

# Rhizonema sequoiae n.gen. n.sp. from Coast Redwood *Sequoia sempervirens* (D. Don) Endl.<sup>1</sup>

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*Abstract:* *Rhizonema sequoiae* n.gen. n. sp. is described from the roots of Coast Redwood, *Sequoia sempervirens* (D. Don) Endl., growing near Lake Lagunitas, Marin County, California. *Rhizonema* females are annulated over their entire bodies, are wholly embedded in host tissue, and secrete an abundant amount of gel material. Mature females do not form a cyst. The vulva is located on a large posterior terminal cone, and the anus is on the dorsal vulval lip. Esophageal glands of the second-stage larvae fill more than half of the body cavity. Tails of the vermiform males are blunt, and a cloacal tubus is present. *Key words:* taxonomy, redwood nematode.

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The family Heteroderidae contains some genera that form a cyst and others that do not. The females of all Heteroderidae are swollen; however, the males have a cylindrical shape with a short, rounded tail and lack caudal alae. Annulation is not apparent over much of the body of females of cyst-forming Heteroderidae, but in the group of genera that do not form cysts, there is partial or total annulation of the cuticle of the mature female. At the present time, the genera of non-cyst-forming Heteroderidae are *Meloidodera* Chitwood et al., 1956 (1); *Verulus* Esser, 1981 (4); *Cryphodera* Colbran, 1966 (2); *Atalodera* Wouts & Sher, 1971 (8); *Hylonema* Luc et al., 1978 (5), and *Thecavermiculatus* Robbins, 1978 (6). These genera differ from one another by the presence or absence of annulation in the posterior portion of the mature female and by the equatorial or terminal position of the vulva.

In 1979, larvae and males of a nematode that appeared to be a member of the Heteroderidae were recovered from roots of

Coast Redwood, *Sequoia sempervirens* (D. Don) Endl., growing near Lake Lagunitas, Marin County Water District, California. Subsequently, mature females were also found in lesser numbers at this same location on Tanbark Oak (*Lithocarpus densiflora* [Hook & Arn.] Rehd), California Bay (*Umbellularia californica* [Hook & Arn.] Nutt.), and Madrone (*Arbutus menziesii* Pursh.). Dissection of the ungalled roots of these trees revealed completely embedded, saccate, white females, which usually had abundant gelatin at their posterior extremity. Morphological and biological differences suggest that the redwood nematode represents a new generic taxon. Detailed morphological and anatomical studies of this nematode were made resulting in the description of *Rhizonema sequoia* n.gen., n.sp.

## MATERIALS AND METHODS

Larvae and male redwood nematodes were obtained by incubating redwood feeder roots for 24 hours in a mist chamber. Mature females were obtained by dissection of redwood roots. These life stages were killed and fixed with Seinhorst's (7) fixative, processed into glycerin, and mounted in glycerin (DeGrise, personal communication). For scanning electron microscope

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study, nematodes were killed and fixed in Seinhorst's fixative. The dehydration was carried out by passage through an ethanol series (20, 30, 40, 50, 60, 70, 80, 90, and 100% ethanol) with 30 min at each concentration and two changes in 100% ethanol. Specimens were then transferred into amyl acetate through a series of solutions of amyl acetate in ethanol going through 25, 40, 50, 70, and 90% into 100% amyl acetate, with 15 min at each concentration. There were two changes in 100% amyl acetate, and nematodes were left overnight in 100% amyl acetate. The following day nematodes were ultrasonicated for 1 min in 100% amyl acetate and processed through critical point drying.

Larvae and males were mounted on stubs with an aluminum foil support and attached with a small drop of glue made by soaking Scotch tape (Minnesota Mining Co.) in benzene. Females were mounted on stubs by using double-coated Scotch tape. Specimens were coated with gold, 400–500 Å, and viewed with a Cambridge Mark II scanning electron microscope using accelerating voltage 10 kV.

## SYSTEMATICS

### Genus: *Rhizonema* n.gen.

*Description:* Family Heteroderidae. Female wholly embedded in host tissue; young and mature females producing abundant gelatinous secretion. Mature female saccate, not forming a cyst. Cuticle annulated on entire body. Vulva located on a large terminal cone, ornamented with small tubercles. Anus on dorsal vulva lip. No fenestration around vulva or anus. Second-stage larvae with low labial disc, hatching within mature female. Esophageal glands fill more than one-half of the body cavity. Phasmids with distinct circular structure intruding into the hypodermal tissue. Males are less than 1.3 mm in length. Their labial disc is not prominent and their cephalic region is separated from the body by a deep constriction. Male tails are short or absent. Cloaca terminal, with a tubus.

*Type species:* *Rhizonema sequoiae* n.sp.

*Rhizonema sequoiae* n.sp.

*Dimensions:* Paratype females (n = 56);

L = 0.51 (0.36–0.65) mm; width = 0.30 (0.17–0.42) mm; a = 1.9 (1.3–3.0); stylet (n = 19) 44 (31–56)  $\mu$ m.

*Holotype* (female): L = 0.57 mm; width = 0.26 mm; a = 2.2; spermatheca = 43  $\mu$ m long and 43  $\mu$ m wide; vulval cone lateral view 104  $\mu$ m wide by 68  $\mu$ m high; vulva to anus 24  $\mu$ m.

*Description:* Females, body asymmetrically swollen (Figs. 1A, 2D); young females with eggs and mature females with larvae. Anterior portion (neck) short, but distinct from rest of body, 77 (54–129)  $\mu$ m in length; posterior extremity with cone 39 (22–69)  $\mu$ m high and 100 (78–121)  $\mu$ m wide. Vulva located on cone formed by dorsal and ventral vulva lips, ornamented by small tubercles. Anus on dorsal lip of vulva, pore-like, 27 (15–33)  $\mu$ m from vulva (Figs. 1B, 2F). Ventral lip slightly narrower 49 (35–62)  $\mu$ m than dorsal lip, 51 (38–67)  $\mu$ m wide. Body without brittle, subcrystalline layer; cuticle finely annulated (Fig. 2E); cuticle thickness varies over body 1.1–8.0  $\mu$ m thick anteriorly and 5.5–17.6  $\mu$ m thick at mid-body and posterior portions. Labial region separated from body by constriction. First lip annule distinct. Cephalic framework weak, hexaradiate. Stylet slender, flexible. Basal stylet knobs wider than high (1.59  $\times$  0.96  $\mu$ m), anteriorly slightly concave with short, blunt, anteriorly directed tips, convex posteriorly. Dorsal esophageal gland duct opening approximately 5.5  $\mu$ m posterior to stylet base. Procorpus short, massive, constricted at junction with metacorpus, median bulb enlarged and nearly spherical (22–42  $\mu$ m)  $\times$  (18–34  $\mu$ m); valve plates well developed. Isthmus short, basal glandular portion globular and extending 26–55  $\mu$ m over the intestine. Excretory pore obscure, in four females 108–153  $\mu$ m from anterior end at level of basal gland of esophagus (Fig. 1C). Spermatheca, observed in three females, round and thick walled, 35–61  $\mu$ m long and 40–64  $\mu$ m wide. Spermatozoa were observed.

Eggs vary in number from 14 to 69; egg shell hyaline without visible markings. Eggs in segmentation (n = 18): length 133 (120–140)  $\mu$ m, width 59 (54–61)  $\mu$ m. Eggs with second-stage larvae (n = 20): length 139 (120–149)  $\mu$ m, width 56 (54–63)  $\mu$ m.

*Paratype males* (n = 30): L = 0.96

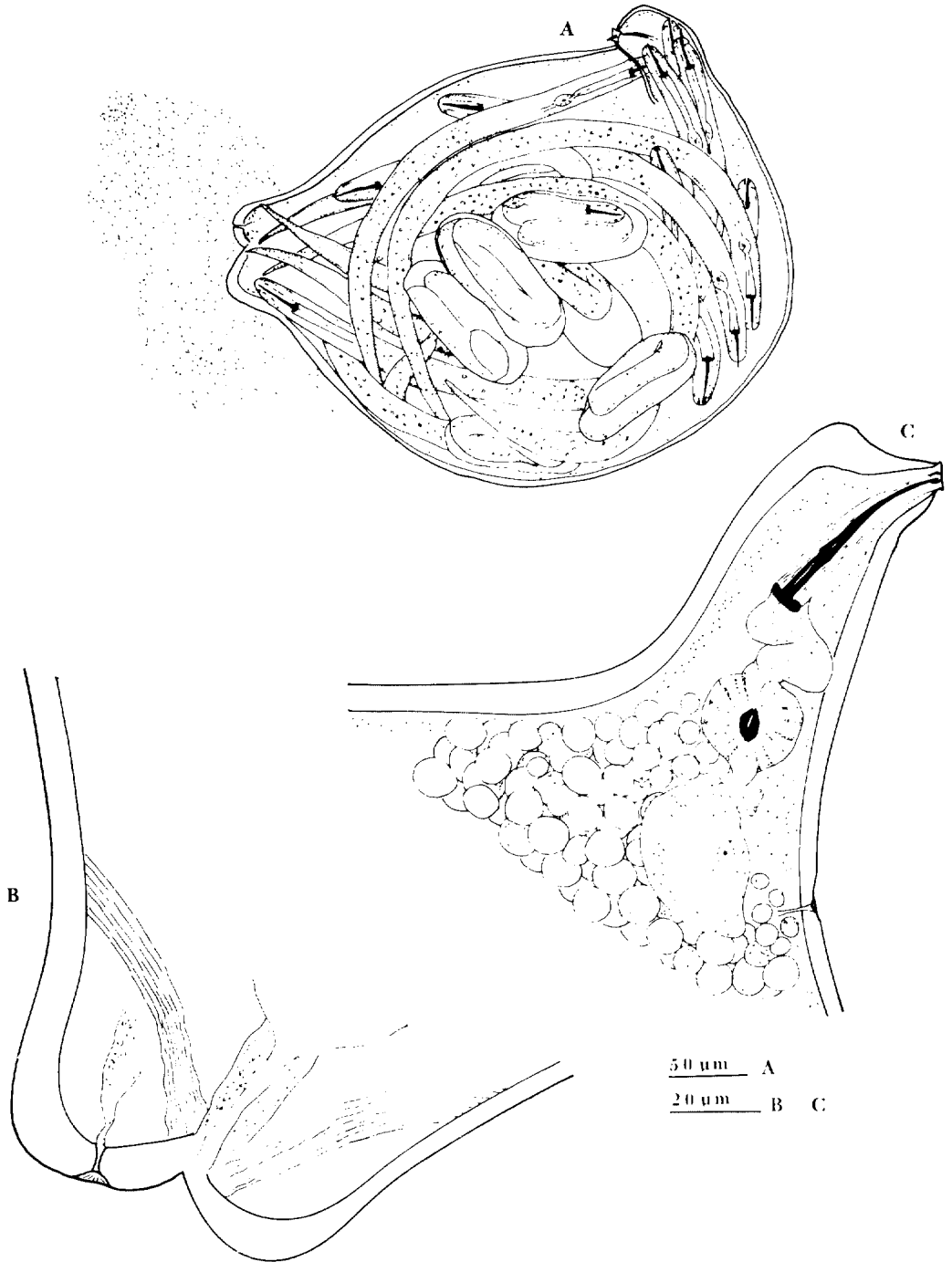


Fig. 1. *Rhizonema sequoiae* n.gen. n.sp., female A) Adult female with second-stage larvae. B) Vulval region (lateral view). C) Oesophageal region.

(0.57–1.27) mm; a = 40 (24–50); b (n = 24) = 8.2 (5.7–10.4); b' (n = 22) = 5.2 (4.6–6.4); stylet = 34 (27–39)  $\mu\text{m}$ ; stylet knobs (height  $\times$  width) = 2.8 (2.2–3.3)  $\mu\text{m} \times$  5.3 (4.4–6.6)  $\mu\text{m}$ ;  $\theta$  = 11.0 (5.7–16.0); spicules = 28.5 (22–34)  $\mu\text{m}$ ; gubernaculum (n = 18) = 8.2 (4.4–11.0)  $\mu\text{m}$ ; median bulb (n = 24) (length  $\times$  width) = 15.4 (13.2–18.7)  $\mu\text{m} \times$  9.4 (7.7–11.0)  $\mu\text{m}$ ; distance from anterior to center of median bulb = 87 (69–

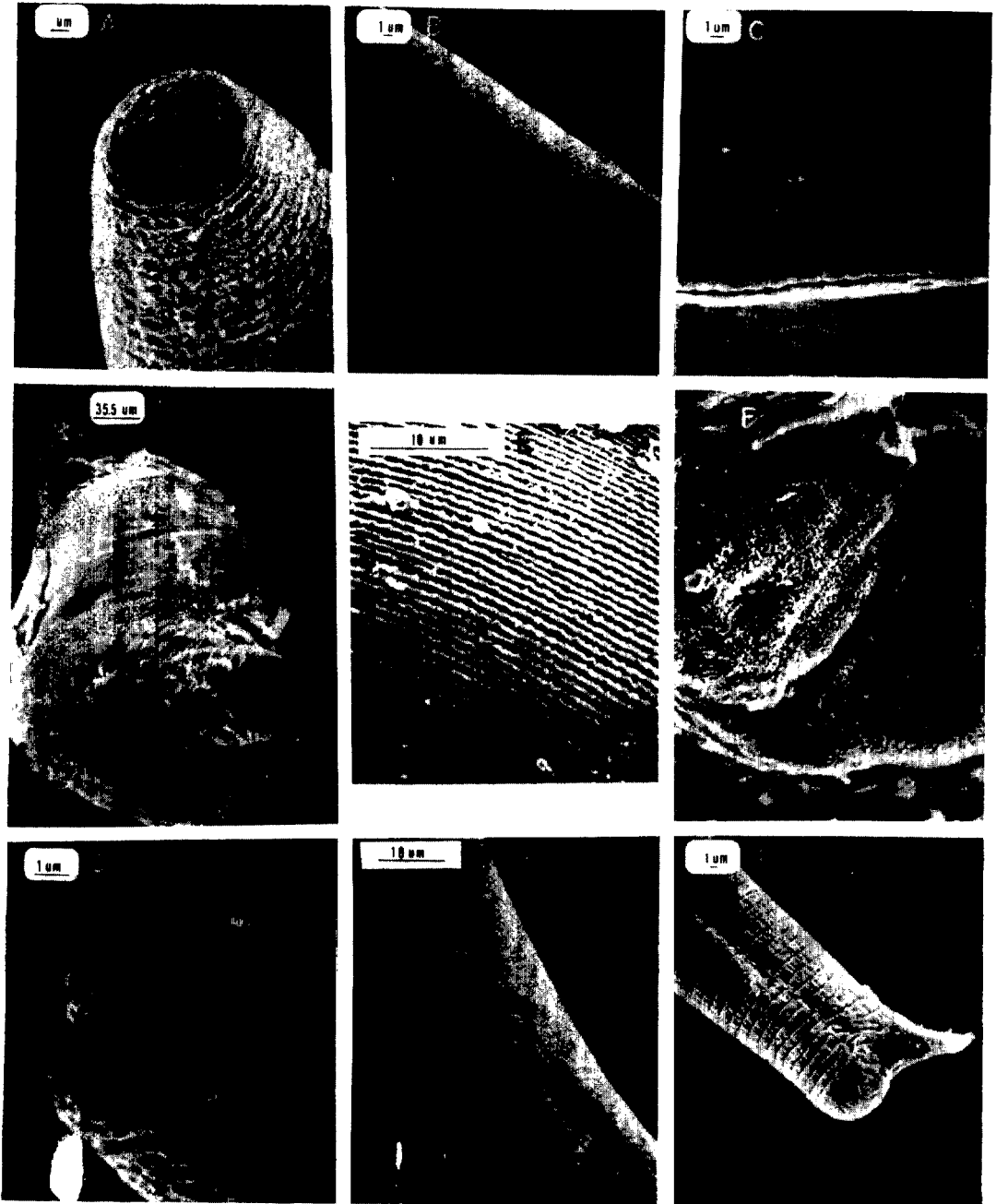


Fig. 2. Scanning electron micrographs of *Rhizonema sequoiae* n.gen. n.sp. A-C) Second-stage larvae. A) Face view. B) Lateral field (at mid body). C) Posterior part (ventral view). D-F) Female. D) Adult female. E) Cuticular annules. F) Vulva-anal close-up (636  $\times$ ). G-I) Male. G) Face view. H) Lateral field (at mid body). I) Posterior part (lateral view).

116)  $\mu\text{m}$ ; T = 35% (21-50).

Body cylindrical, elongate, tapered anteriorly, slightly curved. Cuticle distinctly annulated, about 2  $\mu\text{m}$  wide at mid body. Lateral field with four equally spaced in-

cisures diminishing to one at the tail. Aerolation present (Fig. 2H).

Cephalic region rounded, 4 (3-5)  $\mu\text{m}$  high and 8 (7-9)  $\mu\text{m}$  wide; with five annules; separated from the rest of the body

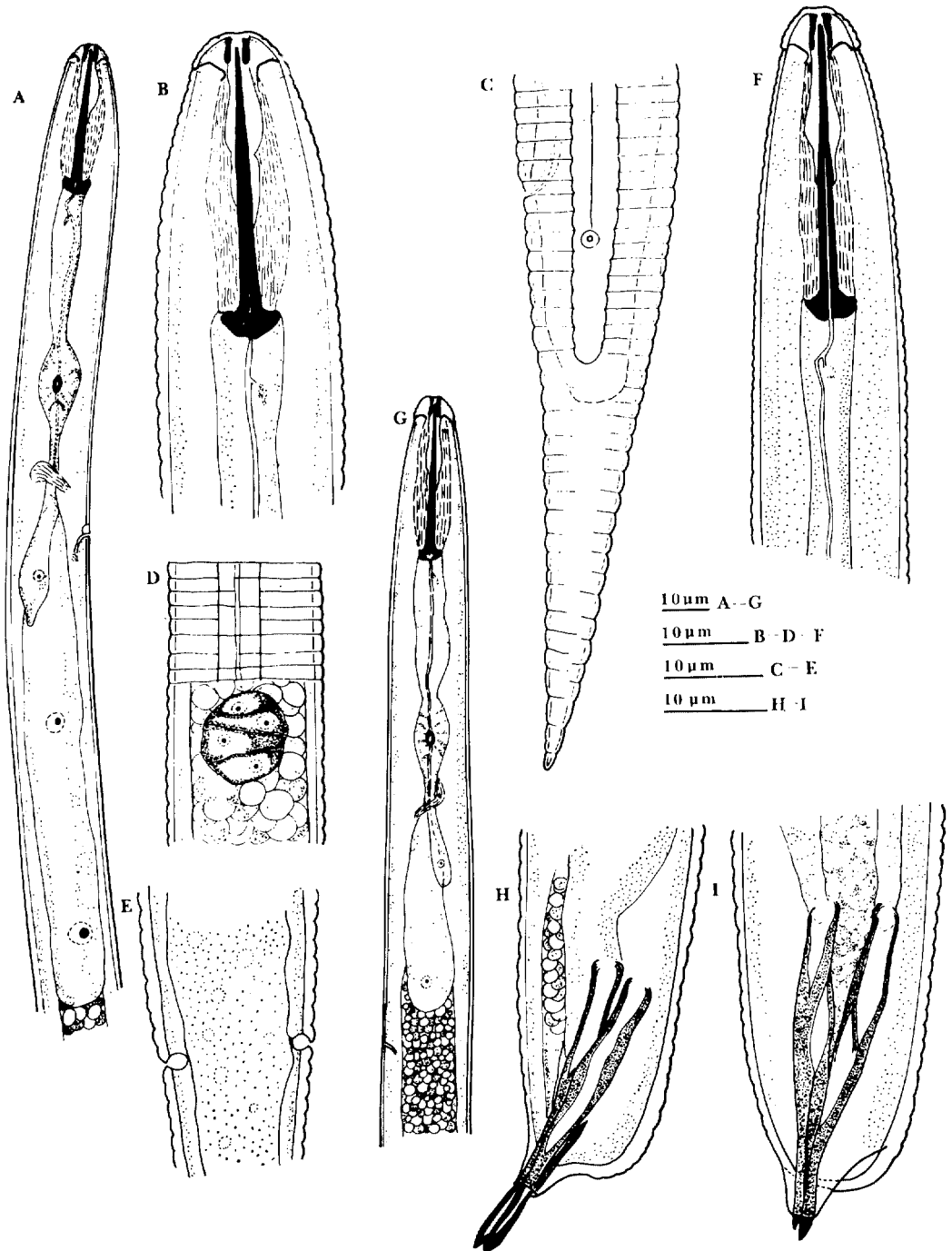


Fig. 3. *Rhizonema sequoiae* n.gen. n.sp. A-E) Second-stage larvae. A) Esophageal region. B) Head. C) Tail. D) Lateral field, intestine and genital primordium. E) Phasmids, dorso-ventral. F-I) Male. F) Head. G) Esophageal region. H) Posterior part (lateral view). I) Posterior part (ventral view).

by distinct constriction (Fig. 2G). Labial disc present with circular shape and low profile. Cephalic sclerotization strong; basal plate convex, slightly angular, and not ex-

tending below level of constriction. Stylet straight, rigid, and thick, with the shaft shorter than the cone; basal knobs slightly concave anteriorly with a short, blunt an-

terior projection, and convex posteriorly (Fig. 3F). Base of stylet to dorsal esophageal gland orifice, 3.6 (2.2–5.5)  $\mu\text{m}$ . Cephalids observed in one specimen; anterior cephalid four annules behind cephalic constriction and posterior cephalid ten annules behind constriction.

Esophagus with median bulb oblong 15.3 (13.2–18.7)  $\mu\text{m}$  long and 9.4 (7.7–11.0)  $\mu\text{m}$  wide; valve less developed than in females (Fig. 3G). Isthmus thin and short. Glands elongated, occupying nearly the entire width of the body cavity, overlapping ventrally and ventro-laterally. Excretory pore situated 134 (75–166)  $\mu\text{m}$  from the anterior end. Hemizonid observed in five specimens, 130 (110–147)  $\mu\text{m}$  from the anterior end and four annules anterior to excretory pore. Testis single, outstretched or with flexures. Spermatozoa globular. Spicules thick, slightly curved ventrally, dilated at capitulum end, thickened at middle, pointed at distal end. Gubernaculum thin, slightly curved. Spicular sheath (cloacal tubus) present (Figs. 3H, 1). Tail absent to short 2.2 (0–5.5)  $\mu\text{m}$ , with 0–4 annules. Phasmids not observed. (Fig. 2I).

*Paratype second-stage larvae:* ( $n = 33$ ):  $L = 0.55$  (0.41–0.77) mm;  $a = 24.2$  (19.3–32.0);  $b$  ( $n = 23$ ) = 2.5 (2.1–6.2);  $b' = 1.9$ –2.8; tail length ( $n = 24$ ) = 60 (50–86)  $\mu\text{m}$ ;  $c = 9.2$  (6.7–12.5);  $c' = 3.7$  (2.4–6.8); stylet = 36 (27–39)  $\mu\text{m}$ ; stylet knobs (height  $\times$  width) = 3.4 (2.2–4.4)  $\times$  7.2 (5.5–9.0)  $\mu\text{m}$ ;  $0$  ( $n = 19$ ) = 15.1 (10.8–19.3); median bulb (length  $\times$  width) = 13.2–15.4  $\times$  9.9  $\mu\text{m}$ ; distance from the anterior end to center of median bulb 76.6 (71–112)  $\mu\text{m}$ ; hyaline terminal portion of tail ( $n = 16$ ) = 35.6 (29.7–51.6)  $\mu\text{m}$ .

Body straight, elongated, cylindrical, tapering gradually posteriorly, with a moderate cephalic constriction. Cuticle with transverse annulations averaging 1.6  $\mu\text{m}$  in width at mid body. Lateral field with four incisures, aerolated at mid body (Fig. 2B); anteriorly and posteriorly the two inner incisures close together (Fig. 3D) forming three incisures anterior to phasmids and terminating in two incisures posterior to phasmids (Fig. 3C). Phasmids situated 3–6 annules posterior to anus (Fig. 2C) and 52 (44–82)  $\mu\text{m}$  from terminus, circular in ventral view (Fig. 2E). Labial disc oval in

shape (Fig. 2A), without visible sensilla (papillae), cephalic region 4.4–5.5  $\mu\text{m}$  high, 9.9–11.0  $\mu\text{m}$  wide, separated from rest of body proper by moderate constriction. Four annules present on cephalic region; two posterior annules wider than anterior two. En face view of labial framework reveals six branches, forming well-developed high arches. In lateral view, basal plate appears slightly convex, not extending posteriorly beyond constriction. Width of cuticle at labial disc equal to width near basal plate. Cephalids not observed (Fig. 3B). Stylet straight, rigid, strong, posterior portion slightly shorter than anterior, stylet knobs well developed, slightly pointed anteriorly and convex posteriorly (Fig. 3A, B). Base of stylet to dorsal esophageal gland orifice, 4.6 (2.0–6.0)  $\mu\text{m}$ . Esophagus with anteriorly enlarged procorpus. Median bulb ovoid with conspicuous valve. Isthmus narrow, short. Basal portion massive, elongated 120 (78–155)  $\mu\text{m}$ , ventrally or ventro-laterally overlapping anterior portion of intestine, occupying almost all of body cavity, three gland nuclei present. Excretory pore situated 126 (78–170)  $\mu\text{m}$  from anterior end. Hemizonid lens-like, anterior to excretory pore (Fig. 3A). Hemizonion not observed. Genital primordium oval 9.9–20  $\mu\text{m}$  long and 7.7–12  $\mu\text{m}$  wide, situated 240–384  $\mu\text{m}$  from anterior end, composed of four cells with large conspicuous nuclei (Fig. 3D). Tail elongate-conoid with very narrowly rounded terminus. Aberrant tail shapes encountered occasionally.

*Holotype (female):* Deposited at University of California Nematode Collection, Davis, USA, UCNC Slide No. 2033.

*Paratypes:* 10 females, slide 2034; 7 males, slide 2035; 7 second-stage larvae, slide 2036; at UCNC, Davis, USA; and

1) 10 females, 5 males, and 9 second-stage larvae at Museum Nat. d'Histoire, Paris, France;

2) 5 females, 1 male, and 6 second-stage larvae at Rothamsted Experimental Station, Nematology Department, Harpenden, Herts, England;

3) 9 females, 5 males, and 6 second-stage larvae at Laboratorium voor Nematologie, Landbouwhogeschool, Wageningen, Netherlands;

4) 8 females, 1 male, and 3 second-stage

larvae at USDA Nematode Collection, Beltsville, Maryland, USA;

5) 3 females, 6 males at Instituto de Biología, Lab. Helmintología. UNAM. Mexico;

6) 11 females, 5 males, and 2 second-stage larvae at Colegio de Postgraduados, Centro de Fitopatología, Chapingo, Mexico.

*Type host:* Coast Redwood, *Sequoia sempervirens* (D. Don) Endl.

*Type locality:* Lagunitas Lake, Marin County Water District, California, USA.

### DIAGNOSIS

The genus *Rhizonema* shares characteristics with most of the other genera in the family Heteroderidae. *Rhizonema* resembles *Cryphodera* Colbran, 1966 because the female does not become a cyst, the cuticle is annulated over the entire body, and eggs are retained within the body. The redwood nematode can be distinguished from *Cryphodera* by the more strongly developed vulval cone in *Rhizonema*, by the position of the anus on the dorsal lip, and the ornamentation of the vulval lips. Males of *Rhizonema* can be distinguished from those of *Cryphodera* by the presence of a cloacal tubus, shape of spicules, and the inconspicuous labial disc. Second-stage larvae of *R. sequoiae* are larger than those of *Cryphodera*.

The redwood nematode is also close to *Atalodera* Wouts and Sher, 1971 because no cyst stage is formed and anus and vulva are terminal on a protuberance. The female redwood nematode can be distinguished from *Atalodera* because the *Rhizonema* cuticle is annulated over the entire body and lacks a lace-like pattern. *Atalodera* females are annulated only in the anterior portion. Second-stage larvae differ from those of *Atalodera* in that the esophageal glands fill more than one-half the body cavity and by the shape of the basal plate.

The redwood nematode resembles *Sarisodera* Wouts & Sher, 1971 in the presence of a vulval cone, with anus on the dorsal vulval lip, the presence of a cloacal tubus in the males, shape of spicules, and the relative thickness of the cuticle at the labial region and basal plate in larvae and males. However, the redwood nematode can be distinguished by the annulation of the

body cuticle and the absence of cyst formation.

In addition to these morphological characteristics, *R. sequoiae* has a unique life history. The females live completely endoparasitically, producing a great amount of gelatin. The second-stage larvae hatch inside the female and the feeding of *R. sequoiae* incites uninucleate giant cells in the vascular tissues (unpublished observations).

### KEY TO GENERA OF THE FAMILY HETERODERIDAE

1. Female cuticle not regularly annulated over whole body ..... 2  
    Female cuticle annulated over whole body ..... 9
2. Second-stage larvae with slender or thin stylet ..... 3  
    Second-stage larvae with robust stylet 4
3. Anus and vulva subterminal, in a hollow depression; tail of second-stage larvae long, filiform .....  
    ..... *Hylonema* Luc, Taylor & Cadet, 1978  
    Anus and vulva terminal, on a very slight terminal prominence; tail of second-stage larvae short-conoid .....  
    ..... *Thecavermiculatus* Robbins, 1978
4. Female not forming a cyst after death. Larval oesophageal gland filling less than half of the body width .....  
    ..... *Atalodera* Wouts & Sher, 1971  
    Female forming cyst after death. Larval oesophageal gland filling more than half the body width ..... 5
5. Vulva sunken in a terminal cone; male without tail, cloacal tubus present .....  
    ..... *Sarisodera* Wouts & Sher, 1971  
    Vulva not sunken; male tail present and cloacal tubus absent ..... 6
6. Cyst lemon-shaped; vulval cone present .....  
    ..... *Heterodera* Schmidt, 1871  
    Cyst round or pear-shaped; vulval cone absent ..... 7
7. Cyst spherical. Perineal tubercles present .....  
    ..... *Globodera* (Skarbilovich, 1959) Mulvey & Stone, 1976  
    Cyst elongate or pear-shaped. Perineal tubercles absent ..... 8
8. Cyst elongate. Anus without fenestrae .....  
    ..... *Dolichodera* Mulvey & Ebsary, 1980  
    Cyst pear-shaped. Anus circumfenestrate .....  
    ..... *Punctodera* Mulvey & Stone, 1976

- 9. Vulva subequatorial ..... 10
- Vulva terminal ..... 11
- 10. Female elongate. Ovoid-shaped. Vulval lips not protruding ..... *Meloidodera* Chitwood, Hannon, & Esser, 1956
- Female saccate, sausage to reniform-shaped. Vulval lips strongly protruding ..... *Verutus* Esser, 1981
- 11. Vulval cone poorly developed; anus subterminal. Males with cloacal tubus absent ..... *Cryphodera* Colbran, 1966
- Vulval cone strongly developed, anus terminal. Males with cloacal tubus present ..... *Rhizonema* n.gen.

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