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Morphological Differences Between *Radopholus citrophilus* and *R. similis*[1]

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Abstract: SEM observations of the external morphology of populations of *Radopholus citrophilus* and *R. similis* revealed several diagnostic differences. The cloaco-spicular orifice on males of *R. citrophilus* had three to seven genital papillae (anterior hypoptygmata), whereas males of *R. similis* were either smooth or had one or two shorter genital papillae (anterior hypoptygmata). Females of *R. citrophilus* had four annules in the region of the vulval opening, but *R. similis* had five annules in the same region. The labial disc and lateral lips appeared to be of diagnostic significance, but these areas were more susceptible to artifacts due to fixation. An unknown population of *Radopholus* from Puerto Rico with a chromosome number of n = 4 was morphologically similar to *R. similis*. These morphological differences provide additional support that *R. citrophilus* and *R. similis* are distinct species.

Key words: burrowing nematode, cytogenetics, chromosome, scanning electron microscope, taxonomy, *Radopholus citrophilus, R. similis,* morphology.

A 1978 SEM study of the external morphology of the then designated "citrus race" of the burrowing nematode suggested possible morphological characters that could be used to distinguish it from populations of the "banana race" (1). Previous studies had failed to reveal morphometric differences between the two races (13,15, 16). Differences had been detected, however, in chromosome number (5,10), isozyme patterns (7,8), mating behavior (6), and host preference (3), and in 1984 the two races were separated into sibling species (9). The banana race retained the name *Radopholus similis* (Cobb, 1893) Thorne, 1949, and the citrus race was named *R. citrophilus* Huettel, Dickson & Kaplan, 1981 (9).

The objective of this study was to extend the morphological observations detected in the 1978 study. Males and females of both species, from different regions of the world, were used for these observations.

Materials and Methods

Nematode populations: Populations of *R. citrophilus* (Tylenchida: Pratylenchidae) were obtained from established carrot disc cultures of populations originally isolated from infested *Anthurium andreaenum* L. collected in Hawaii and from infested *Citrus autrantium* collected in Florida. Populations were previously characterized by karyotype, mating behavior, isozyme patterns, and morphometrics (10). Nematodes were transferred from carrot discs and maintained aseptically on *Zea mays* cv. Iowa Chief root explants on Gamborg's B-5 medium (Grand Island Biological Co., Grand Island, NY) (11).

One population of *R. similis* was obtained from established carrot disc cultures originally isolated from *Musa* sp. in Florida. This population had been previously characterized by the same methods described above. An unknown population of *Radopholus* sp. was obtained from Puerto Rico and isolated from infested banana roots, *Musa* sp. (Jesse Roman, pers. comm.). This population was established in carrot disc culture, transferred to corn root explants, and maintained as described for the above populations. It was characterized by chromosome numbers as described by Huettel and Dickson (5).

Nematodes for SEM: Nematodes were obtained for scanning electron microscope

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(SEM) studies by extracting the mixed life stages from the root explants with a modified Baermann funnel. Males and females were separated by hand. Specimens were fixed by adding 4% glutaraldehyde–2% formalin (buffered in 0.1 M sodium cacodylate, pH 7.2) in a step-wise manner every 30 minutes until a final concentration of 2% glutaraldehyde–1% formalin was reached. After fixation for 4 days at 4 C, the specimens were rinsed in 0.1 M sodium cacodylate and postfixed in 2% osmium tetroxide for 12 hours at 4 C (4). The specimens were then dehydrated in an ethanol series, dried by a critical point dryer with CO_2, and sputter coated with 20–30 nm gold-palladium. Specimens were observed and photographed on a Hitachi SEM operated at 15 kV (5).

RESULTS

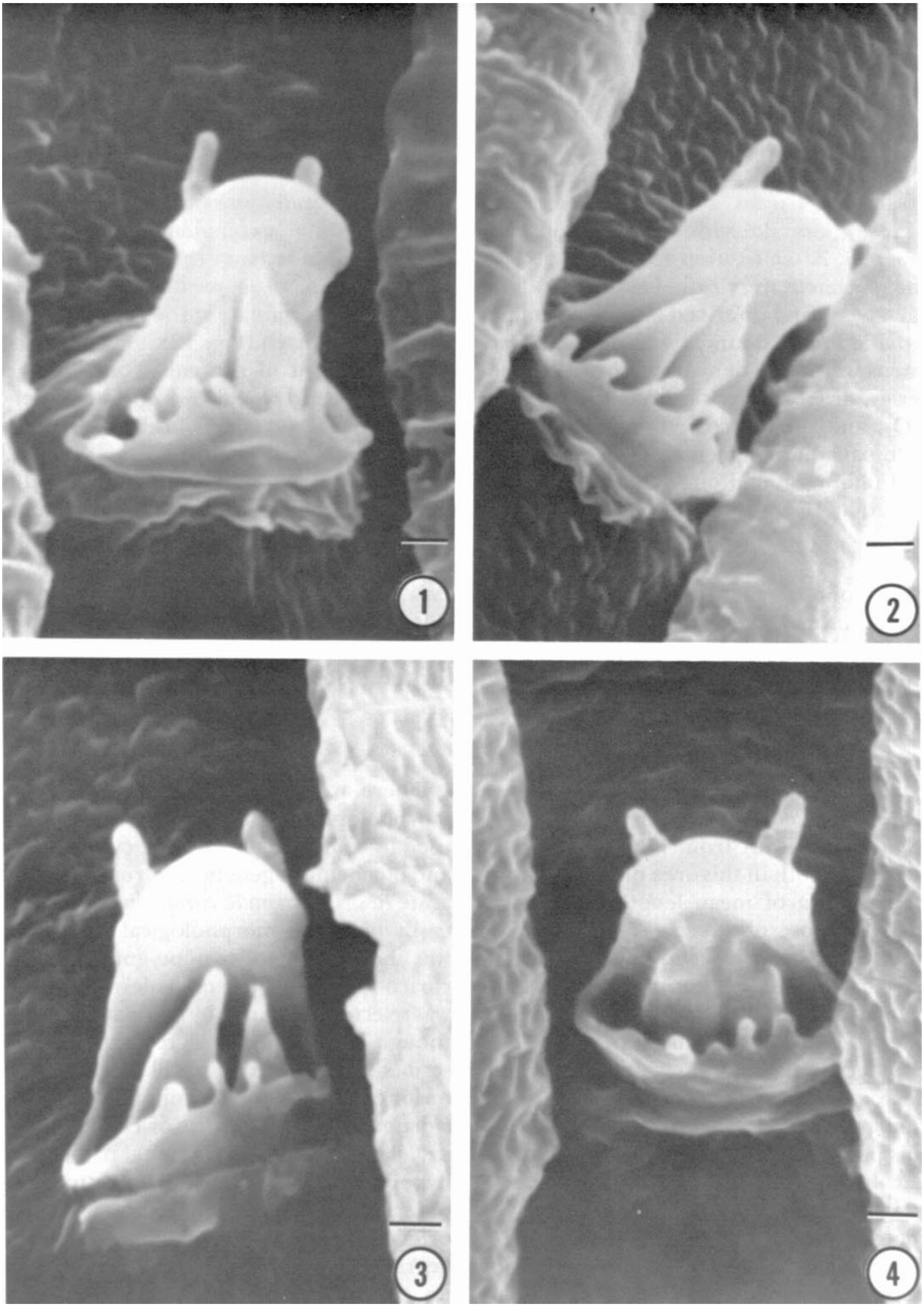
Morphological differences were observed in males of both species in the region of the cloaco-spicular orifice above the spicules. All specimens of *R. citrophilus* had genital papillae on the anterior cloacal aperture, referred to as anterior hypoptygmata. The mean length of the hypoptygmata was 0.43 μm, and three to seven were observed per specimen (Figs. 1–4). *Radopholus similis* from Florida was completely smooth in this area or had only one hypoptygma of mean length 0.24 μm in 14% of those observed (Figs. 5–8). The population of *Radopholus* sp. from Puerto Rico had a smooth anterior cloaco-spicular orifice, or up to 33% of those observed had one or two hypoptygmata (Figs. 9–12). This population also had a chromosome number of n = 4, which confirms that it was similar to *R. similis* (Figs. 13, 14). Neither this population nor *R. similis* from Florida had hypoptygmata as observed in the *R. citrophilus* populations. The number of specimens observed were 75 males of *R. citrophilus*, 44 males of the Puerto Rico population, and 65 males of *R. similis*, Florida.

Morphological differences between females of the two species were observed in the head region and labial discs. The labial disc was more rounded in populations of *R. citrophilus* (Fig. 15), whereas *R. similis* was more hexagonal (Fig. 16). The female medial and lateral lips appeared to be more fused in *R. citrophilus* than in *R. similis* (Figs. 15, 16). The lateral lips appear to terminate differently depending upon the species. In populations of *R. citrophilus*, the lateral lip did not completely terminate at the third annule from the head (Fig. 17). In populations of *R. similis*, the lateral lip appeared to terminate at the bottom of the third annule itself (Fig. 18).

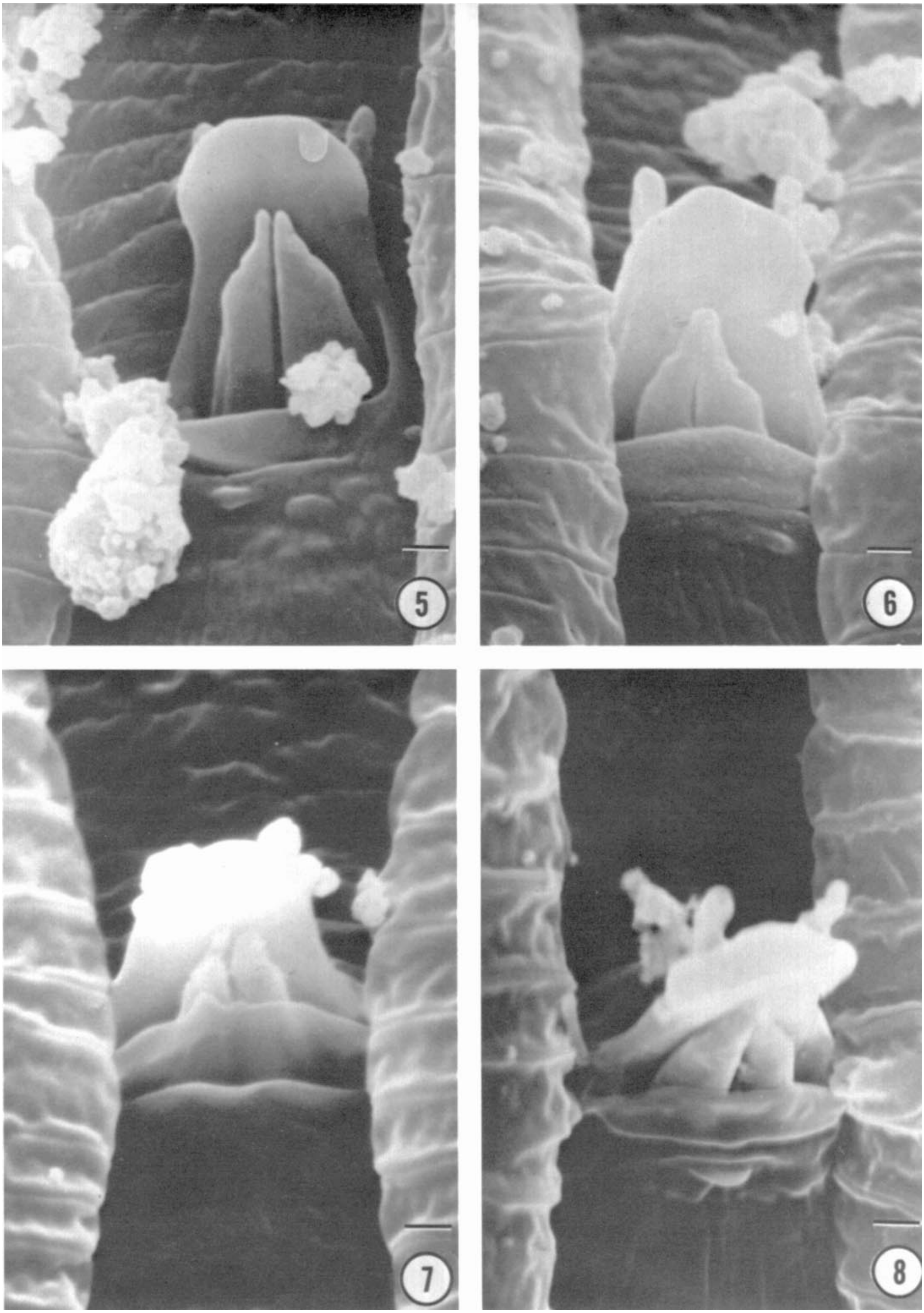
Observations of the vulval area of the two species also revealed diagnostic differences. *Radopholus citrophilus* had three annules terminating at the vulva (Fig. 19), whereas *R. similis* had two annules in the same area (Fig. 20). The vulval opening generally appeared to be more protruding in *R. citrophilus* populations (Fig. 19); however, this could have been an artifact resulting from fixation. Approximately 45 females from each population were used for these observations.

DISCUSSION

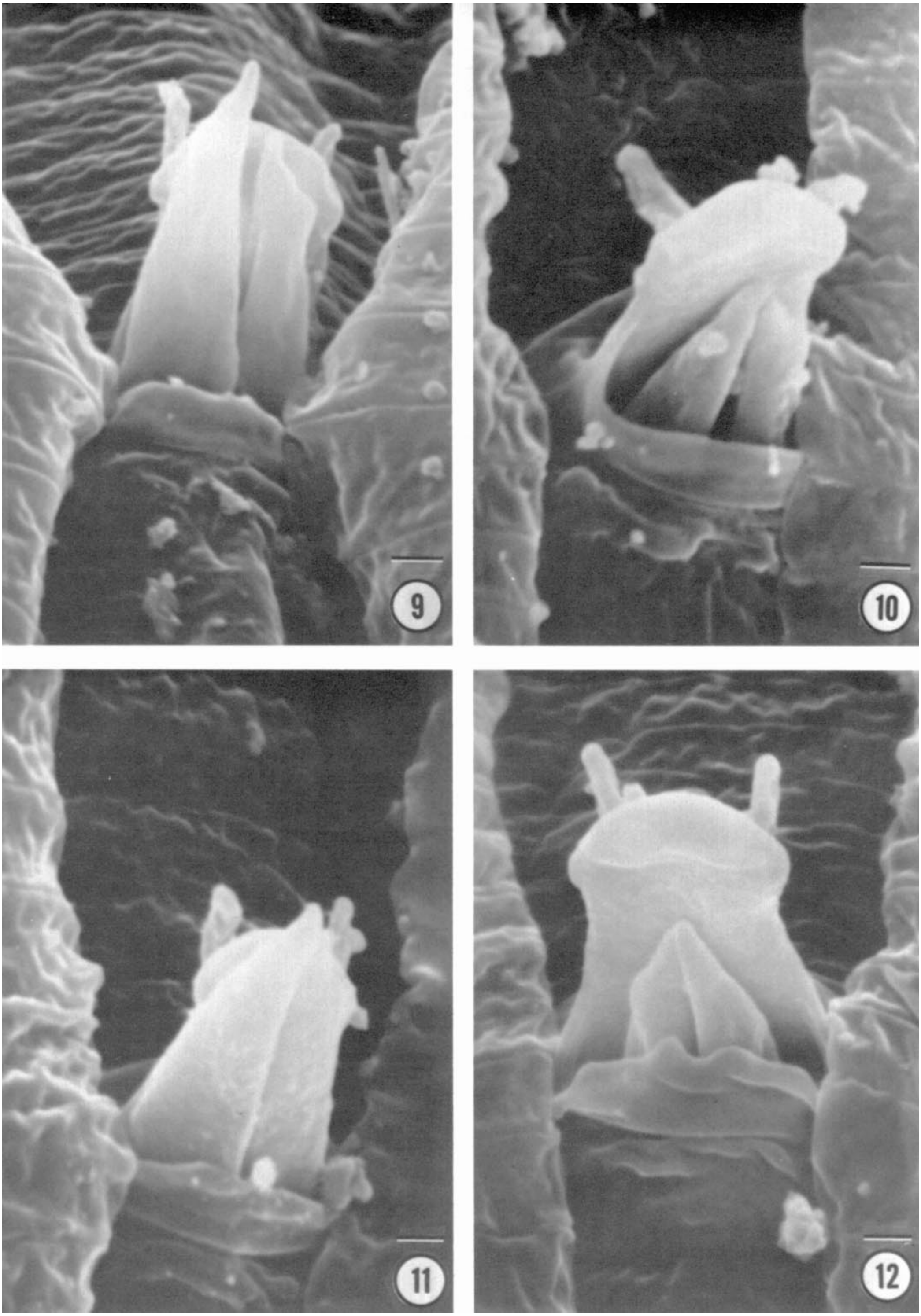
There are several minor but consistent morphological characteristics that can be used in combination with the cytogenetics and biochemical genetic differences to separate *R. similis* from *R. citrophilus*. The most easily observed morphological difference was the number of hypoptygmata on the cloaco-spicular orifice on males. This term was selected as defined by Siddiqi (14). Even though hypoptygmata were originally described for the genital papillae on the posterior cloacal lips of Merliniinae, this is also a diagnostic characteristic of Hoplolaimoidea (14). The posterior genital papillae or hypoptygmata appear to be similar to those observed on the anterior cloaco-spicular orifice of *Radopholus* sp. males. This appears to be a cuticularized area that did not seem to be subject to fixation artifacts. Differences in the vulval region also appeared to be diagnostic but are more difficult to observe in all the specimens because of fixation. The head region and



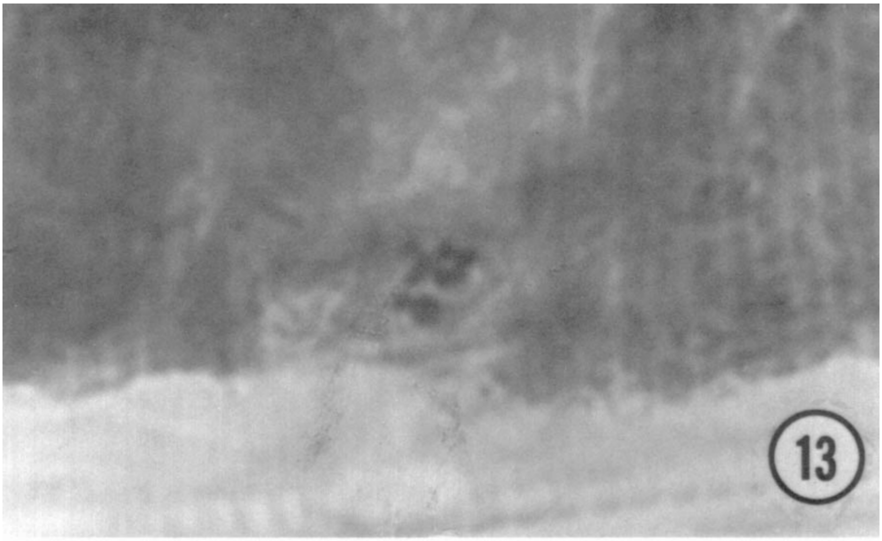
FIGS. 1–4. SEM micrographs of the cloaco-spicular orifice of male *Radopholus citrophilus* posterior region, ventral view. 1, 2) From Hawaii. 3, 4) From Florida. Scale: Bar = 0.5 μm.

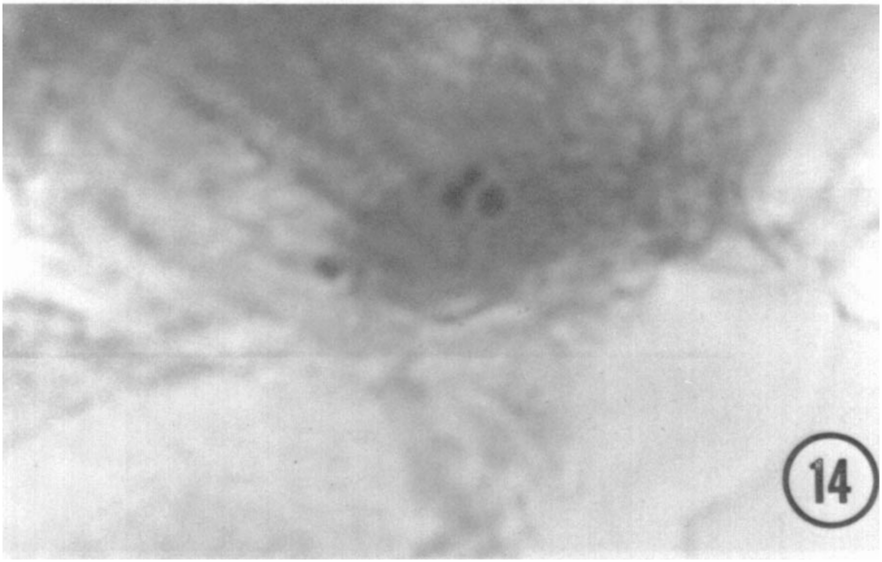


FIGS. 5–8. SEM micrographs of the cloaco-spicular orifice of male *Radopholus similis* from Florida, posterior region, ventral view. Scale: Bar = 0.5 μm.



FIGS. 9–12. SEM micrographs of the cloaco-spicular orifice of male *Radopholus similis* from Puerto Rico posterior region, ventral view. Scale: Bar = 0.5 μm.





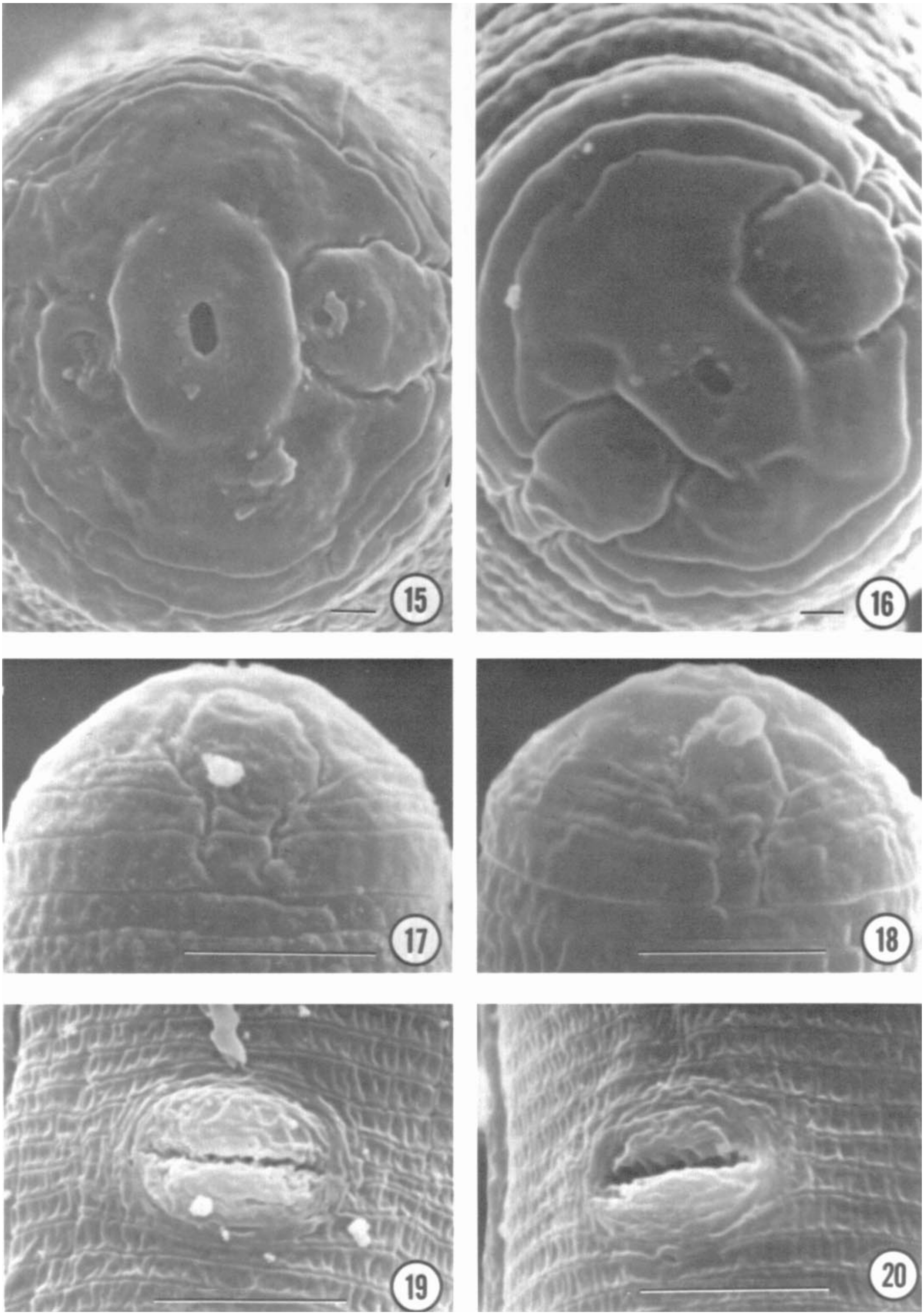
FIGS. 13, 14. Light micrographs of chromosomes of *Radopholus* sp. from Puerto Rico. 13) $2n = 8$, oogonial metaphase. 14) $n = 4$, metaphase I.

lateral lips were also characteristic for each of the species.

There was a marked difference in the critical point drying preparation of *R. similis* and *R. citrophilus. Radopholus similis* males were much more susceptible to fixation, which caused most specimens to collapse or become distorted, whereas most *R. citrophilus* specimens were well preserved following fixation.

It is important to use several criteria for identifying unknown populations of *Radopholus* sp. The Puerto Rico population was previously reported to have $n = 5$ chromosomes (12); however, examination of more than 100 females revealed that the chromosome number is $n = 4$. We have found it important to remove the entire reproductive system from females before beginning the staining procedure. The SEM morphological studies confirmed that this population was more similar to *R. similis* than to *R. citrophilus.*

The two populations of *R. citrophilus*



FIGS. 15–20. SEM micrographs of lips, head, and vulva region of female *Radopholus citrophilus* and *R. similis.* 15) *R. citrophilus* en face view. 16) *R. similis* en face view. 17) *R. citrophilus* labial lips, anterior region. 18) *R. similis* labial lips, anterior view. 19) *R. citrophilus* vulva, ventral view. 20) *R. similis* vulva, ventral view. Scale: 15, 16) Bar = 0.5 μm. 17–20) Bar = 5 μm.

studied originated from different hosts and were geographically separated. Although the population from Hawaii does not infect citrus (10), it has morphological characteristics similar to the Florida population that infects citrus. At present, host preference tests are the only available technique for determining biotypes of *R. citrophilus.*

The degree of protein evolution as previously demonstrated in *R. similis* and *R. citrophilus* is not necessarily associated with the degree of anatomical changes in these organisms. Differences observed in several morphological areas between the two species indicates two genetic events may have occurred. Mutation in regulatory genes resulting in anatomical change could occur separately from mutations at the structural gene level resulting in protein changes (2,17). These results support the theory that organisms with relatively simple anatomical features will have few or no morphological differences between them, although they may differ greatly in the amount of protein change (16). This appears to be the case in *Radopholus* spp. (8,10).

Even though similarities were observed within populations of each species, more populations from other locations should be observed. Since these morphological differences are difficult to impossible to observe by light microscopy, they should be considered minor and additional support for the sibling species concept. However, if these morphological differences are consistently observed within other populations of the two species, then the "sibling species concept" should be redefined.

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