Stauratostoma shelleyi n. gen., n. sp. (Nematoda: Rhabditida: Thelastomatidae) from Appalachian Polydesmid Millipedes (Polydesmida: Xystodesmidae)

Abstract

Stauratostoma shelleyi n. gen., n. sp. is described from the midgut and hindgut of nine species of the millipede family Xystodesmidae collected in the southern Appalachian regions of North Carolina, Tennessee and Alabama. Specimens of S. shelleyi were morphologically examined with differential interference contrast, phase contrast, and scanning electron microscopy. The head of S. shelleyi differs from other thelastomatid nematodes in having a head region mushroom-shaped in profile; cruciform stomatal opening formed from four flaps; greatly expanded labial disc; and eight-sectored annule-like column supporting the labial disc. Thirteen nematodes from various hosts were sequenced for 28S LSU rDNA and compared with other millipede-inhabiting nematodes. Stauratostoma shelleyi is the sister group to the few Thelastoma spp. that have been molecularly characterized using the D2–D3 expansion segments of the 28S LSU rDNA.

Key words
Alabama, Appalachia, Host-parasitic relationship, New genus, North Carolina, Scanning electron microscopy, Taxonomy, Tennessee, Thelastoma, Stauratostoma, 28S rDNA.

The nematode fauna living within the gastrointestinal tract of North American millipedes is not well documented (Carreno, 2007). North American millipedes are understudied hosts of oxyuridomorph and rhigonematomorph nematodes, and records of their distribution in North America are scant. Most studies of nematodes that parasitize diplopods have originated from tropical areas (Carreno et al., 2013). Joseph Leidy, the father of American parasitology, was one of the first researchers to document the existence of nematodes living inside the intestine of millipedes in temperate North America (Leidy, 1849, 1850, 1851, 1853). The first known thelastomatid nematode recorded by Leidy was Thelastomum (=Thelastoma) attenuatum (Leidy 1849), from the millipede host Julus marginatus (=Narceus americanus, Beauvois, 1817) (Leidy, 1853). Leidy also described Thelastomum (=Thelastoma) labiatum (Leidy 1850, 1853); however, he provided a very superficial anatomical description with only one partial sketch of the T. labiatum type specimen. Based on the lack of sound descriptions of some older specimens of Thelastoma spp., and a lack of specimens for comparisons, the taxonomic understanding of this genus has been challenging (Carreno, 2007). Re-descriptions of older type materials, when available, and a comprehensive survey of nematode fauna of North American millipedes are needed to understand phylogenetic relationships between Thelastoma and other genera of nematodes inhabiting the intestinal tracts of millipedes (Carreno, 2007). Nematodes parasitizing diplopods are taxonomically placed in the infraorders Oxyuridomorpha and Rhigonematormorpha. Their phylogenetic placement has been well established among Clade III nematodes but their sister group has not been entirely resolved, and it has been established that these two infraorders do not form a
monophyletic group (Blaxter et al., 1998; Nadler et al., 2007).

In the course of a recent survey of Appalachian millipedes in Tennessee, North Carolina, and Alabama, as well as other areas from the southeastern and western States, many species of Rhigonematomorpha and Oxyuridomorpha were collected from millipedes. Oxyuridomorphan nematodes are commensal parasites in the intestine of millipedes, scorpions, and several orders of insects, while rhigonematomoran nematodes are found only in millipedes. Among the thelastomatid and rhigonematid nematode taxa collected in the southern Appalachian region of North America was an unusual species that could not be placed in any described genus. A new genus and species of nematode, *Stauratostoma shelleyi* n. gen., n. sp., is described herein.

**Materials and methods**

Between February 2013 and July 2017, a total of 352 xystodesmid millipedes were collected in several locations in the southeastern United States, including several Tennessee and Alabama State Parks. Millipedes were transported back to the lab where they were maintained in enclosures with their natural substrate and fed pieces of cucumber fruit. Prior to dissection, morphometric data were recorded for each millipede, including weight, length, width, and sex. Millipedes with movement activity typical of their species were considered healthy.

Millipedes were dissected by severing the head and epiproct with a razor blade as described by Phillips et al. (2016). The intestine was pulled intact from the body cavity with fine-tip forceps and placed in a Syracuse watch glass containing distilled water. The intestine was sectioned into three parts: foregut, midgut, and hindgut (Crawford et al., 1983). The intestine was sketched and then dissected with the aid of Zeiss Stermi 2000 or Olympus SZ51 stereomicroscopes. Nematodes were removed from intestinal tissue and sorted. Males, females, and juveniles were segregated, counted, and grouped to family or genus-level taxa according to general features. Each dissected millipede was preserved as a voucher specimen in 70% or 95% ethanol.

Specimens were prepared for light microscopy, scanning electron microscopy (SEM) or molecular analysis. Most specimens were killed and fixed in hot (60–70°C) 4% formalin, then later processed to anhydrous glycerin (Seinhorst, 1959) for permanent mounts on glass slides or long-term storage in vials. Slide-mounted specimens were examined with an Olympus BX-63 DIC microscope system and imaged with a 14-megapixel Q-camera. Measurements in Table 1 were made from glycerin-preserved specimens.

To better visualize the lateral field, head region, and phasmid features, several females were fixed in 95% ethanol for 2 days and mounted directly on slides into Hoyer’s medium. Slides were placed in a 50°C oven for 3 days to expand the nematodes and harden the mounting medium, then ringed with red insulating varnish. Features were imaged with a 17-megapixel DP73 camera on an Olympus BX-53 phase-contrast microscope.

For SEM, methods used by Phillips et al. (2016) were followed. Formalin-fixed nematodes were washed in distilled water for 20 min then placed into a 12-mm×30-µm microporous specimen capsule (Electron Microscopy Services, Hatfield, PA). Each capsule was placed in a 5-ml glass well and dehydrated with a graded ethanol series consisting of 25%, 50%, 75%, 95%, and 100% ethanol, each for 20 min. Following the 100% ethanol dehydration step, a 1:1 mixture of 100% ethanol and reagent grade hexamethyldisilazane (HMDS) was used in place of a critical point dryer. The HMDS series consisted of 75%, 100%, and a second 100% dehydration, each for 20 min. Nematodes were placed on carbon tape affixed to a 45°/90° aluminum stub and sputter-coated with gold for 10 sec at 20 µA in a SPI-Module Sputter Coater (West Chester, PA). Specimens were viewed with a Hitachi TM 3030 electron microscope at a voltage of 15 kV.

Total genomic DNA was extracted from representative single specimens with the Qiagen DNeasy Blood and Tissue Kit #69506 (Waltham, MA) differing from the manufacturer’s instructions only in reduction of the final elution volume to 70 µl (2 × 35 µl) from 400 µl (2 × 200 µl). The resulting gDNA was stored at −20°C. Polymerase chain reaction (PCR) was carried out with TaKaRa Ex Taq Hotstart DNA polymerase (Takara Bio, Shiga, Japan) per the manufacturer’s suggested protocol, plus 2 µL of DNA template and 3 µl of 20 µM working stocks of 28S LSU rDNA primers. Several primer pairs were employed, with the greatest success from LSU391F: 5'-AGCGGAGGAAAAAGAAACTA-3' and LSU501R: 5'-TCCAGGACCCAGCTACTA-3' (Nadler et al., 2006). Two other custom internal primer pairs were also used with limited success: 28S 40F: 5'-GGAARCAGATAGATTGAC-3' and 28S 41R: 5'-CTACTAGATGGTTCGATTAGTC-3' and 28S 40NF: 5'-GAGTTCAGAGGGCGTGAAC-3' and 28S 41NR: 5'-CCTCTAATCATTGCCTTTACC-3'. Cycling was done using a GenePro (Bioer Technology Co., Hangzhou,
China) thermal cycler using the following PCR regime: initial 90 sec denaturing step at 94°C, then four cycles of 30 sec at 94°C, 30 sec at 56°C and 75 sec at 72°C, followed by four cycles of 30 sec at 94°C, 25 sec at 52°C, and 75 sec at 72°C, nine cycles of 30 sec at 94°C, 20 sec at 48°C and 75 sec at 72°C and finally, 38 cycles of 30 sec at 94°C, 20 sec at 45°C and 75 sec at 72°C.

PCR products were electrophoresed in 1% agarose gels at 110 V for 30 min. Bands were excised from the gel, cleaned using QiaQuick Gel Extraction Kits and eluted in 37 μL of elution buffer. Cleaned PCR products were used as templates for cycle sequencing (Sanger) reactions using 1.5 μL of PCR primers at a working concentration of 5 μM. Sequencing was performed in both directions using BigDye v3.1 terminators (Applied Biosystems, Carlsbad, CA) in a 1/20th reaction using 0.4 μL Big-Dye and 3 to 5 μL of homemade 5X sequencing buffer cocktail in a 20 μL reaction volume. Centrisep columns (Princeton Separations, Adelphia, NJ) were used to clean the sequencing reactions, which were then dried in a Centrivap Concentrator (LABCONCO, Kansas City, MO). Dried samples were sent to the University of Tennessee Genomics Core for sequencing. Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI) was used to reconcile and verify opposing strands for accuracy. The 28S rDNA sequences of 11 females and two juveniles (n = 13) of S. shelleyi were obtained, with resulting sequence lengths of 821 to 1,038 base pairs due to different forward and reverse primers being used to amplify them. The sequence fragments were invariant and therefore were converted into a single consensus sequence submitted to the National Center for Biotechnology Information (NCBI).

<table>
<thead>
<tr>
<th>Measurements (μm)</th>
<th>Holotype female</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>CV</th>
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<td>2,357</td>
<td>249</td>
<td>1,805–2,683</td>
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<td>10.3</td>
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<td>8.1–12.3</td>
<td>8.3</td>
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<tr>
<td>Second annule width</td>
<td>8.4</td>
<td>7.8</td>
<td>1.2</td>
<td>5.3–9.9</td>
<td>14.9</td>
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<td>486</td>
<td>50.0</td>
<td>347–548</td>
<td>10.3</td>
</tr>
<tr>
<td>Basal bulb length</td>
<td>116</td>
<td>111</td>
<td>8.6</td>
<td>84–126</td>
<td>7.8</td>
</tr>
<tr>
<td>Head to excretory pore</td>
<td>404</td>
<td>389</td>
<td>43.9</td>
<td>274–445</td>
<td>11.3</td>
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<tr>
<td>Vulva to anterior head</td>
<td>991</td>
<td>935</td>
<td>105.3</td>
<td>676–1066</td>
<td>11.3</td>
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<tr>
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<td>1,517</td>
<td>174.1</td>
<td>1,105–1,715</td>
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<td>74.2</td>
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<td>67.2–79</td>
<td>4.5</td>
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<tr>
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<td>49.1</td>
<td>2.7</td>
<td>43.9–55.4</td>
<td>5.4</td>
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<td>90.8</td>
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</tr>
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<td>13.6</td>
<td>2.2</td>
<td>10.1–18.4</td>
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<tr>
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<td>0.43</td>
<td>4.3–5.9</td>
<td>8.9</td>
</tr>
<tr>
<td>c</td>
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<td>2.8</td>
<td>0.2</td>
<td>2.4–3.3</td>
<td>5.5</td>
</tr>
<tr>
<td>V</td>
<td>39.3</td>
<td>39.7</td>
<td>2.4</td>
<td>33.2–44.4</td>
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<tr>
<td>V'</td>
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<td>0.62</td>
<td>0.03</td>
<td>0.53–0.69</td>
<td>5.1</td>
</tr>
</tbody>
</table>

CV = Coefficient of Variation.

Table 1. Morphometrics of female *Stauratostoma shelleyi* n. gen., n. sp. from *Apheloria montana*.
In order to ascertain the phylogenetic position of S. shelleyi, phylogenetic studies were conducted. The consensus sequence of S. shelleyi was aligned with orthologous sequences of 36 additional nematode taxa either obtained during the course of this research or oxyuridomorph and rhigonematomorph sequences obtained from the National Center for Biotechnology Information (NCBI). Alignment was completed using Opal (Wheeler and Kecicioglu, 2007) via the Opalescent package within Mesquite 3.03 (Maddison and Maddison, 2011). Reconstruction of relationships was carried out using maximum parsimony methods as implemented in PAUP* (Swofford, 2002) and Bayesian inference methods implemented within MrBayes v3.2.2 in the Cyber-Infrastructure for Phylogenetic Research (CIPRES) portal v. 3.3 (Miller et al., 2010). The distal outgroup consisted of a single representative of the nematomorphan Gordionus spp. Proximal outgroups included the root knot nematode Meloidogyne incognita as well as two free-living bacteria feeding species, Distolabrellus veechi and Plectus murrayi. Node support was gauged by nonparametric bootstrap resampling (10,000 reps of a single random addition sequence) as well as Bayesian posterior probabilities.

Parsimony analysis was comprised of a heuristic search employing 1,000 random addition sequence replicates using tree bisection and reconnection (TBR) branch rearrangement. Of 1,279 aligned characters, 610 were parsimony informative. A single most parsimonious tree of 3,179 steps (CI: 0.506; RI: 0.625; HI: 0.494) was recovered in 900 of the 1,000 replicates conducted. Prior to Bayesian analysis, jModeltest v. 2.1.10 (Darriba et al., 2012) was used to determine the most appropriate evolutionary model. The best-fit model chosen was GTR + I + G: (-lnL: 16,894.3004). A Bayesian phylogeny was estimated using Markov Chain Monte Carlo methods implemented within MrBayes 3.2.2 (Ronquist et al., 2012) through the online CIPRES Science Gateway (Miller et al., 2010). No partitions within 28S LSU rDNA were recognized. Nucleotide substitution matrix, rate variation, gamma shape parameter, and base frequencies were estimated (nst = 6; rate = invgamma; unlink statefreq = (all); revmat = (all); tratio = (all); shape = (all); pinvar = (all); prset applyto = (all) ratepr = variable). Two runs with six chains each were run for a total of 10 million generations. Markov chains were sampled at intervals of 500 generations and the first 35% of trees discarded as burn-in prior to assembling a 50% majority rule consensus tree. Verification that stationarity had been reached was measured by the standard deviation of split frequencies being less than 0.1, the Potential Scale Reduction Factor approaching 1.0, and the MrBayes output overlay plot depicting no directional trend for either run. Resulting phylogenetic trees were modified using Canvas 8.0.5 (Deneba Systems) to produce publication-grade figures.

Millipede and nematode morphometric data were analyzed with mixed model analysis for a nested design, with nematode length as the response variable and millipede species as the categorical independent variable, and millipede length, width, and weight as numeric independent variables, respectively. Rank data transformation was applied when the diagnostics analysis showed non-normality and unequal variance on residuals. Significant effects were identified at p < 0.05. Data analysis was conducted in SAS9.4 TS1M3 (SAS Institute Inc., Cary, NC).

Results

During this research, 972 millipedes spanning six orders, 16 families, and 53 species were collected from 20 middle Atlantic, southeastern, southwestern, and western states. A total of 66,685 nematodes were extracted and separated into morphotaxa. Of the 972 millipedes dissected, 352 (36.2%) were millipedes from the order Polydesmida. Within the order Polydesmida, only one family (Xystodesmidae) contained S. shelleyi. Stauratostoma shelleyi was found in a total of 12 counties throughout Tennessee, North Carolina, and Alabama.

Specimens in 15 genera of xystodesmid millipedes (Table 2) were dissected to determine the presence or absence of intestinal nematodes. Total nematode loads ranged from 0–418 nematodes/specimen, primarily represented by genera in Thelastomatidae, Rhigonematidae, Aoruridae, and Coronostomatidae. Among these 15 genera, nine (60%) contained specimens of S. shelleyi. Stauratostoma shelleyi was found in 27 of 58 (46.6%) specimens, with 0 to 32 individuals per millipede. Males attributable to this species were not found and juveniles were rarely encountered. This new species was most often located in the hindgut of the millipede host, although there were a few occasions when we observed them in the pyloric region of the midgut; no nematodes were found in the foregut.

Systematics

Thelastomatidae (Travassos, 1929)
Stauratostoma Phillips and Bernard, new genus

Description
Obligatory commensal parasitic inhabitants of the hindgut and midgut of some xystodesmid millipedes.

**Etymology:** *Stauratostoma n. gen.* is developed from the Latin word *staurato-*,-cross-shaped or cruciate, combined with –*stoma* (mouth), reflecting the unique appearance of the anterior end.

**Females:** Body strongly annulated. Tail long, attenuated to fine tip. Anterior end mushroom-shaped in profile. Labial disc greatly expanded, wider than columnar first apparent annule (circumoral annule), with cruciform stomatal opening formed from four smooth, rounded, lip-like flaps; labial papillae obscure. First apparent annule cylindrical, divided into eight sectors by longitudinal grooves (Figs. 1A,B; 2A–D; 3A,B); dorsal and ventral grooves extending entire width of first apparent annule, other six grooves sublateral, not reaching second cephalic annule. Second cephalic annule wider than oral disc (Figs. 1B; 2A–C). Esophagus with long procorpus and distinct basal bulb with grinding valves (Fig. 1A). Secretory-excretory gland massive, transversely oval pore with apparent sub-surface operculum (Figs. 1A,C; 3B,C). Anterior end of intestine swollen (Fig. 1A). Reproductive system amphidelphic, vulva near midbody; both gonads doubly reflexed, spermatheca present in posterior gonad, sperm oval, absent from anterior gonad; both uteri on right side of body; vagina strongly muscled, directed anteriorly (Fig. 1D–G). Phasmid apertures pore-like, on tail (Fig. 1F). Juveniles similar to adult females except in development of reproductive system. Males not encountered.

**Type species:** *Stauratostoma shelleyi* Phillips and Bernard, new species

**Description**

**Female** (*n* = 31): Measurements are listed in Table 1.

**Type locality and habitat:** Holotype female, 12 paratype females, Tennessee, Anderson County, Powell, Powder House Road, 36.054521, −84.11283889, elevation 324 m, rocky, mixed hardwood, and pine ecosystem, collected from the hind and midgut of *Apheloria montana* (Bollman, 1887), 31 March 2013 and 18 additional females collected from *A. montana* at same locality throughout the year except in January (Table 1; Table 2. Genera of xystodesmid millipedes dissected.

<table>
<thead>
<tr>
<th>Species</th>
<th>Millipedes dissected (n)</th>
<th>Millipedes with</th>
<th>Total S. shelleyi</th>
<th>Mean</th>
<th>Range</th>
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<tr>
<td>Aphielia montana</td>
<td>77</td>
<td>59</td>
<td>269</td>
<td>3.5</td>
<td>0–19</td>
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<tr>
<td>Aphielia pigina</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Aphielia virginiensis</td>
<td>56</td>
<td>35</td>
<td>276</td>
<td>4.9</td>
<td>0–31</td>
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<td>0</td>
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<td>4</td>
<td>41</td>
<td>6.8</td>
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<td>2</td>
<td>45</td>
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<tr>
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<td>5</td>
<td>41</td>
<td>0.9</td>
<td>0–23</td>
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<td>Harpaphe haydeniana</td>
<td>29</td>
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<td>10</td>
<td>2</td>
<td>2</td>
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<td>0–1</td>
</tr>
<tr>
<td>Total</td>
<td>352</td>
<td>110</td>
<td>700</td>
<td>2.8</td>
<td>0–32</td>
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</table>
Stauratostoma shelleyi n. gen., n. sp. (Nematoda: Rhabditida: Thelastomatidae) from Appalachian Polydesmid Millipedes

Figs. 1–5). Numerous females collected from the gastrointestinal tract of nine species of xystodesmid millipedes from the following counties: in Tennessee, Anderson, Blount, Campbell, Hamblen, Knox, Loudon, White, Wilson, Union; Haywood and Swain Counties, North Carolina; and Shelby County, Alabama (Fig. 6). The collection localities are located in the southern Appalachian Mountains from North Carolina and Tennessee to Alabama, and in the adjacent Ridge and Valley Province and Cumberland Plateau in Tennessee. This nematode was not found in any xystodesmid millipedes collected elsewhere.

Figure 1: Stauratostoma shelleyi n. gen., n. sp. A, Habitus, lateral view. B, Anterior end, lateral view. C, Secretory-excretory system region, ventral view. D, Reproductive system of non-gravid female, right-side lateral view; intestinal detail provided only for anterior and posterior parts. E, Developing reproductive system of presumed fourth-stage juvenile. F, Posterior region of reproductive system of older gravid female, left-side lateral view; only the posterior end of the digestive system is illustrated. G, Posteriormost portion of posterior gonad of younger female with eggs in single file. Scales: A = 200 μm; B, E, F = 50 μm; C, D, G = 100 μm.
**Type designation and deposition:** Holotype female (T-707t) and 6 paratype females (T-6927p and T-6928p) deposited in the USDA Nematode Collection, Beltsville, MD. Eighteen additional paratype females and many additional *S. shelleyi* specimens collected during this study are deposited in the Entomology & Plant Pathology Department, University of Tennessee, Knoxville, Tennessee.

**Etymology:** It is our pleasure to name *S. shelleyi* n. gen., n. sp. after the renowned millipede expert Dr. Rowland M. Shelley, Adjunct Professor, University of Tennessee, Knoxville, Tennessee.

**Description of females:** Body cylindrical, white, robust, widening from head to vulva, slightly tapering to anus (Fig. 1A) then attenuating at tail. Cuticle strongly annulated. Body without prominent lateral alae; lateral field originating in the esophageal region as a single incisure, then expanding as a flat field beginning at the basal bulb, interrupting the annules but without incised boundaries, running the length of the body past the anus (Fig. 3E,F); tail long, filiform, not annulated (Fig. 1A). Annules prominent, cuticle thicker

**Figure 2:** *Stauratostoma shelleyi* n. gen., n. sp., scanning electron microscope. A, Anterior region, sublateral view. B, Enlargement of 2A, showing lateral labial pit or depression (LP), complete dorsal or ventral groove (CG) and sublateral and lateral incomplete grooves. C, Dorsal or ventral view showing complete groove and reticulated surface between lobes and labial disc. D, Labial disc with amphid aperture (AM) and associated spine (SP). Scales: A = 20 μm; B–D = 10 μm.

**Figure 3:** *Stauratostoma shelleyi* n. gen., n. sp. A, Head end, lateral view; inset shows accessory spine near amphid aperture. B, Scanning electron microscope of anterior region showing large S–E pore. C, S–E pore with operculum. D, Typical egg, with slightly roughened outer layer. E, Lateral field, esophageal region. F, Lateral field, posterior region. Figures 3E, F are phase-contrast images. Scales: A, D = 20 μm; B, F = 50 μm; C = 10 μm; E = 100 μm.
Stauratostoma shelleyi n. gen., n. sp. (Nematoda: Rhabditida: Thelastomatidae) from Appalachian Polydesmid Millipedes

Amphid apertures inconspicuous, small, linear, each with a minute associated guard spine (Fig. 2A–D). Stoma wide; cheilostom thin, sinuous in profile; gymnostom appearing as a thick, curved rod wider at base than at apex; telostom thick, angled, bearing three pairs of pointed teeth, larger teeth appearing less sclerotized than smaller teeth (Fig. 1B). Esophagus with long cylindrical corpus, short isthmus, and pyriform basal bulb containing a grinding valve; terminus of corpus with distinct esophago-intestinal valve. Anterior region of esophagus surrounded by six glands (dorsal, ventral, four sublateral), each with prominent granules and 1 or 2 nuclei (Fig. 1B). Anterior end of intestine swollen (Fig. 1A).

Nerve ring encircling esophagus at middle of procorpus. Secretory-excretory system consisting of massive ampulla and large oval pore located level with anterior end of basal bulb at about the 34th annule (Figs. 1C; 3B,C), canals not seen. Reproductive system amphidelphic, vulva located just anterior to midbody, transverse, anterior lip flap-like; vagina strongly muscular, directed anteriorly; both uteri on right side of body, gonads doubly reflexed in mature females, stretching from anterior intestinal region nearly to anus; anterior gonad without spermatheca, posterior gonad with an axial spermatheca containing small, broadly oval sperm (Fig. 1A,D-G). Egg (Fig. 3D) broadly oval, shell thin, minutely roughened. Newly formed eggs with cytoplasm distributed uniformly inside egg, older eggs with cytoplasm contracted (Fig. 1F,G). Four ventral coelomocytes present, most anterior coelomocyte associated with anterior flexures of gonads, other three coelomocytes in region of anterior uterus (Fig. 1A,D–F). Intestine composed of single layer of polygonal cells (Fig. 1D; rectum about two body annules long, straight, not inflated (Fig. 1F). Anus without prominent flap. Phasmid aperture a minute pore about 80 μm posterior to anus (Fig. 1F).

Males not known.

Juveniles similar to females except for development of reproductive system (Fig. 1E); four coelomocytes distributed as in female: one at anteriormost part of gonads, the other three closer to developing vagina.

Differential diagnosis: The morphology of the head region, with expanded labial disc with four flaps, cruciate stomatal opening and supporting eight-sectored annule-like column, appears to be unique among oxyuridomorph parasites of arthropods, setting this new genus and species apart from the other members of the infraorder. There are now six recognized thelastomatid species known from...
North American diplopods: S. shelleyi, T. attenuatum (Leidy, 1849), Thelastoma collare (Upton et al., 1983), Thelastoma krausi (Carreno, 2007), Thelastoma labiatum (Leidy, 1850), and Thelastoma spicatum (Cobb, 1929). Differentiation of these species in previous papers was based on morphological characters such as the position of the excretory pore and length of the tail (Leibersperger, 1960; Jarry and
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Due to the lack of type material from older specimens such as T. labiatum, coupled with scant drawings and descriptions, additional characters need to be re-evaluated by examination of new specimens.

Phylogenetic analysis of 28s rDNA was conducted using two different methods: maximum parsimony and Bayesian Inference. Both analyses yielded largely congruent trees, differing chiefly in the relationships among the basal-most clades, which were weakly supported. The Bayesian inference is presented as Fig. 5. Both trees recovered S. shelleyi as the sister group to Thelastoma spp.

The length of each specimen of S. shelleyi was measured and compared with the length, width, and weight of the most frequently encountered millipede hosts: Apheloria montana (Bollman, 1887) and A. virginiensis (Drury, 1770) (for taxonomy see Shelley et al., 2017) (Fig. 7).

Figure 6: Stauratostoma shelleyi n. gen., n. sp., known distribution by county in southeastern United States.

Figure 7: Correlation of nematode length and millipede weight for two Apheloria spp., 95% confidence limits shown.
Other host millipede species were excluded due to insufficient specimen numbers for analysis.

Discussion

Stauratostoma n. gen. is distinctive in the unique shape of the anterior end, in which the labial disc is greatly expanded. This disc surmounts an apparent columnar first annule, which appears to be longitudinally divided into eight sectors. Those eight sectors may correspond to what traditionally have been called labiopapillae in Thelastoma spp. This interpretation is based on the location of the amphid apertures, which are near the lateral edges of the disc. In Thelastoma spp., the amphid apertures are in the margin between the labial disc and the papillae. Adamson and van Waerebeke (1992) considered Thelastoma to have two cephalic annules, with eight labiopapillae on the first annule surrounding the labial disc. However, these so-called labiopapillae probably are prominences for the anchoring of longitudinal muscles and lack typical cephalic sensory enervation (Trett and Lee, 1981). We consider the columnar structure to be a pseudoannule derived from these prominences. The labial disc plus this apparent or pseudoannule therefore form the first cephalic annule, with the second annule either absent or present as the apparent first body annule. This arrangement differs from that in other thelastomatid nematodes, in which a more conventional smaller labial disc is surrounded by the wider cephalic annule.

Thelastomatidae has been considered a paraphyletic group (Adamson and van Waerebeke, 1992) without a unifying synapomorphy, a hypothesis supported by a limited molecular survey of thelastomatid species from a cockroach (Jex et al., 2005). The family as currently circumscribed contains about 30 genera following additions and subtractions (Adamson and van Waerebeke, 1992; Jex et al., 2005; Phillips et al., 2016). None of these genera have the cephalic development seen in Stauratostoma, nor do any possess a cruciate stomatic opening. The only other thelastomatid genus with a similarly constricted head region is Cranifera (Kloss, 1960) from the gut of cockroaches and scarab beetles (Chitwood, 1932; Kloss, 1966; Camino and Achinelly, 2012). The lateral view of the head region of C. cranifera (Chitwood, 1932) resembles that of S. shelleyi in being mushroom-shaped, but in C. cranifera the labial disc is surrounded by eight lobes as in typical Thelastomatidae (Carreno and Tuhela, 2011). In S. shelleyi these lobes are subsumed into a column-like false neck annule. In frontal view, the stomatal opening of C. cranifera is circular and without flaps or other projections, whereas S. shelleyi has four distinct flaps that together form a cruciate stomatal opening. The stoma of C. cranifera is cylindrical and apparently without teeth, whereas S. shelleyi has an array of teeth arising from the telostom.

Thelastoma labiatum (Leidy, 1850) was described from a millipede now known as Apheloria virginiensis, one of the common hosts of S. shelleyi. Leidy (1853) provided a small illustration of the anterior part of T. labiatum, which bears some resemblance to S. shelleyi in having the head end set off from the body by a significant constriction. In the brief description Leidy (1850, 1853) stated that the head annule was inflated and had six lobes at the margin. Cobb (1929) described T. myolabiatum from the millipede Fontaria marginata Say1, with eight prominent lobes as in most other Thelastoma spp. Christie (1938) synonymized T. myolabiatum with T. labiatum, an action followed by Basir (1956), van Waerebeke (1987) considered both T. labiatum and T. myolabiatum to be species inquirendae. Regardless of the taxonomic status of these two species, S. shelleyi differs from both in the expanded labial disc with four distinct flaps forming a cruciate oral opening, surmounting a narrower column formed of the eight longitudinal sectors.

One other species shows a possible slight convergence with S. shelleyi. Thelastoma retrovulvaris (Adamson, 1987), described from a large millipede collected in the Seychelles, has a dome-like head end with the eight prominences lying on the lower, wider part of the dome. This character is difficult to assess since the specimens were preserved in ethanol before being studied, and significant shrinkage may have occurred. The two taxa differ further in that the vulva is at midbody in S. shelleyi and just anterior to the anus in T. retrovulvaris.

1Fontaria marginata Say, 1821 is an untraceable name. All of the species placed in Fontaria are now scattered as synonyms among several tribes of Xystodesmidae. Neither Hoffman (1999) nor Marek et al. (2014) list this binomen in their checklists, nor is the name recognized in on-line literature databases such as Web of Science. R. M. Shelley (in litt.) suggested this name may have been a lapsus on the part of Cobb (1929), who might have unaccountably written Fontaria for Narceus, a genus in Spirobolidae (Spirobolida). Narceus marginatus Say, 1821 is a synonym of Narceus annularis Rafinesque, 1820, which is a host for some Thelastomatidae but not for S. shelleyi.
Molecular comparisons

Appalachian representatives of Thelastomatidae have several morphological characteristics in common with S. shelleyi: eight anterior prominences surrounding the labial disc, thin eggshells, and a long, filiform tail. Molecular analysis supported the inclusion of S. shelleyi in the family Thelastomatidae as the sister group to Thelastoma. The partial 28s rDNA BI and MP trees suggested that the Thelastoma + Stauratostoma + Coronostoma clade is the apparent sister group to nematodes inhabiting cockroaches (Leidynema spp. + Cranifera + Suifunema sp. + Hammerschmidtella spp. group). However, the taxonomic ranks of these various groups is uncertain. Coronostoma spp. have major morphological differences from all other Oxyuridomorpha, and were returned by Phillips et al. (2016) to their own superfamily, Coronostomatoidea (Poinar and Willmott, 1977).

Millipede versus nematode morphometrics

The length of each specimen of S. shelleyi was measured and compared with the length, width, and weight of two of the most prominent millipede hosts: Apheloria montana and Apheloria virginiensis to determine if there was any correlation between nematode length and millipede size. Adult nematode length was not affected statistically by millipede length ($p = 0.84$), width ($p = 0.77$), or weight ($p = 0.82$) (Fig. 7). However, the relationship of S. shelleyi length to juvenile millipede morphometrics was not determined, and millipede size or developmental stage could have an effect on when infection can first occur. Almost nothing is known about the host-parasite relationships of any oxyuridomorph nematode parasitic in millipedes.

Acknowledgements

The authors thank Parwinder Grewal and William Klingeman III for their guidance and advice. Claire and Sunny Phillips aided in the collection of millipedes and provided photographs. Paul Callomon, Academy of Natural Sciences of Drexel University, loaned us several Thelastoma spp. and Aorurus spp. from the Leidy collection. Veronica Brown and Joe May, University of Tennessee Genomics Core, provided assistance and advice with DNA sequencing while John Dunlap and Uk Huh assisted us with SEM. We appreciate the lab and field work by Satyendra and Ratnasri Pothula, as well as Chauncey Whitlock. Zafar Handoo provided access numbers for our holotypes and paratypes. The Tennessee State Parks, Roger McCoy, Director of the Division of Natural Areas for the State of Tennessee, issued permits, and Ranger Michael Hodge provided field support.

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