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Article

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Two new nematode species from Saldanha Bay, South Africa: Perepsilonema benguelae sp. nov. and Leptepsilonema saldanhae sp. nov. (Desmodorida, Epsilonematidae)

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Abstract

Perepsilonema benguelae sp. nov. and *Leptepsilonema saldanhae* sp. nov. are described and illustrated from coarse sand sediments in Saldanha Bay, along the west coast of South Africa. *Perepsilonema benguelae* sp. nov. is characterised by a large swollen body in the genital region, the annuli are not clearly orientated into anteriorly and posteriorly directed margins and copulatory thorns are restricted to three pairs in the precloacal region. In *Leptepsilonema saldanhae* sp. nov. the somatic setae in the pharyngeal region are very long and the first ambulatory setae of the external subventral row are short. Other distinguishing features include the shape of the amphidial fovea and the copulatory apparatus, and the presence of six ventro-lateral copulatory thorns around the cloaca. These descriptions are the first for the family Epsilonematidae from the west coast of South Africa.

Key words: Description, morphology, Africa, Benguela Current, marine, Nematoda, taxonomy

Introduction

Although many studies have been conducted on the ecology of sandy shores around South Africa (eg Brown & McLachlan 1990), our understanding of the diversity of meiofauna, especially nematodes, is extremely limited. Inglis (1963, 1964) described a collection of nematodes from muddy environments along the west coast of South Africa, including 26 new species, and Coles (1977) described a further nine species from Saldanha Bay. This study reports on two new species of marine nematodes collected from soft sediments in Saldanha Bay.

Both species described here belong to the family Epsilonematidae, first established by Steiner (1927) and revised by Lorenzen (1973). The family currently comprises 13 genera and 96 species (Neira et al 2005), distributed across the globe in shallow and deep waters. Both species are in the subfamily Epsilonematinae, which are typically associated with coarse sediments (Vanreusel & Vincx 1986).

The genus *Perepsilonema* was erected by Lorenzen (1973) who described *Perepsilonema papulosum* and established the distinguishing features of the genus. The genus is now characterised by four subcephalic setae, one pair of setae close to the amphids, the absence of dorsal thorns posterior to the cephalic capsule and the absence of ambulatory setae (Verschelde & Vincx 1993). Thirteen species were recognized in the latest revision (Gourbault & Decraemer 1996).

The genus *Leptepsilonema* was erected by Clasing (1983), and is characterised by having: eight subcephalic setae; body lacking dorsal thorns posterior to the cephalic capsule; five rows of ambulatory setae positioned anterior to the vulva; and six of the eight subcephalic setae anterior to the amphidial fovea (Clasing, 1983). Ten species were recognised in the latest revision (Decraemer & Gourbault, 2000).

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Materials and methods

Samples were collected at a depth of 20 m in Saldanha Bay (33º S, 18º E) from a sediment of sand and coarse gravel (gravel> 35%; sand >35%), using hand-held corers (10 cm² surface area). Nematodes were extracted from the upper 10 cm, separated by elutriation and washing, then mounted in anhydrous glycerine on microscope slides (Warwick *et al*. 1998). Drawings were made using an Olympus-BH2 compound microscope with Nomarski Differential Interference Contrast Illumination and a camera lucida. Morphometric nomenclature used in this manuscript follows Gourbault and Decraemer (1988; 1994) (Table 1). Holotype male and one paratype female of each species are deposited in the nematode collection of the Department of Zoology, Natural History Museum (NHM), London, while other paratypes are deposited at Iziko South African Museum, Cape Town.

TABLE 1. Abbreviations for morphometric analyses, (after Gourbault & Decraemer 1988, 1994). Measurements in µm.

abd	body diameter at level of anus
amph (%)	diameter of amphid as a percentage of the corresponding head diameter
A sl	length of anteriormost ambulatory seta of external subventral row
cs	length of cephalic setae
gub	length of gubernaculum
hl	length of head
hw	maximum head diameter
L	body length
mbd	maximum body diameter in posterior body region
(mbd)	minimum body diameter
mbd ph	maximum body diameter in pharyngeal region
mbd/(mbd)	maximum body diameter divided by minimum body diameter
N	number of body rings
ph	length of pharynx
spic	length of spicules measured along the median line
SS	length of anteriormost supporting seta
SSph	length of subdorsal somatic seta in pharyngeal region
subc s	length of subcephalic seta
t	length of tail
tmr	length of non-annulated tail region
tmr/t	length of non-annulated tail region divided by tail length
V	distance of vulva from anterior end as a percentage of body length
v	distance of vulva from anterior end
a	body length divided by maximum body diameter
b	body length divided by pharynx length
c	body length divided by tail length
c´	tail length divided by body diameter at anus or cloaca.

Descriptions

***Perepsilonema benguelae* sp. nov.**
(Figs 1, 2)

Measurements: Table 2.

Material examined: Holotype male: South Africa, Saldanha Bay, -33.04800°, 17.98350°, coarse sand in shipping channel, sublittoral (20 m), collected August 1999 by MGJ Hendricks (SCUBA-assisted handheld

corers), NHM accession No. 2008:860. Paratypes: one female NHM accession No. 2008:861, five females Iziko South African Museum accession No. SAM A29471.

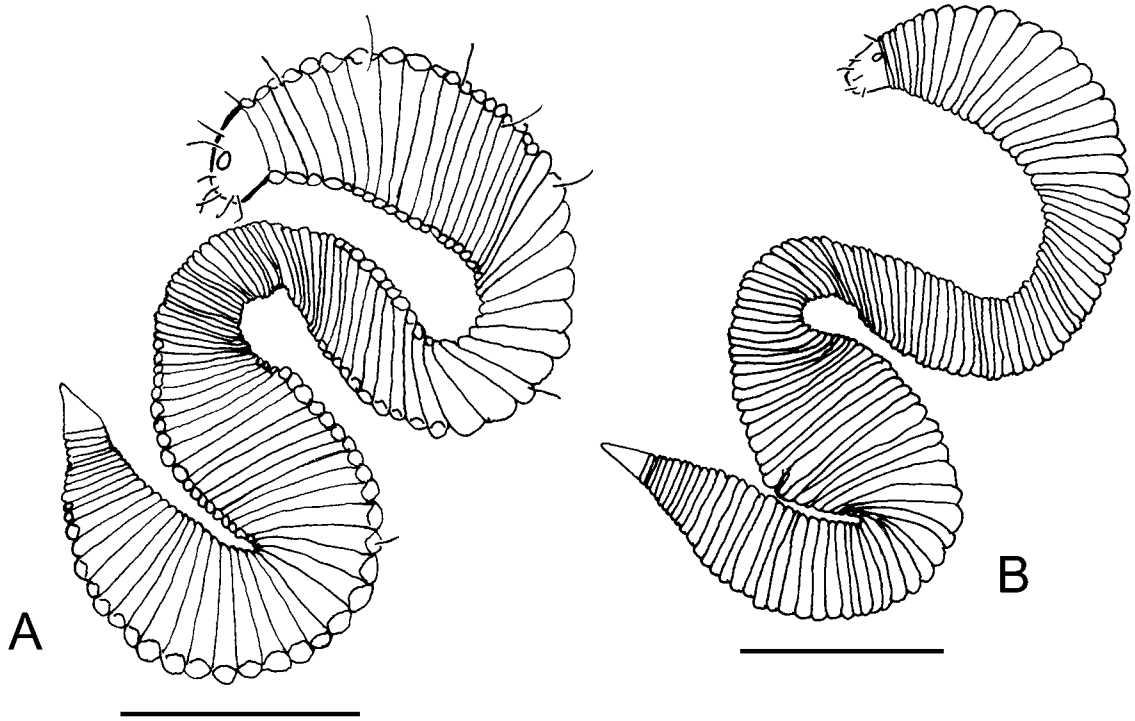

FIGURE 1. *Perepsilonema benguelae* sp.nov. A. Male habitus. B. Female habitus. Scale represents 50 µm.

TABLE 2. Morphometric measurements in µm of *Perepsilonema benguelae* sp. nov. from Saldanha Bay. See Table 1 for abbreviations.

Holotype Male		Females (n = 6)		
		Mean	SD	Range
L	352	321	26.5	266-334
N	118	124	7	112-131
amph	3	3	0.63	2.6-3.9
amph %	37	29	5	23.5-35.3
ph	56	56	2.9	52-59
mbd ph	33	30	2.9	27-36
mbd	39	39	3	33-42
(mbd)	14	15	2.2	12-19
abd	15	16	1.5	15-19
t	21	27	1.8	25-29
tmr	10	11	1.9	8-14
spic	48	-	-	-
gub	7	-	-	-
a	8.9	8.0	0.2	7.7-8.2
b	6.3	5.5	0.3	5.1-6
c	17	11.4	0.9	10.2-12.8
c'	1.3	1.7	0.2	1.3-2
V	-	68	2.9	65-73
mbd/(mbd)	2.9	2.6	0.5	2.2–3.3

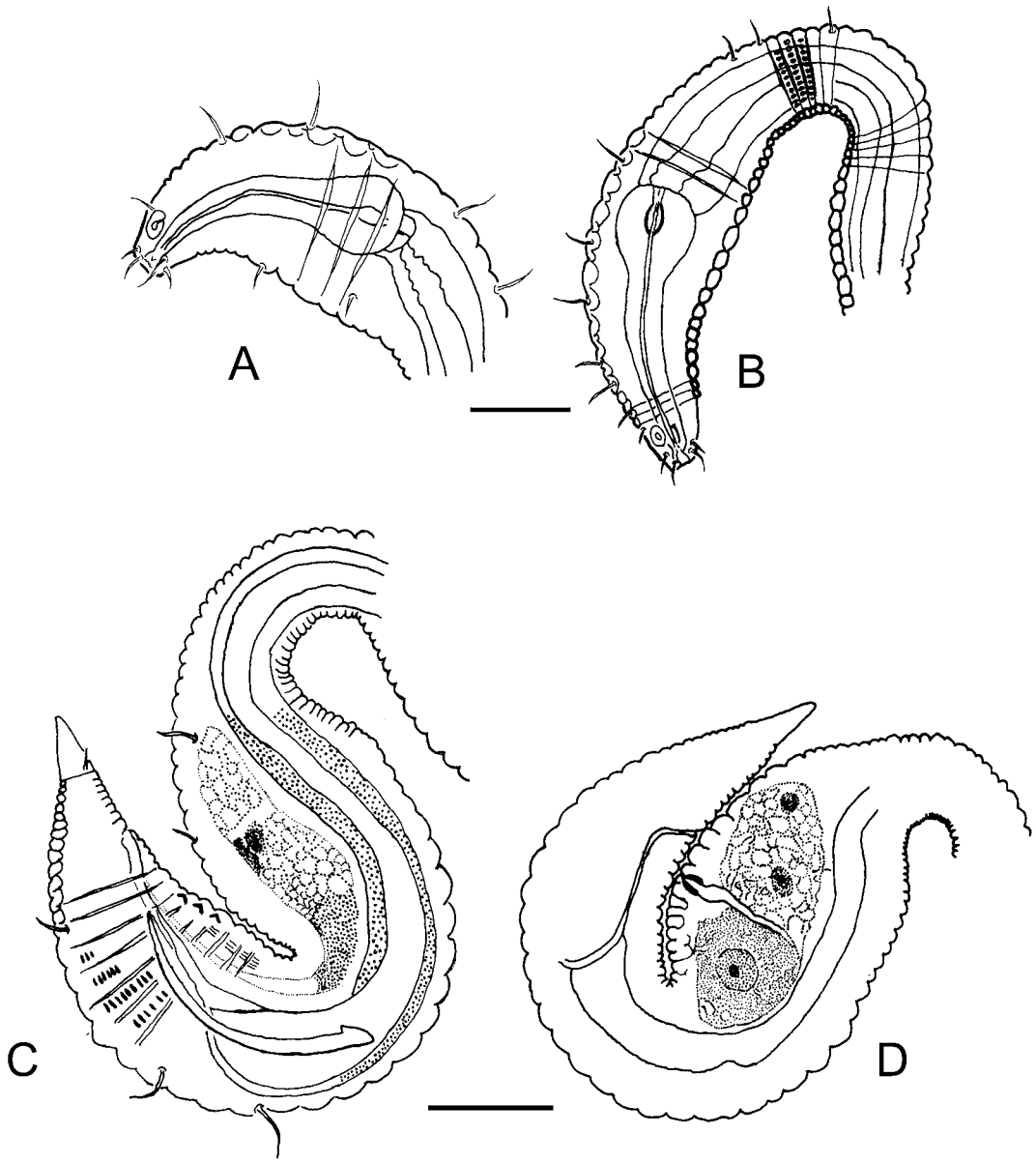

FIGURE 2. *Perepsilonema benguelae* sp.nov. A. Male anterior body region with indication of amphidial fovea and detail of body rings in surface view. B. Female anterior body region with indication of amphidial fovea and detail of body rings in surface view. C. Male posterior body region with reproductive system. D. Female posterior body region with reproductive system. Scale represents 20 µm.

Male. Body epsilon-shaped (Fig. 1A), swollen in cloacal, testis and pharyngeal regions. Cephalic capsule tapering anteriorly, truncated posteriorly; tail short, conical. Body with 118 pronounced annulations (Table 2), not clearly orientated either anteriorly or posteriorly, with prominent box-like vacuoles in swollen portions of body. External layer of cuticle thickened ventrally in testis region, with fine longitudinal markings. Somatic setae (10.3 µm long) regularly spaced in six rows, anterior to the first curvature. Tail short, with one ventrally directed seta (2.6 µm long) on non-annulated section and two short setae (2 µm long) on the last two annulated segments. Three pairs of small, conical, pre-cloacal thorns present.

Cephalic capsule conical, 14 µm in diameter at base and 9 µm long (Fig. 2A). Labial papillae not seen (lip region is retracted in only specimen). Four cephalic setae of equal length (5 µm), not at same level; dorso-lateral pair more anterior to ventro-lateral pair. Eight subcephalic setae on cephalic capsule; one pair present at base of amphidial fovea, longer (6.5 µm) than others (3 µm).

Amphidial fovea at base of cephalic capsule, extending to about 38% of corresponding body diameter, comprising a dorso-ventrally wound loop; canalis situated in centre of spiral fovea. Buccal cavity indistinct, with small tooth; pharynx muscular, 56 µm in length with cuticularised lumen and prominent terminal bulb. Cardia 2.6 µm long, opening into bulbous intestine.

Single outstretched testis ventral to alimentary canal, extending posterior to the narrow middle section of the body (Fig. 2C); vas deferens well defined. Spicules 48 µm long, curved with well-developed "hammer-like" capitulum; velum not observed. Gubernaculum short (7 µm long), parallel to spicules. Two ventral rows of small thorn-like structures present on 7th, 8th and 9th annuli anterior to cloacal opening. Prominent laterally-placed somatic setae (6 µm long) present on 2nd (not shown in Fig. 2C) and 6th annuli anterior to cloaca.

Female. Broadly similar to male in general form, shorter (Table 2). Body wide in anus, ovary and pharynx regions (Fig. 1B). Number and ornamentation of annuli similar to males, but change in orientation pronounced at 88th annulation in the region of the vulva. Cephalic capsule narrower than male, 12 µm wide, 9.5 µm long (Fig. 2B), with amphidial fovea at base. Amphidial fovea different from male, a single coil with canalis situated in centre of spiral fovea; extending to about 29% of corresponding body diameter. Buccal cavity small, one specimen with a minute tooth; pharynx muscular, with prominent terminal bulb.

Reproductive system didelphic, amphidelphic, reflexed; ovaries with tips bent to opposite sides of the intestine (anterior ovary to the right, posterior to the left). Ovarian system ventral to alimentary canal (Fig. 2D). Uterine chamber medially situated, containing a number of sperm cells. Vagina sclerotized along entire length, terminating at uterus, surrounded by constrictor muscle.

Type locality and habitat. Course-grained sediments in Saldanha Bay, at a depth of 20 m.

Diagnosis. *Perepsilonema benguelae* sp. nov. is a medium-sized nematode with eight subcephalic setae; annulations not clearly orientated either anteriorly or posteriorly in the male and with box-like vacuoles, ; tail short, c = 17; short spicules and gubernaculum. The body is similar in length to *P. papulosum*, *P. crassum*, *P. moineaui*, but the annulations fewer than *P. papulosum* and more numerous than *P. moineaui*. copulatory thorns are absent from the mid-body region at level of testis, as in *P. papulosum* and *P. moineaui*, whereas three pairs of copulatory thorns occur in the precloacal region in *P. trauci*. The absence of copulatory thorns at the level of testis in the mid-body region, the presence of three pairs of copulatory thorns in the precloacal region, and the lack of subdorsal post-cloacal caudal spines together with the short spicules and gubernaculum are diagnostic characters for *P. benguelae* sp. nov.

Etymology. The species is named after the Benguela Current, flowing along the west coast of southern Africa.

Genus *Perepsilonema* Lorenzen, 1973

Type species: *Perepsilonema papulosum* Lorenzen, 1973 [Clasing, 1984]
Other species:
Perepsilonema bahiae (Gerlach, 1957) Lorenzen, 1973 syn. *Bathepsilonema bahiae* Gerlach, 1957
P. crassum Lorenzen, 1973
P. trauci Lorenzen, 1973
P. conifer Lorenzen, 1973 *syn. P. conifer lissum* (Lorenzen, 1973) op. Gourbault & Decraemer 1988
P. corsicum Vanreusel & Vincx, 1986
P. mediterraneum Vanreusel & Vincx, 1986
P. longispiculosum Vanreusel & Vincx, 1986
P. coomansi Vanreusel & Vincx, 1986
P. tubuligerum Gourbault & Decraemer 1988
P. kellyae Gourbault & Decraemer, 1988 [Verschelde & Vincx 1994]
P. moineaui Gourbault & Decraemer, 1992
P. ritae Verschelde & Vincx, 1994
P. benguelae sp. nov.

General remarks. A revised taxonomic key to males of the species of the genus is provided: modified from Gourbault and Decraemer (1988).

1 Six subcephalic setae 2
Eight subcephalic setae 3
2 Two fields of poorly developed copulatory thorn-like structures, one in ventral fold, second at level of testis; a few small spines in cloacal region; spicule 68–77 μm long ..... *P. tubuligerum*
Two fields of well-developed copulatory thorn-like structures, one at level of testis, second as precloacal thorns; spicule 31–32 μm long ..... *P. conifer*
3 Six subcephalic setae on cephalic capsule anterior to amphid; two setae at base of amphid inserted on first annulus 4
Eight subcephalic setae anterior to amphid 7
4 Copulatory thorns absent 5
Copulatory thorns present at level of testis or in cloacal region 6
5 Annulations with numerous small vacuoles ..... *P. papulosum*
Annulations with minute vacuoles and additional dorso-longitudinal striations ..... *P. moineaui*
6 Annulations plain; one pair of large copulatory thorn-like structures plus 4 pairs small copulatory thorns in mid-body region ..... *P. kellyae*
Annulations with regular vacuoles; three pairs of copulatory thorn-like structures in precloacal region ..... *P. benguelae* sp. nov.
7 No copulatory thorn-like structures at the enlargement of the median body ..... *P. bahiae*
Copulatory thorn-like structures at the enlargement of the median body 8
8 Two rows of small thorns subdorsally on caudal annulations 9
Two fields of small spines subdorsally on caudal annulations 11
9 Body annulations ornamented with tiny, barely visible vacuoles ..... *P. crassum*
Body annulations with a single row of large vacuoles 10
10 Two fields of subventral copulatory thorn-like structures: 5 pairs at level of testis, and 3 pairs in precloacal region ..... *P. trauci*
Ventral field of tiny spines in region between dorsal and ventral body curvature; two pairs large copulatory thorns at level of testis ..... *P. ritae*
11 Single field of three pairs of subventral copulatory thorn-like structures at level of testis; no precloacal thornlike structures 12
Two fields of copulatory thorn-like structures at level of testis and in precloacal region 13
12 Copulatory thorn-like structures well developed; spicule 64 μm long ..... *P. longispiculosum*
Copulatory thorn-like structures poorly developed; spicule 39 μm long ..... *P. corsicum*
13 Six to seven pairs of subventral copulatory thorn-like structures in testis region; two pairs copulatory thorns in precloacal region; cephalic capsule as wide as long ..... *P. mediterraneum*
Three pairs of subventral copulatory thorn-like structures in the testis region, followed by subventral pair and a single thorn in ventral region anterior to vas deferens, another two pairs subventrally in precloacal region; cephalic capsule longer than wide ..... *P. coomansi*

***Leptepsilonema saldanhae* sp. nov.**
(Figs 3, 4)

Measurements. Table 3.

Material examined. Holotype male: SOUTH AFRICA, Saldanha Bay, -33.04800°; 17.98350, coarse sand in shipping channel, sublittoral (20 m), August 1999 by MGJ Hendricks (SCUBA-assisted handheld corers), NHM accession No. 2008:858. Paratypes: one female NHM accession No. 2008:859, one female Iziko South African Museum accession No. SAM A29472.

Male**.** Body round in cross-section, epsilon–shaped, with swollen pharyngeal and posterior regions (Fig. 3A), and 114 annulations (Table 3) possessing a well-developed hyaline outer layer. Annuli anteriorly directed behind cephalic capsule, changing orientation ventrally at annule 13 (anteriorly to posteriorly), annule 24 (posteriorly to anteriorly) and annule 51 (anteriorly to posteriorly); anteriorly directed annuli change orientation dorsally at annule 23. Size and distribution of vacuoles on annuli variable, indistinct in first annule, of irregular size on anterior swelling, small and distributed as longitudinal bands in narrow

middle region, and as a single row on tail; absent between ambulatory setae. Somatic setae fine, mostly in pharyngeal and posterior regions of body, very long (24–34 µm). Five longitudinal rows of six to 13 ambulatory setae with hooked tips; first seta on annule 69; left external sub-ventral row with 13 collared ambulatory setae (7.4 µm long); left inner sub-ventral row with six setae (6.6 µm long); middle row with 7 setae (6.6 µm long); right inner sub-ventral row with six setae (8.8 µm long); right external sub-ventral row with 13 setae (9.6 µm long). Five thick, ventrally-directed supporting setae occur posterior to the ambulatory setae, arranged as two pairs and a singlet plus a row of three stout lateral supporting setae (3.2 µm long) on the same level as external sub-ventral setae.

TABLE 3. Morphometric measures in µm for *Leptepsilonema saldanhae* sp. nov. from Saldanha Bay. See Table 1 for abbreviations.

	Holotype male	Female NHM	Female SAMA
L	324.6	339	406
N	119	114	126
amph	4.5	3.2	3.9
%	35	21.7	30
cs	7	9	9
subcs	12.3	13	13
hw	14	13	13.6
hl	14.8	13	14.8
phs	32.4		
ss1	31.6	21.3	23.2
ss2	33.6	13	42.7
ss3	23.9	14.7	23.9
ss4	29	22.6	28.7
A sl 1	5.8	11.6	13.6
ph	74	71	85.8
mbd ph	27	27	27
mbd	24.6	18	24.5
(mbd)	16.4	14.8	20
abd	19.4	14.2	15.5
t	29	31	34
tmr	9	12.3	14.8
spic	27		
gub or v	5.9	259	265.6
a	13	16.6	18.8
b	4.4	4.7	4.8
c	11.2	11	12
c’	1.5	2.2	2.2
V		63.7	78.3
mbd/(mbd)	1.5	1.2	1.2

Cephalic capsule truncated, 14.8µm long, 14.2 µm diameter (Fig. 4A), labial region partially extended; with four cephalic setae (7 µm); with six collared subcephalic setae (14 µm), anterior to the amphids. Amphidial fovea dorso-laterally situated, dorsally looped in an inverted open U-shape, dorsal arm stretching into the first annule, diameter about 35% of maximum corresponding body diameter. Buccal cavity lacking

teeth or denticles; pharynx terminating in rounded muscular bulb with strongly cuticularized lumen walls. Tail conical, with 8 annuli; three caudal setae present; no setae on non-annulated region; caudal glands not seen.

Single, outstretched testis with large sperm cells opening into short granular vas deferens (Fig. 4C), ventral and partly to left of intestine in thickened posterior region of the body, behind ventral body curve. Spicules paired, arcuate , relatively slender with enlarged proximal ends forming a capitulum. Gubernaculum short, straight, 5.8 µm long. A field of four ventro-lateral copulatory thorns present on annuli surrounding the cloaca, with short supporting setae; two small subventral precloacal thorns.

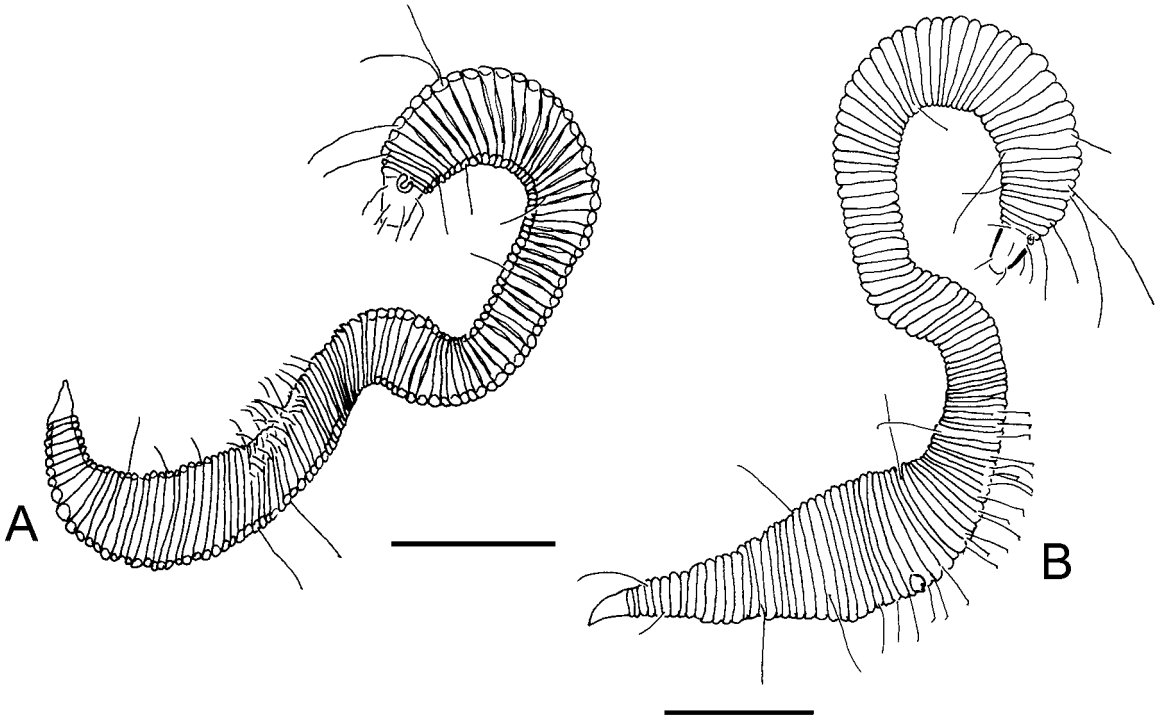

FIGURE 3. *Leptepsilonema saldanhae* sp.nov. A. Male habitus. B. Female habitus. Scale represents 50 µm.

Female. Similar to males in habitus (Fig. 3B). Cuticle with 114 annuli; vacuolar ornamentation generally as males but dorsal spiny projections more pronounced posteriorly. Amphid shape different from male, a ventrally wound spiral with 1.5 turns (Fig. 4B), diameter about 30 % of maximum corresponding body diameter. Reproductive system didelphic, amphidelphic; ovaries reflexed (anterior ovary bent to left side, posterior ovary to right side) ventral to intestine (Fig. 4D). Vagina 12.5 µm long, ending in cuticularized outer part (2 µm long) and larger, weakly cuticularized inner part. Vulva situated ventrally in posterior body half.

Diagnosis**.** *Leptepsilonema saldanhae* sp. nov. is characterised by the following combination of characters: in the male, the shape of the amphid is a dorsally looped inverted U-shape with dorsal arm overlapping the first body annule, whilst in the female it is smaller, and spiral; the anteriormost ambulatory setae of external subventral row (A sl1) are short in the male, and longer in the female; the ambulatory setae are bent; there are six prominent subcephalic setae anterior to, and two setae posterior, to the amphidial fovea, all embedded in a marked collar; the shape of the copulatory apparatus; the six ventro-lateral copulatory thorns on both sides of the cloaca; and the presence of two small postcloacal thorns and a short supporting seta/spine present at the cloacal opening. The long subdorsal somatic setae in the pharyngeal region are also diagnostic. *L. saldanhae* sp. nov. is similar to *L. dauvini* in overall body length and amphidial size, and spicule and gubernaculum lengths, but differs in sexual dimorphism of the amphid, and the number of precloacal thorns. It is similar to *L. filiforme* in body ratios (a, b and c) and amphidial size, but *L. saldanhae* sp. nov. differs from *L. filiforme* in size of spicules and gubernaculum, number of annulations, length of pharynx, and number and arrangement of copulatory structures.

Etymology. The species is named after the type locality, Saldanha Bay, on the west coast of South Africa.

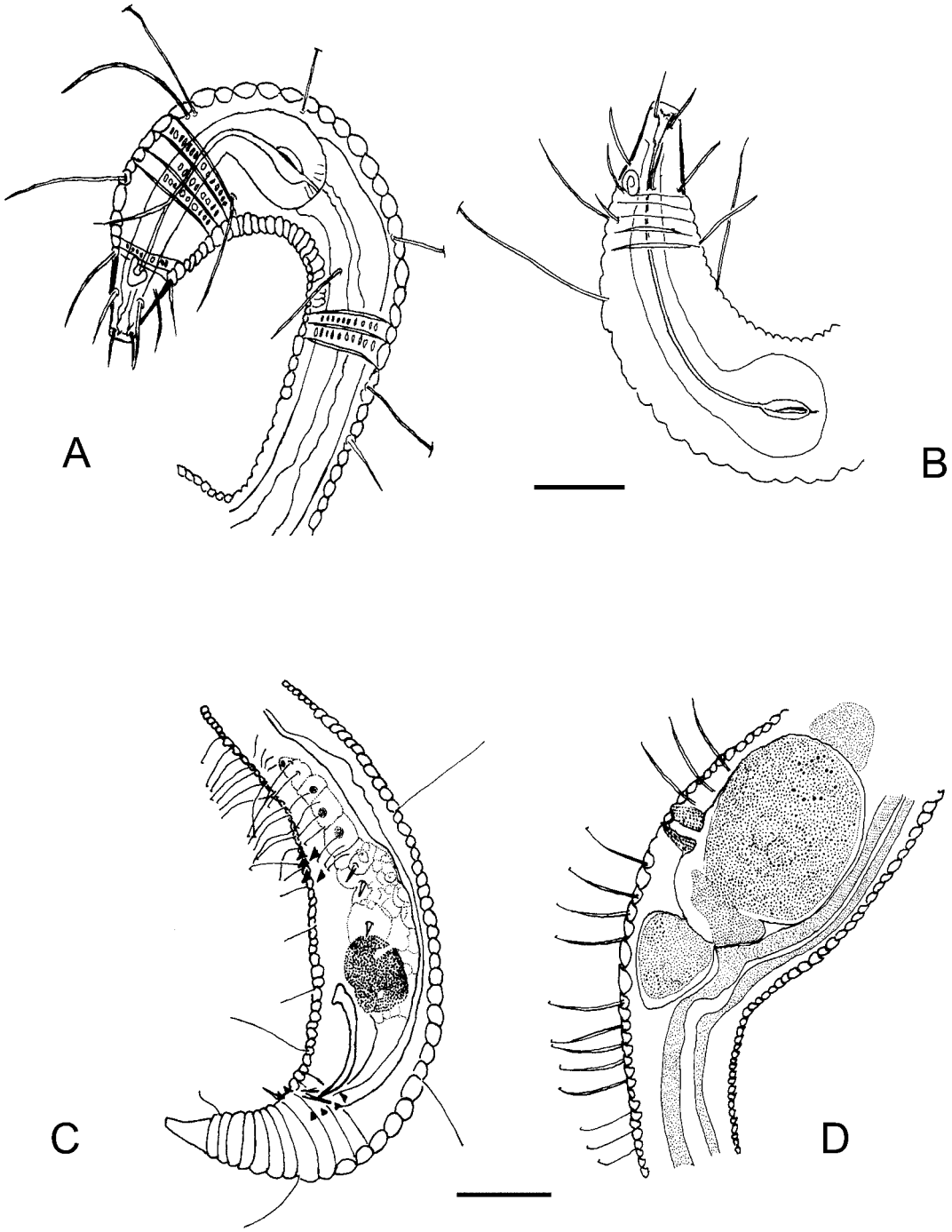

FIGURE 4. *Leptepsilonema saldanhae* sp.nov. A. Male anterior body region with indication of amphidial fovea and detail of body rings in surface view. B. Female anterior body region with indication of amphidial fovea and detail of body rings in surface view. C. Male posterior body region with reproductive system. D. Female posterior body region with reproductive system. Scale represents 20 μm.

Genus *Leptepsilonema* Clasing, 1983

Type species: *Leptepsilonema procerum* Clasing, 1983

Other species:

L. macrum Clasing, 1983

L. exile Clasing, 1983

L. parafiliformе Gourbault & Decraemer 1987
L. filiformе Clasing, 1984 [Gourbault & Decraemer, 1987, 1995]
L. santii Gourbault & Decraemer, 1995
L. richardi Verschelde & Vincx 1992
L. antonioi Decraemer & Gourbault, 2000
L. dauvini Decraemer & Gourbault, 2000
L. horridum Decraemer & Gourbault, 2000
L. saldanhae sp. nov.

General remarks**.** A revised taxonomic key to the males of the species of the genus is provided: modified from Gourbault and Decraemer (1987).

1	Spines present behind cephalic capsule, dorsally	*L. macrum*
	Dorsal spines absent	2
2	Body short (< 400 μm); spicules < 30 μm long	3
	Body long (> 400 μm); spicules > 30 μm long	6
3	Amphid similar in males and females	4
	Amphid sexually dimorphic	5
4	Amphid a curved arch; annulations with heterogeneous vacuoles, rectangular anteriorly, elongated in mid-body region and large and irregular posteriorly; 2 pairs of small copulatory thorns at posterior region of ambulatory setae; without precloacal thorns	*L. parafiliformе*
	Amphid a spiral with flap; annulations with numerous fine vacuoles; field of 5 copulatory thorns at posterior region of ambulatory setae; 5 minute precloacal thorns present	*L. dauvini*
5	Annulations with double row of irregularly shaped vacuoles; anteriormost annulations with a single row of vacuoles; a pair of enormous copulatory thorns at level of ambulatory setae; 3 pairs of small pre-cloacal thorns; without post-cloacal thorns	*L. filiformе*
	Annulations with single row of irregularly shaped vacuoles; vacuoles indistinct in anteriormost annulations; field of 5 copulatory thorns and 3 spines at level of ambulatory setae; 4 subventral thorns flanking cloaca; 2 small subventral precloacal thorns	*L. saldanhae* sp. nov.
6	Copulatory thorns in one field	7
	Copulatory thorns in two or more fields	9
7	Annulations with one row of vacuoles	8
	Annulations with more than one row of vacuoles	*L. exile*
8	Annulations with vacuoles of variable size; 2–5 large copulatory spines at level of ambulatory setae	*L. procerum*
	Annulations with large vacuoles; 7–9 copulatory thorns at level of ambulatory setae	*L. antonioi*
9	Copulatory thorns in two fields	10
	Copulatory thorns in three fields	*L. santii*
10	Annulations with small rectangular vacuoles; 4–5 copulatory thorns in 4 rows at level of ambulatory setae comprising first field; 1 precoacal, 2 post-cloacal thorns present in second field	*L. richardi*
	Annulations with large rectangular vacuoles; 16–30 copulatory thorns in 4 rows at level of ambulatory setae comprising first field; 1–2 copulatory thorns posterior to ambulatory setae in second field, without post-cloacal thorns	*L. horridum*

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