



Two new species of free-living marine nematodes of the family Oxystominidae Chitwood, 1935 (Enoplida) with a review of the genus *Thalassoalaimus* de Man, 1893 from the Argentine coast

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Abstract

Two new free-living marine nematodes of the family Oxystominidae are described from San Antonio Bay (Río Negro) and San José Gulf (Chubut). *Litinium australis* sp. n., is characterized by having a rounded tail, by the first and second crown of cephalic setae with different lengths, gubernaculum with apophysis and by the presence of at least four preloacal papillae; *Thalassoalaimus nestori* sp. n., is characterized by having a conical tail, cephalic setae equal in length, gubernaculum with rounded and dorso-caudally directed apophysis and two preloacal papillae. An emended diagnosis of the genus *Thalassoalaimus* and a key to species are given.

Key words: Patagonia, benthic, description, systematics, key

Introduction

During an ecological and taxonomic study of the meiobenthos from Argentine coasts new free-living marine nematodes were found.

In this work we describe two species belonging to the family Oxystominidae Chitwood, 1935. The species of this family consist of small nematodes, with tiny buccal cavity, polymorphic amphids, and pharynx anteriorly not attached to body cuticle (Smol & Coomans 2006). This family is composed of three subfamilies: Halalaiminae De Coninck, 1965 with the genera *Halalaimus* de Man, 1888 and *Cricohalalaimus* Bussau, 1993; Paroxystomininae De Coninck, 1965 with the genera *Paroxystomina* Micoletzky, 1924 and *Maldivea* Gerlach, 1962; and Oxystomininae Chitwood, 1935 with five genera: *Litinium* Cobb, 1920, *Nemanema* Cobb, 1920, *Oxystomina* Filipjev, 1921, *Thalassoalaimus* de Man, 1893 and *Wieseria* Gerlach, 1956.

The genus *Thalassoalaimus* was established by de Man (1893), with the type species *T. tardus* de Man, 1893. Wieser (1953) carried out the most recent revision of this genus. He provided a key to 10 species: *T. tardus*; *T. egregius* Steiner, 1916; *T. rotundicaudatus* Filipjev, 1927; *T. oxycauda* Filipjev, 1927; *T. septentrionalis* Filipjev, 1927; *T. spissus* Allgén, 1932; *T. filiformis* Allgén, 1933; *T. tenuis* Allgén, 1935; *T. macrosmaticus* Wieser, 1953; and *T. montemari* Wieser, 1953. *T. aquaedulcis* Schneider, 1940 was not included in the revision because it is a freshwater species. Several species have been described since the revision of Wieser: *T. brasiliensis* Gerlach, 1956 and *T. impar* Gerlach, 1962 both from the coast of Brazil; *T. pacificus* Murphy, 1965; *T. setosus* Timm, 1967; *T. pirum* Lorenzen, 1969; *T. aceratus*, *T. brevicaudatus*, *T. longicaudatus*, *T. mediterraneus* and *T. paramontemari* described by Vitiello in 1970 from Golfe du Lion; and *T. lissus* Gagarin, 2009.

Cobb (1920) established the genus *Litinium*, with the type species *L. aequale*. A revision of this genus was carried out recently by Tchesunov *et al.* (2014). They emended the generic diagnosis and presented a pictorial key with 10 species.

We describe one new species of the genus *Litinium* and one new species of the genus *Thalassolaimus*. A list of species of both genera, along with an emended diagnosis and key to species of *Thalassolaimus* are given.

Materials and methods

Description of study sites. Samples were collected from: San Antonio Bay, Río Negro province, during the summer of 2009 and San José Gulf, Chubut province, during the spring of 1984 (Figure 1).

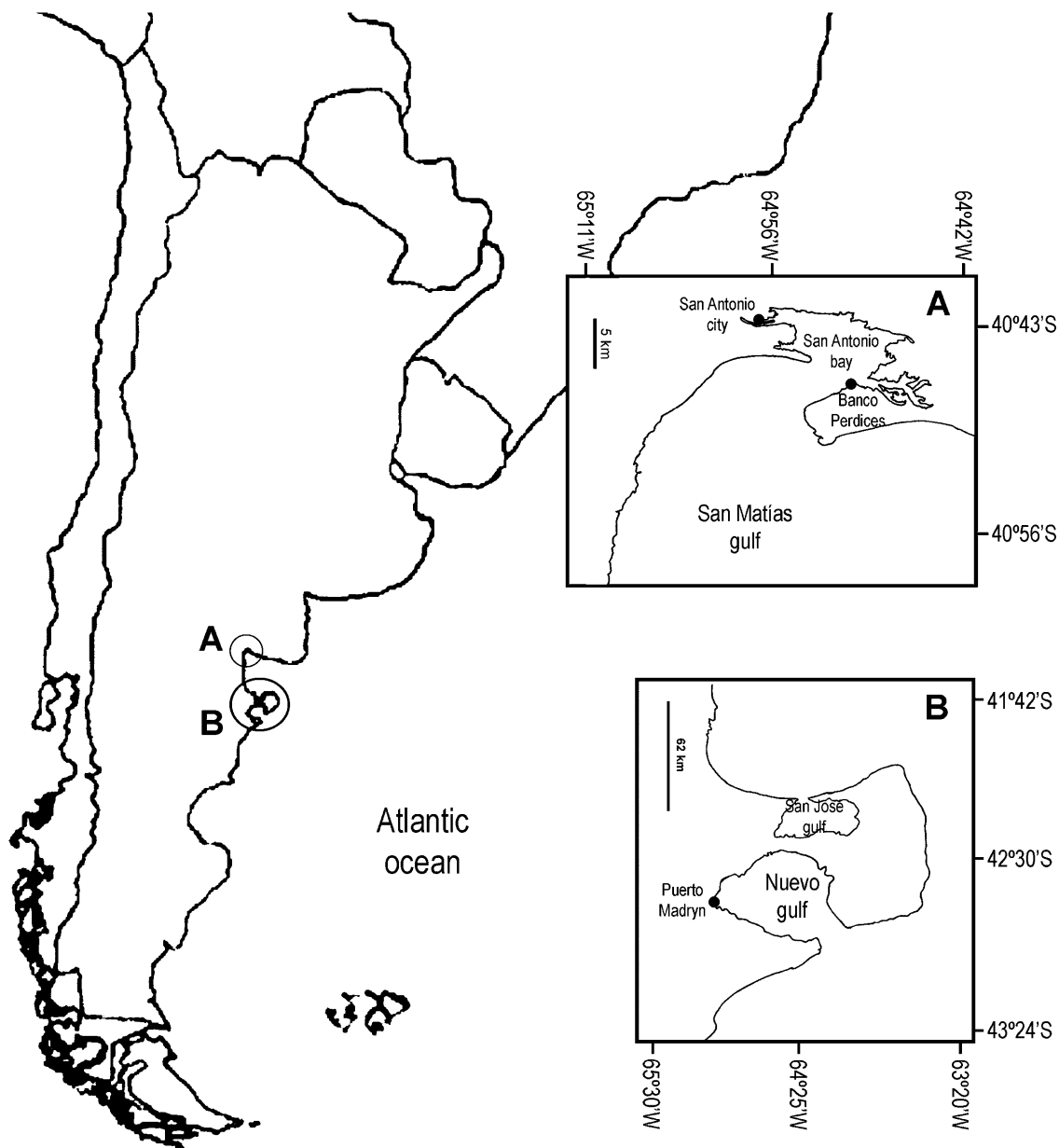


FIGURE 1. Map showing the study sites at San Antonio Bay (A) and San José Gulf (B).

San Antonio Bay is located in the northwest part of San Matías Gulf. The specimens were collected in “Banco Perdices” beach ($40^{\circ}47'S$; $64^{\circ}51'W$) and in front of the city of San Antonio ($40^{\circ}43'S$; $64^{\circ}57'W$).

San José Gulf is located in the northern part of Península Valdés ($42^{\circ}14'$ to $42^{\circ}26'$ S; $64^{\circ}02'$ to $64^{\circ}36'$ W). The Gulf is 43 km long and 20 km wide and has an average depth of 30 m with a maximum depth of 85 m. Outside the entrance to the gulf, the depth increases abruptly to 200 m.

Sample collection and treatment. The littoral samples were collected with a cylindrical Plexiglas corer, 10 cm high and 2.9 cm in diameter. The sublittoral samples were obtained with a van Veen bottom grab sampler. When the grab was on board a vertical cylindrical Plexiglas corer was used to take subsamples (10 cm deep and 1.4 cm in diameter) from the top of the grab. All the samples were preserved in 5% formaldehyde in filtered seawater, and then were sieved through both 500 μm and 50 μm mesh sieves.

The nematodes present on the 50 μm screen were separated and transferred to pure glycerin through a solution of ethanol:water:glycerin in 2:2:1 proportions, and left at least one week in a desiccator. After that they were mounted on glass slides sealed with CANADAX resin.

Specimen analysis. Morphometric data were obtained from camera lucida drawings using a Zeiss Standard WL microscope D-7082 Oberkochen with differential interference contrast (DIC). The measurements are given in microns. Photographs were taken with Olympus microscopy equipment with DIC equipped with a Nikon D80 digital camera. Literature has been obtained from *NeMys* (Guilini *et al.* 2016). Holotypes and some paratypes were deposited in the CNP-NEM collection in the Centro Nacional Patagónico, Chubut, Argentina. Other paratypes were deposited in the National Collection of Invertebrates of the Museo Nacional de Ciencias Naturales ‘Bernardino Rivadavia’, Argentina. De Man’s ratios *a*, *b* and *c* used in this paper were calculated as usual.

Abbreviations: abd, anal body diameter; Amph %, amphid diameter as percentage of corresponding body diameter; Bd, body diameter in μm ; c.d., cephalic diameter; Cs%, cephalic setae length as proportion of head diameter; L, body length in μm .

Results

Thalassoalaimus de Man, 1893, emended diagnosis

Oxystominidae, Oxystomininae. Labial sensilla setiform, in circles of six inner and four or six outer on the anterior end. A circle of four cephalic setae more posteriorly. Amphideal fovea large and pocket-shaped. Males usually have papilliform precloacal supplements. Tail conical or conico-cylindrical. Thick cuticular lining, also called the caudal capsule, at the tail tip. Marine.

Type species: *T. tardus* de Man, 1893 (8 species).

Remarks. As was pointed out by Tchesunov *et al.* (2014), the family Oxystominidae has two morphologically similar genera, *Litinium* and *Thalassoalaimus*. In their emended diagnosis of *Litinium*, one of the most important characters defining the genus is the absence of a caudal capsule. Hence, many putative species of *Thalassoalaimus* seem to be placed in the wrong genus because they have more characters of *Litinium* than of *Thalassoalaimus*. We present here revised species lists for both genera along with a list of *species inquirendae* for *Thalassoalaimus*.

Species list of *Thalassoalaimus*

Thalassoalaimus brasiliensis Gerlach, 1956

Thalassoalaimus lissus Gagarin, 2009

Thalassoalaimus macrosmaticus Wieser, 1953

Thalassoalaimus mediterraneus Vitiello, 1970

Thalassoalaimus nestori **sp. n.** (present paper)

Thalassoalaimus pacificus Murphy, 1965

Thalassoalaimus septentrionalis Filipjev, 1927

Thalassoalaimus tardus de Man, 1893

We consider here *T. oxycauda*, *T. rotundicaudatus*, *T. filiformis*, *T. tenuis*, *T. aquaedulcis*, *T. montemari*, *T. impar* and *T. brevicaudatus*, as *species inquirendae* because all of them are poorly described and/or are based only on one or two female specimens.

Thalassoalaimus septentrionalis spissus Allgén, 1932 and *T. spissus* Wieser, 1953 were considered by Leduc & Gwyther (2008) as synonyms of *T. septentrionalis* Filipjev, 1927. Although not considered synonymous, *T.*

septentrionalis spissus and *T. mediterraneus* are very close to each other. They share equal body and tail length, similar de Man ratios, similar lengths of labial and cephalic setae, and equal distances from the anterior end to the cephalic setae. The reproductive apparatus is also similar: in both species, spicule and gubernaculum lengths are equivalent.

Species list of *Litinium*

Litinium abyssorum Tchesunov, Nguyen & Nguyen, 2014

Litinium aceratus (Vitiello, 1970) comb. n.

Litinium aequale Cobb, 1920

Litinium australis **sp. n.** (present paper)

Litinium bananum Gerlach, 1956

Litinium curticauda Tchesunov, Nguyen & Nguyen, 2014

Litinium egregius (Steiner, 1916) comb. n.

Litinium longicaudatus (Vitiello, 1970) comb. n.

Litinium obtusilobus Bussau, 1993

Litinium paramontemari (Vitiello, 1970) comb. n.

Litinium parmatum Wieser, 1954

Litinium pirum (Lorenzen, 1969) comb. n.

Litinium profundorum Tchesunov, Nguyen & Nguyen, 2014

Litinium quangi Tchesunov, Nguyen & Nguyen, 2014

Litinium setosus (Timm, 1967) comb. n.

Litinium subterraneum Tchesunov, Mokievsky & Thanh, 2010

Litinium volutum Gerlach, 1962

Remarks on the new species of the genus *Litinium*. The new species combinations are made according to the emended *Litinium* diagnosis of Tchesunov *et al.* (2014) respecting the absence or weak development of the caudal capsule, shape of the amphidial fovea, and a non-clavate tail.

Descriptions of new species

Litinium australis **sp. n.**

(Figures 2 & 4 (A–C); Table 1)

Systematic position. Oxystominidae Chitwood, 1935: Oxystomininae Chitwood, 1935: *Litinium* Cobb, 1920

Type material. Holotype: adult male. Registration number CNP-NEM 1528; type locality: San José gulf; coordinates: 42°21'S, 64°08'W; sublittoral, mud sediments (59.8 m water depth). Collected by C.T. Pastor de Ward, 22 August 1984.

Paratype: adult female. Registration number CNP-NEM 1529. Same data as holotype.

Other material: One male and one juvenile. Registration number MACN-In 40898. Same data as holotype.

Etymology. In reference to the name of the sailboat, *El Austral*, with which the sample was collected.

Measurements. See Table 1.

Description. Male (holotype): Cylindrical body, tapering slightly towards the anterior end ($L = 2495 \mu\text{m}$). Cuticle smooth. Somatic setae not seen. Buccal cavity small and narrow. Cephalic sensilla present in $6 + 6 + 4$ circles. Six inner labial setae, $3.2 \mu\text{m}$ long, and six outer labial setae, $4 \mu\text{m}$ long. A crown of four cephalic setae ($4 \mu\text{m}$ in length) at 2 c.d. from anterior end. Amphid and amphidial pouch conspicuous, situated $4 \mu\text{m}$ from anterior end. Amphidial fovea $6 \mu\text{m}$ wide and $9 \mu\text{m}$ long. Cephalic diameter $11 \mu\text{m}$ at level of amphid. Amphid width about 55% of the corresponding head diameter. Excretory pore located at $31 \mu\text{m}$ (3.9 c.d.) from anterior end.

Pharynx cylindrical ($315 \mu\text{m}$ long), ending in a small weakly-developed bulb ($18 \mu\text{m}$ in diameter). Body diameter at the base of pharynx $27 \mu\text{m}$.

TABLE 1. Measurements (μm) of *Litinium australis* sp. n.

	Holotype Male	Paratype Female	Paratype Male	Paratype Juvenile
n	-	1	1	1
L	2495.00	3455.00	2880.00	2935.00
a	95.96	90.92	102.86	97.83
b	7.92	8.97	6.62	7.62
c	86.03	107.97	99.31	91.72
Anterior cephalic setae, length	3.20	3.50	3.50	3.00
Posterior cephalic setae, length	4.00	3.50	4.50	3.50
Sub-cephalic setae, length	4.00	4.50	4.00	4.50
Cephalic diameter	8.00	8.50	8.50	8.00
Cs%	50.00	41.18	52.94	43.75
Amphid from anterior end	4.00	3.00	4.00	not seen
External amphid width	6.00	6.00	6.00	-
External amphid length	9.00	10.00	9.50	-
Bd at amphid level	11.00	10.00	11.00	-
Amph%	54.55	60.00	54.55	-
Nerve ring from anterior end	140.00	140.00	190.00	117.00
Oesophageal bulb diameter	18.00	31.20	28.00	23.00
Bd at oesophageal bulb level	27.50	37.00	34.50	29.00
Pharynx length	315.00	385.00	435.00	385.00
Maximum body diameter	26.00	38.00	28.00	30.00
Anterior end to anus, distance	2466.00	3423.00	2851.00	2903.00
Anal body diameter	21.00	28.00	23.00	25.00
Tail length	29.00	32.00	29.00	32.00
c'	1.38	1.14	1.14	1.28
Spicule length	34.00	-	29.00	-
Gubernaculum length	13.00	-	12.00	-
Vulva from anterior end	-	700.00	-	-
Vulva from anterior end/L (%)	-	20.26	-	-

Reproductive system diorchic, testes opposed. Anterior and posterior testes situated to the left of the intestine. Spicules equal in length, 34 μm long, arcuate and cephalated (1.6 abd). Gubernaculum tubular, corpus 13 μm long, with apophysis, and capitulum 5 μm long. At least four precloacal organs observed, with one seta in each. The two closest to the cloaca are more conspicuous and the others are tiny. The first precloacal organ lies 32 μm anterior to the cloaca and has a seta 3 μm long. The other precloacal organs are 93 μm , 120 μm and 202 μm from the cloaca, each one with a 3 μm long seta. Tail short and round, 29 μm long, without caudal capsule.

Female (paratype): Female larger ($L = 3455 \mu\text{m}$) but similar to male in general body shape. One posterior ovary located on the right side of intestine, antidromously reflexed. Vulval orifice ventral, located at 20% of body length. Uterus and oviduct 2675 μm long and the antidromously reflexed ovary 200 μm in length. Vagina short and not thickened.

Differential diagnosis. *Litinium australis* sp. n. is characterized by the first and the second crown of setae of differing lengths, rounded tail, gubernaculum with apophysis, and by the presence of four precloacal papillae.

Litinium australis sp. n. resembles, in the similar shape and length of the tail (short and rounded): *L. aequale*, *L. curticauda*, *L. obtusilobus*, *L. profundum*, *L. quangi*, *L. volutum* and the recently-transferred species *L. egregius*, *L. paramontemari*, *L. pirum* and *L. setosus*. *L. australis* differs from *L. aequale*, *L. curticauda*, *L. profundum*, *L.*

quangi, *L. egregius*, *L. paramontemari* and *L. setosus* in the number of preloacal organs. *L. pirum* and *L. volutum* have as many preloacal organs as *L. australis*, which differs from these species, and from *L. obtusilobus*, in amphid shape and size and in length of the inner and outer labial setae.

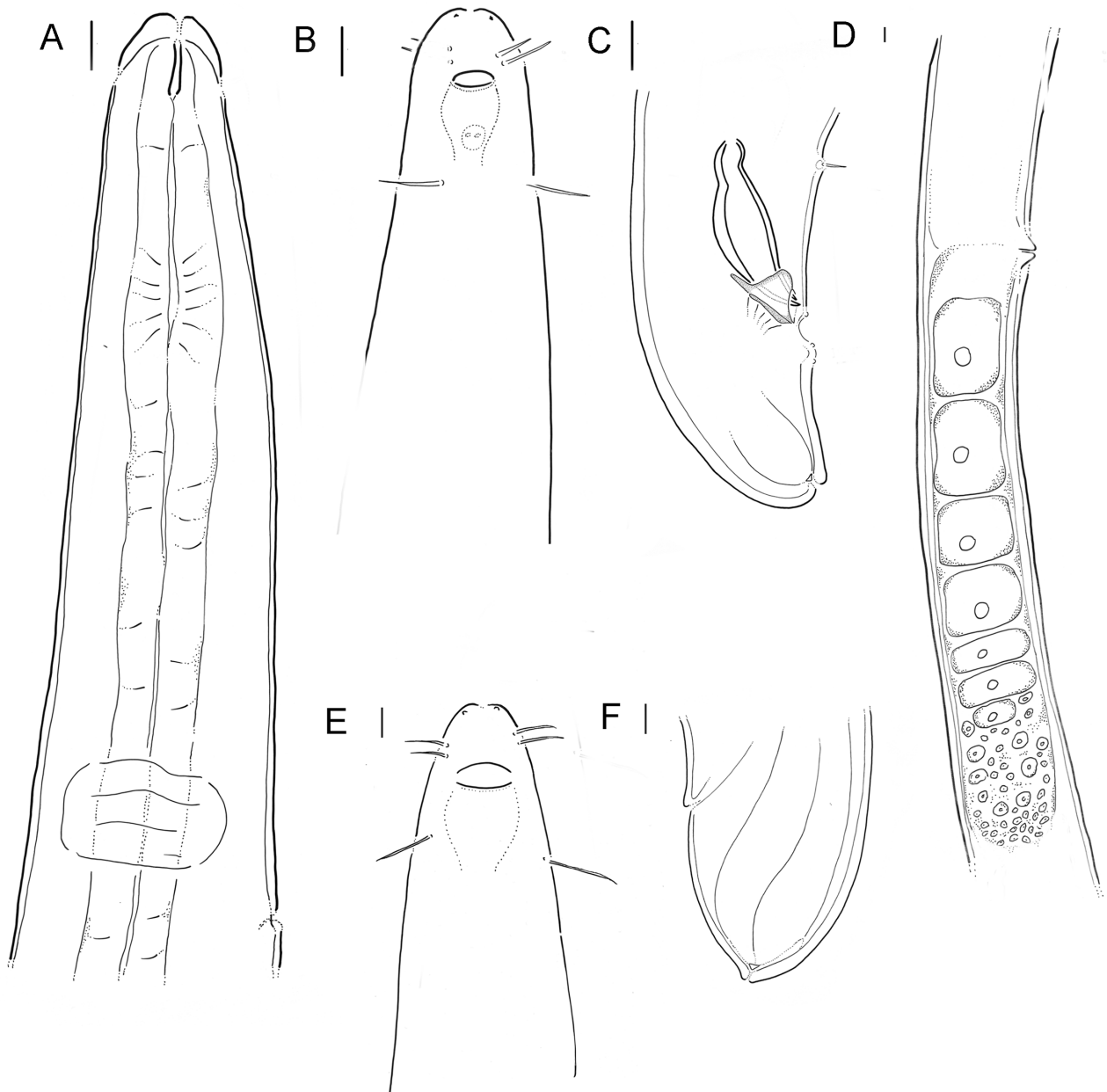


FIGURE 2. *Litinium australis* sp. n.: (A) head region of holotype male (internal); (B) head region of holotype male (external); (C) posterior end of holotype male showing copulatory apparatus; (D) uterus view with vulva of paratype female; (E) head region of paratype female (external); (F) posterior end of paratype female. Scale bars: 20µm.

***Thalassoalaimus nestori* sp. n.**

(Figures 3 & 4 (D–F); Table 2)

Systematic position. Oxystominidae Chitwood, 1935: Oxystomininae Chitwood, 1935: *Thalassoalaimus* de Man, 1893

Type material. Holotype: adult male. Registration number CNP-NEM 17149; type locality: “Banco Perdices” beach; coordinates: 40°47’S; 64°51’W; mid littoral, silty sediments. Collected by V. Lo Russo and G. Villares, 14 February 2009.

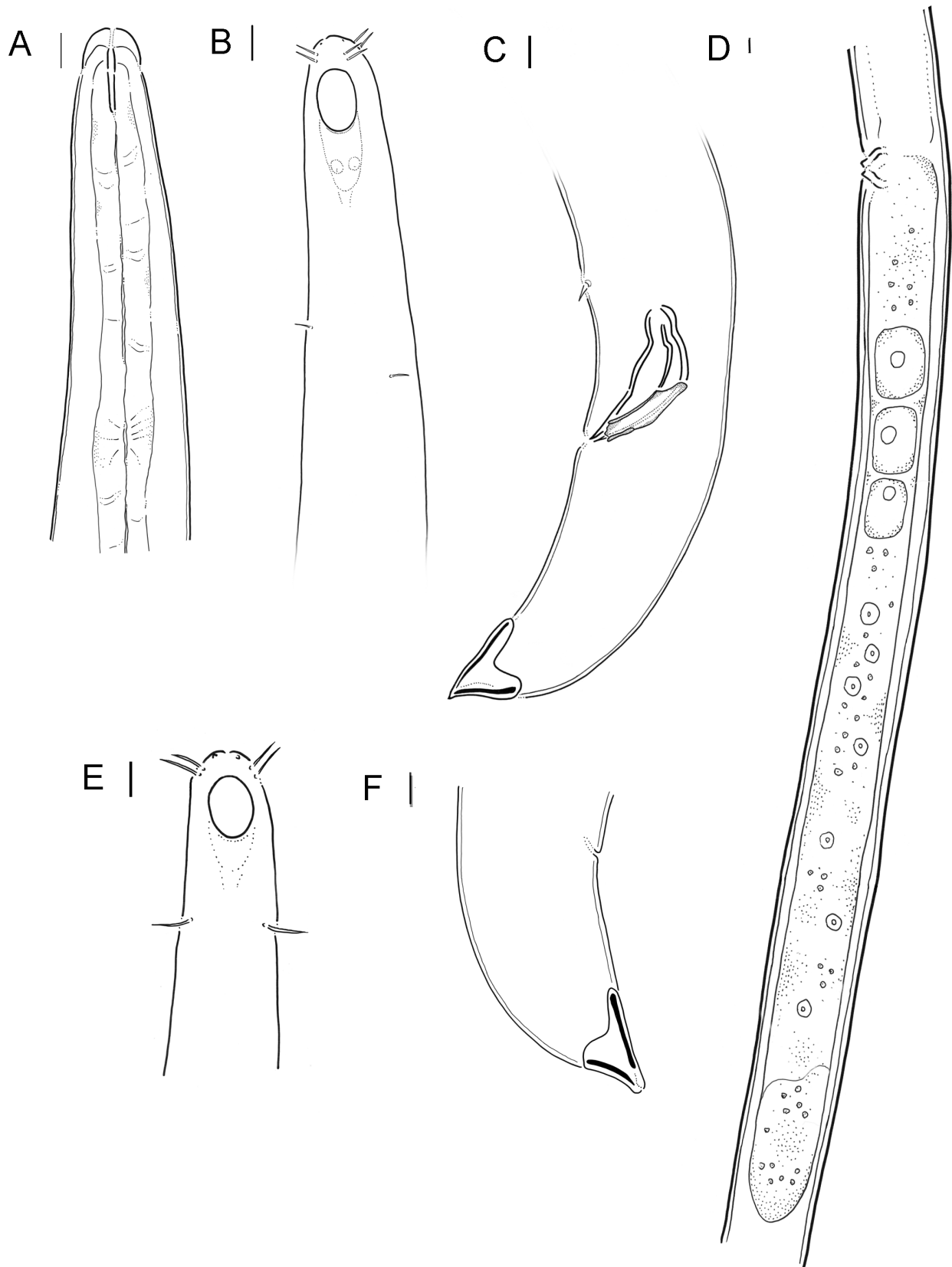


FIGURE 3. *Thalassolaimus nestori* sp. n.: (A) head region of holotype male (internal); (B) head region of holotype male (external); (C) posterior end of holotype male showing copulatory apparatus; (D) uterus view with vulva and ovary of paratype female; (E) head region of paratype female (external); (F) posterior end of paratype female. Scale bars: 20µm.

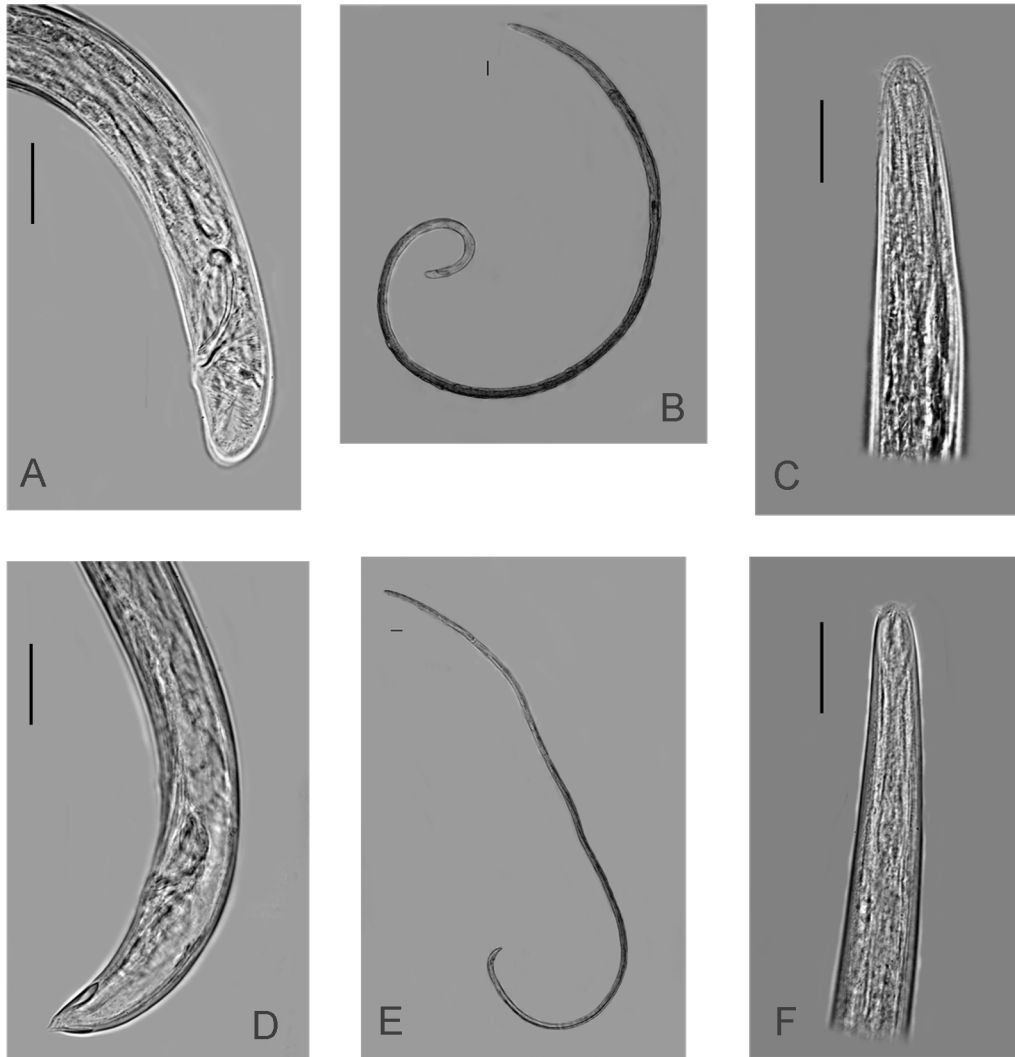


FIGURE 4. *Litinium australis* sp. n.: (A) posterior end of holotype male showing copulatory apparatus; (B) general view of male; (C) head region of holotype male (external). *Thalassoalaimus nestori* sp. n.: (D) posterior end of holotype male showing copulatory apparatus; (E) general view of male; (F) head region of holotype male (external). Scale bars: 20µm.

Paratype: adult female. Registration number CNP-NEM 17192. Same data as holotype.

Other material: Three males. Registration numbers CNP-NEM 18794, 18939 and 19142, locality: “Banco Perdices” beach; coordinates: 40°47’S; 64°51’W; high littoral, fine sand. Collected by V. Lo Russo and G. Villares, 14 February 2009.

One male, one female and two juveniles. Registration numbers CNP-NEM 17135, 17104, 17134 and 17253 respectively. Same data as holotype.

One juvenile. Registration number CNP-NEM 12977; locality: San Antonio city; coordinates: 40°43’S; 64°57’W; mid littoral, mid sand. Collected by V. Lo Russo and G. Villares, 13 February 2009.

Etymology. Dedicated to Néstor J. Cazzaniga in recognition of the help given during this project.

Measurements. See Table 2.

Description. Male (holotype): Cylindrical body (L=2260 µm). Cuticle smooth. Somatic setae observed. Buccal cavity small and narrow. Cephalic sensilla present in 6 + 6 + 4 circles. Six inner and six outer labial setae 3 and 3.5 µm long, respectively. A crown of four cephalic setae (5 µm) at 3.5 c.d. from anterior end. Amphid and amphidial pouch conspicuous, situated at 1.5 µm from anterior end. Amphidial fovea 4 µm wide and 7 µm long. Amphid width about 61% of the corresponding head diameter. Excretory pore at 142 µm (23.7 c.d.) from anterior end.

TABLE 2. Measurements (μm) of *Thalassolaimus nestori* sp. n. including mean values (range).

	Holotype	Paratype	Paratypes	Other	Paratypes
	Male	Female	Males	Female	Juveniles
n	-	-	4	1	3
L	2260.00	2325.00	2205.00 (2050–2260)	2235.00	1498.67 (1135–1800)
a	132.94	86.11	123.17 (105.12–136.36)	111.75	86.20 (81.07–91.82)
b	5.65	5.81	6.54 (5.65–7.26)	5.81	5.12 (4.37–5.78)
c	44.31	40.79	50.96 (46.12–56.25)	42.98	36.87 (26.40–45.91)
Anterior cephalic setae, length	3.00	3.00	2.80 (2.50–3)	2.50	2.17 (2–2.50)
Posterior cephalic setae, length	3.50	3.00	3.13 (3–3.50)	3.00	2.83 (2.50–3)
Sub-cephalic setae, length	5.00	5.00	5.38 (5–6)	5.00	4.67 (4–5)
Cephalic diameter	6.00	6.50	5.63 (5–6)	6.50	5.17 (5–5.50)
Cs%	58.33	46.15	55.91 (50–63.64)	46.15	55.15 (45.45–60)
Amphid from anterior end	1.50	2.00	5.63 (5–6)	2.00	1.83 (1–2.50)
External amphid width	4.00	4.50	3.75 (3–4.50)	4.50	4.00 (3.50–4.50)
External amphid length	7.00	5.00	5.88 (5–7)	5.00	4.83 (4–6)
Bd at amphid level	6.50	7.00	6.63 (5–7.50)	7.00	6.17 (6–6.50)
Amph%	61.54	64.29	57.31 (44.67–69.23)	64.29	65.17 (53.85–75)
Nerve ring from anterior end	113.00	105.00	95.25 (85–108)	106.00	99 (87–120)
Oesophageal bulb diameter	12.00	14.00	11.75 (9–16)	13.50	14.83 (14–15.50)
Bd at oesophageal bulb level	17.00	20.00	17.28 (15.60–21)	18.50	18.17 (16.50–21)
Pharynx length	400.00	400.00	340 (310–400)	385.00	291.67 (260–345)
Maximum body diameter	17.00	27.00	18.13 (15.5–21.5)	20.00	17.33 (14–21)
Anterior end to anus, distance	2209.00	2268.00	2161.50 (2010–2215)	2183.00	1457.33 (1092–1753)
Anal body diameter	17.00	20.00	17.13 (14–22)	16.00	16.17 (13.50–19)
Tail length	51.00	57.00	43.50 (40–49)	52.00	41.33 (34–47)
c'	3.00	2.85	2.59 (2.05–2.88)	3.25	2.59 (2.13–3.19)
Spicule length	25.00	-	22.25 (21–24)	-	-
Gubernaculum length	11.00	-	10.50 (10–12)	-	-
Vulva from anterior end	-	825.00	-	760.00	-
Vulva from anterior end/L (%)	-	35.48	-	34.00	-

Pharynx cylindrical (400 μm long), ending in a small poorly-developed bulb (12 μm in diameter). Body diameter at the base of pharynx 17 μm .

Reproductive system diorchic, testes opposed. Anterior and posterior testes situated to the left of the intestine. Spicules equal in length, 25 μm long, fusiform and with well-developed proximal cephalization. Gubernaculum 11 μm long, rounded at the proximal end. Two precloacal organs with a seta in each one. The first precloacal organ lies 37 μm anterior to the cloaca and has a seta 2 μm long and the second precloacal organ lies at 73 μm and has a seta 2 μm long. Tail conical, 51 μm long, with caudal capsule.

Female (paratype): Female similar to male in general body shape. Body 2325 μm long. One posterior ovary located on the right side of intestine, antidromously reflexed. Vulval orifice ventral, located at 35% of the body length. Uterus and oviduct are 350 μm long, with antidromously reflexed ovary 190 μm in length. Vagina short and conspicuous, walls not thickened.

Differential diagnosis. *Thalassolaimus nestori* sp. n. is characterized by its short and equal cephalic setae, the length and position of the cervical setae, narrow body diameter and tail with conical shape.

T. nestori differs from *T. brasiliensis* in the length of cephalic setae and the shape of the tail (conical vs. rounded in *T. brasiliensis*); *T. septentrionalis* by the length and position of cervical setae and the length and shape

of gubernaculum; *T. pacificus* by the presence of gubernaculum, length of cephalic setae, and the number of precloacal organs; *T. mediterraneus* by the length of cephalic setae and the shape and length of the gubernaculum; and *T. lissus* by the length of the cervical setae, length and shape of gubernaculum and the distance from the cloaca to the first precloacal organ. *T. nestori* is closely related to *T. macrosmaticus*, with similar position and length of cephalic setae, but differs in body length and de Man's ratios *a*, *b* and *c* (2260 µm, 132.9, 5.7 and 44.3 in *T. nestori* vs. 3290 µm, 96.1, 7.9 and 57.7 in the holotype of *T. macrosmaticus*). These species also differ in the position of the precloacal organs, and length of the tail and gubernaculum.

Key to all known species of genus *Thalassoalaimus*

(Based on male characteristics)

1. Tail without pointed tip *T. brasiliensis*
- Tail with pointed tip 2
2. More than 2 precloacal organs *T. pacificus*
- Two precloacal organs. 3
3. Cephalic setae more than 1/2 of the corresponding diameter 4
- Cephalic setae less or equal than 1/2 of the corresponding diameter 5
4. Gubernaculum more than 1/2 spicule length. *T. nestori* **sp. n.**
- Gubernaculum less than 1/2 spicule length. *T. macrosmaticus*
5. Cephalic setae configuration: 6+6+4. 6
- Cephalic setae configuration: 6+4+4. 7
6. Cephalic setae less than 3 times the cephalic diameter from anterior end *T. mediterraneus*
- Cephalic setae more than 3 times the cephalic diameter from anterior end *T. tardus*
7. *c*' less than 2 *T. lissus*
- *c*' greater than 2. *T. septentrionalis*

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