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Morphology and systematics of *Bursaphelenchus gerberae* n. sp. (Nematoda: Parasitaphelenchidae), a rare associate of the palm weevil, *Rhynchophorus palmarum* in Trinidad

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Abstract

Bursaphelenchus gerberae n. sp., a rare associate of the American palm weevil, *Rhynchophorus palmarum* in Trinidad, is described and illustrated. Adults of *B. gerberae* n. sp. were examined with scanning electron microscopy (SEM) for ultrastructural comparisons with other members of the genus. *Bursaphelenchus paracorneolus*, *B. hofmanni* and *B. hylobianum* appear to be the closest related taxa to *B. gerberae* n. sp. based upon shared morphological features of male caudal papillae arrangement, general spicule morphology, female tail shape, and molecular analysis of the near-full length small subunit (SSU) rDNA, D2D3 expansion segments of the large subunit (LSU) rDNA and partial mitochondrial DNA COI. In addition to significant molecular sequence differences in SSU, LSU and mtCOI consistent with separate species status, *B. gerberae* n. sp. can be differentiated from *B. paracorneolus* by differences in bursa and condylus shape of males, and c' in females, from *B. hofmanni* by differences in rostrum and condylus shape and spicule curvature in males, and c' in females, and from *B. hylobianum* by differences in the number of lateral incisures, and rostrum shape, tapering and curvature of the spicule lamina, and cucullus shape in males. *Bursaphelenchus gerberae* n. sp. is also close to *B. corneolus*. Unfortunately, this isolate was not available for sequencing attempts, but differs from *B. gerberae* n. sp. in lack of stylet knobs in both sexes and relative postuterine sac length in females. Population growth of *B. gerberae* n. sp. was measured in a time-course experiment at 23° C in the laboratory on cultures of the fungus, *Monilinia fructicola* grown on lactic acid treated, 5% glycerol-supplemented potato dextrose agar (LGPDA). Nematode counts rapidly increased from 500 to about 600,000 per plate within 14 days and then dropped to and plateaued at about 300,000 for up to 28 days.

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Key words: *Bursaphelenchus gerberae* n. sp., Curculionidae, morphology, Parasitaphelenchidae, phylogeny, *Rhynchophorus palmarum*, scanning electron microscopy, taxonomy

Introduction

A new, mycophagous species of *Bursaphelenchus* was recovered as 160 dauer juveniles (J_{IIIs}) from the dissection of an adult male of the American palm weevil, *Rhynchophorus palmarum* L. from Manzanilla, Trinidad (Gerber & Giblin-Davis 1990A). This nematode is described herein as *Bursaphelenchus gerberae* n. sp. It is the second described species of *Bursaphelenchus* known to be associated with palm weevils in the Neotropics, the other being the causative agent of red ring disease of palms, *B. cocophilus* (Cobb 1919) Baujard 1989. Most *Bursaphelenchus* are known as associates of scolytid bark beetles, cerambycid longhorn beetles, nitidulid beetles, or soil dwelling bees (Ye *et al.* 2006).

The weevil host, *R. palmarum*, is one of about ten species of *Rhynchophorus* known from the world. It is considered the chief vector for the nematode-caused and economically damaging red ring disease of palms which is distributed in Mexico south through Central America into South America and the Lesser Antilles (Giblin-Davis 2001). *Bursaphelenchus gerberae* n. sp. was reported from 5% of the extracted cocoons of *R. palmarum* (n = 76) and from one adult out of 61 (2%) from a study of the association of red ring nematode, *B. cocophilus* with *R. palmarum* in Trinidad (Gerber & Giblin-Davis 1990A). This is relatively rare compared with the >90% association of *B. cocophilus* and >50% association of the phoretic nematodes, *Teratorhabditis palmarum* Gerber & Giblin-Davis 1990B and *Acrostichus* (=*Diplogasteritus*) sp. with *R. palmarum* in that study (Gerber & Giblin-Davis 1990A,B). This low association level suggests that *R. palmarum* may be a surrogate phoretic host of *B. gerberae* n. sp. In some circumstances with lower levels of host synchronization and host specificity, different insects that co-occur in the same micro-environment can serve as surrogate commensal hosts. There are many insects that are associated in dying palms with *R. palmarum* that could be the preferred/normal phoretic host for *B. gerberae* n. sp., including ambrosia beetles in the families Scolytinae and Platypodinae (Howard *et al.* 2002).

A meaningful phylogenetic hypothesis for the relationships within the genus *Bursaphelenchus* has been proposed through 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 providing for the justification of new species status and phylogenetic placement of *B. gerberae* n. sp. (= *B.* sp. ex *Rhyncophorus palmarum* 169 Trinidad) (Giblin-Davis *et al.* 2005; Ye *et al.* 2006).

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

Materials and methods

A culture of *B. gerberae* n. sp. was initiated from dauer juveniles isolated from a newly-emerged adult male *R. palmarum* from Manzanilla, Trinidad (10.49805° N; 61.04824° W) onto *M. fructicola* on potato dextrose agar (PDA). All phylogenetic and taxonomic research are based upon this isolate which has been maintained through sub-culturing on *M. fructicola* on lactic acid treated, 5% (v/v) glycerol-supplemented potato dextrose agar (LGPDA) since 1988 (Gerber & Giblin-Davis 1990A). Nematodes were collected in sterile water from the agar surface, quantified, and aseptically pipetted into 7-day-old cultures of *M. fructicola* on LGPDA. Cultures of *B. gerberae* n. sp. were maintained at 23–25° C and used for all subsequent studies, unless otherwise stated.

Adults of *B. gerberae* n. sp. were collected from 27 to 33-day-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 27 to 33-day-old cultures, fixed in 5% formaldehyde for at least 24 hours, and processed slowly into glycerol before measurement (Southey, 1970). Male spicule terminology used in this description has been previously described (Ryss *et al.* 2005, Yin *et al.* 1989). 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, unless otherwise stated.

For SEM observations, adult males and females of *B. gerberae* n. sp. were collected from culture on a Baermann funnel, heat-killed, and 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 slowly 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 Hitachi S-4000 SEM 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 on LGPDA in 9-cm-diam. petri plates and grown for 7 days at 23° C before inoculation with 500 *B. gerberae* n. sp. in 1 drop of sterile water. Two replicate cultures were subsampled and harvested for 4 to 5 hours on a Baermann funnel at 3 to 7 day intervals for 4 weeks after inoculation and a measured aliquot of the nematodes was counted on a dissecting scope.

Description of *Bursaphelenchus gerberae* n. sp. (Figs. 1–4)

Measurements were made of male and female specimens in temporary water mounts (Table 1).

Morphological description

Males: 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 only described in detail for the male. Cuticle with fine annulation, annules about 0.7-0.8 µm wide at midbody (Figs. 1C, 3A). Lateral field with three incisures (Fig. 1C, 3A), beginning just above level of metacorpus, extending posteriorly to level of ventral preanal papilla; less distinct from ventral preanal papilla to beginning of bursal flap (= caudal alae) at level of subventral pair of postanal papillae (P3). Head distinctly offset from body, lip region in lateral view less than 2.5 times wider than tall (Figs. 1C, 2A). 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 slit on each subdorsal and subventral side (Fig. 2B–C). These slits and indentations may represent the inner labial sensillae. Labial disc surrounded by circular plate comprised of fused lateral, subventral and subdorsal lip sectors, about 1.6-1.7 µm in diameter (Fig. 2). Labial sensillae obscured; a composite of several SEM en face patterns suggests two lateral outer labial sensillae open mediolaterally onto circular lip sector plate (Fig. 2). Circular lip sector plate surrounded by hexaradiate cephalic sectors, two subdorsal, two lateral, and two subventral (Fig. 2). 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 (Fig. 2). Six to eight transverse striae visible on head with SEM (Fig. 2). Stylet two part; cone short, about 2/5 total stylet length, shaft with slight basal thickenings (Fig. 1C). Procorpus cylindrical, about two and one half 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 length above metacorpal valve (Fig. 1C). Esophago-intestinal junction less than one metacorpal valve width behind metacorpus. Postcorpus glandular, overlaps intestine dorsally about 3 metacorpal lengths long (Fig. 1C). Excretory pore posterior to nerve ring, hemizonid about one stylet length behind excretory pore (Fig. 1C). Gonad reflexed, sperm amoeboid (Fig. 1B). Tail arcuate, about 2.6 anal body widths long; terminus claw-like from lateral view (Figs. 1B,H). Spicules separate, ventrally arcuate with gradual lamina taper from calomus to tip, rostrum sharply-pointed to conical, condylus elongate and truncate with slight posterior recurvature, distal ventral end with small cucullus and in SEM with apparent lateral projection (Fig. 3A–B), spicule length measured along its arc/capitulum width (distance between condylus and rostrum) ratio is about 2.1; spicule length measured along its arc/spicule width posterior to rostrum is about 2.4; junction of rostrum and calomus smoothly curved; lamina complex with midpoint widened and rounded; lamina dorsal contour often smoothly and symmetrically curved (Figs. 1H, J–O); ratio of depth of capitulum depression divided by the capitulum width about 0.15. Spicules are recurved so that a line drawn through the anterior-most points of the condylus and rostrum (along the capitulum) and extending the posterior dorsal lamina intersects ventrally at less than a 14° angle (Figs. 1J–O, 4C). Seven preanal and postanal papillae present; one preanal papilla (P1) in ventral midline about 2 µm above cloaca

(Figs. 1G–H, 3A–B), one pair subventral preanal papillae (P2) in line with P1 papilla (Figs. 1G–H, 3A–B), one subventral pair of postanal papillae (P3) at about 55% of a tail length behind cloaca (Figs. 1G–H, 3A), one small ventral pair of papillae (P4) about 22–24 µm from tail terminus (Figs. 1G–H, 3A,C). Occasional asymmetry was observed in P3 and P4 papillae arrangement (Fig. 3A,C). Bursa rounded in ventral view and pointed in lateral view (Figs. 1G–H, 3A,C).

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

Measurement or ratio	Water mounts (males)	Water mounts (females)
n	15	15
Length (mean ± S.D.; range)	603.2 ± 43.7 (520–669)	758.1 ± 43.0 (680–817)
Body width (at vulva for females) (males = GBW; females = VBW)	18.5 ± 1.7 (16–22)	22.2 ± 1.6 (20–25)
Stylet length	13.1 ± 0.4 (12–14)	14.1 ± 0.6 (13–15)
Esophagus length (to end of metacorpus)	61.1 ± 3.4 (55–68)	64.5 ± 4.9 (56–71)
Postuterine sac length	-	66.1 ± 18.0 (45–97)
Vulva to anus distance (VA)	-	130.8 ± 14.7 (103–146)
Spicule length	12.7 ± 0.9 (10–14)	-
Anal body width (ABW)	14.0 ± 0.9 (13–16)	9.2 ± 0.6 (8–10)
Tail length	35.8 ± 2.2 (32–39)	44.0 ± 3.2 (40–50)
a	32.9 ± 4.0 (28–43)	34.3 ± 2.5 (30–40)
b	9.9 ± 0.7 (8–11)	11.8 ± 0.7 (11–13)
c	16.9 ± 1.3 (15–20)	17.1 ± 1.3 (15–19)
c'	2.6 ± 0.2 (2.3–2.9)	4.8 ± 0.3 (4–6)
V (%)	-	77 ± 1.3 (75–79)

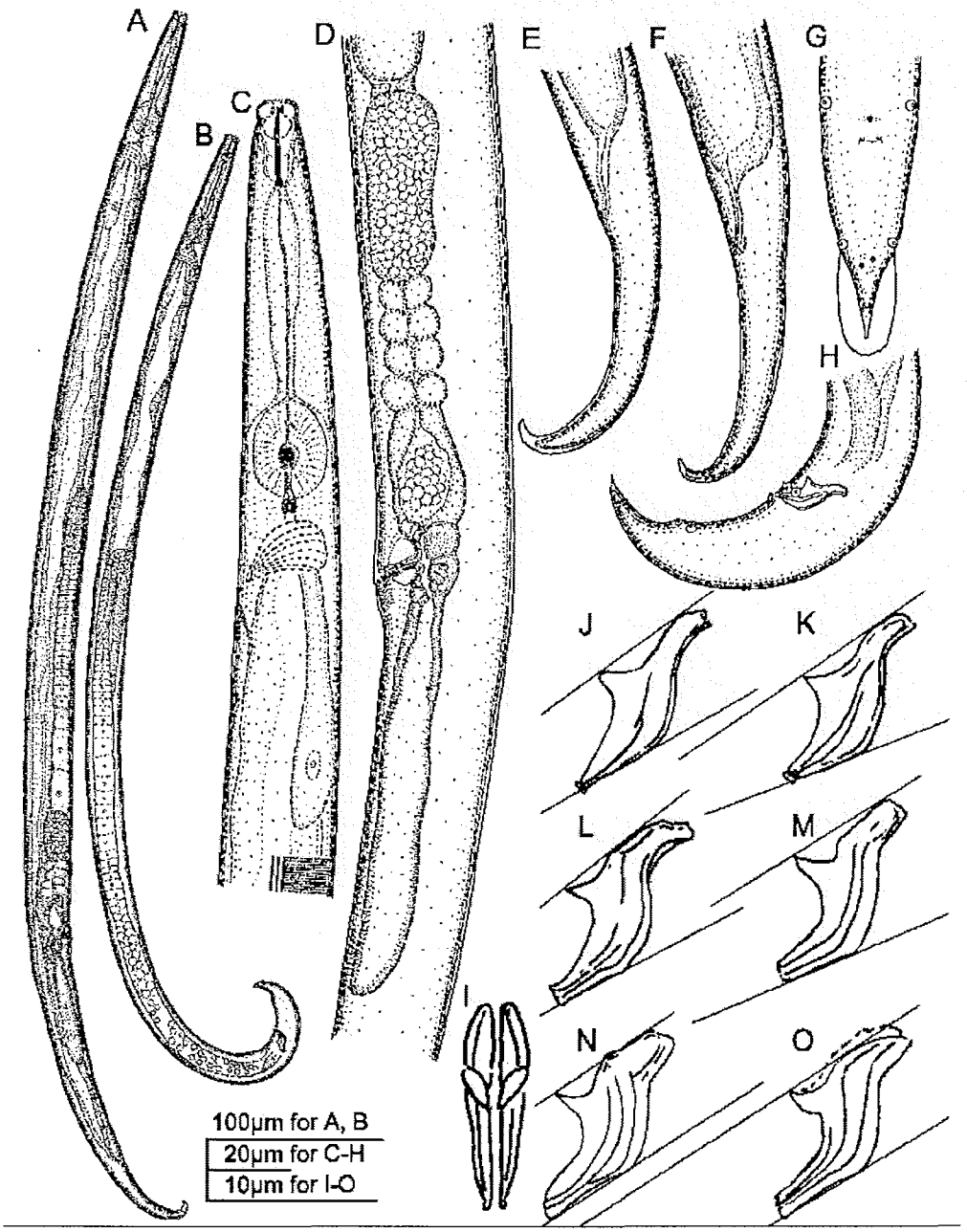

FIGURE 1. Adult females and males of *Bursaphelenchus gerberae* n. sp. in lateral view (except where noted). A) Whole female. B) Whole male. C) Anterior body of female. D) Vulva, vagina, postuterine sac of female reproductive system. E,F) Female tail variability G) Male tail (ventral view). H) Male tail. I) Spicules (ventral view). J–L) Different focal planes through spicules of single male. M–O) Different focal planes through spicules of different male specimen. J–O) Lines drawn through the anterior-most points along the capitulum and extending the posterior dorsal lamina for each spicule are shown.

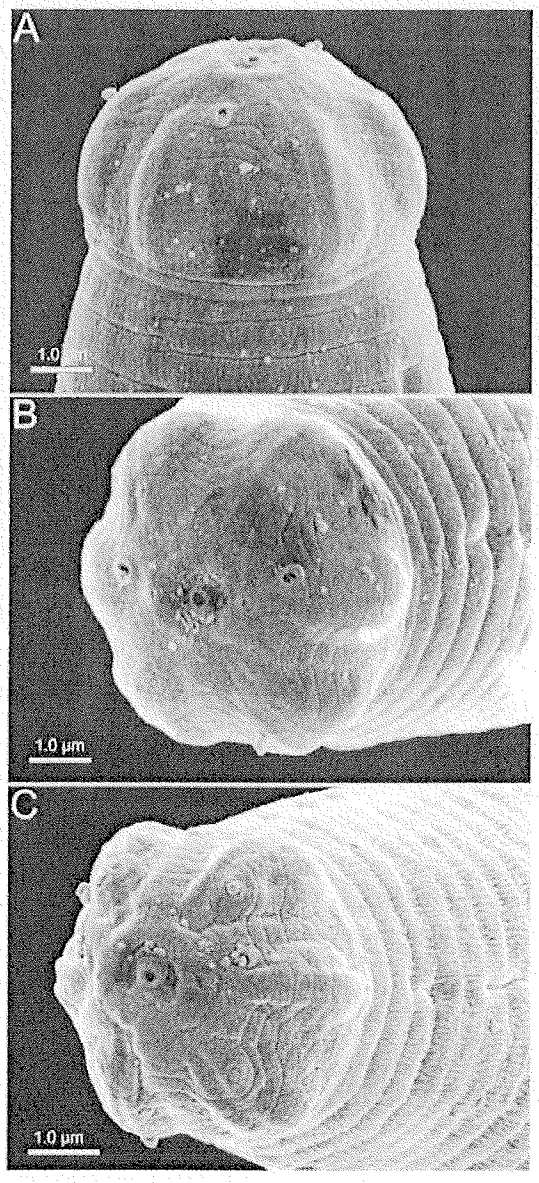

FIGURE 2. Scanning electron micrographs of adult females of *Bursaphelenchus gerberae* n. sp. A) Head, lateral view. B) Almost en face view of same female. C) Almost en face view of different female.

Females: Body ventrally arcuate or straight when killed by heat treatment (Fig. 1A). Lateral field with three incisures; decreases to two incisures posterior to level of anus; extending almost to tail terminus. Ovary single, anteriorly outstretched, oocytes in single file except at anterior half of ovary (Fig. 1A). Vulva dome-shaped slit in ventral view which gives the appearance of a small vulval flap in lateral view, but is not a true vulval flap (Fig. 1D). Vagina straight with anteriorly projected incline in lateral view, paired and

three-celled structures at back of uterus facing vagina (Fig. 1 D). Postuterine sac about 3 vulva body diameters long, about 54% of vulval-anus distance, sometimes filled with sperm (Fig. 1A,D). Anus dome-shaped slit in ventral view. Tail strongly recurved ventrally, about 4.8 times longer than anal body width, and finely rounded to digitate (Figs. 1A, E–F, 4B).

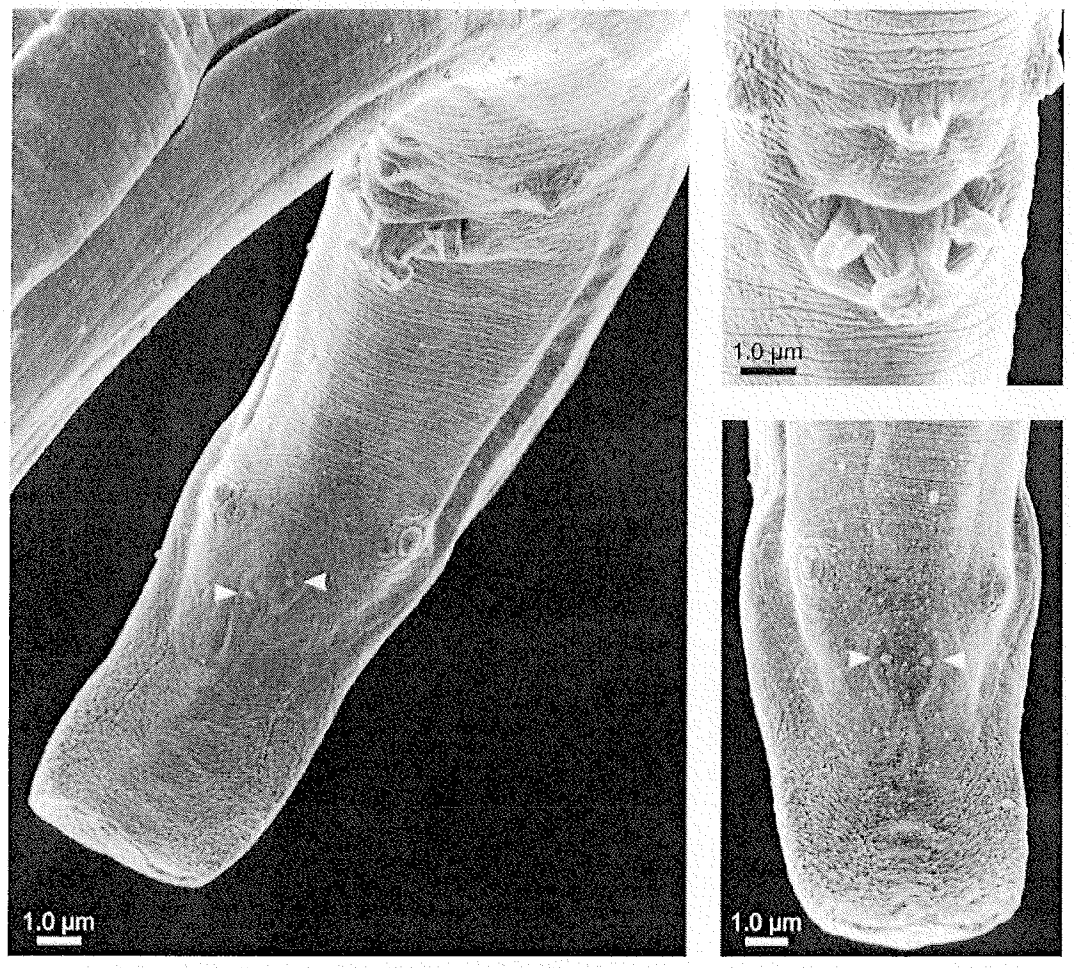

FIGURE 3. Scanning electron micrographs of adult male tail region of *Bursaphelenchus gerberae* n. sp. A) Almost ventral view of tail with protracted spicules with lateral projections and cucullae, asymmetric presentation of paired P3 papillae and P4 "glandpapillae", rounded bursal flap, and lateral incisures, arrows = P4 papillae. B) Almost ventral view of different mail tail with protracted spicules with lateral projections and cucullae and single preanal P1 and preanal pair of P2 papillae. C) Ventral view of different male tail tip showing rounded bursal flap and symmetrical pairs of P3 papillae and P4 "glandpapillae" (= arrows).

Diagnosis

Bursaphelenchus gerberae n. sp. is distinguished from other described species of *Bursaphelenchus* by significant molecular sequence differences in the near-full length

small subunit (SSU) rDNA (GenBank accession number = AY508024), D2D3 expansion segments of the large subunit (LSU) rDNA (AY508092) and partial mitochondrial DNA COI (AY508055) (Giblin-Davis *et al.* 2005; Ye *et al.* 2006). In addition, *B. gerberae* n. sp. can be differentiated from those *Bursaphelenchus* that are closest to it morphologically and molecularly by a combination of character differences in stylet, rostrum, condylus, and lamina shape of male spicules, and c' and post-uterine sac length in females.

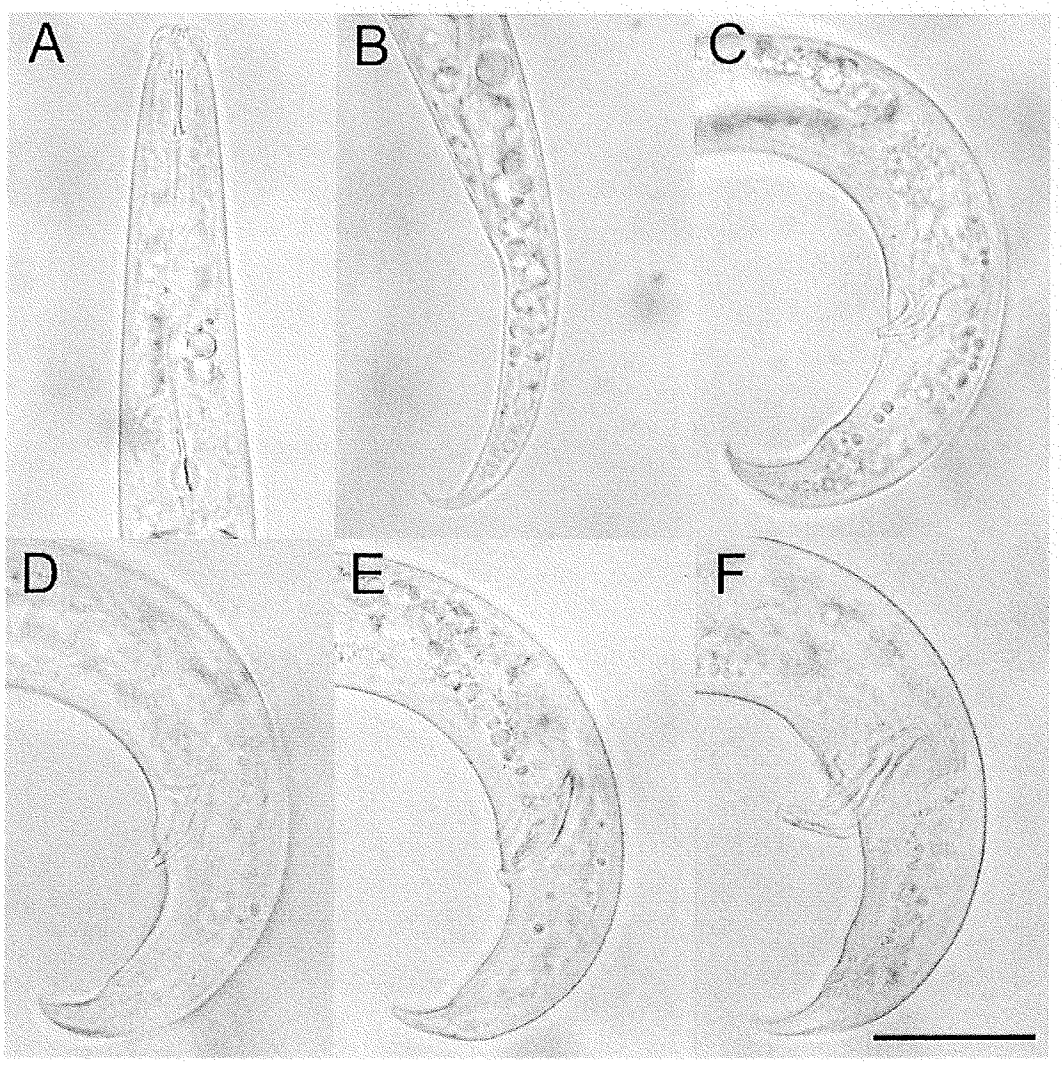

FIGURE 4. Photomicrographs of adults of *Bursaphelenchus* in lateral view. A–C) *Bursaphelenchus gerberae* n. sp. A) Anterior region of female. B) Female tail. C) Male tail with spicules. D) *Bursaphelenchus hofmanni* male tail with spicules. E) *Bursaphelenchus paracorneolus* male tail with spicules. F) *Bursaphelenchus hylobianum* male tail with spicules.

Relationships

Bursaphelenchus gerberae n. sp. is closest to *Bursaphelenchus hylobianum*

Korentchenko 1980, *B. paracorneolus* Braasch 2000 and *B. hofmanni* Braasch 1998 based upon a tree inferred from near full length SSU sequences from 37 isolates of 20 species (Giblin-Davis *et al.* 2005; Ye *et al.* 2006). These species and *B. corneolus* Massey 1966, which has not been sequenced, share the morphological characters of three lateral incisures (except *B. hylobianum* with two and *B. corneolus* with an unknown number), male caudal papillae arrangement (single preanal P1 papilla, pair of pre-/adanal P2 papillae, pair of postanal P3 papillae, and one pair of small P4 papillae at base of bursa), separate mitten-shaped spicules with cucullae, female vulva a dome-shaped slit in ventral view which gives the appearance of a small vulval flap in lateral view, but is not a true vulval flap, and female tail strongly ventrally recurved. In addition to significant molecular sequence differences (Giblin-Davis *et al.* 2005; Ye *et al.* 2006), *Bursaphelenchus gerberae* n. sp., which possesses a stylet with small basal swellings, rounded bursa in ventral view, a pointed rostrum and an elongate and truncate condylus with slight posterior recurvature, spicules recurved so that lines drawn through the anterior-most points along the capitulum and extending the posterior dorsal lamina intersects ventrally at about a 14° angle (Figs. 1J–O, 4C), postuterine sac about 3 vulva body diameters long (54% of vulval-anus distance), and a female tail c' of 4.8 (4.4–5.6), can be differentiated from *B. paracorneolus* because it possesses a bursa with a truncate posterior edge in ventral view, condylus rounded with no posterior recurvature (Fig. 4E), and a female tail c' of less than 3.8. *Bursaphelenchus hofmanni* can be differentiated from *B. gerberae* n. sp. because of the following characteristics; a digitate rostrum and rounded condylus with no posterior recurvature, spicules recurved so that lines drawn through the anterior-most points along the capitulum and extending the posterior dorsal lamina intersects dorsally at 10–29° (Fig. 4D), and a female tail c' of less than 3.4. *Bursaphelenchus hylobianum* can be separated from *B. gerberae* n. sp. because both sexes have two lateral incisures and males possess spicules with a blunt and rounded rostrum and the cucullus shape gives spicule tips a characteristic broadly truncate appearance in lateral view (Fig. 4F). Lastly, *B. corneolus* differs from *B. gerberae* n. sp. because it lacks basal stylet swellings in both sexes and the postuterine sac is about 4.4 vulva body diameters long or about 67% of vulval-anus distance in females.

Biological characteristics

The life history of *B. gerberae* n. sp. is different from all other known species of *Bursaphelenchus* in that it is the only known mycophagous species that is associated with the palm weevil, *Rhynchophorus palmarum* (Gerber & Giblin-Davis 1990A). *Bursaphelenchus cocophilus*, the other *Bursaphelenchus* species known to be associated with *R. palmarum*, is an obligate plant parasite. Although coconut is the plant host, it was a dying tree, which may or may not be important relative to the natural history of this nematode.

Type host and locality

Holotype male and allotype female and paratypes are from a 28-day-old culture on *M. fructicola*. The original culture was started with dauer juveniles of *B. gerberae* n. sp. isolated during a dissection of *R. palmarum* from a red ring diseased coconut palm from Manzanilla, Trinidad (10.49805° N; 61.04824° W) in 1988.

Type designations

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. Karin Gerber, in her honor, for her discovery of this nematode during her postdoctoral research in Trinidad with the first author.

Laboratory culture

Monilinia fructicola was a suitable host for *B. gerberae* n. sp. The mean yields per plate after 7, 14, 21, and 28 days post inoculation were 63,000 ± 26,576, 601,800 ± 14,425, 269,200 ± 49,215, and 351,900 ± 46,188, respectively. This suggests similar population dynamics to what has been reported for other species of *Bursaphelenchus* on *M. fructicola* (Giblin & Kaya 1984).

Discussion

Bursaphelenchus gerberae n. sp. shares several morphological synapomorphies with *B. hylobianum, B. paracorneolus*, *B. hofmanni*, and *B. corneolus*, and molecular data, where available, supports this assertion (Giblin-Davis *et al.* 2005; Ye *et al.* 2006). Unfortunately, the descriptions and diagnoses of *Bursaphelenchus* have been uneven in terms of the elucidation of relevant morphological characters so that important patterns may have been obscured. When possible, examination of type material has proven invaluable, but in many cases, types are not available (Braasch 1998). The main derived characters for this group appear to be three lateral incisures (except *B. hylobianum* with two and *B. corneolus* with an unknown number), characteristic caudal papillae arrangement in males with a total of seven papillae (single preanal P1 papilla, pair of pre-/adanal P2 papillae, pair of postanal P3 papillae, and one pair of small P4 papillae "glandpapillae" on a raised plate at base of bursa), separate mitten-shaped spicules with cucullae, female vulva dome-shaped slit in ventral view which gives the appearance of a small vulval flap in lateral view, but is not a

true vulval flap, and female tail strongly ventrally recurved. This is somewhat consistent with the diagnostic features used by Braasch (2001) for a subset of *Bursaphelenchus* species designated as the *B. hofmanni* group known from conifers in Europe. If we broaden the group to include American (e.g. *B. corneolus*) and Asian species (e.g. *B. hylobianum*) and update the descriptions of a few species where type specimens or cultures are available, the proposed synapomorphies above make more sense. For example, examination of type material of *B. corneolus* (slides 37-L and M) confirms the presence of a P1 adanal male caudal papilla (not reported by Massey 1966, 1974) and a pair of adanal P2 papillae, and two pairs of postanal papillae (P3, P4), with P4 appearing to be very small on a raised plate at the base of the bursa. In addition, the vulval flap described by Massey appears to be consistent with our description for *B. gerberae* n. sp. which appears as a vulval flap laterally but actually represents a dome-shaped slit that leads directly into the vagina when viewed in ventral view. The number of incisures in the lateral field was not observed on the types of *B. corneolus*. Observations of specimens from cultures of *B. hylobianum*, *B. paracorneolus*, and *B. hofmanni* obtained from Dr. H. Braasch confirm the presence of a cucullus on spicules of *B. hylobianum* (not reported in the original description by Korenchenko 1980 or in the recent synopsis of the genus by Ryss *et al.* 2005, but reported in Braasch & Braasch-Bidsak 2002). We also confirmed the presence of what appears laterally as a small vulva flap in *B. hylobianum, B. paracorneolus*, and *B. hofmanni* but is actually a dome-shaped slit that leads directly into the vagina, in ventral view. In lateral view, the region below the vulva is protuberant and dome shaped.

In contrast to Braasch (1998), who reported only two pairs of male caudal papillae for *B. hofmanni*, we were able to confirm the above-mentioned synapomorphic arrangement of seven caudal papillae with P4 as a small pair at the base of the bursa. We were also able to confirm the configuration of seven male caudal papillae in *B. hylobianum* and *B. paracorneolus*. Originally, Korenchenko (1980) described nine caudal papillae (an extra pair of small papillae [="glandpapillae", see Ryss *et al.* 2005] at the base of the bursa) but Braasch & Braasch-Bidasak (2002) reported only five. In lateral view with light microscopy, the synapomorphic P4 pair of papillae (="glandpapillae") at the base of the bursa can be confusing because of their very small size and the presence of a raised cuticular plate, sometimes giving the appearance of two pairs of papillae or they can be missed entirely. SEM clearly identifies this pair of papillae but their status as "glandpapillae" with a secretory or neurosecretory function remains to be confirmed (Fig. 3A,C). However, the shape of the plate in ventral view, is a rounded square giving the appearance in lateral view of a papilla at each corner (sometimes looking like 4 papillae) or given the medial position of the P4 pair (sometimes appearing as six papillae). This situation can be further complicated by the fact that the two papillae on the plate can sometimes be asymmetric (Fig. 3A). An unfortunate consequence of coding the character matrix of *Bursaphelenchus* from original descriptions without reference to type material

(Ryss *et al.* 2005; Tarjan & Baeza-Aragon 1982) is that oversights in these descriptions are perpetuated causing confusion and a loss of potentially important morphological information.

According to an analysis of general spicule similarity by Ryss *et al.* (2005), spicules of *B. corneolus*, *B. paracorneolus*, and *B. hofmanni* cluster in the same general grouping but *B. hylobianum* does not. Braasch & Braasch-Bidasak (2002) suggested that because *B. hylobianum* has two incisures it should be grouped together with *B. abietinus* Braasch & Schmutzenhofer 2000 which also has two incisures. This contrasts with the molecular phylogenetic hypothesis of Giblin-Davis *et al.* (2005) and Ye *et al.* (2006), where there is very strong support in three genes for *B. abietinus* and *B. hellenicus* Skarmoutsos, Braasch & Michalopoulou 1998 (with three incisures) sharing a most recent common ancestor and for *B. hylobianum* and *B. paracorneolus* with differing characters in the lateral field doing the same.

At present, very few *Bursaphelenchus* have been examined using SEM of protracted or excised spicules (Penas *et al.* 2004). The presence of what appears as lateral projections of the lamina of the spicules of *B. gerberae* n. sp. (Fig. 3A,B) may be an autapomorphic character. Currently, the only other species of *Bursaphelenchus* that have been observed in intact specimens with sufficient exposure of the spicules beyond the cloaca are *B. abruptus* Giblin-Davis *et al.* 1993, *B. cocophilus* and *B. xylophilus* (Steiner & Buhrer 1934) Nickle 1970 (Giblin-Davis *et al.* 1989, 1993; Nickle *et al.* 1981). Of these, only *B. abruptus* has clear evidence of similarly-shaped projections (Giblin-Davis et al. 1993). The SEM observations of excised spicules from eleven species of *Bursaphelenchus* by Penas *et al.* (2004) involved use of lactic and acetic acid and did not reveal these lateral projections. More observations are needed to confirm whether these structures are components of the spicules or the spicular pouch, remnants of a gubernaculum, some other manifestation of the male copulatory apparatus, or artifacts.

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