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Prevalence and Distribution of Nematodes from Coastal Sand Dunes in the Iberian Peninsula

Alba N. Ruiz-Cuenca and Joaquín Abolafia *

Department of Animal Biology, Plant Biology and Ecology, University of Jaén, Campus 'Las Lagunillas' s/n, Building B3, 23071 Jaén, Spain; nruiz@ujaen.es
* Correspondence: abolafia@ujaen.es

Abstract: Nematodes are a large and diversified zoological group with a wide global distribution, being able even to be present in habitats with extreme conditions. Although coastal dunes can be considered as an adverse environment for these animals, numerous species are discovered there. In general, these small animals present some morphological characteristics, which provide them a high adaptability to these habitats and the ability to reach a wide distribution. In this study, a total of 222 sample sites of coastal sand dunes have been studied in order to know the nematofauna of these habitats. Thus, 42 coastal dunes from three geographical areas of the Iberian Peninsula coast (Atlantic coast, southern Mediterranean coast, and northern Mediterranean coast) were examined. A total of 120 species of nematodes were found, belonging to eight orders. The results showed the higher prevalence of the species belonging to the order Rhabditida, which were present in 84.2% of the sand dunes studied, most of them belonging to the family Cephalobidae with 42 species, while the order with lower prevalence was the order Enoplida appearing only in one dune (0.9%). The classification of nematofauna by trophic groups showed that bacterial feeders, omnivores, hyphal feeders, and plant feeders shared a high prevalence (83.3%, 40.5%, 34.2%, and 32.8%, respectively), while predators, unicellular eukaryote feeders, and substrate ingesters account for less than 11%. A list of the found species, prevalence, and trophic groups is included.

Keywords: biodiversity; Cephalobidae; Portugal; Rhabditida; SEM; Spain

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1. Introduction

Coastal sand dunes form a specific ecosystem that constitutes a variety of microenvironments due to important factors such as temperature, desiccation, sand accretion, soil salinity, and changes in the organic matter [1]. Furthermore, sand coastal dunes include a wide array of functional types of organisms, including decomposers, mutualistic symbionts, root feeders, and pathogens [2–6], among which appear the nematodes.

Although coastal dunes can be considered an adverse environment for these microscopic animals, many species inhabit these habitats, being the bacteriophage nematodes belonging the family Cephalobidae the most abundant [7]. These small animals present some morphological features that provide them with a high adaptability to these habitats [8], especially the presence of a thickened cuticle with well-developed cuticular annuli that promotes body water retention in these xeric soils [9,10]. Also, they can generate forms of resistance in hostile environments that allow them to be inactive for long periods of time [11], or to carry out the expression of proteins of the head region under stress against certain changes in the environment [12]. Furthermore, some families have modified their labial region developing large and complex lips and labial probolae (or extensions surrounding the oral cavity), which increase the feeding efficiency [13].

Thus, studies of nematodes species present in coastal sand dunes have become important over the years, although there are few who focus on knowing the nematofauna related to dune systems. In spite of this, it is known the possible utility of these animals as quality indicators of these soils [14]. The first recorded studies on nematodes in coastal dunes were carried out in New Zealand [15–18], where 41 new species were described. At a later date, various researchers evidenced the presence of nematodes associated with these types of ecosystems around the world in America [3], Europe [19–25], Asia [7], and Africa [26,27]. With respect to the Iberian Peninsula, the most relevant studies of species of nematodes from sand coastal dunes have been carried out by several authors at the southeast of the peninsula [28–32] and other areas [33–37], describing some new species associated to these sand dunes. Their results show that nematodes belonging to the order Rhabditida are the predominant organisms in these ecosystems since they present adaptations to arid and drought conditions that allow them to survive in this type of environment [38].

Moreover, nematode communities are often sensitive to habitat disturbance, and they show a characteristic sequence of recolonization of disturbed habitats [39]. Nematodes are represented at most trophic levels in the soil and are potentially good indicators of a broad variety of soil properties, being coastal dunes a nutrient-poor ecosystem with very low levels of primary production [40]. These animals are used as bioindicators of soil disturbance, considering their diversity, abundance, and community structure. In contrast to the relatively stable nature of soil, the sand in dune systems is dynamic and always in a state of successional change [41], and therefore changes in the nematode community can help to determine the quality of the soil in these sequences. Some studies carried out to date on the relationship between nematodes and coastal dunes focus on the study of soil quality in these habitats, using these animals as bioindicators, since the same taxa usually appear in this type of environment [7,20,40,42–48]. In addition, due to the large number of species present in the same habitat, the analysis of the biodiversity of nematofauna present in the soil is essential to increase the knowledge of the ecological processes that take place in it [49]. Identifying the families and genera of nematodes present in the community, and determining their prevalence or abundance offers a good opportunity to assess the condition of the ecosystem in relation to the impact of pollutants and other stressors, as well as to monitor changes in the structure and functioning of soil trophic networks [50].

Along the Iberian Peninsula coast appear numerous coastal dunes ecosystems. The objective of this study is to learn the diversity and prevalence of nematodes present in the coastal dunes on the south and east of the Iberian Peninsula, and, more specifically, to identify the most frequent species in this area.

2. Materials and Methods

2.1. Sampling and Nematode Extraction

The soil samples were collected from soil from the rhizosphere of xerophile plants at 5–10 cm depth of soil along 222 sample sites belonging to 42 sand coastal dunes from three littoral regions of the Iberian Peninsula: Atlantic coast (90 samples), southern Mediterranean coast (72 samples) and northern Mediterranean coast (60 samples) (Figure 1). The nematodes were extracted from soil samples (500 g) using Baermann's funnel technique [51] and modified with a stainless steel sieve (100 mm diameter, 100 µm mesh) provided with a disc of thin tissue paper at its bottom [52]. The sieve with the sample is placed on the funnel, filling it with water up to the middle of the sieve. This method uses the hydrophilicity of the nematodes that leave the sample in the water of the funnel and, by gravity, fall to the bottom where the nematodes are collected.

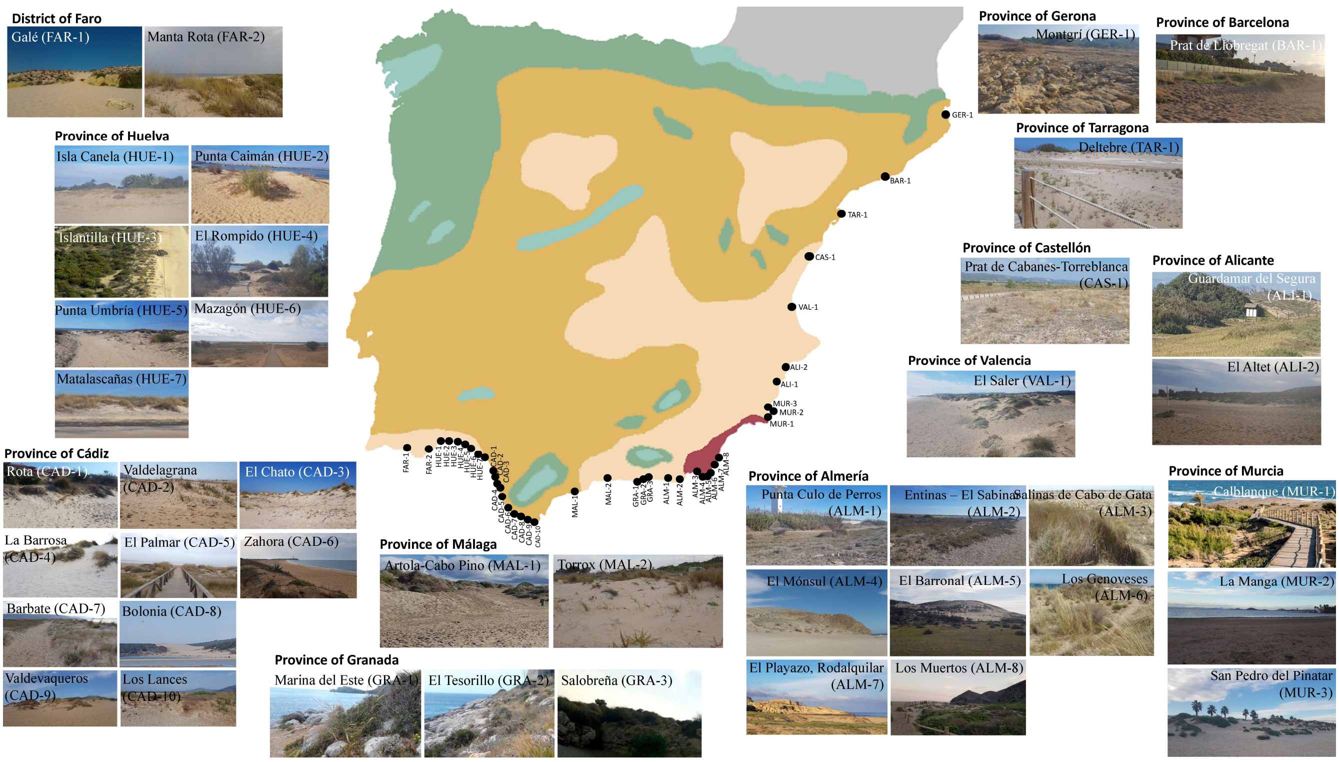

Figure 1. Coastal sand dunes examined in the Iberian Peninsula (map showing the climatological areas).

2.2. Integrative Morphological and Molecular Identification of Nematodes

2.2.1. Morphological and Morphometric Identification

Nematodes extracted were killed by heat and fixed in a 4% formalin solution. A representative number of fixed nematodes, variable according to an abundance of specimens in them, was picked up from each soil sample. The nematodes were processed to anhydrous glycerine according to Siddiqi's method using lactophenol-glycerine solutions [53], and they were permanently mounted on a glass microscope with the glycerine-paraffin method [54], somewhat modified using hot liquid paraffin. Observations were made and measurements were taken using a Nikon Eclipse 80i (Nikon, Tokyo, Japan) microscope provided with a differential interference contrast (DIC) optics. Photomicrographs were taken with the aforementioned microscope equipped with a Euromex sCEMX-6 digital camera (Euromex Microscopen BV, Amhem, The Netherlands) and combined with Adobe® Photoshop® CS v.8.0.1 (Adobe Inc., San José, CA, USA) and figures edited using Microsoft® PowerPoint® v.2016 (Microsoft Corporation, Redmond, DC, USA). For the species identification, several morphometric measurements were taken, and Demanian indices [55] and other ratios [56] were calculated. Furthermore, some specimens mounted in the slides were selected for their observation with an SEM according to Abolafia's method [57]. Accordingly, the nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5 kV) (Zeiss, Oberkochen, Germany).

2.2.2. Molecular Identification

Some fresh specimens were processed using the proteinase K protocol and PCR essays [58] with a few modifications [59]. The specimens were cut in small pieces using a sterilized dental needle on a clean slide [60] with 18 mL of TE (Tris-EDTA) buffer [10 mM Tris-Cl (tris hydrochloride) + 0.5 mM EDTA (ethylene-diamine-tetraacetic acid); pH 9.0], transferred to a microtube and adding 2 µL proteinase K (700 µg/mL) (Roche, Basel, Switzerland), then transferred to −80 °C at least for 15 min. Each tube was left in the freezer for several days and subsequently incubated at 65 °C (1 h), then at 95 °C (15 min).

For DNA amplification, 3 μL of the extracted DNA were transferred to a microtube containing: 0.6 μL of each primer (10 mM), 3 μL Master Mix Taq DNA Polymerase (5× Hot FirePol Blend Master Mix, Solis BioDyne, Tartu, Estonia) and double distilled water (ddH_2O) to a final volume of 20 μL. Amplification of the 18S rRNA gene employed the forward primer 988F (5′-CTCAAAGATTAAGCCATGC-3′) and the reverse primer 1912R (5′-TTTACGGTCAGAACTAGGG-3′) [61]. The primers used for amplification of the D2-D3 region of the 28S rRNA gene were the D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and the D3B (5′-TCGGAAGGAACCAGCTACTA-3′) [62,63]. PCR cycle conditions were as follows: one cycle of 94 °C for 15 min, followed by 35 cycles of 94 °C for 45 s + annealing temperature of 55 °C for 45 s + 72 °C for 45 s, and finally one cycle of 72 °C for 5 min. After DNA amplification, 5 μL of the product were loaded on a 1% agarose gel in 0.5% Tris-acetate-EDTA (40 mM Tris, 20 mM glacial acetic acid. and 2 mM EDTA; pH = 8) to verify the amplification using an electrophoresis system (Labnet Gel XL Ultra V-2, Progen Scientific, London, UK). The bands with the DNA products were stained with SYBR Green I (10,000× concentrate in DMSO; Invitrogen, Waltham, MA, USA) and the DNA-loading buffer 6× (GeneON, Ludwigshafen, Germany). The sequencing reactions of the PCR products were performed at Sistemas Genómicos (Paterna, Valencia, Spain) and Centro de Instrumentación Científico-Técnica (CICT) from the University of Jaén (Jaén, Spain) according to the Sanger method [64]. To confirm the identity of sequences obtained in this study, a Basic local alignment search tool (BLAST) was performed at the National Center for Biotechnology Information (NCBI).

2.2.3. Trophic Structure

The trophic structure of the nematode community was studied by subdividing into seven groups according to feeding type [65]: bacterial feeders (Figure 2a–d), hyphal feeder or mycophagous (Figure 2e,f), omnivores (Figure 2g,h), plant feeder or phytoparasites (Figure 2i,j), predators (Figure 2k,l), unicellular eukaryote feeders and substrate ingested.

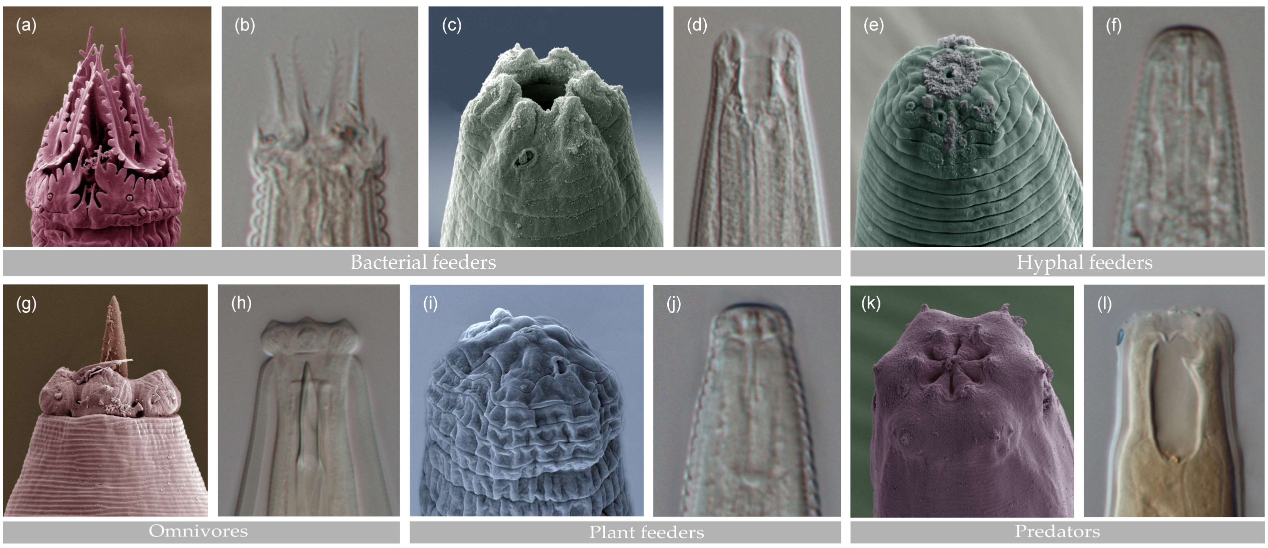

Figure 2. Light microscopy (right) and scanning electron microscopy (left) of the feeder groups found in the coastal sand dunes from Spain. Bacterial feeders: *Nothacrobeles lanceolatus* (**a**,**b**) and *Panagrolaimus superbus* (**c**,**d**); Hyphal feeders: *Aphelenchus avenae* (**e**,**f**); Omnivores: *Aporcelaimellus waenga* (**g**,**h**); Plant feeders: *Tylenchorhynchus aduncus* (**i**,**j**); Predators: *Clarkus papillatus* (**k**,**l**).

2.2.4. Diversity and Prevalence Analysis

The relative proportion of each taxon or group (genera, family, order, or trophic group) was calculated as the proportion of the number of species present in each taxon with respect to the total of species found. The prevalence of different taxa or groups (species, families, genera, orders, and trophic groups) of nematodes present on sand coastal dunes was obtained as the percentage of samples with previously taxon mentioned with respect to the total number of samples [66] using the following equation:

$$\text{Prevalence (\%)} = (\text{Samples with each nematode taxon}/\text{Total samples}) \times 100$$

Data analyses were performed using R v.4.3.1. freeware [67]. Some graphics were created using ggplot2 v.3.4.2 [68]. To study the exclusive and shared species among the three regions sampled, a Venn diagram was conducted using the Venn R package v.0.1.10 [69]. PCA was performed with PAST v.4.13 [70].

3. Results

3.1. Results of Diversity and Prevalence of Species

The study of these coastal dunes revealed a total of 120 species of nematodes belonging to eight orders: Dorylaimida, Enoplida, Isolaimiida, Monhysterida, Mononchida, Plectida, Rhabditida and Triplonchida (Table 1).

Table 1. Orders and species found in coastal dunes in Spain according to their trophic groups: plant feeders (1), hyphal feeders (2), bacterial feeders (3), substrate ingesters (4), predators (5), unicellular eukaryote feeders (6), omnivorous (8); and prevalence.

Orders and Species	Trophic Group	Prevalence
Dorylaimida		
Allodorylaimus sp.	8	1.4%
Aporcelaimellus waenga	8	3.6%
Aporcelaimellus sp.	8	7.2%
Aporcelinus sp.	8	0.9%
Carcharolaimus banaticus	5	0.5%
Chitwoodiellus sp.	2	2.3%
Crassolabium circuliferum	8	0.5%
Crassolabium sp.	8	9.5%
Discolaimium sp.	8	4.1%
Discolaimoides symmetricus	8	0.5%
Discolaimoides sp.	8	0.9%
Dorylaimus sp.	8	1.8%
Ecumenicus monhystera	8	1.8%
Enchodellus sp.	6	0.5%
Eudorylaimus sp.	8	17.6%
Heterodorus sp.	6	2.7%
Longidorella deliblatica	1	4.1%
Longidorella sp.	1	0.9%
Mesodorylaimus brzeskii	8	2.7%
Mesodorylaimus ibericus	8	0.9%
Mesodorylaimus sp.	8	11.3%
Nygolaimus sp.	5	1.4%
Solididens vulgaris	5	1.8%
Solididens sp.	5	0.5%
Takamangai sp.	8	0.9%
Tylencholaimellus minor	2	0.5%
Tylencholaimus sp.	2	1.4%
Xiphinema sp.	1	1.8%
Isolaimiida		
Aulolaimus distortus	3	1.8%
Enoplida		
Cephalanticoma sp.	4	0.5%
Oncholaimus sp.	5	0.5%

Table 1. *Cont.*

Orders and Species	Trophic Group	Prevalence
Monhysterida		
Eumonhystera sp.	3	0.5%
Geomonhystera sp.	3	10.4%
Monhystera sp.	3	2.7%
Mononchida		
Bathyodonthus mirus	3	0.5%
Clarkus papillatus	5	1.4%
Coomansus parvus	5	0.9%
Iotonchus sp.	5	0.5%
Myonchulus sp.	5	0.5%
Rhabditida		
Acrobeles aenigmaticus	3	5.4%
Acrobeles bushmanicus	3	1.4%
Acrobeles ciliatus	3	20.7%
Acrobeles cylindricus	3	14.7%
Acrobeles complexus	3	49.1%
Acrobeles singulus	3	0.5%
Acrobeloides arenicola	3	0.5%
Acrobeloides bodenheimeri	3	48.2%
Acrobeloides nanus	3	12.2%
Acrobeloides sp.	3	0.9%
Acrobeloides tricornis	3	1.8%
Allodiplogaster colobocerca	5	1.4%
Aphelenchoides sp.	1	13.1%
Aphelenchus avenae	2	4.5%
Aphelenchus sp.	2	24.3%
Cephalobus persegnis	3	3.2%
Cervidellus alutus	3	3.6%
Cervidellus neftasiensis	3	1.8%
Cervidellus vexilliger	3	3.6%
Chiloplacus bisexualis	3	2.3%
Chiloplacus demani	3	10.4%
Chiloplacus insularis	3	9.9%
Chiloplacus magnus	3	33.3%
Chiloplacus membranifer	3	0.9%
Chiloplacus minimus	3	0.5%
Chiloplacus mysteriosus	3	0.9%
Chiloplacus symmetricus	3	0.5%
Chiloplacus tenuis	3	5.4%
Chiloplacus trillineatus	3	1.4%
Criconemoides ihlathum	1	0.5%
Crustorhabditis dunicola	3	2.3%
Ditylenchus myceliophagus	2	0.9%
Ditylenchus sp.	1	4.5%
Drilocephalobus moldavicus	3	2.7%
Eucephalobus compsus	3	1.8%
Eucephalobus hooperi	3	7.7%
Eucephalobus mucronatus	3	10.4%
Eucephalobus striatus	3	0.5%
Filenchus sp.	1	0.9%
Gracilacus sp.	1	0.5%
Helichotylenchus sp.	1	3.6%
Heterocephalobellus magnificus	3	1.8%
Macrolaimus crucis	3	4.9%
Mesorhabditis carmenae	3	1.4%
Mesorhabditis minuta	3	5.4%

Table 1. *Cont.*

Orders and Species	Trophic Group	Prevalence
Mesorhabditis sp.	3	3.2%
Nothacrobeles lanceolatus	3	36.5%
Nothacrobeles nanocorpus	3	1.4%
Oscheius tipulae	3	2.3%
Panagrolaimus rigidus	3	26.6%
Panagrolaimus superbus	3	10.4%
Paracrobeles psammophilus	3	5.9%
Paraphelenchus sp.	2	6.8%
Pellioditis sp.	3	0.5%
Pratylenchus sp.	1	1.4%
Protorhabditis sp.	3	1.4%
Pseudacrobeles elongatus	3	0.9%
Pseudacrobeles unguicolis	3	6.3%
Rotylenchus sp.	1	2.3%
Scutylenchus tessellatus	1	2.3%
Stegelleta incisa	3	2.3%
Stegelleta ophioglossa	3	3.6%
Stegelletina devimucronata	3	4.1%
Stegelletina pygmaea	3	1.8%
Stegelletina salinaria	3	0.5%
Stegelletina similis	3	0.5%
Tylenchorhynchus aduncus	1	0.5%
Tylenchorhynchus sp.	1	1.35%
Tylenchus sp.	1	17.6%
Zeldia punctata	3	5.4%
Plectida		
Anaplectus sp.	3	0.5%
Ceratoplecus sp.	3	0.5%
Chiloplectus sp.	3	4.5%
Haliplectus sp.	3	4.5%
Plectus sp.	3	5.9%
Tylocephalobus auriculatus	3	6.8%
Tylocephalobus sp.	3	1.8%
Wilsonema otophorum	3	0.9%
Triplonchida		
Prismatolaimus sp.	3	0.5%
Tripyla sp.	5	0.9%
Tripylina sp.	5	0.9%

The results showed that the order having a higher number of species was the order Rhabditida (58.3%) with 70 species. Also, that this order resulted in the most prevalent, being present in 84.2% of samples studied (Figure 3a,b).

Subsequently, the family Cephalobidae, with 42 species belonging to this order, was the family with the highest number of species (35%), being the family most prevalent in this study (79.7%). Regarding the genera found, the largest number of species belonged to the genus *Chiloplacus,* with 10 species in total (8.3%), while the genus most prevalent was the genus *Acrobeles* being present in 55.4% of the samples studied, both belonging to the family Cephalobidae. Predominant species from the total of the samples studied were *Acrobeles complexus* Thorne, 1925, present in 49.1% of the sand dunes studied followed by *Acrobeloides bodenheimeri* (48.2%), *Nothacrobeles lanceolatus* (36.5%) and *Chiloplacus magnus* (33.3%), all of them belonging to the family Cephalobidae.

The order Dorylaimida, with 28 species, was the second most frequent (23.3%) and prevalent (42.8%) order. The orders with a lower presence in sand dunes samples were Plectida (20.3%), Monhysterida (12.6%), Mononchida (3.2%), Triplonchida (2.7%), Isolaiimida (2.3%) and Enoplida (0.9%), all of them with less than 7% of the species found (Figure 3a,b). Of the total of families obtained, the results showed 16 families represented by only one species, but with different prevalences appearing in different proportions across the samples studied. The second family with the highest number of species was the family Qudsianematidae, which is represented by 10 species (8.3%), and represents 27.5% of the samples studied.

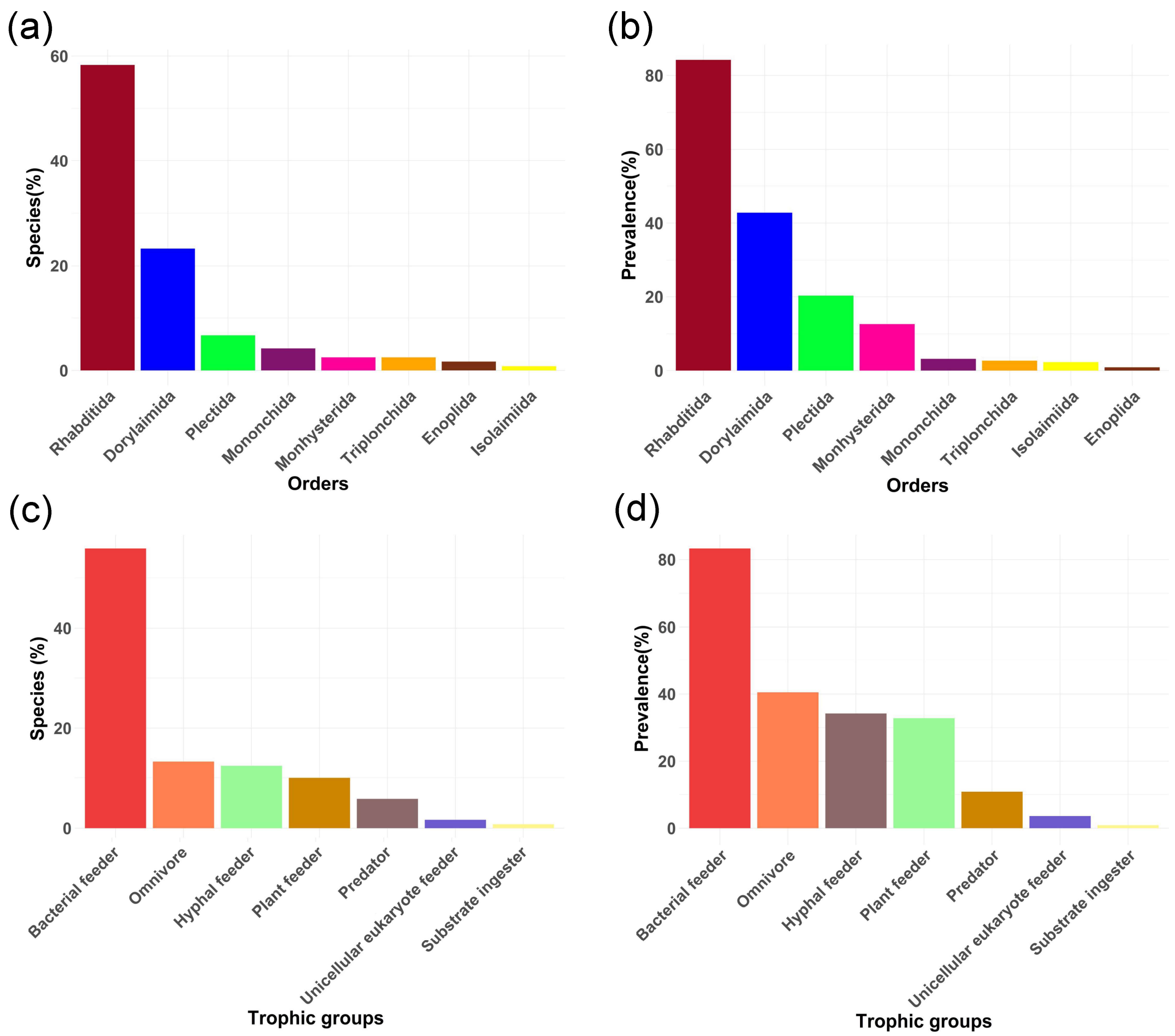

Figure 3. Relative proportion of species and prevalence of orders (**a**,**b**), and relative proportion of species and prevalence of trophic groups (**c**,**d**), found in sand dunes coast.

3.2. Results of Diversity and Prevalence of Trophic Groups

A total of seven trophic groups have been found in the total of the samples studied: bacterial feeders, omnivores, hyphal feeders, plant feeders, predators, unicellular eukaryote feeders, and substrate ingesters (Table 1, Figure 2). The analysis of diversity shows that the nematofauna in this ecosystem is constituted mainly by bacteriophages, represented by 55.8% of the total species found (Figure 3c). Omnivores, plant feeders, and predators represent 13.3%, 12.5%, and 10% of the species studied, respectively. Finally, hyphal feeders, unicellular eukaryote feeders, and substrate ingesters represent less than 6%. Regarding the prevalence of these trophic groups, the largest prevalent trophic group was bacterial feeders present in the 83.3% of samples studied, followed by omnivores (40.5%), hyphal feeders (34.2%) and plant feeders (32.9%). Predators appeared in 10.8% of the sites studied, while unicellular eukaryote feeder and substrate ingesters appeared in 3.6% and 0.9% of the samples studied, respectively (Figure 3d).

3.3. Results of the Distribution of the Species

According to the geographical distribution of species per the three littoral areas, a total of 66 species were found in the Atlantic littoral, 75 species in the southern Mediterranean littoral, and 78 species in the northern Mediterranean littoral, showing the dunes located at the southern Mediterranean coast presented a higher percentage of rhabditid species (73.4%). However, the northern Mediterranean littoral and Atlantic littoral also showed the highest percentage of rhabditid species with respect to the total of species studied (64.1% and 60.6%, respectively) (Figure 4).

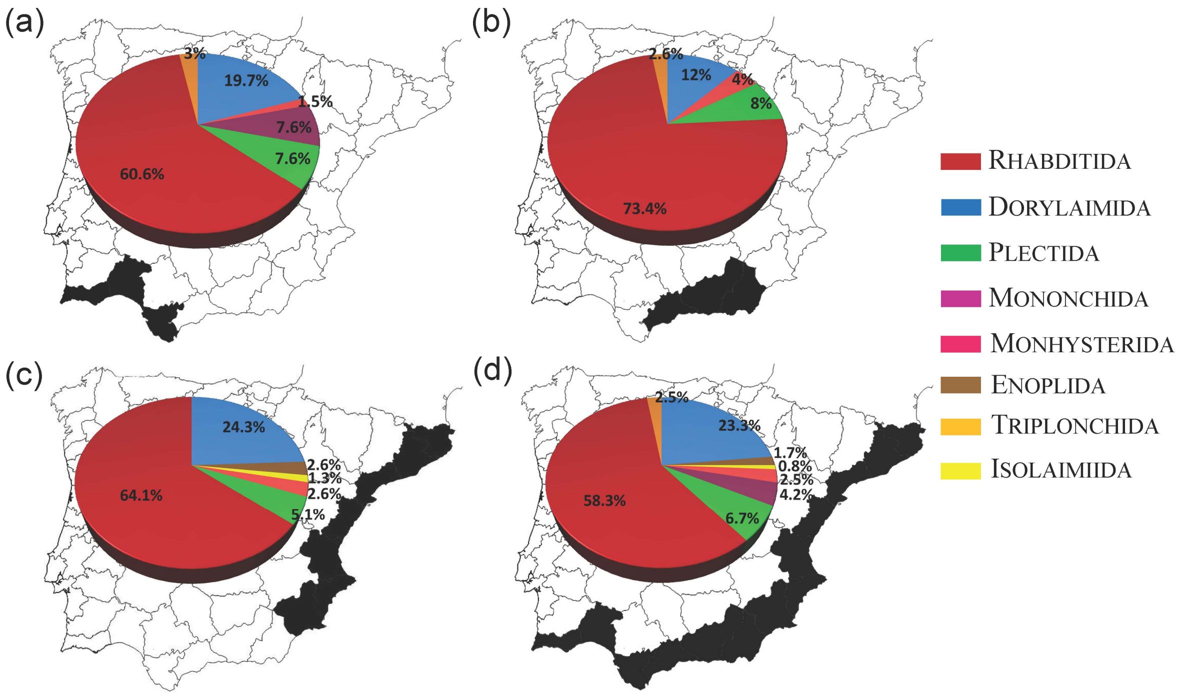

Figure 4. Relative proportion of species of each nematode order in the Iberian coastal dunes grouped per the three littoral area districts. (**a**) Atlantic littoral, (**b**) southern Mediterranean littoral; (**c**) northern Mediterranean littoral; (**d**) total of the coast samples.

Moreover, the prevalence of this order was the highest in southern Mediterranean littoral being present in 97.2% of the samples studied in this area. The prevalence of rhabditid in the northern Mediterranean coast and Atlantic littoral were 70% and 93.3%. The second most frequent order in total of the samples studied, Dorylaimida, continued to be the second most frequently order in three different areas studied, in this case showing the highest number of species in the northern Mediterranean littoral (24.3%), and lower, in Atlantic littoral (19.7%) and southern Mediterranean littoral (12%). The prevalence of this order aligns with the diversity of species being higher in the northern Mediterranean littoral (65%), followed by the Atlantic littoral (38.9%) and southern Mediterranean littoral (31.9%).

The results showed that there are orders that, albeit not prevailing, appear only in one area studied. Thus, the order Mononchida only appears on the Atlantic coast (7.6%) (Figure 4a), and the orders Enoplida and Isolaimiida appear only at the northern Mediterranean dunes in low proportion (2.3% and 1.3%, respectively) (Figure 4c). The order Triplonchida appears south of the Iberian Peninsula (Atlantic coast and southern Mediterranean coast) but does not appear at the northern Iberian Peninsula. In addition, Plectida and Monhysterida appear indistinctly distributed in the three areas but at lower frequenies.

At the species level, in the Atlantic littoral and northern Mediterranean littoral the most prevalent species was *Acrobeles complexus* (35.6% and 61.7%, respectively), while the most prevalent species in the southern Mediterranean littoral was *Acrobeloides bodenheimeri* (59.7%). Furthermore, the results showed that there were 30 species in common between these three studied areas, hence, 25% of the species studied are present along the coast of the Iberian Peninsula (Figure 5).

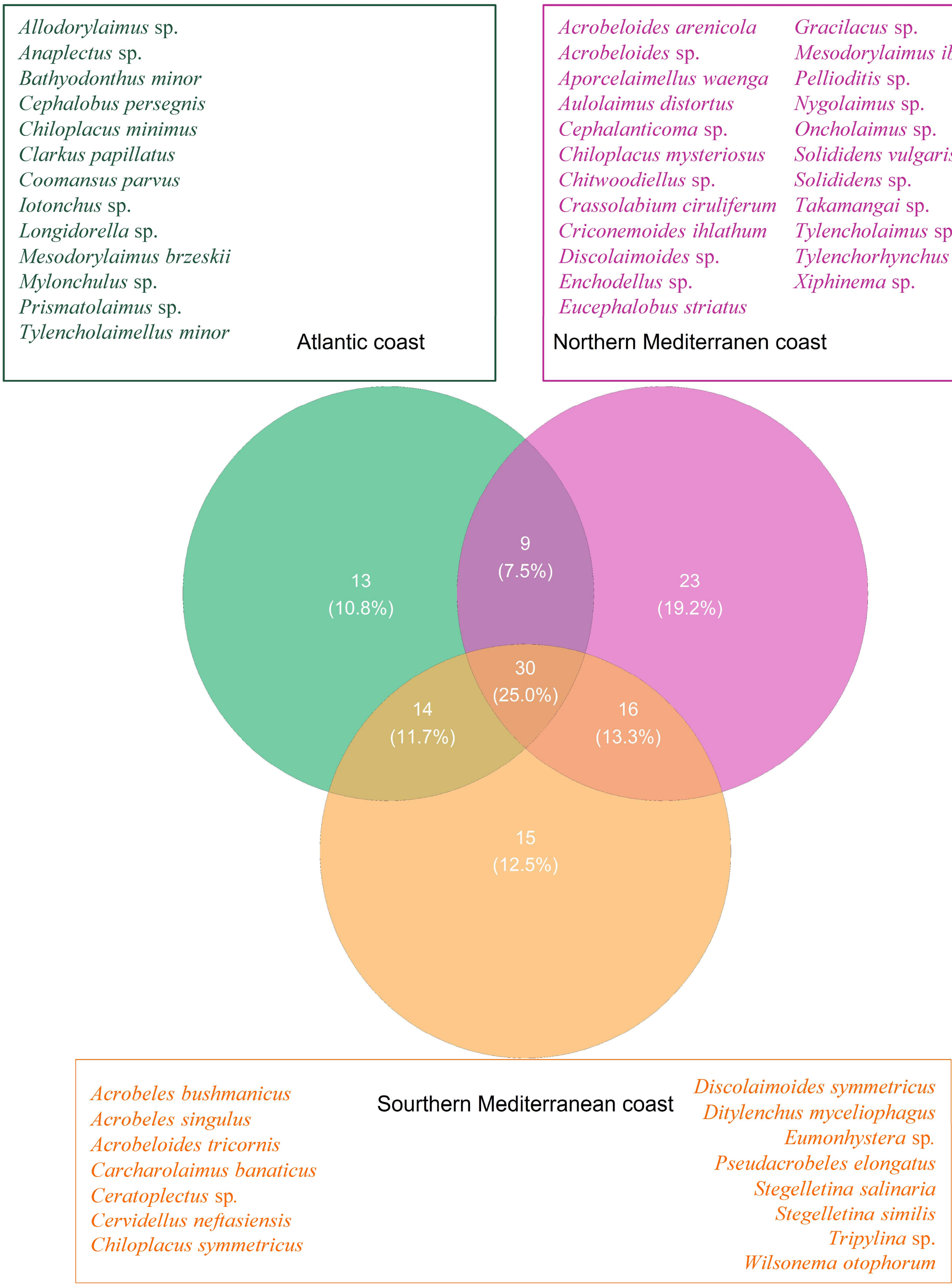

Figure 5. Venn diagram showing exclusive and shared species of nematodes present in the three littoral areas studied (color represents the different areas studied).

Moreover, the Atlantic coast presented 13 species, which only appear in this geographical area, and 14 and 9 species in common with the southern Mediterranean coast and northern Mediterranean coast, respectively. The southern Mediterranean coast showed 15 species, which only appear on there, and 16 species in common with the northern Mediterranean coast. Finally, the northern Mediterranean coast showed 23 species, which only appear in this area.

3.4. Results of Distribution of Trophic Groups

The geographical distribution based on feeding habits shows that the bacteriophages (Isolaimiida, Monhysterida, some Mononchida, Plectida, most Rhabditida, and some Triplonchida) are distributed in all littoral areas studied, the southern Mediterranean coast the one which exhibits a higher percentage of species of bacterial feeder (69.3%) (Figure 6), and the highest prevalence of this trophic group (97.2%). Thus, hyphal feeders (some Dorylaimida and some Rhabditida) appear in the highest percentage in the same area (5.3%), being present in 43.1% of samples of this site. However, omnivores (most Dorylaimida) were found in the highest percentage in the northern Mediterranean coast (15.4%), being present in 61.6% of samples of this area. Plant feeders (some Dorylaimida and some Rhabditida) showed a higher number of species on the northern Mediterranean coast (15.4%), but their prevalence was higher in the southern Mediterranean coast, being present in 43.1% of the samples in this area. Predators (Mononchida, some Dorylaimida, some Rhabditida, and some Triplonchida) appear in the highest proportion of species on the Atlantic coast (9.1%), but appear in more samples on the northern Mediterranean coast (20%). Unicellular eukaryote feeders (some Dorylaimida) showed the highest number of species on the Atlantic coast (1.5%), while the prevalence was highest on the southern Mediterranean coast (5.5%). Finally, substrate ingesters (some Rhabditida) only appear on the northern Mediterranean coast, with a proportion of 1.3% of species studied of this trophic group and being present in 3.3% of the samples studied of this area.

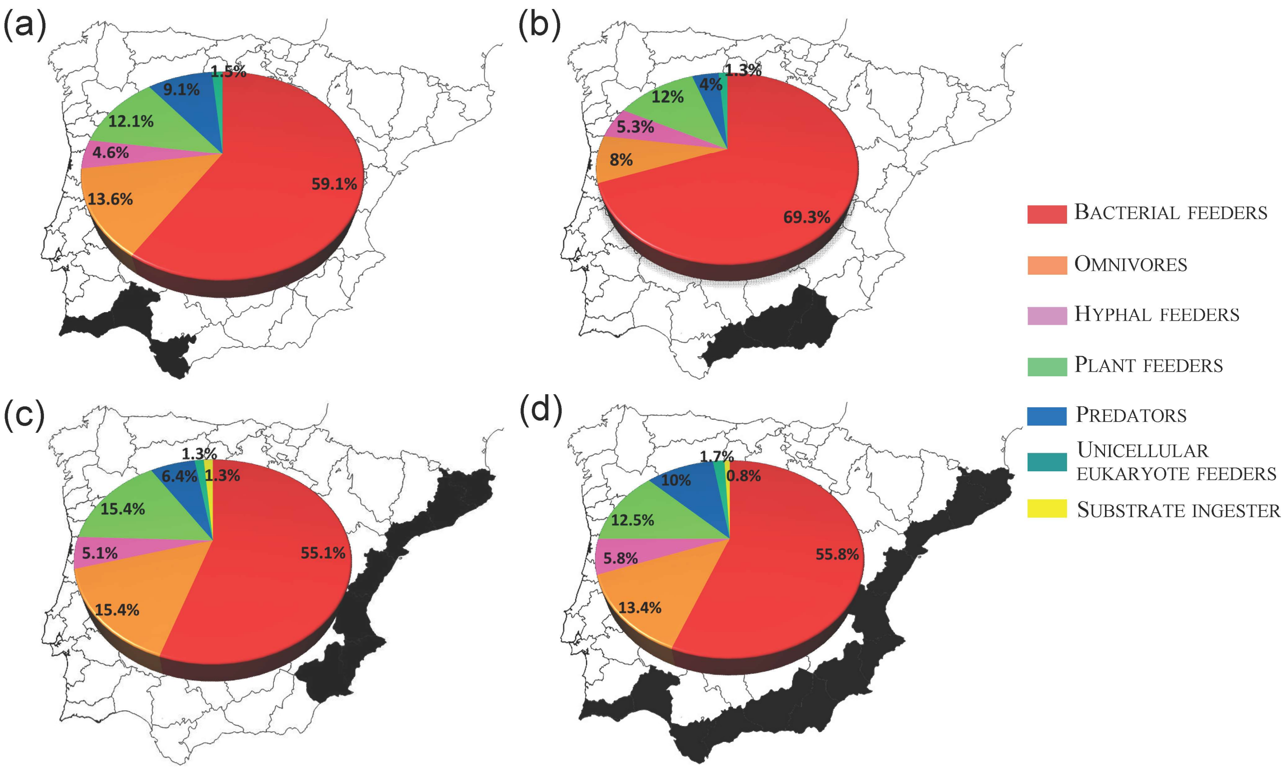

Figure 6. Percentage of species of each nematode trophic group in the Iberian coastal dunes grouped per the three littoral area districts. (**a**) Atlantic littoral, (**b**) southern Mediterranean littoral; (**c**) northern Mediterranean littoral; (**d**) total of the coast samples.

4. Discussion

The members of the nematode order Rhabditida have been the most abundant taxa found in this study, showing the wide distribution of these species and their capacity of adaptability to environmental changes. This result agrees with other previous studies that consider rhabditids as a group of nematodes with a broad ecological range, with species found in all types of soils, and, more specifically, in harmful ecosystems [38,71]. Thus, species belonging to this taxon are considered "r" strategists or opportunistic organisms that can adapt to the different changes that occur in the environment [72,73]. Furthermore, in this study the members of the family Cephalobidae have been the most abundant, agreeing with previous studies [7], which may be due to the evolutionary and morphological adaptations of this family, such as the morphological characteristics, i.e., the structure of their cuticle [38]. Thereby, the most abundant species of this family (*Acrobeles complexus*, *Acrobeloides bodenheimeri,* and *Nothacrobeles lanceolatus*) also match with previous studies that cited these species on sand dunes coast [21,30,34,35,74]. Furthermore, the results suggest that some species found in this study and belonging to the family Cephalobidae could be good indicators of xeric conditions, for example species as *Acrobeles bushmanicus* [26,32], *Acrobeles ciliatus* [17,26,75], *Acrobeloides arenicola* [28], *Chiloplacus insularis* [22,76], *Chiloplacus mysteriosus* [77], *Nothacrobeles lanceolatus* [35,74], *Paracrobeles psammophilus* [22,32,33], *Mesorhabditis carmenae* [78], *Stegelleta ophioglossa* [22,32], *Stegelletina salinaria* [79,80] and *Zeldia punctata* [17,74], have been described previously in this type of environments and are not frequently described at others humid environments. However, it is not concluded that *Acrobeles complexus*, the most frequent species found in this study, can be considered as an indicator of these habitats due to its cosmopolitan distribution, being described throughout all kinds of environments, including wetlands [30]. Regarding plant feeders (order Rhabditida, infraorder Tylenchomorpha), they are not very frequent because an exhaustive study of the roots of the plants has not been carried out.

Looking at the geographical distribution of species, the rhabditid nematodes are the most frequent in all dunes examined (Figure 4), while the presence of other nematode groups is very low. In this respect, the northward dunes are located in a territory with a higher humidity [81], where other nematode orders increase in species number. Secondly, the southeastward dunes, located in the most xeric areas of the Iberian Peninsula, maintain a higher diversity of rhabditid species.

The next abundant taxa have been the members of the order Dorylaimida, which also have a wide distribution [82,83] prevailing the species of the genus *Aporcelaimellus*, morphologically characterized by having a more thickened cuticle. On the other hand, the orders with the lowest diversity were Isolaimiida, Enoplida, Mononchida, Triplonchida, and Monhysterida, all of them with fewer species. This is due to the preference of these taxa for humid habitats [71,83,84]. In the case of the members of the order Mononchida, they appeared only in the Atlantic dunes probably due to the humidity provided by the morning mist, which is frequent in the area. The orders Enoplida and Isolaimiida appeared only at the northern Mediterranean dunes; in this case, the weather is milder, and the rain is more frequent. The rest of the orders found in these coastal dunes (Monhysterida, Plectida, and Triplonchida), appear indistinctly distributed but in lower frequency. In general, all these nematode orders are considered "k" strategists, with slow life cycles, preferring a more stable environment [72,73].

With respect to the trophic structure of these environments, it shows that the bacteriophages nematodes are the most diversified agreeing with previous studies on nematodes in coastal dunes, where bacteriophages constitute the majority of the species found [7,40,45,85]. According to this, the rhabditid nematodes - most of them being bacterial feeding- prevail in all dunes, especially in the most xeric ones (southern Mediterranean coast), while other nematode groups (hyphal feeders, plant feeders, and unicellular eukaryote feeders) appear with less frequency, increasing in dunes with higher humidity environments. Consequently, omnivores, predators, and substrate ingesters appear more frequently on the northern Mediterranean coast.

To compare the three areas studied and see how the nematode orders and their trophic groups are distributed in the three regions, the method of principal component analysis (PCA) was performed. The PCA based on order diversity showed that among the nematode community composition in the three regions, the members of the order Rhabditida are negatively correlated with respect to the members of the orders Dorylaimida and Mononchida. On the other hand, the PCA based on trophic groups (Figure 7b) showed that omnivores, plant feeders, substrate ingesters, and unicellular eukaryote feeders present a similar trend in the northern Mediterranean coastal dunes, while hyphal and bacterial feeders were positively correlated in the southern coast of the Mediterranean coastal dunes. However, the predator nematodes tend to appear on the Atlantic coast. In addition, the PCA shows that the bacterial feeders were negatively correlated with the predators and the omnivores.

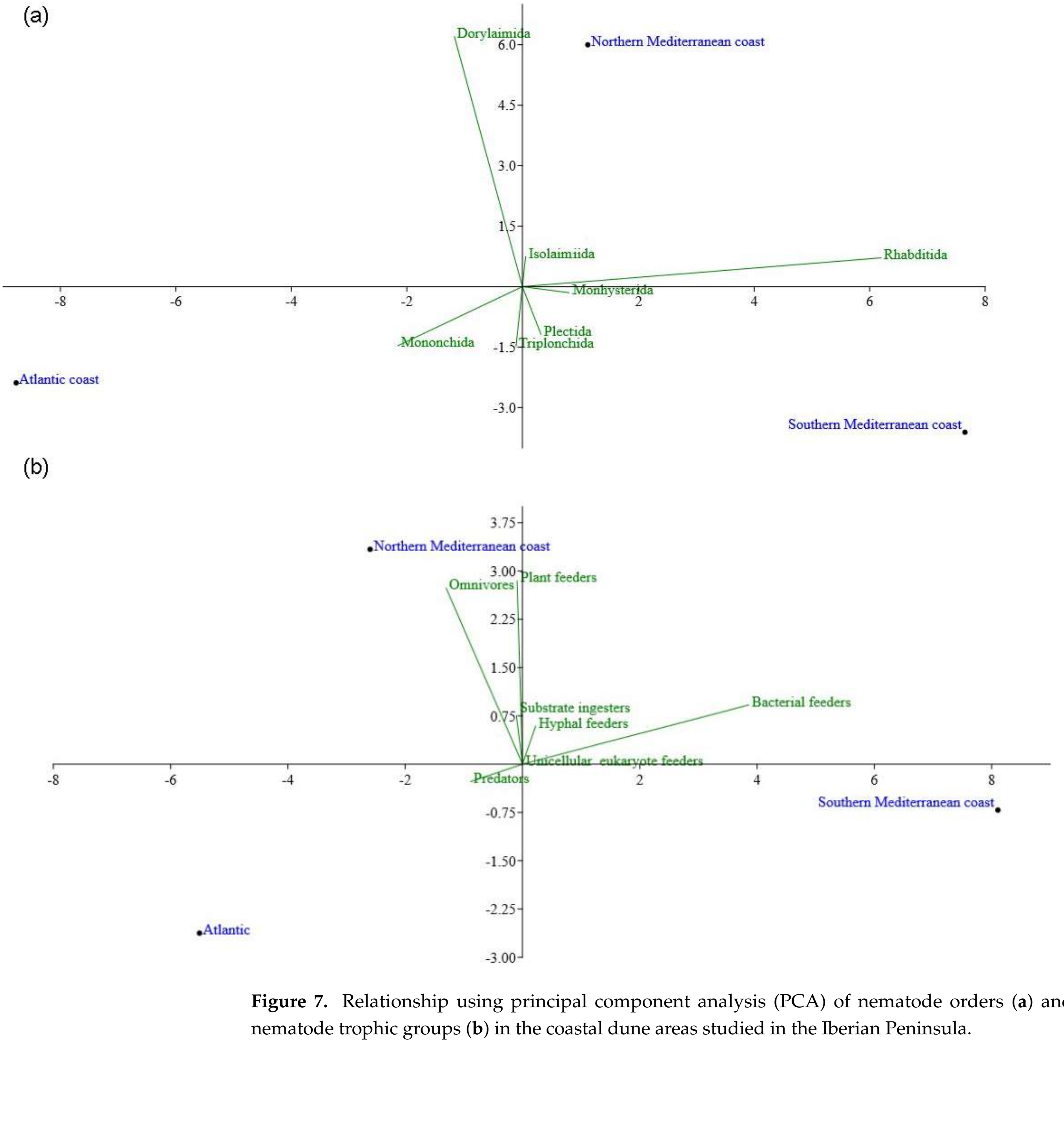

Figure 7. Relationship using principal component analysis (PCA) of nematode orders (**a**) and nematode trophic groups (**b**) in the coastal dune areas studied in the Iberian Peninsula.

5. Conclusions

This study shows the highest prevalence and diversity of the nematode order Rhabditida in sand dunes of the south and the east coasts of the Iberian Peninsula with respect to other nematode taxa. The family Cephalobidae—belonging to the order Rhabditida—is the most frequent and diverse family of nematodes found in sand dunes along the south and the east coasts of the Iberian Peninsula, which could have the highest adaptability to these environments. The species with the highest prevalence in this study is *Acrobeles complexus*, which is a cosmopolitan species. Some species of the family Cephalobidae appear only or preferably in xeric environments, which can be considered as bioindicators of these habitats.

With respect to the distribution of the species, the order Rhabditida is frequently found in xeric geographical areas (southern of the Iberian Peninsula), while other orders appear with higher frequency in environments with more humidity. Finally, with respect to the trophic groups, the bacterial feeders show the highest prevalence along the coasts studied being the more frequent group in this study. Omnivores, plant feeders, and predators represent the following more frequent groups in this study, while hyphal feeders, unicellular eukaryote feeders, and substrate ingesters appear in low frequency.

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