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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - ORIGINAL PAPER

Morphology and molecules resolve the identity and life cycle of an eye trematode, *Philophthalmus attenuatus* n. sp. (Trematoda: Philophthalmidae) infecting gulls in New Zealand

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Abstract
Trematodes of the genus *Philophthalmus* are cosmopolitan parasites that infect the eyes of birds and mammals. They have the potential to affect the survival of their hosts and a few cases of human philophthalmiasis have occurred worldwide. Adults of known *Philophthalmus* species have never been recorded from bird hosts in New Zealand, despite their cercarial stage being a focus of various studies. Here, we describe a new species of *Philophthalmus* infecting New Zealand red-billed and black-backed gulls, *Philophthalmus attenuatus* n. sp. It is distinguished from other marine species of *Philophthalmus* by its long, thin body shape, consistently longer vitelline field on the left, and its body reflexed at the ventral sucker. We use molecular methods to complete the life cycle of this species, matching it with the larval stage infecting the mud whelk, *Zeacumantus subcarinatus*, and present a preliminary *cox1* phylogeny. In addition, we comment on the validity of some taxonomic characters used to differentiate species of this genus, discuss potential colonisation routes to New Zealand and comment on the potential for zoonotic infection.

Keywords *Larus dominicanus* · *Chroicocephalus scopulinus* · New Zealand parasite · *Philophthalmus* new species · Philophthalmiasis · Pathology

Introduction

The genus *Philophthalmus*, Looss 1899 (Echinostoma: Philophthalmidae) is a cosmopolitan group of parasite species that infect the eyes of birds and mammals. *Philophthalmus* species can cause ophthalmic disease in both avian and mammalian species and it has recently been reported that *P. zalophi* Dailey et al. 2005 may affect the survival of its host, juvenile female Galapagos sea lions (Phillips et al. 2018). The importance of this genus for zoonotic concern and its potential role in influencing population dynamics of its host species makes new taxonomic and/or geographical reports significant.

The first report of a *Philophthalmus* species in New Zealand came from Howell (1965), who reported infection in a small intertidal gastropod, *Zeacumantus subcarinatus* (Sowerby, 1855). This *Philophthalmus* sp. has not since been assigned to a species, despite being a subject of studies of its cercarial stage (e.g. Leung et al. 2009; Keeney et al. 2009). Howell (1965) and Keeney et al. (2009) both suggested that the black-backed gull (*Larus dominicanus* Lichtenstein, 1823) may be the definitive host, based on personal observations of philophthalmid specimens extracted from this species in New Zealand. During a survey of the helminth parasites of birds in South Island, New Zealand, we found many specimens of *Philophthalmus* sp. attached beneath the nictitating membranes of red-billed (*Chroicocephalus scopulinus* (Forster, 1844)) and black-backed gulls, which we suspected were the adults of those larval stages known from *Z. subcarinatus*.

The aim of this study is to give a scientific name to describe and complete the life cycle of the *Philophthalmus* sp. previously known to infect New Zealand *Z. subcarinatus*. We use molecular tools to compare the cercarial stage of *Philophthalmus* sp. infecting *Z. subcarinatus*, with the adult

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infecting both *C. scopulinus* and *L. dominicanus*, and to present a preliminary phylogeny for the genus based on available sequences. We use morphological methods to compare the adult *Philophthalmus* specimens from New Zealand to other described marine *Philophthalmus* species, and conclude that the specimens belong to a hitherto undescribed species, which we here describe and name *Philophthalmus attenuatus* n. sp.

Methods

Bird collection and trematode sampling

A total of 26 birds of two gull species, 14 red-billed gulls (*C. scopulinus*) and 12 black-backed gulls (*L. dominicanus*), were examined for *Philophthalmus* between October and November 2018. Birds were frozen less than 12 h after death and kindly donated by the Dunedin Wildlife Hospital for dissection. Birds were defrosted, and eyes with intact nictitating membranes cut out using a scalpel. Eye trematodes were removed from the nictitating membrane using tweezers and preserved in 70% ethanol for whole-mount and 96% ethanol for genetic analyses.

Morphological data

Trematodes fixed for whole mounts were stained using acetic acid iron carmine stain, dehydrated through a graded ethanol series, cleared in clove oil and mounted in permanent preparations with Canada balsam. Measurements were made using ImageJ software (Wayne Rasband, NIH, USA) from photographs taken on an Olympus BX51 compound microscope mounted with DP25 camera attachment. All measurements are in micrometres unless otherwise indicated, with the range followed by the mean. Drawings were made with the aid of a drawing tube mounted on an Olympus compound microscope. Type specimens were deposited in Te Papa Museum, Wellington (Accession no. 21899) and the Otago Museum, Dunedin (Accession no. IV101950-IV101952).

Molecular data and analysis

Genomic DNA was extracted from two single ethanol-fixed specimens of eye trematode (one from a red-billed gull and one from a black-backed gull) using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A partial fragment of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) was amplified (target bp c. 700) using primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) (Bowles et al. 1993) and Trem.cox1.rrnl (5′-AATCATGATGCAAAAGGTA-3′) (Králová-Hromadová et al. 2001). PCRs (polymerase chain reactions) were run in 25 μl reaction mixtures using an Eppendorf Mastercycler Pro thermal cycler. The amplification protocol consisted of an initial denaturation phase (2 min at 95 °C); 40 cycles of denaturation (30 s at 95 °C), primer annealing (40 s at 48 °C), extension (1 min at 72 °C), and a 10 min final extension (72 °C). PCR products were cleaned using EXOSAP-IT™ Express PCR Product Cleanup Reagent (USB Corporation, Cleveland, OH, USA), following manufacturer's instructions. Sanger sequencing by capillary electrophoresis was performed by Genetic Analysis Service, Department of Anatomy, University of Otago (Dunedin, New Zealand).

Sequences were imported into Geneious v8.1.9 (Kearse et al. 2012), trimmed using the trim function with default parameters and manually edited for incorrect or ambiguous base calls. A contiguous sequence was assembled for each sequence and the result was submitted to GenBank under the accession numbers MK482102-MK482103. The generated sequences were aligned in a dataset together with published sequences of identified *Philophthalmus* spp. or closely related species from GenBank. A sequence belonging to the closely related family, Lepocreadiidae (*Stegodexamene anguillae*), was included as outgroup. The dataset and accession numbers are shown in Table 1. The dataset was aligned using MAFT algorithm implemented in Geneious v8.1.9 (auto algorithm using default settings).

To infer the phylogenetic relationships between adult specimens from black-backed and red-billed gulls in New Zealand and cercarial *Philophthalmus* sp. from *Z. subcarinatus*, and downloaded *Philophthalmus* spp. available, Bayesian inference was conducted in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) using the online interface: Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway (Miller et al. 2010). The analysis used random starting trees for two runs (each one cold and three heated chains), employing a Markov Chain Monte Carlo (MCMC) approach for sampling the joint posterior probability distribution across 10,000,000 generations, sampling every 1000 generations. A heating chain value of 0.01 was selected and the first and last 25% of samples were discarded as burning. The resulting phylogeny was summarised in a 50% majority-rule consensus tree with clade credibility support values (Bayesian posterior probability, BPP) and branch length information. BPP higher than 0.95 was considered strong support for nodal positions. Genetic divergence was calculated in MEGA v7 (Kumar et al. 2016).

Results

Eye trematodes were found in five (36%) out of 14 red-billed gulls and six (50%) out of 12 black-backed gulls. The number of trematodes per eye varied from 1 to 6 (mean 3.2)

Table 1 Taxa included in the dataset for *cox1* phylogeny, including host, stage, locality and accession numbers

ID	Host	Stage	Locality	Accession no.	Reference
Philophthalmus attenuatus n. sp. (rgb13tre1)	*Chroicocephalus scopulinus*	Adult	Otago NZ	MK482102	This study
Philophthalmus attenuatus n. sp. (bbg13tre1)	*Larus dominicanus*	Adult	Otago NZ	MK482103	This study
Philophthalmus sp.	*Z. subcarinatus*	Redia	New Zealand	GQ868079	Keeney et al. 2009
Philophthalmus sp.	*Z. subcarinatus*	Redia	Otago, New Zealand	FJ765485	Leung et al. 2009
Philophthalmus sp.	*Z. subcarinatus*	Redia	New Zealand	GQ868094	Keeney et al. 2009
Philophthalmus sp	*Z. subcarinatus*	Redia	New Zealand	GQ868091	Keeney et al. 2009
Philophthalmus lucipetus	*Larus fuscus*	Adult	Portugal	KX925587	Heneberg et al. 2018
Philophthalmus lucipetus	*Larus michahellis*	Adult	Portugal	KX925598	Heneberg et al. 2018
Philophthlalmus lucipetus	*Larus michahellis*	Adult	Portugal	KX925597	Heneberg et al. 2018
Philophthalmus sp.	*Larus fuscus*	Adult	Portugal	KX672820	Heneberg et al. 2018
Philophthalmus gralli	*Tachuris rubrigastra*	Adult	Peru	JQ675731	Literák et al. 2013
Philophthalmus sp.	*Melanoides tuberculata*	Cercaria	Iran	JN621324	Sadjjadi et al. unpublished
Philophthalmus lacrymosus	*Larus fuscus*	Adult	Portugal	KX925599	Heneberg et al. 2018
Philophthalmus sp.	*Larus fuscus*	Adult	Portugal	KX672821	Heneberg et al. 2018
Philophthalmus lacrymosus	*Larus fuscus*	Adult	Portugal	KX925600	Heneberg et al. 2018
Cercaria shikokuensis	*Batillaria cumingi*	Redia	Japan	AY626529	Miura et al. 2005
Cercaria shikokuensis	*Batillaria cumingi*	Redia	Japan	AY626533	Miura et al. 2005
Parorchis sp.	*Austrolittorina antipodum*	Cercaria/redia	New Zealand	KJ868194	O'Dwyer et al. 2014
Parorchis sp.	*Austrolittorina antipodum*	Cercaria/redia	New Zealand	KJ868197	O'Dwyer et al. 2014
Parorchis sp.	*Austrolittorina unifasciata*	Cercaria/redia	Australia	KP903416	O'Dwyer et al. 2015
Echinostomidae sp.	*Cerithidea cingulata*	Cercaria/redia	Kuwait	KC544255	Al Kandari et al. unpublished
Outgroup *Stegodexamene anguillae*	*Gobiomorphus breviceps*	Metacercaria	New Zealand	JN244823	Blasco-Costa et al. 2012

individuals in red-billed gulls and varied from 1 to 31 (mean 12) individuals in black-backed gulls.

Philophthalmus attenuatus n. sp. (Fig. 1) (Table 2).

Other names in the literature: *Philophthalmus* sp. of numerous papers from the laboratory of Robert Poulin at Otago University, New Zealand, since 2008.

Description of adult

Based on 18 stained and mounted mature specimens from black-backed gulls. Body aspinose and elongate with rounded ends; length 3.0–5.4 (4.2); width at level of ventral sucker 0.37–0.78 (0.54). Anterior end of the body narrower than posterior end; maximum width often at level of testes. Forebody naturally reflexed at the level of ventral sucker to an angle of 110–120° (113°) ($n = 4$) when viewed laterally (Fig. 1b, c). Strongly developed oral sucker and pharynx. Oral sucker sub-terminal, 195–304 (244) × 222–357 (286). Pharynx large, globular and muscular 164–302 (233) × 150–278 (225) contiguous with or slightly overlapping posterior edge of oral sucker. Oesophagus 150–180 long. Intestinal bifurcation about midway between oral and ventral suckers. Caeca end level with posterior edge of posterior testis. Genital pore slightly anterior and to left of intestinal bifurcation. Cirrus spinous. Cirrus sac on left side, always extends well beyond posterior edge of ventral sucker; 845–1783 (1089) long. Seminal vesicle oval, 222–414 (285) long. Ventral sucker large, round, 304–488 (405) × 342–452 (411), in anterior third of the body, centre situated at 21–29 (24)% body length. Ventral sucker always larger than oral sucker; ratio of widths 1:1.2–1.7 (1:1.4). Ovary small, round, submedian or to left side, anterior to anterior testis, often obscured by eggs in uterus; 100–182 (130) × 94–135 (113). Testes 2, subcircular, tandem, with entire edges, in extreme posterior end of the body. Anterior testis, 298–536 (419) × 291–610 (437); posterior testis 297–576 (452) × 316–586 (431). Uterine seminal receptacle oval or sausage-shaped, between anterior testis and ovary. Vitellaria extracaecal, covering 49–

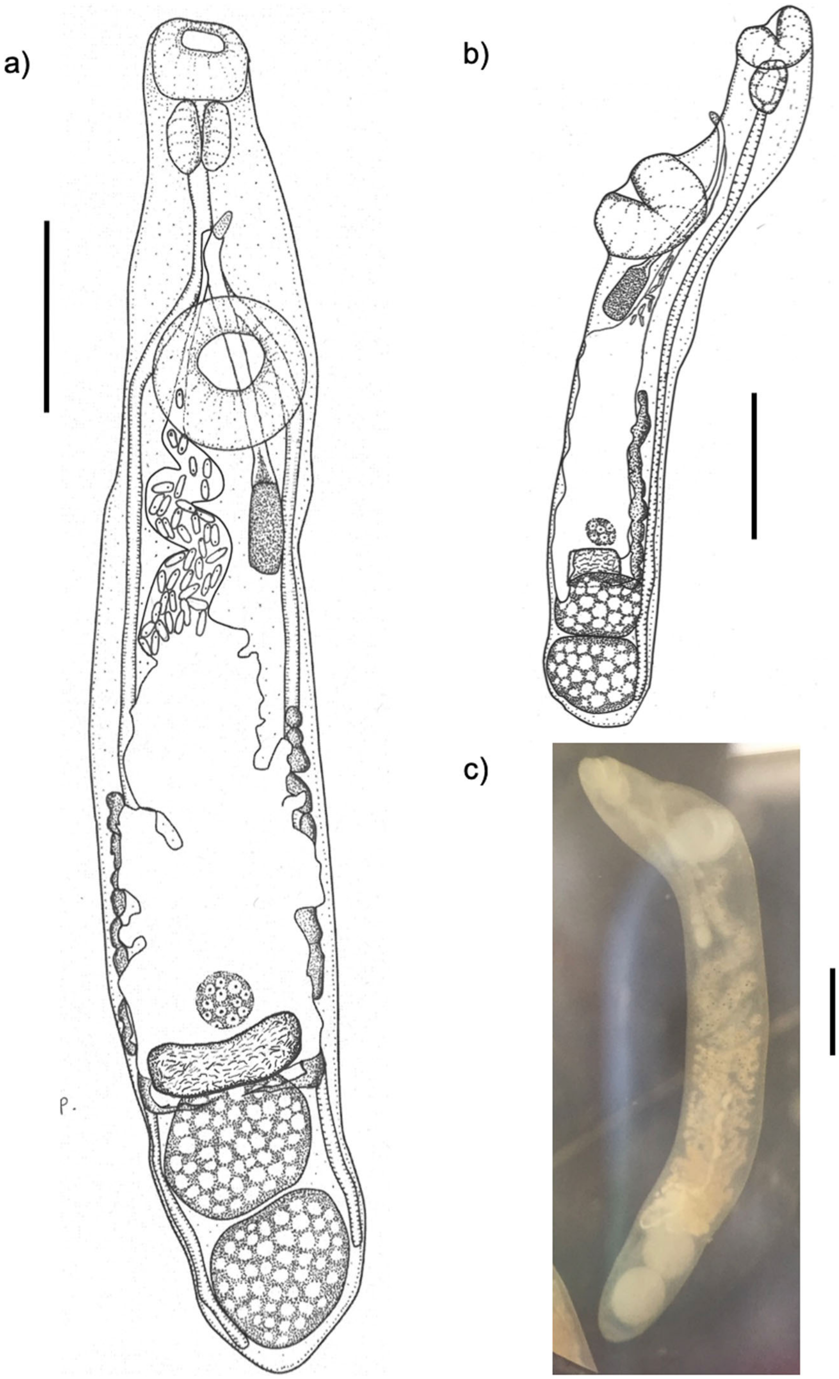

Fig. 1 *Philophthalmus attenuatus* n. sp. from a naturally infected black-backed gull. Ventral view of holotype (**a**). Lateral view of paratype (**b**). Fresh specimen under dissecting microscope (**c**). Scale bars are equal to 500 μm

83 (65)% of distance between ventral sucker and anterior testis. Vitellaria follicular or sometimes tubular (Fig. 2), of unequal length, usually partly obscured by eggs in uterus; when follicular, in 2 rows of 8–9 (left) and 6–7 (right) ($n = 2$) round follicles. Uterine coils occupy space between anterior testis and posterior of cirrus sac, extending posteriorly to the left of anterior testis, and to both sides in specimens with very many eggs. Excretory vesicle Y-shaped, compressed in mature specimens due to posterior testis filling the posterior space. Mature eggs have oculate miracidia with prominent eyespot. Eggs containing miracidia are 60–80 (70) × 25–36 (32); eggs are thin shelled, yellow to brown, and straight-sided oval.

Immature specimens

Measurements are given in Table 2. Immature specimens generally smaller in size than mature ones (< 3 mm long) and, although most contain some eggs, these eggs are not fully developed (i.e. with miracidial eyespots). Features not visible

Table 2 Morphometric comparison of marine species of *Philophthalmus* with *Philophthalmus attenuatus* n. sp. ex black-backed gull from this study, mature and immature specimens

	P. andersoni (Dronen and Penner 1975) CA, USA	*P. hegeneri* (Penner and Fried 1963) FL, USA	*P. larsoni* (Penner and Trimble 1970) FL, USA	*P. zalophi* (Dailey et al. 2005) Ecuador	*P. burrili* (Howell and Bearup 1967) Australia	*Philophthalmus attenuatus* n.sp. New Zealand	*P. attenuatus* n.sp. immature New Zealand
Body size (mm)							
Length	3.5–5.1 (4.2)	1.86–5.46	4.1–6.0 (4.9)	4.0–8.12 (6.0)	2.8–3.7	3.0–5.4 (4.2)	2.0–2.7 (2.4)
Width	0.91–1.15 (1.0)	0.81–1.8	0.93–1.67 (1.66)	0.75–1.8 (1.1)	0.7–1.4	0.37–0.78 (0.54)	0.3–0.5 (0.4)
Oral sucker							
Length	200–210 (205)	250–490	253–560 (321)	230–570 (321)	210–300	195–304 (244)	160–235 (185)
Width	280–350 (303)	360–550	300–700 (412)	260–450 (372)	270–370	222–357 (286)	178–245 (209)
Pharynx							
Length	230–300 (270)	230–360	264–510 (342)	235–430 (331)	250–300	164–302 (233)	137–170 (158)
Width	250–310 (298)	200–360	254–660 (351)	229–420 (316)	240–280	150–278 (225)	137–185 (152)
Ventral sucker							
Length	450–690 (595)	650–930	425–750 (591)	470–830 (616)	400–550	304–488 (405)	252–325 (290)
Width	470–650 (537)	640–1030	529–710 (612)	410–750 (566)	–	342–452 (411)	264–329 (300)
Ovary							
Length	210–270 (227)	150–320	161–370 (241)	120–350 (232)	170–250	100–182 (130)	93–168 (123)
Width	210–260 (240)	180–340	195–380 (275)	125–470 (233)	–	94–135 (113)	106–148 (126)
Anterior testis							
Length	280–430 (390)	220–540	320–660 (436)	350–870 (618)	300–350	298–536 (419)	217–319 (272)
Width	410–600 (562)	250–720	220–840 (461)	370–850 (576)	400–550	291–610 (437)	224–380 (303)
Posterior testis							
Length	260–340 (318)	230–590	290–667 (478)	450–925 (672)	250–400	297–576 (452)	260–393 (318)
Width	360–522 (495)	230–790	260–660 (476)	400–710 (533)	500–550	316–586 (431)	231–389 (298)
Oesophagus							
Length	130–190 (167)	130–410	–	0–80 (50)	150–250	150–180	57–81 (75)
Eggs (w miracidia)							
Length	72–78 (75)	50–75	56–70 (63)	58–102 (78)	90–95	60–80 (70)	–
Width	–	20–35	25–31 (28)	25–46 (33)	50	25–36 (30)	–
Vitellaria							
Right (*n*)	6/7	5–6	4–9 (6)	4–10 (6)	–	6–7	6–7
Left (*n*)	6/7	4–5	2–11 (6)	4–7 (5)	–	8–10	8–9
Distance (%)	*69	35–60	16–84	43–54	68–75	49–83 (65)	67–97 (81)
Sucker-ratio	1:1.8	1:1.8	1:1.5	1:1.8	1:1.4	1:1.2–1.7	1:1.2–1.7
Ovary: testis	1:2.3	1:1.9	1:1.6	1:3.1	1:2.1	1:2.8–3.8	1:1.9–2.8
Body L/W %	33	33–66	28–34	18–33	33–38	9–16	13–16

Range (mean) measurements are shown in micrometres unless indicated otherwise. *Metric calculated from illustration in original description

in mature specimens because of the egg-packed uterus are relatively easy to see in immature specimens, such as the ovary and vitellaria.

Taxonomic summary

Type host: black-backed gull, *Larus dominicanus* Lichtenstein, 1823 (Charadriiformes: Laridae)

Other host: red-billed gull, *Chroicocephalus scopulinus* (Forster, 1844) (Charadriiformes: Laridae)

Site of infection in definitive host: under nictitating membrane and lower eyelid.

Intermediate host: marine intertidal mud whelk, *Zeacumantus subcarinatus* (Prosobranchia: Batilariidae)

Type locality: Otago Harbour, New Zealand

Other localities: Bluff and Christchurch, New Zealand (Lloyd and Poulin 2014).

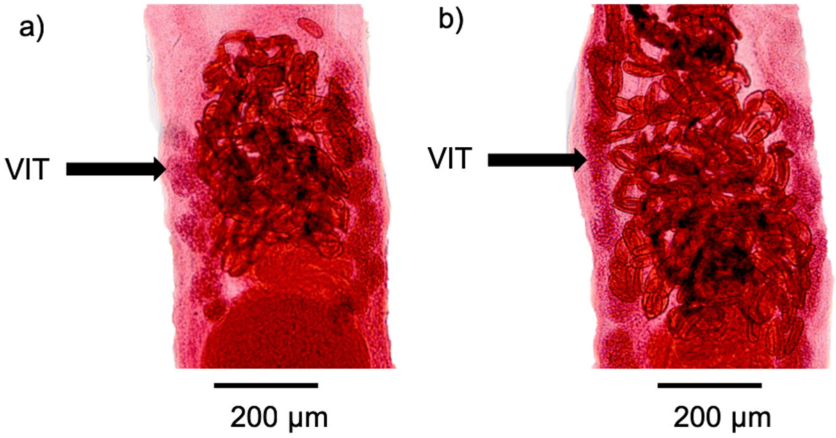

Fig. 2 Ventral view of *P. attenuatus* n. sp. from a naturally infected black-backed gull showing vitelline fields (VIT) in follicular bands (**a**) and vitelline fields as tubular lobes (**b**)

Holotype: Te Papa Museum accession number: 21899 (W.003493)

Paratypes: Otago Museum accession number: IV101950-IV101952 and Te Papa Museum Accession number: 21899 (W.003494-W.003496)

Paragenophore: Te Papa Museum accession number: 21899 (W.003497)

Etymology: the species is named for its attenuated body shape, unique for the genus.

Remarks

Alignment of the DNA sequences, and our *cox1* phylogeny reveals that the *Philophthalmus* specimens infecting red-billed and black-backed gulls have 100% sequence identity with each other and with the redial stages from *Z. subcarinatus*, confirming that they are the same species (Fig. 3). The branch for this clade and sister clade *Philophthalmus lucipetus* is supported by high nodal support (BPP > 0.95) and low sequence similarity (uncorrected pairwise genetic divergence = 0.483). Unfortunately, there are not enough data currently available to draw any conclusions about biogeographical distribution or evolutionary relationships of the genus. We present the sequences and tree here as a starting point for when further molecular data are available from different species around the world.

Comparative morphometrics for all marine species of *Philophthalmus* are given in Table 2. *Philophthalmus attenuatus* n. sp. differs from all other marine species in its long, thin body shape, the width comprising only 9–19% of the body length, compared with 18–66% in other species (see Table 2). In addition, the vitellaria are consistently longer on the left side of the body, whereas in other species, they are either the same length (*P. andersoni* and *P. larsoni*) or the right side is longer (*P. hegeneri* and *P. zalophi*). Another feature seen consistently in *P. attenuatus* n. sp. and not mentioned in the descriptions of any other species is the reflexed body at the level of the ventral sucker (Fig. 1c).

In addition, *P. attenuatus* n. sp. differs from each of the marine species as follows: *Philophthalmus andersoni* has a larger ovary and transversely oval testes which are sometimes lobed. *Philophthalmus hegeneri* has larger oral and ventral suckers and ovary, sometimes lobed testes and cuticular spines. In addition, the cirrus sac does not reach the posterior margin of the ventral sucker. *Philophthalmus larsoni* has larger oral and ventral suckers and ovary, and smaller eggs. *Philophthalmus zalophi* has a larger body size and all associated metrics, lobed testes and a distinctive marine mammal host. *Philophthalmus attenuatus* n. sp. compares most closely to *P. burrili* that infects silver gulls (*Chroicocephalus novaehollandiae* (Stephens, 1826)) in Australia. It is distinguished from *P. burrili* by its smaller ovary and eggs and its attenuated body shape (width 9–16% length versus 33–38% for *P. burrili*) and reflexed forebody.

Discussion

Comparison of the *cox1* DNA sequences from the gulls and the mudsnail has resolved the life cycle of *Philophthalmus attenuatus* n. sp. in New Zealand's marine environment. The life cycle is in accordance with that of other species in the genus: adult *P. burrili* inhabit the nictitating membrane of the bird host (black-backed and red-billed gulls) where sexual maturity is reached. Eggs are released into the environment, via the tears or direct contact of the eye with seawater. The miracidia emerge and penetrate the snail *Z. subcarinatus*, coming to rest within the digestive gland and gonad where the miracidia develop into rediae (Martorelli et al. 2008). The rediae come in two distinct morphs displaying a division of labour (see Leung and Poulin 2011; Lloyd and Poulin 2012). Individuals of the larger morph give rise to the cercariae which leave *Z. subcarinatus* and encyst on hard surfaces such as crustacean carapaces and mollusc shells (Neal and Poulin 2012). Upon ingestion by red-billed or black-backed

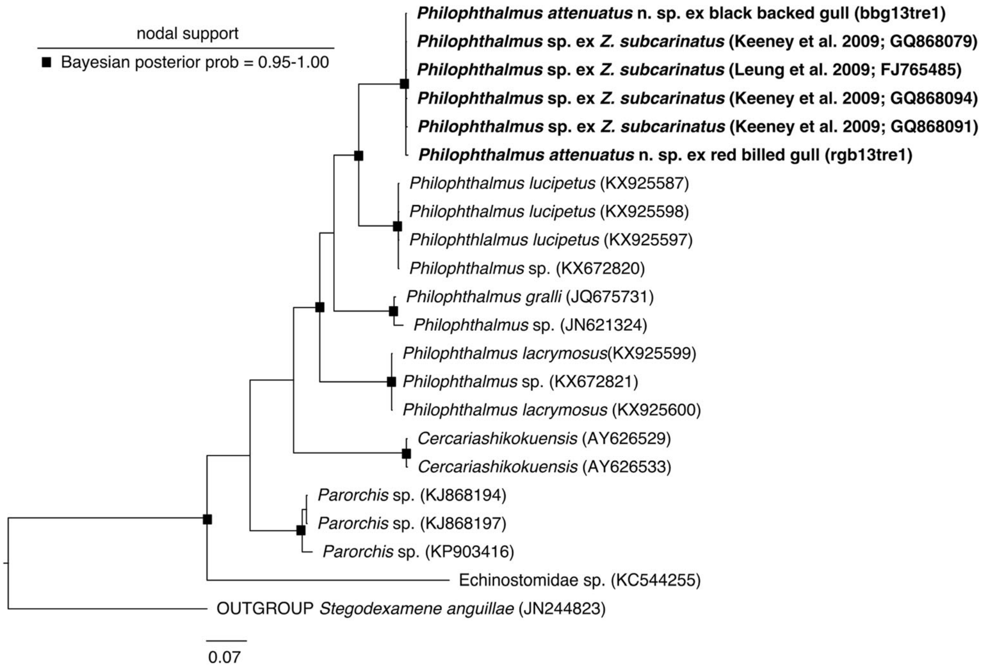

Fig. 3 Bayesian 50% majority-rule inference for the *cox1* gene dataset. The outgroup is *Stegodexamene anguillae* (Lepocreadiidae). The scale bar indicates the number of substitutions per site

gulls, the metacercariae excyst and migrate from the mouth to eye membranes where they mature.

Adult *P. attenuatus* n. sp. specimens from New Zealand are morphologically most similar to *P. burrili* from Australia suggesting that the latter species is its closest relative. The original description of *P. burrili* was from the intermediate host *Batillaria australis* (Quoy & Gaimard, 1834) [then known as *Velacumantus australis*] and silver gulls (Howell and Bearup 1967). Both of these hosts are closely related to their respective New Zealand counterparts, *Z. subcarinatus* and red-billed gulls, so it would be reasonable to assume that *P. attenuatus* n. sp. and *P. burrili* are sister species, one arising from the other with the spread of the host species. The Australian silver gull (*C. novaehollandiae*) gave rise to the New Zealand red-billed gull (*C. scopulinus*) after colonisation from Australia to New Zealand, approximately 130,000 years ago (Given et al. 2005). Conversely, the black-backed gull *L. dominicanus* colonised Australia via New Zealand (Woehler et al. 2014) as recently as the 1940's (McGill 1943) (only c.20 years before Howell and Bearup's 1967 description), and it seems unlikely that the two parasite species have diverged in that short space of time. Therefore, we postulate that if speciation was concurrent with the definitive host, it was most likely to have been in the *Chroicocephalus* gulls.

However, the intermediate host has also had a potentially relevant history. *Zeacumantus subcarinatus* is native to New Zealand but, probably due to trans-Tasman shipping in the 1920–30's, invaded and colonised intertidal areas around Sydney, Australia (Powell 1979). If the ancestor of *P. burrili* and *P. attenuatus* n. sp. accompanied *Z. subcarinatus* to Sydney from New Zealand it is likely that the description of *P. burrili* (Howell and Bearup 1967; Lake Burril and Narrabeen, Sydney) is a species of New Zealand origin, not Australian, and that it came to infect *Velacumantis australis* in a host switching event subsequently. Genetic data are required from specimens of *P. burrili* from Australia in order to confirm the relatedness of the two species and their evolutionary history.

Our specimens from the eyes of gulls showed a large range of sizes, with even the smaller specimens carrying eggs. However, while in mature specimens eggs in at least the anterior uterine field contained developed miracidia (i.e. with visible eyespots), in immature specimens any eggs present were not developed. Specimens thus defined as 'mature' and 'immature' were measured separately from each other herein (Table 2). It is notable that most metrics appear to increase with the length of the worm, except for those of the gonads. While the testes are yet to develop to their full size in immature worms, the ovary is larger at this stage than in mature worms, as illustrated by the ratio of the widths of the anterior testis to the ovary (Table 2). Differential growth patterns of reproductive organs have been demonstrated in *P. andersoni* and *P. larsoni* (Dronen and Fried 2008) with both testes and ovary decreasing in size after 70 days old in *P. andersoni* and continuing to increase in size in *P. larsoni*. In *P. attenuatus* n. sp., it appears that the ovary develops in advance of the testes, an interesting finding that could only be tested by experimental infections in which the exact age of the infection is known.

There has been much discussion in the literature about characters that were traditionally used for species delimitation that have been found in several cases to be highly labile or variable, rendering many species invalid (e.g. Ching 1961;

Penner and Fried 1963; Nollen and Kanev 1995; Dronen and Fried 2008). For instance, the vitelline field has been described as ‘follicular’ or ‘tubular’ and this has traditionally been used to distinguish between species (Nollen and Kanev 1995). However, in our specimens both types of vitellaria were consistently found (Fig. 2). Other authors have shown that the vitellaria start in the tubular state and, as the worm ages, become increasingly follicular (Dronen and Penner 1975; Dronen and Fried 2008). Also important in species delineation has been the extent of the cirrus sac, whether or not it extends below the level of the ventral sucker. It has been demonstrated for some species that this is a character variable according to age, becoming longer as the worm matures (Heneberg et al. 2018), and reducing in size in older specimens (Dronen and Penner 1975). For *P. attenuatus* n. sp., even the most immature specimens have a cirrus sac that extends posterior to the level of the ventral sucker, so this appears to be a reliable diagnostic character for this particular species.

Some species of *Philophthalmus* are known to cause ophthalmic disease due to irritation and secondary infection, resulting in impairment of vision, including blindness, physical weakness (Mukaratirwa et al. 2005) and, in the case of juvenile female Galapagos sea lions, death (Phillips et al. 2018). In other cases, species of *Philophthalmus* cause no clinical signs of eye damage (Heneberg et al. 2018). Heneberg et al. (2018) examined gulls naturally infected with *Philophthalmus lacrymosus* and *P. lucipetus*, and found that *P. lacrymosus* inhabited the conjunctivital sac and caused severe eye damage whereas *P. lucipetus* inhabited the nictitating membrane and had no deleterious effect on the host eye. Like *P. lucipetus*, *P. attenuatus* n. sp. is found in the nictitating membrane and none of the birds examined in this study exhibited signs of eye damage.

National concern was raised by Howell (1965) for human philophthalmiasis, but as of 2018 no case in New Zealand has been reported. Human cases have been reported in Europe, Asia and North America (Marković 1939; Dissanaike and Bilimoria 1958; Kalthoff et al. 1981; Gutierrez et al. 1987; Mimori et al. 1982; Lang et al. 1993), sometimes occurring in localised areas surrounding small streams and water bodies frequented by birds (Dissanaike and Bilimoria 1958; Lang et al. 1993). Despite high numbers of black-backed gulls (Robertson and Bell 1984) and high prevalence of *P. attenuatus* n. sp. in mudsnails (Martorelli et al. 2008 found infection levels of up to 30% in some areas) in New Zealand, the zoonotic risk to humans of this parasite is likely to be low.

In conclusion, we have morphologically compared New Zealand *Philophthalmus* species with descriptions of the other marine species, including the Australian *P. burrili*, and conclude that the specimens found in New Zealand gulls are a new species, *P. attenuatus* n. sp. We have provided molecular evidence that completes the life cycle of *P. attenuatus* n. sp. infecting *Z. subcarinatus* as an intermediate host, and black-backed and red-billed gulls as definitive hosts in New Zealand’s marine environment.

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Compliance with ethical standards

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

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

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