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# Description of *Hemicaloosia graminis* n. sp. (Nematoda: Caloosiidae) Associated with Turfgrasses in North and South Carolina, USA

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*Abstract:* A new nematode species was discovered during a diversity survey of plant-parasitic nematodes on turfgrass conducted in North and South Carolina in 2010 and 2011. It is described herein as *Hemicaloosia graminis* n. sp. and is characterized by two annuli in the lip region, one lateral line, body 610.0–805.0 μm long, stylet 65.0–74.6 μm long, vulva at 84.1% –85.8% of the body , 254–283 annuli, vulva at the 38–53$^{rd}$ annulus from tail terminus, 12–14 annuli between vulva and anus, tail elongate-pointed, 67.5–84.8 μm long in females and spicule straight, 31.0 μm long, caudal alae well developed, two lateral lines in males. The newly described species is morphologically closest to *H. paradoxa*, but has a longer stylet (65.0–74.6 *vs* 61.0–65.0 μm) and a higher V-value (84.1–85.8 *vs* 78.1–84.0%), less RV (38–53 *vs* 50–56), higher RVan (12–14 *vs* 10) in females, and a shorter tail (30.1 *vs* 36.7 μm) and more anteriorly located excretory pore (105.9 *vs* 140.0 μm) in the male. It was easily differentiated from other species based on near-full-length small subunit rRNA gene (SSU) and ITS1 sequences. Phylogenetic analysis from SSU supports placement in a monophyletic clade with the genus *Caloosia*. An identification key and a table of distinguishing characteristics are presented for all seven species of *Hemicaloosia*.
*Key words:* ITS1, molecular phylogeny, morphology, morphometrics, small subunit rRNA (SSU), taxonomy, turfgrass.

Plant-parasitic nematodes are recognized as important pests of turfgrasses in the southeastern United States. Twenty-nine species of plant-parasitic nematodes belonging to 22 genera in 15 families were associated with turfgrasses in a survey conducted in North Carolina (NC) and South Carolina (SC) during 2010 and 2011. Of those, genera from the suborder Criconematina detected were *Mesocriconema, Hemicycliophora, Hemicriconemoides* and *Hemicaloosia*.

*Hemicaloosia* was proposed by Ray and Das (1978) as a new genus within the family Hemicycliophoridae Geraert, 1966, with *H. americana* as the type species. Siddiqi (1980, 2000) placed *Hemicaloosia* together with *Caloosia* Siddiqi & Goodey, 1963 as members of the family Caloosiidae in the superfamily Hemicycliophoroidea, although the validity of the family Caloosiidae is subject to some debates. Siddiqi (2000) listed five species of *Hemicaloosia*: *H. americana* Ray and Das, 1978; *H. luci* Dhanachand and Jairajpuri, 1979; *H. nudata* (Colbran, 1963) Ray and Das, 1978; *H. delpradi* (Maas, 1970) Siddiqi, 1980; and *H. paradoxa* (Luc, 1958) Ray and Das, 1978. Another species *H. psidii* Gambhir and Dhanachand, 1996 was described in 1996. These six species of *Hemicaloosia* have been described from India, Surinam, Ivory Coast and Australia, but none have been reported from North America. A recent survey of plant-parasitic nematodes associated with turfgrasses in NC and SC revealed an undescribed species in this genus. It is herein described as *Hemicaloosia graminis* n. sp. based on morphological characteristics and ribosomal DNA sequences. This species is the first report of the genus *Hemicaloosia* in North America.

## Materials and Methods

*Nematode material:* Samples were collected from turfgrasses in New Hanover County, NC, and Beaufort and Charleston counties, SC, in 2010 and 2011. The nematodes were extracted by a combination of elutriation (Byrd et al., 1976) and centrifugation (Jenkins, 1964) methods. Live nematodes were hand-picked into water for DNA extraction, amplification and sequencing. For measurements by light microscopy, nematodes were heat-killed and placed into FG (formalin:glycerol:d$H_2O$ = 10:5:85) before processing into 100% glycerol for permanent mounts (Southey, 1970).

*Morphological observations:* Drawings, measurements and photomicrographs of nematodes were performed with the aid of a Zeiss video camera (AxioCam MRc5) attached via a C-mount adapter fitted on a Zeiss Imager A1 microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY 10594) and edited using Adobe Photoshop CS4. The morphometric data were analyzed using Microsoft Excel software (Ye, 1996). The morphometric data presented in Table 2 are from the descriptions of type species or other populations of all described six species in the genus *Hemicaloosia*. The measurement parameters were employed from Siddiqi (2000).

*Molecular profiles:* Ten nematodes from New Hanover, NC (Lab ID: 10-27720) were placed into distilled water and their identity was confirmed with light microscopy before being placed into 50-μl AE buffer (10mM Tris-Cl, 0.5mM EDTA; pH9.0) and crushed with a pipette tip. DNA samples were stored at -20°C until used as a PCR template. Primers for SSU amplification were forward primer 18S965 (5'- GGCGATCAGATACCGCCCTAGTT-3') and reverse primer 18S1573R (5'-TACAAAGGGCAGGG ACGTAAT-3') (Mullin et al., 2005), forward primer SSUF07 (5'-AAAGATTAAGCCATGCATG-3') and reverse primer SSUR26 (5'-CATTCTTGGCAAATGCT TTCG-3') (Floyd et al., 2002), and forward primer 18SnF

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(5'-TGGATAACTGTGGTAATTCTAGAGC-3') and reverse primer 18SnR (5'-TTACGACTTTTGCCCGGTTC-3') (Kanzaki and Futai, 2002). Primers for ITS1 amplification were forward primer rDNA2 (5' TTGATTACGTTCC CTGCCCTTT 3') (Vrain et al., 1992) and reverse primer rDNA1.58S (5' ACGAGCCGAGTGATCCACCG 3') (Cherry et al, 1997). The 25 $\mu$l PCR was performed using Apex Taq Red Master Mix DNA polymerase (Genesee Scientific Corporation, San Diego, CA) according to the manufacturer's protocol. The thermal cycler program for PCR was as follows: denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec, and extension at 72°C for 2 min. A final extension was performed at 72°C for 10 min (Ye et al., 2007). PCR products were cleaned using ExoSap-IT (Affymetrix, Inc., Santa Clara, CA) according to the manufacturer's protocol and were sequenced by Genomic Sciences Laboratory at North Carolina State University using an Applied Biosystems 3730 XL DNA Analyzer (Life Technologies, Carlsbad, CA). The resulting ribosomal DNA SSU and ITS1 sequence was deposited in genBank under the accession number JQ446376 and compared with other nematode species in genBank using the BLAST homology search program. The most similar sequences were downloaded for phylogenetic analysis. The DNA sequences were aligned by Clustal W (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). The model of base substitution was evaluated using MODELTEST (Posada and Crandall, 1998; Huelsenbeck and Ronquist, 2001). The Akaike-supported model, the base frequencies, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck and Ronquist, 2001) running the chain for $1 \times 10^6$ generations and setting the "burn in" at 1,000. The MCMC (Markov Chain Monte Carlo) method was used within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using 50% majority rule.

### ***Hemicaloosia graminis* n. sp.**

(Figs.1–2)

Measurements: See Table 1.

Description: *Females*: Body slightly ventrally curved when heat killed, almost uniform in width (21.0–30.0 $\mu$m) anterior to the vulva, but narrowing posteriorly to a pointed, elongate tail. Sheath closely attached to the body, connected only at anterior end. Body and sheath annuli smooth, numbering 254–283, annulus 2.6–3.3 $\mu$m thick at mid-body. Lateral fields on sheath begin from the third annulus, marked by one line running along the body to tail terminus forming a depression. Lip region almost continuous with body contour, 9.0–10.0 $\mu$m wide, bearing two annuli slightly differentiated from the adjoining body annuli. The first annulus bearing a prominent labial disc, 6.0–7.0 $\mu$m wide. Stylet slightly ventrally arcuate, 66.8–74.6 $\mu$m long, conus 82.3% –84.7% of the entire stylet, stylet knobs spheroid, sloping backwards, 4.8–5.9 $\mu$m across and 1.7–2.3 $\mu$m high. Dorsal esophageal gland orifice at 5.0–7.0 $\mu$m from the base of stylet knobs. Hemizonid weakly developed, difficult to see under light microscope, located one annulus anterior to the excretory pore (EP). EP at 122.3–138.1 $\mu$m or 43–54 annuli from anterior end and just at the level of basal bulb base. Vulva at the 38–53$^{rd}$

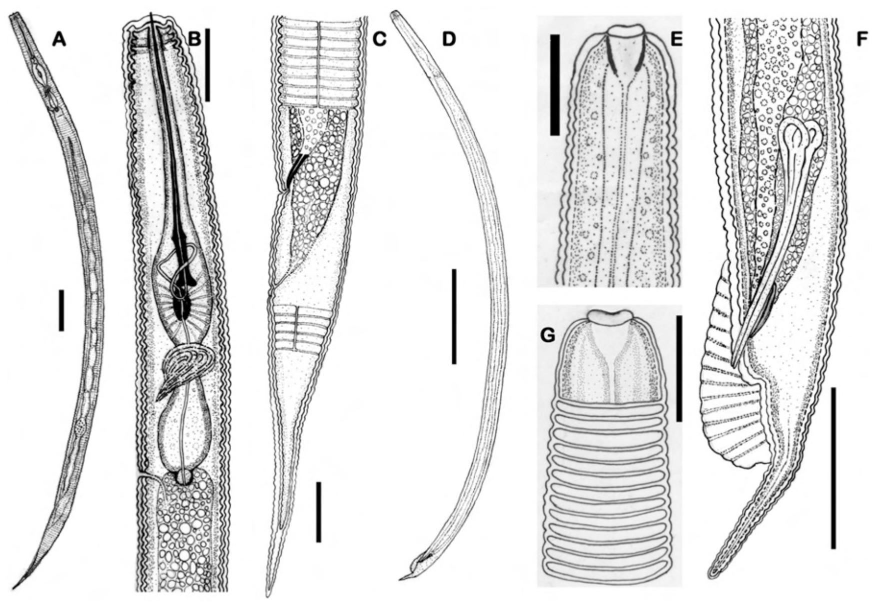


Fig. 1. Drawings of *Hemicaloosia graminis* n. sp. from turfgrassin New Hanover County, NC, USA (Lab ID: 10-22720). A. Female entire body. B. Female esophageal region. C. Vulva and tail region. D. Male entire body. E, G. Male head. F. Male tail. (Scale bars: A=50 μm; D=100 μm; B, C, E–G=20 μm).

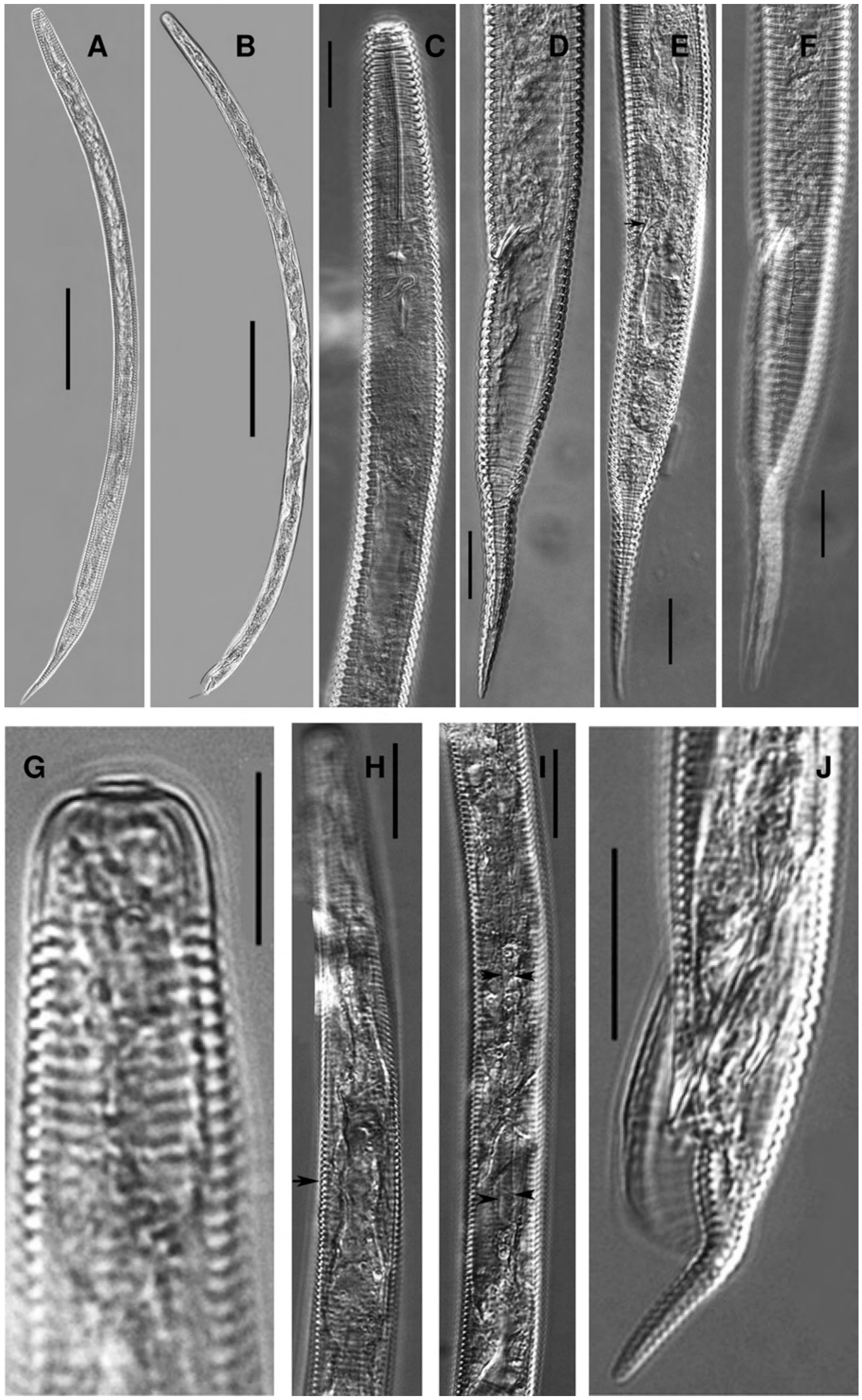


FIG. 2. Micrographs of *Hemicaloosia graminis* n. sp. from turfgrass in New Hanover County, NC, USA (Lab ID: 10-22720). A. Female entire body. B. Male entire body. C. Female esophageal region. D. Vulva and tail region. E. Ventral view of vulva region (arrow refer to overhanging lip). F. Lateral line in females. G. Male head. H. Excretory pore in male. I. Lateral lines in males. J. Male tail. (Scale bars: A, B=100 μm; C–J= 20 μm).

annulus from tail tip, located at 84.1% –85.8% of the entire body. Vulva opening a narrow slit with prominent anterior lip overhanging. Reproductive system monovarial, prodelphic, outstretched. Spermatheca wide oblong, filled with spheroid sperms. Anus at the 26–39$^{th}$ annulus from tail terminus. Tail elongate-conoid, tapering gradually behind anus.

*Male:* Body slightly ventrally curved after fixation, shorter and more slender than female. Head continuous with body. Annuli 1.5–1.6 $\mu$m at mid-body, narrower and finer than female. Head anteriorly degenerated. Labial disc developed, 2.0–3.0 $\mu$m wide. Lateral field plain, about one third of the body width at mid-body, with two longitudinal lines. Stylet absent. Esophagus degenerated.

Table 1. Morphometrics of *Hemicaloosia graminis* n. sp. All measurements in μm and in the format: mean ± S.D. (Range).

| Species | *H. graminis* n. sp. | *H. graminis* n. sp. | *H. graminis* n. sp. | *H. graminis* n. sp. | *H. graminis* n. sp. |
|---|---|---|---|---|---|
| Sex | Female holotype | Female | Male | Female | Female |
| Lab ID | 10-27720 | 10-27720 | 10-27720 | 11-30679 | 11-30766 |
| Host | Turfgrass | Turfgrass | Turfgrass | Bermudagrass | Zoysiagrass |
| Locality | New Hanover, NC, USA | New Hanover, NC, USA | New Hanover, NC, USA | Beaufort, SC, USA | Charleston, SC, USA |
| n | 1 | 6 | 1 | 3 | 1 |
| L | 693.0 | 712.3 ± 70.1 (610.4 – 805.4) | 635.1 | 700.1 ± 10.9 (689.2 – 711.0) | 722.0 |
| a | 29.5 | 28.8 ± 0.9 (27.2 – 29.5) | 36.5 | 28.2 ± 1.0 (27.2 – 29.1) | 28.0 |
| b | 5.8 | 5.8 ± 0.4 (5.1 – 6.2) | 6.2 | 6.0 ± 0.1 (5.9 – 6.1) | 5.3 |
| c | 8.3 | 9.0 ± 0.6 (8.3 – 10.0) | 21.1 | 9.0 ± 0.3 (8.7 – 9.3) | 9.1 |
| c’ | 4.6 | 4.3 ± 0.3 (4.0 – 4.6) | 2.9 | 4.1 ± 0.1 (4.1 – 4.2) | 4.1 |
| V or T | 84.2 | 84.7 ± 0.7 (84.1 – 85.8) | – | 84.3 ± 1.1 (83.2 – 85.4) | 84.0 |
| Body width | 23.5 | 25.3 ± 3.1 (20.9 – 29.6) | 17.4 | 24.9 ± 0.5 (24.4 – 25.4) | 25.8 |
| Stylet length | 66.8 | 69.0 ± 3.3 (66.8 – 74.6) | – | 67.6 ± 2.6 (65.0 – 70.2) | 73.4 |
| Stylet cone length | 56.3 | 57.8± 2.8 (55.3 – 62.5) | – | 59.2 ± 0.1 (59.1 – 59.3) | 60.5 |
| Styl.knobW/H | 3.1 | 2.8 ± 0.4 (2.2 – 3.1) | – | 2.6 ± 0.1 (2.6 – 2.7) | 2.7 |
| Body width at stylet base | 20.9 | 22.2 ± 2.4 (19.4 – 25.9) | – | 22.2 ± 0.7 (21.5 – 22.9) | 27.9 |
| Pharynx length | 119.5 | 123.6 ± 5.9 (119.5 – 133.8) | 101.7 | 116.1 ± 0.3 (115.8 – 116.4) | 135.7 |
| Anal body width | 18.2 | 18.5 ± 1.2 (17.0 – 20.4) | 10.3 | 18.9 ± 0.7 (18.2 – 19.6) | 19.4 |
| Tail length | 83.9 | 79.3 ± 7.0 (67.5 – 84.8) | 30.1 | 78.0 ± 1.8 (76.2 – 79.7) | 79.7 |
| VA | 26 | 29.4 ± 3.6 (26.0 – 33.3) | | 27.5 ± 0.1 (27.5 – 28.0) | 35.8 |
| Excretory pore from anterior end | 127.2 | 131.0 ± 6.5 (122.3 – 138.1) | 105.9 | 121.1 ± 0.9 (120.2 – 122.0) | 139.3 |
| Ring width at mid–body | 2.7 | 2.8 ± 0.1 (2.7 – 2.9) | 1.6 | 2.6 ± 0.0 (2.6 – 2.7) | 2.7 |
| R | 283 | 269.3 ± 11.4 (254.0 – 283.0) | 387 | 269.5 ± 9.5 (260.0 – 279.0) | 274 |
| Rs | 27 | 24.5 ± 1.7 (23.0 – 27.0) | – | 25.0 ± 1.0 (24.0 – 26.0) | 26 |
| Reso | 51 | 45.5 ± 3.4 (42.0 – 51.0) | 57 | 45.5 ± 0.5 (45.0 – 46.0) | 47 |
| Rex | 54 | 48.0 ± 4.1 (43.0 – 54.0) | 63 | 47.5 ± 0.5 (47.0 – 48.0) | 48 |
| RV | 53 | 47.3 ± 5.6 (38.0 – 53.0) | – | 44.3 ± 0.0 (44.0 – 44.0) | 44 |
| Ran | 39 | 34.3 ± 5.1 (26.0 – 39.0) | | 31.5 ± 0.5 (31.0 – 32.0) | 32 |
| RVan | 14 | 13.0 ± 1.0 (12.0 – 14.0) | – | 12.5 ± 0.5 (12.0 – 13.0) | 13 |
| VL/VB | 6.0 | 5.6 ± 0.6 (5.0 – 6.5) | – | 4.9 ± 0.3 (4.6 – 5.2) | 5.2 |
| M | 84.3 | 83.8 ± 0.9 (82.3 – 84.7) | – | 87.8 ± 3.5 (84.2 – 91.3) | 82.4 |
| St% L | 9.6 | 9.8 ± 0.8 (9.1 – 11.0) | – | 9.7 ± 0.2 (9.4 – 9.9) | 10.2 |
| St% Oes | 55.9 | 55.8 ± 0.2 (55.6 – 55.9) | – | 58.2 ± 2.4 (55.8 – 60.6) | 54.1 |
| Hemizonid | | | 104.0 | | |
| Spicule length | – | – | 31.0 | – | – |
| Gubernaculum lenth | – | – | 6.8 | – | – |
| Bursa length (arc.) | – | – | 37.0 | – | – |
| Bursa length (line) | – | – | 32.2 | – | – |

EP lies on the 63rd annulus and 106.0 μm from anterior end. Spicules straight, slender, simple, 31.0 μm long, with slightly cephalated base. Gubernaculum narrow, simple, trough-like, 6.8 μm long. Caudal alae well developed and 37.0 μm long, originating anteriorly at 13.0 μm from cloaca and extending posteriorly up to 16.0 μm from cloaca. Tail short, cylindrically conoid, with a pointed tip.

TYPE HOST AND LOCALITY: *Hemicaloosia graminis* n. sp. was collected from turfgrass in New Hanover County, NC, USA.

OTHER LOCALITIES: The specimens were collected from golf course tees established with *Cynodon dactylon* in Charleston County and *Zoysia* spp. in Beaufort County, SC, USA.

TYPE MATERIAL: Holotype female, one paratype male, one paratype female deposited in the Department of Nematology, University of California, Riverside, CA. Four paratype females deposited at the Nematology Laboratory, USDA, ARS, Beltsville, MD; and one at the Nematode Assay Section, Agronomic Division, NCDA&CS, Raleigh, NC.

*Diagnosis and relationships:* Females of *Hemicaloosia graminis* n. sp. are characterized by the combined characters of cuticular sheath on entire body, two annuli in lip region, one lateral line, body 610.0–805.0 μm long with 254-283 annuli, stylet 65.0–74.6 μm long, vulva at 84.1% –85.8% of the entire body, vulva at the 38–53rd annulus from tail terminus, 12–14 annuli between vulva and anus, 43–54 annuli from excretory pore to anterior end and elongate-pointed tail 67.5–84.8 μm long. Male characters include head not offset, two lateral lines, straight spicule 31.0 μm long and well-developed caudal alae.

The *Hemicaloosia graminis* n. sp. is morphologically closest to *H. paradoxa* (Table 2) from *Pennisetum typhoideum* in Abidjian, Ivory Coast, originally described by Luc (1958), but differes in body size (610.0–805.0 *vs* 680.0–820.0 μm); a and b values (27.2–29.5 *vs* 23.7–29.0 and 5.1–6.2 *vs* 4.9–5.7, respectively); and numbers of R, Rs, Reso and RV (254–283 *vs* 256–263, 23–27 *vs* 23–25, 42–51 *vs* 42–45 and 38–53 *vs* 50–56). However, *H. graminis* n. sp. has a longer stylet (65.0–74.6 *vs* 61.0–65.0 μm), higher RVan (12–14 *vs* 10), shorter tail (67.5–84.8 *vs* 109.1 μm), and a higher V (84.1–85.8 *vs* 78.1–84%) in females, as well as a shorter tail (30.1 *vs* 36.7 μm) and more anteriorly located EP (105.9 *vs* 140.0 μm) in the male.

Compared with the Ivory Coast population of *H. paradoxa* reported by Brzeski (1974), females of *H. graminis* n. sp. have higher R (254–283 *vs* 240–256), RVan (12–14 *vs* 7–11) and VL/VB (4.6–6.5 *vs* 3.8–4.6). Compared with the Nigerian population of *H. paradoxa* reported by Brzeski (1974), females of *H. graminis* n. sp. have a longer stylet (65.0–74.6 *vs* 51.0–56.0 μm) and higher R (254–283 *vs* 240–257) and RVan (12–14 *vs* 7–11). They also have a longer stylet (65.0–74.6 *vs* 50.0–57.0 μm), higher RVan (12–14 *vs* 8–12) and fewer lateral lines (1 *vs* 2) than those of the population from eggplant in Santa Fe, Argentina, reported by Chaves (1983). The Argentinian population might be a different species from *H. paradoxa* since its females have a much shorter body (520.0–670.0 *vs* 680.0–820.0 μm), stylet (50.0–57.0 *vs* 61.0–65.0 μm) and tail (57.0–75.0 *vs* 109.1 μm); less RV (38–47 *vs* 50–56); and more lateral lines (2 *vs* 1) as compared with the African species of Luc (1958). The identity of these species/populations should be examined by DNA sequence data in the future. The new species is also distinguished by its location in the Carolinas, USA and a turfgrass host.

*Hemicaloosia graminis* n. sp. is morphologically similar to the other described species (Table 2). Females differ from those of *H. nudata* by the presence of cuticular sheath on entire body (only on post-vulval part of body in *H. nudata*); a shorter body (610.0–805.0 *vs* 840.0–1097.0 μm); higher a (27.2–29.5 *vs* 22.0–25.0), c (8.3–10.0 *vs* 6.5–8.6) and V (84.1–85.8 *vs* 81.0–84.0%); and presence of continuous line in lateral fields. The male has a smaller spicule (31.0 *vs* 37.0–45.0 μm) and gubernaculum (6.8 *vs* 8.0–8.4 μm) and two lateral lines *vs* none in *H. nudata* males. Compared to *H. delpadi*, females of the new species have a shorter body (610.0–805.0 *vs* 755.0–861.0 μm), higher c (8.3–10.0 *vs* 7.4–8.5) and V (84.1–85.8 *vs* 82.0–83.0%), a shorter stylet (65.0–74.6 *vs* 75.0–78.0 μm), less RV (38–53 *vs* 50–58), and the presence of lateral fields. Compared to *H. luci*, females of the new species have a shorter body and tail (610.0–805.0 *vs* 890.0–1200.0 and 67.5–84.8 *vs* 117.0 μm), lower a (27.2–29.5 *vs* 28.0–40.0) and VL/VB (4.6–6.5 *vs* 6.2–7.1), less R (254–283 *vs* 292–330), and fewer lateral lines (1 *vs* 2). Compared to *H. americana*, females of the new species have a longer stylet (65.0–74.6 *vs* 60.0–64.0 μm) and more anteriorly located EP (120.2–139.3 *vs* 148.0 μm, i.e., at the level of the base of basal bulb *vs* posterior to the base of basal bulb). The male has a smaller spicule (31.0 *vs* 33.0–36.0 μm) and shorter tail (30.1 *vs* 50.4 μm), and the head is not offset. Compared to *H. psidii*, females of the new species have lower c' (4.0–4.6 *vs* 4.9–6.4) and higher V (84.1–85.8 *vs* 78.0%) values, less RVan (12–14 *vs* 20–22), higher Ran (26–39 *vs* 19–23), a longer stylet (65.0–74.6 *vs* 48.0–58.0 μm) and fewer lateral lines (1 *vs* 2). Males of *H. delpradi, H. luci* and *H. psidii* have not been described.

To help identify the species, a key to the species of *Hemicaloosia* female is presented below:

1. Cuticular sheath on post-vulval part of body, stylet more than 90 μm ....................*H. nudata*
   Cuticular sheath on entire body, stylet less than 90 μm................................2
2. Body more than 890 μm, two lateral lines........*H. luci*
   Body less than 890 μm..................................3
3. Stylet more than 75 μm, lateral field absent......*H. delpradi*
   Stylet less than 75 μm, lateral field present........4

TABLE 2. Morphometrics of *Hemicaloosia* spp. All measurements in μm and in the format: mean ± S.D. (Range).

| Species | *H. paradoxa* | *H. paradoxa* | *H. paradoxa* | *H. paradoxa* | *H. paradoxa* | *H. paradoxa* | *H. paradoxa* | *H. americanae* | *H. americanae* | *H. nudata* | *H. nudata* | *H. luci* | *H. delpradi* | *H. psidii* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | Female | Male | Female | Male | Female | Male | Female | Female | Male | Female | Male | Female | Female | Female |
| Reference | Luc, 1958 | Luc, 1958 | Brzeski, 1974 | Brzeski, 1974 | Brzeski, 1974 | Brzeski, 1974 | Chaves, 1983 | Ray &Das, 1978 | Ray &Das, 1978 | Colbran, 1963 | Colbran, 1963 | Dhanachand & Jairajpuri, 1979 | Maasi, 1970 | Gambhir & Dhanachand, 1996 |
| Host | *Pennisetum typhoideum* | *Pennisetum typhoideum* | | | | | Egg-plant | *Agave americana* | *Agave americana* | *Citrus limonia* | *Citrus limonia* | Bushes | Forest vegetation | Guava |
| Locality | Abidjian, Ivory Coast | Abidjian, Ivory Coast | Abidjian, Ivory Coast | Abidjian, Ivory Coast | Nigeria | Nigeria | Santa Fe, Argentina | Orissa, India | Orissa, India | Queensland, Australia | Queensland, Australia | Manipur, India | Kraka, Surinam | Manipur, India |
| n | ? | ? | 8 | 6 | 4 | 2 | 9 | 15 | 7 | 20 | 20 | 20 | 4 | 4 |
| L | 680–820 | 548–663 | 670 (610–700) | 610 (560–630) | 620 (580–660) | 510–550 | 590 (520–670) | 790 (740–845) | 640 (615–660) | 840–1097 | 827–910 | 970 (890 – 1200) | 800 (755–861) | 570–740 |
| a | 23.7–29.0 | 26.4–30.0 | 26 (24–34) | 34 (31–36) | 29 (26–31) | 34–39 | 25 (23–28) | 29 (27–32) | 40 (35–42) | 22–25 | 30–38 | 34 (28 – 40) | 26 (24–28) | 23–36 |
| b | 4.9–5.7 | | 5.4 (5.1–5.7) | 5.6 (4.9–6.2) | 5.6 (5.4–5.8) | 8.3–12.0 | 5.8 (5.5–6.0) | 6.5 (6.2–6.8) | 6.6 (6.1–6.9)? | 5.3–6.0 | ? | 7.1 (6.5 – 9.9) | 5.7 (5.3–6.1) | 5.1–6.5 |
| c | | 12.7–18.5 | 9.4 (8.8–10.2) | 17.2 (16.8–17.8) | 7.5 (7.1–8.4) | 19.0–22.1 | 9 | 7.7 (6.6–10.4) | 12.7 (10.6–14.1) | 6.5–8.6 | 8.5–9.6 | 9.2 (7.2 – 10.8) | 8.1 (7.4–8.5) | 7–10 |
| c' | 4.3* | 2.7* | 4.3* | 2.7* | | | 4.0* | 4.1* | 4.2* | 4.6* | 6.4* | 5.6* | 4.4 (4.2–4.8) | 4.9–6.4 |
| V or T | 78.1– 84.0 | 28.5 | 86 (85–87) | | 83 (82–85) | | 84 (83–85) | 83 (81–87) | – | 81.0–84.0 | 19–26 | 84 (82 – 86) | 82 (82–83) | 78 |
| Stylet length | 61–65 | – | 67 (64–69) | – | 54 (51–56) | – | 53 (50–57) | 61 (60–64) | – | 94.0–109.0 | – | 67 (66 – 70) | 77 (75–78) | 48–58 |
| Tail length | 109.1* | 36.7* | 68.8* | 25.4* | | | 65 (57–75) | 82.8* | 50.4* | 131.4* | 100* | 117.0* | 103.6* | 77* |
| Excretory pore from anterior end | 130.9* | 140* | 100.6* | | | | 109.7* | 148.0 | | 181.4* | | 147.8* | 136.4* | 126.4 |
| R | 256– 263 | | 249 (240–256) | | 247 (240–257) | | 241 (223–260) | 268 (245–283) | | >200 | | 309 (292 – 330) | 270 (261–283) | 237–266 |
| Rs | 23– 25 | | 24* | – | | – | 23 (21–25) | 23* | | 22–25 | | 24* | 24* | |
| Reso | 42–45 | | 48* | | | | 40 (35–44) | | | 39* | | 44* | 36* | |
| Rex | 45* | | 45 (43–47) | | 46 (43–50) | | 42 (39–46) | 50 (48–53) | | 40–44 | | 51 (51 – 54 ) | 45* | 45 |
| RV | 50–56 | – | 207 (198–212)** | – | 204 (195–220)** | – | 43 (38–47) | 41 (28–43) | – | 40 * | – | 261 (243 – 273)** | 55 (50–58) | 217** |
| Ran | 30* | | 32 (28–35) | | 34 (30–37) | | | 30 (18–33) | | 31* | | 30 (23 – 37) | 38 (35–42) | |
| RVan | 10 | | 8 (7–11) | – | 9 (7–11) | – | 10 (8–12) | 11 (10–13) | | 9 * | | 15 (11 – 18) | 16 (13–18) | |
| VL/VB | 4.9* | – | 4.2 (3.8–4.6) | – | 5.8 (5.1–6.2) | – | 4.9* | 5.2* | – | 5.3* | – | 6.6 (6.2 – 7.1) | 5.3* | |
| Hemizonid | 118.2* | 134.5* | 95.3* | | | | 104.5* | 115.4* | | 178.6* | | 144.8* | 114.5* | |
| Spicule length | – | 30–35 | – | 28–30 | – | 22–34 | – | – | 34 (33–36) | – | 37–45 | – | – | – |
| Bursa length (arc.) | – | 35.4* | – | 23.3* | – | | – | – | 42* | – | 51.4* | – | – | – |
| Bursa length (line) | – | 31.9* | – | 21.2* | – | | – | – | 39.7* | – | 47.1* | – | – | – |

* Calculated from figure. ** Counted from anterior end.

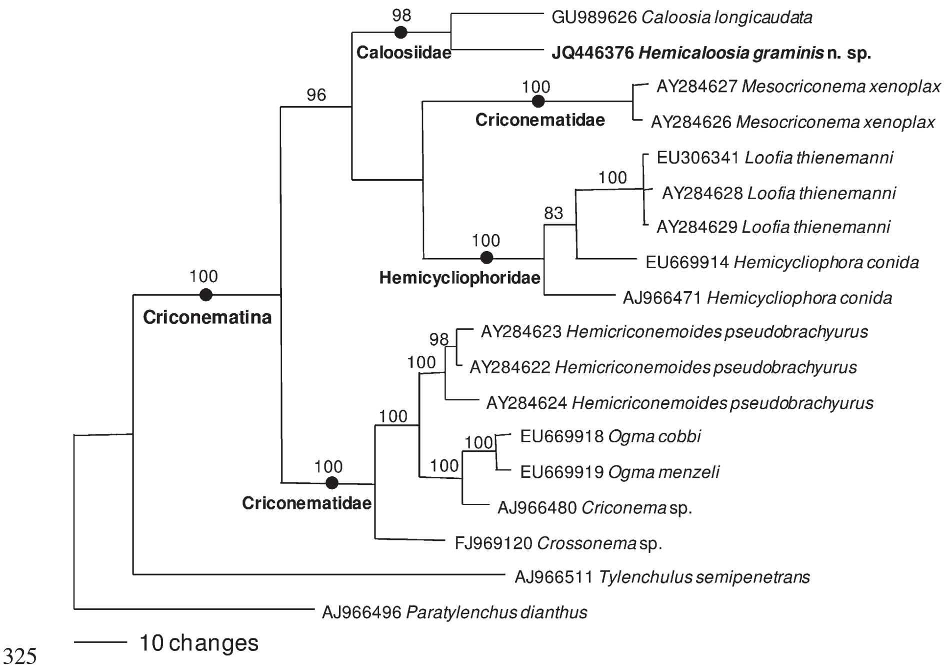


FIG. 3. The 10001st Bayesian tree inferred from ribosomal DNA SSU gene under TIM+I+G model (lnL=4709.6089; AIC=9435.2178; freqA=0.2431; freqC=0.2357; freqG=0.2794; freqT=0.2418; R(a)=1; R(b)=1.8384; R(c)=0.723; R(d)=0.723; R(e)=5.6125; R(f)=1; Pinva=0.6397; Shape=0.6195). Posterior probability values exceeding 50% are given on appropriate clades.

4. V lower than 80%, two lateral lines.............*H. psidii*
   V higher than 80%..........................................5
5. Lateral field occasionally interrupted...............*H. paradoxa*
   Lateral field continuous..........................6
6. Without anastomoses in post-anal region, stylet longer, 65-75 $\mu$m .....*H. graminis* n. sp.
   With anastomoses in post-anal region, stylet shorter, 60-64 $\mu$m ............*H. americana*

*Molecular phylogenetic relationships:* DNA Sequencing of 2121-bp near-full-length SSU and ITS1 for molecular phylogenetic inferences was conducted to determine the relative placement of *Hemicaloosia graminis* n. sp. among closely related species based on blastn search. The tree inferred from SSU (Fig. 3), using *Paratylenchus dianthus* as an outgroup, indicated that i) all the selected taxa from Criconematina are in a monophyletic clade in relation to *Tylenchulus semipenetrans* with 100% support; ii) *Caloosia longicaudatus* from Caloosiidae and *Loofia thienemanni* and *Hemicycliophora conida* from Hemicycliophoridae are in a monophyletic clade with 96% support; iii) *Hemicaloosia graminis* n. sp. is in a highly supported monophyletic clade with its sister genus *Caloosia* which shared a common ancestor with *Hemicycliophora conida* Thorne, 1955; iv) *Mesocriconema xenoplax* in Criconematidae is paraphyletic with many other genera in Criconematidae. This tree is in agreement with the topology inferred from ribosomal DNA large subunit D2D3 by Subbotin et al. (2005). Blast search of the ITS region of *H. graminis* n. sp. yielded no match with any species in genBank; therefore, no phylogenetic analysis was conducted.

The taxonomy of the suborder Criconematina has been quite confusing, particularly with respect to the validity of the family Caloosiidae. Siddiqi (1980) proposed the family Caloosiidae to separate some species from Hemicycliophoridae, but Raski and Luc (1987) did not recognize the family Caloosiidae . Van Den Berg et al. (2011) provided some evidences supporting the validity of the Caloosiidae. This study provided further support for the monophyly of family Caloosiidae and the family Hemicycliophoridae. However, with very limited sequencing data available, further molecular study is needed to test the evolutionary history of this large nematode group.

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