Three New Species of Trichodoridae (Nematoda: Diphtherophorina) with Observations on the Vulva in Paratrichodorus

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Abstract: Trichodorus intermedius n. sp., *Trichodorus dilatatus* n. sp., and *Paratrichodorus (Atlantadorus) grandis* n. sp. are described from soil around native plants of Southern California. The females of the three subgenera of *Paratrichodorus* are further characterized on the basis of the vulva, which is pore-like in *Atlantadorus,* a longitudinal slit in *Paratrichodorus,* and a transverse slit in *Nanidorus. Key Words:* Taxonomy, morphology, *Trichodorus intermedius, Trichodorus dilatatus, Paratrichodorus (Atlantadorus) grandis,* supplementary papillae.

In 1913 Cobb described the genus *Trichodorus* on the basis of *T. obtusus* collected around roots of grasses at Arlington, Virginia (3). Interest in the genus was increased by the discovery by Christie and Perry of the pathogenicity of a species of *Trichodorus* to beets and corn. This species was later described as *T. christei* by Allen in a review of the genus that for the first time clearly identified the morphological characters which could be used for species identification, laying a foundation for the taxonomic study of the genus (1). Siddiqi discussed the systematics and morphology of the genus, proposed the genus *Paratrichodorus,* and erected subgenera (8). He also constructed a key to the species of *Trichodorus* on the basis of male characters. Because males are rare in some species and unknown in others, we undertook a search for characters that would facilitate identification of individual females.

During a study of trichodorids in the nematode collection at the University of California, Riverside, three new species associated with native plants of Southern California were found and are described herein. Additional species were included in

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a study of variation of the morphology of the vulva.

METHODS AND MATERIALS

Specimens used in the description of new species were fixed in formalin, transferred to dilute glycerin, and allowed to dehydrate slowly. Processed specimens were mounted on permanent slides either in dehydrated glycerin or in glycerin jelly (dorsal-ventral mounts). Type materials were used for all species. Measurements were of permanent mounts by means of camera lucida and are expressed as the mean ± 1 standard deviation followed by the range.

Trichodorus intermedius n. sp.

Fig. 1

Measurements: Paratypes 11 ♂♂: L = 0.83 ± 0.07 (0.72-0.95) mm; a = 21.0 ± 2.1 (18-24); b = 5.0 ± 0.59 (3.9-5.9); c = 68 ± 10.2 (59-91); T = 66.8 ± 3.6 (63-72.8); onchiostyle = 69.0 ± 2.5 (62-71.5) μm; spicules = 45.0 ± 3.0 (42-50) μm; gubernaculum = 20.0 ± 1.3 (18-22) μm.

Paratypes 23 ♀♀: L = 0.85 ± 0.1 (0.68-1.13) mm; a = 20.0 ± 1.1 (17.2-22); b = 5.0 ± 0.5 (4.3-6.4); anus subterminal; V = 57.0 ± 2.7 (53-64); onchiostyle = 69.0 ± 3.4 (62-75.2) μm.

Holotype ♂: L = 0.72 mm; a = 20.0; b = 4.6; c = 91; T = 63. Body straight anteriorly, strongly curved ventrad at posterior end. Cuticle not abnormally swollen, subcuticle finely striated. Lip region prominent, somewhat conical. Sixteen papillae arranged in an inner circle of 6 around oral aperture and an outer circle of 10 composed of 2 pairs of subdorsal papillae, 2 pairs of subventral papillae, and 2 single lateral papillae (7). Amphid aperture near level of outer circle of papillae. One ventral cervical papilla anterior to excretory pore, 100 μm from anterior end. Excretory pore 122 μm from anterior end. Onchiostyle 62 μm, tip 30 μm. Collar or "guide ring" 27 μm from anterior end. Nerve ring midway between onchiostyle base and ventral cervical papilla. Esophagus and esophageal gland nuclei position typical of genus. Testis single, outstretched, with large round sperm. Spicules 42 μm, arcuate, slightly cephalated, distal one-third slightly expanded. Proximal two-thirds of retracted spicules appear serrated; when extended, bristle-like structures occur along proximal two-thirds of spicule. Gubernaculum linear, 19 μm, with ventral end expanded. Protractor muscles prominent. Three ventromedian supplementary papillae, 72, 24, and 10 μm from anus. Paired subventral postcloacal papillae. Tail rounded, with slight thickening of cuticle around almost terminal caudal pores.

Allotype ♀: L = 0.73 mm; a = 18.0; b = 4.9; V = 55; onchiostyle = 64 μm. Body straight to slightly curved ventrally. Cuticle, lip region, and esophagus as in male. Excretory pore 110 (78.4-138 in paratypes) μm from anterior end. Gonads paired, reflexed. Spermatheca (present or absent in paratypes) filled with round sperm. Vagina more than one-half vulvar-body-width in length, with cuticularization and musculature prominent. Vaginal cuticularization appearing as two triangles with long bases in lateral view oblique to body axis, with tips characteristically bent toward interior of vagina (Fig. 4-D). Vulva a large transverse slit. Paired lateral body pores about 20 μm posterior to vulva. Tail rounded, with terminal caudal pores. Anus subterminal.

Holotype ♂: Collected by A. Bell on April 22, 1967. Department of Nematology Nematode Collection, University of California, Riverside. Catalog No. 28.

Allotype ♀: Same data as holotype. Catalog No. 29.

Type locality and habitat: Soil around roots of live oak (*Quercus virginiana* Mill) at Oak Grove Campground, San Diego Co., California. Additional specimens have been found associated with the following hosts in Southern California: grass and willow (*Salix* sp.), Dripping Springs near Temecula; *Chenopodium* sp. and *Ambrosia* sp., Santa Ana River, Riverside; *Pinus* sp., Mt. Palomar; *Salvia leucophylla,* south of Camarillo; *Quercus* sp., Fallbrook; *Quercus* sp. and grass, Live Oak Park; also in redwood soil at Big Sur.

T. intermedius was sometimes found mixed with *Paratrichodorus (P.) allius, Paratrichodorus (N.) minor,* or *Trichodorus aequalis.*

Paratypes: 15 ♂♂, 30 ♀♀, 20 jj, same

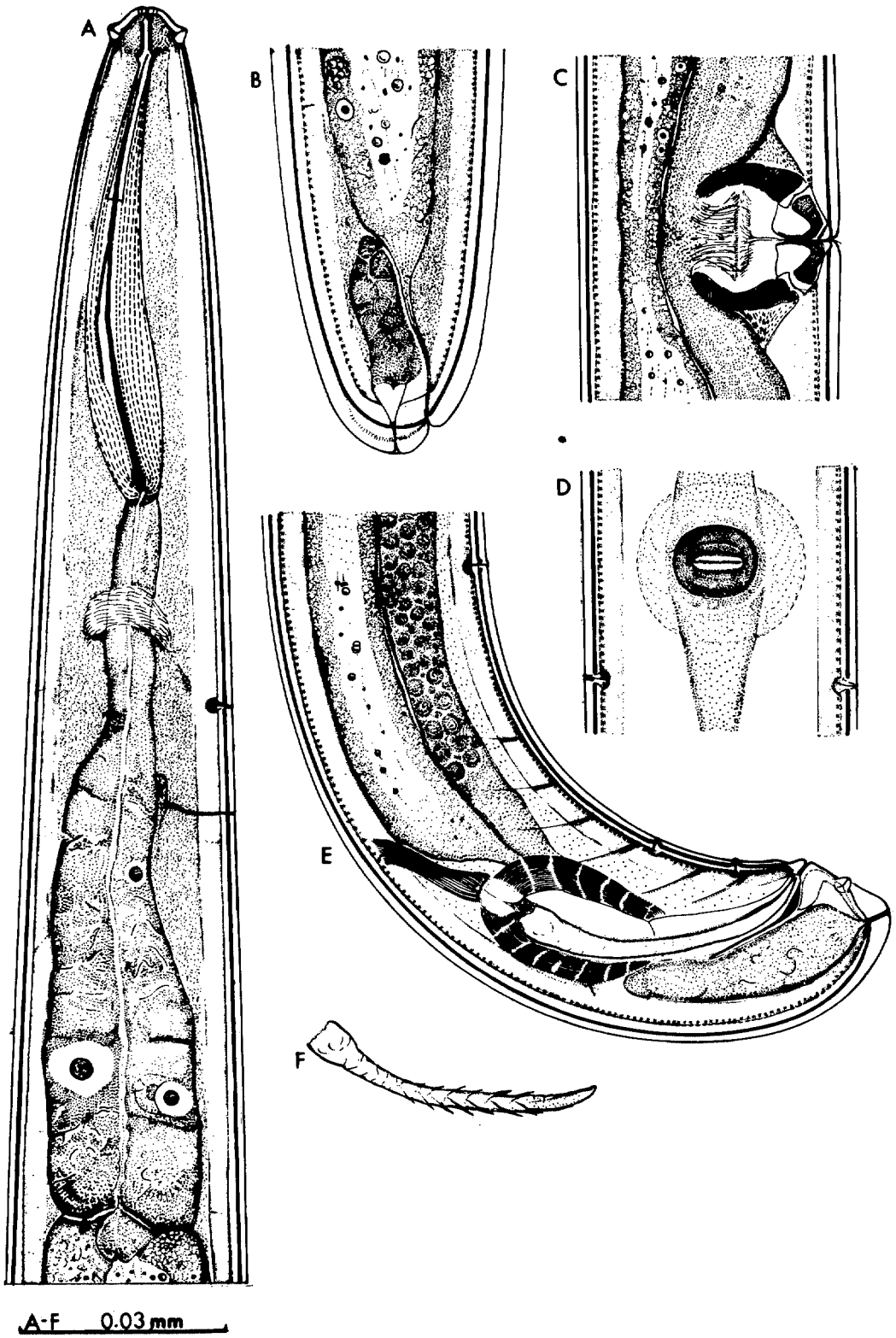


FIG. 1-(A-F). *Trichodorus intermedius* n. sp.: *A)* male anterior region (holotype); *B)* female posterior region (paratype); *C)* vagina and related structures in lateral view; *D)* vulva in ventral view; *E)* male posterior region (holotype); *F)* male spicule (paratype).

data as holotype, distributed as follows: 6 ♂♂, 15 ♀♀, 13 jj, Department of Nematology, University of California, Riverside; 2 ♂♂, 3 ♀♀, Nematode Collection, University of California, Davis; 2 ♂♂, 3 ♀♀, USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland; 1 ♂, 2 ♀♀, Rothamsted Experimental Station, Harpenden, England; 2 ♂♂, 4 ♀♀, 7 jj, Commonwealth Institute of Helminthology, St. Albans, England; 2 ♂♂, 3 ♀♀, Nematology Department, Landbouwhogeschool, Wageningen, The Netherlands.

Diagnosis: By having males with only one ventral cervical papilla, *T. intermedius* is similar to *T. californicus* Allen, 1957 (1) and *T. cottieri* Clark, 1963 (2), but differs in location of ventromedian supplementary papillae and spicule shape. Females of *T. intermedius* can be separated from *T. californicus, T. cottieri,* and *T. obscurus* Allen, 1957 (1), only by shape of vaginal cuticularization (Fig. 4-D).

Trichodorus dilatatus n. sp.

Fig. 2

Measurements: 10 ♂♂ *paratypes:* L = 0.87 ± 0.1 (0.72-1.03) mm; a = 16 ± 1.8 (14-19); b = 5.1 ± 0.4 (4.5-6.1); c = 62.0 ± 10.0 (48-79); T = 59.0 ± 7.0 (50-70); onchiostyle = 53.0 ± 2.3 (63-69) μm; spicules = 53.0 ± 2.3 (48-55) μm; gubernaculum = 12.0 ± 1.1 (11-14) μm.

Paratypes 11 ♀♀: L = 0.87 ± 0.1 (0.72-1.03) mm; a = 16 ± 1.3 (13-17.7); b = 5.3 ± 0.4 (5.6-6.1); anus subterminal; V = 58 ± 2.3 (54-61.7); onchiostyle = 65.0 ± 2.9 (60.5-69) μm.

Holotype ♂: L = 0.90 mm; a = 18.0; b = 4.9; c = 73.0; T = 69.0. Body straight anteriorly, curved ventrally at posterior end. Cuticle not swollen, subcuticle finely striated. Lip region hemispherical to slightly conical, almost continuous with body outline. Amphid aperture at level of outer circle of papillae, immediately posterior to single lateral papilla (7). Right lateral body pore posterior to nerve ring, left lateral body pore slightly anterior to ventral cervical papilla level, 122 μm from anterior end. Excretory pore posterior to ventral cervical papilla, 132 μm from anterior end. Duct of excretory pore perpendicular to body axis, turns anteriorly to disappear near esophagus. Onchiostyle 65 μm, tip 30 μm. Collar or "guide ring" 29 μm from anterior end. Nerve ring 18 μm posterior to onchiostyle base, midway between onchiostyle base and ventral cervical papilla. Esophagus typical of genus; anterior subventral gland nuclei anterior to excretory pore; posterior subventral gland nuclei 20 μm anterior to the esophagointestinal junction; dorsal esophageal gland nucleus larger than nuclei of subventral glands, and located near same level as posterior subventral gland nuclei. Gonad single, outstretched, containing large round sperm. Spicules curved ventrally, expanded at proximal end, then uniformly tapered to distal end. Protruded spicule with fine bristles and ventroterminal opening, 49 μm long. Gubernaculum 19 μm, simple, ventral end expanded, forming a small keel. Ventromedian supplements located 90, 48, and 13 μm anterior to anus. Paired subventral postcloacal papillae. Tail bluntly rounded with almost terminal caudal pores.

Allotype ♀: L = 0.99 mm; a = 17.0; b = 5.7; V = 59; onchiostyle = 68.0 μm. Body almost straight when relaxed. Lip region, cuticle, and esophagus as in male. Excretory pore 140 (94-143 in paratypes) μm from anterior end, near level of anterior subventral esophageal gland nuclei. Gonads typical of genus; spermatheca (present or absent in paratypes) filled with round sperm. Vagina prominent, half or more vulvar-body-width in length; vaginal cuticularization as illustrated (Fig. 4-A). Vulva a large transverse slit. Paired lateral body pores within one body-width posterior to vulva. Tail hemispheric, with subterminal anus and terminal caudal pores.

Holotype ♂: Catalog No. 30. Nematode Collection, Department of Nematology, University of California, Riverside. Collected by O. F. Clarke on June 4, 1975.

Allotype ♀: Same data as holotype. Catalog No. 31.

Paratypes: 10 ♂♂, 11 ♀♀, 5 jj, same data as holotype distributed as follows: 5 ♂♂, 6 ♀♀, 5 jj, Nematode Collection, University of California, Riverside; 1 ♂, 1 ♀, Nematode Collection at University of California, Davis; 1 ♂, 1 ♀, Commonwealth Institute of Helminthology, St. Albans, England; 1 ♂, 1 ♀, Rothamsted Experimental Station, Harpenden, Eng-

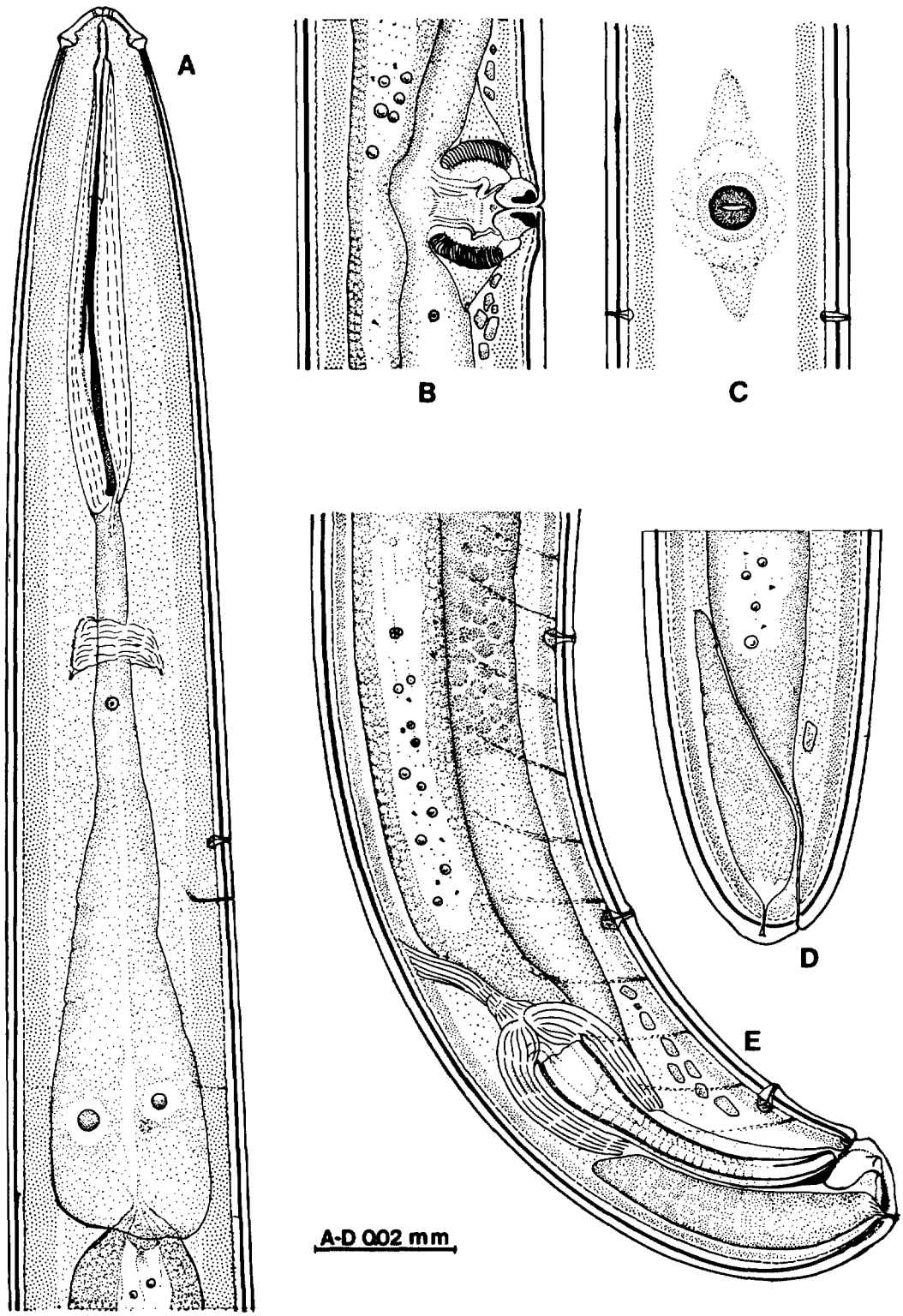


FIG. 2-(A-E). *Trichodorus dilatatus* n. sp.: *A)* male anterior region (holotype); *B)* vagina and related structures in lateral view (paratype); *C)* vulva in ventral view; *D)* female posterior region; *E)* male posterior region (holotype).

land; 1 ♂, 1 ♀, Nematology Department Landbouwhogeschool, Wageningen, The Netherlands; and 1 ♂, 1 ♀, USDA Nematode Collection, Nematology Investigations, Beltsville, Maryland.

Type locality and habitat: Soil around roots of cottonwood (*Populus* sp.), Deep Creek Camp, Hesperia, California. Additional specimens of *T. dilatatus* have been collected from soil around manzanita (*Arctostaphylos* sp.) in San Jacinto Mountains, near Banning, California.

Diagnosis: Males of this species strongly resemble *T. californicus* and *T. cottieri*. *T. dilatatus* differs from *T. cottieri* by the uniform taper of the distal end of spicules and lateral body pores anterior to excretory pore. *T. dilatatus* differs from *T. cottieri* and *T. californicus* by location of supplementary papillae.

Females of *T. dilatatus* are morphologically similar to *T. elegans* Allen, 1957, *T. cottieri*, *T. californicus*, *T. obscurus*, and *T. intermedius*. The only character that can be used to separate females of these species is the shape and size of vaginal cuticularization (Fig. 4).

Paratrichodorus (Atlantadorus) grandis n. sp.

Fig. 3

Measurements: Paratypes 11 ♂♂: L = 0.90 ± 0.08 (0.79-1.04) mm; a = 25.0 ± 1.3 (22.6-27); b = 5.9 ± 0.78 (5.0-7.4); c = 64.0 ± 8.6 (50-80); T = 56.0 ± 2.54 (51.9-60.3); onchiostyle = 70.0 ± 2.2 (64-72) μm; spicules = 40.0 ± 1.4 (39-43) μm; gubernaculum = 14.0 ± 1.41 (12-16) μm.

Paratypes 11 ♀♀: L = 0.96 ± 0.04 (0.90-1.04) mm; a = 26.0 ± 2.47 (23-30); b = 6.1 ± 0.52 (5.1-7.0); anus subterminal; V = 59.0 ± 2.12 (56.4-64); onchiostyle = 68.0 ± 2.2 (65-72) μm.

Holotype ♂: L = 0.92 mm; a = 24.0; b = 6.3; c = 64.0; T = 57.8; onchiostyle = 70 μm; spicules = 42 μm; gubernaculum = 13 μm. Body long and slender, almost straight when relaxed by gentle heat. Cuticle swollen. Lip region hemispherical. Labial papillae inconspicuous. One ventral cervical pore at level of onchiostyle base, 68 μm from anterior end. Paired lateral body pores at level of ventral cervical pore. Excretory pore 113 μm from anterior end. Nerve ring at beginning of esophagus, immediately posterior to pharyngo-esophageal junction. Esophagus with several flexures. Nuclei of posterior subventral glands anterior to esophago-intestinal junction, close to large dorsal gland nucleus. Intestine usually extending slightly dorsolaterally over esophagus base. Testis typical of genus. Ventromedian supplementary papillae at 93, 30, and 15 μm from anus. Spicules slightly curved ventrally at distal end, with fine transverse striations. Gubernaculum inconspicuous, slightly expanded at ventral end. Caudal alae present, extending laterally from slightly anterior to middle supplement to slightly anterior to postcloacal papillae. One pair of sublateral postcloacal papillae. One bilobed flap, easily seen in ventral view, partially covering cloaca. Tail straight and slightly conical, with subterminal caudal pores.

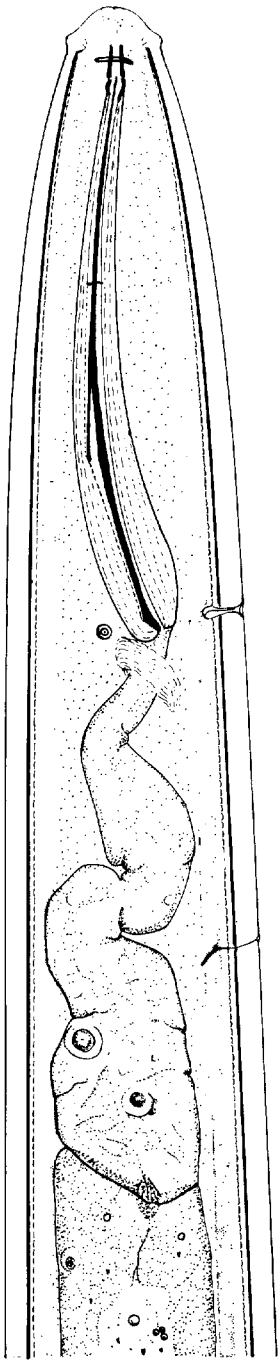
Allotype ♀: L = 1.04 mm; a = 25; b = 6.3; V = 59; onchiostyle = 70.0 μm. Body long and slender, sometimes curved ventrally. Cuticle, lip region, and esophagus as in male. Excretory pore 123 μm from anterior end (102-127 in paratypes). Lateral advulvar body pores present. Vagina conspicuous, about one-fourth vulvar-body-width in length; vaginal cuticularization inconspicuous, appearing as two small triangles, in lateral view, with short bases almost parallel to the body axis. Vulva pore-like, with radial folds. Spermatheca not well defined; something resembling sperm contained along both uteri (Fig. 3-C). Tail slightly conical, with subterminal anus and almost terminal caudal pores.

Holotype ♂: Collected by O. F. Clarke on March 7, 1967. Catalog No. 32. Nematode Collection, Department of Nematology, University of California, Riverside.

Allotype ♀: Same data as holotype. Catalog No. 33.

Type Locality and Habitat: Soil around manzanita (*Arctostaphylos* sp.), Beau Vista Hills Rd., San Jacinto Mountains, near Banning, California.

Paratypes: 21 ♂♂, 20 ♀♀, 24 jj, same data as holotype, distributed as follows: 11 ♂♂, 14 ♀♀, 17 jj, Department of Nematology, University of California, Riverside; 2 ♂♂, 2 ♀♀, 3 jj, Department of Nematology, University of California, Davis; 2 ♂♂, 1 ♀, 2 jj, USDA Nematode Collec-



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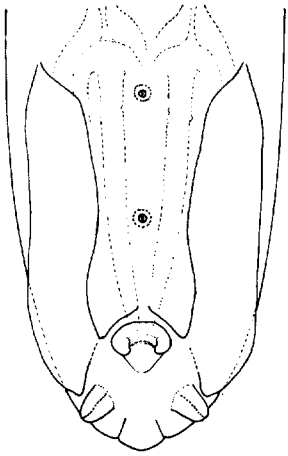

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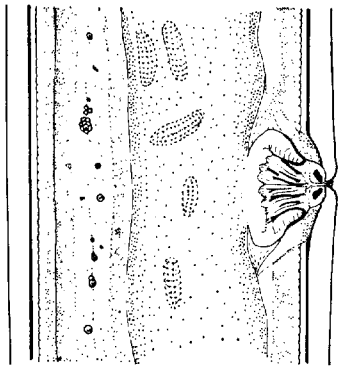

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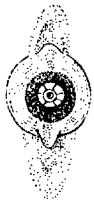

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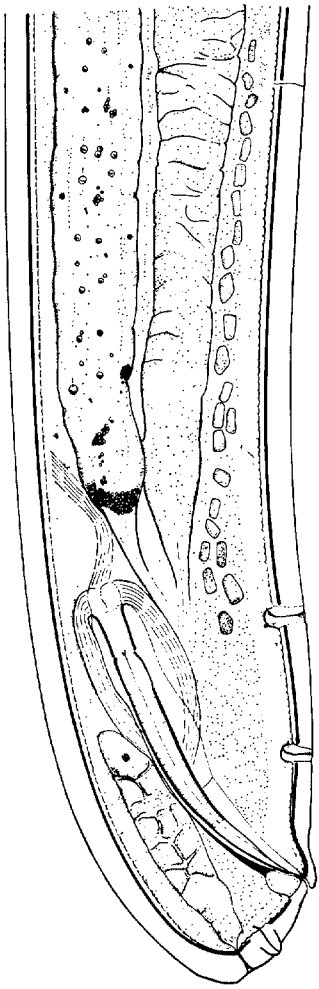

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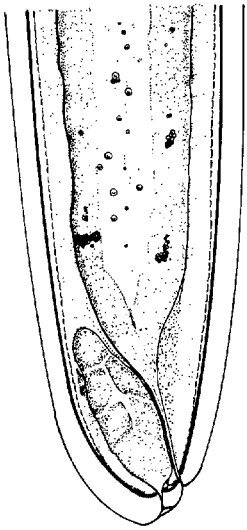

F

A-F 0.03 mm

tion, Nematology Investigations, Beltsville, Maryland; 4 ♂♂, 2 ♀♀, 1 j, Commonwealth Institute of Helminthology, St. Albans, England; 2 ♂♂, 1 ♀, 1 j, Nematology Department, Landbouwhogeschool, Wageningen, The Netherlands.

Diagnosis: Paratrichodorus (A.) grandis closely resembles *P. (A.) atlanticus* (Allen) Siddiqi 1973 (8), but differs in the male by spicule length, position of ventral cervical papilla, and presence of only one pair of submedian cloacal papillae. Females differ in vulva position and by absence of ventrosubmedian pores near vulva.

Discussion: Lateral body pores of *P. (A.) grandis* vary in number and position but always occur posterior to vulva, usually two on right side and one on left side. One paratype had three lateral body pores close together on right side.

The vulva of *P. (A.) atlanticus* was described as a longitudinal slit (1), but a paratype specimen had a pore-like vulva as described for other species of the subgenus.

The species in the subgenus *Atlantadorus* can be separated according to the following keys modified from Loof (4).

KEY TO MALES

1. Onchiostyle 64 μm or more 2
   Onchiostyle 60 μm or less 3
2. Spicule 79-87 μm ..... *P. (A.) atlanticus*
   Spicule 39-43 μm ..... *P. (A.) grandis*
3. Two supplements, both within spicular range, Spicule length 36-39 μm ..... *P. (A.) porosus*
   Three supplements, anterior supplement well anterior to head of retracted spicules; spicules longer than 40 μm 4
4. Tail round, spicule outline irregular, distance from middle supplement to anus 38-64% of spicule length ..... *P. (A.) anemones*
   Tail trapezoid, spicule outline regular, distance from middle supplement to anus 75-94% of spicule length ..... *P. (A.) pachydermus*

KEY TO FEMALES

1. Subventral or ventromedian pores near vulva present 2
   Subventral and ventromedian pores near vulva absent 3
2. Onchiostyle 64 μm or more, one pair of subventral pores posterior to vulva *P. (A.) atlanticus*
   Onchiostyle 50 μm or less, two ventral pores anterior to vulva and two posterior to vulva ..... *P. (A.) porosus*
3. Onchiostyle 64 μm or more ..... *P. (A.) grandis*
   Onchiostyle less than 60 μm 4
4. Sperm contained in spermatheca, 0-2 lateral pores on each side of body posterior to vulva ..... *P. (A.) anemones*
   Sperm distributed throughout uteri, 2-5 lateral pores on each side of body posterior to vulva ..... *P. (A.) pachydermus*

DISCUSSION

The species of *Trichodorus* studied here share several morphological character states. This close similarity of morphological characters is evidenced by the fact that individual females can be distinguished only by shape and size of vaginal cuticularization.

Siddiqi (8) erected the subgenus *Atlantadorus* for species of *Trichodorus* with intestinal diverticulum and similar placement of esophageal gland nuclei. The males of *Atlantadorus* could be identified by the presence of lateral body pores. Loof (4) agreed with Siddiqi in defining females of *Atlantadorus* by the absence of lateral body pores within one body-width from vulva and presence of subterminal caudal pores. Major importance, however, was given to male characters.

All species of *Atlantadorus* except *P. (A.) atlanticus* have been described as having a pore-like vulva. The one paratype female of *P. (A.) atlanticus* available showed the vulva to be pore-like also, rather than a longitudinal slit as in the original description.

All species of the subgenus *Paratrichodorus* (8) have been described with the vulva as a longitudinal slit except for *P. (P.) alleni* (Andrassy) Siddiqi 1973, in which females are unknown, and *P. (P.) rhodesiensis* (Siddiqi and Brown) Siddiqi 1973, in which the vulva was said to be transverse. However, one paratype specimen of *P. (P.) rhodesiensis,* kindly supplied by M. R. Siddiqi, showed the vulva to be a longitudinal slit.

All species of *Nanidorus* (6, 8) except *P. (N.) obesus* Razjivin & Penton 1975 (5) were

FIG. 3-(A-F). *Paratrichodorus (Atlantadorus) grandis* n. sp. *A)* male anterior region (holotype); *B)* male posterior region in ventral view (paratype); *C)* vagina and related structures in lateral view; *D)* vulva ventral view; *E)* male posterior region (holotype); *F)* female posterior region.

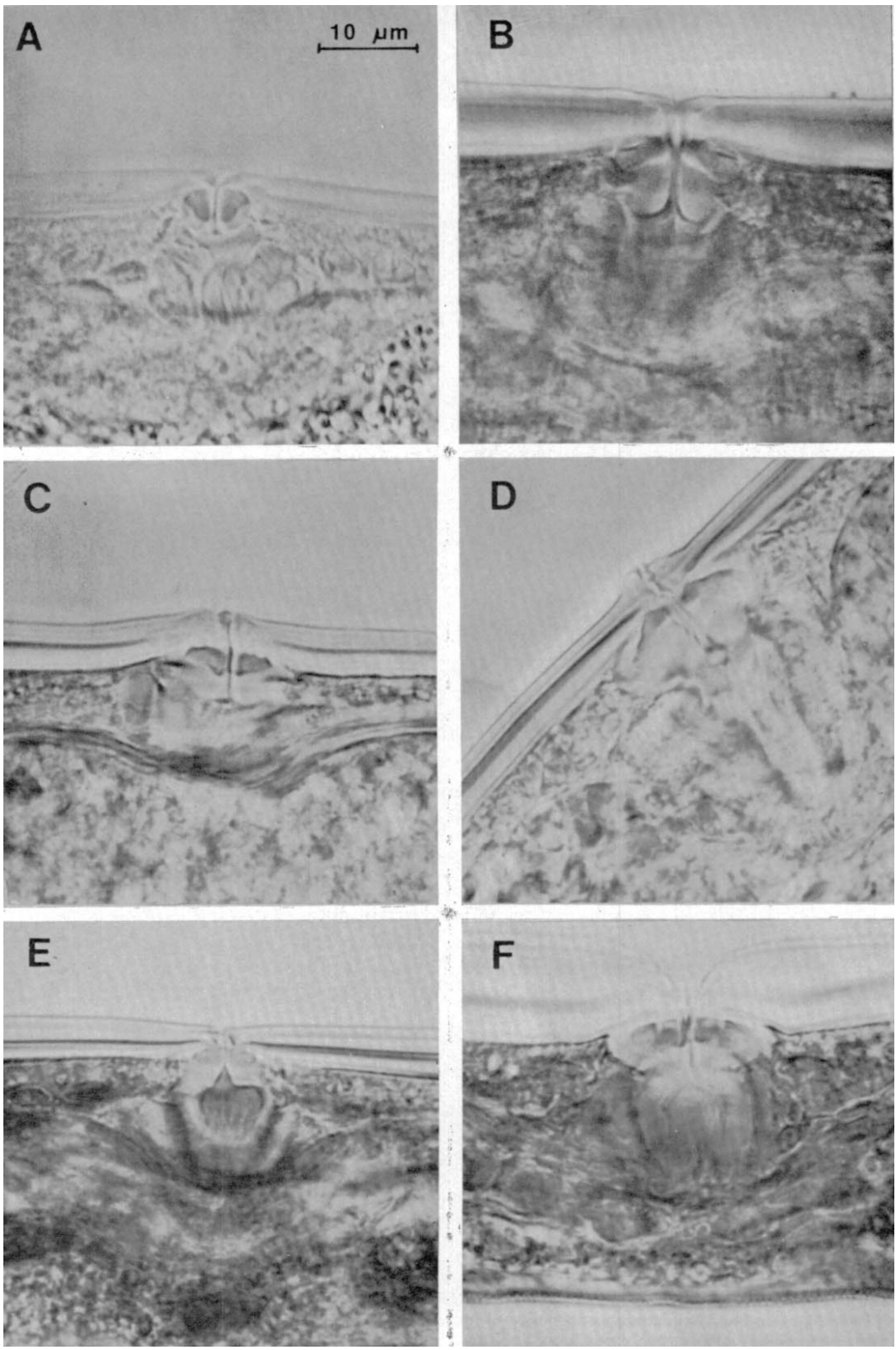

FIG. 4-(A-F). Cuticularization of the vagina. *A) Trichodorus dilatatus* n. sp.; *B) T. elegans; C) T. californicus; D) T. intermedius* n. sp.; *E) T. obscurus; F) T. cottieri.* All photomicrographs of paratypes.

examined and found to have the vulva as a short transverse slit.

Females of the subgenera of *Paratrichodorus* can be characterized on the basis of vulva shape as follows: *Nanidorus* with a short transverse slit, *Paratrichodorus* with a short longitudinal slit, and *Atlantadorus* with a pore-like opening.

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