Description of *Tobriloides loofi* n. sp. from Natal, South Africa (Nematoda: Onchulidae)

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Tobriloides loofi n. sp. has a huge cardia, three whorls of cephalic setae and an onchulid structure of the posterior oesophagus, which place it in the rare genus *Tobriloides* Loof, 1973. It differs mainly from the only other species in this genus, *Tobriloides choii* Loof, 1973, in its large size, the presence of males in *T. loofi* and the position of the vulva (midbody in *T. loofi* and more posterior in *T. choii*). This is the first description of a member of the genus *Tobriloides* from South Africa, as well as the first record of a new species within this genus in 16 years. Line drawings, light- and scanning electron micrographs are included in the text, as well as a short discussion of intergenus relationships of *Tobriloides.*

Tobriloides loofi n. sp. word gekenmerk deur die groot kardia, drie kringe sefaliese setae en die onchuliede (gestratifiseerde) struktuur van die posterior gedeelte van die oesofagus. Hierdie kenmerke plaas *Tobriloides loofi* n. sp., tesame met die enigste ander spesie, *Tobriloides choii* Loof, 1973, in die seldsame genus *Tobriloides* Loof, 1973. Hierdie twee spesies verskil hoofsaaklik van mekaar op grond van die langer liggaam, teenwoordigheid van mannetjies en ekwatoriale ligging van die vulva in *T. loofi*, teenoor die kort liggaam, afwesigheid van mannetjies en die posterior ligging van die vulva in *T. choii.* Hierdie artikel is die eerste beskrywing van 'n spesie van *Tobriloides* uit Suid-Afrika, asook die eerste aanmelding in 16 jaar van 'n nuwe spesie in hierdie genus. Lyntekeninge, lig- en skandeerelektronmikrograwe is ingesluit in die teks, asook 'n kort beskrywing van intergenusverwantskappe van *Tobriloides.*

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Several samples taken from moist soil under indigenous grasses and shrubs at Leisure Bay, Natal South Coast, South Africa, yielded specimens which seemed to represent a new species of *Tobrilus*. On closer examination the huge cardia, onchulid structure of the posterior part of the oesophagus and arrangement of cephalic setae, pointed to the little-known genus *Tobriloides* Loof, 1973, known only from a single species from Korea. This paper is only the second report of the genus *Tobriloides* and presents the first description of males in the genus.

Materials and Methods

Specimens were killed by gentle heat, fixed in FAA, processed to glycerine by Thorne's slow method and mounted on permanent slides. Two paratypes, one female and one male, are deposited in the National Collection of Nematodes, Plant Protection Research Institute, Pretoria. All other mounts are deposited in the collection of the Department of Zoology of the Rand Afrikaans University. Specimens were prepared for scanning electron microscopy (SEM) (Swart & Heyns 1987) and viewed with an ISI SS60 scanning electron microscope. Light microscope photographs were taken with an MC63 photomicrographic camera and differential interference contrast. Measurements and drawings were made with the aid of a Zeiss Standard 18 research microscope equipped with a drawing tube. The body and all curved structures were measured along the median line.

Description

Tobriloides loofi n.sp. (Figures 1A–Q, 2A–F, 3A–D; Table 1)

Female: Body cylindrical, tapering more posteriorly than anteriorly. Body width at mid-body about 1,6 times that of head width. Body curved ventrally when heat-relaxed but some specimens more irregular in posture. Body annules prominent, 1,5–2,5 μm wide. No lateral fields. A lateroventral and laterodorsal row of body setae on either side of body. Setae in each row about 35 μm apart, beginning about 88 μm from anterior end and ending in anal region. No setae observed on tail. One row of ventral setae present, about seven in oesophageal region and again 7–9 in an area from 450 μm anterior of anus to anal region. A few dorsal setae also present, restricted to oesophageal region. Owing to swelling of the cuticle during fixation, the body setae seem to be virtually inside the cuticle (Figure 2F). They are, however, clearly visible in SEM-micrographs.

Head truncate, continuous with body contour. Six large, well-developed lips surrounding the hexagonal oral opening. The incisures around the oral opening are not of equal depth — the ventral and subdorsal incisures being deeper than the dorsal and subventral ones (Figure 1C, 2C). Each lip bears a single short labial seta (Figure 2A). The second circlet of six long (15 μm) cephalic setae are situated posterior to the lips. The third whorl of four cephalic setae (about 6 μm long) occurs more posteriorly, 13–18 μm from anterior, almost at the level of the amphids.

The stoma consists of an anterior, funnel-shaped chamber, about 13,7 μm wide, and a narrower posterior part with two small teeth situated in the subventral walls about 30 μm from the anterior end (Figure 1B & F). These teeth are both forwardly directed. The base of the posterior chamber of the stoma seems to have a tooth-like projection in its dorsal wall (Figure 1B, D & G)

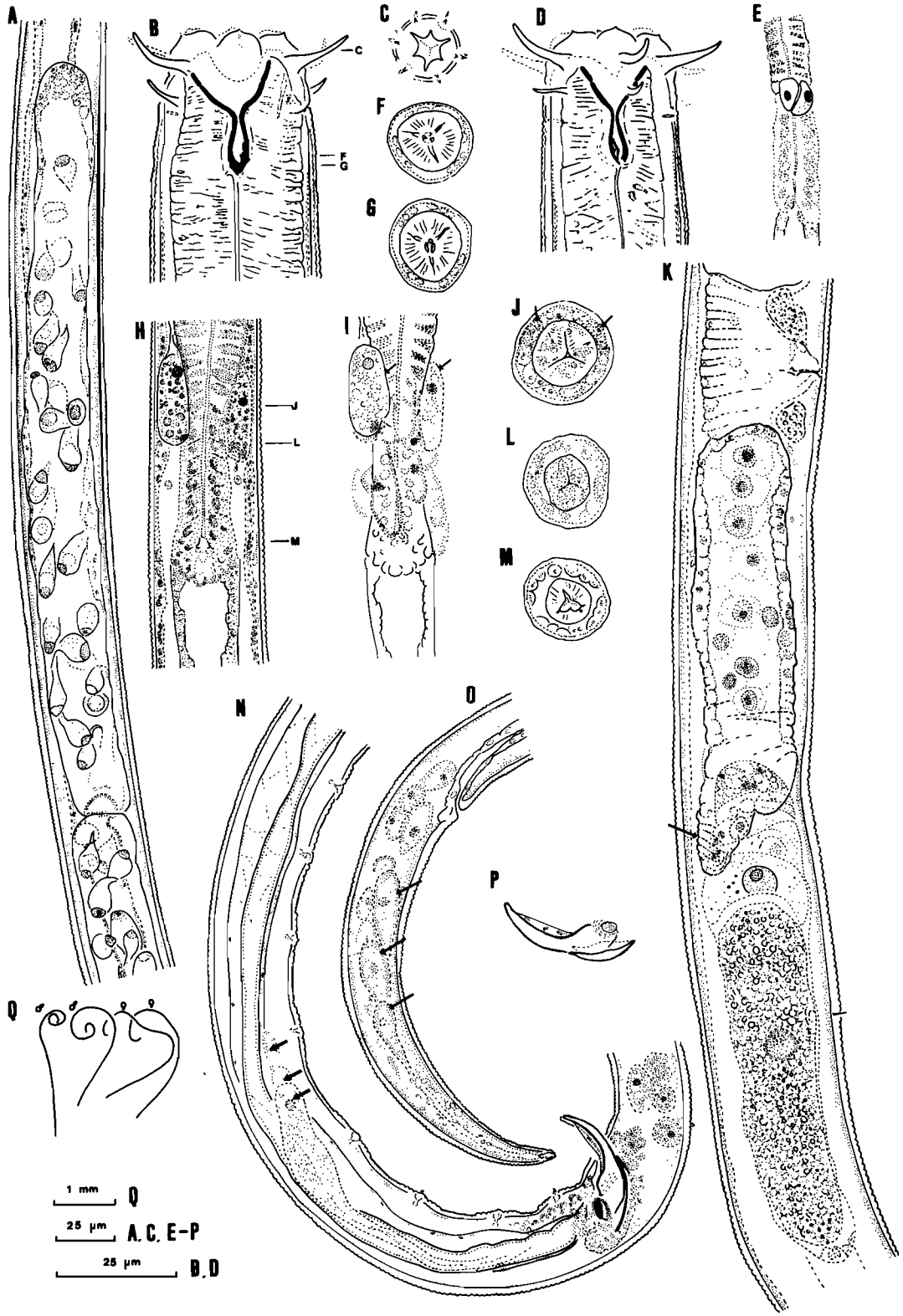

Figure 1 A–D: *Tobriloides loofi* n. sp. A: Anterior testes with spermatozoa, B: Lateral view of female head, C: Face view, showing hexagonal oral opening with longer ventral and subdorsal incisures, D: Lateral view of male head. E: Junction between oesophagus and cardia showing two of the four cardiac glands in *Tobriloides choii* Loof, 1973. F–Q: *Tobriloides loofi* n. sp. F: Cross section at level of two subventral teeth, G: Cross section at level of base of second stomatal chamber, H: Junction between oesophagus and cardia showing glandular cells on each side of junction, I: Junction between oesophagus and cardia of the same individual, showing the glands and cells in this region. Arrows indicate glandular bodies on each side of junction, J: Cross section through body just anterior to junction between oesophagus and cardia. Arrows indicate glandular bodies, K: Posterior branch of female genital track. Arrow indicates oviduct, L: Cross section through cardia showing triradial lumen, M: Cross section through base of cardia showing thickened walls of lumen which is triradiate at this point, N: Male tail region. Arrows indicate ejaculatory glands, O: Female tail. Arrows indicate caudal glands, P: Spicules and gubernaculum, Q: Relaxed body posture.

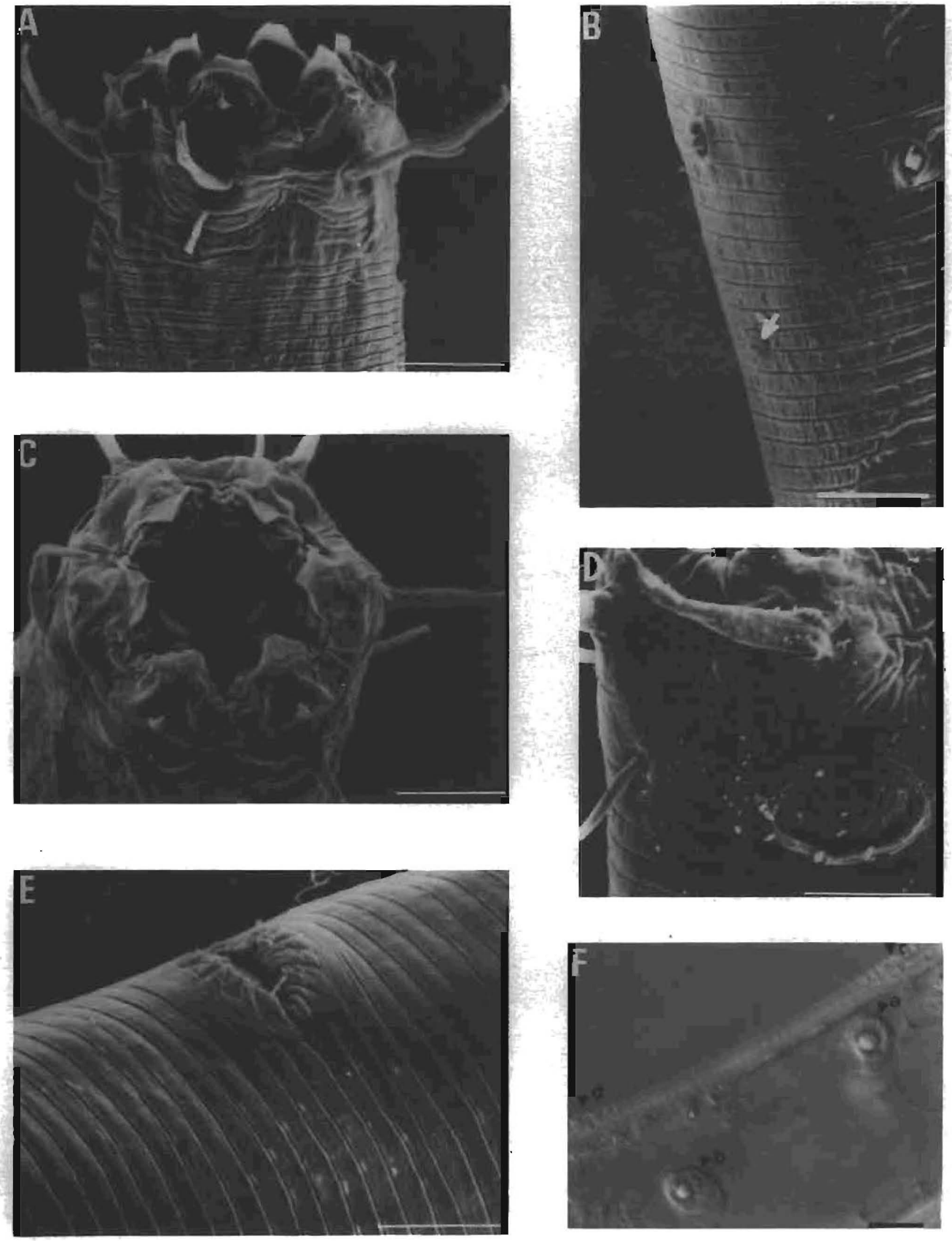

Figure 2 A–F: SEM- and light micrographs of *Tobriloides loofi* n. sp. A: Lateral view of male head, B: Male cuticle in oesophageal region showing the excretory pore (arrow), a ventral papilla and a lateroventral one, C: Face view of female head, showing anterior chamber of stoma, D: Close-up of female head with amphid aperture, long cephalic seta and more posteriorly situated short cephalic seta, E: Vulva, F: Sperm cells (arrows a & b) in female genital tract and retracted body setae (arrows c & d). Bar equals 10 µm.

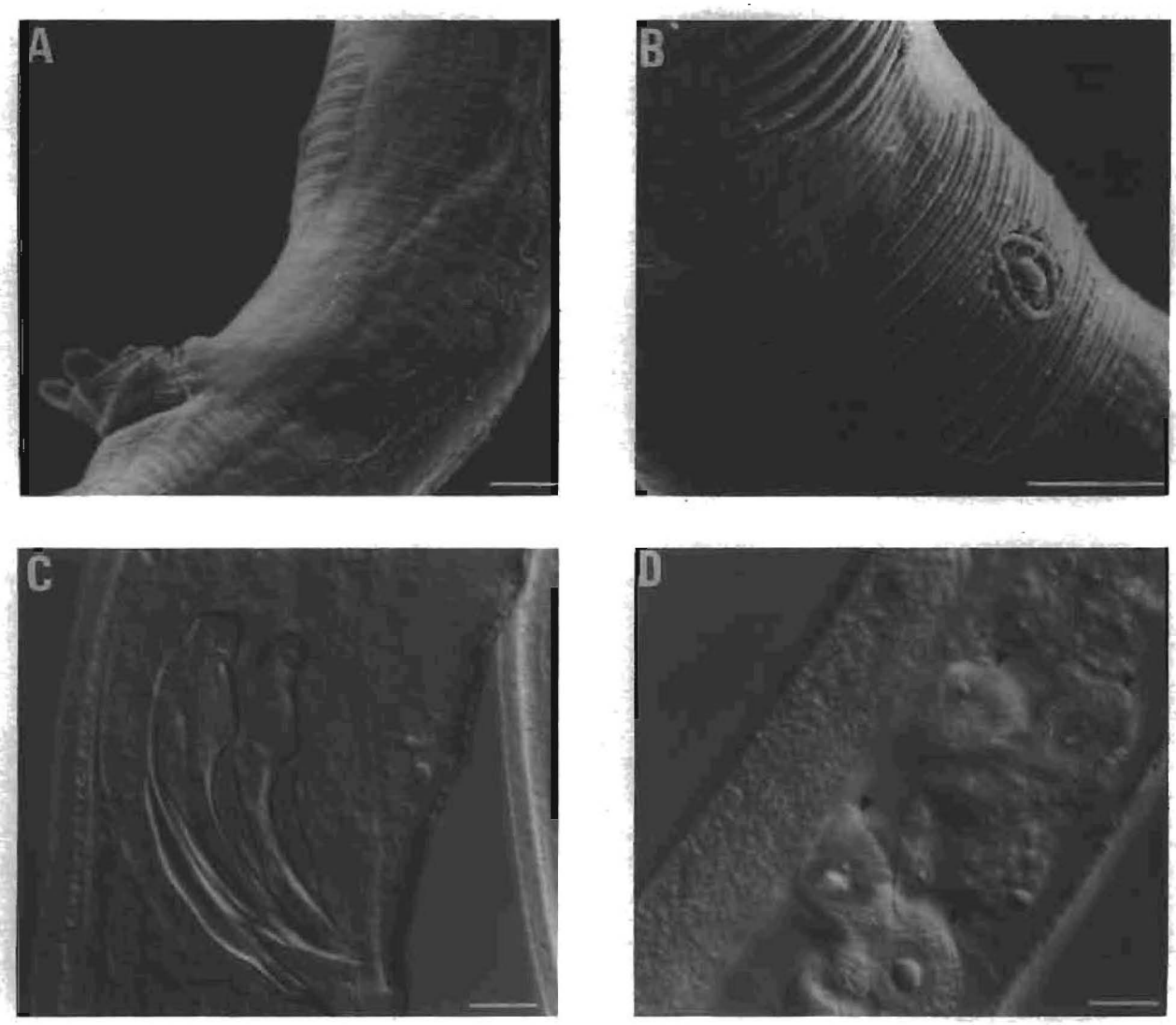

Figure 3 A–D: SEM- and light micrographs of *Tobriloides loofi* n. sp. A: Male tail showing partly protruding spicules and the eighth supplement (S8), B: Subventral view of supplement, C: Spicules, gubernaculum and alae, D: Spermatozoa in testes (arrows). Bar equals 10 μm.

which we interpret as a thickening of the metarhabdions before it fuses with the telorhabdions.

Amphid aperture crescent shaped (Figure 2D), situated about 17,5–20,0 μm from anterior end. Fovea not clearly seen but apparently funnel shaped.

Oesophagus cylindroid, 504–555 μm long, enveloping the stoma. Posterior ⅓ with prominent onchulid structure (transverse muscle bands).

Nerve ring around anterior 23–32% of oesophagus, sometimes difficult to discern. Oesophagus well developed but becoming narrower posteriorly where it fuses with the huge cardia (Figure 1H & I). Cardia about 55–60 μm long, slightly overlapping the intestine (Figure 1H) and containing up to 30 nuclei. Cardia lumen triradiate (Figure 1L), less conspicuous than lumen of oesophagus. The walls of the cardia lumen thicken at the point where it joins the intestine (Figure 1H & M). Some sort of valve may be present at this point as muscles seem to be attached onto the thickened walls of the lumen (Figure 1M).

At the junction between the cardia and the oesophagus, two cell bodies or glands can be seen in more or less lateral positions, 463–517 μm from anterior (Figure 1H & I). These cells have a granular cytoplasm with what seem to be vacuoles. One, and in some individuals, two nuclei can be discerned within the cytoplasm (Figure 1H & I). Up to five vague cells with a much more homogenous cytoplasm were also found surrounding the cardia (Figure 1I). Oesophageal glands could not be seen.

Excretory duct and pore very difficult to see under light microscope (visible in only one individual) but seems to be slightly anterior to nerve ring. Excretory pore seen with SEM, small but well defined (Figure 2B).

Female reproductive system didelphic, amphidelphic. Vulva transverse, oval shaped, with grooved sides (Figure 2E). Vagina short, uterus well developed, usually filled with sperm cells (Figure 1K). Sperm cells large, about 13 μm × 10 μm, their surface covered by minute punctations (Figure 1K & 2F). Oviduct short, consisting of a single row of 6–7 disc-shaped cells (Figure 1K). The oviduct enters the ovary close to the germinal zone (Figure 1K). Growth zone of ovary contains many well-demarcated oocytes in different stages of ripening. No eggs were present in female reproductive system.

Table 1 Morphometrical data of *Tobriloides loofi* n .sp.

	Holotype	Paratypes (n = 3) ♀	Standard deviation	Paratypes (n = 8) ♂	Standard deviation
L	3,0	3,0(2,9–3,2)	0,11	2,9(2,7–3,2)	0,19
a	50,9	57,5(50,9–72,1)	4,57	70,5(66,8–79,1)	4,19
b	5,5	5,8(5,5–6,1)	0,22	5,6(5,2–5,9)	0,23
c	16,2	15,0(14,3–17,4)	1,14	17,4(15,8–19,6)	1,34
c'	5,2	5,7(4,7–6,5)	0,73	4,3(2,9–4,8)	0,67
Tail length (μm)	187,5	192,5(176,3–207,5)	11,71	168,8(153,8–175,0)	8,38
Oesophagus length (μm)	555,5	529,6(503,9–555,5)	20,19	524,9(486,5–552,5)	28,62
Anterior-nerve ring (μm)	130,0	134,8(120,0–166,3)	16,10	130,0(123,8–150,0)	8,31
% Nerve ring	23,4	25,6(23,4–32,2)	3,75	25,6(23,0–30,8)	3,29
Head width (μm)	31,0	31,3(30,0–33,0)	1,03	31,5(30,0–34,0)	1,60
Stoma length (ant. & post. chambers) (μm)	11,0	11,8(8,0–13,0)	2,32	10,8(8,0–12,0)	1,41
Stoma width (μm)	15,0	13,7(7,5–17,0)	3,53	13,5(12,0–17,0)	1,69
Anterior-amphid aperture (μm)	18,5	19,0(17,5–20,0)	0,95	19,4(16,0–21,5)	2,01
Annule width (μm)	2,0	1,9(1,5–2,0)	0,20	2,0(1,5–2,5)	0,34
Cuticle width (μm)	3,1	3,4(3,0–3,5)	0,21	3,0(2,8–3,5)	0,21
T (%)				42,9(39,9–53,7)	4,64
Spiculum length (μm)				54,1(45,0–63,0)	6,09
Gubernaculum length (μm)				30,3(23,0–36,0)	4,29
V%	52,3	51,2(49,5–52,9)	1,35		
OV_1%	41,0	39,8(37,4–41,9)	1,77		
OV_2%	64,1	64,5(61,4–67,4)	2,57		

Tail of female 176–208 μm long, tapering gradually towards the tip, ending in a spinneret (Figure 1O). Tail contains three well developed caudal glands, as well as about eight coelomocytes.

The cuticle 3–3,5 μm thick, appearing in some sections to have a striated subcuticle (Figure 1B & D, 2F, 3C) of which the striations coincide with the annulations on the outer surface.

Male: Description as for female, with the following differences: Body always curved ventrally when heat-relaxed. Posterior ⅓ of body curled into a loose or tight spiral (Figure 1Q). Amphid apertures 16–21,5 μm from anterior end. Oesophagus length 487–553 μm. The two large glandular bodies on either side of junction between cardia and oesophagus 418–506 μm from anterior. Tail length 153–175 μm, slightly shorter than that of female. About five coelomocytes observed in tail region.

Reproductive system diorchic, outstretched (Figure 1A), consisting of two well-developed testes containing numerous spermatozoa (Figure 1A & 3D) and a common spermioduct. Spermatozoa large with a club-like anterior part of 15 μm × 10–15 μm and a tapering posterior part, the length of which is uncertain.

Spermioduct wide and lateral to intestine. No muscular ejaculatory duct present. Spermioduct seems to merge with intestine about 20 μm anterior to anus (Figure 1N).

About six ejaculatory glands can be recognized laterally on either side of the intestine (Figure 1N). The ducts of these glands first run parallel to the intestine, then curve downwards to a more ventral position and open into the cloaca somewhere before the junction between rectum and spermioduct. The exact point of merging is obscured by the spicules and muscles in this area.

Eight, and in one male seven, supplements can be seen which are located midventrally at 31; 60; 95; 131; 163; 196; 229 and 251 μm anterior to the anus (Figure 1N). Each supplement consists of a rounded section about 20 μm long and 15 μm wide with deep transverse grooves (Figure 3A & B). In the centre of this area there is a papillae-like structure surrounded by an elevated ring. In three of the seven males the first supplement (S_1) was only 4 μm in length, whereas S_2–S_8 were each 20 μm long. In the other three males, all supplements, (S_1–S_8) were 20 μm in length (one male had only seven supplements all 20 μm long). The length of the supplements was measured across the rounded section of the supplement along the median line. Under the light microscope a small duct could be seen running inwards from the papilla to a small chamber (Figure 1N & 3C).

Spicules well developed, moderately ventrally curved, 45–54 μm long; lateral alae present; gubernaculum distinct, about 30 μm long. A weak muscular sheath, appearing as a faint ellipsoid ring, surrounds the proximal part of each spicule (Figure 1N). The proximal part of each spicule also surrounded by a cell body which is probably part of the spicule capsule (A. Coomans, pers. comm. 1989).

Holotype: Female on slide RAU 4139.
Paratypes: Four females on slides RAU 4134 and RAU 4139 (collection of RAU) and No. 24730 (National Collection at N.I.P.B.) and eight males on slides RAU 4135, RAU 4137, RAU 4139, RAU 4140 and RAU 4141 (collection at RAU) and No. 24730 (National Collection at P.P.R.I.).

Type locality and habitat: Next to Dolphin Street, Leisure Bay, Natal South Coast, South Africa. Found in moist soil under indigenous grasses, trees and shrubs. Collected by J. Heyns and M. Hutsebaut, September 1988.

Diagnosis

Tobriloides loofi n. sp. can be distinguished from *Tobriloides choii* Loof, 1973 by the following.

The presence of quite a few males in *T. loofi* compared to the absence of males in *T. choii;* the longer female body length (*T. loofi* female: 2,91–3,24 mm compared to *T. choii* female: 1,41–1,77 mm) and the midbody position of the vulva in *T. loofi* (V = 49,5–52,9%) compared to the more posterior position in *T. choii* (V = 56–62%). The tail length also differs: *Tobriloides loofi* has a shorter tail compared to body length (c = 14,26–17,44) against *T. choii* with a c-value of 7,6–9,1. The distal part of the tail in *Tobriloides loofi* is curved in one plain whereas that of *T. choii* is always bent sideways.

When studying specimens of *Tobriloides loofi* n. sp., the authors were surprised to find only one gland cell on either side of the oesophago-cardiac junction, as Loof (1973) observed two pairs (*four* cells) in the same vicinity in *T. choii*. (Fortunately, the authors could examine specimens of *T. choii* and confirmed Loof's observation (Figure 1E). In some individuals of *T. loofi*, however, the two cells each have two nuclei in their cytoplasm (Figure 1H & I) which may suggest a transitional stage between a two-cell state and a four-cell state.

Discussion

The taxonomic position of *Tobriloides* is uncertain because of its similarities to the families Tobrilidae, Onchulidae and Prismatolaimidae (Tsalolikhin 1983). Loof (1973) placed *Tobriloides* in the family Tripylidae Oerley, 1880, subfamily Tobrilinae De Coninck, 1965 because of its cylindrical oesophagus enveloping the stomatal cavity, presence of 'cardiac glands', caudal glands and a spinneret and the shape and anterior location of the amphids. Tsalolikhin (1981) raised the subfamily Tobrilinae to family level. In a revision of this group Tsalolikhin (1983) pointed out that *Tobriloides* has more characters in common with the Onchulidae Andrássy, 1963 than with Tobrilidae and transferred the genus to that family.

The new representative, *Tobriloides loofi* n. sp., adds to the knowledge about the genus, especially since it is the first time males have been found. Some features of the males are close to those of *Kinonchulus sattleri* Riemann, 1972, a member of the Onchulidae. They both possess a non-muscular seminal duct, common to all primitive nematodes (Riemann 1972) and a muscular envelope around the proximal part of each spicule. The club-like spermatozoa with filamentous tails also link the two genera. All these characters place *Tobriloides* firmly in the Onchulidae but its resemblance to Tobrilidae (especially the presence of a spinneret and caudal glands) suggests its descent from an aquatic ancestor.

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References

LOOF, P.A.A. 1973. *Tobriloides choii* n. gen., n. sp. (Tripylidae). *Nematologica* 19: 75–79.

RIEMANN, F. 1972. *Kinonchulus sattleri* n. gen. n. sp. (Enoplida, Tripyloidea), an aberrant freeliving nematode from the lower Amazonas. *Veröff. Inst. Meeresforsch Bremerh.* 13: 317–326.

SWART, A. & HEYNS, J. 1987. Comparative morphology of the head regions of some longidorid nematodes from South Africa using the SEM. *Phytophylactica* 19: 99–101.

TSALOLIKHIN, S.J. 1981. A revision of the genus *Tobrilus* (Nematoda: Tobrilidae). *Zoologischeskii Zhurnal, USSR* 9: 1302–1303.

TSALOLIKHIN, S.J. 1983. World fauna of nematodes of the families Tobrilidae and Tripylidae. Zoological Institute of the Academy of Sciences of the USSR., No. 138. Leningrad, USSR. 232 pp.