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## Three New Species of Nematodes Associated with Endemic Grape (*Vitis*) in California

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**ABSTRACT:** Three new species of nematodes were encountered during a study of natural diversity of nematode associates of native species of *Vitis* L. in California. *Achromadora walkeri* sp. n. was found in rhizosoil of the native California grape *Vitis californica* Benthams and is characterized by the position of the amphid (within the vicinity of both dorsal and ventral teeth), a relatively long stoma, and the absence of a prerectum. The other 2 species were plant parasitic criconematids: *Criconemoides featherensis* sp. n., found in association with roots of *V. californica*, is characterized by possessing strongly retrose annuli posterior to the vulva, a long stylet, the shape of the first annulus of the head, and rare anastomosis of annuli of the body. Specimens of *Hemicycliophora armandae* sp. n. were recovered from the rhizosoil of the desert grape *Vitis girdiana* Munson and are characterized by having 3 cephalic annuli, a lateral field marked by interruption of the striae, a long stylet, and a digitate tail. The study of symbiotic associations of native species of crop plants is important in studies of faunal and floral biodiversity.

**KEY WORDS:** *Achromadora walkeri* sp. n., California, *Criconemoides featherensis* sp. n., *Hemicycliophora armandae* sp. n., *Vitis californica*, *Vitis girdiana*, taxonomy, biodiversity, native plant species.

During a study of the diversity of soil nematode communities associated with native grape (*Vitis*: Vitaceae) of California, several new species were encountered. Nematodes of the family Criconematidae were common in most localities that were sampled, and a new species of *Criconemoides* was found around the roots of *Vitis californica* Benthams in the central valley of California. A second new species of Criconematidae (genus *Hemicycliophora*) was recovered from the rhizosoil of *Vitis girdiana* Munson in desert habitat of southern California. In addition, a new species of *Achromadora* was found in rhizosoil of *Vitis californica* in the coast range west of the Sacramento Valley. Descriptions of these 3 new species are presented herein.

### Materials and Methods

Soil samples from the rhizosphere of the roots of *Vitis* species were collected and transported to the laboratory in plastic bags. Extraction of nematodes from soil followed the sugar floatation–centrifugation method (Niblack and Hussey, 1985) for species of the genera *Criconemoides* and *Hemicycliophora*. Nematodes of the genus *Achromadora* were recovered using both sugar floatation–centrifugation and Baermann funnel methods (Christie and Perry, 1951). Nematodes recovered were killed and fixed in hot buffered formalin. Permanent slides were made using the rapid method of Seinhorst (1959). Nematodes were stained with Rose Bengal. Measurements were taken using both the ocular micrometer and the JAVA® image analysis program. Drawings were made from either permanent mounts or formalin-fixed nematodes using a drawing tube. All measurements are given in micrometers unless otherwise stated; ranges are in parentheses. For the

descriptions, abbreviated measurements are reported as follows: a = ratio of total length to maximum width; b = ratio of total length to esophagus length; c = ratio of total length to length of tail; R = total number of annuli;  $R_{s_1}$  = number of annuli from anterior extremity to base of stylet knobs;  $R_{c_1}$  = number of annuli from anterior extremity to the excretory pore;  $R_v$  = number of annuli from anterior extremity to vulva;  $R_{v_p}$  = number of annuli from vulva to posterior extremity.

### Results

#### *Achromadora walkeri* sp. n.

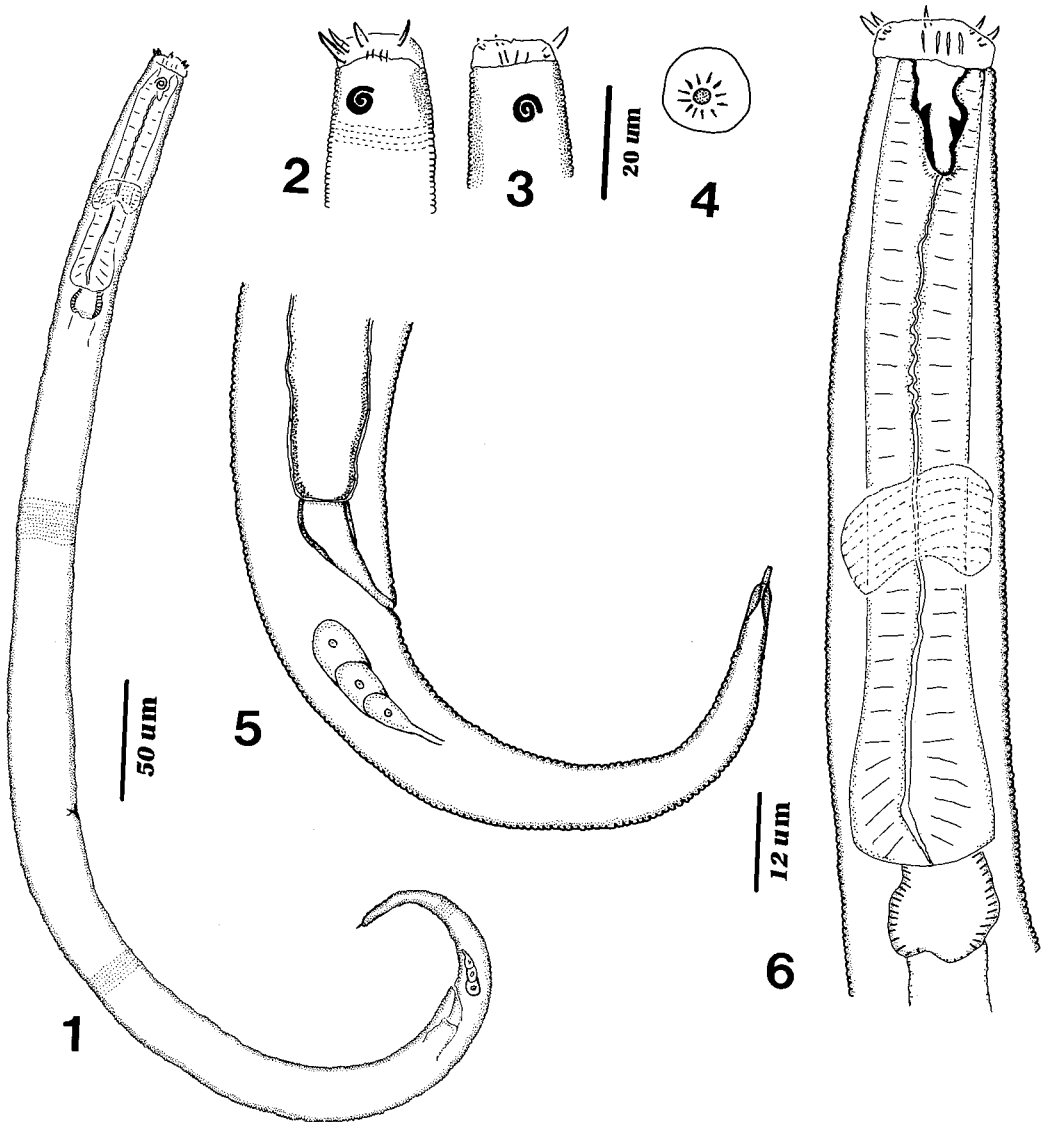
(Figs. 1–6)

#### Description

**HOLOTYPE** (female): Length 698; maximum width 27; esophagus length 105; tail length 77; width at anus 15; rectum length 17; V% = 48; buccal cavity  $13 \times 5$ ; anterior extremity to amphid 8.6; amphid diameter 4.5; a = 26; b = 6.6; c = 8.9.

**FEMALES** ( $N = 6$ ): Length 640 (590–698); maximum width 26 (24–28); esophagus length 101 (91–107); tail length 72 (63–80); width at anus 17 (15–20); rectum length 17 (15–20); V% 47 (43–49); buccal cavity  $12 \times 4.7$  (10–13  $\times$  4–5); anterior extremity to amphid 8.5 (7.5–9); amphid diameter 4.3 (3.4–4.8); a = 25 (23–26); b = 6.4 (5.8–7); c = 8.9 (8–10).

Anterior body straight, posterior half curved then coiled postanal (Fig. 1). Cuticle annuli very fine, transverse rows of punctation exist along the body cuticle (Fig. 2). Head with 12 cephalic setae visible in en face view (Fig. 4). Stoma infundibular, dorsal tooth located in upper third



Figures 1–6. *Achromadora walkeri* sp. n. 1. Female, entire. 2, 3. Female, anterior region, amphid as seen on right-lateral side and left lateral side, respectively (view of body from right-lateral side). 4. Female, en face view of the head showing 12 well-developed setae. 5. Female, posterior region. 6. Female, anterior region showing development of esophagus and buccal capsule.

of buccal cavity (38%). Subventral tooth located posteriorly in buccal cavity (47%). Amphid helical, 8.5 from anterior extremity, diameter approximately 4.3 (Figs. 2, 3). Esophagus with valved posterior bulb, 16% of total body length. Nerve ring approximately 64 from anterior extremity (Fig. 6). Esophago–intestinal valve present. Vulva not protruding, located 43–49% of total body length. Prerectum absent. Tail curved ventrally, tapered to a rounded terminus, with cuticular spinneret (Fig. 5).

MALES: Not found.

TYPE LOCALITY: Mix Canyon (38°24'N, 122°02'W), Solano County, California, U.S.A.

SYMBIOTYPE (see Frey et al., 1992): *Vitis californica* Bentham, University of California Davis, J. M. Tucker Herbarium No. 120481.

SITE: Soil around the roots of *Vitis californica*.

TYPE MATERIAL (holotype): Female on slide, University of California, Davis Nematode Collection (UCDNC) No. 2925.

**PARATYPES** (females): On slide, UCDNC No. 2926.

**ETYMOLOGY:** This nematode was named after Dr. Andrew Walker, who helped obtain samples used in this study.

### Diagnosis

*Achromadora walkeri* sp. n. appears morphologically similar to *A. ruricola* (de Man, 1880), from which it can be differentiated by the position of the amphid. The amphid in *A. ruricola* is located at the base of the stoma (Mulvey, 1969), whereas in *A. walkeri* sp. n. it is more anterior (level of sub-ventral tooth) and the buccal cavity is larger ( $12 \times 4$ ) than that in *A. ruricola* ( $7 \times 4$ ). *Achromadora walkeri* differs from both *A. micoletzkyi* Steiner, 1916, and *A. pseudomicoletzkyi* van der Linde, 1938 (see Mulvey, 1969), in lacking a preectum, and it differs further from *A. micoletzkyi* in having a longer body (0.59–0.7 vs. 0.48–0.61 mm) and larger buccal cavity ( $12 \times 4$  vs.  $8 \times 4$ ) (Mulvey, 1969). *Achromadora walkeri* also differs from *A. pseudomicoletzkyi* in tail length (72 vs. 100) and in vulva position (43–49 vs. 53%) and from *A. semiarmata* Altherr, 1952, in total body length (0.59–0.70 mm vs. 0.45–0.46 mm), length of the stoma (12 vs. 8), c-value (8–10 vs. 5.5–6), and the position of the vulva (43–49 vs. 44%).

### *Criconemoides featherensis* sp. n. (Figs. 7–10)

#### Description

**HOLOTYPE** (female): Length 391; maximum width 35; esophagus length 141; stylet length 101;  $V\% = 84$ ;  $a = 11.2$ ;  $b = 2.8$ ;  $R = 83$ ;  $R_{st} = 27$ ;  $R_{vp} = 13$ .

**FEMALES** ( $N = 15$ ): Length of body 325 (263–391); maximum width 33 (31–35); esophagus length 117 (106–141); stylet length 95 (87–103);  $V\% = 85$  (80–90);  $a = 9.4$  (8.9–11.2);  $b = 2.8$  (2.6–3.2);  $R = 80$  (75–84);  $R_{st} = 27$  (22–31);  $R_{vp} = 13$  (11–14).

Body curved ventrally (open C-shape) (Fig. 10), tapering at posterior extremity. Annuli along body retrose, strongly retrose behind vulva to posterior extremity (Fig. 9). Anastomosis very rare, annuli mostly smooth without interruption. Lip region with 2 annuli (Fig. 7). Labial plate almost flat, slightly elevated. Stylet long, ending with anchor-shaped knobs. Esophagus length 36% of total body length, basal bulb not offset from isth-

mus. Vulval slit-position variable found between annulus numbers 11 and 14 from posterior extremity. Body tapers posterior to vulva to a terminus with protruding knob-like structure.

**MALES:** Not found.

**JUVENILES:** Annuli serrated (Fig. 8).

**TYPE LOCALITY:** Bobelaine Wildlife Preserve, 32 km by road south of Yuba City ( $38^{\circ}55'N$ ,  $121^{\circ}34'W$ ), Placer County, California, U.S.A.

**SYMBIOTYPE:** *Vitis californica* Bentham.

**SITE:** Soil from the rhizosphere of the roots of *Vitis californica*.

**TYPE MATERIAL** (holotype): Female, UCDNC No. 2923.

**PARATYPES** (females): UCDNC No. 2924 from type locality and host.

**ETYMOLOGY:** This nematode was named after the Feather River, near the type locality.

### Diagnosis

*Criconemoides featherensis* sp. n. differs from *C. grassator* Adams and Lapp, 1967, in having very strong retrose annuli on the posterior body. In *C. featherensis*, the first annulus is thick and relatively wide, whereas the first ring in *C. grassator* has edges that project anteriorly. In addition, the tail of *C. featherensis* tapers gradually to a point, whereas that of *C. grassator* tapers to a sharp postanal cone (Adams and Lapp, 1967).

*Criconemoides featherensis* differs from *C. annulifer* (de Man, 1921) and *C. calvus* Raski and Golden, 1966, by having a shorter body length (325 vs. 386 and 390 [mean], respectively, a tail that tapers gradually to a conspicuous knob without attenuation, and a greater number of annuli (80 [mean]) (Raski and Golden, 1966).

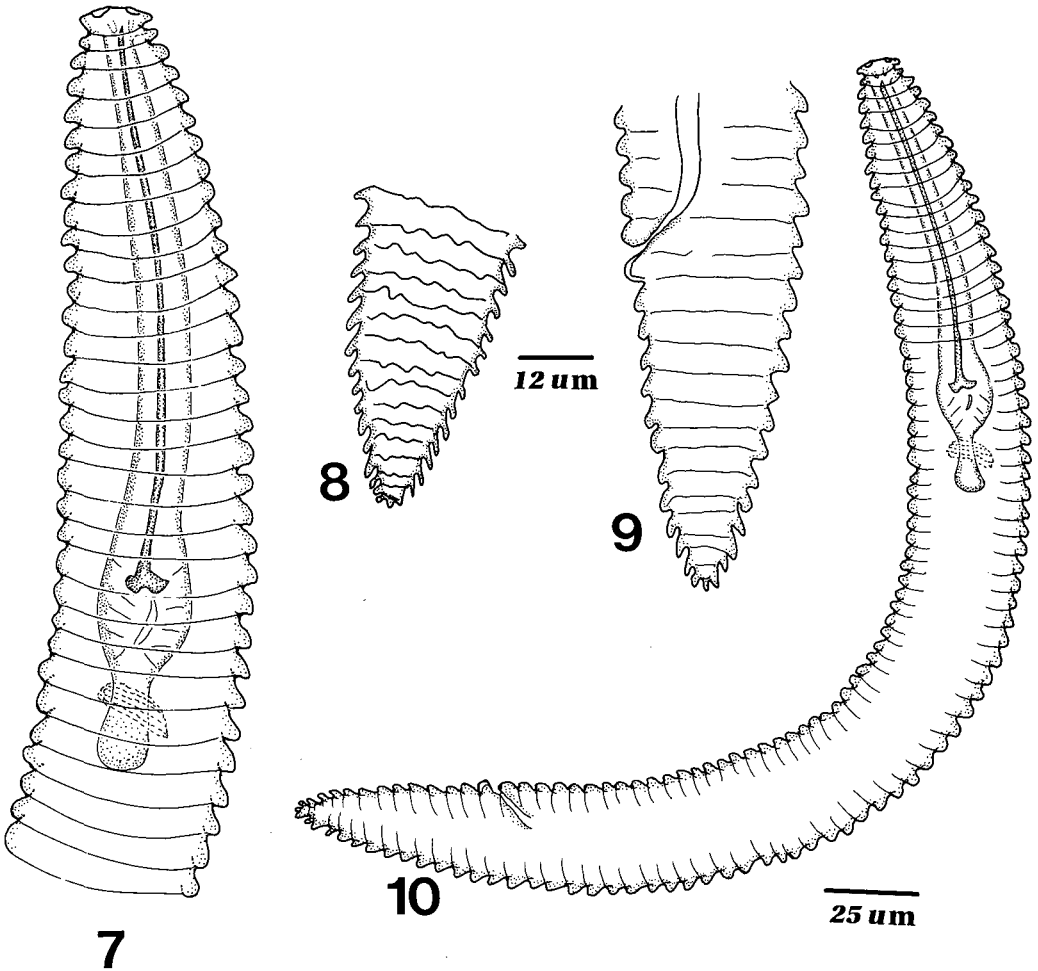
### *Hemicycliophora armandae* sp. n. (Figs. 11–15)

#### Description

**HOLOTYPE** (female): Length 986; maximum width 53; esophagus length 187;  $V\% = 85$ ;  $a = 19$ ;  $b = 5.3$ ; stylet length 112;  $R = 250$ ;  $R_{st} = 27$ ;  $R_{cx} = 56$ ;  $R_v = 233$ .

**FEMALES** ( $N = 8$ ): Length 980 (790–1,090); maximum width 46 (38–54); esophagus length 190 (175–214);  $V\% = 85$  (82–86);  $a = 21$  (19–25);  $b = 5.1$  (4.5–5.7); stylet length 111 (95–119);  $R = 269$  (250–280);  $R_{st} = 29$  (25–30);  $R_{cx} = 54$  (47–60);  $R_v = 220$  (211–233).

Body curved slightly ventrally when killed in hot formalin (Fig. 11). Cuticular sheath close to



Figures 7–10. *Criconemoides featherensis* sp. n. 7. Female, anterior region showing esophagus and stylet. 8. Juvenile, tail. 9. Female, tail. 10. Female, entire.

inner body cuticle. Sheath and body annuli flattened especially on posterior region. Lateral fields marked by breaks in striae (Fig. 15). Cephalic region 16 wide  $\times$  1 high, with 3 annuli. Labial plate raised slightly. Stylet long and thin (95–119  $\times$  1.2). Stylet knobs rounded, sloping slightly posteriad, located usually around annulus 29. Esophagus relatively long 190 (175–214). Esophago–intestinal valve present (Fig. 12). Excretory pore generally 2 annuli posterior to the end of esophagus, 196 (181–220) from the anterior extremity. Female gonad single, anteriorly directed and out-stretched without flexures. Spermatheca oblong, no spermatozoa visible (Fig. 13). Vulval lips modified, anterior lip extending over the posterior (Fig. 14). Body narrowing posterior to vulva but evenly conoid, ending with a

digitate tail with a rounded terminus. Anus located about 16 annuli posterior to vulva.

MALES: Not found.

TYPE LOCALITY: Grapevine Mountain (33°07'N, 116°28'W) in Anza Borego Desert State Park, Riverside County, California, U.S.A.

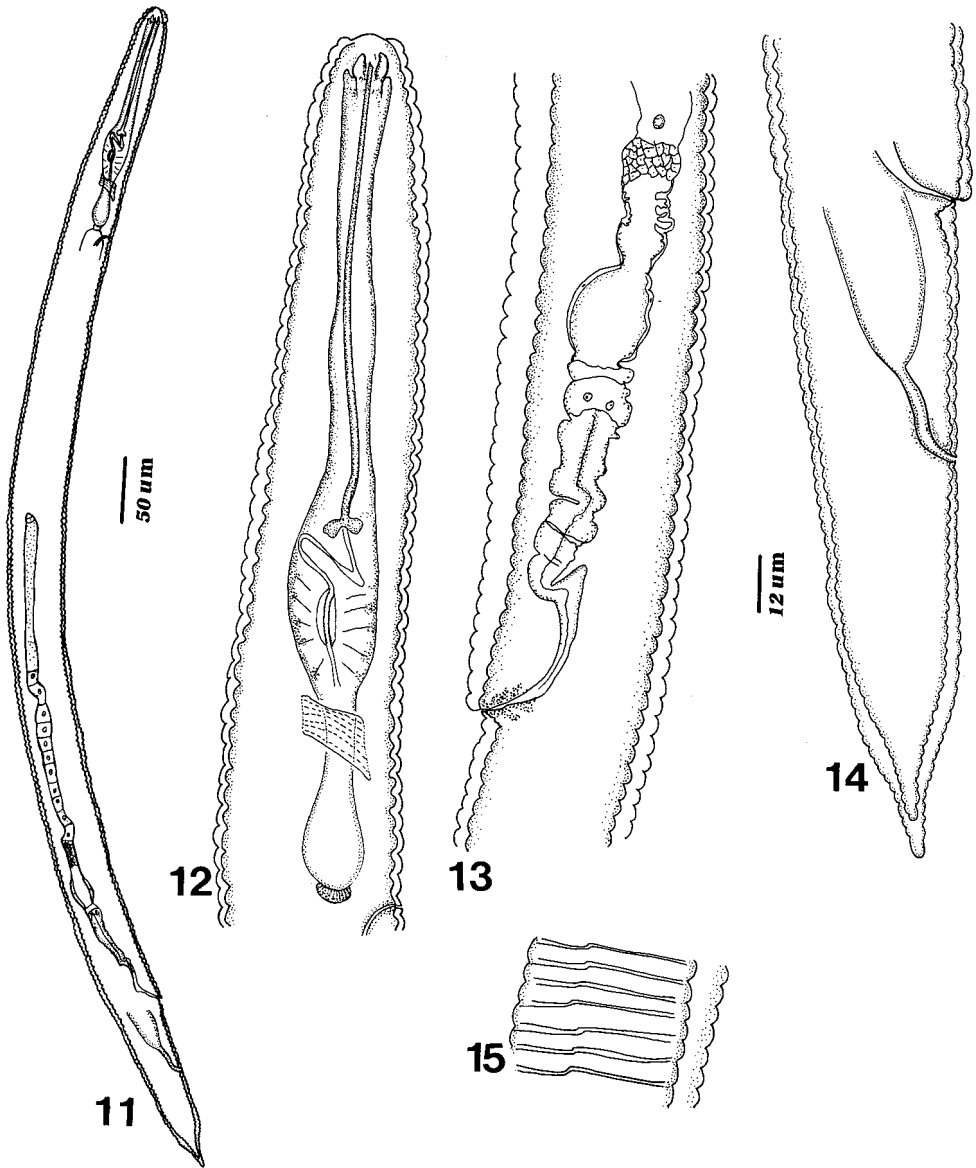
SYMBIOTYPE: *Vitis girdiana* Munson. University of California Davis, J. M. Tucker Herbarium No. 120480.

SITE: Soil around the roots of *Vitis girdiana*.

TYPE MATERIAL (holotype): Female on slide, UCDNC No. 2927.

PARATYPES (females): On slide, UCDNC No. 2928.

ETYMOLOGY: This nematode was named in honor of Dr. Armand Maggenti, a leader in the systematics of nematodes.



Figures 11–15. *Hemicycliophora armandae* sp. n. 11. Female, entire. 12. Female, anterior region. 13. Female, region of vulva. 14. Female, tail. 15. Female, cuticular annulation.

**Diagnosis**

*Hemicycliophora armandae* sp. n. can be recognized as distinct from *H. californica* Brzeski, 1974, *H. halophila* Yeates, 1967, *H. iwia* Brzeski, 1974, *H. minor* Wu, 1966, *H. shepherd* Wu, 1966, *H. similis* Thorne, 1955, and *H. thornei* Goodey, 1963, in having 3 annuli in the cephalic region vs. 2 in all others (Thorne, 1955; Goodey, 1963; Wu, 1966; Yeates, 1967; Brzeski, 1974). The position of the excretory pore of *H. arman-*

*dae* is located within the 47th to the 60th annulus while in the preceding species (except *H. shepherd*), the excretory pore is located between the 38th and 49th annuli (inclusive). In addition, *H. armandae* differs from *H. minor*, *H. shepherd*, and *H. similis* in having a prominent spermatheca, in the shape of the vulval lips, and in the tail terminus. *Hemicycliophora armandae* can be differentiated further from *H. thornei* in having no lateral lines, a longer stylet (111 vs. 63), and a vulva more posteriad (82–86 vs. 80–82%) (see

Goodey, 1963). *Hemicycliophora armandae* differs from *H. californica* in having larger values for R (250–280 vs. 210–236),  $R_v$  (211–233 vs. 172–195), cuticular sheath (close vs. very close to body cuticle), and stylet length (95–119 vs. 85–98) and a lower a-value (19–25 vs. 24–29) and lower V% (82–86 vs. 86–87%). The tail terminus is rounded in *H. armandae* but is sharply pointed in *H. californica* (see Brzeski, 1974) and the vulval lips are more protruding and the contraction behind the vulva is greater than in *H. californica*. *Hemicycliophora armandae* can be differentiated from *H. iwia* in shape of tail terminus (more finger-like in *H. armandae*), by greater total number of annuli (269 vs. 204), and in the absence of lateral lines. *Hemicycliophora armandae* differs from *H. halophila* in having a modified vulva, shorter body length (790–1,090 vs. 1,030–1,210), and greater total number of body annuli (269 vs. 230 [mean]) and in the absence of longitudinal markings; *Hemicycliophora halophila* possesses “delicate longitudinal markings along each edge” (Yeates, 1967).

### Discussion

The diversity and systematic relationships of nematodes parasitic on the majority of plants grown as crops are fairly well known. In contrast, little is known of the symbiotic associates of wild or native relatives of presently cultivated crop plants (e.g., see the volume edited by Nickle, 1984). Despite the economic threat to cultivated crops posed by nematodes worldwide, there are few scientists with sufficient training capable of collecting, identifying, and describing new species of nematodes.

Up to the present time, very little work has been conducted on the nematode associates of native species of plants that are close relatives of presently cultivated crop plants. In the case of grapevine, of the more than 637 published reports of nematodes associated with grapes of the genus *Vitis* L., only 3 studies include data on nematodes of native species of *Vitis* (see González and Valenzuela, 1968; Siddiqui et al., 1973; Al Banna, 1992). The dagger nematode, *Xiphinema index* (Thorne and Allen, 1950), the vector of the grape fan-leaf virus, has a cosmopolitan distribution and has been studied intensively in California (Raski et al., 1983); however, there is no clear picture of the area of origin of this nematode and, therefore, no information is available concerning the community of nematode associates in which *X. index* may have evolved. This

is just one of many examples that demonstrates how little is known of the biological characteristics of nematode associates of native plants in nonagricultural ecosystems.

Because we know so little of the nematode associates of wild native plants, it is difficult to make generalizations concerning the ecological and trophic relationships of nematode associates of cultivated crop plants. We feel that new emphasis should be placed on studies of the relationships among plants and their symbionts.

### Acknowledgments

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### Coccidiosis Conference

The annual Coccidiosis Conference will be held on 31 October 1993 from 2-5 pm in the afternoon of the first day of the joint meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists, in Atlanta, Georgia. The purpose of the conference is to bring together scientists from disparate research areas who are studying mechanisms of immunity against parasites. Topics include developmental stages that induce and are targeted by protective immunity, the role of lymphokines in the immune response, and evasion mechanisms that the parasite may use to evade host immunity. The title of the conference is, "Parasite-Host Interactions: Immunity and Evasion Mechanisms."