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Taxonomic and Molecular Identification of *Hemicaloosia*, *Hemicycliophora, Gracilacus* and *Paratylenchus* Species (Nematoda: Criconematidae)

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Abstract: Populations of *Hemicycliophora epicharoides, H. gigas, H. labiata, H. pruni, H. shepherdi, H. vidua, H. zuckermani, Gracilacus straeleni*, and *Paratylenchus labiosus* were obtained from different geographical areas in the continental United States and characterized morphological and molecularly. Two new species of Hemicycliophorinae: *Hemicaloosia uarki* n. sp from Pinetree, St. Francis County, Arkansas, and *Hemicycliophora wyei* n. sp from Wayne County, North Carolina, are also described. *Hemicaloosia uarki* n. sp. is characterized by having two lip annuli separated from the rest of body and directed anteriorly, a long stylet (106-124 μm), long body length (1,081-1,326 μm) and a single lateral fields demarcated by interruptions of the body annuli. *Hemicycliophora wyei* n. sp. showed a lateral fields demarked by two faint lines with transverse anastomoses and/or breaks of the striae; an elongated not offset conical tail with distinct annulations and a rounded tip and long vulval lips with a vulval sleeve. The molecular characterizations of the new (*H. uarki* n. sp. and *H. wyei* n. sp.) and known species of Criconematidae using the ITS1 rDNA gene sequence and the molecular phylogenetic relationships are provided.

Key words: Gracilacus straeleni, *Hemicaloosia uarki* n. sp., *Hemicycliophora epicharoides*, *Hemicycliophora gigas*, *Hemicycliophora labiata*, *Hemicycliophora pruni*, *Hemicycliophora shepherdi*, *Hemicycliophora vidua*, *Hemicycliophora wyei n. sp.*, *Hemicycliophora zuckermani*, internal transcribed spacer 1, molecular biology, morphology, *Paratylenchus labiosus,* phylogeny.

The classification of Raski and Luc (1987) included in the subfamily Hemicycliophorinae Skarbilovich, 1959 two genera: (i) *Hemicycliophora* De Man, 1921 synonimyzed with *Procriconema* Micoletzky, 1925; *Colbranium* Andrássy, 1979; *Aulospora* Siddiqui, 1980; and *Loofia* Siddiqui, 1980; and (ii) the genus *Caloosia* Siddiqui & Goodey, 1964 (= *Hemicaloosia* Ray & Das, 1978). However, Decraemer and Hunt (2006) and Siddiqi (2000) still recognize *Hemicaloosia* as valid genera in the subfamily Caloosiinae Siddiqi, 1980 and *Colbranium* in Hemicycliophorinae.

Main morphological characters of the subfamily are the presence of nonretrorse body annuli, sometimes with superficial ornamentation appearing as lines or scratches, and the presence of an extra cuticular layer adpressed or loose from the inner cuticle along the body in *Hemicycliophora* or indistinct in some species of *Caloosia*. The lip region has two or three lip annuli that lacks of submedian lobes. A long stylet is greater than 50 μm with rounded to concave knobs posteriorly directed, showing a small, big, or absent cavity at the base where the lumen of the oesophagus connect with the stylet; vulva lips are mostly modified; and the tail is elongated, sometimes offset, filiform, or rounded in some species (Loof, 1976; Raski and Luc, 1987; Siddiqui, 2000).

The genus *Hemicaloosia* is considered a minor synonym of *Caloosia* by Raski and Luc (1987) because the inconsistency in the observation of the outer cuticle and the presence of lateral fields. Recently, the molecular characterization of *Caloosia longicaudata* using sequences of ITS1-rDNA along with D2-D3 fragment of 28S and partial 18S rDNA were reported and the presences of faint longitudinal lines were observed using scanning electron microscopy (Van Den Berg et al., 2011).

Genera *Paratylenchus* Micoletzki, 1922; *Gracilacus* Raski, 1962; and *Cacopaurus* Thorne, 1943 are included at the subfamily Paratylenchinae Thorne, 1949. However, *Gracilacus* is considered a subgenus of *Paratylenchus* by Siddiqi (2000) as he regarded it insufficient to separate the genera based on differences on stylet length and presences of obese females. Subfamily Paratylenchinae is characterized by having a small body, fine body annulations, lateral fields with two to four lines, and typical criconematoid oesophagus with a long and slender isthmus that ends in rounded basal bulb, with some species characterized by of the presence of obese females as sedentary ectoparasites (Raski, 1975a; Raski, 1975b; Raski, 1976; Raski and Luc, 1987).

The ITS-rDNA regions have been used as markers because its low intraspecific variation for species identification in several nematodes. These markers represent a source of valuable information to develop tools

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for diagnostic purposes based on PCR reactions (Gasser, 2001; Subbotin and Moens, 2006).

The objectives of this study were (i) to integrate the morphological and molecular characterization of populations of known of *Hemicaloosia, Hemicycliophora, Gracilacus*, and *Paratylenchus* from different locations in the continental United States; (ii) to characterize morphologically and morphometrically two new species, namely, *Hemicaloosia uarki* n. sp. and *Hemicycliophora wyei* n. sp.; and (iii) to reconstruct the phylogenetic position of these species within the Criconematinae using the molecular analysis of ITS1 rDNA gene.

Materials and Methods

Nematodes were collected from undisturbed natural locations in Arkansas from 2008 to 2011 using a handheld global positional system device (GPS) (*E*trex Garmin, Olathe, KS) was used to identify the locations. Additional populations of nematodes were received from Florida, North Carolina, and Tennessee. Nematodes from others states were received fixed in 3% formaldehyde for morphological purposes or 1 M NaCl solution or 95% ethanol for molecular characterization. Nematodes collected in Arkansas were extracted from soil using Cobb sieving and flotation-centrifugation methods (Jenkins, 1964). Nematodes were killed and fixed in hot 3% formaldehyde, subsequently infiltrated with glycerin using the modified slow method of Seinhorst and mounted for observation (Seinhorst, 1959, 1962). Measurements of specimens were made using an ocular micrometer and drawings with a camera lucida. Abbreviations used are defined by Siddiqi, 2000. Photographs were taken with a Canon EOS Rebel T3i digital camera mounted on a Nikon Optophot-2 compound microscope.

For identification of genus and species, the classification proposed by Raski and Luc (1987). Species of *Hemicycliophora, Hemicaloosia*, and *Caloosia* do not have true lateral fields. For descriptions, we define lateral fields here as the presence of one or two lateral lines, breaks or anastomoses, lateral interruptions of body annuli caused by breaks, or slanted connections of transverse striae. All species reported herein were deposited in the USDA Nematode Collection, Beltsville, MD.

Female specimens of each species populations were grouped to select nematodes for morphological and molecular taxonomic characterization. For molecular analysis adult nematodes were crushed individually in 5 µl of molecular grade water (BDH Chemicals, Chester, PA) and stored at -80°C until use.

PCR: Polymerase chain reaction (PCR) of the ITS1 region was performed using 5 µl of the DNA extraction in a 50-µl PCR reaction mixture. Primers used to perform PCR reaction were rDNA2 (5'-TTGATTACGTC CCTGCCCTTT- 3') (Vrain et al., 1992) and rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA- 3') (Cherry et al., 1997). This PCR primer pair amplified the 3' end of the 18S rDNA gene, the entire ITS1 region, and the 5' end of the 5.8S rDNA gene. The PCR mixture contained 4 µl of dNTP mixture (0.2 mM each) (Qiagen, Valencia, CA), 1 µl of each primer (0.4 µM), 0.4 µl (2 units) *Taq* DNA polymerase (New England Biolabs, Ipswich, MA), and 5 µl 10 X ThermoPol reaction buffer (New England Biolabs, Ipswich, MA). PCR was conducted using a Hybaid Express thermal cycler (Thermo Hybaid, Middlesex, UK) with the follow parameters: denaturation at 94°C for 2 min, then 40 cycles of denaturation at 94°C for 45 sec, annealing at 52 or 56°C for 45 sec and extension at 7°C for 60 sec. A final extension for 5 min at 72°C was performed. Visualization of PCR product was performed using a 5 µl of PCR product and 100 bp DNA ladder (Promega, Madison, WI) subjected to electrophoresis on a 1% agarose gel stained with ethidium bromide. A UV transluminator (BioDoc-it™ system, UVP, Upland, CA) was used to visualize PCR products.

Sequencing: PCR products were purified using Nanosep centrifugal tubes 100k (Pall, Port Washington, NY) in a refrigerated centrifuge at 15°C for 20 min at 13,000 rpm. Samples were sequenced in both directions using an Applied Biosystems Model 3100 genetic analyzer by the DNA sequencing core facility at the University of Arkansas Medical School, Little Rock, AR. Consensus sequences were obtained using BioEdit (Hall, 1999) sequence alignment software and alignment of sequences was performed with MAFFT (Katoh et al., 2002).

Molecular phylogenetic study. The model of base substitution was evaluated using JModeltest 2.1.1 based on Akaike Information Criterion (AIC) (Posada and Crandall, 1998; Posada, 2008; Darriba et al., 2012). The distance matrix and the Bayesian analysis were obtained using MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001) with Geneious Pro 5.6.6 created by Biomatters (http://www.geneious.com). Bayesian analysis was initiated with a random starting tree, running the chain for 1×10^6 generations and setting the "burn in" at 100,000. The Markov Chain Monte Carlo method (MCMC) was used to estimate the posterior probability of the phylogenetics trees using 50% majority rule (Larget and Simon, 1999). Sampling in the Markov chain was made with a frequency of 200 generations. Dataset was supplemented by additional sequences downloaded from GenBank.

Results and Discussion

SYSTEMATICS

Hemicaloosia uarki n. sp.

(Table 1; Figs. 1-2)

Description

Female: Body slightly ventrally arcuate. Body annuli flattened and smooth. Presence of a membranous cuticular sheath, tightly adpressed to the entire body.

TABLE 1. Measurements and ratios of adult females and juveniles of *Hemicaloosia uarki* n. sp. Mean, standard deviation and range in μm.

Character/ratio	Holotype	♀ (n = 5)[1]	♀ (n = 1)[2]	Jv (n = 6)[1]	Jv (n = 7)[2]	Population from Clarkville, AR ♀ (n = 5)
L	1,193.75	1,243.8 ± 102.1 (1,081.3-1,325.5)	937.50	979.2 ± 47.7 (918.8-1062.5)	900.0 ± 51.4 (831.3-993.8)	1,275.8 ± 84.1 (1,162.5-1,366.7)
Oesophagus length	194.88	188.2 ± 8.2 (174.6-194.9)	162.40	155.6 ± 8.8 (142.1-166.5)	172.8 ± 25.3 (142.1-223.3)	191.2 ± 5.8 (184.7-198.9)
Tail	192.85	182.7 ± 47.0 (109.6-227.4)	178.64	159.7 ± 8.2 (146.2-168.5)	152.8 ± 7.5 (146.2-166.5)	193.3 ± 9.8 (182.7-207.1)
Maximum body width	40.60	41.0 ± 3.2 (36.5-44.7)	38.57	34.2 ± 2.4 (32.5-38.6)	36.3 ± 2.5 (32.5-38.6)	37.4 ± 1.8 (36.5-40.6)
a	29.40	30.5 ± 3.2 (26.6-35.4)	24.31	28.7 ± 1.2 (27.5-30.6)	24.9 ± 1.4 (22.8-26.9)	34.2 ± 2.1 (31.8-37.4)
b	6.13	6.7 ± 0.3 (6.2-6.9)	5.77	6.3 ± 0.4 (5.9-6.8)	5.3 ± 0.7 (4.1-6.6)	6.7 ± 0.3 (6.3-7.0)
c	6.19	7.3 ± 2.7 (5.7-12.1)	5.25	6.1 ± 0.5 (5.6-6.8)	5.9 ± 0.2 (5.6-6.2)	6.6 ± 0.2 (6.4-6.8)
Distance lip region end to vulva	917.67	954.0 ± 73.7 (833.6-1,021.0)	819.76	-	-	983.5 ± 76.7 (878.3-1059.6)
Distance lip region end to anus	1,000.90	1,061.2 ± 111.1 (910.7-1,215.9)	758.86	-	-	1,082.6 ± 74.8 (979.8-1159.6)
V	76.87	76.7 ± 0.8 (75.8-77.8)	87.44	-	-	77.0 ± 1.2 (75.6-78.9)
V'	91.68	90.1 ± 3.5 (84.0-92.0)	108.03	-	-	90.8 ± 1.1 (89.6-92.6)
Distance lip region to end oesophageal gland	203.00	192.4 ± 9.3 (182.7-203.0)	166.46	163.1 ± 7.9 (150.2-170.5)	179.2 ± 25.9 (150.2-231.4)	195.3 ± 5.1 (190.8-203.0)
Body width at anus	34.51	34.9 ± 4.8 (28.4-40.6)	30.45	29.8 ± 1.7 (28.4-32.5)	29.6 ± 2.8 (26.4-32.5)	31.7 ± 1.1 (30.5-32.5)
b'	5.88	6.5 ± 0.3 (5.9-6.7)	5.63	6.0 ± 0.3 (5.6-6.4)	5.1 ± 0.7 (3.9-6.2)	6.5 ± 0.3 (6.1-6.9)
c'	5.59	5.3 ± 1.5 (2.7-6.6)	5.87	5.4 ± 0.3 (4.9-5.8)	5.2 ± 0.4 (4.5-5.7)	6.1 ± 0.2 (5.9-6.4)
Distance between vulva and post end of body	276.08	289.9 ± 30.5 (247.7-316.7)	117.74	-	-	292.3 ± 13.8 (284.2-316.7)
Body width at vulva	38.57	40.2 ± 4.2 (36.5-44.7)	32.48	-	-	34.9 ± 3.6 (32.5-40.6)
VL/VB	7.16	7.2 ± 0.7 (6.8-8.6)	3.63	-	-	8.5 ± 1.1 (7.0-9.8)
Rex	55	56 ± 2.6 (54-59)	63	59 ± 3.1 (55-63)	58 ± 2.8 (55-63)	53 ± 2.4 (50-55)
Roes	49	55 ± 5.1 (54-57)	59	56 ± 2.1 (54-59)	56 ± 2.1 (54-59)	54 ± 2.3 (50-56)
Rvan	23	24 ± 2.3 (22-28)	22	-	-	26 ± 3.3 (21-29)
Ran	47	49 ± 0.8 (48-50)	52	-	-	49 ± 3.8 (45-53)
RV	72	74 ± 2.9 (72-79)	75	-	-	75 ± 4.9 (68-81)
R	338	347± 7.4 (339-357)	338.00	312 ± 8.8 (297-322)	314 ± 16.8 (296-342)	368 ± 10.9 (350-377)
Stylet length	118.74	118.1 ± 7.4 (105.6-123.8)	105.56	99.8 ± 5.8 (91.4-103.5)	105.9 ± 4.0 (99.5-109.6)	121.8 ± 5.9 (113.7-127.9)
Length of stylet shaft	20.30	18.7 ± 2.2 (16.2-22.3)	20.30	15.9 ± 1.5 (14.2-18.3)	16.8 ± 4.0 (12.2-22.3)	19.9 ± 0.9 (18.3-20.3)
m	82.76	84.0 ± 1.4 (81.7-85.2)	80.77	84.0 ± 2.3 (80.4-86.3)	84.1 ± 3.6 (79.2-88.7)	83.7 ± 0.5 (82.8-84.1)
Stylet length as percentage of body length	9.86	9.5 ± 0.2 (9.3-9.8)	11.26	10.2 ± 0.5 (9.5-10.8)	11.8 ± 0.6 (10.9-12.2)	9.6 ± 0.7 (9.2-10.8)
Distance between stylet base and D.O.G	10.15	5.3 ± 2.3 (2.0-8.1)	4.06	6.1 ± 1.8 (4.1-8.1)	6.4 ± 2.7 (2.0-10.2)	9.3 ± 4.0 (6.1-16.2)
O	8.62	4.5 ± 2.0 (1.7-6.6)	3.85	6.1 ± 1.9 (3.9-8.7)	6.0 ± 2.6 (1.9-9.4)	7.7 ± 3.4 (5.4-13.8)
Distance lip region-center median bulb	146.16	137.2 ± 7.8 (125.9-146.2)	121.80	117.1 ± 7.9 (105.6-125.9)	124.1 ± 9.0 (117.7-142.1)	136.8 ± 7.6 (125.7-146.2)
MB	75.00	73.8 ± 1.4 (72.1-75.0)	75.00	75.2 ± 0.7 (73.0-76.9)	72.5 ± 5.7 (63.6-82.9)	71.5 ± 2.0 (68.0-73.5)

Host: [1] pine, [2] *Paspalum* sp.

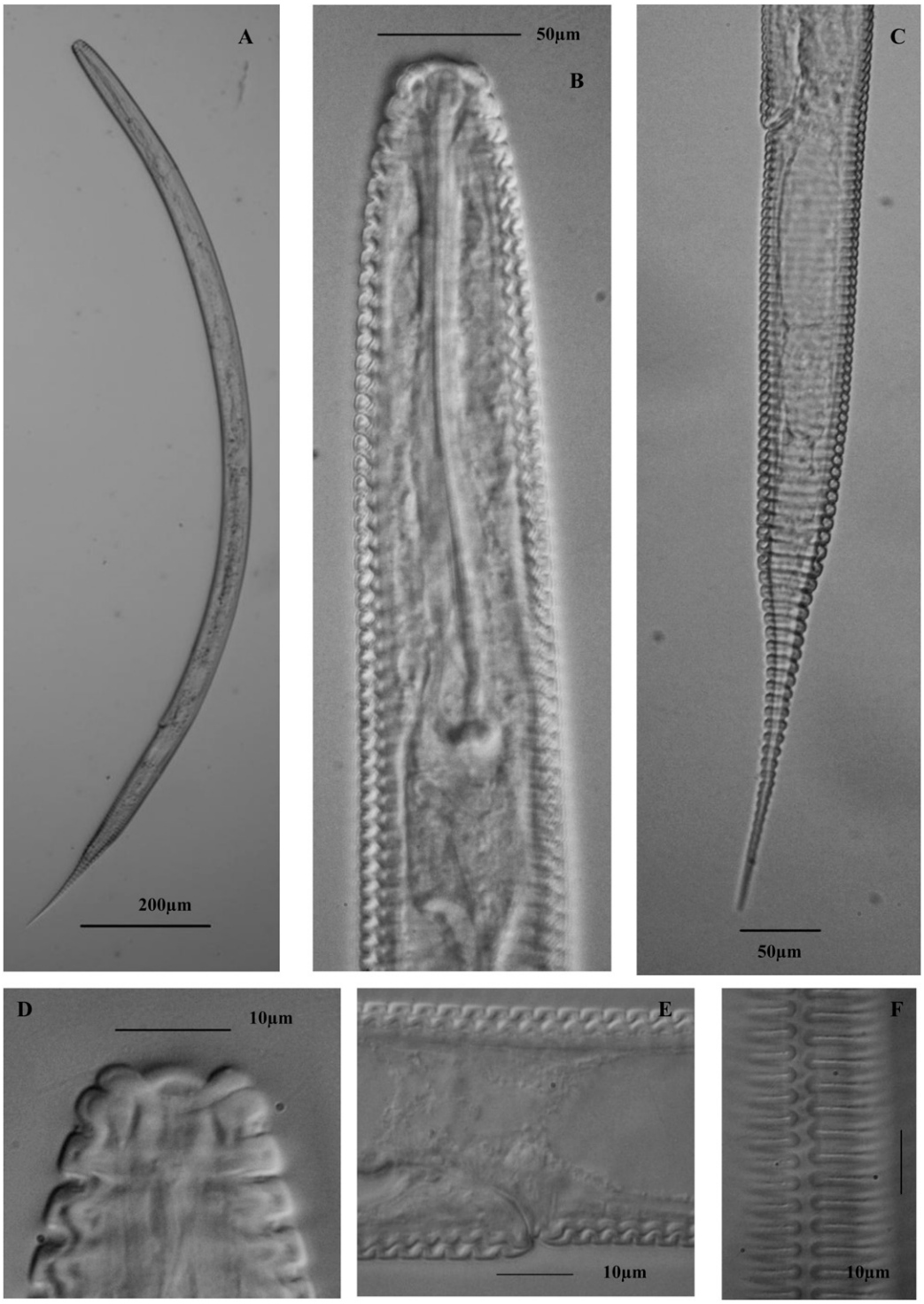

Fig. 1. Light micrographs of *Hemicaloosia uarki* n. sp.: A. Entire female. B. Anterior portion. C. Posterior portion. D. Lip region showing two annuli projected anteriorly. E. Vulva. F. Lateral fields.

Lateral fields marked by interruptions of annuli body without longitudinal lines (Fig 1F), one or two anastomoses observed in lip and tail regions. Labial plate rounded and elevated, pseudolips absent, oral opening indistinct. Lip region continuous with the body, with two annuli: first lip annulus rounded; second lip annulus slightly flattened, both anteriorly directed. Stylet slender, curved, and flexible, with rounded concave knobs. Excretory pore slightly posterior to or at the same level as the oesophageal basal gland. Vulva rounded and closed narrow slit, depressed and flush with body contour, no vulval sleeve present. Vagina curved or slightly curved. Spermatheca round and empty. Tail long and filiform.

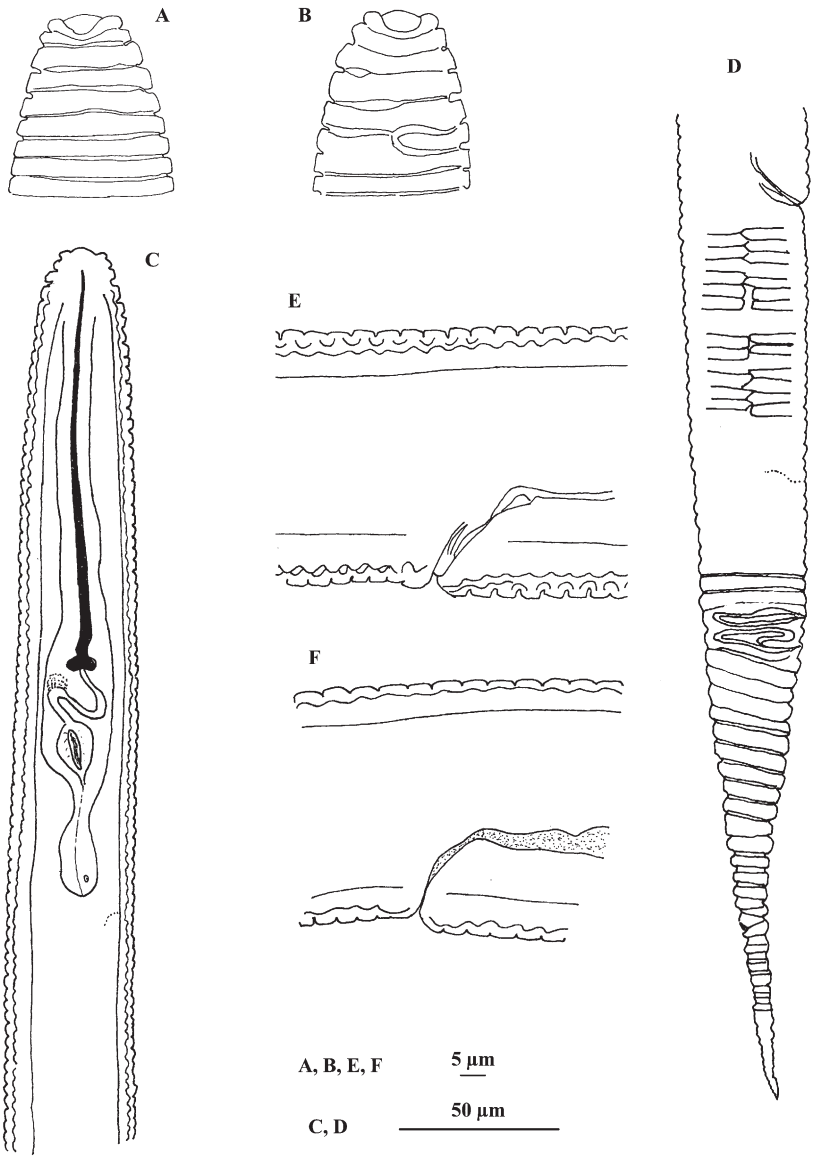

FIG. 2. Camera lucida drawings of *Hemicaloosia uarki* n. sp.: A–B. Lip region with two annuli. C. Anterior portion. D. Posterior region. E–F. Vulva.

Juvenile: Body straight or slightly ventrally arcuate. Resembling female except for lower values of body length, stylet length, and total annuli body, similar number of annuli from anterior end to excretory pore.

Male: Not found.

Type host and locality: Specimens were collected in May to June 2008 by M. Cordero and R. Robbins at Pinetree, AR, designed as type population (GPS coordinates N 35° 07.801 min, -W 090° 58.383 min) from the rhizosphere of small pines and Warren, AR. (GPS coordinates N 33° 30.283 min, -W 092° 11.236 min) from the rhizosphere of *Paspalum* sp. In addition, during August 1983 a population was found associated with a frequently wet hardwood area in Clarkville, AR. These specimens were found in a misidentified slides located at the nematology laboratory at the University of Arkansas.

Type specimens: Holotype (female): Specimen (slide T-658t) deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, MD.

Paratypes (female): Seven female paratypes deposited as in the USDA Nematode Collection, Beltsville, MD; three females paratypes deposited in the Department of Nematology, University of California, Riverside, CA.

Diagnosis: Hemicaloosia uarki n. sp. is characterized by its long body (1,081 to 1,431 µm), surrounded by a membranous cuticular sheath tightly adpressed to the body. Body annuli flattened except for the second annulus in the lip region. Lip region and tail with anastomoses, lateral fields marked by interruptions in body annuli extending from the post labial region to almost immediately posterior to the vulva and a long (106 to 124 µm) and slender stylet. Additional diagnostic characters include lip region annuli directed anteriorly

with the first lip annulus being rounded and the second lip annulus slightly flattened, labial plate is rounded and elevated, without pseudolips, oral aperture is indistinct in lateral view; vulva closed and rounded, without modified vulval lips, anterior vulval lip as a slight depression, without sleeve, continuous with the body; sphermateca round, empty; tail filiform. A specific ITS1 sequence (JQ708156) has been submitted to GenBank and the species has been registered (52D6BFFF-8D46-4597-929D-B352BC1A0270) in ZooBank.

Relationships: The population of *Hemicaloosia uarki* n. sp. is most similar to *H. nudata* Colbran, 1963 and *H. graminis* Zeng, Ye, Martin & Martin, 2012. It differs from *H. nudata* in having a lateral field marked by interruptions or breaks in transverse striae at the midbody region vs. lateral fields without breaks or transverse striae. It differs in having a longer stylet (106-124 vs. 94-109 μm); greater values of Rex (54-59 vs. 40-44); RV (72-79 vs. 37-43); R (339-365 vs. 225-248); and a more anterior vulva V (76-78 vs. 81-84) (Colbran, 1963; Brzeski, 1974). *Hemicaloosia uarki* n. sp. is similar to *H. graminis* in having anastomoses in the post labial region (3th to 5th annulus). However, *H. graminis* does not have anastomoses immediately posterior to the vulva but instead, lateral fields is unmarked and extending to the tail tip. Furthermore, *H. uarki* n. sp. has a longer body (1,081-1,326 vs. 610-805 μm); longer stylet (106-124 vs. 67-74 μm); greater values of Rex (54-59 vs. 43-54); RV (72-79 vs. 38-53); R (339-365 vs. 254-283); a more anterior vulva (76-78 vs. 84-86); and a longer tail (110-227 vs. 68-85 μm).(Zeng et al., 2012).

Etymology: The species epithet is derived from the acronym of the University of Arkansas, UARK.

Hemicycliophora wyei n. sp
(Table 2; Figs. 3-4)

Description

Female: Body straight or slightly ventrally arcuate. Annuli rounded and smooth. Cuticular sheath somewhat detached from inner cuticle, distinctly detached in oesophagus and tail regions. Pattern of lateral fields in diagnosis. Labial plate slightly rectangular, oral disc rounded and slightly elevated, pseudolips not observed. Lip region following the contour of the body, not offset, outer and inner cuticle with two lip annuli. Lip annuli rounded. Stylet slightly curved and flexible, with rounded knobs, slightly directed posteriorly and small cavity present. Excretory pore posterior to the oesophagus basal gland. Vulva closed with modified lips, anterior and posterior vulval lips elongated, vulval sleeve

Table 2. Measurements and ratios of adult females and juveniles of *Hemicycliophora wyei* n. sp Mean, standard deviation and range in μm.

Character/ratio	Holotype	♀ (n = 8)	Jv (n =3)
L	868.75	921.9 ± 86 (800-1056.3)	854.2 ± 47.3 (800-887.5)
Oesophagus length	146.16	154.3 ± 8.7 (146.2-166.5)	136.7 ± 4.7 (134.0-142.1)
Tail	123.83	117.7 ± 14.1 (105.6-142.1)	115.0 ± 8.5 (105.6-121.8)
Maximum body width	46.69	44.7 ± 1.9 (40.6-46.7)	40.6 ± 0.0 (40.6-40.6)
a	18.61	20.7 ± 2.1 (17.9-23.7)	21.0 ± 1.2 (19.7-21.9)
b	5.94	6.0 ± 0.4 (5.5-6.7)	6.2 ± 0.3 (6.0-6.5)
c	7.02	7.9 ± 0.6 (7.2-8.8)	7.4 ± 0.1 (7.3-7.6)
Distance lip region end to vulva	698.23	738.2 ± 79.2 (627.5-869.5)	-
Distance lip region end to anus	744.92	804.1 ± 75.0 (694.4-924.3)	-
V	80.37	80.0 ± 1.8 (78.4-83.4)	-
V'	93.73	91.7 ± 1.6 (90.1-94.1)	-
Distance lip region to end oesophageal gland	150.22	159.4 ± 10.4 (146.2-178.6)	140.7 ± 4.7 (138.0-146.2)
Body width at anus	40.60	36.3 ± 2.5 (32.5-38.6)	33.8 ± 2.3 (32.5-36.5)
b'	5.78	5.8 ± 0.4 (5.3-6.5)	6.1 ± 0.3 (5.8-6.3)
c'	3.05	3.3 ± 0.4 (2.8-4.1)	3.4 ± 0.3 (3.2-3.8)
Distance between vulva and post end of body	170.52	183.7 ± 16.8 (154.3-211.1)	-
Body width at vulva	48.72	45.4 ± 2.9 (40.6-48.7)	-
VL/VB	3.50	4.0 ± 0.3 (3.8-4.4)	-
Rex	47	49 ± 3.9 (43-56)	57.0 ± 1.0 (56.0-58.0)
Roes	48	45 ± 4.3 (40-52)	63.7 ± 9.7 (53.0-72.0)
Rvan	10	18 ± 3.3 (11-22)	-
Ran	40	40 ± 5.6 (32-49)	-
RV	41	58 ± 5.2 (51-66)	-
R	230	228 ± 43 (125-258)	324 ± 16.8 (311-343)
Stylet length	79.17	82.2 ± 4.7 (77.1-89.3)	75.1 ± 2.0 (73.1-77.1)
Length of stylet shaft	16.24	15.2 ± 1.1 (14.2-16.2)	14.9 ± 1.2 (14.2-16.2)
m	79.49	81.5 ± 1.3 (78.9-83.3)	80.2 ± 1.1 (78.9-81.1)
Stylet length as percentage of body length	9.11	9.0 ± 0.7 (8.1-9.9)	8.8 ± 0.5 (8.4-9.4)
Distance between stylet base and D.O.G	6.09	7.9 ± 2.6 (4.1-12.2)	7.4 ± 1.2 (6.1-8.1)
O	7.69	9.7 ± 3.2 (4.7-14.3)	9.9 ± 1.6 (8.1-11.1)
Distance lip region-center median bulb	105.56	107.6 ± 5.7 (101.5-117.7)	94.8 ± 6.2 (89.3-101.6)
MB	72.22	69.8 ± 1.3 (68.3-72.2)	69.3 ± 2.4 (66.7-71.5)

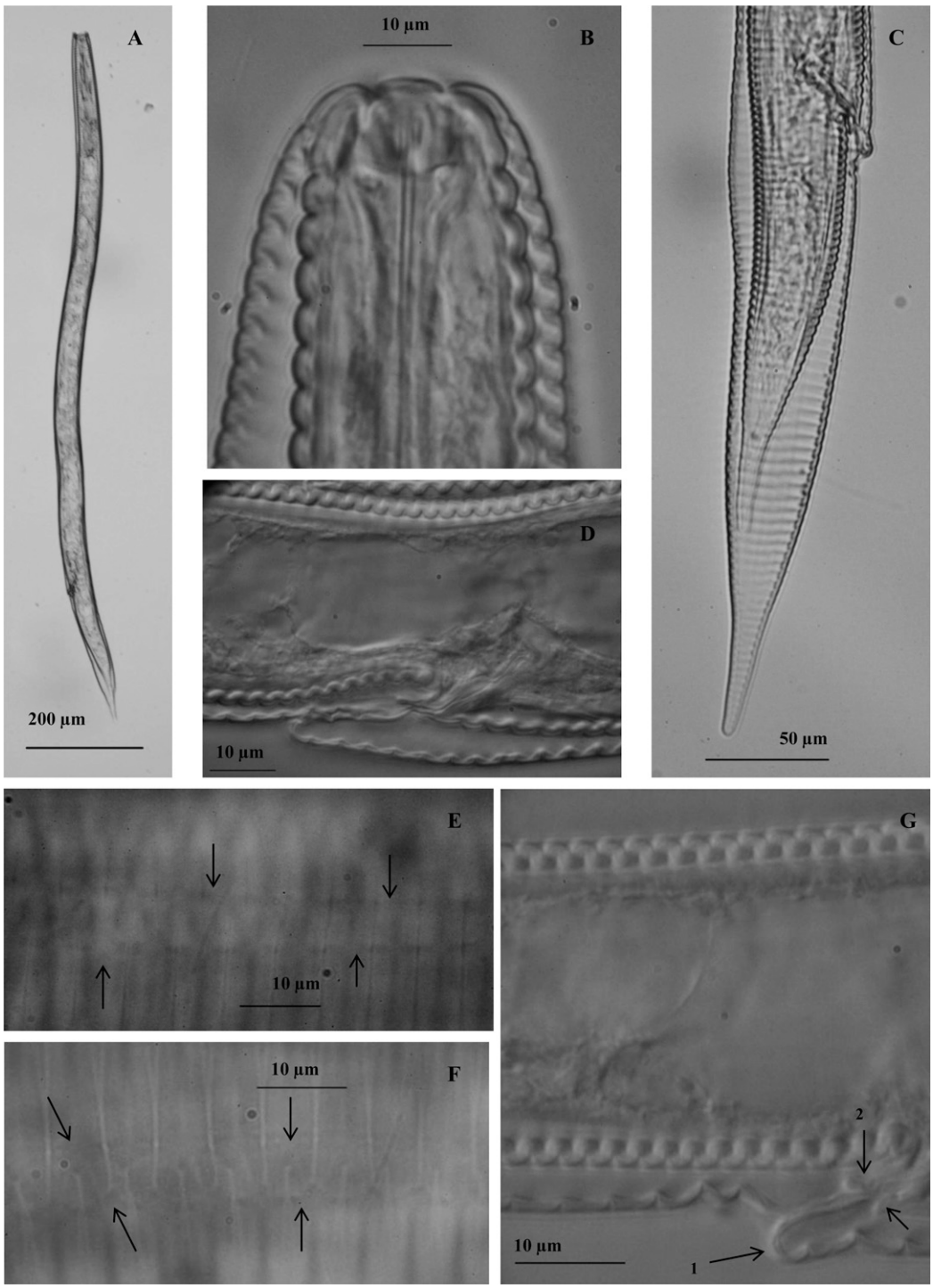

Fig. 3. Light micrographs of *Hemicycliophora wyei* n. sp.: A. Entire female. B. Lip region. C. Posterior portion. D–G. Vulva (1, vulval sleeve; 2, vulval lips). E. Lateral fields (arrows showing two faint lines). F. Lateral fields (arrows showing breaks and anastomoses inside lateral fields).

long, spermatheca rounded and empty. Tail elongated, uniformly conoid, not offset, rounded tip. Tail annulations distinct.

Juvenile: Body straight or slightly ventrally arcuate. Lower values of body length, stylet length, and total annuli body.

Male: Not found.

Type host and locality: Specimens were collected in September 2008 by W. Ye from the rhizosphere of turfgrass. Sample No. 09-22677 from Wayne County, NC. No GPS coordinates provided.

Type specimens: Holotype (female): Specimen (slide T-660t) deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, MD.

Paratypes (female): Three paratypes deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, MD; and single paratypes are deposited as follows: Department of Nematology, Agricultural University,

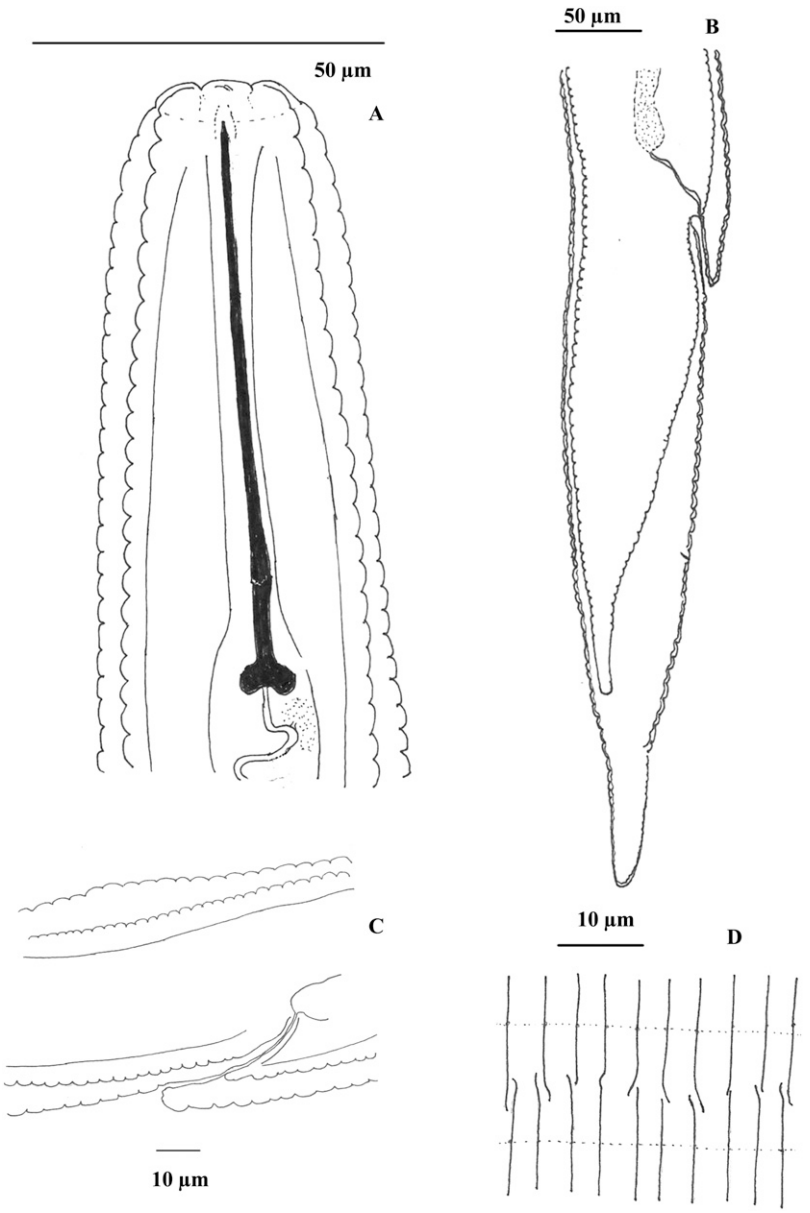

Fig. 4. Camera lucida drawings of *Hemicycliophora wyei* n. sp.: A. Anterior portion. B. Posterior portion. C. Vulva. D. Lateral fields with two lines, break, and anastomoses.

Wageningen, The Netherlands, and Nematode collection of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Diagnosis: Hemicycliophora wyei n. sp. is characterized by an elevated rounded oral disc; two rounded lip annuli visible in the outer cuticle and inner cuticle. Lateral fields demarcated by two faint lines with dot-like structures, equidistant and coincident with the striae of body annuli, revealing a occasionally indistinct elevated ridge or lateral fields sometimes indistinct. Inside the lateral fields, specimens showed the presence of anastomoses and/or breaks of striae in its entire length. Body annuli without markings. Stylet curved and flexible with rounded, slightly posteriorly directed knobs with a small cavity. Vulva with anterior and posterior lips modified and vulval sleeve long. Tail elongated uniformly conoid, not offset, with distinct annuli and rounded tip. A specific ITS1 sequence (JQ708145) has been submitted to GenBank and the species has been registered (E2D41630-CD05-4FC0-A9DE-E54F548C570A) in ZooBank.

Relationships: Hemicycliophora wyei n. sp resembles *H. penetrans* Thorne, 1955 in having a distinct lateral field on the tail region, elongated vulval lips and vulval sleeve. However, it differs by having a lateral fields marked with anastomoses and/or breaks of transverse striae, and demarcated by two faint lines of dot-like structures and smooth annuli outside the lateral fields, whereas, *H. penetrans* has lateral fields formed by two lines with a third faint line running lengthwise, with

transverse lines crossing the lateral fields forming blocks; annuli outside lateral field marked with 60 to 80 longitudinal lines or scratches. In addition, it differs from *H. penetrans* by an empty spermatheca vs. full of sperm. Tail shape (elongated and conoid, not offset with a rounded terminus distinctly annulated vs. an elongated sharply conoid tail) and smaller values of a (18-24 vs. 29-31); c (7.0-9.0 vs. 12-14); VL/VB (4 vs. 5-7); R (125-258 vs. 260-270); and greater Ran (32-49 vs. 22- 27) (Thorne, 1955; Brzeski, 1974; Brzeski and Ivanova, 1978).

Etymology: The species was named after Dr. Weimin Ye who supplied the specimens.

Hemicycliophora epicharoides Loof, 1968
(Table 3; Fig. 5)

Description

Female: Body straight or slightly ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath adpressed more ventrally than dorsally in tail region, attached only at anterior body end. Lateral field marked by one or two longitudinal lines, with frequent anastomoses. Outside lateral fields annuli marked with longitudinal scratches. Labial plate rounded and low, lateral pseudolips at same level of the oral disc, occasionally difficult to observe. Lip region not offset, with two rounded and somewhat flattened lip annuli in outer and inner cuticle. Stylet straight and flexible, with rounded concave knobs directed posteriorly with small cavity. Excretory pore slightly posterior to or at the same level as the oesophagus basal gland. Vulva closed with conspicuous, modified lips, vulval sleeve short. Spermatheca rounded, empty. Tail conoid, more dorsally convex than ventrally, ending in a rounded terminus.

Host and locality: Specimens were collected in June 2008 and September 2011 by M. Cordero and R. Robbins at Illinois River in Washington County, AR (GPS coordinates N 36° 06.068 min, -W 094° 21.517 min); from the rhizosphere of river cane (*Arundinaria* sp.) and Toad Suck Ferry Park, Perry County, AR (GPS coordinates N 35° 04.279 min, -W 092° 32.704 min) from the rhizosphere of willow (*Salis* sp.) and wild strawberries (*Fragaria* sp.).

Diagnosis: Hemicycliophora epicharoides is characterized by lateral fields marked by one or two longitudinal lines, with frequent anastomoses; few scratches on body annuli outside the lateral field; and labial plate rounded and low, with lateral pseudolips present at same level of the oral disc, frequently indistinct. Additionally, vulva with conspicuous, modified lips and a short vulval sleeve, and a conoid tail, more convex dorsally than ventrally ending in a rounded terminus. This population is in agreement with the original description (Loof, 1968) and the redescription of four specimens of the type population (Brzeski, 1974). Specific ITS1 sequences (JQ708146-JQ708151) has been submitted to GenBank.

Relationships: Hemicycliophora epicharoides differs from *H. epicharis* Raski, 1958 by shape of labial disc (no truncate, rounded vs. labial disc truncate, rectangular), vulval sleeve length (short vs. very long), greater RV (40-53 vs. 25-32); and Rex (31-49 vs. 28-32). Also, it closely resembles *H. robusta* Loof, 1968 from which is different by a longer stylet (74-84 vs. 93-108 μm); labial plate (no protuded vs. protruded); vulval lips (modified, elongated vs. not modified, round); and lateral fields (one or two lines vs. breaks or anastomoses of transverse striae) (Loof, 1968; Brzeski, 1974; Brzeski and Ivanova, 1978). Vovlas and Inserra (1980) and Larizza (1995) reported populations of *H. ephicaroides* from Italy characterized by a longer stylet range than the original population; however, morphometrics and morphological characteristics are very close with the original description and redescription. Also, they did not mention differences in labial plate and lateral fields that are herein considered important characters in differentiating *H. ephicaroides* from *H. robusta*.

Hemicycliophora gigas Thorne, 1955
(Table 3; Fig. 6)

Description

Female: Body slightly ventrally arcuate. Body annuli flattened and smooth. Cuticular sheath tightly adpressed to the inner cuticle except at the tail region. Lateral field marked with two rows of round ornamentations between breaks of transverse striae (Fig 6H). Labial plate rounded and elevated, pseudolips not observed. Lip region continuous with the body, with three annuli: first lip annulus rounded, second and third lip annuli slightly flattened. Stylet long, knobs rounded, slightly concave directed posteriorly without cavity. Excretory pore posterior to the oesophagus basal gland. Vulva lips rounded not modified, vulval sleeve absent. Spermatheca rounded, empty. Tail long and filiform, terminal tail annuli indistinct.

Juvenile: Resembles female. Body straight or slightly ventrally arcuate. Lower to similar values of body length, stylet length and total body annuli, similar number of annuli from anterior end to excretory pore.

Male: Not found.

Host and locality: Specimens were collected in May 2008 by M. Cordero and R. Robbins at Pinetree, AR, at the border of a swamp (GPS coordinates N 35° 07.178 min, -W 090° 66.596 min; N 35° 07.188 min, -W 090° 56.591 min); from the rhizosphere of grass, moss, and ash tree (*Fraxinus* sp.), respectively.

Diagnosis: The Arkansas population of *H. gigas* is characterized by having a cuticular sheath tightly adpressed to the inner cuticle except at the postvulvar region; lateral field without longitudinal lines or incisures marked with two rows of round ornamentations between interruptions of body annuli; a rounded and elevated labial plate without pseudolips. Lip region with three annuli, continuous with the body. Vulva lips

TABLE 3. Measurements and ratios of *Hemicycliophora ephicaroides* and adults females and juveniles of *H. gigas*. Mean, standard deviation and range in μm.

Character/ratio	*H. ephicaroides* ♀ (n = 17) Host: River cane	*H. ephicaroides* ♀ (n = 9) Host: Willow/wild strawberry	*H. gigas* ♀ (n = 8)	*H. gigas*Jv (n = 12)
L	881.3 ± 49.8 (781.3-956.3)	917.4 ± 221.9 (756.3-1487.5)	1,368.0 ± 1,69.7 (1068.8-1625)	1,007 ± 170.4 (768.8-1,393.8)
Oesophagus length	153.6 ± 10.6 (129.9-162.4)	149.3 ± 6.7 (140.1-158.3)	198.8 ± 9.1 (182.7-207.1)	170.9 ± 16.8 (146.2-198.9)
Tail	88.0 ± 8.1 (72.9-103.5)	75.5 ± 16.8 (41.4-93.4)	192.3 ± 41.2 (138-276.1)	160.7 ± 16.5 (132-188.8)
Maximum body width	40.1 ± 3.3 (34.9-46.3)	41.6 ± 3.5 (35.7-47.1)	48.0 ± 5.1 (40.6-54.8)	29.8 ± 5.1 (22.3-40.6)
a	22.0 ± 1.4 (20.0-25.1)	22.3 ± 6.5 (16.1-39.0)	28.6 ± 2.6 (24.9-33.4)	33.8 ± 0.7 (33.0-34.3)
b	5.8 ± 0.5 (5.3-7.2)	6.2 ± 1.5 (5.3-10.2)	6.9 ± 0.7 (5.8-7.8)	6.3 ± 0.8 (5.7-7.2)
c	10.1 ± 0.7 (8.8-11.2)	13.2 ± 6.2 (8.1-24.8)	7.2 ± 0.7 (5.9-7.9)	6.8 ± 1.6 (5.1-8.3)
Distance lip region end to vulva	736.4 ± 43.0 (659.5-806.0)	781.7 ± 230.6 (618.2-1381)	1071 ± 125.3 (845.5-1255.5)	-
Distance lip region end to anus	793.2 ± 45.1 (708.4-871.0)	841.9 ± 230.3 (662.9-1427.4)	1175.6 ± 133 (930.7-1348.9)	-
V	83.6 ± 0.7 (82.5-85.0)	84.6 ± 3.2 (81.7-92.8)	78.4 ± 0.7 (77.3-79.2)	-
V'	92.8 ± 0.6 (91.4-93.8)	92.6 ± 2.4 (87.4-96.8)	91.1 ± 0.9 (90.2-93.1)	-
Distance lip region to end oesophageal gland	157.8 ± 10.9 (136.0-168.5)	152.7 ± 6.5 (144.1-160.4)	205.0 ± 10.6 (186.8-215.2)	171.0 ± 25.7 (107.6-203)
Body width at anus	34.2 ± 3.3 (30.0-40.6)	32.9 ± 4.2 (27.6-39.8)	38.8 ± 5.3 (32.5-46.7)	23.0 ± 3.6 (16.2-28.4)
b'	5.6 ± 0.4 (5.2-6.8)	6.0 ± 1.4 (5.2-9.8)	6.7 ± 0.6 (5.7-7.6)	6.1 ± 0.8 (5.4-7.0)
c'	2.6 ± 0.2 (2.1-3.0)	2.3 ± 0.6 (1.1-3.2)	5.0 ± 0.8 (4.3-6.8)	6.6 ± 1.0 (5.9-7.8)
Distance between vulva and post end of body	144.9 ± 9.3 (121.8-156.3)	135.6 ± 15.5 (106.4-156.3)	296.9 ± 44.9 (223.3-369.5)	-
Body width at vulva	40.4 ± 3.1 (34.9-45.5)	38.9 ± 5.0 (29.2-44.7)	43.4 ± 5.0 (36.5-52.8)	-
VL/VB	3.6 ± 0.2 (3.4-4.0)	3.5 ± 0.6 (2.8-4.5)	6.9 ± 1.1 (5.8-9.1)	-
Rex	43 ± 4.6 (31-49)	41 ± 1.6 (41-46)	56 ± 2.9 (50-58)	49 ± 1.6 (47-52)
Roes	42 ± 4.9 (31-47)	43 ± 4.3 (38-53)	54 ± 4.3 (47-59)	43 ± 2.2 (39-47)
Rvan	15 ± 1.7 (13-19)	16 ± 2.2 (12-18)	27 ± 1.8 (24-29)	-
Ran	32 ± 3.4 (26-39)	34 ± 3.6 (30-42)	53 ± 6.7 (45-67)	-
RV	47 ± 3.5 (40-53)	51 ± 3.1 (46-54)	80 ± 4.4 (72-85)	-
R	221 ± 12.5 (197-255)	218 ± 11.3 (196-238)	351 ± 10.6 (335-365)	292 ± 9.3 (270-305)
Stylet length	79.5 ± 3.2 (73.7-84.2)	76.4 ± 3.9 (69.7-83.4)	127.1 ± 5.7 (115.7-134.0)	99.1 ± 9.2 (87.3-113.7)
Length of stylet shaft	14.0 ± 1.4 (10.6-15.4)	13.1 ± 0.9 (12.2-14.6)	18.9 ± 2.8 (13.0-22.3)	16.2 ± 2.7 (10.2-20.3)
m	82.4 ± 1.7 (80.2-87.2)	82.9 ± 1.1 (81.4-84.4)	85.1 ± 2.2 (82.2-90.0)	83.6 ± 2.3 (79.5-88.4)
stylet length as percentage of body length	9.0 ± 0.6 (8.1-10.1)	8.6 ± 1.4 (5.2-10.0)	9.4 ± 0.9 (7.7-10.8)	10.0 ± 1.3 (7.7-11.7)
Distance between stylet base and D.O.G	5.2 ± 1.0 (4.1-6.5)	6.5 ± 1.0 (4.9-8.1)	3.3 ± 2.9 (0.0-8.1)	7.3 ± 2.2 (4.1-10.2)
O	6.5 ± 1.4 (4.9-8.7)	8.5 ± 1.1 (6.7-10.2)	2.6 ± 2.3 (0.0-6.5)	7.4 ± 2.3 (4.0-11.4)
Distance lip region-center median bulb	106.3 ± 7.0 (93.4-121.8)	99.2 ± 3.9 (93.4-105.6)	154.5 ± 16.1 (136.0-190.8)	127.2 ± 13.9 (105.6-150.2)
MB	69.4 ± 4.2 (64.6-77.1)	66.5 ± 1.2 (64.1-68.1)	77.7 ± 7.4 (72.5-94.0)	71.9 ± 1.4 (70.8-94.9)

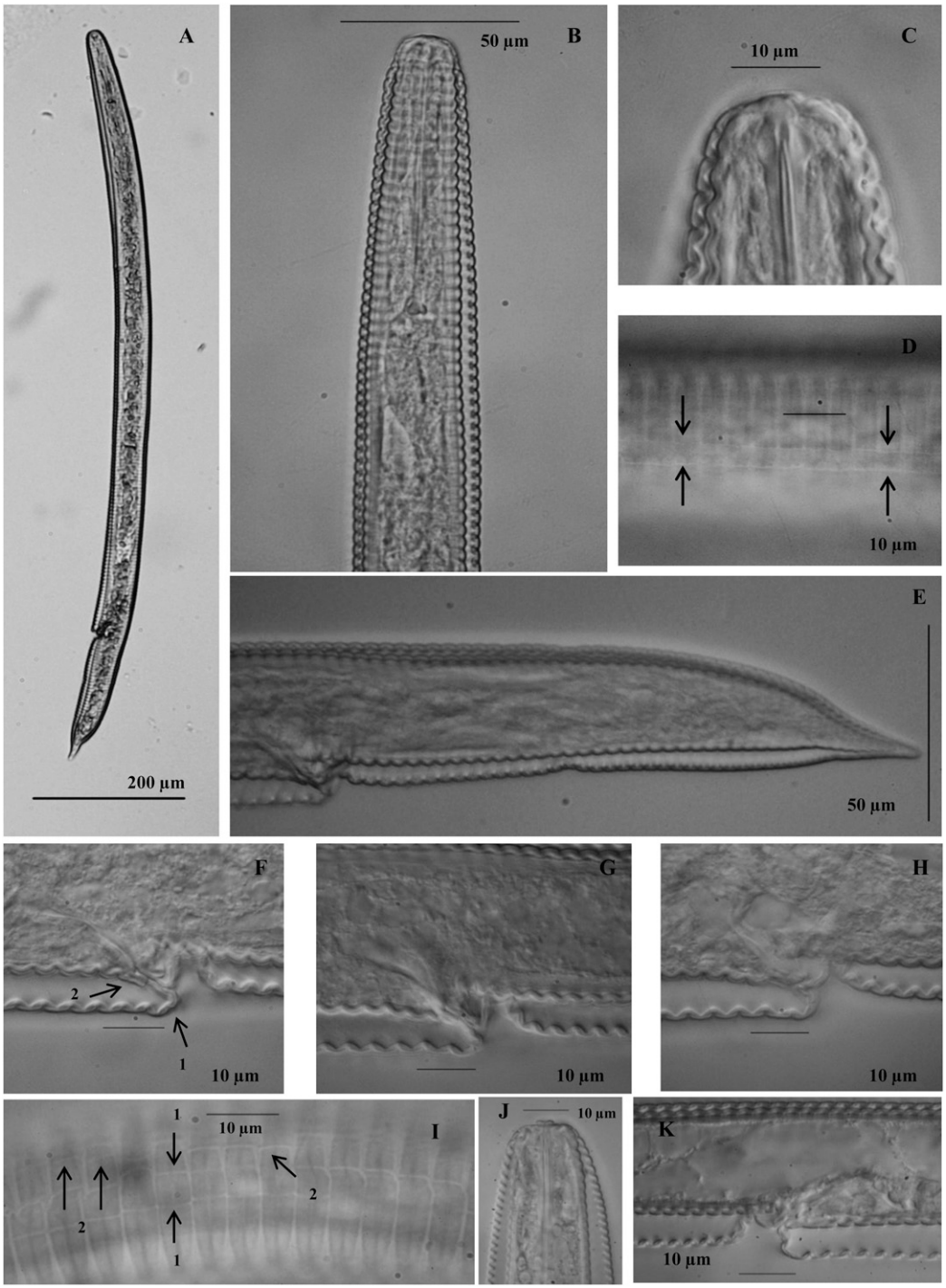

Fig. 5. Light micrographs of *Hemicycliophora ephicaroides*: A. Entire female. B. Anterior region. C. Lip region. D. Lateral fields showing two lines. E. Posterior region. F–G, H–K. Vulva (1, vulval sleeve; 2, vulval lips). I. Lateral fields detail (1, lateral fields lines; 2, scratches outside lateral fields). J. Aberrant lip region.

not modified and sleeve absent. Tail is filiform, with annuli indistinct in its terminal portion.

These populations are in agreement with the original description of the holotype and paratype and one additional specimen from Iowa. (Thorne, 1955; Brzeski, 1974) and a specific ITS1 sequence (JQ708143) has been submitted to GenBank.

Relationships: The Arkansas population of *H. gigas* resembles the following species: *H. gracilis* Thorne, 1955; *H. ovata* Colbran, 1962; *H. tenuis*, Thorne, 1955; *H. vaccinii* Reed and Jenkins, 1963; and *H. uniformis* Thorne, 1955.

Hemicycliophora gigas differs from *H. gracilis* by a lateral field marked with two rows of ornamentations between interruptions of body annuli vs. two longitudinal

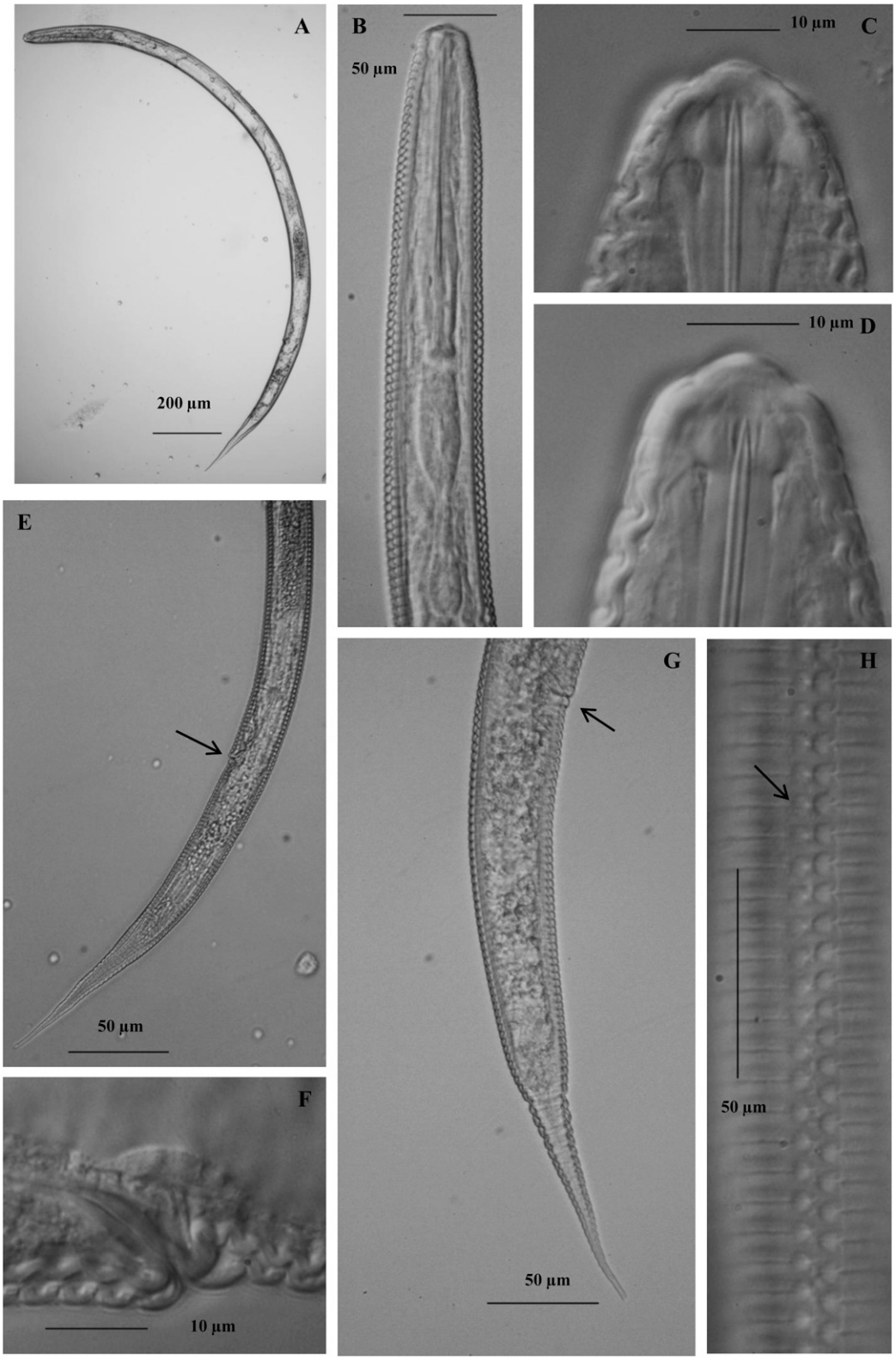

Fig. 6. Light micrographs of *Hemicycliophora gigas*: A. Entire female. B. Anterior region. C–D. Lip region. E–G. Posterior region (arrows showing vulva). F. Vulva. H. Lateral fields with rounded ornamentation.

lines with anastomoses and/or breaks; vulval lips not modified vs. modified; tail shape (filiform vs. slightly conoid); lower Rex (50-58 vs. 68); greater VL/VB (6-9 vs. 6); and lower c (6-8 vs. 10) (Thorne, 1955; Brzeski, 1974; Brzeski and Ivanova, 1978). This species is similar to *H. ovata*, however; *H. gigas* differ by a filiform vs. conical and offset tail (Thorne, 1955; Brzeski, 1974; Brzeski and Ivanova, 1978).

Hemicycliophora gigas differs from *H. tenuis* by having stylet knobs convex vs. rounded; lateral fields marked with two rows of ornamentations between interruptions of body annuli vs. anastomoses and/or breaks of striae; smaller R (335-365 vs.430); slightly smaller V (77-79 vs.82); and a filiform tail vs. elongated and sharply conoid (Thorne, 1955; Brzeski, 1974; Brzeski and Ivanova, 1978).

Hemicycliophora gigas can be differentiated from *H. vaccinii* by having three lip annuli vs. two lip annuli; lateral fields marked by interruptions of body annuli with ornamentation vs. interruption or breaks of the annuli body without ornamentation or occasionally anastomoses; labial disc rounded and elevated vs. rounded. *Hemicycliophora vaccinii* may have a posterior vulval lip bulging. In morphometrics, *H. gigas* has a longer stylet (116-134 vs. 110 μm); greater R (335-365 vs. 284); and lower c (6 vs. 8) (Thorne, 1955; Brzeski, 1974; Brzeski and Ivanova, 1978). *Hemicycliophora gigas* differs from *H. uniformis* by the same characteristics mentioned above for *H. vaccinii* for lateral fields, three lips vs. two lip annuli. A filiform tail vs. elongated and sharply conoid, longer body (L =1,069-1,625 vs. 950 μm); lower c (6-8 vs. 9); longer stylet (116-134 vs. 86 μm); greater R (335-365 vs. 274); greater RV (72-85 vs. 58); VL/VB (6-9 vs. 6); and Ran (45-67 vs. 37) (Thorne, 1955; Brzeski, 1974; Brzeski and Ivanova, 1978).

Hemicycliophora labiata Colbran, 1960
(Table 4; Fig. 7)

Description

Female: Body nematodes straight and curved at tail level or ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath slightly detached from the inner cuticle for the entire body except over tail region. Lateral field marked with longitudinal line running lengthwise, with frequent anastomoses and breaks of transverse straie. Outside the lateral field, annuli marked with scratches. Labial plate somewhat rectangular, labial disc low, lateral pseudolips not observed. Lip region continuous with the contour of the body, with two lip annuli in outer and inner cuticle, somewhat flattened. Stylet straight, basal knobs rounded to concave, slightly posteriorly directed, distinct cavity present. Excretory pore located anteriorly to the oesophagus basal bulb. Vulva lips distinctly modified, posterior vulval lips as long as anterior vulval lip, vulval sleeve small. Spermatheca rounded, containing sperm. Tail conoid, short, slightly offset, dorsally convex.

Host and locality: Specimens were collected by in June 2010 by E. Bernard in the Smoky Mountains, TN, from

TABLE 4. Measurements and ratios of *Hemicycliophora labiata*. Mean, standard deviation and range in μm.

Character/ratio	♀ (n 14) Host: Tulip-poplar	♀ (n = 15) Host: Turfgrass
L	873.7 ± 69.1 (775.0-987.5)	961.7 ± 60.6 (812.5-1075.0)
Oesophagus length	152.0 ± 4.1 (146.2-160.4)	149.4 ± 10.5 (121.8-162.4)
Tail	80.2 ± 11.0 (62.9-95.4)	81.6 ± 10.4 (62.9-101.5)
Maximum body width	39.8 ± 2.4 (34.5-44.7)	42.4 ± 4.1 (36.5-50.8)
a	21.9 ± 1.3 (19.6-23.4)	22.8 ± 1.9 (18.7-26.0)
b	5.8 ± 0.4 (5.2-6.7)	6.5 ± 0.5 (5.6-7.5)
c	11.0 ± 1.3 (9.4-14.4)	11.9 ± 1.5 (9.3-14.5)
Distance lip region end to vulva	733.4 ± 63.6 (645.6-833.2)	822.9 ± 52.1 (686.6-920.7)
Distance lip region end to anus	793.5 ± 62.7 (706.0-902.2)	880.1 ± 56.9 (725.2-973.5)
V	83.9 ± 1.3 (81.3-86.1)	85.6 ± 0.8 (84.5-87.8)
V'	92.4 ± 1.6 (89.8-95.3)	93.5 ± 0.9 (91.6-94.7)
Distance lip region to end oesophageal gland	156.5 ± 4.2 (150.2-164.4)	154.0 ± 10.3 (127.9-166.5)
Body width at anus	31.8 ± 3.0 (26.4-38.6)	32.3 ± 4.7 (22.7-38.6)
b'	5.6 ± 0.4 (5.0-6.5)	6.3 ± 0.5 (5.4-7.2)
c'	2.5 ± 0.3 (2.3-3.4)	2.6 ± 0.4 (2.1-3.3)
Distance between vulva and post end of body	140.2 ± 12.1 (111.7-158.3)	138.7 ± 11.4 (111.7-154.3)
Body width at vulva	39.3 ± 2.6 (34.5-42.6)	38.4 ± 5.6 (28.5-47.9)
VL/VB	3.6 ± 0.3 (3.1-4.0)	3.7 ± 0.4 (3.1-4.6)
Rex	43 ± 4.4 (35-52)	43 ± 2.4 (39-47)
Roes	43 ± 5.7 (37-57)	39 ± 2.9 (32-44)
Rvan	16 ± 2.1 (13-19)	16 ± 2.0 (12-20)
Ran	34 ± 3.3 (29-39)	33 ± 3.0 (28-38)
RV	48 ± 2.6 (44-52)	48 ± 3.4 (40-54)
R	218 ± 11.6 (188-240)	234 ± 5.7 (223-241)
Stylet length	78.7 ± 2.3 (75.3-83.2)	78.0 ± 3.1 (73.7-83.4)
Length of stylet shaft	13.2 ± 0.6 (12.2-14.2)	13.0 ± 1.8 (11.4-17.9)
m	83.2 ± 0.6 (82.3-84.3)	83.4 ± 2.0 (78.4-84.9)
stylet length as percentage of body length	9.1 ± 0.6 (8.3-10.0)	8.1 ± 0.4 (7.4-9.3)
Distance between stylet base and D.O.G	6.4 ± 1.1 (4.1-8.1)	7.1 ± 1.9 (4.1-11.4)
O	8.2 ± 1.4 (5.0-10.7)	9.2 ± 2.5 (5.1-15.1)
Distance lip region-center median bulb	102.7 ± 4.1 (95.4-109.6)	102.0 ± 4.8 (95.4-109.6)
MB	67.6 ± 2.5 (63.5-72.6)	68.5 ± 4.1 (64.0-78.3)

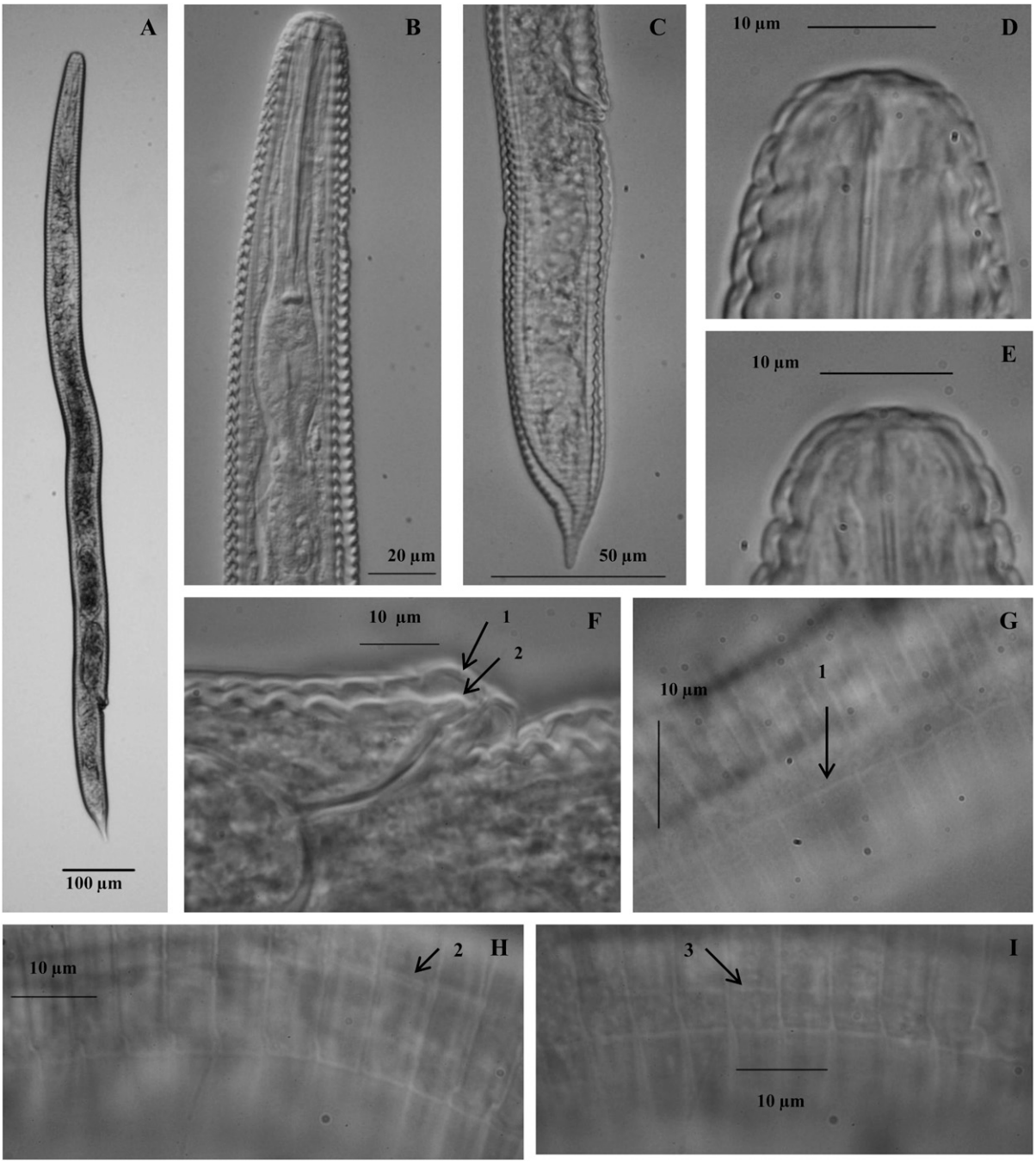

Fig. 7. Light micrographs of *Hemicycliophora labiata*: A. Entire female. B. Anterior region. C. Posterior region. D–E. Lip region. F. Vulva region (1, vulval sleeve; 2, vulval lips). G–I. Lateral fields (1, Line in lateral fields; 2–3, Scratches on body annuli outside the lateral field).

the rhizosphere of tulip-poplar (*Liriodendron tulipifera*); and T. Todd in June 2010 from turfgrass in Kansas. No global coordinates provided.

Diagnosis: Tennessee and Kansas populations of *H. labiata* are characterized by lateral fields marked by a single longitudinal line, with frequent anastomoses and breaks. Annulli with occasional scratches outside the lateral fields. Vulva with distinctly modified lips of equal length, small vulval sleeve, and a slightly offset, short, conoid tail and more convex dorsally than ventrally.

The morphometrics of the two studied populations are in agreement with the species description of topotypes (Brzeski, 1974) and a specific ITS1 sequences (JQ708149 and JQ708150) have been submitted to GenBank.

Relationships: Hemicycliophora labiata can be differentiated from *H. floridensis* Chitwood & Birchfield, 1957 by lateral field marked with lateral line interrupted by anastomoses and breaks vs. lateral fields with two lines forming a groove; shorter stylet (75-83 vs. 95-113 µm); greater RV (44-52 vs. 32-33); and Ran (29-39 vs. 16-23);

vulval sleeve short vs. vulval sleeve slightly long; tail dorsally convex and offset vs. conoid, not offset. The populations of this study have slightly smaller morphometrics than the population described from Namibia, Africa: smaller RV (44-52 vs. 45-71); Ran (29-39 vs. 35-49); tail length (63-95 vs.100-119); and Ran (29-39 vs. 35-49) (Brzeski, 1974; Van Den Berg and Tiedt, 2006).

Hemicycliophora pruni Kirjanova & Shagalina, 1974
(Table 5; Fig. 8)

Description

Female: body slightly ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath somewhat detached from inner cuticle along the entire body. Lateral field marked with a single line with anastomoses and breaks of striae. Labial disc slightly rounded and elevated, pseudolips not observed. Lip region continuous, not offset, outer and inner cuticle with two rounded annuli. Stylet slightly curved with basal knobs rounded to concave directed slightly posteriorly with a large cavity. Excretory pore posterior to oesophageal basal gland, Vulval lips modified, anterior vulval lip long, vulval sleeve long. Spermatheca not observed. Tail elongate, slightly conoid, increasingly convex dorsally and ventrally, with a subacute to rounded tip. Tail annulations distinct.

Male: Not found.

Host and locality: Specimens were collected in July 2008 by W. Ye from the rhizosphere of turfgrass in Wayne, NC. No GPS coordinates provided.

Diagnosis: This North Carolina population of *H. pruni* is distinguished by two rounded lip annuli distinct only in the outer cuticle, lateral fields marked with a longitudinal line with anastomoses and breaks of transverse striae along the body, mostly observed between the oesophagus level and tail, body annuli outside lateral field without scratches. Stylet straight with rounded knobs slightly posteriorly directed with a large cavity. Vulva with a long anterior vulval lip and long vulval sleeve, and an elongated tail, slightly offset dorsally with a subacute or rounded terminus with distinct annulation. The North Carolina population closely agreed with the original description but differs from the original by: greater values of a (21-28 vs. 15-20); b (6-7 vs. 5-6); broader range of Rvan (12-34 vs. 17-21); RV (54-85 vs. 48-61); and a slightly shorter stylet (81-93 vs. 90-103 μm). Based on the original description, this population also differs by the number of longitudinal lines marking the lateral field (one vs. four lines: outer lines crenate, inner lines straight). Also, the excretory pore was observed anterior and/or posterior to the oesophageal basal bulb.

TABLE 5. Measurements and ratios of *Hemicycliophora pruni* and *H. shepherdi*. Mean, standard deviation and range in μm.

Character/ratio	*H. pruni* ♀ (n = 20)	*H. shepherdi* ♀ (n = 11)
L	1,008.3 ± 47.2 (850-1,062.5)	1,002.3 ± 99.6 (825.0-1,175.0)
Oesophagus length	163.0 ± 5.5 (150.2-170.5)	166.1 ± 11.9 (140.1-182.7)
Tail	107.2 ± 14.3 (73.1-134)	133.6 ± 11.7 (109.6-150.2)
Maximum Body width	40.0 ± 2.4 (36.5-44.7)	41.5 ± 3.0 (37.4-47.1)
a	25.3 ± 1.6 (21.3-28.0)	24.2 ± 2.8 (19.4-28.6)
b	6.2 ± 0.2 (5.5-6.5)	6.0 ± 0.4 (5.1-6.7)
c	9.6 ± 1.4 (7.7-14.2)	7.5 ± 0.4 (7.1-8.4)
Distance lip region end to vulva	818.3 ± 42.1 (679.5-869.7)	796.0 ± 81.1 (654.5-949.7)
Distance lip region end to anus	901.1 ± 44.9 (762.7-964.4)	868.7 ± 90.1 (709.3-1034.9)
V	81.1 ± 0.7 (79.7-82.1)	79.4 ± 1.4 (77.3-81.3)
V'	90.8 ± 1.3 (87.6-93.2)	91.7 ± 1.4 (89.7-94.2)
Distance lip region to end oesophageal gland	169.1 ± 6.1 (154.3-178.6)	171.1 ± 11.6 (144.1-186.8)
Body width at anus	32.1 ± 3.0 (26.4-38.6)	33.4 ± 2.1 (30.0-36.5)
b'	6.0 ± 0.2 (5.4-6.2)	5.9 ± 0.4 (5.1-6.4)
c'	3.3 ± 0.4 (2.1-3.9)	4.0 ± 0.4 (3.1-4.7)
Distance between vulva and post end of body	190.0 ± 7.9 (170.5-203.0)	206.3 ± 24.4 (170.5-241.6)
Body width at vulva	38.2 ± 3.5 (30.5-46.7)	39.8 ± 3.7 (32.5-46.3)
VL/VB	5.0 ± 0.5 (3.9-6.2)	5.2 ± 0.7 (4.3-6.5)
Rex	52 ± 2.8 (47-58)	68 ± 6.5 (61-80)
Roes	47 ± 2.4 (43-54)	69 ± 7 (61-80)
Rvan	23 ± 4.7 (12-34)	27 ± 4.1 (19-35)
Ran	41 ± 5.8 (31-52)	61 ± 4.7 (52-67)
RV	65 ± 7.3 (54-85)	88 ± 6.8 (75-98)
R	267 ± 10.4 (248-286)	389 ± 38.6 (334-461)
Stylet length	89.1 ± 2.6 (81.2-93.4)	99.8 ± 3.2 (93.2-103.5)
Length of stylet shaft	16.8 ± 1.6 (14.2-20.3)	15.9 ± 2.0 (12.2-18.7)
m	81.1 ± 1.9 (76.7-84.1)	84.1 ± 1.8 (82.0-87.5)
stylet length as percentage of body length	8.9 ± 0.3 (8.3-9.6)	10.0 ± 0.8 (8.6-11.6)
Distance between stylet base and D.O.G	7.7 ± 2.3 (4.1-12.2)	5.8 ± 1.2 (4.1-8.1)
O	8.7 ± 2.7 (4.4-13.9)	5.8 ± 1.2 (4.3-8.1)
Distance lip region-center median bulb	116.1 ± 9.7 (105.6-154.3)	120.7 ± 11.5 (89.3-129.9)
MB	71.3 ± 6.3 (66.7-97.4)	72.6 ± 4.1 (63.8-80.0)

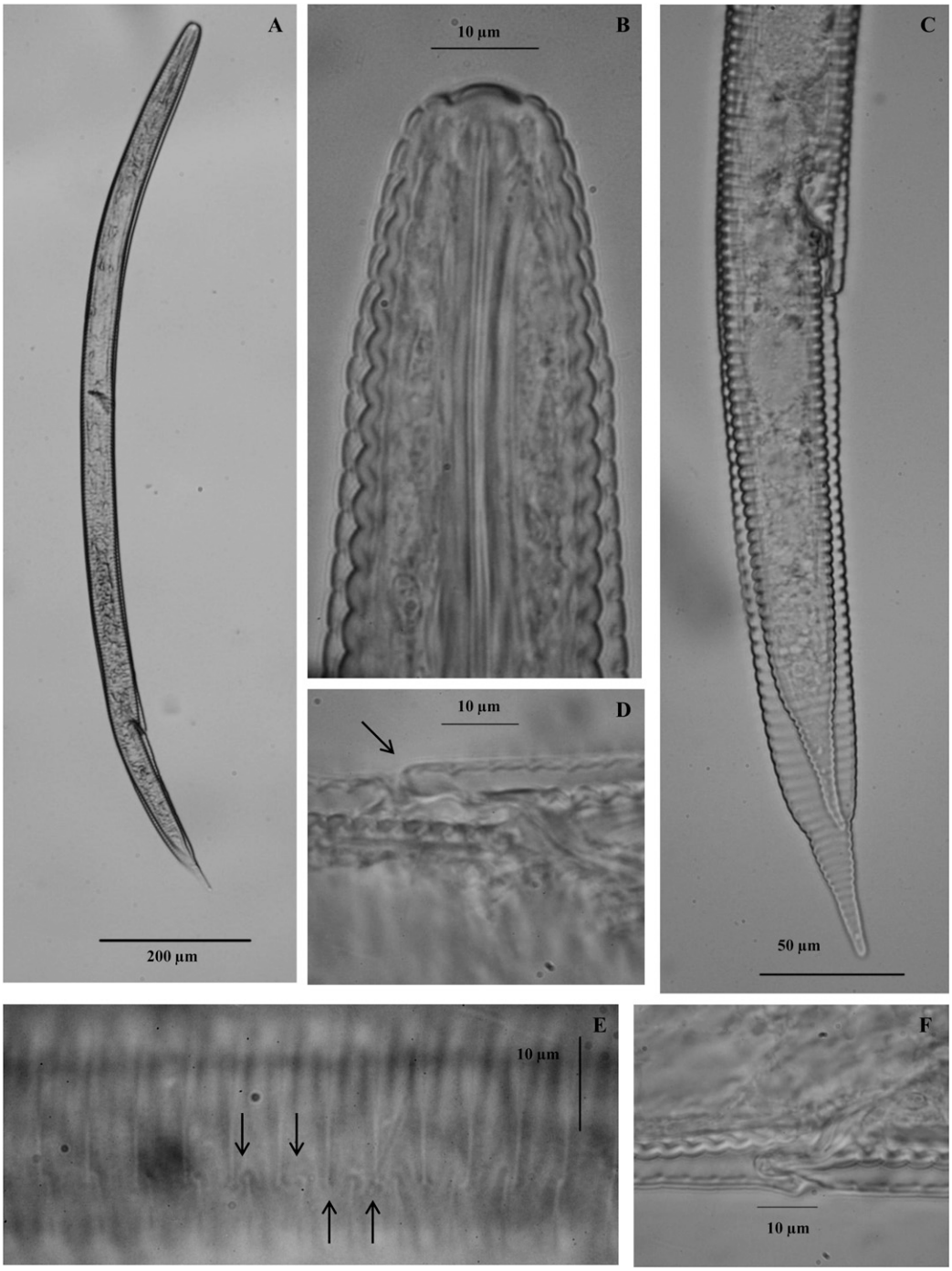

Fig. 8. Light micrographs of *Hemicycliophora pruni*: A. Entire female. B. Anterior region and lip region. C. Posterior region. D. Vulva region (arrow showing vulva sleeve). E. Lateral fields (arrows showing anastomoses). F. Aberrant vulva.

(Kirjanova and Shagalina, 1974; Brzeski and Ivanova, 1978) and a specific ITS1 sequence (JQ708144) has been submitted to GenBank.

Relationships: The closest related species to *H. pruni* are *H. oostenbrinki* Luc, 1958 and *H. penetrans* Thorne, 1955. The three species have long vulval lips and long vulval sleeves. Vulval lips and vulval sleeve are similar in *H. pruni* and *H. penetrans* as they are flattened and follow the contour of the body whereas in *H. oostenbrinki* the anterior lip is wider and the posterior lip has a slight anterior projection. Annuli of *H. pruni* and *H. oostenbrinki* do not show longitudinal lines or scratches outside the lateral field while *H. penetrans* has many of them. Lateral fields in this population of *H. pruni* are not present instead, a single longitudinal line with anastomoses and breaks of the striae was observed whereas in *H. oostenbrinki* lateral fields are marked by two longitudinal lines and a third faint line visible at the

tail. In the original description of *H. pruni* four longitudinal lines are described with the outer ones crenate and inner ones straight. On the other hand, lateral fields in *H. penetrans* demarcated by two longitudinal lines intersected by transverse striae. Morphometrically, the studied population of *H. pruni* differed from *H. oostenbrinki* by a longer stylet (81-93 vs. 70-72 μm); and greater Rex (47-58 vs. 42-47). Differences between *H. pruni* and *H. penetrans* are a longer stylet (81-93 vs. 71-85 μm); and greater RV (54-85 vs. 41-53). (Thorne, 1955; Kirjanova and Shagalina, 1974; Brzeski and Ivanova, 1978).

Hemicycliophora shepherdi Wu, 1966
(Table 5; Fig. 9)

Description

Female: Body straight or ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath loosely

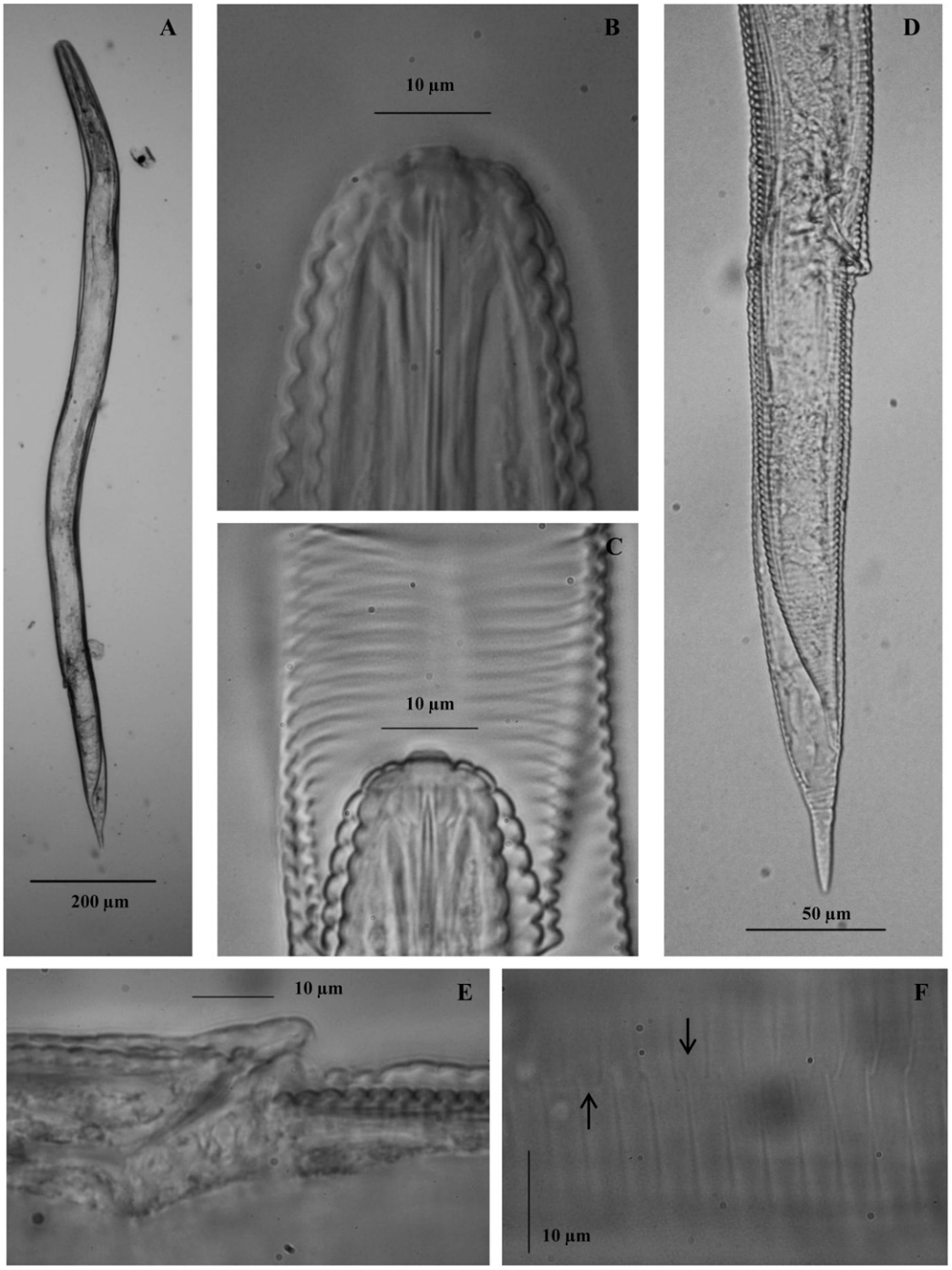

Fig. 9. Light micrographs of *Hemicycliophora shepherdi*: A. Entire female. B–C. Lip region. D. Posterior region. E. Vulva. F. Lateral fields (arrows showing anastomoses).

fitting. Lateral fields marked by longitudinal line with frequent anastomoses and breaks; occasionally, annuli marked with one or two scratches outside the lateral field. Labial disc somewhat rectangular to rounded, elevated, small lateral pseudolips. Lip region continuous, outer and inner cuticle with two rounded to somewhat flattened lip annuli. Stylet straight, with rounded basal knobs, slightly posteriorly directed, with small cavity. Excretory pore at the base of the oesophagus basal bulb. Vulval lips modified, no vulval sleeve present. Spermatheca rounded with or without sperm. Tail conoid, uniformly narrowing, more convex dorsally than ventrally, tail end slightly offset.

Male: Not found.

Host and locality: Specimens were collected in September 2011 by R. Robbins at Toad Suck Ferry Park, Perry County, AR (GPS coordinates N 35° 04.279 min, -W 092° 32.704 min) from the rhizosphere of willow (*Salis* sp.), grass and wild strawberries (*Fragarie* sp.).

Diagnosis: This Arkansas population of *H. shepherdi* is characterized by lateral fields marked with a longitudinal line with frequent anastomoses and breaks, occasionally, one or two scratches outside the lateral field, labial disc somewhat rectangular to rounded, elevated with presence of small lateral pseudolips, two rounded to somewhat flattened lip annuli in the outer and inner cuticle, vulva with distinctly modified lips but without vulval sleeve and a conoid tail, uniformly narrowing and more convex dorsally than ventrally, with a slightly offset terminus.

This population is in agreement with the original description, although no longitudinal lines were reported originally (Wu, 1966; Brzeski, 1974) and a specific ITS1 sequence (JQ911744) has been submitted to GenBank.

Relationships: Hemicycliophora shepherdi is related to *H. similis* Thorne, 1955, but differ by having a labial plate round to rectangular vs. rounded, oral disc elevated vs. oral disc slightly elevated following lip region contour, greater values for Ran (52-67 vs. 30-40); R (334-461 vs. 276-305); Rex (61-80 vs. 49-56); V (77-81 vs. 84-87); smaller c (7-8 vs. 10-11); tail terminus annulation indistinct, outer cuticle detached vs. distinct and adpressed. Vulval lips are modified in both species, however; the posterior lip in *H. shepherdi* is shorter than in *H. similis*. The tail in *H . shepherdi* is more dorsally convex, conoid, and offset whereas in *H. similis* is dorsally ventrally convex, conoid, but not offset. *Hemicycliophora shepherdi* also resembles *H. zuckermani* Brzeski, 1963 but differs form it by smaller (L = 825-1,175 vs. 1,100-1337 μm), and a more elevated and distinct vulval lip compared with *H. zuckermani* that is flat and posteriorly directed. Also the tail in *H. shepherdi* is more dorsally convex, conoid and offset than in *H. zuckermani* (Thorne, 1955; Wu, 1966; Brzeski, 1974; Brzeski and Ivanova, 1978).

Hemicycliophora vidua Raski, 1958
(Table 6; Fig. 10)

Description

Female: Body slightly ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath detached from inner cuticle along entire body. Lateral field marked with a longitudinal line and with frequent anastomoses and breaks of transverse striae. Labial plate rectangular, oral disc rounded and slightly elevated, pseudolips separated, indistinct. Lip region continuous, outer and inner cuticle with two rounded lip annuli. Stylet curved, with rounded basal knobs, directed posteriorly, large cavity present. Excretory pore slightly anterior to the oesophagus basal gland. Vulva with modified lips, short vulval sleeve. Spermatheca rounded, empty when distinct. Tail elongated, dorsally convex, slightly offset, acute terminus with distinct annulation.

Male: Not found.

Host and locality: Specimens were collected in June 2008 by P. Agudelo in Clemson, SC, from the rizosphere of *Camellia* sp. No GPS coordinates provided.

Diagnosis: The South Carolina population of *H. vidua* was characterized by lateral fields marked by a single longitudinal line with frequent anastomoses and breaks; rectangular labial plate with oral disc high, rounded, and slightly elevated; pseudolips separated but occasionally indistinct; vulva with modified lips and a short vulval sleeve; and an elongated tail, slightly offset, and dorsally convex with an acute end with distinct annulation. This population is in agreement with the original description and others populations although no longitudinal lines were reported previously (Raski, 1958; Wu, 1966; Brzeski, 1974) and a specific ITS1 sequence (JQ708147) has been submitted to GenBank.

Relationships: Hemicycliophora vidua is related to *H. zuckermani* Brzeski, 1963 but is different by a longer body L (887-1,025 vs. 670-980 μm); absence of scratches outside the lateral field, a longer stylet (114-124 vs.87-106 μm); greater R (278-343 vs. 239-296); RV (60-84 vs. 56-65); and Ran (39-60 vs. 23-43). *Hemicycliophora vidua* is also close to *H. shepherdi* Wu, 1996 but differentiated from it by a slightly more anterior vulva, V (79-82 vs. 85-87); round vs. convex knobs, and tail annulations distinct vs. indistinct. Also, it is very close to *H. sheri* Brzeski, 1974 but differs from it by a rectangular vs. rounded labial disc, and a longer stylet (114-124 vs. 92-101 μm) (Wu, 1966; Brzeski, 1974; Brzeski and Ivanova, 1978).

Hemicycliophora zuckermani Brzeski, 1963
(Table 6; Fig. 11)

Description

Female: Body slightly ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath loosely fitting entire body. Lateral fields marked by two longitudinal lines with occasional anastomoses of transverse striae. Short lines mark annuli outside lateral fields. Labial

TABLE 6. Measurements and ratios of adult females of *Hemicycliophora vidua* and *H. zuckermani*. Mean, standard deviation and range in μm.

Character/ratio	*H. vidua* ♀ (n = 9)	*H. zuckermani* ♀ (n = 13) type b Host: Maple	*H. zuckermani* ♀ (n = 19) type c Host: River cane	*H. zuckermani* ♀ (n = 19) type a Host: Sycamore	*H. zuckermani* ♀ (n = 17) type d Host: Oat grass	*H. zuckermani* ♀ (n = 16) type d Host: Oak
L	969.4 ± 52.6 (887.5-1025.0)	1,246.2 ± 69.9 (1,100-1,337.5)	1,256 ± 117.2 (1,081.3-1,550)	1,160 ± 116.3 (950-1,437.5)	1,079.8 ± 61.3 (937.5-1,156)	1,049 ± 66.3 (918.8-1,131.3)
Oesophagus length	183.1 ± 9.2 (170.5-203.0)	197.4 ± 7.3 (178.7-207.1)	195.1 ± 9.8 (182.7-215.2)	189 ± 10.3 (170.5-207.1)	180.2 ± 7.8 (170.5-196.9)	176.1 ± 4.9 (168.5-182.7)
Tail	123.2 ± 9.5 (111.7-134.0)	153.2 ± 8.7 (138.0-166.5)	155.5 ± 13.0 (129.9-182.7)	146 ± 16.2 (111.7-174.6)	139.7 ± 8.4 (121.8-154.3)	139.9 ± 15.0 (103.5-160.4)
Maximum body width	43.3 ± 4.1 (37.4-49.5)	45.8 ± 2.9 (40.6-48.7)	46.5 ± 3.8 (40.6-56.8)	44.1 ± 3.1 (40.6-52.8)	42.6 ± 2.7 (36.5-46.7)	42.1 ± 1.7 (38.6-46.7)
a	22.5 ± 1.8 (20.2-25.3)	27.3 ± 1.1 (25.4-29.0)	27.0 ± 1.6 (24.2-30.8)	26.4 ± 2.5 (23.4-35.4)	25.4 ± 1.0 (23.7-27.5)	24.9 ± 1.0 (23.1-26.2)
b	5.3 ± 0.3 (5.0-5.7)	6.3 ± 0.2 (5.8-6.6)	6.4 ± 0.4 (5.8-7.3)	6.1 ± 0.4 (5.4-6.9)	6.0 ± 0.3 (5.3-6.4)	6.0 ± 0.4 (5.3-6.5)
c	7.9 ± 0.6 (7.0-9.0)	8.1 ± 0.3 (7.7-9.0)	8.1 ± 0.4 (7.3-8.8)	8.0 ± 0.7 (6.9-10.3)	7.7 ± 0.4 (7.2-8.4)	7.6 ± 0.6 (6.6-9.2)
Distance lip region end to vulva	774.6 ± 40.4 (708.9-815.9)	1,022.5 ± 58.1 (905.1-1,093.3)	1,028.4 ±102 (886.4-1,294.2)	953.8 ± 107 (767.3-1,194)	897.0 ± 70.8 (744.7-1044.6)	856.7 ± 58.0 (738.6-920.1)
Distance lip region end to anus	846.3 ± 49.5 (767.7-911.3)	1,093.0 ± 63.5 (962.0-1,171.0)	1,100.4 ±106.6 (947-1,367.3)	1,014 ± 104.8 (816-1,262.9)	940.1 ± 55.9 (815.7-1010.1)	909.3 ± 56.4 (795.4-983.1)
V	79.9 ± 0.8 (78.6-81.5)	82.1 ± 0.6 (80.7-83.3)	81.9 ± 1.1 (79.4-83.5)	82.1 ± 2.2 (80.0-90.4)	83.0 ± 4.1 (79.4-90.3)	81.6 ± 1.1 (78.8-83.1)
V'	91.6 ± 1.5 (88.4-93.6)	93.6 ± 0.4 (92.7-94.3)	93.4 ± 0.8 (91.2-94.7)	94.0 ± 2.3 (91.6-103.1)	95.4 ± 4.8 (91.3-104.3)	94.2 ± 1.2 (92.5-96.1)
Distance lip region to end oesophageal gland	190.8 ± 9.1 (178.6-211.1)	202.4 ± 7.2 (182.7-211.1)	201.2 ± 9.5 (186.8-219.2)	194.7 ± 10.8 (178.6- 215)	184.5 ± 7.8 (174.6-198.9)	180.5 ± 4.7 (174.6-190.8)
Body width at anus	37.9 ± 16.5 (26.0-81.2)	37.0 ± 2.2 (32.5-40.6)	38.9 ± 2.6 (36.5-44.7)	36.4 ± 4.9 (18.3-40.6)	37.6 ± 3.1 (30.5-42.6)	36.4 ± 1.9 (32.5-40.6)
b'	5.1 ± 0.3 (4.8-5.5)	6.2 ± 0.2 (5.7-6.5)	6.2 ± 0.4 (5.5-7.1)	5.9 ± 0.3 (5.3-6.7)	5.9 ± 0.3 (5.2-6.2)	5.8 ± 0.3 (5.2-6.3)
c'	3.5 ± 0.8 (1.7-4.6)	4.1 ± 0.3 (3.8-4.6)	4.0 ± 0.3 (3.5-4.4)	4.1 ± 1.0 (2.8-8.0)	3.7 ± 0.2 (3.1-4.0)	3.8 ± 0.4 (3.0-4.4)
Distance between vulva & post end of body	194.9 ± 14.7 (178.6-219.2)	223.6 ± 14.8 (194.9-251.7)	227.5 ± 20.6 (190.8-272.0)	206.2 ± 27.5 (119.8-247.7)	182.8 ± 44.9 (105.6-231.4)	192.5 ± 14.3 (166.5-215.2)
Body width at vulva	47.4 ± 20.7 (32.5-101.5)	44.3 ± 2.9 (38.6-48.7)	46.1 ± 2.7 (40.6-52.8)	44.1 ± 3.4 (36.5-52.8)	42.3 ± 2.1 (38.6-44.7)	41.6 ± 2.0 (38.6-46.7)
VL/VB	4.5 ± 1.0 (2.0-5.5)	5.1 ± 0.4 (4.7-6.0)	4.9 ± 0.4 (4.3-6.1)	4.7 ± 0.6 (2.7-6.0)	4.3 ± 1.0 (2.4-5.2)	4.6 ± 0.3 (4.3-5.3)
Rex	57 ± 3.0 (50-60)	66 ± 5.1 (61-81)	62 ± 3.6 (57-70)	62 ± 3.2 (58-69)	61 ± 2.5 (57-65)	60 ± 3.0 (51-64)
Roes	63 ± 5.5 (53-72)	63 ± 5.5 (57-79)	61 ± 2.4 (57-65)	60 ± 2.8 (56-66)	61 ± 3.2 (55-66)	62 ± 4.1 (58-72)
Rvan	24 ± 3.8 (19-31)	24 ± 2.9 (19-29)	22 ± 2.6 (15-26)	21 ± 2.1 (18-25)	21 ± 2.0 (16-24)	17 ± 3.9 (13-28)
Ran	47 ± 7.2 (39-60)	63 ± 6.5 (52-75)	57 ± 5.1 (46-65)	60 ± 7.4 (48-77)	53 ± 4.5 (46-59)	53 ± 3.5 (45-60)
RV	72 ± 6.9 (60-84)	87 ± 7.1 (72-97)	78 ± 5.2 (65-85)	81 ± 8.3 (68-100)	75 ± 4.9 (66-81)	71 ± 4.6 (60-79)
R	309 ± 17.1 (278-343)	360 ± 23.2 (303-388)	339 ± 19.1 (306-364)	330 ± 21.0 (301-374)	323 ± 7.9 (309-334)	321 ± 11.4 (289-338)
Stylet length	118.2 ± 3.2 (113.7-123.8)	112.9 ± 4.1 (103.5-117.7)	113.6 ± 5.9 (103.5-123.8)	108.9 ± 6.2 (101.5-123.8)	103.6 ± 3.8 (97.4-109.6)	100.9 ± 3.4 (93.4-105.6)
Length of stylet shaft	19.4 ± 1.5 (16.2-20.3)	19.4 ± 1.6 (16.2-22.3)	20.7 ± 1.8 (16.2-24.4)	18.3 ± 1.6 (16.2-22.3)	18.6 ± 2.1 (16.2-22.3)	18.3 ± 1.7 (16.2-20.3)
m	83.6 ± 1.1 (82.5-86.0)	82.9 ± 1.1 (80.4-84.3)	81.8 ± 1.6 (78.4-84.6)	83.2 ± 0.9 (81.7-85.2)	82.0 ± 1.8 (78.0-84.0)	81.9 ± 1.7 (79.2-84.6)
stylet length as percentage of body length	12.2 ± 0.5 (11.4-13.0)	9.1 ± 0.3 (8.7-9.5)	9.1 ± 0.6 (7.6-10.1)	9.4 ± 0.6 (7.6-10.7)	9.6 ± 0.4 (9.0-10.5)	9.6 ± 0.4 (9.0-10.4)
Distance between stylet base and D.O.G	5.4 ± 2.4 (0.8-8.9)	10.0 ± 3.9 (6.1-20.3)	8.7 ± 1.5 (6.1-12.2)	8.0 ± 1.5 (6.1-10.2)	7.6 ± 2.5 (4.1-12.2)	8.2 ± 2.8 (4.1-14.2)
O	4.6 ± 2.0 (0.7-7.5)	8.9 ± 3.4 (5.3-17.9)	7.7 ± 1.3 (5.2-11.1)	7.4 ± 1.5 (4.9-10.0)	7.4 ± 2.4 (3.8-11.8)	8.2 ± 3.1 (4.0-15.2)
Distance lip region-center median bulb	140.7 ± 4.5 (134.0-146.2)	140.5 ± 4.8 (129.9-146.2)	142.1 ± 6.8 (129.9-154.3)	137.6 ± 7.6 (125.9-150.2)	129.0 ± 5.9 (121.8-142.1)	126.5 ± 3.4 (121.8-134.0)
MB	77.0 ± 3.0 (70.0-81.0)	71.2 ± 1.6 (69.4-74.5)	72.8 ± 1.6 (69.8-76.0)	72.8 ± 1.2 (70.8-76.2)	71.6 ± 1.3 (69.9-73.8)	71.8 ± 0.9 (70.6-73.8)

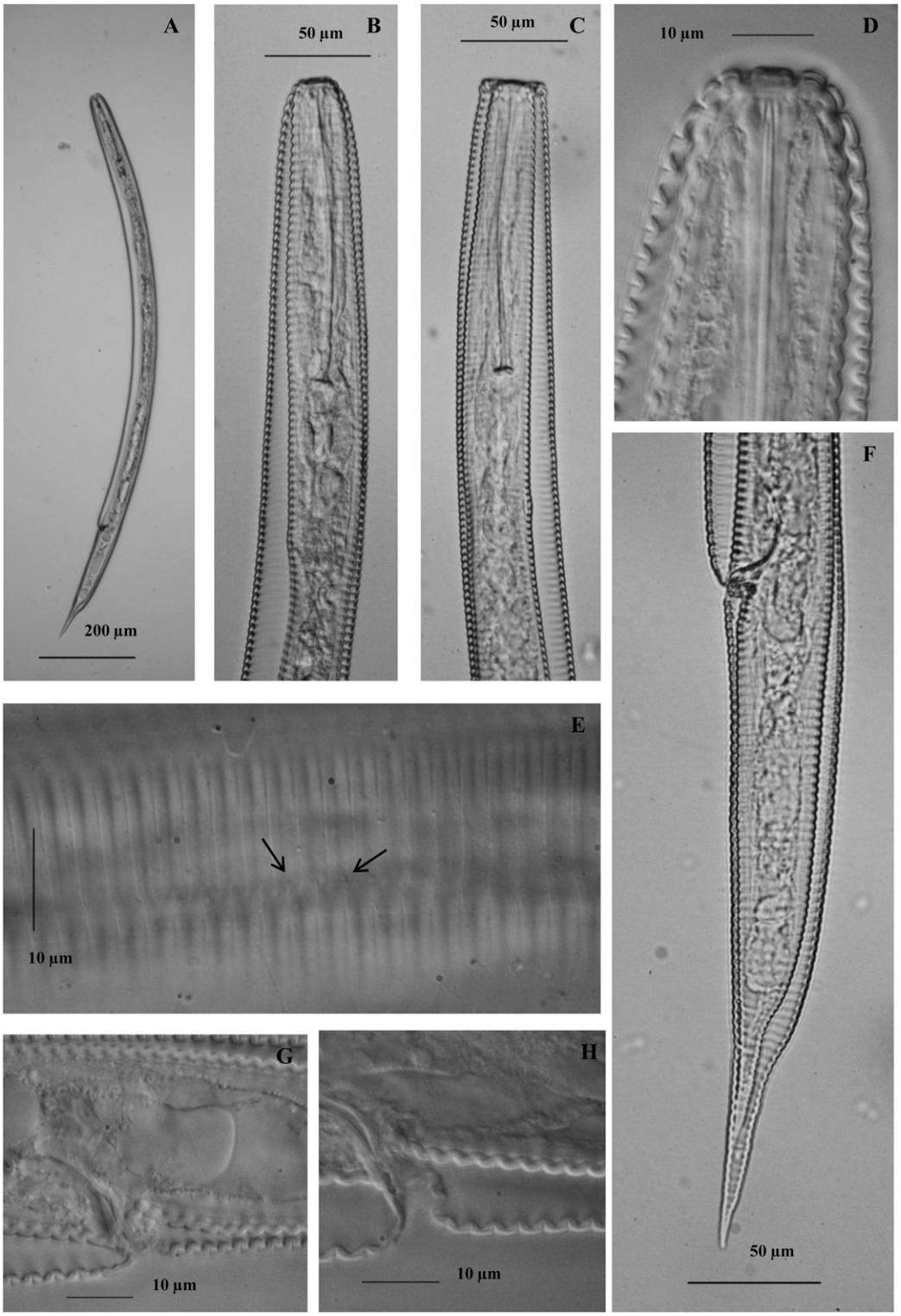

Fig. 10. Light micrographs of *Hemicycliophora vidua*: A. Entire female. B–C. Anterior region. D. Lip region. E. Lateral fields. (arrows showing anastomoses). F. Posterior region. G. Vulva. H. Aberrant vulva.

plate slightly rounded, oral disc rounded and slightly elevated, pseudolips separated indistinct. Lip region continuous, outer and inner cuticle with two rounded annuli. Stylet curved with rounded concave basal knobs directed posteriorly, small cavity present. Excretory pore slightly posterior or at the same level of the oesophagus posterior terminus. Vulva with modified lips, anterior vulval lip somewhat overlapping. Spermatheca rounded, empty. Tail long and progressively convex tapering to an acute terminus.

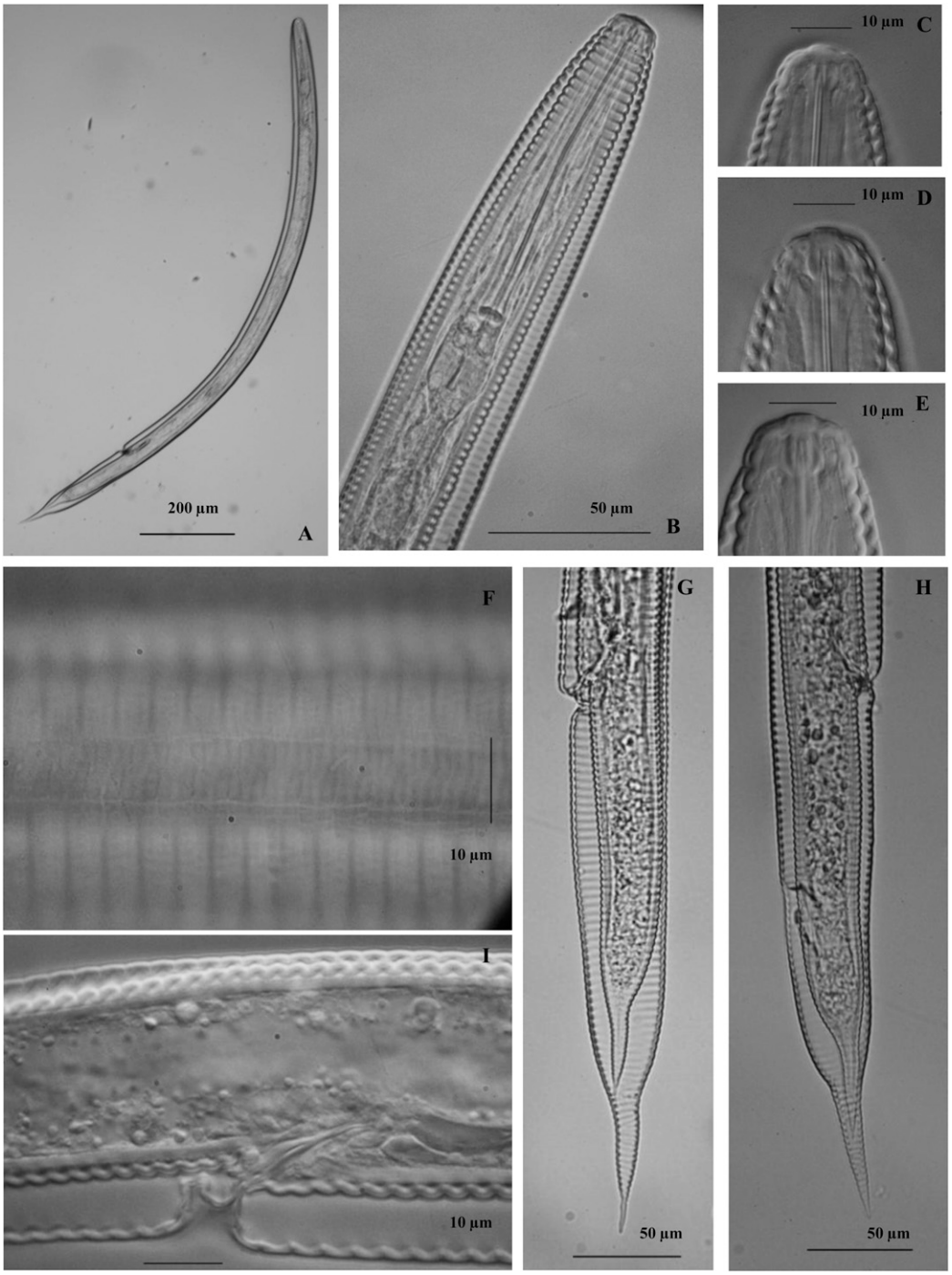

FIG. 11. Light micrographs of *Hemicycliophora zuckermani*: A. Entire female type d. B. Anterior region type d. C–E. Lip region of type a, type d, and type b. F. Lateral fields of type d. G–H. Posterior region of type b and type d. I. Vulva of type d.

Male: Not found.

Host and locality: Specimens were collected in August 2008–2009 by M. Cordero and R. Robbins at Washington County, AR (GPS coordinates N 36° 06.190 min, -W 094° 20.666 min); from the rhizosphere of maple (*Acer* sp.) and river cane (*Arundinaria* sp.) *H. zuckermani* type b and type c; N 36° 06.312 min, -W 094° 20.558 min) from sycamore (*Platanus occidentalis*) type a; and Fayetteville, AR (GPS coordinates N 36° 06.308 min, -W 094° 09.959 min) from the rhizosphere of oak (*Quercus* sp.) and oat grass (*Arrhenatherum* sp.) *H. zuckermani* type d.

Diagnosis: Hemicycliophora zuckermani was characterized by lateral fields marked with two longitudinal lines and occasional anastomoses, sporadic short lines are present close to and outside of the lateral field, vulva with modified lips, the anterior vulval lip somewhat overlapping, and an elongated, progressively convex tail with an acute tip. These populations are in agreement

with the original description (Brzeski, 1974) and specific ITS1 sequences (JQ708142; JQ708148; JQ708152; JQ708153) have been submitted to GenBank.

Relationships: Hemicycliophora zuckermani is similar to *H. shepherdi* Wu, 1964 but differs by a slightly more anterior vulva V (79-84 vs. 85-87); greater RV (65-85 vs. 42-48); and a longer stylet (97-110 vs. 94-101 μm). *Hemicycliophora zuckermani* is differentiated from *H. vidua* Raski, 1958, by its shorter stylet (97-110 vs. 115-119 μm); stylet knob convex projected posteriorly vs. stylet knobs slightly flat, posteriorly directed; vulval sleeve short vs. absent and posterior vulval lip short vs. prominent (Raski, 1958; Wu, 1966; Brzeski, 1974).

Gracilacus straeleni (Wu, 1964) Raski, 1976
(Table 7; Fig. 12)

Description

Female: Body slender and ventrally arcuate. Body annuli rounded and smooth. Lateral field with four lines running lengthwise. Labial plate not visible. Lip region smooth, with indistinct annuli, lip annuli rounded continuous with contour of body. Stylet curved and flexible, with rounded knobs strongly developed, flattened at the base. Excretory pore posterior to stylet knobs and at the midpoint of the isthmus of the oesophagus. Vulva closed with lips nonprotruded, vulval flaps present. Vagina straight. Female genital tract monodelphic, prodelphic, outstretched, reaching half of the body nematode length, rounded spermatheca full of sperm. Tail long, conoid, with strong annulations, becoming progressively finer nearing the terminus.

Male: Not found.

Host and locality: Specimens were collected in June 2009 by M. Cordero at Fayetteville, AR (GPS coordinates N 36° 05.968 min, -W 094° 10.107 min) from the rhizosphere of maple (*Acer* sp.).

Diagnosis: The Arkansas population of *Gracilacus straeleni* is characterized by having a lateral field with four lines, indistinct labial plate, lip annuli rounded, smooth, with indistinct annulations, vulva closed with nonprotruding lips, and distinct vulval flaps present. Vagina straight, rounded spermatheca full of sperm, and long conoid tail with strong annulations, becoming progressively finer nearing the terminus. This population is in agreement with the description of *Paratylenchus sarissa* Tarjan, 1960; collected in California and later synonimized with *Gracilacus straeleni*, along with populations reported in Czech Republic, Spain, and Romania. (Raski, 1962; Castillo and Gomez Barcina, 1988; Brzeski and Háněl, 1999; Ciobanu et al., 2003;) and a specific ITS1 sequence (JQ708155) has been submitted to GenBank.

Relationships: Gracilacus straeleni is very close to *G. ivorensis* (Luc and De Guiran, 1962) Raski, 1976 but is separated by a more posterior vulva (V = 77-84 vs. 73-77); slightly higher value of b (3-4 vs. 3); and presence of spermatheca. The current species differs from *G. aculenta* (Brown, 1959) Raski, 1962 in having four vs. three lines in the lateral field, and presence of vulva flaps that are absent in *G. aculenta* (Luc and De Guiran, 1962; Raski, 1976).

Paratylenchus labiosus Anderson & Kimpinski, 1977
(Table 7; Fig. 12)

Description

Female: Body slender straight or ventrally arcuate, and somewhat spiral-shaped. Body annuli rounded and smooth. Lateral field with four lines. Labial plate with rounded and elevated lips. Lip region concave and conoid without distinct fine annulations, continuous with the body. Stylet straight and robust, with rounded knobs slightly posteriorly directed. Excretory pore posterior to stylet knobs and at the same level as the oesophagus basal bulb. Vulva closed with lips nonprotruded, vulval flaps present. Vagina straight. Female genital tract monodelphic, prodelphic, outstretched, reaching half of the body nematode length. Spermatheca round, with sperm. Tail long, conoid with strong annulation progressively finer at terminus.

Male: Not found.

Host and locality: Specimens were collected in June 2009 by M. Cordero at Washington County, AR (GPS coordinates N 36° 06.244 min, -W 094° 20.270 min) from the rhizosphere of elm (*Ulmus* sp.) and grass.

Diagnosis: The Arkansas population of Paratylenchus labiosus was characterized by a female body slender,

TABLE 7. Measurements and ratios of *Gracilacus straeleni* and *Paratylenchus labiosus*. Mean, standard deviation and range in μm.

Character/Ratio	*Gracilacus straeleni* ♀ (n = 25)	*Paratylenchus labiosus* ♀ (n = 16)
L	321.7 ± 23.3 (274.2-369.7)	348.1 ± 32.5 (295.5-390.9)
a	21.3 ± 2.6 (15.3-25.3)	27.2 ± 6.5 (18.2-38.1)
b	3.4 ± 0.4 (2.8-4.4)	3.7 ± 0.2 (3.4-4.0)
c	9.1 ± 1.4 (6.7-12.2)	9.9 ± 2.9 (6.2-15.8)
V	80.7 ± 1.6 (77.3-84.1)	82.2 ± 2.6 (76.1-84.9)
V'	90.9 ± 2.5 (87.1-97.3)	92.2 ± 2.4 (90.6-98.2)
Stylet length	52.9 ± 2.3 (48.2-58.4)	18.3 ± 0.9 (16.7-20.3)
Stylet length as percentage of body length	16.5 ± 1.2 (14.0-19.3)	5.4 ± 0.5 (4.3-5.8)
Distance from anterior end to excretory pore	93.9 ± 8.3 (80-108.0)	84.8 ± 6.0 (74.7-97.6)
Position of excretory pore as percentage of the body length	29.2 ± 2.6 (21.7-31.7)	24.5 ± 1.5 (22.1-26.4)

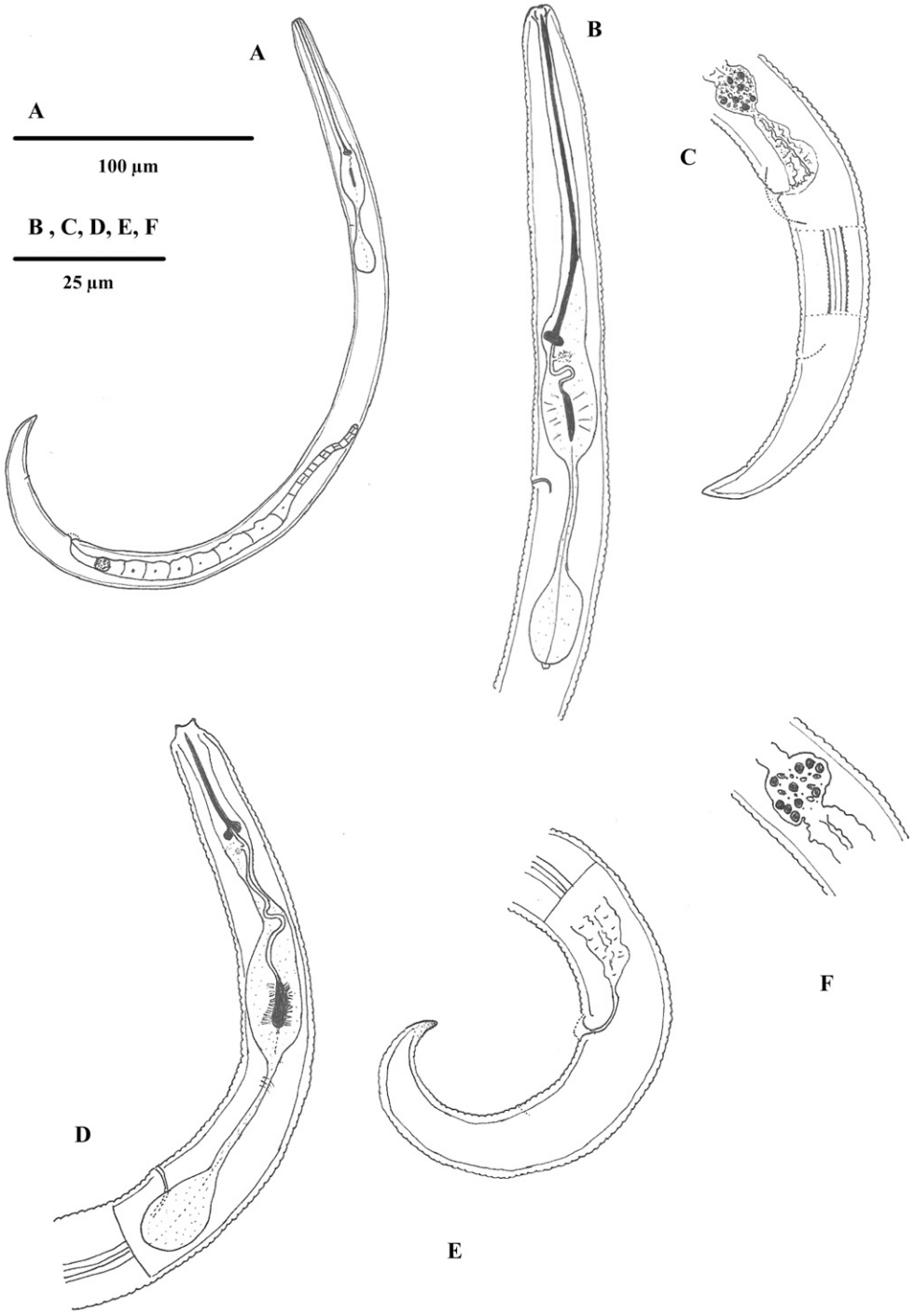

Fig. 12. Camera lucida drawings of *Gracilacus straleni*: A. Entire female. B. Anterior region. C. Tail. *Paratylenchus labiosus*. D. Anterior region. E. Tail. F. Spermatheca.

straight or ventrally arcuate, and somewhat spiral-shaped, lateral field with four lines, labial plate with rounded and elevated lips, vulva closed with non-protruded lips and vulval flaps present, vagina straight and spermatheca with large rounded sperm. This population is in agreement with the original description of the species (Anderson and Kimpinski, 1977) and a specific ITS1 sequence (JQ708154) has been submitted to GenBank.

Relationships: Paratylenchus labiosus is closely related to *P. tateae* Wu & Townsend, 1973 and *P. projectus* Jenkins, 1956 by having a very similar lip region shape and labial plate. *Paratylenchus labiosus* shares elevated lips with *P. tateae* whereas lips in the labial plate of *P. projectus* are conoid and flattened without elevate lips. Main differences between *P. labiosus* and *P. tateae* are the presence of spermatheca with sperm vs. absence of spermatheca and a more slightly anteriorly located vulva (76 to 85 vs.

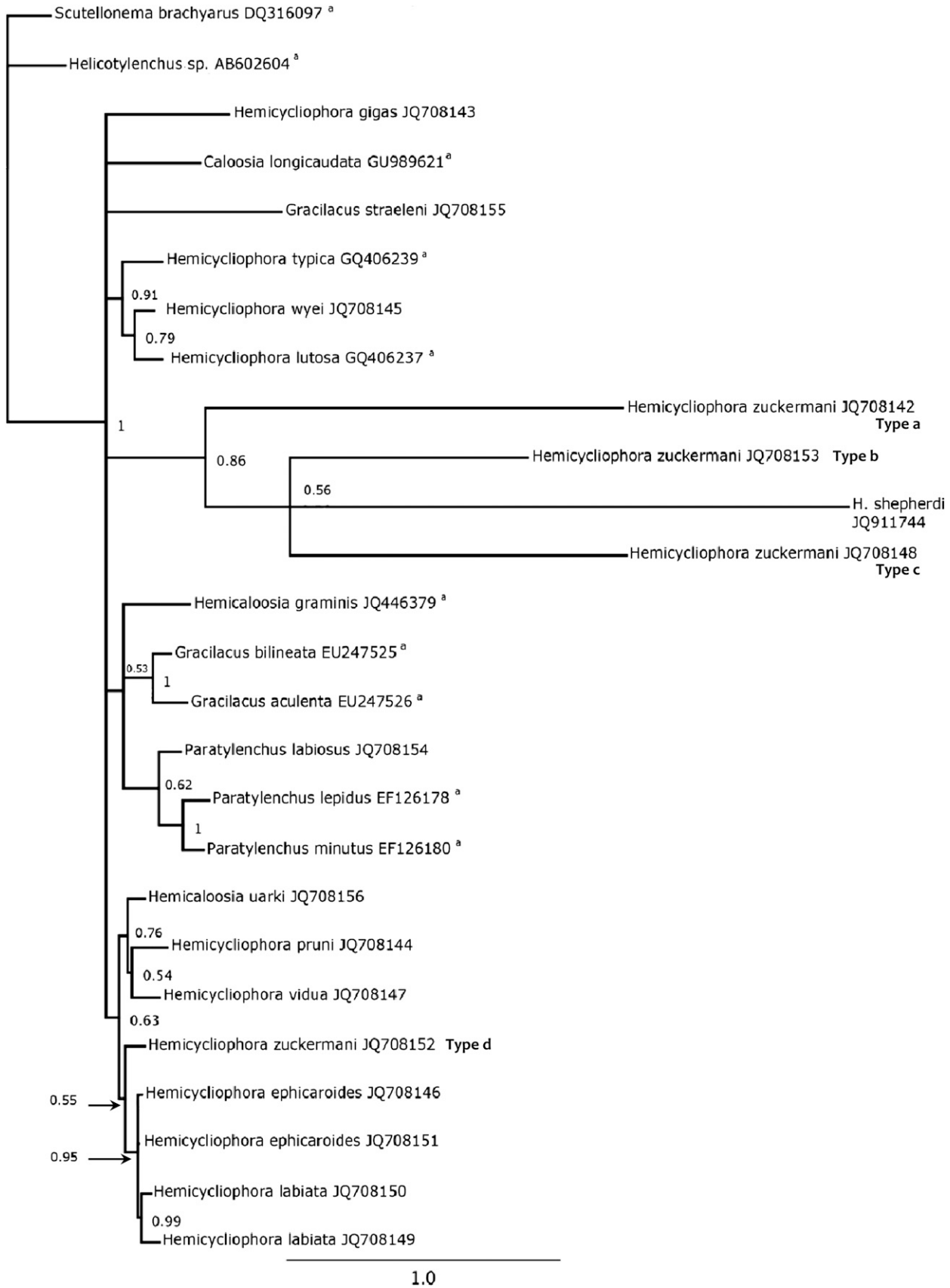

FIG. 13. Bayesian inference 50% majority consensus tree for the ITS1-rDNA region of *Hemicaloosia, Hemicycliophora, Gracilacus,* and *Paratylenchus* under GTR+G model (-Ln likelihood = 6408.5645; AIC = 12931.1290; K = 57; freqA = 0.2487; freqC = 0.2755; freqG = 0.2525; freqT = 0.2233; R(a) [AC]=0.6673; R(b) [AG] = 1.6688; R(c) [AT] = 1.2256; R(d) [CG] = 0.8370; R(e) [CT] = 1.1056; R(f) [GT] = 1.000; Gamma shape = 0.8900). Numbers at nodes are posterior probabilities values. [a] Supplemental sequences taken from GenBank.

81 to 85) (Wu and Townsend, 1973; Raski, 1975a; Raski, 1975b; Wu, 1975; Anderson and Kimpinski, 1977).

Molecular phylogenetic analysis: The length of the PCR product ranged between 600 bp to 940 bp for the species of *Hemicaloosia, Hemicycliophora, Gracilacus* and *Paratylenchus*. After manual correction and alignment the internal transcribed spacer 1 length used for phylogenetic analysis was 658 bp. JModeltest estimated the GTR+G model (-Ln likelihood = 6408.5645; AIC = 12931.1290; K = 57; freqA = 0.2487; freqC = 0.2755; freqG = 0.2525; freqT = 0.2233; R(a)[AC] = 0.6673; R(b)[AG] = 1.6688; R(c)[AT] = 1.2256; R(d)[CG] = 0.8370; R(e)[CT] = 1.1056; R(f)[GT] = 1.000; Gamma shape = 0.8900) (Fig. 13).

Hemicycliophora wyei n. sp. and *H. lutosa* showed a genetic divergence 17%, being similar in the tail shape although *H. wyei* has a more rounded terminus and showed a close vulva with long modified lips with a longer vulval sleeve.

Hemicaloosia uarki n. sp was placed as sister species with *H. pruni* and *H. vidua* and showed a genetic divergence of 20% and 17% with these species, respectively. Genetic divergence of *H. uarki* n. sp. with *H. gigas* was 38%. Position of H. gigas in this analysis was not resolved. All these species has two lip annuli except for *H. gigas* that showed three lip annuli. *Hemicaloosia graminis* showed a genetic divergence with *H. uarki* n. sp. and *Caloosia longicaudata* of 40% and 43%, respectively. A genetic divergence of 49% was found between *H. graminis* and *C. longicaudata*. The position of *Hemicaloosia graminis* and *Caloosia longicaudata* was not resolved in this analysis. Low genetic diversity (10% to 11%) was found among the species *H. labiata* and *H. ephicharoides*.

All specimens from four populations identified as *H. zuckermani*, morphologically and morphometrically meets the original values and characteristics of the species *H. zuckermani*. However, based on our analysis of the ITS1- rDNA gene sequences, these populations probably belong to different biological species. For the present, the specimens of these four populations remain under the name *H. zuckermani* but in different genotype codes as reference for future studies of the genus and this species.

The genetic divergence of *Paratylenchus labiosus* with *P. lepidus* and *P. minutus* was 30% as well as between *G. bilineata* and *G. aculenta*. The position of *Gracilacus straeleni* was not resolved in this analysis. Low support values in this group suggest that additional species have to include for future analysis.

The use of markers as ITS1-rDNA will be useful to confirm the taxonomical identification of species and possible lineages within subfamily Hemicycliophorinae Skarbilovich, 1959 and family Tylenchulidae Skarbilovich, 1947 and to establish the status of family Caloosiidae Siddiqi, 1980 and genera *Caloosia* Siddiqi & Goodey, 1964 and *Hemicaloosia* Ray & Das, 1978 (Raski and Luc, 1987; Siddiqi, 2000).

Molecular information and a correct taxonomical identification are essential to avoid confusion and help to detect relationships and ITS1 differences could be caused by possible different lineages or different rates of multiple substitutions or mutations events within the group. Several examples of the usefulness of the ITS1 rDNA can be cited. Sequences of *Xiphinema* and *Longidorus* reported genetic variation between *X. chambersi* and *L. crassus* of 38.6%; 3.8% between *X. diversicaudatum* and *X. bakeri*, *X. chambersi* and *X. italiae* 29.9%; *L. crassus* and *L. grandis* 8.9% and *L. fragilis* and *L. diadecturus* 32.4% (Ye et al., 2004). The genetic variation between different species of Punctoderinae and Heteroderinae ranged from 0.0% to 31.4% and 0.3% to 14.7% within each subfamily (Subbottin et al., 2001). The genetic variation of ITS1 sequences between *Paratrichodorus macrostylus* and *Trichorus primitivus* was 65% and 21.7% between *P. macrostylus* and *P. pachydermus*. (Boutsika et al., 2004).

Useful information after using the nuclear ITS1 ribosomal region had been obtained. Presence of *Heterodera avenae, H. glycines, H. hordecalis, H. latipons, H. schachtii, H. trifolii, H. elachista, H. turcomanica, H. mothi*, and *Cactodera cacti* were confirmed and identified from Iran (Tanha Maafi et al., 2003). Likewise, Reid et al. (2003) were able to differentiate populations of *Naccobus aberrans* from Peru from those previously studied in Mexico and Argentina, to characterize two different populations of the nematode from Argentina and found similarities between populations of *N. aberrans* from Peru and Bolivia. Also, analysis of ITS1-rDNA confirmed in 2007 the presence of *Globodera pallida* in Idaho (Skantar et al., 2007).

Recently, Powers et al. (2010), using morphology studies and sequences of ITS1 and cytochrome b markers of *Discocriconemella inarata* Hoffmann, 1974, *M. curvatum*, *M. rusticum*, and *M. xenoplax*, confirmed *D. inarata* close related with *Mesocriconema* species and distant relationship to *Discocriconemella* species.

Authors are in agreement with the opinion of several researchers that DNA sequence data from a study involving molecular diagnostics or molecular phylogenetics should be integrated with morphological identification in order to avoid confusion when morphology and biology relationships need to be studied (Luc et al., 2010). Further researches are needed to have a more clear idea about the relationships between taxonomic and molecular identification and the phylogeny of Criconematoidea.

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