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Morphological and molecular characterisation and diagnostics of some species of *Scutellonema* Andrássy, 1958 (Tylenchida: Hoplolaimidae) with a molecular phylogeny of the genus

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Summary – *Scutellonema* spp. are widely distributed across tropical and subtropical regions of the world and are associated with numerous agricultural and horticultural crops. Identification of many *Scutellonema* species is not always reliable, in part because many species share very similar diagnostic characters. In this study, we provide morphological and molecular characterisation of *S. brachyurus* from the USA and South Africa, *S. bradys* from Nigeria and three unidentified species from California, USA, New Zealand and Burkina Faso. Morphological descriptions, measurements, light and scanning electron microscopic photos and drawings are given for *S. brachyurus*. Females of *S. brachyurus* from the USA (type A) and South Africa (type B) showed a significant variation in the number of sectors and blocks on the lip annuli, ranging from about 4-12 and from 8-20, respectively. Molecular analysis using the D2-D3 of 28S rRNA, ITS rRNA and the COI mtDNA gene sequences revealed two distinct genotypes within *S. brachyurus* samples: type A (samples from USA, Italy, Korea, Taiwan) and type B (South Africa). Multivariate analyses determined that *S. brachyurus* from the USA and Taiwan (type A) differed from that from South Africa (type B) mainly in body, tail and DGO lengths, and ratios b′, c′, c and V. 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. PCR-RFLP diagnostic profiles and PCR with species-specific primers are developed for the studied *Scutellonema* species.

Keywords – 28S-rRNA, COI mtDNA, diagnostics, ITS rRNA, morphology, morphometrics, multivariate analysis, PCR-RFLP, PCR with specific primers, *Scutellonema brachyurus*, *Scutellonema bradys*, *Scutellonema truncatum*, SEM, taxonomy.

Scutellonema Andrássy, 1958 contains 46 valid species (Siddiqi, 2000). *Scutellonema* species are widely distributed in the tropical and subtropical regions of the world with the greatest number of species recorded from Africa (Sher, 1964). These nematodes primarily feed ectoparasitically on roots and are associated with a range of

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agricultural and horticultural crops. Three species, however, *S. bradys* (Steiner & LeHew, 1933) Andrássy, 1958, *S. cavenessi* Sher, 1964 and *S. brachyurus* (Steiner, 1938) Andrássy, 1958, are considered as agricultural pests, whereas other species cause no known or little economic damage to crops. The yam nematode, *S. bradys*, feeds as an endoparasite on yam (*Dioscorea* spp.), and is responsible for significant tuber losses in West Africa, especially during storage, causing a condition known as dry rot disease (Bridge *et al.*, 2005; Coyne *et al.*, 2006). This nematode also poses a potential pest risk in West Africa for potato (*Solanum tuberosum* L.) (Coyne *et al.*, 2011) and sweet potato (*Ipomoea batatas* (L.) Lam) (Coyne, unpubl.), and has been found parasitising various crops from the Americas and Asia (Bridge *et al.*, 2005). *Scutellonema brachyurus* has a worldwide distribution. In the USA, *S. brachyurus* is reported from fields of alfalfa, cotton, soybean, tobacco, turf and many other crops in native areas of North and South Carolina, California, Arkansas, Florida and other states, and from glasshouses where it is associated with tropical plants. There are several pathogenicity reports of this nematode on amaryllis, some ornamentals, tobacco and lilyturf (Agudelo & Harshman, 2011). In South Africa, *S. brachyurus* is the most common species of the genus, especially from natural vegetation, but has also been found associated with temperate, subtropical and tropical fruits, vegetables, fibre crops, fodder crops, oilseed crops, nut crops, sugarcane, cereals, ornamental plants, grain legumes, prickly pear, hop, ginger, date palm and tree plantations and natural forests in all provinces of South Africa (Kleynhans *et al.*, 1996). It has economic importance when present in large densities in soil. It is also a relatively common species on cassava, sweet potato and yam in Uganda, East Africa (Coyne *et al.*, 2003). *Scutellonema brachyurus* is morphologically similar to *S. unum* Sher, 1964 and could easily be misidentified with this species. *Scutellonema unum* is mainly found in Africa on crops such as maize, coffee, sugar cane, tea, tobacco, pineapple, citrus, *Ficus* sp., root crops, *etc.*, but has also been reported from China (Germani *et al.*, 1985; Coyne *et al.*, 2003) and from yam in Korea (Park & Khan, 2007).

Morphological identification of *Scutellonema* species is difficult and relies on many characters. Because *Scutellonema* species share very similar diagnostic characters, species boundaries are not well established. Some characters have broad overlapping ranges and high intraspecific variability. Phylogenetic and sequence analysis of rRNA and other genes provide attractive solutions to validate morphology-based identifications and resolve some of the difficulties experienced in the traditional systematics of *Scutellonema.* Phylogenetic relationships of *Scutellonema* with other hoplolaimids were studied using the D2-D3 of 28S rRNA (Subbotin *et al.*, 2007; Bae *et al.*, 2009; Cantalapiedra-Navarrete *et al.*, 2013) and 18S rRNA (Meldal *et al.*, 2007) gene sequences. Molecular analysis of several hoplolaimid sequences in which only two species, *S. brachyurus* and *S. bradys*, were included confirmed a monophyly for this genus. However, because of unrepresentative *Scutellonema* sampling in these studies, the relationships between species in the genus remain unknown.

The main objectives of the current study were therefore to: *i*) conduct a morphological and morphometric analysis of *S. brachyurus* from South Africa and the USA, and two unidentified species from California, USA and Burkina Faso, respectively; *ii*) characterise *S. brachyurus* and *S. bradys* and three unidentified *Scutellonema* species from the USA, New Zealand and Burkina Faso using ITS rRNA, D2-D3 expansion segments of 28S rRNA gene and COI of mtDNA gene sequences; *iii*) study phylogenetic relationships within *Scutellonema* using nuclear rRNA genes and COI gene sequences; and *iv*) develop PCR-RFLP diagnostic profiles and design a PCR protocol with species-specific primers for identification of *S. brachyurus* and *S. bradys* and other *Scutellonema* species.

Materials and methods

Nematode populations

Nematode populations used in this study were obtained from soil samples collected from different locations (Table 1). Six samples of *S. brachyurus* were collected in South Africa and included in this analysis: *i*) three from maize experimental plots on Gourton farm 10 km west of Winterton in the Kwazulu-Natal Province (plot I 10F, plot II 21 CR, plot III 44F); *ii*) one from a soybean field, previously sown to maize, on Mooihoek farm near Kestell in the Orange Free State Province; and *iii*) one from a maize plot on the ARC-Zeekoegat Experimental station north of Pretoria in the Gauteng Province. Six USA samples of *S. brachyurus* were included in this study, five from ornamental plants shipped to California from other states and one from rose roots collected in Escondido, California. One sample of *S. bradys* came from infected yam tubers collected from Ibadan market in Nigeria. Two unidentified *Scutellonema* species were collected, one each from California, USA and New Zealand. A few specimens of

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

Species	Locality	Host	Sample code	GenBank accession numbers			Source
				D2-D3 of 28S rRNA	ITS rRNA	COI	
S. brachyurus type A	USA, MS	Unknown plant	CD574	JX472038, JX472045	JX472069 JX472070,	–	Subbotin, S.A.
S. brachyurus type A	USA, FL, Aventura	*Sansevieria laurentii*	CD657	JX472044, JX472046	JX472072	JX472089	Subbotin, S.A.
S. brachyurus type A	USA, FL, Mount Dora	*Sansevieria* sp.	CD650	JX472043, JX472047	JX472075, JX472076	JX472091	Subbotin, S.A.
S. brachyurus type A	USA, FL, Mount Dora	*Sansevieria* sp.	CD633	JX472039, JX472042	–	JX472092	Subbotin, S.A.
S. brachyurus type A	USA, CA, Escondido	*Rose* sp.	CD672A	JX472040, JX472041	JX472073, JX472077	–	Ploeg, A.
S. brachyurus type A	USA, LA	Unknown plant	CD583	JX472037	JX472071, JX472074	JX472090, JX472093	Subbotin, S.A.
S. brachyurus type B	South Africa, Winterton KwaZulu-Natal	Maize	CD722; N823	JX472048, JX472049	JX472082, X472085	JX472095	Van den Berg, E.
S. brachyurus type B	South Africa, Winterton KwaZulu-Natal	Maize	CD723, N822	JX472050, JX472051	JX472083, JX472084	JX472097	Van den Berg, E.
S. brachyurus type B	South Africa, Kestell Orange Free State	Soybean/maize	CD830, OVS343	JX472052	–	JX472098	Van den Berg, E.
S. brachyurus type B	South Africa, Zeekoegat, Pretoria, Gauteng	Maize	CD767, TVL2054	JX472053	–	JX472094	Van den Berg, E.
S. brachyurus type B	South Africa, Winterton Kwazulu-Natal	Maize	CD585, N803-16	JX472054, JX472055	JX472080. JX472081	JX472096	Van den Berg, E.
S. brachyurus type B	South Africa, Winterton Kwazulu-Natal	Maize	CD549, N803	JX472056, JX472057	–	–	Van den Berg, E.
S. bradys	Nigeria, Ibadan	Yam	CD663	JX472035, JX472036	JX472067, JX472068	JX472088	Coyne, D.L., Adewuyi, W.
Scutellonema sp. A	USA, CA, Riverside, Fairmount Park	Grasses	CD741, CD729	JX472058, JX472050	JX472078, JX472079	JX472099, JX472100	Subbotin, S.A.
Scutellonema sp. A	USA, CA, Escondido	*Rosa* sp.	CD672B	–	–	JX472101, JX472102	Ploeg, A.
Scutellonema sp. B	New Zealand, Himatangi Beach, sand dunes	*Ammophila arenaria*	CD564	JX472060, JX472061	JX472086, JX472087	JX472106	Yeates, G.W.
Scutellonema sp. D	Burkina Faso, Leguema	Unknown plant	CA173	JX472033, JX472034	JX472066	JX472103	Roberts, P.A.
Scutellonema sp. D	Burkina Faso, Farako-Ba	Unknown plant	CA174	JX472031, JX472032	JX472065	JX472104, JX472105	Roberts, P.A.

an identified *Scutellonema* were also obtained from Burkina Faso and were studied under the light microscope and molecularly (Table 1). Unidentified species of *Telotylenchus*, *Trichotylenchus* and *Histotylenchus* collected in Africa were also included in the molecular study.

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 observations

South African *Scutellonema* specimens were extracted from soil using the rapid centrifugal-flotation method (Jenkins, 1964), killed and fixed in FPG (Netscher & Seinhorst, 1969), transferred to anhydrous glycerin (De Grisse, 1969) and mounted on permanent slides. Other specimens were killed by heating, fixed in 4% formalin and then temporarily mounted in 4% formalin for measurements. Light micrographs were taken with an automatic Infinity 2 camera attached to a compound Olympus BX51 microscope equipped with a Nomarski differential interference contrast. Measurements were made with a research microscope (Nikon Labophot-2) equipped with a drawing tube.

For scanning electron microscopy (SEM), fixed specimens were dehydrated in increasing concentrations of amyl acetate in pure alcohol and finally in pure amyl acetate. Following conventional critical point drying and gold/palladium coating (15 nm) specimens were viewed with a FEI ESEM Quanta 200 scanning electron microscope at 10 kV.

Multivariate analyses

A multivariate factor analysis was performed on the various *S. brachyurus* populations from the different locations in South Africa and the USA to determine their morphometric similarities. The analyses were based on raw data of morphometric characters of *S. brachyurus* populations shown in Tables 3 and 4. Moreover, to compare morphometric characters of the populations in our study with those of *S. brachyurus* available in the literature, an additional factor analysis was performed on our data, together with that of populations of *S. brachyurus* from Taiwan, for which ITS rRNA sequence is available (Chen *et al.*, 2006).

Factor analyses were performed with the FACTOR procedure of SAS (Statistical Analysis System, version 9.2; SAS Institute). This analysis produced a set of variables (factors) that were linear combinations of the original variables. The new variables (factors) were independent of each other and ranked according to the amount of variation accounted for. After the initial factor extraction by the principal component method, an orthogonal varimax raw rotation was used to estimate the factor loadings. Only factors with an eigenvalue > 1 were extracted.

Additionally, a stepwise canonical linear discriminant analysis was performed to determine which morphometric characters could be used to discriminate between the South African *S. brachyurus* populations and those from the USA. First, the STEPDISC procedure of SAS was used to eliminate variables within the model that did not provide additional information or were redundant as determined by the Wilks' lambda method, as well as to add variables outside the model that contribute most to the model (Khattree & Naik, 2000). The DISCRIM procedure of SAS was then used to generate a discriminant function capable of determining the classification accuracy of the two *S. brachyurus* populations, based on the pooled covariance matrix and the prior probabilities of the classification groups. The data obtained from the stepwise analysis were further subjected to canonical analysis using the CANDISC procedure of SAS to separate classification variables (*S. brachyurus* populations in this analysis) based on linear combinations of the quantitative variables (morphometric characters). The linear combination of variables (canonical root) was then correlated with the original groups. Means of canonical roots (centroid values) were then calculated for each classification variable and significance between means of both groups was determined using Mahalanobis distance. The model was cross-validated using the 'leave-one-out' method (Khattree & Naik, 2000).

DNA extraction, PCR and sequencing

DNA was extracted from several specimens of each population using the proteinase K protocol. DNA extraction, PCR and cloning protocols were as described by Tanha Maafi *et al.* (2003). The PCR temperature profile for amplification of COI of the mtDNA gene was: 7 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 50°C and 2 min at 72°C, followed by a final step of 10 min at 72°C. The primer sets for amplification of the nuclear ribosomal RNA (D2-D3 of 28S rRNA, ITS rRNA) and mitochondrial (COI mtDNA) genes are given in Table 2. The PCR

Table 2. Primer sets used in the present study.

Primer code	Sequences (5′ → 3′)	Amplified gene	Amplicon length (bp)	References
TW81	GTT TCC GTA GGT GAA CCT GC	ITS-rRNA	1060-1130	Tanha Maafi *et al.* (2003)
AB28	ATA TGC TTA AGT TCA GCG GGT			
D2A	ACAAGTACCGTGAGGGAAAGTTG	D2-D3 of 28S rRNA	780-790	Subbotin *et al.* (2006)
D3B	TCGGAAGGAACCAGCTACTA			
TW81	GTTTCCGTAGGTGAACCTGC	ITS-rRNA	Approx. 110	This study
S_brachyurus type B	CATTGCCCTCAACAGACTAC			
TW81	GTTTCCGTAGGTGAACCTGC	ITS-rRNA	Approx. 185	This study
S_brachyurus type A	GCTGAAGTGACAGCCCAACTT			
TW81	GTTTCCGTAGGTGAACCTGC	ITS-rRNA	Approx. 250	This study
S_bradys	GTGATGGCTAAACCACATTC			
TW81	GTTTCCGTAGGTGAACCTGC	ITS-rRNA	Approx. 500	This study
Scut_D	CAAATGTTTGCACATGGGTCC			
TW81	GTTTCCGTAGGTGAACCTGC	ITS-rRNA	Approx. 300	This study
Scut_A	AGAGGTCACATACATGCGTG			
JB3	TTTTTTGGGCATCCTGAGGTTTAT	COI of mtDNA	Approx. 440	Derycke *et al.* (2010)
JB4	TAAAGAAAGAACATAATGAAAATG			

products were purified using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. Sequences were obtained directly from PCR products or from one or more clones. Sequencing was conducted at the Davis Sequencing Center, Davis, CA, USA. The newly obtained sequences were submitted to the GenBank database under accession numbers (JX472031-JX472106) as indicated in Table 1.

PCR-D2-D3 of 28S rDNA-RFLP

Five to 7 μl of purified PCR product of D2-D3 of 28S rDNA was digested by one of the following restriction enzymes: *Dde*I or *Tsp*RI in the buffer stipulated by the manufacturer. The digested DNA was run on a 1.4% TAE buffered agarose gel, stained with ethidium bromide, visualised on UV transilluminator and photographed. The length of each restriction fragment from the PCR products was obtained by a virtual digestion of the sequences using WebCutter 2.0 (www.firstmarket.com/cutter/cut2.html) or estimated from a gel.

PCR with species-specific primers

Species-specific primers for five *Scutellonema* species (Table 2) were designed using the sequence alignment of the ITS rRNA gene. The PCR mixture was prepared as described by Tanha Maafi *et al.* (2003). The PCR amplification profile consisted of 4 min at 94°C; 30 cycles of 1 min at 94°C, 45 s at 57°C and 45 s at 72°C, followed by a final step of 10 min at 72°C. Two μl of the PCR products were run on a 1.4% TAE buffered agarose gel, stained and photographed. All *Scutellonema* samples were used to test the specificity of PCR with newly designed species-specific primers.

Sequence and phylogenetic 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 default parameters with their corresponding published gene sequences (Chen *et al.*, 2006; Subbotin *et al.*, 2006, 2007; Bae *et al.*, 2009; Lee & Williamson, unpubl.). 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. BI analysis for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. The log-likelihood values of the sample points stabilised after approximately 1000 generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for

further analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. 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

Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958 USA populations, type A (Figs 1, 2, 7A-F; 8A-F)

These populations were obtained from different locations and states of the USA (Table 1) and designated as 'type A' based on the results from the rRNA and mtDNA gene sequence and phylogenetic analyses.

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Body form ranging from an open C-shape to a closed spiral. Lip region broadly rounded, well set off with 4-6 annuli. SEM photographs showing great variation in number and arrangement of longitudinal lines on lip annuli with some blocks regular but some very irregular. Blocks on basal annulus ranging from *ca* 4-12, not always easy to count but certainly more than six in many specimens. Labial disc rounded with small amphid openings laterally. Labial framework moderate. Anterior and posterior cephalids rarely seen, five and 11-12 annuli posterior to base of lip region. Stylet robust with stylet knobs rounded posteriorly and slightly indented anteriorly. Metenchium shorter than telenchium. Median bulb slightly longer than wide, not filling body cavity. Pharyngeal glands overlap distinctly dorsally with three gland nuclei. Excretory pore located from opposite anterior part of basal pharyngeal lobe to about middle of lobe. Hemizonid 2-3 annuli long situated from opposite to three annuli anterior to excretory pore. Hemizonion indistinct. Annuli on body distinct. Spermatheca indistinct, otherwise small, round, thick-walled and without sperm cells. Vaginal glands small and oblong. Epiptygma appearing double and folded into vagina or over vulval opening. Scutellum size moderate, situated opposite or just anterior to anus. Lateral fields with four lines, areolated anteriorly opposite pharyngeal area and mostly opposite scutellum, rarely without any areolation around scutellum. Intestine not overlapping rectum. Tail rounded, more so on dorsal side with 10-15 ventral annuli, those on posterior tip sometimes slightly larger than on rest of body.

Male

Not found.

Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958 South African populations, type B (Figs 3-5, 7I, J; 8I, J)

These populations were obtained from different locations in South Africa (Table 1) and named as 'type B' based on the results of the rRNA and mtDNA gene sequence and phylogenetic analyses.

MEASUREMENTS

See Table 4.

DESCRIPTION

Female

Body form ranging from an open to closed spiral. Lip region rounded, slightly set off with distinct labial disc with mostly three but also four lip annuli when seen laterally, labial disc rounded with small amphid openings laterally. SEM photographs showing mainly three, rarely 4-5 lip annuli, great variation in number and arrangement of longitudinal and oblique lines on lip annuli but mostly confined to basal lip annulus. *Circa* 8-20 regular and irregular blocks seen on basal annulus. Lip region slightly set off from body. Labial framework moderate. Anterior and posterior cephalids not seen. Stylet robust with stylet knobs rounded posteriorly and slightly hollow anteriorly. Metenchium shorter than telenchium, rarely of equal length. Median bulb slightly longer than wide, not filling body cavity. Pharyngeal gland overlapping dorsally with three gland nuclei. Excretory pore situated from rarely opposite middle of isthmus to mostly opposite basal pharyngeal lobe, as far posterior as opposite posterior part of pharyngeal lobe. Hemizonid two annuli long, situated from opposite excretory pore to five annuli

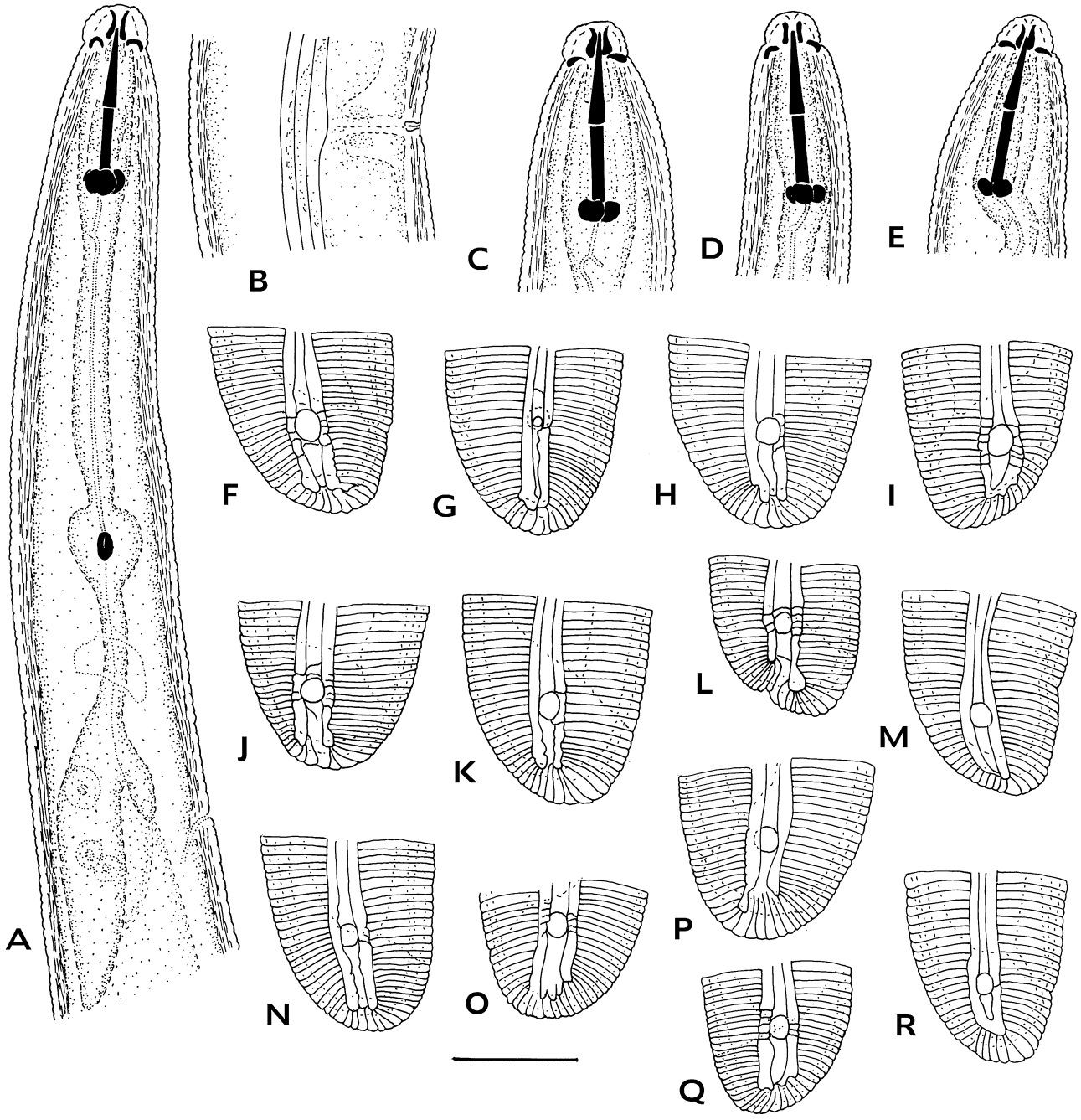

Fig. 1. *Scutellonema brachyurus* type A, four USA populations. Female. A: Anterior part of body; B: Body at vulva with distinct lateral field; C-E: Lip regions; F-R: Selection of tails from all localities showing various tail and lateral field endings. (Scale bar = 20 μm.)

anterior to excretory pore. Hemizonion indistinct in most specimens, in one specimen it was one annulus long and situated 13 annuli posterior to hemizonid. Annuli on body distinct. Spermatheca visible in most specimens but small, round and empty. Vaginal glands small, oblong, not very distinct. Epiptygma appearing double and folded into vagina or over vulval opening. Intestine mostly not overlapping rectum. Scutellum size moderate, situated from three annuli anterior to three annuli posterior to anus. Lateral fields with four lines, areolated anteriorly opposite

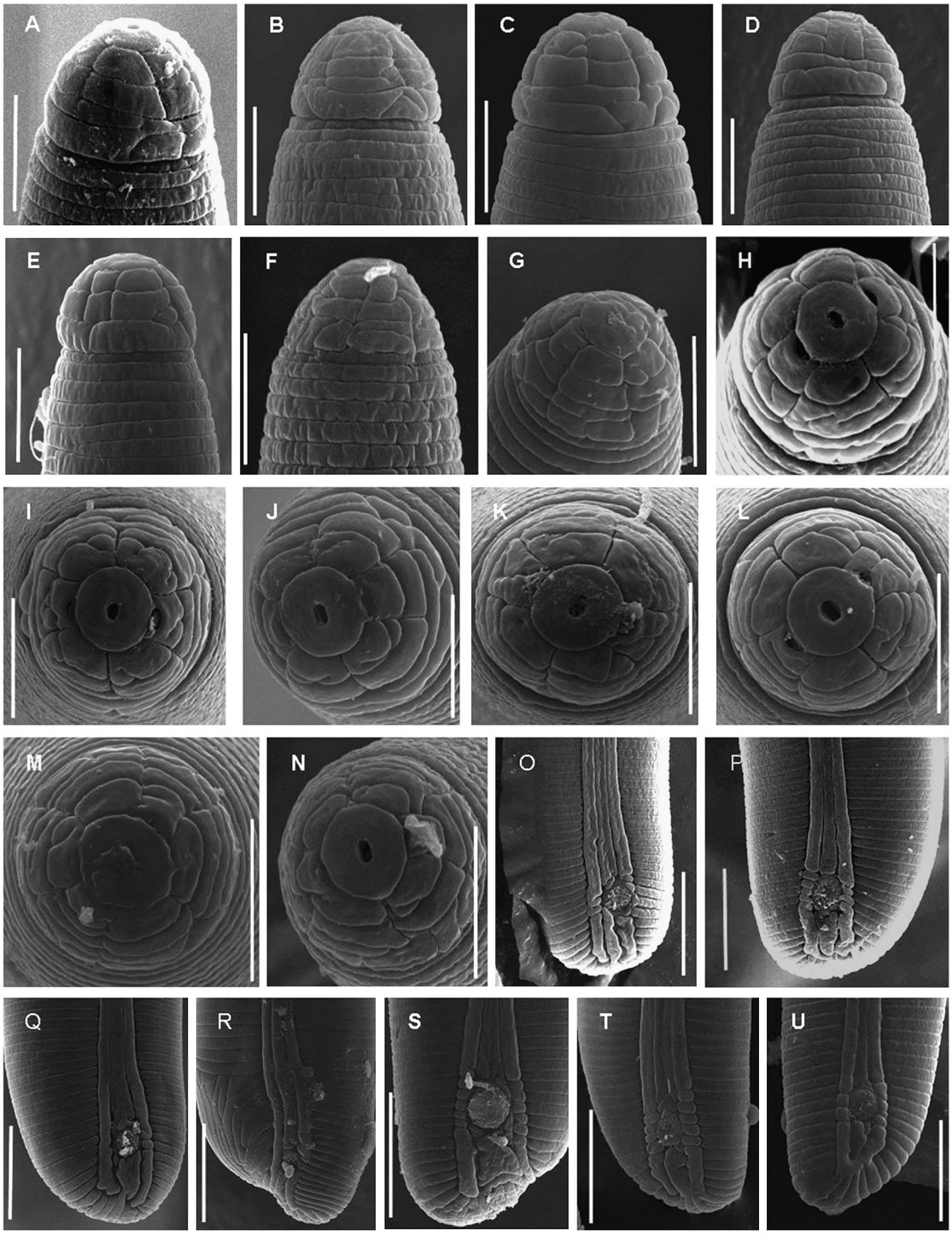

Fig. 2. *Scutellonema brachyurus* type A, four USA populations (FL, USA, CD633, CD650, CD657; CA, USA, CD672A). Female. A-G: Lateral views of female lip regions from various populations showing variation in lip annulation and areolation of annuli; H-N: *En face* views of various females showing variation in areolation of lip annuli; O-U: Tails of various females. (Scale bar: A-N = 5 μm; O-U = 10 μm.)

Table 3. Measurements of *Scutellonema brachyurus* type A from American localities. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Code and locality			
	CD 633 FL, Mount Dora	CD 650 FL, Mount Dora	CD 657 FL, Aventura	CD 672A CA, Escondido
n	11	6	2	4
L	734 ± 45.2 (656-805)	756 ± 30.5 (704-789)	740; 798	715 ± 84.2 (611-787)
a	21 ± 0.9 (19.5-22.9)	21.5 ± 1.1 (20.3-23)	19.9; 20.9	21.7 ± 1 (20.8-23.1)
b	5.0 ± 0.3 (4.6-5.6)	5.2 ± 0.4 (4.7-5.9)	5.4; 5.3	4.6; 5.9
b′	6.1 ± 0.5 (5.5-6.8)	6.3 ± 0.5 (5.9-6.9)	5.9; 6.2	5.8; 7.4
c	69 ± 11.3 (59.4-95.3)	62.6 ± 5.0 (56.5-67.4)	72.5; 72.3	66.5 ± 7 (59.3-75.8)
c′	0.5 ± 0.06 (0.4-0.6)	0.5 ± 0.05 (0.5-0.6)	0.5	0.6
o	21.6 ± 2.6 (17.9-26)	16.4 ± 5.0 (10-22.2)	22; 22.2	17.9
DGO	6.0 ± 0.7 (5-7)	5.0 ± 1.6 (3.0-6.5)	6.5	5
V	59.5 ± 1 (58-61)	59.5 ± 1.2 (57.5-61.5)	61.5; 59	58.5 ± 2.5 (56.5-62)
OV^1	24 ± 3.1 (18-27.5)	27 ± 3.5 (21.5-32)	26; 24	23.7 ± 3.3 (21-27.5)
OV^2	26 ± 4.3 (21-34.5)	24 ± 3.3 (21-27.5)	24; 25.5	25 ± 1.7 (23-26.5)
Stylet length	29 ± 0.8 (28.5-30.5)	29 ± 1.2 (27-30)	30	28.5 ± 1.4 (27-30.5)
Metenchium length	14.5 ± 0.5 (13.5-15.5)	14 ± 1.0 (12-14.5)	14.5; 14	13.5 ± 0.7 (12.5-14.5)
Telenchium length	14.5 ± 0.5 (13.5-15)	15 ± 0.3 (14.5-15.5)	15.5; 16	15 ± 0.7 (14.5-16)
m	50.2 ± 0.8 (49-51)	47.9 ± 1.4 (45.1-49.3)	48.8; 47	46.6 ± 0.6 (46-47.3)
Stylet knob height	3.5 ± 0.3 (3.0-3.5)	3.5 ± 0.3 (3.0-3.5)	3.5	3.5 ± 0.3 (3-3.5)
Stylet knob width	6.0 ± 0.6 (5-7)	5.5 ± 0.6 (5.0-6.5)	6; 6.5	5.5 ± 0.5 (5-6)
Pharynx length	147 ± 6.4 (139-156)	147 ± 7.5 (134-156)	137; 152	133; 134
Excretory pore from ant. end	125 ± 6.5 (111-136)	127 ± 3.6 (121-132)	128; 130	109 ± 4.7 (103-114)
Pharyngeal overlap	27.5 ± 5.9 (20-37)	26 ± 4.3 (18.5-31)	10.5; 23.5	27
Diam. at mid-body	35 ± 2.6 (31.5-38)	35 ± 2.3 (32.5-37.5)	37.5; 38	33 ± 3.2 (29.5-37)
Diam. at anus	22 ± 2.1 (18.5-24.5)	22.5 ± 1.8 (20-25)	22; 23.5	18.5 ± 1.5 (17-20.5)
Median bulb length	12.5 ± 1.0 (11-14.5)	13 ± 0.7 (12.5-14)	13; 14	11.5; 12±
Median bulb diam.	10 ± 0.6 (9-11)	10.5 ± 0.7 (9.5-10.5)	11.5; 12	9.5; 10.5±
MB valve length	3.0 ± 0.4 (2.5-3.5)	3.0 ± 0.3 (3.0-3.5)	3.5; 4	3.3
MB valve width	2.5 ± 0.3 (2-3)	2.5 ± 0.3 (2-3)	3.5; 4	2.9
Lip region diam.	10 ± 0.5 (9-11)	10 ± 0.5 (9.5-11)	9.5; 10.5	9.5 ± 0.3 (9-10)
Lip region height	5.5 ± 0.3 (5-6)	5.5 ± 0.4 (5-6)	6.5; 6	5.5 ± 0.5 (5-6)
Annulus width	1.5 ± 0.2 (1-2)	1.5 ± 0.1 (1.5-2.0)	1.5; 2	1.5 ± 0.2 (1.5-2)
Lateral field width	5.5 ± 0.6 (4.5-6)	6.0 ± 0.5 (5.5-6.5)	5; 5.5	5 ± 0.3 (5-5.5)
Tail length	11 ± 1.6 (7.5-12.5)	12 ± 1.1 (11-14)	10.5; 11	11 ± 0.7 (10-12)
Scutellum length	4.0 ± 0.5 (3.5-5.0)	4.0 ± 0.5 (3.5-4.5)	4.5; 5	4 ± 0.6 (3.5-5)
Scutellum width	3.5 ± 0.3 (3.5-4.0)	3.5 ± 0.4 (3-4)	4; 4.5	3 ± 0.2 (3-3.5)
Spermatheca length	8.5 ± 1.3 (6.5-10.5)	9.5 ± 1.8 (7.0-11.5)	10; 8.5	6; 9
Spermatheca width	8 ± 0.9 (6.5-9.5)	8.5 ± 1.2 (6.5-9.5)	10.5	9; 10

pharyngeal region and mostly opposite scutellum, rarely without any areolation around scutellum. Tail rounded, more so on dorsal side with 9-17 annuli, those on posterior tip slightly larger than on rest of body.

Male

Not found.

***Scutellonema* sp. A**
(Figs 6, 7G, H; 8G, H)

This species was found among grass roots collected at Fairmount Park, Riverside, CA, USA, and from a soil sample collected from roses in Escondido, CA, USA, in the mixture with *S. brachyurus* type A.

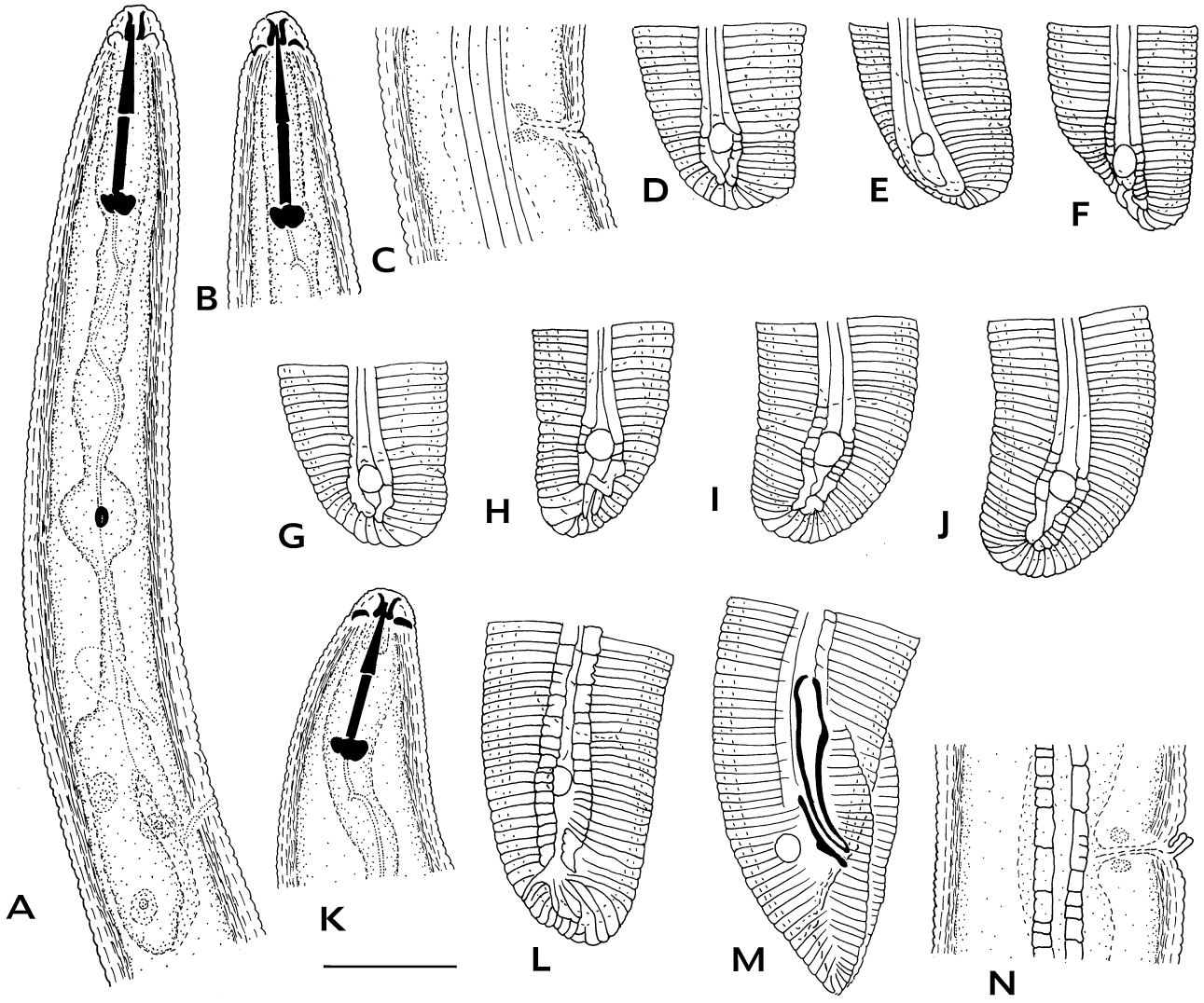

Fig. 3. *Scutellonema brachyurus* type B, five South African populations. Females. A: Anterior part of body; B: Lip region of another female; C: Body at vulva with distinct lateral field; D-J: Tails of various females showing various areolations at scutellum. *Scutellonema* sp. D. Female. K: Lip region; L: Tail; N: Body at vulva with areolated lateral field. Male. M: Tail. (Scale bar = 20 μm).

MEASUREMENTS

Female (n = 3): L = 739 (744-826) μm; a = 27.7 (25.9-29.8); b = 5.1 (4.8-5.4); c = 56.6 (50-63.3); c′ = 0.75 (0.7-0.8); o = 20.2 (15-25.7); V = 57 (56.5-57.5); G_1 = 23 (n = 1); G_2 = 23 (n = 1); stylet = 28 (25.5-29.5) μm; stylet knob height = 3 μm; stylet knob width = 4.5 μm; metenchium = 13.5 (13-14) μm; telenchium = 16 (15.5-16) μm; m = 46.2 (44.9-47.6); lip region diam. = 9 (8-9.5) μm; lip region height = 5 μm; DGO = 5.5 (4.5-6.5) μm; median bulb length = 13 μm; median bulb diam. = 12 μm; excretory pore from anterior = 118 (114-134) μm; pharynx length = 155 (151.5-157.5) μm; diam. at mid-body = 28.5 (27-30) μm; diam. at anus = 19 (17.5-21) μm; lateral fields width = 6 (5-7) μm; scutellum length = 4.5 (3.5-5) μm; scutellum width = 4 (3.5-5) μm; tail length = 14 (12-16.5) μm.

Male

Not found.

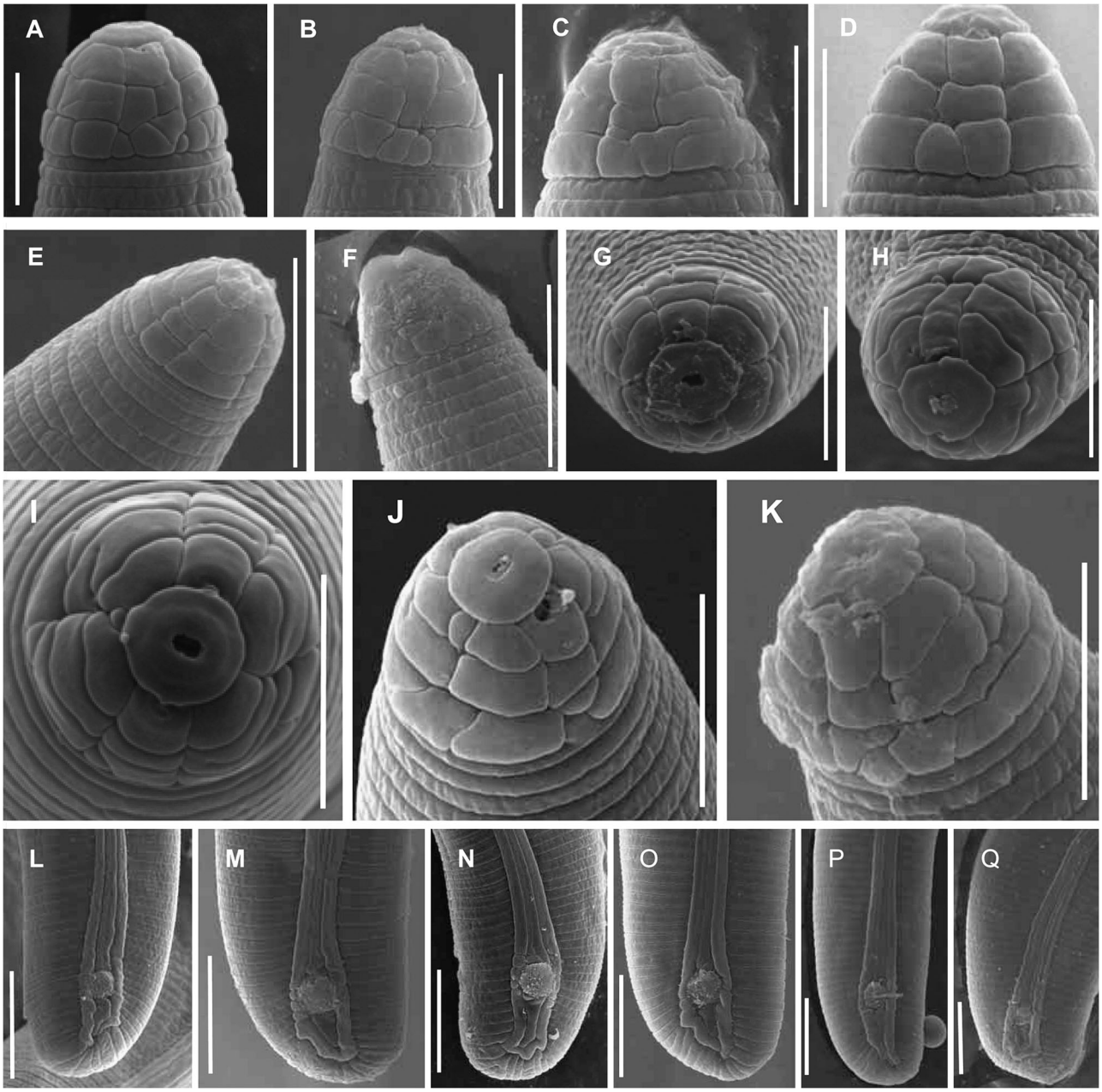

Fig. 4. *Scutellonema brachyurus* type B, three South African populations (TVL2054, OVS343, N823). Females. A-F: Lateral views of various lip regions showing variation in lip annulation and areolation of annuli. G-K: *En face* views of various females showing variation in areolation of lip annuli; L-Q: Tails of various females showing various areolations at scutellum. (Scale bar: A-K = 5 μm; L-Q = 10 μm.)

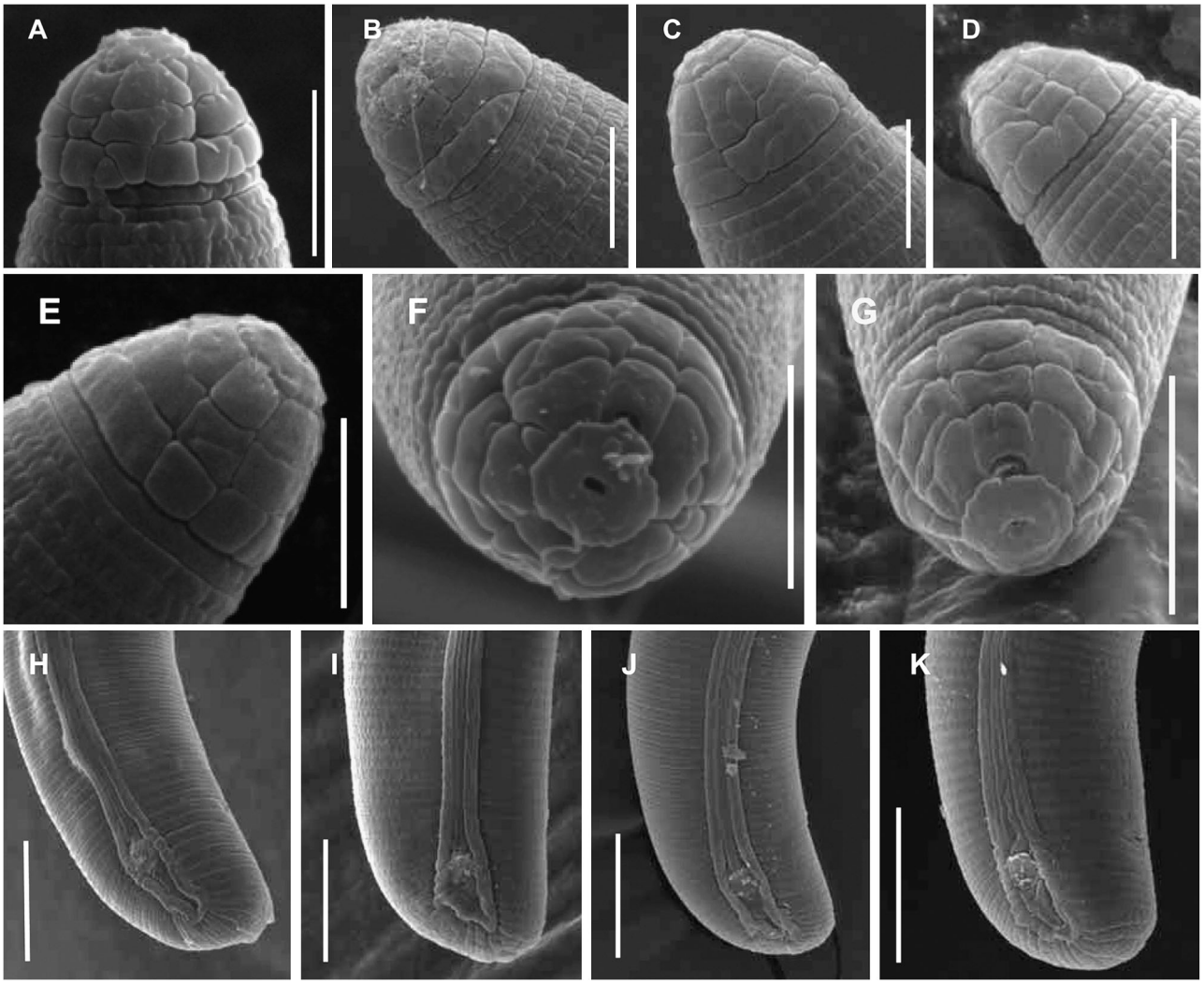

Fig. 5. *Scutellonema brachyurus* type B, two South African populations (N803, N822). Females. A-E: Lateral views of various lip regions showing variation in lip annulation and areolation of annuli; F, G: *En face* views of two lip regions showing variation in areolation of lip annuli; H-K: Tails of various females showing various areolations at scutellum. (Scale bar: A-G = 5 μm; H-K = 10 μm.)

DESCRIPTION

Female

Body curved ventrad. Lip region rounded, slightly set off with 3-4 annuli. SEM photographs showing more than eight blocks on basal annulus. Labial disc rounded with small amphid openings laterally. Labial framework moderate. Anterior and posterior cephalids not seen. Stylet robust with stylet knobs rounded posteriorly and slightly indented anteriorly. Metenchium shorter than telenchium. Pharyngeal glands overlapping dorsally with three gland nuclei. Excretory pore, situated from opposite anterior end to opposite middle of pharyngeal lobe. Hemizonid two annuli long, situated from directly anterior to three annuli anterior to excretory pore. Hemizonion one annulus long situated nine annuli posterior to hemizonid. Body annuli distinct, 2 μm wide. Spermatheca small, indistinct. Vaginal glands small and oblong. Epiptygma protruding outside body or folded into vagina. Scutellum situated just posterior to anus. Lateral field with four lines, areolated anteriorly and opposite excretory pore. Tail rounded, more so on dorsal side with 10-11 annuli.

Table 4. Measurements of *Scutellonema brachyurus* type B from South African localities. All measurements are in μm and in the form: mean ± s.d.(range).

Character	Code and locality				
	N 803 Winterton, Kwazulu-Natal	N 822 Winterton, KwaZulu-Natal	N 823 Winterton, KwaZulu-Natal	TVL 2054 Zeekoegat, Pretoria, Gauteng	OVS 343 Kestell, Orange Free State
n	15	11	11	22	25
L	783 ± 47.3 (692-876)	810 ± 76.3 (697-920)	777 ± 36.6 (726-846)	798 ± 49.2 (702-885)	820 ± 49.5 (715-890)
a	27.6 ± 1.6 (25.4-31.7)	26.3 ± 1.3 (24.1-28.7)	26.5 ± 2 (23-29.8)	28.5 ± 1.5 (25.7-31)	27.6 ± 2.3 (22.2-31.8)
b	5.8 ± 0.3 (5.3-6.5)	5.8 ± 0.4 (5.1-6.3)	6 ± 0.4 (5.5-6.6)	6.2 ± 0.4 (5.5-6.9)	6.3 ± 0.5 (5.4-7.5)
b′	7 ± 0.5 (6.3-7.9)	7 ± 0.4 (6.2-7.7)	7.1 ± 0.5 (6.5-7.9)	7.5 ± 0.4 (6.2-8.4)	7.3 ± 0.7 (6.2-8.8)
c	55.5 ± 9.6 (42.9-74.8)	55.5 ± 10.1 (44.7-76.2)	54.1 ± 7 (44.6-69.8)	67.5 ± 12 (46.3-95.5)	49.7 ± 5.1 (36.6-58.9)
c′	0.7 ± 0.01 (0.5-0.9)	0.75 ± 0.1 (0.55-1.0)	0.8 ± 0.1 (0.6-0.9)	0.7 ± 0.1 (0.5-0.9)	0.9 ± 0.2 (0.7-1.1)
o	17.8 ± 2.4 (13-21.5)	18 ± 2.4 (12.5-20.2)	18.8 ± 2.9 (13.3-22.5)	21.6 ± 2.1 (18.4-26.1)	18.0 ± 2.1 (14.8-23.1)
DGO	5.1 ± 0.8 (3.5-6.0)	5.0 ± 0.7 (3.7-6.0)	5 ± 0.8 (4-6.5)	6.0 ± 0.5 (5.0-6.5)	5 ± 0.6 (4.5-6.5)
V	57.5 ± 1.5 (54-60)	58 ± 0.9 (56.7-59.5)	57 ± 1.1 (55.5-59)	57 ± 1.2 (53-59)	57 ± 1.4 (54-59.5)
OV^1	21.5 ± 4.9 (19-28)	22.7 ± 4.6 (13-27)	21 ± 3.4 (15.5-27)	21.5 ± 3.7 (12.5-25.5)	23 ± 2.6 (18.5-27)
OV^2	19.1 ± 3.4 (15-26)	19 ± 2.6 (13-22)	23 ± 2.3 (19.5-25.5)	21 ± 2.3 (17-25)	22 ± 2.6 (19-28.5)
Stylet length	28.5 ± 1.0 (26.5-30)	29 ± 0.9 (27-30)	29 ± 1 (27.5-30.5)	28 ± 1.2 (25.5-30)	29.5 ± 1 (27-32)
Metenchium length	14 ± 0.6 (12.5-14.5)	14 ± 0.8 (12.9-14.7)	14 ± 0.3 (13.5-14.5)	13 ± 0.7 (11.5-14)	14 ± 0.6 (13-15.5)
Telenchium length	15 ± 0.5 (14-15.5)	15 ± 0.3 (14.5-16)	15 ± 0.7 (14-16)	15 ± 0.6 (13-16)	15 ± 0.6 (13.5-16.5)
m	48 ± 1 (45.5-50)	47.2 ± 1.6 (44-49)	48.4 ± 0.8 (47-49)	46.3 ± 2.0 (42-49.5)	48.4 ± 1.1 (46.2-50)
Stylet knob height	3.0 ± 0.3 (2.0-3.5)	3.0 ± 0.3 (2.5-4.0)	3 ± 0.4 (2-3.5)	3.0 ± 0.4 (2.0-3.5)	3 ± 0.4 (2-3.5)
Stylet knob width	5.0 ± 0.3 (4.5-5.5)	5.0 ± 0.7 (3.5-6.0)	5 ± 0.5 (4.5-6)	5.0 ± 0.3 (4.0-5.5)	5 ± 0.5 (4.5-6.5)
Pharynx length	135 ± 10.3 (114-153)	140 ± 11.7 (129-165)	130 ± 10.3 (116-154)	128 ± 6.9 (115-138)	133 ± 8.7 (118-151)
Excretory pore from ant. end	122 ± 9.7 (105-134)	125 ± 12.6 (101-141)	115 ± 7.1 (109-126)	119 ± 6.7 (106-131)	127 ± 8 (108-150)
Pharyngeal overlap	21.5 ± 4 (12.5-26.5)	23.5 ± 5.3 (17-33)	19.5 ± 3.4 (14-24)	20.5 ± 4.6 (10.5-26.5)	20.5 ± 5.2 (12-34.5)
Diam. at mid-body	28 ± 3 (19.5-31)	31 ± 2.3 (26.5-34)	29.5 ± 2.9 (25.5-35)	28 ± 2.4 (23.5-32.5)	30 ± 2.9 (23-38)
Diam. at anus	21 ± 1.6 (18.5-22.5)	19.5 ± 2.7 (14-23.5)	19 ± 1.3 (17.5-20.5)	18 ± 1.6 (14.5-21.5)	20 ± 1.7 (16-22)
Median bulb length	13.5 ± 1 (12-14.5)	13 ± 1.2 (11-14.5)	13.5 ± 1.1 (12-15.5)	13 ± 0.8 (11-14.5)	12.5 ± 0.8 (11.5-14)
Median bulb diam.	11 ± 0.8 (9.5-12.5)	11 ± 0.9 (9.5-12.5)	11.5 ± 1 (9.5-12.5)	10 ± 1.0 (6.5-12)	10 ± 0.8 (8.5-12.5)
MB valve length	–	3.0 ± 0.5 (3.0-4.5)	–	3.5 ± 0.4 (3.0-4.5)	3.5 ± 0.5 (2.5-5)
MB valve width	–	2.5 ± 0.3 (2-3)	–	2.5 ± 0.3 (2.0-3.5)	2.5 ± 0.2 (2-3)
Lip region diam.	8.0 ± 0.6 (7.5-9.0)	8.5 ± 0.6 (7.5-9.2)	8.5 ± 0.4 (8-9.5)	8.5 ± 0.4 (7.5-9.0)	8.5 ± 0.6 (7.5-10.5)
Lip region height	5.0 ± 0.3 (4.5-6.0)	5.0 ± 0.5 (5.0-6.5)	5 ± 0.5 (4.5-6)	5.0 ± 0.5 (4.5-6.0)	5 ± 0.4 (4.5-6)
Annulus width	1.5 ± 0.1 (1.5-2.0)	1.5 ± 0.2 (1.5-2.0)	1.5 ± 0.2 (1.5-2)	1.5 ± 0.2 (1-2)	1.5 ± 0.1 (1.5-2)
Lateral field width	6.0 ± 0.6 (5.0-6.5)	5.5 ± 0.6 (5.0-6.5)	6 ± 0.5 (5-6.5)	6.0 ± 0.6 (4.5-7.5)	5.5 ± 1 (5-7.5)
Tail length	14.5 ± 2.7 (10.5-19.5)	15 ± 3.6 (10-20.5)	14.5 ± 1.9 (11-18)	12.5 ± 2.5 (7.5-19)	16.5 ± 2.2 (12.5-23)
Scutellum length	4.0 ± 0.5 (3.0-4.5)	4.5 ± 0.6 (3.0-4.5)	3.5 ± 0.6 (3-4.5)	4.0 ± 0.4 (3.5-5.0)	4 ± 0.5 (3.5-5)
Scutellum width	3 ± 0.4 (2.5-3.5)	4.0 ± 0.7 (3-5)	3.5 ± 0.5 (3-4.5)	3.5 ± 0.5 (2.5-4.0)	3.5 ± 0.5 (3-4.5)
Spermatheca length	–	–	9 ± 2.3 (8-12) (n = 3)	9.0 ± 1.4 (6.5-11)	9 ± 1.4 (8-11)
Spermatheca width	–	–	8 ± 1.8 (7-10) (n = 3)	9.0 ± 0.6 (8-10)	8.5 ± 1.4 (6-10.5)

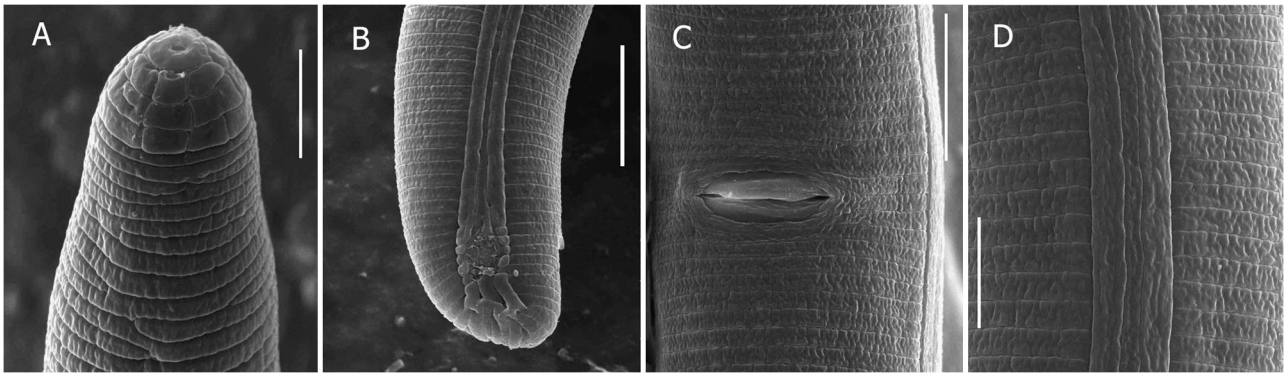

Fig. 6. *Scutellonema* sp. A, CA, USA (CD741). Females. A: Lateral view of female lip region; B: Tail; C: Vulva region; D: Lateral field. (Scale bar: A, B = 5 μm; C, D = 10 μm.)

Scutellonema sp. B

This nematode was collected among the roots from a marram grass (*Ammophila arenaria*) at Himatangi Beach, New Zealand, in the type locality of *S. magna* Yeates, 1967. Smit (1971) considered *S. magna* as a synonym of *Morulaimus geniculatus* Sauer, 1966. Germani *et al.* (1985) and other authors (Siddiqi, 1986; Fortuner & Luc, 1987) accepted this synonymisation. A comparative detailed morphological study of *Scutellonema* sp. B from New Zealand is needed to establish its identity conclusively.

Scutellonema sp. C

The ITS rRNA gene sequence of the nematodes collected from *Bambusa* sp. in Taiwan and identified as *S. truncatum* Sher, 1964 was published by Chen *et al.* (2006). This sequence was also included in our analysis. *Scutellonema truncatum* was originally described from a virgin clay soil in South Africa. It was subsequently recovered from various plants from other areas in South Africa, Zimbabwe, and also from Mozambique. *Scutellonema truncatum* was also reported from Manipur, India (Dhanachand, 2000) and Taiwan (Chen *et al.*, 2006). Detailed study of the article by Chen *et al.* (2006) established that the nematode they described as *S. truncatum* was not, in fact, *S. truncatum*, but a species with a rounded, annulated lip region, whereas specimens of the type and other populations of *S. truncatum* have a typical conical and non-annulated lip region. Unfortunately, the authors were not able to get any slides of this nematode to confirm identification, and thus the sequence of this species is given here under the name *Scutellonema* sp. C (Fig. 11).

Scutellonema sp. D
(Fig. 3K-N)

These specimens were collected in two locations of Burkina Faso from fields (Table 1).

Measurements

Female (n = 1): L = 601 μm; a = 20.5; b = 5.4; c = 27.7; c′ = 1; o = 23.8; V = 53.9; stylet = 24.5 μm; stylet knob height = 3 μm; stylet knob width = 5 μm; metenchium length = 11.5 μm; telenchium length = 13 μm; m = 46.3; lip region diam. = 10 μm; lip region height = 5 μm; DGO = 6 μm; median bulb length = 14.5 μm; median bulb diam. = 12 μm; MB valve length = 4.5 μm; MB valve width = 3 μm; excretory pore from anterior = 78 μm; pharynx length = 110 μm; diam. at mid-body = 29.5 μm; lateral field width = 7.5 μm; scutellum length = 4.5 μm; scutellum width = 3.5 μm; spermatheca length = 17.5 μm; spermatheca width = 15 μm; tail length = 21.5 μm.

Male (n = 1): L = 659 μm; a = 26.4; c = 28.0; c′ = 1.5; stylet = 22.5 μm; spicules = 27 μm; gubernaculum = 14 μm; scutellum length = 6 μm; scutellum width = 4.5 μm.

Description

Female

Body slightly curved ventrad into open C-shape. Lip region broadly rounded, slightly flattened anteriorly and

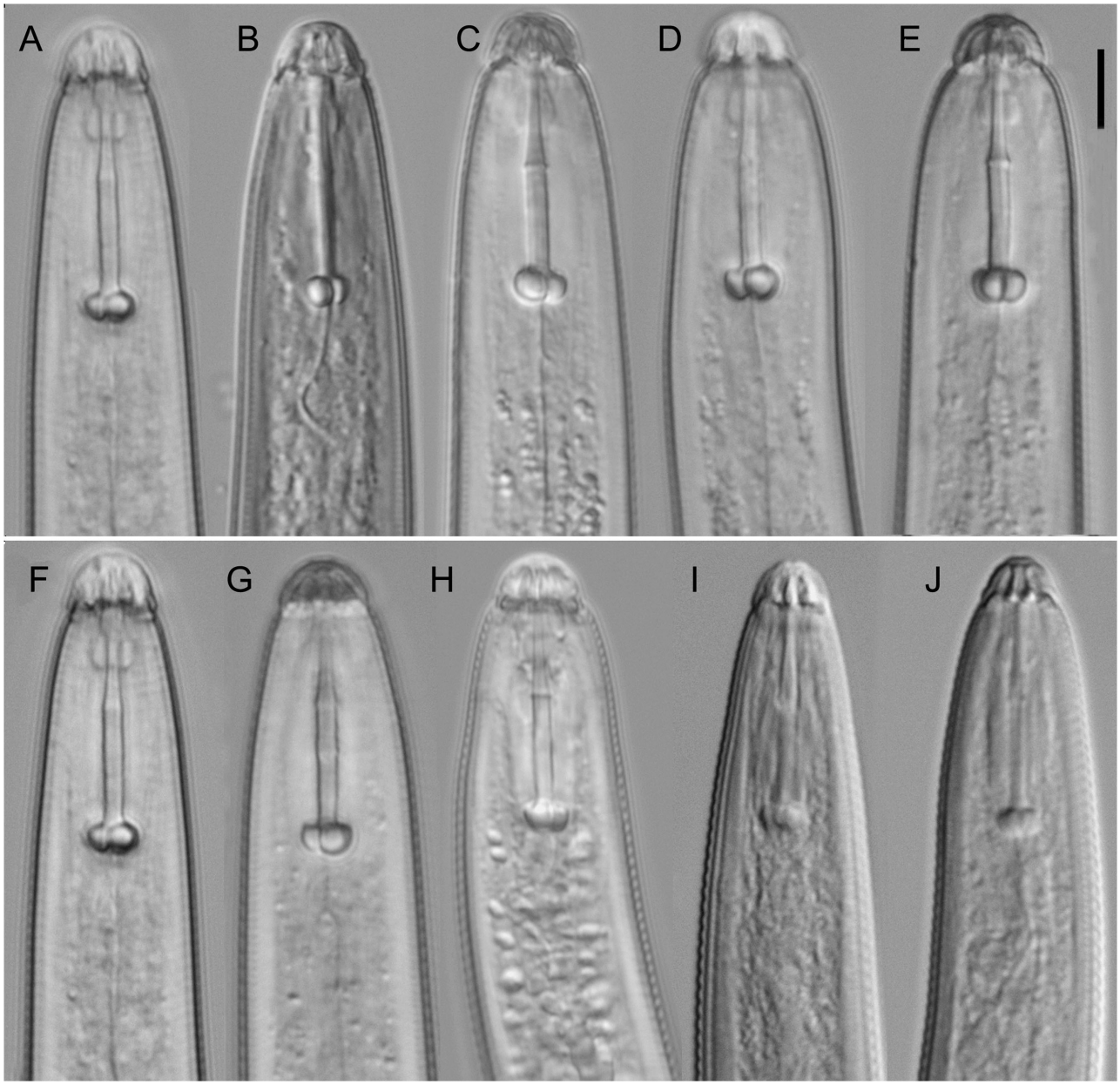

Fig. 7. *Scutellonema* species. Lip region. A, F: *S. brachyurus* type A, MS, USA. (CD574); B: *S. brachyurus* type A, LA, USA (CD583); C: *S. brachyurus* type A, FL, USA (CD633); D, E: *S. brachyurus* type A, FL, USA (CD650); G, H: *Scutellonema* sp. A, CA, USA (CD741, CD729); I, J: *S. brachyurus* type B, South Africa. (Scale bar = 10 μm.)

slightly set off from body with seven annuli. Basal annulus without any incisures. Labial framework very well developed stretching one annulus posterior from basal plate. Anterior and posterior cephalids not observed. Stylet well developed. Stylet knobs rounded posteriorly and flattened and slightly indented anteriorly, 5 μm wide and 3 μm high. Metenchium shorter than telenchium. Median bulb rounded. Excretory pore situated opposite anterior part of isthmus. Hemizonid two annuli long, situated opposite excretory pore. Hemizonion not observed. Lateral fields completely areolated opposite pharyngeal area, then irregular over whole length of body and completely areolated

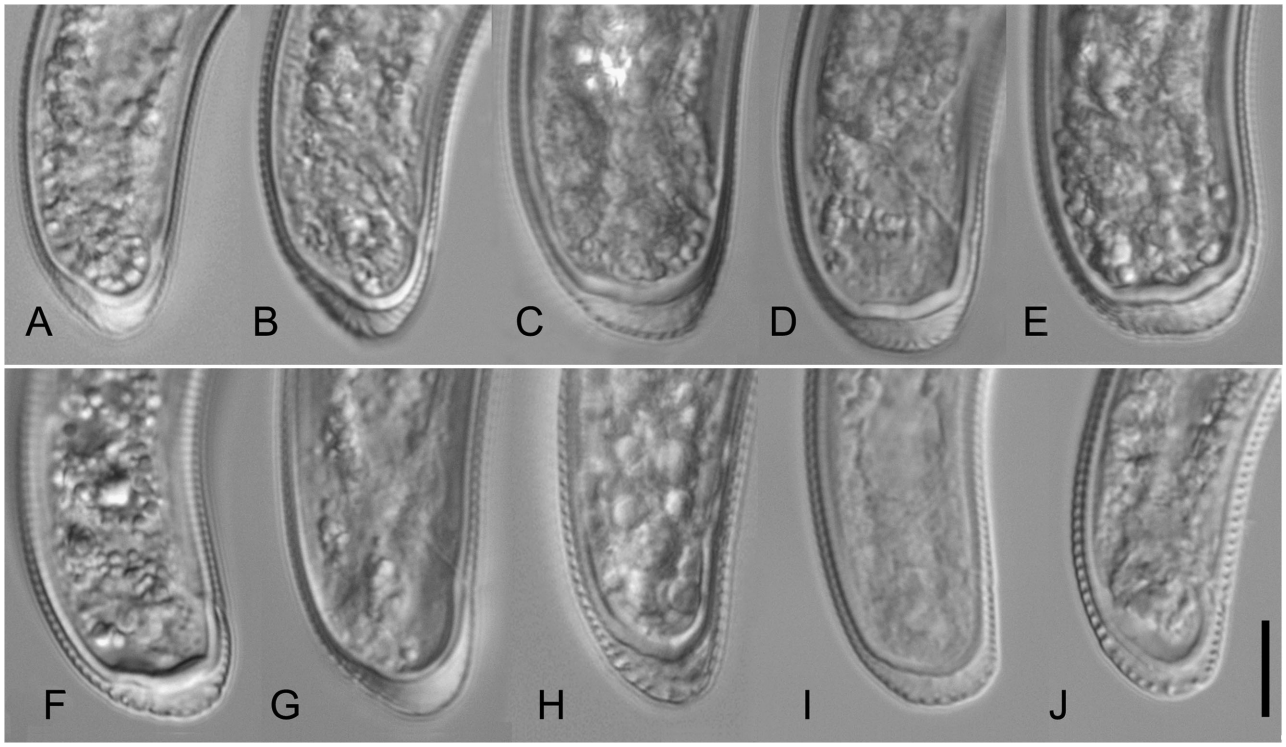

Fig. 8. *Scutellonema* species. Variations of tails. A: *S. brachyurus* type A, MS, USA. (CD574); B: *S. brachyurus* type A, LA, USA (CD583); C, E: *S. brachyurus* type A, FL, USA (CD633); D, F: *S. brachyurus* type A, FL, USA (CD650); G, H: *Scutellonema* sp. A, CA, USA (CD741, CD729); I, J: *S. brachyurus* type B, South Africa. (Scale bar = 15 μm.)

again from anterior of scutellum to ending of lateral fields on tail. Spermatheca thick-walled and empty. Epiptygma double, protruding from vulva. Intestine not overlapping rectum. Scutellum situated opposite anus. Caudalid not observed. Tail with 15 ventral annuli, curved more on dorsal side.

Male

Similar to female. Lateral fields well areolated over pharyngeal area and irregular over whole length of body. Scutellum situated opposite anus. Spicules and gubernaculum well developed, slightly curved ventrad. Gubernaculum protruding from cloacal opening. Bursa crenate, stretching to tail tip.

NOTE

The two specimens available for the present study are very similar to *S. cavenessi* Sher, 1964 and *S. clathricaudatum* Whitehead, 1959, for which descriptions are given by various authors, *viz*. Sher (1964), Van den Berg & Heyns (1973), Germani *et al.* (1985), Baujard *et al.* (1990), Sakwe & Geraert (1992). It is necessary to conduct additional studies with more material to make a more precise identification of the nematodes from this sample.

MULTIVARIATE ANALYSES

The analyses were based on raw data of morphometric characters of *S. brachyurus* populations included in Tables 3 and 4. The additional factor analysis was based on mean values of common morphometric characters available for the two groups of *S. brachyurus* populations and the measurements of six populations of *S. brachyurus* from Taiwan published by Chen *et al.* (2006).

In the first factor analysis, which included populations of *S. brachyurus* from different locations of South Africa and the USA, the first four factors (eigenvalue > 1) accounted for 54.1% of the total variance of morphometric characters of the 103 *S. brachyurus* specimens from the eight populations included in the analysis (Table 6). This table includes the eigenvalues for the four factors extracted, which were a combination of all characters in

Table 5. American and South African *Scutellonema brachyurus* and *S. unum* compared with world literature. All measurements are in μm and in the form: (median) range.

Character	American populations of *S. brachyurus*	South African populations of *S. brachyurus*	*S. brachyurus* from literature[a]	*S. unum* from literature[b]
	Four localities	Five localities		
L	(715-756) 611-805	(777-820) 692-920	500-1000	480-920
a	(21-21.7) 19.5-23.1	(26.3-28.5) 23-31.8	16.7-36	17.1-34.2
b	(5.0-5.2) 4.6-5.9	(5.8-6.3) 5.1-7.5	4.9-10.3	4-7.5
b′	(6.1-6.3) 5.5-7.4	(7-7.5) 6.2-8.8	–	4-7.6
c	(62.6-69) 56.5-95.3	(49.7-67.5) 42.9-95.5	37.2-127.7	40-105.5
c′	(0.5) 0.4-0.6	(0.7-0.9) 0.5-1.0	0.4-0.9	0.4-0.92
o	(16.4-21.6) 10-26	(17.8-21.6) 12.5-26.1	6.8-35.5	10-52.6
DGO	(5-6) 3-7	(5-6) 3.5-6.5	4-7	–
V	(58.5-59.5) 56.5-62	(57-58) 53-60	51.9-67	52.6-63
G_1	(23.7-27) 18-32	(21-23) 12.5-28	13-41	24
G_2	(24-26) 21-34.5	(19-23) 13-28.5	17-39	27
Stylet length	(28.5-29) 27-30.5	(28-29.5) 25.5-32	21-31	22.8-33.1
Metenchium length	(13.5-14.5) 12-15.5	(13-14) 11.5-15.5	–	–
Telenchium length	(14.5-15) 13.5-16	(15) 13-16.5	–	–
m	(46.6-50.2) 45.1-51	(46.3-48.4) 42-50	42-52.3	42-60
Stylet knob height	(3.5) 3.0-3.5	(3) 2-4	2-3	-
Stylet knob width	(5.5-6.0) 5-7	(5) 3.5-6.5	3.7-6.6	4-6.6
Pharynx length	(147) 133-156	(128-140) 114-165	104-122	–
Excretory pore from ant. end	(109-127) 103-136	(115-127) 101-150	79-150	93-122
Pharyngeal overlap	(26-27.5) 10.5-37	(19.5-23.5) 10.5-34.5	–	–
Diam. at mid-body	(33-35) 29.5-38	(28-31) 19.5-38	22-27	–
Diam. at anus	(18.5-22.5) 17-24.5	(18-21) 14-23.5	–	–
Median bulb length	(11.5-13) 11-14.5	(12.5-13.5) 11-15.5	–	–
Median bulb width	(10-10.5) 9-12	(10-11.5) 6.5-12.5	–	–
MB valve length	(3) 2.5-4	(3.0-3.5) 2.5-5.0	–	–
MB valve width	(2.5) 2-4	(2.5) 2.0-3.5	–	–
Lip region diam.	(9.5-10) 9-11	(8-8.5) 7.5-10.5	7-8	–
Lip region height	(5.5) 5-6.5	(5) 4.5-6.5	4-5	–
Annulus width	(1.5) 1-2	(1.5) 1-2	1.0-1.8	1.5-2.2
Lateral field width	(5-6) 4.5-6.5	(5.5-6) 4.5-7.5	–	–
Tail length	(11-12) 7.5-14	(12.5-16.5) 7.5-23	7.0-19.1	5.1-14.7
Scutellum length	(4) 3.5-5	(3.5-4.5) 3-5	3-6	2.6-6.5
Scutellum width	(3-3.5) 3-4.5	(3-5) 2.5-5.0	–	–
Spermatheca length	(8.5-9.5) 6.5-11.5	(9) 6.5-11	–	–
Spermatheca width	(8-8.5) 6.5-10.5	(8-9) 6.0-10.5	–	–

[a] Composite measurements as documented by Siddiqi (1974); Germani *et al.* (1985); Fortuner (1991); Van den Berg & Heyns (1973); Van den Berg (1998).

[b] Sher (1964); Van den Berg & Heyns (1973); Ali *et al.* (1974); Germani *et al.* (1985); Melillo & Troccoli (1993); Park & Khan (2007).

the analysis, and the corresponding values in the eigenvectors for each character that were used to interpret the significance of the factors. Factor 1 is dominated by high positive weights (eigenvector $>$ 0.60) for ratio V, stylet knob width, pharynx length, diam. at mid-body and anus, and lip region diam.; and high negative weights (eigenvector $=$ 0.79) for ratio a (Table 6; Fig. 9A). Factor 2 is dominated by high positive weights (eigenvector $>$ 0.64) for body length (L), ratio c′ and stylet length; and high negative weights (eigenvector $=$ 0.76) for ratio c (Table 6; Fig. 9A). Factor 3 is dominated by high negative weights (eigenvector $>$ 0.87) for ratio o and DGO (Table 6) and

Table 6. Eigenvector and eigenvalues of factor analysis derived from nematode morphometric characters for populations of *Scutellonema brachyurus* from different locations in South Africa and the USA.

Character	Factor			
	F1	F2	F3	F4
L	−0.170	0.745*	−0.217	0.391
a	−0.788*	0.162	0.068	0.254
b	−0.574	0.553	0.041	−0.176
b′	−0.475	−0.077	−0.300	0.388
c	0.157	−0.756*	−0.300	0.088
c′	−0.521	0.641*	0.230	0.014
o	−0.013	−0.185	−0.891*	−0.110
DGO	0.122	−0.008	−0.870*	−0.133
V	0.618*	−0.179	0.056	−0.147
OV^1	0.447	0.082	0.032	−0.134
OV^2	0.404	−0.124	−0.114	−0.473
Stylet length	0.465	0.715*	0.204	−0.003
Metenchium length	0.516	0.551	0.158	−0.365
Telenchium length	0.222	0.569	0.219	0.480
m	0.384	0.150	0.046	−0.632*
Stylet knob height	0.565	0.176	0.035	0.068
Stylet knob width	0.607*	0.046	0.091	−0.087
Pharynx length	0.756	0.182	−0.004	0.241
Excretory pore from ant. end	0.358	0.583	−0.128	0.193
Pharyngeal overlap	0.478	−0.112	0.030	0.164
Diam. at mid-body	0.754*	0.254	−0.224	−0.047
Diam. at anus	0.636*	0.482	−0.047	−0.019
Median bulb length	0.211	0.181	−0.026	0.615*
Median bulb diam.	0.306	0.199	0.199	0.435
Lip region diam.	0.682*	0.042	−0.299	−0.155
Lip region height	0.471	0.036	−0.298	0.054
Annulus width	−0.049	0.010	0.174	0.426
Lateral field width	−0.141	0.159	−0.185	0.277
Tail length	−0.190	0.879*	0.185	0.050
Scutellum length	0.297	0.531	−0.228	0.138
Scutellum width	0.237	0.502	−0.144	0.183
Eigenvalues	6.98	5.37	2.35	6.61
% of total variance	22.52	17.34	7.58	6.61
Cumulative % of total variance	22.52	39.86	47.44	54.05

* Values of morphometric and morphological characters dominating factors 1 to 4 (eigenvector > 0.6).

factor 4 by high positive weights (eigenvector = 0.62) for median bulb length and high negative weights (eigenvector = 0.63) for m (Table 6). The remaining morphological characters are not highly associated (eigenvector < 0.6) with any of the four factors extracted. Results of factor analyses are represented graphically in Cartesian plots in which *S. brachyurus* populations were projected on the plane of factors 1 and 2 (Fig. 9A). Along the *x*-axis (factor 1), *S. brachyurus* populations from South Africa are located on the left, whereas those from the USA are located on the right. Thus, South African populations compared to that from the USA have a higher ratio a, and lower values for the remaining characters highly associated with factor 1 indicated above. Similarly, according to their relative position along the *y* axis (factor 2), populations from South Africa are located along the full length of this axis, which indicates a wide range of variation of all morphometric characters associated with this factor for these populations. In contrast, populations from the USA are located mostly in the bottom part of the axis and are therefore characterised by a short body, stylet and tail length, but high ratio c values (Fig. 9A).

Similarly, in the factor analysis performed to compare published morphometric data of *S. brachyurus* from Taiwan (Chen *et al.*, 2006) to those from South Africa and the USA obtained in this study, the first three factors (eigenvalue > 1) accounted for 88.5% of the total variance of morphometric characters of the 14 *S. brachyurus* populations included in the analysis (Table 7). Factor 1 is dominated by high positive weights (eigenvector > 0.70) for body and tail length, and ratios b′ and c′; and high negative weights (eigenvector > 0.67) for ratios c and o, DGO and V (Table 7; Fig. 9B). Factor 2 is dominated by high positive weights (eigenvector > 0.72) for stylet length and diam. at anus; and high negative weights (eigenvector > 0.65) for DGO and ratios a, b and o (Table 7; Fig. 9B). Factor 3 is dominated by high positive weights (eigenvector > 0.77) for excretory pore from anterior end and scutellum length (Table 7). All morphometric characters used are highly associated with at least one of the three factors extracted. Figure 9B is a biplot display representing *S. brachyurus* populations projected on the plane of factors 1 and 2. In this plot, all populations from the USA and Taiwan are located on the left of the *x*-axis (factor 1), while those from South Africa are located on the right. Thus, according to their relative position along the *x*-axis (factor 1) populations from South Africa, when compared to that of our study, have higher L and tail lengths, and higher ratios b′ and c′, but lower values for c, o, DGO and V (Fig. 9B). Moreover, populations from South Africa show intermediate values for all morphometric characters associated with the *y*-axis (factor 2) when compared to populations from the USA

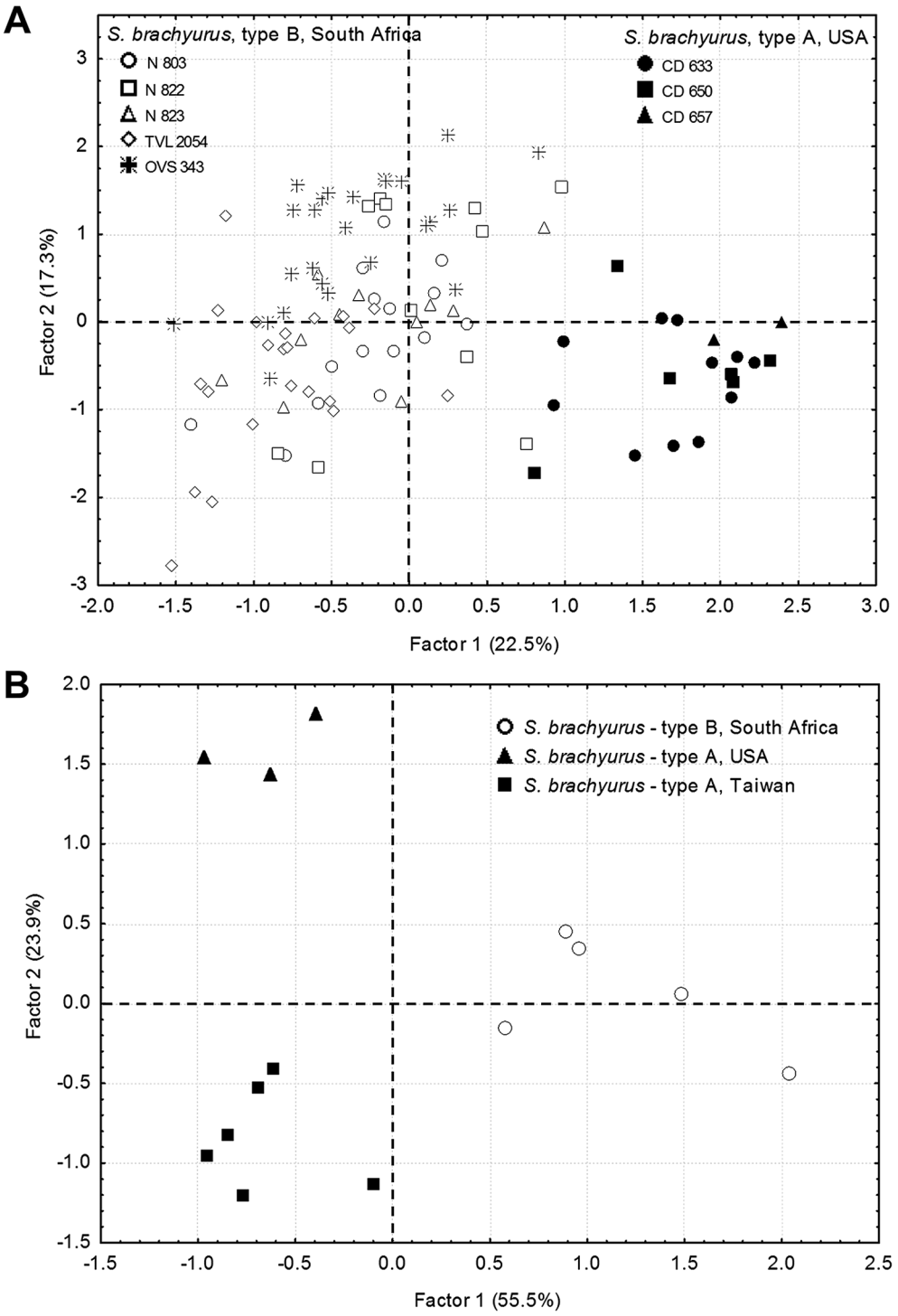

Fig. 9. Factor analysis of morphometric characters used to characterise populations of *Scutellonema brachyurus*. A: Projection of *S. brachyurus* populations from South Africa (type B) and USA (type A) on the plane of factors 1 and 2; B: Projection of *S. brachyurus* populations from South Africa (type B), USA and Taiwan (type A) on the plane of factors 1 and 2. Data of populations from Taiwan were obtained from Chen *et al.* (2006).

Table 7. Eigenvector and eigenvalues of factor analysis derived from nematode morphometric characters for populations of *Scutellonema brachyurus* from different locations in South Africa, the USA and Taiwan.

Character	Factor		
	F1	F2	F3
L	0.852*	0.222	0.145
a	0.158	−0.927*	−0.276
b	−0.364	−0.895*	0.091
b′	0.704*	0.433	−0.347
c	−0.852*	−0.338	0.126
c′	0.953*	−0.229	−0.119
o	−0.672*	−0.670*	0.121
DGO	−0.6788	−0.657*	0.193
V	−0.859*	−0.167	0.394
Stylet length	0.432	0.727*	0.456
Excretory pore from ant. end	−0.050	0.200	0.854*
Diam. at anus	0.175	0.934*	0.228
Tail length	0.953*	0.240	−0.032
Scutellum length	−0.218	0.057	0.775*
Eigenvalues	7.77	3.35	1.27
% of total variance	55.53	23.93	9.06
Cumulative % of total variance	55.53	79.46	88.52

Morphometric data of *S. brachyurus* populations from South Africa and the USA were obtained in this study, and those of *S. brachyurus* from Taiwan were obtained from Chen *et al.* (2006).
* Values of morphometric and morphological characters dominating factors 1 to 3 (eigenvector > 0.6).

and Taiwan, which showed the extreme values for those same characters, respectively (Fig. 9B).

Canonical discriminant analysis proved effective in differentiating populations of *S. brachyurus* from South Africa and the USA, confirming results obtained in the Factor analysis. The degree to which these two groups are separated measured by Mahalanobis distance between centroid values of each group was statistically significant ($F = 145.45$, $P < 0.001$). The morphometric characters with the greatest discrimination power, respectively, are ratio a (Wilks' Lambda = 0.346, $P < 0.0001$), ratio c′ (Wilks' Lambda = 0.231, $P < 0.0001$), V (Wilks' Lambda = 0.169, $P < 0.0001$), and lip region diam. (Wilks' Lambda = 0.144, $P < 0.0001$). The fit between the specimens considered as belonging to each of the two locations and those predicted by the discriminant function was 100%. According to the discriminant model, specimens of populations from the USA have significantly lower ratios a and c′, but higher V and lip region diam.

Molecular characterisation and phylogenetic relationships within *Scutellonema* species

The D2-D3 of 28S rRNA gene sequence alignment was 690 bp in length and contained 39 sequences of *Scutellonema* and three outgroup taxa. Intraspecific ITS sequence variation for *S. brachyurus* type A samples was 0-18 bp (0-2.7%), for *S. brachyurus* type B it was 0-5 bp (0-0.6%), for *S. bradys* it was 6-14 bp (0.8-2.7%), and for *Scutellonema* sp. D. it was 2-15 bp (0.3-2.3%). Interspecific differences between *S. brachyurus* type A and *S. brachyurus* type B were 35-45 bp (5.9-6.7%) and between *S. bradys* and *Scutellonema* sp. D 46-52 bp (7.0-9.1%). Phylogenetic relationships between *Scutellonema* species are given in Figure 10. There are three major clades: *i*) *Scutellonema* sp. D; *ii*) *S. bradys*; and *iii*) *S. brachyurus* type A + *S. brachyurus* type B + *Scutellonema* sp. A + *Scutellonema* sp. B.

The ITS rRNA gene sequence alignment was 1261 bp in length and contained 30 sequences of *Scutellonema* and two *Helicotylenchus multicinctus* sequences as outgroups. Intraspecific ITS sequence variation for most *S. brachyurus* type A samples (excluding CD672A, clone 2) was 0-23 bp (0-2.9%), for *S. brachyurus* type B it was 4-25 bp (0.4-2.5%) and for *S. bradys* it was 0-29 bp (0-3.6%). Interspecific differences between *S. brachyurus* type A and *S. brachyurus* type B were 135-167 bp (14-17%), between *S. brachyurus* type A and *Scutellonema* sp. A were 135-170 bp (13-17%) and between *S. bradys* and *Scutellonema* sp. D were 156-179 bp (18-20%). In the ITS BI phylogenetic tree (Fig. 11), *Scutellonema* sequences formed three major highly supported clades: *i*) *Scutellonema* sp. C in a basal position of the tree; *ii*) *S. bradys* + *Scutellonema* sp. D; and *iii*) *S. brachyurus* type A + *S. brachyurus* type B + *Scutellonema* sp. A + *Scutellonema* sp. B. *S. brachyurus* type A and *S. brachyurus* type B clustered together with PP = 82%.

The COI gene sequence alignment was 391 bp in length and contained 20 sequences of *Scutellonema* and one *Pratylenchus vulnus* sequence as an outgroup. Intraspecific ITS sequence variation for *S. brachyurus* type A samples was 3-16 bp (0.7-4.1%), for *S. brachyurus* type B was 0-9 bp (0-2.3%), for *S. bradys* was 0 bp (0%) and for *Scutellonema* sp. D was 2 bp (0.5%). Interspecific differences between *S. brachyurus* type A and *S. brachyurus* type B were 31-52 bp (10.2-13.4%) and between *S.*

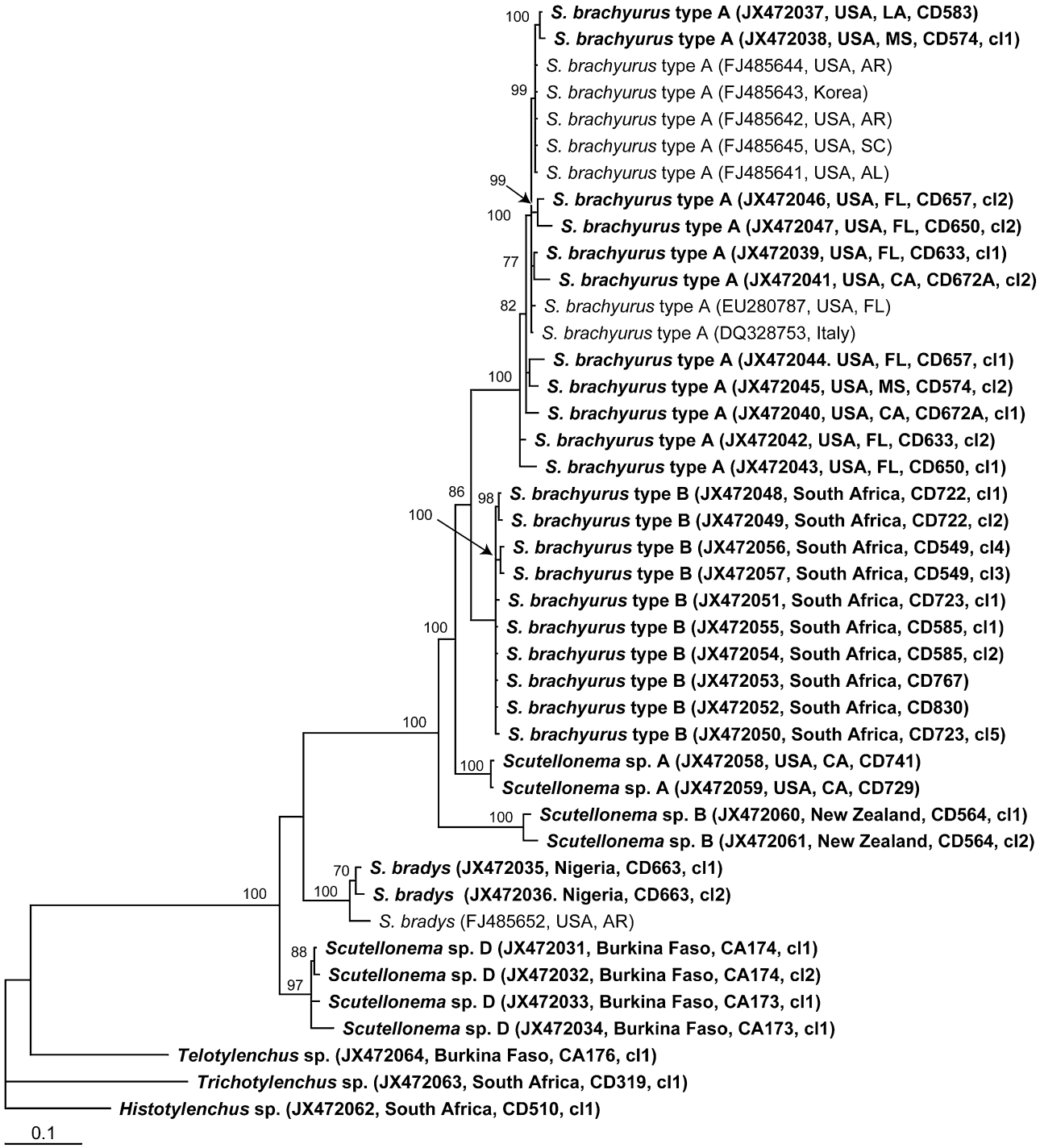

Fig. 10. Phylogenetic relationships within *Scutellonema* populations and species: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated by bold font.

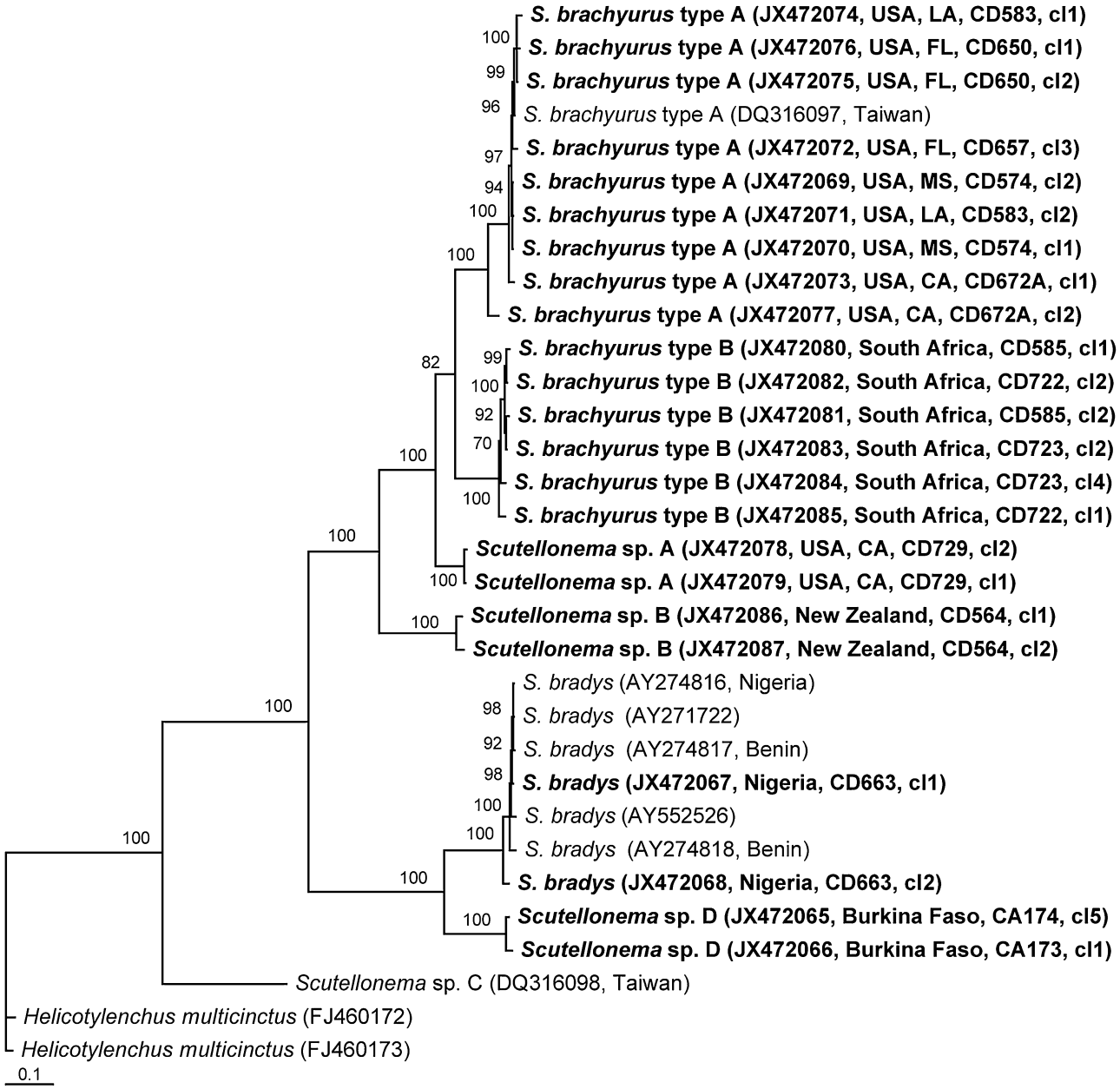

Fig. 11. Phylogenetic relationships within *Scutellonema* populations and species: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated by bold font.

bradys and *Scutellonema* sp. D were 80-86 bp (20-24%). Phylogenetic relationships between *Scutellonema* species are shown in Figure 12. There are three major clades: *i*) *S. bradys*; *ii*) *Scutellonema* sp. D; and *iii*) *S. brachyurus* type A + *S. brachyurus* type B + *Scutellonema* sp. B + *Scutellonema* sp. A.

Molecular diagnostics of *Scutellonema* species

PCR-D2-D3-RFLP profiles generated by two restriction enzymes for two genotypes of *S. brachyurus*, *S. bradys* and three unidentified *Scutellonema* species are given in Figure 13 and Table 8. The enzymes *Dde*I or

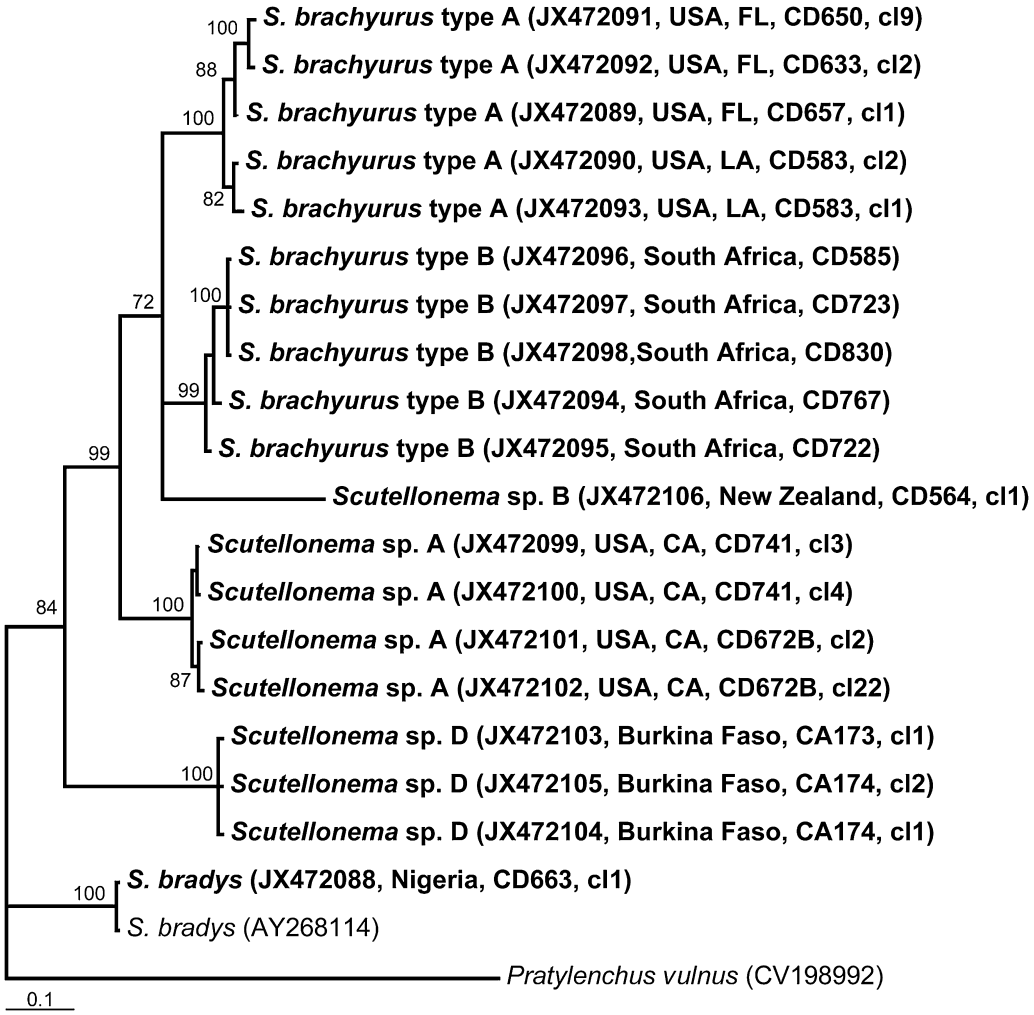

Fig. 12. Phylogenetic relationships within *Scutellonema* populations and species: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the COI mtDNA gene sequence alignment under the GTR + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated by bold font.

*Tsp*RI enable the studied species to be distinguished from each other and also distinguish *S. brachyurus* type A from type B.

Species-specific primers were developed for five *Scutellonema* species based on differences in the ITS rRNA gene sequences (Table 2). Results of PCR with the species-specific primers are given in Figure 14. The combination of the universal primer TW81 with the corresponding species-specific primers yielded a single PCR product of *ca* 300 bp for *Scutellonema* sp. A, 185 bp for *S. brachyurus* type A, 250 bp for *S. bradys*, 110 bp for *S. brachyurus* type B and 500 bp for *Scutellonema* sp. D. PCR with the specific primers were tested with all *Scutellonema* samples. Two amplicons with lengths of 185 bp and 250 bp were obtained from the sample CD672, CA, USA (data not shown) which might indicate that this sample contained two species, *Scutellonema* sp. A and *S. brachyurus* type A (Table 1).

Discussion

Sequence and phylogenetic analysis allowed seven distinct groups to be distinguished within the studied *Scutellonema* species. These groups correspond to seven phylogenetic and biological species: *i*) *S. brachyurus* type

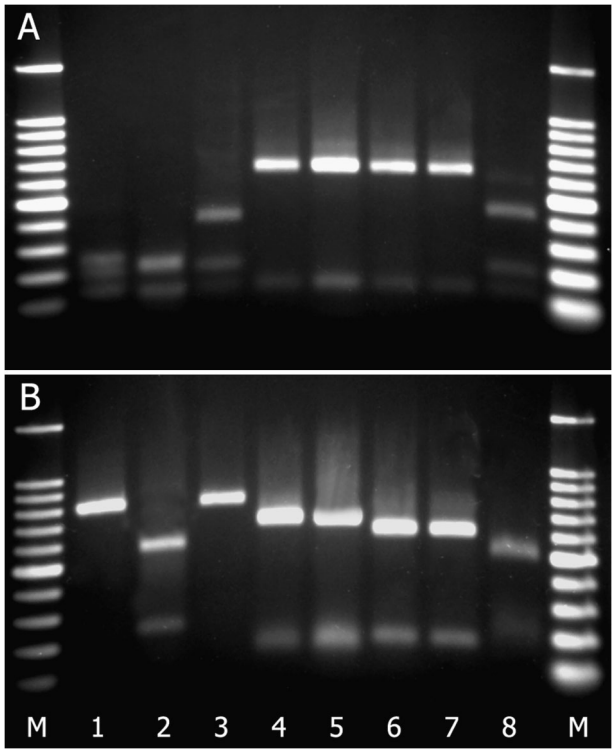

Fig. 13. PCR-D2-D3 of 28S rDNA-RFLP for *Scutellonema* species and populations. A: *Dde*I; B: *Tsp*RI. Lanes: M = 100 bp DNA ladder (Promega); 1 = *S. bradys* (CD663); 2 = *Scutellonema* sp. B (CD564); 3 = *Scutellonema* sp. D (CA174); 4 = *S. brachyurus* type B (CD585); 5 = *S. brachyurus* type B (CD549); 6 = *S. brachyurus* type A (CD657); 7 = *S. brachyurus* type A (CD650); 8 = *Scutellonema* sp. A (CD741).

A; *ii*) *S. brachyurus* type B; *iii*) *Scutellonema* sp. D; *iv*) *S. bradys*; *v*) *Scutellonema* sp. A; *vi*) *Scutellonema* sp. B; and *vii*) *Scutellonema* sp. C. Although multivariate analyses discriminated between the two genotypes of *S. brachyurus* based on several morphometric characters,

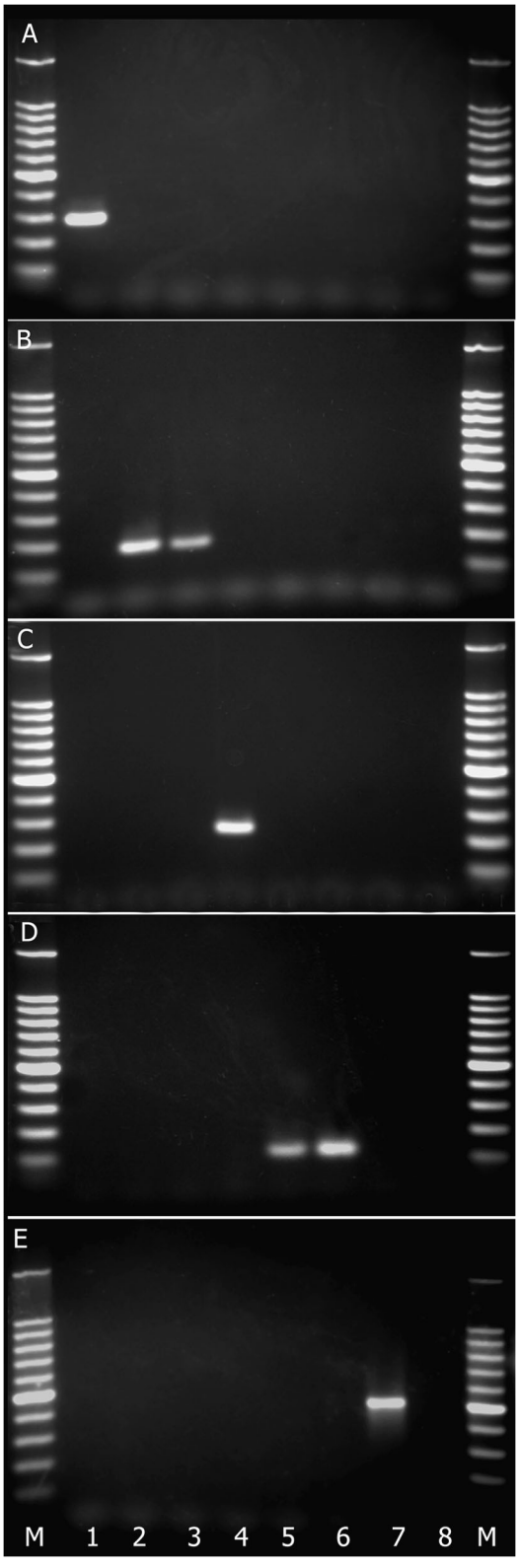

Fig. 14. The gel with specific amplicons obtained in the results of PCR with species-specific primers. A: PCR with the *Scutellonema* sp. A specific primer (TW81 + Scut_A); B: PCR with the *S. brachyurus* type A specific primer (TW81 + S_brachyurus_A); C: PCR with the *S. bradys* specific primer (TW81 + S_bradys); D: PCR with the *S. brachyurus* type B specific primer (TW81 + S_brachyurus_B); E: PCR with the *Scutellonema* sp. D specific primer (TW81 + Scut_D). Lanes: M = 100 bp DNA ladder (Promega); 1 = *Scutellonema* sp. A (CD741, CA, USA); 2 = *S. brachyurus* type A (CD574); 3 = *S. brachyurus* type A (CD657); 4 = *S. bradys* (CD663); 5 = *S. brachyurus* type B (CD585); 6 = *S. brachyurus* type B (CD549); 7 = *Scutellonema* sp. D (CA173); 8 = control without DNA.

Table 8. Approximate lengths (bp) for fragments of PCR-D2-D3-28S of rDNA-RFLP for *Scutellonema* species and populations.

Species	Unrestricted PCR product (bp)	Restriction enzyme	
		*Dde*I	*Tsp*RI (*Tsc*AI)
S. bradys	791	255, 204, 145, 127, 32, 28	791
S. brachyurus type A	782	627, 127, 28	600, 182
S. brachyurus type B	783	628, 127, 28	651, 132
Scutellonema sp. A	785	428, 202, 127, 28	467, 166, 132
Scutellonema sp. B	781	233, 203, 127, 123, 67, 28	558, 223
Scutellonema sp. D	789	396, 204, 127, 34, 28	789

in this paper we keep these two groups under the same specific name until molecular characterisations of African species similar to *S. brachyurus*, including *S. unum*, are conducted.

When comparing the present specimens with those of *S. brachyurus* described in the literature they fit very well except for having 3-6 lip annuli compared to 3-7 (Goodey, 1952; Sher, 1964; Edward & Rai, 1970; Ali *et al.*, 1973; Van den Berg & Heyns, 1973). The lip annuli are profusely marked with longitudinal and oblique lines with the basal annulus having 4-20 regular and irregular blocks compared to six in *S. brachyurus* and 15-22 in *S. unum* according to the literature (Germani *et al.*, 1985; Melillo & Troccoli, 1993). SEM photographs in this study show a high variation in the areolation and configuration of blocks on the lip region. This variation in the number of blocks on the basal lip annulus shows that this character is highly variable and needs to be used with caution in the genus. It is often difficult to determine the exact number of blocks on the basal annulus under the light microscope. Germani *et al.* (1985) stated that the only difference between *S. brachyurus* and *S. unum* is the number of blocks on the basal lip annulus. Lip shape was also described as highly variable, varying from obscurely truncate and not offset (Ali *et al.*, 1973) and roughly hemispherical, varying somewhat in height and width, broadly hemispherical to higher in others (Van den Berg & Heyns, 1973). Tail shape was described as varying from almost pointed to bluntly rounded with the dorsal surface more convex than the ventral (Van den Berg & Heyns, 1973). The close similarity in morphometrics of specimens in the current study with those of literature of *S. brachyurus* and *S. unum* is shown in Table 5. *Scutellonema unum* is not formally synonymised with *S. brachyurus*, but studies of additional populations and molecular characterisation of the type material of *S. unum* are needed to determine this. Further SEM and molecular studies of some other species, described as being close to *S. brachyurus* and *S. unum* could provide evidence to test the validity of *S. brevistyletum* Siddiqi, 1972 and *S. sanwali* Lal, 1995. The absence or presence of areolations at the level of the scutellum should also be used with caution as a number of the present specimens had tails without any areolation around the scutellum. Abebe & Geraert (1995) moreover state that it is difficult to count the longitudinal striations on the basal lip annulus and it is therefore necessary to introduce the use of SEM photographs in descriptions. They also state that there is a high degree of variability in geographically isolated populations within *Scutellonema* and these observations cast some doubt on the morphological characteristics used in the classification of the genus to species level. The current study also shows that as many specimens as possible should be photographed and drawn to account for the actual variation within one population.

The results of the present study suggest that molecular characterisation using nuclear rRNA and mtDNA genes should become an important step for verification of morphologically identified samples and that the development of such molecular tools could usefully serve for rapid and reliable diagnostics of *Scutellonema* species.

Acknowledgements

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