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Description of *Mesocriconema ericaceum* n. sp. (Nematoda: Criconematidae) and notes on other nematode species discovered in an ericaceous heath bald community in Great Smoky Mountains National Park, USA

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Description of *Mesocriconema ericaceum* n. sp. (Nematoda: Criconematidae) and notes on other nematode species discovered in an ericaceous heath bald community in Great Smoky Mountains National Park, USA

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Abstract
A new species of *Mesocriconema* and a unique assemblage of plant-parasitic nematodes was discovered in a heath bald atop Brushy Mountain in Great Smoky Mountains National Park. *Mesocriconema ericaceum* n. sp., a species with males, superficially resembles *M. xenoplax*. DNA barcoding with the mitochondrial COI gene provided evidence of the new species as a distinct lineage. SEM revealed significant variability in arrangement of labial submedian lobes, plates, and anterior and posterior annuli. Three other nematodes in the family Criconematidae were characterized from the heath bald. *Ogma seymouri*, when analyzed by statistical parsimony, established connections with isolates from north-eastern Atlantic coastal and north-western Pacific coastal wet forests. *Criconema loofi* has a southern Gulf Coast distribution associated with boggy soils. *Criconema* cf. *acriculum* is known from northern coastal forests of California. Understanding linkages between these species and their distribution may lead to the broader development of a terrestrial soil nematode biogeography.

Keywords – All Taxa Biodiversity Inventory, biogeography, *Criconema* cf. *acriculum*, *Criconema loofi*, Criconematina, DNA barcode, molecular, morphology, morphometrics, *Ogma seymouri*, phylogeography, plant-parasitic nematode, statistical parsimony, taxonomy.

A new *Mesocriconema* species and an unusual nematode assemblage was discovered as part of a larger survey of North American members of the plant-parasitic suborder Criconematina. The larger nematode survey, focused on comparing nematode species across different ecoregions, included a subset of samples collected from the All Taxa Biodiversity Inventory (ATBI) sites located in Great Smoky Mountains National Park. These ATBI sites were established in 1999 in an effort to catalogue all living organisms in 19 plots representing a replicated set of sites from each of the major vegetation communities in the park (Sharkey, 2001; Nichols & Langdon, 2007). Among these sites is a unique heath bald community located atop Brushy Mountain (1463 m a.s.l.).

The heath bald is a treeless plant community broadly referred to as the South Appalachian Mountain Laurel Bald community (White *et al.*, 2003). It is composed primarily of mountain laurel (*Kalmia latifolia* L.), Catawba rhododendron (*Rhododendron catawbiense* Mischx.), mountain fetterbush (*Pieris floribunda* (Pursh) Benth. & Hook), and highbush blueberry (*Vaccinium corymbosum* L.), all members of the family Ericaceae Juss. The plant community creates a dense, nearly impenetrable assemblage, approximately 2.5 m in height. The soil is extremely acidic with high levels of organic matter (White *et al.*, 2003). There are an estimated 421 heath balds in the Great Smoky Mountains, collectively averaging 1.8 ha in size (White *et al.*, 2001). In the Great Smoky Mountains, heath balds are most often associated with old growth conditions, burned sites, and an acidic rock type known as the Anakeesta formation (White *et al.*, 2001). Ericoid mycorrhizal fungi are an important microbial component of the soil and facilitate nutrient uptake by ericaceous plant species in these acidic soils (Perotto *et al.*, 2002).

In soil samples from the Brushy Mountain bald we discovered a criconematid nematode assemblage which included a species of *Mesocriconema* Andrássy, 1965 that we believe is new to science. Evidence for its distinct status was initially derived from its position on a mitochondrial cytochrome oxidase subunit 1 (COI) phylogenetic tree composed of 550 *Mesocriconema* nucleotide sequences. This criconematid taxonomic framework has been developed through the combined morphological and molecular analysis of individual nematodes, first documented by differential interference microscopy and morphological measurement, and then 'barcoded' by amplification and nucleotide sequencing of a 721 bp region of COI (Powers *et al.*, 2014, 2016). In select specimens COI has been sequenced along with ribosomal small subunit 18S rDNA and the internal transcribed spacer 1 (ITS1).

A second notable feature of this sampling site was the presence of at least two different species of criconematid males. Criconematid males compared to females are thinner and structurally degenerate, lacking a defined stylet, pharynx, and cuticular modifications such as scales that might aid their taxonomic placement (Wouts, 2006). Males are infrequently recorded in criconematid descriptions, possibly due to a combination of their rarity and their radically different morphology. Linking these males to their corresponding females requires DNA analysis. In addition to the *Mesocriconema* species, females from three other criconematid nematodes were recorded from these soils. These three species morphologically conformed to the descriptions of *Ogma seymouri* (Wu, 1965) Siddiqi, 1986, *Criconema loofi* (De Grisse, 1967) Raski & Luc, 1985, and *C. acriculum* (Raski & Pinochet, 1976) Raski & Luc, 1985. By assessing the genetic relationships and distribution of these additional nematodes, we may gain insight into the factors that explain the diversity and distribution of this assemblage of plant-parasitic nematodes. In this manuscript we describe the *Mesocriconema* species and provide taxonomic information about the three other criconematid species.

Materials and methods

Collection Methods

Soil samples were obtained using an Oakfield tube to extract cores from a maximum depth of 25 cm. Cores of 2.5 cm diam. from within a 40 × 40 m plot were bulked and stored in a 8°C cold room until a 200 ml subsample was processed for nematode isolation by a combination of sieving and sugar centrifugation (Jenkins, 1964). Two separate plots established within the Brushy Mountain heath bald were sampled on two separate occasions, 17 July 2014 and 3 August 2015. Collection data associated with the specimens in this study are presented in Table 1.

Isolation and Examination

Nematodes were picked out from a 200 cm^3 soil extract using a dissecting microscope. When possible, five individuals from each morphotype in the extract were individually mounted on glass slides, measured, and digitally imaged with a Leica DC 300 videocamera on a Leica DMLB microscope using Differential Interference Contrast optics. After documentation the slide was dismantled, the nematode crushed in 18 μl of water with a transparent micropipette tip, and added to a PCR microfuge tube. Methods for PCR amplification and DNA sequencing have been previously described (Powers *et al.*, 2014). The COI primer sequences were: COI-F5 5′- AATWTWGGTGTTGGAACTTCTTGAAC-3′ and COIR9 5′-CTTAAAACATAATGRAAATGWGCWACWACA TAATAAGTATC-3′.

The PCR reactions produced a *ca* 790 bp amplification product that, after removal of primers from consideration, provided 721 bp of sequence for genetic analysis. Nematodes were prepared for scanning electron microscopy (SEM) by fixation in 4% formalin followed by dehydration in a graded series of alcohol to 100% EtOH, critical point drying, mounting on SEM specimen stubs, and coating with gold. Images were obtained on a Hitachi S-3000N scanning electron microscope. Nematodes prepared for SEM were selected from conspecific specimens from the same soil sample that were measured and analyzed molecularly.

Phylogeny

A reference maximum likelihood tree for *Mesocriconema* has been previously published (Powers *et al.*, 2014). A schematic of an updated version of the *Mesocriconema* tree is presented in Figure 1. Colored blocks of taxa derived from major branches in that tree are labelled as either described species, haplotype groups, or collections of haplotype groups reflecting a common ecology or host association. The position of the *Mesocriconema* from Brushy Mountain and the sister taxon are shown uncolored in the exploded view.

Table 1. Specimen collection data for *Mesocriconema ericaceum* n. sp. and other criconematids.

NID	Species	Stage	Locality	Ecoregion name	Plant community/Host	Marker	GenBank Accession No.
N2625	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290521
N2627	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290522
N2627	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	18S	KX290602
N2628	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290523
N2629	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290524
N2630	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290525
N2637	M. *ericaceum* n. sp.	J	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290526
N2638	M. *ericaceum* n. sp.	J	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290527
N2639	M. *ericaceum* n. sp.	J	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290528
N2640	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290529
N2641	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290530
N2642	M. *ericaceum* n. sp.	M	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290531
N5953	M. *ericaceum* n. sp.	M	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290532
N5958	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290533
N5959	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290534
N5960	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290535
N5961	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290536
N5962	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290537
N5963	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290538
N5964	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290539
N5965	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290540
N5975	M. *ericaceum* n. sp.	J	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290541
N5976	M. *ericaceum* n. sp.	J	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290542
N5976	M. *ericaceum* n. sp.	J	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	18S	KX290603
N5985	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290543
N5986	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290544
N5987	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290545
N5988	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290546
N5989	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290547
N5990	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290548
N5990	M. *ericaceum* n. sp.	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	18S	KX290604

Table 1. (Continued.)

NID	Species	Stage	Locality	Ecoregion name	Plant community/Host	Marker	GenBank Accession No.
N993	*Criconema* cf. *acriculum*	F	Albright Grove, GRSM, TN	Appalachian/Blue Ridge Forests	Rhododendron	COI	KX290549
N2725	*C.* cf. *acriculum*	F	Gregory Bald, GRSM, NC	Appalachian/Blue Ridge Forests	Grassy Bald	COI	KX290550
N2878	*C.* cf. *acriculum*	F	Snake Den, GRSM, TN	Appalachian/Blue Ridge Forests	Eastern Hemlock/ Rhododendron	COI	KX290551
N3388	*C.* cf. *acriculum*	F	Albright Grove, GRSM, TN	Appalachian/Blue Ridge Forests	Rhododendron	COI	KX290552
N3406	*C.* cf. *acriculum*	F	Gregory Bald, GRSM, NC	Appalachian/Blue Ridge Forests	Grassy Bald	COI	KX290553
N3407	*C.* cf. *acriculum*	F	Gregory Bald, GRSM, NC	Appalachian/Blue Ridge Forests	Grassy Bald	COI	KX290554
N3411	*C.* cf. *acriculum*	F	Gregory Bald, GRSM, NC	Appalachian/Blue Ridge Forests	Grassy Bald	COI	KX290555
N3413	*C.* cf. *acriculum*	F	Gregory Bald, GRSM, NC	Appalachian/Blue Ridge Forests	Grassy Bald	COI	KX290556
N5978	*C.* cf. *acriculum*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	18S	KX344496
N5979	*C.* cf. *acriculum*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290557
N5980	*C.* cf. *acriculum*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290558
N5992	*C.* cf. *acriculum*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290559
N2724	*C. longulum*	J	Gregory Bald, GRSM, NC	Appalachian/Blue Ridge Forests	Grassy Bald	18S	KX344495
N1483	*C. loofi*	F	Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney Woods Forests Cypress	Swamp	COI	KX290560
N1488	*C. loofi*	F	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	Hardwood Forest	COI	KX290561
N1490	*C. loofi*	F	Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney Woods Forests	Cypress Swamp	COI	KU236639
N2634	*C. loofi*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290562
N2635	*C. loofi*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290563
N2635	*C. loofi*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	18S	KX344497
N2669	*C. loofi*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290564
N2670	*C. loofi*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290565
N3104	*C. loofi*	F	Ponce de Leon State Park, FL	Southeastern Conifer Forests	Pine Flatwoods	COI	KX290566
N3148	*C. loofi*	F	Ponce de Leon State Park, FL	Southeastern Conifer Forests	Pine Flatwoods	COI	KX290567
N3149	*C. loofi*	F	Ponce de Leon State Park, FL	Southeastern Conifer Forests	Pine Flatwoods	COI	KX290568
N3150	*C. loofi*	F	Ponce de Leon State Park, FL	Southeastern Conifer Forests	Pine Flatwoods	COI	KX290569
N3151	*C. loofi*	F	Ponce de Leon State Park, FL	Southeastern Conifer Forests	Pine Flatwoods	COI	KX290570
N3152	*C. loofi*	J	Ponce de Leon State Park, FL	Southeastern Conifer Forests	Pine Flatwoods	COI	KX290571
N3652	*C. loofi*	F	Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney Woods Forests	Cypress Swamp	COI	KX290572
N5666	*C. loofi*	F	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	Hardwood Forest	COI	KX290573
N5667	*C. loofi*	F	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	Hardwood Forest	COI	KX290574
N5673	*C. loofi*	J	Big Sandy Creek Unit,	Piney Woods Forests	Hardwood Forest	COI	KX290575

Table 1. (Continued.)

NID	Species	Stage	Locality	Ecoregion name	Plant community/Host	Marker	GenBank Accession No.
N2767	*Ogma seymouri*	F	Brown Mtn., Tongass NF, AK	Northern Pacific Coastal Forests	Western hemlock	COI	KX290576
N5977	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290577
N5982	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290578
N5983	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290579
N5993	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290580
N308	*O. seymouri*	F	Pauchaug State Forest, CT	Northeastern Coastal Forests	Rhododenderon	COI	KX290581
N1031	*O. seymouri*	F	West Point, GRSM, TN	Appalachian/Blue Ridge Forests	Mountain Laurel	COI	KX290582
N1033	*O. seymouri*	M	West Point, GRSM, TN	Appalachian/Blue Ridge Forests	Mountain Laurel	COI	KX290583
N2631	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290584
N2632	*O. seymouri*	M	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290585
N2633	*O. seymouri*	M	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290586
N2636	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	COI	KX290587
N2636	*O. seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian/Blue Ridge Forests	Heath Bald	18S	KX344498
N2736	*O. seymouri*	F	Dude Mtn. Trail, Tongass NF, AK Muskeg	Northern Pacific Coastal Forests	Sub-alpine Meadow,	COI	KX290588
N2737	*O. seymouri*	F	Dude Mtn. Trail, Tongass NF, AK Muskeg	Northern Pacific Coastal Forests	Sub-alpine Meadow,	COI	KX290589
N2738	*O. seymouri*	F	Dude Mtn. Trail, Tongass NF, AK Muskeg	Northern Pacific Coastal Forests	Sub-alpine Meadow,	COI	KX290590
N2750	*O. seymouri*	F	Dude Mtn. Trail, Tongass NF, AK Muskeg	Northern Pacific Coastal Forests	Sub-alpine Meadow,	COI	KX290591
N2751	*O. seymouri*	J	Deer Mtn., Tongass NF, AK	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290592
N2752	*O. seymouri*	F	Deer Mtn., Tongass NF, AK	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290593
N2753	*O. seymouri*	F	Deer Mtn., Tongass NF, AK	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290594
N2754	*O. seymouri*	F	Deer Mtn., Tongass NF, AK	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290595
N2769	*O. seymouri*	F	Brown Mtn. Road, Tongass NF, AK	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290596
N2772	*O. seymouri*	J	Brown Mtn. Road, Tongass NF, AK	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290597
N2773 NF, AK	*O. seymouri*	M	Brown Mtn. Road, Tongass	Northern Pacific Coastal Forests	Western Hemlock	COI	KX290598
N2779	*O. seymouri*	F	Ward Lake, Tongass NF, AK	Northern Pacific Coastal Forests	Mossy Muskeg	COI	KX290599
N2780	*O. seymouri*	F	Ward Lake, Tongass NF, AK	Northern Pacific Coastal Forests	Mossy Muskeg	COI	KX290600
N2781	*O. seymouri*	F	Ward Lake, Tongass NF, AK	Northern Pacific Coastal Forests	Mossy Muskeg	COI	KX290601

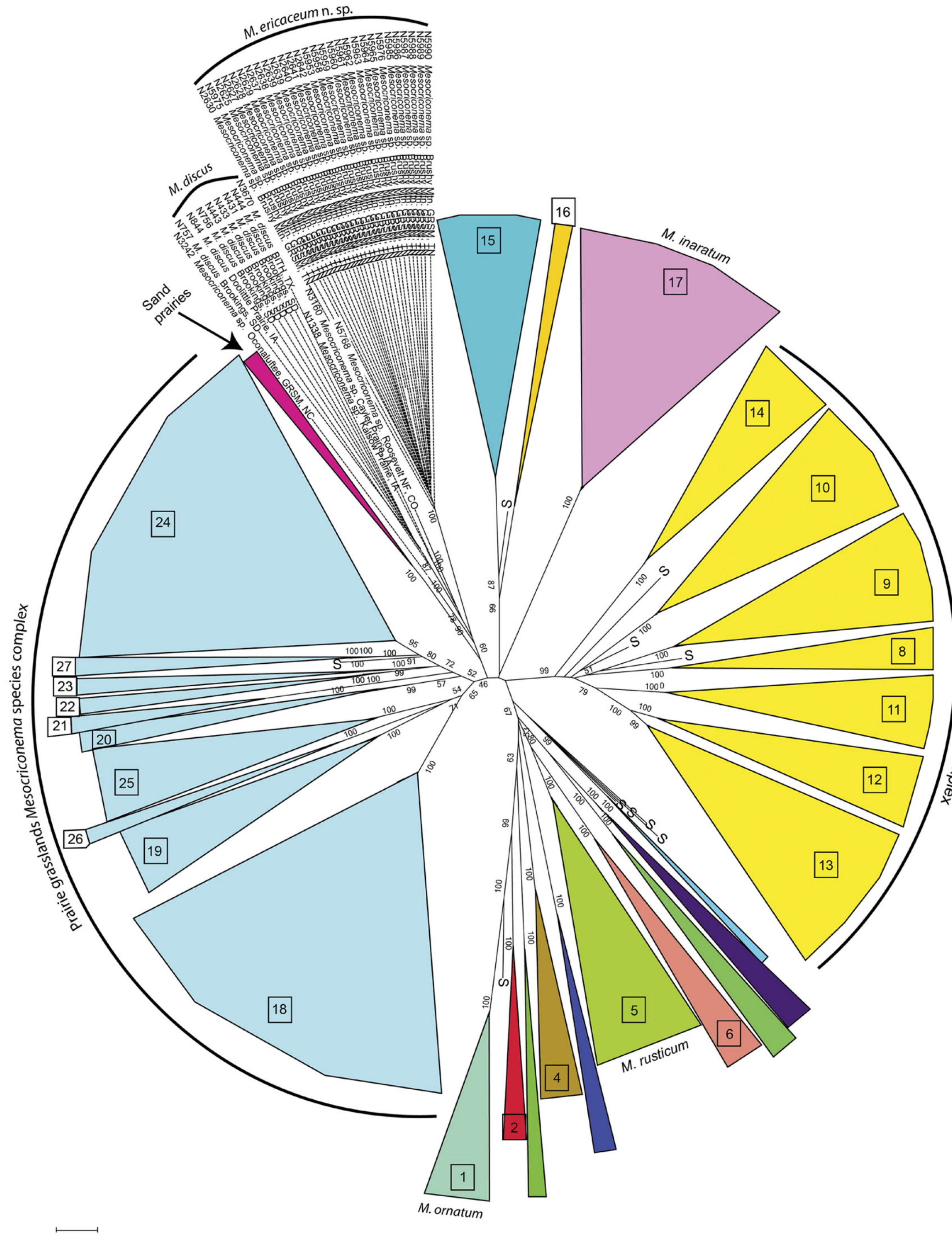

Fig. 1. Neighbor joining tree of 550 *Mesocriconema* COI DNA sequences with 1000 bootstrap replication values located at branch nodes. *Mesocriconema ericaceum* n. sp. specimens are shown without color together with the nearest neighbor haplotype group, *M. discus*. Other *Mesocriconema* haplotype groups are indicated by colored blocks. Numbers associated with colored haplotype groups refer to taxon labels in Powers *et al.* (2014). Haplotype groups that coincide with species names are labelled on the periphery of the circular tree. Nematode Identification Numbers (NID) are associated with specimen labels and refer to individual specimens in the text, tables and figures. Singletons are designated by the letter 'S'.

Network-TCS network analyses (Clement *et al.*, 2000) were used to determine haplotype relationships associated with population level divergences that allow for non-bifurcating genealogical information. The statistical parsimony analysis by TCS used an absolute distance matrix from pairwise comparisons of haplotypes to calculate the probability of parsimony at 95 and 90% connection levels.

Results

*Mesocriconema ericaceum** n. sp.

(Figs 2-8)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Female body generally straight when relaxed by heat. Post-vulval region slightly inclined ventrally. Annuli smooth at their posterior margins with an occasional hint of crenation in tail region. Number of body annuli ranging from 82 to 96. Average width of annuli at mid-body 6.3 μm. Generally 0-2 anastomoses on body but as many as eight observed. In labial region, four labial plates arranged around oral disc, punctuated by four comma-shaped submedian lobes appearing rounded in lateral view. Submedian lobes not conspicuously protruding above cephalic contour in lateral view, but readily observed when labial region slightly tilted. Scanning electron microscopy revealing a relatively high degree of morphological variation in arrangement of submedian lobes, lateral plates and first lip annulus. Lobes, plates and first annulus fusing with each other at times, appearing like a partially formed labial annulus, or may divide forming additional cuticular components surrounding oral disc. Second body annulus usually 4-5 μm wider in profile than first annulus. Stylet averaging 63 μm in length, with robust stylet knobs approximately twice as wide as high. Slight anterior projections observed on knobs of most specimens. Excretory pore located 22-26 annuli from anterior end, usually coinciding with end of pharynx or 1-3 annuli posterior to base of pharyngeal bulb. Reproductive system terminating in a sigmoid vagina, with posterior portion of cuticle-lined canal orientated parallel to body axis for length of a single annulus. Vulva with a low and smooth hemispherical anterior flap that most often lacks projections, although a single specimen with a pair of low, pointed projections was observed. Spermatheca rounded. Tail annulation variable, with multiple anastomoses or discontinuous annulation.

Male

Body approximately same length as in female but only half as wide and with approximately twice as many body annuli. Lateral field with four lines. Anterior region featureless and without recognizable stylet or other pharyngeal structures. Excretory pore located 53 and 57 annuli from anterior end on two males examined. Spicules straight, gubernaculum straight and relatively short. Body immediately posterior to cloacal aperture narrowing and then expanding 5-6 annuli from terminus. Bursa spanning narrow region of tail and apparently not extending to tail terminus.

Juvenile

Body similar to that of female. Annuli with crenate margins extending across entire body. Total number of annuli approximately equal to that of adult females, but annulus width 2-3 μm less. Body and stylet length for juveniles (n = 5) ranging from 303 to 385 μm and 41 to 55 μm, respectively. No more than a single anastomosis observed on juvenile specimens.

TYPE HABITAT AND LOCALITY

Mesocriconema ericaceum n. sp. was only found in the Brushy Mountain ATBI site, despite relatively intense sampling in the park (54 soil samples). The species was not found on the two other surveyed balds in the park, Andrews Bald and Gregory's Bald. Several of the potential plant hosts on Brushy Mountain, particularly mountain laurel and rhododendron, are widespread in the ATBI sites. Measurements of pH and OM at the Brushy Mountain site were 3.9 and 52.1%, respectively, not extremely different from some of the forested ATBI sites (Jenkins, 2007).

The host plant community includes mountain laurel (*K. latifolia*), Catawba rhododendron (*R. catawbiense*),

* The specific epithet refers to the host plant family Ericaceae, and the general term for the ericaceous soil type.

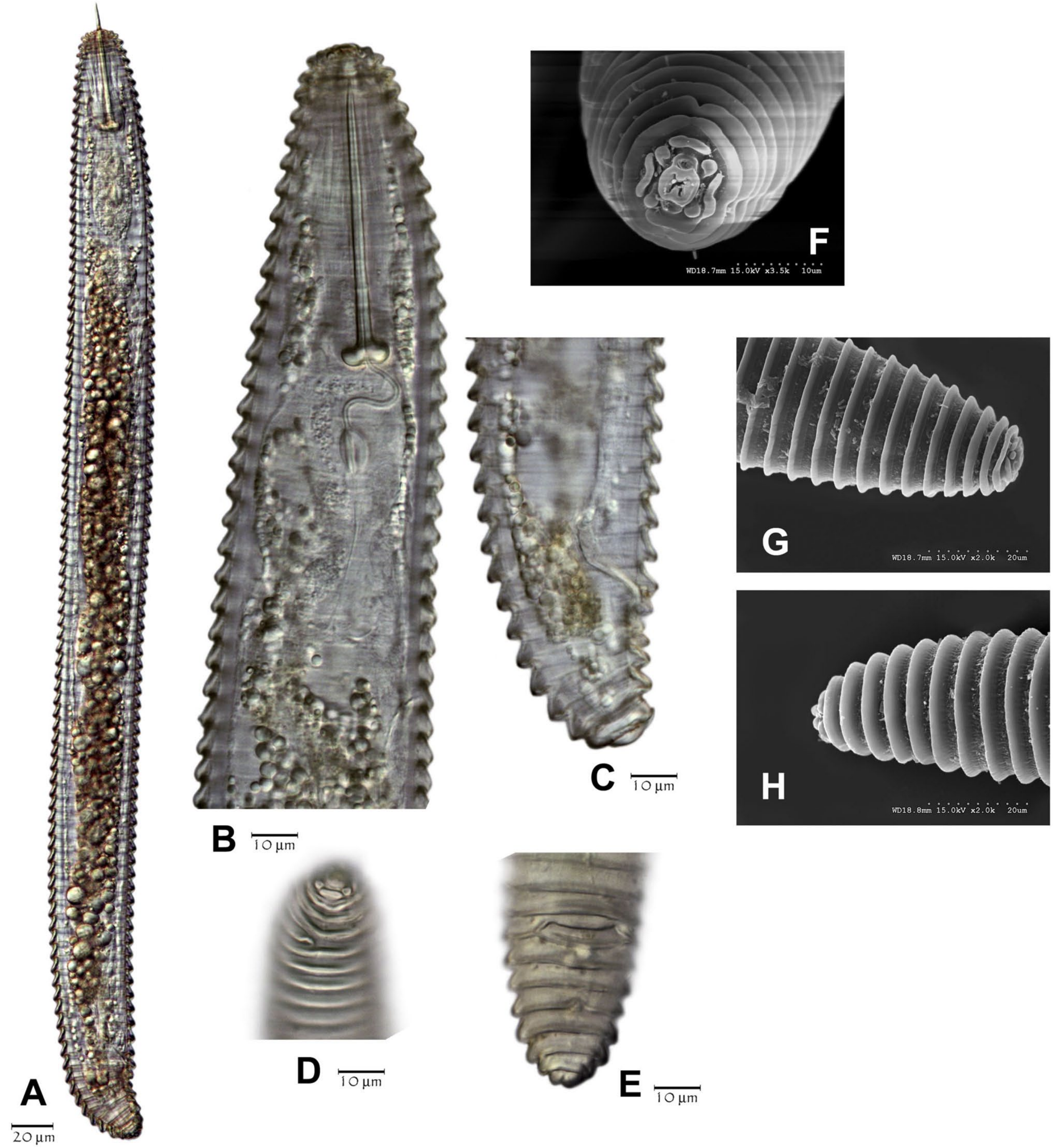

Fig. 2. Images of *Mesocriconema ericaceum* n. sp., females from heath bald on Brushy Mountain, GRSM. A-E, light microscope images, F-H, SEM images. A: Entire body, NID 2629; B: Head and pharynx, NID 5961; C: Tail and S-shaped vagina, NID 5958; D: Head, face view with oral disc, two submedian lobes, and labial plate, NID 5958; E: Tail with low, arcuate vulval flap, ventral view, NID 5985; F: Face view with oral disc flanked by amphid apertures, four submedian lobes and four labial plates, NID 4641; G: Head, profile view, NID 4642; H: Tail, NID 4642.

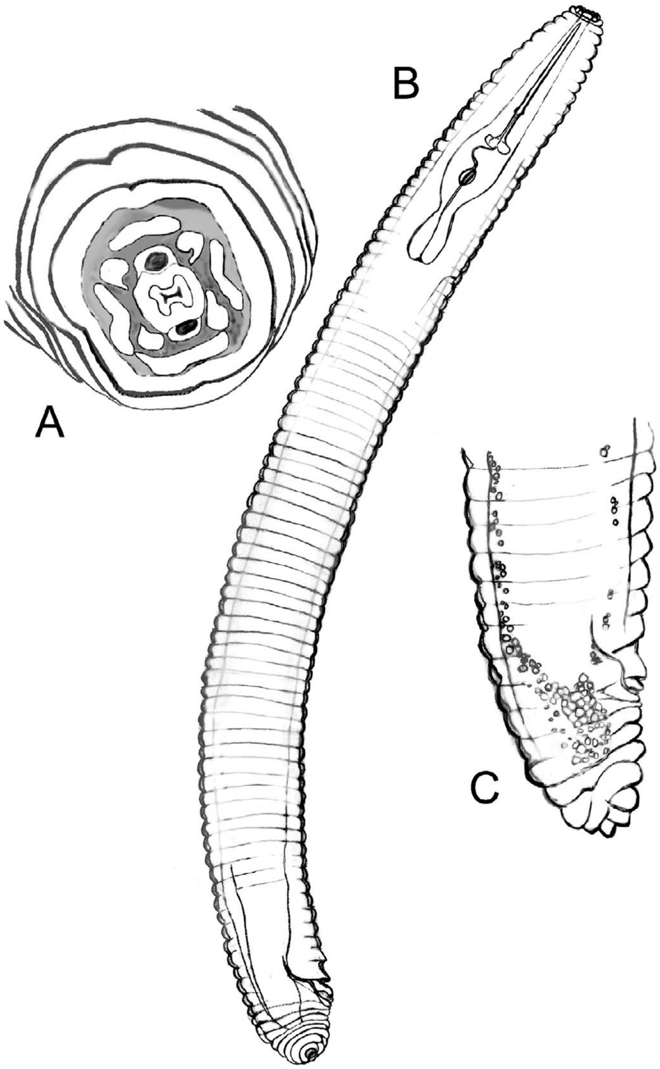

Fig. 3. Drawing of *Mesocriconema ericaceum* n. sp. female. A: Face view, B: Entire body, C: Tail region.

mountain fetterbush (*P. floribunda*), and highbush blueberry (*V. corymbosum*) all members of the family Ericaceae.

Mesocriconema ericaceum n. sp. type location elevation is 1463 m a.s.l., latitude is 35.678018 decimal degrees and longitude – 83.430282 decimal degrees.

TYPE MATERIAL

The holotype specimen of *M. ericaceum* n. sp. and one paratype slide with four specimens are deposited in the Nematode Collection of USDA, Beltsville, MD, USA. Six other paratype slides with two specimens apiece are deposited in the University of Nebraska Parasitology Collection at the Nebraska State Museum, Lincoln, NE, USA.

DIAGNOSIS AND RELATIONSHIPS

Mesocriconema ericaceum n. sp. females, when relaxed, have a straight body with a slightly ventrally inclined tail region. Body annuli are smooth, and the labial region is characterized by four comma-shaped submedian lobes and four interspersed labial plates. Merging of plates, submedian lobes and first annulus is common. Stylet length averages 63 μm with robust stylet knobs and slight anterior projections. The vulva has a low hemispherical anterior flap and generally lacks projections. The post-vulval region is conoid with a bluntly rounded terminus. The male body is narrow compared to the female, possessing four lateral lines, straight spicules and bursa with a subterminal ending.

On the first of two rapid identification charts for *Mesocriconema* (Fig. 79 in Geraert, 2010), the characters of body length and number of annuli (*R*-value) are ordinated on *x*- and *y*-axes. *Mesocriconema ericaceum* n. sp. charts closest to *M. kirjanovae* (Andrássy, 1962) Loof & De Grisse, 1989 from which it clearly differs based on female tail shape. The female tail of *M. kirjanovae* is drawn out to a more attenuated tip compared to the more rounded terminus seen in specimens of *M. ericaceum* n. sp. The second rapid identification chart (Fig. 80 in Geraert, 2010) compares RV and stylet length. On this chart *M. ericaceum* n. sp. falls closest to *M. surinamense* (De Grisse & Maas, 1970) Loof & De Grisse, 1989. This species possesses very large flattened submedian lobes, similar to *M. discus* (Thorne & Malek, 1968) Loof & De Grisse, 1989, and unlike the smaller comma-shaped lobes commonly observed on *M. ericaceum* n. sp. specimens. In the *Mesocriconema* key of Geraert (2010), *M. ericaceum* n. sp. keys to *M. pelerentsi* (Sakwe & Geraert, 1991) Brzeski, Loof & Choi, 2002 if in the first couplet, one accepts the character state of occasional anastomoses instead of the alternate choice, multiple anastomoses or lateral field interruptions. *M. pelerentsi* was originally found on cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.) in North Province, Cameroon. *M. ericaceum* n. sp. differs from *M. pelerentsi* in lacking sub-cuticular punctuations and bands, as well as possessing a lip region that is less compact with regards to labial plates and submedian lobes. The stylet knobs of *M. pelerentsi* have projections that are more strongly directed anteriorly and values of *R* and Rex that are smaller. If instead, one recognizes multiple (*i.e.*, >5) anastomoses

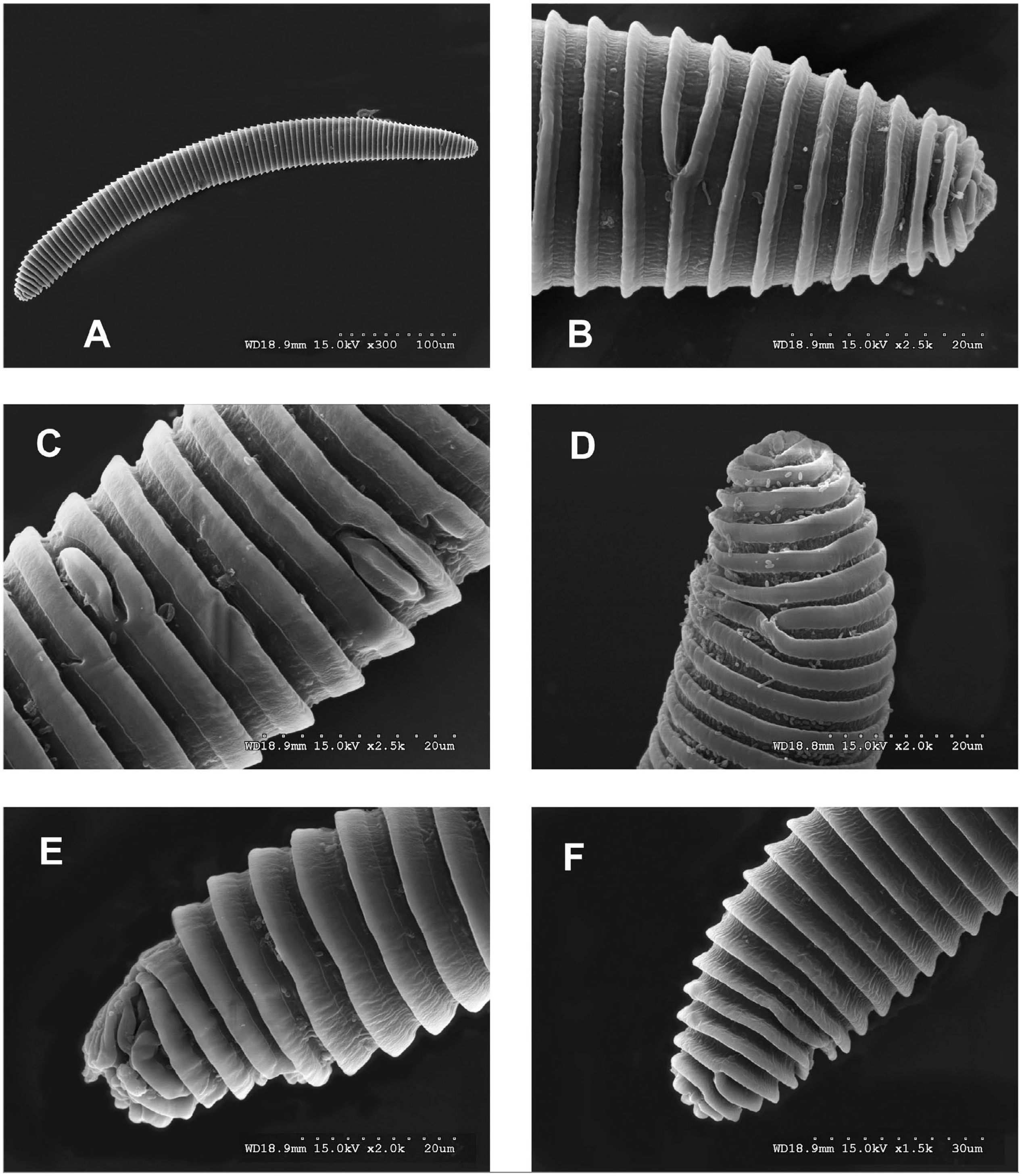

Fig. 4. Scanning electron micrographs of female *Mesocriconema ericaceum* n. sp. from Brushy Mountain, GRSM. A: Entire body; B-F: Variation in anastomoses and cuticle anomalies. A: NID 4633; B: Anastomosis near head, NID 4633; C: Unusual disruptions of annuli near mid-body, NID 4634; D: Anastomosis near tail, NID 4650; E: Convoluted tail annuli, NID 4634; F: Tail with hint of crenation on annulus margin, NID 4633.

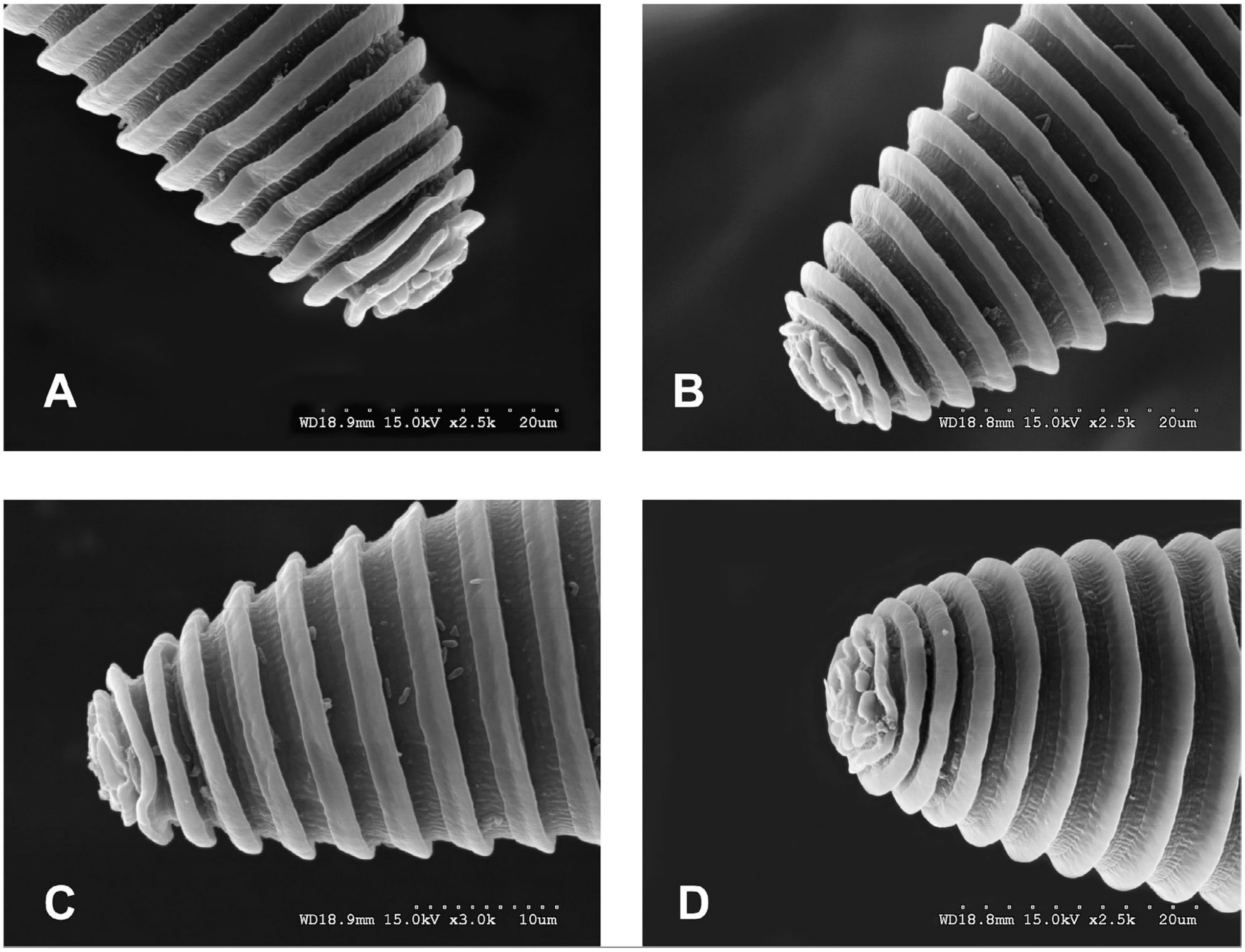

Fig. 5. Scanning electron micrographs of *Mesocriconema ericaceum* n. sp. from Brushy Mountain, GRSM. All are female heads, profile view. First labial annulus often divided. A: NID 4637; B: NID 4643; C: NID 4634; D: NID 4635.

in the first couplet of Geraert's key, then the key settles on *M. involutum* (Loof, 1987) Loof, 1989. The Brushy Mountain specimens, however, lack the involuted tail (see paratype specimen in Fig. 9) and the modified tail annulus that contains the anal opening. *Mesocriconema vadense* (Loof, 1964) Loof & De Grisse, 1989 has been compared to *M. involutum* in Loof (1989) and *M. vadense* has recently been recorded from grass and pine in Arkansas, USA (Cordero *et al.*, 2012). *Mesocriconema ericaceum* n. sp. differs from *M. vadense* in the following measurements from the original description: larger *R* (82-86 *vs* 70-81), longer body (462-642 *vs* 360-540 μm), and male spicule (38 *vs* 30 μm). The male tail shape in *M. vadense* is quite pointed without a dramatic reduction in postcloacal diam. as seen in *M. ericaceum* n. sp. The male bursa of *M. vadense* extends to the tail terminus whereas it appears to end subterminally in *M. ericaceum* n. sp. The female tail terminus in *M. vadense* is said to be a single, projecting annulus whereas a more complex and variable arrangement of terminal annuli is observed in *M. ericaceum* n. sp. (Figs 4, 7). *Mesocriconema ericaceum* n. sp. differs morphologically from *Mesocriconema xenoplax* (Raski, 1952) Loof & De Grisse, 1989 in mean stylet length of 63 *vs* > 70 μm for *M. xenoplax*. *Mesocriconema ericaceum* n. sp. has a smaller Rex value and the vulval flap is a low, smooth hemisphere without projections.

The phylogenetic position of *M. ericaceum* n. sp. relative to other *Mesocriconema* species is depicted on the *Mesocriconema* COI tree (Fig. 1). *Mesocriconema ericaceum* n. sp. forms a distinct lineage, with minimal bootstrap support for grouping it in a clade that includes *M. discus*. The raw (*p*-value) distance between *M. discus* and *M. ericaceum* n. sp. is 10.8% for the 721 bp portion of the COI gene. In addition to genetic distance, six diagnostic nucleotides were identified in the COI nucleotide sequence (Table 3). These diagnostic nucleotides were found in all

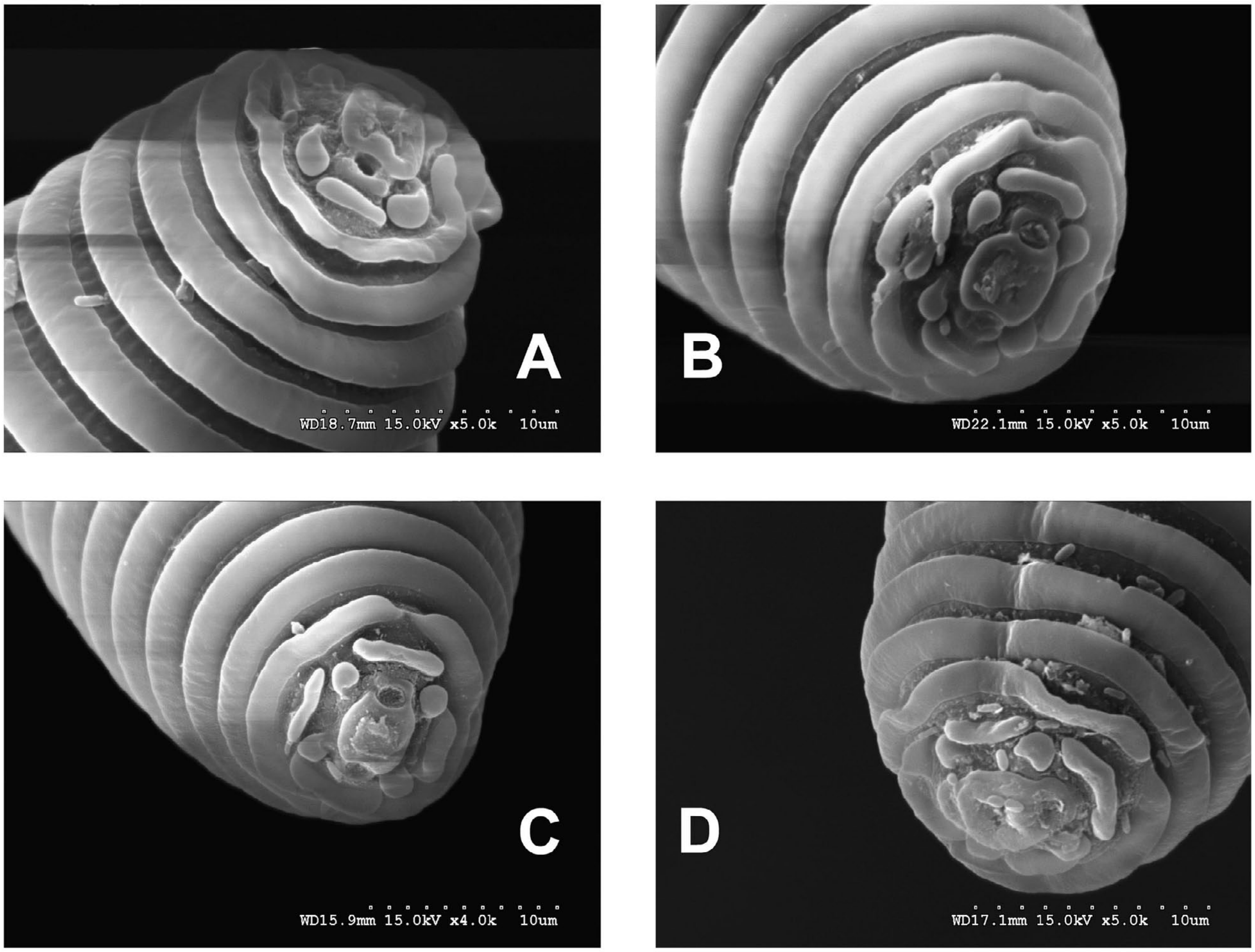

Fig. 6. Scanning electron micrographs of *Mesocriconema ericaceum* n. sp. females from Brushy Mountain, GRSM illustrating variation in face views. A: First labial annulus incomplete, NID 4649; B: First lip annulus merging with labial plate, NID 4646; C: 'Normal' face view, NID 4640; D: NID 4644.

specimens of *M. ericaceum* n. sp., but did not appear in 530 other *Mesocriconema* specimens. There are 18 amino acid differences between *M. ericaceum* n. sp. and *M. discus*, the closest described species (Table 4). The pairwise genetic distance to nine common *Mesocriconema* species found in North America is presented in Table 5. In a pairwise comparison a 5′-947 bp portion of 18S rDNA exhibits a three nucleotide divergence from *M. xenoplax*, GenBank accession number AY284625.1.

Other Criconematid Nematodes on Brushy Mountain

Three other criconematid nematode species were collected from Brushy Mountain heath bald soils. These are dealt with below.

Ogma seymouri (Wu, 1965) Siddiqi, 1986

= *Criconema seymouri* Wu, 1965

= *Crossonema* (*Seriespinula*) *seymouri* (Wu, 1965) Mehta & Raski, 1971

= *Seriespinula seymouri* (Wu, 1965) Khan, Chawla & Saha, 1976

(Fig. 10)

MEASUREMENTS

See Table 6.

DESCRIPTION

Ogma seymouri is a robust-bodied species with 8-10 rows of scales that are either bi- or tri-furcate distally, usually connected at base. Scales seen on entire length of body including all annuli on tail. Terminal annulus may appear

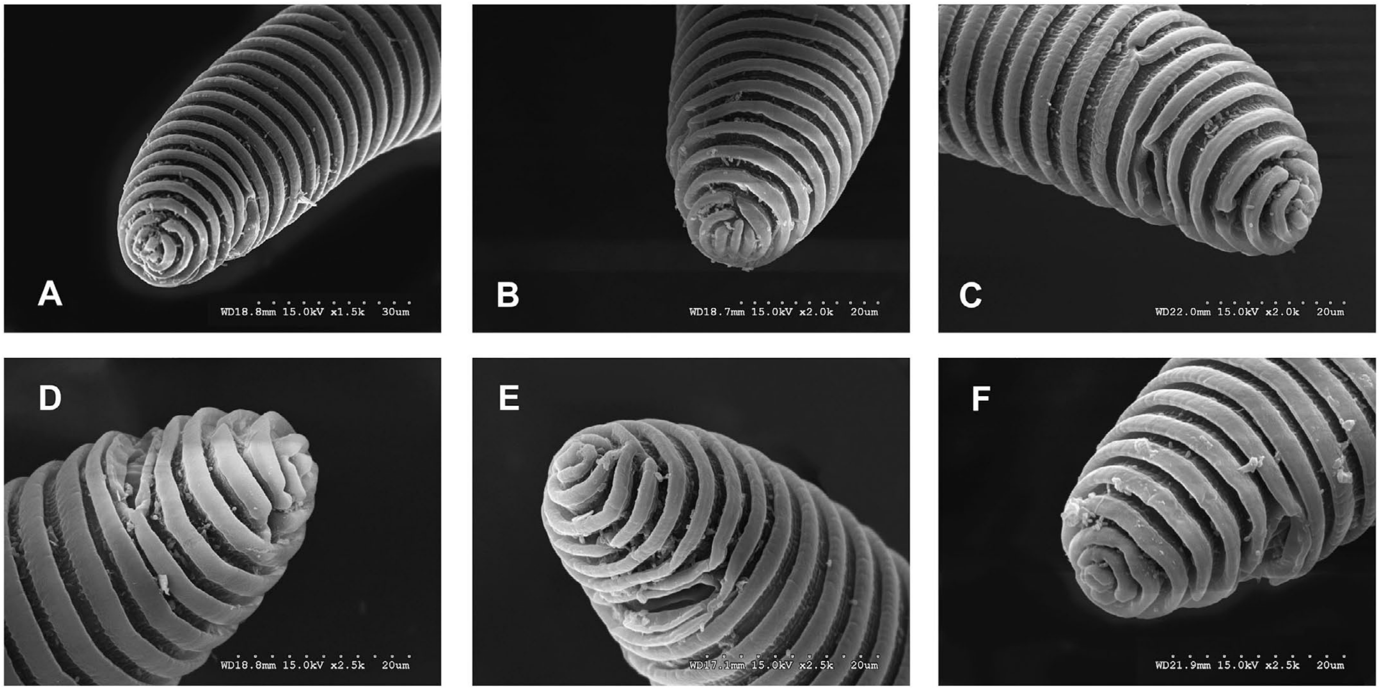

Fig. 7. Scanning electron micrographs of *Mesocriconema ericaceum* n. sp. females from Brushy Mountain, GRSM illustrating tail terminus variation and vulval flap. A: NID 4637; B: NID 4641; C: NID 4647; D: NID 4649; E: NID 4644; F: NID 4648.

forked or multifurcate. Lip region consisting of two labial annuli with crenate to wavy margins. First annulus *ca* 5 μm wider than second. First two body annuli having scales that are nearly continuous, with subsequent annuli possessing scales divided into longitudinal rows. Males were obtained from soil beneath mountain laurel at West Point, a second ATBI site less than a kilometer from Brushy Mountain and at the Brushy Mountain heath bald. The male tail is pointed, drawn out to an acute terminus with a subterminal bursa. Spicules arcuate, 45 μm in length. Three lateral lines on body.

DISTRIBUTION

Brushy Mountain is part of the Appalachian/Blue Ridge Forest ecoregion (Olson *et al.*, 2004). In addition to the Brushy Mountain and West Point sites, *O. seymouri* was collected from two other ecoregions during the North American Ecoregion survey of Criconematina. The species was collected from a native Rosebay rhododendron (*Rhododendron maximum* L.) preserve, growing in a threatened Atlantic white cedar (*Chamaecyparis thyoides* (L.) Britton, Stearns & Poggenb.) swamp in Pachaug State Forest in Connecticut. The forest is part of the Northeastern Coastal Forests ecoregion (Olson *et al.*, 2004). Four collection sites were within Tongass National Forest in Alaska, part of the Northern Pacific Coastal Forest ecoregion. The Deer mountain site was 1206 km up the Pacific coast from Mount Seymour, the type locality for *O. seymouri* (Wu, 1965) which is located just outside Vancouver, Canada. The type host was western red cedar (*Thuja plicata* Don *ex* D. Don). One common vegetative component to all four of the Alaskan collection sites was the presence of one or two species of *Vaccinium* L., *V. oxycoccos* L. (bog cranberry) and *V. uliginosum* L. (bog blueberry). Common tree species at the sites included western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western red cedar (*Thuja plicata*), mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.), yellow cedar (*Chamaecyparis nootkatensis* (D. Don) Spach) and common juniper (*Juniperis communis* L.).

A statistical parsimony network depicting *O. seymouri* haplotype relationships among the three geographic regions is presented in Figure 11. An approximately equivalent number of mutational steps is necessary to link either haplotypes from Alaska or haplotypes from Connecticut with the Great Smoky Mountains haplotypes. This pattern is consistent with the Great Smoky Mountains serving as a glacial refugium during the ice ages, followed by northeastern and north-western recolonization routes, but other explanations of this population structure cannot be ruled out.

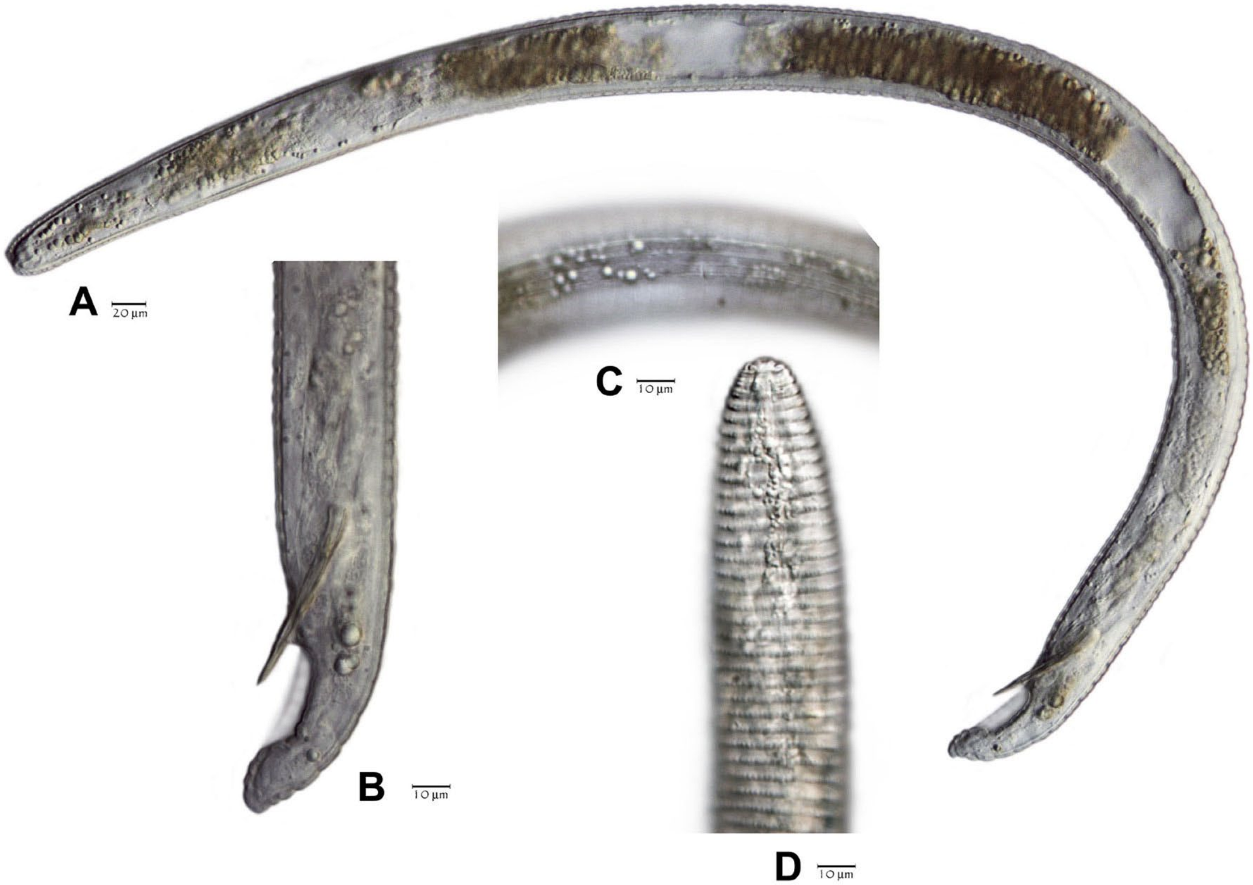

Fig. 8. Light microscope images of male and juvenile stages, *Mesocriconema ericaceum* n. sp. A:Male entire body, NID 5953; B: Male tail with straight spicule, subterminal bursa and expanded terminal annuli, NID 5953; C: Male mid-body with four lateral lines, NID 5953; D: Juvenile anterior with crenate annuli, NID 2638.

***Criconema loofi* (De Grisse, 1967) Raski & Luc, 1985**

= *Nothocriconema loofi* De Grisse, 1967

= *Criconemoides loofi* (De Grisse, 1967) Luc, 1970

(Fig. 12)

MEASUREMENTS

See Table 6.

DESCRIPTION

The notable feature of this species is the presence of a sheath on the tail annuli. Size of sheath varying among specimens from barely perceptible to conspicuous. Body appearing coarse due to low number of body annuli (*R* = 58-69) and mid-body annuli that average 10.6 μm in width. Lip-region dome-shaped with first annulus wider than second, which appears thickened and collar-like. Tail tapering to an acute point with sheath occupying as many as four terminal annuli.

DISTRIBUTION

Criconema loofi was collected from two other locations besides Brushy Mountain. In Ponce de Leon State Park, located in northwest Florida, it was collected near a freshwater spring. Vegetation in that collection site included Sweetbay magnolia (*Magnolia virginiana* L.), black gum (*Nyssa biflora* Walter), bald cypress (*Taxodium distichum* (L.) Rich), American holly (*Ilex opaca* Aiton), saw-palmetto (*Serenoa repens* (Bartram) J.K. Small), water oak (*Quercus nigra* L.), loblolly pine (*Pinus taeda* L.), sparkleberry (*Vaccinium arboretum* Marshall), azalea (*Rhododendron austrinum* (Small) Rehder), and elm (*Ulmus*

Table 2. Morphometrics of *Mesocriconema ericaceum* n. sp. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Female		Male
	Holotype	Paratypes	Topotypes*
n	–	21	2
L 5	70	527 ± 53.6 (445-642)	440, 620
a	9.5	12.5 ± 0.9 (10.7-14.3)	21.0, 22.1
b	4.7	4.3 ± 0.4 (3.7-5.5)	–
c	–	–	13.3, 19.4
c′	–	–	2.3, 1.7
V	96	93.8 ± 0.7 (92.4-95.2)	–
R	93	87.6 ± 3.7 (82-96)	180, 178
Rv	6	6.5 ± 0.6 (6-8)	–
Rex	25	24.3 ± 1.3 (22-26)	57, 53
Body annulus width	7.0	6.4 ± 0.6 (5.5-7.7)	3.1, 3.8
1st lip annulus width	15	17.5 ± 1.1 (15.0-19.0)	7, 9
2nd annulus width	20	–	–
Stylet	66	63 ± 3.1 (56-68)	–
Stylet shaft	15	–	–
Stylet cone	51	–	–
Stylet knob width	12	10.9 ± 0.5 (10.0-12.0)	–
Stylet knob height	5	–	–
Anterior end to vulva	548	495 ± 51.7 (415-608)	–
Spicule length	–	–	34, 38
Tail length	–	–	33, 32
Gubernaculum			–, 5
Max. body diam.	60	42 ± 3.1 (36-49)	21, 28
Vulval body diam.	35	33 ± 2.2 (28-37)	–
Anal body diam.	–	–	14.5, 19
Pharynx	122	122 ± 12.5 (90-145)	–
DGO	7	–	–
No. anastomoses	0	2.2 ± 2.3 (0-8)	–

* As there are only two topotype specimens, NID 2642 and NID 5953, respectively, all data are presented. Both specimens were photographed and processed for DNA analysis.

sp.). In Big Thicket National Preserve, *C. loofi* was collected in a lowland bald cypress slough in the Jack Gore Baygall Unit, and in a American beech (*Fagus grandifolia* Ehrh)-magnolia-loblolly pine slope plant community bordering a bald cypress slough in the Big Sandy Creek Unit. The ecological connection between North American collection sites of *C. loofi* and that of the type locality, from sandy dune soil around the roots of grass plants from Middelkerke, Belgium (De Grisse, 1967), is obscure. The genetic distances between the North American haplotypes exceed the 90% connection limit in the TCS network analysis (Fig. 11). Instead the raw (*p*-distance) between haplotypes was calculated and is shown in Figure 11. These distances, which range from 3.1 to 4.7%, indicate divergences initiated at a much earlier time than those observed in *O. seymouri*.

***Criconema* cf. *acriculum* (Raski & Pinochet, 1976) Raski & Luc, 1985**

= *Nothocriconema acriculum* Raski & Pinochet, 1976

= *Nothocriconemella acricula* (Raski & Pinochet, 1976) Ebsary, 1981 (Fig. 13)

MEASUREMENTS

See Table 6.

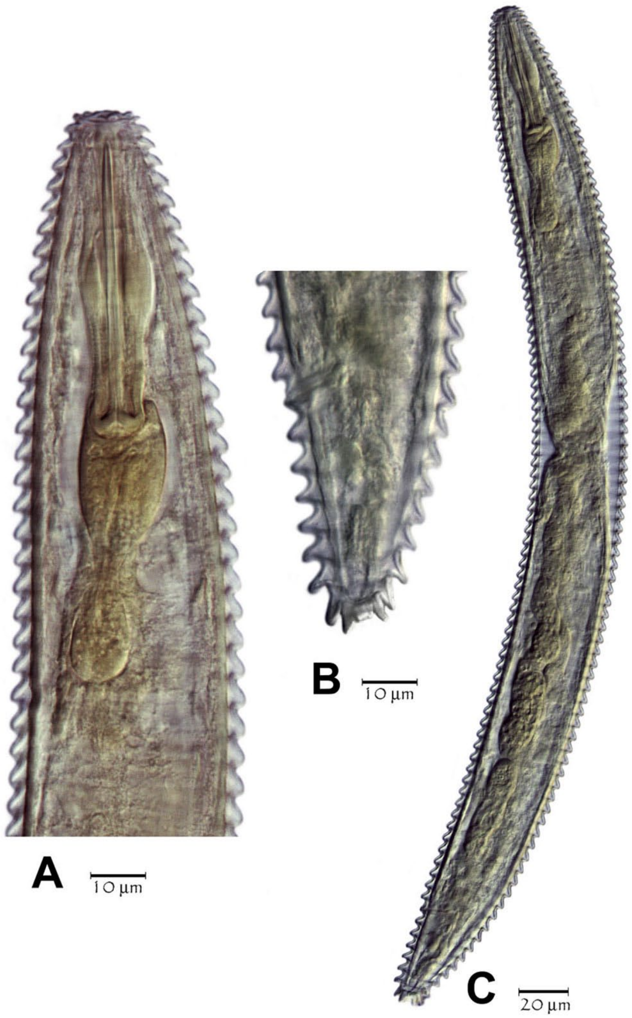

Fig. 9. Light microscope images of *Mesocriconema involutum*, paratype female from USDA Beltsville National Nematology Collection slide T-3920, P.A.A. Loof, collector. A: Head, B: Tail, C: Entire body.

DESCRIPTION

The exact species designation for these specimens requires confirmation from topotype collections. *Criconema* cf. *acriculum* is a small *Criconema* species with a delicate appearance. It has a short stylet, two, similar-sized, labial annuli, and an annulated tail drawn out into a pointed, hyaline terminus. The measurements and observed morphological features of the specimens could also support an identification of *C. longulum* Gunhold, 1953. The key in Geraert (2010) leads to *C. acriculum* because of the shorter stylet and presence of "small irregularities" on the posterior edge of the annuli (Fig. 13E).

DISTRIBUTION

It has only been recorded once in the scientific record, on California laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) north of San Francisco, California (Raski & Pinochet, 1976). In GRSM it was collected from three other ATBI sites, Snake Den and Albright Grove, two similar mid-elevation primary Hemlock forests (*Tsuga canadensis* (L.) Carr.) with a dense understory of *Rhododendron maximum*, and at Gregory's Bald, a grassy bald with scattered blueberry shrubs.

We also analyzed the more general nematode community from which *M. ericaceum* n. sp. was taken. The criconematid species discussed above make up approximately 7% of the total number of nematodes examined and identified to at least the genus level (n = 150 out of approximately 1100 total nematodes in the extracted sample). The most numerous (20% of the total, or 30 individuals) herbivorous nematodes belong to a likely undescribed species of *Helicotylenchus* Steiner, 1945 similar to *H. erythrinae* (Zimmerman, 1904) Golden, 1956 and/or *H. exallus* Sher, 1966, but with a significantly shorter tail (higher c ratio) and more posterior vulva than either of those two species. No other known plant-feeding taxa were seen, although members of the plant-associated genera *Aglenchus* Andrássy, 1954, *Coslenchus* Siddiqi, 1978, *Filenchus* Andrássy, 1954, *Lelenchus* Andrássy, 1954, and *Malenchus* Andrássy, 1968 were fairly numerous, accounting collectively for nearly 17% of the overall assemblage. Another 15% of the total community consists of individuals of the predaceous genus *Tripyla* Bastian, 1865. Other predatory genera observed include *Aporcelaimellus* Heyns, 1965, *Clarkus* Jairajpuri, 1970, *Crassolabium* Yeates, 1967, *Epidorylaimus* Andrássy, 1986, *Eudorylaimus* Andrássy, 1959, *Mylonchulus* (Cobb, 1916) Altherr, 1953, and *Nygolaimus* Cobb, 1913, all with six or fewer occurrences. Altogether, predators make up approximately 25% of the total nematode community, an unusually high percentage in comparison with other GRSM samples that we have examined. Slightly more than 23% of the observed nematodes are microbivores, dominated by representatives of *Cephalobus* Bastian, 1865 and *Plectus* Bastian, 1865. Small numbers of *Alaimus* de Man, 1880, *Bunonema* Jagerskjold, 1905, *Eucephalobus* Steiner, 1936, *Pseudacrobeles* Steiner, 1938, and *Wilsonema* Cobb, 1913 were also present. Fungivores are the least observed trophic category, at 9%, with the genera *Aphelenchoides* Fischer, 1894, *Diphtherophora* de Man, 1880, *Ditylenchus* Filipjev, 1936, *Pseudhalenchus* Tarjan, 1958, and *Tylencholaimus* de Man, 1876 all represented.

Table 3. Diagnostic, fixed nucleotides in COI alignment for *Mesocriconema ericaceum* n. sp. relative to *Mesocriconema* alignment (Powers *et al.*, 2014). Diagnostic nucleotides indicated by boxes.

	Position in alignment
	54 163 187 192 220 641
	T G C T A C
	1 54
N756 *M. discus* Brookings, SD GB-KJ787870	A TTA TAT CCT CCA CTT AGA ACA ATA GGA CAA GTT GGT TTT GCT TCT ATT TTA A$\boxed{G}$T ATT TTT AGT TTA CAT TTT GCT GGT
N2625 *M. ericaceum* n. sp. GRSM, TN	A A.. ..C ..A ... ..A ... .T ..T ... A.A ..A ... ... G.. ..G .$\boxed{T}$A G.. ... ..C ... ...
N2630 *M. ericaceum* n. sp. GRSM, TN	A A.. ..C ..A ... ..A ... .T ..T ... A.A ..A ... ... G.. ..G .$\boxed{T}$A G.. ... ..C ... ...
N5975 *M. ericaceum* n. sp. GRSM, TN	A A.. ..C ..A ... ..A ... .T ..T ... A.A ..A ... ... G.. ..G .$\boxed{T}$A G.. ... ..C ... ...
	80
N756 *M. discus* Brookings, SD GB-KJ787870	ATT AGT TCT ATT TTA GGA AGT ATT AAT TTT ATA AGA ACA ATT AAA AAA ATT AAA TTT AAT TTT TTA AAA ATT ATT AGT
N2625 *M. ericaceum* n. sp. GRSM, TN	G.. ... ...C ..T ... ... .CC ... C.. ... ... .A.
N2630 *M. ericaceum* n. sp. GRSM, TN	G.. ... ...C ..T ... ... .CC ... C.. ... ... .A.
N5975 *M. ericaceum* n. sp. GRSM, TN	G.. ... ...C ..T ... ... .CC ... C.. ... ... .A.
	158 163 187 192 220
N756 *M. discus* Brookings, SD GB-KJ787870	ATT TC$\boxed{T}$ TTA TTT ATT TGA TCT ATT TTT AT$\boxed{T}$ ACT A$\boxed{C}$T TTT TTA TTA ATT TTA TCT TTG CCT GT$\boxed{T}$ TTA GCT TCT TGT TTA
N2625 *M. ericaceum* n. sp. GRSM, TN	G.. ..$\boxed{G}$ ... ...G ... G.. ... G.$\boxed{C}$ ..A .$\boxed{T}$. ... ..G ..G ... .A ..A ..A ..$\boxed{A}$ ... ...
N2630 *M. ericaceum* n. sp. GRSM, TN	G.. ..$\boxed{G}$ ... ...G ... G.. ... G.$\boxed{C}$ ..A .$\boxed{T}$. ... .G ... .A ..A ..A ..$\boxed{A}$ ... ...
N5975 *M. ericaceum* n. sp. GRSM, TN	G.. ..$\boxed{G}$ ... ...G ... G.. ... G.$\boxed{C}$ ..A .$\boxed{T}$. ... ..G ..G ... .A ..A ..A ..$\boxed{A}$ ... ...
	236
N756 *M. discus* Brookings, SD GB-KJ787870	ACT ATA TTA TTA ACA GAT AAA TTA TTA GGC TCA TCT TTT TTT AAT TCA ATT GGA GGT GGA AAT CCA ATT ATA TTT CAA
N2625 *M. ericaceum* n. sp. GRSM, TN	... ... C.. ..T ... ...T ... ...T ... ..T ... ..T ..C ..T ... ...
N2630 *M. ericaceum* n. sp. GRSM, TN	... ... C.. ..T ... ...T ... ...T ... ..T ... ..T ..C ..T ... ...
N5975 *M. ericaceum* n. sp. GRSM, TN	... ... C.. ..T ... ...T ... ...T ... ..T ... ..T ..C ..T ... ...
	314
N756 *M. discus* Brookings, SD GB-KJ787870	CAT TTG TTT TGA TTT TTT GGT CAT CCA GAA GTT TAT ATT TTA ATT TTA CCT GCT TTT GGA ATT ATT AGT TAT AGG GTT
N2625 *M. ericaceum* n. sp. GRSM, TN	... ..A ... ..G ... ...T ... ...T ...
N2630 *M. ericaceum* n. sp. GRSM, TN	... ..A ... ..G ... ...T ... ...T ...
N5975 *M. ericaceum* n. sp. GRSM, TN	... ..A ... ..G ... ...T G.. ... ...T ...
	392
N756 *M. discus* Brookings, SD GB-KJ787870	TTA GTT TTA ACA GGA AAA AAT AAA ACT TTT GGT CCA GTA GGC ATA CTT TTT GCT ATT TTT AGT ATT GGT TTG GTT GGT
N2625 *M. ericaceum* n. sp. GRSM, TN	... A.. ... ...C ... ...A ..T A.. ..A ... T.A ... ...A ..A
N2630 *M. ericaceum* n. sp. GRSM, TN	... A.. ... ...C ... ...A ..T A.. ..A ... T.A ... ...A ..A
N5975 *M. ericaceum* n. sp. GRSM, TN	... A.. ... ...C ... ...A ..T A.. ..A ... T.A ... ...A ..A
	470
N756 *M. discus* Brookings, SD GB-KJ787870	TGT TTA GTT TGA GCT CAT CAT ATA TTT GTT GTT GGT ATA GAT ATT GAT TCT CGA GTT TAT TAT ATA AGA GCA ACA ATA
N2625 *M. ericaceum* n. sp. GRSM, TN	... ...G ... ...A ... ...
N2630 *M. ericaceum* n. sp. GRSM, TN	... ...G ... ...A ... ...
N5975 *M. ericaceum* n. sp. GRSM, TN	... ...G ... ...A ... ...
	548
N756 *M. discus* Brookings, SD GB-KJ787870	ATT ATT GCT GTT CCT ACT GGT ATT AAA ATT TAT AGT TGA TTA TTA ACA ATT AAT GGT TTT ATT TTA AAT TTT TCT GTT
N2625 *M. ericaceum* n. sp. GRSM, TN	... ...A ... ...C ... ... TG. ... ... TC.
N2630 *M. ericaceum* n. sp. GRSM, TN	... ...A ... ...C ... ... TG. ... ... TC.
N5975 *M. ericaceum* n. sp. GRSM, TN	... ...A ... ...C ... ... TG. ... ... TC.

Table 3. (Continued.)

	Position in alignment					
	54	163	187	192	220	641
	T	G	C	T	A	C

	626 ... 641
N756 *M. discus* Brookings, SD GB-KJ787870	TTA TTT TTT TGG GTT $\boxed{\mathrm{T}}$TA GGT TTT ATT TTT ATA TTT ACT TTA GGA GGT TTA ACT GGT TTA GTT TTA TCA AAT ATA ATT
N2625 *M. ericaceum* n. sp. GRSM, TN	C.G ... ... $\boxed{\mathrm{C}}$.T ... ...G ... ..A C.. ..T ... .A ..A ... .G ..T ... ...
N2630 *M. ericaceum* n. sp. GRSM, TN	C.G ... ... $\boxed{\mathrm{C}}$.T ... ...G ... ..A C.. ..T ... .A ..A ... .G ..T ... ...
N5975 *M. ericaceum* n. sp. GRSM, TN	C.G ... ... $\boxed{\mathrm{C}}$.T ... ...G ... ..A C.. ..T ... .A ..A ... .G ..T ... ...
	704 ... 721
N756 *M. discus* Brookings, SD GB-KJ787870	TTA GAT ATT AAC CTT CAT
N2625 *M. ericaceum* n. sp. GRSM, TN	... ...T ... ...
N2630 *M. ericaceum* n. sp. GRSM, TN	... ...T ... ...
N5975 *M. ericaceum* n. sp. GRSM, TN	... ...T ... ...

Table 4. COI amino-acid sequence for *Mesocriconema discus* and *M. ericaceum* n. sp.

N756 *M. discus* Brookings, SD GB-KJ787870	LYPPLSTMGQ	VGFASILSIF	SLHFAGISSI	LGSINFMSTI	NNINFNFLNI	ISISLFIWSI	FITTFLLILS	LPVLASCLTM
N2625 *M. ericaceum* n. sp. GRSM, TN	M......I..	M....V.MV.	......V...	....	..T.L.....	.NV......V	.V.I......	....
N2630 *M. ericaceum* n. sp. GRSM, TN	M......I..	M....V.MV.	......V...	....	..T.L.....	.NV......V	.V.I......	....
N5975 *M. ericaceum* n. sp. GRSM, TN	M......I..	M....V.MV.	......V...	....	..T.L.....	.NV......V	.V.I......	....
N756 *M. discus* Brookings, SD GB-KJ787870	LLTDNLLGSS	FFNSIGGGNP	IMFQHLFWFF	GHPEVYILIL	PAFGIISYSV	LVLTGNNNTF	GPVGMLFAIF	SIGLVGCLVW
N2625 *M. ericaceum* n. sp. GRSM, TN	....	....	....	....	....	.I........	..M.......	....
N2630 *M. ericaceum* n. sp. GRSM, TN	....	....	....	....	....	.I........	..M.......	....
N5975 *M. ericaceum* n. sp. GRSM, TN	....	....	....	....	....V.....	.I........	..M.......	....
N756 *M. discus* Brookings, SD GB-KJ787870	AHHMFVVGMD	IDSRVYYMSA	TMIIAVPTGI	NIYSWLLTIN	GFILNFSVLF	FWVLGFIFMF	TLGGLTGLVL	SNMILDINLH
N2625 *M. ericaceum* n. sp. GRSM, TN	....	....	....	....	....C..S..	....	....	....
N2630 *M. ericaceum* n. sp. GRSM, TN	....	....	....	....	....C..S..	....	....	....
N5975 *M. ericaceum* n. sp. GRSM, TN	....	....	....	....	....C..S..	....	....	....

Table 5. Pairwise COI Tamura-Nei genetic distances.

Species	Specimen ID	NID	Percent similarity to *M. ericaceum* n. sp. NID 2639
Lobocriconema thornei	Topotype	NID 2524	80.58
Mesocriconema xenoplax	Topotype	NID P74053	86.27
M. inaratum	Topotype	NID 9	81.83
M. discus	Topotype	NID 431	89.04
M. rusticum		NID 447	86.55
M. ornatum	Topotype	NID 500	79.06
M. curvatum		NID 122	84.88
M. onoense		NID 3657	83.08
Xenocriconemella macrodora	Topotype	NID 1213	78.73

Percent similarity between *Mesocriconema ericaceum* n. sp. and other criconematid species. Detailed geographic information on these specimens can be found in Powers *et al.* (2014).

Discussion

Mesocriconema species are found in a wide range of native plant communities in North America. In this manuscript we have described a new species that may be limited to the specific ecological conditions found on Brushy Mountain's heath bald. Endemicity in the southern Appalachian Mountains is not unusual, and two of the plant species on Brushy Mountain, Catawba rhododendron and mountain fetterbush are considered southern Appalachian endemics. *Mesocriconema ericaceum* n. sp. was not found in Andrew's and Gregory's Balds, two other geographically similar ATBI sites. Both of these latter balds are predominantly grassy balds with scattered shrubby vegetation and moderately acidic soil. Mountain laurel and Rosebay rhododendron were sampled widely within and outside Great Smoky Mountains National Park without recovering *M. ericaceum* n. sp. Two of the members of the Brushy Mountain heath bald criconematid community exhibited distinctly different biogeographic patterns. *Criconema loofi* displayed a Gulf Coast distribution, occupying wet lowland habitats with a *Rhododendron* or *Vaccinium* understory. *Ogma seymouri* may also be associated with *Vaccinium*, but its distribution from Brushy Mountain extends northeastward along the Appalachian Mountains and northwest towards coastal Alaska (Fig. 11). Additional sampling is required to determine if *O. seymouri* has a discontinuous North American distribution or a transcontinental boreal distribution similar to species of spruce and fir (de Lafontaine *et al.*, 2010). Network analysis supports a Great Smoky Mountains glacial refugium for *O. seymouri*, but alternative refugia and processes other than post-glacial recolonization may be responsible for present-day geographic patterns.

The low level of haplotype variation in the *M. ericaceum* n. sp. population suggests that the species may have been subjected to a genetic bottleneck during the last 10,000 years. Although glaciers did not extend to the Great Smoky Mountains during the Wisconsin Glaciation (last glacial maxima 18,000 years ago), it is likely that many of the alpine mountain peaks were covered with ice year around, or maintained a tundra plant community (Linzey, 2008). One possible factor influencing plant diversity on the Brushy Mountain heath bald was a series of fires in the 1920s (White *et al.*, 2001). Sampling additional health balds in the Appalachian Mountains should help determine if the criconematid nematode community on Brushy Mountain is a random assemblage of nematode species or if it is a remnant of an ancient nematode community adapted to the unique ecological conditions of Appalachian heath balds.

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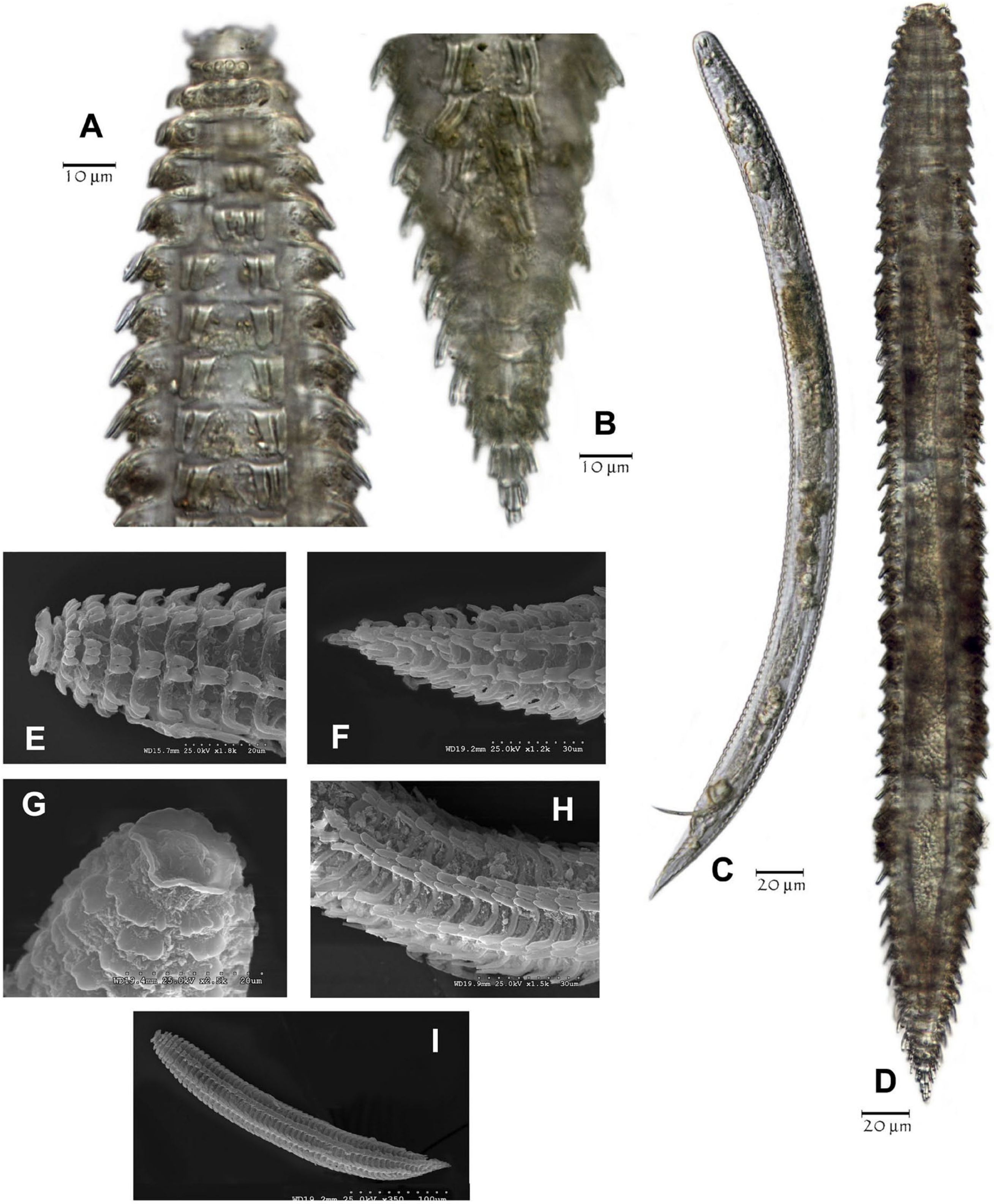

Fig. 10. Light (A-D): and scanning (E-I) micrographs of *Ogma seymouri* from Brushy Mountain, GRSM. A: Female head and anterior paired scales on annuli, NID 2631; B: Female tail, NID 2631; C: Male entire body, NID 2632; D: Female entire body, NID 2631; E: Female head, profile view, NID 4662; F: Female tail, NID 4669; G: Female head, face view, NID 4661; H: Female mid-body cuticle with longitudinal rows of scales, NID 4660; I: Female entire body, NID 4665.

Table 6. Morphometrics of female *Ogma seymouri*, *Criconema loofi* and *Criconema* cf. *acriculum* from Brushy Mountain, GRSM. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*Ogma seymouri*	*Criconema loofi*	*Criconema* cf. *acriculum*
n	19	15	11
L	539 ± 49.7 (425-610)	597 ± 53.3 (477-712)	369 ± 33.6 (315-422)
a	10.3 ± 0.9 (8.2-11.4)	11.3 ± 1.0 (9.4-13.0)	12.1 ± 2.3 (8.9-15.7)
b	4.6 ± 0.7 (3.6-5.9)	4.2 ± 0.3 (3.7-4.8)	3.9 ± 0.5 (3.3-4.6)
V	85.3 ± 1.4 (83.0-89.0)	87.9 ± 1.5 (86.5-91.9)	86.5 ± 1.3 (84.4-88.5)
R	59.4 ± 4.2 (53-67)	62.1 ± 3.2 (58-69)	77.8 ± 6.8 (64-88)
Rv	11.9 ± 1.4 (9-14)	9.6 ± 0.6 (9-11)	12.2 ± 1.4 (10-14)
Rex	–	18.1 ± 1.0 (16-19)	24.1± 2.2 (21-26)
Body annulus width	9.5 ± 0.7 (7.8-10.3)	10.6 ± 0.7 (9.0-11.5)	5.1 ± 0.7 (4.0-6.1)
1st lip annulus width	19.1 ± 1.3 (17-22)	23.5 ± 1.3 (21.0-25.0)	11.5 ± 0.8 (10.0-13.0)
Stylet	63 ± 3.0 (56-68)	94 ± 6.8 (81-106)	55 ± 4.6 (49-67)
Stylet knob width	8.9 ± 0.8 (8-10)	13.2 ± 1.0 (12.0-15.0)	8.0 ± 1.1 (7.0-10.0)
Anterior end to vulva	461 ± 43.5 (367-513)	525 ± 49.7 (415-632)	318 ± 28.8 (275-362)
Max. body diam.	52 ± 3.6 (46-61)	53 ± 2.4 (49-58)	31 ± 4.3 (25-37)
Vulval body diam.	41.8 ± 4.5 (33-48)	41.9 ± 2.1 (39.0-45.0)	24.7 ± 4.2 (18.0-30.0)
Pharynx	119 ± 16.5 (93-150)	144 ± 10.7 (125-167)	95 ± 7.3 (85-108)

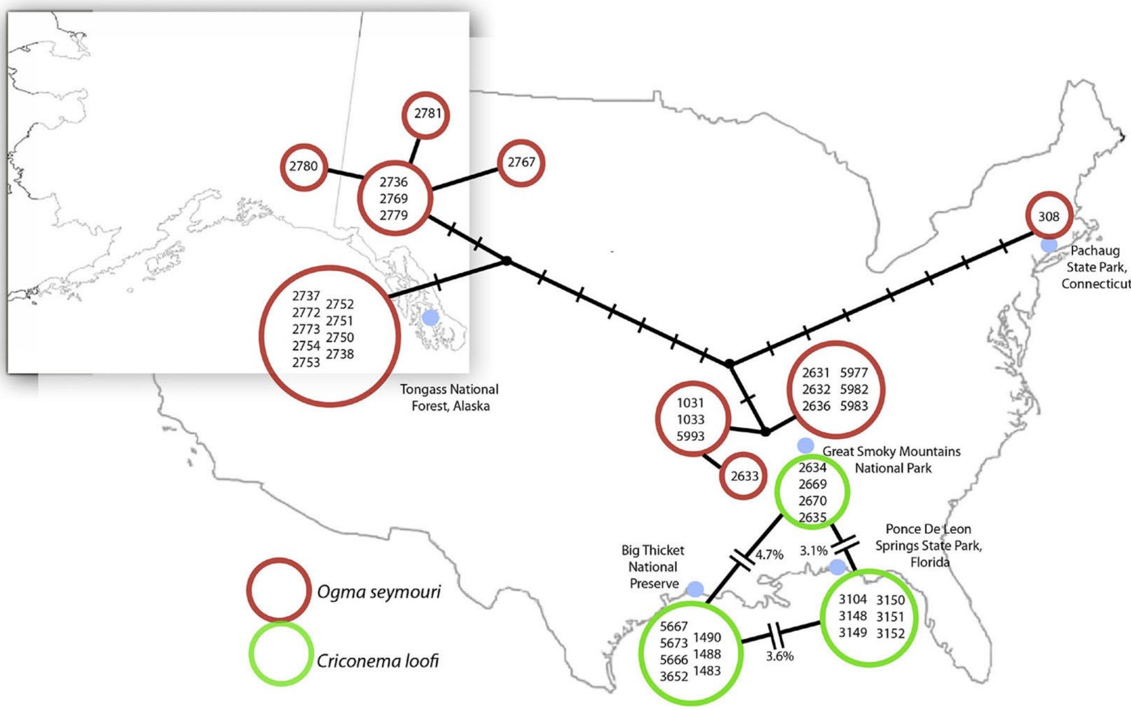

Fig. 11. A TCS statistical parsimony network of COI haplotype relationships in *Ogma seymouri* and *Criconema loofi*. Blue dots indicate collection sites. Red circles denote unique COI haplotypes for *O. seymouri*. Green circles denote unique haplotypes for *C. loofi*. Hash marks in *O. seymouri* network indicate hypothesized intermediate haplotypes separated by a single substitution. Break marks between *C. loofi* haplotypes indicate genetic distance as raw uncorrected *p*-values. NID numbers representing each haplotype are inside colored circles.

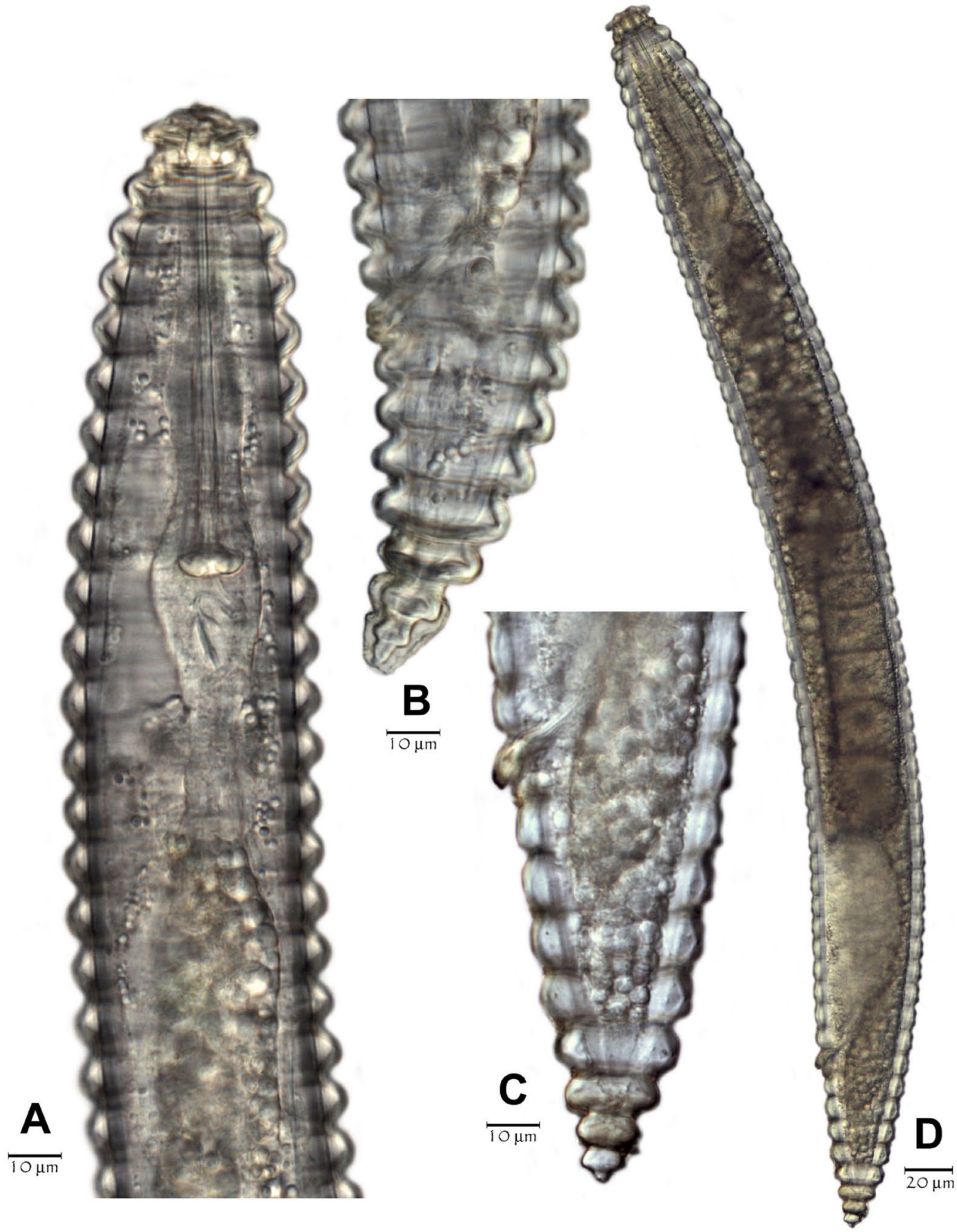

Fig. 12. Light microscope images of *Criconema loofi* from Brushy Mountain, all females. A: Head, NID 2635; B: Tail with terminal three annuli surrounded by sheath, NID 2635; C: Tail, lacking terminal sheath, NID 2634; D: Entire body, NID 2634.

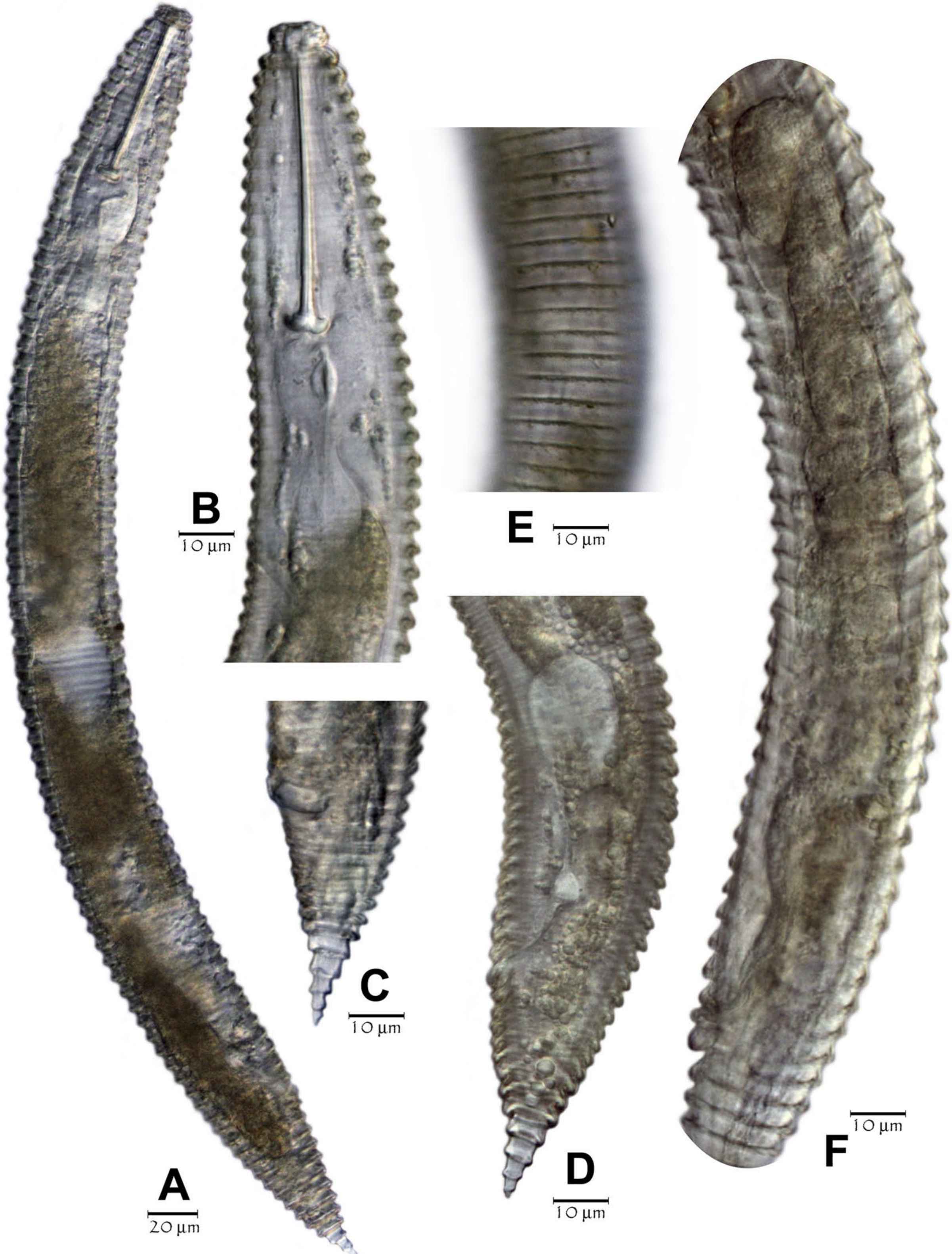

Fig. 13. Light microscope images of *Criconema* cf. *acriculum* from Brushy Mountain, all females. A: Entire body, NID 5992; B: Head with pair of similar sized labial annuli, NID 5979; C: Tail and rectangular vulval flap, NID 5980; D: Tail, NID 5979; E: Mid-body cuticle, NID 5980; F: Reproductive tract with terminal portion of ovary reflexed, NID 5991.

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