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Description of *Hemicycliophora biosphaera* n. sp. from Arizona (Nemata: Criconematidae)[1]

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Abstract: *Hemicycliophora biosphaera* n. sp. (Nemata: Criconematidae) was found in soil from a fallow field plot within the Biosphere 2 Center, Oracle, Arizona. The nematode species is characterized by continuous and irregular breaks in transverse striae in the lateral field, smooth annules, a rounded-truncate lip region with rounded anterior margins, three lip annules, first labial annule elevated and widened laterally, dome-shaped and elevated labial disc, stylet length (76–97 µm), VA%T value (30–59), 234–273 body annules, and tail with a terminus offset, cylindrical to slightly conoid digit. *Hemicycliophora biosphaera* n. sp. most closely resembles *H. armandae* but differs from it in body width (30–39 vs. 38–54 µm), stylet length (76–97 vs. 95–119 µm), greater number of annules between the excretory pore and esophagus base (4–16 vs. 2), length of the tail terminal spike (16–28 vs. 32 µm), lower Rvan value (9–15 vs. 16), and indistinct spermatheca vs. distinct spermatheca.

Key words: Arizona, Biosphere 2, Criconematidae, *Hemicycliophora, armandae, Hemicycliophora biosphaera,* morphology, nematode, new species, sheath nematode, taxonomy.

In June 1995, specimens of a *Hemicycliophora* sp. with a conical, digitate tail were extracted from soil samples collected from a 4-month fallow plot within the Biosphere 2 agricultural mesocosm, called the Intensive Agricultural Biome (IAB), in Oracle, Arizona. Prior to being fallow, the plot had been cultivated with white potato. Almost a year later, in March 1996, additional soil samples collected from the same fallow plot contained large numbers of the same nematode species. Further observations with light and scanning electron microscopy (SEM) as well as comparison with other *Hemicycliophora* species having conical, spiked tails indicated the presence of a new species, which is described herein. The specific name, *Hemicycliophora biosphaera* n. sp., refers to its geographic origin.

MATERIALS AND METHODS

Soil samples were collected from the IAB, which comprised a soil volume of about 2,000 m^3 of local desert origin maintained under semi-tropical climate conditions operated by mechanical systems. Soil and plants within the IAB had been exposed to high levels of atmospheric carbon dioxide, about 500–2000 ppmv from 1994 to 1996. Soil moisture was maintained at near field capacity with recycled water enriched in salts and nitrate content comparable to levels in natural rain water (Mahato et al., unpubl.). Juvenile and adult specimens of *H. biosphaera* n. sp. were extracted from soil and initially preserved in 2.5% formaldehyde. For light microscopy, specimens were fixed in double-strength FAA with half the amount of distilled water added, and processed to dehydrated glycerin according to Seinhorst's method (Seinhorst, 1959). Measurements were made of specimens in glycerin. Length of the posterior spike was measured as a straight line drawn from the beginning of the distal taper of the tail to its tip. The term "cavity" is used in reference to the "space" present between the posteriorly angular stylet knobs, and is distinct when viewed laterally through a light micro-

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scope. Although the use of this term may not be consistent in taxonomic literature, the cavity is consistently present in the new species and, therefore, is used here as a reliable diagnostic character.

Specimens fixed in FAA were processed for scanning electron microscopy (SEM) as described by Chitambar (1992). Specimens were attached to aluminum foil on stubs and sputtered with 300 Å gold-palladium, then examined with a JEOL JSM-6300 SEM at 12 kV. Two paratype females of *H. armandae* Al Banna & Gardner, 1993, mounted in glycerin, were made available for this study. Of these, one female specimen was processed for SEM examination. This specimen was sputtered with 300 Å gold-palladium and examined at 8 kV.

SYSTEMATICS

Hemicycliophora biosphaera n. sp. (Figs. 1 & 2)

Description

Morphometrics of the holotype female and paratype females are given in Table 1.

Female: Body distinctly ventrally arcuate when heat-killed. Sheath fitting closely, attached at lip region and vulva. Lateral field without longitudinal incisures; transverse striae with continuous, diagonally continuous, and irregular breaks (Fig. 1E). Annules smooth, without longitudinal markings. Lip region rounded-truncate with rounded anterior margins and three annules: first annule widened and elevated laterally, faintly visible in most specimens with light microscopy, distinct with SEM, usually narrower than second and third annules in lateral view (Fig. 2B). Labial disc dome-shaped in lateral view, oval in face view, distinctly elevated above lip annule in most specimens, attached dorsally and ventrally at base to first lip region annule. Amphid apertures covered by elevated, rectangular amphidial shields (Fig. 2A). Stylet knobs sloping posteriorly, rounded to rectangular, occasionally with slightly anteriorly directed tips; cavity present between basal knobs (Fig. 1D). Excretory pore always posterior to esophagointestinal junction, usually by more than 4 annules (16 in one specimen). Esophagointestinal valve cone-shaped. Upper and lower vulval lips equally protruded, sleeve short or absent (Fig. 2D). Spermatheca indistinct, collapsed in one specimen. Tail cylindrical, tapering equally and almost abruptly to a slightly rounded posterior portion bearing a digit or spike ending in a rounded tip. Digit offset, cylindrical in most specimens (36), slightly conoid in 7 specimens (Fig. 2 E-H).

Males: Not found.

Type host and locality

Type specimens extracted from soil samples collected in June 1995 and March 1996 from a fallow field plot within the IAB of Biosphere 2 in Oracle, Arizona.

Type designations

Holotype female and 14 paratype females deposited in the University of California, Davis Nematode Collection (UCDNC), Department of Nematology, University of California, Davis; five paratypes deposited in the University of California, Riverside Nematode Collection (UCRNC), Department of Nematology, University of California, Riverside; 10 paratypes deposited in the USDA Nematode Collection, Beltsville, Maryland; 10 paratypes deposited in the Wageningen Agricultural Nematode Collection, Department of Nematology, Wageningen Agricultural University, Wageningen, The Netherlands; and additional paratypes deposited in the California Department of Food and Agriculture permanent nematode slide reference collection.

Diagnosis

Hemicycliophora biosphaera n. sp. is characterized by the pattern of transverse striae in the lateral field; smooth annules; rounded-truncate lip region with rounded anterior margins and three annules, first labial annule widened and elevated laterally, labial disc dome-shaped, elevated; stylet length (76–97 µm); VA%T value (30–59); number of body annules (234–273); and tail with an

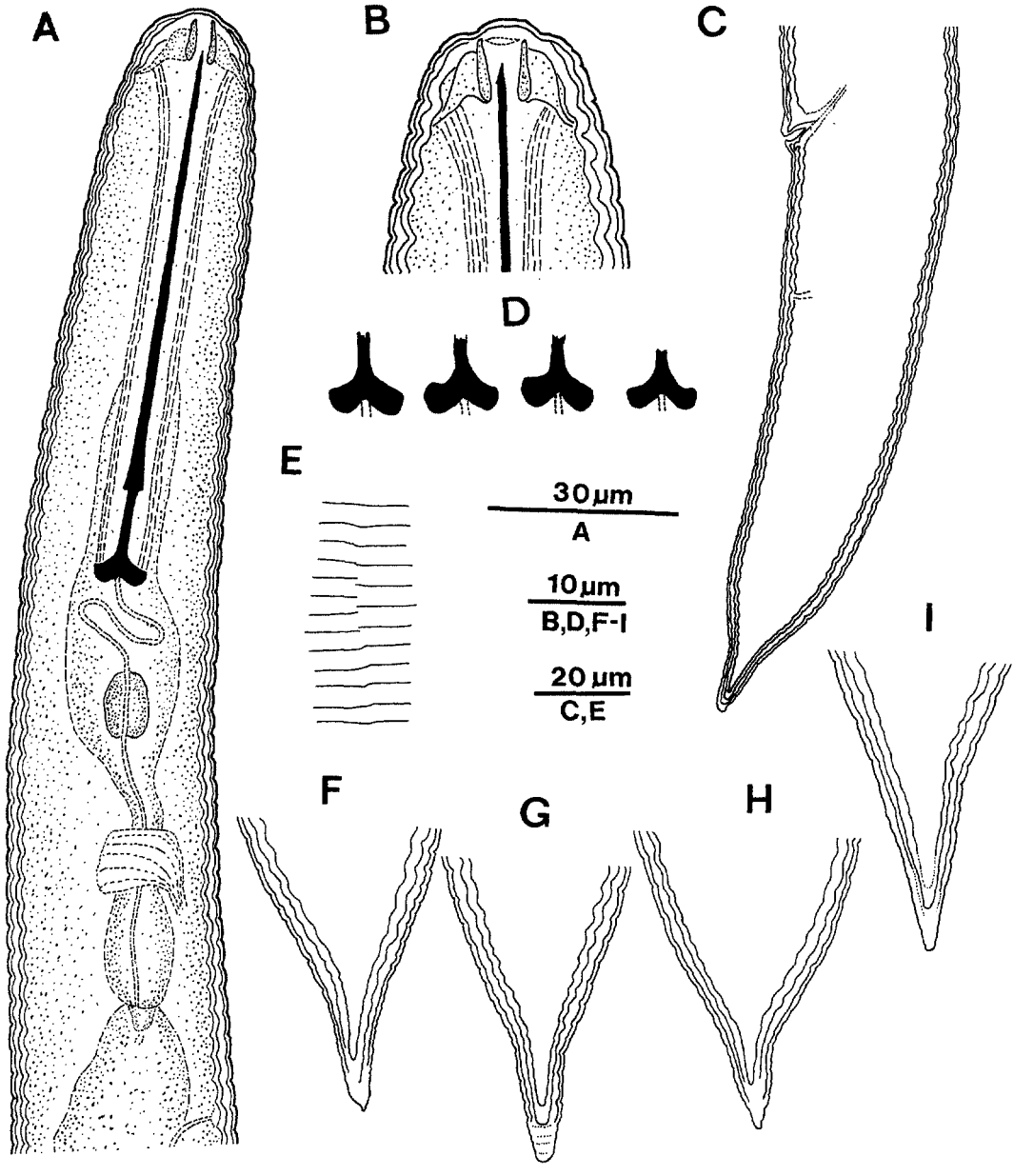

FIG. 1. *Hemicycliophora biosphaera* n. sp. females. A) Anterior body. B) Anterior end. C) Posterior body. D) Stylet knobs. E) Lateral field pattern. F-I) Tail tips. (Scale bars: A = 30 µm, B, D, F-I = 10 µm, C, E = 20 µm).

offset, cylindrical to slightly conoid terminal digit.

Relationships

Hemicycliophora biosphaera n. sp. is similar to several other species with digitate to conoid tails and most closely resembles *H. armandae* and *H. iranica* Loof, 1984 (Loof, 1984). It differs from *H. armandae* in body width (30–39 µm in *H. biosphaera* n.sp. vs. 38–54 µm in *H. armandae*), shorter stylet (76–97 µm vs. 95–119 µm), shape of the first labial annule (widened and elevated laterally vs. not widened or elevated laterally), annules smooth vs. annules with short longitudinal lines, excretory pore 4–16 annules posterior to the base of esophagus vs. 2 annules posterior, shorter tail digit/spike (16–

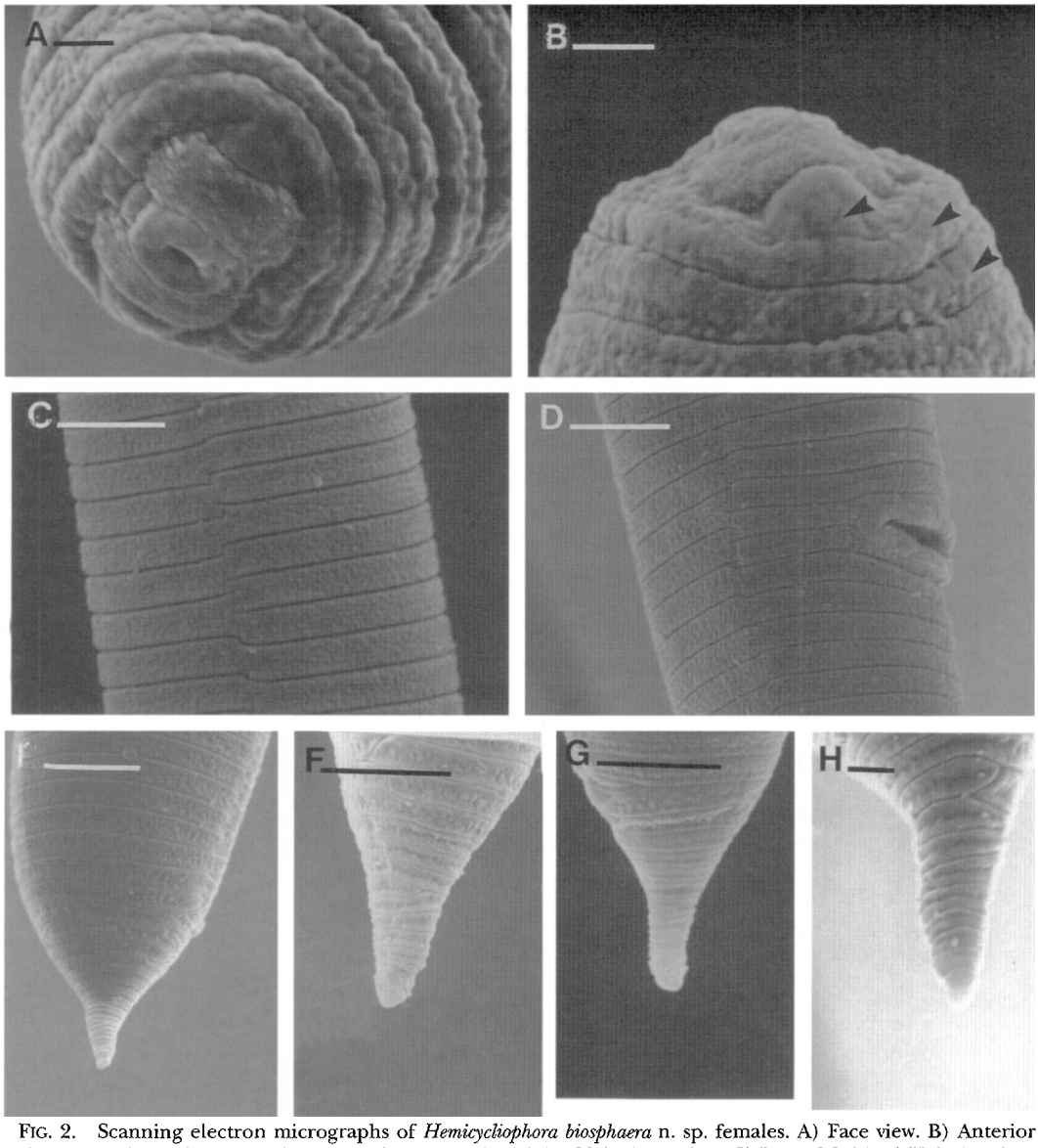

FIG. 2. Scanning electron micrographs of *Hemicycliophora biosphaera* n. sp. females. A) Face view. B) Anterior end, sublateral view (arrowheads indicate first, second, and third labial annules). C) Lateral field. D) Vulval region, subventral view. E-H) Posterior tail ends showing variation in digit shape. (Scale bar: A, B, H = 2 μm; C-G = 10 μm).

28 μm vs. 32 μm), lower Rvan (9–15 annules vs. 16 annules), vulval lips of equal length vs. anterior lip longer than posterior, and indistinct spermatheca vs. prominent.

Hemicycliophora biosphaera n. sp. differs from *H. iranica* by having its amphid apertures covered by shields vs. amphid apertures open and crescent-shaped; lip region with 3 annules vs. lip region with 2 annules; spermatheca indistinct vs. spermatheca distinct and filled with sperm; greater Rex (47–58 vs. 38–48); greater number of annules from esophageal base to excretory pore (4–16 vs. 2); tail with an offset, cylindrical to slightly conical distal digit vs. tail with an elongate, broadly conical distal. (Note: in the original description by Loof [1984] the plate with Fig. 2C and 2D on page 26 showing SEM face view and lateral field was incorrectly titled as representing *H. iranica,* when, in fact, the correct plate was Figs. 9C and 9D [Anonymous, 1984]).

TABLE 1. Morphometrics of female *Hemicycliophora biosphaera* n. sp.

	Holotype	Paratypes (n = 41) Mean ± SE	Range
Measurements in µm			
L	933	924 ± 8.7	762–1,038
Lip region height	8.8	8.5 ± 0.1	7–10
Lip region width	16.0	15.8 ± 0.1	14–18
Stylet length	94	91 ± 0.8	76–97
Stylet knob height	3.6	3.3 ± 0.1	2–4
Stylet knob width	7.2	7.2 ± 0.1	5–9
Body width at stylet base	29	29 ± 0.2	27–32
Maximum body width	35	34 ± 0.3	30–39
Body width at anus	27	28 ± 0.3	24–34
Annule width	4.0	4.0 ± 0.1	3–5
Anterior end to nerve ring	138	133 ± 1.2	100–151
Anterior end to excretory pore	206	188 ± 1.6	162–208
Tail length	87	86 ± 1.2	66–101
Tail spike/length	21	22 ± 0.5	16–28
Ratios			
a	27	27.1 ± 0.3	23–31
b	5.6	5.6 ± 0.1	5.0–6.5
c	10.7	10.9 ± 0.1	9.4–13.0
c′	3.2	3.0 ± 0.1	2.6–3.7
Percentages			
V	88	86.8 ± 0.1	85–88
Stylet cone as percentage of stylet length	83	82.9 ± 0.2	80–85
Vulva-anus distance as percentage of tail length	41	41.9 ± 1.0	30–59
Number of annules			
Body annules	242	250 ± 1.4	234–273
Number of annules from:			
Labial disc to stylet base	25	26 ± 0.2	23–29
Labial disc to esophago-intestinal valve	43	45 ± 0.3	42–49
Labial disc to excretory pore	54	53 ± 0.3	47–58
Labial disc to vulva	205	207 ± 1.2	192–229
Esophagus base to excretory pore	8	7 ± 0.3	4–16
Vulva to anus	11	12 ± 0.2	9–15
Tail terminus to vulva	38	43 ± 0.4	38–49
Tail terminus to anus	27	31 ± 0.3	26–36

Hemicycliophora biosphaera n. sp. also resembles *H. californica* Brzeski, 1974 (Brzeski, 1974); *H. thornei* Goodey, 1963 (Goodey, 1963); *H. shepherdi* Wu, 1966 (Wu, 1966); *H. similis* Thorne, 1955 (Thorne, 1955); *H. hesperis* Raski, 1958, (Raski, 1958); *H. minora* Wu, 1966 (Wu, 1966); and *H. halophila* Yeates, 1967 (Yeates, 1967) but differs from these species by having 3 annules in the lip region instead of 2. In addition, the following characteristics of *H. biosphaera* n. sp. separate it from these species: body length 762–1,038 µm (1,130–1,220 µm in *H. hesperis;* 1,200 µm in *H. thornei*); stylet length 76–97 µm (96–145 µm in *H. halophila,* 103–109 µm in *H. hesperis* (Reay, 1984; Yeates, 1967), 95–106 µm in *H. minora,* 98–105 µm in *H. shepherdi*); conical tail with an offset cylindrical to slightly conical digit (conical tail with an elongate, conical terminus in *H. halophila, H. hesperis, H. shepherdi,* and *H. similis*); Rex 47–58 (38–46 in *H. californica,* 33–45 in *H. halophila,* 41–49 *H. minora*); Rv from anterior end 192–229 (134–162 in *H. californica;* 153–197 in *H. minora*); Rv from tail terminus 38–49 (59 in *H. hesperis;* 48–66 in *H. similis*); RVan 9–15 (15–22 in *H. minora;* 20 in *H. similis*); stylet knob cavity present (cavity absent in *H. californica, H. shepherdi*). Further, *H. biosphaera* n. sp. differs from *H. similis* by having a more posterior vulva (85–88% vs. 79%), from *H. hesperis* by lower Ran value (26–36 vs. 39) and lip annules not distinctly separated vs. distinctly separated; from *H. thornei* by greater "a" value (23–31 vs. 20); from *H. shepherdi* by shorter vulval lips (less than 1 annule vs. 2 annules); from *H. halophila* by smooth annules vs. presence of frequent short longitudinal lines on annules.

Hemicycliophora armandae Al Banna & Gardner, 1993 (Fig. 3)

Two paratype specimens were examined. Morphometrics of the examined specimens agreed with the values reported in the original description. Light microscope and SEM examination of the species provided additional morphological information not included in the original description but useful

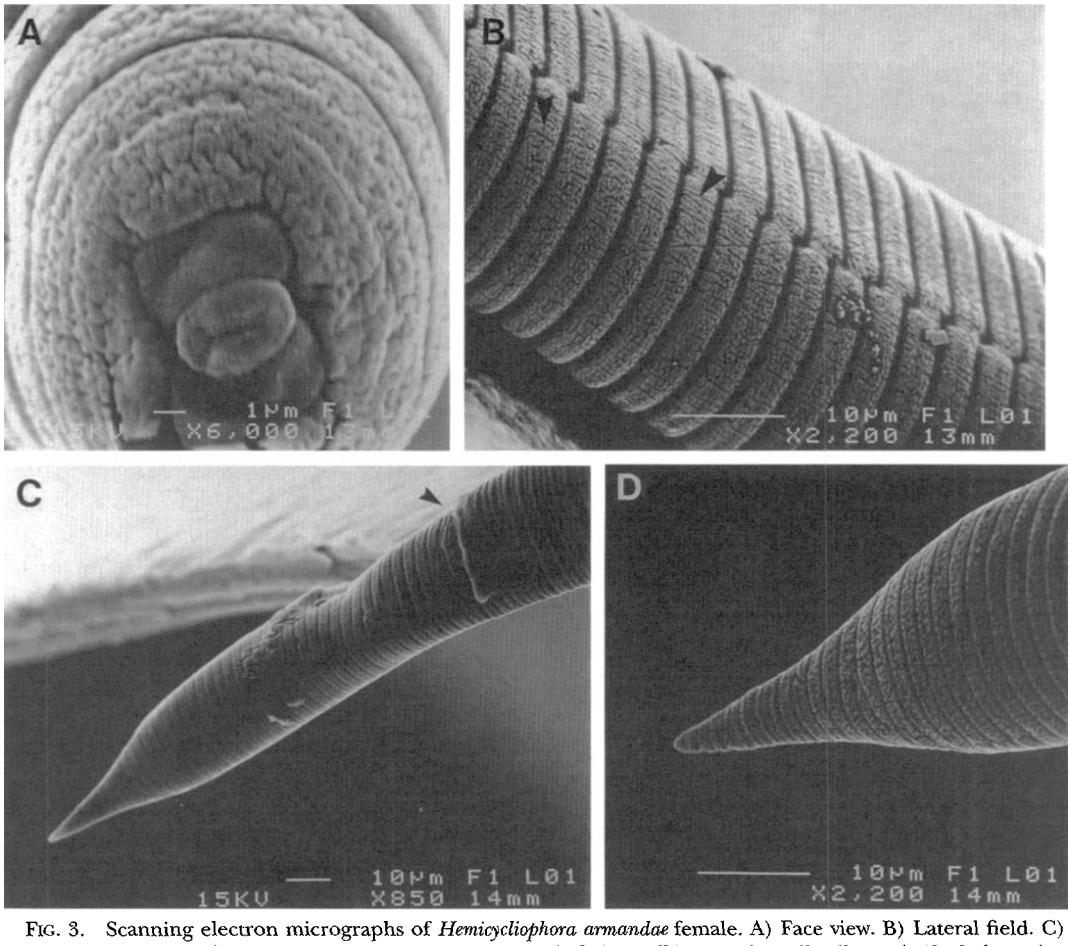

FIG. 3. Scanning electron micrographs of *Hemicycliophora armandae* female. A) Face view. B) Lateral field. C) Posterior body, ventral view showing overlapping upper vulval sleeve. D) Posterior tail spike end. (Scale bars: A = 1 µm, B-D = 10 µm).

for distinguishing *H. armandae* from closely related species such as *H. biosphaera* n. sp. *Hemicycliophora armandae* (n = 2) has a lip region = 9–12 µm high and 18 µm wide; 2 lip annules in one specimen (Fig. 3A); lip annules of equal width in lateral view, first annule not widened or elevated laterally; amphids covered by sunken, rectangular shields (Fig. 3A); sheath annules marked with short longitudinal lines (Fig. 3B); anterior vulval lip longer than posterior lip (Fig. 3C); vulva-anus distance as percentage of tail length = 55% and 76%; length of posterior terminal tail spike/digit = 32 µm.

DISCUSSION

The precise geographic origin of *H. biosphaera* n. sp. may be difficult to prove. Prior to the discovery of this new species in 1995, the nematode microbiota of IAB soil was unknown. In fact, neither soil nor plants were examined for nematodes or other microbiota prior to being placed within Biosphere 2 in 1989. Instead, Biosphere 2 agricultural planners considered it more important to place within Biosphere 2 a highly organic soil that would promote plant health and vigor. As a result, soil placed 1 meter deep within the IAB was of local desert origin and comprised approximately 35% organic matter in volume (cattle pond soil, coarse peat, and commercial mulch), 10% very fine sand, and 55% montmorillonite clay. This soil mixture may seem an unlikely source for any plant-parasitic nematode. However, in a report submitted in 1993 and 1995 to Space

Biospheres Ventures, the presence of few other plant-parasitic nematodes from the same IAB site (*Helicotylenchus dihystera, Criconemella sp., Meloidogyne incognita, M. javanica, M. hapla, and M. arenaria*) was reported. Whether the new species is endemic to Biosphere 2, or was imported in soil or white potato almost 9 years since its discovery may remain unknown. However, the presence of *H. biosphaera* n. sp. adults and larvae in soil kept fallow for more than year implies a direct effect of closed, controlled environments on nematode biology and, therefore, warrants further study.

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