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Morphology and Description of *Bursaphelenchus platzeri* n. sp. (Nematoda: Parasitaphelenchidae), an Associate of Nitidulid Beetles

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Abstract: *Bursaphelenchus platzeri* n. sp., an associate of nitidulid beetles in southern California, is described and illustrated. Adult males and females of *B. platzeri* n. sp. were examined by scanning electron microscopy for ultrastructural comparisons with other members of the genus. *Bursaphelenchus cocophilus* (red ring nematode) appears to be the closest related taxon to *B. platzeri* n. sp. based upon shared morphological features of the fused spicules, female tail shape, phoresy with non-scolytid beetles, and molecular analysis of the near full-length small subunit (SSU) rDNA. Unfortunately, sequence data from the D2D3 expansion segments of the large subunit (LSU) rDNA and partial mitochondrial DNA COI did not help resolve the relationship of nearest relative. In addition to significant molecular sequence differences in SSU, LSU, and COI, *B. platzeri* n. sp., which is an obligate fungal feeder, can be differentiated from *B. cocophilus* because it is an obligate parasite of palms. *Bursaphelenchus platzeri* n. sp. can be differentiated from all other species of *Bursaphelenchus* by the length and shape of the female tail and spicule morphology. The spicules are fused along the ventral midline and possess unfused cucullae; the fused unit appears to function as a conduit for sperm. Population growth of *B. platzeri* n. sp. was measured in a time-course experiment at 25°C in the laboratory on cultures of the fungus *Monilinia fructicola* grown on 5% glycerol-supplemented potato dextrose agar (GPDA). Nematode population densities rapidly increased from 25 to approximately 200,000/culture within 14 d and then plateaued for up to 28 d.

Key words: *Bursaphelenchus platzeri* n. sp., *Carpophilus humeralis*, Coleoptera, morphology, mycophagy, nematode, Nitidulidae, Parasitaphelenchidae, phylogeny, scanning electron microscopy, systematics, taxonomy.

A new species of *Bursaphelenchus* was recovered as dauer juveniles (J_{III}) in the median oviducts and ovipositor sacs of adult females and the internal sacs of adult males of the pineapple beetle, *Carpophilus humeralis* (Nitidulidae), from Riverside, California (Giblin et al., 1984; Giblin, 1985). This nematode is described herein as *Bursaphelenchus platzeri* n. sp. It is the only described species of *Bursaphelenchus* known to be associated with nitidulid beetles (Giblin, 1985).

The beetle host, *C. humeralis*, is one of many species of nitidulids (dried-fruit and sap beetles) that are economically important pests of a variety of fresh and dried fruits and vegetables (Okumura and Savage, 1974). These beetles cause damage by direct infestation or transmission of plant-pathogenic fungi to fruits where the beetles mate, lay eggs, and complete their life cycle (Okumura and Savage, 1974). Dauer juveniles of *B. platzeri* n. sp. are carried phoretically by their nitidulid hosts and can be transferred to a new breeding site during oviposition or when the host beetle dies (Giblin, 1985). Once in the new environment, *B. platzeri* n. sp. dauers molt to the propagative phase and feed and develop on fungi such as *Monilinia fructicola* and *Penicillium* sp. that can be isolated from nitidulid-infested fruit (Giblin et al., 1984). The dauer juvenile stage of *B. platzeri* becomes more prevalent as fungal cultures age or the host environment deteriorates, and they infest newly emerged adult male and female beetles (Giblin, 1985). *Carpophilus humeralis* and four other species of nitidulids (*C. hemipterus*, *C. mutilatus*, *Haptonchus luteolus*, and *Stelidota geminata*) were experimentally infested with *B. platzeri* n. sp. dauer juveniles in the laboratory (Giblin, 1985).

Recent molecular analyses of the near full-length small subunit (SSU) rDNA, D2D3 expansion segments of the large subunit (LSU) rDNA, and partial mitochondrial DNA COI have provided a meaningful phylogenetic framework for the genus *Bursaphelenchus*, including *B. platzeri* n. sp. (as *B.* sp. ex *Carpophilus humeralis* 171 California), helping to justify its species status and placement relative to other nominal species (Giblin-Davis et al. 2005; Ye et al., 2006).

In addition to the taxonomic description of *B. platzeri* n. sp. using light (LM) and scanning electron microscopy (SEM) for morphological observations, this paper reports population dynamics of *B. platzeri* n. sp. cultured monoxenically on *M. fructicola*.

Materials and Methods

A culture of *B. platzeri* n. sp. (designated BNUH 1 and subsequently called RGD 171) was initiated from a single fertilized female from a culture that was started from dauer juveniles isolated from *C. humeralis* onto *M. fructicola* on potato dextrose agar (Giblin et al., 1984). All taxonomic work is based upon this isolate, which has been maintained through subculturing on *M. fructicola* on 5% (v/v) glycerol-supplemented potato dextrose agar (GPDA). Monoxenic cultures of *B.*

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platzeri were established as follows: Nematodes were harvested from cultures, collected on a Baermann funnel for 4 to 5 hr and surface-disinfected in a centrifuge tube with 0.1% merthiolate (w/v) (sodium ethylmercurithiosalicylate) for 10 min, aseptically concentrated on a sterile 20-µm nitex filter (obtained from Tetko Co., Elmsford, NY), and allowed to migrate through an antibiotic-antimycotic mixture in low gelling-temperature agarose as described by Giblin and Platzer (1987). Nematodes were collected in sterile water from the agar surface, quantified, and pipeted aseptically into 7-d-old cultures of *M. fructicola* on GPDA. Monoxenic cultures of *B. platzeri* n. sp. were maintained at 25°C and used for all subsequent studies, unless otherwise stated.

Adults of *B. platzeri* n. sp. were collected from 14-d-old cultures on *M. fructicola* and heat-killed for measurements in temporary water mounts. All nematodes were drawn and measured with the aid of a camera lucida and a stage micrometer. Type specimens were from 14-d-old cultures fixed in TAF (triethanolamine formaldehyde) for 24 hr and processed slowly into glycerol before measurement (Southey, 1970). Male spicule terminology used in this description has been described previously (Yin et al., 1988; Ryss et al., 2005). Unless otherwise stated, spicule length is the distance between the condylus and the posterior-most point of the cucullus measured in a straight line, and spicule width is the length of the capitulum.

J_{III} of *B. platzeri* n. sp. from a 28-d-old culture and J2 from a 7-d-old culture were washed from their respective cultures, heat-killed, fixed in formalin-acetic acid (FAA), and stained in 1% acetic orcein for 24 hr for visualization of gonad primordia (Southey, 1970). These measurements were made in temporary mounts in acetic orcein.

For SEM observations, adult males and females of *B. platzeri* n. sp. were collected from cultures using Baermann funnels, heat-killed, placed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2) for several days, and postfixed in 2% OsO_4 overnight. Specimens were dehydrated into 100% ethanol, critical point-dried using carbon dioxide, mounted on stubs, sputter-coated with 20 nm of gold/palladium, and observed with a JOEL 35C scanning electron microscope at 15 kV. Cephalic and lip region terminology used in this description is as proposed by Giblin-Davis et al. (1989).

Monilinia fructicola was subcultured aseptically onto 16 GPDA petri dishes and grown for 7 d at 25°C before inoculation with 25 ± 6 SD *B. platzeri* n. sp. in 1 drop of sterile water. Nematode inoculum was collected and disinfested as described above. Two dishes each of GPDA and nutrient agar (NA) were inoculated and included as contamination checks. Three to four culture dishes were harvested for 4 to 5 hr on a Baermann funnel at 1, 2, 3, and 4 wk after inoculation, and a measured aliquot of the nematodes was counted using a dissecting scope. Cultures that were inoculation failures (no nematodes recovered) were disregarded.

Description

Bursaphelenchus platzeri n. sp. (Figs. 1–6)

Measurements were made of the holotype male and allotype female in glycerol and of other specimens in temporary water mounts in Table 1.

Male (n = 20): Body cylindrical, tapered at both ends, J-shaped when heat-killed (Fig. 1B). The anterior regions (exterior and interior) of adult males and females were the same and are described in detail only for the male. Cuticle with fine annulation, annules about 0.7–0.8 µm wide at midbody (Figs. 1C,5C). Lateral field with three incisures (Fig. 1C), beginning just above level of metacorpus, extending posteriorly to level of ventral preanal papilla; less distinct from ventral preanal papilla to bursal flap = caudal alae. Head not distinctly offset from body, lip region in lateral view about three times wider than tall (Figs. 1C,5B). En face pattern (SEM) consisting of a clearly defined circular oral aperture (about 0.03 µm) surrounded by a dorsoventrally elongated labial disc, with a long indentation on each lateral side, and a small indentation on each dorsal and ventral side (Figs. 4,5A-B). Inner labial sensillae not resolved on or at the periphery of the labial disc, but may be integrated somehow with indentations. Labial disc surrounded by circular plate comprised of fused lateral, subventral, and subdorsal lip sectors, about 2–2.5 µm in diam. (Figs. 4,5A-B). Labial sensillae obscured; a composite of several SEM en face patterns suggests two lateral outer labial sensillae open mediolaterally onto circular lip sector plate (Figs. 4,5A-B). Circular lip sector plate surrounded by six hexaradiate cephalic sectors - two subdorsal, two lateral, and two subventral (Figs. 4,5A-B). Pore-like amphidial apertures, dorso-medially located on lateral cephalic sectors and a slightly elevated cephalic papilla clearly resolved on each subdorsal and subventral cephalic sector (Figs. 4,5A-B). Six to eight transverse striae visible on head with SEM (Figs. 4,5A-B). Stylet two part; cone short, about one third total stylet length, shaft with basal thickenings (Fig. 1C). Procorpus cylindrical, about 2.5 stylet lengths long ending in well developed metacorpus (Fig. 1A-C). Dorsal esophageal gland orifice opens into lumen of metacorpus less than one metacorpal valve width above metacorpal valve (Fig. 1C). Esophagointestinal junction less than one metacorpal valve width behind metacorpus. Postcorpus glandular overlaps intestine dorsally about 3.7 metacorpal lengths long (Fig. 1C). Excretory pore usually posterior to metacorpus, at or above level of nerve ring, hemizonid obscure, about three stylet lengths behind excretory pore (Fig. 1C). Gonad reflexed, sperm amoeboid (Fig.

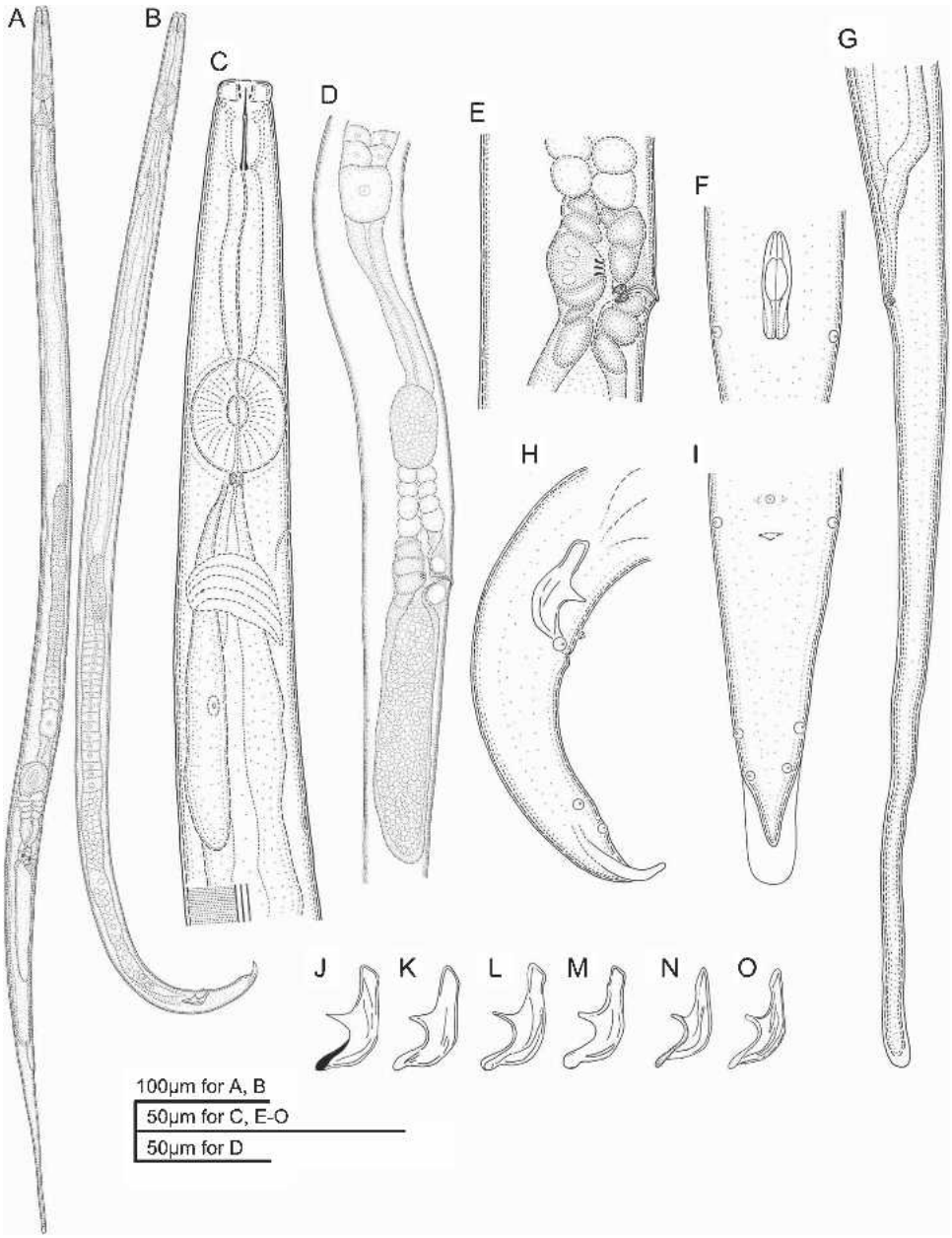

FIG. 1. Adult females and males of *Bursaphelenchus platzeri* n. sp. in lateral view (except where noted). A) Whole female. B) Whole male. C) Anterior body of female. D) Posterior region of female reproductive system. E) Vulva and vagina. F) Spicules (ventral view). G) Female tail. H) Male tail. I) Male tail (ventral view). J–M) Different focal planes through spicules of single male. N–O) Two focal planes through different male specimen.

1B). Tail arcuate, about 2.5–3.5 anal body widths long; terminus claw-like from lateral view (Fig. 1B,H). Bursal flap (= caudal alae) envelops tail terminus, flap usually elliptically shaped in ventral view (Figs. 1I,6G). Occasionally, tail filiform with no bursal flap. Spicules ventrally arcuate with ventrally fused lamina, rostrum prominent, distal ventral end expanded in lateral view with paired and separate cucullae, best seen in ventral view (Figs. 1F,J–O,6A–F), spicule length measured along its arc/capitulum width (distance between condylus and rostrum) ratio is about 1.7; spicule length measured along its arc/spicule width posterior to rostrum is about 3.4–3.6; rostrum sharply conical to digitate; condylus truncate to rounded in shape, junction of rostrum and calomus smoothly curved; lamina complex with midpoint widened and rounded; lamina dorsal contour smoothly and symmetrically curved (Figs. 1H,J–O,4); ratio of depth of capitulum depression divided by the capitulum width about 0.21–0.27. Fused spicule is recurved so that a line drawn through the anterior-most points of the condylus and rostrum (along the capitulum) and projected ventrally will intersect ventrally at about a 45° angle. Seven preanal and postanal papillae present; one preanal papilla (P1) in ventral midline about 2 µm above cloaca (Figs. 1H–I,6A,B,C,E), one pair subventral preanal papillae (P2)

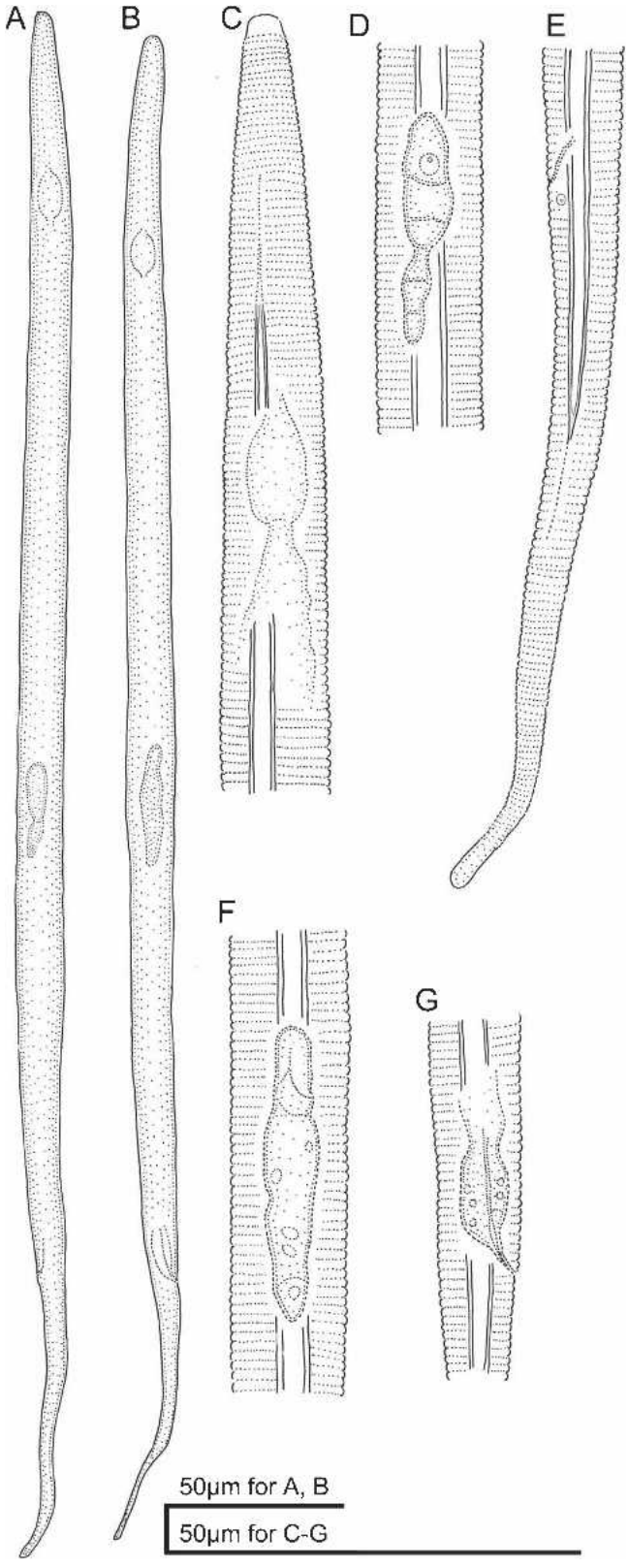

FIG. 2. *Bursaphelenchus platzeri* n. sp. dauer juveniles in lateral view. A) Whole female. B) Whole male. C) Anterior region of female. D) Female gonad. E) Female tail. F) Male gonad. G) Male anus.

at or about 1 µm above level of cloaca (Figs. 1H–I,6A,B,C,E), one subventral pair of postanal papillae (P3) at about 55% of a tail length behind cloaca (Figs.

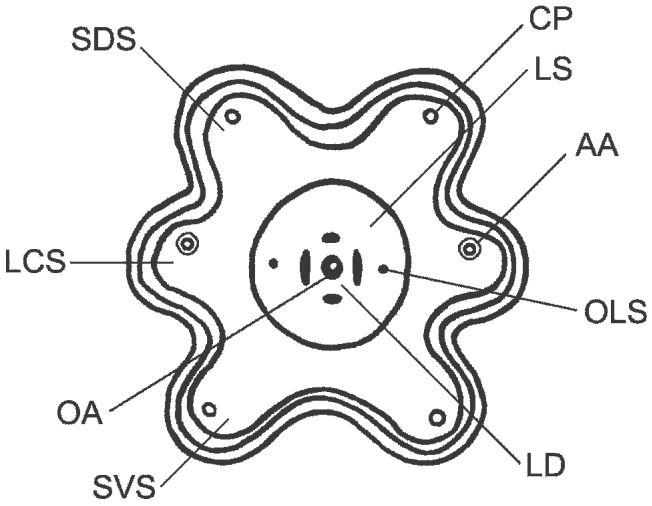

FIG. 3. Diagrammatic representation of the en face pattern of a *Bursaphelenchus platzeri* n. sp. female from scanning electron micrographs. AA = Amphid aperture, CP = cephalic papilla, LCS = lateral cephalic sector, LD = labial disc, LS = lip sector plate, OA = oral aperture, OLS = outer labial sensilla, SDS = subdorsal cephalic sector, and SVS = subventral cephalic sector.

1H–I,6G), one ventral pair of papillae (P4) about 6–12 µm from tail terminus (Figs. 1 H–I, 6G). Occasionally, an additional pair of almost lateral preanal papillae (about 8 µm above the P2 papillae, just ventral to the lateral field) are present (Fig. 6B). Bursa rounded in ventral view and pointed in lateral view (Figs. 1H–I,6G).

Female (n = 20): Body ventrally arcuate or straight when heat-killed (Fig. 1A). Lateral field with three incisures; decreases to two incisures posterior to level of anus; extending to tail terminus (Figs. 1C,5D–E). Ovary single, outstretched anteriorly, oocytes in single file except at anterior half of ovary (Fig. 1A,D). Vulva transverse slit externally in ventral view, vagina curved or straight in lateral view, paired and three-pronged cuticular structures at back of uterus facing vagina (Figs. 1D–E,5C). Postuterine sac 2.4–4.9 vulva body diam. (mean = 3.9; $n = 20$), 46% to 87% (mean = 68%; $n = 20$) of vulval-anus distance, often filled with sperm (Fig. 1A,D). Anus dome-shaped slit in ventral view (Fig. 5D). Tail long, 12.2 times longer than anal body width (range = 8.5–15.9; $n = 20$) and uniformly conoid to the terminus which is knobbed, digitate, or pointed (Figs. 1G,5E).

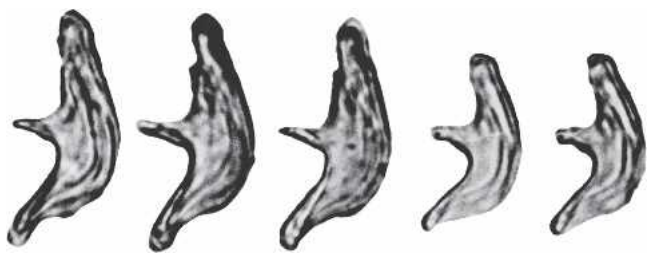

FIG. 4. Light micrographs of spicules of *Bursaphelenchus platzeri* n. sp. First three spicule photographs from left are different focal planes through the same specimen, and last two spicule photographs are of another specimen.

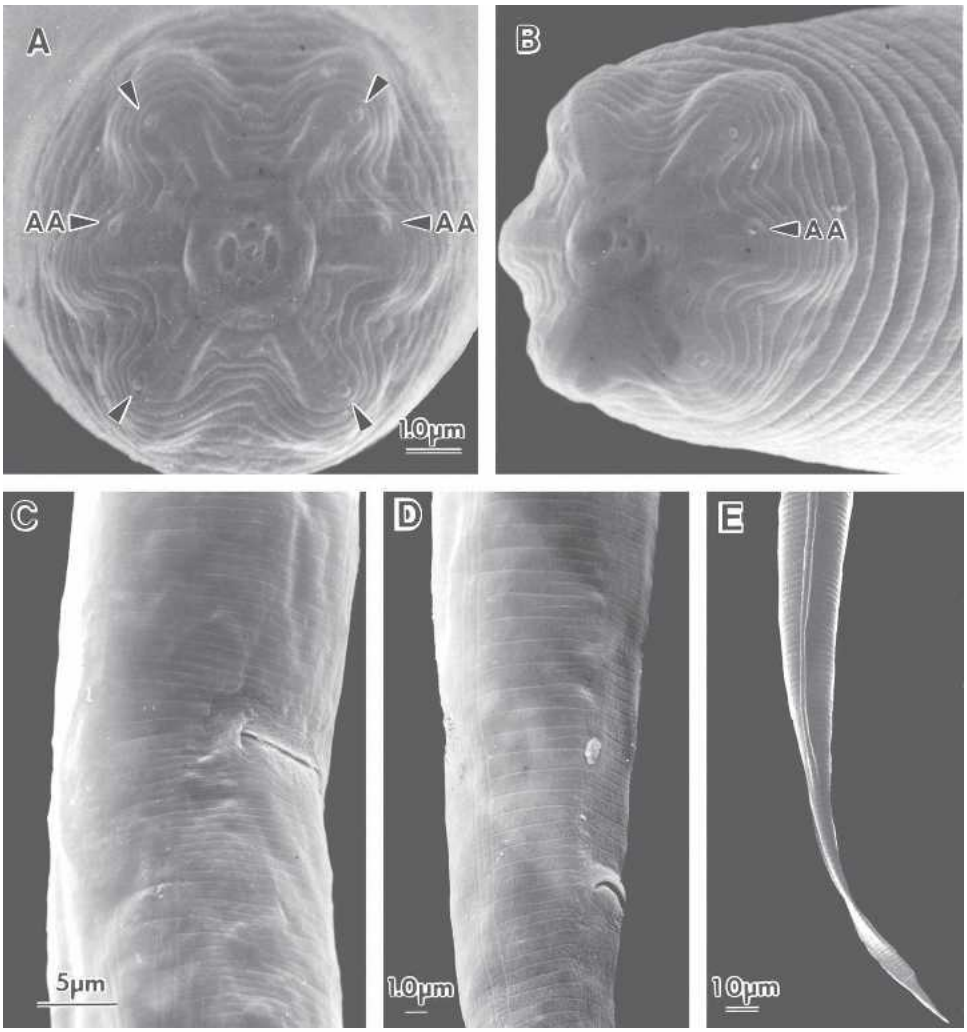

FIG. 5. Scanning electron micrographs of adult females of *Bursaphelenchus platzeri* n. sp. A) En face view, AA = amphidial aperture, arrows = cephalic papillae. B) Head, nearly lateral view. C) Vulval region in lateral view. D) Anus in ventrosublateral view. E) Tail in lateral view.

Dauer juvenile (designated J_{III} vs. J_3 for propagative third-stage juvenile) (n = 10): High, dome-shaped head, lips not defined. Stylet and esophagus indistinct (Fig. 2A–C). Body filled with granular material. Lateral field with four incisures (Fig. 1C–G). Gonad variable in length (Table 2). Tail conoid, 8–10 anal body widths long, terminus usually knobbed (Fig. 1E). J_{III} males differ from J_{III} females by possessing spicule primordia (Fig. 1A–B,E–G).

Type host and locality

Holotype male and allotype female are from 14-d-old cultures on *M. fructicola*. The original culture was started with J_{III} of *B. platzeri* n. sp. isolated from the reproductive tract of an adult female of *C. humeralis* from a rotting grapefruit on the ground from Riverside, San Bernardino County, California (33.92039° N; 117.44075° W) in 1984.

Type specimens

Holotype male and allotype female and additional material deposited at the University of California-Riverside Nematode Collection. Paratypes (males and females same data as holotype) deposited at the University of California, Davis; USDA Nematode Collection, Beltsville, Maryland; and the Nematology Department, Rothamsted Experiment Station, Harpenden Herts., England.

Etymology

This species name is derived from the family name of Dr. Edward G. Platzer, in his honor, for his mentoring of the first author and a distinguished career in nematology and parasitology at the University of California, Riverside.

Diagnosis

Bursaphelenchus platzeri n. sp. is distinguished from all other described species of *Bursaphelenchus* by the unique morphology of the spicules in males. Also, the difference in the number of lines in the lateral field between propagative forms (three lines) and J_{III} (four lines) appears to be unique.

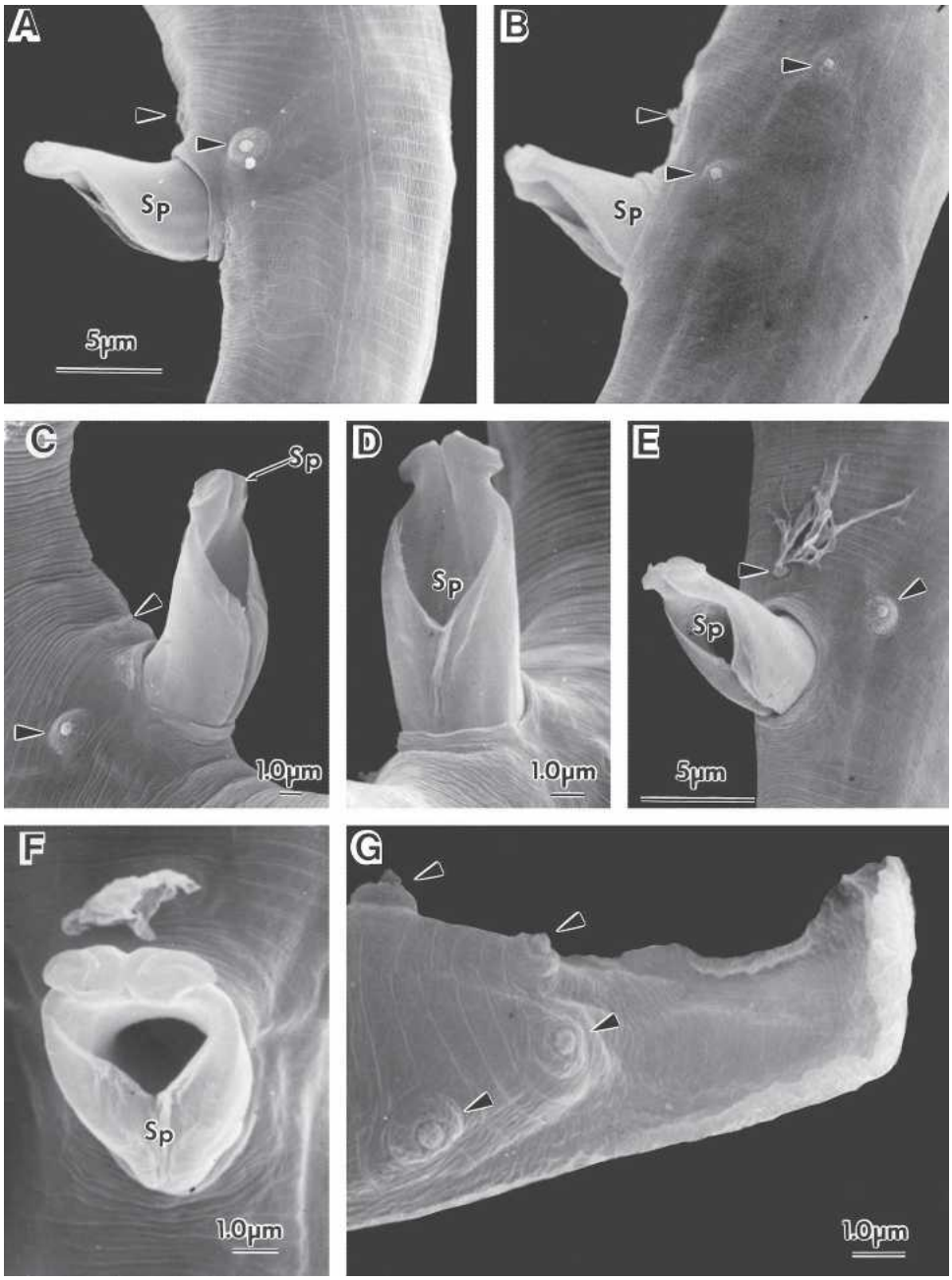

FIG. 6. Scanning electron micrographs of adult male tail region of *Bursaphelenchus platzeri* n. sp. A) Cloaca with fused spicules (Sp) protracted and lateral field in lateral view, arrows = single and first pair of preanal papillae. B) Rare specimen with extra pair of preanal papillae = top arrow, in lateral view. C) Cloaca with protracted fused spicules in subventral view, arrows = single and first pair of preanal papillae. D) Protracted fused spicules in posterior view. E) Cloaca with protracted fused spicules in subventral view, arrows = single preanal papilla with exudate and first pair of preanal papillae. F) Protracted tip of fused spicules in ventral view. G) Tail terminus with bursal flap (caudal ala) in nearly ventral view, arrows = second and third pairs of postcloacal caudal papillae.

Relationships

Bursaphelenchus platzeri n. sp. is closest to the red ring nematode, *B. cocophilus* (Cobb, 1919) Baujard, 1989, based upon trees inferred from SSU and combined SSU, LSU, and mtCOI 0-fold degenerate sequences and distantly allied with the xylophilus, the hunti, and the kevini groups (Giblin-Davis et al., 2005; Ye et al., 2006). These two species share characters such as anteriorly flattened and non-offset head regions, the apparent fusion and thickening of the distal ventral limbs of the spicules (Figs. 6,7), female tail shape elongate and subcylindrical with a rounded terminus, and phoresy with non-scolytid beetles. They can be separated by a more compact lip region in lateral view, with *B. platzeri* n. sp. possessing a width/height ratio of about 3.00 vs. 1.75 in *B. cocophilus*; the presence of three lines in propagative stages but four lines in J_{III} of *B. platzeri* n. sp. as opposed to *B. cocophilus*, which has four lateral lines in all stages; the lack of a vulval flap in females of *B. platzeri* n. sp.; and a discrete difference in the ratio a, which is less than 40 in adults of *B. platzeri* n. sp. but greater than 60

TABLE 1. Morphometrics of holotype and allotype in glycerol and male and female specimens each of *Bursaphelenchus platzeri* n. sp. in temporary water mounts (measurements in µm).

Measurement or ratio	Holotype (male)	Water mounts (males)	Allotype (female)	Water mounts (females)
n	1	20	1	20
Length (mean ± S.D.; Range)	979	759 ± 141.6 (521–1,032)	1,170	932 ± 153.6 (739–1,174)
Body width (at vulva for females) (males = GBW; females = VBW)	26	25 ± 4.2 (18–32)	47	27 ± 4.9 (20–37)
Stylet length	16	13 ± 0.9 (12–15)	17	14 ± 0.9 (13–16)
Esophagus length	89	75 ± 4.3 (68–84)	92	75 ± 3.6 (69–82)
Postuterine sac	—	—	52	103 ± 16.4 (76–137)
Vulval-to-anus distance (VA)	—	—	203	154 ± 25.9 (114–195)
Spicule length	29	19 ± 2.4 (16–23)	—	—
Spicule width	10	10 ± 1.2 (9–13)	—	—
Anal body width (ABW)	23	16 ± 2.1 (13–20)	18	13 ± 1.8 (10–16)
Tail length	62	49 ± 6.8 (39–66)	61	153 ± 22 (101–183)
a	38	31 ± 2.5 (25–34)	25	34 ± 2.5 (29–39)
b	11	10 ± 1.6 (8–13)	13	12 ± 2.0 (10–16)
c	16	15 ± 1.7 (12–19)	9	6 ± 1.0 (5–9)
V	—	—	77	67 ± 2.6 (62–71)

in *B. cocophilus*, indicating the thinner nature of the latter species. *Bursaphelenchus platzeri* n. sp. also is an obligate fungal feeder associated with nitidulid beetles but differs from *B. cocophilus* which is associated chiefly with the palm weevil *Rhynchophorus palmarum* and the only known obligate plant parasite of the genus.

TABLE 2. Morphometrics of second-stage (J_2) and dauer juveniles (J_{III}) of *Bursaphelenchus platzeri* n. sp. in temporary mounts in 1% acetic orcein or glycerol, respectively, from culture (measurements in µm).

Measurement or ratio	J_2	Dauer (J_{III}) male	Dauer (J_{III}) female
n	10	10	10
Length (mean ± S.D.; Range)	267 ± 21.7 (239–297)	471 ± 12.7 (455–496)	467 ± 16.6 (444–496)
Body width	11 ± 0.7 (10–12)	13 ± 0.4 (13–14)	13 ± 0.9 (12–15)
Stylet	10 ± 0.4 (10–11)	—	—
Esophagus length	39 ± 1.9 (36–42)	59 ± 2.8 (55–64)	59 ± 4.8 (50–67)
Gonad length	14 ± 2.3 (11–19)	32 ± 4.6 (22–38)	31 ± 3.7 (25–36)
Anal body width (ABW)	7.6 ± 0.5 (7–8)	9 ± 0.7 (8–10)	9 ± 0.7 (8–10)
Tail length	50 ± 4.6 (43–58)	88 ± 3.8 (82–93)	87 ± 3.1 (81–91)
Tail length/ABW	7 ± 0.7 (6–8)	10 ± 1.0 (8–12)	10 ± 1.0 (8–11)
a	24 ± 1.7 (20–27)	36 ± 1.6 (33–38)	36 ± 2.3 (31–39)
b	7 ± 0.5 (6–8)	8 ± 0.4 (7–8)	8 ± 0.5 (7–9)
c	5 ± 0.4 (5–6)	5 ± 0.2 (5–6)	5 ± 0.3 (5–6)

Biology

The life history of *B. platzeri* n. sp. is different from all other known species of *Bursaphelenchus* in that it is the only known species that is synchronously associated with nitidulid beetles from rotting fruit and soil environments (Giblin et al., 1984; Giblin, 1985).

Laboratory culture

Monilinia fructicola was a suitable host for *B. platzeri* n. sp. Control cultures remained clean of contaminants throughout the experiment. The mean yields per culture 7, 14, 21, and 28 d post inoculation were 24,631 ± 31,514 SD (range = 8,610–70,500), 195,766 ± 7,548 (range 187,100–200,900), 226,805 ± 75,550 (range 139,802–275,844), and 178,000 ± 44,027 (range 130,100–216,700), respectively. This suggests population dynamics similar to those reported for other species of *Bursaphelenchus* on *M. fructicola* (Giblin and Kaya, 1984).

DISCUSSION

Bursaphelenchus platzeri n. sp. has a haploid chromosome number of four ($2n = 8$) (Bolla and Boschert, 1993). The isolate cited in Bolla and Boschert (1993)

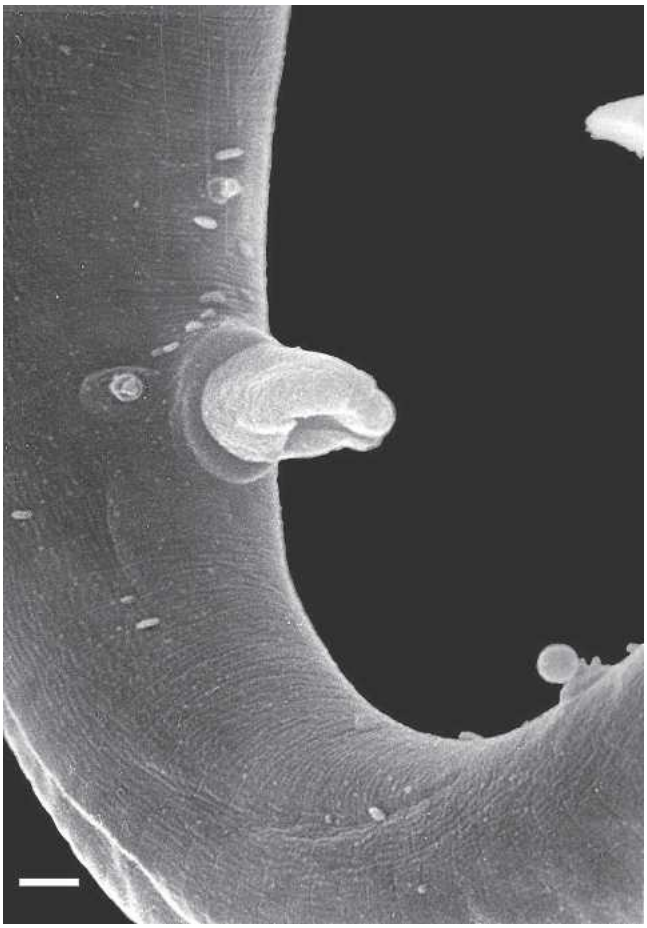

Fig. 7. Scanning electron micrograph of cloaca with protracted tip of fused spicules of an adult male of *Bursaphelenchus cocophilus* in subventral view (Scale bar = 1 μm).

was derived from the same culture stock as described herein (BNUH 1, subsequently called RGD 171). Unfortunately, *B. platzeri* n. sp. was incorrectly and alternately referred to in the tables and text of Bolla and Boschert (1993) as *B. nitidulans* (pp. 228, 231), *B. kevini* (p. 229, Table 1) and *B. nitidulus* (p. 234, Table 5) and incorrectly cited as being isolated from Sonoma County, California (p. 229, Table 1). *Bursaphelenchus platzeri* n. sp., *B. seani* Giblin and Kaya, 1983 ($2n = 16$), and *B. abruptus* Giblin-Davis, Mundo-Ocampo, Baldwin, Norden, and Batra, 1993 ($2n = 28$) were not compatible in crossing attempts with each other (excluding self-crosses that were successful) or with *B. mucronatus* Mamiya and Enda, 1979 ($2n = 10$) and diploid ($2n = 6$) and tetraploid ($2n = 12$) isolates of *B. xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970 (Bolla and Boschert, 1993). These results are not surprising based on molecular phylogenetic analyses (Giblin-Davis et al., 2005; Ye et al., 2006) and morphological data presented herein. *Bursaphelenchus platzeri* n. sp. is clearly within a clade that corresponds to the genus *Bursaphelenchus* but is only distantly allied with the xylophilus and hunti groups. In addition, *B. abruptus* has been shown to be basal in molecular phylogenetic trees of *Bursaphelenchus* (Kanzaki and Futai, 2002; Giblin-Davis et al., 2005; Ye et al., 2006), suggesting that morphological similarities to the xylophilus group represent character convergence (Giblin-Davis et al., 1993). Based upon the biological species concept and all that we know about *B. platzeri* n. sp., it should be reproductively incompatible with all nominal species of *Bursaphelenchus*. That interbreeding potential exists between some isolates of *B. mucronatus* and *B. xylophilus* confirms recent common ancestry of these two species within the xylophilus group (Bolla and Boschert, 1993).

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