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Morphological and molecular characterisation of *Criconemoides brevistylus* Singh & Khera, 1976 and *C. obtusicaudatus* Heyns, 1962 from South Africa (Nematoda: Criconematidae) with first description of a male *C. obtusicaudatus* and proposal of new synonyms

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Summary – During recent collections in South Africa, two species of *Criconemoides* were found. *Criconemoides brevistylus* is described and illustrated from a golf course in KwaZulu-Natal Province. It is compared with several closely related species, and three previously described species (*C. helicus*, *C. onostris* and *C. paronostris*) are regarded as junior synonyms of *C. brevistylus*. *Criconemoides obtusicaudatus* is described and illustrated from a maize field in Limpopo Province. The male of the species is described for the first time. Molecular characterisation of *C. brevistylus* and *C. obtusicaudatus* using the D2-D3 expansions segments of 28S rRNA and ITS rRNA gene sequences are provided. Phylogenetic relationships of these species with other *Criconemoides* are discussed.

Keywords – *Criconemoides helicus* n. syn., *Criconemoides obtusicaudatus*, *Criconemoides onostris* n. syn., *Criconemoides paronostris* n. syn., description, ITS1-5.8S-ITS2, molecular, morphology, morphometrics, new synonym, phylogeny, SEM, taxonomy.

Several *Criconemoides* Taylor, 1936 specimens were recently collected from two localities in South Africa. One group of specimens came from a grass species on the San Lameer Golf Estate between the towns of Marina Beach and Trafalgar, KwaZulu-Natal Province, whilst the other was collected from sandy soil in a maize field near Bela-Bela, Limpopo Province. The specimens from the maize field are regarded as belonging to *C. obtusicaudatus* Heyns, 1962 while the specimens from the golf course are regarded as belonging to *C. brevistylus* Singh & Khera, 1976. Both species are morphologically described using light and scanning electron microscopy and characterised molecularly using the D2-D3 of 28S rRNA, ITS1-5.8S-ITS2 rRNA sequences. In comparing the latter species with other *Criconemoides* species it was found that three species, namely *C. helicus* Eroshenko & Nguent Vu Tkhan', 1981, *C. onostris* (Phukan & Sanwal, 1980) Hunt, Luc & Manzanilla-López, 2005 and *C. paronostris* (Deswal & Bajaj, 1987) Hunt, Luc & Manzanilla-López, 2005, are very close to *C. brevistylus*. Until further morphological and molecular studies show differently, they are herein proposed as junior synonyms of *C. brevistylus*.

In this study the classification of Hunt *et al.* (2005) is used for *Criconemoides* following the reinstatement of validity of the genus by the ICZN. *Mesocriconema* Andrássy, 1965 is regarded as a junior synonym.

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Materials and methods

Nematode population, light and scanning microscopy

Specimens of *C. brevistylus* (21 females and six juveniles) were collected from a golf course in the KwaZulu-Natal Province (National Collection of Nematodes code – N825, DNA code CD768) whilst specimens of *C. obtusicaudatus* (25 females, six juveniles and one male) were collected from a maize field in the Limpopo Province (National Collection of Nematodes code Tvl 2053, DNA code CD892). Specimens were extracted from soil 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. For scanning electron microscopy nine females of *C. brevistylus* and 11 females of *C. obtusicaudatus* were studied. Fresh material was 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, 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, were amplified. The following primers were used for amplification in the present study: ITS-rRNA, TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) (Tanha Maafi *et al.*, 2003); D2-D3 of 28S rRNA, D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.*, 2006). The obtained sequences were submitted to the GenBank database under the following accession numbers: *C. obtusicaudatus*, JQ231186, JQ231187, JQ231189, JQ231190; *C. brevistylus*, JQ231183, JQ231185, JQ231188.

Phylogenetic analyses

The newly obtained sequences for each gene were aligned using ClustalX 1.83 with default parameters with corresponding published gene sequences of *Criconemoides* and *Hemicycliophora* (Subbotin *et al.*, 2005, 2006; Powers *et al.*, 2010; Van den Berg *et al.*, 2011). Out-group 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.

Results

***Criconemoides brevistylus* Singh & Khera, 1976**
= *Macroposthonia brevistylus* (Singh & Khera, 1976) Ebsary, 1979
= *Criconemella brevistylus* (Singh & Khera, 1976) Luc & Raski, 1981
= *Mesocriconema brevistylus* (Singh & Khera, 1976) Loof & De Grisse, 1989
= *C. helicus* Eroshenko & Nguent Vu Tkhan', 1981 n. syn.
= *Criconemella helica* (Eroshenko & Nguent Vu Tkhan', 1981) Raski & Luc, 1987
= *C. onostris* (Phukan & Sanwal, 1980) Hunt, Luc & Manzanilla-López, 2005 n. syn.
= *Macroposthonia onostre* Phukan & Sanwal, 1980
= *Criconemella onostris* (Phukan & Sanwal, 1980) Ebsary, 1982
= *Mesocriconema onostre* (Phukan & Sanwal, 1980) Loof & De Grisse, 1989
= *C. paronostris* (Deswal & Bajaj, 1987) Hunt, Luc & Manzanilla-López, 2005 n. syn.
= *Macroposthonia paronostris* Deswal & Bajaj, 1987
= *Criconemella paronostris* (Deswal & Bajaj, 1987) Raski & Luc, 1987
= *Mesocriconema paronostre* (Deswal & Bajaj, 1987) Ebsary, 1991
(Figs 1, 2)

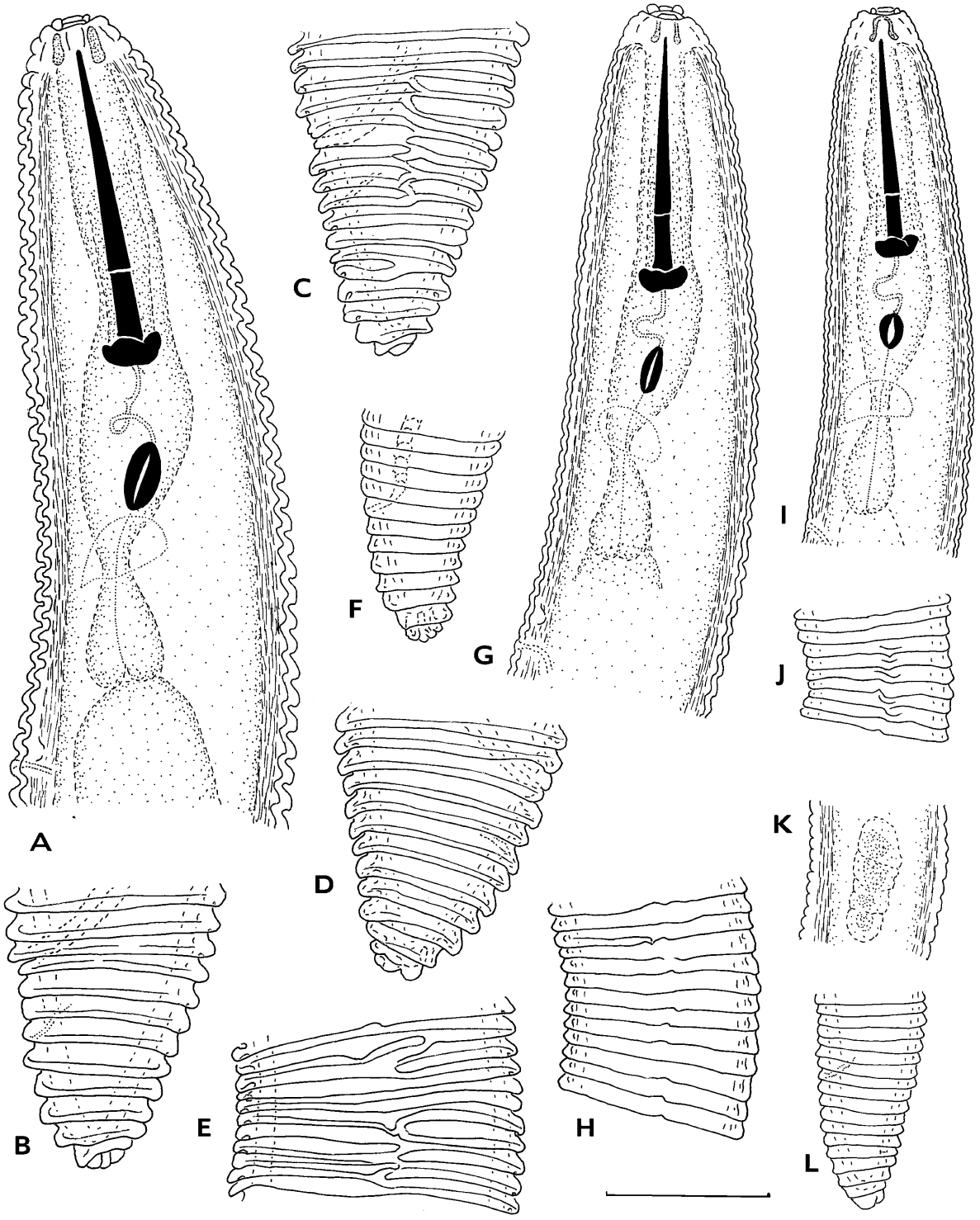

Fig. 1. Criconemoides brevistylus. *A-E: Female. A: Anterior part of body; B-D: Tail region; E: Lateral field at mid-body; F-H: Fourth-stage juvenile. F: Tail region; G: Anterior part of body; H: Lateral field at mid-body; I-L: Second-stage juvenile. I: Anterior part of body; J: Lateral field at mid-body; K: Ovary primordium; L: Tail region. (Scale bar: A-L = 30 μm.)*

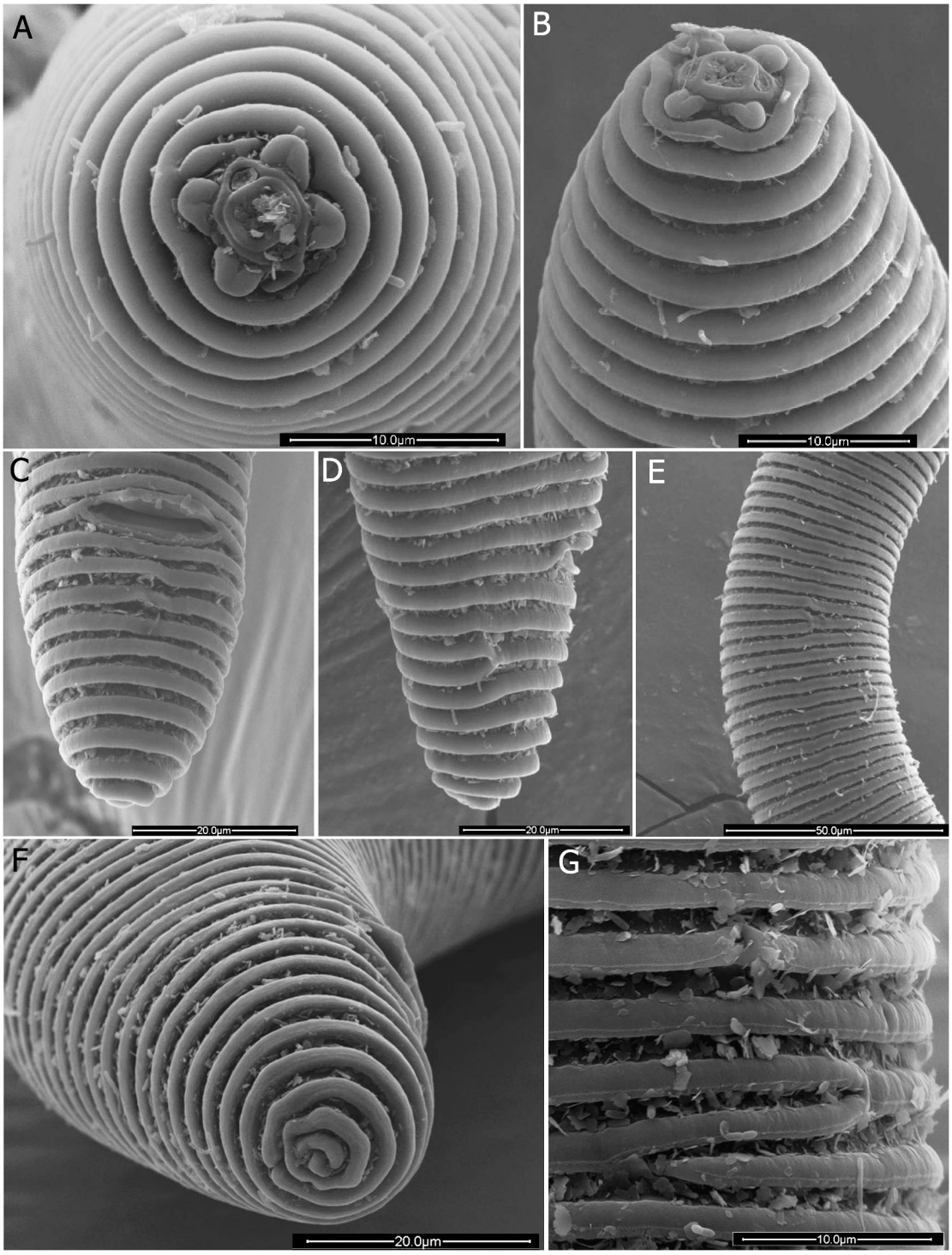

Fig. 2. Criconemoides brevistylus. *Female. A, B: Lip region of two females,* en face *view; C, D: Tail region, ventral and lateral views; E: Lateral field area at mid-body; F: Tail tip showing dorsally directed last annuli; G: Ventral view with excretory pore and ventral anastomosis.*

Measurements

See Tables 1, 2.

Description

Female

Body form ranging from slightly curved ventrad to a closed letter C. Lip region rounded in profile, continuous with body contour, with three rounded annuli, first smaller than second and second smaller than third, all three smaller than first body annulus. First lip annulus not divided into a dorsal and ventral half with distinct sub median lobes and smaller labial plates. Scanning electron microscope photographs showing a squarish labial disc raised slightly above an oval area including distinct amphidial openings laterally. Small, irregular labial plates present and four large sub median lobes visible. Cephalic framework distinct, stretching over first three annuli. All body annuli well retrorse with smooth margins, at most slightly irregular. Lateral field demarcated with none to at most six anastomoses, sometimes short stretches of irregularities occurring. Some anastomosis noticed on dorsal and ventral margins. Hemizonid frequently not seen, otherwise one annulus long and situated from opposite to two annuli anterior to excretory pore. Excretory pore situated from two annuli anterior to two annuli posterior to base of pharynx. Stylet well developed with cupped basal knobs. Spermatheca indistinct, small, round, without sperm cells. Vagina straight. Vulva open. Dorsal vulval lip straight, not overhanging, at most with two small lobes. Tail tapering to a rounded tip which is slightly projecting dorsally. Scanning electron microscope photos showing last two annuli as rounded circles, longer on ventral side than dorsal, thus appearing as curving dorsally when viewed laterally.

Juveniles

J2 similar to female and J4; J4 very similar to female, with a dorsally curved tail but tail appearing longer and more slender.

Male

Not found.

Relationships

These specimens come close to a number of species in the genus with a large number of body annuli with anastomoses in the lateral field and a stylet length of 54.5-61 μm. The present specimens are closest to *C. brevistylus* described by Singh and Khera (1976) from West Bengal, India. Except for lip annuli with slightly larger diameters they are almost identical and are here regarded as belonging to *C. brevistylus*.

Criconemoides brevistylus is compared with several closely related species: *i*) *C. annulatus* Cobb *in* Taylor, 1936; Choi *et al*. (2000) summarised the most important characters of this species. The present specimens differ mainly in having a shorter stylet (54.5-61 *vs* 65-108 μm), slightly more body annuli (R = 137-161 *vs* 113-157), large submedian lobes present *vs* lacking, open vulva *vs* closed and a rounded dorsally curved tail *vs* rounded to broadly rounded, not curving dorsally. The scanning electron microscope photographs of the *en face* view of the lip region presented by Peneva *et al*. (2000) also differ completely from the present specimens; *ii*) *C. hawangiensis* (Choi & Geraert, 1995) Siddiqi, 2000, described from Korea (Choi & Geraert, 1995). Although the number of annuli are very similar to *C. brevistylus* (R = 124-154), differences include a longer stylet (59-73 *vs* 54.5-61 μm), vulva situated more anteriorly (V = 80-90 *vs* 90-94), submedian lobes absent *vs* present, annuli margins crenate *vs* smooth, larger RV value (12-15 *vs* 9-13), longer tail (38.5 μm, as calculated from figure, *vs* 20-31 μm), and a more drawn out narrow tail tip with the last few annuli longer and more irregular than those preceding; *iii*) *C. komabaensis* (Imamura, 1931) Taylor, 1936 (= *C. paragoodeyi* Choi & Geraert, 1975), described from Japan. Minagawa (1995) gave a redescription of this species from Japan, Korea and Iran. These specimens differ from *C. brevistylus* in the following characters: lip region completely different with no submedian lobes and an oral disc with a very distinct outer ridge, shorter body (L = 350-460 *vs* 453-625 μm), smaller c value (10-17.2 *vs* 18-27.5), more anterior vulva (V = 85-90 *vs* 90-94), shorter stylet (45-56 *vs* 54.5-61 μm), larger RV and Ran values (13-21 *vs* 9-13 and 8-15 *vs* 6-9, respectively), tail elongated, conical with narrow tip not directed dorsally and a spermatheca filled with sperm cells; *iv*) *C. oblongata* (Renubala, Dhanachand & Ghambir, 1991) Hunt, Luc & Manzanilla-López, 2005, described from India (Renubala *et al*., 1991). Corresponds with *C. brevistylus* in having 134-148 body annuli with anastomoses and a dorsally curved tail according to the illustrations but differs in being shorter (L = 390-400 *vs* 453-625 μm), smaller stylet (41.6-44.8 *vs* 54.4-61 μm, smaller RSt value (14-17 *vs* 17-23), shorter pharynx (102.4-104 *vs* 111-134 μm) and smaller a value (7.2-7.6 *vs* 9.2-14.5). Most other measurements are also smaller. Nothing is mentioned

Table 1. *Morphometrics of* Criconemoides brevistylus *from South Africa compared with those from original description. All measurements are in μm and in the form: mean ± s.d. (range).*

Character	South Africa			India (Singh & Khera, 1976)
	Female	J4	J2	Female
n	21	5	1	8
L	565 ± 48.2 (453-625)	453 ± 34.9 (410-497)	341	580-670
a	11.3 ± 1.4 (9.2-14.5)	12.5 ± 0.8 (11.5-13.8)	12.5	11-14
b	4.6 ± 0.4 (3.5-5.1)	4.3 ± 0.4 (3.9-4.7)	3.7	4.3-5.5
c	22.2 ± 2.8 (18-27.5)	18.8 ± 1.6 (17.5-21.8)	14.7	18.5*
o	11.8 ± 1.4 (9-14.2)	12.3 ± 1.7 (10.4-13.4)	–	–
DGO	7.0 ± 0.7 (5.0-7.5)	6.0 ± 0.8 (5.0-6.5)	–	8-10
V	93 ± 0.9 (90-94)	–	–	91-94
G_1	38.5 ± 5.2 (34-52)	–	–	50-70
Ovary primordium	–	102 ± 25.9 (83-145.5)	23	–
Stylet length	58 ± 2.2 (54.5-61)	47.5 ± 2.4 (43.5-50)	39.5	56-60
Metenchium length	41 ± 2.2 (38-45.5)	33.5 ± 2.7 (29.5-37)	27	–
Telenchium length	17 ± 1.1 (15-20)	14 ± 0.7 (13-14.5)	12.5	–
M	70.9 ± 2.2 (66.6-73.8)	70.7 ± 2.4 (67.5-73.5)	68.5	–
Stylet knob height	5 ± 0.6 (3.5-6)	3.5 ± 0.4 (3-3.5)	3.5	–
Stylet knob width	10.5 ± 0.6 (9.5-12)	8 ± 0.8 (7.5-9)	7.5	9-10
Excretory pore from anterior	133 ± 15 (100.5-153.5)	115 ± 12 (100-126.5)	98	130-137
Diam. at mid-body	50.5 ± 5.4 (40.5-61.5)	36.5 ± 4.0 (30-41)	27	60*
Annulus width	4.0 ± 0.4 (3.5-5)	3.5 ± 0.4 (3-3.5)	3	4-5
Tail length	25.5 ± 3.2 (20-31)	24 ± 2.3 (21.5-27)	23	23.5*
Pharynx length	124 ± 6.8 (111-134)	104 ± 1.6 (102-106)	92	119-145
Vulva to anus	14 ± 3.1 (9.5-22)	–	–	11.5*
First lip annulus diam.	13 ± 1.2 (11-15.5)	10 ± 1 (9-12)	9.5	7-9
Second lip annulus diam.	16.5 ± 1.1 (14-18.5)	13.5 ± 1.0 (12-14.5)	12	10-11
Third lip annulus diam.	19.5 ± 1.4 (17-22)	16 ± 1.4 (14-18)	13	14-15
First body annulus diam.	23 ± 1.6 (19.5-26)	18 ± 1.2 (16-19)	14	–
Second body annulus diam.	25.5 ± 2.0 (21.5-30)	20 ± 1.4 (17.5-21)	15.5	–
R	147 ± 5.5 (137-161)	147 ± 2.7 (144-151)	158	140-156
RSt	19 ± 1.4 (17-23)	20 ± 0.4 (19-20)	23	19*
ROes	36 ± 3.1 (32-45)	38 ± 2.9 (35-41)	44	37*
Rex	39 ± 3.4 (34-43)	41.5 ± 1.7 (39-43)	47	35-41
Rhem	38 ± 3.5 (30-43)	40 ± 0.6 (40-41)	–	–
RV	10.5 ± 1.2 (9-13)	–	–	9-10
RVan	3.0 ± 0.8 (1-4)	–	–	2-3
Ran	7.0 ± 0.8 (6-9)	8.0 ± 0.7 (7-9)	11	6-7
VL/VB	1.2 ± 0.1 (0.9-1.4)	–	–	0.9*
St%L	10.4 ± 1.0 (9.3-12.7)	10.5 ± 0.5 (10.5-11.5)	11.7	11.4*

* Calculated from figures of Singh and Khera (1976).

Table 2. Criconemoides brevistylus *compared with the closely related species* C. helicus*,* C. onostris *and* C. paronostris*. All measurements are in µm and in the form: mean ± s.d. (range).*

Character	*C. brevistylus* Singh & Khera, 1976	*C. helicus* Eroshenko & Nguent Vu Tkhan', 1981[a]	*C. onostris* (Phukan & Sanwal, 1980) Hunt, Luc & Manzanilla-Lopéz, 2005[b]	*C. paronostris* (Deswal & Bajaj, 1987) Hunt, Luc & Manzanilla-Lopéz, 2005[c]
n	21	?	10	20
L	565 ± 48.2 (453-625)	440-530	553 (518-608)	460-530
a	11.3 ± 1.4 (9.2-14.5)	9-11	11.5 (10.1-12.4)	10.7-12.2
b	4.6 ± 0.4 (3.5-5.1)	3.9-4.9	4.5 (4.3-4.9)	4.5-5
c	22.2 ± 2.8 (18-27.5)	16-20	20 (18-22)	17-20
o	11.8 ± 1.4 (9-14.2)	–	–	–
DGO	7.0 ± 0.7 (5.0-7.5)	–	6-8	7.5-10
V	93 ± 0.9 (90-94)	91-94	93 (92-96)	91-94
G_1	38.5 ± 5.2 (34-52)	–	–	–
Stylet length	58 ± 2.2 (54.5-61)	53-59	57 (54-61)	53-60
Metenchium length	41 ± 2.2 (38-45.5)	42*	43 (39-48)	43-45
Telenchium length	17 ± 1.1 (15-20)	16*	–	10.5-13.5
M	70.9 ± 2.2 (66.6-73.8)	72.5*	–	75-80
Stylet knob height	5.0 ± 0.6 (3.5-6.0)	–	–	4-5
Stylet knob width	10.5 ± 0.6 (9.5-12)	–	8.0-11.6	10.5-13.5
Excretory pore from anterior	133 ± 15 (100-153)	109*	–	112-121
Diam. at mid-body	50.5 ± 5.4 (40.5-61.5)	–	44*	–
Annulus width	4.0 ± 0.4 (3.5-5)	3.5	4.3	3-4
Tail length	25.5 ± 3.2 (20-31)	35.5*	22-29	26-29
Pharynx length	124 ± 6.8 (111-134)	109	112-127	104-115
Vulva to anus	14 ± 3.1 (9.5-22)	–	–	–
First lip annulus diam.	13 ± 1.2 (11-15.5)	14*	10-13	8-10
Second lip annulus diam.	16.5 ± 1.1 (14-18.5)	17.5*	13.5-15.4	12-15
Third lip annulus diam.	19.5 ± 1.4 (17-22)	21*	–	15-18
First body annulus diam.	23 ± 1.6 (19.5-26)	23	–	18-21
Second body annulus diam.	25.5 ± 2.0 (21.5-30)	25.5	–	–
R	147 ± 5.5 (137-161)	120-140	138 (133-147)	119-150
RSt	19 ± 1.4 (17-23)	18-21	21 (20-22)	18-21
ROes	36 ± 3.1 (32-45)	28-38	35 (35-38)	32-39
Rex	39 ± 3.4 (34-43)	28-36	37 (36-38)	35-43
Rhem	38 ± 3.5 (30-43)	–	–	–
RV	10.5 ± 1.2 (9-13)	7-9	8 (7-9)	9-10
RVan	3.0 ± 0.8 (1-4)	1-3	2 (1-3)	2-3
Ran	7.0 ± 0.8 (6-9)	4-8	7 (6-8)	6-7
VL/VB	1.2 ± 0.1 (0.9-1.4)	1*	1-1.1	0.9-1
St%L	10.4 ± 1.0 (9.3-12.7)	–	10.5*	10.6-12.8
Number of lip annuli	3	3-4	3 on fig.	3
Sub median lobes	Distinct, large	Distinct and large	Distinct	Distinct
Anastomosis	0-6; patches of irregularities	Frequently found	Not found	Patches of 2-9 over whole body
Spermatheca	Empty	Empty	Empty	Empty
Tail	Always curved dorsally	Always curved dorsally	Always curved dorsally	Curved dorsally

[*] Calculated from the illustrations.
[a] Eroshenko & Nguent Vu Tkhan' (1982).
[b] Hunt *et al.* (2005).
[c] According to Rahaman *et al*. (1996).

in the original description about the structures on the *en face* view of the lip region; *v*) *C. onoensis* Luc, 1959, described from the Ivory Coast and since reported worldwide. The present specimens were compared with the descriptions of *C. onoensis* by Luc (1959), Raski and Golden (1966), Luc (1970), Van Doorsselaere and Samsoen (1982), Rashid *et al*. (1987), Decraemer and Geraert (1991), Van den Berg and Cadet (1991) and Crozzoli and Lamberti (2001).

Females

Stylet shorter (38-55 *vs* 54.5-61 μm), excretory pore situated closer to anterior end (71-134 *vs* 100.5-153.5 μm), shorter pharynx (82-124 *vs* 111-134 μm), fewer body annuli (R = 110-138 *vs* 137-161), smaller Rex value (27-37 *vs* 34-43), tail generally truncate or more broadly rounded and last two annuli not curved dorsally, filled and empty spermathecae reported. Female tails described by Van den Berg and Cadet (1991) are narrower at the tip but lack dorsally curved annuli.

J4

Reported by Decraemer and Geraert (1991) and Crozzoli and Lamberti (2001), they have a shorter body (L = 314-420 *vs* 410-497 μm), shorter stylet (36-40 *vs* 43.5-50 μm), shorter metenchium (26-29 *vs* 29.5-37 μm), shorter pharynx (85-100 *vs* 102-106 μm), shorter distance of excretory pore from anterior end (79-101 *vs* 100.5-153.5 μm), fewer body annuli (R = 117-141 *vs* 137-161), lower ROes value (31-32 *vs* 32-45), lower Rex value (31-34 *vs* 34-43), shorter tail (22-23 *vs* 20-31 μm), shorter ovary primordium (62-64 *vs* 83-145.5 μm) and tail tip not directed dorsally. In many of the descriptions the lip region is drawn with two lip annuli but nothing is mentioned about the lip annuli in the species descriptions; and *vi*) *C. ripariensis* (Eroshenko & Volkova, 1997) Hunt, Luc & Manzanilla-López, 2005, described from Russia, has 125-150 body annuli, distinct submedian lobes and a dorsally curved tail terminus. This species comes close to *C. brevistylus* but can be separated by its longer stylet (64-73 *vs* 54.5-61 μm), dorsal pharyngeal gland opening closer to stylet base (3.5-5 *vs* 7.5-10 μm), longer tail (35-40 *vs* 20-31 μm), more annuli between vulva and anus (RVan = 4-6 *vs* 1-4) and a more sharply pointed tail.

Three species of *Criconemoides* (all originally described from India), namely *C. helicus* Eroshenko & Nguent Vu Tkhan', 1981, *C. onostris* (Phukan & Sanwal, 1980) Hunt, Luc & Manzanilla-López, 2005 and *C. paronostris* (Deswal & Bajaj, 1987) Hunt, Luc & Manzanilla-López, 2005, are very similar to *C. brevistylus* (also originally described from India) in having body annuli ranging from 119-161, body length ranging from 440 to 625 μm, stylet length ranging from 53 to 61 μm, having three lip annuli, no males, none to several anastomoses and patches of irregularities opposite the lateral field area and, especially, in having the last two or three tail annuli typically curved dorsad when viewed laterally (see Table 2). As a consequence, these three species are considered as junior synonyms of *C. brevistylus*. According to Geraert (2010), *C. paronostris* cannot be separated from *C. brevistylus* and Rahaman *et al.* (1996) states that it is very close to *C. onostris* and *C. onoensis*. However, *C. onoensis* can be separated on the basis of a few characters (see above). The first lip annulus of *C. helicus* and *C. onostris* is described as forwardly directed or not retrorse. The illustrations show more square lip regions while those of the present specimens appear to be more rounded, although the SEM photographs of the lip region of *C. paronostris* reported by Rahaman *et al*. (1996) are almost identical to those of the present specimens. Until more morphological and molecular data prove differently we regard *C. helicus*, *C. onostris* and *C. paronostris* as synonyms of *C. brevistylus*.

Criconemoides obtusicaudatus **Heyns, 1962**
= *Macroposthonia obtusicaudata* (Heyns, 1962) De Grisse & Loof, 1965
= *Criconemella obtusicaudata* (Heyns, 1962) Ebsary, 1982
= *Mesocriconema obtusicaudatum* (Heyns, 1962) Loof & De Grisse, 1989
(Figs 3, 4)

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Body curved slightly ventrad. Lip region with two annuli, first of smaller diam. than second. First lip annulus mostly pointing outward and upward, distinctly divided into a dorsal and ventral half, second annulus retrorse, mostly not, but sometimes with slight indentation laterally. Labial area raised well above first lip annulus, with four small bud-shaped submedian lobes, four smaller additional lobes sometimes observed. Labial plates not always distinct. Cephalic framework moderately sclerotised, extending over first three annuli. All body annuli

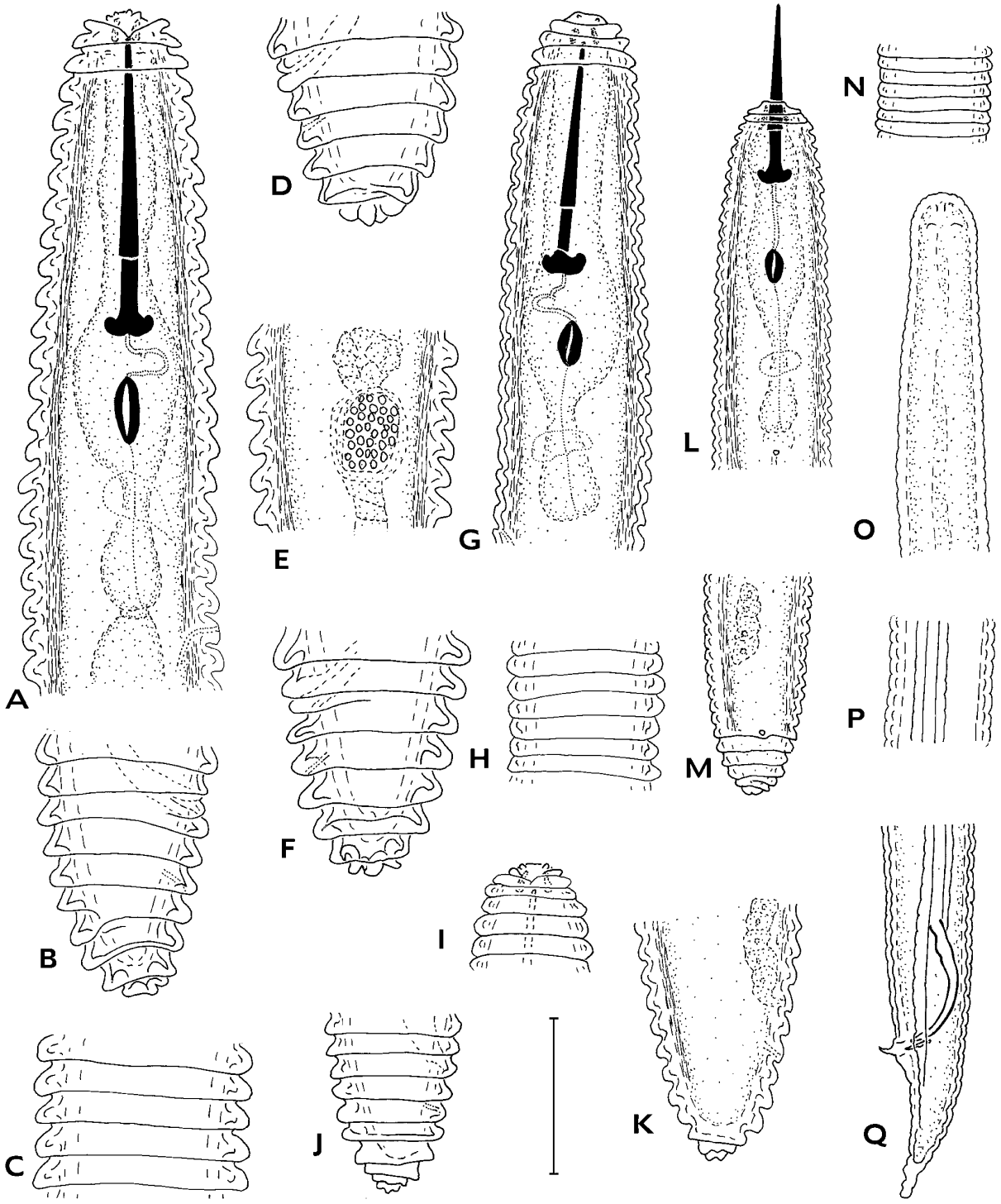

Fig. 3. Criconemoides obtusicaudatus. *A-F: Female. A: Anterior part of body; B, D, F: Tail region; C: Lateral field at mid-body; E: Spermatheca area; G-K: Fourth-stage juvenile. G: Anterior part of body; H: Lateral field at mid-body; I: Another lip region; J: Tail region; K: Tail region, internal view; L-N: Second-stage juvenile. L: Anterior part of body; M: Tail region with ovary primordium; N: Lateral field at mid-body; O-Q: Male. O: Anterior part of body; P: Lateral field at mid-body; Q: Tail region. (Scale bar: A-Q = 30 μm.)*

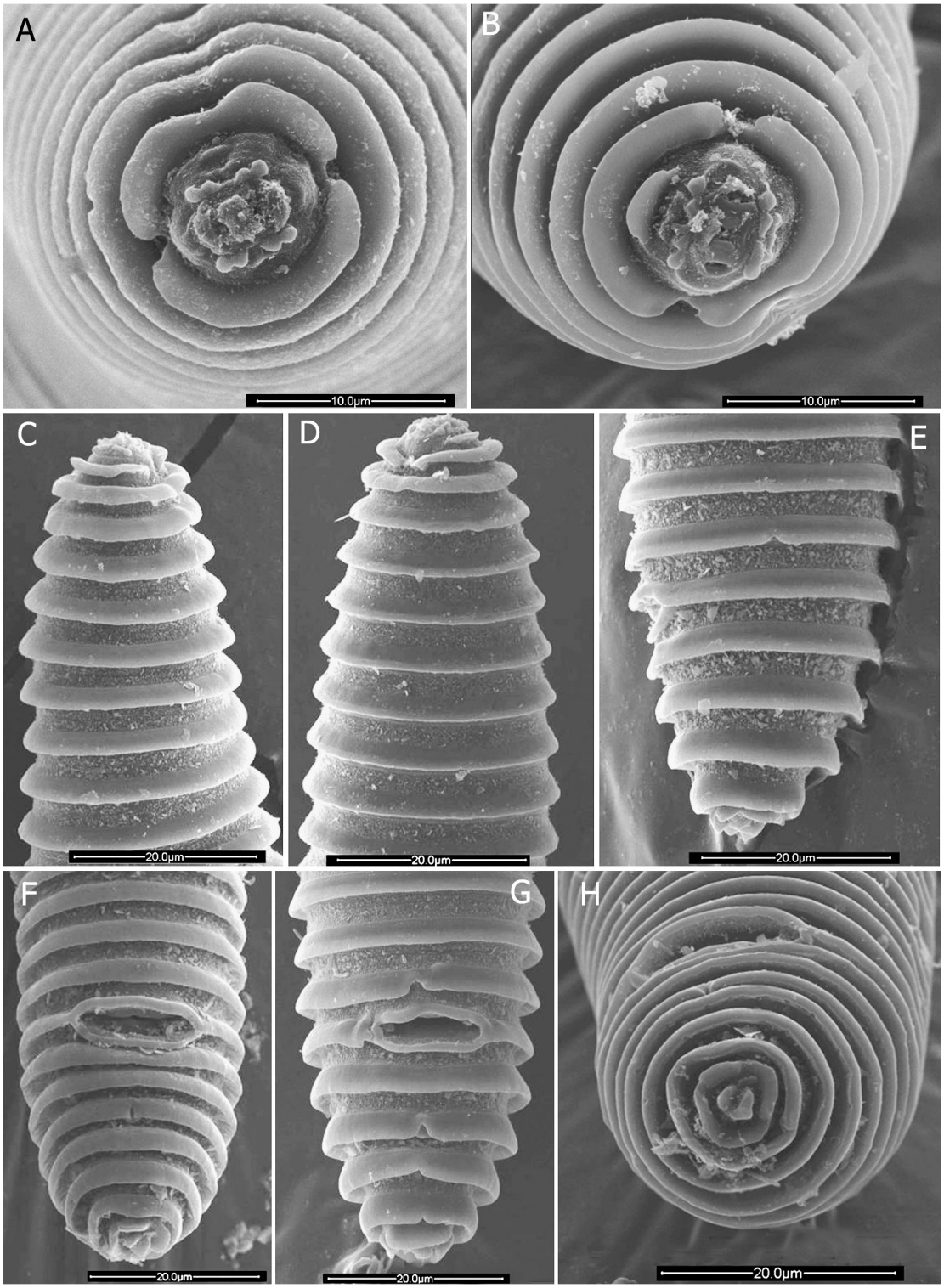

Fig. 4. Criconemoides obtusicaudatus. *Female. A, B: Lip region,* en face *view; C, D: Lip region, lateral view; E-G: Tail region, lateral and ventral views; H: Posterior tip of tail.*

Table 3. *Morphometrics of* Criconemoides obtusicaudatus *compared with the literature. All measurements are in μm and in the form: mean ± s.d. (range).*

Character	Female	J4	J2	Male	Literature[a]
n	25	5	1	1	–
L	432 ± 36.1 (349-510)	331 ± 19.8 (302-351)	190	447	288-470
a	10.3 ± 2.3 (8.9-12.9)	10.3 ± 1.0 (9.0-11.8)	9.6	–	7.7-12.3
b	4.2 ± 0.2 (3.6-4.6)	3.7 ± 0.3 (3.5-4.0)	3	4.1	3.2-4.9
c	27.8 ± 5.3 (21.1-41.3)	26.5 ± 6.8 (19-36.2)	15.2	15.5	22.4-52.5
o	10.3 ± 1.8 (7.1-13.3)	11.5 ± 1.0 (10.4-12.5)	11.4	–	–
DGO	5.2 ± 1.0 (4-7.5)	4.5 ± 0.4 (4.5-5.0)	3.5	–	2.9-6.6
V	93 ± 0.8 (91.5-94.5)	–	–	–	92-96
G_1	65 ± 16.6 (45.5-109.5)	–	–	–	43-55
Ovary primordium	–	65 ± 6.2 (57-71)	14.5	–	–
Stylet length	50 ± 2.6 (46-55.5)	42 ± 1.0 (40.5-42.5)	32.5	–	41-57
Metenchium length	36.5 ± 2.0 (33-40.5)	30.5 ± 0.8 (29.5-31.5)	21.5	–	27-36.5
Telenchium length	13.5 ± 0.8 (12.5-15.5)	11 ± 0.4 (11-12)	11	–	9-15
Stylet knob height	4.0 ± 0.4 (3-4)	3.0 ± 0.3 (2.5-3)	3	–	3.0-4.5
Stylet knob width	8.5 ± 0.5 (5.0-7.5)	7.0 ± 0.4 (6.5-7.5)	6	–	7.5-9.5
Excretory pore from front	114 ± 12.4 (91-135.5)	93 ± 5.0 (86.5-98.5)	64	110	–
Diam. at mid-body	40.5 ± 3.5 (34.5-46)	32 ± 4.6 (27-38.5)	20	20	–
Annulus width	6.0 ± 0.5 (5.0-7.5)	4.0 ± 0.4 (3.0-4.5)	2	2.5	3.5-7.0
Tail length	16 ± 2.6 (10-20)	13 ± 2.9 (9.5-17)	12.5	31	7.0-15.5
Pharynx length	104 ± 7.0 (96-124)	90 ± 8.5 (77-99)	63	108	–
Spermatheca length	13.5 ± 2.9 (9.0-20.5)	–	–	–	–
Spermatheca width	11.5 ± 1.8 (8.5-15.5)	–	–	–	–
Vulva to anus	15 ± 2.2 (11-20)	–	–	–	–
First lip annulus diam.	14.5 ± 2.1 (10.5-18.5)	12.5 ± 1.1 (10-12.5)	9.5	–	10-17.5
Second lip annulus diam.	19.5 ± 1.8 (16.5-23.5)	15 ± 1.0 (14-16)	12.5	–	15-22
First body annulus diam.	22.5 ± 1.8 (20-26)	17 ± 0.7 (16-17.5)	14	–	18-26.5
Second body annulus diam.	25 ± 1.9 (21-28)	19 ± 0.9 (17.5-20)	14.5	–	–
Third body annulus diam.	27 ± 2.2 (23-31.5)	20.5 ± 0.9 (19-21)	16	–	–
R	78 ± 2.8 (73-83)	84 ± 3.7 (78-88)	82	–	59-85
RSt	11 ± 0.9 (9-12)	13 ± 0.4 (13-14)	-	–	9-13
ROes	20 ± 1.0 (18-22)	25 ± 2.0 (22-27)	25	–	17-23
Rex	22 ± 1.7 (19-25)	25 ± 1.3 (23-26)	26	–	18-23
Rhem	22 ± 1.3 (19-23)	25 (n = 1)	–	–	–
RV	6.0 ± 0.7 (5-8)	–	–	–	6-8
RVan	2.0 ± 0.6 (1-3)	–	–	–	2.0-4.5
Ran	4.0 ± 0.6 (2-5)	4.0 ± 1.1 (3-6)	5	–	2-4
VL/VB	1.0 ± 0.1 (0.6-1.2)	–	–	–	0.7-0.9
St%L	11.6 ± 1.0 (10.0-13.7)	12.5 ± 0.6 (12-13.5)	17	–	11.8-16.6
T	–	–	–	29	–
Testis length	–	–	–	129.5	–
Penial tube length	–	–	–	3.5	–
Hyaline part of tail	–	–	–	9.5	–
Spicule length	–	–	–	27	–
Gubernaculum length	–	–	–	5	–
Lip region diam.	–	–	–	12	–
Lip region height	–	–	–	7	–
Lateral field width	–	–	–	5	–

[a] Heyns (1962, 1970), Van den Berg (1980), Renubala *et al*. (1991).

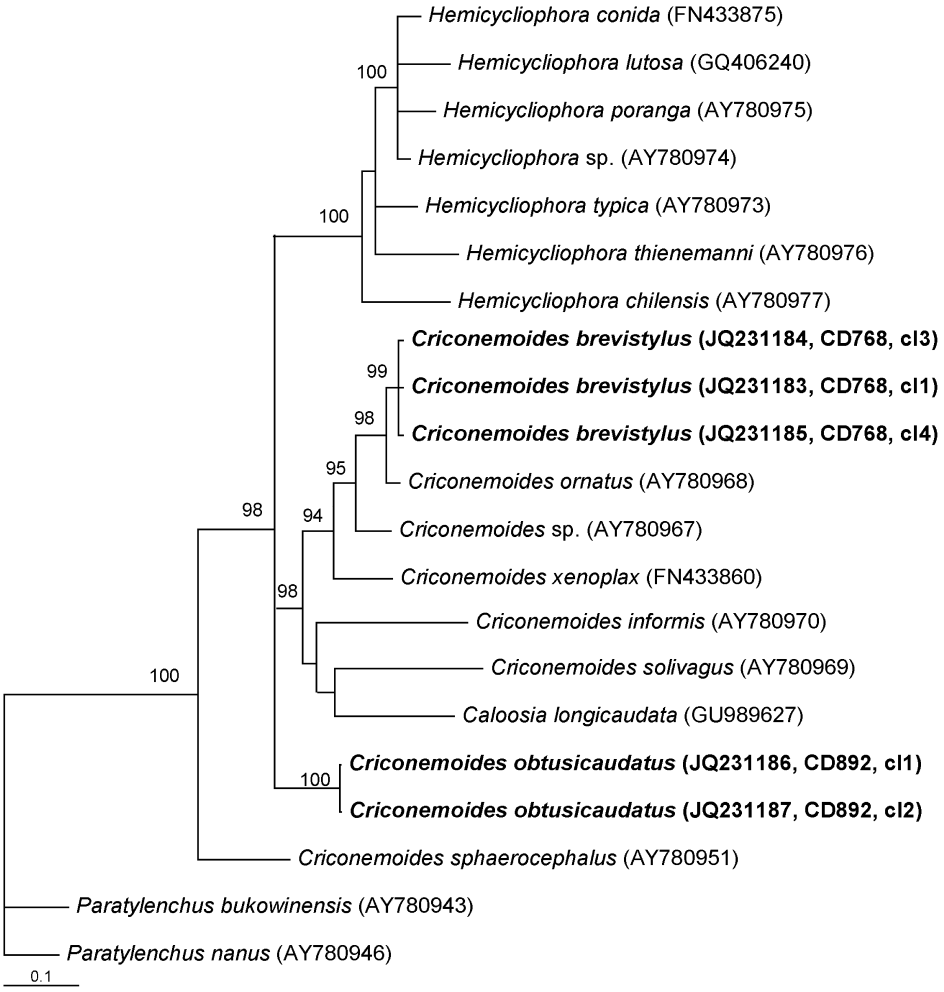

Fig. 5. *Phylogenetic relationships within representatives of* Criconemoides *and related criconematids as inferred from Bayesian analyses of sequences of the D2-D3 of 28S rRNA gene using GTR + I + G model of DNA evolution. Posterior probability values more than 70% are given on appropriate clades. The newly obtained sequence is indicated in bold.*

slightly retrorse with smooth posterior margins. Lateral field not demarcated but an irregularity rarely observed. Hemizonid indistinct, where seen *ca* one annulus long and situated from one annulus anterior to one annulus posterior to excretory pore. Excretory pore situated from two annuli anterior to five annuli posterior to pharynx base. Stylet well developed with cupped basal knobs. Spermatheca distinct, varying from small to large, round to oblong, mostly filled with sperm. Vagina straight. Vulva open with mostly two slightly rounded lobes on anterior lip. Tail tapering to a slightly to a rounded tip, last annulus either with two or three lobes or sometimes with a single button-shaped lobe.

Male

Body slender, slightly curved ventrad. Lip region rounded with five or six annuli, not set off. Labial framework weak. Lateral field with four lines, outer ones with occasional crenations. Excretory pore situated 53 annuli from anterior end, three annuli posterior to base of degenerate pharynx. Pharynx situated 50 annuli from front end. Hemizonid and hemizonion not seen. Stylet absent. Tail 14 annuli long, tapering to a finely rounded tip. Penial tube with a long sharp pointed posterior tip. Bursa low, straight. Spicules slender, slightly curved.

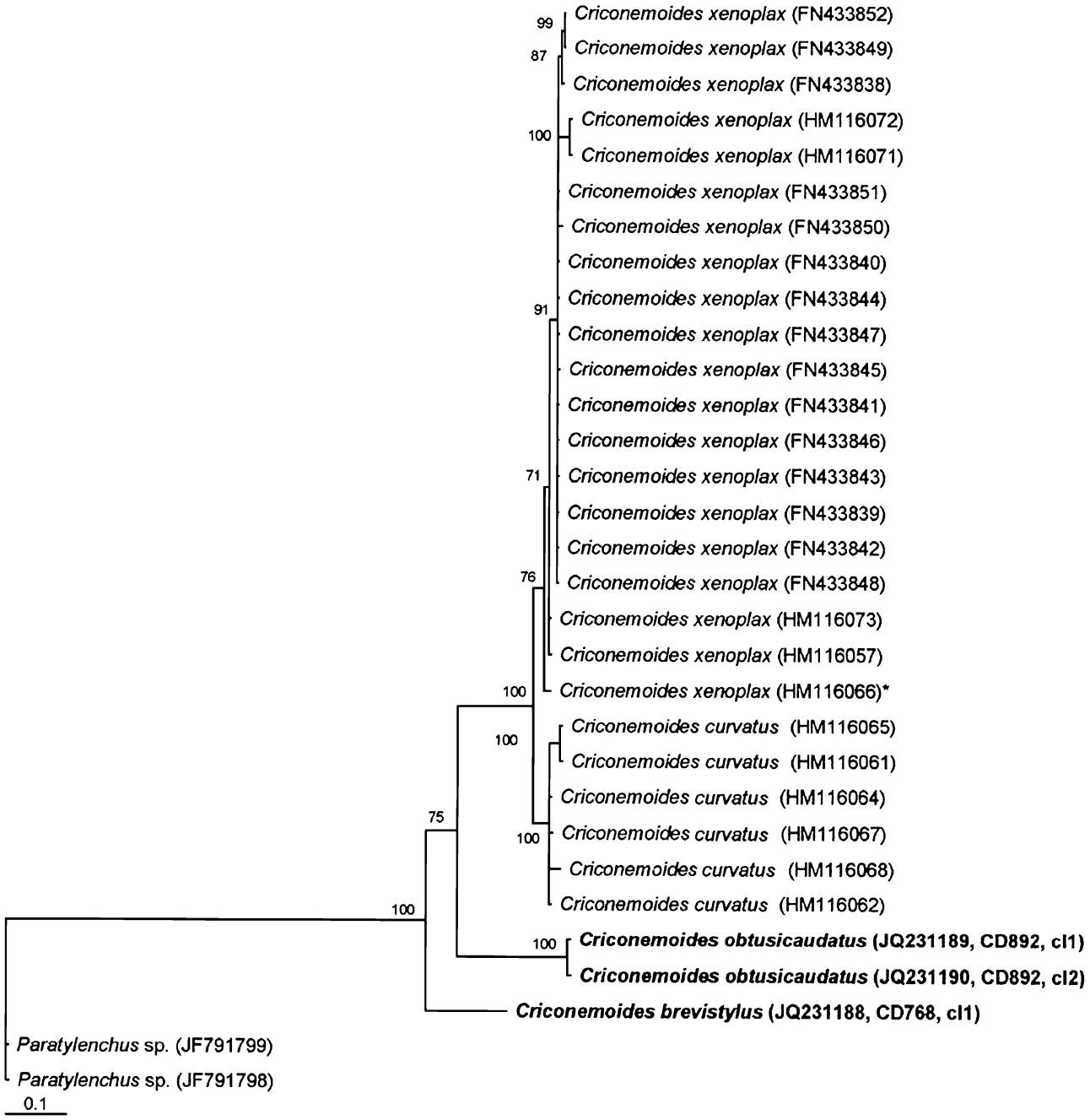

Fig. 6. *Phylogenetic relationships within representatives of* Criconemoides *as inferred from Bayesian analyses of sequences of the ITS1-5.8S rRNA-ITS2 genes using GTR + I + G model of DNA evolution. Posterior probability values more than 70% are given on appropriate clades. The newly obtained sequence is indicated in bold. * – originally identified as* Criconemoides curvatum.

Juveniles

J2 with four primordial ovarial cells. Similar to female. Annuli margins smooth, at most very finely crenate. Two lip annuli not divided into dorsal and ventral half. Stylet robust. Tail rounded with two rounded lobes on last annulus. J4 (n = 5). Similar to female. First and second lip annuli mostly well divided into dorsal and ventral half, rarely not. Four small submedian lobes present. Labial plates not very distinct. Cephalic framework moderately sclerotised. All body annuli retrorse with smooth margins. No anastomoses present. Stylet well developed with cupped basal knobs. Hemizonid seen in one specimen only, one annulus long, opposite excretory pore. Excretory pore situated from one annulus anterior to two annuli posterior to base of pharynx. Distinct primordial ovarium. Tail rounded with last annulus having 1-3 small rounded lobes.

COMMENT

The present specimens compare well with *C. obtusicaudatus* specimens described by Heyns (1962, 1970) and Van den Berg (1980). The male is the first ever found although the above authors mentioned the presence of sperm cells in spermathecae.

MOLECULAR CHARACTERISATION AND PHYLOGENETIC RELATIONSHIPS WITHIN *CRICONEMOIDES* SPECIES

Amplification of DNA from *Criconemoides* samples yielded a single fragment of *ca* 800 and 760 bp in length for the ITS rRNA gene and D2-D3 of 28S rRNA gene, respectively. A phylogenetic tree reconstructed by the BI method for the D2-D3 of 28S rRNA gene sequences is presented in Figure 5. The phylogenetic relationships within this group were generally congruent with those reconstructed by Subbotin *et al.* (2005) and Van den Berg *et al.* (2011) in the tree for the suborder Criconematina. *Criconemoides brevistylus* formed a highly supported clade (PP = 98) with *C. ornatus. Criconemoides obtusicaudatus* formed a separate clade from other *Criconemoides.* The phylogenetic tree reconstructed by the BI method for the ITS rRNA gene sequences is given in Figure 6. Only sequences from four *Criconemoides* species are presently available and these were included in this analysis. *Criconemoides xenoplax* clustered with high support (PP = 100) with *C. curvatus*, whereas the relationships between *C. obtusicaudatus* and *C. brevistylus* were not well resolved. It is necessary to include rRNA gene and other gene sequences from more *Criconemoides* species to obtain a clearer picture of the relationships between the species within this genus.

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