Morphological and molecular characterisation of *Hoplolaimus smokyensis* n. sp. (Nematoda: Hoplolaimidae), a lance nematode from Great Smoky Mountains National Park, USA

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Summary – *Hoplolaimus smokyensis* n. sp. is a new species of lance nematode collected in Great Smoky Mountains National Park, USA. Females of *H. smokyensis* n. sp. have a labial region characterised by six, occasionally five, annules. The basal lip annule is subdivided by about 24 longitudinal striae. The stylet averages 47 μm long with robust, tulip-shaped stylet knobs bearing anterior projections. The hemizonid is ca 4 μm anterior to the excretory pore. The lateral field is incompletely areolated and has four continuous incisures from the metacarpus region to the tail region. There are three pharyngeal gland nuclei. Vulval epiptygma are absent. The scutellate phasmids are located one anterior and one posterior to the vulva. The male is shorter than the female and the head region is higher and more rounded than that of the female. The bursa extends to the tail tip and the gubernaculum is large and protrusible and has titillae and a capitulum. Morphologically, *H. smokyensis* n. sp. is most similar to *H. galeatus* and *H. stephanus*, but can be distinguished by differences such as the number of annules and longitudinal striae on the lip region and morphometric values. *Hoplolaimus smokyensis* n. sp. is also genetically distinct from other species according to comparisons of ribosomal and mitochondrial DNA sequences. Phylogenetic analyses based on ribosomal and mitochondrial gene sequences suggest that *H. smokyensis* n. sp. is a lineage distinct from related *Hoplolaimus* species.

Keywords – *Acer* sp., Eastern hemlock, *Halesia carolina*, lance nematode, maple, morphology, morphometrics, new species, phylogeny, plant-parasitic nematode, silverbell, taxonomy, *Tsuga canadensis*.

During a survey of fauna of Great Smoky Mountains National Park, along the Tennessee–North Carolina border in the south-eastern USA, a *Hoplolaimus* von Daday, 1905 species was isolated from a mixed forest sample of maple (*Acer* sp.), Eastern hemlock (*Tsuga canadensis* (L.) Carrière) and silverbell (*Halesia carolina* L., 1759). This species was initially distinguished from other reported lance nematodes based on a BLAST (Basic Local Alignment Search Tool) search result of internal transcribed spacer 1 (ITS1) sequences at the NCBI (National Center for Biotechnology Information). Its position was isolated on a phylogenetic tree composed of all available unique ITS1 sequences of *Hoplolaimus* species from GenBank. A phylogenetic analysis of mitochondrial cytochrome ox-

idase subunit I (*COI*) sequences supported this result, ensuring that molecular information of this undescribed lance nematode had not yet been reported. Females, males and juveniles were examined for morphological characteristics, morphometrics and phylogenetic relationships. We name the species *Hoplolaimus smokyensis* n. sp. in reference to the locality where it was first found.

Hoplolaimus smokyensis n. sp. is closest to *H. galeatus* (Cobb, 1913) Thorne, 1935 and *H. stephanus* Sher, 1963, but can be distinguished by minor morphological differences, such as the numbers of annules and longitudinal striae in the lip region, morphometric values, and absence of epiptygma. In this study, we applied two genetic markers, mitochondrial (*COI*) and ribosomal DNA

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(ITS1), to obtain an analysis of higher resolution on taxonomic relationships, which support the delimitation of *H. smokyensis* n. sp. DNA sequences were aligned with BLAST in GenBank. The highest similarity (89% on both *COI* and Internal Transcribed Spacer (ITS1) markers) was with *H. magnistylus* Robbins, 1982 sequences. Alignment analyses using MUSCLE (Edgar, 2004) showed unique molecular characteristics possessed by *H. smokyensis* n. sp. Phylogenetic trees generated with MrBayes (Huelsenbeck & Ronquist, 2001) indicated *H. smokyensis* n. sp. as a distinct clade.

Materials and methods

NEMATODE ISOLATION

Female, male and juvenile specimens were sampled from Great Smoky Mountains National Park in July 2006. Nematodes were extracted from soil with a combination of sieving-decanting and sucrose centrifugal-flotation (Jenkins, 1964). Specimens were killed and fixed, processed to glycerin and permanently mounted on slides as described by Ye & Robbins (2003).

MORPHOLOGICAL OBSERVATION AND MICROGRAPHY

Permanently mounted specimens were examined and imaged. Some live specimens were imaged as well. Most images were produced with a 14-megapixel Q-camera on an Olympus BX-63 differential interference-contrast microscope system. Measurements were made on a Nikon Optiphot II compound microscope with the aid of a drawing tube or ocular micrometer. Mean annule width was calculated by measuring a series of ten annules at mid-body.

MOLECULAR PROFILES AND PHYLOGENY

DNA was extracted from individual nematodes using Sigma Extract-N-Amp kit (XNAT2) (Sigma). The manufacturer's protocol was modified by reducing volumes to one eighth of the recommended amount (Ma *et al.*, 2011). The ITS1 was amplified with primers Hoc-1f (5'-AACCTGCTGCTGGATCATTA-3') and LSUD3r (5'-TATGCTTAAGTTCAGCGGGT-3') following Bae *et al.* (2008, 2009); and a portion of cytochrome c oxidase subunit I (*COI*) sequence was amplified with primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB5 (5'-AGCACCTAAACTTAAACATAATGAAA-3') (Derycke *et al.*, 2005). For *COI*, the initial denaturation

was set at 95°C for 3 min, followed by 33 cycles of 95°C for 45 s, 50°C for 1 min 15 s, 72°C for 2 min and final extension at 72°C for 10 min (Holguin *et al.*, 2015a). The amplified products were loaded onto a 1.5% agarose gel and visualised with GelRed™ (Biotium). PCR products for both regions were purified using magnetic beads and sequenced in both directions with the ABI 3730 capillary sequencer (Applied Biosystems) in the DNA Laboratory (School of Life Sciences) at Arizona State University. Sequencing results were edited and assembled in Sequencher 5.1 (Genes Code). Consensus DNA sequences were searched in GenBank using BLAST, then aligned with MUSCLE (Edgar, 2004).

A best-fit model of nucleotide substitution was selected using the GTR + I + G model with the Akaike Information Criterion (AIC) among 56 different models using ModelTest v 3.7 (Posada & Crandall, 1998). Bayesian inference was implemented for each gene separately using the MrBayes 3.1.2 program (Huelsenbeck & Ronquist, 2001) running the chain for 1×10^7 generations with the Markov chain Monte Carlo (MCMC) method, a sample frequency of 100 and burn-in value of 2500. We estimated the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50% majority rule. The phylogenetic trees were viewed on phylo.io (Robinson *et al.*, 2016) and iTOL (Interactive tree of life v3) (Letunic & Bork, 2016).

Results

*Hoplolaimus smokyensis** n. sp. (Figs 1-4)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Female body generally cylindrical, vermiform, tapering slightly at each end. Head set off, with massive cephalic framework, usually bearing six lip annules and the oral disc, but sometimes with five annules (six individuals out of 30 specimens), basal annule tessellated, divided into *ca* 20 equal blocks (Fig. 1A, B). Oral disc surrounded by a lip annule separated into six sections: two subdorsal, two

* The specific epithet refers to the locality where the species was first found.

Table 1. Morphometrics of *Hoplolaimus smokyensis* n. sp. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	–	30	17
L	1409	1335 $\pm$ 174 (997-1870)	1096 $\pm$ 87 (972-1261)
a	34.7	30.1 $\pm$ 3.3 (23.5-36.9)	30.5 $\pm$ 2.3 (26.1-34.5)
b	9.9	9.8 $\pm$ 1.6 (7.1-14.9)	8.1 $\pm$ 0.7 (7.1-9.3)
b'	7.3	6.8 $\pm$ 0.9 (4.9-8.9)	5.8 $\pm$ 0.5 (5.2-6.7)
c	115.7	114 $\pm$ 15.8 (91.3-154)	36.8 $\pm$ 3.7 (28.9-42.3)
c'	0.7	0.7 $\pm$ 0.1 (0.5-1.0)	1.5 $\pm$ 0.2 (1.3-1.9)
V	54.4	56.5 $\pm$ 2.6 (49.7-62.1)	–
Max. body diam.	41	44.4 $\pm$ 4.1 (34.5-52.8)	36.1 $\pm$ 3.4 (30.5-42.6)
Lip annulus	6	5.8 $\pm$ 0.4 (5-6)	5.8 $\pm$ 0.4 (5-6)
Lip height	8	8.1 $\pm$ 0.3 (7.1-9.1)	8.1 $\pm$ 0.2 (7.1-8.7)
Lip diam.	16	15.8 $\pm$ 1.0 (14.2-18.3)	13.9 $\pm$ 0.6 (12.2-14.2)
Conus	24	25.8 $\pm$ 1.6 (22.3-28.4)	23.8 $\pm$ 1.5 (22.3-26.4)
Stylet length	47	47.9 $\pm$ 1.9 (44.7-50.8)	43.4 $\pm$ 2.1 (40.6-48.7)
Pharynx	142	138 $\pm$ 16.7 (79-183)	136 $\pm$ 6.4 (126-152)
Anterior end to pharyngeal gland tip	193	199 $\pm$ 11.2 (168-223)	189 $\pm$ 10.7 (173-207)
Tail length	26	24.9 $\pm$ 4.0 (20.3-34.5)	30.0 $\pm$ 3.4 (24.4-36.5)
Anal body diam.	37	35.5 $\pm$ 3.1 (28.4-40.6)	20.2 $\pm$ 1.3 (18.3-22.3)
Hemizonid from anterior end	162	157 $\pm$ 14.2 (140-207)	150 $\pm$ 8.8 (132-166)
Excretory pore from anterior end	166	164 $\pm$ 14.7 (144-217)	156 $\pm$ 8.6 (136-173)
Dorsal pharyngeal gland orifice	12	11.7 $\pm$ 0.9 (10.2-12.2)	7.0 $\pm$ 1.0 (6.1-8.1)
Vulva from anterior end	766	753 $\pm$ 100 (602-1112)	–
Spicule length	–	–	41.1 $\pm$ 2.5 (36.5-46.7)
Gubernaculum	–	–	10.5 $\pm$ 0.6 (10-12)

subventral, and two reduced lateral sectors. Stylet long and robust, basal knobs tulip-shaped (Fig. 1C-E). Dorsal pharyngeal gland orifice *ca* 12 μm from base of stylet knobs. Pharyngeal glands with three nuclei, one nucleus in the dorsal gland. Pharyngo-intestinal junction at 6-12% of body length from anterior end. Pharyngeal lobe terminus at 12-16% of body length from anterior end. Excretory pore prominent (Fig. 1F), at 10-15.5% of body length from anterior end. Hemizonid large, spanning about two annules, just anterior to excretory pore (Fig. 1G). Lateral field with four incisures, incompletely areolated (Fig. 2A), narrowing to two incisures at level of metacarpus, ending near level of stylet base; posteriorly, lateral field abruptly ending by joining of the outer incisures at level of anus (Fig. 2E, F). Phasmids large, scutelliform, conspicuous, variable in position, right phasmid located anterior to vulva, left one located posterior, anterior one at 20-40% and posterior one at 60-90% of body length from anterior end. Vulva prominent, located near mid-body, with deep transverse slit, epiptygma absent (Fig. 2B). Ovaries two, outstretched. Spermatheca round to oval, usually with many sperm (Fig. 2C). Cuticular annulation at mid-body

distinct, annule width 2 μm , subcuticular annulation distinct, about half as wide as outer cuticular annules. Tail hemispherical to conoid-hemispherical (Fig. 2E, F).

Male

Body shape similar to female, cylindroid, vermiform. Body length generally shorter than female. Head set off, labial region exhibiting sexual dimorphism, higher and rounder than females when viewed laterally (Fig. 4A, B). Head region usually bearing six lip annules and the oral disc, occasionally five annules (four individuals out of 17 specimens). Stylet knobs tulip-shaped. Excretory pore at 12-14% of body length from anterior end, 2-3 annules posterior to hemizonid. Cuticular annules at mid-body *ca* 2 μm wide. Lateral field areolated, with four incisures. Testis one, outstretched anteriorly. Spicules and gubernaculum long and prominent, bursa large and conspicuous, extending to tail tip. Gubernaculum large, protrusible, with prominent capitulum and titillae (Figs 3B; 4D).

Fig. 1. Micrographs of *Hoplolaimus smokyensis* n. sp. females from Great Smoky Mountains National Park. A, B: Head of the same female specimen showing lip annules and longitudinal striae; C-E: Stylet view with different focusing; F, G: Pharyngeal region of the same female specimen showing excretory pore (F) and hemizonid (G) indicated by arrows.

Fig. 2. Micrographs of *Hoplolaimus smokyensis* n. sp. females from Great Smoky Mountains National Park. A: Vulval region with no epiptygma; B: Incomplete areolation in lateral field; C: Ovary with sperm; D: Oocytes; E, F: Tail of same female specimen showing lateral field incisures, areolation and anus (arrow).

Fig. 3. Micrographs of *Hoplolaimus smokyensis* n. sp. from Great Smoky Mountains National Park. A: Female head with stylet, dorsal pharyngeal gland orifice and median bulb; B: Male tail; C: Habitus of male and female.

Fig. 4. Micrographs of *Hoplolaimus smokyensis* n. sp. males from Great Smoky Mountains National Park. A, B: Head of same male specimen showing head lip region (A) and stylet (B); C: Ventral view of an anterior phasmid; D: Ventral view of male tail.

TYPE LOCALITY

All specimens were obtained from Great Smoky Mountains National Park, Sevier County, TN, USA, Laurel

Falls Trail, 35°40.874'N, 83°36.149'W, elevation 1008 m a.s.l., in a mixed maple (*Acer* sp.), Eastern hemlock (*Tsuga canadensis*) and silverbell (*Halesia carolina*) forest.

TYPE MATERIAL

Holotype female (T-702t) and five paratype slides (T-6864p to T-6868p containing seven females and seven males) deposited in the Nematology Laboratory Collection, USDA, ARS, Beltsville, MD, USA. Five other paratype slides (28506-28510), including ten females, seven males and five juveniles, deposited in the Department of Nematology, University of California, Riverside, CA, USA.

DIAGNOSIS AND RELATIONSHIPS

Females of *H. smokyensis* n. sp. have a labial region characterised by six, occasionally five, annules. The basal lip annule is subdivided with about 24 longitudinal striae. The stylet averages 47 μm long with robust, tulip-shaped stylet knobs bearing anterior projections. The hemizonid is ca 4 μm anterior to the excretory pore. The lateral field is incompletely areolated and has four continuous incisures from the metacarpus region to the tail region. There are three pharyngeal gland nuclei. Vulval epiptygma are absent. The scutellate phasmids are located one anterior and one posterior to the vulva. The male is shorter than the female. In lateral view the male head region is higher and more rounded than that of the female. The bursa extends to the tail tip and the

gubernaculum is large and protrusible and has titillae and a capitulum.

Hoplolaimus smokyensis n. sp. keys to couplet 8 in Handoo & Golden (1992) but does not fit either species. It resembles *H. galeatus* in sometimes having five labial annules but has 20-24 longitudinal incisures on the basal lip annule vs 32-36 in *H. galeatus*. The number of longitudinal incisures is similar to that of *H. stephanus* but the latter has only four labial annules rather than five or six. *Hoplolaimus smokyensis* n. sp. differs from other species with four lateral incisures as follows: body length longer than that of *H. aorolaimoides* Siddiqi, 1972 ($L = 1.25$ (1.1-1.4) vs 0.85 (0.8-0.92) mm); phasmids located as one anterior and one posterior to the vulva, differing from those in *H. californicus* Sher, 1963 or *H. igualaensis* Cid Del Prado Vera, 1994, (both posterior to vulva); 5-6 labial annules vs 4 in *H. clarissimus* Fortuner, 1974, 3 or 4 in *H. tylenchiformis* von Daday, 1905 or 3 in *H. sacchari* (Shamsi, 1979) Luc, 1981; juvenile tail not pointed as in *H. concaudajuvenecus* Golden & Minton, 1970; and stylet shorter than in *H. magnistylus* (47 (41-51) vs 55.7 (52-61) μm).

MOLECULAR PROFILES AND PHYLOGENY

Molecular sequences obtained in this study are deposited in the GenBank database with accession num-

Table 2. Origin and GenBank accession numbers of *Hoplolaimus* species ITS1 gene sequences used in this study.

Species	Origin	Host	Accession number
<i>H. columbus</i>	Pierce County, GA	Soybean, <i>Glycine max</i>	KP835333
<i>H. columbus</i>	Barnwell County, SC	Sorghum, <i>Sorghum bicolor</i>	KP835315
<i>H. concaudajuvenecus</i>	Dallas County, TX	Bentgrass, <i>Agrostis</i> sp.	KP303685
<i>H. concaudajuvenecus</i>	Dallas County, TX	Bentgrass, <i>Agrostis</i> sp.	KP303686
<i>H. galeatus</i>	Baldwin County, AL	Bermuda, <i>Cynodon dactylon</i>	KP303596
<i>H. galeatus</i>	St Johns, FL	St Augustine, <i>Stenotaphrum secundatum</i>	KP303607
<i>H. magnistylus</i>	Weakley County, TN	Corn, <i>Zea mays</i>	KP303681
<i>H. magnistylus</i>	Massac County, IL	Soybean, <i>G. max</i>	KP303634
<i>H. seinhorsti</i>	Alachua County, FL	Peanut, <i>Arachis hypogaea</i>	EU515327
<i>H. seinhorsti</i>	Fujian, China	Turfgrass	KF486504
<i>Hoplolaimus</i> sp. 1	Smoky Mountains, TN	—	EU515329
<i>Hoplolaimus</i> sp. 2	University of Illinois, IL	—	EU515330
<i>Hoplolaimus</i> sp. 2	Manhattan, KS	Corn, <i>Zea mays</i>	EU515331
<i>Hoplolaimus</i> sp. 3	Clemson, SC	Birch tree, <i>Betula</i> sp.	EU515332
<i>Hoplolaimus</i> sp. 3	Limestone County, IA	Cotton, <i>Gossypium</i> sp.	EU515333
<i>H. stephanus</i>	Sargent County, ND	Soybean, <i>G. max</i>	KX347888
<i>H. stephanus</i>	Riley County, KS	Bentgrass, <i>Agrostis</i> sp.	KP303646
<i>H. stephanus</i>	Warren County, OH	Bentgrass, <i>Agrostis</i> sp.	KP303664
<i>H. smokyensis</i> n. sp.	Sevier County, TN	Maple, <i>Acer</i> sp.	KP303683
<i>H. smokyensis</i> n. sp.	Sevier County, TN	Maple, <i>Acer</i> sp.	KP303684

Table 3. Origin and GenBank accession numbers of *Hoplolaimus* species *COI* gene sequences used in this study.

Species	Origin	Host	Accession number
<i>H. columbus</i>	Barnwell County, SC	Sorghum, <i>Sorghum bicolor</i>	KP864583
<i>H. columbus</i>	Pierce County, GA	Soybean, <i>Glycine max</i>	KP864611
<i>H. concaudajuvenus</i>	Dallas County, TX	Bentgrass, <i>Agrostis</i> sp.	KP230667
<i>H. concaudajuvenus</i>	Dallas County, TX	Bentgrass, <i>Agrostis</i> sp.	KP230668
<i>H. galeatus</i>	St Johns, FL	St Augustine, <i>Stenotaphrum secundatum</i>	KP230564
<i>H. galeatus</i>	Baldwin County, SC	Bermuda, <i>Cynodon dactylon</i>	KP230554
<i>H. magnistylus</i>	Massac County, IL	Soybean, <i>G. max</i>	KP230588
<i>H. magnistylus</i>	Weakley County, TN	Corn, <i>Zea mays</i>	KP230657
<i>H. stephanus</i>	Riley County, KS	Bentgrass, <i>Agrostis</i> sp.	KP230593
<i>H. stephanus</i>	Warren County, OH	Bentgrass, <i>Agrostis</i> sp.	KP230626
<i>H. smokyensis</i> n. sp.	Sevier County, TN	Maple, <i>Acer</i> sp.	KP230658
<i>H. smokyensis</i> n. sp.	Sevier County, TN	Maple, <i>Acer</i> sp.	KP230659

[illegible]

Fig. 5. Alignment of ITS1 sequences for *Hoplolaimus smokyensis* n. sp. and the four closest *Hoplolaimus* species. Positions without an asterisk indicate polymorphisms, or differences between *H. smokyensis* n. sp. and at least one of the other four species.

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H_smokyensis_n.sp. GGAAAAAAAAAATTGTTGGTCATTTAGGGATAATTTATGCTATGCTTTCTATTGGGTTT
H_galeatus          GGAAAAAAAAAATTATTTGGACATTTGGGAATAATTTATGCGATAATTGCTATTGGTTTT
H_stephanus         GGAAAAAAGAAATTGTTGGACATTTGGGGATAATTTATGCTATAATTTCTATTGGGTTT
H_magnistylus       GGAAAAAAAAAATTGTTGGTCATTTAGGAATGATTTATGCTATAATTGCTATTGGTTTT
H_concaudajuvenus   GGAAAAAAAAAATTATTTGGTCATTTAGGTTGGTTTATGCTATGTTAGCTATTGGTTTT
***** ***** ***** ***** ** * ***** ** * ***** ***

H_smokyensis_n.sp. ATTGGTTGTTTAGTTTGGGCTCATCATATATTTGTAGTTGGAATAGATTAGATAGTCGA
H_galeatus          ATTGGGTGTTTGGTTTGGGCCATCATATGTTTGTGGTTGGGATGGATTGGATAGCCGG
H_stephanus         ATTGGTTGTTTGGTTTGGGCTCATCATATGTTTGTGGGAATAGATTTGGATAGCCGG
H_magnistylus       ATTGGTTGTTTAGTTTGGGCTCATCATATGTTTGTGGTAGGAATAGACTGGATAGACGT
H_concaudajuvenus   ATTGGTTGTTTGGTTTGGGCTCATCATATATTTGTTGTTGGAATGGATTAGATAGACGT
***** ***** ***** ** ***** ***** ** ** ** ** ** ** ***** **

H_smokyensis_n.sp. GCTTATTTTAGTGCTGCTACAATGATTATCGCAGTACCTACTGGTATTAAGGTTTTTCT
H_galeatus          GCTTATTTTAGGGCGGCTACTATAATTATTGCGGTTCCGACTGGGATTAAGGTTTTTCTC
H_stephanus         GCTTATTTTAGTGAGCTTCTATAATTATTGCTGTTCCAACCTGGTATTAAGGTTTTTCTC
H_magnistylus       GCTTATTTTAGGGCAGCAACGATGATTATTCAGTTCCCTACTGGTATTAAGGTTTTTCTC
H_concaudajuvenus   GCTTATTTTAGTGCTGCTACTATAATTATTGCTGTTCCCTACTGGTATTAAGGTTTTTCTC
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H_smokyensis_n.sp. TGATTAATAACATTACATGCTTCTGTTTTATTTAATAGTTATTTATATGATTGGGTAATA
H_galeatus          TGGTTAATAACTTTGTTTCTTCGATTTTTTTTAAACAGTTACCTTTTGATTGGGTGATA
H_stephanus         TGGTTAATAACTTTATATTCTTCGGTTTTGTTTGATAGATTTTATTTGAATGAGTAATA
H_magnistylus       TGAATAATAACTTTATATGCTTCAGTTTTATTTAATAGTTATTTATATTATTTGGGTTTTG
H_concaudajuvenus   TGATTGATGACTTTATATTCTTCAATTTTATTTACTAGTTATTTATTTGAATGGGTAATA
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H_smokyensis_n.sp. GGTTTTATTTATTTATTTACTATAGGGGGTTTAAGAGGTTTAGTTTTAAGTAATGCTAGT
H_galeatus          GGTTTTATTTATTTGTTTACAATTGGGGGTTTAAAGTGGTTTAAATTTGAGGAACGCAAGT
H_stephanus         GGTTTTGTTTATTTATTTACTTTAGGTGGTTTGACTGGTTTGGTTTGGAGTAATGCTAGT
H_magnistylus       GGATTTATTTATTTGTTTACTTTGGGAGGTTTAAAGTGGATTAATTTAAGTAATGCTAGT
H_concaudajuvenus   GGTTTTATTTATTTGTTTACTTTTGGTGGTTTAAAGAGGTTTAGTTTTGAGAAATGCAAGT
** *** ***** ***** * ** ***** * ** ** ***** ** ** **

H_smokyensis_n.sp. TTGGATTATTATTACATGATAC
H_galeatus          TTGGATTGTTATTACATGACAC
H_stephanus         TTAGATTATTGTTACATGATAC
H_magnistylus       TTAGATTATTACTTCATGATAC
H_concaudajuvenus   TTGGATTATTATTACATGATAC
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Fig. 6. Alignment of *COI* gene sequences for *Hoplolaimus smokyensis* n. sp. and the four closest *Hoplolaimus* species. Positions without an asterisk indicate polymorphisms, or differences between *H. smokyensis* n. sp. and at least one of the other four species.

bers KP230658, KP230659, KP303683 and KP303684. All specimen information and GenBank accession numbers of *Hoplolaimus* species used in this study are listed in Table 2 (ITS1 sequences) and Table 3 (*COI* sequences). Sequences were first aligned with BLAST in GenBank. The BLAST results suggested *H. magnistylus* as the closest species to *H. smokyensis* n. sp. with 89% identity (of both ITS1 and *COI* sequences) when query cover is 100%.

Sequences from all available lance nematode species with four lateral incisures were analysed. Alignments of ITS1 and *COI* sequences for *H. smokyensis* n. sp. and the four closest *Hoplolaimus* species are presented (Figs 5, 6). Positions without an asterisk indicate polymorphisms, or differences between *H. smokyensis* n. sp. and at least one of the other four species. Phylogenetic analyses (Figs 7, 8) based on the ribosomal and mitochondrial gene sequences

suggest *H. smokyensis* n. sp. as a distinct lineage from *H. galeatus*, *H. stephanus*, *H. magnistylus*, and *H. concaudajuvenus* (Bayesian posterior probability 100% in ITS1 tree and 100% in *COI* tree) and separate from other *Hoplolaimus* spp. such as *H. columbus*. Compared with other reported species, the phylogenetic results also suggested: i) *H. smokyensis* n. sp. is classified into a unique clade; and ii) in the ITS1 tree, *H. smokyensis* n. sp. is grouped with all other morphologically similar species in a large clade (four lateral incisures, three pharyngeal gland nuclei), and is separated from both *H. columbus* or *H. seinhorsti*, which have six pharyngeal gland nuclei and only a single lateral incisure.

Fig. 7. Molecular phylogeny of *Hoplolaimus* species based on unique ITS1 DNA sequences. *Heterodera glycines* ITS1 sequence was included as an outgroup. Bayesian Inference tree obtained with MrBayes. Model: GTR + I + G. MCMC = 1×10^7 generations.

Discussion

Hoplolaimus species are found feeding on the roots of a diversity of monocotyledonous and dicotyledonous plants. The genus is widely distributed in the USA (Wrather *et al.*, 1992; Martin *et al.*, 1994; Gazaway & McLean, 2003), and there are records from Canada, South America, Central America, and India on a variety of hosts (Fortuner, 1991). Based on differences in morphological characters, Siddiqi (2000) suggested dividing *Hoplolaimus* spp. into three subgenera according to lateral field incisures and the number of pharyngeal gland cell nuclei. With four incisures and three nuclei in the pharyngeal gland lobe, *H. smokyensis* n. sp. belongs to the subgenus *Hoplolaimus*.

To date, seven *Hoplolaimus* spp. have been reported from the south-eastern USA, including *H. columbus* Sher, 1963, *H. concaudajuvenus*, *H. galeatus*, *H. magnistylus*,

H. stephanus, *H. seinhorsti* Luc, 1958, and *H. tylenchiformis*. Of these, *H. columbus*, *H. galeatus*, and *H. magnistylus* have been shown to be economically important and can cause serious damage to agronomic crops including cotton (*Gossypium hirsutum*), corn (*Zea mays*) and soybean (*Glycine max*) (Fassuliotis, 1974; Nyczepir & Lewis, 1979; Robbins *et al.*, 1987, 1989; Henn & Dunn, 1989; Noe, 1993).

In this study we have described *H. smokyensis* n. sp. from a mixed forest sample of maple, hemlock, and silverbell tree in Great Smoky Mountains National Park. Pathogenicity of *H. smokyensis* n. sp. has not been reported from any agricultural field yet, although its similarity to *H. galeatus* may have caused it to be overlooked in agricultural nematode surveys. Several lance nematode species, *H. stephanus*, *H. magnistylus* and *H. concaudajuvenus*, which have been reported to infect trees, are known to also feed on crops such as corn, cotton, soybean,

Fig. 8. Molecular phylogeny of *Hoplolaimus* species based on unique sequences of *COI* gene DNA. *Heterodera glycines* *COI* sequence was included as an outgroup. Bayesian Inference tree obtained with MrBayes. Model: GTR + I + G. MCMC = 1×10^7 generations.

and turfgrass (Ma *et al.*, 2011, Holguin *et al.*, 2015b). *Hoplolaimus galeatus* is also a prevalent pathogen of turfgrasses such as St Augustinegrass (*Stenotaphrum secundatum*) and bermudagrass (*Cynodon dactylon*) in Florida (Henn & Dunn, 1989). These species are in subgenus *Hoplolaimus* with similar morphological characteristics to *H. smokyensis* n. sp. Identification and description of this new species will contribute to studies of comparative biology and evolutionary biology of lance nematodes.

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- ## Acknowledgement
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