PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

ESTUDO DA NEMATOFAUNA NO BIOMA MATA ATLÂNTICA

Carla Aristonara Müller

DISSERTAÇÃO DE MESTRADO

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SUMÁRIO

Dedico minha dissertação a Angélica.
Minha querida amiga Angel que virou estrela no céu.
Tenho certeza que você está me guiando aí de cima.
Te amo amiga.

AGRADECIMENTOS

Agradeço...

Aquela que é meu anjo da guarda, que eu gostaria que fosse eterna. Que coloca asas em todos os meus sonhos. Te amo mãe!

Aquele que eu brigo incansavelmente e amo incondicionalmente, afinal sou sua cópia. Tu é impar. Te amo mais que tudo, orelhudo!

Aquela que é minha amiga, mestre e orientadora. Responsável não só pela minha formação profissional, mas também como pessoa. Beijos Ale, obrigada por juntas termos acreditado em um novo mundo!

Ao Carlos, meu orientador! Que confiou na idéia e sempre me leva a pensar de forma diferente. Meu imenso carinho!

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A minha segunda família, meus amigos que também são fonte de inspiração para o meu intelectual, vivem me animando e torcendo pelo meu sucesso. Amo vocês!

À instituição, Pontifícia Universidade Católica do Rio Grande do Sul, pela infraestrutura fornecida durante a pesquisa, em especial aos professores por terem contribuído de forma grandiosa com meu crescimento e formação.

Ao CNPq pelo apoio financeiro.

RESUMO

O filo Nematoda é bastante diverso e ocorre em vários ecossistemas, ocupando uma posição central na cadeia trófica de solos. O conhecimento das espécies e distribuição destas pode contribuir em estudos sobre a ecologia e funcionamento do solo, incluindo a dinâmica de população e controle biológico não só dos próprios nematódeos, mas também do ecossistema ali presente. Este trabalho teve como objetivo descrever a nematofauna ocorrente na área do Centro de Pesquisas e Conservação da Natureza PRÓ-MATA-RS, e classificá-la taxonomicamente e de acordo com seu grupo trófico. A identificação foi realizada através de microscopia óptica baseada em chaves taxonômicas evidenciando os caracteres diagnósticos de cada grupo. Mil e quinhentos nematódeos foram encontrados em 200 cm^3 de solo. 63% pertencentes à classe Chromadoria e 12% à classe Enoplea, classificados em quatro ordens, oito famílias e 13 gêneros. Quanto aos hábitos alimentares dos nematódeos foram classificados como 35% parasitos de plantas, 28% bacterívoros, 7% onívoros e 5% como predadores. Após isolamento dos nematódeos, foi realizada extração de DNA para a amplificação das regiões V4 e V9 do gene 18S do DNA (rDNA). O produto amplificado foi sequenciado, obtendo-se 281.400 sequências. Dentre o filo Nematoda, as famílias classificadas foram: Cephalobidae, Chromadoridae, Criconematidae, Plectidae, Ironidae, Prismatolaimidae, Rhabdolaimidae e Tripylidae.

ABSTRACT

Nematofauna study in the Atlantic Forest Biome

The Phylum Nematoda is extremely diverse and occurs in several ecosystems, thus occupying a key role in the soil trophic webs. The knowledge of nematode species and their patterns of distribution in soils habitats may contribute to our understanding of soil ecology and functioning. The aims of this work was to describe the nematofauna occurring in the Center of Research and Environmental Conservation - PRÓ-MATA-RS and classifies the nematodes according to their feeding habits thus provide additional information about the Pró Mata soil diversity. Identification was performed through light microscopy and following taxonomic keys evidencing the diagnostic characters of each group. In total, 1.500 nematodes were found into 200 cm^3 of soil analyzed. The Chromadorea class was represented by 63% of the individuals, whereas the class Enoplea class by only 12%. Four orders, eight families and 13 genera were recovered. As for feeding trophic groups, were represented plant parasites by 35%, bacterial feeders by 30%, omnivores by 7% and predators by 5%. After extracting nematodes from soil, DNA extraction for amplification of the V4 and V9 regions of the 18S gene of ribosomal DNA was performed. The amplified product was sequenced, obtaining 281,400 sequences passed. Based on the DNA sequences, seven orders, 15 families, and 16 nematode genera were recovered.

APRESENTAÇÃO

A presente dissertação intitulada "Estudo da nematofauna no bioma Mata Atlântica" é um dos requisitos indispensáveis à obtenção do grau de Mestre, exigido pelo Programa de Pós-Graduação em Zoologia, da Faculdade de Biociências, da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Este estudo teve como principal objetivo conhecer a nematofauna de solo nativo do Rio Grande do Sul em área de conservação do bioma Mata Atlântica. Subsequente i) Descrever a nematofauna ocorrente em solo nativo do RS; ii) Identificar taxonomicamente os grupos e iii) Classificar os grupos de acordo com cada grupo trófico.

Os resultados aqui apresentados foram gerados no Laboratório de Biologia Parasitaria e Parasitologia Molecular da PUCRS através de suporte financeiro do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) concedido na forma de bolsa de mestrado.

Esta dissertação será apresentada no formato de artigo científico, sendo precedida de uma introdução geral ao tema abordado, com suas respectivas referências ao final do documento. O manuscrito encontra-se formatado de acordo com as normas de submissão do periódico Soil Research.

INTRODUÇÃO GERAL

O solo é constituído por um mosaico de materiais inorgânicos resultantes da erosão das rochas, e material orgânico que é produto da decomposição das plantas e outros organismos. Desta forma, o solo reflete a diversidade dos compostos presentes em determinada região e será diferente de acordo com as influências a que é submetido (Wall *et al*., 2013).

Microrganismos que vivem no solo podem ser classificados de acordo com a capacidade de interferência no ecossistema, são os chamados grupos funcionais. Estes grupos funcionais podem ser definidos como um conjunto de espécies que têm efeitos similares sobre um processo biogeoquímico ou biofísico de nível de ecossistema específico (Lavelle 1997; Swift, Izac et al. 2004; Barrios 2007; Kibblewhite 2008). Três categorias agrupam estes indivíduos: "engenheiros químicos" onde se encontram os organismos decompositores e transformadores, os "reguladores biológicos" que são organismos capazes de promover alguma regulação de outros organismos e por fim os "engenheiros de ecossistema" que são organismos que poderiam atuar na manutenção da estrutura do solo pela formação de redes de poros e bio-estruturas, na agregação ou transporte de partículas (Lavelle 1997; Swift *et al*., 2004; Barrios 2007; Kibblewhite 2008)

Dentre os "reguladores biológicos" de solo, estão classificados os nematódeos. Estes atuam na regulação da atividade microbiana do solo e também nas interações parasitárias ou mutualísticas com outros microrganismos ou invertebrados. Essas interações controlam a abundância de populações nas cadeias alimentares do solo, juntamente com a oferta de recursos conforme disponibilizado pelos "engenheiros químicos" (Young & Crawford 2004).

O filo Nematoda atualmente apresenta cerca de 23.000 espécies já descritas e com cerca de um milhão de espécies ainda possíveis de descrever (Blaxter, 2011). Até o momento 2271 gêneros já foram descritos agrupados em 256 famílias (Anderson, 2006). A abundância, diversidade e importância dos nematódeos contrasta-se com o insignificante volume de conhecimentos taxonômicos acumulados sobre tais organismos e também com o baixo número de pesquisadores envolvidos com essa área. A maioria dos estudos em nematologia no Brasil concentra-se na área de fitopatologia, incluindo muitas instituições de pesquisa e ensino, como a Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) que realiza importantes estudos com nematódeos patógenos de solo e de plantas (Huang & Cares, 2006), devido a forte atividade econômica derivada da produção de insumos agrícolas. No sul do Brasil, os estudos realizados em microbiologia de solos, não incluíram a investigação sobre nematódeos de vida livre. Rieff *et al*., (2014) por exemplo, estudaram a dinâmica de ácaros e colêmbolos no Rio Grande do Sul, como potenciais bioindicadores de qualidade de solo. Em comparação ao sistema de classificação de outros metazoários, a classificação dos nematódes encontra-se em um estágio pouco avançado, principalmente pelas limitações dos caracteres morfológicos e morfométricos obtidos por meio da microscopia ótica e de varredura.

O presente trabalho descreveu a nematofauna ocorrente em solo do bioma Mata Atlântica no RS utilizando análises taxonômicas e moleculares. Esse foi o primeiro estudo que evidencia não só as espécies que causam perdas econômicas na agricultura, mas também leva em consideração toda a nematofauna existente naquele solo. Estudos realizados apenas em sítios de atividade agrícola, não refletem a composição total da fauna nativa de uma determinada região. Como esses achados representam a nematofauna de solo de área de conservação, abrem perspectivas para explorações acerca de danos relativos a ecossistemas de solo em áreas que sofreram cultivo recente na mesma região.

Com o conhecimento da nematofauna do solo do RS adquiro, e sabendo que essa diversidade interfere diretamente na velocidade da decomposição e na disponibilidade de nutrientes no solo (Yeates *et al*., 2003) poder se inferir que determinadas espécies de nematódeos podem ser indicadores ecológicos da estrutura e funcionamento da cadeia alimentar daquele solo. Além disso, o estudo das espécies ocorrentes em solo de área de conservação podem proporcionar melhor entendimento das relações interespecíficas mutualísticas ou parasitárias que representam uma população em equilíbrio e futuramente intervenções com o intuito de bioremediação em solos danificados poderão ser aplicadas.

Capítulo I

Diversidade de nematódeos existentes em área de conservação do Bioma Mata Atlântica

Soil Nematode Diversity from a Natural Reserve in the Atlantic Forest, South of Brazil.

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A ser submetido ao periódico Soil Research.

Abstract

The Phylum Nematoda is extremely diverse and occurs in several ecosystems, thus occupying a key role in the soil trophic webs. The knowledge of nematode species and their patterns of distribution in soils habitats may contribute to our understanding of soil ecology and functioning. The aims of this work was to describe the nematofauna occurring in the Center of Research and Environmental Conservation - PRÓ-MATA-RS and classifies the nematodes according to their feeding habits thus provide additional information about the Pró Mata soil diversity. Samples were randomly collected for nematode identification and soil physicochemical analyzes. Identification was performed through light microscopy and following taxonomic keys evidencing the diagnostic characters of each group. In total, 1.500 nematodes were found into 200 cm^3 of soil analyzed. The Chromadorea class was represented by 63% of the individuals, whereas the class Enoplea class by only 12%. Four orders, eight families and 13 genera were recovered. As for feeding trophic groups, were represented plant parasites by 35%, bacterial feeders by 30%, omnivores by 7% and predators by 5%. After extracting nematodes from soil, DNA extraction for amplification of the V4 and V9 regions of the 18S gene of ribosomal DNA was performed. The amplified product was sequenced, obtaining 281,400 sequences, of which 99.99% passed through the quality filter and were submitted to UPARSER algorithm. The taxonomic assignment was obtained using the QIIME v1.8 program through the Naive Bayesian Classifier RDP Algorithm using the SILVA database (v111). 20% of the Eukaryotes were classified at Phylum level using the V4 region and 55% using the V9 region. Based on the DNA sequences, seven orders, 15 families, and 16 nematode genera were recovered.

Introduction

Soils provide an immense array of niches that contain a vast and still largely unknown biodiversity. Much has been done to describe and understand the progressive transformation of bedrocks into soils, including the distribution, nature and specific characteristics of mineral and organic components (Lavelle & Spain, 2001; Buol *et al*., 2003; Coleman *et al.* 2004; Bardgett *et al.,* 2005). Nevertheless, analyzing the function of soil as a habitat is a more recent emphasis, including the way that organisms live in it.

The Nematoda is the most abundant phylum in the Metazoan, which successfully inhabits almost all kind of environments (Ferris *et al*., 2001). In soil, nematodes override to all microfauna organisms such as Protozoa and Rotifera (Wall *et al*., 2013). Amongst these three groups, many thousands of species are known globally, but it is expected that this is only a fraction of the number of species actually present on the globe. The number of total species described of soil nematodes is around 25,000 species comprising about only 6% of the known soil biota (Procter, 1984; Boag and Yeates, 1998; Groombridge and Jekins, 2002; Hillebrand, 2004; Wall *et al*., 2014). In soil, nematodes play a central position in the food web, covering several trophic levels, including**,** bacterial and fungal feeders, parasites, predators and omnivores species, which specialized mouthparts, are used for taxonomic classification (Yeates *et al*., 1993).

The occurrence of specific free-living nematodes is dependent on changes in each ecological niche**,** trophic groups and soil particular characteristics. Thus, nematodes are considered biomarkers and have the potential to provide insights related to the structure and functioning of the soil food web becoming of extreme importance for plant health, since its diversity, directly affects the rate of decomposition and availability of nutrients (Freckman, 1988; Ferris *et al*., 1999; Ritz and Trudgill, 1999; Hoy *et al*., 2014).

Thus, the knowledge on the species and their distribution in particular soil may contribute for better understanding of soil ecology including population dynamics and biological control. In Brazil, few studies have covered nematode identification. The very first was the publication of Goeldi (1887), describing *Meloidogyne* sp., a root-knot nematode parasitic of many plants, especially those with agricultural importance. Since then, free-living nematodes received less attention, with a few records in the Amazon region and central States (Huang *et al*., 2000). Many other researchers have investigated nematode species focused on those with economic importance. In southern Brazil, most of the studies in soil microbiology did not include research on free-living nematodes. Rieff *et al*. (2014) for example, studied the dynamics of mites and springtails in Rio Grande do Sul, as potential biomarkers of soil quality.

In this study we investigated the nematode fauna in the soil of the Conservation Area of Pró-Mata-PUCRS in São Francisco de Paula, Rio Grande do Sul, Brazil. This work is the pioneering approach accessing native species of nematode fauna, which may help better diagnose ecological interactions and biological implications of nematodes in soil fitness of conservational areas.

Materials and methods

1) Sample collection

The location of this study was the Center for Environmental Research and Conservation Pró-Mata, in the municipality of São Francisco de Paula, Rio Grande do Sul (29°26’17”to 29°34’42” S, and 50°08’14” to 50°14’18” W). 15 soil samples were randomly collected in different areas with old mature thick forest, without araucaria, with the presence of big and old trees. These soil was collected in depth from zero to 15 cm, and pooled together in order to better represent the area of study. The soil was sieved through a 5 mm mesh to remove large detricts and root fragments before gently homogenized. The soil was stored at 4°C until use and 500g of collected soil were used for chemical-physical analysis. The air temperature varied between maximum of 30°C and minimum of 19°C. Air humidity ranged between 88% - 71% and had wind speed of 15 km/h these information were obtained at the Brazilian Meteorological Institute (Instituto Nacional de Meteorologia).

Physicochemical analysis of the soil show acid pH (4.2) and has high concentration of organic matter (>6,7%). Values of macronutrients and micronutrients were quite varied. Sulfur, Manganese and Zinc were found in higher concentrations in soil, while Boron and Copper had the lowest concentration. All measured parameters are in Table 1.

Table 1. Physicochemical analysis.

Pró Mata	M.O	Argil	pH	P	K	Al	Ca	Mg	S	B	Mn	Zn	Cu
	%	%	Water	mg / dm^3		cmol / dm^3			mg / dm^3				
	>6,7	21,6	4,2	22,8	182	3,2	3,1	1,6	18	0,3	42	3,06	0,72

M.O = Organic Matter; P = Phosphor; K = Potassium; Al = Aluminum; Ca = Calcium; Mg = Magnesium; S = Sulfur; B = Boron; Mn = Manganese; Zn = Zinc; Cu = Copper.

2) Nematode extraction

Terrestrial nematodes have a specific gravity of 1.08 and can be recovered by flotation on a solution with a specific gravity of 1.15 (Hooper, 1986). After sieving, the remaining sediment was soaked in water and centrifuged at 1,800 x g to obtain a compact pellet, and eliminate the supernatant. The pellet was suspended in 20 ml of Percoll, centrifuged again for 1 minute at same rotation. Nematodes were collected at the interface between the top and bottom of supernatant. The nematodes were collected in a new tube containing fixing solution (Formalin 40%, glycerin 2% and distilled water).

3) Molecular Identification

18s gene of ribosomal RNA was amplified and sequenced for barcode identification according to Floyd *et al*. (2001). The 15 soil samples were processed as described above. For DNA extraction Power Soil-DNA Isolation kit (MO BIO laboratories, Inc.) was used. The sequences were submitted to quality control that retained sequences with a minimum length of 100 bp and were trimmed to remove low-quality bases for the minimum Phred score of 30 using PRINSEQ (Schmieder and Edwards, 2011). The remaining sequences were dereplicated and sorted by decreasing read abundance using USEARCH v7.0.1090 (Edgar, 2010). The OTUS were obtained at 99% of identity using algorithm UPARSE (Edgar, 2013). For the removal of chimeras was utilized the program UCHIME (Edgar, 2011) using RDP gold database (http://drive5.com/uchime/gold.fa) as reference. The taxonomic assignment was obtained using QIIME v1.8 (Caporaso et al., 2010) through of the algorithm RDP Naive Bayesian Classifier (Walg et al., 2007) with 1.0 confidence score and using as reference database the GreenGenes 13.8 (DeSantis et al., 2006)

4) **Fresh Slide Mounts**

Fresh slides were prepared according to the methodology of Seinhorst (1959). Briefly, a small drop of the fixative solution was added in the center of a clean glass slide and a single nematode was selected and put on it. A glass wool was added (about 5 mm in length) near to the edge of the drop. A cover glass (18 mm wide) was gently placed over the drop (using a needle to support it). The slides were observed under a microscope for morphological identification.

5) **Morphological identification**

The morphological identification was made by identifying the mouth parts, for trophic groups and functional guilds according to Yeates *et al*. (1993) and Ferris *et al*. (2001). Reproductive organs (Andrássy, 1983; Kiontke, 1996) and morphological characters of each taxon were used for diagnosis based on taxonomic keys described by Sudhaus (2011) and Kiontke *et al*. (2011).

Results

Two hundred cm³ of pooled soil was examined with the yield of 1,500 nematodes isolated. The majority of specimens found were classified into Chromadorea class (63%), followed by Enoplea class representing 21% of the total specimens observed, 25% (370 individuals) of specimens could not be classified at this time. All nematode taxa found are in Table 1.

Representatives of all feeding habits were found in the examined soil (Figure 1). Plant Parasites were represented by 35% with 522 individuals, being the genus *Discrocriconemella* the most abundant representative, follows by bacterial feeders (28%) with 426 specimens, being the majority of genus *Rhabditis.* Omnivores (7%) with 108 individuals, all classified into Dorylaimida order and predators (5%) with 74 specimens belonging to Mononchida order. Of whole sample 25% could not be classified due to lack poor preservation of their mouthparts.

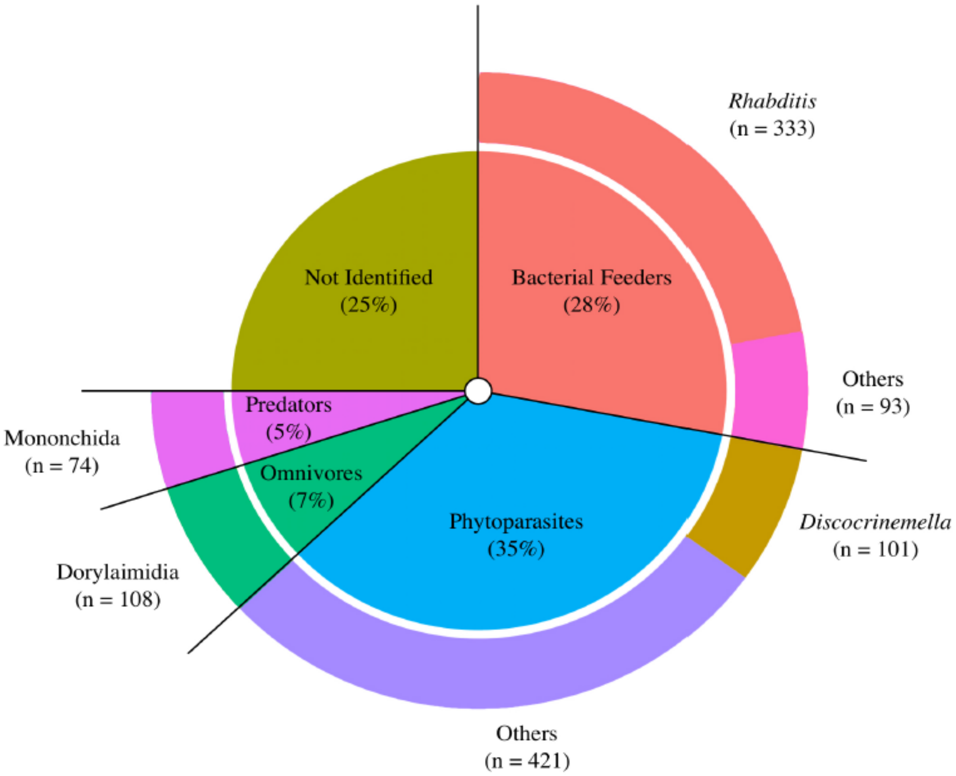

Figure 1. Nematofauna composition according to their feeding habits.

From a total of 948 specimens classified into class Chromadorea, the predominant order was Rhabditida, suborder Tylenchida and infraorder Tylenchomorpha with 522 specimens, classified in 11 genera: *Mesocriconema* (69), *Hemicycliophora* (33), *Discocriconemella* (101), *Helicotylenchus* (75), *Scutellonema* (52), *Aoralaimus* (24), *Rotylenchus* (17), *Aphelenchoides* (34), *Tylenchus* (48), *Ditylenchus* (15) and *Meloidogyne* (54). Order Rhabditida comprised others 426 specimens being 93 classified as family Cephalobiodae. Family Hoplolaimidae included highest genus diversity, *Heliocotylenchus*, *Scutellonema*, *Aorolaimus* and *Rotylenchus*.

Class Enoplea, accounted for 182 specimens, all classified into subclass Dorylaimia and belonging to the orders Dorylaimida and Mononchida, with 108 and 74 specimens, respectively.

Two variables V4 and V9, of the ribosomal DNA 18s were amplified from soil sample. From V4 were obtained 141,218 sequences, and 140,182 sequences from V9 were analyzed by bioinformatic tools, where 56% of Eukaryota sequences remained unclassified, followed by Rhizaria (15,2%), Fungi unclassified (6,6%), Metazoa unclassified (4,2%), Chytridiomycota (2,0%), Nematoda (2,4%), Chrysophyceae (2,0%), Ciliophora (1,7%), Basidiomycota (1,4%) and Ascomycota (1,2%).

Among phylum Nematoda, the class Chromadorea was represented by the following taxa: *Rhabdolaimus terrestres*, *Plectus minumus*, *Prodesmodora circulata*, *Acrobeloides buetschlii* and *Choriorhabditis dudichi* and at the genus level was possible to identify *Discocriconemella* and *Gracilacus*. All these taxa are included in four orders (Araeolamida, Desmodorida, Rhabditida and Tylenchida), seven families (Rhabdolaimidae, Plectidae, Microlamidae, Cephalobidae, Rhabditidae, Criconematidae and Tylenchulidae) and seven genera (*Rhabdolaimus*, *Plectus*, *Prodesmodora*, *Acrobeloides*, *Choriorhabditis. Discocriconemella and Gracilacus*) all included in Table 2.

Enoplea class had six taxa identified at species level: *Dorylaimellus virginianus*, *Xiphinema brasiliense*, *Xiphinema herakliense*, *Amblydorylaimus isokaryon*, *Prismatolaimus dolichurus* and *Prismatolaimus intermedius*. And five taxa were identified to the genus level: *Aporcelaimellus*, *Eudorylaimus*, *Ironus*, *Odontolaimus* and *Tripyla*. These taxa belong to three orders (Dorylaimida, Enoplida and Triplonchida), eight families (Aporcelaimidae, Belondiridae, Qudsianematidae and Longidoridae) and nine genera (*Aporcelaimellus, Dorylaimellus, Eudorylaimus, Xiphinema, Amblydorylaimus, Ironus, Odontolaimus, Prismatolaimus* and *Tripyla*), all showed in Table 2.

Table 2. Nematofauna from Center for Environmental Research and Conservation (Pró-Mata).

Class	Order	Family	Genus	Species
Chromadorea	Araeolaimida	Rhabdolaimidae	*Rhabdolaimus*	*Rhabdolaimus terrestris**
		Plectidae	*Plectus*	*Plectus minimus**
	Desmodorida	Microlamidae	*Prodesmodora*	*Prodesmodora circulata**
	Rhabditida	Cephalobidae	*Acrobeloides*	*Acrobeloides buetschlii**
			Acrobeles	
		Rhabditidae	*Chariorhabditis*	*Chariorhabditis dudichi**
			Rhabditis	
		Anguinidae	*Ditylenchus*	
	Tylenchida	Aphelenchoidedae	*Aphelenchoides*	
		Criconematidae	*Discocriconemella*	
			Hemicycliophora	
			Mesocriconema	
		Hoplolaimidae	*Aorolaimus*	
			Helicotylenchus	
			Rotylenchus	
			Scutellonema	
		Meloidogynidae	*Meloidogyne*	
		Tylenchidae	*Tylenchus*	
		Tylenchulidae	*Gracilacus**	
Enoplea	Dorylaimida	Aporcelaimidae	*Aporcelaimellus**	
		Belondiridae	*Dorylaimellus*	*Dorylaimellus virginianus**
		Qudsianematidae	*Amblydorylaimus*	*Amblydorylaimus isokaryon**
			*Eudorylaimus**	
		Longidoridae	*Xiphinema*	*Xiphinema brasiliense**
				*Xiphinema herakliense**
	Enoplida	Ironidea	*Ironus**	
		Oxystominidae	*Odontolaimus**	
	Mononchida			
	Triplonchida	Prismatolaimidae	*Prismatolaimus*	*Prismatolaimus dolichurus**
				*Prismatolaimus intermedius**
		Tripylidae	*Tripyla**	

* Identified by molecular analysis only.

Discussion

The State of Rio Grande do Sul, Brazil is characterized by strong economic activity derived from the production of agricultural products. The highest diversity of nematodes occurring in a native area compared to agrosystems has been clearly established (Yeates & King, 1997; Mattos, 1999; Andrade *et al*., 2004; Cares & Andrade, 2006; Korenko & Schmidt, 2007), therefore, those studies conducted only in farming sites, should not reflect the composition of the native fauna of Rio Grande do Sul State.

At present study, nematode diversity was evident since representative individuals of 8 orders, 21 families, 27 genera (Table 2). This is in accordance with other studies (Goulart *et al*., 2003; Silva, 2008; Mondino *et al*., 2009; Arieira, 2012) which could be possibly related to plant diversity (Cares, 1984; Cares and Huang, 1991; Iminoto, 2008) since Pró-Mata is covered mostly with native grasslands, mountain forests with *Araucaria* and a complex mosaic of vegetation in different stages of ecological succession.

Although Pró-Mata soil showed a high diversity of nematodes, the abundance was low. According to Liang & Steinberger (2001), moisture difference in soil can determine differences in the abundance of the nematode communities. In dry areas as in the Brazilian Caatinga where the average humidity is 50% the abundance of nematode per cm^3 is about of 500 individuals (Caixeta, 2015). The other extreme may also affect the abundance of nematodes, since the high humidity found in Pró-Mata soil (88%) may have affected nematode abundance since, only 7,5 individuals were present per cm^3. However, the low abundance found here is in accordance with other study in the biome Atlantic Forest, with occurrence of 3.7 nematodes per cm^3 of soil (Caixeta, 2015).

In addition, high abundance of a particular nematode is an indicative of soil disturbance or ecological imbalance (Huang & Cares 2000 a,b; Huang and Cares, 2006; Iminoto, 2008), suggesting that Pró-Mata may be at optimum ecological equilibrium.

Furthermore, the occurrence of representative individuals of different genera from the suborder Tylenchida and orders Mononchida and Dorylaimida in the soil of Pró-Mata is an indicative of soil preservation. These groups have been found extremely sensitive to soil and environmental disturbances, especially those due to farming activity (Bongers, 1999), the infraorder Tylenchida has plant parasites individuals how hasn't these characteristics. Also, the genera *Discocriconemella* and *Dorylaimellus* found here, have been mostly identified in areas of native forest and not in searched farming sites (Goulart et al. 2003; Tomazini 2008).

Other genera found in this study, *Gracilacus* and *Scutellonema*, have been described only in Atlantic Forest (Caixeta, 2015). *Mononchus* and *Aporcelaimus*, both present in our analysis, was classified with higher value of c-p, this means, they are persistent organisms, and this fact can indicate stability of the system analyzed, being absent in areas of environmental disturbance.

Looking at the feeding habits, the plant-parasitic nematodes were the most abundant, representing 35% of the whole sample analyzed. These results are in accordance with previous studies in Amazonian and Savannah regions (Huang & Cares 2006), where 50,71 % of nematode found were plant parasites.

This first characterization of physicochemical and micronutrients components of Pró-Mata soil contributes for better knowledge of the ecological niche conditions of these nematodes, which may serve for futures investigations of nematode behavior and laboratory maintenance.

Inomoto (1995) described the presence of Meloidogyne javanica in fragments of Atlantic Forest in the city of Piracibaca – São Paulo. Lima et al (2005) found the same nematode when analyzed the soil of Rio de Janeiro state. In the Brazilian Cerrado many species of Meloidogyne genus was found in native vegetation (Cares & Huang, 1991; Silva et al., 2012). The soil of Atlantic Forest presence in Pro Mata, also registrated the Meloidogyne genus, in low concentrations, 0,27 per cm³. These fact supports the idea proposes per Caixeta (2015), the idea suggest that this genus is native contributor of Brazilian biomes. The excessive increase of a plant parasites species is due observed in areas where there is an ecological imbalance promoted by the agrosystems, mainly as monocultures (Schimitt & Norton, 1972). Evidencing that the environments where occur a great variety of vegetation can support different species of nematodes to the same ecological niche (Cares & Huang, 1991). Thus, the presence of all nematode trophic groups including may suggest that the soil habitats in the PROMATA natural reserve are in equilibrium. Together with sequencing analysis showed a high diversity of organisms being represented by Rhizaria, Chytridiomycota, Chrysophyceae, Ciliophora, Basidiomycota, Ascomycota. However, many of the DNA sequences were only assigned to unclassified higher groups (e.g. unclassified Ascomycota) and all of them have an important role in the soil diversity and the soil ecosystem. Patterns of abundance and diversity for soil nematodes in natural areas such as the Pro-Mata natural reserve have been extremely poor explore. The present study is the first in characterizing the soil nematofauna present in the Pró Mata soil. This study contributes to the taxonomic knowledge of the phylum Nematoda, it shows that natural reserves might have a higher diversity, that this dataset can be used as a baseline for future studies.

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