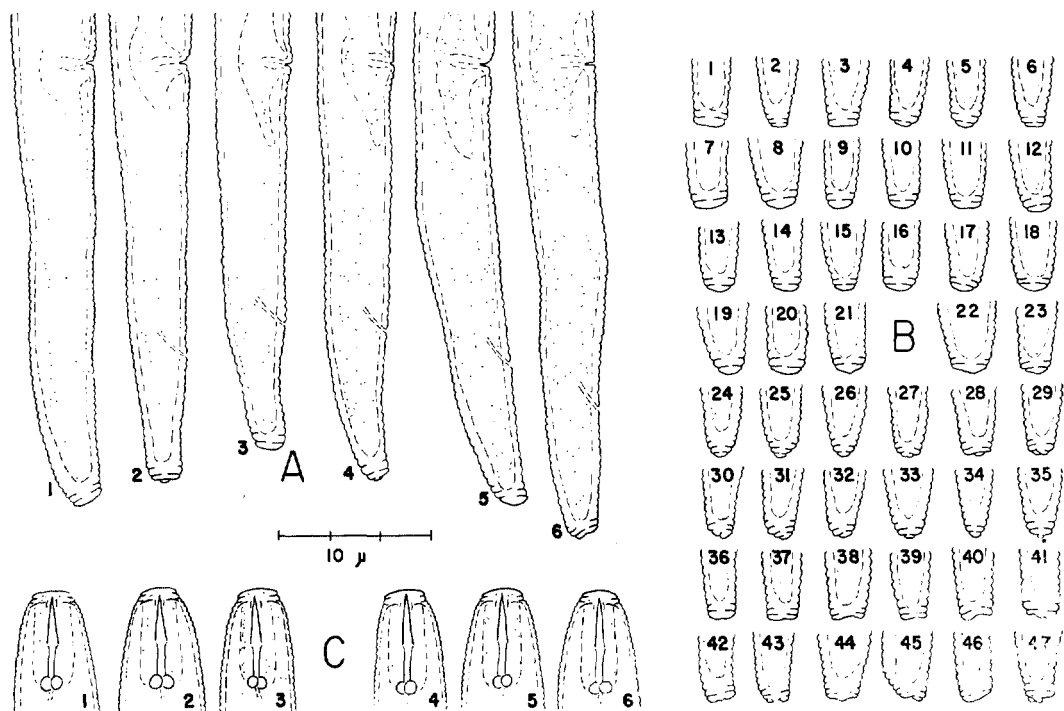


PLATE I (A-C). *Pratylenchus brachyurus* originating from Indiantown, Florida, cultured on black nightshade, *Solanum nigrum*, for 26 months in the greenhouse. All drawings are of adult females. A) Vulva-anal regions of body. B) Tail termini. C) Labial regions.

TABLE 2. Morphometrics on females from three populations of *Pratylenchus coffeae* originating from single females and cultured on sour orange seedlings.

Source	N ^a	Part measured (μm)	\bar{x}^f (μm)	Range (μm)	SD ^g	CV ^h
Orlovista (Culture #1)	54	ST ^b	15.5	15-17	0.54	3.48
		PU ^c	24.0	16-37	5.48	22.83
		TA ^d	30.0	22-40	4.17	13.90
		VA ^e	87.8	70-104	7.67	8.74
Orlovista (Culture #2)	85	ST	15.6	15-17	0.52	3.33
		PU	24.7	17-47	5.96	24.13
		TA	30.2	20-40	3.86	12.78
		VA	89.6	70-127	10.49	11.71
Lake Alfred	57	ST	15.9	15-17	0.58	3.65
		PU	22.7	8-38	5.69	25.07
		TA	29.2	22-38	4.38	15.00
		VA	85.4	65-113	11.49	13.45

^aN = number of females measured.

^bST = stylet length.

^cPU = posterior uterine sac length.

^dTA = length of tail.

^eVA = length of body between vulva and anus.

^f \bar{x} = arithmetical mean.

^gSD = standard deviation from the mean.

^hCV = coefficient of variation in percent.

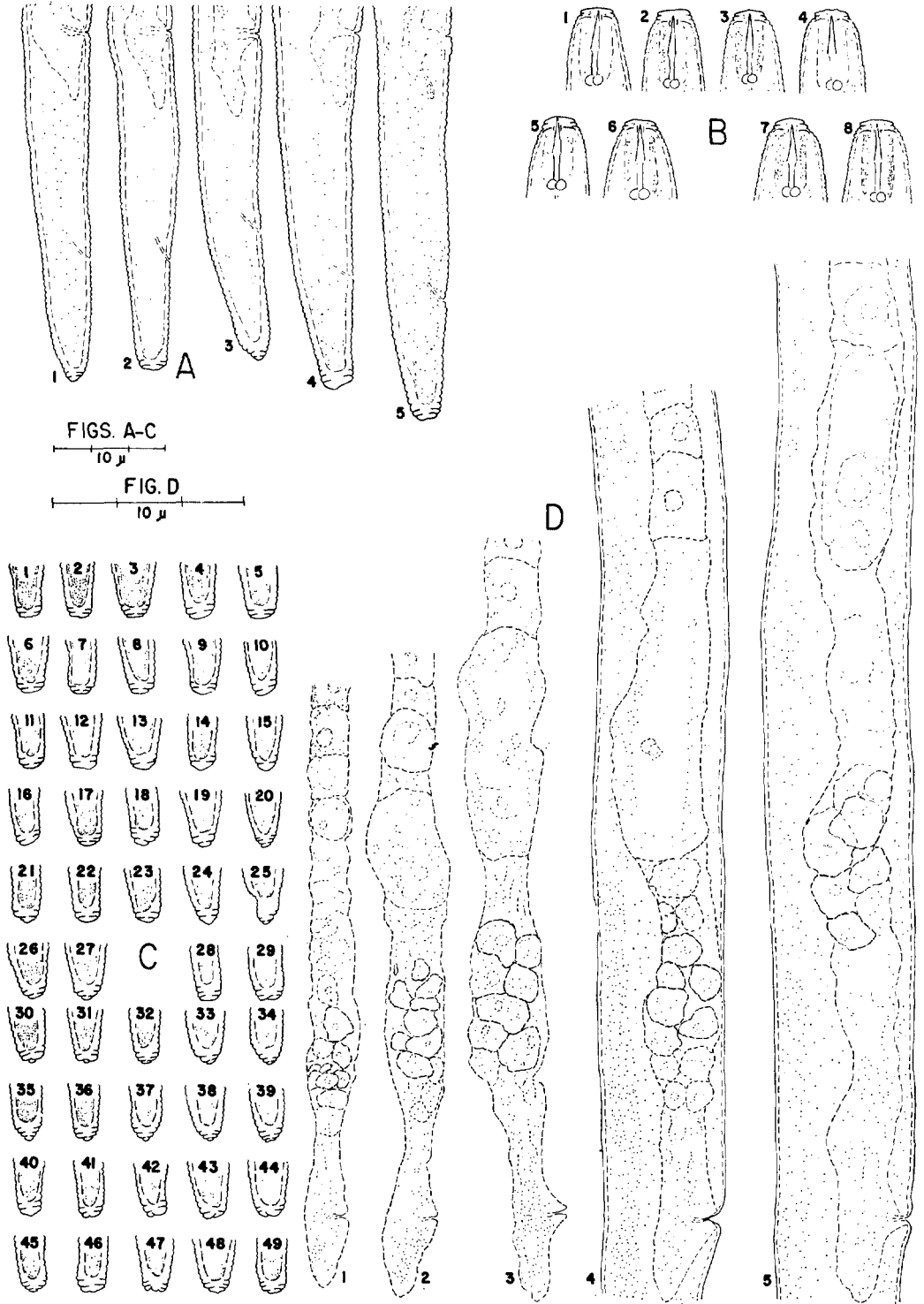


PLATE II (A-D). *Pratylenchus brachyurus* from Indiantown, Florida, cultured on rough lemon, *Citrus jambhiri*, for 42 months in the greenhouse. All drawings are of adult females. A) Vulva-anal regions of body. B) Labial regions. C) Tail termini. D) Female gonads.

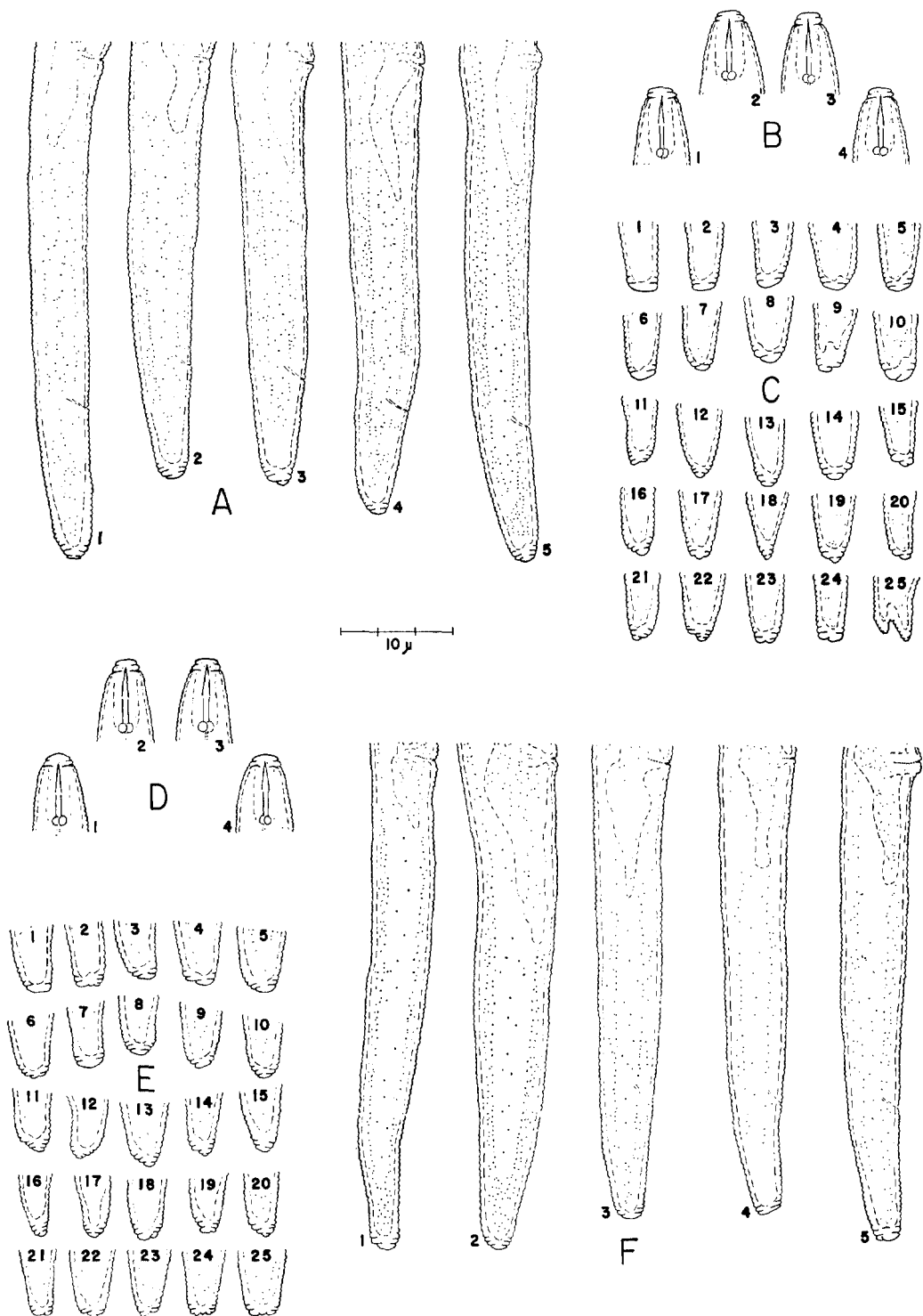


PLATE III (A-F). *Pratylenchus coffeae* originating from Orlovista, Florida, cultured on *Citrus aurantium* for 48 months in the greenhouse. All drawings of adult females from two separate replicate populations (A-C and D-F). A & F) Vulva-anal regions of body. B & D) Labial regions. C & E) Tail termini.

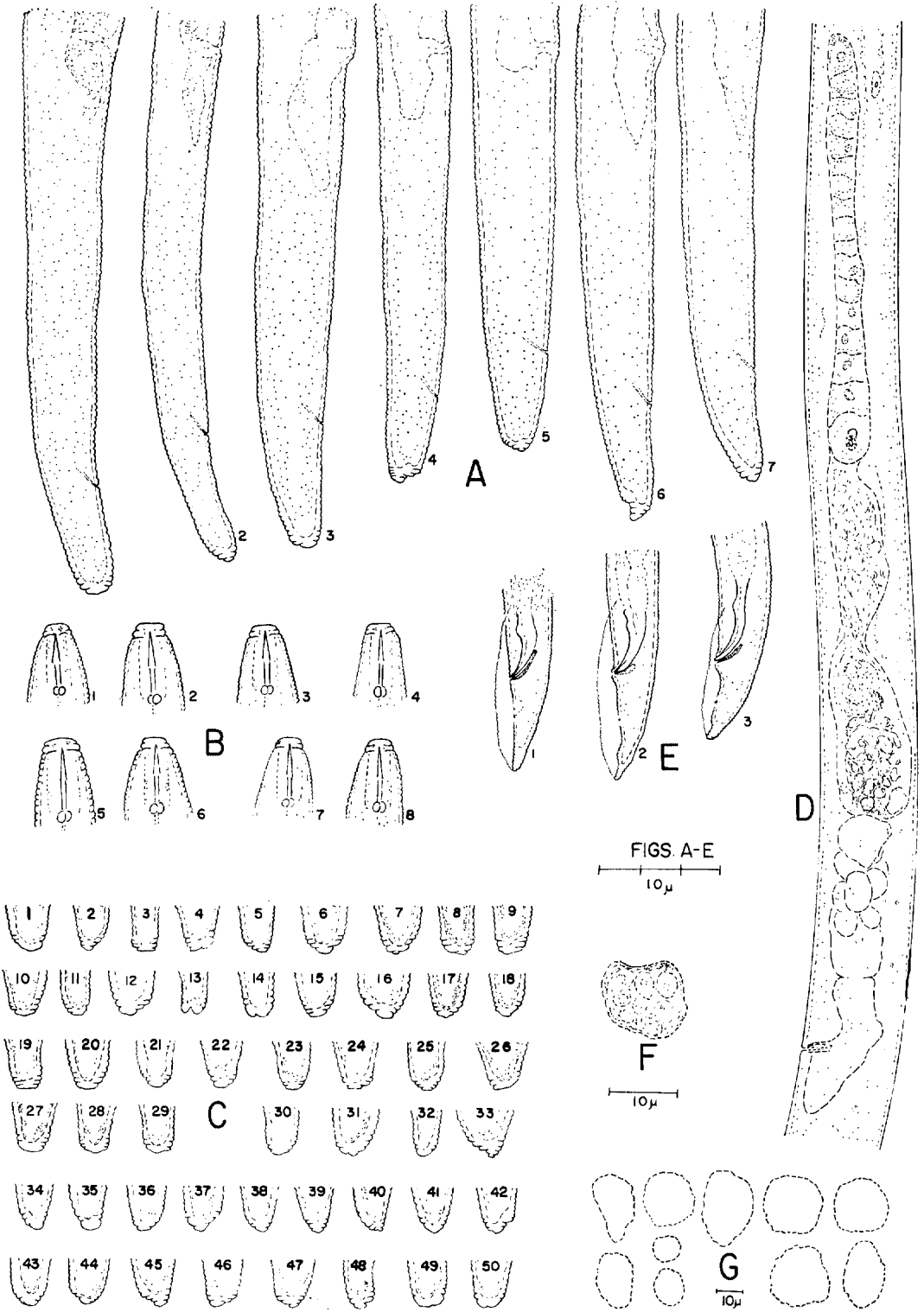


PLATE IV (A-G). *Pratylenchus coffeae* from Lake Alfred, Florida, cultured on *Citrus aurantium* for 44 months in the greenhouse. Drawings are of adult females, except for Fig. E. A) Vulva-anal regions. B) Labial regions. C) Tail termini. D) Female gonad. E) Male tails. F) Spermatheca. G) Variation in spermatheca shapes.

uterus and tricolumella, distinct spermatheca, oviduct, and ovary (Pl. IV-D). Spermathecae are irregularly spherical to oval (Pl. IV-F, G). Males are common and possess prominent caudal alae which completely envelop the tail (Pl. IV-E).

DISCUSSION

On the basis of the investigations conducted and using a low standard deviation (SD) or coefficient of variation (CV) as qualifying criteria, stylet length appeared to be the only statistic obtained that could be regarded as invariable and thus a sound characteristic of the species. If one discounts the importance of SD and CV and, despite the latitude of the ranges shown, accepts arithmetical mean as being equally important because of the large number of specimens studied, then all of the morphological measurements obtained were distinctive. The tail data in Table 2 had a CV of 12.78-15.00% and a range of 20-40 μm . Yet the arithmetical means for measurement of 54, 57, and 85 females were respectively 30.0, 29.2, and 30.2 μm . This difference indicates that the degree of variance on each side of the mean exhibited within populations was relatively constant. The statistics of SD or CV assume most importance when only one to a few specimens are to be identified.

Both *Pratylenchus brachyurus* and *P. coffeae* populations exhibited tails which showed considerable variation in shape and length. The *P. brachyurus* tail terminus has been reported as being rounded and smooth (9) or bluntly rounded (1), which certainly is true for the majority of specimens examined in this study. *Pratylenchus coffeae* exhibited considerably more variation in tail terminus, the extreme of which is the truncate type of tail, usually associated with *P. brachyurus* (Pl. III-C, Figs. 1, 2) to a subacute conical tail quite atypical for the species (Pl. III-C, Fig. 18).

Labial region shapes likewise were noticeably variable within and between the two species. Shapes which could be considered "low," "angular," "flattened," and "rounded" were found within all populations examined. The large variation observed in these populations originating from single females is similar to that ob-

served by Tarté and Mai (14) in *P. penetrans*.

Román and Hirschmann (9) previously had conducted the most thorough study of the two species investigated in this study. They measured stylets on 50 specimens of *P. brachyurus* and obtained an arithmetic mean of 18.4 μm ; we measured stylets in 216 specimens and obtained a mean of 17.9 μm . They found that stylets on 25 specimens of *P. coffeae* had a mean of 15.5 μm ; we measured 196 specimens and found the stylet had a mean length of 15.7 μm . The ranges for the values obtained on tail length, vulva-anus distance, and posterior uterine sac length given in Tables 1 and 2 were similar to, though more extensive than, those presented by Román and Hirschmann (9). In essence, the two investigations effectively complemented each other.

In the absence of males or discernible spermathecae in females of *P. coffeae* that may be mixed with *P. brachyurus*, the species can be separated through morphometric data including stylet length, postvulval uterine sac length, and vulva-anus distance. There was a consistent 2- μm difference in mean stylet length (Tables 1, 2) between *P. brachyurus* (17.8 and 18.0 μm) and *P. coffeae* (15.5, 15.6, and 15.9 μm). The mean posterior uterine sac length was shorter for *P. brachyurus* (13.7 and 15.9 μm) than for *P. coffeae* (22.7, 24.0, and 24.7 μm). The mean distance between the vulva and anus was much shorter for *P. brachyurus* than for *P. coffeae* (56.0 and 57.0 μm ; 85.4, 87.8, and 89.6 μm). No differences were detected in tail lengths.

LITERATURE CITED

1. BERG, E. VAN DEN. 1971. The root-lesion nematodes of South Africa (Genus *Pratylenchus* Family Hoplolaimidae). Dept. Agr. Tech. Svs. Rep. So. Africa Tech. Comm. 99, 13 pp.
2. BROOKS, T. L., and V. G. PERRY. 1967. Pathogenicity of *Pratylenchus brachyurus* to citrus. Plant Dis. Reprtr. 51:569-573.
3. CHITWOOD, M. B. 1957. Intraspecific variation in parasitic nematodes. Syst. Zool. 6:19-23.
4. CORBETT, D. C. M. 1969. *Pratylenchus pinguicaudatus* n. sp. (*Pratylenchinae*: Nematoda) with a key to the genus *Pratylenchus*. Nematologica 15:550-556.
5. GOODEY, J. B. 1952. The influence of the host on the dimensions of the plant parasitic

- nematode, *Ditylenchus destructor*. *Ann. Appl. Biol.* 39:468-474.
6. LOOF, P. A. A. 1960. Taxonomic studies on the genus *Pratylenchus* (Nematoda). *Tijdschr. Plantenziekten.* 66:29-90.
 7. O'BANNON, J. H., A. C. TARJAN, and F. W. BISTLINE. 1974. Control of *Pratylenchus brachyurus* on citrus and tree response to chemical treatment. *Proc. Soil Crop Sci. Fla.* 33:65-67.
 8. O'BANNON, J. H., and A. T. TOMERLIN. 1973. Citrus tree decline caused by *Pratylenchus coffeae*. *J. Nematol.* 5:311-316.
 9. ROMÁN, J., and H. HIRSCHMANN. 1969. Morphology and morphometrics of six species of *Pratylenchus*. *J. Nematol.* 1:363-386.
 10. SHER, S. A., and M. W. ALLEN. 1953. Revision of the genus *Pratylenchus* (Nematoda: Tylenchidae). *Univ. Calif. Publ. Zool.* 57: 441-470.
 11. STURHAN, D. 1970. Intraspezifische Variabilität und Mutationen bei Nematoden. *Zesz. Prob. Post. Nauk Roln.* 92 (Proc. IX Int. Nem. Symp. (Warsaw, 1967)):209-220.
 12. TARJAN, A. C. 1967. Variability of diagnostic characters among some plant and soil nematodes. [Polish text]. *Prace Nauk. Inst. Ochrony Roslin* 9(1):105-116.
 13. TARJAN, A. C., and J. H. O'BANNON. 1969. Observations on meadow nematodes (*Pratylenchus* spp.) and their relation to declines of citrus in Florida. *Plant Dis. Rep.* 53(9):683-686.
 14. TARTÉ, R., and W. F. MAI. 1976. Morphological variation in *Pratylenchus penetrans*. *J. Nematol.* 8:185-195.
 15. THORNE, G., and M. W. ALLEN. 1959. Variation in nematodes. pgs. 412-418. *In* Holton, C. S. ed., *Plant pathology problems and progress, 1908-1958.* Univ. Wisconsin Press, 588 pp.