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Description of *Parasitorhabditis frontali* n. sp. (Nemata: Rhabditida) from *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae)

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Abstract: A new *Parasitorhabditis* species with males and females was discovered from the southern pine beetle *Dendroctonus frontalis* and its galleries in loblolly pine, *Pinus taeda*, growing in Mississippi. Females of the new species have a cupola-shaped tail with a small spike; males possess a 2 + (3+2) + 3 ray pattern on the tail fan with ray 10 reaching the margin, and a distinctive stomatal tooth. *Parasitorhabditis frontali* n. sp. has some similarities to *P. hylurgi* Massey, 1974 from *Hylurgops pinifex* in New York, USA, *P. terebranus* Massey, 1974 from *D. terebrans* (Olivier, 1795) in Texas USA, *P. ligniperdae* Fuchs, 1915 from *Hylergops ligniperda* (Fabricius, 1787) and *P. dendroctoni* Rühm, 1956 from *D. micans* (Kugelann, 1794) in Europe, *P. ateri* Fuchs, 1915 isolated from the beetle *Hylastes ater* (Paykull, 1800) in Germany, and *P. malii* Devdariani and Kakulia,1970 from *Scolytus mali* (Bechstein, 1805) within the republic of Georgia. Morphometrics for 44 species of *Parasitorhabditis* are provided to update older keys. *Parasitorhabditis frontali* n. sp. was initially grown on Malt Extract (ME) agar with its own microbial contaminants that included a bacterium and fungus. The nematode also grew and reproduced after slices of ME agar with nematodes and microbial contaminants were transferred to water agar. It was killed by *E. coli* on NGM agar plates commonly used to raise other Rhabditida. Drawings of diagnostic anatomy and low-temperature SEM images of bodies, heads, and tails are provided for cultured specimens from pine beetle frass.

Key words: morphology, nematode, *Parasitorhabditis frontali* n. sp., SEM, southern pine beetle, taxonomy, trophic interaction.

A rhabditid nematode with males and females was discovered from the southern pine beetle *Dendroctonus frontalis* and its galleries in loblolly pine *Pinua taeda* growing in Mississippi. It was determined to be a new species of *Parasitorhabditis* Fuchs, 1937, a genus of parasitic or phoretic nematodes associated with bark beetles. These nematodes are generally benign to their hosts but at least two instances of damage to cerambycid beetles (Chapman, 1964) and scolytid beetles (Tomalak et al., 1989) were reported. Beetles of the genera *Ips* De Geer, 1775, *Dendroctonus* Erichson, 1836, *Dryocoetes* Eichhoff, 1864, *Hylurgus* Latreille, 1807, *Hylurgops* LeConte, 1876, *Hylastes* Erichson, 1836, *Pityogenes* Bedel, 1888, *Cryphalus* Erichson, 1836, *Crypturgus* Erichson, 1836, some Cerambycidae (Sudhaus, 1974) *Phloeosinus* Chapuis, 1869, *Polygraphus* Erichson, 1836 and *Scolytus* Geoffroy, 1762 (Massey, 1974) have had *Parasitorhabditis* associates. The U. S. Forest Service publication of Calvin Massey (Massey, 1974) listed 6 U.S. species of *Parasitorhabditis* sp. known at the time from various bark beetles, including one *Parasitorhabditis* sp. among the 18 nematode species listed from *D. frontalis.* Within other beetle species there were 19 unspeciated *Parasitorhabditis* isolates listed in the index. Much of the literature has been within Russian or German journals that may be difficult to access. Since the compendia of Blinova (1982) and Andrássy (1984), only one other new species, *P. platidontus* Reboredo and Camino, 2000, has been described. Approximately 44 species are currently known in this genus (Blinova, 1982; Andrássy, 1984, Reboredo and Camino, 2000). Some of these are *species inquirenda* due to inadequate descriptions. Nematodes identified in the U. S. and Canada are also designated (Table 1).

Species within *Parasitorhabditis* have one of the most variable male tail ray patterns among genera of Rhabditida (Sudhaus and Fitch, 2001). GenBank accessions for large and small subunit ribosomal RNA sequence exist for only one morphologically characterized species, *Parasitorhabditis obtusa* (Fuchs, 1915) Chitwood and Chitwood, 1950, the type species. Phylogenetically this species branches between *Mesorhabditis* (Osche, 1952) Dougherty, 1953 and *Teratorhabditis* (Osche, 1952) Dougherty, 1955 in a well-resolved tree of ribosomal DNA and large subunit RNA polymerase II (Kiontke, et al., 2007). This position differed from expectations based on stomatal similarities presumed to be ancestral that grouped *Parasitorhabditis* with *Protorhabditis* (Osche, 1952) Dougherty, 1953 and *Prodontorhabditis* Timm, 1961 within Protorhabditinae Dougherty, 1955 (Sudhaus, 1976; Andrássy, 1984). However, current rhabditid molecular phylogeny shows these last two genera branch immediately outside *Caenorhabditis* (Osche, 1952) Dougherty, 1953, three clades removed from *Parasitorhabditis* (Kiontke et al., 2007). The close genetic relationship of *Parasitorhabditis* with *Mesorhabditis* is interesting since these and only two other genera of Rhabditidae, *Cephaloboides* (Rahm, 1928) Massey, 1974 and *Bunonema* Jägerskiöld, 1905, were identified as bark beetle associates in the U.S. (Massey, 1974).

Parasitorhabditis species have been cultured from juveniles to adults on Malt agar (Massey, 1945) or on water agar or potato dextrose agar supporting fungi (Hunt and Poinar, 1971). The morphology and culture of the new *P. frontali* n. sp. are described below, including a diagnosis of related species with a mosaic distribution of similar characters.

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Table 1. Measurements of Female *Parasitorhabditis* Fuchs, 1937. Range lengths in μm, a - V ratios.

Species[a]	Body L	Stoma L	a	b	c	V
acanthocini	(500 – 1092)	(16 – 19)	(14 – 26)	(3.6 – 5.7)	(36 – 84)	(94 – 97)
acuminati	(494 – 1045)	(15 – 19)	(14 – 25)	(3.9 – 6.3)	(34 – 67)	(93 – 96)
ali[f]	(950 – 1170)	16	20	6.1	27	91
ateri	(433 – 990)	(16 – 25)	(15 – 24)	(3.4 – 6.6)	(20 – 31)	(91 – 94)
autographi[i]	(720 – 780)	(20 – 24)	(20 – 21)	(4.2 – 5.4)	(22 – 32)	(92 – 95)
bellifonti	(560 – 705)	(14 – 16)	(19 – 27)	(3.8 – 5.0)	(25 – 37)	(92 – 94)
bicoloris	568	-	22	3.3	26	90
bidentati	(984 – 1190)	(18 – 19)	(24 – 27)	(6.0 – 6.6)	(57 – 70)	(95 – 96)
cerembraei[bf]	936	14	15	7.6	67	96
chalcographi	(594 – 922)	(14 – 18)	(19 – 24)	(4.7 – 5.6)	(62 – 105)	(95 – 97)
cluniculus[h]	(990 – 1000)	20	(20 – 22)	(5.1 – 5.4)	(56 – 58)	95
crenati[bdf]	656	16	18	5.0	47	95
cryphalophila	(690 – 1110)	(14 – 16)	(16 – 17)	(4.7 – 6.6)	(28 – 40)	(93 – 94)
crypturgophila	(705 – 795)	(18 – 19)	(17 – 18)	(4.5 – 4.9)	(28 – 29)	(92 – 93)
curvidentis	(660 – 780)	(14 – 17)	(17 – 19)	(4.5 – 5.4)	(20 – 22)	(91 – 92)
dendroctoni	(885 – 1050)	(25 – 28)	(15 – 19)	(4.2 – 4.8)	(21 – 32)	(93 – 94)
erosus	670	-	19	4.5	13	90
fuchsi	(570 – 990)	(14 – 20)	(11 – 28)	(3.3 – 6.1)	(38 – 69)	(93 – 98)
gracilis[h]	800	14	25	5.0	27	93
hastulus[h]	(670 – 750)	17	(19 – 21)	(4.4 – 4.8)	(42 – 46)	95
hectographi	(779 – 782)	(21 – 27)	(17 – 24)	(4.5 – 5.2)	(25 – 27)	(79 – 92)
hylurgi[h]	(890 – 900)	20	(17 – 19)	(4.8 – 5.3)	(24 – 25)	92
ipini[h]	(660 – 950)	19	(20 – 23)	(4.4 – 5.1)	(75 – 108)	95
ipsophila[j]	(735 – 1082)	20	(18 – 24)	(4.0 – 5.3)	(43 – 77)	(94 – 96)
ligniperdae	(648 – 1440)	(27 – 28)	(18 – 20)	(4.8 – 5.2)	(23 – 28)	(89 – 92)
malii	(680 – 756)	13	(14 – 17)	(5.0 – 5.3)	(30 – 67)	(93 – 97)
masseyi[bch]	-	18	-	-	-	-
obtusa[hi]	(711 – 1177)	(15 – 22)	(15 – 24)	(4.0 – 5.6)	(44 – 84)	(93 – 99)
opaci	(825 – 1035)	(17 – 19)	18	(4.4 – 5.2)	(24 – 25)	(92 – 93)
palliati	(614 – 1100)	(18 – 24)	(15 – 22)	(4.0 – 6.9)	(20 – 31)	(92 – 94)
pini[f]	(502 – 590)	(15 – 16)	(16 – 17)	(4.3 – 4.9)	(27 – 30)	92
piniperdae	(605 – 1049)	(13 – 22)	(14 – 31)	(4.1 – 8.2)	(32 – 65)	(93 – 95)
platidontus	(588 – 690)	(13 – 17)	18.5	5.3	11	(78 – 83)
poligraphi	757	14	24	5.8	108	96
proximi	(540 – 1050)	(14 – 20)	(16 – 35)	(3.6 – 7.0)	(42 – 93)	(93 – 98)
sexdentati	(660 – 1430)	(14 – 24)	(14 – 29)	(4.1 – 7.1)	(25 – 105)	(90 – 97)
slankisi	(830 – 950)	-	(24 – 25)	(4.4 – 5.6)	(53 – 63)	(93 – 95)
subelongati[ch]	(418 – 1164)	(16 – 20)	(11 – 22)	(3.0 – 6.3)	(35 – 97)	(93 – 97)
terebranus[h]	(770 – 810)	-	20	(4.2 – 4.3)	(26 – 27)	93
thornei[eh]	(800 – 1100)	-	25	5.0	56	95
villosi	(552 – 716)	(15 – 21)	(16 – 17)	(4.5 – 4.9)	(32 – 39)	(92 – 93)
welchi	864	21	28	5.8	81	96

Measurements of Male *Parasitorhabditis* Fuchs, 1937. Range Lengths in μm, a - c ratios.

Species[a]	Body L	Stoma L	Spicule L	a	b	c
acanthocini	(625 – 780)	(13 – 17)	(37 – 41)	(15 – 24)	(4.0 – 5.8)	(20 – 26)
acuminati	(350 – 1020)	(13 – 19)	(30 – 40)	(18 – 27)	(3.8 – 5.6)	(22 – 33)
ali[f]	(800 – 950)	18	(27 – 30)	20	5.4	31
ateri	(359 – 855)	(14 – 24)	(24 – 42)	(14 – 22)	(3.0 – 5.7)	(19 – 21)
autographi[i]	(563 – 705)	(18 – 24)	(31 – 38)	(20 – 24)	(3.6 – 4.7)	(22 – 34)
bellifonti	(435 – 590)	(14 – 16)	(37 – 47)	(20 – 26)	(3.6 – 4.4)	(20 – 24)
bicoloris	692	16	30	31	5.8	29
bidentati	(797 – 901)	(16 – 18)	(32 – 37)	(26 – 31)	(5.1 – 5.2)	(26 – 28)
cerembraei[bf]	587	12	23	22	5.0	21
chalcographi	(488 – 795)	(12 – 17)	(24 – 33)	(21 – 29)	(4.4 – 5.1)	(22 – 31)
cluniculus[h]	(780 – 810)	-	33	(23 – 24)	(4.5 – 4.8)	(18 – 21)
cryphalophila	(630 – 840)	(14 – 16)	(40 – 51)	(18 – 22)	(5.1 – 5.7)	(24 – 26)
crypturgophila	(525 – 600)	(16 – 19)	(32 – 33)	19	(3.9 – 4.3)	(23 – 25)
curvidentis	(570 – 675)	(16 – 19)	(30 – 32)	(16 – 19)	(4.2 – 4.8)	(21 – 27)
dendroctoni	(750 – 976)	(25 – 28)	(41 – 42)	(19 – 20)	(4.0 – 4.8)	(31 – 35)
erosus	625	-	35	-	-	-
fuchsi	(484 – 970)	(12 – 20)	(30 – 40)	(12 – 31)	(3.5 – 6.2)	(14 – 26)
gracilis[h]	760	-	(34 – 41)	29	5.1	29

(*Continued*)

TABLE 1. Continued.

Species[a]	Body L	Stoma L	Spicule L	a	b	c
hastulus[h]	(620 – 670)	-	39	(25 – 26)	(4.2 – 4.6)	(21 – 24)
hectographi	(665 – 783)	(17 – 23)	(32 – 35)	(19 – 21)	(4.7 – 4.9)	25
hylurgi[h]	(770 – 830)	-	40	(19 – 22)	4.4	(22 – 29)
ipini[h]	(610 – 750)	-	(34 – 35)	21	(3.9 – 5.0)	(19 – 23)
ipsophila	(700 – 890)	(17 – 22)	(40 – 48)	(22 – 26)	(4.3 – 5.1)	(18 – 23)
ligniperdae	(885 – 1110)	(27 – 30)	(41 – 49)	(19 – 20)	(4.6 – 5.1)	(23 – 28)
malii	(565 – 635)	-	(28 – 37)	(16 – 29)	(4.4 – 4.6)	(18 – 20)
masseyi[bch]	-	-	31	-	-	-
obtusa[hi]	(639 – 886)	(15 – 21)	(20 – 34)	(19 – 26)	(3.7 – 5.2)	(18 – 30)
opaci	(720 – 840)	(17 – 19)	32	(19 – 20)	(4.0 – 4.5)	(27 – 29)
palliati	(630 – 839)	(16 – 23)	(34 – 44)	(16 – 22)	(4.0 – 6.2)	(20 – 38)
piniperdae	(464 – 1065)	(10 – 22)	(25 – 46)	(16 – 30)	(3.6 – 6.1)	(15 – 30)
platidontus	(560 – 660)	(16.5 – 21.2)	(37.6 – 44.6)	17.9[g]	4.7 [g]	35.7 [g]
poligraphi	645	11	23	27	6.3	27
proximi	(430 – 920)	(13 – 18)	(24 – 37)	(17 – 35)	(3.6 – 6.1)	(19 – 43)
sexdentati	(636 – 1051)	(14 – 22)	(36 – 50)	(15 – 33)	(4.0 – 6.1)	(19 – 43)
slankisi	(825 – 900)	-	(35 – 46)	-	-	-
subelongati[h]	(418 – 927)	(15 – 20)	(30 – 37)	(15 – 28)	(3.1 – 5.2)	(12 – 37)
terebranus[h]	750	-	34	20	4.1	27
thornei[eh]	(600 – 700)	-	-	29	4.5	26
villosi	(562 – 715)	(18 – 21)	(34 – 37)	(22 – 23)	(4.2 – 5.1)	(23 – 24)
welchi	720	21	38	24	4.0	15

[a] *Parasitorhabditis acanthocini* Lasarevskaja, 1961, *P. acuminati* (Fuchs, 1915) Skriabin et al., 1954, *P. ali* Kakulia (1963), *P. ateri* Fuchs, 1915, *P. autographi* Fuchs, 1937, *P. alistina* Fuchs, 1937, *P. bellifonti* Lieutier and Laumond, 1978, *P. bicoloris* Devdariani and Maglakelidze, 1970, *P. bidentati* Rühm, 1954, *P. cembraei* Fuchs, 1937, *P. cluniculus* Massey, 1974, *P. chalcographi* (Fuchs, 1937) Rühm, 1954, *P. crenati* Fuchs, 1937, *P. cryphalophila* Rühm, 1956, *P. crypturgophila* Rühm, 1956, *P. curvidentis* Fuchs, 1915, *P. dendroctoni* Rühm, 1956, *P. erosus* Kurashvili, Kakuliya, and Devdariani, 1980, *P. fuchsi* Blinova and Gurando, 1974, *P. gracilis* Massey, 1974, *P. hastulus* Massey, 1974, *P. hectographi* Rühm and Chararas, 1957, *P. hylurgi* Massey, 1974, *P. ipini* Massey, 1974, *P. ipsophila* Lieutier and Laumond, 1978, *P. ligniperdae* Fuchs, 1915, *P. malii* Devdariani and Kakulia, 1970, *P. masseyi* Sudhaus, 1976, *P. obtusa* (Fuchs, 1937) Chitwood and Chitwood, 1950, *P. opaci* Rühm, 1956, *P. palliati* (Fuchs, 1937) Rühm, 1956, *P. piniperdae* (Fuchs, 1937) Rühm,1956, *P. platidontus* Reboredo and Camino, 2000, *P. poligraphi* Fuchs, 1937, *P. proximi* (Fuchs, 1937) Skriabin at al., 1954, *P. sexdentati*, Rühm, 1960, *P. slankisi* Kurashvili, Kakuliya, and Devdariani, 1980, *P. subelongati* Slobodjanjuk, 1973, *P. terebranus* Massey, 1974, *P. thornei* Sudhaus, 1976, *P. villosi* Rühm, 1956, *P. welchi* Devdariani, 1974; Not listed in table: *P. amitini* Fuchs, 1915, and *P. montani* (Fuchs, 1915) Sudhaus, 1976 are *species inquirendae* of Blinova, 1982, Andràssy, 1984; *P. minoris* (Fuchs, 1937) Rühm, 1956 is *species inquirenda* of Andràssy, 1984.

[b] *Species inquirenda* of Blinova, 1982.

[c] *P. masseyi* Sudhaus, 1976 was synonymized with *P. subelongati* Slobodjanjuk, 1973 (Andràssy, 1984).

[d] *Species inquirenda* of Sudhaus, 1976.

[e] Identified from description of *Rhabditis obtusa*, Massey, 1956.

[f] *Species inquirenda* of Andràssy, 1984.

[g] Ratios 'a' – 'c' of *P. platidontus* derived from average measurements in Reboredo and Camino, 2000.

[h] Present in the U.S. (Sudhaus, 1976, Massey, 1974, Andràssy, 1984).

[i] Present in Canada (Tomalak et al, 1989).

[j] Synonym of *P. thornei* Sudhaus, 1976 per Andràssy, 1984.

Materials and Methods

Nematode isolation: Loblolly pine logs (*Pinus taeda* L.) infested with the southern pine beetle (*Dendroctonus frontalis*) were collected in the Homochitto National Forest near Meadville, MS, USA in June, 2008. One or two types of nematodes, including an aphelenchid, were active in the mycangial area at the prothorax and in mouth parts of beetle adults, and in the head area of larvae.

Nematode culture: Bark frass containing nematodes were applied to Malt Extract (ME) plates. After nematodes and their microbial associates began to reproduce, plates were maintained by cutting slices of the medium with nematodes using a flamed spatula to new plates. They were refrigerated for several months with good viability. However, nematodes died within a day when transferred to plates of Nematode Growth Medium (NGM) agar with *E. coli* used to support other soil and insect-associated nematodes (Stiernagle, 1999). Slices of ME agar were also applied to 4% water agar plates with some fungal mycelium carried from the ME frass culture that supported moderate, sustained growth and egg-laying of the nematodes. There were sufficient numbers of clean adult nematodes for low-temperature scanning electron microscopy (LT-SEM), however nematodes in the culture died before DNA could be extracted.

Nematode imaging, drawing and measuring: Most measurements and photomicrographs of live and fixed specimens were taken on a Zeiss Ultraphot with DIC (differential interference contrast) (Carl Zeiss, Inc., Jena, Germany/Baltimore Instrument Co., Baltimore MD, USA) and a Q-I Micropublisher 5 camera (Q-Imaging Inc., Austin, TX, USA). Some measurements of annules and phasmids were made from LT-SEM micrographs. Scale bars and increased contrast were applied in Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA, USA). Drawings were made from images and specimens at multiple planes. Morphological conventions were based on Sudhaus and Fitch, 2001. Stoma illustration style was based on Massey, 1974.

LT-SEM: Approximately 50 *Parasitorhabditis frontali* n. sp. adult specimens were gently lifted from 4% water agar with a pig's eyelash and placed in a BPI dish of M9 buffer. The nematodes were refrigerated overnight, after which, excess M9 buffer was removed and sterile deionized water was replaced three times. Nematodes were placed in 1.5 ml microcentrifuge tubes and concentrated at the base by gravity. In order to optimize the likelihood that nematodes would turn their heads upward before flash-freezing, specimens were mounted in water on leaves with a variety of elevated structures, as well as the standard filter paper. Rectangles 25 mm x 14 mm were cut from the dorsal and ventral surfaces of fresh azalea (*Rhododendron* sp.) leaves or filter paper and tacked onto rough-surfaced, rectangular, copper plates (1.5-cm-wide, 3.0-cm-long and 1.0-mm thick) with tissue mounting medium (Triangle Biomedical Sciences, Durham, NC). Nematodes were pipetted onto the leaf or filter paper surface and excess moisture was wicked away with absorbent paper under a dissecting microscope (Carta et al., 2003). A single fungus colony grown on water agar was sampled both at the edge and toward the center with a forceps holding sticky, round, ultra smooth carbon adhesive tabs (Electron Microscopy Sciences, Hatfield, PA) pressed onto the agar. The other sides of the sticky tabs were peeled away and pressed onto rough-surfaced, rectangular, copper plates (1.5-cm-wide, 3.0-cm-long and 1.0-mm thick). The mounted specimens were placed on a –196 °C liquid nitrogen-precooled 14-mm square brass tube within a 20.5 cm x 15 cm x 4 cm styrofoam box on a lab bench. This contact freeze immobilization method cryofixes the nematodes milliseconds after contact with the metal surface and preserves their natural biological positioning and actions (Wergin et al., 2000). Samples were mounted on a specimen holder and transferred into a Polaron CryoPrep PP2000T (Energy Beam Sciences, East Granby, CT) Cryotrans system. Samples were etched at –90 °C to remove surface water and coated with platinum. Samples were observed in a Hitachi S-4700 field emission SEM.

Results and Discussion

Parasitorhabditis frontali n. sp.

(Table 2, Figs. 1-4)

Adult Females (n = 10): (Fig. 1 A - D, Fig. 2 A - D; Fig. 3 A, Table 2). Stoma 3.2 ± 0.3 (2.9 – 3.5) µm wide, bearing a single small thin tooth visible in lateral view; convex walls slightly broader at base with a small bifurcation at the anterior end of stoma column and slight flare at basal walls (Fig. 1A), about 1 µm thick. No stomatal collar, glottoid apparatus nor punctations on cuticle of head region. Pseudolips of six sectors bearing three types of sensory structures: apical, labial papillae recessed within striated, open pustules; cephalic papillae at base of lips, and obscure amphid pore on base of lips (Fig. 2A). Lip height 2.5 µm (Fig. 2B). Prominent annulations with 1.3 – 1.4 µm interval between head annules (Fig. 2B). Trasverse ridging in lumen of procorpus, characteristic of

Table 2. Morphometrics of *Parasitorhabditis frontali* n. sp. (LKC43) Measures in µm unless measure is a ratio or %. Length = L, Gub = Gubernaculum

Measure	*P. frontali* n. sp. Female fixed n = 10	*P. frontali* n. sp. Female live n = 2	*P. frontali* n. sp. Male Holotype	*P. frontali* n. sp. Male fixed n = 7	*P. frontali* n. sp. Male live n = 3
L	582 ± 52 (498 – 693)	635 ± 59 593, 677	592	596 ± 58.9 (503 – 661)	635 ± 76 (547 – 683)
a	18.9 ± 1.8 (16.5 – 21.9)	13.7 ± 1.3 12.8, 14.6	23.1	21.9 ± 3.0 (18.5 – 22.7)	19.1 ± 3.6 (15 – 21.4)
b	4.6 ± 0.4 (4.1 – 5.1)	4.9 ± 0.7 4.4, 5.4	5.0	4.6 ± 0.4 (4.0 – 5.0)	5.8 ± 0.3 (5.5 – 6.0)
c	26.5 ± 2.6 (25 – 30.6)	26.7 ± 3.4 24.3, 29.1	25.5	24.8 ± 0.42 (18.5 – 27.2)	21.7 ± 5.9 (15.1 – 26.4)
c' ratio//Fan papillae	1.1 ± 0 (0.5 – 1.7)	0.93 ± 0.03 0.9, 1.0	1.3 2+(4+1)+3	2+(3+2)+3 2+(4+1)+3 2+5+(1+2)	2+(3+2)+3
V//Spicule L	92 ± 0.01 (89.6 – 93.5)	93.3 ± 4.7 90, 96.6	35.5	32.5 ± 3.0 (28 – 36)	29.3 ± 3.2 (25.6 – 31.4)
V-A Dist//Gub Length	25.2 ± 2.8 (22.7 – 27.2)	36.1 ± 1.6 34.9, 37.2	12.8	12.1 ± 0.81 (11.1 – 12.8)	12.8
Stoma Length	13.9 ± 1.5 (13 – 17)	17.5 ± 1.6 16.3, 18.6	12.8	13.7 ± 1.2 (12.8 – 16.3)	(11.6 – 13.9)
Stoma Width	3.2 ± 0.3 (2.9 – 3.5)	3.5	2.9	2.9 ± 0.4 (2.3 – 3.5)	2.9
Body Width	31.5 ± 3.5 (29.4 – 34.0)	46.5 ± 0 46.5	25.6	28.3 ± 2.4 (24.9 – 31.7)	(25.5 – 45.2)
Pharynx Length	130 ± 6.5 (113 – 136)	129 ± 6.6 124.4, 133.7	118.6	127 ± 7.8 (109 – 131)	(100 – 116)
Tail Length	22.4 ± 1.7 (20.4 – 24.9)	24 ± 0.8 23.3, 24.4	23.3	24.1 ± 2.1 (22.7 – 27.2)	(23.2 – 32.5)
Gonad Length	357 ± 33.5 (322 – 416)	-	347.7	349 ± 52.0 (276 – 403)	-
Reflexed Gonad arm	176 ± 29.3 (150 – 213)	-	76.7	78.5 ± 16.4 (50 – 91)	-
Gonad arm/Gonad L %	50 ± 7 (39 – 63)	-	22	23 ± 5 (18 – 31)	-
Gonad L/Body L %	61 ± 4 (55 – 66)	-	59	58 ± 4 (55 – 65)	-

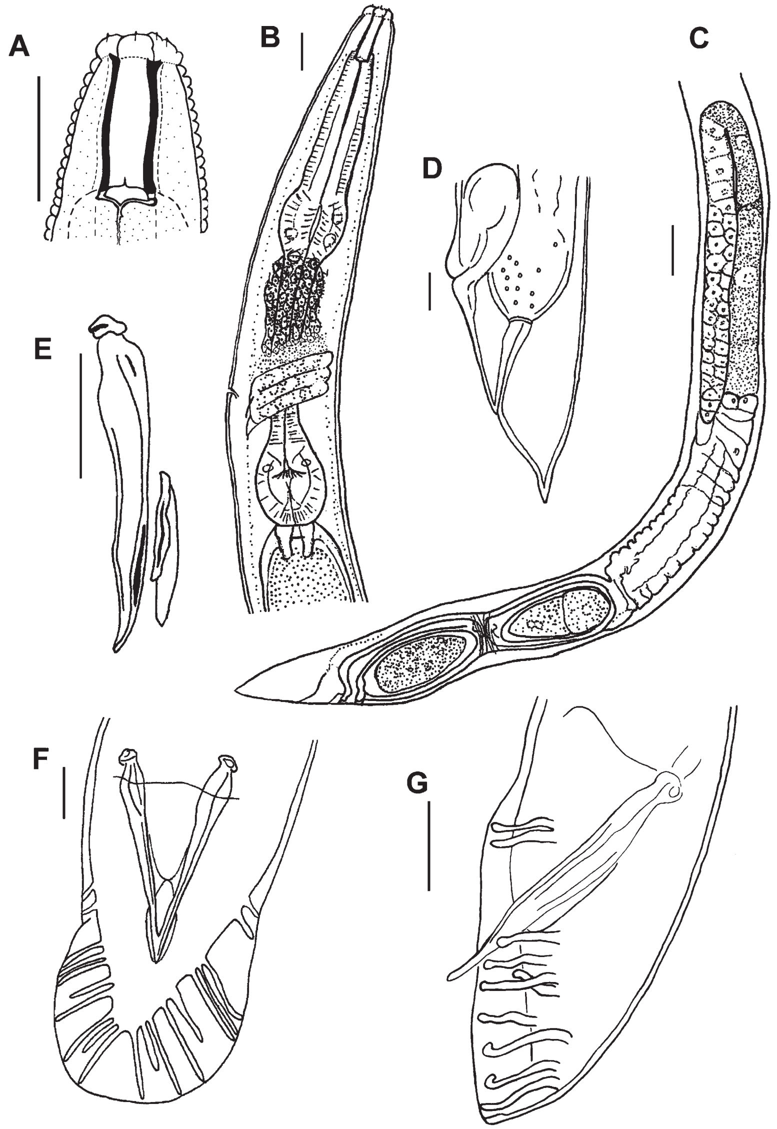

FIG. 1. *Parasitorhabditis frontali* n. sp. Drawings with scale bars of 10 μm for A, B, D – F, and 20μm for C. A. Female stoma, lateral view, B. Female pharynx, lateral view, C. Female reproductive system, lateral view, D. Female tail, lateral view, E. Male spicule, slipper-shaped gubernaculum, lateral view, F. Male tail with spicules, ventral view, open bursal fan, fused spicule tips, 10 rays, 2 + (3 + 2) + 3 ray pattern. G. Male tail with spicules, lateral view, 10 rays, 2 + (4 + 1) + 3, with ray 6 trapped under ray 5 so that ray tip order is reversed from typical pattern.

genus. Pharynx procorpus with moderately swollen median bulb and isthmus about equal in length, each about twice the length of the basal bulb; proportion of procorpus length divided by length of long isthmus plus basal bulb, 69 ± 9% (60 – 80%) (n = 5) (Fig. 1B). Nerve ring 60 – 75 % of pharynx at mid- to posterior mid-isthmus. Excretory pore obscure (n = 5), 96.2 ± 10.7 (90 - 114) μm from lips, 74 ± 8 (68 - 87%) of pharynx length at posterior isthmus. Single anteriorly-directed gonad extends up to 2/3 body length, with prominent crustaformeria often containing 1 – 2 embryos (Fig. 1C). Vulva a transverse slit below anterior lip annule, above semi-circular posterior lip region flanked laterally by oblique stress folds (Fig. 2C), variably protruding. Vagina short, oblique with muscular walls (Fig. 1C, D). Vulval-anal distance in flash-frozen specimens about 17 - 24 μm, with anus 10 - 19 annules below vulval opening, and phasmid 10 - 11 annules below anus, about 11.5 - 19 μm (Fig. 2C, D). Vulval-anal distance in fixed specimens slightly longer than tail length. Interval between major annules 1.7 μm on tail (Fig. 2C). Tail cupola-shaped, with short spike often curled in live specimens (Fig. 2D). Body plump with relatively steep angle from region of stoma to pharynx (Fig. 3A), with posture sinusoidal to straight when dead.

Adult Males (n = 7): (Fig. 1 E-F, Fig. 3 B-D, Table 2). Stoma as in female. Pharynx proportion of procorpus length divided by length of long isthmus plus basal bulb 60 – 70% (n = 4). Nerve ring 65 – 75 % of pharynx, just posterior to mid-isthmus. Excretory opening as in female. Testis extends up to 2/3 of body length, with packed hexagonal sperm diameter of 7 μm. Lateral field extends down to about the third annule of fan.

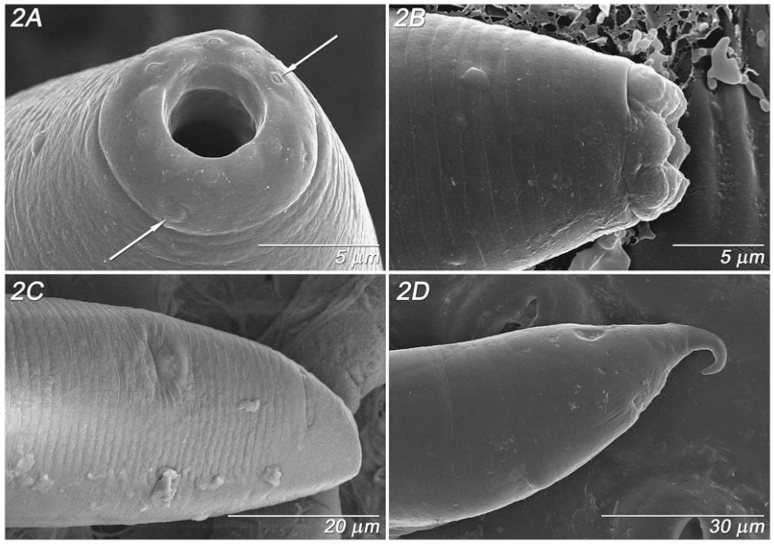

FIG. 2. *Parasitorhabditis frontali* n. sp. LT-SEM images of female morphology. A. Pseudolips, face view, with cephalic papillae (left arrow) labial papillae (right arrow), B. Pseudolips, lateral view, C. Tail, ventro-lateral view with vulval slit (upper middle surface) and anal slit (upper right surface), D. Tail, lateral view with vulval slit (lower middle surface) and anal slit (upper right surface), curved tail spike.

Spicule has nearly straight to minimally curved axis and very short distal fusion near acute, bluntly rounded tip, not curved (Fig. 1E). Gubernaculum slipper-shaped with posterior lateral thickening, 33 - 46% (n = 8) of spicule length (Fig. 1E). Open peloderan tail with bursal fan with 2 + (3+2) + 3 typical ray pattern (Fig. 1F), exceptionally 2 + 5 + (1 + 2) or 2 + (4+1) + 3 (Fig. 1G), with rays 1 and 2 preanal, rays 3, 6 and 9 retracted from edge of fan and opening dorsally, 6 generally thickened at base, and 10 extending to edge of fan (Figs. 3C, D). In one large male rays 6 and 9 extended close to the edges of the fan. A few punctuations visible by light microscopy on the fan, especially toward the posterior end. Body plump with relatively steep angle from region of stoma to pharynx, with posture sinusoidal to (Fig. 3B) straight when dead. Papillar phasmids at ventral junction of tail body and fan (Fig. 3C). Interval between major annules 1.5 µm above tail (Fig. 3C).

Eggs: (Fig. 4 A) 52 – 61 µm length x 23 – 29 µm width (n = 4). Egg shell with slightly elevated, interrupted network pattern.

Type host and locality: Southern pine beetle (*Dendroctonus frontalis*) frass from loblolly pine (*Pinus taeda* L.) trees located within the Homochitto National Forest near Meadville, MS, USA.

Type specimens (Adult Females and Males): Subcultured on agar culture after isolation from frass of *Dendroctonus frontalis* on type plant host and locality, male holotype:

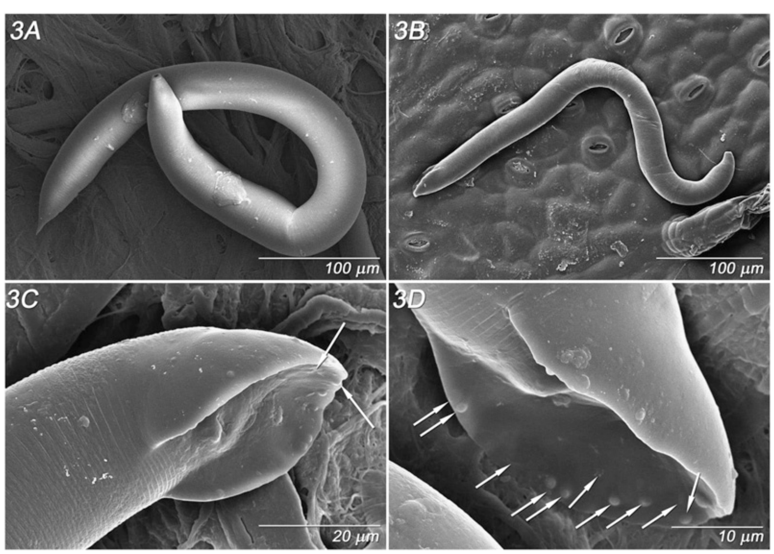

FIG. 3. *Parasitorhabditis frontali* n. sp. LT-SEM images of female and male morphology A. Female body, filter paper background, B. Male body, leaf background, C. Male tail, latero-ventral view, phasmid (upper arrow), and ray 10 tip (lower arrow), D. Male tail, ventro-lateral view, positions of rays 1 – 10 indicated by arrows from anterior to posterior.

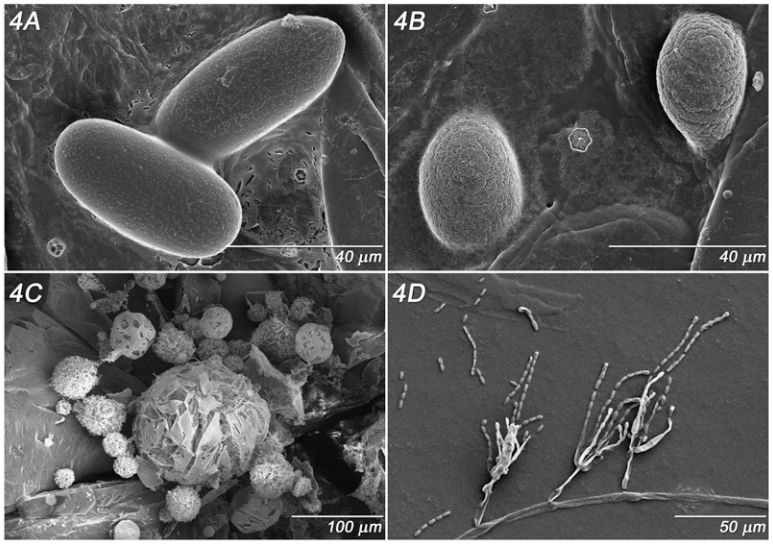

FIG. 4. *Parasitorhabditis frontali* n. sp. LT-SEM images from culture A. Eggs, B. Microbial residue in water, C. Actinomycete-like structures from bacterial colonies on agar substrate, D. *Gliocladium* sp. hyphae and conidiospores on sticky tab surface.

one of three specimens on T-5884p, female paratypes: T-5880p, T-5881p, T-5882p, and male paratypes: T-5883p, T-5884p, T-5885p, T-5886p, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland. A female paratype slide deposited at the National Russian Collection of Nematodes, Moscow, Russia.

Diagnosis and Relationships: (Tables 2, 3.) *Parasitorhabditis frontali* n. sp. LKC43 female with broad, cupola-shaped tail with short spike, sometimes curved, and single thin tooth at stoma base. Diagnostic morphology for males was the 2 + (3+2) + 3 ray pattern on the tail fan with rays 3, 6 and 9 opening dorsally, with retraction from fan edge in rays 3, retracted, often thickened ray 6, and extension of ray 10 to edge of fan, and a straight, bluntly pointed spicule tip. In comparison with other similar species there was a shorter female body and stoma length, and the last three male rays generally formed an exclusive group (Blinova, 1982; Devdariani, 1974; Devdariani and Kakuliya, 1970; Fuchs, 1915, Massey, 1974). Compared to *P. hylurgi*, there were shorter female and male body lengths and stoma lengths, widths, lower 'c' ratio (0.5 – 1.7 *vs* 2.3), female procorpus divided by isthmus plus terminal bulb ratio of 0.69 *vs* 0.95, and lack of obvious head punctations. Compared to *P. terebranus*, presence of a single thin stomatal tooth *vs* 4 teeth, shorter female and male body lengths and stoma lengths and widths, a procorpus-median bulb divided by isthmus plus terminal bulb ratio of 0.69 *vs* 1, and separation of male ray pattern. Compared to the morphometrically similar *P. ligniperdae* having similar 'a – c' ratios and slightly overlapping female body lengths, the new species had a small tooth *vs* no tooth in the shorter stoma (13 – 17 *vs* 27 - 28 µm), a discretely

TABLE 3. Supplemental morphometrics of *Parasitorhabditis* spp. for diagnosis. * denotes derivation from published drawing. Vulval-anal distances from Blinova, 1982.

Measure	*P. ateri*	*P. dendroctoni*	*P. ligniperdae*	*P. malii*	*P. hylurgi*	*P. terebranus*
FEMALE						
Terminal tail shape	Cupola, long spike	Cupola, short spike	Broadly conoid-acute	Cupola, short knob spike	Conoid - acute	Cupola, short spike
c'	1.6*	1.1*	1.9*	0.9*	2.3	1.9*
Vulval-anus Dist.	(16 – 35)	35	(35 – 37)	(10 – 15)	30	23
Stoma W Base	1 small tooth	No tooth	No tooth	5 1 small tooth	4.1* 1 small tooth	6.9 1 large tooth
MALE						
Spicule tip	Curved	Curved, blunt	Curved	Straight, spicate	Straight	Straight, spicate
Gubernaculum	(10 – 21)	46% Spicule (18 – 20)	(21 – 27)	15 (14 – 16)	21	17
Fan Papillae	2 + 4 + 4 2 + 3 + 5	2 + 4 + 4	2+ 4 + 4 2+(3+2)+3	2+(3+2) +1+2	2+3+1+3	2+3+1+2+1
Hosts	*Hylastes ater, cunicularius*	*Dendroctonus micans*	*Hylergops ligneperda*	*Scolytus mali*	*Hylurgops pinifex*	*Dendroctonus terebrans*
Locality	Germany	Germany	Germany	Republic of Georgia	US , NY	US, TX, Nacogdoches

shorter pharynx (113 – 136 *vs* 193 – 217 μm), shorter vulval-anal distance (23 – 27 *vs* 35 – 37 μm), shorter male body lengths (503 – 661 *vs* 885 – 1110 μm), shorter spicule (28 - 36 *vs* 41- 49 μm) and gubernaculum (11 – 13 *vs* 21 – 27 μm), relatively shorter gubernaculum relative to spicule (35 - 40 *vs* 55%), a retracted ray 3, and ray 10 reaching the edge of the fan, 2 + 4 + 4 ray pattern not seen, and larger eggs (52 – 61 x 23 – 29 *vs* 44 – 49 x 20 – 26 μm). Compared to *P. dendroctoni* the new species had a thin stomatal tooth *vs* no tooth, shorter female and male body lengths and stoma lengths and widths, a discretely shorter pharynx (113 – 136 *vs* 189 – 205 μm), vulval-anal distance (23 – 27 *vs* 35 μm), and 2 + 4 + 4 ray pattern not seen. Compared to *P. ateri* there were shorter female and male body lengths and stoma lengths, widths, and male rays had a 2 + (3+2) + 3 pattern *vs* 2 + 4 + 4 or 2 + 3 + 5. There was a longer vulval-anal distance (23 – 27 *vs* 10 – 15 μm), and a retracted ray 3 and separation of male ray 8 from 9-10 on the male tail compared to *P. malii.* The presence of a tooth on the stoma base, shorter female stoma length (13 – 17 *vs* 21 μm), shorter vulva position (up to 93.5 *vs* 97% of body), shorter spicule (32.5 *vs* 38 μm), and retracted ray 3 distinguished the new species from *P. welchi.*

Microbes in Water Agar Culture: (Fig. 4 B - D) The microbial residue from rinsing nematodes from plates (Fig. 4B) is present as small to large, oval globules that may be produced from a biofilm-forming *Rahnella* Izard et al. 1981 sp. bacteria in the bark. The microbial flora will be further characterized in another publication. It is possible that the fine markings on the eggs (Fig. 4A) may be a result of this microbial sludge, since at least one other species lacks egg markings (Reboredo and Camino, 2000). What appeared to be typical bacterial colonies on agar were relatively dense but fragmentable actinomycete-like organisms (Fig. 4C). An unidentified species of *Gliocladium* Corda, 1840 also grew and produced spores within both ME and water agar (Fig. 4D).

Microbial Ecology: Nematodes in bark beetles often have complex trophic associations with fungi and other organisms (Klepzig et al., 2001; Cardoza et al., 2006; Scott et al., 2008; Klepzig et al., 2009). Inability of *Parasitorhabditis* LKC43 to grow on bacteria yet survive with a fungus was also seen in another *Parasitorhabditis* sp. that fed on the bluestain fungus *Ceratocystis minor* Sydow and P. Sydow, 1919 (Hunt and Poinar, 1971), now known as a junior synonym of *Ophiostoma minus* Sydow and P. Sydow (Hedgcock). *Ophiostoma minus* was also present in the bark associated with this nematode in the wild (Scott et al., 2008). The *Gliocladium* Corda, 1840 fungus with asexual spores (Fig. 4D) found in these culture plates is unrelated to those ascomycetous wood fungi.

Remarks: Some of the measurements of fixed specimens, particularly pharyngeal length and body width differ somewhat from those of live material (Table 2) which may be due in part to sampling variation, but in the case of the pharynx may be due to shrinkage from fixation. Since relatively few measurements of fixed material are given in the literature, with almost no mention of any differences from live material, it is difficult to assess the true range of measurements for comparison. *Parasitorhabditis frontali* n. sp. is part of a species complex with *P. hylurgi, P. terebranus* (U. S. species from NY and TX), *P. ligniperdae*, and *P. dendroctoni* (species found in *Dendroctonus*), since they share a mosaic distribution of similar features. Some transitional morphometrics and qualitative morphological characters may yet be demonstrated to allow synonymy of these currently distinct morphotaxa.

An early coevolutionary phylogenetic scheme for four major groups of species within genus *Parasitorhabditis* (Rühm, 1956) included the *Obtusa, Chalcographi, Autographi* and *Ateri* groups of nematode species. While loosely associated with beetle genera, these nematode assemblages lacked unifying characters to distinguish them (Sudhaus and Fitch, 2001). The species compared in the diagnosis fall near the *Ateri* group, the left pole of the transitional *Parasitorhabditis* group spectrum. These nematodes are associated with *Hylastes* and *Hylurgops* bark beetles, with a subgroup containing *P. ligniperdae* and *P. dendroctoni* associated with *Hylurgus* and *Dendroctonus* beetles (Sudhaus and Fitch, 2001).

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