

***Paraiontonchium muscadomesticae* n. sp.**
(Tylenchida: Iotonchiidae) a Parasite of the House Fly
(*Musca domestica*) in Brazil and a Key to Species of the
Genus *Paraiontonchium*¹

R. R. COLER² AND K. B. NGUYEN³

Abstract: *Paraiontonchium muscadomesticae* n. sp., a parasite of the house fly, *Musca domestica* L., is described and illustrated from material collected in Brazil. The life cycle of *P. muscadomesticae* is similar to that of *P. autumnale* (Nickle), consisting of alternating gamogenetic and parthenogenetic generations. *Paraiontonchium muscadomesticae* n. sp. can be distinguished from *P. nicholasi* Slobodyanyuk, *P. autumnale* (Nickle) Slobodyanyuk, and *P. crassirostris* (Yatham & Rao) Siddiqi by the shorter body length of young heterosexual females, 652 μ m (530–709) for *P. muscadomesticae* compared to 750 μ m or more (801–1,050) for the others. *Paraiontonchium muscadomesticae* is close to *P. nicholasi* but differs from it by V ratio and spicule length (V = 80–84; spicule = 16–21 μ m in *P. muscadomesticae* compared to V = 73–78; spicule = 25–35 μ m in *P. nicholasi*). *Paraiontonchium muscadomesticae* and *P. nicholasi* differ from all species of this genus by the absence of a bursa on males of these two species.

Key words: biological control, Brazil, Diptera: Muscidae, entomogenous nematode, life cycle, light microscopy, morphology, *Musca domestica*, nematode, *Paraiontonchium muscadomesticae*, parasite, scanning electron microscopy, taxonomy, Tylenchida: Iotonchiidae.

More than a decade ago, Coler et al. (1) reported on a parasitic nematode of the genus *Heterotylenchus* Bovien (Tylenchida: Sphaerulariidae) parasitizing house flies (Diptera: Muscidae: *Musca domestica* L.) in northeastern Brazil. Recently, the nematode has been reisolated and transported to the University of Florida for further studies.

The genus *Heterotylenchus* was revised by Slobodyanyuk (8), who erected a new genus, *Paraiontonchium*, into which he transferred *Heterotylenchus autumnalis* Nickle (4), *H. crassirostris* Yatham & Rao (10), and *H. xanthomelas* Reddy & Rao (5). To date all members of this genus—*P. autumnale*, *P. nicholasi*, *P. osiris*, *P. crassirostris*, and *P. xanthomelas*—are parasites of *Musca* L. (6–9). Examination of additional live material collected from Brazil and reared in Gainesville, Florida, in the quarantine facility of the Florida Department of Agriculture and Consumer Services has shown

that the nematode found parasitizing house flies in Brazil is a new species belonging to the genus *Paraiontonchium*. This new species is herein described as *Paraiontonchium muscadomesticae*.

MATERIALS AND METHODS

Collection of type material: Nematode specimens were obtained from infected house flies collected near the municipal outdoor market of Serra Talhada, Pernambuco. Wild flies were caged and transported to a laboratory situated at the “Centro de Treinamento e Pesquisa em Pequena Irrigação,” an experimental research facility funded jointly by various federal organizations, including the Universidade Federal Rural de Pernambuco and the Centro Nacional de Pesquisas. Nematodes dissected from parasitized flies were used to infect colonies of house fly larvae. Parasitized house fly pupae were transported to the United States, and after reaching the proper stage of development, the nematodes were dissected from the Brazilian house flies and transferred to a colony of flies established in Florida. All parasitized hosts were maintained in the quarantine facility of the Biological Control Laboratory of the Division of Plant Industry, Gainesville, Florida.

Received for publication 2 February 1994.

¹ Florida Agricultural Experiment Station Journal Series No. R-03636.

² Formerly at the University of Florida; currently with Coler and Colantonio, Inc., 2405 NW 66th Court, Gainesville, FL 32606

³ Entomology and Nematology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0620

Specimen preparation: The four adult forms of *Paraiontonchium muscadomesticae* n. sp. are parasitic and include the male, the young heterosexual female (the infective stage), the mature heterosexual female (larger in size), and parthenogenetic female. Heterosexual males and females were obtained 24–48 hours after they emerged from parasitized flies immobilized on a thin layer of 1% agar in a 60 × 15 mm petri dish. Mature heterosexual and parthenogenetic females were dissected directly from adult flies 5–20 days old.

Light microscopy: Males and young heterosexual females were examined live by sealing them in physiological saline on a glass slide under a supported coverslip. Live specimens of mature heterosexual and parthenogenetic females could not be observed in this way because the unusually sticky cuticle adhered to the transfer needle, microscope slide, and coverglass, resulting in disfigured specimens. Sticky specimens were relaxed and killed in warm FAA (2) for 5 minutes (until cuticle was no longer sticky), then transferred to warm lactophenol for 1–2 hours. Specimens in lactophenol were dehydrated in a desiccator for 3 days before mounting them in glycerin on glass slides.

Scanning electron microscopy (SEM): Live specimens dissected from flies were transferred to cacodylate buffer (pH 7) and processed with a sequential fixation technique modified from Nguyen and Smart (3). Specimens were fixed with refrigerated glutaraldehyde in a process that increased the concentration of fixative from 0.9 to 1.8 to 3% at hourly intervals, after which they were left in 3% glutaraldehyde at 20 C for 12 hours.

After fixation, the nematodes were rinsed five times (15-minute intervals) in sodium cacodylate buffer and postfixed with 2% osmium tetroxide solution for 12 hours at 20 C. The osmium tetroxide was removed from the specimens by rinsing them five times (10-minute intervals) in sodium cacodylate buffer. Specimens were dehydrated in a graded ethanol series,

transferred to a 1:1 mixture of ethanol: amyl acetate for 10 minutes, then placed into 100% amyl acetate. Finally, the specimens were critical-point-dried with liquid CO₂, mounted on SEM stubs, coated with gold, and examined with a Hitachi S-570 SEM at 15 KV.

SYSTEMATICS

Paraiontonchium muscadomesticae n. sp. (Figs. 1–5)

Holotype (young heterosexual female): Length = 659 µm; greatest body width = 20 µm; stylet length = 19 µm; distance from head to excretory pore = 152 µm; distance from anterior end to base of esophagus = 384 µm; a = 33.0; b' = 1.7; c = 9.4; V = 81.6; distance between vulva and anus divided by maximum body width 2.6; tail length = 70 µm.

Young heterosexual female: Measurements of 40 specimens in Table 1. Body straight or slightly curved ventrally when killed with heat (Fig. 1A). Cuticle finely annulated. Lateral field consisting of two marginal ridges delimiting a broad band marked by irregular longitudinal lines (Fig. 2C). Head continuous with body, perioral disc elevated with a protruding central cylinder (Fig. 2A). Stylet without knobs but usually slightly thickened at its base (Fig. 1C). Dorsal esophageal gland opening located approximately 1.3 stylet lengths posterior to stylet base. Subventral gland openings prominent, approximately 1–2 body widths anterior to the excretory pore. Esophagus with cylindrical procorpus narrowing to isthmus a short distance posterior to subventral gland openings. Esophageal glands lobe-like, with three cells extending posteriorly beyond mid-body (Fig. 1A), overlapping intestine dorsally. Nerve ring 1–1.5 body widths anterior to excretory pore. Deirid in lateral field near hemizonid (Fig. 2B).

Anterior portion of excretory duct well cuticularized. Hemizonid one half body width anterior to excretory pore, sometimes obscure. Vulva in posterior fourth of

TABLE 1. Morphometrics of males and young heterosexual females of *Paraiotonchium muscadomesticae* n. sp. ($n = 40$).

Character	Young heterosexual female			Male		
	Mean	(SD)	Range	Mean	(SD)	Range
Body length (μm)	652	(87)	530–709	615	(58)	500–691
Greatest width (μm)	23	(0.7)	20–25	20	(1.2)	19–23
Stylet length (μm)	20	(0.7)	16–23	15	(1.4)	12–17
EP† (μm)	140	(25)	117–152	132	(13.9)	97–153
ES‡ (μm)	384	(35)	325–472			
a	29.1	(3.1)	25.2–34.0	30.6	(4.1)	22.7–36.4
b'	1.57	(0.08)	1.4–1.75			
c	9.9	(1.1)	8.6–11.1	11.0	(0.6)	9.7–12.5
V (%)	81.9	(1.4)	79.5–83.9			
AV§	2.4	(0.2)	1.8–3.0			
Spicule length (μm)				18	(1.0)	16–21
Tail length (μm)	65.7	(2.1)	57–78	56	(5.3)	47–66

† distance from anterior end to excretory pore.

‡ distance from anterior end to base of esophagus.

§ distance between vulva and anus divided by maximum body width.

body; vulval opening circular to semicircular, lips slightly protruding. Vagina weakly cuticularized, perpendicular to body wall or directed anteriorly. Gonad prodelphic with large cylindrical uterus, narrow oviduct, ovary with three cells (three prominent nuclei), and terminal cap cell (Fig. 1D). Spermatheca not clearly defined; sperm observed in uterus. Postvulval uterine sac absent. Anus small, obscure. Phasmids not observed. Tail tapering gradually to a small, round terminus (Fig. 2D).

Allotype (male): Length = 563 μm ; greatest body width = 16 μm ; stylet length = 14 μm ; distance from head to excretory pore = 133 μm ; a = 35.2; c = 12.5; spicule length = 21 μm ; tail length = 45 μm .

Male: Measurements of 40 specimens in Table 1. Sexual dimorphism pronounced in anterior part. Body thinner and shorter than that of female. Anterior region of body straight or slightly curved dorsally, posterior region more strongly curved dorsally, especially at tail (Figs. 1B;3C). Cuticle finely annulated. Lateral field a central band margined by two ridges and marked by continuous and discontinuous longitudinal lines (Fig. 3A,B). Head continuous with body, shape similar to that of female. Stylet shorter than that of female, very thin (ca. half the width of female stylet), no knobs or basal thickening ob-

served. Esophagus partially degenerate. Procorpus similar to that of female but much thinner and less prominent. Dorsal esophageal gland and subventral gland orifices as well as nerve ring similar in position to those of female. Esophageal glands present, posterior two obscured by gonad (Fig. 1B). Anterior portion of excretory duct well cuticularized. Hemizonid reduced, less than one body width anterior to excretory pore, sometimes obscure or not observed. Gonad monorchic, outstretched; extending anteriorly beyond midbody (Fig. 1B) with testis in lateral view composed of two rows of cells (Fig. 1E). Vas deferens with sperm. Spicule with anterior portion straight and posterior portion curved ventrally (Fig. 1B,F). Distal portion of spicule swollen, spatula-like, tapering to a point (Figs. 1G;3D). Gubernaculum absent. Bursa absent. Phasmids not observed. Tail tapered with a characteristic constriction in the posterior fourth and strongly curved dorsally (Figs. 1B;2C) forming, with the body, an angle of 137° (122–150°, $n = 10$). Tail tip narrower than that of female.

Mature heterosexual female: Measurements of 20 individuals in Table 2. Body larger than young heterosexual female and strongly curved ventrally, forming "C" or "O" shape (Fig. 5A). Cuticle of varying

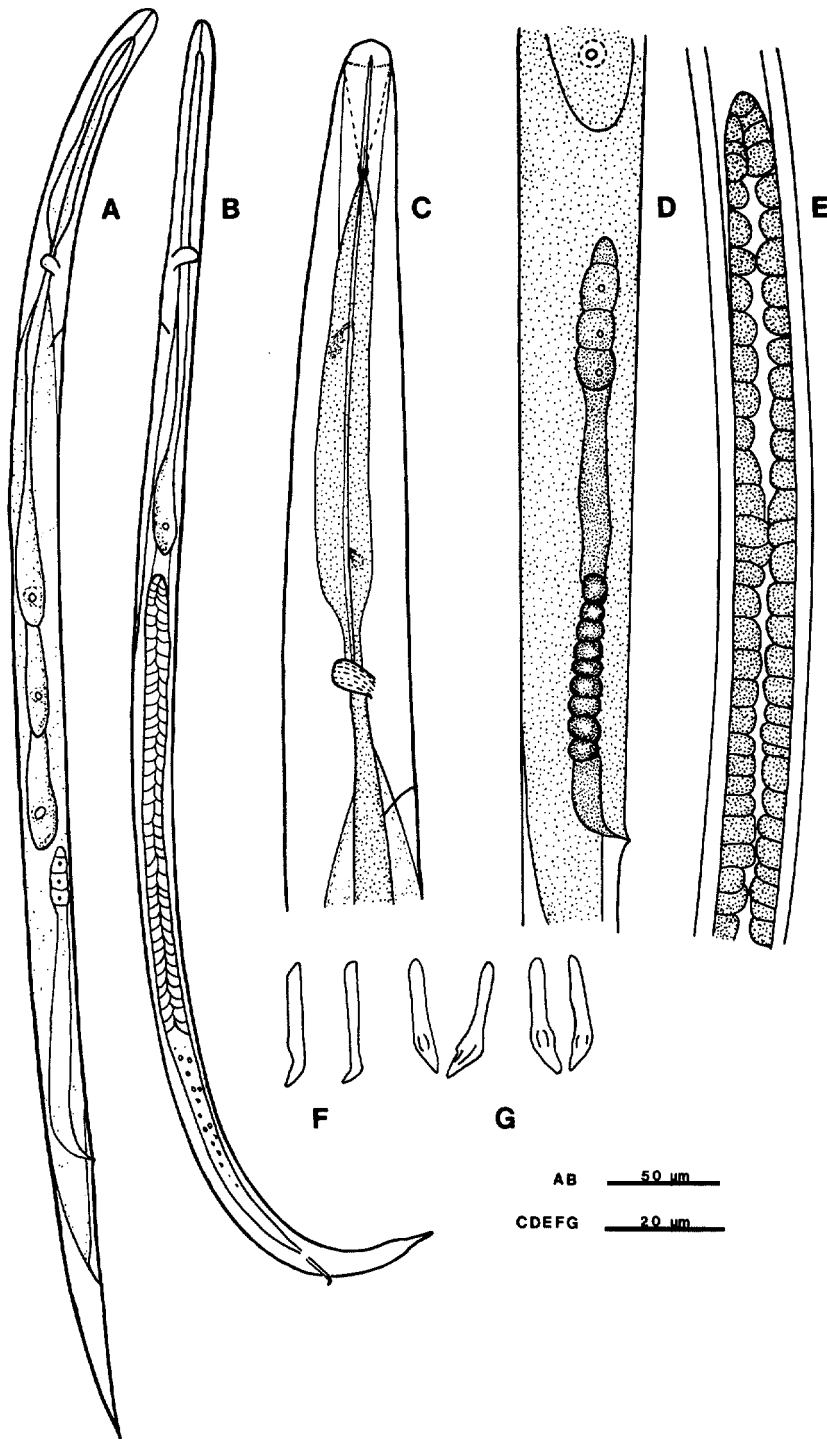


FIG. 1. *Paraio-tonchium muscadomesticae* n. sp. male and young heterosexual female. A) Entire body of female. B) Entire body of male. C) Anterior region of female. D) Female gonad showing anterior portion of ovary with three nuclei and terminal cap cell. E) Anterior portion of male gonad showing two rows of spermatocytes. F) Lateral view of spicules showing straight anterior portion followed by a distinct bend in the posterior region. G) Ventral and dorsal view of spicules showing the swollen spatula-like area anterior to narrow tip of terminus.

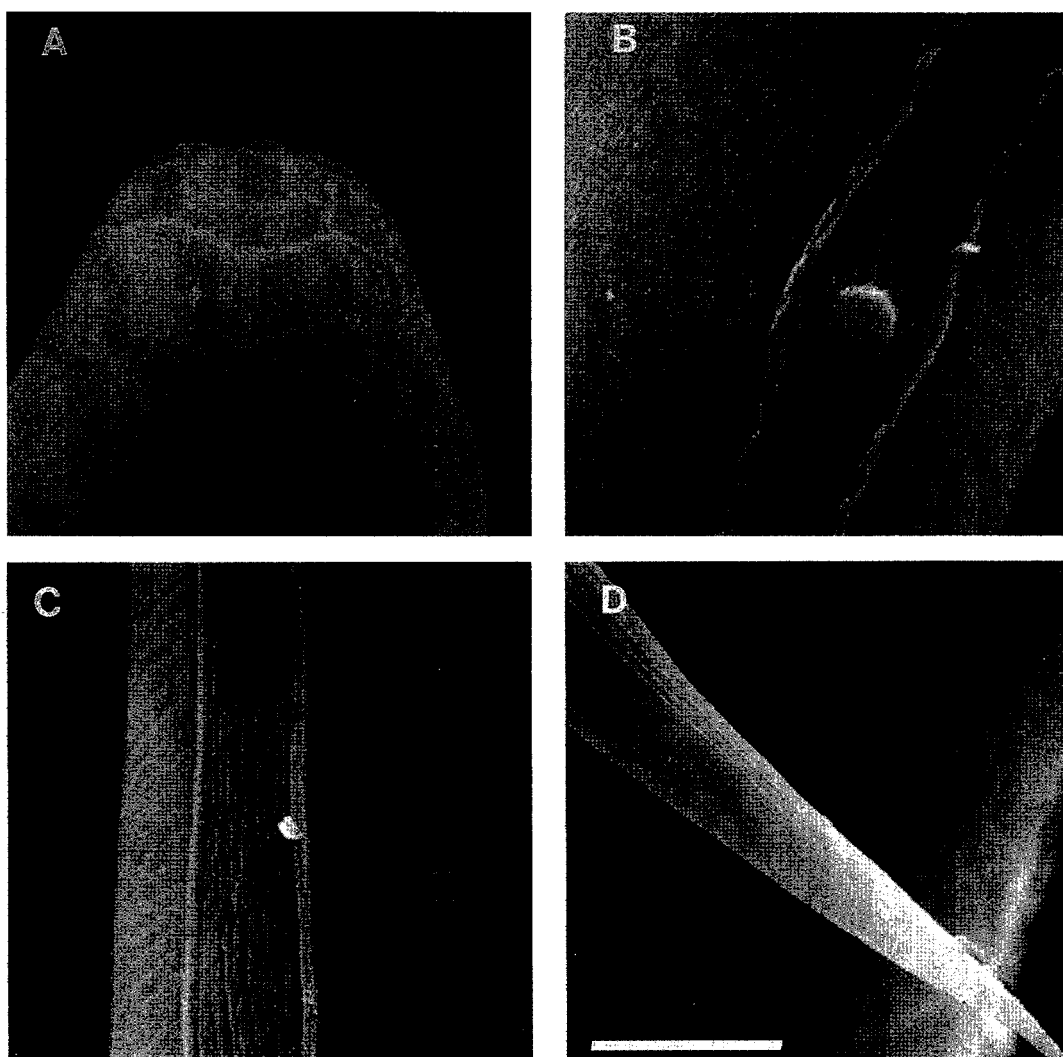


FIG. 2. *Paraionchium muscadomesticae* n. sp. SEM of young heterosexual females. A) Perioral disk surrounding a protruding central cylinder. B) Deirid within lateral field. C) Lateral field composed of one mid-band characterized by broken longitudinal lines margined by two ridges. D) Lateral view of tail showing gradual tapering to a small, rounded terminus. All magnifications based on scale bar in D: A = 2.13 μm , B = 3.8 μm , C = 4.3 μm , D = 13.6 μm .

thickness, mean 9.1 μm (6–13, $n = 9$), thicker at the anterior and vulval regions. Cuticle surface smooth, sometimes with fine cross lines, subcuticle striated. Lateral field not observed. Somatic musculature with prominent nuclei. Head region truncate to rounded. Perioral disk elevated with protruding central cylinder (Fig. 4A). Labial papillae not observed. Stylet as described in young heterosexual female. Esophagus degenerate. Dorsal esophageal gland and gland openings as in young het-

erosexual female. Subventral esophageal glands obscure. Excretory pore present, excretory duct not cuticularized, hemizonid not observed. Intestine large, dark in color, nuclei of intestinal cells prominent, larger than those of somatic muscles and gonads. Rectum and anus distinct. Vulva a transverse slit, slightly protruding from body surface (Fig. 4C). Gonad prodelphic, outstretched, extending beyond midbody of less developed individuals. In older mature females, gonads longer, in

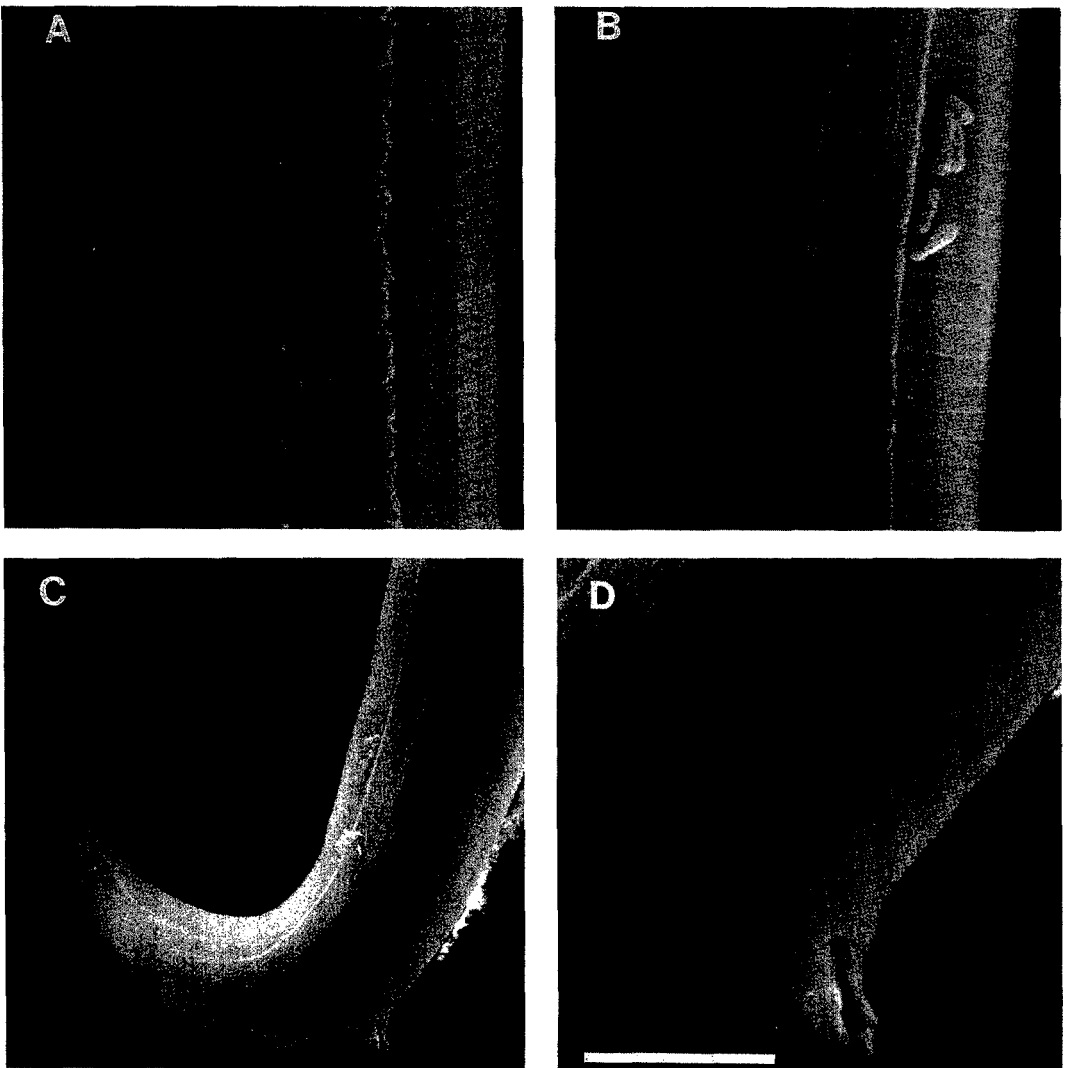


FIG. 3. *Paraiotonchium muscadomesticae* n. sp. SEM of males. A,B) Lateral field composed of A) A central band of longitudinal lines margined by two ridges, varying in texture from a discontinuous "pleated" pattern also seen under light microscope or B) a continuous "smooth" pattern. C) Posterior portion of a male showing distinct dorsal bend and gradual tapering of tail marked by abrupt narrowing near terminus. D) Posterior portion of spicules protruding from cloaca. All magnifications based on scale bar in D: A and B = $4.3\text{ }\mu\text{m}$, C = $3.8\text{ }\mu\text{m}$, D = $13.7\text{ }\mu\text{m}$.

some individuals reflexed but never extending to base of esophagus. Width of ovary in anterior portion mostly constant, occasionally tapering gradually. Germinal zone of ovary narrow, up to 3–4 oocytes wide (Fig. 5A). Eggs and sperm observed in uterus. Tail curved ventrally, spicate with tip skewed dorsally, directed ventrally (Fig. 5A).

Parthenogenetic female: Measurements of

20 individuals in Table 2. When heat-killed, body forming a spiral with 0.8–1.3 coils (Fig. 5B). Cuticle of varying thickness (as in mature heterosexual form), either striated or smooth with subcuticular striations. Lateral field not observed. Somatic musculature with prominent nuclei. Small preoral cap (stylet support) flanked by two lateral amphids (Fig. 4B). Stylet very thin, approximately $10\text{ }\mu\text{m}$ in length (9–13, $n =$

TABLE 2. Morphometrics of mature heterosexual and parthenogenetic females of *Paratontochium muscadomesticae* n. sp. (n = 20).

Character	Mature heterosexual female			Parthenogenetic female		
	Mean	(SD)	Range	Mean	(SD)	Range
Body length (μm)	1,990	(345)	1,209–2,576	2217	(352)	1,762–3,079
Greatest width (μm)	108	(12.4)	81–134	140	(13.0)	116–175
Stylet length (μm)	22	(3.1)	18–31	10	(1.2)	9–13
a	18.3	(2.4)	14.5–22.3	15.9	(2.8)	11.9–23.0
c	21.1	(3.8)	14.5–30.4	15.0	(1.3)	12.9–18.7
V (%)	86.2	(1.7)	82.5–89.2	89.1	(1.2)	86.1–90.9
AV†	1.6	(0.2)	1.2–2.1	0.7	(0.2)	0.4–1.2
Tail (μm)	96	(19.0)	66–145	149	(26.2)	120–236

† distance between vulva and anus divided by maximum body width.

20). Esophagus greatly reduced. Dorsal and subventral esophageal gland openings obscure. Excretory pore, duct, and hemizonid not observed. Intestine as in mature heterosexual form, large with nuclei of intestinal cells clearly defined and more prominent than nuclei of somatic muscles and ova. Vulva a transverse slit, similar in shape to that of mature heterosexual form, protrusion reduced (Fig. 4D). Gonad prodelphic, wider and longer than mature heterosexual form, extending anteriorly beyond base of esophagus; in most individuals extending into head region, becoming reflexed (Fig. 5B). Posteriorly, gonad with looped oviduct (Fig. 5B) and a large uterus. Ovarian width variable within individual, tapering gradually at tip. Germinal zone of ovary larger than that of mature heterosexual female, up to 9–10 oocytes wide (Fig. 5B). Eggs packed in uterus of mature individuals. Tail tapered and bluntly rounded with fine terminal mucro (Fig. 4D).

Biology. The life cycle of *P. muscadomesticae* is similar to that of *P. autumnale* (Nickle) (4), consisting of alternating gamogenetic and parthenogenetic generations. The infective nematode (heterosexual females) enters the insect through the body wall. The nematode then develops into the mature parasitic form, which begins to produce eggs (10–15) of the parthenogenetic generation approximately 5 days after adult fly emergence. This sec-

ond generation then begins to produce eggs (500–1,000) of the third generation (heterosexual) approximately 10 days after adult fly emergence.

Type host and locality: House fly (*Musca domestica*) collected from the municipal marketplace of Serra Talhada, in the state of Pernambuco, in northeast Brazil.

Type specimens

Holotype (Young heterosexual female): This free-living form was reared from the J4 stage, which was obtained from adult *Musca domestica* at least 15 days old. Holotype in glycerin, slide number T-504t, deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

Allotype (Heterosexual adult male): Same data as holotype. Slide number T-505t, deposited in the USDANC, Beltsville, Maryland.

Paratypes (Adult: males and young heterosexual females, mature heterosexual and mature parthenogenetic stages): Same data as holotype. Males and females in lactophenol, vial number T-363p, deposited in the USDANC, Beltsville, Maryland.

Etymology: To derive the species name, the two components of the host's binomen, *Musca domestica*, were united to form a compound noun in the nominative case, feminine gender, and first declension. *Muscadomestica*, the compound noun, was then made the epithet of the name of the

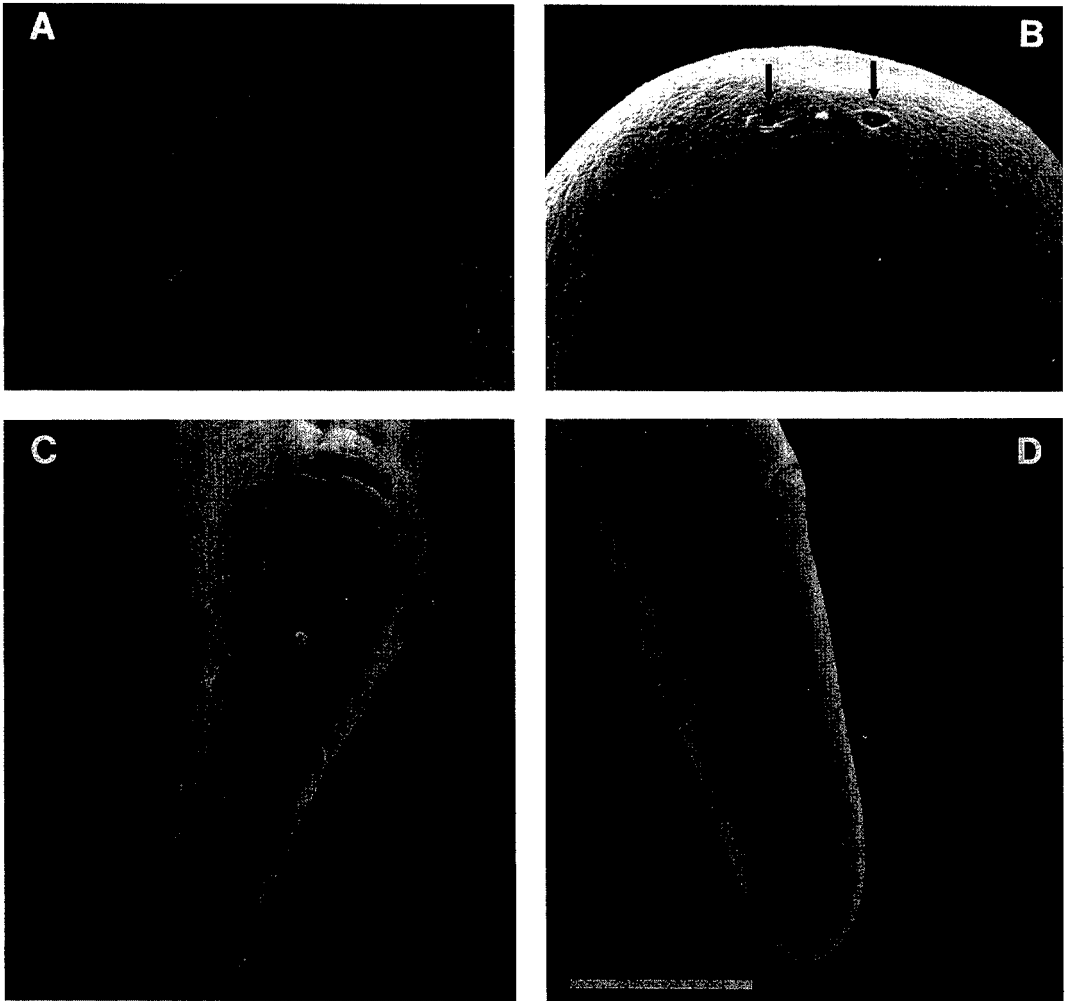


FIG. 4. *Paraiotonchium muscadomesticae* n. sp. SEM of mature heterosexual and parthenogenetic females. A) Head region of mature heterosexual form with perioral disc. B) Head region of parthenogenetic form with small preoral cap (stylet support) flanked by amphids (arrows). C) Tail portion of mature heterosexual form with swollen vulval region and spicate tail. D) Vulva and tail region of parthenogenetic form. All magnifications based on scale bar in D: A = 6 μm , B = 23.1 μm , C = 43 μm , D = 60 μm .

new species, *Paraiotonchium muscadomesticae*, by changing its suffix to the genitive (possessive) case.

Diagnosis: Young heterosexual females 652 μm (530–705) long. Stylet without knobs but thickening usually present. Hemizonid and deirid present. Gonad single with large cylindrical uterus, narrower oviduct and ovary consisting of three cells and a terminal cap cell. Male with reduced

stylet and esophagus. Bursa absent. Spicules with anterior portion straight and posterior portion curved and swollen, then tapering to a point.

Mature heterosexual females “C”-shaped. Cuticle thick. Vulva a transverse slit, situated on a ventral protuberance.

Parthenogenetic females spiral-shaped, larger than mature heterosexual females, 2,217 μm vs. 1,990 μm in length. Vulval

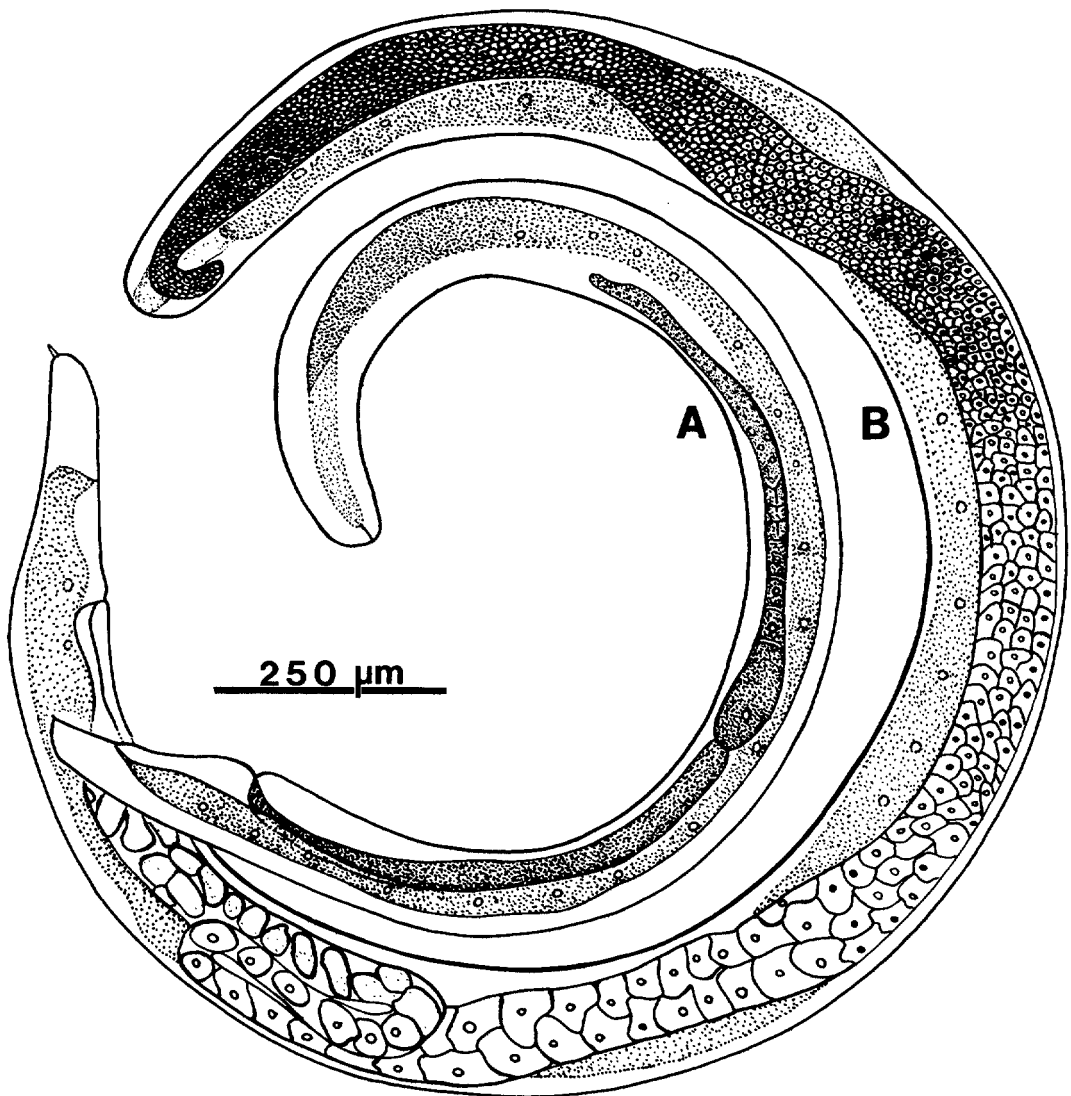


FIG. 5. *Paraiontonchium muscadomesticae* n. sp. mature heterosexual and parthenogenetic females. A) Entire body of mature heterosexual female with less developed reproductive tract. B) Entire body of parthenogenetic form showing enlarged reproductive tract dominating system.

area not protruding as that of mature heterosexual form. Ovary long, extending anteriorly beyond base of esophagus. Tail subcylindrical with a fine terminal mucro.

Relationships: *Paraiontonchium muscadomesticae* n. sp. can be distinguished from *P. nicholasi*, *P. autumnale*, and *P. crassirostris* by the shorter body length of the young heterosexual females, 652 μm (530–709) for *P. muscadomesticae* compared to 750 μm

or more (820–1,050) for the others. *Paraiontonchium muscadomesticae* is close to *P. nicholasi* but differs from it by V ratio and spicule length ($V = 80\text{--}84$, spicule = 16–21 μm in *P. muscadomesticae* compared to $V = 73\text{--}78$, spicule = 25–35 μm in *P. nicholasi*). *Paraiontonchium muscadomesticae* and *P. nicholasi* differ from all the species of this genus including *P. xanthomelas* (Reddy & Rao) Remillet and Laumond and *P. osiris* Slobodyanyuk by the absence of a bursa.

KEY TO SPECIES OF THE
GENUS *PARAIOTONCHIUM*

Identification based on adult male and young heterosexual female nematodes (all measurements in μm)

1. Body length of young heterosexual female greater than 750 (820–1,050) ----- 2
Body length of young heterosexual female less than 750 (474–702) ---- 4
2. Bursa absent in male, originally described as a parasite of the bush fly, *Musca vetustissima* Walker -----
----- *P. nicholasi* Slobodyanyuk
Bursa present in male ----- 3
3. Mean body length of male 823 (749–900), stylet 18.9 (18.8–19.0), spicule 33.6 (30.2–35.7), described as a parasite of the face fly, *Musca autumnalis* DeGeer -----
---- *P. autumnale* (Nickle) Slobodyanyuk
Mean body length of male 625 (607–717), stylet 5 (4–6), spicule 43 (40–45), originally described as a parasite of the fly *Musca crassirostris* Stein -----
--- *P. crassirostris* (Yatham & Rao) Siddiqi
4. Bursa absent in male, originally described as a parasite of the house fly, *Musca domestica* Linnaeus -----
----- *P. muscadomesticae* n. sp.
Bursa present in male ----- 5
5. Mean body length of male 534 (502–566), spicules of unequal length, bursa small (shorter than spicule length) described as a parasite of *Musca xanthomelas* Wiedemann -----
----- *P. xanthomelas* (Reddy & Rao)
Remillet & Laumond
Mean body length of male 621 (469–804), spicules equal in length,

bursa large (longer than or equal to the length of spicules), described as a parasite of *Musca osiris* Wiedemann ----- *P. osiris* Slobodyanyuk

LITERATURE CITED

1. Coler, R. R., J. G. Stoffolano Jr., and S. P. Barreto. 1980. *Heterotylenchus* sp. (Nematoda: Sphaerulariidae), a nematode parasite of house fly, *Musca domestica* L. (Diptera: Muscidae) in northeastern Brazil. Proceedings of the Helminthological Society of Washington 47:135–136.
2. Hooper, D. J. 1986. Handling, fixing, staining and mounting nematodes. Pp. 59–80 in J. F. Southey, ed. Laboratory methods for work with plant and soil nematodes. 6th ed. (HMSO, reference book no. 402.) London: HMSO Publication Center.
3. Nguyen, K. B., and G. C. Smart, Jr. 1993. Scanning electron microscope studies of *Steinernema anomali* Kozodoi, 1984. Journal of Nematology 25:486–492.
4. Nickle, W. R. 1967. *Heterotylenchus autumnalis* sp. n. (Nematoda: Sphaerulariidae), a parasite of the face fly, *Musca autumnalis* De Geer. Journal of Parasitology 53:398–401.
5. Reddy, Y. N., and P. N. Rao. 1987. Studies on *Heterotylenchus xanthomelas* sp. n. parasitic in *Musca xanthomelas* Wiedemann (Muscidae: Diptera). Indian Journal of Nematology 17:180–183.
6. Remillet, M., and C. Laumond. 1991. Sphaerularioid nematodes of importance in agriculture. Pp. 967–1024 in W. R. Nickle, ed. Manual of agricultural nematology. New York: Marcel Dekker.
7. Siddiqi, M. R. 1986. Suborder Hexatylini; section D: superfamily Iotonchioidea. Pp. 554–574 in M. R. Siddiqi, ed. Tylenchida: Parasites of plants and insects. Slough, UK: CAB Commonwealth Institute of Parasitology.
8. Slobodyanyuk, O. V. 1975. Basis for the new genus *Paraitionchium* gen. n. (Nematoda: Sphaerulariidae) and additions to the description of a species of this genus, *P. autumnale*. Trudy Gel'mintologicheskoi Laboratorii 25:156–168.
9. Slobodyanyuk, O. V. 1976. *Paraitionchium osiris* (Iotonchiidae: Tylenchida) a new nematode species from the zoophilous fly *Musca osiris*. Parazitologiya 10:30–39.
10. Yatham, N. R., and P. N. Rao. 1981. Studies on *Heterotylenchus crassirostris* sp. n. parasitic in *Musca crassirostris* Stein and *Stomoxys calcitrans* L. Indian Journal of Nematology 11:19–24.