

Characterisation of some *Scutellonema* species (Tylenchida: Hoplolaimidae) occurring in Botswana, South Africa, Costa Rica and the USA, with description of *S. clavicaudatum* sp. n. and a molecular phylogeny of the genus

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Summary – The genus *Scutellonema* contains more than 40 species of spiral nematodes with enlarged phasmids called scutella. In this study, we provide morphological and molecular characterisation of *S. clavicaudatum* sp. n., *S. brachyurus*, *S. bradys*, *S. cavenessi*, *S. transvaalense*, *S. truncatum* and *Scutellonema* sp. A. from North and Central America, and Africa. The new species, *S. clavicaudatum* sp. n., was found on sugarcane in South Africa and is characterised by a lack of lip annuli as in *S. africanum*, *S. siamense* and *S. truncatum*. The lip region, in both males and females, is conical and marked by six large rectangular blocks separated or fused with the submedian and lateral lip sectors, which surround a round and distinct labial disc. Females of this new species also have large vaginal glands, a functional spermatheca, the lateral field posterior to the scutellum ending in a bluntly pointed shape and a clavate tail. Morphological descriptions, measurements, light and scanning electron microscopic photos and drawings are also given for *S. bradys*, *S. cavenessi*, *S. transvaalense* and *S. truncatum*. The study of spiral nematode samples from Florida, USA, confirmed the presence of a morphologically and genetically atypical populations of *S. bradys*. The morphology of the *S. bradys* population from Bermuda grass in pasture land from central Florida fits that of type specimens of this species, but differs in having a truncate tail terminus rather than round and also a prominent spermatheca filled with flagellate spermatozoa. Other Florida *Scutellonema* samples analysed in this study belonged to *S. cavenessi*, a species native to West Africa. This is the first report of *S. cavenessi* in Florida, where it parasitises the ornamental plant *Sansevieria trifasciata*. Our study showed a high level of intraspecific variation for *Scutellonema* rRNA and mtDNA genes, which can reach 5.6% for the D2-D3 of 28S rRNA, 12.9% for the ITS rRNA genes and 14.4% for the *COI* gene. Phylogenetic relationships within *Scutellonema* are given as inferred from the analyses of the D2-D3 of 28S rRNA, ITS rRNA and the *COI* mtDNA gene sequences.

Keywords – 28S rRNA, California, *COI* mtDNA, description, Florida, ITS rRNA, morphology, morphometrics, *Sansevieria trifasciata*, *Scutellonema bradys*, *Scutellonema cavenessi*, *Scutellonema transvaalense*, *Scutellonema truncatum*, SEM, spiral nematodes, taxonomy.

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The spiral nematodes of the genus *Scutellonema* Andrassy, 1958, are primarily characterised by enlarged phasmids, called scutella, which are opposed and located near the anus. Presently, the genus contains more than 40 species. These nematodes are widely distributed in tropical and subtropical regions with the greatest number of species recorded from Africa (Sher, 1964; Siddiqi, 2000). Three species, the yam nematode *S. bradys* (Steiner & LeHew, 1933) Andrassy, 1958, *S. cavenessi* Sher, 1964, and the Carolina spiral nematode *S. brachyurus* (Steiner, 1938) Andrassy, 1958, are considered as agricultural pests, whereas other species either cause no known, or little, economic damage to crops.

Fourteen *Scutellonema* species are currently reported from South Africa: *S. africanum* Smit, 1971, *S. bizanae* Van den Berg & Heyns, 1973, *S. brachyurus*, *S. cavenessi*, *S. clathricaudatum* Whitehead, 1959, *S. commune* Van den Berg & Heyns, 1973, *S. dreyeri* Van den Berg & De Waele, 1990, *S. nigermontanum* Van den Berg, 1990, *S. sofiae* Van den Berg & Heyns, 1973, *S. sorghi* Van den Berg & De Waele, 1990, *S. transvaalense* Van den Berg, 1981, *S. truncatum* Sher, 1964, *S. tsitsikamense* Van den Berg, 1976 and *S. unum* Sher, 1964 (M. Marais, pers. comm.). In South Africa, *S. brachyurus* has a wide geographical distribution and is the only species of the genus that has been molecularly characterised so far in the country (Van den Berg *et al.*, 2013). The South African populations of *S. brachyurus* are genetically different from those from USA and other world regions and also show some differences in their morphometrics.

Presently, eight *Scutellonema* species are reported in the USA. For many years, *S. brachyurus* and *S. bradys* have been the only species found in the USA. Several reports indicate that the most numerous records of *Scutellonema* species in the USA are from Florida and California (Sher, 1964; Van den Berg *et al.*, 2013). According to Lehman (2002), four *Scutellonema* species have been detected in Florida in addition to *S. bradys* and *S. brachyurus*. They include *S. africanum*, *S. clathricaudatum*, *S. conicephalum* Sivakumar & Selvasekaran, 1982 and *S. grande* Sher, 1964. However, the identification of these species has not been confirmed by subsequent morphological and molecular analyses. Another species, *S. cephalidum* Anderson, Handoo & Townshend, 1984, described in Canada from *Ficus elastica* Roxb., 1819 exported from the Pensacola area of Florida, is morphologically and morphometrically very similar to *S. brachyurus* and may be considered as a synonym of this species. During a recent nematode survey, a population of *S. bradys*

with a truncate tail terminus was found in a pasture in central Florida. In the USA, three records of *S. bradys* were reported from different localities in Arkansas from soybean (Robbins *et al.*, 1987), blackberry (Wehunt *et al.*, 1991) and tomato (Bae *et al.*, 2009).

Recent studies of *Scutellonema* samples from California by Van den Berg *et al.* (2013) have provided evidence of the presence of two species: *S. brachyurus* and a putatively new species referred to as *Scutellonema* sp. A, which was also found together with *S. brachyurus* in some samples. In Florida, *S. brachyurus* occurs commonly on ornamentals, including snake plants (*Sansevieria trifasciata* Prain) and, in some cases, our observations revealed that it is associated with another *Scutellonema* species having numerous males, which are reportedly absent in *S. brachyurus*. The *Scutellonema* samples with males have often been detected by the CDFA Nematology laboratory in plant shipments from Florida traded with California. The representatives of these samples were considered to be morphologically similar to *S. cavenessi*. Previous to this paper, there was only a single report, supported by morphometrics, of *S. cavenessi* from cotton from an unknown location in the USA (Elmiligy, 1970).

A correct characterisation of *Scutellonema* species is important for regulatory purposes and also to improve the knowledge concerning the diversity of *Scutellonema* species infesting American and African cultivated and non-cultivated plants. The main objectives of our study were: *i*) to describe a new South African species of *Scutellonema* found on sugarcane using both morphological and molecular analyses; *ii*) to characterise morphologically and molecularly Florida samples of *Scutellonema* spp.; *iii*) to analyse morphologically and molecularly both *S. transvaalense* and *S. truncatum*, neither previously molecularly characterised; and *iv*) to study phylogenetic relationships within *Scutellonema* using nuclear rRNA gene sequences and *COI* gene sequences.

Materials and methods

NEMATODE POPULATIONS

Nematode populations used in this study were from Botswana, Costa Rica, USA (Florida and California), and geographically diverse locations in South Africa (Table 1). Florida populations of *Scutellonema* were collected from pasture land and the rhizosphere of *S. trifasciata* using sampling tubes in the upper 10-40 cm soil. Other populations from other hosts were collected using a

Table 1. Species and populations of *Scutellonema* used in the study.

Species	Locality	Host	Sample code	GenBank accession numbers			Source
				D2-D3 of 28S rRNA	ITS rRNA	COI	
<i>S. brachyurus</i>	USA, Florida, Mount Dora	<i>Sansevieria trifasciata</i>	CD1943, N15-01326-3	KX959281, KX959283	KX959305	C.L. Spriggs, R. Inseira	
<i>S. brachyurus</i>	Costa Rica	Unknown	CD1380	-	KX959306	S.A. Subbotin	
<i>S. brachyurus</i>	USA, California, Riverside	<i>Ficus nitida</i>	CD1510	-	-	S.A. Subbotin	
<i>S. brachyurus</i>	USA, California, Riverside, White Park	<i>Umbellularia californica</i>	CD1804	-	-	S.A. Subbotin	
<i>S. brachyurus</i>	USA, Florida, Zellwood (intercepted by CDFA, USA)	<i>Sansevieria</i> sp.	CD1771	KX959261	-	S.A. Subbotin	
<i>S. bradyi</i>	USA, Florida, Sanford	<i>Cynodon dactylon</i>	CD1941, N15-0708-3, N16-00978-4	KX959258	KX959299	R. Inseira	
<i>S. cavenessi</i>	USA, Florida, Mount Dora	<i>Sansevieria</i> sp.	CD1908, N15-01178-4	KX959253	KX959300	R. Inseira	
<i>S. cavenessi</i>	USA, Florida, Homestead (intercepted by CDFA, USA)	<i>Sansevieria</i> sp.	CD1470	KX959256	KX959301	S.A. Subbotin	
<i>S. cavenessi</i>	USA, Florida, Lake Worth (intercepted by CDFA, USA)	<i>Sansevieria</i> sp.	CD1542	KX959255	KX959303, KX959304	S.A. Subbotin	
<i>S. cavenessi</i>	USA, Florida (intercepted by CDFA, USA)	<i>Sansevieria</i> sp.	CD1771	KX959252, KX959257	-	S.A. Subbotin	
<i>S. cavenessi</i>	USA, Florida, Mount Dora	<i>S. trifasciata</i>	CD1942, N15-01326-2	KX959254	KX959302	C.L. Spriggs, R. Inseira	
<i>S. clavicaudatum</i> sp. n.	South Africa, Mpumalanga, Komatiport	<i>Saccharum officinarum</i>	CD1853, TVL2063	KX959264- KX959269	-	E. Van den Berg	
<i>S. transvaalense</i>	South Africa, Limpopo, Pont Drift (topotype)	Grasses	TVL2065	-	-	E. Van den Berg	
<i>S. transvaalense</i>	South Africa, Limpopo, Tzaneen	<i>Abelmoschus esculentus</i>	CD1852, LP17	KX959250, KX959251	-	E. Van den Berg	
<i>S. truncatum</i>	Botswana, Sherwood	<i>Brassica oleracea</i> , <i>Solanum tuberosum</i>	CD1917, Bot21	KX959270- KX959272	KX959307, KX959308	E. Van den Berg	
<i>Scutellonema</i> sp. A	USA, California, Riverside, White Park	<i>U. californica</i>	CD1804	-	-	S.A. Subbotin	

similar procedure. Nematodes were extracted from soil by the rapid centrifugal-flotation method (Jenkins, 1964).

Species delimitation of the *Scutellonema* used in this study was undertaken using an integrated approach that considered morphological and morphometric evaluation combined with molecular-based phylogenetic inference (tree-based methods) and sequence analyses (genetic distance methods) (Sites & Marshall, 2004).

LIGHT AND SCANNING ELECTRON MICROSCOPE STUDIES

Scutellonema specimens were killed and fixed in FPG (Netscher & Seinhorst, 1969), transferred to anhydrous glycerin and mounted on permanent slides (De Grisse, 1969). Florida specimens of *S. bradys* were narcotised by gently heating and then mounted in water agar for measurements and photographs (Esser, 1986). Nematodes were studied at the ARC-Plant Protection Laboratory, Nematology Section, Pretoria, South Africa, using a Nikon Labophot 2 microscope with drawing tube. Light micrographs of nematodes were taken in the nematology laboratories of the Nematology Section, FDACS, DPI, Gainesville, FL, USA and CDFA, Sacramento, CA, USA, with an automatic Infinity 2 camera attached to a compound Olympus BX51 microscope equipped with a Nomarski interference contrast.

For scanning electron microscopy (SEM), the samples were killed and fixed in 70% ethanol for at least 12 h and then dehydrated in an ethanol series of 80, 90 and 100% for 15 min each. The samples were critical point-dried using liquid carbon dioxide in a critical point dryer. The dried samples were mounted on SEM stubs with double-sided carbon tape and sputter-coated with 15 nm gold/palladium (66/33%). The coated samples were viewed under a FEI Quanta FEG 250 SEM under high vacuum mode at 5-10 kV.

DNA EXTRACTION, PCR AND SEQUENCING

DNA was extracted from several specimens of each sample using the proteinase K protocol. DNA extraction, PCR and cloning protocols were as described by Tanha Maafi *et al.* (2003) and Van den Berg *et al.* (2013). The following primer sets were used for PCR: the forward D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and the reverse D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') primers (Subbotin *et al.*, 2007) for amplification of the D2-D3 expansion segments of 28S rRNA gene; the forward TW81 (5'-GTT TCC GTA GGT GAA CCT

GC-3') and the reverse AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') primers (Tanha Maafi *et al.*, 2003) or the forward F194 (5'-CGT AAC AAG GTA GCT GTA G-3') and the reverse 26S (5'-TTT CAC TCG CCG TTA CTA AGG-3') primers for amplification of the ITS of rRNA gene; the forward JB3 (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and the reverse JB4 (5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') primers (Derycke *et al.*, 2010) for amplification of the partial *COI* gene of mtDNA. The PCR products were purified using QIAquick (Qiagen) Gel or PCR extraction kits and submitted for direct sequencing or cloned using pGEM-T Vector System II kit (Promega). One to several clones were sequenced from each sample. Sequencing was conducted at the Davis Sequencing Center, Davis, CA, USA, or Quintara Biosciences. The newly obtained sequences were submitted to the GenBank database under accession numbers: KX959250-KX959308 as indicated in Table 1 and the phylogenetic trees.

PHYLOGENETIC AND SEQUENCE ANALYSIS

The newly obtained sequences for each gene (D2-D3 of 28S rRNA, ITS rRNA and the *COI* mtDNA) were aligned using ClustalX 1.83 (Thompson *et al.*, 1997) with their corresponding published gene sequences (Chen *et al.*, 2006; Subbotin *et al.*, 2006, 2007; Bae *et al.*, 2009; Van den Berg *et al.*, 2013; Humphreys-Pereira *et al.*, 2014; Coyne *et al.*, 2016; Tzortzakakis *et al.*, 2016 and others). Outgroup taxa for each dataset were chosen based on previously published data (Subbotin *et al.*, 2006). Sequence datasets were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) under the GTR model as described by Van den Berg *et al.* (2013). Sequence analyses of alignments were performed with PAUP* 4b10 (Swofford, 2003). Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance values based on whole alignment, with adjustment for missing data.

Results

SPECIES IDENTIFICATION AND DELIMITING

By integrating traditional morphological taxonomic characters and molecular criteria we distinguished the following species within the studied samples: *S. clavicaudatum* sp. n., *S. brachyurus*, *S. bradys*, *S. cavenessi*,

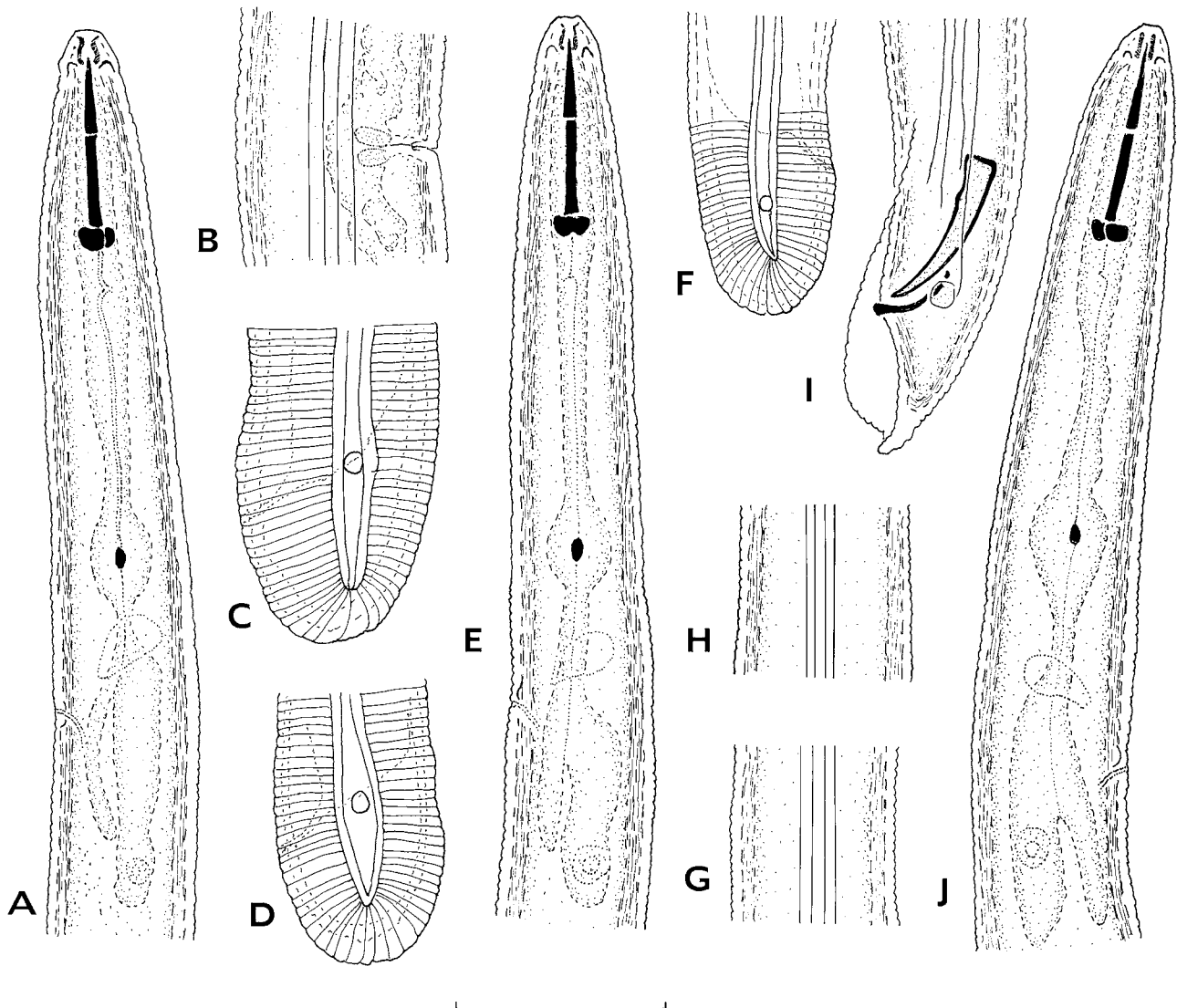


Fig. 1. *Scutellonema clavicaudatum* sp. n. (TVL2063). Female. A: Anterior region of body; B: Vulval region; C, D: Tail variations. Juvenile. E: Anterior region of body; F: Tail; G: Lateral field at mid-body. Male. H: Lateral field at mid-body; I: Tail region; J: Anterior region of body. (Scale bar = 30 μ m.)

S. transvaalense, *S. truncatum* and *Scutellonema* sp. A. Samples of *S. brachyurus* from Costa Rica, Florida and California and *Scutellonema* sp. A from California were only analysed molecularly. The soil samples examined in this study were mostly monospecific, although two samples contained a mixture of two species: *S. cavenessi* and *S. brachyurus* from Florida; and *S. brachyurus* and *Scutellonema* sp. A from California, USA. Morphological descriptions with illustrations (Figs 1-21) and morphometric values (Tables 2-9) for five species are given below.

***Scutellonema clavicaudatum** sp. n.**
(Figs 1, 2, 19A; 20A, B; 21A)

Specimens were collected from sugarcane in the Komatipoort area, Mpumalanga Province on 2 July 2012 by personnel of the South African Sugar Research Institute.

* Specific epithet derived from the Latin term *clava* = club, and the adjective of the Latin term *cauda* = tail.

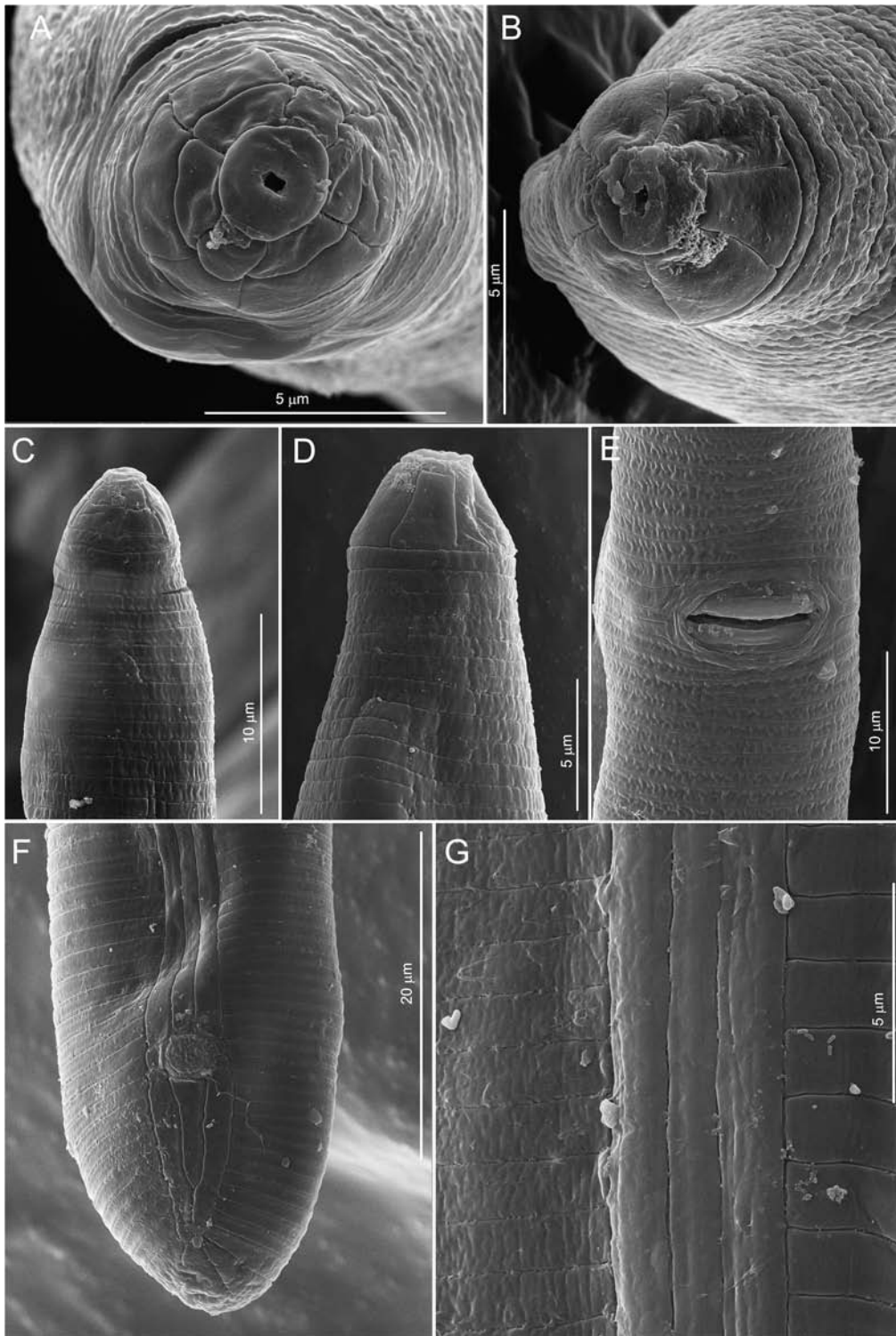


Fig. 2. *Scutellonema clavicaudatum* sp. n. (TVL2063). Female. A, B: *En face* views of lip region; C, D: Lateral views of lip region; E: Vulval region; F: Tail; G: Lateral field at mid-body.

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Body form varying from slightly arcuate ventrad, open or closed letter C and a complete circle, sometimes with the lip region and the tail region crossing. Under LM, lip region conical, labial disc not so distinct. Labial disc well defined with SEM. Basal part slightly wider than that of adjoining body, but not distinctly set off. On most specimens no annuli seen on lip region. In SEM, labial disc well defined and round, in some specimens surrounded by large trapezoidal submedian lip sectors and by two small, almost semicircular lateral lip sectors containing amphidial apertures. Lip annuli absent and replaced by six large rectangular blocks (Fig. 2A). In other specimens, rectangular blocks fused with submedian and lateral lip sectors forming six trapezoidal elongated blocks surrounding labial disc, as in *S. truncatum* (Fig. 2B). Labial framework moderately developed, stretching posteriorly one or two annuli from basal plate. Cephalids not observed. Stylet well developed with knobs rounded posteriorly and mostly flattened to slightly hollow anteriorly. Metenchium shorter than telenchium. Median bulb not unusually large and basal pharyngeal lobe crossing over intestine as normal. Excretory pore situated from opposite middle of isthmus to opposite posterior part of basal pharyngeal lobe. Hemizonid two annuli long, situated over excretory pore or immediately anterior. Hemizonion not seen. Lateral field consisting of four lines delimiting three equal bands. No areolation observed in anterior region nor opposite scutellum. One female with a few crenations in outer lines just posterior to stylet region and in some one or two or fragments of lines visible opposite scutellum. Lateral field continuing for 8-12 annuli posterior to scutellum, usually narrowing at tip and ending in a bluntly pointed shape. Spermatheca varying from small, round and filled with sperm cells, to large, oblong and filled with sperm cells. Epiptygma mostly folded into vagina and not clearly seen. Vaginal glands large and well developed. Intestine not overlapping rectum. Scutellum 2.0-4.5 μm wide, situated from directly anterior to anus to four annuli anterior to anus. Caudalid not seen. Tail rounded and mostly clavate, widening from just anterior of scutellum with 9-14 annuli, a few annuli on tip sometimes wider than on rest of body.

Male

Very similar to female with regard to most characters. Scutellum situated from opposite to just posterior to cloacal aperture. Tail ending in a finger-like hyaline tip. Bursa not enveloping tail tip.

Juvenile

One found. Very similar to female but tail not clavate.

TYPE HOST AND LOCALITY

Associated with sugarcane *Saccharum officinarum* L. rhizosphere in the Komatipoort area, Mpumalanga Province, South Africa.

TYPE MATERIAL

Holotype female, 11 paratypes of females and seven of males are deposited in the South African Plant Parasitic Nematode Survey collection PPRI, Pretoria, South Africa. Holotype female and male slide number 50516; all other paratypes on slides 50517-50522.

DIAGNOSIS AND RELATIONSHIPS

Scutellonema clavicaudatum n. sp. is characterised by females having a conical lip region without lip annuli, these being replaced by six large rectangular blocks separated or fused with the submedian and lateral lip sectors surrounding a round and distinct labial disc. Females also have large vaginal glands, a functional spermatheca, lateral field posterior to the scutellum ending in a bluntly pointed shape, and a clavate tail.

When following the key of Sher (1964), the specimens of *S. clavicaudatum* sp. n. key out at *S. truncatum*. However, females of *S. clavicaudatum* sp. n. can immediately be separated from those of *S. truncatum* in having a clavate tail but less convex dorsally, lacking areolations in the anterior part of the body and opposite the scutellum, lip region less conical, and also longer body of 898 (786-977) vs 600 (500-800) μm and stylet = 32 (28.5-35.5) vs 25.7 (21.3-29) μm . Males are separated by the longer body of 834.5 (786-919) vs 552 (544-562) μm , stylet = 30 (29.5-31) vs 22 (22-23) μm , tail = 25 (22-27) vs 10 (7-12) μm , and spicules = 30 (27-32) vs 20 μm .

When following the key of Smit (1971) and Germani *et al.* (1985), *S. clavicaudatum* sp. n. can key out at *S. africanum* or *S. siamense* Timm, 1965. *Scutellonema clavicaudatum* sp. n. can be differentiated from *S. africanum* by having a lip region conical and without annuli vs

Table 2. Morphometrics of female and male *Scutellonema clavicaudatum* sp. n. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Female		Male	Juvenile
	Holotype	Paratypes	Paratypes	Paratype
n	1	11	7	1
L	977	898 \pm 63.5 (786-977)	834 \pm 54 (768-919)	726
a	34.1	28.5 \pm 3.5 (23.0-34.1)	32.2 \pm 1.2 (30.6-33.8)	28.2
b	6.4	6.8 \pm 0.6 (5.8-8.1)	6.3 \pm 0.8 (5.3-7.7)	5.3
b'	7.7	8.3 \pm 0.7 (7.3-9.0)	7.4 \pm 1 (6.3-9.1)	6.6
c	63.3	61.7 \pm 7.7 (50.9-74.9)	33.4 \pm 3.4 (29-37.9)	31.9
c'	0.7	0.6 \pm 0.09 (0.4-0.7)	1.6 \pm 0.2 (1.3-1.7)	1.1
o	17.1	14.4 \pm 2.9 (10.5-18.6)	18.5 \pm 4.9 (13-25)	17.5
DGO	5.0	4.5 \pm 0.8 (3.5-6.0)	5.5 \pm 1.5 (4.0-7.5)	5.0
V/T	60.5	55.5 \pm 2.3 (53.0-60.5)	36.3 \pm 11.2 (26.8-59.0)	–
OV ¹	20.0	20.5 \pm 3.7 (16.5-25.5)	301 \pm 91.8 (217-492)	–
OV ¹ length	194	186 \pm 29 (137-215)	–	–
OV ²	16.5	20 \pm 3.6 (16-26)	–	–
OV ² length	162	179 \pm 21.3 (148-203)	–	–
Stylet length	30	32 \pm 1.8 (28.5-35.5)	30 \pm 0.7 (29.5-31)	29
Metenchium length	13	14 \pm 1.2 (12-16)	13 \pm 0.9 (12-14)	12
Telenchium length	17	17 \pm 0.8 (16-19)	17 \pm 0.8 (16-18)	17
m	43.9	45.0 \pm 1.7 (42.8-47.1)	44.7 \pm 2.7 (39.1-47.6)	42.5
Stylet knob height	3.5	3.5 \pm 0.3 (3.0-3.5)	3.0 \pm 0.4 (3.0-4.0)	3.5
Stylet knob width	5.0	6.0 \pm 1.1 (5.0-8.5)	5.0 \pm 0.6 (4.5-6.0)	5.0
Pharynx length	153	132 \pm 10.5 (115-153)	134 \pm 8.1 (119-143)	136
Pharyngeal overlap	25	21 \pm 5.6 (12-29)	19 \pm 3.3 (14-23)	26
Excretory pore from anterior	112	109 \pm 8.4 (98-122)	108 \pm 6.5 (99-117)	108
Diam. at mid-body	28	32 \pm 4.1 (28-41)	26 \pm 1.1 (24-27)	25
Diam. at anus	22	24 \pm 3.1 (20-29)	17 \pm 1.8 (15-21)	20
Median bulb length	16.0	13.5 \pm 2.1 (9.5-16)	12.5 \pm 0.7 (12-13)	13.0
Median bulb diam.	10.5	10.5 \pm 1.2 (9-12)	9.0 \pm 1.2 (7.5-10.0)	11.0
Median bulb valve length	3.0	3.0 \pm 0.5 (2.5-3.5)	3.0	3.0
Median bulb valve width	2.5	2.0 \pm 0.5 (1.5-3.0)	1.5, 2.0 (n = 2)	2.0
Lip region diam.	9.0	9.5 \pm 0.6 (9.0-10.5)	8.5 \pm 0.6 (7.5-9.0)	9.0
Lip region height	5.0	5.5 \pm 0.6 (5.0-6.5)	5.5 \pm 0.4 (5.0-6.0)	5.0
Annulus width	1.5	1.5	1.5	2.0
Lateral field width	5.0	5.5 \pm 0.5 (5.0-6.5)	5.0 \pm 0.5 (4.5-6.0)	5.0
Tail length	15	15 \pm 2.1 (11-16)	25 \pm 2.2 (22-27)	23
Scutellum width	2.5	3.0 \pm 0.7 (2.0-4.5)	3.5 \pm 0.5 (2.5-3.5)	3.0
Spermatheca length	14	16 \pm 3.2 (14-22)	–	–
Spermatheca width	11	12 \pm 1.1 (11-14)	–	–
Spicule length	–	–	30 \pm 1.6 (27-32)	–
Gubernaculum length	–	–	14.5 \pm 0.7 (13.0-15.5)	–
h	–	–	9.5 \pm 1.2 (8.0-12.0)	–

rounded and annulated, lateral fields not areolated anteriorly *vs* areolated, a longer body (898 (786-977) *vs* 700 (500-810) μm), and a longer stylet (32.0 (28.5-35.5) *vs* 25.5 (22.8-28.3) μm). The males of *S. clavicaudatum* sp. n. are separated from *S. africanum* by having a conical non-annulated lip region *vs* rounded and annulated; a bursa not enveloping the tail tip *vs* bursa with posterior margins passing the tail tip and forming two wings, and by the longer tail of 22-27 *vs* 7.4-13.5 μm (Smit, 1971; Van den Berg & Heyns, 1973; Germani *et al.*, 1985).

Scutellonema clavicaudatum sp. n. can be separated from *S. siamense* by the longer females (898 (786-977) *vs* 650 (590-720) μm) with a longer stylet (32.0 (28.5-35.5) *vs* 27.0 (26.0-27.5) μm), smaller o value (10.5-18.6 *vs* 27-32) and greater stylet knob width (5.0-8.5 *vs* 4.0 μm). Furthermore, the lip region is non-annulated *vs* annulated, the lateral field is not areolated anteriorly or opposite the scutellum *vs* areolated, and by the longer and clavate tail *vs* not clavate. The males are longer (834.5 (768-919) *vs* 600 (510-660) μm) and have a smaller c value of 33.4 (29-37.9) *vs* 41 (36-47), longer stylet (30 (29.5-31) *vs* 20-25 μm), longer spicules (30 (27-32) *vs* 20-27 μm), and a smaller o value (13-25 *vs* 28-32) (Timm, 1965; Ratanaprapa & Boonduang, 1975; Boonduang & Pliansinchai, 1980; Germani *et al.*, 1985; Rahaman & Ahmad, 1995).

There are quite a few *Scutellonema* species lacking areolation opposite the pharyngeal area and/or scutellum, as in *S. clavicaudatum* sp. n., although most have a rounded, slightly set off, annulated lip region and none has a clavate tail. *Scutellonema clavicaudatum* sp. n. differs from similar species such as *S. coffeae* Giribabu & Saha, 2002 by the presence of males and lateral field lacking crenations *vs* lack of males and crenate outer lines of the lateral field over the entire length of the body in *S. coffeae*. It differs also from *S. commune* by the presence of males and by having a non-areolated lateral field anteriorly *vs* absence of males and areolated lateral field anteriorly. *Scutellonema clavicaudatum* sp. n. can be separated from *S. dreyeri* by the longer stylet of 32.0 (28.5-35.5) *vs* 25.0 (23.5-27.5) μm , and by the more anterior position of the scutellum in both females and males *vs* a more posterior position on the tail in *S. dreyeri*. *Scutellonema clavicaudatum* sp. n. also differs from *S. labiatum* Siddiqi, 1972 by the longer females (898 (786-977) *vs* 610 (570-770) μm) and males (834 (768-919) *vs* 580 (540-640) μm), both sexes having a longer stylet of 32 (28.5-35.5) *vs* 22.5 (20.0-25.0) μm and 30.0 (29.5-31.0) *vs* 21.5 (20.0-25.0) μm , respectively. The bursa in *S.*

clavicaudatum sp. n. does not envelop the tail tip whereas in *S. labiatum* it envelops the tail tip to form two lobes posterior to the tail tip (Germani *et al.*, 1985). Finally, *S. clavicaudatum* sp. n. differs from *S. paralabiatum* Siddiqi & Sharma, 1994, by the presence of males *vs* absence in *S. paralabiatum*, and by having females with a longer body (898 (786-977) *vs* 780 (650-870) μm) and stylet (32.0 (28.5-35.5) *vs* 26.7 (25.0-28.0) μm) (Siddiqi & Sharma, 1994; Van den Berg *et al.*, 2003).

***Scutellonema bradys* (Steiner & LeHew, 1933) Andrássy, 1958**

(Figs 3, 19B, C; 20C-E; 21B)

The sample of *S. bradys* collected from pasture land in central Florida consisted of females, males and a few juveniles. The number of specimens observed was variable and, in some cases, reached 53 specimens per 100 cm³ soil.

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Morphology of Florida specimens agreeing with description provided by Sher (1964). Body slightly arcuate or straight with a hemispherical and deeply set off lip region marked by 7-9 annuli. Basal lip annulus without longitudinal striations. Body annuli 1.4 μm wide. Stylet robust with round basal knobs. Pharyngeal gland lobe overlapping intestine dorsally. Pharyngeal overlap variable in length. Excretory pore at level of terminal portion of pharyngeal gland lobe. Hemizonid not visible. Spermathecae distinct, oval, filled with sperm. Spermatozoa appearing flagellate with round heads collectively orientated towards uterus and with flagellate tails orientated in opposite direction towards oviduct. Vaginal glands small, often indistinct. Vulval slit with thickened edges. Epiptygma barely visible, not protruding from vagina. Scutellum round. Tail cylindrical with truncate terminus marked by 18 annuli. Lateral field posterior to scutellum appearing as two elongate, areolated and finger-like bands extending posteriorly from scutellum and enclosing a third, small, pointed band.

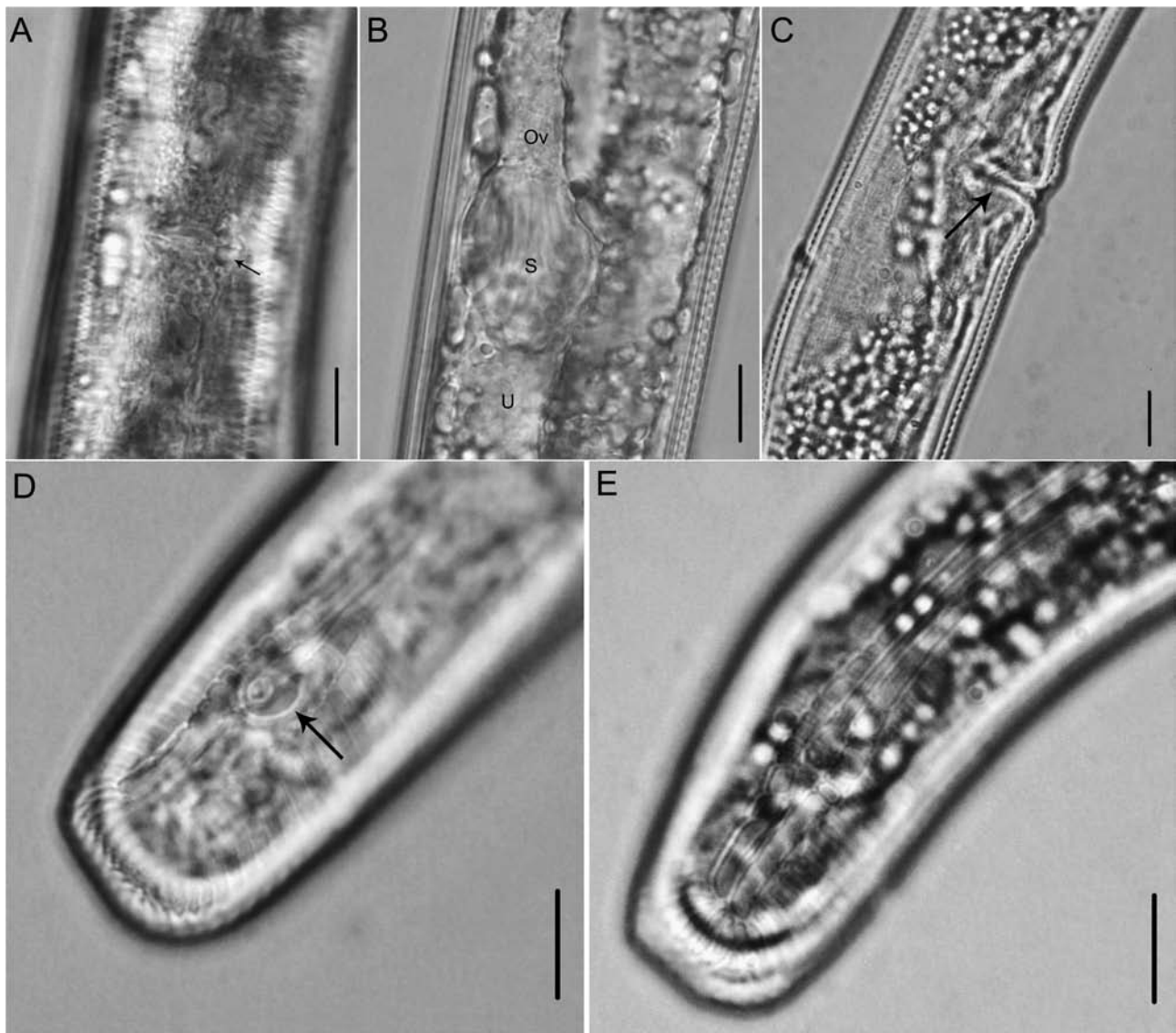


Fig. 3. *Scutellonema bradys* from central Florida. Female. A: Ventral view of vulval slit with sclerotised edges (arrow); B: Oval spermatheca (S) filled with sperm. Note flagellate spermatozoa with rounded heads collectively orientated toward the uterus (U) and flagellate tails orientated in the opposite direction toward the oviduct (Ov); C: Vagina (arrow); D, E: Tail with truncate terminus. Note the round scutellum (arrow) in D and the lateral field posterior to the scutellum ending as two elongate, areolated and finger-like bands enclosing a small third band in E.

MALE

Similar to female except for sexual characters. Scutellum situated more or less at mid-tail.

REMARKS

The specimens of this Florida population compare very well with those described by Sher (1964) for an-

other population, which was also from Florida. They showed the most important characters of this species, such as the sclerotised sides of the vulva, the distinctly rounded, offset lip region with 7-9 annuli and an inconspicuous epiptygma. The size of their body was similar to that of type specimens. Female body length ranged from 871 to 1154 vs 1200 μm as reported by Steiner & LeHew (1933) and 860 to 1110 μm as reported by

Table 3. Morphometrics of female and male *Scutellonema bradyi* from central Florida, USA, compared with those in the literature. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Florida population (N16-00978-4)		Florida population (Sher. 1964)		Jamaica population (Germani <i>et al.</i> , 1985)		Florida population (N16-00978-4)		Florida population (Sher. 1964)		Jamaica population (Germani <i>et al.</i> , 1985)	
	Females	n	Females	20	Females	14	Males	6	Males	10	Males	15
n		9										
L	1030 \pm 102 (871-1154)		780-1160		1000 (860-1110)		981 \pm 57.4 (890-1054)		720-810		880 (660-960)	
a	26.7 \pm 1.5 (23.2-28.3)		21-32		26.1 (23.6-30.3)		29.4 \pm 2 (26-31.4)		22-28		27.5 (23.6-33)	
b	6.3 \pm 0.8 (4.7-7.4)		6.3-9.4		5.2-6.7		5.9 \pm 0.3 (5.4-6.3)		5.9-7		5.2 (4.1-6.8)	
b'	8.1 \pm 0.7 (6.7-9)		5.7-7.4		-		7.5 \pm 0.4 (7-8.2)		4.5-5.6		-	
c	44.4 \pm 4.7 (37.4-49.8)		35-54		43.1 (35-51)		29.6 \pm 1.9 (27.3-33)		25-30		30.5 (26.5-39.6)	
c'	0.8 \pm 0.1 (0.6-1.0)		-		0.95 (0.7-1.3)		1.4 \pm 0.1 (1.3-1.6)		-		1.4 (1.1-1.6)	
o	24.4 \pm 4.6 (18-29.9)		18-26		23.6 (17.2-26.9)		28.6 \pm 3.1 (24.4-32.2)		-		23.8 (16.3-35.3)	
DGO	6.5 \pm 1.2 (4.9-8.0)		-		-		7.6 \pm 0.8 (6.4-8.5)		-		-	
V	56.1 \pm 1.2 (54.3-58.6)		53-61		50.8 (48.1-53.8)		-		-		-	
OV ¹	23.6 \pm 3.3 (19.3-28)		-		-		-		-		-	
OV ¹ length	239 \pm 39.3 (200-322)		-		-		-		-		-	
OV ²	22.9 \pm 3.4 (18-28.3)		-		-		-		-		-	
OV ² length	231 \pm 33.2 (192-277)		-		-		-		-		-	
Stylet length	26.9 \pm 0.9 (24.7-27.5)		24-30		27.9 (26.0-29.5)		26.4 \pm 0.3 (26.2-27.0)		24-29		26.5 (25.0-28.0)	
Metenchium length	13.0 \pm 0.5 (12.3-13.5)		-		-		13.0 \pm 0.2 (12.8-13.3)		-		-	
Telenchium length	13.8 \pm 0.7 (12.4-15.0)		-		-		13.3 \pm 0.3 (13.1-13.8)		-		-	
m	48.6 \pm 1.6 (45.2-50.9)		-		-		49.5 \pm 0.7 (48.8-50.3)		-		-	
Stylet knob height	3.6 \pm 0.3 (3.3-4.0)		-		-		3.1 \pm 0.1 (3.0-3.4)		-		-	
Stylet knob width	6.1 \pm 0.2 (5.9-6.4)		-		-		5.2 \pm 0.2 (4.9-5.4)		-		-	
Pharynx length	126 \pm 8.3 (112-139)		-		-		129 \pm 2.7 (127-133)		-		-	
Pharyngeal overlap	37.4 \pm 11 (18-53.4)		-		-		35.9 \pm 3 (31.6-39.6)		-		-	
Pharyngeal gland lobe from anterior	163 \pm 15.7 (134-183)		-		-		164 \pm 3.3 (160-169)		-		-	
Excretory pore from anterior	141 \pm 10.5 (129-156)		-		-		146 \pm 8 (139-160)		-		-	
Diam. at mid-body	38.5 \pm 3 (33.6-42.6)		-		-		33.3 \pm 1.5 (31.6-35.6)		-		-	
Diam. at anus	29.3 \pm 2.8 (23.7-32.6)		-		-		22.2 \pm 0.7 (21.3-23.2)		-		-	
Median bulb length	17.2 \pm 1.6 (13.8-19)		-		-		17.0 \pm 1.6 (15.3-19.8)		-		-	
Median bulb diam.	14.8 \pm 1 (12.4-15.8)		-		-		12.0 \pm 0.8 (11.0-12.8)		-		-	
Median bulb valve length	3.7 \pm 0.3 (3.2-4.0)		-		-		3.4 \pm 0.3 (3-3.8)		-		-	
Median bulb valve width	2.7 \pm 0.3 (2.1-3.0)		-		-		2.5 \pm 0.3 (2.2-2.9)		-		-	
Lip region diam.	12.0 \pm 0.6 (11.3-12.9)		-		-		11.6 \pm 0.4 (10.8-11.9)		-		-	
Lip region height	6.8 \pm 0.7 (5.8-7.9)		-		-		6.8 \pm 0.2 (6.3-6.9)		-		-	
Annulus width	1.4 \pm 0.2 (1.0-1.7)		-		-		1.4 \pm 0.1 (1.3-1.6)		-		-	
Lateral field width	7.3 \pm 0.7 (6.1-8.8)		-		-		7.1 \pm 0.4 (6.7-7.7)		-		-	

Table 3. (Continued.)

Character	Florida population N16-00978-4 (Sher, 1964)		Jamaica population (Germani <i>et al.</i> , 1985)		Florida population N16-00978-4 (Sher, 1964)		Jamaica population (Germani <i>et al.</i> , 1985)	
	Females	Males	Females	Males	Females	Males	Females	Males
Tail length	23.5 ± 4.1 (17.5-30.6)	-	-	33 ± 2.3 (30.6-36.5)	-	-	-	-
Scutellum width (external diam.)	3.4 ± 0.7 (2.1-4.4)	-	2.1 (1.5-3.5)	3.5 ± 0.4 (3.0-3.9)	-	-	2.0 (1.5-2.5)	-
Scutellum width (internal diam., ampulla)	6.0 ± 0.6 (5.0-6.9)	-	4.1 (3.0-5.0)	5.6 ± 0.6 (5.0-6.7)	-	-	3.7 (3.0-4.0)	-
Spermatheca length	21.1 ± 2.6 (15.8-23.7)	-	-	-	-	-	-	-
Spermatheca width	19.9 ± 3.4 (15.3-24.5)	-	-	-	-	-	-	-
Tail annuli	18 ± 2.0 (16-22)	-	-	44.8 ± 3.9 (37.7-48)	-	-	-	-
T length	-	-	-	440 ± 35.9 (381-484)	-	-	-	-
Spicule length	-	-	-	33.9 ± 0.7 (32.8-34.6)	-	-	26-33	32.5 (27.5-35.5)
Gubernaculum length	-	-	-	16.7 ± 1 (15.8-18.3)	-	-	13-16	13.6 (10.5-16.5)

Siddiqi (1972) for an African population. However, the tail of female specimens showed a truncate terminus and a pronounced oval spermatheca with spermatozoa having round heads and flagellate tails. The biology of the Florida *S. bradys* is not well known. This species has been known in the state for many decades (Sher, 1964; Lehman, 2002) in association with bushes and trees grown in non-cultivated land, including azalea (*Rhododendron* spp.), castor bean (*Ricinus communis* L.), oak (*Quercus virginiana* Mill.), and also vegetable crops such as cabbage, *Brassica oleracea* L., without any clear evidence that these plants are suitable hosts for the nematode. These records do not include the most economically important host of this species, yam (*Dioscorea* spp.), as this vegetable is not grown commonly or commercially grown in Florida. Our studied population from central Florida was found in soil particles adhering to the surface of Bermuda grass, *Cynodon dactylon* (L.) Pers. roots in pasture land.

***Scutellonema cavenessi* Sher, 1964**

(Figs 4, 5, 6A-F; 7-12; 19F-H; 20 H-L; 21C, D)

Specimens collected from various nurseries and localities from Florida, USA (Table 1). This species has been consistently found on *S. trifasciata* and other *Sansevieria* species. The population levels were 15 specimens (100 cm³ soil)⁻¹.

MEASUREMENTS

See Tables 4-6.

DESCRIPTION

Female

Body form varying from slightly arcuate ventrad to an open or closed letter C. Lip region broadly rounded, well set off with 6-7 lip annuli, basal annulus without longitudinal striations First lip annulus (labial plate) almost square in shape, consisting of a distinct rounded labial disc surrounded by enlarged and thickened submedian lip sectors and small lateral lip sectors which contain circular amphidial apertures. Labial framework well developed, stretching posteriad one annulus from basal plate. Stylet well developed with stylet knobs rounded posteriorly and mostly slightly hollow anteriorly, knobs occasionally higher and appearing almost tulip-like. Mentenchium mostly shorter than telenchium (m = 42-50%).

Anterior and posterior cephalids seen in a very few specimens, anterior cephalids 2-4 and posterior cephalids 8-9 annuli posterior to base of lip region. Basal pharyngeal bulb crossing over intestine dorsally. Excretory pore situated from opposite anterior part to opposite posterior part of pharyngeal lobe, usually just anterior or posterior to junction of pharynx with intestine. Hemizonid two annuli long, situated from opposite excretory pore to two annuli anterior to excretory pore. Hemizonion not seen. Lateral field starting as two or three lines a few annuli posterior to lip region, them becoming four lines with three equal bands. All bands well areolated in pharyngeal area and on tail, but occasionally areolated on rest of body, outer lines sometimes with a few crenations. Areolation on tail sometimes very irregular around scutellum with inner band also containing a few irregular blocks around scutellum. Spermatheca varying from small to large, round to oblong and empty or filled with rounded sperm cells. In some specimens sperm very distinct but not seen in others. Flagellate spermatozoa observed in a few specimens. Epiptygma prominently projecting outward or with two lips folded over each other on outside of body, very seldom (5-10% of observed specimens) folded into vagina. Vaginal glands indistinct or small and rounded. Intestine usually not extending posterior to anus but in a few specimens a very slight overlap visible. Caudalid not seen. Tail with 11-22 mostly broadly to slightly narrowly rounded annuli. Annuli on tail sometimes larger or irregular. Scutellum situated from one annulus anterior to two annuli posterior to anus but mostly opposite anus.

Male

Similar to female except for sexual characters. Scutellum situated more or less at mid-tail.

Juvenile

A few juveniles were found of which stages could only be judged by measurements. Very similar to female, but in some specimens lateral field at mid-body appearing to be slightly more areolated than in female and scutellum situated from 4-9 annuli posterior to anus. Tail with 16-20 annuli.

REMARKS

The Florida specimens compare very well with those reported in the literature. When following the key of Germani *et al.* (1985) this species is very close to *S.*

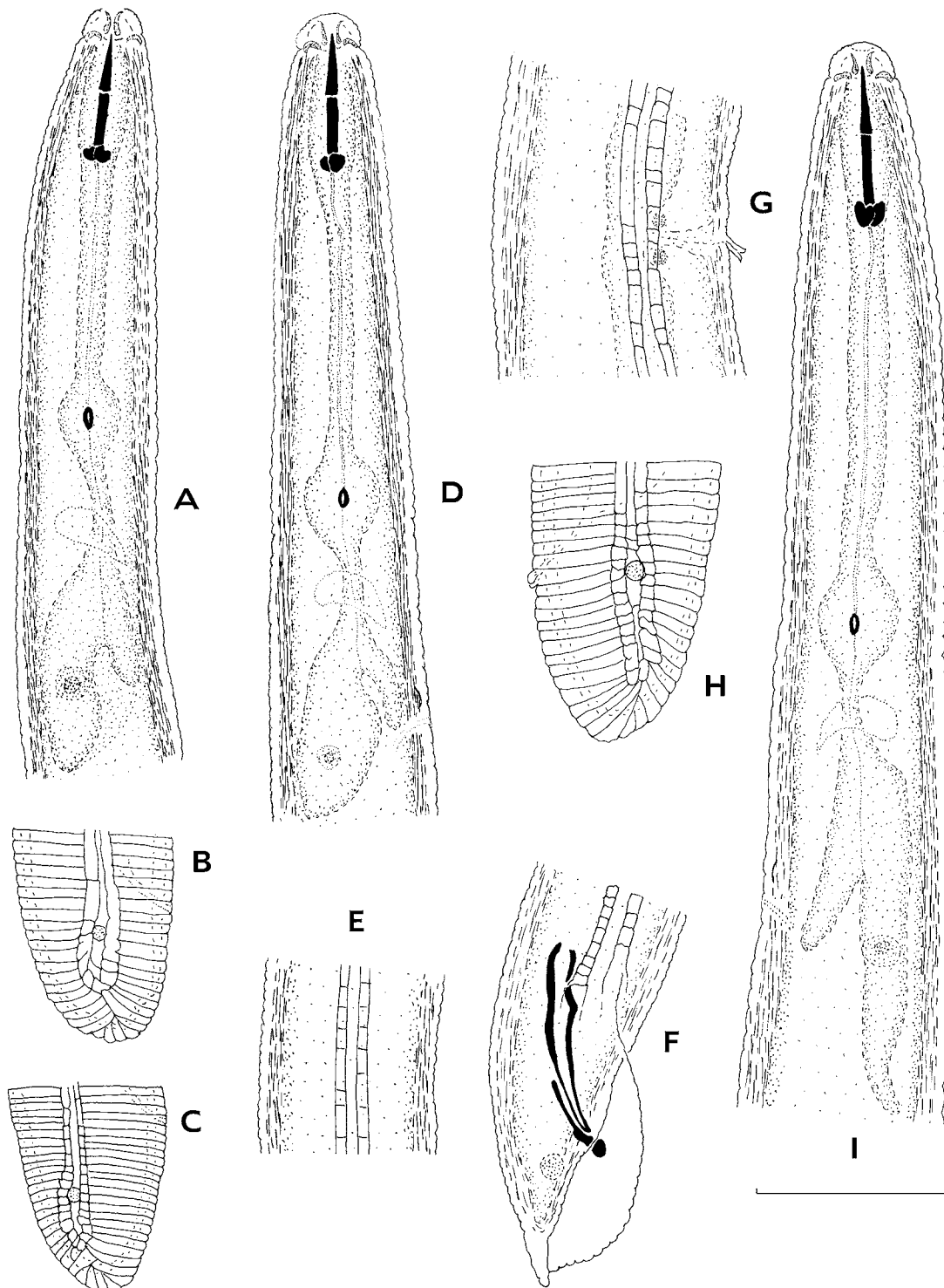


Fig. 4. *Scutellonema cavenessi* (CD1470). Female. A: Anterior region of body; B, C: Tail variations. Male. D: Anterior region of body; E: Lateral field at mid-body; F: Tail. *Scutellonema cavenessi* (CD1771). Female. G: Vulval area with protruding epiptygma; H: Tail. I: Anterior region of body. (Scale bar = 30 μm .)

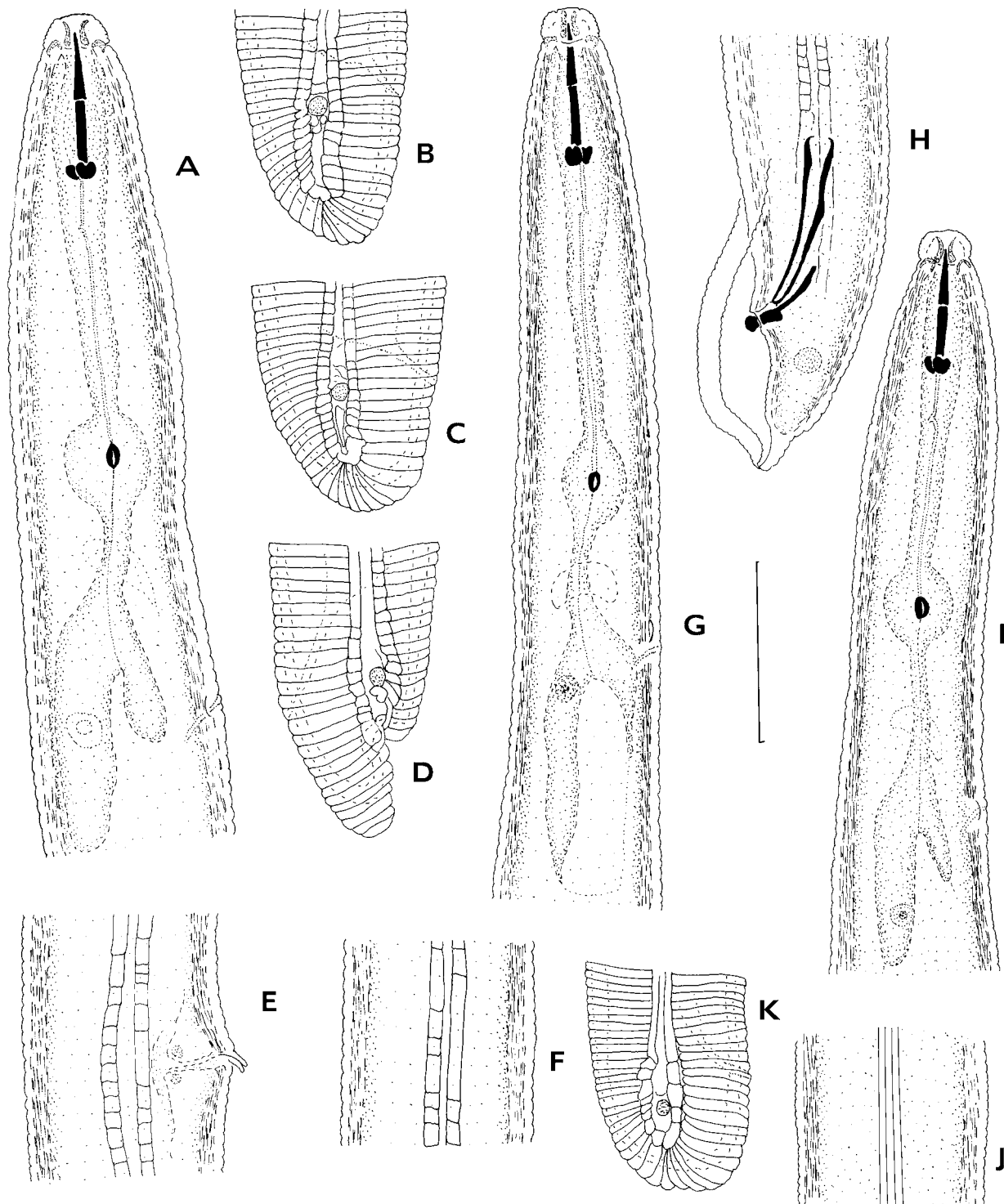


Fig. 5. *Scutellonema cavenessi* (CD1542). Female. A: Anterior region of body; B-D: Tail variations; E: Vulval region with protruding epiptygma. Male. F: Lateral field at mid-body; G: Anterior region of body; H: Tail. Juvenile. I: Anterior region of body; J: Lateral field at mid-body; K: Tail. (Scale bar = 30 μ m.)

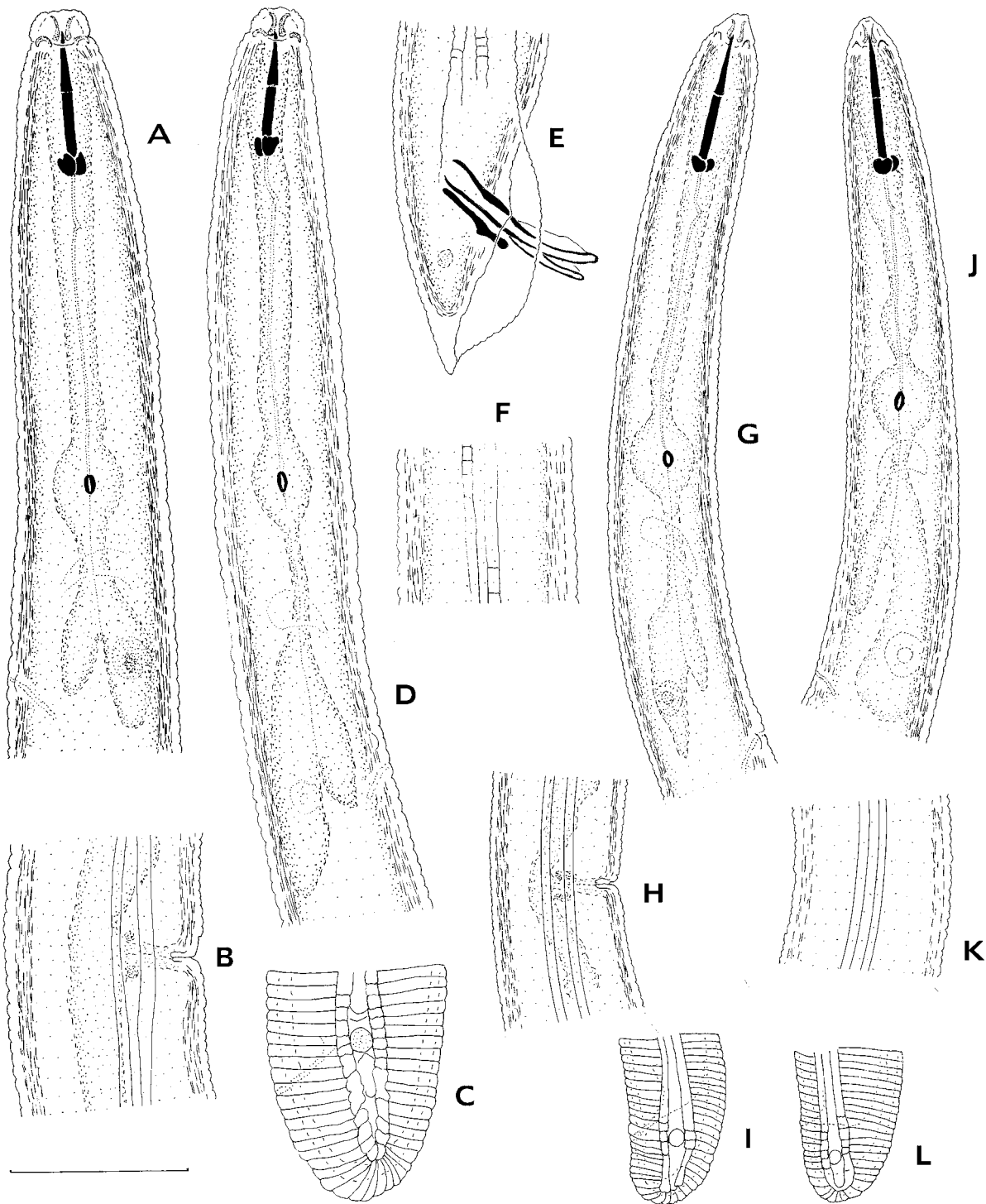


Fig. 6. *Scutellonema cavenessi* (CD1908). Female. A: Anterior region of body; B: Vulval region with epiptygma folded in; C: Tail. Male. D: Anterior region of body; E: Tail; F: Lateral field at mid-body. *Scutellonema truncatum*. (Bot21). Female. G: Anterior region of body; H: Vulval region with infolded epiptygma; I: Tail. Juvenile. J: Anterior region of body; K: Lateral field at mid-body; L: Tail. (Scale bar = 30 μ m.)

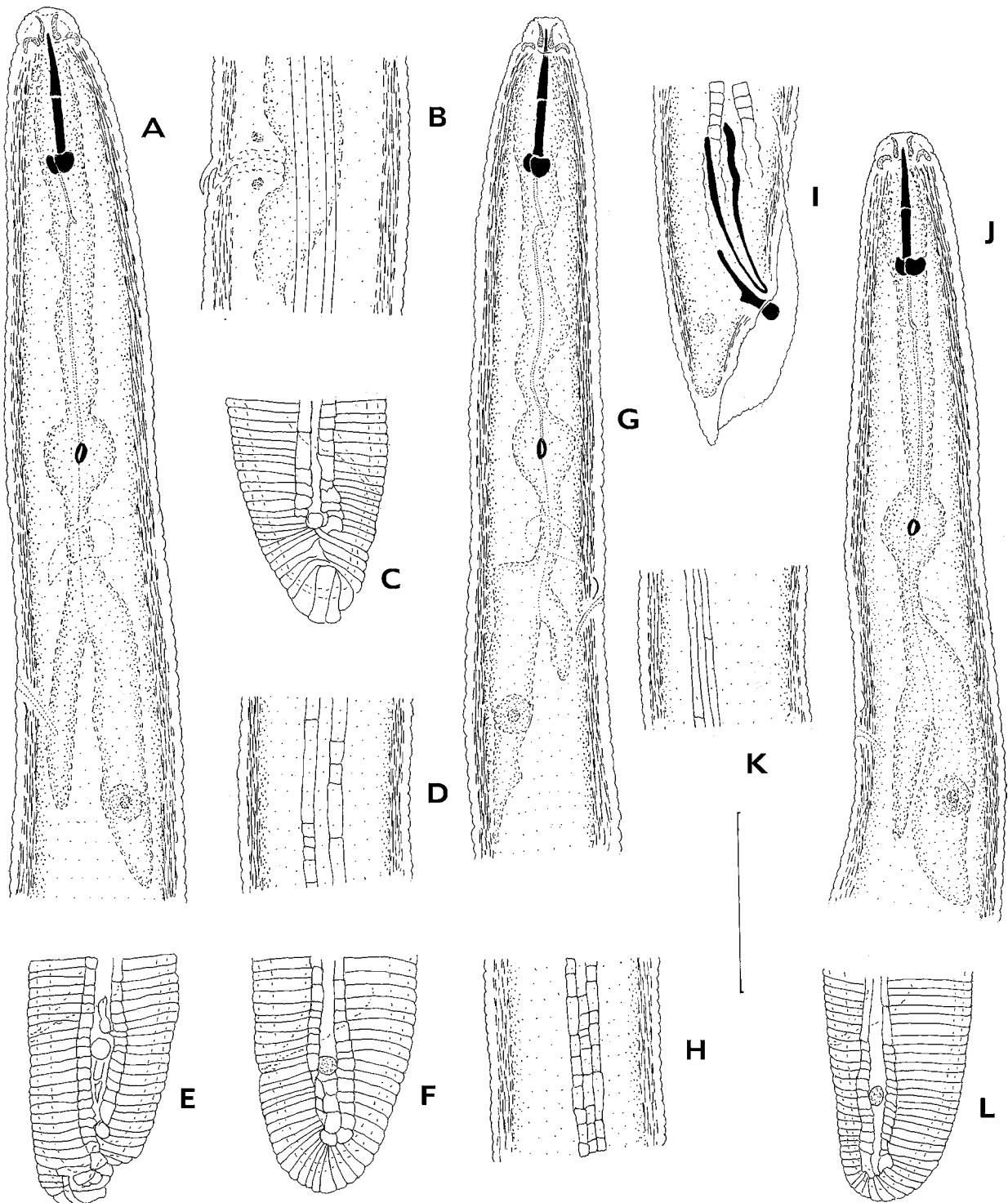


Fig. 7. *Scutellonema cavenessi* (CD1942, N15-01326-2). Female. A: Anterior region of body; B: Vulval region with protruding epiptygma; C: Tail; D: Lateral field at mid-body; E, F: Tail variations. Male. G: Anterior region of body; H: Lateral field at mid-body; I: Tail. Juvenile. J: Anterior region of body; K: Lateral field at mid-body; L: Tail. (Scale bar = 30 μ m.)

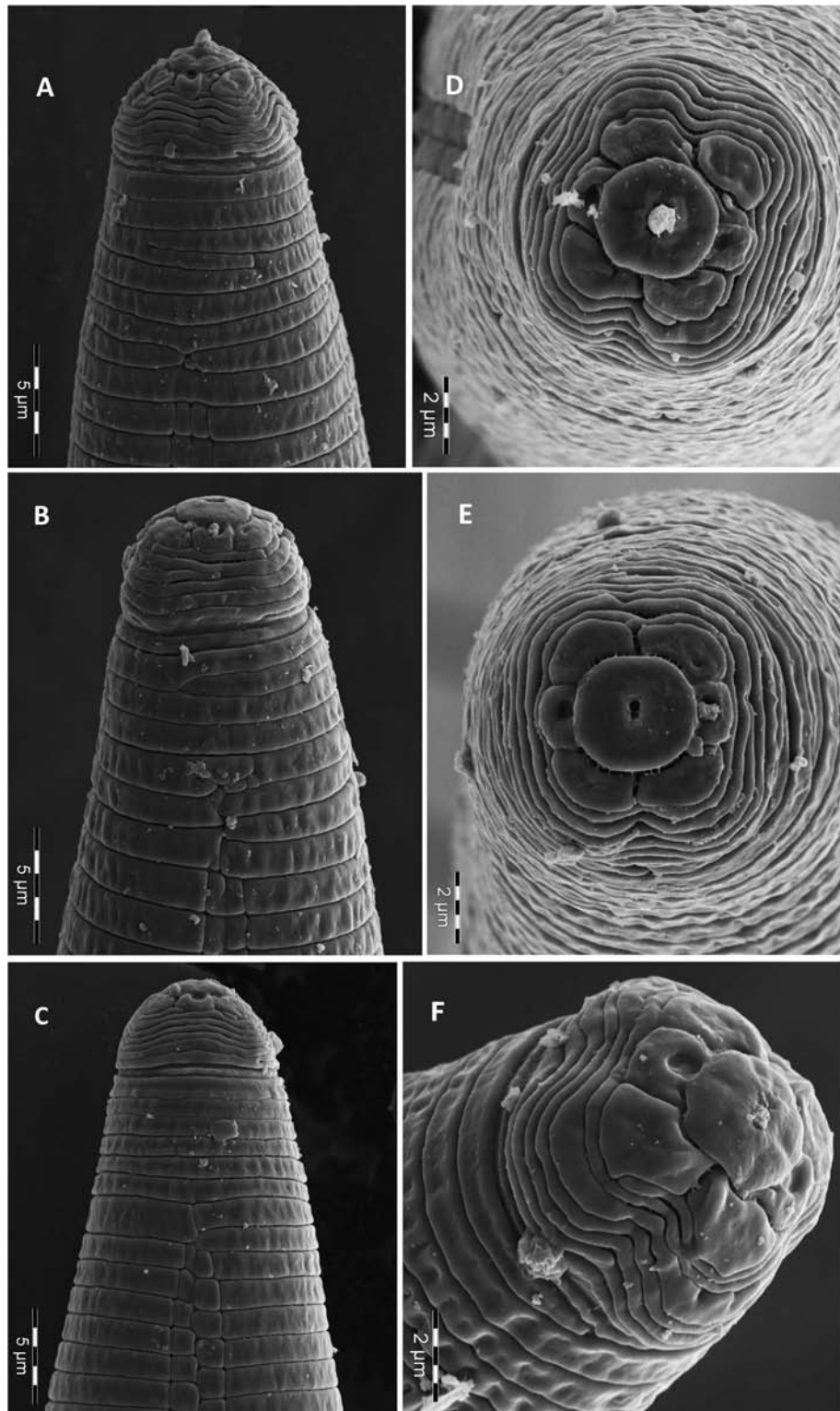


Fig. 8. *Scutellonema cavenessi* (CD1542). Female. A-C: Lateral views of lip region; D-F: *En face* views of lip region.

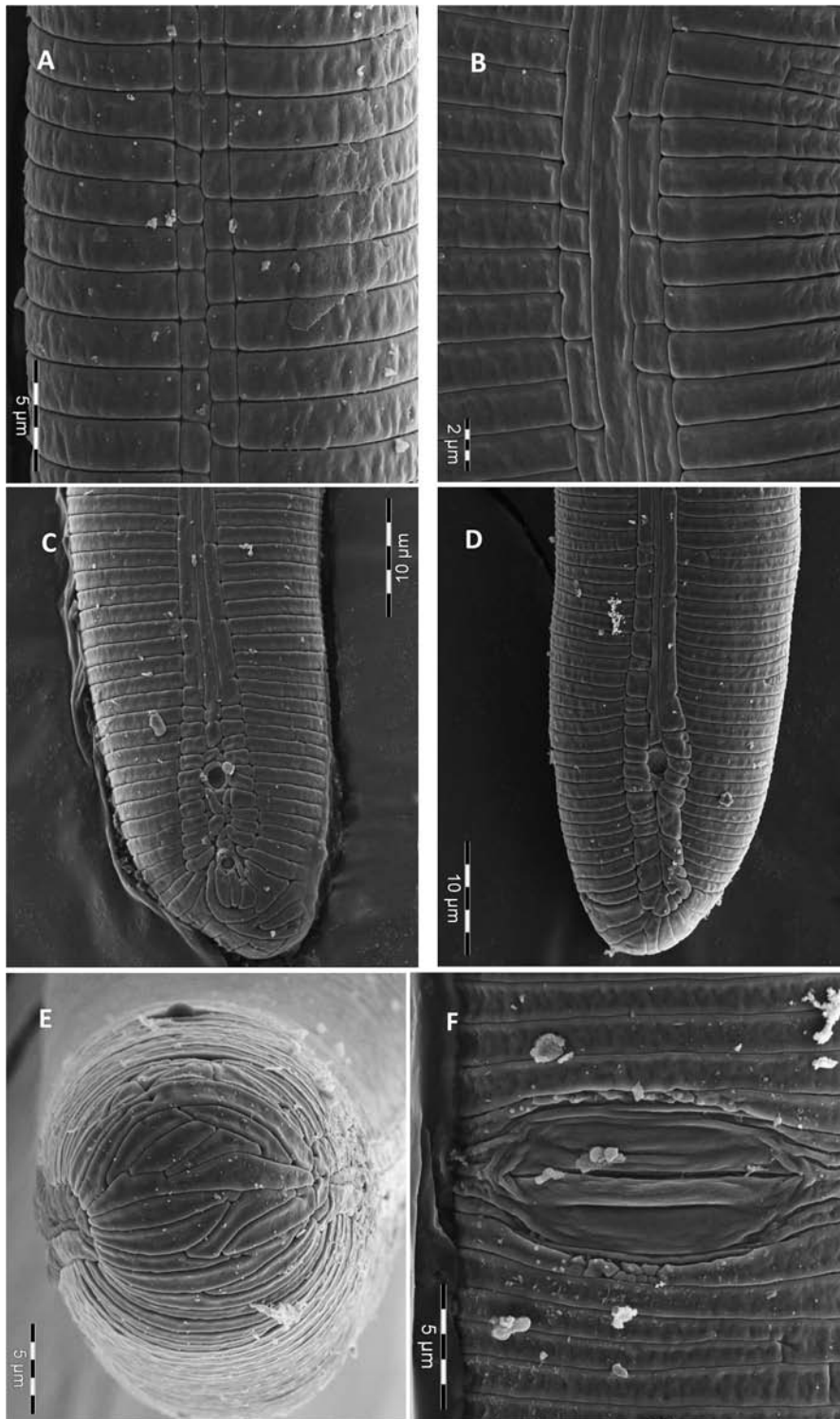


Fig. 9. *Scutellonema cavenessi* (CD1542). Female. A: Lateral field in pharyngeal area; B: Lateral field at mid-body; C, D: Tail variations; E: Posterior view of tail tip; F: Vulval region lacking protruding epitygma.

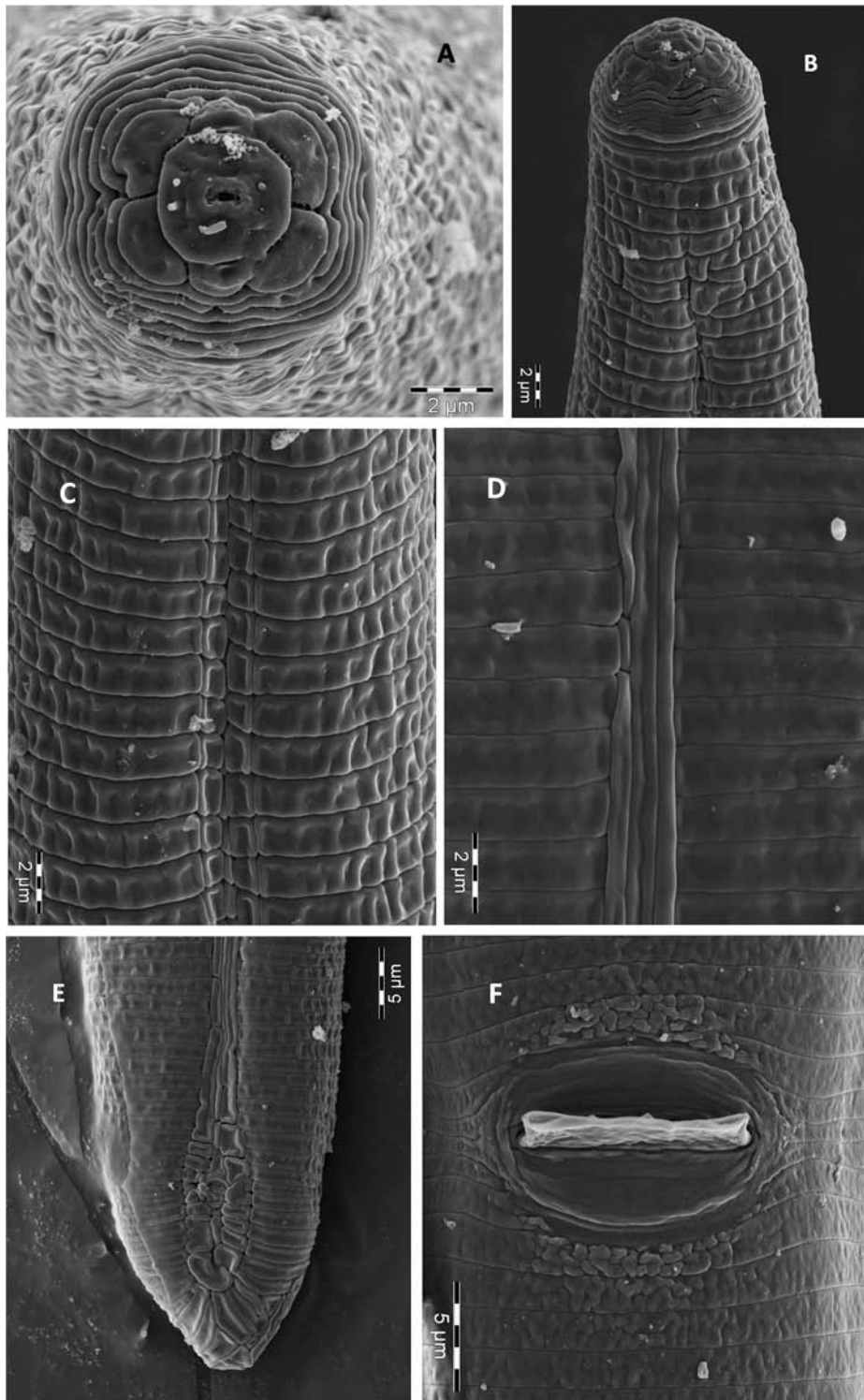


Fig. 10. *Scutellonema cavenessi* (CD1542). Juvenile. A: En face view of lip region; B: Lateral view of lip region; C: Lateral field at mid-body; D: Lateral field in pharyngeal area. Female. E: Tail; F: Vulval region with protruding epitygma.

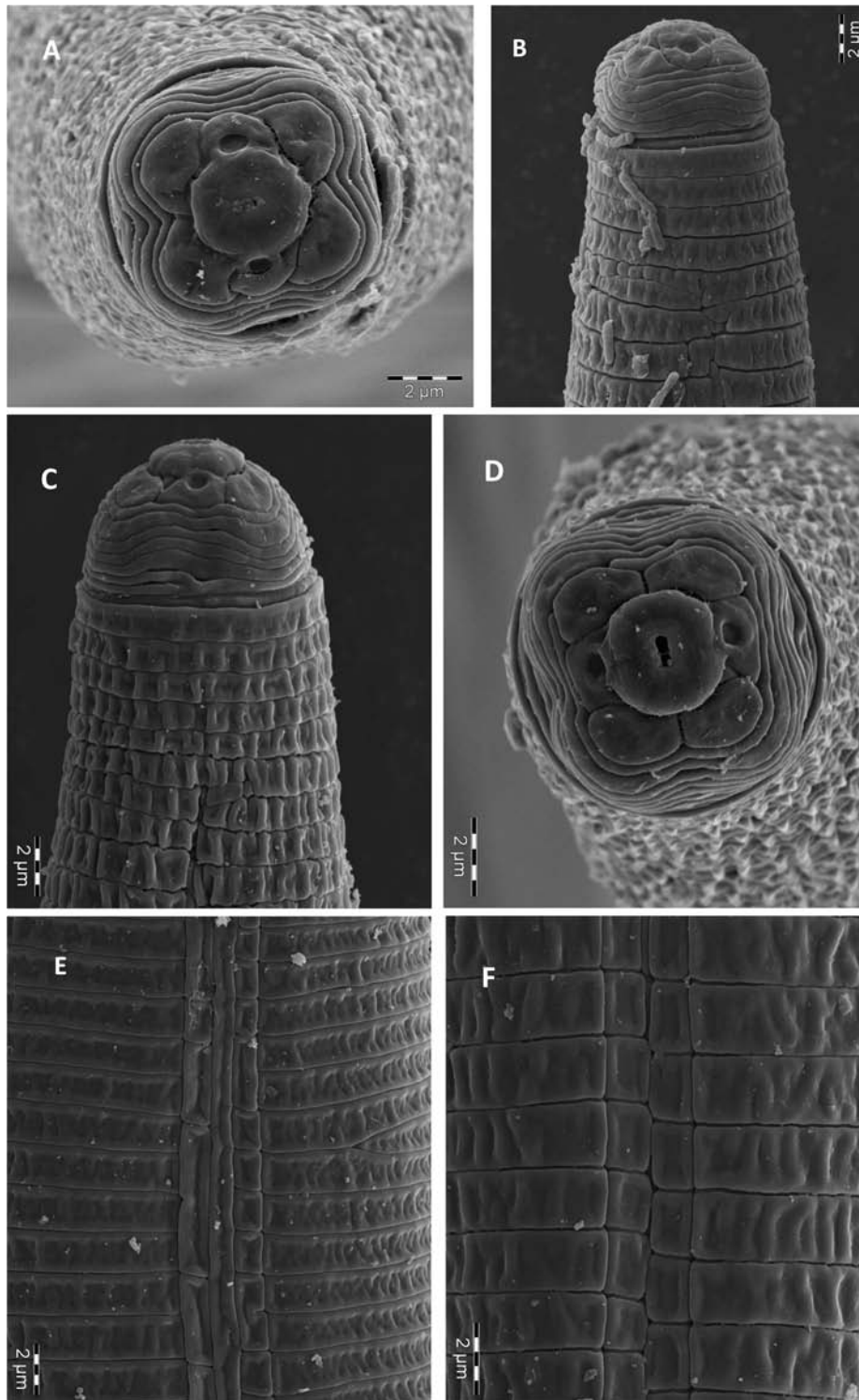


Fig. 11. *Scutellonema cavenessi* (CD1542). Male. A, D: *En face* views of lip region; B, C: Lateral view of lip region; E: Lateral field near mid-body; F: Lateral field in pharyngeal region.

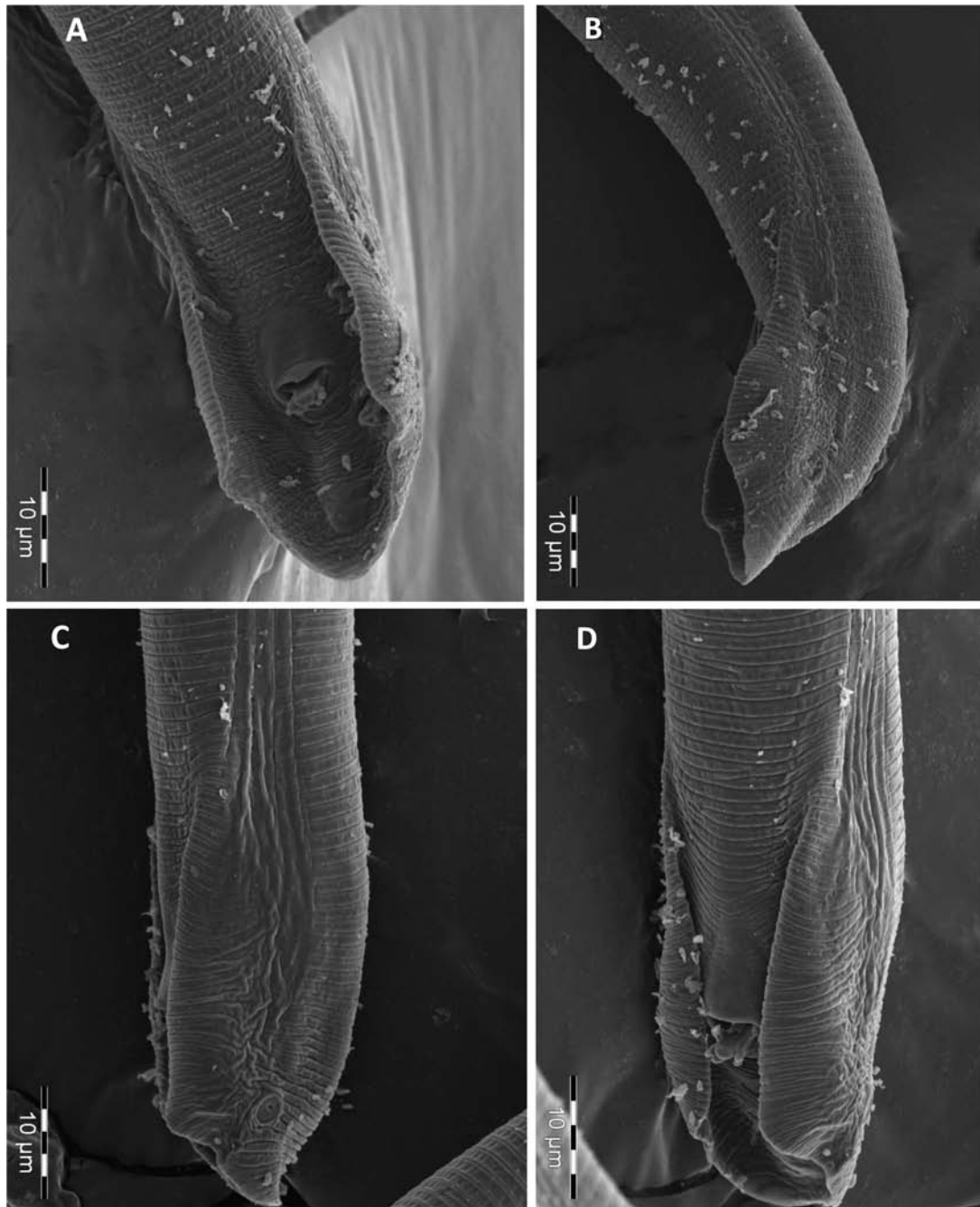


Fig. 12. *Scutellonema cavenessi* (CD1542). Male. A-D: Various views of male tail.

bradys, which shares a similar lip pattern configuration. However, the *S. bradys* female shows distinctly more sclerotised vaginal walls and inconspicuous epitygma that are barely visible and which do not pro-

trude from the vagina. In *S. cavenessi*, the vulval lips are not thickened and the epitygma are mostly outside the vagina and projecting outward, or folded and protruding partially from the vulva. The males of *S. bradys*

Table 4. Morphometrics of female *Scutellonema cavenessi* from various localities in Florida, USA, compared with those in the literature. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	CD1542	CD1771	CD1908	CD1942, N15-01326-2	Literature*
n	14	4	1	11	115
L	851 \pm 84.8 (710-999)	916 \pm 72.4 (824-1000)	927	861 \pm 47 (771-913)	600-945
a	25.2 \pm 4.4 (17.9-34)	26.9 \pm 3.9 (21.5-30.9)	27.5	25.8 \pm 3.1 (21.0-29.3)	17-30
b	5.9 \pm 0.7 (5.1-7.4)	6.2 \pm 0.7 (5.7-7.1)	–	6 \pm 0.5 (5.2-6.8)	5.2-8.5
b'	7.2 \pm 0.6 (6.5-8.4)	7.5 \pm 0.8 (6.4-8.3)	–	7.4 \pm 0.5 (6.4-7.9)	4.5-7.3
c	40 \pm 5.1 (39.9-48.2)	33.9 \pm 3.9 (28.9-38.3)	50.5	40.1 \pm 7 (31-52.2)	22.8-58.6
c'	0.8 \pm 0.1 (0.6-0.9)	0.9 \pm 0.2 (0.8-1.2)	0.7	0.9 \pm 0.2 (0.5-1.2)	0.57-1.2
o	31.5 \pm 3.9 (25.7-40.6)	37.3 \pm 1.3 (36.4-38.2)	29.4	29.8 \pm 3.3 (24.2-34.4)	8.9-34.7
DGO	8.0 \pm 0.8 (6.6-9.6)	9.0 \pm 0.6 (9.0-9.5)	7.5	7.5 \pm 0.8 (6.0-8.0)	5-7
V	57.5 \pm 1.2 (56.0-60.0)	58.0 \pm 1 (57.0-59.0)	61	57.0 \pm 2.4 (55.0-63.0)	52-62
OV ¹	23.4 \pm 3.6 (17-30)	22.0 \pm 5.2 (19.0-26.0)	–	19.5 \pm 8.9 (18.5-31)	–
OV ¹ length	197 \pm 35.4 (164-240)	216 \pm 60.8 (173-259)	–	187 \pm 60.5 (143-276)	–
OV ²	25.0 \pm 4.4 (19.5-35.5)	19 \pm 0.7 (18.0-20.0)	–	27.5 \pm 5.4 (20.0-31.0)	–
OV ² length	211 \pm 33.9 (173-263)	181 (n = 2)	–	233 \pm 56 (160-313)	–
Stylet length	24.0 \pm 1.1 (22.0-26.5)	24.5 \pm 0.4 (24.0-2.05)	25.0	25.0 \pm 1.2 (23.5-27.0)	20.6-29.0
Metenchium length	11 \pm 0.7 (10-12)	11	11	12 \pm 0.6 (11-12)	–
Telenchium length	13 \pm 0.8 (12-15)	14 \pm 0.6 (13-15)	14	13 \pm 0.8 (12-14)	–
m	45.0 \pm 1.9 (42.4-49.1)	43.7 \pm 1.8 (41.2-45.5)	44	46.5 \pm 1.5 (44.5-50.0)	42-57
Stylet knob height	3.0 \pm 0.4 (2.0-4.0)	3.0 \pm 0.4 (3.0-4.0)	2.5	3.0 \pm 0.4 (2.5-3.5)	–
Stylet knob width	5.0 \pm 0.5 (4.0-6.0)	5 \pm 0.5 (5.0-6.0)	5	5 \pm 0.6 (4.5-6.0)	–
Pharynx length	146 \pm 7.3 (135-156)	147 \pm 19.4 (128-174)	–	142 \pm 8.7 (129.5-152)	115-142
Pharyngeal overlap	27 \pm 7.5 (15-40)	25 \pm 12.6 (14-39)	–	29 \pm 4.2 (18.5-33)	–
Excretory pore from ant. end	119 \pm 8.2 (104-130)	132 \pm 11.3 (118-143)	120	118 \pm 6.7 (105-130)	95-125
Diam. at mid-body	34 \pm 4.5 (29-42)	34 \pm 2.6 (32-38)	34	34 \pm 2.4 (30-37)	–
Diam. at anus	28 \pm 2.3 (23-31)	29	27	26 \pm 2.1 (23-30)	–
Median bulb length	15.5 \pm 0.9 (14.0-17.0)	15.5 \pm 1.3 (14.0-17.0)	–	15.0 \pm 1 (13.0-17.0)	–
Median bulb diam.	12.5 \pm 1 (11.0-14.0)	11.0 \pm 1.4 (9.5-12.5)	–	11.5 \pm 0.7 (10.5-12.5)	–
Median bulb valve length	4 \pm 1.0 (3.0-6.0)	3.5 \pm 0.3 (3.0-4.0)	–	3.0 \pm 0.5 (2.5-4.0)	–
Median bulb valve width	3 \pm 0.4 (2.0-4.0)	3.0 \pm 0.5 (2.0-3.5)	–	2.5 \pm 0.2 (2.0-3.0)	–
Lip region diam.	11.0 \pm 0.8 (10.5-12.0)	11.0 \pm 1 (10.5-12.5)	11.0	10.5 \pm 0.5 (9.5-11.0)	–
Lip region height	6.0 \pm 0.7 (4.5-7.0)	6.0 \pm 0.2 (5.5-6.0)	5.0	5.5 \pm 0.6 (5.0-7.0)	–
Annulus width	2.0 \pm 0.2 (2.0-3.0)	2.5 \pm 0.2 (2.0-3.0)	2.5	2.0 \pm 0.5 (1.5-3.0)	1.1-2.6
Lateral field width	6.0 \pm 0.9 (4.0-7.0)	6.0 \pm 0.6 (5.5-7.0)	6.5	7.0 \pm 0.7 (6.5-9.0)	6.2-6.9
Tail length	21 \pm 2.7 (17-25)	27 \pm 4.8 (24-34)	18.5	22 \pm 4.5 (16-29)	11-31
Scutellum width	3.5 \pm 0.5 (3.0-4.5)	4.0	4.0	4.0 \pm 0.8 (3.0-6.0)	1.3-5.0
Spermatheca length	20.5 \pm 3.1 (18.0-26.0)	–	–	20.0 \pm 3.4 (14.0-24.0)	–
Spermatheca width	15.5 \pm 1.3 (14.0-17.5)	–	–	14.5 \pm 1.4 (13.0-17.0)	–

* Sher (1964); Van den Berg & Heyns (1973); Germani *et al.* (1985); Sakwe & Geraert (1992).

have a lower, smoothly contoured bursal margin whereas in *S. cavenessi* they are more prominently lobed. Female and male morphometrics of the Florida populations were in the range of those reported in the literature and also of an American population from cotton (Elmiligy, 1970), although the female body of the Florida population was longer than that of females as-

sociated with cotton (L = 1030 (871-1154) vs 720 (680-760) μm).

***Scutellonema transvaalense* Van den Berg, 1981**
(Figs 13-16; 19I; 20M; 21E)

Scutellonema transvaalense was described from several localities in the northern part of Limpopo Province, South

Table 5. Morphometrics of male *Scutellonema cavenessi* from various localities in Florida, USA, compared with those in the literature. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	CD1542	CD1771	CD1908	CD1470	CD1942, N15-01326-2	Literature*
n	13	1	3	1	6	76
L	805 \pm 87.6 (661-975)	847	867 \pm 12.7 (853-878)	701	775 \pm 43.9 (718-821)	500-800
a	25.1 \pm 2.7 (22-32.5)	28.1	26.3 \pm 3.6 (22.7-29.9)	22.7	25.5 \pm 2.7 (22.2-28.6)	17.9-30
b	5.5 \pm 0.4 (4.8-6.3)	5.8	5.5	5.5	5.7 \pm 0.5 (5.1-6.4)	5.8-8.2
b'	6.9 \pm 0.7 (6.1-8.6)	–	7.1 \pm 0.2 (6.9-7.3)	6.7	7.3 \pm 0.6 (6.7-8.3)	4.6-6.8
c	30.0 \pm 3.7 (23.5-37.5)	32.9	32.3 \pm 2.8 (29.1-34.1)	27.3	29.0 \pm 2.7 (26.2-31.6)	21.0-55.2
c'	1.5 \pm 0.2 (1.1-1.8)	1.4	1.3 \pm 0.2 (1.2-1.5)	1.6	1.5 \pm 0.1 (1.3-1.6)	–
o	31.9 \pm 3.6 (26.7-37.3)	41.9	33 \pm 2.1 (31.4-34.4)	31.3	30.6 \pm 2.1 (28.2-32.8)	–
DGO	7.0 \pm 0.8 (6.0-8.0)	9.5	8.0	7.5	7.5 \pm 0.6 (6.5-8.0)	–
Stylet length	23 \pm 1.1 (21-25)	23	24 \pm 1 (23-25)	23	24 \pm 0.7 (23-24)	20-25
Metenchium length	11 \pm 0.8 (9-12)	10.5	11 \pm 0.8 (10-12)	11.5	11 \pm 0.7 (10-11)	–
Telenchium length	12 \pm 0.6 (12-13)	12.5	13 \pm 0.9 (12-14)	12	13 \pm 0.7 (12-14)	–
m	45.4 \pm 2 (41.3-48.2)	45.2	46.4 \pm 1.2 (45.2-47.6)	48.5	45.7 \pm 2.7 (41.4-48.6)	–
Stylet knob height	3.0 \pm 0.5 (2.0-4.0)	3.0	3.5 \pm 0.5 (3.0-4.0)	3.0	3.0 \pm 0.5 (2.5-4.0)	–
Stylet knob width	5.0 \pm 0.5 (4.0-5.0)	4.5	5.0	4.5	4.0 \pm 0.8 (3.5-5.5)	–
Pharynx length	147 \pm 8.8 (136-165)	147	154 \pm 5.5 (148-159)	126	137 \pm 8.4 (123-146)	–
Pharyngeal overlap	31 \pm 5.6 (22-45)	18	31 \pm 4.8 (26.5-36)	21	33 \pm 8.8 (23-40)	–
Exc. pore from ant. end	119 \pm 13 (102-145)	123.5	126 \pm 6.2 (122-133)	111	106 \pm 6.5 (99-117)	–
Diam. at mid-body	32 \pm 2.6 (28-36)	30	33 \pm 4.1 (29-37)	31	31 \pm 1.9 (28-34)	–
Diam. at anus	18 \pm 1.3 (16-20)	18.5	19 \pm 2.8 (16-21)	16	19 \pm 0.6 (18-20)	–
Median bulb length	14 \pm 1.4 (12.5-18)	15	14 \pm 1.5 (13-16)	–	13.5 \pm 0.9 (13-15)	–
Median bulb width	11 \pm 0.8 (10-12)	10	9 \pm 0.5 (9-9)	–	11 \pm 0.9 (9-12)	–
Median bulb valve length	3.0 \pm 0.4 (2.5-4.0)	2.0	3.0 \pm 0.4 (2.5-3.5)	–	3.0 \pm 0.5 (2.5-4.0)	–
Median bulb valve width	2.5 \pm 0.4 (2.0-3.0)	2.0	2.0	–	2.5 \pm 0.5 (1.5-3.0)	–
Lip region diam.	10 \pm 0.7 (8.5-11)	11	10.5 \pm 0.6 (10-11)	9	10 \pm 0.8 (8-10)	–
Lip region height	5.0 \pm 0.7 (4.5-6.5)	4.5	5.0 \pm 0.8 (4.5-6)	4.5	5.0 \pm 0.4 (5.0-6.0)	–
Annulus width	2.5 \pm 0.2 (2.0-3.0)	2.0	2.5 \pm 0.2 (2-3)	2.0	2.0	–
Lateral field width	6.0 \pm 0.8 (4.5-6.5)	5	5.5 \pm 0.5 (5.0-6.0)	6	6.0 \pm 0.5 (5.5-7.0)	–
Tail length	27.0 \pm 2.8 (23.0-33.0)	26.0	27.0 \pm 2.1 (26.0-29.5)	2.06	27.5 \pm 1.9 (25.0-30.0)	13.6-28.7
Scutellum width	3.5 \pm 0.3 (3.0-4.0)	4.5	3.5 \pm 0.6 (3.0-4.0)	3.0	3.5 \pm 0.6 (3.0-4.5)	1.5-3.5
T	46.9 \pm 10.1 (34.2-74.7)	47.0	49.1 \pm 6.8 (42.2-55.7)	48.6	42.1 \pm 6.9 (32.4-51.2)	–
T length	371 \pm 75 (270-581)	398.5	426 \pm 64.8 (360-489)	341	326 \pm 54.5 (256-397)	–
Spicule length	31 \pm 1.5 (29-34)	32	32 \pm 0.8 (31-33)	33	33 \pm 1.3 (31-34)	13-35
Gubernaculum length	16 \pm 1.1 (13-17)	15	16 \pm 1.3 (14-17)	15.5	16 \pm 0.6 (14-16)	7-17

* Sher (1964); Van den Berg & Heyns (1973); Germani *et al.* (1985).

Africa (Van den Berg, 1981). Herein, two collections, also from Limpopo Province, were found and identified as *S. transvaalense*: sample TVL2065 was found in natural veldt near Pont Drift on 10 February 2013 and characterised only morphologically, whilst sample LP17 was found on 5 February 2015 from okra roots near Tzaneen and characterised both morphologically and molecularly.

MEASUREMENTS

See Tables 7, 8.

DESCRIPTION

Female

Body almost straight to slightly arcuate ventrad and extremely obese in some specimens. Lip region broadly rounded, flattened, well set off from body with annuli very indistinct although 6-7 apparently present. SEM photo showing lip region marked by no less than six annuli, despite specimen being covered with bacteria. Basal annulus appearing to have no longitudinal striations. Labial framework moderately developed, stretching posteriad one or

Table 6. Morphometrics of juvenile *Scutellonema cavenessi* from various localities of Florida, USA. All measurements are in μm and in the form: mean \pm s.d. (range). Where only two individuals are present the values for each specimen are separated by a comma.

Character	CD1542	CD1470		CD1942, N15-01326-2
	?4 th stage	?1 st stage	?3 rd stage	?4 th stage
n	5	1	2	2
L	640 \pm 86.3 (566-781)	287	562, 568	625, 743
a	21.2 \pm 4.1 (17.1-27.9)	10.7	17.8, 18.4	20.7, 23.5
b	4.9 \pm 0.7 (4.2-5.9)	2.5	4.2, 4.6	4.6, 6
b'	6.1 \pm 0.8 (5.3-7.3)	3.2	5.3, 5.5	7.1
c	26.8 \pm 2.9 (22.0-29.5)	15.0	20.7, 25.7	25.0, 33.7
c'	1.0 \pm 0.2 (0.8-1.4)	1.1	1.1, 0.9	1.1, 0.9
o	35.4 \pm 5.9 (27.1-40.7)	44.0	36.7, 44.1	31.7, 31.2
DGO	7.5 \pm 1.2 (6.0-9.0)	8.0	8.0, 9.5	7.5
Genital primordium	134 \pm 32.4 (96-155)	6 cells	–	–
Stylet length	21 \pm 0.5 (20-22)	18	22, 21	23, 23
Metenchium length	9 \pm 0.6 (9-10)	8	9.5, 10	11, 12
Telenchium length	12 \pm 0.4 (11-12)	10	12	12
m	44.1 \pm 2.1 (42.4-47.5)	44.0	45.0, 44.0	47.6, 50.0
Stylet knob height	2.0 \pm 0.5 (2.0-3.5)	2.0	3.5, 3.0	2.0
Stylet knob width	4.5 \pm 0.5 (4.0-5.0)	4.0	4, 5	5.0
Pharynx length	131 \pm 8.3 (121-142)	114	134, 124	135, 123
Pharyngeal overlap	26 \pm 8.5 (16-38)	24.5	28, 21	23.5, 18.5
Excretory pore from anterior	106 \pm 5.7 (98-114)	103	107, 111	105
Diam. at mid-body	30 \pm 1.9 (28-33)	27	31, 31	30, 30
Diam. at anus	25.5 \pm 6 (18.0-34.0)	17.5	25.0, 26.0	23.5, 18.5
Median bulb length	14 \pm 0.8 (13-15)	11	13	14, 15
Median bulb diam.	10 \pm 1 (8-11)	9	11	9, 12
Median bulb valve length	3.0	3.0	3.0	3.0, 4.0
Median bulb valve width	2.5 \pm 0.3 (2.0-3.0)	2.0	2.0	2.0, 2.5
Lip region diam.	9.5 \pm 0.3 (9.0-10.0)	8.0	9.0, 10.5	10.0, 11.0
Lip region height	5.0 \pm 0.9 (4.5-6.5)	4.0	5.0, 6.0	4.0, 4.5
Annulus width	2.0 \pm 0.9 (1.5-2.5)	2.0	–	2.0
Lateral field width	4.5 \pm 0.5 (4-5)	2.0	4.0, 4.5	4.4, 6.0
Tail length	24 \pm 2.5 (21-26)	19	27, 22	25, 22
Scutellum width	3.0 \pm 0.5 (2-3)	2.0	2.0	2.0

two annuli from basal plate Stylet robust with spear knob rounded posteriorly and rounded, flattened to slightly hollow anteriorly. Metenchium ranging from slightly shorter to slightly longer than telenchium. Median bulb broadly rounded with basal pharyngeal bulb wide and short appearing as a cap over anterior part of intestine, overlap mostly about 12.5-13.5 μm long but ranging from 7 to 21 μm . Excretory pore situated from just anterior to 12 annuli posterior to base of pharynx. Hemizonid 2-4 annuli long and mostly situated opposite excretory pore. Hemizonion not seen. Lateral field comprising four lines over entire length of body, areolated in pharyngeal area (although sometimes very difficult to see) and well areo-

lated opposite scutellum. Lateral field posterior to scutellum consisting of an areolated and semicircular band surrounding scutellum, unlike that of *S. bradys* and *S. cavenessi*, which appear as two elongate, areolated and finger-like bands extending posteriorly from scutellum and enclosing a third and small pointed band. Spermatheca varying from small to large, rounded to oblong, mostly empty in TVL 2065 population but filled with sperm cells in LP17 population. Epiptygma not seen. Two small, oblong vaginal glands seen in all specimens. Intestine with no, or only a very slight, overlap over rectum. Scutellum 2.0-4.5 μm wide, situated from two annuli anterior to six

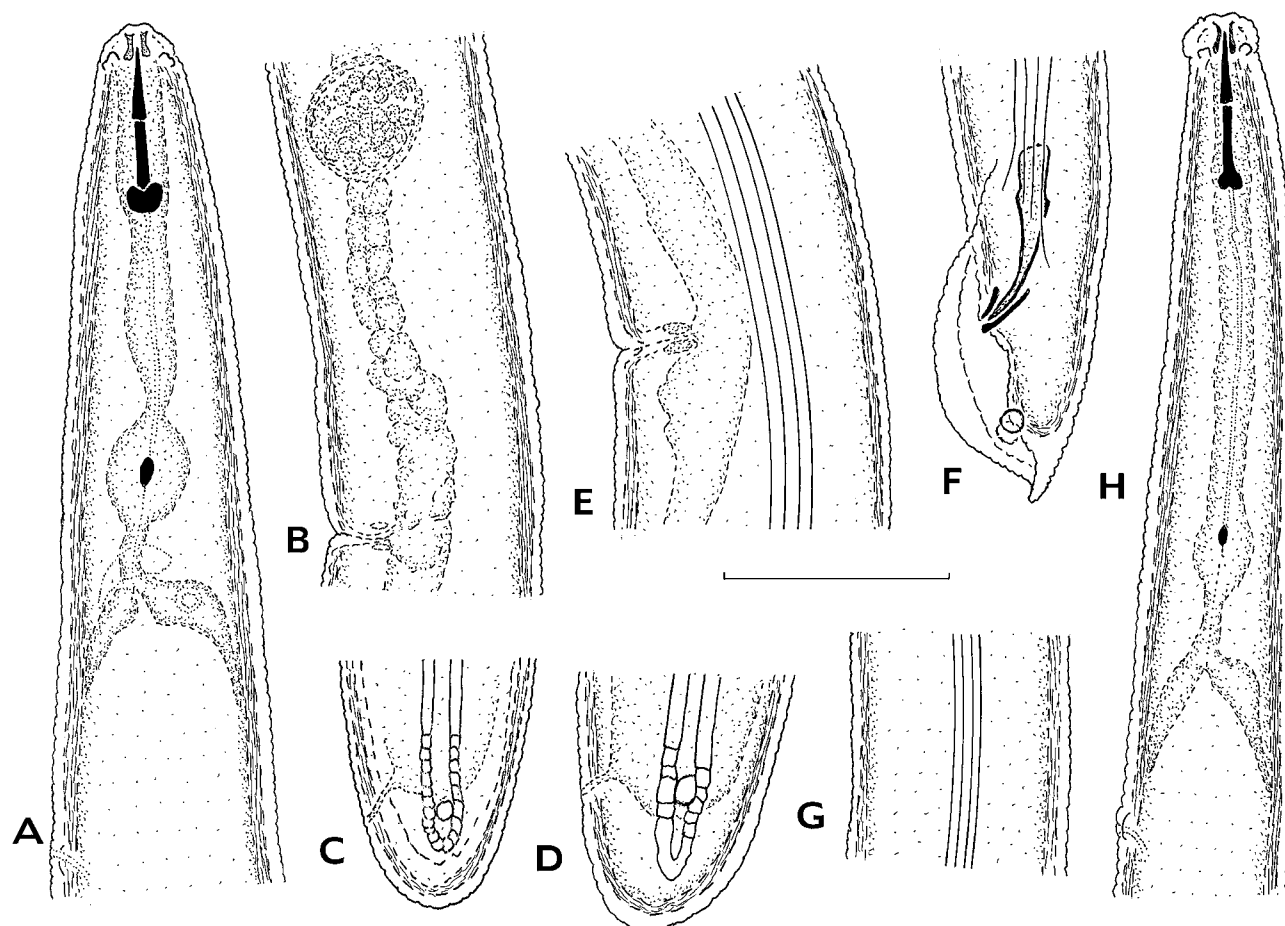


Fig. 13. *Scutellonema transvaalense* (LP17). Female. A: Anterior region of body; B: Vulval region with anterior spermatheca filled with sperm; C, D: Tail variations; E: Vulval region. Male. F: Tail region; G: Lateral field at mid-body; H: Anterior region of body. (Scale bar = 30 μ m.)

annuli posterior to anus. Caudalid not seen. Tail round to broadly rounded with 7-12 annuli.

Male

Body slightly arcuate ventrad, irregularly curved or curved in form of a letter C. Male not as obese as female. Lip region broader than adjoining body, SEM showing an almost square lip region with a rounded labial disc and labial area divided into three blocks dorsally and ventrally with small amphidial openings visible in between. Stylet not so well developed as in female with knobs mostly sloping anteriorly. Metenchium ranging from slightly shorter to, or slightly longer than, telenchium. Basal pharyngeal lobe longer and overlap longer than in female. Excretory pore situated from opposite anterior part of pharyngeal lobe to just posterior to its base. Hemizonid

about two annuli long and situated from just anterior, to just posterior, to excretory pore. Hemizonion not seen. Lateral field with four lines. Areolated anteriorly but not on tail. Scutellum 2.0-3.5 μ m wide and situated far back on tail where hyaline portion of tail starts. SEM not showing scutellum as featuring much on outside of the bursa but more on inside. Tail ending in a long finger-like hyaline tip with bursa not enveloping tail tip.

Juvenile

Only one fourth-stage found, very similar to female in morphology.

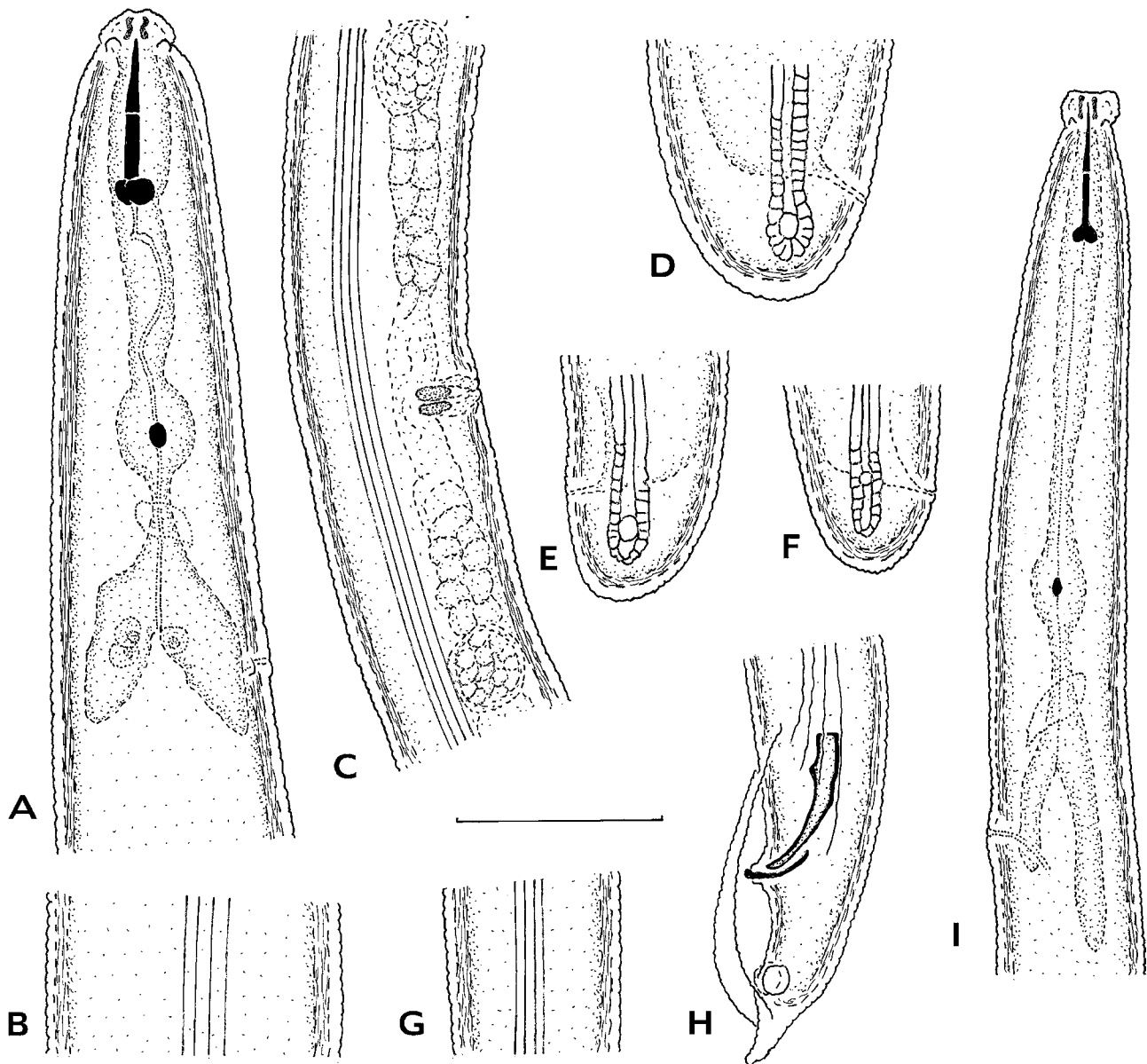


Fig. 14. *Scutellonema transvaalense* (TVL2065). Female. A: Anterior region of body; B: Lateral field at mid-body; C: Vulval region with both spermathecae filled with sperm; D-F: Tail variations. Male. G: Lateral field at mid-body; H: Tail region; I: Anterior region of mid-body. (Scale bar = 30 μ m.)

REMARKS

Although the present female and male specimens are slightly shorter than those of the original description, the pharynx is slightly shorter, the excretory pore located nearer the anterior end and the tail is slightly shorter, other characters, such as the female and male lip region form, correspond. The cap-like overlap of the pharynx

over the intestine in the female and the positioning of the scutellum far posterior on the male tail, and also the fact that the present specimens were found in the same region, points to the fact that they belong to the same species. In the original description, the species was well separated from *S. cavenessi* and *S. bradys*. *Scutellonema transvaalense* differs from the Florida *S. cavenessi* in the

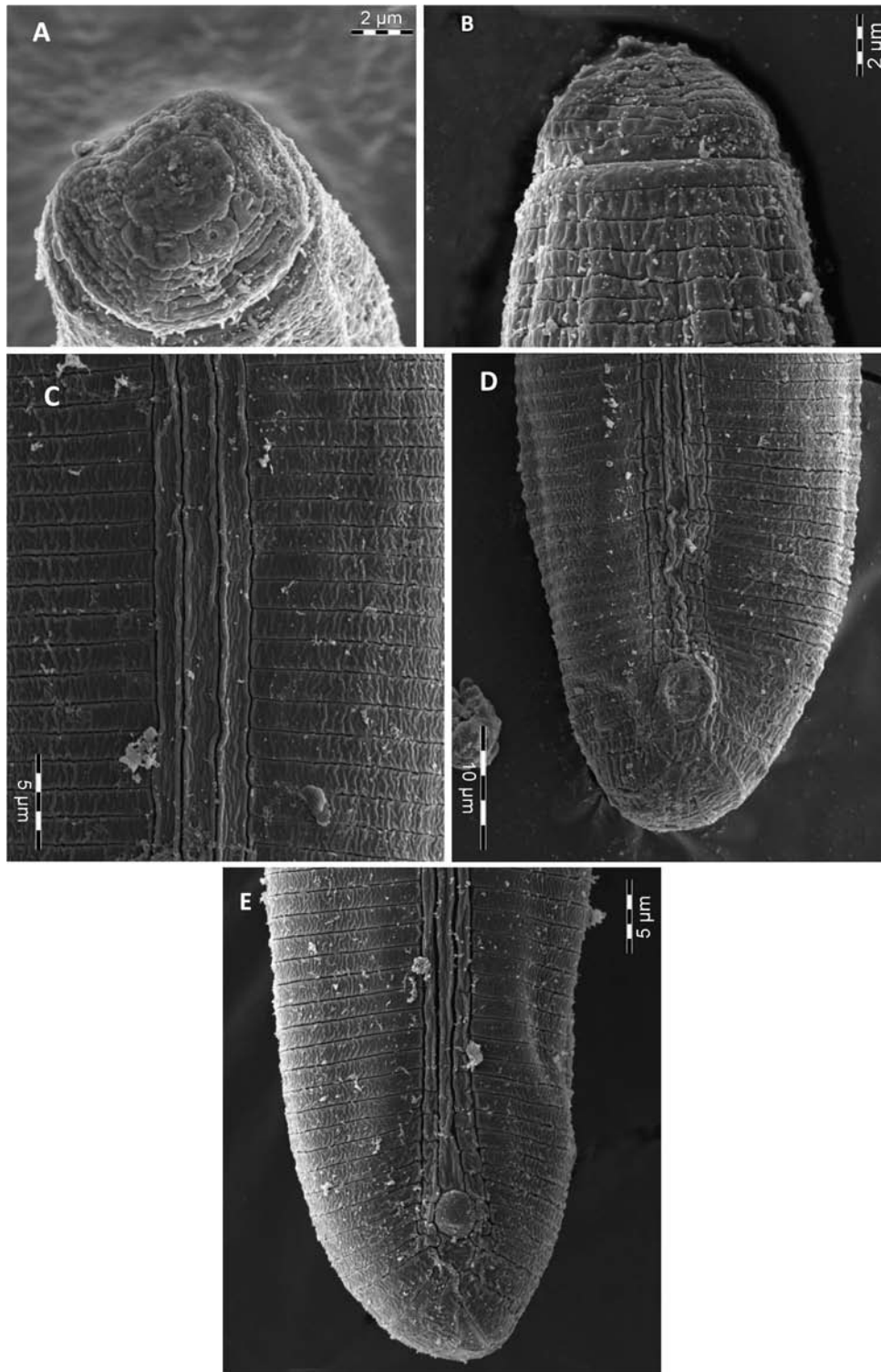


Fig. 15. *Scutellonema transvaalense* (TVL2065). Female. A: *En face* view of lip region; B: Lateral view of lip region; C: Lateral field at mid-body; D, E: Tail variations.

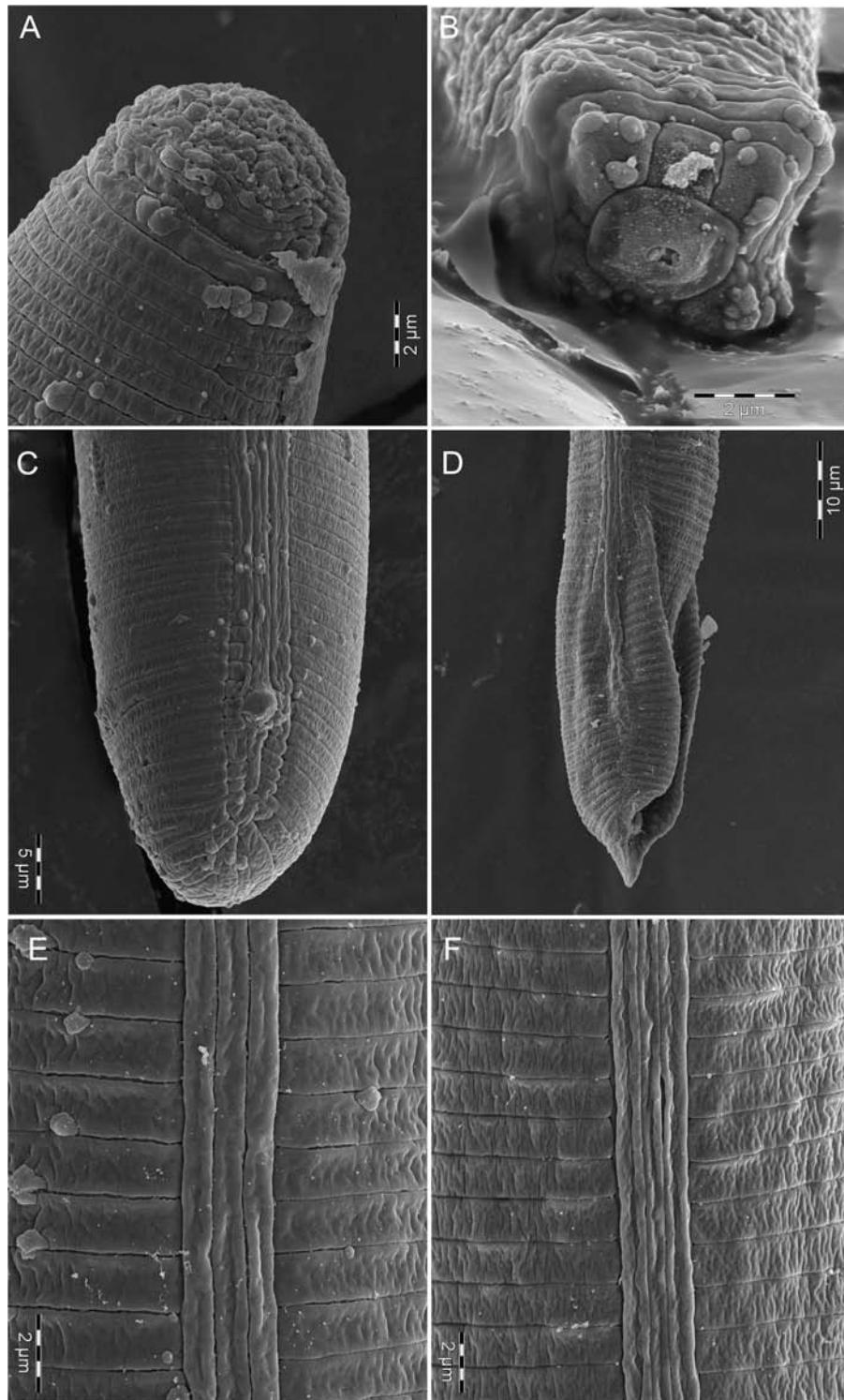


Fig. 16. *Scutellonema transvaalense* (TVL2065). Female. A: Lateral view of lip region; C: Tail region; E: Lateral field at mid-body. Male. B: *En face* view of lip region; D: Tail region; F: Lateral field at mid-body.

Table 7. Morphometrics of female and juvenile *Scutellonema transvaalense* from various localities in South Africa compared with those of the original description. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	<i>S. transvaalense</i> (TVL2065)	<i>S. transvaalense</i> (LP17)	<i>S. transvaalense</i> (TVL2065)	<i>S. transvaalense</i> (Van den Berg, 1981)
	Females	Females	Juvenile 4 th stage	Females
n	11	18	1	9
L	730 \pm 136.1 (609-1010)	753 \pm 134 (546-1009)	982	961 (772-1244)
a	20.9 \pm 2.7 (16.9-26.4)	22.5 \pm 3.6 (14.9-32.3)	24.3	21.8 (19.6-24.9)
b	7.3 \pm 0.8 (6.0-8.4)	7.6 \pm 1 (6.0-9.6)	9	8.7 (7.1-10.3)
b'	8.5 \pm 0.9 (7.3-9.9)	8.8 \pm 1.2 (6.6-11.3)	–	–
c	55.7 \pm 8 (42.7-68.7)	56.9 \pm 9.8 (43.3-75.4)	53.4	73.2 (31.2-164.7)
c'	0.6 \pm 0.9 (0.5-0.8)	0.7 \pm 0.1 (0.4-0.9)	0.8	0.6 (0.3-0.8)
o	15.7 \pm 2.9 (10.8-21.9)	21 \pm 4.3 (14.5-30)	15.7	20.9 (16.9-27.4)
DGO	4.0 \pm 0.6 (3.0-5.0)	5.0 \pm 0.9 (3.0-6.5)	4.0	–
V	56.0 \pm 3.9 (49-61)	56.0 \pm 2.8 (49.5-59.5)	–	54.0 (49.0-59.0)
OV ¹	23 \pm 4.5 (14-29)	31 \pm 6.6 (21-40)	–	29 (26-30)
OV ¹ length	176 \pm 51.7 (120-291)	228 \pm 57.5 (131-366)	–	–
OV ²	26 \pm 5.3 (18-34)	31 \pm 6 (21-42)	–	28 (24-32)
OV ² length	189 \pm 59.2 (112-294)	229 \pm 42.9 (152-317)	–	–
Stylet length	25 \pm 2.4 (21-28)	23 \pm 1.7 (20-26)	26	27 (24-30)
Metenchium length	12.0 \pm 1.1 (10.5-14.0)	11.5 \pm 1.1 (9.5-14.0)	12.0	–
Telenchium length	13.0 \pm 1.4 (10.5-14.5)	12.0 \pm 1 (10.5-13.0)	14.0	–
m	49.1 \pm 2 (46.5-52.3)	48.3 \pm 2.5 (43.8-54.3)	45.7	44-48
Stylet knob height	3.5 \pm 0.6 (2.0-4.5)	3.0 \pm 0.4 (3.0-3.5)	4.0	3.5 (2.2-4.4)
Stylet knob width	5.5 \pm 0.6 (5.0-7.0)	4.5 \pm 0.7 (3.5-5.5)	6.5	6.1 (4.0-8.1)
Pharynx length	96 \pm 10.6 (81-109)	96 \pm 8.1 (81.5-112.5)	109	102 (85-121)
Pharyngeal overlap	13.5 \pm 4.7 (7.4-20.6)	12.5 \pm 3.8 (7-21)	–	–
Excretory pore from anterior	105 \pm 12.6 (87-126)	109 \pm 9.6 (94-126)	114	136 (96-168)
Diam. at mid-body	35.5 \pm 8.5 (25.5-48.5)	34.0 \pm 6.1 (28.0-48.5)	–	–
Diam. at anus	21.0 \pm 5.1 (14.5-30.0)	20.0 \pm 3.6 (15.0-27.0)	2.07	–
Median bulb length	12.5 \pm 1.4 (10.5-15)	12.0 \pm 1.4 (10.0-15.0)	13.5	14.8 (12.1-16.5)
Median bulb width	10.5 \pm 0.9 (9.0-11.0)	10.0 \pm 1.2 (8.0-12.5)	11.0	12.0 (7.7-14.0)
Median bulb valve length	3.0 \pm 0.3 (3.0-3.5)	3.0 \pm 0.7 (2.0-5.0)	3.0	–
Median bulb valve width	2.0 \pm 0.4 (2.0-3.0)	2.0 \pm 0.4 (1.5-3.0)	2.0	–
Lip region diam.	10 \pm 1 (9-12)	10 \pm 1 (7.5-11)	9	9 (8-10)
Lip region height	4.0 \pm 0.3 (3.5-4.5)	4.5 \pm 0.7 (3.5-6.0)	4.5	3.8 (3.7-4.4)
Annulus width	2.0 \pm 0.5 (1.5-3.0)	2 \pm 0.3 (1.5-2.0)	1.5	1.6 (1.3-2.2)
Lateral field width	6.0 \pm 1.1 (4.5-7.5)	6.0 \pm 1.1 (4.5-8.0)	7.5	6.4 (5.9-8.1)
Tail length	13.0 \pm 2 (10.0-15.5)	13.0 \pm 3.3 (10.5-17.5)	18.5	17.4 (5.2-22.1)
Scutellum width	2.5 \pm 0.6 (2.0-4.0)	3.0 \pm 0.6 (2.0-4.5)	2.0	3.7 (2.9-4.4)
Spermatheca length	14.5 \pm 4.8 (11.0-21.5)	18.0 \pm 3.9 (14.0-26.0)	–	–
Spermatheca width	13.5 \pm 4.4 (10.5-20.0)	16.0 \pm 3.6 (11.0-24.5)	–	–

more posterior location of the scutellum in both female (7-8 annuli from tail tip vs 9-14) and male (end of the alae vs slightly posterior to cloacal aperture) and the shape of the lateral field posterior to the scutellum. The diagnosis of the original description of this well-defined species was reported in its entirety by Germani *et al.* (1985) in a revision of the genus *Scutellonema*.

***Scutellonema truncatum* Sher, 1964** (Figs 6G-L; 17; 18; 19J; 20N, O)

Scutellonema truncatum was described as a species without males from South Africa and also reported from Zimbabwe by Sher (1964). Since then it has been found to be widespread in South Africa on various hosts and

Table 8. Morphometrics of male *Scutellonema transvaalense* from various localities in South Africa compared with those of the original description. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	<i>S. transvaalense</i> (TVL2065)	<i>S. transvaalense</i> (LP17)	<i>S. transvaalense</i> (Van den Berg, 1981)
n	1	11	24
L	838	641 \pm 89.7 (522-765)	835 (690-1005)
a	30.8	27.9 \pm 3 (23.1-32.5)	28.9 (22.4-35.1)
b	7.2	5.9 \pm 0.8 (4.2-6.9)	6.2 (5.0-7.3)
b'	8.4	7.2 \pm 0.9 (5.7-8.1)	–
c	29.2	30 \pm 5.3 (24-40.8)	28.1 (22.8-34.3)
c'	2.0	1.5 \pm 0.3 (1.0-1.9)	1.8 (1.4-2.4)
o	17.9	22.1 \pm 3.7 (15.7-28.6)	19.3 (13.5-31)
DGO	3.5	4.5 \pm 1 (3.0-6.5)	–
T	50.7	46.5 \pm 6.3 (34.5-56.5)	–
T length	424	297 \pm 47.4 (225-371)	–
Stylet length	20.5	20.5 \pm 1.8 (17.5-23.0)	22.8 (18.8-25.7)
Metenchium length	11.0	10.5 \pm 0.9 (9.0-12.0)	–
Telenchium length	9.5	10 \pm 1 (9.0-11.5)	–
m	53.4	50.8 \pm 1.5 (48.2-53.9)	47-53
Stylet knob height	2.0	2.0 \pm 0.4 (1.5-3.0)	2.3 (1.5-2.9)
Stylet knob width	2.5	3.0 \pm 0.3 (3.0-3.5)	3.4 (2.6-4.4)
Pharynx length	117	109 \pm 7.2 (100-125)	125 (108-142)
Pharyngeal overlap	17.5	15.0 \pm 7 (12.0-21.0)	–
Excretory pore from anterior	112	99 \pm 7.7 (91-115)	126 (96-149)
Diam. at mid-body	27	23 \pm 3.8 (18-30)	–
Diam. at anus	17	15 \pm 2.7 (12-22)	–
Median bulb length	10.5	9.5 \pm 1.7 (7.5-12.0)	–
Median bulb width	6.5	6.5 \pm 1.3 (5.0-9.0)	–
Median bulb valve length	–	2 \pm 0.5 (2.0-3.0)	–
Median bulb valve width	–	1.5	–
Lip region diam.	8.0	9 \pm 1.2 (8-12)	10.6 (9.2-11.8)
Lip region height	5.0	5.0 \pm 0.5 (4.0-6.0)	–
Annulus width	2.0	1.75 \pm 0.3 (1.5-2.0)	–
Lateral field width	5.0	4.0 \pm 0.6 (3.0-4.5)	–
Tail length	28.5	22 \pm 3.9 (17-25.5)	29.7 (23.5-34.2)
Scutellum width	–	3.0 \pm 0.5 (2.0-3.5)	3.1 (2.6-3.3)
Spicule length	25	22 \pm 2.4 (19-26)	24.9 (21.3-27.2)
Gubernaculum length	15.5	9.5 \pm 1.1 (7.5-11)	11 (9.6-14.7)
Capitulum length	6.5	–	7.7 (7-8.8.0)

crop plants. In 1993, a population with males was reported from pumpkin in Mozambique for the first time (Van den Berg & Van den Oever, 1993). A sample from farm soil near Sherwood, Botswana, was sent for analysis on 10 October 2015, the previous crops being millet, cabbage and potatoes. This population lacked males, as reported for other populations. The morphological details of the *S. truncatum* from Botswana are reported for comparison with the morphology of the similar *S. clavicaudatum* sp. n.

MEASUREMENTS

See Table 9.

DESCRIPTION

Female

Body form ranging from an open letter C to a figure 6 and very often a complete or double circle. Lip region conical with a distinct labial disc, not distinctly set off, lacking annuli but with six longitudinal sectors. Rarely

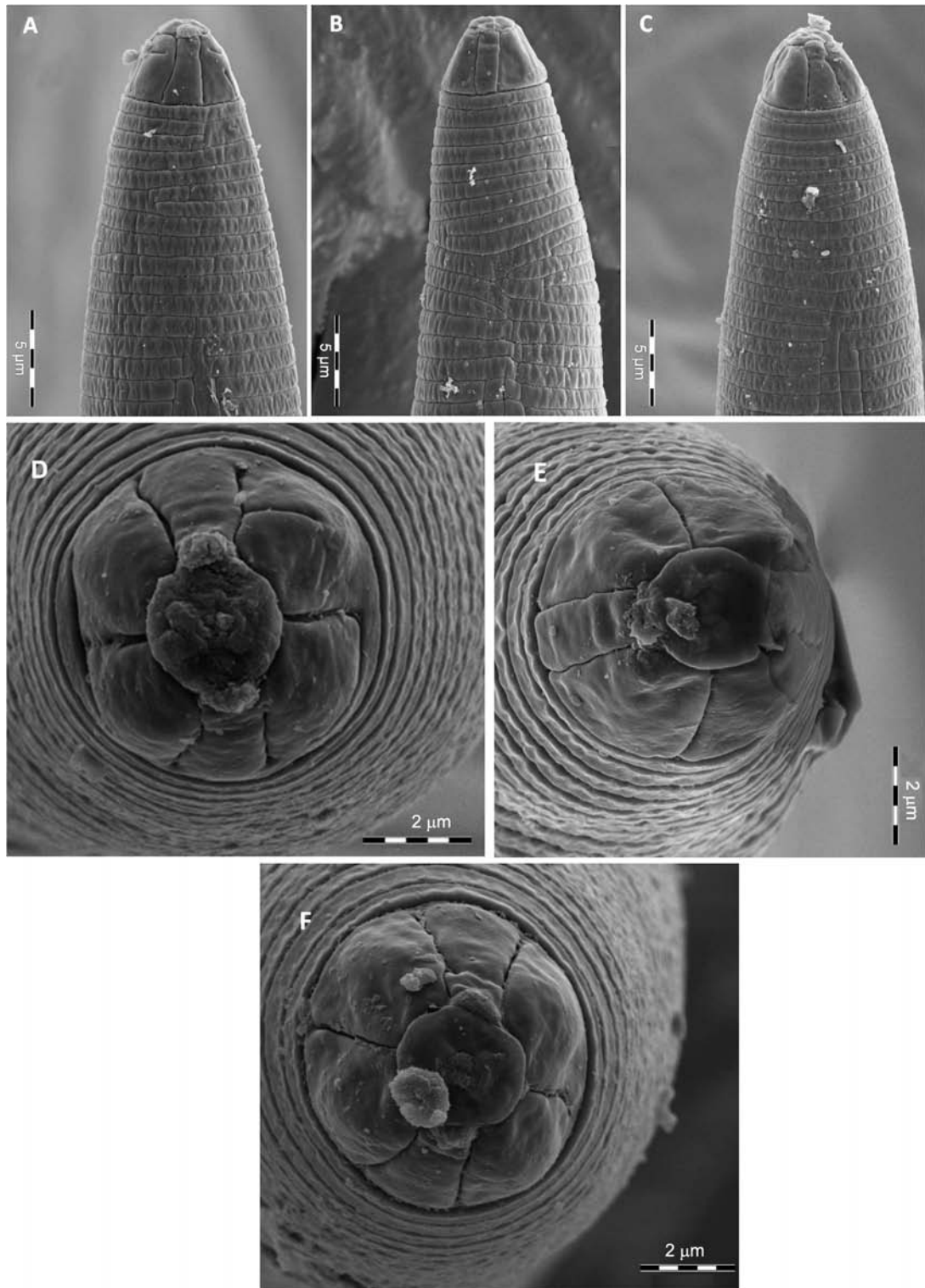


Fig. 17. *Scutellonema truncatum* (Bot21). Female. A-C: Lateral views of lip region; D-F: *En face* views of lip region.

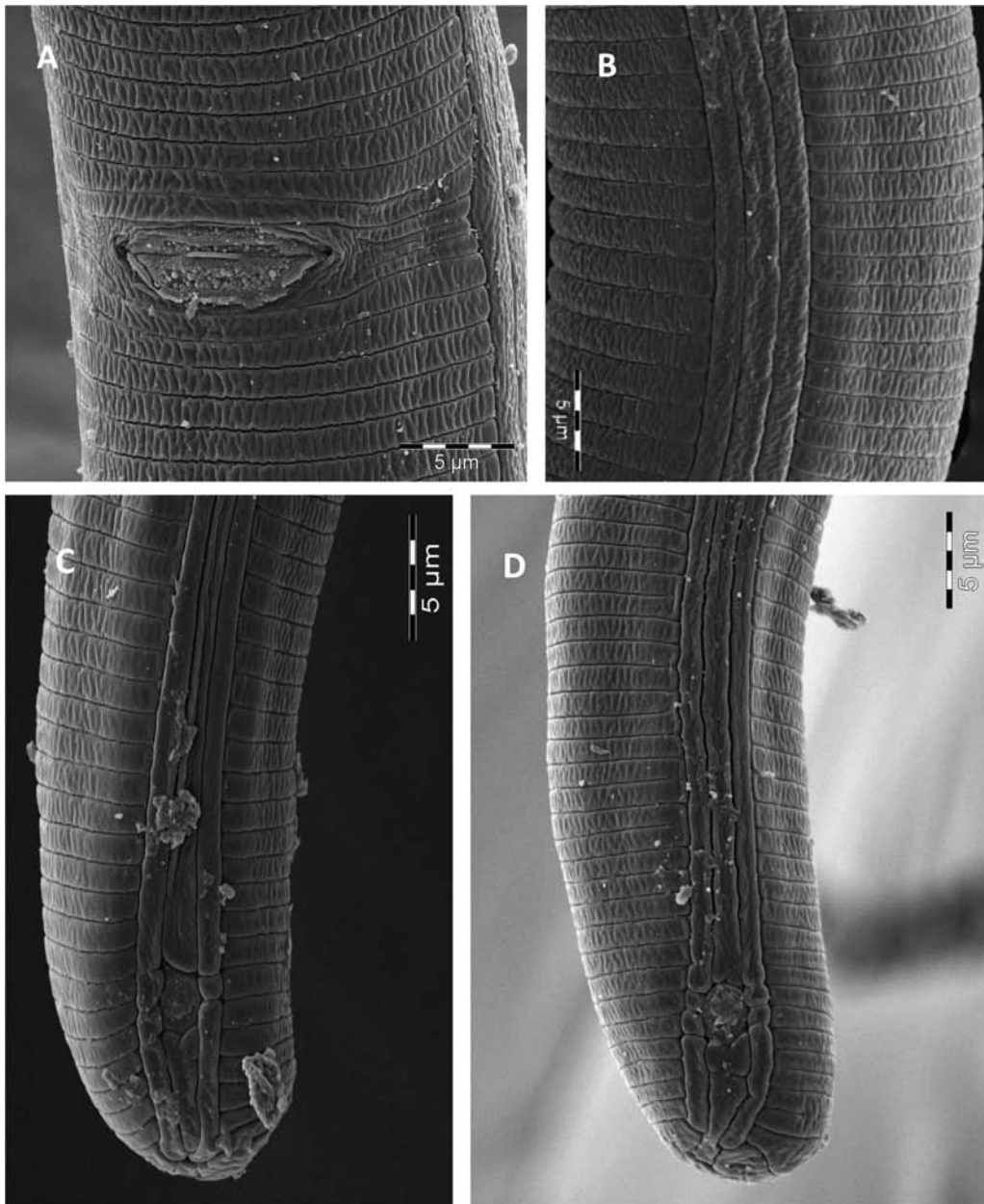


Fig. 18. *Scutellonema truncatum* (Bot21). Female. A: Vulval region with protruding epiptygma; B: Lateral field at mid-body; C, D: Tail variations.

one or two of these sectors may have a short line forming an irregular block but no regular annuli seen. Labial framework moderately developed. Cephalids very rarely seen, situated four and 12 annuli posterior to base of lip region. Stylet well developed, flattened to slightly hollow anteriorly and rounded posteriorly. Metenchium

varying from shorter to slightly longer than telenchium. Median bulb not unusually large and pharyngeal lobe crossing over intestine normally. Excretory pore situated from opposite anterior part to mostly opposite posterior part of pharyngeal overlap. Hemizonid two annuli long and situated from immediately anterior to excretory pore

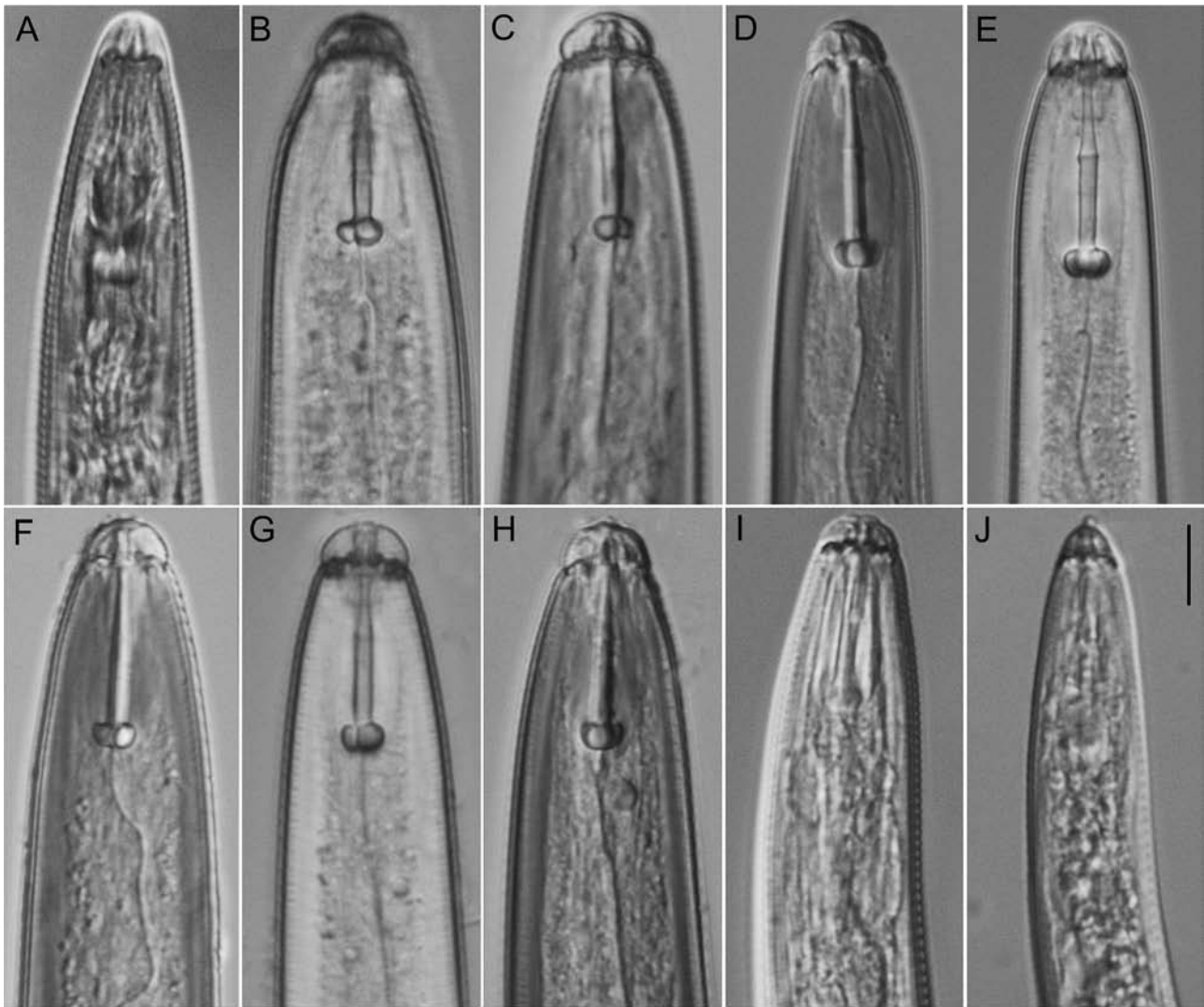


Fig. 19. *Scutellonema* species. Female lip region. A: *S. clavicaudatum* sp. n., South Africa (TVL2063); B, C: *S. bradys*, Florida, USA (CD1941); D, E: *S. brachyurus*, Costa Rica (CD1380); F: *S. cavenessi*, Florida, USA (CD1908); G, H: *S. cavenessi*, Florida, USA (CD1470); I: *S. transvaalense*, South Africa (LP17); J: *S. truncatum*, Botswana (Bot21). (Scale bar = 10 μ m.)

to three annuli anterior to it. Hemizonion seen in only a few specimens, one annulus long and situated 10-12 annuli posterior to hemizonid. Lateral field comprising four lines with three equal bands, areolated anteriorly and opposite scutellum. Posteriorly to scutellum bands ending in a V shape, sometimes with an additional block or two in middle band. Spermatheca indistinct or, where seen, small, rounded and empty. Epiptygma folded into vagina. Vaginal glands small, round to oval. Intestine not overlapping rectum. Scutellum 2.0-3.5 μ m wide, situated from two annuli anterior to anus to five annuli posterior

to anus. Caudalid not seen. Tail rounded, more convex dorsally with 7-14 annuli, annuli on tip slightly larger than on rest of body.

Male

Not found.

Juvenile

Morphology of juveniles similar to that of female.

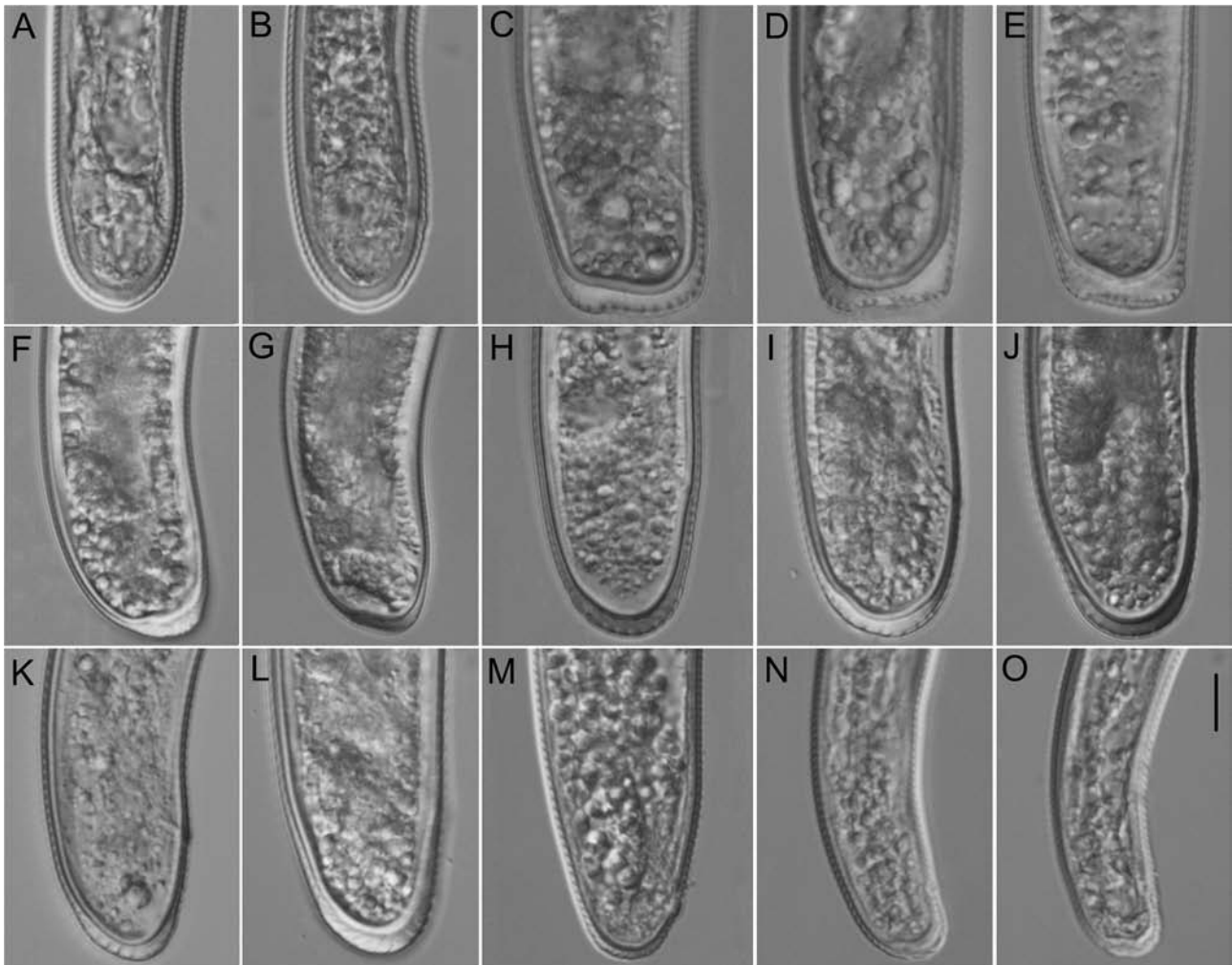


Fig. 20. *Scutellonema* species. Female tail. A, B: *S. clavicaudatum* sp. n., South Africa (TVL2063); C-E: *S. bradys*, Florida, USA (CD1941); F, G: *S. brachyurus*, Costa Rica (CD1380); H, I: *S. cavenessi*, Florida, USA (CD1908); J, K: *S. cavenessi*, Florida, USA (CD1470); L: *S. cavenessi*, Florida, USA (CD1542); M: *S. transvaalense*, South Africa (LP17); N, O: *S. truncatum*, Botswana (Bot21). (Scale bar = 10 μm .)

REMARKS

Except for being slightly longer, these specimens compare very well with the previous descriptions of the species. *Scutellonema truncatum* males were described by Van den Berg & Van den Oever (1993) from Mozambique. In addition, male *S. clavicaudatum* sp. n. have a longer body at 834 (768-919) vs 552 (544-562) μm , higher c' value of 1.6 (1.3-1.7) vs 0.8 (0.6-1), longer stylet of 30 (29.5-31) vs 22 (22-23) μm , longer tail of 25 (22-27) vs 10 (7-12) μm , and longer spicules and gubernaculum at

30 (27-32) vs 20 μm , and 14.5 (13.0-15.5) vs 8 (7-9) μm , respectively.

Molecular characterisation and phylogenetic relationships of *Scutellonema* species

The D2-D3 of 28S rRNA gene sequence alignment was 700 bp long and contained 65 sequences of *Scutellonema* and three sequences of outgroup taxa. Twenty-three new sequences of the D2-D3 of 28S rRNA gene from six species were obtained in this study. Intraspecific D2-

Table 9. Morphometrics of *Scutellonema truncatum* from Botswana (Bot21) compared with those in the literature. All measurements are in μm and in the form; mean \pm s.d. (range).

Character	<i>S. truncatum</i> (Bot21)		<i>S. truncatum</i> (from literature*)
	Females	Juveniles	Females
n	16	3	155
L	827 \pm 66.9 (715-955)	672-730	500-800
a	34.4 \pm 2.1 (31.2-37.4)	27.7-32.1	17.9-34.4
b	6.0 \pm 0.4 (5.0-6.6)	5.4-5.7	5.0-8.6
b'	7.2 \pm 0.7 (6.2-8.0)	6.4-6.8	4.1-8.7
c	70.0 \pm 8.4 (59.6-89.3)	37.1-57.2	22-156
c'	0.7 \pm 0.07 (0.6-0.8)	0.7-1.2	0.3-0.72
o	14.8 \pm 3.6 (10.3-20.9)	11.1-17.6	5.7-31.0
DGO	4.0 \pm 0.9 (3.0-5.0)	3-4	–
V	62.0 \pm 1.9 (58-65)	66-86 (primordium)	55-63
OV ¹	19 \pm 5 (12-28)	–	18-28
OV ¹ length	150 \pm 37.5 (113-232)	–	–
OV ²	18 \pm 2.4 (15-22)	–	17-26
OV ² length	147 \pm 22.3 (125-190)	–	–
Stylet length	27 \pm 1.1 (25-28.5)	25-26	21-29
Metenchium length	13 \pm 0.6 (12-14)	12-13	12-13
Telenchium length	14 \pm 0.9 (12-15)	12-14	13-14
m	49 \pm 1.8 (46-52)	47-53	43-55
Stylet knob height	2.5 \pm 0.6 (1.5-3.5)	2.5-3.0	2.0-3.0
Stylet knob width	5.0 \pm 0.6 (4.5-5.0)	4.5-6.0	4.5-5.0
Pharynx length	137 \pm 6.1 (124-147)	125-132	81
Pharyngeal overlap	23 \pm 6.7 (12-33)	14.7-28.7	–
Excretory pore from anterior	128 \pm 7.6 (119-143)	118-126	89-104
Diam. at mid-body	24 \pm 2.4 (19-27)	22-24	21-25
Diam. at anus	16 \pm 1.3 (13-18.5)	16-17	–
Median bulb length	12 \pm 0.9 (11-14)	11-12	11
Median bulb width	10 \pm 1.1 (7.5-12)	9-11	10
Median bulb valve length	3.5 \pm 0.4 (3.0-4.0)	3.0-3.5	–
Median bulb valve width	2.5 \pm 0.4 (2.0-3.0)	2-3	–
Lip region diam.	8.0 \pm 0.5 (6.5-8.5)	7.5-8.0	5.0-7.0
Lip region height	5.0 \pm 0.4 (4.0-5.5)	4.5-6.0	4.0-5.0
Annulus width	2.0 \pm 0.2 (1.5-2.25)	2.0-3.0	1.0-2.0
Lateral field width	5.5 \pm 0.7 (5.0-7.5)	5.0-6.0	5.0-6.0
Tail length	12.0 \pm 1.5 (9.5-14.5)	12.0-19.0	4.0-16.2
Scutellum width	3.0 \pm 0.5 (2.0-3.5)	2.0-2.5	1.6-3.7
Spermatheca length	12.5 \pm 1.5 (10.5-14.0)	–	–
Spermatheca width	10.5 \pm 1.1 (8.5-12.0)	–	–

* Sher (1964); Van den Berg & Heyns (1973); Germani *et al.* (1985); Van den Berg & Van den Oever (1993).

D3 variation for *S. clavicaudatum* sp. n. was 0-5.6% (0-35 bp); *S. brachyurus* type A 0-3.2% (0-21 bp); *S. brachyurus* type B 0-0.7% (0-5 bp); *S. bradys* 0.1-2.7% (1-15 bp); *S. cavenessi* 0.1-0.7% (1-6 bp); *S. truncatum* 0-0.8% (0-6 bp); and *S. transvaalense* 0.2% (2 bp). Phylogenetic relationships between *Scutellonema* species are given in Figure 22. *Scutellonema clavicaudatum* sp. n.

has a sister relationship with *S. brachyurus* type A and its sequence differs in 6.0-8.5% (39-50 bp) from that of this species. Interspecific variation between *S. cavenessi* and *Scutellonema* sp. D was 2.9-4.0% (13-26 bp).

The ITS rRNA gene sequence alignment was 1444 bp long and contained 79 sequences of *Scutellonema* and two sequences of *Helicotylenchus multicinctus* (Cobb,

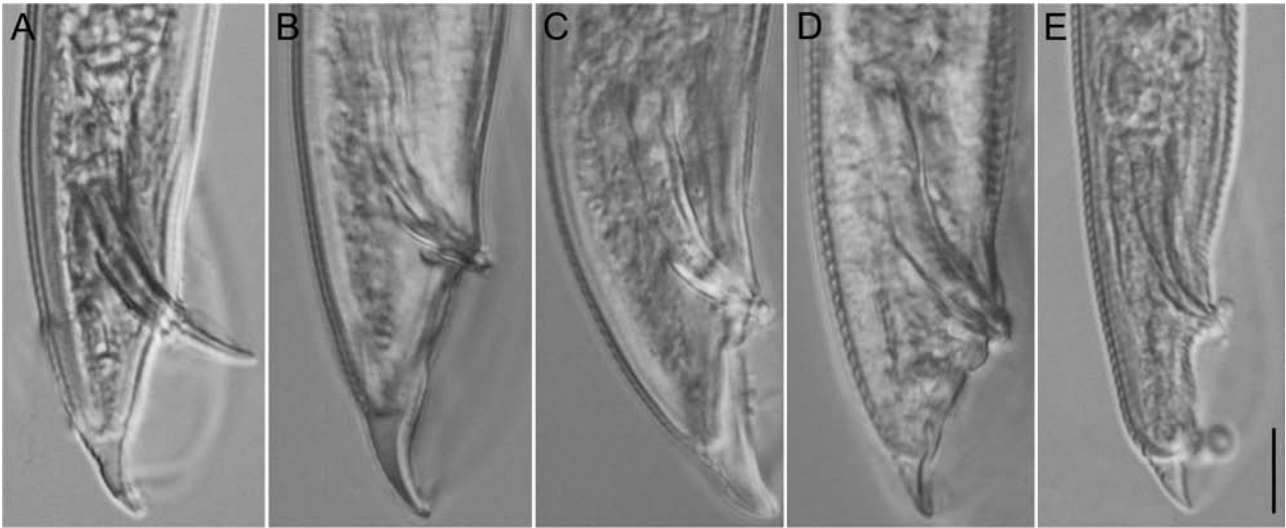


Fig. 21. *Scutellonema* species. Male tail. A: *S. clavicaudatum* sp. n., South Africa (TVL2063); B: *S. bradys*, Florida, USA (CD1941); C: *S. cavenessi*, Florida, USA (CD1542); D: *S. cavenessi*, Florida, USA (CD1942); E: *S. transvaalense*, South Africa (LP17). (Scale bar = 10 μ m.)

1893) Golden, 1956 used as outgroups. Twenty-six new sequences of the ITS rRNA gene from six species were obtained in this study. Intraspecific ITS variation for *S. clavicaudatum* sp. n. was 0.4-12.9% (4-130 bp); *S. brachyurus* type A 0-9.4% (0-91 bp); *S. brachyurus* type B 0.4-2.5% (4-25 bp); *S. bradys* 0-9.5% (0-89 bp); and *S. cavenessi* 0.4-2.4% (4-23 bp). Phylogenetic relationships between *Scutellonema* species are given in Figure 23. *Scutellonema clavicaudatum* sp. n. has a sister relationship with *Scutellonema* sp. A and differs in 11.0-12.9% (109-128 bp) of the ITS rRNA gene sequences from that of this species. Interspecific variation between *S. cavenessi* and *Scutellonema* sp. D was 9.1-10.4% (84-98 bp).

The *COI* gene sequence alignment was 367 bp long and contained 32 sequences of *Scutellonema* and two sequences of *Pratylenchus* sequences used as an outgroup. Ten new sequences of *COI* gene from four species were obtained in this study. Intraspecific *COI* variation for *S. brachyurus* type A was 0.2-5.5% (1-20 bp); *S. brachyurus* type B 0-2.4% (0-9 bp); *S. bradys* 0-14.4% (0-53 bp); and *S. cavenessi* 0-2.8% (0-10 bp). Interspecific variation between *S. cavenessi* and *Scutellonema* sp. D was 15.4-16.1% (53-59 bp). Phylogenetic relationships between *Scutellonema* species are given in Figure 24.

Discussion

In the results of this study, using an integrative approach, we distinguished six valid and four still unidentified species within all molecularly characterised *Scutellonema* samples. For species demarcation in *Scutellonema* using molecular markers, we took into account the higher level of intraspecific variation of representatives of this genus for the rRNA and mtDNA genes than that observed for other studied nematodes. In our analysis the intraspecific variation for *Scutellonema* reached 5.6% for the D2-D3 of 28S rRNA, 12.9% for the ITS rRNA genes and 14.4% for *COI* gene.

The study confirms the presence of morphologically and genetically atypical populations of *S. bradys* in Florida. Our population from central Florida differed genetically from the other populations from Africa and Central America (Costa Rica), but is similar with a population from Arkansas characterised molecularly by Bae *et al.* (2009). These findings suggest that USA populations may have a different origin to those from Africa and Central America, the latter possibly sharing a common origin. The morphology of the Florida *S. bradys* fits that of type specimens of this species yet differs in having a truncate tail terminus rather than round and also a prominent oval spermatheca filled with flagellate spermatozoa. Similar flagellate spermatozoa were observed in some Florida specimens of *S. cavenessi*.

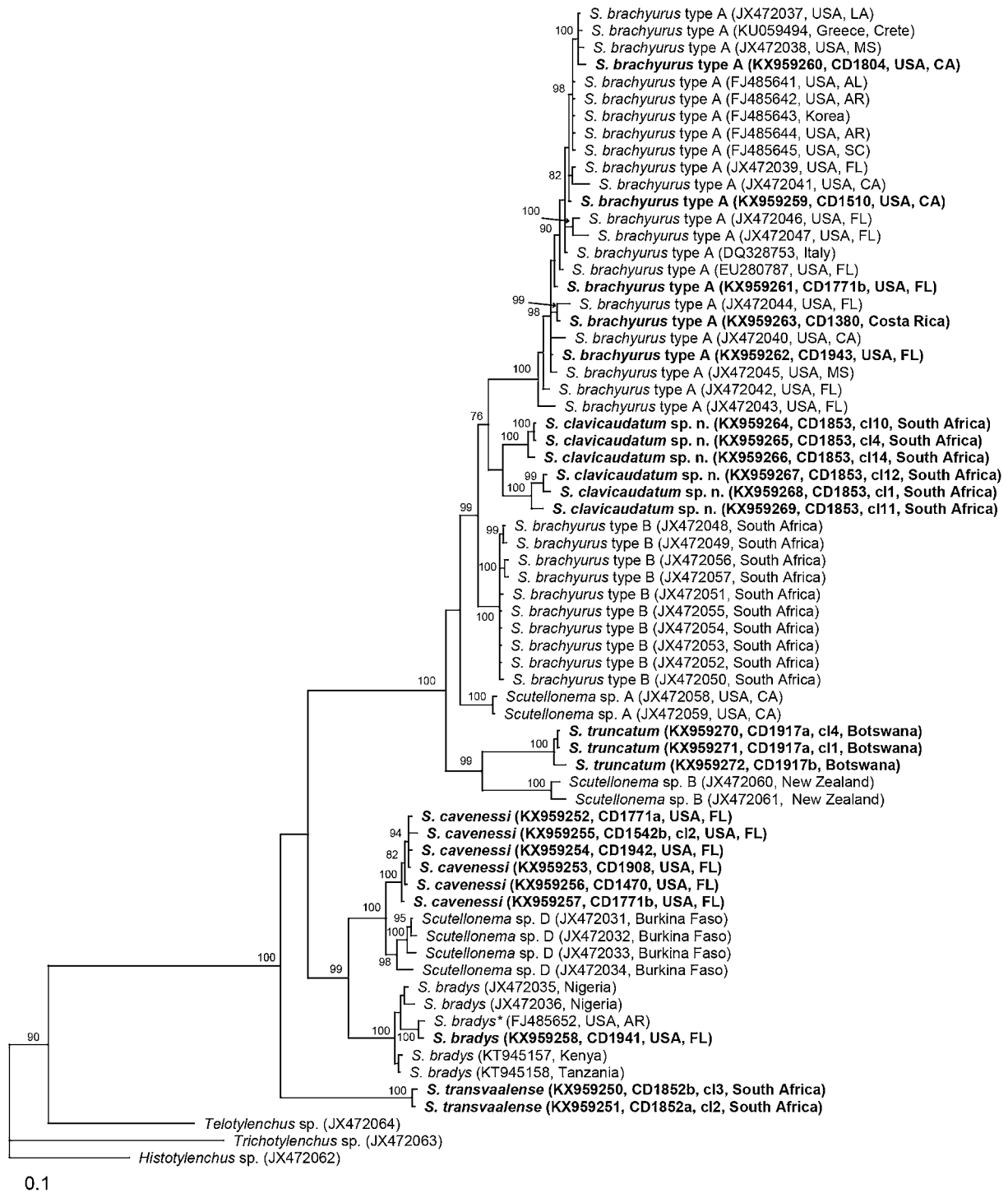


Fig. 22. Phylogenetic relationships within *Scutellonema* populations and species: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. Original sequences are indicated by bold font. * – identified as *S. cavenessi* in GenBank and as *S. bradys* in the article by Bae *et al.* (2009).

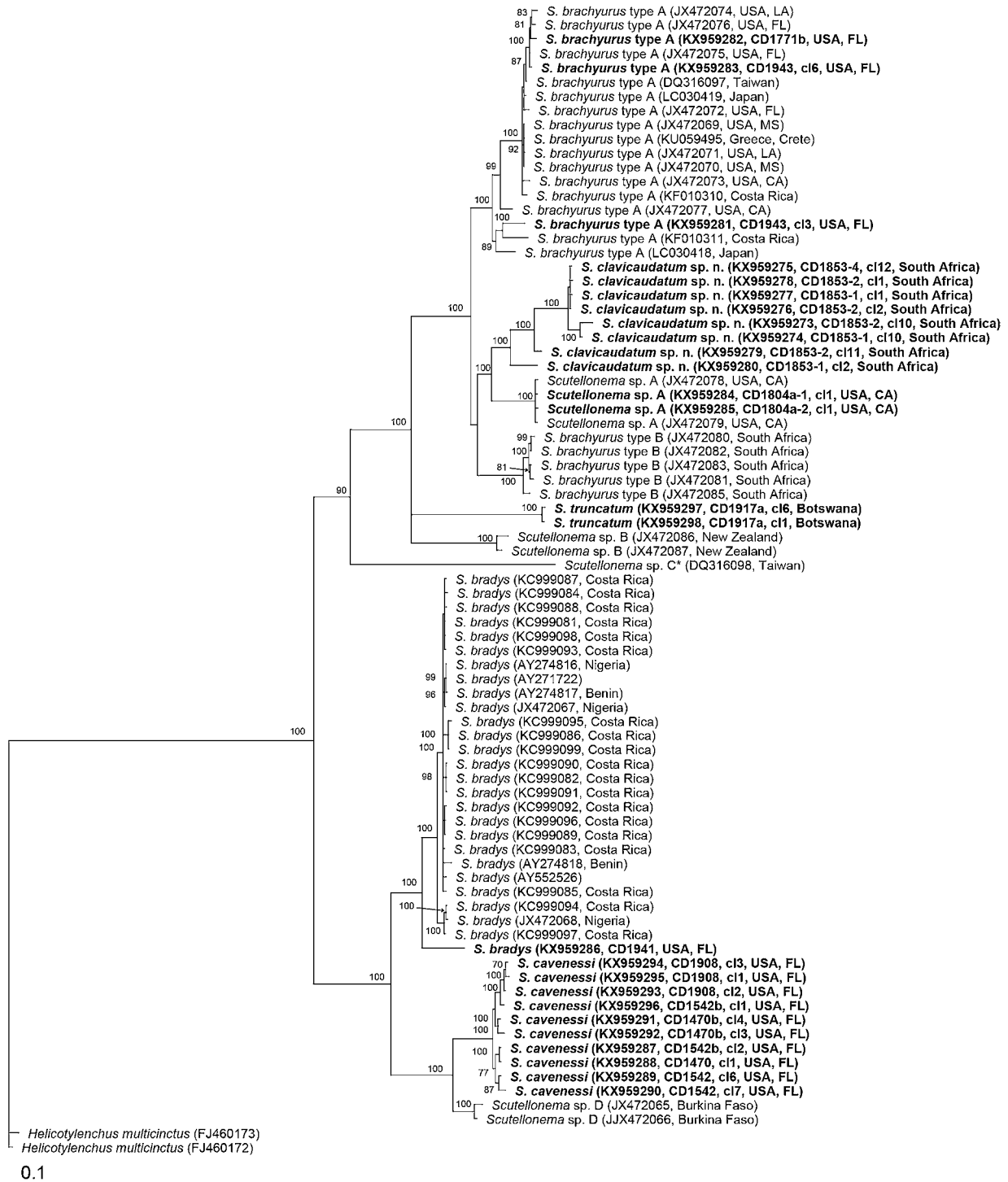


Fig. 23. Phylogenetic relationships within *Scutellonema* populations and species: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. Original sequences are indicated by bold font. * – identified as *S. truncatum* in the article by Chen *et al.* (2006) and named as *Scutellonema* sp. C by Van den Berg *et al.* (2013).

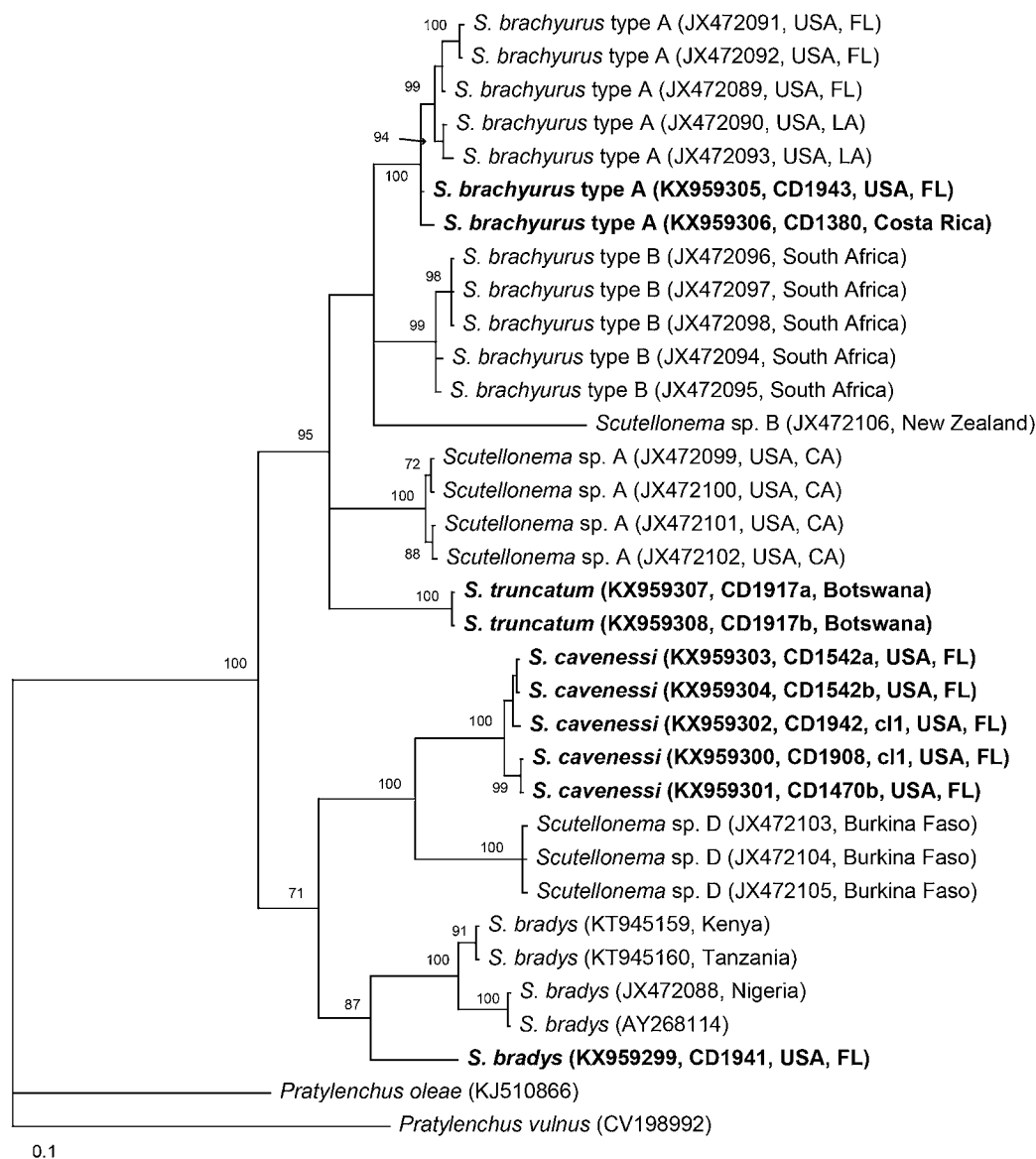


Fig. 24. Phylogenetic relationships within *Scutellonema* population and species: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the *COI* gene sequence alignment under the GTR + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. Original sequences are indicated by bold font.

They were also drawn by Sher (1964) in the description of *Aorolaimus helicus* Sher, 1964. Flagellate spermatozoa were not visible in improperly fixed specimens. The truncate tail terminus is a consistent character of Florida populations of this species and it is not known whether *S. bradys* populations from soybean and tomato in Arkansas also have a truncate tail terminus. We would like to emphasise that our examination of *S. bradys* populations

from Florida and other geographical areas indicates that the vaginal glands in this species are inconspicuous, not prominent and large as reported in the literature (Sher, 1964). Vaginal glands have been confused with the vaginal wall thickenings, thickenings that are prominent in this species. The host range of Florida *S. bradys* is not known. In this study, the nematode was found in soil particles adhering to the surface of roots of Bermuda

grass. It is important to note that *in silico* analysis of ITS sequences shows that specific primers recently developed for detection of *S. bradys* by Van den Berg *et al.* (2013) and Humphreys-Pereira *et al.* (2014) might not work for populations from the USA.

Our study also provided evidence of the presence in Florida of *S. cavenessi*, a species not previously reported in the state. Snake plants (*S. trifasciata*) are a good host of this species which has been spread through the trade of this ornamental. *Scutellonema cavenessi* has been possibly confused in routine identifications with *Peltamigratus christiei* (Golden & Taylor, 1959) Sher, 1964 because both species have numerous males, and females with a distinct epitygma. The molecular characterisation of Florida populations of *S. cavenessi* will facilitate the correct identification of this species in other areas of the USA outside of Florida, since the report of this species from cotton in an undetermined locality in the USA by Elmiligy (1970) remains unconfirmed. It is interesting to note that the Florida *S. cavenessi* clustered with *Scutellonema* sp. D from Burkina Faso (West Africa) in all phylogenetic trees. The unidentified species *Scutellonema* sp. D is very similar to *S. cavenessi* (Van den Berg *et al.*, 2013) and may be conspecific. The genetic similarity between these two *Scutellonema* populations from Florida and West Africa may be explained by the fact that they have the same geographical origin, since *S. cavenessi* arrived in Florida with the trade of snake plants from Tropical West Africa where *S. trifasciata* is an indigenous component of the local flora.

Several species were characterised from South Africa. The new morphological and molecular data on the South African species *S. transvaalense* confirms the validity of this species and improves the diagnostics of the species of *Scutellonema*. The description of *S. clavicaudatum* sp. n. provided in this study is based on robust morphological characters and is supported by the results of molecular analyses, which clearly distinguished this species from other *Scutellonema* species. The identification of South African *S. brachyurus* type B, which is molecularly different from *S. brachyurus* type A, remains unresolved and requires further study and comparison with other African *Scutellonema* species, including *S. unum*.

Acknowledgements

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South African Plant-Parasitic Nematode Survey (SAPPNS) database and Dr J.J. Chitambar (CDFA, USA) for help in morphological characterisation of *S. bradys*. We also thank L. Violet and C.L. Spriggs, Florida Department of Agriculture and Consumer Services, for their field assistance.

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