

# Diversity and abundance of free-living nematodes from Carlini station, Isla 25 de Mayo/King George Island, Antarctica: A case study in pristine and disturbed soils

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# Abstract

This study describes the soil nematodes of King George Island (Isla 25 de Mayo) in Antarctica through molecular identification, abundance, and structure analysis. Nematodes are key organisms in terrestrial ecosystems, with important roles in nutrient cycling and trophic networks. Despite the harsh conditions in the extreme environment of Antarctica, these organisms have managed to adapt and survive. Soil samples were collected from pristine and disturbed areas, and morphological and molecular analyses were conducted to identify the nematode species present. Abundance, trophic groups, maturity indices, and colonizer-persister values (*cp*) were analyzed and compared between the sampling sites. The molecular results revealed four genera of Antarctic nematodes (*Coomansus*, *Calcaridorylaimus*, *Eudorylaimus*, and *Plectus*), and the phylogenetic relationships of these nematodes with others found in Antarctica were established. ANOSIM and SIMPER statistical analysis showed significant differences in nematode abundance between pristine and disturbed areas. The analysis of the nematode assemblage through *Mi*, *Ei*, and *Si* showed less disturbance at the pristine sites than at the anthropic sites. Further studies should be carried out at the Carlini Station and 25 de Mayo/King George Island to learn more about the structure of the nematode community and how they are affected by anthropic practices in Antarctic environments.

## Introduction

The ecosystem services provided by edaphic organisms in their natural environments favor interspecific relationships and balance in ecosystems. Free-living nematodes are key organisms in terrestrial ecosystems, performing numerous roles, such as cycling nutrients, decomposing organic matter, and making these nutrients available to other organisms (di Montanara et al. 2022). Their feeding habits are diverse, and they include bacteriophagous and fungivorous nematodes (primary consumers), herbivores, omnivores and predators, thus representing all the links in the trophic web.

Edaphic nematodes live and move through the film of water and humidity that is generated on the surfaces and interstices of the soil, so they need a certain amount of oxygen to enable them to survive (Iqbal and Jones 2017). Their survival is impossible in anoxic and extreme conditions, but these organisms have adapted to a remarkable variety of environments and climates, making them the most abundant metazoans in terrestrial habitats (Machado and von Reuss 2022). Due to their adaptability, they are widely used as environmental bioindicators to assess the degree of disturbance in certain environments, including those impacted by anthropogenic intervention (Salas and Achinelly 2020).

Antarctica is one of the harshest environments on Earth, with extreme cold, high winds, freeze-thawing cycles, and limited resources, posing significant life challenges. Despite these conditions, a surprising diversity of organisms have adapted to survive in this environment, including nematodes. The Antarctic 25 de Mayo/King George Island, located in the South Shetland archipelago, has an approximate size of 1250 km<sup>2</sup> and has been a popular research site for scientists from around the globe (Dziembowski and Bialik 2022). Nematodes have been studied on this island over the last 30 years. Janiec (1993) and Bölter

et al. (1997) collected samples with nematodes at the water-sediment interface in marine and nearshore ponds, moss banks, streams, and station buildings. Mouratov et al. (2001) studied the negative correlation between the abundance of nematodes and soil moisture. Dalto et al. (2010) studied the fauna associated with *Prasiola crispa* (Chlorophyta), a terrestrial green alga, finding the *Plectus* nematode in great abundance. Ileva-Makulec et al. (2009) studied the abundance and trophic diversity of nematodes which increased with the age of ice-free areas. Nematodes have been studied as bioindicators of climate change and of the effect of anthropic practices on the Antarctic meiofauna, exposing the degrees of stress they present through the analysis of ecological indices (Gheller and Corbisier 2022; Kang et al. 2022).

The Carlini Station, located within Potter Cove to the south of the 25 de Mayo/King George Island, is the main scientific station of Argentina and various environmental studies have been conducted there. Although the scientific community has provided information on several species of organisms through monitoring and field samples, records on Antarctic nematodes are practically nil. Concerning free-living nematodes, Chaves (1990) morphologically described the species *Coomansus gerlachei*, found in association with samples of *Deschampsia antarctica*, and Camino et al. (2017) described the sporadic finding of *Coomansus* sp. in nests of Wilson's storm petrel, *Oceanites oceanicus*. Other groups have analyzed the fauna of parasitic nematodes associated with bird hosts in Antarctica (Vidal et al 2012; Diaz et al. 2013; Fusaro et al. 2015, 2018, 2023, Diaz et al. 2016;).

The study of Antarctic nematodes is important not only for understanding the biodiversity of this unique ecosystem but also to gain insights into how organisms can adapt to extreme environments. Nematodes are also ecologically important, as they play key roles in nutrient cycling and food webs. Recent advances in molecular techniques have made it possible to study the diversity and ecology of nematodes in more detail, providing new opportunities to investigate the biology of these organisms in the Antarctic. This research paper aims to describe, through abundance, and structure, the current status of free-living soil nematode assemblages associated with pristine and disturbed soils on 25 de Mayo Island/King George Island, Antarctica. It also reports the first molecular and phylogenetic analysis of free-living nematodes in the region of the Carlini scientific base.

## Materials and methods

### Sampling sites

Within the framework of the 2019–2020 Summer Antarctic Campaign of the Argentine Antarctic Institute, soil samples associated with the vascular plants *Deschampsia antarctica* and *Colobanthus quitensis* were taken in two regions of Isla 25 de Mayo/King George, Antarctica (Fig. 1). The samples were from six pristine areas (PZ1-PZ6) within the Antarctic Specially Protected Area 132, Stranger Point (62°14'S – 58°39'W), and in five areas with anthropic intervention (AZ1-AZ5) within the Carlini Station (Fig. 2). Area 132 is characterized as a sympatria breeding site for two penguin species, *Pygoscelis adeliae* and *Pygoscelis papua*, as well as of the mammal *Mirounga leonina* and *Arctocepalus gazella* colonies.

Anthropic areas are affected by different degrees of disturbance that include the transit of people and vehicles, occasional fuel spills, and the release of effluents (Table 1).

A 15 cm long PVC tube auger sampler was used to extract samples at a depth of approximately 10 centimeters, or less when the substrate did not allow for greater depth. At each sampling point, three samples were collected, labeled, and placed in containers. They were then stored at 6°C in the Argentine Laboratory of the Carlini Base before being transferred to the Laboratory of Free-Living Nematodes and Agronomic Importance at the Center for Parasitological and Vector Studies (CEPAVE-CONICET-UNLP), in La Plata, Buenos Aires, Argentina.

## **Extraction and morphological determination of nematodes**

For nematode extraction, 100 cm<sup>3</sup> of each sample was processed using the centrifugation and modified flotation technique (Caveness and Jensen 1955; Salas et al. 2021). Two centrifugation cycles were applied to each sample at 1210 g for three minutes (Macro Centrifuga Giumelli® Z-29): a cycle with distilled water to discard the supernatant with impurities and unwanted material; and a second cycle with sucrose solution (484 g sucrose: 1L distilled water). The final supernatant was poured into a 40 µm sieve and rinsed with water to obtain the final nematodes. The nematode specimens were morphologically determined using the keys described by Manzanilla-López and Marbán-Mendoza (2012) and Chaves et al. (2019), in collaboration with the NEMA-AGRIS Nematology Laboratory, La Plata, Buenos Aires, Argentina.

## **Molecular analysis**

To confirm the morphological identification, molecular analyses were performed on the nematode specimens at the molecular laboratory of the Center for Parasitological and Vector Studies (CEPAVE, CONICET-UNLP). For genomic DNA extraction, five specimens of each nematode genus were individually processed using 100 µl of a 5% suspension of Chelex in deionized water and 2 µl of 10 mg/ml proteinase K, followed by overnight incubation at 56°C, boiling at 90°C for 8 minutes, and centrifugation at 14,000 rpm for 10 minutes. An aliquot of 1 µl of the supernatant was utilized as a template for polymerase chain reaction (PCR). The 18S rRNA partial sequences were amplified using the Nem18SF (5'-CGCGAATRGCTCATTACAACAGC-3') and Nem18SR (5'-GGGCGGTATCTGATCGCC-3') primers and Go Taq Master Mix (Promega) (Singh et al. 2013). The thermocycler conditions were: 94°C for 15 min; 35 cycles of 94°C denaturation for 30 s, annealing at 52°C for 40 s and extension at 72°C for 60 s, and a single final extension period of 72°C for 10 min. Final products were analyzed by electrophoresis on agarose gels (1%) and visualized by staining with ethidium bromide. Amplicons were sequenced in MacroGen Inc. (Korea) and edited with the CHROMAS and MEGA software (Tamura et al. 2021). The consensus sequences obtained were compared with sequences of Antarctic nematodes in the BLAST tool available in National Center for Biotechnology Information (NCBI). The phylogeny tree of the Antarctic nematodes based on 18S rDNA was realized through maximum likelihood (Tamura et al. 2021). The sequences of genomic-DNA were submitted to the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov>).

## **Ecological analysis**

The nematode community was studied based on the abundance of individuals per taxon, which was used in turn to calculate the structure and diversity indices of the ecosystem. To determine significant differences between the nematode populations of anthropic sites versus pristine sites, an ANOSIM analysis was performed (Clarke and Gorley 2006). ANOSIM provides a *p-value* (significance level < 0.05) and an *R-value* (range 0 to 1) to evaluate for statistical differences in the abundance of nematodes (Clarke and Warwick 2001). The SIMPER (SIMilarity PERcentages) test was used to determine which nematodes contributed to differentiating assemblage composition between sites (Clarke and Gorley 2006).

Free-living soil nematodes can behave as opportunistic colonizers of enrichment (*r-strategists*) or as persistent organisms sensitive to environmental disturbances (*k-strategists*). Bongers (1990) developed a classification system for nematode families with values (*cp-value*) from 1–5. The *cp1* are colonizer nematodes, present a short life cycle, are tolerant of environmental changes, and are dominant in disturbed or enriched soils. The *cp2* nematodes have longer life cycles and less fertility than *cp1*; they are very tolerant against polluting conditions, indicating polluted soils. The *cp3*, *cp4*, and *cp5* have even longer life cycles and are considered persistent nematodes sensitive to disturbed soils. Taking this scale into account, the nematodes found were classified according to their *cp-value*, and were also differentiated according to their trophic group following the classification proposed by Yeates et al. (1993).

Using the NINJA (Nematode INDicator Joint Analysis) software (Sieriebriennikov et al. 2014), community indices were determined: the maturity index ( $M$ ) =  $\sum (v_i \times f_i)/n$  (where  $v_i$  = colonizer-persistent value (*cp*) assigned to the family,  $f_i$  = frequency of family  $i$  in the sample,  $n$  = the total number of individuals in the sample); and the enrichment index ( $E$ ) and the structure index ( $S$ ) proposed by Ferris et al. 2001. Studying the functional guilds of nematodes can be valuable in characterizing the condition of the trophic network. This approach enables the evaluation of the fertility, response to disturbances, and decomposition pathways, of matter using the enrichment ( $E$ ) and structure ( $S$ ) indices (Biswal 2022). By considering the presence or absence of specific nematode guilds, these indices can establish a profile of the nematode fauna in the ecosystem. This information allows us to determine whether the nematode community is in a basal, enriched, or structured state, and provides insight into the soil condition.

## Results

### Molecular and phylogenetic analysis

Through the 18S ribosomal RNA gene molecular analysis, it was possible to determine the genera of *Coomansus*, *Calcaridorylaimus*, *Eudorylaimus*, *Plectus* nematodes, and specimens of the Rhabditidae family. The sequences generated from this study were submitted to the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>) and can be accessed using the GenBank accession number: *Coomansus* 25 de Mayo (OQ626766), *Calcaridorylaimus* 25 de Mayo (OQ676577), *Eudorylaimus* 25 de Mayo (OQ676578), *Plectus* 25 de Mayo (OQ676576). The phylogenetic tree analysis conducted with MEGA

Software identified four main nematode clades (Fig. 3). Regarding *Coomansus* 25 de Mayo, a high coverage and identity was observed with three sequences of *C. gerlachei* (KM092523; LC457639/LC457655) ranging between 90–100% and 99.74–100% respectively. *Eudorylaimus* 25 de Mayo presented a query cover of 95% and percent identity of 99.75% with sequences of the genus *Eudorylaimus* sp (HQ270134) and was related to the species *Eudorylaimus coniceps* (LC457645/LC457662) with a query cover of 90% and percent identity of 99.09%. *Calcaridorylaimus* 25 de Mayo specimens coincided with the species *C. signatus* with a query cover of 90% and percent identity of 99.36%, respectively. *Plectus* 25 de Mayo shows a query cover of 99% and a percent identity of 100% with the species *P. antarcticus*, *P. belgicae*, *P. murrayi* (Fig. 3).

In the anthropic zone (sites AZ1-AZ5), only *Calcaridorylaimus* and *Plectus* species were found, along with juveniles of the Rhabditidae family. In the pristine zone (PZ1-PZ6), the four mentioned species were found and no specimens of the Rhabditidae family. The abundance values for each sampling site can be seen in Table 2. Results of the global ANOSIM ( $R = 0.157$  and  $p = 0.006$ ) showed significant differences in nematode abundance between anthropic and pristine sites. The SIMPER analysis showed that the nematodes that most contributed to the dissimilarity between sites were individuals of the genus *Plectus* (39.22% contribution).

**Table 1.** Numbering of the anthropic (AZ) and pristine (PZ) zones where soil samples were collected corresponding to the 2019-2020 Summer Antarctic Campaign of the Argentine Antarctic Institute. The description shows the type of anthropic intervention and the presence of other organisms. The coordinates correspond to the exact points where samples were taken from the Carlini Station (anthropic areas) and the Antarctic Specially Protected Areas 132 (pristine areas).

Sampling site	Description	GMS coordinates
AZ1	fuel storage area	S 62° 14' 17.74" O 58° 40' 8.472"
AZ2	water intake box	S 62° 14' 16.692" O 58° 40' 2.186"
AZ3	water treatment plant	S 62° 14' 14.996" O 58° 40' 1.768"
AZ4	main house	S 62° 14' 15.205" O 58° 40' 2.719"
AZ5	computer building; <i>D. antarctica</i> and mosses present	S 62° 14' 14.73" O 58° 39' 44.517"
PZ1	<i>Arctocephalus gazella</i> colonies, <i>D. antarctica</i> and mosses present	S 62° 15' 32.231" O 58° 36' 34.937"
PZ2	temporary freshwater body; <i>D. antarctica</i> and mosses present	S 62° 15' 32.897" O 58° 36' 35.863"
PZ3	boulder beach; mosses present	S 62° 15' 33.887" O 58° 36' 39.416"
PZ4	<i>Mirounga leonina</i> colonies and moss present	S 62° 15' 19.213" O 58° 37' 55.135"
PZ5	<i>Pygoscelis adeliae</i> breeding site	S 62° 15' 18.115" O 58° 37' 55.365"
PZ6	<i>Pygoscelis papua</i> breeding site	S 62° 15' 18.259" O 58° 37' 56.373"

Table 2

Average abundance, standard deviation, minimum and maximum values of abundance for each nematode taxon found in the sampling sites with anthropic intervention (AZ1-AZ5) and in the pristine sites (PZ1-PZ6). Data corresponding to soil and moss samples from Isla 25 de Mayo/King George Island, Antarctica, January 2020.

	<i>Calcaridorylaimus</i>	<i>Coomansus</i>	<i>Eudorylaimus</i>	<i>Plectus</i>	Rhabditidae
AZ1	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	80 ± 26.96 (62-111)
AZ2	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	2 ± 2 (0-4)	2 ± 3.46 (0-6)
AZ3	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	7 ± 2 (5-9)	7 ± 2 (5-9)
AZ4	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	40 ± 15.09 (26-56)	0 ± 0 (0-0)
AZ5	40 ± 14.42 (28-56)	0 ± 0 (0-0)	0 ± 0 (0-0)	40 ± 17.43 (28-60)	0 ± 0 (0-0)
PZ1	0 ± 0 (0-0)	10 ± 2 (8-12)	0 ± 0 (0-0)	200 ± 17.77 (186-220)	0 ± 0 (0-0)
PZ2	10 ± 2 (8-12)	10 ± 8.71 (0-16)	0 ± 0 (0-0)	20 ± 17.08 (2-36)	0 ± 0 (0-0)
PZ3	5 ± 5.19 (2-11)	0 ± 0 (0-0)	5 ± 1 (4-6)	0 ± 0 (0-0)	0 ± 0 (0-0)
PZ4	0 ± 0 (0-0)	0 ± 0 (0-0)	4 ± 3.6 (1-8)	4 ± 1.73 (2-5)	0 ± 0 (0-0)
PZ5	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	2 ± 2 (0-4)	0 ± 0 (0-0)
PZ6	0 ± 0 (0-0)	0 ± 0 (0-0)	0 ± 0 (0-0)	45 ± 19.07 (25-63)	0 ± 0 (0-0)

The ecological results through the NINJA software allowed us to classify the nematodes according to their trophic group as bacteriophages (*Plectus* and Rhabditidae specimens), omnivores (*Calcaridorylaimus* and *Eudorylaimus*), and predators (*Coomansus*) (Table 3). According to their life strategy, the presence of opportunistic enrichment bacteriophage nematodes, *cp1*, was observed in three of the five anthropic zones. These nematodes, represented by juveniles of the Rhabditidae family, were absent from all the pristine sites sampled. The *Plectus* bacteriophage genus, belonging to the *cp2* category, showed a more equal distribution, with specimens found in all the sampled sites except one anthropic site and one pristine site. Omnivorous and predatory nematodes (*Calcaridorylaimus* and *Eudorylaimus* and *Coomansus*, respectively) sensitive to disturbances, represented by the *cp4* category, were mostly found in pristine sites (Table 3).

**Table 3.** Values of colonizer-persister category, feeding types and isolation source of each nematode genus found in the sampling sites with anthropic intervention and in the pristine sites corresponding to soil and moss samples from Isla 25 de Mayo/King George Island, Antarctica, in January 2020. Accession number of the genomic-DNA sequences submitted to the GenBank database are shown.



	<i>cp class</i>	Feeding type	Acession number
<i>Calcaridorylaimus</i>	4	Omnivore	OQ676577
<i>Coomansus</i>	4	Predator	OQ626766
<i>Eudorylaimus</i>	4	Omnivore	OQ676578
<i>Plectus</i>	2	Bacterivore	OQ676576
Rhabditidae	1	Bacterivore	-

The indices of maturity, enrichment, and structure gave us a general overview of the ecosystem services offered by the nematodes present in the edaphic system studied. The value of *MI* increased in the anthropic area the further the sampling sites were from the center of the station where the fuel storage area was (AZ1). In the pristine site, the highest values of *MI* were found at point PZ3 (boulder beach), decreasing in value towards the sites located to the east and west of it (Fig. 4). The highest *EI* values were found at the AZ1 site, located in the fuel storage area of the station. The next highest values were found at the AZ3 site, next to the water treatment plant, and at site AZ2, next to the water intake box (Fig. 4). The highest value of *SI* was at the AZ5 site, which was the only site within the anthropic area to present structure values in the nematode community. The pristine sites PZ1, PZ2, and PZ3 showed similar structure values (Fig. 4).

## Discussion

The results presented in this case study constitute the largest record for the country of free-living nematodes in Antarctica. Four genera (*Plectus*, *Calcaridorylaimus*, *Eudorylaimus*, and *Coomansus*) of nematodes from soil samples, associated with vascular plants and mosses, from different areas with anthropic intervention and pristine areas, were molecularly determined. These genera could be linked, thanks to bioinformatics analyses, to genomic sequences of other Antarctic nematode specimens.

Regarding *Coomansus* 25 de Mayo, a 100% bootstrap value was obtained for the clade formed by three sequences of the species *Coomansus gerlachei* (KM092523; LC457639/LC457655) cited by Elshishka et al. (2015) and Kagoshima et al. (2019), respectively. This genus of predatory nematodes was found only at the sites furthest away from the pristine zone, at points PZ1 and PZ2. As no associated microfauna were prospected, it was not possible to determine the diet of this *cp4* nematode, although it has been cited as a predator of other nematodes (Devi and George 2018; Khan and Kim 2007). Due to the high abundance of *Plectus* observed in the same area, further analyses may be conducted to establish if there is an association between this nematode and the *Coomansus* diet.

For the specimens of *Calcaridorylaimus* 25 de Mayo, the genomic analyses showed a 96% bootstrap value with the species *C. signatus* (LC457648.1) described by Kagoshima et al. (2019). In this study, this *cp4* nematode belonging to the trophic group of omnivores was the second most abundant in the samplings carried out and was found in the pristine site and in the anthropic zone furthest from the station (AZ5). Molecular determination of *Calcaridorylaimus* is necessary to avoid misinterpretations of

the results, since this nematode, when classified according to morphological data, is synonymized with the genera *Mesodorylaimus* and *Aporcelaimus* (Velasco-Castrillón et al. 2014).

*Eudorylaimus* 25 de Mayo was 97% related to GenBank sequences of the genus *Eudorylaimus* sp. (HQ270134.1) determined within 25 de Mayo/King George Island, confirming its identification with this genus (Raymond et al. 2014). It was 22% related with the clade formed by the species *Enchodeloides signyensis* (KY881720), also isolated on 25 de Mayo/King George Island, and with *E. coniceps* (LC457645/ LC457662) (Elshishka et al. 2017; Kagoshima et al. 2019). Due to their sharing a low bootstrap value, a clear phylogenetic relationship with the species of this group could not be determined. In the present study, *Eudorylaimus* 25 de Mayo was found in low abundance, agreeing with other results of this nematode on the island (Raymond et al. 2014). In turn, it was only isolated in pristine sites, so its absence in sites with anthropic intervention could be considered indicative of sensitivity to anthropic disturbance (Ayres et al. 2008). *Eudorylaimus* was also reported on Isla 25 de Mayo/King George Island in freshwater and soil samples (Janiec 1993; Ileva-Makulec et al. 2009).

The phylogenetic analysis of *Plectus* 25 de Mayo through 18S showed a 100% bootstrap value for the clade of species determined in Antarctica of *P. murrayi*, *P. antarcticus*, *P. belgicae* and *P. cf. meridianus* (Kagoshima et al. 2012; Raymond et al. 2014; Kagoshima et al. 2019). However, the lack of differentiation between the specimens found and those belonging to the NCBI database suggests that the 18S small subunit and the primers used may not be entirely effective for determining species within *Plectus*. Therefore, future genomic studies using different set of primers should be performed with new isolated specimens. *Plectus* 25 de Mayo was the most abundant nematode and the most distributed at all sampling points. Its feeding habits and life cycle position it as an opportunistic pioneer nematode (*cp2*), with the capacity to colonize extreme environments such as the Antarctic soil (Borgmeier et al. 2022). This nematode has also been dominant in other studies within 25 de Mayo/King George Island and other Antarctic sites (Janiec 1993; Ileva-Makulec et al. 2009). In this case study, the mere presence of *Plectus* would not suffice to make inferences about the degree of disturbance in pristine and anthropic soils due to its uniform distribution throughout the sampled sites.

The specimens of the Rhabditidae family found at Carlini Station could not be morphologically identified at the genus level because only juvenile stages were found. However, its high abundance would reflect the degree of colonization and permanence of these opportunistic *cp1* organisms in disturbed sites (Mokuah et al. 2023).

The indices of maturity and structure of the nematode community allow a description of its trophic state, reflecting the health of the habitat in which these organisms are found. *MI* is an index that decreases with environmental contamination (heavy metals, waste or treated water, fuel) (Bongers 1990). In this work, the lowest *MI* values (< 2) were observed in the areas with the highest anthropic activity AZ1, AZ2, and AZ3 (fuel storage area, water intake box, and water treatment plant, respectively). These results of *MI* values match the descriptions of other authors in the maturity analysis of nematode communities on 25 de Mayo Island/King George Island (Gheller et al. 2022) and what has been described in other works from

Antarctica where a weak organization in the nematode community structure was observed (Ileva-Makulec et al. 2009). In contrast, the highest values of  $MI(2-4)$  were found in the PZ2, PZ3 and PZ4 sites where the anthropic presence is practically nil.

The results of  $EI$  were congruent with the maturity values obtained from the trophic webs in the nematode community. The AZ1 site presents the highest degree of anthropic activity in the station, due to circulation on foot and with heavy machinery, and because it is the fuel exchange site. The highest enrichment value ( $EI=100$ ) was found in this site due to the exclusive presence of an abundant population of *cp1* nematodes belonging to the Rhabditidae family, known to be very apt colonizers of disturbed soils (Salas and Achinelly 2020, Du Preez et al. 2022). The other anthropic sites where enrichment values were obtained were AZ2 and AZ3, where *cp2* nematodes were found, represented by *Plectus* populations. The presence of these nematodes in these sites where wastewater and treated water are handled could indicate a certain degree of environmental response that should be studied in greater depth in future samplings within the Carlini Station.

The equilibrium of an edaphic community is given by the balance within the structure of the trophic web where the organisms in its highest levels control the growing populations of the primary producers. In this context, the  $SI$  is used to see the current state of the assemblage of nematodes in the soils studied. The AZ5 anthropic site was the furthest and with the least (almost nil) presence of people moving in the area and was the only one in the station that presented structure values due to the presence of the *cp4* nematode *Calcaridorylaimus*. Within the pristine sites, the highest  $SI$  value was found in PZ2 due to the presence of this genus, together with the predatory nematode *Coomansus*. The omnivorous nematode *Calcaridorylaimus* was previously isolated from the Antarctic maritime sector on the Galíndez and Signy islands (Maslen and Convey 2006; Velasco-Castrillón et al. 2014, Kagoshima et al. 2019), and from continental soils (Peneva et al. 2009). In our work, the sequences of *Calcaridorylaimus* 25 de Mayo (found in the continental area) were closely related to *C. signatus* (LC457648) (Kagoshima et al. 2019) from the Antarctic maritime sector of Signy Island. *Calcaridorylaimus* 25 de Mayo was exclusively associated with mosses and with the vascular plant *Deschampsia antarctica* (site AZ5.), in agreement with Peneva et al. (2009), although these authors did not perform molecular analyses, and therefore we do not know whether they are phylogenetically related to our specimens. Further sampling should be carried out to determine the distribution of these nematodes sensitive to disturbances, considered as one of the key pieces within the structure of the edaphic community.

Conducting research and gathering data on nematodes in Antarctica is crucial for comprehending the biodiversity of this exceptional environment, discovering how organisms can adjust to extreme conditions, and examining how human activities affect the ecosystem. In this study, it was possible to observe through the analysis of the nematode assemblage that the pristine sites showed less disturbances than the anthropic sites. Future studies at Carlini Station and 25 de Mayo/King George Island should continue investigating the nematode community, placing emphasis on obtaining physicochemical data to establish more precise distinctions between environments that have been disturbed by human activity and those that remain pristine.

# Declarations

## Acknowledgments

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## Author contributions

AS and BF conceived and designed research. AS, JMR, MR, EC and FA analyzed the soil samples and morphologically determined the nematodes. AS and DB performed the molecular analysis of nematodes. AS and DS analyzed the data. FA, LR and MA supervised sampling and provided financial support. AS, BF, DS and FA wrote the manuscript. All authors read and approved the manuscript.

## Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Conflict of interest

The authors declare that no conflicts of interest exist.

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## Ethical Standards

All samples were taken in compliance with all appropriate research permissions given by the Environmental Management and Tourism Program of the National Directorate of the Antarctic (DNA), Argentina.

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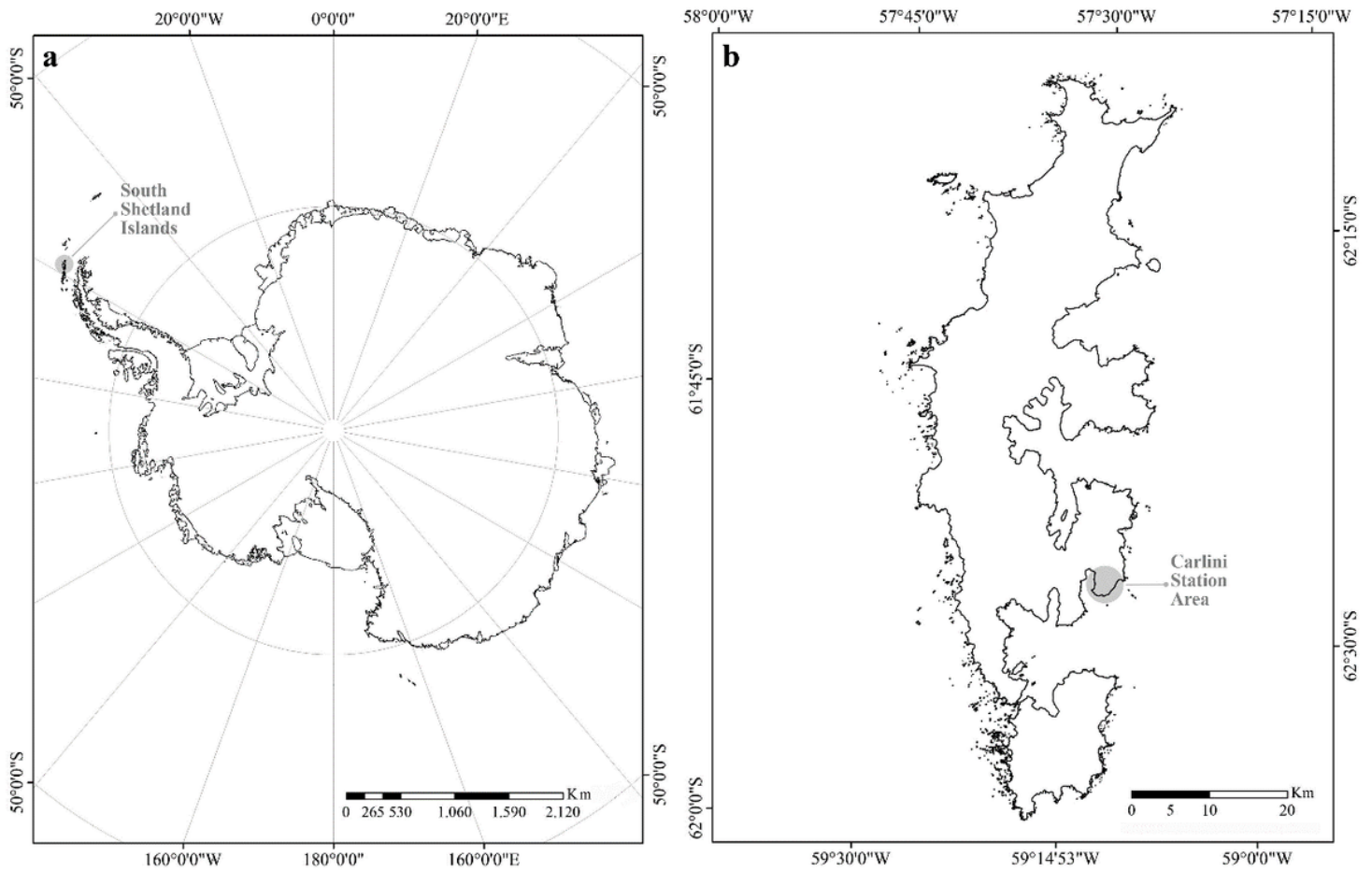
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## Figures



**Figure 1**

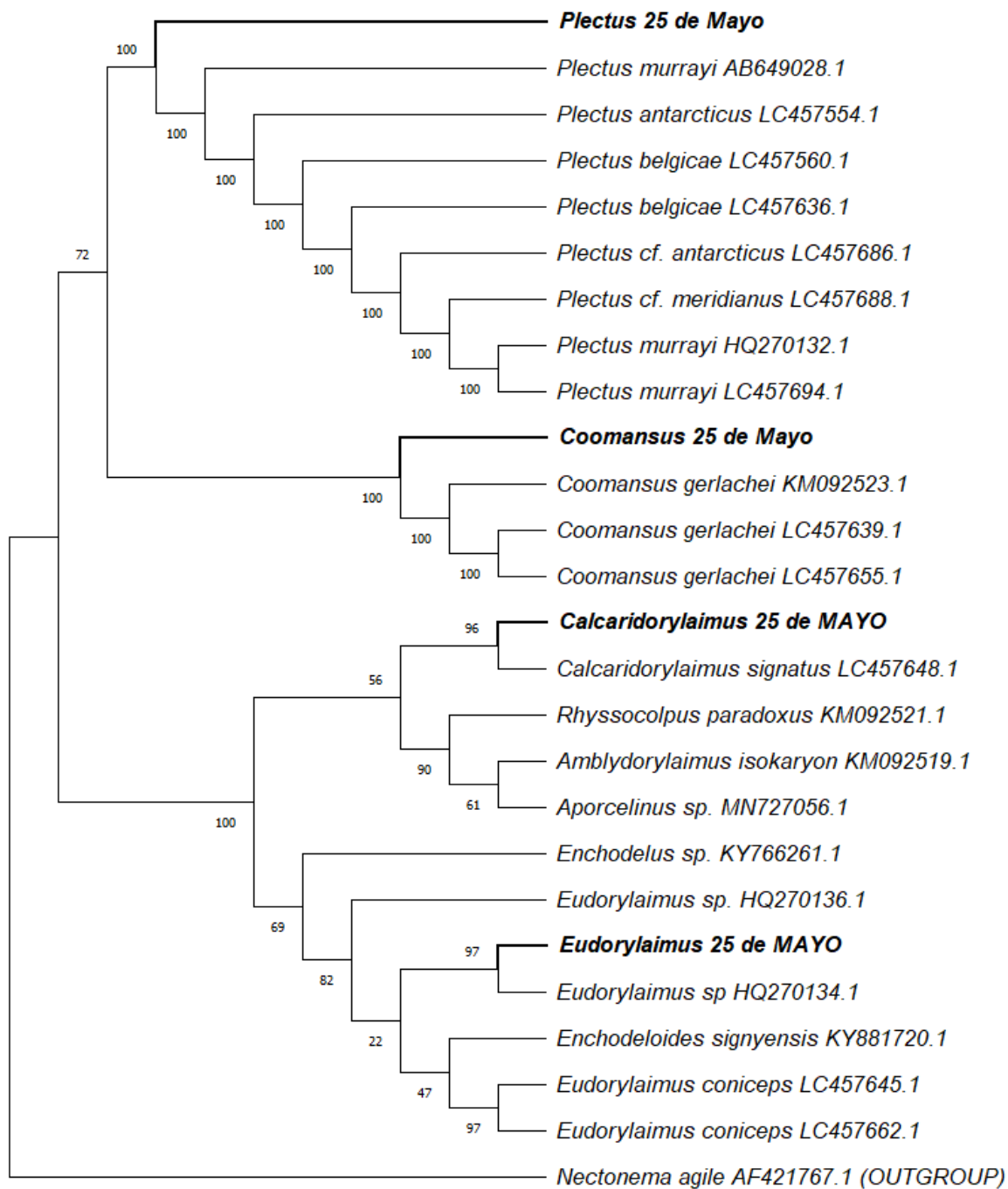
Geographic location of the sampling zone corresponding to the 2019-2020 Summer Antarctic Campaign of the Argentine Antarctic Institute. General map of Antarctica showing the archipelago of the South Shetland Islands in a gray circle (a). Isla 25 de Mayo/King George Island and Caletta Potter in a gray circle showing the area corresponding to the Carlini Station and the pristine coast (b). Map modified from QGIS, 2023.





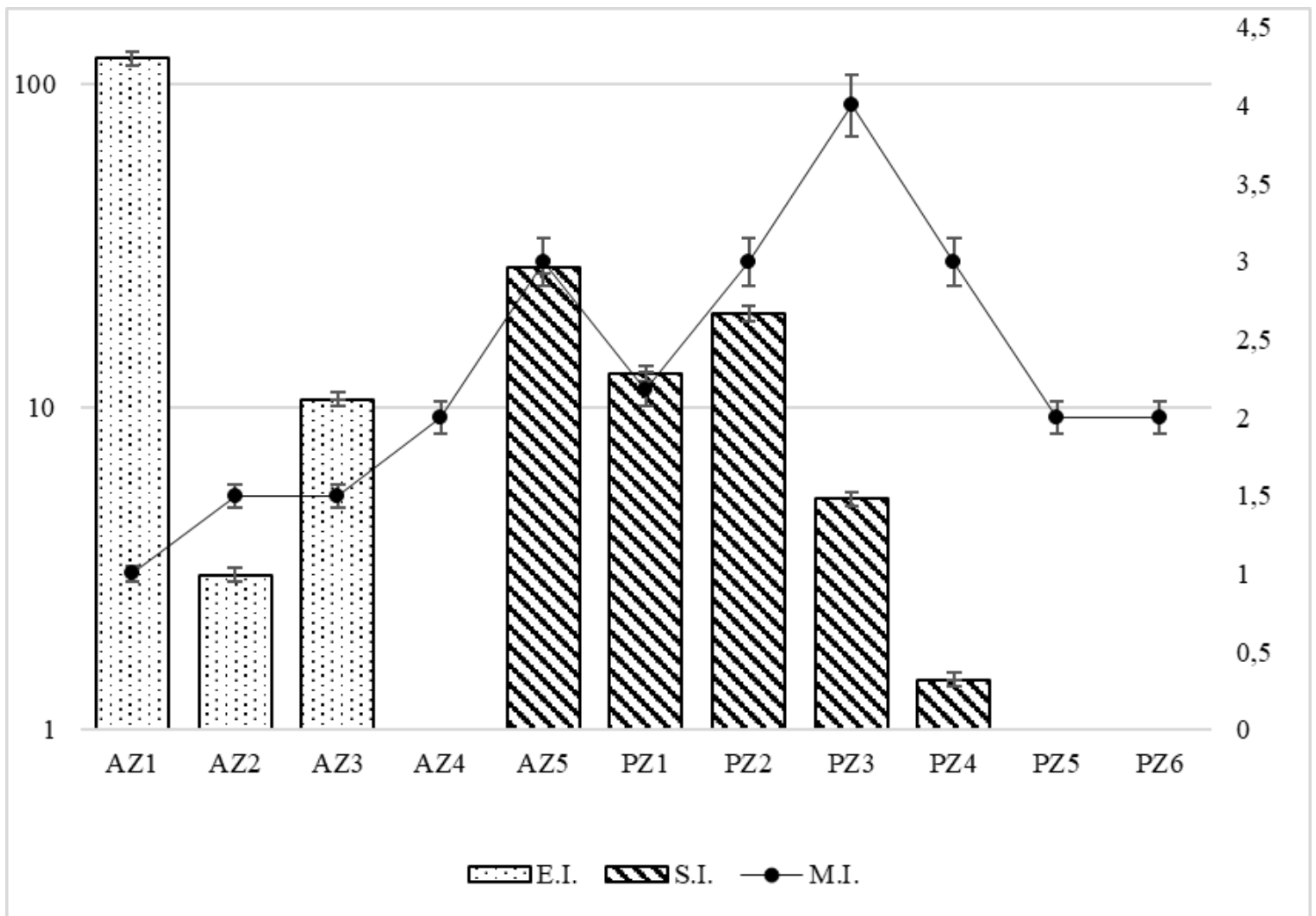
**Figure 2**

Satellite image of the Carlini Station corresponding to the sampling area with anthropic intervention of the 2019-2020 Summer Antarctic Campaign of the Argentine Antarctic Institute. AZ1: fuel storage area; AZ2: water intake box; AZ3: water treatment plan; AZ4: Main house; AZ5: Computer Building. Map modified from Google Maps, 2023.



**Figure 3**

Phylogenetic results of the molecularly identified genera of nematodes found in this work (*Plectus* ARG 25 de Mayo, *Coomansus* ARG 25 de Mayo, *Calcaridorylaimus* ARG 25 de Mayo, *Eudorylaimus* ARG 25 de Mayo) corresponding to soil and moss samples from Isla 25 de Mayo/King George Island, Antarctica, January 2020. Results were obtained using the Neighbor-Joining method (Saito and Nei, 1987). The optimal tree is shown. Next to the branches, the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown (Felsenstein, 1985). Evolutionary analyses were conducted in MEGA11 Software (Tamura et al 2021).



**Figure 4**

Values of enrichment index (*E*), structure index (*S*) and maturity index (*M*) of the Antarctic nematodes found in anthropic sites (AZ1-AZ5) and pristine sites (PZ1-PZ6). Data corresponding to soil and moss samples from Isla 25 de Mayo/King George Island, Antarctica, January 2020.