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Larval parasite gene sequence data reveal cryptic trophic links in life cycles of porbeagle shark tapeworms

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ABSTRACT: The lack of information on marine tapeworm life cycles can be addressed with knowledge derived from trophic interactions in marine environments. These trophically transmitted parasites exploit transmission routes involving predator–prey interactions. Porbeagle sharks *Lamna nasus* are apex predators feeding on a wide range of organisms, including teleosts and cephalopods. Although the biology of this shark species is relatively well studied, there is a surprising lack of information about the trophic interactions involving this species that lead to the acquisition of tapeworms. Recently, the use of molecular tools, combined with phylogenetics, has proven useful in identifying trophic links involved in the transmission of marine tapeworms. In the present study, we used sequence data from the D2 domain of the large subunit ribosomal DNA to link adult tapeworms of the species *Clistobothrium* cf. *montaukensis* and *Dinobothrium* sp. parasitising porbeagle sharks to larvae recovered from the squid *Doryteuthis gahi*. To the best of our knowledge, it is the first to provide empirical evidence for a trophic link between porbeagle sharks and *D. gahi* as a definitive route for the successful transmission of these tapeworms. Furthermore, our data suggest an ontogenetic shift in diet away from squid. Parasite abundance data in *D. gahi* indicate that the abundance of porbeagle sharks can be significant in some years.

KEY WORDS: Life cycle · *Lamna nasus* · Tetraphyllidea · *Clistobothrium* · *Dinobothrium* · *Doryteuthis gahi* · D2 domain · Large subunit ribosomal DNA · Falkland Islands

INTRODUCTION

Knowledge of trophic interactions within a study system can provide clues for identifying the various transmission routes exploited by trophically transmitted parasites. The presence of an adult parasite in the gut of the definitive host and of conspecific larvae in an intermediate host provides convincing evidence for a trophic link between both hosts. Until recently, our understanding of life cycles of marine tapeworms has been hindered by our inability to identify larvae because of the lack of suitable morphological features necessary for their specific identification (Olson & Tkach 2005). With the notable exception of the Trypanorhyncha, larval tapeworms possess rudimentary features that make it difficult to assign them confidently to species or even genera (see Jensen & Bullard 2010). The advent of molecular tools has enabled us to overcome these difficulties when sequence data from adult voucher material are available (Olson & Tkach 2005). For instance, Jensen & Bullard (2010) sampled numerous marine vertebrate and invertebrate taxa for tapeworm larvae, encompassing a variety of potential prey items of elasmobranch fishes in the Gulf of Mexico. Using a molecular approach, they successfully identified organisms that played a role in the trophic transmission of these tapeworms to their definitive hosts. However, a major limitation of this approach is the paucity of molecular data for most adult marine tapeworms (Brickle et al. 2001, Agusti et al. 2005, Aznar et al. 2007, Jensen &

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Bullard 2010). As of August 2010, fewer than 20% of described marine tapeworm taxa have been characterised molecularly (authors' unpubl. obs.). The probability of finding sequence matches between larvae and adults is therefore relatively low. Consequently, there are some larval sequences in GenBank that are unmatched to adult forms (Brickle et al. 2001, Agusti et al. 2005, Aznar et al. 2007, Jensen & Bullard 2010). For instance, one plerocercoid and one merocercoid from the squid *Doryteuthis gahi* were molecularly characterised at the 5′ end of the large subunit ribosomal DNA (D1–D3 LSU rDNA), but neither matched those of adult forms (Brickle et al. 2001).

Brickle et al. (2001) surveyed nearly 1100 individual squid *Doryteuthis gahi* and revealed the presence of larval helminths. Most of these consisted of larval tetraphyllideans and, for reasons mentioned above, these were not identifiable to species. Using molecular tools combined with a phylogenetic approach, one larval type showed close affinity to the genus *Clistobothrium* Dailey & Vogelbein, 1990 and the other to *Ceratobothrium* Monticelli, 1892 (Brickle et al. 2001). However, their specific identification could not be confirmed because of the lack of representative taxa available from GenBank. Nevertheless, both these genera are restricted to sharks of the family Lamnidae Müller and Henle, 1838 (Ruhnke 1993). It thus became clear that these tetraphyllideans use lamnid sharks (e.g. great white, mako and porbeagle) as definitive hosts and it was further hypothesised that porbeagle sharks *Lamna nasus*, the only lamnid known from the southern Patagonian Shelf (P. Brickle pers. obs. in Brickle et al. 2001), were the definitive host for these larval parasites. At this time, it was not known to the Falkland Islands Government Fisheries Department (FIFD) that these sharks may be a predator of *D. gahi*.

The porbeagle shark *Lamna nasus* (Bonnaterre, 1788) is a large predatory pelagic shark. This endothermic shark is capable of maintaining its body temperature 7 to 10°C above ambient water temperatures (Carey & Teal 1969), thus allowing this species to inhabit cold temperate waters ranging between 1 and 18°C (Compagno et al. 2005). Its diet consists primarily of teleosts and cephalopods (Ellis & Shackley 1995, Compagno 2001, Joyce et al. 2002, Francis et al. 2008). Because of its small litter size and long maturation time, this shark is susceptible to overfishing (Jensen et al. 2002, Natanson et al. 2002). Accordingly, the International Union for Conservation of Nature (IUCN) lists this species as globally threatened (Stevens et al. 2006), although regional assessments vary from endangered in the northwestern Atlantic to critically endangered in the northeastern Atlantic and Mediterranean Sea (Stevens et al. 2006). Little is known about the stock status for this species in the Southern Hemisphere (Francis et al. 2008).

The objectives of the present study were to: (1) identify the tapeworms from porbeagle sharks on the Falkland Islands shelf; (2) determine whether larval tapeworms sequenced by Brickle et al. (2001) from the squid *Doryteuthis gahi* do indeed match the tapeworms recovered from porbeagle sharks sequenced as part of this study; and (3) gain insights into the life cycles of tapeworms infecting porbeagle sharks and the importance of the shark–squid (predator–prey) trophic link. Because there are no gene-sequence data available from adult tapeworms from porbeagle sharks, we sequenced the adult worms to determine whether they match any of the sequence data from unidentified larvae available on GenBank.

MATERIALS AND METHODS

Parasite material was recovered from the spiral valves of 11 porbeagle sharks (7 males, 3 females and 1 unsexed individual), ranging in size (total length) from 132 to 218 cm (n = 10), collected in waters sur-

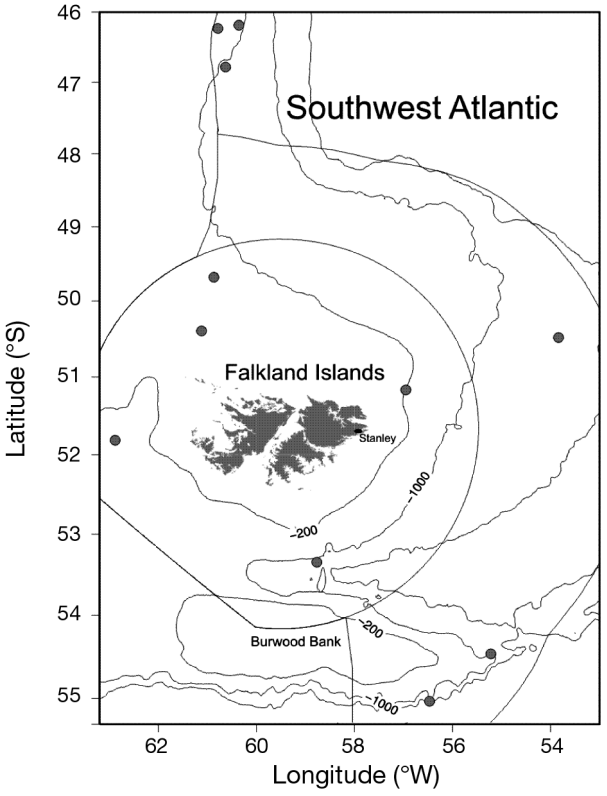

Fig. 1. Locations (circles) around the Falkland Islands where the 11 porbeagle sharks were collected for this study between 15 May 2003 and 8 March 2009. Contour lines illustrate bathymetry in metres and the lines surrounding the Falkland Islands illustrate the position of the Falkland Islands Interim Conservation Zone (inner) and the Falkland Islands Outer Conservation Zone (outer)

rounding the Falkland Islands (Fig. 1) as bycatch from commercial long-liners and trawlers between 15 May 2003 and 8 March 2009. The spiral valves were removed from the sharks while at sea by FIFD fisheries observers, bagged individually and frozen. Prior to examination for parasites, spiral valves were thawed in a refrigerator overnight and then opened with a mid-ventral incision through the whorls of mucosa, from the rectum to the pyloric portion of the stomach along the ventral blood vessel, and cut into 4 to 12 pieces (depending on the size of the specimen). Each piece was placed in a 1 l container filled with saline (2 parts seawater:9 parts tapwater) to which was added 1 tablespoon of sodium bicarbonate to free the parasites from mucus. The containers were vigorously shaken and allowed to rest for at least 4 h. The piece of spiral valve, solution and sediment were examined using a binocular dissecting microscope. Parasites were cleaned in saline prior to being processed. Parasites used for molecular analyses and scanning electron microscopy (SEM) were fixed in 95% ethanol (n = 675) and 4% buffered formalin solution (n = 283), respectively. SEM material (7 tapeworms) was post-fixed in 1% osmium tetraoxide for 2 h prior to being dehydrated through a graded series of ethanols, critical point dried in a Bal-Tec CPD-030 critical point dryer in carbon dioxide, mounted on stubs using double-sided adhesive carbon tape and sputter coated with Au/Pd to a thickness of 12 nm using an Emitech K575X Peltier-cooled high resolution sputter coater (EM Technologies) fitted with an Emitech 250X carbon coater. Specimens prepared for SEM were examined with a Cambridge 360 scanning electron microscope fitted with a Dindima Image Slave frame grabber at the Otago Centre for Electron Microscopy (OCEM, University of Otago, New Zealand).

Molecular characterization and analysis. Molecular sequence data were obtained from 4 individual tapeworms. Genomic DNA was extracted from the anterior portion of the strobila using standard techniques (Devlin et al. 2004). The region encompassing the D2 domain of the LSU was amplified with primers T16 (5′-GAG ACC GAT AGC GAA ACA AGT AC-3′) and T30 (5′-TGT TAG ACT CCT TGG TCC GTG-3′) (Harper & Saunders 2001) using BioLine DNA polymerase (Total Lab Systems) with a ca. 750 bp target length. The amplification protocol consisted of an initial 5 min denaturation phase (94°C); 38 cycles of denaturation (30 s at 94°C), primer annealing (30 s at 50°C) and extension (2 min at 72°C), and a 7 min final extension (72°C). The amplicons were gel-purified using the Omega Ultra-Sep gel extraction kit (Ngaio Diagnostics), cycle-sequenced using the ABI PRISM® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit v.3.1 and bidirectionally sequenced using the PCR primers on a 96 capillary 3730XL DNA Analyzer (Applied Biosystems). Sequence data were edited using Sequencher 4.9™ (GeneCodes) and screened using BLASTn (McGinnis & Madden 2004) to confirm orthology with the LSU of tetraphyllidean tapeworms. Sequences are available from GenBank under accession numbers JF436969 to JF436972. The scoleces and, when available, posterior region of the strobilae were preserved in 95% ethanol to serve as hologenophore (sensu Pleijel et al. 2008) and deposited with the Natural History Museum, London, UK (NHMUK 2011.3.17.1, NHMUK 2011.3.17.12, NHMUK 2011.3.17.23, and NHMUK 2011.3.17.25, respectively). Vouchers were deposited with the Natural History Museum, London, UK (NHMUK 2011.3.17.2-11, NHMUK 2011.3.17.13-22, NHMUK 2011.3.17.24, and NHMUK 2011.3.17.26-35), and the New Brunswick (NB) Museum, Saint John, NB, Canada (NBM-010218-NBM 010221).

The parasite alignment consisted of the 4 new LSU sequences, from adult tetraphyllideans parasitising *Lamna nasus*, encompassing the D2 domain (622 bp) combined with 20 previously published sequences of tetraphyllidean tapeworms, including 5 larval gene sequences from tetraphyllideans parasitising *Doryteuthis gahi* and cetaceans, which have yet to be matched with those from adult tapeworms (Brickle et al. 2001, Aznar et al. 2007). The outgroup consisted of 2 species of *Acanthobothrium* Van Beneden, 1850. Sequences were subsequently aligned using MacClade 4.07 (Maddison & Maddison 2005). Ambiguous regions, or those containing gaps across most sequences, were excluded from the data set. Modeltest 3.7 (Posada & Crandall 1998, Posada & Buckley 2004) determined the best nucleotide-substitution model for the data. The generalized time-reversible (GTR) model with a proportion of invariable site (I) was determined to provide the best fit to the data based on Akaike's Information Criterion (AIC). Bayesian inference was performed using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001) using the covarion option according to a GTR+I nucleotide-substitution model with no initial values assigned and with empirical nucleotide frequencies. Four separate Markov chains were used to estimate posterior probabilities over 2×10^6 generations, sampling the Markov chains at intervals of 100 generations. The first 4000 trees were discarded as burn-in and then a 50% majority-rule tree was constructed from the subsequent trees. Nodal support was estimated as the mean posterior probabilities (Huelsenbeck et al. 2001) using the sumt command.

RESULTS

A total of 958 helminths were recovered from the spiral valves of 10 of the 11 porbeagle sharks; thus 1 indi-

vidual was free of parasites. These were assignable to 3 different tapeworm species and 1 nematode species. One of these tapeworms was assigned to the genus *Clistobothrium*. Although no complete strobilae were recovered because of the advanced state of decay, these worms were unequivocally assigned to this genus on the basis of scolex morphology, i.e. 4-stalked bothridia with a cruciform apical region (Fig. 2A) and distinguished from *C. montaukensis* Ruhnke 1993, the latter possessing a dome-shaped apical region (Ruhnke 1993). A second species was assigned to the genus *Dinobothrium* Van Beneden, 1889 on the basis of scolex morphology and host. It is identified, herein, as *Dinobothrium* sp. in the absence of suitable material from which to describe the reproductive anatomy for comparison with other species of the genus (Fig. 2B). The third tapeworm was identified as *Dinobothrium septaria* Van Beneden, 1889 on the basis of its size, scolex morphology and host (see Baylis 1950) (Fig. 2C). The nematode species has yet to be identified. The prevalence and intensity of infection (range) data for each species are presented in Table 1.

The alignment included 622 sites, of which 17 were excluded. Both *Clistobothrium* cf. *montaukensis* individuals shared LSU rDNA sequences that were identical at the D2 region and identical to those of plerocercoids recovered from *Doryteuthis gahi* (Brickle et al. 2001) (Fig. 3). These showed close affinity with *C.* cf. *montaukensis*. Furthermore, the dissimilarity between sequences encompassing the D2 domain within the *Clistobothrium* clade, expressed as percentage of nucleotide difference, was between 0.00 and 1.61%. The sequence from *Dinobothrium* sp. was identical to that of the merocercoid recovered from *D. gahi* by Brickle et al. (2001), whereas the *D. septaria* sequence differed from that of the merocercoid by 1 A–T transversion and 1 C–T transition (0.32%). Both *Dinobothrium* species showed close affinity to *Ceratobothrium xanthocephalum* Monticelli, 1892. Interrelationships between tetraphyllidean taxa, based on Bayesian inference (Fig. 3), were similar to those obtained by Aznar et al. (2007) and Randhawa (2011).

Spearman's rank correlation analyses reveal that the abundance of *Clistobothrium* cf. *montaukensis* was significantly negatively correlated with porbeagle total length ($r_S = -0.705$, $p = 0.0268$; Fig. 4A), whereas *Dinobothrium* sp. abundance was weakly negatively correlated with porbeagle total length ($r_S = -0.328$, $p = 0.3487$; Fig. 4B). Low abundance of *D. septaria* prevented us from performing similar analyses using this taxon.

DISCUSSION

The molecular evidence of the present study demonstrates empirically that the squid *Doryteuthis gahi* harbours larval tapeworms assignable to *Clistobothrium* cf. *montaukensis* and *Dinobothrium* sp. infecting porbeagle sharks. Our survey of helminth parasites of porbeagle sharks off the Falkland shelf recovered 3 tapeworm species: *C.* cf. *montaukensis*, *Dinobothrium* sp. and *D. septaria.* Based on a molecular approach using phylogenetic methods, we found that 2 larval

Table 1. Summary of the prevalence (proportion of infected individuals) and intensity of infection (mean number of parasites per infected individual) of helminths recovered from porbeagle sharks

Parasite species	Prevalence (%)	Intensity of infection (range)
Clistobothrium cf. *montaukensis*	81.8 (9 of 11)	22.6 (2–70)
Dinobothrium sp.	81.8 (9 of 11)	75.7 (4–204)
D. septaria	27.3 (3 of 11)	3.3 (1–8)
Nematoda gen. sp.	45.5 (5 of 11)	7.0 (1–26)

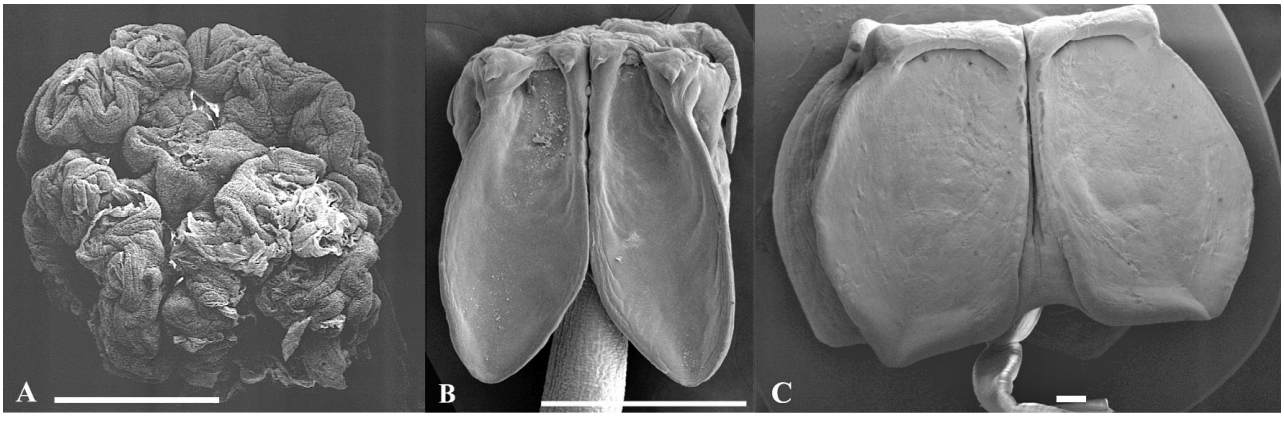

Fig. 2. Scanning electron micrographs of the scoleces of tapeworms from porbeagle sharks: (A) *Clistobothrium* cf. *montaukensis*, (B) *Dinobothrium* sp., and (C) *D. septaria*. Scale bars = 0.5 mm

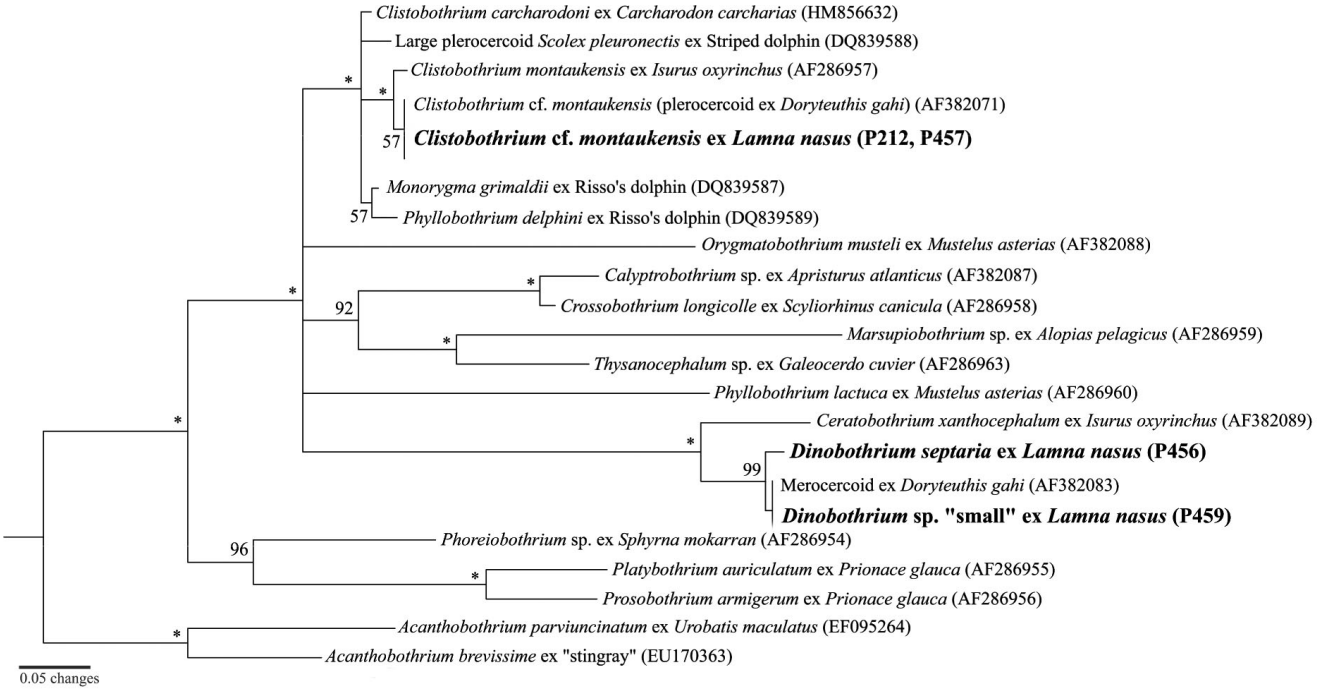

Fig. 3. Bayesian inference tree (50% majority-rule tree) based on DNA sequence data (622 bp) of the D2 region of the LSU gene, showing close affinities of *Clistobothrium* cf. *montaukensis*, *Dinobothrium* sp. and *D. septaria* (ex *Lamna nasus*) with tetraphyllidean larvae collected by Brickle et al. (2001). Nodal support is expressed as posterior probabilities, with asterisks indicating 100% posterior probability

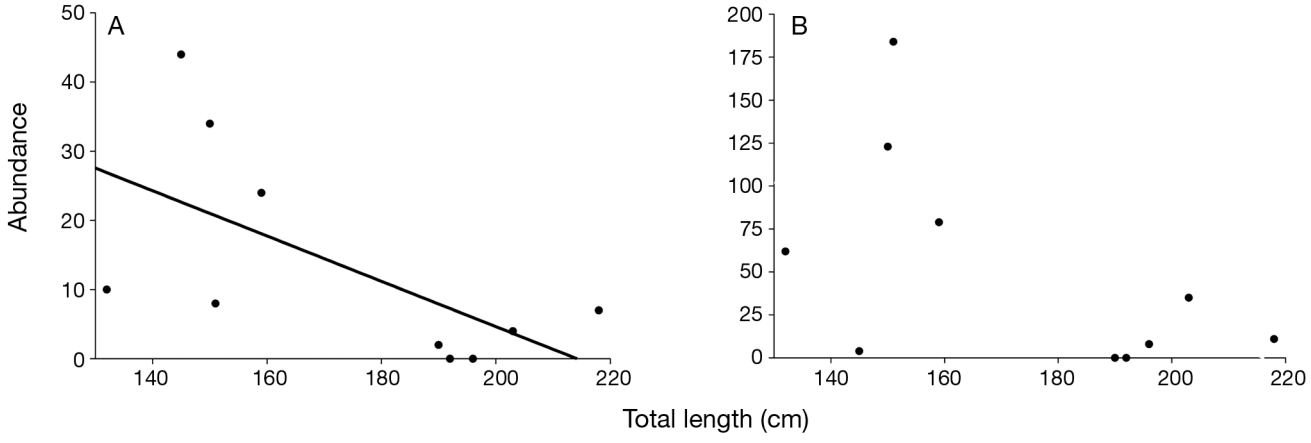

Fig. 4. Relationship between tapeworm abundance and porbeagle shark total length: (A) *Clistobothrium* cf. *montaukensis* and (B) *Dinobothrium* sp. The line in (A) represents the best-fit relationship from a linear regression. Note that total length data for one shark were unavailable; thus analyses were performed on 10 of the 11 porbeagle sharks

tapeworms recovered from *D. gahi* (Brickle et al. 2001) share identical LSU rDNA sequences at the D2 domain with *C.* cf. *montaukensis* and *Dinobothrium* sp. from porbeagle sharks. This suggests conspecificity between larvae and the respective adult forms sharing common sequences. To the best of our knowledge, the present study is the first to provide empirical evidence for a trophic link between *D. gahi* and porbeagle sharks that leads to the transmission of tetraphyllidean tapeworms. The third tapeworm species recovered from porbeagle sharks, *D. septaria*, did not match any larval sequences available in GenBank, but did show close affinity to *Dinobothrium* sp., further confirming the congeneric nature of both species.

Furthermore, we confirm the hypothesis put forth by Brickle et al. (2001) that porbeagle sharks are indeed the definitive hosts for these tapeworms. This implies that porbeagle sharks are the primary source of infec-

tion of these tapeworms in *Doryteuthis gahi*. Despite the absence of specific identifications of tapeworm larvae from squid, their role in the transmission of marine tapeworms has been well documented (e.g. Linton 1922, Brown & Threlfall 1968, Pascual et al. 1996; also reviewed by Hochberg 1990). Squid are an important food source for porbeagle sharks (Joyce et al. 2002, Francis et al. 2008). However, in the northwestern Atlantic, the number and frequency of occurrence of squid in their diet decreases in larger sharks (Joyce et al. 2002). Using parasites as a proxy, our data suggest that the same is true off the Falkland shelf. The abundance of both *Clistobothrium* cf. *montaukensis* and *Dinobothrium* sp., mutually found in *D. gahi*, was negatively correlated with porbeagle total length, albeit only significantly so for the former (Fig. 4A,B). This may be an artefact of low sample size (n = 10 porbeagle sharks; missing total length data for the eleventh shark). Despite this small sample size, the negative relationship between the abundance of these 2 tetraphyllidean parasites and porbeagle total length suggests that these tapeworms are shorter-lived than their host and there is an ontogenetic shift in diet away from squid. Assuming these tapeworms lived as long as their porbeagle hosts, we would have expected an accumulation of these parasites, reaching a plateau corresponding to the shift in diet away from squid. The relationships shown in Fig. 4 indicate that some of these tapeworms — either tapeworms acquired just prior to the ontogenetic shift in diet, i.e. older worms, or newly acquired tapeworms — infected larger sharks. Porbeagle sharks are opportunistic feeders and larger individuals will occasionally feed on squid when these are available (Joyce et al. 2002). The diet of porbeagle sharks is akin to that of the shortfin mako shark (Stillwell & Kohler 1982) and their parasite communities are similar: shortfin makos harbour species assignable to *Clistobothrium* and *Ceratobothrium* (Schmidt 1986, Hine et al. 2000).

Many marine organisms have evolved strategies for enhancing their fitness by moving between ecosystems (meta-ecosystems) for food and reproduction (Arkhipkin & Laptikhovsky 2010). Extreme examples of species that utilize distant ecosystems for food and reproduction include tunas, billfish, large sharks and cetaceans (Marti 1980, Takahashi et al. 2003, Bonfil et al. 2005, Bailey et al. 2009). Little is known about the migration patterns of porbeagle sharks, but recent work from archival transmission tags has shown that females can migrate up 2300 km over winter from cold temperate waters of the North Atlantic to pupping grounds in the subtropical Sargasso Sea, whilst male and immature sharks of both sexes remain in cool temperate waters (Campana et al. 2010). Another recent study suggests that porbeagle shark populations around Ireland, the UK, northwest Africa and the Mediterranean are connected and that there may be regular mixing between these locations (Saunders et al. in press). There is some evidence of transoceanic and transhemispheric migration based on the population genetics of porbeagle sharks taken from the Falkland Islands, the North Atlantic and New Zealand (L. Noble pers. comm.). If, indeed, this is correct, then examining the tetraphyllidean fauna of porbeagle sharks at a population level from different locations throughout its species range may shed some light on the transmission of these species across ecosystems.

Since the Brickle et al. (2001) *Doryteuthis gahi* study, porbeagle sharks have been shown to feed quite extensively on *D. gahi* (FIFD unpubl. data) and anecdotal evidence suggests that increased abundances of tetraphyllidean larvae in squid in the second season (15 July to 30 September) are coincident with an increased abundance of porbeagle sharks in the fishery. However, modelling this relationship is difficult because most of the vessels fishing in Falkland Islands waters are bottom trawlers targeting demersal finfish and squid and therefore only occasionally catch these fast-swimming pelagic sharks. Natural mortality in the *D. gahi* fishery is calculated using Hoenig's empirical equation based on longevity, as argued in Hewitt & Hoenig (2005), to arrive at a value of approximately 0.0133, with longevity set as the maximum observed age (Roa-Ureta & Arkhipkin 2007). Currently, predation in the fishery is not taken into consideration in the calculation of natural mortality. Ideally, modelling the proportion of the population removed by predators, including porbeagle sharks, should be incorporated into the management of this fishery, e.g. through diet studies, to take into account the wider ecosystem of the southern Patagonian Shelf.

The squid *Doryteuthis gahi* harbours other larval parasites (Brickle et al. 2001) that do not typically use lamnid sharks as definitive hosts, i.e. anisakid nematodes *Anisakis* spp., parasites of marine mammals (Anderson 2000), and the trypanorhynch tapeworm *Grillotia* sp., a cosmopolitan parasite not known from lamnid sharks (Palm 2004). *D. gahi* is preyed upon by a variety of marine mammals (Clarke & Goodall 1994, Clarke 1996, Klages 1996, Schiavini et al. 1997, Alonso et al. 1998) and may play a role in the transmission of *Anisakis* spp. as an intermediate or paratenic host. Additionally, several skates of the genus *Bathyraja* Ishiyama, 1958 are known as predators of *D. gahi* (Brickle et al. 2003, Laptikhovsky et al. 2010) and as hosts to *Grillotia* sp. (P. Brickle pers. obs. in Brickle et al. 2001), which may be acquired by feeding on this squid. The involvement of *D. gahi* in 3 different trophic networks suggests that this species plays multiple ecological roles in marine ecosystems and highlights the

potential use of parasites in uncovering potentially cryptic trophic links.

Our data provide empirical evidence for a transmission pathway for *Clistobothrium* cf. *montaukensis* and *Dinobothrium* sp. involving the trophic interaction between *Doryteuthis gahi* and porbeagle sharks. However, the early stages of these life cycles remain unresolved. Using *D. gahi* stomach content analyses, we can make informed suggestions about organisms likely to act as potential first intermediate hosts in the early stages of these life cycles. Brickle et al. (2001) concluded that euphausids and the amphipod *Themisto gaudichaudi* Guérin, 1825 were the most abundant prey items of *D. gahi*, and suggested that they may be promising targets for elucidating another step in these life cycles. However, *D. gahi* is not the only organism to target these crustaceans as sources of food. The teleosts *Micromesistius australis* Norman, 1937 (southern blue whiting) and *Macruronus magellanicus* Lönnberg, 1907 (hoki) feed almost exclusively on these crustaceans (Brickle et al. 2009) and are themselves preyed upon by porbeagle sharks (FIFD unpubl. data). This suggests a potential alternative pathway for the transmission of *C.* cf. *montaukensis* and *Dinobothrium* sp. Furthermore, adult body size in a parasite is generally correlated with its size at other stages of its life cycle (Poulin & Latham 2003, Poulin et al. 2003). The large tetraphyllidean reaching at least 18 cm in length (Lönnberg 1899) and possessing a large scolex (Fig. 2C), *D. septaria*, was not recovered from the relatively small squid *D. gahi*. Because the main features of the tetraphyllidean scolex, such as size, are laid down early in the development of the parasite (Williams 1960), larger tetraphyllideans are expected to have larger scoleces and be restricted to larger intermediate hosts. Consequently, the transmission pathway of *D. septaria* may involve the aforementioned teleosts.

In summary, our data provide empirical evidence for a trophic link between the squid *Doryteuthis gahi* and porbeagle sharks that leads to the successful transmission of the tetraphyllideans *Clistobothrium* cf. *montaukensis* and *Dinobothrium* sp. Furthermore, using ecological parameters of the parasites, such as intensity of infection (Fig. 4), we have provided data that suggest an ontogenetic shift in diet in porbeagle sharks from waters off the Falkland shelf. Examinations of greater numbers of porbeagle shark spiral valves will shed light on the dynamics of *D. septaria* infections and, indeed, a greater number of stomach samples for diet studies will help validate these suggested ontogenetic shifts in diet. In a broad context, studies elucidating marine tapeworm life cycles can provide useful clues to identify cryptic trophic links that lead to potential transmission of parasites; this information is also applicable to fisheries management.

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