VNIVERSITAT DE VALÈNCIA

Facultat de Ciències Biològiques

INSTITUT CAVANILLES DE BIODIVERSITAT I BIOLOGIA EVOLUTIVA

Taxonomy and ecology of metazoan parasites of otariids from Patagonia, Argentina: adult and infective stages

TESIS DOCTORAL POR:

Jesús Servando Hernández Orts

Codirectores
Francisco Javier Aznar Avendaño
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Valencia, mayo 2013

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PROGRAMA DE DOCTORADO 119 A

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FRANCISCO JAVIER AZNAR AVENDAÑO, Profesor Titular de la Facultad de Ciencias Biológicas de la Universitat de València,

FRANCISCO ESTEBAN MONTERO ROYO, Profesor Contratado Doctor de la Facultad de Ciencias Biológicas de la Universitat de València, y

ENRIQUE ALBERTO CRESPO, Investigador Principal del CONICET y Profesor Titular de Ecología de la Universidad Nacional de la Patagonia, República Argentina.

CERTIFICAN: que Jesús Servando Hernández Orts ha realizado bajo nuestra dirección, y con el mayor aprovechamiento, el trabajo de investigación recogido en esta memoria, y que lleva por título: 'Taxonomy and ecology of metazoan parasites of otariids from Patagonia, Argentina: adult and infective stages', para optar al grado de Doctor en Ciencias Biológicas.

Y para que así conste, en cumplimiento de la legislación vigente, expedimos el presente certificado en Paterna, a 31 de mayo de 2013

Francisco Javier Aznar Avendaño　　　　Francisco Esteban Montero Royo

Enrique Alberto Crespo

A MI OSO PARDO

Foto principal de portada: Laboratorio de Mamíferos Marinos, Centro Nacional Patagónico, CONICET

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SUMMARY

SUMMARY

At present, the metazoan parasite fauna of most species of otariids is generally poorly known, in part because these marine mammals are mostly protected and, therefore, sampling is limited to specimens stranded on the coast or captured as by-catch in fisheries. Similar problems also occur for the larval stages of gastrointestinal helminths of otariids. For most of these parasite species, the specific identity of the intermediate/paratenic of hosts is unknown and, therefore, many stages of their life cycles remain to be described. Similarly, little is known about the routes of transmission of these parasites between intermediate/paratenic hosts to their otariids definitive hosts.

The present thesis is committed to improving the knowledge on these aspects, characterizing for the first time, the intestinal helminth fauna of 56 South American sea lions *Otaria flavescens* (Shaw, 1800), and 5 South American fur seals *Arctocephalus australis* (Zimmerman, 1783), from the Patagonian coast of Argentina. Additionally, a total of 542 specimens of 20 marine fish species collected in the same locality, were analysed for helminths, identifying and quantifying the larval forms of parasite infecting otariids. The large dataset obtained provided the opportunity to describe the larval forms and to assess pathways of transmission of these parasites between intermediate/paratenic fish hosts and their definitive otariid hosts. Finally, the large number of larval specimens from several fish species collected in the course of this study allowed us to know essential aspects to understand the population dynamics of these parasites, as the effects of the different host species on some life history traits of the larvae, such as growth patterns or sex ratio, or the potential role of the host in the transmission of the parasite.

This study targeted the following objectives:

1. To quantify and describe the intestinal metazoan parasite communities of *O. flavescens* and *A. australis* off northern Patagonia, Argentina, based on a detailed morphological and taxonomical study. This information is used to ascertain the role of parasite host specificity in shaping helminth community diversity in otariids.

2. To characterize the component populations of cystacanths of *Corynosoma australe* (Acanthocephala: Polymorphidae) in paratenic fish hosts off the Patagonian coast of Argentina. The main goal is to elucidate the pathways of transmission of this species between paratenic hosts and definitive otariid hosts, and to assess the effect of

different fish hosts on growth, body size, fitness and sex ratio of the cystacanths of *C. australe.*

3. To describe, for the first time, the temporal allocation of investment on holdfast structures (trunk spines) between cystacanths and adults of two congeneric species of acanthocephalans (*Corynosoma cetaceum* and *C. australe*), and investigating the factors that may account for the patterns of trunk spine growth.

4. To carry out a taxonomic identification and description of third-stage larvae of species of *Pseudoterranova* (Nematoda: Ascaridoidea) from various fish species of Patagonia using sequence data for the mitochondrial cytochrome *c* oxidase subunit 1 (cox 1) gene and a detailed morphological study. This evidence is then used to describe the component populations of third-stage larvae of species of *Pseudoterranova* in fishes, assessing the role of different fish hosts on the microhabitat selection, transmission strategies and infection parameters of the third-stage larvae.

A total of 97,325 helminth specimens were collected from *O. flavescens* from the Patagonian coast in Argentina. The intestinal helminth fauna of sea lions in this locality comprised 11 taxa (1 trematode, 1 cestode, 5 nematodes and 4 acanthocephalans). Gravid individuals were represented by 6 species: *Ascocotyle* (*Ascocotyle*) *patagoniensis*, *Contracaecum ogmorhini* (*s.s.*), *Corynosoma australe*, *Diphyllobothrium* spp., *Pseudoterranova cattani* and *Uncinaria hamiltoni*. Third-stage larvae of *Anisakis* sp. type I and *Contracaecum* sp., and juvenile specimens of *Andracantha* sp., *Corynosoma cetaceum* and *Profilicollis chasmagnathi* were also collected. Four of these parasites species, *Andracantha* sp., *A.* (*A.*) *patagoniensis*, *C. ogmorhini* (*s.s.*) and *P. chasmagnathi* represent new host records. A total of 1,516 helminth specimens were collected from the intestine of *A. australis*. The intestinal helminth fauna of fur seals comprises 7 parasite taxa (2 cestodes, 3 nematodes and 2 acanthocephalans). Gravid individuals were represented by 4 species of parasites: *C. ogmorhini* (*s.s.*), *C. australe*, *Diphyllobothrium* spp., and *P. cattani*. Third-stage larvae of *Contracaecum* sp. and juvenile specimens of *C. cetaceum* were also collected. *Corynosoma australe* was the most prevalent and abundant parasite in both hosts, accounting for >90% of all specimens.

SUMMARY

In northern Patagonia, sea lions and fur seals harbour the intestinal helminth communities that could be predicted for otariids worldwide, *i.e.* the combination of species of the genera *Corynosoma*, *Diphyllobothrium*, *Pseudoterranova*, *Contracaecum* and, in pups, *Uncinaria*. The estimation of helminth community parameters in sea lions and fur seals, especially species richness at component community level, was affected by the inclusion or exclusion of parasites for which both species of otariids are putative non-hosts (*i.e.* hosts in which the parasite is unable to reproduce). This study demonstrates that the inclusion of these taxa can exert a significant influence on some community parameters. Information about the reproductive status of helminth species is often lacking in parasitological surveys on otariids and other marine vertebrates, but it is of significance to improve precision in parascript studies or ecological meta-analyses.

A new species of a heterophyid trematode was described from the intestine of South American sea lions. A detailed morphological and morphometrical analysis of specimens of *Ascocotyle* (*Ascocotyle*) *patagoniensis* Hernández-Orts, Montero, Crespo, García, Raga and Aznar, 2012 suggests that this trematode can be distinguished from the other species of the subgenus by the number of circumoral spines, which are arranged in 2 rows of 18 to 23, by having a gonotyl without papillae, and by their widest seminal receptacle. Species of the subgenus *Ascocotyle* usually infect fish-eating birds or mammals in freshwater or brackish habitats. *Ascocotyle* (*A.*) *patagoniensis* is the first species of the subgenus described from a marine mammal. However, no metacercariae of *Ascocotyle* spp. were found in 542 marine teleosts from 20 species collected along the Patagonian Shelf. The absence of metacercariae in marine fish inhabiting this area could be related to the fact that the life cycle of this trematode is restricted to littoral waters. Nevertheless, more fishes should be analysed to confirm this hypothesis as the small metacercariae could have been overlooked, mainly in host species with small sample sizes.

A total of 1,367 cystacanths of *C. australe* was collected in 18 species of marine fish from the Patagonian coast. The most infected fish species with n ≥ 15 were as follows: *Acanthistius patachonicus*, *Paralichthys isosceles*, *Prionotus nudigula*, *Raneya brasiliensis* and *Xystreurys rasile*. Eight fish species, *i.e. A. patachonicus*, *Brama brama*, *Congiopodus peruvianus*, *Cottoperca gobio*, *Genypterus blacodes*, *Patagonotothen ramsayi*, *Seriolella porosa* and *Stromateus brasiliensis* represent new host records for *C. australe*. Results of this study demonstrate that cystacanths of *C.*

australe are able to infect and colonize a wide array of fish species, which would act as paratenic hosts. The ubiquity of this acanthocephalan through the trophic web would guarantee infections to their definitive hosts through alternative pathways. Nevertheless, this study suggest that *R. brasiliensis,* is one of the prey that most likely contributes to the transmission of cystacanths of *C. australe* in this area, due to both the high prevalence in this fish species, and its high relative importance in the diet of sea lions and fur seals.

There were significant differences in the levels of infection of cystacanths of *C. australe* between fish inhabiting different zones of the water column, being the ones associated with benthic zone those with highest cystacanth infections. This study suggests that at least 2 main factors could be directly promoting differences in the infection levels of *C. australe* between fish from different zones: *1*) distribution of the invertebrate intermediate hosts; and *2*) patterns of transmission of cystacanths between paratenic fish hosts through food webs.

The overall sex ratio of cystacanths of *C. australe* infecting fish hosts was slightly, but significantly, female-biased and no significant differences were found among fish species. This suggests that the sex ratio would begin to be biased before individuals of *C. australe* infect the definitive host, in which the sex ratio is known to become strongly female-biased because females have a longer life span. In other words, part of the biased sex ratio that we observe in the definitive hosts would be already transferred from paratenic hosts. In theory, 3 factors could be involved in generating the sex ratio biases in our sample, namely, sampling error, differential sampling of female and male larvae, and/or differential mortality between the sexes.

This study analyses, for the first time, the potential costs that trophically-transmitted helminths may face in paratenic-to-paratenic transmission. The results suggest that some fish species, in particular *Acanthistius patachonicus,* might actually be unsuitable paratenic hosts for *C. australe* since most cystacanths found in this species were not viable. Also, a slight, but statistically significant, tendency to decrease body size of cystacanths was observed as the trophic level of fish species increased. This tendency, which was not related to crowding effects, appears to suggest that *C. australe* may incur in non-negligible energetic costs when experiencing putative paratenic-to-paratenic transmission. The implications of this finding cannot be underestimated, since this negative consequence may have an important role on the population dynamics of trophically-transmitted helminths.

Acanthocephalans have evolved a hooked proboscis and some taxa have trunk spines to attach to their definitive hosts. These structures are generated before being used, thus a key question is how investment in attachment could optimally be allocated through the ontogeny. The number and arrangement of hooks and spines are never modified in the definitive host, but it is unclear whether these structures grow during adult development. The present study compared, for the first time using inferential statistics, the size of holdfast structures between cystacanths and adults of acanthocephalans. The results suggest that the size of trunk spines grows between cystacanths and adults of *C. australe* and an allied species infecting cetaceans, *C. cetaceum*, but only in females, which also had significantly larger spines than males. However, this sexual dimorphism did not result from pure allometry since the body of females was smaller, and did not grow more than that of males. Nevertheless, females have longer lifespan, and therefore this factor would induce different investment and development schedules for spines, in order to withstand the extreme flow conditions prevailing in marine mammals for longer time.

Unexpectedly, the patterns of spine growth appear also to differ between both species of *Corynosoma*. In *C. cetaceum* fore-trunk spines and hind-trunk spines grew, whereas in *C. australe* only fore-trunk spines differed between cystacanths and adults. An explanation of these differences is that females of *C. cetaceum* fine-tune the size of spines during the development in the definitive hosts because they achieve a larger adult size, a trait that correlates with stronger dislodging forces and, possibly, with a longer lifespan. This study sheds light on the question of whether or not the holdfast of acanthocephalans is fully developed prior to entering the definitive host. It suggests that temporal allocation of investment in attachment structures may differ, not only between congeneric species, but also between sexes of the same species, possibly due to the different selective pressures that each population subset faces.

A total of 635 encapsulated third-stage larvae of *Pseudoterranova* (sealworm larvae) were collected from 12 species of marine fish from the Patagonian coast. The most infected fish species with sealworm larvae was *P. nudigula*, followed by *A. patachonicus*, *P. isosceles*, *Percophis brasiliensis* and *Pseudopercis semifasciata*. Five

species of fish, *i.e. C. gobio*, *Nemadactylus bergi*, *M. argentinae*, *P. brasiliensis* and *P. nudigula* represent new host records for larval sealworms.

Sequences obtained for the mitochondrial cytochrome c oxidase subunit 1 gene (cox 1) of sealworms from the red searobin, *P. nudigula*, formed a reciprocally monophyletic lineage with published sequences of *P. cattani* from definitive hosts. A detailed morphological and morphometrical description of larvae of *P. cattani* from the red searobin is provided. On the other hand, sealworm larvae from other fish species did not differ morphologically from L3 of *P. cattani* from the red searobin. However, the results of the comparative morphometric analyses carried out on larvae from different fish hosts indicated significant differences in some distances. However, we provisionally identified all larvae as *P*. cf. *cattani*, awaiting further identification based on molecular genetic markers.

The results of this study suggest that the main microhabitat for sealworm larvae infecting fish hosts from Patagonia is the muscle (principally the epaxial musculature, followed by the hypaxial muscles), and to a lesser degree, in the mesenteries and liver. The lines of evidence obtained in this study suggest that most important fish prey of otariids inhabiting the Patagonian coast presented low infection levels of sealworm larvae. Given that *P. cattani* is specific to otariids, transmission of this nematode appears to rely on the catholic diet of both sea lions and fur seals, which include a number of specimens of many fish species from the benthic realm, where transmission most likely occurs.

RESUMEN

1. Introducción general

1.1. La familia Otariidae

Los otáridos pertenecen a la superfamilia Pinnipedia, dentro del orden Carnivora. Son mamíferos marinos que habitan y se alimentan en un amplio rango de hábitats marinos, tanto en regiones subpolares como tropicales de ambos hemisferios. Tradicionalmente la familia Otariidae se subdivide en Otariinae (comúnmente llamados lobos marinos de un pelo), y Arctocephalinae (comúnmente llamados lobos marinos peleteros o de dos pelos) (Brunner, 2003; Rice, 1998). Los lobos marinos se caracterizan por poseer un pelaje relativamente escaso, mientras que los lobos marinos peleteros presentan un pelaje mucho más espeso (Berta *et al.*, 2006). En la actualidad la familia comprende 15 especies vivas y 1 especie extinta (**Tabla 1**).

Los otáridos se distinguen principalmente de otros pinnípedos por la presencia de oídos externos y por su habilidad de rotar sus aletas anteriores, lo cual les permite caminar, correr o escalar en tierra firme. Además, todas las especies de otáridos son polígamas y sexualmente dimórficas, siendo los machos normalmente más grandes que las hembras. Una característica importante de todas las especies de otáridos, que las diferencian de otras especies de pinnípedos, es que las hembras continúan alimentándose mientras están lactando. Durante la lactancia, las hembras realizan visitas breves y regulares a la costa para alimentar a sus cachorros.

Los otáridos son nadadores vigorosos, que se impulsan en el agua mediante sus aletas anteriores (Berta *et al.*, 2006). Son considerados buceadores muy eficientes, llegando algunas especies a alcanzar profundidades superiores a los 400 m y/o permanecer en apnea durante aproximadamente 15 minutos (Costa *et al.*, 2004; Schreer & Kovacs, 1997). Dada su gran movilidad en el agua, los otáridos son capaces de alimentarse de una gran variedad de presas, por lo que se les ha considerado depredadores bastante generalistas de peces y cefalópodos principalmente. Sin embargo, su comportamiento durante el forrajeo y su hábitos alimenticios están muy influenciados por la abundancia de las distintas presas y/o por características oceanográficas.

Desde finales del siglo XVIII hasta mediados del siglo XX, la mayoría de las especies de otáridos fueron explotadas en todo el mundo principalmente por su piel. A mediados del siglo XX, se decretaron nuevas leyes de protección que prohibieron la caza de la mayoría de las especies. Sin embargo, otras amenazas antropomórficas, tales como contaminación, caza ilegal, capturas accidentales o la competencia por los recursos con pesquerías, han reducido considerablemente las poblaciones de algunas especies (p. ej. Plagányi & Buterworth, 2009; Reijnders *et al.*, 2009). En la actualidad, la mayoría de las especies de otáridos están asignadas como "Especie bajo preocupación menor" en la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza (IUCN, 2012) (**Tabla 1**).

1.2. Otáridos de la costa atlántica de Argentina

En la actualidad, las dos especies de otáridos más abundantes en la costas argentinas, con un gran número de apostaderos y de colonias reproductivas, son el lobo marino de un pelo *Otaria flavescens* (Shaw, 1800) y el lobo marino de dos pelos *Arctocephalus australis* (Zimmerman, 1783) (Crespo *et al.*, 2008a, 2008d).

El lobo marino de un pelo *O. flavescens* es el único miembro del género *Otaria*. Esta especie presenta una amplia distribución, desde Recife das Torres en Brasil en el Atlántico hasta Zorritos al norte de Perú sobre el Pacífico (Crespo, 1988; Vaz-Ferreira, 1982). En la actualidad, se han citado más de 120 colonias de esta especie a lo largo de la costa argentina (Dans *et al.*, 2004), con aproximadamente un total de unos 45000 individuo en la costa patagónica (Crespo *et al.*, 2012). El lobo marino de un pelo es una especie que presenta un gran dimorfismo sexual (**Fig. 1**), siendo los machos adultos mucho más grandes que las hembras.

En las costas sudamericanas, el lobo marino de un pelo se alimenta principalmente de peces o cefalópodos y en menor medida de crustáceos, gasterópodos, poliquetos, esponjas o tunicados (Hückstädt *et al.*, 2007; Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). Concretamente, en la costa patagónica argentina, su dieta incluye un amplio rango de especies de peces y cefalópodos, siendo las más consumidas (con un porcentaje en número mayor al 10%): la merluza argentina, *Merluccius hubbsi*; la raneya, *Raneya brasiliensis*; la anchoíta, *Engraulis anchoita*, y los calamares *Illex argentinus* y *Loligo gahi* (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011).

El lobo marino de dos pelos *A. australis* presenta una distribución geográfica que coincide en gran medida con la del lobo marino de un pelo (Túnez *et al.*, 2008b). En la costa patagónica argentina, se han citado al menos 17 colonias con aproximadamente un total de unos 20000 individuos (Crespo *et al.*, 1999). La mayor concentración de estos otáridos en Patagonia se encuentra en la Isla Rasa y en la Isla Escondida en la Provincia de Chubut (Crespo *et al.*, 2008a; Túnez *et al.*, 2008b). El lobo marino de dos pelos presenta también un gran dimorfismo sexual (**Fig. 2**), sin embargo, comparado con el lobo marino de un pelo este dimorfismo es considerablemente más pequeño.

El lobo marino de dos pelos se alimenta principalmente de peces, cefalópodos, crustáceos y gasterópodos a lo largo de la costa sudamericana (Jefferson *et al.*, 1993; Ponce de León & Pin, 2006). Actualmente los hábitos alimenticios de este otárido en la costa patagónica argentina son muy poco conocidos. Sin embargo, estudios isotópicos recientes sugieren que, en esta área, la dieta de los lobos de dos pelos incluye peces y calamares, tales como la merluza argentina, *M. hubbsi*, la anchoíta, *E. anchoíta*, y los calamares pelágicos *I. argentinus* y *L. gahi* (Vales *et al.*, 2012).

1.3. Diversidad parasitaria en otáridos

En la actualidad, la fauna de parásitos metazoos de la mayoría de las especies de otáridos es poco conocida. Esto se debe principalmente a que la mayoría de las especies están actualmente protegidas, por lo que los estudios parasitológicos se restringen a ejemplares varados muertos en la costa o capturados accidentalmente por las pesquerías. A pesar de estos problemas, a día de hoy, se han registrado especies de parásitos metazoos pertenecientes a los phyla Platyhelminthes, Acanthocephala, Nematoda y Arthropoda en varias especies de otáridos por todo el mundo (Aznar *et al.*, 2001b; Raga *et al.*, 2009) (**Fig. 3**). En este caso, todos los parásitos platelmintos, acantocéfalos y nematodos encontrados son exclusivamente endoparásitos, infectando principalmente los sistemas respiratorio y circulatorio y/o el tracto gastrointestinal (Dailey, 2005; Raga *et al.*, 2009).

1.3.1. Helmintos gastrointestinales de otáridos

1.3.1.1. Phylum Platyhelminthes

1.3.1.1.1. Clase Trematoda

Dentro de la clase Trematoda, la subclase Digenea incluyen unas 18000 especies descritas en aproximadamente 2700 géneros (Cribb *et al.*, 2001). La característica morfológica más distintiva de estos parásitos, es la presencia en la mayoría de las especies, de dos ventosas musculares en las formas adultas, una alrededor de la boca y la otra posterior en la parte ventral (**Fig. 3A**). La mayoría de las especies de digeneos son hermafroditas, presentando un ciclo de vida complejo. El ciclo de vida de estos parásitos incluye formas de vida libre y parásitas. También presentan generaciones alternas en casi todas las especies, dos asexuales en invertebrados y una sexual en vertebrados. El ciclo de vida de las especies de digeneos que infectan a otáridos es actualmente desconocido, sin embargo, tomando como modelo el ciclo conocido en especies próximas, es posible que incluyan 3 hospedadores: otáridos como hospedador definitivo, moluscos como primer hospedador intermediario y peces como segundo hospedador intermediario (**Fig. 4**). En otáridos se han citado especies de las familias Heterophyidae Leiper, 1909 y Notocotylidae Lühe, 1909 (p. ej. Dailey, 1969; George-Nascimento & Carvajal, 1981; Shults, 1978).

1.3.1.1.2. Clase Cestoda

Los cestodos incluyen aproximadamente unas 5000 especies divididas en dos subclases: Cestodaria y Eucestoda (Olson & Tkach, 2005; Smyth, 1994). Las formas adultas de estos parásitos infectan casi exclusivamente el sistema digestivo de vertebrados. Sus características morfológicas más importantes son la ausencia del sistema digestivo y la presencia de un tegumento con epitelio sincitial cubierto por microtriquias (Bush *et al.*, 2001; Caira & Reyda, 2005). En la actualidad en otáridos únicamente se han citado ejemplares adultos pertenecientes a la subclase Eucestoda, concretamenta a los órdenes Diphyllobothriidea y Tetrabothriidea (p. ej. Delyamure & Parukhin, 1968; Hoberg & Adams, 1992; Shults, 1986). Las características morfológicas más importantes de estos cestodos son el órgano de fijación anterior o escólex (**Figs 3B y D**), un cuello no segmentado y el cuerpo posterior segmentado (o estróbilo) compuesto por una serie de eslabones o proglótides (**Fig. 3C**) (Bush *et al.*, 2001; Smyth, 1994). Todos estos cestodos son polizoicos, es decir, presentan uno o más juegos de órganos masculinos y femeninos en cada proglótide (Caira & Reyda, 2005). Aunque el ciclo de vida de muchos cestodos que parasitan otáridos continúa siendo desconocido, basándonos en las especies de ciclo conocido parece que en general sus ciclos son complejos e incluyen dos, o en ocasiones tres, hospedadores: otáridos como hospedadores definitivos, copépodos como primeros hospedadores intermediarios y peces como segundos hospedadores intermediarios y/o hospedadores paraténicos (**Fig. 5**).

1.3.1.2. Phylum Nematoda

Los nematodos comprenden aproximadamente 24783 especies incluidas en 2829 géneros (Hodda, 2011), de los cuales se considera que un 33% son parásitos de vertebrados (Anderson, 2000). Una de las características morfológicas más importantes de estos helmintos es su cuerpo cilíndrico cubierto por una cutícula acelular, la cual necesitan mudar cuatro veces antes de alcanzar la madurez sexual. Todas las especies de nematodos que infectan el tracto digestivo de los otáridos son dioicas y ovíparas, y en general, pertenecientes a la clase Chromadorea y a los órdenes Rhabditida y Spirurida (Dailey, 2005; McClelland, 2005).

Dentro del orden Rhabditida, las especies del género *Uncinaria* (familia Ancylostomidae) se encuentran en el intestino de otáridos juveniles. Una característica morfológica importante de estos nematodos, es la presencia de una cápsula bucal esclerotizada, frecuentemente armada con placas para cortar (**Fig. 3E**). También presentan una bolsa copuladora amplia en la parte posterior de los machos (Roberts & Janovy, 2009). El ciclo de vida de estos nematodos es directo, es decir, no utilizan otros hospedadores intermediarios y/o paraténicos (**Fig. 6)**.

Por otro lado, los nematodos espirúridos del suborden Ascaridina se caracterizan por la presencia de tres labios prominentes, así como papilas labiales externas y numerosas papilas caudales. Dentro de este suborden, la formas larvales y adultos de especies de los géneros *Anisakis*, *Contracaecum*, *Phocascaris* y *Pseudoterranova* (Anisakidae) han sido citadas comúnmente en el tracto digestivo de otáridos (Dailey, 2001; Mattiucci & Nascetti, 2008). El ciclo de vida de los nematodos anisáquidos que infectan otáridos es complejo, incluyendo fases de vida libre y parasitaria (**Fig. 7**). Estos nematodos incluyen otáridos como hospedadores definitivos, copépodos como primeros hospedadores intermediarios, y macroinvertebrados y peces como segundos hospedadores intermediarios y/o paraténicos (**Fig. 7**).

1.3.1.3. Phylum Acanthocephala

Los acantocéfalos son un grupo de endoparásitos estrictos con aproximadamente 1115 especies adscritas a 125 géneros (Verweyen *et al.*, 2011). Las principales características morfológicas de estos parásitos son una probóscide retráctil e invaginable con una serie de filas de ganchos curvos y un sistema lacunar en la pared corporal. Todos los acantocéfalos son dioicos, exhibiendo comúnmente un gran dimorfismo sexual en el tamaño corporal (Miller & Dunagan, 1985). El ciclo de vida de los acantocéfalos que infectan otáridos incluye dos especies obligatorias: artrópodos como hospedadores intermediarios y otáridos como hospedadores definitivos. Sin embargo, muchas de estas especies pueden incluir en su ciclo a peces como hospedadores paraténicos para superar la brecha trófica entre los hospedadores intermediarios y definitivos (**Fig. 8)**. Hasta la fecha, las únicas especies de acantocéfalos registradas en otáridos pertenecen a los géneros *Corynosoma* (**Fig. 3G**) y *Bolbosoma* (Polymorphidae) (p. ej. Aznar *et al.*, 2006; Kuzmina *et al.*, 2012; Zdzitowiecki, 1984).

1.4. Asociación y transmisión de helmintos gastrointestinales en otáridos

1.4.1. Origen de la asociación parásito-hospedador en otáridos

La estructura de las comunidades parasitarias actuales de otáridos se originó posiblemente a partir de dos tipos de asociaciones: *1*) *asociación por descendencia*, en la cual los ancestros de los hospedadores y parásitos presentaban interacciones previas; y *2*) *asociación por colonización*, en donde el parásito se originó en otro hospedador pero colonizó a los otáridos. La asociación por descendencia implica coevolución, y asume una asociación a largo plazo entre los parásitos y sus hospedadores, con un alto grado de coespeciación y coadaptación (Brooks & McLennan, 1991). Este tipo de asociación sería el caso de los nematodos ancilostómidos (*Uncinaria* spp.) o de los artrópodos que parasitan otáridos, que poseen un ancestro terrestre (p. ej. Kim, 1985; Nadler *et al.*, 2000). Por otro lado, la asociación por colonización (o cambio de hospedador), depende de dos factores: *1*) la probabilidad de encuentro, que está regulada por factores ecológicos, biogeográficos y/o etológicos; y *2*) la compatibilidad entre el nuevo hospedador y el parásito, que depende de barreras morfológicas, fisiológicas y/o inmunológicas del hospedador (Raga *et al.*, 2009). En el medio marino, la asociación por colonización ocurre frecuentemente debido a que los parásitos entran en contacto con hospedadores inusuales que pertenecen al mismo nivel trófico que el de sus hospedadores comunes. A escala ecológica, los parásitos comúnmente no pueden superar las barreras de compatibilidad de los hospedadores inusuales (p. ej. Aznar *et al.*, 2012). Sin embargo, las oportunidades para que una colonización favorable ocurra aumentan continuamente, debido a que el contacto entre el parásito y el hospedador inusual ocurren en largos períodos de tiempo, lo que puede dar lugar a nuevas asociaciones parásito-hospedador. Esto es particularmente viable cuando los nuevos hospedadores están filogenéticamente emparentados con los “hospedadores donantes”, ya que ambos proporcionarán condiciones similares para la supervivencia y reproducción del parásito. Un ejemplo de asociación por colonización son los cestodos del género *Anophryocephalus* que se diversificaron en fócidos a partir de un ancestro que posiblemente ocurrió en arcosaurios, y que posteriormente colonizó otáridos (Hoberg & Brooks, 2008).

1.4.2 Transmisión de parásitos gastrointestinales hacia otáridos como hospedadores definitivos

Las rutas de transmisión de los parásitos gastrointestinales de otáridos a través de los distintos hospedadores implicados en su ciclo de vida son poco conocidas. Con la excepción de los nematodos ancilostómidos que poseen un ciclo de vida directo, los demás helmintos gastrointestinales son transmitidos hacia lobos marinos mediante la red trófica del medio marino. Sin embargo, para la mayoría de estas especies de parásitos, la identidad de los hospedadores intermediarios y/o paraténicos continúa siendo desconocida, por lo que muchas etapas de su ciclo de vida no han podido ser descritas. No obstante, una de las predicciones generales que podrían aplicarse a la mayoría de estos parásitos es la baja especificidad hacia sus hospedadores intermediarios y/o paraténicos (p. ej. Laskowski *et al.*, 2008; Moles, 2007; Palm *et al.*, 1994). La capacidad de infectar una amplia gama de hospedadores parece ser una adaptación que garantiza la transmisión hacia sus hospedadores definitivos a través de varias presas potenciales, lo que amplía el riesgo de infección entre un gran número de especies hospedadoras (Bush, 1990).

El espectro tan amplio de hospedadores intermediarios y/o paraténicos infectados por helmintos gastrointestinales de otáridos, así como la gran diversidad de formas larvarias, hacen de este grupo de parásitos un modelo ideal para investigar los patrones de especificidad de distintos hospedadores, así como para describir la forma en que estos parásitos utilizan las redes tróficas para garantizar su transmisión. Diversos estudios (p. ej. Aznar *et al.*, 2012; George-Nascimento *et al.*, 1992) han proporcionado información sobre el efecto de diferentes hospedadores definitivos en la especificidad de parásitos gastrointestinales de otáridos. Por lo tanto, efectos similares se podrían esperar para distintas especies de hospedadores intermediarios y/o paraténicos.

1.5. El presente estudio

Esta tesis doctoral se llevó a cabo bajo el marco de dos proyectos: "*Estudio de las amenazas para la conservación de mamíferos marinos de Patagonia*" financiado por la Fundación BBVA (BIOCON 04) y "*Historia evolutiva de la familia Polymorphidae (Acanthocephala) en aves acuáticas y mamíferos marinos: diversidad comparada, biogeografía y ecomorfología*" financiado por el Ministerio de Ciencia e Innovación, España (CGL2007-63221/BOS).

En particular, el presente trabajo aborda los siguientes estudios: *1*) En primer lugar, se examinó parasitológicamente una amplia muestra de intestinos de los lobos marino de uno y dos pelos, recolectados entre el período 1994–2009 a lo largo de la costa patagónica argentina. Esta muestra proporciona una oportunidad única para caracterizar, por primera vez, la fauna de helmintos intestinales de ambas especies de otáridos de esta región. *2*) En segundo lugar, durante el período 2006-2007, se recolectaron 542 ejemplares pertenecientes a 20 especies de peces marinos de la costa patagónica argentina para ser examinados parasitológicamente, con el objetivo de identificar y cuantificar las larvas de parásitos que infectan estos otáridos. La base de datos obtenida de estos estudios parasitológicos, proporcionó una gran oportunidad para describir algunas formas larvarias desconocidas en esta área, además de evaluar las posibles vías de transmisión de estos parásitos entre peces hospedadores intermediarios/paraténicos y sus hospedadores otáridos definitivos. *3*) En tercer lugar, el gran número de larvas recolectadas en diferentes especies de peces durante este estudio, permitió evaluar el efecto de diferentes especies hospedadoras sobre algunas características del ciclo biológico de estas larvas, como por ejemplo los patrones de crecimiento o la proporción de sexos, los cuales son aspectos esenciales para comprender la dinámica poblacional de estos parásitos.

2. Justificación y objetivos

El presente estudio tiene dos objetivos generales: *1*) Incrementar el conocimiento sobre la diversidad y la composición de las comunidades de parásitos helmintos de dos especies de otáridos, el lobo marino de un pelo, *O. flavescens* y el lobo marino de dos pelos, *A. australis* a lo largo de la costa patagónica argentina; *2*) Investigar diferentes aspectos sobre la morfología, estructura poblacional, características del ciclo de vida y estrategias de transmisión de las formas larvarias de estos helmintos en peces que actúan como hospedadores paraténicos o intermediarios.

Los objetivos específicos del presente estudio son:

2.1. Cuantificar y describir las comunidades de parásitos metazoos intestinales de *O. flavescens* y *A. australis* de la costa norpatagónica argentina, mediante un estudio morfológico y taxonómico detallado. Utilizar los resultados obtenidos para determinar el papel que tiene la especificidad parásito-hospedador en la configuración de la diversidad en comunidades de helmintos en otáridos.

2.2. Caracterizar las poblaciones componentes de cistacantos de *Corynosoma australe* (Acanthocephala: Polymorphidae) en diferentes especies de peces hospedadores paraténicos de la costa patagónica argentina. Clarificar las vías de transmisión de este acantocéfalo entre peces hospedadores paraténicos y otáridos hospedadores definitivos, así como evaluar el efecto de diferentes especies de peces en el crecimiento, tamaño corporal, eficacia biológica y proporción de sexos en los cistacantos de *C. australe*.

2.3. Describir, por primera vez, la estrategia ontogenética de inversión en las estructuras de fijación (espinas del tronco) entre cistacantos y adultos de dos especies congenéricas de acantocéfalos (*C. australe* y *C. cetaceum*), investigando los factores que pueden explicar los patrones de crecimiento de las espinas del tronco.

2.4. Realizar una identificación y descripción taxonómica de larvas en estado 3 de *Pseudoterranova* sp. (Nematoda: Ascaridoidea) procedentes de varias especies de peces del litoral patagónico, mediante secuencias obtenidas del gen del citocromo *c* oxidasa subunidad 1 (cox 1), así como un estudio morfológico detallado. Utilizar los datos obtenidos para describir las poblaciones componentes de larvas 3 de *Pseudoterranova* sp. en peces, además de evaluar el papel de las distintas especies de peces hospedadores en la selección del microhábitat, estrategias de transmisión y parámetros de infección de estas larvas.

3. Materiales y métodos

3.1. Pinnípedos

Para el desarrollo del presente estudio se analizaron los intestinos de 56 lobos marinos de un pelo, *O. flavescens*, y 5 lobos marinos de dos pelos, *A. australis*. Los lobos marinos de ambas especies se obtuvieron durante el período 1994–2009 de la costa norpatagónica (40°43'–43º20'S, 63°04'–65º07'O; **Fig. 9**). Los ejemplares fueron recolectados a partir de varamientos en la costa (*O. flavescens*, n = 48, y *A. australis*, n = 4) o como captura accidental de pesquerías (*O. flavescens*, n = 8, y *A. australis*, n = 1).

Cada ejemplar de lobo marino fue medido (longitud estándar) y necropsiado por el personal del Laboratorio de Mamíferos Marinos (LAMAMA) del Centro Nacional Patagónico (Puerto Madryn, Chubut, Argentina) en el campo o en el laboratorio. El intestino fue retirado de la cavidad corporal y posteriormente congelado a –20ºC. Después de descongelarse, cada intestino fue pesado, extendido y medido. Todos los intestinos fueron procesados de acuerdo a la metodología propuesta por Aznar *et al.* (2004): divididos en 5 secciones (n = 15) o en 30 secciones (n = 41) de igual longitud. El contenido de cada sección se lavó por separado a través de una serie de tamices con una luz de malla de entre 0,2–0,5 mm. Finalmente, la pared del intestino de cada sección fue examinada visualmente para recoger parásitos que pudieran permanecer enganchados.

La edad de todos los ejemplares de lobo marino fue estimada mediante el recuento de las marcas de crecimiento de la dentina o del cemento de los caninos superiores (ver Grandi *et al.*, 2010, para más información). Los datos biológicos de cada ejemplar de lobo marino de un pelo o de lobo marino de dos pelos analizados en el presente estudio se resumen en la **Tabla 2** y en la **Tabla 3** respectivamente.

3.2. Teleósteos

Se recolectaron ejemplares pertenecientes a 20 especies de peces marinos mediante barcos de pesca comerciales de arrastre durante 3 muestreos en diferentes zonas que rodean el área de veda (AV) establecida como de protección de juveniles de merluza argentina, *M. hubbsi* (**Fig. 12**): “Muestreo 1” al sur del AV y en las cercanías del Golfo San Jorge (47º00'–47º20'S; 64º17'–65º00'O; Marzo 2006; rango de profundidad: 82–102 m); “Muestreo 2” principalmente al sur y sureste de la AV, en aguas de la plataforma patagónica (47º00'–47º19'S; 61º59'–64º25'O; Marzo 2007; rango de profundidad: 101–119 m); “Muestreo 3” al norte de la AV, en aguas próximas a la península Valdés (42º45'–42º59'S; 61º09'–62º58'O; Octubre 2007; rango de profundidad: 72–88 m).

Las especies de peces fueron seleccionadas en base a su abundancia en las áreas de muestreo (Bezzi *et al.*, 2000) y su presencia en la dieta de los otáridos (Koen-Alonso *et al.*, 2000). En este último caso se seleccionaron individuos cuyo tamaño corporal se encontraba dentro del rango citado en la dieta de lobos marinos de la costa patagónica (Koen-Alonso *et al.,* 2000). Se recolectaron 542 ejemplares pertenecientes a 20 especies de peces marinos (**Tabla 4**). Todos los peces se mantuvieron en hielo hasta su desembarque, y en el LAMAMA fueron identificados, medidos y pesados. Algunos peces fueron analizados en fresco, mientras que el resto fueron congelados a –20ºC para su análisis posterior. Antes de ser analizados, se examinó visualmente la piel y las aletas con el fin de detectar parásitos superficiales. A continuación, se retiraron los órganos internos de la cavidad corporal y se colocaron en placas Petri con solución salina fisiológica. La cavidad corporal también fue analizada visualmente. El contenido del estómago, intestino y ciegos intestinales fueron lavados y diluidos en solución salina fisiológica, y posteriormente examinados con una lupa binocular (6–40x). El cerebro, vesícula biliar, agallas, gónadas, corazón, riñón, hígado, mesenterio, bazo, vejiga natatoria, músculo (epiaxial e hipoaxial), así como las paredes del estómago, intestino y ciegos intestinales también fueron examinados analizados para la obtención de parásitos.

Cada una de las especies de peces fueron asignadas a un grupo ecológico según su posición en la columna de agua de acuerdo a Koen-Alonso *et al.* (2000), Romero *et al.* (2011) y Romero *et al.* (2012). Los grupos ecológicos considerados en el presente estudio fueron los siguientes: bentónicos (peces estrictamente relacionados con el fondo del mar), pelágicos (peces que viven en la columna de agua, alejados del fondo marino) y demersales (peces que viven en la columna de agua, cerca del fondo marino). Este último grupo fue subdividido en demersal-pelágico (peces con un patrón de migración diario, dispersándose en la columna de agua durante la noche y permaneciendo cerca del fondo durante las horas de luz) y demersal-bentónico (peces que no realizan migraciones verticales y que no están estrictamente relacionados con el fondo del mar).

3.3. Conservación y determinación taxonómica de los helmintos

El contenido intestinal y los parásitos de cada ejemplar de lobo marino fueron fijados en etanol al 70% o 100%. Los parásitos recolectados en las distintas especies de peces fueron lavados en solución salina, contados y fijados en etanol 70%. Todos los cistacantos y nematodos (larvas 3) fueron extraídos de su cápsula antes de ser fijados.

La identificación morfológica de todos los parásitos se realizó en la Unidad de Zoología Marina del Instituto Cavanilles de Biodiversidad y Biología Evolutiva de la Universidad de Valencia. Las técnicas específicas de tinción, transparentación y montaje para cada taxón, así como las técnicas microscópicas e histológicas, se describen específicamente en cada capítulo. Todas las ilustraciones y mediciones se realizaron a través de un tubo de dibujo unido a un microscopio óptico. Las instituciones donde el material tipo y vouchers fueron depositados, así como su número de acceso en las distintas colecciones, se describen específicamente en cada capítulo.

Los análisis moleculares para la identificación de las larvas 3 de los nematodos anisáquidos del género *Pseudoterranova* se realizaron en el Laboratorio de Helmintología perteneciente al Instituto de Parasitología de la Academia de Ciencias de la República Checa. Los procedimientos de extracción del ADN, PCR, secuenciación y alineamiento se describen específicamente en el capítulo correspondiente.

3.4. Terminología, parámetros de infección y análisis estadísticos

En el presente estudio se siguió la terminología propuesta por Bush *et al.* (1997) sobre ecología parasitaria. Los parámetros de infección se estimaron de acuerdo con Rózsa *et al.* (2000) mediante el software Quantitative Parasitology v3.0 (Reiczigel & Rózsa, 2005). La mayoría de los análisis se llevaron a cabo con el programa de análisis estadístico SPSS v17 y la significación estadística se estableció en $P < 0,05$.

4. Helmintofauna intestinal del lobo marino de un pelo, *Otaria flavescens* y del lobo marino de dos pelos, *Arctocephalus australis* de Patagonia, Argentina

En este estudio se describe, por primera vez, las comunidades de helmintos intestinales del lobo marino de un pelo y del lobo marino de dos pelos de la costa norpatagónica argentina. Asimismo, se evalúa la importancia de la especificidad en la conformación de la diversidad en las comunidades de helmintos. En este contexto, estudios recientes (Aznar *et al.*, 2012; Mateu *et al.*, 2011) han señalado que muchos posibles hospedadores para parásitos de mamíferos marinos transmitidos tróficamente en realidad actúan como “no-hospedadores” (es decir aquellos hospedadores en los que el parásito no puede establecerse y/o desarrollarse). Este fenómeno puede tener importantes repercusiones al evaluar el papel de posibles “no-hospedadores” en la dinámica poblacional de helmintos, y al definir la “verdadera” comunidad de helmintos de una especie hospedadora concreta. Por último, este estudio proporciona también el contexto para comparar la riqueza de especies y la composición de las comunidades de helmintos de ambas especies con las de otras especies de otáridos.

Todos los ejemplares de lobo marino de un pelo (n = 56) presentaron helmintos intestinales. Los parámetros de infección se presentan en la Tabla 1 de la página 47. Se recolectaron 97325 ejemplares de helmintos: 88998 acantocéfalos, 3684 nematodos, 4589 digeneos y 54 cestodos. Se encontraron ejemplares grávidos pertenecientes a seis especies de helmintos: *Ascocotyle* (*Ascocotyle*) *patagoniensis*, *Diphyllobothrium* spp., *Contracaecum ogmorhini* (*s.s.*), *Pseudoterranova cattani*, *Uncinaria hamiltoni* y *Corynosoma australe*. Además, se hallaron larvas 3 de los nematodos *Contracaecum* sp. y *Anisakis* sp. tipo I, junto con individuos juveniles de los acantocéfalos *Andracantha* sp., *Profilicollis chasmagnathi* y *Corynosoma cetaceum*. Cuatro especies de parásitos, *A.* (*A.*) *patagoniensis*, *C. ogmorhini* (*s.s.*), *Andracantha* sp. y *P. chasmagnathi*, representan nuevos registros para este hospedador.

En el lobo marino de dos pelos, se recolectaron helmintos únicamente en los machos (n =4). Los parámetros de infección se presentan en la Tabla 2 de la página 48. Se recolectaron 1516 ejemplares de helmintos: 1408 acantocéfalos, 99 nematodos y 9 cestodos. Los ejemplares adultos correspondieron a tres especies de helmintos: *Diphyllobothrium* spp., *C. ogmorhini* (*s.s.*), y *C. australe*. Además, se encontraron larvas 3 del nematodo *Contracaecum* sp., larvas en estado 4 de *P. cattani*, juveniles de *C. cetaceum* y ejemplares inmaduros de cestodos identificados como Tetrabothriidae gen. sp.

La comunidad componente de helmintos intestinales del lobo marino de un pelo está compuesta por 11 especies. A nivel de infracomunidad, la riqueza de especies varió de 1 a 6 especies (media ± desviación estándar: 3,1 ± 1,1 especies). Un total de 3 lobos marinos de un pelo (5,4%) estaban infectados con 1 especie de helminto, 14 (25,0%) con 2, 20 (35,7%) con 3, 14 (25,0%) con 4, 4 (7,1%) con 5 y un solo lobo (1,8%) con 6. Por otro lado, la comunidad componente de helmintos intestinales del lobo marino de dos pelos está compuesta de 7 especies. A nivel de infracomunidad, la riqueza de especies varió de 1 a 5 especies (2,2 ± 1,9 especies). Un hospedador (20%) estaba infectado con 1 especie de helminto, 1 (20%) con 2, 1 (20%) con 3 y un solo lobo

(20%) con 5. No se detectaron diferencias en la riqueza de la infracomunidad entre lobos marinos de uno y dos pelos.

Los valores de la riqueza de especies de helmintos en ambas especies de lobos marinos se ven claramente influenciados por los taxones que fueron encontrados únicamente en formas larvarias o juveniles. Cuando estos taxones se excluyen, la comunidad componente de lobo marino de un pelo se reduce a 6 especies, mientras que la riqueza de especies varía de 1 a 5 especies (2,6 ± 0,9). Un total de 5 lobos marinos de un pelo (8,9%) estaban infectados con 1 especie de helminto, 20 (35,7%) con 2, 22 (39,3%) con 3, 8 (14,3%) con 4 y un solo lobo (1,8%) con 5. En el caso del lobo marino de dos pelos la comunidad componente (excluyendo larvas y juveniles) está compuesta por 4 especies de helmintos (1,75 ± 1,0). Tres lobos de dos pelos (60%) estaban infectados con 1 especie de helminto, mientras que un único lobo (20%) presentó 4 especies.

Los otáridos que habitan el litoral norpatagónico parecen ser hospedadores adecuados para 6 o 7 especies de helmintos (dependiendo si hay una o dos especies de *Diphyllobothrium*), 5 especies pertenecen a géneros cuyas especies infectan otáridos en todo el mundo (ver Tabla 4 y referencias allí citadas, página 52), es decir, especies de los géneros *Diphyllobothrium*, *Corynosoma*, *Pseudoterranova*, *Contracaecum* y *Uncinaria*. La presencia global de este grupo predecible de especies de helmintos en pinnípedos sugiere que estas asociaciones pudieron establecerse con anterioridad a la división de los tres principales clados de pinnípedos (Hoberg & Adams, 2000) aunque *Uncinaria* está restringido exclusivamente a otáridos (George-Nascimento *et al.*, 1992).

La omisión de los taxones parásitos para los cuales los lobos marinos de uno y dos pelos no parecen ser hospedadores adecuados tiene un impacto significativo en la estimación de la riqueza de especies, particularmente al nivel de comunidades componentes, y puede llevar a una comparación más precisa entre especies de otáridos. Desafortunadamente, la información relacionada sobre el estado reproductivo de los helmintos no se proporciona frecuentemente en los estudios parasitológicos de otáridos, o se asume que cualquier parásito encontrado en un hospedador es parte de su helmintofauna. Sin embargo el presente estudio resalta las diferencias cuantitativas de incluir o excluir a los parásitos encontrados en hospedadores considerados no adecuados. Por lo tanto, se recomienda siempre proveer la información apropiada sobre el estado de madurez de los parásitos, ya que pueden ser de gran utilidad para futuros estudios filogenéticos sobre interacciones parásito-hospedador o para metaanálisis.

5. Una nueva especie de *Ascocotyle* (Trematoda: Heterophyidae) del lobo marino de un pelo, *Otaria flavescens*

Una nueva especie de heterófido, *Ascocotyle* (*Ascocotyle*) *patagoniensis* Hernández-Orts, Montero, Crespo, García, Raga y Aznar, 2012, se describió del intestino del lobo marino de un pelo, *O. flavescens*. La comparación morfológica de *A.* (*A.*) *patagoniensis* con otras especies del subgénero *Ascocotyle* (*Ascocotyle*) sugiere que la nueva especie puede distinguirse de las demás por el número de espinas circunorales (18 a 23 espinas en cada fila), por poseer un gonotilo sin papilas así como por tener el receptáculo seminal más amplio descrito en una especie de este subgénero. Las principales diferencias taxonómicas entre la nueva especie y el resto de especies del subgénero se presentan en la Tabla II, página 63.

El número de espinas circunorales es el carácter morfológico más útil para diferenciar a las especies dentro del subgénero *Ascocotyle* (Santos *et al.*, 2007; Sogandares-Bernal & Lumsden, 1963). Las espinas circunorales son a menudo difíciles de contar debido a su pequeño tamaño o a la superposición visual de estas estructuras en los ejemplares montados. Asimismo, muchas espinas pueden perderse debido a la descomposición post-mortem del tegumento (Santos *et al.*, 2007). En este estudio, las espinas de las filas superior e inferior solamente se encontraron completas en 10 y 3 ejemplares respectivamente de los 629 montados. Esta especie es única por poseer 2 círculos completos de 18 a 23 espinas cada uno (ver Figs. 2 a 6, página 61).

Los ciclos de vida de las especies del subgénero *Ascocotyle* involucran a 3 hospedadores, es decir, un caracol hidróbido, un teleósteo y un ave ictiófaga o un mamífero en hábitats de agua dulce o salobre (Font *et al.*, 1984; Ostrowski de Núñez, 2001). Por lo tanto, la presencia de *A.* (*A.*) *patagoniensis* en lobos marinos representa el primer registro de una especie de este subgénero en un hospedador definitivo marino. Desafortunadamente, las otras etapas en el ciclo de vida (p. ej. metacercarias) de este trematodo continúan siendo desconocidas. En el presente estudio, ninguna metacercaria de *Ascocotyle* spp. fue encontrada en 542 peces marinos pertenecientes a 20 especies recolectados a lo largo de la plataforma patagónica. La ausencia de estas formas larvarias en peces marinos de esta área puede estar relacionada con el hecho de que el ciclo de vida de este trematodo esté restringido a aguas litorales. Sin embargo, dado el pequeño tamaño de las formas adultas de este trematodo, no se puede descartar que en la muestra de peces analizados, algunas metacercarias fueran pasadas por alto. Asimismo, para algunas especies de teleósteos, en las que se analizaron pocos especímenes, es posible que las metacercarias no fueran encontradas debido al número tan pequeño ejemplares estudiados.

6. Patrones de transmisión de *Corynosoma australe* en peces hospedadores paraténicos

Un gran número de especies de helmintos se transmiten tróficamente, utilizando a menudo hospedadores paraténicos como vías alternativas para reducir la brecha trófica y facilitar la transmisión entre los hospedadores obligatorios, es decir, los intermediarios y definitivos (Marcogliese, 2002). Los hospedadores paraténicos se distinguen fundamentalmente de otros hospedadores en que en ellos las formas larvales de los parásitos no se desarrollan ni crecen esencialmente (Beaver, 1969; Bush *et al.*, 2001; Roberts & Janovy, 2009). Asimismo, estos hospedadores se distinguen de los hospedadores accidentales “no adecuados” en que, únicamente en los hospedadores paraténicos, las formas larvales continuarán su desarrollo una vez transferidos a un hospedador adecuado (Kennedy, 2006).

En la actualidad, los hospedadores paraténicos han recibido menor atención que los hospedadores obligatorios, ya que usualmente son considerados hospedadores facultativos (Schmidt, 1985). Sin embargo, desde el punto de vista ecológico, juegan un papel importante en la estructura y dinámica poblacional de los helmintos, ya que transportan y acumulan un gran número de larvas, contribuyendo a su transmisión y dispersión (Médoc *et al.*, 2011), además de mantenerlos en el medio ambiente y protegerlos de amenazas externas (Marcogliese, 2002; Poulin, 1998). Dentro de los hospedadores paraténicos, los parásitos se encuentras comúnmente enquistados o encapsulados, facilitando su supervivencia por largos períodos de tiempo, además de permitirles movimientos hacia nuevos hospedadores o hábitats, lo que aumenta la probabilidad de nuevas interacciones parásito-hospedador (Marcogliese, 2007; Médoc *et al.*, 2011).

Sin embargo, el uso de hospedadores paraténicos también puede tener un efecto negativo en la biología y la estructura poblacional de los parásitos. La transmisión entre varios hospedadores paraténicos expone a los parásitos a varias respuestas de los sistemas inmunes de los hospedadores, lo que implica la necesidad de desarrollar múltiples adaptaciones contra los diferentes tipos de respuesta inmune, además de padecer la acumulación de daños provocados por las sucesivas exposiciones a distintas defensas. En otros casos, la transmisión desde algunos hospedadores paraténicos es poco probable, por lo que pueden actuar como un sumidero en el ciclo de vida de los parásitos, afectando a su dinámica poblacional (Holmes, 1979; Rohde, 2005).

La paratenia ocurre en muchos grupos de parásitos (Médoc *et al.*, 2011; Parker *et al.*, 2009); sin embargo es rara en trematodos (p. ej. Latham *et al.*, 2003; Madhavi, 1978) y cestodos (Morand *et al.*, 1995), y más frecuente en nematodos (Anderson, 2000) y acantocéfalos (Schmidt, 1985). En los acantocéfalos, la paratenia ocurre entre el 10 al 20% de las aproximadamente 1100 especies descritas (Parker *et al.*, 2009). En acantocéfalos, los hospedadores paraténicos son normalmente vertebrados poiquilotermos (p. ej. teleósteos, anfibios o reptiles) (Nickol, 1985; Schmidt, 1985). En general, después de ser reclutados por estos hospedadores, los cistacantos se activan y atraviesan la pared intestinal para ocupar posiciones extra intestinales (mesenterios o musculatura) donde son encapsulados (Petrochenko, 1956; Taraschewski, 2000).

A día de hoy, la mayor parte de los estudios de acantocéfalos marinos que infectan hospedadores paraténicos se han desarrollado con especies del género *Corynosoma*. Estos acantocéfalos son parásitos cosmopolitas que habitan principalmente el intestino de pinnípedos (Aznar *et al.*, 2006; Aznar *et al.*, 2012). A partir de los estudios disponibles sobre el ciclo de vida de varias especies de *Corynosoma*, parece ser que utilizan anfípodos bentónicos como hospedadores intermediarios (p.ej. Laskowski *et al.*, 2010; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986, 2001). Sin embargo, debido a que el nivel trófico de los hospedadores intermediarios y definitivos es muy distante, estos acantocéfalos utilizan una gran variedad de especies de peces como hospedadores paraténicos para superar la brecha trófica entre sus hospedadores obligatorios (p. ej. Valtonen, 1983; Sinisalo & Valtonen, 2003; Timi *et al.*, 2011b; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986).

Este estudio proporciona nuevos datos sobre las interacciones parásito-hospedador, estructura poblacional y costos/efectos del paso por los hospedadores paraténicos en acantocéfalos. En particular se analizan cuestiones biológicas, morfológicas y ecológicas, con especial atención sobre los efectos negativos de la paratenia en cistacantos de *C. australe* infectando varias especies de peces hospedadores paraténicos de la costa patagónica en Argentina. *Corynosoma australe* es un acantocéfalo euritópico, que madura y se reproduce en el intestino de pinnípedos en Sudamérica (Aznar *et al*., 2012; Hernández-Orts *et al.*, 2013; Sardella *et al.*, 2005). La identidad de los hospedadores intermediarios de esta especie de acantocéfalo se desconoce, aunque posiblemente pueda ser un invertebrado bentónico como en otras especies del género. Además, los cistacantos de *C. australe* han sido comúnmente citados como componentes integrales de la comunidades helmínticas de peces hospedadores paraténicos (p. ej. Alves *et al*., 2003; Iannacone *et al.*, 2011; Oliva & Luque, 2002; Santos *et al*., 2008; Timi, 2007; Timi *et al*., 2011b). Únicamente en la costa argentina, *C. australe* ha sido reportado en al menos 24 especies de peces marinos pertenecientes a 19 familias (**Tabla 5**). Por lo tanto, esta especie de acantocéfalo parece ser un modelo ideal para estudiar las interacciones parásito/hospedador y aspectos biológicos/ecológicos entre helmintos transmitidos tróficamente y sus hospedadores paraténicos.

Los objetivos de este estudio fueron tres. *1*) Obtener los parámetros de infección de *C. australe* en 20 especies de peces con lo que se describió su estructura poblacional en la costa patagónica argentina. Asimismo se exploró la distribución de este acantocéfalo en la columna de agua, con lo que se describió la circulación, patrones de transmisión y los factores que afectan la distribución espacial de este parásito. También se investigó la importancia relativa de cada especie de pez en la transmisión de *C. australe* hacia el lobo marino de un pelo *O. flavescens* con el objetivo de identificar a las especies claves en el área de estudio. *2*) Examinar la proporción de sexos de los cistacantos de *C. australe* infectando teleósteos. El objetivo de este estudio fue explorar si la proporción de sexos es distinta al valor esperado de 1:1, También se investigó, si ya existía el tipo de sesgo hacia hembras adultas de *C. australe* observado en pinnípedos por Aznar *et al.* (2004). Asímismo se discutieron los posibles factores que provocan este sesgo en los hospedadores paraténicos. *3*) Analizar, por primer vez, los costos potenciales que la paratenia puede suponer en *C. australe*. Se prestó especial atención al costo energético que los cistacantos de este acantocéfalo pueden afrontar en la transmisión entre hospedadores paraténicos. Si los acantocéfalos no se desarrollan ni crecen esencialmente en los hospedadores paraténicos, las reservas energéticas, y por lo tanto el tamaño corporal, de *C. australe* tenderá a disminuir cuando ocurran transferencias entre hospedadores paraténicos. Esta hipótesis fue evaluada al comparar, el volumen corporal de *C. australe* y el nivel trófico de diferentes especies de peces de donde los cistacantos fueron colectados. También se exploró si la intensidad de infección pudo influir en el tamaño del parásito a través de efectos de hacinamiento (Lotz *et al.*, 1995). Estos resultados se discutieron en relación a las consecuencias negativas que la transición entre hospedadores paraténicos pueden ejercer en helmintos transmitidos tróficamente.

6.1. Patrones de circulación y transmisión de* C. australe *en peces hospedadores paraténicos

Un total de 1367 cistacantos de *C. australe* fueron recolectados en 18 especies de peces marinos de la costa patagónica argentina. Los parámetros de infección de cistacantos de *C. australe* en las distintas especies de peces se presentan en la **Tabla 6**. Ocho especies de peces, *Acanthistius patachonicus*, *Brama brama*, *Congiopodus peruvianus*, *Cottoperca gobio*, *Genypterus blacodes*, *Patagonotothen ramsayi*, *Seriolella porosa* y *Stromateus brasiliensis* representan nuevos registros de hospedador para *C. australe*. La comparación en la abundancia de cistacantos de *C. australe* entre las especies de peces reveló diferencias significativas (**Tabla 7**), mostrando que *Raneya brasiliensis* albergó significativamente más cistacantos que cualquier otra especie de pez, seguida por *Paralichthys isosceles* y, en tercer lugar, por el grupo formado por *A. patachonicus*, *Nemadactylus bergi*, *Percophis brasiliensis*, *Prionotus nudigula*, *Scomber japonicus* y *Xystreurys rasile*. Por otra parte, el grupo formado por las especies *C. peruvianus*, *C. gobio*, *Helicolenus lahillei*, *Merluccius hubbsi* y *S. brasiliensis* albergó significativamente menos cistacantos que cualquiera de las otras especies de peces. Los resultados sugieren una tendencia monotónica significativa hacia la disminución de la prevalencia de *C. australe* de peces bentónicos hacia los pelágicos en la costa patagónica argentina (**Fig. 14**).

Los resultados del presente estudio sugieren que las infecciones de *C. australe* en peces hospedadores paraténicos no son aleatorias y que la transmisión de este acantocéfalo ocurrirá con mayor probabilidad en peces bentónicos y demersal-bentónicos. Este tipo de transmisión seguramente este asociada con la distribución del hospedador intermediario, posiblemente un anfípodo bentónico, como ha sido reportado en otras especies de *Corynosoma* (p. ej. Laskowski *et al.*, 2010; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986, 2001). Esta idea se apoya por la observación de que *R. brasiliensis*, una de las especies más infectadas por *C. australe*, se alimenta casi exclusivamente de pequeños invertebrados bentónicos, principalmente poliquetos y anfípodos (Barraza Bernardas, 2009). Sin embargo, *C. australe* también infecta a un gran número de especies de peces de diferentes zonas en la columna de agua, lo que sugiere que la transmisión entre hospedadores paraténicos también debe de ocurrir. Este tipo de transmisión sería responsable, en gran medida, de la ubicuidad de *C. australe* en una amplia variedad de especies de peces.

La hipótesis propuesta sobre la transmisión “bentónica” de *C. australe* en este estudio, explicaría porque dos especies de presas claves en la dieta del lobo marino, *E. anchoita* y *M. hubbsi*, no presentan altos parámetros de infección por *C. australe*. Ambas especies de peces, se alimentan principalmente en zonas pelágicas o demersal-pelágicas (p. ej. Hansen, 2000; Ocampo Reinaldo *et al.*, 2011), por lo que no estarían expuestos a los cistacantos de *C. australe*. Sin embargo, este estudio reportó una anomalía en esta hipótesis, debido a que *S. japonicus*, una especie que se alimenta principalmente de organismos planctónicos y peces pelágicos pequeños (Pájaro, 1993), presentó altos niveles de infección de *C. australe*. Esto sugiere que otros factores, como la longevidad del parásito o los cambios en los hábitos alimenticios de los peces durante su ontogenia, podrían regular los niveles de infección de *C. australe* en otras especies de peces.

Para calcular la importancia relativa de las distintas especies de peces en la transmisión de *C. australe* hacia el lobo marino de un pelo, se utilizaron los datos sobre la dieta de esta especie de otárido obtenidos de dos muestras independientes en la costa patagónica por Koen-Alonso *et al*. (2000) (incluyendo datos adicionales del Koen-Alonso, 1999) y de Romero *et al.*, (2011). Los resultados mostraron que *M. hubbsi* fue la presa más importante en términos numéricos y de biomasa, incluso sobre otras presas importantes como los cefalópodos. Sin embargo, las infecciones por cistacantos de *C. australe* son anecdóticas en *M. hubbsi* (**Tablas 5 y 6**). La segunda especie más importante en la dieta de los lobos marinos, *R. brasiliensis*, es una de las especies de pez más infectadas con *C. australe* (**Fig. 15**). Además de estas especies de peces, los lobos marinos consumen otras especies de peces en niveles de importancia variables, pero ninguna de estas presas (p. ej. *Engraulis anchoita*, *S. porosa* y *S. brasiliensis*), parecen tener un papel significativo en la transmisión de *C. australe* debido a su bajos niveles de infección (ver **Tablas 5 y 6**).

En base a los estudios sobre los hábitos alimenticios de *O. flavescens*, *E. anchoita*, *R. brasiliensis* y, especialmente, *M. hubbsi* son las especies de teleósteos principalmente consumidas. Sin embargo *E. anchoita* y *M. hubbsi* son hospedadores excepcionales para *C. australe* (**Tablas 5 y 6**) y difícilmente contribuirán de manera significativa a su transmisión. En cambio, *R. brasiliensis* promoverá el reclutamiento continuo de cistacantos de *C. australe*, por lo que tendrá un papel importante en la transmisión de este acantocéfalo en el área de estudio. Muchas otras especies de peces citadas en la dieta del lobo marino han sido registradas como hospedadores paraténicos de *C. australe* (**Tablas 5 y 6**), por lo que la ubicuidad de este parásito en la red trófica incrementará sus posibilidades de transmisión, independientemente de los posibles cambios en los hábitos alimenticios de los lobos marinos. Además, muchas especies de peces con altos niveles de infección de *C. australe*, particularmente las asociados al fondo del mar, contribuirán con una gran cantidad de cistacantos cuando son consumidos ocasionalmente.

Es importante destacar dos factores que no fueron incluidos en el presente estudio y que podrían tener un papel importante en la dinámica poblacional de *C. australe* en esta la costa patagónica: *1*) Los cefalópodos, como por ejemplo el pulpo colorado *Enteroctopus megalocyathus*, y los calamares *I. argentinus* y *L. gahi*, también son parte fundamental en la dieta de *O.* flavescens (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). El papel que tienen en la transmisión de *C. australe* es desconocido, debido, principalmente, a que los estudios parasitológicos sobre estos hospedadores son escasos. La evidencia disponible sugiere, que en general, los cefalópodos no son hospedadores adecuados para acantocéfalos (Hochberg, 1990), por lo que es posible que tengan un papel menor en la transmisión de *C. australe*. *2*) Otro hospedador definitivo para *C. australe*, el lobo marino de dos pelos *A. australis* (ver Aznar *et al.*, 2004), también habita la costa patagónica argentina. La evidencia previa sugiere que el *A. australis* es un hospedador más adecuado para *C. australe*, debido a que las hembras de este acantocéfalo en este otárido presentan un mayor tamaño corporal, así como una mayor fecundidad, que las de *O. flavescens* (George-Nascimento & Marin, 1992). Sin embargo, dado que datos sobre hábitos alimenticios del lobo de dos pelos son escasos en la costa patagónica, habrá que desarrollar futuros estudios para conocer con mayor detalle el papel del lobo de dos pelos en las dinámicas de transmisión de *C. australe* en esta área.

6.2. Proporción de sexos de cistacantos de **C. australe**

La proporción de sexos se calculó como el porcentaje de machos [es decir, n^{o} de machos/(n^{o} de machos + n^{o} de hembras)] de *C. australe.* El número total de cistacantos machos y hembra, y la proporción de sexos estimada para cada especie de pez se presenta en la **Tabla 8**. La mayoría de los cistacantos pudieron ser sexados en casi todos los peces, con excepción de *A. patachonicus,* donde la mayor parte de los cistacantos se encontraron en diferentes estados de degradación (**Tabla 8**). La proporción de sexos de *C. australe* entre las distintas especies de peces no fue significativamente diferente, aunque en la mayoría de las especies de peces la proporción se encontró sesgada hacia las hembras (**Fig. 16**). Por otra parte, la proporción global de sexos de *C. australe* en esta área se halló ligeramente sesgada hacia las hembras (porcentaje de hembras: 43,9%, Intervalos de confianza al 95%: 39,0–48,8) (**Fig. 16**). La diferencia global entre machos y hembras a nivel de infrapoblación fue altamente significativa.

En los acantocéfalos, el sexo se determina cromosómicamente y se establece durante la fertilización (Crompton, 1985), por lo tanto, la proporción de sexos es probable que sea 1:1 en los cigotos. Sin embargo, en las formas adultas de acantocéfalos, la proporción de sexos ha sido encontrada muy sesgada hacia las hembras, incluso en varias especies de *Corynosoma* (p. ej. Aznar *et al*., 2001c, 2004; Nickol *et al*., 2002; Sinisalo *et al*., 2004). Este patrón se ha explicado generalmente por la mayor longevidad de las hembras adultas respecto a la de los machos, ya que las hembras requieren más tiempo para producir la descendencia (Crompton, 1985). Contrariamente a la situación observada en los adultos, en las larvas de acantocéfalos la proporción de sexos tendería a ser similar a la observada en los cigotos. Los resultados de este estudio sugieren que en los cistacantos de *C. australe* aparece una ligera desviación en la proporción de sexos hacia las hembras, independientemente de la especie de pez en la que ocurren. En teoría tres factores pueden estar influenciando en este sesgo hacia las hembras en los cistacantos de *C. australe*: errores de muestreo, muestreo sesgado de larvas machos y hembras, y/o diferente mortalidad entre ambos sexos. Independientemente de la razón por la cual la proporción de sexos está sesgada hacia las hembras de *C. australe* en los hospedadores paraténicos, una cuestión interesante es que un parte de este sesgo será transferido a los hospedadores definitivos. Este sesgo hacia las hembras posiblemente no tenga un impacto significativo en la dinámica poblacional de *C. australe* en sus hospedadores definitivos, pero aporta una nueva evidencia sobre otro factor que puede influir en la proporción de sexos observada en los adultos infectando otáridos.

6.3. Costos relacionados con la infección de hospedadores paraténicos

Por lo general, la expansión de las fases infectivas a través de la red trófica se considera una adaptación cuando los hospedadores definitivos tienen una gran plasticidad en sus hábitos alimenticios. Sin embargo, un aspecto poco estudiado en esta idea es el tipo de costes que la infección a hospedadores paraténicos o la transmisión entre estos hospedadores pueden suponer al parásito. Los costos más obvios son aquellos asociados a la infección de hospedadores inadecuados. Muchas especies de pez pueden actuar como "sumidero" para la población de *C. australe* (p. ej. los caballitos de mar *Hippocampus patagonicus,* ver **Tabla 5**) ya que son raramente consumidas por otáridos. Otras especies de pez de gran tamaño corporal pueden acumular un gran número de cistacantos de *C. australe,* sin embargo existen limitaciones físicas relacionadas con el tamaño de las presas que los otáridos pueden ingerir (Koen-Alonso *et al.*, 2000), lo que sugiere que estos peces probablemente también actúen como "sumidero" para *C. australe*. Finalmente, existe la posibilidad de que todas las especies de peces no sean fisiológica o inmunológicamente adecuados para *C. australe*. El sistema inmune de los hospedadores paraténicos pueden implicar costos para los cistacantos de *C. australe.* Los resultados de este estudio sugieren que *A. patachonicus* podría actuar como un hospedador no adecuado, debido al alto número de cistacantos degradados que se observaron únicamente en esta especie de pez. Sin embargo, habrá que desarrollar estudios histopatológicos para resolver esta cuestión.

6.4 Influencia del nivel trófico del pez en el tamaño de **C. australe**

El nivel trófico de las mayoría de las especies de peces, es decir, su posición en la red trófica determinado por el número de pasos en la transferencia de energía hasta ese nivel, se obtuvo de Barraza Bernardas (2009), Timi *et al.* (2011b) y Froese & Pauly (2013). Para la mayoría de los cistacantos de *C. australe* se obtuvo la longitud del tronco y el diámetro del disco (**Fig. 13**). A continuación se calculó el volumen corporal asumiendo que el cuerpo tiene una forma cónica (ver Hernández-Orts *et al.*, 2012). Los valores promedio de las variables que están relacionadas con el tamaño del cuerpo, medidas en los cistacantos de *C. australe* en cada especie de pez, se muestran en la **Tabla 9**. Para este estudio, el efecto del nivel trófico y la intensidad de infección sobre el tamaño corporal de *C. australe* fue investigado utilizando modelos mixtos (Paterson & Lello, 2003). El análisis de estos modelos indicó que, basados en los valores del criterio de Akaike (AIC), el mejor modelo incluyó únicamente 2 parámetros, la media general (origen) y la variación aleatoria asociada a las especies de hospedador (**Tabla 10**). Sin embargo el modelo que incluyo también el nivel trófico como un factor fijo generó un ΔAIC bajo (**Tabla 10**), sugiriendo que este modelo también recibió un apoyo considerable. Este último modelo indicó un efecto débil, pero significativo, del nivel trófico. Todos los demás modelos, incluyendo aquellos con supuestos efectos de la intensidad de infección, recibieron menor soporte empírico (**Tabla 10**).

Los resultados de este estudio sugieren que los costes energéticos que *C. australe* afronta durante la transmisión entre hospedadores paraténicos son sutiles. En general, se ha sugerido que los cistacantos no crecen ni se desarrollan dentro de los hospedadores paraténicos (Kennedy, 2006; Schmidt, 1985). Los factores que llevan a los cistacantos a dejar de crecer o desarrollarse en estos hospedadores son desconocidos. Sin embargo, los beneficios energéticos y los costos durante la infección en hospedadores paraténicos pueden ser descritos teóricamente (**Fig. 19**). Después de la activación del cistacantos en el sistema digestivo del hospedador paraténico, existe la posibilidad de que la larva absorba nutrientes o no. En cualquiera de los casos, la larva tiene que incurrir en un costo energético al migrar hacia una posición extra intestinal, donde los nutrientes son posiblemente poco disponibles (Crompton, 1973) y encapsularse. Los cistacantos encapsulados entrarán eventualmente en un periodo de latencia, en el cual el consumo de energía es mínimo (Petrochenko, 1956). En teoría cuatro combinaciones de resultados son posibles (ver **Fig. 19**): (**A**) El parásito absorbe nutrientes en el hospedador paraténico. En este caso ***1***) la energía adquirida puede ser mayor a los costos durante la migración y encapsulación, por lo que el parásito debería de crecer, lo que podría ser detectable en la medida en que las reservas no se agotan durante el período de latencia o ***2***) la energía adquirida es menor a los costos de migración y encapsulación, por lo que el tamaño corporal debería disminuir. (**B**) El parásito no absorbe nutrientes y utiliza sus propias reservas energéticas para realizar la migración y encapsulación. En este caso ***1***) no deberían de observarse cambios notables en el tamaño del parásito si el costo es insignificante o ***2***) debería de ocurrir una reducción en el tamaño corporal si los costos durante la migración y encapsulación son significantes.

Estos escenarios se vuelven más complejos cuando existe transmisión entre hospedadores paraténicos. En particular, los efectos en el tamaño corporal del parásito en los escenarios "**A1**", "**A2**" y "**B2**" se amplificarían en cada paso trófico. La ligera, pero estadísticamente significativa tendencia a la disminución del tamaño corporal de los cistacantos de *C. australe*, que no está relacionada con efectos de hacinamiento, sugiere que este acantocéfalo incurren en costes energéticos no despreciables cuando se transfieren entre hospedadores paraténicos (escenarios "**A2**" y "**B2**"). Las implicaciones de este hallazgo no deben ser subestimados, ya que esta consecuencia negativa puede tener un papel importante en la dinámica poblacional de helmintos transmitidos tróficamente.

7. Patrones de crecimiento de espinas del tronco en dos especies congenéricas de acantocéfalos

Durante su historia evolutiva los parásitos han desarrollado una gran variedad de mecanismos de fijación para anclarse exitosamente cuando son reclutados por sus hospedadores y minimizar así los riesgos de desprenderse posteriormente (Poulin, 2009; Randhawa & Poulin, 2010). Los acantocéfalos, en particular, han desarrollado una probóscide armada con ganchos para fijarse al tejido de su hospedador definitivo (Taraschewski, 2000). Adicionalmente, muchas otras especies de acantocéfalos poseen espinas en el tronco (parte posterior del cuerpo) que se enganchan a la superficie del tejido del hospedador y que juegan un papel muy importante en la fijación de algunas especies (Aznar *et al.*, 1999, 2002; Van Cleave, 1952).

En los acantocéfalos, la probóscide y las espinas del tronco se generan mucho antes de ser utilizadas para fijarse al hospedador definitivo, lo que ha originado un gran interés sobre la estrategia ontogenética de inversión en estas estructuras por estos parásitos. Los estudios preliminares han sugerido que las estructuras de fijación están completamente desarrolladas en las larvas (cistacantos) (Petrochenko, 1956; Van Cleave, 1952). Sin embargo, el grado en que la probóscide, los ganchos de la probóscide o las espinas del tronco crecen durante el desarrollo hacia las formas adultas es desconocido, ya que la información disponible actualmente es especialmente contradictoria (p. ej. Amin, 1986, 1987; Amin *et al.*, 1995, 2004; Podesta & Holmes, 1970).

Por otro lado, es muy probable que las estrategias de inversión en las estructuras de fijación durante la ontogenia entre especies de acantocéfalos difieran dependiendo de su tamaño corporal. En el tracto digestivo de su hospedador definitivo, los acantocéfalos adultos están sujetos al flujo de los alimentos digeridos generados por movimientos peristálticos (Poulin, 2007). Teóricamente, los acantocéfalos deben de experimentar 3 tipos de fuerza que tenderán a desprender a los parásitos del tejido del hospedador donde están anclados y que están relacionadas con el flujo: arrastre por fricción, arrastre por presión y reacción por aceleración. Estas fuerzas son proporcionales, a su vez, al área de superficie, área de sección y volumen del cuerpo de los parásitos respectivamente (ver Koehl, 1984, para más detalles). Por lo tanto, las fuerzas que tienden a desprender a los acantocéfalos aumentarán de forma desproporcionada a medida que el cuerpo del parásito crezca, por lo que cabría esperar que los acantocéfalos con un tamaño corporal mayor necesiten ajustar de forma más precisa sus estructuras de fijación durante el crecimiento de los adultos, especialmente si experimentan un cambio corporal mayor entre los cistacantos y las formas adultas (Poulin *et al.*, 2003).

En este estudio se comparan el tamaño de las espinas del tronco entre cistacantos y adultos de dos especies congenéricas de acantocéfalos del hemisferio sur: *C. australe* y *C. cetaceum*, que se diferencian en su tamaño corporal (ver Fig. 1, página 108). Ambas especies de acantocéfalos parasitan diferentes microhábitats y especies de hospedadores. *C. australe* habita el intestino, principalmente en el íleon y el yeyuno de pinnípedos, mientras que *C. cetaceum* se encuentra en el estómago y la parte anterior del duodeno de cetáceos pequeños (Aznar *et al.*, 2001b, 2004, 2012; Sardella *et al.*, 2005). Este trabajo se centró en las espinas del tronco debido a que estas estructuras tienen un papel importante en la fijación de las especies de *Corynosoma* (Aznar *et al.*, 1999, 2001b; Van Cleave, 1952) y pueden ser medidas en cualquier espécimen. Los objetivos de este estudio han sido dos: *1*) se obtuvo, por primera, vez evidencia estadística sobre el crecimiento de las espinas durante el desarrollo de las formas adultas de acantocéfalos, y *2*) se investigaron los factores que pueden explicar los patrones de crecimiento de las espinas, incluyendo el tamaño corporal.

Para cada espécimen de acantocéfalo se calcularon cuatro variables que están relacionadas con el tamaño del cuerpo y con la probabilidad de ser desprendidos del tejido del hospedador: *1*) área de disco, *2*) área de sección, *3*) área de superficie, y *4*) volumen. También se midió la longitud de las espinas en tres sitios: *1*) el borde del disco, *2*) la región entre los dos pliegues que se forman típicamente en el tronco de las especies del género *Corynosoma* y *3*) la región posterior del tronco (ver Fig. 2, página 109, para más detalles).

Los resultados de este estudio sugieren que los machos de ambas especies de acantocéfalos son significativamente más grandes que las hembras. El dimorfismo sexual es mucho más pronunciado en *C. cetaceum* que en *C. australe* (ver, Fig. 4, página 112). Por otro lado, la comparación intraespecífica en el crecimiento corporal relativo entre cistacantos y adultos mostró diferencias altamente significativas, sugiriendo que durante el desarrollo los ejemplares de *C. cetaceum* (especialmente los machos) crecen comparativamente más que los de *C. australe.*

Con respecto a las espinas del tronco, los ejemplares de *C. cetaceum* tuvieron espinas más grandes que los de *C. australe*, independientemente del estado de desarrollo y del sexo. Sin embargo, las espinas fueron mucho más largas en las hembras que en los machos en ambas especies de acantocéfalos, a pesar que las hembras son significativamente más pequeñas. Los patrones de crecimiento de las espinas presentaron diferencias significativas entre sexos y especies. No se observaron diferencias en el tamaño de las espinas entre cistacantos y adultos en los machos. Sin embargo, las espinas fueron significativamente más largas en las hembras adultas de ambas especies comparadas con las de las hembras cistacantos. En las hembras de *C. cetaceum* todas las espinas crecieron significativamente, mientras que únicamente las espinas del área del disco crecieron en las hembras de *C. australe* (ver Fig. 5, página 113).

En lo que se refiere a la relación entre el tamaño corporal y el tamaño de la espinas, no se detectó ninguna relación significativa entre estas dos variables en cistacantos y adultos machos de ambas especies. Por otro lado, únicamente en las hembras de *C. cetaceum* se observó una relación significativa entre la longitud de la espina y el tamaño corporal, tanto en cistacantos como adultos, pero sólo en las espinas de la parte posterior del tronco (ver Fig. 6, página 114). Esto sugiere que las hembras de

C. cetaceum ajustan exclusivamente el tamaño de las espinas posteriores del tronco con respecto a su tamaño corporal final.

Las diferencies sexuales en el tamaño de las estructuras de fijación no parecen estar únicamente asociadas a las espinas del tronco en ambas especies de *Corynosoma*. Los resultados de este estudio sugieren también que otras estructuras (p. ej. área del disco, tamaño de la probóscide o de sus ganchos, etc.) son de igual o menor tamaño en los machos que los observados en las hembras. Esto sugiere que las estructuras de fijación están menos desarrolladas en los machos, mientras que las hembras invierten más en estas estructuras, principalmente en las etapas finales del desarrollo.

El desarrollo de estructuras de fijación más eficientes en las hembras parece estar asociado a otros factores distintos al tamaño corporal, los cuales ejercerán una alta presión selectiva. En este contexto, Petrochenko (1956) propone que las hembras adultas de acantocéfalos necesitan desarrollar estructuras de fijación más grandes que los machos, ya que deben permanecer más tiempo dentro del hospedador definitivo, para producir y liberar los huevos. Siguiendo este argumento, el mayor tamaño de las espinas en las hembras, podría ser una adaptación para reducir la probabilidad de desprenderse debido al paso de los alimentos digeridos generados por los movimientos peristálticos (Poulin, 2009). Esta hipótesis parece estar sustentada por dos líneas de evidencia: *1*) las hembras de ambas especies de *Corynosoma* parecen tener una mayor longevidad que los machos, por la sesgada proporción de sexos hacia las hembras en sus hospedadores definitivos (Aznar *et al.*, 2001b, 2004); *2*) las condiciones de flujo tan extremas en el tracto digestivo de los mamíferos marinos carnívoros, podrían haber generado distintas estrategias ontogenéticas de inversión y desarrollo en las estructuras de fijación para machos y hembras de estos acantocéfalos.

Este estudio también sugiere que los patrones de crecimiento de las espinas difieren entre las hembras de ambas especies de *Corynosoma*. Los distintos microhábitats y especies de hospedadores en los que cada especie de *Corynosoma* encuentra podrían explicar las diferencias encontradas entre *C. australe* y *C. cetaceum*. Sin embargo, en la actualidad no existen datos cuantitativos sobre variables críticas de sus microhábitats (p. ej. viscosidad del quimo o la velocidad del flujo de los alimentos digeridos) o de sus regímenes ecológicos (p. ej. alteración física o disponibilidad de alimento) que permitan realizar conclusiones contrastables sobre estos aspectos. Por lo tanto, los factores que influyen sobre la morfología y los patrones de crecimiento entre estas especies de acantocéfalos continúan siendo desconocidos. Sin embargo este estudio sugiere que existe una mayor necesidad de ajustar el tamaño de la espina en hembras de *C. cetaceum*, la especie con una mayor tasa de crecimiento corporal y que por lo tanto, estará sujeta a mayores fuerzas que tenderán a desprenderla del tejido del hospedador (véase más arriba), así como una posible mayor longevidad.

En conclusión, en este trabajo se proporcionó por primera vez, una evidencia estadística sobre el crecimiento de las estructuras de fijación durante el desarrollo en el hospedador definitivo de dos especies de acantocéfalos. Asimismo, sugiere que las estrategias ontogenéticas de inversión en las estructuras de fijación pueden diferir no sólo entre especies congenéricas, sino también entre sexos de una misma especie, posiblemente por las diferentes presiones selectivas a las que se enfrentan cada subconjunto de la población.

8. Descripción, microhábitat y patrones de infección de larvas pertenecientes al complejo de especies de *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) en peces de Patagonia, Argentina

Los nematodos anisáquidos pertenecientes al complejo de especies *Pseudoterranova decipiens*, comprende en la actualidad 6 especies que maduran y se reproducen en el tracto gastrointestinal de pinnípedos (Mattiucci & Nascetti, 2007, 2008; McClelland, 2002; Zhu *et al.*, 2002). En Sudamérica, larvas 3 de *Pseudoterranova* spp. se han registrado en más de 40 especies de peces marinos (**Tabla 11**), muchas de las cuales poseen un alto valor comercial. Estos nematodos están asociados a enfermedades zoonóticas, cuando los humanos consumen pescado crudo o poco cocinado (McClelland, 2002). No obstante, a pesar de la importancia económica y zoosanitaria de estos parásitos, la identificación específica de las larvas de *Pseudoterranova* mediante técnicas morfológicas y/o moleculares continúa siendo escasa en este subcontinente.

En este estudio se proporcionan por primera vez datos moleculares, morfológicos y ecológicos de larvas 3 de *Pseudoterranova* sp., recolectadas en 12 especies de peces marinos de la costa patagónica argentina. En primer lugar, se realizó una identificación molecular de las larvas, seguida por una descripción morfológica detallada. A continuación, se compararon datos morfométricos de larvas 3 recolectadas en distintas especies de peces para investigar patrones de variabilidad morfológica. Por último se citan los niveles de infección y los patrones de distribución (microhábitat) de estas larvas 3 en las distintas especies de peces.

Las secuencias obtenidas del gen del citocromo c oxidasa subunidad 1 (cox 1) de 3 especímenes de larvas 3 de *Pseudoterranova* sp. recolectadas en *P. nudigula* formaron un clado común bien soportado junto con las secuencias de ejemplares adultos de *Pseudoterranova cattani* del lobo de un pelo en Chile, a su vez distinto de las otras 5 especies de *Pseudoterranova* (**Fig. 21** y **Tabla 13**). La divergencia genética media entre las especies oscilaron entre 5,5 y 11,9%. Por otro lado, la divergencia genética intraespecífica media varió entre 1,1 y 1,9%, teniendo con *P. cattani* un 1,4 ± 0,4% de divergencia (incluyendo las 3 secuencias obtenidas de larvas 3 recolectadas en *P. nudigula*).

Los datos morfométricos obtenidos de larvas 3 de *P. cattani* recolectadas en *P. nudigula* y de larvas 3 de *Pseudoterranova* sp. recolectadas en *P. brasiliensis* y *A. patachonicus* mostraron diferencias altamente significativas entre ellos. Las variables morfométricas que se diferenciaron significativamente entre los nematodos de diferentes especies de peces fueron: longitud corporal, anchura del cuerpo, distancia de la parte anterior del cuerpo al anillo nervioso, longitud del esófago, longitud del ventrículo glandular y longitud del ciego intestinal. La comparación morfométrica de las larvas recolectadas en peces de Patagonia con otras larvas 3 de *P. cattani* de otras especies de peces marinos de Chile se presentan en la **Tabla 14.** En general, no se observaron diferencias morfométricas claras entre las larvas de *Pseudoterranova* de ambas áreas geográficas, excepto que algunos ejemplares recolectados en *A. brasilianus* eran aparentemente más pequeños.

Los datos moleculares obtenidos de las larvas 3 recolectadas en *P. nudigula* sugieren que estas larvas pueden ser identificadas como *P. cattani.* En la actualidad, *P. cattani* es la única especie dentro del complejo de especie *P. decipiens* identificada mediante técnicas moleculares en Sudamérica (Mattiucci & Nascetti, 2008; Zhu *et al.*, 2002). Según un estudio molecular preliminar, en la costa atlántica argentina, se identificaron larvas de *P. cattani* infectando diversas especies de peces marinos (.Timi *et al.*, 2011a) Asimismo, formas adultas de esta especie de anisáquido han sido citada recientemente en otáridos de la costa patagónica argentina (Hernández-Orts *et al.*, 2013), por lo que la identificación molecular de las larvas 3 de esta especie en *P.*

nudigula recolectadas en localidades próximas es congruente con la distribución geográfica de las formas adultas.

Por otro lado, las larvas 3 de *Pseudoterranova* recolectadas en otras especies de peces en el presente estudio probablemente también correspondan a la especie *P. cattani*. Sin embargo, en la actualidad la identificación correcta de las formas larvarias de estos nematodos se basa principalmente en marcadores genéticos. Por lo tanto, estas larvas fueron identificadas provisionalmente como *P.* cf. *cattani* hasta que futuros estudios morfológicos y moleculares sean desarrollados.

En este estudio, se recolectaron un total de 635 larvas 3 pertenecientes a especies del género *Pseudoterranova* en 12 especies de peces marinos de la costa patagónica argentina. Los parámetros de infección de larvas 3 de *Pseudoterranova* en las distintas especies de peces se presentan en la **Tabla 15**. Cinco especies de peces marinos, *C. gobio*, *N. bergi*, *M. argentinae*, *P. brasiliensis* y *P. nudigula*, representan nuevos registros de hospedador para estos nematodos anisákidos. La comparación en la abundancia de larvas 3 de *Pseudoterranova* entre las especies de peces reveló diferencias altamente significativas, indicando que únicamente *P. nudigula* albergó significativamente más larvas de *Pseudoterranova* que cualquier otra especie de pez. La mayor parte de las larvas se recolectaron en el músculo (principalmente la musculatura epiaxial, seguida por la musculatura hipoaxial), y en menor medida, en los mesenterios y el hígado (**Tabla 15**). Estos resultados sugieren que el microhábitat principal de las larvas de *Pseudoterranova* sp. en peces de la costa patagónica es la musculatura, en particular la región epiaxial para las larvas 3 de *P. cattani* en *P. nudigula*.

En las costa patagónica argentina *P. nudigula* parece actuar como hospedador primario en el ciclo de vida de *P. cattani.* Los peces hospedadores primarios son, en general consumidores bentónicos, que adquieren el parásito directamente de hospedadores invertebrados, y son esenciales en la dispersión temporal y espacial de las larvas (McClelland, 2002). Por otro lado, los resultados sugieren que *P. brasiliensis* puede ser considerado como hospedador secundario, debido a la alta intensidad de larvas encapsuladas en la musculatura. Este tipo de hospedadores son generalmente grandes peces demersales que adquieren los parásitos al alimentarse de peces más pequeños (McClelland, 2002).

El presente estudio sugiere que las presas más importantes en la dieta del lobo de un pelo, es decir *E. anchoita*, *M. hubbsi* y *R. brasiliensis*, presentan niveles bajos de larvas de *Pseudoterranova*. Sin embargo, la trasmisión y reclutamiento de estos nematodos parecen estar garantizados en el lobo de un pelo, debido al alto número de especímenes de cada una de estas especie de peces citados en su dieta, especialmente en el caso de *M. hubbsi* (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). Asimismo, los lobos pueden infectarse ocasionalmente con estos nematodos al alimentarse de pequeños peces bentónicos (con altas prevalencias) o grandes peces pelágicos (con altas intensidades). Estos resultados sugieren que la transmisión de las larvas 3 de *P. cattani* en la costa patagónica argentina parece estar relacionada con la amplia variedad de presas consumidas por los lobos marinos, los cuales incluyen un gran número de especies de peces asociadas a la zona bentónica, donde se produce probablemente la transmisión de este nematodo en esta área.

9. Conclusiones

En el presente estudio, se caracterizaron por primera vez las helmintofaunas intestinales de 56 lobos marinos de un pelo *Otaria flavescens* (30 machos y 26 hembras) y 5 lobos marinos de dos pelos *Arctocephalus australis* (4 machos y 1 hembra) procedentes de la costa norpatagónica en Argentina. Adicionalmente, se analizaron parasitológicamente un total de 542 peces marinos de 20 especies, también recolectados en la costa patagónica, para obtener formas larvales de estos parásitos de lobos marinos. Como resultado de las investigaciones realizadas en el presente estudio, se obtuvieron las siguientes conclusiones:

9.1. La helmintofauna intestinal de *O. flavescens* de la costa patagónica en Argentina comprendió 11 taxones (1 trematodo, 1 cestodo, 5 nematodos y 4 acantocéfalos). Se encontraron individuos grávidos en 6 especies: *Ascocotyle* (*Ascocotyle*) *patagoniensis*, *Contracaecum ogmorhini* (*s.s.*), *Corynosoma australe*, *Diphyllobothrium* spp., *Pseudoterranova cattani* y *Uncinaria hamiltoni*. También se recolectaron larvas 3 de los nematodos *Anisakis* sp. tipo I y *Contracaecum* sp., así como especímenes juveniles de *Andracantha* sp., *Corynosoma cetaceum* y *Profilicollis chasmagnathi*. Cuatro especies de parásitos, *Andracantha* sp., *A.* (*A.*) *patagoniensis*, *C. ogmorhini* (*s.s.*) y *P. chasmagnathi*, representan nuevos registros para este hospedador.

9.2. En los intestinos de *A. australis* de la costa patagónica, se recolectaron parásitos pertenecientes a 7 taxones (2 cestodos, 3 nematodos y 2 acantocéfalos). Cuatro especies de parásitos incluían individuos grávidos: *C. ogmorhini* (*s.s.*), *C. australe*, *Diphyllobothrium* spp., y *P. cattani*. También se recolectaron larvas 3 de *Contracaecum* sp. y especímenes juveniles de *C. cetaceum*.

9.3. En la costa norpatagónica, los lobos marinos de uno y dos pelos albergaron las comunidades de helmintos intestinales predecibles para otáridos en todo el mundo, es decir, la combinación de especies de los géneros *Corynosoma*, *Diphyllobothrium*, *Pseudoterranova* y *Contracaecum* en los adultos, y en los cachorros adicionalmente *Uncinaria*.

9.4. La estimación de los parámetros de las comunidades de helmintos en lobos marinos de uno y dos pelos, especialmente la riqueza de las comunidades componentes, se ha visto afectada significativamente por la inclusión o exclusión de los parásitos para los cuales ambas especies de otáridos no parecen ser hospedadores adecuados (es decir aquellos en los que el parásito es incapaz de reproducirse).

9.5. Una nueva especie de trematodo heterófido se ha descrito del intestino del lobo marino de un pelo, *Ascocotyle* (*Ascocotyle*) *patagoniensis*. La descripción morfológica detallada, junto con el análisis morfométrico, sugiere que este trematodo puede distinguirse de otras especies del subgénero por el número de espinas circunorales, (dispuestas en 2 filas de 18 a 23 espinas), por tener un gonotilo sin papilas y por su amplio receptáculo seminal.

9.6. Ninguna metacercaria de *Ascocotyle* spp. fue encontrada en 542 peces marinos pertenecientes a 20 especies marinos recolectados a lo largo de la plataforma patagónica argentina. La ausencia de metacercarias en peces marinos habitando esta área de la plataforma puede estar relacionada con el hecho de que el ciclo de vida de este trematodo esté restringido a aguas litorales. Sin embargo, para confirmar esta hipótesis se recomienda analizar más peces, ya que las metacercarias son minúsculas y podrían haber pasado desapercibidas, especialmente en las especies en las que el tamaño muestral fue bajo.

9.7. Un total de 1367 cistacantos de *Corynosoma australe* fueron recolectados en 18 especies de peces marinos procedentes de la costa patagónica. Las especies de peces más infectadas fueron: *A. patachonicus*, *P. isosceles*, *P. nudigula* y *X. rasile*. Ocho especies de peces: *A. patachonicus, B. brama*, *C. peruvianus*, *C. gobio, G. blacodes*, *P. ramsayi*, *S. porosa* y *S. brasiliensis* representan nuevos hospedadores para *C. australe*.

9.8. Los resultados del presente estudio demostraron que los cistacantos de *C. australe* son capaces de infectar y colonizar una gran variedad de especies de peces, que actúan como hospedadores paraténicos. La ubicuidad de este acantocéfalo a través de la cadena trófica garantizaría la infección de sus hospedadores definitivos a través de varias presas alternativas. Sin embargo, este estudio sugiere que *R. brasiliensis* es una de las presas que más contribuye en la transmisión de los cistacantos de *C. australe* en esta área, debido a la alta prevalencia de este acantocéfalo en este pez, así como por su alta importancia relativa como presa en la dieta de ambas especies de lobos marinos.

9.9. Se han encontrado diferencias significativas en los niveles de infección de *C. australe* entre peces de diferentes zonas de la columna de agua, siendo las especies asociadas a la zona bentónica las que presentaron las mayores infecciones por estos cistacantos. Este estudio sugiere que al menos dos factores pueden estar provocando directamente diferencias en los niveles de infección de *C. australe* entre peces de diferentes zonas: *1*) la distribución de los hospedadores intermediarios invertebrados y *2*) los patrones de transmisión de los cistacantos entre peces hospedadores paraténicos a través de las redes tróficas.

9.10. La proporción global de sexos de los cistacantos de *C. australe* infectando hospedadores teleósteos estuvo ligera, pero significativamente, sesgada hacia las hembras. Asimismo, no se encontraron diferencias significativas entre la proporción de sexos de estos cistacantos entre las diferentes especies de peces. Esto sugiere que la proporción de sexos se encuentra sesgada mucho antes de que los cistacantos de *C. australe* infecten al hospedador definitivo, en donde la proporción de sexos se encuentra considerablemente sesgada hacia las hembras debido a su mayor longevidad. En otras palabras, parte de este sesgo en la proporción de sexos que se observa en los hospedadores definitivos sería transferido desde los hospedadores paraténicos. En teoría tres factores pueden estar influenciando en la proporción de sexos sesgada hacia las hembras en los cistacantos de *C. australe*: errores de muestreo, muestreo sesgado de larvas machos y hembra, y/o diferente mortalidad entre ambos sexos.

9.11. Este estudio analiza, por primera vez, los costos potenciales que los helmintos transmitidos tróficamente afrontan durante la transmisión entre hospedadores paraténicos. Los resultados sugieren que algunas especies de peces, en particular *Acanthistius patachonicus*, en realidad podrían actuar como hospedadores paraténicos inadecuados para *C. australe*, debido a que la mayoría de los cistacantos encontrados en esta especie de pez no eran viables. Además, se observó una ligera, pero estadísticamente significativa tendencia a la disminución del tamaño corporal de los cistacantos a medida que el nivel trófico de las especies de peces se incrementaba. Esta tendencia sugiere que *C. australe* puede incurrir en costos energéticos no despreciables cuando se trasmite entre hospedadores paraténicos. Las implicaciones de este hallazgo no deben ser subestimadas, ya que esta consecuencia negativa puede tener un papel importante en la dinámica poblacional de helmintos transmitidos tróficamente.

9.12. El presente estudio ha comparado, el tamaño de las estructuras de fijación entre cistacantos y adultos de acantocéfalos utilizando, por primera vez, estadística inferencial. Los resultados sugieren que el tamaño de las espinas aumenta entre cistacantos y adultos de *C. australe* y *C. cetaceum* (parásito de cetáceos), pero únicamente en las hembras, que presentan espinas significativamente más largas que los machos. Este dimorfismo sexual no es un resultado alométrico, ya que el cuerpo de las hembras es más pequeño y no crece más que el de los machos. No obstante, las hembras son más longevas y, por lo tanto, este factor podría inducir diferencias en las estrategias de inversión y desarrollo de las espinas con el fin de soportar durante más tiempo las extremas condiciones de flujo en el tracto digestivo de los mamíferos marinos.

9.13. Inesperadamente, los patrones de crecimiento de las espinas han diferido aparentemente entre ambas especies de *Corynosoma*. En *C. cetaceum* todas las espinas crecieron, mientras que en *C. australe* solo las espinas del área del disco difirieron entre cistacantos y adultos. Una explicación de estas diferencias es que las hembras de *C. cetaceum* ajustan el tamaño de las espinas con respecto a su tamaño durante su desarrollo en el hospedador definitivo, ya que éstas adquieren un tamaño adulto mayor. Esta característica está relacionada con mayores fuerzas de arrastre y, posiblemente, con una mayor longevidad.

9.14. Este estudio ha aportado evidencias que indican que los mecanismos de fijación en los acantocéfalos parecen estar completamente desarrollados antes de infectar a los hospedadores definitivos. Asimismo, sugiere que la estrategia ontogenética de inversión en las estructuras de fijación no difiere solamente entre especies congenéricas, sino también entre sexos de una misma especie. Este hecho es debido, posiblemente, a las diferentes presiones selectivas a las que se enfrenta cada subconjunto de la población.

9.15. Un total de 635 larvas 3 de nematodos anisáquidos pertenecientes a especies del género *Pseudoterranova* fueron recolectadas en 12 especies de peces marinos procedentes de la costa patagónica. Los peces más infectados con larvas de *Pseudoterranova* fueron *P. nudigula* seguida de *A. patachonicus*, *P. isosceles*, *P. brasiliensis* y *P. semifasciata*. Cinco especies de peces, *C. gobio*, *N. bergi*, *M. argentinae*, *P. brasiliensis* y *P. nudigula* representan nuevos hospedadores para larvas de *Pseudoterranova*.

9.16. Las secuencias obtenidas del gen citocromo c oxidasa subunidad 1 (cox 1) de las larvas de *Pseudoterranova* recolectadas en *P. nudigula*, formaron un linaje monofilético recíproco con las secuencias publicadas de *P. cattani* recolectados de hospedadores definitivos. En este estudio se proporcionó una descripción morfológica y morfométrica detallada de las larvas de *P. cattani* de *P. nudigula*. Otras larvas de *Pseudoterranova* recolectadas en otras especies de peces no se diferenciaron morfológicamente de las larvas 3 de *P. cattani* de *P. nudigula*. Sin embargo, los resultados de los análisis morfométricos comparativos realizados entre las larvas 3 de *Pseudoterranova* de diferentes especies de peces indicaron diferencias significativas en algunas dimensiones. A pesar de esto, estas larvas 3 se identificaron provisionalmente como *P.* cf. *cattani*, en espera de futuras identificaciones moleculares basadas en marcadores genéticos.

9.17. Los resultados de este estudio han sugerido que el principal microhábitat para las larvas de *Pseudoterranova* de peces de la costa patagónica es el músculo (principalmente epiaxial) y, en una menor medida, los mesenterios y el hígado.

9.18. Las evidencias obtenidas en este estudio han sugerido que los peces que actúan como presas más importantes para los otáridos que habitan la costa patagónica presentan niveles de infección bajos de larvas de *Pseudoterranova*. Sin embargo dado que *P. cattani* es específico de otáridos, la transmisión de este nematodo parece estar relacionada con la amplia variedad de presas consumidas por los lobos marinos, las cuales incluyen un gran número de especies de peces asociadas a la zona bentónica, donde se produce probablemente la transmisión de este nematodo en esta área.

1. GENERAL INTRODUCTION

1.1. PINNIPEDS, THE FIN-FOOTED MAMMALS

Pinnipeds, also known as fin-footed mammals, are included in the suborder Caniformia Kretzoi, 1938 within the order Carnivora Bowdich, 1821 (see Wilson & Reeder, 2011). They are considered to have originated in the North Pacific during the late Oligocene (32-24 mya), from an early aquatic carnivore with well-developed paddle-shaped limbs and feet (Berta, 2009a; Berta *et al.*, 2006; Higdon *et al.*, 2007; and references therein). Pinnipedia (Illiger, 1811) is composed of 3 (monophyletic) families: Otariidae Gray, 1825 (eared or walking seals), Phocidae Gray, 1821 (true or earless seals) and Odobenidae Allen, 1880 (walruses) (Berta, 2009b; Wilson & Reeder, 2011). Pinnipeds are amphibious and highly specialized aquatic carnivores, living and feeding in a wide range of marine and freshwater habitats. One unifying feature of the group is that all of them must return to a solid substrate (land or ice) to bear their pups (Jefferson *et al.*, 1993).

1.1.1. The family Otariidae

Traditionally, the family Otariidae has been subdivided into the Otariinae (von Boetticher, 1934) or sea lions, and the Arctocephalinae (von Boetticher, 1934) or fur seals (Brunner, 2003; Rice, 1998). However, new morphological and molecular data suggest that this subdivision is invalid (Berta & Churchill, 2012). Moreover, the alpha level taxonomy is controversial or poorly known for some species, suggesting that the number of species and subspecies may change as new morphological or molecular data are gathered (Berta & Churchill, 2012; Brunner, 2003; Rice, 1998). Nevertheless, morphologically, sea lions are characterized and readily distinguished from fur seals by their sparse pelage, whereas fur seals are named due their thick dense fur (Berta *et al.*, 2006). Modern otariids comprise 15 living species and 1 extinct species (**Table 1**). Otariids occupy a broad geographic distribution in both the Northern and Southern Hemispheres, from subpolar to tropical seas (**Table 1**).

Table 1. Species and subspecies of otariids according to Jefferson *et al.* (1993) and Wilson & Reeder (2005). The conservation status (CS) of each species is based on the red list of threatened species developed by the IUCN (2012)*.

Class Mammalia	Common name	Distribution	CS
Order Carnivora Bowdich, 1821			
Family Otariida Gray, 1825			
***Arctocephalus* Geoffroy Saint-Hilaire and Cuvier, 1826**			
A. pusillus (Schreber, 1775)	Brown fur seal		LC
A. pusillus doriferus (Wood Jones, 1925)	Australian fur seal	Southeastern Australia	
A. pusillus pusillus (Schreber, 1775)	South African fur seal	Southern African coastal regions	
A. australis (Zimmerman, 1783)	Southern fur seal	Coasts of Argentina, Brazil, Chile, Falkland Islands, Peru and Uruguay	LC
A. forsteri (Lesson, 1828)	New Zealand fur seal	Australia, New Zealand and nearby subantarctic islands	LC
A. galapagoensis Heller, 1904	Galapagos fur seal	Galapagos Islands (Ecuador)	EN
A. gazella (Peters, 1875)	Antarctic fur seal	Islands south of Antarctic Convergence	LC
A. philippii (Peters, 1866)	Juan Fernandez fur seal	Juan Fernandez and San Felix Islands (Chile)	NT
A. townsendi Merriam, 1897	Guadalupe fur seal	Guadalupe Island, Mexico and USA	NT
A. tropicalis (Gray, 1872)	Subantarctic fur seal	Islands north of Antarctic Convergence	LC
***Callorhinus* Gray, 1859**			
C. ursinus (L.)	Northern fur seal	North Pacific coastal regions in Canada, China, Japan, Mexico, Russia and USA	VU
***Eumetopias* Gill, 1866**			
E. jubatus (Schreber, 1776)	Steller's sea lion	Northern Pacific coastal regions of Canada, China, Japan, Russia, USA	NT
***Neophoca* Gray, 1866**			
N. cinerea (Péron, 1816)	Australian sea lion	Australian coastal region	EN
***Otaria* Péron, 1816**			
O. flavescens (Shaw, 1800) (syn. *O. byronia* de Blainville, 1820)	South American sea lion	Coasts of Argentina, Brazil, Chile, Peru, Uruguay and Falkland Islands	LC
***Phocarctos* Peters, 1866**			
P. hookeri (Gray, 1844)	New Zealand sea lion	New Zealand subantarctic islands	VU
***Zalophus* Gill, 1866**			
Z. californianus (Lesson, 1828)	California sea lion	Northeastern Pacific coastal regions of Canada, Mexico and USA	LC
Z. japonicus (Peters, 1866)	Japanese sea lion	Historical range: Sea of Japan, Russia and South Korea	E
Z. wollebaeki Sivertsen, 1953	Galapagos sea lion	Galapagos Islands (Ecuador)	EN

*Abbreviations: E, extinct; EN, endangered; LC, least concern; NT, near threatened; VU, vulnerable.

1.1.2. Biology of otariids

One of the main anatomic features that distinguish otariids from the other pinnipeds is the presence of external ear flaps or pinnae, hence their common name, “eared seals”. Another typical feature of sea lions and fur seals is their ability to rotate their rear flippers forward to walk, run or climb on land. In water they swim with their front large fore flippers, while the rear flippers trail behind and are only used in turning and stopping (Gentry, 2009; Jefferson *et al.*, 1993). Being able to move on land and water, otariids are considered truly amphibious: they feed at sea but rest, mate and rear their young on land. Other diagnostic osteological and soft anatomical characters of the family Otariidae are summarized in Berta *et al.* (2006).

All the species of sea lions and fur seals are polygamous and sexually dimorphic. Males are normally much larger than females and often have other secondary sex characteristics (*e.g.* a thick mane). During the breeding season males form and defend breeding territories on land where aggregations of females and pups occur. Pups are born few days after the arrival of the mother to mainland, and mating occurs after 4 to 11 days of perinatal nursing period (Gentry, 2009). Otariids differ from phocids in that the females of all species continue feeding while they are lactating (*i.e.* they are ‘income’ breeders). Females make a series of brief and regular nursing visits to shore to feed their pups. Once pups are able to swim, they continue feeding on milk until weaning, which lasts approximately 4 months at low latitudes, or 9 to 12 month at high latitudes (Gentry, 2009; Riedman, 1990).

Sea lions and fur seals are vigorous swimmers, propelling through water using paired flipper movements. Swimming speed varies among otariid species, with velocities ranging from 0.6 to 2.4 m/s under natural conditions (Boyd *et al.*, 1995; Crocker *et al.*, 2001; Ponganis *et al.*, 1990). Sea lions and fur seals are also efficient divers when they are foraging; some species can reach depths greater than 400 m and hold their breath for 15 min (Costa *et al.*, 2004; Schreer & Kovacs, 1997). Distance and time of foraging trips also vary interspecifically (*e.g.* Francis *et al.*, 1998; Kirkwood *et al.*, 2006; Riet-Sapriza *et al.*, 2012; Thompson *et al.*, 2003; Weise *et al.*, 2006), with some species traveling more than 1,000 km during several days before returning to a solid substrate (Chilvers *et al.*, 2005). Moreover, interspecific spatial segregation between sexes and developmental stages has been reported in species of otariids (Campagna *et al.*, 2001; Merrick & Loughlin, 1997; Staniland & Robinson, 2008).

A large number of prey species have been reported in the diet of sea lions and fur seals (*e.g.* Dellinger & Trillmich, 1999; Hume *et al.*, 2004; Koen-Alonso *et al.*, 2000; Lowry *et al.*, 1991; Sinclair & Zeppelin, 2002). Otariids are considered to be generalist predators that commonly feed underwater mainly on fish and cephalopods. Some species also consume crustaceans (mainly krill) and, to a lesser extent, birds or other seals (Berta *et al.*, 2006; Harcourt, 1993). Otariids' foraging behaviour is influenced by the abundance of prey and oceanographic features. Additionally, intrinsic factors such as age and sex can also affect diet of individuals, as their foraging tactics and behaviour can change over time, reflecting increased physiological capabilities and learning (Bowen *et al.*, 2009). It has been also suggested that sexual dimorphism could affect diet composition because body size influence the diving skills; larger individuals are capable of longer and deeper foraging dives (Drago *et al.*, 2009a; and references therein). Finally, recent studies suggest that females can change their foraging strategy after parturition to reduce the foraging trip duration, hence the time pups remain unattended on land (Drago *et al.*, 2010).

1.1.3. Exploitation and conservation

From late 18th to mid-20th centuries, most species of sea lions and fur seals were exploited for their pelts and fat on a massive scale. Overhunting pushed some populations to critical levels and, when acting together with other anthropomorphic threats, provoked the extinction of some species. After the protection laws in mid-Twentieth century, hunting was prohibited on most species of sea lions and fur seals. However, other anthropomorphic threats such as pollution, illegal hunting, incidental catches or resource competition with fisheries, have been causing a severe population decline of many otariid species (Crespo *et al.*, 1997; Plagányi & Buterworth, 2009; Reijnders *et al.*, 2009; Robertson & Chilvers, 2011). Moreover, natural threats, like changes on climate patterns, have been also suggested to compound conservation problems (Stevens & Boness, 2003; Trillmich & Limberger, 1985; Trites *et al.*, 2007).

At present, most otariid species are assigned a “Least Concern” label according to Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) (IUCN, 2012). Some species, however, are currently considered as “threatened” (see **Table 1**). On the other hand, species of the genus *Arctocephalus* are included in the Appendices I (*A. townsendi*) and II (other *Arctocephalus* spp.) of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, which prohibits trade between signatory countries (CITES, 2013).

1.2. OTARIIDS FROM THE ATLANTIC COAST OF ARGENTINA

At present, the most abundant otariid species in Argentina are the South American sea lion, *O. flavescens*, and South American fur seal, *A. australis* (Crespo *et al.*, 2008a, 2008d). Breeding colonies of both species are currently distributed all along the Argentine coast (Crespo & Pedraza, 1991; Crespo *et al.*, 1999; Dans *et al.*, 2004; Petracci *et al.*, 2010; Reyes *et al.*, 1999; Schiavini *et al.*, 2004; Túnez *et al.*, 2008a, 2008b). Two additional species, the Antarctic fur seal, *A. gazella*, and the Subantarctic fur seal, *A. tropicalis*, have also been reported along the Argentinean coast (Crespo *et al.*, 2008c), but records of these species in this region seem to correspond to wandering individuals outside their breeding and feeding areas.

1.2.1. The South American sea lion

The South American sea lion is the only member of the genus *Otaria*. Today, its specific name remains controversial and two names are commonly used: *O. byronia* (Blainville, 1820) and *O. flavescens*. Although the specific name of this species has been reviewed on several times (Brunner, 2003; Cabrera, 1940; Oliva, 1988; Rodriguez & Bastida, 1993), there is no general consensus on which is the valid name for this species. In this study, the traditional and more usual denomination for this species, *O. flavescens*, will be used throughout, following Wilson & Reeder (2005), in order to avoid confussions.

Sea lions are distributed from Recife das Torres (southern Brazil) to Zorritos (northern Peru) (Crespo, 1988; Vaz-Ferreira, 1982). Along the Argentine coast, more than 120 colonies have been reported (Dans *et al.*, 1996, 2004; Reyes *et al.*, 1999; Schiavini *et al.*, 2004; Túnez *et al.*, 2008a), with the highest concentrations occurring in the mainland and islands of the Patagonian region (Crespo, 1988). The current population of sea lions along the Patagonian coast of Argentina have been estimated to be about 45,000 individuals (Crespo *et al.*, 2012). Sea lion populations were heavily exploited during the first half of the Twentieth century (Godoy, 1963), particularly in northern Patagonia and Tierra del Fuego (Crespo & Pedraza, 1991). Since 1974, sea lions have been protected in the Argentinean coasts, and after several decades of stagnation (Reyes *et al.*, 1999; Schiavini *et al.*, 2004), the population is currently growing at an annual rate of increase of 5.7% (Dans *et al.*, 2004).

The South American sea lion is a highly dimorphic species (**Fig. 1**), with adult males reaching a maximum length of 266 cm and 300–350 kg in weight, while adult females, a maximum length of 204 cm and 100–150 kg in weight (Cappozzo & Perrin, 2009; Grandi *et al.*, 2010; Rosas *et al.*, 1993; Vaz-Ferreira, 1979b). The maximum age recorded for males and females are 19 and 21 years, respectively. Males become sexually mature at 9 years, and females at mature at 4.8 years, having their first birth between 4 and 5 years old (Grandi *et al.*, 2010).

The main events of the reproductive biology of *O. flavescens* in Argentina have been summarized by Cappozzo & Perrin (2009). The breeding and pupping season begins in mid-December and extends to early February. Adult males and females arrive at the breeding rookeries during the first half of December. Most of the pups are born in January, usually 2–3 days after the mother arrival at the rookery. Copulation occurs on land 6 days after parturition, and during the breeding season, males will attempt to mate with as many females as possible. Mothers stay with their pups for 2–3 days more and then go to forage offshore for 1–4 days; each foraging trip is followed by 2 days of nursing bouts on land. Lactation continues for 8–10 months.

Figure 1. Adult male and female, and pups of South American sea lion, *Otaria flavescens* during the reproductive season on the Patagonian coast of Argentina. (Courtesy from the Laboratorio de Mamíferos Marinos, Centro Nacional Patagónico).

South American sea lions feed mainly on fishes, cephalopods, crustaceans, gastropods, polychetes, sponges and tunicates (Hückstädt *et al.*, 2007; Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011; Suarez *et al.*, 2005). They are considered opportunistic and broad-spectrum feeders that are able to change their food habits according to their developmental stage, sex, or depending on prey availability and distribution (Drago *et al.*, 2009a, 2009b; George-Nascimento *et al.*, 1985; Koen-Alonso *et al.*, 2000).

Along the Patagonian coast, satellite tracking and dead-reckoning technology suggest that sea lions tend to forage close to the seabed and in the middle of the water column, with males exploiting deeper habitat near the shelf break and females being more restricted to coastal areas (Campagna *et al.*, 2001; Müller, 2004; Werner & Campagna, 1995). Notwithstanding, recent stable isotope analyses from skull bone fragments of sea lion from Patagonia suggest that both sexes preferentially consume benthic prey items, except in the case of senile males (13–19 years) which feed mainly on pelagic prey (Drago *et al.*, 2009a). In Patagonia, the diet of sea lions include a wide range of fish and cephalopod species; some of the dominant species (with a percent number higher than 10%) are the Argentine hake, *Merluccius hubbsi* Marini, 1933, banded cusk eel, *Raneya brasiliensis* (Kaup, 1856), Argentine anchovy, *Engraulis anchoita* Hubbs and Marini, 1935, Argentine shortfin, squid *Illex argentinus* (Castellanos, 1960), and Patagonian squid, *Loligo gahi* (Orbigny, 1835) (see Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011).

1.2.2. The South American fur seal

The geographical distribution of the South American fur seal, *A. australis*, largely overlaps with that of the South American sea lion (Túnez *et al.*, 2008b). On the Atlantic coast, over 80% of the populations are distributed on islands off the coast of Uruguay (Ponce de León & Pin, 2006). Along the Patagonian coast of Argentina, at least 17 colonies have been reported, with a total of 20,000 individuals (Crespo *et al.*, 1999). The highest concentrations occur on Isla Rasa and Isla Escondida, Chubut Province (Crespo *et al.*, 2008b; Túnez *et al.*, 2008b). Fur seals were also heavily exploited in the Patagonian coast since the 18th century (Carrara, 1952). Fortunately, the population in this region is increasing (Crespo *et al.*, 2008b).

South American fur seals also are a highly dimorphic species (**Fig. 2**) but, compared with South American sea lions, they are considerable smaller. Adult male reaches 190 cm in length and 120–200 kg in weight, whereas adult females are about 140 cm in length and 40–50 kg in weight (Jefferson *et al.*, 1993). The maximum age recorded for males and females of this species is 15.5 and 18 years, respectively (Molina-Schiller & Pinedo, 2004; Ponce de León & Pin, 2006). Males become sexually mature approximately at the age of 7 years, and females at 3 years (Vaz-Ferreira, 1979a).

Breeding and pupping season begins in mid-October and extends to January, with most of the pups being born in late December (Ponce de León & Pin, 2006). Copulation occurs few days after parturition and gestation takes approximately one year (Vaz-Ferreira, 1979a). Like other otariids, mothers stay and feed their pups for some days after parturition, and thereafter, make short foraging trips of a few hours in proximal areas to the breeding site (Thompson *et al.*, 2003). Lactation lasts from approximately six months to one year (Vaz-Ferreira, 1979a).

Fish, cephalopods, crustaceans and gastropods are the main prey reported from fur seals on the coast of South America (Jefferson *et al.*, 1993; Ponce de León & Pin, 2006; Vaz-Ferreira, 1979a). On the Peruvian and Uruguayan coasts, fur seals consume mainly small pelagic fishes (engraulids and clupeids) (Majluf, 1989; Naya *et al.*, 2002; Szteren *et al.*, 2004; Zavalga *et al.*, 1998). The food habits in the Patagonian coast of Argentina are currently unknown. However, recent isotopic analysis suggests that, in this area, the dietary basis of fur seals also are pelagic fish and cephalopods such as the Argentine hake, Argentine anchovy, Argentine shortfin and Patagonian squid (Vales *et al.*, 2012).

Figure 2. Adult males, females, and pups of South American fur seal, *Arctocephalus australis* during the breading and reproductive season at Isla Escondida, Chubut Province, Argentina. (Courtesy from the Laboratorio de Mamíferos Marinos, Centro Nacional Patagónico).

1.3. PARASITE DIVERSITY IN OTARIIDS

Despite their sanitary and economic importance, parasites represent a substantial part of biodiversity that still has to be evaluated in detail (Hoberg *et al.*, 1997; Windsor, 1995). In the case of otariids, the metazoan parasitic fauna of most species of sea lions and fur seals is generally poorly known, in part because these mammals are currently protected and, therefore, sampling is largely limited to specimens stranded on the coast or captured at by-catch in fisheries. As a result, most parasitological studies consist of single-species records (*e.g.* Aznar *et al.*, 2012; Dailey & Brownell, 1972; Delyamure, 1955; Margolis & Dailey, 1972; Zdzitowiecki, 1991), taxonomic issues (*e.g.* Dailey, 1969; Dailey *et al.*, 2002; Delyamure *et al.*, 1985; Rausch *et al.*, 2010; Timi *et al.*, 2003; Zdzitowiecki, 1984), or surveys on helminth parasites from specific organs or systems (*e.g.* George-Nascimento & Carvajal, 1981; Kelly *et al.*, 2005; Sepulveda & Alcaino, 1993; Shults, 1986), Only a few surveys have provided a full report of the metazoan parasitic fauna of sea lions and fur seals (see Dailey, 1975; Dailey & Hill, 1970; Machida, 1969; Morgades *et al.*, 2006; Stroud & Dailey, 1978). There are also population studies of some parasites species (*e.g.* Aznar *et al.*, 2004, 2009; George-Nascimento *et al.*, 1992; George-Nascimento & Marin, 1992; Olsen & Lyons, 1965).

At present, species belonging to the metazoan phyla Acanthocephala, Arthropoda, Platyhelminthes 59 and Nematoda have been commonly reported as parasites of sea lions and fur seals (Aznar *et al.*, 2001b; Dailey, 2001, 2005; Raga *et al.*, 2009) (see **Fig. 3**). Sucking lice (Echinophthiriidae Enderlein, 1904; **Fig. 3H**) and mites (Halarachnidae Oudemans, 1906) are arthropods that inhabit the fur and the respiratory tract, respectively. The other three phyla are endoparasites infecting the respiratory and circulatory systems or the gastrointestinal tract (Dailey, 2005; Raga *et al.*, 2009).

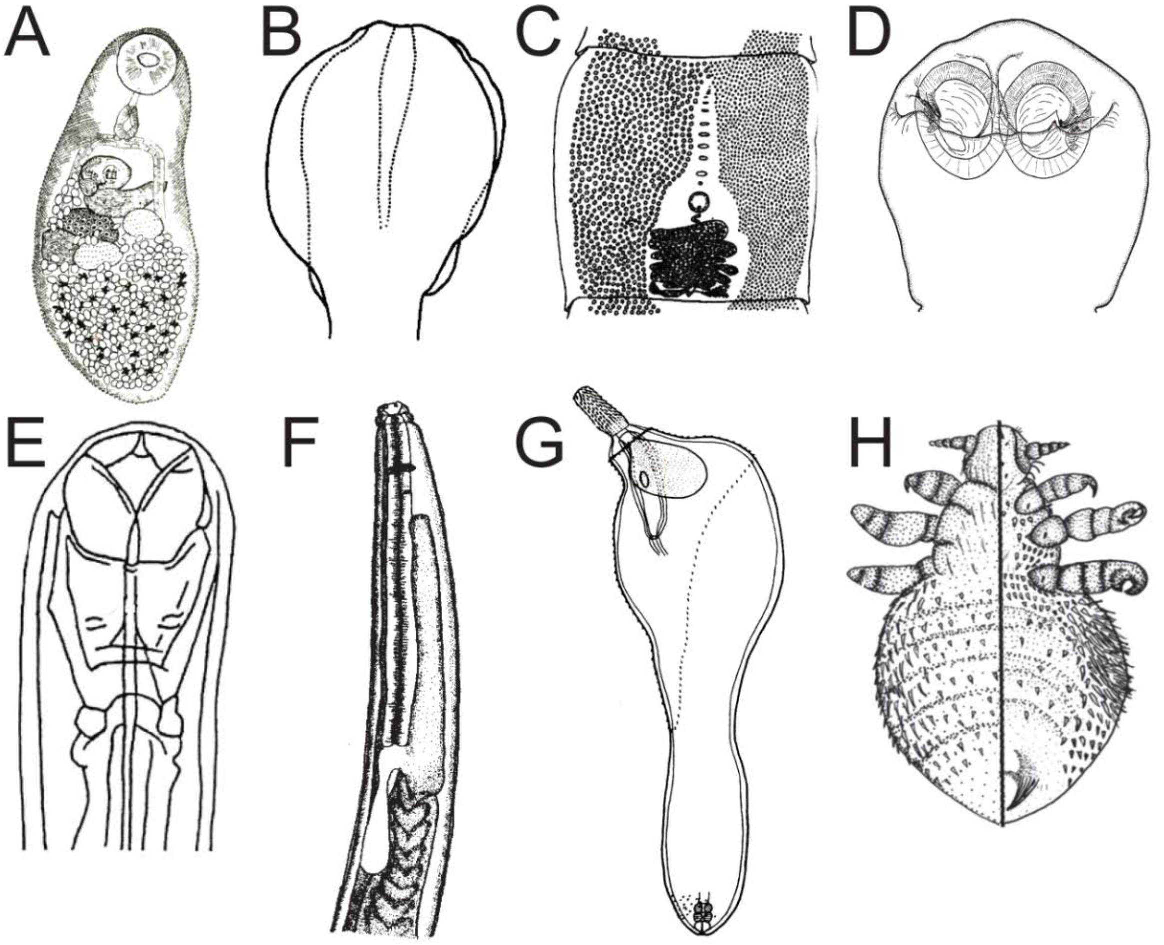

Figure 3. Some representative parasites of otariids. Digeneans: **(A)** *Stictodora ubelakeri* Dailey, 1969 from *Z. californianus* [drawing from Dailey (1969)]; Cestodes: **(B)** Scolex of *Diphyllobothrium pacificum* (syn. *D. arctocephalinum* Johnston, 1937 according to Scholz *et al.* (2009)) from *A. forsteri* [drawing from Delyamure *et al.* (1985)], **(C)** Mature proglotid of *D. pacificum* (syn. *D. arctocephalinum*) from *A. forsteri* [drawing from Delyamure *et al.* (1985)], **(D)** Scolex of *Anophryocephalus eumetopii* Hoberg, Adams and Rauch, 1991 from *E. jubatus* [drawing from Hoberg *et al.* (1991)]; Nematodes: **(E)** Anterior end of *Uncinaria* sp. from *P. hookeri* [drawing from Castinel *et al.* (2006)], **(F)** Anterior end of *Contracaecum ogmorhini sensu stricto* Johnston and Mawson, 1914 from *A. australis* [drawing from Timi *et al.* (2003)]; Acanthocephalans: **(G)** *Corynosoma arctocephali* Zdzitowiecki, 1984 from *A. gazella* [drawing from Zdzitowiecki (1984)]**;** Arthropods: **(H)** *Antarctophthirus microchir* (Trouessart and Neumann, 1888) from *O. flavescens* [drawing from Leonardi *et al.* (2009)].

1.3.1. Gastrointestinal helminths of sea lions and fur seals

1.3.1.1. Phylum Platyhelminthes

1.3.1.1.1. Class Trematoda

Trematodes or flukes comprise two subclasses, the Aspidogastrea and the Digenea. Aspidogastreans are a small group of parasites infecting freshwater and marine molluscs or teleosts and freshwater turtles, whereas digeneans are parasites which, as adults, infect all vertebrate groups, rarely invertebrates (Gibson, 2002; Rohde, 2002). Digeneans are probably the largest group of internal metazoan parasites, with approximately 18,000 described species belonging to 2,700 genera (Cribb *et al.*, 2001).

The general morphology and life cycles of the Digenea are summarised in Smyth (1994), Gibson (2002) and Cribb (2005). Morphologically, digeneans commonly have an unremarkable shape, as they can be elongate-oval, tubular, filamentous, helical or spherical. Most species have two muscular suckers in their adult form, one at the anterior end surrounding the mouth, and the other posterior, on the ventral side (**Fig. 3A**). Digeneans have a syncytial tegument, smooth or armed with spines, through which nutrients can be absorbed. The mouth is situated at the anterior body end and, in most species, the digestive system is well developed and comprise a prepharynx, pharynx, oesophagus and commonly a pair of blind caeca. They also have an excretory system which opens through the excretory pore at the posterior end of body. Except for two families whose species are dioecious (Schistomatidae Stiles and Hassall, 1898 and some Didymozoidae Monticelli, 1888), most of the digeneans are hermaphrodites.

The life cycle of digeneans is likely the most complex of those from the helminths inhabiting the digestive tract of otariids (**Fig. 4**). Digenean life cycles include free-living and parasitic stages and there are alternate generations in almost all the species; two asexual ones in molluscs and a single sexual one in vertebrates. In the definitive host, adults produce eggs that are released with host’s faeces. In water, the miracidium hatches from the egg, swims and penetrate a molluscan first intermediate host. In the mollusc, the miracidium develops into a mother sporocyst or “first intramolluscan generation”. The mother sporocyst is a simple sac that lacks any trace of feeding structures or gonads and asexually produces a second intramolluscan generation. The generation produced by the mother sporocyst comprises either multiple daughter sporocysts (which usually resemble the mother sporocyst) or multiple rediae (which develop a mouth, pharynx and short saccular gut). Daughter sporocysts and rediae can reproduce asexually and produce cercariae. Cercarie emerge actively from the mollusc and usually swim using a tail. In most digenean species infecting marine mammals, the cercaria encysts inside other host (probably a fish) which is a potential food source of the definitive host. In this second intermediate host, the encysted stage becomes a juvenile worm (the metacercaria). Metacercariae infect definitive hosts via trophic transmission, develop into adults, and reproduce sexually.

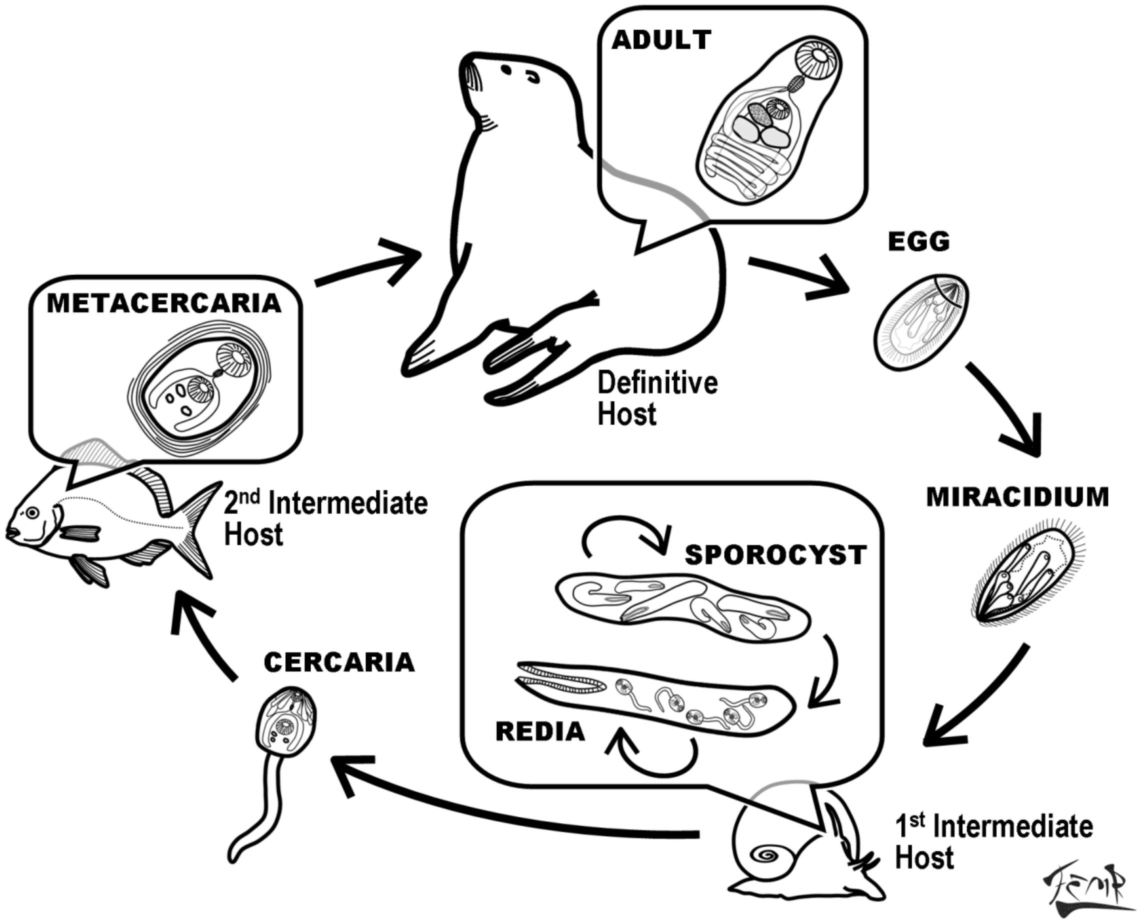

Figure 4. Generalized life cycle for digeneans infecting sea lions and fur seals (modified from Cribb, 2005).

Species of two families of digeneans, namely, Heterophyidae Leiper, 1909 [including species of *Apophallus* Luhe, 1909 (syn. *Pricetrema* Ciurea, 1933), *Ascocotyle* Looss, 1899, *Stictodora* Looss, 1899 (**Fig. 3A**) and *Phocitrema* Goto and Ozaki, 1930] and Notocotylidae Lühe, 1909 (including species of *Ogmogaster* Jägerskiöld, 1891), have been reported as parasites of otariids (Dailey, 1969; Dailey *et al.*, 2002; Dailey & Hill, 1970; George-Nascimento & Carvajal, 1981; Morgades *et al.*, 2006; Sepulveda & Alcaino, 1993; Shults, 1978, 1986; Stroud & Dailey, 1978). In South America, only *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920 and *O. heptalineatus* Carvajal, Durán and George-Nascimento, 1983 have been described from the intestine of sea lions collected in the Uruguayan and Chilean coasts, respectively (Carvajal *et al.*, 1983; Morgades *et al.*, 2006).

1.3.1.1.2. Class Cestoda

The cestodes, or tapeworms, include *ca*. 5,000 species divided into two subclasses, Cestodaria and Eucestoda (or true tapeworms) (Olson & Tkach, 2005; Smyth, 1994). Adults almost exclusively infect the digestive system of vertebrates. A morphological feature common to all cestodes is the lack of a digestive system; nutrients are absorbed through the neodermis, a specialised syncytial microtrich-covered tegumental surface (Bush *et al.*, 2001; Caira & Reyda, 2005). Currently, only adult specimens of the Eucestoda, belonging to orders Diphyllobothriidea Kuchta, Scholz, Brabec and Bray, 2008 and Tetrabothriidea Baer, 1954 have been recorded in otariids (see below). Therefore, the general morphology and life cycle of cestodes described hereafter corresponds to species from these orders.

The main morphological traits of cestodes are a distinct anterior holdfast organ or scolex (**Figs. 3B & D**), an unsegmented neck, and a segmented posterior body (strobili) composed of a linear series or sets of proglotiids (**Fig. 3C**) (Bush *et al.*, 2001; Smyth, 1994). The youngest proglotiids are formed immediately behind the neck and, as new proglotiids develop, the previous ones develop into mature and gravid proglotiids and move posteriorly in a continuous process (Bush *et al.*, 2001). Cestodes are polyzoic, *i.e.* most of the species present one or more sets of male and female organs in each proglotiid (Caira & Reyda, 2005).

The life cycles of cestodes infecting otariids have been summarised by Hoberg (1994), Caira & Reyda (2005) and Roberts & Janovy (2009). In otariids, life cycles are complex and involve two, or sometimes three, hosts **(Fig. 5).** Adult worms inhabit the intestine and release operculated eggs with host's faeces. In water the embryogenesis continues within the egg until a free-swimming larva, named coracidium (in diphyllobothriidean) or hexacanth (in tetrabothriidean) hatch. In diphyllobothriids, the free-swimming coracidum must be eaten by a copepod (first intermediate host), where it develops into a procercoid larvae stage in the hemocoel. Thereafter, the copepod must be eaten by a fish (second intermediate host), in which the procercoid develops into a plerocercoid larvae. In some species of *Diphyllobothrium* fish paratenic hosts may also be involved in their life cycles. On the other hand, the life cycle of tetrabothriideans is currently unknown, but it is thought to involve crustaceans, cephalopods, and /or teleosts as intermediate and paratenic hosts. Available data suggest that the infective larval stage of tetrabothrideans that infects the definitive host is a uniacetabulate plerocercoid (Hoberg, 1987). In both diphyllobothriids and tetrabothriideans, the intermediate / or paratenic hosts must be ingested by otariids, in which the plerocercoid develops rapidly into the adult stage.

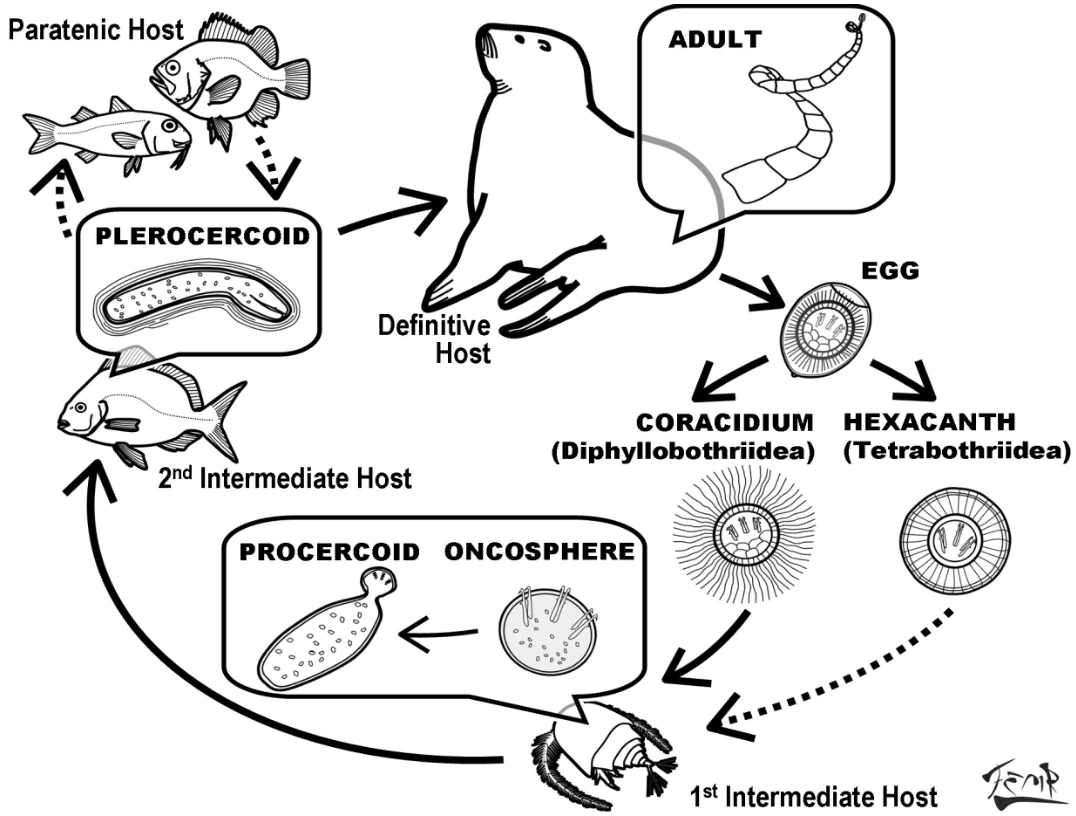

Figure 5. Generalized life cycle for cestodes infecting sea lions and fur seals (modified from Caira & Reyda, 2005)

Adult tapeworms belonging to the family Diphyllobothriidae Lühe, 1910 (species of *Diphyllobohrium* Cobbold, 1858 and *Diplogonoporus* Lönnberg, 1892) and from the family Tetrabothriidae Linton, 1891 (species of *Anophryocephalus* Baylis, 1922) have been recorded occurring in the intestine of sea lions and fur seals (*e.g.* Delyamure & Parukhin, 1968; Delyamure & Skrjabin, 1960; Hoberg & Adams, 1992; Hoberg *et al.*, 1991; Rausch *et al.*, 2010; Shults, 1986) (**Figs 3B, C & D**). In sea lions and fur seals inhabiting the coasts of South America, only *D. pacificum*, *Diphyllobothrium* sp. and Tetrabothriidae gen. sp. have thus far been reported (George-Nascimento & Carvajal, 1981; Morgades *et al.*, 2006).

1.3.1.2. Phylum Nematoda

Nematodes or roundworms, comprise at least 24,783 species included in 2,829 genera (Hodda, 2011), of which *ca.* 33% of genera occur as parasites of vertebrates (Anderson, 2000). Nematodes have a cylindrical body tapered at both ends. They possess a pseudocoel, a complete digestive system and a secretory-excretory system. A common morphological feature of all nematodes is the possession of a noncellular cuticle that covers the body; all species have to moult it four times before reaching maturity. Most of the nematodes are dioecious and oviparous, but some species are also ovoviviparous (Roberts & Janovy, 2009; Smyth, 1994).

Currently, the higher classification of the Nematoda is unstable, with frequent nomenclatural and organizational changes based on recent morphological and molecular evidence (*e.g.* Anderson *et al.*, 2009; De Ley & Blaxter, 2002, 2004; Hodda, 2007, 2011; Yamaguti, 1961). To avoid confusion, the higher classification of the Phylum Nematoda in the present study follows Hodda (2011). The main nematode groups infecting the digestive tract of otariids belong to the Class Chromadorea, *i.e.* species of the orders Rhabditida Chitwood 1933 and Spirurida Railliet 1914 (Dailey, 2005; McClelland, 2005; see below).

Within the Rhabditida, species of *Uncinaria* Froelich, 1789 (family Ancylostomidae Looss 1905) are commonly known as hookworms and typically occur in the intestine of juvenile land-breeding seals. These parasites may cause high mortalities in young otariids due to haemorrhagic enteritis and anemia (Dailey, 2005). Common morphological traits of these nematodes are a large and heavily sclerotized buccal capsule, usually armed with cutting plates (**Fig. 3E**), and a broad copulatory bursa at the posterior end of males (Roberts & Janovy, 2009).

Hookworms have a direct life cycle (**Fig. 6**). According to Olsen & Lyons (1965), adult worms mature and reproduce in the intestine of young otariid pups. Third-stage larvae (L3) are released within the eggs with pup's faeces and hatch on the soil. The free-living L3 infects hosts of all age groups, entering mainly through the flippers, and migrate via the circulatory system into the blubber of the belly region and, in females, into mammary glands and milk cisterns. Infection of young pups occurs when the L3 is passed along with the milk.

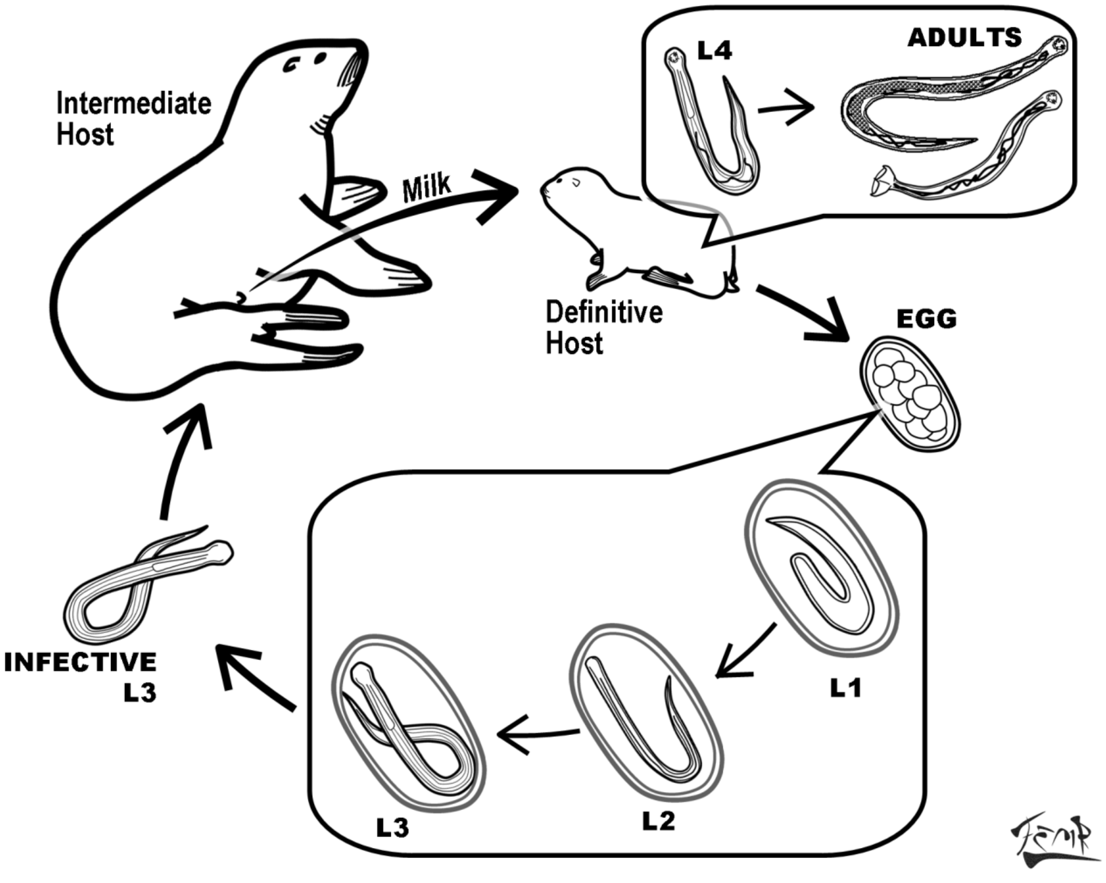

Figure 6. Life cycle of *Uncinaria* spp. (Nematoda: Ancylostomidae) infecting sea lions and fur seals (modified from Dailey, 2005).

Spirurid nematodes of the Suborder Ascaridina Inglis 1983 are characterised by the presence of three prominent lips, external labial papillae and numerous caudal papillae. In the digestive tract of otariids, there are reports of adults and larval forms of species of the family Anisakidae Railliet and Henry 1912 belonging to the genera *Anisakis* Dujardin, 1845, *Contracaecum* Railliet and Henry, 1913 (**Fig. 3F**), *Phocascaris* Höst, 1932, and *Pseudoterranova* Krabbe, 1878 (see Dailey, 2001; Delyamure & Skrjabin, 1960; Mattiucci & Nascetti, 2008).

The life cycle of anisakid is heteroxenous and has been summarised from McClelland (2002) and Klimpel *et al.* (2004) (**Fig. 7)**. Adult forms inhabit the stomach of pinnipeds where they release eggs that pass through the faeces to the marine realm. Within the egg, the embryos develop and moult; the number of moults performed in the egg is uncertain, since the available evidence suggests that larva could moult once to second-stage larvae (L2) or twice to L3. Free-living L2 (L3) hatched from the egg are ingested by a microinvertebrate (mainly copepods), and penetrate to the haemocoel where it begins to grow. The infected copepod is later ingested by larger crustaceans (*e.g.* euphausiids, gammarids, decapods, etc.) where the larvae continue growing, and develop the anatomical features of infective L3. Infected large crustaceans can be then be consumed by fish, which can act as paratenic (transport) hosts. Larvae within the invertebrate host are able to infect otariids, but fish are essential for the temporal and spatial dispersion of the larvae, by increasing the likelihood of ingestion by the definitive hosts. Within the fish, the L3 grows and typically encyst in internal organs or musculature. Third-stage larvae can pass through one or more fish hosts, which acquire the parasite by preying on smaller fish. Finally, infected fish are ingested by otariids, where the L3 moult into the fourth-stage larva and then into adult.

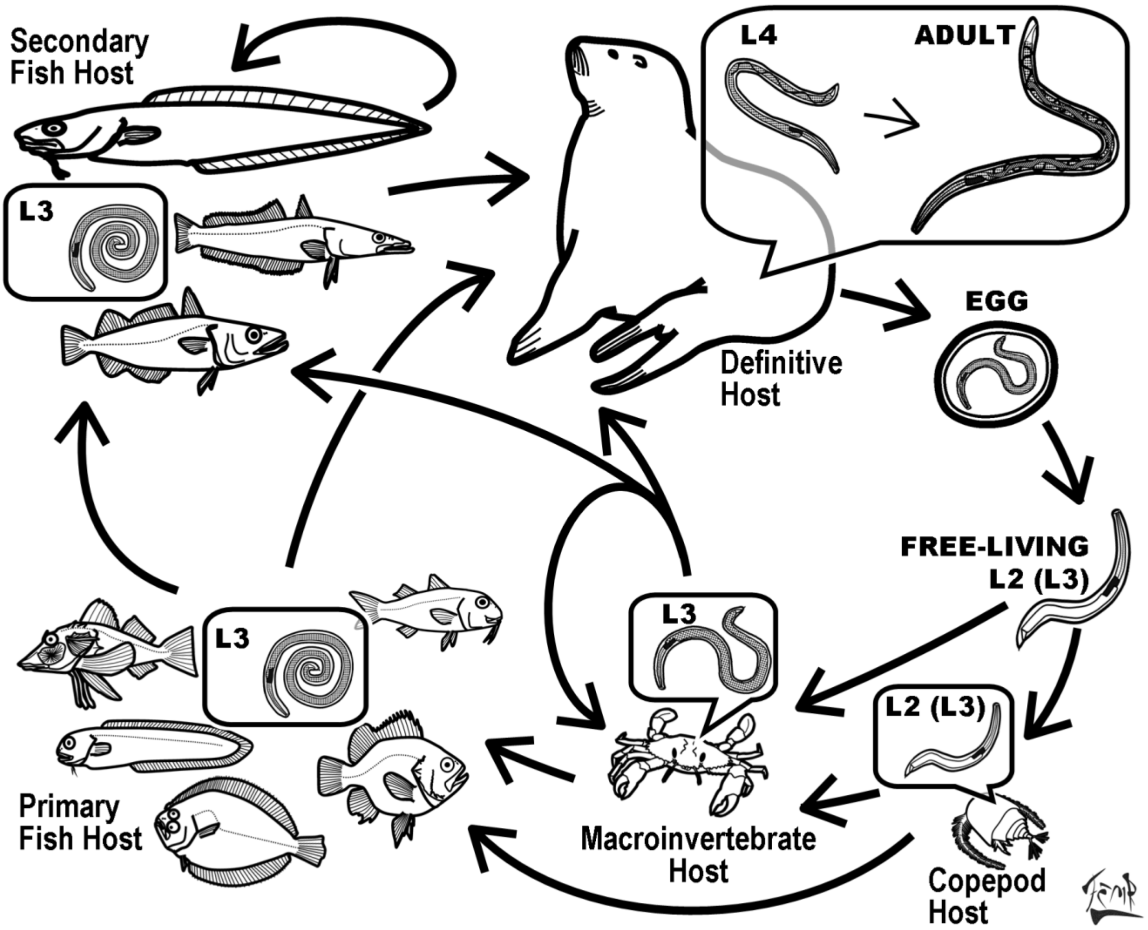

Figure 7. Life cycle of *Pseudoterranova* spp. (Nematoda: Anisakidae) infecting sea lions and fur seals (modified from McClelland, 2005).

Specimens of *U. hamiltoni* Baylis, 1933 and *Uncinaria* sp. have been reported from South American sea lions and fur seals pups in South America (Berón-Vera *et al.*, 2004; George-Nascimento *et al.*, 1992; Katz *et al.*, 2012; Morgades *et al.*, 2006). Adult specimens of the anisakids *C. ogmorhini sensu stricto* Johnston et Mawson, 1941 (**Fig. 3F**), *Contracaecum* sp. and *P. cattani* George-Nascimento and Urrutia, 2000, and larval stages of *Anisakis* sp. have been recorded from the stomach of both species of otariids (George-Nascimento & Carvajal, 1981; George-Nascimento & Urrutia, 2000; Morgades *et al.*, 2006; Timi *et al.*, 2003).

1.3.1.3. Phylum Acanthocephala

Acanthocephalans, or thorny-headed worms, are endoparasitic organisms with approximately 1,115 species described in 125 genera (Verweyen *et al.*, 2011). The main diagnostic traits of this phylum are the invaginable and retractile proboscis with rows of recurved hooks, and a body wall with a lacunar system. Acanthocephalans have a cylindrical or slightly flattened body and are dioecious, exhibiting a variable degree of sexual dimorphism in body size (Miller & Dunagan, 1985). They have no mouth, intestine or conventional circulatory system, thus nutrient assimilation is performed through the body surface (Starling, 1985). Some species also present trunk spines in the body surface which play a significant role in attachment to their hosts (Van Cleave, 1952).

The life cycle of acanthocephalans includes an arthropod intermediate host, a vertebrate definitive host and, in some species, there also are paratenic hosts (Schmidt, 1985). In otariids, the life cycle could be summarised as follows (**Fig. 8**): Adult acanthocephalans live and reproduce in the alimentary tracts of vertebrates. The eggs are released through the host faeces and are then ingested by an arthropod (the intermediate host) in which the first larval stage, or acanthor, hatches and passes through 2 subsequent stages, the acanthella and the cystacanth. At the cystacanth stage all the structures of the adult are developed, and therefore the larva becomes infective. However, some species require fish paratenic hosts to bridge the trophic gap between intermediate and definitive hosts. In the paratenic hosts, cystacanths encyst in the body cavity, awaiting ingestion by the definitive host (Skorobrechova & Nikishin, 2011).

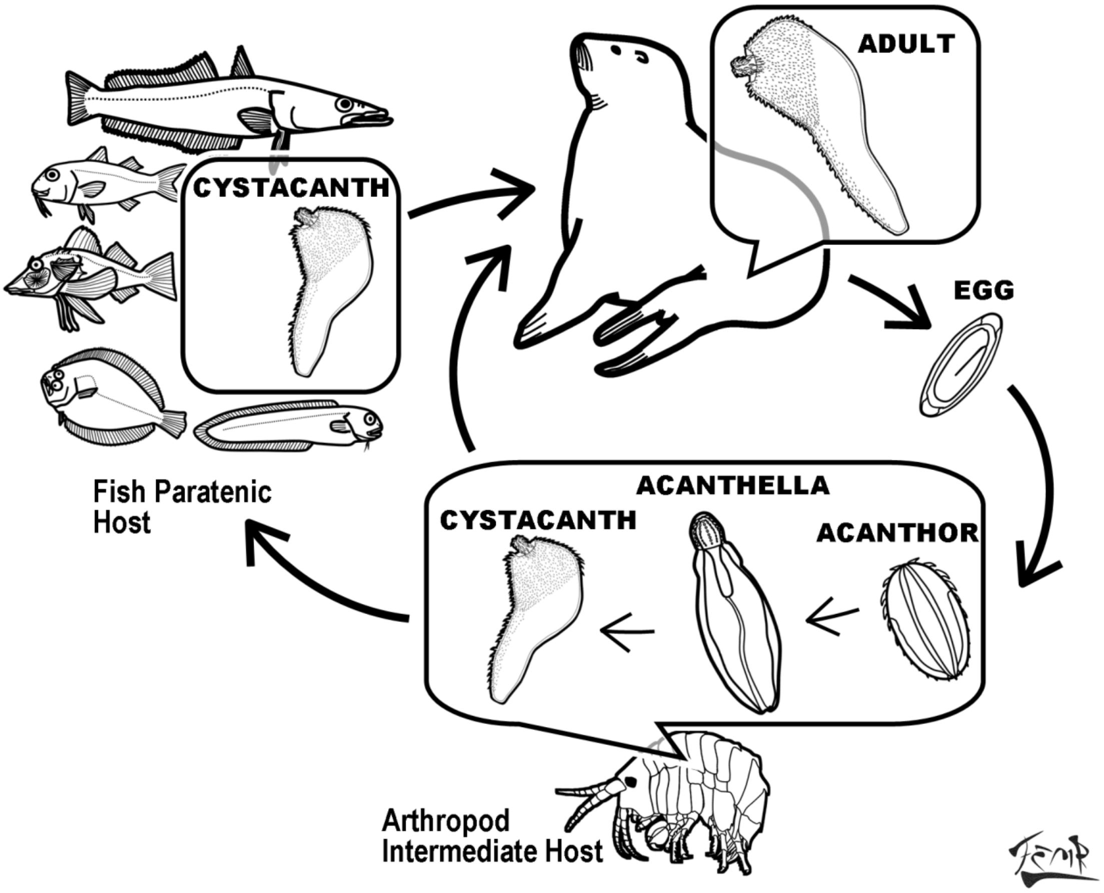

Figure 8. Life cycle of *Corynosoma* spp. (Acanthocephala: Polymorphidae) infecting sea lions and fur seals (modified from Bush *et al.*, 2001).

Records from adult and juvenile acanthocephalans from the intestine of fur seals and sea lions from both Hemispheres include species of polymorhid acanthocephalans of the genera *Corynosoma* and *Bolbosoma* (*e.g.* Aznar *et al.*, 2006; Ionita *et al.*, 2008; Kuzmina *et al.*, 2012; Lincicome, 1943; Zdzitowiecki, 1984) (**Fig. 3G**). Specimens of *C. australe*, *C. cetaceum* and *Corynosoma* sp. have been reported from the sea lions and fur seals from the eastern and western coasts of South America (Aznar *et al.*, 2004, 2006, 2012; George-Nascimento & Carvajal, 1981; George-Nascimento & Marin, 1992; Katz *et al.*, 2012; Morgades *et al.*, 2006; Sardella *et al.*, 2005).

1.4. ASSOCIATIONS AND TRANSMISSION OF GASTROINTESTINAL HELMINTHS OF OTARIIDS

1.4.1. Origin of host-parasite associations in otariids

The structure of the current parasite communities of sea lions and fur seals arises from two types of possible associations: *i*) *association by descent*, in which the ancestor of both host and parasites interacted; and *ii*) *association by colonisation,* in which the parasites emerged in another host and colonised the otariid species (Aznar *et al.*, 2001b; Raga *et al.*, 2009). Association by descent implies co-evolution, and assumes a long-term association of parasites and their hosts with a high degree of co-speciation and co-adaptation between them (Brooks & McLennan, 1991). This type of association could be advocated at least in the case of ancylostomid nematodes (*Uncinaria* spp.) or arthropods parasitizing otariids, which also have a terrestrial ancestor (*e.g.* Durette-Desset *et al*., 1994; Kim, 1985; Light *et al*., 2010; Nadler *et al*., 2000).

On the other hand, association by colonisation (hosts switching), depends on two factors: *i*) the probability of encounter (which is regulated by behavioural, ecological, or biogeographical factors); and *ii*) the compatibility between the new hosts and the parasite (which depends on morphological, physiological and/or immunological host barriers) (Raga *et al.*, 2009). In the marine realm, association by colonisation seems to occur frequently because parasites are put in contact with unusual hosts that belong to the same trophic guild. At ecological scale, parasites cannot overcome compatibility barriers posed by uncommon host (*e.g.* Aznar *et al.*, 2012), but the opportunities for successful colonisation are continuously arising, and a long-term contacts between the parasite and the target host can end-up in new host-parasite associations (Aznar *et al.*, 2001b). This is particularly true when target hosts are phylogenetically close to ‘donors’, as both will provide similar conditions for the survival and reproduction of the parasite. A good example of association by colonisation is that of tapeworms of the genus *Anophryocephalus* which diversified in phocids from an ancestor occurring in archosaurians, and which subsequently colonized otariids (Hoberg & Brooks, 2008).

1.4.2. Transmission of gastrointestinal parasites to sea lions and fur seals

Little is known about the routes of transmission of gastrointestinal helminths of otariids. With the exception of ancylostomid nematodes, which have a monoxenous life cycle, parasitic worms of sea lions and fur seals are transmitted through the food web via predator-prey interactions in the marine realm. However, for most of these parasite species, the specific identity of the intermediate/paratenic of hosts is unknown, and, therefore, many stages of their life cycles remain to be described. Nevertheless, one of the general predictions that could be applied to most of these parasites is the low specificity for intermediate/paratenic hosts (see George-Nascimento, 1987; Laskowski *et al.*, 2008; Moles, 2007; Moles & Heintz, 2007; Palm *et al.*, 1994). The ability to infect several hosts seems to be an adaptation to guarantee transmission to the definitive hosts through several potential preys by spreading the risk among a large number of host species (Bush, 1990).

The broad spectrum of intermediate/paratenic hosts infected by gastrointestinal helminths of otariids and the diversity of larval forms make this parasite group an ideal model to investigate patterns of host specificity and to shed light on the way parasites use food webs to ensure transmission. Several studies (Aznar *et al.*, 2012; George-Nascimento *et al.*, 1992; George-Nascimento & Marin, 1992) have commented on the effect of different definitive hosts on the specificity of gastrointestinal parasites in sea lions and fur seals. Similar effects could be expected in for intermediate/paratenic hosts.

1.5. THIS STUDY

The present study has been carried out within the framework of two projects "A study of threats to marine mammal conservation in Patagonia" supported by the BBVA Foundation (BIOCON 04), and "Evolution of the family Polymorphidae (Acanthocephala) in aquatic birds and marine mammals: diversity, biogeography and ecomorphology" from the Spanish Ministry of Science and Innovation (CGL2007-63221/BOS).

In particular, this study addressed the following questions. Firstly, a reasonably large sample of the intestines of South American sea lions and fur seals collected in 1994-2009 from the Patagonian coast of Argentina was examined for parasites. This sample provided the unique opportunity to characterize, for the first time, the intestinal helminth fauna of both species of otariids in this locality. Secondly, a total of 542 specimens of 20 marine fish species were collected in 2006–2007 off the Patagonian coast and examined for identification and quantification of larval forms from the parasites infecting otariids. The large dataset obtained provided an excellent opportunity to describe some unknown larval forms in this locality and to assess pathways of transmission of these parasites between intermediate/paratenic fish hosts and their definitive otariid hosts. Thirdly, the large number of larval specimens from several fish species collected in the course of the study allowed an assessment of the effect of different host species on some life history traits of the larvae, such as growth patterns or sex ratio, and their potential role in the transmission, all of which are essential aspects to understand the population dynamics of these parasites.

2. AIM AND OBJECTIVES

2.1. AIM

The aim of the present study is twofold. First, to increase knowledge regarding the diversity and community composition of the helminth fauna in two otariids hosts, the South American sea lion, *Otaria flavescens* and the South American fur seal, *Arctocephalus australis* off Patagonian coasts of Argentina. Second, to investigate the population structure, life history traits and transmission strategies of larval forms of these helminths in paratenic/intermediate fish hosts.

2.2. OBJECTIVES

In particular, the specific objectives of this study are:

2.2.1. To quantify and describe the intestinal metazoan parasite communities of *O. flavescens* and *A. australis* off northern Patagonia, Argentina, based on a detailed morphological and taxonomical study. This information is used to ascertain the role of parasite host specificity in shaping helminth community diversity in otariids.

2.2.2. To characterize the component populations of cystacanths of *Corynosoma australe* (Acanthocephala: Polymorphidae) in paratenic fish hosts off the Patagonian coast of Argentina. The main goal is to elucidate the pathways of transmission of this species between paratenic hosts and definitive otariid hosts, and to assess the effect of different fish hosts on growth, body size, fitness and sex ratio of the cystacanths of *C. australe.*

2.2.3. To describe, for the first time, the temporal allocation of investment on holdfast structures (trunk spines) between cystacanths and adults of two congeneric species of acanthocephalans (*Corynosoma cetaceum* and *C. australe*), investigating the factors that may account for the patterns of trunk spine growth.

2.2.4. To carry out a taxonomic identification and description of third-stage larvae of species of *Pseudoterranova* (Nematoda: Ascaridoidea) from various fish species of Patagonia using sequence data for the mitochondrial cytochrome *c* oxidase subunit 1 (cox 1) gene and a detailed morphological study. This evidence is then used to describe the component populations of third-stage larvae of species of *Pseudoterranova* in fishes, assessing the role of different fish hosts on the microhabitat selection, transmission strategies and infection parameters of the third-stage larvae.

3. GENERAL MATERIALS AND METHODS

3.1. HOST SAMPLING

3.1.1. Pinnipeds

The intestines of 56 South American sea lions *Otaria flavescens* (Shaw, 1890) and 5 South American fur seals *Arctocephalus australis* (Zimmerman, 1783) obtained during the period 1994-2009 were analysed. Sea lions and fur seals were collected in northern Patagonia (40°43'–43º20'S, 63°04'–65º07'W; **Fig. 9**), found either dead stranded on the coast (*O. flavescens*, n = 48; and *A. australis*, n = 4; **Fig. 10A**) or as by-catch in fisheries (*O. flavescens*, n = 8; and *A. australis*, n = 1; **Fig. 10B**).

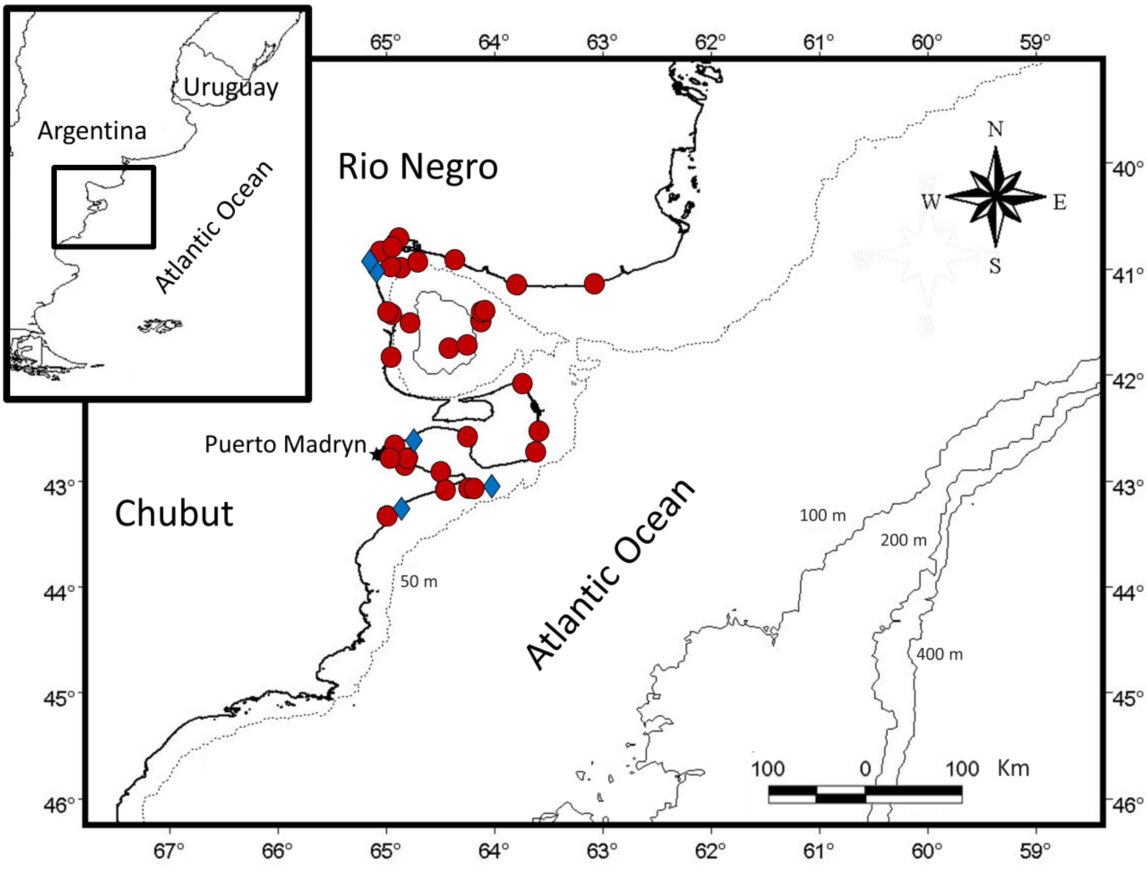

Figure 9. Map of north Patagonia showing the stranding or by-catch locations of South American sea lions, *Otaria flavescens* (red dots), and South American fur seals, *Arctocephalus australis* (blue diamond).

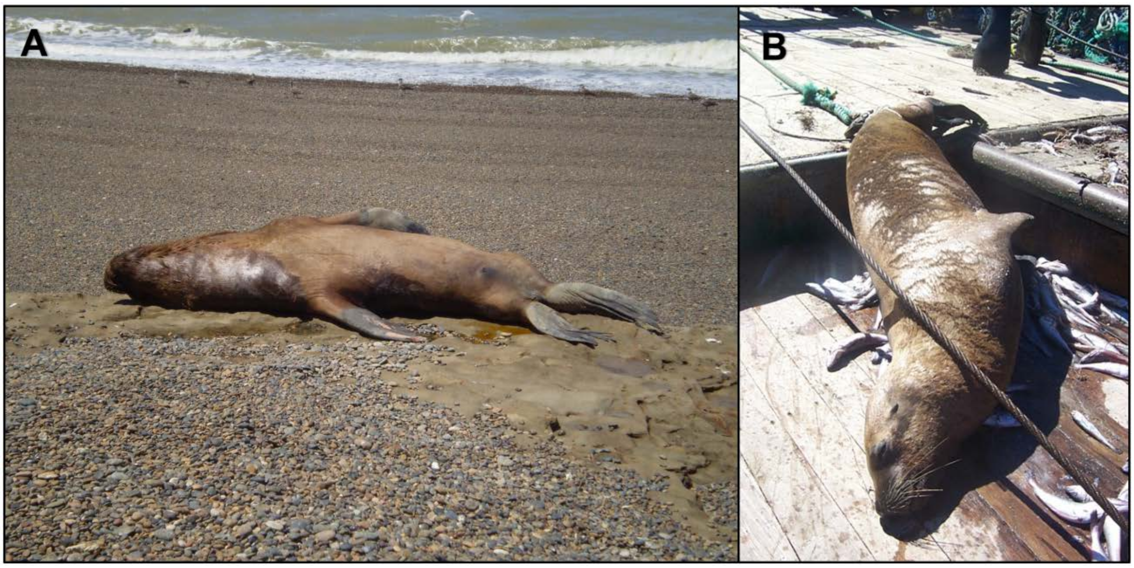

Figure 10. (**A**) Adult male of South American sea lion, *Otaria flavescens* found dead at Punta León Beach, Chubut in 2005. (**B**) Young female of *O. flavescens* drawn in an Argentine hake trawler in 2008. (Courtesy from the Laboratorio de Mamíferos Marinos, Centro Nacional Patagónico).

The standard body length was measured to the nearest cm using standard methods (Committee on Marine Mammals, 1967). Animals were then necropsied according to the procedure described in Geraci & Lounsbury (1993) by the staff of the Laboratorio de Mamíferos Marinos (LAMAMA) of the Centro Nacional Patagónico (Puerto Madryn, Chubut, Argentina), either in the field or under laboratory conditions. The intestine was removed from the carcass and kept frozen at –20ºC. After thawing, the intestine was weighted, extended and measured. Each intestine was processed according to Aznar *et al.* (2004), *i.e.* it was divided into 5 sections (n = 15) or 30 sections (n = 41) of equal length, and contents of each section were separately flushed through sieves of either 0.2 or 0.5 mm mesh. Finally, the intestine wall of each section was examined to collect attached worms.

The age, to the nearest year, of each pinnipeds individual was estimated based on counts of incremental growth layers in the dentine of tooth section or in the cementum of upper canines (see Grandi *et al.*, 2010). Biological data of South American sea lions and fur seals are summarized in **Table 2** and **Table 3,** respectively.

Table 2. Data of the South American sea lions *Otaria flavescens* from northern Patagonia, Argentina, between 2000 to 2009 analysed in this study. Abbreviations: F, female; IL, intestine length; M, male; SBL, standard body length; SMG, San Matías Gulf.

Code	**Collection date**	**Stranding locality or vessel name**	**Sex**	**Age (year)**	**SBL (cm)**	**IL (m)**
464	10/08/2003	Punta León, Chubut	F	1	119	13.1
536	08/25/2000	Barco Roca Beach, Chubut	M	5	212	22.1
549	08/25/2000	Barco Roca Beach, Chubut	M	10	212	18.6
574	03/27/2001	Puerto Madryn, Chubut	M	1	97	14
589	01/23/2002	Punta León, Chubut	F	8	160	17.9
591	01/30/2002	Puerto Pirámide, Chubut	M	12	239	20.3
602	02/13/2002	Puerto Madryn, Chubut	M	1	90	17.4
603	06/09/2002	Puerto Madryn, Chubut	F	8	151	22.7
605	07/20/2002	Punta Loma, Chubut	M	5	168	20.9
606	07/31/2002	El Doradillo Beach, Chubut	M	1	108.5	13.6
607	07/31/2002	El Doradillo Beach, Chubut	M	2	122	11.7
615	11/30/2002	Puerto Madryn, Chubut	M	0	96	13.0
620	03/12/2003	Puerto Madryn, Chubut	F	1	110	16.0
649	02/04/2004	Punta León, Chubut	F	17	194	23.7
650	03/06/2004	Puerto Madryn, Chubut	F	12	170	22.5
662	02/23/2006	Punta Norte, Chubut	F	18	174	22.9
670	08/14/2005	Cerro Avanzado, Chubut	F	3	122	11.1
682	07/18/2007	Pardelas Beach, Chubut	M	2	120	16.3
706	12/01/2005	Punta Este, Chubut	M	0	101	17.7
762	12/11/2005	Punta Delgada, Chubut	F	15	170	23.2
788	02/06/2006	Puerto Madryn, Chubut	F	15	190	21.4
796	04/30/2006	Colombo Beach, Chubut	F	10	175	18.8
800	06/01/2006	San Antonio Oeste, Río Negro	M	13	228	10.3
817	09/18/2006	Siempre Salvador Vessel,	M	9	220	30.4
829	12/31/2006	Piedras Coloradas, Río Negro	M	0.9	109	17.0
830	01/20/2007	Punta Bermeja, Río Negro	F	21	180	32.0
831	01/24/2007	Promontorio Belén, Río Negro	F	12	173	18.6
838	01/28/2007	Los Hornitos, Río Negro	F	10	157	20.0
851	06/29/2006	Puerto Madryn, Chubut	M	15	220	32.3
854	08/02/2006	Los Tamariscos Beach, Chubut	F	1	119.5	14.8
855	08/17/2006	Puerto Pirámide, Chubut	M	0.6	109.5	18.3
886	05/05/2007	Kaiser Beach, Chubut	F	0.4	109	13.6
888	07/09/2007	Punta Cuevas, Chubut	M	9	189	21.5
892	03/27/2007	La Pastosa Island, Río Negro	M	11	–	12.3
924	04/29/2007	Colombus Vessel, SMG	M	6	181	26.2
931	07/07/2007	San Antonio Oeste, Río Negro	F	19	168	25.2
932	07/13/2007	Islote Lobos, Río Negro	F	19	200	29.1
933	08/10/2007	San Antonio Oeste, Río Negro	F	1	109	14.9
936	09/28/2007	Siempre Salvador Vessel, SMG	M	7	200	28.0
943	11/05/2007	Don Raúl Vessel, SMG	M	4	156	18.8
947	02/22/2008	Nueva Neptunia I Vessel, SMG	F	9	181	17.2
949	05/08/2008	Las Grutas, Río Negro	M	11	210	28.0
950	05/23/2008	Nueva Neptunia I Vessel, SMG	M	3	142	14.1
951	06/27/2008	Las Grutas, Río Negro	F	14	181	24.0

Table 2. continued.

Code	Collection date	Stranding locality or vessel name	Sex	Age (year)	SBL (cm)	IL (m)
954	01/26/2008	Promontorio Belén, Río Negro	M	2	150	20.7
964	09/30/2007	Viernes Santo Vessel, SMG	M	13	232	28.4
965	09/30/2007	Viernes Santo Vessel, SMG	M	9	222	28
966	10/08/2007	San Salvador Vessel, SMG	M	4	165	20
969	08/20/2008	Barranca Final, Río Negro	M	12	218	14.5
1035	04/18/2008	Cracker Bay, Chubut	M	12	187	23.5
1038	07/25/2008	Cerro Avanzado, Chubut	M	8	181	27.7
1041	12/13/2008	Puerto Madryn, Chubut	F	16	168	25.1
1058	01/10/2009	Puerto Madryn, Chubut	F	2	143	16.7
1067	04/29/2009	Paraná Beach, Chubut	F	1	127	16.8
1068	05/03/2009	Punta Loma, Chubut	F	6	153	20.6
1071	11/26/2009	Unión Beach, Chubut	F	21	195	25.1

Table 3. Data of South American fur seal *Arctocephalus australis* analysed from northern Patagonia, Argentina, between 1994 to 2008. Abbreviations: F, female; IL, intestine length; M, male; SJG, San Jorge Gulf; SBL, standard body length.

Code	Collection date	Stranding locality or vessel name	Sex	Age (year)	SBL (cm)	IL (m)
05	06/01/1994	Antártida Vessel, SJG	M	6.5	147	22.3
15	09/13/1996	Escondida Island, Chubut	M	10.5	166	14.5
47	09/04/2006	Punta Flecha, Chubut	F	1	78	11.6
53	12/21/2007	Las Grutas, Río Negro	M	1.5	68	10.4
64	08/29/2008	La Madre Selva Beach, Río Negro	M	8	176	23.2

3.1.2. Teleosts

Teleost fishes of 20 species were collected by Argentine hake trawlers (**Figs. 11A & B**) from 3 zones surrounding the closed fishing area (CFA) for the protection of juveniles specimens of Argentine hake *Merluccius hubbsi* Marini, 1933 along the coasts of Chubut and Santa Cruz in Argentina (see Resolución de la Secretaría de Agricultura, Ganadería, Pesca y Alimentación (SAGPyA) de la Argentina N° 26/2009 for more details): “Sampling 1”, south of the CFA, offshore the area of San Jorge Gulf (47º00'–47º20'S; 64º17’–65º00’W; March 2006; depth range: 82–102 m; vessel: “Cabo San Juan”); “Sampling 2”, mostly south and southeast of the CFA, in waters of the Patagonian shelf (47º00'–47º19'S; 61º59'–64º25'W; March 2007; depth range: 101–119 m; vessel: “Cabo San Juan”); and “Sampling 3”, north of the CFA, in the vicinity of Valdes Peninsula (42º45'–42º59'S; 61º09'–62º58'W; October 2007; depth range: 72–88 m; vessel: “Cabo Buen Tiempo”) (**Fig. 12**).

Figure 11. Hake trawling along the Patagonian shelf. (**A**) Hake trawling vessel operating in northern Patagonia. (**B**) Net of "Cabo Buen Tiempo" vessel after the trawling maneuver in October 2008. (Photo by Jesús Hernández-Orts).

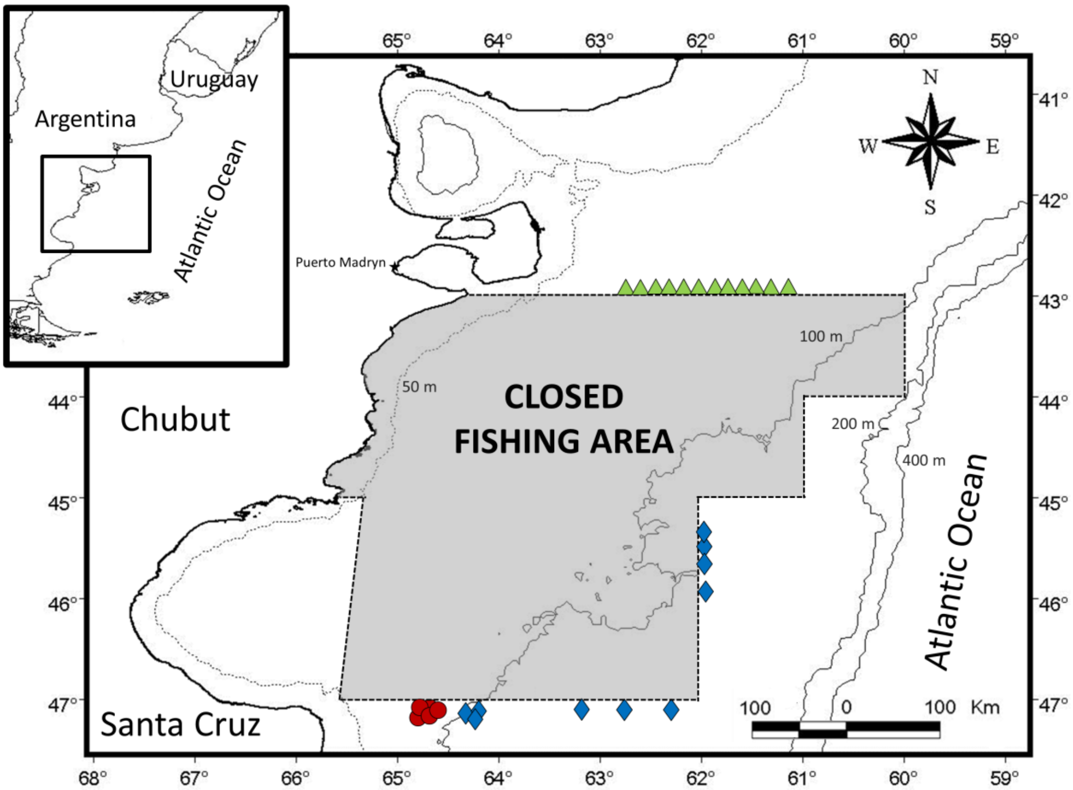

Figure 12. Geographic location of the sampling area for fishes along Patagonian coast of Argentina. "Sampling 1", south of the CFA (red dots); "Sampling 2", south and southeast of the CFA (blue diamonds); and "Sampling 3", north of the CFA (green triangles).

Fish species were selected based on their abundance in the sampling areas (Bezzi *et al.*, 2000) and according to their body size, *i.e.* fish whose size were within the range of those reported in the diet of sea lions and fur seals from the study area (Koen-Alonso *et al.*, 2000; Néstor A. García, pers. comm.). A total of 542 fishes from 20 species were collected (see **Table 4**). All the specimens were kept on ice until landing. In the LAMAMA, fishes were identified according to Menni *et al.* (1984), sized (total length) and weighted. Specimens were then either examined fresh or frozen in plastic bags at –20ºC for later examination. Before been analysed, the skin and fins of each fresh and thawed fish specimen were naked eye examined for parasites. The internal organs were removed from the carcass and separately placed on Petri dishes with saline solution 9% (w/v) and the body cavity was naked-eye examined for parasites. The contents of the stomach, intestine and intestinal caeca were washed and cleared in saline and examined under a stereomicroscope (6–40x). Finally, the brain, gallbladder, gills, gonads, heart, kidney, liver, mesentery, spleen, swim bladder, epaxial and hypaxial muscle, and the walls of the stomach, intestine and intestinal caeca were pressed between Petri dishes and subsequently examined with a stereomicroscope with transmitted light (6–40x).

Based on Koen-Alonso *et al.* (2000), Romero *et al.* (2011) and Romero *et al.* (2012), each fish species was assigned to an ecological group according its position in the water column as follows: benthic (*i.e.* fish species strictly related to the sea floor), pelagic (*i.e.* fish living near the surface) and demersal (*i.e.* fish living in the water column, near the sea floor). The latter group was divided into demersal-pelagic (*i.e.* fish species with a daily migration pattern, dispersing in the water column at night and staying near the bottom during daylight hours), and demersal-benthic (*i.e.* fish species not performing vertical migration, but not strictly related to the sea floor).

Table 4. Biological data of the 20 fish species sampled from the Patagonian coast of Argentina. Fish common names according to Froese & Pauly (2013). Abbreviations: N, number of specimens analysed; NA, not assigned; TL, total length; SD, standard deviation; W, weight.

Order / Family	Species	Common name	N	TL ± SD (cm)	W ± SD (gr)	Fish sex ♀	♂	NA
SAMPLING I								
Gadiformes								
Merlucciidae	*Merluccius hubbsi* Marini, 1933	Argentine hake	30	30.2 ± 2.6	189.4 ± 46.0	8	2	20
Perciformes								
Nototheniidae	*Patagonotothen ramsayi* (Regan, 1913)	Longtail southern cod	31	25.1 ± 2.5	193.0 ± 64.1	7	–	24
Stromateidae	*Stromateus brasiliensis* Fowler, 1906	Southwest Atlantic butterfish	30	27.3 ± 2.6	291.0 ± 73.8	26	2	2
Pleuronectiformes								
Paralichthyidae	*Xystreurys rasile* (Jordan, 1891)	Flounder	7	38.3 ± 4.5	725.4 ± 236.7	6	–	1
SAMPLING II								
Gadiformes								
Merlucciidae	*Macruronus magellanicus* Lönnberg, 1907	Patagonian grenadier	2	65.0 ± 25.5	801.0 ± 847.1	1	–	1
	Merluccius hubbsi Marini, 1933	Argentine hake	21	23.9 ± 5.0	93.7 ± 58.0	1	–	20
Ophidiiformes								
Ophidiidae	*Genypterus blacodes* (Forster, 1801)	Pink cusk-eel	13	51.8 ± 6.6	601.2 ± 210.1	2	3	8
Perciformes								
Bovichtidae	*Cottoperca gobio* (Günther, 1861)	Channel bull blenny	1	52.0	1500	–	1	–
Bramidae	*Brama brama* (Bonnaterre, 1788)	Atlantic pomfret	2	60.5 ± 2.1	2600.0 ± 282.8	1	–	1
Centrolophidae	*Seriolella porosa* Guichenot, 1848	Choicy ruff	3	41.2 ± 1.4	796.6 ± 54.0	2	1	–
Cheilodactylidae	*Nemadactylus bergi* (Norman, 1937)	Castaneta	1	22.4	130.0	–	1	–
Nototheniidae	*Patagonotothen ramsayi* (Regan, 1913)	Longtail southern cod	21	24.8 ± 3.6	196.8 ± 94.0	5	2	14
Scombridae	*Scomber japonicus* Houttuyn, 1782	Chub mackerel	13	42.7 ± 5.0	304.6	6	6	1
Stromateidae	*Stromateus brasiliensis* Fowler, 1906	Southwest Atlantic butterfish	12	27.9 ± 4.3	312.7 ± 74.1	10	2	–
Pleuronectiformes								
Paralichthyidae	*Xystreurys rasile* (Jordan, 1891)	Flounder	5	34.4 ± 1.7	435.6 ± 71.8	3	2	–

Table 4. continued.

Order / Family	Species	Common name	N	TL ± SD (cm)	W ± SD (gr)	Fish sex ♀	 ♂	 NA
Scorpaeniformes								
Congiopodidae	*Congiopodus peruvianus* (Cuvier, 1829)	Horsefish	15	23.9 ± 2.0	202.1 ± 49.4	8	7	–
Sebastidae	*Helicolenus lahillei* Norman, 1937	Rubio	6	28.8 ± 2.6	419.7 ± 108.3	4	2	–
SAMPLING III								
Gadiformes								
Merlucciidae	*Macruronus magellanicus* Lönnberg, 1907	Patagonian grenadier	1	40.1	2004.0	–	–	1
	Merluccius hubbsi Marini, 1933	Argentine hake	28	29.1 ± 2.0	169.3 ± 36.1	5	5	18
Ophidiiformes								
Ophidiidae	*Genypterus blacodes* (Forster, 1801)	Pink cusk-eel	31	34.7 ± 4.6	159.6 ± 74.3	4	5	22
	Raneya brasiliensis (Kaup, 1856)	Banded cusk-eel	16	21.2 ± 1.4	49.3 ± 11.4	13	1	2
Perciformes								
Bovichtidae	*Cottoperca gobio* (Günther, 1861)	Channel bull blenny	7	27.0 ± 3.2	294.1 ± 98.4	1	1	5
Centrolophidae	*Seriolella porosa* Guichenot, 1848	Choicy ruff	31	32.2 ± 5.2	355.7 ± 170.0	20	7	4
Cheilodactylidae	*Nemadactylus bergi* (Norman, 1937)	Castaneta	31	25.7 ± 5.5	229.8 ± 122.0	9	6	16
Mullidae	*Mullus argentinae* Hubbs and Marini, 1933	Argentine goatfish	2	20.7 ± 0.4	125.3 ± 1.1	–	1	1
Nototheniidae	*Patagonotothen ramsayi* (Regan, 1913)	Longtail southern cod	32	24.9 ± 4.2	173.1 ± 86.1	5	2	25
Percophidae	*Percophis brasiliensis* Quoy and Gaimard, 1825	Brazilian flathead	8	45.3 ± 4.9	392.3 ± 136.9	3	4	1
Pinguipedidae	*Pseudopercis semifasciata* (Cuvier, 1829)	Argentinian sandperch	31	26.5 ± 2.7	211.2 ± 61.0	10	4	17
Serranidae	*Acanthistius patachonicus* (Jenyns, 1840)	Seabass	16	30.0 ± 2.6	482.7 ± 139.8	8	7	1
Stromateidae	*Stromateus brasiliensis* Fowler, 1906	Southwest Atlantic butterfish	31	27.6 ± 4.2	269.2 ± 90.9	20	9	2
Pleuronectiformes								
Paralichthyidae	*Paralichthys isosceles* Jordan, 1891	Flounder	15	27.2 ± 5.3	270.9 ± 165.1	13	–	2
	Xystreurys rasile (Jordan, 1891)	Flounder	17	30.1 ± 5.5	309.9 ± 177.5	16	–	1
Scorpaeniformes								
Triglidae	*Prionotus nudigula* Ginsburg, 1950	Red searobin	32	23.1 ± 2.8	148.3 ± 46.0	18	13	1

3.2. PARASITE FIXATION AND TAXONOMIC DETERMINATION

The intestinal contents and parasites from each pinniped specimen were fixed in 70% or 100% ethanol. Parasites collected from fishes were washed in saline, counted, and fixed and conserved in 70% ethanol. Acanthocephalan cystacanths and nematodes (third-stage larvae) were carefully removed from their capsule before being fixed.

Morphological identification of parasites was carried out at the Marine Zoology Unit of the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia. The specific staining, clearing, and mounting techniques that were used for each taxon will be explained in each chapter, as will additionally microscopical or histological techniques. Illustrations and measurements were made using a drawing tube attached to a bright field microscope. Specimens were identified following the taxonomic criteria provided by specific keys or specific references for each taxonomic group which will also be detailed in each chapter.

Molecular characterization of larval forms of anisakid nematodes was carried out at the Laboratory of Helminthology of the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic. DNA extraction, PCR, sequencing and alignment procedures will be indicated in in their corresponding chapter.

Type specimens and vouchers were deposited in parasite collections of renowned institutions: the Natural History Museum, London, United Kingdom; the Helminthological Collection of the Institute of Parasitology (IPCAS), Biology Centre ASCR, České Budějovice, Czech Republic; the United States National Parasite Collection, Beltsville, Maryland, United States of America, and the National Museum of Natural Sciences, Madrid, Spain. Additional voucher specimens are deposited at the Collection of the Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Valencia, Spain.

3.3. INFECTION PARAMETERS AND STATISTICAL ANALYSES

Ecological terms follow Bush *et al.* (1997). Infection parameters were estimated following Rózsa *et al.* (2000) with the statistical software Quantitative Parasitology v3.0 (Reiczigel & Rózsa, 2005). Most statistical analyses were carried out using the statistical package SPSS v17 (IBM). Specific analyses developed for each study, or other statistical programs used, will be indicated in each chapter. Statistical significance was set at $P < 0.05$, unless otherwise stated.

4. INTESTINAL HELMINTH FAUNA OF THE SOUTH AMERICAN SEA LION *OTARIA FLAVESCENS* AND FUR SEAL *ARCTOCEPHALUS AUSTRALIS* FROM NORTHERN PATAGONIA, ARGENTINA

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Intestinal helminth fauna of the South American sea lion *Otaria flavescens* and fur seal *Arctocephalus australis* from northern Patagonia, Argentina

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Abstract

We report on the intestinal helminth fauna of 56 South American sea lions, *Otaria flavescens*, and 5 South American fur seals, *Arctocephalus australis*, from northern Patagonia, Argentina. A total of 97,325 helminth specimens were collected from sea lions. Gravid individuals were represented by 6 species of parasites: 1 digenean (*Ascocotyle* (*Ascocotyle*) *patagoniensis*), 1 cestode (*Diphyllobothrium* spp.), 3 nematodes (*Uncinaria hamiltoni*, *Contracaecum ogmorhini s.s.*, *Pseudoterranova cattani*) and 1 acanthocephalan (*Corynosoma australe*). In addition, third-stage larvae of 2 nematodes (*Contracaecum* sp. and *Anisakis* sp. type I) and 3 juvenile acanthocephalans (*Andracantha* sp., *Profilicollis chasmagnathi* and *Corynosoma cetaceum*) were also collected. *Andracantha* sp., *C. ogmorhini s.s.* and *P. chasmagnathi* represent new host records. A total of 1516 helminth specimens were collected from fur seals. Gravid individuals were represented by three species of parasites, namely, *Diphyllobothrium* spp., *C. ogmorhini s.s.* and *C. australe*. In addition, larvae of *Contracaecum* sp. and *P. cattani*, juveniles of *C. cetaceum* and immature cestodes (Tetrabothriidae gen. sp.) were also collected. *Corynosoma australe* was the most prevalent and abundant parasite in both hosts, accounting for >90% of all specimens. Sea lions and furs seals from northern Patagonia harbour the intestinal helminth communities that could be predicted for otariids, i.e. the combination of species of the genera *Corynosoma*, *Diphyllobothrium*, *Pseudoterranova*, *Contracaecum* and, in pups, *Uncinaria*. Additionally, both species of otariid are apparently unsuitable hosts (i.e. non-hosts) for as many as five parasite taxa. The inclusion or exclusion of these species affects estimation of species richness at both component community (11 versus 6 species in sea lions; 7 versus 3 species in fur seals) and infracommunity (mean: 3.1 versus 2.6 in sea lions; 2.2 versus 1.7 species) levels. Information about the reproductive status of helminth species is often lacking in parasitological surveys on otariids and other marine vertebrates, but it is of significance to improve precision in parascript studies or ecological meta-analyses.

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Introduction

The South American sea lion, *Otaria flavescens*, and the South American fur seal, *Arctocephalus australis*, are common pinnipeds along the eastern and western coasts of South America. The South American sea lion and the South American fur seal are distributed from Peru to southern Brazil (Vaz-Ferreira, 1982; Crespo, 1988; Sanfelice *et al.*, 1999; Túnez *et al.*, 2008). Along only the Argentine coast, over 100 colonies of sea lion and 17 of fur seal have been reported to date (Crespo *et al.*, 1999; Dans *et al.*, 2004). Not surprisingly, there is a great deal of information about the feeding ecology, life history parameters and population dynamics for both species of otariid (Crespo *et al.*, 1997, 1999; Lima & Páez, 1997; Koen Alonso *et al.*, 2000; Naya *et al.*, 2002; Suarez *et al.*, 2005; Cappozzo & Perrin, 2009, and references therein).

Several studies have also addressed diverse aspects about the helminth fauna of South American sea lions and fur seals, including single-species records (Southwell & Walker, 1936; Dailey, 1975; Lauckner, 1985; Zdzitowieki, 1991; Aznar *et al.*, 2012, and references therein), taxonomic issues (Zdzitowiecki, 1986; George-Nascimento & Urrutia, 2000; Timi *et al.*, 2003; Sardella *et al.*, 2005) and population studies of some parasite species (George-Nascimento & Marin, 1992; George-Nascimento *et al.*, 1992; Aznar *et al.*, 2004; Berón-Vera *et al.*, 2004). What is lacking, however, is a community perspective in the study of the helminth fauna from both species of pinnipeds. This integral, quantitative approach is fundamental to address key questions about the factors that determine the diversity and composition of parasite faunas in carnivores (Lindenfors *et al.*, 2007) or vertebrates in general (Poulin, 1995). To our knowledge, only the study by George-Nascimento & Carvajal (1981) has provided a partial quantitative account of the gastrointestinal helminth fauna of the South American sea lion based on a sample of seven male individuals collected on the coast of Chile.

In this study we report, for the first time, on the intestinal helminth communities of the South American sea lion and the South American fur seal from northern Patagonia, Argentina. In particular, we assess the importance of specificity in shaping community diversity. Recently, some authors (Mateu *et al.*, 2011; Aznar *et al.*, 2012) have pointed out that many putative hosts for trophically transmitted parasites from marine mammals could actually be non-hosts in which the parasite passes through the gut without further development (i.e. the parasite never establishes). This phenomenon may have important implications: (1) to assess the role of putative non-hosts in the population dynamics of helminths; and (2) to define the ‘true’ community of helminths of a given host species. This discussion provides the context to compare species richness and composition of the helminth communities of both species with those from other otariids.

Materials and methods

Collection and examination of sea lions and fur seals

A total of 56 South American sea lions and 5 South American fur seals were collected in northern Patagonia (40°43′–43°20′S, 63°04′–65°07′W), either stranded on the coast (48 sea lions and 4 fur seals) or as by-catch in fisheries (8 sea lions and 1 fur seal), between 1998 and 2009. For each specimen, the standard body length (SL) was measured to the nearest centimetre (Committee on Marine Mammals, 1967). The age has been estimated from counts of the incremental growth layers in the dentine of tooth sections or in the cementum growth layers (Grandi *et al.*, 2010). Thirty out of the 56 specimens of sea lion were males (SL, mean ± SD [range]: 168 ± 47.9 [90–239]; age (years), 6.4 ± 4.7 [0.3–15]) and 26 were females (SL: 159.4 ± 31.8 [109–228]; age, 10 ± 7.1 [0.4–21]). Four out of the 5 fur seals were males (SL: 139.3 ± 49.0 [68–176]; age, 6.6 ± 3.8 [1.0–10.5]) and 1 was a female (SL: 78; age, 1 year).

Following post-mortem examination, the intestine of each host was removed from the carcass and kept frozen at −20°C. After thawing, the intestine was opened following the procedure described in Aznar *et al.* (2004). Intestinal contents were washed with tap water through sieves of either 0.2 or 0.5 mm mesh. Additionally, the intestine wall was examined to collect attached worms. Intestinal contents were later examined under a stereomicroscope (40–80×). Parasites were fixed in 70% ethanol.

Parasitological procedures

For parasite identification, trematodes were stained with iron acetocarmine or alum carmine, dehydrated through an ethanol series, cleared in clove oil and mounted in Canada balsam. In the case of cestodes of the genus *Diphyllobothrium*, gravid proglottids ($n = 3$) were positioned in sagittal view alongside in cassettes, dehydrated and embedded in acrylic resin. Then, sections (2 μm) were obtained and stained with 1% toluidine blue. Also, gravid proglottids ($n = 5$) were stained in alum carmine and cut by hand using a microtome blade to obtain sagittal sections. Sections were then later dehydrated through an ethanol series, mounted in Canada balsam and examined in profile for species identification according to Rausch *et al.* (2010). Nematodes and acanthocephalans were cleared in glycerin or lactophenol. Additionally, some acanthocephalans were stained in Mayer’s carmine, dehydrated through an ethanol series, cleared in methyl salicylate and mounted in Canada balsam. The stage of development of anisakids, i.e. third-stage, fourth-stage and adult, was determined according to Berland (1961), Kagei (1969), George-Nascimento & Urrutia (2000) and Timi *et al.* (2001, 2003). Mounted or cleared specimens were examined with a compound microscope using bright field and differential interference contrast optics (400–1000×). Drawings and morphometric measurements were taken with the aid of a drawing tube. All morphometric measurements are given in micrometres (μm) and expressed as the mean followed by SD, with the range in parentheses. Voucher specimens are deposited in the Natural History Museum, London, UK (accession numbers for parasites from *O. flavescens*: *Diphyllobothrium* spp. (2012.5.15.40–65), *Anisakis* sp. (2012.5.15.23–24), *Contracaecum* sp. (2012.5.15.25–34), *Contracaecum ogmorhini s.s.* (2012.5.15.77–92), *Pseudoterranova cattani* (2012.5.15.66–76), *Uncinaria hamiltoni* (2012.5.15.35–39), *Corynosoma australe* (2012.5.15.1–20) and *Corynosoma cetaceum* (2012. 5.15.21–22); accession numbers for parasites from *A. australis*: *Diphyllobothrium* spp. (2012.5.15.140–141), Tetrabothriidae gen. sp. (2012.5.15.141–143), *Contracaecum* sp. (2012.5.15.125–139), *C. ogmorhini s.s.* (2012.5.15.173), *C. australe* (2012.5.15.93–122) and *C. cetaceum* (2012.5.15.123–124)) and the Collection of the Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

Some cestode, nematode and acanthocephalan specimens were also examined using scanning electron microscopy. Specimens were dehydrated through an ethanol series, critical point dried and coated with gold–palladium alloy to a thickness of 250 nm. Specimens were then examined at the Central Service of the Support to the Experimental Research (SCSIE) of the University of Valencia with a Hitachi 4100 FE scanning electron microscope operating at 20 kV.

Infection parameters were estimated following Bush *et al.* (1997) and Rózsa *et al.* (2000). The 95% confidence intervals (CI) for prevalence were set with Sterne's exact method (Reiczigel, 2003); the 95% CIs of mean intensity and mean abundance were estimated with 20,000 bootstrap replications with the statistical software Quantitative Parasitology v.3 (Reiczigel & Rósza, 2005).

Results

All South American sea lions harboured intestinal helminths; infection parameters are shown in table 1. A total of 97,325 helminth specimens were collected; 88,998 were acanthocephalans, 3684 were nematodes, 4589 were digeneans and 54 were cestodes. Adult specimens were ascribed to six species, namely, *Ascocotyle* (*Ascocotyle*) *patagoniensis*, *Diphyllobothrium* spp., *Contracaecum ogmorhini s.s.*, *Pseudoterranova cattani*, *Uncinaria hamiltoni* and *Corynosoma australe*. In addition, five forms were found either as third-stage larvae (the nematodes *Contracaecum* sp. and *Anisakis* sp. type I) or juvenile individuals (*Andracantha* sp., *Profilicollis chasmagnathi* and *Corynosoma cetaceum*).

In the South American fur seals, helminths were collected from the four males; the female was uninfected (table 2). From a total of 1516 helminth individuals, 1408 specimens were acanthocephalans, 99 were nematodes and 9 were cestodes. Adult specimens corresponded to three species, namely, *Diphyllobothrium* spp., *C. ogmorhini s.s.* and *C. australe*. In addition, four forms were found either as third-stage larvae (*Contracaecum* sp.), fourth-stage larvae (*P. cattani*), juvenile (*C. cetaceum*) or sexually immature individuals (Tetrabothriidae gen. sp.).

A single female specimen of an unidentified species of *Andracantha* was found in the rectum of one South American sea lion. The specimen, which was poorly preserved, had numerous ovarian balls. The pipe-shaped trunk was 2594 long and 1574 wide (disc diameter). The disc was covered with spines, except in a small circle on the anterior part where spines became scattered, or even disappeared in a small ventral area (fig. 1). A continuous field of faint spines was also observed along the hind trunk, but spines did not reach the vicinity of the genital pore (fig. 1). The proboscis was partly invaginated and its whole morphological features could only be ascertained by transparency. It had a conical shape, widest at its base, 670 long and 270 wide, with 12 hooks, the 4 basal ones being rootless.

Specimens of *Diphyllobothrium* spp. collected from the South American fur seal had a rounded–ellipsoidal scolex in lateral view ($n = 2$; fig. 2A). Gravid proglottids were 1944 ± 214 (1667–2292, $n = 13$ from two specimens) long and 3391 ± 274 (3042–3833) wide. Width–length ratio of mature proglottids was 1:1.8 (1:1.3–2.2). Transverse tegumentary recesses and pits were observed extending ventrally from the anterior margin of proglottid to the anterior margin of genital atrium on the midline of proglottid. Eggs were 56.9 ± 2.6 (52.1–59.6) long and

Table 1. Infection parameters of helminths found in 56 South American sea lions (*Otaria flavescens*) from northern Patagonia, Argentina.

Species	Prevalence (%) (95% CI)	Mean abundance (95% CI)	Mean intensity (95% CI)	Range
Trematoda				
Ascocotyle (*Ascocotyle*) *patagoniensis*[a]	–	–	–	48–4541
Cestoda				
Diphyllobothrium spp.	26.8 (17.8–41.9)	1.0 (0.5–2.6)	3.4 (1.8–7.9)	1–24
Nematoda				
Uncinaria hamiltoni	3.6 (0.6–12.2)	1.4 (0.0–4.9)	38.0	14–62
Contracaecum ogmorhini[b]	62.5 (49.1–74.2)	8.3 (5.5–12.8)	13.3 (9.3–19.6)	1–70
Pseudoterranova cattani	66.1 (54.7–77.9)	9.8 (3.5–34.5)	14.8 (5.5–55.8)	1–313
Contracaecum sp. (L3)	69.9 (56.3–80.5)	45.6 (21.9–121.1)	65.5 (32.0–169.1)	1–1022
Anisakis sp. (L3)	28.6 (17.6–41.9)	0.6 (0.4–1.2)	2.3 (1.5–3.8)	1–9
Acanthocephala				
Corynosoma australe	100 (93.3–100)	1589 (1177.4–2215.1)	1589 (1182.9–2233.9)	3–10,489
Corynosoma cetaceum	8.9 (3.6–19.4)	0.21 (0.1–0.7)	2.4 (1.0–4.8)	1–7
Profilicollis chasmagnathi[b]	0.2 (0.0–1.0)	0.02 (0.0–0.05)	1.0	1
Andracantha sp.[b]	0.2 (0.0–1.0)	0.02 (0.0–0.05)	1.0	1

CI, confidence interval; L3, third-stage larvae.

[a] Infection parameters could not be calculated for the entire sample (see Materials and methods). The range corresponds to two hosts for which a complete census of specimens was obtained.

[b] New host record.

Table 2. Infection parameters of helminths found in five South American fur seals (*Arctocephalus australis*) from northern Patagonia, Argentina.

Species	Prevalence (%) (95% CI)	Mean abundance (95% CI)	Mean intensity (95% CI)	Range
Cestoda				
Diphyllobothrium spp.	20 (0.0–71.7)	0.4 (0.0–0.8)	2	–
Tetrabothriidae gen. sp.	20 (0.0–71.7)	1.4 (0.0–2.8)	7	–
Nematoda				
Contracaecum ogmorhini	20 (0.0–71.7)	5.0 (0.0–10.0)	25	–
Pseudoterranova cattani	20 (0.0–71.7)	0.4 (0.0–0.8)	2	–
Contracaecum sp. (L3)	20 (0.0–71.7)	14.4 (0.0–28.8)	72	–
Acanthocephala				
Corynosoma australe	80 (28.4–99.5)	279.6 (100.0–459.2)	349.5 (162.3–523.5)	149–567
Corynosoma cetaceum	40 (5.3–85.4)	2 (0.0–5.6)	5 (1.0–5.0)	1–9

CI, confidence interval; L3, third-stage larvae.

39.6 ± 2.0 (36.2–43.3) wide ($n = 25$, from six proglottids of two specimens). No other structures were discernible.

Specimens of *Diphyllobothrium* spp. from the South American sea lion had a lanceolate scolex ($n = 54$; fig. 2B). Gravid proglottids were 2874 ± 763 (1750–4042, $n = 15$ from two specimens) long and 2056 ± 263 (1667–2500, $n = 15$) wide. Width–length ratio of mature proglottids was 1:0.78 (1:0.42–1.33). Transverse tegumentary recesses or pits could not be observed in these specimens, probably because the tegument was poorly preserved. Eggs were 55.8 ± 2.2 (54.0–60.4) long and 35.2 ± 2.0 (33.3–37.5) wide ($n = 18$, from five proglottids of three specimens). No other structures were discernible.

Specimens of Tetrabothriidae gen. sp. from the South American fur seal had a round scolex ($n = 7$; fig. 3A) with four round, sucker-like bothridia; 184 ± 51 (81–226, $n = 10$) long and 166 ± 36 (93–196) wide, each with a small appendage located in antero-lateral to anterio-medial position (fig. 3A and B). Remains of a sucker-like structure were observed at the apex of the scolex in two specimens (fig. 3A). Numerous fragments of strobili with non-mature proglottids were observed.

The intestinal component community of helminths from the South American sea lion was composed of 11 species. At infracommunity level, species richness ranged from 1 to 6 species (mean ± SD: 3.1 ± 1.1 species). A total of 3 hosts (5.4%) were infected with 1 helminth species; 14 (25.0%) with 2; 20 (35.7%) with 3; 14 (25.0%) with 4; 4 (7.1%) with 5, and a single host (1.8%) with 6. In contrast, the intestinal component helminth community of the South American fur seal was composed of seven species. At infracommunity level, species richness ranged from 1 to 5 species (2.2 ± 1.9). One host (20%) was infected with 1 helminth species, 1 (20%) with 2, 1 (20%) with 3, and 1 host (20%) with 5. A single host (a 1-year-old female) was uninfected. There were no significant differences of infracommunity richness between sea lions and fur seals (Mann–Witney test, $U = 91.5$, $P = 0.209$).

Values of helminth species richness are strongly influenced by the taxa that were found only as larvae or juvenile individuals. When these taxa are excluded, the component community of the South American sea lion dropped to six species, and mean infracommunity species richness to 2.6 ± 0.9 species (range 1–5). A total of 5 hosts (8.9%) were infected with 1 helminth species; 20 (35.7%)

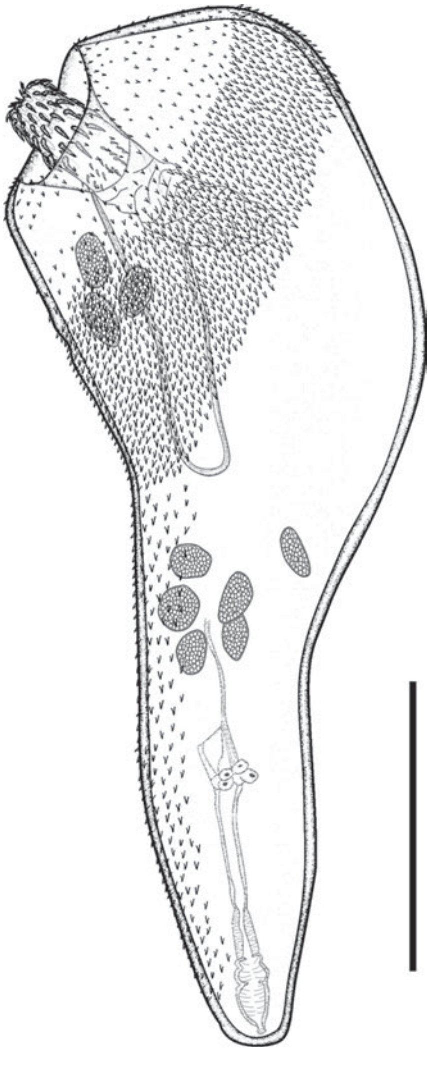

Fig. 1. *Andracantha* sp. from the intestine of the South American sea lion, *Otaria flavescens* from northern Patagonia, whole-mount lateral view. Scale bar: 1000 μm.

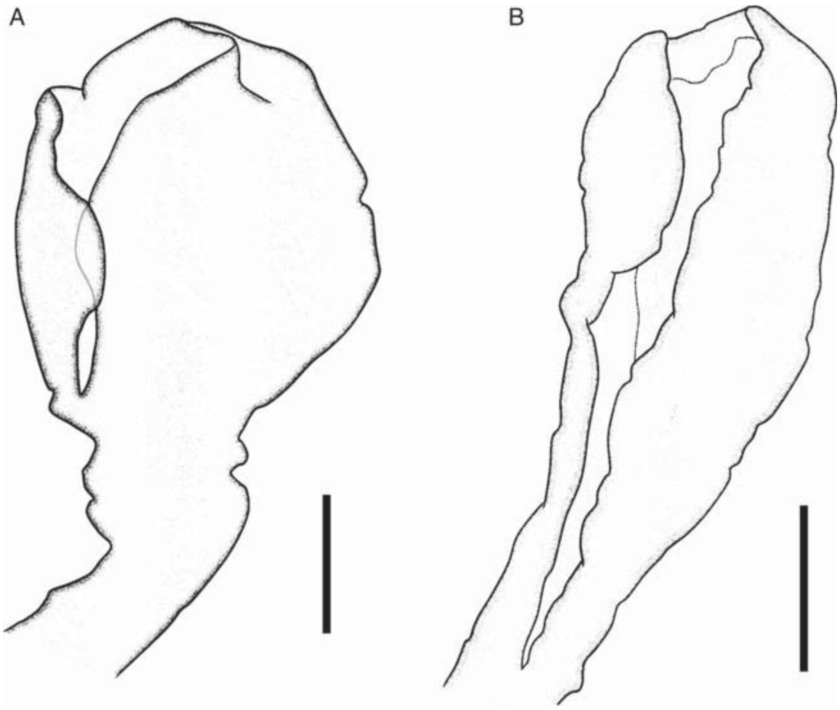

Fig. 2. Scolices of *Diphyllobothrium* spp. collected from the intestine of otariids from northern Patagonia, Argentina, lateral view. (A) *Diphyllobothrium* spp. from the South American fur seal, *Arctocephalus australis*. (B) *Diphyllobothrium* spp. from the South American sea lion, *Otaria flavescens*. Scale bars: 500 μm.

with 2; 22 (39.3%) with 3; 8 (14.3%) with 4 and 1 (1.8%) with 5. In the South American fur seal, the component community excluding larvae and juveniles was composed of four species (mean infracommunity species richness 1.75 ± 1.0). A single host (20%) harboured 4 helminth species and 3 (60%) harboured 1.

Discussion

Parasite composition

Ascocotyle (*A.*) *patagoniensis* was recently described from the South American sea lion (Hernández-Orts *et al.*, 2012). Species of the subgenus *Ascocotyle* infect birds and

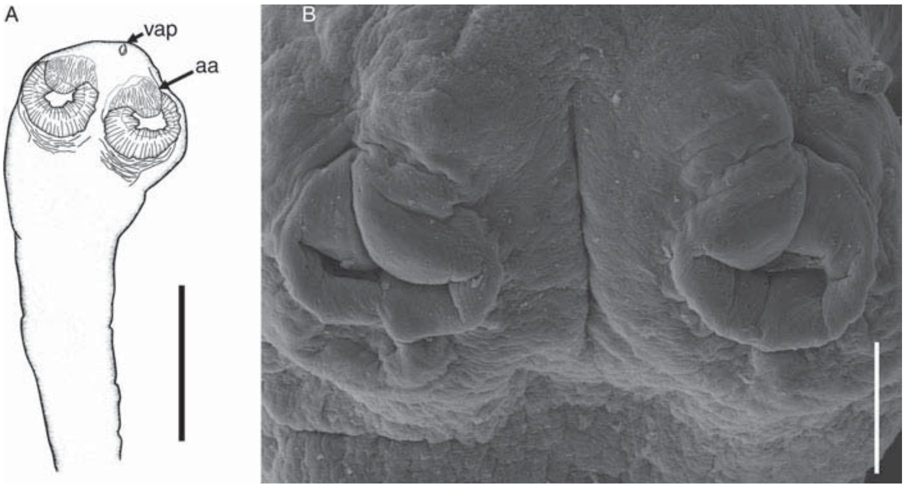

Fig. 3. Tetrabothriidae gen. sp. from the South American fur seal, *Arctocephalus australis*. (A) Scolex, lateral view. (B) Scanning electron micrographs of the round, sucker-like bothridia, lateral view. Scale bars: 400 μm (A) and 60 μm (B). vap, vestiges of apical sucker; aa, anterior appendage.

mammals of freshwater or brackish habitats (Font *et al.*, 1984; Ostrowski de Nuñez, 2001). Therefore, this is the first record of a species from this subgenus in a marine habitat and in a marine mammal. However, the life cycle of *A.* (*A.*) *patagoniensis* is yet to be elucidated; no metacercariae of this species were found in 542 individuals from 20 marine teleost species (Hernández-Orts *et al.*, 2012), nor have specimens of *Ascocotyle* spp. been reported in marine fish from Patagonia (Timi & Poulin, 2003; Sardella & Timi, 2004; Vales *et al.*, 2011, and references therein). Although the high parasite burdens indicated that it was not an accidental parasite, infection parameters of *A.* (*A.*) *patagoniensis* could not be determined reliably because we could not rule out that an indeterminate number of specimens had been lost during sieving, due to the small size of the parasite. It is therefore advisable that sieves <0.2 mm mesh are used in future parasitological surveys of sea lions or other pinnipeds.

Species of *Diphyllobothrium* are common intestinal parasites of fur seals and sea lions (Rausch *et al.*, 2010). Two species have been reported frequently in otariids from the southern hemisphere, i.e. *D. pacificum* and *D. arctocephalinum*, in the Pacific and Atlantic Oceans, respectively (Rausch *et al.*, 2010). The specimens of *Diphyllobothrium* spp. collected in the present study were very poorly preserved, which prevents identification at the species level. Based on some morphological traits (e.g. scolex shape, proglottids and size of eggs) specimens of *Diphyllobothrium* spp. from South American fur seals resemble both *D. pacificum* and *D. arctocephalinum* (table 3). Similarities are less evident in specimens collected from South American sea lions (table 3). Molecular data and morphological examination based on suitable material will be necessary for a reliable identification of specimens of species of *Diphyllobothrium* infecting sea lions and fur seals in northern Patagonia.

Species of Tetrabothriidae occur as parasites of marine birds and marine mammals and are assigned to six genera based on features of the scolex and the reproductive system (Hoberg, 1994). No gravid proglottids were observed in any of the specimens collected from fur seals in our study. Nor did the structure of the scolex allow us to make a reliable generic allocation of our specimens. In tetrabothriids, the presence of vestiges of the larval apical sucker suggests that the ontogeny of the scolex is almost complete (Hoberg, 1987; Hoberg *et al.*, 1991; Hoberg & Measures, 1995). Accordingly, our specimens probably represented a relatively recent infection and/or they could not progress development further. In any event, the structure of the scolex would preclude the assignation of our specimens to *Tetrabothrius*, which should exhibit clear, laterally directed auricular appendages at this stage of development (Hoberg, 1987). The type of anterior appendages that we observed is reminiscent of, but not similar to, those from species of *Anophryocephalus* or *Trygonocotyle* (Hoberg, 1994, and references therein). New material should therefore be made available to perform further morphological and molecular analyses in order to identify these cestodes.

Species of *Uncinaria* have been reported in fur seals and sea lions worldwide, including the South American sea lion in the study area (Berón-Vera *et al.*, 2004). Adults

Table 3. Comparison of taxonomic data between *Diphyllobothrium pacificum*, *D. arctocephalinum* and unidentified species of *Diphyllobothrium* collected from sea lions and fur seals in northern Patagonia, Argentina. Measurements in micrometres.

	D. pacificum	*D. arctocephalinum*	*Diphyllobothrium* spp.	*Diphyllobothrium* spp.
Reference	Rausch *et al.* (2010)	Rausch *et al.* (2010)	Present study	Present study
Host	Otariids	Otariids	South American sea lion	South American fur seal
Distribution	Pacific Ocean (northern and southern hemispheres)	Pacific Ocean (southern hemisphere), southern Atlantic coast of South Africa	Southern Atlantic, coast of Argentina	Southern Atlantic, coast of Argentina
Scolex morphology (lateral view)	Rounded–ellipsoidal	Rather ellipsoidal	Lanceolate	Rounded–ellipsoidal
Pits anterior to genital atrium	Present	Present	Could not be observed	Present
Length/width ratio of gravid proglottids	1:4–5	1:1.5	1:0.42–1.33	1:1.3–2.2
Egg size (range)	48.7–56.0 × 38.9–48.7	41.4–56.0 × 36.5–43.8	54.0–60.4 × 33.3–37.5	52.1–59.6 × 36.2–43.3

reproduce in the intestine of pups, and evidence suggests that pups become infected through the milk of mothers carrying third-stage larvae (Lyons *et al.*, 2000 and references therein). In our sample, individuals of *U. hamiltoni* were collected from the two youngest pups, 5 and 7 months old, but were not found in any of ten pups aged 8–10 months. The latter were probably in the weaning process (Cappozzo & Perrin, 2009) and had no further opportunity of (re)infection. Therefore, these pups either had never been infected or had cleared previous infections. The latter possibility would confirm that most infections occurred soon after parturition, as has been suggested for species of *Uncinaria* infecting otariids in the northern hemisphere; in these species, the estimated life-span of the parasite in pups was 3–8 months (Lyons *et al.*, 2000).

Contracaecum ogmorhini s.s. and *P. cattani* have been reported in the stomach of pinnipeds in South America (George-Nascimento & Urrutia, 2000; Timi *et al.*, 2003). In this study, fourth-stage larvae and adult specimens of both species were frequently found in the intestine of sea lions, and in 1 out of 5 fur seals. Most specimens, especially those collected from the middle and posterior intestine, were degraded, suggesting that they likely were senescent worms from the stomach that were passing throughout the gut. However, whether or not the upper intestine could be a suitable microhabitat for these nematodes is an open question.

Third-stage larvae of *Contracaecum* sp. were the most abundant nematodes collected from the intestine (tables 1 and 2). Larvae of *Contracaecum* sp. cannot be identified to the species level based on morphological traits. We believe that our material probably contains not only third-stage larvae from *C. ogmorhini s.s.*, but also from sympatric species of *Contracaecum* that typically mature in marine birds or other pinnipeds, which come from digested fishes. Apart from *C. ogmorhini s.s.* at least four species of *Contracaecum* have been reported in northern Patagonia: *C. pelagicum*, from the Magellan penguin, *Spheniscus magellanicus*; *C. chubutensis* from the Imperial cormorant, *Phalacrocorax atriceps*; *C. osculatum s.l.* and *C. mirounga*e from the elephant seal, *Mirounga leonina* (Mattiucci *et al.*, 2003; Garbin *et al.*, 2007, 2008).

Species of *Anisakis* are typical parasites of cetaceans (Mattiucci & Nascetti, 2008). The morphology of the third-stage larvae of *Anisakis* sp. collected from South American sea lions conforms to Berland's (1961) type I, which corresponds to the larvae of *A. simplex s.l.* (Nascetti *et al.*, 1986). These larvae are widespread in fishes from northern Patagonia (Timi & Poulin, 2003; Sardella & Timi, 2004; Timi & Lanfranchi, 2009, and references therein), whereas adults have been recorded from species of toothed whales from the same locality (Dans *et al.*, 1999; Berón-Vera *et al.*, 2007, 2008).

Corynosoma australe was the most prevalent and abundant parasite from the intestine of the South American sea lion and the South American fur seal, accounting for $>90\%$ of all individual parasites found in both species. This acanthocephalan is a common parasite of pinnipeds (principally otariids) from the southern hemisphere, including sea lions (George-Nascimento & Marin, 1992; Aznar *et al.*, 2012) and fur seals (Aznar *et al.*, 2004; Sardella *et al.*, 2005). In Uruguay, *C. australe* is known to readily infect the South American fur seal because the parasite seems to be ecologically ubiquitous, occurring in many species of both pelagic and demersal fish (Aznar *et al.*, 2004). The same situation appears to occur in northern Patagonia, where cystacanths of this species have been found in at least 17 pelagic, benthopelagic and benthic fish species (Hernández-Orts, unpublished data); there are additional records in other parasitological surveys (Timi & Poulin, 2003; Sardella & Timi, 2004; Vales *et al.*, 2011, and references therein). Not surprisingly, *C. australe* appeared in all sea lions analysed, even in pups aged 4, 5 or 7 months.

Juvenile specimens of three additional acanthocephalan species, i.e. *C. cetaceum, P. chasmagnathi* and *Andracantha* sp. were found in this study. It has been suggested that *C. cetaceum* is specific to cetaceans, but juvenile specimens also occur in different pinniped species, including both species of otariids from South America (Aznar *et al.*, 2001, 2012). Our data confirm that *C. cetaceum* is apparently unable to mature in sea lions and fur seals. Definitive hosts for species of *Profilicollis* are marine fish-eating birds (Zdzitowiecki, 1991). *Profilicollis chasmagnathi* is the only species from this genus that has been reported from the Atlantic coast of South America. There are records of cystacanths in grapsid crabs from Uruguay (Holcman-Spector *et al.*, 1977). Adult worms have been recorded in Olrog's gull, *Larus atlanticus,* from Bahía Blanca (La Sala & Martorelli, 2007) and in the kelp gull, *Larus dominicanus*, from northern Patagonia (Diaz *et al.*, 2011).

Species of *Andracantha* also reproduce in marine birds, especially cormorants, worldwide (García-Varela *et al.*, 2009). In Argentina, *Andracantha* sp. had only been reported from nototheniid fishes in the Beagle Channel (Laskowski & Zdzitowiecki, 2009). We assigned our specimen to this genus based on the observed pattern of trunk spination, which conforms to the generic concept as defined by Zdzitowiecki (1989), i.e. the possession of conspicuous spines arranged in two circular fields separated from each other by either a bare zone or a zone covered with smaller spines. Unfortunately, we were unable to make a reliable specific assignation of our immature specimen because it, too, was in poor condition and had a partly inverted proboscis. Interestingly, our specimen closely resembles immature specimens of an unidentified species of *Andracantha* that was recently found in a Franciscana dolphin, *Pontoporia blainvillei*, in Buenos Aires Province (Aznar *et al.*, 2012). Overall, specimens of *Andracantha* collected from marine mammals in Argentina are most similar to *A. phalacrocoracis*, but the combination of a singular pattern of trunk armature and proboscis traits does not preclude the possibility that they might represent a new species (see Schmidt, 1975; Zdzitowiecki, 1989).

Parasite diversity

Otariids from northern Patagonia seem to be suitable hosts for 6–7 species (depending on whether there are one or two species of *Diphyllobothrium*), five of which belong to genera whose species infect otariids worldwide (table 4), i.e. *Diphyllobothrium*, *Corynosoma*, *Pseudoterranova*, *Contracaecum* and *Uncinaria* (Dailey, 1975;

Table 4. Richness and composition of intestinal heminth fauna of otariid species obtained from surveys worldwide. N, host sample size; C, number of helminth taxa in the total sample (component community); I, number of taxa per individual host (infracommunity) calculated as the sum of prevalences expressed on a per unit basis.

					Number of species				
Species	Locality[a]	N	C	I	*Diphyllobothrium*	*Corynosoma*	*Contracaecum*	*Pseudoterranova*	Other species (genera)
Callorhinus ursinus	Northern Japan[1]	50	6	NA	1	1	1	1	1 (*Anisakis*)
	Komandor Islands[2]	109	12	NA	3	2	1	1	5 (*Anisakis, Phocascaris*, Anisakidae gen.)
	Okhotsk Sea[3]	38	6	1.2	2	–	–	1	3 (*Bolbosoma*, Anisakidae gen.)
Eumetopias jubatus	California[4]	9	3	0.4	1	1	–	–	1 (*Apophallus*)
	Oregon[5]	9	7	1.3	3	2	–	1	1 (*Apophallus*)
	Gulf of Alaska[6]	67	9	4.2	1	2	1	–	5 (*Diplogonoporus, Anophryocephalus, Apophallus, Phocitrema, Bolbosoma*)
	Bering Sea[6]	7	7	2.6	1	3	1	–	3 (*Diplogonoporus, Anophryocephalus*)
Zalophus californianus	California[4]	14	6	1.6	1	1	1	–	3 (*Apophallus, Stictodora, Uncinaria*)
	Oregon[5]	7	3	0.7	–	–	–	1	2 (*Apophallus, Nanophyetus*)
Arctocephalus philippii	Juan Fernández I.[7]	17	3	1.9	1	1	–	–	1 (*Ogmogaster*)
Arctocephalus pusillus	Namibia[8]	8	3	NA	1	1	–	–	1 (Tetrabothriidae gen.)
Otaria flavescens	Chile[9]	7	3	NA	1	1	–	–	1 (*Ogmogaster*)
	Argentina[10]	56	11	3.1	1	2	2	1	5 (*Ascocotyle, Anisakis, Profilicollis, Andracantha, Uncinaria*)
Arctocephalus australis	Argentina[10]	5	7	2.2	1	2	2	1	1 (Tetrabothriidae gen. sp.)

NA, Not available.

[a] Source: [1]Machida (1969); [2]Delyamure & Skrjabin (1960); [3]Krotov & Delyamure (1952); [4]Dailey & Hill (1970); [5]Stroud (1978); [6]Shults (1986); [7]Sepulveda & Alcaino (1993); [8]Delyamure & Parukhin (1968); [9]George-Nascimento & Carvajal (1981); [10]Present study.

Lauckner, 1985; Nascetti *et al.*, 1986; Aznar *et al.*, 2006; Castinel *et al.*, 2006; Rausch *et al.*, 2010, and references therein). The global occurrence of this predictable group of species in pinnipeds suggests that associations might have been established prior to the splitting of the three major pinniped clades (Hoberg & Adams, 2000); nevertheless, *Uncinaria* is almost exclusively associated with otariids (George-Nascimento *et al.*, 1992). However, the coevolutionary history between these five parasite taxa and otariids may be complex, including independent associations of the parasites with fur seals and sea lions during the expansion to the southern hemisphere (Hoberg & Adams, 2000).

There are additional taxa that appear to have become associated with otariids at a more regional geographical scale. This might be the case of species of *Anophryocephalus*, *Phocitrema* and *Stictodora* in the northern hemisphere, and species of *Ogmogaster* and *Ascocotyle* in the southern hemisphere (see references in table 4). These associations are probably linked to a history of host-switching, with geographical restriction resulting, at least in part, from the temporal limits of the initial colonization event (Hoberg & Adams, 2000; see also Hoberg, 1995, for a detailed account of the history of *Anophryocephalus* spp.). Finally, there is another group of species whose consideration as actual community members is more doubtful. As many as six parasite taxa found in this study have cetaceans, marine birds and even fish as definitive hosts, pinnipeds being apparently unsuitable hosts for these taxa. This might also be the case at least for species of *Anisakis*, Tetrabothriidae, *Bolbosoma* and *Nanophyetus* reported in other otariids (table 4).

The exchange of parasites species is not particularly surprising in hosts that belong to the same trophic level, because many infective stages of parasites can end up in the 'wrong' hosts (Hoberg, 1987, 1996; Hoberg & Adams, 2000; Raga *et al.*, 2009). This process is apparent in guilds of hosts with close phylogenetic affiliation; for instance, Antarctic pinnipeds share several species of *Corynosoma*, but not all species are able to mature in all pinniped species (Zdzitowiecki, 1991). The point is, however, the extent to which species that do not reproduce in a given host can be qualified as members of its helminth community or assemblage with regard to taxonomic composition or diversity (e.g. species richness) of the assemblage, or the potential for interspecific interactions. For instance, the helminth component community of the South American sea lion is close to the upper limit of values reported for otariids worldwide (table 4). However, this does not result just from an artefact due to a high sampling effect, but also from the inclusion of parasites for which sea lions are putative non-hosts. Obviously, the category 'non-host' is based on the assumption that the absence of sexually mature worms is related to their inability to reproduce in sea lions and/or fur seals, rather than to the fact that these infections are recent (Mateu *et al.*, 2011; Aznar *et al.*, 2012). This assumption appears to be reasonably supported in parasite taxa that are typical in other definitive hosts, i.e. *Andracantha* sp. and *P. chasmagnathi* from birds, *C. cetaceum* and *Anisakis* sp. from cetaceans. However, the caveat remains that larger host sample sizes collected over longer time frames could be necessary to confirm categorization of sea lions and fur seals as 'non-hosts' for specific parasites.

Omitting the parasite taxa for which sea lion and fur seals are putative non-hosts can have a significant impact on the estimation of species richness, particularly at component community level, and can lead to more accurate comparisons among otariid species (table 4). Unfortunately, information about the reproductive status of helminths is frequently lacking from parasitological surveys of otariids, or it is merely assumed that any parasite found in a host is part of their helminth fauna (see references in table 4). However, data from our study highlight the quantitative difference of including or excluding parasites obtained from non-hosts (see Aznar *et al.*, 2012). We urge researchers to always provide appropriate information on maturity status of the parasites, if they are to be informative for parascript studies (e.g. Hoberg & Adams, 2000) or meta-analyses (e.g. Poulin, 1995; Lindenfors *et al.*, 2007).

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5. A NEW SPECIES OF *ASCOCOTYLE* (TREMATODA: HETEROPHYIDAE) FROM THE SOUTH AMERICAN SEA LION, *OTARIA FLAVESCENS*, OFF PATAGONIA, ARGENTINA

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A NEW SPECIES OF *ASCOCOTYLE* (TREMATODA: HETEROPHYIDAE) FROM THE SOUTH AMERICAN SEA LION, *OTARIA FLAVESCENS*, OFF PATAGONIA, ARGENTINA

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ABSTRACT: We describe a new heterophyid species, *Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp., based on specimens collected from the intestines of the South American sea lion *Otaria flavescens* from Patagonia (Argentina). *Ascocotyle* (*A.*) *patagoniensis* n. sp. is distinguished from the other species of the subgenus by the number of circumoral spines, which are arranged in 2 rows of 18 to 23. The new species also differs from the other species in having a gonotyl without papillae. The specimens exhibited the widest seminal receptacle described for a species of this subgenus. Species of the subgenus *Ascocotyle* usually infect fish-eating birds or mammals in freshwater or brackish habitats. *Ascocotyle* (*A.*) *patagoniensis* n. sp. is the first species of the subgenus described from a marine mammal. However, no metacercariae of *Ascocotyle* spp. were found in 542 marine teleosts from 20 species collected in the same locality. The life cycle of the marine species from the *Ascocotyle*-complex infecting pinnipeds remains elusive.

During a survey of intestinal parasites of the South American sea lion *Otaria flavescens* (Shaw, 1890) (Carnivora: Otariidae) from the Argentine Patagonian coast, a number of heterophyid digenean specimens were collected. The worms appeared to represent a new species of the *Ascocotyle*-complex sensu Sogandares-Bernal and Lumsden (1963) that we describe herein. As it is the first species of *Ascocotyle* (Ascocotyle) Looss, 1899, infecting a marine mammal (see below), a parasitological survey of fish species from the study area was also conducted in an attempt to identify potential intermediate hosts for this species.

MATERIALS AND METHODS

Fifty-six South American sea lions (30 males and 26 females) were collected from 2000 to 2009 in northern Patagonia (40°43′–43°20′S, 63°04′–65°07′W); animals were found as by-catch in fisheries or stranded on the coast. At necropsy, intestines were removed and kept frozen at −20 C. After thawing, intestines were opened and the contents washed with tap water through sieves of either 0.2- or 0.5-mm mesh size. Intestinal contents were later examined using a stereomicroscope; a large number of minute digeneans (ca. 0.6 × 0.2 mm) were found entangled in the mucous contents of 2 host specimens. Given the small size of the parasite, we believe that a significant number of specimens could have been lost during sieving.

The flukes were fixed and preserved in 70% ethanol. A total of 629 specimens was stained with iron acetocarmine (n = 184) or alum carmine (n = 445), dehydrated through an ethanol series, cleared in clove oil, and mounted in Canada balsam. Specimens were examined with a compound microscope using bright field and differential interference contrast optics. Measurements were taken from drawings made with the aid of a drawing tube. Measurements are in micrometers and are shown as the mean followed by standard deviation (SD), with the range in parentheses and the number of measured specimens or structures.

Specimens from the type series are deposited in the Natural History Museum (NHMUK), London, U.K., the United States National Parasite Collection (USNPC), Beltsville, Maryland, and the National Museum of Natural Sciences (MNCN), Madrid, Spain; voucher specimens are deposited in the Collection of the Marine Zoology Unit (MZU), the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

A total of 542 individual fish from 20 species was examined for helminths including metacercariae (Table I). Fishes were collected from 2 zones of the Argentinean shelf: north (42°45′S–42°59′S, 61°09′W–62°58′W) and central Patagonia (47°00′S–47°19′S, 61°59′W–64°25′W). Of these species, 16 fish are common prey of the South American sea lion (Koen-Alonso et al., 2000; N. A. García, pers. comm.). Fish were sampled onboard Argentine hake trawlers during 2006–2007 from the same area where sea lions were collected. Samples of fish were frozen at −20 C for later examination or kept on ice to be examined fresh upon arrival at the laboratory. The skin, epaxial and hypaxial muscles, abdominal cavity, liver, intestine and intestinal ceca, stomach, swimbladder, gonads, heart, head, brain, and gills were examined for helminths by stereomicroscopy. Typical microhabitats for metacercariae in species of the subgenus *Ascocotyle* in fish, i.e., walls of the stomach, intestine, and other visceral organs (Martin and Steele, 1970; Font et al., 1984; Ostrowski de Núñez, 2001), bulbus arteriosus (Schroeder and Leigh, 1965; Ostrowski de Núñez, 2001; Santos et al., 2007), and muscle (Font et al., 1984) were pressed between 2 Petri dishes and subsequently examined using a stereomicroscope.

DESCRIPTION

Ascocotyle (*Ascocotyle*) *patagoniensis* n. sp.
(Figs. 1–8)

Diagnosis (based on 18 specimens for general morphology and 12 additional specimens for morphology of circumoral spines): With characters of *Ascocotyle* Looss, 1899, subgenus *Ascocotyle*, sensu Sogandares-Bernal and Lumsden (1963). Body tear-shaped, 622 ± 112 (489–860, n = 18) long and 211 ± 35 (160–302, n = 18) wide, maximum width at level of ovary (Figs. 1, 7, 8). Tegument spinose. Preoral lobe well-developed, 29 ± 10 (17–43, n = 14) long. Oral sucker subterminal, 45 ± 11 (26–68, n = 16) long and 49 ± 12 (22–68, n = 16) wide. Oral sucker surrounded by 2 rows of circumoral spines (Figs. 2–6). Anterior row with 17–23 spines (n = 17); 10 specimens with complete anterior rows, with a distribution of spines per specimen as follows: 18 (n = 6; Fig. 2); 19 (n = 1; Fig. 3); 20 (n = 2; Fig. 5); and 23 (n = 1; Fig. 6). Posterior row with 17–20 spines (n = 8); 3 specimens with complete posterior row, with a distribution of spines per specimen as follows; 18 (n = 2; Figs. 2, 4) and 19 (n = 1; Fig. 3). Spines in anterior row longer than in posterior row, 15 ± 2 (13–17, n = 28 from 5 specimens) long; spines in posterior row 11 ± 2 (10–13, n = 18 from 3 specimens) long (Figs. 2–6). Oral sucker with conical posterior prolongation, prolongation 88 ± 30 (48–149, n = 14) long, more elongated in long-necked specimens (Figs. 1, 7, 8). Prepharynx 117 ± 43 (56–200, n = 16) long. Pharynx strongly muscular, oval, 57 ± 7 (47–72, n = 20) long and 37 ± 8 (20–49, n = 20) wide. Esophagus not observed. Intestinal ceca short, wide, ending anterior to ventral sucker (Figs. 7, 8). Ventral sucker oval, medial, slightly postequatorial, 57 ± 9 (47–77, n = 9) long and 59 ± 8 (43–68, n = 9) wide; opening of ventral sucker small.

Testes irregularly ellipsoidal, symmetrical, situated near posterior extremity, similar in size, 51 ± 9 (43–72, n = 15) long and 69 ± 15 (39–89, n = 15) wide. Seminal vesicle voluminous, 75 ± 14 (43–96, n = 12) long and 111 ± 28 (79–170, n = 12) wide, sinuous with dextral dorso-ventral loop connecting 2 large sacs, ventral more elongated than dorsal (Figs. 7, 8). Ejaculatory duct dorsal, curved proximally. Ventro-genital sac with slit-like opening; gonotyl simple muscular, situated antero-laterally (dextrally) to ventral sucker, without papillae, 12 ± 1 (11–13, n = 3) long and 34 ± 7 (28–43, n = 3) wide (Fig. 7). Ovary oval, tenuous, situated medially between seminal vesicle and seminal receptacle (Figs. 7, 8), 37 ± 7

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TABLE I. Sampling details of fish species that were examined for the presence of species of *Ascocotyle* in Patagonia, Argentina (measurements in centimeters).

Host family	Scientific name	n	Total length (mean ± SD)
Bramidae	*Brama brama* (Bonnaterre, 1788)	2	60.5 ± 2.1
Bovichtidae	*Cottoperca gobio* (Günther, 1861)	8	30.2 ± 9.3
Centrolophidae	*Seriolella porosa* Guichenot, 1848	34	33.0 ± 5.6
Cheilodactylidae	*Nemadactylus bergi* (Norman, 1937)	32	25.6 ± 5.5
Congiopodidae	*Congiopodus peruvianus* (Cuvier, 1829)	15	23.9 ± 2.0
Merlucciidae	*Macruronus magellanicus* Lönnberg, 1907	3	56.7 ± 23.0
	Merluccius hubbsi Marini, 1933	79	28.1 ± 4.2
Mullidae	*Mullus argentinae* Hubbs and Marini, 1933	2	20.7 ± 0.4
Nototheniidae	*Patagonotothen ramsayi* (Regan, 1913)	84	24.9 ± 3.5
Ophidiidae	*Genypterus blacodes* (Forster, 1801)	44	42.1 ± 9.9
	Raneya brasiliensis (Kaup, 1856)	16	21.2 ± 1.4
Paralichthyidae	*Paralichthys isosceles* Jordan, 1891	15	27.2 ± 5.5
	Xystreurys rasile (Jordan, 1891)	29	32.8 ± 5.9
Percophidae	*Percophis brasiliensis* Quoy and Gaimard, 1825	8	45.3 ± 4.9
Pinguipedidae	*Pseudopercis semifasciata* (Cuvier, 1829)	31	26.5 ± 2.7
Scombridae	*Scomber japonicus* Houttuyn, 1782	13	42.7 ± 5.0
Sebastidae	*Helicolenus lahillei* Norman, 1937	6	28.8 ± 2.6
Serranidae	*Acanthistius brasilianus* (Cuvier, 1828)	16	30.0 ± 2.6
Stromateidae	*Stromateus brasiliensis* Fowler, 1906	73	27.5 ± 3.6
Triglidae	*Prionotus nudigula* Ginsburg, 1950	32	23.1 ± 2.8

(30–43, n = 3) long and 49 ± 5 (43–52, n = 3) wide. Seminal receptacle voluminous, round to ellipsoidal, pretesticular or slightly sub-median (dextral), 77 ± 28 (51–128, n = 6) long and 107 ± 14 (86–123, n = 6) wide (Fig. 7). Vitelline follicles small, scattered, extending into lateral fields from anterior margin of ventral sucker to posterior body end (Figs. 1, 7, 8). Transverse vitelline ducts joining at ovary level. Uterus tubular, forming several loops from region between pharynx and ventral sucker to posterior margin of body (Figs. 1, 7, 8). Eggs ellipsoidal, operculated, 19 ± 1 (17–22, n = 61 from 20 specimens) long and 11 (10–15, n = 61 from 20 specimens) wide. Excretory vesicle Y-shaped with pretesticular lateral branches. Excretory pore sub-terminal, slightly dorsal.

Taxonomic summary

Type host: South American sea lion *Otaria flavescens* (Shaw, 1890) (Carnivora: Otariidae).

Type locality: North Patagonia (40°43′–43°20′S, 63°04′–65°07′W), Chubut, Argentina.

Site in host: Intestine.

Specimens studied: Thirty mounted specimens.

Type specimens: Holotype (NHMUK 2012.2.13.1), 5 paratypes (NHMUK 2012.2.13.2–6), 4 paratypes (USNPC 105290), 6 paratypes (MNCN 4.02/52–4.02/57), 14 vouchers (MZU, EF2 11592–11605).

Infection parameters: Infection parameters could not be reliably determined (see Materials and Methods); about 4,500 specimens were collected from 2 host specimens.

Etymology: The epithet *patagoniensis* indicates the geographical region where the parasite was collected.

Remarks

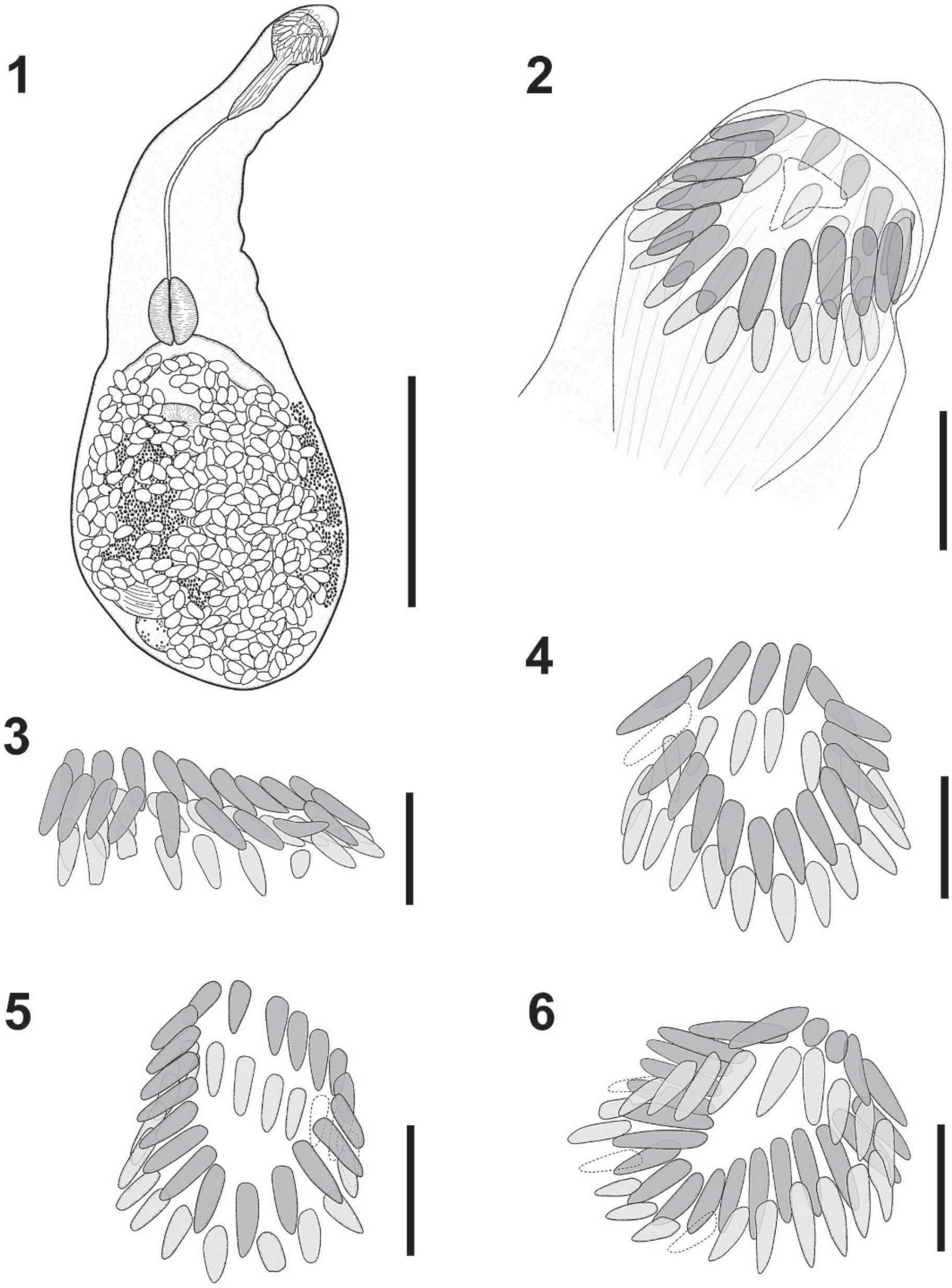
Currently, 10 valid species are recognized in *Ascocotyle* (*Ascocotyle*): *Ascocotyle* (*A.*) *branchialis* Timon-David, 1961; *Ascocotyle* (*A.*) *coleostoma* Looss, 1896; *Ascocotyle* (*A.*) *felippei* Travassos, 1928; *Ascocotyle* (*A.*) *gemina* Font, Heard and Overstreet, 1984; *Ascocotyle* (*A.*) *leighi* Burton, 1956; *Ascocotyle* (*A.*) *pachycystis* Schroeder and Leigh, 1965; *Ascocotyle* (*A.*) *paratenuicollis* Nasir, Lemus de Guevara and Díaz, 1970; *Ascocotyle* (*A.*) *secunda* Ostrowski de Núñez, 2001; *Ascocotyle* (*A.*) *sexidigita* Martin and Steele, 1970; and *Ascocotyle* (*A.*) *tertia* Ostrowski de Núñez, 2001 (see Santos et al., 2007; Table II). *Ascocotyle* (*A.*) *patagoniensis* can be distinguished morphologically from these species by the number of circumoral spines (18 to 23 per row; 17 spines were found only in apparently incomplete rows). *Ascocotyle* (*A.*) *paratenuicollis* has only 11 spines per row, whereas 5 species have 16 spines per row and 4 species ≥22 spines per row (maximum number 32 in *A.* (*A.*) *gemina*) (Table II). The number of spines per row of *A.* (*A.*) *patagoniensis* only overlaps with that of *A.* (*A.*) *pachycystis*, but they differ from one another in the maximum number of spines (23 and 29, respectively) and the seminal receptacle size. *Ascocotyle* (*A.*) *patagoniensis* has the widest seminal receptacle of all described species, both in absolute and relative terms (Table II). The new species also differs from *A.* (*A.*) *gemina*, *A.* (*A.*) *secunda*, *A.* (*A.*) *sexidigita*, and *A.* (*A.*) *tertia* in lacking papillae in the gonotyl (Table II).

No metacercariae of *Ascocotyle* spp. were found in any of the fish examined.

DISCUSSION

Species belonging to the subgenus *Ascocotyle* possess 2 rows of circumoral spines, vitellarium extending to ventral sucker, uterus mainly confined to the area posterior to ventral sucker, and parapleurolophocercous cercariae (Sogandares-Bernal and Lumen, 1963). These diagnostic traits are shared with *A.* (*A.*) *patagoniensis*, although the type of cercaria is unknown for the new species. Additionally, apart from the morphological differences, the new species is the first in this subgenus found from a marine mammal. The other species occur mainly in fish-eating birds in freshwater habitats or salt marshes; 2 species have also been reported in terrestrial carnivores (raccoons) feeding on aquatic prey (Table II).

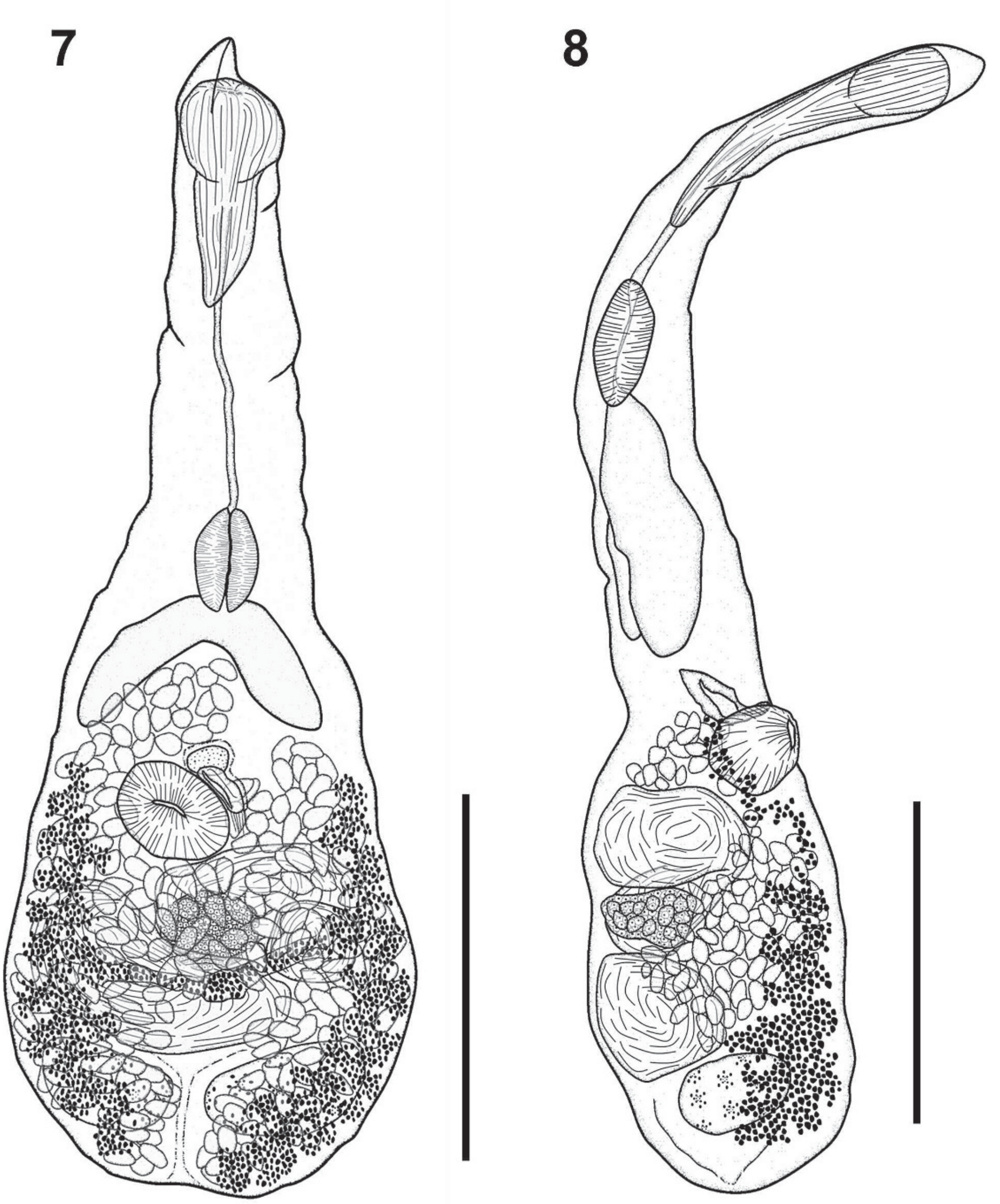
The number of circumoral spines is the most useful morphological character to differentiate species within the subgenus *Ascocotyle* (Sogandares-Bernal and Lumsden, 1963; Santos et al., 2007). *Ascocotyle* (*A.*) *patagoniensis* is unique in having 2 complete circles of 18 to 23 spines each (Figs. 2, 3). Travassos (1928) described *A.* (*A.*) *felippei* as also having 18 spines per row. Santos et al. (2007) re-examined the original specimens described by Travassos (1928) (who did not designate a holotype), synonymized with other species as *A.* (*A.*) *puertoricensis* and *A.* (*A.*) *tenuicollis*, and concluded that no specimen possessed more than 16 spines per row. Circumoral spines are often difficult to



FIGURES 1–6. *Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp. (**1**) Holotype. Whole worm, dorsal view. (**2**) Holotype. Detail of oral sucker showing the double row of 18 spines each. (**3–6**) Paratypes. Details of the circumoral spines with different combinations of numbers per rows (possible missing spines have been reconstructed with dotted lines): 19 spines each (3); 17 spines in anterior row and 18 in posterior row (4); 20 spines in anterior row and 17 in posterior row (5); and 23 spines in anterior row and 20 in posterior row (6). Scale bars: Figure 1 = 200 μm; Figures 2–6 = 50 μm.

count due to their small size and visual overlapping in mounted specimens. Moreover, spines may be missing because of the post-mortem decomposition of the tegument (Santos et al., 2007). Many specimens of *A.* (*A.*) *patagoniensis* were in relatively poor condition, and the upper and lower rows of circumoral spines were complete in just 10 and 3, respectively, of 629 mounted specimens (Figs. 2–6). The problem of post-mortem maceration is widespread in digeneans of marine mammals because the host is usually difficult to obtain and process because, frequently, it may have been dead for a long time, or frozen, before worms can be collected (Gibson, 2005). In fact, spines are often lost in other typical structures of digeneans, e.g., the tegument, the cirrus pouch, or the metraterm (Adams and Rausch, 1989).

Other structural traits, e.g., the morphology of the gonotyl, meristic, the number and morphometric character of gonotyl papillae (or both), the size of the body, relative position of testes,



FIGURES 7–8. *Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp. (**7**) Paratype. Diagrammatic representation of whole worm, ventral view. (**8**) Paratype. Diagrammatic representation of worm, lateral view. Scale bars = 200 μm.

and extension of vitellaria have been used to separate species within the subgenus *Ascocotyle* (Schroeder and Leigh, 1965; Martin and Steele, 1970; Font et al., 1984; Ostrowski de Núñez, 2001, Santos et al., 2007). In addition, we suggest that the voluminous seminal receptacle of *A* (*A.*) *patagoniensis* could be a useful feature that separates it from other species in the subgenus because this structure was markedly consistent in fully developed specimens. Unfortunately, the seminal receptacle has rarely been measured in older descriptions; therefore, we generally obtained its dimensions from original drawings (Table II). However, even a cursory examination of drawings clearly reveals that the seminal receptacle is comparatively much more developed in *A.* (*A.*) *patagoniensis* than in any other species. The seminal vesicle was also voluminous in this species, with a particular looped shape. A folded or looped seminal vesicle has only been described in 3 other species of this subgenus, i.e., *A.* (*A.*) *pachycystis*, *A.* (*A.*) *secunda*, and *A.* (*A.*) *sexidigita* (Schroeder and Leigh, 1965; Martin and Steele, 1970; Ostrowski de Núñez, 2001). Nevertheless, the

TABLE II. Taxonomic data on the species of *Ascocotyle*, subgenus *Ascocotyle* (measurements in micrometers).

Species of *Ascocotyle* and References:	*Ascocotyle* (*Ascocotyle*) *branchialis* Timon-David, 1961; Santos et al., 2007	*Ascocotyle* (*Ascocotyle*) *coleostoma* Travassos, 1930; Santos et al., 2007	*Ascocotyle* (*Ascocotyle*) *gemina* Font et al., 1984; Scholz et al., 2001	*Ascocotyle* (*Ascocotyle*) *felippei* Santos et al., 2007	*Ascocotyle* (*Ascocotyle*) *leighi* Burton, 1956; Kennedy, 1988; Forrester and Spalding, 2003; Kinsella et al., 2004	*Ascocotyle* (*Ascocotyle*) *pachycystis* Schroeder and Leigh, 1965; Kennedy, 1988; Underwood, 1990
Definitive host	Pigeon*	Fish-eating birds	Fish–eating birds	Fish-eating birds, raptors	Fish-eating birds, raccoon	Raccoon, clapper rail
Second intermediate host	Edible frog	Freshwater, brackish fish	Freshwater, brackish fish	Freshwater, brackish fish	Freshwater, brackish fish	Freshwater, brackish fish
Locality	France	Palearctic	North America	America	United States	United States
Body length × width	332–475 × 147–199	700–800 × 250	402–902 × 103–284	361–655 × 123–218	283–402 × 84–122	470–679 × 127–158
No. circumoral spines (no. per row)	32 (16)	32 (16)	55–61 (27–32)	32 (16)	48–52 (24–26)	44–58 (22–29)
Gonotyl	Unknown	Unknown	With 7–10 papillae	Simple	Simple	Simple†
Seminal vesicle (length × width)	Unknown	23 × 55†	49 × 37†	32–39 × 39–55†	19 × 53†	74 × 63† ‡
Seminal receptacle (length × width)	30 × 33†	30 × 40†	69 × 82†	25–48 × 34–60	22 × 22†	37 × 33†

Species of *Ascocotyle* and References:	*Ascocotyle* (*Ascocotyle*) *paratenuicollis* Nasir et al., 1970	*Ascocotyle* (*Ascocotyle*) *secunda* Ostrowski de Núñez, 2001	*Ascocotyle* (*Ascocotyle*) *sexidigita* Martin and Steele, 1970	*Ascocotyle* (*Ascocotyle*) *tertia* Ostrowski de Núñez, 2001	*Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp. Present study
Definitive host	Chicken*	Chicken*	Chicken*	Chicken*	South American sea lion
Second intermediate host	Freshwater, brackish fish	Freshwater, brackish fish	Brackish, marine fish	Freshwater, brackish fish	Unknown
Locality	Venezuela	Argentina	United States	Argentina	Argentina
Body length × width	429 × 143	377–678 × 119–264	350–810 × 140–240	377–590 × 138–220	489–860 × 160–302
No. circumoral spines (no. per row)	22 (11)	32 (16)	? (29–30)	32 (16)	36–46 (18–23)
Gonotyl	Simple	With 9 papillae	With 6 papillae	With 10 papillae	Simple
Seminal vesicle (length × width)	65 × 38†‡	54 × 81†	60 × 138†	60 × 127†	43–96 × 79–170
Seminal receptacle (length × width)	50 × 54†	36 × 36†	62 × 74†	25 × 22†	51–128 × 86–123

* Adult specimens obtained only from experimental infections.
† Obtained from figures in species descriptions.
‡ Seminal vesicle not differentiated from ejaculatory duct.

morphology of these 2 saccular structures must be considered carefully, as they are thin-walled and can change shape depending on the state and position of the worm.

Because the adult stage of as many as 5 species from the subgenus *Ascocotyle* are still known only from experimental infections in chickens and pigeons (Table II), it is relevant to note that the parasites herein described were obtained from wild hosts. Moreover, 2 additional species, i.e., *A.* (*A.*) *gemina* and *A.* (*A.*) *leighi*, have been reported from fish-eating birds or mammals under natural conditions (Table II), but descriptions are available only from experimental hosts, namely chickens and ducks (Burton, 1956; Font et al., 1984). Host species have been shown to induce significant effects on morphometric traits and allometric relationships in digeneans (e.g., Kinsella, 1971; Pérez-Ponce de León, 1995, Mateu et al., 2011), and it would not be surprising that the effect is more pronounced in specimens obtained from unnatural hosts. Therefore, re-descriptions of most species of *Ascocotyle* (*Ascocotyle*) based on specimens collected from natural hosts would be required for a proper comparison of morphometric differences among species. Moreover, a genetic comparison of specimens from different hosts would be necessary to ensure the validity of the species of the subgenus *Ascocotyle* because there are no available sequences to date.

Life cycles of *Ascocotyle* (*Ascocotyle*) species involve 3 hosts, i.e., a hydrobiid snail, a teleost, and a fish-eating bird or mammal in freshwater or brackish habitats (Font et al., 1984; Ostrowski de Núñez, 2001). Thus, the occurrence of *A.* (*A.*) *patagoniensis* in sea lions represents the first record of a species from this subgenus in a marine definitive host. The low prevalence in sea lions of the present study could indicate that *O. flavescens* is not the principal definitive host of this species; however, we cannot argue this conclusively because the intensities of gravid parasites were high and, as previously mentioned, many worms could have been lost due to their small size.

Unfortunately, the detection of other stages of the life cycle of this species has hitherto been elusive. South American sea lions feed principally on marine teleosts and cephalopods (Koen-Alonso et al., 1999; N. A. García, pers. comm.). However, in the study area, we failed to find metacercariae of *Ascocotyle* spp. in 542 fish specimens from 20 species, including common fish prey for South American sea lions along the Argentine coast. Moreover, there are no records in other parasitological surveys (Timi and Poulin, 2003; Sardella and Timi, 2004; Vales et al., 2011, and references therein). Given the small size of adult worms, we cannot rule out that, in our fish sample, some metacercariae have been overlooked, particularly in microhabitats that are difficult to examine, e.g., in muscles. However, we thoroughly surveyed, by transparency, the typical microhabitats that have been reported for other species of *Ascocotyle* (*Ascocotyle*), so it is hard for us to believe that metacercariae were systematically missed. For some species, few specimens could be collected (see Table I) and, therefore, metacercariae might not have been recorded due to low sample size. Because our samples were obtained 80–330 km from the coastline, one possibility is that the life cycle is restricted to more coastal waters. Alternatively, the fish intermediate host of *A.* (*A.*) *patagoniensis* could be a euryhaline species that serves as prey for other potential definitive hosts, such as salt marsh or marine birds, and that it is seldom consumed by sea lions. In fact, Morgades et al. (2002) reported thousands of adult *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920, in South American sea lions from Uruguay, and *A.* (*P.*) *longa* is known to be a typical parasite from freshwater and marine birds (Scholz, 1999).

Ascocotyle (*A.*) *patagoniensis* is the fourth species of the *Ascocotyle*-complex that has been reported in pinnipeds, with the other 3 belonging to the subgenus *Phagicola* Faust, 1920. Apart from the record of *A.* (*P.*) *longa* Ransom, 1920, in the South American sea lions noted above, *Ascocotyle* (*Phagicola*) *sinoecum* Ciurea, 1933 has been reported in the Caspian seal *Phoca caspica* (Gmelin, 1788) (Raga, 1992; Demidenko and Korolev, 2004) and *Ascocotyle* (*Phagicola*) *septentrionalis* Van Den Broek, 1967, in the harbor seal *Phoca vitulina* (L.) from the North Sea (Van Den Broek, 1967; Borgsteede et al., 1991). The 2 latter species are apparently specific to their respective hosts, with few records in natural conditions, other than seals in the case of *A.* (*P.*) *sinoecum*. This evidence suggests the potential for host switching between birds and mammals in freshwater and marine environments, with or without subsequent speciation of the parasite in the new hosts (see Hoberg and Brooks, 2008). The same phenomenon seems to occur in other heterophyids as well, e.g., *Cryptocotyle lingua* (Creplin, 1825) in harbor seals (see Borgsteede et al., 1991) and species of *Galactosomum* Looss, 1899 in sea lions (see Dailey, 1969; Dubois and Angel, 1976; Dailey et al., 2002). In conclusion, although *A.* (*A.*) *patagoniensis* could be harbored by definitive hosts other than South American sea lions, its unique morphological traits clearly indicate that this is a new species within *Ascocotyle*.

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6. TRANSMISSION PATTERNS OF *CORYNOSOMA AUSTRALE* IN FISH PARATENIC HOSTS

6.1. INTRODUCTION

6.1.1. Paratenic hosts, a trophic bridge to the definitive hosts

Trophically-transmitted helminths show a remarkable diversity in modes of life, types of hosts and life history stages. Most of these helminths develop complex life cycles, involving one or several intermediate hosts to accomplish their cycle after the ingestion by the definitive hosts. In most of these cases, high predation rates on previous hosts are necessary for the transmission of the parasite between obligatory hosts (namely intermediate and definitive ones) (Parker *et al.*, 2009). However, for some helminth taxa, trophic links between intermediate and definitive hosts can be unlikely, making parasite transmission extremely difficult, or in some cases at relatively low rates. In these scenarios, paratenic hosts are used as alternative pathways to bridge the trophic gap and ease transmission between obligatory hosts (Marcogliese, 2002). Both intermediate and paratenic hosts take part in parasite transmission (Parker *et al.*, 2009), but the fundamental distinction between both could be that in paratenic hosts essential growth or development of the parasite is absent (Anderson, 2000; Beaver, 1969; Bush *et al.*, 2001; Nicholas, 1967; Roberts & Janovy, 2009). Paratenic hosts can also be distinguished from accidental non-suitable hosts, in that only in the former hosts the larval parasitic stage will resume its development when transferred to suitable hosts (Kennedy, 2006).

In helminths with complex life cycles, paratenic hosts have usually received less attention than obligatory hosts since they are usually considered as facultative (Schmidt, 1985). However, from an ecological perspective, paratenic hosts play an important role in the helminth population structure and dynamics, since they accumulate high numbers of larvae (*e.g.* Laskowski *et al.*, 2012; Morand *et al.*, 1995; Zdzitowiecki, 1991) and contribute to their transmission and dispersion (Bush *et al.*, 2001; Marcogliese, 2007). Moreover, paratenic hosts seem to maintain parasites in the environment (Marcogliese, 2002) and protect them from external threats (Poulin, 1998). Parasites are usually latent, encapsulated in these hosts, which also facilitate survival of the parasites for very long periods, allowing movements into new habitats and exposure to new potential hosts, increasing the probability of new host-parasite interactions (Marcogliese, 2007; Médoc *et al.*, 2011).

However, the use of paratenic hosts may also have a negative effect on the biology and population structure of the parasites. Transmission through several paratenic hosts exposes the parasite to different hosts' immune systems (*e.g.* Nikishin & Skorobrechova, 2007; Skorobrechova & Nikishin, 2011), which imply both multiple adaptations against different types of host immune responses and the accumulation of damages provoked by the immune defences of each successive parasitized host. In other cases, the transmission from some paratenic hosts is unlikely and, therefore, they may act as a sink in the life cycle of parasites, thus affecting the population dynamics of trophically-transmitted helminths (*e.g.* Anderson, 2000; Holmes, 1979; Rohde, 2005; Taraschewski, 2000).

Paratenicity occurs in most of the parasitic groups (Médoc *et al.*, 2011; Parker *et al.*, 2009); however, it is rare in trematodes (*e.g.* Latham *et al.*, 2003; Madhavi, 1978) and cestodes (Morand *et al.*, 1995), and more frequent in nematodes (Anderson, 2000) and acanthocephalans (Schmidt, 1985). However, despite the biological and ecological importance of paratenic hosts in these two groups of parasites, paratenicity has been poorly studied and any further discussion on this topic is required in order to accurately assess the role of these hosts in the life cycles of trophically-transmitted helminths.

6.1.2. Paratenicity in acanthocephalans

Acanthocephalans display a complex two-host life cycle requiring arthropods as intermediate hosts for the larval stages (acanthella and cystacanth) and vertebrates as definitive hosts for adults (Bush *et al.*, 2001; Kennedy, 2006; Petrochenko, 1956; see **Chapter 1**, **Fig. 8**). In acanthocephalans, paratenicity is not common, since from the *ca.* 1,100 species described, paratenicity seems to occur in almost 10–20% of species (Parker *et al.*, 2009). In these parasites, paratenic hosts are mainly vertebrates, usually poikilotherms (*e.g.* teleosts, amphibians or reptiles) (Nickol, 1985; Schmidt, 1985). After being recruited by the paratenic host, cystacanths (infective stages) are activated and pass through the intestinal wall to locate in extra-intestinal sites, e.g. mesenteries and muscles, where they are encapsulated (Kennedy, 2006; Nicholas, 1967; Petrochenko, 1956; Taraschewski, 2000).

Little is known about the specific identity of paratenic hosts for many species of acanthocephalans (Kennedy, 2006). Additionally, other epizootiological aspects related to paratenic hosts are even less clear: host-parasite interactions, transmission strategies, and/or relative importance of paratenic hosts in life cycles (Nickol, 1985; Taraschewski, 2000). Available evidence suggests that paratenicity can be facultative for many species and their life cycles do not strictly depend on these hosts (Nickol, 1985; Petrochenko, 1956; Schmidt, 1985; Taraschewski, 2000). However, for a few species that reproduce in carnivores, cetaceans or birds (*e.g.* those from *Andracantha*, *Corynosoma*, *Centrorhynchus* or *Oncicola*) their transmission strongly depends on paratenic hosts to reach their definitive hosts (Nickol, 1985; Petrochenko, 1956; Taraschewski, 2000).

At present, most of the research related to marine acanthocephalans infecting paratenic hosts has been performed with species belonging to the polymorphid genus *Corynosoma*. These acanthocephalans are cosmopolitan parasites, mainly found within the intestine of pinnipeds (Aznar *et al.*, 2006, 2012). Although the life cycles of most species are currently unknown, it seems that most of them likely use benthic amphipods as intermediate hosts (Hoberg, 1986; Laskowski *et al.*, 2010; Sinisalo & Valtonen, 2003; Valtonen & Crompton, 1990; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986, 2001; Zdzitowiecki & Presler, 2001). In this case, the trophic levels of intermediate and definitive hosts are very distant, and these acanthocephalans require several paratenic hosts to bridge this trophic gap, thus a wide array of fish have been reported worldwide as paratenic hosts (*e.g.* Laskowski & Zdzitowiecki, 2005; Schmidt, 1985; Sinisalo & Valtonen, 2003; Timi *et al.*, 2011b; Valtonen, 1983; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986).

Available studies on *Corynosoma* spp. in fish paratenic hosts have provided information including species records (Carballo *et al*., 2011, Laskowski & Zdzitowiecki, 2005; Moles & Heintz, 2007; Zdzitowiecki *et al.*, 1999), taxonomic issues (Braicovich *et al*., 2005; Mašová & Baruš, 2013; Sardella *et al*., 2005), host–parasite histopathological interactions (Nikishin & Skorobrekhova, 2007; Skorobrechova & Nikishin, 2011), developmental (Hernández-Orts *et al.*, 2012), or ecological studies (Sinisalo & Valtonen, 2003; Valtonen, 1983; Valtonen & Niinimaa, 1983). However, the negative consequences of paratenic transmission on the population dynamics of *Corynosoma* species are the missing elements of all these studies. This information is fundamental to explain key questions about the types of costs that paratenicity can entail for the parasite, affecting the spectrum of suitable hosts, population structure, or even the life-span of the parasites (see below).

6.1.3. **Corynosoma australe** ***Johnston, 1937***

The present study attempts to provide novel data on the host-parasite interactions, population structure and costs/effects of infecting fish paratenic hosts for acanthocephalans. In particular, we explore biological, morphological and ecological issues, with special attention on the negative consequences of paratenicity in cystacanths of *Corynosoma australe* infecting several species of fish paratenic hosts from Patagonia, Argentina. *C. australe* is an eurytopic acanthocephalan, maturing and reproducing in the intestine of pinnipeds from the South American coasts, *i.e.* South American sea lion *Otaria flavescens* and South American fur seal *Arctocephalus australis* (Aznar *et al.*, 2004, 2012; Hernández-Orts *et al.*, 2013; Sardella *et al.*, 2005; Silva *et al.*, 2013). In this area, juvenile *C. australe* have been also reported infecting other hosts, including elasmobranchs (Knoff *et al.*, 2001), fish eating birds (Brandão, 2013) and cetaceans (Aznar *et al.*, 2012; Berón-Vera *et al.*, 2008).

The specific identity of the invertebrate intermediate hosts of *C. australe* is currently unknown; however, as in other species of the genus, it seems likely that the intermediate host could be a benthic invertebrate (Hoberg, 1986; Laskowski *et al.*, 2010; Sinisalo & Valtonen, 2003; Valtonen & Crompton, 1990; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986, 2001; Zdzitowiecki & Presler, 2001). On the other hand, cystacanths of *C. australe* have been commonly recorded as integral components of the helminth communities of fish paratenic hosts (*e.g.* Alves *et al*., 2003; Alves & Luque, 2001; Braicovich *et al*., 2012; Iannacone *et al.,* 2011; Luque *et al*., 2002; Oliva & Luque, 2002; Salinas *et al*., 2008; Santos *et al*., 2008; Timi, 2007; Timi *et al*., 2011b). Only taking into account surveys along the coast of Argentina, *C. australe* have been reported in at least 24 species of marine fish belonging to 19 families (**Table 5**). Therefore, in this area, *C. australe* seems to be an ideal model for studying host-parasite interactions and biological/ecological issues between trophically-transmitted helminths and their paratenic hosts.

Table 5. Records of cystacanths of *Corynosoma australe* in species of marine fishes in the Argentine Sea. Abbreviations: n, the number of host analysed; MI, number of worms per infected host; P, Prevalence; TL, fish total length ± standard deviation (or range).

Order / Family	Host	Latitude	Longitude	TL (cm)	n	P	MI	Source*
Anguilliformes								
Congridae	*Conger orbignianus*	37º30'S	56º39'W	–	50	95.4	18297	1
		38º45'S	62º15'W	(48.8–80.5)	81	37.03	61	2
Atheriniformes								
Atherinopsidae	*Odontesthes argentinensis*	37º32'S	57º19'W	33.9 ± 6.1	42	14.2	75	3
	Odontesthes smitti	38º03'S	57º32'W	25.7 ± 1.6	59	54	65	4
		42º25'S	64º07'W	22.6 ± 1.2	32	31	22	4
		42º25'S	64º07'W	28.3 ± 2.3	31	48	25	4
		42º47'S	65º02'W	26.0 ± 2.1	23	17	5	4
		42º47'S	65º02'W	21.7 ± 1.6	32	3	1	4
Batrachoidiformes								
Batrachoididae	*Porichthys porosissimus*	38º45'S	62º15'W	(11.5–29.7)	166	3.01	5	5
Clupeiformes								
Clupeidae	*Brevoortia aurea*	37º32'S	57º19'W	36.3 ± 4.3	19	20.0	21	3
Engraulidae	*Engraulis anchoita*	35–37ºS	–	> 14.0	585	0.51	4	6
		34–40ºS	–	> 14.0	715	3.78	35	6
		40–43ºS	–	> 14.0	504	0.59	4	6
		43–46ºS	–	> 14.0	241	0.41	1	6
Gadiformes								
Merlucciidae	*Merluccius hubbsi*	35º30'–38º00'Sº	53º30'–56º00'W	40.5 ± 5.5	66	27.3	92	7
		41º40'–42º10'S	63º50'–65º00'W	38.7 ± 4.5	83	1.2	1	7
		46º30'S	66º30'W	40.4 ± 4.8	80	0.0	0	7
		45º50'–47º10'S	60º20'–63º10'W	39.9 ± 3.6	115	5.2	23	7
		45º00'–47º00'S	61º00'–62º00'W	(36.7–74.9)	19	0	0	8
		45º00'–47º00'S	61º00'–62º00'W	(32.0–63.2)	14	0	0	8
		45º00'–47º00'S	61º00'–62º00'W	(34.0–59.7)	22	0	0	8
		45º00'–47º00'S	61º00'–62º00'W	(30.9–62.9)	32	0	0	8
Ophidiiformes								
Ophidiidae	*Genypterus blacodes***	36º–56ºS	–	(20.0–>100)	101	–	–	9
	*Genypterus brasiliensis***	45–47ºS	–	(40.0–79.0)	31	–	–	9

Table 5. continued

Order / Family	Host	Latitude	Longitude	TL (cm)	n	P	MI	Source*
	Raneya brasiliensis	38º00'S	56º00'W	23.0 ± 2.7	26	100	191	10
		37º50'S	56º54'W	24.7 ± 2.1	30	86.7	174	10
		38º04'S	56º40'W	23.2 ± 1.3	20	95.0	101	10
		46º00'S	65º00'W	24.5 ± 1.7	107	95.3	686	10
Perciformes								
Carangidae	*Trachurus lathami*	36º44'S	55º44'W	19.4 ± 0.8	42	88.1	349	11
		36º44'S	55º44'W	19.6 ± 0.9	36	88.9	335	11
		38º34'S	58º03'W	19.8 ± 0.7	49	87.8	265	11
		38º34'S	58º03'W	20.1 ± 0.7	46	89.1	547	11
		38º34'S	58º03'W	20.1 ± 0.8	50	96	470	11
Cheilodactylidae	*Nemadactylus bergi*	38º27'S	57º90'W	32.4 ± 1.8	35	97.1	1257	12
		38º27'S	57º90'W	34.7 ± 4.2	15	100	333	12
		38º27'S	57º90'W	35.1 ± 2.1	19	94.7	228	12
		38º27'S	57º90'W	36.2 ± 2.8	15	100	383	12
		38º27'S	57º90'W	35.2 ± 4.4	16	93.8	320	12
Percophidae	*Percophis brasiliensis*	34º30'–36º30'S	53º30'–56º00'W	49.4 ± 6.7	35	100	3353	13
		38º08'S	57º32'W	52.2 ± 3.7	59	100	5452	13
		39.00'–41º00'S	60º00'–62º00'W	50.0 ± 3.7	51	96.1	1250	13
		41º40'–42º10'S	63º50'–65º00'W	52.9 ± 6.7	32	75.0	176	13
Pinguipedidae	*Pinguipes brasilianus*	38º27'S	57º90'W	33.3 ± 3.1	54	92.6	1335	14
		42º00'S	65º10'W	32.7 ± 3.4	52	61.5	102	14
		42º09'S	64º05'W	37.1 ± 3.1	50	50.0	40	14
	Pseudopercis semifasciata	37º15'Sº	57º23'W	67.5 ± 6.3	20	100	1598	15
		38º03'S	57º30'W	71.2 ± 3.5	30	96.7	2019	15
		42º00'–42º45'S	–	67.2 ± 7.2	50	90	225	15
Mullidae	*Mullus argentinae*	38º27'S	57º90'W	19.5 ± 1.9	75	38.67	50	16
Sciaenidae	*Cynoscion guatucupa*	38º08'S	57º32'W	27.0 ± 2.9	24	41.7	26	17
		38º08'S	57º32'W	40.7 ± 5.3	136	86.0	1427	17
Scombridae	*Scomber japonicus*	37º00'–38º00'S	56º00'–57º00'W	(30.9–53.4)	90	64.4	956	18
		39º00'–41º00'S	60º00'–61º00'W	(29.7–44.0)	83	49.4	518	18

Table 5. continued

Order / Family	Host	Latitude	Longitude	TL (cm)	n	P	MI	Source*
Serranidae	*Serranus auriga*	–	–	–	61	–	–	19
Sparidae	*Pagrus pagrus*	–	–	–	123	–	–	19
Pleuronectiformes								
Paralichthyidae	*Paralichthys isosceles*	38º52'S	58º10'W	28.0 ± 2.1	51	92.1	749	20
	Paralichthys orbignyanus	37º32'S	57º19'W	39.8 ± 11.7	26	26.9	42	3
	Paralichthys patagonicus	38º52'S	58º10'W	35.2 ± 2.6	51	94.1	324	20
	Xystreurys rasile	38º52'S	58º10'W	29.3 ± 2.6	48	89.6	443	20
Sebastidae	*Helicolenus lahillei*	–	–	17.4 ± 2.5	120	–	–	19
Scorpaeniformes	*Sebastes capensis*	43º45'S	–	26.0	60	16.7	16	21
Triglidae	*Prionotus nudigula*	38º27'S	57º90'W	19.7 ± 3.0	101	85.1	394	22
Syngnathiformes								
Syngnathidae	*Hippocampus* sp.	40º47'S	64º54'W	(2.1–15.1)	88	6.8	12	23

*1, Timi & Lanfranchi (2013); 2, Tanzola & Guagliardo (2000); 3, Alarcos & Etchegoin (2010); 4, Carballo *et al.* (2012); 5, Tanzola *et al.* (1997); 6, Timi & Poulin (2003); 7, Sardella & Timi (2004); 8, Mackenzie & Longshaw (1995); 9, Sardella *et al.* (1998); 10, Vales *et al.* (2011); 11, Braicovich *et al.* (2012); 12, Rossin & Timi (2010); 13, Braicovich & Timi (2008); 14, Timi *et al.* (2008); 15, Timi & Lanfranchi (2009b); 16, Lanfranchi *et al.* (2009); 17, Timi *et al.* (2005); 18, Cremonte & Sardella (1997); 19, Timi *et al.* (2011b); 20, Alarcos & Timi (2012); 21, González *et al.* (2006); 22, Timi & Lanfranchi (2009a); 23, Braicovich *et al.* (2005).

**The presence of *C. australe* was confirmed by the authors.

The aim of this study is three-fold:

- To provide infection parameters of *C. australe* in 20 fish hosts and to describe its population structure in the neritic zone of the Patagonian coast of Argentina. We explore the distribution of the hosts of *C. australe* in the water column in order to elucidate circulation, transmission patterns, and factors leading the spatial distribution of this parasite. We also investigate the relative importance of each fish species for the transmission of *C. australe* to the South American sea lion, *O. flavescens*, identifying key species in the study area.

- To examine the sex ratio of cystacanths of *C. australe* in fish. We explore whether the sex ratio departs from the expected value 1:1. If so, we investigate whether sex ratio is biased towards females, which could mean that this property is transferred to the adult stage infecting pinnipeds, where sex ratio has been observed to be strongly female-biased (Aznar *et al*., 2004; J. S. Hernández-Orts *et al.*, unpublished data). The possible factors involved in generating sex ratio biases are also discussed.

- To analyse, for the first time, potential costs that paratenicity may entail for *C. australe*. We pay special attention to the energetic costs that the cystacanths of *C. australe* may face in paratenic-to-paratenic transmission. If no further growth and development occurs in acanthocephalans infecting paratenic hosts, energy reserves and, therefore, body size, of *C. australe* should decrease when several switches between paratenic hosts occur. This hypothesis is evaluated by comparing, through inferential statistics, the body volume of *C. australe* and the trophic level of different fish species where the cystacanths were collected. We also explore another factor, *i.e.* intensity, which can also influence the body size of the parasite through crowding effects (see Lotz *et al*., 1995). These results are discussed in relation to the negative consequences of paratenic-to-paratenic transmission for trophically-transmitted helminths.

6.2. MATERIALS AND METHODS

6.2.1. Collection and examination of cystacanths

Cystacanths of *Corynosoma australe* were obtained from the mesentery of 542 fish from 20 species (see **Chapter 3** for details). Cystacanths were placed in physiological saline, removed from their capsule and fixed in 70% ethanol. All the cystacanths were examined using a stereo microscope (up to 80×), and identified following the taxonomic criteria of Zdzitowiecki (1984), Zdzitowiecki (1991) and Sardella *et al.* (2005).

6.2.2. Infection patterns

Ecological terms follow Bush *et al.* (1997) and Rózsa *et al.* (2000). The prevalence, mean abundance and mean intensity are followed by the 95 % confidence intervals (C.I.) in parentheses. The 95 % C.I. for prevalence was set with Sterne's exact method (Reiczigel, 2003), whereas the 95 % C.I.s for mean abundance and mean intensity were estimated with 20,000 bootstrap replications. A preliminary analysis indicated that there were no significant differences on the abundance of cystacanths of *C. australe* between species of fish collected in different sampling sites (Mann–Whitney tests, $P > 0.05$). Therefore, infection parameters and statistical analyses were calculated for pooled data. Differences in the intensity of *C. australe* among fish species were investigated with a Kruskal-Wallis test followed by post hoc comparisons between fish species (Conover, 1999). For this analysis, only species of fish with a sample size ≥ 5 individuals were considered.

As noted at the Introduction, the intermediate host for *C. australe* usually is a benthic amphipod. Therefore, we hypothesized that infection levels should decrease according to the way fish species exploit resources in the water column, *i.e.* from benthic to pelagic species. To test the hypothesis, we used prevalence data of *C. australe* from both the sample of fish species analysed in this study and other published surveys in Patagonia. When more than one survey per species was available, a weighted average prevalence was obtained based on sample size. Fish species was assigned to four categories according its position in the water column, *i.e.* 'benthic', 'demersal-benthic', 'demersal-pelagic' and 'pelagic' (see **Chapter 3**) a. A non-parametric

Jonckheere-Terpstra test was used to explore whether there was a significant monotonotic decrease of prevalence among the 4 categories.

6.2.3. Relationship between infection patterns and sea lion diet

To explore the relative importance of different fish species in the transmission of *C. australe* to South American sea lions we used dietary data from sea lions obtained in the study area. Two independent samples were dealt with, namely, that from Koen-Alonso *et al.* (2000) (including additional data from Koen-Alonso, 1999), which was based on stomach contents obtained from 59 sea lions (28 males, 31 females) in northern and central Patagonia between 1982–1998, and that from Romero *et al*. (2011), which was based on 33 sea lions (17 males, 16 females) collected in northern Patagonia (San Matías Gulf) between 2006–2009. Our hypothesis was that infection levels of *C. australe* should be higher in those fish species that represent the bulk of sea lion's diet.

We used two parameters to measure the relative importance of each fish species in the diet, which could be gathered from the dietary surveys described above:

- Percentage by number (*%N*), which is calculated as percent of prey individuals of fish species *i* in the overall sample of individual prey regardless of prey species. This parameter is somehow analogous to a "mean abundance" of fish species per sea lion and is relevant from a parasitological point of view because each individual fish represent a potential 'packet' of *C. australe* recruits.

- Index of Relative Importance (*IRI*), which is calculated as $(\%N_{\mathrm{i}}+\%W_{\mathrm{i}})*\%FO_i$, where *%N* is as defined above, *%W* is the percentage wet weight of fish species *i* in the overall sample (*i.e.* it is a measure of biomass), and *FO* is the frequency of occurrence of fish species *i* in the sample of sea lions (*i.e.* it is analogous to a 'prevalence'). The *IRI* is important as it combines 3 parameters that potentially have a direct relationship with the likelihood of infection.

Prevalence and mean abundance were used as alternative infection parameters; note that precision of the latter is more dependent on sample size because of the aggregated nature of parasite populations (Rózsa *et al.*, 2000). We attempted to gather the most accurate estimation on infection levels and, therefore, parasitological data obtained in this study was completed with published information from fish species in the same area (**Table 5**). For some species, there were several parasitological surveys and, therefore, weighed averages of mean or median abundance per survey were used.

The association between "*%N*" or "*IRI*", and prevalence or mean abundance were investigated with one-tailed Spearman's correlation tests. We are aware that data from each fish species are not truly independent since fish species are related through phylogenetic relationships (Poulin, 1995). For this reason, our correlational analysis must be considered as a preliminary step in the search for potential trends.

6.2.4. Sex ratios

Sex ratio was calculated as the percentage of males [*i.e.* no. of males/(no. of males + no. of females)] of *C. australe* in each fish species with n ≥ 5 infected individuals, and for the overall sample of fish. The 95% C.I. was set based on 20,000 bootstrap replicates using the bias-corrected percentile method (Efron & Tibshirani, 1993). Data on sex ratio of *C. australe* from individual fish were used to investigate differences among fish species based on the Kruskal-Wallis test followed by post hoc comparisons. Again, only species of fish with a sample size ≥ 5 infected individuals were considered for this analysis.

To investigate whether there are significant departures for the theoretical sex ratio of 1:1 a Wilcoxon signed rank test for paired samples was employed (Conover, 1999; Neuhäuser, 2004). Note that the chi-square test might be not appropriate because individual parasites likely are not recruited independently. In other words, recruitment in clusters violates the assumption of independence among observations (Garson & Moser, 1995; Kramer & Schmidhammer, 1992), and sampling from aggregated distributions usually inflate the type I error when the chi-square test is used (Garson & Moser, 1995; Rao & Scott, 1992).

6.2.5. Influence of fish trophic level on parasite size

The trophic level (TL) of most fish species, *i.e.* its position in the food web, determined by the number of energy-transfer steps to that level, was obtained from Barraza Bernardas (2009), Timi *et al.* (2011b) and Froese & Pauly (2013). In these species, TL had been estimated based on trophic ecology studies that collate data from several samples. There were 2 species, *Acanthistius patachonicus* and *Seriolella porosa*, for which TL was not available and, therefore, it was obtained from its closest sympatric relative (*Acanthistius brasilianus* (Cuvier, 1828) and *Seriolella brama* (Günther, 1860), respectively) (Froese & Pauly, 2013). Also, for *Cottoperca gobio* and *Patagonotothen ramsayi*, TL could only be obtained from fragmentary data about single food items (not entire diets) and, therefore, TL position was considered to be tentative (see Froese & Pauly, 2013 for details).

Body volume was considered as an appropriate surrogate of body size for *C. australe*. Cystacanths of *C. australe* were drawn in profile with the aid of a drawing tube. Trunk length and disk diameter were measured using homologous landmarks (**Fig. 13**). Then, body volume was calculated assuming a conical body shape (Hernández-Orts *et al.*, 2012, see **Chapter 7**).

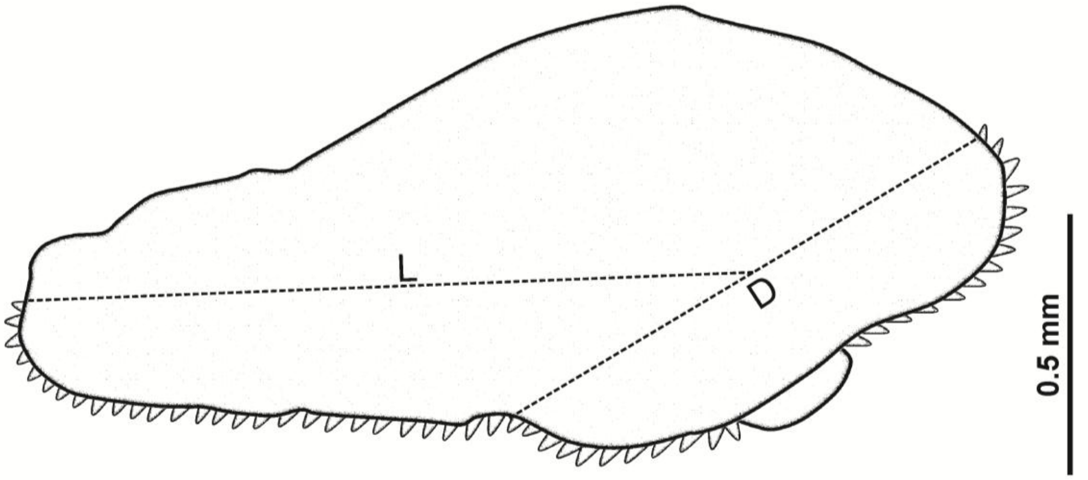


Figure 13. Morphometric measurements taken from the profile of cystacanths of *Corynosoma australe*. L, trunk length; D, disk diameter.

A preliminary analysis indicated that there were no significant differences for body volumes between males and females of *C. australe* and, therefore, data from both sexes were pooled in subsequent analyses.

According to the arguments given in the Introduction, our main hypothesis was that the body volume of *C. australe* should decrease in fish species with higher trophic levels. Note, however, that intensity can also influence body volume through potential crowding effects (Lotz *et al*., 1995) and, therefore, intensity was included as a second covariate. The effects of both TL and intensity were investigated using mixed models (Paterson & Lello, 2003). Mean volume of individuals of *C. australe* per individual fish was used as dependent variable. The fixed part of the most complex model included 'intensity' and 'TL' as covariates, and the random part of the model, 'fish species' and 'intensity', assuming an unstructured covariance. In so doing, we allowed that intercepts and slopes of intensity effects may vary depending on the fish species, and that intercepts and slopes are correlated (Singer, 1998). Suitability of alternative (*i.e.* simpler) models was compared according to the AIC criterion (Burnham & Anderson, 2002). Differences of AIC with respect to the best model (least AIC) were evaluated according to the following criteria: 0-2, substantial empirical support; 4-7, considerably less support; >10, essentially no support (Burham & Anderson, 2002). Importance of individual fixed predictors was also evaluated according to *P*-values (Paterson & Lello, 2003). Note that all test involving fixed factors were one-tailed because we established the predicted effects beforehand.

6.2.6. Statistical software

Confidence intervals for infection parameters were calculated with the free software Quantative Parasitology 3.0 (Reiczigel & Rózsa, 2005). Bootstrap replicates for 95% I.C.s, corrected by the bias-corrected percentile method, in sex ratio analyses were calculated with SPSS v20. Other statistical analyses were carried out with SPSS v19. Statistical significance was set at $P < 0.05$.

6.3. RESULTS

6.3.1. Infection patterns

A total of 1,367 cystacanths of *C. australe* were collected from 18 marine fish species from the Patagonian coast in Argentina (**Table 6**). Eight species, *i.e. A. patachonicus*, *Brama brama*, *Congiopodus peruvianus*, *C. gobio*, *Genypterus blacodes*, *P. ramsayi*, *S. porosa* and *Stromateus brasiliensis* represent new host records for *C. australe*. The smallest fish infected was an individual of *P. ramsayi* 14.7 cm long (intensity, 1), whereas the largest was an individual of *Macruronus magellanicus* 83.0 cm long (intensity, 1). A specimen of *Paralichthys isosceles* 33.2 cm long presented the highest infection level (intensity, 138).

The abundance of cystacanths of *C. australe* was significantly correlated with body length in 6 marine fish species: *A. patachonicus* (Spearman rank correlation: r_s = 0.668, n = 16, one-tailed P = 0.005), *G. blacodes* (r_s = 0.558, n = 44, one-tailed P < 0.001), *Nemadactylus bergi* (r_s = 0.609, n = 32, one-tailed P < 0.001), *P. isosceles* (r_s = 0.817, n = 15, one-tailed P < 0.001), *P. ramsayi* (r_s = 0.259, n = 84, one-tailed P = 0.018) and *Pseudopercis semifasciata* (r_s = 0.381, n = 31, one-tailed P = 0.034).

There were highly significant differences in the abundance of *C. australe* among fish species (Kruskal-Wallis test, X^2 = 262.16, 16 d.f., P < 0.001). The post hoc comparison (**Table 7**) indicated that *Raneya brasiliensis* harboured significantly more cystacanths of *C. australe* than any other fish species, followed by *P. isosceles*; a third group with lower intensities included *A. patachonicus*, *N. bergi*, *Percophis brasiliensis*, *Prionotus nudigula*, *Scomber japonicus*, and *Xystreurys rasile*. Finally, the group formed by *C. peruvianus*, *C. gobio*, *Helicolenus lahillei*, *Merluccius hubbsi*, and *S. brasiliensis* harboured significantly less cystacanths than any other fish species above. It is remarkable that the 2 fish species with the highest abundance of *C. australe* were demersal-benthic (*R. brasiliensis*) and benthic (*P. isosceles*) (**Table 6 & 7**). Except for a single species (*S. japonicus*), demersal-pelagic species (*e.g. M. hubbsi*, *S. porosa* and *S. brasiliensis*) were characterized by low abundances of *C. australe* (**Table 6 & 7**).

Table 6. Infection parameters of cystacanths of *Corynosoma australe* in 20 fish species from the Patagonian coast of Argentina. The ecological group according to water column position for each species is shown in parentheses after the host name. The 95 % CI was estimated only for fish species with n ≥ 15. Abbreviations: B, benthic; CI, confidence intervals; DB, demersal–benthic; DP, demersal–pelagic; n, number of specimens analysed; P, pelagic.

Host	n	Prevalence (%) (95% CI)	Mean abundance (95% CI)	Mean intensity (95% CI)	Range
A. patachonicus (DB)	16	75.0 (50.0–90.1)	13.9 (4.8–44.8)	18.6 (6.8–57.2)	1–131
B. brama (DP)	2	50.0	0.5	1	1
C. peruvianus (DB)	15	6.7 (0.4–30.2)	0.1 (0.0–0.2)	1	1
C. gobio (DB)	8	12.5	0.9	7.0	7
G. blacodes (DB)	44	45.5 (31.2–60.3)	1.1 (0.7–1.9)	2.4 (1.7–4.0)	1–11
H. lahillei (DB)	6	–	–	–	–
M. magellanicus (DP)	3	33.3	0.3	1	1
M. hubbsi (DP)	79	–	–	–	–
M. argentinae (DB)	2	50.0	1.5	3	3
N. bergi (DB)	32	65.6 (47.3–80.0)	5.0 (3.2–7.7)	7.6 (5.5–11.3)	1–29
P. ramsayi (DB)	84	22.6 (14.7–32.7)	0.3 (0.2–0.5)	1.4 (1.1–1.8.0)	1–4
P. isosceles (B)	15	80.0 (53.4–94.3)	26.5 (13.6–53.0)	33.1 (18.3–64.0)	2–138
P. brasiliensis (DB)	8	75.0	3.5	4.7	1–12
P. nudigula (B)	32	75.0 (57.7–87.9)	3.3 (2.3–5.0)	4.4 (3.3–6.5)	1–18
P. semifasciata (DB)	31	32.3 (17.2–50.0)	0.4 (0.2–0.6)	1.1 (1–1.3)	1–2
R. brasiliensis (DB)	16	100 (79.2–100)	4.4 (3.3–5.7)	4.4 (3.3–5.7)	2–9
S. japonicus (P)	13	76.9	4.4	5.5	2–14
S. porosa (DP)	34	20.6 (9.9–38.1)	0.8 (0.2–2.7)	4.0 (1.6–10.0)	1–16
S. brasiliensis (DP)	73	1.4 (0.1–7.3)	0.0 (0.0–0.04)	1	1
X. rasile (B)	29	79.3 (60.5–90.6)	6.9 (4.1–15.0)	8.7 (5.3–18.7)	1–67

Table 7. Post hoc comparisons of a Kruskal–Wallis test comparing the abundance of cystacanths of *Corynosoma australe* between fish species from Patagonia, Argentina. Theoretical critical values ($P = 0.05$) and observed values are presented above and below the diagonal, respectively. Statistically significant comparisons are indicated in bold.

	A. patachonicus	*N. bergi*	*C. peruvianus*	*C. gobio*	*G. blacodes*	*H. lahillei*	*M. hubbsi*	*P. isosceles*	*P. ramsayi*	*P. brasiliensis*	*P. nudigula*	*P. semifasciata*	*R. brasiliensis*	*S. japonicus*	*S. porosa*	*S. brasiliensis*	*X. rasile*
A. patachonicus	–	47.7	56.0	67.5	45.5	74.7	42.8	56.0	42.5	67.5	47.7	48.0	55.1	58.2	47.3	43.0	48.6
N. bergi	31.2	–	48.8	61.6	36.2	69.4	32.7	48.8	32.4	61.6	39.0	39.3	47.7	51.3	38.4	33.1	40.0
C. peruvianus	**210.4**	**179.2**	–	68.3	46.6	75.3	43.9	56.9	43.7	68.3	48.8	49.0	56.0	59.1	48.3	44.2	49.6
C. gobio	**184.4**	**153.1**	26.1	–	59.9	84.2	57.9	68.3	57.7	78.0	61.6	61.8	67.5	70.1	61.3	58.1	62.3
G. blacodes	**116.4**	**84.9**	**94.4**	**68.3**	–	67.9	29.3	46.6	29.0	59.9	36.2	36.6	45.5	49.2	35.6	29.8	37.3
H. lahillei	**223.9**	**192.6**	13.4	39.4	**107.7**	–	66.0	75.3	65.9	84.2	69.4	69.6	74.7	77.0	69.1	66.2	69.9
M. hubbsi	**223.9**	**192.6**	13.4	39.4	**107.7**	0.0	–	43.9	24.4	57.9	32.7	33.1	42.8	46.7	32.0	25.3	33.9
P. isosceles	35.2	**66.4**	**245.6**	**219.6**	**151.3**	**259.0**	**259.0**	–	43.7	68.3	48.8	49.0	56.0	59.1	48.3	44.2	49.6
P. ramsayi	**175.4**	**144.3**	35.0	8.9	**59.4**	48.3	**49.3**	**210.7**	–	57.7	32.4	32.8	42.5	46.5	31.7	25.0	33.6
P. brasiliensis	18.8	12.4	**191.6**	**165.6**	**97.3**	**205.0**	**205.0**	54.0	**156.7**	–	61.6	61.8	67.5	70.1	61.3	58.1	62.3
P. nudigula	19.7	11.5	**190.8**	**164.7**	**96.4**	**204.1**	**204.1**	**54.9**	**155.8**	0.9	–	39.3	47.7	51.3	38.4	33.1	40.0
P. semifasciata	**157.8**	**126.6**	**52.7**	26.6	**41.7**	66.0	**66.0**	**193.0**	17.9	**139.0**	**138.1**	–	48.0	51.5	38.7	33.4	40.3
R. brasiliensis	**55.4**	**86.6**	**265.8**	**239.8**	**171.5**	**279.2**	**279.2**	20.2	**230.9**	**74.2**	**75.1**	**213.8**	–	58.2	47.3	43.0	48.6
S. japonicus	1.63	29.6	**208.8**	**182.7**	**114.4**	**222.2**	**222.2**	36.9	**173.8**	17.2	18.0	**156.1**	57.0	–	50.9	46.9	52.1
S. porosa	**172.6**	**141.4**	37.9	11.9	**56.5**	51.2	**51.2**	**207.8**	2.9	**153.8**	**159.9**	14.8	**228.0**	**171.0**	–	32.4	39.4
S. brasiliensis	**221.0**	**189.9**	10.6	36.7	**105.0**	2.8	2.8	**256.3**	**45.6**	**202.3**	**201.4**	**63.3**	**276.4**	**219.4**	**48.5**	–	34.2
X. rasile	0.06	31.3	**210.9**	**184.4**	**116.1**	**223.8**	**223.8**	35.2	**175.5**	18.8	19.7	**157.8**	**55.3**	1.69	**172.6**	**221.1**	–

There was a significant monotonic trend of decrease of prevalence from benthic to pelagic fish in the Patagonian region (Jonckheere-Terpstra test, T = 190.5, n = 27, one-tailed *P* = 0.002) (**Fig. 14**).

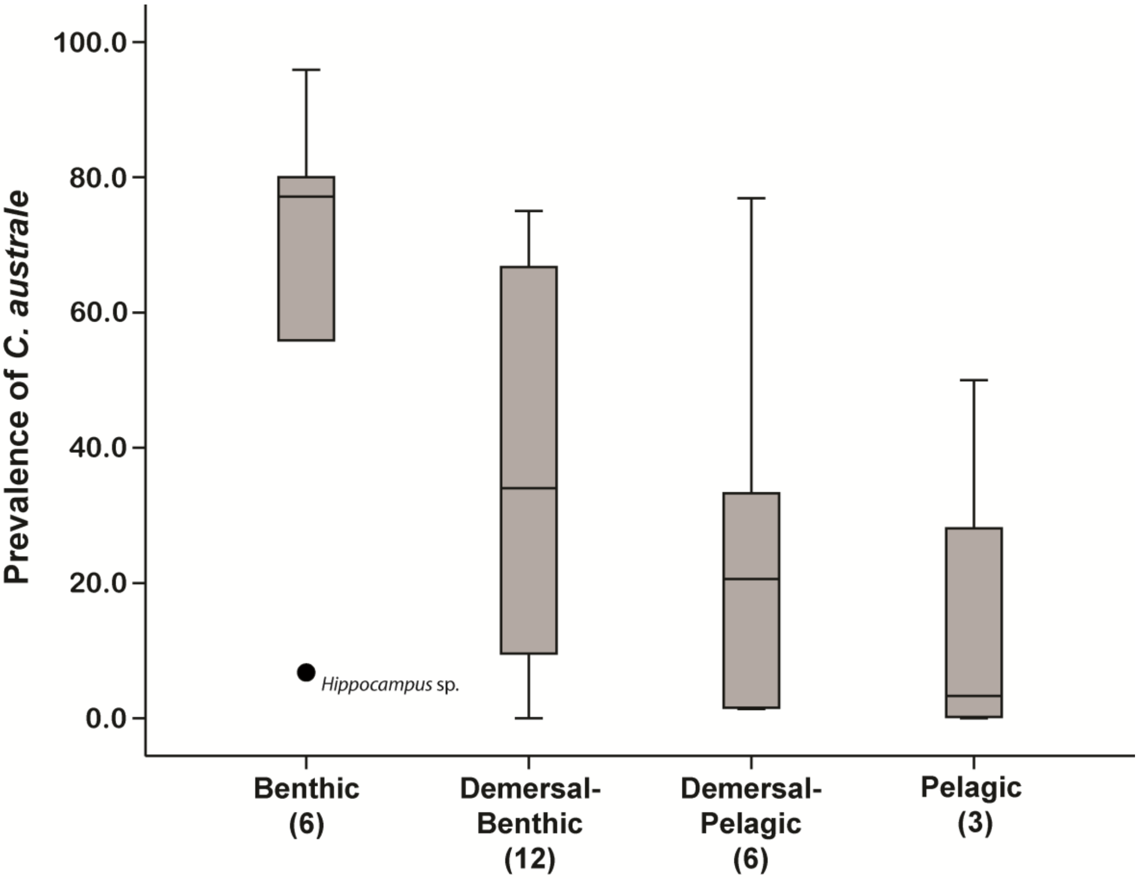


Figure 14. Box plot of prevalence of cystacanths of *Corynosoma australe* from paratenic fish hosts according to their ecological group assigned to its position in the water column. Numbers under ecological groups indicate the number of fish species included in each category.

6.3.2. Relationship between infection patterns and sea lion diet

None of the correlations between infection levels and relative importance of fish in the diet of sea lions was statistically significant. Results for Koen-Alonso *et al.* (2000)'s and sample were as follows: *%N* and prevalence: r_s = 0.000, n = 13, one-tailed *P* = 0.50; *%N* and mean abundance: r_s = 0.28, n = 13, one-tailed *P* = 0.536; *IRI* and prevalence: r_s = -0.094, n = 13, one-tailed *P* = 0.62; *IRI* and mean abundance: r_s = -0.171, n = 13, one-tailed *P* = 0.712. Results for Romero *et al.* (2011) were as follows: *%N* and prevalence: r_s = -0.350, n = 9, one-tailed *P* = 0.82; *%N* and mean abundance: r_s = -0.576, n = 9, one-tailed *P* = 0.95; *IRI* and prevalence: r_s = -0.267, n = 9, one-tailed *P* = 0.76; *IRI* and mean abundance: r_s = -0.559, n = 9, one-tailed *P* = 0.94.

In the studies of Koen-Alonso *et al.* (2000) and Romero *et al.* (2011), *M. hubbsi* was, by far, the most important prey, in terms of number and biomass, even including non-fish prey such as cephalopods (*%IRI* were 39.7% and 44.4% in each study, respectively). However, infections of *C. australe* in *M. hubbsi* were anecdotal in all parasitological studies hitherto carried out, not only in the study area, but also in other Argentine localities (**Tables 5 & 6**; **Fig. 15**). *Raneya brasiliensis* was found to be the second fish species in importance in the diet of sea lions (*%IRI* were 7.3% and 15.1%, respectively), and was one of the fish species most heavily infected with *C. australe* (**Fig. 15**; see above). Apart from these predictable elements in the diet, sea lion consume other fish in variable degrees, but none of the most important fish prey, *i.e. Engraulis anchoita* Hubbs and Marini, 1935, *S. porosa* or *S. brasiliensis* (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011), seems to play a significant role in the transmission of *C. australe* based on their infection levels (**Tables 5 & 6**; see above).

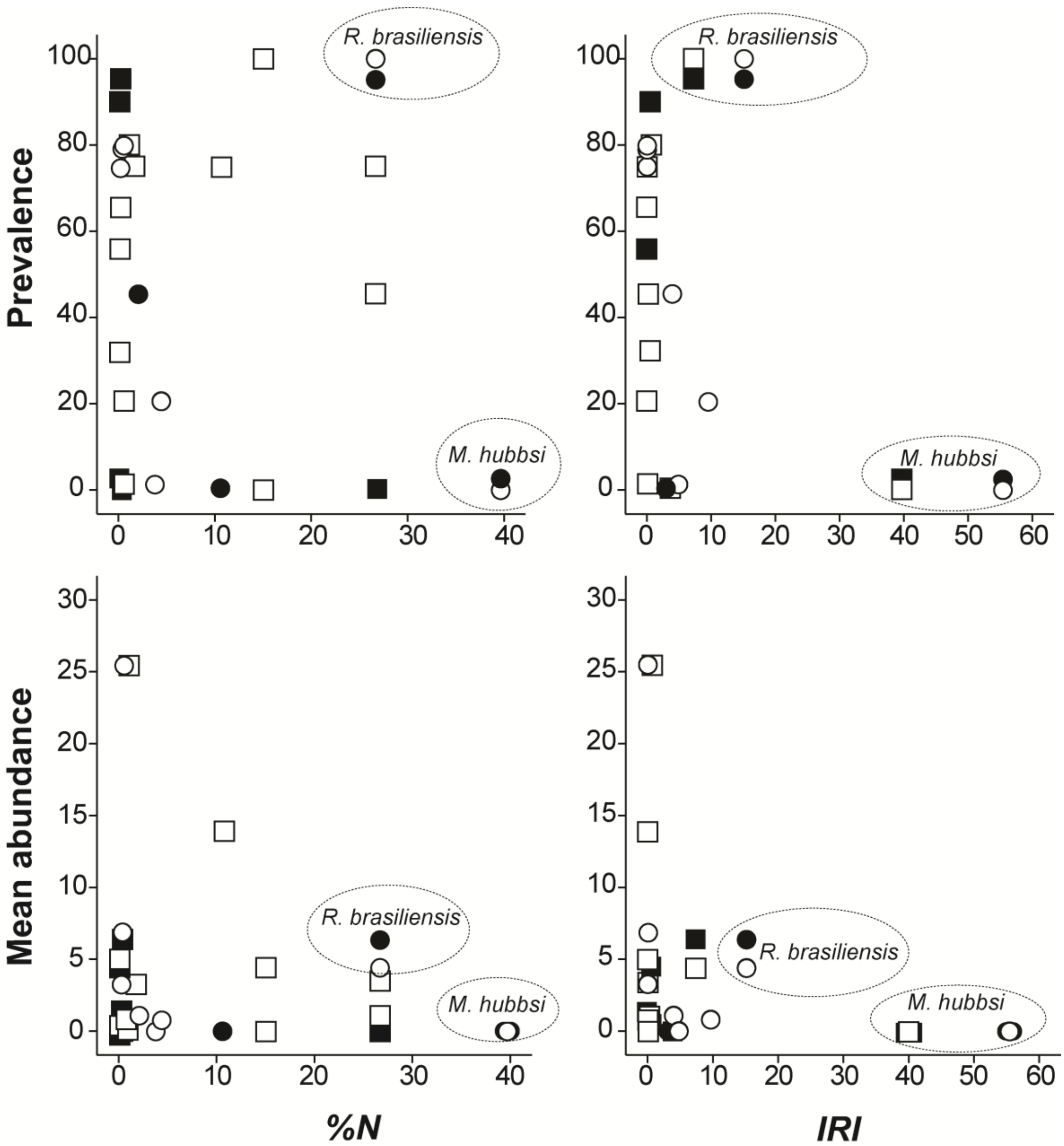


Figure 15. Relationship between infections levels of cystacanths of *Corynosoma australe* obtained from the present study (empty dots) and from other fish parasitological surveys (solid dots) and percentage by number (*%N*) or Index of Relative Importance (*IRI*) of individual fish species in the diet of South American sea lions, *Otaria flavescens*. Dietary data were obtained from Koen-Alonso *et al.* (2000) (squares) and Romero *et al.* (2011) (circles).

6.3.3. Sex ratio

Total number of cystacanth males and females, and estimated sex ratio (percent males) of *C. australe* in each fish species are shown in **Table 8**. Most worms could be sexed in all fish species, except in the case of *A. patachonicus*, where most worms were observed to be different stages of degradation through a granulomatous inflammatory response (F. E. Montero, pers. comm.). Although the range of values of sex ratio was apparently wide (30% to 51.4%; **Table 8**, **Fig. 16**), differences among fish species were not significant (Kruskal-Wallis test, $X^2 = 5.456$, df = 11, $P > 0.05$). The overall sex ratio for cystacanths of *C. australe* in this area was slightly biased towards females (percentage of females: 43.9%, 95% CI: 39.0–48.8) (**Fig. 16**). However, the difference of males *vs.* females at infrapopulation level (regardless of host species) was highly significant (Wilcoxon test, $Z = -3.146$, n = 182, $P = 0.002$). In **Fig. 17** it is shown the intensity of *C. australe* in individual fish plotted against estimated sex ratio, with theoretical 95% CIs superimposed. Only slight departures of expected sex ratio were found in 3 fish (**Fig. 17**). Removing them, the overall sex ratio was 43.8%, 95% CI: 38.8–48.8.

Table 8. Total number of cystacanth males and females of *Corynosoma australe* from 18 species of fish paratenic hosts from Patagonia, Argentina. Values of sex ratio (percent males) and 95% CIs were estimated only for fish species with n ≥ 5. Information about worms that could not be sexed because they were broken or degraded is also provided. Abbreviations: n, total number of cystacanths collected; B, number of cystacanths broken; NE, number of cystacanths necrotic and encapsulated.

Host	*C. australe*					
	n	**♂**	**♀**	**Sex ratio (95% CI)**	**B**	**NE (%)**
A. patachonicus	223	17	24	47.3% (32.2–63.7)	–	182 (81.6%)
B. brama	1	–	–	–	1	–
C. peruvianus	1	–	1	–	–	–
C. gobio	7	2	5	–	–	–
G. blacodes	47	22	24	48.6% (30.5–66.5)	1	–
M. magellanicus	1	–	1	–	–	–
M. argentinae	3	1	2	–	–	–
N. bergi	159	55	98	40.2% (29.2–51.5)	2	4 (2.5%)
P. ramsayi	26	13	12	51.4% (31.9–70.8)	1	–
P. isosceles	397	198	190	48.5% (36.8–58.6)	5	4 (1.0%)
P. brasiliensis	28	14	14	50.0% (27.8–72.2)	–	–
P. nudigula	106	44	60	49.3% (30.6–55.7)	2	–
P. semifasciata	11	3	8	30.0% (10.0–60.0)	–	–
R. brasiliensis	70	34	35	45.8% (33.4–57.8)	1	–
S. japonicus	57	21	32	34.7% (22.9–46.0)	4	–
S. porosa	28	8	19	38.6% (10.1–71.4)	–	1 (3.6%)
S. brasiliensis	1	1	–	–	–	–
X. rasile	201	91	105	45.7% (34.3–57.8)	5	–

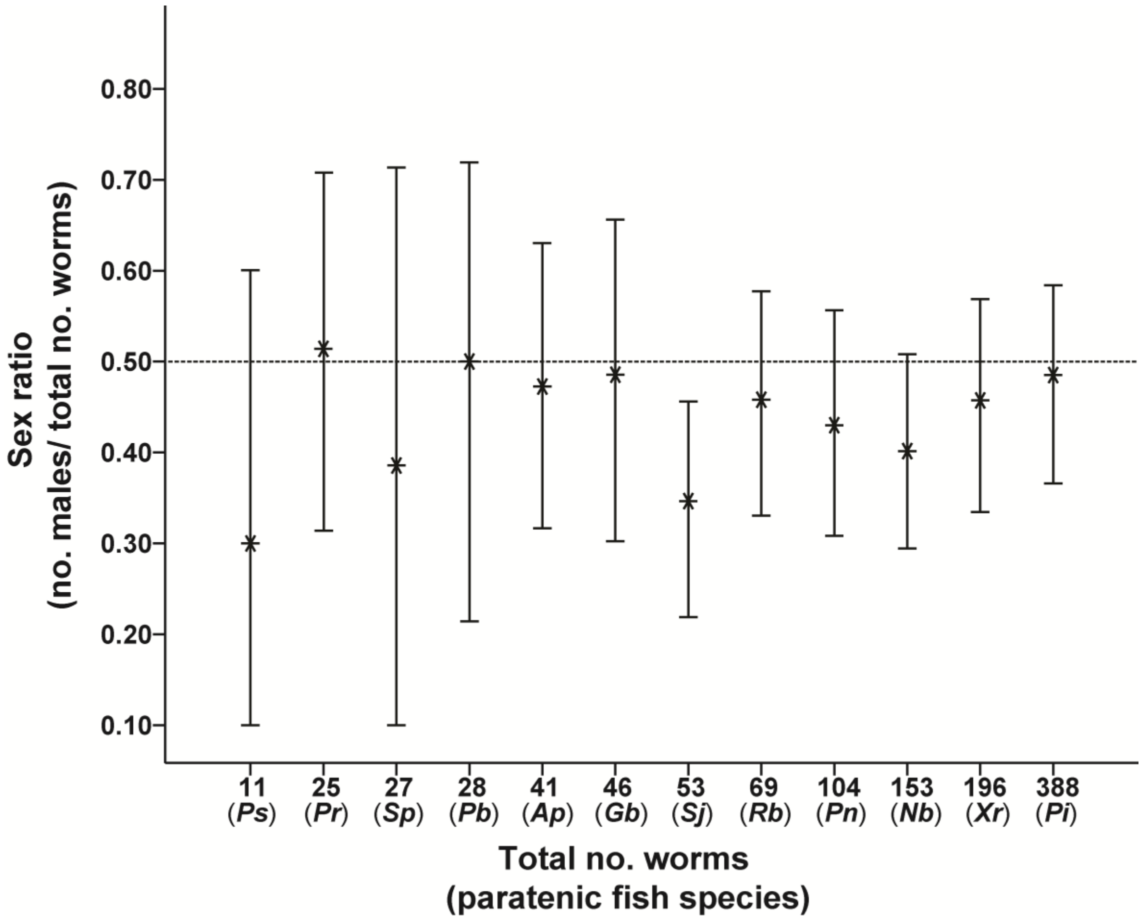


Figure 16. Sex ratio values of *Corynosoma australe* from 12 marine fish species from Patagonia, Argentina. The asterisk indicates the empirical value obtained using the total of worms from each fish species; the bar through the asterisk is the estimated mean value for 20,000 bootstrap replicates; the segment represents the 95% confidence interval calculated by the bootstrap procedure (see Materials and Methods for details). Abbreviations: *Ap*, *A. patachonicus*; *Gb, G. blacodes*; *Nb, N. bergi*; *Pb, P. brasiliensis*; *Pi, P. isosceles*; *Pn, P. nudigula*; *Pr, P. ramsayi*; *Ps, P. semifasciata*; *Rb, R. brasiliensis*; *Sj, S. japonicus*; *Sp, S. porosa*; *Xr, X. rasile*.

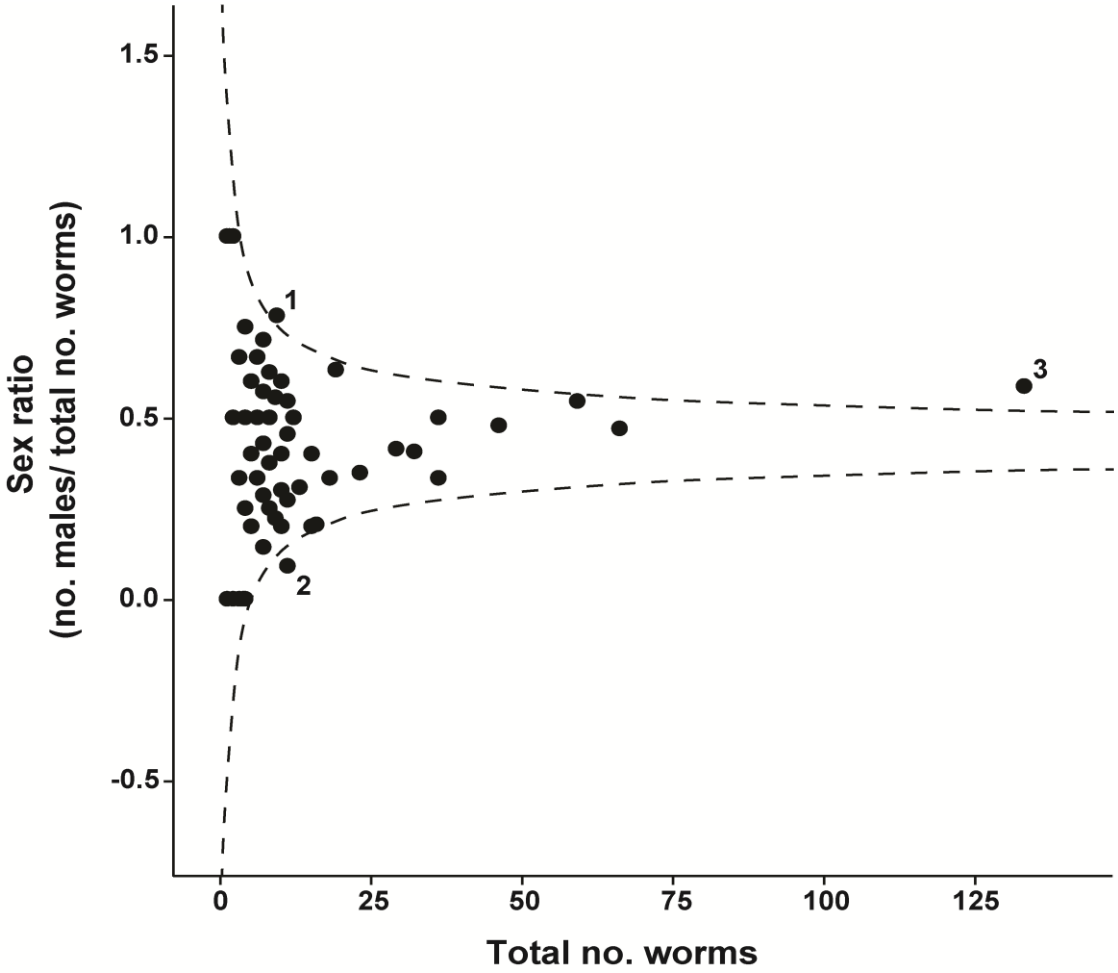


Figure 17. Relationship between sex ratio and intensity value of cystacanths of *Corynosoma australe* for each infected fish collected from Patagonia, Argentina. The 95% confidence interval (broken lines) for a theoretical sex ratio value of 43.9% is also shown (see the text for details). Numbers indicate the 3 fish individuals that depart from the expected sex ratio.

6.3.4. Influence of fish trophic level on parasite size

Average values of body volume of *C. australe* in each fish species are shown in **Table 9**. Mixed model analysis indicated that, based on AIC values, the best model only included 2 parameters, *i.e.* the grand mean (intercept) and random variation associated to host species (**Table 10**). However, the model including also 'trophic level' as a fixed factor generated ΔAIC = 1.13, suggesting that this model also received substantial support (**Table 10**). In this model, inference based on the 'frequentist' approach also indicated a weak but significant effect of trophic level (t= -2.075, one-tailed $P = 0.020$). The estimated parameter was -0.056 (95% IC: -0.109, -0.003), namely, a negative effect of fish trophic level the volume of individuals of *C. australe* (**Fig. 18**). All the remaining models, including those with putative intensity effects, received substantially less empirical support (ΔAIC > 5).

Table 9. Mean values ± S.D. (range) of body dimensions obtained from cystacanths of *Corynosoma australe* collected from 13 fish species from Patagonia, Argentina. Measurements in mm (trunk length and disk diameter) and mm^3 (volume).

		♂			♀			♂ & ♀
Host	**TL**	**n**	**Trunk Length**	**Disk diameter**	**n**	**Trunk Length**	**Disk diameter**	**Volume**
A. patachonicus	4.01	17	1.37 ± 0.24 (0.95–1.81)	0.90 ± 0.15 (0.58–1.09)	24	1.3 0 ± 0.22 (0.90–1.70)	0.92 ± 0.15 (0.68–1.23)	0.31 ± 0.14 (0.08–0.67)
C. gobio	4.26	–	–	–	3	1.33 ± 0.21 (1.12–1.53)	0.88 ± 0.05 (0.84–0.93)	0.27 ± 0.07 (0.21–0.35)
G. blacodes	4.34	22	1.38 ± 0.14 (1.13–1.63)	0.87 ± 0.10 (0.69–1.05)	17	1.43 ± 0.19 (1.13–1.72)	0.93 ± 0.12 (0.74–1.14)	0.31 ± 0.11 (0.15–0.58)
N. bergi	3.45	53	1.38 ± 0.15 (1.04–1.68)	0.87 ± 0.10 (0.60–1.09)	90	1.38 ± 0.15 (0.87–1.75)	0.91 ± 0.11 (0.55–1.11)	0.30 ± 0.09 (0.7–0.53)
P. ramsayi	3.49	11	1.42 ± 0.19 (1.15–1.86)	0.94 ± 0.11 (0.71–1.12)	8	1.40 ± 0.21 (1.09–1.75)	0.98 ± 0.10 (0.84–1.13)	0.35 ± 0.11 (0.17–0.61)
P. isosceles	4.04	184	1.46 ± 0.22 (0.17–2.05)	0.90 ± 0.14 (0.44–1.19)	173	1.38 ± 0.21 (0.78–1.80)	0.86 ± 0.14 (0.53–1.26)	0.31 ± 0.12 (0.05–0.76)
P. brasiliensis	4.33	11	1.36 ± 0.32 (0.70–1.98)	0.88 ± 0.22 (0.56–1.34)	14	1.40 ± 0.22 (0.80–1.61)	0.85 ± 0.13 (0.61–1.11)	0.30 ± 0.17 (0.06–0.93)
P. nudigula	3.77	35	1.39 ± 0.16 (1.05–1.92)	0.88 ± 0.14 (0.57–1.29)	56	1.35 ± 0.18 (0.82–1.75)	0.90 ± 0.14 (0.55–1.17)	0.30 ± 0.12 (0.09–0.68)
P. semifasciata	3.88	–	–	–	5	1.25 ± 0.12 (1.16–1.45)	0.83 ± 0.10 (0.72–0.93)	0.23 ± 0.72 (0.16–0.33)
R. brasiliensis	3.20	22	1.43 ± 0.13 (1.26–1.68)	0.91 ± 0.11 (0.77–1.15)	35	1.42 ± 0.17 (0.97–1.74)	0.92 ± 0.13 (0.69–1.26)	0.32 ± 0.11 (0.15–0.72)
S. japonicus	3.09	11	1.35 ± 0.16 (1.06–1.61)	0.85 ± 0.16 (0.60–1.00)	11	1.40 ± 0.13 (1.18–1.62)	0.87 ± 0.14 (0.70–1.14)	0.28 ± 0.11 (0.11–0.52)
S. porosa	3.30	6	1.38 ± 0.15 (1.20–1.59)	0.85 ± 0.66 (0.77–0.94)	19	1.33 ± 0.11 (1.11–1.60)	0.91 ± 0.15 (0.51–1.12)	0.29 ± 0.09 (0.09–0.44)
X. rasile	3.29	56	1.55 ± 0.14 (1.10–1.80)	0.99 ± 0.12 (0.55–1.17)	82	1.49 ± 0.21 (0.89–2.15)	1.00 ± 0.15 (0.61–1.26)	0.41 ± 0.13 (0.09–0.72)

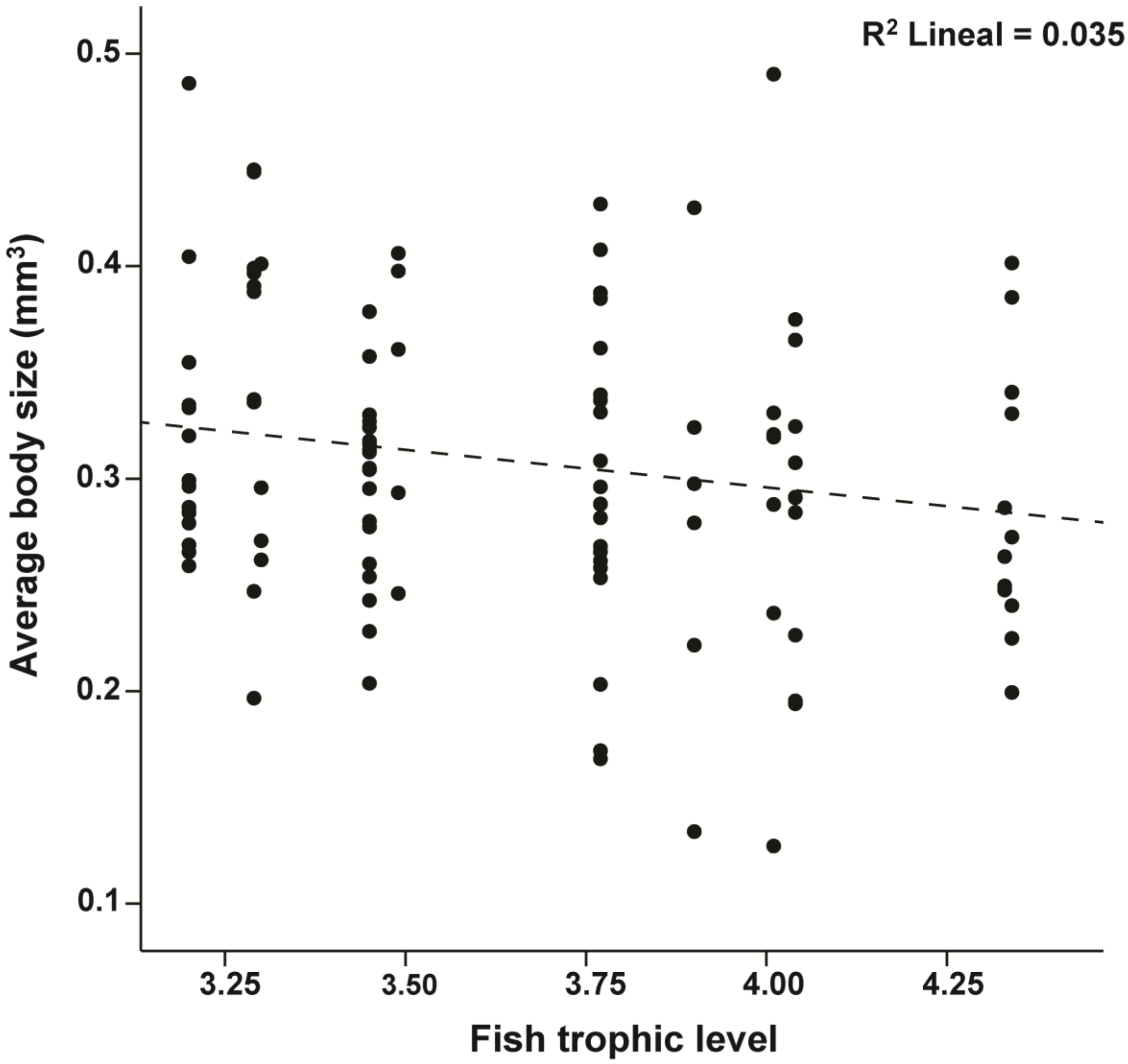


Figure 18. Relationship between the average volume of cystacanths of *Corynosoma australe* and trophic level of 11 marine fish species from Patagonia, Argentina.

Table 10. Mixed models accounting for the effect of fish species (factor) and intensity and fish trophic level (covariates) on body volume of cystacanths of *Corynosoma australe* collected from 12 fish species from Patagonia. Models are arranged by increase of Akaike information criterion (AIC). The probability associated to each fixed effect (excluding the intercept) is also given. Abbreviations: BV, Worm body volume; FS, Fish species; I, Intensity; TL, Trophic level; Un, Unstructured covariance; Vc, Variance components.

Model			**ΔAIC**	**Predictor**	**t**	**One-tailed *P***
Fixed effects	**Random effects**	**Covariance structure**				
Intercept	FS	Vc	0.00	–	–	–
Intercept + TL	FS	Vc	1.13	TL	-2.075	**0.020**
Intercept + I	FS	Vc	5.27	I	0.419	0.338
Intercept + TL + I	FS	Vc	6.36	TL	-2.087	**0.019**
				I	0.509	0.306
Intercept + I	FS + I	Vc	6.97	I	0.553	0.295
Intercept + TL + I	FS + I	Vc	8.36	TL	-2.087	**0.019**
				I	0.509	0.306
Intercept + I	FS + I	Un	9.30	I	0.421	0.487
Intercept + TL + I	FS + I	Un	10.37	TL	-2.730	0.136
				I	0.505	0.349

6.4. DISCUSSION

6.4.1 Infection patterns of C. australe *in paratenic fish hosts*

6.4.1.1. Circulation of C. australe *in the trophic web*

The life cycle of species of *Corynosoma* includes an arthropod (*i.e.* amphipods) as the intermediate host, teleosts as paratenic hosts and homeothermic vertebrates (*i.e.* carnivores, rodents, cetaceans and fish eating birds) as definitive hosts (Aznar *et al.*, 2006). In the south western Atlantic, the specific identities of the arthropod intermediate hosts for *C. australe* are still unknown, but a wide range of fish species has been reported as paratenic hosts (**Tables 5 & 6**), and 2 otariid species act as definitive hosts (Aznar *et al*., 2012; Hernández-Orts *et al.*, 2013, see **Chapter 4**).

Results from the present study suggest that infections of *C. australe* in paratenic fish hosts are not random, since significant differences of infections levels were found among fish species. In particular, evidence would suggest that transmission of *C. australe* from arthropod to fish varies along the water column, with infection most likely occurring in benthic and benthic-demersal fish (**Fig. 14**). We submit that this pattern is probably associated with the distribution of the arthropod intermediate host of this acanthocephalan. Benthic amphipods have been reported as their intermediate hosts for at least 6 species of *Corynosoma* (see Hoberg, 1986; Laskowski *et al.*, 2010; Sinisalo & Valtonen, 2003; Valtonen, 1983; Valtonen & Niinimaa, 1983; Zdzitowiecki, 1986, 2001; Zdzitowiecki & Presler, 2001), and therefore, it seems plausible that similar invertebrates could act as intermediate hosts for *C. australe*. This idea is supported by the fact that *R. brasiliensis*, which was the fish species most heavily infected with *C. australe*, feeds almost exclusively on small benthic invertebrates, mainly polychaetes and amphipods (Barraza Bernardas, 2009).

However, *C. australe* also infects a number of fish species from different zones of the water column, thus strongly suggesting that transmission between paratenic hosts must also occur. Transfer of *Corynosoma* spp. between fish species in the Baltic region has also been invoked to explain their occurrence in ichthyophagous fish (Valtonen, 1983; Valtonen & Julkunen, 1995). To our knowledge, there is little experimental evidence on transmission of cystacanths of any acanthocephalan between paratenic hosts linked by predator-prey relationships, but this phenomenon is usually implied in life-cycles where many paratenic hosts are not directly consumed by definitive hosts (*e.g.* Santoro *et al.*, 2012, 2013). Also, recent studies have suggested that cystacanths of *Corynosoma strumosum* (Rudolphi, 1802), collected from fish are able to re-encapsulate in the body cavity of lizard predators (Skorobrechova *et al.*, 2012). Furthermore, post-cyclic transmission of adult specimens between different definitive hosts is a frequent phenomenon in acanthocephalans (Kennedy, 1999; Nickol, 1985, 2003). These lines of evidence would support the idea that these phenomena are possible, although an open question is whether, and to what extent, they are costly (see below).

According to the paratenic-to-paratenic transmission hypothesis, trophic interactions should account for the ecological ubiquity of *C. australe* in a wide array of neritic fish species in Patagonia. It would also be one of the most important factors leading the spatial distribution of the parasites, and will account for the high infections of benthic or benthic-demersal fish, particularly if they include *R. brasiliensis* in their diet, *e.g. A. patachonicus*, *G. blacodes* or *P. semifasciata* (see Elias & Rajoy, 1992; Galván *et al.*, 2009; Goldstein & Cousseau, 1987; Sánchez & Prenski, 1996). However low infections were found in demersal-pelagic and pelagic fish, which feed mainly on planktonic prey, *e.g. M. hubbsi* (<30 cm total length), *E. anchoita*, *S. brasiliensis, P. ramsayi* or *S. porosa* (see Laptikhovsky, 2004; Mianzan *et al.*, 1996; Ocampo Reinaldo *et al.*, 2011; Pájaro, 2002; Sabatini, 2004) (**Tables 5 & 6**).

There are two key fish species in the diet of sea lions (see below) that were hardly infected with *C. australe, i.e. E. anchoita* and, particularly, *M. hubssi*. According to the 'benthic' transmission hypothesis, low infection levels found in *E. anchoita* are not particularly surprising as this is a planktivorous epi-pelagic fish (Hansen, 2000; Leonarduzzi *et al.*, 2010) that would hardly be exposed to infective stages of *C. australe*. This would also be the case of *M. hubbsi*. Young individuals of this species (<35 cm of total length) are planktophagous opportunistic predators (Cousseau & Perrota, 2008; Sánchez & García de la Roza, 1999). Larger individuals (>35 cm) mainly feed on pelagic and demersal-pelagic species, e.g, the pelagic shrimp, *Peisos petrunkevitchi* Burkenroad, 1945, *E. anchoita*, conespecifics and cephalopods (Ocampo Reinaldo *et al.*, 2011; Ruiz & Fondacaro, 1997; Sabatini, 2004; Sánchez, 2009; Sánchez & García de la Roza, 1999). It is therefore likely that most prey consumed by *M. hubbsi*, being pelagic, are not infected with *C. australe*. Ruiz & Fondacaro (1997) and Sánchez (2009) reported only low numbers (<6.0 percent no. items) of a key fish species for transmission, *R. brasiliensis*, (see below) in the diet for *M. hubbsi* from proximal localities to our study area.

There is, however, a clear 'anomaly' to the above hypothesis, namely, the high infections levels observed in *S. japonicus* (Cremonte & Sardella, 1997; this study). *S. japonicus* is a pelagic fish that feeds mostly on planktonic organisms or small pelagic fish (Angelescu, 1980; Pájaro, 1993). This suggests that additional factors may regulate infection levels of *C. australe* in particular fish species. For instance, cystacanths of

Corynosoma are considered to be long-lived, with a lifespan spanning over several years in fish (Comiskey & Mackenzie, 2000; Valtonen, 1983). Accordingly, cystacanths of *C. australe* could tend to accumulate in large, long-lived fish species that are rarely consumed by definitive hosts. This is a case in point for *S. japonicus*, a relatively long-lived species (10 to 13 years, see Perrota, 2000) that have never been reported as a prey of South American sea lions in Patagonia, contrary to most fish species analysed in this study (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). Another relevant factor that should be considered is that, in most fish species, food habits change throughout ontogeny. This may influence transmission patterns of *C. australe* among fish that feed on benthic invertebrates mostly during their juvenile stage (Elias & Rajoy, 1992; García, 2007; Renzi, 1986).

6.4.1.2. Transmission of **C. australe** *to definitive hosts*

For a given reproductive effort, trophic-transmitted parasites face a potential trade-off between spreading infections through the trophic web, which would result in many paratenic host species being slightly infected, or infecting specific taxa that are likely consumed by definitive hosts, which would result in more reduced set of species but more heavily infected. Obviously, transmission is only partially, if at all, under parasite’s control (*e.g.* Lafferty, 1999; Poulin, 1998, 2010); it also depends strongly on temporal dynamics of trophic relationships between potential hosts (Lafferty, 1999; Poulin & Leung, 2011). Given this constraint, natural selection should tend to maximize transmission from paratenic to definitive hosts with minimum losses, *i.e.* to optimize the ratio of potential paratenic species that are consumed *vs.* those that are not consumed (and therefore act as a population ‘sink’) (Holmes, 1979; Koehler & Poulin, 2010).

These theoretical considerations are fundamental to interpret potential transmission patterns of *C. australe* to South American sea lions. Contrary to our expectations, not all fish species that are mostly consumed by sea lions are heavily infected with *C. australe*. At least in a period of 27 years (1982-2009), the diet of sea lions has been dominated by 3 fish species, *i.e. E. anchoita*, *R. brasiliensis* and, especially, *M. hubbsi* (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). As noted above, both *E. anchoita* and *M. hubbsi* are exceptional hosts for *C. australe* (MacKenzie & Longshaw, 1995; Sardella & Timi, 2004; Timi & Poulin, 2003; present study), and would hardly contribute significantly to transmission. In contrast, intake of *R. brasiliensis* by sea lions would likely promote a continuous recruitment of *C. australe*. In other words, it is likely that *R. brasiliensis* have been played a key role for the transmission of *C. australe* to sea lions in the study area over the last decades. Indeed, *R. brasiliensis* could play a primary link in the transmission of *C. australe* throughout the trophic web since it has also been reported as an important prey item in wide array of marine birds and fishes (Gosztonyi *et al.*, 2007 and references therein), including other pinniped species inhabiting the study area (Vales *et al.*, 2012).

The fact that the bulk of the sea lions' diet is derived from only a few species is in agreement with previous studies on other pinniped species (Naya *et al.*, 2002). However, at an ecological-time scale, it is unclear that, quantitatively, sea lions' diet is so predictable at the level of prey species. Just in the study area 23 of fish species and 7 cephalopod species have been reported as prey for South American sea lions (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011), and sea lions are considered opportunistic and broad-spectrum feeders that are able to change their food habits depending on prey availability and distribution (Drago *et al.*, 2009a, 2009b; Koen-Alonso *et al.*, 2000). Although no specific data on decadal changes in sea lion's diet exist, strong short-term dietary plasticity has been reported in otariid species in response to changes in prey availability, *e.g.* the inter-year changes reported in the diet of South American fur seals from Uruguay (Naya *et al.*, 2002). In this context, most fish species included in the diet of sea lions in Patagonia has been reported as paratenic host for *C. australe* (**Tables 5 & 6**). This ecological ubiquity through the trophic web would therefore increase the chances of transmission to sea lions regardless of potential dietary changes associated, *e.g.* to prey availability in different seasons or years (Aznar *et al.*, 2004). In other words, many fish species with high infection levels of *C. australe*, particularly those associated to the sea floor (*i.e.* demersal-benthic and benthic fish), will contribute with a large amount of cystacanths even when they are occasionally consumed.

There are other elements in Patagonian food webs that make up a sizeable part of the diet of sea lions but were not included in the present study, *i.e.* cephalopods. In particular, the red octopus, *Enteroctopus megalocyathus* (Gould, 1852), the Argentine shortfin squid, *Illex argentinus* (Castellanos, 1960), and the Patagonian squid, *Loligo gahi* (Orbigny, 1835), have *%IRI* ranging from 1.2 to 25.6, 4.6 to 13.9 and 0.2 to 5.7, respectively (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). Biological and ecological information about acanthocephalans found in cephalopods is currently scarce (Hochberg, 1990). However, available evidence indicates that infections of acanthocephalans in cephalopods are infrequent and usually represent accidental or transitory infections (Nickol, 1985). For instance, no cystacanths of any acanthocephalan species have ever been reported in cephalopods of commercial interest worldwide (González *et al*., 2003; Hochberg, 1990; Pascual & Hochberg, 1996). In this context, the question that arises is whether cephalopods rarely contact infective stages of *C. australe* or the parasite is unable to establish in them. At least *I. argentinus* is considered to be an unsuitable host for this acanthocephalan (González & Kroeck, 2000; Nigmatullin & Shukhgálter, 1990; Sardella *et al.*, 1990; Threlfall, 1970). In summary, evidence would suggest that cephalopods probably play a minor role for the transmission of *C. australe*.

In any event, it is also important to emphasize that the above discussion has been based only on one of the two definitive hosts inhabiting Patagonia. South American fur seals are also readily infected with *C. australe* in southwestern Atlantic (Aznar *et al*., 2004, Hernández-Orts *et al*., 2013; see **Chapter 4**). In the study area, the abundance of fur seals is about one-half from that of sea lions (Crespo *et al*., 1999, 2012; see **Chapter 1**), but evidence suggest that the former are more suitable hosts for *C. australe*. Two independent studies have revealed that female worms from fur seals are both larger and more fecund that those from sea lions (George-Nascimento & Marin, 1992; J.S. Hernández-Orts *et al*., unpublished data). Dietary data of fur seals inhabiting the Patagonian coast are still scanty, but at least 2 species of cephalopods and 4 of fish, including *M. hubbsi*, *R. brasiliensis* and *E. anchoita* have been reported as their prey (Vales *et al.*, 2012; Néstor A. García unpublished data). Future studies should complete the picture about the transmission dynamics of *C. australe* in Patagonia by addressing the role of fur seals as definitive hosts of this species.

6.4.2 Sex ratio in cystacanths of **C. australe**

In acanthocephalans, sex determination is chromosomal and is established during the fertilization process (Crompton, 1985). Accordingly, sex ratio is likely to be 1:1 at the zygotic stage. Significant departures of this ratio have been reported in many species at later stages of development. In particular, a common observation is that sex ratio is female-biased at the adult stage (in the case of *Corynosoma* spp., see Aznar *et al.*, 2001c; George-Nascimento & Marin, 1992; Helle & Valtonen, 1980; Nickol *et al.*, 2002; Sinisalo *et al.*, 2004; Valtonen & Helle, 1988; see also Aznar *et al*., 2004 for the case of adult *C. australe* in fur seals). This pattern is generally accounted for by life-span differences between sexes, with adult females living longer than males (Crompton, 1985; Parshad & Crompton, 1981; Poulin, 1997). As the argument goes, females would require more time to produce the offspring (presumably more than males to inseminate females). However, this hypothesis does not explain why males do not accrue the benefits of living longer as well, thus having more chances to fertilize a higher number of females. Perhaps within the typical polygynous mating systems of acanthocephalans, costs associated with male-to-male competition are responsible, at least in part, for the reduced lifespan of males (Poulin & Morand, 2000; Sasal *et al.*, 2000).

Contrary to the situation at the adult stage, acanthocephalans are expected to conserve the zygotic sex ratio at the larval stage. This assumption is considered to be so well-established that the degree of sex ratios departure from 1:1 in the definitive hosts (due to the process described above) has been used as an index of the ‘age’ of infection (*i.e.* more female-biased infections would be older, *e.g.* Helle & Valtonen, 1980, 1981; Itämies *et al*., 1980; Valtonen & Helle, 1982), or to infer that some hosts are actually unsuitable (*i.e.* sex ratios close to 1:1 in senescent worms would indicate that parasites have been unable to establish, see Aznar *et al*., 2012). The assumption of a 1:1 sex ratio at the larval stage is supported by several studies. In freshwater amphipod intermediate hosts, Dezfuli & Giari (1999) and Steinauer & Nickol (2003) reported no significant departures from 1:1 for cystacanths of *Polymorphus minutus* (Goeze, 1782) and *Leptorhynchoides thecatus* (Linton, 1891), respectively. In the Baltic region, Valtonen & Niinimaa (1983) reported no significant departures from 1:1 in cystacanths of *Corynosoma semerme* (Forssell, 1904), infecting paratenic hosts, *i.e.* teleosts.

Some studies, however, have reported clear departures from 1:1 in the larvae of some acanthocephalan species. Based on a reasonable sample size (n = 246), Amin *et al*. (1980) found a female-biased sex ratio in *Acanthocephalus parksidei* Amin, 1987, from freshwater isopod intermediate hosts. Likewise, Dimitrova (2009) reported strongly female-biased sex ratios (percent males: 30%) in a sample of 524 individuals of

Plagiorhynchus (*Prosthorhynchus*) *cylindraceus* (Goeze, 1782) collected for terrestrial isopods, but sex ratios close to 50% (50.5%) in another sample (n= 786) from the same region. As far as we are aware, no mechanisms leading to sex ratio biases have been proposed in larval acanthocephalans (Amin *et al*., 1980).

Our results suggest that the sex ratio in cystacanths of *C. australe* is also slightly but significantly biased towards females, and this bias is seemingly independent of the species of fish where cystacanths occur. In theory, 3 factors could be involved in generating the sex ratio biases in our sample, namely, sampling error, differential sampling of female and male larvae, and/or differential mortality between the sexes. Regarding the first factor, our analysis suggest that sampling biases can hardly account for a biased sex ratio because the sample size is large and data conform to the theoretical binomial distribution except for 3 cases; when these cases were removed, sex ratio bias was even more pronounced. With regard to the second factor, differential sampling can result in biased sex ratios of free-living organisms when males and females do not follow random distributions in space (*e.g.* Johnson, 2003; Morgan & Trippel, 1996; Pájaro *et al.*, 2005), or when different sampling methods are used (*e.g.* Price & Welch, 2009; Ream & Ream, 1966). However, in the case of a trophically-transmitted parasite there are few realistic scenarios in which amphipods, fish, or we researchers, have 'sampled' female and male larvae of *C. australe* differently. An obvious possibility is that probability of infection (to amphipods or to fish) is sex-dependent. This would be realistic, for instance, if transmission of female *vs*. male larvae is enhanced through adaptive or side-effects of the former upon the host's phenotype (*e.g.* Benesh *et al*., 2009). Finally, there is the possibility that female and male *C. australe* suffer differential mortality at the acanthor, acanthella or cystacanth stages. For instance, Benesh & Valtonen (2007) found that of female *Acanthocephalus lucii* (Müller, 1776), had a much stronger relationship with intermediate host size and survived longer in an experimental culture medium.

Regardless of the reason why female-biased sex ratios of *C. australe* occur in paratenic hosts, an interesting point is that such a biased sex ratio will be 'transferred' to definitive hosts (Bush *et al*., 1993; Lotz *et al.*, 1995; Poulin, 1998). We do not expect this slight bias have a significant impact on the population dynamics of *C. australe* in their pinniped hosts but certainly it challenges the conventional wisdom that the observed sex ratio bias in definitive host depends only on processes operating in those hosts.

6.4.3 Costs related to paratenic host infections

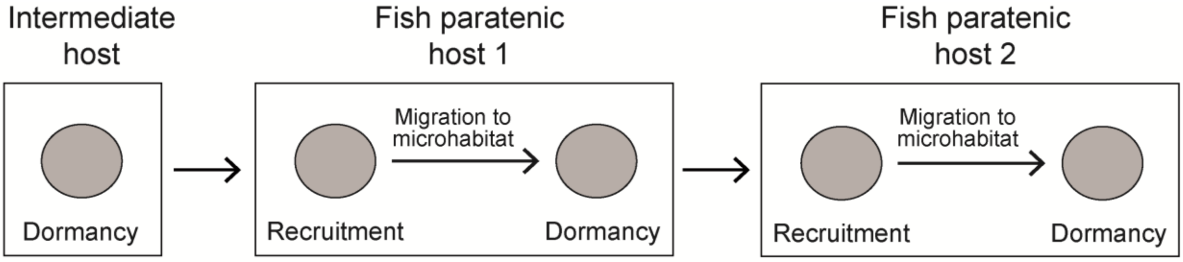
We argued above that paratenicity, and trophic interactions between paratenic hosts, affect the transmission to obligatory hosts by defining their spectrum of potentially infected prey. Usually, expansion of infective stages throughout the trophic web is considered adaptive when the definitive host(s) have great plasticity in dietary habits (see above). However, a relatively neglected facet of this argument is the type of costs that infections to paratenic hosts, and paratenic-to-paratenic transmission, may also entail for the parasite. The most obvious costs are those associated with infection of unsuitable hosts. The role of unsuitable hosts in the population dynamics of trophically-transmitted parasites has been dealt with in the case of the adult stage (Holmes *et al*., 1977). Aznar *et al*. (2001a) suggested that this type of analysis should also be carried out in other stages of the life cycle, but we are unaware of any study that has actually addressed it.

We previously pointed out that some fish can act as population ‘sinks’ for *C. australe* because these fish will rarely be consumed, if at all, by definitive hosts; *e.g.* this might be the case of seahorses, *Hippocampus patagonicus* Piacentino and Luzzatto, 2004 (see Braicovich *et al*., 2005). Also, we found that in 6 fish species that are prey of sea lions, larger fish accumulate higher loads of *C. australe*. However, there are physical limits to the size of fish that sea lions can handle and consume: in fact, sea lions apparently consume mainly small fish (<35 cm TL), occasionally medium-sized fish (>50 cm TL) and rarely large fish (>65 cm TL) (Koen-Alonso *et al*., 2000; N. A. García unpublished data). Accordingly, most infections in large fish will probably be dead-ends in the life cycle.

Finally, there is the open question of whether all fish species are physiologically/immunologically suitable as hosts for *C. australe*. In particular, host immune mechanisms in paratenic hosts can entail obvious costs for cystacanths of *C. australe*. In an allied species, *C. strumosum*, there is evidence of differences in immune and cellular response to the structure of the capsule surrounding cystacanths depending on the fish species (Nikishin & Skorobrechova, 2007; Skorobrechova & Nikishin, 2011). This would suggest distinct suitability of paratenic hosts for this acanthocephalan. Interestingly, we observed a high number of necrotic, encapsulated cystacanths only in the Argentine seabass, *Acanthistius patachonicus* (see **Table 8**). This is a demersal-benthic species that feeds on a wide range of soft-bottom invertebrates and fish species in Patagonian waters (Galván *et al*., 2009), and seems to be readily infected with cystacanths of *C. australe*. Two non-exclusive factors could account for the exceptionally high number of necrotic cystacanths in this fish. First, most worm could be necrotic simply because they are senescent, *i.e.* they were recruited when fish was young. Note that, according to Rubinich & González (2001), the age of our specimens of *A. patachonicus* was from *ca*. 7 to 15 years. However, this hypothesis is at odds with the virtual absence of necrotic worms in other fish species. Alternatively, *A. patachonicus* might actually be an unsuitable host for *C. australe*. Histopathological and developmental studies on the morphology of the capsule surrounding the cystacanths of C. *australe* are necessary to shed light on this issue (Skorobrechova & Nikishin, 2011). In any event, this is an interesting phenomenon that, to our knowledge, had rarely been reported in potential paratenic hosts.

Other potential costs associated to paratenicity are more subtle. In acanthocephalans it has been suggested that no further growth and development occurs in cystacanths infecting paratenic hosts (Kennedy, 2006; Nicholas, 1967; Schmidt, 1985). However, factors that lead cystacanths to stop growing or developing in paratenic hosts are currently unknown. In intermediate hosts, theoretical predictions suggest that parasite mortality increases with parasite growth, and this mortality would define the size and/or time at which a parasite should stop growing (growth arrest) (Ball *et al.*, 2008). In other words, too much growth would be at the expense of the host, which would decrease its survival, hence that of the parasite itself (Parker *et al.*, 2009). Nevertheless, why growth arrest continues in paratenic hosts is unclear, since space and nutrients could be available for the parasite to grow larger (Parker *et al*., 2009).

In **Fig. 19**, theoretical scenarios describing energetic benefits and costs associated to infection of paratenic hosts are shown. Let us assume that an individual of *C. australe* is recruited to a fish paratenic host from the amphipod intermediate host. After activation of the cystacanth, which seems to be a highly energy-consuming process (Taraschewski, 2000), there is then the possibility that this individual actually absorbs nutrients (*e.g.* in the host's intestine) or not. In any event, the larvae must incur in energetic costs as it has to migrate to host's mesenteries and produce a capsule. Available evidence suggests that, in paratenic hosts, *C. semerme* generates a thick layer of glycocalyx on its surface (Skorobrechova & Nikishin, 2011). Encapsulation may effectively isolate the larva, reducing its mortality by averting host immune response and allowing its survival for a very long duration (Parker *et al.*, 2009). Encapsulated individuals (cystacanths) will eventually enter a period of dormancy in which energy consumption is minimal (Nikishin & Skorobrechova, 2007; Petrochenko, 1956). Note that encapsulation of cystacanths commonly occurs in extraintestinal positions (*e.g.* mesenteries), where nutrients presumably are not available for the parasite (Crompton, 1973).



Scenario	Benefit – cost			Balance (Growth)
A1	Nutrient absorption	>	Cost of migration and encapsulation	+
A2	Nutrient absorption	<	Cost of migration and encapsulation	–
B1	No nutrient absorption	&	Little cost	0
B2	No nutrient absorption	&	Cost of migration and encapsulation	–

Figure 19. Theoretical scenarios describing energetic benefits and costs associated to infection of paratenic hosts by *Corynosoma australe*. See text for details.

Theoretically, then, 4 outcomes are possible (**Fig. 19)**: (**A**) the parasite absorbs nutrients in the paratenic host. In this case (**1**) energy intake can be higher than costs of migration and encapsulation and, therefore, net growth of the parasite should occur, and it could be detectable insofar as reserves are not depleted during the period of dormancy; (**2**) energy intake is lower than costs of migration and encapsulation and, therefore, a decrease of body volume should follow; (**B**) the parasite does not absorb nutrients, *i.e.* it relies on endogenous reserves to carry out migration and encapsulation. In this case, (**1**) there would be no noticeable change in size if costs are negligible or (**2**) there would be a decrease of body volume if costs of migration and encapsulation were significant. The scenario becomes more complex when there is paratenic-to-paratenic transmission. In particular, effects on parasite body size under scenarios **A1, A2** and **B2** would be amplified in every trophic step.

Empirical support for the assumptions of the models depicted in **Fig. 19** is extremely scanty. Indeed, there is circumstantial evidence that cystancanths may grow after transmission to the paratenic host. In *Corynosoma pseudohamanni* Zdzitowiecki, 1984, Mašová & Baruš (2013) reported ranges of trunk length from 1.1 to 1.9 mm, and from 1.8 to 2.7 mm, in cystacanths collected from amphipods and fish, respectively. This pattern is compatible with scenario **A1** (**Fig. 19**).

In contrast, our analysis suggests that cystacanths of *C. australe* may incur in non-negligible energetic costs when experiencing putative paratenic-to-paratenic transmission. Certainly, the use of trophic levels (TLs) is just a gross surrogate of the actual host switch events that individual parasites may have suffered. Still, there is a slight, yet statistically significant tendency to decrease body volume of *C. australe* in fish with higher TLs (also note that some estimations of TL are approximate). This pattern would conform to scenarios **A2** or **B2** (**Fig. 19**), and could hardly be explained by other causes. For instance, there is no evidence that fish with higher TLs are less consumed by sea lions, making individuals of *C. australe* to be closer to senescence (and small size due to energy depletion) in these hosts (Koen-Alonso *et al*., 2000; Romero *et al*., 2011; **Table 9**). The implications of this finding cannot be underestimated. An immediate question that arises is whether individuals of *C. australe* that have undergone several switches between paratenic hosts have lower fitness because either larval lifespan, or survival in the definitive hosts, or both, are reduced. Future studies should pay more attention to the negative consequences of paratenic-to-paratenic transmission of trophically-transmitted helminths.

7. PATTERNS OF TRUNK SPINE GROWTH IN TWO CONGENERIC SPECIES OF ACANTHOCEPHALAN: INVESTMENT IN ATTACHMENT MAY DIFFER BETWEEN SEXES AND SPECIES

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Patterns of trunk spine growth in two congeneric species of acanthocephalan: investment in attachment may differ between sexes and species

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SUMMARY

Acanthocephalans have evolved a hooked proboscis and some taxa have trunk spines to attach to their definitive hosts. These structures are generated before being used, thus a key question is how investment in attachment could optimally be allocated through the ontogeny. The number and arrangement of hooks and spines are never modified in the definitive host, but it is unclear whether these structures grow during adult development. A comparison of the size of trunk spines between cystacanths and adults of *Corynosoma cetaceum* and *C. australe* indicated that spines grow in both species, but only in females, which also had significantly larger spines than males. This sexual dimorphism did not result from pure allometry because the body of females was smaller, and did not grow more than that of males. However, having a longer lifespan, females would need to withstand the extreme flow conditions prevailing in marine mammals for longer, inducing different investment and development schedules for spines. Patterns of spine growth also differed between species: fore-trunk spines grew in both species, but hind-trunk spines did only in *C. cetaceum*. In conclusion, investment strategies on attachment may differ, not only between congeneric species of acanthocephalan, but also between sexes of the same species.

Key words: Acanthocephala, *Corynosoma australe*, *Corynosoma cetaceum*, trunk spine, investment strategy, ontogeny, attachment.

INTRODUCTION

Parasites have evolved a wide array of holdfast mechanisms that maximize the likelihood of successful attachment upon recruitment to their hosts and minimize the risk of subsequent dislodgment (Poulin, 2009; Randhawa and Poulin, 2010). Selective pressures on morphology are especially strong in parasites living in the lumen of the gastrointestinal tract, where physical disturbance in the form of peristalsis and food movement can exert powerful drag on attached parasites (Poulin, 2009). Acanthocephalans, in particular, have developed a proboscis armed with hooks that anchor to the gut of their definitive host (Taraschewski, 2000). Many species also have trunk spines that engage on the gut surface, sometimes playing a significant role in attachment (Van Cleave, 1952; Aznar *et al*. 1999*a*, 2002*a*). It has been argued that investment in these primary holdfast structures is optimized for the species of host and the particular microhabitat where each species of acanthocephalan lives (Poulin, 2007). A possible reason is that attachment structures are costly to produce and, therefore, it would not be advantageous for a worm to produce them larger than the size necessary to ensure attachment (Poulin, 2007). Also, depending on the size of the animal, the size of holdfast structures should also be bounded within certain limits to ensure that attachment performance is functional (Van Cleave, 1952; see also Koehl, 1996).

Interestingly, both the proboscis and trunk spines of acanthocephalans are generated prior to being used for attachment, and this raises the question of how investment in such structures could optimally be allocated through ontogeny. The first larval stage, the acanthor, hatches from the egg and passes through 2 subsequent stages, the acanthella and the cystacanth, within an intermediate arthropod host; many acanthocephalans may also use a paratenic host (usually a vertebrate) in which the cystacanth gets

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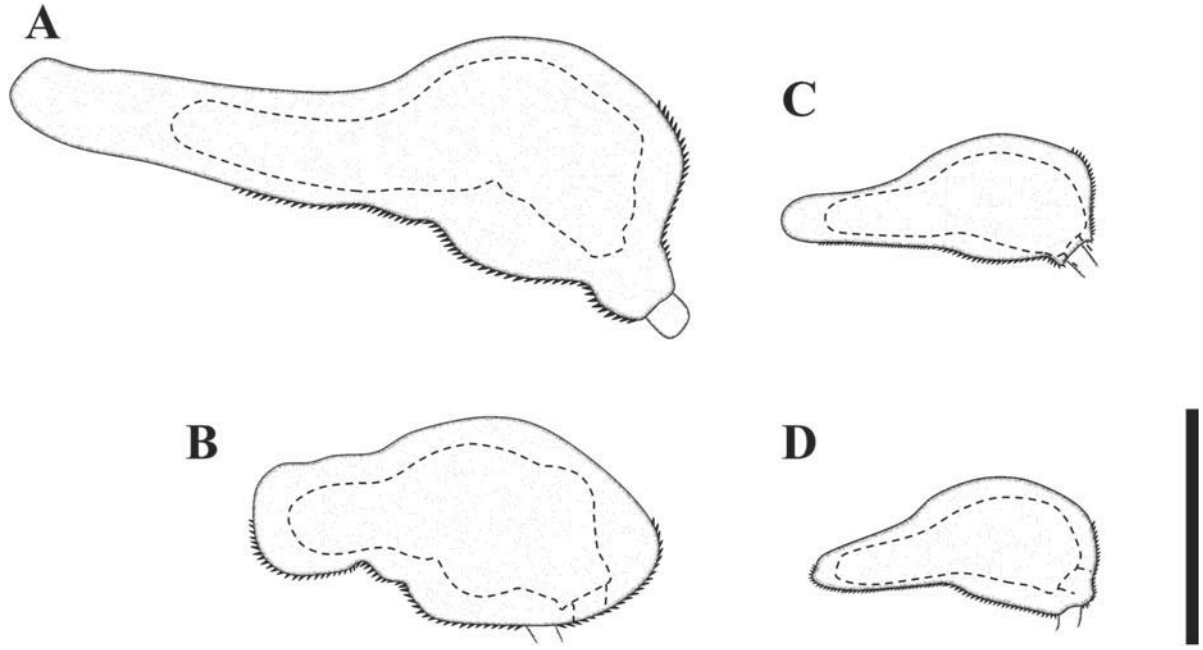

Fig. 1. Diagrammatic comparison of the body size and spine coverage in two species of *Corynosoma*. (A) Male *Corynosoma cetaceum*, (B) Female *C. cetaceum*, (C) Male *C. australe*, (D) Female *C. australe*. Dashed lines indicate the relative body size of cystacanths. Scale bar = 2 mm.

encysted in the mesentery without further development (Schmidt, 1985). The cystacanth is the infective stage that is consumed by the definitive vertebrate host and already has all the primary attachment structures of the adult. Van Cleave (1952) and Petrochenko (1956) suggested that, in most species, attachment structures are fully formed at the cystacanth stage, perhaps as an investment priority of the developing worm to secure successful establishment upon arrival to the definitive host. Indeed, to the best of our knowledge, the number and arrangement of hooks in the proboscis and the extension of spines on the trunk are never modified in the definitive host (Van Cleave, 1952). However, the extent to which the proboscis, proboscis hooks, and trunk spines grow during the adult development is an open question. Some authors reported no changes in the size of proboscis and/or proboscis hooks between cystacanths and adults of some species (Podesta and Holmes, 1970; Amin *et al*. 1995, 2004). Other authors, however, noted an increase in the size of proboscis hooks or trunk spines in adults of different species compared to cystacanths (Podesta and Holmes, 1970; Amin *et al*. 1995), or juveniles i.e. recently recruited worms in the definitive host (Amin, 1986, 1987).

In any of the above studies it is difficult to separate the putative growth of the holdfast from measurement error because none used inferential statistics. However, it seems likely that the timing of growth of attachment structures may differ among species of acanthocephalan depending on their body size. Adult acanthocephalans are subject to the unsteady flow of digested food generated by peristalsis (Poulin, 2007). Although the physical properties of the flow of digesta are far from clear (see Schulze, 2006), acanthocephalans are theoretically expected to experience 3 types of dislodging forces i.e. frictional drag, pressure drag, and acceleration reaction, which are proportional to the surface area, sectional area, and volume of the body, respectively (see Koehl, 1984, for details). Thus, everything else being equal, dislodging forces should increase disproportionately as the body grows, and larger acanthocephalans could therefore need a finer adjustment of their holdfast structures during the adult growth, particularly if they experience a greater change of body size from the cystacanth to the adult stage (see Poulin *et al*. 2003).

In this study we compared the size of trunk spines between cystacanths and adults of 2 congeneric species of acanthocephalans from the Southern Hemisphere that clearly differ in size, namely *Corynosoma cetaceum* and *C. australe* (Fig. 1). Individuals of *C. cetaceum* inhabit the stomach and upper duodenum of small cetaceans, whereas *C. australe* is found in the intestine, mainly in the ileum and jejunum, of pinnipeds (Aznar *et al*. 2001, 2004, 2012; Sardella *et al*. 2005). We focused on trunk spines because they play a key role in the attachment of species of *Corynosoma* (Van Cleave, 1952; Aznar *et al*. 1999*a*; 2001) and can be measured in any specimen; the proboscis is rarely found fully evaginated in adult specimens, and cannot be induced to withdraw because worms are collected dead from hosts. The goals of our study were 2-fold. First, we obtained, for the first time, statistical evidence on whether spines grow during the adult development

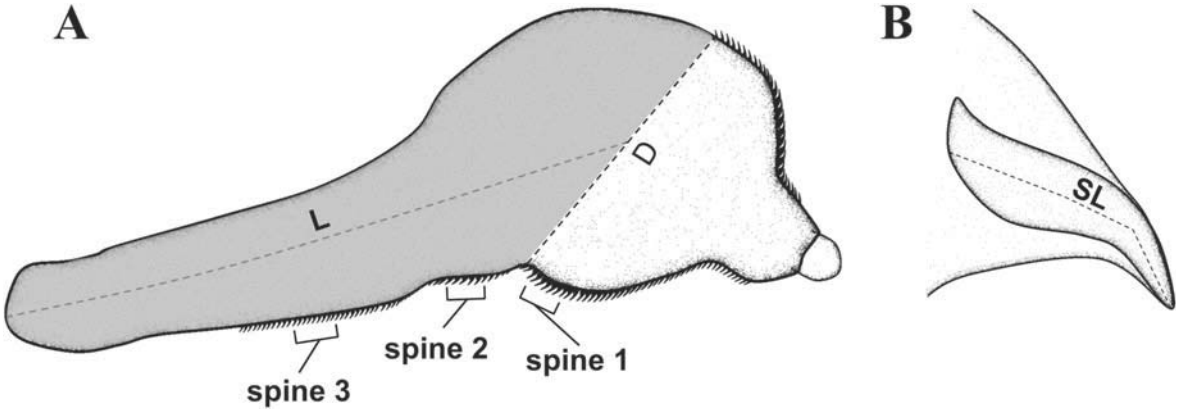

Fig. 2. Morphometric measurements taken in specimens of *Corynosoma cetaceum* and *C. australe*. L, trunk length; D, disk diameter; SL, spine length. The shadowed area is sectional area. Regions where spines were measured are also indicated (see Materials and Methods section for details).

of an acanthocephalan. Second, we investigated the factors that may account for patterns of spine growth, including body size.

MATERIALS AND METHODS

Data collection

Specimens of *Corynosoma cetaceum* were collected in several localities along the coast of Argentina. Cystacanths (20 females and 26 males) were obtained from the mesentery of 2 individuals of Argentine sandperch *Pseudopercis semifasciata* in the neighbourhood of Península Valdés (42°00′ – 42°45′S). Adults (43 females and 42 males) were collected from the pyloric stomach of 5 franciscana dolphins, *Pontoporia blainvillei*, that were found drowned in shark fishery gillnets in Necochea (38°27′S, 58°50′W) and Claromecó (38°52′S, 60°05′W). Sampling of *Corynosoma australe* was conducted in the north coast of Patagonia (42°45′S, 62°30′W): cystacanths (33 females and 24 males) were collected from the mesentery of 11 individuals of the flounder *Paralichthys isosceles*, whereas adults (35 females and 35 males) were collected from the intestine of 3 South American sea lions, *Otaria flavescens* stranded on Patagonian beaches. Acanthocephalan specimens were generally washed in saline and fixed and conserved in 70% ethanol. Cystacanths of *C. cetaceum* were fixed in 4% buffered formaldehyde and preserved in 70% ethanol. No significant morphometric differences were found between cystacanths fixed in ethanol or formaldehyde (MANOVA, $P >> 0{\cdot}05$).

Acanthocephalans were examined under a stereomicroscope (X100) and identified following the taxonomic criteria of Aznar *et al*. (1999*b*) and Sardella *et al*. (2005). Then, each specimen was drawn in profile with the aid of a drawing tube (Fig. 2). Trunk length (L) and disk diameter (D) were measured using homologous landmarks that were unaffected by the degree of fore-trunk invagination (Fig. 2). Four body size variables directly related to attachment performance were obtained from each specimen as follows. (1) Disk area. In species of *Corynosoma*, the disk covered with spines is used as a key attachment device (Van Cleave, 1952; Aznar *et al*. 1999*a*, 2006). The disk surface is roughly circular, thus its area was estimated as the area of a circle. (2) Sectional area (Fig. 2). This variable is related to pressure drag (Koehl, 1984). To obtain it, the drawing in profile of each specimen was scanned and the area was calculated using Image Tool v. 3.0 (UTHSCSA). (3) Surface area. This variable is related to skin friction drag (Koehl, 1984). The body of species of *Corynosoma* can faithfully be reproduced just by bending a cone (Aznar *et al*. *unpublished data*; see Fig. 1). Therefore, surface area can be approximated using the formula for a cone surface, without considering the area of the disk (the disk is attached to the intestine so it is not exposed to drag). (4) Body volume. This variable is related to 'virtual buoyancy', a lifting force proportional to the mass of fluid displaced by the body (Koehl, 1984). Volume was calculated assuming a conical body shape.

To measure spines, each specimen was cut with a razor blade through the sagittal plane and one half was temporarily mounted on a slide with lactic acid to clear the tegument. Using this procedure, specimens could be re-accommodated, if necessary, for spines to be drawn in profile minimizing tilt-related error. Three spines were drawn under a light microscope (X1000) from each of the 3 sites indicated in Fig. 2 i.e. the disk border, the interfold area, and the posterior hind-trunk (see Aznar *et al*. 2002*a* for details). For brevity, we will refer to the spines from these sites as Spines 1, 2 and 3, respectively. Spine length was measured as indicated in Fig. 2, and the values taken from 3 spines randomly selected from each site were averaged to obtain a single value per site and specimen.

Statistical analyses

A preliminary analysis indicated that the factor 'host individual' did not have a significant effect on average values of morphometric variables either in paratenic or definitive hosts (MANOVA, $P > 0{\cdot}05$ in all 4 tests), thus, this factor was not considered in further analyses.

The effect of developmental stage, sex, and species on body size variables was examined with MANOVA, using disk area, sectional area, surface area and volume as dependent variables. The 3 factors were considered as fixed. Concerning the 'species' effect, we were specifically interested in the interaction of 'species' with 'developmental stage' and 'sex' because this analysis allowed investigation of whether patterns of body growth differed between species, a point that was relevant for the interspecific differences observed in spine growth (see the Results section).

Multivariate analysis of covariance (MANCOVA) was used to examine patterns of spine growth within each species. Values of Spines 1, 2 and 3 were treated as dependent variables and 'developmental stage' and 'sex' as fixed factors. In addition, we used principal component analysis on the 4 body variables to obtain scores on the first axis (PC1) i.e. a multivariate measure of body size (Klingerberg, 1996). The scores in PC1 were then included in the model as a co-variate. The inclusion of PC1 is pertinent to explore the relationships between static and ontogenetic allometry in spine size growth (see Klingerberg, 1996). Static allometry results from co-variation between morphometric traits among individuals of the same age or developmental stage; in our case cystacanths or adults (Fig. 3A). Ontogenetic allometry deals with co-variation between morphometric traits during growth i.e. the population of cystacanths and adults considered as a whole (Fig. 3A). Both allometric patterns are usually, but not necessarily, similar (Cock, 1966; Klingerberg, 1996). In our model, the way to compare allometric patterns was by examining the interaction between PC1 and developmental stage: if the interaction was significant, this would mean that static and ontogenetic allometries did not coincide. In other words, the relationship between body size and spine size would differ between cystacanths and adults, thus indicating changes in relative growth rate during the adult development in the definitive host (Fig. 3B, C). When interaction terms with the co-variate were not significant, they were removed from models to increase the sensitivity of the analysis and to correctly interpret main effects (Engqvist, 2005).

MANCOVA models were also used to explore whether variability in spine size within sites (i.e. the disk border, the interfold area, and the posterior hind-trunk) differed between sexes and developmental stages; PC1 was used as a co-variate. For each specimen, the coefficient of variation (CV) of each set of 3 spines was calculated (i.e. for Spines 1, 2 and 3). These CVs were treated as dependent variables in the MANCOVA models.

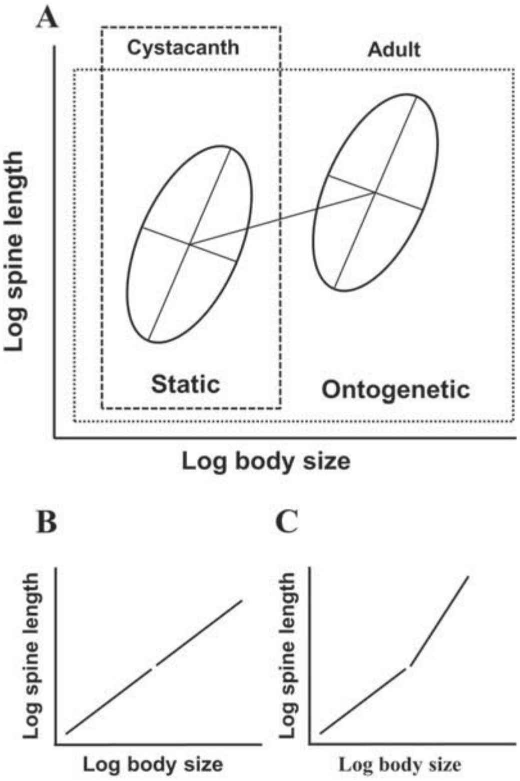

Fig. 3. Theoretical relationships between static and ontogenetic allometry. (A) Levels of co-variation between spine length and body size in 2 developmental stages of an acanthocephalan i.e. cystacanth and adult (redrawn from Klingenberg, 1996). Static allometry (dashed rectangle) refers to co-variation among individuals of the same developmental stage (e.g. cystacanth). Ontogenetic allometry (dotted rectangle) refers to co-variation due to growth from the cystacanth to the adult stage. (B) Hypothetical relationship between static and ontogenetic allometry in which relative growth rate do not change between the cystacanth and the adult stage. (C) Hypothetical relationship between static and ontogenetic allometry in which both levels of allometry differ because the relative growth of spines changes during the adult development.

Statistical analyses were carried out with SPSS v. 17. Statistical significance was set at $P < 0{\cdot}05$.

RESULTS

Patterns of body growth

Data on morphometric variables are shown in Table 1. In *C. cetaceum*, highly significant differences were found in body dimensions, not only between developmental stages, but also between sexes. Also, a highly significant interaction 'developmental stage * sex' was observed (Table 2). Univariate ANOVAs revealed that disk area did not differ

Table 1. Mean values (S.D.) [Coefficient of Variation] of body dimensions and length of trunk spines in cystacanth and adult specimens of the acanthocephalans *Corynosoma cetaceum* and *C. australe*

(Measurements in mm (trunk length and disk diameter), mm^2 (disk, sectional and surface areas), mm^3 (volume) and μm (spine length).)

			Body dimensions						Spine length		
Species	Group	*N*	Trunk length	Disk diameter	Disk area	Sectional area	Surface area	Volume	Spine 1	Spine 2	Spine 3
C. cetaceum	Cystacanth female	20	1·98 (0·23) [0·12]	1·43 (0·15) [0·10]	1·63 (0·32) [0·20]	1·58 (0·36) [0·23]	3·15 (0·60) [0·19]	1·09 (0·28) [0·26]	60·0 (5·5) [0·09]	53·5 (5·1) [0·09]	49·8 (4·2) [0·08]
	Adult female	43	2·86 (0·37) [0·13]	2·20 (0·36) [0·16]	3·91 (1·32) [0·34]	4·18 (1·20) [0·28]	6·90 (1·93) [0·28]	3·87 (1·85) [0·48]	66·5 (4·6) [0·07]	63·4 (5·5) [0·09]	56·0 (5·8) [0·10]
	Cystacanth male	26	2·55 (0·42) [0·16]	1·33 (0·15) [0·12]	1·42 (0·33) [0·23]	1·96 (0·45) [0·23]	4·15 (0·97) [0·23]	1·21 (0·37 [0·30])	60·1 (3·1) [0·05]	55·1 (3·4) [0·06]	50·6 (3·6) [0·07]
	Adult male	42	5·02 (1·01) [0·20]	2·21 (0·35) [0·16]	3·91 (1·25) [0·32]	5·34 (2·10) [0·40]	14·4 (5·22) [0·36]	6·93 (3·54) [0·51]	59·2 (5·3) [0·09]	54·6 (5·8) [0·11]	49·7 (5·5) [0·11]
C. australe	Cystacanth female	35	1·51 (0·27) [0·17]	0·91 (0·14) [0·16]	0·67 (0·19) [0·30]	0·69 (0·14) [0·20]	1·61 (0·42) [0·26]	0·73 (0·18) [0·24]	46·6 (3·7) [0·09]	37·8 (3·3) [0·09]	40·5 (3·8) [0·10]
	Adult female	34	1·99 (0·31) [0·17]	1·33 (1·17) [0·15]	1·40 (0·33) [0·30]	1·47 (0·40) [0·27]	3·03 (0·80) [0·29]	1·40 (0·35) [0·28]	49·1 (3·0) [0·08]	37·8 (3·3) [0·11]	40·4 (4·0) [0·11]
	Cystacanth male	23	1·49 (0·20) [0·13]	0·95 (0·08) [0·09]	0·70 (0·13) [0·19]	0·66 (0·11) [0·16]	1·62 (0·30) [0·19]	0·74 (0·12) [0·17]	47·0 (2·9) [0·06]	36·3 (2·5) [0·07]	38·3 (3·5) [0·13]
	Adult male	33	2·06 (0·38) [0·19]	1·30 (0·20) [0·16]	1·36 (0·40) [0·30]	1·39 (0·43) [0·33]	3·15 (0·99) [0·33]	1·43 (0·44) [0·32]	47·8 (3·0) [0·09]	36·0 (2·5) [0·07]	37·1 (2·3) [0·06]

Table 2. Results from a multivariate analysis of variance that examines the effects of sex and developmental stage (cystacanth and adult) on 4 body variables i.e. disk area, sectional area, surface area and volume in the acanthocephalans *Corynosoma cetaceum* and *C. australe*

(Statistically significant effects are in bold.)

Factor	D.F.	Wilks' lambda	F	*P*
C. cetaceum				
Stage	4	0·267	85·134	**<0·001**
Sex	4	0·201	123·256	**<0·001**
Stage * Sex	4	0·536	26·829	**<0·001**
Error	124			
C. australe				
Stage	4	0·318	63·152	**<0·001**
Sex	4	0·863	4·674	**0·002**
Stage * Sex	4	0·962	1·157	0·333
Error	118			

between sexes ($F_{(1,127)}=1{\cdot}864$, $P=0{\cdot}175$), but males had a significantly larger sectional area ($F_{(1,127)}=14{\cdot}427$, $P<0{\cdot}001$), surface area ($F_{(1,127)}=81{\cdot}202$, $P<0{\cdot}001$) and body volume ($F_{(1,127)}=17{\cdot}469$, $P<0{\cdot}001$) than females (Fig. 4). Significant univariate differences concerned surface area and volume (interaction 'developmental stage * sex': surface area, $F_{(1,127)}=16{\cdot}607$, $P<0{\cdot}001$; body volume, $F_{(1,127)}=8{\cdot}287$, $P<0{\cdot}005$). These variables grew comparatively faster in males than in females (Fig. 4). In *C. australe*, significant differences in body dimensions were also found between developmental stages and sexes (Table 2). However, sexual dimorphism was slight because none of the univariate ANOVAs was found to be significant (minimum nominal $P=0{\cdot}221$) (Fig. 4). Also, the interaction 'developmental stage * sex'was not significant (Table 2).

As an interspecific comparison, we tested whether the relative amount of growth from cystacanth to adult differed between *C. cetaceum* and *C. australe*. In males, the multivariate interaction 'developmental stage * species' was highly significant (Wilks' Lambda $=0{\cdot}279$, $F_{(4,117)}=75{\cdot}556$, $P<0{\cdot}001$), as were interactions of these factors for each dependent variable ($P<0{\cdot}001$). Average relative size of adult males compared to cystacanths was as follows (*C. cetaceum vs C. australe*): disk area: 175% *vs* 94%; sectional area: 172% *vs* 111%; surface area: 247% *vs* 94%; and volume: 473% *vs* 93% (see the Table 1). In females, a highly significant interaction 'developmental stage * species' was also detected (Wilks' Lambda $=0{\cdot}354$, $F_{(4,125)}=57{\cdot}031$, $P<0{\cdot}001$), but significant univariate differences concerned sectional area and volume only (sectional area, $F_{(1,128)}=6{\cdot}500$, $P<0{\cdot}012$; volume, $F_{(1,128)}=22{\cdot}603$, $P<0{\cdot}001$). Sectional area and volume in adult females of *C. cetaceum* increased 164% and 255%, respectively, compared to cystacanths; however, in *C. australe*, these figures were just 113% and 91% (see the Table 1). In summary, during adult development individuals of *C. cetaceum* grew comparatively more than those of *C. australe*.

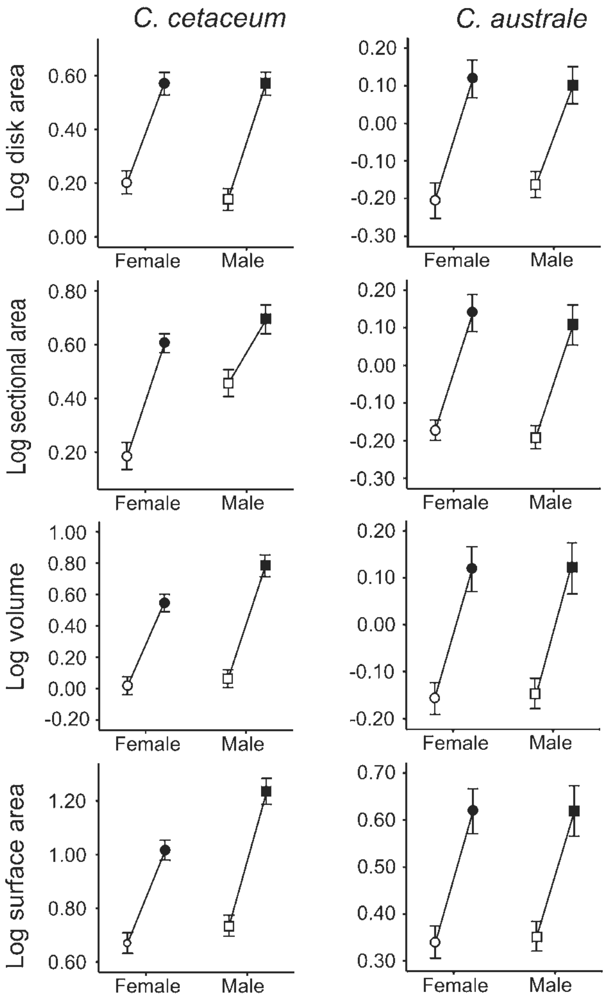

Fig. 4. Mean values (bars: standard error) of 4 body size variables in cystacanths (open symbols) and adults (solid symbols) of individuals from each sex of *Corynosoma cetaceum* and *C. australe*.

Patterns of spine growth

Individuals of *C. cetaceum* had larger spines than those of *C. australe* regardless of developmental stage and sex (Fig. 5; see also the Table 1).

In *C. cetaceum*, spine length significantly differed between developmental stages and sexes, and the overall relationship between spine size and body size was not significant (Table 3). However, a highly significant interaction 'developmental stage * sex' was found and, therefore, analyses were carried out for each sex separately to tear apart the effects of developmental stage (ontogenetic allometry) and body size (static allometry). In females, the full factorial MANCOVA indicated that adults had longer spines than cystacanths (Fig. 5A) but neither

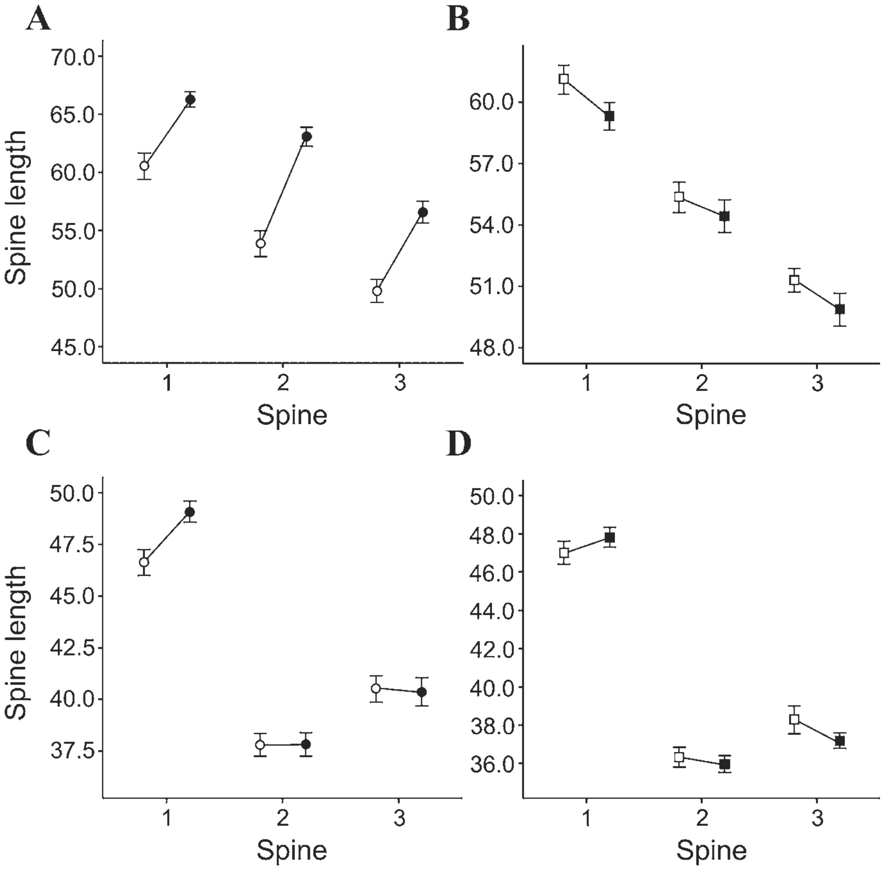

Fig. 5. Mean length (bar: standard error) of spines measured at 3 sites (see Fig. 2) in cystacanths (open symbols) and adults (solid symbols) of both sexes in 2 species of *Corynosoma*. (A) Female *C. cetaceum*; (B) Male *C. cetaceum*; (C) Female *C. australe*; (D) male *C. australe*.

the overall effect of PC1 on spine size nor the interaction 'developmental stage *PC1' were significant (Table 3). After removing the interaction term, a significant main effect of PC1 was found (Table 3). Univariate ANOVAs indicated that PC1 correlated significantly ($P < 0{\cdot}05$) with spine length only in Spines 2 and 3 (Fig. 6). In males of *C. cetaceum*, there was no significant difference in spine length between cystacanths and adults, nor was there any indication of a significant relationship between body size and spine size in cystacanths or adults (Table 3, Fig. 5B). In summary, (1) females of *C. cetaceum* had longer spines than males; (2) all spines were longer in adults, but only in females, and (3) there was a significant relationship between spine length and body size only in females (both cystacanths and adults), and only for Spines 2 and 3 (hind-trunk spines).

In *C. australe*, spine length significantly differed between both developmental stages and sexes (Table 4). Again, a significant interaction 'developmental stage * sex' was found and, therefore, separate analyses were performed for each sex. In females, spine length differed between cystacanths and adults (Table 4); the univariate ANOVAs revealed that only Spine 1 was significantly larger in adults ($P = 0{\cdot}003$) (Fig. 5C). However, the effect of PC1 on spine size was not significant, even after removing the interaction 'developmental stage *PC1' in the model (Table 4). In males, none of the predictors of spine length was significant in any model (Table 4; Fig. 5D). In summary, (1) females of *C. australe* had longer spines than males; (2) disk spines (Spine 1) were longer in adults than in cystacanths, but only in females, and (3) there was no significant pattern of static allometry between spine length and body size in either sex or developmental stage.

None of the MANCOVA models for each species involving CVs of Spines 1, 2 and 3 revealed significant effects of sex or developmental stage on spine variability; an overall MANOVA using 'species' as a single factor also did not(results not shown).

DISCUSSION

Results from this study provide, for the first time, statistical evidence that trunk spines of 2 species of acanthocephalan grow during the worm development in the definitive host. Unexpectedly, spines appear to grow only in females and exhibit a different pattern of growth depending on the species. A preliminary question that must be addressed is whether there are

Table 3. Models of multivariate analysis of covariance that examine the effects of developmental stage (cystacanth and adult), sex, and a multivariate measure of body size (PC1, the first principal component of the 4 morphometric variables indicated in Table 1) on the length of trunk spines from 3 sites in the acanthocephalan *Corynosoma cetaceum*

(Statistically significant effects are in bold.)

Factor	D.F.	Wilks' lambda	F	*P*
Full factorial model				
Stage	3	0·832	8·119	**< 0·001**
Sex	3	0·843	7·496	**< 0·001**
PC1	3	0·970	1·253	0·294
Stage * Sex	3	0·742	14·040	**< 0·001**
Stage * PC1	3	0·986	0·552	0·647
Sex * PCA	3	0·985	0·634	0·595
Stage * Sex * PC1	3	0·968	1·329	0·268
Error	121			
Females				
Full factorial model				
Stage	3	0·478	20·745	**< 0·001**
PC1	3	0·924	1·566	0·208
Stage * PC1	3	0·936	1·301	0·283
Error	157			
Main effects model				
Stage	3	0·484	20·639	**< 0·001**
PC1	3	0·854	3·151	**0·031**
Error	58			
Males				
Full factorial model				
Stage	3	0·962	0·806	0·495
PC1	3	0·987	0·267	0·849
Stage * PC1	3	0·974	0·550	0·650
Error	62			
Main effects model				
Stage	3	0·963	0·803	0·497
PC1	3	0·978	0·483	0·697
Error	63			

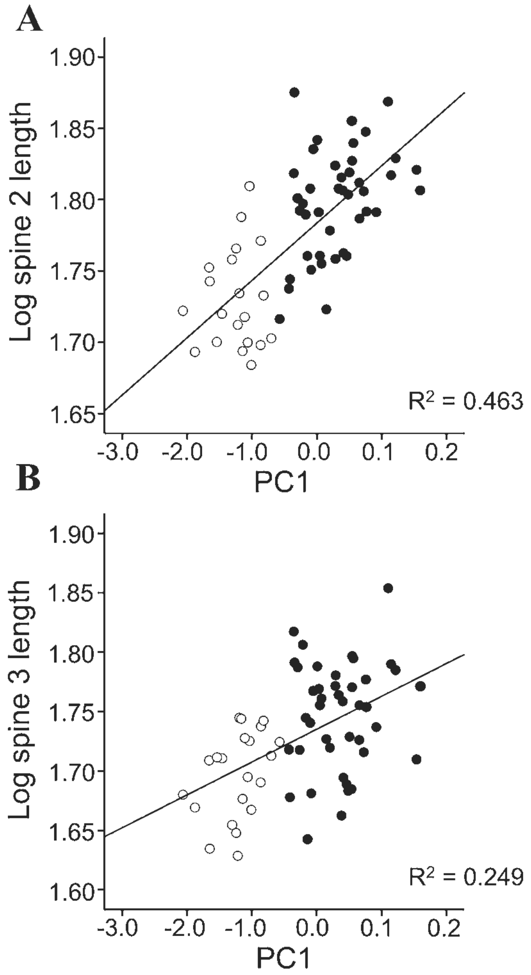

Fig. 6. Regression lines of spine length on the first principal component of 4 body variables (as indicated in Table 2) in cystacanth (open dots) and adult (solid dots) females of *Corynosoma cetaceum*. (A) Spine 2; (B) Spine 3 (see Fig. 2 for location of these spines on the body).

sampling and/or measurement artifacts that could confound these results. First, cystacanths and adults of *C. cetaceum* could not be sampled in the same locality, but in places 600 km apart. Since there is evidence of morphological divergence between populations of *C. cetaceum* from South America and Australia (Aznar *et al.* 1999*b*), perhaps some degree of divergence might also occur at the geographical scale covered in our study, thus potentially affecting the morphometrical comparison between developmental stages. This does not appear to be the case because the morphology of all specimens of *C. cetaceum* thus far collected along the coast of southwestern Atlantic from Uruguay to Patagonia is very uniform (Aznar *et al.* 1999*b*, 2002*b*). Second, spines of *C. australe* were clearly smaller than those of *C. cetaceum*, and small structures may exhibit greater levels of variability just because their measurement is less precise (see Aznar *et al.* 2002*a*). Although all spines had been measured at the same magnification regardless of species, coefficients of variation were very similar between *C. australe* and *C. cetaceum*. Therefore, the smaller size effect that was observed for spine growth in *C. australe* could hardly be accounted for by higher measurement error.

According to our results, both females and males of *C. australe* are roughly equal in size and grow at a similar rate from the cystacanth to the adult stage, whereas females of *C. cetaceum* are clearly smaller and grow less than males. However, females of both species have longer spines, and only in females do spines grow significantly during the adult development. Therefore, spine growth does not seem to follow simple allometric rules, nor does it conform to simple biomechanical principles i.e. females are not predicted to suffer stronger dislodgment forces than males according to their body size (Koehl, 1984; Poulin, 2007, 2009). So why do spines grow only in females? One hypothesis is that males require no further growth of spines beyond the cystacanth stage

Table 4. Models of multivariate analysis of covariance that examine the effects of developmental stage (cystacanth and adult), sex, and a multivariate measure of body size (PC1, the first principal component of the 4 morphometric variables indicated in Table 1) on the length of trunk spines from 3 sites in the acanthocephalan *Corynosoma australe*

(Statistically significant effects are in bold.)

Factor	D.F.	Wilks' lambda	F	*P*
Full factorial model				
Stage	3	3·429	3·429	**0·020**
Sex	3	0·843	6·733	**<0·001**
PC1	3	0·770	0·770	0·513
Stage * Sex	3	0·875	3·532	**0·017**
Stage * PC1	3	0·996	0·152	0·557
Sex * PC1	3	0·982	0·695	0·927
Stage * Sex * PC1	3	0·986	0·539	0·657
Error	114			
Females				
Full factorial model				
Stage	3	0·864	3·246	**0·028**
PC1	3	0·960	0·859	0·467
Stage * PC1	3	0·980	0·416	0·742
Error	62			
Main effects model				
Stage	3	0·865	3·267	**0·027**
PC1	3	0·955	0·996	0·400
Error	63			
Males				
Full factorial model				
Stage	3	0·941	1·042	0·382
PC1	3	0·964	0·631	0·598
Stage * PC1	3	0·984	0·265	0·850
Error	62			
Main effects model				
Stage	3	0·941	1·062	0·373
PC1	3	0·958	0·741	0·533
Error	63			

because they develop other attachment devices (i.e. the proboscis, the disk) more than females during late ontogeny. We could not provide an overall test for this hypothesis because most adult specimens had an invaginated proboscis. However, our results clearly indicate that the area of the attachment disk does not differ between sexes. Also, information obtained from other datasets indicate that, in both species of *Corynosoma*, the proboscis and hooks are significantly smaller in adult males, and the field of spines covers a roughly similar extension of the trunk in both sexes (Hernández-Orts *et al. unpublished data*; see also Aznar *et al.* 1999*b*; Sardella *et al.* 2005). Thus, adult males appear to have a less-developed holdfast than females.

A second hypothesis would suggest that factors other than body size exert stronger overall selective pressures on females to develop more efficient attachment devices, including spines. In this context, Petrochenko (1956) argued that adult females of acanthocephalans need to develop larger attachment structures than males because they must stay in the definitive host for longer to produce and release the eggs. Following this argument, the larger size of spines could be viewed as an adaptation of females to reduce the likelihood of being ripped loose by peristaltic movements and passing food (see Poulin, 2009). Females would also require a fine-tuned adjustment of the spine size to the specific micro-habitat conditions they encounter during the adult development. Note that the latter strategy is not unusual: after recruitment to the definitive host, females, but not males, of the polymorphid *Filicollis anatis* inflate the anchored proboscis as a device that obviously improves attachment performance (Van Cleave, 1952; Petrochenko, 1958).

The hypothesis mentioned above is supported by 2 lines of evidence. First, females of *C. cetaceum* and *C. australe* appear to have indeed a longer lifespan than males, as indicated by the strongly female-biased sex ratios observed in the definitive host (Aznar *et al.* 2001, 2004). A longer lifespan of females has also been recorded in other species of *Corynosoma* using controlled infections in experimental hosts (Valtonen and Helle, 1982; Castro and Martínez, 2004). Unfortunately, we lack direct quantitative data from natural hosts, although information obtained from an allied species of comparable size, *Polymorphus minutus*, suggests that the lifespan of females could be at least 1·5-fold than that of males (see data from Crompton and Whitfield, 1968).

Second, it is likely that lifespan differences between sexes may have a selective impact on attachment devices because carnivorous marine mammals are hosts that impose very harsh conditions for a gut-dwelling helminth (Petrochenko, 1956). Both cetaceans and pinnipeds have higher metabolic rates than terrestrial mammals of comparable size (Williams *et al.* 2001), and high metabolic rates are often associated with high rates of food intake and short transit times of food along the gut (Karasov and Diamond, 1985). With regard to food intake, carnivorous marine mammals need to feed often (Kastelein *et al.* 1997*a*), and on prey that are patchily distributed in the environment, so that large quantities of food are consumed when the occasion arises (Gaskin, 1978; Williams *et al.* 2001). Accordingly, acanthocephalans must suffer the frequent but unpredictable passing of a great amount of digested food. On the other hand, marine mammals have comparatively long alimentary tracts associated with their elevated metabolic rates (Williams *et al.* 2001), but the transit time of food is generally shorter than that of terrestrial mammals of similar size (Kastelein *et al.* 1997*b*; Hall-Aspland *et al.* 2011). Therefore, the flow of digesta must be, not only frequent, but fast. In summary, we believe that the need to withstand extreme flow conditions for periods of different extent might have driven a different investment and development schedule of holdfast structures in males and females of *C. cetaceum* and *C. australe*.

Another non-exclusive hypothesis is also compatible with the observed sexual differences in investment and development schedule of spines in species of *Corynosoma* i.e. males and females differ in sexual behaviour. The mating system of acanthocephalans appears to be polygamous; males have a more active role in copulation than females, seeking and mating with several females (Parshad and Crompton, 1981). In the intestine of Saimaa ringed seals (*Phoca hispida saimensis*), Sinisalo *et al.* (2004) found evidence of significant competition between males of *Corynosoma magdaleni* for the access to females, with large-sized males firstly approaching non-mated females. Therefore, sexual selection could favour strong, permanent attachment in females of *Corynosoma*, but only short-term attachment in males as they need to move in search of mates.

Our study also indicates that patterns of spine growth differ between females of each species of *Corynosoma*. Attempting to infer adaptation in this 2-species comparison inevitably involves the confounding of independent variables (Garland and Adolph, 1994). In other words, each species lives within a different species of host and selects a different microhabitat and, therefore, each species is subject to different ecological regimes, including the degree of physical disturbance and food availability, which have never been quantified in the system under study. Therefore, we have no reasonable clue about the actual factors that account for the differences in morphology and growth patterns between species. Nonetheless, it is worth noting that spines on the disk border are the ones that grow in both species. Perhaps this is not surprising because the disk is a major attachment device in *Corynosoma* (Van Cleave, 1952), with the disk border exerting a wedge-like force against the host tissue (Aznar *et al.* 1999*a*). In contrast, hind-trunk spines are apparently used only as a secondary holdfast (Aznar *et al.* 2002). On the other hand, it seems clear that females of *C. cetaceum* fine-tune the size of spines during the development in the definitive host more than *C. australe*. All else being equal, this might be adaptive because (i) the relative increment in volume from cystacanth to adult in *C. cetaceum* is almost 3-fold that of *C. australe*, and (ii) females of *C. cetaceum* also achieve a larger adult size, a trait that correlates with stronger dislodging forces (Koehl, 1984; Poulin, 2007, 2009) and, possibly, with a longer lifespan (see Sorci *et al.* 1997).

Rather surprisingly, we found no significant patterns of static allometry between body size and the size of spines on the disk border in females of either *C. cetaceum* or *C. australe*. However, co-variation was significant for hind-trunk spines in females of *C. cetaceum*. This suggests that the final size of spines may or may not match the adult body size achieved by each individual worm depending on the body region where the spine grows. Again, it seems premature to speculate on the reasons for these differences as we lack information about the factors that control spine morphogenesis (Aznar *et al.* 2002), and the specific attachment performance of disk or hind trunk spines (see Koehl, 1996). It should be pointed out, however, that narrow co-variation between spine size and final body size must not functionally be required if slight increases in spine size suffice for secure attachment within a range of body sizes (see Poulin, 2009).

In conclusion, this study sheds light on the question regarding whether or not the holdfast of acanthocephalans is fully developed prior to entering the definitive host. In particular, it suggests that temporal allocation of investment in attachment structures may differ, not only between congeneric species, but also between sexes of the same species, possibly due to the different selective pressures that each population subset faces. Future studies should address whether life span and body size are also relevant factors affecting development of other attachment structures (e.g. the proboscis) in a multi-species context.

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8. DESCRIPTION, MICROHABITAT SELECTION AND INFECTION PATTERNS OF SEALWORM LARVAE (*PSEUDOTERRANOVA DECIPIENS* SPECIES COMPLEX, NEMATODA: ASCARIDOIDEA) IN FISH FROM PATAGONIA, ARGENTINA

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CHAPTER 8

Description, microhabitat selection and infection patterns of sealworm larvae (*Pseudoterranova decipiens* species complex, Nematoda: Ascaridoidea) in fish from Patagonia, Argentina

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ABSTRACT

Twenty fish species from the Patagonian coast of Argentina were examined for sealworm larvae (*Pseudoterranova decipiens* species complex, Nematoda: Ascaridoidea). A total of 635 encapsulated sealworm larvae were collected from 12 fish species. The most infected fish species was *Prionotus nudigula*, followed by *Acanthistius patachonicus*, *Paralichthys isosceles*, *Percophis brasiliensis* and *Pseudopercis semifasciata*. Sequences obtained for the mitochondrial cytochrome c oxidase subunit 1 gene (cox 1) of sealworms from *P. nudigula* formed a reciprocally monophyletic lineage with published sequences of adult specimens of *Pseudoterranova cattani* from the South American sea lion *Otaria flavescens.* A detailed morphological description, including drawings and scanning electron microscopy photomicrographs of the third stage larvae of *P. cattani* is provided. On the other hand, sealworm larvae collected from other fish species did not differ in their diagnostic traits from the larvae of *P. cattani.* However a discriminant analysis suggests that specimens from *P. nudigula* were significantly larger than those from other fish. We provisionally identified these larvae as *P.* cf. *cattani*, pending further molecular studies. In the Patagonian coast, most of the sealworms were collected from the muscles (mainly in the epaxial musculature of *P. nudigula*) and, to a lesser degree, in the mesenteries and liver. In this study, we also investigated some aspects of the life cycle of sealworms in the Patagonian coast. Interestingly our results suggest that the most important fish prey for South American sea lion (definitive host) inhabiting this area presented relatively low infection levels of sealworm larvae. Therefore, transmission and survival of these nematodes to South American sea lions seems to be ensured by the high ingestion of slightly infected fish prey and, to a lesser extent, by occasionally foraging on small benthic fish with high prevalences, or on large demersal fish with high intensities of sealworm larvae.

Keywords

Anisakidae, sealworms, taxonomy, ecology, cox 1, marine fish, southwestern Atlantic

8.1. INTRODUCTION

Anisakid nematodes belonging to the *Pseudoterranova decipiens* species complex (also known as sealworms or codworms) mature and reproduce in the digestive tract of pinnipeds (Lauckner, 1985; Mattiucci & Nascetti, 2007, 2008). As far as it is known, the life cycle of species of *Pseudoterranova* also includes crustaceans as the first hosts, and fish as second hosts. The *P. decipiens* complex is composed of 6 sibling species, with 4 species occurring in the Northern Hemisphere, namely, *P. azarasi* (Yamaguti and Arima, 1942), *P. bulbosa* (Cobb, 1888), *P. decipiens sensu stricto* (*s.s.*) (Krabbe, 1868) and *P. krabbei* Paggi, Mattiucci, Gibson, Berland, Nascetti, Cianchi and Bullini, 2000; and 2 species in the Southern Hemisphere: *P. cattani* George-Nascimento and Urrutia, 2000, and *P. decipiens* E of Bullini, Arduino, Cianchi, Nascetti, D'Amelio, Mattiuci, Paggi, Orecchia, Plötz, Berland, Smith and Brattey, 1997 (Bullini *et al.*, 1997; George-Nascimento & Urrutia, 2000; Gibson, 1983; Mattiucci & Nascetti, 2008; Mattiucci *et al.*, 1998; Paggi *et al.*, 2000).

The third stage larvae (L3) of sealworms have commonly been reported in marine teleosts worldwide (George-Nascimento, 1987; Mattiucci & Nascetti, 2008; McClelland *et al.*, 1990; Palm *et al.*, 1994). Just along the South America coasts, sealworm larvae have been reported in at least 40 species of marine fish belonging to 21 families and 10 orders (see **Table 11**). In this region, sealworm larvae infect the flesh of economically important fishes (*e.g.* Herreras *et al.*, 2000; Oliva *et al.*, 1996; Torres *et al.*, 2000) and cause zoonotic diseases when humans consume raw or undercooked fish (Cabrera *et al.*, 2003; Torres *et al.*, 2007).

Table 11. Check list of records of third stage larvae of *Pseudoterranova* spp. in fish from South America. Abbreviations: Bc, body cavity; Li, liver; MA, host muscles analysed for parasites; MH, microhabitat; Me, mesenteries; MS, mean number of sealworms; Mu, muscle; N.S., not specified; NI, number of infected hosts; TL, total length ± standard deviation (or range).

Order/Family	**Species**	**Locality***	***n***	**TL (cm)**	**MA**	**MH**	**NI**	**MS**	**References**
Anguilliformes									
Congridae	*Conger orbignianus*	North Argentina^{a}	50	–	Yes	Me	1	1	Timi & Lanfranchi (2013)
Atheriniformes									
Atherinopsidae	*Odontesthes nigricans*	Patagonia, Argentina^{a}	125	(16.3–16.6)**	No	Bc	–	–	Carballo *et al.* (2011)
	Odontesthes smitti	Patagonia, Argentina^{a}	118	(21.7–28.3)**	No	Bc	1	10	Carballo *et al.* (2012)
Batrachoidiformes									
Batrachoididae	*Aphos porosus*	Central Chile^{b}	8	28.3 ± 1.5	N.S.	–	6	10	Torres *et al.* (1993)
		Central Chile^{a}	30	26.8 ± 3.9	Yes	Bc, Me, Mu	24	128	Cortés & Muñoz (2008)
		South Chile^{c}	13	–	Yes	Mu	6	10	Torres *et al.* (1983)
	Porichthys porosissimus	North Argentina^{a}	166	(11.5–29.7)	No	Bc	26	39	Tanzola *et al.* (1997)
Beloniformes									
Belonidae	*Tylosurus acus acus*	Brazil^{a}	31	(58.0–105.5)	N.S.	Me	3	4	Tavares *et al.* (2004)
Gadiformes									
Gadidae	*Micromesistius australis*	South Chile^{a}	61	(26.0–57.0)	N.S.	–	3	4	Chávez *et al.* (2012)
Macrouridae	*Nezumia pulchella*	North Chile^{a}	167	(26.6–32.1)**	No	–	2	2	Salinas *et al.* (2008)
		Central Chile^{a}	50	31.1 ± 2.3	No	–	1	1	Salinas *et al.* (2008)
Merlucciidae	*Macruronus magellanicus*	South Chile^{c}	288	–	Yes	Mu	9	29	Torres *et al.* (1983)
		Central Chile^{b}	3	134.3 ± 55.7	N.S.	–	1	1	Torres *et al.* (1993)
		South Chile^{b}	4	(22.0–28.0)	Yes	Mu	1	1	Torres *et al.* (2000)
	Merluccius australis	South Chile^{b}	685	(45.0–95.0)	No	Bc, Me, Mu	76	99	George-Nascimento & Arancibia (1994)
	Merluccius gayi gayi	Central Chile^{b}	1051	(42.7–46.6)**	No	Me	211	897	George-Nascimento (1996)
		South Chile^{b}	121	45.4 ± 10.1	No	Me	42	213	George-Nascimento (1996)
		South Chile^{c}	34	–	Yes	Mu	19	71	Torres *et al.* (1983)
		South Chile^{b}	17	(36.0–50.0)	Yes	Mu	4	8	Torres *et al.* (2000)

Table 11. continued.

Order/Family	Species	Locality*	*n*	TL (cm)	MA	MH	NI	MS	References
	Merluccius hubbsi	Patagonia, Argentina[a]	278	(38.7–40.4)**	No	Me	2	2	Sardella & Timi (2004)
		Patagonia, Argentina[a]	42	(57.0–78.0)	Yes	Mu	4	8	Herreras *et al.* (2000)
Mugiliformes									
Mugilidae	*Mugil cephalus*	Central Chile[c]	107	(15.5–46.0)	No	Hi	1	1	Fernández (1987)
Ophidiiformes									
Ophidiidae	*Genypertus* sp.	South Chile[c]	1	60	N.S.	Li, Me	–	–	Torres & González (1978)
	Genypterus blacodes	Argentina[b]	101	(24.0–127.0)	No	Me	4	20	Sardella *et al.* (1998)
		South Chile[c]	5	–	Yes	Mu	2	36	Torres *et al.* (1983)
	Genypterus brasiliensis	Patagonia, Argentina[b]	31	(40.0–75.0)	No	Me	6	8	Sardella *et al.* (1998)***
		Brazil[a]	55	29.0–70.0	N.S.	Me	3	5	Alves *et al.* (2002)***
		Brazil[b]	74	(41.5-93)	Yes	Me	4	5	Knoff *et al.* (2007)***
		Brazil[a]	74	(41.5-93)	Yes	Me	8	22	Knoff *et al.* (2007)***
	Genypterus chilensis	Central Chile[c]	80	(43.6-73.5)	Yes	Li, Mu	5	22	Vergara & George-Nascimento (1982)
		South Chile[c]	7	–	Yes	Mu	4	27	Torres *et al.* (1983)
		South Chile[b]	18	(41.0–55.0)	Yes	Mu	9	12	Torres *et al.* (2000)
	Raneya brasiliensis	Patagonia, Argentina[a]	107	24.5 ± 1.7	Yes	–	1	1	Vales *et al.* (2011)
Perciformes									
Blenniidae	*Hypsoblennius sordidus*	South Chile[a]	12	(4.8–10.2)	No	Mu	1	1	Sepúlveda *et al.* (2004)
Carangidae	*Caranx hippos*	Brazil[a]	60	(27.0–64.0)	N.S.	–	6	9	Luque & Alves (2001)
	Caranx latus	Brazil[a]	55	(26.0–43.0)	N.S.	–	18	401	Luque & Alves (2001)
	Trachurus murphyi	North Chile[b]	600	36.3 ± 2.9	No	–	2	3	George-Nascimento & Arancibia (1992)
		Central Chile[b]	600	33.9 ± 2.3	No	–	17	28	George-Nascimento & Arancibia (1992)
		South Chile[c]	35	–	Yes	Mu	10	60	Torres *et al.* (1983)
		South Chile[b]	16	(34.0–47.0)	Yes	Mu	5	12	Torres *et al.* (2000)
		Chile–Oceanic[b]	183	43.2 ± 2.1	No	–	3	3	George-Nascimento & Arancibia (1992)
Pinguipedidae	*Pinguipes brasilianus*	Patagonia, Argentina[a]	102	(32.7–37.1)**	No	Me	5	7	Timi *et al.* (2008)

Table 11. continued.

Order/Family	Species	Locality*	*n*	TL (cm)	MA	MH	NI	MS	References
	Pseudopercis semifasciata	North Argentina[a]	50	(67.5–71.2)**	No	Me	41	547	Timi & Lanfranchi (2009b)
		Patagonia, Argentina[a]	50	67.2 ± 7.2	No	Me	38	220	Timi & Lanfranchi (2009b)
Pomatomidae	*Pomatomus saltatrix*	Brazil[c]	55	(32.0–52.0)	N.S.	–	8	33	Luque & Chaves (1999)
Priacanthidae	*Priacanthus arenatus*	Brazil[a]	58	(14.0–54.0)	N.S.	–	5	23	Tavares *et al.* (2001)
Sciaenidae	*Cilus gilbert*	Central Chile[d]	57	(51.6–75.5)	No	–	36	153	Garcías *et al.* (2001)
		Central Chile[d]	29	(32.5–75.0)	No	–	23	139	Garcías *et al.* (2001)
		South Chile[c]	11	–	Yes	Mu	6	15	Torres *et al.* (1983)
	Micropogonias furnieri	Brazil[a]	100	(10.0–66.0)	N.S.	Me	2	3	Alves & Luque (2001)
Scombridae	*Scomber japonicus*	North Argentina[a]	173	(29.7–53.4)	No	Me	13	4	Cremonte & Sardella (1997)
		Brazil[c]	50	(20.0–25.0)	No	Me	5	–	Rego & Santos (1983)
Sparidae	*Pagrus pagrus*	Brazil[a]	90	(16.0–50.0)	N.S.	Me	6	15	Paraguassú *et al.* (2002)
Trichiuridae	*Trichiurus lepturus*	Brazil[a]	55	(108.0–148.0)	N.S.	–	21	46	Silva *et al.* (2000)
Pleuronectiformes									
Paralichthyidae	*Hippoglossina macrops*	South Chile[c]	21	–	Yes	Mu	3	4	Torres *et al.* (1983)
	Hippoglossina montemaris	South Chile[c]	8	–	Yes	Mu	1	1	Torres *et al.* (1983)
	Paralichthys adspersus	North Chile[b]	179	(31.2–34.2)	Yes	Bc, Mu	16	47	Oliva *et al.* (1996)
	Paralichthys isosceles	North Argentina[a]	51	28.0 ± 2.1	Yes	Me, Mu	1	1	Alarcos & Timi (2012)
	Paralichthys microps	South Chile[b]	10	(26.0–38.0)	Yes	Mu	7	14	Torres *et al.* (2000)
	Paralichthys patagonicus	North Argentina[a]	51	35.2 ± 2.6	Yes	Me, Mu	9	13	Alarcos & Timi (2012)
	Xystreurys rasile	North Argentina[a]	48	29.3 ± 2.6	Yes	Me. Mu	1	3	Alarcos & Timi (2012)
Scorpaeniformes									
Normanichthyidae	*Normanichthys crockeri*	South Chile[a]	11	9.0 ± 0.2	No	Mu	1	1	Sepúlveda *et al.* (2004)
Sebastidae	*Helicolenus lengerichi*	Central Chile[a]	30	28.1 ± 4.5	No	–	1	1	George-Nascimento & Iriarte (1989)
		Central Chile[a]	56	30.9 ± 2.3	No	–	2	2	Balboa & George-Nascimento (1998)
	Sebastes capensis	Central Chile[a]	42	(27.5–27.8)**	No	–	10	13	Balboa & George-Nascimento (1998)

*Specific identification of sealworms larvae: [a]*Pseudoterranova* sp.; [b]*P. decipiens*; [c]*Phocanema* sp.; [d]*P. cattani*; **Range calculated from host total length means; ***Fish identified as *Genypterus brasiliensis* by the authors.

Despite the wide range of hosts infected with sealworms along the South American coast, morphological and molecular characterization of species of *Pseudoterranova* is still scarce. In the southeastern Pacific, Torres & Gonzalez (1978) provided the first biometrical and morphological data of the L3 of *Pseudoterranova* (=*Phocanema*) sp. from the liver of *Genypterus* sp. Later, Cattan & Carvajal (1981) described adult specimens of *Pseudoterranova* (=*Phocanema*) *decipiens sensu lato* (*s.l.*) collected from the stomach of the South American sea lion *Otaria flavescens* (Shaw, 1890), hereinafter referred to as sea lion. George-Nascimento & Llanos (1995) reported biometrical, morphological, and electrophoretic data from both L3 and adult specimens of *Pseudoterranova* sp. collected from marine fish and sea lions, respectively, in the southeastern Pacific. Subsequently, George-Nascimento & Urrutia (2000) described *P. cattani* from sea lions, and identified the sealworm larvae reported by George-Nascimento & Llanos (1995) as the L3 of this species. With regard to the southwestern Atlantic, Hernández-Orts *et al.* (2013) recently confirmed the occurrence of fourth stage larvae and adults of *P. cattani* in sea lions and South American fur seals *Arctocephalus australis* (Zimmerman, 1783) along the Patagonian coast of Argentina. Preliminary evidence also reports the occurrence of L3 of *P. cattani* in marine fish caught off Argentina (Timi *et al.*, 2011a).

In this paper we provide, for the first time, molecular, morphological, and ecological data on the L3 of *Pseudoterranova* sp. in the Patagonian coast of Argentina based on an extensive parasitological survey on 20 fish species. We firstly carried out a molecular identification of larvae, followed by a morphological description of specimens, including examination by scanning electronic microscopy. Second, we compared morphometric data of L3s collected from different fish species to investigate patterns of morphological variability. Finally, we report on infection level among fish species and examine the distribution of larvae in fish, to provide a better understanding of the ecology of sealworms in the Patagonian coasts of Argentina.

8.2. MATERIALS AND METHODS

8.2.1. Sample collection

A total of 542 individual fish from 20 species were examined for sealworm larvae (**Table 12**). Fish were caught by commercial bottom trawling vessels during 2006–2007 along 2 areas of the Patagonian coast of Argentina: north (42º45'–42º59'S, 61º09'–62º58'W; depth range: 72–88 m) and central Patagonia (47º00'–47º19'S, 61º59'–64º25'W; depth range: 82–119 m) (**Fig. 20**). Fish were kept on ice on board and, after arrival to the laboratory, identified according to Menni *et al.* (1984). Fish scientific names were validated according to Froese & Pauly (2013). Specimens were then either examined fresh or frozen in plastic bags at –20ºC for later examination. Fresh or thawed fish were dissected, and internal organs were removed from the carcass. The body cavity was examined by naked eye, whereas the epaxial and hypaxial muscles regions, internal organs (liver, stomach, intestine and intestinal caeca, swim bladder, gonads) and mesenteries were pressed between Petri dishes and examined under a stereomicroscope (up to 40×) to detect encapsulated nematodes. Sealworm larvae were removed from their capsules, washed in saline and fixed in 70% ethanol.

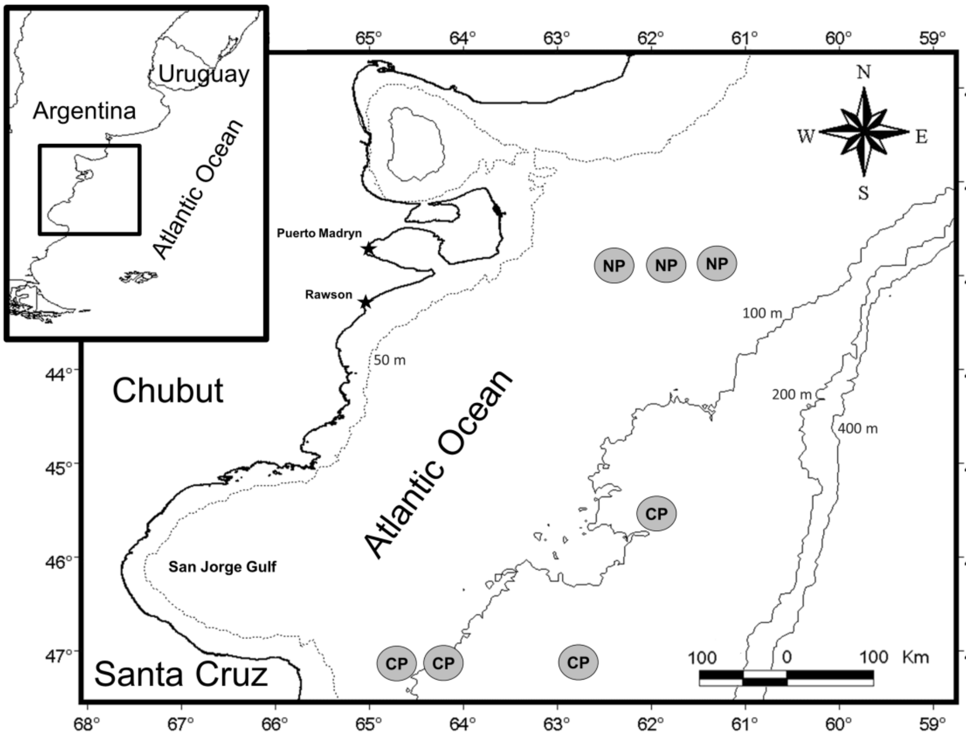

Figure 20. Map of the Patagonian coast of Argentina showing the study area and the sampling sites (grey circles). Abbreviation: CP, central Patagonia; NP, north Patagonia.

Table 12. Biological data of the fish species examined for the presence of larvae of *Pseudoterranova* sp. from the Patagonian coast of Argentina. Fish total length in centimeters, presented as the mean followed by S.D. and the range in parentheses.

Host	**n**	**Total length**
Gadiformes: Merlucciidae		
Macruronus magellanicus Lönnberg, 1907	3	56.7 ± 23.0 (40.1–83.0)
Merluccius hubbsi Marini, 1933	79	28.1 ± 4.2 (16.5–34.2)
Ophidiiformes: Ophidiidae		
Genypterus blacodes (Forster, 1801)	44	39.7 ± 9.4 (24.7–58.5)
Raneya brasiliensis (Kaup, 1856)	16	21.2 ± 1.4 (18.2–23.3)
Perciformes: Bramidae		
Brama brama (Bonnaterre, 1788)	2	60.5 ± 2.1 (59.0–62.0)
Perciformes: Bovichtidae		
Cottoperca gobio (Günther, 1861)	8	30.2 ± 9.3 (22.0–52.0)
Perciformes: Centrolophidae		
Seriolella porosa Guichenot, 1848	34	33.0 ± 5.6 (22.7–42.7)
Perciformes: Cheilodactylidae		
Nemadactylus bergi (Norman, 1937)	32	25.6 ± 5.5 (11.4–34.6)
Perciformes: Mullidae		
Mullus argentinae Hubbs and Marini, 1933	2	20.7 ± 0.4 (20.4–21.0)
Perciformes: Nototheniidae		
Patagonotothen ramsayi (Regan, 1913)	84	24.9 ± 3.5 (14.7–31.7)
Perciformes: Percophidae		
Percophis brasiliensis Quoy and Gaimard, 1825	8	45.3 ± 4.9 (37.1–51.8)
Perciformes: Pinguipedidae		
Pseudopercis semifasciata (Cuvier, 1829)	31	26.5 ± 2.7 (22.3–32.2)
Perciformes: Scombridae		
Scomber japonicus Houttuyn, 1782	13	42.7 ± 5.0 (32.5–48.0)
Perciformes: Serranidae		
Acanthistius patachonicus (Jenyns, 1840)	16	30.0 ± 2.6 (24.1–34.2)
Perciformes: Stromateidae		
Stromateus brasiliensis Fowler, 1906	73	27.5 ± 3.6 (13.7–36.4)
Pleuronectiformes: Paralichthyidae		
Paralichthys isosceles Jordan, 1891	15	27.2 ± 5.3 (17.9–34.4)
Xystreurys rasile (Jordan, 1891)	29	32.8 ± 5.8 (21.9–42.6)
Scorpaeniformes: Congiopodidae		
Congiopodus peruvianus (Cuvier, 1829)	15	23.9 ± 2.0 (21.0–28.0)
Scorpaeniformes: Sebastidae		
Helicolenus lahillei Norman, 1937	6	28.8 ± 2.6 (25.9–32.5)
Scorpaeniformes: Triglidae		
Prionotus nudigula Ginsburg, 1950	32	23.1 ± 2.8 (16.7–27.8)

8.2.2. Molecular analysis

The central part of the body of 3 specimens of *Pseudoterranova* sp. from the most infected fish species, the red searobin *Prionotus nudigula* Ginsburg, 1950 (see **Table 15**) was used in the molecular analysis. Specimens had been fixed in 70% ethanol, and the anterior and posterior ends of each specimen were deposited as voucher specimens. DNA extractions consisted of placing individual isolates into 1.5 ml tubes in 300 µl of 5% chelex containing 0.1 mg/ml proteinase K, incubating at 60ºC overnight, boiling at 90ºC for 8 min and centrifuging at 15,000 g for 10 min. The mitochondrial cytochrome c oxidase subunit 1 gene (cox 1) is a commonly used molecular marker for barcoding and prospecting species in numerous groups including some parasitic helminths (Detwiler *et al.*, 2010; Ferri *et al.*, 2009). We selected this marker because there exist already cox 1 sequences of for *Pseudoterranova* spp. in Genbank that we could compare with and, at the same time, it would allow us to rule out the possibility of cryptic species. We amplified partial cox 1 sequences using primers JB3 (forward 5’-TTTTTTGGGCATCCTGAGGTTTAT-3’) and JB4 (reverse 5’-TAAAGAAAGAACATAATGAAAATG-3’) (Bowles *et al.*, 1993). Polymerase chain reaction (PCR) amplifications were performed with 25µl reactions containing 2.5µl of extraction supernatant, 1X PCR buffer (16mM (NH4)2SO4, 67 mM Tris-HCl at pH 8.8), 2 mM MgCl2, 200 µM of each dNTP, 0.5 mM each primer, and 0.7 unit MyFi DNA polymerase (Bioline Ltd.). The following thermocycling profile was used for amplification: denaturation of DNA (95ºC for 3 min); 35 cycles of amplification (94ºC for 40 s, 50ºC for 30 s and 72ºC for 45 s); and 4 min extension hold at 72ºC. PCR products were purified using PCR Product Pre-Sequencing Kit™ (Affymetrix / USB corporation). PCR primers were used for sequencing and PCR amplicons were cycle-sequenced from both strands using ABI BigDye™ Terminator v3.1 Ready Sequencing Kit, ethanol-precipitated, and run on an ABI 3730xl automated sequencer. Contiguous sequences were assembled and edited using MEGA 5.0 (Tamura *et al.*, 2011).

In order to examine the affinity of our isolates with other species of *Pseudoterranova* newly obtained sequences for cox 1 were aligned together with 15 sequences available from Genbank obtained by Cao *et al.* (2005) (Genbank accession numbers for these sequence were not provided in their study): *P. azarasi, P. bulbosa, P. cattani, P. decipiens* (*s.l.*), *P. decipiens* (*s.s.*), *P. decipiens* and *P. krabbei* (see **Table**

13), using MUSCLE implemented in MEGA with default parameter values. Sequences of two species of *Contracaecum* Railliet and Henry, 1913 were used as outgroups (**Table 13**). Bayesian Inference analysis was performed in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) using Markov chain Monte Carlo searches on two simultaneous runs of four chains during 10^7 generations, sampling trees every 10^3 generations. The evolutionary substitution model GTR (general time-reversible model) was applied and the parameter gamma was allowed to accommodate among-site rate variation. The first 10^3 trees sampled were discarded as 'burn-in', as determined by stationarity of lnL assessed using Tracer v. 1.4 (Rambaut & Drummond , 2007), and a consensus topology and nodal support estimated as posterior probability values (Huelsenbeck *et al.*, 2001) were calculated from the remaining trees. Mean genetic distances (raw p-distance) between and within species were calculated on a total of 364 unambiguously aligned positions and standard deviation (S.D.) estimates were calculated on 500 replicates bootstrap.

Table 13. Sequence information of species of *Pseudoterranova* and *Contracaecum* used in the molecular analysis in the present study.

Species	Host name	Developmental stage	Locality	GenBank cox1
P. azarasi	*Eumetopias jubatus*	Adult	Iwanai, Japan	AJ891139 AJ891140
P. bulbosa	*Erignathus barbatus*	Adult	Newfoundland, Canada	AJ891141 AJ891142
P. cattani	*Otaria flavescens* (syn. *Otaria byronia*)	Adult	Concepcion, Chile	AJ891143 AJ891144
	Prionotus nudigula	Larva	Patagonia, Argentina	########** ########** ########**
P. decipiens (*s.s.*)*	*Phoca vitulina*	Adult	Newfoundland, Canada	AJ891145
P. decipiens (*s.l.*)*	*Chaenocephalus aceratus*	Larval	Elbe estuary, Germany	AJ891146 AJ891147 AJ891148 AJ891149
	Osmerus eperlanus	Larval	Elbe estuary, Germany	AJ891150
P. krabbei	*Halichoerus grypus*	Adult	Froya Island, Norway	AJ891151 AJ891152 AJ891153
C. osculatum	–	–	Australia/Antarctic	AJ405315
C. rudolphii C	–	–	Florida, US	FJ866816

*Species epithets for *P. decipines* isolates according to Cao *et al*. (2005); **Sequences obtained in the present study.

8.2.3. Morphological analyses

Larvae were cleared in lactophenol and examined under stereomicroscope, bright field microscope, or differential interference contrast microscope. For the description of the larvae found in *P. nudigula,* measurements were taken from drawings made with the aid of a drawing tube and are expressed in millimetres. For the morphological description of sealworm larvae, measurements are presented as the mean followed by S.D., with the range and sample size in parentheses. Morphometric data of sealworm larvae were also obtained, by the same procedure mentioned above, from other 2 fish species, *i.e.* the Brazilian flathead, *Percophis brasiliensis* Quoy and Gaimard, 1825, and the Patagonian grouper, *Acanthistius patachonicus* (Jenyns, 1840). Voucher specimens are deposited in the Natural History Museum, London, UK (accession numbers: 2012.5.15.144-172), and the Helminthological Collection of the Institute of Parasitology (IPCAS), Biology Centre ASCR, České Budějovice, Czech Republic (accession numbers: N-1013).

Also, some larvae from *P. nudigula* were studied externally and internally with scanning electron microscopy (SEM). Three larvae fixed in 70% ethanol were dissected and the body wall partially removed to examine the morphology of the anterior part of the digestive tract. All the specimens were dehydrated through an ethanol series, critical point dried, and coated with a gold-palladium alloy to a thickness of 250 nm. Specimens were examined with a Hitachi 4100 FE scanning electron microscope, operating at 20 kV, from the Central Service for the Support to Experimental Research of the University of Valencia.

Morphometric variation of sealworm larvae from different fish species was examined through a discriminant analysis, based on canonical distances. Morphometric data were obtained as described above. Multivariate statistical analysis were performed on 8 metrical variables: body length, body width, distance from anterior end to nerve ring, distance from distal end of excretory gland to posterior end, muscular oesophagus length, glandular ventriculus length, intestinal caecum length and tail length. The canonical discriminant functions were calculated using all variables simultaneously. Statistical analysis was carried out with SPSS v19. Statistical significance was set at $P < 0.05$.

8.2.4. Ecological analyses

Ecological terms follow Bush *et al.* (1997) and Rózsa *et al.* (2000). The prevalence, mean abundance and mean intensity are followed by the 95 % confidence intervals (C.I.) in parentheses. The 95 % C.I. for prevalence was set with Sterne's exact method (Reiczigel, 2003), whereas the 95 % C.Is. for the mean abundance and mean intensity were estimated with 20,000 bootstrap replications using the statistical software Quantitative Parasitology v3.0 (Reiczigel & Rózsa, 2005).

A preliminary analysis indicated no significant differences on the abundances of sealworm larvae between species of fish collected in different sampling sites (Mann–Whitney test, $P > 0.05$, **Fig. 20**). Therefore, infection parameters and statistical analyses were calculated for the pooled data.

Differences in the intensity of L3 of *Pseudoterranova* between species of fish were investigated with the Kruskal-Wallis test followed by post hoc comparison between fish species (Conover, 1999). For this analysis we considered only fish species with a sample size > 5 individuals.

8.3. RESULTS

8.3.1. Molecular identification

Species of *Pseudoterranova* formed a monophyletic clade with high support (**Fig. 21**). The three newly sequenced isolates from *P. nudigula* formed a well supported clade together with representative sequences of adult specimens of *P. cattani* from sea lion of Chile, and distinct from the additional 5 species of *Pseudoterranova* (**Table 13**). Mean genetic divergence among species ranged between 5.5–11.9%. Intraspecific mean genetic divergence ranged between 1.1–1.9% with *P. cattani* having 1.4 ± 0.4%, including the 3 isolate sequences from *P. nudigula*. Overall, molecular results suggest that sealworm larvae from *P. nudigula* belong to *P. cattani*.

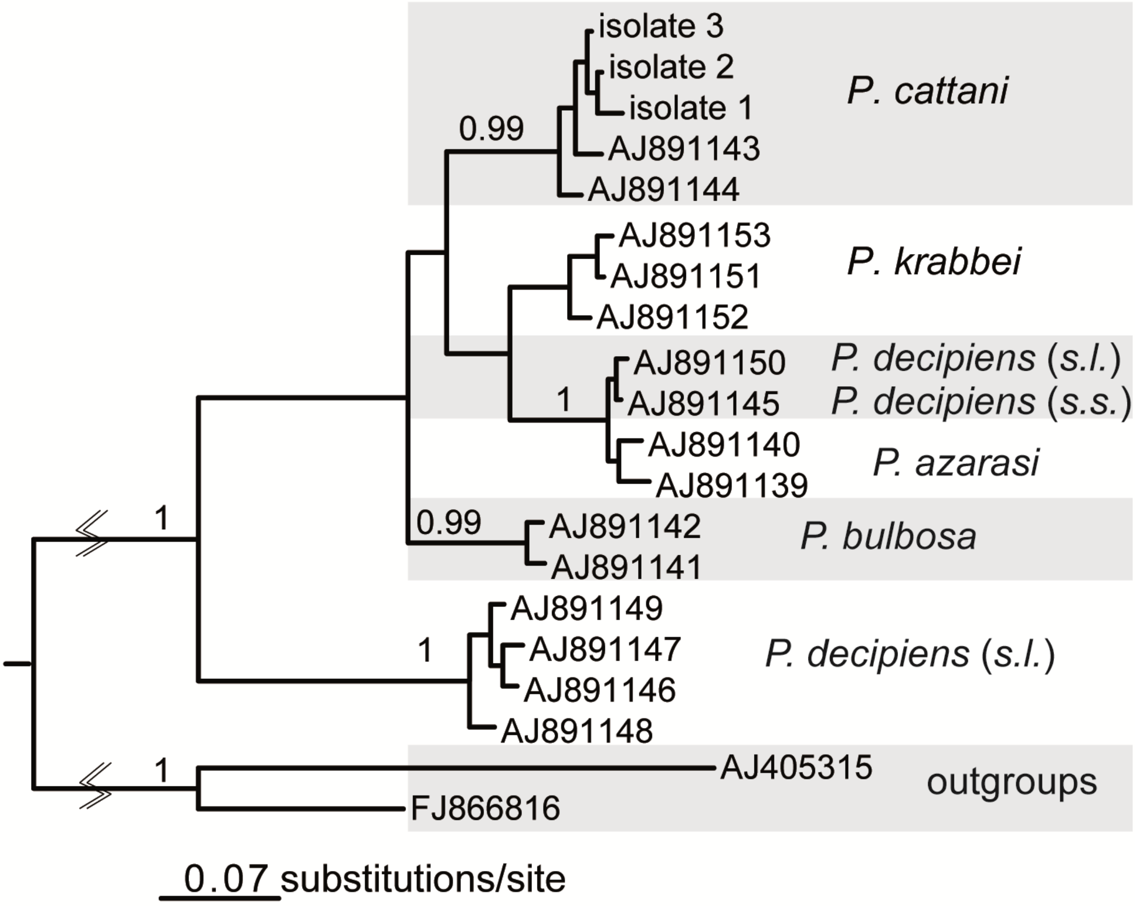

Figure 21. Phylogram derived from Bayesian analysis of cox 1 sequences (364 bp) of six *Pseudoterranova* spp. Sequences of *Contracaecum rudolphii* and C. *osculatum* were used as outgroups. Posterior probabilities are indicated at the nodes (<0.95 were omitted). Species epithets for *P. decipiens* isolates according to Cao et al. (2005).

Incidentally, one isolate labelled as *P. decipiens* (*s.s.*) and another as *P. decipiens* (*s.l.*) (see Table 1 in Cao *et al.* (2005) but not specified in the genbank records), clustered as sister to two isolates of *P. azarasi*, being distant from the clade formed by 4 other *P. decipiens* (*s.l.*) isolates (**Fig. 21**). The low mean divergence between *P. decipiens* (*s.s.*) isolate AJ891145 and *P. decipiens* (*s.l.*) isolate AJ891150 and isolates of *P. azarasi* (1.4 ± 0.4%) falls within the range of intraspecific divergence for the group, suggesting that these four isolates may be conspecific.

8.3.2. Morphological description of **P. cattani** *from* **P. nudigula**

8.3.2.1. Third stage larvae **(Figs. 22 & 23).**

Description based on 80 third-stage larvae examined by light microscopy and 7 specimens by SEM. Body yellowish to reddish, medium-sized, elongate, 31.1 ± 3.6 (23.8–43.2, n = 80) long by 0.9 ± 0.1 (0.7–1.4, n = 80) wide (**Fig. 22A**), with transverse striations along the whole body (**Figs. 23A & B**). Anterior end rounded. Cuticle covering the triangular mouth aperture (**Fig. 23B**), with 3 lips (2 ventro-lateral and 1 dorsal) of approximately equal size (**Figs. 23A & B**). Each lip with a pair of soft swellings of the cuticle at level of papillae. Boring tooth antero-ventral, between the ventro-lateral lips (**Figs. 23A & B**). Excretory pore opening ventrally (**Figs. 23A & B**), below boring tooth. Nerve ring at 0.4 ± 0.1 (0.2–0.6, n = 80) from anterior body end (**Figs. 22A & B**). Deirids lateral, posterior to nerve ring, about 0.7 ± 0.1 (0.5–1.0, n = 16) from anterior body end (**Fig. 22B**). Muscular oesophagus 2.0 ± 0.2 (1.4–2.4, n = 80) long (**Figs. 22A and B**). Oesophagus / body length ratio 0.1 ± 0.01 (0.04–0.1, n = 80). Glandular ventriculus 1.2 ± 0.2 (0.7–1.5, n = 80) long (**Figs. 22A, 22B & 23C**). Glandular ventriculus / body length ratio 0.04 ± 0.01 (0.02–0.1, n = 80). Intestinal caecum 1.1 ± 0.2 (0.4–1.7, n = 80) long (**Figs. 22A, 22B & 23C**). Intestinal caecum / body length ratio 0.04 ± 0.01 (0.02–0.5, n = 80). Intestinal caecum shorter (n = 39), equal (n = 18) or slightly longer (n = 23) than glandular ventriculus. Intestinal caecum / glandular ventriculus length ratio 1.0 ± 0.1 (0.6–1.3, n = 80). Rectum surrounded by three rectal glands, one ventral and two dorsal (**Fig. 22C**). Tail short, conical, pointed, 0.2 ± 0.03 (0.1–0.2, n = 80) long (mucron not included) (**Figs. 22C & 23D**). Distance from distal end of excretory gland to posterior body end 0.2 ± 0.1 (0.1–0.4, n = 79). Mucron 0.02 ± 0.00 (0.01–0.04, n = 71) long (**Figs. 22C & 23E**).

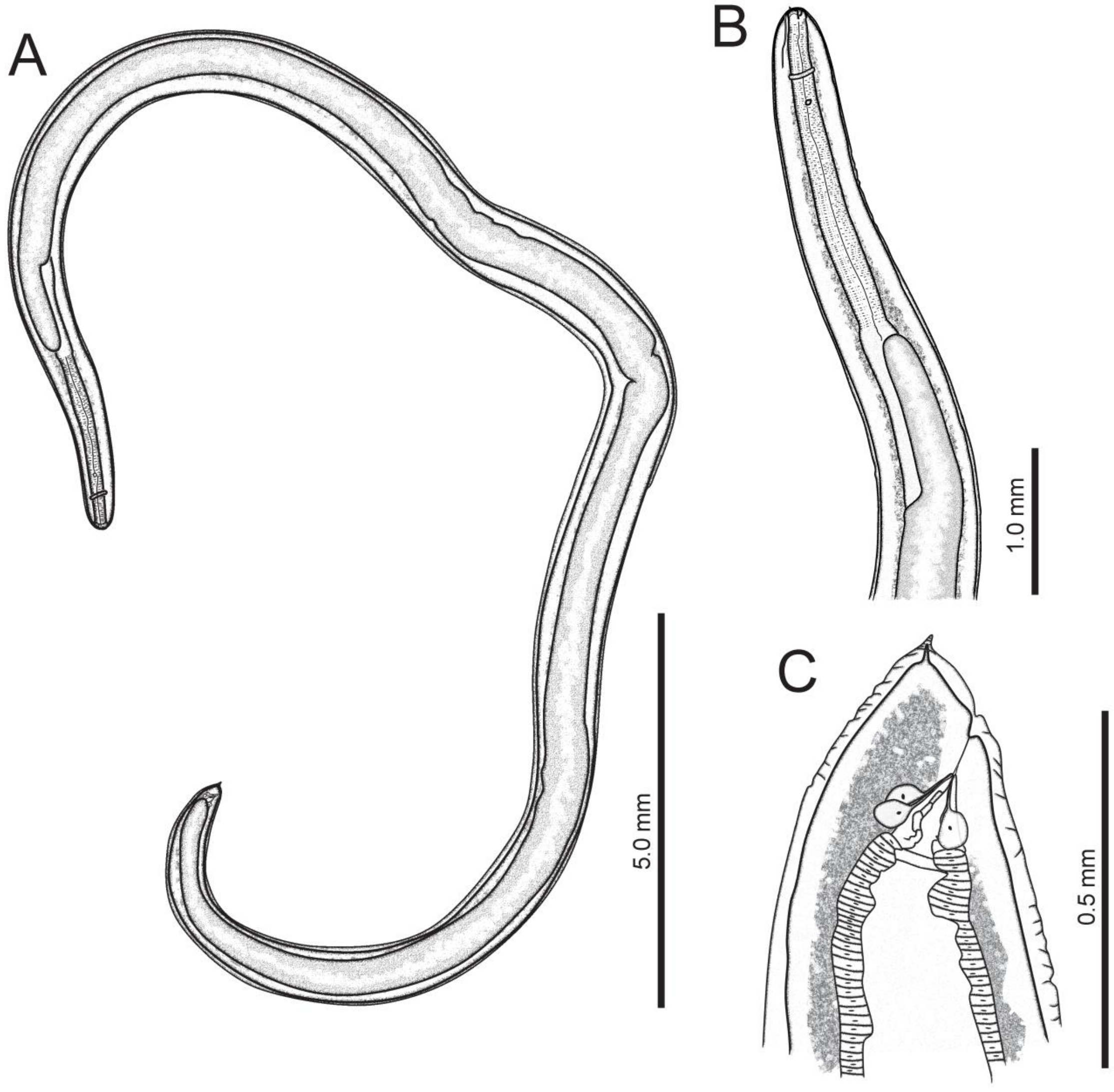

Figure 22. Third stage larvae of *Pseudoterranova cattani* collected from the red searobin, *Prionotus nudigula*. (**A**) Whole worm, lateral view. (**B**) Anterior end, lateral view. (**C**) Posterior end, lateral view.

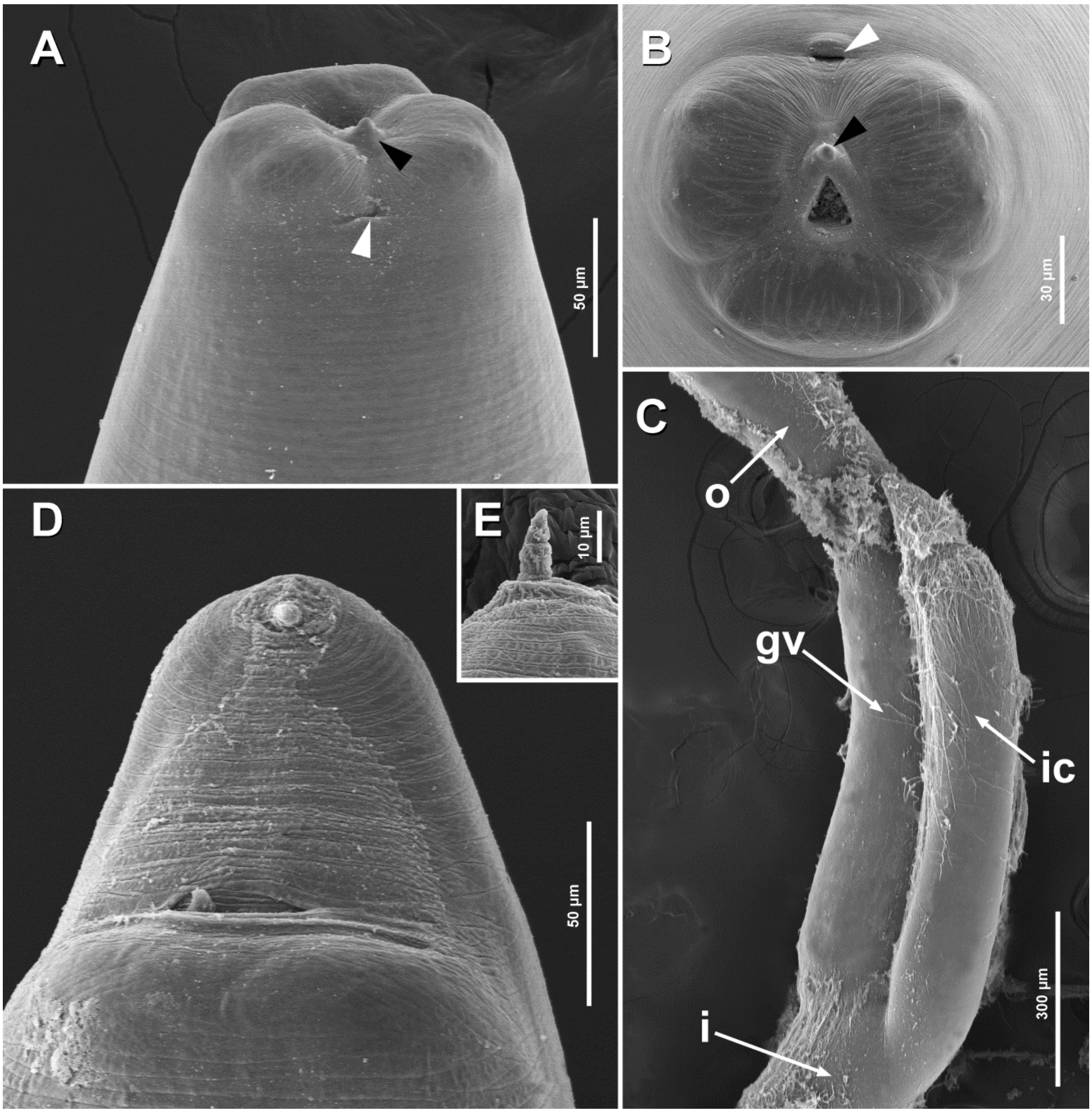

Figure 23. Scanning electron micrographs of the third stage larvae of *Pseudoterranova cattani* collected from the red searobin, *Prionotus nudigula*. (**A**) Anterior end, ventral view. (**B**) Anterior end, apical view. (**C**) Detail of the area of the glandular ventriculus and intestinal caecum of a dissected worm, lateral view. (**D**) Posterior end, ventral view. (**E**) Mucron lateral view. Black arrowheads point to the boring tooth and white arrowheads point to the excretory pore. *Abbreviations*: i, intestine; ic, intestinal caecum; gv, glandular ventriculus; o, oesophagus.

8.3.2.2. Taxonomic summary

Fish host: red searobin *Prionotus nudigula* Ginsburg, 1950 (Scorpaeniformes: Triglidae).
Locality: north Patagonia, Chubut Province, Argentina (42º45'–42º59'S, 61º09'–62º58'W).
Site on the host: liver, mesenteries and muscles.
Infection parameters: prevalence, 100% (89.5-100); abundance, 16.2 (12.5-20.9); intensity, 16.2 (12.5-20.9) (see **Table 15**).

8.3.3. Morphometric comparison of larvae among fish species

Morphometric data obtained from L3 of *P. cattani* from *P. nudigula* and sealworm larvae from *P. brasiliensis* and *A. patachonicus* are presented in **Table 14**. Overall morphometric differences of larvae from the 3 fish species from the Patagonian coast of Argentina were highly significant. Univariate tests indicated that 6 out of the 8 variables significantly differ among groups, *i.e.* body length (Wilks' $\lambda = 0.650$, $F_{(2,110)} = 29.645$, $P < 0.001$), body width (Wilks' $\lambda = 0.663$, $F_{(2,110)} = 0.27.932$, $P < 0.001$), distance from anterior end to nerve ring (Wilks' $\lambda = 0.717$, $F_{(2,110)} = 21.657$, $P < 0.001$), muscular oesophagus length (Wilks' $\lambda = 0.668$, $F_{(2,110)} = 27.306$, $P < 0.001$), glandular ventriculus length (Wilks' $\lambda = 0.699$, $F_{(2,110)} = 23.646$, $P < 0.001$), and intestinal caecum length (Wilks' $\lambda = 0.755$, $F_{(2,110)} = 17.894$, $P < 0.001$).

Functions 1 and 2 of the discriminant analysis accounted for 82.3% (eigenvalue = 1.168) and 17.7% (eigenvalue = 0.252) respectively (**Fig. 24**). The variables showing high absolute values of standardized coefficients along the first function were the glandular ventriculus length (0.44), distance from anterior end to nerve ring (0.41) and body width (0.35). Although the specimens of *P. cattani* from *P. nudigula* mostly overlap with sealworms from *P. brasiliensis* in the first function (**Fig. 24**), these variables tend to be relatively larger to that those of sealworm larvae from other fish. For the second function, the variables showing high absolute values of standardized coefficients were the body length (-0.75), distance from anterior end to nerve ring (0.45) and the body width (0.43). These suggest that sealworms from *P. brasiliensis* will tend to show relatively lower body length than those of sealworms from the other two fish.

Table 14. Morphometric data of third stage larvae of *P. cattani* and *P.* cf. *cattani* in different fish species from South America. Measurements in millimetres. Abbreviations: BL, body length; BW, body width; GLV, glandular ventriculus length; ICL, intestinal caecum length; OL, oesophagus length; TL, tail length.

Reference	George-Nascimento & Llanos (1995)[*]				Present study		
Hosts	*Merluccius gayi gayi*	*Genypterus maculatus*	*Paralichthys microps*	*Cilus gilberti*	*Prionotus nudigula*	*Acanthistius brasilianus*	*Percophis brasiliensis*
Locality	Talcahuano, Chile	Talcahuano, Chile	Talcahuano, Chile	Talcahuano, Chile	Chubut, Argentina	Chubut, Argentina	Chubut, Argentina
Species	*P. cattani*	*P. cattani*	*P. cattani*	*P. cattani*	*P. cattani*	*P.* cf. *cattani*	*P.* cf. *cattani*
n	15	15	15	15	80	8	27
BL	28.5 ± 4.3	30.6 ± 5.8	29.0 ± 5.8	30.1 ± 3.6	31.1 ± 3.6 (23.8–43.2)	18.3 ± 5.6 (8.4–23.8)	28.2 ± 5.1 (17.1–40.5)
BW	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.1 (0.7–1.4)	0.6 ± 0.2 (0.5–0.8)	0.8 ± 0.1 (0.5–0.9)
OL	1.6 ± 0.3	1.7 ± 0.2	1.6 ± 0.2	1.7 ±0.2	2.0 ± 0.2 (1.4–2.4)	1.5 ± 0.3 (1.0–1.8)	1.8 ± 1.2 (1.4–2.1)
GVL	0.8 ± 0.3	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.2 (0.7–1.5)	0.8 ± 0.2 (0.5 – 1.0)	1.0 ± 0.1 (0.7–1.3)
ICL	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	1.1 ± 0.2 (0.4–1.7)	0.7 ± 0.1 (0.5–0.8)	1.0 ± 0.2 (0.6–1.7)
TL	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0 (0.1–0.2)	0.1 ± 0.0 (0.1–0.2)	0.1 ± 0.0 (0.1–0.2)
BL/BW	35.4 ± 6.7	42.0 ± 8.4	42.4 ± 6.7	41.3 ± 6.9	34.3 ± 5.3 (22.8–47.9)	28.6 ± 5.4 (16.8–34.0)	37.8 ± 5.1 (22.1–50.6)
BL/OL	18.4 ± 4.1	17.8 ± 2.9	17.9 ± 3.3	17.5 ± 2.3	15.5 ±2.3 (11.5–22.4)	11.8 ± 2.1 (8.4–14.9)	15.7 ± 2.2 (10.9–20.8)
BL/GVL	37.5 ± 11.6	34.3 ± 5.2	29.9 ± 5.1	30.6 ± 4.7	27.3 ± 4.8 (20.5–40.3)	25.7 ± 8.5 (15.4–36.8)	27.4 ± 4.5 (18.4–36.8)
BL/ICL	42.1 ± 15.6	44.0 ± 8.6	39.3 ± 9.8	38.0 ± 6.5	29.0 ± 6.8 (20.2–59.8)	28.8 ± 9.5 (18.0–45.2)	28.8 ± 4.8 (18.3–37.5)
GVL/ICL	1.2 ± 0.5	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.1 ± 0.2 (0.8–1.6)	1.2 ± 0.2 (0.9–1.4)	1.1 ± 0.1 (0.8–1.3)
BL/TL	235.3 ± 73.3	289.9 ± 46.3	267.9 ± 127.0	277.1 ± 58.7	205.6 ± 40.4 (114.4–305.0)	133.2 ± 45.6 (76.4–210.0)	199.3 ± 45.4 (134.9–334.7)

[*] Latter classified as *P. cattani* by George-Nascimento & Urrutia (2000).

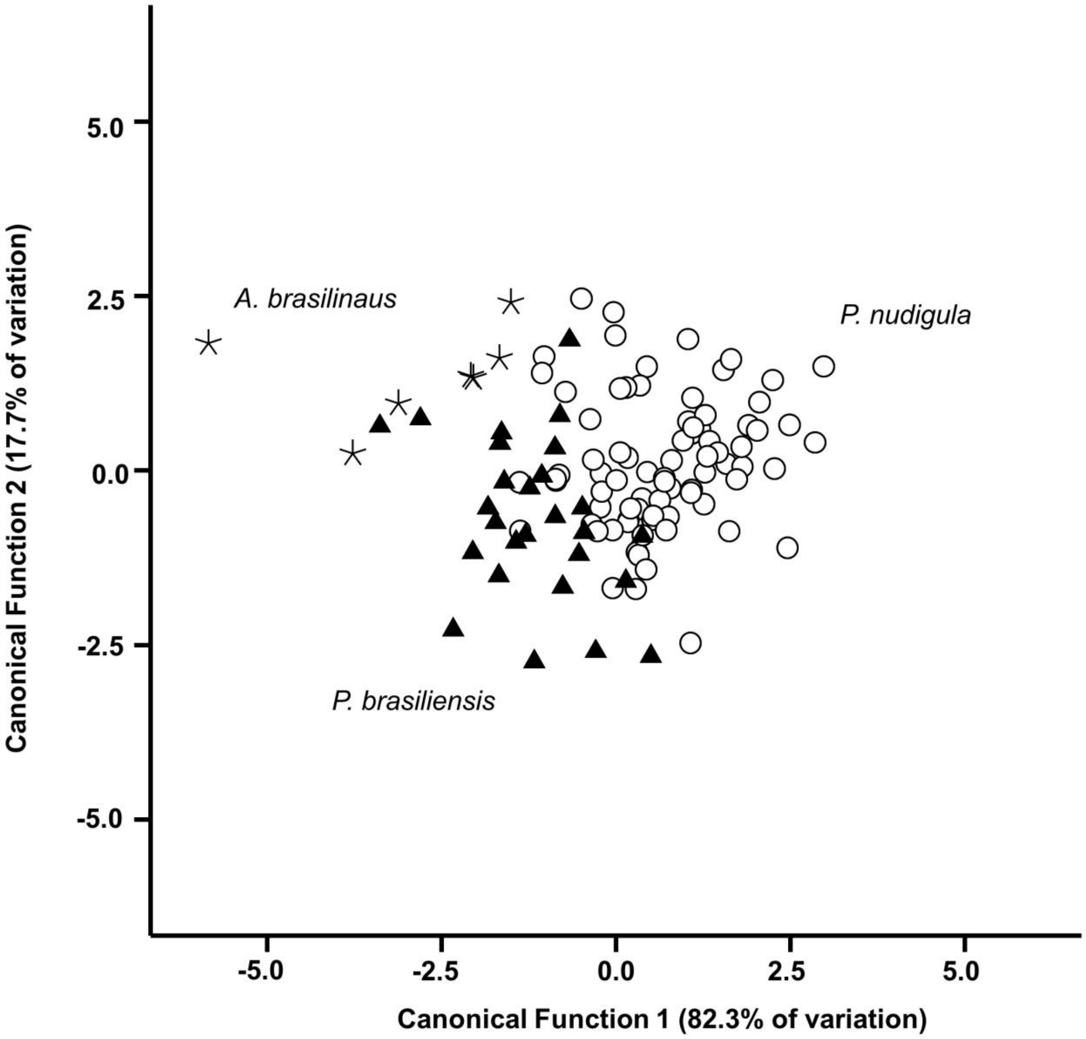

Figure 24. Plot of the 9 metrical variables measured from 80 specimens of L3 of *P. cattani* collected from *Prionotus nudigula* and 35 specimens of L3 of *P.* cf. *cattani* from *Acanthistius patachonicus* (n = 8) and *Percophis brasiliensis* (n = 27), against the first and second canonical discriminant functions.

A comparison of morphometric data from sealworm larvae of the Patagonian coast of Argentina and L3 of *P. cattani* from other fish species of Chile is shown in **Table 14**. Morphometric measurements obtained from sealworm larvae from *P. nudigula* and *P. brasiliensis* overlap with those reported by George-Nascimento & Llanos (1995) from L3 of *P. cattani* infecting 4 marine fish species from the southeastern Pacific coast of Chile (**Table 14**). No clear differences were detected between sealworm larvae from both geographical areas, except that some specimens collected from *A. brasilianus* are apparently smaller.

8.3.4. Ecological patterns

A total of 635 third stage sealworm larvae were collected from 12 marine fish species from the Patagonian coast in Argentina (**Table 15**). Five species of fish, *i.e. Cottoperca gobio* (Günther, 1861), *Nemadactylus bergi* (Norman, 1937), *Mullus argentinae* Hubbs and Marini, 1933, *P. brasiliensis* and *P. nudigula* represent new host records for sealworms. The smallest infected fish with sealworm larvae was a *P. nudigula* 16.7 cm long (intensity, 1), whereas the largest was a *G. blacodes*, 58.0 cm long (intensity, 2). A specimen of *P. nudigula* of 20.7 cm long presented the highest infection (intensity, 50).
A comparison of sealworm abundance between fish species revealed highly significant differences (Kruskal-Wallis test, $\chi^2 = 336.141$, 16 d.f., $P < 0.001$); the post hoc test ($P < 0.05$) indicated that only *P. nudigula* harboured significantly more sealworm larvae than any other fish species. The abundance of sealworm larvae was weakly, but significantly correlated with body length only in *P. nudigula* (Spearman rank correlation: $r_s = 0.324$, $n = 32$, one-tailed $P = 0.035$).

In *P. nudigula*, most of the L3 of *P. cattani* were found encapsulated in the epaxial muscles (number of sealworms collected, 315; mean abundance [95% C.I.], 9.8 [7.3–13.1]) followed by hypaxial muscles (180; 5.6 [4.3–7.2]) and, to a lesser extent, mesenteries (22; 0.7 [0.4–1.1]) and liver (1; 0.03 [0.0–0.1]). Differences in the number of larvae between microhabitats were all highly significant (Friedman test, $\chi^2 = 71.544$, 3 d.f., $P < 0.001$; in all post hoc comparisons, $P < 0.005$). In other species too, sealworm larvae were generally collected from the muscles, and to a lesser extent, the mesenteries and liver (**Table 15**).

Table 15. Occurrence of sealworm larvae in 12 species of marine fish from Patagonia, Argentina. The ecological group for each species is shown in parentheses after the host name. Ecological groups mainly assigned following Koen-Alonso *et al*. (2000) and Romero *et al*. (2011). The 95 % C.I. were only estimated for fish species with n ≥ 15. Abbreviations: B, benthic; DB, demersal–benthic; DP, demersal–pelagic; Li, liver; Me, mesenteries; Mu, muscles; P, pelagic.

Host	**n**	**Microhabitat (n sealworms collected)**	**Prevalence (%) (95% C.I.)**	**Mean abundance (95% C.I.)**	**Mean intensity (95% C.I.)**	**Range**
Acanthistius patachonicus (DB)	16	Li (1), Me (2), Mu (11)	25.0 (9.0-50.0)	0.9 (0.2-2.3)	3.5 (1.5-5.5)	1-7
Brama brama (DP)	2	–	–	–	–	–
Congiopodus peruvianus (DB)	15	–	–	–	–	–
Cottoperca gobio (DB)	8	Mu (4)	12.5	0.5	4	4
Genypterus blacodes (DB)	44	Li (2)	2.3 (0.1-12.1)	0.1 (0.0-0.1)	2.0	2
Helicolenus lahillei (DB)	6	–	–	–	–	–
Macruronus magellanicus (DP)	3	–	–	–	–	–
Merluccius hubbsi (DP)	79	Me (2), Mu (1)	3.8 (1.1-10.6)	0.0 (0.0-0.1)	1.0	1
Mullus argentinae (DB)	2	Me (1), Mu (1)	100	1.0	1.0	1
Nemadactylus bergi (DB)	32	Me (1)	3.1 (0.1-16.6)	0.0 (0.0-0.1)	1.0	1
Patagonotothen ramsayi (DB)	84	–	–	–	–	–
Paralichthys isosceles (B)	15	Me (2), Mu (9)	26.7 (9.7-53.4)	0.7 (0.1-2.3)	2.8 (1.0-5.5)	1-7
Percophis brasiliensis (DB)	8	Mu (67)	25.0	8.4	33.5	29-38
Prionotus nudigula (B)*	32	Li (1), Me (22), Mu (495)	100 (89.5-100)	16.2 (12.5-20.9)	16.2 (12.5-20.9)	1-50
Pseudopercis semifasciata (DB)	31	Li (1), Mu (10)	25.8 (12.6-43.4)	0.4 (0.1-0.6)	1.4 (1.0-1.6)	1-2
Raneya brasiliensis (B)	16	–	–	–	–	–
Scomber japonicus (P)	13	Me (1)	7.7	0.1	1.0	1
Seriolella porosa (DP)	34	–	–	–	–	–
Stromateus brasiliensis (DP)	73	–	–	–	–	–
Xystreurys rasile (B)	29	Mu (1)	3.4 (0.2-16.8)	0.0 (0.0-0.1)	1.0	1

*Molecularly identified as *Pseudoterranova cattani* George-Nascimento & Urrutia (2000).

8.4. DISCUSSION

8.4.1. Identification and morphometric variability of sealworm larvae from Patagonia, Argentina

Morphological characters for species identification in anisakid nematodes are few and are mostly used to differentiate adult specimens. Molecular genetic markers are necessary to reliably identify larval stages (Mattiucci & Nascetti, 2008). At present, most of the studies identifying sealworm larvae from fish using molecular tools have been performed in the Northern Hemisphere (*e.g.* Arizono *et al.*, 2011; Brattey & Davidson, 1996; Kellermanns *et al.*, 2007; Mattiucci & Nascetti, 2007; Mattiucci *et al.*, 1998), as these nematodes are responsible for great economic losses to the fishing industry (McClelland, 2002). In contrast, in the South Hemisphere, and especially along the South American coasts, accurate species-level identification of sealworm larvae infecting fish is still scarce.

Currently *P. cattani* is the only species of sealworm that has been identified using molecular tools in South America (Mattiucci & Nascetti, 2008; Zhu *et al.*, 2002). This species has been reported in 4 species of marine fish in the southeastern Pacific coast of Chile, *i.e.* corvina drum *Cilus gilberti* (Abbott, 1899), black cusk-eel *Genypterus maculatus* (Tschudi, 1846), south Pacific hake *Merluccius gayi gayi* (Guichenot, 1848) and flatfish *Paralichthys microps* (Günther, 1881) (Garcías *et al.*, 2001; George-Nascimento & Llanos, 1995). Preliminary genetic studies indicated that larvae of *P. cattani* also occur in fish from in the southwestern Atlantic coast of Argentina, including *A. patachonicus*, flatfish *Paralichthys patagonicus* Jordan, 1889 and Argentinian sandperch *Pseudopercis semifasciata* (Timi *et al.*, 2011a). Overall, geographical data of infection of *P. cattani* in fish are congruent with reports of adults in Chile (George-Nascimento & Urrutia, 2000), and the Patagonian coast of Argentina (Hernández-Orts *et al.*, 2013).

Molecular data obtained from 3 sealworm specimens collected from the most infected fish species, *P. nudigula*, indicate that these larvae can be identified as *P. cattani*. Larvae of *Pseudoterranova* found in other fish species of this study probably belong to a single species, *i.e. P. cattani*, as diagnostic traits are very similar. However, as mentioned before, a reliable identification of sealworm larvae species must be based principally on molecular genetic markers. Therefore we provisionally identify these larvae as *P*. cf. *cattani* until further morphological and molecular studies could be developed on sealworm larvae from these fish hosts of this area.

In the present study, sealworm larvae from *P. brasiliensis* and specially those collected from *A. patachonicus* were significantly smaller (including some internal organs) than those of *P. cattani* from *P. nudigula*. However, these morphometric differences could be related to three factors: *i*) the small number of specimens studied (8 sealworms from *A. patachonicus vs*. 80 from *P. nudigula*); *ii*) the degree of development of the sealworm larvae, *i.e.* recently recruited sealworm larvae from the invertebrate host are smaller (McClelland, 1995); and *iii*) different fish species could have a significant effect on the morphometric values of sealworm larvae. In this respect, George-Nascimento & Llanos (1995) reported small, but significant morphometric variations in L3 of *P. cattani* collected from different fish species from the Chilean coast. Moreover, experimental evidence revealed that growth rate, and therefore the morphometric variables, of sealworm larvae, differ with host species (McClelland, 1995). Thereafter, morphometric variables must be considered with caution when differentiating sealworm larvae species infecting different fish hosts from proximal localities.

Finally, we would like to stress the advantages of SEM to study dissected anisakids (**Fig. 23C**), and other nematodes in general cannot be underestimated. These SEM micrographs provide novel perspective on morphology and arrangement of the internal organs (*e.g.* the oesophagus and proximal intestine). Additionally, the observation of the internal organs with higher magnification could provide new diagnostic traits for the taxonomy of other nematode species.

8.4.2. Ecology of sealworms from the Patagonian coasts of Argentina

Knowledge on the life cycle of species belonging to the *P. decipiens* complex is scarce, and has mainly been proposed by natural and experimental evidence from sealworms and their hosts from the Northern Hemisphere. The complete life cycle of *P. decipiens* (*s.s.*) was summarized by McClelland (2002), and includes mainly copepods, macro invertebrates (*e.g.* polychaetes and decapods), fish and several species of pinnipeds. Although the host species of the life cycle may differ among sealworm species, we followed McClelland (2002) to elucidate some parts of the life cycle of sealworms inhabiting the Patagonian coast.

To our knowledge, the identity of the invertebrate hosts for sealworms is unknown along the southwestern Atlantic coasts. However, in this area, sealworms could possibly infect a wide range of invertebrates hosts (*e.g.* copepods, mysids, isopods, decapods, etc.), like it has been reported in other species of *Pseudoterranova* from the Northern Hemisphere (Marcogliese, 2001). In the present study, the most infected fish, *P. nudigula* (see **Table 15**), feeds on small benthic invertebrates, mainly crustaceans (Cousseau & Perrota, 2004). Therefore, characterization of the food habits and prey of this fish from the Patagonian coast, could help to elucidate the specific identity of the invertebrate hosts for *P. cattani* in this area.

According to our results, in the Patagonia coast *P. nudigula* seems to act as the primary fish hosts for sealworm larvae. Primary fish hosts are generally benthic consumers which acquire the parasite directly from invertebrate hosts and are essential in the temporal and spatial dispersion of the larvae (McClelland, 2002). In this area, other sympatric benthic fish, *i.e.* the banded cusk eel *Raneya brasiliensis* (Kaup, 1856) and flatfish *Paralichthys isosceles* Jordan, 1891 and *Xystreurys rasile* (Jordan, 1891), lack or have been reported with low intensities of sealworm larvae compared with those of *P. nudigula* (Vales *et al.*, 2011; see **Table 15**). Differences in the levels of sealworm infection on sympatric benthic fish are not surprisingly. Martel & McClelland (1995) reported significant differences in the abundance of sealworm larvae in three sympatric flatfish species from Canada which seems to be related to their food habits. Therefore, this could also be the main factor promoting differences in the infection levels of sealworms in benthic fish from the Patagonian coast.

Our results also suggest that *P. brasiliensis* could be considered as secondary fish host in the Patagonian coast due to the high intensity of sealworm larvae encapsulated in the muscles. Secondary fish hosts of sealworms are commonly large demersal fish which acquire the parasites by preying on small fish (McClelland, 2002). In other parasitological studies of large demersal fish from Patagonia, Timi &

Lanfranchi (2009b) reported high prevalence but low intensity of sealworm larvae in specimens of *P. semifasciata* (>60 cm TL) caught offshore from Península Valdés; while in the pink cusk-eel *Genypterus blacodes* (Forster, 1801) [syn. *G. brasiliensis* according to Froese & Pauly (2013)] (>40 cm TL) and the Brazilian sandperch *Pinguipes brasilianus* Cuvier, 1829 (>35 cm TL), sealworms reached the status of component species (prevalence >10%), although the mean intensities are considerably low (<2) (Sardella *et al.*, 1998; Timi *et al.*, 2008). Interestingly, the economically most important fish caught in the Patagonian waters, the Argentine hake *Merluccius hubbsi* Marini, 1933 also presented low prevalences of sealworms (<10%) in large specimens (>57 cm TL) (Herreras *et al.*, 2000). On the other hand, sealworms have also been reported in demersal cephalopods. Low prevalences of a L3 resembling *Pseudoterranova* sp. were reported from the Argentine shortfin squid *Illex argentinus* from northern Patagonia (González & Kroeck, 2000). Nevertheless, further parasitological surveys of different species of cephalopods are necessary in order to characterize the relative importance of these hosts in the biology and life cycle of sealworms.

Regarding pelagic fish from the Patagonian coast, sealworm larvae were not recorded infecting the Argentine anchovy, *Engraulis anchoita* (see Timi & Poulin, 2003), while low prevalences and intensities have been reported in the Silversides *Odontesthes smitti* (Lahille, 1929) and *Odontesthes nigricans* (Richardson, 1848) (Carballo *et al.*, 2011, 2012), and in the chub mackerel *Scomber japonicus* Houttuyn, 1782 (see **Table 15**). The mild infection of sealworms in pelagic fish could be related with the benthic early stages in the life cycle of these nematodes.

At present, 3 potential definitive hosts of sealworms inhabit the Patagonian coast of Argentina: 2 otariids, sea lion and South American fur seal; and one phocid, the Southern elephant seal *Mirounga leonina* (L.) (see Crespo *et al.*, 1999; Dans *et al.*, 2004; Lewis *et al.*, 1998; Túnez *et al.*, 2008b). In this area, larvae and adults of *P. cattani* have been reported from the intestine of both species of otariids, (Hernández-Orts *et al.*, 2013); while in elephant seals, a single adult specimen of *Pseudoterranova* sp. was collected from the intestine of a young female stranded in northern Patagonia (Hernández-Orts *et al.*, unpublished data). Although the 3 species of pinnipeds seem to be suitable definitive hosts for sealworms, the relative importance of these sympatric pinnipeds in the population dynamics of sealworms in this area is uncertain, as currently the abundance, morphology and fecundity of sealworms in these hosts are unknown.

In the Patagonian coast, pinnipeds will be infected with sealworm through the consumption of infected invertebrates, cephalopods or fish. However, for most of these pinnipeds species inhabiting this area, their food habits are still unknown, and therefore, the transmission strategies of sealworm larvae cannot be elucidated. At present, only the food habits of sea lion have been characterized in detail from the Patagonian coast of Argentina (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011). According to these studies, sea lions feed mainly on *M. hubbsi*, *R. brasiliensis*, *E. anchoita*, and choicy ruff *Seriolella porosa* Guichenot, 1848. Interestingly, sealworm larvae were not recorded, or have been reported in low intensities, in these species of fish along the Patagonian coast (Guagliardo *et al.*, 2009; Herreras *et al.*, 2000; MacKenzie & Longshaw, 1995; Sardella & Timi, 2004; Timi & Poulin, 2003; Vales *et al.*, 2011; **see Table 15**). Moreover, sea lions, consumed mainly smaller fish (<35 cm TL), occasionally medium size fish (>50 cm TL) and rarely large fish (>65 cm TL) (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011; N. A. García unpublished data). Therefore large fish, with higher densities of sealworm larvae, which could be considered as a potential route of transmission for sealworms, likely may act as a significant population ‘sink’ in the parasite life cycle, as they act as physical barrier for their transmission.

The present study suggests that the most important fish prey for sea lion in the Patagonian coast of Argentina, *i.e. E. anchoita*, *M. hubbsi and R. brasiliensis* (Koen-Alonso *et al.*, 2000; Romero *et al.*, 2011), present low levels of sealworm infections. However, for sea lions, transmission and recruitment of sealworms would be ensured by the high quantity of ingested prey [especially in the case of *M. hubbsi*, see Koen-Alonso *et al.* (2000) and Romero *et al.* (2011)], even when they are slightly infected. Nevertheless, sea lions could also acquire heavy sealworm infections sporadically by foraging on small benthic fish with high prevalences [*e.g. P. nudigula* represents the 0.29% of the percentage by number of the diet composition of sea lions according to Romero *et al.* (2011)], or by occasionally preying large fish with high intensities of sealworm larvae. Finally we cannot exclude, to a lesser extent, direct transmission of sealworm larvae between invertebrate hosts to definitive hosts, as there also records of some species of invertebrates in the food habits of sea lions from Patagonia (Koen-Alonso *et al*., 2000; Romero *et al*., 2011).

8.4.3. Microhabitat of sealworm larvae in fish from the Patagonian coasts of Argentina

Distribution in fish host tissue also apparently differs between species of *Pseudoterranova* (see McClelland, 2002). In other localities in the Southern Hemisphere, Palm (1999) reported that the preferred site of infestation was the body cavity and the liver for sealworm larvae in fish from the Antarctic waters. Our results suggest that the main microhabitat of sealworms in Patagonia is the muscle, and particularly those in the epaxial region for L3 of *P. cattani* infecting *P. nudigula*. One could wonder whether larval distribution could be affected by post-mortem migration of sealworms. However, this seems unlikely because, all larvae were found encapsulated. On the other hand, according to the available evidence, apparently the main microhabitat of sealworms in South America is also the muscles; however, these tissues are not systematically analysed for parasites in this area (see **Table 11**). This may cause that the infection parameters of sealworm larvae in many studies could be underestimated. To date, several methods are available to detect larval sealworms in fish muscles (McClelland, 2002), therefore we urge researchers to consider this location in further parasitological studies to provide complete information about the sealworm larvae in the marine fish helminth communities, what moreover will be particularly useful for stock discrimination studies.

8.5. CONCLUSION

In conclusion, in this study we provide an overview of the current state of knowledge on the taxonomy and ecology of sealworm larvae in the southwestern Atlantic. We provided the first molecular identification, description and microhabitat characterisation of sealworm larvae from the Patagonian coast of Argentina. Additionally, we reported the infection levels of sealworms on 20 fish species in order to elucidate the life cycle of these nematodes in this area. We are aware that our ecological results convey a rather static picture of the dispersion of sealworm larvae for a short period of time. Furthermore, for some species of fish, few specimens could be collected (see **Table 12**) and, therefore, sealworm larvae might not have been recorded due to low sample size. Further studies on sealworm from invertebrates, fish and pinnipeds hosts from this area are necessary to understand the systematic, biology and population dynamics of this nematode. However, this study provides a starting point to investigate the life cycle of sealworms in the Argentine Patagonian coast.

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9. CONCLUSIONS

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In the present study, the intestinal helminth fauna of 56 South American sea lions, *Otaria flavescens* (30 males and 26 females), and 5 South American fur seals, *Arctocephalus australis* (4 males and 1 female), from the Patagonian coast in Argentina was characterized for the first time. Additionally, a total of in 542 marine teleosts from 20 species collected from the Patagonian coast were also analysed for helminths, including larval forms of parasites from sea lions and fur seals. As a result of the investigations carried out in the present study, the following conclusions were drawn:

9.1. The intestinal helminth fauna of *O. flavescens* from the Patagonian coast in Argentina comprised 11 taxa (1 trematode, 1 cestode, 5 nematodes and 4 acanthocephalans). Gravid individuals were represented by 6 species: *Ascocotyle* (*Ascocotyle*) *patagoniensis*, *Contracaecum ogmorhini* (*s.s.*), *Corynosoma australe*, *Diphyllobothrium* spp., *Pseudoterranova cattani* and *Uncinaria hamiltoni*. Third-stage larvae of *Anisakis* sp. type I and *Contracaecum* sp., and juvenile specimens of *Andracantha* sp., *Corynosoma cetaceum* and *Profilicollis chasmagnathi* were also collected. Four of these parasites species, *Andracantha* sp., *A.* (*A.*) *patagoniensis*, *C. ogmorhini* (*s.s.*) and *P. chasmagnathi* represent new host records.

9.2. From the intestine of *A. australis* from the Patagonian coast, a total of 7 parasite taxa were collected (2 cestodes, 3 nematodes and 2 acanthocephalans). Gravid individuals were represented by 4 species of parasites: *C. ogmorhini* (*s.s.*), *C. australe*, *Diphyllobothrium* spp., and *P. cattani*. Third-stage larvae of *Contracaecum* sp. and juvenile specimens of *C. cetaceum* were also collected.

9.3. In northern Patagonia, sea lions and fur seals harbour the intestinal helminth communities that could be predicted for otariids worldwide, *i.e.* the combination of species of the genera *Corynosoma*, *Diphyllobothrium*, *Pseudoterranova*, *Contracaecum* and, in pups, *Uncinaria*.

9.4. The estimation of helminth community parameters in sea lions and fur seals, especially species richness at the component community level, was affected by the inclusion or exclusion of parasites for which both species of otariids are putative non-hosts (*i.e.* hosts in which the parasite is unable to reproduce). This study demonstrates that the inclusion of these taxa can exert a significant influence on some community parameters.

9.5. A new species of a heterophyid trematode was described from the intestine of South American sea lions. A detailed morphological and morphometrical analysis of specimens of *Ascocotyle* (*Ascocotyle*) *patagoniensis* Hernández-Orts, Montero, Crespo, García, Raga and Aznar, 2012 suggests that this trematode can be distinguished from the other species of the subgenus by the number of circumoral spines, which are arranged in 2 rows of 18 to 23, by having a gonotyl without papillae, and by their widest seminal receptacle.

9.6. No metacercariae of *Ascocotyle* spp. were found in 542 marine teleosts from 20 species collected along the Patagonian Shelf. The absence of metacercariae in marine fish inhabiting this area shelf could be related to the fact that the life cycle of this trematode is restricted to littoral waters. However, more fishes should be analysed to confirm this hypothesis as the small metacercariae could have been overlooked, mainly in host species with small sample sizes.

9.7. A total of 1,367 cystacanths of *C. australe* was collected in 18 species of marine fish from the Patagonian coast. The most infected fish species with n ≥ 15 were as follows: *Acanthistius patachonicus*, *Paralichthys isosceles*, *Prionotus nudigula*, *Raneya brasiliensis* and *Xystreurys rasile*. Eight fish species, *i.e. A. patachonicus*, *Brama brama*, *Congiopodus peruvianus*, *Cottoperca gobio*, *Genypterus blacodes*, *Patagonotothen ramsayi*, *Seriolella porosa* and *Stromateus brasiliensis* represent new host records for *C. australe*.

9.8. Results of this study demonstrate that cystacanths of *C. australe* are able to infect and colonize a wide array of fish species, which would act as paratenic hosts. The ubiquity of this acanthocephalan through the trophic web would guarantee infections to their definitive hosts through alternative pathways. Nevertheless, this study suggest that

R. brasiliensis, is one of the prey that most likely contributes to the transmission of cystacanths of *C. australe* in this area, due to both the high prevalence in this fish species, and its high relative importance in the diet of sea lions and fur seals.

9.9. There were significant differences in the levels of infection of cystacanths of *C. australe* between fish inhabiting different zones of the water column, being the ones associated with benthic zone those with highest cystacanth infections. This study suggests that at least 2 main factors could be directly promoting differences in the infection levels of *C. australe* between fish from different zones: *1*) distribution of the invertebrate intermediate hosts; and *2*) patterns of transmission of cystacanths between paratenic fish hosts through food webs.

9.10. The overall sex ratio of cystacanths of *C. australe* infecting fish hosts was slightly, but significantly, female-biased and no significant differences were found among fish species. This suggests that the sex ratio would begin to be biased before individuals of *C. australe* infect the definitive host, in which the sex ratio is known to become strongly female-biased because females have a longer life span. In other words, part of the biased sex ratio that is observed in the definitive hosts would be already transferred from paratenic hosts. In theory, 3 factors could be involved in generating the sex ratio biases in our sample, namely, sampling error, differential sampling of female and male larvae, and/or differential mortality between the sexes.

9.11. This study investigates, for the first time, the potential costs that trophically-transmitted helminths may face in paratenic-to-paratenic transmission. The results suggest that some fish species, in particular *Acanthistius patachonicus*, might actually be unsuitable paratenic hosts for *C. australe* since most cystacanths found in this species were not viable. Also, a slight, but statistically significant, tendency to decrease body size of cystacanths was observed as the trophic level of fish hosts species increased. This tendency, which was not related to crowding effects, appears to suggest that *C. australe* may incur in non-negligible energetic costs when experiencing putative paratenic-to-paratenic transmission. The implications of this finding cannot be underestimated, since this negative consequence may have an important role on the population dynamics of trophically-transmitted helminths.

9.12. The present study compared, for the first time using inferential statistics, the size of holdfast structures between cystacanths and adults of acanthocephalans. The results suggest that the size of trunk spines grows between cystacanths and adults of *C. australe* and an allied species infecting cetaceans, *C. cetaceum*, but only in females, which also had significantly larger spines than males. However, this sexual dimorphism did not result from pure allometry since the body of females was smaller, and did not grow more than that of males. Nevertheless, females have longer lifespan, and therefore this factor would induce different investment and development schedules for spines, in order to withstand the extreme flow conditions prevailing in marine mammals for longer time.

9.13. Unexpectedly, the patterns of spine growth appear also to differ between both species of *Corynosoma*. In *C. cetaceum* fore-trunk spines and hind-trunk spines grew, whereas in *C. australe* only fore-trunk spines differed between cystacanths and adults. An explanation of these differences is that females of *C. cetaceum* fine-tune the size of spines during the development in the definitive hosts because they achieve a larger adult size, a trait that correlates with stronger dislodging forces and, possibly, with a longer lifespan.

9.14. This study sheds light on the question of whether or not the holdfast of acanthocephalans is fully developed prior to entering the definitive host. It suggests that temporal allocation of investment in attachment structures may differ, not only between congeneric species, but also between sexes of the same species, possibly due to the different selective pressures that each population subset faces.

9.15. A total of 635 encapsulated third-stage larvae of *Pseudoterranova* (sealworm larvae) were collected from 12 species of marine fish from the Patagonian coast. The most infected fish species with sealworm larvae was *P. nudigula*, followed by *A. patachonicus*, *P. isosceles*, *Percophis brasiliensis* and *Pseudopercis semifasciata*. Five species of fish, *i.e. C. gobio*, *Nemadactylus bergi*, *M. argentinae*, *P. brasiliensis* and *P. nudigula* represent new host records for larval sealworms.

9.16. Sequences obtained for the mitochondrial cytochrome c oxidase subunit 1 gene (cox 1) of sealworms from the red searobin, *P. nudigula*, formed a reciprocally monophyletic lineage with published sequences of *P. cattani* from definitive hosts. A detailed morphological and morphometrical description of larvae of *P. cattani* from the red searobin is provided. On the other hand, sealworm larvae from other fish species did not differ morphologically from L3 of *P. cattani* from the red searobin. However, the results of the comparative morphometric analyses carried out on larvae from different fish hosts indicated significant differences in some distances. However, we provisionally identified all larvae as *P.* cf. *cattani*, awaiting further identification based on molecular genetic markers.

9.17. The results of this study suggest that the main microhabitat for sealworm larvae infecting fish hosts from Patagonia is the muscle (principally the epaxial musculature, followed by the hypaxial muscles), and to a lesser degree, in the mesenteries and liver.

9.18. The lines of evidence obtained in this study suggest that most important fish prey of otariids inhabiting the Patagonian coast presented low infection levels of sealworm larvae. Given that *P. cattani* is specific to otariids, transmission of this nematode appears to rely on the catholic diet of both sea lions and fur seals, which include a number of specimens of many fish species from the benthic realm, where transmission most likely occurs.

10. FURTHER PARASITOLOGICAL STUDIES

10.1. WHAT OTHER ISSUES SHOULD BE ADDRESSED ON HELMINTHS FROM SEA LIONS AND FUR SEALS?

The present study provides ecological data on the intestinal helminth communities of two South American otariids, as well as on morphological and taxonomical aspects of the parasites from these marine mammals. However, although our conclusions were derived from a considerable large sample size of South American sea lions collected over an extended time frame, the structure of their helminth communities is inextricably linked to host age, diet or migration patterns, which may significantly vary in time and space. Therefore, to obtain a more complete picture of their helminth communities, and to study possible changes in its structure, we recommend continuing routinel parasitological analyses of sea lions from the Patagonian coast. These studies could provide an extensive database, which would also allow the use of parasites as biological tags to establish whether sea lions form isolated populations units (stocks), or to elucidate historical changes related to their food habits and differences in the foraging strategy between sexes. Furthermore, these data will provide more information on the wide spectrum of parasites that apparently cannot reproduce in both species of otariids, as well as their recruitment dynamics. On the other hand, it is necessary to continue studying the helminth fauna of South American fur seals, based on much larger sample sizes, to obtain a more complete picture of their parasite communities.

There are other ecological and taxonomical issues on parasites of sea lions and fur seals that were not included in this study but can be carried out in short term. In this respect the following studies are recommended:

- **Ecological issues:**

  1. To increase our knowledge of the gastric helminth communities, especially those of the anisakid nematodes of the genus *Contracaecum* and *Pseudoterranova*, helminth parasites from the stomach of South American sea lions and fur seals should be collected, identified and counted.

2. To complete the results on the ecology of the cystacanths of *C. australe* obtained in the present work, we suggest to study the recruitment, population structure and habitat selection of this acanthocephalan in the intestine of South American sea lions and fur seals, to widen our understanding of the population dynamics of this parasite in paratenic and definitive hosts. In fact, we have already collected data about sex, maturity stage and linear distribution from ca. 30,000 individuals of *C. australe* from 19 South American sea lions and 4 fur seals for future statistical analysis.

3. To assess host-parasite specificity and allow to explore the relative importance of different hosts in the population dynamics of *C. australe*, a comparative study on the abundance, morphological traits and fecundity of this acanthocephalan from South American sea lions, fur seal and other potential definitive hosts, *i.e.* the Southern elephant seal *Mirounga leonina* L. and the Magellanic penguin *Spheniscus magellanicus* (Forster, 1781) should be carried out. In fact, data have already been gathered from all these hosts, in collaboration with Brazilian researchers, and will be analysed soon. These studies should also be performed for other groups of parasites, like nematodes (*e.g. Pseudoterranova* spp.) or cestodes (*e.g. Diphyllobothrium* spp.) which infect different species of definitive hosts in the area.

- **Taxonomical issues**

1. To establish whether specimens of the genus *Anisakis* are able to mature and reproduce in otariid hosts, as well as to assess host–parasite specificity of this nematode in pinnipeds, a detailed morphological and molecular study on specimens of *Anisakis* spp. infecting the stomach of both species of otariids should be carried out.

2. Because of the conservation and fixation methods used to preserve the specimens of *Ascocotyle* (*A.*) *patagoniensis* analysed in the present study, some morphological traits could not be described in detail (*e.g.* the reproductive system or the gonotyl). A morphological study, including scanning electron micrographs of the circumoral spines, from new material

collected from fresh sea lions would provide new data on the morphology and taxonomy this species.

3. Clearly, more research is needed on the taxonomic status of cestodes infecting South American sea lions and fur seals, *i.e. Diphyllobothrium* spp. and Tetrabothriidae gen. sp. in the southwestern Atlantic coast. The collection of morphological, ecological and molecular data from new material would allow ascertaining the identity of the larval an adult forms of these tapeworms.

10.2. FURTHER STUDIES ON LARVAL FORMS OF HELMINTHS FROM OTARIIDS INFECTING INTERMEDIATE/PARATENIC HOSTS

A high diversity of species of cephalopods and fish inhabit the Patagonian coast of Argentina. However their helminth fauna is unknown or has been poorly studied for most of them, especially those species considered unmarketable by the fisheries. Therefore, parasitological analysis of these hosts should provide useful data to increase our knowledge on the population dynamics and the relative importance of each host species for the transmission of infective stages of parasite from pinnipeds. We strongly suggest to perform a more comprehensive parasitological analysis the helminth fauna of the fish species examined in the present study, in order to 1) detect changes in the intensity and/or frequency of these larval forms during a longer periods of time, and 2) establish infection parameters with more precision, particularly in those species with small sample size in the present study.

Other studies that could be performed in intermediate/paratenic hosts are the following:

1. A parasitological analysis of octopuses, such as southern red octopus, *Enteroctopus megalocyathus* or the small Patagonian octopus *Octopus tehuelchus* d'Orbigny, 1834, both of which are essential part of the diet of Patagonian otariids and from which their metazoan parasite fauna is unknown.

2. Parasitological studies are recommended in fish from brackish and coastal waters to explore whether the life cycles of helminth species infecting sea lions or fur seals (*e.g.* trematodes of the genus *Ascocotyle*) are linked to not strictly marine habitats.

3. Morphological and molecular data from third stage-larvae of species of *Contracaecum* from various fish species should be collected to get a complete picture of the actual diversity of species of these nematodes in fish from the Patagonian coast of Argentina, and to describe the possible route of transmission of *C. ogmorhini* s.s. to their otariid definitive hosts.

4. Specificity of species *Corynosoma* to different paratenic fish host could be investigated by studying the morphology of the capsules surrounding the cystacanth when they infect the teleost (see Skorobrechova & Nikishin, 2011 for more details). Furthermore, we also recommend carrying out a histological study to describe the degradation process of encapsulated cystacanths of *C. austral*, which has been observed exclusively in the Patagonian grouper, *A. patachonicus.*

5. More research is needed to clarify the negative consequences of the paratenic-to-paratenic transmission of trophically-transmitted helminths. Our suggestion would be exploring, through inferential statistics, the relation between size of the cystacanths (and, by extension, of any larva of trophically-transmitted helminth) and the trophic level of the different paratenic species where the cystacanths (or other larvae) were collected. This study will allow confirming whether the tendency of body size decrease, thus indicating the putative energetic costs associated to paratenic-to-paratenic transmission. Additionally, we also recommend carrying out experimental research on the transmission of helminths (e.g. cystacanths of *C. australe*) between fish paratenic hosts in order to obtain: *1*) experimental evidence on transmission of cystacanths between paratenic hosts; *2*) further evidence bearing on the issue that growth or development do not occur in acanthocephalans infecting paratenic hosts; and *3*) whether there are decreases in body size under controlled conditions.

6. A detailed study of the food contents from fish which acts as intermediate/paratenic hosts for otariid helminths would help to elucidate the specific identity of the other hosts and thus facilitate their sampling.

10.3. OTHER STUDIES ON GASTROINTESTINAL HELMINTHS FROM OTARIIDS

One of the major issues that should be addressed in upcoming studies has to do with the early stages in the life cycle of helminths from sea lions and fur seals. In particular, we are eager to obtain basic data, including a the morphological and molecular characterization of all larval stages of all parasite species, in order to elucidate their life cycles, which is a basic step to track transmission patterns through the marine food web. Therefore sampling efforts should primarily be focused on collecting pelagic and benthic invertebrates and examine them for larval forms using morphological and molecular methods. Nevertheless, given low prevalence reported in other putative helminth species infecting intermediate invertebrate hosts in the marine realm (*e.g.* Gregori *et al*., 2012; Laskowski *et al.*, 2010; Marcogliese, 2001; Martell & McClelland, 1995; Zdzitowiecki & Presler, 2001), laboratory research should not be ruled out in order to explore the ontogeny and transmission of these parasites.

Finally, the collection larval stages of acanthocephalans of the genus *Corynosoma* from the invertebrate intermediate hosts would allow obtaining novel data on the ontogeny of these parasites. This would help answering fundamental questions about patterns of growth of holdfast structures, and about the processes that may govern these patterns before paratenic hosts are infected.

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11. REFERENCES

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