Morphological and molecular characterisation of some Criconematidae (Nematoda, Tylenchida): *Ogma decalineatus* (Chitwood, 1957) Andrássy, 1979, *Criconema silvum* (van den Berg, 1984) Raski & Luc, 1985 and *Neobakernema variabile* (Raski & Golden, 1966) Ebsary, 1981 from South Africa and the USA

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Summary. During recent nematological surveys *Ogma decalineatus* and *Criconema silvum* were found in South Africa and *Neobakernema variabile* was found in California, USA. These three species are morphologically and morphometrically described. The SEM photos are also given for the species. Molecular characterisations of these three species using the D2-D3 expansions segments of 28S rRNA, ITS rRNA and *COI* mtDNA gene sequences are provided.

Key words: *COI*, D2-D3 28S rRNA, ITS rRNA, phylogeny, SEM.

During nematological surveys several criconematid species were found in South Africa and California, USA. Samples were collected along the Maretlwana River, near Mooinooi in the Magaliesberg Mountains, and from a swamp forest on the shores of Lake Mgobezeleni in the Isimangaliso Wetland Park, KwaZulu Natal, South Africa and from a willow tree growing in Napa County, California, USA. The objectives of this work were to: *i*) carry out a morphological and morphometric characterisation of these criconematids; *ii*) provide molecular characterisation of these species using sequences of the D2-D3 expansion segments of the 28S nuclear ribosomal RNA, the ITS of rRNA gene and partial *COI* gene; *iii*) analyse phylogenetic relationships within criconematid nematodes using these genes.

MATERIAL AND METHODS

Light and scanning electron microscopy. Specimens were extracted from soil samples using the rapid centrifugal-flotation method (Jenkins, 1964), fixed in FPG (Netscher & Seinhorst, 1969), transferred to anhydrous glycerin (De Grisse, 1969) and mounted on Cobb slides. Measurements were made with a research microscope (Nikon Labophot-2) equipped with a drawing tube. Light micrographs were taken with an automatic Infinity 2 camera attached to a compound Olympus BX51 microscope equipped with Nomarski differential interference contrast.

For scanning electron microscopy specimens were transferred to TAF (40% formalin, triethanolamine, distilled water), then dehydrated in increasing concentrations of alcohol in distilled water and finally into pure alcohol. 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.

DNA extraction, PCR and sequencing. DNA was extracted from several dead specimens of each species using the proteinase K protocol. Detailed protocols for DNA extraction, PCR, cloning and sequencing were as described by Tanha Maafi *et al.* (2003). Two rRNA gene fragments, ITS rRNA and D2-D3 expansion segments of 28S rRNA gene and

partial *COI* gene were amplified. The following primers were used for amplification in the present study: ITS rRNA gene – TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') (Tanha Maafi *et al.*, 2003); D2-D3 of 28S rRNA gene – D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GA GGA ACC AGC TAC TA-3') (Subbotin *et al.*, 2006) and partial *COI* gene – COIF5 (5'-AAT WTW GGT GTT GGA ACT TCT TGA AC-3') and COIR9 (5'-CTT AAA ACA TAA T GR AAA TGW GCW ACW ACA TAA TAA GTA TC-3') (Powers *et al.*, 2014). The obtained sequences were submitted to the GenBank database under the following accession numbers: MF683230-MF683242.

Phylogenetic analysis. The newly obtained sequences for each gene were aligned using ClustalX 1.83 with default parameters with corresponding published gene sequences of criconematids and related genera (Subbotin *et al.*, 2005, 2006; Powers *et al.*, 2010, 2014, 2016a, b; van den Berg *et al.*, 2012). Outgroup taxa for each dataset were chosen according to the results of previously published data (Subbotin *et al.*, 2005, 2006). Sequence datasets were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). BI analysis under the GTR + I + G model 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.

DESCRIPTIONS

***Ogma decalineatus* (Chitwood, 1957) Andrássy, 1979**

= *Criconema decalineatum* Chitwood, 1957

= *Criconema (Variasquamata) decalineatum* (Chitwood, 1957) Mehta & Raski, 1971

= *Variasquamata decalineatum* (Chitwood, 1957) Kahn, Chawla & Saha, 1976

= *Criconema coffeae* Edward, Misra & Rai, 1970

= *Ogma coffeae* (Edward, Misra & Rai, 1970) Andrássy, 1979

= *Criconema (Variasquamata) gracile* Mehta & Raski, 1971

= *Variasquamata gracile* (Mehta & Raski, 1971) Khan, Chawla & Saha, 1976

Ogma gracile (Mehta & Raski, 1971)

(Figs 1A-C & 2)

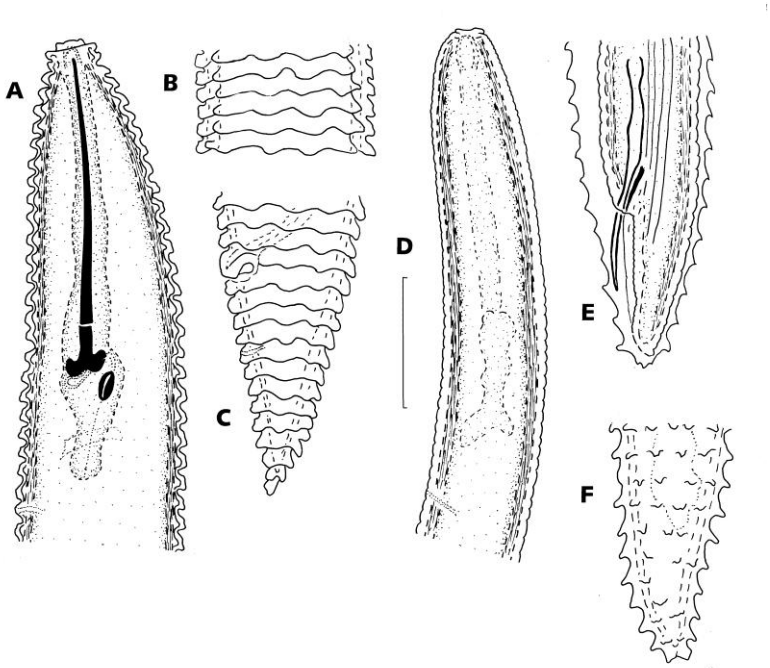

Fig. 1. *Ogma decalineatus*. Female. A: anterior part of body; B: annuli at mid-body; C: tail region. *Neobakernema variabile*. Male. D: anterior part of body. Juvenile. E: juvenile cuticle with male tail inside; F: tail of another juvenile. Scale bar = 30 μm.

Table 1. Measurements of females of *Ogma decalineatus* from Mooinooi (Tvl 1970), South Africa compared with measurements from some literature. All measurements are in µm and in the form: mean ± s.d. (range).

Character / Population	*O. decalineatus* Mooinooi (Tvl 1970)	*O. decalineatus* a	*O. decalineatus* b
n	15	26	40
L	357 ± 32.3 (311-408)	309-451	310-451
a	8.4 ± 0.7 (7.3-9.5)	7.1-12.1	9.2-13.9
b	3.2 ± 0.2 (2.9-3.5)	3.1-4.8	3.1-4.3
c	12.6 ± 1.4 (10.4-15.1)	11.5-21.5	10-27.7
c’	1.3 ± 0.2 (0.9-1.5)	–	1.5-1.7
o	8 ± 1.5 (5.6-10.3)	6-9.5	–
DGO	6 ± 1 (4.5-7.5)	4.5-8.1	–
V	86.5 ± 1.3 (85-89)	85-90	84-91
OV1	71 ± 24.2 (57.5-95.5)	30-78.5	–
OV length	271.5 ± 54.9 (181.5-363)	–	–
Stylet length	75 ± 3.4 (71.5-84)	46.3-93	65-78
Metenchium	63 ± 3.5 (59.5-72)	37.5-79	54-65
Telenchium	12 ± 0.7 (10.5-13)	8.5-14	–
M	84.2 ± 1.2 (81.5-86)	–	83-88
Stylet knob height	3 ± 0.4 (2.5-4)	2.2-4	3-4.7
Stylet knob width	8.5 ± 0.8 (7.5-9.5)	6-9	7.7-9.3
Excretory pore from front	123 ± 8.7 (108-134.5)	104-144	91-117
Width at mid-body	42.5 ± 2.8 (38-48)	28.7-49	–
Width at anus	21.5 ± 2.3 (19-25)	–	–
Annulus width	4.5 ± 0.4 (4-5.5)	3.7-7	–
Tail length	28.5 ± 3.9 (20.5-33)	19.5-33	27-33
Pharynx length	112 ± 8.5 (100-128)	93-130.5	83-100
First lip annulus width	13 ± 0.9 (11.5-14.5)	10.7-15	12-14.7
Second lip annulus width	13.5 ± 0.8 (12.5-15)	10-15	13-16
First body annulus width	17.5 ± 1.1 (16-20)	14-21	–
Second body annulus width	21 ± 1.1 (19-23)	20-26.5	–
R	83 ± 1.9 (80-87)	75-94	75-84
RSt	18.5 ± 1.5 (15-22)	13-21	15-18
ROes	26.5 ± 2.4 (23-29)	20-26	20-26
Rex	28 ± 1.3 (26-30)	23-30	23-28
Rhem	27 ± 1 (26-28)	26-28	–
RV	14 ± 1.1 (12-16)	11-16	10-14
RVan	4.5 ± 0.6 (3-5)	3-6	3-6
Ran	9 ± 1 (7-10)	5-10	5-10
VL/VB	1.5 ± 0.2 (1.2-1.8)	1.3-1.9	–
St% L	21.2 ± 1.6 (18.9-23.5)	11.9-24.9	–

a) van den Berg *et al.* (2004); van den Berg (1983); van den Berg & Spaull (1985); b) Minagawa (1988); Crozzoli & Lamberti (2002).

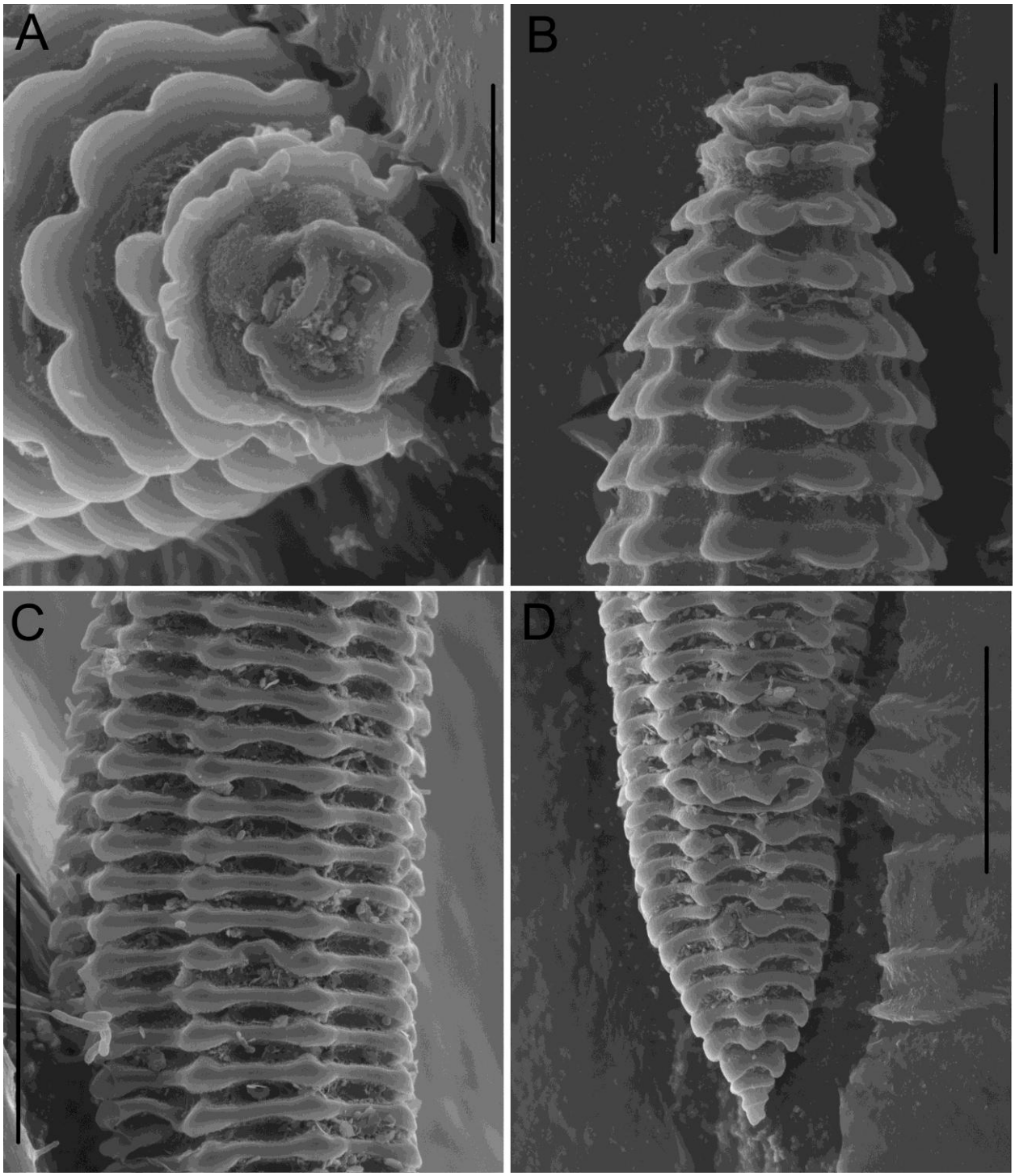

Fig. 2. *Ogma decalineatus*. Female. A: *en face* view of lip region. B: lateral view of anterior part of body. C: annuli at mid-body; D: ventral view of posterior part of body with vulva. Scale bars: A, C, D = 20 µm, B = 10 µm.

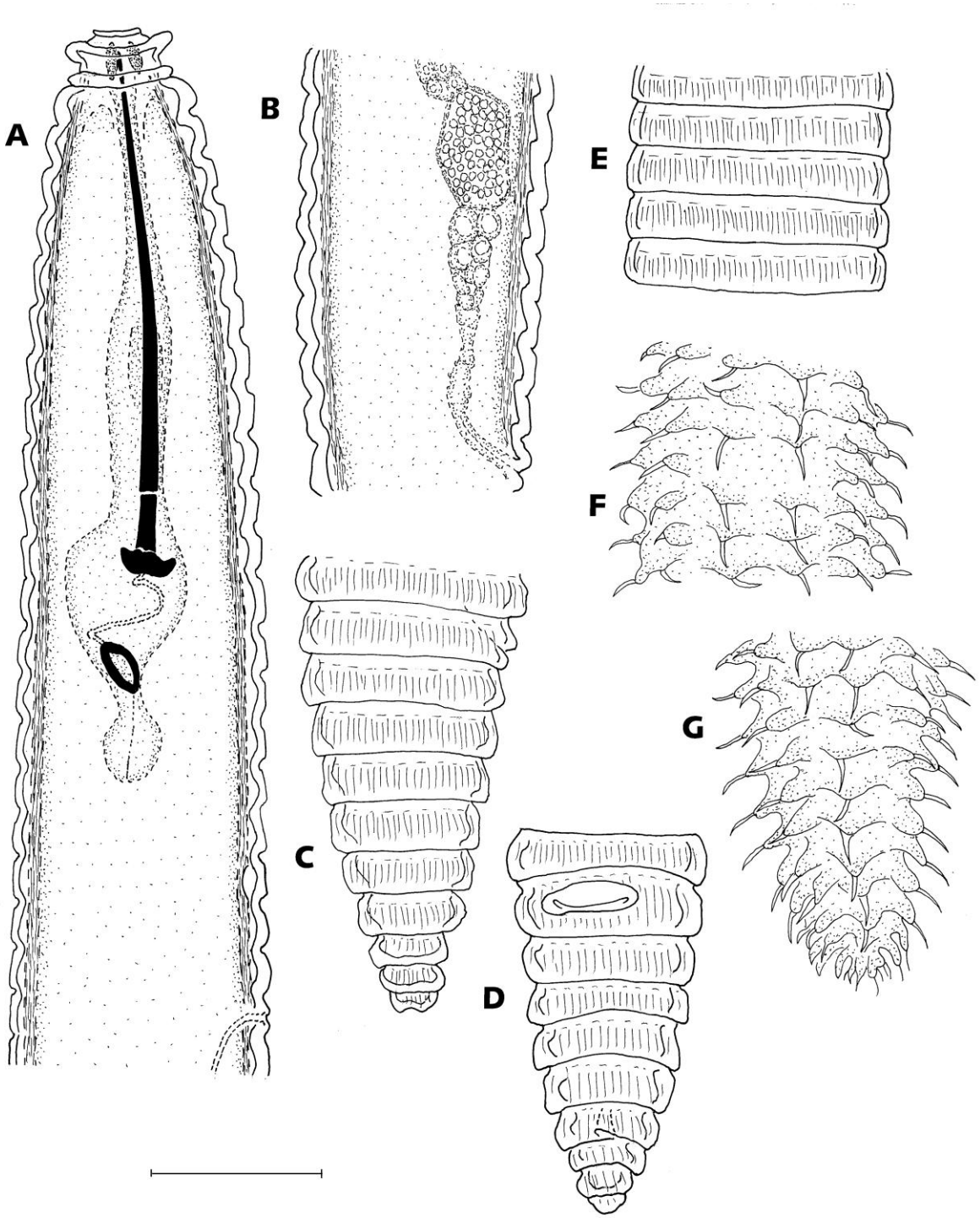

Fig. 3. *Criconema silvum*. Female. A: anterior part of body; B: vulval area with spermatheca; C, D: lateral and ventral views of tail region; E: annuli at mid-body. Juvenile. F: annuli at mid-body; G: tail area. Scale bar = 30 µm.

This species was described from *Ficus elastica* Roxb. ex Hornem. 1819 roots from Florida, USA and since has been reported from various countries in the world (Geraert, 2010). It is very common in South Africa and found very often on various plants and biomes. S. Neser collected the present specimens from sandy soil underneath various trees along the Maretlwana River, near Mooinooi (Tvl 1970, sample CD555) in the Magaliesberg Mountains, South Africa on 22.11.2008.

Measurements. See Table 1.

Female. Body slightly arcuate ventrad. Lip region with two annuli with irregularly lobed margins; first annulus directed more forward and second one more outward with rounded lobes on margin corresponding to that of the first body annulus. Two lip annuli slightly separated from the body annuli, directed posteriorly. Labial disc protruding. SEM shows a rectangular labial disc, rimmed with a narrow ridge with each corner pointing prominently outward (submedian lobes). Labial plates not seen. Amphids small, slit-like, laterally. Labial framework not very distinct. All body annuli thick and well retrorse with mostly ten longitudinal rows of rounded scales. In some specimens, twelve were counted at mid-body. Posterior margins of scales smooth. No anastomosis observed except for one or two on tail tip. Hemizonid seen in two specimens only, one annulus long and situated from directly anterior to two annuli anterior to excretory pore. Excretory pore situated from three annuli anterior to six annuli posterior to base of pharynx. Stylet long and slender with cupped basal knobs. Spermatheca small, round to oval and mostly filled with rounded sperm cells. Vagina straight to slightly sigmoid with rounded lips not projecting beyond the profile of the body.

Anterior lip bi-lobed, overlapping posterior lip slightly. Tail tapering to a small rounded knob-like terminus.

Male. Not found.

Juvenile. Not found.

Remarks. The present specimens compare very well with all the various descriptions of the species such as Chitwood (1957), van den Berg (1983), van den Berg & Spaull (1985), Vovlas (1986), Minagawa (1988), van den Berg & Cadet (1991), Crozzoli & Lamberti (2002) and van den Berg *et al.* (2004).

Criconema silvum (van den Berg, 1984) Raski & Luc, 1985

= *Lobocriconema silvum* van den Berg, 1984

(Figs 3, 4 & 5)

Originally, this species was described as *Lobocriconema silvum* (van den Berg, 1984b) from subtropical growth in the Dukuduku Forst Reserve, near St. Lucia Bay, KwaZulu Natal Province, South Africa. A further collection from the same site in 1985 produced 30 females and in 1989 13 females were collected from young pine trees in the Kwambonambi State Forest described by van den Berg (1992). In 2014 specimens were collected from swamp forest on the shores of Lake Mgobezeleni in the Isimangaliso Wetland park, KwaZulu Natal (KZN4; sample CD1851), South Africa. These specimens were studied morphologically and now also molecularly for the first time. Andrássy (1979) already synonymised *Lobocriconema* with *Criconema* on various grounds. Raski and Luc (1985) discussed various Criconematidae genera and the characters separating them and finally made nine genera synonyms of *Criconema*, one of which was *Lobocriconema*. During the last decades, many of the species were moved to different genera and backed by Geraert (2010), Siddiqi (2000), Brzeski *et al.* (2002), Wouts (2006) and Andrássy (2007). We regard these present species as belonging to the genus *Criconema* as the SEM photos show that there are no real submedian lobes present.

Measurements. See Table 2.

Female. Body slightly curved ventrad. Lip region with one annulus, saucer shaped and pointing outward or slightly backward. First body annulus with a slightly smaller diameter. Second and third body annuli with larger diameters. SEM show a rectangular labial disc, but no distinct submedian lobes seen. All body annuli slightly retrorse covered with fine longitudinal lines, frequently slightly irregular or broken, margins very finely scalloped. Space between the annuli coarse with irregular nodules. No anastomosis seen except two irregular ones posterior to the vulva on one specimen. Hemizonid seen in two specimens only, two annuli posterior to excretory pore. Excretory pore situated from one annulus anterior to five annuli posterior to base of pharyngeal lobe. Stylet long, slightly curved dorsad with cupped basal knobs. Spermatheca present, varying from small to large, round to oblong and empty to be filled with rounded sperm cells. Vagina straight. Vulval lips not protruding beyond body profile. Dorsal lip straight or with two small rounded lobes and not overlapping posterior lip. Tail tapering gradually to last annulus with a small rounded lobe, sometimes this lobe is slightly irregular.

Male. Not found.

Juvenile. One female was found with a piece of the 4th stage juvenile cuticle attached to the posterior part of the body. There appeared to be 8 to 10 long pointed scales on the cuticle each ending in a sharp pointed seta.

Remarks. The present specimens fit the specimens previously described well except for having less anastomosis and fewer lobes on the tail tip and no real submedian lobes in the labial area.

Table 2. Measurements of females of *Criconema silvum* compared with newly collected specimens from KZN4. All measurements are in µm and in the form: mean ± s.d. (range).

Character / Population	*C. silvum* South Africa (KZN4)	*C. silvum* a	*C. silvum* b
n	33	3	43
L	560 ± 59.4 (413-657)	564 (551-577)	314-625
a	12.7 ± 1.7 (9.4-17.6)	10.5 (8.6-12.2)	6.7-14.7
b	3.8 ± 0.3 (3.2-4.6)	4 (3.9-4.1)	2.8-4.4
c	49.7 ± 11.6 (30.4-76.6)	65.2 (59.1-68.2)	35.1-110.2
c’	0.7 ± 0.1 (0.5-0.9)	–	–
o	5.8 ± 0.8 (4.2-6.9)	6.3 (6.1-6.5)	4.2-10.6
DGO	5.5 ± 0.5 (4-6.5)	5.7 (5.5-5.9)	4-9.6
V	90 ± 2.1 (85-92)	91 (91-92)	88-92
OV1	42.4 ± 11.2 (26.9-79.9)	–	–
OV length	239 ± 66.8 (153-424)	–	–
Stylet length	95 ± 4.9 (82.5-107.5)	91.3 (90.4-92.6)	74.2-101.1
Metenchium length	79.5 ± 4.1 (69-90)	74 (73.5-75)	60.6-86.7
Telenchium length	15 ± 2.3 (9-19)	17.3 (16.9-17.6)	12.9-19.9
M	84.1 ± 2.5 (80.4-94)	–	–
Stylet knob height	3.5 ± 0.5 (3-5)	4.8 (4.4-5.2)	2.6-5.5
Stylet knob width	9.5 ± 1 (8-11)	11.8 (11.4-12.5)	8.1-12.5
Exc. pore from front	174 ± 16.1 (142-197)	177 (172-181)	99-204
Width at midbody	45 ± 4 (36-55)	49.6 (47.5-51.5)	38.2-51.5
Width at excretory pore	–	48.3 (45.6-50)	365.8-52.9
Width at anus	18 ± 2.8 (13-25)	–	
Annulus width	9 ± 1.1 (6-12)	9.3 (8.8-9.6)	5.1-10.7
Tail length	12 ± 3.1 (7.5-17)	8.7 (8.1-9.6)	4-15.8
Pharynx length	148 ± 12.6 (123-178)	–	–
1st lip annulus diam.	22 ± 2.1 (18-26.5)	25 (23.9-25.7)	18-29
1st body annulus diam.	19.5 ± 2.6 (17.5-24.5)	22.9 (21.3-24.3)	16.5-27.9
2nd body annulus diam.	26 ± 2.6 (20.5-33)	31.2 (30.1-33.1)	22.1-34.6
3d body annulus diam.	29.5 ± 2.8 (23-37.5)	–	–
Spermatheca length	18 ± 3.5 (12-27)	–	–
Spermatheca width	12.5 ± 2 (10-18)	–	–
R	67 ± 2.8 (61-74)	65-67	57-68
RSt	12 ± 1.1 (9-14)	11-13	11-19
ROes	18 ± 1.6 (15-22)	16-17	14-25
Rex	20 ± 1 (19-22)	19-22	18-24
Rhem	17; 20 (n = 2)	–	–
RV	9 ± 0.8 (7-10)	7-8	6-9
RVan	4 ± 1.4 (1-6)	3-5	3-5
Ran	3 ± 0.7 (2-5)	2-3	1-3
VL/VB	1.6 ± 0.2 (1-2)	1.3 (1.1-1.4)	0.9-1.7
St% L	16.6 ± 1.3 (14-21.1)	16.2 (16-16.5)	14.6-29.6

a) van den Berg (1984a); b) van den Berg (1992).

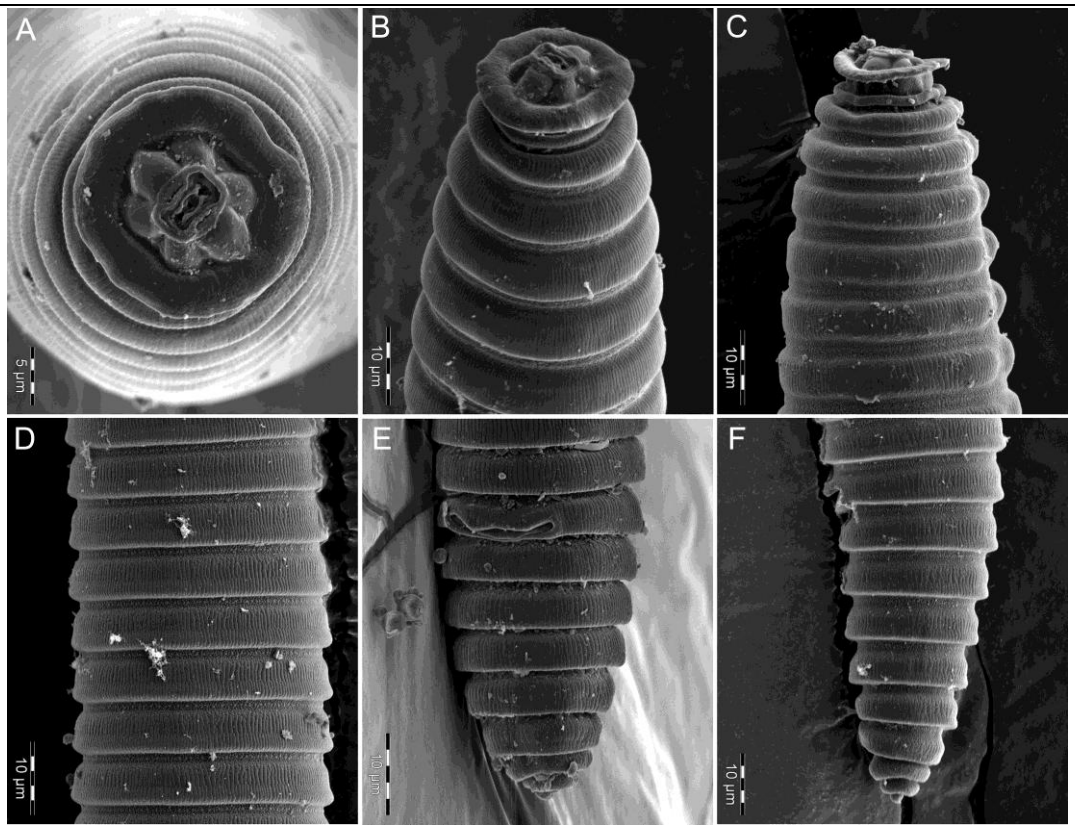

Fig. 4. *Criconema silvum*. Female. A, B: *en face* view of two lip regions; C: lateral view of anterior part of body; D: mid-body; E, F: lateral and ventral view of posterior part of body.

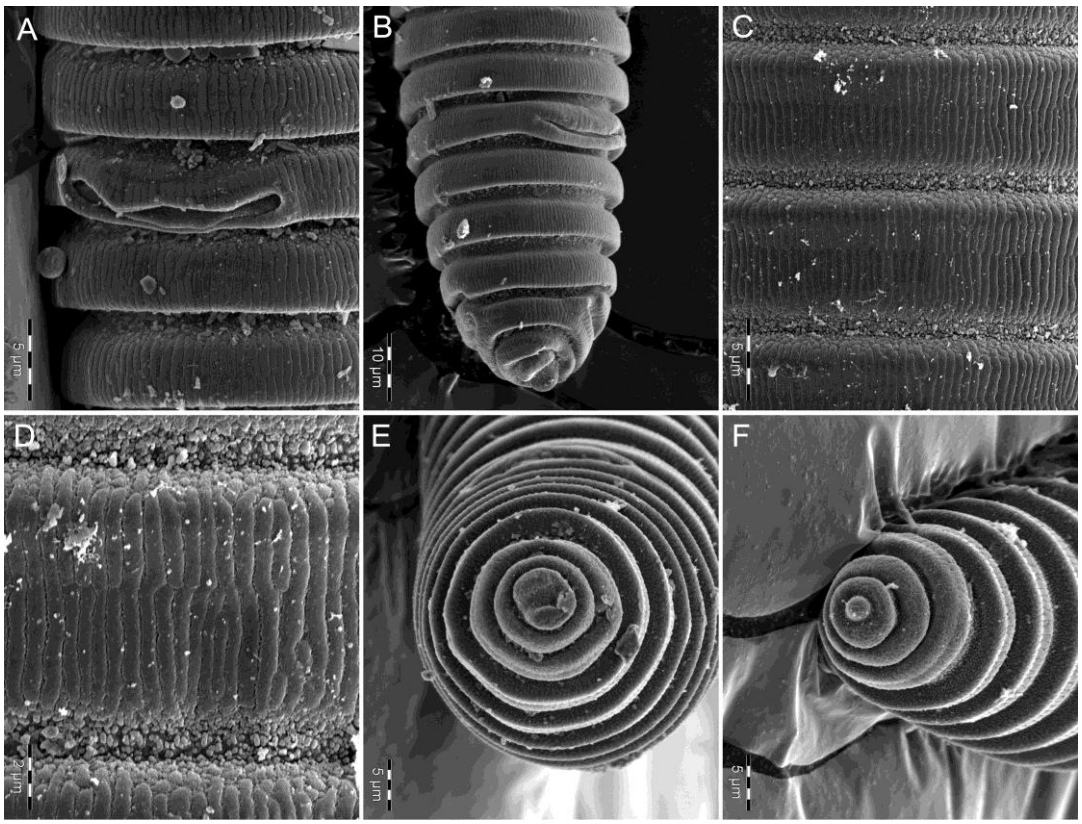

Fig. 5. *Criconema silvum*. Female. A: close up view of vulva; B: ventral view of posterior part of body with vulva; C, D: enlargement of annuli at mid-body; E, F: tail tip endings.

***Neobakernema variabile* (Raski & Golden, 1966) Ebsary, 1981**

= *Bakernema variabile* Raski & Golden, 1966

= *Criconemella variabilis* (Raski & Golden, 1966) Raski & Luc, 1987

= *Mesocriconema variabile* (Raski & Golden, 1966) Loof & De Grisse, 1989

= *Macroposthonia variabilis* (Raski & Golden, 1966) Siddiqi, 2000

(Figs 1D-F, 6-9)

Raski and Golden (1966) originally described this species as *Bakernema variabile* from various hosts and localities in California, USA. The present specimens were collected from Napa County, California, USA (Capell Valley Road crossing with Wragg Canyon Road, host – *Salix* sp., sample CD848) and studied for the first time molecularly and with SEM.

Measurements. See Table 3.

Female. Body form ranging from slightly curved ventrad to an open letter C. Lip region flattened anteriorly, first annulus margins rounded and directed more forward while second annulus is directed backward. In the SEM photos the labial area is unfortunately covered with bacteria but labial disc appears rectangular with four flattened, indistinct rounded lobes on the corners, two dorsally and two ventrally. Amphids not distinctly seen. Labial framework not very distinct. All body annuli well retrorse with wavy fringe at the posterior end. This fringe represents the fine extra cuticular layer this species possesses. The annuli appears angular when seen laterally. No anastomosis observed. Hemizonid seen in one specimen only, about one annulus long and situated one annulus anterior to excretory pore. Excretory pore situated from 13 annuli anterior to four annuli posterior to base of a pharyngeal lobe. Stylet very long, robust with cupped basal knobs. Spermatheca large, round to oblong and mostly filled with rounded sperm cells. Vagina straight to slightly sigmoid. Vulva with anterior lip slightly bilobed but not overlapping posterior lip; both lips not projecting beyond profile of body. Tail tapering slightly to a bluntly rounded tip with two to three coarsely infolded lobes. Fringe of second outer cuticle more prominent on tail with slightly longer angular projections.

Male. Five males found still within the fourth stage juvenile cuticle. Body form slightly curved ventrad. Lip region not set off but number of annuli not clear. First annulus seems to project outward. Pharynx degenerate. Stylet absent. Excretory pore seen in one specimen only, about 54 annuli from anterior end. Lateral field with four lines, outer ones appearing slightly wavy. Annuli on body distinct. Spicules long and curved slightly irregular due to still being in the juvenile cuticle. Tail ending and number of annuli not distinct because of being cramped into the juvenile cuticle.

Juvenile. Three juveniles found, two fourth-stage juveniles with males inside. Stage of third juvenile unknown, probably third-stage juvenile. Body slightly curved ventrad. Lip region very similar to female. Annuli margins with small rounded, projections, not in longitudinal rows, difficult to count, probably about 20 present on each annulus. Tail tapering to a narrow irregular tip.

Remarks. Described as *Bakernema variabile* by Raski & Golden (1966). Ebsary (1981) stated that the genus *Bakernema* contains two species differing in several characters and created a new genus, *Neobakernema* for the species *variabile*, which has an open vulva, straight vagina and spined juveniles. Siddiqi (1986) regarded *Neobakernema* as a valid genus. Later, Siddiqi (2000) regarded *Neobakernema* as a synonym of *Macroposthonia* de Man, 1880. Raski and Luc (1987) gave a detailed description of the close relationship of a few genera of the Criconematidae such as *Bakernema*, *Ogma*, *Blandicephalanema* and *Pateracephalanema*. They stated that *N. variabile* has a closer relationship with *Criconemella* and, thus, transferred the species to *Criconemella*. Loof and De Grisse (1989) discussed the plesiomorphic and apopmorphic states of the characters of several genera and synonymised *Neobakernema* with *Mesocriconema*, a synonymy accepted by Brzeski *et al.* (2002). Geraert (2010) noted that the morphometrics of the seven *Neobakernema* species clearly separate them from *Mesocriconema* and regarded this genus as a valid one. The present specimens fit the descriptions of the various authors very well.

Molecular characterisation and phylogeny. The D2-D3 of 28S rRNA gene alignment included 20 sequences of criconematids and three sequences of *Paratylenchus* selected as outgroup taxa and was 710 bp in a length. Five new sequences were obtained in the present study. Phylogenetic analysis resulted in a majority consensus BI tree with two major highly supported clades (Fig. 10).

The ITS1 rRNA gene alignment included 21 sequences of criconematids and two sequences of *Paratylenchus* selected as outgroup taxa and was 422 bp in a length. Five new sequences were obtained in the present study. Phylogenetic relationships within some criconematids as inferred from BI analysis are given in Fig. 11. Sequences of *Ogma decalineatus* from South Africa and USA clustered together and differed in 10.1% (35 bp).

Table 3. Measurements of females, males and juveniles of *Neobakernema variabile* (CD848) from the USA. All measurements in µm and in the form: mean ± s.d. (range).

Character / Population	*Neobakernema variabile*				*N. variabile* Raski & Golden (1965)	
	Females	**Juveniles (?J4)**	**Juvenile (?J3)**	**Males**	**Females**	**Males**
n	13	2	1	5	67	8
L	723 ± 54.7 (647-829)	643-681	568	635 ± 45.7 (579-702)	720 (530-990)	630 (550-790)
a	9.9 ± 1.9 (8.1-12.7)	–	14.3	22.8 ± 2.6 (20.9-27.3)	12.4 (10-15)	22.3 (12-27)
b	3.7 ± 0.3 (3.2-4.2)	–	3.9	6.9 (n = 1)	3.7 (2.5-4.7)	–
c	28 ± 6.6 (18.4-39.3)	20.4	–	14.5 ± 0.7 (13.5-15.5)	22.7 ± (18-27)	14.6 (12-21)
c^1	0.7 ± 0.1 (0.6-0.9)	0.8	0.8	2.1 ± 0.4 (1.7-2.6)	–	–
o	5.2 ± 1.5 (3-8.6)	–	11.7	–	–	–
DGO	6 ± 1.8 (4-10.5)	–	8	–	–	–
V	93.5 ± 0.9 (92-95)	–	–	–	92 (89-95)	–
OV1	48 ± 9.9 (35-63)	–	–	–	–	–
OV length	347.5 ± 77.7 (249.5-460.5)	–	–	–	–	–
Stylet length	114 ± 5 (106.5-120.5)	70-73.5	70	–	113 (97-142)	–
Metenchium length	88 ± 5 (80-95)	–	53.5	–	89 (74-97)	–
Telenchium length	26 ± 3 (17.5-29.5)	–	15.5	–	–	–
M	77.3 ± 2.1 (75-83.2)	–	77.7	–	–	–
Stylet knob height	7.5 ± 0.8 (6-8)	–	3	–	–	–
Stylet knob width	17 ± 1 (15-18.5)	–	8	–	15	–
Exc. pore from front	183 ± 30.4 (138-236)	–	141	141 ± 16.3 (119-155)	183-217	155-166
Width at midbody	78 ± 4.9 (66-84)	68.5	40	28.5 ± 1.2 (26.5-29.5)	–	–

Table 3. (continued)

Character / Population	*Neobakernema variabile*				*N. variabile* Raski & Golden (1965)	
	Females	Juveniles (?J4)	Juveniles (J3?)	Males	Females	Males
Width at anus	43.5 ± 4.8 (36-50)	34.5-40	–	21 ± 4 (16-27)	–	–
Annulus width	8.5 ± 0.7 (7.5-9.5)	7.5-8	6	4	7-8	4.1-6.3
Tail length	27 ± 6.1 (18-41)	32-34.5	–	44 ± 5.2 (39-52)	–	–
Pharynx length	197 ± 17.7 (170-224)	–	146.5	–	–	–
1st lip annulus diam.	18 ± 1.1 (16-20)	13-14	12	–	–	–
2nd lip annulus diam.	24.5 ± 1.7 (22-27)	17-18.5	15	–	–	–
1st body annulus diam.	29.2 ± 2 (26-32.5)	23-23.5	17.5	–	–	–
2nd body annulus diam.	35 ± 2.1 (32-38)	27-28	20	–	–	–
3 d body annulus diam.	40.5 ± 2.2 (37-44)	–	–	–	–	–
Lip region width	–	–	–	15	–	–
Lip region height	–	–	–	8	–	–
R	94 ± 4.7 (86-102)	91-94	91	–	100 (61-108)	–
RSt	20 ± 3.6 (17-29)	–	16	–	–	–
ROes	31 ± 4.9 (24-41)	–	30	–	–	–
Rex	27 ± 1.8 (23-30)	–	29	–	26-29	–
Rhem	25 (n = 1)	–	–	–	–	–
RV	7 ± 0.7 (5-7)	–	–	–	6-10	–
Rvan	1-2	–	–	–	–	–
Ran	4 ± 0.8 (3-6)	5-6	–	–	3-6	–
VL/VB	0.9 ± 0.1 (0.7-1.1)	–	–	–	–	–
St% L	15.2 ± 2.3 (13.7-18.3)	–	12.2	–	–	–
Spermatheca length	31.5 ± 2.2 (27-34.5)	–	–	–	–	–
Spermatheca width	22.5 ± 3.7 (15-26.5)	–	–	–	–	–
Spiculum length	–		–	49 ± 6.3 (41-55)	–	56 (55-66)
Gubernaculum length	–		–	9; 9.5 (n = 2)	–	9.5 (9-11)

J3 – third-stage juvenile; J4 – fourth-stage juvenile.

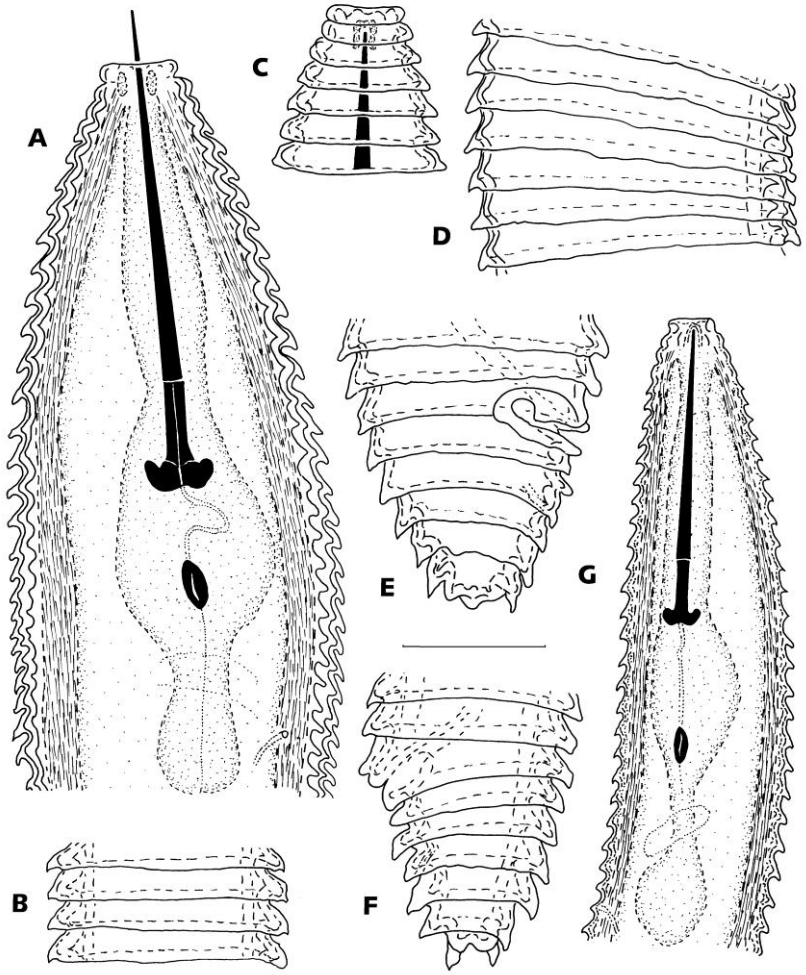

Fig. 6. *Neobakernema variabile*. Female. A: anterior part of body; C: another view of lip region; B, D: annuli at mid-body; E, F: tail area of two females. Juvenile. G: anterior part of body. Scale bar = 30 µm.

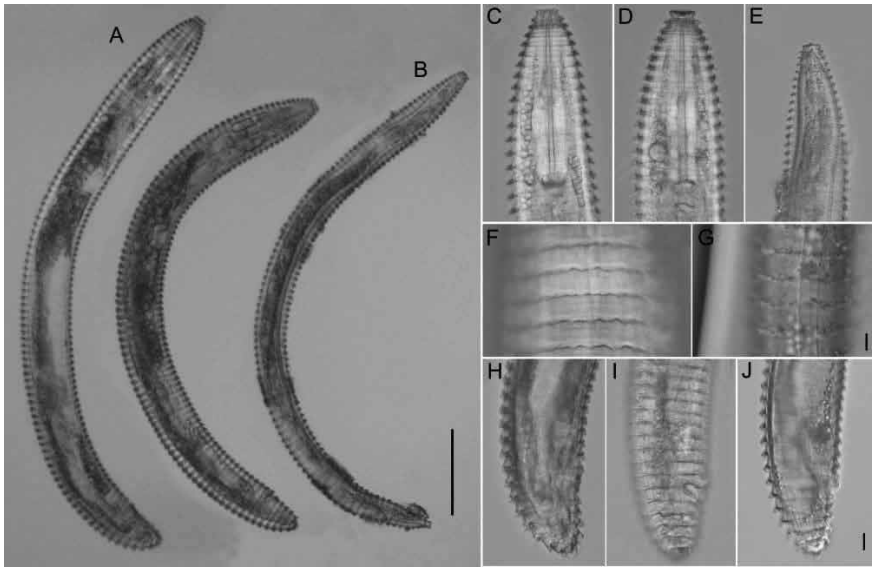

Fig. 7. *Neobakernema variabile*. A: entire body of two females; B: entire body of male; C, D: female anterior regions; E: male anterior region; F: female body annulation; G: male lateral field; H, I: female posterior regions; J: male posterior region. Scale bars: A, B = 100 µm, C-E, H-J = 10 µm, F, G = 5 µm.

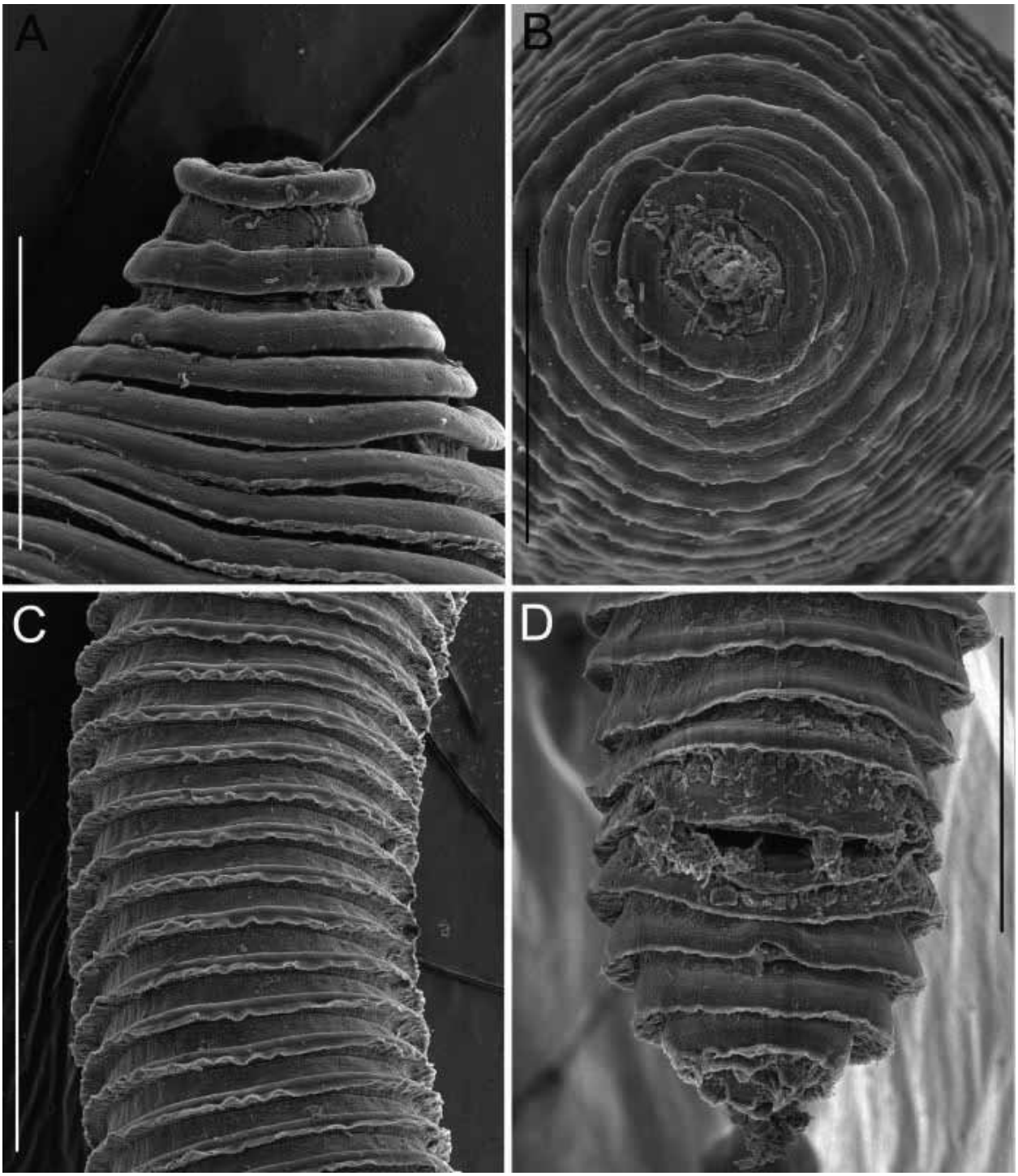

Fig. 8. *Neobakernema variabile*. Female. A: lateral view of lip region; B: *en face* view of lip region; C: annuli at mid-body; D: tail area with vulva. Scale bars: A, B = 20 µm, C = 50 µm, D = 30 µm.

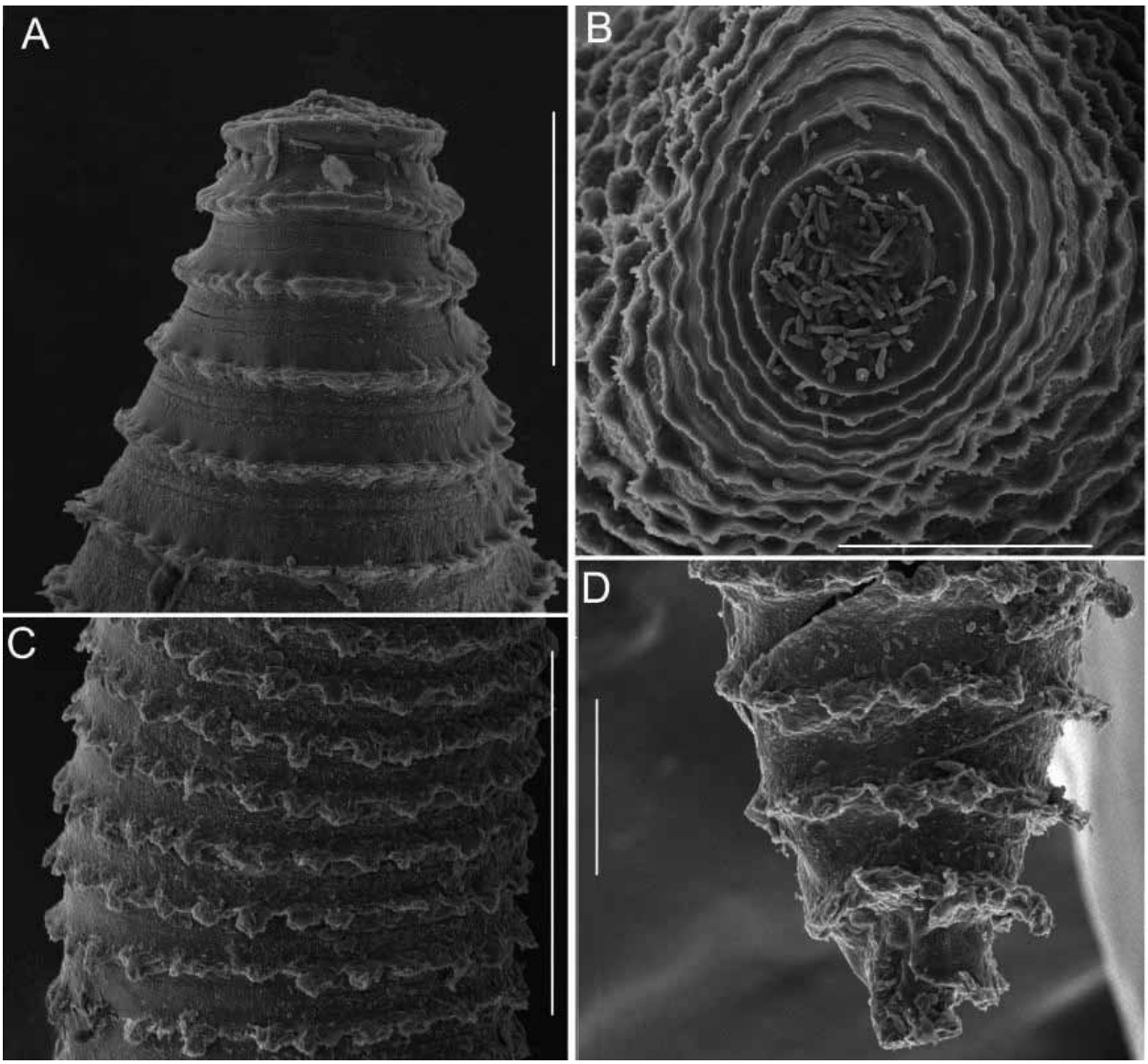

Fig. 9. *Neobakernema variabile*. Juvenile. A: lateral view of lip region; B: *en face* view of lip region; C: annuli at mid-body; D: tail region. Scale bars: A, B, D = 10 µm, C = 30 µm.

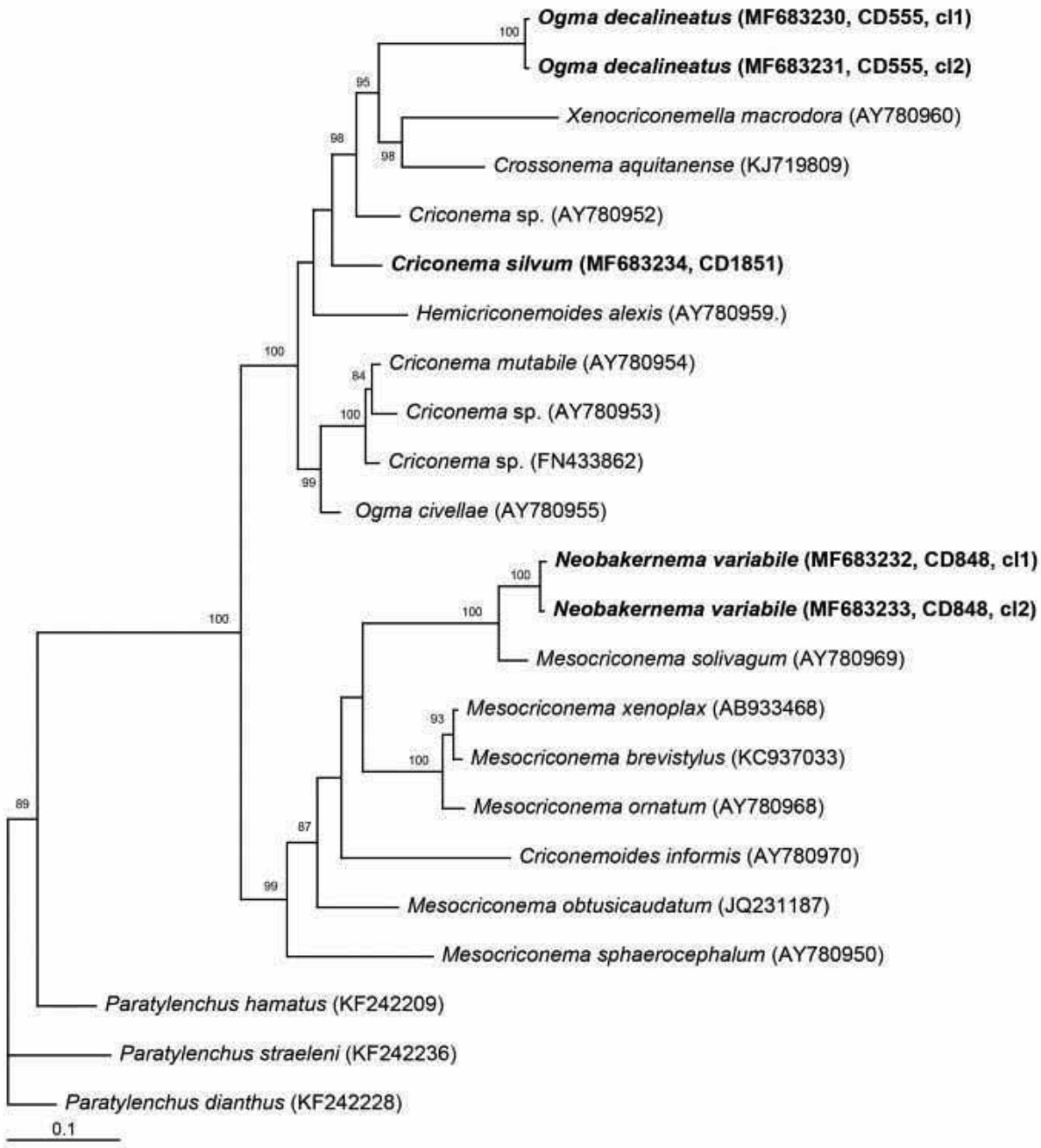

Fig. 10. Phylogenetic relationships within populations and species of Criconematidae as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

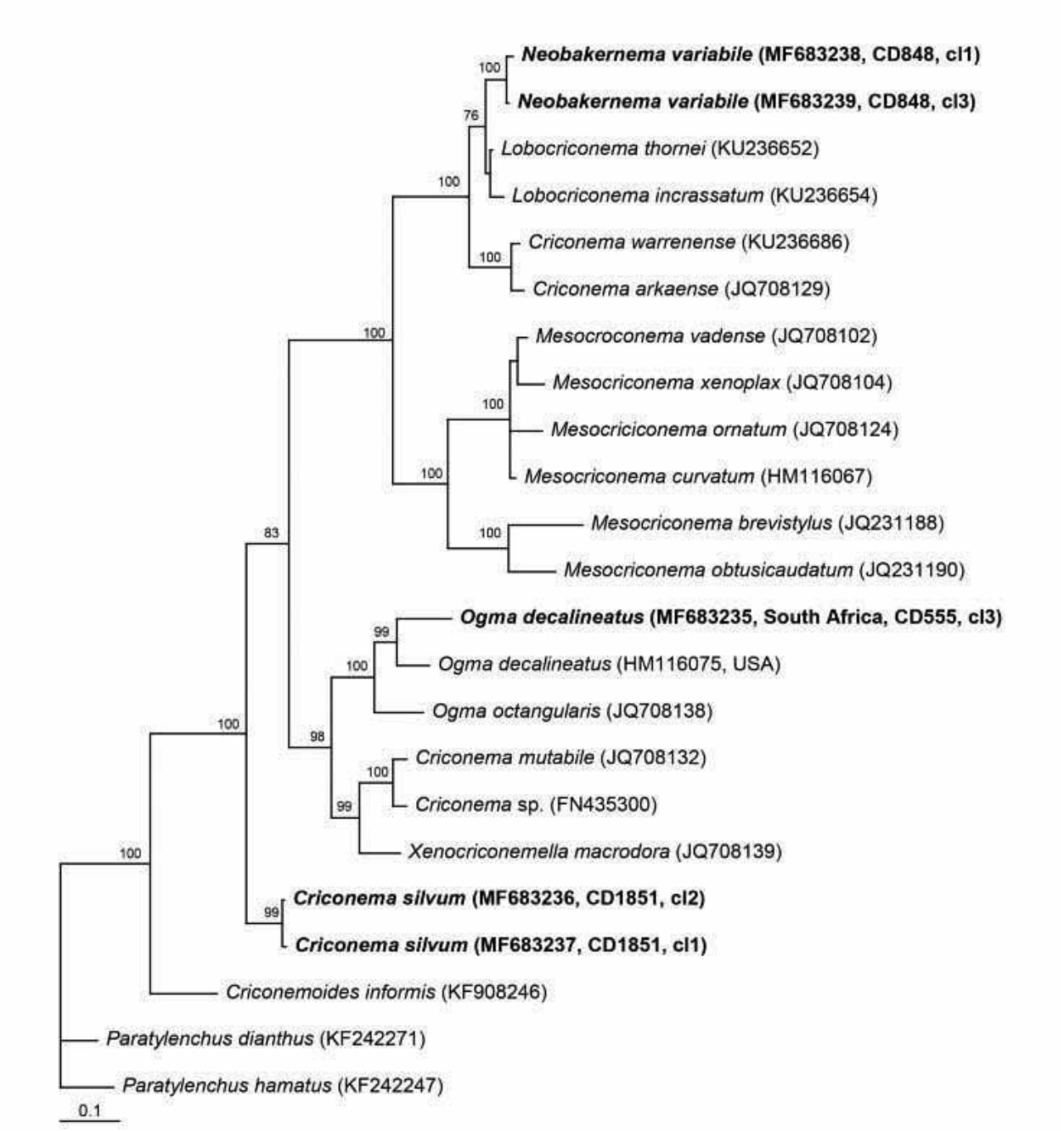

Fig. 11. Phylogenetic relationships within populations and species of Criconematidae as inferred from Bayesian analysis using the ITS1 rRNA gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

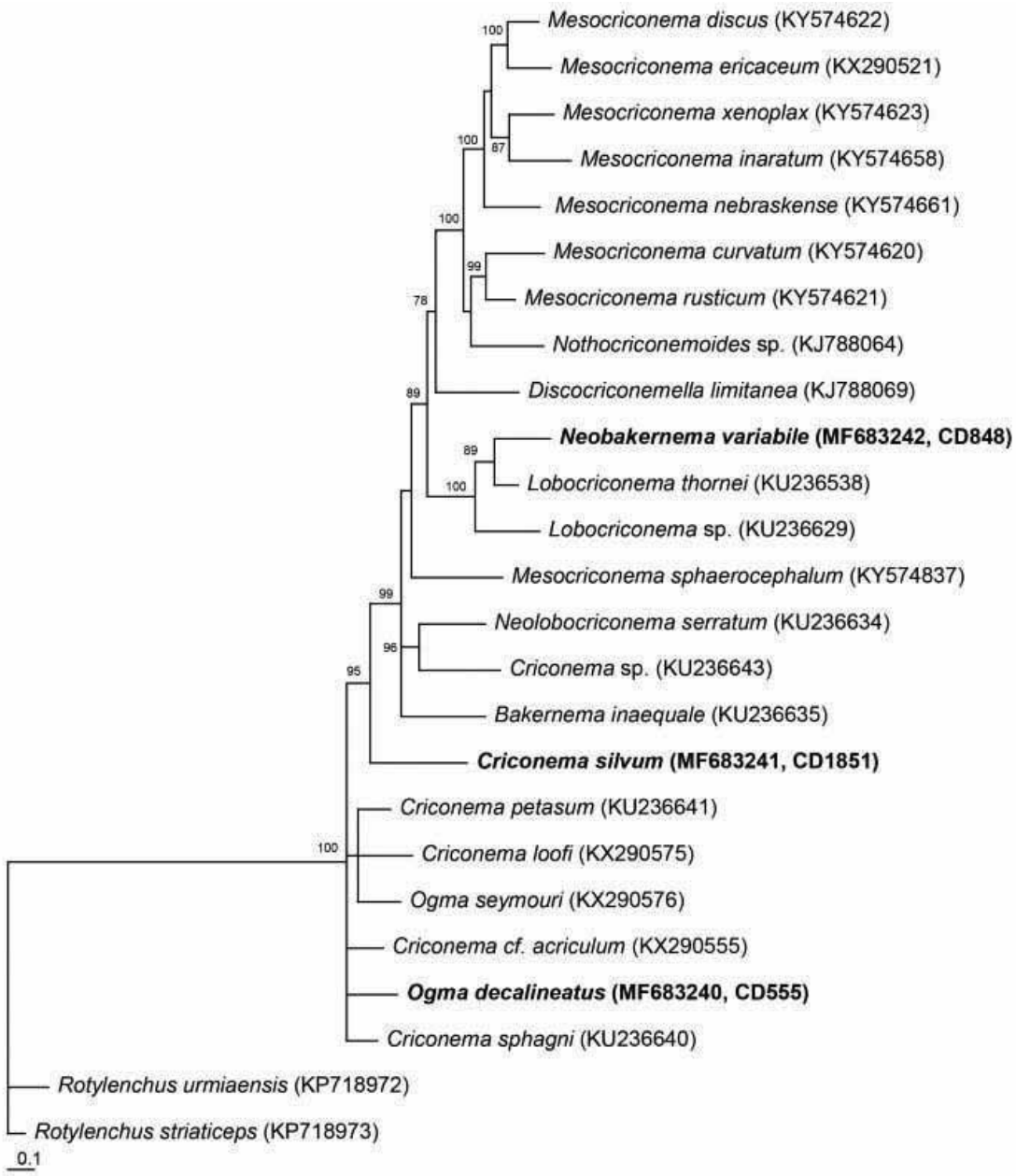

Fig. 12. Phylogenetic relationships within populations and species of Criconematidae as inferred from Bayesian analysis using the partial *COI* gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

The *COI* gene alignment included 23 sequences of criconematids and two sequences of *Rotylenchus* used as outgroup taxa and was 721 bp in a length. Three new sequences were obtained in this study. Phylogenetic relationships within some criconematids as inferred from BI analysis are given in Fig. 12.

In the present study, we provided sequences for several representatives of Criconematina; however, relationships within some genera remain unresolved. Greater phylogenetic resolution will require representatives of additional genera and species. Reconstruction of the phylogenetic relationships within criconematids is essential to developing a consistent classification as well as ultimately to understand genes and processes involved in plant parasitism (Subbotin *et al.*, 2005).

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E. van den Berg, L. R. Tiedt and S.A. Subbotin. Морфологическая и молекулярная характеристика некоторых Criconematidae (Nematoda, Tylenchida): *Ogma decalineatus* (Chitwood, 1957) Andrássy, 1979, *Criconema silvum* (van den Berg, 1984) Raski & Luc, 1985 и *Neobakernema variabile* (Raski & Golden, 1966) Ebsary, 1981 из Южной Африки и США.

Резюме. В результате последних нематологических обследований Ogma decalineatus и Criconema silvum были обнаружены в Южной Африке и вид Neobakernema variabile был обнаружен в Калифорнии, США. Эти три вида морфологически и морфометрически описаны в статье. Фотографии с использованием электронной сканирующие микроскопии также дается в описании для этих видов. Представлены молекулярные характеристики этих трех видов, сделанные с использованием последовательностей генов сегментов D2-D3 28S рРНК, ITS и COI.