5-2013

Morphological and Molecular Taxonomic Identification and Phylogenetics of Criconematoidea

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MORPHOLOGICAL AND MOLECULAR TAXONOMIC IDENTIFICATION AND PHYLOGENETICS OF CRICONEMATOIDEA

MORPHOLOGICAL AND MOLECULAR TAXONOMIC IDENTIFICATION AND PHYLOGENETICS OF CRICONEMATOIDEA

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Plant Science

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May 2013
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ABSTRACT

The superfamily Criconematoidea has been studied since 1886. It is composed of two families: Criconematidae (subfam. Criconematinae, Hemicycliophorinae) and Tylenchulidae (subfam. Tylenchulinae, Paratylenchinae and Tylenchocriconematinae). Multiple species in genera have been identified and differences and similarities have been found. Species belonging to genera *Mesocriconema* and *Criconemoides* show very few differences making their identification difficult. Seventy two populations were studied. They were collected in Arkansas and/or received from the following states: California, Florida, Kansas, Missouri, North Carolina and Tennessee. Populations of the following species were identified: *Mesocriconema curvatum, M. kirjanovae, M. onoense, M. ornatum, M. sphaerocephala, M. surinamense, M. vadense, M. xenoplax, Criconemoides informis, Bakernema inaequale, C. petasum, C. sphagni, C. mutabile, Ogma octangulare, Xenocriconemella macrodora, Hemicriconemoides chitwoodi, Hemicycliophora epicharoides, H. gigas, H. labiata, H. typica, H. pruni, H. shepherdi, H. vidua, H. zuckermani, Gracilacus straeleni and Paratylenchus labiosus.* The new species reported are *Mesocriconema ozarkiense* n. sp., *Criconema arkaense* n. sp., *Criconema warrenense* n. sp., *Hemicaloosia uarki* n. sp and *Hemicycliophora wyei* n. sp. In addition, species were characterized morphologically and molecularly using the conserved region 18S for some species and the Internal transcriber spacer 1, ITS1, from ribosomal DNA for all. Phylogenetic studies were performed using both rDNA amplicons to study the relationship among genera and species rejecting the hypothesis of a common ancestor.

This dissertation is approved for recommendation
To the Graduate Council.

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ACKNOWLEDGMENTS

Its have been four years of intense and rewarding work at Cralley – Warren Laboratory at Arkansas Agricultural Research and Extension Center in Fayetteville.

I would like to express my sincere gratitude to my mayor advisor Dr. Robert T. Robbins for his direction, assistance, guidance and friendship. My deepest gratitude to Dr. Allen Szalanski for help me with the molecular techniques and software used in this research. Equally, I want to thank to the rest of my committee members: Dr. Terrence L. Kirkpatrick, Dr. Craig Rothrock and Dr. John Clark for their advice and guidance.

Special thanks to the Department head of the Plant Pathology Department Dr Rick Bennett and the staff at campus: Cindy Morley, Debby Huey and Kim Keeney and my appreciation to Pat Nelson for her friendship and help at the beginning of my studies.

Thank you very much to the personnel at Cralley-Warren Laboratory: Larry Jackson, Devine Crippen, Mary Thomas and Virgil Piazza

The author would like to thanks also to all the graduate students at the Department of Plant Pathology for your friendship and support.

Special thanks to Robin Roggio at Interlibrary loan at Mullins Library for her help finding valuable papers that help me to reach my objectives.

To conclude, I would to thanks my father, my mother, my sister and brother for their invaluable support.

DEDICATORY

To my grandparents Candelaria, Nacha, Juan Antonio, and Enrique.

TABLE OF CONTENTS

INTRODUCTION

The history of the superfamily Criconematoidea began in 1882-1883 at the international expedition to Hoste Island, Chile from which *Criconema giardi* (Certes, 1889) Micoletsky, 1925 was described. Two schemes of classification for Criconematoidea have been proposed: A) the superfamily Criconematoidea was raised one level to the suborder Criconematina by Siddiqi with three families Criconematoidea, Hemiciclyophoroidea and Tylenchuloidea and B) the scheme by Raski and Luc which proposed the superfamily Criconematoidea consisting of two families Criconematidae and Tylenchulidae. The morphological character that clusters the superfamiliy Criconematoidea is the typical criconematoid esophagus. However, the group shows diverse degrees of variation on morpho-anatomical characters among the species which frequently makes their identification complex.

Molecular phylogenetics is an excellent method to determine relationships among taxa based on the information resulting from different molecular markers and morphological identification. The nuclear ribosomal genes, 18S, 5.8S and 28S, which have low variability (i.e. low rate of evolution), are important genetic markers currently used in phylogenetic studies on different organisms in the same taxa that diverged a long time ago. Conversely, the ITS1-rDNA and ITS2 -rDNA regions have a high rate of evolution because of mutations. These markers show greater similarities within species and less among species.

Therefore the combination of morphological and taxonomic identification along with the use of nuclear ribosomal 18S and internal transcribed spacer 1 (ITS1) are promising tools to recognize and understand true relationships between the species belonging to the superfamily Criconematoidea.

The major objectives of this study were: i) to integrate the morphological and morphometrical characterization of populations of known and unknown Criconematoidea species in the United States; ii) to characterize molecularly Criconematoidea species using ITS1 rRNA gene; and iii) reconstruct the phylogenetic position of these species in the Criconematinae using the analysis of this gene.

TAXONOMIC AND MOLECULAR IDENTIFICATION OF *Mesocriconema* and *Criconemoides* SPECIES (NEMATODA: CRICONEMATIDAE)

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The authors thank Keith Striegler, University of Missouri; Weimen Ye, North Carolina Department of Agriculture; Tim Todd, Kansas State University; Ernest Bernard, University of Tennessee and Howard Ferris, University of California-Davis for providing specimens for this study and Mourad Benamara, Institute for Nanoscience and Engineering, University of Arkansas, Fayetteville for his support with the scanning electron microscope.

This paper was edited by

Running head: TAXONOMIC AND MOLECULAR IDENTIFICATION OF SPECIES OF *Mesocriconema* and *Criconemoides* (NEMATODA: CRICONEMATIDAE)

Abstract:

Populations of *Mesocriconema curvatum, M. kirjanovae, M. onoense, M. ornatum, M. sphaerocephala, M. surinamense, M. vadense, M. xenoplax,* and *Criconemoides informis* from different geographical areas in the continental United States were characterized morphologically and molecularly. A new ring nematode from Washington County, Arkansas, is also described and named *Mesocriconema ozarkiense* n. sp., This new species is characterized by females with small flattened submedian lobes, lower than or at the same level as the labial disc, vagina straight, very well developed spermatheca without sperm, no more than one anastomoses, L= 379-512 μm, V=89-93, stylet length = 49-61 μm, R=107-119, annuli with slightly crenate margins on tail portion and a simple anterior vulval lip. The molecular characterization of *M. ozarkiense* n. sp. using the ITS rRNA gene sequence and the phylogenesis relationship of this new species with the ring nematodes included in this study are provided.

Key words: Criconematidae, *Criconemoides*, *Criconemoides informis,* internal transcribed spacer 1, *Mesocriconema, Mesocriconema ozarkiense* n. sp. *M. crenatum, M. curvatum, M. kirjanovae, M. onoense, M. ornatum, M. sphaerocephala, M. surinamense, M. vadense, , M. xenoplax*, molecular biology, morphology, phylogenesis, taxon.

Ring nematodes of the genera *Criconemoides* Taylor 1936 and *Mesocriconema* Andrassy, 1965 are damaging root ectoparasites of many economical important crops. Proper identification of these nematodes is critical for their management and development of germplasm resistant to these pests.

The taxonomic status of the genera *Criconemoides* Tylor 1936 and *Mesocriconema* Andrassy, 1965 is controversial and taxonomists have not reached a consensus of opinion about the validity and species composition of these genera. Many taxonomists including Brezski et al. (2002 a,b) consider these two genera valid, however, others, such as Siddiqi (2000), list *Mesoscriconema* as a junior synonym of *Macroposthonia* de Man, 1880. In a recent classification of plant parasitic nematodes by Decraemer and Hunt (2006) the genus *Mesocriconema* is synonymized with *Criconemoides*. In this paper, we follow the classification proposed by Brzeski et al. (2002 a,b). According to these authors the species of the genus *Criconemoides* are characterized morphologically by annuli more or less retrorse, first and second annuli separated from succeeding annuli, presence of six pseudolips on the first annulus, consisting of two lateral ones reduced to a connection with the four more developed and pronounced submedian lips; a closed vulva with a non-ornamented anterior lip; postvulval body short, conoid with a terminus rounded, conoid or acute. The species of the genus *Mesocriconema* are characterized by a cuticle with retrorse annuli with margin smooth or crenate; first annulus seldom separated; the four submedian lips are reduced and showing each a prominent outgrowth or true submedian lobes; an open vulva with often ornamented anterior lip; postvulval body short with terminus round or truncate.

Morphological studies concerning *Criconemoides* and *Mesocriconema* species are numerous in the literature, but data on the molecular characterization of these ring nematodes is

insufficient and necessary in order to validate their taxonomic status and infer phylognetic relationships among the species of these genera. Molecular information derived from the high variable, D2-D3 expansion segment of the 28s rRNA gene of representatives of Criconematina was recently provided by Subbotin et al. (2005) based on the classification of Siddiqi (2000). The results of their phylogenetic analysis based on D2-D3 domain indicated monophyly among *Mesocriconema, Hemicriconemoides,* and *Criconema* and showed that a representative of the genus *Criconemoides* clustered together with *Mesocriconema* species. The nuclear rDNA internal transcriber regions (ITS) have been used as markers because its low intraspecific variation for species identification in several nematodes, representing useful information in order to develop tools for diagnostic purposes based on PCR reactions (Gasser, 2001). In a recent study by Powers et al. 2010, sequences of the nuclear ribosomal ITS1 were obtained for *M. curvatum* (Raski, 1952) Loof & De Grisse, 1989, *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989 and *M. xenoplax* (Raski, 1952) Loof & de Grisse, 1989.

The major objectives of this study were: i) to integrate the morphological and morphometrical characterization of populations of known *Mesocriconema* and *Criconemoides* species in the continental United States and describe a new species namely, *Mesocriconema ozarkiense* n. sp.: ii) to characterize molecularly *M. ozarkiense* and other ring nematodes included in this study using ITS1 rRNA gene; and iii) reconstruct the phylogenetic position of these species in the Criconematinae using the analysis of this gene. This is the first part of four intended to clarify and identify species of the superfamily Criconematoidea following the classification of Brzeski et al. (2002 a,b) and Raski and Luc (1987). The second part will provide the taxonomical and molecular identification of *Bakernema, Criconema, Hemicriconemoides,Ogma, Xenocriconemella* (subfamily Criconematinae), the third part

Caloosia and *Hemicycliophora* (subfamily Hemicycliophorinae), *Gracilacus* and *Paratylenchus* (Family Tylenchulidae) and a final study about the phylogenesis relationships of Criconematoidea species.

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) (*E*trex Garmin, Olathe, KS) was used to identify the location. Additional populations of nematodes were obtained from California, Kansas, Missouri, North Carolina and Tennessee. Nematodes from others States were received fixed in 3% formaldehyde for morphological purposes or they were preserved in a 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, and subsequently infiltrated with glycerin using Seinhorst's modified slow method (Seinhorst, 1959; Seinhorst, 1962) and mounted on slides for observation and preservation. 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. Nematodes were fixed and gold coated before examination using a FEI Nano lab 200 Workstation scanning electron microscope at the Institute for Nanoscience and Engineering at University of Arkansas in Fayetteville, Arkansas.

Specimens of all populations of this study are deposited in the USDA Nematode Collection, Beltsville, MD. Morphometrics of related species to those identified in this work are included using data reported by Brzeski et al., 2002a,b.

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 analyses were crushed individually in 5µl of molecular grade (BDH Chemicals, Chester, PA) water and storage at -80^{o}C until use.

PCR: Polymerase chain reaction (PCR) of the ITS1region 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'-TTGATTACGTCCCTGCCCTTT- 3') (Vrain et al., 1992) and rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA- 3') (Cherry et al., 1997). This PCR primer pair ampliflied 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 (0.2mM 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 ^{o}C for 2 minutes, then 40 cycles of denaturation at 94 ^{o}C for 45 seconds, annealing at 52 or 56 ^{o}C for 45 seconds and extension at 72 ^{o}C for 60 seconds. A final extension for 5 minutes at 72 ^{o}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^{o}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.

Alignment of sequences was performance with CLUSTAL W (Thompson et al., 1994) and consensus sequences were obtained using BioEdit (Hall 1999) sequence alignment software.

Molecular phylogenetic study. The distance matrix option of PAUP* 4.010 (Swofford, 2002) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura, 1980) of sequence evolution. Maximum likelihood and unweighted maximum parsimony analysis on the alignments were performed using PAUP* 4.010 (Swofford, 2002). Gaps were treated as missing characters for all analyses and the reliability of the trees was tested by a bootstrap test (Felsenstein, 1985). Parsimony bootstrap analysis included 1,000 resamplings using the branch and bound algorithm of PAUP*. The maximum likelihood parameter (Yang, 1994), the default likelihood parameter settings of PAUP* were used (HKY85 6-parameter model of nucleotide substitution, empirical base frequencies, and transition/transversion ratio set to 2:1). These parameters were employed to perform a heuristic search using PAUP*, using either the single most parsimonius tree as the starting tree or step-wise addition. Sequences of *Mesocriconema xenoplax* HM116073 and HM116057; *M. curvatum* HM 116066 and *Heterorhabditis indica* JQ178381 were obtained from GenBank and used for the phylogenetic analysis.

Results and Discussion

SYSTEMATICS

Mesocriconema ozarkiense n. sp.

(Table 1; Figs. 1-2-3)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth to irregular margins, crenate at the tail level. Not more than one anastomoses observed. Lip region not offset, tapering, slightly conical. First annulus with no constriction, retrorse. Labial plate minute and visible. Lip region with small submedian lobes, flattened and visible at same level or lower than labial plate. Stylet slender, robust, with knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore slightly anterior to or at the same level as the oesophagus basal gland, 27-34 annuli from the anterior end. Vulva open with anterior vulva lip simple. Vagina slightly curved or straight. Female genital tract monodelphic, prodelphic, outstretched, empty spermatheca, sometimes reaching more than ¾ of the nematode length (stylet knobs level). Tail uniformly conical decreasing to a pointed terminus of a single truncated annulus in most cases or small rounded end annulus, slightly dorsally arcuate.

Host and locality

Specimens were collected in August 2009 by M. Cordero in the Ozark National Forest at Illinois river in Washington County, Arkansas (GPS coordinates N 36^{o} 09.979 min-W 094^{o} 26.061 min) from the rhizosphere several *Paspalum* spp. (grasses).

Type specimens

Holotype (*female*): Specimen (T-656t) are deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

*Paratype*s (*females*): Two paratypes are deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland; and four paratypes are deposited as follows 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

Mesocriconema ozarkiense n. sp is characterized by small, flattened submedian lobes, smooth to irregular annuli body margins, except for those of the tail which are slightly irregular to slightly crenate which were visible with the compound microscope but indistinct with the scanning electron microscope. The vulva is open with a simple anterior vulval lip, straight vagina, tail conical with last annulus truncated or with a very small rounded dorsally arcuate tip and a specific ITS1 sequence (JQ708122) has been submitted to GenBank.

Relationships

Mesocriconema ozarkiense is related to several species which have a conical tail shape, stylet length about 40- 65 µm, number of body annuli around 70 to 120, and the absence or presence of anastomoses. There are differences in annuli margin appearance, shape of submedian lobes, shape of the vagina, and type of anterior vulval lip. The closest related species to *Mesocriconema ozarkiense* n.sp. is *M. kirjanovae* (Andrássy, 1962) Loof & De Grisse, 1989 which are similar in labial region with small labial plates, similar body length (378-512 *vs*. 350-790 µm), bigger value of c (13-23 *vs*. 12-13) similar stylet length (49-61 *vs*. 51-54 µm) similar V value (89-93 % *vs*. 88-90 %) but has a different tail shape, conical tail vs. conical-acute tail, respectively. Main features to differentiate *M. ozarkiense* from *M. kirjanovae* are small flattened submedian lobules reaching the border of the labial disc or lower (flattened) vs. rounded and elevated submedian lobes, a higher number of annuli (107-119 µm *vs*. 79-89), an anterior vulval

lip simple, lacking lobes vs. rounded or thorn like-projections (Andrássy, 1962). Annuli margins in *M. ozarkiense* are smooth or irregular vs. smooth to finely crenate in *M. kirjanovae*. Annuli from vulva to posterior end are crenate and the last annulus in the tail is truncated or with a delicate rounded annulus instead of an acute end.

Mesocriconema ozarkiense differs from others related species because: *M. citricola* (Siddiqui, 1965) Loof & De Grisse, 1989 has lower R= (107-119 *vs*. 73-78), no or at most one anastomose vs. few anastomoses, annuli margins smooth to irregular vs. crenate, vulval lip simple vs. vulval lip with lobes, both species have flat submedian lobes and a straight vagina; *M. denoudeni* (De Grisse, 1967) Loof & De Grisse, 1989 has no or at most one anastomose vs. 0-4 anastomoses, smooth to irregular annuli margins vs. smooth annuli margins, submedian lobes flattened vs. submedian lobes rounded, anterior vulval lip simple *vs*. anterior vulval lip with lobes, both species have a straight vagina; *M. jessiense* (Van der Berg, 1992) Van der Berg, 1994, has a smaller R (107-119 *vs*. 88-102), has no or at most one anastomose *vs*. few anastomoses, similar annuli margins which appear smooth to irregular, submedian lobes flattened *vs*. rounded, anterior vulval lip simple *vs*. anterior vulval lip flap, both species have a straight vagina; *M. ornicauda* (Vovlas, Inserra, & Esser, 1991) has no or at most one anastomose vs. few anastomoses, annuli margins smooth to irregular *vs*. annuli margins smooth, submedian lobes flattened vs. submedian lobes rounded, vagina straight *vs*. vagina sigmoidal and anterior vulval lip simple *vs*. anterior vulval lip with lobes; *M. paradenoudeni* (Rashid, Geraert, & Sharma, 1987) Loof & De Grisse, 1989 has higher R (107-119 *vs*. 102-130), lower RV (10-14 *vs*. 8-10), lower Ran (6-10 *vs*. 4-7), no or at most one anastomose *vs*. 0-5 anastomoses, annuli margins smooth to irregular *vs*. annuli margins smooth, submedian lobes flattened *vs*. submedian lobes rounded, both species have a straight vagina and a simple anterior vulval lip; *M. parareedi*

(Ebsary, 1981) Loof & De Grisse, 1989, has no or at most one anastomose *vs*. no to few anastomoses, annuli margins smooth to irregular *vs*. annuli margins smooth, submedian lobes flattened *vs*. submedian lobes rounded, straight vagina *vs*. sigmoid vagina and anterior vulval lip simple *vs*. anterior vulval lip with lobes; *M. reedi* (Diab & Jenkins, 1966) Loof & De Grisse, 1989 has no or at most one anastomose *vs*. 0-5 anastomoses, annuli margins smooth to irregular *vs*. annuli margins smooth, submedian lobes flattened *vs*. submedian lobes rounded, straight vagina *vs*. sigmoid vagina, both species have a simple anterior vulval lip; *M. sigillarium* (Eroshenko & Volkova, 1997) has a shorter stylet length (49-61 vs. 46-51 µm), no or at most one anastomose vs. many anastomoses, annuli margins smooth to irregular *vs*. annuli margins crenate, submedian lobes flattened *vs*. submedian lobes rounded, both species have a straight vagina and a simple anterior vulval lip (Brzeski *et al*, 2002a; Diab and Jenkins, 1966).

Etymology

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

Mesocriconema crenatum (Loof, 1964b) Andrássy, 1962.

(Table 2; Fig. 4)

Description

Female nematodes ventrally arcuate. Annuli retrorse, crenate margins. Anastomoses not observed. Lip region not off set, submedian lobes small, rounded, visible. Labial plate minute. Stylet robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory

pore slightly posterior to oesophagus basal gland, 28-33 annuli from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca not observed. Vulva open, simple without lobes. Tail conical, tip rounded.

Host and locality

Specimens were collected in August 2008 by K. Striegler from the rhizosphere of grape vines (*Vitis vinifera*) var. Chambourcin in Hermam, MO. No GPS coordinates provided.

Diagnosis

Mesocriconema crenatum has crenate body annuli, simple vulva without lobes, or spine like projections, or ornamentation. This population is in agreement with the original description (Loof, 1964b) and a specific ITS1 sequence (JQ708125) has been submitted to GenBank.

Relationships

This population of *Mesocriconema crenatum* is compared to populations of *M. crenatum* reported in Belgium (De Grisse, 1969) but has a longer stylet (71-83 µm *vs*. 38-51 µm), more body annuli (101-114 *vs*. 73-84), and a smaller c value (17-24. *vs*. 22-56). Populations from Romania were similar in c value (17-24 *vs*. 24-28), have a smaller stylet (71-83 µm *vs*. 38- 40 µm) and a smaller number of body annuli (101-114 *vs*. 80-81) (Popovici and Ciobanu, 2000). *Mesocriconema crenatum* is similar to *M. ornatum* but differs in having crenate annuli margins. However, differences in morphometrics of populations of *M. crenatum* described in Belgium and Romania suggested another species different from *M. crenatum.*

Mesocriconema curvatum (Raski, 1952) Loof & De Grisse, 1989.

(Table 2; Fig. 5)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth margins. Anastomoses occasionally observed throughout the body. Lip region not offset, submedian lobes obvious, rounded. Labial plates minute or obvious. Stylet robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore slightly posterior to oesophagus basal gland, 23-26 annuli from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca rarely observed, if so empty of sperm. Vulva open, anterior lip with two round lobes variable in size. Tail conical, tip rounded.

Host and locality

Nematodes were collected in August 2008 by M. Cordero in the Ozark National Forest at Illinois river in Washington County, AR (GPS coordinates N 36^{o} 09.979 min-W 094^{o} 26.061 min and N 36^{o} 05.900 min-W 094^{o} 10.686 min.) from the rhizosphere of river cane (*Arundinaria* sp.), oak (*Quercus robur*) and turfgrass.

Diagnosis

Mesocriconema curvatum is characterized by body annuli with smooth margins, presence of anastomoses (1 to 3), rounded submedian lobes and anterior vulval lip with two rounded lobes. All the morphometric values of the specimens are in agreement with the ranges of the

original description (Raski, 1952, Loof & De Grisse, 1989) and a specific ITS1 sequence (JQ708123) has been submitted to GenBank.

Relationships

Mesocriconema curvatum does not have either a high elevated or an emarginated first lip annule which is a main difference from *M. xenoplax* (Raski, 1952) Loof, 1989 and *M. ornatum* (Raski, 1958) Loof & De Grisse, 1989. It also has a straight vagina and smooth annuli margins. However, these three species have a labial disc that is somewhat elevated and obvious, with lateral submedian lobes. *Mesocriconema curvatum* and *M. ornatum* share a straight vagina and smooth annuli margins while *M. xenoplax* has a sigmoid vagina and smooth to irregular annuli margins (Raski, 1952; Brzeski et al., 2002a).

Mesocriconema kirjanovae (Andrássy, 1962) Loof & De Grisse, 1989.

(Table 3; Fig. 6)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth to slightly crenate margins. Anastomoses either absent or only one present. Lip region not off set, slightly conical. Submedian lobes small, rounded and visible. Labial plate minute. Stylet robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore always posterior to the oesophagus basal gland, 26-31annuli from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca full of sperm. Anterior vulval lip with two rounded projections of moderate size. Tail conical uniformly decreasing, tip acute.

Host and locality

Specimens were collected in May 2008 by R. T. Robbins and M. Cordero in the border of a swamp area near Pine Tree, AR (GPS coordinates N 35° 07.161 min-W 090° 66.581 min.) from the rhizosphere of young pine trees, hickory (*Carya* sp.) and grass (unidentified spp.). This is the first report of *M. kirjanovae* in the United States.

Diagnosis

Mesocriconema kirjanovae exhibited two projections from the anterior lip of the vulva, although they were sometimes difficult to observe. Crenate and smooth rings were observed in the margins of the annuli. This feature is highly variable among populations of this species (Brzeski, 1998; Castillo and Vovlas, 1992). Numbers of annuli from anterior end to the excretory pore, length of the sylet, ratios a, b and V are similar to the original population and those examined as *M. annulatiformis* (Andrássy, 1962). *M. annulatiformis* was later synonymized as the current species, even though the population from Arkansas was longer in body length and R (Andrássy, 1962; Brzeski, 1998; De Grisse and Loof, 1967). All morphometrics values of the specimens are in agreement with the original description (Andrássy, 1962) with the exceptions mentioned above and a specific ITS1 sequence (JQ708100) has been submitted to GenBank.

Relationships

This population of *Mesocriconema kirjanovae* has a slightly greater number of annuli in the body than the original description (98-115 *vs*. 71-105), similar stylet length (48-61 *vs*. 51-54 µm), Rex (26-31 *vs*. 26-27), RV (8-11 *vs*. 10-12), RVan (2 *vs*. 2-3), a (9-14 *vs*. 9-10), b (4-6 *vs*.

4-4-), m (69-78 *vs*. 74-75) and bigger value of c (12-26 *vs*. 12-13) compared with the original description. *Mesocriconema kirjanovae* has a conical-acute tail as *M. bareilli* (Misra & Edward, 1972); *M. bilaspurense* (Gupta & Gupta, 1981) Loof & De Grisse, 1989; *M. calvatum* (Eroshenko, 1981) Loof & De Grisse, 1989, *M. reedi* (Diab & Jenkins, 1966) Loof & De Grisse, 1989 and *M. ripariensis* (Eroshenko & Volkova, 1997) (Brzeski et al, 2002a). This species has lobes in its anterior vulval lip as *M. calvatum* and it is the only species of the above mentioned that has smooth to crenate annuli margins throughout the body. The remaining species vary from smooth margins in, *M. bareilli, M. bilaspurense* and *M. reedi* to crenate margins in *M. calvatum* and *M. ripariensis.*

Mesocriconema ornatum (Raski, 1958) Loof & De Grisse, 1989.

(Table 4; Fig. 7)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth margins. Anastomoses present but no more than two randomly distributed in the body. Lip region not well off set, large submedian lobes, rounded and visible. Labial plate minute, slightly developed and, anteriorly projected. Stylet robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 25-28 annuli from the anterior end. Female genital tract monodelphic, prodelphic, outstretched, empty spermatheca. Vulva open. Anterior vulval lip with two spicate projections of moderate size. Tail conical, tip rounded and somewhat truncated with last annulus folded.

Host and locality

Specimens were collected in 2009 by T. Todd, Kansas State University, from the rhizosphere of turfgrass. No Global positioned coordinates provided.

Diagnosis

This population of *M. ornatum* presented body annuli with smooth margins, one or two anastomoses along the body, lip region not so offset, submedian lobes prominent and rounded, labial plate slightly projected anteriorly and anterior vulval lip with two spicate projections. All the morphometric values of the specimens are in agreement with the ranges of the original description (Raski, 1952; Raski, 1958) and a specific ITS1 sequence (JQ708124) has been submitted to GenBank.

Relationships

The population of *Mesocriconema ornatum* reported here has a similar morphometrics compared with the original description as stylet length (52-59 *vs*. 43-46 µm), R (96-106 *vs*. 94-100), Rex (25-28 *vs*. 25-27), RV (9-12 *vs*. 7-9). *Mesocriconema ornatum* is very similar to *M. crenatum* (Loof, 1964) Andrássy, 1965 although margins of the annuli in *M. ornatum* are not crenate. Anastomoses, if present, no more than one in the entire body vs. *M. ornatum* does not show anastomoses at the posterior end of the body (Brzeski et al., 2002a). Previous descriptions of *M. ornatum* are similar to those reported from Argentina (Chaves, 1983) China (Ye, et al., 1997) Spain (Escuer and Bello, 1996) USA (Jaffe et al., 1987) and Venezuela (Loof 1964b; Crozzoli and Lamberti, 2001).

Mesocriconema onoense (Luc, 1959) Loof & De Grisse, 1989.

(Table 4; Fig. 8)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth margins. Anastomoses occasionally observed in the body. Lip region not offset and tapering slightly anteriorly. Submedian lobes rounded, surrounded tightly by the first lip annulus, sometimes difficult to observe. Labial plate minute. Stylet robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore slightly posterior to the oesophagus basal gland, 32-39 annuli from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca present and full of sperm. Vulva open, simple with lobes. Tail rounded, tip rounded. Last annulus folded.

Host and locality

Specimens were collected in July 2008 from grass and maple (*Acer saccharum*) near Savoy, Washington County AR. by M. Cordero. GPS coordinates N 36^{o} 06.246 min-W 094^{o} 20.278 min.

Diagnosis

Mesocriconema onoense belongs to a group of species within the genus with a high number of annuli in the body, R= 106-143 similar to *M. multiannulatum* (Doucet, 1982) Loof & De Grisse, 1989, R= (143-150); *M. oblongatum* R= 134-148; *M. onostre* (Phukan & Sanwal, 1981) Loof & De Grisse, 1989 R= (133-147); and *M. paranostre* (Deswal & Bajaj, 1987) Loof

& De Grisse, 1989 R= 117-150. M*esocriconema onoense* has a very low lip region with small submedian lobes almost covered by the first lip annulus but visible. Spermatheca full of sperm in most specimens and a last tail annulus surrounded by the previous one. All the morphometric values of the specimens are in agreement with the ranges of the original description and redescription. (De Grisse and Loof, 1965; Luc, 1959; Loof and De Grisse, 1989) and a specific ITS1 sequence (JQ708120) has been submitted to GenBank.

Relationships

Mesocriconema onoense is similar to *M. vadense* (Loof, 1964) Loof & De Grisse, 1989 in the anterior portion, but submedian lobes in *M. onoense* are rounded while *M. vadense* has flattened submedian lobes. Last annulus is folded in *M. onoense*, a feature which is shared with *M. ornatum* (Raski, 1958) Loof & De Grisse, 1989; *M. antipolitanum* (De Guiran, 1963) Loof & De Grisse, 1989; and *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989.

Mesocriconema onoense is closely related also to *M. onostre* but can be differentiated by having small submedian lobes *vs*. large and obvious submedian lobes, RV = 9-11 *vs*. 7-9 for *M. onostre*, long conical rounded tail *vs*. a conical tail, anterior vulval lip simple with lobes vs. simple anterior vulval lip. In the description as *M. onostris* by (Phukan and Sanwal) 1980 it was mentioned that *M. onoense* has a broken first annulus that was considered to be a feature to differentiate between both species. However, after review of the original description of *M. onoense* (Luc, 1959) both species share an unbroken first lip annulus (Brzeski *et al*, 2002a).

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Mesocriconema vadense (Loof, 1964b) Loof & De Grisse, 1989.

(Table 5; Fig. 9)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth margins. Anastomoses frequently observed throughout the body in groups of 3 or separately along with some interruptions in some annuli. Tapering slightly anteriorly, lip region not offset. Submedian lobes small, rounded and oriented in the same direction as the labial plate. Labial plate obvious. Stylet robust, knobs concave or anchor shape. Typical criconematoid oesophagus. Excretory pore frequently far posterior to the oesophagus basal gland, 30-45 annuli from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca empty if observed. Vulva open with two small lobes and/or rounded spines in the anterior annulus. Tail conical, tip rounded without unfolded annuli.

Host and locality

Arkansas populations were collected in August 2008 by M. Cordero and R. T. Robbins in pine in Pine Tree, AR Saint Francis County, and Fayetteville, Washington County, AR. from grass at coordinates N 35^{o} 07.004 min-W 090^{o} 58.370 min and N 36^{o} 05.918 min-W 094^{o} 10.708 min., respectively.

Diagnostic

Mesocriconema vadense is characterized by having body annuli with smooth margins, frequent anastomoses throughout the body which tapers anteriorly with lip region not offset and anterior vulva lip with small lobes or rounded spines. All the morphometrics values of the specimens are in agreement with the original description and redescription (Loof, 1964b) Loof &

De Grisse, 1989. However, a population of the species in Belgium (De Grisse, 1969) sometimes showed lobes at the anterior annuli of the vulva whereas others did not. The features at the cephalic portion, labial plate and the shape and orientation of the submedian lobes are typical for the species and specific ITS1 sequences (JQ708102 and JQ708121) have been submitted to GenBank

Relationships

Mesocriconema vadense and *M. curvatum* are similar and difficult to separate morphologically. Shape and length of the tail and shape and orientation of the submedian lobes are the main features used to separate them. Tail shape in *M. curvatum* is rounded *vs.* a conical tail in *M. vadense*. Anastomoses are common in *M. vadense*, with 3 or 4 in the body *vs*. one in *M. curvatum*. Submedian lobes of *M. vadense* are small and rounded, similar to those observed on *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989. The cephalic portion of *Mesocriconema curvatum* appears flattened whereas the cephalic portion in *M. vadense* is not flattened (Ivanova, 1976; Brzeski et al, 2002a).

Mesocriconema sphaerocephala (Taylor, 1936) Loof, 1989.

(Table 6; Fig. 10)

Description

Female nematodes small, ventrally arcuate. Annuli retrorse, margins finely crenate. Numerous anastomoses present throughout the body forming a zig-zag pattern. Lip region not off set, slightly tapering anteriorly. Submedian lobes small, rounded, barely visible. Labial plate not

visible. Stylet robust, knobs concave or anchor shape. Typical criconematoid oesophagus. Excretory pore in most occasions anterior to the posterior end of the oesophagus basal gland, 15-25 annule from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca empty. Vulva open and simple. Tail conical, tip rounded without unfolded annuli.

Host and locality

Specimens were collected in July 2008 from three different locations from the rhizosphere of turfgrass and daylily (*Hemerocallis* sp.) in Johnston, Sampsom, and Beaufort Counties in North Carolina by W. Ye. No GPS coordinates were provided.

Diagnostics

Mesocriconema sphaerocephala is characterized by small body size (294-406 µm) with body annuli margins finely crenate, tapering anteriorly, minute submedian lobes and numerosus anastomoses in the body. All the morphometric values of the specimens are in agreement with the ranges of the original description and redescription (De Grisse, 1967; Loof, 1989; Raski and Golden, 1965) and a specific ITS1 sequence (JQ708103) has been submitted to GenBank

Relationships

Mesocriconema sphaerocephala is characterized by the presence of high numbers of anastomoses throughout the body, annuli crenate and a conical-rounded tail. Presence of such numbers of anastomoses is present in *M. brevistylus* (Singh & Khera,1976) Loof & De Grisse, 1989; *M. caelatum* (Raski & Golden, 1966) Loof & De Grisse, 1989; *M. paronostre* (Deswal & Bajal, 1987) Loof & De Grisse, 1989; *M. pseudosolivagum* (De Grisse, 1964b) Andrássy, 1965;

M. raskiensis (De Grisse, 1964) Andrássy, 1965; *M. sigillarium* (Eroshenko &Volkova, 1997), *M. sphaerocephala* (Taylor, 1967) Loof, 1989 and *M. thabaum* Van den Berg, 1996. However, the closest species related with M. *sphaerocephala* is *M. sphaerocephaloides* (De Grisse, 1967) Loof &De Grisse, 1989, showing variations in small submedian lobes vs. large and obvious submedian lobes, conical-rounded tail *vs*. rounded blunt tail, Sty%L (13-18 *vs*. 22), Rex (15-25 *vs*. 27), RV (4-7 *vs*.7), R (61-71 *vs*. 82) and annuli smooth to crenate vs. smooth to irregular (Brzeski et al., 2002a; De Grisse, 1967).

Mesocriconema surinamense (De Grisse & Maas, 1970) Loof & De Grisse, 1989.

(Table 7; Fig. 11)

Description

Female nematodes ventrally arcuate. Annuli retrorse, smooth margins. Anastomoses rare, no more than one in the body and sometimes present in the tail region. Lip region not offset, tapering and flattened anteriorly. Submedian lobes large and flattened. Labial plate visible. Stylet robust, knobs concave or anchor shape. Typical criconematoid oesophagus. Excretory pore at the posterior end of the oesophagus basal gland, 24-29 annuli from anterior end. Female genital tract monodelphic prodelphic, outstretched, spermatheca empty of sperm. Vulva open and simple with two small lobes sometimes difficult to observe in lateral view. Tail conical, tip rounded without unfolded annuli, unilobed.

Host and locality

Specimens were collected in August 2008 by M. Cordero in the Ozark National Forest and Savoy, Washington County, AR. from the rhizosphere of grass and maple (*Acer saccharum*)

at GPS coordinates N 36^{o} 09.969 min-W 094^{o} 26.061 min and N 36^{o} 06.246 min-W 094^{o} 20.278 min., respectively.

Diagnostics

Mesocriconema surinamense is characterized by having a large, obvious and flattened submedian lobes, anastomoses rare or no more than one, annuli margins smooth, anterior vulva lip with two small lobes and last annulus unfolded. All the morphometric values of the specimens are in agreement with the ranges of the original description and redescription (De Grisse and Maas, 1970; Loof, and DeGrisse, 1967; Loof and DeGrisse, 1989) and a specific ITS1 sequence (JQ708101) has been submitted to GenBank

Relationships

Mesocriconema surinamense belongs to a group of *Mesocriconema* that have flattened submedian lobes of different size: *M. antipolitanum* (De Guiran, 1963) Loof & De Grisse, 1989; *M. caballeroi* (Cid del Prado, 1978) Luc & Raski, 1981 synonym of *M. surinamenese*; *M. vadense* (Loof, 1964b) Loof & De Grisse, 1989; *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989 and *M. yossifovich* (Krnjaic, 1968) Luc & Raski, 1981). *Mesocriconema surinamense* is very similar to M. *yossifovich,* but the submedian lobes are not fused as in *M. yossifovich* where they form a plate with four lobes that surround the oral opening (Vovlas, 1984). In lateral view of *M. surinamense* a separation is observed between the two submedian lobes and the labial disc whereas *M. yossifovich* in lateral view shows a flat anterior end (Brzeski et al, 2002a; Cid del Prado, 1979; De Grisse & Maas, 1970).

Mesocriconema xenoplax (Raski, 1952) Loof, 1989.

(Table 8; Fig. 12)

Description

Female nematodes slightly ventral arcuate. Annuli retrorse, smooth to irregular margins. Labial disc elevated surrounding the oral opening. Anastomoses rare, no more than one in the body. Lip region not off set, large, rounded, conspicuous submedian lobes, equidistant of labial disc, anteriorly projected. First annulus indented. Stylet robust, knobs concave or anchor shape. Typical criconematoid oesophagus. Excretory pore at the posterior end of the oesophagus, 22-28 annuli from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca empty of sperm. Vulva open, two sharp projections in anterior anule. Vagina sigmoid. Tail conical, tip rounded and unilobed

Host and locality

Specimens were collected in June – August 2008 by M. Cordero at various locations in Washington county, AR including: Farmington (GPS coordinates N 36^{o} 01.530 min-W 094^{o} 19.274 min); Fayetteville (N 36^{o} 10.223 min-W 094^{o} 16.444 min and N 36^{o} 05.918 min-W 094^{o} 10.708 min.), near Savoy N (36^{o} 06.246 min-W 094^{o} 20.278 min), from the rhizosphere of oak (*Quercus robur*), pine (*Pinus* sp.), elm (*Ulmus* sp.) river cane (*Arundinaria* sp.), and grass. Nematodes from North Carolina were associated with the rhizosphere of bermuda grass (*Cynodon dactylon*), peach (*Prunus persica*) and turfgrass. Populations from California were sent by Dr. Howard Ferris - University of California at Davis and were collected in the rhizosphere of grapes vines (*Vitis vinifera*) at Ripon, Parlier, Los Alamos, Russian River,

Mendocino, Fresno and Livingston. No global positioned coordinates were provided for California and North Carolina populations.

Diagnosis

Mesocriconema xenoplax is the type species of the genus characterized by body annuli margins smooth to irregular, submedian lobes large and anteriorly projected, first cephalic annulus elevated and indented and vulva sigmoid. All the morphometric values of the specimens are in agreement with the ranges of the original description (Raski, 1952) and redescription (Loof and DeGrisse, 1989) and specific ITS1 sequences (JQ708104 to JQ708117 and JQ708119) have been submitted to GenBank.

Relationships

Mesocriconema xenoplax is different from other species in its elevated labial disc and first cephalic annulus are indented or projected anteriorly. *Mesocriconema xenoplax* is closer to *M. rusticum* and *M. ornatum*. *Mesocriconema xenoplax* has a stylet longer (65 -80 µm *vs*. 50-60 µm) than for *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989 and *M. ornatum* (Raski, 1958) Loof & De Grisse, 1989 (65-80 µm *vs*. 44-56 µm). Submedian lobes in *M. xenoplax* and *M. ornatum* are rounded while *M. rusticum* has flattened submedian lobes and a tapering anterior end. The tail in *M. xenoplax* is rounded and conical while *M. rusticum* has a rounded tail terminus. *M. ornatum* has a smaller body length (324-736 *vs*. 330-520 µm), lower labial plate, annuli margins that are smooth, anterior annulus of the vulva with lobes, straight vs. sigmoid vagina and a similar conoid-rounded tail shape in comparison with *M. xenoplax.* According to Brzeski et al (2002a) these three species are frequently misidentified. Population from Russian

River, Ca. showed variations in submedian lobes which appeared in some cases flattened along with a lower labial disc and a longer and conical tail, as compared with the others 6 populations studied. See tables 4 and 5 to compare with morphometrics of *M. ornatum* and *M. rusticum.*

Criconemoides informis (Micoletzky, 1922) Taylor, 1936.

(Table 9; Fig.13)

Description

Female nematodes straight or dorsally arcuate. Annuli retrorse, smooth to irregular margins. Anastomoses absence. Lip region not offset, without submedian lobes. Labial disc elevated surrounding the oral opening. First lip annulus sometimes anteriorly projected, smaller than the second one. Second lip annulus smaller than rest of body annuli. Stylet robust, knobs concave or anchor shaped. Typical criconematoid oesophagus. Excretory pore posterior to the oesophagus basal gland, 19-22 annules from anterior end. Female genital tract monodelphic, prodelphic, outstretched, spermatheca full of sperm. Vulva closed as a simple narrow slit located at 2–4 annuli from the posterior end. Tail conical, tip rounded and unilobed

Host and locality

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

Diagnostic

Criconemoides informis has a lip region no off set without submedian lobes, first annulus elevated and anteriorly projected and vulva close as a simple narrow slit. All the morphometric values of the specimens are in agreement with the ranges of the original description (De Grisse, 1969) and a specific ITS1 sequence (JQ708118) have been submitted to GenBank.

Relationships

Criconemoides informis has a conical tail shape as *C. mongolensis* Andrássy, 1964 and *C. morgensis* (Hofmänner *in* Hofmänner & Menzel, 1914) Taylor, 1936. Both species have an elevated labial plate that surrounds the oral opening. The submedian lobes are either absent or are not developed in both species. *Criconemoides informis* has a shorter stylet than *C. morgensis* (45- 50 *vs*. 68-108 µm). The stylet in *C. informis* is robust when compared with the stylet of *C. mongolensis* Andrássy, 1964 which is slender and delicate (Choi et al., 2000). Recently, populations of *C. informis* found in Iran exhibited a longer stylet (45- 50 *vs*. 64-87 µm), a similar position of the excretory pore Rex (15-22 *vs*. 18-25), a longer tail (8-16 *vs*. 16-31 µm), and a similar body length (415-506 *vs*. 440-600 µm). This last population was divided in females with or without sperm in the spermatheca but the purpose for this division wasn't mentioned and no significant measurement differences were found (Brzeski et al., 2002a; Eskandari, 2010).

Molecular phylogenetic analysis

For the species of *Mesocriconema* and *Criconemoides* studied the length of the PCR product ranged between 560 bp to 680 bp. The portion of internal transcribed spacer 1 length used for phylogenetic analysis was 387 bp with 7 characters constant (7%) and 332 characters parsimony-informative (85%). The population group have an average nucleotide composition of 24.1% (A), 25.2% (C), 26.8 (G) and 23.8 (T). The nucleotide composition of the ITS1 region for

each species showing similarities and differences as percentages of bases among them is shown in Table 10.

Only one most parsimonious tree was obtained from *Mesocriconema* and *Criconemoides* data (Fig. 14). (Length = 1396; C.I =0.58). Two clades were originated. The first clade has two clusters that are mainly conformed by populations of *M. xenoplax*, *M. curvatum*, *M. ornatum*, *M. crenatum*, *M. kirjanovae*, *M. vadense*, *M. ozarkiense* n.sp. and *M. sphaerocephala* as sister species with a 92% bootstrap support. The second clade included 4 populations: *M. onoense*, *M. surinamensis*, *M. xenoplax* and *Criconemoides informis* with 74% bootstrap support. The maximum likelihood tree included the species in two clades as well (Fig. 15) (-Ln likelihood = 5362.01162), Topology of maximum parsimony and maximum likelihood trees kept the same clades among the species including the clade with 89% bootstrap support which clustered *M. vadense*, *M. curvatum* and *M. ozarkiense* n.sp. however, *M. sphaerocephala* was clustered as a species close related with *M. ornatum* in the maximum likelihood tree.

Genetic variation among *M. xenoplax* populations and *M. curvatum*, *M. ornatum*, *M. crenatum*, *M. kirjanovae*, *M. vadense* ranged from 0.7% to 33%. Genetic divergence between *M. vadense* and *M. curvatum*, two species difficult to separate morphologically, ranged between 27% to 32%. Maximum likelihood showed a close relationship of *M. sphaerocephala* with *M. ornatum* with 43% of genetic divergence. Morphologically, these two species have very low submedian lobes and a cylindrical body. Morphological differences of *M. sphaerocephala* with the rest of the species are evident having a small body length average (354 ± 29 µm), very small submedian lobes, labial plates no evident, numerosus anastomoses and vulva simple. These differences seen to agree with the genetic variation mentioned above. *Mesocriconema ornatum* do not have anastomoses, has a larger body length and it is morphologically most close to *M.*

xenoplax. The genetic variation between *M. ornatum* and *M. xenoplax* population ranged from 5% to 15.1%, except for the population of *M. xenoplax* from North Carolina which showed a higher genetic variation of 58%. *Mesocriconema ozarkiense* showed a genetic divergence of 30% with *M. vadense* and one population of *M. curvatum.* The populations of *M. xenoplax* obtained and studied from California showed a genetic variation of 0.5% to 2.8%

Criconemoides informis showed the typical morphological differences that separate the genus from *Mesocriconema* species and a range of genetic divergence of 55-60% between both genera. Besides, ITS1 DNA sequences were able to show similarities with those species of *Mesocriconema* that have similar molecular structure but are different from the *M. xenoplax* group and to separate species with notorious morphological differences as *M. onoense, M. sphaerocephala* and *M. surinamense* with a range of genetic divergence with the others species of 59- 62%, 42%-54% and 52-55%, respectively.

The topology of maximum parsimony and maximum likelihood showed monophyletic and paraphyletic relationships with different rates of substitutions in the ITS1sequences and possibly different evolutionary histories.

A recent proposal to synonymize genera *Criconemella, Macroposthonia* and *Mesocriconema* as *Criconemoides* (Decraemer and Hunt, 2006) is not shared by the authors because the proposal did not take into consideration the clear differences mentioned early in this work regarding the presence of true submedian lobes and open vulva in *Mesocriconema* and the absence of true submedian lobes and closed vulva in *Criconemoides*, as important characters of diagnostic extensively studied by Brzeski et al (2002a,b) and before them by Loof and De Grisse (1967) . Therefore, *Criconemoides* and *Mesocriconema* are considered here as valid genera of the subfamily Criconematinae.

Genetic variation in the nuclear rDNA ITS1 region could be the results of different lineages or multiple substitutions because mutations events evolving at different rates within the group according with genetic variation percentages. These molecular differences among *Mesocriconema* spp. and *Criconemoides* sp. are important in order to determine barcodes for identification and diagnostic purposes for those species with many similarities and just a few differences even though, the known high variability of the internal transcribed spacer 1.

Accurate morphological and taxonomical identification is essential to avoid confusion and help to detect real relationships and possible lineages among species when molecular information is obtained. Ye et al. (2004) using ITS1 sequences reported genetic variation between *Xiphinema chambersi* and *Longidorus crassus* was 38.6%; *X. diversicaudatum* and *X. bakeri* 3.8%, *X.chambersi* and *X. italiae* 29.9%; *L.crassus* and *L. grandis* 8.9% and *L. fragilis* and *L. diadecturus* 32.4%. 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 (Subbotin 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 characterization of the nuclear ITS1 ribosomal region using PCR-RFLP had been obtained. Variation within individuals and between isolates from US and India of *Heterodera zeae* and, between isolates of *H. goettingiana* from North Ireland and US (Szalanski et al., 1997); 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); populations of *Naccobus aberrans* from Peru were differentiated from those studied in Mexico and Argentina. Furthermore, two different populations from Argentina were detected and similarities

between populations of the species from Peru and Bolivia were found (Reid et al., 2003) and presence of *Globodera pallida* in Idaho in 2007 was confirmed using ITS1 sequence (Skantar, et al, 2007) Recently, morphology studies and sequences of ITS1 of *Discocriconemella inarata* Hoffmann, 1974, *M. curvatum*, *M. rusticum* and *M. xenoplax* allowed to confirm that *D. inarata* was morphological different from the others *Mesocriconema* species however, molecular information showed a close relation with *Mesocriconema* species but distantly related to *Discocriconemella* species (Powers et al, 2010).

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 need to be 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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TABLES

Table 1. Measurements and ratios of *Mesocriconema ozarkiense* n.sp. Morphometrics of related species are presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*M. ozarkiense* *Holotype*[1]	*M. ozarkiense* (n=19) *Paratypes*[1]	*M. citricola*[a]	*M. denoudeni*[a]
L	412.12	424.9 ± 38.9(378.8-512.1)	380-470	390-570
Oesophagus length	93.38	94.0 ± 6.9(81.2-111.7)	-	-
Tail	27.61	24.7 ± 3.1(18.7-30.0)	-	-
Maximum Body width	43.04	41.8 ± 2.8(36.5-48.7)	-	-
a	9.58	10.2 ± 0.9(8.4-11.9)	-	-
b	4.41	4.5 ± 0.2(4.2-4.9)	-	-
c	14.93	17.4 ± 2.2(13.2-22.5)	-	-
Distance lip region to vulva	370.71	388.2 ± 37.8(340.6-475.6)	-	-
Distance lip region to anus	384.51	400.2 ± 37.9(353.6-483.7)	-	-
V	89.95	91.3 ± 1.0(88.7-92.9)	90-92	90-94
V'	96.41	97.0 ± 0.6(96.0-98.3)	-	-
Distance lip region to end oesophageal gland	97.44	98.4 ± 7.7(87.3-121.8)	-	-
Body width at anus	25.17	23.2 ± 2.1(20.3-27.6)	-	-
b'	4.23	4.3 ± 0.2(4.0-4.7)	-	-
c'	1.10	1.1 ± 0.1(0.9-1.2)	-	-
Distance between vulva & post end of body	41.41	36.6 ± 3.8(30.0-44.7)	-	-
Body width at vulva	31.67	29.7 ± 2.1(26.0-34.1)	-	-
VL/VB	1.31	1.2 ± 0.1(1.0-1.4)	1.1-1.4	1.0-1.3
Rex	29.00	29.2 ± 1.6(27.0-34.0)	23-26	32-37
Roes	30.00	29.2 ± 1.4(26.0-31.0)	-	-
Rvan	3.00	2.9 ± 0.5(2.0-4.0)	2-3	0-2
Ran	8.00	7.2 ± 0.8(6.0-10.0)	4-5	6-9
RV	12.00	11.1 ± 1.0(10.0-14.0)	8-9	8-11
R	114.00	111.6 ± 3.1(107.0-119.0)	73-78	102-127
Stylet length	58.87	55.4 ± 3.1(48.7-60.9)	48-51	53-59
Length of stylet shaft	14.21	14.0 ± 1.0(12.2-16.2)	-	-
m	75.86	74.8 ± 1.3(72.6-77.3)	-	-
Stylet length as percentage of body length	14.28	13.1 ± 1.0(11.2-14.9)	-	-
Distance between stylet base and D.O.G	0.81	2.1 ± 0.8(0.8-4.1)	-	-
O	1.38	3.8 ± 1.6(1.4-8.1)	-	-
Distance lip region-centre median bulb	62.93	67.1 ± 4.5(62.9-81.2)	-	-
MB	67.39	71.5 ± 4.0(59.6-77.5)	-	-

Table 1. Continued

Character/Ratio	*M. jessiense*[a]	*M. ornicauda*[a]	*M. paradenoudeni*[a]	*M. parareedi*[a]	*M. reedi*[a]	*M. sigillarium*[a]
L	440-560	370-460	320-470	380-480	360-470	370-450
Oesophagus length	-	-	-	-	-	-
Tail	-	-	-	-	-	-
Maximum Body width	-	-	-	-	-	-
a	-	-	-	-	-	-
b	-	-	-	-	-	-
c	-	-	-	-	-	-
Distance lip region to vulva	-	-	-	-	-	-
Distance lip region to anus	-	-	-	-	-	-
V	90-93	88-93	90-94	88-90	90-92	87-90
V'	-	-	-	-	-	-
Distance lip region to end oesophageal gland	-	-	-	-	-	-
Body width at anus	-	-	-	-	-	-
b'	-	-	-	-	-	-
c'	-	-	-	-	-	-
Distance between vulva & post end of body	-	-	-	-	-	-
Body width at vulva	-	-	-	-	-	-
VL/VB	0.8-1.1	1.1-1.6	0.8-1.4	1.3-1.6	1.1-1.3	1.3-1.6
Rex	22-26	24-33	38-35	31-34	26-29	35-39
Roes			-	-		
Rvan	1-2	1-2	2-4	-	1-3	5-7
Ran	6-7	6-9	4-7	3-5	5-7	5-9
RV	8-9	9-11	8-10	12-13	9-10	11-15
R	88-102	92-111	102-130	111-121	104-112	115-127
Stylet length	56-61	43-50	39-52	59-66	51-62	46-51
Length of stylet shaft	-	-	-	-	-	-
m	-	-	-	-	-	-
Stylet length as percentage of body length	-	-	-	-	-	-
Distance between stylet base and D.O.G	-	-	-	-	-	-
O	-	-	-	-	-	-
Distance lip region-centre median bulb	-	-	-	-	-	-
MB	-	-	-	-	-	-

1. Host: *Paspalum* spp. [a] (Brzeski et al., 2002a).

Table 2. Measurements and ratios of *Mesocriconema curvatum and M. crenatum.* Original morphomentrics of *M. crenatum* and species related are presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*M.crenatum*[1] (n=7)	*M.curvatum*[2] (n=34)
L	490.0 ±33.2(427.3-533.3)	474.6±48.0(381.8-557.6)
Oesophagus length	120.4±5.5(109.6-125.9)	104.4±6.8(89.3-121.8)
Tail	23.2±3.9(20.3-30.5)	21.6±3.5(13.0-27.6)
Maximum Body width	46.4±4.1(40.6-52.8)	43.3±3.3(36.5-48.7)
a	10.6±0.5(10.0-11.2)	11.0±0.9(8.9-12.4)
b	4.1±0.2(3.8-4.4)	4.5±0.4(3.7-5.3)
c	21.4±2.6(16.8-24.3)	22.5±4.1(16.2-36.6)
Distance lip region to vulva	457.8±28.5(400.9-492.7)	443.9±46.0(350.1-525.1)
Distance lip region to anus	466.8±31.0(407.0-506.9)	453.0±47.2(360.7-534.0)
V	93.5±0.9(92.1-94.5)	93.5±0.7(91.7-94.7)
V'	98.1±0.7(97.2-99.1)	98.0±0.7(96.5-99.4)
Distance lip region to end oesophageal gland	127.0±6.3(113.7-132.0)	110.8±6.7(95.4-129.9)
Body width at anus	31.3±3.5(24.4-34.5)	30.6±2.8(26.8-35.7)
b'	3.9±0.2(3.6-4.1)	4.3±0.4(3.5-4.9)
c'	0.7±0.1(0.6-0.9)	0.7±0.1(0.5-0.9)
Distance between vulva & post end of body	32.2±6.1(26.4-40.6)	30.7±3.5(23.6-38.2)
Body width at vulva	35.4±3.1(30.5-40.6)	35.1±2.9(31.7-47.1)
VL/VB	0.9±0.1(0.8-1.1)	0.9±0.1(0.7-1.0)
Rex	29.4±1.7(28.0-33.0)	24.8±3.6(19.0-30.0)
Roes	28.6±1.9(25.0-31.0)	23.7±4.7(17.0-30.0)
Rvan	1.4±0.5(1.0-2.0)	0.6±0.7(0.0-2.0)
Ran	4.9±0.7(4.0-6.0)	6.8±1.5(4.0-9.0)
RV	6.4±0.8(6.0-8.0)	8.3±1.6(5.0-11.0)
R	109.1±4.5(101.0-114.0)	91.2±13.1(71.0-111.0)
Stylet length	77.7±3.8(71.1-83.2)	54.8±2.8(50.3-61.7)
Length of stylet shaft	17.4±4.2(12.2-22.3)	14.4±0.7(13.0-16.2)
m	77.7±4.9(71.8-84.6)	73.7±1.6(69.3-77.8)
Stylet length as percentage of body length	15.9±0.8(14.8-17.1)	11.6±1.0(10.0-14.3)
Distance between stylet base and D.O.G	2.6±1.0(2.0-4.1)	3.7±1.0(1.6-4.9)
O	3.3±1.1(2.6-5.1)	6.7±1.7(3.0-9.7)
Distance lip region-centre median bulb	91.6±5.2(81.2-95.4)	73.3±5.7(60.9-83.2)
MB	76.2±3.0(72.1-79.7)	70.2±3.5(60.0-76.1)

Table 2. Continued

Character/Ratio	*M. crenatum*[a]	*M. ornatum*[a]	*M. xenoplax*[a]
L	210-400	330-520	400-750
Oesophagus length	-	-	-
Tail	-	-	-
Maximum Body width	-	-	-
a	-	-	-
b	-	-	-
c	-	-	-
Distance lip region to vulva	-	-	-
Distance lip region to anus	-	-	-
V	92-98	92-96	92-96
V'	-	-	-
Distance lip region to end oesophageal gland	-	-	-
Body width at anus	-	-	-
b'	-	-	-
c'	-	-	-
Distance between vulva & post end of body	-	-	-
Body width at vulva	-	-	-
VL/VB	0.7-11	0.7-1.2	0.7-1.3
Rex	20-26	25-27	26-30
Roes	-	-	-
Rvan	1-2	0-2	0-4
Ran	4-6	5-8	4-7
RV	6-8	7-9	6-11
R	73-84	78-94	77-114
Stylet length	38-51	44-56	54-87
Length of stylet shaft	-	-	-
m	-	-	-
Stylet length as percentage of body length	-	-	-
Distance between stylet base and D.O.G	-	-	-
O	-	-	-
Distance lip region-centre median bulb	-	-	-
MB	-	-	-

Host: [1] *Vitis vinifera* [2]River cane-turfgrass. Oak. [a] (Brzeski et al., 2002a)

Table 3. Measurements and ratios *Mesocriconema kirjanovae* .Morphometric of species related are presented for comparison. Mean standard deviation and range in µm.

Character/Ratio	*Mesocriconema kirjanovae*[1] (n=38)	*M. bareilli*[a]	*M. bilaspurense*[a]
L	441.1±46.3(363.6-569.7)	280-350	350-410
Oesophagus length	97.8±6.3(87.3-111.7)	-	-
Tail	25.5±5.4(16.2-36.5)	-	-
Maximum Body width	40.4±2.9(36.5-48.7)	-	-
a	10.9±1.1(9.2-13.8)	-	-
b	4.5±0.4(3.6-5.8)	-	-
c	18.0±3.8(12.2-26.0)	-	-
Distance lip region to vulva	404.0±45.6(329.5-527.5)	-	-
Distance lip region to anus	415.7±44.9(340.9-538.0)	-	-
V	91.5±1.5(89.1-94.4)	91-95	92-93
V'	97.2±1.0(94.1-99.1)	-	-
Distance lip region to end oesophageal gland	105.5±15.0(91.4-186.8)	-	-
Body width at anus	26.4±2.5(21.9-32.5)	-	-
b'	4.2±0.5(2.2-5.6)	-	-
c'	1.0±0.2(0.6-1.3)	-	-
Distance between vulva & post end of body	37.1±6.3(24.4-48.7)	-	-
Body width at vulva	30.7±2.2(26.8-34.9)	-	-
VL/VB	1.2±0.2(0.9-1.6)	1.0	1.1-1.3
Rex	29.0±1.4(26.0-31.0)	28-29	29-31
Roes	29.1±2.6(23.0-37.0)	-	
Rvan	1.3±0.6(0.0-2.0)	-	2-3
Ran	7.4±1.0(5.0-9.0)	6-8	7
RV	9.5±1.0(8.0-11.0)	7-9	9-10
R	110.3±5.3(98.0-121.0)	90-100	93-97
Stylet length	54.7±2.6(47.9-60.9)	35-50	40-47
Length of stylet shaft	14.3±1.2(13.0-18.3)	-	-
m	73.8±2.0(69.2-78.1)	-	-
Stylet length as percentage of body length	12.5±1.3(9.9-15.3)	-	-
Distance between stylet base and D.O.G	2.7±1.4(0.0-6.5)	-	-
O	4.9±2.7(0.0-13.6)	-	-
Distance lip region-centre median bulb	68.7±7.0(60.9-101.5)	-	-
MB	70.3±5.7(60.8-92.6)	-	-

Table 3. Continued

Character/Ratio	*M. calvatum*[a]	*M. reedi*[a]	*M. ripariense*[a]
L	570-620	360-470	500-580
Oesophagus length	-	-	-
Tail	-	-	-
Maximum Body width	-	-	-
a	-	-	-
b	-	-	-
c	-	-	-
Distance lip region end to vulva	-	-	-
Distance lip region end to anus	-	-	-
V	92-94	90-92	87-90
V'	-	-	-
Distance lip region to end oesophageal gland	-	-	-
Body width at anus	-	-	-
b'	-	-	-
c'	-	-	-
Distance between vulva & post end of body	-	-	-
Body width at vulva	-	-	-
VL/VB	0.9	1.1-1.3	1.3
Rex	18-21	26-29	35-40
Roes			
Rvan	1-2	1-3	4-6
Ran	2-3	5-7	8-10
RV	4	9-10	12-16
R	60-65	104-112	125-150
Stylet length	100-110	51-62	64-73
Length of stylet shaft	-	-	-
m	-	-	-
Stylet length as percentage of body length	-	-	-
Distance between stylet base and D.O.G	-	-	-
O	-	-	-
Distance lip region-centre median bulb	-	-	-
MB	-	-	-

Host: [1.] Hickory-grass. [a] (Brzeski et al., 2002a)

Table 4. Measurements and ratios *Mesocriconema ornatum* and *M. onoense*. Morphometric of species related are presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*Mesocriconema ornatum*[1] (n=19)	*M.ornatum*[b]	*Mesocriconema onoense*[2] (n=12)
L	504.5±27.7(445.5-557.6)	363-442	580.6±62.2(500.0-681.8)
Oesophagus length	111.8±5.3(101.5-121.8)	-	112.3±6.1(99.5-119.8)
Tail	26.5±3.1(21.9-32.5)	-	25.2±4.7(18.7-33.3)
Maximum Body width	42.6±3.1(36.5-47.9)	-	44.3±2.7(40.6-48.7)
a	11.9±1.0(10.0-13.7)	10.1-12.8	13.1±1.3(11.4-15.8)
b	4.5±0.2(4.1-4.9)	3.5-44	5.2±0.5(4.2-5.8)
c	19.3±1.9 (15.2-22.4)	15.9	23.4±2.7(19.7-29.2)
Distance lip region end to vulva	466.4±25.7(412.2-514.5)	-	545.0±57.9(465.1-641.2)
Distance lip region end to anus	478.1±26.3(423.5-527.5)	-	555.3±58.3(476.5-648.5)
V	92.4±0.9(90.6-93.7)	90.3-93.8	93.9±0.5(93.0-94.6)
V'	97.6±0.8(95.4-99.3)	-	98.1±0.4(97.5-98.9)
Distance lip region to end oesophageal gland	117.6±5.2(105.6-127.9)	-	117.4±6.4(103.5-125.9)
Body width at anus	29.6±2.2(26.0-33.3)	-	31.2±2.1(28.4-34.9)
b'	4.3±0.2(3.9-4.7)	-	4.9±0.5(4.1-5.6)
c'	0.9±0.1(0.7-1.1)	-	0.8±0.1(0.7-1.0)
Distance between vulva & post end of body	38.1±4.9(30.9-47.1)	-	35.5±5.0(28.4-43.9)
Body width at vulva	34.5±2.1(30.9-39.0)	-	35.8±1.9(32.5-39.0)
VL/VB	1.1±0.1(0.9-1.3)	0.7-1.2	1.0±0.1(0.8-1.1)
Rex	26.7±1.0(25.0-28.0)	25-27	36.9±2.2(32.0-39.0)
Roes	26.5±1.1(25.0-28.0		35.7±2.0(30.0-38.0)
Rvan	1.5±0.8(0.0-3.0)	0-2	1.0±0.6(0.0-2.0)
Ran	7.8±1.0(6.0-9.0)	6-8	7.9±0.5(7.0-9.0)
RV	10.3±1.2(9.0-12.0)	7-9	9.9±0.7(9.0-11.0)
R	100.9±2.8(96.0-106.0)	87-92	135.9±10.1(106.0-143.0)
Stylet length	55.9±2.0(52.0-59.3)	48-56	58.7±2.7(54.4-65.0)
Length of stylet shaft	15.3±2.0(8.1-17.1)	-	16.2±0.8(15.4-17.9)
m	72.7±3.6(69.7-84.8)	-	72.3±1.6(69.4-75.0)
Stylet length as percentage of body length	11.1±0.7(9.9-12.0)	-	10.2±1.2(8.2-11.7)
Distance between stylet base and D.O.G	4.7±1.7(0.0-8.1)	-	4.3±1.3(2.4-6.5)
O	8.4±3.1(0.0-15.2)	-	7.4±2.1(4.2-11.3)
Distance lip region-centre median bulb	76.1±3.5(69.0-85.3)	-	77.5±3.7(71.1-83.2)
MB	68.1±2.1 (64.3-72.0)	-	69.0±2.6(64.4-73.1)

Table 4. Continued

Character/Ratio	*M. multiannulatum*[a]	*M. oblongatum*[a]	*M. onostre*[a]	*M. paranostre*[a]
L	540-670	390-400	520-610	380-570
Oesophagus length	-	-	-	-
Tail	-	-	-	-
Maximum Body width	-	-	-	-
a	-	-	-	-
b	-	-	-	-
c	-	-	-	-
Distance lip region to vulva	-	-	-	-
Distance lip region to anus	-	-	-	-
V	93-94	91	92-96	90-94
V'	-	-	-	-
Distance lip region to end oesophageal gland	-	-	-	-
Body width at anus	-	-	-	-
b'	-	-	-	-
c'	-	-	-	-
Distance between vulva & post end of body	-	-	-	-
Body width at vulva	-	-	-	-
VL/VB	1.0-1.2	0.8	1.0-1.1	09-1.2
Rex	30-32	27-28	36-38	31-43
Roes				
Rvan	2-3	6-8	1-3	1-3
Ran	8-9	2	6-8	4-7
RV	10-12	8-10	7-9	6-10
R	143-150	134-148	133-147	117-150
Stylet length	52-59	42-45	54-61	51-62
Length of stylet shaft	-	-	-	-
m	-	-	-	-
Stylet length as percentage of body length	-	-	-	-
Distance between stylet base and D.O.G	-	-	-	-
O	-	-	-	-
Distance lip region-centre median bulb	-	-	-	-
MB	-	-	-	-

Host: 1. Turfgrass 2. Grass-Maple. [a] (Brzeski et al., 2002a). [b] Raski, 1952

Table 5. Measurements and ratios of *Mesocriconema vadense.* Morphometric of *M. rusticum* as related species is presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*M. vadense*[1] (n=42) Fayetteville, AR	*M. vadense*[2] (n=20) Pinetree, AR	*M. rusticum*[a]
L	511.1±40.5(421.2-597)	429.2±37.3(354.5-487.9)	340-520
Oesophagus length	102.8±11.2(39.8-117.7)	94.2±7.6(83.2-111.7)	-
Tail	23.3±4.3(14.6-32.5)	26.2±2.9(20.3-30.5)	-
Maximum Body width	41.3±2.1(37.4-44.7)	41.9±2.5(38.6-46.7	-
a	12.4±1.0(10.3-14.2)	10.2±0.6(9.2-11.5)	-
b	5.1±1.2(4.1-12.3)	4.6±0.3(4.1-5.1)	-
c	22.6±4.4(16.1-36.3)	16.5±1.8(13.8-19.9)	-
Distance lip region to vulva	477.6±38.8(390.4-561.2)	389.7±34.8(324.1-445.2)	-
Distance lip region to anus	487.8±38.8(403.4-566.1)	403.1±36.3(334.2-459.5)	-
V	93.4±0.6(96.8-95.1)	90.8±0.6(89.6-92.0)	92-95
V'	97.7±0.7(96.8-99.3)	96.7±0.8(95.4-98.4)	-
Distance lip region to end oesophageal gland	110.1±4.8(99.5-121.8)	100.3±7.2(91.4-117.7)	-
Body width at anus	28.0±2.4(25.2-33.3)	29.1±2.3(24.4-32.5)	-
b'	4.6±0.3(3.9-5.3)	4.3±0.3(3.9-4.9)	-
c'	0.8±0.1(0.5-1.1)	0.9±0.1(0.7-1.1)	-
Distance between vulva & post end of body	33.5±3.5(24.4-40.6)	39.6±3.6(30.5-44.7)	-
Body width at vulva	32.5±2.2(28.4-37.4)	34.2±2.1(30.5-36.5)	-
VL/VB	1.0±0.1(0.8-1.3)	1.2±0.1(1.0-1.3)	0.7-1.2
Rex	28±1.2(25.0-30.0)	26.5±1.6(24.0-29.0)	24-32
Roes	25.1±1.8(23.0-30.0)	26.3±1.3(24.0-28.0)	
Rvan	1.4±0.7(0.0-3.0)	2.3±0.7(1.0-4.0)	0-2
Ran	7.5±0.9(6.0-9.0)	6.8±0.8(5.0-9.0)	4-9
RV	9.9±0.8(8.0-11.0)	9.1±0.5(8.0-10.0)	7-10
R	103.2±4.0(92.0-111.0)	101.0±2.9(94.0-105.0)	81-107
Stylet length	50.9±2.3(43.9-56.0)	52.9±2.5(48.7-56.8)	50-60
Length of stylet shaft	13.9±0.9(12.2-16.2)	14.7±1.7(12.2-18.3)	-
m	72.7±1.6(67.8-75.4)	72.2±2.7(66.7-76.9)	-
Stylet length as percentage of body length	10.0±0.9(8.0-12.7)	12.4±1.0(11.1-14.9)	-
Distance between stylet base and D.O.G	3.9±0.8(2.4-5.7)	4.0±2.1(2.0-10.2)	-
O	7.6±1.6(4.7-10.9)	7.4±3.8(3.7-18.5)	-
Distance lip region-centre median bulb	71.1±3.1(65.0-79.2)	69.0±4.1(62.9-79.2)	-
MB	70.8±17.2(59.6-178.6)	73.4±3.0(69.4-79.5)	-

Host: Host: 1 grass 2. Turfgrass. [a] (Brzeski et al., 2002a)

Table 6. Measurements and ratios of *Mesocriconema sphaerocephala*. morphometrics of *M. sphaerocephaloides* as related species is presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*M. sphaerocephala*[1] (n=22)	*M. sphaerocephaloides*[a]
L	353.9±28.9(293.9-406.1)	320
Oesophagus length	101.2±6.2(87.3-113.7)	-
Tail	17.9±3.0(12.2-24.4)	-
Maximum Body width	35.7±1.2(34.5-38.6)	-
a	9.9±0.8(8.5-11.8)	-
b	3.5±0.3(3.1-4.1)	-
c	20.2±3.6(14.8-30.6)	-
Distance lip region to vulva	327.1±27.4(269.6-373.6)	-
Distance lip region to anus	335.9±28.1(277.7-385.8)	-
V	92.4±0.7(91.1-93.6)	95
V'	97.4±0.5(96.5-98.2)	-
Distance lip region to end oesophageal gland	106.8±5.7(95.4-117.7)	-
Body width at anus	24.0±1.9 (20.3-28.4)	-
b'	3.3±0.2(3.0-3.8)	-
c'	0.7±0.1(0.5-0.9)	-
Distance between vulva & post end of body	26.8±2.9(22.3-32.5)	-
Body width at vulva	28.6±2.2(24.4-32.5)	-
VL/VB	0.9±0.1(0.7-1.1)	0.6
Rex	20.6±2.5(15.0-25.0)	27
Roes	22.0±1.5(20.0-24.0)	
Rvan	1.4±0.5(1.0-2.0)	1
Ran	4.0±0.7(3.0-5.0)	5
RV	5.4±0.7(4.0-7.0)	7
R	65.7±2.5(61.0-71.0)	82
Stylet length	51.8±2.2(46.7-54.8)	51
Length of stylet shaft	14.8±1.8(10.2-18.3)	-
m	71.5±2.8(65.4-79.2)	-
Stylet length as percentage of body length	14.7±1.1(13.0-16.6)	-
Distance between stylet base and D.O.G	3.8±1.4(2.0-6.1)	-
O	7.3±2.7(3.7-11.5)	-
Distance lip region-centre median bulb	72.2±3.8(65.0-79.2)	-
MB	71.5±3.4(64.3-78.7)	-

Host: 1. Turfgrass-dailylily. [a] (Brzeski et al., 2002a)

Table 7. Measurements and ratios of *Mesocriconema surinamense.* Morphometric of *M. yossifovichi* as related species is presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*Mesocriconema surinamense*[1] (n=40)	*M. yossifovichi*[a]
L	537.3 ± 60.6(424.2-639.4)	480-600
Oesophagus length	112.9 ± 6.7(99.5-123.8)	-
Tail	22.8 ± 3.7(16.2-30.9)	-
Maximum Body width	48.5 ± 3.8(42.2-56.8)	-
a	11.1 ± 1.2(8.3-14.4)	-
b	4.8 ± 0.5(3.9-5.9)	-
c	24.1 ± 4.4(16.5-35.6)	-
Distance lip region to vulva	500.5 ± 58.9(393.4-600.6)	-
Distance lip region to anus	514.4 ± 59.6(404.8-618.5)	-
V	93.1 ± 0.7(91.7-95.4)	92-94
V'	97.3 ± 0.7(96.2-99.6)	-
Distance lip region to end oesophageal gland	118.8 ± 6.7(104.6-132.0)	-
Body width at anus	29.1 ± 3.0(21.9-34.5)	-
b'	4.5 ± 0.5(3.7-5.5)	-
c'	0.8 ± 0.1(0.5-1.1)	-
Distance between vulva & post end of body	36.7 ± 3.8(28.4-44.7)	-
Body width at vulva	34.7 ± 2.8(29.2-41.4)	-
VL/VB	1.1 ± 0.1(0.8-1.4)	1.4-1.6
Rex	26.9 ± 1.4(24.0-29.0)	25-29
Roes	26.7 ± 1.9(24.0-32.0)	-
Rvan	2.0 ± 0.8(0.0-4.0)	1
Ran	5.9 ± 1.0(4.0-8.0)	7-8
RV	9.0 ± 1.0(7.0-11.0)	9-10
R	102.8 ± 3.3(96.0-110.0)	95-108
Stylet length	69.6 ± 4.4(58.5-76.1)	61-74
Length of stylet shaft	15.6 ± 2.6(8.1-21.9)	-
m	77.5 ± 3.9(66.3-88.9)	-
Stylet length as percentage of body length	13.1 ± 1.4(10.6-17.0)	-
Distance between stylet base and D.O.G	3.1 ± 1.1(0.8-4.9)	-
O	4.5 ± 1.5(1.1-7.1)	-
Distance lip region-centre median bulb	85.9 ± 4.8(77.1-95.4)	-
MB	76.1 ± 3.5(67.8-82.7)	-

Host: 1. Grass-maple. [a] (Brzeski et al., 2002)

Table 8. Measurements and ratios of *Mesocriconema xenoplax*. Mean, standard deviation and range in µm.

Character/Ratio	*M. xenoplax* (n=20)[1] Farmington, AR	*M. xenoplax* (n=20)[2] Fayetteville, AR	*M. xenoplax* (n=10)[5] Fayetteville,AR
L	571.8 ± 66.1(478.8-736.4)	564.8 ±42.6(475.8- 639.4)	577.6±39.7(533.3-666.7)
Oesophagus length	140.3± 9.1(121.8-158.3)	145.0 ± 7.6(132.0-162.4)	127.7 ± 6.2(117.7-136.0)
Tail	30.1± 7.8(18.3-48.7)	29.8 ± 4.4(24.4- 36.5)	22.2 ± 4.4(16.2-30.9)
Maximum Body width	47.3 ± 2.6(42.6-50.8)	50.6 ± 2.9(44.7- 54.8)	50.1 ± 4.1(43.0-56.0)
a	12.1 ± 1.0(10.6-15.1)	11.2 ± 0.6(9.8 -12.3)	11.6 ± 1.0(10.0-13.2)
b	4.1 ± 0.3(3.6-4.7)	3.9 ± 0.3(3.5-4.3)	4.5 ± 0.3(4.2-5.0)
c	19.7 ± 3.7(13.9-28.0)	19.3 ± 3.0(15.0-24.0)	26.7 ± 4.1(21.6-33.2)
Distance lip region to vulva	532.2 ± 60.3(442.2-687.6)	525.5 ± 41.7(435.2-598.8)	543.1± 36.2(500.9-623.6)
Distance lip region to anus	541.7± 60.5 (454.4-695.8)	535.0 ± 42.2(451.4-606.9)	555.4± 36.0(517.1-635.8)
V	93.1 ± 1.1(91.4-94.8)	93.0 ± 1.0(89.9-94.8)	94.1 ± 0.3(93.5-94.7)
V'	98.2 ± 0.9(96.1-99.4)	98.2 ± 1.3(93.8-99.3)	97.8 ± 0.4(96.9-98.3)
Distance lip region to end oesophageal gland	146.7 ± 8.8(127.9-164.4)	152.4 ± 7.8(140.1-170.5)	136.0 ± 7.3(123.8-146.2)
Body width at anus	35.6 ± 4.5(24.4-42.6)	36.7 ± 2.6(32.5-42.6)	33.9 ± 2.6(29.2-38.2)
b'	3.9 ± 0.3(3.5-4.6)	3.7 ± 0.3(3.3-4.2)	4.2 ± 0.2(4.0-4.7)
c'	0.8 ± 0.2(0.6-1.1)	0.8 ± 0.1(0.7-0.9)	0.6 ± 0.1(0.5-0.8)
Distance between vulva & post end of body	39.6 ± 8.5(26.4-56.8)	39.4 ± 5.6(30.5-58.9)	34.4 ± 3.9(28.4-43.0)
Body width at vulva	39.2 ± 5.8(28.4-56.8)	39.9 ± 2.5(36.5-46.7)	39.8 ± 2.2(36.5-43.0)
VL/VB	1.0 ± 0.2(0.7-1.4)	1.0 ± 0.1(0.8-1.4)	0.9 ± 0.1(0.8-1.0)
Rex	26.5 ± 2.4(23.0-30.0)	25.7 ± 1.5(23.0-29.0)	26.2 ± 1.6(25.0-29.0)
Roes	24.8 ± 1.8(20.0-27.0)	25.4 ± 1.8(23.0-29.0)	24.4 ± 1.8(21.0-27.0)
Rvan	1.3 ± 0.6(1.0-3.0)	1.4 ± 0.5(1.0-2.0)	1.0 ± 0.0(1.0-1.0)
Ran	5.7 ± 1.2(4.0-8.0)	4.6 ± 0.7(3.0-5.0)	5.6 ± 0.8(4.0-7.0)
RV	6.9 ± 1.2(5.0-10.0)	6.0 ± 0.5(5.0-7.0)	7.6 ± 0.8(6.0-9.0)
R	94.2 ± 7.2(75.0-113.0)	92.3 ± 3.9(86.0-99.0)	96.5 ± 4.2(91.0-106.0)
Stylet length	90.3 ± 22.4(69.0-182.7)	86.1 ± 3.6(79.2-91.4)	72.2 ± 4.2(65.6-80.2)
Length of stylet shaft	21.4 ± 1.8(16.2-24.4)	20.8 ± 1.6(18.3-24.4)	17.8 ± 1.4(16.2-20.3)
m	74.7 ±3.1(70.3-81.1)	75.8 ± 1.8(70.7-78.6)	75.4 ± 1.3(73.8-77.7)
Stylet length as percentage of body length	15.7 ± 2.4(13.3-24.8)	15.3 ± 1.2(12.8-17.5)	12.5 ± 0.3(12.0-13.1)
Distance between stylet base and D.O.G	4.2 ± 1.8(2.0-8.1)	5.5 ± 2.7(2.0-12.2)	4.1 ± 1.1(2.4- 6.5)
O	4.8 ± 2.1(2.2-9.3)	6.4 ± 3.3(2.3-14.3)	5.6 ± 1.4(3.7- 8.7)
Distance lip region-centre median bulb	101.9 ± 10.8(83.2-121.8)	108.6 ± 4.3(101.5-115.7)	93.8 ± 5.1(87.3-101.5)
MB	72.6 ± 5.4(60.9-80.0)	75.0 ±, 3.1(69.9-81.8)	73.5 ± 1.9(70.3-76.9)

Table 8. continued

Character/Ratio	*M. xenoplax* (n=10)[5] Fayetteville, AR	*M. xenoplax* (n=20)[6] Fayetteville, AR
L	581.8 ±42.8(527.3-675.8)	567.9 ± 48.3(497.0-657.6)
Oesophagus length	131.1 ± 6.8(121.8-142.1)	134.3 ± 7.8(119.8-146.2)
Tail	23.1 ± 3.2(17.9-27.6)	24.5 ± 3.2(17.1-30.0)
Maximum Body width	46.8 ± 4.1(42.2-56.0)	47.9 ± 3.2(41.4-55.2)
a	12.5 ± 0.9(11.2-13.9)	11.8 ± 0.6(10.4-13.1)
b	4.4 ± 0.3(4.1-5.0)	4.2 ± 0.2(3.8-4.7)
c	25.5 ± 3.5(20.5-32.6)	23.5 ± 3.1(17.4-32.3)
Distance lip region to vulva	545.9 ±41.3(493.2-636.8)	532.4 ± 46.8(464.5-620.2)
Distance lip region to anus	558.7 ±41.4(506.2-649.8)	543.4 ± 47.1(475.0-631.6)
V	93.8 ± 0.5(93.2-94.7)	93.7 ± 0.6(92.6-95.1)
V'	97.7 ± 0.5(97.1-98.8)	98.0 ± 0.5(97.0-99.5)
Distance lip region to end oesophageal gland	137.6 ± 6.9(129.9-150.2)	141.1 ± 8.2(123.8-152.3)
Body width at anus	31.0 ± 3.5(26.0-36.5)	33.0 ± 2.5(28.4-37.4)
b'	4.2 ± 0.3(3.9-4.8)	4.0 ± 0.2(3.6-4.4)
c'	0.7 ± 0.1(0.6-0.9)	0.7 ± 0.1(0.6-1.1)
Distance between vulva & post end of body	35.9 ± 3.0(30.9-39.0)	35.4 ± 3.2(26.8-41.4)
Body width at vulva	36.3 ± 4.3(30.9-43.9)	37.2 ± 2.5(31.7-41.4)
VL/VB	1.0 ± 0.1(0.8-1.1)	1.0 ± 0.1(0.8-1.1)
Rex	28.2 ± 1.1(27.0-30.0)	27.2 ± 2.7(22.0-34.0)
Roes	26.0 ± 1.2(23.0-28.0)	25.5 ± 2.1(22.0-30.0)
Rvan	1.6 ± 0.8(0.0-3.0)	2.1 ± 0.4(1.0-3.0)
Ran	6.0 ± 0.9(4.0-7.0)	5.4 ± 0.6(4.0-6.0)
RV	8.6 ± 1.0(7.0-10.0)	8.5 ± 0.7(7.0-10.0)
R	103.7 ± 2.6(99.0-107.0)	99.8 ± 3.9(94.0-109.0)
Stylet length	71.5 ± 3.4(65.0-76.1)	74.0 ± 3.4(67.2-81.0)
Length of stylet shaft	17.2 ± 0.8(15.4-17.9)	18.3 ± 0.9(16.2-19.5)
m	75.9 ± 0.8(74.7-76.8)	75.2 ± 0.8(73.4-76.2)
Stylet length as percentage of body length	12.3 ± 0.8(10.7-13.2)	13.1 ± 0.7(12.0-15.2)
Distance between stylet base and D.O.G	4.3 ± 1.0(3.3-6.5)	3.9 ± 1.5(0.8-6.1)
O	6.0 ± 1.4(4.5-8.8)	5.2 ± 2.0(1.0-7.8)
Distance lip region-centre median bulb	94.8 ± 5.0(87.3-103.5)	98.2 ± 5.2(87.3-105.6)
MB	72.3 ± 2.5(68.6-76.1)	73.2 ± 3.0(68.6-79.7)

Table 8. continued

Character/Ratio	*M. xenoplax* (n=20)[6] Fayetteville, AR	*M. xenoplax* (n=3)[7] Fayetteville, AR	*M. xenoplax* (n=20)[5] Nashville, NC
L	567.9 ± 48.3(497.0-657.6)	507±29.4(481.8-539.4)	555.8 ±59.3(481.8-690.9)
Oesophagus length	134.3 ± 7.8(119.8-146.2)	105.6 ±5.4(101.5-111.7)	111.3 ± 7.2(91.4-121.8)
Tail	24.5 ± 3.2(17.1-30.0)	21.4 ± 3.4(18.7-25.2)	28.6 ± 4.8(22.3-40.6)
Maximum Body width	47.9 ± 3.2(41.4-55.2)	46.3 ± 2.4(43.9-48.7)	43.5 ± 2.5(38.6-46.7)
a	11.8 ± 0.6(10.4-13.1)	11.0 ± 0.1(10.8-11.1)	12.8 ± 1.3(11.0-15.5)
b	4.2 ± 0.2(3.8-4.7)	4.8 ± 0.1(4.7-4.9)	5.0 ± 0.5(4.4-6.1)
c	23.5 ± 3.1(17.4-32.3)	24.2 ± 4.3(19.1-26.8)	19.7± 2.6(16.1-25.6)
Distance lip region to vulva	532.4 ± 46.8(464.5-620.2)	473.8±30.5(446.9-506.9)	519.1 ±57.6(448.3-646.2)
Distance lip region to anus	543.4 ± 47.1(475.0-631.6)	485.7 ±31.5(456.6-519.1)	527.1 ±56.5(456.4-650.3)
V	93.7 ± 0.6(92.6-95.1)	93.4 ± 0.6(92.8-94.0)	93.4 ± 0.7(92.3-95.2)
V'	98.0 ± 0.5(97.0-99.5)	97.6 ± 0.4(97.1-97.9)	98.4 ± 0.7(97.2-99.6)
Distance lip region to end oesophageal gland	141.1 ± 8.2(123.8-152.3)	111.7 ± 5.4(107.6-117.7)	118.0 ± 6.3(101.5-127.9)
Body width at anus	33.0 ± 2.5(28.4-37.4)	29.8 ± 0.5(29.2-30.0)	31.3 ± 2.0(28.4-34.5)
b'	4.0 ± 0.2(3.6-4.4)	4.5 ± 0.1(4.5-4.6)	4.7 ± 0.5(4.2-5.8)
c'	0.7 ± 0.1(0.6-1.1)	0.7 ± 0.1(0.6-0.8)	0.9 ± 0.1(0.8-1.3)
Distance between vulva & post end of body	35.4 ± 3.2(26.8-41.4)	33.3 ± 1.4(32.5-34.9)	36.6 ± 3.9(32.5-46.7)
Body width at vulva	37.2 ± 2.5(31.7-41.4)	34.9 ± 1.6(33.3-36.5)	34.3 ± 2.2(30.5-38.6)
VL/VB	1.0 ± 0.1(0.8-1.1)	1.0 ± 0.1(0.9-1.0)	1.1 ± 0.1(0.9-1.3)
Rex	27.2 ± 2.7(22.0-34.0)	19.3 ± 1.2(18.0-20.0)	22.6 ± 2.0(18.0-25.0)
Roes	25.5 ± 2.1(22.0-30.0)	17.3 ± 0.6(17.0-18.0)	21.7 ± 2.7(13.0-24.0)
Rvan	2.1 ± 0.4(1.0-3.0)	0.7 ± 0.6(0.0-1.0)	0.8 ± 0.5(0.0-2.0)
Ran	5.4 ± 0.6(4.0-6.0)	5.3 ± 0.6(5.0-6.0)	6.0 ± 0.5(5.0-7.0)
RV	8.5 ± 0.7(7.0-10.0)	6.7 ± 0.6(6.0-7.0)	7.7 ± 0.5(7.0-8.0)
R	99.8 ± 3.9(94.0-109.0)	72.7 ± 2.5(70.0-75.0)	90.1 ± 3.2(84.0-97.0)
Stylet length	74.0 ± 3.4(67.2-81.0)	56.0 ± 0.8(55.2-56.8)	54.1 ± 1.8(50.8-56.8)
Length of stylet shaft	18.3 ± 0.9(16.2-19.5)	14.6 ± 0.8(13.8-15.4)	14.2 ± 1.7(10.2-16.2)
m	75.2 ± 0.8(73.4-76.2)	73.9 ± 1.3(72.5-75.0)	73.8 ± 2.9(69.2-80.0)
Stylet length as percentage of body length	13.1 ± 0.7(12.0-15.2)	11.1 ± 0.5(10.5-11.5)	9.8 ± 0.9(7.8-11.3)
Distance between stylet base and D.O.G	3.9 ± 1.5(0.8-6.1)	3.5 ± 1.7(1.6-4.9)	4.4 ± 1.8(2.0-8.1)
O	5.2 ± 2.0(1.0-7.8)	6.3 ± 3.0(2.9-8.6)	8.1 ± 3.3(3.6-15.4)
Distance lip region-centre median bulb	98.2 ± 5.2(87.3-105.6)	77.8 ± 6.5(73.1-85.3)	76.7± 3.8(69.0-81.2)
MB	73.2 ± 3.0(68.6-79.7)	73.7 ± 2.9(70.6-76.4)	69.1 ± 3.3(64.2-77.8)

Table 8. continued

Character/Ratio	*M. xenoplax* (n=9)[3] Carteret, NC	*M. xenoplax* (n=8)[8] Ripon,CA	*M. xenoplax* (n=11)[8] Parlier, CA
L	402.4 ±47.3(324.2-490.9)	691.5 ± 59.2(624.2- 781.3)	658.6 ± 44.8(603.0-737.5)
Oesophagus length	93.6 ±7.6(83.2-103.5)	145.9 ± 7.2(136.0-158.3)	142.8 ± 7.1(129.9-154.3)
Tail	21.7 ± 3.5(16.2-28.4)	29.2 ± 5.0(22.3-36.5)	25.5 ± 3.9(20.3-32.5)
Maximum Body width	40.8 ± 2.0(38.6-44.7)	53.5 ± 2.0(50.8-56.3)	51.5 ± 2.8(46.7-54.8)
a	9.9 ± 1.0(8.4-11.0)	12.9 ± 1.1(11.6-14.8)	12.8 ± 0.6(11.5-13.5)
b	4.3 ± 0.2(3.9-4.7)	4.7 ± 0.3(4.4-5.3)	4.7 ± 0.3(4.2-5.0)
c	18.7 ± 1.7(15.0-20.9)	24.1 ± 3.3(18.8-28.5)	26.3 ± 3.8(19.9-32.4)
Distance lip region to vulva	369.1± 45.4(293.8-454.4)	646.9 ± 55.5(577.6-726.4)	621.4 ± 49.9(553.3-692.8)
Distance lip region to anus	380.7± 44.7(308.0-466.5)	662.3 ± 56.5(593.8-748.8)	639.1 ± 49.9(575.6-709.1)
V	91.7 ± 1.0(90.1-92.8)	93.5 ± 0.8(92.3-94.6)	93.4 ± 0.9(91.3-94.2)
V'	96.9 ± 1.1(95.3-98.5)	97.7 ± 0.4(97.0-98.3)	97.2 ± 0.6(96.1-98.1)
Distance lip region to end oesophageal gland	99.9 ± 7.3(91.4-109.6)	152.0 ± 7.0(144.1-162.4)	148.4 ± 7.8(136.0-162.4)
Body width at anus	31.1 ± 2.9(26.4-34.5)	35.5 ± 2.4(32.5-40.6)	32.3 ± 3.1(26.4-36.5)
b'	4.0 ± 0.3(3.5-4.6)	4.5 ± 0.3(4.2-5.1)	4.5 ± 0.2(4.0-4.7)
c'	0.7 ± 0.1(0.6-0.8)	0.8 ± 0.1(0.6-1.1)	0.8 ± 0.1(0.7-0.9)
Distance between vulva & post end of body	33.3 ± 3.9(28.4-40.6)	44.7 ± 6.9(34.5-54.8)	42.1 ± 4.7(36.5-52.8)
Body width at vulva	35.5 ± 2.0(32.5-38.6)	42.1 ± 2.1(40.6-44.7)	39.7 ± 1.9(36.5-42.6)
VL/VB	0.9 ± 0.1(0.8-1.1)	1.1 ± 0.1(0.9-1.2)	1.1 ± 0.1(0.9-1.2)
Rex	23.6 ± 1.4(20.0-25.0)	24.1 ± 1.4(22.0-26.0)	24.5 ± 1.9(21.0-28.0)
Roes	22.4 ± 1.8(18.0-24.0)	19.8 ± 1.2(18.0-21.0)	21.5 ± 1.1(20.0-23.0)
Rvan	1.0 ± 0.5(0.0-2.0)	0.9 ± 0.4(0.0-1.0)	1.1 ± 0.5(0.0-2.0)
Ran	5.0 ± 0.7(4.0-6.0)	5.5 ± 0.5(5.0, 6.0)	5.3 ± 0.6(4.0-6.0)
RV	6.1 ± 0.7(5.0-7.0)	7.0 ± 0.8(6.0-8.0)	7.3 ± 0.8(6.0-9.0)
R	87.0 ± 2.8(81.0-91.0)	86.5 ± 3.2(84.0-93.0)	93.0 ± 2.8(88.0-97.0)
Stylet length	51.4 ± 2.4(46.7-54.8)	81.7 ± 4.8(71.1-87.3)	78.8 ± 4.0(71.1-83.2)
Length of stylet shaft	15.8 ± 1.3(14.2-18.3)	17.3 ± 1.9(14.2-20.3)	17.7 ± 1.8(14.2-20.3)
m	69.1 ± 3.0(64-73.1)	78.9 ± 1.4(76.7-80.5)	78.0 ± 2.2(75.0-82.1)
Stylet length as percentage of body length	12.9 ± 1.1(10.8-14.4)	11.9 ± 1.1(10.7-13.3)	11.9 ± 1.2(10.6-13.7)
Distance between stylet base and D.O.G	4.1 ± 1.4(2.0-6.1)	5.6 ± 2.8(0.0-10.2)	6.5 ± 2.5(0.0-10.2)
O	7.9 ± 2.6(3.8-12.0)	6.8 ± 3.4(0.0-12.2)	8.7 ± 1.1(7.5-10.3)
Distance lip region-centre median bulb	67.8 ± 4.6(60.9-73.1)	107.8 ± 7.0(101.5-119.8)	102.4 ± 5.4(95.4-109.6)
MB	72.6 ± 3.0(68.2-78.0)	73.9 ± 2.6(69.9-78.7)	72.0 ± 2.8(68.1-75.4)

Table 8. continued

Character/Ratio	*M. xenoplax* (n=4) [8] Los Alamos, AR	*M. xenoplax* (n=10) [8] Russian River, CA	*M. xenoplax* (n=2) [8] Mendocino, CA
L	573.5 ±59.3(527.3-660.6)	554.2±85.9(397.0-703.0)	598.5 ± 19.3(584.8-612.1)
Oesophagus length	141.1 ± 7.9(134.0-152.3)	124.3±11.3(105.6-138.0)	141.1 ± 7.2(136.0-146.2)
Tail	21.8 ± 3.0(20.3-26.4)	28.6 ± 5.2(20.3-34.5)	24.4 ± 2.9(22.3-26.4)
Maximum Body width	49.7 ± 4.2(44.7-54.8)	45.1 ± 2.8(40.6-48.8)	47.7 ± 1.4(46.7-48.7)
a	11.5 ± 0.5(10.9-12.1)	12.0 ± 1.6(9.8-15.1)	12.5 ± 0.0(12.5-12.6)
b	4.1 ± 0.2(3.9-4.3)	4.4 ± 0.5(3.8-5.2)	4.2 ± 0.1(4.2-4.3)
c	26.4 ± 1.1(25.0-27.3)	19.8 ± 3.1(16.5-25.1)	24.8 ± 3.7(22.2-27.4)
Distance lip region to vulva	542.0 ±57.3(490.7-624.1)	497.7 ±80.3(362.5-646.2)	558.9 ± 20.7(544.2-573.6)
Distance lip region to anus	551.7 ±56.4(507.0-634.2)	516.8 ±83.5(374.6-670.6)	574.1 ± 22.2(558.5-589.8)
V	94.5 ± 1.1(93.1-95.6)	91.4 ± 0.9(89.9-92.3)	93.4 ± 0.5(93.1-93.7)
V'	98.2 ± 1.0(96.8-99.2)	96.3 ± 0.6(95.4-97.2)	97.4 ± 0.1(97.2-97.5)
Distance lip region to end oesophageal gland	148.2 ±10.2(138.0-162.4)	129.5 ±10.9(109.6-142.1)	147.2 ± 7.2(142.1-152.3)
Body width at anus	34.0 ± 4.2(28.4-38.6)	29.5 ± 1.8(26.4-32.5)	31.5 ± 1.4(30.5-32.5)
b'	3.9 ± 0.1(3.8-4.1)	4.2 ± 0.5(3.6-5.1)	4.1 ± 0.1(4.0-4.1)
c'	0.6 ± 0.1(0.6-0.7)	0.9 ± 0.1(0.7-1.1)	0.8 ± 0.1(0.7-0.9)
Distance between vulva & post end of body	31.5 ± 6.1(24.4-36.5)	47.8 ± 7.9(34.5-56.8)	39.6 ± 1.4(38.6-40.6)
Body width at vulva	37.6 ± 3.5(34.5-42.6)	36.8 ± 2.8(32.5-40.6)	36.5 ± 0.0(36.5-36.5)
VL/VB	0.8 ± 0.2(0.7-1.1)	1.3 ± 0.2(1.1-1.7)	1.1 ± 0.0(1.1-1.1)
Rex	27.3 ± 1.0(26.0-28.0)	31.4 ± 2.5(27.0-36.0)	27.0 ± 1.4(26.0-28.0)
Roes	25.8 ± 1.5(25.0-28.0)	28.8 ± 3.8(23.0-36.0)	22.5 ± 0.7(22.0-23.0)
Rvan	0.5 ± 0.6(0.0-1.0)	2.7 ± 0.5(2.0-3.0)	1.0 ± 0.0(1.0-1.0)
Ran	5.5 ± 0.6(5.0-6.0)	6.8 ± 1.0(6.0-9.0)	4.5 ± 0.7(4.0-5.0)
RV	7.0 ± 0.8(6.0-8.0)	10.4 ± 1.2(9.0-13.0)	6.5 ± 0.7(6.0-7.0)
R	94.3 ± 2.6(92.0-98.0)	107.8 ± 4.6(99.0-114.0)	94.0 ± 1.4(93.0-95.0)
Stylet length	82.7 ± 1.9(81.2-85.3)	67.7 ± 6.6(60.9-77.1)	77.1 ± 5.7(73.1-81.2)
Length of stylet shaft	19.8 ± 1.0(18.3-20.3)	17.1 ± 1.5(14.2-18.3)	18.3 ± 2.9(16.2-20.3)
m	76.0 ± 1.7(75.0-78.6)	75.0 ± 3.3(70.0-78.4)	76.4 ± 2.0(75.0-77.8)
Stylet length as percentage of body length	14.5 ± 1.1(12.9-15.4)	12.8 ± 1.7(10.4-15.3)	12.9 ± 0.5(12.5-13.3)
Distance between stylet base and D.O.G	4.1 ± 1.7(2.0-6.1)	5.5 ± 2.8(2.0-10.5)	9.1 ± 1.4(8.1-10.2)
O	4.9 ± 1.9(2.4-7.1)	7.6 ± 4.6(3.0-17.2)	11.9 ± 2.7(10.0-13.9)
Distance lip region-centre median bulb	102.0 ± 5.3(97.4-109.6)	84.4 ± 7.3(73.1- 95.4)	100.5 ± 7.2(95.4-105.6)
MB	72.3 ± 0.3(72.0-72.7)	67.9 ± 2.4(64.6-71.2)	71.2 ± 1.5(70.1-72.2)

Table 8. continued

Character/Ratio	*M. xenoplax* (n=15) [8] Fresno,CA	*M. xenoplax* (n=2) [8] Livingston,CA	*M. xenoplax* (n=2) [8] Mendocino, CA
L	629.6 ±37.1(572.7-715.2)	650.0 ± 2.1(648.5-651.5)	598.5 ± 19.3(584.8-612.1)
Oesophagus length	141.4 ± 9.1(115.7-154.3)	147.2 ± 7.2(142.1-152.3)	141.1 ± 7.2(136.0-146.2)
Tail	25.6 ± 3.6(20.3-32.5)	26.4 ± 5.7(22.3-30.5)	24.4 ± 2.9(22.3-26.4)
Maximum Body width	48.4 ± 2.3(44.7-52.8)	49.7 ± 1.4(48.7-50.8)	47.7 ± 1.4(46.7-48.7)
a	13.2 ± 0.8(11.8-14.1)	13.1 ± 0.4(12.8-13.4)	12.5 ± 0.0(12.5-12.6)
b	4.5 ± 0.2(4.3-4.9)	4.4 ± 0.2(4.3-4.6)	4.2 ± 0.1(4.2-4.3)
c	26.5 ± 3.5(21.9-32.4)	25.2 ± 5.4(21.4-29.0)	24.8 ± 3.7(22.2-27.4)
Distance lip region to vulva	588.8 ±41.1(534.2-672.5)	611.4 ± 3.6(608.9-614.0)	558.9 ± 20.7(544.2-573.6)
Distance lip region to anus	605.8 ±41.6(548.4-688.8)	623.6 ± 3.6(621.1-626.2)	574.1 ± 22.2(558.5-589.8)
V	93.5 ± 0.5(92.7-94.3)	94.1 ± 0.9(93.5-94.7)	93.4 ± 0.5(93.1-93.7)
V'	97.2 ± 0.5(96.2-97.6)	98.0 ± 0.0(98.0-98.1)	97.4 ± 0.1(97.2-97.5)
Distance lip region to end oesophageal gland	147.8 ± 7.1(129.9-160.4)	153.3 ± 7.2(148.2-158.3)	147.2 ± 7.2(142.1-152.3)
Body width at anus	32.1 ± 1.9(28.4-36.5)	35.5 ± 1.4(34.5-36.5)	31.5 ± 1.4(30.5-32.5)
b'	4.3 ± 0.2(4.1-4.6)	4.2 ± 0.2(4.1-4.4)	4.1 ± 0.1(4.0-4.1)
c'	0.8 ± 0.1(0.7-0.9)	0.7 ± 0.1(0.6-0.8)	0.8 ± 0.1(0.7-0.9)
Distance between vulva & post end of body	41.0 ± 3.2(34.5-46.7)	38.6 ± 5.7(34.5-42.6)	39.6 ± 1.4(38.6-40.6)
Body width at vulva	38.4 ± 1.4(36.5-40.6)	39.6 ± 1.4(38.6-40.6)	36.5 ± 0.0(36.5-36.5)
VL/VB	1.1 ± 0.1(0.9-1.2)	1.0 ± 0.1(0.9-1.1)	1.1 ± 0.0(1.1-1.1)
Rex	27.3 ± 1.8(25.0-31.0)	26.0 ± 1.4(25.0-27.0)	27.0 ± 1.4(26.0-28.0)
Roes	23.9 ± 1.2(22.0-26.0)	22.5 ± 0.7(22.0-23.0)	22.5 ± 0.7(22.0-23.0)
Rvan	1.1 ± 0.5(0.0-2.0)	0.5 ±, 0.7(0.0-1.0)	1.0 ± 0.0(1.0-1.0)
Ran	5.3 ± 0.8(4.0-7.0)	4.5 ± 0.7(4.0-5.0)	4.5 ± 0.7(4.0-5.0)
RV	7.4 ± 0.7(6.0-9.0)	6.0 ± 0.0(6.0-6.0)	6.5 ± 0.7(6.0-7.0)
R	99.6 ± 3.0(92.0-104.0)	91.5 ± 6.4(87.0-96.0)	94.0 ± 1.4(93.0-95.0)
Stylet length	82.4 ± 4.0(73.1-91.4)	82.2 ± 1.4(81.2-83.2)	77.1 ± 5.7(73.1-81.2)
Length of stylet shaft	18.5 ± 1.3(16.2-20.3)	19.3 ± 1.4(18.3-20.3)	18.3 ± 2.9(16.2-20.3)
m	76.5 ± 2.4(72.2-80.0)	76.5 ± 2.2(75.0-78.0)	76.4 ± 2.0(75.0-77.8)
Stylet length as percentage of body length	13.1 ± 0.4(12.7-13.7)	12.6 ± 0.2(12.5-12.8)	12.9 ± 0.5(12.5-13.3)
Distance between stylet base and D.O.G	6.0 ± 1.8(2.0-8.1)	6.1 ± 5.7(2.0-10.2)	9.1 ± 1.4(8.1-10.2)
O	7.1 ± 2.8(2.8-10.3)	7.3 ± 6.9(2.5-12.2)	11.9 ± 2.7(10.0-13.9)
Distance lip region-centre median bulb	104.7 ± 5.4(91.4-113.7)	108.6 ± 4.3(105.6-111.7)	100.5 ± 7.2(95.4-105.6)
MB	74.4 ± 2.2(71.4-78.9)	73.8 ± 0.7(73.3-74.3)	71.2 ± 1.5(70.1-72.2)

Host: 1.Oak 2. Pine 3. Bermuda grass 4. Peach 5.Grass 6. Elm 7. River cane 8. Grapes vines

Table 9. Measurements and ratios of *Criconemoides informis.* Morphometrics of *C. mongolensis* and *C. morgensis* as related species is presented for comparison. Mean, standard deviation and range in µm.

Character/Ratio	*C. informis* (n=20)[1]	*C. mongolensis*[a]	*C. morgensis*[a]
L	459.4 ± 21.5(415.2-506.1)	380-470	510-700
Oesophagus length	92.0 ± 2.6(87.3-95.4)	-	-
Tail	12.6 ± 2.3(8.1-16.2)	-	-
Maximum Body width	31.7 ± 1.6(28.4-34.9)	-	-
a	14.5 ± 0.8(12.9-16.8)	-	-
b	5.0 ± 0.3(4.4-5.5)	-	-
c	37.9 ± 8.0(28.9-54.9)	-	-
Distance lip region to vulva	428.7 ± 20.6(385.1-475.2)	-	-
Distance lip region to anus	446.8 ± 20.7(402.2-493.1)	-	-
V	93.3 ± 0.5(92.5-94.2)	87-89	90-94
V'	95.9 ± 0.4(95.0-96.8)	-	-
Distance lip region to end oesophageal gland	97.5 ± 5.5(91.4-117.7)	-	-
Body width at anus	19.1 ± 1.7(15.4-21.9)	-	-
b'	4.7 ± 0.3(3.9-5.2)	-	-
c'	0.7 ± 0.1(0.5-0.8)	-	-
Distance between vulva & post end of body	30.7 ± 2.3(26.0-34.1)	-	-
Body width at vulva	25.9 ± 1.2(22.7-28.4)	-	-
VL/VB	1.2 ± 0.1(1.0-1.3)	1.62	0.9-1.8
Rex	20.6 ± 0.9(19.0-22.0)	18	28-39
Roes	17.0 ± 1.8(15.0-22.0)	-	-
Rvan	2.2 ± 0.5(1.0-3.0)	2	1-7
Ran	3.4 ± 0.7(2.0-4.0)	5	5-8
RV	6.5 ± 0.5(6.0-7.0)	8	7-13
R	71.3 ± 2.4(67.0-75.0)	57-61	100-133
Stylet length	47.4 ± 1.3(44.7-49.5)	70-76	74-91
Length of stylet shaft	12.1 ± 2.0(10.6-20.3)	-	-
m	74.5 ± 3.8(59.0-78.3)	-	-
Stylet length as percentage of body length	10.3 ± 0.5(9.5-11.1)	-	-
Distance between stylet base and D.O.G	3.9 ± 0.6(2.4-4.9)	-	-
O	8.1 ± 1.3(5.3-10.9)	-	-
Distance lip region-centre median bulb	63.8 ± 1.9(60.9-67.0)	-	-
MB	69.5 ± 2.2(63.8-74.4)	-	-

[1]. Host: Tulip-Poplar; [a] (Brzeski, 2002b).

Table 10. Nucleotides composition of the nuclear ITS1 ribosomal region (387 bp) of the populations of *Mesocriconema* and *Criconemoides* obtained in this study and those sequences obtained from GenBank.

Species	%A	%C	%G	%T	%G+C	%A+T
Mesocrionema xenoplax HM116057	24.03	25.58	26.87	23.51	52.45	47.55
Mesocrionema xenoplax HM116073	24.03	25.32	27.13	23.51	52.45	47.55
Mesocrionema xenoplax Ripon CA	23.77	25.06	26.61	24.29	51.68	48.06
Mesocrionema xenoplax Parlier CA	23.83	24.87	27.20	24.09	52.07	47.93
Mesocrionema xenoplax Los Alamos CA	23.77	25.58	27.13	23.26	52.71	47.03
Mesocrionema xenoplax Russian River CA	24.03	25.32	27.39	23.26	52.71	47.29
Mesocrionema xenoplax Mendocino CA	23.51	27.13	27.13	21.96	54.26	45.48
Mesocrionema xenoplax Fresno CA	23.77	26.36	27.13	22.74	53.49	46.51
Mesocrionema xenoplax Livingston CA	23.77	25.06	27.13	24.03	52.20	47.80
Mesocrionema xenoplax AR	23.26	21.45	27.65	27.65	49.10	50.90
Mesocrionema xenoplax AR	23.77	26.36	27.39	22.48	53.75	46.25
Mesocrionema xenoplax NC	23.77	25.32	27.39	23.51	52.71	47.29
Mesocrionema xenoplax AR	23.77	25.32	27.13	23.77	52.45	47.55
Mesocrionema xenoplax AR	23.77	25.32	26.87	24.03	52.20	47.80
Mesocrionema xenoplax AR	23.77	25.58	26.61	24.03	52.20	47.80
Mesocrionema xenoplax AR	22.80	27.46	29.53	20.21	56.99	43.01
Mesocrionema curvatum HM116066	23.77	25.32	26.87	24.03	52.20	47.80
Mesocrionema curvatum AR	24.55	25.06	24.55	25.84	49.61	50.39
Mesocrionema ornatum KS	24.81	23.51	27.13	24.55	50.65	49.35
Mesocrionema crenatum MO	24.74	25.78	26.30	23.18	52.08	47.92
Mesocrionema vadense AR	24.29	24.55	27.39	23.77	51.94	48.06
Mesocrionema vadense AR	24.81	25.32	26.36	23.51	51.68	48.32
Mesocrionema kirjanovae AR	25.32	25.06	27.91	21.45	52.97	46.77
Mesocrionema ozarkiense AR	23.26	23.26	27.65	25.84	50.90	49.10
Mesocrionema surinamense AR	21.71	27.91	28.94	21.45	56.85	43.15
Mesocrionema onoense AR	28.94	26.36	25.06	19.64	51.42	48.58
Mesocrionema sphaerocephala NC	24.55	22.22	25.84	27.13	48.06	51.68
Criconemoides informis TN	25.77	28.53	22.70	22.70	51.23	48.47
Heterorhabditis indica JQ178381	24.87	22.51	24.87	27.75	47.38	52.62

FIGURES

Fig. 1 Light micrographs of *Mesocriconema ozarkiense* n. sp. A) Entire female. B, C, D) Anterior body portion showing lip region pattern and submedian lobes. E) Annuli margins. F, G, H) Posterior portion showing vulva, vagina and tail shape. Arrows showing crenate annuli margins in tail.

Fig. 2 SEM micrograghs. A) Lateral view of lip region showing submedian lobes. B,C) Face view of lip region showing submedian lobes and labial plates. D) Posterior region. E) Detail of anterior vulval lip and anus. F) Tail end annuli.

Fig. 3 Camera lucida drawings of *Mesocriconema ozarkiense* n. sp. A) Entire female. B) Anterior body portion. C, D) Posterior body portion showing vulva, vagina, tail shape and crenate annuli.

Fig. 4 Light micrographs of *Mesocriconema crenatum*. A) Entire female. B) Anterior body portion showing lip region pattern and submedian lobes. C) Posterior body portion showing open vulva and tail shape. D) Annuli margins crenate.

Fig. 5 Light micrographs of *Mesocriconema curvatum*. A) Entire female. B) Anterior body portion showing lip region pattern and submedian lobes. C) Posterior body portion showing open vulva and tail shape. D) Annuli margins.

Fig. 6 Light micrographs of *Mesocriconema kirjanovae*. A) Entire female. B) Anterior body portion showing lip region pattern and submedian lobes. C) Posterior body portion showing open vulva and tail shape. D) Annuli margins. E) Anastomoses. F) Vulva detail. G) Anterior vulva lip and lobe.

Fig. 7 Light micrographs of *Mesocriconema ornatum*. A) Entire female. B) Anterior body portion showing lip region pattern and submedian lobes. C) Posterior body portion showing vulva, vagina, tail shape and folded annulus. D) Body annuli margins.

Fig. 8 Light micrographs of *Mesocriconema onoense*. A) Entire female. B) Anterior body portion showing lip region pattern. Arrows showing submedian lobes. C) Posterior body portion showing vulva, vagina and tail shape. Arrows showing last annulus folded for the previous annulus

Fig. 9 Light micrographs of *Mesocriconema vadense*. A) Entire female. B, C, D) Anterior body portion showing lip region pattern, submedian lobes and labial plates. Arrows showing submedian lobes E, F, G, H, I) Posterior body portion showing vulva, vagina, tail shape. J) Margins annuli. K) Anastomoses.

Fig. 10 Light micrographs of *Mesocriconema sphaerocephala* A) Entire female. B) Anterior body portion lip region pattern and showing submedian lobes. C) Anastomoses. D) Posterior body portion showing vulva and tail shape E) anastomoses in tail.

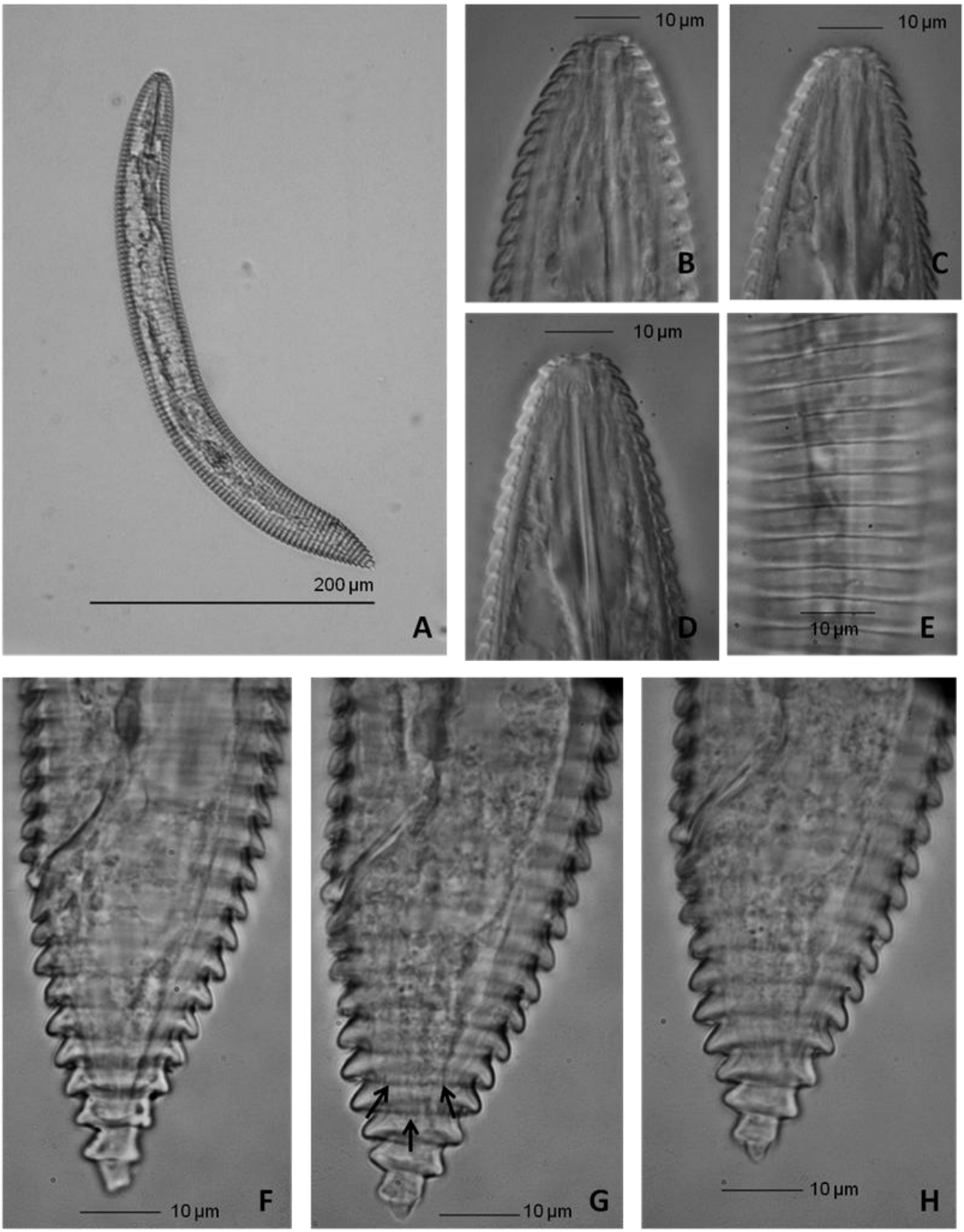
Fig. 11 Light micrographs of *Mesocriconema surinamense*. A) Entire female. B, C, D, E,) Anterior body portion showing lip region pattern and submedian lobes and labial plates. F, G, H) Posterior body portion showing vulva, vagina, tail shape.

Fig. 12 Light micrographs of *Mesocriconema xenoplax*. A) Entire female. B) Anterior portion. C, F, G, H,J) Anterior body portion showing lip region pattern and submedian lobes, first lip annulus and labial plates. D,E,) Posterior body portion showing vulva, vagina and tail shape. I) Margins annuli and anastomoses.

Fig. 13 Light micrographs of *Criconemoides informis*. A) Entire female. B, C, D, E) Anterior body portion showing first annulus and oral opening. F, G) Posterior body portion showing vulva, vagina and tail shape.

Fig. 14 Consensus tree from the maximum parsimony bootstrap analysis for ITS1-rDNA region of *Mesocriconema* and *Criconemoides*. The percentages of bootstrap replicates supporting the clades are indicated at the branch points.

Fig. 15 Best maximum likelihood tree for ITS1-rDNA region of *Mesocriconema* and *Criconemoides*. Changes lengths are proportional to the number of inferred changes.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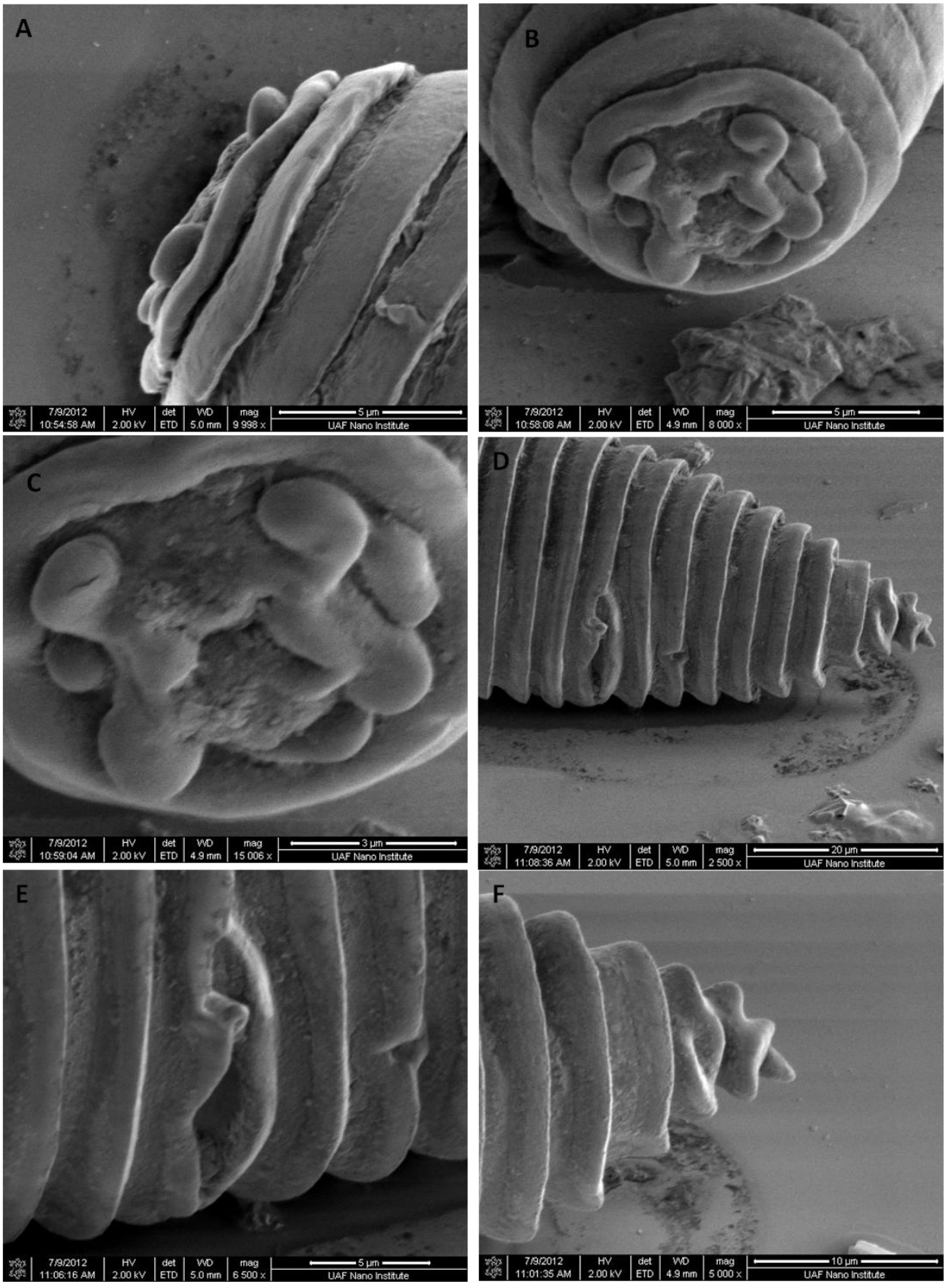
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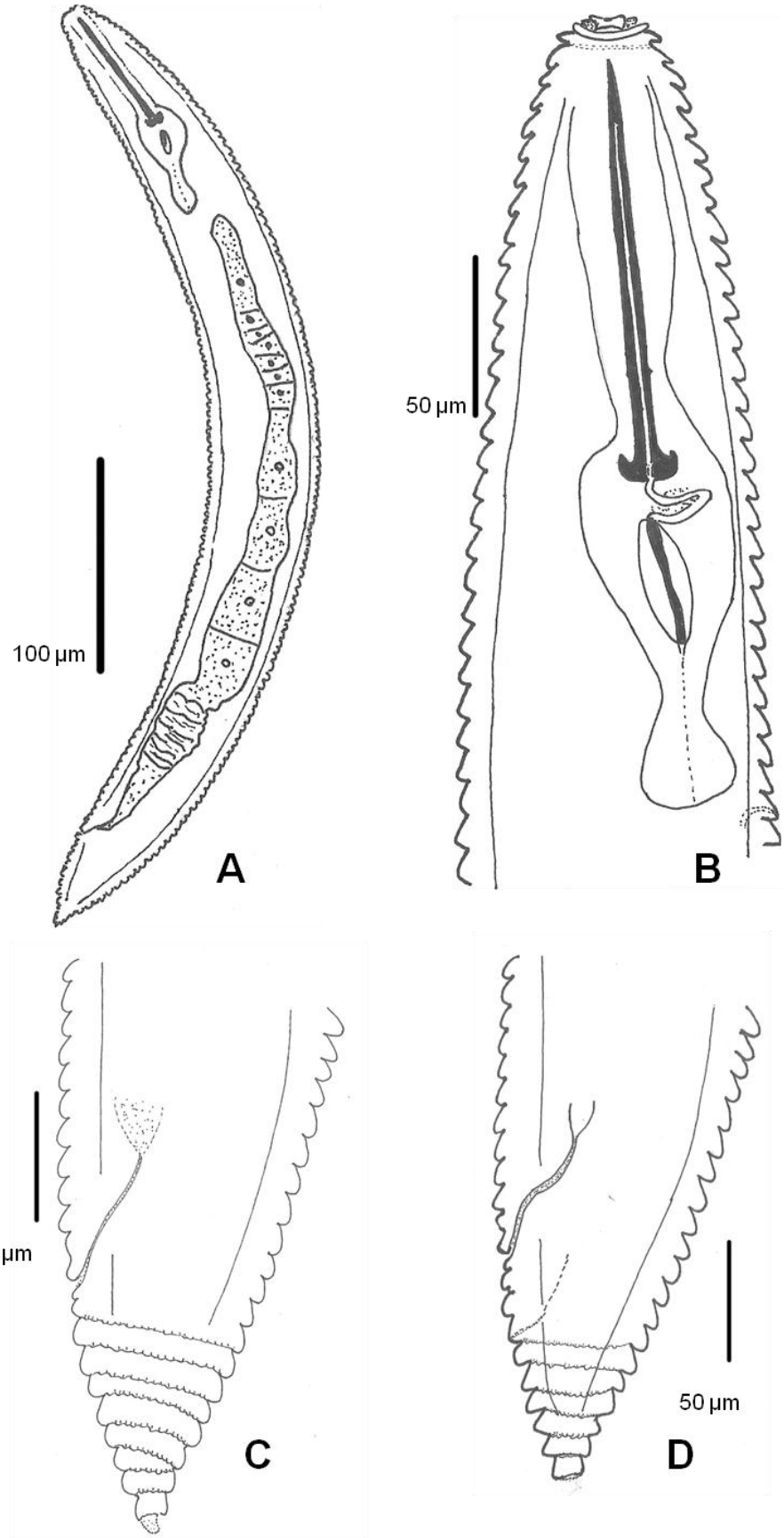
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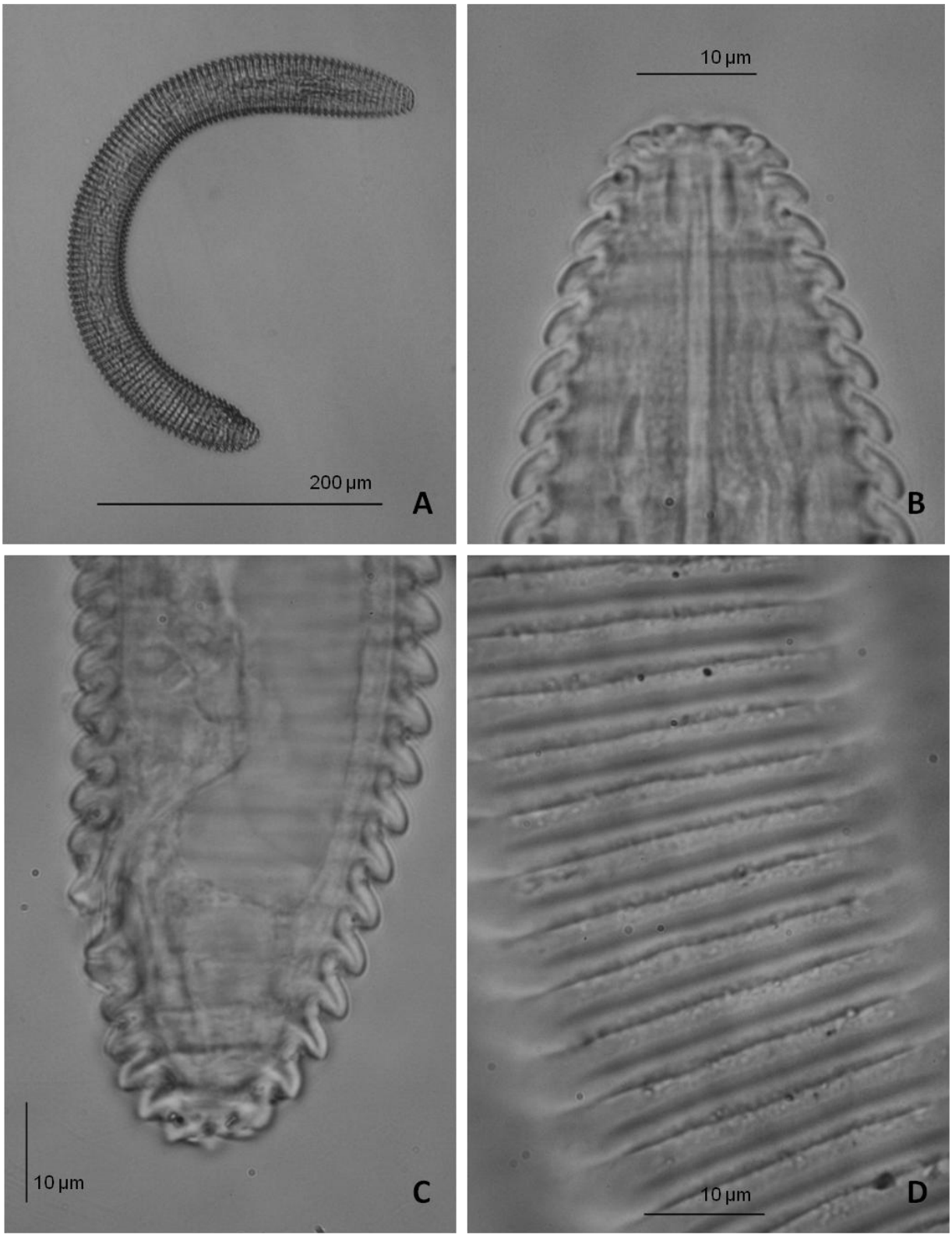
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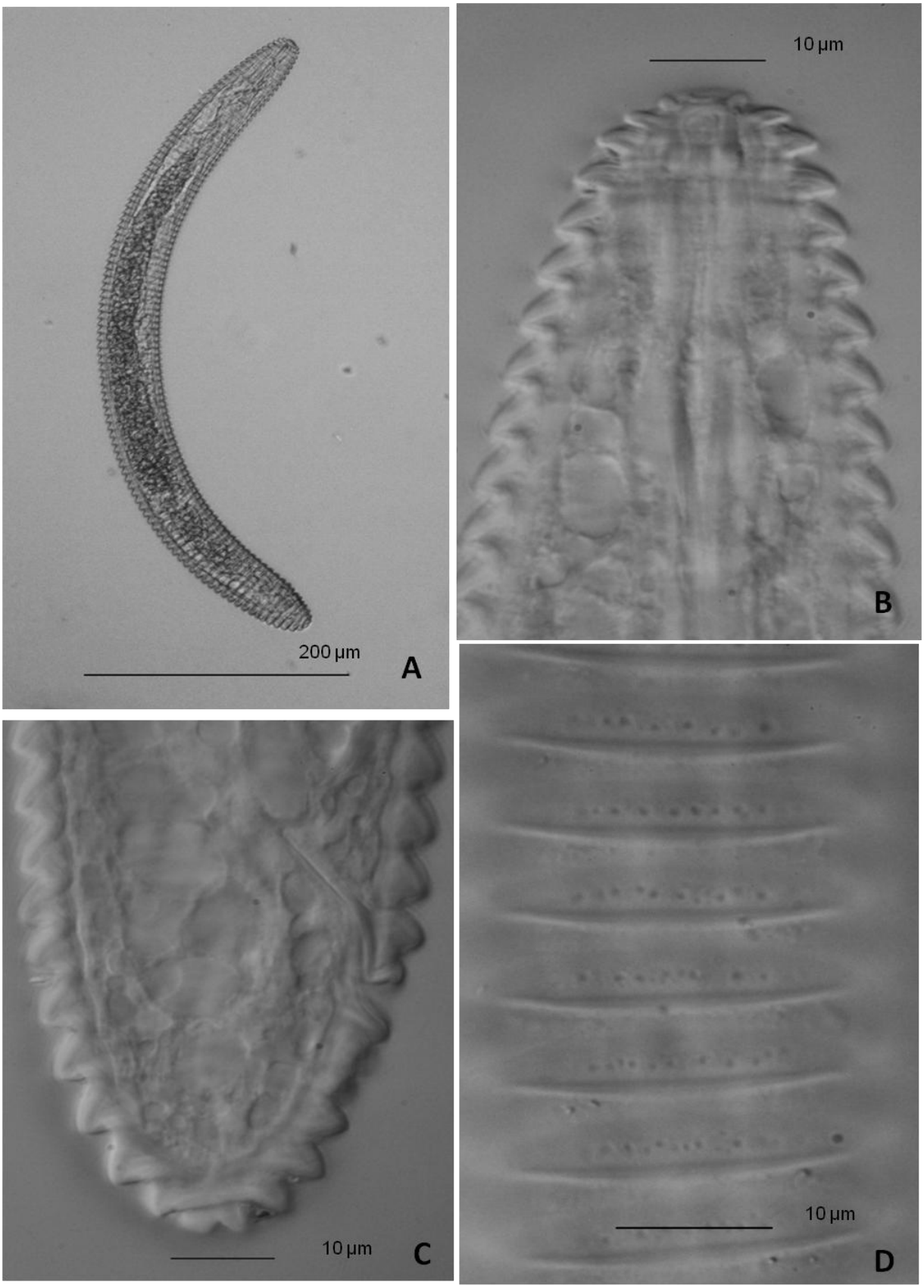
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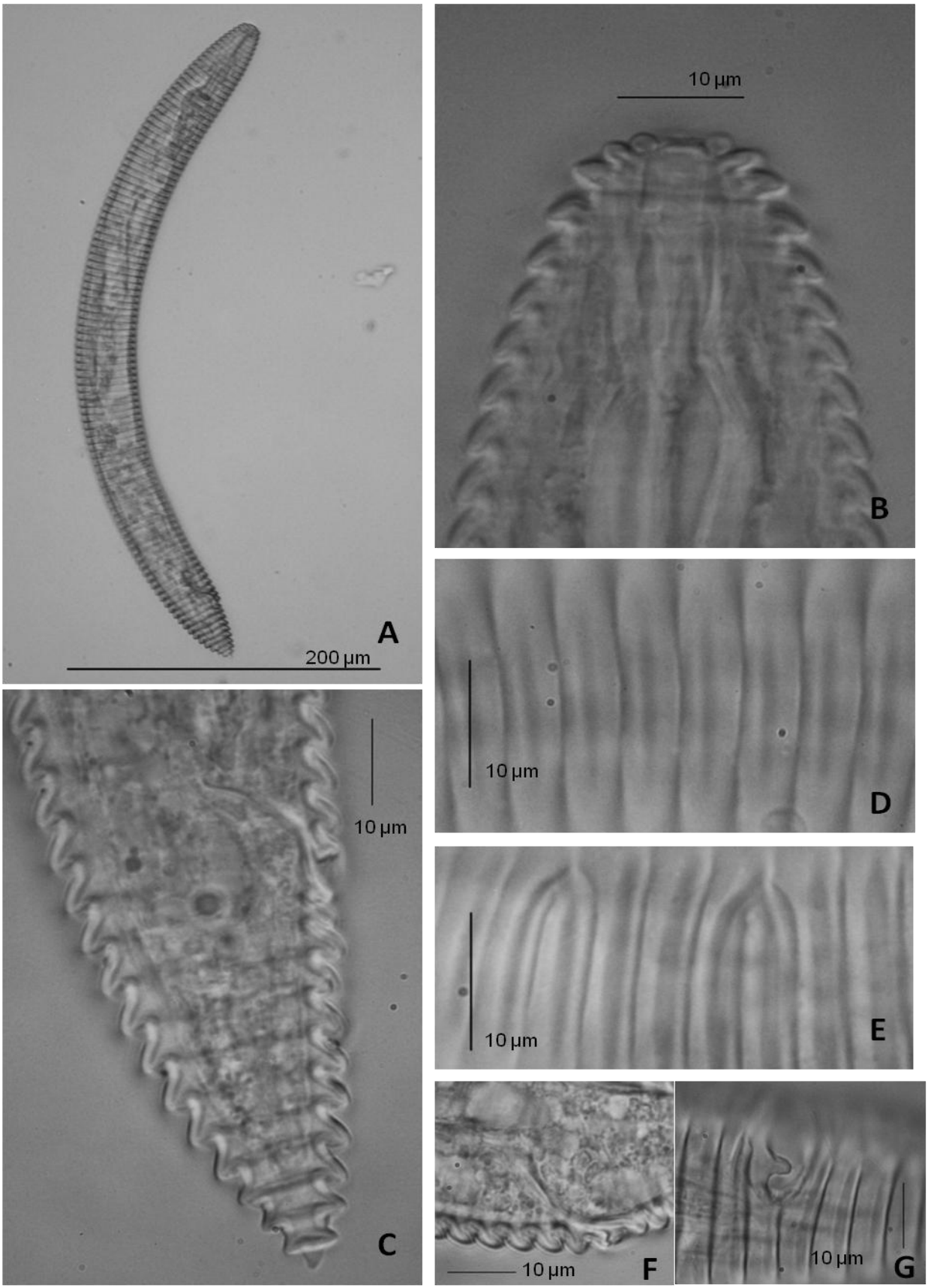
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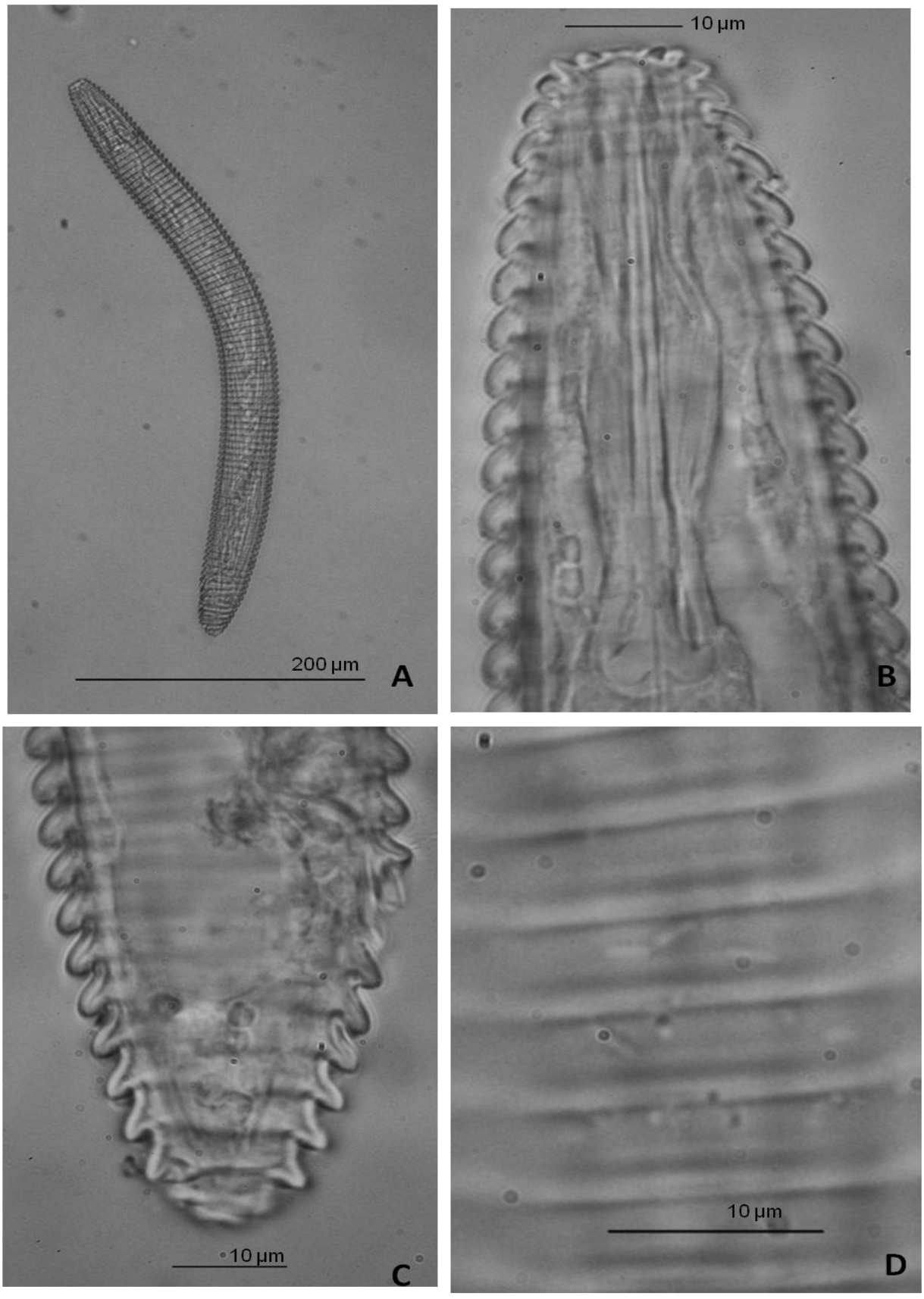
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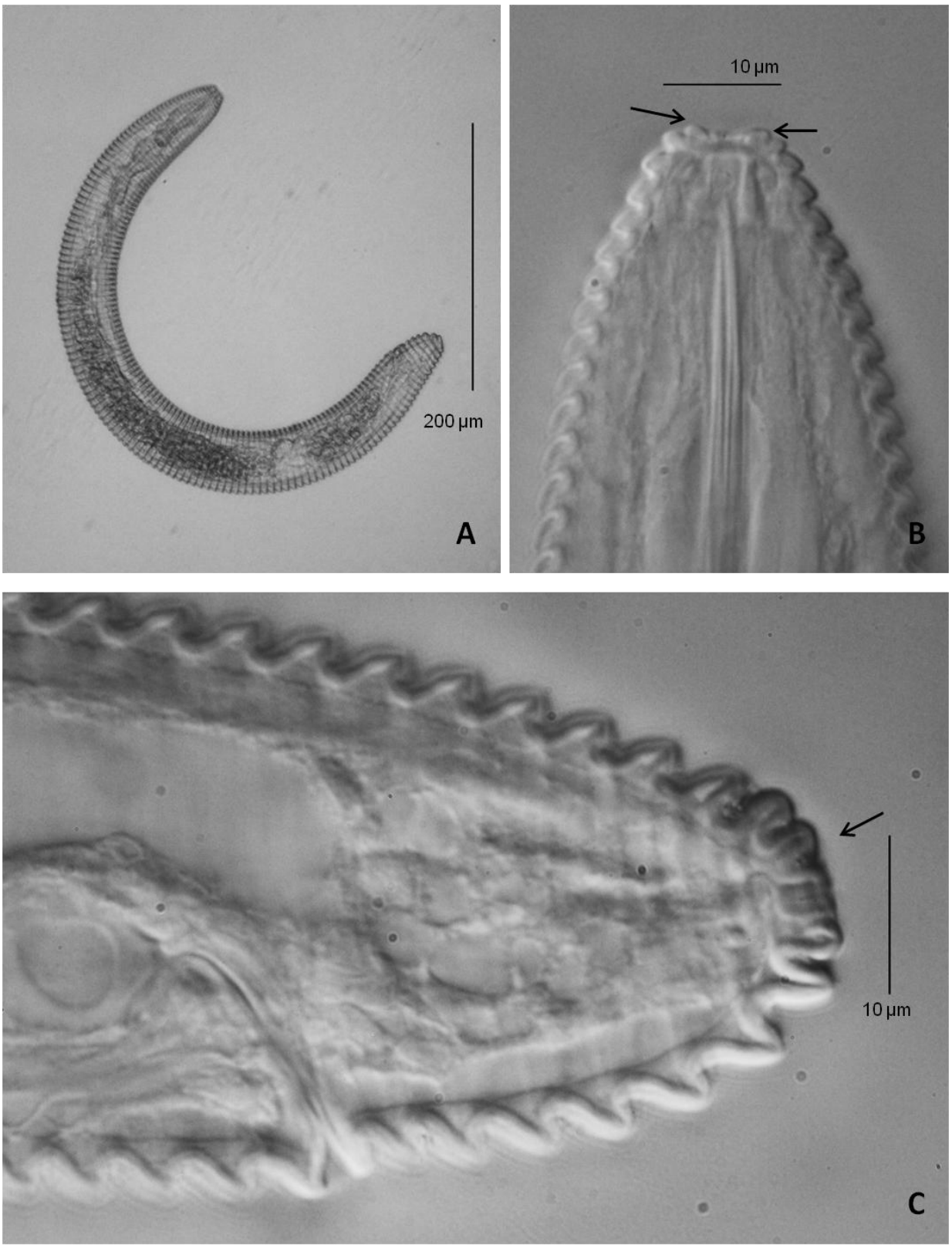
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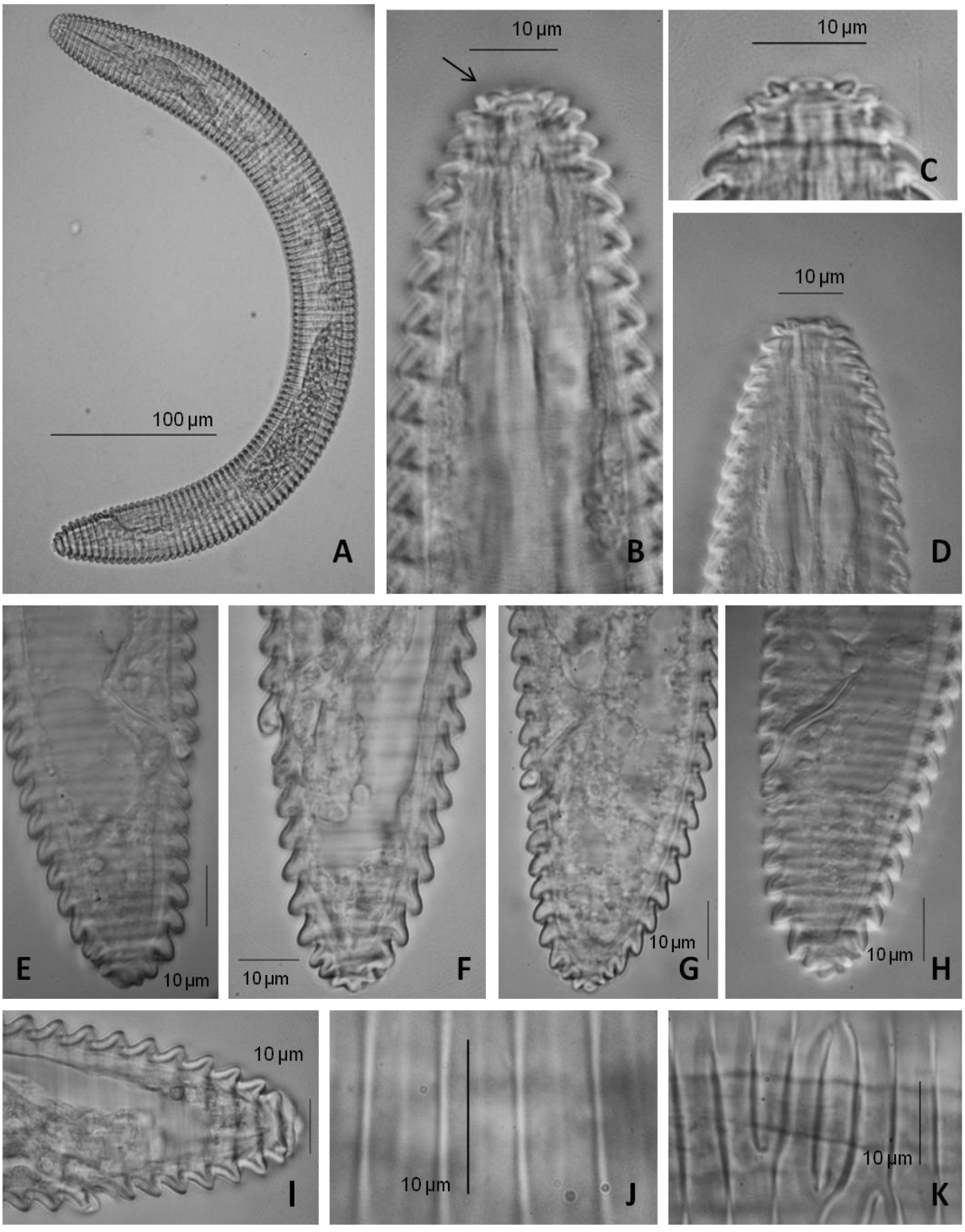
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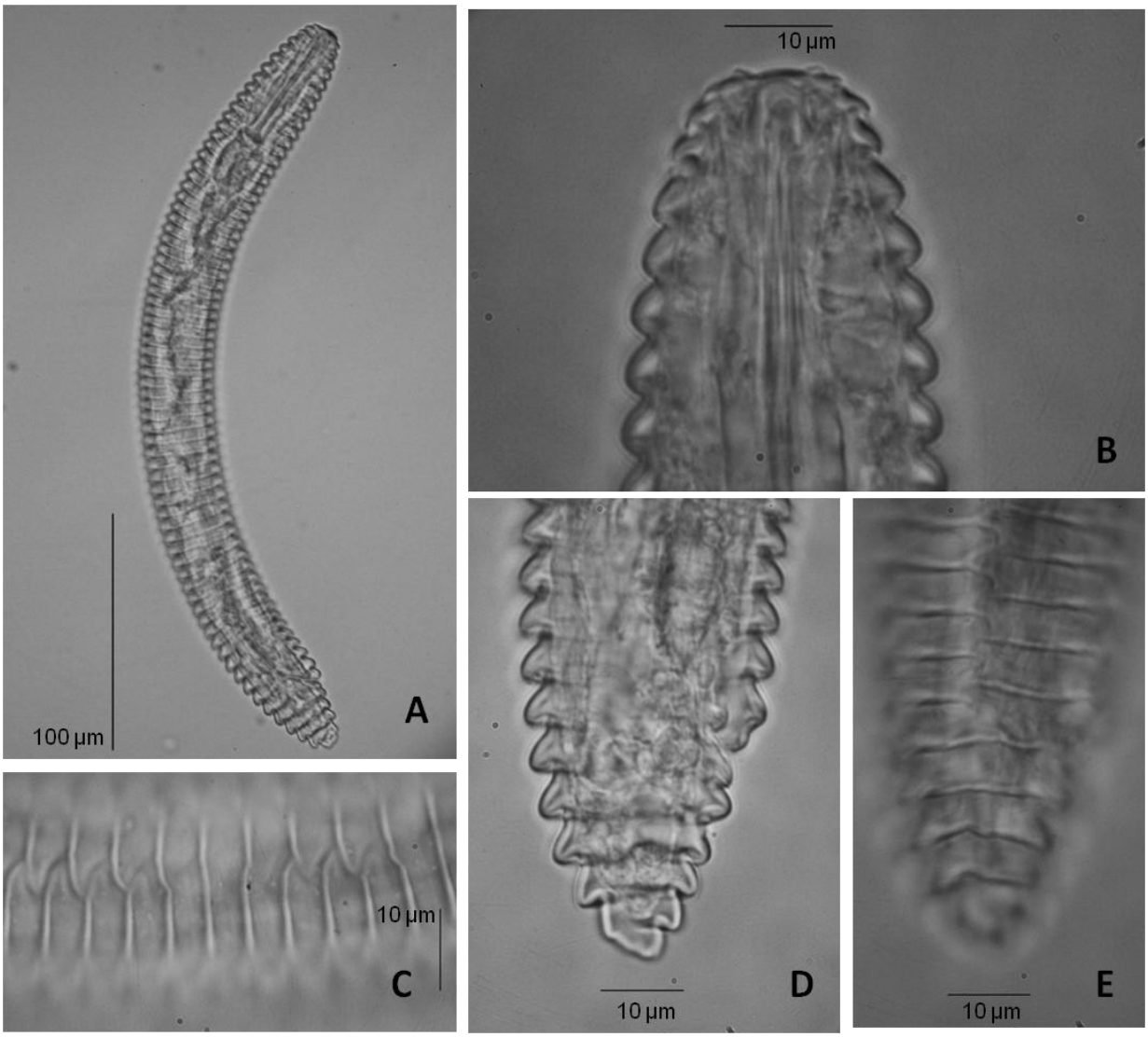
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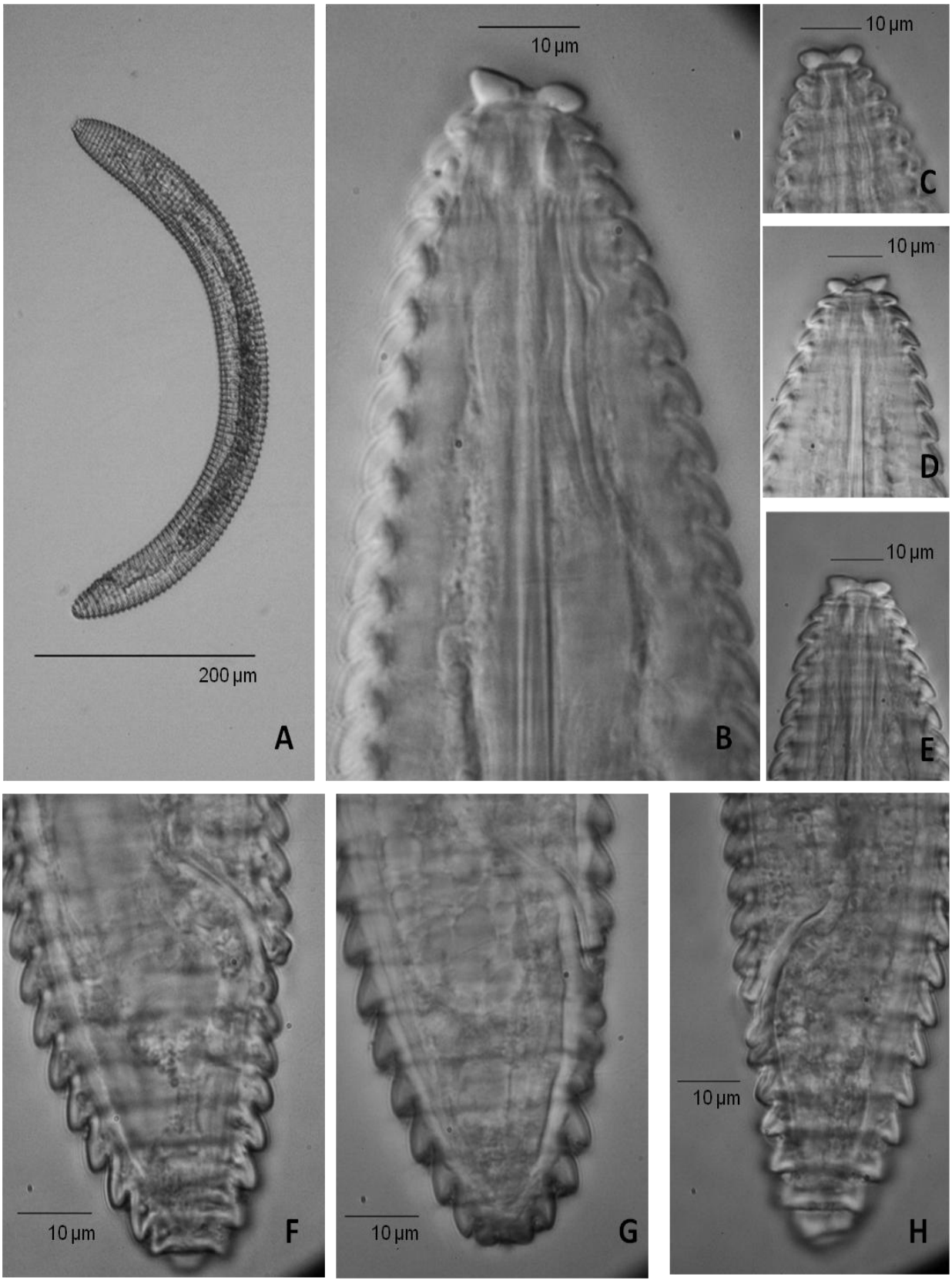
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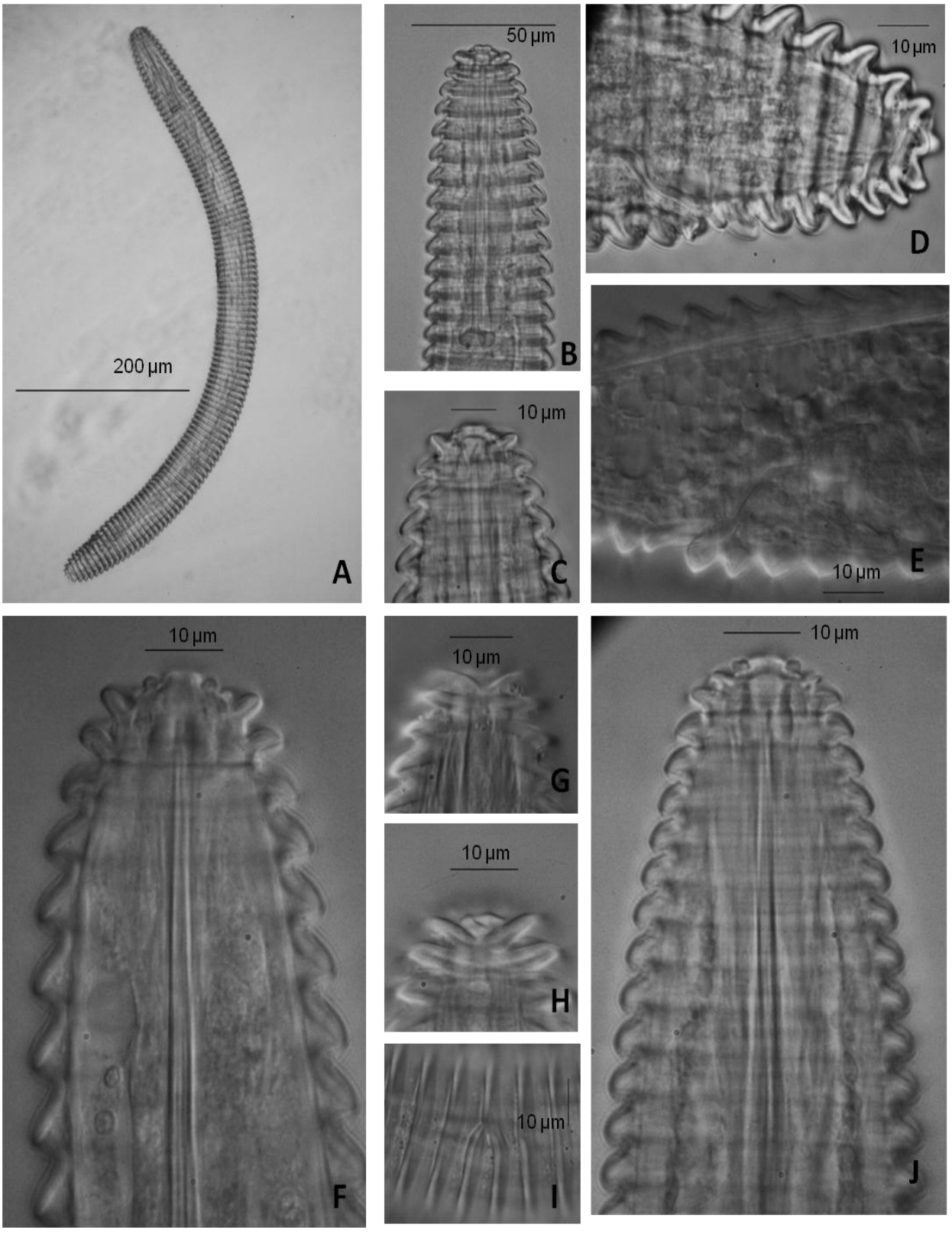
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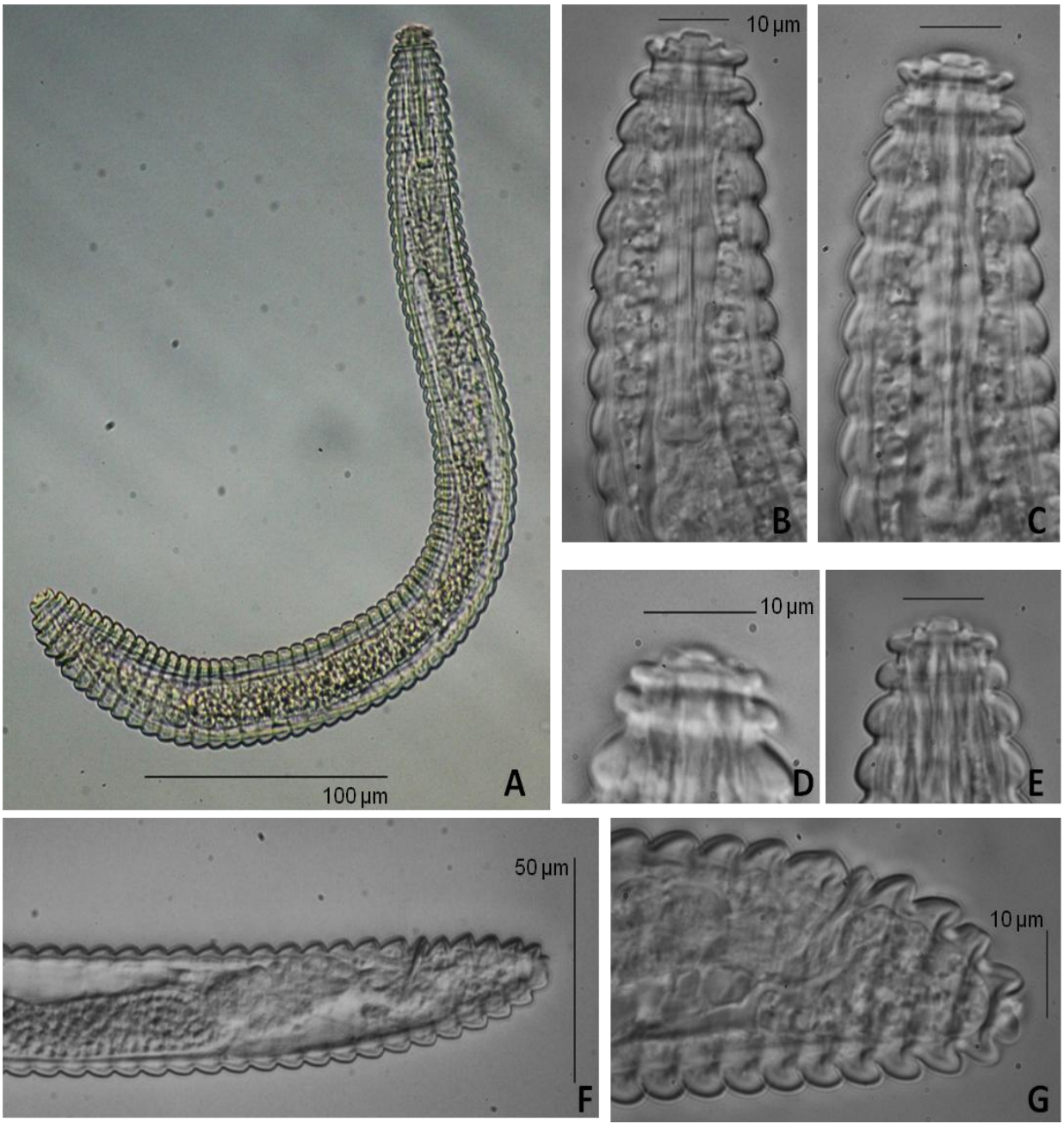
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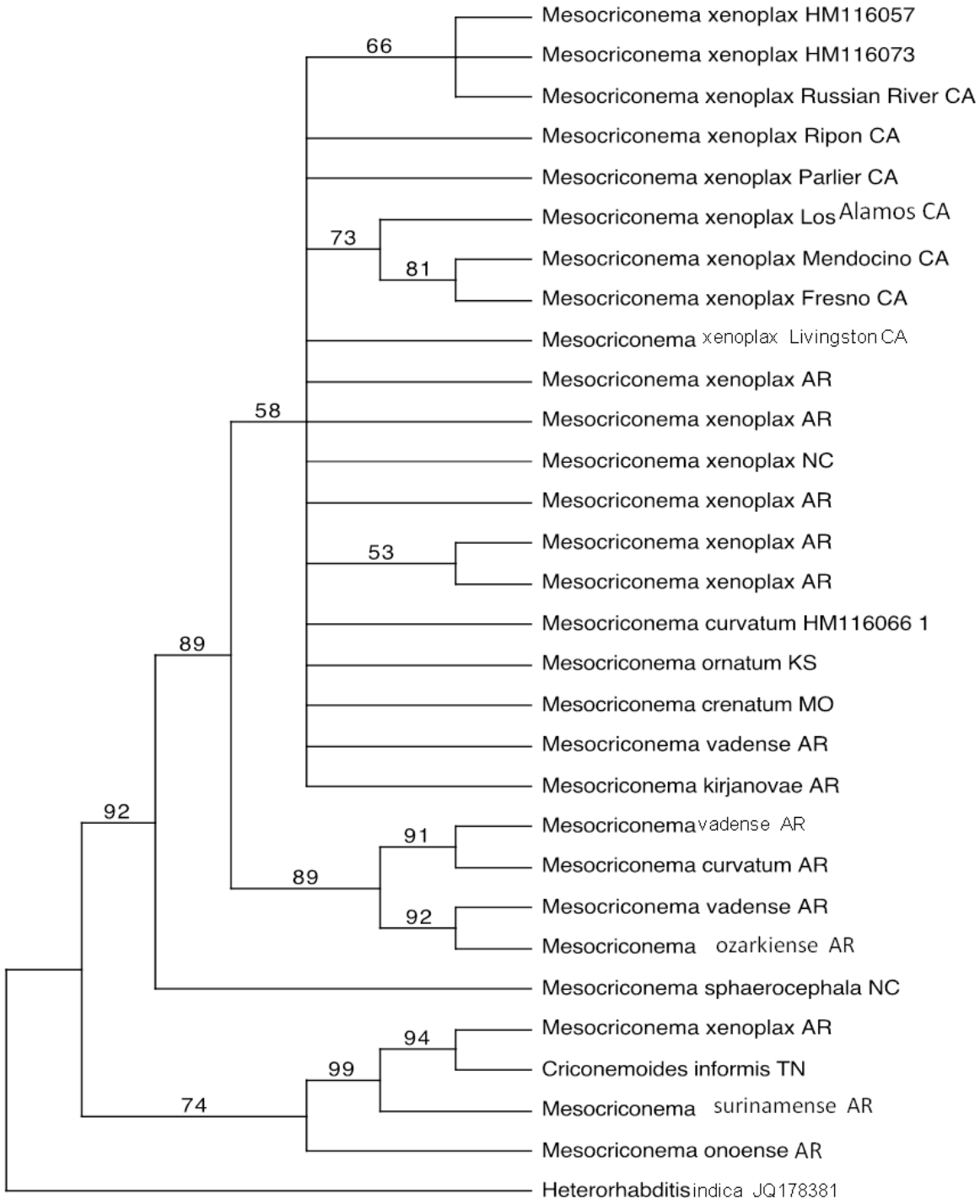
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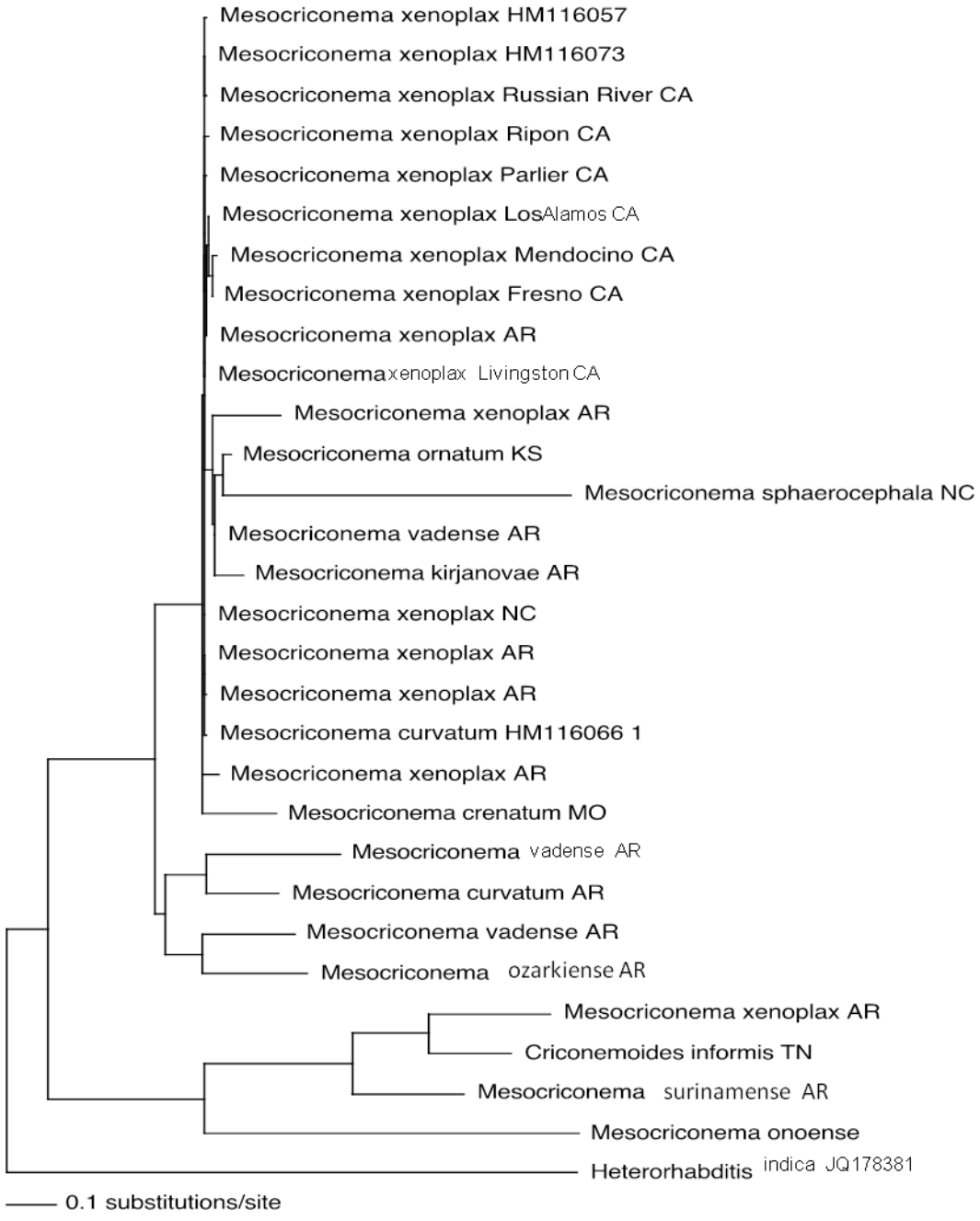
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APPENDIX

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TAXONOMIC AND MOLECULAR IDENTIFICATION OF *Bakernema*, *Criconema*, *Hemicriconemoides*, *Ogma* and *Xenocriconemella* SPECIES (NEMATODA: CRICONEMATIDAE)

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The authors thank Ernest Bernard, University of Tennessee; Jason Stanley, Florida Department of Agriculture and consumer services-Division of Plant Industry; Paula Agudelo, Clemson University; and Weimen Ye, North Carolina Department of Agriculture for providing specimens for this study.

This paper was edited by
Running head: TAXONOMIC AND MOLECULAR IDENTIFICATION OF (NEMATODA: CRICONEMATIDAE) SPECIES

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 characterize 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 cuticule 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; *Blandicephalenema* Mehta & Raski, 1971; *Pateracephalanema* Mehta & Raski, 1971 and *Hemicriconemoides* Chitwoodi & Birchfield, 1957.

Regardless of the previous study, Loof (1988), Sidiqui (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) reconstruct the phylogenetic position of these species in the Criconematinae using the analysis of this gene. Known species previously identified in early years have been redescribed 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) (*E*trex Garmin, Olathe, KS) was used to identify the location. 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; 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 5µl of molecular grade water (BDH Chemicals, Chester, PA) and stored at -80^{o}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'-TTGATTACGTCCCTGCCCTTT-3') (Vrain et al., 1992) and rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA- 3') (Cherry et al., 1997). This PCR primer pair ampliflied 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 (0.2mM 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 ^{o}C for 2 minutes, then 40 cycles of denaturation at 94 ^{o}C

for 45 seconds, annealing at 52 or 56 ^{o}C for 45 seconds and extension at 72 ^{o}C for 60 seconds. A final extension for 5 minutes at 72 ^{o}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^{o}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 performanced 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 x 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% mayority 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. stricthatecus* 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 indistint, 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 ¾ of the nematode length close to stylet knobs. Tail slightly conoid to bluntly rounded surrounded by a thin cuticular sheath. Anus subterminal.

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 distint 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^{o} 08.075 min-W 094^{o} 21.511 min; N 36^{o} 09.979 min-W 094^{o} 26.061 min; N 36^{o} 06.190 min -W 094^{o} 20.666 min.; N 36^{o} 06.319 min-W 094^{o} 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^{o} 43.452 min-W 090^{o} 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.

*Paratype*s (*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 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 ¾ 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^{o} 35.655 min-W 092^{o} 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.

*Paratype*s (*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.

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 synonimized 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 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 ¾ of the nematode length close to metacorpus. 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 redescription (Ebsary, 1978b; Wu, 1965) and a specific ITS1 sequence (JQ708136) 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.

Criconema mutabile (Taylor, 1936) Raski & Luc, 1985.

(Tabla 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 ¾ 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^{o} 08.108 min-W 094^{o} 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 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, prodelphic, outstretched, spermatheca full of sperm, sometimes reaching more than ¾ 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^{o} 08.053 min-W 094^{o} 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.

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 retrorse 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 ¾ 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 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 that 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 ¾ 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 indistint. 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 ¾ of the nematode length close to stylet knobs with one or two flexures. Tail sharply 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 tulip-poplar (*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^{o} 06.190 min-W 094^{o} 20.666 min and N 36^{o} 06.309 min-W 094^{o} 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 monodelphic, prodelphic, outstretched, spermatheca full of sperm, sometimes reaching more than ¾ 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 analize 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 decalineatum, 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 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 subterminal anus.

Population of *Hemicriconemoides chitwoodi* from Arkansas was cluster together with *H. californianum* with a genetic divergence 6%. Genetic divergence between populations of *H. chitwoodi* form 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 µm (Sty%L=15-18), body length 318- 418 µm in *C. mutabile* and stylet length 71-100 µm (Sty%L=28-40) and body length 182-312 µm 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) perfomed 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 *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).

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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TABLES

Table 1. Measurements and ratios of paratypes and holotypes of *Criconema arkaense n.sp*. and *C. warrenense* n.sp. Mean, standard deviation and range in µm.

Character/Ratio	*C. arkaense* Host: hackberry (n=19)	*C. arkaense* Host: *Paspalum* sp.(n=20)	*C. arkaense* Host: oat grass (n=16) Type population
L	529.8 ± 36.0(459.4-609.4)	458.5 ± 47.6(381.8-578.8)	507.9± 50.3 (427.3-593.9)
Oesophagus length	121.3 ± 10.6(81.2-132.0)	119.1 ± 8.6(107.6-140.1)	126.0± 8.1 (111.7-140.1)
Tail	7.5 ± 2.9(3.3-13.0)	17.2 ± 3.0(10.2-22.3)	8.6 ± 2.4(4.1-13.8)
Maximum Body width	49.9 ± 5.2(40.6-56.8)	53.2 ± 2.4(46.7-56.8)	48.6 ± 3.1 (44.7-57.7)
a	10.7 ± 1.2(9.5-14.3)	8.6 ± 1.0(7.5-10.6)	10.5± 1.0(8.5-12.2)
b	4.4 ± 0.7(3.8-6.7)	3.9 ± 0.3(3.6-4.8)	4.0 ± 0.3(3.5-4.6)
c	80.5 ± 28.7(38.5-145.2)	27.8 ± 5.3(20.9-35.6)	64.1 ± 23.5(37.1-135.8)
Distance lip region end to vulva	500.5 ± 34.3(437.0-576.9)	434.9 ± 53.1(381.7-552.4)	475.2± 47.8 (402.9-559.8)
Distance lip region end to anus	522.2 ± 36.2(454.5-600.4)	443.2 ± 54.4(387.8-562.5)	499.3± 50.2 (419.2-582.6)
V	94.5 ± 1.2(90.9-96.6)	94.5 ± 0.7(93.3-95.4)	93.5 ± 0.6(92.5-94.4)
V'	95.8 ± 1.3(91.8-97.6)	98.1 ± 0.3(97.7-98.6)	95.2 ± 0.7(93.6-96.1)
Distance lip region to end oesophageal gland	128.0 ± 11.8(85.3-142.1)	125.3 ± 8.6(111.7-146.2)	131.7 ± 8.6(115.7-146.2)
Body width at anus	20.1 ± 4.8(13.8-28.4)	34.5 ± 2.6(28.4-38.6)	19.5 ± 4.8(13.0-28.4)
b'	4.7 ± 2.5(3.5-14.6)	3.7 ± 0.3(3.3-4.5)	3.9 ± 0.3(3.3-4.5)
c'	0.4 ± 0.2(0.2-0.8)	0.5 ± 0.1(0.4-0.6)	0.5 ± 0.1(0.2-0.7)
Distance between vulva & post end of body	29.3 ± 6.8(18.3-50.8)	25.1 ± 2.9(20.3-30.5)	32.7 ± 4.0(24.4-39.0)
Body width at vulva	38.8 ± 3.9(30.5-44.7)	41.4 ± 1.9(36.5-44.7)	37.5 ± 2.7(32.5-43.9)
VL/VB	0.8 ± 0.1(0.5-1.3)	0.6 ± 0.0(0.5-0.7)	0.9 ± 0.1(0.7-1.0)
Rex	17 ± 1.3(13-19)	17 ± 0.9(15-18)	18 ± 0.9(16-19)
Roes	15 ± 1.0(12-16)	17 ± 1.4(14-20)	17 ± 1.0(15-18)
Rvan	1 ± 0.5(1-2)	2 ± 0.4(1-2)	2 ± 0.0(2-2)
Ran	1 ± 0 (1-1.)	1 ± 0(1-1)	2 ± 0.5(1-2)
RV	4 ± 0.6(3-5)	4 ± 0.5(3-4)	5 ± 0.5(4-5)
R	54 ± 4.1(49-67)	53 ± 3.0(50-62)	54 ± 3.2(48-58)
Stylet length	79.3 ± 6.6(71.1-99.5)	81.0 ± 5.3(69.0-89.3)	82.3 ± 3.6(77.0-89.1)
Length of stylet shaft	20.0 ± 2.1(14.2-22.3)	19.9 ± 1.4(16.2-22.3)	19.9 ± 1.3(17.9-21.9)
m	74.7 ± 2.8(70.3-81.1)	75.3 ± 1.3(72.5-76.9)	75.8 ± 1.0(74.0-77.5)
stylet length as percentage of body length	15.0 ± 1.4(13.2-19.9)	17.9 ± 1.5(15.1-20.4)	16.3 ± 1.4(14.8-19.7)
Distance between stylet base and D.O.G	3.8 ± 1.9(2.0-10.2)	2.8 ± 1.4(2.0-6.1)	2.8 ± 1.4(0.8-5.7)
O	4.9 ± 2.5(2.0-13.2)	3.3 ± 1.3(2.3-5.3)	3.4 ± 1.7(1.0-7.4)
Distance lip region-centre median bulb	92.5 ± 4.9(83.2-103.5)	93.7 ± 5.9(85.3-105.6)	95.5 ± 6.6(77.1-105.6)
MB	77.0 ± 9.3(68.3-112.5)	79.4 ± 1.8(75.4-83.0)	75.9 ± 3.4(69.1-82.0)

Table 1. continued

Character/Ratio	*C. arkaense* Host: maple (n=20)	*Criconema warrenense* (n=17)	*Criconema arkaense* Holotype	*Criconema warrenense* Holotype
L	507.7 ± 48.7(427.3-593.9)	469.9 ± 54.7(384.8-548.5)	503.03	475.75
Oesophagus length	125.4 ± 8.3(111.7-140.1)	112.2 ± 5.1(103.5-119.8)	115.71	115.71
Tail	8.6 ± 2.4(4.1-13.8)	27.2 ± 3.2(22.3-32.5)	7.31	26.39
Maximum Body width	49.0 ± 3.4(44.7-57.7)	47.2 ± 2.5(42.6-50.8)	55.22	46.69
a	10.4 ± 1.1(8.5-12.2)	10.0 ± 1.4(7.9-11.7)	9.11	10.19
b	4.1 ± 0.3(3.5-4.6)	4.2 ± 0.5(3.5-4.9)	4.35	4.11
c	64.4 ± 22.8(37.1-135.8)	17.3 ± 2.4(14.6-23.1)	68.81	18.03
Distance lip region end to vulva	474.9 ± 46.3(402.9-559.8)	433.2 ± 55.1(352.4-511.9)	470.55	443.27
Distance lip region end to anus	499.1 ± 48.6(419.2-582.6)	442.1 ± 55.9(358.5-518.0)	495.72	449.36
V	93.5 ± 0.6(92.5-94.4)	92.2 ± 0.8(91.2-93.3)	93.54	93.17
V'	95.2 ± 0.7(93.6-96.1)	98.0 ± 0.5(97.5-98.8)	94.92	98.64
Distance lip region to end oesophageal gland	131.0 ± 8.8(115.7-146.2)	117.2 ± 5.3(107.6-123.8)	119.77	121.80
Body width at anus	19.4 ± 4.6(13.0-28.4)	35.4 ± 2.0(32.5-38.6)	17.86	34.51
b'	3.9 ± 0.3(3.3-4.5)	4.0 ± 0.5(3.3-4.8)	4.20	3.91
c'	0.4 ± 0.1(0.2-0.7)	0.8 ± 0.1(0.7-0.9)	0.41	0.76
Distance between vulva & post end of body	32.7 ± 3.9(24.4-39.0)	35.9 ± 3.8(30.5-40.6)	32.48	32.48
Body width at vulva	37.9 ± 3.1(32.5-43.9)	38.9 ± 1.8(36.5-42.6)	43.85	38.57
VL/VB	0.9 ± 0.1(0.7-1.0)	0.9 ± 0.1(0.7-1.1)	0.74	0.84
Rex	18 ± 0.9(16-19)	16 ± 2.0(12-20)	18	12
Roes	17 ± 0.9(15-18)	14 ± 1.7(12-18)	16	12
Rvan	2 ± 0.0(2-2)	3 ± 0.0(3-3)	2	3
Ran	2 ± 0.5(1-2)	1 ± 0.0(1-1)	1	1
RV	5 ± 0.5(4-5)	5 ± 0.5(4-5)	4	4
R	54 ± 3.1(48-58)	48 ± 1.7(45-51)	54	45
Stylet length	82.7 ± 3.8(77.0-89.1)	75.3 ± 5.4(65.0-81.2)	89.10	79.17
Length of stylet shaft	20.0 ± 1.3(17.9-21.9)	16.9 ± 3.6(10.2-22.3)	21.11	16.24
m	74.4 ± 1.3(72.9-76.2)	77.5 ± 4.1(71.1-84.8)	76.31	79.49
stylet length as percentage of body length	16.4 ± 1.4(14.8-19.7)	16.1 ± 1.9(12.6-19.5)	17.71	16.64
Distance between stylet base and D.O.G	2.8 ± 1.3(0.8-5.7)	2.7 ± 1.3(2.0-6.1)	3.25	2.03
O	3.4 ± 1.7(1.0-7.4)	3.7 ± 1.8(2.5-7.5)	3.65	2.56
Distance lip region-centre median bulb	94.8 ± 7.0(77.1-105.6)	86.1 ± 6.0(75.1-95.4)	83.23	91.35
MB	75.6 ± 3.5(69.1-82.0)	76.8 ± 7.3(63.8-92.2)	71.93	78.95

Table 2. Measurements and ratios of males of *Criconema arkaense* from the type population. Mean, standard deviation and range in µm.

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

Table 3. Measurements and ratios of *Criconema petasum*, *Criconema mutabile*, *Criconema sphagni and Bakernema inaequali*. Mean, standard deviation and range in µm.

Character/Ratio	*Criconema petasum* Tulip-poplar (n=9)	*Criconema mutabile* Host: oat grass Arkansas (n=20)	*Criconema sphagni* Host: oak Arkansas (n=24)
L	523.5 ± 74.4(481.8-706.3)	364.2 ± 22.5(318.2-418.2)	390.9±34.4(300-445.5)
Oesophagus length	115.5 ± 13.1(105.6-144.1)	91.1 ± 4.4(83.2-99.5)	105.6 ± 4.8(93.4-117.7)
Tail	56.2 ± 5.2(45.7-60.9)	18.4 ± 3.3(13.0-23.6)	25.1 ± 3.9(17.9-34.1)
Maximum Body width	61.7 ± 4.5(54.8-69.0)	29.5 ± 2.1(25.2-33.3)	38.3 ± 2.6(34.1-45.5)
a	8.4 ± 0.9(7.7-10.2)	12.3 ± 0.6(11.2-13.2)	10.2 ± 0.8(8.9-11.6)
b	4.5 ± 0.3(4.1-4.9)	4.0 ± 0.3(3.7-4.6)	3.7 ± 0.3(3.1-4.3)
c	9.5 ± 2.5(7.9-15.5)	20.2 ± 3.2(15.9-27.8)	16.2 ± 2.1(13.1-21.7)
Distance lip region end to vulva	435.8 ± 67.1(402.6-600.7)	340.4 ± 16.2(320.9-384.1)	337.2 ±25.9(274.4-383.7)
Distance lip region end to anus	467.3 ± 78.8(420.9-660.6)	352.1 ± 16.2(332.2-395.4)	368.2 ±27.9(297.9-411.4)
V	83.2 ± 1.3(81.5-85.1)	91.8 ± 0.5(90.8-92.6)	85.8 ± 0.9(84.1-87.9)
V'	93.4 ± 1.9(90.9-96.2)	96.7 ± 0.5(95.6-97.3)	91.6 ± 1.0(88.9-93.3)
Distance lip region to end oesophageal gland	123.8 ± 14.9(111.7-156.3)	95.7 ± 4.2(89.3-101.5)	110.7 ± 5.0(99.5-123.8)
Body width at anus	46.7 ± 2.6(42.6-50.4)	20.0 ± 1.9(16.2-23.6)	21.6 ± 1.7(17.9-25.2)
b'	4.2 ± 0.2(3.8-4.5)	3.8 ± 0.2(3.6-4.4)	3.5 ± 0.3(2.9-4.0)
c'	1.2 ± 0.1(0.9-1.3)	0.9 ± 0.1(0.6-1.2)	1.1 ± 0.2(0.8-1.5)
Distance between vulva & post end of body	87.7 ± 9.2(77.0-105.6)	30.9 ± 3.0(26.0-37.4)	55.7 ± 6.1(43.7-68.9)
Body width at vulva	53.1 ± 4.1(46.7-60.9)	25.1 ± 1.8(21.9-28.4)	35.1 ± 2.0(30.0-38.2)
VL/VB	1.7 ± 0.2(1.4-2.0)	1.2 ± 0.1(1.0-1.4)	1.6 ± 0.1(1.3-1.8)
Rex	15 ± 1.0(13-16)	33 ± 1.5(30-36)	22 ± 1.2(20-24)
Roes	13 ± 0.7(12-14)	31 ± 2.0(27-34)	20 ± 1.2(18-23)
Rvan	3 ± 0.0(3-3)	3 ± 0.7(2-4)	4 ± 0.5(3-5)
Ran	7 ± 0.5(6-8)	7 ± 1.1(4-9)	8 ± 0.8(6-9)
RV	11 ± 0.6(10-12)	11 ± 1.0(9-13)	12 ± 0.8(11-14)
R	51 ± 1.1(49-52)	119 ± 5.4(108-130)	67 ± 1.6(65-72)
Stylet length	76.6 ± 3.2(72.9-83.2)	62.9 ± 2.1(60.1-66.4)	79.4 ± 2.7(74.5-85.1)
Length of stylet shaft	24.9 ± 11.7(17.1-52.8)	10.0 ± 1.2(8.1-14.2)	12.1 ± 1.1(10.6-14.6)
m	67.4 ± 15.9(29.2-76.6)	84.1 ± 2.5(76.7-86.3)	84.8 ± 1.2(81.8-86.8)
stylet length as percentage of body length	14.8 ± 1.3(11.8-15.8)	17.0 ± 0.8(15.1-18.3)	20.3 ± 1.6(17.6-24.9)
Distance between stylet base and D.O.G	1.9 ± 1.7(0.0-4.1)	2.6 ± 0.9(0.8-4.1)	1.4 ± 0.7(0.8-3.3)
O	2.5 ± 2.2(0.0-5.2)	4.5 ± 1.6(1.3-6.7)	1.8 ± 1.0(1.0-4.1)
Distance lip region-centre median bulb	89.3 ± 5.5(83.2-101.5)	74.2 ± 2.4(71.1-77.1)	88.1 ± 3.4(81.2-95.4)
MB	78.0 ± 8.0(59.2-84.9)	81.3 ± 2.7(75.5-85.4)	83.5 ± 3.1(79.3-93.9)

Table 3. continued

Character/Ratio	*Criconema sphagni* Host: Tulip-poplar Tennessee (n=16)	*Bakernema inaequali* Host: Tulip-poplar Tennessee (n=18)
L	386.9 ± 43.4(324.2-463.6)	518.2 ± 33.2(457.6-578.8)
Oesophagus length	144.5 ± 8.9(132.0-156.3)	116.2 ± 6.0(105.6-125.9)
Tail	34.3 ± 6.5(24.4-51.2)	27.0 ± 3.5(20.3-34.1)
Maximum Body width	42.0 ± 6.4(36.5-58.9)	56.3 ± 3.4(52.0-62.5)
a	9.3 ± 1.4(6.4-11.4)	9.2 ± 0.6(8.3-10.5)
b	2.7 ± 0.2(2.5-3.1)	4.5 ± 0.2(4.1-4.9)
c	11.5 ± 1.4(8.1-14.3)	19.4 ± 2.0(16.0-22.5)
Distance lip region end to vulva	330.9 ± 37.3(273.5-396.6)	482.0 ± 31.1(430.0-544.7)
Distance lip region end to anus	352.6 ± 39.1(293.8-423.0)	491.2 ± 31.2(437.3-548.7)
V	85.5 ± 1.1(83.7-87.2)	93.0 ± 0.7(91.4-94.1)
V'	93.8 ± 0.8(92.6-95.5)	98.1 ± 0.6(97.0-99.3)
Distance lip region to end oesophageal gland	149.3 ± 9.3(136.0-162.4)	123.0 ± 5.3(113.7-134.0)
Body width at anus	24.3 ± 2.1(20.3-29.2)	35.2 ± 4.4(24.4-40.6)
b'	2.6 ± 0.2(2.4-3.0)	4.2 ± 0.2(3.9-4.7)
c'	1.4 ± 0.2(1.0-1.8)	0.8 ± 0.1(0.6-1.0)
Distance between vulva & post end of body	56.0 ± 7.5(44.7-67.2)	36.1 ± 4.5(27.6-44.7)
Body width at vulva	32.2 ± 2.1(28.4-35.7)	42.7 ± 2.5(39.0-47.9)
VL/VB	1.7 ± 0.2(1.4-1.9)	0.8 ± 0.1(0.7-1.0)
Rex	31 ± 3.7(27-39)	19 ± 0.9(17-20)
Roes	34. ± 2.6(30-38)	17 ± 1.0(15-19)
Rvan	4 ± 0.7(2-5)	1 ± 0.5(1-2)
Ran	10 ± 1.1(8-13)	3 ± 0.4(3-4)
RV	14 ± 0.8(13-16)	4.4 ± 0.5(4-5)
R	86 ± 2.7(79-89)	65 ± 4.1(60-79)
Stylet length	114.8 ± 7.2(103.5-123.8)	64.0 ± 2.4(58.9-68.0)
Length of stylet shaft	14.4 ± 3.1(12.2-21.1)	16.3 ± 3.0(8.1-18.3)
m	87.4 ± 2.7(80.7-90.2)	74.5 ± 4.8(69.0-86.8)
stylet length as percentage of body length	29.9 ± 2.0(26.3-33.5)	12.4 ± 0.7(11.3-13.5)
Distance between stylet base and D.O.G	2.5 ± 1.0(0.8-4.1)	3.6 ± 0.5(2.4-4.1)
O	2.2 ± 0.8(0.7-3.3)	5.6 ± 0.7(4.0-6.6)
Distance lip region-centre median bulb	123.5 ± 8.3(111.7-134.0)	84.5 ± 3.6(79.2-91.4)
MB	85.4 ± 1.7(81.7-88.7)	72.8 ± 2.8(67.4-77.8)

Table 4 Measurements and ratios of *Hemicriconemoides chitwoodi*. Mean, standard deviation and range in µm.

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

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 µm.

Ch/Ratio[a]	*Ogma octangulare* Host: bahia grass Arkansas (n=20)	*Ogma octangulare* Host:Maple Arkansas (n=19)	*Ogma octangulare* Host: tulip-Poplar Tennessee (n=10)
Character/Ratio	376.4 ± 36.6(309.1-430.3)	372.6± 25.9(324.2- 439.4)	399.7 ± 20.3(378.8-442.4)
L	92.4 ± 5.6(83.2-103.5)	95.2 ± 3.8(89.3-105.6)	92.6 ± 4.1(87.3-99.5)
Oesophagus length	27.0 ± 4.3(20.3-37.6)	26.9 ± 3.4(20.3-32.5)	31.6 ± 6.1(18.3-38.6)
Tail	39.9 ± 2.0(36.5-43.9)	41.0 ± 1.8(35.7-43.9)	40.7 ± 4.2(30.5-44.7)
Maximum Body width	9.4 ± 0.8(8.0-11.2)	9.1 ± 0.5(8.3-10.0)	9.9 ± 1.2(8.8-12.5)
a	4.1 ± 0.3(3.5-4.4)	3.9 ± 0.2(3.5-4.3)	4.3 ± 0.2(4.0-4.7)
b	14.2 ± 1.9(9.7-17.5)	14.0 ± 1.7(11.6-17.1)	13.2 ± 3.2(10.4-21.6)
c	325.7 ± 34.8(262.8-376.1)	320.7 ± 24.2(273.9-382.6)	344.7 ± 19.1(319.9-383.6)
Distance lip region end to vulva	349.4 ± 35.2(288.8-401.3)	345.7 ± 24.6(296.6-408.5)	368.1 ± 18.8(346.3-405.9)
Distance lip region end to anus	86.4 ± 1.1(84.4-88.0)	86.1 ± 1.0(84.1-88.1)	86.2 ± 1.2(84.5-88.7)
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)
V'	97.8 ± 6.1(87.3-109.6)	99.7 ± 3.4(95.4-109.6)	98.0 ± 4.5(93.4-105.6)
Distance lip region to end oesophageal gland	21.7 ± 1.4(18.7-25.2)	20.8 ± 1.6(17.1-23.6)	22.5 ± 2.1(18.7-26.0)
Body width at anus	3.8 ± 0.3(3.3-4.2)	3.7 ± 0.2(3.4-4.1)	4.1 ± 0.2(3.8-4.3)
b'	1.2 ± 0.2(0.9-1.7)	1.3 ± 0.2(1.0-1.6)	1.4 ± 0.2(0.8-1.6)
c'	50.7 ± 3.2(44.7-58.5)	51.8 ± 3.8(45.5-58.5)	55.0 ± 4.9(44.7-58.9)
Distance between vulva & post end of body	33.9 ± 1.7(30.9-37.4)	33.9 ± 2.0(28.4-36.5)	32.6 ± 2.5(28.4-34.5)
Body width at vulva	1.5 ± 0.1(1.3-1.7)	1.5 ± 0.1(1.3-1.8)	1.7 ± 0.2(1.3-1.9)
VL/VB	20.1 ± 1.1(18.0-22.0)	21.7 ± 1.4(19.0-25.0)	20.4 ± 1.8(17.0-24.0)
Rex	17 ± 1.2(16-20)	19 ± 1.4(16-22)	19 ± 1.4(16.0-21)
Roes	4 ± 0.6(2-4)	4 ± 0.6(3-5.0)	3 ± 0.8(2-5)
Rvan	8 ± 0.7(7-9)	8 ± 0.6(7-9)	9 ± 1.1(7-10)
Ran	12 ± 0.7(11-14)	13 ± 0.8(12-15.0)	13 ± 0.6(12-14)
RV	67 ± 2.5(62-71)	70 ± 2.5(64-76)	71 ± 1.8(69-74)
R	63 ± 2.2(59-66)	63 ± 2.0(59-69)	62 ± 1.9(59-65)
Stylet length	14.4 ± 0.9(13.0-16.2)	14.1 ± 0.9(12.2-15.4)	14.4 ± 1.2(12.2-16.2)
Length of stylet shaft	77.0 ± 1.1(75.0-79.2)	77.5 ± 1.5(74-80.5)	76.7 ± 1.5(74.4-79.3)
m	16.8 ± 1.6(14.3-20.0)	16.8 ± 1.0(15.1-18.5)	15.5 ± 0.6(14.7-16.6)
stylet length as percentage of body length	2.8 ± 0.8(1.6-4.1)	2.9 ± 0.8(0.8- 4.1)	2.9 ± 1.9(2.0-8.1)
Distance between stylet base and D.O.G	4.5 ± 1.4(2.5-6.9)	4.7 ± 1.3(1.3-6.6)	4.6 ± 2.8(3.2-12.5)
O	74.2 ± 3.6(67.0-79.2)	75.5 ± 6.3(62.9-95.4)	73.1 ± 4.4(65.0-79.2)
Distance lip region-centre median bulb	80.4 ± 3.3(75.0-86.0)	79.3 ± 5.5(67.4-97.9)	79.0 ± 4.0(72.7-83.7)

Table 5. continued

Character/Ratio	*Xenocriconemella macrodora* Host: box elder North Carolina (n=7)
L	268.0 ± 44.2(181.8-312.1)
Oesophagus length	111.1 ± 8.3(95.4-119.8)
Tail	11.1 ± 3.0(7.3-14.6)
Maximum Body width	26.7 ± 2.9(21.9-30.9)
a	10.0 ± 1.1(8.3-11.7)
b	2.4 ± 0.3(1.9-3.0)
c	25.3 ± 7.3(19.7-38.4)
Distance lip region end to vulva	247.7 ± 40.5(170.4-296.7)
Distance lip region end to anus	256.8 ± 43.0(172.9-304.0)
V	92.5 ± 1.7(90.1-95.1)
V'	96.5 ± 1.3(94.9-98.6)
Distance lip region to end oesophageal gland	115.4 ± 8.7(99.5-123.8)
Body width at anus	14.2 ± 2.2(10.6-17.9)
b'	2.3 ± 0.3(1.8-2.9)
c'	0.8 ± 0.2(0.5-1.0)
Distance between vulva & post end of body	20.3 ± 6.3(11.4-28.4)
Body width at vulva	20.2 ± 2.2(16.2-22.3)
VL/VB	1.0 ± 0.2(0.7-1.3)
Rex	38 ± 2.9(34-43)
Roes	46 ± 6.5(42-60)
Rvan	3 ± 0.7(2-4)
Ran	7 ± 1.4(4-8)
RV	10 ± 1.7(7-12)
R	101 ± 7.0(89-112)
Stylet length	90.5 ± 10.1(71.1-99.5)
Length of stylet shaft	12.0 ± 1.9(8.9-14.2)
m	86.5 ± 3.5(80-90.6)
stylet length as percentage of body length	34.3 ± 4.4(28.0-39.9)
Distance between stylet base and D.O.G	1.9 ± 1.7(0.8-5.7)
O	2.1 ± 1.9(0.8-6.2)
Distance lip region-centre median bulb	96.0 ± 10.4(75.1-107.6)
MB	86.3 ± 5.3(78.7-94.6)

FIGURES

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.

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.

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.

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.

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.

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

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

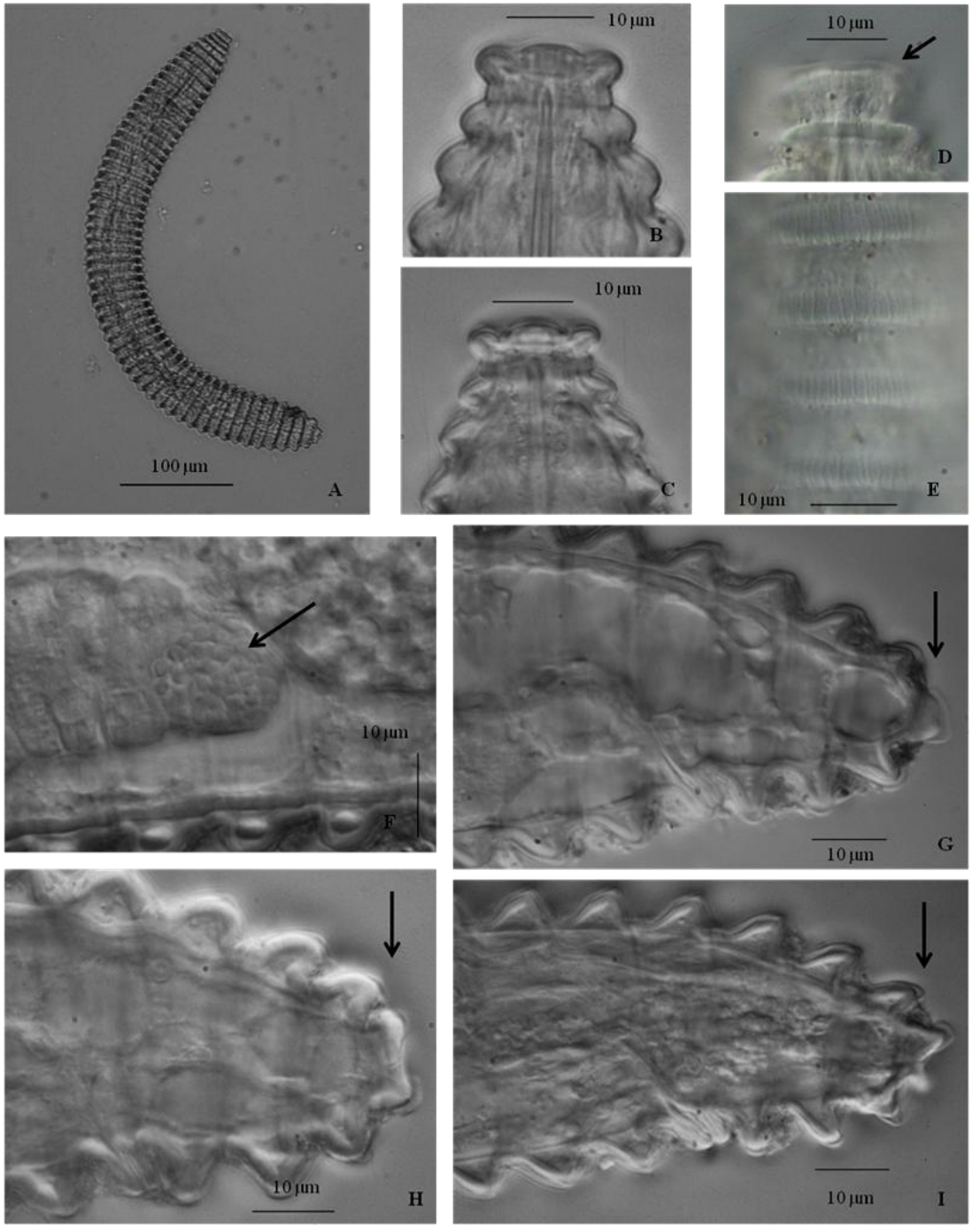
Fig 8. Light micrographs of *Bakernema inaequali*. A) Entire female. B) Anterior region. C) Lip region. Arrows showing submedian lobes. D) Posterior region. Arrows showing spermatheca. E) Scales. F) Tail. Arrows showing vulva and anus.

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

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.

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

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 boostrap support values. New species are in bold.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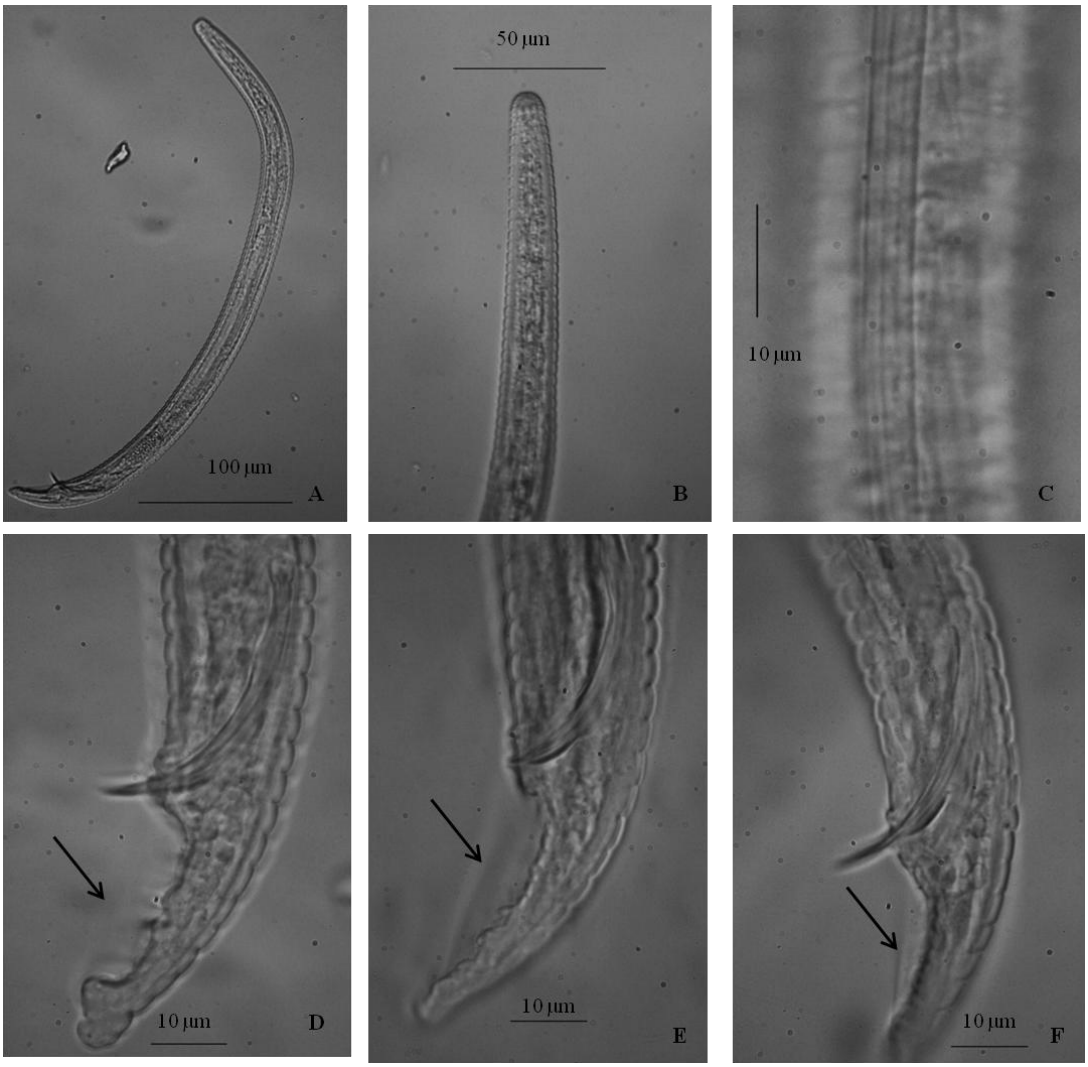
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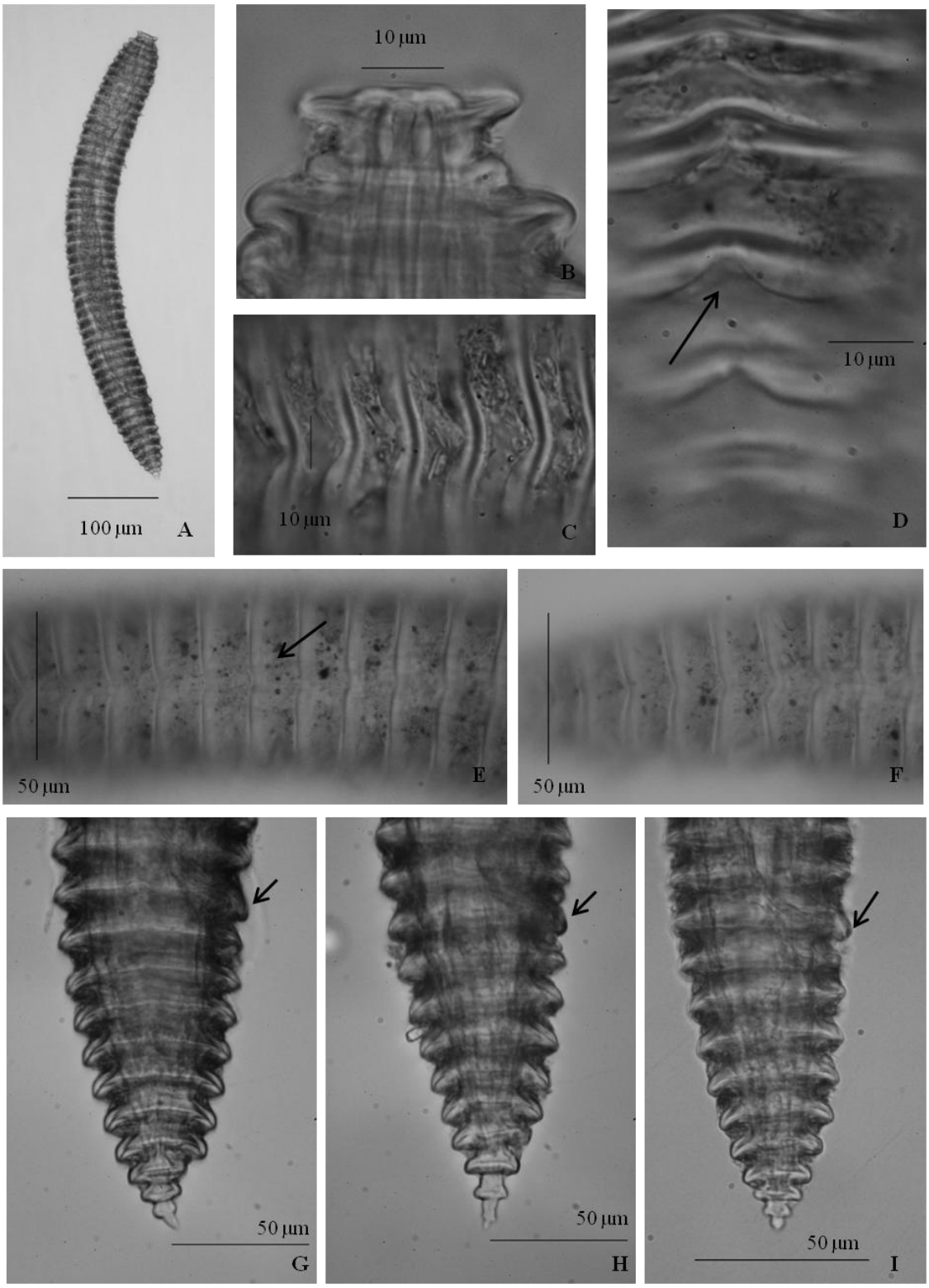
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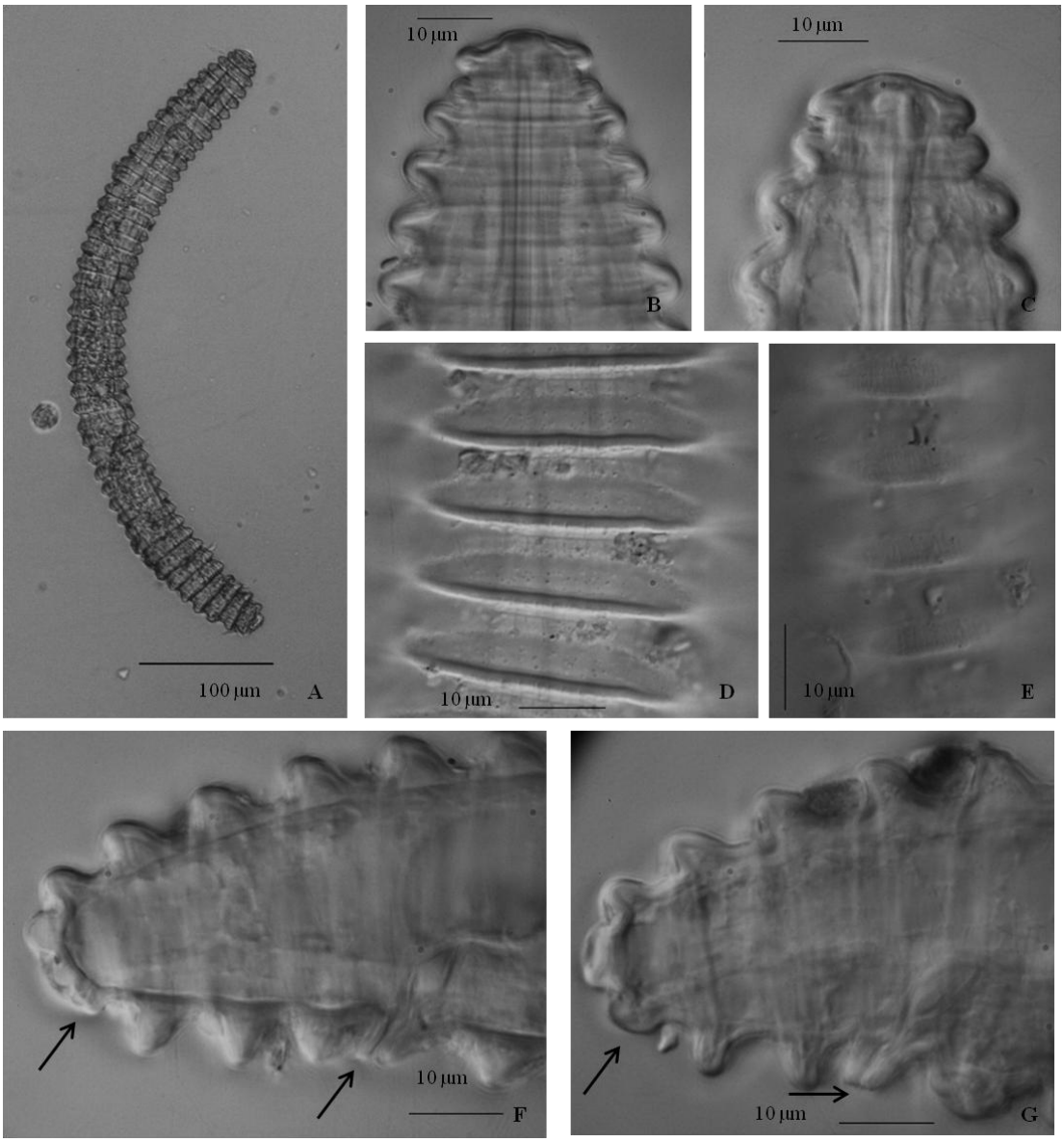
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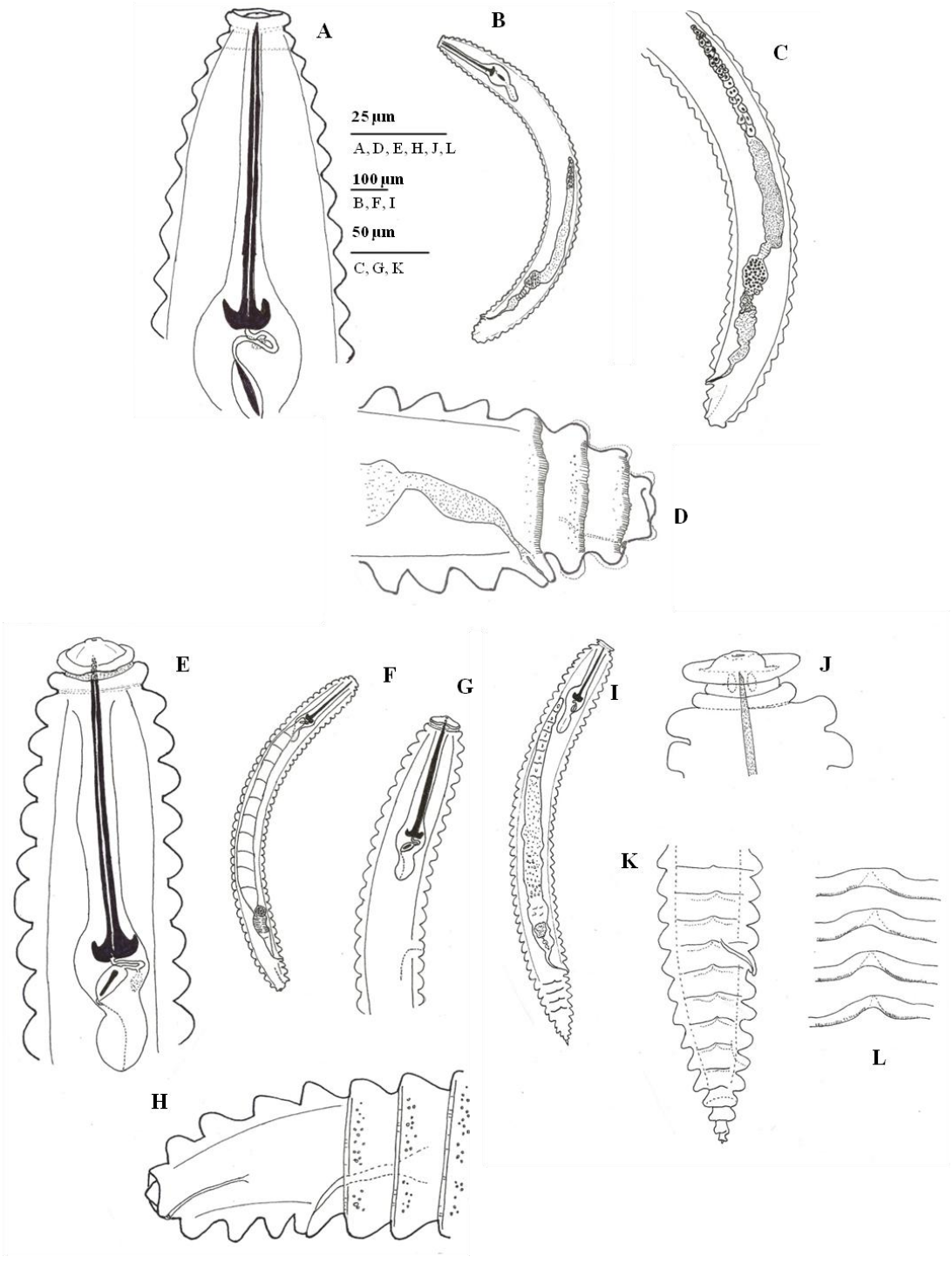
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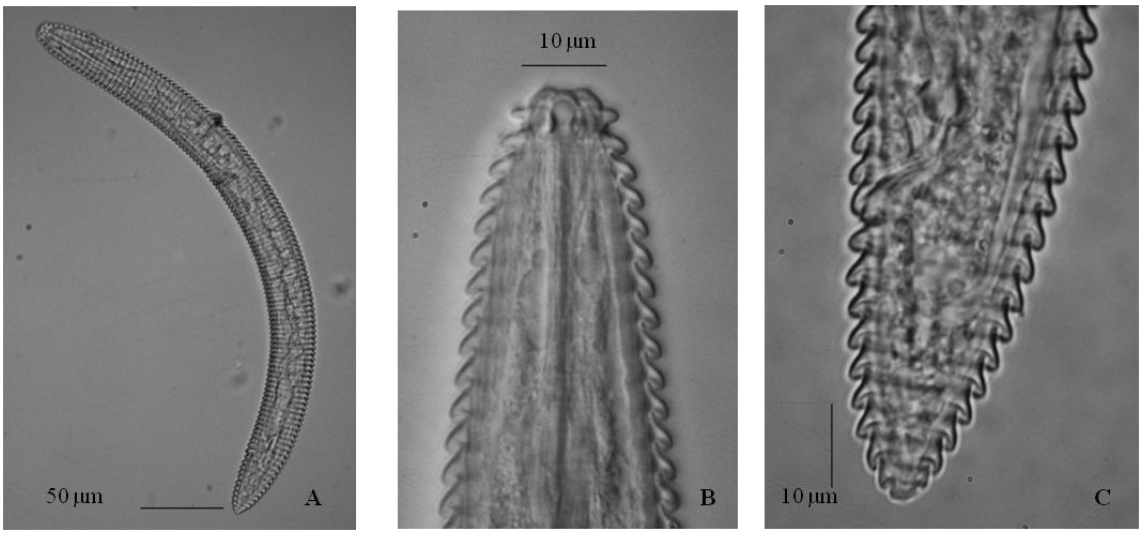
C, G, K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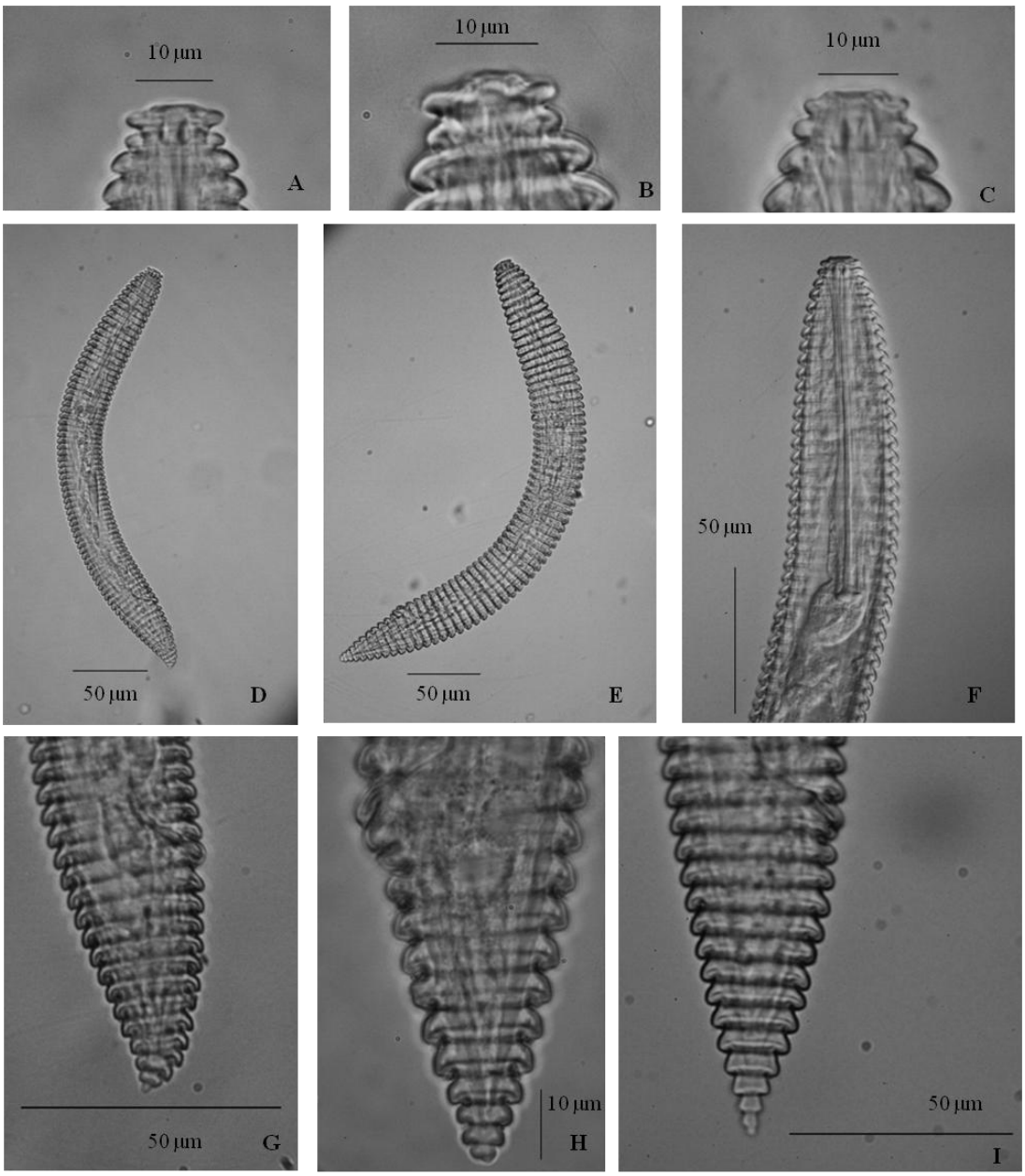
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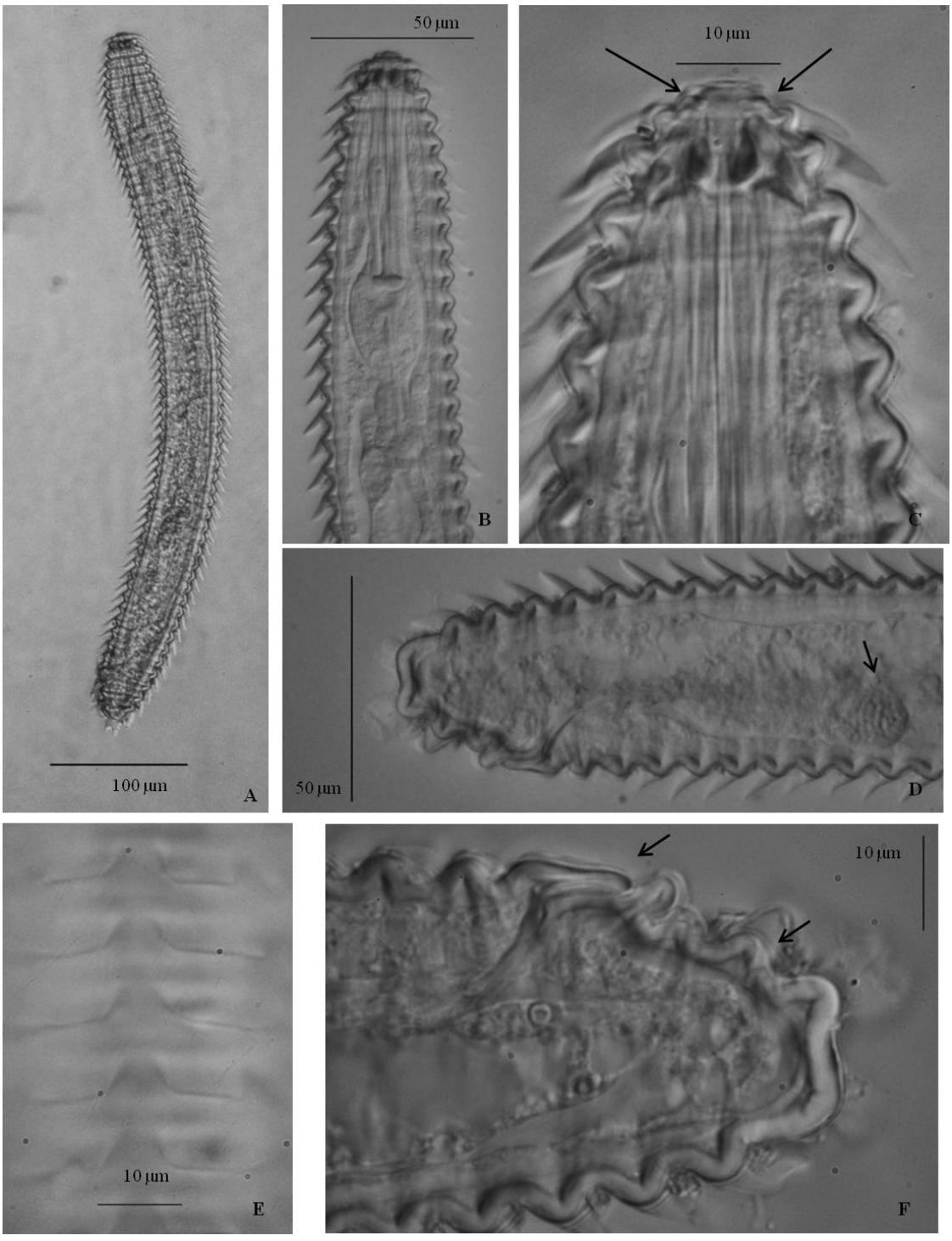
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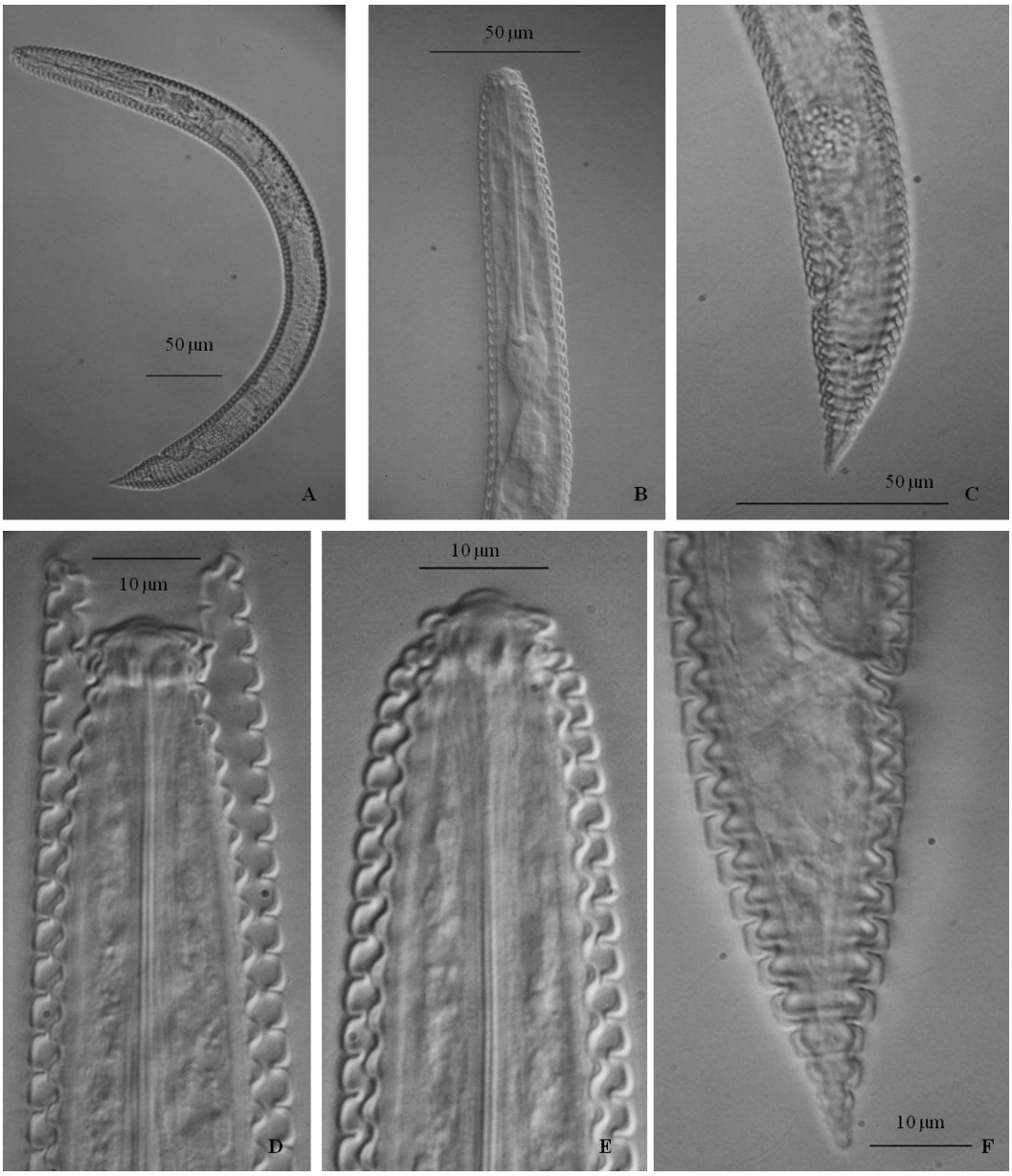
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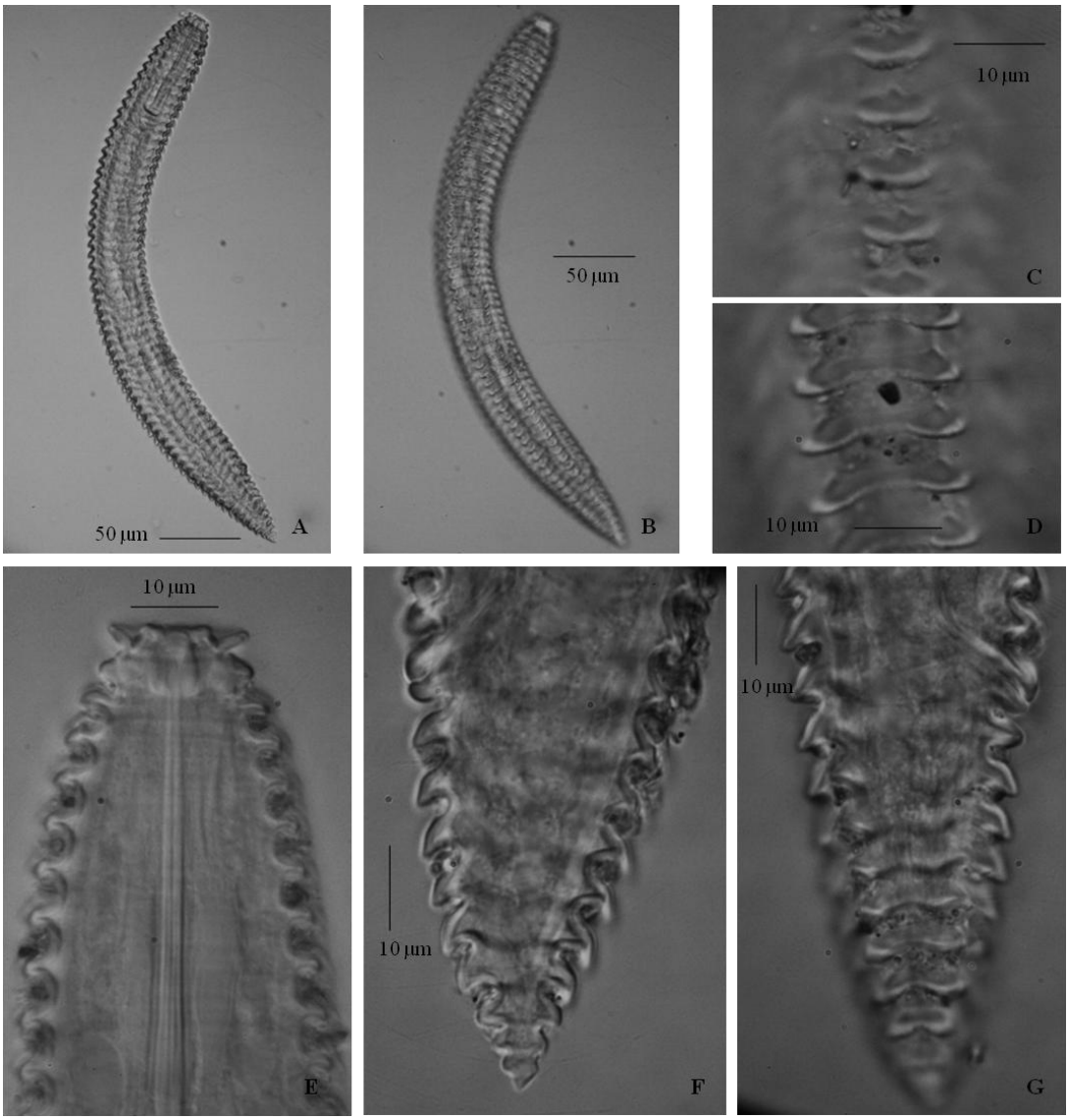
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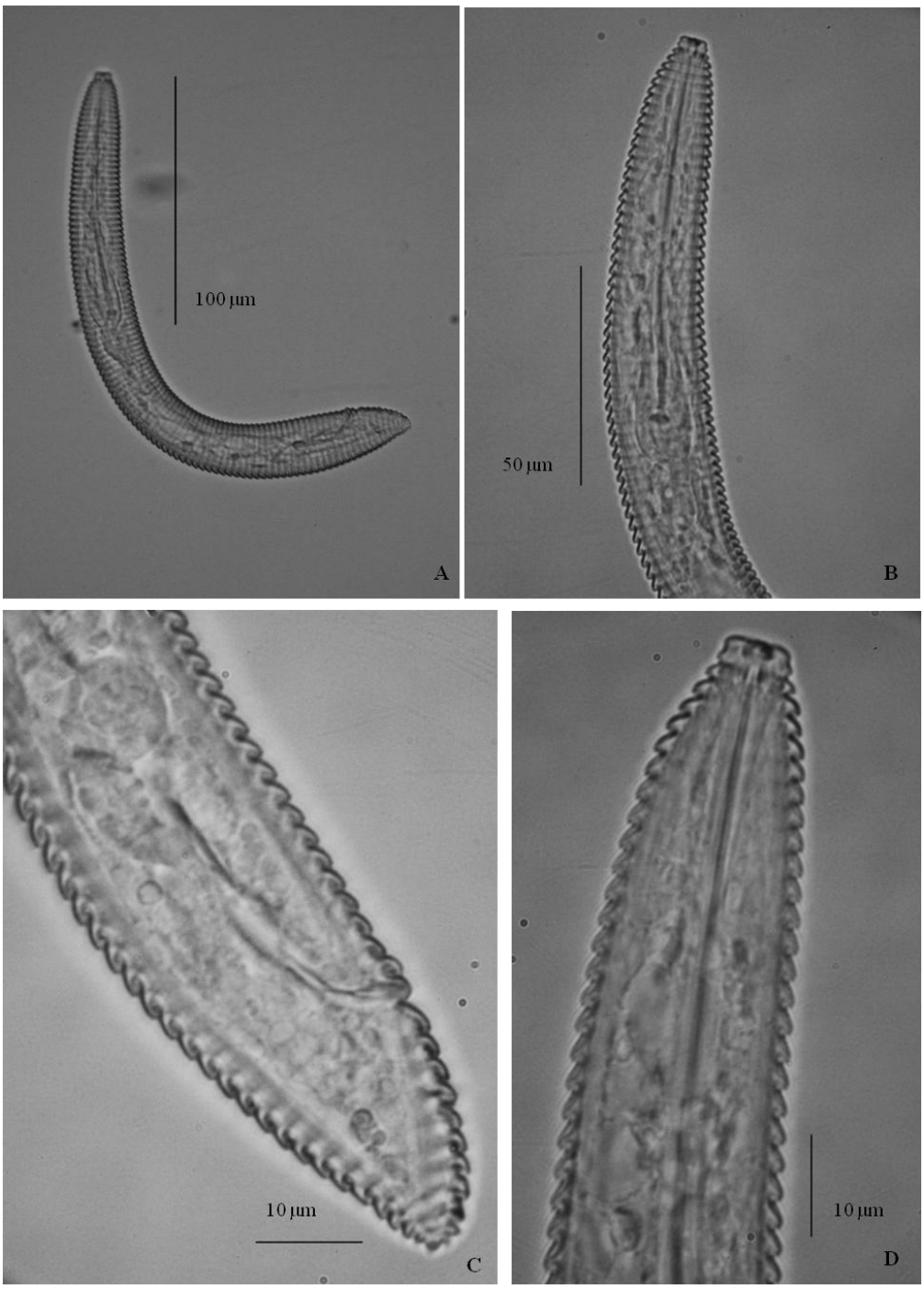
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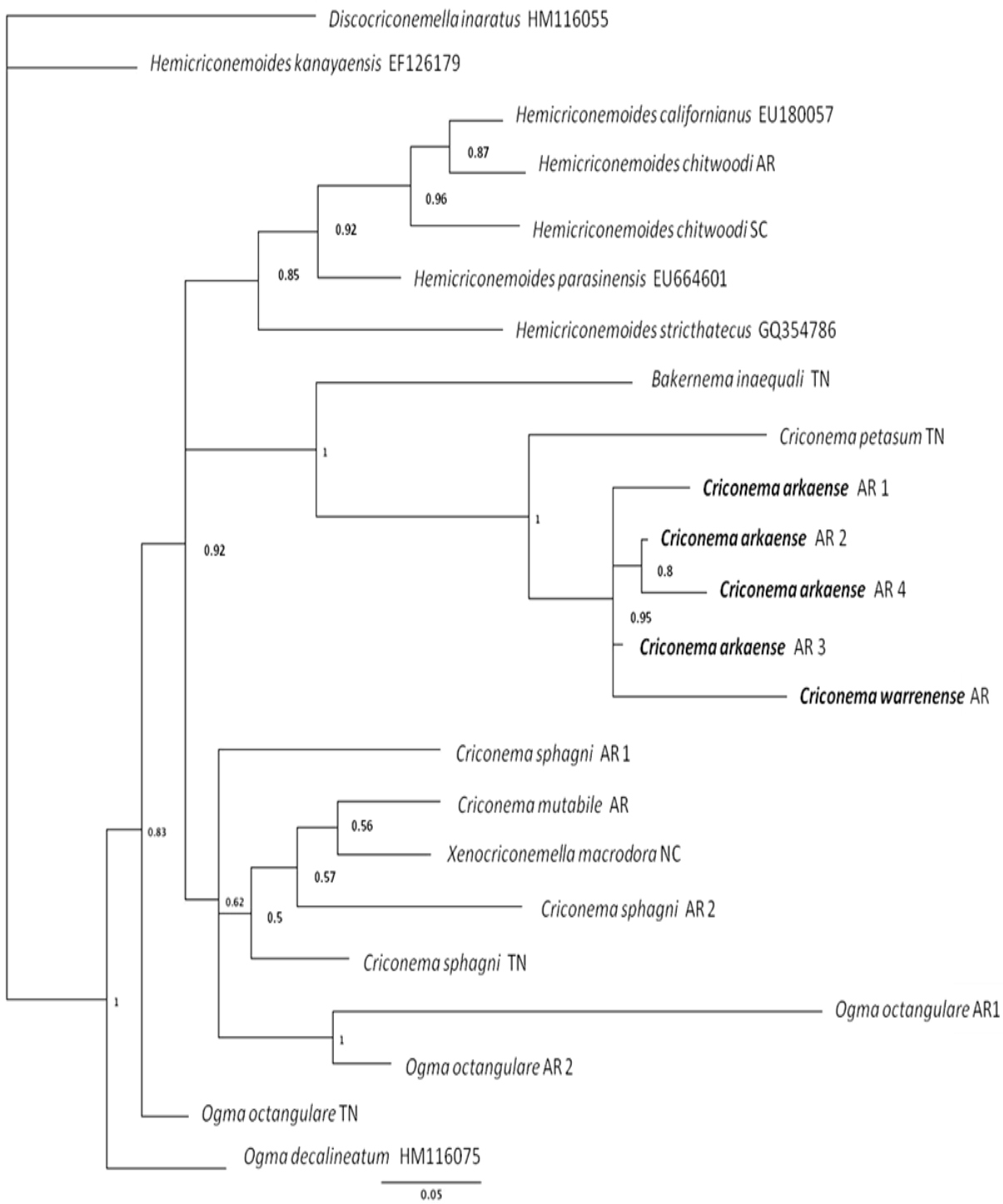
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Criconema arkaense AR 1
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Ogma octangulare AR 2
Ogma octangulare TN
Ogma decalineatum HM116075
0.05

APPENDIX

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sipes.brent@gmail.com on behalf of Brent Sipes [sipes@hawaii.edu]
Sent: Tuesday, February 12, 2013 11:30 AM
To: Nancy Burelle, JON Editor in Chief [nancy.burelle@ars.usda.gov]; Marco A. Cordero Lopez
Cc: Andrea Skantar, SON Secretary [andrea.skantar@ars.usda.gov]

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808-956-7813 (voice) | 808-956-2832 (fax)

TAXONOMIC AND MOLECULAR IDENTIFICATION OF *Hemicaloosia*, *Hemicycliophora*, *Gracilacus* and *Paratylenchus* SPECIES (NEMATODA: CRICONEMATIDAE)

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The authors thank Ernest Bernard, University of Tennessee; Paula Agudelo, Clemson University; Tim Todd, Kansas State University and Weimen Ye, North Carolina Department of Agriculture for providing specimens for this study and Sergei Subbotin for his assistance for the molecular analysis

This paper was edited by

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 (106-124µm), 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.

Keywords: *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, morphology, molecular biology, *Paratylenchus labiosus,* phylogeny.

The classification of Raski and Luc (1987) included in the subfamily Hemicycliophorinae Skarbilovich, 1959 two genera: *Hemicycliophora* De Man, 1921 synonimyzed with *Procriconema* Micoletzky, 1925; *Colbranium* Andrássy, 1979; *Aulospora* Siddiqui, 1980 and *Loofia* Siddiqui, 1980; and 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 non-retrorse body annuli, sometimes with superficial ornamentation appearing as lines or scratches, 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 which lacks of submedian lobes. A long stylet over 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 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 sub-genus of *Paratylenchus* by Siddiqi (2000) as he regarded it insufficient to separate the genera based on differencies 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 for diagnostic purposes based on PCR reactions (Gasser, 2001; Subbotin and Moens, 2006).

The objectives of this study were to: 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, USA 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; Seinhorst, 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* don't 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 5µl of molecular grade water (BDH Chemicals, Chester, PA) and stored at -80^{o}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'-TTGATTACGTCCCTGCCCTTT- 3') (Vrain et al., 1992) and rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA- 3') (Cherry et al., 1997). This PCR primer pair ampliflied 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 (0.2mM 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 ^{o}C for 2 minutes, then 40 cycles of denaturation at 94 ^{o}C for 45 seconds, annealing at 52 or 56 ^{o}C for 45 seconds and extension at 72 ^{o}C for 60 seconds. A final extension for 5 minutes at 72 ^{o}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^{o}C for 20 minutes 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) (Darriba et al., 2012; Posada and Crandall, 1998; Posada, 2008). 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 x 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% mayority 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

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

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

Males: not found.

Type host and locality

Specimens were collected in May – June 2008 by M. Cordero and R. Robbins at Pinetree, AR designed as type population (GPS coordinates N 35^{o} 07.801 min-W 090^{o} 58.383 min) from the rhizosphere of small pines and Warren, AR. (GPS coordinates N 33^{o} 30.283 min-W 092^{o} 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 misindentified 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, Maryland.

*Paratype*s (*females*): Seven female paratypes deposited as in the USDA Nematode Collection, Beltsville, Maryland; three females paratypes deposited in The Department of Nematology, University of California, Riverside.

Diagnosis

Hemicaloosia uarki n. sp. is characterized by its long body (1,081-1,431 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 (106-124 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, continouos 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) (Brzeski, 1974; Colbran, 1963). *Hemicaloosia uarki* n. sp. is similar to *H. graminis* in having anastomoses in the post labial region (3^{th}-5^{th} annulus). However, *H. graminis* does not have anastomoses inmediately 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

Females: 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 elongate, vulval sleeve long, spermatheca rounded and empty. Tail elongated, uniformly conoid, not offset, rounded tip. Tail annulations distinct.

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

Males: 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, North Carolina. No GPS coordinates provided.

Type specimens

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

*Paratype*s (*females*): Three paratypes deposited in U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland; and single paratypes are deposited as follows: Department of Nematology, Agricultural University, 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 indistintct. 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.

Relationship

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 *vs*. 12-14), VL/VB (4 *vs*. 5-7), R (125-258 *vs*. 260-270) and greater Ran (32-49 *vs*. 22-27) (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955).

Etymology

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

Hemicycliophora epicharoides Loof, 1968

(Table 3; Fig. 5)

Description

Females: 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^{o} 06.068 min-W 094^{o} 21.517 min); from the rhizosphere of river cane (*Arundinaria* sp.) and Toad Suck Ferry Park, Perry County, AR (GPS coordinates N 35^{o} 04.279 min-W 092^{o} 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, labial plate rounded and low, with lateral pseudolips present at same level of the oral disc, frequently indistinct. Aditionally, 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 4 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, elongate *vs*. not modified, round), and lateral fields (one or two lines *vs*. breaks or anastomoses of transverse striae) (Brzeski, 1974; Brzeski and Ivanova, 1978; Loof, 1968). 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 which are herein considered important characters in differentiating *H. ephicaroides* from *H. robusta*.

Hemicycliophora gigas Thorne, 1955

(Table 3; Fig. 6)

Description

Females: 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.

Juveniles: resemble females. 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.

Males: 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^{o} 07.178 min-W 090^{o} 66.596 min; N 35^{o} 07.188 min-W 090^{o} 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 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. (Brzeski, 1974; Thorne, 1955) 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 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) (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955). This species is similar to *H.ovata* however, *H. gigas* differ by a filiform *vs*. conical and off set tail. (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955).

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.* elongate and sharply conoid (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955).

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 ocasionally 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) (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955). *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*. elongate 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) (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955).

Hemicycliophora labiata Colbran, 1960

(Table 4; Fig. 7)

Description

Females: 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 off set, dorsally convex.

Host and locality

Specimens were collected by in June 2010 by E. Bernard in the Smoky Mountains from the rhizosphere of tulip-poplar (*Liriodendron tulipifera*) and T. Todd in June 2010 from turfgrass, in Tennessee and Kansas, respectively. 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 distintly modified lips of equal length, small vulval sleeve, and a slightly off set, 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

Females: 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 sub acute to rounded tip. Tail annulations distinct.

Males: 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 sub acute 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 (1 vs. 4 lines: outer lines crenate, inner lines straight). Also, the excretory pore was observed anterior and/or posterior to the oesophageal basal bulb.

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

Relationship

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). (Brzeski and Ivanova, 1978; Kirjanova and Shagalina, 1974; Thorne, 1955)

Hemicycliophora shepherdi Wu, 1966

(Table 5; Fig. 9)

Description

Females: body straight or ventrally arcuate. Body annuli rounded and smooth. Cuticular sheath loosely 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 off set.

Males: 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^{o} 04.279 min-W 092^{o} 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, altrough no longitudinal lines were reported originally (Brzeski, 1974; Wu, 1966) 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 to *H. zuckermani* which is flat and posteriorly directed. Also the tail in *H.shepherdi* is more dorsally convex, conoid and offset than in *H. zuckermani* (Brzeski, 1974; Brzeski and Ivanova, 1978; Thorne, 1955; Wu, 1966).

Hemicycliophora vidua Raski, 1958

(Table 6; Fig. 10)

Description

Females: 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 continous, 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.

Males: not found.

Host and locality

Specimens were collected in June 2008 by P. Agudelo in Clemson, South Carolina 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 elongate 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 (Brzeski, 1974; Raski, 1958; Wu, 1966) 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) (Brzeski, 1974; Brzeski and Ivanova, 1978; Wu, 1966).

Hemicycliophora zuckermani Brzeski, 1963

(Table 6; Fig. 11)

Description

Females: 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 plate slightly rounded, oral disc rounded and slightly elevated, pseudolips separated indistinct. Lip region continous, 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.

Males: 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^{o} 06.190 min-W 094^{o} 20.666 min); from the rhizosphere of maple (*Acer* sp.) and river cane (*Arundinaria* sp.) *H. zuckermani* type b and type c; N 36^{o} 06.312 min-W 094^{o} 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 . (Brzeski, 1974; Raski, 1958; Wu, 1966).

Gracilacus straeleni (Wu, 1964) Raski, 1976

(Table 7; Fig. 12)

Description

Females: 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 non-protruded, 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.

Males: not found.

Host and locality

Specimens were collected in June 2009 by M. Cordero at Fayetteville, AR (GPS coordinates N 36^{o} 05.968 min-W 094^{o} 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 non-protruding 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. (Brzeski and Háněl.

1999; Castillo and Gomez, 1988; Ciobanu et al., 2003; Raski, 1962) 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 which are absent in *G. aculenta* (Luc and De Guiran, 1962; Raski, 1976).

Paratylenchus labiosus Anderson & Kimpinski, 1977

(Table 7; Fig. 12)

Description

Females: 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, continous 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 non-protruded, 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.

Males: not found.

Host and locality

Specimens were collected in June 2009 by M. Cordero at Washington County, AR (GPS coordinates N 36^{o} 06.244 min-W 094^{o} 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, 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 (76-85 *vs.* 81-85) (Anderson and Kimpinski, 1977; Raski, 1975a; Raski, 1975b; Wu and Townsend, 1973; Wu, 1975).

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 sub family 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 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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TABLES

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]
L	1,193.75	1,243.8 ± 102.1(1,081.3-1,325.5)	937.50
Oesophagus length	194.88	188.2 ± 8.2(174.6-194.9)	162.40
Tail	192.85	182.7 ± 47.0(109.6-227.4)	178.64
Maximum Body width	40.60	41.0 ± 3.2(36.5-44.7)	38.57
a	29.40	30.5 ± 3.2(26.6-35.4)	24.31
b	6.13	6.7 ± 0.3(6.2-6.9)	5.77
c	6.19	7.3 ± 2.7(5.7-12.1)	5.25
Distance lip region end to vulva	917.67	954.0 ± 73.7(833.6-1,021.0)	819.76
Distance lip region end to anus	1,000.90	1,061.2 ± 111.1(910.7-1,215.9)	758.86
V	76.87	76.7 ± 0.8(75.8-77.8)	87.44
V'	91.68	90.1 ± 3.5(84.0-92.0)	108.03
Distance lip region to end oesophageal gland	203.00	192.4 ± 9.3(182.7-203.0)	166.46
Body width at anus	34.51	34.9 ± 4.8(28.4-40.6)	30.45
b'	5.88	6.5 ± 0.3(5.9-6.7)	5.63
c'	5.59	5.3 ± 1.5(2.7-6.6)	5.87
Distance between vulva & post end of body	276.08	289.9 ± 30.5(247.7-316.7)	117.74
Body width at vulva	38.57	40.2 ± 4.2(36.5-44.7)	32.48
VL/VB	7.16	7.2 ± 0.7(6.8-8.6)	3.63
Rex	55	56 ± 2.6(54-59)	63
Roes	49	55 ± 5.1(54-57)	59
Rvan	23	24 ± 2.3(22-28)	22
Ran	47	49 ± 0.8(48-50)	52
RV	72	74 ± 2.9(72-79)	75
R	338	347± 7.4(339-357)	338.00
Stylet length	118.74	118.1 ± 7.4(105.6-123.8)	105.56
Length of stylet shaft	20.30	18.7 ± 2.2(16.2-22.3)	20.30
m	82.76	84.0 ± 1.4(81.7-85.2)	80.77
Stylet length as percentage of body length	9.86	9.5 ± 0.2(9.3-9.8)	11.26
Distance between stylet base and D.O.G	10.15	5.3 ± 2.3(2.0-8.1)	4.06
O	8.62	4.5 ± 2.0(1.7-6.6)	3.85
Distance lip region-centre median bulb	146.16	137.2 ± 7.8(125.9-146.2)	121.80
MB	75.00	73.8 ± 1.4(72.1-75.0)	75.00

Table 1. continued

Character/Ratio	Jv (n=6)[1]	Jv (n=7)[2]	Population from Clarkville, AR ♀ (n=5)
L	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	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	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	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	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	6.3 ± 0.4(5.9-6.8)	5.3 ± 0.7(4.1-6.6)	6.7 ± 0.3(6.3-7.0)
c	6.1 ± 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	-	-	983.5 ± 76.7(878.3-1059.6)
Distance lip region end to anus	-	-	1,082.6 ± 74.8(979.8-1159.6)
V	-	-	77.0 ± 1.2(75.6-78.9)
V'	-	-	90.8 ± 1.1(89.6-92.6)
Distance lip region to end oesophageal gland	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	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'	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'	5.4 ± 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 & post end of body	-	-	292.3 ± 13.8(284.2-316.7)
Body width at vulva	-	-	34.9 ± 3.6(32.5-40.6)
VL/VB	-	-	8.5 ± 1.1(7.0-9.8)
Rex	59 ± 3.1(55-63)	58 ± 2.8(55-63)	53 ± 2.4(50-55)
Roes	56 ± 2.1(54-59)	56 ± 2.1(54-59)	54 ± 2.3(50-56)
Rvan	-	-	26 ± 3.3(21-29)
Ran	-	-	49 ± 3.8(45-53)
RV	-	-	75 ± 4.9(68-81)
R	312 ± 8.8(297-322)	314 ± 16.8(296-342)	368 ± 10.9(350-377)
Stylet length	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	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	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	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	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	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-centre median bulb	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	75.2 ± 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.

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 & 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-centre 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)

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
L	881.3 ± 49.8(781.3-956.3)	917.4 ± 221.9(756.3-1487.5)
Oesophagus length	153.6 ± 10.6(129.9-162.4)	149.3 ± 6.7(140.1-158.3)
Tail	88.0 ± 8.1(72.9-103.5)	75.5 ± 16.8(41.4-93.4)
Maximum Body width	40.1 ± 3.3(34.9-46.3)	41.6 ± 3.5(35.7-47.1)
a	22.0 ± 1.4(20.0-25.1)	22.3 ± 6.5(16.1-39.0)
b	5.8 ± 0.5(5.3-7.2)	6.2 ± 1.5(5.3-10.2)
c	10.1 ± 0.7(8.8-11.2)	13.2 ± 6.2(8.1-24.8)
Distance lip region end to vulva	736.4 ± 43.0(659.5-806.0)	781.7 ± 230.6(618.2-1381)
Distance lip region end to anus	793.2 ± 45.1(708.4-871.0)	841.9 ± 230.3(662.9-1427.4)
V	83.6 ± 0.7(82.5-85.0)	84.6 ± 3.2(81.7-92.8)
V'	92.8 ± 0.6(91.4-93.8)	92.6 ± 2.4(87.4-96.8)
Distance lip region to end oesophageal gland	157.8 ± 10.9(136.0-168.5)	152.7 ± 6.5(144.1-160.4)
Body width at anus	34.2 ± 3.3(30.0-40.6)	32.9 ± 4.2(27.6-39.8)
b'	5.6 ± 0.4(5.2-6.8)	6.0 ± 1.4(5.2-9.8)
c'	2.6 ± 0.2(2.1-3.0)	2.3 ± 0.6(1.1-3.2)
Distance between vulva & post end of body	144.9 ± 9.3(121.8-156.3)	135.6 ± 15.5(106.4-156.3)
Body width at vulva	40.4 ± 3.1(34.9-45.5)	38.9 ± 5.0(29.2-44.7)
VL/VB	3.6 ± 0.2(3.4-4.0)	3.5 ± 0.6(2.8-4.5)
Rex	43 ± 4.6(31-49)	41 ± 1.6(41-46)
Roes	42 ± 4.9(31-47)	43 ± 4.3(38-53)
Rvan	15 ± 1.7(13-19)	16 ± 2.2(12-18)
Ran	32 ± 3.4(26-39)	34 ± 3.6(30-42)
RV	47 ± 3.5(40-53)	51 ± 3.1(46-54)
R	221 ± 12.5(197-255)	218 ± 11.3(196-238)
Stylet length	79.5 ± 3.2(73.7-84.2)	76.4 ± 3.9(69.7-83.4)
Length of stylet shaft	14.0 ± 1.4(10.6-15.4)	13.1 ± 0.9(12.2-14.6)
m	82.4 ± 1.7(80.2-87.2)	82.9 ± 1.1(81.4-84.4)
stylet length as percentage of body length	9.0 ± 0.6(8.1-10.1)	8.6 ± 1.4(5.2-10.0)
Distance between stylet base and D.O.G	5.2 ± 1.0(4.1-6.5)	6.5 ± 1.0(4.9-8.1)
O	6.5 ± 1.4(4.9-8.7)	8.5 ± 1.1(6.7-10.2)
Distance lip region-centre median bulb	106.3 ± 7.0(93.4-121.8)	99.2 ± 3.9(93.4-105.6)
MB	69.4 ± 4.2(64.6-77.1)	66.5 ± 1.2(64.1-68.1)

Table 3. Continued

Character/Ratio	*H.gigas* ♀(n=8)	*H. gigas* Jv (n=12)
L	1,368.0 ± 1,69.7(1068.8-1625)	1,007 ± 170.4(768.8-1,393.8)
Oesophagus length	198.8 ± 9.1(182.7-207.1)	170.9 ± 16.8(146.2-198.9)
Tail	192.3 ± 41.2(138-276.1)	160.7 ± 16.5(132-188.8)
Maximum Body width	48.0 ± 5.1(40.6-54.8)	29.8 ± 5.1(22.3-40.6)
a	28.6 ± 2.6(24.9-33.4)	33.8 ± 0.7(33.0-34.3)
b	6.9 ± 0.7(5.8-7.8)	6.3 ± 0.8(5.7-7.2)
c	7.2 ± 0.7(5.9-7.9)	6.8 ± 1.6(5.1-8.3)
Distance lip region end to vulva	1071 ± 125.3(845.5-1255.5)	-
Distance lip region end to anus	1175.6 ± 133(930.7-1348.9)	-
V	78.4 ± 0.7(77.3-79.2)	-
V'	91.1 ± 0.9(90.2-93.1)	-
Distance lip region to end oesophageal gland	205.0 ± 10.6(186.8-215.2)	171.0 ± 25.7(107.6-203)
Body width at anus	38.8 ± 5.3(32.5-46.7)	23.0 ± 3.6(16.2-28.4)
b'	6.7 ± 0.6(5.7-7.6)	6.1 ± 0.8(5.4-7.0)
c'	5.0 ± 0.8(4.3-6.8)	6.6 ± 1.0(5.9-7.8)
Distance between vulva & post end of body	296.9 ± 44.9(223.3-369.5)	-
Body width at vulva	43.4 ± 5.0(36.5-52.8)	-
VL/VB	6.9 ± 1.1(5.8-9.1)	-
Rex	56 ± 2.9(50-58)	49 ± 1.6(47-52)
Roes	54 ± 4.3(47-59)	43 ± 2.2(39-47)
Rvan	27 ± 1.8(24-29)	-
Ran	53 ± 6.7(45-67)	-
RV	80 ± 4.4(72-85)	-
R	351 ± 10.6(335-365)	292 ± 9.3(270-305)
Stylet length	127.1 ± 5.7(115.7-134.0)	99.1 ± 9.2(87.3-113.7)
Length of stylet shaft	18.9 ± 2.8(13.0-22.3)	16.2 ± 2.7(10.2-20.3)
m	85.1 ± 2.2(82.2-90.0)	83.6 ± 2.3(79.5-88.4)
stylet length as percentage of body length	9.4 ± 0.9(7.7-10.8)	10.0 ± 1.3(7.7-11.7)
Distance between stylet base and D.O.G	3.3 ± 2.9(0.0-8.1)	7.3 ± 2.2(4.1-10.2)
O	2.6 ± 2.3(0.0-6.5)	7.4 ± 2.3(4.0-11.4)
Distance lip region-centre median bulb	154.5 ± 16.1(136.0-190.8)	127.2 ± 13.9(105.6-150.2)
MB	77.7 ± 7.4(72.5-94.0)	71.9 ± 1.4(70.8-94.9)

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 & 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-centre 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)

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 & 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-centre 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)

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
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
Rex	57 ± 3.0(50-60)	66 ± 5.1(61-81)	62 ± 3.6(57-70)
Roes	63 ± 5.5(53-72)	63 ± 5.5(57-79)	61 ± 2.4(57-65)
Rvan	24 ± 3.8(19-31)	24 ± 2.9(19-29)	22 ± 2.6(15-26)
Ran	47 ± 7.2(39-60)	63 ± 6.5(52-75)	57 ± 5.1(46-65)
RV	72 ± 6.9(60-84)	87 ± 7.1(72-97)	78 ± 5.2(65-85)
R	309 ± 17.1(278-343)	360 ± 23.2(303-388)	339 ± 19.1(306-364)
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)
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)
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)
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)
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)
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)
Distance lip region-centre 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)
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)

Table 6. continued

Character/Ratio	*H. zuckermani* ♀(n=19) type a Sycamore	*H. zuckermani* ♀(n=17) type d Host:Oat grass	*H. zuckermani* ♀(n=16) type d Host:Oak
L	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	189 ± 10.3(170.5-207.1)	180.2 ± 7.8(170.5-196.9)	176.1 ± 4.9(168.5-182.7)
Tail	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	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	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	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	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	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	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	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'	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	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	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'	5.9 ± 0.3(5.3-6.7)	5.9 ± 0.3(5.2-6.2)	5.8 ± 0.3(5.2-6.3)
c'	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	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	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	4.7 ± 0.6(2.7-6.0)	4.3 ± 1.0(2.4-5.2)	4.6 ± 0.3(4.3-5.3)
Rex	62 ± 3.2(58-69)	61 ± 2.5(57-65)	60 ± 3.0(51-64)
Roes	60 ± 2.8(56-66)	61 ± 3.2(55-66)	62 ± 4.1(58-72)
Rvan	21 ± 2.1(18-25)	21 ± 2.0(16-24)	17 ± 3.9(13-28)
Ran	60 ± 7.4(48-77)	53 ± 4.5(46-59)	53 ± 3.5(45-60)
RV	81 ± 8.3(68-100)	75 ± 4.9(66-81)	71 ± 4.6(60-79)
R	330 ± 21.0(301-374)	323 ± 7.9(309-334)	321 ±11.4(289-338)
Stylet length	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	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	83.2 ± 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	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	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	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-centre median bulb	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	72.8 ± 1.2(70.8-76.2)	71.6 ± 1.3(69.9-73.8)	71.8 ± 0.9(70.6-73.8)

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)

FIGURES

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.

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.

Fig 3. Light micrographs of *Hemicycliophora wyei* n.sp. A) Entire female. B) Lip region. C) posterior portion. D-G) Vulva. 1. Vulval sleeve and 2.Vulval lips. E) Lateral fields. Arrows showing two faint lines. F) Lateral fields. Arrows showing breaks and anastomoses inside lateral fields.

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.

Fig 5. Light micrographs of *Hemicycliophora ephicaroides*. A) Entire female. B) Anterior region. C) Lip region. D) Lateral fields showing 2 lines. E) Posterior region. F-G-H-K) Vulva. 1. Vulval sleeve and 2.Vulval lips. I) Lateral fields detail. 1. Lateral fields lines. 2. Scratches outside lateral fields. J) Aberrant lip region.

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.

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 and 2. Vulval lips. G-H-I) Lateral fields. 1) Line in lateral fields. 2-3) Scratches on body annuli outside the lateral field.

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.

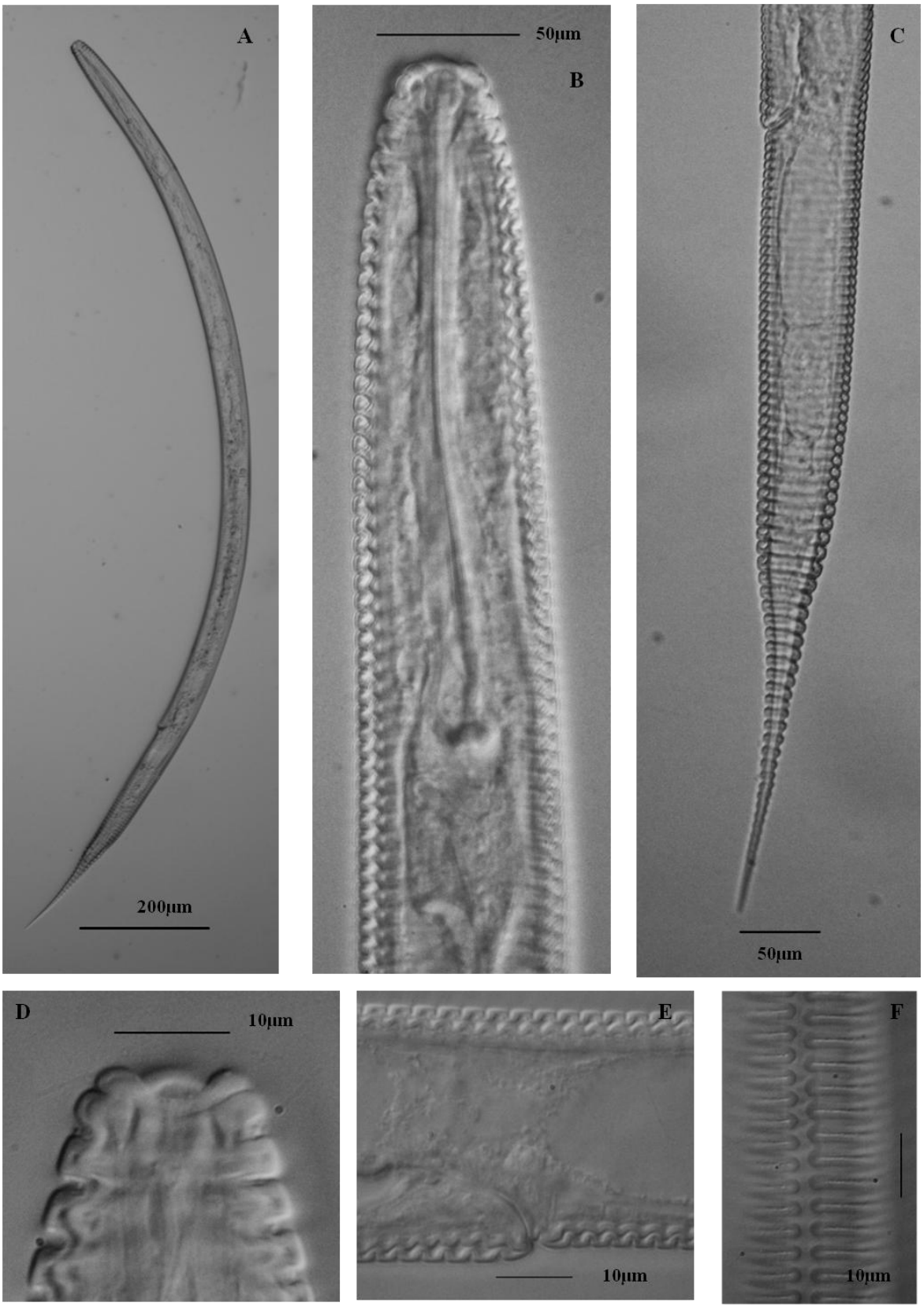
Fig 9. Light micrographs of *Hemicycliophora shepherdi.* A) Entire female. B-C) Lip region. D) Posterior region. E) Vulva. F) Lateral fields. Arrows showing anastomoses.

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.

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

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

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.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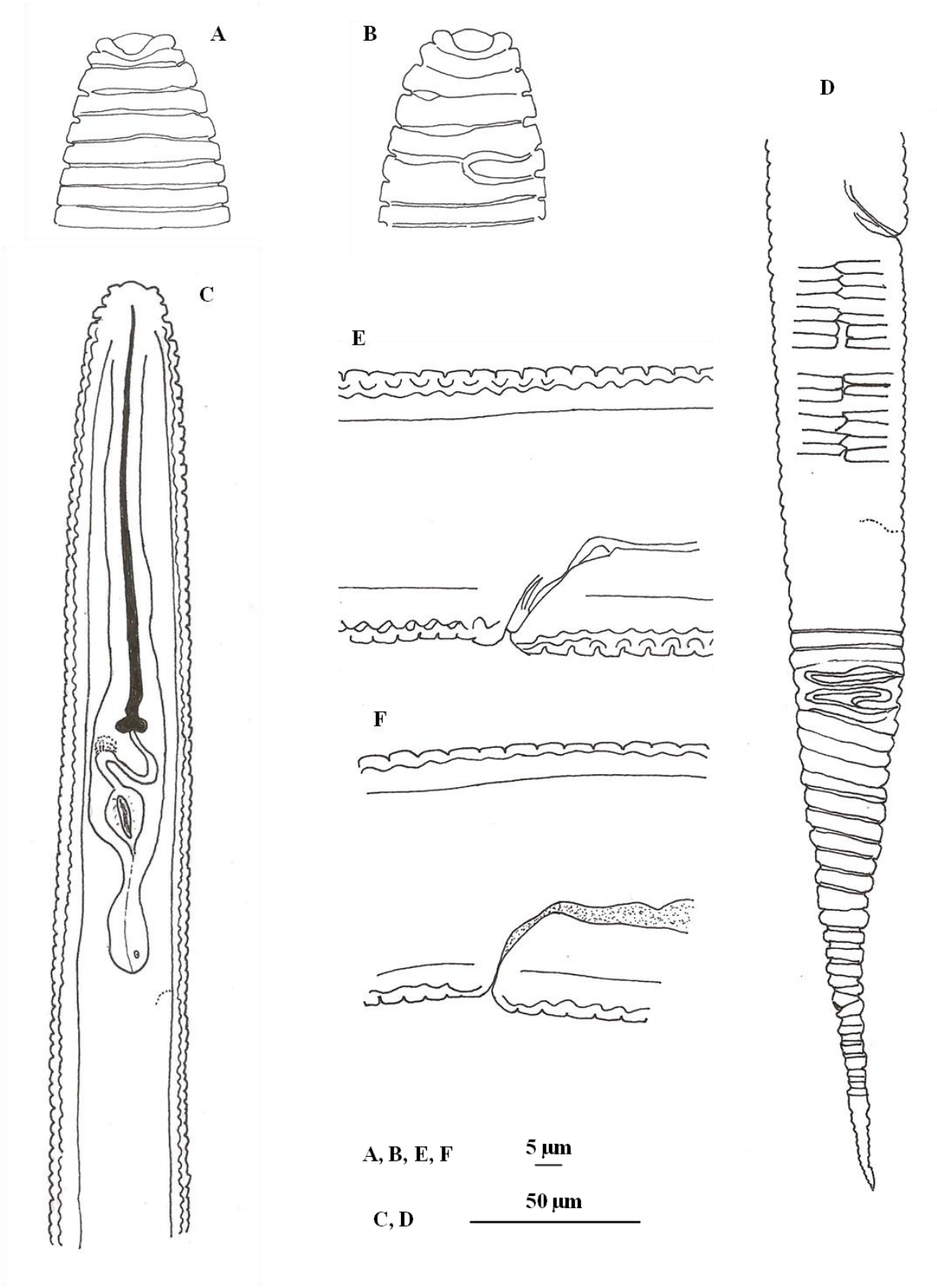
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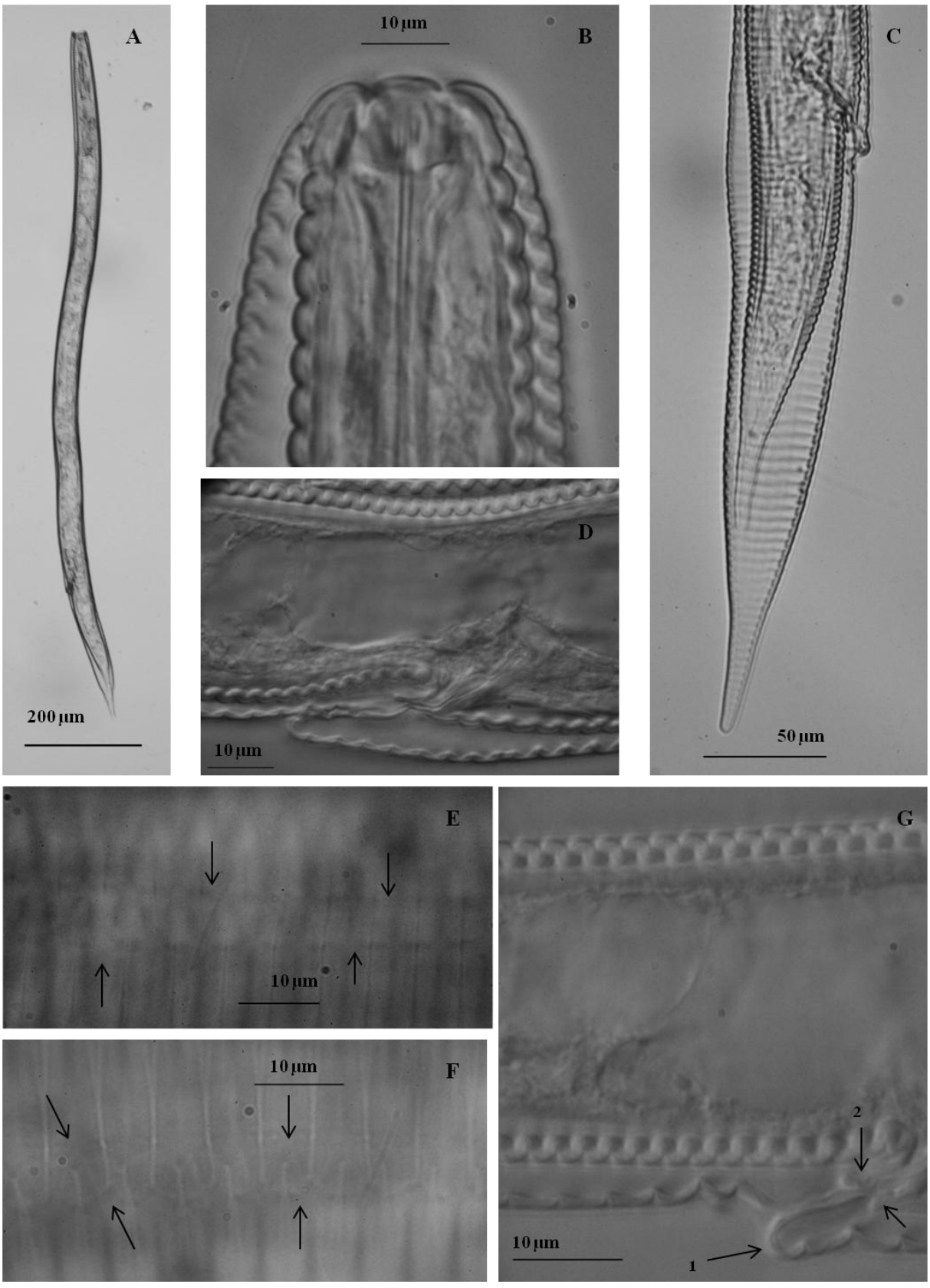
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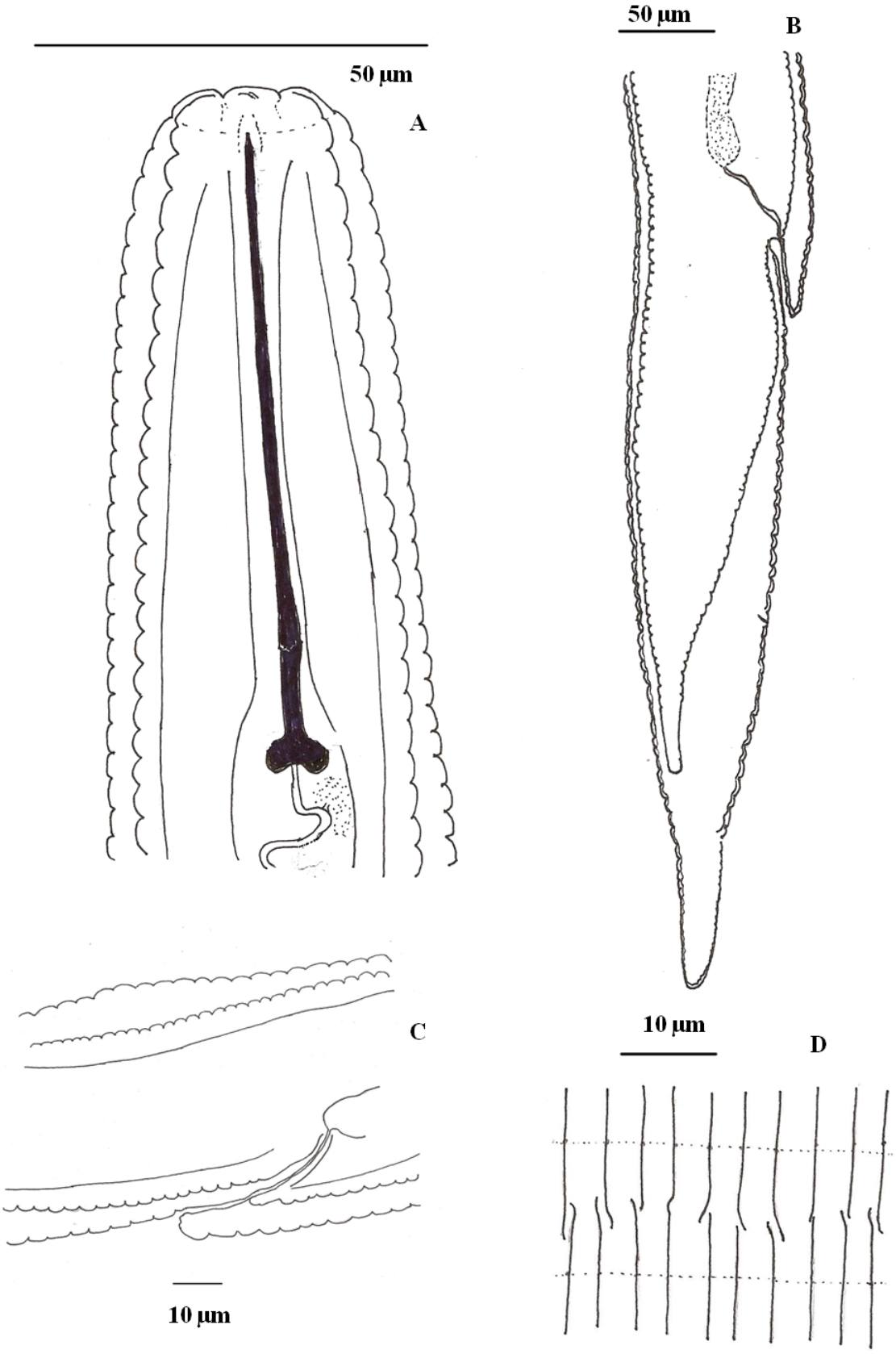
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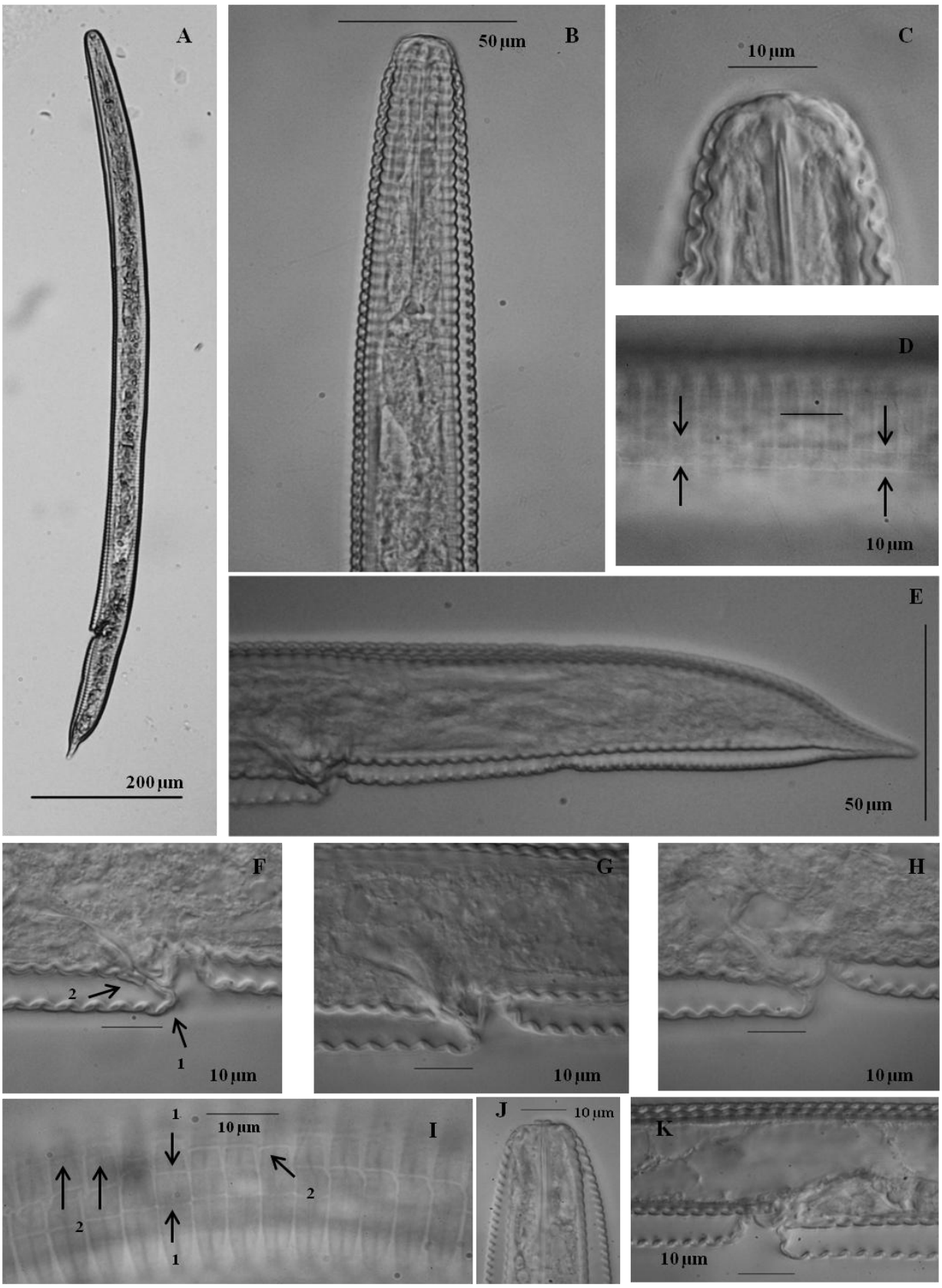
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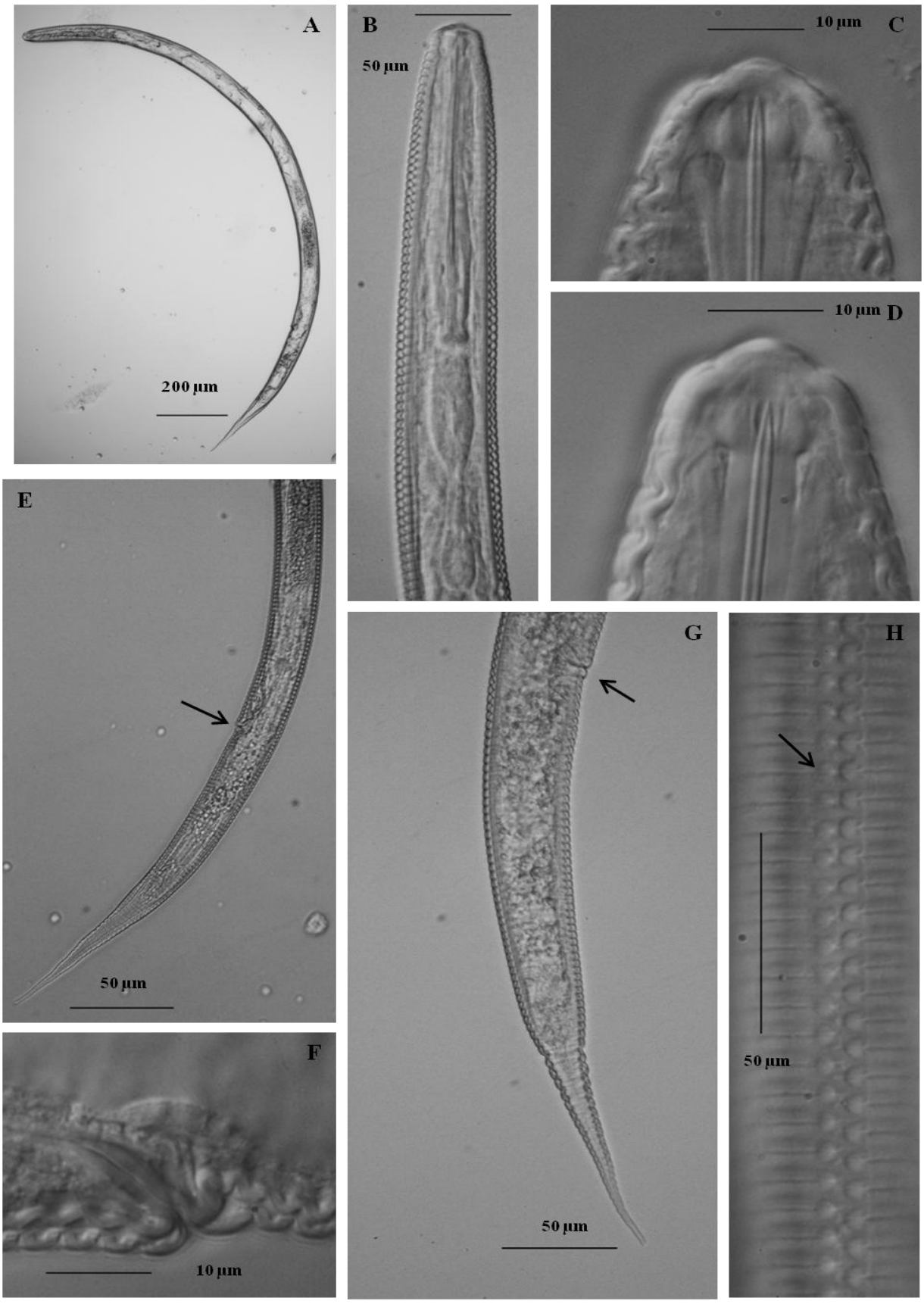
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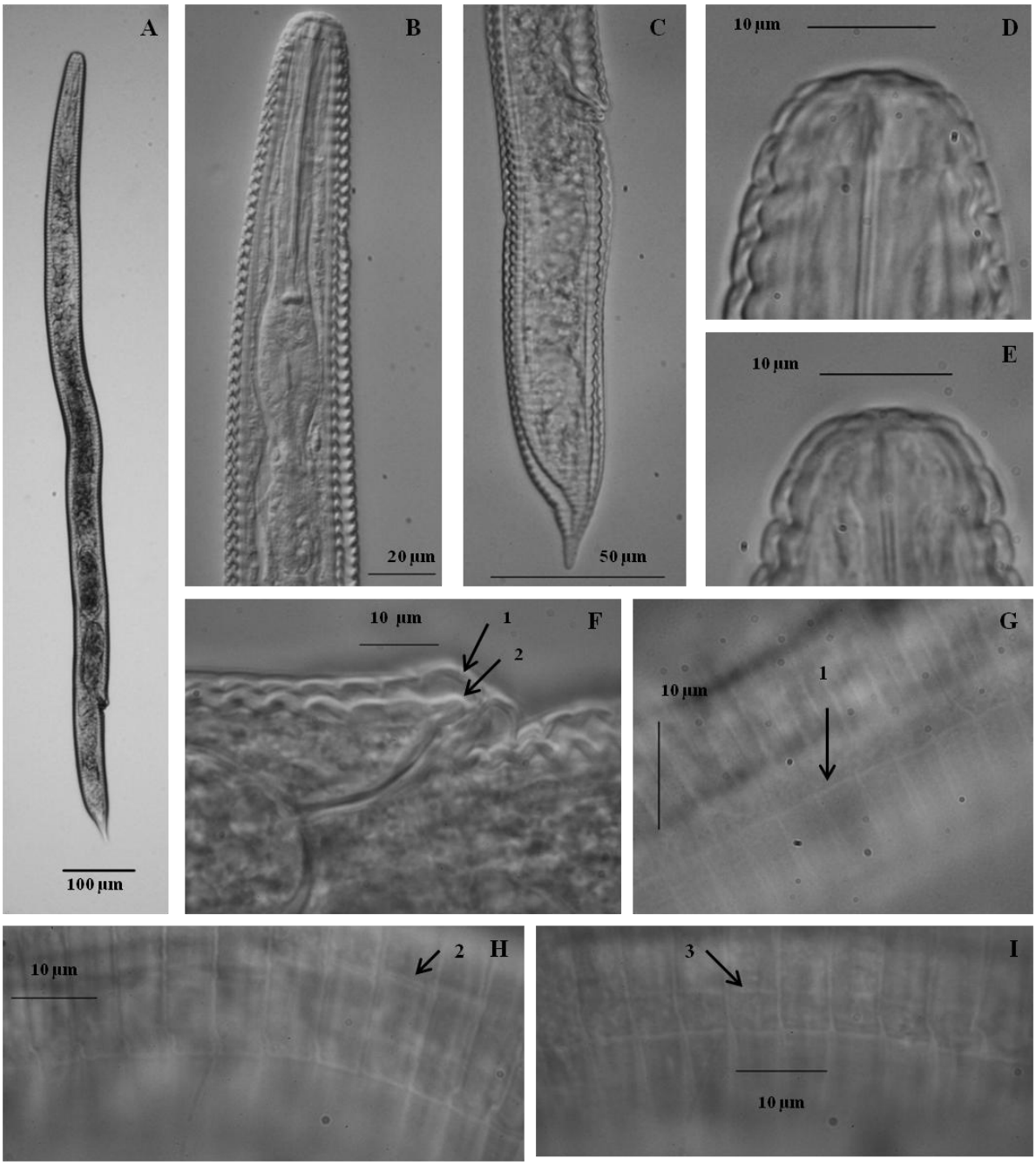
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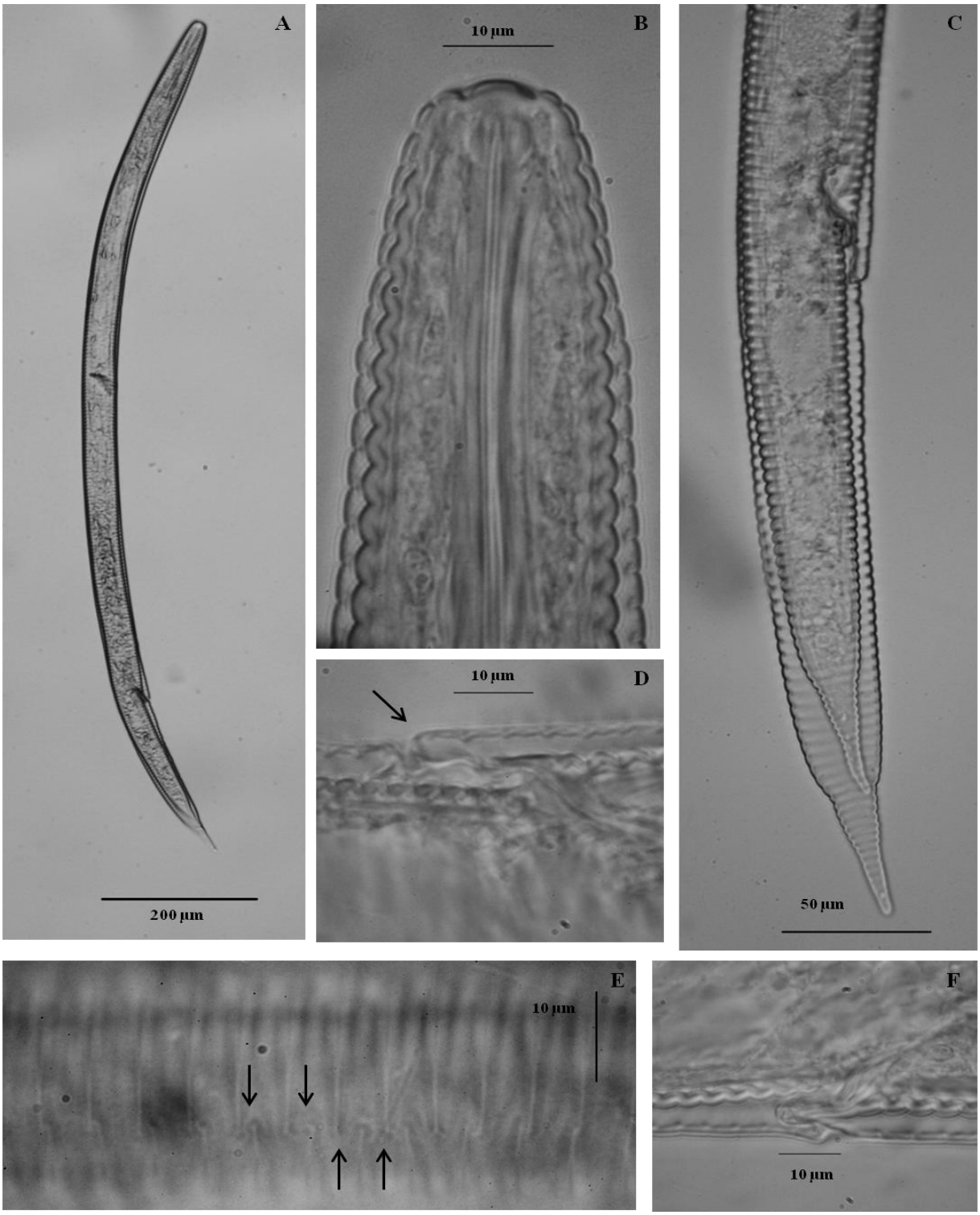
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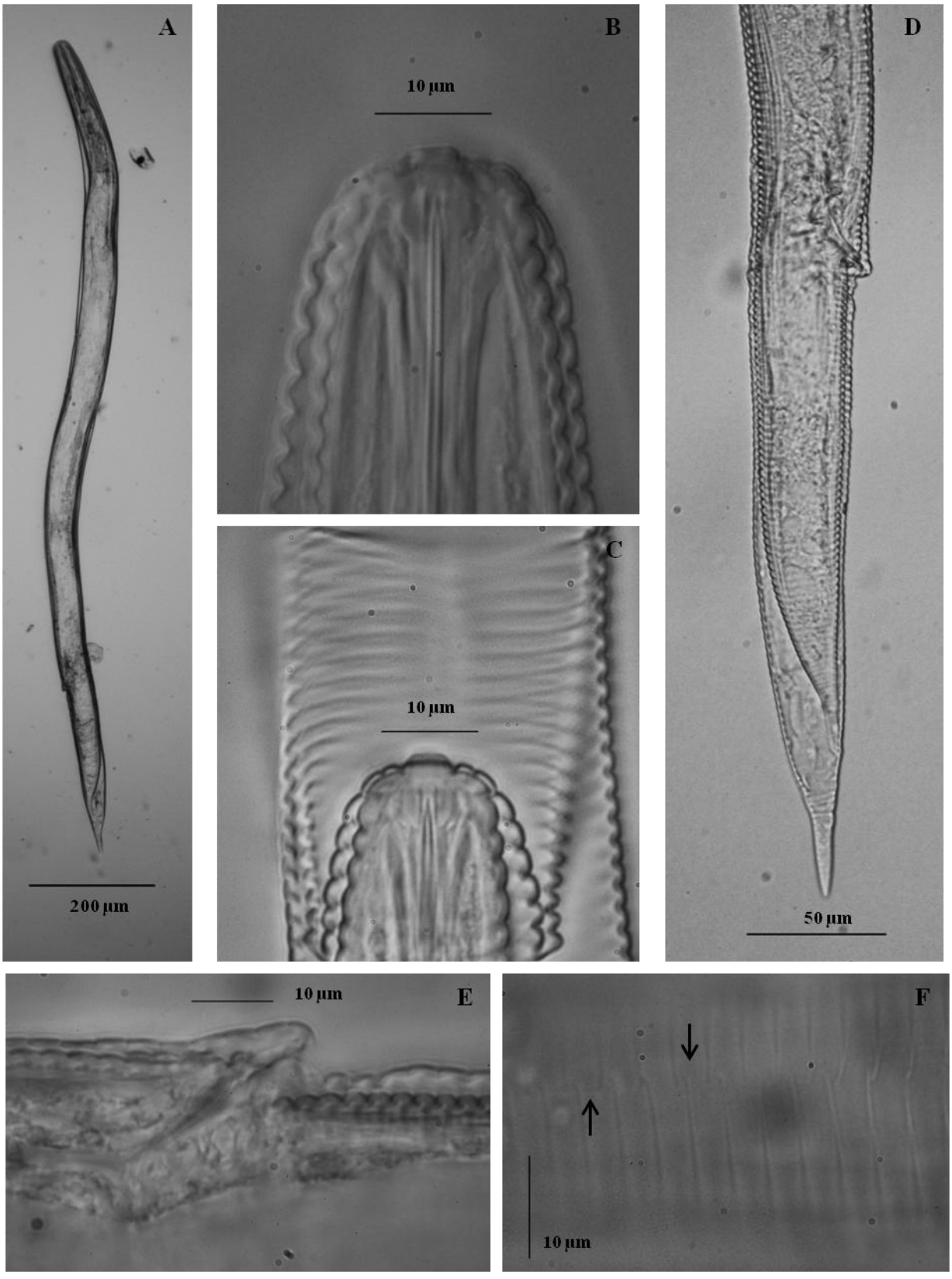
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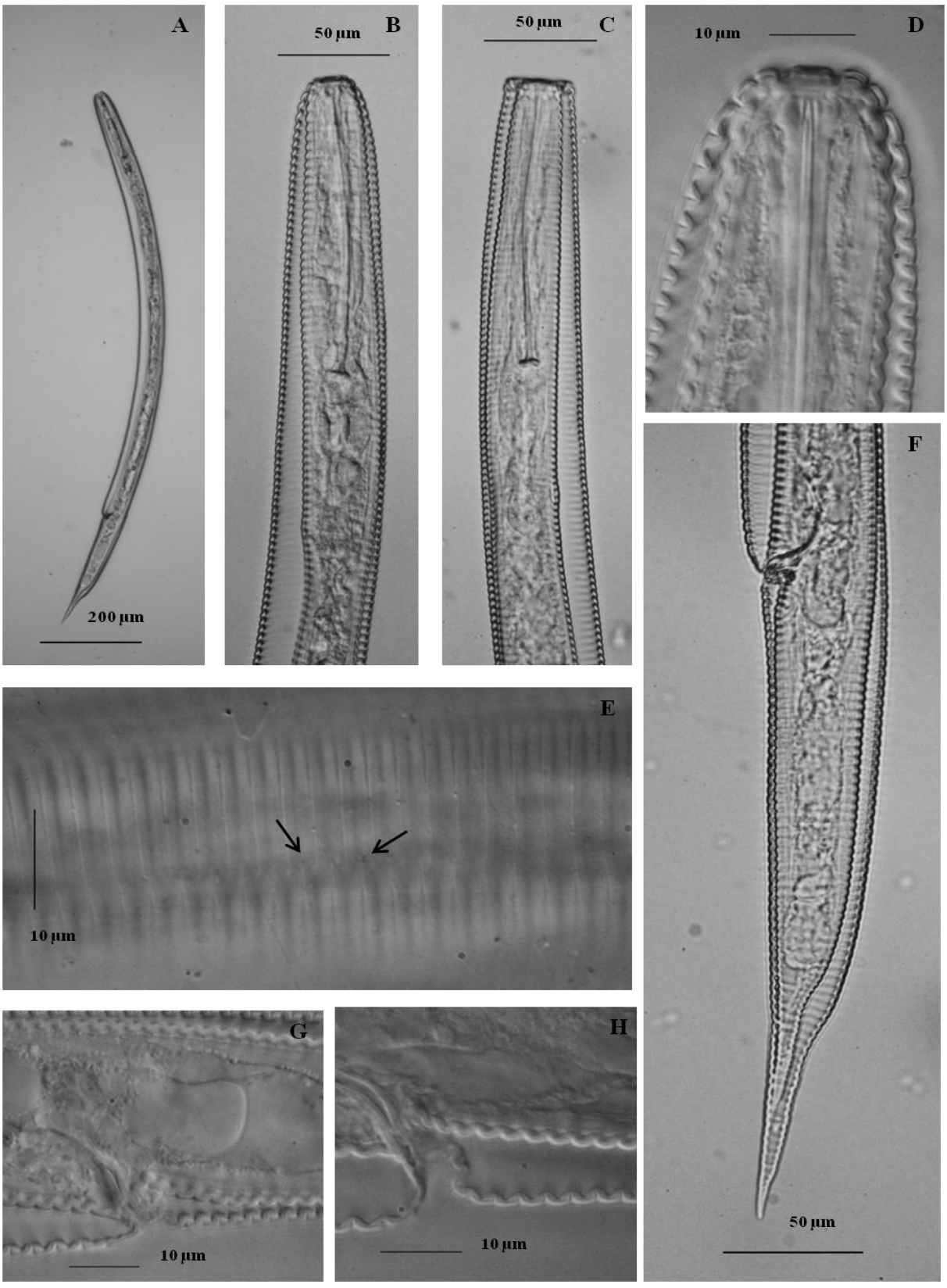
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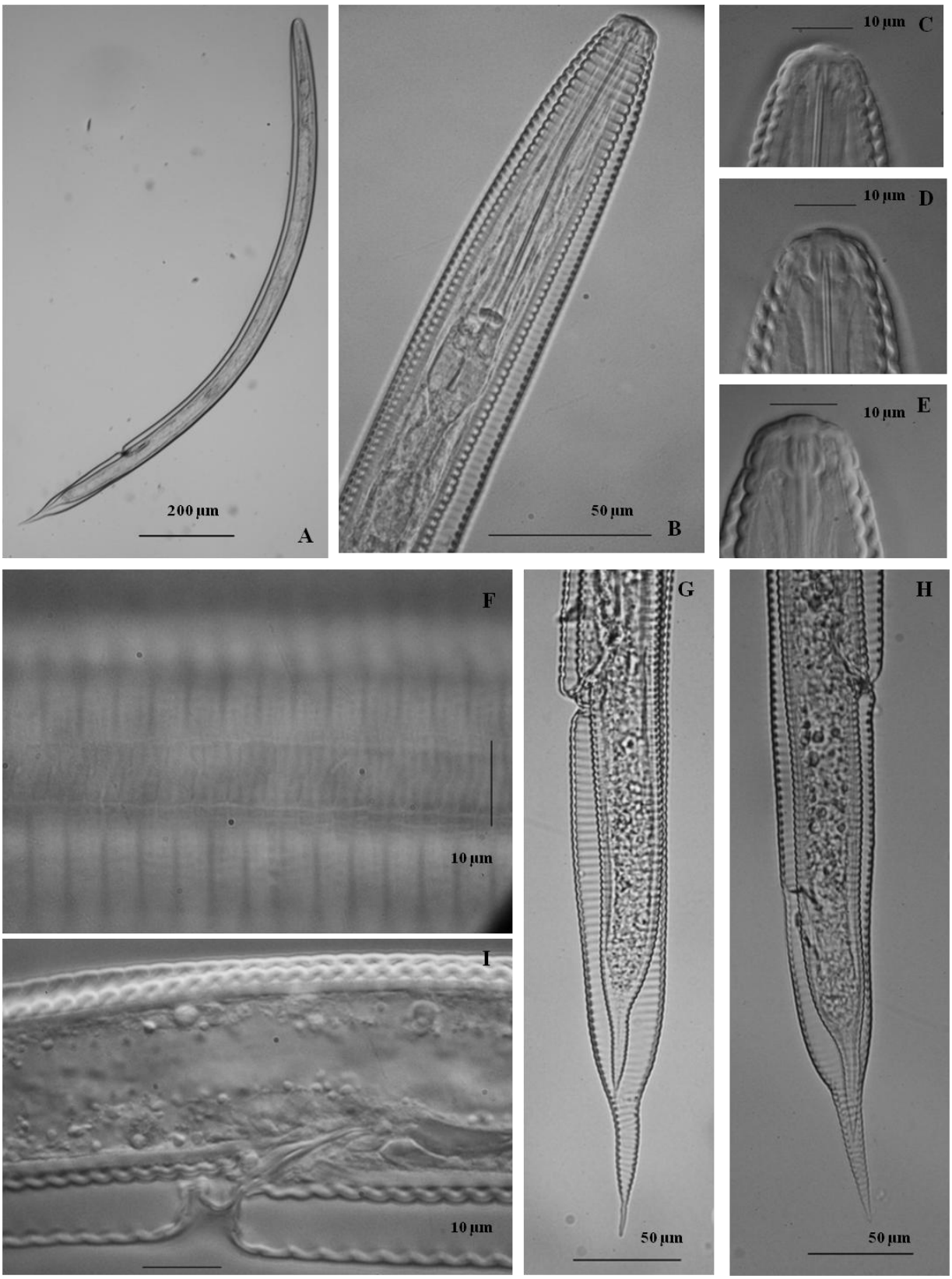
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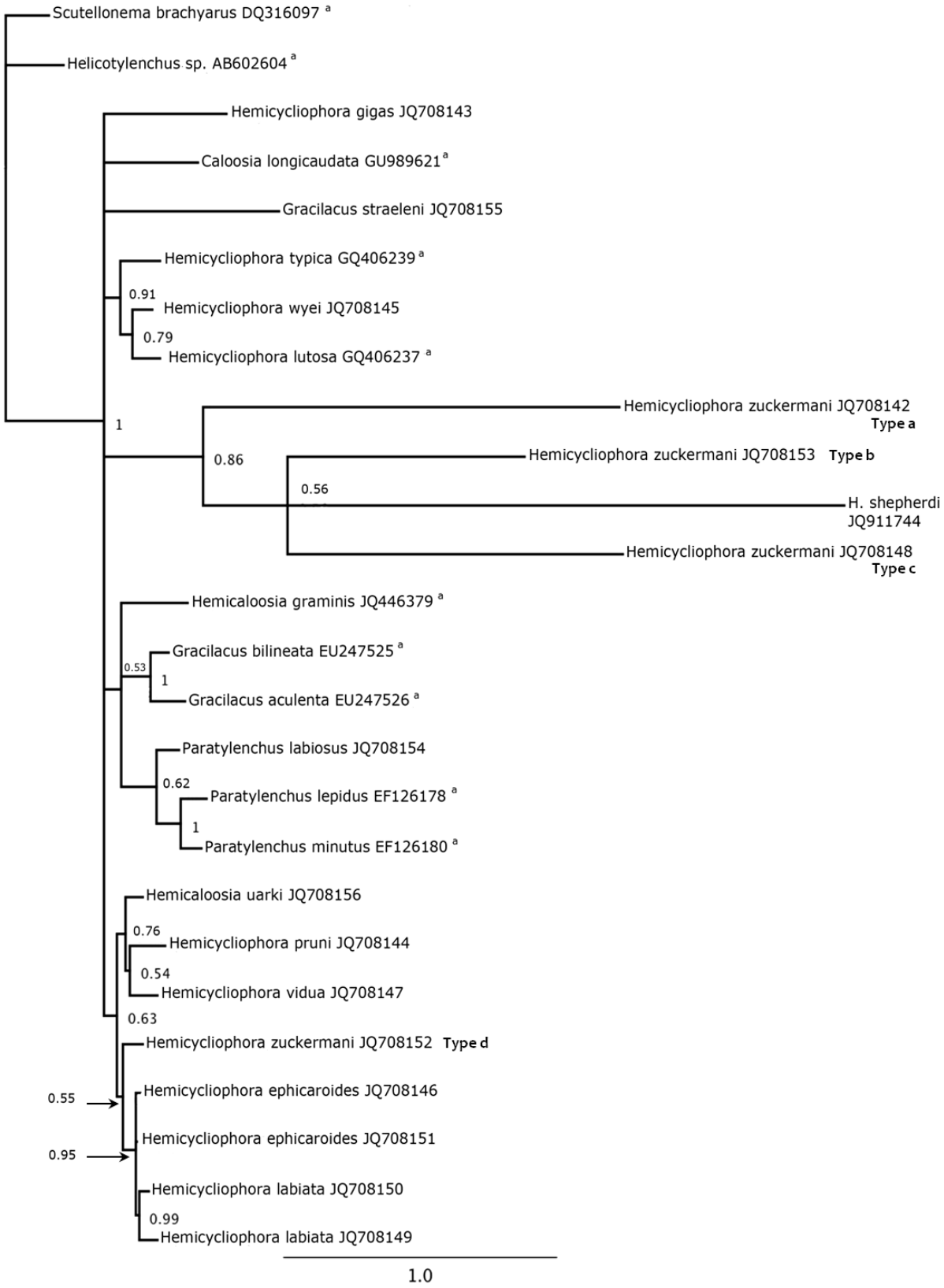
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APPENDIX

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Sent: Tuesday, February 12, 2013 11:30 AM
To: Nancy Burelle, JON Editor in Chief [nancy.burelle@ars.usda.gov]; Marco A. Cordero Lopez
Cc: Andrea Skantar, SON Secretary [andrea.skantar@ars.usda.gov]

To Whom it may concern,

As President of the Society of Nematologists, the publisher of the Journal of Nematology, I grant permission for Marco A. Cordero Lopez to submit electronically the content of the dissertation.

Brent Sipes
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Society of Nematologists

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MOLECULAR BASED-PHYLOGENETIC RELATIONSHIPS IN THE SUPERFAMILY CRICONEMATOIDEA

Abstract:

Populations of nematodes of the superfamily Criconematoidea were obtained and identified morphologically from different geographical areas in the continental United States in order to study their phylogenetic relationship based on the DNA sequences of the nuclear 18S and first internal transcribed spacer ribosomal regions. Phylogenetic analysis of the ITS1-rDNA region showed monophyletic groups in Criconematidae: A) species of *Mesocriconema* and *Criconemoides*; B) *Bakernema, Criconema Hemicriconemoides* and *Xenocriconemella* species and C) Subfamily Hemicycliophorinae, (*Caloosia, Hemicaloosia* and *Hemicycliophora)* and family Tylenchulidae (*Paratylenchus* and *Gracilacus*) were clustered together, respectively with some variation among Hemicycliophorinae species. Molecular phylogenetic analysis using the ITS1-r-DNA marker rejects the hypothesis of a common ancestor for criconematids with double sheath or cuticle among others characters. This molecular phylogenetic study showed different rates of substitution in ITS1 rDNA sequences.

Keywords: 18S, Criconematoidea, genetic variation, internal transcribed spacer 1, phylogenesis, ribosomal DNA, maximum parsimony, maximum likelihood.

The history of the superfamily Criconematoidea Taylor, 1936 began in 1882-1883 at the international expedition to Hoste Island, Chile from which a juvenile of *Criconema giardi* (Certes, 1889) Micoletsky, 1925 was described (Raski et al., 1984; Raski and Luc, 1985). Two systems of classification for this group have been proposed. In one system, the superfamily Criconematoidea was raised one level to the suborder Criconematina by Siddiqi (1980, 2000) with three superfamilies Criconematoidea, Hemiciclyophoroidea and Tylenchuloidea. In the other hand, Raski and Luc (1987) proposed the superfamily Criconematoidea consisting of only two families Criconematidae and Tylenchulidae. Morphological characters that describe the superfamiliy Criconematoidea are the typical criconematoid oesophagus characterized by having a median bulb or metacorpus enormously developed, with a large median valvular apparatus, metacorpus and a broad procorpus are amalgamated and surrounded the basal region of the stylet. At postcorpus, the isthmus could be long and off set from the basal bulb or short and broad and fused with a small basal bulb, containing three oesophageal glands (Raski and Luc, 1987). However, the group shows diverse degrees of variation on morphological characters among the species which frequently makes their identification difficult (Geraert, 2010; Raski and Luc, 1987; Siddiqui, 2000).

Molecular phylogenetic is an excellent method to determine relationships among taxa based on the information resulting from different molecular markers as well as morphological identification. The nuclear ribosomal genes, 18S and 28S, have low variability (i.e. low rate of evolution) with the 28S gene less conserved than 18S. These two important genetic markers are currently used to phylogenetic studies on different organisms in the same taxa that diverged long ago. Conversely, the ITS1 and ITS2 regions of rDNA have a high rate of evolution because of mutations. Similarities in the ITS sequence regions tend to be greater within species than among

species, with exception of *Meloidogyne* species which has intraspecific variation too high for the marker to be reliable for species discrimination (Gasser, 2001; Powers, 2004; Blaxter, 2001; Subbotin and Moens, 2006). In recent years, evidence of intra-specific and intra-individual variation in nematode nuclear ribosomal DNA sequences, including ITS, has been mounting (Cutillas et al., 2004; Hugall et al., 1999; Mes and Cornelissen, 2004; Porazinska et al., 2010).

Because of their low intraspecific variation, nuclear rDNA transcriber regions have been used as markers for species identification in several nematodes, representing useful information in order to develop tools for diagnostic purposes based on PCR reactions (Gasser, 2001). A recent phylogenetic analysis in Criconematoidea based on the D2-D3 expansion a segment which is a less conserved region of the 28S-rDNA gene, named divergent domains, supported monophyly of the genera *Mesocriconema*, *Hemicriconemoides* and *Criconema* (Subbotin 2005). In addition, a single origin of criconematids with single or double cuticle was rejected showing the usefulness of this marker to discriminate among characters that result from common ancestry versus those that are homoplasious. (Subbotin 2005). Recently, in a study by Powers et al. (2010), sequences of the nuclear ribosomal ITS1 region were obtained for *Discocriconemella inarata* Hoffman, 1974; *M. curvatum* (Raski, 1952) Loof & De Grisse, 1989; *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989 and *M. xenoplax* (Raski, 1952) Loof & de Grisse, 1989.They found evidence for the paraphyly of *Discocriconemella* including placement of *D. inarata* separate from other species of the genus and instead with species of *Mesocriconema*.

This study followed the classification system for the superfamily Criconematoidea of Raski and Luc (1987) and Maggenti et al. (1988). The genera *Mesocriconema* Andrássy, 1965 and *Criconemoides* Taylor, 1936 are used in accordance with their re-establishment and validation, respectively (Loof and De Grisse, 1967; Loof and De Grisse, 1989).

The objective of this study was to further test and clarify molecular based-phylogenetic relationships among different genera and species of the superfamily Criconematoidea using 18S-rDNA and ITS1-rDNA sequences and to consider molecular phylogenies in relation to classical morphological features of the group.

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) (*E*trex Garmin, Olathe, KS) was used to identify and record the location. Additional populations of nematodes were received in 1M NaCl from California, Florida, Kansas, Missouri, North Carolina and Tennessee. Nematodes collected in Arkansas were extracted from soil by Cobb sieving and flotation-centrifugation methods (Cobb, 1918; Jenkins, 1964). Specimens of each population were separated in two groups: 1) nematodes for morphological identification and 2) nematodes for molecular analysis. For morphological identification, nematodes were fixed in hot 3% Formaldehyde for one week and later infiltrated with glycerine using the modified slow method of Seinhort (Seinhorst, 1959; Seinhorst 1962). A range of 5 to 10 nematodes for molecular work were used for each population. Nematodes were crushed individually in 5µl of PCR water (BDH Chemicals, Chester, PA) and store at -80^{o}C until use.

PCR: Polymerase chain reaction (PCR) was performed using 5 µl of a DNA extraction in a 50-µl PCR reaction mixture. Primers used to amplify a 3' portion of the 18S gene were: 18S1.2 (5'- GGCGATCAGATACCGCCCTAGT -3'); and 18Sr2b (5'-TACAAAGGGCAGGGACGT-3') (Mullin et al., 2005). Primers used to amplified the 3' end of

the 18S rDNA gene, the entire ITS1 region and the 5' end of the 5.8S rDNA gene were rDNA2 (5'-TTGATTACGTCCCTGCCCTTT- 3') (Vrain et al., 1992) and rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA- 3') (Cherry et al., 1997). Primers amplified a PCR product of 600 bp for both markers. The PCR mixture contained 4 µl of dNTP-mixture (0.2mM 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 ^{o}C for 2 minutes, then 40 cycles of denaturation at 94 ^{o}C for 45 seconds, annealing at 52 and 56 ^{o}C for 45 seconds and extension at 72 ^{o}C for 60 seconds. A final extension for 5 minutes at 72 ^{o}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. An UV transluminator (BioDoc-it ™ system, UVP, Upland, CA) was used to visualize PCR products. For 18S-rDNA amplification, five specimens representing each genus were selected from each population. ITS1 amplification was performed previously using the same procedure (Cordero et al., 2012a,b,c)

Sequencing: PCR products were purified using Nanosep centrifuge tubes 100k (Pall, Port Washington, NY) in a refrigerated centrifuge at 15^{o}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. Pairwise alignment of forward and reverse sequences was performed to obtain consensus sequences of either 18S or ITS1 amplicon using BioEdit alignment software (Hall, 1999). Alignment of 18S sequences was performed using Geneious aligner with Geneious Pro 5.6.6

created by Biomatters (http://www.geneious.com) and alignment of ITS1 sequences was performed using 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) parameters (Dariba et al., 2012; Posada and Crandall, 1998; Posada, 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% mayority rule (Larget and Simon, 1999). Sampling in the Markov chain was made with a frequency of 200 generations. Sequences of 18S-rDNA obtained in this study were submitted to GenBank (JQ708157 to JQ708179). Dataset was supplemented by additional sequences downloaded from GenBank (Table 1).

Results and discussion

Phylogenetic study of 18S and ITS1 regions

The length of the 18S-rDNA PCR amplicon was 600 bp for the species. After correction and alignment an internal transcribed spacer 1 length was 375 bp. JModeltest estimated the HKY+G model as the best fit (-Ln likelihood = 2762.0794; AIC= 5682.1588; K=79; freqA =0.2371; freqC=0.2310; freqG=0.2810; freqT=0.2508; Kappa=2.0750 [(ti/tv=1.053)] Gamma shape=0.8310) for the analysis (Fig. 1).

The length of the ITS1-rDNA PCR amplicon was 640 bp. After alignment of all the consensus sequences, the ITS1 size used for phylogenetic analysis was 549 bp. JModeltest estimated the GTR+G model as the best fit (-Ln likelihood = 15770.6970; AIC= 31867.3939; K=163; freqA =0.2323; freqC=0.2740; freqG=0.2758; freqT=0.2179; R(a)[AC]=0.8263; R(b)[AG]=1.7400; R(c)[AT]=1.2319; R(d)[CG]=0.9083; R(e)[CT]=2.2622; R(f)[GT]=1.000; Gamma shape=2.3370) for the analysis (Fig. 2).

According to data obtained from the analysis of the conserved 18S-rDNA region, this marker established a condition of common ancestry among the genera of Criconematoidea. A population of *Tylenchulus* sp. juveniles found in Missouri associated with vineyards was identified and clustered with *Trophotylenchus* and *Tylenchulus semipenetrans.*

Conversely, ITS1 data confirmed the monophyly of *Mesocriconema* and *Criconemoides* and the monophyly of spine nematodes *Bakernema, Criconema*, *Ogma, Xenocriconemella* and sheathoid nematodes, *Hemicriconemoides*. Three species of *Hemicycliophora zuckermani* showing high genetic divergence were clustered together with *H. shepherdi*. *Paratylenchus* and *Gracilacus* were clustered in two groups but their monophyly was not confirmed. A population of *M. xenoplax* which showed the highest genetic divergence (57%) among the rest of the *M. xenoplax* group was clustered along with the most dissimilar species: *M. onoense*, *M. surinamensis* and *Criconemoides informis.* The position of *Hemicaloosia graminis* and *Caloosia longicaudata* were not resolved in this study.

Molecular phylogenetic analysis using the ITS1-rDNA region rejects the hypothesis of single origin for genera with a double cuticle or double cuticular sheath in *Hemicycliophora* and *Hemicriconemoides*. Even though, this analysis did not show monophyly of *Hemicycliophora*

because the high genetic divergence of ITS1 sequences of the 3 species previously mentioned, we agree in the monophyly of *Hemicycliophora* previously shown by Subbotin et al., (2005).

Rejection of the hypothesis of a single origin for genera with a double cuticle includes other important characters such as body length; fine, smooth and/or coarse body annuli; presence of ornamentation in body annuli; presence or absence of sub-median lobes; variations on the criconematoid oesophagus such as length of isthmus and size of basal glands in postcorpus; regular ectoparasitism; ectoparasitism with sedentary obese females (Family Paratylenchinae: *Paratylenchus*, *Gracilacus*) and sedentary obese females with or without presence of immature females showing endo or semi-endoparasitism in their life cycle (Family Tylenchulinae: *T. semipenetrans*, *Trophotylenchus*). (Geraert, 2010; Raski and Luc, 1987; Siddiqui, 2000). Moreover, the position of *Mesocriconema sphaerochepala* with a genetic divergence ranged between 42-56% with others species of *Mesocriconema* was not resolved. These results previously mentioned: rejection of a single origin for Criconematoidea and the uncertainty of the position of *M. sphaerochepala* are in agreement with the results of the analysis of the D2-D3 expansion segments of the 28S-rRNA by Subbotin et al. (2005).

Discocriconemella inarata was placed as a sister species with the spines nematodes in this group however, Powers et al., (2010) found decisive arguments to establish the position of this species as part of *Mesocriconema* group, using ITS1 and cytochrome b.

The high nucleotide similarity of the 18S- rDNA conserved region erroneously accepted the hypothesis of a single origin for Criconematoidea. However, this marker was useful to clarify the position at the family level of an unidentified population of juveniles of *Tylenchulus* sp. found in Missouri

The use of nuclear ribosomal 18S-rDNA and ITS1-rDNA for phylogenetic purposes has proved its usefulness to integrate morphological and molecular information. Mullin et al., (2005) using 18S-rDNA concluded that the suborders Nygolaimina and Dorylaimina were monophylectic lineages with a possible paraphyletic relationship within Nygolaimina. Species of *Ekphymatodera thomasoni* and *Bilobodera flexa*, non-forming cyst nematodes, were clustered with cyst forming nematodes when 18S and ITS1-rDNA were used to study their phylogenetic relationships (Ferris et al., 2004). Tanha Maafi et al.,(2003) using ITS1-rDNA defined group of cyst nematodes: *Avenae, Sacchari*, *Schatchtii*, *Humuli*, *Cyperi* and *Goettingiana* according to morphological and molecular information. Powers et al., (2010) using ITS1-rDNA information showed that *Discocriconemella inarata* which morphologically showed lack of submedian lobes and an anterior vulval lip with two small lobes is closely related to *Mesocriconema* species and distantly related to species of the genus *Discocriconemella.*

To perform a phylogenetic analysis, it is necessary to analyze at least two markers with different variability in order to detect true relationships among them. Highly conserved markers such as 18S-rDNA can be used it to determine the position of an organism at higher taxonomic rank e.g. family level. On the other hand, ITS1-rDNA was useful to determine relationships to genus and species level. In the particular case of the family Tylenchulidae, more ITS1 species sequences have to be added to the data set to get a better resolution of their positions.

More populations of Criconematoidea need to be incorporated in order to have a better understanding of the relationships based on morphology, biology and molecular information derived from the ITS1 ribosomal DNA region. The ITS1-rDNA region is informative enough to identify populations at species level and to characterize different populations of the same species with variation within the marker e.g. single nucleotide polymorphism (SNP) and/or short tandem

repeats. 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 need to be studied. Further research is needed in order to have a clearer idea about the relationships between taxonomic and molecular identification and the phylogeny of Criconematoidea.

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TABLES

Table 1. Supplemental 18S and ITS1 sequences rDNA of Criconematoidea obtained from GenBank for the analysis.

18S-rDNA Species Sequences	GenBank number	Host	Origin
Aphelenchoides besseyi	AY508035	Unknown	Florida, USA
Bakernema inaequali	HM116043	Unknown. Tallgrass prairies	USA
Criconema sp.	FJ489592	Unknown. Rain forest	Costa Rica
Criconemoides sp	FJ489592	Unknown. Rain forest	Costa Rica
Gracilacus latescens	AY912039	Unknown. Forest	Konza, KS. USA
Hemicriconemoides sp.	JF972471	Unknown	USA
Hemicriconemoides wessoni	JF972467	Unknown	USA
Hemicycliophora sp	FJ489588	Unknown. Rain forest	Costa Rica
Hemicycliophora typica	JF972475	Unknown	USA
Mesocriconema rustica	FJ489582	Unknown. Rain forest	Costa Rica
Ogma decalineatum	AY919222	Unknown	USA
Paratylenchus dianthus	AJ966496	Unknown	Belgium
Paratylenchus microdorus	AY284633	Unknown	The Netherlands
Paratylenchus straeleni	AY284631	Unknown	The Netherlands
Trophotylenchus sp	AY146455	Unknown. Forest	Konza, KS. USA
Tylenchocriconema sp.	FJ489544	Unknown. Rain forest	Costa Rica
Tylenchus davainei	AY146459	Unknown	USA
ITS1-rDNA Species Sequences			
Caloosia longicaudata	GU989621	Unknown	Hawaii, USA
Discocriconemella inaratus	HM116055	Unknown. Tallgrass prairies	USA
Gracilacus aculenta	EU247526	Bamboo	Taiwan
Gracilacus bilineata	EU247525	Bamboo	Taiwan
Helicotylenchus sp	AB602604	Bermuda grass	Japan
Hemicaloosia_graminis	JQ446376	Turfgrass	USA
Hemicriconemoides californianus	EU180057	tea	Taiwan
Hemicriconemoides kanayaensis	EF126179	tea	Taiwan
Hemicriconemoides parasinensis	EU664601	Grape	Taiwan
Hemicriconemoides stricthatecatus	GQ354786	Unknown	Taiwan
Hemicycliophora lutosa	GQ406237	Fallow soil	South Africa
Hemicycliophora typica	GQ406239	Sugar cane	South Africa
Mesoscriconema curvatum	HM116066	Unknown. Tallgrass prairies	USA
Mesoscriconema xenoplax	HM116057	Unknown. Tallgrass prairies	USA
Mesoscriconema xenoplax	HM116073	Unknown. Tallgrass prairies	USA
Ogma decalineatum	HM116075	Unknown. Tallgrass prairies	USA
Paratylenchus lepidus	EF126178	tea	Taiwan
Paratylenchus minutus	EF126180	tea	Taiwan
Scutellonema brachyurum	DQ316097	Unknown	Taiwan

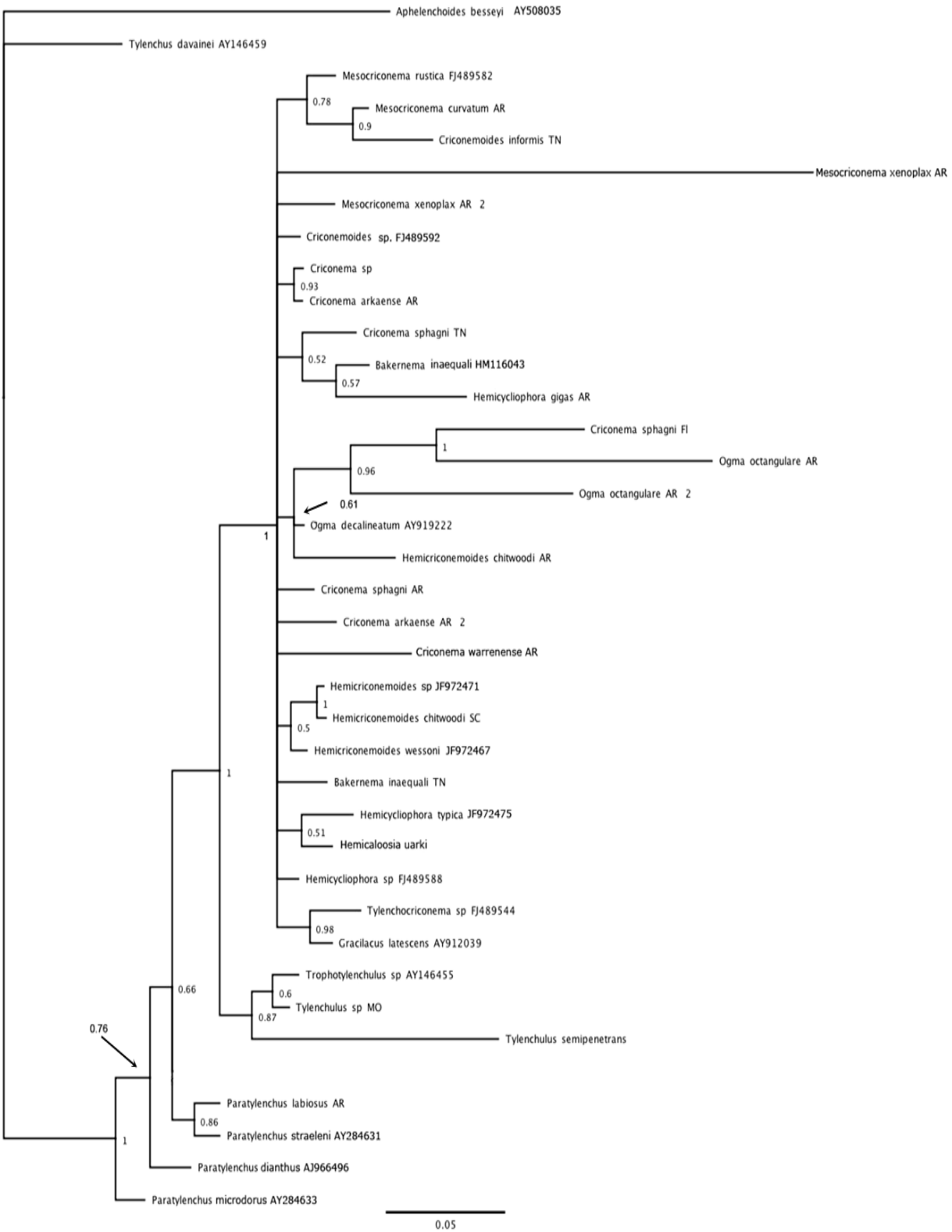
FIGURES

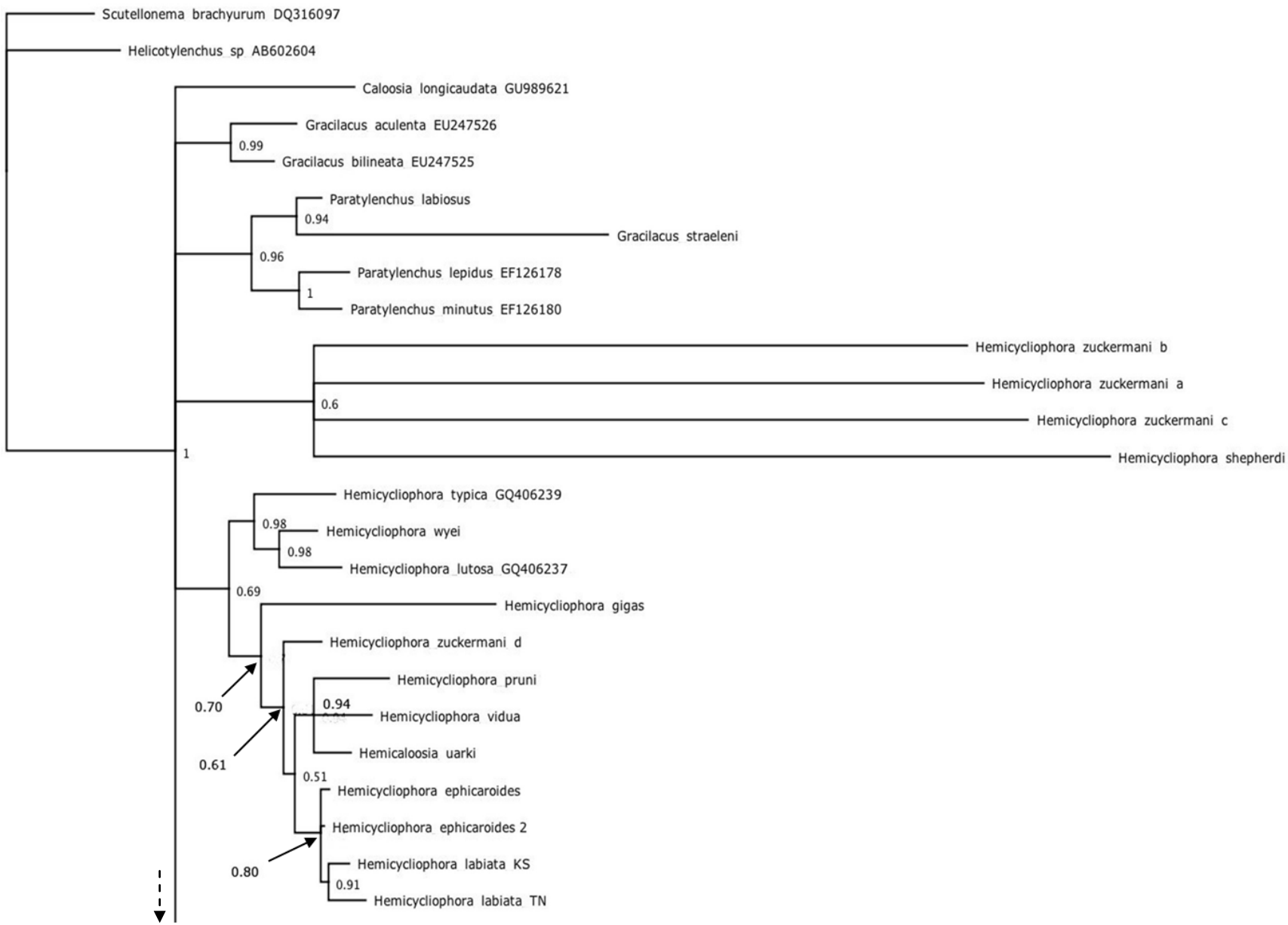
Fig 1. Bayesian inference 50% majority rule consensus tree of 18S-rDNA region under HKY+G model (-Ln likelihood = 2762.0794; AIC= 5682.1588; K=79; freqA =0.2371; freqC=0.2310; freqG=0.2810; freqT=0.2508; Kappa=2.0750 [(ti/tv=1.053)] Gamma shape=0.8310.) Numbers at nodes are posterior probability values.

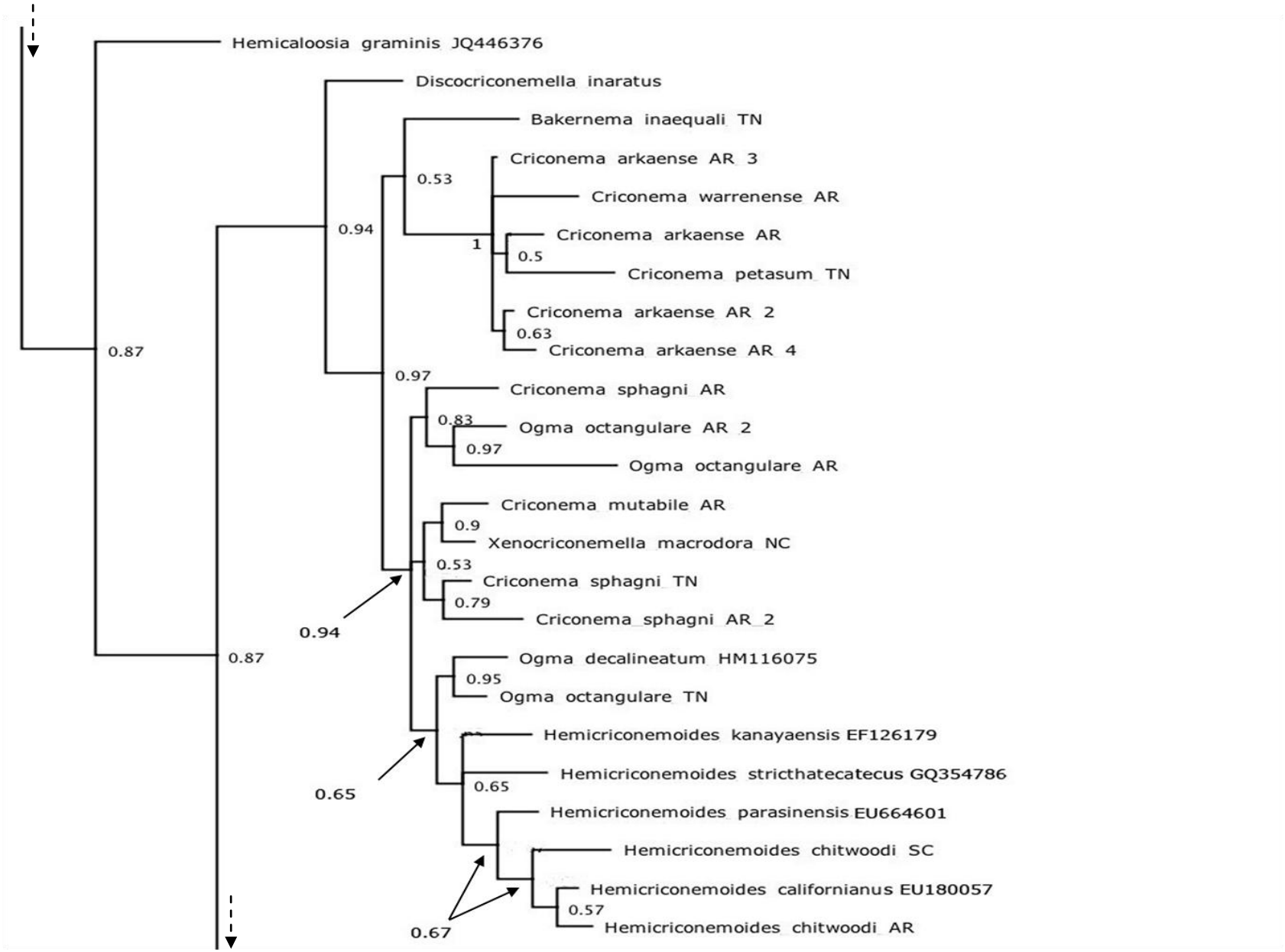
Fig 2a. Bayesian inference 50% majority rule consensus tree of ITS1-rDNA region under the GTR+G model (-Ln likelihood = 15770.6970; AIC= 31867.3939; K=163; freqA =0.2323; freqC=0.2740; freqG=0.2758; freqT=0.2179; R(a)=0.8263; R(b)=1.7400; R(c)=1.2319; R(d)=0.9083; R(e)=2.2622; R(f)=1.000; Gamma shape=2.3370) Numbers at nodes are posterior probability values.

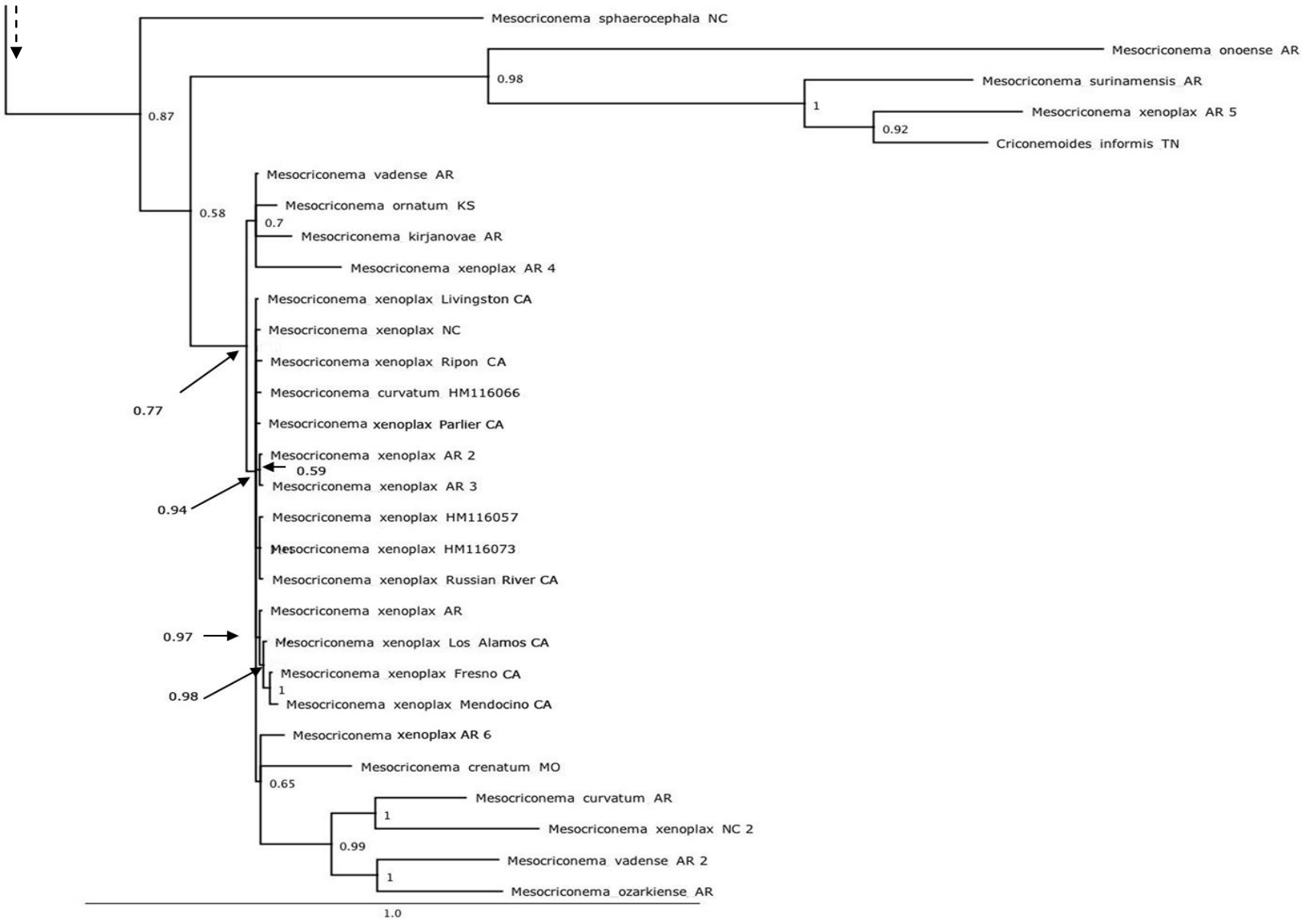
Fig 2b. Bayesian inference 50% majority rule consensus tree of ITS1-rDNA region…continued

Fig 2c. Bayesian inference 50% majority rule consensus tree of ITS1-rDNA region…continued


Aphelenchoides besseyi AY508035
Tylenchus davainei AY146459
Mesocriconema rustica FJ489582
0.78
Mesocriconema curvatum AR
0.9
Criconemoides informis TN
Mesocriconema xenoplax AR
Mesocriconema xenoplax AR 2
Criconemoides sp. FJ489592
Criconema sp
0.93
Criconema arkaense AR
Criconema sphagni TN
0.52
Bakernema inaequali HM116043
0.57
Hemicycliophora gigas AR
Criconema sphagni Fl
1
Ogma octangulare AR
0.96
Ogma octangulare AR 2
0.61
Ogma decalineatum AY919222
1
Hemicriconemoides chitwoodi AR
Criconema sphagni AR
Criconema arkaense AR 2
Criconema warrenense AR
Hemicriconemoides sp JF972471
1
Hemicriconemoides chitwoodi SC
0.5
Hemicriconemoides wessoni JF972467
1
Bakernema inaequali TN
Hemicycliophora typica JF972475
0.51
Hemicaloosia uarki
Hemicycliophora sp FJ489588
Tylenchocriconema sp FJ489544
0.98
Gracilacus latescens AY912039
Trophotylenchulus sp AY146455
0.6
Tylenchulus sp MO
0.87
Tylenchulus semipenetrans
0.66
0.76
Paratylenchus labiosus AR
0.86
Paratylenchus straeleni AY284631
1
Paratylenchus dianthus AJ966496
Paratylenchus microdorus AY284633
0.05


Scutellonema brachyurum DQ316097
Helicotylenchus sp AB602604
Caloosia longicaudata GU989621
Gracilacus aculenta EU247526
0.99
Gracilacus bilineata EU247525
Paratylenchus labiosus
0.94
Gracilacus straeleni
0.96
Paratylenchus lepidus EF126178
1
Paratylenchus minutus EF126180
Hemicycliophora zuckermani b
Hemicycliophora zuckermani a
0.6
Hemicycliophora zuckermani c
Hemicycliophora shepherdi
1
Hemicycliophora typica GQ406239
0.98
Hemicycliophora wyei
0.98
Hemicycliophora lutosa GQ406237
0.69
Hemicycliophora gigas
Hemicycliophora zuckermani d
Hemicycliophora pruni
0.70
0.94
Hemicycliophora vidua
0.61
Hemicaloosia uarki
0.51
Hemicycliophora ephicaroides
Hemicycliophora ephicaroides 2
0.80
Hemicycliophora labiata KS
0.91
Hemicycliophora labiata TN


Hemicaloosia graminis JQ446376
Discocriconemella inaratus
Bakernema inaequali TN
Criconema arkaense AR 3
Criconema warrenense AR
Criconema arkaense AR
Criconema petasum TN
Criconema arkaense AR 2
Criconema arkaense AR 4
Criconema sphagni AR
Ogma octangulare AR 2
Ogma octangulare AR
Criconema mutabile AR
Xenocriconemella macrodora NC
Criconema sphagni TN
Criconema sphagni AR 2
Ogma decalineatum HM116075
Ogma octangulare TN
Hemicriconemoides kanayaensis EF126179
Hemicriconemoides stricthatecatecus GQ354786
Hemicriconemoides parasinensis EU664601
Hemicriconemoides chitwoodi SC
Hemicriconemoides californianus EU180057
Hemicriconemoides chitwoodi AR
0.53
0.94
1
0.5
0.63
0.87
0.97
0.83
0.97
0.9
0.53
0.79
0.94
0.87
0.95
0.65
0.65
0.57
0.67


Mesocriconema sphaerocephala NC
Mesocriconema onoense AR
0.98
Mesocriconema surinamensis AR
1
Mesocriconema xenoplax AR 5
0.92
Criconemoides informis TN
0.87
Mesocriconema vadense AR
Mesocriconema ornatum KS
0.58
0.7
Mesocriconema kirjanovae AR
Mesocriconema xenoplax AR 4
Mesocriconema xenoplax Livingston CA
Mesocriconema xenoplax NC
Mesocriconema xenoplax Ripon CA
0.77
Mesocriconema curvatum HM116066
Mesocriconema xenoplax Parlier CA
Mesocriconema xenoplax AR 2
0.59
Mesocriconema xenoplax AR 3
0.94
Mesocriconema xenoplax HM116057
Mesocriconema xenoplax HM116073
Mesocriconema xenoplax Russian River CA
Mesocriconema xenoplax AR
0.97
Mesocriconema xenoplax Los Alamos CA
Mesocriconema xenoplax Fresno CA
0.98
1
Mesocriconema xenoplax Mendocino CA
Mesocriconema xenoplax AR 6
Mesocriconema crenatum MO
0.65
Mesocriconema curvatum AR
1
Mesocriconema xenoplax NC 2
0.99
Mesocriconema vadense AR 2
1
Mesocriconema ozarkiense AR
1.0

GENERAL CONCLUSION

The thirty-three populations of species of the superfamily Criconematoidea were identified and described: *Mesocriconema curvatum, M. kirjanovae, M. onoense, M. ornatum, M. sphaerocephala, M. surinamense, M. vadense, M. xenoplax, Criconemoides informis, Bakernema inaequale, C. petasum, C. sphagni, C. mutabile, Ogma octangulare, Xenocriconemella macrodora, Hemicriconemoides chitwoodi, Hemicycliophora epicharoides, H. gigas, H. labiata, H. typica, H. pruni, H. shepherdi, H. vidua, H. zuckermani, Gracilacus straeleni and Paratylenchus labiosus*. The new species *Mesocriconema ozarkiense* n. sp., *Criconema arkaense* n. sp., *Criconema warrenense* n. sp., *Hemicaloosia uarki* n. sp and *Hemicycliophora wyei* n. sp were described.

Mesocriconema sphaerocephala, M. surinamense and *M. onoense* showed the highest percentage of genetic variability compared with the rest of the species of this group. Equally, some populations of *M. xenoplax* showed enough genetic diversity to be differentiated from other groups of the same species and from those closely related like *M. vadense.* Different lineages of *Hemicriconemoides chitwoodi* were detected between populations of this species from Arkansas and South Carolina. A close genetic divergence relationship was obtained between *Criconema mutabile* and *Xenocriconemella macrodora.* Within this group, only *Bakernema inaequali* and *Criconema petasum* species showed a genetic variation above the range of the group.

Members of the subfamily Hemicycliophorinae showed a very close relationship among species, especially *H. labiata,* and *H. ephicaroides; Hemicaloosia uarki, H. gigas, H. pruni* and *H. vidua.* The species *Gracilacus straeleni* showed a high genetic variation and *Hemicycliophora*

shepherdi was closely related to three populations of *Hemicycliophora zuckermani.* Similar to *G. straeleni, Caloosia longicaudata* was genetically close to *Paratylenchus* and *Gracilacus* species but distant to *Hemicaloosia uarki*.

The 18S-rDNA data showed the monophyly of the superfamiliy Criconematoidea and accepted erroneously the theory of a single origin for genera which have a double cuticle as *Hemicriconemoides* and *Hemicycliophora*. Molecular phylogenetic analysis using the ITS1-rDNA region rejects the hypothesis of a single origin for genera with a double cuticle or double cuticular sheath in *Hemicycliophora* and *Hemicriconemoides*. Even though, this analysis did not show monophyly of *Hemicycliophora* because the high genetic divergence of ITS1 sequences of 3 species previously mentioned, we agree in the monophyly of *Hemicycliophora.* Rejection of the hypothesis of a single origin for genera with a double cuticle includes other important characters such as body length; fine, smooth and/or coarse body annuli; presence of ornamentation in body annuli; presence or absence of sub-median lobes; variations on the criconematoid oesophagus such as length of isthmus and size of basal glands in postcorpus; regular ectoparasitism; ectoparasitism with sedentary obese females (Family Paratylenchinae: *Paratylenchus*, *Gracilacus*) and sedentary obese females with or without presence of immature females showing endo or semi-endoparasitism in their life cycle (Family Tylenchulinae: *T. semipenetrans*, *Trophotylenchus*).

Highly conserved markers as 18S-rDNA can be used to determine the position of an organism at higher taxonomic rank e.g. family level. On the other hand, ITS1-rDNA was useful to determine relationships to genus and species level. In the particular case of the family Tylenchulidae, more ITS1 sequences have to be added to the data set to get a better resolution of their phylogenetic position.