Observations on the morphology of the red ring nematode, *Rhadinaphelenchus cocophilus* (Nemata : Aphelenchoididae)[1]

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SUMMARY

The morphology of adult males and females of the red ring nematode, *Rhadinaphelenchus cocophilus* (Cobb, 1919) Goodey, 1960, was examined with light (LM) and scanning electron microscopy (SEM). Nematodes were isolated and compared from stem tissue of red ring nematode-diseased coconut, *Cocos nucifera* L., from a plantation in Manzanilla, Trinidad and from infested oil palm, *Elaeis guineensis* Jacquin, from San Felipe, Venezuela. No differences in morphology were observed between the adults from either palm host. The heads of both males and females were domed with fine annular striations. *En face* patterns exhibited an oral aperture surrounded by a modified labial disc, six lips, and a dorso-ventrally flattened cephalic region with six lip sectors. Inner and outer labial sensillae were not observed but two amphid apertures and four cephalic papillae were resolved with SEM. A vulval flap was present in all females examined. Both sexes had transverse annular striations of the cuticle and the lateral field had four incisures. Seven caudal papillae were observed in males : a newly observed ventral preanal papilla, one pair of subventral preanal or adanal papillae, and two pairs of subventral postanal papillae. The distal ends to the spicules were separate and appeared heavily sclerotized. The caudal alae formed a spade-shaped flap (= bursal flap) which was annulated dorsally. The similarities of the morphology of *R. cocophilus* to species of *Bursaphelenchus* and other aphelenchoids are discussed.

RÉSUMÉ

Observations sur la morphologie du nématode de l'anneau rouge du cocotier, Rhadinaphelenchus cocophilus *(Nemata : Aphelenchoididae)*

La morphologie des mâles et des femelles du nématode de l'anneau rouge du cocotier, *Rhadinaphelenchus cocophilus* (Cobb, 1919) Goodey, 1960, a été étudiée en microscopies optique et électronique à balayage (MEB). Des comparaisons ont été faites entre nématodes extraits *i)* de troncs de cocotiers, *Cocos nucifera* L., atteints de l'anneau rouge, provenant d'une plantation à Manzanilla, Trinidad, et *ii)* de palmiers à huile, *Elaeis guineensis* Jacquin, infestés provenant de San Felipe, Venezuela. Aucune différence morphologique n'a été observée entre les individus adultes de ces deux provenances. Chez les deux sexes, la tête est en forme de dôme et porte de fines stries annulaires. La face présente une ouverture buccale entourée par un disque labial modifié, six lèvres et une région céphalique aplatie comportant six secteurs labiaux. Les sensilles labiales internes et externes n'ont pas été observées, mais le MEB permet la localisation des quatre papilles céphaliques et des deux ouvertures amphidiennes. Des volets vulvaires sont présents chez toutes les femelles. Chez les deux sexes, la cuticule est annelée et le champ latéral comporte quatre incisures. Sept papilles caudales sont présentes chez le mâle : une papille ventrale préanale, observée pour la première fois, une paire de papilles subventrales (préanales ou adanales) et deux paires de papilles subventrales postanales. Les extrémités distales des spicules ne sont pas soudées et paraissent fortement sclérotisées. Les ailes caudales (bourse) sont en forme de pelle et annelées sur la face dorsale. Les ressemblances morphologiques entre *R. cocophilus* et les espèces appartenant au genre *Bursaphelenchus* sont discutées.

Rhadinaphelenchus cocophilus (Cobb, 1919) Goodey, 1960, the red ring or coconut palm nematode, causes the red ring disease of coconut palm, *Cocos nucifera* L., and the oil palm, *Elaeis guineensis* Jacquin and is reported to occur naturally in wild palms such as *Oenocarpus distichus* Mart. (Schuiling & van Dinther, 1982). In addition, the red ring nematode has been associated with « little leaf » symptomology in coconut and oil palms (Hoof & Seinhorst, 1962). The nematode is vectored in the juvenile stage by the palm weevil, *Rhynchophorus palmarum* (L.), and transmission to a new host occurs during oviposition by a nematode-infested female weevil (Griffith, 1987). The nematode causes reddish lesions to form in the stem which gradually enlarge and often form

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a characteristic red ring when the cut stem is viewed in cross-section (Griffith, 1987). In Trinidad, red ring diseased coconut palms usually die within two months of infection (Griffith 1987).

Rhadinaphelenchus cocophilus is a long, thin nematode; the females and males have been reported to be 60-96 and 65-179 times longer than wide, respectively, with the greatest body width being less than 15.5 µm (Cobb, 1919; Lordello & Zamith, 1954; Goodey, 1960; Thorne, 1961; Nickle, 1970; Brathwaite & Siddiqi, 1975). Most of the common diagnostic characters for the family Aphelenchoididae are difficult to see in *R. cocophilus* with a light microscope (LM) because of fixation problems and the thinness of the nematode. The lip region is very narrow and fragile which makes it difficult to resolve potentially significant taxonomic features with LM or scanning electron microscopy (SEM).

Our preliminary observations of adults of *R. cocophilus* from Trinidad indicated possible contradictions with previously published observations and descriptions of *R. cocophilus* (Cobb, 1919; Lordello & Zamith, 1954; Goodey, 1960; Thorne, 1961; Nickle, 1970; Brathwaite & Siddiqi, 1975). In addition, we have not found any SEM observations of this monotypic genus published in the literature. The objective of this project was to examine the morphology of adult males and females of *R. cocophilus* with SEM and LM from two palm species from different geographical regions for comparison with previous observations and to serve as a reference for future comparisons with geographical and (or) host isolates of the red ring nematode.

Materials and methods

Red ring nematode-diseased coconut palms (three to five years old) were collected from the Cocal plantation in Manzanilla, Trinidad. Oil palms (29 years old) were collected from San Felipe, Venezuela. Palm stems without apparent damage by weevil larvae were cut longitudinally. Tissue possessing pink lesions that had not coalesced was chopped into thin, 3.0 × 3.0 × 0.5 cm chunks with a cutlass. The tissue was soaked in tap water for *ca* 4 h and decanted over nos. 12 and 400 USA standard testing sieves. The nematodes were backwashed off the no. 400 sieve into a large Baermann funnel apparatus with a piece of cotton positioned at the stem base of the funnel. The apparatus was allowed to sit overnight. Adults often represented more than 5 % of the specimens in these preparations. Some of the adult males and females of *R. cocophilus* were hand-picked from the suspension into a counting dish and a weak ammonia solution (0.3 N) was added to induce stylet and spicule protrusion (Hooper, 1977). The nematodes were heat-killed and placed either into 5 % formaldehyde or 3 % glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2). Fixed specimens were transported to the University of California, Riverside where they 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 JEOL 35C SEM at 15 kV. Some specimens were postfixed in 2 % OsO_4 to improve preservation of the lip region.

Specimens in 5 % formaldehyde were processed into lactophenol for permanent mounts (Esser, 1973) for photomicrographs and for LM observations. Photomicrographs were taken with an Olympus PM-10-A automatic camera attached to a Zeiss standard microscope. Scale was determined by photographing a stage micrometer.

Results

LM and SEM observations did not reveal any morphological differences between adults of *R. cocophilus* taken from red ring diseased coconut or oil palms. Consequently, all of the micrographs in Figs 2 to 5 are of *R. cocophilus* that were extracted from red ring diseased coconut palms from Trinidad.

Head morphology

En face patterns were identical among females, males, and juveniles of *R. cocophilus*. The pattern consisted of a labial disc surrounded by six lips (= lip sectors) which, in turn, was surrounded by six cephalic sectors (Figs 1; 2 A, B). The *en face* patterns of *R. cocophilus* included an oral aperture surrounded by a labial disc which was specialized by dorso-ventral elongation and small indentations on each lateral side (Figs 1; 2 A, B). Inner labial sensillae were not resolved on the labial disc. The labial disc was surrounded by six lips; two subdorsal, two subventral, and two lateral. The lateral lips were larger than the subdorsal and subventral lips and the lip region

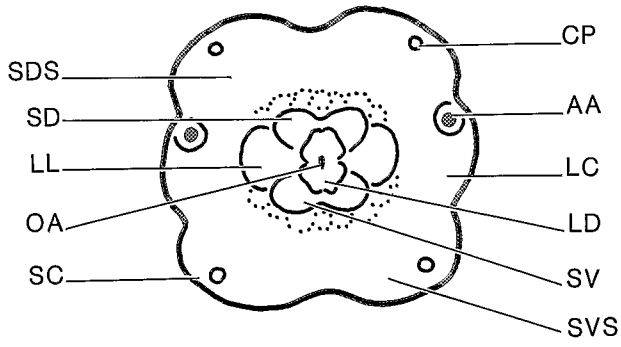

Fig. 1. *Rhadinaphelenchus cocophilus*. Diagramatic representation of the *en face* pattern. LD = Labial disc; OA = oral aperture; LL = lateral lip sectors; SD, SV = subdorsal and subventral lip sectors; AA = amphid aperture; CP = cephalic papilla; LS = lateral cephalic sector; SDS, SVS = subdorsal and subventral cephalic sectors.

was dorso-ventrally flattened. Outer labial sensillae were not resolved on the lip region. In most specimens the lip region was surrounded by a ring of material (secretions?) which could have been produced by the amphids (Figs 1; 2 A, B). The cephalic region surrounding the lip region was resolved into six sectors and also was dorso-ventrally flattened. The two amphid apertures were resolved in a slightly dorso-lateral position; four cephalic papillae occurred submedially (Figs 1; 2 A, B).

Transverse striae, amphidial apertures, and labial and cephalic papillae were not visible with LM on the domed heads of males or females of *R. cocophilus*. SEM observations demonstrated that the heads of both males and females of *R. cocophilus* had about seven fine annular striations (0.14-0.25 µm wide) (Fig. 2 C, D). The heads of both sexes of *R. cocophilus* were not offset by a constriction but were narrower than the body (Fig. 2 C, D).

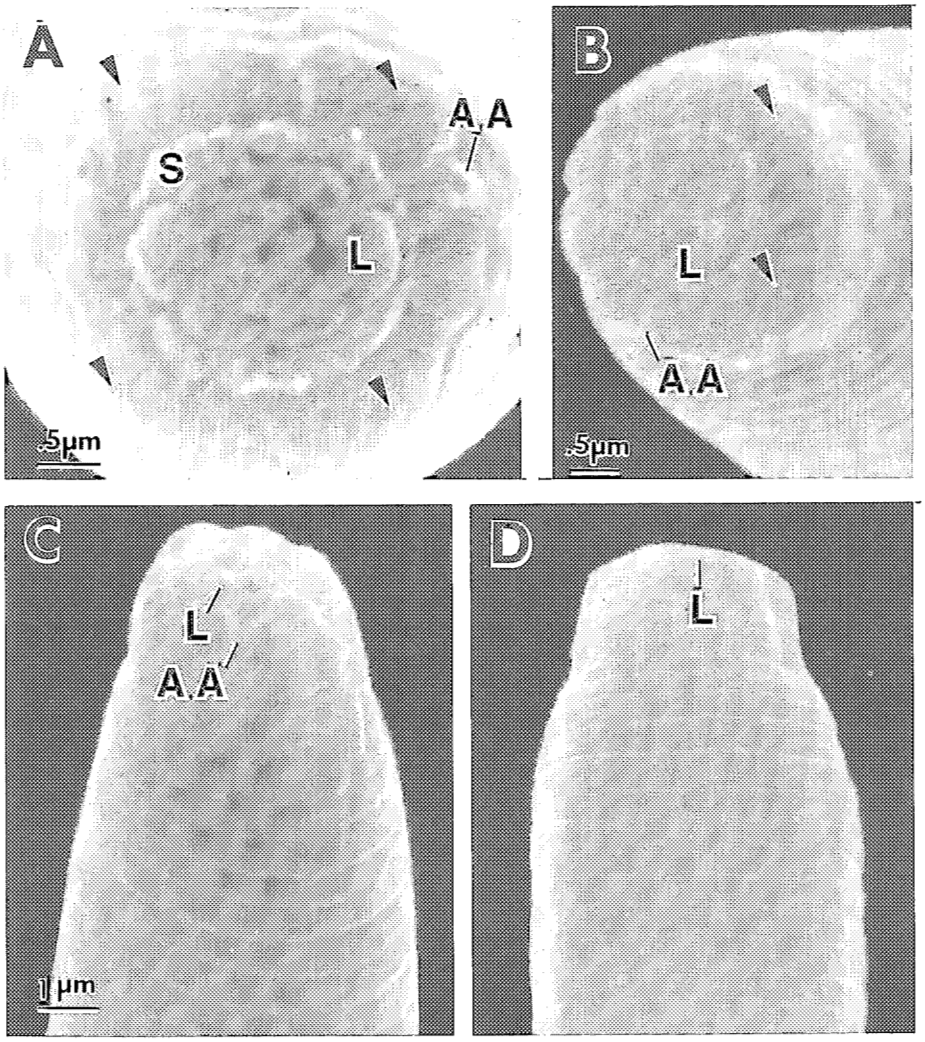

Fig. 2. *Rhadinaphelenchus cocophilus*. Scanning electron micrographs of the anterior end. A : *En face* view of adult female. Arrows = cephalic papillae; S = secretion?; B : *En face* view of adult male; C : Nearly lateral view of adult female showing one amphid aperture; D : Lateral view of adult male (same scale as C).

Morphology of male caudal region

Morphology of the posterior end of males of *R. cocophilus* was elucidated through a combination of LM (Fig. 3) and SEM (Fig. 4). Investigation of spicule structure was aided by successful use of Hooper's (1977) technique for protrusion. We observed seven caudal papillae in SEM of *R. cocophilus* (Fig. 4). A single

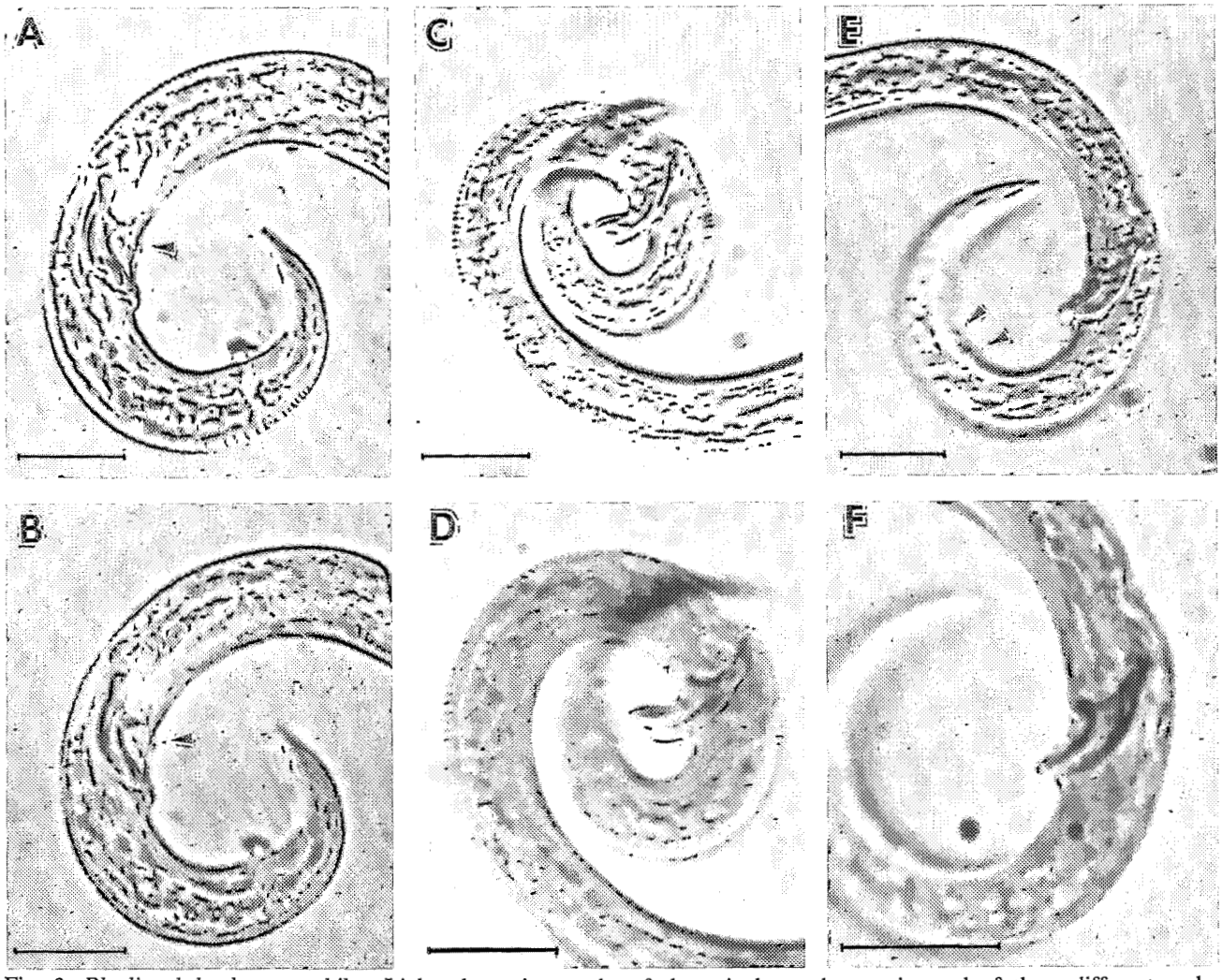

Fig. 3. *Rhadinaphelenchus cocophilus.* Light photomicrographs of the spicules and posterior end of three different males (Bar = 10 µm).

ventral papilla was located about 3 µm above the open cloaca (Fig. 4 A, B). This preanal papilla appeared as a central pore within a concavity (Fig. 4 A, B). This papilla was difficult to see, but visible in most specimens that were examined with LM. The other three pairs of caudal papillae appeared as small mounds with a central pore in SEM (Fig. 4 A-D). The most anterior occurring pair of subventral papillae in *R. cocophilus* was located at or slightly above the level of the open cloaca (Fig. 4 A, B, G). This pair of preanal or adanal papillae was difficult to see with LM. Two pairs of postanal subventral papillae near the base of the caudal alae (= bursal flap) were easily seen with SEM (Fig. 4 C, D) and the LM.

The spicules of *R. cocophilus* overlapped and were appressed along the dorsal and ventral midline forming a hollow conduit for sperm transfer. However, the distal tips were not fused (Fig. 4 E-G). The distal portion of the spicules was heavily sclerotized (Fig. 3) and protruded farther on the anterior than the posterior side making the combined spicules appear notched on the side nearest the tail (Fig. 4 E-G). When the spicules of *R. cocophilus* were retracted the cloacal opening appeared circular (Fig. 4 A, B), but this may have been due to an artifact of the ammonia treatment.

The caudal alae (= bursal flap) were fused together forming a spade-shaped flap with a tapering terminus; the dorsal surface was annulated (Fig. 4 A, C, G). The caudal alae began anteriorly at the level of the last pair of subventral caudal papillae (Fig. 4 A, G).

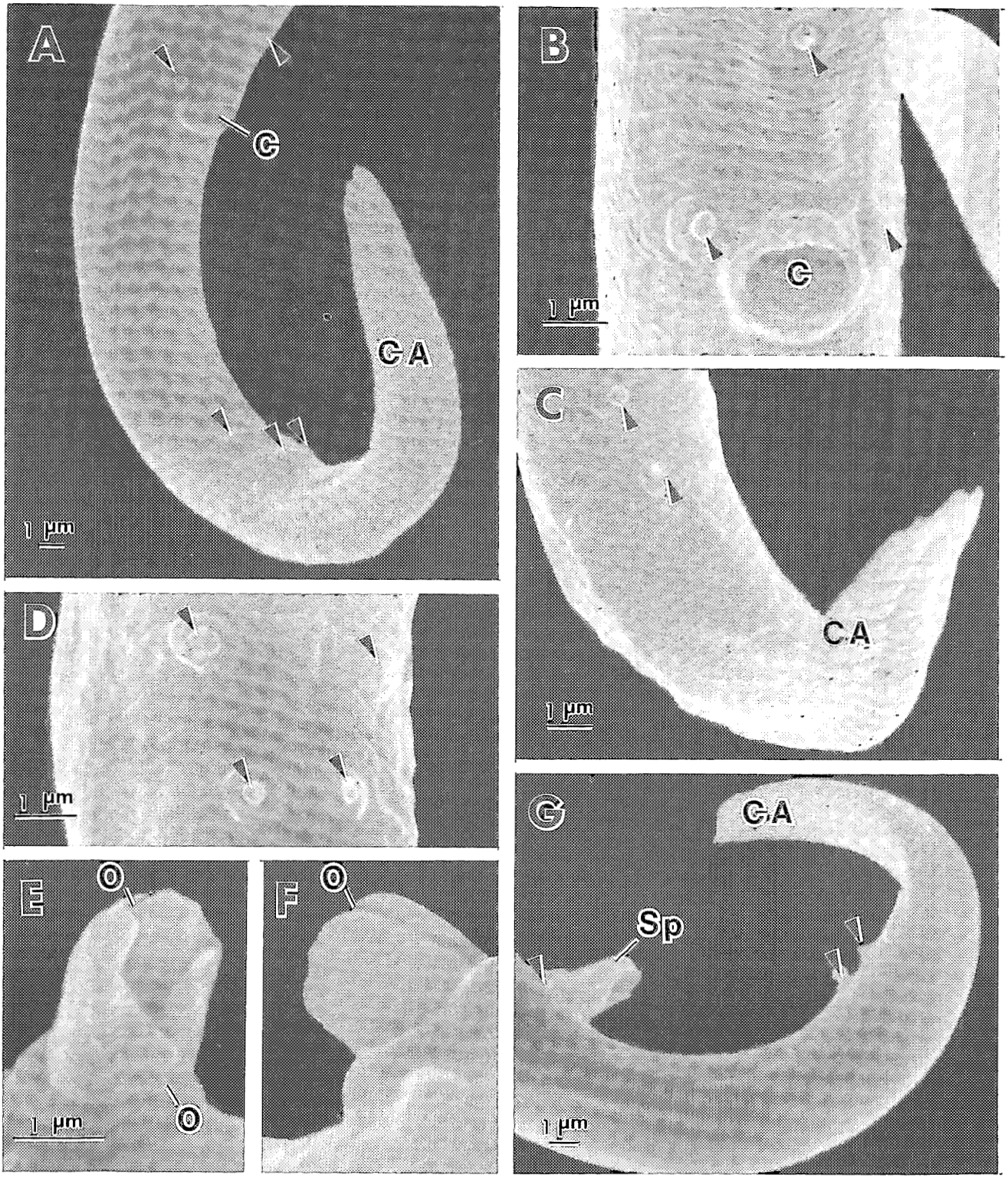

Fig. 4. *Rhadinaphelenchus cocophilus.* Scanning electron micrographs of the posterior region of adult males. A : Subventral view of caudal region; C = cloacal opening; arrows = caudal papillae; CA = caudal alae; B : Enlarged ventral view from A showing position and detail of preanal papillae (arrows) and cloacal opening; C : Subventral view of postanal papillae (arrows) and subdorsal view of caudal alae; D : Ventral view of postanal papillae; E : Posterior view of the tips of the protracted spicules showing areas of separation and overlap (O); Enlargement of G; F : Nearly anterior ventro-lateral view of the tips of the protracted spicules showing areas of separation and overlap (O); Scale same as E; G : Dorso-lateral view of tail with the tips of the protracted spicules.

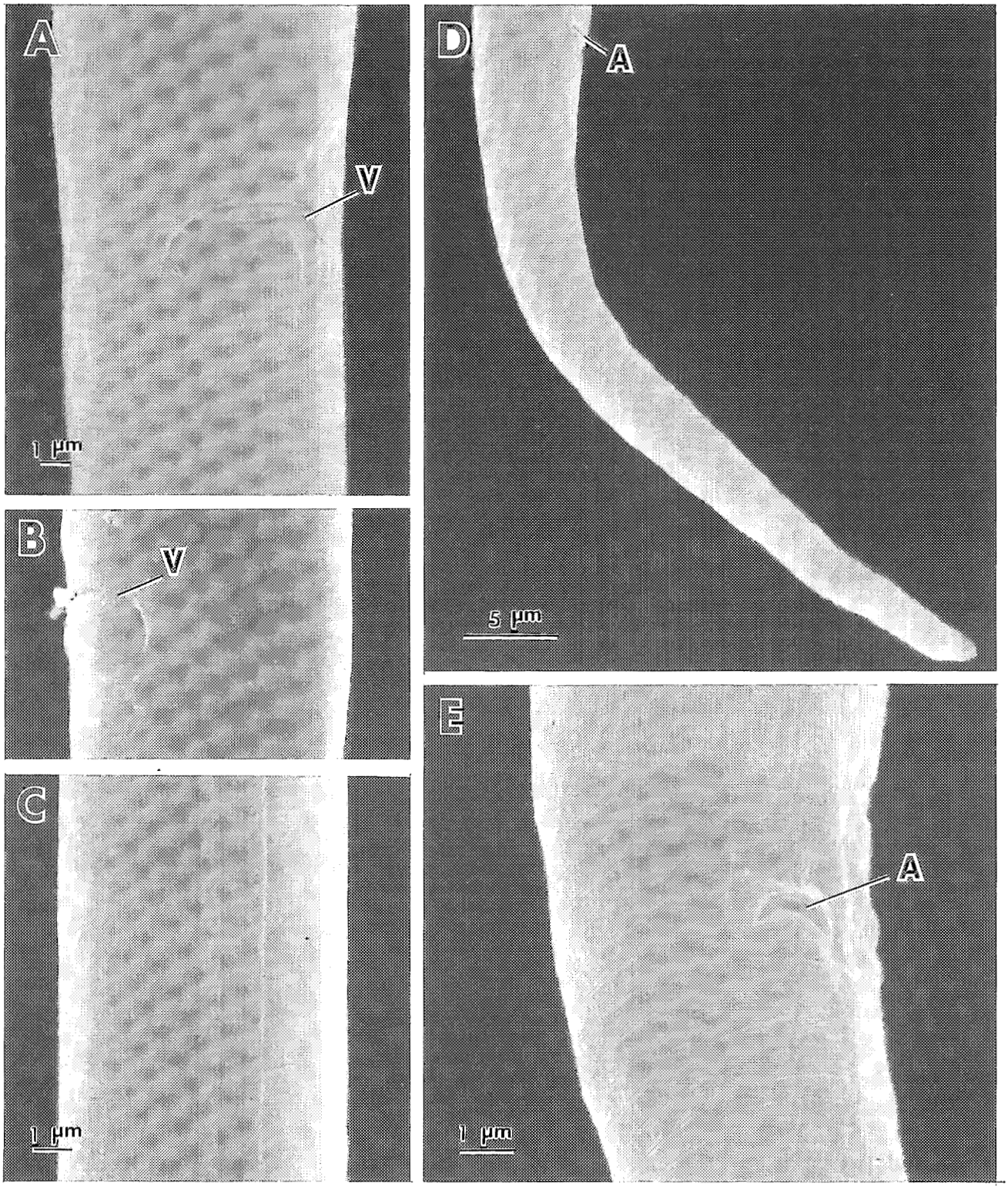

Fig. 5. *Rhadinaphelenchus cocophilus*. Scanning electron micrographs of the vulval and posterior region of adult females. A : Ventral view of the vulval slit; V = vulval slit; B : Lateral view of the vulval slit; C : Midbody region with four lines in the lateral field; D : Lateral view of tail; A = anus; E : Subventral view of anus.

MORPHOLOGY OF FEMALE VULVAL AND CAUDAL REGIONS

Females of *R. cocophilus* possessed a vulval flap that was crescent-shaped posteriorly in ventral view (Fig. 5 A, B). The posterior lip of the vulva was heavily sclerotized (Fig. 5 A, B). The cuticles of both sexes had transverse annular striations and the lateral field, when observed had four incisures (Fig. 5 C). We rarely observed the lateral fields in LM observations. The anus of the female was crescent-shaped posteriorly in ventral view (Fig. 5 D, E) and the tail was elongate with a rounded terminus (Fig. 5 D).

Discussion

The terminology used in this paper for the *en face* pattern of *R. cocophilus* differs slightly from that proposed for Aphelenchida by Hooper and Clark (1980) but is necessary to represent possible homologies with the more readily distinguished labial disc and lip sectors of Tylenchida (Baldwin, Luc & Bell, 1983). Hooper and Clark (1980) apparently recognized this likely homology with the following sentence, " Encircling them [the inner labial papillae] are six large protuberances which we think represent the lips *(sensu stricto)* and which seem to correspond with the position of the outer papillae... " Nevertheless, Hooper and Clark (1980) extend the labial disc to include what we consider the lips. The " cephalic sectors " (Fig. 1) of this study correspond to the " lip sectors " of Hooper and Clark (1980).

One problem in relating lip terminology of tylenchs to aphelenchs is due to the relative position of the anterior sensory structures. In tylenchs, amphid openings are typically at the periphery of the labial disc and cephalic papillae occur on the submedial lips. Whereas in aphelenchs, amphid openings and cephalic papillae occur more posteriorly making homology of the labial disc and lips potentially less reliable. Questions of the homology of the highly specialized lip patterns of aphelenchs and alternative terminology must be tested with additional investigation. New evidence of homology may be provided by transmission electron microscope (TEM) observations of underlying structures as well as by recognizing transformation series of the lip patterns of additional representatives of other aphelenchoids.

The *en face* pattern of *R. cocophilus* is similar to that of other aphelenchoids with respect to the relatively posterior position of the amphids and cephalic papillae (Hooper & Clark, 1980). However, the shape and morphology of the labial disc, and the lip region as well as the dorso-ventral flattening of the cephalic region may be diagnostic characters for the genus or species.

That *R. cocophilus* adults possess fine annulations on the head is similar to almost all SEM observations of aphelenchoids reported previously (Hooper & Clark, 1980; Yik & Birchfield, 1981; Giblin & Kaya, 1983; Giblin, Swan & Kaya, 1984). In fact, only *Aphelenchoides helicosoma* Maslen, 1979 has been confirmed with SEM to be without fine annular striations on the head (Hooper & Clark, 1980).

Previous workers reported the arrangement of caudal papillae in males of *R. cocophilus* to be one pair of preanal papillae *ca* 1/2 spicule length anterior to the cloaca, and two pairs of ventrosubmedian papillae near the base of the caudal alae (Cobb, 1919; Lordello & Zamith, 1954; Goodey, 1960; Thorne, 1961; Nickle, 1970; Brathwaite & Siddiqi, 1975). Another pair of ventro-submedian papillae just behind the cloaca were reported as obscure (Thorne, 1961; Nickle, 1970; Brathwaite & Siddiqi, 1975). It is clear from our observations that these reports were not accurate. Instead of a pair of preanal subventral papillae, there is a single ventral preanal papilla *ca* 3 μm above the cloacal opening (Fig. 4 A, B). This single papilla is probably homologous to the single preanal papilla found in members of the genus *Bursaphelenchus*, eg. *B. xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970 (Yik & Birchfield, 1981), *B. seani* Giblin & Kaya, 1983 (Giblin & Kaya, 1983), and *B. kevini* Giblin, Swan & Kaya, 1984 (Giblin, Swan & Kaya, 1984).

The three pairs of caudal papillae that we observed with SEM (Fig. 4 A) were similar in structure to the anterior two pairs of subventral papillae described by Clark and Shepherd (1977) for *Aphelenchoides blastophthorus* Franklin, 1952. The caudal papillae of *A. blastophthorus* were examined by TEM and described tentatively as chemosensory sensillae that were open to the exterior through a pore (Clark & Shepherd, 1977). The most anterior occurring pair of subventral papillae that we observed in SEM were preanal or adanal (Fig. 4 A, B, G), and probably correspond to the pair of postanal subventral papillae reported previously (Thorne, 1961; Nickle, 1970; Brathwaite & Siddiqi, 1975). Arrangement of the caudal papillae in males of *R. cocophilus* is typical for some species of *Bursaphelenchus*, eg. *B. xylophilus* (Yik & Birchfield, 1981) and the *B. hunti* (Steiner, 1935) Giblin & Kaya, 1983 group (Giblin & Kaya, 1983).

Cobb (1919) emphasized the thickening of the distal end of the spicule in his original drawing of *R. cocophilus*. Some of the subsequent reports have not mentioned this feature (Lordello & Zamith, 1954; Nickle, 1970; Brathwaite & Siddiqi, 1975). In lateral view, the spicules of *R. cocophilus* resemble those of some species of *Parasitaphelenchus* (Hunt & Hague, 1974) and *Bursaphelenchus* (*B. hunti* group) (Giblin & Kaya, 1983). Spicules of *Parasitaphelenchus oldhami* Rühm, 1956 are fused along the dorsal midline and open ventrally (Hunt & Hague, 1974) whereas the spicules of members of the *B. hunti* group are separate (Giblin & Kaya, 1983). If TEM confirms that the spicules of *Rhadinaphelenchus* are fused proximally, they may be unique among the Aphelenchoididae.

The caudal alae of *R. cocophilus* are similar to those described for most members of the genus, *Bursaphelenchus*, eg., *B. xylophilus* (Yik & Birchfield, 1981), *B. seani* (Giblin & Kaya, 1983), and *B. kevini* (Giblin, Swan & Kaya, 1984).

The vulval flap of females of *R. cocophilus* is similar in appearance to the vulval flap in *B. xylophilus* (Yik & Birchfield, 1981). The body annulation and lateral fields of both sexes of *R. cocophilus* are similar to other members of the genus, *Bursaphelenchus*, eg., *B. xylophilus* (Yik & Birchfield, 1981), *B. seani* (Giblin & Kaya, 1983), and *B. kevini* (Giblin, Swan & Kaya, 1984).

Conclusions

Our observations have established that *Rhadinaphelenchus* shares many characters with the genus *Bursaphelenchus*. The *en face* pattern of *R. cocophilus* is similar to that of other aphelenchoids with respect to the posterior position of the amphids and cephalic papillae but appears to be distinctive in the shape of the labial disc and lip region, and the dorso-ventral compression of the cephalic region. Arrangement and morphology of the caudal papillae, caudal alae in the males, body annulation, lateral incisures in both sexes, and vulval flap in the females of *R. cocophilus* are very similar to the corresponding features of species in the genus *Bursaphelenchus*. The primary morphological feature that distinguishes *Rhadinaphelenchus* from other genera in the family Aphelenchoididae is the very long and slender shape of both sexes. If confirmed by future TEM investigations, the proximal fusion of the spicules would be unique among the aphelenchoids.

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