

# Taxonomic and Molecular Identification of *Bakernema*, *Criconema*, *Hemicriconemoides*, *Ogma* and *Xenocriconemella* Species (Nematoda: Criconematidae)

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**Abstract:** Populations of *Bakernema inaequale*, *C. petasum*, *C. sphagni*, *C. mutabile*, *Ogma octangulare*, *Xenocriconemella macrodora* and *Hemicriconemoides chitwoodi* were identified and re-described from different geographical areas in the continental United States and molecularly characterized. Two new species of spine nematodes *Criconema arkaense* n. sp. from Washington County and Lee County, Arkansas and *Criconema warrenense* n. sp. from Warren, Bradley County, Arkansas are also described and named. *Criconema arkaense* is characterized by having a conspicuous lip region offset from the body with two annuli, short rounded tail with a thin cuticular sheath and subterminal anus. *Criconema warrenense* n. sp. has two lip region annuli about the same width, first annulus directed posteriorly, separated by a narrow neck annulus and a short conoid tail, unilobed non-folded annulus. The molecular characterization of *Criconema arkaense* and *Criconema warrenense* using ITS1 rDNA gene sequence and the molecular phylogenetic relationships of these new species along with the known spines nematodes are provided.

**Key words:** *Bakernema inaequale*, Criconematidae, *Criconema*, *Criconema arkaense* n.sp., *Criconema mutabile*, *Criconema petasum*, *Criconema sphagni*, *Criconema warrenense* n.sp., *Hemicriconemoides*, *Hemicriconemoides chitwoodi*, internal transcribed spacer 1, morphology, molecular biology, *Ogma*, *Ogma octangulare*, phylogenesis, taxon, *Xenocriconemella*, *Xenocriconemella macrodora*.

The origin of Superfamily Criconematoidea goes back to 1889 with the description of the first specimen of criconematids under the name *Eubostrichus guernei* described by Certes in 1889 from a population of juveniles. Later this species was re-described as *Criconema giardi* (Certes, 1889) Micoletzky 1925, and became the type species of *Criconema* Hofmänner & Menzel, 1914 (Raski et al., 1984; Maggenti et al., 1988).

The subfamily Criconematinae Taylor, 1936 has several spine and sheathoid nematodes morphologically different to *Mesocriconema* and *Criconemoides*. These species are characterized by having a lip region offset from the body with the presence of one or two lip annuli of different widths, presence or absent of submedian lobes, annuli margins smooth, crenate or with ornamentation like scales/spines or having an extra cuticle or a sheath covering the whole body as in *Hemicriconemoides*. Males of this species are degenerate with oesophagus absent or rudimentary, lacking stylet, with three to five lateral lines throughout the body length and round annuli without ornamentation (Raski et al., 1984; Raski and Luc, 1987).

After an comprehensive revision by Raski and Luc (1987), valid genera of ring nematodes in this subfamily are *Criconema* Hofmänner & Menzel, 1914; *Ogma* Southern, 1914; *Criconemella* De Grisse & Loof, 1965; *Discocriconemella* De Grisse & Loof, 1965; *Nothocriconemoides* Maas, Loof & De Grisse, 1971; *Bakernema* Wu, 1964;

*Blandicephalanema* Mehta & Raski, 1971; *Pateracephalanema* Mehta & Raski, 1971 and *Hemicriconemoides* Chitwoodi & Birchfield, 1957.

Regardless of the previous study, Loof (1988), Sidiqi (2000) and Decraemer and Hunt (2006) still consider *Lobocriconema* De Grisse & Loof, 1965, *Neolobocriconema* Mehta & Raski, 1971, and *Pateracephalanema* Mehta & Raski, 1971 as valid genera in Criconematoidea.

The nuclear rDNA internal transcriber regions (ITS) have been used as markers because it has low intraspecific variation for species identification in several nematodes, representing useful information in order to develop tools for diagnostic purposes based on PCR reactions. However, for some species of *Meloidogyne* this intraspecific variation is too high that the use of this marker is not reliable for species discrimination (Gasser, 2001; Powers, 2004; Subbotin and Moens, 2006).

The major objectives of this study were to: i) To integrate the morphological and morphometrics characterization of populations obtained of known *Bakernema*, *Criconema*, *Hemicriconemoides*, *Ogma* and *Xenocriconemella* species in the continental United States and describe two new species namely *C. arkaense* n.sp., and *C. warrenense* n.sp.; ii) To characterize molecularly *C. arkaense* n.sp. and *C. warrenense* n.sp. and other spines nematodes included in this study using ITS1 rDNA gene; and iii) re-construct the phylogenetic position of these species in the Criconematinae using the analysis of this gene. Known species previously identified in early years have been re-described with the intention of enhance the taxonomic background for this study and to facilitate our understanding of their phylogenetic relationships.

## MATERIALS AND METHODS

Nematodes were collected from undisturbed natural locations in Arkansas, USA from 2008 to 2011 and a handheld global positional system device (GPS) (*Étrex*

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Garmin, Olathe, KS) was used to identify the location. Additional populations of nematodes were received from Florida, North Carolina and Tennessee. Nematodes from other 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; Seinhorst, 1962). Measurements of specimens were made with an ocular micrometer and drawings with a camera lucida. Abbreviations used are defined by Siddiqi, 2000. Photographs were taken with Canon EOS Rebel T3i digital camera mounted on a Nikon Optophot-2 compound microscope. In terms of identification of genus and species, the classification proposed by Raski and Luc (1987) was followed. Specimens of all populations were deposited in the USDA Nematode Collection, Beltsville, MD.

Female specimens of each population were grouped and visibly checked for identification to select nematodes for morphological and molecular taxonomy characterization. Adult female nematodes for molecular analysis were crushed individually in 5  $\mu$ l 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  $\mu$ l of the DNA extraction in a 50- $\mu$ l PCR reaction mixture. Primers used to perform PCR reaction were rDNA2 (5'-TTGATTACG TCCCTGCCCTTT-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  $\mu$ l of dNTP-mixture (0.2mM each) (Qiagen, Valencia, CA), 1  $\mu$ l of each primer (0.4  $\mu$ M), 0.4  $\mu$ l (2 units) *Taq* DNA polymerase (New England Biolabs, Ipswich, MA) and 5  $\mu$ l 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 following parameters: denaturation at 94 °C for 2 minutes, then 40 cycles of denaturation at 94 °C for 45 seconds, annealing at 52 or 56 °C for 45 seconds and extension at 72 °C for 60 seconds. A final extension for 5 minutes at 72 °C was performed. Visualization of PCR product was performed using a 5  $\mu$ l 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 transilluminator (BioDoc-it™ system, UVP, Upland, CA) was used to visualize PCR products.

**Sequencing:** PCR products were purified using Nano-sep centrifugal tubes 100k (Pall, Port Washington, NY)

in a refrigerated centrifuge at 15°C for 20 minutes at 13,000 rev. 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 sequence alignment software (Hall, 1999) and alignment of sequences was performed using Geneious alignment with Geneious Pro 5.6.6 (<http://www.geneious.com>).

**Molecular phylogenetic study:** The model of base substitution was evaluated using JModeltest 2.1.1 based on Akaike Information Criterion (AIC) (Dariba et al., 2012; Posada and Crandall, 1998). The distance matrix and the Bayesian analysis were obtained using MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001) with Geneious Pro 5.6.6 (<http://www.geneious.com>). Bayesian analysis was initiated with a random starting tree, running the chain for  $2 \times 10^5$  generations and setting the "burn in" at 20,000. 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. Sequences of *Discocriconemella inarata* HM116055, *Hemicriconemoides californianus* EU180057, *H. kanayaensis* EF126179, *H. parasinensis* EU664601, *H. strichatecus* GQ354786 and *Ogma decalineatum* HM116075 were obtained from GenBank and used for the phylogenetic analysis.

## RESULTS AND DISCUSSION

### SYSTEMATICS

*Criconema arkaense* n.sp.  
(Table 1-2; figure 1-2-5)

#### Description

Female nematodes slightly to significantly ventrally arcuate. Body annuli crenated, somewhat retrorse. Labial plate elevated, six pseudolips indistinct, absence of submedian lobes. Lip region offset, with two lip annuli separated by a narrow constriction. First lip annulus anteriorly directed, narrower than the second lip annulus and the last narrower than the first body annulus. Lip annuli margins crenate. Stylet, robust, with concave knobs or anchor shaped. Typical criconematoid oesophagus. Excretory pore slightly posterior to or at the same level of the oesophagus basal gland, 16-21 annuli from the anterior end. Vulva closed as a simple narrow slit, directed posteriorly, anterior vulval lip non-overlapping. Vagina straight. Female genital tract monodelphic, prodelphic, outstretched, spermatheca empty, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs. Tail slightly conoid to bluntly rounded surrounded by a thin cuticular sheath. Anus subterminal.

TABLE 1. Measurements and ratios of paratypes and holotypes of *Criconeema arhaense* n.sp. and *C. warrenense* n.sp. Mean, standard deviation and range in  $\mu\text{m}$ .

Character/Ratio	<i>C. arhaense</i> Host: hackberry (n=19)	<i>C. arhaense</i> Host: <i>Paspalum</i> sp.(n=20)	<i>C. arhaense</i> Host: oat grass (n=16) Type population	<i>C. arhaense</i> Host: maple (n=20)	<i>Criconeema warrenense</i> (n=17)	<i>Criconeema arhaense</i> Holotype	<i>Criconeema warrenense</i> Holotype
L	529.8 $\pm$ 36.0(459.4-609.4)	458.5 $\pm$ 47.6(381.8-578.8)	507.9 $\pm$ 50.3 (427.3-593.9)	507.7 $\pm$ 48.7(427.3-593.9)	469.9 $\pm$ 54.7(384.8-548.5)	503.03	475.75
Oesophagus length	121.3 $\pm$ 10.6(81.2-132.0)	119.1 $\pm$ 8.6(107.6-140.1)	126.0 $\pm$ 8.1 (111.7-140.1)	125.4 $\pm$ 8.3(111.7-140.1)	112.2 $\pm$ 5.1(103.5-119.8)	115.71	115.71
Tail	7.5 $\pm$ 2.9(3.3-13.0)	17.2 $\pm$ 3.0(10.2-22.3)	8.6 $\pm$ 2.4(4.1-13.8)	8.6 $\pm$ 2.4(4.1-13.8)	27.2 $\pm$ 3.2(22.3-32.5)	7.31	26.39
Maximum Body width	49.9 $\pm$ 5.2(40.6-56.8)	53.2 $\pm$ 2.4(46.7-56.8)	48.6 $\pm$ 3.1 (44.7-57.7)	49.0 $\pm$ 3.4(44.7-57.7)	47.2 $\pm$ 2.5(42.6-50.8)	55.22	46.69
a	10.7 $\pm$ 1.2(9.5-14.3)	8.6 $\pm$ 1.0(7.5-10.6)	10.5 $\pm$ 1.0(8.5-12.2)	10.4 $\pm$ 1.1(8.5-12.2)	10.0 $\pm$ 1.4(7.9-11.7)	9.11	10.19
b	4.4 $\pm$ 0.7(3.8-6.7)	3.9 $\pm$ 0.3(3.6-4.8)	4.0 $\pm$ 0.3(3.5-4.6)	4.1 $\pm$ 0.3(3.5-4.6)	4.2 $\pm$ 0.5(3.5-4.9)	4.35	4.11
c	80.5 $\pm$ 28.7(38.5-145.2)	27.8 $\pm$ 5.3(20.9-35.6)	64.1 $\pm$ 23.5(37.1-135.8)	64.4 $\pm$ 22.8(37.1-135.8)	17.3 $\pm$ 2.4(14.6-23.1)	68.81	18.03
Distance lip region end to vulva	500.5 $\pm$ 34.3(437.0-576.9)	434.9 $\pm$ 53.1(381.7-552.4)	475.2 $\pm$ 47.8 (402.9-559.8)	474.9 $\pm$ 46.3(402.9-559.8)	433.2 $\pm$ 55.1(352.4-511.9)	470.55	443.27
Distance lip region end to anus	522.2 $\pm$ 36.2(454.5-600.4)	443.2 $\pm$ 54.4(387.8-562.5)	499.3 $\pm$ 50.2 (419.2-582.6)	499.1 $\pm$ 48.6(419.2-582.6)	442.1 $\pm$ 55.9(358.5-518.0)	495.72	449.36
V	94.5 $\pm$ 1.2(90.9-96.6)	94.5 $\pm$ 0.7(93.3-95.4)	93.5 $\pm$ 0.6(92.5-94.4)	93.5 $\pm$ 0.6(92.5-94.4)	92.2 $\pm$ 0.8(91.2-93.3)	93.54	93.17
V'	95.8 $\pm$ 1.3(91.8-97.6)	98.1 $\pm$ 0.3(97.7-98.6)	95.2 $\pm$ 0.7(93.6-96.1)	95.2 $\pm$ 0.7(93.6-96.1)	98.0 $\pm$ 0.5(97.5-98.8)	94.92	98.64
Distance lip region to end oesophageal gland	128.0 $\pm$ 11.8(85.3-142.1)	125.3 $\pm$ 8.6(111.7-146.2)	131.7 $\pm$ 8.6(115.7-146.2)	131.0 $\pm$ 8.8(115.7-146.2)	117.2 $\pm$ 5.3(107.6-123.8)	119.77	121.80
Body width at anus	20.1 $\pm$ 4.8(13.8-28.4)	34.5 $\pm$ 2.6(28.4-38.6)	19.5 $\pm$ 4.8(13.0-28.4)	19.4 $\pm$ 4.6(13.0-28.4)	35.4 $\pm$ 2.0(32.5-38.6)	17.86	34.51
b'	4.7 $\pm$ 2.5(3.5-14.6)	3.7 $\pm$ 0.3(3.3-4.5)	3.9 $\pm$ 0.3(3.3-4.5)	3.9 $\pm$ 0.3(3.3-4.5)	4.0 $\pm$ 0.5(3.3-4.8)	4.20	3.91
c'	0.4 $\pm$ 0.2(0.2-0.8)	0.5 $\pm$ 0.1(0.4-0.6)	0.5 $\pm$ 0.1(0.2-0.7)	0.4 $\pm$ 0.1(0.2-0.7)	0.8 $\pm$ 0.1(0.7-0.9)	0.41	0.76
Distance between vulva & post end of body	29.3 $\pm$ 6.8(18.3-50.8)	25.1 $\pm$ 2.9(20.3-30.5)	32.7 $\pm$ 4.0(24.4-39.0)	32.7 $\pm$ 3.9(24.4-39.0)	35.9 $\pm$ 3.8(30.5-40.6)	32.48	32.48
Body width at vulva	38.8 $\pm$ 3.9(30.5-44.7)	41.4 $\pm$ 1.9(36.5-44.7)	37.5 $\pm$ 2.7(32.5-43.9)	37.9 $\pm$ 3.1(32.5-43.9)	38.9 $\pm$ 1.8(36.5-42.6)	43.85	38.57
VL/VB	0.8 $\pm$ 0.1(0.5-1.3)	0.6 $\pm$ 0.0(0.5-0.7)	0.9 $\pm$ 0.1(0.7-1.0)	0.9 $\pm$ 0.1(0.7-1.0)	0.9 $\pm$ 0.1(0.7-1.1)	0.74	0.84
Rex	17 $\pm$ 1.3(13-19)	17 $\pm$ 0.9(15-18)	18 $\pm$ 0.9(16-19)	18 $\pm$ 0.9(16-19)	16 $\pm$ 2.0(12-20)	18	12
Roes	15 $\pm$ 1.0(12-16)	17 $\pm$ 1.4(14-20)	17 $\pm$ 1.0(15-18)	17 $\pm$ 0.9(15-18)	14 $\pm$ 1.7(12-18)	16	12
Rvan	1 $\pm$ 0.5(1-2)	2 $\pm$ 0.4(1-2)	2 $\pm$ 0.0(2-2)	2 $\pm$ 0.0(2-2)	3 $\pm$ 0.0(3-3)	2	3
Ran	1 $\pm$ 0(1-1)	1 $\pm$ 0(1-1)	2 $\pm$ 0.5(1-2)	2 $\pm$ 0.5(1-2)	1 $\pm$ 0.0(1-1)	1	1
RV	4 $\pm$ 0.6(3-5)	4 $\pm$ 0.5(3-4)	5 $\pm$ 0.5(4-5)	5 $\pm$ 0.5(4-5)	5 $\pm$ 0.5(4-5)	4	4
R	54 $\pm$ 4.1(49-67)	53 $\pm$ 3.0(50-62)	54 $\pm$ 3.2(48-58)	54 $\pm$ 3.1(48-58)	48 $\pm$ 1.7(45-51)	54	45
Stylet length	79.3 $\pm$ 6.6(71.1-99.5)	81.0 $\pm$ 5.3(69.0-89.3)	82.3 $\pm$ 3.6(77.0-89.1)	82.7 $\pm$ 3.8(77.0-89.1)	75.3 $\pm$ 5.4(65.0-81.2)	89.10	79.17
Length of stylet shaft	20.0 $\pm$ 2.1(14.2-22.3)	19.9 $\pm$ 1.4(16.2-22.3)	19.9 $\pm$ 1.3(17.9-21.9)	20.0 $\pm$ 1.3(17.9-21.9)	16.9 $\pm$ 3.6(10.2-22.3)	21.11	16.24
m	74.7 $\pm$ 2.8(70.3-81.1)	75.3 $\pm$ 1.3(72.5-76.9)	75.8 $\pm$ 1.0(74.0-77.5)	74.4 $\pm$ 1.3(72.9-76.2)	77.5 $\pm$ 4.1(71.1-84.8)	76.31	79.49
stylet length as percentage of body length	15.0 $\pm$ 1.4(13.2-19.9)	17.9 $\pm$ 1.5(15.1-20.4)	16.3 $\pm$ 1.4(14.8-19.7)	16.4 $\pm$ 1.4(14.8-19.7)	16.1 $\pm$ 1.9(12.6-19.5)	17.71	16.64
Distance between stylet base and D.O.G	3.8 $\pm$ 1.9(2.0-10.2)	2.8 $\pm$ 1.4(2.0-6.1)	2.8 $\pm$ 1.4(0.8-5.7)	2.8 $\pm$ 1.3(0.8-5.7)	2.7 $\pm$ 1.3(2.0-6.1)	3.25	2.03
O	4.9 $\pm$ 2.5(2.0-13.2)	3.3 $\pm$ 1.3(2.3-5.3)	3.4 $\pm$ 1.7(1.0-7.4)	3.4 $\pm$ 1.7(1.0-7.4)	3.7 $\pm$ 1.8(2.5-7.5)	3.65	2.56
Distance lip region-centre median bulb	92.5 $\pm$ 4.9(83.2-103.5)	93.7 $\pm$ 5.9(85.3-105.6)	95.5 $\pm$ 6.6(77.1-105.6)	94.8 $\pm$ 7.0(77.1-105.6)	86.1 $\pm$ 6.0(75.1-95.4)	83.23	91.35
MB	77.0 $\pm$ 9.3(68.3-112.5)	79.4 $\pm$ 1.8(75.4-83.0)	75.9 $\pm$ 3.4(69.1-82.0)	75.6 $\pm$ 3.5(69.1-82.0)	76.8 $\pm$ 7.3(63.8-92.2)	71.93	78.95

1. Knobloch and bird, 1978; 2. De Grisse, 1969.

TABLE 2. Measurements and ratios of males of *Criconema arkaense* from the type population. Mean, standard deviation and range in  $\mu\text{m}$ .

Character/Ratio	Host: grass (n=5)
L	510.3 $\pm$ 38.7(457.6-551.5)
Tail	31.7 $\pm$ 1.4(29.2-32.5)
Maximum Body width	22.9 $\pm$ 0.8(22.3-24.4)
c	16.1 $\pm$ 0.7(15.4-17.0)
Distance from lip region end to anus	478.6 $\pm$ 37.6(428.3-519.0)
Body width at anus	15.4 $\pm$ 0.6(14.6-16.2)
c'	2.1 $\pm$ 0.1(1.9-2.2)
Rex	45 $\pm$ 0.5(45-46)
R	132 $\pm$ 1.9(130-135)
Distance from the cloacal aperture to anterior end of testis	169.9 $\pm$ 16.3(143.5-183.5)
T	33.3 $\pm$ 2.6(30.4-36.9)
Number of annuli from the anterior end of the testis-anterior end to the body	85 $\pm$ 3.4(82-91)
Number of annuli from the anterior end of the testis to posterior end to the body	47 $\pm$ 2.5(44-51)
Distance from the anterior end of the testis to anterior end to the body	201.6 $\pm$ 17.4(172.7-215.2)
Distance from the anterior end of the testis to posterior end to the body	308.7 $\pm$ 30.7(281.8-351.5)
Spicule	45.1 $\pm$ 2.0(43.4-48.2)
Gubernaculum	10.8 $\pm$ 0.7(10.2-12.0)

*Males*: Body slender ventrally arcuated, annuli body visible. Three lateral fields present, without areolation, originate from the 5th anterior annulus. Lip region not offset from the body. Stylet absent, oesophagus region distinct with clear differentiation between oesophagus and intestine. Tail conoid, tip rounded, bursa present. A single testis anteriorly directed, spicule slightly curved.

#### Type host and locality

Specimens were collected August 2008 and August 2009 by M. Cordero at Washington County, AR. (GPS coordinates N 36° 08.075 min-W 094° 21.511 min; N 36° 09.979 min-W 094° 26.061 min; N 36° 06.190 min-W 094° 20.666 min.; N 36° 06.319 min-W 094° 20.565 min.) from the rhizosphere of hackberry (*Celtis occidentalis*), *Paspalum* sp. and maple (*Acer saccharum*), and the type population at Lee county, Marianna, AR. (GPS coordinates N 34° 43.452 min-W 090° 44.214 min.) from the rhizosphere of oatgrass (*Arrhenatherum* sp.) and a unknowtree.

#### Type specimens

*Holotype (female)*: Specimen (slide T-575t) has been deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

*Paratypes (females and males)*: Four female (slide T-575p) and 5 male (slide T-576p) paratypes have been deposited as in the U SDA Nematode Collection, Beltsville, Maryland; four females paratypes deposited in each of the following locations: Department of Nematology, University of California, Riverside; CABI Bioscience,

UK Centre, Surrey, UK; Department of Nematology, Agricultural University, Wageningen, The Netherlands and Nematode collection of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

#### Diagnosis

*Criconema arkaense* is mainly characterized by having two lips annuli crenate without appendages or ornamentation, first lip annulus is anteriorly directed and narrower than the second lip annulus. Both lip annuli are separated by a constriction and the first body annulus wider than the second lip annulus. Body annuli are slightly retrorse with highly crenated margins. Specimens showed a simple vulva slit, posteriorly directed with an anterior vulval lip non-overlapping and a straight vagina. Tail slightly conoid to bluntly rounded with a subterminal anus, surrounded by a thin cuticular sheath on the last annuli and specific ITS1 sequence (JQ708128 to JQ708131) have been submitted to GenBank.

#### Relationships

*Criconema arkaense* is closest related with *Criconema lamellatum* (Raski & Golden, 1966) Raski & Luc, 1985 but is different by having a conspicuous lip region off set *vs.* a lip region not offset, two lip annuli *vs.* one lip annulus, a tail slightly conoid to bluntly rounded with anus subterminal with cuticular sheath *vs.* a conoid tail with last annulus folded by the anterior annulus. Presence of a cuticular sheath on the tail is only shared with *Criconema loofi* (De Grisse, 1967) Raski & Luc, 1985 however; *C. loofi* has a conical pointed tail (De Grisse, 1969; Ebsary, 1981a) *Criconema arkaense* is very similar to *Criconema (Lobocriconema) thornei* Knobloch and bird, 1978. Specimens of *C. arkaense* lack of submedian lobes, strong crenate body annules margins and cuticular sheath in last tail annules while *C. thornei* show big and prominent submedian lobes around the oral opening, smooth to faint ornamentation like lines or dots on body annules margins and lack of cuticular sheath in tail (Knobloch and bird, 1978).

#### Etymology

The species epithet is derived from the state of Arkansas the latin suffix *ense*, meaning belonging to or from.

*Criconema warrenense* n.sp.

(Table 1; figure 4-5)

#### Description

Female nematodes slender, straight or slightly ventrally arcuate. Body annuli not retrorse and slightly crenate. Labial plate elevated, pseudolips indistinct, absence of submedian lobes. Lip region partially offset with two lip annuli of the same size, separated by a narrow constriction. First lip annulus sometimes

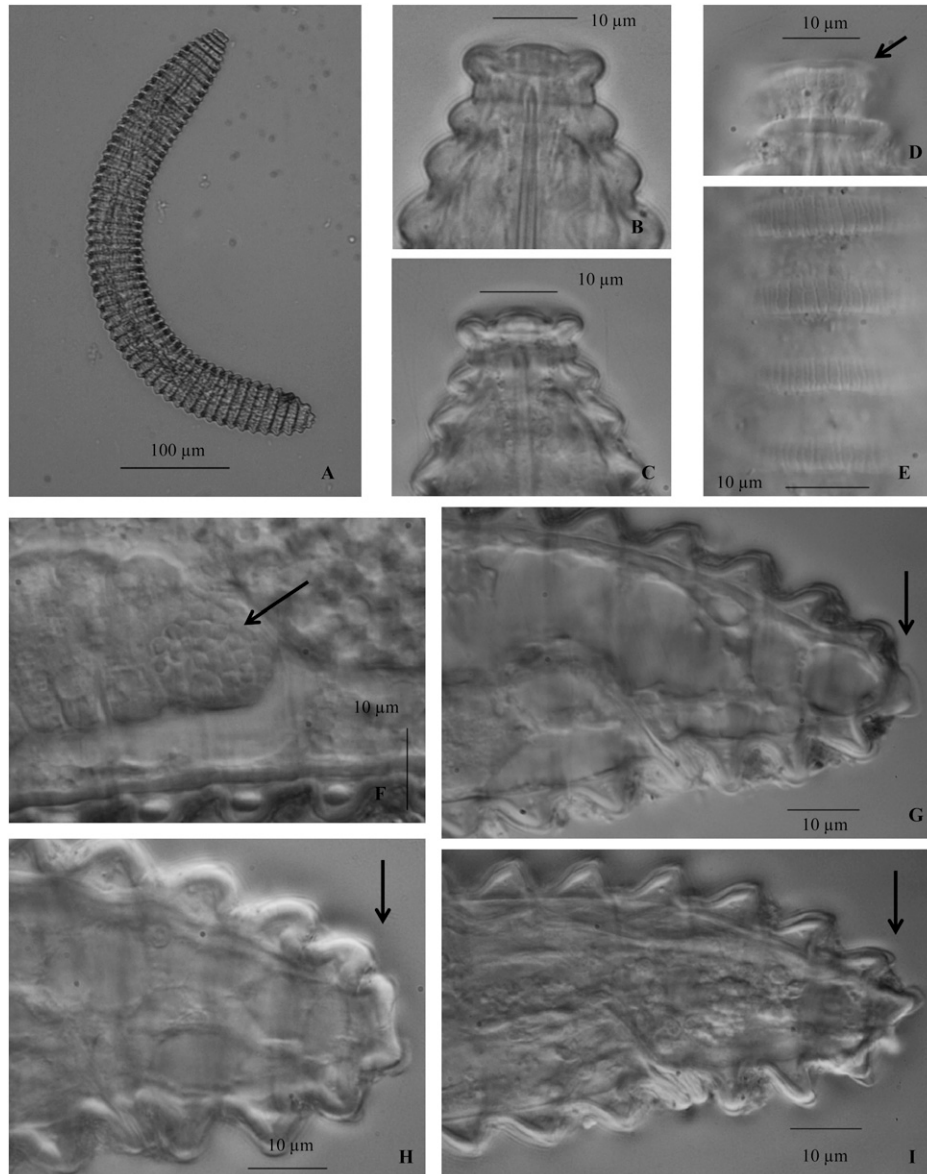


FIG. 1. Light micrographs of *Criconema arkaense* n. sp. A) Entire female. B, C, D) Lip region. Arrow showing crenate margins. E) Body annuli margins. F) Arrow showing spermatheca. G, H, I) Posterior region. Arrows showing cuticular sheath.

slightly posteriorly directed and the second lip annulus anteriorly directed. Stylet slender, robust, with knobs anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 12-20 annuli from the anterior end. Vulva closed as a simple narrow slit, posteriorly directed, anterior vulva lip non-overlapping, located at 2 annuli from posterior end. Vagina straight. Female genital tract monodelphic, prodelphic, outstretched, spermatheca empty, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs. Anus subterminal. Tail rounded conoid without cuticular sheath.

#### *Type host and locality*

Specimens were collected in June 2009 by M. Cordero in Warren, Bradley County, Arkansas (GPS coordinates

N 33° 35.655 min-W 092° 06.941 min) from the rhizosphere of *Paspalum* sp.

#### *Type specimens*

*Holotype (female)*: Specimen (slide T-658t) has been deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

*Paratypes (females)*: five paratypes (slide T-578p) have been deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland; and three paratypes are deposited as follows: CABI Bioscience, UK Centre, Surrey, UK; Department of Nematology, Agricultural University, Wageningen, The Netherlands and Nematode collection of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

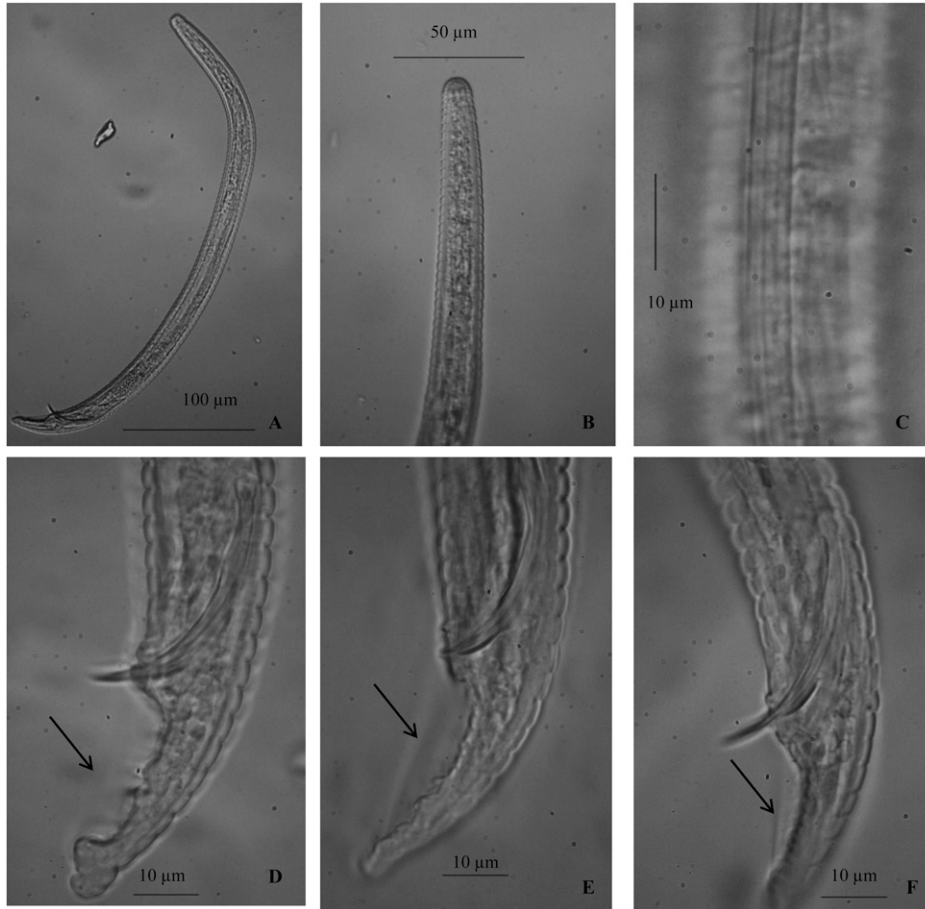


FIG. 2. Light micrographs of males of *Criconema arkaense* n. sp. A) Entire male. B) Anterior region. C) Lateral fields. D,E,F) Posterior region, spicule and arrows showing bursa.

*Diagnosis*

*Criconema warrenense* is characterized by its slender body and an elevated lip region with a visible oral disc. The lip region has two smooth annuli of the same size separated by a narrow constriction. The two lip annuli are slightly directed in opposite direction; however, the second annulus showed a more obvious tendency to be anteriorly directed. Body annuli (R=45-51) are not retrorse, with marked crenations randomly distributed in their surfaces. The tail is conoid-rounded, unilobed without folded annulus or cuticular sheath or subterminal anus and a specific ITS1 sequence (JQ708127) has been submitted to GenBank.

*Relationships*

*Criconema warrenense* is closely related to those species previously classified as *Nothocriconema* and later synonymized as *Criconema* (De Grisse, 1969; Raski and Luc, 1984). *Criconema warrenense* is different from *Criconema braziliensis* (Raski & Pinochet, 1975) Raski & Luc, 1985, by having two lip annuli of the same size *vs.* two different lip annuli, first lip annulus wider than the second lip annulus, body annuli not retrorse *vs.* body annuli retrorse; absence of scales *vs.* two or more row

of bilobulate scales. *Criconema lamellatum* (Raski & Golden, 1966) Ebsary 1981 and *C. warrenense* can be separated by the presence of one lip annulus *vs.* two lip annuli, tail conoid rounded unilobed *vs.* conoid rounded tail with the last annulus folded. *Criconema crassianulatum* (De Guiran, 1963) Raski & Luc, 1985 resembles *C. lamellatum* in the lip region but is different from *C. warrenense* in having an open vulva *vs.* closed vulva. The three species, *C. warrenense*, *C. lamellatum* and *C. crassianulatum* have an elevated lip region, similar stylet length (65-81 µm; 80-84 µm; 68-75 µm) and a subterminal anus. *Criconema sheperdae* Jairajpuri & Southey, 1984 is also related to *C. warrenense* but is different in having one lip annulus *vs.* two lip annuli; a closed vulva with anterior vulval lip with a pair of spines slightly overlapping the posterior lip *vs.* vulva closed as a simple narrow slit not overlapping and presence of protuberances resembling fine crenate margins *vs.* finely crenate body annuli margins. *Criconema annuliferum* (De Man, 1921) De Grisse & Loof, 1965 resembles *C. warrenense* in the lip region. However, *C. annuliferum* has the first lip annulus wider than the second lip annulus *vs.* two lip annuli with the same width; tail conoid with a not folded pointed terminus *vs.* tail conoid with rounded terminus and anus not subterminal *vs.* anus

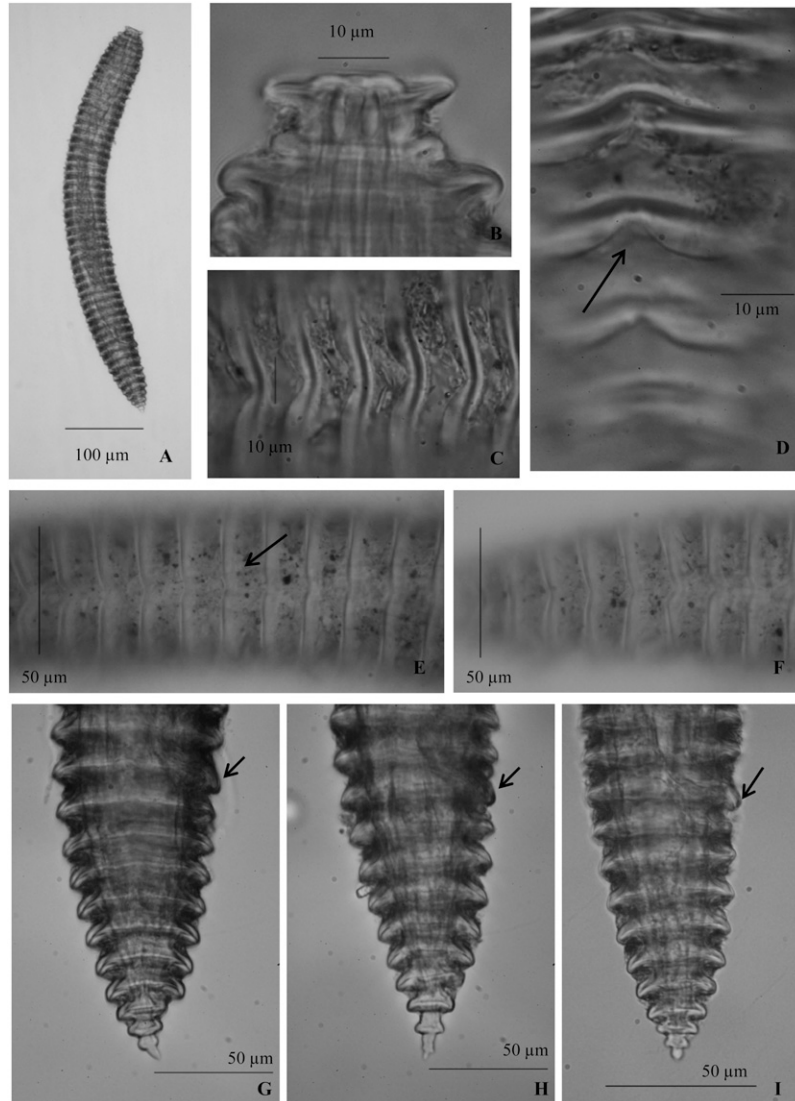


FIG. 3. Light micrographs of *Criconema petasum* A) Entire female. B) Lip region. C, D, E) Body annuli margins. Arrow showing interruptions in wave-like pattern. F) Wave-like pattern in tail. G, H, I,) Tails showing vulva position. Arrows showing vulva.

subterminal (Ebsary, 1981a; Jairajpuri and Southey, 1984; Peneva, et al., 2000; Rashid et al., 1986; Van der Berg, 1992).

#### Etymology

The species epithet is derived from Warren, AR. the location where it was found in Arkansas, USA and the latin suffix *ense*, meaning belonging to or from.

*Criconema petasum* Wu, 1965  
(Table 3; figure 3-5)

#### Description

Female nematodes slightly ventrally arcuate. Annuli body somewhat retrorse, smooth margins. In lateral view, body annuli with wave-like pattern that interrupt the body annuli margins in the middle of the body. Labial plate slightly elevated, six pseudolips present, submedian lobes absent. Lip region offset, with two lip

annuli separated by a wide constriction, first lip annulus wider than the second lip annulus, second annulus narrower than the first body annulus. Lip annuli margins smooth. Stylet, robust, with concave knobs or anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 13-16 annuli from the anterior end. Vulva closed, strongly curved and directed posteriorly as a simple narrow slit, anterior vulval lip overlapping. Vagina curved, not sigmoid. Female genital tract monodelphic, prodelphic, outstretched, spermatheca empty, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to metacarpus. Tail elongated sharply conoid ending in a single pointed lobe.

All the morphometrics values of the specimens are in agreement with the original description and re-description (Ebsary, 1978b; Wu, 1965) and a specific ITS1 sequence (JQ708136) has been submitted to GenBank.

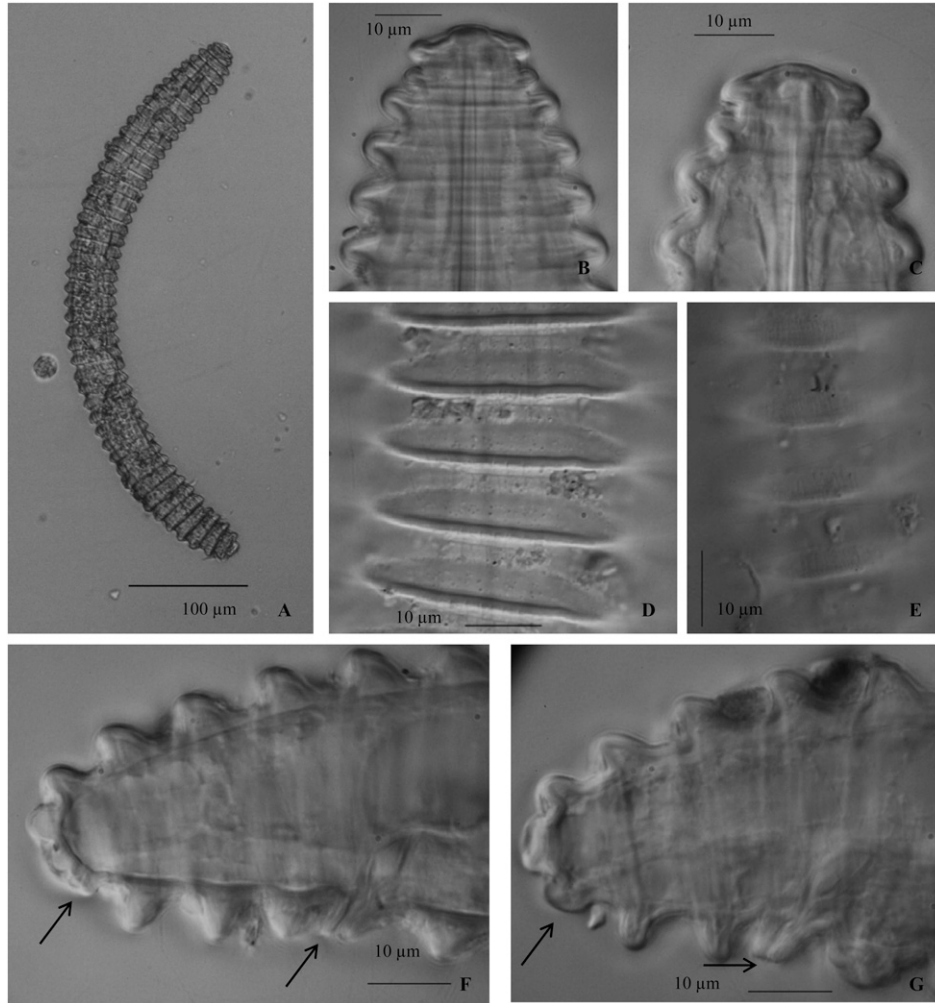


FIG. 4. Light micrographs of *Criconema warrenense* n. sp. A) Entire female. B, C) Lip region. D, E) Body annuli margins. F, G) Posterior region showing vulva and subterminal anus.

*Host and locality*

Specimens were collected in June 2010 by E. Bernard in the Smoky Mountains from the rhizosphere of tulip-poplar (*Liriodendron tulipifera*). No GPS coordinates provided.

*Criconema mutabile* (Taylor, 1936) Raski & Luc, 1985.  
(Table 3; figure 6)

*Description*

Female nematodes straight ventrally arcuate, slightly tapering anteriorly. Body annuli finely crenate and retrorse. Labial plate high, with six prominent pseudolips, submedian lobes absent. Lip region with one lip annulus, offset, separated by a narrow constriction from body annuli. Stylet long and flexible with knobs anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 30-36 annuli from the anterior end. Vulva closed as a simple narrow slit, directed posteriorly and anterior vulval lip not overlapping. Vagina straight. Female genital tract monodelphic, prodelphic, outstretched, spermatheca

empty if observed, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs. Tail slightly conoid and bluntly rounded.

All the morphometric values of the specimens are in agreement with the ranges of the original description (Edward and Misra, 1964; Raski, 1952) and a specific ITS1 sequence (JQ708132) has been submitted to GenBank.

*Host and locality*

Specimens were collected in Illinois River near to Savoy, AR in August 2008 by M. Cordero (GPS coordinates N 36° 08.108 min-W 094° 21.513 min) from the rhizosphere of oatgrass, *Arrhenatherum* sp.

*Criconema sphagni* Micoletzky, 1925  
(Table 3; figure 7)

*Description*

Female nematodes straight or ventrally arcuate, slightly tapering anteriorly. Body annuli finely crenate and retrorse. Labial plate low, truncate with six pseudolips, absence of submedian lobes. Lip region offset



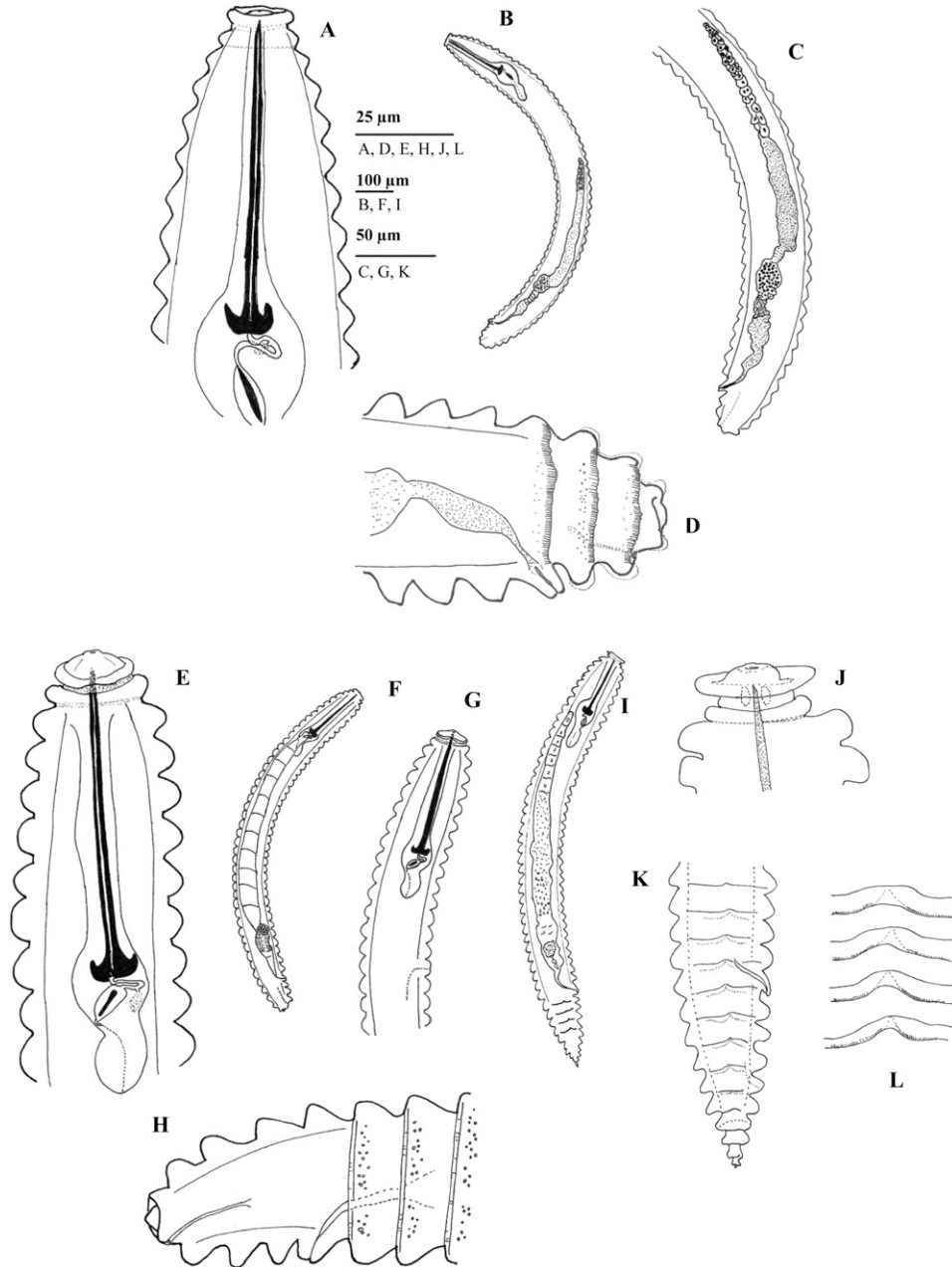


FIG. 5. Camera lucida drawings of *Criconema arkaense* n. sp. A) Lip region. B. Entire female. C. Posterior region. D) Tail. *Criconema warrenense* n. sp. E) Lip region. F) Entire female. G) Anterior region. H) Tail. *Criconema petasum*. I) Entire female. J) Lip region. K) Posterior region. L) Body annuli margins.

with two lip annuli of same size separated by a narrow constriction. Stylet long and flexible with knobs anchor shaped. Typical criconematoid oesophagus. Excretory pore anterior to the oesophagus basal gland, 24-26 annuli from the anterior end. Vulva closed with anterior vulval lip overlapping without spines. Vagina straight. Female genital tract monodelphic, prodelfic, outstretched, spermatheca full of sperm, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs. Tail sharply conoid tapering uniformly to a small pointed terminus, sometimes dorsally arcuated.

All the morphometric values of the specimens are in agreement with the ranges of the original description.

(De Grisse and Loof, 1965; Ebsary, 1978a) and a specific ITS1 sequences (JQ708133 to JQ708135) have been submitted to GenBank.

#### *Host and locality*

Specimens from Arkansas were collected Ozark National Park, Washington County in August 2008 by M. Cordero (GPS coordinates N 36° 08.053 min-W 094° 21.545 min) from the rhizosphere of Oak trees, *Quercus* sp. and oatgrass *Arrhenatherum* sp. The population from Tennessee was collected by E. Bernard from Tulip-Poplar (*Liriodendron tulipifera*) No GPS coordinate provided.

TABLE 3. Measurements and ratios of *Criconeima petasum*, *Criconeima mutabile*, *Criconeima sphagni* and *Bakernema inaequali*. Mean, standard deviation and range in  $\mu\text{m}$ .

Character/Ratio	<i>Criconeima petasum</i> (n=9)		<i>Criconeima mutabile</i> Host: oat grass Arkansas (n=20)		<i>Criconeima sphagni</i> Host: oak Arkansas (n=24)		<i>Criconeima sphagni</i> Host: Tulip-poplar Tennessee (n=16)		<i>Bakernema inaequali</i> Host: Tulip-poplar Tennessee (n=18)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
L	523.5	± 74.4	364.2	± 22.5	390.9	± 34.4	386.9	± 43.4	518.2	± 33.2
Oesophagus length	115.5	± 13.1	91.1	± 4.4	105.6	± 4.8	144.5	± 8.9	116.2	± 6.0
Tail	56.2	± 5.2	18.4	± 3.3	25.1	± 3.9	34.3	± 6.5	27.0	± 3.5
Maximum Body width	61.7	± 4.5	29.5	± 2.1	38.3	± 2.6	42.0	± 6.4	56.3	± 3.4
a	8.4	± 0.9	12.3	± 0.6	10.2	± 0.8	9.3	± 1.4	9.2	± 0.6
b	4.5	± 0.3	4.0	± 0.3	3.7	± 0.3	2.7	± 0.2	4.5	± 0.2
c	9.5	± 2.5	20.2	± 3.2	16.2	± 2.1	11.5	± 1.4	19.4	± 2.0
Distance lip region end to vulva	435.8	± 67.1	340.4	± 16.2	337.2	± 25.9	330.9	± 37.3	482.0	± 31.1
Distance lip region end to anus	467.3	± 78.8	352.1	± 16.2	368.2	± 27.9	352.6	± 39.1	491.2	± 31.2
V	83.2	± 1.3	91.8	± 0.5	85.8	± 0.9	85.5	± 1.1	93.0	± 0.7
V'	93.4	± 1.9	96.7	± 0.5	91.6	± 1.0	93.8	± 0.8	98.1	± 0.6
Distance lip region to end oesophageal gland	123.8	± 14.9	95.7	± 4.2	110.7	± 5.0	149.3	± 9.3	123.0	± 5.3
Body width at anus	46.7	± 2.6	20.0	± 1.9	21.6	± 1.7	24.3	± 2.1	35.2	± 4.4
b'	4.2	± 0.2	3.8	± 0.2	3.5	± 0.3	2.6	± 0.2	4.2	± 0.2
c'	1.2	± 0.1	0.9	± 0.1	1.1	± 0.2	1.4	± 0.2	0.8	± 0.1
Distance between vulva & post end of body	87.7	± 9.2	30.9	± 3.0	55.7	± 6.1	56.0	± 7.5	36.1	± 4.5
Body width at vulva	53.1	± 4.1	25.1	± 1.8	35.1	± 2.0	32.2	± 2.1	42.7	± 2.5
VL/VB	1.7	± 0.2	1.2	± 0.1	1.6	± 0.1	1.7	± 0.2	0.8	± 0.1
Rex	15	± 1.0	33	± 1.5	22	± 1.2	31	± 3.7	19	± 0.9
Roes	13	± 0.7	31	± 2.0	20	± 1.2	34	± 2.6	17	± 1.0
Rvan	3	± 0.0	3	± 0.7	4	± 0.5	4	± 0.7	1	± 0.5
Ran	7	± 0.5	7	± 1.1	8	± 0.8	10	± 1.1	3	± 0.4
RV	11	± 0.6	11	± 1.0	12	± 0.8	14	± 0.8	4.4	± 0.5
R	51	± 1.1	119	± 5.4	67	± 1.6	86	± 2.7	65	± 4.1
Stylet length	76.6	± 3.2	62.9	± 2.1	79.4	± 2.7	114.8	± 7.2	64.0	± 2.4
Length of stylet shaft	24.9	± 11.7	10.0	± 1.2	12.1	± 1.1	14.4	± 3.1	16.3	± 3.0
m	67.4	± 15.9	84.1	± 2.5	84.8	± 1.2	87.4	± 2.7	74.5	± 4.8
stylet length as percentage of body length	14.8	± 1.3	17.0	± 0.8	20.3	± 1.6	29.9	± 2.0	12.4	± 0.7
Distance between stylet base and D.O.G	1.9	± 1.7	2.6	± 0.9	1.4	± 0.7	2.5	± 1.0	3.6	± 0.5
O	2.5	± 2.2	4.5	± 1.6	1.8	± 1.0	2.2	± 0.8	5.6	± 0.7
Distance lip region-centre median bulb	89.3	± 5.5	74.2	± 2.4	88.1	± 3.4	123.5	± 8.3	84.5	± 3.6
MB	78.0	± 8.0	81.3	± 2.7	83.5	± 3.1	85.4	± 1.7	72.8	± 2.8

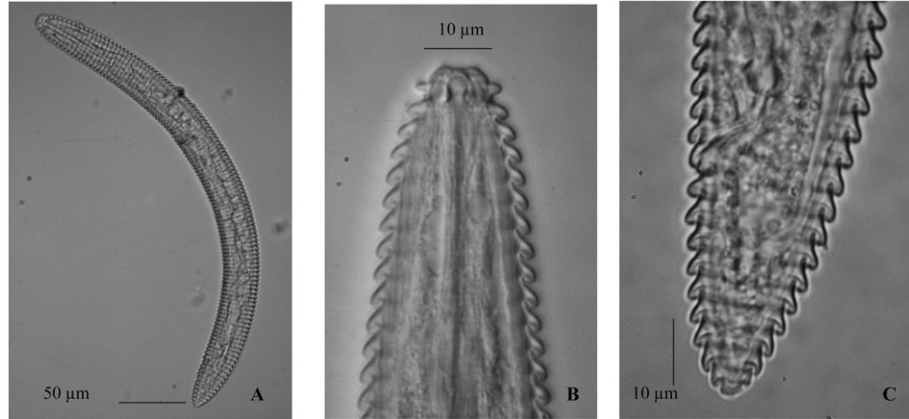


FIG. 6. Light micrographs of *Criconema mutabile*. A) Entire female. B) Lip region. C. Tail.

*Bakernema inaequale* (Taylor, 1936)  
Mehta & Raski, 1971  
(Table 3; figure 8)

*Description*

Female nematodes straight or slightly ventrally arcuate. Annuli rounded not retrorse, with membranous

thick cuticular outgrowths which appear in lateral view as spine-like structures. Each annulus has at least 10-12 cuticular outgrowths in the middle of the body and their numbers decrease for annuli at both ends of the body. Cuticular outgrowths are broad and flag-like structures in the posterior end. Lip region not offset, without constriction, slightly conical, with three non

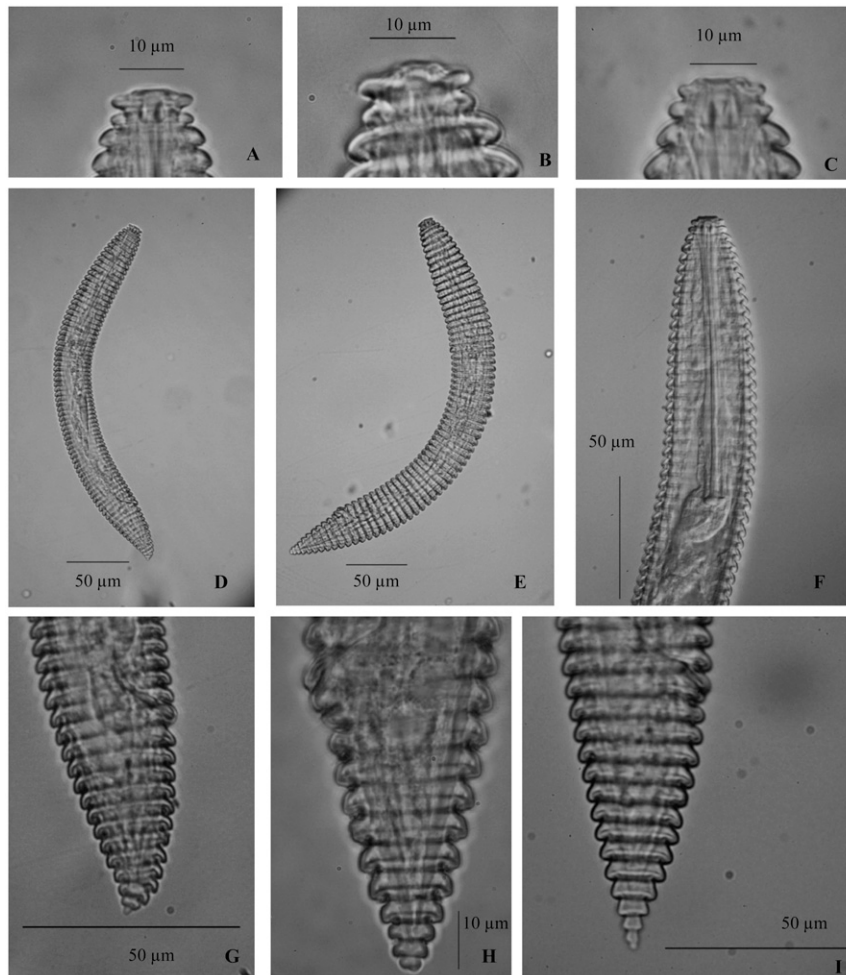


FIG. 7. Light micrographs of *Criconema sphagni*. A, B, C) Lip region. D, E) Entire females. F) Anterior region. G, H, I) Tails.

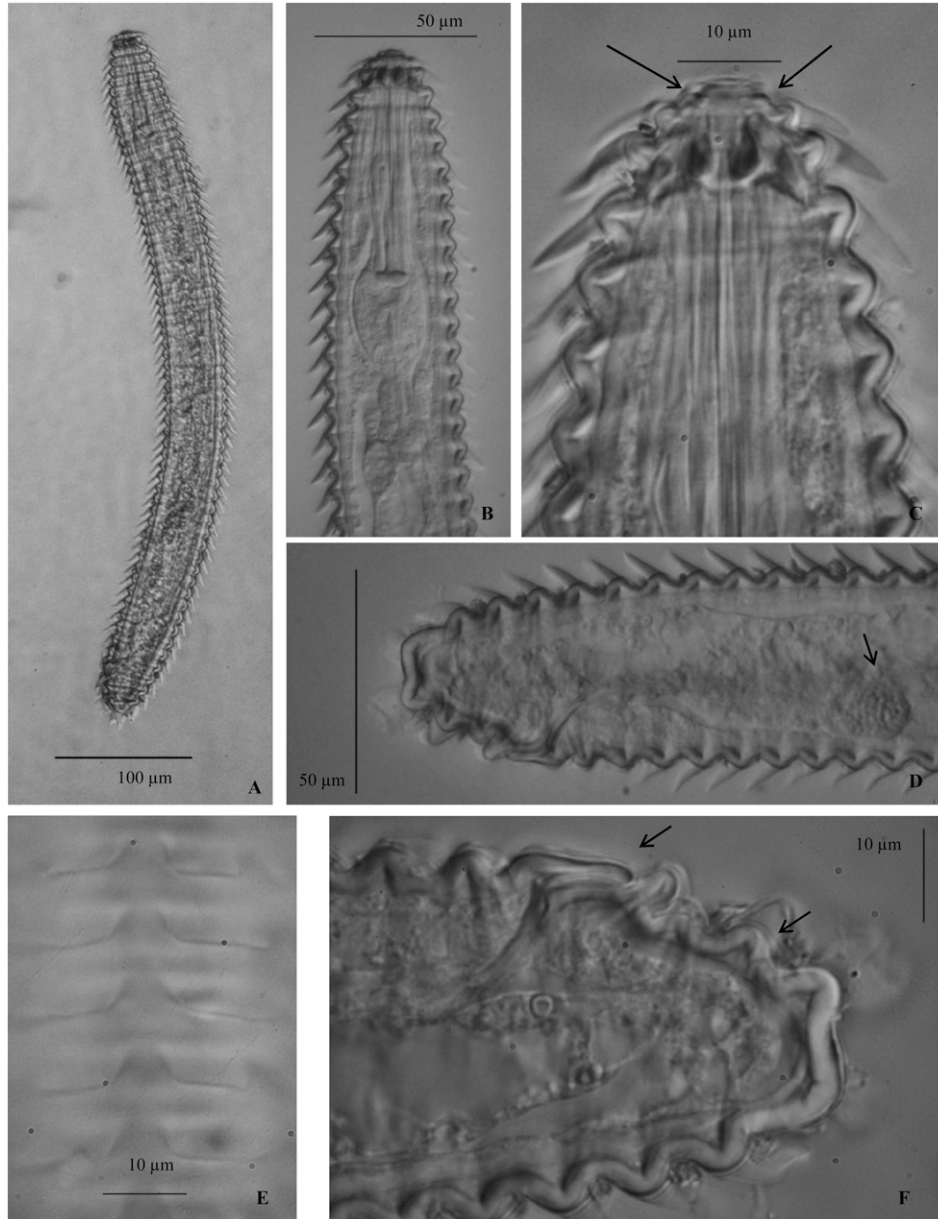


FIG. 8. Light micrographs of *Bakernema inaequali*. A) Entire female. B) Anterior region. C) Lip region. Arrows showing submedian lobes. D) Posterior region. Arrows showing spermatheca. E) Scales. F) Tail. Arrows showing vulva and anus.

retorse lip annuli anteriorly directed. Labial disc visible. Lip region with small, rounded submedian lobes on the labial plate. Stylet strongly developed, robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to oesophagus basal gland, 17-20 annuli from the anterior end. Vulva closed with anterior vulval lip strongly developed and overlapping. Vagina sigmoid. Female genital tract monodelphic, prodelphic, outstretched, spermatheca full of sperm, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to posterior end of oesophagus. Tail rounded and blunt.

All the morphometric values of the specimens are in agreement with the ranges of the original description (Ebsary, 1981b; Wu, 1964a; Wu, 1964b) and a specific

ITS1 sequence (JQ708126) has been submitted to GenBank.

*Host and locality*

Specimens were collected in June 2010 by E. Bernard in the Smoky Mountains from the rhizosphere of Tulip-Poplar (*Liriodendron tulipifera*). No GPS coordinates provided.

*Hemicriconemoides chitwoodi* Esser, 1960  
(Table 4; figure 9)

*Description*

Female nematodes straight or ventrally arcuate. Body annuli covered by a cuticular sheath, sheath annuli

TABLE 4. Measurements and ratios of *Hemicriconemoides chitwoodi*. Mean, standard deviation and range in  $\mu\text{m}$ .

Character/Ratio	Host: Camellia South Carolina (n=20)	Host: Maple Arkansas (n=20)
L	503.9 $\pm$ 40.1(442.4-606.1)	485.8 $\pm$ 46.5(381.8-575.8)
Oesophagus length	122.0 $\pm$ 4.6(113.7-132.0)	122.8 $\pm$ 8.2(97.4-138.0)
Tail	28.9 $\pm$ 3.5(20.3-34.9)	28.4 $\pm$ 2.6(23.6-32.5)
Maximum Body width	31.4 $\pm$ 1.4(29.2-34.9)	28.6 $\pm$ 1.3(26.4-30.5)
a	16.0 $\pm$ 1.1(14.3-18.2)	17.0 $\pm$ 1.5(13.4-20.0)
b	4.1 $\pm$ 0.3(3.8-4.8)	4.0 $\pm$ 0.3(3.3-4.9)
c	17.7 $\pm$ 2.7(14.7-24.3)	17.2 $\pm$ 1.3(14.1-19.2)
Distance lip region end to vulva	459.4 $\pm$ 38.4(400.2-551.7)	441.5 $\pm$ 43.7(346.1-525.0)
Distance lip region end to anus	475.0 $\pm$ 39.5(412.4-571.1)	457.4 $\pm$ 44.9(358.3-545.3)
V	91.1 $\pm$ 0.7(89.7-92.5)	90.9 $\pm$ 0.6(89.7-91.8)
V'	96.7 $\pm$ 0.6(95.6-97.8)	96.5 $\pm$ 0.5(95.2-97.3)
Distance lip region to end oesophageal gland	127.5 $\pm$ 4.5(119.8-136.0)	128.3 $\pm$ 7.8(103.5-142.1)
Body width at anus	21.6 $\pm$ 1.3(19.5-24.4)	19.7 $\pm$ 1.5(16.2-22.3)
b'	4.0 $\pm$ 0.3(3.6-4.7)	3.8 $\pm$ 0.3(3.1-4.7)
c'	1.3 $\pm$ 0.2(0.8-1.7)	1.4 $\pm$ 0.1(1.3-1.7)
Distance between vulva & post end of body	44.5 $\pm$ 3.8(38.2-54.4)	44.2 $\pm$ 4.0(35.7-50.8)
Body width at vulva	26.4 $\pm$ 1.3(23.6-28.4)	25.2 $\pm$ 1.4(22.3-28.4)
VL/VB	1.7 $\pm$ 0.1(1.5-2.0)	1.8 $\pm$ 0.2(1.5-2.1)
Rex	33 $\pm$ 1.6(30-36)	37 $\pm$ 1.8(33-41)
Roes	31 $\pm$ 2.5(27-36)	35 $\pm$ 3.0(27-39)
Rvan	3 $\pm$ 0.7(2-5)	4 $\pm$ 0.6(2-4)
Ran	10 $\pm$ 1.0(8-12)	11 $\pm$ 0.8(9-13)
RV	14 $\pm$ 1.1(12-16)	15 $\pm$ 0.9(13-17)
R	119 $\pm$ 3.8(113-127)	124 $\pm$ 4.7(118-135)
Stylet length	88.2 $\pm$ 3.4(82.6-94.8)	89.9 $\pm$ 3.1(81.8-93.4)
Length of stylet shaft	10.1 $\pm$ 1.4(8.1-14.6)	18.3 $\pm$ 2.6(12.2-22.3)
m	88.6 $\pm$ 1.5(83.6-90.5)	79.6 $\pm$ 2.9(75.0-86.4)
stylet length as percentage of body length	17.6 $\pm$ 1.4(14.3-19.4)	18.6 $\pm$ 1.5(15.9-21.4)
Distance between stylet base and D.O.G	3.5 $\pm$ 0.8(2.4-4.9)	4.3 $\pm$ 2.4(0.8-10.2)
O	4.0 $\pm$ 0.9(2.6-5.6)	4.8 $\pm$ 2.6(0.9-11.4)
Distance lip region-centre median bulb	99.3 $\pm$ 4.1(91.4-107.6)	98.5 $\pm$ 7.2(71.1-105.6)
MB	81.4 $\pm$ 2.0(77.4-84.7)	80.2 $\pm$ 3.5(72.9-86.0)

flattened and smooth. Labial plate rounded, with six pseudolips and absence of submedian lobes. Lip region partly offset with two lip annuli, first lip annulus laterally directed and wider than the second lip annulus. Stylet long and flexible, knobs anchor shaped or anteriorly directed. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 33-41 annuli from the anterior end. Vulva open without vulva sheath, anterior vulval lip not overlapping. Vagina straight, sometimes slightly curved. Female genital tract monodelphic, prodelphic, outstretched, spermatheca full of sperm, reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs with one flexure. Tail sharply conoid tapering to an acute tip.

All the morphometric values of the specimens are in agreement with the ranges of the original description (Esser, 1960) and a specific ITS1 sequences (JQ708140 and JQ911743) have been submitted to GenBank.

#### *Host and locality*

Specimens were collected in June 2008 by P. Agudelo in Clemson, SC from the rhizosphere of camellia (*Camellia* sp.). No GPS coordinates provided.

*Ogma octangulare* (Cobb, 1914) Schuurmans, Stekhoven & Teunissen, 1938  
(Table 5; figure 10)

#### *Description*

Female nematodes straight or slightly ventrally arcuate, tapering slightly anteriorly. Body annuli strongly retrorse. Annuli body in anterior portion showing five to six rows of scales, eight rows in the middle of the body and three rows in the tail. Scales semicircular to triangular wedge-shaped with smooth to irregular margins. Lip region flattened and truncate. Presence small submedian lobes around oral disc, mostly indistinct. Lip region off set, two smooth lip annuli of same size, first lip annulus plate-like directed forward. Second lip annulus wider than the first lip annulus, rounded and not retrorse. Stylet strong with knobs anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 19-25 annuli from the anterior end. Vulva closed with anterior vulval lip overlapping. Vagina straight. Female genital tract monodelphic, prodelphic, outstretched, spermatheca full of sperm, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs with one or two flexures. Tail sharply

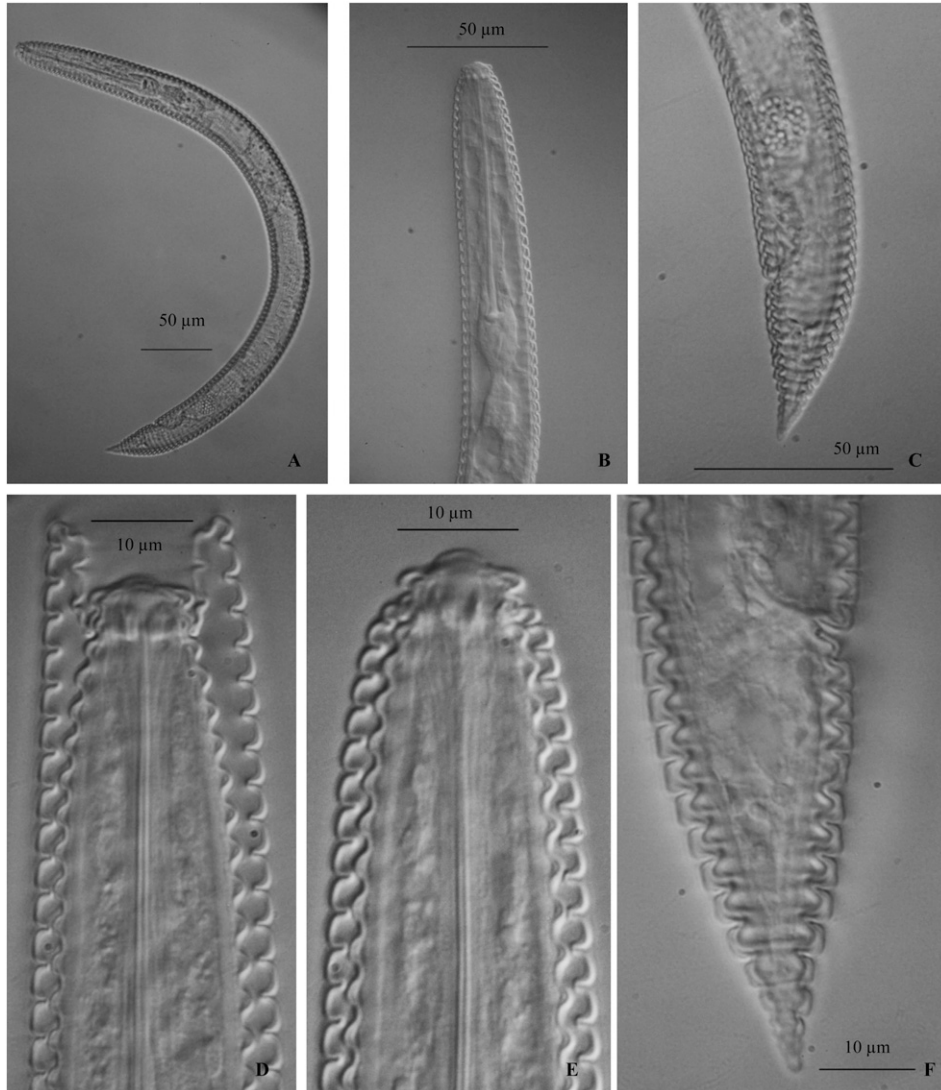


FIG. 9. Light micrographs of *Hemicriconemoides chitwoodi*. A) Entire female. B) Anterior region. C) Posterior region. D, E) Lip region. F) Tail.

conoid tapering uniformly to a small slightly pointed terminus.

All the morphometric values of the specimens are in agreement with the ranges of the original description (Ivanova, 1976; Mehta and Raski, 1971) and a specific ITS1 sequences (JQ708137, JQ708138 and JQ708141) have been submitted to GenBank.

*Host and locality*

Specimens were collected in June 2010 by E. Bernard in the Smoky Mountains from the rhizosphere of tulipoplar (*Liriodendron tulipifera*). No global coordinates provided. Populations from Arkansas were collected by M. Cordero in near to Savoy, AR and Fayetteville, AR (GPS coordinates N 36° 06.190 min-W 094° 20.666 min and N 36° 06.309 min-W 094° 09.961) from rizosphere of bahia grass (*Paspalum notatum*) and Maple (*Acer* sp.), respectively.

*Xenocriconemella macrodora* (Taylor, 1936)  
De Grisse & Loof, 1965  
(Table 5; figure 11)

*Description*

Female nematodes ventrally arcuate, tapering anteriorly. Annuli body smooth and retrorse. Labial plate low, pseudolips not visible, submedian lobes absent. Lip region with two annuli, not offset, not separated from body annuli, first lip annulus partially covering the second lip annulus, second lip annulus retrorse and slightly wider than first annulus. Stylet thin, long and flexible, occupying 1/3 of the body length, knobs slightly rounded, concave and anteriorly directed. Typical criconematoid oesophagus. Excretory pore anterior to the oesophagus basal gland, 34-43 annuli from the anterior end. Vulva closed as a simple slit, directed out of the contour of the body, anterior vulval lip non- overlapping. Vagina straight. Female genital tract

TABLE 5. Measurements and ratios of *Ogma octangulare* and *Xenocriconemella macrodora*. Morphometrics of related species are presented for comparison. Mean, standard deviation and range in  $\mu\text{m}$ .

Character/Ratio	<i>Ogma octangulare</i> Host:		<i>Ogma octangulare</i> Host:		<i>Xenocriconemella macrodora</i> Host:	
	babia grass Arkansas (n=20)	Maple Arkansas (n=19)	tulip-Poplar Tennessee (n=10)	box elder North Carolina (n=7)		
L	376.4 ± 36.6(309.1-430.3)	372.6 ± 25.9(324.2-439.4)	399.7 ± 20.3(378.8-442.4)	268.0 ± 44.2(181.8-312.1)		
Oesophagus length	92.4 ± 5.6(83.2-103.5)	95.2 ± 3.8(89.3-105.6)	92.6 ± 4.1(87.3-99.5)	111.1 ± 8.3(95.4-119.8)		
Tail	27.0 ± 4.3(20.3-37.6)	26.9 ± 3.4(20.3-32.5)	31.6 ± 6.1(18.3-38.6)	11.1 ± 3.0(7.3-14.6)		
Maximum Body width	39.9 ± 2.0(36.5-43.9)	41.0 ± 1.8(35.7-43.9)	40.7 ± 4.2(30.5-44.7)	26.7 ± 2.9(21.9-30.9)		
a	9.4 ± 0.8(8.0-11.2)	9.1 ± 0.5(8.3-10.0)	9.9 ± 1.2(8.8-12.5)	10.0 ± 1.1(8.3-11.7)		
b	4.1 ± 0.3(3.5-4.4)	3.9 ± 0.2(3.5-4.3)	4.3 ± 0.2(4.0-4.7)	2.4 ± 0.3(1.9-3.0)		
c	14.2 ± 1.9(9.7-17.5)	14.0 ± 1.7(11.6-17.1)	13.2 ± 3.2(10.4-21.6)	25.3 ± 7.3(19.7-38.4)		
Distance lip region end to vulva	325.7 ± 34.8(262.8-376.1)	320.7 ± 24.2(273.9-382.6)	344.7 ± 19.1(319.9-383.6)	247.7 ± 40.5(170.4-296.7)		
Distance lip region end to anus	349.4 ± 35.2(288.8-401.3)	345.7 ± 24.6(296.6-408.5)	368.1 ± 18.8(346.3-405.9)	256.8 ± 43.0(172.9-304.0)		
V	86.4 ± 1.1(84.4-88.0)	86.1 ± 1.0(84.1-88.1)	86.2 ± 1.2(84.5-88.7)	92.5 ± 1.7(90.1-95.1)		
V'	93.1 ± 1.3(90.8-96.1)	92.8 ± 0.8(90.8-94.1)	93.6 ± 1.2(92.1-95.4)	96.5 ± 1.3(94.9-98.6)		
Distance lip region to end oesophageal gland	97.8 ± 6.1(87.3-109.6)	99.7 ± 3.4(95.4-109.6)	98.0 ± 4.5(93.4-105.6)	115.4 ± 8.7(99.5-123.8)		
Body width at anus	21.7 ± 1.4(18.7-25.2)	20.8 ± 1.6(17.1-23.6)	22.5 ± 2.1(18.7-26.0)	14.2 ± 2.2(10.6-17.9)		
b'	3.8 ± 0.3(3.3-4.2)	3.7 ± 0.2(3.4-4.1)	4.1 ± 0.2(3.8-4.3)	2.3 ± 0.3(1.8-2.9)		
c'	1.2 ± 0.2(0.9-1.7)	1.3 ± 0.2(1.0-1.6)	1.4 ± 0.2(0.8-1.6)	0.8 ± 0.2(0.5-1.0)		
Distance between vulva & post end of body	50.7 ± 3.2(44.7-58.5)	51.8 ± 3.8(45.5-58.5)	55.0 ± 4.9(44.7-58.9)	20.3 ± 6.3(11.4-28.4)		
Body width at vulva	33.9 ± 1.7(30.9-37.4)	33.9 ± 2.0(28.4-36.5)	32.6 ± 2.5(28.4-34.5)	20.2 ± 2.2(16.2-22.3)		
VL/VB	1.5 ± 0.1(1.3-1.7)	1.5 ± 0.1(1.3-1.8)	1.7 ± 0.2(1.3-1.9)	1.0 ± 0.2(0.7-1.3)		
Rex	20.1 ± 1.1(18.0-22.0)	21.7 ± 1.4(19.0-25.0)	20.4 ± 1.8(17.0-24.0)	38 ± 2.9(34-43)		
Roes	17 ± 1.2(16-20)	19 ± 1.4(16-22)	19 ± 1.4(16.0-21)	46 ± 6.5(42-60)		
Rvan	4 ± 0.6(2-4)	4 ± 0.6(3-5.0)	3 ± 0.8(2-5)	3 ± 0.7(2-4)		
Ran	8 ± 0.7(7-9)	8 ± 0.6(7-9)	9 ± 1.1(7-10)	7 ± 1.4(4-8)		
RV	12 ± 0.7(11-14)	13 ± 0.8(12-15.0)	13 ± 0.6(12-14)	10 ± 1.7(7-12)		
R	67 ± 2.5(62-71)	70 ± 2.5(64-76)	71 ± 1.8(69-74)	101 ± 7.0(89-112)		
Stylet length	63 ± 2.2(59-66)	63 ± 2.0(59-69)	62 ± 1.9(59-65)	90.5 ± 10.1(71.1-99.5)		
Length of stylet shaft	14.4 ± 0.9(13.0-16.2)	14.1 ± 0.9(12.2-15.4)	14.4 ± 1.2(12.2-16.2)	12.0 ± 1.9(8.9-14.2)		
m	77.0 ± 1.1(75.0-79.2)	77.5 ± 1.5(74-80.5)	76.7 ± 1.5(74.4-79.3)	86.5 ± 3.5(80-90.6)		
stylet length as percentage of body length	16.8 ± 1.6(14.3-20.0)	16.8 ± 1.0(15.1-18.5)	15.5 ± 0.6(14.7-16.6)	34.3 ± 4.4(28.0-39.9)		
Distance between stylet base and D.O.G	2.8 ± 0.8(1.6-4.1)	2.9 ± 0.8(0.8-4.1)	2.9 ± 1.9(2.0-8.1)	1.9 ± 1.7(0.8-5.7)		
O	4.5 ± 1.4(2.5-6.9)	4.7 ± 1.3(1.3-6.6)	4.6 ± 2.8(3.2-12.5)	2.1 ± 1.9(0.8-6.2)		
Distance lip region-centre median bulb	74.2 ± 3.6(67.0-79.2)	75.5 ± 6.3(62.9-95.4)	73.1 ± 4.4(65.0-79.2)	96.0 ± 10.4(75.1-107.6)		
MB	80.4 ± 3.3(75.0-86.0)	79.3 ± 5.5(67.4-97.9)	79.0 ± 4.0(72.7-83.7)	86.3 ± 5.3(78.7-94.6)		

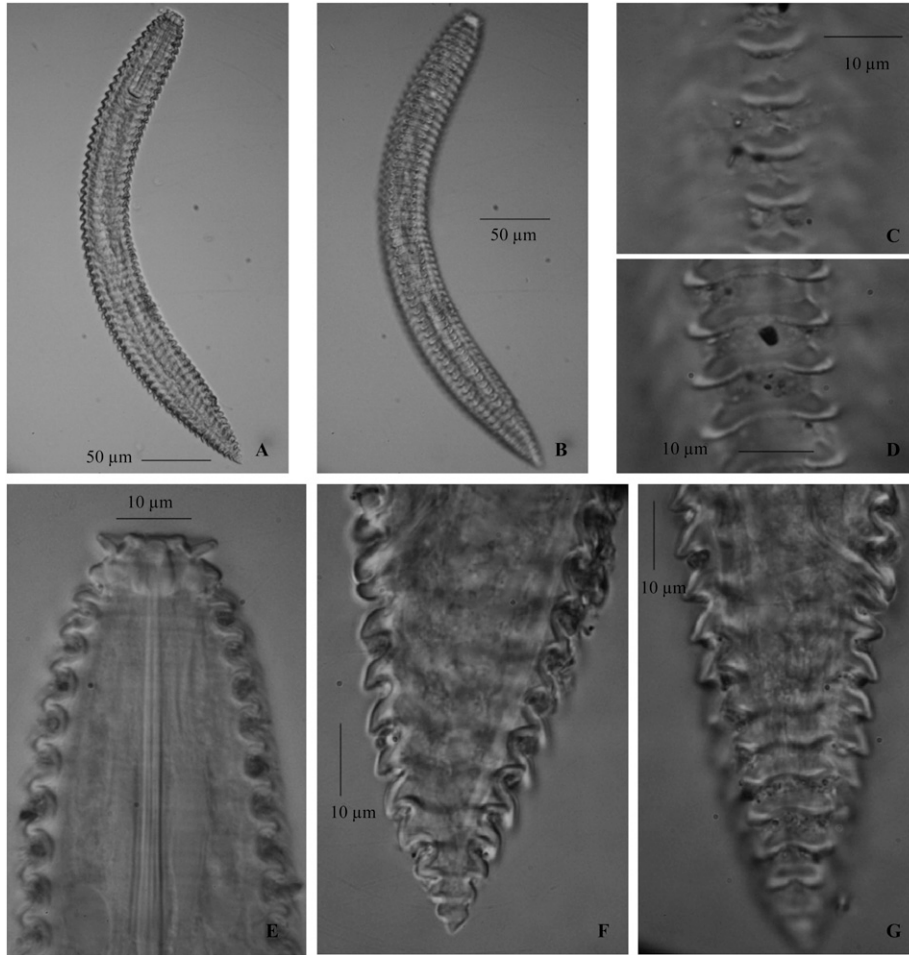


FIG. 10. Light micrographs of *Ogma octangulare*. A, B) Entire female. C, D) Rows of scales in the body. E) Lip region. F, G) Tail.

monodelphic, prodelfhic, outstretched, spermatheca full of sperm, sometimes reaching more than  $\frac{3}{4}$  of the nematode length close to stylet knobs, sometimes with one flexure. Tail conoid and bluntly rounded, tip upwardly directed.

All the morphometric values of the specimens are in agreement with the ranges of the original description (De Grisse & Loof, 1965; Taylor, 1936) and a specific ITS1 sequence (JQ708139) has been submitted to GenBank.

*Host and locality*

Specimens were collected in Guilford, North Carolina by W. Ye from the rhizosphere of Box Elder (*Acer negundo*). No global coordinates provided.

*Molecular phylogenetic analysis*

The length of the PCR product ranged between 560 bp to 680 bp for species of *Bakernema*, *Criconema*, *Hemicriconemoides*, *Ogma* and *Xenocriconemella*. After correction and alignment an internal transcribed spacer 1 length of 299 bp was obtained. JModeltest estimated the TPM3+G model (-Ln likelihood = 2548.7351; AIC= 5191.4702; K=47; R(a)=0.7034;

R(b)=1.4088; R(c)=1.000; R(d)=0.7034; R(e)=1.4088; R(f)=1.000; Gamma shape=0.6040.) as the best fit to present the molecular data. However, because this recent version of JModeltest includes new models, the closest best fit model, K80+G (-Ln likelihood = 2551.2892; AIC= 5194.5784), was selected to analyze the molecular data set (Dariba et al., 2012; Posada, 2008). The Bayesian inferred tree included the entire group of species in a very strong supported cluster (Fig 12). *Ogma decalimeatum*, *O. octangulare* from Tennessee and *Hemicriconemoides kanayaensis* were placed as sister species. The group that includes species of *Criconema sphagni*, *C. mutabile* and *Xenocriconemella macrodora* showed the lowest posterior probabilities values. *Bakernema inaequali* and *Criconema petasum* were clustering together as sister species with *C. arkaense* n.sp. and *C. warrenense* n. sp. with a strong support. In addition, species of *Hemicriconemoides* were clustered with good support with the exception of *H. kanayaensis*.

Molecularly, *B. inaequali* showed a genetic diversity ranged from 22 to 30% with the rest of the group. *Bakernema inaequali* is morphologically, the most dissimilar species of the group by having three lip region annuli, small submedian lobes and 10 to 12 cuticular



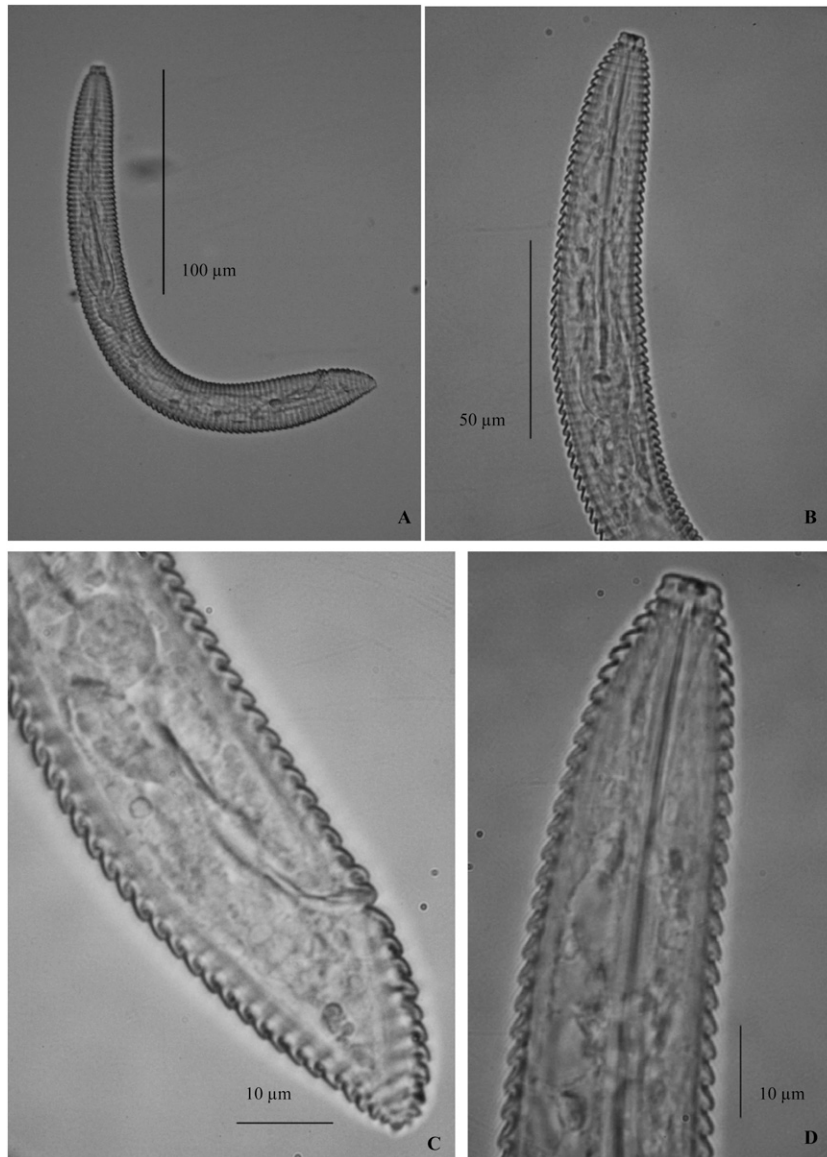


FIG. 11. Light micrographs of *Xenocriconemella macrodora*. A) Entire female. B) Anterior region. C) Posterior region. D) Lip region.

membranous outgrowths by annulus which look alike spines laterally with a strongly develop overlapping anterior vulval lip (Raski and Luc, 1987). *Criconema petasum* keeps most of the characteristics of the group with the exception of the two lip region annuli separated by a wide constriction. Genetic diversity of *C. petasum* with the clade ranged from 28% to 38%. Genetic diversity of *Discocriconemella inaratus* Hoffman, 1974 ranged from 21 to 47% with the group. This species has one lip annulus as a cup shape, anteriorly directed without submedian lobes and anterior vulval lip with two small spicate projections (Hoffmann, 1974b, Powers, 2010).

The new species, *C. arkaense* and *C. warrenense* are close related morphologically and molecularly. Genetic divergence of *C. warrenense* and populations of *C. arkaense* ranged from 10 to 14%. Morphologically, these

two species showed different conformation at lip region. *Criconema arkaense* has two lip region annuli, the first lip annuli is anteriorly directed, separated by a wide constriction from a second lip annulus which is posteriorly directed, body annuli margins are noticeably crenate, and has a cuticular sheath present in the last annuli of the tail. *Criconema warrenense* has a slender body, two lip region annuli separated by a narrow constriction, the first lip annulus is posteriorly directed and the second is anteriorly directed. Body annuli showed a more delicate crenate margins and do not show a cuticular sheath at tail level. Both species showed a vulva close in a single slit directed posteriorly and a sub-terminal anus.

Population of *Hemicriconemoides chitwoodi* from Arkansas was cluster together with *H. californianum* with a genetic divergence 6%. Genetic divergence between

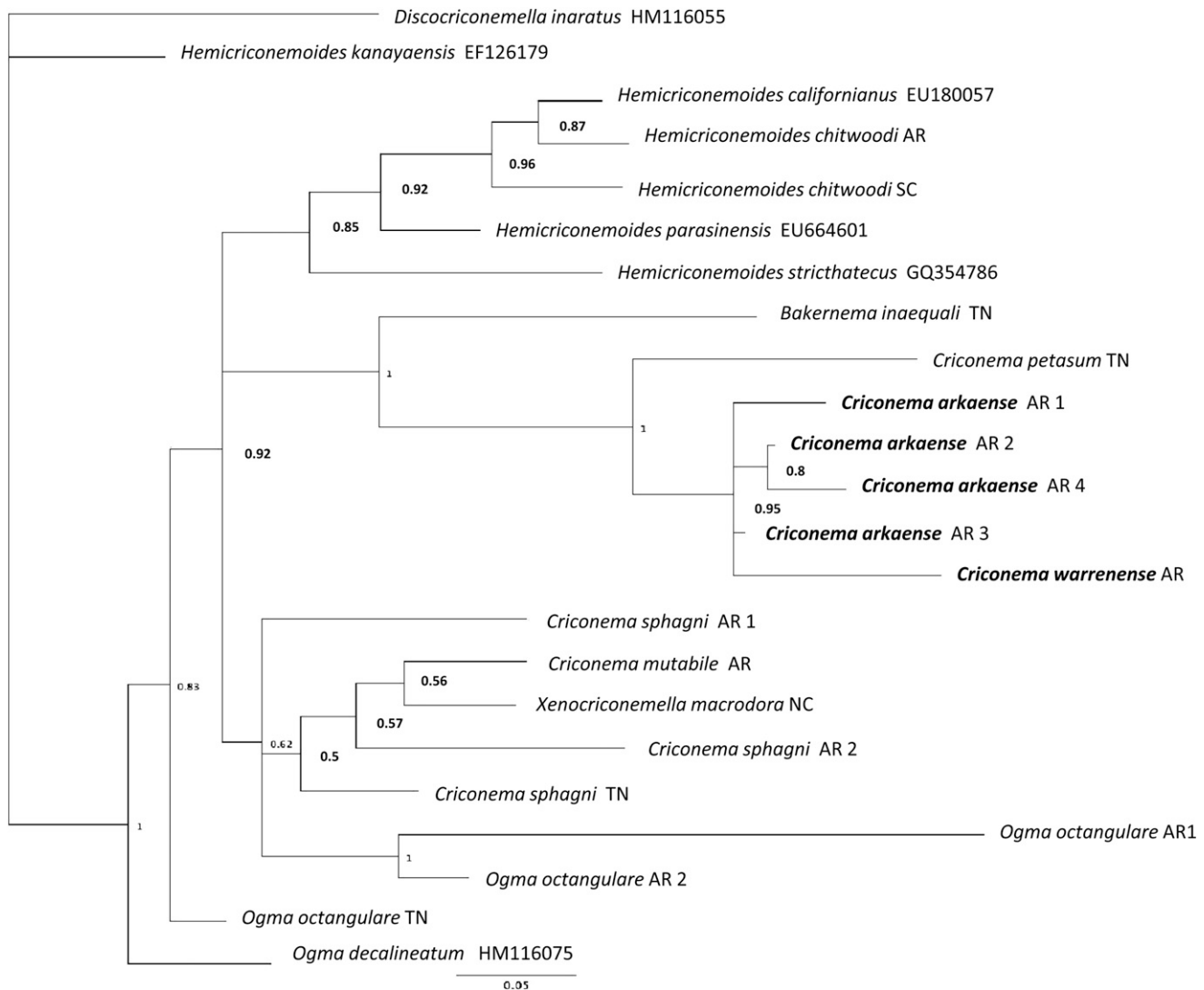


FIG. 12. Bayesian inference 50% majority rule consensus tree of ITS1-rDNA region under K80+G model (-Ln likelihood = 2551.2892; AIC=5194.5784; K=46; Kappa=1.6791 [ti/tv=0.8396]; Gamma shape=0.6080). Numbers at nodes are bootstrap support values. New species are in bold.

populations of *H. chitwoodi* from Arkansas and South Carolina was 14%.

*Criconema mutabile* and *Xenocriconemella macrodora* showed a very close relationship with 8% of genetic divergence. Morphologically, both species has a short and rounded tail with a close vulva in a single slit slightly directed posteriorly, a long and delicate stylet 60-66  $\mu\text{m}$  (Sty%L=15-18), body length 318- 418  $\mu\text{m}$  in *C. mutabile* and stylet length 71-100  $\mu\text{m}$  (Sty%L=28-40) and body length 182-312  $\mu\text{m}$  in *X. macrodora*. The lip region in *C. mutabile* shows a labial plate with six prominent pseudolips, one lip annulus separate by a narrow constriction from body annuli while *X. macrodora* has two annuli which are not separated by a neck annulus and first annulus is partially covering a slightly wider second annulus.

*Ogma octangulare* obtained from Tennessee is closer related molecularly to *O. decalineatum* with a genetic divergence of 5%. However, this population of *O. octangulare*

was clustered as a sister species with the entire group. Both populations of *O. octangulare* from Arkansas clustered together with good support and 21% of genetic divergence. *Ogma decalineatum* has 10 longitudinal rows of scales in the body annuli and both lip annuli are crenated while *O. octangulare* has 8 longitudinal rows of scales in the body annuli and both lip annuli are smooth. (Mehta and Raski, 1971).

Specimens of populations named as *Lobocriconema*, *Neolobocriconema*, and *Crossonema* accepted by Loof (1988), Siddiqi (2000) and Decraemer and Hunt (2006) and *Pateracephalanema* a valid genus for Raski and Luc (1987) were not found in this study therefore, morphological and ITS1 rDNA information of these species is needed to clarify their real position.

Molecular information and correct taxonomical identification are essential to avoid confusion and help to detect and/or differentiate relationships that lead to different lineages or multiple substitutions because

of mutations events evolving at different rates within the group. There are some examples that show the value of the ITS1-rDNA as a tool to differentiate species of plant parasitic nematodes. Ye et al. (2004) using ITS1 sequences reported genetic variation between *Xiphinema chambersi* and *Longidorus crassus* was 39%; *X. diversicaudatum* and *X. bakeri* 4%, *X. chambersi* and *X. italiae* 30%; *L. crassus* and *L. grandis* 9% and *L. fragilis* and *L. diadecturus* 32%. The genetic variation between different species of Punctoderinae and Heteroderinae ranged from 0 to 31% and 0.3 to 15% within each subfamily (Subbottin et al., 2001). The genetic variation of ITS1 sequences between *Paratrichodorus macrostylus* and *Trichorus primitivus* was 65% and 22% between *P. macrostylus* and *P. pachydermus*. (Boutsika et al., 2004).

Tanha Maafi et al. (2003) performed an analysis of ITS1-rDNA to confirm the presence of *Heterodera avenae*, *H. glycines*, *H. hordecalis*, *H. latipons*, *H. schachtii*, *H. trifolii*, *H. elachista*, *H. turcomanica*, *H. mothi* and *Cactodera cacti* in Iran. Likewise, Reid et al. (2003) were able to differentiate populations of *Nacobus 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).

Identification of species of Criconematoidea using morphology had been difficult because the presence of groups that share similar anatomical characteristics. The use of taxonomy and DNA sequence comparison is now the best way to find true taxonomic relationships among nematodes. Recently, Powers (2010) in order to clarify the taxonomic position of *Discocriconemella inarata* analyzed 18S, ITS1-rDNA and cytochrome b markers of the last species along with *D. limitanea*, *Mesocriconema xenoplax* and *M. curvatum*. In this study, the 18S sequences of *D. inarata* showed an exact match with *M. xenoplax*. However, when this sequence was compared with sequences of *Discocriconemella limitanea* a few differences in nucleotides were found. After compared ITS1-rDNA and cytochrome b sequences of *D. inarata* with *Mesocriconema* species, the markers showed a strong and moderate likelihood-ratio support, respectively. This last comparison confirmed that *D. inarata* is different from *Mesocriconema* species but part of the *Mesocriconema* species group and different from *Discocriconemella*.

In this study, the use of ITS1-rDNA as a marker was useful to identify correctly species of Criconematoidea, to confirm relationships among species and to detect possible species lineages. This information will help taxonomists in further investigations to understand associations between taxonomic and molecular data of Criconematoidea and others members of Tylenchida.

Authors are in agreement with the opinion of several researchers (Luc et al., 2010) 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 are studied. Further researches are needed in order to have a more clear idea about the relationships between taxonomic and molecular identification and the phylogeny of Criconematoidea.

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