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SHORT COMMUNICATION

Metazoan parasites from odontocetes off New Zealand: new records

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Abstract Information about the parasite fauna of spectacled porpoises and cetaceans from New Zealand waters in general is scarce. This study takes advantage of material archived in collections of the Otago Museum in Dunedin and Massey University in Auckland, sampled from cetacean species found stranded along the New Zealand coastline between 2007 and 2014. Parasites from seven species of cetaceans (spectacled porpoise, *Phocoena dioptrica* (n = 2 individuals examined); pygmy sperm whale (n = 1); long-finned pilot whale, *Globicephala melas* (n = 1); Risso's dolphin, *Grampus griseus* (n = 1); short-beaked common dolphin, *Delphinus delphis* (n = 7); striped dolphin, *Stenella coeruleoalba* (n = 3) and dusky dolphin, *Lagenorhynchus obscurus* (n = 2)) from the respiratory and gastro-intestinal tract, cranial sinus, liver, urogenital and mammary tract, fascia and blubber were investigated. Ten parasite species were identified, belonging to the Nematoda (*Stenurus minor*, *Stenurus globicephalae*, *Halocercus* sp. (Pseudaliidae), *Anisakis* sp. (Anisakidae), *Crassicauda* sp. (Crassicaudidae)), Cestoda (*Phyllobothrium delphini* and *Monorygma grimaldii* (Phyllobothriidae)), Trematoda (*Brachicladium palliata* and *Brachicladium delphini* (Brachicladiidae)) and Crustacea (*Scutocyamus antipodensis* (Cyamidae)). Some of the parasite species encountered comprises new records for their host. Although the material was not sampled within a systematic parasitological survey, the findings contain valuable new information about the parasite fauna of rare, vagile and vulnerable marine wildlife from a remote oceanic environment.

Keywords Cetaceans · Parasites · Lungworms · Ectoparasitic crustaceans · Anisakidae · Brachicladiidae · Phyllobothriidae · *Crassicauda* sp.

Introduction

Although almost half of the world's marine mammal species occur in New Zealand waters, information about their parasites is scarce, and little is known of parasite diversity, prevalence and impact on the health of odontocetes (Duignan 2003; Stockin et al. 2009). For spectacled porpoises (*Phocoena dioptrica*), which occur in cold-temperate waters throughout the southern hemisphere (Jefferson et al. 1993) and are seldom found stranded, there are few parasitological records (Berón-Vera et al. 2008; Nikolov et al. 2010). Risso's (*Grampus griseus*), short beaked common (*Delphinus delphis*), striped (*Stenella coeruleoalba*) and dusky (*Lagenorhynchus obscurus*) dolphins, as well as long-finned pilot (*Globicephala melas*) and pygmy sperm (*Kogia breviceps*) whales, have been found stranded along the New Zealand coast and been subjected to post mortem investigations (Duignan 2003; Stockin et al. 2009), including a few reports of parasitological findings. In a reappraisal of the conservation status of New Zealand (NZ) marine mammals (Cetacea and Pinnipedia), none of the listed 56 taxa was considered to have an improved status (Molloy 2002; Townsend et al. 2008). Furthermore, three endemic NZ marine mammals, i.e.

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NZ sea lion (*Phocarctos hookeri*), Hector's dolphin (*Cephalorhynchus hectori hectori*) (both endangered) and Maui's dolphin (*Cephalorhynchus hectori maui*) (nationally critical) are considered threatened. Thirteen taxa are considered data deficient (Baker et al. 2010).

In 1988, the New Zealand Whale Stranding Data Base (NZWSDB) was established under the Marine Mammal Protection Act (1978) by the Department of Conservation (DOC) (Brabyn 1991). This study uses marine mammal parasite samples collected within this network and stored in archives at the Otago Museum (OM) and Massey University (MU) to gain more information about the diversity of parasites in marine mammals as a necessary first step toward assessing their impact on host ecology (Poulin et al. 2016). Marine mammal parasites are valuable biological indicators of host-habitat use, diet, migration and population dynamics (Balbuena and Raga 1994; Aznar et al. 1995; Marcogliese 2002), as well as their health and exposure to pollutants (Pascual and Abollo 2005; Aznar et al. 2005; Siebert et al. 1999, 2001, 2007). Parasitology increasingly complements marine ecology to further our understanding of ecosystem dynamics (Poulin et al. 2016), but so far little is known about parasite biodiversity in New Zealand marine mammals (Poulin 2004).

The new information presented here underlines the important role of archived samples in museum and university collections and the New Zealand stranding network (DOC) in monitoring cetacean health and ecology.

Material and methods

Collection of parasites

Parasites were collected during necropsies performed on stranded cetaceans (spectacled porpoise, *P. dioptrica* (n = 2 individuals examined); pygmy sperm whale (n = 1); long-finned pilot whale, *Globicephala melas* (n = 1); Risso's dolphin, *Grampus griseus* (n = 1); short-beaked common dolphin, *D. delphis* (n = 7); striped dolphin, *S. coeruleoalba* (n = 3) and dusky dolphin, *L. obscurus* (n = 2)) found along the coasts of New Zealand between 2007 and 2014 (Table 1). Animals were dissected after storage at −20 °C for up to 6 months; however, a few were dissected while fresh. Parasites were preserved in 70–90% ethanol; some tissue samples with parasites were preserved in formalin and stored in the collections of the OM (Dunedin) or at MU. Endoparasites were cleared in glycerol or lactophenol and identified based on morphological characteristics (Baylis and Daubney 1925; Arnold and Gaskin 1975; Delyamure 1955; Sarmiento and Tantalean 1991; Agustí et al. 2005). Measurements were taken using a digital camera (Olympus DP25) attached to a stereo microscope (Olympus SZ61) or compound microscope (Olympus CX41) with Olympus DP2-BSW software on a personal computer. Ectoparasites (whale lice) were identified according to Leung (1967) and Lincoln and Hurley (1974, 1980); their length was measured from the anterior border of the head to the posterior border of the last segment of the pereon, and their width was measured at pereon 5 in females and pereon 6 in males.

The samples investigated in this study are deposited at the OM collection, Dunedin, New Zealand (Host ID beginning with a number) or at Massey University, Auckland, New Zealand (Host ID beginning with KS) and can be accessed by their host ID code (see Table 1).

DNA isolation, amplification and sequencing

Where possible, parasite identification was also achieved using gene sequence data. Genomic DNA was isolated from specimens of pseudaliid nematodes using a QIAamp Tissue Kit (Qiagen, Hilden, Germany). The ribosomal DNA (rDNA) ITS-2 was amplified from one individual of *Stenurus* and *Halocercus* sp. originating from a spectacled porpoise, long-finned pilot whale, Risso's dolphin and short-beaked common and striped dolphins. In some cases, samples had been stored in formalin prior to Ethanol, so that molecular identification was not feasible. DNA concentrations and purity were determined using a Nanodrop ND-1000 spectrophotometer. Approximately 600 bp of the rDNA ITS-2 was amplified using oligonucleotide primers 5′-GCA GAC GCT TAG AGT GGT GAA A-3′ and 5′-ACT CGC CGT TAC TAA GGG AAT C-3′ as described by Lehnert et al. (2010). Reactions were performed in a total volume of 25 ml, comprising MyTaq Red DNA polymerase (Bioline) and 5 ml of MyTaqTM Red reaction buffer (Bioline (Aust) Pty Ltd., Alexandria, New South Wales, Australia), primers at 0.5 mM each, MyTaqTM Red DNA Polymerase (Bioline) at 0.025 units/ml and 2 μl of DNA template in an Eppendorf Mastercycler ProS (Eppendorf, Hamburg, Germany). PCR products were visualized in a 2% agarose gel using SYBRSafe DNA Gel stain on a UVITEC system (Total Lab Systems Ltd., Cambridge). Electrophoretic bands were gel-extracted and purified using the MEGAquick-spin Total fragment DNA purification kit (iNtRON). Purified PCR products were sent for nucleotide sequencing to the Department of Anatomy at Otago University with 3.2 μM primer concentrations in a 5 μl volume containing 2 ng DNA/100 bp. Nucleotide reactions were performed using ABI PRISMH Big DyeTM TerminatorCycle Sequencing Ready Reaction Kit v.3.1 run on an ABI 3730XL Analyser (Applied Biosystems). The closest match to the sequence was determined using BLASTn on GenBank.

Table 1 Host ID and animal background data, infected organs and parasites species identified

Host	Species	Location	Date	Lung	Cranial sinus, pterygoid sinus, tympanic bullae	Oesophagus/stomach/ intestine	Liver	Urogenital area, mammary gland	Muscle	Fascia	Blubber	Ectoparasites
140024	*G. melas*	Kaka Point	21 February 2014		*S. globicephalae*							
130074	*G. griseus*	Big River, Kahurangi National Park	23 June 2013		*S. globicephalae*							
140130	*K. breviceps*	Moeraki Beach	09 February 2010			*Anisakis* sp.			*C. magna*			
140087	*P. dioptrica*	Caroline Bay	02 October 2014			*Anisakis* sp.					*P. delphini*	*S. antipodensis*
140117	*P. dioptrica*	Pipikaretu	17 September 2014		*S. minor*							
KS07-13Dd	*D. delphis*	Kawaa Kawaa Bay	19 December 07	*Halocercus* sp.								
KS10-09Dd	*D. delphis*	Bethell's Beach, AKL	23 March 10				*B. delphini*					
KS11-13Dd	*D. delphis*	Battey's Beach, Warkworth	07 May 11				*B. palliata*					
KS11-50Dd	*D. delphis*	Sandspit Estuary, Green's Point	16 November 11								*P. delphini*	
KS12-14Dd	*D. delphis*	Shakespeare Park	21 July 12				*B. palliata*					
KS13-08Dd	*D. delphis*	Buckland's Beach, Howick	27 July 13							*Crassicauda* sp.		
KS14-38Dd	*D. delphis*	Waiake Beach, AKL	01 August 14				*B. palliata*					
KS11-51Lo	*L. obscurus*	Portobello Bay, Otago Harbour	26 November 11								*P. delphini*	
KS11-03Lo	*L. obscurus*	At sea (off of Kaikoura)	18 December 10								*P. delphini*	
KS14-46Sc	*S. coeruleoalba*	Carter's Beach, Te Akau	06 October 14	*H. delphini*				*M. grimaldii*			*P. delphini*	
KS12-12-Sc	*S. coeruleoalba*	Kawera Parade, Paramon East	28 June 12					*M. grimaldii*				
KS11-47Sc	*S. coeruleoalba*	Muriwai Beach	16 October 11	*Halocercus* sp.							*M. grimaldii*	

Results

Nineteen ectoparasitic crustaceans (whale lice) on a male spectacled porpoise found stranded in Caroline Bay, New Zealand, were determined as *Scutocyamus antipodensis* (Cyamidae; Amphipoda). Six females, 12 males and one juvenile male were identified and measured. Their length ranged from 1.603–2.164 mm (mean 1.796 mm) in males (n = 12) to 2.821–3.283 mm (mean 3.080 mm) in females (n = 6); the single juvenile measured 1.178 mm. Their width ranged from 0.965–1.211 mm (mean 1.083 mm) in males to 1.812–2.077 mm (mean 1.942 mm) in females; the juvenile measured 0.673 mm.

Lungworm samples from striped dolphins (n = 2) and short-beaked common dolphin (n = 1) contained pseudaliid nematodes of the genus *Halocercus* (Metastrongyloidea). The 631 bp ITS-2 sequence obtained confirmed this identification, the closest match when blasted in GenBank being *Halocercus invaginatus* (Sequence ID: FJ787301.1) with 93% identity. Lungworms in one striped dolphin sample were morphologically identified as *Halocercus delphini* (Pseudaliidae; Metastrongyloidea). The nematodes from the pterygoid sinuses of a long-finned pilot whale (n = 1) and Risso's dolphin (n = 1) where determined as *Stenurus globicephalae* (Pseudaliidae; Metastrongyloidea); this was supported by molecular identification using ITS-2 sequences obtained for specimens from both hosts. The 617 bp ITS-2 sequence derived from the Risso's dolphin was 95% and the 617 bp sequence derived from the long-finned pilot whale was 99% identical to a published *S. globicephalae* ITS-2 sequence (Sequence ID: FJ787303.1). In a nematode sample from the cranial and pterygoid sinus and tympanic cavity of a spectacled porpoise, *Stenurus minor* (Pseudaliidae; Metastrongyloidea) was identified morphologically and provided an 87% match with a published *S. minor* sequence (GenBank Sequence ID: FJ787302.1).

The liver trematodes in short-beaked common dolphins were identified morphologically as belonging to the genus *Brachicladium* (Brachicladiidae) and as the species *Brachicladium palliata* in four host individuals and as *Brachicladium delphini* in one host.

Anisakid nematodes were found from the stomach and intestine of one male spectacled porpoise and the mouth of a pygmy sperm whale. In the spectacled porpoise, only larval anisakids were found. The nematodes from the mouth of the pygmy sperm whale contained two adult males with short ventriculus and short and thick subequal spicules, which measured 345 and 347 μm in length and 64 μm in width, respectively. They probably belong to *Anisakid paggiae*, *Anisakid physeteris* or *Anisakid brevispiculata*.

The posterior end of a male nematode *Crassicauda magna* (Crassicaudidae) was recovered from the neck area of a pygmy sperm whale (*K. breviceps*). The fragment was > 20 cm long and displayed the characteristic coiled tail with two unequal spicules. A nematode fragment belonging to *Crassicauda* sp. was recovered from the fascia of a short-beaked common dolphin.

The merocercoid *Phyllobothrium delphini* (Phyllobothriidae) from the subcutaneous blubber was recorded from seven cetaceans: one spectacled porpoise, two dusky dolphins, two striped dolphins, one short beaked common dolphin and the pygmy sperm whale. Merocercoids of *Monorygma grimaldii* were found in the mammary and urogenital region and blubber of three striped dolphins.

Discussion

Our findings add three new records of previously known parasite taxa in additional host species and contribute to the scarce, but growing, knowledge of parasites in New Zealand marine mammals. The morphology of the whale lice recovered from a spectacled porpoise corresponds to the description of *S. antipodensis* by Lincoln and Hurley (1980). The characteristics that distinguish *S. antipodensis* from the closely related *Scutocyamus parvus* (Lincoln and Hurley 1974), e.g. spinose processes on pereon segments 6 and 7, prolonged posterolateral angles of pereon segment 3, and outer marginal spines on the merus of pereopods 5, 6 and 7 (Lincoln and Hurley 1980) were observed in our specimens. The size of the 12 adult male specimens available here closely matched the three males on which the first description is based (Lincoln and Hurley 1980), although the six females investigated here are slightly larger than the female type specimens (n = 3) described by Lincoln and Hurley (1980). This is the first record of this cyamid on a spectacled porpoise and only its second record after it was first described in 1980 on a Hector's dolphin off New Zealand (Lincoln and Hurley 1980). Cyamids parasitise other odontocetes and have a worldwide distribution on coastal and oceanic hosts as they were recorded on pilot whales off the Faroe Islands (Balbuena and Raga 1991; *Isocyamus delphinii*), harbour porpoises (Lehnert et al. 2007; *I. delphinii*) and white-beaked dolphins in the North Sea (Lincoln and Hurley 1974; *Syncyamus parvus*), striped dolphins in the Mediterranean (Aznar et al. 2005; *Syncyamus aequus*) and a pygmy sperm whale off Australia (Sedlak-Weinstein 1992; *Isocyamus kogiae*).

The pseudaliid nematodes from the respiratory tract of common and striped dolphins, from the pterygoid sinuses of a long-finned pilot whale and Risso's dolphin and from the tympanic cavity of a spectacled porpoise have been found before in delphinids and porpoises in both hemispheres (Lehnert et al. 2005; Tomo et al. 2010). *Halocercus* spp. have been documented in the lungs of short-beaked common dolphins around Australia (Tomo et al. 2010) as well as bottlenose dolphins from Florida (Fauquier et al. 2009) and striped and common dolphins from the North Atlantic (Gibson et al. 1998). *S. globicephalae* has been described

from the tympanic cavity of e.g. pilot whales found stranded in the North Sea (Gibson et al. 1998; Lehnert et al. 2010), from stranded Risso's dolphins (Abollo et al. 1998; Fernández et al. 2003) in the Mediterranean and from a false killer whale in Uruguay (Zylber et al. 2002). To the best of our knowledge, *S. minor* found here in the tympanic cavities of a spectacled porpoise comprises a new host and geographic record for this species in the southern hemisphere. The 87% match with an ITS-2 sequence of *S. minor* from harbour porpoises from the North Atlantic supports the classification as *Stenurus* spp.; however, further analyses of additional loci may reveal genetic differences between pseudaliid nematodes of porpoises from the northern and southern hemisphere. *S. minor* is commonly found in the cranial sinuses and tympanic cavities of odontocetes (Fraija-Fernández et al. 2016), e.g. harbour porpoises in the North Sea and around Canada (Gibson et al. 1998, Faulkner et al. 1998, Lehnert et al. 2005), as well as Dall's (Jefferson 1988) and Burmeister's porpoise (Corcuera et al. 1995). *Stenurus australis* and *Stenurus* sp. have been described from Burmeister's porpoises from Peru and Chile (Sarmiento and Tantalean 1991, Torres et al. 1994, Reyes and Van Waerebeek 1995), but the specimens we observed lacked the distinct dorsal lobe described for the male bursa in *S. australis* and ventrally flexed caudal end of the female. The results reinforce the notion that the evolutionary old group of pseudaliid nematodes have developed life-cycle strategies that allow them to infect pelagic and neritic/coastal host species in both hemispheres.

Trematodes of the Brachicladiidae family parasitise the bile ducts and sometimes the pancreas of porpoises and delphinids (Gibson et al. 1998; Lehnert et al. 2005) and have been reported e.g. in common dolphins from Canada (Adams et al. 1998). *B. delphini* were found in common dolphins of the Mediterranean (Pillerei and Horning 1969) and *B. palliata* were observed in a common dolphin off the UK (Gibson et al. 1998) and in the Black Sea (Delyamure 1955). Brachicladiids have not been recorded from New Zealand cetaceans before. The findings indicate that these trematodes rely on pelagic cetaceans with long-range movements, as shown previously for e.g. short-beaked common dolphins and their digenean parasite *Pholeter gastrophilus* (Fraija-Fernández et al. 2017).

Anisakid nematodes are common parasites of cetaceans (Mattiucci et al. 1997; Nadler et al. 2005). They are usually found in the gastric compartments or oesophagus, causing ulcers at the site of attachment to the mucosa (Siebert et al. 2001). *A. physeteris* and *A. brevispiculata* have been described from e.g. pygmy sperm whales off Canada (McAlpine et al. 1997) and Spain (Abollo and Pascual 2002). Mattiucci et al. (2005) described *A. paggiae* parasitizing pygmy and dwarf sperm whales off the coast of Florida. This shows the wide geographic range in which these generalist parasite species and their hosts occur (Abollo et al. 1998). While the anisakid nematodes found here from the stomach contents and intestine of the spectacled porpoise were not identified to the species level, the two male specimens recovered from the pygmy sperm whale exhibit short, thick and subequal spicules and a short ventriculus characteristic of *A. physeteris*, *A. paggiae* or *A. brevispiculata*.

Crassicauda (Spirurida) nematodes parasitise the muscles and organs of whales and dolphins. Most are reported from the urogenital tract, e.g. *Crassicauda boopis* in fin whales (Lambertsen 1985, 1986). Because of their large size, entire specimens are difficult to recover from carcasses, and so far, mostly heads or tails have been described (Johnston and Mawson 1939; Dollfus 1966). A recent finding providing morphological and molecular characteristics of *C. magna* from a pygmy sperm whale stranded in Queensland, Australia (Jabbar et al. 2015) supports the identification of the incomplete specimen found in this study. Previous reports of *C. magna* from pygmy sperm whales include Australian, US (Johnston and Mawson 1939; Keenan-Bateman et al. 2016) and Mediterranean (Abollo et al. 1998) waters, suggesting a worldwide distribution. The unusual location of the specimen around the cervico-thoracic region of the pygmy sperm whale in this study is supported by a recent study observing *Crassicauda* individuals close to a newly detected exocrine gland unique to *K. breviceps* (Keenan-Bateman et al. 2016). *Crassicauda* spp. have been reported also in short-beaked common dolphins (Stockin et al. 2009) and beaked whales (*C. boopis* in *Ziphius cavirostris*) (Duignan 2003) stranded in New Zealand.

P. delphini and *M. grimaldii* are larval cestodes infecting marine mammals and belonging to the Phyllobothriidae (Ruhnke 2011). While *P. delphini* infects the subcutaneous blubber, *M. grimaldii* is mostly found encysted in the peritoneum/abdominal cavity of its odontocete hosts, often near the urogenital and reproductive tract. These merocercoids are located within a liquid-filled bladder and can be differentiated by their scolex and filament morphology (Agustí et al. 2005). They are frequently reported from cetaceans (Delyamure 1955; Gibson et al. 1998; Lehnert et al. 2014) worldwide and are believed to use cetaceans as intermediate or paratenic hosts to infect shark definitive hosts (Agustí et al. 2005; Aznar et al. 2007; Randhawa 2011). Recent molecular evidence suggests that cestode merocercoids infecting marine mammals are assignable to the '*Clistobothrium*' clade (Aznar et al. 2007; Randhawa 2011; Randhawa and Brickle 2011), which includes the cestodes *C. carcharodoni* and *C. tumidum* (e.g. great white sharks), *C. montaukensis* (e.g. mako sharks), and *C.* cf. *montaukensis* (e.g. porbeagle sharks). Although Ruhnke (2011) treated *Clistobothrium* as members of the Phyllobothriidae, the inclusion of this genus in this family and the order Phyllobothriidea is likely, but not unequivocal (Caira et al. 2014). Here, *P. delphini* merocercoids were found in the subcutaneous blubber of striped, short-beaked common and dusky dolphins, as well as in spectacled porpoise and a

pygmy sperm whale, and in the urogenital and reproductive area of two striped dolphins, possibly because multiple shark species inhabit the waters off New Zealand as potential definitive hosts. *P. delphini* has been reported previously in short-beaked common dolphins from these waters (Stockin et al. 2009) and in striped and common dolphins from the Oregon coast (Dailey and Stroud 1978). *M. grimaldii* has been reported from striped dolphins in the UK and Mediterranean (Gibson et al. 1998; Agustí et al. 2005), and short-beaked common dolphins has been reported around New Zealand (Stockin et al. 2009). *P. delphini* was also described from a pygmy sperm whale off New Brunswick, Canada (McAlpine et al. 1997) and in dusky dolphins off Peru, Chile and Argentina (Van Waerebeek et al. 1993; Loizaga de Castro et al. 2014), while this is the first record of *P. delphini* from a spectacled porpoise.

Most parasites found in this study have not been reported previously in cetaceans from New Zealand (Duignan 2003; Stockin et al. 2009). Some of them, e.g. *S. antipodensis*, *S. minor* and *P. delphini*, comprise new host and/or geographic records for their cetacean host. Although the material was not obtained from a dedicated parasitological survey, the findings contain valuable data about the parasites of rare and vulnerable species from a remote oceanic environment. The data indicate that many marine mammal parasite species have adapted to the highly vagile lifestyle of their oceanic hosts in this remote environment and that species diversity in marine mammals around New Zealand and their phylogeography needs to be investigated further. The information derived from archived samples underlines how important museum collections and the NZ stranding network are in monitoring cetacean health and ecology. In future, full investigations of parasite infections during post mortem investigations would enable systematic analyses of parasite diversity and its impact on marine mammal health, as well as provide scientifically organized data on which to base management decisions for their conservation.

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