



# Tracking life cycles of parasites across a broad taxonomic scale in a marine ecosystem

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## ABSTRACT

Parasitic helminths exhibit remarkable diversity in their life cycles, although few parasite species have their whole life cycles resolved. Owing to the fact that parasite life stages within hosts are often not comparable using morphological data, genetic data provides convincing evidence of transmission pathways between intermediate and definitive hosts. We took this approach to an ecosystem level, genetically matching parasite (acanthocephalan, cestode, nematode and trematode) life stages across a broad taxonomic range of intermediate and definitive hosts (invertebrates, seabirds, elasmobranchs and teleost fish) in Otago's (New Zealand) coastal marine ecosystem. We identified which transmission routes are utilized by the most parasite species and assessed which intermediate hosts are most important in facilitating the transmission of parasites in this ecosystem. Our findings reveal 59 new records of larval parasites infecting their respective intermediate hosts and 289 transmission pathways utilized by 35 helminth species to complete their life cycles. Sprat, triplefin and arrow squid all hosted the highest number of larval parasite species, suggesting they play important roles as intermediate hosts. We then used the new life cycle data to provide a synthetic overview of the life cycles known for various parasite groups in New Zealand. This study highlights how studying parasite life cycles can enhance our understanding of the ecology and evolution of parasites and hosts in natural systems, beyond simply resolving life cycles.

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## 1. Introduction

Parasite life cycles are extraordinarily diverse, complex, and fascinating. In one generation, an individual parasite must either find and penetrate or be consumed by up to four different host species in sequential order before it is able to reproduce. If any of a parasite's hosts are eaten by a predator that is not the correct next host, that parasite will not survive to reproduce. Clearly, strong evolutionary pressures have favoured the reliance of parasites on predictable host feeding interactions for their survival. The evolution of complex parasite life cycles has occurred independently throughout the tree of life for different groups of parasites (Weinstein and Kuris, 2016), and as a consequence they differ in their life cycle characteristics (e.g. number of hosts, morphological stages, host specificity, site of infection). Despite the ubiquitous and biodiverse nature of parasites (Poulin and Morand, 2004), little is known about parasite life cycles, especially in the marine envi-

ronment where it is estimated that fewer than 5% of marine helminth life cycles are known (Blasco-Costa and Poulin, 2017).

Resolving marine parasite life cycles can have implications extending beyond parasite species identification (Blasco-Costa and Poulin, 2017). Life cycle resolution can provide insights into predator–prey interactions as most parasites transfer between hosts through trophic transmission. This is the process by which one infected host is consumed by the correct next host in the life cycle which then becomes infected. With the marine realm being a notoriously difficult environment in which to study predator–prey interactions between species, helminth life cycle data can indirectly fill the gaps in our knowledge of food web dynamics (Sukhdeo, 2010; Valtonen et al., 2010; Dunne et al., 2013). Resolution of life cycles can also inform taxonomy of species as some larval stages exhibit morphological features meaningful for delineation of species beyond the morphology of the adult form (e.g. some trematode cercariae have morphological characters that are unique to the cercarial life stage, and to their family (Schell, 1970)). Resolving life cycles also provides an insight into the coevolutionary history of interacting species. Hypotheses on the evolution of helminth life histories have been informed by life cycle resolution (Benesh, 2016). In addition, the coevolutionary histories of parasites and their hosts together have been elucidated

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**Table 1**

Larval parasites obtained from intermediate/paratenic/accidental hosts in Otago, New Zealand with life stage and associated GenBank accession numbers.

Parasite species	Life stage	Isolate	Intermediate host	Molecular marker and GenBank accession number
<b>Acanthocephala</b>				
<i>Andracantha sigma</i>	Cystacanth	SPRaca1_42	Sprat sp. 1 <i>Sprattus antipodum</i>	28S, <b>OQ407810</b>
<i>Bolbosoma balaenae</i>	Cystacanth	SPR1aca1_37	Sprat sp. 1 <i>S. antipodum</i>	28S, <b>OQ407811</b>
	Acanthella/cystacanth	AMP1aca1	Amphipod sp. 1 <i>Themisto</i> sp.	28S, <b>ON661298</b>
<i>Corynosoma hanna</i>	Cystacanth	FF3Baca1_17	Lemon sole <i>Pelotretis flavilatus</i>	28S, <b>OQ407812</b>
	Cystacanth	Tara1aca1_37	Tarahiki <i>Nemadactylus macropterus</i>	28S, <b>OQ407813</b>
	Cystacanth	SWR1aca3_24	Scarlet wrasse <i>Notolabrus celidotus</i>	28S, <b>OQ407814</b>
	Cystacanth	BWR1aca1_23	Banded wrasse <i>Pseudolabrus fucicola</i>	28S, <b>OQ407815</b>
	Cystacanth	BCD2aca1_24	Blue cod <i>Parapercis colias</i>	28S, <b>OQ407816</b>
	Cystacanth	SPR1aca2_37	Sprat sp. 1 <i>S. antipodum</i>	28S, <b>OQ407817</b>
	Cystacanth	PIG1aca1_42	Pigfish <i>Congiopodus leucopaecilus</i>	28S, <b>OQ407818</b>
	Cystacanth	SCG6aca1_41	Scaly gurnard <i>Lepidotrigla brachyotpera</i>	28S, <b>OQ407819</b>
	Cystacanth	CBF1aca1_42	Crested bellowsfish <i>Notopogon lilliei</i>	28S, <b>OQ407820</b>
	Cystacanth	STGAaca1_41	Stargazer <i>Genyagnus monopterygius</i>	28S, <b>OQ407821</b>
	Cystacanth	FF1aca2_10	NZ sole <i>Peltorhamphus novaezeelandiae</i>	28S, <b>OQ407822</b>
	Cystacanth	FF2aca1_17	Brill <i>Colistium guntheri</i>	28S, <b>OQ407823</b>
<i>Plagiorhynchus allisonae</i>	Cystacanth	AMP4aca1_42	Amphipod sp. 2 <i>Transorchestia serrulata</i>	28S, <b>OQ407824</b>
<i>Proflicollis novaezeelandensis</i>	Cystacanth	SMC1aca1_29	Stalk-eyed mud crab <i>Hemiplax hirtipes</i>	28S, <b>OQ407825</b>
	Cystacanth	PCS4aca1_14	Purple shore crab <i>Hemigrapsus sexdentatus</i>	28S, <b>OQ407826</b>
<b>Cestoda</b>				
<i>Acanthobothrium</i> sp. 1	Metacestode, plerocercoid type	Fish2ces3	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407768</b>
	Metacestode, plerocercoid type	OPL1ces1	Opalfish <i>Hemerocoetes monopterygius</i>	28S, <b>OQ407769</b>
	Metacestode, plerocercoid type	LIN2ces5_41	Ling <i>Genypterus blacodes</i>	28S, <b>OQ407770</b>
<i>Acanthobothrium</i> sp. 2	Metacestode, plerocercoid type	SPR1ces1B_37	Sprat sp. 1 <i>S. antipodum</i>	28S, <b>OQ407771</b>
<i>Acanthobothrium wedli</i>	Metacestode, plerocercoid type	SPRAces4_42	Sprat sp. 1 <i>S. antipodum</i>	28S, <b>OQ407772</b>
	Metacestode, plerocercoid type	TF2ces2_22	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407773</b>
<i>Anomotaenia</i> sp. 1	Metacestode, cysticeroid type	TRC_ces1	Amphipod sp. 2 <i>T. serrulata</i>	28S, <b>ON661306</b>
<i>Anonchocephalus chilensis</i>	Metacestode, plerocercoid type	Fish2ces1A	Opalfish <i>H. monopterygius</i>	28S, <b>OQ407774</b>
	Metacestode, plerocercoid type	FF2Bces3	NZ sole <i>P. novaezeelandiae</i>	28S, <b>OQ407775</b>
<i>Anthobothrium</i> sp. 1	Metacestode, plerocercoid type	FF2ces4_10	Lemon sole <i>P. flavilatus</i>	28S, <b>OQ407776</b>
	Metacestode, plerocercoid type	BCD1ces2	Blue cod <i>P. colias</i>	28S, <b>OQ407777</b>
	Metacestode, plerocercoid type	TRP1ces3	Thornfish <i>Bovichtus variegatus</i>	28S, <b>OQ407778</b>
	Metacestode, plerocercoid type	BAR2ces8_41	Barracouta <i>Thyrsites atun</i>	28S, <b>OQ407779</b>
	Metacestode, plerocercoid type	SPR1ces1A_37	Sprat sp. 1 <i>S. antipodum</i>	28S, <b>OQ407780</b>
	Metacestode, plerocercoid type	NOS5ces7_41	Arrow squid <i>Nototodarus sloanii</i>	28S, <b>OQ407781</b>
<i>Bothriocephalus scorpii</i>	Metacestode, plerocercoid type	FF2Bces4	NZ sole <i>P. novaezeelandiae</i>	28S, <b>OQ407782</b>
<i>Bothriocephalus</i> sp. 1	Metacestode, plerocercoid type	ANC5ces2_41	Anchovy <i>Engraulis australis</i>	28S, <b>OQ407783</b>
<i>Crossobothrium</i> sp. 1	Metacestode, plerocercoid type	BAR2ces9_41	Barracouta <i>T. atun</i>	28S, <b>OQ407784</b>
	Metacestode, plerocercoid type	Tera3ces1_41	Tarakihi <i>N. macropterus</i>	28S, <b>OQ407785</b>
<i>Eutetrarhynchidae</i> gen. sp. 1	Metacestode, plerocercus type	PAG1ces1_33	Hermit crab <i>Mixtopagurus spinosus</i>	28S, <b>ON661299</b>
	Metacestode, plerocercus type	PCB4ces1	Stalk-eyed mud crab <i>H. hirtipes</i>	28S, <b>ON661302</b>
	Metacestode, plerocercus type	MCB1ces1_33	Paddle crab <i>Ovalipes catharus</i>	28S, <b>ON661303</b>
	Metacestode, plerocercus type	Pillces1_23	Pillbox crab sp. 1 <i>Halicarcinus varius</i>	28S, <b>ON661301</b>
	Metacestode, plerocercus type	Octces1	NZ octopus <i>Macroctopus maorum</i>	28S, <b>ON661300</b>
	Metacestode, plerocercus type	HCB1ces1_36	Chameleon shrimp <i>Hippolyte</i> sp.	28S, <b>ON661304</b>

Table 1 (continued)

Parasite species	Life stage	Isolate	Intermediate host	Molecular marker and GenBank accession number
<i>Hepatoxylon trichiuri</i>	Metacystode, plerocercoid type	NOS1ces2_13	Arrow squid <i>N. sloanii</i>	28S, <b>ON661313</b>
<i>Lacistorhynchus dollfusi</i>	Metacystode, plerocercoid type	BAR2ces1_41	Barracouta <i>T. atun</i>	28S, <b>OQ407786</b>
<i>Microsomacanthus</i> sp. 1	Metacystode, cysticeroid type	AMP1ces1_30	Amphipod sp. 1 <i>Themisto</i> sp.	28S, <b>ON661305</b>
<i>Molicola thrysites</i>	Metacystode, plerocercoid type	BAR3ces1_41	Barracouta, <i>T. atun</i>	28S, <b>OQ407787</b>
<i>Nybelinia</i> sp. 1	Metacystode, plerocercoid type	NOS1ces1_13	Arrow squid <i>N. sloanii</i>	28S, <b>ON661311</b>
<i>Parorygmatobothrium</i> sp. 1	Metacystode, plerocercoid type	BAR2ces13_41	Barracouta <i>T. atun</i>	28S, <b>OQ407788</b>
Rhinebothriidae gen. sp. 1	Metacystode, plerocercoid type	BCD1ces3	Blue cod <i>P. colias</i>	28S, <b>OQ407789</b>
	Metacystode, plerocercoid type	TF6ces1_20	Triplefin sp. 1 <i>Forsterygion lapillum</i>	28S, <b>OQ407790</b>
	Metacystode, plerocercoid type	TF2ces2_20	Triplefin sp. 2 <i>Forsterygion capito</i>	28S, <b>OQ407791</b>
	Metacystode, plerocercoid type	TF10ces1	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407792</b>
	Metacystode, plerocercoid type	SPWrAces1	Spotted wrasse <i>Notolabrus calidodus</i>	28S, <b>OQ407793</b>
Rhinebothriidae gen. sp. 2	Metacystode, plerocercoid type	TF11ces	Triplefin sp. 1 <i>F. lapillum</i>	28S, <b>OQ407794</b>
	Metacystode, plerocercoid type	TF3ces1_20	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407795</b>
Rhinebothriidae gen. sp. 3	Metacystode, plerocercoid type	TF4ces1_37	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407796</b>
Tentaculariidae gen. sp. 1	Metacystode, plerocercoid type	NOS4ces2	Arrow squid <i>N. sloanii</i>	28S, <b>ON661312</b>
	Metacystode, plerocercoid type	BCDAces3	Blue cod <i>P. colias</i>	28S, <b>OQ407797</b>
	Metacystode, plerocercoid type	BAR2ces2_41	Barracouta <i>T. atun</i>	28S, <b>OQ407798</b>
Tentaculariidae gen. sp. 2	Metacystode, plerocercoid type	FF1ces1_10	NZ sole <i>P. novaezeelandiae</i>	28S, <b>OQ407799</b>
	Metacystode, plerocercoid type	FF2ces3	Lemon sole <i>P. flavilatus</i>	28S, <b>OQ407800</b>
	Metacystode, plerocercoid type	PPF2ces1B_42	Puffer fish <i>Contusus richiei</i>	28S, <b>OQ407801</b>
	Metacystode, plerocercoid type	NOS7ces2_41	Arrow squid <i>N. sloanii</i>	28S, <b>OQ407802</b>
<i>Tetrabothrius</i> sp. 3	Unknown	ARS5hel1_17	Arrow squid <i>N. sloanii</i>	28S, <b>OQ407803</b>
<i>Trilocularia</i> sp. 1	Metacystode, plerocercoid type	CLI1ces1_18	Clingfish <i>Gastroscyphus hectoris</i>	28S, <b>OQ407804</b>
	Metacystode, plerocercoid type	WAR2ces2_41	Blue warehou <i>Seriotelele brama</i>	28S, <b>OQ407805</b>
	Metacystode, plerocercoid type	NOS8ces5_41	Arrow squid <i>N. sloanii</i>	28S, <b>ON661314</b>
	Metacystode, plerocercoid type	SWH5ces5_41	Silver warehou <i>Seriotelele punctata</i>	28S, <b>OQ407806</b>
<i>Yamaguticestus</i> sp. 1	Metacystode, plerocercoid type	NOS1ces4_41	Arrow squid <i>N. sloanii</i>	28S, <b>ON661307</b>
	Metacystode, plerocercoid type	TF2ces2	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407807</b>
	Metacystode, plerocercoid type	Fish3ces1_31	Opalfish <i>H. monopterygius</i>	28S, <b>OQ407808</b>
<i>Yamaguticestus squali</i>	Metacystode, plerocercoid type	NOS8ces1_41	Arrow squid <i>N. sloanii</i>	28S, <b>ON661308</b>
	Metacystode, plerocercoid type	SWH3ces1_41	Silver warehou <i>S. punctata</i>	28S, <b>OQ407809</b>
<b>Nematoda</b>				
<i>Anisakis simplex</i> s.l. of Bennett et al. (2022c)	L3 encysted	BAR1nem1_17	Barracouta <i>T. atun</i>	<i>cox1</i> , <b>OP431142</b>
	L3 encysted	Fish3Cnem1_17	Scarlett wrasse <i>N. celidotus</i>	<i>cox1</i> , <b>OP431143</b>
	L3 encysted	NOS2nem1_14	Arrow squid <i>N. sloanii</i>	<i>cox1</i> , <b>OP431144</b>
	L3 encysted	Mull2nem1_39	Mullet <i>Aldrichetta forsteri</i>	<i>cox1</i> , <b>OP431146</b>
	L3 encysted	SWH2nem1_24	Silver warehou <i>S. punctata</i>	<i>cox1</i> , <b>OP431147</b>
	L3 encysted	SWR1nem1_24	Scarlett wrasse <i>N. celidotus</i>	<i>cox1</i> , <b>OP431148</b>
	L3 encysted	BCD1nem1_38	Blue cod <i>P. colias</i>	<i>cox1</i> , <b>OP431149</b>
	L3 encysted	SWR3nem1_18	Tarahiki <i>N. macropterus</i>	<i>cox1</i> , <b>OP431150</b>
	L3 encysted	ORF1nem1_14	Olive rockfish <i>Acanthoclinus fuscus</i>	<i>cox1</i> , <b>OP431153</b>
	L3 encysted	SPM2nem1_41	Sprat sp. 2 <i>Sprattus mulleri</i>	<i>cox1</i> , <b>OP431154</b>
	L3 encysted	Fish4Anem1_17	Banded wrasse <i>P. fucicola</i>	<i>cox1</i> , <b>OP431155</b>

(continued on next page)

Table 1 (continued)

Parasite species	Life stage	Isolate	Intermediate host	Molecular marker and GenBank accession number
<i>Contracaecum rudolphii</i> E	L3 encysted	Mull1nem1_10	Mullet <i>A. forsteri</i>	<i>cox1</i> , <b>OP431156</b>
	L3 encysted	PIGCnem1_41	Pigfish <i>C. leucopaecilus</i>	<i>cox1</i> , <b>OP431157</b>
	L3 encysted	BAR1nem2_17	Barracouta <i>T. atun</i>	<i>cox1</i> , <b>OP431159</b>
	L3 encysted	SWR1nem1_38	Scarlett wrasse <i>N. celidotus</i>	<i>cox1</i> , <b>OP431161</b>
	L3 encysted	TRP1nem1_18	Thornfish <i>B. variegatus</i>	<i>cox1</i> , <b>OP431162</b>
	L3 encysted	SPR4nem2_28	Sprat sp. 1 <i>S. antipodum</i>	ITS1, <b>OP470855</b>
	L3 encysted	BCD1nem2	Blue cod <i>P. colias</i>	ITS1, <b>OP470856</b>
	L3 encysted	SPR1nem1_28	Sprat sp. 1 <i>S. antipodum</i>	18S, <b>OP458411</b>
	L3 encysted	TF2nem1_37	Triplefin spp., Tripterygiidae gen. spp.	18S, <b>OP458410</b>
	<i>Hysterothylacium aduncum</i>	L3 encysted	FF2anem1_10	Lemon sole <i>P. flavilatus</i>
L3 encysted		WIT1nem1	Witch, <i>Arnoglossus</i> sp.	18S, <b>OP455095</b>
L3 encysted		Mull9nem1_41	Mullet, <i>A. forsteri</i>	18S, <b>OP455096</b>
L3 encysted		CBFnem1	Crested bellowsfish, <i>N. lilliei</i>	18S, <b>OP455097</b>
L3 encysted		Fish2nem1	Triplefin spp., Tripterygiidae gen. spp.	18S, <b>OP455099</b>
<i>Hysterothylacium deardorffoverstreeterum</i>	L3 encysted	SCG4nem1_41	Scaly gurnard <i>L. brachyoptera</i>	18S, <b>OP455098</b>
	L3 encysted	CLI1nem1_18	Clingfish <i>G. hectoris</i>	18S, <b>OP455093</b>
	L3 encysted	PIG1nem1_10	Pigfish <i>C. leucopaecilus</i>	18S, <b>OP455100</b>
	L3 encysted	SH1nem1	Seahorse <i>Hippocampus abdominalis</i>	18S, <b>OP455092</b>
	L3 encysted	OPF1nem1_17	Opalfish <i>H. monopterygius</i>	18S, <b>OP455091</b>
	L3 encysted	ANC4nem1_41	Anchovy <i>E. australis</i>	18S, <b>OP455101</b>
	L3 encysted	SPR5nem1_28	Sprat sp. 1 <i>S. antipodum</i>	18S, <b>OP455102</b>
	L3 encysted	SPM1nem1_41	Sprat sp. 2 <i>S. mulleri</i>	18S, <b>OP455103</b>
	L3 encysted	TF3nem1_21	Triplefin sp. 2 <i>F. capito</i>	18S, <b>OP455105</b>
	<b>Trematoda</b>			
<i>Acanthoparyphium</i> sp. 1 A	Cercariae	Zea1tre1_14	Mud snail 1 <i>Zeacumantus subcarinatus</i>	28S, <b>ON661319</b>
	Metacercariae	LIMAtre1_42 CHI1tre3_40	Estuarine limpet <i>Notoacmea scapha</i> Snakeskin chiton <i>Sypharochiton pelliserpentis</i>	28S, <b>OQ407744</b> 28S, <b>OQ407745</b>
<i>Acanthoparyphium</i> sp. <i>Cardiocephaloides ovicarpus</i>	Metacercariae	CHI2tre1_36	Green chiton <i>Chiton glaucus</i>	28S, <b>OQ407746</b>
	Metacercariae	WCL1tre1_40	Wedge clam <i>Macomona liliiana</i>	28S, <b>OQ407747</b>
	Metacercariae	COC1tre1_42	Cockle <i>Austrovenus stutchburyi</i>	28S, <b>OQ407748</b>
	Cercariae	Lim2tre1_40	Estuarine limpet <i>Notoacmea scapha</i>	28S, <b>ON661320</b>
	Metacercariae	TF3tre1_37	Triplefin spp., Tripterygiidae gen. spp.	28S, <b>OQ407749</b>
<i>Copiatestes thrysiatae</i> <i>Galactosomum otepotiense</i> <i>Gymnophallus</i> sp. 1 <i>Himasthla</i> sp. 1	Metacercariae	TFBtre2_42	Triplefin sp. 1 <i>F. lapillum</i>	28S, <b>OQ407750</b>
	Metacercariae	TFAtre2_42	Triplefin sp. 2 <i>F. capito</i>	28S, <b>OQ407751</b>
	Metacercariae	EUP1tr3A_38	Euphausiid <i>Nyctiphanes australis</i>	28S, <b>OQ407752</b>
	Cercariae	Zea1tre2	Mud snail 1 <i>Z. subcarinatus</i>	28S, <b>OQ407753</b>
	Metacercariae	BML1tre2_40	Blue mussel <i>Mytilus edulis</i>	28S, <b>OQ407754</b>
<i>Lecithochirium</i> sp. 1 <i>Levinseniella</i> sp. 1 <i>Maritrema novaezealandense</i>	Metacercariae	Gas1tre2_40	Wedge clam <i>M. liliiana</i>	28S, <b>ON661321</b>
	Metacercariae	BML3tre1_40	Blue mussel <i>M. edulis</i>	28S, <b>ON661322</b>
	Cercariae	CHI1tre1_40	Limpet 1 <i>Sigapatella novaezealandiae</i>	28S, <b>ON661323</b>
	Metacercariae	MTS1tre1_10	Mantis shrimp <i>Heterosquilla armata</i>	28S, <b>ON661318</b>
	Metacercariae	PCS1tre1_14	Purple shore crab <i>H. sexdentatus</i>	28S, <b>OQ407755</b>
	Cercariae	Zea1tre3	Mud snail 1 <i>Z. subcarinatus</i>	28S, <b>ON661324</b>
	Metacercariae	AMP1tre1_35	Amphipod sp. 3 <i>Paracallioppe novizealandiae</i>	28S, <b>ON661326</b>
	Metacercariae	SWIAtre1_42	Green seaweed isopod <i>Batedotea elongata</i>	28S, <b>OQ407756</b>
	Metacercariae	SLC1tre1	Seaweed isopod <i>Paridotea unguulate</i>	28S, <b>ON661325</b>
	Metacercariae	ISO1tre1_40	Isopod sp. 1 <i>Euryllana</i> sp.	28S, <b>ON661327</b>
<i>Microphallus</i> sp. 1	Metacercariae	HHCAtre1_35	Hairy handed crab <i>H. crenulatus</i>	28S, <b>OQ407757</b>
	Metacercariae	Pil1tre1_38	Pillbox crab <i>H. varicus</i>	28S, <b>OQ407758</b>
	Metacercariae	PCS5treA_42	Purple shore crab <i>H. sexdentatus</i>	28S, <b>OQ407759</b>
	Metacercariae	PCSAtreB	Purple shore crab <i>H. sexdentatus</i>	28S, <b>OQ407760</b>
	Cercariae	Zea1treA_42	Mud whelk 1 <i>Z. subcarinatus</i>	28S, <b>OQ407761</b>
	Metacercariae	ANC5tre1_41	Anchovy <i>E. australis</i>	28S, <b>OQ407762</b>
	Cercariae	Dil1tre1B_34	Top shell 1 <i>D. substratum</i>	28S, <b>OQ407763</b>
	Metacercariae	Mix1tre1	Hermit crab <i>M. spinosus</i>	28S, <b>ON661328</b>
	Metacercariae	Cru1tre1_35	Euphausiid <i>N. australis</i>	28S, <b>ON661329</b>
	Cercariae	Zea1trePhil	Mud snail 1 <i>Z. subcarinatus</i>	28S, <b>OQ407764</b>
<i>Renicola</i> sp. 1 Schistosomatidae gen. sp. 1 <i>Stephanostomum</i> sp. 1	Metacercariae	ANC1tre1A_41	Anchovy <i>E. australis</i>	28S, <b>OQ407765</b>
	Cercariae	Lim1tre1_40	Limpet 1 <i>S. novaezealandiae</i>	28S, <b>ON661331</b>
	Metacercariae	Brillstrigeid	Brill <i>C. guntheri</i>	28S, <b>OQ407766</b>
Metacercariae	TF3tre2_20	Triplefin sp. 1 <i>F. lapillum</i>	28S, <b>OQ407767</b>	

28S, 28S rDNA gene, *cox1*, cytochrome c oxidase subunit 1 gene; ITS1, internal transcribed spacer 1 gene; 18S, 18S rDNA gene.

by life cycle data (Cribb et al., 2003). These basic biological data regarding the parasitic way of life provide a better understanding of how natural systems are structured and function.

There are several reasons for our deficient knowledge of marine helminth life cycles. Firstly, parasites are small organisms that are often hidden within their host's much larger body. This makes them seemingly insignificant at first glance, and they are therefore often unrecognised or neglected in science, even unnoticed. Secondly, larval stages of parasites often do not possess any morphological characteristics that would allow them to be matched with their adult counterparts. There are many reports of parasites in the literature from both intermediate and definitive hosts, at multiple life stages, all impossible to match using morphology alone. Lastly, despite invertebrates undoubtedly being essential in the life cycle of almost all parasites, parasitology, like many other disciplines is biased towards studying vertebrate taxa (Leung et al., 2015). This means that, in combination with our limited knowledge of marine parasite life cycles, that of invertebrate parasites is even more limited. In New Zealand, a recent review of marine parasites revealed that fewer than 1% of invertebrates expected to host helminths have records of parasites, compared with about 10% of vertebrate taxa (Bennett et al., 2022c).

Despite the associated challenges, significant advances in resolving life cycles are now possible with the use of genetic data. An example would be: a gene sequence is obtained from a larval parasite within a fish host and compared with sequence data from an adult parasite within a seabird host. If the two sequences are identical or within the expected level of intraspecific genetic divergence, this provides convincing evidence that, (i) these parasites from two hosts represent the same species at two life stages, and (ii) there is a feeding link or transmission pathway between the fish and seabird, i.e. the bird eats this fish species. Of course, there are exceptions to this rule, such as trematodes with free-living stages that are transmitted from the first intermediate host to the second one by seeking and contacting the latter, and nematodes that have single-host life cycles. The success of matching adult and larval stages also depends on the specific gene locus being used, with highly conserved regions being less likely to resolve parasite identity to genus or species level. Nevertheless, as a result of genetic studies, over the last 20 or so years, a number of larval and adult counterparts have been successfully genetically matched (e.g. Jensen and Bullard 2010; Cribb et al., 2011; Alcántar-Escalera et al., 2013).

The aim of this study was to resolve transmission routes and life cycles of helminths using molecular data from intermediate and definitive hosts within an entire coastal marine ecosystem, that of the Otago region (South Island, New Zealand). Our research spans all major helminth taxa and all major invertebrate and vertebrate host taxa. Here, we identify which transmission routes are utilized by the most parasite species and assess which intermediate host species and taxonomic groups are most important in facilitating the transmission of marine helminth parasites. Lastly, we use the new insights gained here to provide a synthetic overview of the life cycles known for various parasite groups for New Zealand and marine waters.

## 2. Material and methods

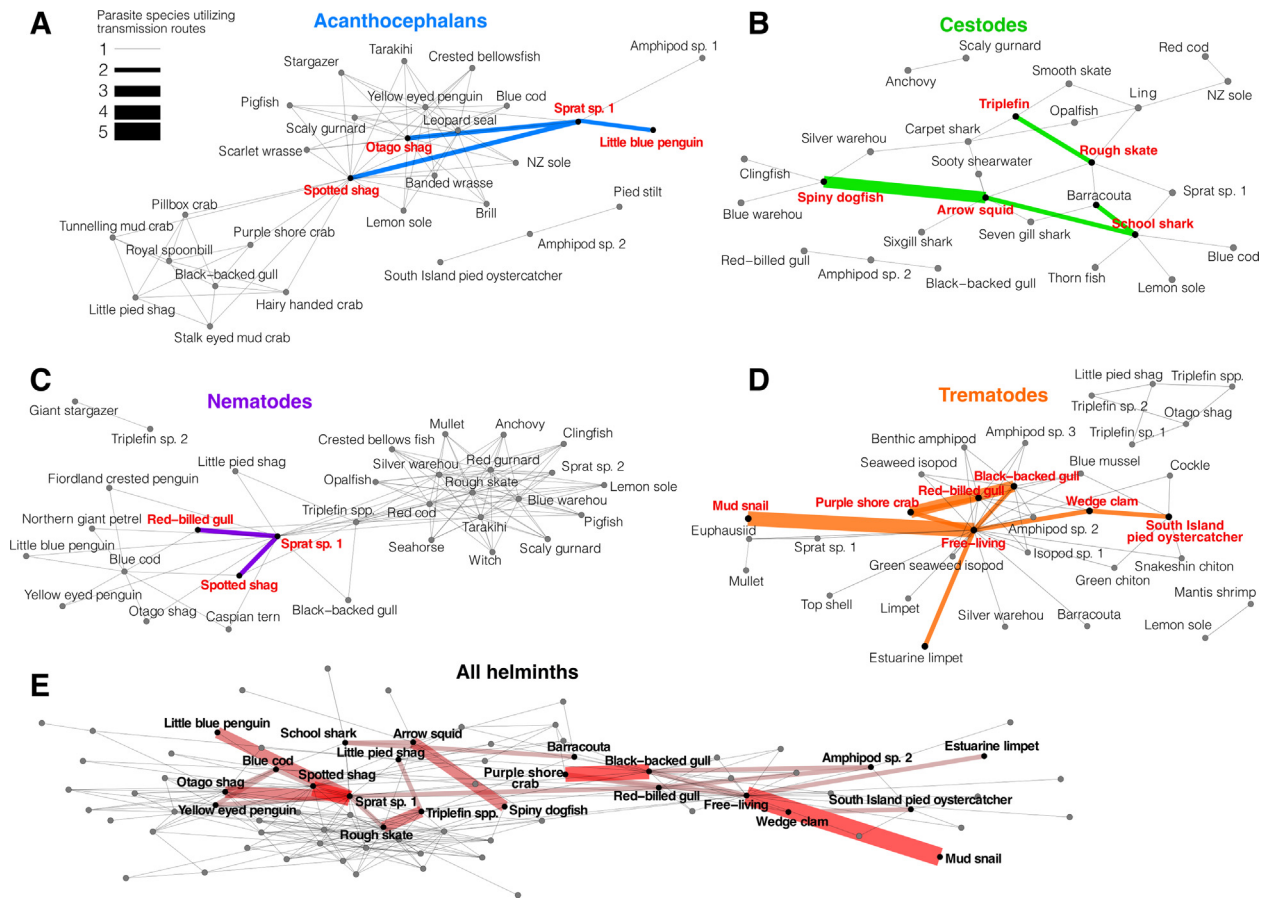
The marine animals examined for parasitic helminths in this study were obtained from a range of sources and locations around Otago's coastal marine ecosystem (OCME), as defined by Otago Regional Council (2012). All animals were provided deceased as by-catch or as a by-product of other research, except for a few inter- or sub-tidal fish and invertebrate species that were collected using hand nets and buckets, and some plankton that was collected

on plankton tows. Euthanasia of these animals was done under University of Otago, NZ animal use protocol AUP-19-190 (<https://www.otago.ac.nz/council/committees/committees/otago000865>). All animals were obtained between June 2019 and August 2021. The host taxa investigated here included 81 species of vertebrates (31 seabird species, 40 teleost fish species, nine elasmobranch species and one marine mammal species, see Table 1 of Bennett et al. (2022b)) and 87 invertebrate species (parasites recovered from invertebrates are detailed and listed by Bennett et al. (2022a)).

To resolve transmission routes and life cycles of parasites, we used molecular data to compare gene sequences of larval and adult parasites to confirm whether different stages belong to the same species. For acanthocephalans, cestodes and trematodes, the partial 28S rDNA gene was targeted using primers T16 and T30 (Harper and Saunders 2001) (see Bennett et al. (2019) for PCR protocol), and for nematodes, partial small subunit 18S rDNA gene, cytochrome c oxidase subunit 1 (*cox1*) gene or internal transcribed spacer 1 (ITS1) gene were targeted, depending on the taxon (18S for members of Acuariidae and *Hysterothylacium*, ITS1 for *Contraecium*, and *cox1* for representatives of *Anisakis*; see Bennett et al. (2022b) for details on nematode PCR protocols). Sequences of each parasite group (acanthocephalans, cestodes, nematodes and trematodes) were compared in MEGA v.7.0.26 using uncorrected pairwise genetic divergence calculations. If sequences from two life stages showed uncorrected pairwise genetic divergence of 0.00, they were considered one species. Some genetic divergence was allowed for some taxa, but the species cut-off threshold differed between parasite clades depending on the presence of species complexes and expected genetic variation in molecular markers as defined by previous studies. It is also possible that distinct species could be subsumed as one using these criteria due to the relatively small number of loci targeted. In total, 19 larval acanthocephalan sequences, 55 larval cestode sequences, 34 larval nematode sequences and 37 larval trematode sequences were produced (Table 1). Of the 54 larval parasite species identified by these sequences, 28 were genetically matched to adult stages within definitive hosts. Of those, only two species exhibited any apparent intraspecific genetic variation including *Corynosoma hanna* (0.018% difference within partial 28S) and Tentaculariidae gen. sp. 1 (0.007% difference within partial 28S).

The parasite fauna present in a given host individual (at least trophically transmitted species) has been shown to reflect well that host's diet (Williams et al., 1992; Valtonen et al., 2010). We assumed that if a larval parasite within an intermediate host was genetically matched to an adult parasite within a definitive host there was a transmission link between the hosts. In some instances, this may not be the case, especially where a parasite has low host specificity and the definitive host in question may have acquired infection of the same parasites from a different intermediate host. However, this is likely to be rare because the majority of parasites identified here exhibited relatively high levels of host specificity. Therefore, all trophic transmission links observed here are considered genuine. Once transmission routes were identified by genetic data, an edge list was created combining all potential transmission routes, for acanthocephalans, cestodes, nematodes, and trematodes separately and for all helminths combined. These edge lists also included transmission routes between first-intermediate hosts and free-living stages (Edge list including all helminth associations is available in Supplementary Table S1). Using R with packages Tidyverse (Wickham et al., 2019), Network (Butts, 2008), igraph (Csardi and Nepusz, 2006), tidygraph (Pedersen, 2022) and ggraph (Pedersen, 2020) we created transmission networks to visualise all potential transmission pathways in Otago.

For groups of parasites where new life cycle data was obtained, these data were combined with what is currently known to pro-



**Fig. 1.** Transmission routes utilized by parasites in Otago, New Zealand, separately for (A) acanthocephalans, (B) cestodes, (C) nematodes, (D) trematodes and also for (E) all helminths combined. Thickness of transmission routes indicates number of parasite species that use these routes. Highlighted routes represent the transmission pathways used by multiple parasite species. Bold text highlights taxa of interest.

vide an overview of the current knowledge of parasite life cycles in New Zealand. The new insights into helminth life cycles ranged in taxonomic levels, involving one acanthocephalan (Family Polymorphidae), six cestode (Order Cyclophyllidea, Tetrabothriidea, Trypanorhyncha, “Tetraphyllidea” and Phyllobothriidea, Onchoproteocephalidea and Bothriocephalidea) and seven trematode (Family Microphallidae, Himasthliidae, Opecoelidae, Hemiuridae, Syncoelidae, Schistosomatidae and Genus *Cardiocephaloides*) taxonomic groups. Life cycle data for “Tetraphyllidea” and Phyllobothriidea are combined due to the uncertainty of their phylogenetic positionings within Eucestoda (Caira et al., 2014, 2017). All new nematode life cycle data from this survey are presented in Bennett et al. (2022b).

### 3. Results

Between June 2019 and August 2021, specimens of 167 marine species were dissected with the aim to genetically match helminth parasite life stages from different hosts. In total, 57 animal species hosted larval parasites (Table 1). Of those, 48 intermediate hosts had parasites that were genetically matched to another life stage within a different host (typically matched to a definitive host, except for a few cases of larval trematodes within first intermediate hosts genetically matching infections in second intermediate host). This encompassed 34 parasite species: five acanthocephalan, 14 cestode, four nematode and 11 trematode species. Supplementary Table S2 presents uncorrected pairwise intraspecific genetic divergence calculated for each parasite species recovered from at

least two life stages within different host species. Based on the obtained genetic evidence for parasite transmission between hosts (or the ‘free-living’ environment in the case of some trematodes), the following section provides an overview of the transmission pathways utilized by helminth parasites in the Otago marine system.

#### 3.1. Transmission pathways utilized by Otago parasites

Within the acanthocephalan transmission network, we identified 75 potential routes that five species utilize in Otago to complete their life cycles (Fig. 1A). Of those, three pathways are exploited by more than one acanthocephalan species, namely pathways from sprat sp. 1 *Sprattus antipodum* to three seabirds, little blue penguin *Eudyptula novaehollandiae*, Otago shag *Leucocarbo chalconotus* and spotted shag *Phalacrocorax punctatus*. The host species with the highest number of potential encounters with acanthocephalans is the spotted shag, for which 18 possible transmission pathways from intermediate hosts were identified.

Within the cestode transmission network, there were far fewer transmission routes compared with other helminth groups (36 transmission routes involving 14 cestode species) (Fig. 1B). All transmission routes were between the intermediate and definitive hosts. Of the trophic transmission routes resolved, four were utilized by more than one cestode species (Fig. 1B). The transmission pathway between arrow squid *Nototodarus sloanii* and spiny dogfish *Squalus acanthias* was utilized by the most cestode species (three cestodes utilize this link). The transmission pathway from

**Table 2**

Number of larval parasites recovered from each intermediate host in Otago's coastal marine ecosystem, New Zealand.

Host taxa	Number of larval parasite species recovered
<b>Teleost fish</b>	
Anchovy <i>Engraulis australis</i>	4
Banded wrasse <i>Pseudolabrus fucicola</i>	2
Barracouta <i>Thyrsites atun</i>	8
Blue cod <i>Paraperchis colias</i>	6
Blue warehou <i>Serirolella brama</i>	1
Brill <i>Colistium guntheri</i>	2
Clingfish <i>Gastrosicyphus hectoris</i>	2
Common roughy <i>Paratrachichthys trailli</i>	1
Crested bellowsfish <i>Notopogon lilliei</i>	2
Lemon sole <i>Pelotretis flavilatus</i>	4
Ling <i>Genypterus blacodes</i>	1
Mullet <i>Aldrichetta forsteri</i>	3
NZ sole <i>Peltorhamphus novaezealandiae</i>	4
Olive rockfish <i>Acanthoclinus fuscus</i>	1
Opalfish <i>Hemerocoetes monoptygius</i>	4
Pigfish <i>Congiopodus leucopaecilus</i>	3
Puffer fish <i>Contusus richiei</i>	1
Scaly gurnard <i>Lepidotrigla brachyotpera</i>	2
Scarlett wrasse <i>Pseudolabrus miles</i>	4
Seahorse <i>Hippocampus abdominalis</i>	1
Silver warehou <i>Serirolella punctata</i>	3
Spotted wrasse <i>Notolabrus celidotus</i>	1
Sprat sp. 1 <i>Sprattus antipodum</i>	9
Sprat sp. 2 <i>Sprattus mulleri</i>	2
Stargazer <i>Genyagnus monoptygius</i>	1
Tarakihi <i>Nemadactylus macropterus</i>	3
Thornfish <i>Bovichtus variegatus</i>	2
Triplefin sp. 1 <i>Forsterygion lapillum</i>	4
Triplefin sp. 2 <i>Forsterygion capito</i>	3
Triplefin spp., Trypterugiidae gen. spp.	9
Witch <i>Amoglossus</i> sp.	1
<b>Invertebrates</b>	
Amphipod sp. 1 <i>Themisto</i> sp.	2
Amphipod sp. 2 <i>Transorchestia serrulata</i>	2
Amphipod sp. 3 <i>Paracallioppe novizealandiae</i>	1
Arrow squid <i>Nototodarus sloanii</i>	10
Blue mussel <i>Mytilus edulis</i>	2
Chameleon shrimp <i>Hippolyte</i> sp.	1
Cockle <i>Austrovenus stutchburyi</i>	1
Estuarine limpet <i>Notoacmea scapha</i>	2
Euphausiid krill <i>Nyctiphanes australis</i>	2
Green chiton <i>Chiton glaucus</i>	1
Green seaweed isopod <i>Batedotea elongata</i>	1
Hairy handed crab <i>Hemigrapsus crenulatus</i>	1
Hermit crab <i>Mixtopagurus spinosus</i>	2
Isopod sp. 1 <i>Eurylana</i> sp.	1
Limpet sp. 1 <i>Sigapatella novaezealandiae</i>	2
Mantis shrimp <i>Heterosquilla tricarinata</i>	1
Mud snail <i>Zeacumantus subcarinatus</i>	5
NZ octopus <i>Macroctopus maorum</i>	1
Paddle crab <i>Ovalipes catharus</i>	1
Pillbox crab <i>Halicarcinus varius</i>	2
Purple shore crab <i>Hemigrapsus sexdentatus</i>	4
Seaweed isopod <i>Paridotea unguolata</i>	1
Snakeskin chiton <i>Sypharochiton pelliserpentis</i>	1
Stalk-eyed mud crab <i>Hemiplax hirtipes</i>	2
Top shell <i>Diloma substratum</i>	1
Wedge clam <i>Macomona liliana</i>	2

arrow squid to school shark *Galeorhinus galeus*, and barracouta *Thyrsites atun* to school shark, both involved two cestode species. Additionally, the trophic transmission route between triplefin, Tripterugiidae gen. sp. and rough skate *Zearaja nasuta* was also utilized by two cestode species. The host species with the highest number of potential cestode transmission routes is arrow squid, whose seven larval cestode parasite species were genetically matched to seven definitive hosts.

The nematode transmission network held the highest number of potential transmission routes ( $n = 103$ ) (Fig. 1C). However, only two transmission pathways were utilized by more than one nematode species: the predation link between sprat sp. 1 and red-billed gull *Chroicocephalus scopulinus*, and sprat sp. 1 and spotted shag (Fig. 1C). Four of the five nematode species recovered from both larval and adult stages used the same intermediate host, sprat sp. 1.

Within the trematode network, 65 potential pathways used by trematodes to complete their life cycles were identified (Fig. 1D). Of those, eight pathways were used by multiple trematode species, including the most widely used link from mud snail *Zeacumantus subcarinatus* to free-living in the water column ( $n = 5$ ). Three trematode species utilize the transmission route between purple shore crab *Hemigrapsus sexdentatus* intermediate hosts and black-backed gull definitive hosts.

When transmission networks of all helminth groups are combined, additional transmission pathways utilized by multiple parasite species are revealed (Fig. 1D). For instance, the transmission route between purple shore crab to black-backed gull is utilized by not only three trematodes (Microphallidae, *Maritrema novaezealandense*, *Levinseniella* sp. 1 and *Microphallus* sp. 1), but also the acanthocephalan *Profilicollis novaezealandensis*. Another example involves the life cycle pathway between sprat sp. 1 and yellow-eyed penguin *Megadyptes antipodes* which, when all helminth data are combined, is utilized by multiple parasite groups (including the acanthocephalan *Corynosoma hanna* and nematode *Contraecum rudolphii* E; Fig. 1E).

### 3.2. Use of intermediate hosts

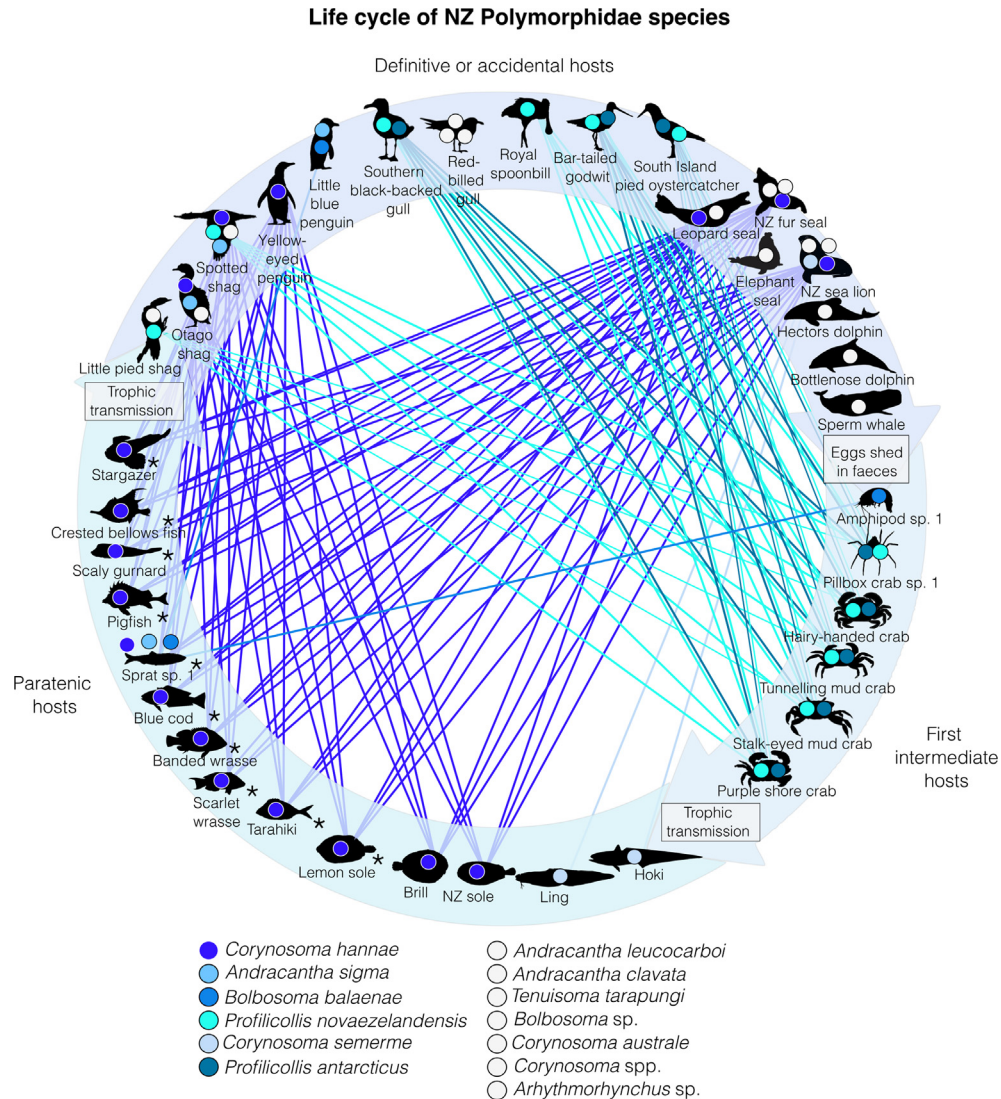
In Otago's coastal marine ecosystem, arrow squid, triplefin spp. and sprat sp. 1 hosted the highest number of larval parasite species (Table 2), being infected with 10, nine and nine species respectively. Most invertebrates hosted one or two larval parasite species whereas teleost intermediate hosts typically hosted between three and six species each.

### 3.3. New insights into New Zealand parasite life cycles

Supplementary Table S3 presents a list of host common names and scientific names used in Figs. 2–8 pertaining to section 3.3. No information is presented here for nematodes life cycles as any new data obtained in this study are presented in Bennett et al. (2022b).

#### 3.3.1. Acanthocephalans

The Otago marine ecosystem is home to at least 13 species of acanthocephalans belonging to Family Polymorphidae, and new data recovered here contributes to the life cycle knowledge of the group (Fig. 2). Polymorphid life cycles follow a two or three host life cycle where eggs in the water column are ingested by the first intermediate host where a cystacanth develops. The first intermediate host is always an arthropod, but genera differ in which type of arthropod they use. For example, species within *Corynosoma* utilize amphipods and species of *Profilicollis* utilize brachyuran crustaceans (García-Varela et al., 2013). First intermediate hosts are then consumed by the mammal or seabird definitive hosts where they develop into adults within the



**Fig. 2.** Overview of Polymorphidae life cycles known in New Zealand. Life cycle includes definitive or accidental, first intermediate and paratenic hosts. Black silhouettes represent host species and circles represent acanthocephalan infections. Circles filled in shades of grey (or blue in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host record obtained from this study. (For interpretation of the reference to colour in this figure legend, the reader is referred to the online version of this article.)

gastrointestinal tract. Some genera, such as *Corynosoma*, *Bolbosoma* and *Andracantha* have an additional host step in their life cycle which includes paratenic hosts (García-Varela et al., 2013). Here, the first intermediate host is consumed by a fish, but the cystacanth does not develop to maturity. Paratenic hosts often serve as trophic connectors between the first intermediate and definitive hosts. In Otago, *Corynosoma hannaie* was recovered from over 30% of all teleost fish species dissected (12/40), including 10 species which constitute new host records. This demonstrates the relatively low host specificity of *C. hannaie* for its paratenic hosts. Furthermore, *C. hannaie* is recognised as a parasite of pinnipeds, yet was recovered here as an immature adult free in the intestines of a few bird species. These birds, although accidental hosts, may also act as paratenic hosts if the birds themselves are preyed upon by pinniped definitive hosts. In fact, direct observations and scat remains have provided evidence of predation by NZ sealions on both yellow-eyed penguins and spotted shags, including between 20–30 direct observations per year (Lalas, 2007). Species such as *Andracantha sigma* and *Bolbosoma balaenae* exhibited relatively

strict host specificity as they were only recovered from one paratenic host species, a sprat.

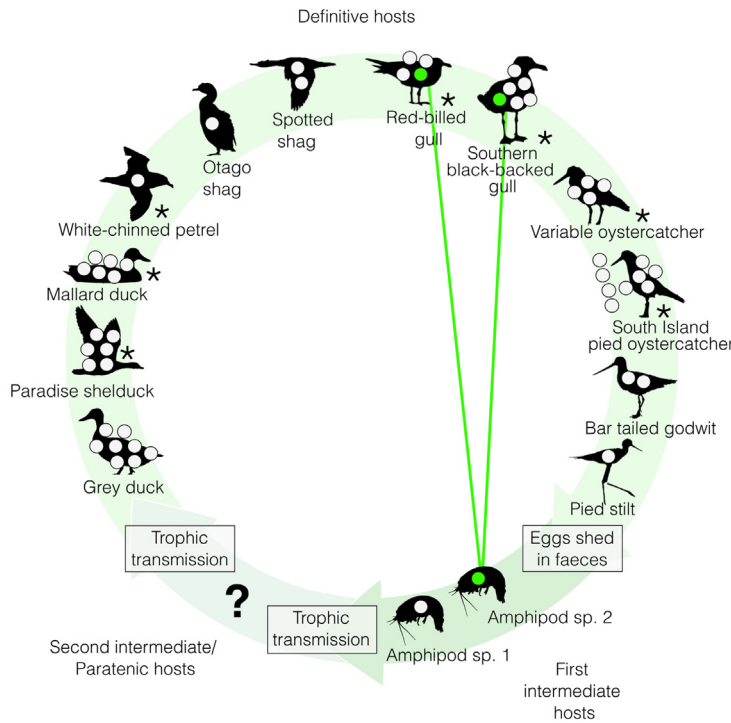
### 3.3.2. Cestodes

New Zealand marine cyclophyllideans (Order Cyclophyllidea) currently comprise 32 species and of those, two species have been reported from first intermediate hosts, without genetic match to respective adult stages within definitive hosts (Lagrue et al., 2016; Bennett et al., 2022c). Here, we genetically matched larval *Anomotaenia* sp. 2 (Family Dilepididae) from an amphipod first intermediate host to seabird definitive hosts (red-billed and black-backed gulls) (Fig. 3A). In this case, the newly generated data completes the life cycle of *Anomotaenia* sp. 2 as species of Dilepididae have two life stages.

Considering that species of order Tetrabothriidea have been known for over 200 years (Schmidt, 1986) and they constitute a dominant group of cestodes occurring in all orders of seabirds, some cetaceans and in pinnipeds (Temirova and Sкриabin, 1978), very few records of larval stages within intermediate or paratenic hosts exist. We recovered a single larval tetrabothriid (*Tetraboth-*



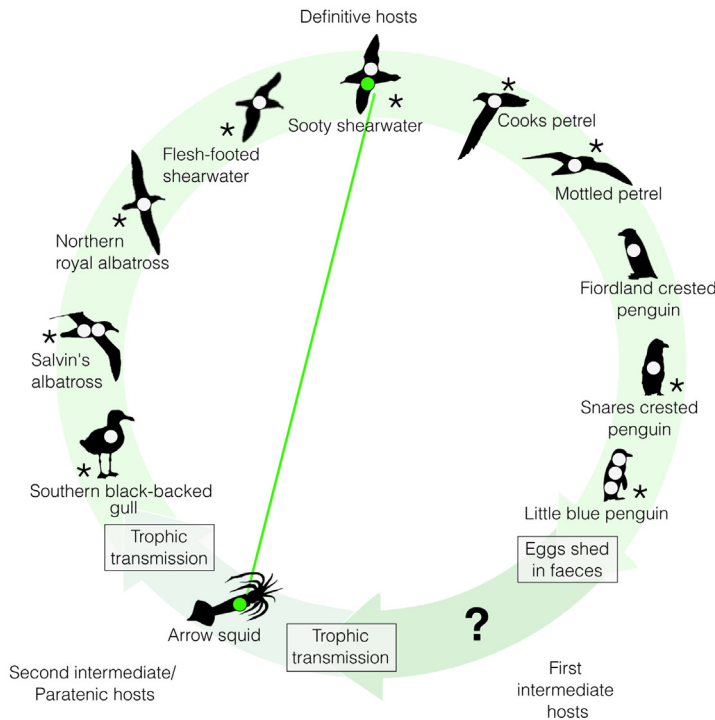
**A Life cycle of NZ Cyclophyllidea species**



Parasite species

- *Anomotaenia* sp. 2
- *Anomotaenia* sp. 1
- *Anomotaenia ciliata*
- *Aploparaksis furcigera*
- *Capsulata edenensis*
- *Cladogynia latovarium*
- *Dicranotaenia coronula*
- Dilepididae gen. sp. 1
- Dilepididae gen. sp. 2
- Dilepididae gen. sp. 3
- *Dilepis* cf. *undula*
- *Diorchis inflata*
- *Diorchis flavescens*
- *Diplophallus polymorphus*
- *Fimbraria fasciolaris*
- Hymenolepididae gen. sp. A
- Hymenolepididae gen. sp. 1
- Hymenolepididae gen. sp. 2
- Hymenolepididae gen. sp. 3
- *Hymenolepis megalops*
- *Hymenolepis alaskensis*
- *Microsomacanthus alaskensis*
- *Microsomacanthus* sp. 1
- *Microsomacanthus* sp. 2
- *Nadejdolepis laurei*
- *Ophryocotyle proteus*
- *Paradilepis urcenia*
- *Proterogynotaenia dougi*
- *Proterogynotaenia haematopodis*
- *Sobolevicanthus gracilis*
- *Wardium haematopodis*
- *Wardium clandestina*

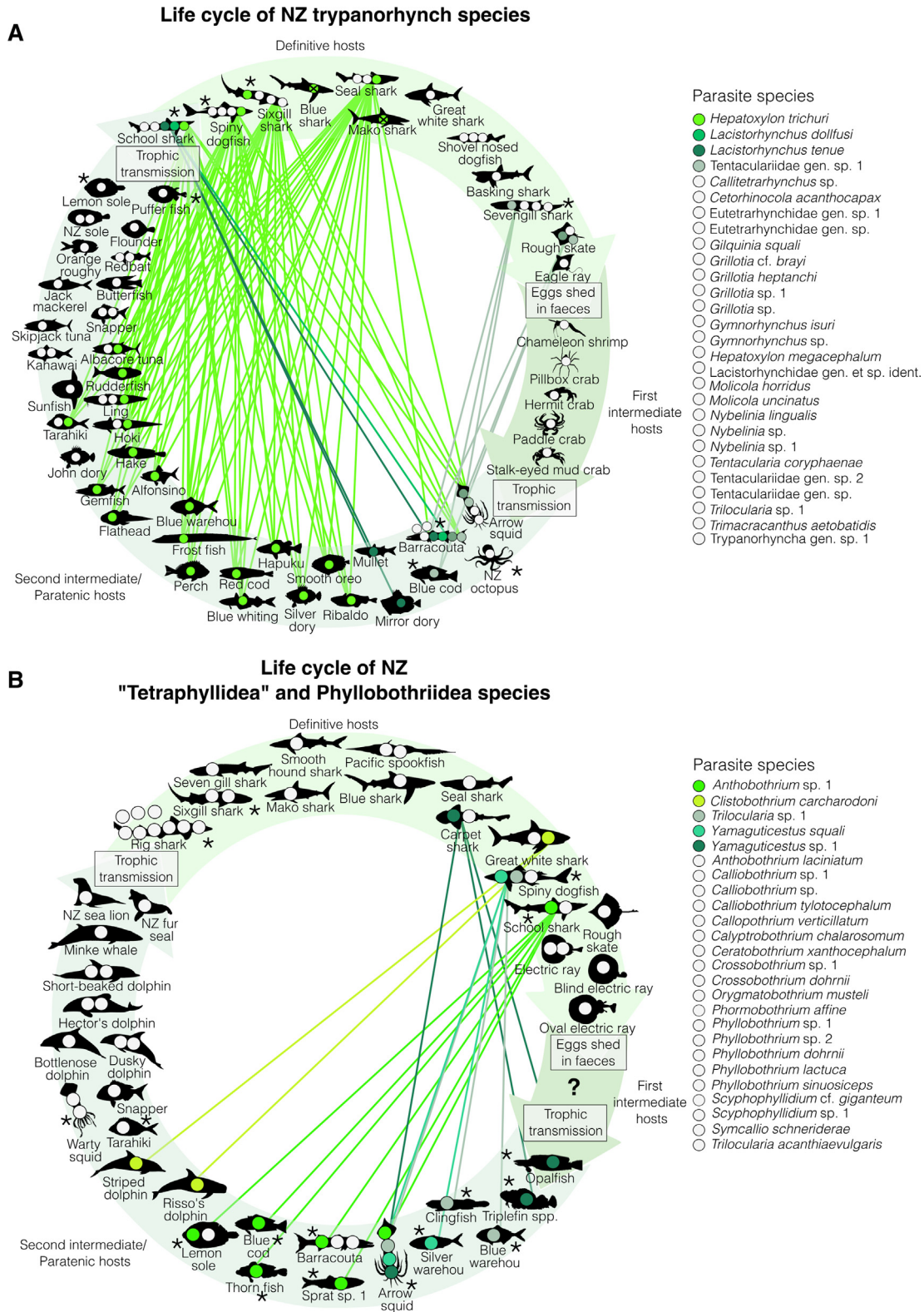
**B Life cycle of NZ Tetrabothriidea species**



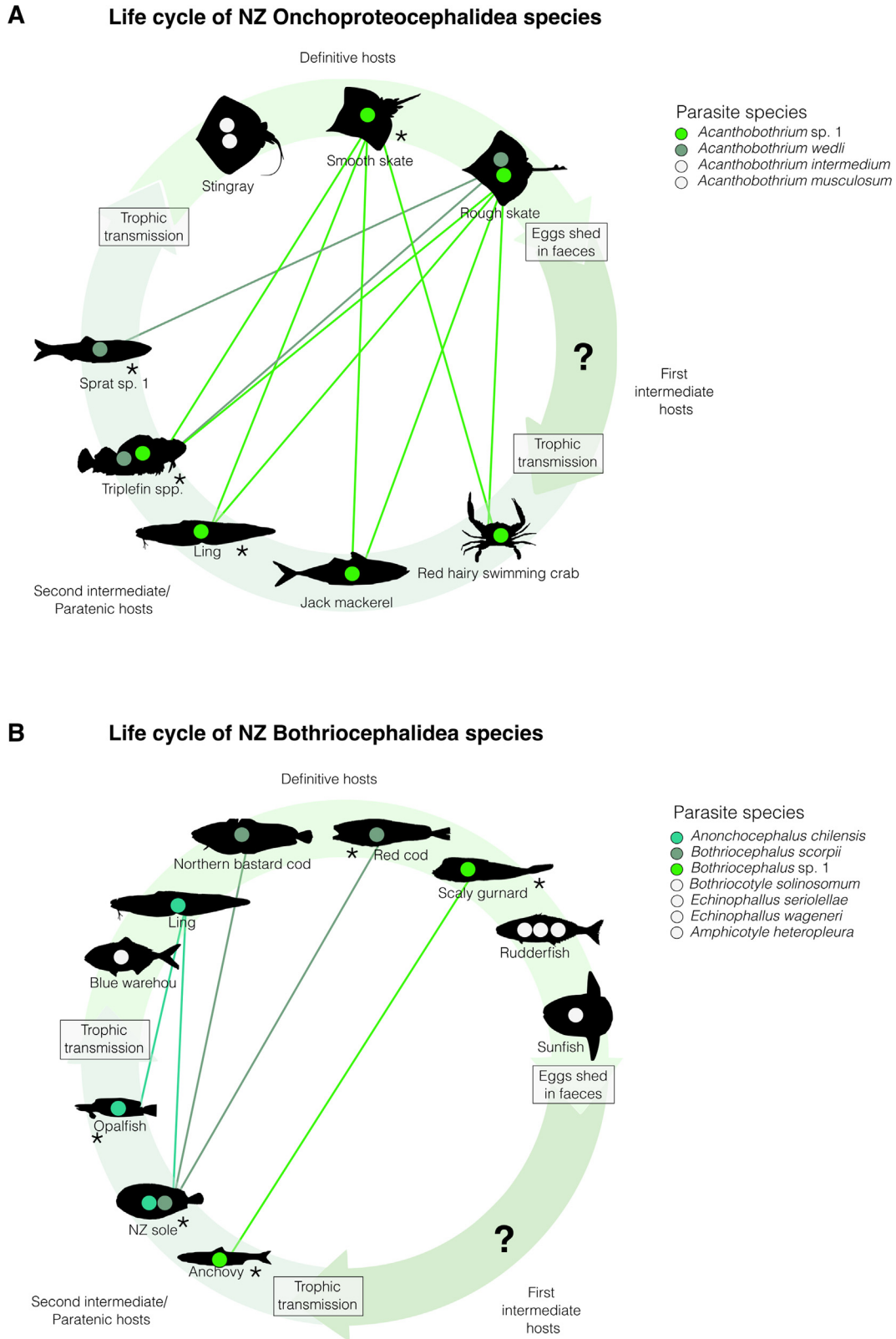
Parasite species

- *Tetrabothrius* sp. 3
- *Tetrabothrius* sp. 1
- *Tetrabothrius* sp. 2
- *Tetrabothrius* sp. 4
- *Tetrabothrius* sp. 5
- *Tetrabothrius* sp. 6
- *Tetrabothrius* sp. 7
- *Tetrabothrius* sp. 8
- *Tetrabothrius* sp. 9
- *Tetrabothrius* sp. 10
- *Tetrabothrius lutzi*

**Fig. 3.** Overview of (A) Cyclophyllidea and (B) Tetrabothriidea life cycles known in New Zealand. Life cycles include definitive, first and second intermediate, and/or paratenic hosts. Black silhouettes represent host species and circles represent cestode infections. Circles filled in grey (or green in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host records from this study.



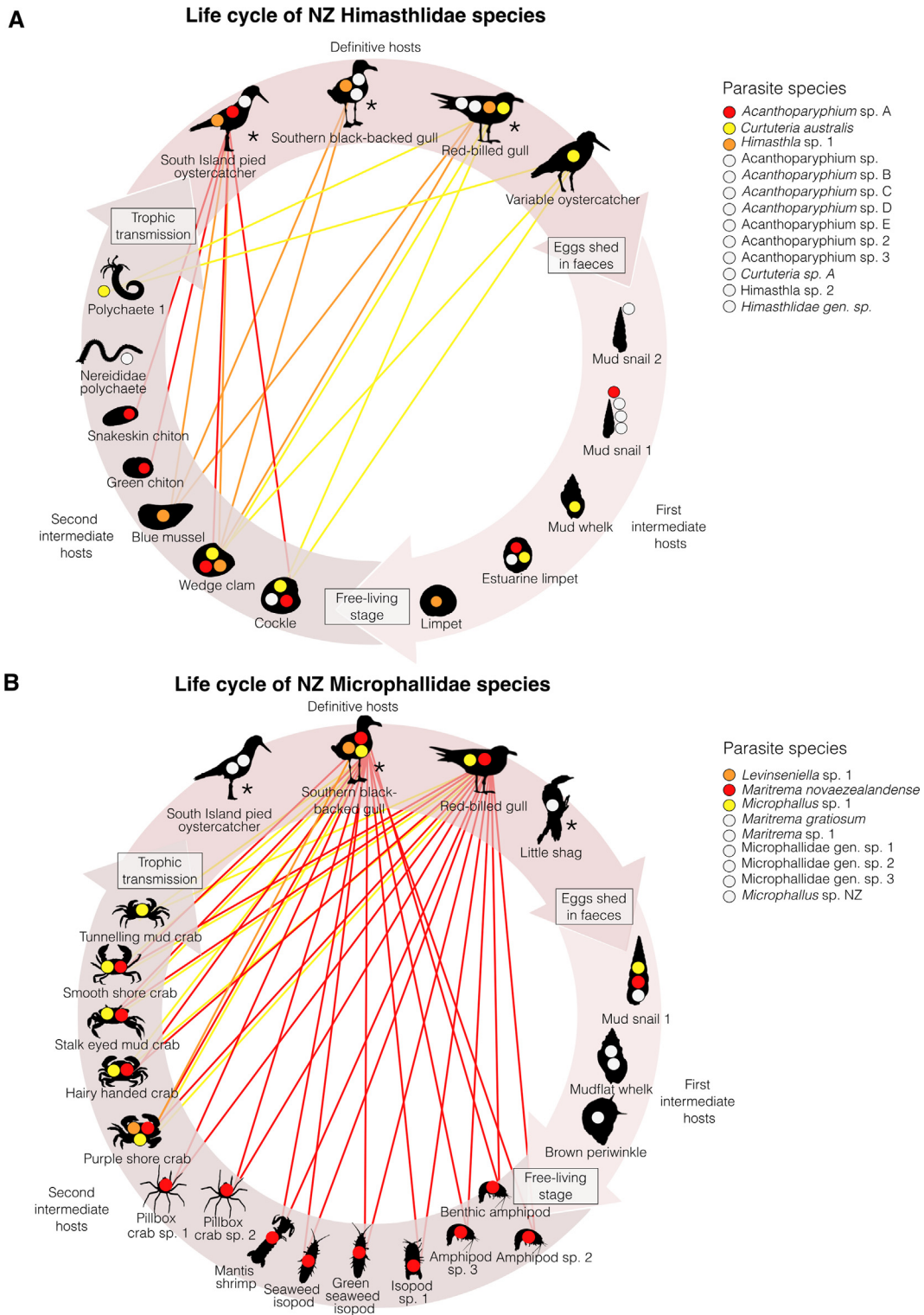
**Fig. 4.** Overview of (A) Trypanorhyncha and (B) "Tetraphyllidea" and Phyllobothriidea life cycles known in New Zealand. Life cycles includes definitive, first and second intermediate, and/or paratenic hosts. Black silhouettes represent host species and circles represent cestode infections. Circles filled in shades of grey (or green in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host records from this study. Circles crossed out represent probable definitive hosts infected with larval stages. (For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this article.)



**Fig. 5.** Overview of (A) Onchoproteocephalidea and (B) Bothriocephalidea life cycles known in New Zealand. Life cycles include definitive, first and second intermediate, and/or paratenic hosts. Black silhouettes represent host species and circles represent cestode infections. Circles filled in shades of grey (or green in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host records from this study.

*rius* sp. 3), infecting arrow squid which genetically matched an adult stage within a sooty shearwater definitive host (Fig. 3B).

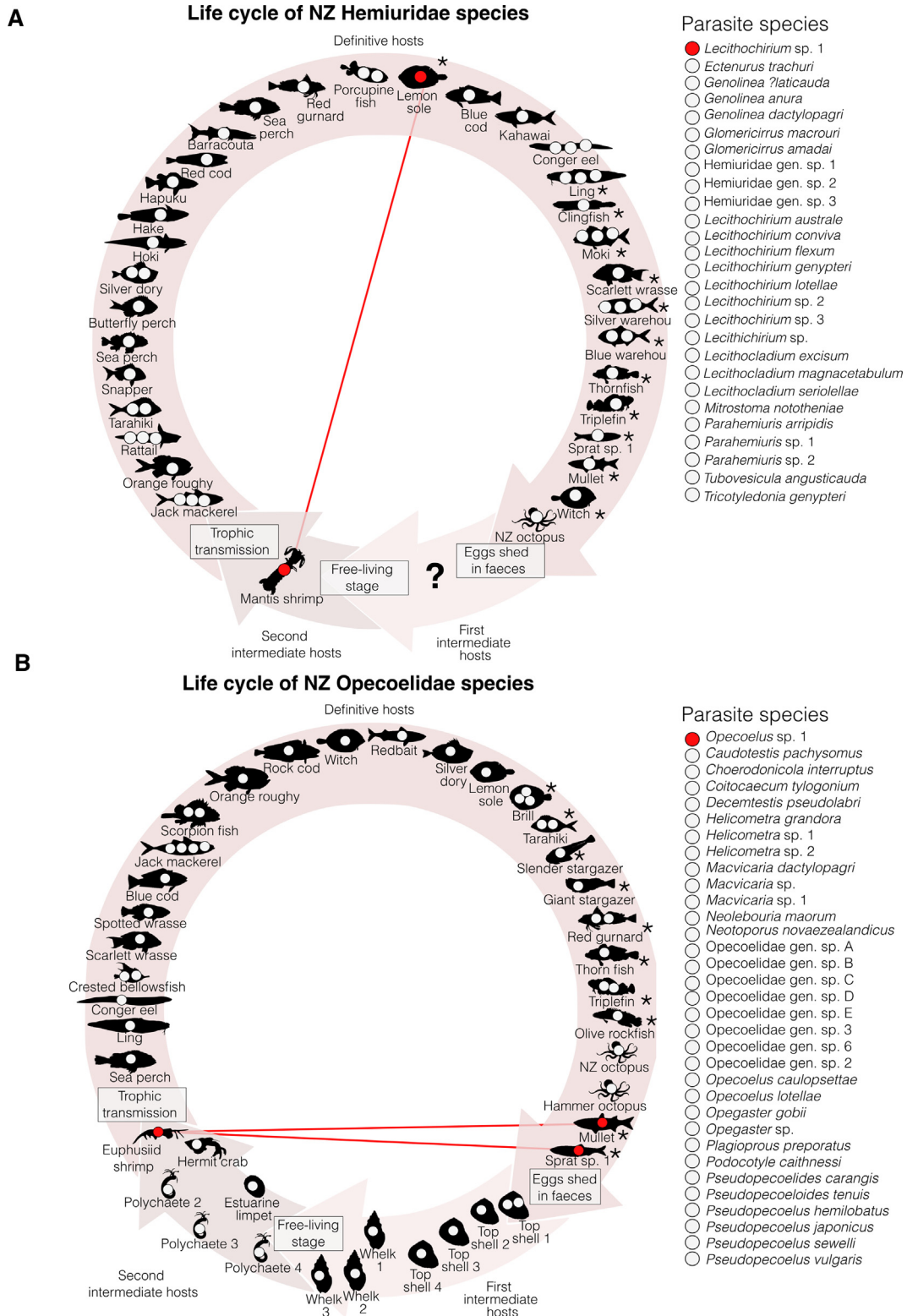
Unfortunately, no photos were taken of the larval stage before it was sacrificed for DNA sequencing, so no morphological data are



**Fig 6.** Overview of (A) Himasthidae and (B) Microphallidae life cycles known in New Zealand. Life cycles include definitive, first and second intermediate hosts. Black silhouettes represent host species and circles represent trematode infections. Circles filled in shades of grey (or yellow–red in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host records from this study.

available to assess which type of larval cestode it represented. Based on the life cycle of closely related orders, it is assumed that two intermediate hosts are involved in the life cycle, probably including crustaceans, cephalopods and (or) teleosts as intermediate and paratenic hosts (Hoberg, 1986; Mariaux et al., 2017). How-

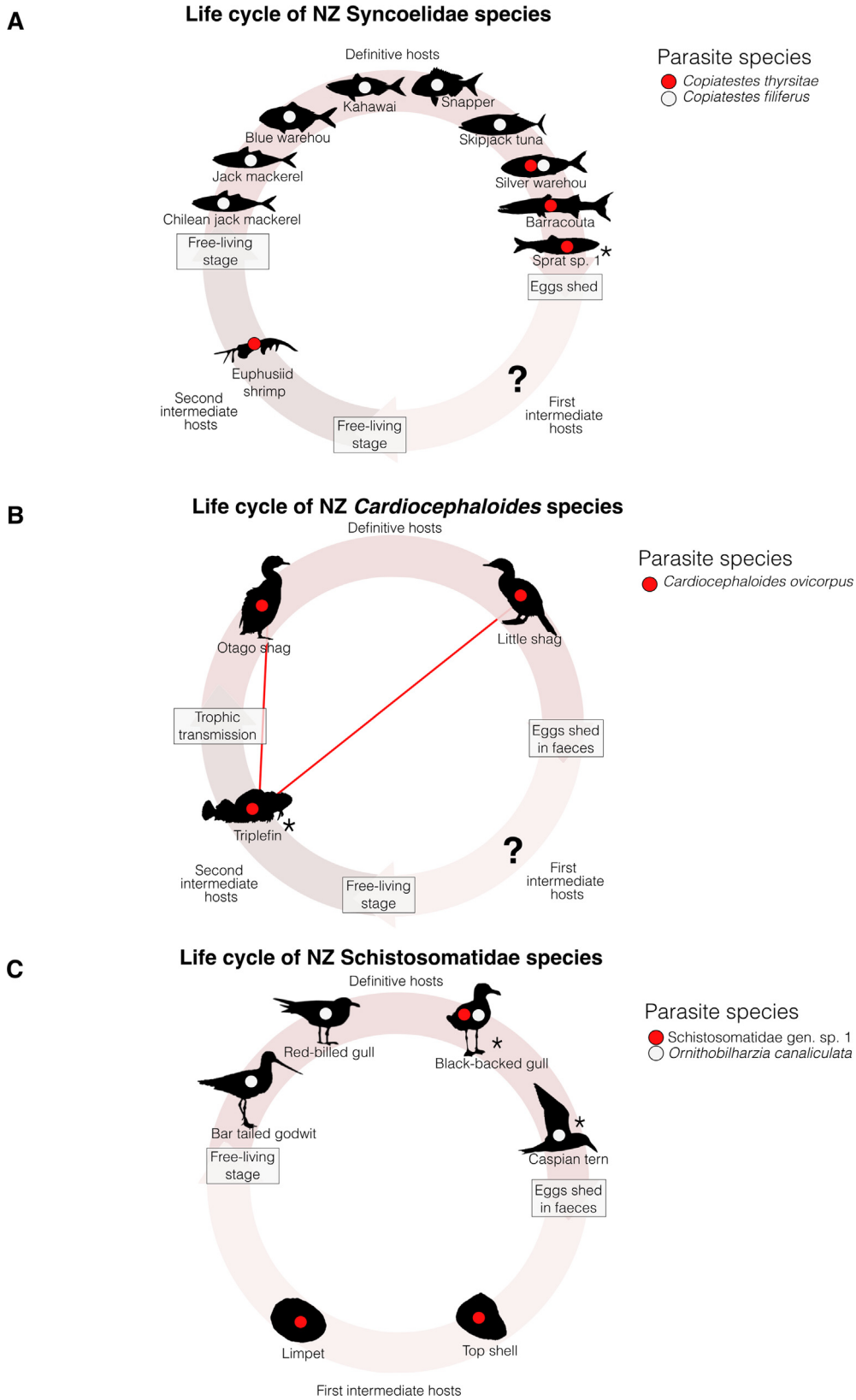
ever, it is not possible to make any inference regarding whether the squid is a paratenic or second intermediate host in this case, only to confirm that tetrabothriids can use squid to complete their life cycles.



**Fig. 7.** Overview of (A) Hemiuridae and (B) Opcoelidae life cycles known in New Zealand. Life cycles include definitive, first and second intermediate, and/or paratenic hosts. Black silhouettes represent host species and circles represent trematode infections. Circles filled in grey (or red in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host records from this study.

New transmission routes between intermediate and definitive hosts for three species belonging to Order Trypanorhyncha were uncovered (Fig. 4A). Some trypanorhynch species infected only one intermediate/paratenic host, e.g. *Lacistorhynchus dollfusi* recov-

ered only from barracouta, whereas other trypanorhynch species infected a large range of intermediate hosts, such as *Hepatoxylon trichiuri* which infected arrow squid from Otago and has been previously reported to infect a range of other fish from records around



**Fig. 8.** Overview of (A) Syncoelidae, (B) *Cardiocephaloides*, and (C) Schistosomatidae life cycles known in New Zealand. Life cycles include definitive, first and second intermediate, and/or paratenic hosts. Black silhouettes represent host species and circles represent trematode infections. Circles filled in grey (or red in the online version) are known from at least two stages within intermediate and definitive hosts, whereas white filled circles are known from only one life cycle stage. Lines between hosts illustrate potential trophic transmission routes (predator–prey interactions) utilized by each species. Host species with \* denote new host records from this study.

New Zealand. Palm and Caira (2008) demonstrated that host specificity of trypanorhynch larval stages, especially that of the last stage before the adult stage, tends to be lower than that of the adults within definitive hosts. Trypanorhynch life cycles generally involve a crustacean first intermediate host, in which the proceroid develops, the second intermediate host in which the larva develops further to a pleuroceroid, plerocercus or merocercoid, and finally, consumption of the second intermediate host by an elasmobranch definitive host where the larva develops into an adult (Palm, 2004).

Adult cestodes from orders “Tetrphyllidea” and Phyllobothriidea develop within chondrichthyan definitive hosts, and although there are currently 25 known species in New Zealand, previous to this study only one species *Calyptrobothrium chalarosomum*, was known as a larval stage within intermediate hosts and an elasmobranch definitive host. We expand the current knowledge of their life cycles through identification of eight larval species within intermediate/paratenic hosts, of which four genetically matched adult stages within definitive hosts (Fig. 4B). Arrow squid was an intermediate/paratenic host for all four species and other intermediate/paratenic hosts comprised a range of teleost fish.

Of the four onchoproteocephalidean cestodes known in New Zealand, all species of *Acanthobothrium*, we identified trophic transmission pathways for two species (Fig. 5A). The New Zealand species belong to the elasmobranch-hosted members of the order, Onchoproteocephalidea II, as defined by Caira et al. (2017). No full life cycle of *Acanthobothrium* has been resolved, but it is believed to comprise molluscs as first and second intermediate hosts and crustaceans and teleost fish as intermediate/paratenic hosts (Caira and Reyda, 2005; Bennett et al., 2019).

We recovered transmission routes between intermediate and definitive hosts for three species belonging to Order Bothriocephalidea (Fig. 5B). Both larval and adult forms were found in teleost fish. The proceroids are thought to develop in copepod first intermediate hosts with fish acting as second intermediate and definitive hosts (Kutcha et al., 2008).

### 3.3.3. Trematodes

There are currently 13 species of Himasthliidae present in Otago animals, and of those, three are known from multiple life stages, including new definitive host records provided here (Fig. 6A). The life cycle of himasthliid trematodes includes gastropod first intermediate hosts where ingested eggs develop into cercariae within the host's gonad. Here, asexual reproduction produces cercariae that emerge into the water column and penetrate bivalves, polychaetes and chitons as second intermediate hosts. After penetration, cercariae develop into encysted metacercariae in the gills, hepatopancreas, appendages or free within the body cavity of the second intermediate host. Definitive hosts, usually shorebirds, consume infected second intermediate hosts, and metacercariae excyst and mature into adults within the intestine of the definitive host.

Within Family Microphallidae, we provide new life cycle data for three seabird definitive hosts (Fig. 6B). The life cycles of microphallid trematodes are similar to those of Himasthliidae, although the second intermediate hosts are exclusively crustaceans.

There are many species of Hemiuridae known to infect New Zealand fish definitive hosts (37 species from here and from Hine et al. (2000)). However, no first known intermediate hosts have been identified for any species in New Zealand, and only one record of a second intermediate host is known, a mantis shrimp (Fig. 7A).

There are over 30 species of Opcoelidae known from New Zealand, although only one species has been identified from multiple life stages within intermediate and definitive hosts (Fig. 7B). There exist various records of larval infections within first and second

intermediate hosts from the literature (e.g. Donald et al., 2004), but their genetic sequences do not match any other life stage within another host. In this study we genetically matched an opcoelid from euphausiid shrimp second intermediate host and two fish definitive hosts. The first intermediate hosts of this group consist of gastropods from which cercariae are released into the water column. After penetrating polychaetes, crustaceans or limpets they develop into encysted metacercariae and await their host being eaten by the definitive host, which is almost always a teleost fish, except for *Plagioporus maorum* which infects an octopus as its definitive host.

We recovered *Copiatestes thyrstae* (Family Syncoelidae) from various life stages and provide insight into the life cycles of Syncoelidae species (Fig. 8A). First, metacercarial specimens were recovered from their second intermediate crustacean host, a euphausiid shrimp, with an estimated prevalence of about 1% ( $n = 5$  infections from just over 500 euphausiid individuals). Secondly, we recovered multiple free-living *C. thyrstae* metacercariae from the water column, in a plankton tow from Otago Harbour. Lastly, we recovered the adult stage from definitive fish host gills, including barracouta, silver warehou and sprat fish. Around the world, elasmobranchs are also reported as definitive hosts, and site of infection can range from skin, buccal cavities as well as gills. No first intermediate hosts have been identified as of Gibson and Bray (1977). Euphausiids are known as second intermediate hosts in other areas around the world (Marcogliese, 1995, Busch et al., 2012).

We provide new data regarding the life cycle of Genus *Cardiocephaloides* for New Zealand (Fig. 8B). Only one species was found, *Cardiocephaloides ovicorpus*, and this presumably infects a yet to be investigated gastropod species as first intermediate host based on what is currently known for other species around the world (e.g. *Cardiocephaloides longicollis* infects Nassariidae gastropods in the Mediterranean and Black Sea (Born-Torrijis et al., 2016)). When cercariae are shed into the water column they penetrate triplefin fish in which the trematodes migrate to a position alongside the brain and develop into encysted metacercariae. Unlike other species in the genus *C. ovicorpus* appears to have high host specificity for its second intermediate host as all other coastal fish species investigated had no apparent infections. Metacercariae within triplefin await predation by the definitive hosts, which in this case are two cormorant species where the metacercariae develop into adults directly below the stomach in the duodenum.

We recovered only the second species from Family Schistosomatidae ever recorded in New Zealand's marine environment (Fig. 8C). Schistosomes follow a two-host life cycle infecting gastropod first intermediate hosts (cercariae are produced and released into the water column), and seabird definitive hosts (cercariae burrow into the skin of their definitive hosts and develop into adult worms in venous and arterial vessels or in the nasal tissues) (Brant et al., 2006). In the case of the newly discovered species Schistosomatidae gen. sp. 1, it infects two gastropod species as first intermediate hosts and then black-backed gulls as definitive hosts.

## 4. Discussion

Genetically matching adult and larval parasite forms is considered the next step in resolving the ecology and evolution of complex life cycle parasites (Blasco-Costa and Poulin, 2017). This study applies such an approach to a whole ecosystem, incorporating multiple host and parasite taxa. We uncovered 289 transmission routes between intermediate and definitive hosts and this in turn revealed which transmission pathways, host species and host taxa are the most important for each helminth group to complete

their life cycles within Otago's coastal marine ecosystem. We provide insights into the ecology and evolution of life history characteristics of helminth parasites with complex life cycles. The implications of the life cycle data gathered here extend beyond knowledge for Otago or New Zealand, as we report the first known instances of larval parasites within intermediate hosts for various parasite higher taxa whose intermediate hosts have never been or are seldom reported on in the literature.

#### Transmission pathways

Due to the phylogenetically diverse range of parasite groups considered, each requires investigation both on its own as well as in combination with the others for an overall view of life cycle patterns in the Otago ecosystem. Each helminth group differed in the number and frequency of transmission routes utilized to complete its life cycle. Cestodes and trematodes tended to use fewer but species-rich transmission pathways, whereas nematodes and acanthocephalans typically had a generalized approach to which transmission routes are taken. Many species within each parasite group are relatively phylogenetically distant too, differing in evolutionary histories and life cycle characteristics requiring investigation at an individual species level.

Nematodes had the fewest species with life cycle data recovered but comprised the highest number of transmission routes observed (Fig. 1C). This high number of potential transmission routes reflects the generalist nature of both adult and larval stages for the most common nematode recovered from multiple life stages, *Hysterothylacium aduncum* (Family Raphidascarididae) which infected 30% of fish intermediate host species examined. The three other nematodes with life cycle data, *Hysterothylacium deardorffoverstreetorum* (Family Raphidascarididae), *Contraecaecum rudolphii* E (Family Anisakidae) and *Cosmocephalus jaenschii* (Family Acuariidae), exhibited relatively high host specificity at least in their larval stages within intermediate hosts. All three infected only one or two fish intermediate host species each, all of which comprised small bodied species; triplefin and sprat. For *C. rudolphii* E and *C. jaenschii*, these intermediate host records complement observations of sprat in the diet of their definitive shorebird hosts, as reported by Lalas (1983). A similar pattern is observed within the acanthocephalan transmission network where a high number of transmission routes were identified which belonged to only five parasite species. This also likely reflects the low host specificity of one species in particular, *C. hanna*e which infected 13 fish paratenic hosts in Otago.

Cestodes and trematodes tended to utilize fewer total transmission links, with some pathways being utilized by multiple parasite species. Multiple parasite species (including between and within parasite groups) may be converging on specific predator–prey interactions to complete their life cycles. For example, when considering all parasite transmission pathways in Otago, the predator–prey interaction between sprat and little blue penguins is utilized by at least three parasite species, including two acanthocephalans and one nematode. Such pathways may reflect the consistent availability of specific predator–prey interactions over time, which multiple parasite taxa have convergently evolved to exploit (Combes, 2001; Rossiter and Sukhedo, 2011). If a predator–prey interaction is stable and predictable over evolutionary time, selection may favour those parasites utilizing it (Price, 1980; Thompson, 1982). These widely used transmission routes may also represent the stronger trophic links within the food web, along which the most matter and energy flow from prey to predator, also representing optimal pathways for life cycle completion (Thompson et al., 2013).

Alternatively, such species-rich pathways, if exploited by closely related parasites may simply reflect evolutionary constraints where ancestral parasites evolved to use specific transmission pathways (Brooks, 1988), and transmission pathways of extant clo-

sely related species may reflect historical host use. We see this occurrence within species of Microphallidae recovered here, of which numerous closely related species typically infect mudsnail, *Zeacumantus subcarinatus*, purple shore crab, *Hemigrapsus sexdentatus* and black-backed gulls, *Larus dominicanus* as hosts. Thus, certain trophic transmission routes, however advantageous they may be, are likely unachievable for many parasite taxa due to such constraints (Cirtwill et al., 2017).

The predator–prey interactions utilized by the most parasite species could also reflect host diet breadth. At first glance, it might be assumed that if a host specializes on one prey item, this interaction may provide parasites with a high probability of life cycle success. However, natural 'boom-and-bust' dynamics for the predator–prey pair may result in significant extinction risk for the parasite species involved, especially considering their sensitivity to secondary extinctions compared with that of their hosts (Lafferty and Kuris, 2009). Rossiter and Sukhedo (2011) identified downward asymmetry as the most likely predator–prey interaction which parasites should evolutionarily converge towards. This scenario includes a single definitive host predator and various prey species, only one of which facilitates parasite transmission. Here, parasites have increased probability of successful transmission and reduced extinction risk as the predator–prey interaction achieves stability with the predator having multiple prey options. This idea also fits well with the fact that generalist predators tend to have more parasite species than specialist predators (Vásquez et al., 2005; Chen et al., 2008). Although the various transmission routes identified here facilitate the persistence of multiple parasite species, none are species-rich enough to provide strong evidence for convergence toward use of specific predator–prey interactions. We must consider also the fact that although a large effort was employed here towards matching larval and adult life stages to identify transmission pathways, a high proportion of parasite species recovered do not have life cycle data yet, concealing true patterns of transmission within Otago. Unfortunately, until further resolution is achieved, it will not be possible to identify which specific predator–prey interactions are being utilized in a nonrandom nature, or why. This study, however, does provide a step in the right direction towards understanding why some transmission routes are favored over others, and an example of how this question could be approached in future.

#### Important intermediate hosts

Presumably, animals within an ecosystem that host the greatest number of larval parasite species should also be the most important species for transmitting parasites and life cycle completion. Here, the intermediate hosts with the highest number of larval parasites include arrow squid, *Nototodarus sloanii*, sprat sp. 1, *Sprattus antipodum* and triplefin spp., *Forsterygion* spp., which hosted 10, nine, and nine larval parasite species respectively. The species composition of helminths in each of these three hosts differed greatly, therefore their importance likely differs for different parasite groups and species.

Arrow squid are apparently very important for the transmission of cestodes in Otago. We recovered nine cestodes and one nematode species from arrow squid, eight of which were matched with adult stages infecting elasmobranch definitive hosts. Additionally, the host specificity of half those species was relatively high (four of the eight cestodes were recovered from arrow squid and only one other fish species), further indicating the importance of arrow squid for the completion of cestode life cycles in Otago. Although cephalopods tend to receive little attention from parasitologists, they may play significant roles as hosts for many parasites (Tedesco et al., 2020; Bennett et al., 2022a). Cephalopods often serve as trophic connectors, meaning they consume low trophic level prey and are consumed by higher trophic level species. In New Zealand, arrow squid comprise one of the country's largest



commercial fisheries, accounting for 43 million dollars in economic value yearly (on average between 2010–2015; Williams et al., 2017). Despite this, little is known about their parasites. Additionally, only a handful of the 200 plus species of cephalopods known in New Zealand have parasite records (Bennett et al., 2022a,c). Based on our findings, future investigations into the role of cephalopods in parasite life cycles appear well overdue.

Triplefin species (genus *Forsterygion*) were infected by 11 larval parasite species including six cestodes, three nematodes and two trematodes. These are small benthic inter- to sub-tidal fish that spend most of their time under large rocks, opportunistically consuming benthic invertebrates, particularly small crustaceans (Feary et al., 2009). Some triplefin larval parasites have particularly interesting life history characteristics; some provide insights into predator–prey interactions between triplefin and definitive hosts and some are potentially disease-causing. All cestodes recovered from triplefin were elasmobranch specific, suggesting that triplefin are likely often consumed by coastal elasmobranchs, especially species that visit shallow tidal areas. Larval *Stephanostomum* sp. 1 was found encysted in the body cavity of triplefin, constituting the first known record of this species in Otago, and from this host. Species of *Stephanostomum* have previously been associated with mass mortality events in aquaculture host species (e.g. McGladdery et al., 1990; Olsen and Pierce 1991). McGladdery et al. (1990) found that increased temperatures and low oxygen conditions may provoke severe host immune responses to infection by this parasite. Therefore, the identification of this species in Otago's common, coastal triplefin species constitutes a significant find for monitoring this potentially disease-causing parasite. However, definitive fish host species potentially at risk of this disease remain unknown for now.

*Sprattus antipodum*, another intermediate host with a high number of larval parasites, occupy a different niche to triplefin and arrow squid, being coastal pelagic schooling fish (Whitehead et al., 1988). Probably as a consequence, they also host a different suite of helminth species. Sprat were infected with three larval acanthocephalans, two larval cestodes and four larval nematode species. Of the acanthocephalans, two species, *Andracantha sigma* and *Bolbosoma balaenae*, were recovered only from sprat and no other paratenic fish host of all species investigated. This suggests that sprat may be essential for the transmission of both acanthocephalan species to their definitive hosts. Although sprat and triplefin hosted quite different parasite assemblages, two parasites (one nematode and one cestode) were shared between them. These were *C. jaenschi* and *Acanthobothrium* sp. 1, both of which were relatively highly host specific, utilizing only these two fish species as second intermediate/paratenic hosts, although both species require different definitive hosts for life cycle completion (one involves coastal seabirds and the other elasmobranchs).

It is surprising that only three invertebrate species were found hosting larval nematodes as all those recovered from vertebrate definitive hosts must undoubtedly utilize invertebrate first intermediate hosts. Considering that over 60% of fish species were infected with nematodes, often at high intensities, it is perhaps more surprising that no L1 or L2 larval nematodes were recovered from any invertebrate species. The 'paradox of infected plankton' was first posed by Marcogliese (1995) who pondered on how parasites accumulate in such high numbers in their fish hosts when prevalence within invertebrate first intermediate hosts is so low. Indeed, here, despite the relatively large dissection effort undertaken, these results only scratch the surface toward understanding how parasites utilize transmission routes between first and second intermediate hosts. To overcome this disparity in invertebrate versus vertebrate parasite recovery, future research focusing on specific invertebrate host groups, especially planktonic invertebrates, or predictive models on host–parasite population dynamics may shed

light on some missing links (Marcogliese, 1995). Of the planktonic copepods, amphipods and ostracods examined in this study, all of which are thought to be extremely important first intermediate hosts for a range of helminths, only one species of amphipod was found acting as a first intermediate host for larval acanthocephalans and cestodes (*Amphipod* sp. 1, *Themisto* sp.). Rates of infections in zooplankton within marine environments are often reported as extremely low (ranging between 0.01% and 1.0% in some cases; Marcogliese, 1995). For example, larval *Anisakis simplex* sensu lato infecting euphausiids, *Nyctiphanes australis*, has previously been reported in New Zealand at a prevalence of 0.0003% (Hurst, 1984). Therefore, we may have missed several larval infections of small crustaceans despite our relatively high search effort.

#### Further insights

Large scale biodiversity surveys hold considerable benefits for elucidating parasite life cycles (Jensen and Bullard, 2010). Here, it would not have been possible to make inference on the host range and specificity of parasites without the incorporation of multiple host taxa. For example, prior to this study, *Corynosoma hanna* was thought to occur in two fish paratenic hosts (Hernández-Orts et al., 2017). Here, we recovered *C. hanna* from over 10 additional fish species suggesting it is more generalist in host range than previously thought. In comparison, paratenic hosts of *Andracantha sigma* and *Bolbosoma balaenae* included only one of the 41 fish species investigated in Otago. Thus, as well as allowing increased resolution regarding the host range of particular species, our data also allows comparisons of host specificity of closely related species. Studies such as this provide the framework for understanding how parasites have evolved to exploit specific hosts or a range of hosts in order to complete their life cycles.

Blasco-Costa and Poulin (2017) asked "Who knows which helminth parasite of wildlife, known today from its adult only, will tomorrow become a disease agent of concern to conservation biologists?". The increased knowledge of parasite life cycles gained here may provide a starting framework for estimating the risk of spread or emergence of potentially problematic parasites. Such parasites identified here include zoonotic taxa, such as *Anisakis*, *Contracaecum*, *Corynosoma* and schistosomes, and potentially pathogenic species such as *Stephanostomum* and *Copiatestes*. Management of each disease-causing agent requires that, first, all host species required for their life cycle are identified (Thompson, 2013). The identification of larval stages within intermediate hosts may in some cases provide an alternative way to monitor the presence of these diseases. For logistical and ethical reasons, it may not be appropriate to obtain vertebrate definitive hosts, so alternatively monitoring parasite infections within the intermediate hosts may provide an insight into disease dynamics without impacting the focal vertebrate definitive hosts.

In many cases, the findings of this study may be directly applied to other ecosystems. Phylogenetic conservatism of host–parasite interactions means that the intermediate hosts of congeneric parasites found in other ecosystems should be closely related to the intermediate hosts of their relatives from this ecosystem (Brooks et al., 2006). Similarly, life cycle pattern conservatism may apply too, where transmission routes identified here may help narrow down the range of likely transmission pathways for parasites elsewhere, thus facilitating their discovery.

The broad sampling approach taken in this study and the use of genetic data has provided a unique insight into parasite life cycles at an ecosystem level. Seldom are multiple parasite and host taxa investigated together at this scale, probably due to practicality and ethical bounds of obtaining host specimens, and because the identification of parasites can require some level of taxonomic expertise. Here, we recovered 145 host–parasite associations between larval parasites and their intermediate hosts, 59 of which constitute new host records. Genetic matching of adult and larval

stages identified 289 potential transmission routes used by parasites to complete their life cycles in one localised ecosystem, highlighting which routes and host species are most important for life cycle completion.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2023.02.004>.

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