UNIVERSIDADE FEDERAL DE PELOTAS
Programa de Pós-Graduação em Parasitologia

Dissertação

IDENTIFICAÇÃO DA COMUNIDADE COMPONENTE DE HELMINTOS, GASTROINTESTINAIS, HEPÁTICOS, PULMONARES, CARDÍACOS E RENAIS DE *Otaria flavescens* (Shaw, 1800) LEÃO- MARINHO-DO-SUL, NO LITORAL SUL DO BRASIL

Eliane Machado Pereira

Pelotas, 2012

ELIANE MACHADO PEREIRA

IDENTIFICAÇÃO DA COMUNIDADE COMPONENTE DE HELMINTOS, GASTROINTESTINAIS, HEPÁTICOS, PULMONARES, CARDÍACOS E RENAIS DE *Otaria flavescens* (Leão-marinho-do-sul), NO LITORAL SUL DO BRASIL

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RESUMO

PEREIRA, ELIANE MACHADO. **Identificação da comunidade componente de helmintos gastrointestinais, hepáticos, pulmonares, cardíacos e renais de** *Otaria flavescens* **(Shaw, 1800) Leão-marinho-do-sul, no litoral sul do Brasil.** 2012.70f. Dissertação (Mestrado) – Programa de Pós-graduação em Parasitologia. Universidade Federal de Pelotas.

Este estudo analisou os helmintos parasitos gastrointestinais, pulmonares, cardíacos e renais do *O. flavescens* no litoral sul do Rio Grande do Sul, Brasil. Foram necropsiados 29 leões-marinhos, cujas carcaças apresentavam baixo estado de decomposição. Os órgãos coletados mediante necropsia a campo para análise em laboratório foram 24 intestinos delgado e grosso, 24 fígados incluindo parênquima e vesícula biliar e 29 estômagos, 24 corações e 24 pares de rins. Os órgãos foram congelados a –20^{o}C até o seu processamento. Para a triagem dos parasitos foi usada peneira com malha de 150 µm e todo o conteúdo retido foi analisado sob microscópio estereoscópico. Os helmintos foram fixados em AFA, corados com Carmin e clarificados com creosoto de Faia. Os intestinos delgados foram divididos em três segmentos que foram analisados separadamente para registrar a distribuição dos helmintos por sítios de preferência. Teste de Kolmorogov-Smirnov foi utilizado para verificar tipo de distribuição dos dados. A comparação da abundância média de infecção entre classes etárias foi realizada através do teste de Teste de Wilcoxon usando nível de significância de 0.05. As correlações entre intensidade de infecção, sexo, comprimento total do individuo e comprimento dos intestinos delgado e grosso foram verificadas usando a Correlação de Pearson. Para as análises estatísticas usou-se o software Action® versão 1.1. De 29 espécimes de *O. flavescens* 23 eram machos, três fêmeas e em três indivíduos o sexo não pode ser determinado. A média do comprimento total dos

animais foi 2,14±0,31m (1,58 - 2,64m), sendo 13 subadultos e 16 adultos. Foram registrados 996 espécimes de *Contracaecum ogmorhini* presentes principalmente no estômago, (prevalência 10%). Registrou-se 42.145 espécimes de *Corynosoma australe* (Prev. 100%) e 512 de *Bolbosoma turbinella* (Prev. 50%). Duas espécies de trematódeos foram coletados: *Stephanophrora uruguayense* (Prev. 4.17%) e *Ascocotyle (Phagicola) longa* (Prev. 33.33%) totalizando 1.988.202 espécimes. Cestódeos foram encontrados em apenas um hospedeiro (Prev. 4,16%) que apresentou 4 escóleces. Macroscopicamente, fígado, vesícula biliar, coração, pulmões e rins examinados não estavam parasitados. Nenhuma correlação significativa foi observada entre a intensidade de infecção, sexo, comprimento total ou comprimento dos intestinos. Este é primeiro registro de *Diphyllobothrium* sp., *Bolbosoma turbinella* e *Contracaecum ogmorhini* em *O. flavescens* em águas brasileiras. No que se diz respeito à fauna parasitária de *O. flavescens*, as espécies encontradas no presente estudo não são as mesmas previamente citadas para leão-marinho-do-sul da costa pacífica da América do Sul.

Palavras-chave: *Otaria flavescens*, helmintos, fauna parasitária, leão-marinho-do-sul.

Abstract

PEREIRA, ELIANE MACHADO. Identification of component community of helminths in gastrointerstinal tract, liver, lungs, heart and kidneys of *Otaria flavescens* **(Shaw, 1800) southern sea lion, in southern coast of Brazil. 2012. 70f. Dissertation (Master in Science) – Postgraduate Program in Parasitology. Federal University of Pelotas, Pelotas – RS.**

This study verified helminth parasites infection in gastrointestinal tract, lungs, heart, and kidneys of southern sea lions, *Otaria flavescens*, from south coast of Rio Grande do Sul State, Brazil. Twenty-nine sea-lions were found dead on the beaches, whose carcasses had mild state of decomposition, were necropsied. The organs were collected during field necropsies for laboratory analyses were 24 small and large intestines, 24 livers including parenchyma and gall bladder, 29 stomachs, 24 hearts, and 24 pairs of kidneys. The organs were maintained frozen at -20°C until their processing. A sieve with 150µm mesh was used for screening the parasites. All content retained was analyzed under stereomicroscope. The helminthes were collected, counted, fixed in AFA, stained with carmine, and clarified in beechwood creosote. The small intestines were divided into three segments that were separately analyzed to record the distribution of helminthes by preference sites. Kolmorogov-Smirnov test was used to verify the type of data distribution. Comparison of mean abundance of infection between age classes was performed through Wilcoxon test at significant level of 0.05. Correlations between infection intensity, sex, total length of the individual, and length of small and large intestines were determined using Pearson's Correlation. Action® software version 1.1 was applied for statistical analyses. Among 29 specimens of *O. flavescens* 23 were males, three females, and three individuals whose sex could not be determined. The average length of the animals was 2.14±0.31m (1.58 to 2.64m) including 13 sub-adults and 16 adults. A total of 996 specimens of *Contracaecum ogmorhini* were recorded, especially in the stomach (10.34% of prevalence), 42,145 specimens of *Corynosoma australe* (100% of prevalence) and 512 of *Bolbosoma turbinella* (50% of prevalence) were found. Two species of trematodes were found: *Stephanophrora uruguayense* (Prev. 4.17%) and *Ascocotyle (Phagicola) longa* (Prev. 33.33%), a estimated total of 1,988.202 specimens. Cestodes were found in only one of the hosts (4.16% of prevalence) which presented four scoleces. Macroscopically, liver, gall bladder, heart, lungs, and kidneys did not contain parasites. No significant

correlation was observed between infection intensity, mean abundance, sex, total length of the host, or length of intestines. Infections levels were similar between sub-adults and adults sea lions. This is the first record of *Diphyllobothrium* sp., *Bolbosoma turbinella*, *Contracaecum ogmorhini, Ascocotyle (Phagicola) longa,* and *Stephanoprora uruguayense* in *O. flavescens* in Brazilian waters. As regards parasite fauna of *O. flavescens,* our data are different from those previously reported for specimens from Pacific coast of South America.

Key-words: *Otaria flavescens,* helminthes, parasitic fauna, sea lion.

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Sumário

1.Introdução Geral

1.1. Mamíferos Marinhos

Dentre as espécies integrantes da classe Mammalia existe um grupo de animais que, por terem seu habitat comum e suas características morfológicas, fisiológicas e comportamentais semelhantes, são chamados de mamíferos aquáticos. Dentre eles, os mamíferos marinhos sempre despertaram interesse e curiosidade, talvez por serem ainda pouco conhecidos quanto a seus hábitos e comportamento, o que se deve a dificuldade de observação no meio em que vivem. Várias espécies estão incluídas em três ordens diferentes: Cetácea, que inclui os golfinhos e baleias, Sirenia, que inclui peixes-boi e dugongos e a Carnivora, a qual pertence a Sub-ordem Pinnipedia que inclui as focas, lobos e leões-marinhos. As espécies dessas ordens possuem adaptações essenciais para a vida aquática que evoluíram independentemente, sendo as principais: forma do corpo alongada para deslocamentos na água, camada de gordura sub-cutânea que envolve todo o corpo para suportar as baixas temperaturas da água e orifícios respiratórios situados em posições estratégicas de modo a serem facilmente emersos da água no momento da respiração (HEWER, 1974; PINEDO et al., 1982).

1.2. Pinípedes

O termo “pinípedes” provém do formato dos membros torácicos e pelvianos de um grupo de mamíferos marinhos pertencentes à subordem Carnivora, os quais são constituídos por nadadeiras (Fig.1) (*pinna*=pena; *podos*= pés) “pé em forma de pena” devido a presença de extensas membranas interdigitais usadas para locomoção na água (PINEDO et al., 1982; BARRETO, 2001). Dentre os mamíferos marinhos, os pinípedes são um grupo que destaca-se por apresentar ampla distribuição geográfica e uma grande diversidade, incluindo as focas (família Phocidae), morsas (família Odobenidae), lobos e leões marinhos (família Otaridae). São carnívoros aquáticos extremamente especializados que estão distribuídos pelas regiões ártica, antártica, tropicais e subtropicais (RIEDMAN,1990, PINEDO et al., 1982, BARRETO, 2001).

Os pinípedes apresentam corpo fusiforme e totalmente coberto de pêlos, que são anualmente renovados, a epiderme é grossa e na derme existem glândulas sebáceas

sudoríparas. Os membros torácicos e pelvianos são diferenciados dos demais carnívoros pela existência de dedos alongados e unidos por espessas membranas. Possuem orifíco respiratório na frente do focinho, as orelhas ou pavilhões auditivos estão presentes em otarideos e ausentes em odobenideos e focídeos. A cauda é reduzida, não parecendo exercer uma função é ma estrutura vestigial, herança dos ancestrais terrestres. As fêmeas possuem um ou dois pares de mamas no abdômen. Nos machos, o pênis é interno e retrátil, localizado entre o ânus e o umbigo possuindo osso peniano (báculo) (PINDEDO et al., 1982).

Figura 1. Nadadeira de *O. flavescens*, com membranas interdigitais. Fonte: Ivipora Roga, 2008.

Em geral a cópula ocorre em terra e as fêmeas parem um filhote a cada ano, a lactação pode durar de 3 a 24 semanas dependo da espécie. A temperatura corporal é mantida em torno de 36º a 37º C e para isso espécies de regiões frias possuem uma grossa camada de gordura e pelos. A alimentação dos pinípedes é variada, geralmente composta de peixes, lulas, crustáceos e outros animais marinhos (HEWER, 1974; PINDEDO et al., 1982). Estimativas de abundância indicam a existência total de 50 milhões de pinípedes, entretanto sua maioria são focídeos (RIEDMAN, 1990). Apesar de sua grande abundância, somente duas espécies são encontradas nos trópicos as quais compõe populações pequenas e ameaçadas de extinção (BARRETO, 2001). Por outro lado, os lobos e leões-marinhos são abundantes nas áreas tropicais e subtropicais sendo que os lobos apresentam maior

ocorrência no Hemisfério sul e os leões em ambos os hemisférios (RIEDMAN, 1990; SANTOS et al., 1992).

A ocorrência de pinípedes em águas brasileiras é registrada em maiores números na região sul, durante os meses de inverno e primavera (IBAMA, 2001). Não existem colônias reprodutivas no litoral do Brasil, sendo que a maioria dos espécimes encontrados é representada por machos e indivíduos jovens (ESTIMA, 2002). Até o presente já foram registrada ocorrência de sete espécies de pinípedes, na costa do Brasil, são elas: Leão-Marinho-do-Sul, *Otaria flavescens,* o Lobo-marinho-do-sul, *Arctocephalus australis*, lobo-marinho-subantártico, *Arctocephalus tropicalis,* o lobo-marinho-antártico, *Arctocephalus gazella*, o elefante-marinho-do-sul, *Mirounga leonina*, a foca-caranguejeira *Lobodon carcinophagus,* e a foca-leopardo, *Hydrurga leptonyx* (PINEDO et al., 1992). As espécies mais freqüentes que utilizam o sul do Brasil para descanso e alimentação, são o Leão-Marinho-do-Sul, *Otaria flavescens,* e o Lobo-marinho-do-sul, *Arctocephalus australis*. As demais espécies ocorrem de forma esporádica (PINEDO et al., 1992).

1.3. Família Otariidae

Pertencem a esta família lobos e leões–marinhos. Membros torácicos adaptados ao meio aquático com carpos flexionados lateralmente formando grandes nadadeiras. (Fig.2). Possuem nadadeiras com palmas e solas desprovidas de pêlos, nadadeiras posteriores com garras mais desenvolvidas que as anteriores e com flexão para frente, participando assim da locomoção na terra. Machos normalmente são maiores que as fêmeas (Fig. 3), os testículos são extra-abdominais e em alguns casos os machos adultos podem apresentar juba (nos leões-marinhos). O pavilhão auricular esta presente e em forma de um pequeno tegumento cilíndrico afilado (Fig.4). Fêmeas possuem dois pares de mamas abdominais. (PINEDO et al., 1982).

Figura 2 – Membros torácicos adaptados ao meio aquático com carpos flexionados lateralmente formando grandes nadadeiras Fonte: Patrick Gries. Museum of natural history, Paris.

Figura 3. Espécimes de *O. flavescens*, machos adultos. Fonte: Silvia Gastal,2012.

Figura 4. Espécime *Otaria flavescens*, macho juvenil. Pavilhão auricular de forma cilíndrica e afilada. Fonte: Silvia Gastal, 2012.

1.4. Sistemática do hospedeiro

Reino: Animalia

Filo: Chordata

Classe: Mammalia

Ordem: Carnivora

Sub-ordem : Pinnipedia

Família: Otariidae

Gênero: *Otaria* Espécie: ***O. flavescens*** (Shaw,1800)

1.5. *Otaria flavescens*

O nome científico da espécie vulgarmente conhecida como leão-marinho-do-sul tem sido alvo de várias discussões por ser reconhecida como *Otaria flavescens* (SHAW, 1800) e também *Otaria byronia* (BLAINVILLE, 1820). Segundo Rosas (1989) o holótipo no qual Blainville baseou-se para descrição da espécie foi destruído na II Guerra Mundial Considerando todas as dificuldades inerentes à busca da identidade correta da espécie os autores tem divergido fortemente sobre este assunto, porém neste trabalho adotamos *O. flavescens* segundo Campagna (2008).

O leão-marinho-do-sul é uma espécie que habitualmente vive em profundidades menores que 50 m (VAZ-FERREIRA, 1981). Distribuem-se pela região costeira de ambas as costas da América do Sul, agrupando-se em locais rochosos. No Atlântico, a área mais ao norte com agrupamento desta espécie é o sul do Brasil, estendendo-se meridionalmente até a Terra do Fogo, Argentina e continuando ao norte pelo Pacífico até o Peru (VAZ-FERREIRA, 1981; ROSAS et al., 1994).

Alimentam-se preferencialmente em águas rasas, a uma distância de cinco milhas da costa e seu alimento mais comum são os peixes, crustáceos e moluscos (VAZ-FERREIRA, 1981; ROSAS, 1989). Segundo Falco (2008), em estudo realizado caracterizando a dieta de *O. flavescens* no litoral sul do Rio Grande do Sul, foram identificados no conteúdo de 70 estômagos as seguintes taxóns: Teleósteos: - *Macrodon ancylodon* (pescadinha ou pescada real), *Micropogonias furnieri* (corvina), *Paralonchurus brasiliensis* (maria-luiza), *Trichiurus lepturus* (peixe-espada), *Cynoscion guatucupa* (pescada-olhuda), *Urophycis brasiliensis*, *Umbrina conosai*, *Porichthys porosissimus*, *Stellifer rastrifer*, *Menticirrhus* sp. Elasmobrânquios: *Sympterigia acuta*, *Raja agassizi*, *Mustelus schmitii*, *Symptergia bonapartii*, *Myliobatis* sp. Cefalópodes: *Loligo sanpaulensis*, *IIIex argentinus* e *Octopus vulgaris.* Crustáceos: *Penaeus* sp, *Loxopagurus loxochelis*, e *Artemisia longinaris*.

Os principais predadores naturais da espécie são os tubarões e a orca (*Orcinus orca*) (VAZ-FERREIRA, 1981). Espécimes são mortos regularmente por pescadores, devido aos danos nas redes de pescarias (SILVA,2004).

A maturidade sexual ocorre após os 3 anos para as fêmeas e após 5 anos para os machos. A cópula ocorre em terra, fêmeas parem um filhote por ano (PINEDO et al., 1992;

VAZ-FERREIRA, 1981; ROSAS, 1989) após uma gestação que dura aproximadamente um ano. Longevidade é considerada cerca de 20 anos e as taxas de mortalidade de adultos são desconhecidas (VAZ-FERREIRA, 1981; CAMPAGNA, 2008). Machos ocupam-se da formação de haréns, sendo usualmente grandes grupos de fêmeas reunidos pelos machos mais velhos e fortes (PALAZZO JR., 1988). Os machos adultos atingem 2,6 m de comprimento e pesam de 300 a 350 kg; as fêmeas atingem 2 m e em média 150 kg. Ao nascer, os filhotes pesam 11-15 kg e medem 75-85 cm de comprimento. Os filhotes nascem pretos, freqüentemente com tons de laranja acinzentado no lado inferior (CAMPAGNA, 2008). Morfologicamente *O. flavescens* possui corpo fusiforme e coberto por uma única camada de pêlos, por este motivo, em vários países sul-americanos de idioma hispânico, a espécie é chamada "lobo marino de un pelo". A coloração dos animais é marrom claro ou escuro, variando bastante de acordo com a idade. Os machos são mais escuros e de porte mais robusto, apresentando uma "juba" proeminente de pêlos longos, que se desenvolve a partir do quinto ano de vida do animal (PINEDO et al., 1992).

Segundo a Lista Vermelha da IUCN de espécies ameaçadas (CAMPAGNA, 2008), *Otaria flavescens*, encontra-se em um nível pouco preocupante de extinção. A população mundial está estimada em mais de 250.000 espécimes. Estimativas de abundância em alguns países indicam que no Atlântico há cerca de 12.000 animais na costa do Uruguai, na Argentina continental este número não é inferior a 100.000 e que nas Ilhas Malvinas são estimados aproximadamente 6.000 indivíduos. No Pacífico, as estimativas populacionais são de 90.000-100.000 no Chile e 60.000 no Peru (CAMPAGNA, 2008).

As duas maiores concentrações do *Otaria flavescens* no litoral brasileiro estão no Rio Grande do Sul: o Refúgio da Vida Silvestre do Molhe Leste da Lagoa dos Patos e a Reserva Ecológica da Ilha dos Lobos, em Torres (ROSAS et al.,1994; IBAMA, 2001). Considera-se que a partir do estado de Santa Catarina até a Bahia a espécie pode aparecer ocasionalmente, através de incursões de indivíduos isolados nos meses de inverno e primavera, que se deslocam em busca de alimentação (SILVA, 2004). No Atlântico sul, colônias de reprodução ocorrem ao longo da costa do Uruguai, Argentina e Ilhas Malvinas (PINEDO et al., 1992). Durante a estação reprodutiva, ou seja, no verão, formam-se grupo de reprodução onde um macho pode reproduzir com até 15 fêmeas (VAZ-FERREIRA, 1981). Nos haréns, os machos reprodutores ocupam lugares definidos nos locais de reprodução, fixando limites nos seus territórios através da vocalização, rituais de combate e

brigas, e nele mantêm as fêmeas. Os não reprodutores ocupam os extremos das colônias reprodutivas, sendo compostos de indivíduos de diferentes idades ou apenas de indivíduos velhos (VAZ-FERREIRA, 1981).

Otaria flavescens é citada por Pinedo (1986) como a espécie de maior ocorrência na costa do Rio Grande do Sul, porém Santos et al. (1996) mencionam como a segunda espécie de pinípede mais freqüentemente encontrada, concordando com os resultados de Silva (2004) após monitoramento de praia no litoral do Rio Grande do Sul. Segundo Vaz-Ferreira (1981), o leão-marinho pode penetrar algumas vezes em estuários e rios, fato este confirmado através da ocorrência de um macho adulto no Rio Guaíba no extremo norte da Lagoa dos Patos, e de um macho juvenil na Colônia de Pesca Z3, no Município de Pelotas. Considerando toda a extensão do litoral do Rio Grande do sul, segundo Silva (2004) os encalhes de leões-marinhos-do-sul, ocorrem todo ano, sendo reduzido nos meses de dezembro, janeiro e fevereiro. A grande maioria de animais encontrados nas praias refere-se a animais mortos, classificados como machos adultos e subadultos, incluindo uma pequena quantidade de fêmeas (SILVA, 2004).

1.6. Helmintos de *Otaria flavescens*

Alguns helmintos já são conhecidos por infectar *Otaria flavescens* em sua ampla área de distribuição ao longo das costas da América do Sul. No Pacífico, foram registrados no litoral chileno os nematóides *Phocanema decipien* e *Anisakis* sp. (CATTAN et al., 1980) e, *Pseudoterranova cattani* parasitando estômagos de animais encontrados na porção centro-sul da costa (GEORGE-NASCIMENTO & URRUTIA, 2000). Na mesma região, o trematódeo *Ogmogaster heptalineatus* é conhecido por infectar o intestino delgado de filhotes (CARVAJAL et al. 1983). No Peru, Tantaleán et al. (2005) registraram o acantocéfalo *Corynosoma obtuscens* presentes no intestino. No Atlântico, na costa argentina são conhecidas infecções pelo nematóide *Uncinaria hamiltoni* (Nematoda: Ancylostomatidae) em espécimes jovens capturados em Punta León, Província de Chubut (BÉRON-VERA, 2004) e por *Bolbosoma* sp. (Acanthocephala: Polymorphidae) (HOLCMAN-SPECTOR et al., 1977).

Mais recentemente, Aznar et al. (2011) em estudo realizado em 252 amostras de fezes de *O. flavescens* na província de Buenos Aires, citam a presença *de Corynosoma*

australe e *C. cetaceum*. No Uruguai, Morgades et al. (2006) estudando leões-marinhos de diversas faixas etárias encontraram *Uncinaria* sp. no intestino delgado de um filhote; *Diphyllobothrium* sp. (Cestoda: Diphyllobothriidae) no intestino grosso de juvenis. O trematodeo *Ascocotyle (Phagicola) longa* (Trematoda: Heterohyidae) foi encontrado no intestino delgado e grosso de leões adultos e juvenis. Os acantocéfalos *Corynosoma australe* e *Corynosoma* sp. foram encontrados nos estômagos, intestino delgado e grosso de leões juvenis e somente no intestino delgado e grosso de animais adultos. No mesmo trabalho os autores citam a presença de anisaquídeos não identificados e *Contracaecum* sp. coletados do estômago e intestinos de animais juvenis e adultos. *Stephanoprora* sp. (Trematoda: Echinostomatidae) esteve presente somente no intestino delgado de espécimes adultos.

No Brasil, Andrade et al. (1999) analisaram somente o trato gastrointestinal de leões-marinhos encontrados mortos no litoral sul do Rio Grande do Sul. Estes autores encontraram *Corynosoma australe* (Acanthocephala: Polymorphidae) e o nematóide *Contracaecum* sp. parasitando o intestino e estômago, respectivamente, de animais adultos.

2. Objetivos

Esta dissertação teve como meta a realização de pesquisa parasitológica sistemática de helmintos em sistemas orgânicos do leão-marinho, *Otaria flavescens*, com o seguinte objetivo:

2.1. Objetivo Geral

Identificar a comunidade componente de helmintos, gastrointestinais, hepáticos, pulmonares, cardíacos e renais de *Otaria flavescens* (leão-marinho-do-sul) no litoral sul do Rio Grande do Sul, Brasil.

2.2. Objetivos Específicos

1) Verificar a presença, identificar e quantificar as infecções por trematódeos gastrointestinais, pulmonares, cardíacos e hepáticos de *O. flavescens* do litoral sul do Rio Grande do Sul, Brasil;
2) verificar a presença, identificar e quantificar as infecções por nema gastrointestinais, pulmonares, cardíacos e hepáticos de *O. flavescens* do sul do Rio Grande do Sul, Brasil;
3) verificar a presença, identificar e quantificar as infecções por acantocéfalos e cestóides gastrointestinais, pulmonares, cardíacos e hepáticos de *O. flavescens* do litoral sul do Rio Grande do Sul, Brasil;
4) definir o sítio de infecção de cada espécie parasita encontrada e valorar os seus níveis de infecção em relação à classe etária e sexo do hospedeiro.

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Artigo 1

Digenetic Trematodes in South American sea lions from southern Brazilian waters.

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DIGENETIC TREMATODES IN SOUTH AMERICAN SEA LIONS FROM SOUTHERN BRAZILIAN WATERS

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ABSTRACT: The aim of this work was to perform a systematic study to detect and quantify the digenetic trematode infections in south American sea lion from Southern Brazilian coast. Twenty-four south American sea lions, *Otaria flavescens* (Carnivora: Otaridae), found dead along the coast of Rio Grande do Sul State, Brazil, between June 2010 and September of 2011 were collected. The animals were necropsied on the beach, and the esophagus, stomach, small and large intestines, liver and gallbladder, heart, lungs and respiratory tract, and kidneys were inspected for the presence of flukes. Only two trematodes species were found in the intestines, *Stephanophrora uruguayense* (Digenea: Echinostomatidae) and *Ascocotyle (Phagicola) longa* (Digenea: Heterophyidae). No trematodes were found in the other organs studied. *Ascocotyle (P.) longa* was found in the small intestine of eight sea lions, prevalence of 33.33% and mean intensity of 24.8500 worms whereas *S. uruguayense* showed prevalence of 4.17% and mean intensity of 202 trematodes, also in the intestine. Mullets that commonly harbor Heterophyidae matacercarae, may be an important cause of infection by these trematodes in sea lions. The present work is the first report of digenetic trematode infecting *O. flavescens* in Brazil. Trematodes from Echinostomatidae and Heterophydae may have a zoonotic potential and their presence in sea lions suggests caution in feeding unbaked fish from the studied area because fish species (specially mullets) preyed by the sea lions are also consumed by humans.

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Most adult digenetic trematodes are parasites found in all classes of vertebrates. These helminths always have indirect life cycles, with four hosts in a few species (Bush et al., 2002). Many pinnipeds species are definitive hosts of trematodes where they cause infections in various organs. *Zalophotrema hepaticum* are common parasites of liver, gallbladder and pancreas of the California sea lion, *Zalophus californianus* (Dailey, 2001). Eye flukes, *Philophthalmus* sp. (Echinostomata: Philophthalmidae) have been reported in Galapagos sea lions, *Zalophus wollebaeki* from Ecuador (Dailey et al., 2005). In the common seal, *Phoca vitulina*, from Wadden Sea, Strauss et al. (1991) recorded two species, *Cryptocotyle lingua* and *Phagicola septentrionalis*. In *Z. californianus* from Pacific cost of central América, trematodes found in the gastrointestinal system consisted of species in 10 genera (*Cryptocotyle*, *Galactosomum*, *Rossicotrema*, *Phagicola*, *Stictodera*, *Phocitrema*, *Pricetrema*, *Microphallus*, *Maritrema*, and *Ogmogaster*) (Colom-Llavina, 2005).

The South American sea lion is a widely-distributed pinniped with occurrence from north coast of Peru (Pacific ocean) to southern Brazil (Atlantic ocean), where occurs with major frequency at the coast of Rio Grande do Sul State (Vaz- Ferreira, 1981; Rosas et al., 1994). In general, parasitological works with *O. flavescens* are scarce (Cattan and Carvajal, 1980*;* Morgades et al. 2006; Aznar et al., 2011*)*. In the Pacific coast of South America, only one study mentioned the presence of trematodes, *Ogmogaster heptalineatus* (Carvajal et al., 1983). In Atlantic South Occidental waters, researches focusing parasitic fauna of *O. flavescens* have been performed since 1977 when Holcman-Spector et al. (1977) described the presence of acanthocephalans in Uruguayan coast. In the 90s, Andrade et al. (1999) studying sea lions from Rio Grande do Sul coast, Brazil, mentioned the occurrence of nematodes and acanthocephalan but not trematodes. Currently, only in the short Uruguayan coast, there are two records of flukes infecting the gastrointestinal tract of sea lion from South Atlantic Ocean (Morgades et al., 2006). Probably all pinnipeds are susceptible to flukes infections but the occurrence of a certain parasite species is also dependent of an association of biotic and abiotic conditions in the geographical area where they inhabits. On the other hand, trematodes may be very small and therefore lost or not seen if a specific methodology is not employed.

The aim of this work was to perform a systematic study to find, identify, and quantify the digenetic trematode infections in South American Sea Lion from Southern Brazilian Waters.

MATERIAL AND METHODS

Twenty-four South American sea lions, *Otaria flavescens* (Carnivora: Otariidae), found dead between June 2010 and September of 2011 along the coast of Rio Grande do Sul State, 31°21'38''S - 33°44'35"S, Brazil, were inspected to trematode presence. Dead animals were collected with IBAMA license number 16586-2 and registry number 2031900. The sea lions identification and biometry followed Pinedo et al. (1982). The animals were necropsied on the beach and the organs were collected and frozen at -20^0 until their analysis in laboratory. Only carcasses in low degree of decomposition (level 2 and 3, Geraci and Lounsbury, 2005) were used. The organs inspected included esophagus, stomach, small and large intestines, liver and gallbladder, heart, lungs and respiratory tract, and kidneys. Each organ was washed on a sieve with mesh size of 150µm. The intestines were removed from mesentery; the length was measured and divided in three equivalent parts. Parenchymatous organs were sectioned every two centimeters previous to washing. Flukes retained in the mesh or in the tissue were removed using a microscope steoreoscope, washed in tape water, counted and fixed in ethylic alcohol 70^0 or AFA (Amato et al., 1991). Carmin and Hematoxilyn were used to stain the worms that were cleared in beechwood creosote or lactophenol solution, and mounted in Canada balsam. Morphological identification was based in specific literature. Phase contrasted microscopy was used to visualize keratinized structures. Parasitological terms of prevalence, abundance and mean intensity followed Bush et al., (1997). The number of trematode specimens with size smaller than 1mm was estimated. The total of worms found was diluted in 500ml of alcohol 70^0, mixed for 1 minute in a magnetic mixer device (TE 089, Marconi – Piracicaba, SP) and removed a subsample of 0,25ml in three replicas in which total flukes was counted and proportionally calculated to the total sample.

RESULTS

Sea lions studied were preponderantly adult and males. Mean of total length was 218.10±29.95cm for males (n=20) and 170.00±14.42cm for females (n=3). Distribution of total body length and sex are presented in the Figure 1. Sex could not be identified in only one animal.

Trematodes were found only in the small and large intestines. A total of 1.988.202 worms were collected from eight sea lions. Two species of trematodes, *Ascocotyle (Phagicola) longa* Ransom, 1920 (Heterophyidae) and *Stephanoprora uruguayense* Holcman et Olagüe, 1989 (Echinostomatidae) (Figures 1 and 2) and were identified. The infection levels are presented in the Table 1. Both species were represented by adult specimens with egg production. No food content was present in the host organs where parasites were found. *Stephanoprora uruguayense* was found

only in one sea lion. Higher abundance of this parasite (135 flukes) was observed in the last third of the small intestine (jejune and ileum) (Table 1). *Ascocotyle (P.) longa* was found in the small intestine of eight sea lions. Only in one sea lion this parasite was also collected from the large intestine. Very high mean abundance was observed, 82,667 worms. The site of infection was along whole intestines (Table 1) but in sea lions with lower intensity of infection the flukes were concentrated in the second and last thirds of small intestine.

Table I. Infection levels and sites of infection of the trematodes found in South American Sea Lions from coast of Rio Grande do Sul State, Brazil.

	Infection levels				**Site of Infection**			
Trematodes	Prevalence (%)	n	Mean Intensity	Mean abundance	Mean Intensity by Infection site			
					(SI)1st	(SI)2nd	(SI)3rd	Large intestine
Stephanoprora uruguayense	4.17	1	202	8,42	0*	9*	193*	0*
Ascocotyle (Phagicola) longa	33.33	8	248,500±539,140	82,833±320,601	300,333± 419,345	94,800± 164,961	61,333± 78,946	6,000*

SI – Small intestine, * only one sea lion infected in this site.

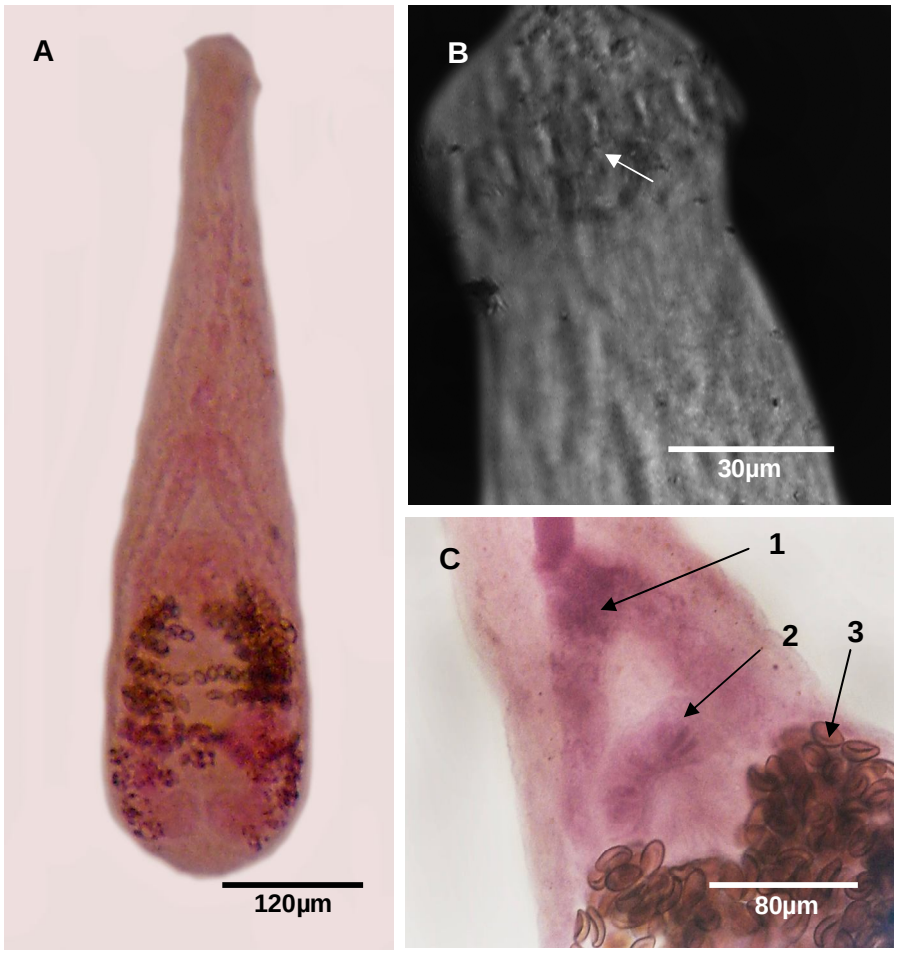

Figure 1. *Ascocotyle (Phagicola) longa* from *O. flavescens.* A.Ventral view of a specimen.; B, Anterior end with a single row of 16 circumoral spines (arrow indicates spine); C, 1. Intestinal ceca bifucation, 2. Bipartite gonotyl composed of two pad-like lobes. 3. Eggs.

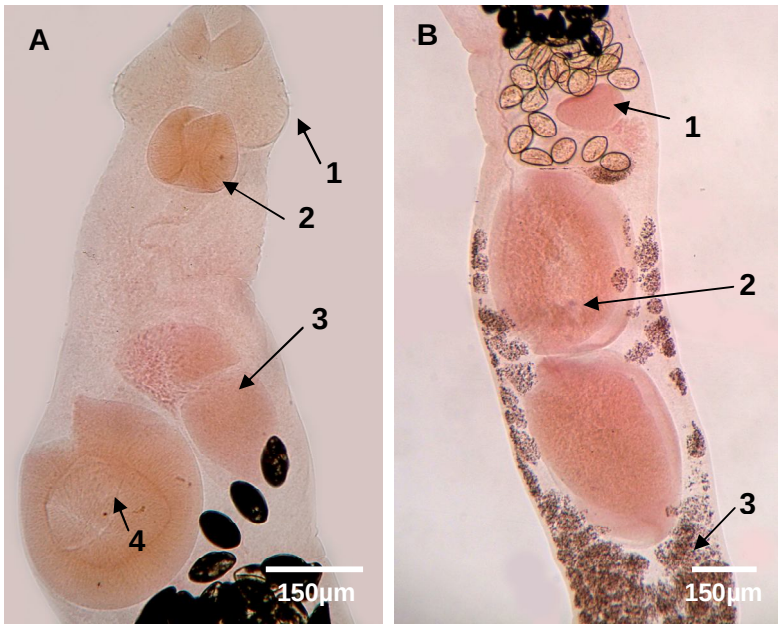

Figure 2. *Stephanoprora uruguayense* from *Otaria flavescens*. A, Anterior third of the body. 1. Head collar; 2. Pharynx; 3. Seminal vesicle; 4. Ventral sucker. B, Medium third of the body. 1.Ovary; 2.Anterior testis; 3. Vitelline glands.

DISCUSSION

Infections by *Stephanoprora* spp. in mammals have been reported mainly in cats eating fish infected with metacercariae. Most infected animals are asymptomatic for the disease, and adult parasites can be found in duodenum and jejunum (Sohn and Chai, 2005). Morgades et al. (2006) studying parasitological infections in pinnipeds from Uruguay, found infection with *Stephanoprora* sp. in one specimen of *O. flavescens*, intensity of 25 flukes, and in 4 juveniles of South American fur seals, *Arctocephalus australis,* that were infected with a maximum of 1,200 worms. The present work is the first record of *S. uruguayense* infecting *O. flavescens* in Brazilian waters. The morphology of the flukes found in the present study coincided with those cited for adults of the species (Ostrowski de Nuñez, 1998; Ostrowski de Nuñez, 2007). Unlike for most digenetic trematodes, the life cycle of *S. uruguayense* was experimentally resolved. The cycle was artificially reproduced using cercariae collected from the snail *Heleobia parchappei* that experimentally infected fishes *Cnesterodon decemmaculatus* (Poecilidae) as second hosts and chicks and mices as the definitive ones (Ostrowski de Nuñez, 2007).

Ascocotyle (Phagicola) longa was reported in the intestines of 4 from 14 *O. flavescens* studied in Uruguay (Morgades et al., 2006). Abundance was estimated only for one juvenile sea lion which was infected by approximately 45,000 worms. In our work we have found this

trematode in 8 from 24 analyzed sea lions with mean intensity as high as 248,500 specimens. *Ascocotyle (P.) longa* is a widespread parasite recorded from the Americas, Europe, Africa and Middle East. It is considered to be one of the causative agents of heterophyiasis, an emerging fish Muller-borne disease of humans (Muller, 2001; Scholz et al., 2001, Fried et al., 2004). The adult parasites are found in the intestine of fish-eater birds and mammals, and the metacercariae are found mainly in mullets (*Mugil* spp.) (Scholz, 1999). In the studied area, lebranch mullet, *Mugil liza* (Actinopterygii: Mugilidae) and correlated species (for other details see Fraga et al., 2007; Heras et al., 2009; Menezes et al., 2010) are an important component in the diet of sea lions, and the interaction between the artisanal fishery of the mullet and sea lions is frequent (Estima, 2002). The taxonomic status of the *Ascocotyle* species has been discussed recently. Scholz et al. (2006) verified the morphology of the worms and proposed as a junior synonym of *Ascocotyle (Phagicola) angeloi*. The authors based on similarities in morphology and morfometry of both species and that the only difference between *A.(P.)rara* and *A.(P.) angeloi* was the absence of circumoral spines in the former, which could be lost after the death of the worm. The same author also performed a taxonomic study of *A. (P.) longa* and related taxa (Scholz, 1999). In most species, general morphology is quite similar which could generate some misunderstanding. According to Scholz (1999) *A. (P.) longa* is related to *Ascocotyle* species that show bipartite gonotyl and a single crown of 16 circumoral spines. Morphological structures of the parasites collected in our study coincided with the description of *A. (P.) longa* published by Scholz (1999). Only the tegument spinose was not clearly observed in our specimens. It could be lost due to the use of dead worms collected from no fresh carcasses as suggested by (Scholz et al., 2006). The life cycle of *A.(P.) longa* has been studied by Simões et al. (2010) and is related to estuaries and coastal lagoons where the recruitment of mugilid juveniles occurs. This work is the first record of the trematode *Ascocotyle (P.) longa* infecting *O. flavescens* in Brazilian waters.

Concerning the geographic distribution of *O. flavescens*, trematodes species found in South Brazilian Coast are quite similar to those previously reported from Uruguay but completely different from those recorded in South American sea lions from Pacific coast of the South America. (Tantaleán et al., 2005).

The presence of adult specimens of *A.(P.) longa* and *S. uruguayense* suggest an adaptation of these species to the parasitism in pinnipeds. Trematodes from Echinostomatidae and Heterophydae may have a zoonotic potential (Simões et al., 2010) and their presence in sea lions suggest caution in feeding unbaked fish from the studied area because the fish species (especially mullets) preyed by the sea lions are also consumed by humans.

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Artigo 2

Contracaecum ogmorhini (Nematoda: Ascaridoidea) in southern sea lion , *Otaria flavescens* (Shaw, 1800) from southern Brazil

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Contracaecum ogmorhini Johnston & Mawson, 1941 (Nematoda: Anisakidae) in southern sea lion , *Otaria flavescens* (Shaw, 1800) from southern Brazil

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Abstract

The objective of this study was to identify the assemblage of parasitic nematodes from southern sea lion, *Otaria flavescens*, along of the southern coast of Rio Grande do Sul State, Brazil. Twenty-nine southern sea lions were necropsied, including 23 males, three females, and three individuals whose sex could not be determined found dead on the coast of Rio Grande do Sul State, in the area between Lagoa do Peixe and Arroio do Chuí, from June, 2010 to August, 2011. Gastrointestinal tract, liver, heart, lungs, and kidneys were analyzed.. A total of 996 *Contracaecum ogmorhini* specimens were recorded, especially in O. *flavescens'* stomach. The prevalence was 10.34%, and mean intensity of infection and mean abundance were 332 and 34.34 parasites, respectively. The nematodes found had juvenile and adult forms. There was no difference in mean abundance between sub-adult and adults. Liver, gall bladder, heart, lungs, and kidneys examined showed no macroscopic parasites. With regard to nematode parasitic fauna of *O. flavescens*, our data are different from those cited for specimens from Pacific Coast of South America. This is the first record of *C. ogmorhini* parasitizing *O. flavescens* specimens in Brazilian waters.

Key words: nematodes; Southern American sea lion; helminthes; parasite.

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Introduction

The pinnipeds are aquatic mammals adapted to aquatic and terrestrial life. The southern sea lion, *Otaria flavescens* (Shaw,1800), is a marine species that usually lives at depths less than 50m (Vaz- Ferreira, 1981), and is widely distributed from northern Peru on the Pacific coast of South America to the south of Brazil on the Atlantic coast of this continent (Pinedo et al. 1992). The two high concentrations of the species on the Brazilian coastline are in Rio Grande do Sul State: Refuge of Terrestrial Life in the East pier of Lagoa dos Patos in the municipality of São José do Norte, and Ecological Reserve of Ilha dos Lobos, in Torres (Rosas et al. 1994; Ibama, 2001). In South Atlantic, breeding colonies occur along Uruguay, Argentina, and Malvinas Islands coasts (Pinedo et al. 1992).

Generally, the parasitological study in pinnipeds has been developed more often in northern hemisphere, mainly in seals because of its greater abundance (Lehnert et al. 2007). In South America, the studies on this topic in otariids are conducted in animals inhabitanting Atlantic and Pacific coasts, and also in some islands (Dailey et al. 2005), with the occurrence of a few gastrointestinal and lung nematodes species (Morgades et al. 2006; Dailey, 2009), some of them identified only at genus status (Andrade et al. 1999). In *O. flavescens*, the study of these parasites was mainly developed on the Pacific coast. The presence of nematodes *Phocanema decipien*, *Anisakis* sp. e *Pseudoterranova cattani* is known in Peru and Chile (Tantaleán et al. 2005; Cattan et al. 1996, 1980; George-Nascimento & Urrutia, 2000; Carvajal et al. 1983.). The reports are more scarce and incomplete on Atlantic coast. In Brazil Andrade et al. (1999) cited the presence of only nematodes *Contracaecum* sp., and Morgades et al. (2006) reported the presence of *Uncinaria* sp and *Contracaecum* sp. in Uruguay. In Argentina, Béron-Vera et al. (2004) identified the presence of *Uncinaria hamiltoni* in juvenile specimens captured in Punta León, Chubut Province. Phylum Nematoda show great diversity, infect organisms from different *taxa*, and are known as causes of zoonoses (Cabrera and Trillo-Altamirano, 2004). Two anisakids species are very common in marine mammals: *Pseudoterranova decipiens* e *Anisakis simplex* (Bush et al. 2001). Nematodes anisakids present a complex life cycle that includes invertebrate and vertebrate hosts of different trophic levels. They can be indicators of food chain integrity, and of general biodiversity of marine ecosystems (Mattiucci and Nascetti, 2007).

The study of the parasitic fauna allows to increase knowledge of pinnipeds biological evolution, providing information about its origin, and geographic distribution, and the cause of their deaths (Sepúlveda, 1998). This study has the aim of recording and characterizing the nematodes species found in *O. flavescens* on southern coastline of Rio Grande do Sul state, Brazil, and thus to complete information gap about parasites in this distribution area for this species.

Material and Methods

From June , 2010 to September, 2011, 29 necropsies were performed on specimens of *O. flavescens* founded dead along the coastline of Rio Grande do Sul State, Brazil, in the area between Lagoa do Peixe (31°21'38''S) and Arroio do Chuí (33°44'35"S). The carcasses were necropsied and classified according to specific protocol (IBAMA, 2005). The animals collected were in the stages 2 and 3 of decomposition, according to Geraci and Lounsbury (2005) criterion. Identification of species, sex, and biometrics was according to Pinedo et al. (1992). The hosts were classified into age groups according Rosa et al. (1994) and Silva (2004). Animals reaching total length less than 1.5m were considered as juvenile, sub-adults between 1.5 and 2.1m, and adults those in length longer than 2.1m. The thoracic and abdominal cavities were opened for the removal of organs. Lungs and airways, heart and segments of the great vessels of the heart base, kidneys, and full gastrointestinal tract (including liver) were collected from 24 specimens. Additionally, other five stomach. The organs were placed into plastic bags, identified, numbered, and frozen for further analysis. The organs were opened, washed on a sieve, and inspected internally. The retained material was placed in Petri dishes, and observed in optical stereomicroscope (10-40x) to collect nematodes that were washed with distilled water, counted, and preserved in ethyl alcohol 70GL (Dailey, 1978). Clarification was achieved with beechwood creosote. Identification was performed by evaluation of the morphological structures and morphometry of specimens on temporary slides. Quantitative descriptors such as prevalence, mean abundance, and mean intensity of infection were calculated according to Bush et al. (1997). Comparison of mean abundance between age classes was performed using the Wilcoxon test at 0.05 significant level and Action® software version 1.1.

Results

Twenty-nine host specimens were collected: 23 males, 3 females, and 3 individuals whose sex could not be determined. The average total length of the animals was 2.14±0.31m (1.58 and 2.64m), 13 sub-adults and 16 adults. The stomachs had not content, but in ten animals, all free from helminthes in the stomach, gastroliths were found.

In the 24 sea lions completely analyzed, helminthes were not found in lungs and airways, heart and segments of the great vessels of the heart base, kidneys, and liver. Only one nematode species, *Contracaecum ogmorhini* (Nematoda: Anisakidae), totalizing 996 specimens, was found in the gastrointestinal tract. The morphology of the specimens was consisted with the one mentioned by Fagerholm and Gibson (1986), with the presence of three large lips interspersed with three inter-labia (Figure 1A), cuticle transversely striated, digestive tract consisted of a long esophagus with ventricular appendix, and simple intestine with ascending cecum (Figure 1B and C), cervical papillae rounded, males with conical tail with pre- and post-cloacal papillae, and spicules with similar length (Figure 1D).

From the 29 sea lions were studied, only three contained this nematode whose prevalence was of 10.34%, intensity of infection was 332, and mean abundance 34.34 parasites. There was no significant difference between mean abundance in the infection of sub-adults animals (50.23±181.11) and adult animals (21.44±76.21). Most species were found in the stomach (941 nematodes), but other organs from animals which had the stomach already infected presented *C. ogmorhini*, including a small intestine (2 nematodes) and two esophagus (53 nematodes). *Contracaecum ogmorhini* specimens presented themselves in young and adult forms, including females with egg presence (Figure 2).

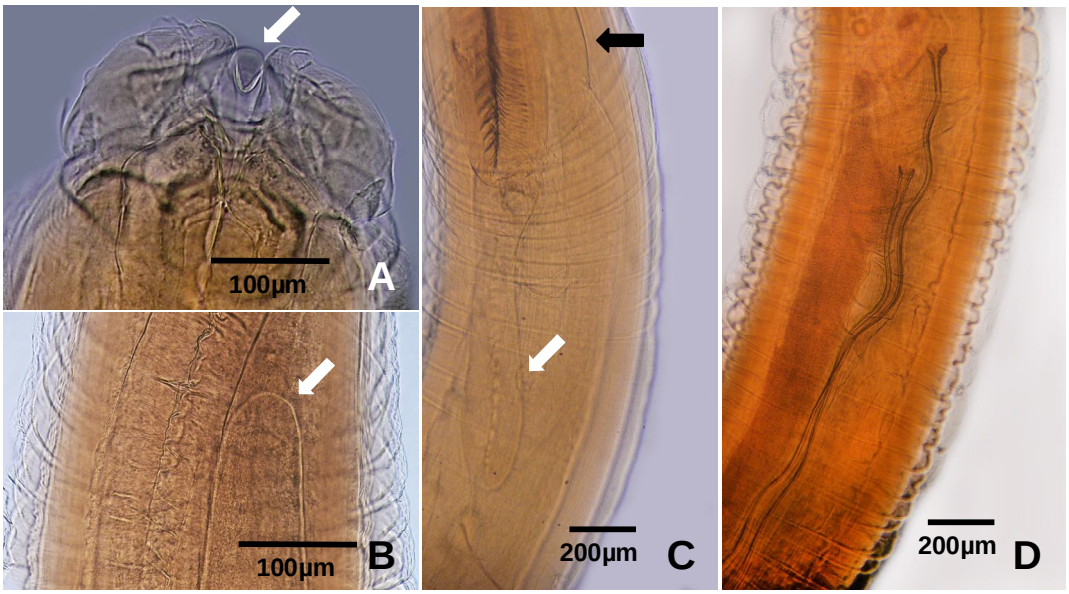

Figure 1. *Contracaecum ogmorhini* from *Otaria flavescens*. A, Anterior end with 3 great lips and interlabia (arrow). B, Anterior end of the ascendent intestinal cecum (arrow). C, Ventricular appendix (write arrow) and intestinal cecum (black arrow). D, Spicules.

Figure 2. Eggs in *Contracaecum ogmorhini* collected from *Otaria flavescens*.

Discussion

The presence of gastroliths in stomachs of southern sea lions is well known (Hamilton, 1934; Vaz-Ferreira, 1981; Drehmer e Oliveira, 2003). Some hypotheses have been discussed about the reason of gastroliths presence in some animals. Among them is the one related to natural control of parasites in which it is believed that the gastrolith could crush the helminth adhered to gastric mucosa, relieving the burning sensation in stomach produced by parasitic spoliation. In this study as well as in the Drehmer and Oliveira's (2003) none of sea lions which contained gastroliths had gastric helminthes. Since the prevalence of *C. ogmorhini* was low even in animals which didn't contain gastroliths, one can't ensure that there is an association between their presence and parasitic levels, which corroborates Koen Alonso et al. (2000). The currently most accepted hypothesis is that the stones could influence the buoyancy of the animals, allowing a better control during the dives to capture prey (Taylor, 1993).

Contracaecum sp. infections have been reported in a great diversity of organisms including fish and mammals (Martins et al. 2003). Due to economic, political, and pathological disruption, knowledge of anisakids infection, including those in *Otaria flavescens*, is important for human beings (Cabrera and Trillo-Altamirano, 2004). Life cycles of these nematodes are indirect, they include marine crustaceans as intermediate hosts, and very often a variety of fish and cephalopods as paratenic hosts (Bush et al. 2001). Human species is not normal host for any of these parasites, but by eating raw or undercooked fish, human being partakes of the food chain of the marine ecosystem, which usually involves organisms such as fish, crustaceans, cephalopods, and marine mammals. When entering the top of the food chain, humans assume the position of marine mammals, and may become infected by consuming the flesh of a fish with infective larvae (Bush et al. 2001). Even in the lake environment, fish species host *Contracaecum* sp. larvae. La Rue et al. (2010), in a recent study conducted in the reservoir Costa e Silva which captures water supply for the municipality of Santa Maria, also in South Brazil, collected 53 'Jundiás', *Rhamdia quelen* (Pisces:Pimelodidae), and one of them had larvae of *Contracaecum* sp. In 50 specimens of 'traíras' *Hoplias malabaricus*, (Pisces, Characidae) from fishmongers in the municipality of Pelotas, south Brazil, Rodrigues et al. (2010) reported parasitism by larvae of *Contracaecum* sp. in 32% of fish examined.

In marine mammals infections by species of *Contracaecum* are more frequent in pinnipeds than in cetaceans, in which the presence of nematode of genus *Anisakis* is common (Andrade et al. 1999), however Luque et al. (2010) reported the presence of *Contracaecum* sp. in the stomach of estuarine dolphin, *Sotalia guianensis,* collected on southern coast of Espírito Santo State. *Contracaecum ogmorhini* seems to be an anisakid with specificity for otarids, since it has already been reported its infection in stomachs of Cape fur seals, *Arctocephalus pusillus pusillus,* collected on the southern coast of Africa (Stewardson e Fourie, 1998). On the other hand, the presence of lung nematode *Parafilaroides normani* (Nematoda: Filaroididae), existing in some species of sea lions *Arctocephalus pusillus doriferus*, *Arctocephalus forsteri* e *Arctocephalus tropicalis* (Dailey, 2009) was not verified in this study. The absence of *P. normani* in the animals studied, could be related to differences in diet and geographic area. According to Dailey (1970) the experimental infection in California sea lions (*Zalophus californianus*) occurred using shallow waters fish, *Girella nigricans*, as an intermediate host. The only report of lung nematodes in *O. flavescens* known is in the north coast of Rio Grande do Sul State, Brazil (Marigo, 2003). The author relates a young male of *O. flavescens* that died because parasitic fibrinous pneumonia showing moderate to severe, associated with extensive areas of edema, hemorrhage, and moderate congestion caused by lung parasites which were seen only in histological preparations. Histological analyses of the lung tissues were not performed in the present study and could be suggested in the future researches.

In regard to nematode parasitic fauna of *Otaria flavescens*, our data are different from those previously mentioned for specimens from Pacific coast of South America, which were recorded on Chilean coastline, *Phocanema decipien* and *Anisakis* sp. (Cattan *et al*. 1976, 1980), and *Pseudoterranova cattani* (George-Nascimento and Urrutia, 2000). In the Atlantic, infections by the nematode *Uncinaria hamiltoni* in *Otaria flavescens* from the Northern Province of Patagonia, Argentina, are known (Béron- Vera, 2004). In the Uruguayan coast, there are also records of *Uncinaria* sp. (Nematoda: Ancylostomatidae) and *Contracaecum* sp. (Nematoda: Anisakidae) in the small intestine and stomach, respectively (Morgades et al. 2006). Interestingly, the nematode *Uncinaria* sp. was not found in the animals from this study which could be related to age of the animals sampled, that included only sub-adults and adults. In Argentina, the specimens studied by Béron-Vera et al. (2004) were 31 young infants which showed a 50% prevalence of this parasite.

In Uruguay, Morgades et al. (2006) analyzed two pups, five sub-adults, and seven adults, of which only one of two pups were parasitized by *Uncinaria* sp. In the same study, nematodes belonging to Anisakidae, including *Contracaecum* sp., were found in four of five sub-adults, and in four of seven adults sea lions. *Uncinaria* sp. was also registered in Falkland Islands (Baylis, 1932), Uruguay (Botto e Mañe-Gazón, 1975), and Chile (George-Nascimento et al. 1992). According to Lyons et al. (2001) they are helminthes frequently found in the intestines of very young sea lions, and rarely in seals pups (George-Nascimento et al. 1992). Similarly to Ancylostomatidae congeneric, e.g., *Ancylostoma caninum*, it is believed that the infection of pup sea lions by *Uncinaria* sp. may occur during the prenatal period when the larvae reach the fetus by the bloodstream of the mother, or by ingestion of colostrums. Adult dogs are rarely infected due to the development of specific immunity at this helminth (Pellon, 1953; Fortes, 2004).

The parasitic fauna of O. *flavescens* was little known until now on the southern coast of Brazil with only data described by Andrade et al. 1999, mentioning the presence of *Contracaecum* sp. This is the first record of *Contracaecum ogmorhini* parasitizing *Otaria flavescens* in Brazilian coast, which agrees with the data presented by Fagerholm and Gibson (1986) who mention the presence of this parasite in *Arctocephalus pusillus* (South Africa fur seal) from southern coast of Africa.

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Artigo 3

Southern Sea Lion (*Otaria flavescens*) infection by acanthocephalans and cestodes on southern coast of Rio Grande do Sul State, Brazil

Revista: Diseases of Aquatic Organisms.

Normas da revista: Anexo 3

Otaria flavescens infection by acanthocephalans and cestodes on southern coast of Brazil

Running head: Acanthocephalans and cestodes from Southern Sea Lion

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Abstract: The parasitological study of marine mammals, especially of *Otaria flavescens* in Brazilian waters is scarce. The objective of this study was to identify the assemblage of cestodes and acanthocephalans in *O. flavescens* on southern coast of Brazil. Twenty-four specimens found dead on the coastline of Rio Grande do Sul, along of 364 km of Atlantic coast (31°21'38''S-33°44'35"S), were necropsied . Gastrointestinal tract, liver including gall bladder, lungs and airways, heart, and kidneys were collected and analyzed. Only the gastrointestinal tract had parasites, where 42,145 specimens of *Corynosoma australe* and 512 of *Bolbosoma turbinella* were found especially in the first two-thirds of small intestine. The cestode *Diphyllobothrium* sp. was found in the intestine of one animal. The mean density of helminthes on the surface of intestinal mucosa was of 0.06±0.08 parasites/cm^2 for *C. australe* and 0.001±0.003 parasites/cm^2 for *B. turbinella*. *Bolbosoma turbinella* showed prevalence of 50%, mean intensity of 42.66, and mean abundance of 21.33 parasites. *Corynosoma australe* infected 100% of the sea lions with mean intensity

and mean abundance equal to 1,756.04 parasites. All specimens of *B. turbinella* presented immature sexual form. Despite the apparent difference between the mean intensity of sub-adults (2,752±5,395. parasites) and adults (981±796 parasites) of *C. australe*, it was not statistically significant. No significant correlation was observed between infection intensity, sex, total sea lion length, or length of the intestines. This is the first record of *Diphyllobothrium* sp. and *Bolbosoma turbinella* in *O. flavescens* in Brazilian coast.

Key-words: *Otaria flavescens*, helminthes, parasites, South American sea lion

Introduction

Acanthocephalans and cestodes are helminthes parasites common in the marine environment (George-Nascimento, 1987). Generally, pinnipeds and cetaceans, for being at the top of the food chain, are definitive hosts, mainly of Polymorphidae acanthocephalans (Aznar et al. 1994, Andrade et al. 1997). The cestodes, at the marine environment, may complete their cycles using fishes, especially cartilaginous, as definitive hosts (Ivanov, 2009; Caira et al, 2011). Larvae of cestodes have been found encysted in the abdominal subcutaneous adipose tissue from some cetacean species (Walker, 2001, Mazzariol et al., 2011).

In the southern hemisphere, the parasite fauna of marine mammals in the Pacific Ocean has been studied for a long time (Delyamure, 1957). However, in the Atlantic the interest in studying parasitology in pinnipeds has been more recent (Holcman-Spector et al. 1977, Berón-Vera et al. 2004, Morgades et al. 2006, Aznar et al. 2011), and information is scarce or incomplete, mainly for the Brazilian coast (Andrade et al. 1999).

Southern Sea Lion, *Otaria flavescens*, is a relatively large pinniped that lives in the Pacific and Atlantic coasts of South America, grouping mainly in rocky places. In the Atlantic, the area further north with clusters of this species is southern Brazil, extending meridionally to Tierra del Fuego, Argentina, and continuing north across the Pacific to Peru (Vaz- Ferreira, 1981; Rosas et al. 1994). Some helminthes are already known for infecting *Otaria flavescens* along South America coasts. In the Pacific, the nematodes *Phocanema decipiens*, *Anisakis* sp. and *Pseudoterranova cattani* (Cattan et al. 1976, 1980; George-Nascimento and Urrutia, 2000), and the tremadode *Ogmogaster heptalineatus* were registered in Chilean coastline, infecting the small intestine of pups (Carvajal et al. 1983). In Peruvian coast, Tantaleán et al. (2005) registered the presence of *Corynosoma obtuscens* (Acanthocephala: Polymorphidae) infecting the intestine. Most of these studies do not present parasitological infection levels, therefore the information on quantification of infections is lacking. In the Atlantic, on the Argentina coast infections by *Uncinaria hamiltoni* (Nematoda: Ancylostomatidae) are known in juvenile specimens captured in Punta León, Chubut Province (Béron-Vera et al. 2004), *Bolbosoma* sp. (Acanthocephala: Polymorphidae) (Holcman-Spector et al. 1977), and *Corynosoma australe* and *Corynosoma cetaceum* in Buenos Aires Province (Aznar et al. 2011). In Uruguay, in a more complete study, Morgades et al. (2006), studying sea lions of various age groups found *Uncinaria* sp. (Nematoda: Ancylostomatidae), *Stephanoprora* sp. (Trematoda: Echinostomatidae), *Diphyllobothrium* sp. (Cestoda: Diphyllobothriidae), *Ascocotyle (Phagicola) longa* (Trematoda: Heterohyidae), *Corynosoma australe* and *Corynosoma* sp. (Acanthocephala: Polymorphidae), *Contracaecum* sp., and anisakides do not identified. In Brazil, only Andrade et al. (1999) analyzed the gastrointestinal tract of sea lions found dead in the southern coast of Rio Grande do Sul State. These authors found only

Corynosoma australe (Acanthocephala: Polymorphidae) and *Contracaecum* sp. (Nematoda: Anisakidae). Given the large gap on the parasitic infections in pinnipeds on Brazilian coast, this study aims to identify the assemblage of cestodes and acanthocephalans presented in *Otaria flavescens* on the southern coast of Brazil.

Material and Methods

Twenty-four specimens of *Otaria flavescens* were sampled. The animals were found dead along the beaches of Rio Grande do Sul State during systematic fieldwork, covering an area of 364Km of Atlantic coast between Lagoa do Peixe (31°21'38''S) and Arroio do Chuí (33°44'35"S). The carcasses found were identified, sexed following Pinedo et al. (1992), analyzed, and classified according to their state of decomposition, following the topics indicated by Geraci and Lounsbury's Protocol (2005). Only the animals considered relatively fresh were sampled, stage 2 an3 so the Protocol. Biometry was performed according to Pinedo et al. (1992), and the animals were classified as young, sub-adults, and adults, using total length as a reference (Rosas et al. 1994). The sea lions were necropsied *in situ*: thoracic and abdominal cavities were opened, and the respiratory tract (trachea, bronchi and lungs), heart, segments of the great vessels of the heart base, kidneys, and gastrointestinal tract, including liver, were removed. After the removal the organs were placed into plastic bags, identified, numbered, and transported to Laboratory of Turtles and Marine Mammals of Department of Oceanography of Fundação Universidade do Rio Grande – FURG, where they were frozen at -20°C until their processing. The organs were defrosted in the Laboratory of Parasitology, Department of Microbiology and Parasitology, Biology Institute, Universidade Federal de Pelotas. The gastrointestinal tract was measured (length and width), and the small intestine was divided into three regions for a better

identification of the sites infection sites. Airways, trachea, and bronchi were opened longitudinally and washed through a sieve. The search for parasites was also made by inspection of the bronchial branches. The lung parenchyma was cut into sectors for observing the parasites or the cysts. The same procedure was performed with the liver, however the gall bladder and ducts were opened separately. The heart and large vessels such as aorta, vena cava and pulmonary arteries and veins, and kidneys were opened, washed through sieve, and inspected internally. Filtration of the content was made in sieve with mesh opening of 150 µm. The material retained on the mesh was washed in running water, put into Petri dish, and observed under optical stereomicroscope (10-40x) for collecting helminth. The mucosa was macroscopically inspected, and under stereomicroscope for collecting parasites adhered or encysted. All acanthocephalans found were counted.

The helminthes were washed with distilled water, fixed, and conserved. The fixation was performed in AFA, and conservation in ethyl alcohol at 70° GL. Worms were stained with Semichon's aceto-carmine, following Amato *et al.* Protocol (1991). Clarification was achieved with beechwood creosote, and the parasites were mounted on slides with Canada balsamo. Identification was performed using systematic keys and updated papers. Measurement of levels of infection included the parasitic prevalence rates, mean intensity of infection, and mean abundance (Bush et al., 1997). Additionally, the number of acanthocephalans was quantified by area of the intestinal mucosa analyzed. The Kolmorogov-Smirnov test was used to verify the type of data distribution. Comparison of mean abundance of infection between age groups was performed using the Wilcoxon test, using the significant level of 0.05. Comparison of mean abundances among different sites along the intestines was performed using ANOVA and Tukey Test with $p<0.05$.

Correlations between intensity of infection, sex, total length of the individual, and length of small and large intestine were verified using the Pearson correlation. For statistical analyses we used the Action ® software version 1.1.

Results

Sea lions total length varied between 1.58 and 2.64m (2.10±0.33m), 19 were males, four were females, and an individual whose sex could not be determined. Among these animals 13 were sub-adults, and 11 were adults. The total length of small intestine varied between 24 and 48.6m (32.12±1.81m). The large intestine varied between 1.15 and 2.75m (5.97± 0.49m).

All sea lions were infected with some species of helminth. Acantocephalans were presents in stomachs of five hosts and in all of intestines analyzed. Only an individual was infected by cestodes. Liver, gall bladder, heart, lungs, and kidneys had no macroscopic parasites.

Two species of acanthocephalans were collected: *Corynosoma australe* (Acanthocephala: Polymorphidae), (**Figure 1**) and *Bolbosoma turbinella* (Acanthocephala: Polymorphidae) (**Figure 2**), and only one of cestode *Diphyllobothrium* sp., present in the small intestine of an adult sea lion. Quantitative distribution of helminth by site of infection in the gastrointestinal tract is shown in the Table 1.

Table 1. Quantitative distribution of *Corynosoma australe* and *Bolbosoma turbinella* along the intestinal tract of 24 southern sea lions collected in southern Rio Grande do Sul State, Brazil. Total number of helminth found is followed by mean abundance and standard deviation (in parentheses).

Acanthocephalans	Site of infection				
	Stomach	SI 1/3	SI 2/3	SD 3/3	LI
C. australe	14	9,727	17,037	14,223	5,570
	(2,80±2,177)	(511,95±1055)	(1002,1±3400)	(711,15±910)	(253,18±295)
B. turbinella	0	261	236	46	1
		(10.88±35.78)	(9.83±30,13)	(1.92±5,44)	(0.04±0.20)

SI- small intestine, LI – large intestine

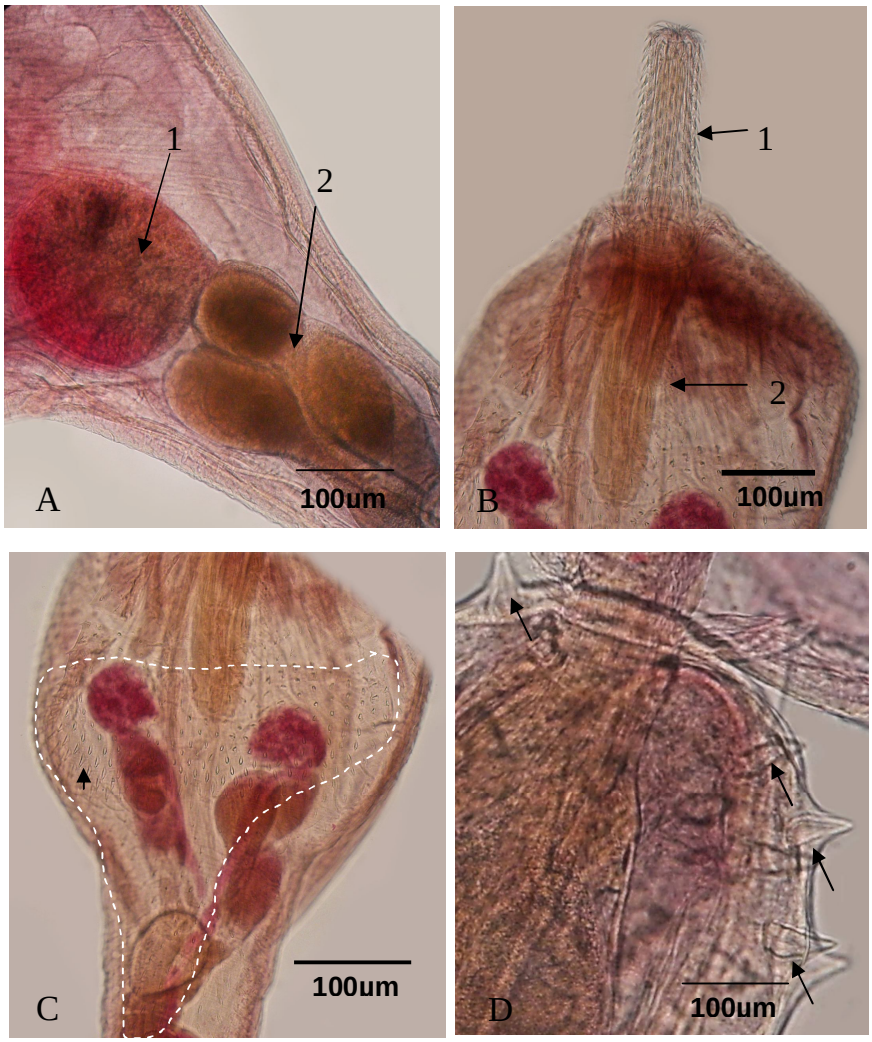

Figura 1: *Corynosoma australe* from *Otaria flavescens*. A ,Close of males genital structures, 1 testis; 2 cement glands; B, Anterior end. 1 Proboscis; 2 Proboscis pouch. C, Body of a male, cuticulares spines area is circunded, arrow indicates a single spine; D, Genital end of a male. Genital spines are indicated by arrows.

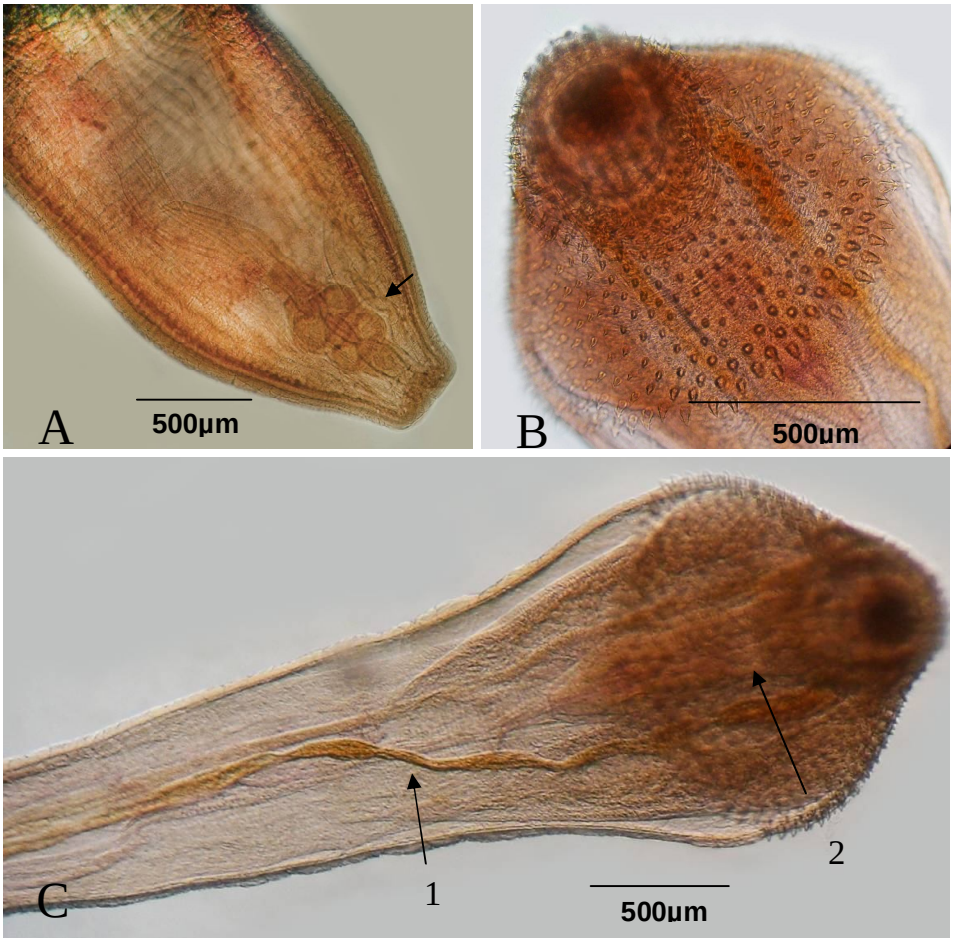

Figura 2. *Bolbosoma turbinella* from *O. flavescens*. A , Close of female genital structures (double sphincter); B, Anterior end bulb-like covered by cuticular spines. C, Anterior end. 1. long lemiscus, 2. proboscis pouch.

Regarding the organs studied, intestine showed the highest prevalence and mean abundance of infection for *C. australe* (Table 1). Significant difference in the mean intensity of infection among the first, second and last third was not present. The presence of 42,145 specimens of this acanthocephalan, and 512 of *Bolbosoma turbinella* were registered mainly in the first two thirds of small intestine. Mean density of helminth on intestinal mucosal surface was 0.06 ± 0.08 parasites/cm^2 for *C. australe,* and 0.001 ± 0.003 parasites/cm^2 for *B. turbinella*. *Bolbosoma turbinella* showed a prevalence of 50%, mean intensity of 42.66, and mean abundance of 21.33 parasites.

Corynosoma australe infected all host studied, prevalence of 100%, mean intensity and mean abundance equal to 1,756.04 parasites. Most of *C. australe* specimens presented adult form, the females showed high egg production, whereas all specimens of *B. turbinella* were sexually immature. Cestodes were found in only one host, prevalence of 4.16%, which showed four scoleces.

As usual, distribution of the parasites did not showed normality, and despite the apparent difference of mean intensity for *C. australe* between sub-adults (2,752.23 ± 5,395.52 parasites) and adults (981.09 ± 796.28 parasites), it was not statistically significant. No significant correlation was observed between intensity of infection, sex, sea lion total length or intestines length.

Discussion

Most helminthes that parasitize marine mammals use food chain for their transmission, using one or two intermediate hosts to achieve the complete sexual development in its definitive host (Bush et al., 2002). Therefore, the success of the parasitic cycle depends on the probability of contact between, prey and predator, so that they can lead infection to the next host, and the parasite may not play some kind of selection pressure. Two publications on the diet of *Otaria flavescens* on the southern coast of Rio Grande do Sul are avaiable (Pinedo and Barros, 1983; Falco et al., 2008). According these authors the diet of sea lion in the region of this study consists mainly of demersal fish Scianidae, represented by 'maria-luisa', *Paralonchurus brasiliensis*, 'pescadinha', *Macrodon ancylodon*, 'pescada', *Cynoscion striatus*, 'corvina', *Micropogonias furnieri*, and Trichiuridae, 'peixe-espada', *Trichiurus lepturus*. Cystacanths classified in the *Corynossoma* are common in marine osteichthyes, that act as intermediate hosts, transport,

or even paratenic, while the adult forms are found in birds and marine mammals (George-Nascimento and Carvajal, 1981). On the coast of Rio Grande do Sul State, larval stages of *Corynosoma* sp. and *C. australe* infect, respectively, 'corvina branca' *Micropogonias furnieri* and 'castanha', *Umbrina canosai* (Pereira Jr. and Neves, 1993, Anderson and Amato, 1998). In marine mammals from the same region of this study, immature specimens of *C. australe* have already been reported in *Pontoporia blainvillei* (Cetacea: Pontoporidae) (Andrade et al. 1997). In pinnipeds occurrence in the South American fur seal, *Arctocephalus australis*, is known (Andrade et al., 1999, Sardela et al. 2005; Aznar et al. 2004; Morgades et al. 2006).

Compared with previous data on helminth fauna of *O. flavescens*, the results of this study are partially different from those previously cited for sea lions from Pacific coast (Carvajal et al. 1983; Cattan et al. 1976) where among the acanthocephalans there is only the recording of *C. obtusens* (Tantaleán et al. 2005), but resemble when compared with the studies in the Atlantic particularly in a biogeographical area very close and similar like the Uruguay coast. In this region, Morgades et al. (2006) registered *Diphyllobothrium* sp., *Corynosoma australe* and another species of *Corynosoma* that was not identified. Studies on helminth expelled in fecal samples of sea lions in the Province of Buenos Aires, Argentina, indicated a mixed infection by *C. australe* and immature forms of *C. cetaceum* (Aznar et al. 2011). In the present study, approximately 400 specimens of *Corynosoma* spp. collected systematically from all sampled lions, were stained and analyzed. In this material were verified morphological characteristics that distinguish *C. australe* from *C. cetaceum*, as distribution of somatic spines, number of hooks per row and presence of genital spines (Sardella et al. 2005) (Figure 2). *C. australe* was found in animals from southern Brazil, but not *C. cetaceum*, as occurred in Argentina. This discrepancy could be

related to methodological differences or a possible difference in density of intermediate host between the two areas that are distant approximately 880 km. *Corynosoma cetaceum* is an acanthocephalan that is part of component community of a small cetacean known as La Plata River Dolphin, *Pontoporia blainvillei*, present in both regions (Raga et al. 1993.; Andrade et al. 1997). Mixed infections by *C. cetaceum* and *C. australe* are recorded in this cetacean, but lack of sexual development in *C. australe* (Andrade, 1997, Aznar et al. 2011), suggesting a high specificity both *C. australe* as *C. cetaceum* for their respective definitive host *Pontoporia blainvilei* and *Otaria flavescens*. So when comparing results obtained from fecal samples with those obtained with samples taken directly from the intestines, it is possible to obtain different information, since the rate of elimination in the feces of a non-specific helminth species to the host is very high. In contrast, it is possible that the intake of preys infected by *C. cetaceum* is more frequent on the coast of Argentina than in the southern Brazil. This hypothesis can be corroborated by differences in infection levels of this helminth in 'franciscanas', its definitive host. La Plata River dolphins collected in Necochea and Claramecó (Province of Buenos Aires, Argentina) (Aznar et al. 1994), are significantly higher infected than those sampled in southern Rio Grande do Sul State (Andrade et al. 1997), principle by which two ecological stocks of this host may be distinguished (Andrade et al. 2000). Among the cestodes that infect fish and humans, those belonging to *Diphyllobothrium* have the highest importance. In the Pacific coast of South America *Diphyllobothrium* spp. was observed in marine mammals and in human beings (Rausch et al. 2010). In this geographic region two species, *Diphyllobothrium pacificum* and *D. arctocephalinum*, use sea lions as final hosts. The taxonomic position of these cestodes is not clearly discernible due to mistakes in its relations, and complexity of synonymys (Rausch et al. 2010). In our study, the site of infection of *Diphyllobothrium* sp.

was the small intestine, different from the study carried out in Uruguay waters, where presence of cestodes occurred in the large intestines (Morgades et al. 2006). Even having already been registered in Atlantic coast waters, this study describes for the first time the presence of *Diphyllobothrium sp.* composing the parasite fauna of *Otaria flavescens,* belonging to southern coastline of Brazil.

Infection of other organs besides digestive tract is common in pinnipeds (Dierauf, 1990; Dailey, 2009). Although the adjustment of the methodology to determine these infections by non gastrointestinal helminths has been made, only the gastrointestinal tract was infected.

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4. Conclusões gerais

Em base as informações geradas nos três artigos apresentados neste documento, concluise:

I. Espécimes de *Otaria flavescens* (Shaw, 1800), subadultos e adultos que chegam à costa sul do Rio Grande do Sul são infectados por acantocéfalos, cestóides, nematódeos e trematódeos;

II. Macroscopicamente, constatou-se que apenas o trato digestório destes animais estava parasitado por helmintos;

III. *Corynosoma australe* (Acanthocephala: Polymorphidae) infecta este hospedeiro com alta prevalência e intensidade de infecção utilizando como sítios de infecção principalmente os intestinos delgado e grosso. Não se observou predileção por algum dos segmentos dos intestinos.

IV. Neste documento registra-se pela primeira vez a ocorrência do acantocéfalo *Bolbosoma turbinella* (Acanthocephala:Polymorphidae) do nematóide *Contracaecum ogmorhini* (Nematoda:Anisakidae), e do trematodeo *Stephanoprora uruguayense* (Digenea:Echinostomatidae) em *O. flavescens*;

V. Registra-se também pela primeira vez a presença dos helmintos *Bolbosoma turbinella*, *Diphyllobothrium* sp. (Cestoda:Diphyllobothriidae), *Contracaecum ogmorhini, Ascocotyle (Phagicola) longa* (Digenea: Heterophyidae) e *Stephanoprora uruguayense* em *O. flavescens* em águas brasileiras;

VI. As espécies de helmintos encontradas no presente estudo diferem daquelas encontradas em *Otaria flavescens* habitantes da costa pacífica do continente sul-americano. Somente em relação à presença de acantocéfalos do gênero *Corynosoma* ocorre similaridade, sendo conhecida a presença de *C. obstrucens* na costa do Peru.

VII. Observou-se uma similaridade de espécies de helmintos que infectam *O. flavescens* entre o litoral sul do Rio Grande do Sul, Brasil e o litoral uruguaio com sobreposição de pelo menos duas espécies, *Ascocotyle (Phagicola) longa e Corynosoma australe*, e três gêneros, *Contracaecum, Diphyllobothrium e Stephanoprora*.

VIII. Diferenças na presença ou ausência de espécies entre o presente estudo e estudos prévios podem estar relacionadas com diferentes metodologias aplicadas, presença ou ausências de fatores abióticos e bióticos (ex. hospedeiros intermediários) compatíveis com o ciclo de vida dos helmintos, assim como fatores co-evolutivos e de colonização do continente americano pelo hospedeiro em questão.