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Morphological and molecular characterisation of several *Paratylenchus* Micoletzky, 1922 (Tylenchida: Paratylenchidae) species from South Africa and USA, together with some taxonomic notes

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Summary – Pin nematodes of the genus *Paratylenchus* are widely distributed across the world and associated with many plant species. Morphological identification of *Paratylenchus* species is a difficult task because it relies on many characters with a wide range of intraspecific variation. In this study we provide morphological and molecular characterisation of several pin nematodes: *Paratylenchus aquaticus*, *P. dianthus*, *P. hamatus*, *P. nanus* and *P. straeleni*, collected in different states of the USA and South Africa. *Paratylenchus aquaticus* is reported from South Africa and Hawaii and *P. nanus* is found from South Africa for the first time. Morphological descriptions, morphometrics, light and scanning electron microscopic photos and drawings are given for these species. Molecular characterisation of nematodes using the D2-D3 of 28S rRNA and ITS rRNA gene sequence revealed that samples morphologically identified as *P. aquaticus*, *P. hamatus* and *P. nanus* indeed represent species complexes containing several species. Sequences of the rRNA genes are also provided for several unidentified *Paratylenchus*. Phylogenetic relationships within the genus *Paratylenchus* are given as inferred from the analyses of the D2-D3 of 28S rRNA and ITS rRNA gene sequences. We present here the most complete phylogenetic analysis of the genus.

Keywords – description, morphology, morphometrics, *Paratylenchus aquaticus*, *Paratylenchus dianthus*, *Paratylenchus hamatus*, *Paratylenchus nanus*, *Paratylenchus straeleni*, phylogeny, rRNA gene, SEM, species complex, taxonomy.

The pin nematodes of the genus *Paratylenchus* Micoletzky, 1922 are characterised by their small body size with length varying from 0.2 to 0.6 mm. They are widely distributed across the world and associated with many plant species. *Paratylenchus* contains more than 120 nominal species (Siddiqi, 2000). Morphological identification of some species of *Paratylenchus* is rather difficult and relies on many characters. Most species share very similar diagnostic characters, which are very difficult to separate. Some characters have broad, overlapping ranges and show high levels of intra-specific variability. Many environmental and other factors (such as temperature, host, population size, *etc.*) might also have an influence on the variations in characters (Fisher, 1965).

Phylogenetic and sequence analysis of rRNA and other genes provides attractive solutions to resolve some of the difficulties in species identification and understanding of relationships between species. Subbotin *et al.* (2005), Chen *et al.* (2008, 2009) and van Megen *et al.* (2009) gave molecular characterisation of *Paratylenchus* species using the D2-D3 expansion segments of 28S rRNA, ITS rRNA and 18S rRNA gene sequences, respectively. However, because only a few species were analysed in those studies,

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the intra- and interspecific variation of rRNA genes as well as the pattern of phylogenetic relationships for this nematode group remained unclear.

The main objectives of this study were to: *i*) conduct identification with a morphological and morphometric study of some *Paratylenchus* species collected in South Africa and several states of the USA; *ii*) provide molecular characterisation of several species using the D2-D3 expansion segments of 28S rRNA and ITS rRNA gene sequences; and *iii*) study phylogenetic relationships within *Paratylenchus* using two fragments of rRNA gene sequences.

Materials and methods

Nematode populations

Nematode samples used in this study were collected from various localities (Table 1). These include species morphologically identified as *P. aquaticus* Merny, 1966 from Hawaii and Kansas, USA, and South Africa, *P. dianthus* Jenkins & Taylor, 1956 female, male and juvenile specimens from South Africa, specimens of *P. hamatus* Thorne & Allen, 1950, *sensu stricto*, collected in the type locality and other places in California, and two closely related species from South Africa and several American localities, species identified morphologically as *P. nanus* Cobb, 1923 from South Africa and USA, and *P. straeleni* (De Coninck, 1931) Oostenbrink, 1960 female specimens from the USA. Sequences of several species are included in the present study and left as unidentified species because of lack of sufficient material.

Species delimitation of *Paratylenchus* 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 microscope observations

The nematodes were extracted from the soil using the rapid centrifugal-flotation method (Jenkins, 1964). The South African specimens were killed 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 and drawings were made with a research microscope (Nikon Labophot-2) equipped with a drawing tube.

For electron microscopy, 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 Phillips FEI Quanta FEG 250 scanning electron microscope at 10 kV.

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 primer sets: D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.*, 2006), TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) (Tanha Maafi *et al.*, 2003) were used for amplification of D2-D3 of 28S rRNA and ITS rRNA, respectively. PCR 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 Davis Sequencing (Davis, CA, USA). The newly obtained sequences were submitted to the GenBank database under accession numbers KF242189-KF242278 as indicated in Table 1 and Figures 16 and 17.

Sequence and phylogenetic analysis

The newly obtained sequences for each gene (D2-D3 of 28S rRNA and ITS rRNA) were aligned using ClustalX 1.83 (Thompson *et al.*, 1997) with their corresponding published gene sequences (Subbotin *et al.*, 2005, 2006; Chen *et al.*, 2008, 2009). 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

Table 1. *Paratylenchus* populations and species sequenced in the present study.

Species	Location	Host	Sample code	rRNA gene sequences		Collector/ Identifier[a]
				D2-D3 of 28S	ITS	
P. aquaticus type A	Waimanalo, HI, USA	Bromeliad (*Neoregelia* sp.)	CD619	KF242239, KF242240	KF242277, KF242278	1, 2
P. aquaticus type B	Washington Marlatt park, Manhattan, KS, USA	Grasses	CD868	KF242241	–	1, 2
P. aquaticus	Durban, South Africa	Grass (*Paspalum* sp.)	N692	–	–	2
P. dianthus	Tarlton, Gauteng, South Africa	Chrysanthemum	CD552, Tvl1976	KF242226-KF242229	KF242271, KF242272	2
P. hamatus, *s. str.*	Planada, California, USA	Fig (*Ficus carica*)	CD1155	KF242212	–	1
P. hamatus, *s. str.*	Live Oak, Sutter county, CA, USA	Peach (*Prunus* sp.)	CD17	KF242207, KF242218	KF242244, KF242253	1
P. hamatus, *s. str.*	Patterson, Stanislaus county, CA, USA	Apricot (*Prunus* sp.)	CD19	KF242205, KF242209, KF242210	KF242254, KF242258	1
P. hamatus, *s. str.*	Kingsburg, Kings county, CA, USA	Peach (*Prunus* sp.)	CD489	KF242204	KF242245, KF242250	1
P. hamatus, *s. str.*	Reedley, Tulare county, CA, USA	Plum (*Prunus* sp.)	CD442	–	KF242249	1
P. hamatus, *s. str.*	Westley, Stanislaus county, CA, USA	Peach (*Prunus* sp.)	CD480	KF242214	KF242246, KF242257	1
P. hamatus, *s. str.*	Maricopa, Kern county, CA, USA	Apricot (*Prunus* sp.)	CD454	KF242206, KF242216, KF242217	KF242247, KF242256	1
P. hamatus, *s. str.*	Maricopa, Kern county, CA, USA	Plum (*Prunus* sp.)	CD455	KF242208, KF242211	KF242248, KF242252	1
P. hamatus, *s. str.*	Wasco, Kern county, CA, USA	Rose (*Rosa* sp.)	CD315	KF242213, KF242215	KF242251, KF242255	1
P. hamatus, *s. str.*	Oak Creek, vicinity of Sedona, AZ, USA	Tree	CD1319	KF242219	–	1
P. hamatus, *s. l.*	Randfontein, Gauteng, South Africa	Peach (*Prunus* sp.)	Tvl1895	–	–	2
P. nanus type A	Niebüll, Germany	–	869	AY780946	KF242269, KF242270	3
P. nanus type A	Marin county, CA, USA	*Festuca* sp.	CD850	KF242192, KF242193	–	1
P. nanus type A	Marin county, CA, USA	Grasses	CD860	KF242191, KF242195	–	1
P. nanus type A	Marin county, CA, USA	Grasses	CD883	KF242196	–	1

Table 1. (Continued.)

Species	Location	Host	Sample code	rRNA gene sequences		Collector/ Identifier[a]
				D2-D3 of 28S	ITS	
P. nanus type A	Riverside, CA, USA	Grasses	CD728	KF242194, KF242197	KF242267, KF242268	1
P. nanus type B	George, Western Cape, South Africa	Bent grass	CD587, KP2214	KF242198, KF242200	KF242263, KF242264	2
P. nanus type B	Gridley, Butte county, CA, USA	Walnut (*Juglans* sp.)	CD137	KF242199	KF242265, KF242266	1
P. nanus type B	Roosevelt, Los Angeles county, CA, USA	Alfalfa (*Medicago sativa*)	CD186	KF242201	–	1
P. straeleni	Mendocino county, CA, USA	–	CD786	KF242235	–	1, 2
P. straeleni	Napa county, CA, USA	Tree	CD899	KF242236	–	1, 2
Paratylenchus sp. 1	Orland, Glenn county, CA, USA	Prune (*Prunus* sp.)	CD57	KF242223, KF242225	KF242261, KF242262	1
Paratylenchus sp. 1	Butte City, Glenn county, CA, USA	Prune (*Prunus* sp.)	CD61	KF242224	KF242259, KF242260	1
Paratylenchus sp. 2	Davis, Yolo county, CA, USA	Grasses under a willow	CD604	KF242220-KF242222	KF242243	1
Paratylenchus sp. 3	Goleta, Santa Barbara county, CA, USA	Lemon (*Citrus* sp.)	CD232	KF242231, KF242232	KF242273, KF242274	1
Paratylenchus sp. 3	Mulberry, AR, USA	Horsetail (*Equisetum hyemale*)	CD1017	KF242230	–	1
Paratylenchus sp. 4	OR, USA	Trees	CD986	KF242203	–	1
Paratylenchus sp. 4	Saint Paul, MN, USA	–	CD1092	KF242202	–	1
Paratylenchus sp. 5	Napa, Napa county, CA, USA	Grape (*Vitis* sp.)	CD106	KF242237, KF242238	KF242275, KF242276	1
Paratylenchus sp. 6	Madera, Madera county, CA, USA	Grasses	CD1223	KF242190	–	1
Paratylenchus sp. 6	Lodi, San Joaquin county, CA, USA	Grasses	CD1288	KF242189	–	1
Paratylenchus sp. 7	UCR Campus, Riverside, CA, USA	–	CD1004	KF242242	–	1
Paratylenchus sp. 8	Strawberry canyon, Berkeley, CA, USA	Grasses	CD1053	KF242233, KF242234	–	1

[a] 1 = S.A. Subbotin; 2 = E. Van den Berg; 3 = D. Sturhan.

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 and discussion

Morphological and molecular analysis revealed the following species and species complexes among studied samples: the *P. aquaticus* species complex with two morphologically similar species but differing in the D2-D3 of 28S rRNA gene sequences, which are named here as 'A' and 'B', *P. dianthus*, the *P. hamatus* species complex with *P. hamatus*, *sensu stricto* and two unidentified species named here as *Paratylenchus* sp. 1 and sp. 2, the *P. nanus* species complex with two morphologically similar, but not closely related species, differing in the D2-D3 of 28S rRNA and the ITS rRNA gene sequences and named here as 'A' and 'B', *P. straeleni* and six unidentified species (*Paratylenchus* spp. 3-8). For these six putative *Paratylenchus* species we do not have enough materials to make morphological and morphometric characterisation and are not able to identify these nematodes at species level.

Morphological descriptions and morphometrics of the species and species complexes with illustrations (Figs 1-15) are given here.

Paratylenchus aquaticus Merny, 1966 species complex

Type A – Sample CD619
Type B – Sample CD868 (USA)
N692 (South Africa)
(Figs 1, 2, 14M-R, 15I-L)

This species was originally described from the Ivory Coast by Merny (1966). Subsequently, it has been described from Kenya and Zaire (Geraert & Ali, 1978) whilst Pinochet & Raski (1977) found it in South Korea and Brzeski (1995) reported it from Mexico and Brazil. This species is also known from other West African countries and the USA. Three *Paratylenchus* samples, two from the USA and one from South Africa, were identified as representatives of *P. aquaticus*. These populations were studied and compared morphologically with each other. We distinguished two genotypes, A and B, from the USA based on molecular differences within this morphospecies. Because these populations were very similar morphologically, except for tail shapes, we give only one description below. These populations slightly differ in tail length, vulva-anus distance and post-uterine branch (PUB) length (Table 2). SEM photos were done on the Durban specimens, South Africa and the specimens from CD868, USA, while molecular characterisation was done on the American specimens only.

Measurements

See Table 2.

Composite description

Female

Heat-relaxed body posture ranging from slightly curved ventrad, an open letter C to a Figure 6. Lip region flattened, not set off, not narrowing much towards tip with small to distinct submedian lobes with three indistinct lip annuli. *En face* view not clear but showing a squarish labial area. Labial framework moderate, stoma walls slightly sclerotised. Stylet small and slender. Stylet knobs sloping anteriorly with outer tips slightly curved upward, rounded posteriorly. Nerve ring encircling isthmus from opposite middle to opposite posterior part of isthmus. Excretory pore situated from opposite posterior part of isthmus to opposite basal margin of basal pharyngeal lobe. Hemizonid 2-3 annuli long situated from just posterior to just anterior to excretory pore. Hemizonion seen in only a few Durban specimens, situated from 12-15 annuli posterior to hemizonid. Lateral field with two distinct outer lines and a faint to more distinct inner line. Two outer bands may be slightly areolated in tail region. Distinct deirid seen from just anterior to five annuli posterior to level of excretory pore. Body only very slightly narrower posterior to vulva in slender specimens narrowing more in slightly more obese specimens. Vulval lips not prominent. Lateral vulval flaps distinct, rounded or slightly flattened, slightly crenate. Spermatheca distinct, ranging from small, rounded to large and oblong, mostly filled with rounded sperm cells while in CD868 specimens all spermathecae were empty except for one individual with a few sperm cells. In all Durban specimens and CD868 a post uterine branch was present along ventral body wall, mostly with a few vestigial ovarial cells

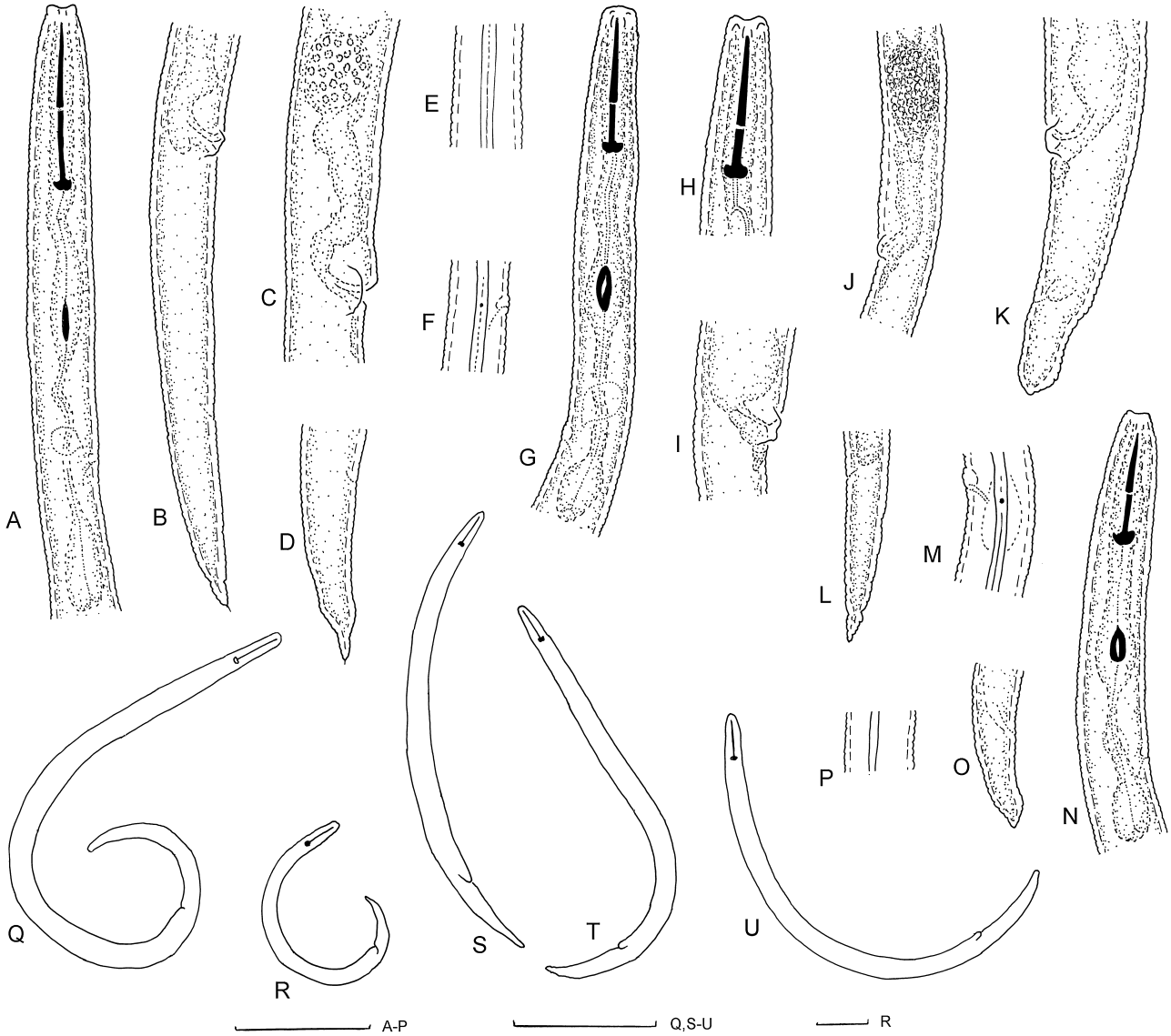

Fig. 1. *Paratylenchus aquaticus* type A, Hawaii population CD619, Female. A: Anterior region; B: Posterior region; C: Vulval area; D: Tail; E: Lateral field at mid-body; F: Excretory pore and deirid. South African population N692, Female. G: Anterior region; H: Another lip region; I, J: Vulval areas; K: Posterior region of female with aberrant tail; L: Tail; M: Excretory pore and deirid. Juvenile J2. N: Anterior region; O: Tail; P: Lateral field at mid-body. Q-U: Female habitus of both localities. (Scale bars: A-P = 20 μm; Q, S-U = 50 μm; R = 50 μm.)

at tip but not so distinct in Hawaiian specimens. Phasmids not observed. Tail with 20-30 annuli, tapering gradually to a finely rounded tip, crenate, digitate with some Hawaiian specimens displaying a small mucro on tip. One Durban female displaying a shortened, broadly rounded, aberrant tail.

Male

Not found.

Juvenile

One second-stage juvenile found in the Durban population. Very similar to female. Stylet present. Tail tip more

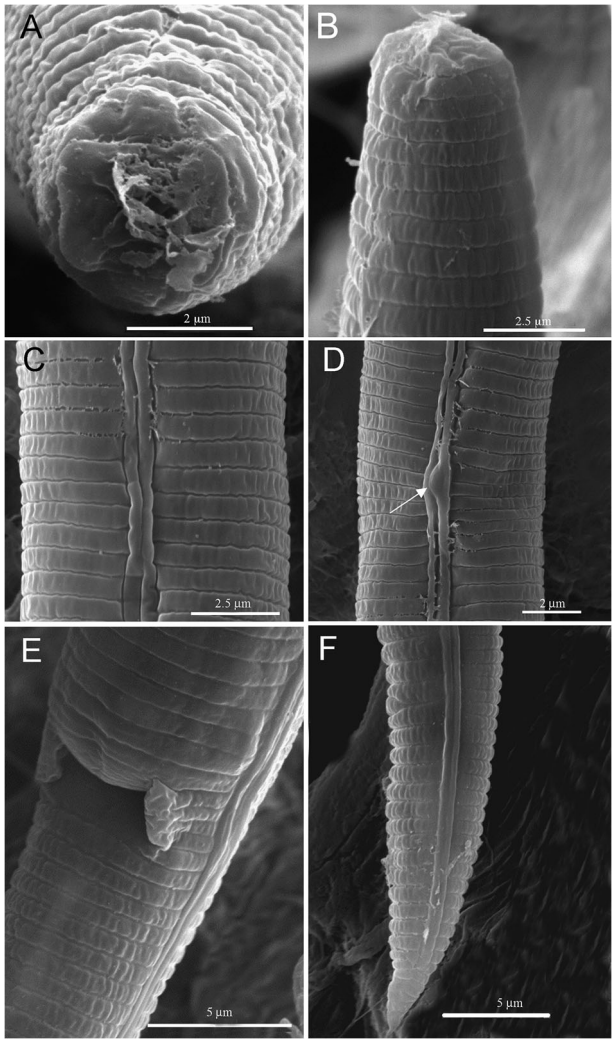

Fig. 2. *Paratylenchus aquaticus*, South African population N692, Female. A: Lip region, *en face* view; B: Lip region, lateral view; C: Lateral field at mid-body; D: Lateral field with deirid (arrow); E: Vulval area; F: Tail.

broadly rounded. Lateral field a narrow band with two distinct lines, no inner line seen but may be very faint.

Note

The specimens from three studied samples fit the various descriptions of the species (Merny, 1966; Pinochet & Raski, 1977; Geraert & Ali, 1978; Brzeski, 1995, 1998) very well. The present specimens are compared in Table 2 with the other species in the genus that have three lines in the lateral field and a stylet of more or less the same length.

Geraert & Ali (1978), Raski & Luc (1987), Ebsary (1991) and Siddiqi (2000) regarded *P. humilis* Raski, 1975a as a synonym of *P. aquaticus* but Raski (1975a, 1991), Esser (1992) and Brzeski (1995, 1998) regarded it as a valid species. With the present larger range of morphometrics for *P. aquaticus*, we regard *P. humilis* as a valid species. Females of *P. humilis* are shorter than *P. aquaticus* (170-210 *vs* 256-409 μm); the distance of the excretory pore from the anterior end is much shorter (43-53 *vs* 58-80 μm). Brzeski (1995) stated that the c′ value of the male needs more study and he noted that the male tail of *P. humilis* is longer than that of *P. aquaticus* ($c' = 1$ *vs* 2.6-3.4); a bursa is present *vs* absent in the illustration of Raski (1975a). The male tail of *P. humilis* appears much shorter in the original description of Raski (1975a) than the illustration of the tail of *P. aquaticus* of Merny (1966). Males of *P. aquaticus* are rare compared with *P. humilis* where 14 males were described in the original description. In the original description of *P. aquaticus*, the male spicule length is given as 21-22 *vs* either 12-14 μm for *P. humilis* (Raski, 1975a) or 16-18 μm (Brzeski, 1995).

Pinochet & Raski (1977) described *P. pandus* from soybean from Korea and separated it from *P. aquaticus* by having a shorter stylet (15-20 *vs* 15-23 μm) and a smaller V value (76-79 *vs* 79-85), also stating that it is most closely related to *P. aquaticus*. With the present range of characters extended by further collections of *P. aquaticus* we regard *P. pandus* as a possible synonym of *P. aquaticus*.

Paratylenchus triincisus Bajaj, 1987, described from Haryana, India, appears very similar to *P. aquaticus*. The only difference is the female having a smaller stylet (16 *vs* 15-23 μm), shorter male spicules (16-18 *vs* 21-22 μm) and longer male tail, 36.9 (calculated from the original illustration) *vs* 21-23 μm for *P. aquaticus* (Merny, 1966). *Paratylenchus triincisus* was, strangely enough, not compared with any of the other species with three lines in the lateral field by Bajaj (1987), but only with *P. nawadus* which has four lines in the lateral field. The morphometrics of *P. aquaticus* and *P. triincisus* corresponds very well. We therefore regard *P. triincisus* as a possible synonym of *P. aquaticus*.

Similarly, *P. variatus* Jairajpuri, 1982, described from sugarcane in Nigeria, is very close to *P. aquaticus*. The illustrations of the female tail displays a little more variation than described for *P. aquaticus* but this could be due to geographic variation. We regard *P. variatus* as a possible synonym of *P. aquaticus*. Although appearing

Table 2. Morphometrics of females of *Paratylenchus aquaticus* from the USA, Hawaii and South Africa compared with those from the literature. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*P. aquaticus* type A Hawaii, USA (CD619)	*P. aquaticus* type B Kansas, USA (CD868)	*P. aquaticus* unknown type Durban, South Africa (N692)	*P. aquaticus* from literature*
n	5	7	11	61
L	366 ± 27.1 (342-409)	301 ± 36.5 (272-378)	288 ± 22.4 (256-331)	270-390
a	30.6 ± 3.8 (24.8-34.8)	20.9 ± 2.5 (18.5-25.7)	23.5 ± 4 (16.2-30.2)	26-39
b	4.2 ± 0.2 (4.0-4.4)	4.2 ± 0.5 (3.6-4.8)	3.9 ± 0.4 (3.5-4.7)	3.5-4.8
c	13.4 ± 0.7 (12.3-14.1)	12.8 ± 0.9 (11.3-14.3)	14.8 ± 2.6 (11.6-19.7)	13.2-18
c′	3.6 ± 0.4 (3.1-4.1)	2.8 ± 0.3 (2.4-3.1)	2.9 ± 0.7 (1.7-4.0)	2.6-3.7
o	–	19.7 ± 2.1 (17.6-23.3)	24 ± 5.4 (15.1-33.3)	–
DGO	–	3.5 ± 0.3 (3.0-3.5)	5 ± 1 (2.5-6.0)	4-8
V	81 ± 1.2 (79-82)	80.5 ± 1.1 (79.5-82.5)	81 ± 2 (79-85)	79-84
OV	30 ± 9.2 (19-39)	34.5 ± 9.1 (23.5-43.5)	38 ± 12.9 (24-57.5)	–
Stylet length	21 ± 1 (20-23)	17 ± 1 (16-18.5)	19.5 ± 1.4 (18-21.5)	14.5-20
Metenchium length	13 ± 0.8 (12-14)	10 ± 0.7 (9-11)	12 ± 1.3 (10-14)	–
Telenchium length	8 ± 0.4 (8-9)	7 ± 0.8 (6-8)	7.5 ± 0.6 (6-8)	–
m	–	60.6 ± 3.8 (53.2-64.7)	62.8 ± 3 (58.2-67.9)	–
Stylet knob height	2 ± 0.4 (1.5-2.0)	2 ± 0.3 (1.5-2.0)	1.75 ± 0.3 (1.5-2)	–
Stylet knob width	3	3 ± 0.5 (3.0-3.5)	3.5 ± 0.3 (3-4)	–
Pharynx length	90 ± 1.9 (89-92)	71 ± 4.4 (66-78)	65.5 ± 6.2 (59-77)	–
Excretory pore from ant. end	68 ± 3.1 (63.5-72)	62.5 ± 4.7 (58-72)	73 ± 4.6 (67-80)	64-81
Diam. at mid-body	12 ± 1.1 (11-14)	14.5 ± 2.2 (11-17)	12.5 ± 2.5 (8.5-17)	–
Lip region diam.	5.5 ± 0.5 (5-6)	9 ± 1.5 (7.5-12)	7 ± 0.5 (6.5-7.5)	–
Lip region height	2.5 ± 0.4 (2-3)	6 ± 0.6 (5-7)	3.5 ± 0.3 (3-4)	–
Annulus width	0.8 ± 0.1 (0.7-1.0)	1 ± 0.2 (0.7-1.0)	1.0 ± 0.2 (1.0-1.5)	0.7-1
Lateral field width	1.8 ± 0.3 (1.5-2.0)	2.5 ± 0.2 (2-2.5)	2 (n = 1)	1.5
Tail length	27 ± 2.2 (25.5-31)	23.5 ± 1.9 (22-26.5)	21 ± 3.7 (14-25)	20-24
EP%L	18.6 ± 0.8 (17.6-19.9)	20.9 ± 1.2 (19.1-22.2)	22 ± 0.9 (21-23)	18.5-23.5
V-anus distance	42 ± 6 (35-51)	30.5 ± 11.3 (29.5-35.5)	35.5 ± 2.9 (30-40)	–
Spermatheca length	13 ± 2 (12-14.5)	10 ± 1.6 (8-12)	11 ± 2.5 (8-15)	–
Spermatheca diam.	6.5 ± 2.0 (5-8)	7 ± 1.3 (5.5-8.5)	7.5 ± 1.5 (6-10)	–
PUB length	2.5 ± 0.5 (2-3)	7.5 ± 1.8 (4.5-10)	8 ± 1.9 (5.5-11)	–

* Literature data are from Merny (1966); Pinochet & Raski (1977); Geraert & Ali (1978); Brzeski (1995, 1998).

similar, *P. leptos* Raski, 1975a, *P. perminimus* Siddiqi, 1996 and *P. rostrocaudatus* Huang & Raski, 1987 females are all much smaller with tail tips more broadly rounded, digitate or beak-like.

The two genotypes, A and B, from the USA might represent two separate *Paratylenchus* species. Morphological and molecular characterisation of *P. aquaticus* from the type locality will provide additional data, which may help to make a revision of this species complex.

Paratylenchus dianthus **Jenkins & Taylor, 1956**

Sample Tvl1976, CD552 (South Africa)
(Figs 3, 4)

The species was originally described from cultivated carnations (*Dianthus caryophilus*) from Maryland by Jenkins & Taylor (1956). Raski (1975b) reported additional specimens from various states in the USA, as well as two from Australia. Van den Berg (1977) described the species from chicory from the Western Cape, South Africa, and it was also reported from Poland (Brzeski & Maciejczyk, 1977), India (Baqri, 1978) and Vietnam (Nguyen *et al.*, 2004).

Measurements

See Table 3.

Description

Female

Heat-relaxed body ranging from almost straight to a Figure 6. Lip region not set off, slightly sloping to a flattened or slightly rounded anterior end with four faint annuli. *En face* view of lip region showing a rectangular labial area with distinct amphid openings with four raised ridges around mouth opening. Labial disc not raised above labial area. Labial framework moderate. Stoma walls slightly sclerotised. Stylet well developed but slender. Stylet knobs ranging from hollow, slightly flattened to slightly sloping anteriorly and rounded posteriorly. Nerve ring encircling isthmus from opposite anterior part to opposite posterior part of isthmus. Hemizonid three annuli long, situated opposite to directly anterior to excretory pore. Hemizonion seen in three specimens only, one annulus long, situated 14-16 annuli posterior to excretory pore. Deirid distinct, situated from four annuli anterior to four annuli posterior to excretory pore. Lateral field four lines, outer ones rarely crenate and outer bands sometimes areolated in posterior half. Body mostly not constricted posterior to vulva in slender specimens but more so in obese specimens. Vulval lips not prominent. Lateral vulval flaps distinct, rounded, sometimes annulated. Small uterine branch present along ventral body wall, sometimes with a few vestigial ovarial cells. Spermatheca small, varying from round, oval to oblong, filled with rounded sperm cells. Phasmids not observed. Tail varying from rarely straight to well curved ventrad with 23-29 annuli. Tail tip ranging from a narrowly rounded tip to a sharply rounded tip, sometimes digitate.

Male

Heat-relaxed body ranging from almost straight to slightly curved ventrad and sometimes straight with only last third curved ventrad. Lip region tapering slightly to rounded or flattened anteriorly, not set off, with four annuli. *En face* view of lip region not distinct but similar to that of female. Stylet absent. Pharynx degenerate ending near excretory pore. Hemizonid distinct, three annuli long, situated opposite or directly anterior to excretory pore. Hemizonion not seen. Deirid distinct, situated from two annuli anterior to one annulus posterior to excretory pore. Lateral field with four lines. Spicules distinct, slightly curved ventrad. Velum seen faintly when spicules protruded. Spicular pouch prominent with longer posterior lip. Bursa absent. Tail with 23-28 annuli, slightly curved ventrad, tapering to a narrowly rounded tip, sometimes slightly digitate. Phasmids not observed.

Juvenile

Very similar to female with less developed stylet with stylet knobs sloping anteriorly, deirid seen in a few specimens, situated 1-2 annuli posterior to excretory pore. Tail tapering to a narrowly rounded tip, annuli not very distinct.

Note

When following the Group III key of Geraert (1965), the keys of Wouts (1966) and Raski (1975b, 1991) and the compendium of Esser (1992) and Brzeski (1998), the present population comes close to the following species: *P. arculatus* Luc & De Guiran, 1962; *P. ciccaronei* Raski, 1975b; *P. curvitatus* Van der Linde, 1938; *P. dianthus* Jenkins & Taylor, 1956; *P. halophilus* Wouts, 1966; *P. hamatus* Thorne & Allen, 1950; *P. lepidus* Raski, 1975b; *P. mexicanus* Raski, 1975b; *P. nanus* Cobb, 1923; *P. neoamblycephalus* Geraert, 1965; *P. projectus*

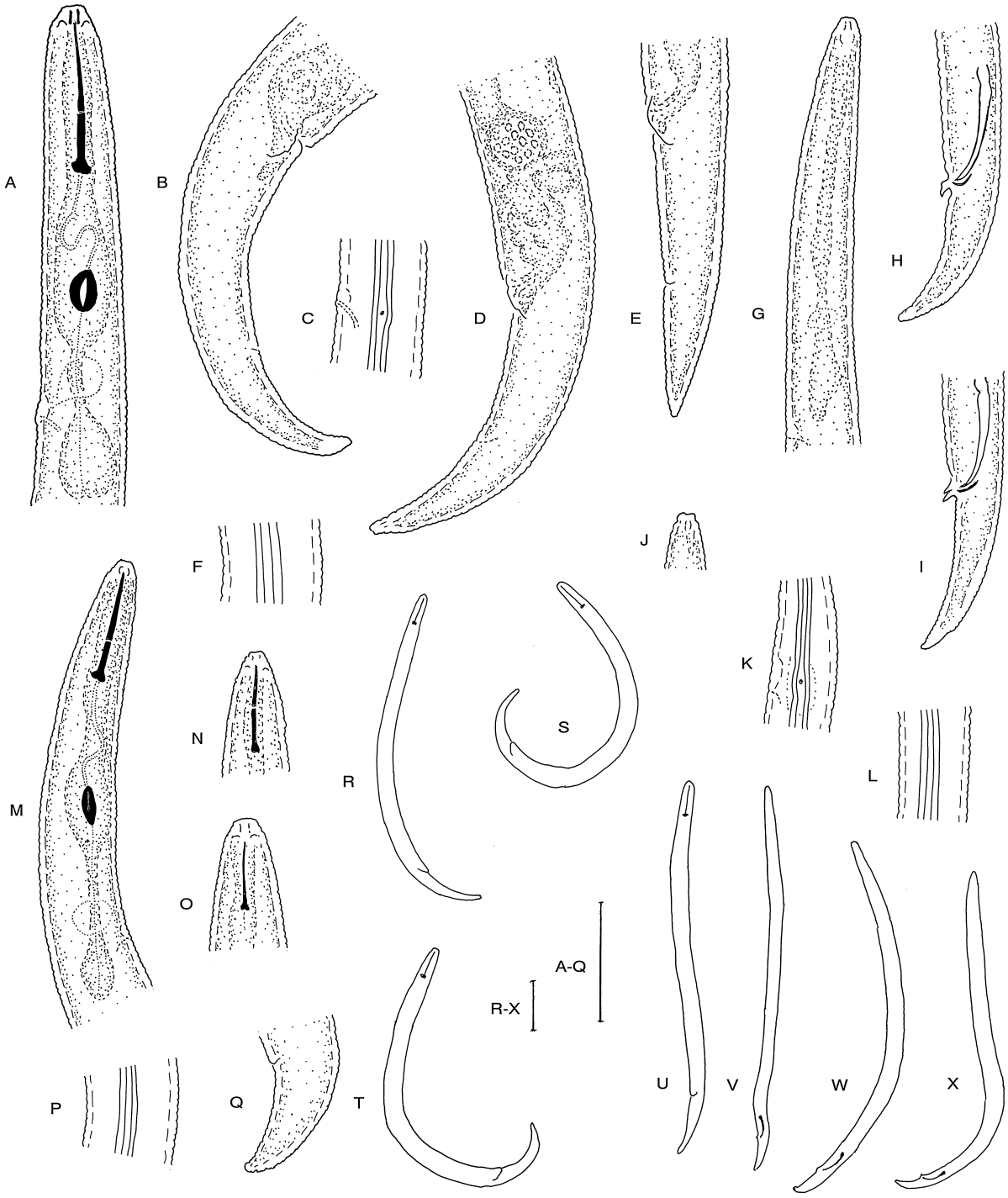

Fig. 3. *Paratylenchus dianthus*, South African population Tvl1976, Female. A: Anterior region; B, D: Posterior regions; C: Excretory pore and deirid; E: Tail region; F: Lateral field at mid-body. Male. G: Anterior region; H, I: Tail regions; J: Lip region of another male; K: Excretory pore and deirid; L: Lateral field at mid-body. J4. M: Anterior region; N, O: Lip regions of two other juveniles showing variation in stylet length; P: Lateral field at mid-body; Q: Tail; R-U: Female habitus; V-X: Male habitus. (Scale bars: A-Q = 20 μm; R-X = 50 μm.)

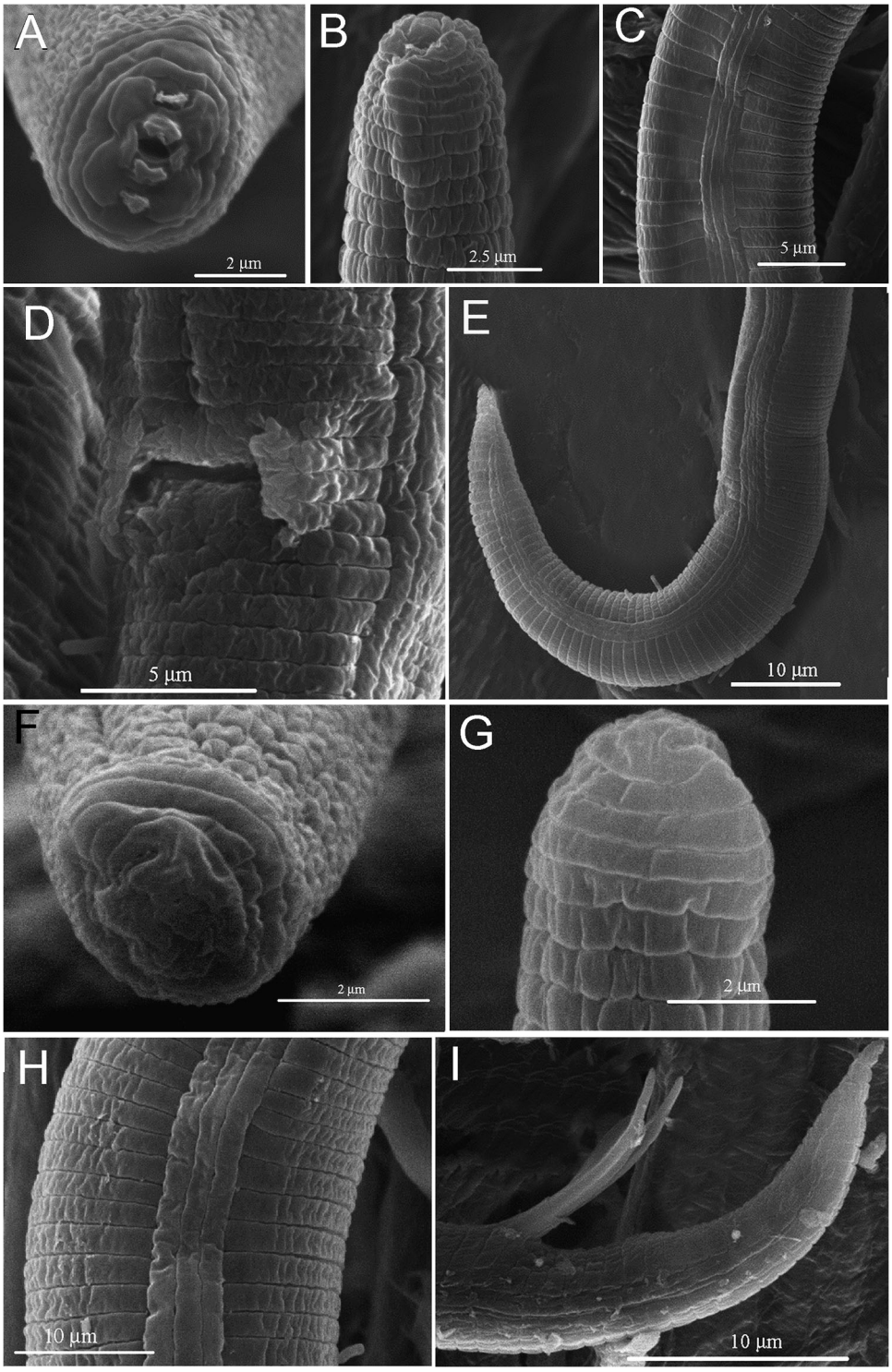

Fig. 4. *Paratylenchus dianthus*, South African population Tvl1976. Female. A: *En face* view of lip region; B: Lip region, lateral view; C: Lateral field at mid-body; D: Vulval area; E: Posterior region. Male. F: *En face* view of lip region; G: Lip region, lateral view; H: Lateral field at mid-body; I: Tail.

Table 3. *Paratylenchus dianthus* from South Africa compared with closely related species. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*P. dianthus* (South Africa, Tvl1976)		*P. dianthus*[a]		*P. nanus*[b]		*P. projectus*[c]		*P. neoamblycephalus*[d]
	Female	Male	Female	Male	Female	Male	Female	Male[e]	Female[e,f]
n	26	8	–	–	–	–	–	–	–
L	326 ± 21 (294-400)	346 ± 13.5 (326-364)	290-440	290-480	270-460	310-390	234-480	306-435	317.7-480
a	21.3 ± 1.8 (17.6-23.8)	27.6 ± 1.9 (25.4-30)	18-26	23.7 (21-29.3)	16.8-28	18-28	14-28	26.1-35.4	26-32; 10.2-13?[g]
b	3.9 ± 0.2 (3.4-4.6)	4.7 ± 0.4 (4-5)	3.5-5	3.1-4.9	3.1-4.6	4.3	3-5	3.7-4.7	3.7-4.5
c	13.1 ± 1.1 (10.8-15.2)	11.3 ± 0.5 (10.5-11.8)	10.2-19	11.7-15.4	9.6-24.4	10-15	11-24	10.9-16.5	11.8-17.1
c′	2.7 ± 0.3 (2.1-3.1)	3.6 ± 0.4 (3.1-3.9)	2.3-3	3	1.8-4.4	2.2	2-3.6	–	–
o	24.1 ± 2.2 (19.8-28.8)	–	–	–	19.8	–	12-23	–	–
DGO	6.5 ± 0.7 (5-8)	–	4.6	–	4-8	–	4-8	–	–
V	82.5 ± 0.8 (81-84.5)	–	80-88	–	79-88	–	80-88	–	76-84.3
OV	39 ± 7 (30-54)	–	–	–	–	–	–	–	–
Stylet length	27.5 ± 1.3 (25-30)	–	25.1 (18-29.5)	–	23-36	–	24-32	–	23-33.7
Metenchium	17.5 ± 1 (15.5-20)	–	–	–	15-19.6	–	15-22	–	–
Telenchium	10 ± 0.6 (8.5-11)	–	–	–	11.5	–	–	–	–
m	64.1 ± 1.7 (61.3-68.4)	–	–	–	57.3-71	–	–	–	–
Stylet knob height	2 ± 0.4 (1.5-2.5)	–	–	–	–	–	–	–	–
Stylet knob diam.	4 ± 0.3 (3-4.5)	–	66.5 (64.5-70.3)	–	3-6.4	–	3-4	–	–
Pharynx length	83 ± 5.5 (73.5-104.5)	74.5 ± 7.6 (66-84)	–	–	82-100.9	–	68-92	–	85.7-102.6
Excretory pore from ant. end	72 ± (65.5-87.5)	73 ± 2.4 (70-76)	79 (72-89)	–	65-103	57-76	56-89	–	63.6-89.6
Diam. at mid-body	15.5 ± 1.7 (13-20.5)	12.5 ± 0.5 (12-13)	–	–	12-22.5	–	12-20	12.1-13.0	–
Lip region diam.	6.5 ± 0.5 (6-7.5)	5 ± 0.3 (4.5-5.5)	–	–	6-7	–	4-6	–	–
Lip region height	3.25 ± 0.4 (3-4)	3 ± 0.5 (2.5-4.0)	–	–	3-3.5	–	3-4	–	–

Table 3. (Continued.)

Character	*P. dianthus* (South Africa, Tvl1976)		*P. dianthus*[a]		*P. nanus*[b]		*P. projectus*[c]		*P. neoamblycephalus*[d]
	Female	Male	Female	Male	Female	Male	Female	Male[e]	Female[e,f]
Annulus width	1.5 ± 0.2 (1.5-2)	1.5 ± 0.2 (1.5-1.8)	1.4-2	–	1.3-1.6	–	0.8-1.5	–	1.5
Lateral field width	3.5 ± 0.4 (2.5-4)	3 ± 0.5 (2.5-4.0)	3.5-3.8	–	–	–	2-3	–	2.5
Tail length	25 ± 1.9 (20-28.5)	30.5 ± 2.1 (27.5-34.5)	–	–	19-29	–	12-25	–	22.7-25.9
EP%L	22 ± 1.4 (20-26.5)	21.2 ± 0.7 (20.3-22.5)	–	–	20.1-24.7	–	19.1-25	–	–
V-anus distance	31 ± 3.5 (25.5-44)	–	–	–	36	–	–	–	–
St%L	8.4 ± 0.5 (7-9.3)	–	–	–	–	–	–	–	–
PUB length	7 ± 1.8 (4-10)	11.7	4-10	–	–	–	–	11.7	–
Spermatheca length	11 ± 2.5 (8-15)	16	15	–	–	–	–	16	–
Spermatheca diam.	10 ± 1.1 (8-12.5)	15	11	–	–	–	–	15	–
Spiculum length	–	22.5 ± 1.4 (20-23.5)	–	22.8 (18-28.5)	–	20-24	–	21-23	–
Gubernaculum length	–	5.5 ± 0.6 (5.0-6.5)	–	4.1	–	4	–	4	–
Penial tube length	–	3	–	–	–	–	–	–	–
Tail annuli	25 ± 1.6 (25-29)	26 ± 1.5 (23-28)	–	–	–	–	–	–	–

[a] Tarjan (1960); Raski (1975b); Brzeski & Maciejczyk (1977); Baqri (1978); Nguyen *et al.* (2004).
[b] Tarjan (1960); Thorne & Smolik (1971); Raski (1975b); Brzeski & Maciejczyk (1977); Brzeski (1995); Talavera *et al.* (1997); Ciobanu *et al.* (2003); Ryss *et al.* (2005).
[c] Reuver (1959); Loof (1975); Brzeski (1995); Van den Berg & Quénéhervé (1999).
[d] Geraert (1965); Raski (1975b); Brzeski & Maciejczyk (1977); Larizza & Lamberti (1995).
[e] Males seem to be scarce.
[f] Tail tip more sharply pointed. Lip region more rounded according to Larizza & Lamberti (1995).
[g] Larizza & Lamberti (1995).

Jenkins, 1956; *P. rotundicephalus* Bajaj, 1978; and *P. tenuicaudatus* Wu, 1961.

Paratylenchus arculatus differs in having smaller females, 180-250 μm long, with large submedian lobes; males have stylets, spicules 11.5-14 μm long and gubernaculum 2.5-2.6 μm long.

Paratylenchus ciccaronei differs in female having a long, slender digitate tail with almost acute terminus and tail length = 34 (27-49) μm. Males have filiform tails with an acute terminus, length = 45 (39-48) μm with c value = 8.5 (7.8-8.8).

Paratylenchus curvitatus – species inquirenda according to Raski (1975b), and Brzeski (1998) did not regard this species as valid. *Paratylenchus dianthus* was regarded a synonym of *P. curvitatus* by Tarjan (1960).

Paratylenchus dianthus – very close, see Table 3.

Paratylenchus halophilus – female vulva more anterior at 76-80%, vulval flaps inconspicuous, DGO shorter = 3.3 μm and fewer (15-20) tail annuli. Males are more slender, 267-310 μm long, excretory pore more anterior at 64 μm, and have a shorter tail of 11 μm with 20 annuli.

Paratylenchus hamatus differs mainly in male having a stylet and female does not appear to narrow markedly posterior to vulva and lateral field narrower at 2.9 (1.8-3.7) μm. No information could be found in the literature on juvenile stylets.

Paratylenchus lepidus – female with indistinct annuli and a longer (35 (29-47) μm) tail (Larizza & Lamberti, 1995), although Raski (1975b) and Bajaj (1987) gave ratio c as 11-16. Males have not been found, but Raski (1975b) in the original description observed sperm cells in the spermatheca. Brzeski (1998) gave the spicule length as 16-17 μm.

Paratylenchus mexicanus – Male and female have a bluntly rounded lip region, tail bluntly rounded in female and bluntly conoid in male. Male tail short, c value = 15 and vulva slightly more posterior with V = 85 (83-87).

Paratylenchus nanus – very close. See Table 3.

Paratylenchus nainianus – synonym of *P. arculatus* (Brzeski *et al.*, 1999).

Paratylenchus neoamblycephalus – only difference seems a value = 26-32 given by Geraert (1965) and Brzeski & Maciejczyk (1977), but Larizza & Lamberti (1995) gives it as 10-13 although their specimens seem to be very obese and the lip region form is also completely different. See Table 3.

Paratylenchus projectus – very close, see Table 3.

Paratylenchus rotundicephalus – male spicules shorter at 17.4 (16-19) μm and female with a straight body form and a larger ratio a of 23-27.

Paratylenchus tenuicaudatus – female mean lengths larger (Wu, 1961; Raski, 1975b), pharyngeal lobe longer (97-124 μm), tail longer (37-58 μm), c′ larger (3.5-5), V-anus longer (40-66 μm) and vulva more anterior at 78-86%. Raski (1975b) reported various males but they are also longer (340-450 μm) with a bluntly rounded head.

Morphometrics and morphology of the Tarlton specimens are compared with *P. dianthus*, *P. nanus*, *P. neoamblycephalus* and *P. projectus* in Table 3. Discussion of the relationships with these species follows:

The Tarlton *Paratylenchus* seems to fit *P. dianthus* the closest morphologically except for the female pharynx which is slightly longer in the South African specimens at 83 (73.5-104.5) *vs* 66.5-70.3 μm.

The female morphometrics of *P. nanus* are very similar to the Tarlton specimens. Only anterior sloping stylet knobs have been reported for *P. nanus*. Very few males have been reported for *P. nanus* and the spermathecae are mostly empty or with only a few sperm cells compared with males numerous and spermathecae all filled in *P. dianthus*. The tail tip of *P. nanus* seems to differ slightly. In *P. dianthus*, the tail tip is very variable and the following forms have been reported: tapering to a rather blunt to sub-acute tip; dorsally indented near tip; pointed, conoid tail, finely rounded or with an acute tip compared with tail tapering gradually to rounded or finely rounded tip. From *P. neoamblycephalus* females, the South African *P. dianthus* females differ in being slightly shorter (294-400 *vs* 318-480 μm); V slightly more posterior (81-84.5 *vs* 76-84.3); and oral aperture on lip not protruding *vs* protruding. Larizza & Lamberti (1995) describe the lip region as more rounded than conoid and they refer to SEM photos by Raski (1975b) which also show the outline to be more rounded in outline; the tail of *P. neoamblycephalus* is more variable in shape and sometimes well digitate. Males of *P. neoamblycephalus* very scarce – measurements not given.

The South African *P. dianthus* differs from *P. projectus* in o value being slightly larger (19.8-28.8 *vs* 12-23 μm); lip region slightly broader (6-7.5 *vs* 4-6 μm); lateral field slightly wider (2.5-4 *vs* 2-3 μm); tail longer (20-28.5 *vs* 12-25 μm); male gubernaculum longer (5-6.5 *vs* 4 μm); lip annuli distinct *vs* indistinct; lip region sometimes described with distinct labial plates *vs* not seen; excretory pore situated from opposite middle of isthmus to base of pharyngeal bulb *vs* opposite isthmus in illustrations;

annuli distinct over whole body *vs* fine striae; males numerous and spermatheca always filled *vs* very rare and spermatheca empty or occasionally with one or two sperm cells; tail tip more constantly rounded or finely rounded *vs* round, smooth, digitate, sub-acute, dorsally flattened and slightly depressed dorsally.

Paratylenchus hamatus Thorne & Allen, 1950 species complex

We distinguish within this species complex the following species: *P. hamatus sensu stricto* (samples CD17, CD315, CD1155 – the type locality, CA, USA) and two putative species: *Paratylenchus* sp. 1 (CD57, CD61 from California, USA), *Paratylenchus* sp. 2 (CD604, California, USA). The two putative species distinguished using molecular criteria have very similar morphology and did not differ from each other and from *P. hamatus sensu stricto*. Additional morphological and molecular studies are required to confirm specific status for these samples. The South African sample (Tvl1895) belongs to this complex. This sample was not molecularly characterised and considered here as *P. hamatus*, *sensu lato*. The illustrations for the samples are given in Figures 5, 6, 7, 8M-V, 14A-C, E, F, K, L and 15A-D.

Paratylenchus hamatus was originally described from fig orchards near Planada, CA, USA, and was later found from vegetable crops, ornamentals and many different fruit trees, grape vine and oak trees in the USA. It is known to have a worldwide distribution and has been reported from countries such as Australia, Belgium, Canada, Cyprus, Estonia, Pakistan, Slovakia, The Netherlands, the Ukraine, *etc*. The first author found it previously from grass under a willow tree in the Golden Gate Highlands National Park in South Africa. The last author collected these nematodes from the type locality for the present study. Females and males of the South African and American populations and putative species of this complex were studied and compared morphologically and LM photos were taken of specimens from the type population. SEM photos were taken from *P. hamatus sensu stricto* specimens collected from the type locality (CD1155) and *Paratylenchus* sp. 2 (CD604). Molecular studies were done on several American samples only.

Because the species of the *Paratylenchus hamatus* complex are very similar morphologically and morphometrically only one description is given below.

Measurements

See Tables 4, 5.

Composite description of American and South African populations

Female

Heat-relaxed body posture ranging from slightly curved ventrad and a Figure 6 to a letter C. Lip region not set off, slightly sloping, flattened anteriorly with small submedian lobes and 4-5 faint lip annuli. *En face* views of lip region showing a rectangular labial area with four submedian lobes and distinct amphid openings and four ridges around mouth opening. Labial disc not markedly raised above labial area. Labial framework moderate, stoma walls slightly sclerotised. Stylet well developed, slender. Stylet knobs ranging from more flattened to slightly sloping with upturned tips anteriorly and rounded posteriorly. Nerve ring encircling isthmus from opposite middle to opposite posterior part of isthmus. Excretory pore situated from opposite middle of isthmus to opposite basal margin of basal pharyngeal lobe. Hemizonid 2-4 annuli long, situated from directly anterior to just posterior to excretory pore. Hemizonion not distinctly seen in American specimens but 1-2 annuli long and situated from 12-24 annuli posterior to hemizonid in South African specimens. Deirid distinct, situated from opposite, to three annuli anterior to two annuli posterior, to level of excretory pore. Lateral field four lines, rarely outer lines slightly crenate and outer bands sometimes areolated in posterior end. Body not constricted posterior to vulva in slender specimens but slightly more so in more obese specimens. Vulval lips not prominent. Lateral vulval flaps distinct, rarely rounded, more flattened with one or two crenations. Small post-vulval sac present along ventral body wall with a few vestigial ovarial cells at tip. Spermatheca varying from round to oblong, small to large, mostly filled with sperm cells. Phasmids not observed. Tail varying from rarely straight to slightly to more prominently curved ventrad with 18-30 annuli. Tail tip ranging from narrowly to slightly more broadly rounded.

Male

Body posture ranging from only very slightly curved ventrad to a letter C. Lip region tapering slightly, flattened anteriorly, mostly not set off with very faint annuli, probably four. *En face* view of lip region similar to that of female. Labial framework weak. Stylet small with

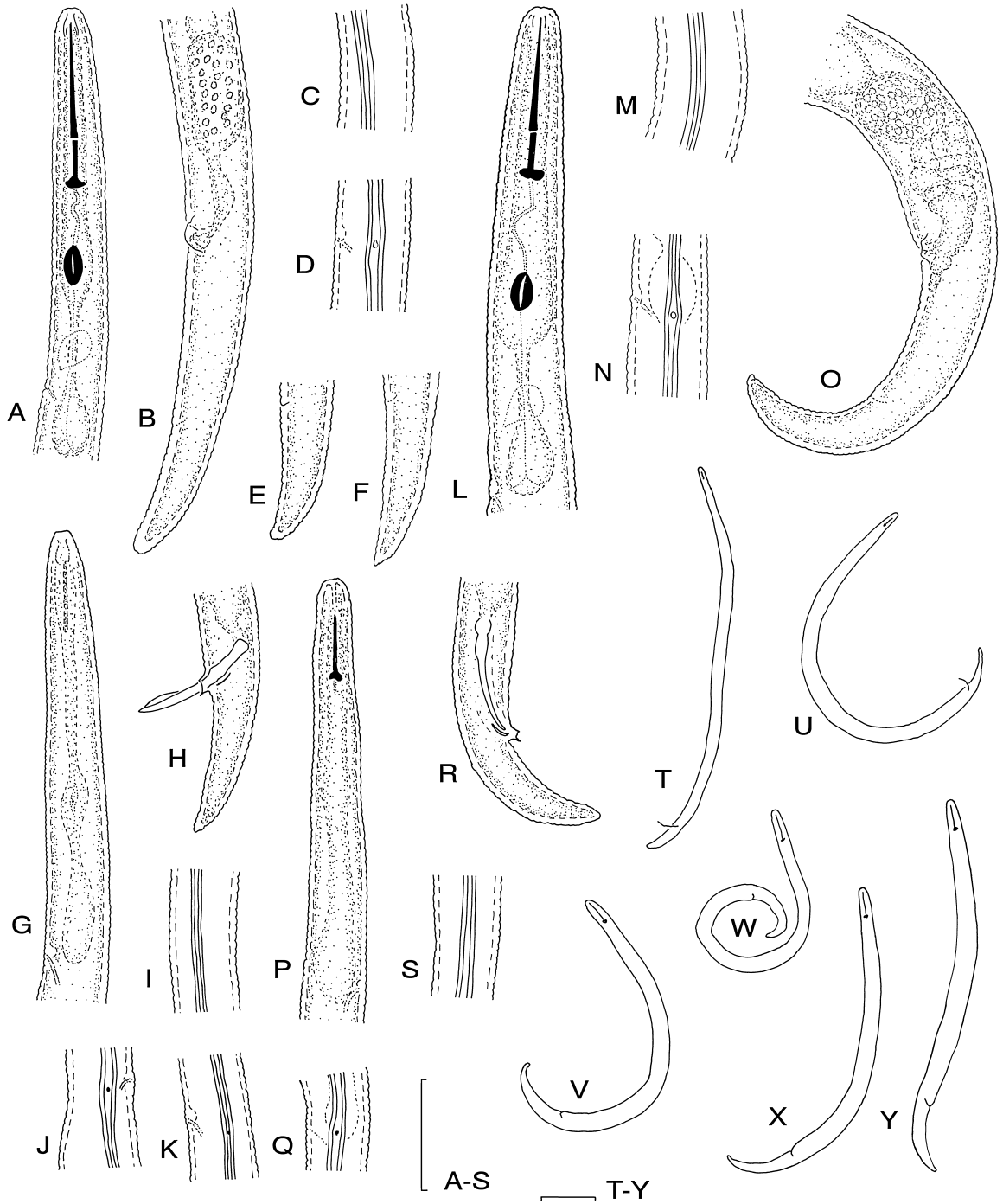

Fig. 5. *Paratylenchus hamatus* species complex. *Paratylenchus* sp. 2, American population CD604. Female. A: Anterior region; B: Posterior region; C: Lateral field at mid-body; D: Excretory pore and deirid; E, F: Tail regions. Male. G: Anterior region; H: Posterior region; I: Lateral field at mid-body; J, K: Excretory pore and deirid of two specimens. *Paratylenchus hamatus*, *sensu lato*. South African population Tvl1895, Female. L: Anterior region; M: Lateral field at mid-body; N: Excretory pore and deirid; O: Posterior region. Male. P: Anterior region; Q: Excretory pore and deirid; R: Posterior region; S: Lateral field at mid-body; T-Y: Female and male habitus of both populations. (Scale bars: A-S = 20 μm; T-Y = 50 μm.)

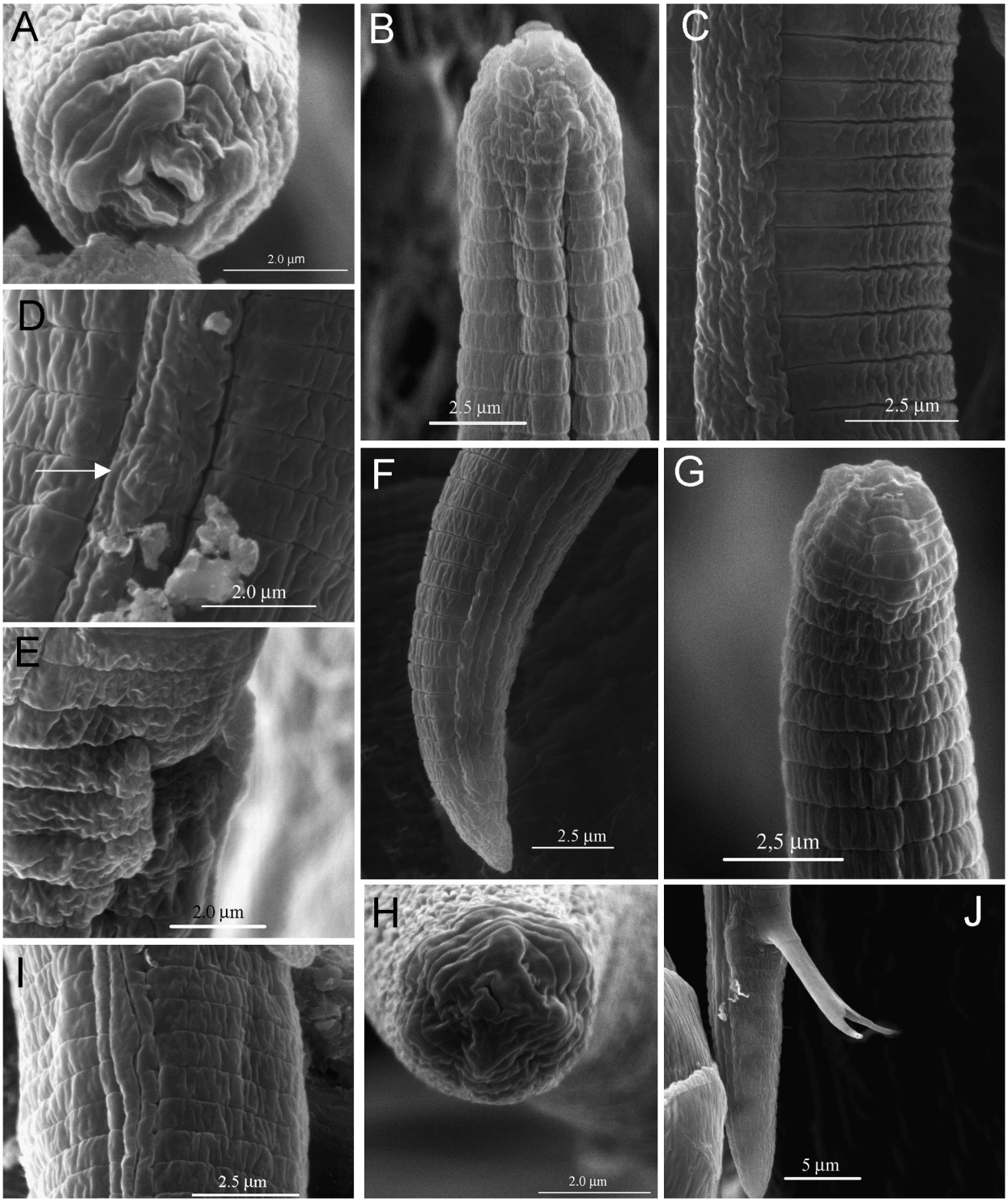

Fig. 6. *Paratylenchus hamatus* species complex. *Paratylenchus* sp. 2, American population CD604. Female. A: *En face* view of lip region; B: Lip region, lateral view; C: Lateral field at mid-body; D: Deirid (arrow); E: Vulval area; F: Tail. Male. G: Lateral view of lip region; H: *En face* view of lip region; I: Lateral field at mid-body; J: Tail.

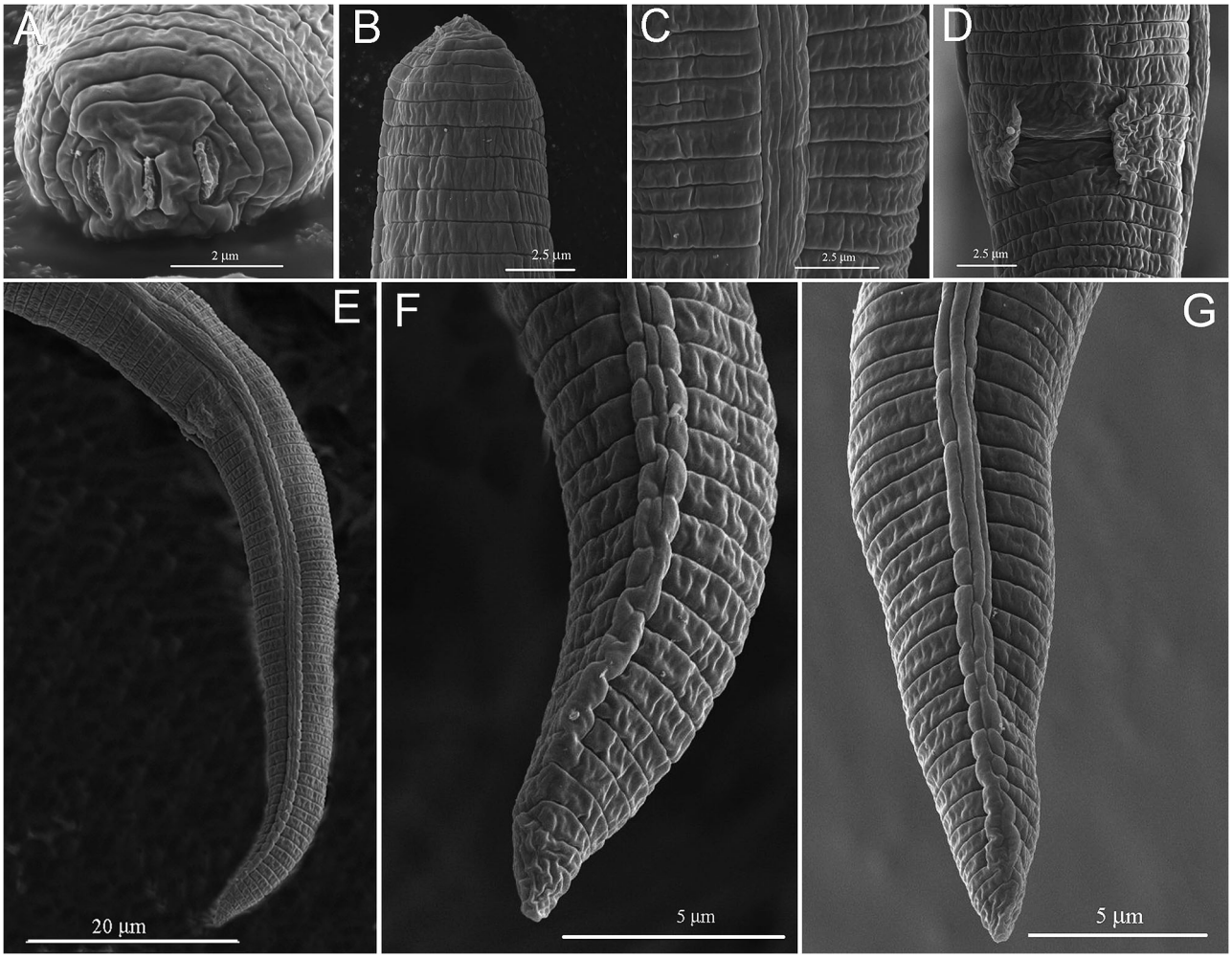

Fig. 7. *Paratylenchus hamatus*, *sensu stricto*. American population CD1155, Female. A: *En face* view of lip region; B: Lip region, lateral view; C: Lateral field at mid-body; D: Vulva, ventral view; E: Posterior region; F, G: Tail tips.

small stylet knobs sloping anteriorly and rounded posteriorly. Pharynx degenerate. Excretory pore situated opposite posterior part of degenerate pharynx. Hemizonid 2-3 annuli long, opposite excretory pore. Hemizonion seen in one specimen, one annulus long, situated seven annuli posterior to hemizonid. Deirid distinct, situated from opposite to two annuli posterior to level of excretory pore. Lateral field with four lines. Spicules distinct, slightly curved ventrad. Velum seen where spicules protrude. Spicular pouch distinct with a longer ventral lip. Bursa absent. Tail almost straight to curved more ventrad, tapering to a narrow, rounded tip. Phasmids not observed.

Juvenile

A few found but not well preserved. Stylet apparently absent.

NOTE

When following the compendium of Esser (1992) carefully, these specimens key out at *P. hamatus* with which they correspond very well. The other species with which they also correspond well is *P. baldacci* Raski, 1975b. Raski (1975b) stated that the two species can really only be separated from each other by the more slender and sharply conoid female tail tip and male tail tips of *P.*

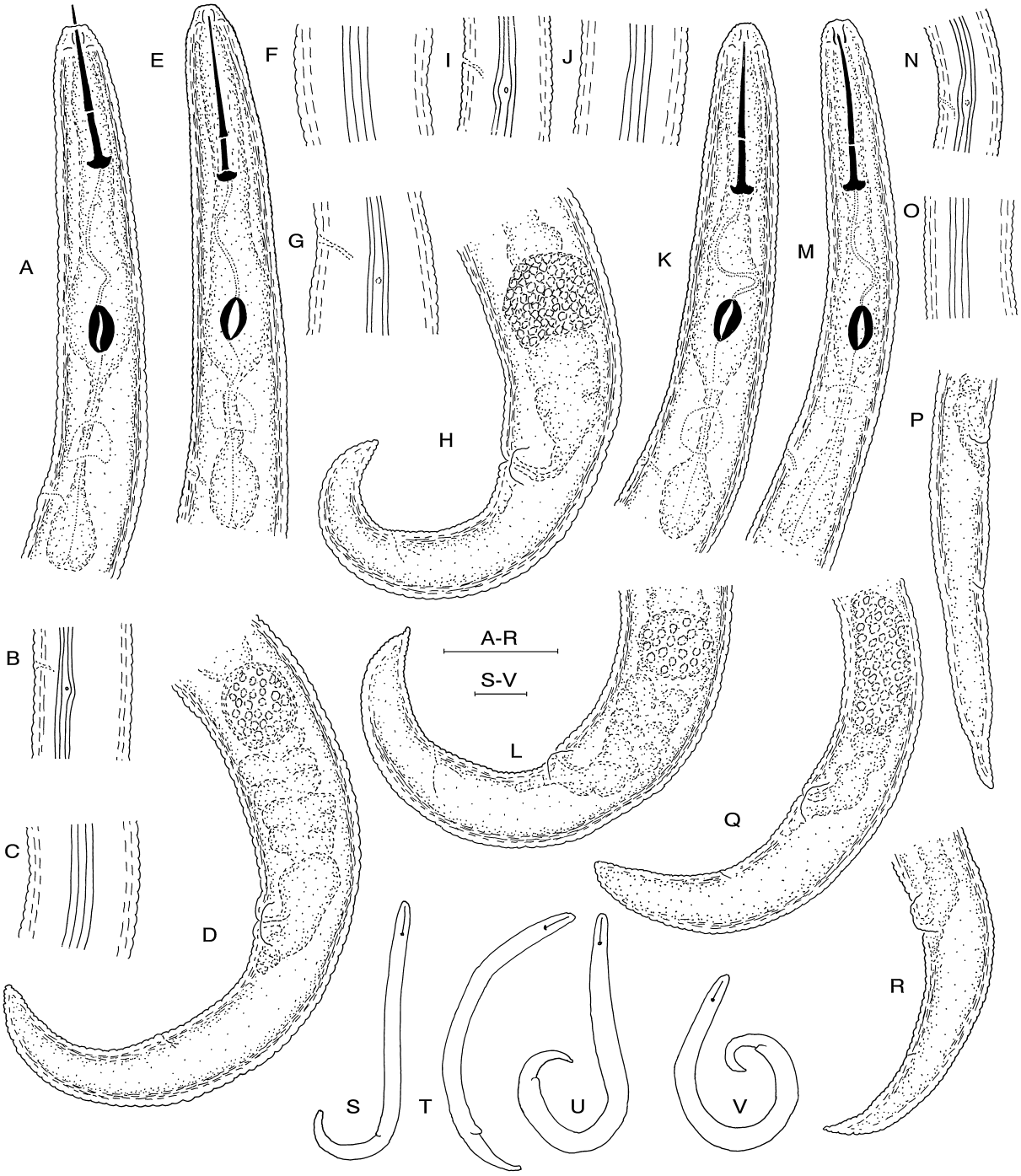

Fig. 8. *Paratylenchus nanus* type A, American population CD850, Female. A: Anterior region; B: Excretory pore and deirid; C: Lateral field at mid-body; D: Posterior region. American population CD860, Female. E: Anterior region; F: Lateral field at mid-body; G: Excretory pore and deirid; H: Posterior region. American population CD883, Female. I: Excretory pore and deirid; J: Lateral field at mid-body; K: Anterior region; L: Posterior region. *Paratylenchus hamatus*, *sensu stricto*. American population CD1155, Female. M: Anterior region; N: Excretory pore and deirid; O: Lateral field at mid-body; P-R: Posterior regions; S-V: Female habitus of all populations. (Scale bars: A-R = 20 μm; S-V = 50 μm.)

Table 4. Morphometrics of females of *Paratylenchus hamatus*, *sensu stricto*, *Paratylenchus* sp. 1, *Paratylenchus* sp. 2. from the USA and *P. hamatus*, *sensu lato* from South Africa, compared with those from the literature and with those of *P. baldacci* Raski, 1975. All measurements are in μm in the form: mean ± s.d. (range).

Character	*P. hamatus*, *sensu stricto* (USA, CD17, CD315, CD1155)	*Paratylenchus* sp. 1 (USA, CD57, CD61)	*Paratylenchus* sp. 2 (USA, CD604)	*P. hamatus*, *sensu lato* (South Africa, Tvl1895)	*P. hamatus* from literature[a]	*P. baldacci* Raski, 1975b from literature[b]
n	10	3	29	17	–	58
L	301-458	331-386	374 ± 27.5 (317-413)	371 ± 15.1 (349-399)	299-470	264-443
a	15.8-28	21.9-27.5	24 ± 2.3 (18.7-28)	21.7 ± 1.8 (18-24)	15.6-29.5	17-29
b	3.4-4.5	3.7-3.9	3.8 ± 0.2 (3.4-4.2)	4.3 ± 0.3 (3.8-4.9)	3.3-4.7	3.2-4.5
c	11.1-17.9	12.1-13.8	13.8 ± 1.4 (11.1-16.4)	13.1 ± 0.8 (11.5-14.7)	11.3-16.2	8.2-19.2
c′	1.7-3.6	2.7-3.6	3 ± 0.3 (2.5-3.6)	3.4 ± 0.4 (2.9-4.2)	2.8-4	2-4.1
o	13.1-31	14.1-20	21.5 ± 3.9 (13.1-31)	20.4 ± 2.7 (14.1-24.5)	15.7-18.8	–
DGO	4.0-8.5	4.5-6.5	6 ± 1.1 (4-8.5)	6 ± 0.8 (4-7.5)	4.8-8.9	4-6
V	79-86.5	79.5-82.5	82 ± 1 (79.5-84.5)	82 ± 0.9 (80.5-83)	78-85	78.5-86
OV	20.5-70	–	33 ± 6.7 (24.5-45)	44.5 ± 7.6 (35-62.5)	29-37	19-25.5
Stylet length	25.5-33	29-31	29.5 ± 1.3 (27-33)	28.5 ± 1.2 (27-31)	23-34	27-35
Metenchium length	15.5-23	19-21.5	19 ± 0.9 (17.5-21.3)	19 ± 1.2 (18-21)	17-24	17-23
Telenchium length	8-12	10.5-12	10.5 ± 0.6 (9.5-12)	9.5 ± 0.6 (9-10)	7-9.2	9.5-11
m	60.5-73.8	64.5-65	64.8 ± 1.1 (62.5-66.7)	67.1 ± 1.3 (65-69.2)	–	–
Stylet knob height	1.5-4.0	2.0-2.5	2 ± 0.3 (1.5-3)	2 ± 0.2 (2-2.5)	1.5-2.2	2
Stylet knob width	3-5	3.5-4.5	4 ± 0.3 (3.5-5)	3.5 ± 0.4 (3-5)	3.3-4.4	3-5
Pharynx length	78.5-115	89-99	99 ± 7.2 (78.5-115)	87 ± 4.8 (76.5-96)	79-97	64-79
Excretory pore from ant. end	61.5-105	73-83	81 5 ± 5.3 (68.5-105)	83 ± 3.6 (78-92)	69-91	58-89
Diam. at mid-body	12.5-27	13-17.5	15.5 ± 2.1 (12.5-22)	17 ± 2 (14.5-20.5)	11.8-17.3	11-14
Lip region diam.	5.5-8.5	6.5	7.5 ± 0.7 (5.5-8.5)	7 ± 0.5 (6.5-8.5)	–	6-6.5
Lip region height	3.0-5.5	3.5-4.0	4 ± 0.5 (3-5)	3 ± 0.5 (2.5-4)	–	2-2.5
Annulus width	1.0-2.5	–	1.5 ± 0.3 (0.7-2)	1.5 ± 0.2 (1.5-2)	1.1-1.5	1.5
Lateral field width	2-6	–	3.5 ± 0.5 (2-4.5)	3 ± 0.3 (2.5-3.5)	1.8-3.7	2-3
Tail length	21.5-37	25.5-29.5	27.5 ± 2.8 (22-37)	28.5 ± 1.2 (25.5-32)	17-33.1	17-24.5
EP%L	17.1-24.7	21.3-22	21.9 ± 1 (20.2-24)	22.5 ± 1 (20.5-24)	–	17.6-24.2
V-anus distance	29.5-50	32.5-43.5	39 ± 4.7 (29.5-50)	39.5 ± 2.8 (35-43.5)	–	–
Spermatheca length	9.5-29.5	–	16 ± 4.5 (10-24.5)	15 ± 2.7 (12.5-21)	8.8-15.1	13
Spermatheca diam.	7.5-17.5	–	10 ± 1.7 (7.5-14)	12 ± 1.3 (9.5-13)	6.6-9.6	8
PUB length	4.5-12.5	–	10 ± 2.7 (4.5-16)	8.5 ± 2.3 (6-16)	–	–

[a] Tarjan (1960); Raski (1975b); Van den Berg (1989); Yu (2009).
[b] Brzeski (1995); Raski (1975b); Van den Berg & Tiedt (2000).

Table 5. Morphometrics of males of *Paratylenchus hamatus*, *sensu lato*, from South Africa and *Paratylenchus* sp. 2 from USA compared with *P. hamatus* from the literature. All measurements are in μm in the form: mean ± s.d. (range).

Character	*P. hamatus*, *sensu lato* (South Africa, Tvl1895)	*Paratylenchus* sp. 2 (USA, CD604)	*P. hamatus* from literature[a]
n	7	2	
L	359 ± 25.4 (331-401)	379; 404	331-410
a	31.1 ± 2.5 (27.6-33.4)	31.2	23.6-35
b	4.9 ± 0.3 (4.6-5.4)	4.4; 4.5	4.2-5.1
c	12.9 ± 1.2 (10.9-14)	11; 13.6	11-15.2
c′	3 ± 0.3 (2.7-3.3)	3.1; 3.6	17
Stylet length	15.5 ± 2.5 (13-19)	12.1	–
Metenchium length	10.7; 12.5 (n = 2)	–	–
Telenchium length	5.9; 6.6 (n = 2)	–	–
Stylet knob height	1 ± 0.4 (0.75-1.5)	–	–
Stylet knob width	2.25 ± 0.4 (2-3)	–	–
Pharynx length	73.5 ± 5.4 (64-80)	85.3; 88.9	–
Excretory pore from ant. end	71 ± 5.6 (61-75.5)	76.4; 80.1	–
Diam. at mid-body	11.5 ± 1.0 (10-12.5)	12	–
Lip region diam.	5 ± 0.4 (5-6)	6	–
Lip region height	3 ± 0.6 (2-4)	3.7; 4.5	–
Annulus width	1.5 ± 0.4 (0.75-2.0)	1; 1.5	–
Lateral field width	2.5 ± 0.5 (2-3)	2;.6	–
Tail length	28 ± 1.8 (26.5-31.5)	27.9; 6.8	–
EP%L	20 ± 1.3 (17.5-21.5)	20.2	–
Spiculum length	21.5 ± 0.9 (20.5-23)	22.1; 3.2	15.5-24.3
Gubernaculum length	4 ± 0.4 (3.5-4.5)	4.4	3.5-5
Penial tube	2	1.8	–
T	30.4 ± 7.2 (19.3-36.8)	–	34-38

[a] Tarjan (1960); Raski (1975b); Yu (2009).

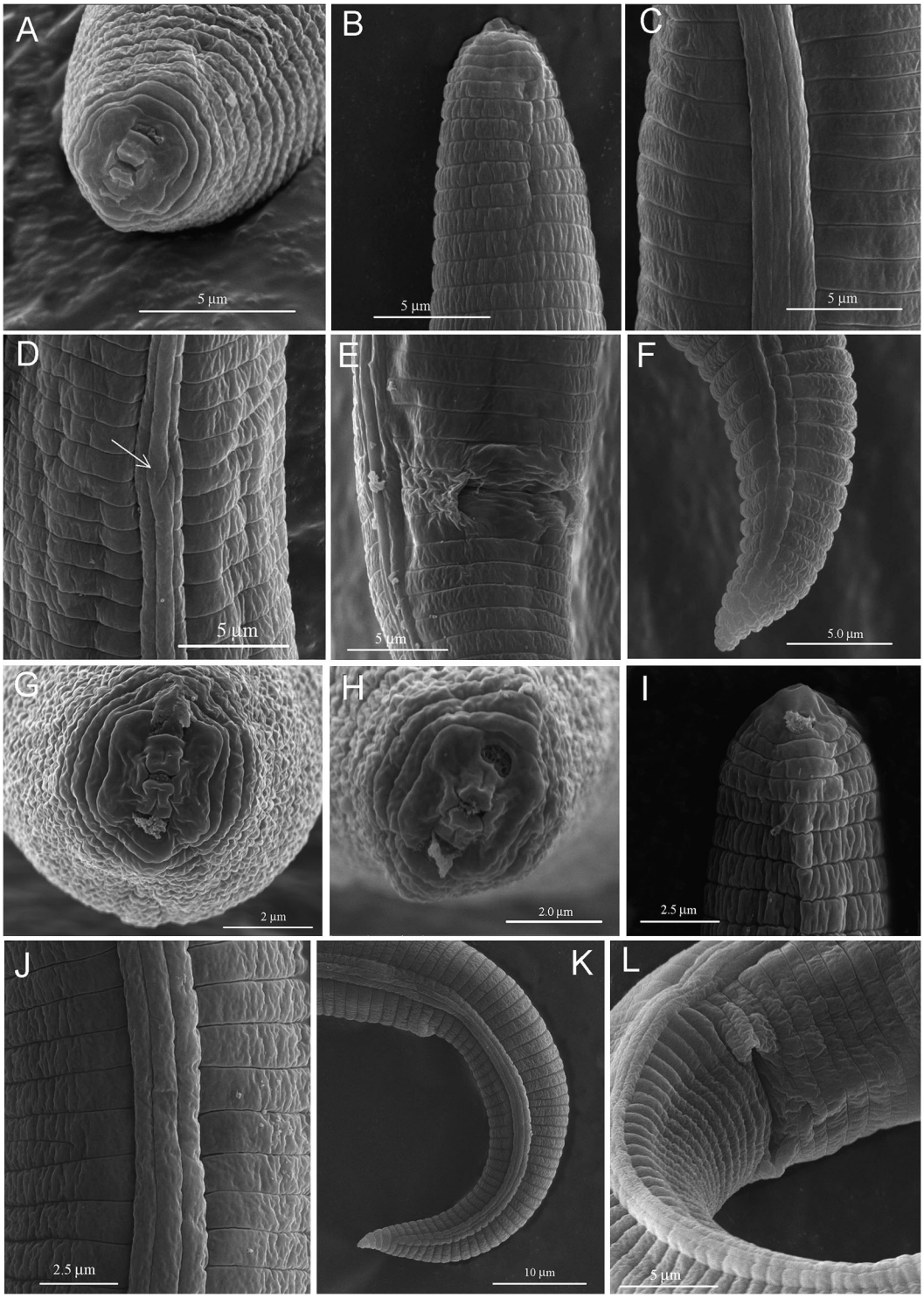

Fig. 9. *Paratylenchus nanus* type A, American population CD850, Female. A: *En face* view of lip region; B: Lip region, lateral view; C: Lateral field at mid-body; D: Deirid (arrow); E: Vulva, ventral view; F: Tail. American population CD883, Female. G, H: *En face* views of lip region of two females; I: Lip region, lateral view; J: Lateral field at mid-body; K: Posterior region; L: Vulva, ventral view.

baldacci which was described as finely rounded to almost acute. This close relationship was confirmed by Brzeski (1995). He also noted that the slightly more rounded tail end of *P. hamatus* falls within the limits of *P. baldacci*. The close morphometric relationship of these two species is shown in Table 4. Because of this close relationship, *P. baldacci* might belong to *P. hamatus sensu stricto* or one of the putative species from this complex. Additional morphological and molecular studies should be done to confirm this hypothesis.

Paratylenchus nanus **Cobb, 1923 species complex**

Type A – Samples CD850, CD860 and CD883 (USA) (Figs 8A-L, 9, 14G-J, 15E-G)
Type B – Sample CD587, KP2214 (South Africa) (Figs 10, 11)

This species was described by Cobb in 1923 from a single female collected near Devil's lake in North Dakota. Tarjan (1960) re-studied the type materials, and provided measurements of additional specimens. Geraert (1965) suggested that *P. nanus* should be a synonym of *P. bukowinensis*. Raski (1975b) gave a detailed report of various collections and descriptions of the species and finally gave a detailed description of topotypes from Falls Church, Virginia, establishing the species as valid.

Our study shows that the morphospecies *P. nanus* might contain two sibling species (type A and type B), which are well separated using molecular criteria, but have very similar morphology.

Measurements

See Table 6.

Description

Female

Body posture ranging from slightly curved ventrad to a complete Figure 6. Lip region not set off, conical rounded or slightly squarish and more flattened with four lip annuli, *en face* view showing a more square to oblong

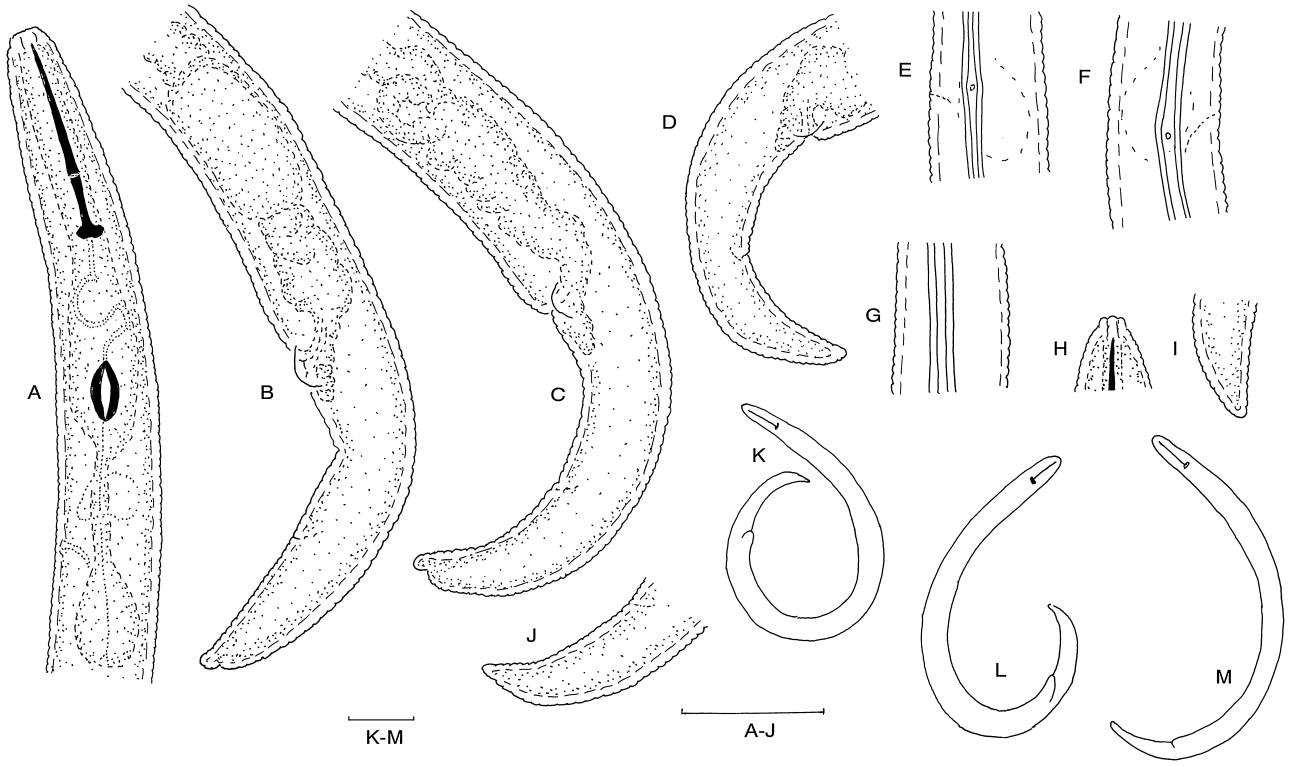

Fig. 10. *Paratylenchus nanus* type B, South African population KP2214, Female. A: Anterior region; B-D: Posterior regions; E, F: Excretory pore and deirid of two females; G: Lateral field at mid-body; H: Lip region of another female; I, J: Tail tips of two other females; K-M: Female habitus. (Scale bars: A-J = 20 μm; K-M = 50 μm.)

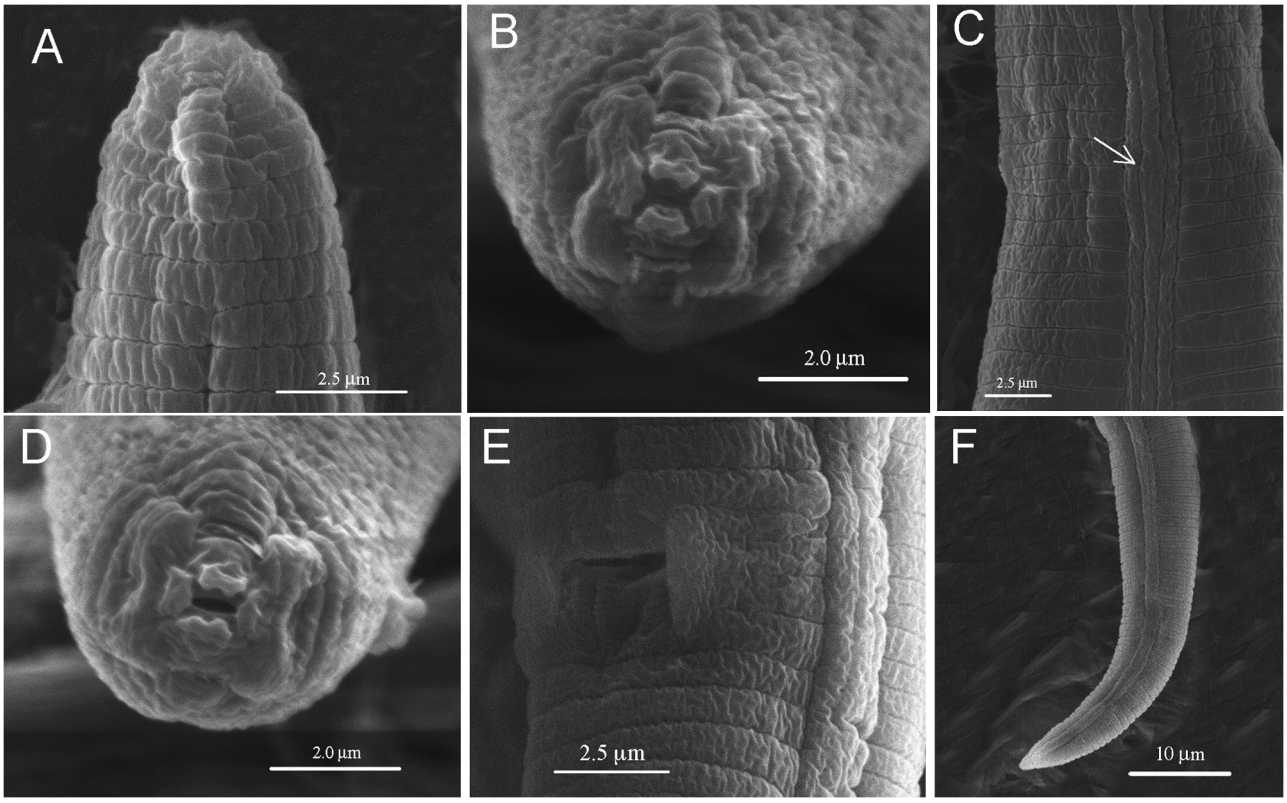

Fig. 11. *Paratylenchus nanus* type B, South African population KP2214, Female. A: Lip region, lateral view; B, D: *En face* view of lip region of two females; C: Lateral field with deirid (arrow); E: Vulva, ventral view; F: Posterior region.

labial area with four rounded submedian lobes not protruding prominently upward or outward. Lateral thickenings around stoma opening and two slit-like amphid openings laterally. Labial framework weak, stoma wall slightly sclerotised. Stylet long and slender with stylet knobs sloping anteriorly with outer tips pointing upward, rounded posteriorly. Nerve ring mainly encircling middle of isthmus, rarely slightly more posterior or anterior. Excretory pore located from opposite middle of isthmus to opposite basal part of pharynx. Hemizonid 2-3 annuli long, situated from directly anterior to directly posterior to excretory pore. Hemizonion seen in one specimen only, situated six annuli posterior to hemizonid. Deirids distinct in middle band of lateral field, situated from three annuli anterior to *ca* three annuli posterior to excretory pore. Lateral field four distinct lines, rarely areolated but then more so in post-vulval area. Body narrowing slightly posterior to vulva in more obese specimens. Lateral vulval flaps distinct, round or flattened, smooth or crenate. Spermatheca small to large, round to oblong, mostly filled with sperm cells. Small post-vulval sac present in all specimens close to ventral body wall with a few vestigial ovarial cells at tip. Phasmids not observed. Tail tapering to a rounded or finely rounded tip, rarely digitate. Posterior part of body mostly curved stronger posterior to vulva but rarely remaining almost straight.

Male

Not found.

Juvenile (J4)

Similar to female with a slight to more developed stylet. Distinct deirid seen in most specimens, situated from 1-2 annuli posterior to level of excretory pore.

Note

When following the various keys of Geraert (1965), Wouts (1966), Raski (1975a, b) and Pinochet & Raski

Table 6. Morphometrics of females and juveniles of *Paratylenchus nanus* type A and type B from USA and South Africa compared with those from the literature. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*P. nanus* type A (USA, CD850, CD860, CD833). Female	*P. nanus* type B (South Africa, CD587, KP2214). Female	*P. nanus* type B (South Africa, KP2214). Juvenile (J4)
n	26	17	10
L	328-458	359 ± 36.3 (300-415)	339.5 ± 35.2 (299-390)
a	17.5-22	22.1 ± 1.4 (19.5-24.6)	20.4 ± 1.6 (17.7-22.9)
b	3.5-4.5	3.9 ± 0.3 (3.5-4.4)	4.1 ± 0.3 (3.7-4.7)
c	12.4-17.9	15.3 ± 1.2 (14-18.5)	13.8 ± 1.9 (12.3-18.9)
c′	1.6-2.9	2.4 ± 0.2 (2.0-2.8)	2.2 ± 0.2 (1.7-2.5)
o	17-26	20.4 ± 2.7 (14.5-23.7)	–
DGO	5.0-7.5	6 ± 0.9 (4.5-7.0)	–
V	81.5-86.5	84 ± 1.2 (82.5-85)	61.5 ± 11.8 (50-75) genital primordium
OV	29.5-70	40 ± 5.5 (30.5-48.5)	–
Stylet length	25.5-31.5	28.5 ± 1.3 (26-31)	10 ± 4.2 (3.5-14.5)
Metenchium length	15.5-20.5	18.5 ± 1.1 (16-20.5)	–
Telenchium length	8.0-11.5	10 ± 0.9 (9-12)	–
m	60.9-70.7	65 ± 2.6 (60.5-69.5)	–
Stylet knob height	1.5-2.0	2	–
Stylet knob width	3.0-4.5	4 ± 0.4 (3.5-5.0)	–
Pharynx length	85-109	92 ± 5.6 (85-110)	83.5 ± 5.6 (72.5-94.5)
Excretory pore from ant. end	61.5-86.5	77.5 ± 3.7 (71-85)	71 ± 4.4 (65-78.5)
Diam. at mid-body	15-25.5	16 ± 1.9 (13-20)	16.5 ± 1.1 (14.5-17.5)
Lip region diam.	6.5-8.5	7 ± 0.3 (6.5-7.5)	6 ± 0.5 (5.0-6.5)
Lip region height	3.5-5.0	3.5 ± 0.3 (3-4)	3.25 ± 0.4 (3-4)
Annulus width	1.5-2.0	1.5 ± 0.2 (1.0-1.5)	1.5 ± 0.2 (1-2)
Lateral field width	3.5-6.0	3 ± 0.4 (2.5-3.5)	2.75 ± 0.3 (2.5-3.0)
Tail length	21.5-31	23.5 ± 3.1 (17.5-29.5)	23 ± 7.5 (20.5-29.5)
EP%L	17.1-22.2	21.5 ± 1.5 (19.9-24.3)	21 ± 1.8 (19.4-24.4)
V-anus distance	29.5-46.5	33.5 ± 5.9 (26-44)	–
St%L	6.5-9.5	8 ± 0.7 (6.8-9.3)	3.6 ± 0.5 (3.2-4.5)
Spermatheca length	9.0-22.5	13.5 ± 5.2 (9-27)	–
Spermatheca diam.	7.5-17.5	9 ± 1.6 (7.0-12.5)	–
PUB length	–	9 ± 1.6 (6.5-12)	–

(1977), the specimens from our samples can belong to the nominal species *P. nanus*, *P. projectus*, *P. curvitatus* group or *P. neoamblycephalus*. They also fit in well with *P. nanus* in the diagnostic compendia of Esser (1992) and Brzeski (1998). They correspond very well with the various descriptions of *P. nanus* (Tarjan, 1960; Thorne & Smolik, 1971; Raski, 1975b; Brzeski & Maciejczyk, 1977; Brzeski, 1995, 1998; Talavera & Tobar Jiménez, 1997; Ciobanu *et al.*, 2003; Ryss *et al.*, 2005), but also with the descriptions of *P. projectus* (Reuver, 1959; Tarjan, 1960; Szczygiel, 1974; Raski, 1975; Wu, 1975; Brzeski & Maciejczyk, 1977; Bajaj, 1987; Van den Berg, 1989; Brzeski, 1995; Loof, 1995; Van den Berg & Quénéhervé, 1999; Ciobanu *et al.*, 2003) (see Table 3).

Geraert (1965) regarded species with a stylet 19-36 μm and a vulva situated at 78-86% as belonging to the *P. curvitatus* group and stated that they are difficult to distinguish from each other. Amongst this group are species such as *P. amblycephalus*, *P. dianthus*, *P. hamatus*, *P. nainianus*, *P. nanus*, *P. neoamblycephalus*, *P. projectus*, *etc*. In 1965, Fisher regarded *P. projectus* as a synonym of *P. nanus* and Raski (1975b) made *P. neoamblycephalus* a synonym of *P. projectus*. These synonymies were not accepted by later authors. Tarjan (1960) said *P. neoamblycephalus* and *P. nanus* are similar. He also stated that *P. projectus* is close to *P. curvitatus* and *P. nanus*. Brzeski & Szczygiel (1963) synonymised *P. neoamblycephalus* with *P. nanus*. The close similarity of *P. nanus* with some of the above species is shown in Table 3.

***Paratylenchus straeleni* (De Coninck, 1931) Oostenbrink, 1960**

Sample CD899 (USA)
(Figs 12, 13)

This species was originally described from moss in Belgium by De Coninck (1931) and later reported by different authors from several other regions in the world such as Canada, various countries in Europe, USA and South Africa (Brown, 1959; Raski, 1976; Castillo & Gomez Barcina, 1988; Brzeski, 1995; Van den Berg & Tiedt, 2001; Ciobano *et al.*, 2003 *etc*.).

MEASUREMENTS

See Table 7.

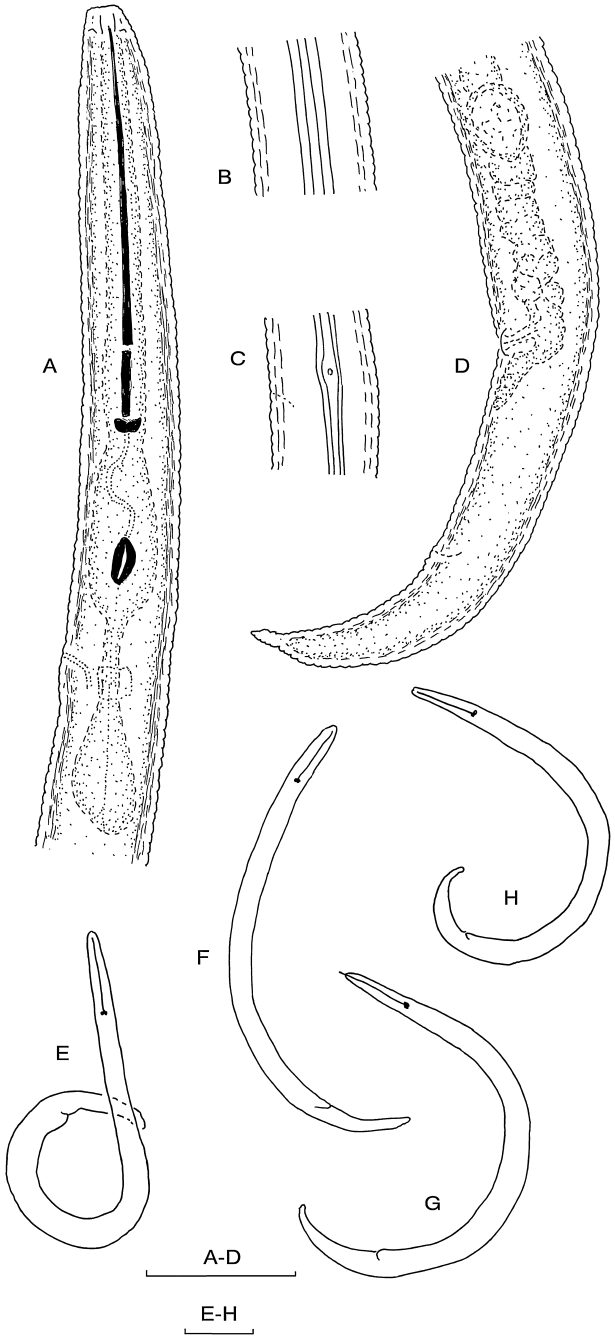

Fig. 12. *Paratylenchus straeleni*, American population CD899, Female. A: Anterior region; B: Lateral field at mid-body; C: Excretory pore and deirid; D: Posterior region; E-H: Female habitus. (Scale bars: A-D = 20 μm; E-H = 50 μm.)

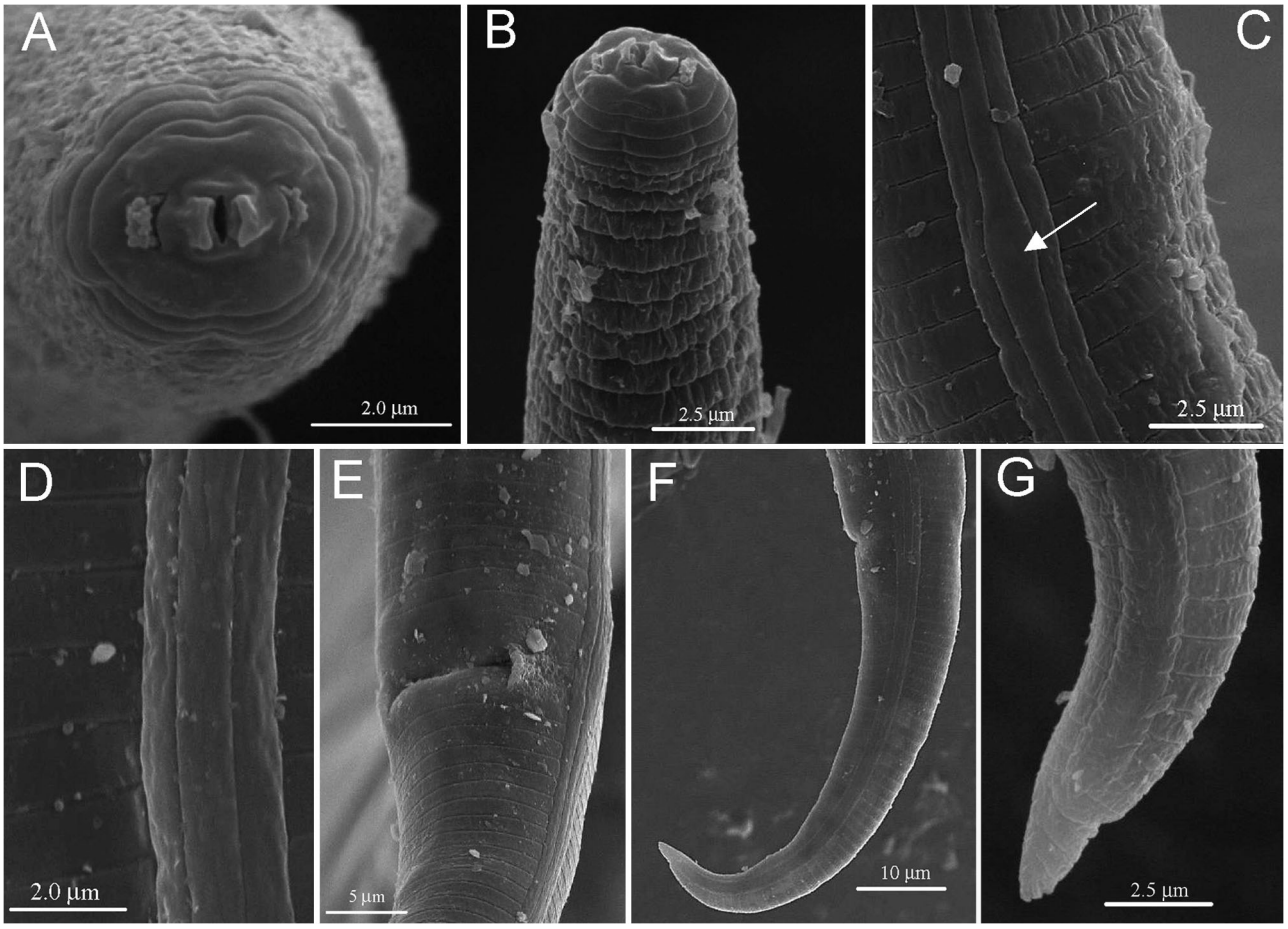

Fig. 13. *Paratylenchus straeleni*, American population CD899, Female. A: *En face* view of lip region; B: Lip region, lateral view; C: Deirid (arrow); D: Lateral field at mid-body; E: Vulva, ventral view; F: Posterior region; G: Tail tip.

Description

Female

Slender nematodes with body habitus ranging from slightly curved ventrad into an open letter C to an open or closed Figure 6. Lip region not set off, narrowing very slightly anteriad with four faint annuli. *En face* view of lip region showing a somewhat rectangular labial disc with two distinct amphid openings, no distinct submedian lobes and mouth opening with two raised ridges laterally. Labial framework not very distinct with only stoma walls slightly sclerotised. Stylet long and slender, slightly curved dorsad. Stylet knobs small, slightly tapering anteriorly with outer tips slightly curved upward, rounded posteriorly. Nerve ring distinct, encircling isthmus from opposite middle to opposite posterior part of isthmus. Hemizonid two annuli long, situated from two annuli posterior to opposite excretory pore. Hemizonion not seen in any specimens. Excretory pore situated from opposite anterior part of isthmus to opposite middle of basal pharyngeal bulb. Deirid distinct, situated from just anterior to just posterior to excretory pore. Lateral field four distinct lines. Vulval lips not conspicuous and body not narrowing much posterior to vulva. Lateral vulval flaps distinct, flattened or rounded and crenate. Spermatheca small round and empty in all specimens except one female where a few sperm cells were seen. Posterior uterine branch present in all specimens along ventral body wall with a few vestigial cells present. Phasmids not seen. Tail with 21-27 annuli, tapering to a narrowly rounded terminus, sometimes slightly digitate.

Male

Not found.

Table 7. Morphometrics of females of *Paratylenchus straeleni* from the USA compared with those from the literature. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*P. straeleni* (USA, CD899)	*P. straeleni* after Brzeski (1995)	*P. straeleni*, after Castillo & Gomez-Barcina (1988)	*P. straeleni*, after Van den Berg & Tiedt (2001)	*P. straeleni*, after Ciobanu *et al.* (2003)
n	7	?	19	14	6
L	398 ± 7.6 (388-407)	280-447	308-447	317-383	324-403
a	25.2 ± (22.1-27)	16-30	17.3-29.8	21.3-27.2	20.7-24.9
b	3.6 ± 0.2 (3.4-3.8)	3.0-4.5	3.0-4.2	3.3-3.8	3.1-3.6
c	12.7 ± 0.9 (12.3-14.9)	9.9-18.6	9.9-18.6	11-13.5	11.9-14.7
c′	3.3 ± 0.4 (2.8-3.9)	2.3-3.8	2.4-3.4	3.0-3.8	–
o	7.6 ± 0.8 (6.6-8.4)	–	10-12.9	6.8-7.3	–
DGO	4 ± 0.4 (3.5-5.0)	–	5.3-6.6	4	–
V	83 ± 0.6 (82-83.5)	79-85	79-84	80-83	80.2-83.4
OV	24.5 ± 3.4 (19-27)	–	23-58	24-50	–
Stylet length	55 ± 1.7 (52.5-57.5)	44-66	47-57	52.5-56	55-58
Metenchium length	42.5 ± 1.5 (41-45)	45-49	35-46	42-48	41-45
Telenchium length	12 ± 0.7 (11-13)	–	–	8-12	12-15
m	77.9 ± 1.5 (75.5-80)	–	73-81	–	74.1-78.6
Stylet knob height	2 ± 0.3 (1.5-2.0)	–	–	1.0-1.5	–
Stylet knob width	3.5 ± 0.3 (3.0-3.5)	–	2.7-3.3	2.5-3.0	–
Pharynx length	109.5 ± 5.1 (105-117)	–	92-129	91.5-102.5	101-114
Excretory pore from ant. end	94 ± 3.3 (89.5-98.5)	71-107	71-107	72.5-85	75-92
Diam. at mid-body	15.5 ± 1.3 (14.5-17.5)	–	13-20	12.5-17	13-18
Diam. at anus	9.5 ± 0.8 (8.0-10.5)	–	8-13	–	–
Lip region diam.	6 ± 0.4 (6.0-6.5)	–	–	6.0-6.5	–
Lip region height	3.5 ± 0.5 (3-4.5)	–	–	3-4	–
Annulus width	1.5 ± 0.1 (1.5-2.0)	1	1.2-1.3	1-2	–
Lateral field width	3.5	–	2.3-4.9	2.5-3.0	–
Tail length	30.5 ± 1.9 (27-32.5)	–	22-34	24-31	22-34
EP%L	23.7 ± 0.9 (22.8-25.4)	–	–	21.6-23.8	–
V-anus distance	37.5 ± 2.3 (35.5-40.5)	–	–	–	–
Spermatheca length	10 ± 1.5 (8-11.5)	9-11	–	–	–
Spermatheca diam.	8 ± 0.8 (7-9)	9	9-13	–	–
PUB length	12.5 ± 2.6 (10.5-17.5)	–	–	–	–
Ovary length	97 ± 13.8 (75-109.5)	–	–	–	–

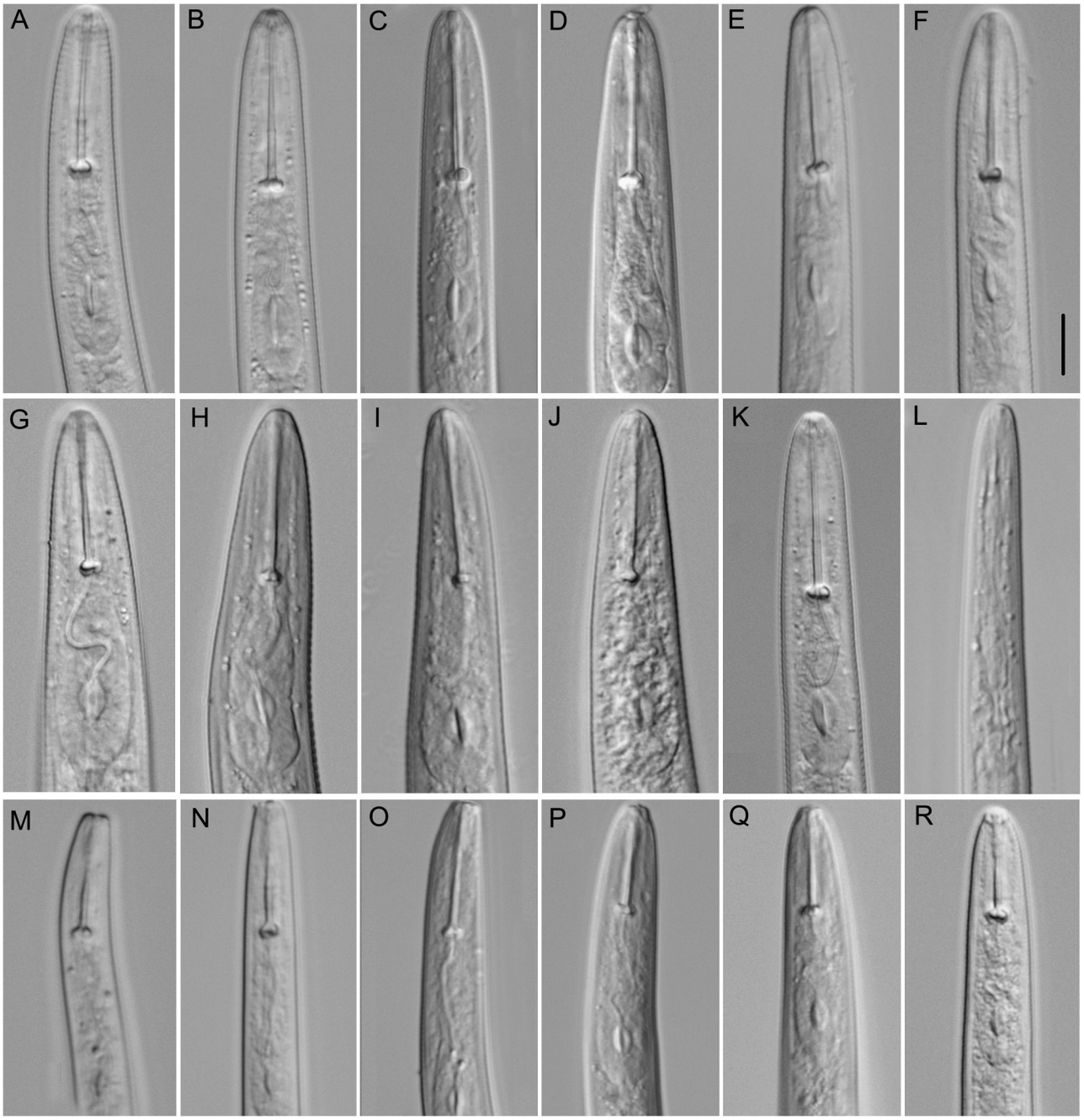

Fig. 14. Heads. Light microscopic photos of *Paratylenchus* species. A-C, K: *Paratylenchus* sp. 2 females (CD604); D: *P. hamatus*, *s. str*. female (CD315); E, F: *P. hamatus*, *s. str*. females (CD1155); G-I: *P. nanus* type A females (CD850); J: *P. nanus* type A female (CD860); L: *Paratylenchus* sp. 2 male (CD604); M-O: *P. aquaticus* type A females (CD619); P-R: *P. aquaticus* type B females (CD868). (Scale bar = 10 μm.)

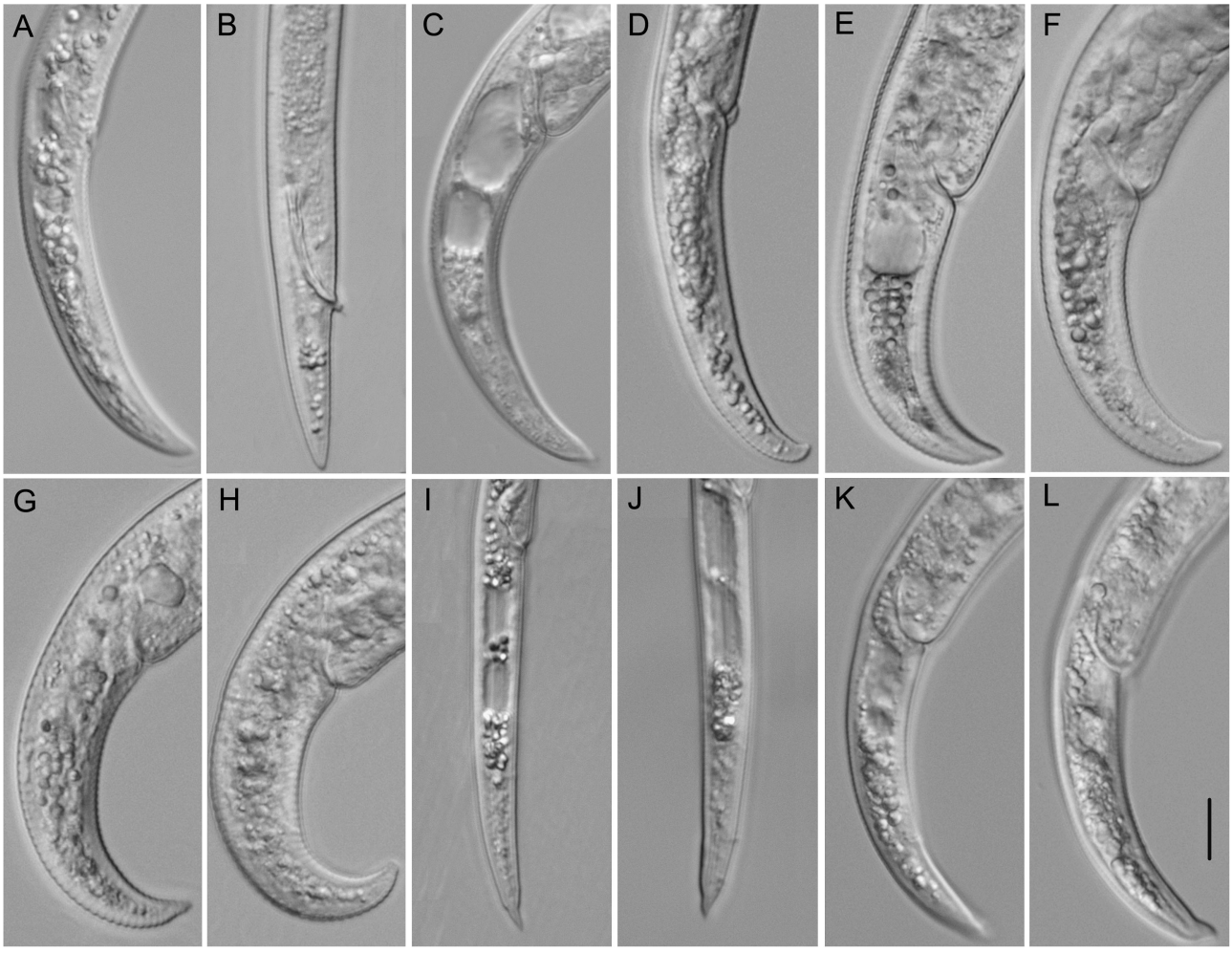

Fig. 15. Tails. Light microscopic photos of *Paratylenchus* species. A: *Paratylenchus* sp. 2 female (CD604); B: *Paratylenchus* sp. 2 male (CD604); C: *P. hamatus*, *s. str*. female (CD315); D: *P. hamatus*, *s. str*. female (CD1155); E, F: *P. nanus* type A females (CD850); G, H: *P. nanus* type A female (CD860); I, J: *P. aquaticus* type A females (CD619); K, L: *P. aquaticus* type B females (CD868). (Scale bar = 10 μm.)

NOTE

The present specimens compare very well morphologically with *P. straeleni* described by authors such as Brown (1959), Tarjan (1960), Raski (1962, 1976), Thorne & Malek (1968), Castillo & Gomez-Barcina (1988), Brzeski (1995), Van den Berg & Tiedt (2001) and Ciobanu *et al.* (2003) (see Table 7). No males were found in the present population, although the spermatheca of one female contained a few sperm cells. In most populations described by the above authors, males were plentiful but Van den Berg & Tiedt (2001) and Ciobanu *et al.* (2003) found no males. The SEM photographs of the present specimens are very similar to those given by Van den Berg & Tiedt (2001).

Molecular characterisation and phylogenetic relationships within *Paratylenchus* species

The D2-D3 of 28S rRNA gene sequence alignment was 730 bp in length and contained 58 sequences of *Paratylenchus* and three outgroup taxa. Intraspecific sequence variations were: for *P. hamatus s. str*. 0-4 bp (0-0.6%); for *P. nanus* type A 0-4 bp (0-0.6%); and for *P. nanus* type B 0-8 (0-1.1%). Interspecific sequence variations be-

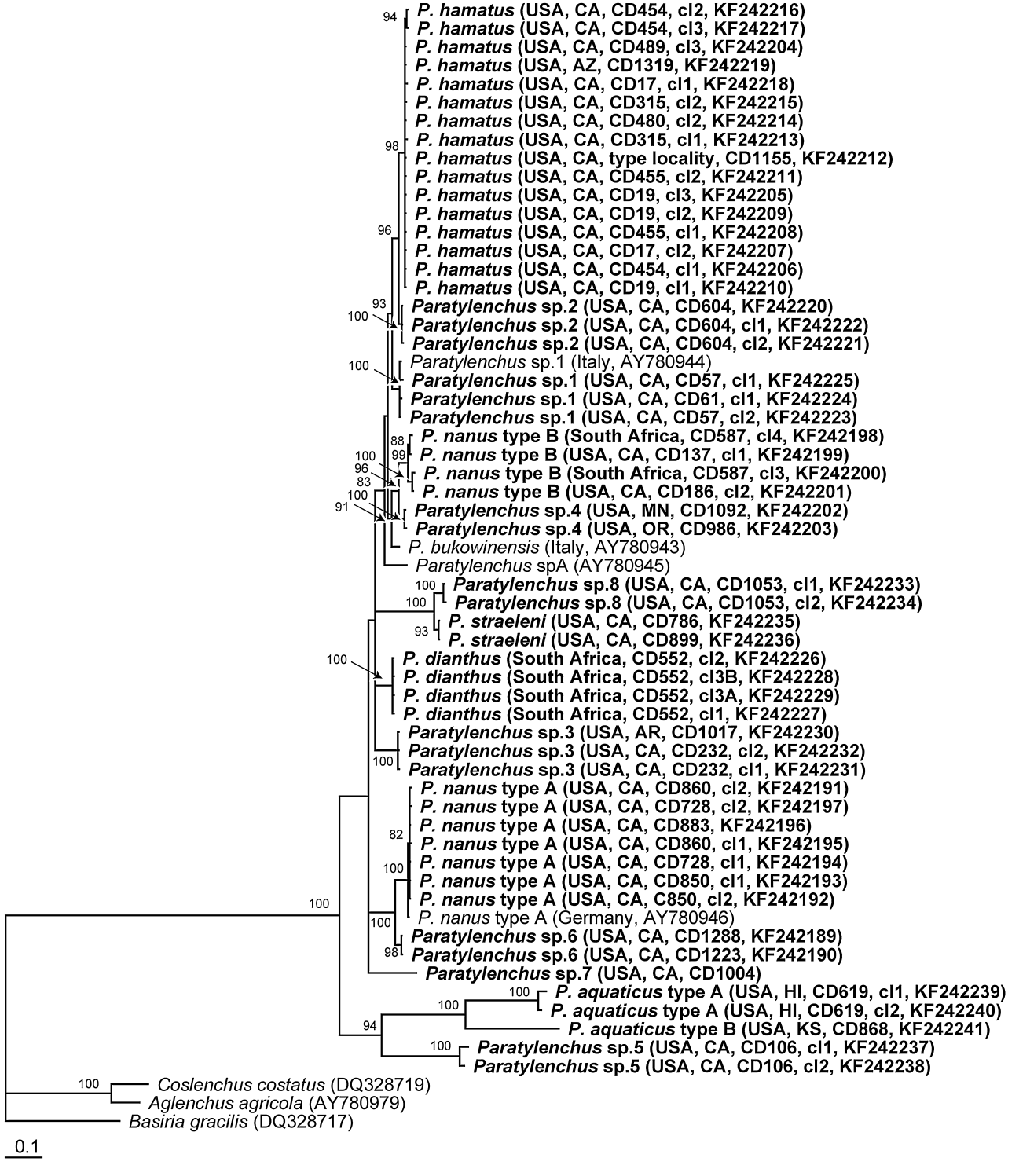

Fig. 16. Phylogenetic relationships within *Paratylenchus* species and populations: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 expansion segments of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated in boldface.

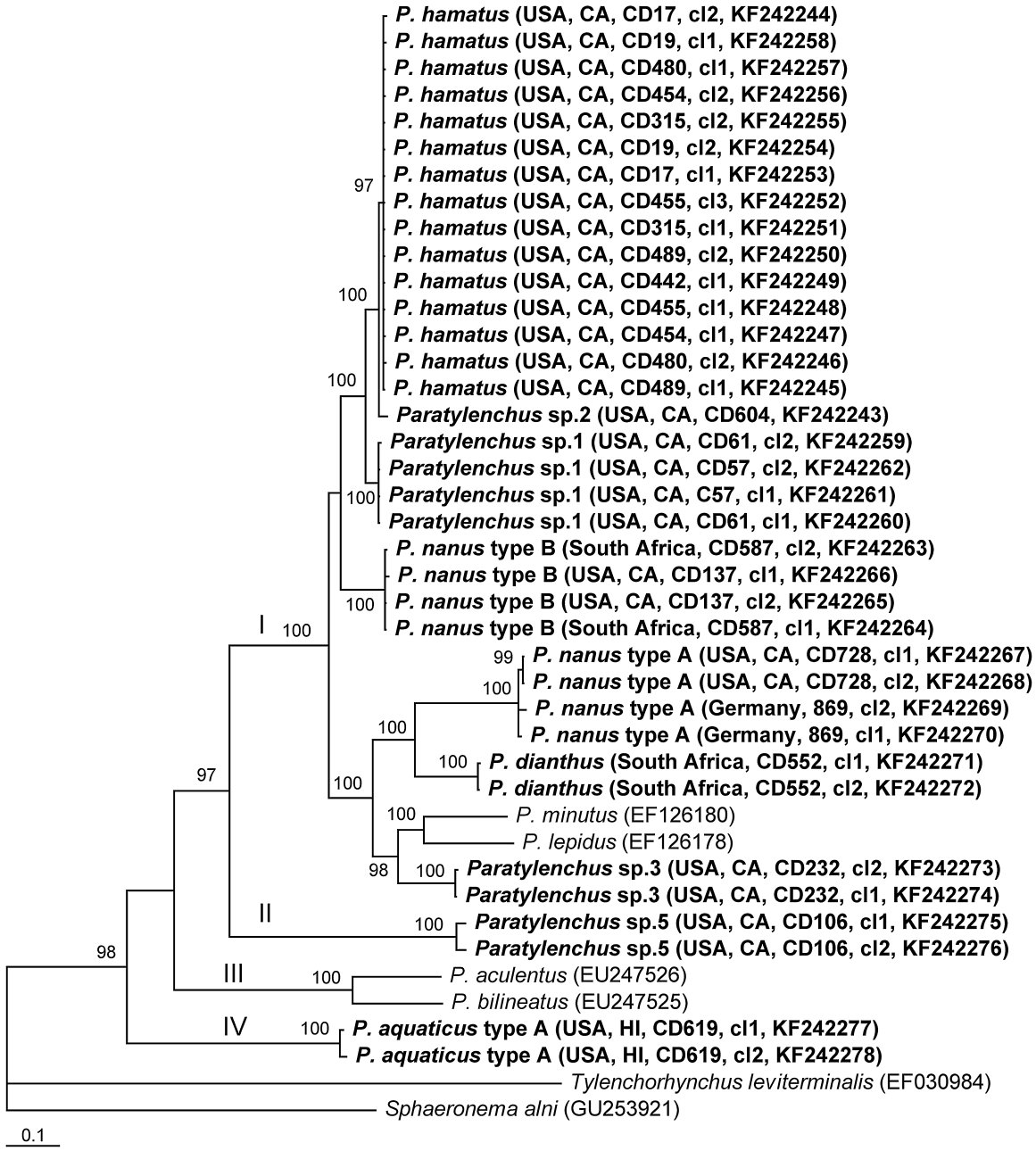

Fig. 17. Phylogenetic relationships within *Paratylenchus* species and populations: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated in boldface.

tween *P. hamatus s. str*. and *Paratylenchus* sp. 1 or *Paratylenchus* sp. 2 were 23-28 bp (3.3-4.0%) or 11-14 bp (1.6-2.0%), respectively. Sequence variations between *P. nanus* type A and type B were 71-75 bp (10-13%) and between *P. aquaticus* type A and type B they were 129-131 bp (19%). Phylogenetic relationships between *Paratylenchus* species are given in Figure 16. Relationships between species or species groups were not resolved.

The ITS rRNA gene sequence alignment was 916 bp in length and contained 40 sequences of *Paratylenchus*

and two outgroup taxa. Intraspecific sequence variations were: for *P. hamatus s. str*. 0-5 bp (0-0.5%); and for *P. nanus* type A 0-21 bp (0-2.5%). Interspecific sequence variations between *P. hamatus s. str*. and *Paratylenchus* sp. 1 and *Paratylenchus* sp. 2 were 35-40 bp (4.5-5.2%) and 14-16 bp (1.8-2.1%), respectively. Phylogenetic relationships between *Paratylenchus* species are given in Figure 17. Four major clades were distinguished in this tree. Relationships between the clades and species were well resolved. Clade I included the *P. hamatus* species complex, *P. nanus* (type A and B), *P. dianthus*, *P. minutus*, *P. lepidus* and *Paratylenchus* sp. 3; Clade II *Paratylenchus* sp. 5; Clade III *P. aculentus* and *P. bilineatus*; and Clade IV *P. aquaticus*.

Subbotin *et al.* (2006) and van Megen *et al.* (2009) included only four *Paratylenchus* species in phylogenetic analysis using the D2-D3 of 28S rRNA gene and 18S rRNA gene sequences, respectively. In our study, we found that the ITS rRNA gene sequences gave better resolution in relationships between species than other rRNA gene fragments. Our present phylogenetic analysis with higher numbers of *Paratylenchus* species included give a new view on congruence of molecular and morphological evolution of this group. After studying numerous *Paratylenchus* slides, Geraert (1965) concluded that there were only a few characters that can be used in species determination for these nematodes, *viz*., stylet length, vulva position, tail and head shape and lateral field, and distinguished ten groups using these characters. These groupings generally correspond to major clades in the phylogenetic trees in our study. In the ITS rRNA gene tree (Fig. 17), Clade I contains representatives of Group III (stylet = 19-36 μm, V = 78-86), namely *P. dianthus*, *P. nanus*, *P. hamatus*, *P. minutus* and *P. lepidus*; Clade IV contains a representative of Group I (stylet = 10-19 μm, V = 79-85) with *P. aquaticus*; and Clade III contains one representative, *P. aculentus*, of Group VI (stylet 54-85 μm; V = 68-79) plus *P. bilineatus* which, however, has a shorter stylet and therefore fits into Geraert's Group V (stylet = 43-50 μm).

According to Siddiqi (2000) *P. aculentus*, *P. bilineatus* and *P. straeleni* belong to the subgenus *Gracilacus*, whereas all other identified species used in the phylogenetic analysis belong to the subgenus *Paratylenchus*. Raski (1962) introduced *Gracilacus* to differentiate those *Paratylenchus* species with a stylet longer than 48 μm. Siddiqi & Goodey (1964) synonymised *Gracilacus* with *Paratylenchus*, although this action was not followed by several authors. In the ITS rRNA gene tree the relationships of the basal clades, in which *P. aculentus* and *P. bilineatus* occurred, are not resolved, and thus the validity of *Gracilacus* cannot be rejected using our datasets.

Geraert (1965) noticed that many of the described *Paratylenchus* species cannot be morphologically distinguished from one another. Our study showed that morphologically similar species might represent a complex of sibling biological species and any synonymisation must be done only after careful molecular comparison. The problems of species identification and delimiting are very acute in nematology and our present molecular study is a starting point for more detail analysis and molecular characterisation of the genus *Paratylenchus*.

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