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GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Revealing trophic transmission pathways of marine tapeworms

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Abstract
Parasites are important components of natural systems, and among their various roles, parasites strongly influence the flow of energy between and within food webs. Over 1000 tapeworm species are known to parasitise elasmobranchs, although full life cycles are resolved for fewer than 10 of them. The lack in resolution stems from the inability to distinguish larval from adult stages using morphology alone. Molecular elucidation of trophic transmission pathways is the next step in understanding the role of hosts and parasites within food webs. We investigated the parasite assemblage of New Zealand's rough skate, *Zearaja nasuta*. Skates and their prey items (obtained from the skates' stomachs) were dissected for the recovery of adult and larval tapeworms, respectively. A fragment of the 28S rDNA region was amplified for worm specimens with the aim to confirm species identity of parasites within rough skates and to uncover trophic transmission pathways that exploit the predation links between rough skates and their prey. We identified seven species of tapeworms from four tapeworm orders. Four trophic transmission pathways were resolved between three prey items from skates stomachs and skates, and one pathway between larval tapeworm sequence from a New Zealand sole and skate, i.e. a genetic match was found between larval tapeworms in prey and adult worms in skates. We report the first case of an adult trypanorhynch parasitising rough skate. These findings contribute to our limited understanding of cestode life cycles as well as providing insights into the importance of predator-prey relationships for parasite transmission.

Keywords Trophic transmission · Tapeworm · *Zearaja nasuta* · 28s rDNA · *Acanthobothrium* · *Echeneibothrium*

Introduction

Understanding the structure and functioning of food webs is fundamental to ecological research (Pascual et al. 2005). Parasites are an important component of natural systems and play many roles in their structure and functioning as they can regulate host population dynamics, mediate species compositions of free-living communities and redirect energy flow within and between food webs (Huxham et al. 1995; Lafferty and Morris 1996; Hudson and Greenman 1998; Thompson et al. 2005; Lafferty et al. 2006; Wood et al. 2007; Hernandez and Sukhdeo 2008; Amundsen et al. 2009; Sato et al. 2012). Many parasites are also intimately connected with their hosts' trophic relationships because they are reliant on their hosts being eaten by the correct next host in their life cycles (Choisy et al. 2003; Parker et al. 2003; Benesh et al. 2014). Despite the well-documented importance of parasites within natural systems, relatively little is known about the life cycles of many parasites that are trophically transmitted through food webs (Cirtwill et al. 2017). Further resolving such pathways between hosts is a next step in understanding how these parasites fit into natural systems (Poulin et al. 2016; Blasco-Costa and Poulin 2017).

Within marine food webs, elasmobranchs occupy high trophic levels (Paine 1980; Crooks and Soulé 1999; Pace et al. 1999; Duffy 2002; Myers et al. 2007; Heithaus et al. 2008;

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Baum and Worm 2009) and are significant definitive hosts to many marine tapeworms (Caira and Healy 2004; Caira and Reyda 2005). Intermediate hosts for such parasites can include teleosts, molluscs, crustaceans and marine mammals as these often accumulate a range of larval stages (Jensen and Bullard 2010). Our knowledge of the transmission of tapeworms between intermediate and elasmobranch definitive hosts is particularly limited, in part due to a deficiency in sampling effort, and because larval stages cannot be reliably identified to species level (Chambers et al. 2000; Chervy 2002; Caira and Reyda 2005; Fyler et al. 2009; Jensen and Bullard 2010; Caira et al. 2014; Caira and Jensen 2017). Larval tapeworms lack distinctive morphological features that would allow them to be properly identified and do not resemble their adult counterparts (with the exception of trypanorhynch tapeworms (Campbell and Beveridge 1994)). The literature is filled with descriptions of larval tapeworms that are often limited to family level and are unmatched to their adult counterparts (see Jensen and Bullard 2010).

Few advances in the matching of adult and larval tapeworms have been accomplished in the past decades (Poulin and Keeney 2008). Genetic sequencing of adult tapeworms, typically with the aim of inferring evolutionary relationships between taxa (e.g. Mariaux 1998; Olson and Caira 1999; Kodedová et al. 2000; Waeschenbach et al. 2007, 2012; Caira et al. 2014), has provided resolution for a few transmission pathways (e.g. Brickle et al. 2001; Agusti et al. 2005; Aznar et al. 2007; Holland and Wilson 2009; Jensen and Bullard 2010; Randhawa 2011; Randhawa and Brickle 2011). The presence of an adult tapeworm within the spiral intestine of an elasmobranch and its conspecific larvae within a prey item of that predator provides convincing evidence for trophic transmission of that parasite (Randhawa and Brickle 2011). Online databases such as GenBank are increasing the availability of tapeworm sequences for larval-adult matching (Brickle et al. 2001; Agusti et al. 2005; Aznar et al. 2007; Jensen and Bullard 2010). As a result of genetic sequencing, substantial inroads have been made in our understanding of the evolution, classification and host associations of marine tapeworms (e.g. Olson et al. 2001; Waeschenbach et al. 2007; Olson et al. 2008; Jensen and Bullard 2010; Olson et al. 2010; Littlewood 2011; Waeschenbach et al. 2012; Jensen et al. 2016). Resolving trophic transmission pathways of elasmobranch tapeworms is beginning to reveal the role that particular intermediate host taxa play in their life cycles (Jensen and Bullard 2010). For these reasons and considering the lack of knowledge surrounding trophically transmitted parasites and their life cycles, it is important to elucidate how parasites fit into food webs and how they have evolved to exploit them (Lafferty et al. 2006). Here, we present a study on the relatively little known parasite assemblage of New Zealand's endemic rough skate, *Zearaja nasuta* (Table 1). Rough skates are commonly found throughout New Zealand waters at depths of a few to 200 m; however, little is known about their predatory role and trophic interactions within the ecosystem. The objective of this study was to resolve trophic transmission pathways used by tapeworms that use rough skate as a definitive host. The 28S rDNA gene is a commonly used molecular marker for tapeworm phylogenetic studies (Zehnder and Mariaux 1999; Olson et al. 2001; de Chambrier et al. 2004; Caira and Reyda 2005; Waeschenbach et al. 2007; Healy et al. 2009; Jensen and Bullard 2010) and for identification of tapeworm larvae (e.g. Brickle et al. 2001; Agusti et al. 2005; Aznar et al. 2007; Holland and Wilson 2009; Randhawa and Brickle 2011). This marker was employed here for genetic matching of larval and adult tapeworm counterparts. This study also provides the first genetic characterisation of the tapeworm assemblage inhabiting the rough skate spiral intestines. Our study resolves trophic transmission pathways through phylogenetic methods, an appropriate next step for exploring of how these parasites fit into natural systems.

Materials and methods

Obtaining adult tapeworms

Rough skates were collected in waters surrounding Nugget Point (approximately 46.4481° S, 169.8147° E) and Cape Saunders (approximately 45.9° S, 170.8° E) off the southeast coast of New Zealand on several occasions by a commercial bottom trawler. Skates from Nugget Point were caught between November 2015 and June 2017, and those from Cape Saunders were caught between September 2008 and January 2011. Spiral valves were removed from skates and opened with a mid-ventral incision along the ventral blood vessel, exposing the surfaces of each spiral chamber. Spiral valves were cut into between three and six sections depending on the total length of each spiral valve, placed in a 1-L container filled with saline (2 parts seawater:9 parts tapwater) and one tablespoon of sodium bicarbonate. The containers were briskly shaken for 1 min and rested for at least 4 h to allow parasites to be freed from intestinal mucus. The spiral valve, mucus and remaining liquid were examined for parasites using a dissecting microscope. To count the number of tapeworms, individuals were counted using the number of scolices found. Parasites were cleaned in saline and fixed in 95% ethanol (EtOH) prior to being processed for molecular analysis. Tapeworms reserved for morphological identification were fixed in hot 70% EtOH, hydrated through an EtOH gradient and placed in a 2% acetic acid Alum Carmine stain for 24 h before being dehydrated through an EtOH gradient to 70% EtOH, destained in acid 70% EtOH alcohol, dehydrated through a further EtOH gradient, cleared in clove oil and mounted in Canada Balsam. When possible, tapeworms were identified to lowest taxonomic level possible based on

Table 1 Known tapeworms and their life stage within New Zealand rough skates

Species	Stage	Reference
Acanthobothrium filicolle var. *benedeni* Lönnberg, 1889	Adult	Alexander (1963)
Acanthobothrium filicolle var. *paulum* Linton, 1890	Adult	Alexander (1963)
Acanthobothrium wedli Robinson, 1959	Adult	Alexander (1963)
Clydonobothrium elegantissimum Lönnberg, 1889	Adult	Alexander (1963)
Clydonobothrium leioformum Alexander 1963	Adult	Alexander (1963)
Phormobothrium affine (Olsson, 1867) Alexander 1963	Adult	Alexander (1963)
Echinobothrium coenoformum Alexander 1963	Adult	Alexander (1963)
Nybelina lingualis (Cuvier, 1817) Dollfus, 1929	Plerocercoid	Palm (2004)

descriptions provided by Alexander (1963). Tapeworms recovered from skates in November 2015 from Nugget Point and from September 2008 through January 2011 from Cape Saunders were labelled with prefix "P". Ethanol and slide vouchers were deposited at the Otago Museum, New Zealand, under accession numbers OMNZIV101750–OMNZIV101753 and OMNZIV101759–OMNZIV101764.

Obtaining larval tapeworms

In order to resolve trophic transmission pathways, we sorted through the prey items within rough skate stomachs and examined them for larval parasites. Prey species were identified to lowest taxonomic ranking using New Zealand reference guides (e.g. Naylor et al. 2005; Tracey et al. 2005; McMillan et al. 2011a, b). Only prey items that were relatively intact were dissected. For crustacean prey items, the carapace was removed and internal organs, such as muscle tissue, stomach and gills, were checked for parasites by flattening tissues between glass plates and scanning under a microscope. For teleost prey items, muscle tissue was macerated with a scalpel and tweezers, the body cavity examined and intestines dissected for larval tapeworms. Any parasites extracted from these prey items were washed in saline and fixed in 95% ethanol for molecular analysis.

Prevalence and intensity calculations

Prevalence and mean intensity of infection were defined as described in Bush et al. (1997). Clopper-Pearson exact 95% confidence limits were calculated for prevalence (Clopper and Pearson 1934), and bias-corrected and accelerated bootstrapped 95% confidence limits (number of bootstrap replicated = 2000) were calculated for mean intensity (Efron 1987). All these calculations were done with the use of Quantitative Parasitology 3.0 software (Reiczigel and Rózsa 2005). Due to the high morphological similarity of *Acanthobothrium* n. sp. and *Acanthobothrium wedli*, they were pooled to estimate their combined prevalence and intensity.

DNA extraction, amplification and sequencing

Genomic DNA was extracted using standard fish buffer techniques for 60% (*N* = 61) of samples (Devlin et al. 2004) and chelex extraction method for 40% (*N* = 40) of the samples (Walsh et al. 1991). A partial fragment of the 28S rDNA was amplified with forward primer T16 (5′-GAG ACC GAT AGC GAA ACA AGT AC-3′) and reverse primer T30 (5′-TGT TAG ACT CCT TGG TCC GTG-3′) (Harper and Saunders 2001) with a 750-bp target length. This region of the LSU has proved informative for both diagnostic and phylogenetic studies of tapeworms and related taxa (e.g. Brickle et al. 2001; Olson et al. 2001; Reyda and Olson 2003; Randhawa et al. 2007; Holland and Wilson 2009; Caira et al. 2014). The amplification protocol consisted of an initial 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). Amplified products were sequenced for only the forward direction (unless otherwise stated), using the same primers as for amplification. PCR product purification and Sanger sequencing by capillary electrophoresis were performed by an international commercial facility (Macrogen Inc., Seoul, Republic of Korea). Previously acquired sequence data (sequences denoted with prefix P in Fig. 1) were obtained as per above (for P959, P961, P962, P965, P1023, P1025, P1026 and P1035) or amplifying using forward primer T01N (5′-GAT GAC CCG CTG AAT TTA AG-3′) and reverse primer T13N (5′-GCA CCT GAG TTG TTA CAC ACT-3′) (Harper and Saunders 2001) with a *c.*1850-bp target length using the amplification protocol described above. Sequences for samples P479 and P484 were generated using both PCR primers, internal forward primers T16, SPF1 (5′-GCG ATG TAG ACT GTG GTG GG-3′) and U25 (5′-GAA AGA TGG TGA ACT ATG C-3′) and reverse primers SPR1 (5′-CTT GAC GCC ACC CGT TTA CC-3′), T30 and T24 (5′-GCA ACT AAT CAT TCG CTT TAC C-3′) (Harper and Saunders 2001, for the latter only). Sequences for samples P482, P486 and P487 were generated using only primers T16 and T30. All sequences were deposited in GenBank under the accession numbers MH913263–MH913276 and MH924004–MH924016.

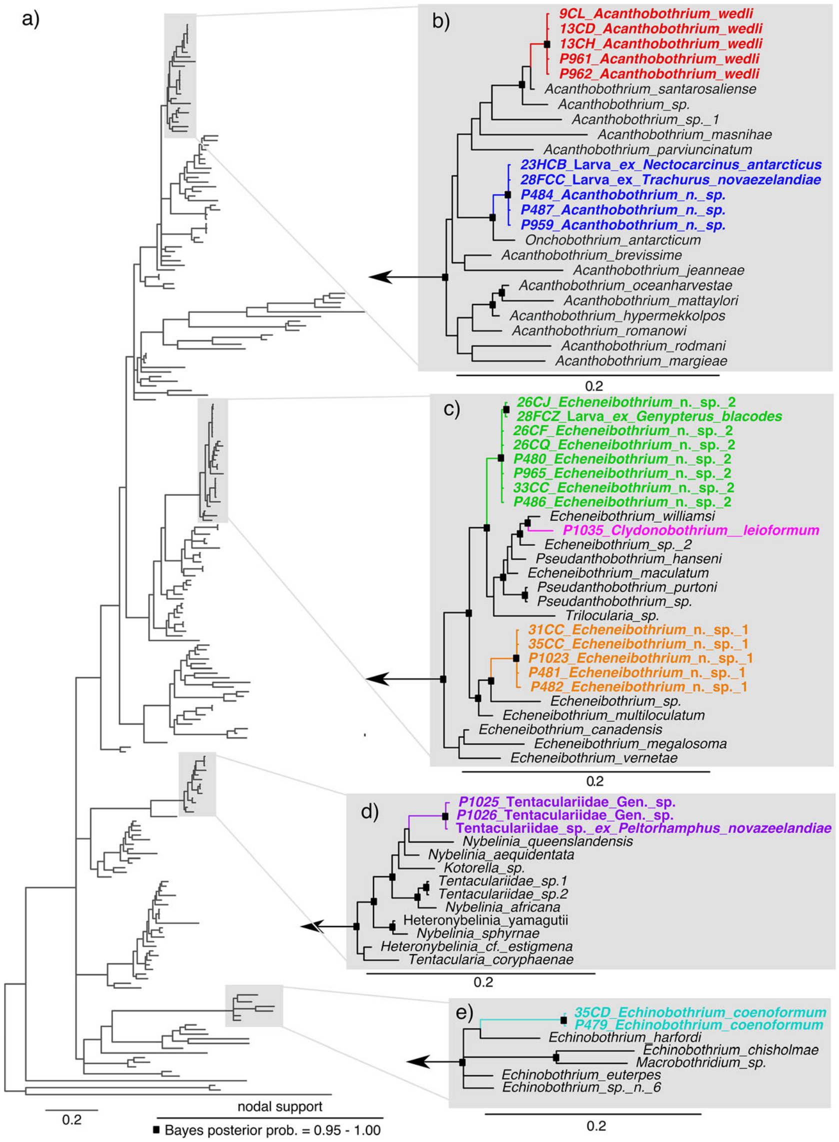

Fig. 1 Bayesian 50% majority-rule inference for the partial 28S rDNA dataset. Shaded rectangles indicate clades including sequences from this study. Colours indicate sequences collected in this study or indicate GenBank sequences that are 100% identical to sequences in this study. **a** Systematic representation of the full tree. **b** Enlarged section of phylogeny representing *Acanthobothrium* genus. **c** Enlarged section of phylogeny representing Echeneibothriidae species. **d** Enlarged section of phylogeny representing Tentaculariidae species. **e** Enlarged section of phylogeny representing *Echinobothrium* genus

Molecular characterisation and phylogenetic analysis

Molecular sequence data was obtained from 101 adult tapeworms parasitising rough skates and three larval tapeworms parasitising prey items. Sequences were imported into Geneious v8.1.9 (Kearse et al. 2012), trimmed using the trim function with default parameters and manually edited for incorrect or ambiguous base calls. If ambiguity occurred in the identity of a base, the reverse sequence was obtained for clarification using the same reverse primer as for amplification. Sequences were uploaded into the BLAST online search tool (http://www.ncbi.nhm.nih.gov, Zhang et al. 2000) to confirm sequence identity as tapeworms. When each sequence was BLAST searched, sequences with greater than 90% similarity between query and subject were downloaded and included as in-groups. Additionally, sequences representing all orders of tapeworms that parasitise elasmobranchs according to recent studies (Waeschenbach et al. 2012; Caira and Jensen 2014; Caira et al. 2014) were also downloaded and used as in-groups in the dataset for phylogenetic analysis. A total of four out-group taxa sequences were selected based on previous studies: *Diclidophora denticulata* (Monogenea), *Gyrocotyle rugosa* (Cestoda), *G. urna* (Cestoda) and *Austramphilina elongata* (Cestoda) (Waeschenbach et al. 2012; Caira and Jensen 2014; Caira et al. 2014).

The final dataset used in the phylogenetic analysis included 25 adult tapeworm sequences (representatives of 101 successfully sequenced) (Table 2) obtained from rough skates, three larval tapeworm sequences from various prey items of rough skates (Table 2), 200 downloaded in-group sequences and four out-group sequences (ESM 1).

Bayesian inferences were conducted in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) using the online interface: Cyberinfrastructure for Phylogenetic Research

Table 2 Sequences included in the phylogenetic analysis obtained from this study. $N = 27$

Taxonomic identification	Accession number
13CD *Acanthobothrium wedli*	MH924012
13CH *Acanthobothrium wedli*	MH924013
23HCB Larva ex *Nectocarcinus antarcticus*	MH924014
26CF *Echeneibothrium* n. sp. 2	MH924004
26CJ *Echeneibothrium* n. sp. 2	MH924007
26CQ *Echeneibothrium* n. sp. 2	MH924005
28FCC Larva ex *Trachurus novaezelandiae*	MH924015
28FCZ Larva ex *Genypterus blacodes*	MH924008
31CC *Echeneibothrium* n. sp. 1	MH924009
33CC *Echeneibothrium* n. sp. 2	MH924005
35CC *Echeneibothrium* n. sp. 1	MH924010
35CD *Echinobothrium coenoformum*	MH924016
9CL *Acanthobothrium wedli*	MH924011
P1023 *Echeneibothrium* n. sp. 1	MH913276
P1025 Tentaculariidae Gen. sp.	MH913274
P1026 Tentaculariidae Gen. sp.	MH913275
P1035 *Clydonobothrium leioformum*	MH913273
P480 *Echeneibothrium* n. sp. 2	MH913263
P481 *Echeneibothrium* n. sp. 1	MH913264
P482 *Echeneibothrium* n. sp. 1	MH913265
P484 *Acanthobothrium* n. sp.	MH913266
P486 *Echeneibothrium* n. sp. 2	MH913267
P487 *Acanthobothrium* n. sp.	MH913268
P959 *Acanthobothrium* n. sp.	MH913269
P961 *Acanthobothrium wedli*	MH913270
P962 *Acanthobothrium wedli*	MH913271
P965 *Echeneibothrium* n. sp. 2	MH913272

(CIPRES) Science Gateway (Miller et al. 2010). To account for uncertainty in the model of DNA substitution, the reversible-jump Markov chain Monte Carlo algorithm was used to integrate over the pool of all 203 possible reversible 4 × 4 nucleotide models. The analysis was performed with random starting trees for two runs (each with one cold and three heated chains), employing a Markov chain Monte Carlo (MCMC) approach for sampling the joint posterior probability distribution across 100,000,000 generation sampling every 10,000 generations. A heating chain parameter value of 0.04 was selected after preliminary analysis.

Mixing and convergence of each run were monitored through the statistics provided in MrBayes [values of standard deviations of partition frequencies (< 0.01), potential scale reduction factors (PSRF) (1.00), effective sample sizes (ESS) (> 200))] and in Tracer v1.6.0 (Rambaut et al. 2014; Tracer v1.6.0, available from http://beast.bio.ed.ac.uk/Tracer). The first 25% of samples were discarded as burnin. Resulting trees were summarised in a 50% majority-rule consensus tree with clade credibility support values (Bayesian posterior probability (BPP)) and branch length information. The consensus tree was visualised in FigTree v1.4.3. (http://www.tree/bio.ed.ac.uk/software/figtree), and figures were created in Inkscape (Albert et al. 1999; Inkscape v0.9, available from http://inkscape.org/). A BPP higher than 0.95 was considered strong support for nodal positions. To calculate the genetic divergence between the sequenced tapeworms from this study and their closest relatives inferred in the Bayesian consensus phylogenetic tree, uncorrected pairwise genetic distances were calculated in MEGA v7 (Kumar et al. 2016).

Results

Rough skates harbour seven tapeworm species (sensu Alexander (1963)) from four orders (Diphyllidea, Onchoproteocephalidea, Rhinebothriidea and Trypanorhyncha) with the resulting phylogeny demonstrating strong Bayesian posterior support for their respective positioning (BPP > 0.95) (Fig. 1). These species include the onchoproteocephalids *Acanthobothrium wedli* Robinson, 1959 and *Acanthobothrium* n. sp., the rhinebothriids *Echeneibothrium* n. sp. 1, *Clydonobothrium leioformum* Alexander, 1963, and *Echeneibothrium* n. sp. 2, the diphyllidean *Echinobothrium coenoformum* Alexander, 1963, and the unidentified trypanorhynch Tentaculariidae Gen. sp. The latter represents the first record for a trypanorhynch infecting the NZ rough skate. Alexander (1963) reported two variants of *A. filicolle* ex *Torpedo marmorata* (*A. f.* var. *benedeni* Lönnberg, 1889 ex *Raja clavata* and *A. f.* var. *paulum* Linton, 1890 ex *Bathytoshia centroura*) that are morphologically similar. Given the geographical isolation of material collected from rough skate and the type material for different variants of *A. filicolle*, and due to the phylogenetic distance between their respective hosts, it is most likely that material collected during this study represents an undescribed species of *Acanthobothrium*, but this is pending a thorough re-description. Hence, we will refer to this material as *Acanthobothrium* n. sp. throughout.

Four cases of trophic transmission in three tapeworm species have been identified in our phylogeny. The larval sequences 23HCB and 28FCC parasitising the crab, *Nectocarcinus antarcticus*, and the mackerel, *Trachurus novaezelandiae*, respectively, have 100% sequence similarity to the adult tapeworm *Acanthobothrium* n. sp. (BPP > 0.95), suggesting trophic transmission of this parasite through these intermediate or paratenic hosts (Fig. 1b). In another instance, the larval tapeworm sequence 28FCZ parasitising *Genypterus blacodes* has 100% sequence similarity to the adult tapeworm *Echeneibothrium* n. sp. 2 (BPP > 0.95) (Fig. 1c). Adult sequences from rough skates P1025 and P1026 morphologically identified as Tentaculariidae Gen. sp. have 100% sequence similarity to a larval Tentaculariidae sp. sequence from the New Zealand sole, *Peltorhamphus novaezeelandiae* (Anglade and Randhawa 2018) (BPP > 0.95) also indicative of trophic transmission (Fig. 1d).

Discussion

Tapeworms are trophically transmitted parasites that exploit feeding links among free-living organisms within food webs to complete their life cycles. Resolving trophic transmission pathways and life cycles of tapeworms is an important next step in advancing our knowledge on how parasites fit into marine food webs (Blasco-Costa and Poulin 2017). The main goal of this study was to provide molecular evidence for trophic transmission of tapeworms that exploit the predation links between New Zealand rough skate and its prey items to complete their life cycles. We revealed four new trophic transmission routes that three tapeworm species exploit. These results allow inferences about the ecological role of both hosts and parasites within New Zealand's marine ecosystem. The inferences in this study were based on a specific fragment of the D2 region of the 28S rDNA gene. While this fragment is relatively short compared to using multiple fragments, the main high-level taxonomic relationships and nodal support between families within the class Cestoda were similar to those previously reported in other studies (e.g. Olson et al. 2001; Waeschenbach et al. 2007; Caira et al. 2014; Ruhnke et al. 2015) (see ESM 2 for Bayesian support).

Feeding link properties influence the ability of parasites to complete their life cycles (Thompson et al. 2013). Bennett and Randhawa (2019) found that *N. antarcticus* makes up the majority of rough skate diet in comparison to *T. novaezelandiae*, which is opportunistically or incidentally consumed. Predation of *N. antarcticus* by rough skates may be the most important transmission route for *Acanthobothrium* n. sp. to complete its life cycle due to its considerably high transmission probability (Canard et al. 2014) and high biomass transfer (Thompson et al. 2013). Further investigation into the prevalence and intensity of infection in prey populations could indicate whether parasitism strengthens certain trophic links over others. For example, if prevalence is greater among individuals that are preyed upon by skates than in the population at large, this would suggest that infection biases the probability of a prey being caught by a predator. Alternatively, *T. novaezelandiae* may be an incidental or paratenic host to *Acanthobothrium* n. sp., especially if this fish preys on infected *N. antarcticus*.

Acanthobothrium wedli and *Acanthobothrium* n. sp. were pooled together for prevalence and intensity estimates (Table 3); however, our personal observations suggest that *A. wedli* was the most prevalent tapeworm due to differences in observed size of strobila between the two. Being the most prevalent tapeworm, it was interesting that *A. wedli* larvae were not found within the prey items investigated. Either (a) the prevalence of *A. wedli* larval stages within prey items is low or (b) the prey items that were investigated were not suitable intermediate hosts for *A. wedli* and true hosts is yet to be determined. It should be noted that *A. wedli* is a large tapeworm relative to the other ones recovered in this study, and given selective pressures placed on larger species due to their size and space constraints of intermediate hosts of differing sizes (Benesh 2010), its plerocercoid most likely requires a larger intermediate host. Among the 11 currently accepted genera of the order Onchoproteocephalidea, *Acanthobothrium* is the most diverse with 188 accepted species (Caira and Jensen 2017). It is not uncommon for multiple *Acanthobothrium* species to parasitise a single elasmobranch species, and these species are often highly host-specific as adults (Caira et al. 2003). Representatives from *Acanthobothrium* parasitise other skates of the genus *Zearaja*, such as *Zearaja chilensis*, the yellow-nose skate, that is host to *A. annapinkiensis* (Carvajal and Goldstein 1971).

All species within Rhinebothriidea are restricted to parasitising batoids (Healy et al. 2009). Our phylogeny includes 14 species from three of the five currently accepted genera of Echeneibothriidae (*Clydonobothrium*, *Echeneibothrium* and *Pseudanthobothrium*). Bueno and Caira (2017) suggested that *Pseudanthobothrium* may be a

Table 3 Prevalence and intensity of adult tapeworms found parasitising rough skates around Nugget Point, New Zealand. Prevalence confidence intervals based on the Clopper-Pearson method and mean intensity confidence intervals based on 2000 bootstrap replicates. Asterisk denotes species that were pooled together due to high morphological similarity

Species ID	Number	Prevalence (CI)	Mean intensity (CI)
Acanthobothrium n. sp./*A. wedli**	125*	77.1 (59.9–89.6)*	4.6 (2.9–7.3)*
Clydonobothrium leioformum	36	25.7 (12.5–43.3)	4.6 (2.8–6.2)
Echeneibothrium n. sp. 1	27	31.4 (16.9–49.3)	2.5 (1.5–4.7)
Echeneibothrium n. sp. 2	11	14.3 (4.8–30.3)	2.2 (1.0–3.4)
Echinobothrium coenoformum	4	5.7 (0.7–19.2)	2.0
Tentaculariidae Gen. sp.	1	2.9 (0.1–14.9)	1.0

synonym of *Echeneibothrium* because of its placement among members of *Echeneibothrium* (Caira et al. 2014; Ruhnke et al. 2015; Bueno 2018). This is consistent with our estimated phylogeny where strong Bayesian support was found for *Pseudanthobothrium* placement within *Echeneibothrium*. Bueno (2018) found that the determining characteristics for the genera *Echeneibothrium* and *Pseudanthobothrium* have been found to be taxonomically uninformative, also supporting the idea that *Pseudanthobothrium* is probably a synonym for *Echeneibothrium*. Our study is the first to incorporate representatives of *Clydonobothrium* into a molecular phylogeny. *Clydonobothrium* is polyphyletic in the estimated phylogeny, suggesting that this genus may also be a synonym of *Echeneibothrium*. Additional molecular sequencing of representatives from Echeneibothriidae would further resolve the taxonomic discrepancies between genera.

Multiple congeners of Echeneibothriidae within one host have been reported in the past (e.g. Williams 1961; Carvajal and Dailey 1975; Keeling and Burt 1996; Randhawa and Burt 2008; Bueno and Caira 2017). For example, *Z. chilensis*, the Chilean yellownose skate, a congener of rough skates, is parasitised by *Echeneibothrium megalosoma*, *Echeneibothrium multiloculatum* and *Echeneibothrium williamsi* (Carvajal and Dailey 1975; Bueno and Caira 2017). Bueno and Caira's (2017) phylogenetic analysis also revealed that these three *Echeneibothrium* species are not each other's closest relatives, suggesting that multiple colonisation events by echeneibothriid tapeworms may have occurred rather than speciation within this host. Recently, Beer et al. (in press) found that evolutionary histories of skates and their tapeworm parasites can be influenced by both the phylogeny and ecology of the host. Including more taxa and amplifying additional molecular markers would add taxonomic resolution to this clade, furthering our inference on tapeworm colonisation histories and diversification patterns and clarifying patterns of host switching and specificity (Caira 2011).

Our study provides the first evidence for an adult trypanorhynch parasitising rough skates. Our Tentaculariidae Gen. sp. sequences (P1025 and P1026) have 100% sequence similarity to a Tentaculariidae sp. found parasitising the New Zealand sole (*Peltorhamphus novezeelandiae*) (Anglade and Randhawa 2018). This genetic match between larval and adult stages suggests that this tapeworm utilises the trophic link between sole and rough skates to complete its life cycle. Indeed, sole have been identified within rough skate stomachs; however, sole in this study were too digested to examine for larval parasites (Bennett and Randhawa 2019). This potential trophic transmission pathway was suggested in Anglade and Randhawa (2018) and can now be confirmed. Trypanorhynch tapeworms are generally considered to exhibit low host specificity at each life stage (Palm 2004). Based on the typical low host specificity and lack of genus or species identification, we cannot comment on the importance of this trophic link for Tentaculariidae Gen. sp.

Using a molecular approach, trophic transmission pathways of tapeworms can be easily identified, thus avoiding the limitations of morphological approaches. When dealing with limited specimens, there is often a trade-off between morphological and molecular information. Our study identified and resolved four transmission pathways among skate feeding links that tapeworms exploit in order to complete their life cycles. We also found the first instance of rough skates being parasitised by an adult trypanorhynch and molecularly characterised the tapeworm assemblage of this elasmobranch host. Parasitological investigations can inform feeding links, the same way that feeding links can help make predictions about life cycles of trophically transmitted parasites. We encourage scientists to utilise predator stomachs by investigating the prey items present for parasites to further resolve trophic transmission pathways. This is an appropriate and cost-effective next step in furthering our understanding of how parasites are transmitted through food webs.

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Compliance with ethical standards

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

Conflict of interest The authors declare that they have no conflict of interest.

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