

## Short Communication

# Fecal Eukaryotic Community of Wild Young South American (*Arctocephalus australis*) and Subantarctic Fur Seals (*Arctocephalus tropicalis*)

Cláudio Marcos Lauer Júnior<sup>1,2</sup>, Aline Weber Medeiros<sup>1</sup>, Adriana Giongo<sup>1,3</sup>, Luiz Gustavo dos Anjos Borges<sup>3</sup>, Derek Blaese de Amorin<sup>4</sup>, Maurício Tavares<sup>4,5</sup>, Jeverson Frazzon<sup>6</sup>, and Ana Paula Guedes Frazzon<sup>1\*</sup>

<sup>1</sup>Department of Immunology and Parasitology, Universidade Federal do Rio Grande do Sul, Brazil

<sup>2</sup>Institute of Health Sciences, Feevale University, Brazil

<sup>3</sup>Institute of Petroleum and Natural Resources, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil

<sup>4</sup>Center for Coastal Studies, Limnology and Marine (Ceclimar), Universidade Federal do Rio Grande do Sul, Brazil

<sup>5</sup>Biosciences Institute, Universidade Federal do Rio Grande do Sul, Brazil

<sup>6</sup>Department of Food Science, Universidade Federal do Rio Grande do Sul, Brazil

## \*Corresponding author

Ana Paula Guedes Frazzon, Department of Microbiology, Immunology and Parasitology, Federal University of Rio Grande do Sul, Rua Sarmento Leite 500/216, Porto Alegre, Rio Grande do Sul, 90050-170, Brazil, Tel: 55215133084505; Email: ana.frazzon@ufrgs.br

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## Abstract

Eukaryotic microbes that reside in the mammalian gut have an important role in maintaining metabolism, digesting nutrients, and regulating the immune system. Therefore, changes in the microbial composition of the gut may generate adverse impacts on animal health. Using high-throughput sequencing, the present study examined the fecal eukaryotic community of wild young South American (*Arctocephalus australis*) ( $n = 2$ ) and Subantarctic fur seals (*Arctocephalus tropicalis*) ( $n = 3$ ). The results indicated there was a distinct and diverse eukaryotic community in the fecal samples of young wild fur seals. Perhaps based on the migratory habits of certain species and the difficulty in obtaining samples, the microbiota of wild animals is poorly understood. This work reports a number of phyla and classes of microorganisms never noticed in the fecal samples of wild fur seals before and provide insight into the fecal eukaryotic community of wild young South American and Subantarctic fur seals.

## INTRODUCTION

The Brazilian coast is influenced primarily by three different current systems: the North Brazil Current, the Brazil Current, and the Malvinas Current [1-3]. The diversity of marine animals on the Brazilian Coast is widely variable among these systems. South American fur seals (*Arctocephalus australis*) (Zimmerman 1783) are frequently observed along the southern Brazilian coast. However, climatic phenomena have increased the prevalence of Subantarctic fur seals (*Arctocephalus tropicalis*) (Gray 1872) [4]. The pelagic habitat of South American and Subantarctic fur seals has contributed to their dispersion outside of the reproductive period, especially young animals. The availability of food, predator-prey relationships, and climate all play significant roles in the natural selection process of these mammals [5].

The large community of microorganisms in the gut is known to be composed of a vast number of prokaryotes, fungi, and

protozoa [6]. Throughout, the composition, and thus the operation of microbiome, can be influenced by a variety of factors, such as environment, food, antibiotics, diseases, and senescence among others. The microbiota is recognized as a major contributor to the digestion and utilization of foods in the gastrointestinal tracts as well as a modulator of the immune system of the host, like, for example, the interactions between the microorganism and Peyer's patches [7]. Relatively little research employing the evaluation of gut microbiota from wild animals has been undertaken, probably because of the migratory behaviour of some species and the difficulty in obtaining samples from wild marine mammals [8-10]. To the best of our knowledge, there are no studies that have offered insight into gut micro-eukaryotic diversity in wild marine animals.

The fecal microbiota in wild animals can provide critical information into host physiology. The analysis of the microbiota in wild animals can highlight important aspects concerning the

ecological and evolutionary relationship between mammals and their gut microbiota, and, in the future, the data can be compared against environmental changes. In the present study, the fecal eukaryotic microbiota obtained from young wild fur seals was investigated using a culture-independent molecular method.

## MATERIALS AND METHODS

Faeces were collected directly from the large intestine using sterilized wooden sticks from young wild South American ( $n = 2$ ) and Subantarctic fur seals ( $n = 3$ ) and DNA extraction was carried out as described by Medeiros [11]. The sampling of young wild fur seals was performed according to the regulations established

by the *Sistema de Autorização e Informação em Biodiversidade* (SISBIO) nº 20185-4, code number 62966211. Total DNA was used as a template for PCR with eukaryotic *18S rRNA* gene-specific primers - Euk1AF and Euk516R [12]. All samples were taken in triplicate in order to minimize PCR bias. Fragments were sequenced as described by Medeiros [11]. Sequences were submitted to the EMBL database under accession number PRJEB22375. The rRNA reads generated were trimmed to a minimum length of 100 bp and minimum Phred score of 20 via PRINSEQ [13]. The remaining sequences were replicated and sorted by decreasing read abundance and then filtered to exclude singletons using USEARCH v7.0.1090 [14] according to

**Table 1:** Relative abundance (estimate in percentage of sequence reads) of eukaryotic DNA sequence obtained from the fecal samples of South American and Subantarctic fur seals in the present study. a. G1537: Male; death by natural causes (Gercaci code 2); b. G1535: Male; death by multiple fractures; c. G1561: Male; death by multiple fractures (Gercaci code 2); d. G1516: Female; death by cervical fracture (Gercaci code 2); e. G1517: Male; death by multiple fractures (Gercaci code 2). \*Infraclass.

Kingdon/phylum/class	Subantarctic			South American		Mean (SD)
	G1537 <sup>a</sup>	G1535 <sup>b</sup>	G1561 <sup>c</sup>	G1516 <sup>d</sup>	G1517 <sup>e</sup>	
<b>Metazoa</b>	94.77	99.89	91.22	84.16	99.33	93.871 (6.484)
<b>Chordata</b>	2.55	85.34	59.77	39.5	69.25	51.280 (31.892)
Mammalia	2.26	81.42	39.63	29.31	61.91	42.90 (30.381)
Actinopterygii		0.003	0.003		0.002	0.0016 (0.001)
Teleostei*	0.29	3.84	19.95	10.18	7.29	8.31 (7.488)
<b>Nematoda</b>	33.81	9.38	29.41	43.88	3.2	23.936 (17.081)
Chromadorea	33.81	9.38	29.41	43.88	3.2	23.936 (17.081)
<b>Platyhelminthes</b>	58.16		0.2		0.35	11.740 (25.948)
Trematoda	58.16		0.2		0.35	11.740 (25.948)
<b>Arthropoda</b>	0.06	0.21	0.13		25.78	5.24 (11.484)
Maxillopoda			0.01		25.78	5.14 (11.492)
Malacostraca	0.01	0.14	0.11			0.052 (0.067)
Insecta	0.05	0.03				0.016(0.023)
<b>Mollusca</b>	0.11	4.4	1.23	0.01	0.03	1.16 (1.884)
Cephalopoda	0.11	4.4	1.23	0.01	0.03	1.156 (1.884)
<b>Acanthocephala</b>					0.02	0.004 (0.008)
<b>Annelida</b>	0.05	0.28			0.06	0.078 (0.116)
<b>Chaetognatha</b>		0.003				0.0006 (0.001)
Sagittoidea		0.003				0.0006 (0.001)
<b>Myxozoa</b>	0.04	0.01	0.01	0.53	0.28	0.172 (0.228)
Myxosporea	0.04	0.01	0.01	0.53	0.28	0.174 (0.228)
<b>Fungi</b>		0.03	0.02	0.01	0.012	0.014 (0.011)
<b>Ascomycota</b>		0.03	0.02	0.01	0.012	0.014 (0.011)
Eurotiomycetes			0.02		0.002	0.0044 (0.013)
Saccharomycetes		0.03		0.01	0.01	0.01 (0.012)
<b>Chromalveolata</b>	0.1	0.003	0.01	0.73	0.24	0.22 (0.302)
<b>Ciliophora</b>			0.01			0.0006 (0.04)
Oligohymenophorea			0.01			0.0006 (0.04)
<b>Apicomplexa</b>	0.04			0.63	0.005	0.13 (0.277)
Conoidasida	0.03			0.63	0.005	0.133 (0.278)
Aconoidasida	0.01					0.002 (0.004)
<b>Dinoflagellata</b>	0.06	0.003		0.1	0.24	0.08 (0.098)
Dinophyceae	0.06	0.003		0.1	0.24	0.08 (0.098)
<b>Chloroplastida</b>				0.02		0.004 (0.008)
Pinophyta				0.02		0.004 (0.008)
Pinopsida				0.02		0.004 (0.008)
<b>Unassigned</b>	5.13	0.08	8.76	15.08	0.42	5.89 (6.263)
Unassigned;Other	5.655	0.7	9.43	15.34	0.95	6.0 (6.161)

the algorithm UPARSE [15]. The RDP Classifier was used for 18S fragment taxonomic assignment on QIIME v1.9 [16]. Taxonomic data was garnered through the Silva database, version 111 [17].

## RESULTS AND DISCUSSION

### South american and subantarctic fur seals 18S rRNA survey

A total of 353,941 reads of 18S rRNA gene sequences were performed with the fecal samples of South American and Subantarctic fur seals. Relative abundance was estimated in terms of percentage of sequence reads (%). Overall, the results showed there to be a diverse eukaryotic community within the fecal samples of young wild South American and Subantarctic fur seals (Table 1). This is the first report of certain phyla and classes in the fecal samples of wild fur seals.

Metazoa was the most abundant (93.87%) followed by Chromoalvelata (0.22%), Fungi (0.014%), Chloroplastida (0.004%), and other unidentified sequences (5.894%). Within the kingdom Metazoa, nine phyla were observed. The Chordata was more frequently seen at an average total of 51.28%. As expected, a large number of DNA sequences (42.90%) representing the mammalian class were detected. The Actinopterygii (0.0016 %) and Teleostei (8.31%) belonging to the Chordata phylum were also observed. The presence of these classes and infraclasses in faeces was directly related to the animals' diets as they included all existent species of fish. These findings corroborate a study evaluating samples of faeces of South American fur seals (*A. australis*) in Isla de Lobos, Uruguay, where the majority of the fish found were Teleostei [18]. Another investigation assessing the fecal samples of Australian fur seals (*Arctocephalus pusillus doriferus*) employing pyrosequencing of prey DNA identified the Actinopterygii [19].

The Nematoda phylum was observed at an average of 23.93% frequency. This was in a different proportion from all animal fecal samples, ranging from 43.88% to 3.20%. Nematodes live in a wide variety of habitats; ecologically, they can be divided into free-living forms and parasitic forms. The Chromadorea class was observed across all fecal samples. This is the major class of nematodes that includes the Rhabditida order [20]. The high prevalence of this class could be related to infection by the *Strongyloides* species in these animals. These parasites have already been observed in the small intestine of South American fur seals from the coast of Uruguay [21,22].

The phylum, Platyhelminthes, was observed in three samples, however only in high proportions in one sample of Subantarctic fur seal (1537). Platyhelminthes are divided into Turbellaria, which are mostly non-parasitic animals, such as planarians, and the Cestoda, Trematoda, and Monogenea that are entirely parasitic groups. In the fecal samples of wild marine fur seals, the class, Trematoda, was found in three samples, with one of the samples presenting an unusually high rate (58.16%) when compared with the others (0.35% and 0.20%). The presence of this class was already established in infected pinnipeds on the Brazilian coast. According to the authors, the species was seen infecting sealions were probably transmitted by means of feeding as these animals prey on mullets [23,24].

The Arthropoda phylum was observed with a frequency of 5.246%. Within Arthropoda, the most abundant class within the fecal samples of wild fur seals was Maxillopoda (5.14%) followed by Malacostraca (0.052%) and Insecta (0.016%). The Maxillopoda and Malacostraca were the most common types of microbiological organisms found in the fecal samples of loggerhead turtles (*Caretta caretta*) [25]. In one study employing metabarcoding to analyse the Australian sealion's diet (*Neophoca cinerea*) in different areas of the Indian Ocean, this class was detected in scat [26]. An interesting finding from this study was the presence of the Insecta class in fecal samples of Subantarctic fur seals, which features species that infest pinnipeds and other mammals. Previous evidence obtained from South American sealion pups (*Otaria flavescens*) from Patagonia, Argentina, infested with louses *Antarctophthirus microchir* demonstrated a clear pattern of habitat selection, such as the host's ventral area for development and copulation and the dorsal area for oviposition. However, they may also be found within the genital and anal orifices, eyelids, and auditory canal and on the head and dorsal neck [27,28]. Thus, the existence of this class in fecal samples might be related to the licking behaviour of the animals.

The Mollusca phylum was identified in all samples at an average rate of 1.16%. The mollusk composes the large phylum, *Mollusca*, of invertebrate animals, of which the majority of the organisms are marine-based. The only class found in the Mollusca phylum was Cephalopoda, which included squid, octopus, or nautilus, and its presence in fur seals was expected as they are the main prey of American and Australian fur seals [18].

The frequency of the Acanthocephala phylum was very low (0.0004%) and found in just one sample from South American fur seals. This phylum contains parasitic worms and adult Acanthocephalans and is found in a variety of bird species along with other vertebrates. Acanthocephala have been reported in mammalian marine animals - *Corynosoma* sp. have been observed in pinnipeds and species of *Bolbosoma* have also been reported in Arctic seals [29]. Aznar et al., described the existence of acanthocephalans in scats from the South American sea lion (*Otaria flavescens*) and rectal faeces from Franciscanas (*Pontoporia blainvillei*) from Buenos Aires Province, concluding that acanthocephalans contact sea lions regularly [30, 31].

The presence of micro-eukaryotes belonging to the phylas, Annelida (0.08%), Chaetognatha (0.003%), Myxozoa (0.174%), Ascomycota (0.072%), Ciliophora (0.0006%), Apicomplexa (0.13%), and Dinoflagellata (0.08%) was detected here for the first time in fecal samples from wild South American and Subantarctic fur seals. The Annelida phylum is found worldwide, from the deepest marine sediments to soils. The species has adapted to a large variety of environments, such as marine zones, hydrothermal vents, fresh water, and moist terrestrial environments. The Chaetognatha exclusively reside in marine environments, these microorganisms being planktonic worms that are very important predators in many food webs [32]. The Sagittoidea interacts with small marine worms and was found in only one sample. Myxosporeans are among the most common class parasites of marine fish, freshwater fish, and, rarely, invertebrates [33,34]. Based on it being a class of parasite

organisms, its life cycle is constituted by vegetative phases across two hosts - one aquatic invertebrate, usually an annelid, and a cold-blooded vertebrate, like fish that are the prey of pinnipeds.

The micro-eukaryotic phylum, Ascomycota, was also observed, and the Eurotiomycetes and Saccharomycetes classes exhibited prevalences that varied between 0.0064% and 0.01%. The presence of these classes matched the data published when evaluating fungi in mammalian faeces. One of these studies, which assessed healthy and sick dogs, observed the presence of both these two classes [35]. Another investigated that examined children's fecal samples detected Saccharomycetes [36]. These classes have also been identified in the contents of intestinal lobsters' larvae [37]. A number of yeast isolated from ocean waters have their origins from terrestrial habitats based on evidence of their existence in fruits, soil, domestic animals, and humans [38]. These yeast were perhaps taken to the sea by means of rivers, sewers, or even strong winds blowing towards the ocean [39,40].

As well, DNA sequences of the micro-eukaryotes from Chromalveolata were detected. With this, the Ciliophora phylum was observed in just one sample. These microorganisms can be ectosymbionts, endosymbionts, and parasites from other organisms. The Apicomplexa phylum of intracellular obligates was observed in three samples and has the ability to efficiently replicate within different host cells and is crucial to the pathogenesis of parasites that cause many important human and veterinary diseases, including malaria, toxoplasmosis, cryptosporidiosis, and coccidiosis [41]. The Conoidasida was the only class present in the fecal samples of both animals. This class features two subclasses - Coccidia and Gregarinia. The Gregarinia tend to behave parasitically with invertebrates and Coccidia predominantly infect vertebrates and act at the intercellular level [42]. In pinnipeds, diseases like toxoplasmosis and sarcoidosis are induced by parasites that belong to the Coccidia class [43]. Keeping this in mind, *Toxoplasma gondii* antibodies have been observed in Southern elephant seals (*Mirounga leonina*), Weddell seals (*Leptonychotes weddellii*), and Antarctic fur seals (*Arctocephalus gazella*) [44]. *Sarcocystis neurona* is capable of infecting pinnipeds and marine cetaceans, and the infection is related to severe encephalitis and mortality of mammals from the Pacific North East [45]. The transmission and dissemination of protozoa diseases in mammals is not well understood as of yet, however the major theory suggests that mussels and crabs exposed to terrestrial fresh water flows that contain oocysts resistant to the environment dumped in domestic feline faeces serve as primary source of infection in South sea otters (*Enhydra lutris nereis*) [45].

The Dinoflagellata phylum was identified in four samples with a total prevalence of 0.403%. These micro-eukaryotes constitute a large group of flagellate, are important primary producers, and are an essential component of the food chain in the marine ecosystem. It is a large group of unicellular Protists, prominent members of plankton in marine ecosystems and freshwater habitats as well as in benthic and sea ice environments. The presence of this phylum in the fecal samples of wild fur seals can be associated with food webs, since Dinoflagellate is very common in fish diets [46].

The Pinophyta phylum and Pinopsida class were determined to be present in only one sample of the South America fur seal (0.02%). This class consists of conifers that are trees or shrubs characterized by carrying reproductive structures called cones. This group of vegetables has already been found in sea turtle faeces [25]. However, this is the first time that the presence of this class in pinnipeds faeces has been established. Apparently, they were moved by humans to areas well outside their natural ranges and only persist where they are cultivated or are endowed with the ability of dispersing pollen and seeds [47].

## CONCLUSION

This study evaluated the fecal eukaryotic community of wild young fur seals. The results showed there to be a dominant eukaryotic phylum, Chordata, and the highest levels of the eukaryotic class, Mammalia, followed by Platyhelminthes, Arthropoda, and Mollusca. In addition, we found other classes of micro-eukaryotes from fecal samples of pinnipeds found in South America that were not yet reported in the literature. Overall, the eukaryotic communities found were heterogeneous, and, in addition, the high-throughput sequencing technology employed herein offers the identification of organisms that are either difficult to detect through morphological analysis of faeces or through direct observational studies.

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