UNIVERSIDADE FEDERAL DO PARANÁ

LAIZA CABRAL DE FARIA

METABARCODING AS A TOOL TO ESTIMATE THE MEIOFAUNAL DIVERSITY IN A TIDAL FLAT

PONTAL DO PARANÁ,

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Trabalho de conclusão de curso apresentada como requisito parcial para obtenção do grau de bacharel em Oceanografia, Centro de Estudos do Mar, Setor de Ciências Da Terra, Universidade Federal do Paraná.

Orientador: Prof. Dr. Maikon Di Domenico

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"Metabarcoding as a tool to estimate the meiofaunal diversity in a tidal flat"

Monografia aprovada como requisito parcial para a obtenção do grau de Bacharel em Oceanografia, da Universidade Federal do Paraná, pela Comissão formada pelos professores:

Profª. Drª. Sónia Andrade - Instituto de Biociências/USP

Prof. Dr. Paulo da Cunha Lana - CEM/UFPR

Prof. Dr. Maikon Di Domenico
Presidente

Pontal do Paraná, 16 de dezembro de 2016

“In every outthrust headland, in every curving beach, in every grain of sand there is the story of the earth.”
Rachel Carson

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RESUMO

Estimar a diversidade marinha é uma tarefa desafiadora. Mais de 250 anos após Lineu começar a descrever a diversidade marinha, é estimado que mais de 70% das espécies ainda não foram descritas. Essa missão é ainda mais difícil para a meiofauna, devido ao seu pequeno tamanho, ao tempo requerido para o estudo desta comunidade polifilética e à necessidade de taxonomistas treinados em cada grupo. Ferramentas moleculares começaram a ser utilizadas com o intuito de facilitar essa missão, mas ainda há limitações por serem uma técnica recente. Neste trabalho utilizamos o gene 18S para sequenciar a meiofauna marinha de uma planície de maré de uma baía subtropical. Em 13 amostras foram encontrados 11 filos, com dominância de Nematoda. O comportamento assimptótico da curva de rarefação indicou que 13 amostras foram suficientes para acessar a diversidade total da área estudada (0,12 km^2). Quando os resultados da riqueza e composição da fauna obtidos por *metabarcoding* e morfologia foram comparados, os padrões de diversidade encontrados foram similares, mas a composição de gêneros foi diferente. A distribuição da diversidade foi significativamente correlacionada com a porcentagem de areia média, o grau de seletividade do sedimento e a concentração mínima de bactérias. O *metabarcoding* se mostrou uma ferramenta útil e eficiente para explorar a diversidade, porém com incompatibilidades na identificação da maioria dos gêneros. O aumento do número de sequencias depositadas em banco de dados virtuais e a construção de livrarias especificas para a área são requisitos essenciais para o aumento da robustez dos dados de diversidade obtidos por metabarcoding. Nosso estudo também enfatiza a necessidade de integrar abordagens moleculares com identificação morfológica e taxonômica para a construção de livrarias.

Palavras-chave: 18S; Ecologia de comunidade; Baía do Araçá; Ecologia bêntica; Canal de São Sebastião.

METABARCODING AS A TOOL TO ESTIMATE THE MEIOFAUNAL DIVERSITY IN A TIDAL FLAT

De acordo com as normas regimentais do curso de Oceanografia da Universidade Federal do Paraná, este trabalho de conclusão de curso é apresentado na forma de artigo.

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ABSTRACT

Estimating marine diversity is still a challenging task, more than 250 years after Linnaeus started to describe it. We estimate that more than 70% of the species are still undescribed. This mission is even harder for meiofaunal animals, because of their small body size, polyphyletic tratis and need of high taxonomical expertise. Molecular tools can make this task easier, but they are still limited. In this study the 18S gene was used to sequence marine meiofauna from a subtropical tidal flat. 11 phyla were found in 13 samples, dominated by nematodes. The asymptotic behaviour of the rarefaction curve indicated that 13 samples were sufficient for diversity estimative for the studied area (0.12 km^2). The richness and faunal composition as estimated by metabarcoding and morphology were congruent, but differed in the composition of genera. Diversity distribution patterns were related to the mean sand percentage, sediment sorting, and the minimum concentration of bacteria. Metabarcoding was useful and efficient to explore diversity, but with mismatches in genera identification. Increasing the number of sequences deposited in virtual databases and building specific libraries to the study area are essential to enhance the robustness of the diversity data obtained by metabarcoding. Our study also emphasizes the urgency to integrate molecular approaches with morphological and sound taxonomical identification by experts.

Key words: 18S; Community ecology; Araçá Bay; Benthic ecology; São Sebastião channel.

SUMÁRIO

1.INTRODUCTION

Meiofauna is a diverse and abundant benthic size-compartment, with thousands of individuals and hundreds of species in a small portion of sediment (Giere, 2009). Meiofauna is a polyphyletic community, with small sized individuals between 42 μm and 1000 μm (Higgins and Thiel, 1988). At least 22 of the 35 known phyla are found within the meiofauna (Higgins and Thiel, 1988; Giere, 2009). Sound meiofauna studies require time and trained taxonomists for each group of organisms (Carugati et al. 2015; Sinniger et al. 2016). On a large scale, meiofaunal distribution and diversity seem be linked to sedimentological (Flach et al. 2002; Semprucci et al. 2010), physical (Atilla et al. 2005; Boeckner et al. 2009) and chemical drivers (Urban-Malinga and Moens 2006; Pusceddu et al. 2009), while on a small scale they are correlated with biotic factors (Papageorgiou et al. 2007; Pusceddu et al. 2016) .

The lack of consistent morphological information (Derycke et al. 2008; Creer et al. 2010), the small-size bodies (Fontaneto et al. 2009), and scarcity of trained taxonomist place meiofauna among the most unknown metazoans (Carugati et al. 2015). Experts assume that less than 30% of meiofaunal species have been described (Appeltans et al. 2012). Then, assessing meiofaunal diversity becomes a challenging task (Carugati et al. 2015; Fontaneto et al. 2015). Due to the difficulties to handle, visualize, and identify the organisms, meiofauna are in the cutting edge of taxonomical, ecological and phylogenetic discussion (Vinther 2015), and molecular technics for a fast and effective knowledge of its diversity are urgent (Carugati et al. 2015; Brannock et al. 2016).

In the last decades, several different views are discussing the possibilities to finish Linnaeus task (Godfray and Charles 2002; Anonymous 2007; Godfray 2007). For instance, some studies regarding DNA barcoding (Cristescu 2014) for species identification support the idea that the global diversity cannot be estimated before it disappears (Godfray and Charles 2002), while other authors support the robustness of the morphological information and the necessity to keep training taxonomist and enhance museological traditions (Carvalho et al. 2005; Ebach and Holdrege 2005). Nowadays, most experts agree that both approaches are complementary and necessary (Pennisi 2003; Stoeckle 2003).

DNA barcoding, a technique that uses individual fragments of DNA (Cristescu 2014), has been used for the identification of organisms since the early 2000s (Floyd et al. 2002; Hebert et al. 2003). Recently, the introduction of the metabarcoding method for the identification of multiple species from environmental DNA (eDNA) raised the promise of representing a faster and more efficient technique for assessing marine biodiversity (Ji et al. 2013; Leray and Knowlton 2015). Due to its versatility, this technique can be applied to evaluate the organisms diet through their gut contents (Leray et al. 2015); microorganism diversity (Grossart and Rojas-Jimenez 2016; Shehzad et al. 2016), and even to explore remote places, such as the deep sea (Guardiola et al. 2015; Sinniger et al. 2016).

This technique has already been used to describe and identify meiofaunal distribution patterns, by assessing the relative levels of richness and diversity patterns in micro- and macro-scale (Fonseca et al. 2010; Fonseca et al. 2014b), as well as to access the spatial distribution patterns of the organisms (Brannock et al. 2016)

However, there is still a need for methodological standardization to optimize the results (Brannock et al. 2016). This study aims to estimate meiofaunal diversity in a tidal flat, using metabarcoding approach. First, we estimate the effectiveness of metabarcoding, contrasting OTUs richness with the number of expected OTUs, then we compared the diversity obtained from metabarcoding and from morphological analyses. Finally, we assessed the relation between diversity parameters, richness and faunal composition, and environmental variables.

2. STUDY AREA

The Araçá Bay (23°49'S, 45°24'W), is a small tidal flat (0.12 km$^{2)}$ near to São Sebastião Harbor and São Sebastião Oil Terminal (Fig 1), is located in São Sebastião, on the northern coast of São Paulo state, Brazil. The bay shelters four beaches: Deodato, Pernambuco, Germano and Topo, apart from two small islands, Pernambuco and Pedroso. During low tides, the exposed area can reach 300 m (Amaral et al. 2010). Araçá Bay is one of the last remaining mangrove areas along the coast of São Sebastião.

The bay presents low hydrodynamics and is protected from wave action by the São Sebastião Island (Dottori et al. 2015). Heterogeneous sediments compose the tidal flat, with mud (silt and clay), sand, and gravel areas (Amaral et al. 2015)

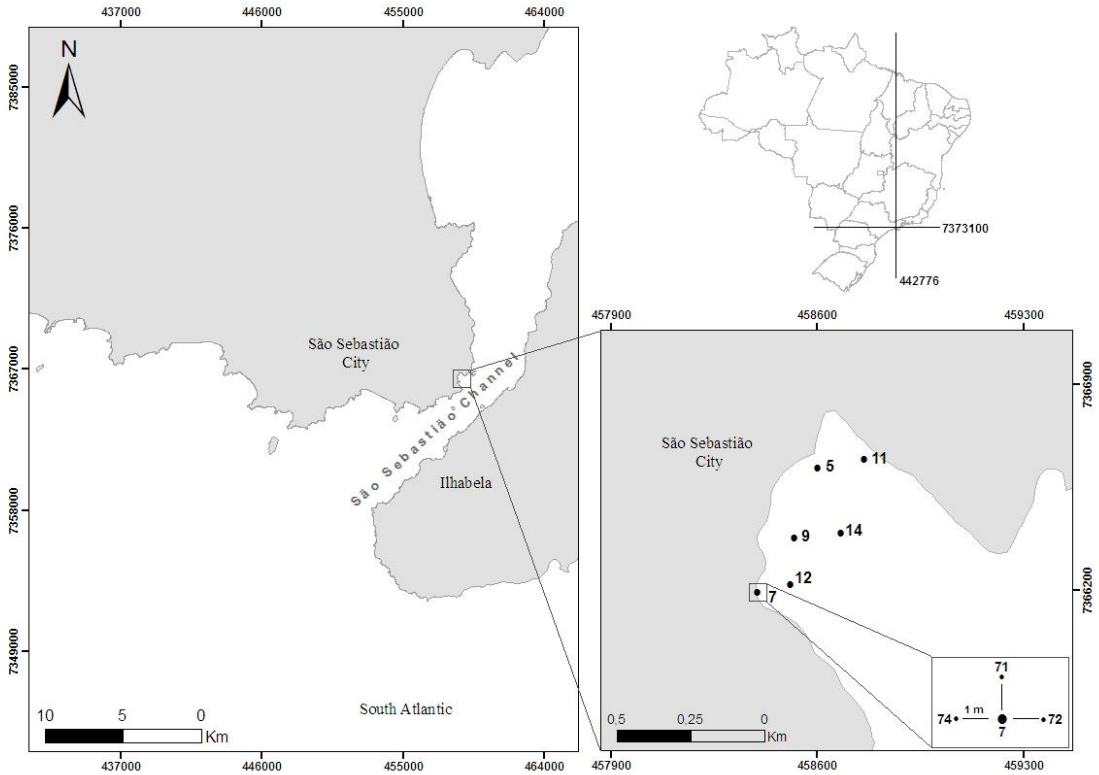

Fig 1. The Araçá Bay and sampling points in detail.

3. MATERIALS AND METHODS

3.1. METABARCODING SAMPLING AND EXTRACTION

Sampling was done in March 2015 with a 2.5 cm diameter cylindrical corer to a 10-cm depth. Sampling was performed in six geo-referenced sites, spaced approximately 50 m from each other. Four samples, with a distance of 1m from each other were taken in each site, totalizing 24 samples. Samples were immediately fixed in absolute ethanol, and stored at -20ºC. Meiofauna were floated from each sample with Ludox TM 50 (specific density 1.18) (Heip et al. 1985), sieved through a 63 µM mesh, washed with distilled H_2O, and stored again with absolute ethanol at -20ºC. Before DNA extraction, ethanol was discarded; samples were washed with distilled H_2O, and centrifuged to settle the organisms.

3.2. MORPHOLOGICAL ANALYSES

For meiofaunal analyses, sediment samples were taken using a cylindrical corer (2.5 cm diameter and 5 cm height) in October 2012, February, June and September 2013. Samples were taken during the low tide from thirty-seven geo-referenced sites arranged on an irregular sampling grid, spaced about 50 m from the neighbour site, from the intertidal zone up to 25 m depth. Samples were collected simultaneously at each sampling site for the investigation of meiofauna, microbiota and grain size.

Samples were immediately fixed in 4% formaldehyde. In the laboratory, samples were processed after sieving through a 45 µM mesh and flotation with Ludox TM 50 (specific density 1.18) (Heip et al. 1985). Meiofauna was counted and identified under a stereomicroscope. Nematodes were identified to genus level and identified into morphospecies.

3.3. ENVIRONMENTAL CHARACTERIZATION

The environmental characterization of Araçá Bay was made along one year before the metabarcoding sampling. Four field surveys were done in October 2012, February, June and September 2013. The parameters evaluated were chlorophyll-a, phaeopigments, cyanobacteria, bacteria, depth, nanophytobenthos, margalef, wave orbital velocity, organic carbon, grain size, percentage of coarse and medium sand, coarse sand, medium sand, fine sand, medium and fine sand, silt and clay, and sorting. Concentration of chlorophyll a, phaeopigments, and nanophytobenthos were obtained according to Plante-Cuny (1978). The cellular density of cyanobacteria was estimated according to Lund et al. (1958). Bacteria density was determinated by direct counting. Titillation of organic carbon followed Gaudette et al. (1974). Margalef's diversity pigment index, was calculated according to Margalef (1974). For sediment texture analyses, samples were dried in a kiln at 60ºC, and then through traditional routines of sieving and pipetting (Suguio 1973)

Average, minimum, maximum and standard deviation values were used as predictors for each parameter.

3.4. DNA EXTRACTION AND AMPLIFICATION

For the metabarcoding analyses, specimens' DNA was extracted from each sample using the *PowerSoil® DNA Isolation Kit*, following the company protocols. The primers SSU_FO4 (5'- GCTTGTCTCAAAGATTAAGCC -3') and SSU_R22 (5'- GCCTGCTGCCTTCCTTGGA -3') were used to amplify approximately 450bp, between the regions V1 and V2 of 18S ribosomal DNA (rDNA). PCR conditions was 2 minutes denaturation at 95ºC, followed by 35 cycles of 1 minute at 95ºC, 45 seconds at 57ºC, 3 minutes at 72ºC and a final extension of 10 minutes at 72ºC (Fonseca et al. 2010).

Primers mlCOIintF (5'-GGWACWGGWTGAACWGTWTAYCCYCC -3') (Leray et al. 2013) and jgHCO2198 (5'- TAIACYTCIGGRTGICCRAARAAYCA -3') (Geller et al. 2013) were needed to amplify a 313bp region of the mitochondrial Cytochrome C oxidase subunit I (COI) region. PCR conditions involved 16 initial cycles: denaturation for 10s at 95°C, annealing for 30s at 62°C (−1°C per cycle) and extension for 60s at 72°C, followed by 25 cycles at 46°C annealing temperature (Leray et al. 2013).

PCR products were purified using *illustra GFX PCR DNA and Gel Band Purification* kit, according to the company protocols. After purification, all of the COI PCR products, and a few of the 18S had concentrations below the minimum recommend to be read on *Illumina MiSeq* sequencer. The remaining thirteen samples were sequenced.

3.5. METABARCODING DATA ANALYSES

A database was created on *Usearch v9.0* software (Edgar 2010) using sequences downloaded from GenBank to predict OTUs (Operational Taxonomic Unit). The sequences were selected according to a species list of nematodes, created by traditional meiofaunal sampling and identification and by a list of species, performed by experts during the "Workshop on Taxonomy and Diversity of Marine Meiofauna, Brazil", held at CEBIMar, the Centre for Marine Biology of the University of São Paulo in São Sebastião, in 2012 (28 October-9 November 2012). During the workshop, the same sites area were sampled and prosed by several experts.

All sequences generated by *Illumina* were merged on *Pear 0.9.10* software (Zhang et al. 2014), selecting only sequences with length between 380 bp e 420 bp. *Usearch v9.0* was used to procedure quality filtering, to find uniques, clustering with chimeras filtering, predict OTUs, and alignment sequences with 80% of similarity (Edgar 2016).

3.6. DIVERSITY ESTIMATIVES

Statistical analyses were performed with the *R* language (R Development Core Team, 2016). The number of genera (S) per area for the metabarcoding sampling were calculated using rarefaction curves with the function *specaccum*. The number of expected genera (ES) was calculated by Chao, Jackknife 1, Jackknife 2, and Bootstraping with the function *poolaccum*. The ratio of ES/S was used to evaluate the representativeness of the metabarcoding method. The package *vegan* was used in both cases (Oksanen et al. 2016). The same approach was used to compare the congruency between metabarcoding and morphology. To generate phylum diversity plot, the packages *reshape*, *ggplot2* and *scales* were necessary (Wickham 2007, 2009, 2016).

To test the response of the association obtained by metabarcoding and by traditional methods for nematode composition a permutational multivariate analysis was performed with *adonis* functio*n*, using the *Mountford* index of similarity (Mountford 1962). We used studies (Metabarcoding, Morphology) and sites and the interaction among then as predictors variables. A PCoA (Principal Coordinates Analysis), using *ape* package, was used to visualize the similarities between studies (Paradis et al. 2004).

The presence-absence matrix of diversity and richness of OTUs generated by metabarcoding was used as response variable to assess patterns of distribution and environmental correlations. The multicollinearity of the environmental variables was tested by the variance inflation factor (VIF), package *car* (Fox and Weisberg, 2011). Then, the function *adonis* (*Mountford* index), and a generalized linear model (GLM) were used to assess the multi- and univariate response. Richness are counted data and a Poisson model as used to fit the GLM models.

To avoid Type-I error generated by the geo-spatial distance of the samples and points in the GLM (Diniz-Filho et al. 2003), and make the results more realistic, a spatial autocorrelation by SARerr was applied. We used the function *sarerrr* in *spdep* package (Bivand and Piras 2015). The spatial weights were constructed with 0.005 neighbourhood distance, coding style "W". The model selection and model averaging was performed with the package *MuMIn* (Barton 2016).

After this, a Redundancy analysis (RDA), to summarise correlation between OTUs and environmental variables was applied. An ANOVA was used to test the significance of the RDA axes (Borcard et al. 2011)

4. RESULTS

4.1 METABARCODING DIVERSITY

A summary of the number of reads for each sample, merged and filtered sequences, uniques and singletons, chimera and OTUs is given in Appendix 1. 115 genera were found in the 13 samples, separated into 60 families, 24 orders, 12 classes, and 11 phyla for a total of 8,040,154 reads generated by *Illumina*, (Appendix 2). The rarefaction curve shows that the registered number of OTUs is close to the expected for Araçá Bay (Fig 2.). The ratio of ES/S indicated that between 78% (Jack2) and 91% (Bootstrap) of total diversity was reported.

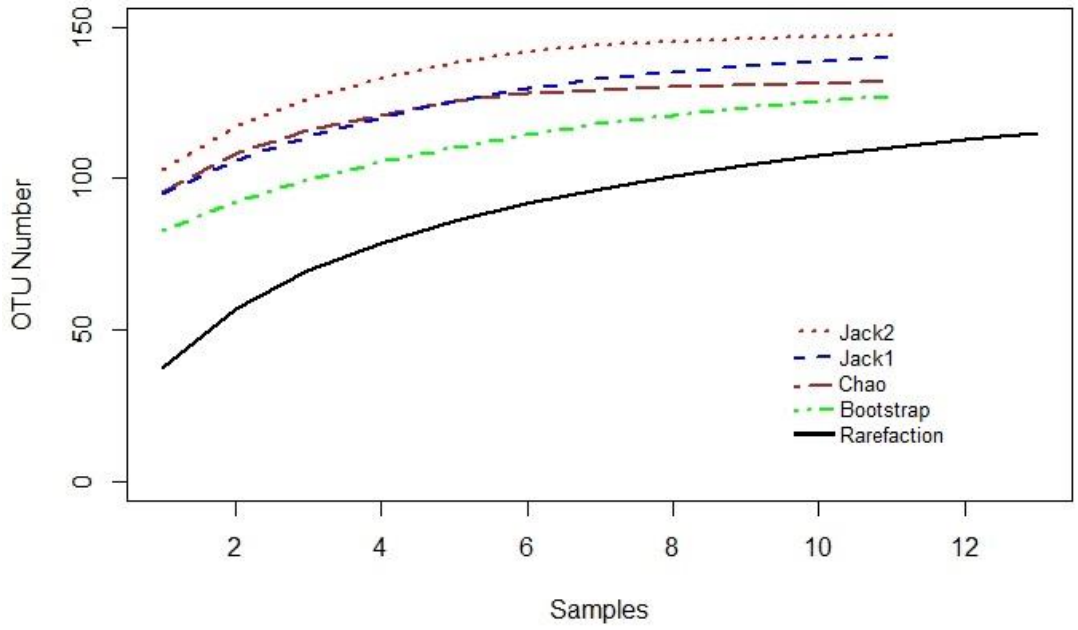

Fig 2. Rarefaction curves showing mean expected OTU number (Jack 2, Jack 1, Chao and Bootstrap) as a function of sample size, and what was found with metabarcoding on Araçá Bay (black line).

The phylum Nematoda was the most representative in all samples, followed by Arthropoda and Annelida (Fig 3). Platyhelminthes and Gastrotricha were also reported in all samples. Nematode OTUs summed up for more than 50% of the OTUs in nine samples. Tardigrada, Xenacoelomorpha, and Kinorhyncha were reported in one, three and four samples, respectively. Samples 141 and 142 showed the highest number of different phyla (9 phyla), with 73 and 63 OTUs respectively. Sample 112 had the lowest number of phyla (5), but also 73 OTUs.

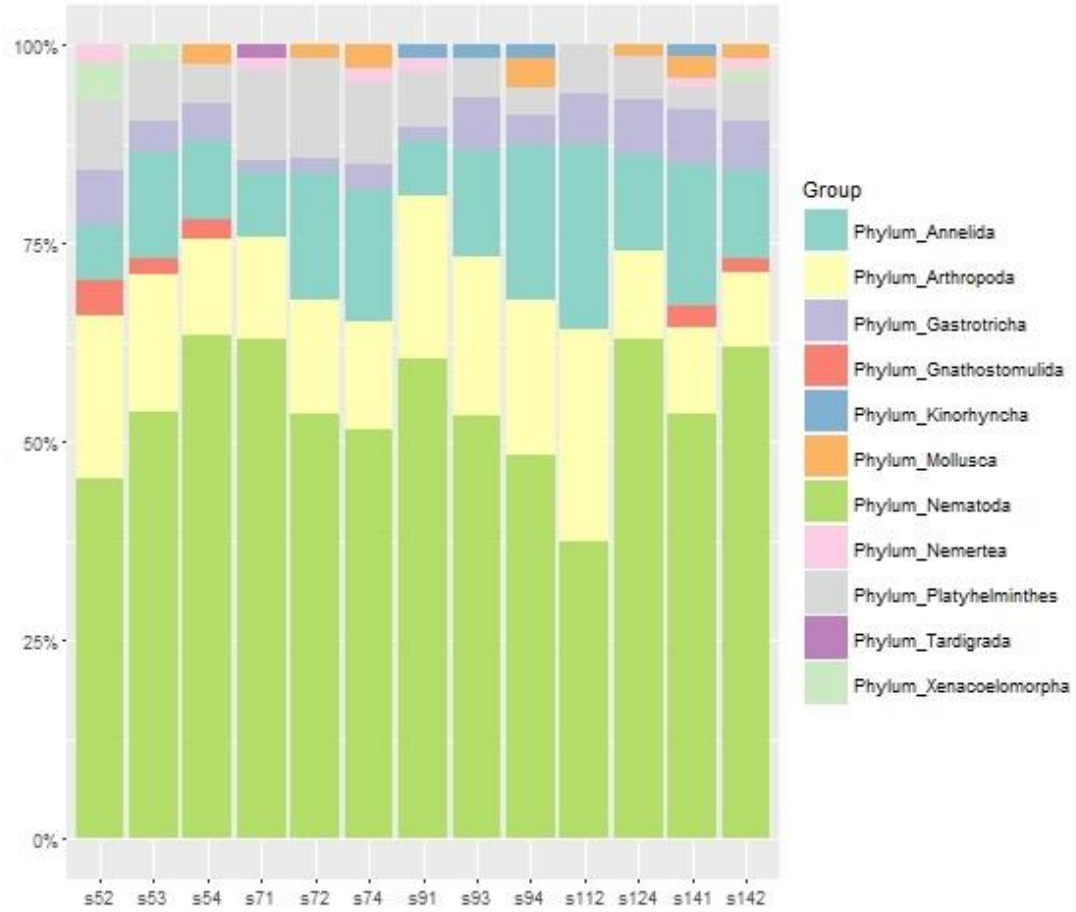

Fig 3. Percentage of OTUS per phylum for each sample (s).

4.2. NEMATODE DIVERSITY: MORPHOLOGICAL VS. MOLECULAR APPROACHES

When contrasting the metabarcoding and morphological methods, the rarefaction curve of morphospecies and number of OTUS showed a similar slope (Fig 4). The asymptotic behaviour of the curve shows that approximately five samples are sufficient to assess the total diversity of nematodes in Araçá Bay. Despite the similar number of morphospecies and OTUs, the permutational multivariate analysis showed significant differences between the two methodologies (Table 1).

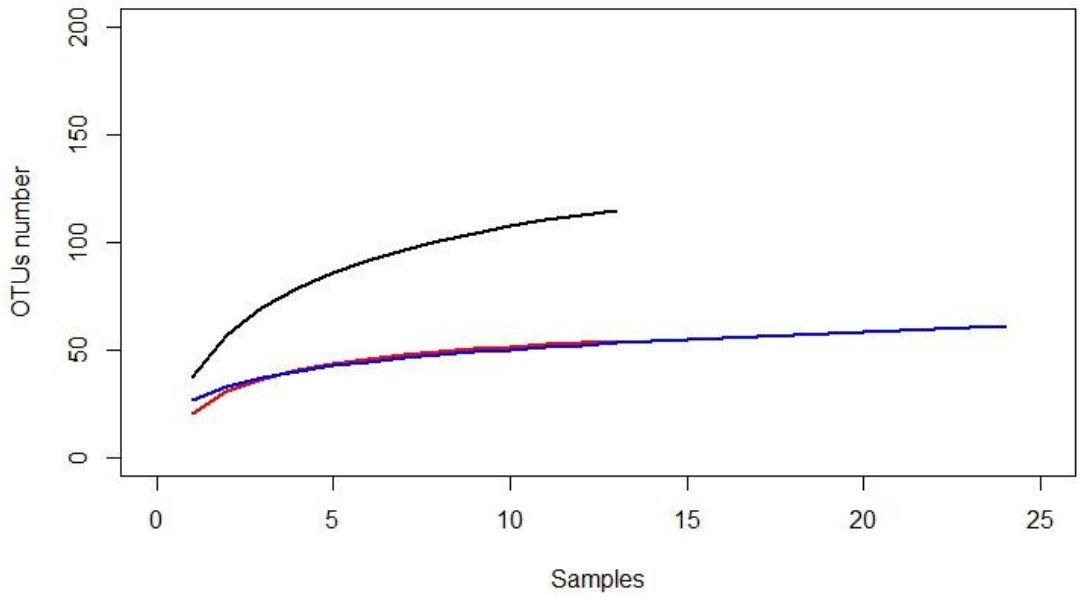

Fig 4. Black line represents the total diversity found using metabarcoding. The blue line is the nematode diversity found using morphology, and the red line represent the nematode diversity found with metabarcoding.

Table 1. The Pr(>F) near to zero shows a significant difference between studies, between sites, and between studies and sites.

	Df	SumOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Study	1	2.4001	2.40011	14.9796	0.20688	1e-04***
Site	5	3.4016	0.68031	4.2460	0.29320	1e-04***
Study:Site	5	1.7940	0.35881	2.2394	0.15464	2e-04***
Residuals	25	4.0056	0.16023		0.33527	
Total	36	11.6013			1.00000	

The PCoA also shows that OTUs composition for metabarcoding and morphology are different (Fig 5.). PCoA1 divides the approaches, metabarcoding on the left side, and morphology on the right side. Nevertheless, PCoA2 shows a tendency: sites 5 and 7 from both studies are located at the same side, while point 9 is located on the other side. Sites 11, 12 and 14 were randomly spread among the sites 5 and 9.

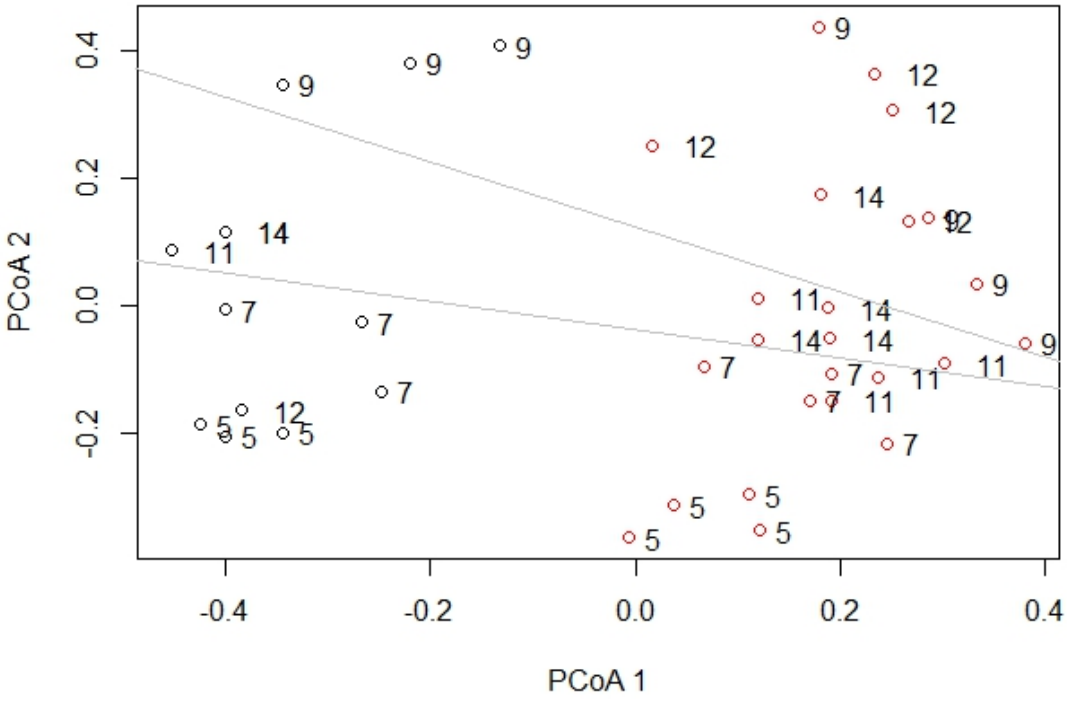

Fig 5. PCoA 1 and PCoA 2, metabarcoding approach is represented by black circles, and morphology by the red circles.

4.3. ENVIRONMENTAL VARIABLES VS. DIVERSITY

The multicollinearity test selected only five among all the environmental variables: medium sand average, sorting standard deviation, minimum bacteria record, maximum of orbital velocity and nanophytobenthos. A GLM was applied using these five variables, producing 13 candidate models.

From the 13 candidate models, five better explained the OTU richness (Table 2). The model with average of medium sand and standard deviation of sorting was selected as the best model. The remaining models showed a Delta value between 0 and 5.3, and an Akaike weight between 0.046 and 0.654.

Table 2: Selection of GLM and SARerr models for richness of OTUs. MS (medium sand average), SORT (sorting standard deviation), BACMIN (minimum bacteria record), maximum of orbital velocity (Ov.max) and nanophytobenthos (NA_M).

	(Int)	MS	BACMIN	NA_M	Ov.max	SORT	df	logLik	AICc	delta	weight
Sem.nb1.5.w2	66.31	-0.4607				-31.82	5	-30.075	78.7	0.00	0.654
Sem.nb1.5.w9	11.08		37.12			10.74	5	-31.835	82.2	3.52	0.112
Sem.nb1.5.w4	48.88	-0.3092					4	-34.682	82.4	3.64	0.106
Sem.nb1.5.w8	59.90			-83.23			4	-35.450	83.9	5.18	0.049
Sem.nb1.5.w7	21.90		30.74	-5.057			5	-32.720	84.0	5.29	0.046

Genera distribution was correlated with MS (medium sand average), SORT (sorting standard deviation), and BACMIN (minimum bacteria record) (Fig 6.). The RDA was formed by two significant axes (Table 3), which together explained about 27% of the total variability between genera, sites and environmental variables. From that 27%, 39% is explained by RDA1, which separated site 5 from sites 9, 11, 12 and 14. MS and BACMIN were correlated with the first RDA axe. RDA2 explain 35% from the 27%, and separate sites 7 and 9, from sites 5, 11, 12 and 14. SORT was correlated with this second axe.

The Gnathostomulida *Austrognathia*, Xenacoelomorpha *Proposus*, and Nematoda *Paradesmodora* and *Axonolaimus* were positively related with higher amount of medium sand. Poorly sorted sediments were linked to the Nematoda genera *Sabatieria*, *Spilophorella*, *Paraphanolaimus*, *Bathylaimus*, *Halalaimus*, *Paralinhomoeus*, and Gastrotricha of the genus *Urodasys*. Some Platyhelminthes, as *Gyatrix*, *Itaipusa*, and *Mesorhynchus*, Annelida, as *Protodrilus*, *Saccocirrus*, and *Protodriloides*, and the Nematoda *Terschellingia* were related with higher minimum bacteria record.

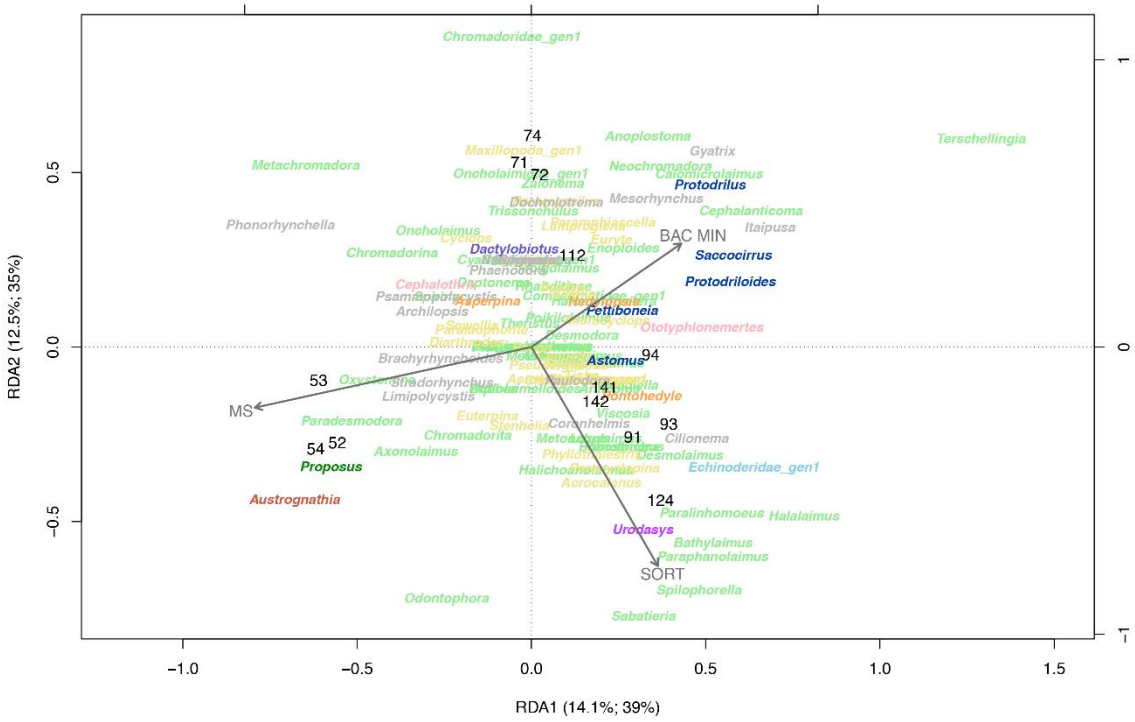

Fig 6. Canonical Redundancy Analysis (RDA) ordination plots of genera, sample site, environmental variables. Arrows represent the environmental variables MS (medium sand average), SORT (sorting standard deviation), and BACMIN (minimum bacteria record). All genera from same phylum are with the same colour, being dark green= Xenacoelomorpha, light green= Nematoda, dark blue= Annelida, light blue= Kinorhyncha, grey= Platyhelminthes, rose= Nemertea, yellow= Arthopoda, orange= Mollusca, purple= Gastrotricha, violet= Tardigrada and coral= Gnathostomulida.

Table 3. Summary of ANOVA shows the significance of RDA1 and RDA2.

	Df	Variance	F	Pr(>F)
RDA1	1	1.4859	1.9745	0.003
RDA2	1	1.3226	1.7574	0.009
RDA3	1	0.9763	1.2973	0.125
Residual	9	6.7730		

5. DISCUSSION

5.1 METABARCODING DIVERSITY

Our intensive survey in a small tidal flat area (0.12 km^2) yielded an effective estimate of total meiofaunal diversity. A total of 768 OTUs belonging to 115 genera showed an asymptotic behaviour in the rarefaction curve, and 10 samples per area may be enough to assess the diversity of the intertidal area of Araçá, a subtropical Bay. Comparing our study with Leray & Knowlton (2015), we found ten time less OTUs, but around 25% of their OTUs were unidentified, and several groups (such as Gastrotricha, Nematoda, Nemertea, Platyhelminthes, Tardigrada, and Xenacoelomorpha) were classified as "other animals". This observation may be explained by the fact that our study let several non-meiofaunal groups out as Bryozoa, Cnidaria, Rotifera, and Fungi.

In general, Nematoda is the dominant marine meiofaunal phyllum, followed by Platyhelminthes and Arthropoda (Fonseca et al. 2010; Fonseca et al. 2014b; Lallias et al. 2015). Nematodes are so diverse and abundant probably because of their rapid generation time and capacity to adapt to new environmental conditions, especially in interstitial areas (Fonseca et al. 2010). Our results showed a similar pattern, except that Platyhelminthes were less representative than Annelida. This observation can be a reflex of some groups of Platyhelminthes, such Proseriata, being more abundant in sediments from high-energy areas (Swedmark 1964; Curini-Galletti 2014) and because Araçá Bay is a protected area (Dottori et al. 2015).

Xenacomelomorpha, Gastrotricha, Gnathostomulida are not commonly reported in metabarcoding studies. The morphological identification by experts before the metabarcoding study in the Araçá Bay would be helpful to generate a more reliable matrix of species and phyla.

5.2. NEMATODE DIVERSITY: MORPHOLOGICAL VS. MOLECULAR APPROACHES

Differences in association composition were found when comparing the nematode genera obtained by metabarcoding and morphological methods. Differences between the methods may be a methodological artifact probably generated by the nucleotide dataset deposited on NCBI (Noppe and Guilini 2015; Holovachov 2016). The estimated diversity of marine nematodes is estimated in about 61,400 species, but only 11,400 are currently described and accepted (Appeltans et al. 2012). Robustness of metabarcoding is closely dependent on available libraries, and related to the amount of the target gene (18S and COI) deposited and correctly assigned to a species (Fonseca et al. 2010; Leray et al. 2016).

Nematode genera retrieved by metabarcoding probably were misidentified wrong identification because of the lack of information on this group. Studies with deep-sea nematodes showed a similar mismatch with the traditional taxonomical routines. Morphological and metabarcoding identification only matched at the order-family level (Dell'Anno et al. 2015). Genera that were only detected with metabarcoding may also be explained because morphology methods consider just body complete animals, while metabarcoding can identify body-damaged animals.

These results indicate that the metabarcoding approach may be a powerful tool for environmental studies, although it stills need to be improved by the background complements of the experts and morphology (Sinniger et al. 2016).

5.3 ENVIRONMENTAL VARIABLES AS DRIVERS OF LOCAL DIVERSITY

The correlation between the diversity descriptors, richness and meiofauna composition emphasised the utility of the metabarcoding to detected patterns of distribution of these organisms. Richness showed a tendency to decrease when the percentage of medium sand increased. As nematodes were the most diverse group, a close relation between medium sand and nematode richness was expected. However, nematode distribution does not not seem to be directly correlated to sediment grain size in the literature (Vanaverbeke et al. 2011; Fonseca et al. 2014a).

Gnasthotomulida, Gastrotricha and Annnelida also contributed to explain meiofaunal distribution patterns. Richness was at the lowest in poorly sorted sediments. Nevertheless, some groups, as Gastrotricha, presented higher richness in this kind of habitat (Garraffoni et al. 2016), corroborating our results regarding the presence of *Urodasys*. Sorting is associated with transport dynamics and deposition forces, and a high standard deviation of the degree of sorting is likely a result of mixed of forces, such as tidal forces, waves, river discharge, and wind (Wright et al., 1999). The group Gnathostomulida is mostly associated with fine sands and hypoxic sediments (Giere, 2009). Our results showed a strong relation between *Austrognathia* and medium sand.

The effect of the minimum amount of bacteria on the richness and meiofauna composition is probably explained the trophic cascade effect (Pace et al. 1999). Bacteria are an important source of food for meiofaunal organisms (Moens and Vincx 1997; Vanaverbeke et al. 2011) which may regulate the distribution of niches, and consequently the total diversity. We found that the richness decreases with decreasing concentration of bacteria. The annelids, some platyhelminths (*Gyatrix, Itaipusa* and *Mesorhynchus*) and *Terschellingia* are related with higher amount of minimum bacteria (Moens and Vincx 1997).

5.4. CONCLUSIONS

This is the first DNA metabarcoding study specifically applied for meiofauna in the Brazilian coast. We found this approach reliable to be used on the estimative of the meiofauna diversity and its distribution, especially emphasizing in small areas, such as the Araçá Bay. We also found that meiofauna and nematodes diversity estimated by metabarcoding may be indicative of environmental conditions. On the other hand, metabarcoding is still fragile on identifying organism on more specific levels, as genus. Further studies combining molecular biology and morphology are highly encouraged to achieve the Linnaeus saga, and better understanding the complexity of the ecological process and patterns among the interstitial spaces.

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Appendix 1.

Mean numbers of merged and filtered sequences, number of uniques and singletons, chimera and OTUs per sample.

Sample	Number of sequences merged	Number of sequences filtered	Number of uniques/singletons	Number of chimera	Number of OTUs
52	352711/423634 (83.258%)	204349 (57.9%)	69584/58632 (84.3%)	2744	165
53	246495/366997 (67.165%)	140856 (57.1%)	53801/45201 (84%)	1793	302
54	126113/188128 (67.036%)	28727 (22.8%)	11877/10091 (85%)	538	142
71	414988/521135 (79.632%)	236596 (57%)	80255/67806 (84.5%)	3814	310
72	336117/421637 (79.71%)	185912 (55.3%)	68822/58313 (84.7%)	3244	275
74	333689/441372 (75.603%)	184158 (55.2%)	60503/50351 (83.2%)	2526	357
91	117919/381352 (30.921%)	66967 (56.8%)	36531/32042 (87.7%)	2194	152
93	318443/445095 (71.545%)	181134 (56.9%)	67195/56438 (84%)	3127	325
94	244632/407167 (60.081%)	133429 (54.5%)	53126/44986 (84.7%)	2610	362
112	274286/463471 (59.181%)	145965 (53.2%)	71830/62558 (87.1%)	6138	349
124	277756/401667 (69.151%)	150304 (54.1%)	49171/41314 (84%)	2063	245
141	282755/412373 (68.568%)	162997 (57.6%)	66966/57003 (85.1%)	3367	361
142	262261/399978 (65.569%)	144082 (54.9%)	53590/45312 (84.6%)	2228	348

Appendix 2.

List of OTUs found by metabarcoding on Araçá Bay.

	52	53	54	71	72	74	91	93	94	112	124	141	142
Phylum Nematoda													
Class Chromadorea													
Order Chromadorida													
Family Cyatholaimidae	0	0	0	0	0	1	0	0	0	0	0	0	0
Genus Longicyatholaimus	1	1	1	1	1	1	1	1	1	1	1	1	1
Genus Praeacanthonchus	1	1	1	1	1	1	1	1	1	1	1	1	1
Genus Gomphionema	1	1	1	1	1	1	1	1	1	1	1	1	1
Family Selachinematidae													
Genus Halichoanolaimus	1	0	1	0	1	0	0	1	0	1	2	1	1
Genus Bendiella	0	0	0	0	0	1	0	1	0	0	1	0	0
Family Chromadoridae	0	0	0	1	2	1	0	0	0	0	0	0	0
Genus Chromadorita	1	2	0	1	0	1	1	0	1	0	1	0	1
Genus Chromadorina	0	1	1	1	1	1	0	0	0	0	1	0	0
Genus Spilophorella	0	0	1	0	0	0	1	1	1	0	1	1	1
Genus Neochromadora	0	0	0	1	0	1	1	0	0	1	0	1	1
Genus Punctodora	0	0	0	0	0	0	0	0	0	0	1	1	0
Order Monhysterida													
Family Comesomatidae	0	0	0	0	0	0	0	0	0	1	0	1	0
Genus Sabatieria	4	3	4	2	2	2	7	3	5	3	6	5	6
Family Xyalidae													
Genus Theristus	1	2	2	3	1	2	1	1	0	2	4	3	3
Genus Daptonema	1	2	1	2	1	1	1	1	1	2	1	1	1
Genus Metadesmolaimus	0	0	0	0	0	0	0	0	0	0	0	1	0
Family Linhomoeidae													
Genus Desmolaimus	0	1	1	1	1	0	1	1	1	1	2	1	2
Genus Terschellingia	0	0	0	3	3	4	2	2	2	1	4	1	4
Genus Paralinhomoeus	0	0	0	0	0	0	0	1	1	0	1	0	1
Family Monhysteridae													
Genus Diplolaimelloides	0	2	0	1	0	1	0	1	1	0	1	0	0
Genus Halomonhystera	0	0	0	0	0	1	0	1	0	0	0	1	0
Family Sphaerolaimidae													
Genus Sphaerolaimus	0	0	0	0	0	0	0	0	0	0	0	1	1
Family Siphonolaimidae													
Genus Astomonema	0	0	0	0	0	0	0	0	0	0	0	0	1
Order Desmodorida													
Family Desmodoridae													

	Genus Metachromadora	1	1	1	2	1	1	0	0	0	0	0	1	1
	Genus Desmodora	1	1	1	1	2	1	2	2	1	1	1	1	1
	Genus Robbea	0	1	0	0	1	0	0	0	0	0	1	0	0
	Genus Spirina	0	1	0	1	0	0	0	0	0	0	0	0	0
	Genus Paradesmodora	0	1	1	0	0	0	0	0	0	0	0	0	0
	Genus Zalonema	0	0	0	1	1	0	0	0	0	0	0	0	1
	Genus Stilbonema	0	0	0	1	0	0	0	0	0	0	0	0	0
	Genus Laxus	0	0	0	0	0	0	0	0	0	0	1	0	0
	Genus Eubostrichus	0	0	0	0	0	0	0	0	0	0	1	1	0
Family Microlaimidae														
	Genus Calomicrolaimus	0	0	0	1	1	1	0	0	1	1	1	2	0
	Genus Molgolaimus	0	0	0	0	0	1	0	0	0	0	0	1	0
Order Araeolaimida														
Family Axonolaimidae														
	Genus Axonolaimus	1	0	1	0	0	0	0	0	0	0	0	1	1
	Genus Odontophora	1	0	2	0	0	0	1	1	0	0	1	0	0
Family Leptolaimidae														
	Genus Paraphanolaimus	0	0	0	0	0	0	1	1	0	0	2	0	0
Order Rhabditida														
Family Rhabditidae														
	Genus Poikilolaimus	1	1	1	1	1	1	1	1	1	2	1	2	1
	Genus Rhabditinae	0	1	0	1	0	1	1	0	1	0	0	0	1
Class Enoplea														
Order Enoplida														
Family Thoracostomopsidae														
	Genus Enoploides	1	1	0	3	1	1	1	0	1	1	2	2	1
Family Oxystominidae														
	Genus Oxystomina	1	1	2	1	1	1	1	1	0	0	1	1	1
	Genus Halalaimus	0	0	0	0	0	0	3	2	1	0	0	1	1
Family Oncholaimidae		0	0	0	0	1	1	0	0	0	0	0	0	0
	Genus Oncholaimus	1	1	1	2	1	1	0	1	0	1	1	1	1
	Genus Viscosia	0	0	0	0	0	0	1	1	0	0	0	0	0
	Genus Metoncholaimus	0	0	0	0	0	0	0	0	0	0	1	0	0
Family Enchelidiidae														
	Genus Bathyeurystomina	1	1	1	1	1	1	1	1	1	1	1	1	1
	Genus Calyptronema	0	0	0	1	0	0	0	0	0	0	0	0	0
Family Ironidae														
	Genus Trissonchulus	0	1	1	1	1	1	1	1	1	1	0	0	1
Family Anticomidae														
	Genus Cephalanticoma	0	0	0	1	1	0	1	1	1	1	0	1	1
	Genus Anticoma	0	0	0	0	0	0	0	0	1	0	0	1	0
Family Anoplostomatidae														
	Genus Anoplostoma	0	0	0	1	1	2	1	1	1	0	0	0	0

Family Tripyloididae														
	Genus Bathylaimus	0	0	0	0	0	0	1	1	0	0	2	0	0
Phylum Arthropoda														
Class Maxillopoda		0	0	1	0	1	1	0	0	1	2	0	0	0
Order Poecilostomatoida														
Family Ergasilidae														
	Genus Sinergasilus	1	1	0	1	0	1	1	1	0	1	1	1	1
	Genus Pseudergasilus	1	1	1	1	1	1	1	2	1	1	1	1	1
	Genus Paraergasilus	0	0	0	0	0	1	0	0	0	1	0	0	0
	Genus Ergasilus	0	0	0	0	0	0	0	0	1	0	0	0	0
Family Chondracanthidae														
	Genus Chondracanthus	0	0	0	0	0	0	1	0	0	0	0	0	0
Order Siphonostomatoida														
Family Sphyriidae														
	Genus Paeon	1	1	1	1	1	1	1	1	1	1	1	1	1
Family Asterocheridae		0	0	0	0	0	0	1	0	0	0	0	0	0
Order Harpacticoida														
Family Miraciidae														
	Genus Paramphiascella	1	0	0	1	1	1	0	1	1	1	1	0	0
	Genus Stenhelia	1	0	0	0	0	0	0	1	0	0	0	1	0
Family Dactylopusiidae														
	Genus Diarthrodes	1	0	1	1	0	0	0	1	1	1	0	0	0
	Genus Sewellia	0	1	0	0	0	0	0	0	0	1	0	0	0
Family Tisbidae														
	Genus Tisbe	1	0	0	0	0	0	1	0	0	1	0	1	1
Family Laophontidae														
	Genus Paralaophonte	0	1	0	0	0	1	0	1	0	0	0	0	0
Family Euterpinidae														
	Genus Euterpina	0	0	1	0	0	0	1	0	0	0	0	0	0
Family Canthocamptidae														
	Genus Itunella	0	0	0	0	1	0	0	0	1	0	0	0	0
Family Tachidiidae														
	Genus Tachidius	0	0	0	0	0	1	0	0	0	0	0	0	0
Family Harpacticidae														
	Genus Harpacticus	0	0	0	0	0	0	1	0	0	0	0	0	0
Family Ectinosomatidae														
	Genus Bradya	0	0	0	0	0	0	0	0	0	1	0	0	0
Family Thalestridae														
	Genus Phyllothalestris	0	0	0	0	0	0	0	0	0	0	1	0	0
Family Canuellidae														
	Genus Canuella	0	0	0	0	0	0	0	0	0	0	0	1	0
Order Calanoida														
Family Paracalanidae														

	Genus Acrocalanus	1	1	0	0	1	0	1	1	1	1	1	0	1
Family Temoridae														
	Genus Temora	0	0	0	0	0	0	0	0	1	0	0	0	0
Order Cyclopoida														
Family Cyclopettidae														
	Genus Paracyclopina	1	1	0	1	0	0	0	2	1	1	1	1	1
Family Lernaeidae														
	Genus Lamproglena	0	1	0	1	1	0	1	1	0	1	0	1	0
	Genus Lernaea	0	0	0	0	0	0	0	0	0	1	1	0	0
Family Cyclopidae														
	Genus Cyclops	0	1	0	0	0	1	0	0	0	1	0	0	0
	Genus Euryte	0	0	0	1	1	0	1	0	1	0	0	0	0
	Genus Microcyclops	0	0	0	0	0	0	1	0	0	1	0	0	0
Phylum Gnathostomulida														
Class Gnathostomulida														
Order Bursovaginoidea														
Family Austroghathiidae														
	Genus Austrognathia	2	1	1	0	0	0	0	0	0	0	0	2	1
Phylum Annelida														
Class Polychaeta														
Order Polychaeta														
Family Saccocirridae														
	Genus Saccocirrus	1	1	3	1	5	4	3	2	3	4	3	6	2
Family Protodrilidae														
	Genus Protodrilus	2	5	0	3	2	4	0	4	6	9	3	6	2
	Genus Protodriloides	0	0	0	0	1	1	0	1	1	1	1	0	1
	Genus Astomus	0	0	0	0	0	1	0	0	0	0	1	0	1
Order Eunicida														
Family Dorvilleidae														
	Genus Pettiboneia	0	1	1	1	1	1	1	1	1	1	1	1	1
Phylum Gastrotricha														
Class Gastrotricha														
Order Macrodasyida														
Family Macrodasyidae														
	Genus Urodasys	3	2	2	1	1	2	1	4	2	4	5	5	4
Phylum Platyhelminthes														
Class Rhabditophora														
Order Rhabdocola														
Family Polycystididae														
	Genus Stradorhynchus	1	0	0	0	0	0	0	0	0	0	0	0	0
	Genus Phonorhynchella	1	1	1	0	1	1	0	0	0	1	0	0	0
	Genus Gyatrix	2	0	0	3	1	3	2	1	1	1	1	2	2

	Genus Psammopolycystis	0	1	0	1	0	0	0	0	0	0	0	0	0
	Genus Limipolycystis	0	0	1	0	0	0	0	0	0	0	0	0	0
	Genus Brachyrhynchoides	0	1	0	0	0	0	0	0	0	0	0	0	0
	Genus Mesorhynchus	0	0	0	1	0	1	0	1	0	0	0	0	0
	Genus Polycystis	0	0	0	0	1	0	0	0	0	0	0	0	0
	Genus Rogneda	0	0	0	0	1	0	0	0	0	0	0	0	0
	Genus Neopolycystis	0	0	0	0	1	0	0	0	0	0	0	0	0
	Genus Paulodora	0	0	0	0	0	0	1	0	0	0	0	0	0
Family Koinocystididae														
	Genus Itaipusa	0	0	0	1	1	1	1	1	1	1	1	0	1
Order Rhabdocoela														
Family Typhloplanidae														
	Genus Phaenocora	0	0	0	1	0	0	0	0	0	0	0	0	0
	Genus Dochmiotrema	0	0	0	0	1	0	0	0	0	1	0	0	0
Family Promesostomidae														
	Genus Coronhelmis	0	0	0	0	0	0	0	0	0	0	1	0	0
	Genus Cilionema	0	0	0	0	0	0	0	0	0	0	1	0	0
Order Proseriata														
Family Monocelididae														
	Genus Archilopsis	0	1	0	0	0	1	0	0	0	0	0	0	0
Phylum Xenacoelomorpha														
Class Acoela														
Order Acoela														
Family Proporidae														
	Genus Proposus	2	1	0	0	0	0	0	0	0	0	0	0	1
Phylum Nemertea														
Class Anopla														
Order Palaeonemertea														
Family Cephalothricidae														
	Genus Cephalothrix	1	0	0	0	0	1	0	0	0	0	0	0	0
Class Enopla														
Order Monostilifera														
Family Ototyphlonemertidae														
	Genus Ototyphlonemertes	0	0	0	1	0	0	1	0	0	0	0	1	1
Phylum Mollusca														
Class Gastropoda														
Order Acochlidiacea														
Family Asperspinidae														
	Genus Asperpina	0	0	1	0	1	1	0	0	0	0	1	0	0
Family Hedylopsidae														

Genus Hedylopsis	0	0	0	0	0	1	0	0	1	0	0	1	0
Family Microhedylidae													
Genus Pontohedyle	0	0	0	0	0	0	0	0	1	0	0	1	1
Phylum Tardigrada													
Class Eutardigrada													
Order Parachela													
Family Macrobiotidae													
Genus Dactylobiotus	0	0	0	1	0	0	0	0	0	0	0	0	0
Phylum Cephalorhynca													
Class Kinorhyncha													
Order Cyclorhagida													
Family Echinoderidae	0	0	0	0	0	0	1	1	1	0	0	1	0