BRILL

Nematology 26 (2024) 1115-1125

Nematology
brill.com/nemy

Leptolaimus petrelense n. sp. (Plectida: Leptolaimidae), a new nematode species from the Petrel lagoon, central Chile

Brynelly BASTIDAS [1], Patricia MORALES-MONTERO [1,2], Oleksandr HOLOVOCHOV [3], Yoelvis SULBARÁN [4] and Ernesto SAN-BLAS [1,2,*]

[1] *Centre of Systems Biology for Crop Protection (BioSaV), Universidad de O'Higgins, San Fernando, Chile*
[2] *Laboratory of Nematology, Institute of Agri-food, Animal and Environmental Sciences (ICA3), Universidad de O' Higgins, San Fernando, Chile*
[3] *Department of Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden*
[4] *Laboratory of Entomology, Institute of Agri-food, Animal and Environmental Sciences (ICA3), Universidad de O' Higgins, San Fernando, Chile*
ORCID iDs: Bastidas: 0000-0003-0990-8501; Morales-Montero: 0009-0000-4813-1411; Holovochov: 0000-0002-4285-0754; Sulbarán: 0009-0005-4887-0691; San-Blas: 0000-0002-4676-9945

Received: 19 September 2023; revised: 11 August 2024
Accepted for publication: 1 October 2024; published online: 6 November 2024

Summary – During a survey on the biodiversity of nematodes in the Petrel lagoon (central Chile), a population of *Leptolaimus* was found in sediment samples. Morphological, morphometric and molecular data indicated that the recovered nematode is a new species. This nematode belongs to the group in which the males only have four pre-cloacal tubular supplements present, and females have no supplements. The new species can be separated from the other species of the group by the length of both males and females (843-1060 μm), the tail length (118-208 μm), the shape of the gubernaculum, and the arrangement of the caudal setae in males (a pair of ventral precloacal setae at level of spicules, a pair of dorsal post-cloacal setae, two subventral and two subdorsal caudal pairs. The differences from closely related species *L. gerlachi*, *L. praeclarus*, *L. octavus*, *L. hydrothermalis*, *L. vipriensis*, and *L. hadalis* are discussed. The recovered population is described herein as *Leptolaimus petrelense* n. sp.

Keywords – bacterial feeder, description, morphology, morphometry.

The Petrel lagoon is a coastal lagoon located in Pichilemu (O'Higgins region, Chile). It has a large amount of sediments, which, together with its Mediterranean hydrology, determines that in the dry season (October-May) a bar is formed that blocks its mouth from the adjacent sea, forming a lagoon. During the rainy season (June-September), this bar opens again with the seasonal floods. Because of this it contains a high percentage of seawater (Caamaño, 2012). It has an area of approximately 25 ha (0.25 km^2) and is seen as a widening of the bed of the Petrel estuary. Its depth is 1.1 m on average and its volume is 0.165 hm in the pre-rainy period. The lagoon includes a small islet of approximately 42 000 m^2 (4.2 ha) formed near the southern shore of the lagoon, currently covered by a shrub forest. In 2018, a survey on the eutrophication state of the Petrel lagoon was performed by the General Water Board of Chile (Direccion General de Aguas, DGA), concluding that it presents a hypereutrophic condition during the summer (determined by its chlorophyll suspension) and is eutrophic during the spring, mainly on the surface (DGA, 2017). The sewage from the surrounding urban areas of Pichilemu was deposited in the lagoon until the installation of a water treatment plant in 2009. This measure produced a cleaning effect on the lagoon and improved conditions for the local flora and fauna, which still remained under pressure from anthropic activities such as silviculture, animal farming and agriculture. In 2021, the Petrel lagoon was declared by the Ministry of the Environment as a protected urban wetland in order to safeguard its ecological characteristics and its operation.

* Corresponding author, e-mails: esanblas@uoh.cl; esanblas@yahoo.com

Published with license by Koninklijke Brill BV | DOI: 10.1163/15685411-bja10362
 | ISSN: 1388-5545 (print) 1568-5411 (online)

The genus *Leptolaimus* de Man, 1876 *sensu lato* (Holovachov & Boström, 2010) is a widely distributed genus of Plectida Gadea, 1973 commonly found in marine and brackish habitats worldwide. At present, there is a total of 67 valid species identified in the world (Nemys, 2024). In a survey done in January 2023, sediment and soil samples were taken for a broad study of the health state of the Petrel lagoon and its subsidiary creeks. Among other things, the nematode community structure was studied and we observed a high number of *Leptolaimus* nematodes all around the wetland. Using morphological examination, we determined that this nematode was an undescribed species, therefore described herein as *Leptolaimus petrelense* n. sp.

Materials and methods

Sampling procedures

Leptolaimus petrelense n. sp. was isolated from bottom sediment and soil samples in several locations over the Petrel lagoon in January 2023. The nematodes were extracted from the samples using sugar flotation (Jenkins, 1964) and the collected nematodes were fixed immediately in TAF (Courtney *et al*., 1955) and processed in glycerin by slow evaporation for measurements and morphologic characterisation.

Morphologic characterisation

For light microscope observations, live and processed specimens were observed. For the morphological description, 20 males and females were mounted on permanent slides. Measurements and drawings were done using a Leica DM2500 compound microscope fitted with a differential interference contrast system and analysed using LAS X software.

Molecular characterisation

DNA was extracted from an individual nematode. Each nematode was transferred to a sterile 1.5 ml Eppendorf tube containing 100 μl of nuclease-free water. The nematodes were homogenised with a silicone pestle, ensuring complete homogenisation. The homogenate was centrifuged at 4000 *g*, and the supernatant was used as template for molecular analyses. To amplify the 18S rRNA ribosomal region, the primer pair MN18F (forward) (5′-CGCGAATRGCTCATTACAACAGC-3′) (IDT, USA) and Nem_18S_R (reverse) (3′-GGGCGGTATCTGATC GCC-5′) (Bhadury *et al*., 2006) was used. The PCR mix consisted of 1X Sapphire master mix (13 μl), forward and reverse primers (2.5 μl each), ddH_2O (4.5 μl), and 5 μl of DNA. The PCR profile was as follows: 1 cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 60 s, 54°C for 60 s, 72°C for 60 s, and a final extension at 72°C for 10 min. Afterward, DNA integrity was assessed by 1% agarose gel electrophoresis; concentration and quality were evaluated with the Qubit RNA IQ assay kit (Thermo Fisher Scientific). Once prepared, the samples were sent to Macrogen for sequencing.

Quality control and assembly of the Sanger sequences were performed using the sangeranalyseR package in R (Chao *et al*., 2021). The PCR products were deposited in the NCBI GenBank under the following accession number: PQ432145. The sequences were then compared to reference sequences using the Basic Local Alignment Search Tool (BLASTn) from the National Center for Biotechnology Information (NCBI). Alignments with 18S rRNA sequences of the genus *Leptolaimus* were performed using the multiple sequence alignment tool MAFFT (Katoh *et al*., 2019). The phylogeny of the aligned sequences was inferred with FastTree v2.1.11 (Price *et al*., 2010) using a generalised time-reversible model, with *Caenorhabditis elegans* used as the outgroup. The resulting tree was visualised and annotated using the online tool iTOL v6.9.1 (Letunic & Bork, 2024).

Results

Leptolaimus petrelense n. sp.

(Figs 1-3; Table 1)

Description

Adults

Body slender and tapering anteriorly in pharyngeal region and posteriorly on tail (Fig. 2A). Maximum body diam. at vulva in female, male body more cylindrical. Cuticle annulated, annuli 1.5-1.8 μm wide at mid-body, with no ornamentation. Lateral field present, consists of single ala (two incisures), 2.1-2.3 μm wide at mid-body, originating just below the level of the basal bulb (in the pharynx-intestine junction) and extending posteriorly to the first third of the tail length (Fig. 2C). Distinct pores in sublateral position scattered over the body (between 10-12 pairs). Anteriormost body pore very distinct and always located below the amphidial aperture without seta, all other pores having seta (Fig. 2C). Labial region set off,

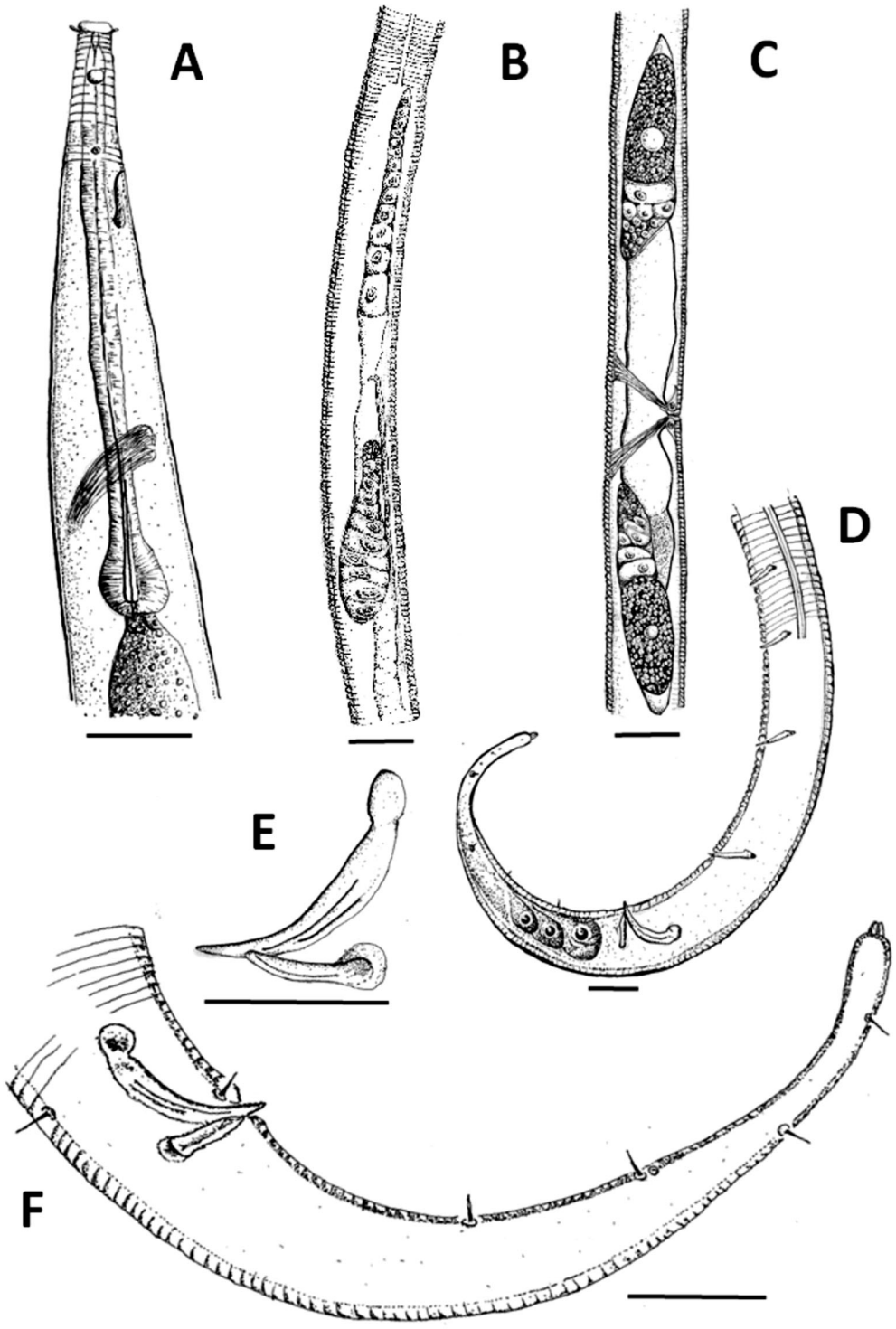

Fig. 1. Drawings of *Leptolaimus petrelense* n. sp. A: Pharyngeal region; B: Male gonads; C: Female reproductive system; D: Posterior portion of male, showing spicules, gubernaculum and precloacal supplements; E: Spicules and gubernaculum; F: Posterior portion of male showing setae arrangement. (Scale bars = 20 μm.)

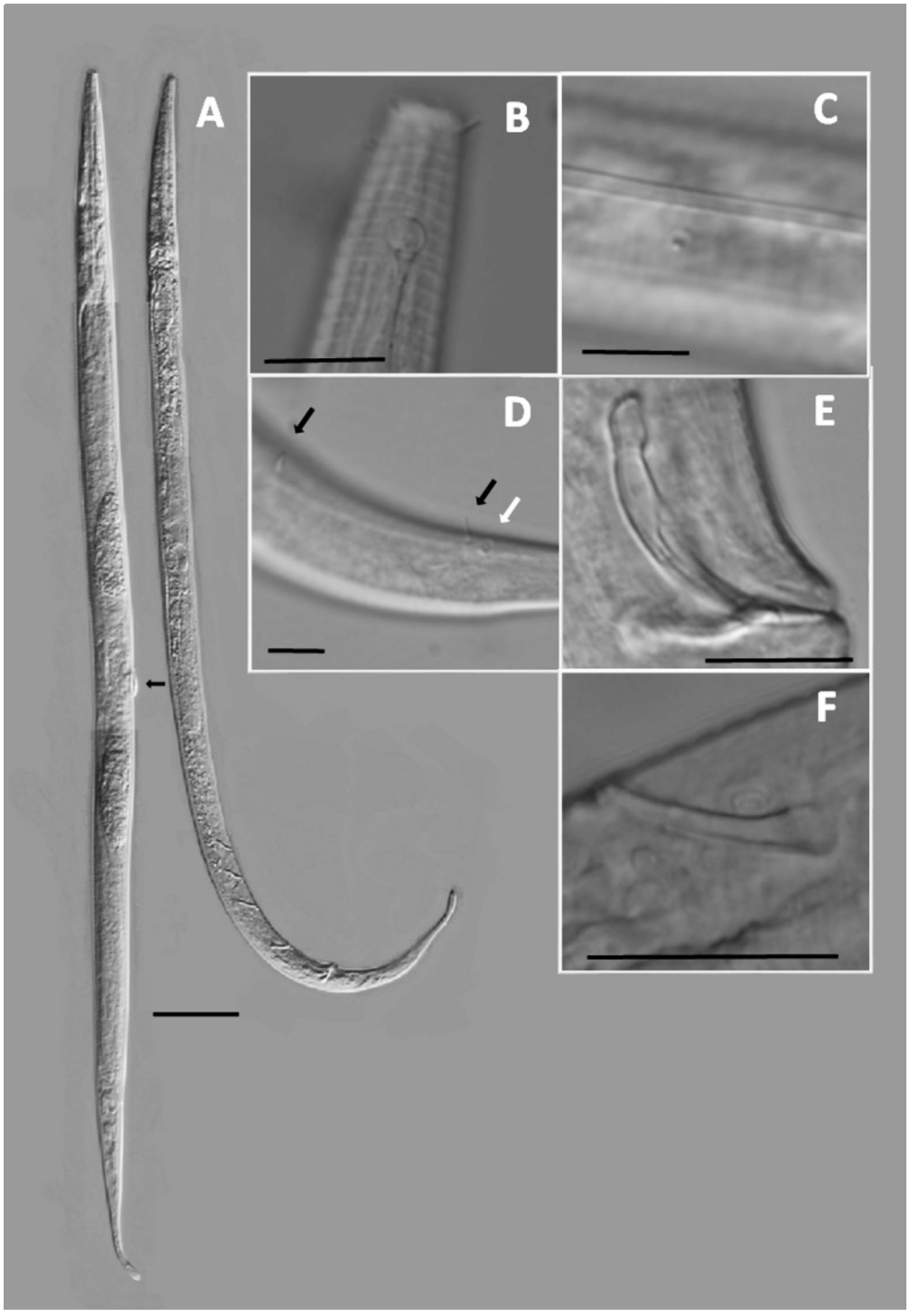

Fig. 2. Microphotography of *Leptolaimus petrelense* n. sp. A: Female (left) and male (right) *in toto*, arrow showing copulatory plug; B: Anterior end showing cephalic papillae, amphideal fovea and anulli; C: Lateral ala, and pore with seta; D: Subventral caudal setae (black arrow) and pore-like structure at the base of the second setae (white arrow); E: Spicules and gubernaculum; F: Male supplement. (Scale bars = 20 μm.)

Fig. 3. Petrel lagoon and wetland. Sampling site and points (P1-P12, white dots).

rounded in the edges but somehow flattened at the top, lips fused. Inner and outer labial sensilla indistinct (Fig. 1A; 2B). Cephalic sensilla setiform, 2.0-3.3 μm in length. Buccal cavity uniformly tubular: cheilostom and gymnostom short, undifferentiated, stegostom tubular, Amphidial fovea rounded, located 9-13 μm from anterior end. Nerve ring surrounding pharynx at level of the posterior half part of isthmus (Fig. 1A). Hemizonid not seen. Secretory-excretory system indistinct. Pharynx muscular, having uniformly thickened lumen with distinct oval basal bulb, valvular apparatus absent. Anterior cylindrical part of pharynx subdivided by three breaks, metacorpus cylindrical and isthmus narrow. Pharyngeal glands observed in a few specimens and their orifices indistinct. Cardia cylindrical, its posterior part embedded in the intestine in some specimens. Tail similar in shape in both sexes (curved ventrally in male and straight in females after fixation), elongate-conoid proximally and sub-cylindrical distally. Three caudal glands present, their nuclei in caudal. Spinneret functional.

Males

Body slender, 877-1043 μm long, ventrally curved upon fixation. Somatic setae in the posterior region as follows: a pair of subventral precloacal setae at level of spicules, a pair of dorsal post-cloacal setae, two consecutive subventral and two consecutive subdorsal terminal pairs (Fig. 2F). Distinct pore located at base of the second subventral seta (Fig. 1F; 2D) on each body side. Reproductive system diorchic with anterior testis outstretched and posterior reflexed (Fig. 1B). Spicules paired, symmetrical, lamina slightly curved ventrally, calomus short and ovoid to rounded manubrium. Gubernaculum straight and weakly cuticularised, with dorsal plate-like apophysis (Figs 1D-F; 2E). Accessory apparatus composed of four pre-cloacal tubular supplements (Fig. 1D; 2A), alveolar supplements absent. Tubular supplements almost straight, proximally cephalated and distally pointed (Fig. 2F).

Table 1. Morphometrics of *Leptolaimus petrelense* n. sp. All measurements are in μm and in the form: mean ± SD (range).

Character	Males		Females
	Holotype	Paratype	Paratype
n	-	19	20
L	1007	977 ± 50 (877-1043)	912 ± 63 (843-1060)
a	33.1	34 ± 2.2 (31-38.)	32 ± 1.5 (29-36)
b	5.9	6 ± 0.3 (5-6)	6 ± 0.3 (5-6)
c	7.7	7 ± 0.7 (5-8)	7 ± 0.4 (6-8)
c′	4.9	6 ± 0.6 (5-8)	7 ± 0.7 (6-8)
Diam. at cephalic setae	7.4	7 ± 0.4 (5-8)	7 ± 0.4 (6-8)
Diam. at amphids	9.2	9 ± 0.4 (8-10)	9 ± 0.4 (8-10)
Length of cephalic setae	3.1	3 ± 0.2 (2-3)	3 ± 0.4 (2-3)
Amphid length	3.8	3 ± 0.4 (3-4)	3 ± 0.3 (2-4)
Amphid width	3.8	3 ± 0.4 (3-4)	3 ± 0.3 (2-4)
Amphid width/cdb (%)	41.7	42 ± 9.7 (28-68)	32 ± 3.3 (25-38)
Amphid from anterior end	9.55	11 ± 0.7 (9-12)	10 ± 1.1 (9-13)
First body pore from anterior end	31.4	31 ± 2.5 (25-35)	30 ± 3.5 (26-43)
Buccal cavity length	3.7	4 ± 0.7 (3-5)	4 ± 0.8 (2-5)
Nerve ring (NR)	111	111 ± 14 (79-135)	120 ± 18 (90-142)
NR cdb	26.8	24 ± 1.6 (20-27)	24 ± 1.4 (21-27)
Pharynx (ES)	171	167 ± 7 (150-178)	164 ± 5 (154-174)
ES cdb	27.4	26 ± 1.3 (24-28)	25 ± 1.9 (21-29)
Basal bulb length	18.5	22 ± 2.3 (16-25)	22 ± 2.8 (18-31)
Basal bulb diam.	15	15 ± 1.1 (13-16)	15 ± 1.1 (13-18)
Maximum body diam.	30.5	28 ± 2.1 (25-32)	29 ± 2.5 (26-36)
Spicule length (straight)	26.2	24 ± 1.4 (21-27)	–
Spicule length (arc)	29.4	28 ± 2 (25-32)	–
Gubernaculum length	13.7	13 ± 1.0 (7.0-16)	–
Cloacal/anal body diam. (cdb)	26.6	24 ± 1.6 (22-27)	19 ± 2.3 (16-24)
Tail length	130	136 ± 17.7 (121-208)	135 ± 7.1 (118-152)
V	–	–	462 ± 35 (415-526)
%V	–	–	50 ± 0.1 (49-53)
Vulval body diam.	–	–	28 (24-33)
Number of tubular supplements	4	4	0
Distance supplement 1 and 2*	31	27 ± 3.3 (21-31)	–
Distance supplement 2 and 3	30	31 ± 3.2 (27-41)	–
Distance supplement 3 and 4	40	41 ± 3.3 (36-47)	–
Distance supplement 4 to cloaca	35	37 ± 2.8 (33-43)	–
Egg width	–	–	21 ± 2.6 (17- 25)
Egg length	–	–	54 ± 9.1 (41-71)

* Precloacal supplements counted from anterior end.

Female

Similar to males but straight when relaxed (Fig. 2A). Reproductive system with two opposed reflexed ovaries (Fig. 1C), vulva situated at mid-body 50% (48.5-52.7%). Supplements absent, lacking somatic setae, large single egg or two eggs often present in the uterus (Fig. 2A), spermatheca indistinct, uterus a wide tube, vagina straight, vulva midventral, epiptygmata and sensory structures around vulva (advulval sensilla) absent. Some individuals showed copulatory plugs (Fig. 2A).

Type locality

Leptolaimus petrelense n. sp. was isolated from bottom sediment and soil samples in several locations over the Petrel lagoon, Estero San Antonio (river) and their banks (34.381696°S, 71.998171°W) in central Chile in January 2023 (Fig. 3).

Type material

Holotype male, four paratype males and five paratype females were deposited in the National Museum of Natural Sciences (Madrid, Spain). Five paratype males and five paratype female were deposited in the University of California Riverside Nematode Collection (UCRNC), another set of paratypes (five females and five males) were deposited in the invertebrate collection of the Department of Zoology, Swedish Museum of Natural History (Stockholm, Sweden). The rest of the specimens, five males and five females, remain in the laboratory of nematology at the Universidad de O' Higgins, Chile. As required by the International Commission on Zoological Nomenclature, the ZooBank registration number for the new Linnaean binomials is LSID urn:lsid:zoobank.org:pub:49AAAF3E-3F7F-4708-B9F7-DFC68CFC9619.

Etymology

Specific epithet refers to 'from Petrel', a local name of a coastal area in central Chile.

Diagnosis and relationships

Leptolaimus petrelense n. sp. belongs to the group in which only males have four pre-cloacal tubular supplements and have no pharyngeal supplements, sharing this characteristic with *L. gerlachi* Murphy, 1966, *L. praeclarus* Timm, 1961, *L. octavus* Holovachov & Bostöm, 2013, *L. hydrothermalis* Tchesunov, 2015, *L. vipriensis* Gagarin & Nguyen, 2005 and *L. hadalis* Leduc, 2021. Apart from measurements, *L. petrelense* n. sp. can be separated from the above-mentioned species by their male setae arrangement from pre-cloaca to tail terminus (pairs) (Table 2).

Leptolaimus petrelense n. sp. differs from the above-mentioned species in body size (877-1043 μm) being the closest to *L. gerlachi* with 760-840 μm. The a value also differs between both species (31-38 *vs* 22-28 respectively), indicating that the new species is longer and more slender than *L. gerlachi*. The tail is longer in *L. petrelense* n. sp. also (121-208 *vs* 103-111 μm). The shape of the labial region in the new species is flat at the top and distinctively set off, but in *L. gerlachi* the labial region is rounded and faintly set off. The tubular supplements of males of the new species are clearly cephalated proximally, contrary to those of *L. gerlachi*. Males of *L. petrelense* n. sp. lack both the prominent ventral papilla located just anterior to the anus and the two long, ventrally directed terminal setae described for *L. gerlachi*. Additionally, the females of this new species do not have somatic setae along their bodies, unlike *L. gerlachi*; it differs from *L. praeclarus* by its longer body (877-1043 *vs* 442-518 μm), longer tail (121-208 *vs ca* 60 μm), greater a value in both males and females (31.0-38.1 *vs* 27 and 29.4-36.2 *vs* 20-22, respectively) and greater b value (5.0-6.4 *vs* 4.6).The males of *L. petrelense* n. sp. lack cervical setae below the amphids, while the males of *L. praeclarus* have a couple of them. *Leptolaimus petrelense* n. sp. can be separated from *L. octavus* easily by lacking a bursa-like expansion of the lateral field along the proximal part of the tail (*vs* present in both females and males) (Holovachov & Boström, 2013). Furthermore, both species differ in body length (877-1043 *vs* 541-638 μm), tail length of males (121-208 *vs* 73-89 μm) and females (118-152 *vs* 73-91 μm), length of cephalic setae (2.3-3.3 *vs* 4.0-4.5 μm), greater a value (31.0-38.1 *vs* 16-24), greater b value (5.0-6.4 *vs* 4.4-5.0) and greater c′ value of males and females (4.9-7.7 *vs* 3.8-4.3 and 6.2-8.4 *vs* 4.3-5.0, respectively).

The new species can be easily separated from *L. hydrothermalis* because the latter species has "accumulations of dark pigment granules scattered beneath cuticle in the epidermis, not only within the lateral fields, but in the entire body circumference" (Tchesunov, 2015). Also, the amphideal fovea is rounded in *L. petrelense* n. sp. but pear-shaped in *L. hydrothermalis*. In addition, *L. petrelense* n. sp. differs from *L. hydrothermalis* by its longer body (877-1043 *vs* 561-755 μm).

Leptolaimus petrelense n. sp. differs from *L. vipriensis* in its longer body (877-1043 *vs* 683-812 μm), longer tail in males (121-208 *vs* 78-95 μm) and in females (118-152 *vs* 98-104 μm), shorter cephalic setae (2.3-3.3 *vs* 4.0-4.5 μm). Also, the distance between the two anterior most supplements is more or less equal to other supplements in the row in the new species whereas in *L. vipriensis* the distance between the two anterior most supplements is noticeably shorter than between other supplements in the row.

Leptolaimus petrelense n. sp. can be separated from *L. hadalis* easily by longer body (877-1043 *vs* 612-741 μm), longer tail in males (121-208 *vs* 90-111 μm) and in females (118-152 *vs* 101-110 μm). Males of *L. petrelense* n. sp. have a straight gubernaculum and a very distintic ventral precloacal seta whereas males of *L. hadalis* have gubernaculum with dorsal apophysis

Table 2. Diagnostic measurements (italicised) and characteristics separating *Leptolaimus petrelense* n. sp. from other closely related species in the genus having four tubular supplements in males.

Character	*L. petrelense* n. sp	*L. gerlachi* (Murphy, 1966)	*L. praeclarus* (Timm, 1961)	*L. octavus* (Holovachov & Boström, 2013)	*L. vipriensis* (Gagarin & Nguyen, 2005)	*L. hydrothermalis* (Tchesunov, 2015)	*L. hadalis* (Leduc, 2021)
L	843-1060	*760-840*	*442-518*	*541-638*	*683-812*	*561-755*	*587-741*
a	29-38	*22-28*	*20-27*	*16-24*	22-36	27-32	27-37
c′ (male)	5-8	6.0	*3.7*	*3.8-4.3*	*3.7-4.5*	–	–
c′ (female)	6-8	7.2	*3.0*	*4.3-5.0*	4.9-6.1	–	–
Length of cephalic setae	2-3	3.0	–	1.5-2.0	*4.0-4.5*	2.5-3	2.0
Gubernaculum length	7-16	–	–	–	–	*3-5*	7-11
Tail length (male)	121-208	*103-111*[1]	*approx. 60*[1]	*73-89*	*78-95*	–	–
Tail length (female)	118-152	110-123[1]	*approx. 69*[1]	*73-91*	*98-104*	–	–
Distance supplement 1 and 2	21-31	–	–	–	–	44	–
Distance supplement 2 and 3	27-41	–	–	–	–	28-31	–
Distance supplement 3 and 4	36-47	–	–	–	–	23-24	–
Male setae arrangement from pre-cloaca to tail terminus (pairs)	one pre-cloacal subventral one post-cloacal dorsal two consecutive post-cloacal subventral and a pair of pores just posterior to second setatwo consecutive post-cloacal subdorsal	one pre-cloacal subventral[2] one post-cloacal subdorsal[2] one post-cloacal lateral[2] two post-cloacal consecutive dorsolateral[2] one long terminal on tail	one pre-cloacal subventral[2] two post-cloacallateral at the end of the lateral field[2] one post-cloacal dorsoventral[2]	one pre-cloacal subventralone post-cloacal lateroventralone post-cloacal dorsolateral	one pre-cloacal subventral[3] one post-cloacal subdorsal *ca* 1CBD[3] Two consecutive post-cloacal subventral (first at *ca* 1/5 and second *ca* 2/5 tail length) and a pair of pores just posterior to second seta)[3] One post-cloacal subdorsal at 3/4 tail length[3] One post-cloacal dorsosublateral where spinneret ampulla becomes distinct[3]	one pre-cloacal subventralone post-cloacal subdorsalthree consecutive post-cloacal lateroventral two close consecutive alveolar pits in the middle of the tail. two consecutive dorsolateral	one pre-cloacal subventral[2] two consecutive post-cloacal lateroventral[2] three consecutive dorsolateral[2]
Presence of somatic setae in females	no	yes	no[2]	no	not reported	yes[2]	yes

[1] Calculated from drawings or data in the original description.
[2] From drawings in the original description.
[3] After revising the original slides by Holovachov (this work), the original description is incorrect in setae arrangement.

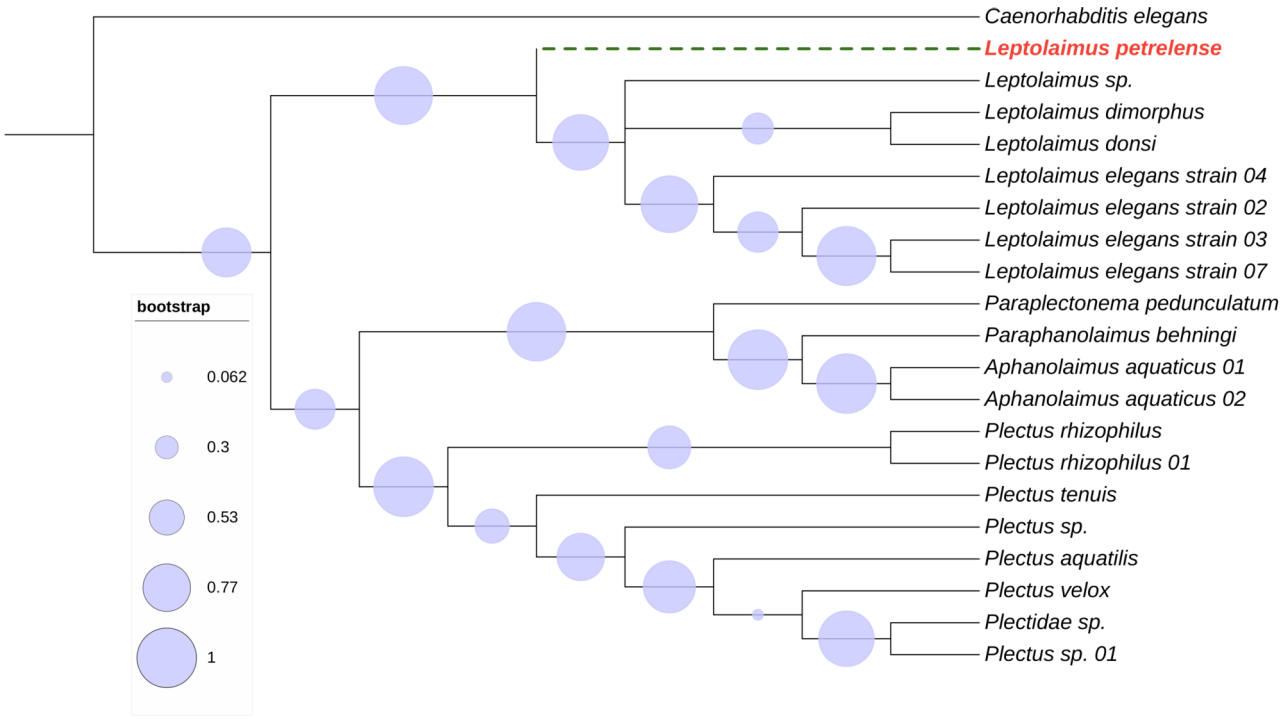

Fig. 4. Phylogenetic tree based on 18S rRNA gene sequences of phylotypes of *Leptolaimus petrelense* n. sp. isolated from the Petrel lagoon. The tree was constructed using the maximum likelihood method with the general time-reversible (G + T + R) model and was rooted with *Caenorhabditis elegans*. The scale bar represents changes per nucleotide position, and branch support values were assessed through bootstrap analysis with 1000 replicates.

with strongly curved distal portion and lack ventral precloacal seta Additionally, females of *L. petrelense* n. sp. do not have somatic setae unlike the females of *L. hadalis*.

Molecular characterisation and phylogenetic analysis

Electrophoresis and sequencing of the 18S rRNA gene from *Leptolaimus* collected in the Petrel lagoon revealed a fragment approximately 1750 bp in length. Comparison with reference sequences showed that the 18S sequence of *L. petrelenses* n. sp. differs by 300-400 bp from other species within the *Leptolaimus* group. Furthermore, phylogenetic analysis demonstrated that *Leptolaimus petrelenses* n. sp. do not cluster with any known nematode species, instead forming a distinct branch within the *Leptolaimus* group, separate from any previously described species (Fig. 4). These findings are supported by high bootstrap values, confirming the robustness of this separation and suggesting that the observed divergence is statistically significant. Both molecular and morphological data validate that *L. petrelenses* n. sp. represents a new species, as defined by the phylogenetic and evolutionary species concepts.

Ecological remarks

The species of the genus *Leptolaimus* are considered bacterial feeders based on the structure of their digestive system (Yeates *et al*., 1993; Moens & Vincx, 1997). Many individuals of *L. petrelense* n. sp. were found feeding inside some unidentified soft larvae cadavers, possibly as scavengers (Fig. 5) that can be colonised by bacteria, but further investigation on the feeding habits of this species must be done.

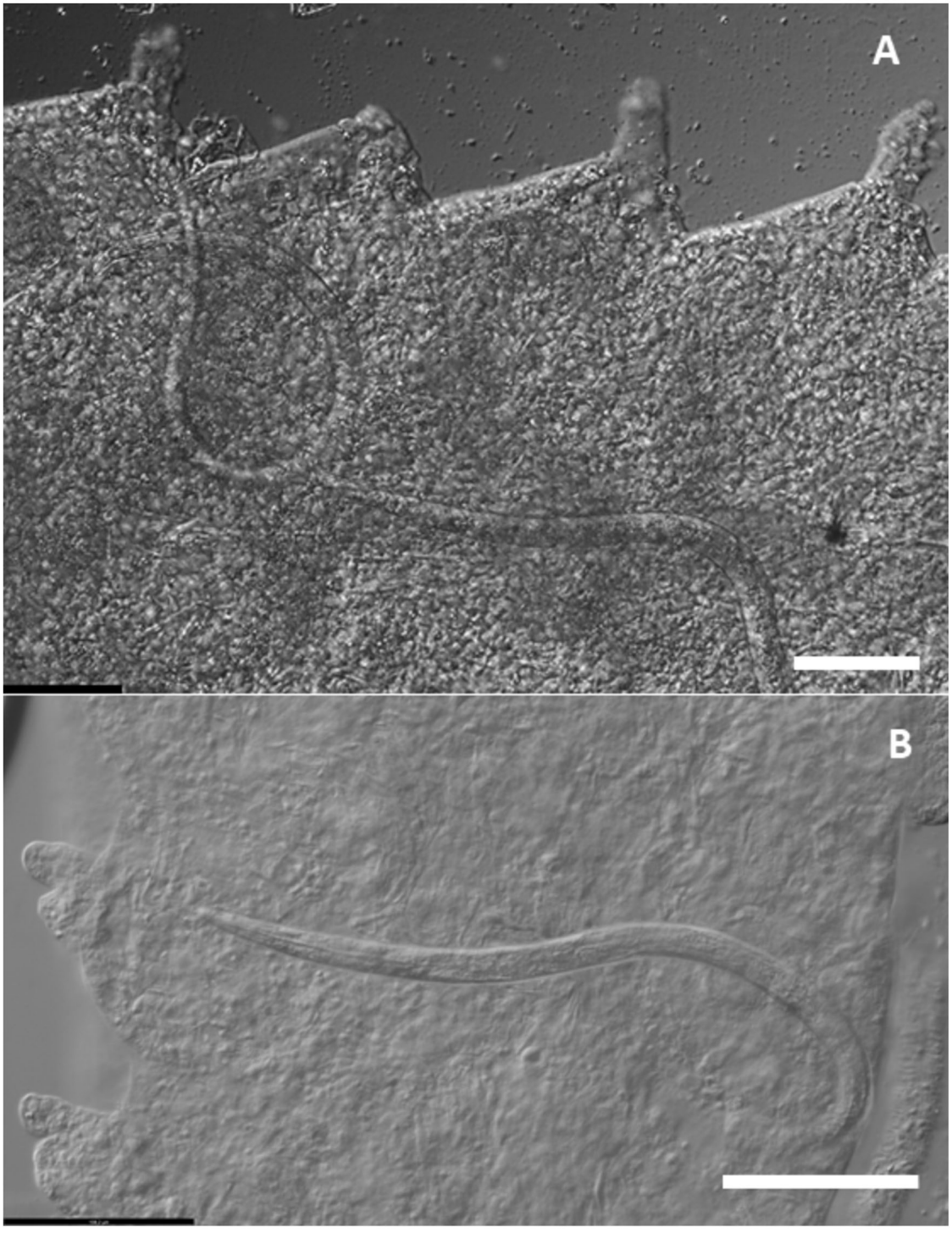

Fig. 5. *Leptolaimus petrelense* n. sp. inside unidentified dead larva. A: 100× magnification; B: Female inside the cadaver. (Scale bars = 100 μm.)

Acknowledgements

This work was partially supported by the project 'Sistema Articulado de Investigación en Cambio Climático y Sustentabilidad de Zonas Costeras de Chile (URO RED21992)' founded by the Ministry of Education of Chile.

References

Bhadury, P., Austen, M.C., Bilton, D.T., Lambshead, P.J., Rogers, A.D. & Smerdon, G.R. (2006). Development and evaluation of a DNA-barcoding approach for the rapid identification of nematodes. *Marine Ecology Progress Series* 320, 1-9.

Caamaño, A. (2012). *Humedales costeros de Chile: Aportes científicos a su gestión sustentable*. Santiago, Chile, Editorial UC.

Chao, K.-H., Barton, K., Palmer, S. & Lanfear, R. (2021). sangeranalyseR: Simple and interactive processing of Sanger sequencing data in R. *Genome Biology and Evolution* 13, evab028. DOI: 10.1093/gbe/evab028

Courtney, W.D., Polley, D. & Miller, V.L. (1955). TAF, an improved fixative in nematode technique. *Plant Disease Reporter* 39, 570-571.

DGA (Dirección General de Aguas) (2017). Website. Available online at https://bibliotecadigital.ciren.cl/items/830fa56c-ccd4-48c3-afc2-fcb6cd9aa605 (accessed 7 October 2023).

Gagarin, V.G. & Nguyen, V.T. (2005). New species of free-living nematodes of the family Leptolaimidae from the Cam river, Vietnam. *Zoologicheskii Zhurnal* 84, 771-777.

Holovachov, O. & Boström, S. (2010). *Identification of Plectida (Nematoda)*. Riverside, CA, USA, EUMAINE, Ghent and Nematology, University of California Riverside.

Holovachov, O. & Boström, S. (2013). Swedish Plectida (Nematoda). Part 4. The genus *Leptolaimus* de Man, 1876. *Zootaxa* 3739, 1-99. DOI: 10.11646/zootaxa.3739.1.1

Jenkins, W.R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48, 692.

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualisation. *Briefings in Bioinformatics* 20, 1160-1166. DOI: 10.1093/bib/bbx108

Leduc, D. (2021). New free-living nematode species and records (Chromadorea: Plectida and Desmodorida) from the edge and axis of Kermadec Trench, Southwest Pacific Ocean. *PeerJ* 9, e12037. DOI: 10.7717/peerj.12037

Letunic, I. & Bork, P. (2024). Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research* 52, W78-W82. DOI: 10.1093/nar/gkae268

Moens, T. & Vincx, M. (1997). Observations on the feeding ecology of estuarine Nematodes. *Journal of the Marine Biological Association of the United Kingdom* 77, 211-227. DOI: 10.1017/S0025315400033889

Murphy, D.G. (1966). An initial report on a collection of Chilean marine nematodes. *Mitteilungen Hamburgisches Zoologisches Museum und Institut* 63, 29-50.

Nemys (2024). Nemys: World database of nematodes. Available online at https://nemys.ugent.be (accessed 11 August 2024).

Price, M.N., Dehal, P.S. & Arkin, A.P. (2010). FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5, e9490. DOI: 10.1371/journal.pone.0009490

Tchesunov, A.V. (2015). Free-living nematode species (Nematoda) dwelling in hydrothermal sites of the North Mid-Atlantic Ridge. *Helgoland Marine Research* 69, 343-384. DOI: 10.1007/s10152-015-0443-6

Timm, R.W. (1961). The marine nematodes of the Bay of Bengal. *Proceedings of the Pakistan Academy of Sciences* 1, 25-88.

Yeates, G.W., Bongers, T., De Goede, R.G., Freckman, D.W. & Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera-an outline for soil ecologists. *Journal of Nematology* 25, 315-331.